An Australian Government Initiative

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Dissolved copper in freshwater

Technical brief

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The default guideline values (DGVs) and associated information in this technical brief should be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (www.waterquality.gov.au/anz-guidelines).

Copper is widely distributed in the earth's crust and is an essential trace element for micro-organisms, plants and animals. It is commonly used in roofing, plumbing, electrical wiring and electronics. Copper is also used as a biocide in antifouling paints, wood preservatives, fungicides and algaecides. The major anthropogenic sources of copper for freshwater environments include municipal wastewater discharges, mining and mineral processing, metal and electrical manufacturing, fungicide use, and stormwater.

Since the last revision of the freshwater copper DGVs (i.e. ANZECC/ARMCANZ 2000), new data have become available, including high quality local species data. Furthermore, there is increased understanding of the effects of dissolved organic carbon (DOC), hardness and other toxicity modifying factors on copper toxicity. Increases in DOC reduce the aquatic toxicity of copper, as copper binds to DOC, decreasing the available free copper concentrations. Previously used hardness corrections for copper were not protective of sensitive invertebrate and algal species (Markich et al. 2005). Consequently, this update of the copper DGVs replaces water hardness corrections with DOC corrections.

Very high reliability DGVs for dissolved copper in freshwater were derived from chronic toxicity data for 59 species (comprising 15 fish, 18 molluscs, 11 crustaceans, two insects, one rotifer, one cnidarian, three macrophytes, six green microalgae, one fungus and one bacterium), with a good (visual) fit of the species sensitivity distribution (SSD) to the toxicity data. Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values lists all chronic toxicity data used in the derivation. The DGVs (at DOC of 0.5 mg/L) for 99%, 95%, 90% and 80% species protection are 0.20 µg/L, 0.47 µg/L, 0.73 µg/L and 1.3 µg/L, respectively. The DGVs can be adjusted to the DOC concentrations of ambient waters (up to 30 mg/L DOC). The 95% DGV for dissolved copper in freshwater is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

The Australian and New Zealand guidelines for fresh and marine water quality (ANZG 2018) provides guidance for evaluating monitoring data against DGVs and recommends a decision scheme that includes consideration of the bioavailable fraction. Such guidance for metals, presented as a decision tree, is provided in Appendix B: Actions to assess the bioavailable fraction of a metal.

1 Introduction

Copper is a naturally occurring metallic element. It is an abundant trace element, present in the Earth's crust at approximately 50 ppm (Landner & Reuther 2004). It is found as the native metal but predominantly in the form of: sulfide minerals chalcopyrite ($CuFeS_2$), chalcocite (Cu_2S) and bornite (Cu_5FeS_4); copper carbonates azurite ($Cu_3(CO3)_2(OH)_2$) and malachite ($Cu_2CO_3(OH)_2$); and the Cu^+ oxide mineral cuprite (Cu_2O). The major copper mines are in the Americas (particularly Chile), Australia and Europe (particularly Russia, Poland and Sweden) (Landner & Reuther 2004, Schoeters et al. 2008).

Copper has been used for centuries as jewellery, vessels, currency and in tools (Landner & Reuther 2004). Current uses include architectural structures, roofing, plumbing and electronics (European Copper Institute 2008). Copper is also used as a biocide in antifouling paints, wood preservatives, fungicides and algaecides (European Copper Institute 2008). The major anthropogenic sources of copper in aquatic environments are municipal wastewater discharges, mining and mineral processing, metal and electrical manufacturing, anti-fouling paints, fungicide use, and stormwater (which can include copper from sources such as architectural surfaces (Pennington & Webster-Brown 2008) and vehicle brake linings (McKenzie et al. 2009)).

Copper is a D-block element and a transition metal—as such, it has more than one oxidation state (Stumm & Morgan 1996, European Copper Institute 2008). The principal states are cuprous (Cu^+) and cupric (Cu^{2+}). These are found as salts such as Cu^{2+} sulfate pentahydrate ($CuSO_4$ $5H_2O$) and Cu^+ oxide (Cu_2O). Cu^+ is unstable in aqueous media and will oxidise to Cu^{2+}, which typically binds to inorganic and organic ligands, such as iron oxides or humic acids. In water, sediment and soil, the binding affinities of Cu^{2+} with inorganic and organic matter depend on pH, the oxidation-reduction potential in the local environment, and the presence of competing metal ions and inorganic anions.

Background concentrations of copper in freshwater can be extremely low; in some New Zealand lakes and rivers, concentrations measure 0.04–0.5 µg/L (Ahlers et al. 1991, Reid et al. 1999, Sander et al. 2013)—low by global standards (Reid et al. 1999). However, concentrations in urban streams range from <1 µg/L to 15 µg/L and higher during storm events (Gadd et al. 2014, Allinson et al. 2017, Shi et al. 2019). In freshwater streams receiving untreated discharges from historical and contemporary mines, concentrations can exceed 100 µg/L (Smith & Williamson 1986, Mudd & Patterson 2010, Mackay & Taylor 2013) and, in some cases, can be found in the hundreds of milligrams per litre range (e.g. NTEPA 2014).

This technical brief provides updated default guideline values (DGVs) for dissolved copper in freshwater, which supersede the ANZECC/ARMCANZ (2000) DGVs. The current derivation has added new data published since 2000, including chronic data for Australasian species. The hardness corrections applied to the ANZECC/ARMCANZ (2000) DGVs have been replaced with dissolved organic carbon (DOC) corrections. The rationale for this change is detailed in Section 3 and also in Gadd (2021).

2 Aquatic toxicology

2.1 Mechanisms of toxicity

Copper is an essential nutrient for plants, animals and humans. It is required in at least 12 major proteins, and deficiency of copper can lead to impaired metabolic functions and reduced growth (IPCS 1998). This is observed in copper deficient soils, with adverse effects on livestock occurring in the absence of dietary copper supplementation. However, at higher concentrations (e.g. ≥microgram per litre concentrations) copper is acutely and chronically toxic, particularly to aquatic organisms. In addition to acute effects such as mortality, chronic exposure to excess copper can alter brain function, enzyme activity, blood chemistry, and metabolism, which lead to adverse effects on growth, reproduction and survival.

Copper toxicity occurs because copper can: block essential functional groups of biomolecules; displace essential metal ions in biomolecules; and form free oxyradicals causing oxidative stress (IPCS 1998).

For fish and invertebrates, acute and chronic copper toxicity are primarily caused by disturbance of sodium ion regulation, with the gill and gut tissues the primary targets for metal uptake, binding and toxicity. In acute exposures, this results in loss of sodium (and chloride), which creates an osmotic imbalance and leads to cardiovascular collapse and death (Van Sprang et al. 2008). In chronic exposures, organisms can (to an extent) recover their ionoregulatory balance; however, there is a metabolic cost of this, which may reduce growth and reproduction (Meyer et al. 2007). In fish, copper also affects the olfactory system, which can alter a fish's ability to avoid water with high copper concentrations.

For unicellular algae, the mechanism of copper toxicity is different to fish and is thought to be through changes in membrane potential and permeability or through competition with essential metals for binding and uptake (Stauber & Davies 2000, Van Sprang et al. 2008). Within algal cells, copper can inhibit enzymes and change intracellular pH (Stauber & Davies 2000). In algae and plants, copper is an inhibitor of photosynthesis and growth; thus, it has been widely used in herbicides.

As there are different mechanisms of copper toxicity between fish, invertebrates, plants and algae, copper toxicity datasets may exhibit bimodality or multimodality (Section 4.2).

2.2 Acute and chronic toxicity

Acute toxicity values (LC50) for freshwater species range from 0.5 µg/L for *Daphnia magna* mortality (Brix et al. 2001) to 84 600 µg/L for the fish *Notemigonus crysoleucas* (USEPA 2007). The cladoceran taxonomic group is one of the most sensitive to copper, with species mean acute values of 2.7 µg/L, 5.9 µg/L and 6.0 µg/L reported for *D. pulicaria*, *Ceriodaphnia dubia* and *D. magna*, respectively, based on based on copper data normalised to pH of 7.5, calcium of 14 mg/L, magnesium of 12 mg/L (hardness of 84 mg/L as $CaCO_3$) and DOC of 0.5 mg/L using the biotic ligand model (BLM) (USEPA 2007) (see Section 3 for details of factors affecting copper toxicity).

