Toxicant default guideline values for aquatic ecosystem protection

Iron in freshwater

Technical brief

September 2025

Cataloguing data
This publication (and any material sourced from it) should be attributed as: ANZG 2025, *Toxicant default guideline values for aquatic ecosystem protection: Iron in fresh water.* Australian and New Zealand Guidelines for Fresh and Marine Water Quality. CC BY 4.0. Australian and New Zealand Governments and Australian state and territory governments, Canberra, ACT, Australia.

This publication is available at the Toxicant default guideline values technical briefs website.

Contact
Australian Government Department of Climate Change, Energy, the Environment and Water
GPO Box 858 Canberra ACT 2601
Switchboard +61 2 6272 3933 or 1800 900 090
Email waterquality@dcceew.gov.au

Contents

Figures

Tables

Appendix tables

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).

Iron, in the form of iron(II) (Fe^{2+}) and iron(III) (Fe^{3+}), is an essential element for aquatic biota but has low solubility at the pH of natural waters. Iron(II) is considered to be more bioavailable than iron(III) because it is more soluble. Iron(III) is the dominant form of iron in oxygenated waters, while iron(II) dominates iron speciation in anoxic waters. Dissolved iron, typically defined by filtration (< 0.45 µm), includes colloidal iron forms.

Although biologically essential, iron can be toxic at elevated concentrations. Toxicity is largely associated with iron(II) and iron(III) in dissolved, colloidal and precipitated forms (operationally defined by size fractionation). The mechanisms of toxicity of iron are unclear but may occur via direct chemical or physical mechanisms involving oxidative damage to DNA and cell membranes, or through the coating of respiratory structures, thereby reducing oxygen diffusion. Adverse effects of iron may also be indirect, such as the degradation of habitat and food quality for benthic macroinvertebrates by iron flocs coating benthic surfaces and periphyton communities.

Since ANZECC AND ARMCANZ (2000), sufficient data have become available to enable the derivation of DGVs for iron in freshwater. Chronic toxicity appears to occur in the range of hundreds to thousands of micrograms per litre. Water-quality parameters that modify iron toxicity are primarily pH and dissolved organic carbon. The influence of hardness on iron toxicity is not well understood. Aged iron(III) hydroxide precipitate is less toxic to aquatic biota than fresh precipitate, suggesting that transient forms of iron also contribute to toxicity.

Very high reliability DGVs for iron in freshwater were derived based on chronic toxicity data for 20 species from 10 taxonomic groups, comprising 3 fungi, one microalga, one macrophyte, one rotifer, one annelid, one planarian, 2 insects, 3 crustaceans, one amphibian and 6 fish. Chronic toxicity values ranged from 192 µg/L to 50,000 µg/L total iron across a range of different endpoints and exposure durations. The DGVs for 99%, 95%, 90% and 80% species protection are 140 µg/L, 280 µg/L, 430 µg/L and 730 µg/L, respectively. The 95% species-protection level of 280 µg/L (total iron) is recommended when assessing ecosystems that are slightly to moderately disturbed. The DGVs are relatively well supported by available mesocosm and field studies on the effects of iron on freshwater biota. When comparing iron concentrations in water samples to the DGVs, the iron concentration should be based on either total iron or, preferably, the potentially bioavailable fraction (i.e. pH 2 extractable) of chromium (III). Additional guidance is provided on the fractions of iron to measure in water samples for comparison with the DGVs.

1 Introduction

Iron is the fourth-most common element in the Earth's crust and is an essential trace element for aquatic biota. It is a key constituent in the enzymatic pathways of chlorophyll and protein synthesis, and it modulates processes such as DNA and RNA synthesis and oxygen metabolism and transport (Norman et al. 2014). Although iron is an essential element, it has low bioavailability due to its speciation, to the point of becoming limiting for aquatic biota in freshwater lakes (Norman et al. 2014). To deal with iron limitation, algae and bacteria have evolved the ability to harvest iron from the surrounding water by secreting organic chelators (siderophores) that complex iron(III) and transport it back into the cell where it is enzymatically reduced to iron(II) (Haese 2006).

Natural sources of iron include weathering and leaching of iron-rich sedimentary rocks, such as hematite (Fe_2O_3) and magnetite (Fe_3O_4), basalt and acid-sulfate soils, and sediments containing pyrite (FeS_2). Anthropogenic releases of iron are mainly due to burning of fossil fuels, acid mine drainage, industrial waste discharges, and corrosion of iron and steel. Dissolved/colloidal concentrations of iron in non-impacted lakes range from 0.03 µg/L Fe to 17 µg/L Fe (Norman et al. 2014). Rivers globally have an average concentration of 67 µg/L Fe (Chester and Jickells 2012). Total and dissolved iron concentrations in freshwaters are highly variable and are a result of the geology and land use of the surrounding catchment. Total iron measurements are confounded by the presence of mineralised sedimentary iron.

Iron exists in 2 oxidation states – iron(II) (Fe^{2+}) and iron(III) (Fe^{3+}). Iron(III) dominates under oxic conditions. Under anaerobic conditions, iron(II) dominates and has higher water solubility. Iron(II) rapidly oxidises to form iron(III), which then rapidly hydrolyses to form amorphous iron(III) hydroxide precipitates and colloidal iron oxyhydroxides, which readily complex with organics, especially humic substances. These may remain in suspension or flocculate and deposit over time. Size-fractionated speciation of iron into categories of truly soluble, dissolved, colloidal and total iron is operationally defined by the filter pore size, and dissolved (< 0.45-µm) iron includes colloidal fractions. Analytical techniques for iron oxidation-state speciation in natural waters and their limitations have been described by Pehkonen (1995).

The speciation of iron is controlled largely by the solubility limits for iron(II) and iron(III). The solubility of iron(III) hydroxide (0.048 µg/L at 18°C) is markedly lower than that of iron(II) hydroxide (1,420 µg/L at 20°C) (Haese 2006). Iron speciation is influenced by water-quality parameters such as pH, redox potential (Eh), dissolved oxygen, carbon dioxide, hardness, sulfur species, natural organic matter, photo-reduction of organic iron complexes and microbial activity (Norman et al. 2014). At alkaline pH values, the hydroxy complexes of iron(II) and iron(III) dominate under reducing and oxygenated conditions, respectively.

The low solubility of iron(III) hydroxides at neutral pH in oxygenated water means that toxicity testing of most aquatic organisms is confounded by the presence of dissolved, colloidal and precipitated iron forms, each differing in their modes of action and contribution to toxicity. Dissolved iron(II) is thought to be more toxic than iron(III) due to its higher solubility. However, the conditions under which iron(II) dominates (i.e. low pH, low oxygen) means that aquatic biota are either not present or are stressed by the inherent physicochemical conditions (Bury et al. 2011). The complex speciation of

iron corresponds to complex mechanisms of toxicity. Limited iron may result in adverse effects on biota due to deficiencies in biological requirements or, alternatively, excess dissolved iron may result in oxidative damage to membranes or disruption of metabolic processes. Precipitated iron may coat respiratory surfaces (gills), egg pores or digestive surfaces and impede uptake of nutrients (see section 2.1). It can also have indirect effects by altering the quality of benthic habitats and food resources. There is no evidence for bioaccumulation of iron in aquatic invertebrates and fish but it does bioaccumulate in algae and higher plants (Johnson et al. 2007). It is therefore necessary to consider toxicity associated with both dissolved/colloidal and precipitated forms of iron in freshwater systems.

