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Toxicant default guideline values for aquatic ecosystem protection

Zinc in freshwater

Technical brief

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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 (www.waterquality.gov.au/anz-guidelines).

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) DGV for zinc was 8 µg/L Zn. This DGV applied to 95% species protection in freshwaters at a hardness of 30 mg/L of calcium carbonate ($CaCO_3$) and was based on chronic toxicity data for 19 species from 5 taxonomic groups. 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 level and biotic ligand models (BLMs). These can be used to derive bioavailability-based DGVs 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 DOC on the toxicity of zinc.

Very high reliability DGVs for zinc in freshwater were derived from chronic (long-term) toxicity data for 31 species, comprising one amphibian, 6 fish, 6 crustaceans, 2 insects, 12 molluscs, one rotifer and 3 green microalgae. Appendix A lists all chronic toxicity data used in the derivation. DGVs for 99%, 95%, 90% and 80% species protection are provided for waters of different pH (6.5–8.1), hardness (23–370 mg/L $CaCO_3$) and DOC (0.5–15 mg/L) values. The DGVs for 99%, 95%, 90% and 80% species protection at the index water-quality condition (pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC; representative of conditions where zinc would be highly bioavailable) are 1.5 µg/L, 4.1 µg/L, 6.8 µg/L and 12 µg/L, respectively. The 95% species-protection level DGV should be used when assessing ecosystems that are slightly disturbed to moderately disturbed. Where ambient water data for pH, hardness (Ca and Mg) or DOC are not available, the value for the index condition should be used in the interim. The DGVs for zinc reported here supersede the ANZECC and ARMCANZ (2000) DGVs for zinc in freshwater.

1 Introduction

Zinc 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 ppm (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 oxidised 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 (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) DGVs 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 $CaCO_3$.

These DGVs were considered high reliability, derived using the species sensitivity distribution (SSD) method based on 85 values from 21 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 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 revised Australian and New Zealand DGVs for zinc in freshwater that supersede the ANZECC and ARMCANZ (2000) DGVs. The revision incorporates data published since 2000, including chronic data for Australasian species. The hardness correction applied to the ANZECC and ARMCANZ (2000) DGVs has been replaced with 3 MLR bioavailability models at the species level and trophic level, developed to account for the influence of pH, hardness and DOC on the toxicity of zinc. The DGV derivation process and the data used are described in section 4.

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, toxicities ranged from 140 µg/L to 9,600 µg/L (Bacher and O'Brien 1990; Skidmore and Firth 1983). 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 1, resulting 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 EC10s of 19–228 µg/L (all data normalised to pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC; references in Appendix A). Data for rainbow trout (*Oncorhynchus mykiss*) ranged from 63 µg/L (LC10, 30-day juvenile survival; De Schamphelaere and Janssen 2004) to 228 µg/L (LC10, 72-day embryo survival; Cairns et al. 1982), depending on the life stage, effect and endpoint. Based on the species' geometric mean, *Cottus bairdi* was the most sensitive fish, with an EC10 of 19 µg/L from a 30-day test on juvenile survival (Brinkman and Woodling 2005). An EC10 of 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). This equates to an EC10 of 77 µg/L when normalised to the index condition of pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC. However, the hardness and pH values in the test waters were outside of the range for the models used to normalise the data to the index condition (pH > 6.5 and hardness > 23 mg/L $CaCO_3$; see section 3).

3 Factors affecting toxicity

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 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 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, below).

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).

Of the factors that affect zinc toxicity, the influence of 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 occurs in algae, invertebrates and fish following either acute or chronic exposures, although there are far more data documenting this relationship for acute 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 (*Salmo 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, 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 effect of hardness was 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:

- 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 (decreasing toxicity at low pH).

Many studies conducted with varying pH and no ligands (where artificial or filtered water was used) have shown the expected increasing toxicity with increasing pH, including studies on 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. 2005b; Schubauer-Berigan et al. 1993) and 2 green algal species, *R. subcapitata* (De Schamphelaere et al. 2005a) and *Chlorella* sp. (Price et al. 2021; Wilde et al. 2006). However, in other studies, the reverse or no effect has been found (Heijerick et al. 2003, 2005). In natural waters or waters with ligands added (such as dissolved organic matter), the effect of pH is less clear. For *C. dubia*, an increase in toxicity of only 1.7-fold was found with an increase in pH of 3 units (Belanger and Cherry 1990). In contrast, Heijerick et al. (2003) did not find a clear pattern between pH and zinc toxicity.

Alkalinity (usually due to carbonate) affects zinc toxicity by reducing the concentration of Zn^{2+} in water via the formation of zinc carbonate complexes. In general, studies have shown lower toxicity at higher alkalinity. 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, 2 showed that alkalinity had no influence on acute zinc 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.

Dissolved organic matter, typically referred to as DOC as it contains ~50% carbon by mass (Duarte et al. 2016), affects zinc toxicity primarily through formation of zinc complexes which 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. 2005a; Oikari et al. 1992; Paulauskis and Winner 1988) and fathead minnow larvae (Bringolf et al. 2006). DOC also reduces toxicity in chronic tests as shown for *Daphnia* sp. (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 algae *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 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.

3.1 Accounting for toxicity modifying factors

The inverse relationship between water hardness and toxicity was the basis of a hardness function in the US EPA ambient water-quality criterion from 1984 to 2007 (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 zinc guideline value 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. 2001) and its more recent 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 model developed by Santore et al. (2002). A unified version based on the average across BLMs for individual species and studies has also been used to develop water-quality criteria for zinc (DeForest and Van Genderen 2012; Van Sprang et al. 2009) but 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 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 predicted no-effect concentration (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 was used by Environment Canada in developing zinc (and, more recently, copper) guidelines for freshwater (CCME 2018). In developing their zinc MLR, Environment Canada reviewed the toxicity modifying factors (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 biomass). 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 guideline value 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.

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. Toxicity in New Zealand waters ranged < 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 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 full in Gadd et al. (in prep.) 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, including those used by CCME (2018) for the Canadian zinc guideline value. Based on the assessment and as detailed in Gadd et al. (in prep.), a suite of 4 MLR models (Table 1) was selected for adjusting the ecotoxicity dataset as described in section 4.2.

Table 1 Summary of bioavailability models used for deriving the guideline values for zinc in freshwater

		Coefficients for toxicity modifying factors				Range for toxicity modifying factors			
Taxonomic group (phylum or clade)	**Model species**	**pH**	**Hardness**	**Dissolved organic carbon**	**Interaction term (from the multiple linear regression)**	**pH**	**Hardness**	**Dissolved organic carbon**	**Reference**
Chordata (fish)	*Oncorhynchus mykiss*	-0.815	0.947	0.398	No interaction	6.5–8.13	23–399	0.3–23	CCME (2018)
Arthropoda (crustacean)	*Daphnia magna*	-0.52	0.31	-1.4	0.24 (dissolved organic carbon × pH)	6–8.5	26–370	0.3–40	Gadd et al. (in prep.)
Chlorophyta (green microalgae)									
Chlorella sp.	*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)
Other microalgae	*Raphidocelis subcapitata*	-0.865	0	0.209	No interaction	5.6–8.5	7–529	0.3–22	DeForest et al. (2023)

4 Default guideline value derivation

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

4.1 Collation and screening of toxicity data

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 2023) 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 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 could not be included in the DGV derivation. Studies that did not report DOC were included in the derivation as it was assumed that most standard laboratory synthetic test waters had low DOC (for the purpose of the derivation, this was assumed to be 0.5 mg/L). Furthermore, studies were not included if conducted in test waters where either hardness, pH or DOC were outside the boundaries of the MLR model used for that species (see Table 1), with some margins applied. As all TMF measurements are subject to variability, and TMFs vary during toxicity testing, a margin of error was allowed when determining whether data could be included (Table 2).

