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Toxicant default guideline values for aquatic ecosystem protection

Copper in freshwater

Technical brief

September 2026

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Summary

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 (ANZG 2018).

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 and in vehicle brake pads. 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 and ARMCANZ 2000), new data have become available, including high quality data for local species. Furthermore, there is increased understanding of the effects of dissolved organic carbon (DOC), hardness and other toxicity modifying factors (TMFs) 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. The DGVs correspond to a high bioavailability reference condition, determined based on DOC data from across Australia and New Zealand, where DOC is ≤ 0.5 mg/L, and can be adjusted using the DOC correction to bioavailability-adjusted guideline values (BAGVs) based on DOC concentrations in local waterbodies.

Very high reliability DGVs for dissolved (0.45 µm filtered) copper in freshwater were derived from chronic toxicity data for 52 species (comprising 13 fish, 16 molluscs, 9 crustaceans, 3 insects, 1 rotifer, 1 cnidarian, 2 macrophytes, 5 green microalgae, 1 fungus and 1 bacterium), with a good (visual) fit of the species sensitivity distribution (SSD) to the toxicity data. The DGVs (at DOC of ≤ 0.5 mg/L) for 99%, 95%, 90% and 80% species protection are 0.53 µg/L, 0.73 µg/L, 0.89 µg/L and 1.4 µg/L, respectively. The DGVs can be adjusted to the DOC concentrations of ambient waters (up to 20 mg/L DOC), measured as non-purgeable organic carbon (NPOC) in 0.45 µm filtered samples. The 95% DGV for dissolved copper in freshwater is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems. Where ambient water data for DOC are not available and a BAGV cannot be calculated, the DGV should be used in the interim. The DGVs supersede the ANZECC and ARMCANZ (2000) DGVs for copper in freshwater.

The ANZG (2018) Guidelines provide a tiered assessment framework for assessing copper bioavailability and toxicity so that the effects of DOC on copper bioavailability can be taken into account. The tiered assessment framework represents a decision tree to guide the user through the process of accounting for copper bioavailability when comparing monitoring data with guideline values, and includes a metals bioavailability tool to adjust DGVs to BAGVs based on local water chemistry.

1 Introduction

Copper (Cu) is a naturally occurring metallic element. It is an abundant trace element, present in the Earth's crust at approximately 50 mg/kg (Landner and 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 ($Cu_3(CO_3)_2(OH)_2$) and malachite ($Cu_2CO_3(OH)_2$); and the copper(I) oxide mineral cuprite (Cu_2O). The major copper mines are in the Americas (particularly Chile), Australia and Europe (particularly Russia, Poland and Sweden) (Landner and Reuther 2004, Schoeters et al. 2008).

Copper has been used for centuries as jewellery, vessels, currency and in tools (Landner and Reuther 2004). Current uses include architectural structures, roofing, plumbing and electronics (Schoeters et al. 2008). Copper is also used as a biocide in antifouling paints, wood preservatives, fungicides and algaecides (Schoeters et al. 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 (including run-off from architectural surfaces (Pennington and 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 and Morgan 1996, Schoeters et al. 2008). The principal states are cuprous (Cu^+) and cupric (Cu^{2+}). These are found as salts such as copper(I) oxide (Cu_2O) and copper(II) sulfate pentahydrate ($CuSO_4.5H_2O$). 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 dissolved 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 and Williamson 1986, Mudd and Patterson 2010; Mackay and Taylor 2013) and, in some cases, can be found in the hundreds of milligrams per litre range (e.g. NT EPA 2014).

This technical brief provides updated default guideline values (DGVs) for dissolved copper in freshwater that supersede the ANZECC and ARMCANZ (2000) DGVs. The current derivation has added new chronic toxicity data published since 2000, including chronic data for Australasian species. The hardness corrections applied to the ANZECC and ARMCANZ (2000) DGVs have been replaced with dissolved organic carbon (DOC) corrections. The rationale for this change is detailed in Section 3 and 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, when above natural background concentrations, copper has the potential to be acutely and chronically toxic, particularly to aquatic organisms.

For fish and invertebrates, acute copper toxicity is 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 other mechanisms of action can occur. Intra-cellular copper can generate reactive oxygen species, which can then go on to damage DNA and proteins (Brix et al. 2022; Grosell and Wood 2002). Copper ions can also displace essential metal ions in biomolecules and bind to some proteins with high affinity, leading to their dysfunction (Grosell and Wood 2002). These molecular pathways, as well as the requirement to maintain the copper homeostasis, increase the energy demands and decrease the energy available for growth and reproduction (Brix et al. 2022; Meyer et al. 2007). In fish, copper also affects the olfactory system (Thomas et al. 2016), which can alter the ability of fish to avoid water with elevated copper concentrations (Meyer and Adams 2010). Thus, chronic exposure to excess copper can, at a cellular and organ level, alter enzyme activity, blood chemistry and development of organs, and damage tissues, and at an individual level, lead to reduced growth, reproduction and survival.

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 and Davies 2000, Van Sprang et al. 2008). Within algal cells, copper can inhibit enzymes and change intracellular pH (Stauber and 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* (US EPA 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 *Daphnia pulicaria*, *Ceriodaphnia dubia* and *D. magna*, respectively, 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) (US EPA 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 US EPA (2007) were mainly in the range of 2:1 to 6:1, though higher ratios were also reported (e.g. 171:1) for the freshwater snail *Campeloma decisum*. The chronic toxicity data compiled for the US EPA (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 US EPA (2007) date from the 1970s and 1980s, with only 2 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 (EU) 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 an 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 EU 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 (and in culture medium containing chelators and adsorbents), 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 (US EPA 2007). In 58-d chronic tests, the embryo life stage of *Oncorhynchus 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 3 North American species, 28-d test IC10 values ranged 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 6 freshwater mussel species based on cell closure in 72-hour exposure tests, while Clearwater et al. (2014) reported 48-hour 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 (US EPA 2007). Algal isolates from Papua New Guinea are the most sensitive to copper in 3-d growth rate 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 rate 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 experimental factors (e.g. initial cell density) 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. Apart from lipid-soluble complexes of copper, freely dissolved copper is the most toxic form as it is the most bioavailable, whereas most inorganic and organic (water soluble) 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 guideline values (i.e. US EPA 1996, ANZECC and ARMCANZ 2000) have recommended that, rather than comparing total copper concentrations 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 and 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. Most of these are less toxic than free Cu^{2+}, although $CuOH^-$ and $CuCO_3$ are considered bioavailable, particularly for chronic exposures (De Schamphelaere and Janssen 2004; Van Sprang et al. 2008). In natural water, the organic ligands are the most important ligands for copper binding (Stumm and 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 increase and as the concentrations of competing ions decrease (Stumm and 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 chemistries (Section 3.2).

3.2 Toxicity modifying factors

Factors affecting dissolved copper toxicity typically involve changes to copper speciation or competition with copper at biological uptake sites. Compared to acute copper toxicity data, there are fewer studies that examine the influence of toxicity modifying factors (TMFs) such as pH, hardness, alkalinity and DOC on chronic toxicity. The influence of TMFs on chronic toxicity may not be the same

as that on acute toxicity due to differences in the mechanism of action, and the ability of organisms to regulate internal copper concentrations in longer exposures. Therefore, the information provided here is based only on studies of chronic toxicity, where available.

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 cation competition is demonstrated by the reduction in dissolved 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 and Hansen (1996) and Paquin et al. (2002)). However, water hardness does not influence copper toxicity to all species, particularly for chronic exposures, as demonstrated for the cladoceran *D. magna* (De Schamphelaere and 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 for dissolved copper (e.g. as pH increases, NOEC values increase and toxicity decreases) that were statistically significant for *P. promelas*, marginally statistically significant for *D. pulex*, and not significant for *D. magna.* In individual studies for *D. magna* (De Schamphelaere and Janssen 2004), as pH increased, the EC50 (and NOEC) values increased (i.e. toxicity decreased) when copper was expressed as dissolved copper. When expressed as copper(II) activity to account for the different copper species present in solution, there was a negative relationship between toxicity and pH, demonstrating the competition effect. Recent tests also indicate positive relationships between pH and EC10 (or EC50) values for dissolved copper for *C. dubia*, a more sensitive cladoceran species (Weighman and De Schamphelaere 2023). Studies with freshwater algae indicate the reverse association: dissolved copper toxicity values are lower (i.e. higher toxicity) at higher pH (De Schamphelaere and Janssen 2004; Van Sprang et al. 2008).

Increases in alkalinity decrease acute copper toxicity, based on studies with fish and cladocerans (Hyne et al. 2005; Meyer et al. 2007) including some species native to Australia (Daly et al. 1990), but not others (Reithmuller et al. 2000). However, the effect of alkalinity in modifying chronic copper toxicity is less clear. *P. promelas* is the only species for which a clear, positive relationship between alkalinity and chronic dissolved copper toxicity values has been reported (Meyer et al. 2007). In many studies reviewed by Meyer et al. (2007), pH (and sometimes hardness) covaried with alkalinity in toxicity tests, and the influence of alkalinity could not be differentiated from pH (or hardness). This reduces the amount of useful information available on which to explore the effect of pH or alkalinity on copper toxicity, particularly for sensitive and/or native species. Also, the relationships observed for acute toxicity may not be applicable to chronic toxicity.

Water temperature may affect metal toxicity due to increased metabolic rates and increased respiratory inflows (Khangarot and 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 dissolved 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 dissolved copper to the Australian freshwater shrimp *Paratya australiensis* reduced approximately 2-fold as the concentration of DOC increased 2-fold (Daly et al. 1990). Markich et al. (2003) reported greater sensitivity of the freshwater mussel *Hyridella depressa* to dissolved copper at low DOC. Studies with freshwater algae (*Chlorella* sp. and *R. subcapitata*) also indicate that DOC reduces toxicity of dissolved copper (De Schamphelaere and Janssen 2006, Macoustra et al. 2019); for example, copper toxicity was reduced by 10- to 20-fold when DOC was increased from < 1 mg/L to 10–13 mg/L (Macoustra et al. 2019).

While all TMFs discussed above have been demonstrated to affect dissolved copper toxicity in one or more studies, the effect of each TMF can vary between species. DOC is considered the most influential factor for determining copper toxicity (OECD 2017). The greater effect of DOC on copper toxicity compared to other TMFs is shown in Appendix A using data for *O. mykiss*, *D. magna*, *Chlorella vulgaris* and *R. subcapitata*. DOC is also the only factor that demonstrates a consistent effect across species based on the available data (e.g. Appendix A) (Meyer et al. 2007). By contrast, studies demonstrate that hardness mitigates toxicity for some invertebrates and fish but not all, while pH influences algal toxicity differently to fish and invertebrates (Meyer et al. 2007).

3.3 Accounting for toxicity modifying factors

The available information suggests that the effect of pH, hardness and/or alkalinity on copper toxicity is unlikely to be a simple relationship across all water chemistry and across all species/trophic levels. The hardness adjustment used for the ANZECC and ARMCANZ (2000) DGVs is no longer considered appropriate for all species and for a generic derivation, as it was based on acute toxicity data (mainly for fish). Conflicting results have been published about the effect of hardness on the toxicity of copper to various species (e.g. De Schamphelaere and Janssen, 2004), including the more 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.

Biotic ligand models (BLMs) have been developed to account for the effect of multiple TMFs on copper toxicity, 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). All BLMs consist of a geochemical speciation component (e.g. CHESS, with WHAM V to describe binding to dissolved organic matter), which accounts for the speciation of metals and complexation to

inorganic and organic ligands; and a biotic ligand reactions component, which accounts for the binding of the metal to biotic ligands in the presence of competing cations (e.g. Ca^{2+}, Mg^{2+}, Na^{+}, H^{+}).

DOC affects copper toxicity through the abiotic mechanism – i.e. via complexation of copper. This influence is well-understood through measurements of copper using ion selective electrodes and through speciation modelling (e.g. Apte et al. 2005). It is implemented in the BLM solely within the chemical speciation component of the model. By contrast, while pH influences copper speciation (an abiotic process) – with higher free copper concentrations at low pH – there are also biotic components represented in the BLMs (both acute and chronic versions). The H^{+} ions compete with Cu^{2+} for binding sites on the biotic ligand, as represented through the log K_{HBL} values in the biotic component of the model. Similarly, the effect of hardness is primarily through competition of Ca^{2+}, Mg^{2+} and Na^{+} for binding sites (log $K_{CaBL,}$ log K_{MgBL} log K_{NaBL}).