There were no ANZECC AND ARMCANZ (2000) default guideline values (DGVs) for iron in freshwater. However, an interim indicative working level of 300 µg/L was recommended, based on a Canadian water-quality guideline value for long-term exposure to total iron (CCREM 1987). Other international jurisdictions have developed freshwater guideline values for iron, deriving separate guideline values for dissolved and total iron, short-term and long-term exposures, and protection of sensitive species versus whole communities (Appendix A, Table A1). Different approaches to deriving guideline values have been used. Shuhaimi-Othman et al. (2012) and Johnson et al. (2007) applied acute-to-chronic ratios or assessment factors to the lowest toxicity value available based on results from single-species laboratory bioassays. Another approach has been to apply non-linear quantile regression analysis to macroinvertebrate community field data to derive a threshold value that will protect the 90th quantile of all aquatic biota, based on field-based evidence that macroinvertebrates (particularly mayflies) are the most sensitive trophic level (EPRI 2004; Crane et al. 2007; Linton et al. 2007; Peters et al. 2011a, 2011b).

The DGV-derivation approach reported below uses published toxicity data from laboratory-based single-species bioassays of acceptable quality in a species sensitivity distribution (SSD) to derive DGVs that provide protection against chronic toxicity of iron to freshwater species (as per Warne et al. 2018). Moreover, supporting information from 2 iron toxicity mesocosm studies was used to help validate the DGVs. Given that both dissolved and precipitated iron can result in toxicity, it is recommended that the application of the DGVs be based on a measurement of these potentially bioavailable forms. Guidance on this is provided in Appendix B. The updated DGVs reported here supersede the ANZECC AND ARMCANZ (2000) interim indicative working-level value.

2   Aquatic toxicology

The complexity of iron speciation is problematic for attributing toxicity to either dissolved/colloidal iron or amorphous/precipitated iron(II) and iron(III) forms, when all forms may be present simultaneously or may change in relative contributions to toxicity over time. For example, iron(II) rapidly oxidises to iron(III), and amorphous iron transitions to precipitated iron over time. Attributing toxicity to iron(II) versus iron(III) is also confounded by the preferred low-pH and reduced-redox conditions under which dissolved iron(II) dominates, which may be outside the physiological limits of tolerance for many biota. There is insufficient evidence to attribute toxicity to a single form of iron and, therefore, all forms of iron are considered in the current derivation of DGVs.

2.1   Mechanisms of iron toxicity

Direct mechanisms of iron toxicity attributed to dissolved iron(II) are associated with oxidative stress via free radical production causing damage to DNA, and damage to cellular membranes as a result of lipid peroxidation resulting in subsequent leakage of potassium (Bury et al. 2011; EPRI 2004; Vuori 1995). Dietary uptake of precipitated iron(III) oxyhydroxides is associated with cell membrane damage to the digestive system (Gerhardt 1992). Direct physical toxicity of iron(III) hydroxide is evident from histopathological damage to fish and macroinvertebrate gills, where the precipitate accumulates on the gill surface and prevents oxygen diffusion without being bioaccumulated (Gerhardt 1992; Vuori 1995; Dalzell and Macfarlane 1999). Teien et al. (2008) found toxicity to fish and effects on fish gills associated with transient forms of iron (speculated by Peters et al. 2011a to be $FeOH^{2-}_{(aq)}$ and $Fe(OH)_2^-{}_{(aq)}$) going from low-molecular-weight to high-molecular-weight iron forms correlated with a decrease in iron(II) and increase in iron(III). Small (2-µm) precipitates of iron(III) hydroxide have been found to reduce fish (fathead minnow [*Pimephales promelas*]) egg hatch success by clogging the pores of the egg chorion (Smith et al. 1973).

Indirect toxic effects have been observed primarily in field studies and manifest as iron hydroxides and iron–humus precipitates that coat benthic surfaces and alter the habitat and food quality, which impacts macroinvertebrate communities and subsequent higher trophic levels. These precipitates have been observed to restrict the distribution, abundance and diversity of fish and benthic invertebrates (Peters et al. 2011a; Vuori 1995).

2.2   Chronic effects of iron(II) on aquatic biota

Iron(II) is considered more bioavailable than iron(III) due to its higher solubility and rapid ligand exchange kinetics that facilitate iron trans-membrane transport (Sunda and Huntsman 1998). However, due to the lack of iron(II) and iron(III) speciation measurements, there is limited experimental evidence for this hypothesis. Gerhardt (1992) conducted chronic toxicity tests using iron(II) with the acid-tolerant mayfly (*Leptophlebia marginata*) at pH 4.5 where iron(II) was dominant and at pH 7 where iron(III) was dominant. Mayfly survival, motility and feeding behaviour over 30 days were reduced when exposed to iron(II) and were unaffected by iron(III). It was suggested that iron(II) acted on the gut membrane to prevent food absorption so that the mayflies starved. This study was able to discern a difference between chronic effects of iron(II) and iron(III) because the

test organism was compatible with low pH and iron(II) was measured, but this has not been replicated with other species.

2.3   Chronic effects of iron(III) as Fe(OH)₃ on aquatic biota

A series of studies (Sykora et al. 1972a; Smith et al. 1973; Sykora et al. 1972b; Sykora et al. 1975; Smith and Sykora 1976) assessed the chronic toxicity of iron(III) hydroxide precipitate (obtained by neutralisation of iron(II) sulfate with calcium hydroxide and aeration) to invertebrates (the amphipod *Gammarus minus* and the caddisfly *Cheumatopsyche* sp.) and fish ([*P. promelas*], brook trout [*Salvelinus fontinalis*] and coho salmon [*Oncorhynchus kisutch*]). Most of the data from these studies did not pass the data-quality assessment criteria for use in the current DGV-derivation process due to a failure to state the test acceptability criteria or to infer acceptability criteria by stating a standard method (Warne et al. 2018). However, they are worth commenting on because of the long exposure durations (3.5 months to 2 years) and the measurement of iron speciation and physicochemistry throughout the exposure period. The precipitate was maintained in suspension with an aerated flow-through system. Iron speciation measurements demonstrated that iron was predominantly in the iron(III) form – iron(II) comprised 0.3% to 9% of the total iron. The diluent consisted of well water and the test solution hardness and pH ranged from 112 mg/L CaCO₃ to 256 mg/L CaCO₃ and 6.9–8.1 pH, respectively. The hardness gradient was due to the addition of calcium hydroxide for the neutralisation process. Iron hydroxide precipitates caused 100% amphipod mortality after the third week of exposure to 100,000 µg/L total iron and after 14 weeks of exposure to 4,000 µg/L total iron. Freshly prepared Fe(OH)₃ solutions were 3 times more toxic to amphipods than aged (6.5 hours) solutions (Sykora et al. 1972a). Emergence of the caddisfly larvae was low and variable in controls (30% to 60% emergence) and iron treatments (0% to 50% emergence) but declined to 0% emergence at greater than 12,000–25,000 µg/L fresh or aged Fe(OH)₃ (Sykora et al. 1972a). Smith et al. (1973) found that 2,000 µg/L total iron was protective of chronic effects on juvenile *P. promelas* survival, growth and egg-hatch success. Juvenile brook trout were less sensitive – the 35-week growth NOEC was 13,420 µg/L (Sykora et al. 1972b), and no effects on hatch success were observed up to the highest test concentration of 9,000 µg/L total iron (Sykora et al. 1975). Similarly, egg hatch success of coho salmon was not affected by Fe(OH)₃ at the highest test concentration of 10,000 µg/L total iron (Smith and Sykora 1976). However, survival of the juvenile (30-day post-hatch) coho salmon declined to 69% at concentrations ≥ 6,000 µg/L, suggesting higher sensitivity to Fe(OH)₃ than brook trout but lower sensitivity than *P. promelas*.