Table 2 Boundaries applied to each multiple linear regression model in selecting toxicity data to include in the derivation of the default guideline values

TMF	Lower boundary	Upper boundary	Justification
pH	Within 0.2 pH units	Within 0.2 pH units	Based on allowable variation in toxicity tests (e.g. US EPA 2002)
Hardness	Within 5 mg/L $CaCO_3$	Within 120% of the model boundary	Based on likely accuracy of hardness test methods at lower end and allowing for some flexibility at the upper end
Dissolved organic carbon	within 1 mg/L	Within 120% of the model boundary	Based on likely accuracy of test methods for dissolved organic carbon at lower end and allowing for some flexibility at the upper end

The above data-exclusion rules resulted in some chronic data for Australasian species not being included in the derivation (Table 3). 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 boundaries 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 21 and 18 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.

Table 3 Summary of chronic toxicity values for Australasian species that were excluded from the derivation of the default guideline values for zinc in freshwater

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

NR = not reported.

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.

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 were included.

There were 300 chronic toxicity values for 31 species that were suitable quality for use in the DGV derivation and within the range of the MLR models. Of these, approximately half (145 values for 23 species) were of the most preferred type of toxicity estimates (i.e. NECs, ECx/ICx/LCx values where x ≤ 10, and bounded-effect concentrations [BECs] where the effect is ≤ 10%, as per Warne et al. 2018). Six values were NECs and 139 were EC/IC/LC10s. Less preferred toxicity estimates included one LC1, 31 EC/LC11–20s and 123 NOEC/NOELs from 21 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 22 species from 6 taxonomic groups. However, to increase the number and diversity of species represented in the dataset used for the DGV derivation, the preferred data were supplemented with EC10–20 and NOEC data. This resulted in data for 31 species, including 12 species native to Australia and New Zealand from 7 taxonomic groups. There was an increase of only 1.2–1.3-fold in the protective concentrations (i.e. 80%, 90%, 95%, 99% species protection) when using the 31 species dataset compared to the 22 species data set (see Appendix B). Thus, the larger dataset with greater species diversity was selected to derive the DGVs.

The trophic-level-specific MLRs from Table 1 were used to predict negligible-effect (i.e. EC10/NOEC) values for each of the 31 species at an index water-chemistry condition. The index condition is a specific combination of water-chemistry parameters, representing environmentally realistic conditions of high metal bioavailability. The index condition for Australia and New Zealand was agreed to by a panel of experts to be: pH 7.5, 6 mg/L Ca and 4 mg/L Mg (i.e. hardness of approximately 30 mg/L $CaCO_3$) and 0.5 mg/L DOC (Stauber et al. 2021). The trophic-level-specific MLRs were applied to the toxicity data for the 31 species based on their taxonomic group, as described in Table 1. The predicted negligible-effect values for the index condition were then summarised to single-species values for use in the SSD, by either calculating geometric means or selecting the toxicity value associated with the most sensitive endpoint, life stage and toxicity test duration for each species, based on Warne et al. (2018).

A summary of the toxicity data (one value per species, at the index condition) used to calculate the DGVs for zinc in freshwater is provided in Table 4Table 4. The 31 species included in the SSD were from 7 taxonomic groups: one amphibian, 6 fish, 6 crustaceans, 2 insects, 12 molluscs, one rotifer and 3 green microalgae. The toxicity values in the SSD ranged over more than 3 orders of magnitude, from 0.91 µg/L for *Chlorella* sp. (green alga; Papua New Guinea isolate) to 5,530 µg/L for *Faxonius virilis* (reclassified from *Orconectes virilis* [freshwater crayfish]). Notably, the *Chlorella* sp. (Papua New Guinea isolate) value represents a geometric mean from 28 normalised EC10 values ranging from 0.4 µg/L to 103 µg/L from 3-day population growth rate tests (Price et al. 2021; Price et al. 2022). Further details of the water-quality parameters for each single-species value used to calculate the DGVs are presented in Appendix A. Details of the data-quality assessment and the data that passed the quality assessment are provided as supporting information.

Table 4 Summary of single chronic toxicity values for all species used to derive default guideline values for zinc in freshwater; toxicity values normalised to index water chemistry of pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Normalised toxicity value (µg/L Zn)
Chordata (amphibian)	*Bufo boreas*	Larvae	28	NOEC (development)	75
Chordata (fish)	*Cottus bairdii*	Recently hatched	30	EC10 (mortality)	19
	Oncorhynchus clarkii	Larvae (fry)	30	EC20 (mortality)	181
	Oncorhynchus mykiss	Juvenile	30	LC10 (mortality)	63
	Pimephales promelas	Larval (< 24 hours old)	7	IC10 (growth)	43
	Prosopium williamsoni	Eyed egg to fry	90	IC10 (growth)	82
	Salmo trutta	Embryo/larval	58	NOEC (growth)	57
Arthropoda (crustacean)	*Ceriodaphnia dubia*	Neonates (< 24 hours old)	7	EC10 (reproduction)	16
	Ceriodaphnia reticulata	Neonates (< 24 hours old)	7	NOEC (survival and reproduction)	50
	Daphnia magna	< 48 hrs old	21	EC10 (reproduction)	42
	Daphnia thomsoni	Neonates (< 24 hours old)	21	EC10 (reproduction)	22
	Hyalella azteca	< 1 week old	70	NOEC (mortality)	45
	Orconectes virilis	Adult	14	LC10 (mortality)	5,530
Arthropoda (insect)	*Neocloeon triangulifer*	Neonates (< 24 hours old)	14	EC20 (reproduction)	7.3
	Rhithrogena hageni	Nymph	10	EC10 (development)	2,200
Mollusca (mollusc)	*Alathyria profuga*	Larvae	3	NEC (development)	14
	Cucumerunio novaehollandiae	Larvae	3	NEC (development)	8.4

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Normalised toxicity value (µg/L Zn)
	Dreissena polymorpha	Adult/juvenile	70	LC10 (mortality)	95
	Echyridella menziesii	Larvae	2	EC20 (mortality)	76
	Hyridella australis	Larvae	3	NEC (development)	8.7
	Hyridella depressa	Larvae	3	NEC (development)	10
	Hyridella drapeta	Larvae	3	NEC (development)	11
	Lampsilis siliquoidea	Juvenile (2 months old)	28	IC10 (growth)	40
	Lymnaea stagnalis	21 days old	28	EC10 (growth)	171
	Physa gyrina	Adult/juvenile	30	NOEC (mortality)	357
	Potamopyrgus antipodarum	Juvenile	77–112	NOEC (growth)	17
	Velesunio ambiguus	Larvae	3	NEC (development)	15
Rotifera (rotifer)	*Brachionus calyciflorus*	< 2 hours old	2	EC10 (population growth rate)	83
Chlorophyta (green microalga)	*Chlorella* sp. (Papua New Guinea isolate)	Exponential growth phase	3	EC10 (population growth rate)	0.91
	Chlorella sp. (Kakadu isolate)	Exponential growth phase	3	EC10 (population growth rate)	570
	Raphidocelis subcapitata	Exponential growth phase	3	EC10 (population growth rate)	17