The BLM model used in the US EPA ambient water quality criteria for copper (US EPA 2007) was developed from and assessed for acute toxicity data for 6 species (2 fish and 4 water fleas). This model is used in the US EPA criteria to adjust the acute toxicity values, and in accordance with the US process, the acute data are used to calculate the chronic criteria.

The EU environmental quality standard (EQS) derivation process uses chronic toxicity data for its BLM (Van Sprang et al. 2008). The acute BLM model was not adequate for predicting chronic toxicity, as demonstrated for *D. magna* (De Schamphelaere et al. 2005). A different BLM version based on chronic *D. magna* data was developed (and validated) for invertebrates (1 rotifer *Brachionus calyciflorus,* 2 molluscs *L. siliquoidea* and *H. depressa,* and an amphipod *Hyalella azteca*). The acute *D. magna* model (with different coefficients to the chronic model) was considered suitable for predicting bioavailability effects of chronic copper toxicity to fish, and used for all fish species. A third model based on algae is used for all algae and higher plants.

In Canada, the US EPA BLM was adapted to predict chronic copper toxicity, and used across all fish and invertebrates (ECCC 2021). Furthermore, a plant BLM is also used in the Canadian derivation and is used for all plant and algal species.

An alternative approach using multiple linear regression (MLR) models to account for toxicity modifiers has also been proposed (Brix et al. 2017, 2020, 2021). These models are developed from toxicity data, specifically, the statistical relationships between toxicity estimates (e.g. EC10 values) and concentrations of different TMFs in the test solutions. MLR models have been promoted as being more transparent and easier to use than the BLMs (Brix et al. 2020). No copper criteria, standards or guidelines are currently based on MLRs, although MLRs have been used in developing guidelines for other metals, such as aluminium (US EPA 2018) and zinc (CCME 2018).

3.4 Accounting for DOC in the current DGVs

A review of the three available BLMs (summarised in Section 3.3) indicated key differences between the models – particularly regarding the effect of hardness on copper toxicity. The US EPA model is based on acute toxicity data and increased hardness results in higher guideline values (i.e. a protective effect). The Canadian model also includes this protective effect. In contrast, in the EU model, increased hardness results in lower guideline values. The strengths and weaknesses of each BLM, and their applicability to Australian and New Zealand native species, would need to be

evaluated before any model could be used for deriving DGVs. Similarly, any MLR model would need to be evaluated for its applicability to Australian and New Zealand native species before being adopted for use in for deriving ANZG guideline values. Such evaluations require significant resources and have not been undertaken to date. Consequently, a more simplistic approach was sought to incorporate bioavailability into the copper freshwater DGVs, noting that the option of not incorporating TMFs would result in overly conservative (i.e. overly protective) DGVs for many natural waters.

A DOC-only approach was adopted for the current DGVs as DOC is the only one of the 3 key TMFs (the others being pH and hardness) for which the effect on copper toxicity is consistent across species, given that the effect occurs through an abiotic mechanism. DOC reduces copper toxicity through formation of copper-organic matter complexes, and therefore it mitigates toxicity across all (biological) species. Further, the available data suggest the effect of DOC is stronger than the effect of pH, hardness and/or alkalinity (Appendix A). By contrast, although pH has an abiotic effect (on copper speciation), it also has biotic effects (through competition for binding sites and possibly other mechanisms for algae). Use of an abiotic model for DOC only is consistent with the approach used by OECD (2012), whereby an abiotic correction is recommended when a full bioavailability model is not available (or in this case, not validated for Australian and New Zealand species).

The calculation of the DOC adjustment for the copper freshwater DGVs followed the procedure used by US EPA for calculating hardness adjustments. This means the use of a DOC adjustment is very similar to the previously used hardness correction for the ANZECC and ARMCANZ (2000) copper DGVs. The adjustment was calculated using toxicity data from toxicity tests that assessed the effects of different DOC concentrations on copper toxicity (described in more detail in Appendix B). The overall adjustment factor of 0.70 was used to standardise the toxicity values in the final toxicity database to a test water with low DOC concentration (0.5 mg/L; see Section 4.3 for determination of this DOC concentration) for the derivation of the DGVs. The adjustment factor is also used to calculate bioavailability-adjusted guideline values (BAGVs) at differing concentrations of DOC to match site-specific water chemistry (Section 4.5).

4 Default guideline value derivation

The DGVs for dissolved copper in freshwater were derived in accordance with the method described in Warne et al. (2018), except that the ssdtools package (version 2.3.0, Thorley et al. 2025) for R (R Core Team 2024) was used instead of the Burrlioz 2.0 software, as recommended by Warne et al. (2025). Additional steps that were required to derive bioavailability-based DGVs for copper are also captured in this Section 4.3.

4.1 Collation and screening of toxicity data

The DGVs 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 and ARMCANZ (2000); ECOTOX database (US EPA n.d.); 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. While some studies reported concentrations as total copper, all copper was assumed to be in the 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). In addition, tests at very low hardness (< 5 mg as $CaCO_3$/L) were excluded, as high sensitivity can be observed in waters with very low ionic strength, and the bioavailability model used in this derivation (DOC only) did not adjust for low hardness. Only 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.

Not all studies reported the DOC concentration in the test waters. If DOC was not reported but toxicity data were available for the same species with accompanying DOC data, these toxicity data were generally used in favour of the toxicity data that were not accompanied by a DOC concentration. If a study did not report DOC, it was included if the tests were conducted in high purity (deionised or reverse osmosis) waters, as it was assumed to have low DOC (≤ 0.5 mg/L), as would be the case for standard laboratory test control or diluent waters. This approach was consistent with that used by Canada, the EU and US for their copper guideline values. In a few cases, the DOC had been estimated by others (e.g. for use in EU risk assessment). These DOC concentrations and toxicity values were retained where the DOC was < 2 mg/L. Values above that would have higher uncertainty in the DOC estimate, which would result in increased uncertainty in the adjusted EC10 value (using the equation in Figure 1 in Section 4.2).

Data from tests in waters with DOC > 10 mg/L were excluded from the derivation. The adjustment of EC10 values from such waters resulted in 10-fold or greater change in EC10 values that would warrant further validation of the DOC model.

The exclusion rules resulted in some chronic data for some sensitive Australasian species being excluded from the DGV derivation (Table 1). The most sensitive of the species excluded were the green alga *Chlorella* sp., a tropical cladoceran *Moinodaphnia macleayi* and the New Zealand freshwater mussel *E. menziesii*.

Data for several Australian tropical species were included in the derivation despite exposure durations less than that 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 (Markich 2017) were included despite test durations of 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-hour 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 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
Green microalga	*Chlorella* sp.	Exponential growth phase	3	EC10 (growth rate)	0.6	Hardness very low [a]	Franklin et al. (2000)
Crustacean	*Moinodaphnia macleayi*	Neonate	6	EC10 (reproduction)	1.0	Hardness very low	Trenfield et al. (2022)
Green microalga	*Chlorella* sp.	Exponential growth phase	3	EC10 (growth rate)	1.6	Hardness very low	Trenfield et al. (2022)
Mollusc	*Echyridella menziesii*	Larva	2	EC20 (mortality)	1.2–2.7	Issues with DOC from nanopure water	Clearwater et al. (2014)
Cnidarian	*Hydra viridissima*	Budding adult	4	EC10 (population growth rate)	2.5	Hardness very low	Trenfield et al. (2022)
Mollusc	*Potamopyrgus antipodarum*	Juvenile / adult	77–112	NOEC (growth)	5	No hardness data	Dorgelo et al. (1995)
Mollusc	*Amerianna cumingi*	Adult	4	EC10 (reproduction)	5.7	Hardness very low	Trenfield et al. (2022)
Macrophyte	*Lemna aequinoctialis*	—	4	EC10 (population growth rate)	6.8	Hardness very low	Trenfield et al. (2022)
Fish	*Mogurnda mogurnda*	Larva	7	EC10 (growth)	9.6	Hardness very low	Trenfield et al. (2022)
Crustacean	*Paratya australiensis*	Juvenile	21	EC50 (growth)	59	Only EC50 data	Bacher and O'Brien (1990)

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value (µg/L)	Reason for exclusion	Reference
						reported, no NOEC	
Macrophyte	*Ipomoea aquatica*	Seedling	14	NOEC (growth)	2,500	Nominal data only; water chemistry not reported	Wu and 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)

[a] Very low hardness of < 5 mg $CaCO_3$/L.

4.2 Toxicity data used in derivation

Most of the data sourced from the ANZECC and 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 US EPA WQC (US EPA 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 320 chronic toxicity values for 52 species that were of suitable quality for use in the DGV derivation. Of these, approximately 45% (145 values for 29 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): 6 were NECs, 137 were EC/IC/LC10s and 2 were BEC10s. Lesser preferred toxicity estimates included 66 EC/LC11–20s, 92 NOECs and 17 minimum detectable effect concentrations (MDECs) from 33 species.

Rather than strict inclusion and exclusion criteria for studies based on whether or not they measured DOC, a preferential approach was used whereby toxicity data were selected according to a hierarchy of DOC conditions in the test waters. The guiding principle was to maximize consistency with the reference condition of low DOC (≤ 0.5 mg/L; Section 4.3), while ensuring sufficient coverage across species. Studies reporting low measured DOC concentrations (≤ 0.5 mg/L) in test waters were preferred over toxicity data without DOC measurements or with higher DOC concentrations that would require adjustment. If no such data were available for a species, studies without reported DOC were used provided that a low DOC concentration could reasonably be assumed (e.g. tests in synthetic waters with no added DOC). Where neither of these options existed, data from studies with DOC > 0.5 mg/L were included, after adjusting the toxicity values to the reference condition of

DOC 0.5 mg/L using a slope of 0.697 (Figure 1). Based on these preferential selection rules, DOC was reported for 60% of the toxicity data used in the DGVs derivation.

$$Normalised\ EC10 = EC10 \div \left(\frac{DOCtest}{0.5}\right)^{0.697}$$

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

Warne et al. (2018) recommend using only preferred chronic toxicity values (e.g. NEC, ECx where x≤10) where there are sufficient (e.g. >8) such values. The dataset based on only the preferred values comprised 26 species from 7 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 (3 species) and NOEC data (23 species). This resulted in data for 52 species, including 21 species native to Australia and/or New Zealand, from 10 taxonomic groups. The protective concentrations (i.e. 80%, 90%, 95%, 99% species protection) derived from the datasets for 26 species were 1.2-fold to 1.5-fold lower than those with 52 species. This level of difference was considered to be small given the overall uncertainties in the derivation process and, thus, the larger dataset with the greater taxonomic representation was selected to derive the DGVs.

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). The 52 species included in the final dataset were from 10 taxonomic groups: bacteria (1 species), cnidarians (1 species), rotifers (1 species), fungi (1 species), insects (3 species), macrophytes (2 species), green microalgae (5 species), crustaceans (9 species), fish (13 species) and molluscs (16 species). The toxicity values in the final dataset ranged over approximately 3 orders of magnitude, from 0.5 µg/L for *M. arcuatum* (3-d population growth tests) (McKnight et al. 2023) to 180 µg/L for a groundwater-isolated strain of *Penicillum* (21-d growth test) (Lategan and Hose 2014). Further details of the water quality parameters for each single species value used to calculate the DGVs are presented in Appendix C. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

4.3 Selection of default guideline values that represent a high bioavailability condition

Adopting a bioavailability-based approach for the copper freshwater DGVs enables the adjustment of the DGVs to account for DOC in local waters. This requires the DGVs to be set at a conservative, high bioavailability water chemistry condition, as the DGVs can then be conservatively applied to situations where there are no local DOC data. The process for doing this for copper is described in detail by Gadd et al. (2026a, 2026b) and summarised below.

The high bioavailability condition upon which the DGVs were based was determined based on an extensive collation and analysis of data from freshwaters around Australia and New Zealand. There were more than 18,000 and 10,000 DOC data available for Australia and New Zealand, respectively. Details of the DOC datasets are provided in Gadd et al. (2026a, 2026b).