Several recent papers, including Cadmus et al. (2018a, 2018b), Cardwell et al. (2023) and Kotalik et al. (2019), have contributed significantly to the knowledge on iron toxicity to freshwater biota. Cadmus et al. (2018a) undertook 30-day chronic tests on brown trout (*Salmo trutta*), mountain whitefish (*Prosopium williamsoni*), an oligochaete (*Lumbriculus variegatus*), a mayfly (*Hexagenia limbata*), boreal toad tadpoles (*Bufo boreas*) and a planarian (*Dugesia dorotocephala*), while Cadmus et al. (2018b) undertook a 10-day mesocosm experiment where responses of a range of insect groups were examined, including the mayfly *Epeorus* spp., the caddisfly *Micrasema* spp. and tanytarsinid chironomids (note that the toxicity values for the Cadmus et al. 2018b mesocosm study were actually reported in Cadmus et al. 2018a). Single-species tests EC20 (see 'Glossary and acronyms' for definitions) values (total iron) ranged from 870 µg/L for *Lumbriculus variegatus* to > 40,000 µg/L for *D. dorotocephala*, while the insect EC20s (total iron) from the mesocosm study ranged from 234 µg/L for Tanytarsini to > 14,073 µg/L for *Rithrogena* spp. and *Ephemerella* spp. (Cadmus et al. 2018a).

Kotalik et al. (2019) undertook a 14-day mesocosm experiment to assess the effects of precipitated iron on macroinvertebrate and algal communities. Community composition was significantly altered at or below 1 mg/L total iron. While green algal and diatom community colonisation (measured as chlorophyll-*a*) were reduced at very low total iron levels, cyanobacteria were stimulated with increasing iron concentration. Effects on algal communities were primarily attributed to iron deposition and smothering. Some insect taxa were also very sensitive to iron exposure/deposition – effects on some taxa/life stages were observed at iron concentrations well below 100 µg/L. Overall, Kotalik et al. (2019) reported EC20 values for total iron ranging from 0.4 µg/L for chlorophyll-*a* to 1,009 µg/L for Chironomidae adult abundance. It was notable that the EC20 values based on biomass data for some communities were typically in the range of 1–16 µg/L, while other biomass and abundance endpoints were in the range of 261–1,009 µg/L. However, the r^2 values for the linear models used to test for significance for the endpoints that resulted in low EC20s (1–16 µg/L) ranged from 0.02 to 0.44, indicating very high uncertainty regarding these effects. In contrast, the corresponding r^2 values for the endpoints that resulted in higher EC20s (261–1,009 µg/L) ranged from 0.27 to 0.94 (with two-thirds being greater than 0.5). Kotalik et al. (2019) also reported that the adult life stages of some insect groups were more sensitive than their larvae. Notwithstanding the uncertainty of some of the results, the study generally suggested higher sensitivity of insect taxa to iron than that reported by Cadmus et al. (2018b), despite the studies being done at the same facility with similar methodologies.

Cardwell et al. (2023) comprehensively examined the effects of pH, hardness and dissolved organic carbon on chronic toxicity to the green alga *Raphidocelis subcapitata*, the cladoceran *Ceriodaphnia dubia* and the fish *P. promelas*. As advised by the primary author of Cardwell et al. (2023) (Allison Cardwell, personal communication), the toxicity values presented in this paper represented updated values from previously published studies by her co-authors (CIMM 2011; OSU 2013; Arbildua et al. 2017). The findings from these latest papers were used by Brix et al. (2023) to develop multiple linear regression (MLR) models for predicting chronic iron toxicity.

A range of species show sensitivity to iron at > 400 µg/L, dependent on solution conditions. Insects appear to be the most sensitive taxa under a range of conditions (Brix et al. 2023). Dissolved organic carbon and pH consistently affected toxicity to all species, while hardness had a lesser effect on *P. promelas* and *R. subcapitata*, and less again with *C. dubia*. At pH 7.2 and 2.5 mg/L dissolved organic carbon (DOC), the mayfly *Epiorus* spp., caddisfly *Microsema* spp. and chironomid tribe Tanytarsini had EC20s of 335 g/L, 356 µg/L and 234 µg/L, respectively (Cadmus et al. 2018a). However, in an earlier study, the mayfly *Leptophlebia marginata*, appeared to be insensitive to iron – its 30-day NOEC was 50,000 µg/L measured total iron (Gerhardt 1992). It should be noted that DOC was not measured by Gerhardt (1992), but the diluent stream water was described as being 'humus-rich', which may have reduced iron toxicity.

For pH 6 and low DOC, the most sensitive species was *P. promelas*, which had an EC10 of 192 µg/L (Cardwell et al. 2023). Other fish species were less sensitive. For example, the brook trout *Salvelinus fontinalis* and medaka *Oryzias latipes* had 14-day NOEC and 30-day NOEC values of 13,420 µg/L and 25,000 µg/L measured as total iron, respectively (Brix et al. 2023). *C. dubia* at pH 6 and low DOC had an EC10 of 383 µg/L (Cardwell et al. 2923).

Peters et al. (2011b) estimated effects-based thresholds for iron in freshwater based on field data for macroinvertebrate communities in the United Kingdom. Matched chemical (dissolved, particulate and total iron) and ecological monitoring data for 1,830 samples were used to assess the effects of

iron on benthic macroinvertebrate communities based on 3 key environmental-quality indices relative to a reference condition. The EC10 values for the environmental-quality indices and across the 3 different fractions of iron were generally similar, ranging from 1,250 µg/L to 2,460 µg/L. These values were considered to represent a threshold for iron concentrations below which good ecological status is likely to be achieved, despite some sensitive species being potentially affected. Notably, these thresholds are higher than toxicity values for numerous macroinvertebrate taxa.

No acute data were used in the derivation of the DGVs, as the minimum data requirements were met with chronic data alone (see section 4.1). However, acute iron toxicity has been reviewed elsewhere (Johnson et al. 2007; Shuhaimi-Othman et al. 2012; EPRI 2004; Phippen et al. 2008).

3   Factors affecting toxicity

Factors that influence the solubility and speciation of iron will also affect its toxicity. The solubility of iron(III) is greatly enhanced by the presence of natural organic matter, particularly humic substances, which stabilise dissolved colloidal iron. This form of iron may be more or less bioavailable to aquatic biota, depending on the organism (Norman et al. 2014). Increased hardness was also found to increase iron(III) solubility (Phippen et al. 2008). Dissolved organic carbon may stabilise iron(II) in solution, thereby reducing the rate of oxidation to iron(III). Sulfate, nitrate and chloride ions may also inhibit oxidation of iron(II) (Vuori 1995). Oxidation of iron(II) is accelerated by increased pH and temperature, the presence of trace metals, phosphate, fluoride and bacteria, and adsorption to particles (e.g. complexation with surface hydroxyl groups, such as surfaces of iron(III) hydroxides) (Teien et al. 2008). Mechanisms of reductive dissolution of iron(III) to release iron(II) include reduction of iron(III) hydroxides by dissolved sulfides, photo-induced dissolution, and the reductive enzymatic reactions on surfaces of phytoplankton (Vuori 1995). Increasing age of iron(III) hydroxide precipitates is associated with decreased toxicity. Iron(III) hydroxide decreased in toxicity to the cladoceran *Daphnia pulex* when iron stocks were aged for 0, 3 and 6 days (Birge et al. 1985) and to amphipods when test solutions were aged for 6.5 hours (Sykora et al. 1972b). Teien et al. (2008) demonstrated that in situations where iron is undergoing transformation (possibly due to hydrolysis), it may be considerably more toxic than in aged solutions. Different sets of fish were exposed to iron(III) (500 µg/L total iron) that had been left to react for different periods of time, and this resulted in greater mortality and accumulation of iron hydroxides on fish gills after the first 0.5 minutes of reaction time than after 5 minutes or 20 minutes of reaction time (Peters et al. 2011a; Teien et al. 2008). The conditions with the highest potential for iron toxicity to freshwater macroinvertebrate communities at neutral pH were estimated to be low DOC (1.1 mg/L) and low hardness (14 mg/L $CaCO_3$) (Peters et al. 2011a).