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). A visual assessment of the final toxicity dataset (31 species) suggested potential bimodality, with a break in the data between 22 µg/L and 41 µg/L (Figure 1). However, this break was less than a 2-fold difference, and the 5 taxonomic groups represented in the lower subset of values were also represented in the upper subset of values. Although there is a cluster of sensitive molluscs with normalised EC10 values of 8–17 µg/L, there were also some of lower sensitivity, with EC10 values from 40 µg/L to 360 µg/L. Other taxonomic groups were more evenly spread throughout the SSD (Figure 1). The bimodality coefficient value for the log-transformed dataset was 0.31, which is less than the indicative threshold criterion for bimodality of 0.55. Therefore, the dataset was deemed to be unimodal, and all the toxicity data (i.e. from 31 species) were used for the derivation.

4.3 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution based on the 31 chronic toxicity data for zinc in freshwater (Table 4) is presented in Figure 1. The SSD was plotted using Burrlioz 2.0 software. The fit of the model was good.

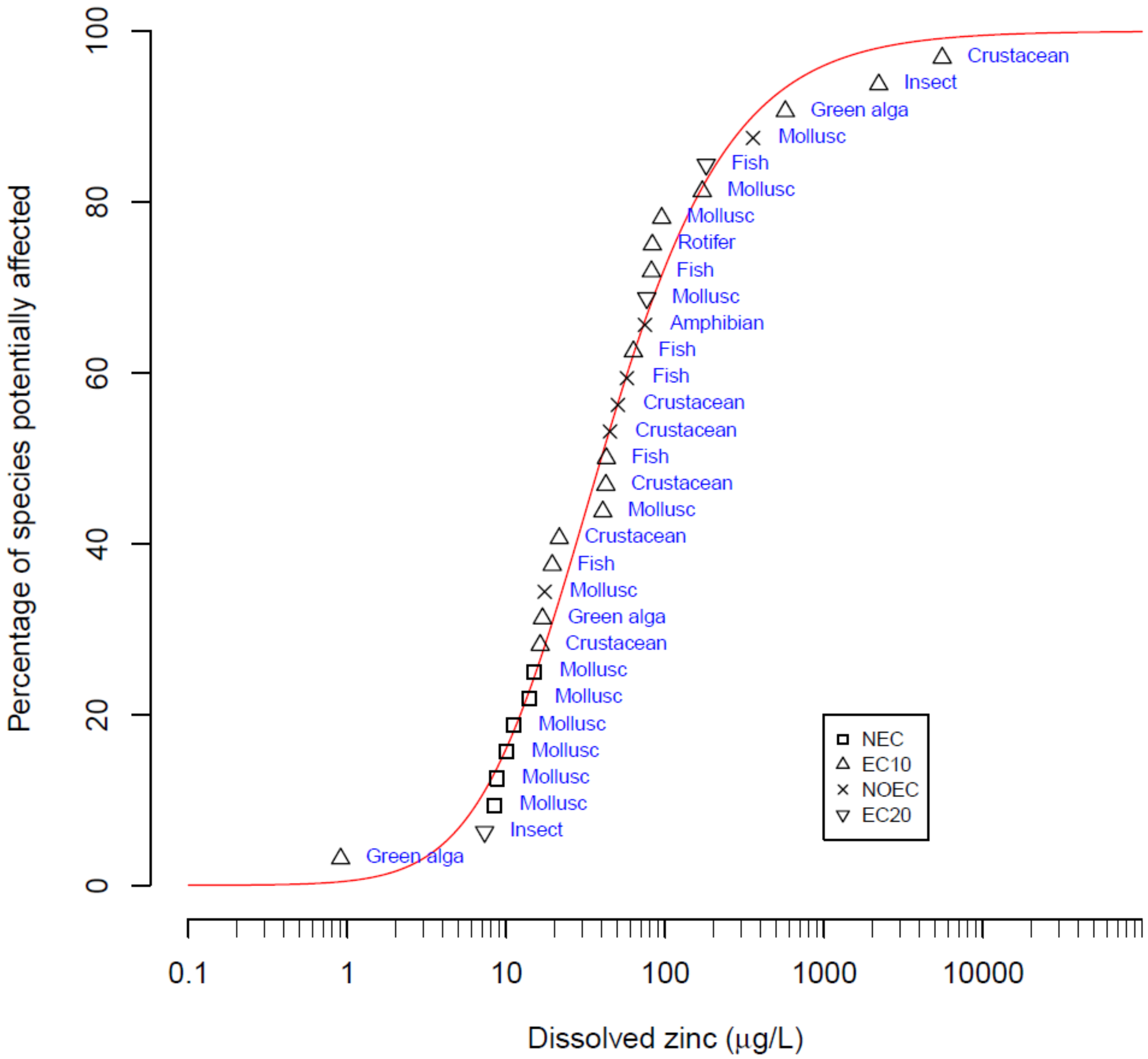

Figure 1 Species sensitivity distribution for zinc in freshwater, normalised to the index condition of pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC

4.4 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).

With the DGVs being adjustable based on the pH, hardness (Ca and Mg) and DOC values of ambient waters, the zinc freshwater DGVs represent a range of values over wide ranges of these water-quality parameters. The DGVs for 99%, 95%, 90% and 80% species protection at the index water-quality condition are listed in Table 5, with DGVs for waters of different pH, hardness and DOC listed in Appendix C. The DGVs apply to the < 0.45 µm-filtered fraction of zinc for waters with pH of 6.2–8.3, hardness of 20–440 mg/L $CaCO_3$ and ≤ 0.5–15 mg/L DOC. These are the ranges within which the MLR models used have been derived.

The 95% species-protection level DGV should be used when assessing ecosystems that are slightly disturbed to moderately disturbed. These DGVs supersede the ANZECC and ARMCANZ (2000) DGVs for zinc in freshwater. For freshwaters where pH, hardness or DOC are consistently outside the ranges above, the MLR models may not be suitable and, therefore, the DGVs may not be reliable. Site-specific guideline values may need to be derived. This may include modelling of metal speciation or toxicity testing in site-specific waters.

Table 5 Toxicant default guideline values (DGVs) for zinc in freshwater at the index condition (pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC), with very high reliability

Level of species protection (%)	DGV for zinc in freshwater (µg/L)[a]
99	1.5
95	4.1
90	6.8
80	12

[a] Default guideline values were derived using Burrlioz 2.0 software and based on data normalised to a pH of 7.5, hardness of 30 mg/L $CaCO_3$ and 0.5 mg/L DOC using trophic-level multiple linear regression models. All DGVs have been rounded to 2 significant figures.

4.5 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 – 31 species from 7 taxonomic groups (preferred)
- type of toxicity data – chronic
- SSD model fit – good (Burr Type III model).

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.
BEC	Bounded-effect concentration
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'.
DOC	Dissolved organic carbon
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.
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.