Using the DOC-adjustment equation in Figure 1, bioavailability-adjusted protective concentrations (BAPCs) for 99%, 95%, 90% and 80% species protection were calculated for each available water chemistry sample (where DOC was measured) for all sites in each state and mainland territory in Australia and each region in New Zealand. The 99, 95, 90 and 80% species protection level BAPCs corresponding to the DOC concentration at the weighted 5th percentile of the BAPCs in the most sensitive state/territory in both Australia (Northern Territory) and the most sensitive region in New Zealand (Bay of Plenty, Canterbury and Otago) were selected as the DGVs. Full details of the process are provided in Gadd et al. (2026b).

The above approach aims to ensure that the DGVs are protective of 95% of waters in the most sensitive region in each country and, therefore, more than 95% of waters in each country. The high bioavailability water chemistry condition is termed the 'reference condition' and corresponds to a DOC concentration of ≤ 0.5 mg/L for both Australia and New Zealand. The final chronic toxicity dataset described in Section 4.2, normalised to the Australian and New Zealand DOC reference condition of 0.5 mg/L (using the equation in Figure 1), is shown in Table 2.

Table 2 Summary of single chronic toxicity values normalised to the reference condition of ≤ 0.5 mg/L DOC, 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 and 28	NOEC (mortality)	8.5 [a]
	Cyprinella monacha	Larva	30	IC10 (growth)	23
	Etheostoma fonticola	Larva	30	IC10 (biomass)	8.1 [a]
	Galaxias maculatus	Juvenile	21	NOEC (mortality)	18
	Gobiomorphus cotidianus	Juvenile	18	NOEC (mortality)	10
	Melanotaenia splendida inornata	Adult	30	NOEC (reproduction)	12
	Oncorhynchus mykiss	Juvenile	30	LC10 (mortality)	7.2 [a]
	Pelteobagrus fulvidraco	Juvenile	42	NOEC (growth)	71
	Perca fluviatilis	Juvenile	30	NOEC (growth)	24
	Pimephales promelas	Larva	30	IC10 (growth)	8.4 [a]
	Prosopium williamsoni	Juvenile	30 and 60	NOEC (mortality, growth)	1.5
Mollusc	*Alathyria profuga*	Larva	3	NEC (development)	1.2
	Cucumerunio novaehollandiae	Larva	3	NEC (development)	0.7
	Epioblasma capsaeformis	Juvenile	28	IC10 (mortality)	3.1
	Fluminicola sp.	Adult/juvenile	28	NOEC (mortality)	5.8
	Fontigens aldrichi	Adult/juvenile	28	EC10 (mortality)	8.1
	Hyridella australis	Larva	3	NEC (development)	0.8

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value normalised to 0.5 mg/L DOC (µg/L)
	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	—	14	EC10 (growth)	2.8
	Physa gyrina	Juvenile	28	EC10 (growth)	11
	Pomacea paludosa	Adult/juvenile	60	NOEC (growth)	8.8
	Pyrgulopsis robusta	Larva/juvenile	28	EC10 (mortality)	5.2
	Taylorconcha serpenticola	Adult/juvenile	28	LC20 (mortality)	11
	Velesunio ambiguus	Larva	3	NEC (development)	1.3
	Villosa iris	Adult/juvenile	28	EC10 (growth)	6.2
Crustacean	*Ceriodaphnia dubia*	Neonate	7	EC10 (survival)	10 [a]
	Daphnia ambigua	Neonate	10	EC10 (reproduction)	24
	Daphnia longispina	Neonate	21	NOEC (population growth rate)	15 [a]
	Daphnia magna	Neonate	21	EC20 (reproduction)	9.5 [a]
	Daphnia thomsoni	Neonate	14	NOEC (reproduction)	6.4
	Gammarus pulex	—	100	NOEC (population growth rate)	6.8
	Hyalella azteca	Juvenile	15 and 35	NOEC (mortality/ reproduction)	19
	Paracalliope fluviatilis	Juvenile	32	NOEC (mortality)	10
	Paranephrops planifrons	Juvenile	14	LC10 (mortality)	175
Insect	*Chironomus riparius*	Larva	10	NOEC (growth)	17
	Clistorina magnifica	Larva	240	NOEC (life cycle)	4.3
	Paratanytarsus parthenogeneticus	—	16	NOEC (growth/ reproduction)	40
Rotifer	*Brachionus calyciflorus*	Larva	2	EC20 (reproduction)	4.4 [a]
Cnidarian	*Hydra viridissima*	Budding adult	4	BEC10 (growth rate)	0.9 [a]
Macrophyte	*Lemna minor*	—	7	EC10 (root growth)	0.7 [a]
	Zizania palustris	Seedling	14	NOEC (growth – weight)	54
Green microalga	*Chlamydomonas reinhardtii*	Exponential growth phase	10	NOEC (growth rate)	22
	Chlorella sp. (PNG)	Exponential growth phase	3	EC10 (growth rate)	0.8 [a]
	Chlorella vulgaris	Exponential phase	3	EC10 (growth rate)	11.3 [a]
	Monoraphidium arcuatum	Exponential growth phase	3	EC10 (growth rate)	0.5
	Raphidocelis subcapitata	Exponential growth phase	3	NOEC (cell yield)	13.5

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value normalised to 0.5 mg/L DOC (µg/L)
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.

[a] Geometric mean

The different mechanisms of copper toxicity suggest that the dataset may exhibit bimodality or multimodality; hence, the dataset was assessed for 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 species (Figure 2). The dataset did not indicate bimodality based on histogram statistics, a bimodality coefficient of 0.34 for the log-transformed dataset (less than the threshold criterion for bimodality of 0.55), and the spread of taxa (from toxicity studies spanning 4 decades) in the SSD (Figure 3). Therefore, the dataset was deemed to be unimodal, and all the toxicity data (i.e. from 52 species) were used for the derivation.

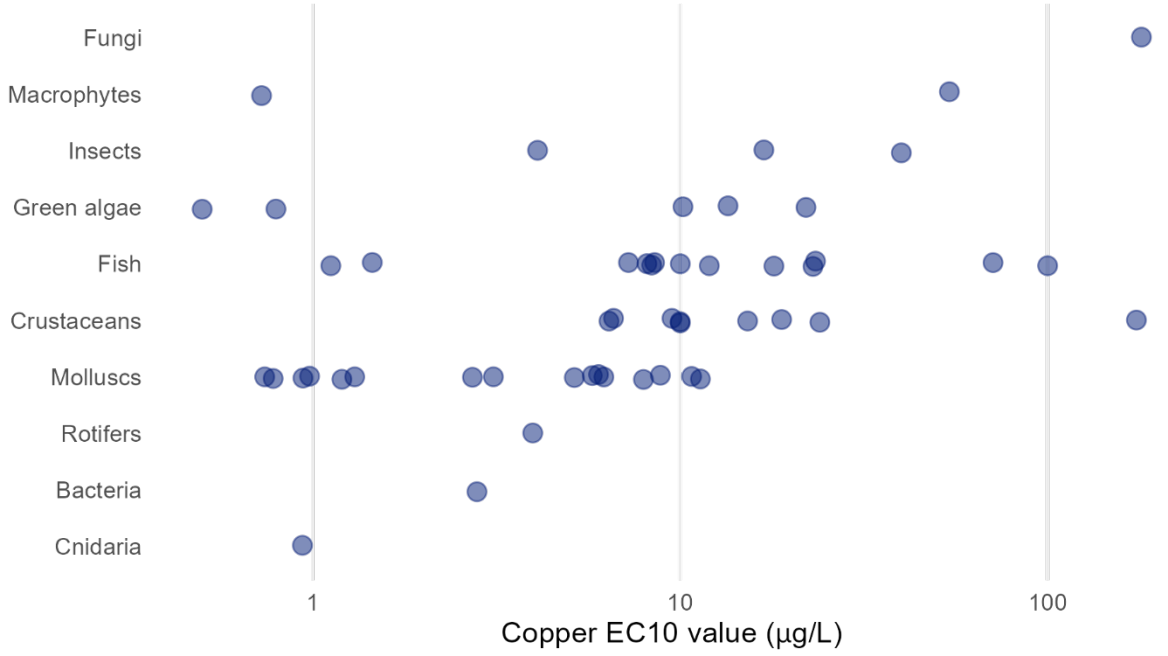


Figure 2 Comparison of dissolved copper toxicity between taxonomic groups. Copper toxicity data normalised to the Australian and New Zealand reference condition of 0.5 mg/L DOC.

4.4 Species sensitivity distribution

The SSD based on the 52 chronic toxicity data for dissolved copper in freshwater at the reference condition of ≤ 0.5 mg/L DOC (Table 2) is shown in Figure 3. Only one SSD was required for both Australia and New Zealand because the reference condition was the same for both countries. The SSD was modelled using the ssdtools package (Version 2.3.0) (Thorley et al. 2025) using the function to fit the 6 distributions recommended for the ANZG toxicant DGVs (Gamma, log-Gumbel, log-logistic, log-normal, log-normal/log-normal and Weibull) (Fox et al. 2024), with the model averaging method used to calculate the DGVs. The model was judged to provide a good fit to the data (Figure 3). Further details on the SSD fitting process are provided in Appendix D. A sensitivity analysis confirmed that the final dataset of 52 species with measured and estimated DOC concentrations was an appropriate dataset to derive DGVs for copper (Appendix E).

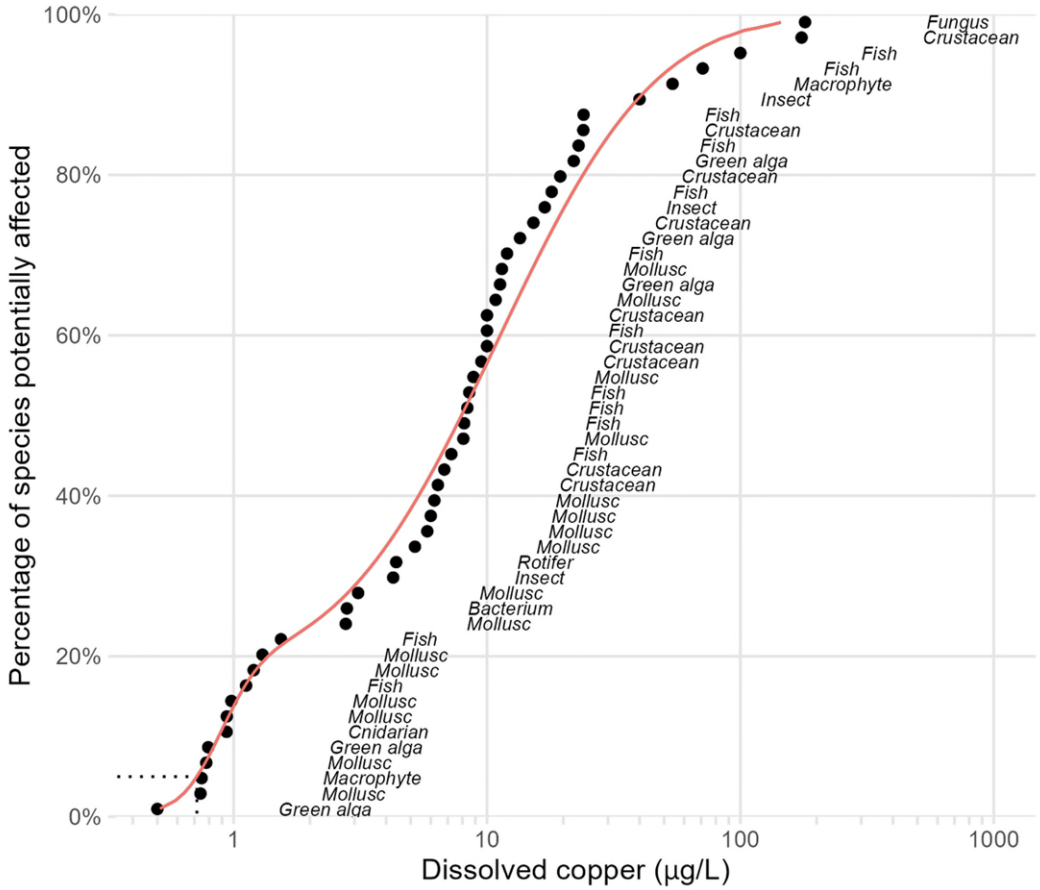


Figure 3 Species sensitivity distribution, dissolved copper in freshwater (at the reference condition of ≤ 0.5 mg/L dissolved organic carbon).