As already noted, modification of chronic iron toxicity by pH, DOC and hardness has been investigated in studies with *R. subcapitata*, *C. dubia*, and *P. promelas* (Cardwell et al. 2023), all of which are represented in the database used to derive the DGVs (see Appendix C).

Cardwell et al. (2023) measured survival and reproduction of *C. dubia* over 7 days in static renewal of media at pH 6.3, hardnesses of 25 mg/L $CaCO_3$, 84 mg/L $CaCO_3$, 252 mg/L $CaCO_3$ and DOC of 0 mg/L and 4 mg/L at 5 iron (iron(III) as $Fe_2(SO_4)_3$) concentrations ranging from 200 µg/L to 10,000 µg/L measured total iron. Survival was unaffected at all iron concentrations and all hardness and DOC conditions. In the absence of DOC, toxicity to *C. dubia* reproduction was reduced, but not consistently (EC10 = 570 µg/L, 1,200 µg/L and 900 µg/L total iron), as respective hardness concentration increased. The 95% confidence limits for the EC10 values overlapped, suggesting a statistically non-significant trend. However, the presence of DOC (4 mg/L) was significantly more effective at reducing reproductive toxicity (EC10s), reducing toxicity by a factor of 2 at 25 mg/L hardness and by a factor of 5 at 84 mg/L hardness (Cardwell et al. 2023), respectively. There was no detectable dissolved (< 0.22-µm) iron present in the absence of DOC regardless of the hardness concentration. When DOC was added, dissolved iron ranged from 2% to 14%, 0.005% to 6%, and 0% of total iron at 25 mg/L $CaCO_3$, 84 mg/L $CaCO_3$ and 252 mg/L $CaCO_3$ hardness, respectively. Therefore, DOC increased the solubility of iron(III) but this was counteracted by increased hardness.

This suggests that the precipitated form of iron(III) was chronically toxic to *C. dubia* reproduction and this was primarily mitigated by DOC and secondarily through interactions with hardness.

Cardwell et al. (2023) also measured survival and growth (dry biomass) of *P. promelas* in 7-day chronic tests with juveniles (< 24 hours old) in a static renewal system at pH 6 and 8; hardnesses of 10.6 mg/L $CaCO_3$, 84 mg/L $CaCO_3$ and 252 mg/L $CaCO_3$; and DOC of 0 mg/L, 2 mg/L and 4 mg/L (as Suwannee River natural organic matter) at iron (iron(III) as $Fe_2(SO_4)_3$) concentrations ranging from 128 µg/L to 62,000 µg/L measured total iron. Iron solutions were equilibrated for 3 hours prior to use, and buffers were added to maintain pH, which subsequently contributed to the DOC (332– 490 mg/L) but, due to low binding capacities, the buffer contribution of DOC is unlikely to have influenced iron speciation. Chronic fish survival was only affected at the highest total iron test concentration of 62,000 µg/L (with survival reduced to 85%). However, increasing iron reduced growth in all treatments. The exception was when DOC (4 mg/L) was added in pH-6 test solutions, and no effects were detected at the highest total iron concentration of 7,450 µg/L. The effect of iron on growth was greater at pH 6 compared to pH 8, regardless of hardness level and DOC (0 mg/L and 2 mg/L). Iron effects on growth were partially mitigated by increasing DOC and hardness at pH 6 and pH 8 but these factors had less influence on iron toxicity at pH 8. There was no detectable dissolved (< 0.2-µm) iron in the absence of DOC, regardless of hardness or pH. Dissolved iron was influenced more by increased DOC than hardness, regardless of pH. Therefore, chronic toxicity to *P. promelas* (based on growth) was greatest in the presence of precipitated iron(III) and this was mitigated in the presence of DOC by the formation of more soluble species of iron. Hardness had the least effect on reducing iron toxicity and influencing solubility of iron. Overall, pH had the strongest influence on iron toxicity (based on growth of *P. promelas*) – the greatest toxicity occurred at pH 6. Dissolved organic carbon and hardness were most effective in partially mitigating toxicity.

The available data indicate that pH and DOC (listed in order of their influence on iron toxicity) likely interact with the dissolved and precipitated forms of iron present to modify toxicity. Hardness did not have consistent effects on iron toxicity to *R. subcapitata, C. dubia* or *P. promelas*.

Brix et al. (2023) developed MLR models for 3 species (*R. subcapitata, C. dubia* and *P. promelas*) using the data of Cardwell et al. (2023). A pooled model was not possible because of differences between species, so the *R. subcapitata* model was applied to plants, the *P. promelas* model was applied to fish, and the *C. dubia* model was applied to all invertebrates, including insects. There was uncertainty about the applicability of the *C. dubia* model to insects because of a lack of knowledge regarding the pH dependence of insect responses and a lack of validation for molluscs and other crustaceans (e.g. *Daphnia magna*).

Although the Brix et al. (2023) MLR models were recently used by Canada to develop bioavailability-based guideline values for iron in freshwater (ECCC 2024), these models have not been adopted in the current version of the iron DGVs for Australia and New Zealand. This is because the models are yet to be validated for local species and water-quality conditions. As noted, there is still considerable uncertainty surrounding the applicability of the *C. dubia* MLR model for other invertebrates (Brix et al. 2023), and none of the existing models have been validated for very soft, acidic waters (Kevin Brix, EcoTox, pers comm, 28 March 2024), which are not uncommon in Australia.

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 Toxicity data used in derivation

A summary of the toxicity data (one value per species) used to calculate the DGVs for iron in freshwaters is provided in **Error! Reference source not found.**. Further details on the data that passed the screening and quality-assurance schemes, including those used to derive the single-species values used to calculate the DGVs, are presented in Appendix C, Table C1. Details of the data-quality assessment and the data that passed the quality assessment are provided as supporting information.