Term	Definition
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.
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: toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table A1 Summary of chronic toxicity data used to derive the default guideline values for zinc in freshwater[a]

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
Chordata (amphibian)	*Bufos boreas*	Larvae	28	Development	NOEC	57	7.2	0.5	172	75	Davies and Brinkman (1999)
		Larvae	42	Development	NOEC	57	7.2	0.5	172	75	Davies and Brinkman (1999)
		Larvae	14	Growth	NOEC	57	7.2	0.5	172	75	Davies and Brinkman (1999)
		Larvae	14	Mortality	NOEC	57	7.2	0.5	404	175	Davies and Brinkman (1999)
		Larvae	28	Mortality	NOEC	57	7.2	0.5	404	175	Davies and Brinkman (1999)
		Larvae	42	Mortality	NOEC	57	7.2	0.5	404	175	Davies and Brinkman (1999)
										75	**Lowest value used in species sensitivity distribution**
Chordata (fish)	*Cottus bairdi*	Recently emerged	30	Mortality	EC10	154	7.5	1.9	156	19	Brinkman and Woodling (2005)
		Recently emerged	30	Mortality	NOEC	154	7.5	1.9	172	21	Brinkman and Woodling (2005)
										19	**Lowest value used in species sensitivity distribution**
	Oncorhynchus clarkii	Larvae (fry)	30	Mortality	EC20	31	7.2	0.5	129	181	Brinkman and Hansen (2004)
		Larvae (fry)	30	Mortality	EC20	149	7.5	0.5	1,515	181	Brinkman and Hansen (2004)
										181	**Lowest value used in species sensitivity distribution**
	Oncorhynchus mykiss	Embryo	72	Mortality	LC10	25	7.0	1.6	458	228	Cairns et al. (1982)
		Juvenile	30	Mortality	LC10	29	6.7	0.3	99	63	De Schamphelaere and Janssen (2004)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
		Juvenile	30	Mortality	LC10	30	7.5	0.3	38	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	28	7.6	0.3	74	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	102	7.6	0.3	171	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	29	7.6	0.3	35	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	29	7.7	0.3	46	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	396	7.7	0.3	337	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	190	7.9	0.3	290	63	De Schamphelaere and Janssen (2004)
		Juvenile	30	Mortality	LC10	104	7.8	23	902	63	De Schamphelaere et al. (2005a)
		Juvenile	30	Mortality	LC10	176	8.1	6.2	578	63	De Schamphelaere et al. (2005a)
		Juvenile	30	Mortality	LC10	28	6.8	3.9	185	63	De Schamphelaere et al. (2005a)
		Juvenile	30	Mortality	LC10	32	7.1	2.8	219	63	De Schamphelaere et al. (2005a)
		Eyed egg	69	Growth	EC10	20	6.8	0.5	199	197	Mebane et al. (2008)
		Eyed egg	69	Growth	EC10	20	6.8	0.5	300	197	Mebane et al. (2008)
		Eyed egg	69	Mortality	EC10	20	6.8	0.5	88	71	Mebane et al. (2008)
										63	**Value used in species sensitivity distribution (geometric mean of LC10s)**
	Pimephales promelas	Larval (< 24 hours old)	7	Growth	IC10	48	7.6	1.1	84	43	Norberg and Mount (1985)
										43	**Value used in species sensitivity distribution**
	Prosopium williamsoni	Eyed egg to fry	90	Growth	IC10	48	6.8	1.9	380	82	Brinkman and Vieira (2008)
										82	**Value used in species sensitivity distribution**
	Salmo trutta	Eyed egg	80	Growth	NOEC	54	7.4	1.4	416	146	Brinkman and Woodling (2014)
		Eyed egg		Mortality	NOEC	54	7.4	1.4	416	146	Brinkman and Woodling (2014)
		Eyed egg		Mortality	NOEC	54	7.4	1.4	416	146	Brinkman and Woodling (2014)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
		Eyed egg		Mortality	NOEC	54	7.4	1.4	416	146	Brinkman and Woodling (2014)
		Embryo	58	Growth	NOEC	48	7.6	1.9	141	57	Davies et al. (2002)
		Embryo	58	Mortality	NOEC	48	7.6	1.9	1,090	444	Davies et al. (2002)
										57	**Lowest value used in species sensitivity distribution**
Arthropoda (crustacean)	*Ceriodaphnia dubia*	Neonates (< 24 hours old)	3 broods	Survival and reproduction	EC10	40	7.5	0.5	47	43	Naddy et al. (2015)
		Neonates (< 24 hours old)	7	Reproduction	EC10	23	7.7	2.0	83	16	Stauber et al. (2022)
		Neonates (< 24 hours old)	7	Reproduction	EC10	213	8.3	16	73	16	Nys et al. (2017)
		Neonates (< 24 hours old)	7	Reproduction	EC10	226	8.1	9.5	111	16	Nys et al. (2017)
		Neonates (< 24 hours old)	7	Reproduction	EC10	80	8.0	13	64	16	Nys et al. (2017)
		Neonates (< 24 hours old)	7	Reproduction	EC10	46	7.2	4.9	89	16	Nys et al. (2017)
		Neonates (< 24 hours old)	7	Reproduction	EC10	103	7.2	4.8	98	16	Nys et al. (2017)
		Neonates (< 24 hours old)	7	Reproduction	EC10	46	7.8	4.7	14	16	Nys et al. (2017)
										16	**Value used in species sensitivity distribution (geometric mean of EC10s)**
	Ceriodaphnia reticulata	Neonates (< 24 hours old)	7	Survival and reproduction	NOEC	376	7.9	0.5	58	50	Carlson and Roush (1985)
		Neonates (< 24 hours old)	7	Survival and reproduction	NOEC	362	7.7	0.5	140	50	Carlson and Roush (1985)
										50	**Lowest value used in species sensitivity distribution**
	Daphnia magna	< 48 hrs old	21	Reproduction	IC10	65	7.7	0.5	68	61	Munzinger and Monicelli (1991)
		Neonates	21	Reproduction	EC10	370	8.0	9.7	90	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	8.5	21	634	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	370	6.5	32	341	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	7.3	21	331	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	35	7.3	21	328	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	7.3	21	502	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	6.0	21	423	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	7.3	21	394	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	7.3	2.0	179	42	Heijerick et al. (2003)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
		Neonates	21	Reproduction	EC10	370	8.0	32	600	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	110	8.0	9.7	233	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	110	6.5	32	313	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	240	7.3	40	911	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	370	6.5	9.7	114	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	110	6.5	9.7	277	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	110	8.0	32	557	42	Heijerick et al. (2003)
		Neonates	21	Reproduction	EC10	122	8.4	4.2	59	42	De Schamphelaere et al. (2005a)
		Neonates	21	Reproduction	EC10	122	6.8	17	387	42	De Schamphelaere et al. (2005a)
		Neonates	21	Reproduction	EC10	196	8.2	2.3	126	42	De Schamphelaere et al. (2005a)
		Neonates	21	Reproduction	EC10	189	8.0	7.5	171	42	De Schamphelaere et al. (2005a)
		Neonates	21	Reproduction	EC10	26	7.3	2.5	93	42	De Schamphelaere et al. (2005a)
		Neonates	21	Reproduction	EC10	183	8.0	9.9	265	42	De Schamphelaere et al. (2005a)
		Neonates	21	Reproduction	EC10	250	7.2	0.3	196	42	De Schamphelaere et al. (2005a)
		Neonates	14	Reproduction	EC10	250	7.6	4.0	84	42	Muyssen and Janssen (2007)
		Neonates	21	Reproduction	EC10	250	7.6	4.0	85	42	Muyssen and Janssen (2007)
		Neonates	21	Reproduction	EC10	91	8.1	4.3	109	42	Van Regenmortel et al. (2017)
										42	**Value used in species sensitivity distribution (geometric mean of EC10s)**
	Daphnia thomsoni	Neonates	21	Reproduction	EC10	33	8.1	0.7	55	22	Stauber et al. (2022)
		Neonates	21	Reproduction	EC10	61	7.9	6.6	36	22	Stauber et al. (2022)
		Neonates	21	Reproduction	EC10	90	8.2	4.0	46	22	Stauber et al. (2022)
										22	**Value used in species sensitivity distribution (geometric mean of EC10s)**
	Hyalella azteca	< 1 week old	42	Mortality	NOEC	130	8.3	0.5	108	115	Borgmann et al. (1993)
		< 1 week old	70	Mortality	NOEC	130	8.3	0.5	42	45	Borgmann et al. (1993)
										45	**Lowest value used in species sensitivity distribution**
	Orconectes virilis	Adult	14	Mortality	LC10	26	7.1	1.6	9,920	5,533	Borgmann et al. (1993)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
										5,533	**Value used in species sensitivity distribution**
Arthropoda (insect)	*Rhithrogena hageni*	Nymph	10	Development	EC10	44	7.8	0.5	2,069	2,205	Brinkman and Johnston (2008)
										2,205	**Value used in species sensitivity distribution**
	Neocloeon triangulifer	< 24 hours old	14	Growth	EC20	27	6.8	41	55	7	Besser et al. (2021)
		< 24 hours old	14	Growth	EC20	323	7.1	5.0	25	7	Besser et al. (2021)
										7	**Value used in species sensitivity distribution (geometric mean of EC20s)**
Mollusca (mollusc)	*Alathyria profuga*	Larvae	3	Mortality	NEC	42	7.0	0.1	14	14	Markich (2017)
										14	**Value used in species sensitivity distribution**
	Cucumerunio novaehollandiae	Larvae	3	Mortality	NEC	42	7.0	0.1	8.4	8.4	Markich (2017)
										8.4	**Value used in species sensitivity distribution**
	Dreissena polymorpha	Adult/juvenile	70	Mortality	LC10	268	7.9	6.7	517	95	Kraak et al. 1994
										95	**Value used in species sensitivity distribution**
	Echyridella menziesii	Larvae	2	Mortality	EC20	30	7.8	2.5	155	76	Clearwater et al. (2014)
		Larvae	2	Mortality	EC20	30	7.9	2.5	281	76	Clearwater et al. (2014)
		Larvae	2	Mortality	EC20	30	7.8	2.5	56	76	Clearwater et al. (2014)
										76	**Value used in species sensitivity distribution (geometric mean of EC20s)**
	Hyridella australis	Larvae	3	Mortality	NEC	42	7.0	0.1	8.7	8.7	Markich (2017)
										8.7	**Value used in species sensitivity distribution**
	Hyridella depressa	Larvae	3	Mortality	NEC	42	7.0	0.1	10	10	Markich (2017)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
										10	**Value used in species sensitivity distribution**
	Hyridella drapeta	Larvae	3	Mortality	NEC	42	7.0	0.1	11	11	Markich (2017)
										11	**Value used in species sensitivity distribution**
	Lampsilis siliquoidea	Juvenile (2 months old)	28	Growth	IC10	48	8.0	0.5	55	40	Wang et al. (2010)
		Juvenile (2 months old)	28	Growth	IC10	49	7.8	0.5	24	40	Wang et al. (2010)
		Juvenile (2 months old)	28	Mortality	IC10	48	8.0	0.5	127	155	Wang et al. (2010)
		Juvenile (4 months old)	28	Mortality	IC10	49	7.8	0.5	125	132	Wang et al. (2010)
										40	**Value used in species sensitivity distribution (geometric mean of IC10s for growth)**
	Lymnaea stagnalis	21 days old	28	Growth	EC10	256	7.8	13	1,629	171	De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	225	7.9	7.8	910	171	De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	38	7.4	2.9	200	171	De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	41	6.8	1.5	244	171	De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	40	8.3	1.7	330	171	De Schamphelaere and Janssen (2010)
		21 days old	28	Growth	EC10	296	8.3	1.5	719	171	De Schamphelaere and Janssen (2010)
										171	**Value used in species sensitivity distribution (geometric mean of EC10s)**
	Physa gyrina	Adult/juvenile	30	Mortality	NOEC	36	6.9	0.5	570	357	Nebeker et al. (1986)
										357	**Value used in species sensitivity distribution**
	Potamopyrgus antipodarum	Juvenile	77–112	Growth	NOEC	238	8.0	4.3	72	17	Dorgelo et al. (1995)
										17	**Value used in species sensitivity distribution**