Dotted line indicates the copper concentration at which there is 95% species protection and 5% of species are potentially affected (0.73 µg/L).

4.5 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 and at the reference condition of DOC ≤ 0.5 mg/L are shown in Table 3. These DGVs supersede the ANZECC and ARMCANZ (2000) DGVs for copper in freshwater and should be applied in accordance with the tiered assessment framework for copper in freshwater (Section 4.6).

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	0.53
95	0.73
90	0.89
80	1.4

[a] DGVs were derived using the ssdtools package in R (Thorley et al. 2025) and apply to water with a DOC of ≤ 0.5 mg/L, a pH range of 6–8.5 and hardness range of 5–400 mg $CaCO_3$/L. DGVs have been rounded to 2 significant figures. DGVs can be adjusted for different DOC concentrations.

As noted in Section 4.3, the DGVs are expected to be protective of at least 95% of waters in both Australia and New Zealand. The DGVs apply to dissolved copper (operationally defined as the < 0.45 μm filtered measurement) and in water with pH between 6.0 and 8.5 and hardness between 5 mg/L and 400 mg/L as $CaCO_3$. Although the DGVs cannot be adjusted for pH or hardness, they may not be valid outside the above-specified applicability ranges as the toxicity test data used to either derive the DGVs or develop the DOC slope adjustment did not extend beyond these ranges. The range for pH is based solely on the toxicity dataset used for the derivation, whereas the range for hardness is based on both the toxicity dataset used for the derivation (i.e. 5–200 mg/L as $CaCO_3$, restricted to high bioavailability data) as well as the data used to develop the DOC slope adjustment (i.e. 20–400 mg/L as $CaCO_3$). The 95% species protection DGV and BAGVs are recommended for application to slightly-to-moderately disturbed ecosystems. The DGVs should be applied in accordance with the tiered assessment framework for copper in freshwater (Section 4.6).

The DGVs at 0.5 mg/L DOC may be close to analytical detection limits routinely used by commercial laboratories (e.g. 1 μg/L); however, when DOC is 1 mg/L or more, the associated BAGVs are likely to be above typical limits of detection. ANZG (2018) provides advice on data analysis where the DGV is less than the limit of detection.

Under the tiered assessment framework, if the dissolved copper concentration exceeds the relevant DGV, the DGV can be adjusted to a BAGV based on the DOC concentrations of ambient water (up to 20 mg/L), using either the equation in Figure 4 or the ANZG metals bioavailability tool. To illustrate how the BAGVs change across the DOC range, BAGVs for a selection of different DOC concentrations are provided in Table 4. DGVs should not be adjusted above a DOC of 20 mg/L, as this is outside the range of the data used to develop the adjustment and the models of copper toxicity and DOC are uncertain above this concentration. If DOC exceeds 20 mg/L, a BAGV based on adjustment to 20 mg/L should be used. DOC should be measured in filtered samples after first acidifying then purging CO_2 (sometimes reported as non-purgeable organic carbon (NPOC)). The alternative method of measuring total carbon, and inorganic carbon, then subtracting to calculate organic carbon, has high measurement error at the low concentrations generally found in surface waters.

$$DOC\ adjusted\ BAGV = DGV_{0.5} \times \left(\frac{DOC}{0.5}\right)^{0.697}$$

Figure 4 Equation for calculating bioavailability-adjusted GVs for dissolved copper in freshwater

Table 4 Toxicant default guideline values and bioavailability-adjusted guideline values, dissolved copper in freshwater at different dissolved organic carbon concentrations

DOC concentration (mg/L)	Guideline value for dissolved copper in freshwater, different levels of species protection (μg/L)			
	99%	**95%**	**90%**	**80%**
			Default	
≤0.5	**0.53**	**0.73**	**0.89**	**1.4**
			Bioavailability adjusted	
1	0.86	1.2	1.4	2.2
2	1.4	1.9	2.3	3.6
4	2.3	3.1	3.8	5.8
8	3.7	5.1	6.1	9.4
12	4.9	6.7	8.1	12
16	5.9	8.2	9.9	15
20	6.9	9.6	12	18

For freshwater with DOC > 20 mg/L and/or outside the specified ranges for pH (6.0–8.5) and hardness (5–400 mg/L), site-specific factors affecting toxicity should be considered to develop site-specific guideline values. This could include modelling of metal speciation or toxicity testing in relevant water chemistry.

In low DOC waters where other aspects of water chemistry such as high pH or hardness may affect copper speciation and/or bioavailability, existing speciation, MLR or BLM models could be used to adjust toxicity data for site-specific water chemistry and develop site-specific guideline values, provided their use can be justified and supported by other site-specific data including toxicity data. Note that this recommendation does not extend to using a BLM-based water quality guideline value from other jurisdictions, as these use different toxicity datasets that may not have been derived using chronic toxicity data or be relevant to Australian and New Zealand ecosystems.

4.6 Use of the DGVs in the tiered assessment framework

The DGVs should be applied in accordance with the tiered assessment framework for dissolved copper in freshwater. The tiered assessment framework, depicted as a flow chart in Appendix F, represents a decision tree to guide the user through the process of accounting for copper bioavailability when comparing monitoring data with guideline values, and includes a metals bioavailability tool to adjust DGVs to BAGVs based on local water chemistry. Details about the tiered

assessment framework can be found in Stauber et al. (2026) and ANZG (2018). Briefly, the tiered assessment framework comprises a screening level at Tier 1, where the measured dissolved copper concentration in a sample should be compared with the relevant DGV. If the DGV is exceeded at Tier 1, then at Tier 2, metal bioavailability for the local water quality conditions can be taken into account. This can be done either by (i) calculating a copper BAGV based on the sample's DOC concentration and comparing the dissolved copper concentration in the sample to the BAGV, or (ii) adjusting the copper concentration in the sample to represent the bioavailable fraction, and comparing this concentration with the DGV. If the sample still exceeds the relevant guideline value, then, at Tier 3, additional lines of evidence are required to determine the implications of the exceedance, which may include toxicity testing or bioavailability modelling.

4.7 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—52 (preferred)
- type of toxicity data—chronic (EC10, IC10, LC10, EC15, EC20, NEC, NOEC)
- SSD model fit—good.

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 (ACR)	The species mean acute value (LC50/EC50) divided by the chronic value (e.g. NOEC or EC10) for the same species.
ANZG	Australian and New Zealand Guidelines
BAGV (bioavailability-adjusted guideline value)	A default guideline value that has been adjusted to account for one or more specific toxicity modifying factors using one or more established models.
BLM (biotic ligand model)	A model or set of models for metal toxicity in aquatic organisms that accounts for differing bioavailability of individual metals in waters with differing chemistry (particularly DOC, pH, and cations).
CHESS (chemical equilibria in soils and solutions)	Model used to predict metal speciation (Santore and Driscoll 1995).
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.
DGV (default guideline value)	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)	Refers specifically to the mass of carbon in the dissolved organic material, operationally defined as that which can pass through a filter with a pore size typically between 0.22 and 0.7 µm.
DOM (dissolved organic matter)	A complex heterogeneous mixture of humic substances (humic and fulvic acids), carbohydrates, proteins, and amino acids and is formed via the decomposition of plant and animal 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.

Term	Definition
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.
MLR (multiple linear regression)	A statistical technique that uses 2 or more explanatory variables to model a single dependent variable.
NEC (no effect concentration)	The maximum concentration of a toxicant that causes no adverse effect in a target organism.
Negligible effect	Corresponding to an estimated no-effect or acceptable-effect level; negligible-effect concentrations typically include the NEC, NSEC, BEC10, ECx/ICx where x ≤ 10, and NOEC, but if necessary, can also include the ECx/ICx where x is > 10 and ≤ 20.
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.
Reference condition	The high metal bioavailability water chemistry condition at the default guideline values. At least 95% of waters in Australia and New Zealand would have lower bioavailability conditions than the reference condition. It is based on an extensive collation and analysis of data from freshwaters around Australia and New Zealand.
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.
Slightly-to-moderately disturbed	ANZG narrative descriptor for 95% protection level .
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.
WHAM V (Windermere humic acid model, version V)	Model (Version V, 1994) to predict metal speciation in soil and water, with an emphasis on binding to humic acid (UK CEH 2021).
WQC	Water quality criterion, from US EPA (2007).

Appendix A: Effect of water chemistry on copper toxicity

The effect of water chemistry on copper toxicity is most apparent in studies with a suite of toxicity tests where a single TMF is varied, while other modifying factors are held constant. This approach was used by Crémazy et al. (2017) in a study with rainbow trout and De Schamphelaere and Janssen (2004, 2006) in studies with the cladoceran *Daphnia magna*, and the green algae *Chlorella vulgaris* and *Raphidocelis subcapitata*. Data from these studies are shown in Figure A 1 to Figure A 4 to demonstrate the clear effect of DOC on toxicity, and that DOC has a greater effect on toxicity than pH or hardness.

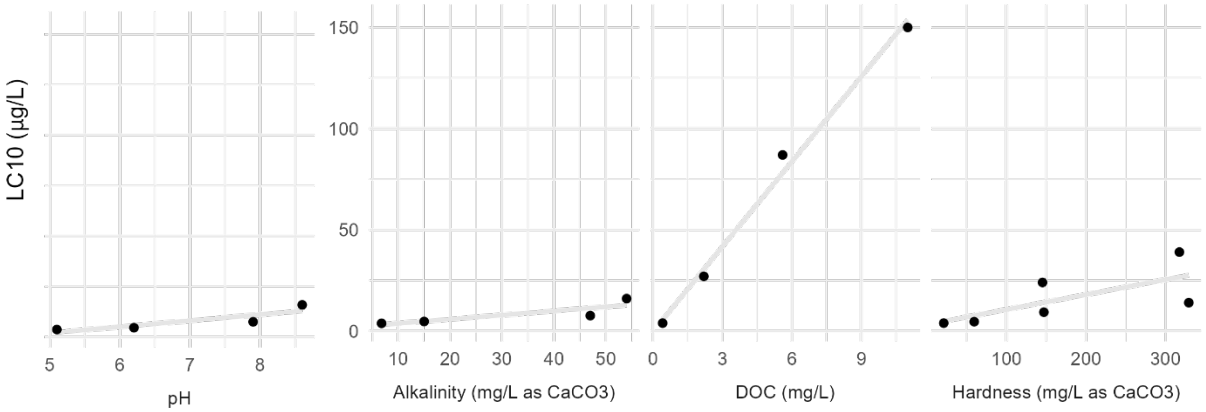


Figure A 1 Effect of different TMFs on toxicity values for rainbow trout, when each are varied individually across ranges commonly found in natural waters

Data from Crémazy et al. (2017) for 30-d mortality tests with rainbow trout (*Oncorhynchus mykiss*). Note that alkalinity and pH change simultaneously in these tests (the 2 plots demonstrate the same data).

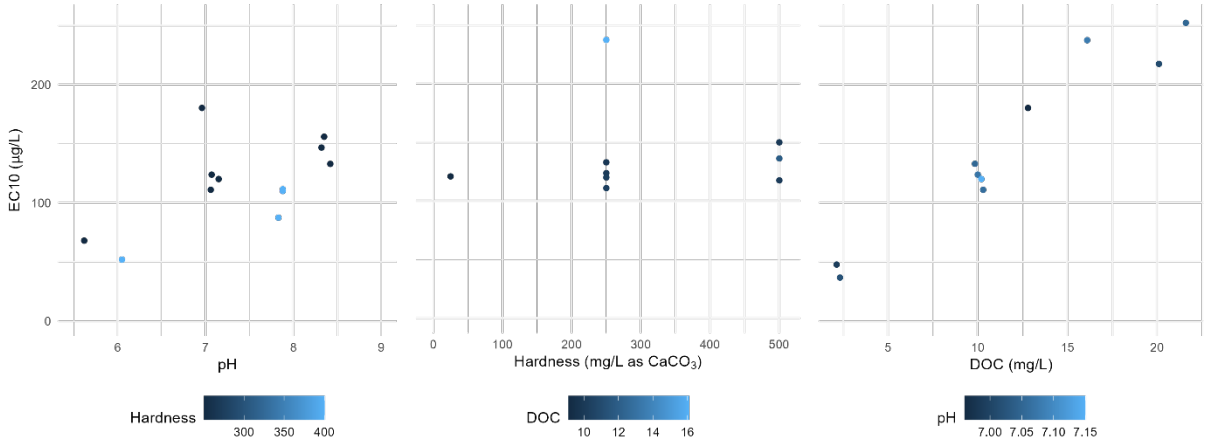


Figure A 2 Effect of different TMFs on toxicity values for the cladoceran *Daphnia magna*, when each are varied individually across ranges commonly found in natural waters

Colour of point depends on hardness, DOC or pH value, as shown in the legend for each plot. Data from De Schamphelaere and Janssen (2004) for 21-d reproduction tests. DOC in the pH set was 5–12 mg/L; pH in the hardness set was 7.0–7.2; water hardness in the DOC set was consistently 250 mg $CaCO_3$/L.