Table 1. Summary of single chronic toxicity values for all species used in the derivation of default guideline values for iron in freshwater

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Final toxicity value (µg/L)
Fungus	*Alatospora acuminata*	NR	21	NOEC (growth)	6,900
Fungus	*Articulospora tetracladia*	NR	21	NOEC (growth)	6,900
Fungus	*Tetrachaetum elegans*	NR	21	NOEC (growth)	6,900
Microalga	*Raphidocelis subcapitata*		3	EC10 (yield)	442[a]
Macrophyte	*Phragmites australis*	Seedling	64	NOEC (growth)	1,000[a]
Rotifer	*Euchlanis dilitata*	Neonate	5	LC10 (reproduction)	957
Annelid	*Lumbriculus variegatis*	Worm	35	EC10 (reproduction)	470
Planarian	*Dugesia dorotocephala*		30	EC10 (growth)	40,000
Insect	*Hexagenia limbata*	Nymph	30	EC10 (survival)	7,863
Insect	*Leptophlebia marginata*	Larvae	30	NOEC (immobility)	50,000
Crustacean	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	383[a]
Crustacean	*Daphnia magna*	Neonate	21	EC16 (reproduction)	4,380
Crustacean	*Daphnia pulex*	Neonate	21	NOEC (reproduction)	852
Amphibian	*Bufo boreas*	Tadpole	35	EC10 (biomass)	2,607
Fish	*Oncorhyncus kisutch*	Larvae	7	NOEC converted to EC10 (mortality)	3,040
Fish	*Oryzias latipes*	Larvae	7	NOEC (mortality)	25,000
Fish	*Pimephales promelas*	Larvae	7	EC10 (growth)	192[a]
Fish	*Prospium williamsoni*	Egg	78	EC10 (biomass)	868
Fish	*Salmo trutta*	Egg	79	EC20 (biomass)	5,000
Fish	*Salvelinus fontinalis*	3 months	245	NOEC (growth)	10,280

NR = not reported.

[a] The lowest toxicity value from different endpoints from a single species was used.

Chronic toxicity values of acceptable quality were available for 20 species from 10 taxonomic groups, comprising 3 fungi, one microalga, one macrophyte, one rotifer, one annelid, one planarian, 2 insects, 3 crustaceans, one amphibian and 6 fish. Toxicity data consisted mainly of chronic EC10 and EC20 values (one EC16) in addition to 7 chronic NOECs (**Error! Reference source not found.**). Quality-assessment scores for the toxicity values used to derive the DGVs ranged from 50% to 96%.

Cadmus et al. (2018a) reported chronic EC20 values for 15 insect taxa (one species, 12 genera, one sub-family and one tribe), mainly derived from a mesocosm experiment undertaken by Cadmus et al. (2018b). As noted by Brix et al. (2023), the mesocosm data increased the representation of insect taxa that are typically significantly under-represented relative to natural aquatic communities. For a limited number of these insect taxa, Brix et al. (2023) recalculated EC10 and EC20 values from the original data of Cadmus et al. (2018a, 2018b), as well as for an older study of fish species by Smith and Sykora (1976), as indicated in the listing in Appendix C, Table C2. The Brix et al. (2023) EC10 values are tabulated in the Supplementary Information attached to Brix et al. (2023).

Despite the use of the Cadmus et al. (2018a, 2018b) mesocosm data for the insect genera, sub-family and tribe by Brix et al. (2023), only species-level data can be used in Australia and New Zealand for deriving DGVs (Warne et al. 2018). Notably, there was ambiguity over whether some of the toxicity values from the mesocosm study were based on a single or multiple unidentified species from a genus. Advice on this matter from a co-author of Cadmus et al. (2018b) was that some results might have been for a single species while others were based on multiple species (Professor Will Clements, personal communication, 1 August 2024). Given the uncertainty, it was decided to derive the DGVs from the dataset without the mesocosm insect species and to use the mesocosm data to help validate the DGVs, as recommended by Warne et al. (2018). The mesocosm study had a 10-day exposure duration which, given that most of the organisms counted would have been larval/nymph forms, would be classified as chronic (Warne et al. 2018). Therefore, the mesocosm study was appropriate for validation purposes (see section 4.3).

Data from the Kotalik et al. (2019) mesocosm study were also not used for the derivation of the DGVs, because the endpoints were not based on individual species. Instead, the results from this study were also used to help validate the DGVs (see section 4.3).

Where there were data for the same species, endpoint and test duration, but the physicochemical conditions were different (e.g. different pH, hardness, DOC), toxicity values from the most bioavailable condition were used in the SSD. For example, the data reported by Cardwell et al. (2023) for *R. subcapitata, P. promelas* and *C. dubia* covered a range of pH, hardness and DOC concentrations (Appendix C). Given that the Brix et al. (2023) MLR models have not been used in the current derivation, the lowest toxicity value for each of the above species was used in the SSD, in accordance with the recommendations in Warne et al. (2018) (see Appendix C).

A number of the results were reported as greater-than (>) values. These were accepted as the actual value, following the recommendations of Warne et al. (2018).

Iron salts used to prepare the test solutions consisted of iron(II) as $FeSO_4$ and iron(III) as $FeCl_3.6H_2O$ or $Fe_2(SO_4)_3$. This ensured that the total iron measurements included freshly precipitated iron, i.e. forms that are bioavailable, not including the mineralised forms that might be present in natural water samples.

An assessment of whether the data selected for the derivation of the DGVs were unimodal or multimodal in distribution was conducted according to the weight-of-evidence approach described in Warne et al. (2018). Because iron is an essential element used in many cellular processes, there is no one specific mode of toxicity and no one particular taxonomic group more sensitive than the others that would indicate multimodality. A visual inspection of the distribution of data in the SSD did not suggest multimodality. Supporting this, the bimodality coefficient (Freeman and Dale 2013) calculated on the log-10-transformed values used in the SSD was 0.38, which was below the 0.555 threshold for indicating that the data may exhibit bimodality. Therefore, the dataset was considered to be unimodal.

The low solubility of iron in oxygenated systems meant that dissolved/colloidal and particulate iron were the dominant forms of iron giving rise to these toxicity data. A DGV based only on dissolved iron would be overly conservative and neglect the direct physical toxic effects of precipitated iron that occurs readily in the environment and is also present in the bioassays used in the derivation process. When both dissolved/colloidal and precipitate forms occur in the natural environment, the toxicity associated with those forms needs to be represented in the DGVs.

As pH is an influential water-quality parameter for iron toxicity, all data selected for DGV derivation came from toxicity tests where the pH was between 6 and 8, representing the physiological limits of many aquatic biota. There is currently insufficient evidence of the influence of DOC and hardness on iron toxicity to screen data on the basis of these water-quality parameters, and no hardness or DOC bioavailability-based algorithms are available for application to a wide range of biota. Therefore, toxicity values obtained under a range of hardness (8–252 mg/L $CaCO_3$) and DOC (0–4 mg/L added) conditions were included in the derivation process. Where environmental water quality is outside the pH, hardness and DOC ranges above, it may be beneficial to undertake site-specific assessments in order to determine site-specific guideline values.

4.2 Species sensitivity distribution

The cumulative frequency SSD of the 20 freshwater iron chronic toxicity data reported in Table 1 is shown in Figure 1. The SSD was plotted using the Burrlioz 2.0 software. The model was judged to provide a good fit to the data.