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
	Velesunio ambiguus	Larvae	3	Mortality	NEC	42	7.0	0.1	15	15	Markich (2017)
										15	**Value used in species sensitivity distribution**
Rotifera (rotifer)	*Brachionus calyciflorus*	< 2 hours old	2	Population growth	EC10	255	7.8	8.9	550	83	De Schamphelaere and Janssen (2010)
		< 2 hours old	2	Population growth	EC10	46	7.4	2.8	197	83	De Schamphelaere and Janssen (2010)
		< 2 hours old	2	Population growth	EC10	47	6.9	1.2	142	83	De Schamphelaere and Janssen (2010)
		< 2 hours old	2	Population growth	EC10	42	8.1	1.7	66	83	De Schamphelaere and Janssen (2010)
		< 2 hours old	2	Population growth	EC10	311	8.2	1.5	453	83	De Schamphelaere and Janssen (2010)
										83	**Value used in species sensitivity distribution (geometric mean of EC10s)**
Chlorophyta (green microalga)	*Chlorella* sp. (Papua New Guinea isolate)	Exponentially growing	3	Population growth	EC10	85	7.5	0.5	28	1	Johnson et al. (2007)
		Exponentially growing	3	Population growth	EC10	93	6.7	0.7	4.5	0.9	Price et al. (2021)
		Exponentially growing	3	Population growth	EC10	93	7.1	0.4	1.8	0.9	Price et al. (2021)
		Exponentially growing	3	Population growth	EC10	94	7.7	0.6	0.8	0.9	Price et al. (2021)
		Exponentially growing	3	Population growth	EC10	94	8.0	0.6	4.1	0.9	Price et al. (2021)
		Exponentially growing	3	Population growth	EC10	93	8.3	0.7	3.2	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	5.0	6.7	0.5	1.5	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	5.0	7.6	0.5	1.8	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	5.0	8.3	0.5	0.9	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	31	6.7	0.5	3.3	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	31	7.6	0.5	2.1	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	31	8.3	0.5	1.3	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	402	6.7	0.5	5.3	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	402	7.6	0.5	4.4	0.9	Price et al. (2022)
		Exponentially growing	3	Population growth	EC10	402	8.3	0.5	3.9	0.9	Price et al. (2022)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
		Exponentially growing	3	Population growth	EC10	90	7.7	0.5	1.6	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	2.5	2.0	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	5.4	3.5	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	10	4.5	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	15	6.1	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	6.7	5.5	2.7	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	8.3	5.5	2.8	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	2.0	1.8	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	4.6	2.3	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	8.8	2.9	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	7.6	13	3.4	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	6.7	4.9	2.2	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	90	8.3	4.9	2.0	0.9	Price et al. (2023a)
		Exponentially growing	3	Population growth	EC10	355	8.1	4.2	145	0.9	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	3.0	6.4	6.0	27	0.9	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	11	7.5	0.5	6.6	0.9	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	11	8.0	0.5	6.3	0.9	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	18	7.1	5.3	193	0.9	Stauber et al. (2022)
										0.9	**Value used in species sensitivity distribution (geometric mean of EC10s)**
	Chlorella sp. (Kakadu isolate)	Exponentially growing	3	Population growth	EC10	3.5	6.4	1.4	286	570	Trenfield et al. (2023)
										570	**Value used in species sensitivity distribution**
	Raphidocelis subcapitata	Exponentially growing	3	Population growth	EC10	27	6.3	2.5	109	17	De Schamphelaere et al. (2005a)
		Exponentially growing	3	Population growth	EC10	27	6.4	3.7	79	17	De Schamphelaere et al. (2005a)
		Exponentially growing	3	Population growth	EC10	144	7.4	22	136	17	De Schamphelaere et al. (2005a)
		Exponentially growing	3	Population growth	EC10	239	8.0	5.9	27	17	De Schamphelaere et al. (2005a)
		Exponentially growing	2	Population growth	EC10	75	7.0	9.5	89	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	45	7.3	12	36	17	Van Regenmortel et al. (2017)

