An Australian Government Initiative

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Zinc in freshwater

Technical brief

September 2026

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Cataloguing data
This publication (and any material sourced from it) should be attributed as: ANZG (2026) *Toxicant default guideline values for aquatic ecosystem protection: Zinc in freshwater,* Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australian and New Zealand governments and Australian state and territory governments, Canberra, ACT, Australia.

This publication is available at waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants.

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Acknowledgements
These default guideline values (DGVs) were derived by Dr Jennifer Gadd (NIWA, Hydrotoxy, New Zealand, Dr Chris Hickey (RMA Science, New Zealand), Dr Jenny Stauber and Dr Lisa Golding (CSIRO Environment, Lucas Heights, NSW) and Dr Aleicia Holland (La Trobe University). The DGVs were peer reviewed by three anonymous reviewers and by contracted technical advisors, Dr Rick van Dam, Alicia Hogan and Dr Melanie Trenfield. The DGVs were also reviewed and approved by jurisdictional technical and policy oversight groups and the National Water Committee prior to being published. The DGVs also underwent a public consultation process, the comments from which were addressed and used to improve the final DGVs.

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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).

Zinc (Zn) is widely distributed in the Earth's crust and is an essential trace element for microorganisms, plants and animals. It is mostly used in galvanised products, including roofing and other building products. The major anthropogenic sources of zinc into freshwater environments include stormwater (particularly from tyre wear and runoff from galvanised iron roofs), metal processing and mining, and discharges from municipal wastewater treatment plants.

The ANZECC and ARMCANZ (2000) 95% species protection DGV for zinc was 8 µg/L. This DGV was based on chronic toxicity data for 20 species from 6 taxonomic groups and was normalised to a hardness of 30 mg/L as calcium carbonate ($CaCO_3$). However, other water-quality parameters apart from hardness (i.e. calcium [Ca] and magnesium [Mg] concentrations), particularly pH and dissolved organic carbon (DOC), also play important roles in controlling zinc bioavailability and toxicity in freshwater aquatic systems. Bioavailability models have been developed for zinc, including multiple linear regressions (MLRs) specific to species and trophic levels, and biotic ligand models (BLMs). These models can be used to derive bioavailability-based guideline values that account for a wider range of water chemistry parameters. Since 2000, a large body of chronic toxicity data has also become available, including for many local species, from which updated DGVs have been derived. The DGVs reported here employ MLR bioavailability models at the species level and trophic level, developed to account for the influence of pH, hardness and/or DOC on the toxicity of zinc.

Very high reliability DGVs for dissolved zinc (operationally defined as the < 0.45 µm filtered measurement) in freshwater were derived from chronic toxicity data for 25 species, comprising 4 fish, 5 crustaceans, 2 insects, 10 molluscs, 1 rotifer and 3 green microalgae. The DGVs (shown in the table below) are based on high bioavailability reference conditions for Australia (pH 7.6, hardness 39 mg/L $CaCO_3$ and DOC 1.0 mg/L) and New Zealand (pH 7.5, hardness 28 mg/L $CaCO_3$ and DOC 0.7 mg/L), and can be adjusted to bioavailability-adjusted guideline values (BAGVs) based on pH, hardness and DOC concentrations in local waterbodies. BAGVs for 99%, 95%, 90% and 80% species protection are provided for waters of different pH (from 6.0 to 8.3), hardness (from 26 mg/L as $CaCO_3$ to 370 mg/L as $CaCO_3$) and DOC (from 0 mg/L to 15 mg/L) values.

Default guideline values (DGVs) for dissolved zinc in freshwater, for Australia and New Zealand

Level of species protection (%)	DGVs for zinc in freshwater for Australia (µg/L)	DGVs for zinc in freshwater for New Zealand (µg/L)
99	1.9	1.5
95	5.2	4.1
90	8.2	6.4
80	15	11

The 95% species protection DGVs or BAGVs for dissolved zinc are recommended for application to slightly-to-moderately disturbed ecosystems. Where ambient water data for pH, hardness (Ca and Mg) or DOC are not available and a BAGV cannot be calculated, the DGV should be used in the interim. The DGVs for dissolved zinc reported here supersede the ANZECC and ARMCANZ (2000) DGVs for zinc in freshwater.

The ANZG (2018) Guidelines provide a tiered assessment framework for assessing zinc bioavailability and toxicity so that the effects of pH, hardness and DOC on zinc bioavailability can be taken into account. The tiered assessment framework represents a decision tree to guide the user through the process of accounting for zinc 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

Zinc (Zn) is a naturally occurring metallic element with an atomic number of 30. It is an abundant trace element, present in the Earth's crust at approximately 10–300 mg/kg (Malle 1992), similar to chromium, copper and nickel (Landner and Reuther 2004). It is not naturally found as the native metal – it is predominantly present in the form of sulfide minerals, particularly sphalerite (ZnFeS) (IPCS 2001). Carbonates and oxides of zinc occur less frequently (Stumm and Morgan 1996). There are large deposits in Australia, Canada, the United States, Peru, China and Iran (IPCS 2001; Landner and Reuther 2004).

Brass, an alloy of zinc and copper, has been used since around the Bronze Age (~3,000 BC). However, zinc as a metal was not used until the 12th century. It is now the fourth-most commonly used metal, after iron, aluminium and copper (IPCS 2001). Zinc is mostly used to galvanise iron and steel, accounting for nearly 50% of global zinc use (IPCS 2001). Pure zinc has low strength, so it is alloyed with other metals, such as copper to produce brass (the most widely used), or with aluminium, nickel, titanium and magnesium for various uses such as casting and bearings (IPCS 2001; Landner and Reuther 2004). Zinc is also used as a reinforcing agent in rubber and as zinc oxide pigments for paint. Various zinc compounds are also used for dentistry, medicinal and household products (IPCS 2001; Landner and Reuther 2004). Sources of zinc in freshwater aquatic environments include stormwater, particularly from tyre wear in road runoff and runoff from galvanised iron roofs (Kennedy and Sutherland 2008; Timperley et al. 2005), metal processing and mining, and discharges from municipal wastewater treatment plants.

Zinc is a transition metal and, like other transition metals, has more than one oxidation state, with +2 oxidation state being the most common (Stumm and Morgan 1996). At pH 4–7, the free zinc ion (Zn^{2+}) is by far the predominant form. At pH 8–9, zinc carbonate ($ZnCO_3$) also becomes important (Stumm and Morgan 1996). Hydroxide forms are less prevalent, and chloride complexes are insignificant in freshwaters (Stumm and Morgan 1996). Zinc will also complex to organic ligands, such as humic acids. Partitioning to suspended particulate matter in oxic neutral to alkaline environments is an important mechanism of removal from the aqueous phase (Stumm and Morgan 1996).

Background concentrations of zinc in freshwaters can be extremely low. Filtered (< 0.45 µm) zinc concentrations in undisturbed lakes and rivers in New Zealand and Australia have been reported to range between 0.04 µg/L and 1.6 µg/L (Ahlers et al. 1991; Ellwood et al. 2001; Reid et al. 1999; Sander et al. 2013; Stauber et al. 2023; Trenfield et al. 2023). These concentrations are lower than reported in other jurisdictions (Reid et al. 1999). At the other end of the scale, concentrations in urban streams can range from 5 µg/L to 200 µg/L during baseflow (Gadd et al. 2020; Shi et al. 2019) and are highest during storm events, reaching concentrations of 500–800 µg/L (Gadd et al. 2019; McDonald et al. 2022). In freshwater streams receiving untreated discharges from mines (particularly historical workings), concentrations have been reported to exceed 1,000 µg/L (Smith and Williamson 1986; Edraki et al. 2005).

The ANZECC and ARMCANZ (2000) default guideline values (DGVs; formerly known as trigger values) for zinc in freshwaters for 99%, 95%, 90% and 80% species protection were 2.4 µg/L, 8 µg/L, 15 µg/L and 31 µg/L, respectively, at a hardness of 30 mg/L $CaCO_3$. Those DGVs were considered high

reliability, derived using the species sensitivity distribution (SSD) method based on 85 values from 20 species from 6 taxonomic groups (fish, amphibians, crustaceans, insects, molluscs and annelids). All data were from chronic studies. Most values were EC50s/LC50s and LOECs, all of which were converted to negligible-effect estimates by dividing by 5 for EC50s/LC50s and 2.5 for LOECs (see 'Glossary and acronyms' for definitions). Eighteen of the available values were NOECs. The lowest values were for the insect midge (NOEC of 5 µg/L for *Tanytarsus dissimilis*) and a crustacean (5.5 µg/L for *Ceriodaphnia reticulata*, converted from an LC50 of 27.5 µg/L). While the ANZECC and ARMCANZ (2000) DGVs could be adjusted for hardness, other water-quality parameters, particularly pH and DOC, also play important roles in controlling zinc bioavailability and toxicity in freshwater aquatic systems.

This technical brief provides updated DGVs for dissolved zinc in freshwater that supersede the ANZECC and ARMCANZ (2000) DGVs. The current derivation incorporates chronic toxicity data published since 2000, including for Australasian species. The hardness correction applied to the ANZECC and ARMCANZ (2000) DGVs has been replaced with four multiple linear regression (MLR) bioavailability models at the species- and trophic-level, developed to account for the influence of pH, hardness and DOC on the toxicity of zinc. The rationale for this change is detailed in Section 3 and in Gadd (2023) and Stauber et al. (2023). The DGV derivation process and the toxicity data used are described in Section 4, while the selection of the DGVs based on high bioavailability waters is described in Gadd et al. (2026a, b).

2 Aquatic toxicology

2.1 Mechanisms of toxicity

Zinc is an essential trace element at all trophic levels, as it has fundamental roles in the structure and function of numerous proteins and in the maintenance of plasma membrane stability (IPCS 2001). Zinc is found in all tissues of mammals, fish and invertebrates. Zinc deficiencies can lead to disorders, which are well documented in humans and terrestrial animals, and also observed in terrestrial plants. Deficiencies in zinc are relatively rare in aquatic organisms but may be observed in phytoplankton in the open ocean (IPCS 2001). Zinc deficiencies have also been reported in laboratory experiments, where zinc-free water reduces survival, growth and reproduction of freshwater algae, sponges, water fleas and rainbow trout (Eisler 1993).

Zinc toxicity can occur at excess concentrations. In aquatic organisms, this is due to disruption of the internal calcium balance, leading to hypocalcaemia (Clifford and McGeer 2009; Hogstrand et al. 1995). This is thought to occur through interference with calcium-transport systems, particularly in the uptake pathways of the gill (Hogstrand et al. 1996), and is predominantly an acute effect (De Schamphelaere and Janssen 2004). Zinc also interferes (to a lesser extent) with sodium and chloride fluxes (Spry and Wood 1984). Meyer et al. (2007) concluded that bioaccumulation and tissue accumulation of zinc are neither related to zinc toxicity nor good predictors of it.

2.2 Acute toxicity

Reviews of acute toxicity of zinc to freshwater species (expressed as an EC50/LC50) reported ranges for North American species of 51–81,000 µg/L at a hardness of 50 mg/L $CaCO_3$ (US EPA 1987, 1996). Cladocerans were the most sensitive group, followed by several fish species, including striped bass (*Morone saxatilis*), longfin dace (*Agosia chrysogaster*), salmon and trout species. For Australian species, LC50 values ranged from 140 µg/L to 9,600 µg/L, where hardness was either 54 mg/L to 60 mg/L $CaCO_3$ (Skidmore and Firth 1983) or unspecified (Bacher and O'Brien 1990). For New Zealand species, EC50 values ranged from 350 µg/L to > 6,900 µg/L for invertebrates and 1,010 µg/L to 7,700 µg/L for native fish, at a standard hardness of 50 mg/L $CaCO_3$ (Hickey 2000). The aquatic crustaceans *Ceriodaphnia dubia* and *Paracalliope fluviatilis* were the most sensitive species reported by Hickey (2000), compared to insects, snails and fish (including eels).

2.3 Chronic toxicity

Acute-to-chronic ratios (ACRs) for zinc calculated by US EPA (1996) mainly ranged from < 1:1 to 7:1, with one higher ratio of 41:1 for the flagfish *Jordanella floridae*. US EPA (1996) used an average ACR of 2 to calculate the chronic water-quality criterion. Note that because the acute water-quality criterion was divided by a 'safety factor' of 2, this resulted in its chronic water-quality criterion being equal to its acute water-quality criterion.

Compilations of chronic zinc toxicity data (CCME 2018; DeForest et al. 2023; Munn et al. 2010) indicate that most zinc toxicity values vary from 10 µg/L to 1,000 µg/L, although there are values up to 10,000 µg/L for fish, invertebrate, macrophyte and algal species (CCME 2018; DeForest et al.

2023). The relative sensitivity to zinc of different taxonomic groups is somewhat unclear, as zinc toxicity varies with water chemistry.

Markich (2017) reported that Australian native freshwater mussel larvae were very sensitive to zinc, with chronic NECs (72-hour glochidial survival) of 8.4–15 µg/L for the 6 species tested. However, the native New Zealand freshwater mussel (*Echyridella menziesii*) may be less sensitive, with reported EC20 values of 56–281 µg/L from 48-hour glochidial survival tests (Clearwater et al. 2014). Wang et al. (2010) reported that early life stages of freshwater mussels were only moderately sensitive to zinc, compared to other freshwater species.

Munn et al (2010) suggested that freshwater unicellular algae may be more sensitive to zinc than invertebrates and fish. More recent data confirm that some unicellular algae are sensitive to zinc, but that sensitivity both within and between species can vary markedly. Tests with the Papua New Guinea isolate of the green microalga *Chlorella* sp. indicate that it can be very sensitive to zinc, with 72-hour growth rate inhibition EC50 values ranging from 6.2 µg/L to 184 µg/L, depending on the pH and hardness of the waters (Price et al. 2022). Similarly, EC10 values for the alga *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*) ranged from 6 µg/L to 109 µg/L, depending on the water chemistry (De Schamphelaere et al. 2005a; Stauber et al. 2023; Van Regenmortel et al. 2015).

Chronic toxicity data for fish species include negligible effect values (i.e. NEC, EC10–20, LC10–20, NOEC) from 29 µg/L to ~1000 µg/L, depending on species and water chemistry. Data for rainbow trout (*Oncorhynchus mykiss*) ranged from 35 µg/L to 974 µg/L (LC10, 30-day juvenile survival; De Schamphelaere and Janssen 2004), depending on the water chemistry. The lowest EC10 (29 µg/L) was reported for the tropical northern trout gudgeon (*Mogurnda mogurnda*) from a 7-day growth test in waters with very low hardness (3.5 mg/L $CaCO_3$), low DOC (1.4 mg/L) and pH 6.7 (Trenfield et al. 2023). *Salmo trutta* was also sensitive when tested as eggs, with NOEC values of 56 and 61 µg/L reported for a 120-day test on egg/larval development in waters with low pH (6.5-6.7) and very low hardness (6.5–8.6 mg/L as $CaCO_3$/L) (Källqvist et al. 2003).

3 Factors affecting toxicity

3.1 Zinc speciation

The toxicity of zinc depends on its form – whether it is freely dissolved, an inorganic complex, an organic complex or associated with particulates. The Zn^{2+} species is the most bioavailable and potentially toxic form (Allen et al. 1980; Mebane et al. 2020; Meyer et al. 2007). The hydroxide form ($ZnOH^{+}$) is also likely to cause toxicity, while carbonates (e.g. $ZnHCO_3^{-}$) are generally considered to have low toxicity. However, hydroxide and carbonate species are generally correlated (both increasing as pH increases) and it is possible that both contribute to toxicity.

The speciation of zinc, and thus the concentration of Zn^{2+}, in a waterbody is affected by the physico-chemical properties of the water, including pH, hardness, alkalinity, dissolved organic matter (DOM) and suspended particulate matter. In addition to effects on speciation, water chemistry can also affect bioavailability and toxicity through competition between zinc and other cations with biotic ligands of organisms (see discussion on the BLMs in Section 3.2 and Section 3.3).

Within natural waterbodies, a large proportion of zinc is found partitioned to suspended solids, with around 80% of zinc present in particulate form in rivers with low metal concentrations and high suspended solids (Windom et al. 1991). Particulate-bound zinc has low bioavailability. However, zinc may be released from particulates under reducing or acidic conditions (Stumm and Morgan 1996), increasing the concentration of dissolved zinc. Based on the low bioavailability of particulate zinc, previous water-quality guideline values (ANZECC and ARMCANZ 2000; US EPA 1996) have recommended that the < 0.45 µm-filtered fraction of zinc should be used, rather than comparing total zinc to numeric criteria.

In addition to adsorption to particles, zinc can also form inorganic complexes with iron and manganese oxides and hydroxides, and complexes with organic acids such as humic and fulvic acids (Stumm and Morgan 1996). This further reduces bioavailability and, therefore, toxicity. The extent of this complexation varies strongly with pH (Waller and Pickering 1991) and tends to be lower than some other metals – for example, copper, which has very high affinity for humic acids (Stumm and Morgan 1996).

3.2 Toxicity modifying factors

Factors affecting dissolved zinc toxicity typically involve changes to zinc speciation or competition with zinc at biological uptake sites. Of the various toxicity modifying factors (TMFs) for zinc, water hardness (due to calcium and magnesium) is the best studied. The toxicity of zinc generally decreases as water hardness increases. This is attributed to competition between zinc and calcium cations for binding sites on biological tissues (Bradley and Sprague 1985; Heijerick et al. 2002). This effect was first documented with acute exposures in algae, invertebrates and fish; however, it has also been demonstrated with chronic exposures. Examples from chronic exposures include studies with the cladoceran *Daphnia magna*, where a 4-fold increase in the hardness of the water, from 50 mg/L to 200 mg/L $CaCO_3$, resulted in a 6-fold increase in the NOEC for reproduction (Paulauskis and Winner 1988). For brown trout (*S. trutta*), a 5-fold increase in hardness, from 37 mg/L to 200 mg/L $CaCO_3$,

resulted in a 2-fold increase in the LC50 (30-day survival tests), from ~1,000 µg/L to ~2,300 µg/L (Davies and Brinkman 1999). Similar results have been reported for rainbow trout and Colorado River cutthroat (*Oncorhynchus aters pleuriticus*) (Brinkman and Hansen 2004). In some studies with *D. magna*, the effects of hardness were not linear, and there was little additional protective effect at hardness exceeding 100–250 mg/L $CaCO_3$ (Chapman et al. 1980; Heijerick et al. 2003). Furthermore, in many studies, the test waters with higher hardness also had higher alkalinity (and sometimes pH), thus confounding the protective effect of hardness (e.g. with *C. dubia*; Belanger and Cherry 1990).

The pH of a waterbody can influence zinc toxicity by three mechanisms:

- influencing zinc speciation, with higher concentrations of Zn^{2+} occurring at low pH (increasing toxicity at low pH)
- influencing binding of zinc to biotic ligands through direct competition with H^+ ions (decreasing toxicity at low pH)
- modifying the affinity between zinc and membrane binding sites due to conformational changes in binding sites (decreasing toxicity at low pH).

Many studies conducted with varying pH in laboratory waters (with no ligands) have shown increasing toxicity with increasing pH, attributed to competition between H^+ ions and zinc for binding sites. This relationship was reported in studies with rainbow trout (Cusimano et al. 1986; De Schamphelaere and Janssen 2004), fathead minnow (*Pimephales promelas*; Mount 1966; Schubauer-Berigan et al. 1993), *D. magna* (Chapman et al. 1980), *C. dubia* (Hyne et al. 2005a; Schubauer-Berigan et al. 1993; Nys et al. 2017), and the green algal species, *Chlorella* sp. (Price et al. 2021; Wilde et al. 2006), most of which were acute exposures. However, in other studies, the reverse effect has been reported, or no clear effect has been found (Heijerick et al. 2002). This is particularly so for chronic studies, or those in natural waters, or with organic ligands present. For *C. dubia*, an increase in toxicity of only 1.7-fold was found with an increase in pH of 3 units from pH 6 to 9 (Belanger and Cherry 1990), noting that this range extends outside of the range of most natural waters. In tests with *D. magna*, Heijerick et al. (2003) found no clear pattern between pH and zinc toxicity. The effect of pH on zinc toxicity appears to differ between taxonomic groups. The effect of pH on zinc toxicity is illustrated in Appendix A and discussed further in CCME (2018).

Alkalinity (usually due to carbonate) affects zinc toxicity by reducing the concentration of Zn^{2+} in water via the formation of zinc carbonate complexes. Some studies have shown lower toxicity at higher alkalinity (e.g. Holcombe and Andrew 1978). However, in most cases, the hardness of the water and pH vary alongside alkalinity, and changes in toxicity cannot be solely attributed to any single characteristic. Of the few studies that compared alkalinity while maintaining constant water hardness, two showed that alkalinity had no influence on acute zinc uptake or toxicity to rainbow trout at or below pH 7 (Barron and Albeke 2000; Bradley and Sprague 1985). A third study suggested that both hardness and alkalinity influenced the acute toxicity of zinc to rainbow trout and brook trout (*Salvelinus fontinalis*) (Holcombe and Andrew 1978). There were no studies where the influence of alkalinity on the chronic toxicity of zinc was assessed in the absence of other factors.

DOM, typically quantified as DOC as it contains ~50% carbon by mass (Duarte et al. 2016), affects zinc toxicity primarily through formation of zinc-DOC complexes that are of low bioavailability, thus reducing the toxicity of zinc in waters with high DOC. This has been observed in acute toxicity studies using various cladoceran species (Clifford and McGeer 2009; Hyne et al. 2005b; Oikari et al. 1992;

Paulauskis and Winner 1988) and fathead minnow larvae (Bringolf et al. 2006). DOC also reduces zinc toxicity in chronic tests as shown for several *Daphnia* species (Heijerick et al. 2003; Winner and Gauss 1986). For these species, the effect of DOC appears to be strongest in waters of soft to moderate hardness (< 200 mg/L $CaCO_3$; Winner and Gauss 1986) and at DOC concentrations > 5–10 mg/L, although some protective effect has been shown with 1.5 mg/L of humic acids (Paulauskis and Winner 1988). Furthermore, studies have reported reduced chronic zinc toxicity to the green alga *R. subcapitata* (De Schamphelaere et al. 2005a) and *Chlorella* sp. (Price et al. 2023a) in the presence of DOC. Price et al. (2023a) demonstrated that the effect of DOC on zinc toxicity was dependent on the source of DOC, i.e. where it was collected and its associated biochemical composition.