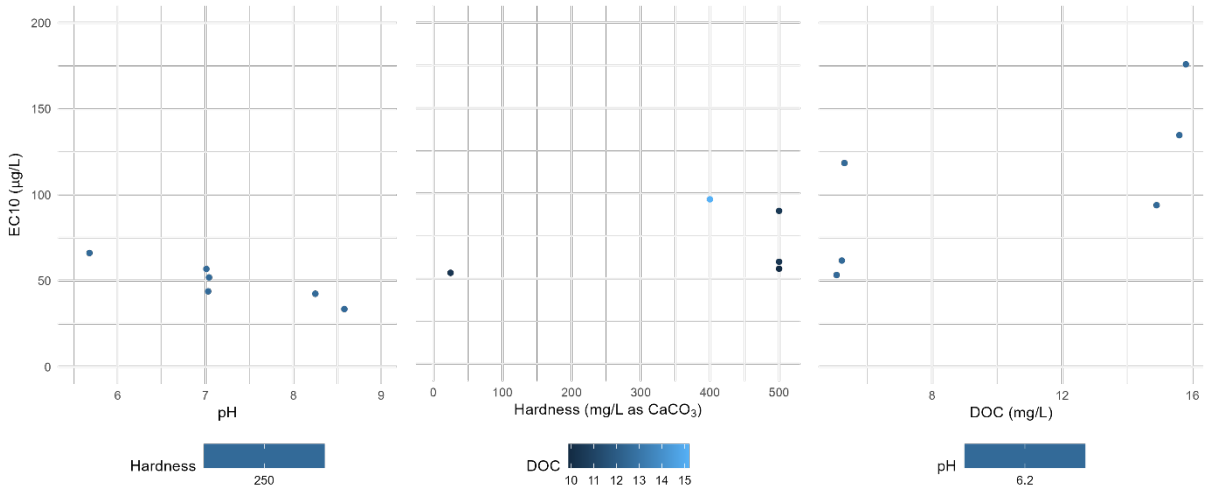


Figure A 3 Effect of different TMFs on toxicity values for *Raphidocelis subcapitata* [1], when each are varied individually across ranges commonly found in natural waters

Data from De Schamphelaere and Janssen (2004) for 3-d population growth tests. DOC in the pH set was 9.8–10.5 mg/L; pH in the hardness set was 7.0–7.8; water hardness in the DOC set was consistently 100 mg $CaCO_3$/L.

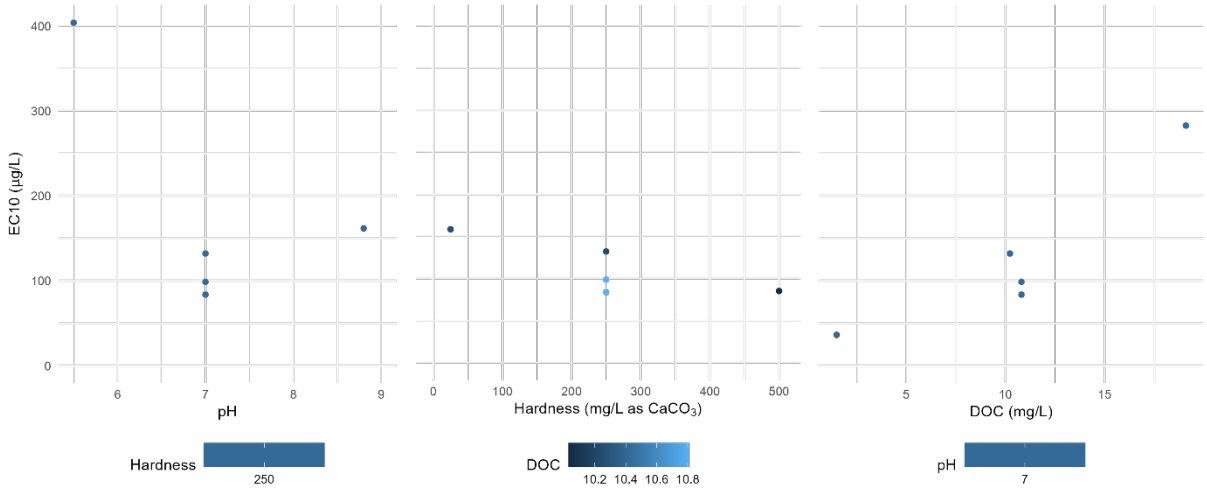


Figure A 4 Effect of different TMFs on toxicity values for the alga *Chlorella vulgaris*, when each are varied individually across ranges commonly found in natural waters

Data from De Schamphelaere and Janssen (2004) for 3-d population growth tests. DOC in the pH set was 9.9–10.8 mg/L; pH in the hardness set was 7.0; water hardness in the DOC set was consistently 250 mg $CaCO_3$/L. The outlier with an EC10 of 400 µg/L is shown at pH 5.5 (hardness 250 mg/L and DOC 10 mg/L) and outside the applicability range for dissolved copper DGVs.

[1] The original paper refers to this species as *Pseudokirchneriella subcapitata*, as it was known at that time.

Appendix B: Calculation of DOC adjustment

To develop a DOC adjustment, data were collated from studies that assessed the effects of differing DOC concentrations on chronic copper toxicity. Such data were available for 10 different species, including 3 green microalgae, 1 macrophyte, 2 crustaceans, 1 rotifer, 1 mollusc and 2 fish (Table B 1). However, not all data were suitable for calculating slopes with DOC. Studies were not included if tested at only 2 DOC concentrations or if DOC was estimated (e.g. where values were reported as below the limit of detection). Furthermore, similar rules to those established by Stephan et al. (1985) for hardness adjustment were used. This required the highest DOC to be at least 3 times, and at least 2 mg/L greater than the lowest DOC. Reproduction tests with *Ceriodaphnia dubia* by Wang et al. (2011) were not included as the authors reported that the metals were not adequately equilibrated with the DOC solutions and that this affected the reproduction results (but not the survival data).

Table B 1 Chronic toxicity data available at different DOC values

Species	Data type	Reference	Suitability for calculating slopes
Chlorella sp.	EC10 and EC50	Macoustra et al. (2019)	Suitable, multiple DOC concentrations (and types of DOC)
Chlorella vulgaris	EC10 and EC50	De Schamphelaere and Janssen (2006)	Suitable, DOC 1.5 to 15 mg/L
Raphidocelis subcapitata	EC10 and EC50	De Schamphelaere et al. (2003)	Suitable, DOC 1.3 to 20 mg/L
Zizania palustris	NOEC	Nimmo et al. (2003)	No, low DOC reported as < 1 mg/L, insufficient data
Brachionus calyciflorus	EC20	De Schamphelaere et al. (2006)	No, tests at only 2 DOC concentrations
Ceriodaphnia dubia	EC10 and EC20	Wang et al. (2011)	Survival data included (DOC 0.4 to 10 mg/L n=4), reproduction data not used as test solutions not equilibrated which affected reproduction results
Ceriodaphnia dubia	EC20	Schwartz and Vigneault (2007)	Suitable, DOC 0.4 to 33 mg/L
Daphnia magna	EC10 and EC50	Villavicencio et al. (2011)	No, DOC range too small (0.2 to 2.0 mg/L)
Daphnia magna	EC20 and EC50	De Schamphelaere and Janssen (2004)	Suitable, DOC 1.7 to 22 mg/L
Hyridella depressa	EC20 and EC50	Markich et al. (2003)	No, valve closure not an accepted endpoint for slope factor
Villosa iris	EC10 and EC20	Wang et al. (2011)	Suitable, DOC 0.6 to 10 mg/L (n=3)
Oncorhynchus mykiss	LC10 and LC50	Crémazy et al. (2017)	Suitable, DOC 0.6 to 10 mg/L (n=3)
Pimephales promelas	NOEC	Welsh (1996)	No, tests at only 2 DOC concentrations

Typically, EC50 values would be the preferred toxicity values for calculating slope factors as they have narrower confidence intervals than EC10 or EC20 values. However, EC50 values were not available from all studies and using only EC50 values would have reduced the number of species for which slopes could be calculated from 7 to 5. Where EC20 values were available for a study, they were used in preference over EC10 values to reduce the uncertainty in the slopes associated with the higher uncertainty of EC10 values.

Slopes between toxicity values (EC20, EC10) and DOC were calculated for each species with sufficient and suitable data. Slopes were calculated for each study or study-subset individually (Table B 2) rather than pooling across studies. There were several reasons for this:

- Different pH and hardness between studies and/or between tests reported in a paper. While these may not be the dominant TMF for copper, they may influence some species. Where there was variation across pH and hardness, tests with similar pH (within 0.5 pH units) and hardness (within a factor of 1.6) were pooled, with slopes calculated for different datasets (e.g. tests at pH 7, tests at pH 8).
- Possible study-specific species sensitivity.

Slopes were calculated using log10 derivatives. Slopes using EC50 values were also calculated (where available) and are shown in Table B 2 for comparison with the slopes from EC20 or EC10 values.

Species geometric mean slopes were calculated and then an overall geometric mean for all species was calculated, in a method analogous to that used by US EPA (1996) for hardness adjustments (Table B 2). The species geometric mean slopes ranged from 0.49 for *V. iris* to 1.09 for *O. mykiss*. The overall geometric mean slope was 0.697. This slope is considered suitable for adjustment of toxicity data and BAGVs up to a DOC of 20 mg/L. This is based on the range of DOC available for deriving the slope, whereby there were multiple points around 20 mg/L and data for only one species above this.

Table B 2 Slopes between DOC and toxicity values

Species	Data sources	Test details (H= hardness)	EC50 slope	Species selected or geometric mean slope	Statistic	EC10/20 slope	R^2	Species selected or geometric mean slope
Chlorella sp.	Macoustra et al. (2019)	pH 7.3, H 85, DOC 0.3-13	0.908	0.908	EC10	0.915	0.68	**0.915**
Chlorella vulgaris	De Schamphelaere and Janssen (2006)	pH 7.0, H 250, DOC 1.5-19	0.794	0.794	EC10	0.686	0.81	**0.686**
Raphidocelis subcapitata	De Schamphelaere et al. (2003)	pH 7, H 250, DOC 2.0-18	0.990	—	EC10	0.821	0.93	—
Raphidocelis subcapitata	De Schamphelaere et al. (2003)	pH 8, H250-400, DOC 1.3-16	0.256	0.503	EC10	0.473	0.38	**0.623**
Ceriodaphnia dubia	Wang et al. (2011)	Survival data, pH 8.3, H100, DOC 0.4-10	NA	—	EC20	0.811	0.91	—

Species	Data sources	Test details (H= hardness)	EC50 slope	Species selected or geometric mean slope	Statistic	EC10/20 slope	R^2	Species selected or geometric mean slope
Ceriodaphnia dubia	Schwartz and Vigneault (2007)	Reproduction, pH 7.3-7.6, H 23-35, DOC 0.4-33	NA	—	EC20	0.450	0.87	**0.604**
Daphnia magna	De Schamphelaere and Janssen (2004)	pH 6.1, H 100, DOC 4.8-17	0.913		EC20	0.838	0.90	—
Daphnia magna	De Schamphelaere and Janssen (2004)	pH 7.1, H 250, DOC 1.7-22	0.796	—	EC20	0.736	0.95	—
Daphnia magna	De Schamphelaere and Janssen (2004)	pH 8.3, H 250, DOC 5.2-18	0.621	0.767	EC20	0.425	0.78	**0.640**
Villosa iris	Wang et al. (2011)	Growth (weight), pH 8.2-8.3, H 98-101, DOC 0.6-10	NA	—	EC20	0.480	0.62	—
Villosa iris	Wang et al. (2011)	Growth (biomass), pH 8.2-8.3, H 98-100, DOC 0.6-10	NA	—	EC20	0.444	0.60	—
Villosa iris	Wang et al. (2011)	Survival, pH 8.2-8.3, H 98-100, DOC 0.6-10	NA	—	EC20	0.542	0.97	**0.487**
Oncorhynchus mykiss	Crémazy et al. (2017)	pH 7-7.3, H 20-24, DOC 0.4-11	0.99	0.995	EC20	1.09	0.997	**1.086**
					Overall geometric mean slope			**0.697**

NA Not applicable

Evaluation of the derived slope factor

The predictiveness and protection of the slope factor was investigated where there were suitable data, to evaluate the derived slope factor.