Chronic toxicity (e.g. as expressed by NOECs for long-term tests) is typically less than 10-fold higher than acute toxicity. Acute-to-chronic ratios calculated by USEPA (2007) were mainly in the range of 2:1 to 6:1, though higher ratios were also reported (e.g. 171:1 for freshwater snail *Campeloma decisum*). The chronic toxicity data compiled for the USEPA (2007) water quality criterion (WQC) for copper ranged from 2.8 µg/L (EC20, survival) for the cladoceran *Daphnia pulex* to 60 µg/L (maximum acceptable toxicant concentration, biomass) for the northern pike *Esox lucius*. Most of the data used in USEPA (2007) date from the 1970s and 1980s, with only two studies conducted more recently (i.e. 1990–2001). Several higher values, including a NOEC of 138 µg/L for *Chlorella vulgaris* (based on growth in a 3 d test), were included in the data compiled for the European Union risk assessment (Van Sprang et al. 2008), which included species outside of North America and studies undertaken in the 1990s and 2000s. The most recent data compilation (Brix et al. 2017) included lower NOEC values of 1.3 µg/L for *C. dubia* (reproduction), 1.9 µg/L for *Pimephales promelas* (early life stage test on growth), <2.0 µg/L for the freshwater mussel *Lampsilis siliquoidea* (survival, 28 d test on juveniles), and an EC20 of 2.7 µg/L for the fish *Acipenser transmontanus* (biomass, 28 d test on larvae).

The available toxicity data indicate that there is marked overlap in the sensitivity to copper between different taxonomic groups. Molluscs appear to be consistently sensitive, with negligible effect values typically below 10 µg/L. Cladocerans are the most sensitive organisms in some studies but not others. The European Union compilation of NOEC values (Van Sprang et al. 2008) suggested that algae and plants are less sensitive than invertebrates and fish; however, the majority of the algae data is from tests with DOC of ≥5 mg/L, whereas other tests on invertebrates and fish mostly used lower DOC.

For fish, the genus *Oncorhynchus* has been reported as the most sensitive fish genus based on acute toxicity (USEPA 2007). In 58 d chronic tests, the embryo life stage of *O mykiss* appeared to be more sensitive than the juvenile life stage, with EC10 values for growth of 10 µg/L and >22 µg/L, respectively (Besser et al. 2001). However, larvae of *A. transmontanus* were considerably more sensitive in chronic tests (EC10 of 1.2 µg/L in 53 d growth test (USGS 2014)), as were larvae of *Etheostoma fonticola* (IC10 of 7–9 µg/L, 30 d survival and biomass tests (Besser et al. 2001)).

Studies with freshwater mussels (Order: Unionidae) have shown that the larval (glochidia) and juvenile life stages are extremely sensitive to copper, and can be more sensitive than other species commonly used in toxicity testing, including cladocerans. For three North American species, 28 d tests provided IC10 values from <3.1 µg/L to 4.9 µg/L for survival and 5.5 µg/L to 6.3 µg/L for growth (Wang et al. 2007). Tests with Australian and New Zealand species suggest they are more sensitive than North American species: Markich (2017) reported NECs of 0.74–1.3 µg/L for six freshwater mussel species based on cell closure in 72 h exposure tests, while Clearwater et al. (2014) reported 48 h EC20s for mortality of 1.2–2.7 µg/L for the glochidial life stage of the native New Zealand freshwater mussel *Echyridella menziesii*.

Algae and plants are known to be sensitive to copper, although there is a considerable variation in the toxicity values, which range from 1 µg/L to 8 000 µg/L (USEPA 2007). Algal isolates from Papua New Guinea are the most sensitive to copper in 3 d growth tests, with EC10 values of 0.45 µg/L and 0.9 µg/L for *Monoraphidium arcuatum* and *Chlorella* sp., respectively (Adams et al. 2018). Other studies have found *Chlorella* sp. to be slightly less sensitive than this, with IC50s for cell division ranging from 3.4 µg/L to 46 µg/L (Langdon et al. 2009). For the Norwegian isolates of the green

microalga *Raphidocelis subcapitata*, NOEC values for growth rate have been reported between 0.3 µg/L (Levy et al. 2009) and >100 µg/L (De Schamphelaere et al. 2003, Franklin et al. 2004, Heijerick et al. 2005) depending on the testing laboratory and the chemistry of the test water. Although there have been fewer studies examining toxicity to vascular plants, the available studies suggest some vascular plants are as sensitive as unicellular algae and invertebrates. For example, an EC10 value of 15 µg/L was reported for *Lemna minor* for a 7 d growth test (Naumann et al. 2007), though other researchers testing this species report values >100 µg/L (Sobrero et al. 2004).

3 Factors affecting toxicity

3.1 Copper speciation

The toxicity of copper depends on its form—whether it is freely dissolved, an inorganic complex, an organic complex, or associated with particulates. Freely dissolved copper is the most toxic form as it is the most bioavailable, whereas most inorganic and organic complexes are less bioavailable and, hence, less toxic. Particulate-associated copper has low bioavailability, particularly to bacteria and phytoplankton. Based on this, previous water quality guidelines (i.e. USEPA 1996, ANZECC/ARMCANZ 2000) have recommended that, rather than comparing total copper to numeric criteria, the dissolved form of copper should be used (operationally defined as the <0.45 µm filtered fraction).

Dissolved copper includes 'free' copper, which exists predominantly as the cupric ion (Cu^{2+}) weakly associated with water molecules ($Cu.nH_2O^{+2}$), but this species is usually a small percentage of dissolved copper at pH >6.5 (Stumm & Morgan 1996). At more alkaline pH, typically over 90% of dissolved copper is bound to inorganic or organic ligands such as humic acids, fulvic acids, hydroxides, and carbonates, which are less toxic than free Cu^{2+} (Van Sprang et al. 2008). In natural water, the organic ligands are the most important ligands for copper binding (Stumm & Morgan 1996); complexation with dissolved organic matter (DOM; typically referred to as DOC as it contains ~50% carbon by mass (Duarte et al. 2016)) increases as the pH and concentration of DOC are increased, and as the concentrations of competing ions are decreased (Stumm & Morgan 1996). As the speciation of dissolved copper is dependent on aspects of water chemistry (e.g. pH, humic acids, carbonates), the toxicity of copper varies between waterbodies with different water chemistry (see Section 3.2).

3.2 Toxicity modifying factors

Factors affecting copper toxicity typically involve changes to copper speciation or competition with copper at biological uptake sites, as discussed below.

Competition between copper and other cations (e.g. calcium and magnesium—together represented by water hardness) for biological uptake sites can reduce the uptake of copper by some organisms. The effect of competition is demonstrated by the reduction in copper toxicity as water hardness increases, as shown for many species (e.g. rainbow and bull trout (Hansen et al. 2002), or see reviews in Hunt (1987), Campbell (1995), Allen & Hansen (1996) and Paquin et al. (2002)). However, water hardness does not influence copper toxicity to all species, as demonstrated for the cladocerans *C. dubia* (Hyne et al. 2005, Markich et al. 2005) and *D. magna* (De Schamphelaere & Janssen 2002),

the macrophyte *Ceratophyllum demersum* (Markich et al. 2006) and the green microalgae *R. subcapitata* (De Schamphelaere et al. 2003) and *Chlorella* sp. (Markich et al. 2005).

The pH of a waterbody can affect metal toxicity through two opposing mechanisms:

- pH influences metal speciation, usually resulting in increased concentrations of bioavailable metal species at lower pH
- the H^+ ion, which is more abundant at lower pH, competes with metals for biological uptake sites.

These mechanisms can result in contradictory effects of pH on the aquatic toxicity of metals. In a meta-analysis of chronic copper toxicity data, Meyer et al. (2007) found positive correlations between pH and chronic toxicity values (i.e. as pH decreases, toxicity increases) that were statistically significant for *P. promelas*, marginally statistically significant for *D. pulex*, and not significant for *D. magna.* In fact, in individual studies for *D. magna*, the EC50 values increased (i.e. toxicity decreased) as pH decreased (De Schamphelaere & Janssen 2004). In many studies, the effects of pH are confounded by covarying alkalinity and/or hardness that may also affect copper bioavailability and toxicity. As changes in pH may also release metals sorbed to particulate phases or remove metals from the dissolved phase, models that consider the whole system (not only dissolved phases) may be required to better understand the role that pH plays in copper bioavailability and toxicity (Smith et al. 2015).