Increasing water temperature can influence metal toxicity due to increased metabolic rates and increased respiratory inflows (Khangarot and Ray 1989). In their meta-analysis of the water chemistry effects on metal toxicity, Meyer et al. (2007) found that, in chronic tests with zinc, fathead minnow mortality increased as water temperature increased. However, few other studies have investigated temperature effects under chronic exposures. Some studies have demonstrated increases in acute toxicity of zinc at higher temperatures for *D. magna* (Cairns et al. 1978) and rainbow trout (Lloyd and Herbert 1962), while other studies have reported no significant change in acute toxicity for rainbow trout (Cairns et al. 1978; Hansen et al. 2002). Overall, there are insufficient data to incorporate the effects of temperature on zinc toxicity into the DGVs.

3.3 Accounting for bioavailability

3.3.1 Overseas and ANZECC and ARMCANZ (2000) approaches

The inverse relationship between water hardness and toxicity was the basis of a hardness function reported in the US EPA ambient water-quality criterion for zinc from 1984 (e.g. as published in US EPA 1996), whereby the criterion was higher at higher hardness levels (i.e. criterion continuous concentration = $e^{(0.8473[\ln(\text{hardness})]+0.884)}$). The slope for the hardness equation (0.8473) used in the US EPA's 1995 derivation (US EPA 1996) was adopted for the ANZECC and ARMCANZ (2000) zinc in freshwater DGVs, in the form of Equation 1.

Equation 1 Adjustment of the ANZECC and ARMCANZ (2000) zinc DGVs for different levels of hardness

$$Hardness\ adjusted\ GV = GV \times \left(\frac{H}{30}\right)^{0.85}$$

Increased understanding of mechanisms of toxicity for zinc to freshwater species led to the development of the BLM for assessing acute zinc toxicity (Santore et al. 2002) and its subsequent extension to chronic toxicity (De Schamphelaere and Janssen 2004; Heijerick et al. 2005). The most important constituents in those models are calcium, magnesium, pH, DOC and, in some cases, sodium and potassium (Clifford and McGeer 2009; De Schamphelaere et al. 2004; De Schamphelaere et al. 2005b; Heijerick et al. 2005). A BLM to predict chronic HC_5 (5% hazardous concentration) values for zinc is freely available from Windward Environmental (BLM Freshwater and Marine version 3.41.2.45) based on the unified Zn BLM described by DeForest and Van Genderen (2012). The unified BLM is yet to be adopted in any regulatory guidelines. The US EPA water-quality criterion for zinc in freshwater has not been updated since 1995 and continues to use the US EPA (1996) hardness function.

The European risk assessment of zinc and zinc compounds used the BLM method for fish, invertebrates and algae (based on individual BLMs for rainbow trout, *D. magna* and *R. subcapitata*, respectively) to derive a predicted no-effect concentration (PNEC) under high bioavailability conditions (Munn et al. 2010). This can be implemented at a site of interest (for differing water chemistry) by using a simple zinc bioavailability tool to calculate the bioavailable zinc concentration for comparison to the PNEC (Bio-met 2022).

An MLR approach has been suggested as a simpler method than BLMs for deriving guideline values (Brix et al. 2017; DeForest et al. 2018) and has been used in Canada in developing zinc and, more recently, aluminium guidelines for freshwater (CCME 2018; ECCC 2022). In developing its zinc MLR, Environment Canada reviewed the TMFs for zinc in chronic (long-term) tests and found the most important factors to be hardness, pH and DOC (CCME 2018). Data were collected for 3 species with sufficient available data for developing models: a cladoceran (*D. magna,* 21-day EC10s for reproduction), rainbow trout (30-day LC10s) and a green microalga (*R. subcapitata*, 72-hour EC50s for population growth). The algal dataset included only pH and hardness data, as there were no tests with varying DOC at that time. Three separate MLRs were developed based on the data. The MLR models for *D. magna* and *R. subcapitata* included only DOC and pH, respectively, as statistically significant predictors of zinc toxicity. Other TMFs (hardness and pH for *D. magna*, hardness for *R. subcapitata*) were not statistically significant (p-values > 0.05). The MLR for rainbow trout included all 3 TMFs and was subsequently used in the CCME (2018) guideline derivation. The rainbow trout MLR had performed best in predicting the measured LC10 values, had a high adjusted R^2, covered a broad range of hardness, DOC and pH values, and was shown to be protective of 96% of the species in the SSD (CCME 2018). It was subsequently applied to all trophic levels and species to derive the long-term exposure guideline value.

3.3.2 Approach for the current default guideline values

In 2019, a project initiated by CSIRO and NIWA and funded by the International Zinc Association assessed zinc toxicity in natural waters collected in Australia and New Zealand with varying water characteristics (pH, hardness, DOC, etc). The project assessed existing BLMs and MLRs against Australian and New Zealand species and natural water-quality conditions to determine the most suitable models for deriving the DGVs for zinc in freshwater. The results indicated that the effect of water chemistry on toxicity was species dependent (Stauber et al. 2023). Zinc toxicity (based on EC10s) varied by up to 30-fold for *Chlorella* sp. but less than 2-fold for *C. dubia* in the same waters. The range in toxicity in New Zealand waters was < 10-fold for *R. subcapitata* and *Daphnia thomsoni* (New Zealand native cladoceran). Based on these data, a bioavailability approach for the zinc DGVs was technically justified. Stauber et al. (2023) showed that no single trophic-level-specific MLR was always the best predictor of toxicity to the Australian and New Zealand algae or invertebrates. Zinc toxicity to algae was relatively difficult to predict in natural waters, even when using a newly developed MLR for *Chlorella* sp. to predict toxicity to *Chlorella* (Price et al. 2023b). Both the new *Chlorella* sp. MLR and existing *R. subcapitata* MLRs predicted zinc toxicity within a factor of 2 for about 50% of the natural waters tested.

Following the results of Stauber et al. (2023), a detailed assessment was undertaken to identify the best bioavailability models for deriving the DGVs for zinc in freshwater. This assessment is described in Gadd (2023) and considered qualitative and quantitative factors, including:

- **ease of use**, particularly for regulators, and accessibility of models within a reasonable timeframe
- **consideration of the model structure and rigour**, ensuring it considers the TMFs identified by laboratory and mechanistic studies as most important, with model formulations consistent with current understanding of metal bioavailability and uptake, and that the model adequately describes the calibration datasets (auto-validation)
- **a preference for species-level or trophic-level models over a unified model**, given potential differences between organisms in the way TMFs influence toxicity (De Schamphelaere et al. 2005a; Meyer et al. 2007; Price et al. 2022; Price et al. 2023a)
- **consideration of the ranges of the TMFs in the model(s)**, their coverage of the ecotoxicity dataset and relevance to Australian and New Zealand waters
- **local validation of the model(s)** using species found in Australia and New Zealand, including sensitive native species, and in water chemistry for Australia and New Zealand
- **assessing the likely protection** of sensitive and native species with the use of the model to derive zinc water-quality guideline values.

The suite of models assessed included BLMs, pooled MLRs, and trophic-level and multiple species-level MLRs derived by multiple authors. Based on the assessment and as detailed in Gadd (2023), a suite of 4 MLR models (Table 1) was selected for adjusting the ecotoxicity dataset. That suite of models was later amended by replacing the initially selected model for fish (based on that used by CCME (2018) for the Canadian zinc guideline value), with a model with no relationship between pH and zinc toxicity (see Appendix A). The four models included in Table 1 were used to (i) standardise the toxicity values in the final toxicity database to a high bioavailability water chemistry (i.e. pH, hardness, DOC) condition (see Section 4.3 for determination of this condition) for the derivation of the DGVs, and (ii) calculate bioavailability-adjusted guideline values (BAGVs) at different water chemistry combinations to match site-specific water chemistry (see Section 4.5).

Table 1 Summary of multiple linear regression (MLR) bioavailability models used for deriving the default guideline values for zinc in freshwater

	Coefficients for toxicity modifying factors				Range for toxicity modifying factors			
Model species	**pH**	**Hardness (as $\log_e$[Hardness])**	**Dissolved organic carbon (as $\log_e$[DOC])**	**Interaction term (from the MLR)**	**pH**	**Hardness (mg/L $CaCO_3$)**	**Dissolved organic carbon (mg/L)**	**Reference**
Oncorhynchus mykiss	Not significant	0.837	0.271	No interaction	6.2–8.1	23–399	0.3–23	See Appendix A
Daphnia magna	-0.524	0.311	-1.40	0.244 (DOC × pH)	6.0–8.5	26–370	0.3–40	Stauber et al. (2023)
Chlorella sp. (Papua New Guinea isolate)	-0.359	0.673	0.351	No interaction	6.7–8.3	5–402	0.5–15	Price et al. (2023b)
Raphidocelis subcapitata	-0.865	Not significant	0.209	No interaction	5.6–8.5	7–529	0.3–22	DeForest et al. (2023)

4 Default guideline value derivation

The DGVs for dissolved zinc 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. 2024) 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 zinc are also described in Sections 4.2, 4.3 and 4.4.

4.1 Collation and screening of toxicity data

Since ANZECC and ARMCANZ (2000), numerous significant publications and data reviews have been published about the aquatic toxicity of zinc. These were the primary sources of data for the DGV derivation. They include new guideline value derivations by Environment and Climate Change Canada (CCME 2018), the European Union zinc risk assessment (Munn et al. 2010) and a case study for bioavailability model evaluation and selection (Van Genderen et al. 2020). Toxicity data were also collated from ANZECC and ARMCANZ (2000), the ECOTOX database (US EPA n.d.) and compilations of Australasian toxicity data (Langdon et al. 2009; Markich et al. 2002). Additional international data were collated through searches using the journal abstracting service Web of Science for studies published during 2015–16 that were not included in the ECOTOX database. Additional Australian and New Zealand toxicity data (from 2009 to 2020) were collated through internet searches for data contained within grey literature, theses or unpublished reports and by targeted emails to local researchers.

Although there is an extensive set of published data on zinc toxicity, not all data met the preferred requirements and associated acceptability criteria for the derivation of DGVs. The toxicity dataset was restricted to chronic toxicity studies, following details in Warne et al. (2018). Data were only included for studies that had measured the zinc concentrations in the test solutions, or in the stock solutions used to produce the test solutions and if a clear concentration-response relationship was observed or stated. Although some studies reported concentrations as total zinc, all zinc 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 zinc concentrations.

The minimum data requirements were met with chronic negligible-effects data (i.e. NEC, EC/LC10–20 and NOEC data) alone. Therefore, the dataset used to derive the DGVs did not need to be supplemented with converted chronic (e.g. LOEC, EC50) or acute toxicity values. Some toxicity data used by other jurisdictions to derive zinc guideline values (e.g. CCME 2018) were not included for various reasons, most commonly due to the type of reported statistic (e.g. EC50 value) or the age of the study (data from studies prior to 1980 are not recommended by Warne et al. 2018).

Because test data for pH, hardness and DOC were required to adjust each toxicity value for bioavailability, studies that did not report pH and either hardness or the concentrations of calcium and magnesium were not included in the DGV derivation. Fewer studies have reported DOC, especially older tests, and requiring DOC to be measured in all studies would have excluded many species, including native species. Studies that did not report DOC were included in the derivation under some conditions. A preferential approach was adopted for selecting toxicity data in relation to

DOC. Studies where DOC was measured and reported in the original paper (primary data) were prioritised for each species. If no primary data were available for a species, then secondary data were used. Secondary data were those where DOC was estimated. These included tests where the DOC was reported in other studies that measured DOC in the same waters and where the DOC was < 2 mg/L[1]; or where media was prepared from deionised or distilled water and a low DOC value of 0.5 mg/L was adopted. If DOC could not be reliably estimated, the data were excluded. These rules excluded some species that were included in zinc guideline value derivations by other jurisdictions (e.g. CCME 2018).

Furthermore, studies were not included if conducted in test waters where either hardness, pH or DOC were outside the boundary limits[2] of the MLR model used for that species (see Table 1).

The above data-exclusion rules resulted in some chronic data for Australasian species not being included in the derivation (Table 2). This included high-quality data for 4 Australian tropical species tested in water with low hardness (3.5–4.3 mg/L $CaCO_3$) that was outside the hardness boundary limits of the relevant MLRs (Trenfield et al. 2023). Note that, although the *Chlorella* sp. (Kakadu isolate) data are also from waters of low hardness, the *Chlorella* MLR had a lower boundary of < 1 mg/L $CaCO_3$, compared to 18 mg/L and 21 mg/L $CaCO_3$ for the fish and invertebrate MLRs, respectively. Three freshwater vascular plants native to Australia (*Ipomoea aquatica, Landolitia punctata* and *Lemna aequinoctialis*) were also excluded, as the studies did not report the required information on water hardness or pH, pH was below 6.5 (often as low as 4–4.5), or the studies did not measure the concentrations of zinc in the test waters or stock solutions. Consequently, there were no suitable macrophyte data for inclusion in the derivation, for either local or international species.

Data for several Australian tropical species were included despite exposure durations being less than recommended by Warne et al. (2018) for chronic tests. The test duration recommendations are for temperate species, and Warne et al. (2018) acknowledged that there is scope to relax them for tropical species. Tests on the larval (glochidial) stage of freshwater mussels (Clearwater et al. 2014; Markich 2017) were also included despite the test durations being 48–72 hours with lethality as an endpoint. 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 exposure duration was greater than or equal to the 48-hour minimum for early life stage larval development/metamorphosis tests as required by Warne et al. (2018), these tests were accepted as chronic.

[1] DOC in natural waters can fluctuate significantly (e.g. 2-fold or more), especially seasonally, leading to greater variability in toxicity and hence greater uncertainty in normalised toxicity values. In contrast, at low DOC concentrations (< 2 mg/L) differences of 0.5 mg/L or less make little difference to the predicted toxicity values.

[2] Boundary limits differ from the range limits of the TMFs used to derive the MLRs (Table 1). The boundary limits provide a degree of variability in pH, hardness and DOC values in the toxicity tests that could be considered acceptable for inclusion in DGV derivation. For each of the four MLR models (fish, invertebrate and two microalgae), the boundary limits for pH were within 0.2 pH units of the upper and lower range limits; the hardness boundary limits were within 5 mg/L as $CaCO_3$ of the lower range limit and within 120% of the upper range limit; and the DOC boundary limits were within 1 mg/L of the lower range limit and within 120% of the upper range limit.

Table 2 Summary of chronic toxicity values for Australasian species that were excluded from the derivation of the default guideline values for zinc in freshwater. Species are ordered from most to least sensitive based on the reported toxicity value.

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Toxicity value (µg/L)	pH	Hardness (mg/L as $CaCO_3$)	Dissolved organic carbon (mg/L)	Reason for exclusion	Reference
Mollusca (snail)	*Amerianna cumingi*	Adult	4	EC10 (reproduction)	27	6.8	3.8	1.4	Hardness outside of boundary range for invertebrate MLR model	Trenfield et al. (2023)
Chordata (fish)	*Mogurnda mogurnda*	Juvenile (fry)	7	EC10 (growth)	29	6.7	3.5	1.4	Hardness outside of boundary range for fish MLR model	Trenfield et al. (2023)
Arthropoda (crustacean)	*Moinodaphnia macleayi*	Neonates	6	EC10 (reproduction)	40	7	3.7	1.5	Hardness outside of boundary range for invertebrate MLR model	Trenfield et al. (2023)
Cnidaria (hydra)	*Hydra viridissima*	Hydroids	4	EC10 (population growth rate)	53	6.8	3.5	1.4	Hardness outside of boundary range for invertebrate MLR model	Trenfield et al. (2023)
Arthropoda (crustacean)	*Paratya australiensis*	Juvenile	21	LC50 (mortality)	100	6.9	19	NR	Only LC50 reported	Bacher and O'Brien (1990)
Cnidaria (hydra)	*Hydra vulgaris*	Non-budding	7	NOEC (population growth rate)	< 250	7.3–7.5	19–30	NR	Nominal data only	Holdway et al. (2001)
Magnoliophyta (macrophyte)	*Lemna aequinoctialis*	Vegetatively reproducing	4	EC10 (growth rate)	320	6.7	3.5	1.4	Did not pass acceptability test; hardness outside of boundary range for MLR model	Trenfield et al. (2023)
Magnoliophyta (macrophyte)	*Ipomoea aquatica*	Seedling	14	NOEC (growth)	10,000	NR	NR	NR	Zinc not measured, no water chemistry data	Wu and Sun (1998)
Magnoliophyta (macrophyte)	*Landolitia punctata*	Adult	7	EC50 (frond production/ biomass)	193,400 413,500	4.6–4.8	NR	NR	Zinc and hardness not measured; pH outside of boundary range for MLR model	Lahive et al. (2011)

NR = not reported.

4.2 Toxicity data used in derivation

Data sourced from ANZECC and ARMCANZ (2000), the Australasian Ecotoxicology Database (Langdon et al. 2009; Markich et al. 2002), the European Union Risk Assessment (Munn et al. 2010) and the Canadian Council of Ministers for the Environment Guideline (CCME 2018) had already been assessed for quality and so were deemed acceptable. All remaining data were assessed for quality based on Warne et al. (2018), and only acceptable quality data (scoring > or = 50%) were included.

There were 151 chronic toxicity values for 25 species that were of acceptable quality for use in the DGV derivation and within the boundary of the MLR models. The dataset included 11 species native to Australia and New Zealand from 6 taxonomic groups. Most of the values (133 values for 17 species) were of the most preferred type of toxicity estimates (i.e. NECs and ECx/ICx/LCx values where x ≤ 10, as per Warne et al. 2018). Six values were NECs (6 species) and 133 were EC/IC/LC10s (11 species). Less preferred toxicity estimates included 6 EC/LC11–20s (3 species) and 12 NOEC values (5 species).

Warne et al. (2018) recommend using only preferred chronic toxicity values where there are sufficient (i.e. > 8) values. The dataset based on only the preferred values comprised 17 species from 6 taxonomic groups, 8 species fewer than the full acceptable dataset. A comparison of the protective concentrations (i.e. for 80%, 90%, 95%, 99% species protection) derived from both datasets indicated there were only minor differences that were well within the uncertainty of the estimates (see Appendix B). Therefore, the larger dataset with greater species diversity was selected to derive the DGVs.

The trophic-level-specific MLRs were applied to the accepted toxicity data for the 25 species to calculate sensitivity coefficients for each species and data point. The sensitivity coefficient is calculated as the difference between the observed (experimental) value ($\log_e EC10_{expt}$) and the MLR predicted value ($\log_e EC10_{pred}$) for each species at each water chemistry combination tested. For the purposes of calculating the guideline values, any DOC concentration < 0.5 mg/L was treated as being 0.5 mg/L. The toxicity modification of DOC below 0.5 mg/L is expected to be minimal. Differences in the reported concentrations at DOC of 0.5 mg/L are typically due to instrumental sensitivity (i.e. with standard instruments the DOC may be reported as 0.5 mg/L whereas with a more sensitive instrument, the same DOC may be reported as 0.1 mg/L). Adjustment for toxicity modification is therefore not justifiable within this low range.

A mean sensitivity coefficient was calculated across studies with replicate tests with the same endpoint, life stage and toxicity test duration to determine study-specific sensitivity coefficients. Single-species sensitivity coefficients were then calculated by selecting the value associated with the most sensitive endpoint, life stage and toxicity test duration for each species, based on Warne et al. (2018) or, where there were multiple values by calculating means[3] across all available studies for that endpoint, life stage and toxicity test duration. Note that there are two algal taxa described as *Chlorella* sp., which have only been identified to genus level, not species level. These are unlikely to be the same species, having been isolated from different locations. Mean values were only calculated

[3] Means were used instead of geometric mean because the sensitivity coefficients are already log-transformed.

across taxa that can be confirmed as the same species. EC10 values used in the SSD were then calculated at the target water chemistry using the formulae in Table 3.

Table 3 Trophic-level-specific Multiple Linear Regression (MLR) equations

Trophic level	MLR [a]
Fish	$Log_e(EC10) = Sensitivity^b + 0.84 \times log_e[Hardness] + 0.27 \times log_e[DOC]$
Invertebrates	$Log_e(EC10) = Sensitivity - 0.52 \times pH + 0.31 \times log_e[Hardness] - 1.4 \times log_e[DOC] + 0.24 \times log_e[DOC] \times pH$
Chlorella sp.	$Log_e(EC10) = Sensitivity - 0.36 \times pH + 0.67 \times log_e[Hardness] + 0.35 \times log_e[DOC]$
Other algae	$Log_e(EC10) = Sensitivity - 0.86 \times pH + 0.21 \times log_e[DOC]$

[a] The parameters in the MLR equations (Table 1) have been rounded to 2 decimal places.

[b] Sensitivity is a species-specific coefficient calculated as the difference between the observed (experimental) value (log_eEC10_{expt}) and the MLR predicted value (log_eEC10_{pred}) for each species at each water chemistry combination tested (see Table 2).

The 25 species included in the SSD were from 6 taxonomic groups: 4 fish, 5 crustaceans, 2 insects, 10 molluscs, 1 rotifer and 3 green microalgae. The toxicity values in the SSD ranged over nearly 3 orders of magnitude, from 6.2 µg/L and 4.2 µg/L for *Chlorella* sp. (green alga; Papua New Guinea isolate) to 2,910 and 1,920 µg/L for *Rhithrogena hageni* (mayfly). Notably, the value for the most sensitive species *Chlorella* sp. (Papua New Guinea isolate) was calculated from 28 EC10 values that ranged from 0.8 µg/L to 193 µg/L from 3-day population growth rate tests (Price et al. 2021; Price et al. 2022). The other organism described as *Chlorella* sp. is much less sensitive, while the third green algal species is moderately sensitive compared to all other species (Table 4). 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 zinc freshwater DGVs enables the adjustment of the DGVs to account for pH, hardness and 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 water chemistry data. The process for doing this for zinc is described in detail by Gadd et al. (2026a, b) and, thus, is only summarised below.