Figure 1. Species sensitivity distribution for iron in freshwater
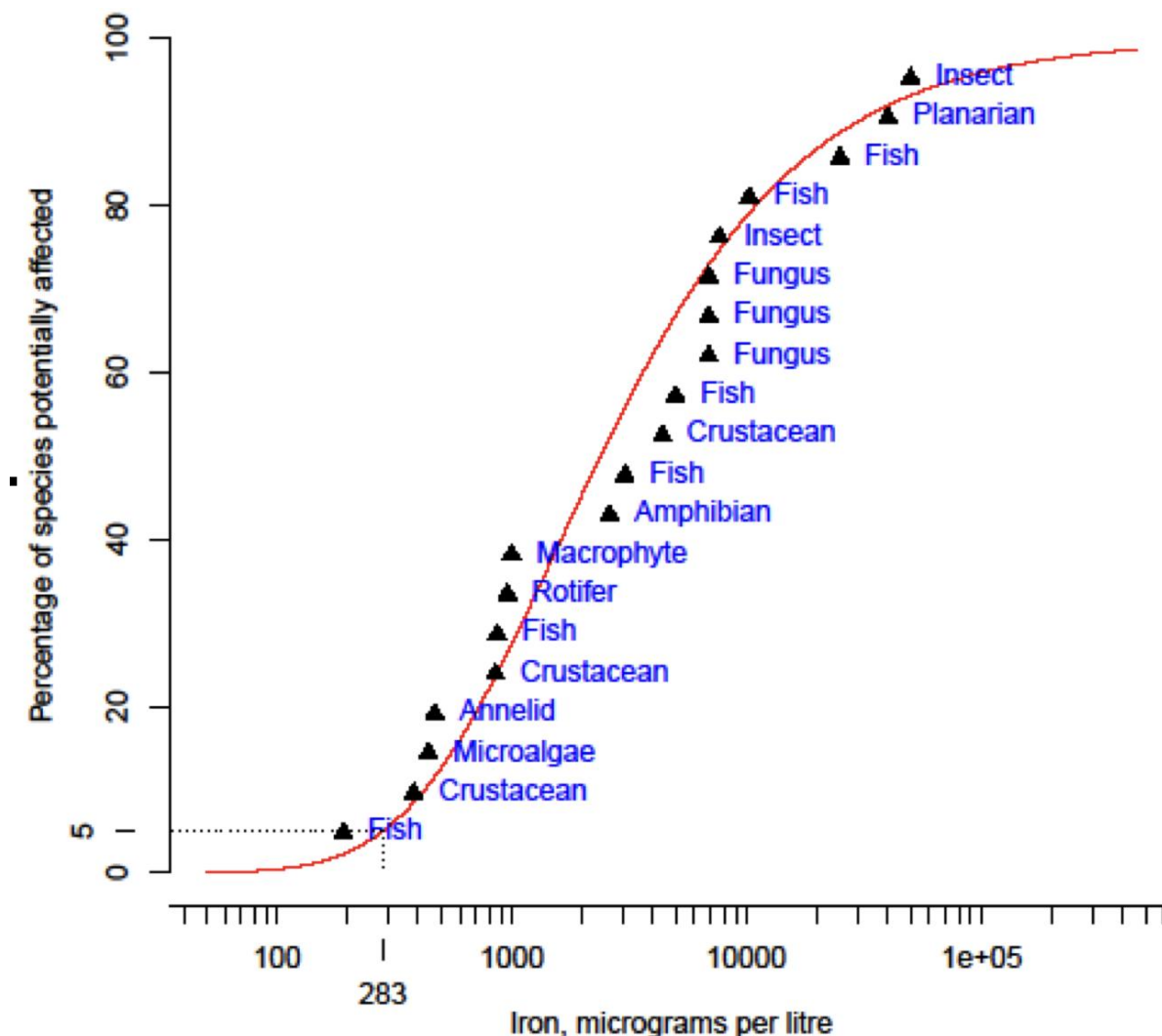
4.3   Default guideline values

It is important that the DGVs (Table 2) 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 are shown in Table 2. The 95% species-protection DGV of 280 µg/L iron is recommended when assessing ecosystems that are slightly to moderately disturbed. When applying the DGV for iron to an unfiltered water sample, it is recommended to distinguish the iron associated with mineralised forms in suspended sediment, which is not considered bioavailable, from the potentially bioavailable iron in colloidal/precipitated and surface-adsorbed forms. To do this, a weak-acid (pH-2) extraction of the unfiltered water sample is recommended, as detailed in ANZG (2025). The weak-acid extractable fraction will contain dissolved/colloidal and precipitated forms without a large contribution from mineralised particulate iron. Although the total iron fraction of the unfiltered water sample can be compared with the DGV, if this fraction exceeds the DGV, it will not be possible to identify the relative proportions of non-

bioavailable and bioavailable iron in the sample. Further details on applying the DGVs to metals of low solubility, including iron(III), are presented in Appendix B.

Table 2. Toxicant default guideline values for iron in freshwater with very high reliability

Level of species protection (%)	DGV for iron in freshwater (µg/L)[a,b]
99	140
95	280
90	430
80	730

[a] The default guideline values (DGVs) were derived using the Burrlioz 2.0 software and based on data from toxicity tests conducted for a pH range of 6–8, hardness range of 8–252 mg/L $CaCO_3$ and dissolved organic carbon range of 0–4 mg/L. They have been rounded to 2 significant figures.

[b] The total iron concentration or, preferably, the potentially bioavailable fraction (i.e. weak-acid extract) of the unfiltered environmental water sample should be compared with the DGVs. Filtered/dissolved fractions of iron should not be compared with the DGVs. See section 4.3 and Appendix B for further details.

The DGVs were compared with the results of the mesocosm studies of Cadmus et al. (2018a, 2018b) and Kotalik et al. (2019) and the field assessment of Peters et al. (2011b). A summary follows:

- Based on the criteria detailed in Warne et al. (2018), the 2 mesocosm studies were considered to be of acceptable quality. The key limitations included the fact that they focused only on phytoplankton and insect communities and only just met exposure-duration criteria for a chronic exposure to larval/nymph aquatic insects (10–14 days).

- Cadmus et al. (2018a, 2018b) – The 99% species-protection DGV is protective of all the insect taxa listed, while the 95% species-protection DGV is protective of all but the Tanytarsini (Chironomidae) taxon. Thus, the DGVs appear to be appropriately protective based on these data.

- Kotalik et al. (2019) – All the DGVs are higher than a number of EC20s in the range of 1–16 µg/L total iron derived for various endpoints for algae, diatoms and some insects. However, as noted in section 2.3, there was high uncertainty associated with these EC20s. Moreover, these concentrations are likely to be lower than, or at least representative of, natural background concentrations of total iron. Notably, most other EC20s reported by Kotalik et al. (2019) were > 250 µg/L and had lower uncertainty. These values were around or above the 95% species-protection DGV.

- Peters et al. (2011b) – All the DGVs are below the estimated threshold range of 1,250–2,460 µg/L iron for maintaining good ecological status based on field macroinvertebrate data. However, Peters et al. (2011b) also noted that there may be some sensitive taxa that would not be adequately protected by thresholds based on whole-community responses. Consequently, the more conservative DGVs for iron appear to be appropriately protective based on the macroinvertebrate field data.

- On balance, the available mesocosm and field evidence supports the protectiveness of the DGVs.

It is also instructive to compare the DGVs with values derived by other investigations (Appendix A). The most recent of these (from Canada, ECCC 2024) has a 95% species-protection value of 110 µg/L total iron at pH 7.5 and 0.5 mg/L DOC, derived using the shinyssdtools software. As noted earlier, this derivation used the MLR models from Brix et al. (2023). The major driver for the lower Canadian guideline value compared to the 95% species-protection level DGV in Table 2 appears to be due to the Canadian guideline value having been derived based on a very low DOC concentration, as DOC is an influential toxicity-modifying factor. An example of this is 3 different insect EC20 values of 234 µg/L, 335 µg/L and 356 µg/L that were converted to EC10 values at pH 7.5 and 0.5 mg/L DOC of 89 µg/L, 127 µg/L and 135 µg/L, respectively.

In infrequent cases where the background iron concentrations exceed the DGV, and it is deemed that the ecosystem could tolerate increases in concentration above the already naturally elevated background, the recommended approach is to derive a new site-specific guideline value based on background or reference site data (ANZG 2018). In most cases, the 80th percentile of the background concentration becomes the site-specific guideline value. Further guidance on this is provided in ANZG (2018). Note that local jurisdictions should always be consulted when deriving site-specific guideline values.