For each of the datasets with differing DOC, ECx values from tests in high DOC waters were adjusted to the lowest DOC tested in that study, or in that test group (e.g. same DOM type or same pH and hardness). For example, EC10 values for *Chlorella* sp. tested in Appletree Creek DOM at DOC of 9.4 mg/L (Macoustra et al. 2019) were adjusted to a DOC of 2.1 mg/L (the lowest tested). These "predicted" EC10 values were compared to the measured EC10 value (Figure B 1). For the slope factor of 0.70, around 71% of values were within a factor of 2, a factor commonly used to assess model suitability (Di Toro et al. 2001; Garman et al. 2020). About 86% of predictions were within a factor of 3, a range that has been considered more suitable for EC10 data (Price et al. 2022). However, much of the data shown here were outside the range used for DGV calculation as the

hardness was > 200 mg $CaCO_3$/L, or DOC was > 10 mg/L[2]. When those data were removed (leaving 53 values), 62% of values were within a factor of 2 and 83% within a factor of 3. Residuals based on the predicted ECx divided by the measured ECx can be used in evaluating a model's accuracy. A regression of the residuals against measured ECx values was not significant (p-value 0.2, R^2 value = 0.005) and did not show any clear bias.

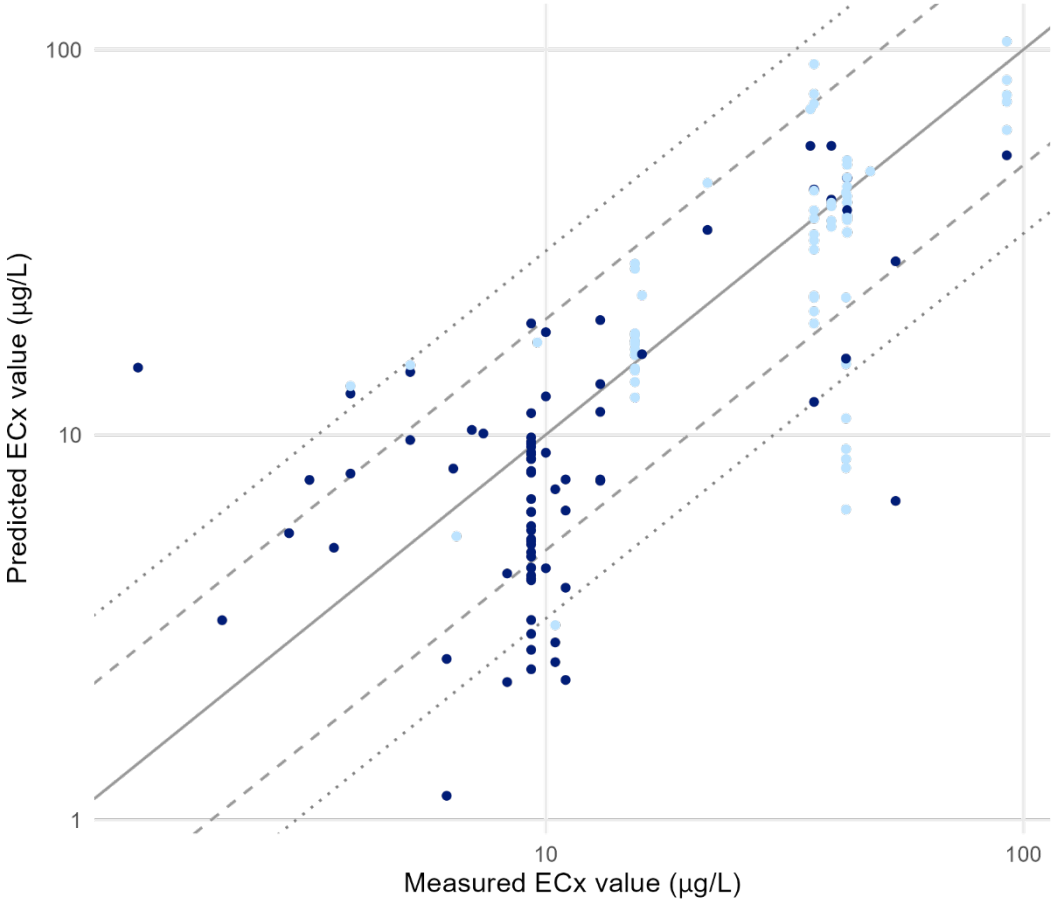


Figure B 1 Comparison of measured toxicity values at lower DOC to predicted values from tests at higher DOC.

Each point represents a single test. Dark blue points represent studies that would be included in the DGV derivation; light blue points represent studies that would be excluded based on high hardness (> 200 mg $CaCO_3$/L) or DOC (> 10 mg/L). Solid grey line represents a 1:1 line and dashed grey lines represent a factor of 2 (and 3) above or below.

Data for Australian and New Zealand species tested at DOC greater than 0.5 mg/L were collated to assess protection (Table B 3). Bioavailability-adjusted guideline values (BAGVs) were calculated at the DOC of the test and compared to the reported EC10 (or NOEC). BAGVs lower than the EC10 indicate that the BAGV at that DOC would protect that species, whereas EC10 values below the BAGV indicate that a species may not be adequately protected.

There were 2 species where one or more of the reported EC10 values were lower than the BAGVs. The first species was a freshwater mussel (*E. menziesii*), where 1 of 3 reported EC10 values was

[2] This cut-off was used to avoid converting values by large amounts, when changing from DOC 10 mg/L to DOC 0.5 mg/L, see Section 4.1.

below the BAGV, while the other 2 values were above the BAGV. Further, the DOC concentrations reported for this test were influenced by resin from the water purification equipment and are unlikely to have any mitigating effect on copper toxicity (Clearwater et al. 2014). The other species, *Moinodaphnia macleayi*, was tested in slightly acidic and very low hardness waters (i.e. 2.8 mg/L as $CaCO_3$, Trenfield et al. 2022), which may affect the bioavailability. As such, this species was not included in the dataset used to derive the DGVs (hardness < 10 mg $CaCO_3$/L) and is close to the range for the BAGV application (hardness ≥ 2 mg $CaCO_3$/L). The remaining species would be protected by the BAGVs, providing further confidence in the use of the slope factor of 0.70.

Table B 3 Toxicity data for native species tested at DOC values greater than 0.5 mg/L

Taxonomic Group	Species	Endpoint	Hardness (mg/L as $CaCO_3$)	pH	DOC	Measured concen-tration (µg/L)	BAGV at reported DOC (µg/L)
Green alga	*Chlorella* sp. (eriss)	Population growth rate	1.9	6.3	2.1	1.6	1.2
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	2.1	7	1.2
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	9.4	10	1.9
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	2.8	7	1.3
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	1.2	16	1.0
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	1.9	3	1.1
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	13	8	2.1
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	2.3	1.4	1.2
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	9.9	44	1.9
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	3	3	1.3
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	8.5	12	1.8
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	1.9	7	1.1
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	8.8	32	1.8
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	2.3	7	1.2
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	9.6	29	1.9
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	3.7	3	1.4
Green alga	*Chlorella* sp. (PNG)	Population growth rate	85	7.3	8.4	14	1.8
Macrophyte	*Lemna aequinoctialis*	Growth	3.1	6.8	2	6.8	1.1
Cnidarian	*Hydra viridissima*	Population	2.7	6.6	2.1	2.5	1.2
Mollusc	*Amerianna cumingi*	Reproduction	2.1	6.7	2.1	5.7	1.2
Mollusc	*Echyridella menziesii*	Mortality	30	7.8	2.45	1.2	1.2
Mollusc	*Echyridella menziesii*	Mortality	30	7.8	2.45	2.7	1.2
Mollusc	*Echyridella menziesii*	Mortality	30	7.8	2.45	1.8	1.2
Crustacean	*Daphnia thomsoni*	Reproduction	20	7.2	4.9	65	1.5
Crustacean	*Moinodaphnia macleayi*	Reproduction	2.8	6.6	2	1.0	1.1
Fish	*Mogurnda mogurnda*	Growth	3.1	6.7	1.4	9.6	1.0

Rows highlighted in orange indicate where the BAGV is greater than the reported EC10 value and where the species may not be protected.