The high bioavailability condition upon which the DGVs were based was determined based on an extensive collation and analysis of water chemistry data from freshwaters around Australia and New Zealand. A matched dataset with pH, DOC and hardness measured in the same sample and within the applicability range for the zinc MLR models included 10,607 values for Australia and 6,436 values for New Zealand. Full details of the water chemistry datasets are provided in Gadd et al. (2026a, b).

The collated water chemistry datasets, together with the zinc freshwater ecotoxicity dataset and the 4 trophic level-specific MLRs as described in Section 4.2, were used in the ssdtools package with model averaging to construct probability distributions of zinc concentrations that were protective of 99%, 95%, 90% and 80% of species in each individual water sample. These bioavailability-adjusted protective concentrations (BAPCs) were calculated for each available water chemistry sample for all

sites in each state and mainland territory in Australia and each region in New Zealand. Only those data within the boundary conditions of the bioavailability models were used to calculate the BAPCs. The 99, 95, 90 and 80% species protection level BAPCs corresponding to the water chemistry conditions at the weighted 5th percentile (weighting by the number of samples at each site) of the BAPCs in the most sensitive state/territory in both Australia (Australian Capital Territory) and the most sensitive region in New Zealand (Bay of Plenty) were selected as the DGVs. Because the DGVs are calculated from weighted percentiles calculated independently for each level of species protection, the matching water chemistry is slightly different for each level of species protection. The reference conditions below are based on the water chemistry for the 95% species protection BAPC. 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 associated, high bioavailability water chemistry condition is termed the 'reference condition', and these are as follows for Australia and New Zealand:

- Australian reference condition – pH 7.6, 39 mg/L as $CaCO_3$ hardness and 1.0 mg/L DOC
- New Zealand reference condition – pH 7.5, 28 mg/L as $CaCO_3$ hardness and 0.7 mg/L DOC

The final chronic toxicity dataset described in Section 4.2, normalised to the Australian and New Zealand reference conditions, is shown in Table 4.

Table 4 Summary of single chronic toxicity values normalised to the Australian and New Zealand reference conditions, used to derive default guideline values for dissolved zinc in freshwater [a]

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Toxicity value normalised to Australian reference condition [b] (µg/L)	Toxicity value normalised to NZ reference condition [c] (µg/L)
Chordata (fish)	*Oncorhynchus mykiss*	Juvenile	30	LC10 (mortality)	120	81
	Pimephales promelas	Larval (< 24 hours old)	7	NOEC (mortality)	100	69
	Prosopium williamsoni	Larvae (fry)	30	IC20 (mortality)	220	150
	Salmo trutta	Embryo/ larval	3, 29 and 80	NOEC (growth and mortality)	290	200
Arthropoda (crustacean)	*Ceriodaphnia dubia*	Neonates (< 24 hours old)	7	EC10 (reproduction)	44	36
	Ceriodaphnia reticulata	Neonates (< 6 hours old)	7	NOEC (survival and reproduction)	48	39
	Daphnia magna	Neonates (< 48 hours old)	21	EC10 (reproduction)	47	39
	Daphnia thomsoni	Neonates (< 24 hours old)	21	EC10 (reproduction)	31	25

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Toxicity value normalised to Australian reference condition [b] (µg/L)	Toxicity value normalised to NZ reference condition [c] (µg/L)
	Hyalella azteca	< 1 week old	42 and 70	NOEC (mortality)	62	51
Arthropoda (insect)	*Neocloeon triangulifer*	Neonates (< 24 hours old)	14	EC20 (growth)	10	8.4
	Rhithrogena hageni	Nymph	10	LC10 (mortality)	2,200	1,800
Mollusca (mollusc)	*Alathyria profuga*	Larvae	3	NEC (mortality)	12	10
	Cucumerunio novaehollandiae	Larvae	3	NEC (mortality)	7.3	6.0
	Echyridella menziesii	Larvae	2	EC20 (mortality)	230	190
	Hyridella australis	Larvae	3	NEC (mortality)	7.6	6.2
	Hyridella depressa	Larvae	3	NEC (mortality)	8.7	7.2
	Hyridella drapeta	Larvae	3	NEC (mortality)	9.6	7.9
	Lampsilis siliquoidea	Juvenile (2 or 4 months old)	28	IC10 (growth and mortality)	34	28
	Lymnaea stagnalis	21 days old	28	EC10 (growth)	240	190
	Physa gyrina	Adult/ juvenile	30	NOEC (mortality)	400	330
	Velesunio ambiguus	Larvae	3	NEC (mortality)	13	11
Rotifera (rotifer)	*Brachionus calyciflorus*	< 2 hours old	2	EC10 (population growth rate)	110	94
Chlorophyta (green microalga)	*Chlorella* sp. (Papua New Guinea isolate)	Exponential growth phase	3	EC10 (population growth rate)	5.7	4.2
	Chlorella sp. (Kakadu isolate)	Exponential growth phase	3	EC10 (population growth rate)	840	610
	Raphidocelis subcapitata	Exponential growth phase	3	EC10 (population growth rate)	20	20

[a] Values shown here are rounded to 2 significant figures. The DGVs are calculated from weighted percentiles calculated independently for each of the protective concentrations. The reference condition is based on the water chemistry that matches the 95% species protection. Calculating other protective concentrations (i.e. 99%, 90% and 80% species protection) from these toxicity data will not result in the same values as the DGVs.

[b] Australian reference condition – pH 7.6, hardness 39 mg/L $CaCO_3$ and DOC 1.0 mg/L.

[c] New Zealand reference condition – pH 7.5, hardness 28 mg/L $CaCO_3$ and DOC 0.7 mg/L.

The different mechanisms of zinc toxicity suggest the potential for the data to exhibit bimodality or multimodality. The toxicity dataset was assessed for modality following the weight-of-evidence approach recommended in Warne et al. (2018). There was no clear evidence of taxa-specific sensitivity (Figure 1). Although there is a cluster of sensitive molluscs with EC10 values of 7.5-13 µg/L (predicted at the Australian reference condition) at the low end of the SSDs, there were also some molluscs of lower sensitivity, with EC10 values from 35 µg/L to 410 µg/L. Other taxonomic groups were more evenly spread throughout the concentration range (Figure 1). Thus, there was no clear pattern of taxa-specific sensitivity. A visual assessment of datasets for the Australian and New Zealand reference conditions showed several minor breaks that could indicate bimodality (Figure 2 and Figure 3). However, none of these breaks were indicative of a strong bimodal response, and the bimodality coefficients (0.43 and 0.41 for Australian and New Zealand data sets, respectively) did not exceed the threshold condition for bimodality (> 0.55, Warne et al. 2018). Therefore, the indications of bimodality were deemed to be due to limitations in data availability, and all the toxicity data (i.e. from 25 species) were used for the derivation.

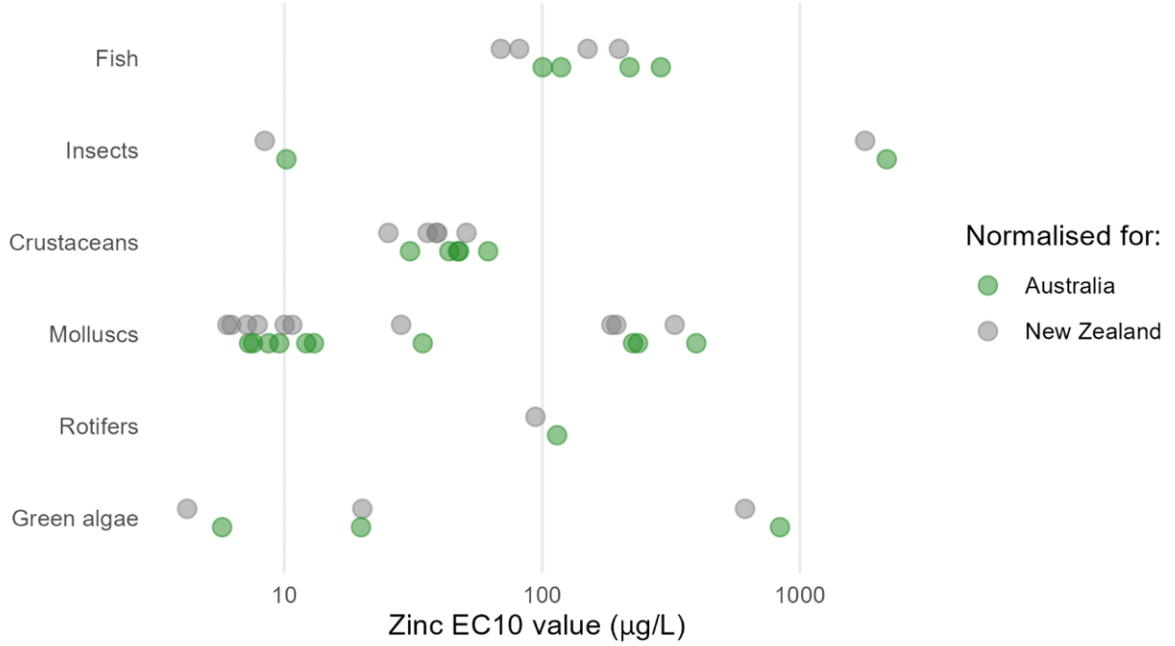


Figure 1 Comparison of dissolved zinc toxicity between taxonomic groups. Zinc toxicity data normalised to the Australian reference condition of pH 7.6, hardness 39 mg/L as $CaCO_3$ and DOC 1.0 mg/L and the New Zealand reference condition of pH 7.5, hardness 28 mg/L as $CaCO_3$ and DOC 0.7 mg/L.

4.4 Species sensitivity distribution

The SSDs based on the 25 chronic toxicity data for dissolved zinc in freshwater (Table 4) at the Australian reference condition (pH 7.6, hardness 39 mg/L as $CaCO_3$ and DOC 1.0 mg/L) and New Zealand reference condition (pH 7.5, hardness 28 mg/L as $CaCO_3$ and DOC 0.7 mg/L) are shown in Figure 2 and Figure 3, respectively. The SSDs were modelled using the ssdtools package (Version 2.4.0) (Thorley et al. 2024) using 5 of the 6 default distributions recommended for the ANZG toxicant DGVs (Gamma, log-Gumbel, log-logistic, log-normal and Weibull; Fox et al. 2024, Warne et al. 2025), with the model averaging method used to calculate the DGVs. The model averaged distributions were judged to provide a good fit to both datasets.

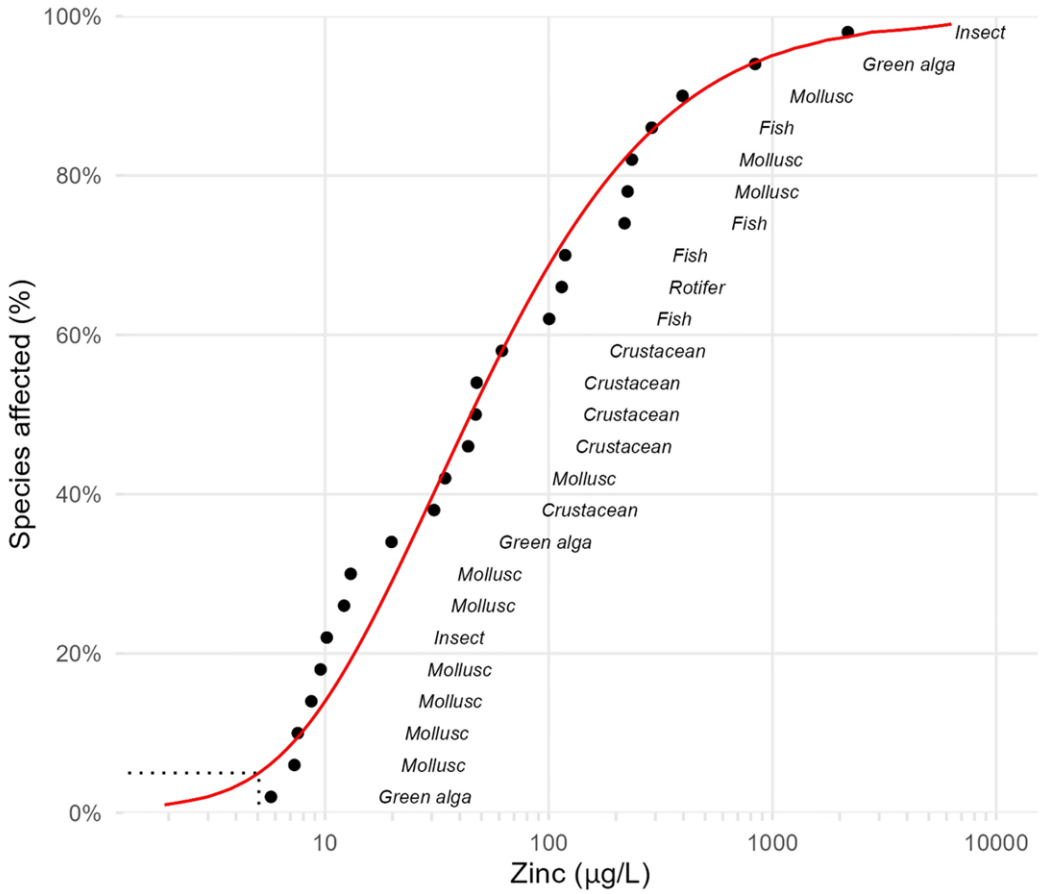


Figure 2 Species sensitivity distribution for dissolved zinc in freshwater, normalised to the Australian reference condition (pH 7.6, hardness 39 mg/L as $CaCO_3$ and DOC 1.0 mg/L)

Dotted line indicates the zinc concentration at which there is 95% species protection and 5% of species are potentially affected (5.2 µg/L).

The log-normal/log-normal distribution was excluded from the default model set for calculating the zinc DGVs/BAGVs. This was due to the occurrence of dataset artefacts (clustering into two or sometimes three groups) that became more or less apparent depending on the water chemistry combination. This clustering occasionally caused the fit of bimodal log-normal/log-normal distribution to change substantially with only minor changes to the species sensitivity data as water chemistry changed. This resulted in differences in the BAGVs calculated from the model averaged fit when the log-normal/log-normal distribution was included that were inconsistent and counter-intuitive with the minor changes in water chemistry. As the log-normal/log-normal distribution's influence on the BAGVs was identified as being (i) due to data artefacts rather than true bimodality, and (ii) inconsistent with the changes in water chemistry and the expected changes in zinc toxicity, the toxicity datasets were modelled using only the 5 default unimodal distributions, and their model-averaged estimates were used to calculate DGVs and BAGVs. This approach was consistent with the guidance provided in Warne et al (2025). Further details on the SSD fitting process are provided in Appendix D.

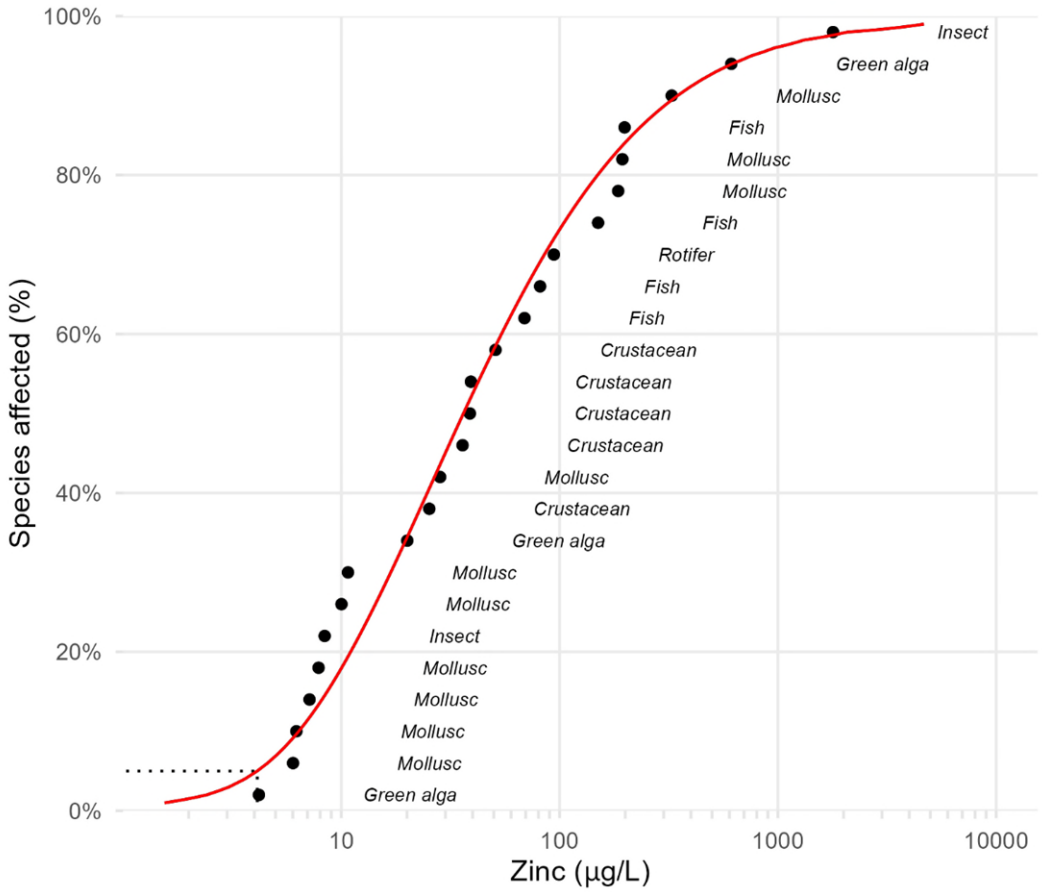


Figure 3 Species sensitivity distribution for dissolved zinc in freshwater, normalised to the New Zealand reference condition (pH 7.5, hardness 28 mg/L as $CaCO_3$ and DOC 0.7 mg/L)

Dotted line represents the zinc concentration at which there is 95% species protection and 5% of species are potentially affected (4.1 µg/L).

4.5 Default guideline values

It is important that the DGVs (Table 5) 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* (ANZG 2018).

The DGVs for 99%, 95%, 90% and 80% species protection and at the reference conditions for Australia and New Zealand are shown in Table 5. As there are differences in the chemistry of freshwaters between Australia and New Zealand (see Gadd et al. 2026a, b), separate DGVs were derived for each country. These DGVs supersede the ANZECC and ARMCANZ (2000) DGVs for zinc in freshwater and should be applied in accordance with the tiered assessment framework for zinc in freshwater (see Section 4.6).

Table 5 Toxicant default guideline values (DGVs) for dissolved zinc in freshwater for Australian and New Zealand reference conditions, with very high reliability [a]

Level of species protection (%)	DGV for zinc in freshwater for Australia (µg/L) [b]	DGV for zinc in freshwater for New Zealand (µg/L) [c]
99	1.9	1.5
95	5.2	4.1
90	8.2	6.4
80	15	11

[a] DGVs were derived using the ssdtools package in R (Thorley et al. 2024) based on species sensitivity data adjusted using trophic-level multiple linear regression models. All DGVs have been rounded to 2 significant figures. Bioavailability-adjusted GVs at other water chemistry combinations are provided in Appendix E.

[b] Australian reference condition – pH 7.6, hardness 39 mg/L $CaCO_3$ and DOC 1.0 mg/L.

[c] New Zealand reference condition – pH 7.5, hardness 28 mg/L $CaCO_3$ and DOC 0.7 mg/L.

As noted in Section 4.3, the DGVs are expected to be protective of at least 95% of waters in each country. The DGVs apply to dissolved zinc (operationally defined as the < 0.45 µm filtered measurement) and can be applied in waters with pH 6.0–8.3, hardness 26–370 mg/L as $CaCO_3$ and DOC 0–15 mg/L. With the exception of pH, the above applicability ranges are based on the range where all MLRs for fish, invertebrates and algae were applicable. The pH applicability range was extended below the lower *Chlorella* model boundary as *Chlorella* sensitivity was lower at low pH, and beyond the fish model boundaries as fish sensitivity did not change with pH. BAGVs can only be calculated for water chemistries within these ranges. When DOC is less than 0.5 mg/L, no further DOC-related adjustment is made to the BAGVs. The 95% species-protection level DGVs and BAGVs are recommended for application to slightly-to-moderately disturbed ecosystems. Where the local water chemistry (pH, hardness, DOC) is outside the above-mentioned applicability ranges, the DGVs and BAGVs may not be applicable, and site-specific guideline values may be needed (Stauber et al. 2026). This includes acidic, low hardness and high DOC waters as found in the Northern Territory of Australia and West Coast of New Zealand. Deriving site-specific guideline values may include modelling of metal speciation and/or toxicity testing in site-specific waters. ANZG (2018) provides guidance on the derivation of site-specific guideline values, and local jurisdictions should always be consulted on appropriate methods.

Under the tiered assessment framework (see Section 4.6), if the dissolved zinc concentration exceeds the relevant DGV, the DGV can be adjusted to a BAGV based on the pH, hardness and DOC concentrations of ambient water, using either the tables in Appendix E or the ANZG metals bioavailability tool.

4.6 Use of the default guideline values in the tiered assessment framework

The DGVs should be applied in accordance with the tiered assessment framework for zinc 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 zinc 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 zinc 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 should be taken into account. This can be done either by (i) calculating a zinc BAGV based on the sample's pH, hardness and DOC concentration and comparing the dissolved zinc concentration in the sample to the BAGV, or (ii) adjusting the zinc 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 should be used to determine the implications of the exceedance, which may include toxicity testing or bioavailability modelling.

4.7 Reliability classification

The zinc freshwater DGVs have a very high reliability classification (Warne et al. 2018) based on the outcomes for the following 3 criteria.