Water temperature may affect metal toxicity due to increased metabolic rates and increased respiratory inflows (Khangarot & Ray 1989, Meyer et al. 2007). For copper, correlations that indicate higher toxicity at higher temperature have been reported for *D. magna* and *P. promelas* but not for *O.* mykiss (Meyer et al. 2007).

DOC in waterbodies reduces copper bioavailability by forming copper-organic complexes that have low or no bioavailability. Lower toxicity in the presence of DOC has been reported for many test organisms including fish (*O. mykiss*, *P. promelas*) and invertebrates (*D. magna, D. pulex*), as reviewed in Van Sprang et al. (2008). The same effect has also been shown for Australasian species. The toxicity of copper to the Australian freshwater shrimp *Paratya australiensis* reduced approximately two-fold as the concentration of DOC increased two-fold (Daly et al. 1990). Markich et al. (2003) reported greater sensitivity of the freshwater mussel *Hyridella depressa* to copper at low DOC.

3.3 Accounting for toxicity modifying factors

The inverse relationship between water hardness and toxicity was the basis of a hardness function in the USEPA ambient WQC from 1984 to 2007, whereby the criterion was higher when hardness levels were higher (criterion continuous concentration = $e^{(0.8545[\ln(\text{hardness})]-1.465)}$). The slope for the hardness equation used in the USEPA (1996) derivation was adopted for the ANZECC/ARMCANZ (2000) copper in freshwater DGV derivation. However, since then, conflicting results have been published about the effect of hardness on the toxicity of copper to various species, including sensitive native cladocerans and green microalgae (Markich et al. 2005). Based on these and other similar findings, there was concern that hardness corrections would not protect sensitive freshwater species; therefore, the current method for deriving DGVs (Warne et al. 2018) recommended that the hardness correction for copper not be used.

Since the early 2000s, biotic ligand models (BLMs) have been developed to account for the effect of toxicity modifying factors, including temperature, hardness, pH and DOC. The BLM incorporates the influences of these factors on speciation and competition by predicting metal accumulation at the gill (or other biological uptake site, known as the biotic ligand) of an aquatic organism (Di Toro et al. 2001), in an adaptation of the gill-surface interaction model originally proposed by Pagenkopf (1983) and the free ion activity model (e.g. Campbell 1995). USEPA (2007) uses a BLM derived from acute toxicity data for fish and cladocerans to calculate the copper WQC (acute and chronic) at site-specific values for 10 factors: temperature, pH, DOC, calcium, magnesium, sodium, potassium, sulfate, chloride, and alkalinity. The European Union also incorporated a BLM approach into its risk assessment for copper (Van Sprang et al. 2008), using BLMs based on chronic toxicity data for fish and cladocerans (with slight differences in the model constants), to derive chronic predicted no effect concentrations . Most recently, Environment and Climate Change Canada has incorporated a BLM adjustment in its copper guideline value, based on chronic toxicity data for fish, cladocerans and algae (ECCC 2021).

Brix et al. (2017, 2020, 2021) have proposed an alternative approach using multiple linear regression (MLR) models to account for toxicity modifiers. MLR models have been used for the proposed Australia and New Zealand water quality DGV for nickel in freshwater (Stauber et al. 2021), the Canadian water quality guideline for zinc in freshwater (CCME 2018), and the USEPA (2018) ambient WQC for aluminium in freshwater.

As no BLM or MLR models for copper toxicity have been validated for Australian or New Zealand water quality conditions and species, an adjustment based on DOC concentration is used for the current DGVs. Of the modifying factors included in the BLMs and MLRs for copper, DOC is the most influential factor for determining copper toxicity, and it is the only factor that is consistent in all models and species (whereas hardness mitigates toxicity for some invertebrates and fish but not all, and pH influences algal toxicity differently to fish and invertebrates) (Gadd 2021). The DOC adjustment is similar to the previously used correction for hardness in ANZECC/ARMCANZ (2000), and it is used to standardise the test waters of the toxicity dataset to a low DOC concentration prior to DGV derivation and to calculate DGVs at differing concentrations of DOC. The basis of the DOC correction is summarised below, with further details provided in Gadd (2021).

The relationship between DGVs and DOC varies at different values of pH and hardness. Slope factors for DOC from log-log relationships between water quality guideline values and DOC were calculated by Gadd (2021) based on the Canadian (ECCC 2021) and European (Van Sprang et al. 2008) BLMs, both of which are based on chronic toxicity data for fish, invertebrates and algae/plants. The Canadian model provided slightly lower, more conservative, slope factors for almost all combinations of pH and hardness. These slope factors were used to normalise all toxicity data to a standard DOC (0.5 mg/L) before calculating a DGV for dissolved copper. A slope factor of 0.977 based on a pH of 6.5 and hardness of 11.6 mg/L was selected for the adjustment of the current DGVs (Gadd 2021; also see Section 4.4).

4 Default guideline value derivation

The DGVs were derived in accordance with the method described in Warne et al. (2018) and using Burrlioz 2.0 software.

4.1 Collation and screening of toxicity data

The default guideline values for copper in freshwater were derived based on data from: recent guideline value derivations by ECCC (2021), BCMECCS (2019), Brix et al. (2021); European copper risk assessment (Van Sprang et al. 2008); ANZECC/ARMCANZ (2000); ECOTOX database (USEPA 2016); and compilations of Australasian toxicity data (Markich et al. 2002, Langdon et al. 2009). Additional international data were collated through searches using the journal abstracting service 'Web of Science' for studies published during 2015–2016 and not included in the ECOTOX database. Australian and New Zealand toxicity data (up to 2020) were included through internet searches for toxicity data contained within grey literature, theses or unpublished reports.

Although there is a large number of published data on copper toxicity, not all data met the preferred requirements and associated acceptability criteria for the DGV derivation. Data were only included for studies that had measured the copper concentrations of the test solutions, or the stock solutions used to produce the test solutions, and that provided clear evidence that a concentration–response relationship was observed. Although some studies reported concentrations as total copper, all copper was assumed to be in dissolved form in the test solutions given that laboratory toxicity test solutions typically have low particulate concentrations; therefore, the DGVs are representative of dissolved copper concentrations.

As there were sufficient chronic negligible effects data (i.e. NEC, EC10–20, LC10–20, NOEC) to meet the minimum data requirements, no acute or chronic data that required conversion to negligible effects values (e.g. LC50, EC50, LOEC) were needed to supplement the toxicity dataset.

Of the chronic negligible effects data collated for the derivations, the following were excluded. Some toxicity data from other jurisdictions (e.g. ECCC 2021) were excluded for reasons such as the type of reported statistic (e.g. EC50 value) or the age of the study (studies prior to 1980 are not recommended by Warne et al. (2018)). Because hardness and pH may affect copper toxicity, studies were excluded if they were conducted under conditions of low bioavailability (i.e. hardness >200 mg/L as $CaCO_3$) or where pH was within a range that may cause the organism stress (<6 or >8.5). Studies that reported hardness, or the concentrations of calcium and magnesium, were included in the derivation. Studies that did not report pH were included in the derivation as it was assumed that most standard laboratory synthetic test waters would have circumneutral pH. The exclusion rules resulted in some chronic data for some sensitive Australasian species being excluded from the DGV derivation (Table 1). The most sensitive species excluded were the New Zealand freshwater snail *Potamopyrgus antipodarum* and the protozoan *Trachelomonas* sp.

Data for several Australian tropical species were included in the derivation despite exposure durations being less than recommended by Warne et al. (2018) for chronic tests. However, these test duration recommendations are for temperate species, and Warne et al. (2018) acknowledged that there was scope to relax them for tropical species. Studies on the larval (glochidial) stage of freshwater mussels (Clearwater et al. 2014, Markich 2017) were also included despite the test durations being 48–72 h. It is reasonable to consider the glochidial stage as a critical early life stage, similar to a larval development effect on an oyster or sea urchin. As the test duration was greater than or equal to the 48 h minimum for early life stage larval development/metamorphosis tests required by Warne et al. (2018), these study data were accepted as chronic.