4.4 Reliability classification

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

- Sample size – 20 (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 due to a short (relative to the organism's life span) exposure to a chemical. Refer to Warne et al. (2018) for examples of acute exposures.
Acute-to-chronic ratio	The species' mean acute value (LC/EC50) divided by the chronic value (e.g. NOEC or EC10) for the same species.
Assessment factor	A unitless number applied to the lowest toxicity figure for a chemical to derive a concentration that should not cause adverse environmental effects. The size of the assessment factor varies with the type of data. Also called 'application factor' or 'safety factor'.
Benthic	Refers to organisms living in or on the sediments of aquatic habitats (e.g. lakes, rivers, ponds).
Bioaccumulation	The process by which chemical substances are accumulated by aquatic organisms by all routes of exposures (dietary and the ambient environment).
Chronic toxicity	A lethal or sub-lethal adverse effect that occurs as the result of exposure to a chemical for a period that is a substantial portion of the organism's life span or an adverse sub-lethal effect on a sensitive early life stage. Refer to Warne et al. (2018) for examples of chronic exposures.
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	Measured attainment response, typically applied to ecotoxicity or management goals.
Guideline value (GV)	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 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.)
Humic substances	Organic substances only partially broken down that occur in water mainly in a colloidal state. Humic acids are large-molecule organic acids that dissolve in water.
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms under specified conditions.
Lowest-observed-effect concentration (LOEC)	The lowest concentration of a chemical used in a toxicity test that has a statistically significant (p ≤ 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.
MLR	Multiple linear regression.
No-observed-effect concentration (NOEC)	The highest concentration of a toxicant used in a toxicity test that does not have a statistically significant (p ≤ 0.05) adverse effect on the exposed population of test organisms as compared with the controls.

Term	Definition
Site-specific	Relating to something that is confined to, or valid for, a particular place. Site-specific trigger values are relevant to the location or conditions that are the focus of a given assessment.
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.
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 concentration of chemical.
Toxicity value	A value defining the concentration of a toxicant that represents an estimate of its toxicity to a species.

Appendix A: summary of protection levels for iron in freshwater

Table A1. Summary of published protection levels derived to protect freshwater biota from iron

Protection level (µg/L)	Iron form	Protection type	Derivation method	Reference
9	Total	Criterion continuous concentration	Assessment factor	Shuhaimi-Othman et al. (2012)
16	Dissolved	Long-term protection	Assessment factor	Johnson et al. (2007)
37.2	Total	Continuous maximum concentration	Assessment factor	Shuhaimi-Othman et al. (2012)
41	Dissolved	Short-term protection	Assessment factor	Johnson et al. (2007)
110	Total	All aquatic biota	SSD + multiple linear regression (shinyssdtools) using EC10s, pH 7.5, 0.5 mg/L DOC	ECCC (2024)
114–200	Total	All aquatic biota	SSD + multiple linear regression using EC10s or EC20s, pH < 7, DOC < 1 mg/L, 15 mg/L hardness	Brix et al. (2023)
210	Total	Sensitive mayflies	Quantile regression	EPRI (2004)
251	Total	All aquatic genera EC20s, including mesocosm insects	Quantile regression	Cadmus et al. (2018a)
280	Total	All aquatic biota	Species sensitivity distribution (Burrlioz 2.0)	This report
300	Total	Interim guideline value	Assessment factor	ANZECC and ARMCANZ (2000)
300	Total	All aquatic biota	Assessment factor	CCREM (1987)
350	Dissolved (< 0.45 µm)	All aquatic biota	Assessment factor	Phippen et al. (2008)
499	Total	All aquatic biota EC20s, excluding mesocosm insects	Quantile regression	Cadmus et al. (2018a)
730	Total	Sensitive taxa	Quantile regression	Peters et al. (2011a, 2011b)
1,000	Total	All aquatic biota	Assessment factor	US EPA (1986)
1,000	Total	All aquatic biota	Assessment factor	Phippen et al. (2008)

Toxicant default guideline values for aquatic ecosystem protection: Iron in freshwater

Protection level (µg/L)	Iron form	Protection type	Derivation method	Reference
1,740	Total	Community	Quantile regression	EPRI (2004)
1,840	Total	Community	Quantile regression	Peters et al. (2011a, 2011b)

Appendix B: water-quality assessment for sparingly soluble metals

A number of metals have low solubility in freshwater and marine water but have been found to be toxic at concentrations above their solubility limit. Therefore, the derivation of guideline values for those metals included data for which toxicity was at least in part due to particulate (precipitated) metals. Examples include iron(III) in marine water (solubility < 0.03 µg/L; Liu and Millero 2002), iron(III) in freshwater (solubility < 0.05 µg/L; Phippen et al. 2008), chromium(III) in freshwater (solubility < 5 µg/L; Rai et al. 1989) and aluminium in marine water (solubility ca 500 µg/L; Angel et al. 2016). The DGVs for these metals are expressed as total metal concentrations.

For iron and chromium, the DGVs are above the solubility limits under oxic conditions and neutral pH. Measuring total metal concentrations to compare with these DGVs requires a method that discriminates between precipitated metals and metals in mineralised forms that are not likely to be bioavailable. This is normally a cold, weak-acid (pH-2) extraction (e.g. as per US EPA 1991) that will solubilise precipitated metal oxyhydroxides, including those that become adsorbed to other substrates, such as mineralised forms or particulate (or colloidal) organic matter (Markich et al. 2001). A total recoverable metals analysis (concentrated acid digestion) is not advisable, as this will overestimate the precipitated metals fraction by also including the digested mineralised forms, potentially leading to false exceedance of the DGV. At least in the case of iron and chromium, 0.45-µm sample filtration is not a recommended step, as it will exclude colloidal and precipitated metal that might be contributing to toxicity, potentially leading to false compliance with the DGV.

A recent study by Balsamo Crespo et al. (2023) demonstrated that a ≥ 4-hour extraction of an unfiltered sample at pH 2 adequately discriminated non-mineralised iron in freshwaters. These results were subsequently confirmed for both freshwater and marine water samples in another study specifically commissioned to address public comments on the iron in freshwater and marine water DGVs relating to the appropriate chemical analysis method, although a 16-hour extraction period was recommended (ANZG 2025). Another study by Rodriguez et al. (2019) reported that, for the analysis of aluminium in freshwater, a pH-4 extractable fraction best correlated with the toxic fraction. However, there is currently no accepted standard analytical method for iron that employs a pH-4 extraction. Moreover, for iron, Balsamo Crespo et al. (2023) found that the pH-2 method performed better than the pH-4 method.