- sample size – 25 species from 6 taxonomic groups (preferred)
- type of toxicity data – chronic (NEC, EC10, IC10, LC10, EC20, NOEC)
- SSD model fit – good.

The bioavailability models used in this derivation include the key TMFs identified in the literature as most important in modifying zinc toxicity. They are based on relevant species and have been assessed for applicability to the groups of species to which they are applied. Where possible, the models have been validated using species found in Australia and New Zealand, and in relevant water chemistry (Price et al. 2023b; Stauber et al. 2023).

Glossary and acronyms

Term	Definition
Acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short (relative to the organism's life span) exposure period to a chemical.
Acute-to-chronic ratio (ACR)	The species' mean acute value (LC50/EC50) divided by the chronic value (NOEC) for the same species.
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.
BAPC (bioavailability-adjusted protective concentration)	A protective concentration value derived from a species sensitivity distribution that has been adjusted to account for one or more specific toxicity modifying factors using one or more established models. These values were derived in order to identify and select the DGVs.
Biotic ligand model (BLM)	A mechanistic model that relates the physico-chemistry of the receiving water to the bioavailability and toxicity of metals to aquatic organisms.
Chronic toxicity	A lethal or sub-lethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
Default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. a site-specific value) in the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. Formerly known as 'trigger values'.
Dissolved organic carbon (DOC)	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.
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, reproduction, 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.)
HC_5	The concentration of a substance in water or sediment that is predicted to be hazardous (i.e. could cause toxicity) to 5% of species.
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.
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms relative to the control response, under specified conditions.
LOEC (lowest-observed-effect concentration	The lowest concentration of a material used in a toxicity test that has a statistically significant ($p \leq 0.05$) adverse effect on the exposed population of test organisms as compared with the controls. All higher concentrations should also cause statistically significant effects.

Term	Definition
Maximum acceptable toxicant concentration	The geometric mean of the NOEC and the LOEC.
Multiple linear regression (MLR) model	An empirical model that relates the physico-chemistry of the receiving water to the bioavailability and toxicity of metals to aquatic organisms.
NEC (no-effect concentration)	Parametric or Bayesian estimate of the highest concentration of a chemical below which no effect occurs.
Negligible effect	Corresponding to an estimated no-effect or acceptable-effect level; negligible-effect concentrations typically include the NEC, NSEC, BEC10, ECx/ICx where $x \leq 10$, 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 ($p > 0.05$) adverse effect on the exposed population of test organisms as compared with the controls. The statistical significance is measured at the 95% confidence interval.
NOEL (no-observed-effect level)	See NOEC.
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.
Species sensitivity distribution (SSD)	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.
TMF	Toxicity modifying factor
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.

Appendix A: Summary of effects of toxicity modifying factors and bioavailability adjustment methods

Effects of toxicity modifying factors (TMFs) on zinc toxicity to fish, invertebrates and algae

The effect of hardness, pH and DOC on zinc toxicity to different species is shown in Figure A 1. An effect of hardness is more evident for fish than for the other taxonomic groups, whereas the effect of pH appears to be more evident for algae. There is a clear relationship between zinc toxicity to fish and DOC, but no distinct relationship for the other taxonomic groups. However, these relationships are complicated by differing values for other TMFs in the tests for many of the species.

Relationships between zinc toxicity and individual TMFs are clearer when other TMFs are held constant, though this type of data is available for fewer species (Figure A 2 – Figure A 4).

Development of multiple linear regression (MLR) model for fish

CCME (2018) developed a MLR model to relate the toxicity of zinc to rainbow trout (*O. mykiss*) based on the three TMFs of pH, hardness and DOC, which was initially considered for deriving the zinc DGVs. However, the model was based on EC10 values and included a test at pH 5.68 – outside the range of applicability for DGVs. That test result was also shown to be leveraging the regression model, resulting in a highly negative slope with pH (-0.815). This slope was used to adjust EC10 values to differing pH values from pH 6.5 to pH 8.1 (CCME 2018). However, the high negative slope is not well supported by studies that indicate a non-monotonic effect of pH on zinc toxicity, with minimal effect between pH 6.7 and 7.6 (De Schamphelaere and Janssen 2004).

Consequently, the MLR for fish was reassessed for the zinc DGVs derivation. For *O. mykiss*, there were 14 tests (all 30-day mortality tests) with differing pH, hardness and/or DOC values excluding tests at pH < 6, or with high sodium and magnesium concentrations (used by De Schamphelaere and Janssen (2004) in developing BLM models). There were insufficient data to include interactions in the MLR model, so models without interactions were fitted. As there were few data, all were used for model development with none excluded for future validation purposes. Other studies that reported LC50 values and pH, hardness and DOC in the test waters (Cairns et al. 1982; Chapman 1978; Lazorchak and Smith 2007; Mebane et al. 2008; Nehring and Goettl 1974) were used to validate the developed model.

EC50 values were used for the model development, consistent with the other models used for the zinc DGVs derivation. Data outside the range of applicability were removed if shown to be leveraging the model. This resulted in an MLR model where pH was only weakly significant (p-value 0.069) in a stepwise regression. On that basis, pH was not included in the final MLR model for *O. mykiss*.

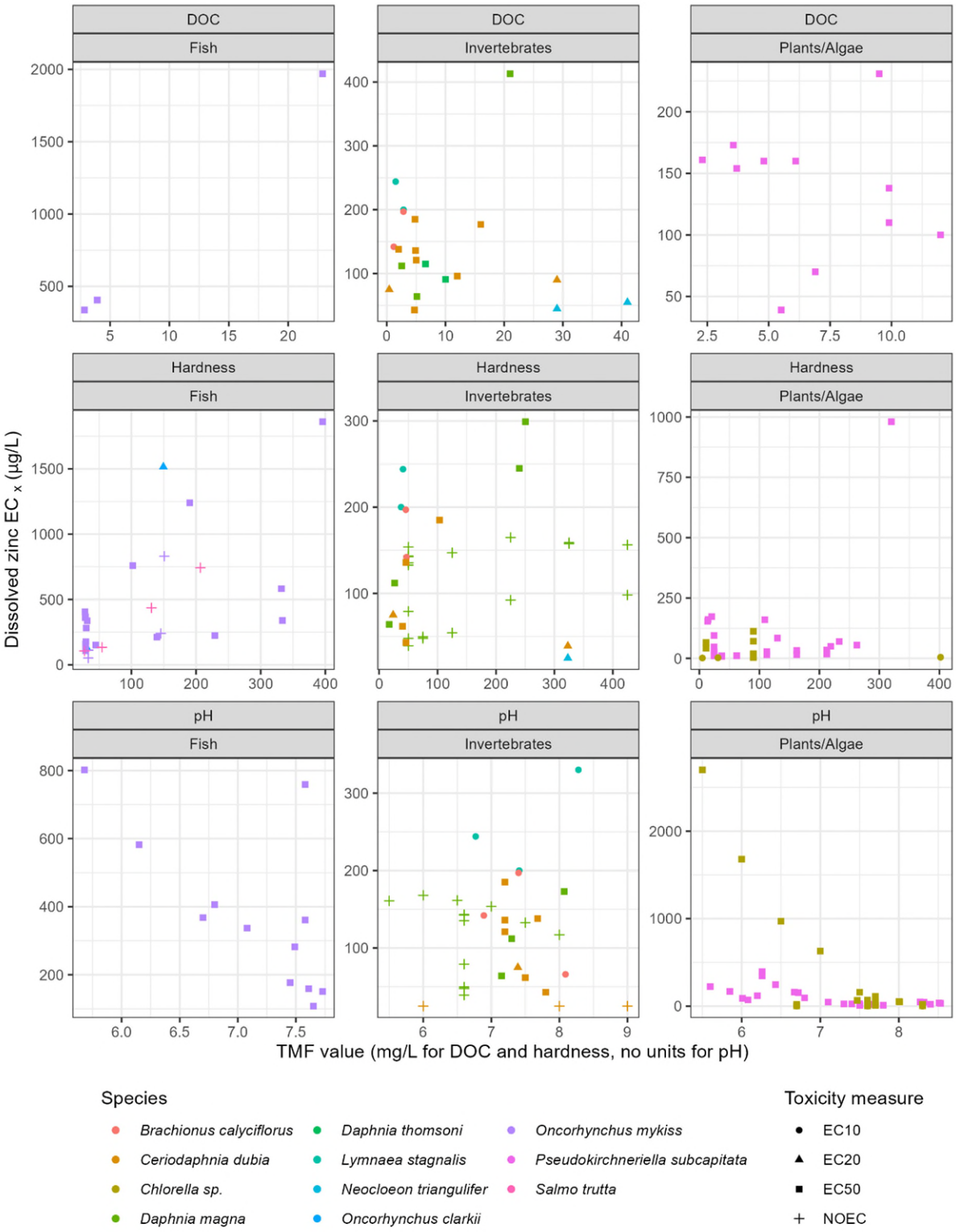


Figure A 1 Influence of DOC, hardness and pH on zinc toxicity for different species (plots split by taxonomic groups). Toxicity measures vary depending on available data, ECx values preferentially used. Relationships with DOC are based on tests at low to moderate hardness (< 140 mg/L) and near neutral pH (6.5–8); relationships with hardness are based on tests at near neutral pH (6.5–8) and low to moderate DOC (< 5 mg/L) and relationships with pH are based on tests at low to moderate hardness (< 140 mg/L) and DOC (< 5 mg/L). Note that *Pseudokirchneriella subcapitata* is currently known as *Raphidocelis subcapitata*).

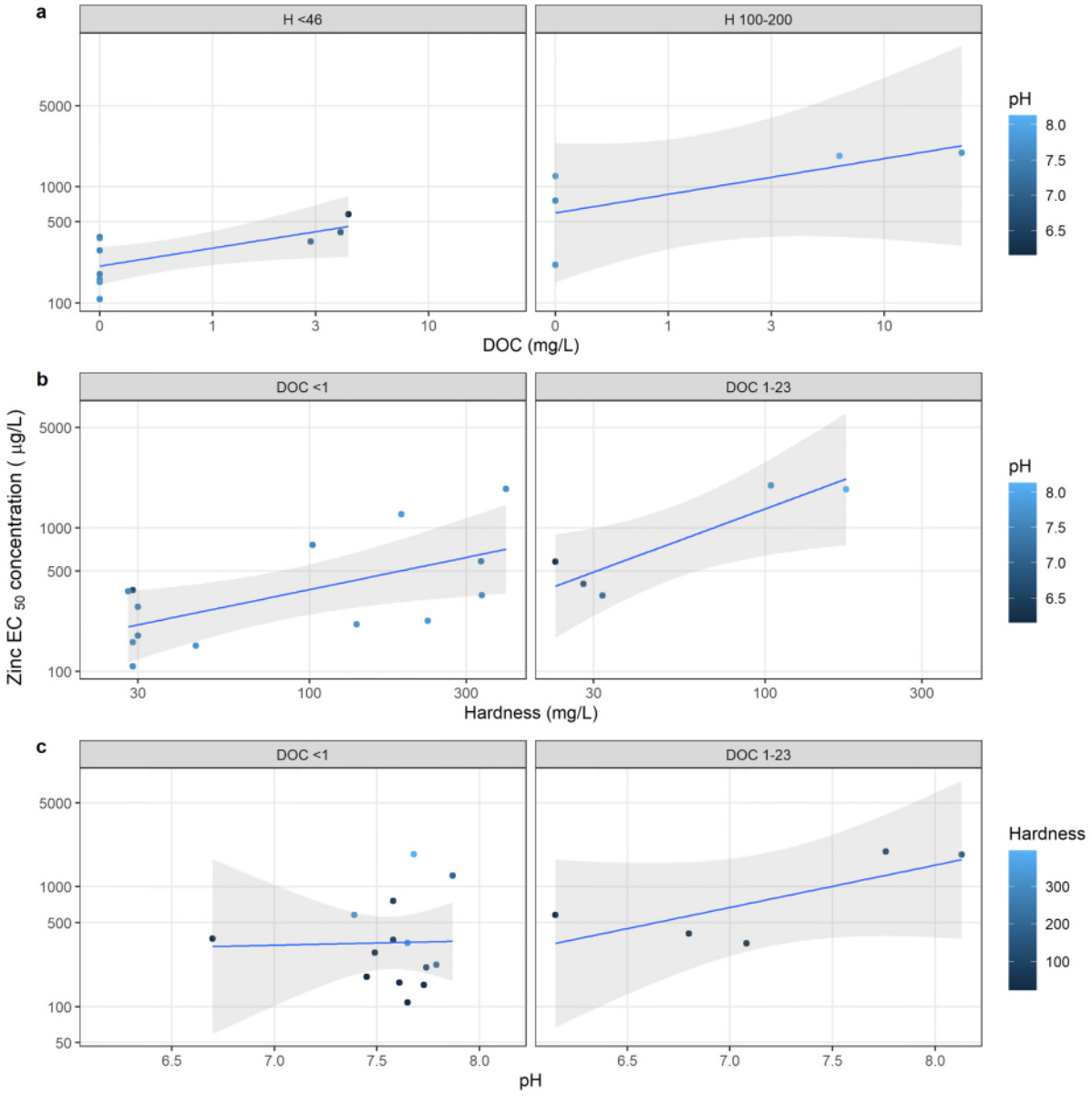


Figure A 2 Influence of (a) DOC, (b) hardness and (c) pH on zinc toxicity (as EC50) to *O. mykiss*. The pH, DOC and hardness vary by column and colour. Linear regression lines shown in blue are for descriptive purposes only to indicate direction and strength of linear relationships. The strength of regressions is illustrated by the R^2 values and the confidence band (grey shaded area): wider bands indicate less confidence/poorer model fit, narrow bands indicate higher confidence/better model fit.

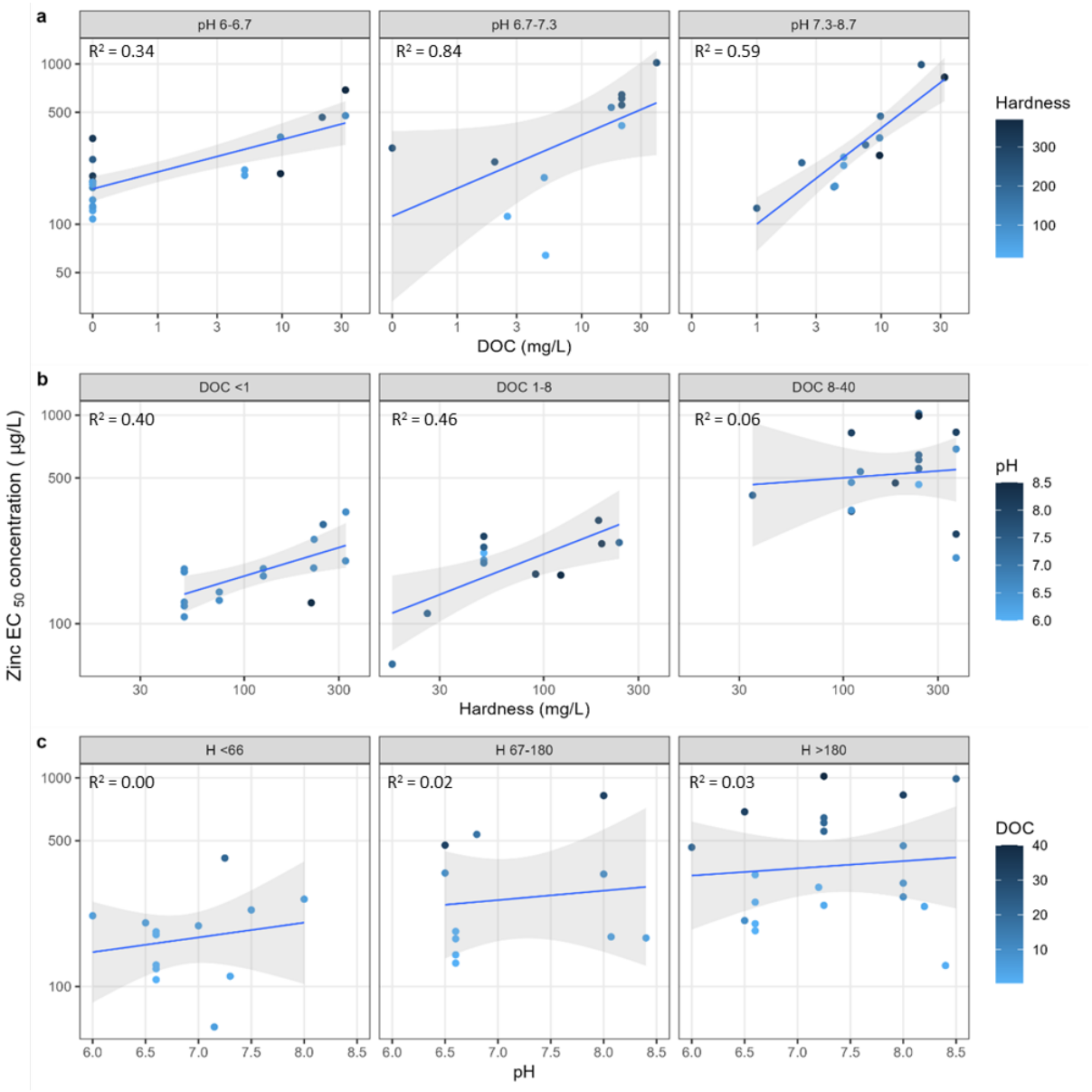


Figure A 3 Influence of (a) DOC, (b) hardness and (c) pH on zinc toxicity to *D. magna* (as EC50). The pH, DOC and hardness vary by column and colour. Linear regression lines shown in blue are for descriptive purposes only to indicate direction and strength of linear relationships. The strength of regressions is illustrated by the R^2 values and the confidence band (grey shaded area): wider bands indicate less confidence/poorer model fit, narrow bands indicate higher confidence/better model fit.

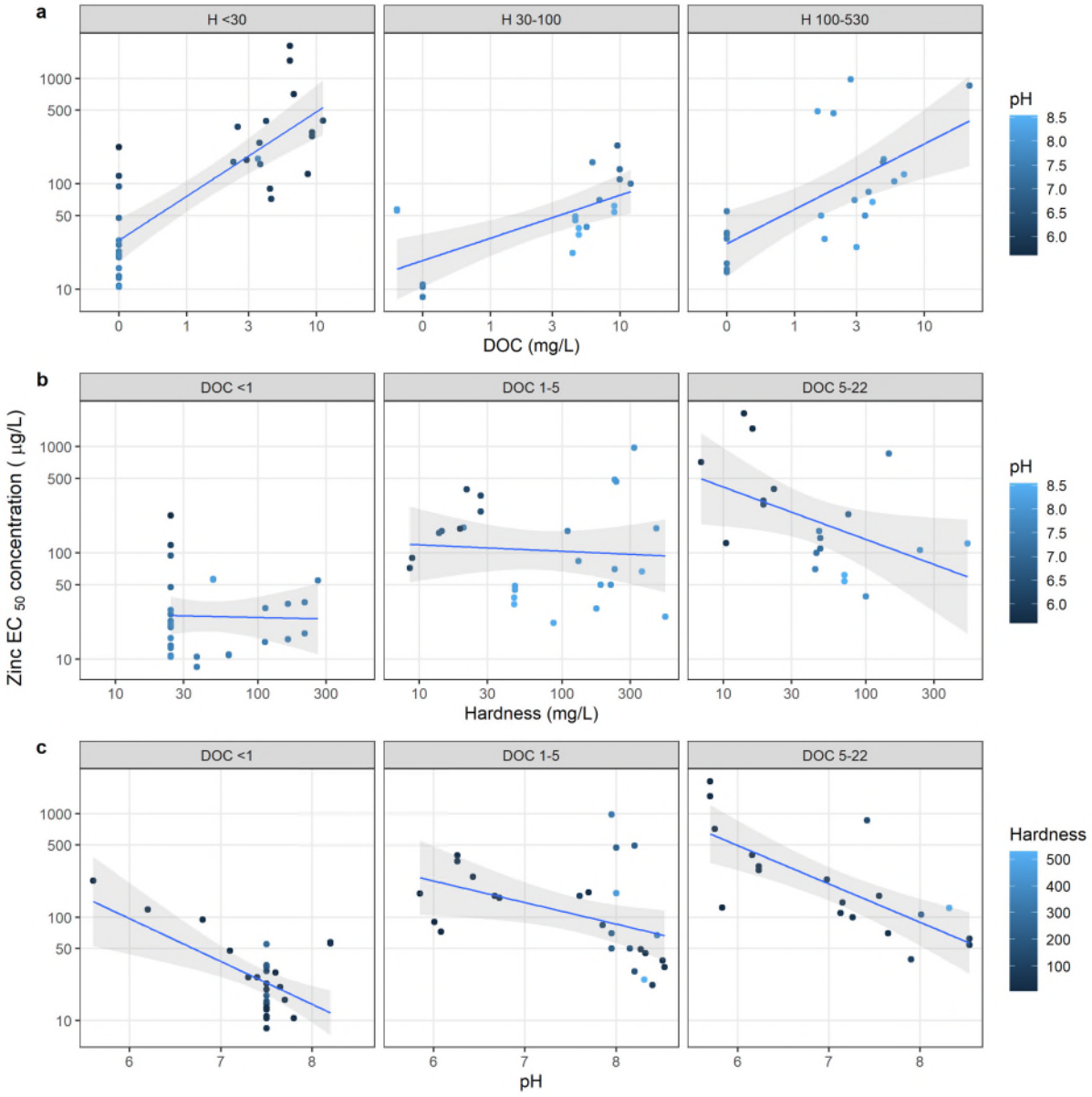


Figure A 4 Influence of (a) DOC, (b) hardness and (c) pH on zinc toxicity to *R. subcapitata* (as EC50).
The pH, DOC and hardness vary by column and colour. Linear regression lines shown in blue are for descriptive purposes only to indicate direction and strength of linear relationships. The strength of regressions is illustrated by the R^2 values and the confidence band (grey shaded area): wider bands indicate less confidence/poorer model fit, narrow bands indicate higher confidence/better model fit.