Table 1 Summary of chronic toxicity values, chronic data for Australasian species excluded from the default guideline value derivation for copper in freshwater

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value (µg/L)	Reason for exclusion	Reference
Mollusc	*Potamopyrgus antipodarum*	Juvenile / adult	77–112	NOEC (growth)	5	No hardness data	Dorgelo et al. (1995)
Crustacean	*Paratya australiensis*	Juvenile	21	EC50 (growth)	59	Only EC50 data reported, no NOEC	Bacher & O'Brien (1990)
Macrophyte	*Ipomoea aquatica*	Seedling	14	NOEC (growth)	2 500	Nominal data only; water chemistry not reported	Wu & Sun (1998)
Green microalga	*Staurastrum chaetoceras*	Exponential growth phase	1–4	EC50 (population biomass)	19–774	Only EC50 data reported; no NOEC	Ivorra et al. (1995)
Protozoan	*Trachelomonas* sp.	Exponential growth phase	2–3	EC50 (population growth rate)	5–10	Only EC50 data reported; no NOEC	Franklin et al. (2004)
Protozoan	*Trachelomonas* sp.	Exponential growth phase	27	NOEC (population biomass)	160	No hardness data	Le Jeune et al. (2007)

4.2 Toxicity data used in derivation

Most of the data sourced from the ANZECC/ARMCANZ (2000) guidelines, the compiled Australasian toxicity data (Markich et al. 2002, Langdon et al. 2009), the European risk assessment (Van Sprang et al. 2008), the USEPA WQC (USEPA 2007), and the Canadian Federal Environmental Quality Guidelines (ECCC 2021) were considered to have already been assessed for quality and considered to be acceptable. All remaining data were quality assessed based on Warne et al. (2018), and only acceptable quality data were included.

There were 396 chronic toxicity values for 59 species that were considered to be of suitable quality for use in the DGV derivation. Of these, approximately 40% (152 values for 34 species) were of the most preferred type of toxicity estimates (i.e. NECs, ECx/ICx/LCx values where x is ≤10, and bounded effect concentrations (BECs) where the effect is ≤10% (Warne et al. 2018)): six were NECs, 143 were EC/IC/LC10s and three were BEC10s. Lesser preferred toxicity estimates included 76 EC/LC11–20s, 151 NOECs and 17 minimum detectable effect concentrations (MDECs) from 36 species.

Warne et al. (2018) recommend using only preferred chronic toxicity values where there are sufficient (e.g. >8) such values. The dataset based on only the preferred values comprised 34 species from seven taxonomic groups. However, to increase the number and diversity of species represented in the dataset used for the DGV derivation, the preferred data were supplemented with EC11–20 and NOEC data. This resulted in data for 59 species, including 21 species native to Australia and/or New Zealand, from 10 taxonomic groups. There was only a 1.2-fold to 1.7-fold difference in the protective

concentrations (i.e. 80%, 90%, 95%, 99% species protection) between the 34 species dataset and the 59 species dataset (Gadd 2021). Thus, the larger dataset was selected to derive the DGVs.

Toxicity data based on DOC >0.5 mg/L were adjusted to a DOC of 0.5 mg/L based on a slope factor closest to the pH and hardness concentration of the test waters (Gadd 2021) using the equation in Figure 1. Data from tests conducted at DOC ≤0.5 mg/L, or where DOC was not reported and was assumed to be ≤0.5 mg/L, were not adjusted (approximately 60%). The slope factors ranged from 0.977 to 1.08 and had the effect of reducing the majority of the toxicity values.

$$Normalised\ EC10 = EC10 \div \left(\frac{DOCtest}{0.5}\right)^{slope\ factor}$$

Figure 1 Equation for adjusting to DOC of 0.5 mg/L based on slope factor

The acceptable toxicity data were summarised to single species values for use in the species sensitivity distribution (SSD), by either calculating geometric means or selecting the value for the most sensitive endpoint, life-stage and toxicity test duration for each species, based on Warne et al. (2018). A summary of the toxicity data (one value per species) used to calculate the DGVs for copper in freshwater is provided in Table 2. The 59 species included in the SSD (see Table 2 and Figure 2) were from 10 taxonomic groups: bacteria (one species), cnidarians (one species), rotifers (one species), fungi (one species), insects (two species), macrophytes (three species), green microalgae (six species), crustaceans (11 species) fish (15 species) and molluscs (18 species). The toxicity values in the SSD ranged over approximately three orders of magnitude, from 0.3 µg/L for *Moinodaphnia macleayi*, normalised from an EC10 of 1.0 µg/L (6 d reproduction tests) (Trenfield et al. 2022) to 180 µg/L for a groundwater-isolated strain of *Penicillum* (21 d growth test) (Lategan & Hose 2014). Further details of the water quality parameters for each single species value used to calculate the DGVs are presented in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

As the different mechanisms of copper toxicity suggest the potential to exhibit bimodality or multimodality, the toxicity dataset was assessed for this following the weight of evidence approach recommended in Warne et al. (2018). Although copper is a well-known algaecide and fungicide, algal and macrophyte toxicity values were spread evenly across the SSD, and the fungal species was the least sensitive of all included (Figure 2). The data were not indicative of bimodality based on histogram statistics, a bimodality coefficient of 0.37 for the log-transformed dataset (less than the threshold criterion for bimodality of 0.55), and the even spread of taxa (from toxicity studies spanning four decades) in the SSD (Figure 2). Therefore, the dataset was deemed to be unimodal, and all the toxicity data (i.e. from 59 species) were used for the derivation.

Table 2 Summary of single chronic toxicity values, all species used to derive default guideline values for copper in freshwater

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value normalised to 0.5 mg/L DOC (µg/L)
Fish	*Acipenser transmontanus*	Larva	53	EC10 (growth)	1.1
	Cirrhinus mrigala	Juvenile	60	NOEC (growth)	100
	Cottus bairdi	Juvenile	21–28	NOEC (mortality)	8.5
	Cyprinella monacha	Larva	30	IC10 (growth)	23
	Etheostoma fonticola	Larva	30	IC10 (biomass)	8.1
	Galaxias maculatus	Juvenile	21	NOEC (mortality)	18
	Gobiomorphus cotidianus	Juvenile	18	NOEC (mortality)	10
	Melanotaenia splendida inornata	Adult	30	NOEC (reproduction)	12
	Mogurnda mogurnda	Larva	7	EC10 (growth)	3.5
	Oncorhynchus kisutch	Juvenile	60	NOEC (growth)	3.6
	Oncorhynchus mykiss	Juvenile	30	LC10 (mortality)	5.5
	Pelteobagrus fulvidraco	Juvenile	42	NOEC (growth)	71
	Perca fluviatilis	Juvenile	30	NOEC (growth)	19
	Pimephales promelas	Larva	30	IC10 (growth)	8
	Prosopium williamsoni	Embryo	90	NOEL (growth)	1.0
Mollusc	*Alathyria profuga*	Larva	3	NEC (development)	1.2
	Amerianna cumingi	Adult	4	EC10 (reproduction)	1.4
	Cucumerunio novaehollandiae	Larva	3	NEC (development)	0.7
	Echyridella menziesii	Larva	2	EC20 (mortality)	0.4
	Epioblasma capsaeformis	Juvenile	28	IC10 (mortality)	3.1
	Fluminicola sp.	Adult/juvenile	28	NOEC (mortality)	5.5
	Fontigens aldrichi	Adult/juvenile	28	EC10 (mortality)	7.1
	Hyridella australis	Larva	3	NEC (development)	0.8
	Hyridella depressa	Larva	3	NEC (development)	0.9
	Hyridella drapeta	Larva	3	NEC (development)	1.0
	Juga plicifera	–	30	NOEC (mortality)	6.0
	Lymnaea stagnalis	–	30	EC20 (growth)	0.7
	Physa gyrina	Juvenile	28	EC10 (growth)	10
	Pomacea paludosa	Adult/juvenile	60	NOEC (growth)	8.8
	Pyrgulopsis robusta	Larva/juvenile	28	EC10 (mortality)	4.9
	Taylorconcha serpenticola	Adult/juvenile	28	LC20 (mortality)	11
	Velesunio ambiguus	Larva	3	NEC (development)	1.3
	Villosa iris	Adult/juvenile	28	EC10 (growth)	1.5