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Test endpoint	Toxicity measure	Water hardness (mg/L $CaCO_3$)	pH	Dissolved organic carbon (mg/L)	Reported zinc concentration (µg/L Zn)	Normalised zinc concentration (µg/L Zn)	Reference
		Exponentially growing	2	Population growth	EC10	22	6.3	4.1	116	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	71	8.5	9.0	17	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	71	8.5	9.0	14	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	23	6.2	11	89	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	48	7.1	9.9	51	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	48	7.2	9.9	55	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	47	8.3	4.5	6.0	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	47	8.3	4.5	10.0	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	46	8.5	4.8	16	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	46	8.5	4.8	16	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	19	6.2	9.3	80	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	19	6.2	9.3	131	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	513	8.3	7.0	34	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	14	6.7	2.3	65	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	14	6.7	3.7	68	17	Van Regenmortel et al. (2017)
		Exponentially growing	2	Population growth	EC10	19	5.9	2.9	62	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	8.6	6.1	4.5	22	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	8.9	6.0	4.4	32	17	Van Regenmortel et al. (2017)
		Exponentially growing	3	Population growth	EC10	18	7.4	0.5	21	17	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	39	7.9	0.5	6.3	17	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	50	8.0	0.4	7.7	17	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	110	8.2	2.4	20	17	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	26	7.2	7.3	43	17	Stauber et al. (2022)
		Exponentially growing	3	Population growth	EC10	72	7.6	4.6	6.9	17	Stauber et al. (2022)
										17	**Value used in species sensitivity distribution (geometric mean of EC10s)**

[a] Data used in the species sensitivity distribution were selected following the selection rules in Warne et al (2018) – i.e. a geometric mean is calculated where there are multiple values at the same endpoint and duration, and the lowest toxicity value is selected for each species.

Appendix B: derivation based on preferred toxicity estimates only

Chronic data using the preferred toxicity estimates of EC/IC/LCx, NEC, BEC10 and EC/IC/LC15–20 are summarised in Table B1. The SSD based on these data is shown in Figure B1. The fit of the Burr III distribution to these data was good. Based on the use of chronic data, the number of species included (22 species; classified as 'preferred') and the good fit of the distribution, guideline values based on these data aloneTable B2 (Table B2) would have very high reliability.

Table B1 Summary of preferred chronic toxicity data values for zinc in freshwater, normalised to the index condition of pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC).

Taxonomic group (phylum or clade)	Species	Life stage	Duration (days)	Toxicity measure (test endpoint)	Normalised toxicity value (µg/L Zn)
Chordata (fish)	*Cottus bairdii*	Recently hatched	30	EC10 (mortality)	19
	Oncorhynchus mykiss	Juvenile	30	LC10 (mortality)	63
	Pimephales promelas	Larval (< 24 hours old)	7	IC10 (growth)	43
	Prosopium williamsoni	Eyed egg to fry	90	IC10 (growth)	82
Arthropoda (crustacean)	*Ceriodaphnia dubia*	Neonates (< 24 hours old)	7	EC10 (reproduction)	16
	Daphnia magna	< 48 hours old	21	EC10 (reproduction)	42
	Daphnia thomsoni	Neonates (< 24 hours old)	21	EC10 (reproduction)	22
	Orconectes virilis	Adult	14	LC10 (mortality)	5,530
Arthropoda (insect)	*Rhithrogena hageni*	Nymph	10	EC10 (development)	2,200
Mollusca (mollusc)	*Alathyria profuga*	Larvae	3	NEC (development)	14
	Cucumerunio novaehollandiae	Larvae	3	NEC (development)	8.4
	Dreissena polymorpha	Adult/juvenile	70	LC10 (mortality)	95
	Hyridella australis	Larvae	3	NEC (development)	8.7
	Hyridella depressa	Larvae	3	NEC (development)	10
	Hyridella drapeta	Larvae	3	NEC (development)	11
	Lampsilis siliquoidea	Juvenile (2 months old)	28	IC10 (growth)	40
	Lymnaea stagnalis	21 days old	28	EC10 (growth)	171
	Velesunio ambiguus	Larvae	3	NEC (development)	15
Rotifera (rotifer)	*Brachionus calyciflorus*	< 2 hours old	2	EC10 (population growth rate)	83
Chlorophyta (green microalga)	*Chlorella* sp. (Papua New Guinea isolate)	Exponential growth phase	3	EC10 (population growth rate)	0.91
	Chlorella sp. (Kakadu isolate)	Exponential growth phase	3	EC10 (population growth rate)	570
	Raphidocelis subcapitata	Exponential growth phase	3	EC10 (population growth rate)	17