In the final regression model, the slopes for log hardness and log DOC were positive, indicating that EC50 concentrations increase (i.e. toxicity decreases) as hardness and/or DOC increase. This is shown by the coefficients in Table A 1 and by the added variable plots (which visually isolate the unique contribution of one predictor in an MLR after accounting for all other predictors) in Figure A 5. The adjusted R^2 was 0.84 indicating that the model explained a large amount of the variance in the EC50 values.

Table A 1 Summary of multiple linear regression model analyses for *O. mykiss*. Shaded row is the model adopted for the DGVs derivation.

Model	Endpoint	No. data points	Input data range			Model coefficients (p-values)			Adjusted R^2	Predicted R^2
			pH	Hardness (mg $CaCO_3$/L)	DOC (mg C/L)	pH	LnH	LnDOC		
CCME (2018)	EC10	14	5.7 – 8.1	23 – 399	0.3 – 23	-0.815 (<0.001)	0.947 (<0.001)	0.398 (<0.001)	0.90	0.80
Final model	EC50	13	6.2 - 8.1	23 - 399	0.3 – 23	Not included	0.837 (<0.001)	0.271 (0.0039)	0.84	0.80

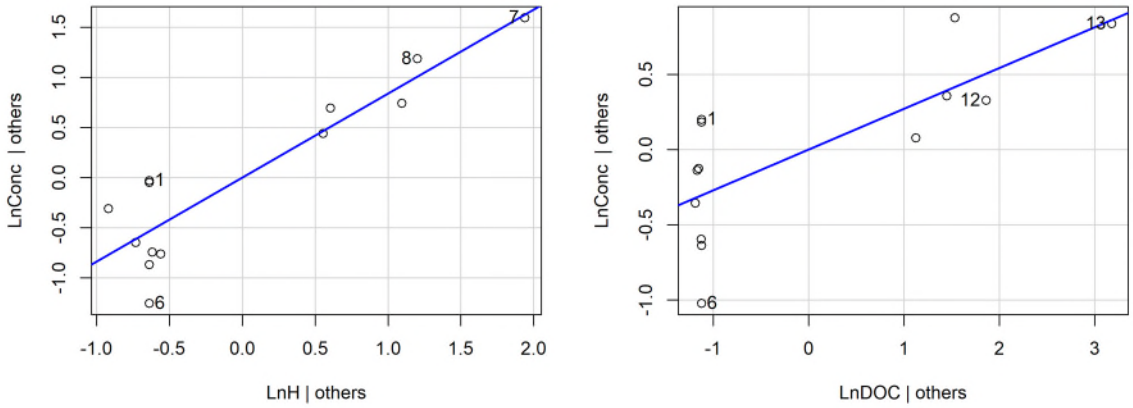


Figure A 5 Added variable plots showing the effect of each TMF in the *O. mykiss* model. This indicates a positive relationship between the EC50 concentration and both hardness and DOC. Labelled points in the plots indicate the possibility of outliers or observations that may have potentially greater influence on the regression (leveraging data points).

A number of statistical diagnostic methods were used to investigate model fits including potential existence of leveraging values or those with high influence on the regression coefficients. There was some departure from normality based on the Q-Q plot (Figure A 6) but the Shapiro-Wilk test did not suggest any non-normality (W = 0.95, p-value 0.58). There was no indication of outliers (no Studentized residuals with Bonferroni < 0.05, maximum was 0.52).

Examination of the residuals indicated a slight bias in the regression when compared to observed EC50 values (Figure A 7), though the R^2 values for these correlations were low. The slope of the regression between the residuals (using the ratio of predicted to observed, Figure A 8) and pH suggests a potential relationship, and that the effect of pH may not be fully explained in this model. However, the wide confidence intervals indicate the strength of this relationship is poor and may be simply due to noise. There was no evidence of bias when examining the residuals against DOC or hardness, suggesting the MLR model does adequately account for these two TMFs. The confidence intervals around these regressions are also wide (Figure A 8).

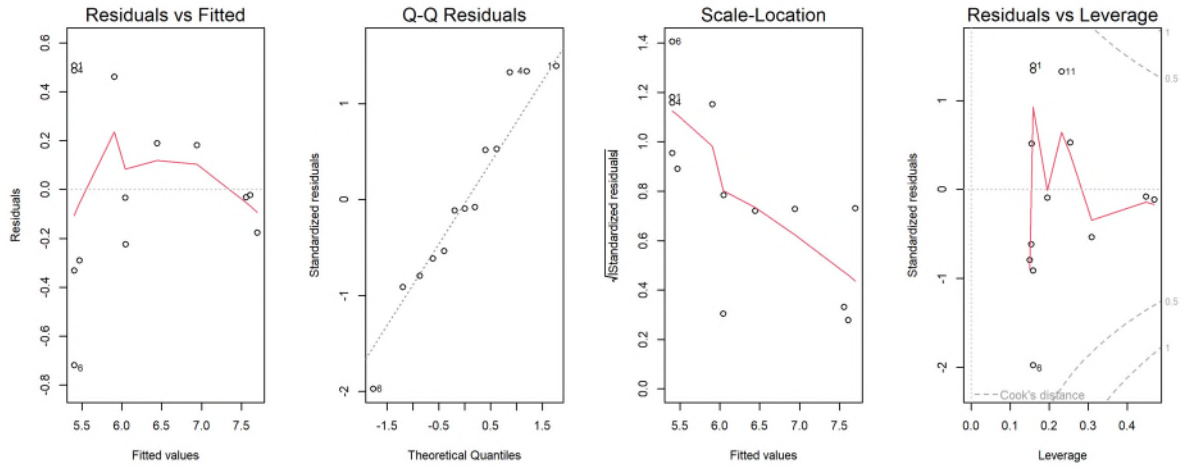


Figure A 6 Diagnostic plots for *O. mykiss* model. Labelled points indicate the possibility of outliers or observations that may have potentially greater influence on the regression (leveraging data points). Note that although the shape of the normal Q-Q plot indicates departure from normality, this S-shape is associated with less spread in the residuals than a normal distribution, is common in small data sets and is not considered an issue in fitting linear regression models.

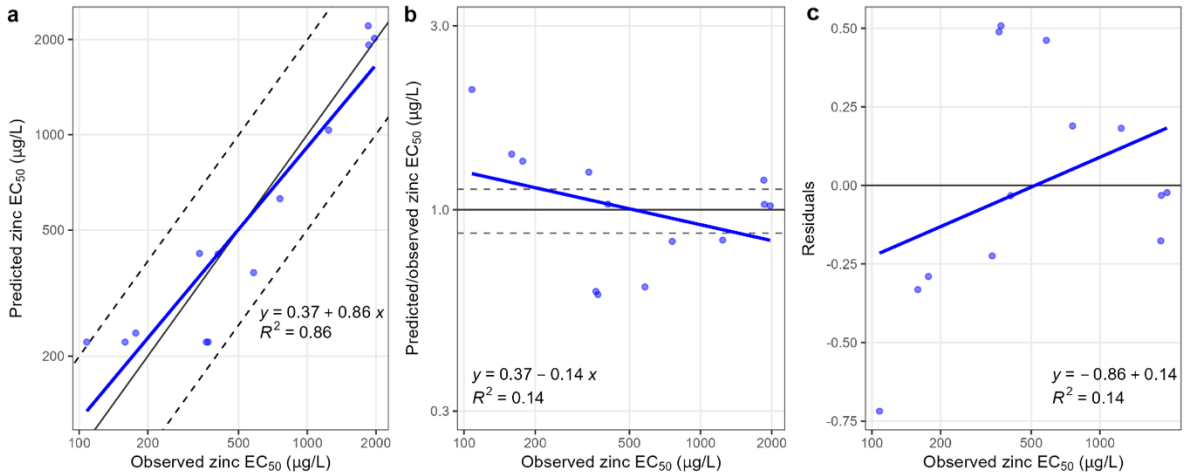


Figure A 7 Comparison of (a) predicted and observed zinc EC50 values for the *O. mykiss* model; (b) the same data plotted as predicted / observed ratio versus observed zinc EC50 values and c) model residuals versus observed zinc EC50 values. Blue line indicates the linear regression between (a) the predicted and observed; (b) the ratio and observed and (c) residuals and observed. Linear regression equation and R^2 value shown on each plot. Black line is the 1:1 line of agreement between model-predicted and observed EC50 concentrations; dashed lines in plots (a) and (b) are at plus or minus a factor of 2 above and below the 1:1 line.

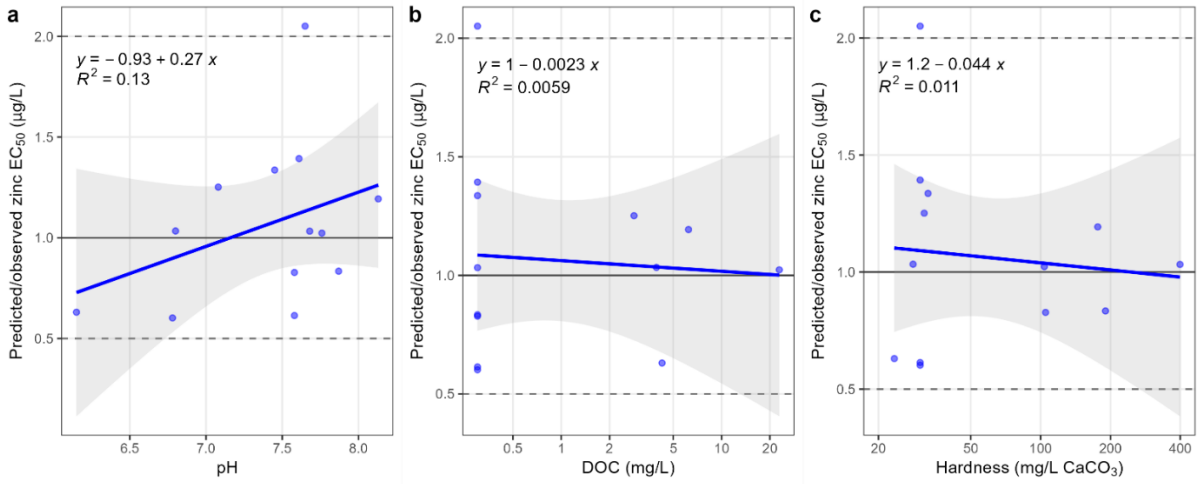


Figure A 8 Residuals (as predicted/observed ratio) against (a) pH and (b) log-transformed DOC and (c) hardness for the *O. mykiss* model. The blue line indicates the linear regression between the residuals and each factor, and the shaded area is the confidence interval for that regression. Linear regression equation and R^2 value are shown on each plot. The black line is the 1:1 line of agreement between model-predicted and observed EC50 concentrations; dashed lines are plus or minus a factor of 2 above and below the 1:1 line.

Predicted ECx values are shown in Figure A 9 for both development and validation datasets, using the data points not used in the model development. There was no adjustment of the intercept to account for differences between studies. About half of the predictions (6 out of 13; 46%) were within a factor of 2, but all were within a factor of 3.

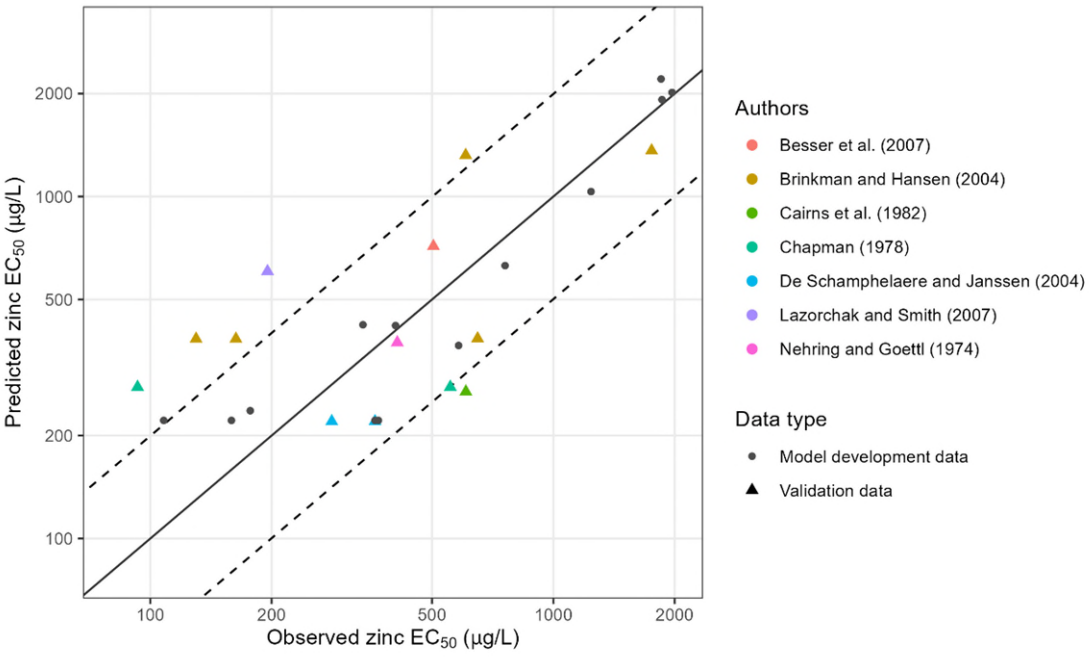


Figure A 9 Comparison of observed zinc EC50 values versus predicted EC50 values for *O. mykiss* plotted showing the model development (black) and validation (coloured) data sets. Black line is the 1:1 line of agreement between model-predicted and observed EC50 concentrations; dashed lines are at plus or minus a factor of 2 above and below the 1:1 line. Note that some data from De Schamphelaere and Janssen (2004) were used in model development and some in model validation.

Predicted effect of toxicity modifying factors on zinc toxicity and on guideline values

The effects of each of the three TMFs on the predicted toxicity to the different taxonomic groups is shown in Figure A 10 to Figure A 13, based on the models used for the derivation of the zinc DGVs and BAGVs. There is no predicted effect of pH on zinc toxicity to fish (Figure A 10), where toxicity decreases (EC10 value increases) with both increasing hardness and DOC concentration, regardless of the pH.

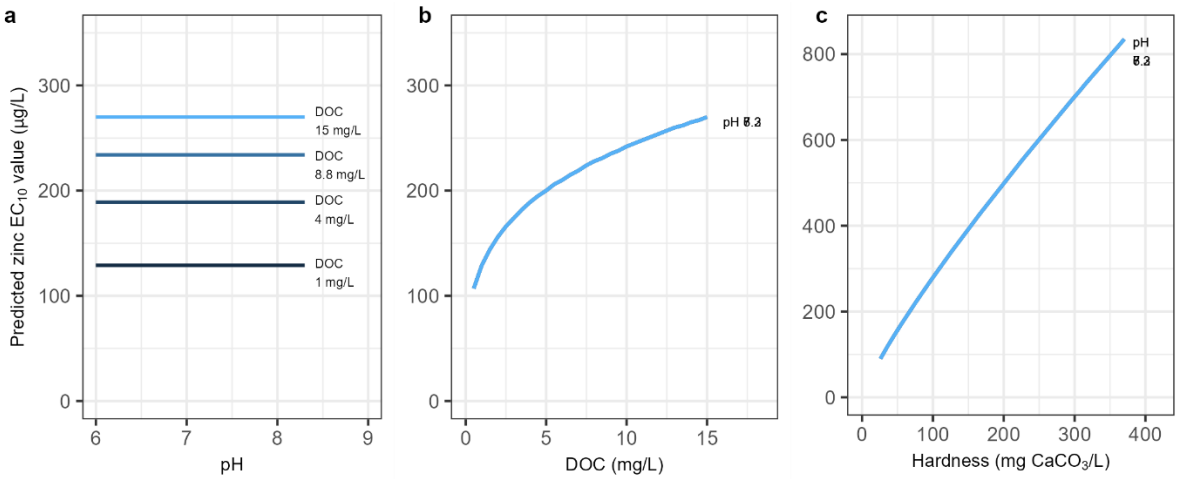


Figure A 10 Predicted toxicity to *O. mykiss* at differing (a) pH, (b) hardness and (c) DOC concentrations. Predicted values are at (a) DOC of 1, 4, 8.8, and 15 mg/L and hardness of 50 mg/L as $CaCO_3$, (b) pH 6.2 and hardness of 50 mg/L as $CaCO_3$, and (c) pH 8.3 and DOC of 2 mg/L.

For invertebrates, the predicted effect of pH depends on the DOC concentration (Figure A 11). At lower DOC concentrations (< 8.8 mg/L), toxicity increases as pH increases (lower EC10 values). This likely reflects that competition by H^+ ions at low pH is the dominant effect of pH in the absence of organic matter. At higher DOC concentrations (> 8.8 mg/L), toxicity decreases as pH increases (higher EC10 values). This likely reflects that at higher DOC, the relative importance of organic ligands increases and their capacity to bind Zn^{2+} increases as they deprotonate at higher pH. Zinc toxicity reduces with increasing hardness regardless of pH or DOC. The effect of DOC on zinc toxicity to invertebrates depends on the pH. At acidic pH (e.g. 6.2), zinc toxicity changes little with increases in DOC concentration, whereas at higher pH values, increases in DOC concentration result in lower toxicity (higher EC10 values).

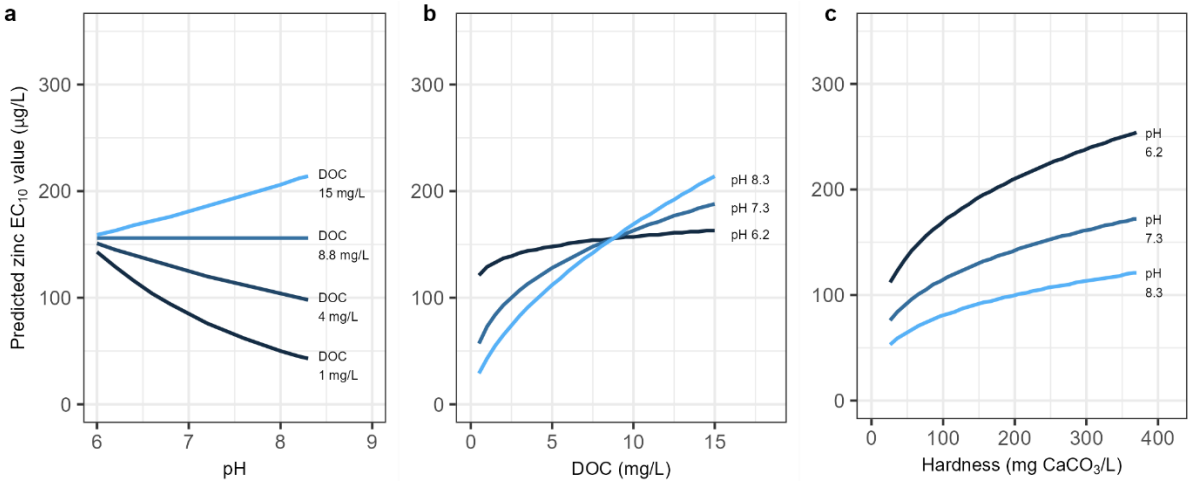


Figure A 11 Predicted toxicity to *D. magna* at differing (a) pH, (b) hardness and (c) DOC concentrations. Note that the slope between EC10 and pH changes direction at DOC of 8.8 mg/L. Predicted values are at (a) DOC of 1, 4, 8.8, and 15 mg/L and hardness of 50 mg/L as $CaCO_3$, (b) pH 6.2, 7.3, and 8.3 and hardness of 50 mg/L as $CaCO_3$, and (c) pH 6.2, 7.3, and 8.3 and DOC of 2 mg/L.

For *Chlorella* sp., all three TMFs affect zinc toxicity (Figure A 12). Zinc toxicity is predicted to increase (EC10 values decrease) as pH increases, regardless of DOC concentration. Zinc toxicity is predicted to decrease (EC10 values increase) as hardness increases and as DOC increases.

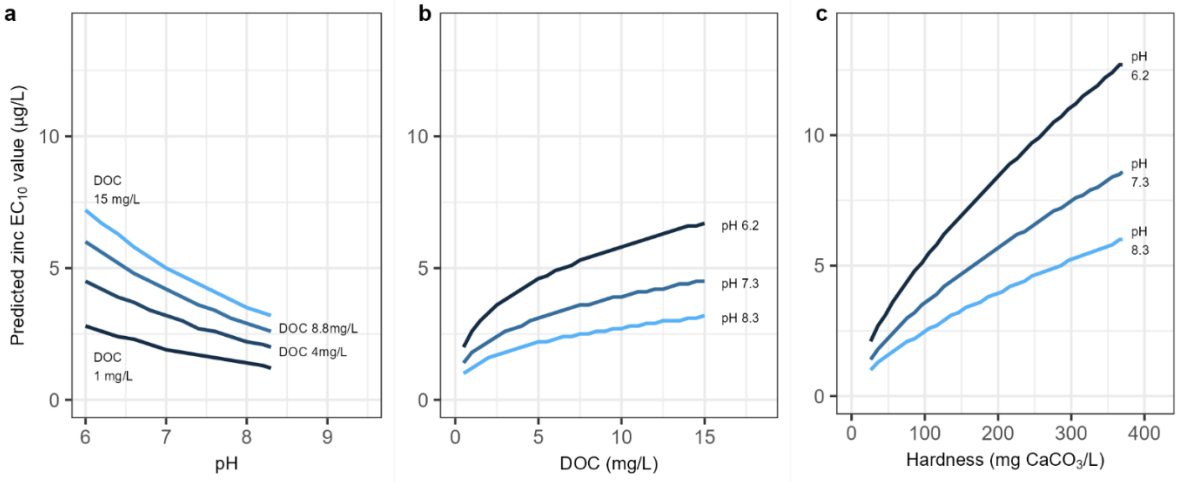


Figure A 12 Predicted toxicity to *Chlorella* sp. at differing (a) pH, (b) DOC and (c) hardness concentrations. Predicted values are at (a) DOC of 1, 4, 8.8, and 15 mg/L and hardness of 50 mg/L as $CaCO_3$, (b) pH 6.2, 7.3, and 8.3 and hardness of 50 mg/L as $CaCO_3$, and (c) pH 6.2, 7.3, and 8.3 and DOC of 2 mg/L.