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value normalised to 0.5 mg/L DOC (µg/L)
Crustacean	*Ceriodaphnia dubia*	Larva	7	EC10 (reproduction)	3.6
	Daphnia ambigua	Larva	10	EC10 (reproduction)	24
	Daphnia longispina	Larva	21	NOEC (population growth rate)	25
	Daphnia magna	Larva	21	EC20 (reproduction)	4.5
	Daphnia thomsoni	Neonate	14	IC10 (reproduction)	5.4
	Gammarus pulex	–	100	NOEC (population growth rate)	5.4
	Hyalella azteca	Juvenile	15–35	NOEC (mortality/ reproduction)	16
	Moinodaphnia macleayi	Neonate	6	EC10 (reproduction)	0.3
	Paracalliope fluviatilis	Juvenile	32	NOEC (mortality)	10
	Paranephrops planifrons	Juvenile	14	LC10 (mortality)	175
	Paratanytarsus parthenogeneticus	–	16	NOEC (growth/ reproduction)	40
Insect	*Chironomus riparius*	Larva	10	NOEC (growth)	17
	Clistorina magnifica	Larva	240	NOEC (life cycle)	3.7
Rotifer	*Brachionus calyciflorus*	Larva	2	EC20 (reproduction)	2.1
Cnidarian	*Hydra viridissima*	Budding adult	4	EC10 (population growth rate)	0.6
Macrophyte	*Lemna aequinoctialis*	–	4	EC10 (population growth rate)	1.8
	Lemna minor	–	7	EC10 (growth)	0.8
	Zizania palustris	Seedling	14	NOEC (growth)	14
Green microalga	*Chlamydomonas reinhardtii*	Exponential growth phase	10	NOEC (population growth rate)	22
	Chlorella sp. (eriss)	Exponential growth phase	3	EC10 (growth rate)	0.4
	Chlorella sp. (PNG)	–	3	EC10 (growth rate)	0.9
	Chlorella vulgaris	Exponential phase	3	EC10 (population growth rate)	6.9
	Monoraphidium arcuatum	–	3	EC10 (growth rate)	0.45
	Raphidocelis subcapitata	Exponential growth phase	3	EC10 (population abundance)	2.0
Fungus	*Penicillium* strain	Spore	21	IC10 (population growth rate)	180
Bacterium	*Isolate 37* Unidentified sp.	–	2	EC15 (population growth rate)	2.8

– : No data / not stated.

4.3 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) based on the 59 chronic toxicity data for dissolved copper in freshwater (Table 2) is shown in Figure 2. The SSD was plotted using the Burrlioz 2.0 software. The model provided a good fit to the data.

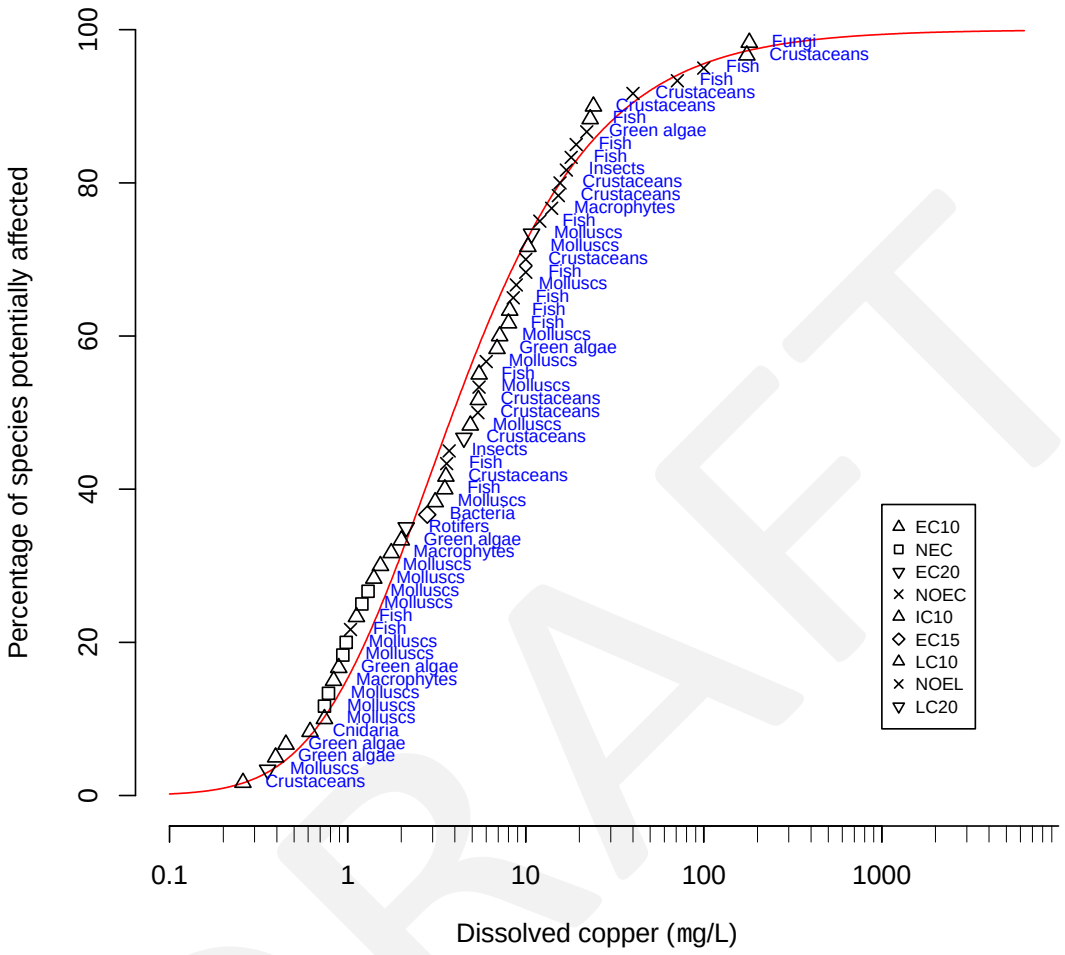

Figure 2 Species sensitivity distribution, dissolved copper in freshwater

4.4 Default guideline values

It is important that the DGVs (Table 3) and associated information in this technical brief are used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (ANZG 2018).

The DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 3. The DGVs apply to dissolved copper (operationally defined as the <0.45 µm filtered measurement) and in water with DOC ≤0.5 mg/L, hardness between 2 mg/L as $CaCO_3$ and 200 mg/L as $CaCO_3$ and pH between 6 and 8.5. The 95% species protection DGV is recommended for application to slightly-to-moderately disturbed ecosystems.

Table 3 Toxicant default guideline values, dissolved copper in freshwater at ≤0.5 mg/L dissolved organic carbon, very high reliability

Level of species protection (%)	DGV for dissolved copper in freshwater (μg/L) [a]
99	0.20
95	0.47
90	0.73
80	1.3

a DGVs were derived using the Burrlioz 2.0 software, and apply to water with a DOC of ≤0.5 mg/L, a pH range of 6–8.5 and hardness range of 2–200 mg $CaCO_3$/L. DGVs have been rounded to one or two significant figures.

The DGVs can be adjusted to the DOC concentrations of ambient water (up to 30 mg/L) based on the equation in Figure 3 or using the DGVs for different DOC concentrations provided in Table 4. For freshwater with DOC >30 mg/L and/or outside the specified pH and hardness ranges, site-specific factors affecting toxicity should be considered, including modelling of metal speciation (see Appendix B: Actions to assess the bioavailable fraction of a metal).

$$DOC\ adjusted\ DGV = DGV_{0.5} \times \left(\frac{DOC}{0.5}\right)^{0.977}$$

Figure 3 Equation for calculating DOC adjusted DGVs

Table 4 Toxicant default guideline values, dissolved copper in freshwater at different dissolved organic carbon concentrations, very high reliability

DOC concentration (mg/L)	DGV for dissolved copper in freshwater, different levels of species protection (μg/L)			
	99%	95%	90%	80%
0.5	0.20	0.47	0.73	1.3
1	0.39	0.93	1.4	2.6
2	0.77	1.8	2.8	5.0
4	1.5	3.6	5.6	9.9
8	3.0	7.1	11	20
12	4.5	10	16	29
16	5.9	14	22	38
20	7.3	17	27	48
25	9.1	21	33	59
30	11	26	40	71

4.5 Reliability classification

The dissolved copper in freshwater DGVs have a very high reliability classification (Warne et al. 2018) based on the outcomes for the following three criteria:

- sample size—59 (preferred)
- type of toxicity data—chronic
- SSD model fit—good (Burr Type III model).