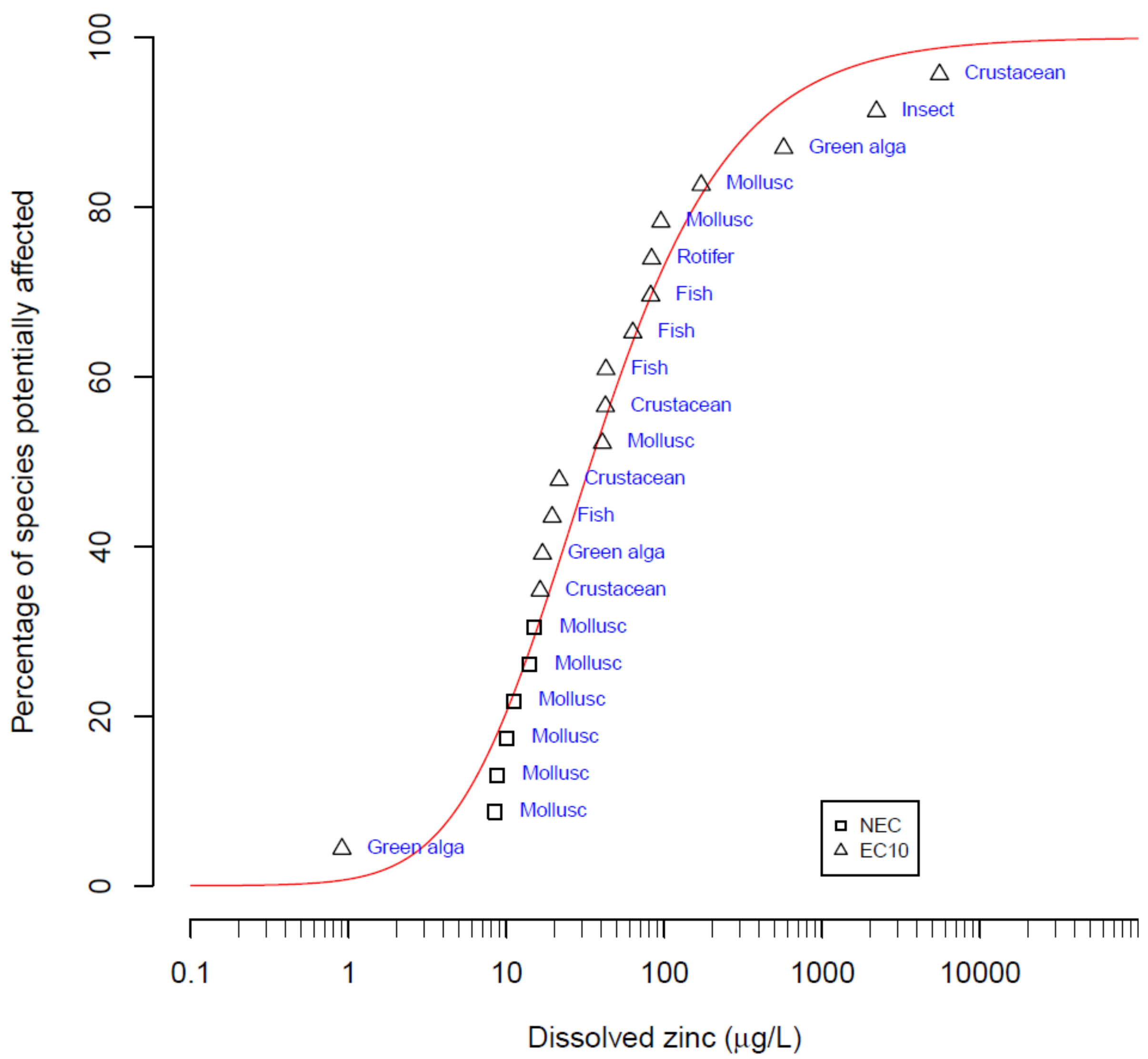

Figure B1 Species sensitivity distribution for zinc in freshwater based on preferred toxicity estimates only, normalised to the index condition of pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC

Inclusion of only EC10 data excludes several species, including 2 molluscs native to New Zealand, for which only EC20 or NOEC data were available. The guideline values derived from only the preferred data would be protective of both species based on 95% level of protection or above, and protective of the other excluded species. However, by including EC20 and NOEC estimates, the number of species included in the SSD increased from 22 to 31, which provides a better taxonomic representation and increased confidence in the guideline values.

Table B2 Toxicant default guideline values (DGVs) for zinc in freshwater at the index condition (pH 7.5, hardness 30 mg/L $CaCO_3$ and 0.5 mg/L DOC), using the preferred toxicity estimates and with very high reliability

Level of species protection (%)	DGV for zinc in freshwater (µg/L)[a]
99	1.2
95	3.1
90	5.5
80	9.7

[a] Default guideline values were derived using Burrlioz 2.0 software and based on data normalised to a pH of 7.5, hardness of 30 mg/L $CaCO_3$ and 0.5 mg/L DOC using trophic-level multiple linear regression models. All guideline values have been rounded to 2 significant figures.

Appendix C: look-up tables for zinc default guideline values for differing pH, hardness and dissolved organic carbon concentrations

Table C1 Guideline values (µg/L Zn) for protection of 99% of species; hardness is in mg/L $CaCO_3$, DOC is in mg/L, and the guideline value at the index condition is highlighted in grey

pH 6.2

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	2.5	2.8	3.6	4.3	4.8	5.9	6.9	7.9
	1	2.7	3.1	4.1	5.0	5.8	7.2	8.5	9.8
	2	3.0	3.5	4.8	5.9	7.0	8.9	11	12
	5	3.5	4.2	6.1	7.7	9.2	12	14	15
	10	4.1	5.0	7.4	9.5	11	14	15	16
	15	4.4	5.5	8.3	11	13	14	16	17

pH 6.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	2.1	2.4	3.1	3.7	4.2	5.2	6.1	6.9
	1	2.4	2.8	3.7	4.5	5.1	6.4	7.6	8.9
	2	2.8	3.3	4.4	5.4	6.3	8.0	9.5	11
	5	3.4	4.0	5.6	7.0	8.4	11	13	14
	10	4.0	4.8	6.9	8.8	10	13	15	16
	15	4.4	5.3	7.8	9.9	12	15	16	17

pH 7.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	1.6	1.9	2.5	3	3.4	4.2	4.9	5.7
	1	2.0	2.3	3.1	3.7	4.3	5.3	6.2	7.1
	2	2.5	2.9	3.8	4.6	5.3	6.6	7.8	8.4
	5	3.2	3.8	5.0	6.1	7.1	9.0	11	12
	10	3.9	4.6	6.3	7.6	8.9	11	14	15
	15	4.5	5.2	7.1	8.7	10	13	16	17

pH 7.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	1.3	1.5	2.0	2.4	2.7	3.4	4.0	4.6
	1	1.6	1.9	2.5	3.0	3.5	4.3	5.1	5.8
	2	2.1	2.5	3.2	3.9	4.4	5.5	6.3	7.3
	5	3.0	3.4	4.5	5.3	6.0	7.4	8.7	9.9
	10	3.8	4.4	5.7	6.7	7.6	9.2	11	12
	15	4.4	5.1	6.5	7.6	8.6	11	12	14

pH 8.0									
	Hardness	**20**	**30**	**60**	**90**	**120**	**180**	**300**	**440**
DOC	**0.5**	0.99	1.2	1.6	1.9	2.2	2.8	3.3	3.7
	1	1.3	1.6	2.1	2.5	2.9	3.5	4.1	4.7
	2	1.8	2.1	2.8	3.2	3.7	4.5	5.3	5.8
	5	2.6	3.1	3.9	4.5	5.0	5.9	6.8	7.5
	10	3.5	4.0	5.1	5.8	6.3	7.3	8.2	9.0
	15	4.0	4.7	5.9	6.6	7.3	8.3	9.1	9.9

pH 8.3									
	Hardness	**20**	**30**	**60**	**90**	**120**	**180**	**300**	**440**
DOC	**0.5**	0.85	1.0	1.4	1.7	1.9	2.4	2.9	3.1
	1	1.2	1.4	1.9	2.2	2.5	3.1	3.7	4.2
	2	1.6	1.9	2.5	2.9	3.3	3.9	4.4	5.0
	5	2.4	2.8	3.6	4.1	4.5	5.2	5.8	6.3
	10	3.1	3.7	4.7	5.3	5.7	6.4	7.0	7.4
	15	3.7	4.4	5.5	6.1	6.6	7.3	7.8	8.3