For *R. subcapitata*, only pH and DOC are predicted to affect zinc toxicity (Figure A 13). Zinc toxicity is predicted to increase (EC10 values decrease) as pH increases, regardless of DOC concentration. This is consistent with the direction of the effect of pH on the other algal species, *Chlorella* sp. Zinc toxicity is predicted to decrease (EC10 values increase) as DOC increases, consistent with the effect on fish, *Chlorella* sp. and invertebrates (at higher pH). In contrast to the other species, zinc toxicity is predicted to not change with hardness.

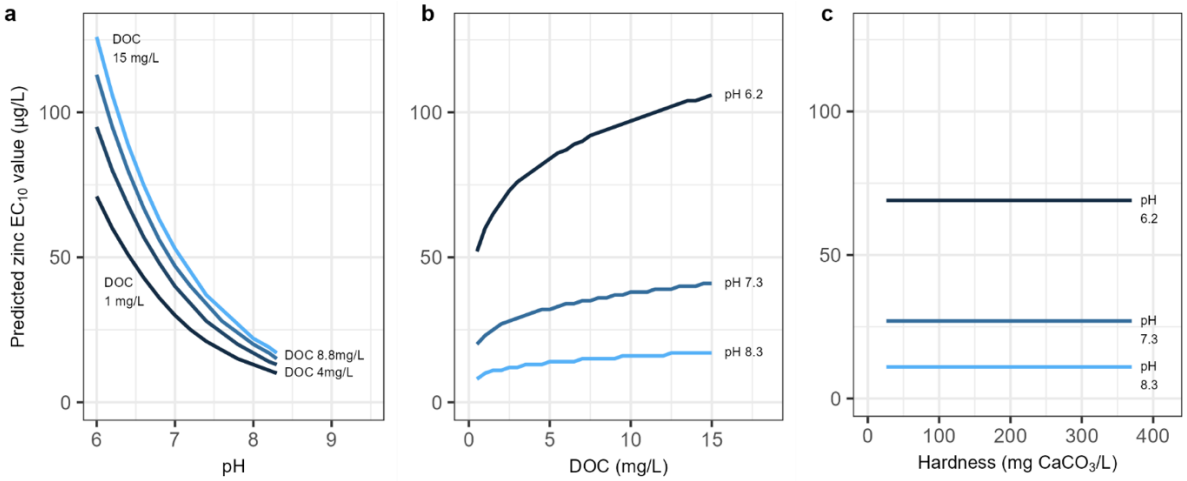


Figure A 13 Predicted toxicity to *R. subcapitata* at differing (a) pH, (b) DOC and (c) hardness concentrations. Predicted values are at (a) DOC of 1, 4, 8.8, and 15 mg/L and hardness of 50 mg/L as $CaCO_3$, (b) pH 6.2, 7.3, and 8.3 and hardness of 50 mg/L $CaCO_3$, and (c) pH 6.2, 7.3, and 8.3 and DOC of 2 mg/L.

The overall effect of these four models on zinc toxicity and the zinc BAGVs is demonstrated in Figure A 14 to Figure A 16. The effect of the four models with DOC is shown in Figure A 14. All four models show decreasing toxicity with increasing DOC, which results in an overall increase in the BAGVs with increasing DOC.

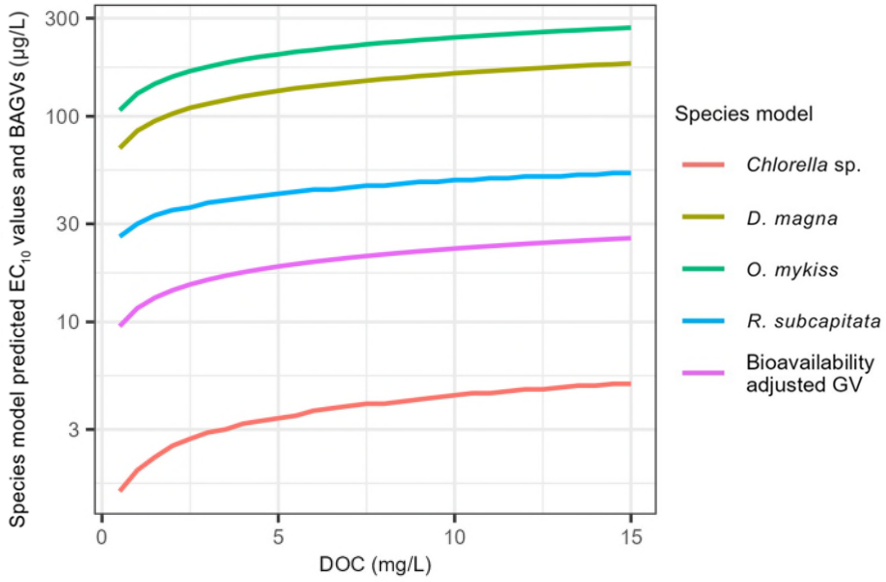


Figure A 14 Effect of DOC on toxicity with individual species models and overall effect on bioavailability-adjusted guideline values (BAGVs). Predicted values at pH 7.0 and hardness of 50 mg/L as $CaCO_3$.

The effect of the four models with hardness is shown in Figure A 15. Three of the models show decreasing toxicity with increasing hardness, though the effect is weaker for the *D. magna* invertebrate model. The *R. subcapitata* model used for green microalgal species does not indicate any change in toxicity with hardness. The overall effect is an increase in the BAGVs with increasing hardness.

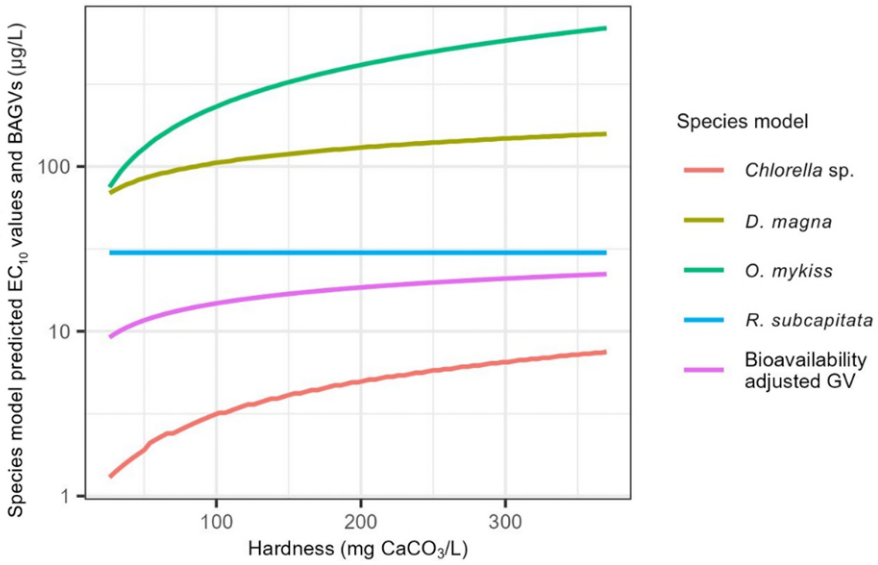


Figure A 15 Effect of hardness on toxicity with individual species models and overall effect on bioavailability-adjusted guideline values (BAGVs). Predicted values are at pH 7.0 and DOC of 1 mg/L.

The effect of the models on the BAGVs is more complex for pH. Three of the models show increasing toxicity with pH at low to moderate DOC, while the model used for fish species does not indicate any change in toxicity with pH (Figure A 16a). At higher DOC the *D. magna* model predicts a *decrease* in toxicity with pH (Figure A 16b). Despite the change in direction for the invertebrate model, BAGVs continue to be lower as pH increases due to the influence of pH in the other models and the number of other species in the SSD (Figure A 16b), up to the maximum DOC of 15 mg/L (though the difference in the BAGVs across the pH range is negligible at this DOC).

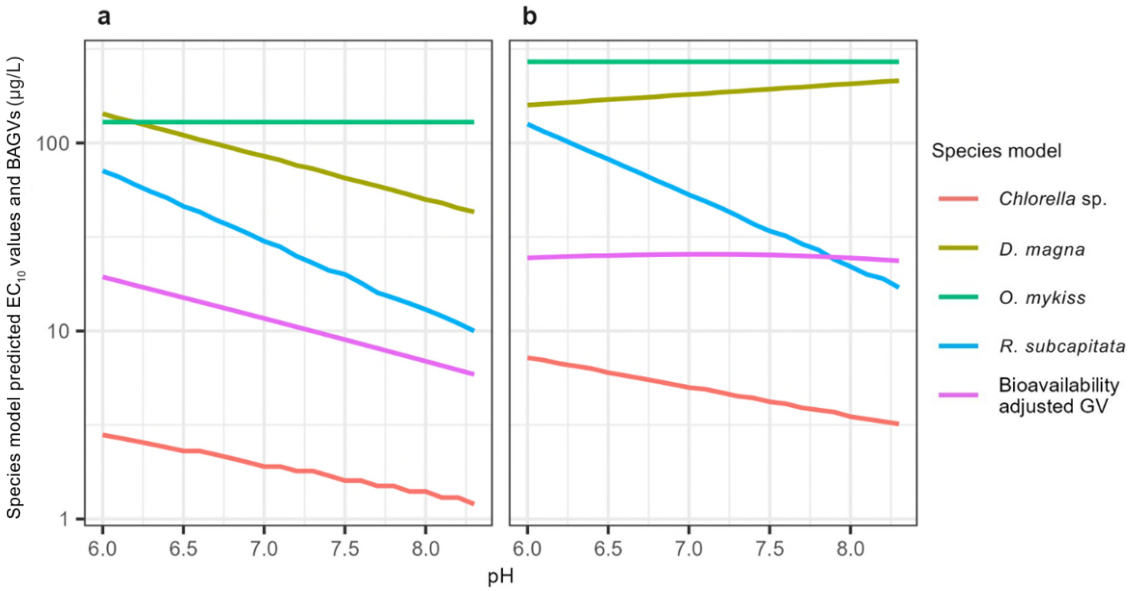


Figure A 16 Effect of pH on toxicity with individual species models and overall effect on bioavailability-adjusted guideline values (BAGVs). Predicted values are at hardness 50 mg/L as $CaCO_3$, and DOC of (a) 1 mg/L, and (b) 15 mg/L.

Summary

The information presented in this appendix shows that the effects of water chemistry on zinc toxicity are complex, varying across species and taxonomic groups. There are limited data for most species to fully assess the complexity and differences between species and groups. The MLR model for invertebrates adjusts for DOC, hardness and pH. The MLR model for fish adjusts for DOC and hardness, but does not vary with pH. The MLR model for green algae based on *R. subcapitata* adjusts for DOC and pH, but not hardness. The overall effect of water chemistry on the DGVs relates to the coefficients of the multiple MLRs, the number of species in each taxonomic group included in the SSD and their relative sensitivities.

Appendix B: Derivation based on preferred toxicity estimates only

Chronic toxicity data for zinc reported as the preferred toxicity estimates of NEC and EC/IC/LC10 are summarised in Table B 1. This dataset comprised data for 17 species from 6 taxonomic groups. The SSDs for the Australian and New Zealand reference conditions based on this dataset are shown in Figure B 1 and Figure B 2, respectively (modelled using the ssdtools package with the model averaging method). The models were judged to provide a good fit to each dataset. Based on the use of only the preferred toxicity estimates, the number of species (17 species; classified as 'preferred') and the good fits of the distributions, the resulting protective concentrations (Table B 2) would have very high reliability.

Table B 1 Summary of preferred chronic toxicity data values for zinc in freshwater, normalised to the Australian and New Zealand reference conditions

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)
Chordata (fish)	*Oncorhynchus mykiss*	Juvenile	30	LC10 (mortality)	120	81
Arthropoda (crustacean)	*Ceriodaphnia dubia*	Neonates (< 24 hours old)	7	EC10 (reproduction)	44	36
	Daphnia magna	Neonates (< 48 hours old)	21	EC10 (reproduction)	47	39
	Daphnia thomsoni	Neonates (< 24 hours old)	21	EC10 (reproduction)	31	25
Arthropoda (insect)	*Rhithrogena hageni*	Nymph	10	LC10 (mortality)	2,200	1,800
Mollusca (mollusc)	*Alathyria profuga*	Larvae	3	NEC (mortality)	12	10
	Cucumerunio novaehollandiae	Larvae	3	NEC (mortality)	7.3	6.0
	Hyridella australis	Larvae	3	NEC (mortality)	7.6	6.2
	Hyridella depressa	Larvae	3	NEC (mortality)	8.7	7.2
	Hyridella drapeta	Larvae	3	NEC (mortality)	9.6	7.9
	Lampsilis siliquoidea	Juvenile (2 or 4 months old)	28	IC10 (growth and mortality)	34	28
	Lymnaea stagnalis	21 days old	28	EC10 (growth)	240	190
	Velesunio ambiguus	Larvae	3	NEC (mortality)	13	11

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)
Rotifera (rotifer)	*Brachionus calyciflorus*	< 2 hours old	2	EC10 (population growth rate)	110	94
Chlorophyta (green microalga)	*Chlorella* sp. (Papua New Guinea isolate)	Exponential growth phase	3	EC10 (population growth rate)	5.7	4.2
	Chlorella sp. (Kakadu isolate)	Exponential growth phase	3	EC10 (population growth rate)	840	610
	Raphidocelis subcapitata	Exponential growth phase	3	EC10 (population growth rate)	20	20

[a] Australian reference condition: pH 7.6, hardness 39 mg/L $CaCO_3$ and DOC 1.0 mg/L. Values reported to 2 significant figures.

[b] New Zealand reference condition: pH 7.5, hardness 28 mg/L $CaCO_3$ and DOC 0.7 mg/L. Values reported to 2 significant figures.

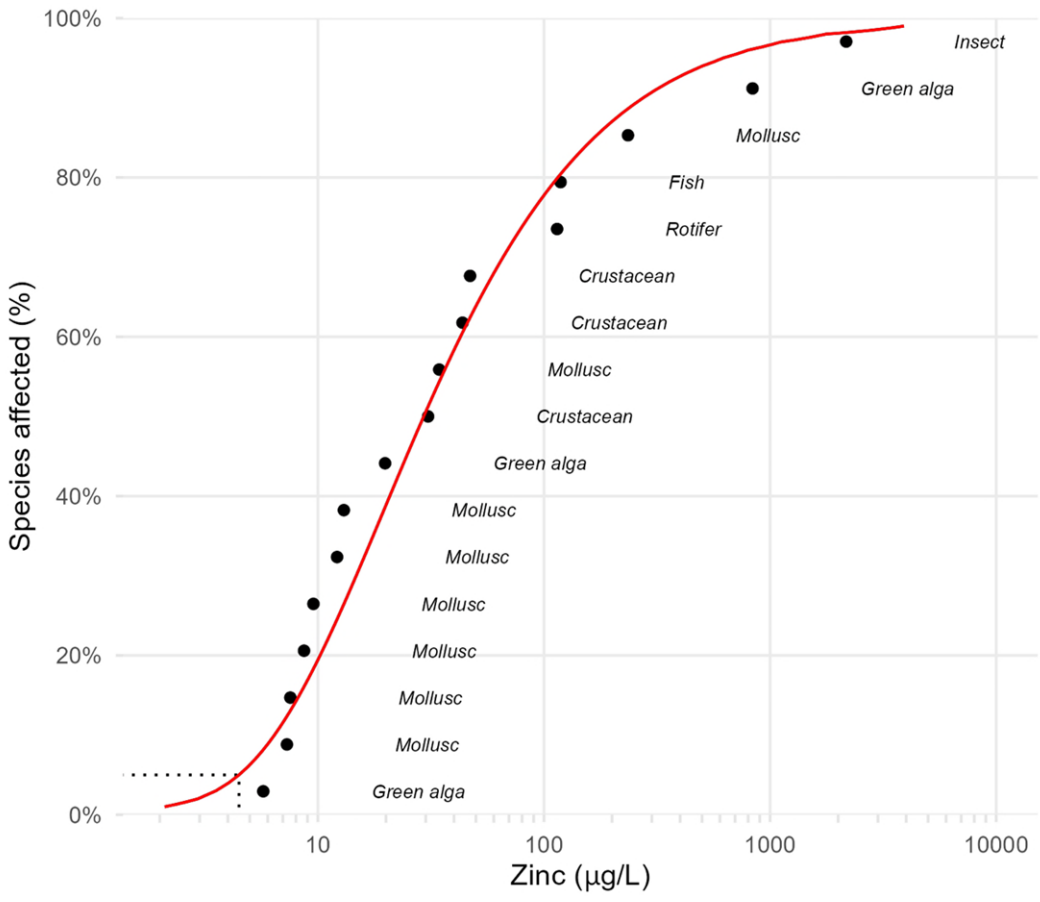


Figure B 1 Species sensitivity distribution for zinc in freshwater based on preferred toxicity estimates only (n = 17), normalised to the Australian reference condition of pH 7.6, hardness 39 mg/L $CaCO_3$ and DOC 1.0 mg/L.

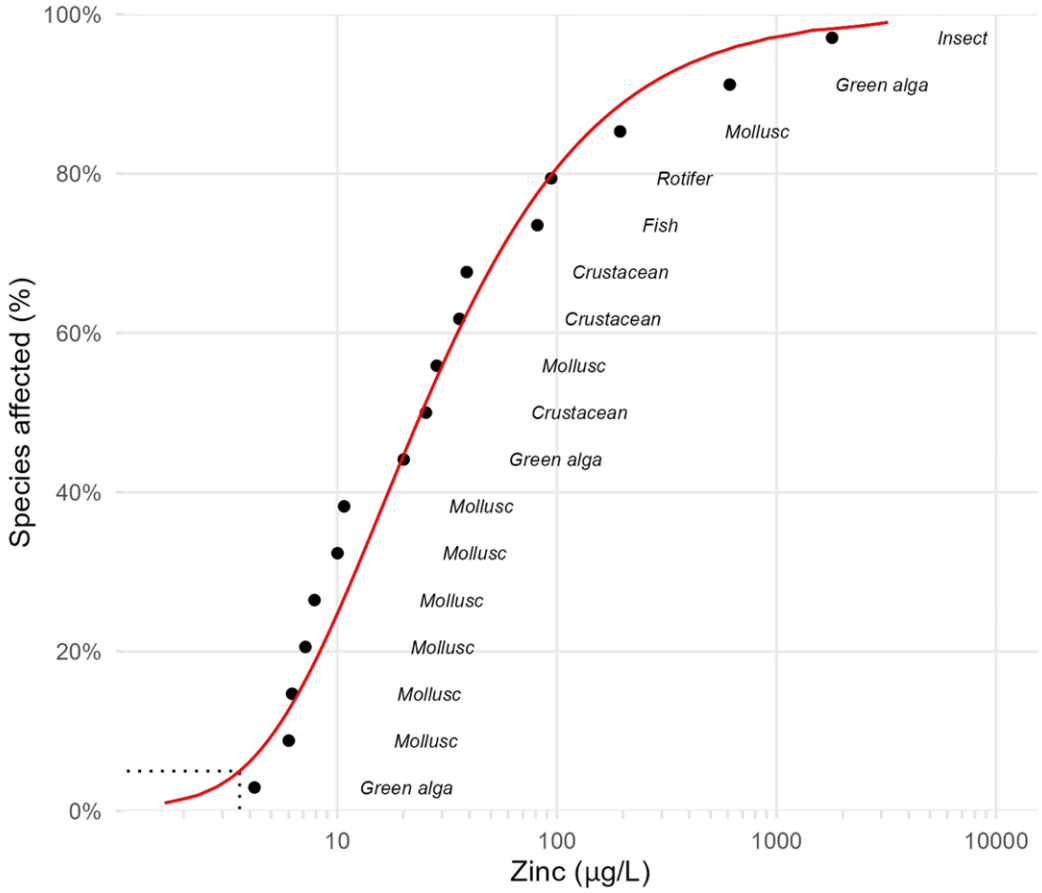


Figure B 2 Species sensitivity distribution for zinc in freshwater based on preferred toxicity estimates only (n = 17), normalised to the New Zealand reference condition of pH 7.5, hardness 28 mg/L $CaCO_3$ and DOC 0.7 mg/L.

However, inclusion of only preferred toxicity estimates excluded data for several species, including 1 mollusc native to New Zealand, for which only EC11–20 or NOEC data were available. It also included only 1 fish species. The protective concentrations derived from only the preferred data (Table D2) would be protective of the native mollusc species based on 95% level of protection or above, and protective of the other excluded species. Overall, the differences between the protective concentrations based on the datasets with only the preferred estimates (n = 17) (Table D2) and the preferred estimates plus the EC11-20 and NOEC values (n = 25) (Table 5) were minor and well within the uncertainty of the estimates. By including the EC11-20 and NOEC estimates, the number of species included in the SSD increased from 17 to 25, including an additional 3 fish species, which provided a better taxonomic representation and increased confidence in the DGVs. Consequently, the full dataset (n = 25) was selected to derive the DGVs.

Table B 2 Protective concentrations for dissolved zinc in Australian and New Zealand freshwaters using only the preferred toxicity estimates (n = 17) [a]

Level of species protection (%)	Protective concentration for zinc in freshwater in Australia (µg/L) [b]	Protective concentration for zinc in freshwater in New Zealand (µg/L) [c]
99	2.1	1.6
95	4.5	3.6
90	6.4	5.2
80	10	8.3

[a] Guideline values were derived using ssdtools with model averaging and have been rounded to 2 significant figures.

[b] Based on data normalised to Australian reference condition of pH 7.6, hardness 39 mg/L $CaCO_3$ and DOC 1.0 mg/L.

[c] Based on data normalised to New Zealand reference condition of pH 7.5, hardness 28 mg/L $CaCO_3$ and DOC 0.7 mg/L.