Glossary

Term	Definition
acute toxicity	A lethal or adverse sublethal effect that occurs as the result of a short exposure period to a chemical relative to the organism's life span.
acute-to-chronic ratio	The species mean acute value (LC50/EC50) divided by the chronic value (e.g. NOEC or EC10) for the same species.
BLM	Biotic ligand model.
chronic toxicity	A lethal or sublethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. site-specific) in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality. Formerly known as 'trigger values'.
DOC	Dissolved organic carbon.
DOM	Dissolved organic matter.
EC50 (median effective concentration)	The concentration of a substance in water or sediment that is estimated to produce a 50% change in the response being measured or a certain effect in 50% of the test organisms relative to the control response, under specified conditions.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
guideline value	A measurable quantity (e.g. concentration) or condition of an indicator for a specific community value below which (or above which, in the case of stressors such as pH, dissolved oxygen and many biodiversity responses) there is considered to be a low risk of unacceptable effects occurring to that community value. Guideline values for more than one indicator should be used simultaneously in a multiple lines of evidence approach. (Also refer to default guideline value and site-specific guideline value.)
ICx	The concentration of a substance in water or sediment that is estimated to produce an x% inhibition of the response being measured in test organisms relative to the control response, under specified conditions.
LC50 (median lethal concentration)	The concentration of a substance in water or sediment that is estimated to be lethal to 50% of a group of test organisms, relative to the control response, under specified conditions.
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms under specified conditions.
LOEC (lowest observed effect concentration)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
MATC (maximum acceptable toxicant concentration)	The geometric mean of the lowest exposure concentration that causes a statistically significant adverse effect (LOEC) and the highest exposure concentration where no statistically significant effect is observed (NOEC).
MDEC (minimum detectable effect concentration)	The effect that produces a minimal detectable response that is statistically significantly different ($p>0.05$) to controls (Ahsanullah & Williams 1991).
MLR	Multiple linear regression.

Term	Definition
NEC (no effect concentration)	The maximum concentration of a toxicant that causes no adverse effect in a target organism.
NOEC (no observed effect concentration)	The highest concentration of a material used in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
site-specific guideline value	A guideline value that is relevant to the specific location or conditions that are the focus of a given assessment or issue.
species (biological)	A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group.
species (chemical)	Most commonly used for metals, chemical species are different forms of a particular chemical that may include different oxidation states, isotopes, complexes with organic ligands (in the case of metals) or with particulate matter.
SSD (species sensitivity distribution)	A method that plots the cumulative frequency of species' sensitivities to a toxicant and fits a statistical distribution to the data. From the distribution, the concentration that should theoretically protect a selected percentage of species can be determined.
toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.
WQC	Water quality criterion, from USEPA (2007).

Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table A 1 Summary of chronic toxicity data used to derive the freshwater copper default guideline values