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

Table C 1 Summary of chronic toxicity data used to derive the freshwater copper default guideline values (Bold values 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 (estimated values in italics)[a]	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
Fish	*Cirrhinus mrigala*	Juvenile	60	NOEC (growth)	26	7.6	135	*0.5*	100	**100**	Mohanty et al. (2009)
Fish	*Cottus bairdi*	Juvenile	21	NOEC (mortality)	–	8.3	101	*0.5*[b]	2.9	2.9	Besser et al. (2007)
		Juvenile	28	NOEC (mortality)	–	8.2	104	*0.5*[b]	25	25	Besser et al. (2007)
										8.5	**Value used in SSD (geometric mean)**
Fish	*Cyprinella monacha*	Larva	30	IC10 (growth)	25	8.3	162	*0.5*	23	**23**	Besser et al. (2005)
Fish	*Etheostoma fonticola*	Larva	30	IC10 (biomass)	23	8.3	170	*0.5*	7.1	7.1	Besser et al. (2005)
		Larva	30	IC10 (biomass)	23	8.3	170	*0.5*	9.3	9.3	Besser et al. (2005)
										8.1	**Value used in SSD (geometric mean)**
Fish	*Galaxias maculatus*	Juvenile	21	NOEC (mortality)	20	7.8	39	*0.5*	18	**18**	Hickey et al. (2000)
Fish	*Gobiomorphus cotidianus*	Juvenile	18	NOEC (mortality)	–	7.0	37	*0.5*	10	**10**	Hickey et al. (2000)
Fish	*Melanotaenia splendida inornata*	Adult	30	NOEC (reproduction)	25	7.5	54	*0.5*	12	**12**	Skidmore (1986)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC (estimated values in italics)[a]	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
Fish	*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	7.2	145	0.5	24	24	Crémazy et al. (2017)
		Juvenile	30	LC10 (mortality)	13	7.1	147	0.5	9.3	9.3	Crémazy et al. (2017)
										7.2	**Value used in SSD (geometric mean)**
Fish	*Pelteobagrus fulvidraco*	Juvenile	42	NOEC (growth)	21.9	7.6	62	*0.5*	71	**71**	Chen et al. (2013)
Fish	*Perca fluviatilis*	Juvenile	30	NOEC (growth)	–	7.8	194	1.0	39	**24**	Collvin (1985)
Fish	*Pimephales promelas*	Larva	30	IC10 (growth)	25	8.3	170	*0.5*	4	4	Besser et al. (2005)
		Larva	30	IC10 (growth)	23	8.3	170	*0.5*	16	16	Besser et al. (2005)
										8.4	**Value used in SSD (geometric mean)**
Fish	*Proposium williamsoni*	Embryo	90	NOEC (mortality)	9.6	6.8	48	1.9	8.3	3.1	Brinkman and Viera (2008)
		Juvenile	30	NOEC (mortality)	9.6	6.8	48	1.9	3.9	1.5	Brinkman and Viera (2008)
		Juvenile	60	NOEC (growth)	9.6	6.8	48	1.9	3.9	1.5	Brinkman and Viera (2008)
										1.5	**Value used in SSD (most sensitive endpoint)**
Mollusc	*Alathyria profuga*	Larva	3	NEC (development)	22	7.0	42	0.1	1.2	**1.2**	Markich (2017)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC (estimated values in italics)[a]	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
Mollusc	*Cucumerunio novaehollandiae*	Larva	3	NEC (development)	22	7.0	42	0.1	0.7	**0.7**	Markich (2017)
Mollusc	*Epioblasma capsaeformis*	Juvenile	28	IC10 (mortality)	20	8.2	162	*0.5*	<3.1	**3.1**	Wang et al. (2007)
Mollusc	*Fluminicola* sp.	Adult/juvenile	28	NOEC (mortality)	–	8.4	166	0.6	6.6	**5.8**	Besser et al. (2009)
Mollusc	*Fontigens aldrichi*	Adult/juvenile	28	EC10 (mortality)	–	8.3	166	0.7	10	**8.1**	Besser et al. (2016)
Mollusc	*Hyridella australis*	Larva	3	NEC (development)	22	7.0	42	0.1	0.8	**0.8**	Markich (2017)
Mollusc	*Hyridella depressa*	Larva	3	NEC (development)	22	7.0	42	0.1	0.9	**0.9**	Markich (2017)
Mollusc	*Hyridella drapeta*	Larva	3	NEC (development)	22	7.0	42	0.1	1.0	**1.0**	Markich (2017)
Mollusc	*Juga plicifera*	–	30	NOEC (mortality)	–	7.1	23	*0.5*	6.0	**6.0**	Nebeker et al. (1986)
Mollusc	*Lymnaea stagnalis*	Larva	28	EC20 (mortality)	21	8.3	170	0.7	9.9	7.6	Besser et al. (2009)
		Larva	28	EC20 (mortality)	21	8.3	170	0.7	16.9	12.9	Besser et al. (2009)
		Larva	28	EC20 (mortality)	21	8.3	170	0.7	22.4	17.1	Besser et al. (2009)
		Larva	28	EC20 (mortality)	21	8.3	170	0.7	18.0	13.7	Besser et al. (2016)
		–	14	EC10 (growth)	21	7.8	116	0.8	3.7	2.8	Crémazy et al. (2018)
										2.8	**Value used in SSD (most sensitive endpoint)**
Mollusc	*Physa gyrina*	Juvenile	28	EC10 (growth)	20	8.3	170	0.6	12	**11**	Besser et al. (2016)
Mollusc	*Pomacea paludosa*	Adult/juvenile	60	NOEC (growth)	28	7.9	70	*0.5*	8.8	**8.8**	Rogevich et al. (2009)
Mollusc	*Pyrgulopsis robusta*	Larva/juvenile	28	EC10 (mortality)	–	8.2	170	0.6	5.9	**5.2**	Besser et al. (2016)
Mollusc	*Taylorconcha serpenticola*	Adult/juvenile	28	LC20 (mortality)	–	8.4	166	0.6	13	**11**	Besser et al. (2016)
Mollusc	*Velesunio ambiguus*	Larva	3	NEC (development)	22	7.0	42	0.1	1.3	**1.3**	Markich (2017)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC (estimated values in italics)[a]	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
Mollusc	*Villosa iris*	Adult/juvenile	28	EC10 (growth)	20	8.3	101	0.5	6.2	**6.2**	Wang et al. (2011)
Insect	*Chironomus riparius*	Larva	10	NOEC (growth)	–	6.9	151	*0.5*	17	**17**	Taylor et al. (1991)
Insect	*Clistorina magnifica*	Larva	240	NOEC (life cycle)	20	7.6	26	*1.3*	8.3	**4.3**	Nebeker et al. (1984)
Insect	*Paratanytarsus parthenogeneticus*	–	16	NOEC (growth)	–	7.0	25	*0.5*	40	40	Hatakeyama and Yasuno (1981)
		–	16	NOEC (reproduction)	–	7.0	25	*0.5*	40	40	Hatakeyama and Yasuno (1981)
										40	**Value used in SSD**
Crustacean	*Ceriodaphnia dubia*	Neonate	7	EC10 (survival)	20	8.4	105	0.4	10	10	Wang et al. (2011)
		Neonate	7	EC10 (reproduction)	20	8.4	105	0.4	11	11	Wang et al. (2011)
		Neonate	7	EC20 (reproduction)	20	7.6	23	0.4	10	10	Schwartz and Vigneault (2007)
										10	**Value used in SSD (most sensitive endpoint)**
Crustacean	*Daphnia ambigua*	Larva	10	EC10 (reproduction)	21	8.0	63	*0.5*	24	**24**	Harmon et al. (2003)
Crustacean	*Daphnia longispina*	Neonate	21	NOEC (population growth rate)	20	7.8	180	*0.5*	5.5	5.5	Agra et al. (2011)
		Neonate	21	NOEC (population growth rate)	20	7.8	180	*0.5*	42	42	Agra et al. (2011)
										15	**Value used in SSD (geometric mean)**
Crustacean	*Daphnia magna*	Neonate	21	EC20 (reproduction)	20	7.9	76	0.3	6.3[c]	6.3	Villavicencio et al. (2011)
		Neonate	21	EC20 (reproduction)	20	8.0	18	0.5	10[c]	10	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 (estimated values in italics)[a]	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Neonate	21	EC20 (reproduction)	20	8.4	70	0.2	11 [c]	11	Villavicencio et al. (2011)
		Neonate	21	EC20 (reproduction)	20	8.2	33	0.5	12 [c]	12	Villavicencio et al. (2011)
										9.5	**Value used in SSD (geometric mean)**
Crustacean	*Daphnia thomsoni*	Neonate	14	NOEC (reproduction)	–	7.2	20	0.3	6.4	**6.4**	Thompson et al. (2021)
Crustacean	*Gammarus pulex*	–	100	NOEC (population growth rate)	11	8.0	103	*1.0*	11	**6.8**	Maund et al. (1992)
Crustacean	*Hyalella azteca*	Juvenile	15	NOEC (mortality)	22	7.6	128	*1.0*	32	19	Othman and Pascoe (2002)
		Juvenile	35	NOEC (reproduction)	22	7.6	128	*1.0*	32	19	Othman and Pascoe (2002)
										19	**Value used in SSD**
Crustacean	*Paracalliope fluviatilis*	Juvenile	32	NOEC (mortality)	20	7.8	39	*0.5*	10	**10**	Hickey et al. (2000)
Crustacean	*Paranephrops planifrons*	Juvenile	14	LC10 (mortality)	17.9	7.8	37	*0.5*	175	**175**	Albert et al. (2021)
Rotifer	*Brachionus calyciflorus*	Larva	2	EC20 (reproduction)	25	6.0	100	4.9	9.6	2.0	De Schamphelaere et al. (2006)
		Larva	2	EC20 (reproduction)	25	7.8	100	4.8	48	9.8	De Schamphelaere et al. (2006)
										4.4	**Value used in SSD (geometric mean)**
Cnidarian	*Hydra viridissima*	Budding adult	4	BEC10 (growth rate)	27	6.0	6.6	*0.5*	0.8	0.8	Riethmuller 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 (estimated values in italics)[a]	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
		Budding adult	4	BEC10 (growth rate)	27	6.0	165	*0.5*	1.1	1.1	Riethmuller et al. (2000)
										0.9	**Value used in SSD (geometric mean)**
Macrophyte	*Lemna minor*	–	7	EC10 (root growth)	25	7.6	163	1.0	2.1	1.3	Antunes et al. (2012)
		–	7	EC10 (root growth)	25	7.9	184	1.0	0.7	0.4	Antunes et al. (2012)
										0.7	**Value used in SSD (geometric mean)**
Macrophyte	*Zizania palustris*	Seedling	14	NOEC (growth - weight)	–	6.8	89	< 1	54	**54**	Nimmo et al. (2003)
Green microalga	*Chlamydomonas reinhardtii*	Exponential growth phase	10	NOEC (growth rate)	24	6.6	25	*0.5*	22	**22**	Schafer et al. (1994)
Green microalga	*Chlorella* sp. (PNG)	Exponential growth phase	3	EC10 (growth rate)	27	–	85	0.5	0.7	0.7	McKnight et al. (2023)
		Exponential growth phase	3	EC10 (growth rate)	27	7.3	85	0.3	0.9	0.9	Macoustra et al. (2019)
										0.8	**Value used in SSD (geometric mean)**
Green microalga	*Chlorella vulgaris*	Exponential growth phase	3	EC10 (growth rate)	25	6.0	100	5.2	108	21.2	De Schamphelaere and Janssen (2006)
		Exponential growth phase	3	EC10 (growth rate)	25	7.9	100	5.3	31	6.0	De Schamphelaere and Janssen (2006)
										11.3	**Value used in SSD (geometric mean)**
Green microalga	*Monoraphidium arcuatum*	Exponential growth phase	3	EC10 (growth rate)	27	–	85	*0.5*	0.5	**0.5**	McKnight et al. (2023)

Taxonomic group	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Temp. (°C)	pH	Water hardness (mg $CaCO_3$/L)	DOC (estimated values in italics)[a]	Reported concentration (µg/L)	Concentration normalised to 0.5 mg/L DOC (µg/L)	Reference
Green microalga	*Raphidocelis subcapitata*	Exponential growth phase	3	NOEC (cell yield)	23	7.8	26	0.5	13.5	**13.5**	Heijerick et al. (2005)
Fungus	*Penicillium* strain	Spore	21	IC10 (population growth rate)	21	7.3	52	*0.5*	180	**180**	Lategan and Hose (2014)
Bacterium	*Isolate 37* Unidentified *sp.*	–	2	EC15 (population growth rate)	30	7.8	60	*0.5*	2.8	**2.8**	Davies et al. (1998)

[a] DOC estimated by van Sprang et al. (2008) as 0.5 mg/L for reconstituted waters, for other waters based on assumptions from Santore et al (2002): 1 mg/L for tap water and spring water and 1.3 mg/L for well water at Western Fish Toxicology Station. [b] Reported as < 1 mg/L. [c] EC20 value derived by Brix et al. (2017) using raw data from original study.

Appendix D: Details of the shinyssdtools SSD fitting process for the copper toxicity dataset

The SSD software package ssdtools (V 2.3.0) uses a model averaging approach to generating a SSD (as described by Fox et al. 2021). Figure D 1 shows the full set of default distributions fitted to the copper freshwater toxicity dataset, while Table D 1 provides the relevant goodness of fit and weighting estimates for each of the distributions. The Gamma distribution, with the highest AIC, was weighted zero for the model averaging and, hence, had no influence on the DGVs. The final model is shown in Figure 3.