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 default guideline values for zinc in freshwater

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
Chordata (fish)	*Oncorhynchus mykiss*	Juvenile	30	Mortality	LC10	Synthetic water	29[c]	6.7[c]	0.3[d]	99			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	30[c]	7.5[c]	0.3[d]	38			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	28[c]	7.6[c]	0.3[d]	74			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	102[c]	7.6[c]	0.3[d]	171			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	29[c]	7.6[c]	0.3[d]	35			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	29[c]	7.7[c]	0.3[d]	46			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	396[c]	7.7[c]	0.3[d]	337			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	″	190[c]	7.9[c]	0.3[d]	290			De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	Natural water	104[c]	7.8[c]	23[c]	902			De Schamphelaere et al. (2005a)
		Juvenile	30	Mortality	LC10	″	176[c]	8.1[c]	6.2[c]	578			De Schamphelaere et al. (2005a)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Juvenile	30	Mortality	LC10	″	28[c]	6.8[c]	3.9[c]	185			De Schamphelaere et al. (2005a)
		Juvenile	30	Mortality	LC10	″	32[c]	7.1[c]	2.8[c]	219			De Schamphelaere et al. (2005a)
										153	**120**	**81**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)[e]**
	Pimephales promelas	Larvae (< 24 h old)	7	Mortality	NOEC	Lake Superior water	48[f]	7.6[g]	1.1[g]	85			Norberg and Mount (1985)
		Larvae	7	Mortality	NOEC	Lake Superior water	48[f]	7.6[g]	1.1[g]	173			Norberg-King (1989)
										129	100	69	Toxicity value calculated from mean of species sensitivity
		Embryo-Larvae	7	Mortality	NOEC	Lake Superior water	48[f]	7.6[g]	1.1[g]	129	108	75	Norberg-King (1989)
										129	**100**	**69**	**Values in bold used in SSDs (Lowest toxicity value)**
	Prosopium williamsoni	Larvae (fry)	30	Mortality	IC20	Dechlorinated Fort Collins tap water	48[c]	6.8[c]	1.9[c]	309			Brinkman and Vieira (2008)
										309	**219**	**151**	**Values in bold used in SSDs**
	Salmo trutta	Eyed egg	80	Growth (length and weight)	NOEC	dechlorinated Fort Collins tap water	54[c]	7.4[c]	1.4[h]	416			Brinkman and Woodling (2014)
		Eyed egg to hatching	3	Mortality	NOEC	″	54[c]	7.4[c]	1.4[h]	416			Brinkman and Woodling (2014)
		Eyed egg to post-hatch to yolk absorption	29	Mortality	NOEC	″	54[c]	7.4[c]	1.4[h]	416			Brinkman and Woodling (2014)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Eyed egg to surviving fry	80	Mortality	NOEC	″	54[c]	7.4[c]	1.4[h]	416			Brinkman and Woodling (2014)
										416	**289**	**199**	**Values in bold used in SSDs (Lowest toxicity value)**
Arthropoda (crustacean)	*Ceriodaphnia dubia*	Neonates (< 24 h old)	3 broods	Reproduction	EC10	Synthetic water	40[c]	7.5[c]	0.5[i]	47			Naddy et al. (2015)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Jingellic Ck, NSW	23[c]	7.7[c]	2.0[c]	83			Stauber et al. (2022)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Regge, The Netherlands (0.2 µm)	213[c]	8.3[c]	16[c]	73			Nys et al. (2017)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Markermeer, The Netherlands (0.2 µm)	226[c]	8.1[c]	9.5[c]	111			Nys et al. (2017)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Voyon, France (0.2 µm)	80[c]	8.0[c]	13[c]	64			Nys et al. (2017)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Brisy, Belgium (0.2 µm)	46[c]	7.2[c]	4.9[c]	89			Nys et al. (2017)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Brisy (added Ca), Belgium (0.2 µm)	103[c]	7.2[c]	4.8[c]	98			Nys et al. (2017)
		Neonates (< 24 h old)	7	Reproduction	EC10	Natural water, Brisy (pH adjusted), Belgium (0.2 µm)	46[c]	7.8[c]	4.7[c]	14			Nys et al. (2017)
										63	**44**	**36**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
	Ceriodaphnia reticulata	Neonates (< 6 h old)	7	Reproduc-tion	NOEC	Natural water (Lake Superior, USA)	376[c]	7.9[c]	1.1[g]	58			Carlson and Roush (1985)
		Neonates (< 6 h old)	7	Survival and reproduc-tion	NOEC	″	362[c]	7.7[c]	1.1[g]	140			Carlson and Roush (1985)
										99	**48**	**39**	**Values in bold used in SSDs (Lowest toxicity value)**
	Daphnia magna	Neonates	21	Reproduc-tion	EC10	Synthetic water	370[j]	8.0[j]	9.7[j]	90			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	8.5[j]	21[j]	634			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	370[j]	6.5[j]	32[j]	341			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	7.3[j]	21[j]	331			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	35[j]	7.3[j]	21[j]	328			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	7.3[j]	21[j]	502			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	6.0[j]	21[j]	423			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	7.3[j]	21[j]	394			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	7.3[j]	2.0[j]	179			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	370[j]	8.0[j]	32[j]	600			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	110[j]	8.0[j]	9.7[j]	233			Heijerick et al. (2003)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Neonates	21	Reproduc-tion	EC10	″	110[j]	6.5[j]	32[j]	313			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	240[j]	7.3[j]	40[j]	911			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	370[j]	6.5[j]	9.7[j]	114			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	110[j]	6.5[j]	9.7[j]	277			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	″	110[j]	8.0[j]	32[j]	557			Heijerick et al. (2003)
		Neonates	21	Reproduc-tion	EC10	Natural water, Voyon, France	122[c]	8.4[c]	4.2[c]	59			De Schamphelaere et al. (2005a)
		Neonates	21	Reproduc-tion	EC10	Natural water, Ankeveen, The Netherlands	122[c]	6.8[c]	17[c]	387			De Schamphelaere et al. (2005a)
		Neonates	21	Reproduc-tion	EC10	Natural water, Rhine, The Netherlands	196[c]	8.2[c]	2.3[c]	126			De Schamphelaere et al. (2005a)
		Neonates	21	Reproduc-tion	EC10	Natural water, Markermeer, The Netherlands	189[c]	8.0[c]	7.5[c]	171			De Schamphelaere et al. (2005a)
		Neonates	21	Reproduc-tion	EC10	Natural water, Brisy, Belgium	26[c]	7.3[c]	2.5[c]	93			De Schamphelaere et al. (2005a)
		Neonates	21	Reproduc-tion	EC10	Natural water, Regge, The Netherlands	183[c]	8.0[c]	9.9[c]	265			De Schamphelaere et al. (2005a)
		Neonates	21	Reproduc-tion	EC10	Synthetic water	250[c]	7.2[c]	0.3[c]	196			De Schamphelaere et al. (2005a)
		Neonates	14	Reproduc-tion	EC10	Synthetic water	250[k]	7.6[c]	4.0[c]	84			Muyssen and Janssen (2007)
		Neonates	21	Reproduc-tion	EC10	Synthetic water	250[k]	7.6[c]	4.0[c]	85			Muyssen and Janssen (2007)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Neonates	21	Reproduc-tion	EC10	Natural water La Dolaizon, France	91[c]	8.1[c]	4.3[c]	109			Van Regenmortel et al. (2017)
										234	**47**	**39**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
	Daphnia thomsoni	Neonates	21	Reproduc-tion	EC10	Natural water (filtered), Clutha, NZ	33[c]	8.1[c]	0.7[c]	55			Stauber et al. (2022)
		Neonates	21	Reproduc-tion	EC10	Natural water (filtered), Hoteo, NZ	61[c]	7.9[c]	6.6[c]	36			Stauber et al. (2022)
		Neonates	21	Reproduc-tion	EC10	Natural water (filtered), Mahurangi, NZ	90[c]	8.2[c]	4.0[c]	46			Stauber et al. (2022)
										45	**31**	**25**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
	Hyalella azteca	< 1 week old	42	Mortality	NOEC	Dechlorinated tap water (Burlington city)	130[c]	8.3[c]	1.1[l]	108			Borgmann et al. (1993)
		< 1 week old	70	Mortality	NOEC	″	130[c]	8.3[c]	1.1[l]	42			Borgmann et al. (1993)
										67	**62**	**51**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
Arthropoda (insect)	*Rhithrogena hageni*	Nymph	10	Mortality	LC10	Mix of onsite well water and reverse osmosis water	44[c]	7.8[c]	1[m]	2,069[n]			Brinkman and Johnston (2008)
										2,069	**2,171**	**1,790**	**Values in bold used in SSDs**
	Neocloeon triangulifer	< 24 h old	14	Growth	EC20	Natural water (1.5 µm), St Louis, USA	27[c]	6.8[c]	41[c]	55			Besser et al. (2021)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		< 24 h old	14	Growth	EC20	Natural water (1.5 µm), Spring, USA	323[c]	7.1[c]	5.0[c]	25			Besser et al. (2021)
										37	**10**	**8.4**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
Mollusca (mollusc)	*Alathyria profuga*	Larvae	3	Mortality	NEC	Reconstituted water	42[c]	7.0[c]	0.1[c]	14			Markich (2017)
										14	**12**	**10**	**Values in bold used in SSDs**
	Cucumerunio novaeholl-andiae	Larvae	3	Mortality	NEC	Reconstituted water	42[c]	7.0[c]	0.1[c]	8.4			Markich (2017)
										8.4	**7.3**	**6.0**	**Values in bold used in SSDs**
	Echyridella menziesii	Larvae	2	Mortality	EC20	Reconstituted water (20% dechlorinated Hamilton City tap water (1.2 µm filtered) and 80% nanopure water)	30[c]	7.8[c]	0.5°	155			Clearwater et al. (2014)
		Larvae	2	Mortality	EC20	″	30[c]	7.8[c]	0.5°	281			Clearwater et al. (2014)
		Larvae	2	Mortality	EC20	″	30[c]	7.8[c]	0.5°	56			Clearwater et al. (2014)
										135	**225**	**164**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
	Hyridella australis	Larvae	3	Mortality	NEC	Reconstituted water	42[c]	7.0[c]	0.1[c]	8.7			Markich (2017)
										8.7	**7.6**	**6.2**	**Values in bold used in SSDs**
	Hyridella depressa	Larvae	3	Mortality	NEC	Reconstituted water	42[c]	7.0[c]	0.1[c]	10			Markich (2017)
										10	**8.7**	**7.2**	**Values in bold used in SSDs**

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
	Hyridella drapeta	Larvae	3	Mortality	NEC	Reconstituted water	42[c]	7.0[c]	0.1[c]	11			Markich (2017)
										11	**9.6**	**7.9**	**Values in bold used in SSDs**
	Lampsilis siliquoidea	Juvenile (2 months old)	28	Growth	IC10	Reconstituted soft water	48[c]	8.0[c]	0.3[p]	55			Wang et al. (2010)
		Juvenile (4 months old)	28	Growth	IC10	″	49[c]	7.8[c]	0.3[p]	24[q]			Wang et al. (2010)
		Juvenile (2 months old)	28	Mortality	IC10	″	48[c]	8.0[c]	0.3[p]	127			Wang et al. (2010)
		Juvenile (4 months old)	28	Mortality	IC10	″	49[c]	7.8[c]	0.3[p]	125			Wang et al. (2010)
										24	**34**	**28**	**Values in bold used in SSDs (Lowest toxicity value)**
	Lymnaea stagnalis	21 days old	28	Growth	EC10	Natural water from Ankeveen, The Netherlands (filtered 0.45 µm)	256[c]	7.8[c]	13[c]	1,629			De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	Natural water from Markeveen, The Netherlands (filtered 0.45 µm)	225[c]	7.9[c]	7.8[c]	910			De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	Natural water from Voyon, France (filtered 0.45 µm)	38[c]	7.4[c]	2.9[c]	200			De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	Natural water from Brisy, Belgium (filtered 0.45 um µm	41[c]	6.8[c]	1.5[c]	244			De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	Natural water from Brisy, Belgium (filtered 0.45 µm)	40[c]	8.3[c]	1.7[c]	330			De Schamphelaere and Janssen (2010)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		21 days old	28	Growth	EC10	Natural water from Brisy, Belgium (filtered 0.45 µm)	296[c]	8.3[c]	1.5[c]	719			De Schamphelaere and Janssen (2010)
										508	**235**	**194**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
	Physa gyrina	Adult, juvenile	30	Mortality	NOEC	Well water	36[c]	6.9[c]	1.1[g]	570			Nebeker et al. (1986)
										570	**396**	**327**	**Values in bold used in SSDs**
	Velesunio ambiguus	Larvae	3	Mortality	NEC	Reconstituted water	42[c]	7.0[c]	0.1[c]	15			Markich (2017)
										15	**13**	**11**	**Values in bold used in SSDs**
Rotifera (rotifer)	*Brachionus calyciflorus*	< 2 h old	2	Population growth	EC10	Natural water, Ankeveen, The Netherlands (filtered 0.45 µm)	255[c]	7.8[c]	8.9[c]	550			De Schamphelaere and Janssen (2010)
		< 2 h old	2	Population growth	EC10	Natural water, Voyon, France (filtered 0.45 µm)	46[c]	7.4[c]	2.8[c]	197			De Schamphelaere and Janssen (2010)
		< 2 h old	2	Population growth	EC10	Natural water, Brisy, Belgium (filtered 0.45 µm);	47[c]	6.9[c]	1.2[c]	142			De Schamphelaere and Janssen (2010)
		< 2 h old	2	Population growth	EC10	Natural water, Brisy, Belgium (filtered 0.45 um)	42[c]	8.1[c]	1.7[c]	66			De Schamphelaere and Janssen (2010)
		< 2 h old	2	Population growth	EC10	Natural water, Brisy, Belgium (filtered 0.45 µm);	311[c]	8.2[c]	1.5[c]	453			De Schamphelaere and Janssen (2010)
										215	**114**	**94**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
Chlorophyta (green microalga)	*Chlorella* sp. (Papua New Guinea isolate)	Exponentially growing	3	Population growth[r]	EC10	Synthetic water	85	7.5	0.5[j]	28			Johnson et al. (2007)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	93[c]	6.7[c]	0.7[c]	4.5			Price et al. (2021)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	93[c]	7.1[c]	0.4[c]	1.8			Price et al. (2021)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	94[c]	7.7[c]	0.6[c]	0.8			Price et al. (2021)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	94[c]	8.0[c]	0.6[c]	4.1			Price et al. (2021)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	93[c]	8.3[c]	0.7[c]	3.2			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	5.0[c]	6.7[c]	0.5[s]	1.5			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	5.0[c]	7.6[c]	0.5[s]	1.8			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	5.0[c]	8.3[c]	0.5[s]	0.9			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	31[c]	6.7[c]	0.5[s]	3.3			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	31[c]	7.6[c]	0.5[s]	2.1			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	31[c]	8.3[c]	0.5[s]	1.3			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	402[c]	6.7[c]	0.5[s]	5.3			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	402[c]	7.6[c]	0.5[s]	4.4			Price et al. (2022)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	402[c]	8.3[c]	0.5[s]	3.9			Price et al. (2022)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water	90	7.7[c]	0.5[s]	1.6			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added natural DOM)	90	7.6[c]	2.5[c]	2.0			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added natural DOM)	90	7.6[c]	5.4[c]	3.5			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added natural DOM)	90	7.6[c]	10[c]	4.5			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added natural DOM)	90	7.6[c]	15[c]	6.1			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added natural DOM)	90	8.3[c]	5.5[c]	2.8			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added Suwanee River DOM)	90	7.85	2.5	6.8			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added Suwanee River DOM)	90	7.85	4.4	15			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Synthetic water (added Suwanee River DOM)	90	7.85	8.7	20			Price et al. (2023a)
		Exponentially growing	3	Population growth[r]	EC10	Natural water, Blackwood River AUS	355[c]	8.1[c]	4.2[c]	145			Price et al. (2023b)
		Exponentially growing	3	Population growth[r]	EC10	Natural water, Magela Creek AUS	3.0[c]	6.4[c]	6.0[c]	27			Price et al. (2023b)
		Exponentially growing	3	Population growth[r]	EC10	Natural water, Ovens River AUS	11[c]	7.5[c]	0.5[s]	6.6			Price et al. (2023b)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Exponentially growing	3	Population growth[r]	EC10	Natural water, Ovens River AUS (pH adjusted)	11[c]	8.0[c]	0.5[s]	6.3			Price et al. (2023b)
		Exponentially growing	3	Population growth[r]	EC10	Natural water, Woronora River AUS	18[c]	7.1[c]	5.3[c]	193			Price et al. (2023b)
										5.0	**5.7**	**4.2**	**Values in bold used in SSDs (Toxicity value calculated from mean of species sensitivity)**
	Chlorella sp. (Kakadu isolate)	Exponentially growing	3	Population growth[r]	EC10	Natural water, Magela Creek, AUS	3.5[c]	6.4[c]	1.4[c]	286			Trenfield et al. (2023)
										286	**837**	**612**	**Values in bold used in SSDs**
	Raphidocelis subcapitata	Exponentially growing	3	Population growth[t]	EC10	Natural water, Brisy, Belgium (filtered 0.45 µm)	27[c]	6.3[c]	2.5[c]	109			De Schamphelaere et al. (2005a)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Voyon, France	27[c]	6.4[c]	3.7[c]	79			De Schamphelaere et al. (2005a)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Ankeveen, The Netherlands	144[c]	7.4[c]	22[c]	136			De Schamphelaere et al. (2005a)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Markermeer, The Netherlands	239[c]	8.0[c]	5.9[c]	27			De Schamphelaere et al. (2005a)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Voyon France (filtered 0.45 µm)	75[c]	7.0[c]	9.5[c]	89			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Brisy, Belgium (filtered 0.45 µm)	45[c]	7.3[c]	12[c]	36			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Bihain, Belgium (filtered 0.45 µm)	22[c]	6.3[c]	4.1[c]	116			Van Regenmortel et al. (2017)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Voyon, France (filtered 0.45 µm)	71[c]	8.5[c]	9.0[c]	17			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Voyon, France (filtered 0.45 µm)	71[c]	8.5[c]	9.0[c]	14			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Bihain, Belgium (filtered 0.45 µm)	23[c]	6.2[c]	11[c]	89			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Brisy, Belgium (filtered 0.45 µm)	48[c]	7.1[c]	9.9[c]	51			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Brisy, Belgium (filtered 0.45 µm)	48[c]	7.2[c]	9.9[c]	55			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Loire, France (filtered 0.45 µm)	47[c]	8.3[c]	4.5[c]	6.0			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Loire, France (filtered 0.45 µm)	47[c]	8.3[c]	4.5[c]	10.0			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Loire, France (filtered 0.45 µm)	46[c]	8.5[c]	4.8[c]	16			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Loire, France (filtered 0.45 µm)	46[c]	8.5[c]	4.8[c]	16			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Bihain, Belgium (filtered 0.45 µm)	19[c]	6.2[c]	9.3[c]	80			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Bihain, Belgium (filtered 0.45 µm)	19[c]	6.2[c]	9.3[c]	131			Van Regenmortel et al. (2017)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Maddon, France (filtered 0.45 µm)	513[c]	8.3[c]	7.0[c]	34			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Taurion, France (filtered 0.45 µm)	14[c]	6.7[c]	2.3[c]	65			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Maulde, France (filtered 0.45 µm)	14[c]	6.7[c]	3.7[c]	68			Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth[t]	EC10	Natural water, Moselotte, France (filtered 0.45 µm)	19[c]	5.9[c]	2.9[c]	62			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Taurion, France (filtered 0.45 µm)	8.6[c]	6.1[c]	4.5[c]	22			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Maulde, France (filtered 0.45 µm)	8.9[c]	6.0[c]	4.4[c]	32			Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth[t]	EC10	Synthetic water (culture media)	18[c]	7.5[c]	<0.3[c]	21			Stauber et al. (2023)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Waihou River, NZ	39[c]	7.9[c]	<0.3[c]	6.3			Stauber et al. (2023)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Clutha River, NZ	50[c]	8.0[c]	<0.5[c]	7.7			Stauber et al. (2023)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Mahurangi River, NZ	110[c]	8.2[c]	2.4[c]	20			Stauber et al. (2023)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Okutua / Maihou mixture, NZ	26[c]	7.2[c]	7.3[c]	43			Stauber et al. (2023)
		Exponentially growing	3	Population growth[t]	EC10	Natural water, Hoteo, NZ	72[c]	7.6[c]	4.6[c]	6.9			Stauber et al. (2023)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Test media	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported toxicity value (µg/L Zn)	Toxicity value normalised to Australian reference condition [a] (µg/L)	Toxicity value normalised to NZ reference condition [b] (µg/L)	Reference
										33	**20**	**20**	**Values in bold used in SSDs (Toxicity values calculated from mean of species sensitivity)**

[a] The reference condition for Australia is a pH of 7.6, hardness 39 mg/L $CaCO_3$ and DOC of 1.0 mg/L. Values rounded to 2 or 3 significant figures.

[b] The reference condition for New Zealand is a pH of 7.5, hardness 28 mg/L $CaCO_3$ and DOC of 0.7 mg/L. Values rounded to 2 or 3 significant figures.

[c] Measured values from original study (or for hardness, calculated from measured calcium and magnesium concentrations) (for Markich (2017) DOC values are 'less than' values).

[d] Measured DOC generally below 1 mg/L with the max value of 1.4 mg/L (De Schamphelaere and Janssen, 2004). Deionised dilution water was ~0.3 mg/L (De Schamphelaere and Janssen, 2002).

[e] Data used in the species sensitivity distribution were selected following the selection rules in Warne et al. (2018) with the lowest toxicity value selected for each species. An arithmetic mean was calculated for the species sensitivity where there are multiple values at the same endpoint and duration. The non-bold value underneath the reported toxicity values represents the geometric mean of the reported toxicity values; this value was not used in the SSD as only MLR-adjusted values were used in the SSD.