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
Fish	*Acipenser transmontanus*	Larva	53	EC10 (growth)	15	8.1	101	0.4	1.1	1.1	USGS 2014
	–									**1.1**	**Value used in SSD**
	Cirrhinus mrigala	Juvenile	60	NOEC (growth)	26	7.6	135	–	100	100	Mohanty et al. (2009)
	–									**100**	**Value used in SSD**
	Cottus bairdi	Juvenile	21	NOEC (mortality)	–	8.3	101	0.5	2.9	2.9	Besser et al. (2007)
		Juvenile	28	NOEC (mortality)	–	8.2	104	0.5	25	25	Besser et al. (2007)
	–									**8.5**	**Value used in SSD (geometric mean)**
	Cyprinella monacha	Larva	30	IC10 (growth)	25	8.3	162	–	23	23	Besser et al. (2005)
	–									**23**	**Value used in SSD**
	Etheostoma fonticola	Larva	30	IC10 (biomass)	23	8.3	170	–	7.1	7.1	Besser et al. (2005)
		Larva	30	IC10 (biomass)	23	8.3	170	–	9.3	9.3	Besser et al. (2005)
	–									**8.1**	**Value used in SSD (geometric mean)**
	Galaxias maculatus	Juvenile	21	NOEC (mortality)	20	7.8	39	–	18	18	Hickey et al. (2000)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
	–									**18**	**Value used in SSD**
	Gobiomorphus cotidianus	Juvenile	18	NOEC (mortality)	–	7.0	37	0.5	10	10	Hickey et al. (2000)
	–									**10**	**Value used in SSD**
	Melanotaenia splendida inornata	Adult	30	NOEC (reproduction)	25	7.5	54	–	12	12	Skidmore (1986)
	–									**12**	**Value used in SSD**
	Mogurnda mogurnda	Larva	7	EC10 (growth)	27	6.7	3.1	1.4	9.6	3.5	Trenfield et al. (2022)
	–									**3.5**	**Value used in SSD**
	Oncorhynchus kisutch	Juvenile	60	NOEC (growth)	–	7.1	32	2.9	21	3.6	Mudge et al. (1993)
	–									**3.6**	**Value used in SSD**
	Oncorhynchus mykiss	Juvenile	30	LC10 (mortality)	13	7.1	21	0.4	3.9	3.9	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	7.1	60	0.5	4.6	4.6	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	6.2	13	0.5	4.7	4.7	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	7.9	11	0.5	7.6	7.6	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	7.1	20	2.2	27	5.4	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	7.0	23	5.6	87	6.3	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	7.0	24	11.0	150	6.7	Crémazy et al. (2017)
	–									**5.5**	**Value used in SSD (geometric mean)**
	Pelteobagrus fulvidraco	Juvenile	42	NOEC (growth)	21.9	7.6	62	–	71	71	Chen et al. (2013)
	–									**71**	**Value used in SSD**
	Perca fluviatilis	Juvenile	30	NOEC (growth)	–	7.8	194	1.0	39	19	Collvin (1985)
	–									**19**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
	Pimephales promelas	Larva	30	IC10 (growth)	25	8.3	170	–	4	4	Besser et al. (2005)
		Larva	30	IC10 (growth)	23	8.3	170	–	16	16	Besser et al. (2005)
	–									**8**	**Value used in SSD (geometric mean)**
	Prosopium williamsoni	Embryo	90	NOEL (growth)	9.6	6.8	48	1.9	3.9	1.0	Brinkman & Vieira (2008)
	–									**1.0**	**Value used in SSD**
Mollusc	*Alathyria profuga*	Larva	3	NEC (development)	22	7.0	42	0.1	1.2	1.2	Markich (2017)
	–									**1.2**	**Value used in SSD**
	Amerianna cumingi	Adult	4	EC10 (reproduction)	29.5	6.7	2.1	2.1	5.7	1.4	Trenfield et al. (2022)
	–									**1.4**	**Value used in SSD**
	Cucumerunio novaehollandiae	Larva	3	NEC (development)	22	7.0	42	0.1	0.7	0.7	Markich (2017)
	–									**0.7**	**Value used in SSD**
	Echyridella menziesii	Larva	2	EC20 (mortality)	20.5	7.8	30	2.5	1.2	0.2	Clearwater et al. (2014)
		Larva	2	EC20 (mortality)	20.5	7.8	30	2.5	2.7	0.5	Clearwater et al. (2014)
		Larva	2	EC20 (mortality)	20.5	7.8	30	2.5	1.8	0.4	Clearwater et al. (2014)
	–									**0.4**	**Value used in SSD (geometric mean)**
	Epioblasma capsaeformis	Juvenile	28	IC10 (mortality)	20	8.2	162	–	<3.1	3.1	Wang et al. (2007)
	–									**3.1**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
	Fluminicola sp.	Adult/juvenile	28	NOEC (mortality)	–	8.4	166	0.6	6.6	5.5	Besser et al. (2009)
	–									**5.5**	**Value used in SSD**
	Fontigens aldrichi	Adult/juvenile	28	EC10 (mortality)	–	8.3	166	0.7	10	7.1	Besser et al. (2016)
	–									**7.1**	**Value used in SSD**
	Hyridella australis	Larva	3	NEC (development)	22	7.0	42	0.1	0.8	0.8	Markich (2017)
	–									**0.8**	**Value used in SSD**
	Hyridella depressa	Larva	3	NEC (development)	22	7.0	42	0.1	0.9	0.9	Markich (2017)
	–									**0.9**	**Value used in SSD**
	Hyridella drapeta	Larva	3	NEC (development)	22	7.0	42	0.1	1.0	1.0	Markich (2017)
	–									**1.0**	**Value used in SSD**
	Juga plicifera	–	30	NOEC (mortality)	–	7.1	23	–	6.0	6.0	Nebeker et al. (1986)
	–									**6.0**	**Value used in SSD**
	Lymnaea stagnalis	–	14	EC10 (growth)	21	7.8	116	0.8	3.7	2.4	Crémazy et al. (2018)
		–	30	EC20 (growth)	24	7.8	56	1.2	1.8	0.7	Brix et al. (2011)
	–									**0.7**	**Value used in SSD**
	Physa gyrina	Juvenile	28	EC10 (growth)	20	8.3	170	0.6	12	10	Besser et al. (2016)
	–									**10**	**Value used in SSD**
	Pomacea paludosa	Adult/juvenile	60	NOEC (growth)	28	7.9	70	–	8.8	8.8	Rogevich et al. (2009)
	–									**8.8**	**Value used in SSD**
	Pyrgulopsis robusta	Larva/juvenile	28	EC10 (mortality)	–	8.2	170	0.6	5.9	4.9	Besser et al. (2016)
	–									**4.9**	**Value used in SSD**
	Taylorconcha serpenticola	Adult/juvenile	28	LC20 (mortality)	–	8.4	166	0.6	13	11	Besser et al. (2016)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
	–									**11**	**Value used in SSD**
	Velesunio ambiguus	Larva	3	NEC (development)	22	7.0	42	0.1	1.3	1.3	Markich (2017)
	–									**1.3**	**Value used in SSD**
	Villosa iris	Adult/juvenile	28	EC10 (growth)	20	8.3	101	0.5	6.2	6.2	Wang et al. (2011)
		Adult/juvenile	28	EC10 (growth)	20	8.2	100	10.0	21	1.0	Wang et al. (2011)
		Adult/juvenile	28	EC10 (growth)	20	8.3	98	3.0	3.8	0.6	Wang et al. (2011)
	–									**1.5**	**Value used in SSD (geometric mean)**
Insect	*Chironomus riparius*	Larva	10	NOEC (growth)	–	6.9	151	0.5	17	17	Taylor et al. (1991)
	–									**17**	**Value used in SSD**
	Clistorina magnifica	Larva	240	NOEC (life cycle)	20	7.6	26	1.1	8.3	3.7	Nebeker et al. (1984)
	–									**3.7**	**Value used in SSD**
Crustacean	*Ceriodaphnia dubia*	Larva	7	EC10 (reproduction)	20	8.4	105	0.4	11	11	Wang et al. (2011)
		Larva	7	EC10 (reproduction)	20	8.3	106	5.8	29	2.3	Wang et al. (2011)
		Larva	7	EC10 (reproduction)	20	8.3	106	10.0	25	1.1	Wang et al. (2011)
		Larva	7	EC10 (reproduction)	20	8.3	103	5.8	46	3.7	Wang et al. (2011)
		Larva	7	EC10 (reproduction)	20	8.3	102	3.0	34	5.4	Wang et al. (2011)
	–									**3.6**	**Value used in SSD (geometric mean)**
	Daphnia ambigua	Larva	10	EC10 (reproduction)	21	8.0	63	–	24	24	Harmon et al. (2003)
	–									**24**	**Value used in SSD**
	Daphnia longispina	Larva	21	NOEC (population growth rate)	20	7.8	180	–	5.5	5.5	Agra et al. (2011)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Larva	21	NOEC (population growth rate)	20	7.8	180	–	85	85	Agra et al. (2011)
		Larva	21	NOEC (population growth rate)	20	7.8	180	–	42	42	Agra et al. (2011)
		Larva	21	NOEC (population growth rate)	20	7.8	180	–	21	21	Agra et al. (2011)
	–									**25**	**Value used in SSD (geometric mean)**
	Daphnia magna	Larva	21	EC20 (reproduction)	20	6.1	100	5.6	42 [a]	3.6	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	6.1	100	16.9	85 [a]	2.4	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	6.1	100	6.2	39 [a]	3.1	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	6.1	100	16.9	96 [a]	2.7	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	6.2	100	5.0	38 [a]	3.7	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	6.2	100	14.5	118 [a]	3.9	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	7.9	100	13.5	225 [a]	7.9	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	7.1	25	9.1	121 [a]	6.5	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	7.9	100	4.8	92 [a]	9.2	De Schamphelaere & Janssen (2004)
		Larva	21	EC20 (reproduction)	20	8.4	21	2.0	28 [a]	5.5	Villavicencio et al. (2011)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Larva	21	EC20 (reproduction)	20	8.4	43	2.0	28 [a]	6.6	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.4	85	2.0	29 [a]	7.0	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.2	21	2.0	16 [a]	3.1	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.1	43	2.0	19 [a]	4.5	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.1	85	2.0	26 [a]	6.2	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.2	169	2.0	20 [a]	4.8	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.6	21	2.0	7.3 [a]	1.5	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.5	43	2.0	9.8 [a]	2.4	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.5	85	2.0	13 [a]	3.3	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.5	169	2.0	19 [a]	4.5	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.5	21	2.0	8.2 [a]	1.7	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.6	21	2.0	15 [a]	3.1	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.3	111	1.0	14 [a]	7.0	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.4	122	0.8	13 [a]	8.3	Villavicencio et al. (2011)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Larva	21	EC20 (reproduction)	20	8.3	109	1.0	16 [a]	7.8	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.9	76	0.3	6.3 [a]	6.3	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	94	1.3	9.3 [a]	3.5	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	32	1.0	5.4 [a]	2.6	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.8	14	0.8	8.5 [a]	5.0	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	18	0.5	10 [a]	10	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.9	10	1.1	9.2 [a]	3.8	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	12	1.1	8.6 [a]	3.4	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	13	1.8	12 [a]	2.6	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	14	1.2	8.6 [a]	3.1	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	26	0.6	9.7 [a]	8.0	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.0	12	0.7	7.9 [a]	5.3	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.4	70	0.2	11 [a]	11	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	8.2	33	0.5	12 [a]	12	Villavicencio et al. (2011)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Larva	21	EC20 (reproduction)	20	7.6	21	2.0	15 [a]	3.3	Villavicencio et al. (2011)
		Larva	21	EC20 (reproduction)	20	7.5	21	2.0	16 [a]	3.3	Villavicencio et al. (2011)
	–									**4.5**	**Value used in SSD (geometric mean)**
	Daphnia thomsoni	Neonate	14	IC10 (reproduction)	–	7.2	20	4.5	65	5.4	Thompson et al. (2021)
	–									**5.4**	**Value used in SSD**
	Gammarus pulex	–	100	NOEC (population growth rate)	11	8.0	103	1.0	11	5.4	Maund et al. (1992)
	–									**5.4**	**Value used in SSD**
	Hyalella azteca	Juvenile	15	NOEC (mortality)	22	7.6	128	1.0	32	16	Othman & Pascoe (2002)
		Juvenile	35	NOEC (reproduction)	22	7.6	128	1.0	32	16	Othman & Pascoe (2002)
	–									**16**	**Value used in SSD**
	Moinodaphnia macleayi	Neonate	6	EC10 (reproduction)	26.5	6.6	2.8	2.0	1.0	0.3	Trenfield et al. (2022)
	–									**0.3**	**Value used in SSD**
	Paracalliope fluviatilis	Juvenile	32	NOEC (mortality)	20	7.8	39	–	10	10	Hickey et al. (2000)
	–									**10**	**Value used in SSD**
	Paranephrops planifrons	Juvenile	14	LC10 (mortality)	17.9	7.8	37	0.5	175	175	Albert et al. (2021)
	–									**175**	**Value used in SSD**
	Paratanytarsus parthenogeneticus	–	16	NOEC (growth)	–	7.0	25	0.5	40	40	Hatakeyama & Yasuno (1981)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		–	16	NOEC (reproduction)	–	7.0	25	0.5	40	40	Hatakeyama & Yasuno (1981)
	–									**40**	**Value used in SSD**
Rotifer	*Brachionus calyciflorus*	Larva	2	EC20 (reproduction)	25	6.0	100	4.9	9.6	1.0	De Schamphelaere et al. (2006)
		Larva	2	EC20 (reproduction)	25	6.0	100	14.5	39	1.3	De Schamphelaere et al. (2006)
		Larva	2	EC20 (reproduction)	25	7.8	100	4.8	48	4.8	De Schamphelaere et al. (2006)
		Larva	2	EC20 (reproduction)	25	7.8	100	14.7	110	3.5	De Schamphelaere et al. (2006)
	–									**2.1**	**Value used in SSD (geometric mean)**
Cnidarian	*Hydra viridissima*	Budding adult	4	NOEC (population growth rate)	26.5	6.6	2.7	2.1	2.5	0.6	Trenfield et al. (2022)
	–									**0.6**	**Value used in SSD**
Macrophyte	*Lemna aequinoctialis*	–	4	EC10 (population growth rate)	29	6.8	3.1	2.0	6.8	1.8	Trenfield et al. (2022)
	–									**1.8**	**Value used in SSD**
	Lemna minor	–	7	EC10 (growth)	25	7.6	163	1.0	2.1	1.0	Antunes et al. (2012)
		–	7	EC10 (growth)	25	7.9	184	1.0	0.7	0.3	Antunes et al. (2012)
		–	7	EC10 (growth)	25	7.5	35	2.0	3.8	0.9	Antunes et al. (2012)
		–	7	EC10 (growth)	25	7.4	96	2.0	5.9	1.5	Antunes et al. (2012)
	–									**0.8**	**Value used in SSD (geometric mean)**
	Zizania palustris	Seedling	14	NOEC (growth)	–	6.8	89	5.0	37	3.7	Nimmo et al. (2003)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Seedling	14	NOEC (growth)	–	6.8	89	7.1	201	14	Nimmo et al. (2003)
		Seedling	14	NOEC (growth)	–	6.8	89	<1	54	54	Nimmo et al. (2003)
	–									**14**	**Value used in SSD**
Green microalga	*Chlamydomonas reinhardtii*	Exponential growth phase	10	NOEC (population growth rate)	24	6.6	25		22	22	Schafer et al. (1994)
	–									**22**	**Value used in SSD**
	Chlorella sp. (eriss)	Exponential growth phase	3	EC10 (growth rate)	29	6.3	1.9	2.1	1.6	0.4	Trenfield et al. (2022)
	–									**0.4**	**Value used in SSD**
	Chlorella sp. (PNG)	–	3	EC10 (growth rate)	27	–	85	0.5	0.9	0.9	Adams et al. (2018)
	–									**0.9**	**Value used in SSD**
	Chlorella vulgaris	Exponential growth phase	3	EC10 (population growth rate)	25	6.0	100	5.2	108	10	De Schamphelaere & Janssen (2006)
		Exponential growth phase	3	EC10 (population growth rate)	25	6.0	100	15.5	407	13	De Schamphelaere & Janssen (2006)
		Exponential growth phase	3	EC10 (population growth rate)	25	7.9	100	5.3	31	2..8	De Schamphelaere & Janssen (2006)
		Exponential growth phase	3	EC10 (population growth rate)	25	7.9	100	15.7	188	5.6	De Schamphelaere & Janssen (2006)
		Exponential growth phase	3	EC10 (population growth rate)	25	7.1	25	10.3	159	7.6	De Schamphelaere & Janssen (2006)
	–									**6.9**	**Value used in SSD (geometric mean)**
	Monoraphidium arcuatum	–	3	EC10 (growth rate)	27	–	85	–	0.5	0.45	Adams et al. (2018)
	–									**0.45**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
	Raphidocelis subcapitata	Exponential growth phase	3	EC10 (population abundance)	25	8.0	100	5.4	18	1.6	De Schamphelaere et al. (2003)
		Exponential growth phase	3	EC10 (population abundance)	25	8.1	100	15.3	68	2.0	De Schamphelaere et al. (2003)
		Exponential growth phase	3	EC10 (population abundance)	25	7.2	25	10.4	53	2.5	De Schamphelaere et al. (2003)
	–									**2.0**	**Value used in SSD (geometric mean)**
Fungus	*Penicillium* strain	Spore	21	IC10 (population growth rate)	21	7.3	52	–	180	180	Lategan & Hose (2014)
	–									**180**	**Value used in SSD**
Bacterium	*Isolate 37* Unidentified *sp.*	–	2	EC15 (population growth rate)	30	7.8	60	–	2.8	2.8	Davies et al. (1998)
	–									**2.8**	**Value used in SSD**