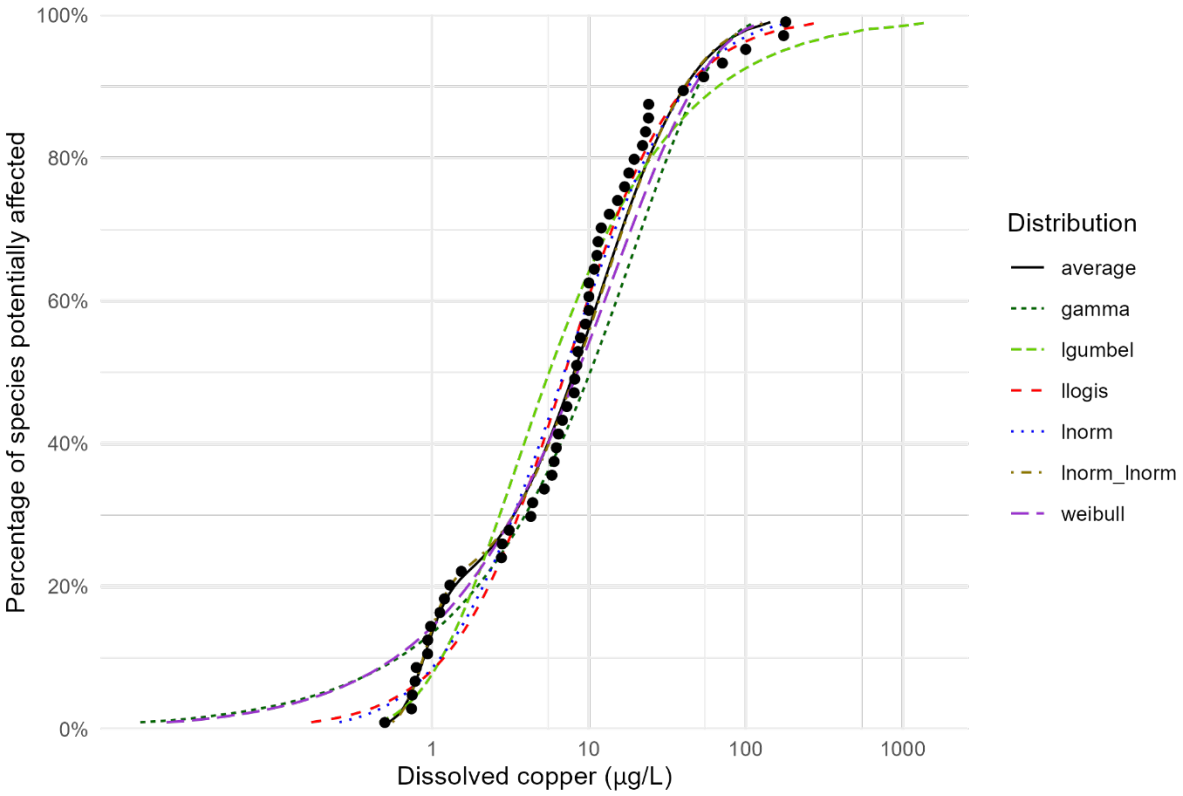


Figure D 1 SSDs for set of six default distributions used to model the copper freshwater toxicity dataset

Table D 1 Goodness of fit and weighting estimates for six default distributions used in model averaging for the copper freshwater toxicity dataset

Distribution	Akaike's Information Criterion (AIC)	Corrected Akaike's Information Criterion (AICc)	Delta	Weight
Gamma	405	405	20	0
Log gumbel	394	394	9.5	0.008
Log logistic	391	392	6.6	0.032
Log normal	390	390	5.3	0.063
Log normal - Log normal	384	385	0	0.896
Weibull	399	399	14.5	0.001

Appendix E: Sensitivity analysis of toxicity data choices and DOC adjustments

E.1 Effect of datasets and DOC adjustments on calculated DGVs

DOC concentrations were not reported in a relatively high proportion of the toxicity data set (60% of acceptable data). The effect of including these data, despite the associated uncertainty, is shown in Table E 1 and Figure E 1 through comparison with protective concentrations calculated when only data with reported DOC are included. Using only data with measured and reported DOC, the number of species with suitable data decreases from 52 to 26 and the distribution of data generally shifts to the left (more sensitive). The calculated protective concentrations increase or decrease marginally, depending on the level of protection.

The SSD model fit for the measured-only dataset remains reasonable based on a visual estimate. This suggests that (from a statistical model fitting perspective) the DGVs could be based solely on data with measured DOC. The comparison indicates negligible to minor differences in the protective concentrations for all four levels of species protection, as there were few differences in the data and model fit at the tail of the distribution. However, exclusion of data with estimated DOC removed many species and all insects, fungi and bacteria included in the derivation. The number of fish decreased from 15 to 4 and the number of crustaceans decreased from 11 to 4 (all cladocerans, no amphipods or crayfish).

In most cases where DOC was not reported, the tests were in high purity (deionised or reverse osmosis) waters and DOC concentration was assumed to be 0.5 mg/L. At this DOC, the toxicity data were used as reported, that is, no estimation of the value at a lower DOC was required. For 4 species, only estimated DOC values were available, and those estimates (by van Sprang et al. 2008) were between 0.5 mg/L and 2 mg/L. These species were:

- *Clistorina magnifica* (caddisfly, EC10 8.3 µg/L, DOC estimated at 1.3 mg/L)
- *Gammarus pulex* (amphipod, EC10 11 µg/L, DOC estimated at 1 mg/L)
- *Hyalella azteca* (amphipod, EC10 value 32 µg/L, DOC estimated at 1 mg/L)
- *Perca fluviatilis* (European perch, EC10 value 39–188 µg/L, DOC estimated at 1 mg/L)

None of these species are particularly sensitive or insensitive species, and their inclusion or exclusion makes minimal difference to the calculated protective concentrations (Table E 1). To increase the number of species (and ecosystem relevance), toxicity data from tests where DOC was estimated were considered acceptable if the DOC was estimated at < 2 mg/L, and if no other data were available for that species. This reduced the uncertainty in normalising data with uncertain DOC concentrations, whilst retaining as many species as possible.

Table E 1 Calculated protective concentrations for dissolved copper in freshwater based on different toxicity data sets and normalisation methods

Option	No. species	Protective concentration for dissolved copper in freshwater (µg/L) [a] at specified level of species protection (%)			
		99	95	90	80
Only including data with measured DOC	26	0.62	0.75	0.84	0.99
Including data with measured DOC, or low estimates (0.5 mg/L)	48	0.52	0.72	0.86	1.2
Finalised version, excluding data where DOC > 10 mg/L or estimated at more than 2 mg/L	52	0.53	0.73	0.89	1.4

[a] Protective concentrations calculated using model averaging method in ssdtools package (Thorley et al. 2025) and rounded to 2 significant figures.

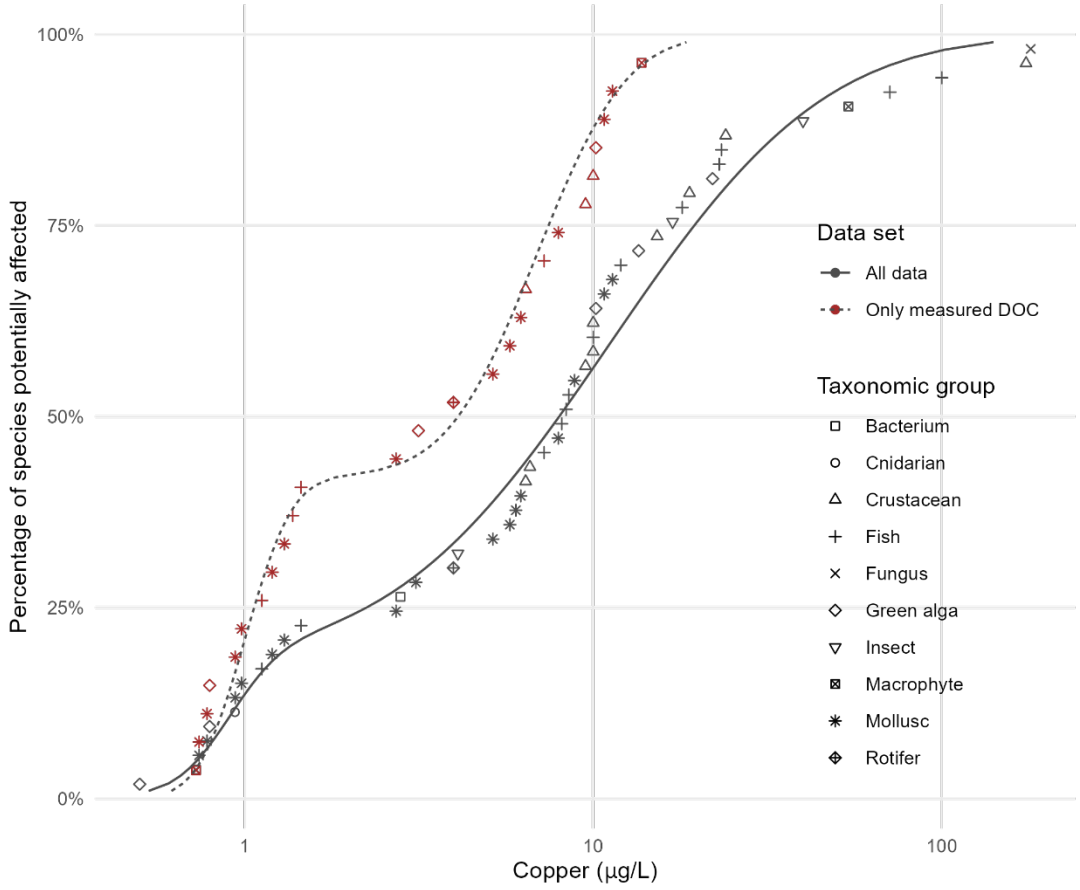
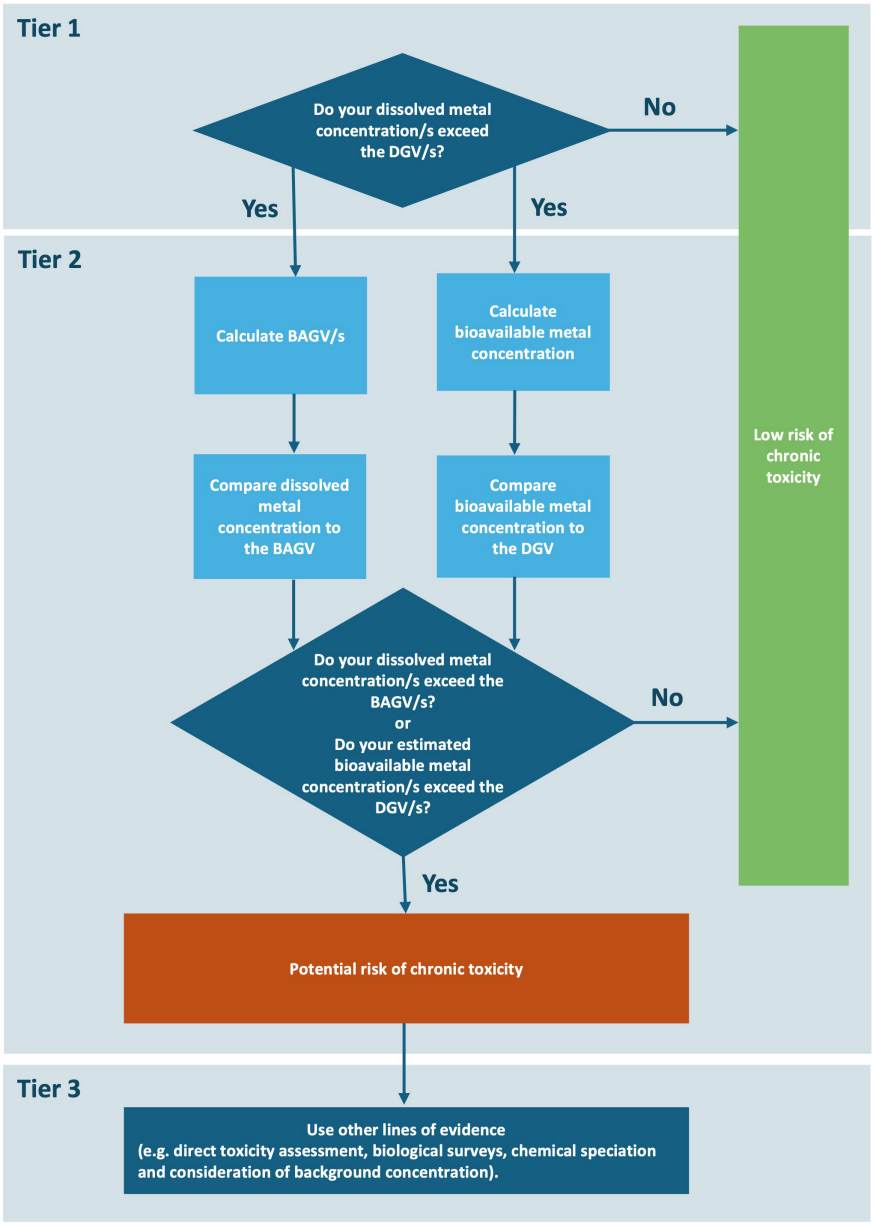


Figure E 1 Comparison of species sensitivity distributions for the DGV (grey symbols, solid line for model fit) and based on only toxicity data where DOC was measured in test solutions (brown symbols, dashed model fit line)

Appendix F: Flow chart of tiered assessment framework for bioavailability-based default guideline values

Figure F 1 depicts the tiered assessment framework for bioavailability-based default guideline values, including those for dissolved copper in freshwater. Refer to Section 4.6, Stauber et al. (2026) and ANZG (2018) for further details of how the framework is implemented.

Figure F 1 The tiered assessment framework for bioavailability-based default guideline values
Adapted from Comber et al. (2008) and EC (2019).



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