[f] Quoted in original paper, but unclear if this is measured (no measurement methods quoted).

[g] Quoted in CCME (2018) Appendix (not in original study). DOC estimate is recommended DOC value for Lake Superior water as per US EPA (2007) AWQC for Copper, Appendix C. The pH estimate is the mean of other pH measurements in studies conducted in Lake Superior water from this database.

[h] Estimated value based on measured DOC reported in Brinkman and Johnston (2012) for the same test water.

[i] Assumed DOC is low as test media is synthetic water. Naddy et al. (2015) study reported approximate water quality composition with organic carbon of < 1 mg/L.

[j] Nominal concentration. Measured concentrations were within defined limits of nominal concentration.

[k] Calculated from data provided.

[l] Secondary DOC value used: Borgmann et al. (2005).

[m] Estimated based on water type, as per US EPA AWQC for Copper (US EPA 2007), Appendix C.

[n] EC/LC10 values calculated by CCME (2018). For *R. hageni*, raw data was provided by the author of the original study.

[o] Reported by authors at 2.5 mg/L, but measurement was affected by resin leaching from water purification system, and not expected to affect zinc bioavailability. Value of 0.5 mg/L used in DGV and BAGV adjustment, consistent with expectations for DOC in synthetic waters.

[p] CCME Appendix spreadsheet quotes a DOC of 0.3 mg/L "DOC estimate from author correspondence, based on measurement in similar test water".

[q] Less than value.

[r] Toxicity tests with *Chlorella* sp. were carried out with low nutrients (1.5 mg NO_3/L, 0.15 mg PO_4/L and 0.5 g/L MOPS buffer for PNG isolate, and 3.63 mg NO_3/L, 0.0345 mg PO_4/L and 1 mM HEPES buffer for Kakadu isolate) and an initial cell density of 10^3 cells/mL. The exception was for the *Chlorella* sp. PNG isolate reported in Johnson et al. (2007) that used higher concentrations of nitrate and phosphate (15 mg NO_3/L and 1.5 mg PO_4/L), no added buffer, and higher initial cell densities (10^4 cell/mL).

[s] Reported as < 1 mg/L, but 0.5 mg/L adopted based on other measurements by authors in synthetic waters.

[t] Toxicity tests with *R. subcapitata* were carried out with nutrients added to test media and with initial cell densities of 10^4 cells/mL.

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

The SSD software package ssdtools (V 2.4.0) uses a model averaging approach to generate a SSD (as described by Fox et al. 2021). Figure D 1 and Figure D 2 show the set of five distributions fitted to the zinc freshwater toxicity datasets normalised to the Australian and New Zealand reference conditions respectively, while Table D 1 provides the relevant goodness of fit and weighting estimates for each of the distributions. The log-normal/log-normal model was excluded from the default model set as it modelled dataset artefacts and had a marked influence on the DGVs (see Section 4.4 for details). For the Australian and New Zealand datasets, the Gamma and Weibull distributions were weighted near to zero for the model averaging, and hence had negligible influence on the DGVs.

The model-averaged SSDs for the Australian and New Zealand reference conditions are shown in Figure 2 and Figure 3 of the main report, based on the distributions, weighted as shown in Table D 1.

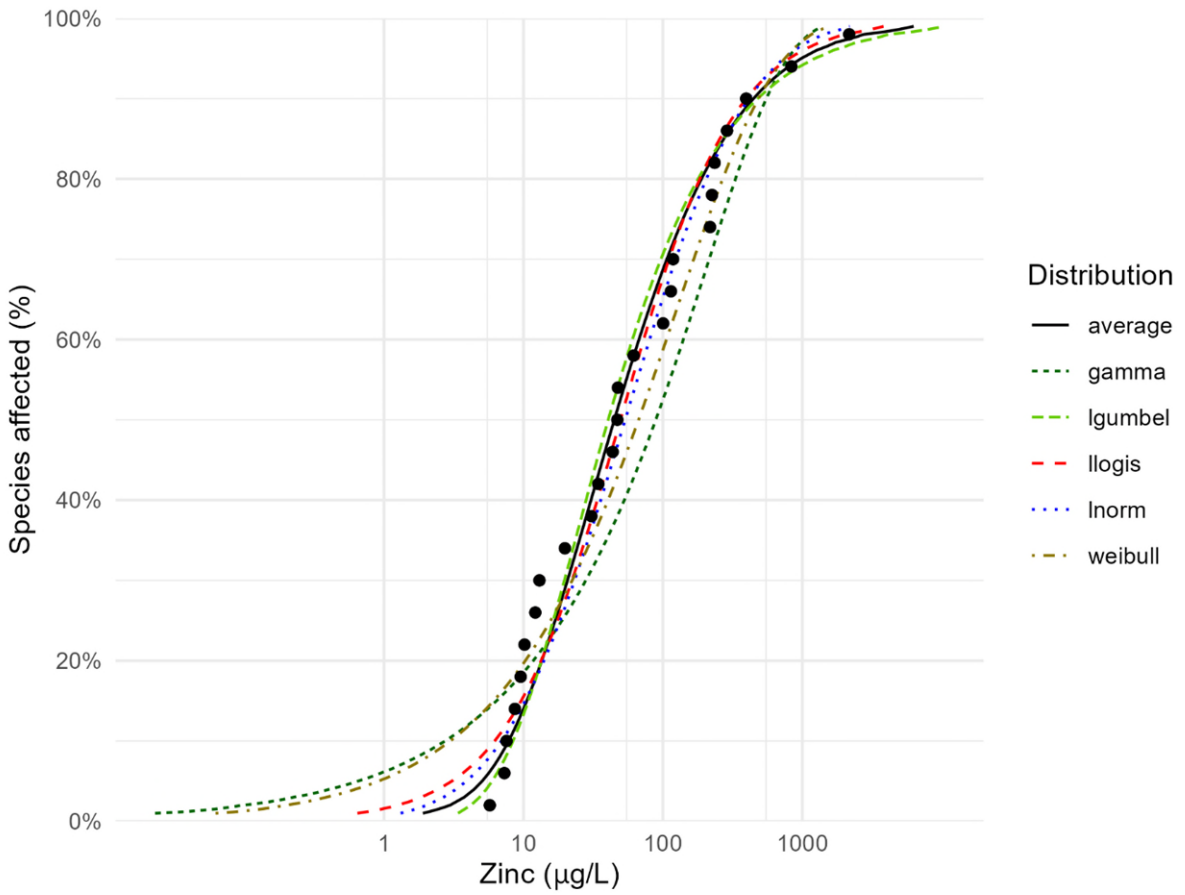


Figure D 1 SSDs for set of five distributions used to model the zinc freshwater toxicity dataset normalised to the Australian reference condition.

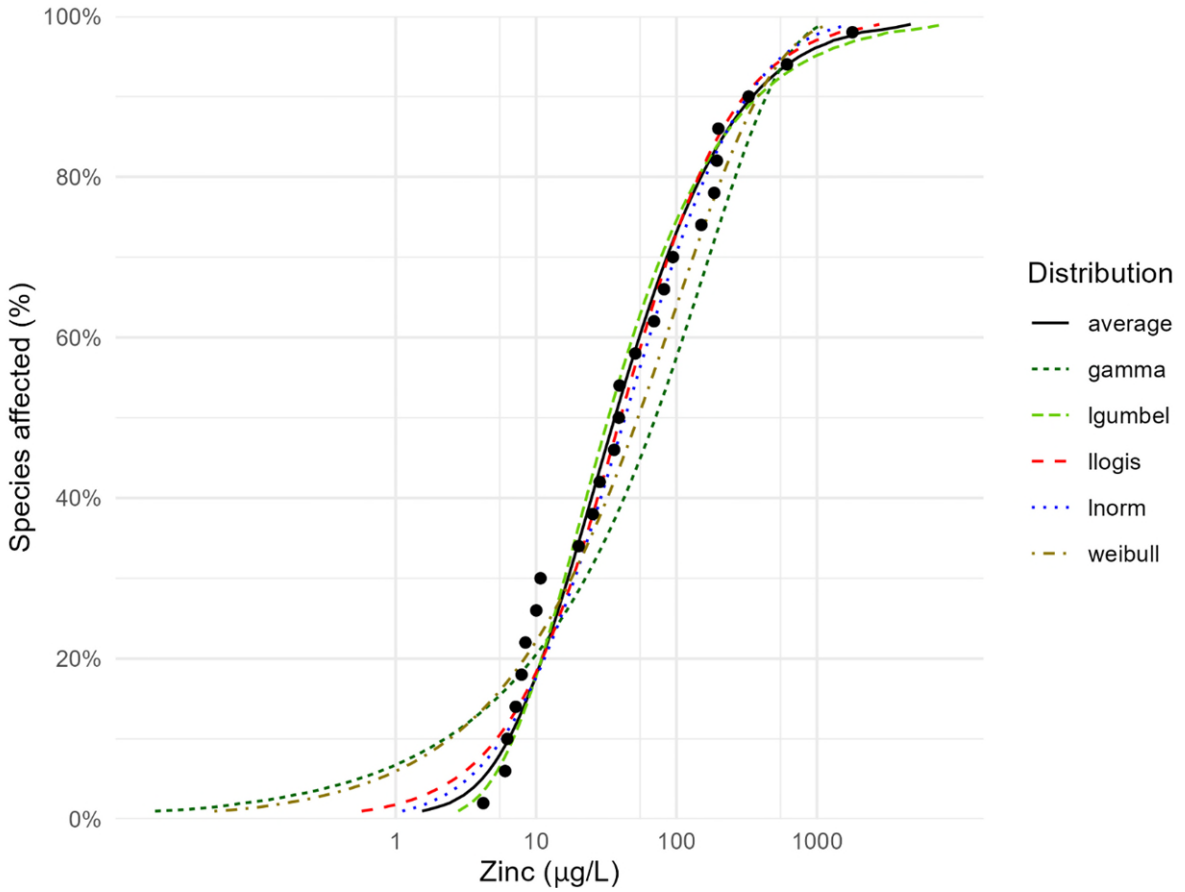


Figure D 2 SSDs for set of five distributions used to model the zinc freshwater toxicity dataset normalised to the New Zealand reference condition.

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

Distribution	Akaike's Information Criterion (AIC)	Corrected Akaike's Information Criterion (AICc)	Delta	Weight
Toxicity dataset normalised to the Australian reference condition				
Log gumbel	296	296	0	0.609
Log normal	297	298	1.74	0.255
Log logistic	299	299	3.25	0.12
Weibull	303	304	7.55	0.014
Gamma	307	308	11.6	0.002
Toxicity dataset normalised to the New Zealand reference condition				
Log gumbel	284	284	0	0.581
Log normal	285	286	1.56	0.266
Log logistic	287	287	2.87	0.139
Weibull	292	292	7.65	0.013
Gamma	296	296	11.9	0.002

Appendix E: Examples of zinc bioavailability-based guideline values (BAGVs) for differing pH, hardness and dissolved organic carbon concentrations

Table E 1 Bioavailability-adjusted guideline values (BAGVs) for dissolved zinc (µg/L) for protection of 99% of species at varying pH, hardness (mg/L $CaCO_3$) and DOC (mg/L)

DOC	pH	Hardness							
		30	50	90	120	180	240	300	370
0.5	**6.0**	3.7	4.8	5.9	6.6	7.6	8.3	8.9	9.5
	6.5	2.7	3.3	4.2	4.6	5.3	5.7	6.1	6.5
	7.0	1.9	2.4	2.9	3.2	3.6	3.9	4.2	4.4
	7.5	1.4	1.7	2.0	2.2	2.5	2.7	2.9	3.0
	8.0	1.0	1.2	1.4	1.5	1.7	1.9	2.0	2.1
	8.3	0.8	0.9	1.1	1.2	1.4	1.5	1.6	1.7
2.0	**6.0**	4.8	5.9	7.0	7.6	8.6	9.4	10	11
	6.5	3.8	4.6	5.6	6.1	6.9	7.5	7.9	8.4
	7.0	3.1	3.7	4.5	4.9	5.5	5.9	6.3	6.6
	7.5	2.5	3.0	3.5	3.8	4.3	4.6	4.8	5.1
	8.0	2.0	2.3	2.8	3.0	3.3	3.5	3.7	3.8
	8.3	1.7	2.0	2.3	2.5	2.8	2.9	3.1	3.2
5.0	**6.0**	5.4	6.4	7.4	8.1	9.1	9.8	10	11
	6.5	4.8	5.6	6.7	7.3	8.2	8.8	9.4	9.9
	7.0	4.2	5.0	6.0	6.5	7.2	7.7	8.1	8.5
	7.5	3.6	4.3	5.1	5.5	6.1	6.5	6.8	7.0
	8.0	3.0	3.6	4.2	4.4	4.8	5.1	5.3	5.4
	8.3	2.7	3.1	3.6	3.8	4.0	4.1	4.2	4.3
15.0	**6.0**	5.8	6.8	7.8	8.4	9.4	10	11	11
	6.5	6.1	7.0	8.3	8.9	9.9	11	11	12
	7.0	6.1	7.0	8.4	9.0	9.9	11	11	12
	7.5	5.6	6.6	7.8	8.4	9.1	9.6	9.9	10
	8.0	4.9	5.7	6.6	6.9	7.4	7.4	7.5	7.7
	8.3	4.3	5.0	5.7	5.9	6.0	6.1	6.1	6.2

Table E 2 Bioavailability-adjusted guideline values (BAGVs) for dissolved zinc (µg/L) for protection of 95% of species at varying pH, hardness (mg/L $CaCO_3$) and DOC (mg/L)

DOC	pH	Hardness							
		30	**50**	**90**	**120**	**180**	**240**	**300**	**370**
0.5	**6.0**	9.5	12	15	16	19	21	22	24
	6.5	7.0	8.6	11	12	13	15	16	17
	7.0	5.1	6.2	7.6	8.3	9.5	10	11	12
	7.5	3.7	4.4	5.4	5.9	6.6	7.2	7.7	8.2
	8.0	2.7	3.1	3.8	4.1	4.7	5.1	5.4	5.7
	8.3	2.2	2.6	3.1	3.3	3.8	4.1	4.3	4.6
2.0	**6.0**	11	14	17	19	21	23	25	26
	6.5	9.4	11	14	15	17	19	20	21
	7.0	7.8	9.4	11	12	14	15	16	17
	7.5	6.4	7.6	9.2	10	11	12	13	14
	8.0	5.2	6.2	7.4	8.1	9.1	9.8	10	11
	8.3	4.6	5.4	6.5	7.0	7.9	8.5	9.0	9.4
5.0	**6.0**	13	15	18	20	22	24	26	28
	6.5	11	14	17	18	20	22	24	25
	7.0	10	12	15	16	18	20	21	22
	7.5	9.1	11	13	14	16	17	18	19
	8.0	8.0	9.5	11	12	14	15	16	16
	8.3	7.3	8.7	10	11	12	13	14	15
15.0	**6.0**	14	16	19	21	24	26	28	29
	6.5	14	17	20	22	25	27	29	30
	7.0	14	17	20	22	25	27	29	30
	7.5	14	17	20	22	24	26	28	29
	8.0	13	16	19	20	22	24	25	26
	8.3	12	15	17	19	20	22	23	24

Table E 3 Bioavailability-adjusted guideline values (BAGVs) for dissolved zinc (µg/L) for protection of 90% of species at varying pH, hardness (mg/L $CaCO_3$) and DOC (mg/L)

DOC	pH	Hardness							
		30	**50**	**90**	**120**	**180**	**240**	**300**	**370**
0.5	**6.0**	14	18	22	25	29	32	34	36
	6.5	11	13	16	18	21	23	24	26
	7.0	7.9	9.5	12	13	15	16	17	18
	7.5	5.7	6.9	8.4	9.2	10	11	12	13
	8.0	4.1	4.9	5.9	6.5	7.4	8.0	8.5	9.1
	8.3	3.4	4.0	4.8	5.3	6.0	6.5	6.9	7.3
2.0	**6.0**	17	21	26	28	32	35	38	40
	6.5	14	17	21	23	27	29	31	33
	7.0	12	14	17	19	22	24	25	27
	7.5	9.8	12	14	16	18	19	21	22
	8.0	8.1	9.6	12	13	14	16	17	18
	8.3	7.2	8.5	10	11	13	14	15	15
5.0	**6.0**	19	23	27	30	34	38	40	43
	6.5	17	21	25	28	31	34	37	39
	7.0	16	19	23	25	28	31	33	35
	7.5	14	17	20	22	25	27	29	31
	8.0	12	15	18	20	22	24	25	27
	8.3	12	14	16	18	20	22	23	24
15.0	**6.0**	21	24	29	32	37	40	43	46
	6.5	21	25	31	33	38	41	44	47
	7.0	21	26	31	34	38	42	45	47
	7.5	21	25	31	34	38	41	44	46
	8.0	20	24	30	32	36	39	41	44
	8.3	20	24	28	31	34	37	39	41

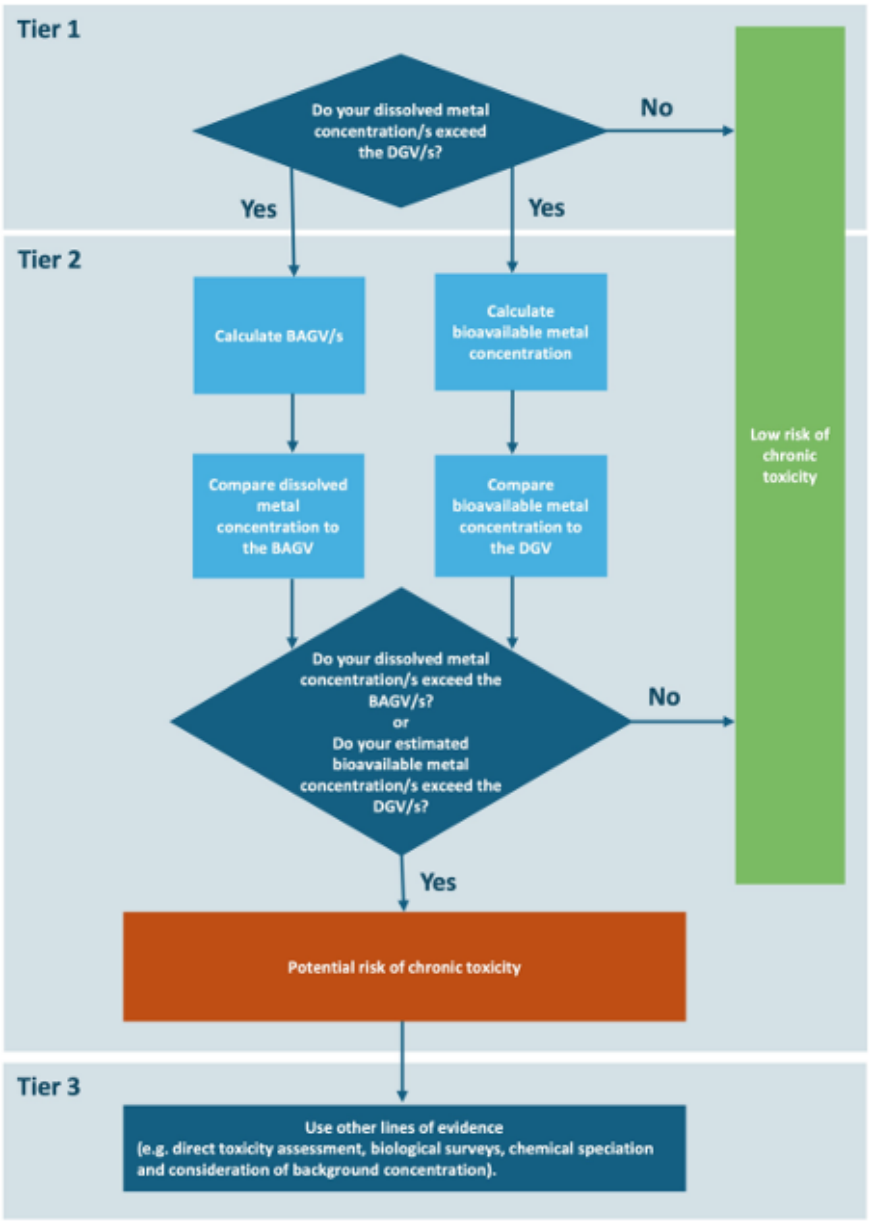
Table E 4 Bioavailability-adjusted guideline values (BAGVs) for dissolved zinc (µg/L) for protection of 80% of species at varying pH, hardness (mg/L $CaCO_3$) and DOC (mg/L)

DOC	pH	Hardness							
		30	**50**	**90**	**120**	**180**	**240**	**300**	**370**
0.5	**6.0**	25	30	38	42	49	54	58	62
	6.5	18	22	28	31	35	39	42	45
	7.0	14	16	20	22	26	28	30	32
	7.5	10	12	15	16	18	20	22	23
	8.0	7.3	8.6	11	12	13	14	15	17
	8.3	6.0	7.1	8.6	9.5	11	12	13	13
2.0	**6.0**	29	35	43	48	55	60	65	70
	6.5	24	29	36	40	45	50	54	58
	7.0	20	24	30	33	38	41	45	48
	7.5	17	20	25	27	31	34	37	39
	8.0	14	17	20	22	26	28	30	32
	8.3	13	15	18	20	23	25	27	28
5.0	**6.0**	31	38	46	51	59	65	70	75
	6.5	29	34	42	47	54	59	63	68
	7.0	26	31	39	43	49	54	58	62
	7.5	24	29	35	39	44	48	52	55
	8.0	22	26	31	35	39	43	46	49
	8.3	20	24	29	32	37	40	43	46
15.0	**6.0**	34	41	50	56	64	70	75	80
	6.5	35	42	52	57	65	72	77	82
	7.0	36	43	53	58	66	73	78	84
	7.5	36	43	53	58	66	73	78	84
	8.0	36	43	52	58	66	72	77	83
	8.3	35	43	52	57	65	72	77	82

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 zinc 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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