a EC20 value derived by Brix et al. (2017) using raw data from original study.

Appendix B: Actions to assess the bioavailable fraction of a metal

A decision tree for evaluating monitoring data against default guideline values (DGVs) or site-specific guideline values for dissolved copper in freshwater, which includes consideration of the bioavailable fraction, is shown in Figure B 1. The outcomes of the process shown here for water chemistry assessment should be used with other lines of evidence (e.g. biodiversity assessment or direct toxicity assessment) in a weight of evidence approach to assess overall water quality.

With respect to the modelling of bioavailable copper, it is necessary to consider simple ionic complexes; however, it is also known that there are colloidal forms and weak ionic complexes that can dissociate and cross biological membranes. Approaches such as the biotic ligand model (BLM) for copper in freshwater (e.g. USEPA 2007, ECCC 2021) are appropriate modelling options. Alternatively, speciation modelling is also an option; for example, the Windermere Humic Aqueous Model (WHAM7), which includes a solution speciation model as well as sub-models for ion binding to humic and fulvic acids, clay and oxides of iron, aluminium, manganese and silica (UKCEH 2021). Bioavailable copper can be measured using a range of techniques designed to measure the 'labile' fraction of metals that has been shown to correlate with the biologically available fraction (see Batley et al. 2004). Currently, the use of Chelex columns and diffusive gradients in thin films (DGT) are the most widely used approaches.

The bioavailable fraction of copper should be compared to the DGV (at a DOC of 0.5 mg/L; i.e. not adjusted for the local DOC concentration).

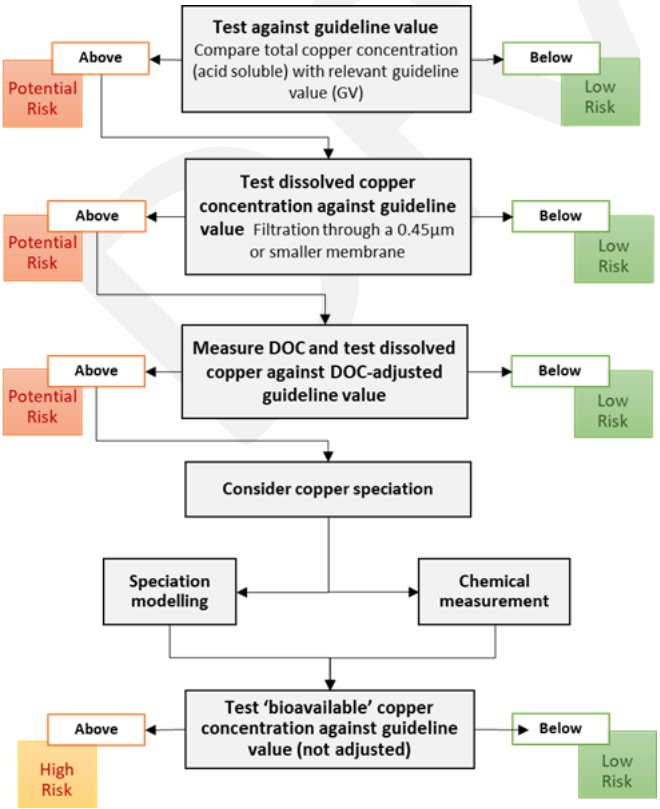

Figure B 1 Actions to assess bioavailable fraction of copper in freshwater

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