Table C2 Guideline values (µg/L Zn) for protection of 95% of species; hardness is in mg/L $CaCO_3$, DOC is in mg/L, and the guideline value at the index condition is highlighted in grey

pH 6.2

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	7.9	9.2	12	13	15	17	19	21
	1	8.7	10	13	15	16	19	21	23
	2	9.6	11	14	16	18	21	24	26
	5	11	13	16	19	21	25	28	30
	10	12	14	18	21	24	28	31	33
	15	13	15	19	23	26	29	32	35

pH 6.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	6.5	7.6	9.7	11	12	14	16	17
	1	7.5	8.7	11	13	14	17	18	20
	2	8.6	10	13	15	17	19	21	23
	5	10	12	15	18	20	23	26	28
	10	12	14	18	21	23	27	30	33
	15	13	15	19	23	25	30	33	35

pH 7.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	4.8	5.6	7.2	8.3	9.2	11	12	13
	1	5.9	6.8	8.7	10	11	13	15	16
	2	7.2	8.3	11	12	14	16	18	19
	5	9.4	11	14	16	18	21	23	25
	10	11	13	17	20	22	26	29	31
	15	13	15	19	22	25	29	32	35

pH 7.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	3.5	4.1	5.3	6.1	6.8	7.9	8.8	9.6
	1	4.5	5.3	6.8	7.9	8.8	10	11	12
	2	5.9	6.9	8.8	10	11	13	15	16
	5	8.3	9.7	12	14	16	18	20	22
	10	11	13	16	18	20	24	26	28
	15	13	15	19	21	24	27	30	33

pH 8.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	2.5	3	3.9	4.5	5.1	5.9	6.6	7.2
	1	3.5	4.1	5.3	6.2	6.9	8	8.9	9.7
	2	4.8	5.6	7.3	8.4	9.3	11	12	13
	5	7.2	8.5	11	13	14	16	18	19
	10	9.7	11	15	17	19	21	23	25
	15	12	14	18	20	22	25	28	30

pH 8.3									
	Hardness	**20**	**30**	**60**	**90**	**120**	**180**	**300**	**440**
DOC	**0.5**	2.1	2.5	3.3	3.8	4.2	5.0	5.5	6.0
	1	3.0	3.5	4.6	5.3	5.9	6.9	7.7	8.4
	2	4.2	5.0	6.5	7.5	8.3	9.5	11	11
	5	6.5	7.7	10	12	13	15	16	17
	10	8.9	11	14	16	18	20	22	23
	15	11	13	17	19	21	24	26	28

Table C3 Guideline values (µg/L Zn) for protection of 90% of species; hardness is in mg/L $CaCO_3$, DOC is in mg/L, and the guideline value at the index condition is highlighted in grey

pH 6.2

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	14	16	21	24	26	30	32	35
	1	15	18	22	26	28	32	35	38
	2	17	19	25	28	31	35	39	41
	5	19	22	27	31	35	39	43	47
	10	21	24	30	34	38	43	48	52
	15	22	25	31	36	40	46	51	55

pH 6.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	11	13	17	19	21	24	27	29
	1	13	15	19	22	24	28	30	32
	2	15	17	22	25	28	31	34	37
	5	18	21	26	30	33	37	41	44
	10	20	23	30	34	37	42	47	51
	15	22	25	32	36	40	46	51	55

pH 7.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	8.0	9.4	12	14	15	18	19	21
	1	9.9	12	15	17	19	21	23	25
	2	12	14	18	21	23	26	28	31
	5	16	18	23	27	29	34	37	40
	10	19	22	29	33	36	41	45	48
	15	22	25	32	37	40	46	50	54

pH 7.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	5.8	**6.8**	8.7	10	11	13	14	15
	1	7.5	8.8	11	13	14	16	18	19
	2	9.8	11	15	17	19	21	23	25
	5	14	16	21	24	26	30	33	35
	10	18	21	27	31	34	39	43	46
	15	21	25	32	36	40	45	50	53

pH 8.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	4.1	4.8	6.3	7.2	8.0	9.1	10	11
	1	5.7	6.7	8.7	10	11	13	14	15
	2	7.8	9.2	12	14	15	17	19	21
	5	12	14	18	21	23	27	29	31
	10	16	19	25	29	32	37	40	43
	15	19	23	30	35	39	44	49	52

pH 8.3									
Hardness		**20**	**30**	**60**	**90**	**120**	**180**	**300**	**440**
DOC	**0.5**	3.4	4.0	5.1	5.9	6.6	7.5	8.3	9.0
	1	4.8	5.7	7.4	8.5	9.4	11	12	13
	2	6.8	8.1	11	12	13	15	17	18
	5	11	13	17	20	22	25	27	29
	10	15	18	24	28	31	35	39	42
	15	18	22	29	34	38	43	48	51

Table C4 Guideline values (µg/L Zn) for protection of 80% of species; hardness is in mg/L $CaCO_3$, DOC is in mg/L, and the guideline value at the index condition is highlighted in grey

pH 6.2

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	26	31	40	46	51	57	63	67
	1	29	34	43	50	55	61	67	72
	2	32	37	47	54	59	66	72	76
	5	36	42	52	59	64	72	79	86
	10	39	45	56	63	69	79	88	95
	15	41	47	58	66	72	83	93	100

pH 6.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	21	25	32	37	41	47	51	54
	1	24	29	37	42	46	52	57	60
	2	28	33	42	48	52	59	64	68
	5	34	39	49	56	61	68	74	80
	10	38	44	55	63	68	76	85	92
	15	41	48	59	67	73	83	92	100

pH 7.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	15	17	23	26	29	33	36	38
	1	18	21	28	32	35	40	43	46
	2	22	26	34	39	43	48	52	57
	5	29	34	44	50	55	62	67	72
	10	36	42	53	61	67	75	81	87
	15	40	47	60	68	75	83	91	98

pH 7.5

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	10	12	16	18	20	23	25	27
	1	14	16	21	24	26	30	33	35
	2	18	21	27	31	35	39	43	46
	5	25	30	39	45	49	56	61	65
	10	33	39	51	58	64	73	80	85
	15	39	46	59	68	75	86	93	100

pH 8.0

	Hardness	20	30	60	90	120	180	300	440
DOC	0.5	7.3	8.6	11	13	14	16	18	19
	1	10	12	16	18	20	23	25	26
	2	14	17	22	25	28	32	35	37
	5	21	26	34	39	44	50	55	59
	10	30	35	47	55	61	71	78	84
	15	36	43	57	67	75	86	95	100

pH 8.3									
	Hardness	**20**	**30**	**60**	**90**	**120**	**180**	**300**	**440**
DOC	**0.5**	5.9	7	9.1	10	12	13	14	16
	1	8.4	10	13	15	17	19	21	22
	2	12	14	19	22	25	28	31	33
	5	19	23	31	36	41	47	52	56
	10	27	33	45	53	59	69	76	82
	15	33	41	55	65	73	86	95	100

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