The same study team that published Balsamo Crespo et al. (2023) has also validated the pH-2 method as a measure of bioavailable iron in freshwater through toxicity tests using the cladoceran *C. dubia*. The results are expected to be published in 2025, but a summary of the currently unpublished data is provided here. Results from reproduction tests with *C. dubia* (US EPA 2002) showed that freshly precipitated iron represents a higher risk than well-mineralised phases – EC10 values were significantly lower for fresh precipitates than crystalline phases. The pH-2 extraction method (Balsamo Crespo et al., 2023, ANZG 2025) was able to describe the dose–response relationship without significant differences between EC10 values from experiments with single and combined exposure to fresh and well-mineralised iron phases. Filtered iron measurements (US EPA 1994, 2002) resulted in at least half the exposure range having readings below the limit of detection

(0.005 mg/L Fe) and, therefore, this operationally defined fraction was deemed unsatisfactory to describe the response of *C. dubia* in single and combined exposure settings. Total recoverable iron determination (US EPA 1994, 2002) was able to describe the response curve for single exposure to freshly precipitated iron, but in combined treatments, the total extraction over-estimated the dose–response relationship on reproduction because it recovered iron from the mineralised fractions that were below the concentration that caused effects in exposures to only the mineralised phase. In turn, this yielded significant differences between EC10 values from models for single and combined exposure to fresh precipitates. In summary, the results support the hypothesis that the bioavailable iron fraction should encompass iron phases of lower crystallinity and, furthermore, the pH-2 method (with an extraction time of 6–16 hours) is a suitable method to predict the chronic effects of iron at low response levels without the interference of well-mineralised iron phases.

Consequently, the recently validated pH-2 extraction method (ANZG 2025) is recommended for use when analysing iron for the purpose of comparing concentrations with the iron DGVs. The method is analogous to US EPA (1991) Method 200.1, which was designed to determine acid-soluble metals but was not validated for iron. There is a low risk of toxicity if the pH-2 extractable fraction does not exceed the DGV, but there is potential for toxicity if the DGV is exceeded. Although it is also possible to compare the total iron concentration of the unfiltered water sample with the DGV, if this fraction exceeds the DGV, it will not be possible to identify the relative proportions of non-bioavailable and bioavailable iron in the sample.

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

Table C1. Summary of the chronic toxicity data that passed the screening and quality assurance processes and were used to derive the iron in freshwater default guideline values

Taxonomic group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	Water hardness (mg/L CaCO$_3$)	pH	DOC (mg/L)	Concentration (µg/L)	Reference
Fungus (Ascomycota)	*Alatospora acuminata*	NR	21	NOEC (growth, biomass)	Basal medium	15	NR	7	NR	> 6,900[a]	Bermingham et al. (1996)
Fungus (Ascomycota)	*Articulospora tetracladia*	NR	21	NOEC (growth, biomass)	Basal medium	15	NR	7	NR	> 6,900	Bermingham et al. (1996)
Fungus (Ascomycota)	*Tetrachaetum elegans*	NR	21	NOEC (growth, biomass)	Basal medium	15	NR	7	NR	> 6,900	Bermingham et al. (1996)
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	25	6.4	0.3	442	Cardwell et al. (2023)
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	25	8.0	0.3	1,559	Cardwell et al. (2023)
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	84	6.4	0.3	653	Cardwell et al. (2023)
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	84	7.9	0.3	2,047	Cardwell et al. (2023)
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	252	6.2	0.3	497	Cardwell et al. (2023)
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	252	7.8	0.3	1,195	Cardwell et al. (2023)

Taxonomic group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	Water hardness (mg/L CaCO$_3$)	pH	DOC (mg/L)	Concentration (µg/L)	Reference
Microalga (Chlorophyta)	*Raphidocelis subcapitata*		3	EC10 (yield)	Reconstituted water	24	255	6.1	4.2	8,625	Cardwell et al. (2023)
Macrophyte (Tracheophyta)	*Phragmites australis*	Seedling	64	NOEC (growth, biomass)	10% Rorison's solution	14–20	NR	6	NR	2,000	Batty and Younger (2002)
Macrophyte (Tracheophyta)	*Phragmites australis*	Seedling	64	NOEC (growth, rhizome biomass)	10% Rorison's solution	14–20	NR	6	NR	2,000	Batty and Younger (2002)
Macrophyte (Tracheophyta)	*Phragmites australis*	Seedling	64	NOEC (growth, shoot biomass)	10% Rorison's solution	14–20	NR	6	NR	2,000	Batty and Younger (2002)
Macrophyte (Tracheophyta)	*Phragmites australis*	Seedling	64	NOEC (growth, root length)	10% Rorison's solution	14–20	NR	6	NR	1,000	Batty and Younger (2002)
Macrophyte (Tracheophyta)	*Phragmites australis*	Seedling	64	NOEC (growth, (root:shoot ratio)	10% Rorison's solution	14–20	NR	6	NR	1,000	Batty and Younger (2002)
Rotifer	*Euchlanis dilitata*	Neonate	5	LC10 (reproduction	Moderately hard water	25	80-100	7.5	—	957	Hernandez-Flores et al. (2020)
Annelid (Annelida)	*Lumbriculus variegatis*	Adult	35	EC10 (reproduction)	Dechlorinated tap water	22	44	7.9	—	470	Cadmus et al. (2018a)
Planarian (Platyhelminthes)	*Dugesia dorotocephala*		30	EC10 (growth)	Dechlorinated tap water	22.6	44	7.2	1.9	> 40,000	Cadmus et al. (2018a)
Insect (Arthropod)	*Hexagenia limbata*	Nymph	30	EC10 (survival)	Dechlorinated tap water	17	44	7.9	1.9	> 7,683	Cadmus et al. (2018a)
Insect (Arthropod)	*Leptophlebia marginata*	Nymph	30	NOEC (immobility)	Stream water	11	NR	7.5	0[b]	50,000	Gerhardt (1992)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	10.6	8.0	0.3	383	Cardwell et al. (2023)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	25	6.3	0.3	1,223	Cardwell et al. (2023)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	84	6.3	0.3	1,369	Cardwell et al. (2023)

Taxonomic group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	Water hardness (mg/L CaCO$_3$)	pH	DOC (mg/L)	Concentration (µg/L)	Reference
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	84	8.0	0.3	2,233	Cardwell et al. (2023)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	84	6.3	0.3	596	Cardwell et al. (2023)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	84	7.9	2	961	Cardwell et al. (2023)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	252	6.3	0.3	776	Cardwell et al. (2023)
Crustacean (Arthropod)	*Ceriodaphnia dubia*	Neonate	7	EC10 (reproduction)	Reconstituted water	25	252	8.4	7.0	4,476	Cardwell et al. (2023)
Crustacean (Arthropod)	*Daphnia magna*	Neonate	21	EC16 (reproduction)	Unfiltered Lake Superior water	18	45.3	7.7	NR	4,380	Biesinger and Christensen (1972)
Crustacean (Arthropod)	*Daphnia pulex*	Neonate	21	EC10 (reproduction)	ASTM synthetic water	20	93.6	7.6	0[b]	852	Birge et al. (1985)
Amphibian (Chordata)	*Bufo boreas*	Tadpole	35	EC10 (biomass)	Dechlorinated tap water	20	44	7.1	1.9	2,607	Cadmus et al. (2018a)
Fish (Chordata)	*Oncorhynchus kisutch*	Eggs and post hatch	90	NOEC converted to EC10 (survival)	Well water	11.6	170	7.9	1.6	3,040	Smith and Sykora (1976)
Fish (Chordata)	*Oryzias latipes*	Larva	14	NOEC (mortality)	Embryo-rearing media	26	NR	7.2	NR	25,000	Chen et al. (2011)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	10.3	6.0	0.3	192	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	11.5	6.0	2.0	266	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	77.2	6.0	0.3	316	Cardwell et al. (2023)

Taxonomic group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	Water hardness (mg/L CaCO$_3$)	pH	DOC (mg/L)	Concentration (µg/L)	Reference
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	231	6.0	0.3	332	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	244	6.0	2.0	2,781	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	16.4	7.9	0.3	533	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	12.2	7.9	4.0	27,086	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	74.6	8.0	0.4	1,704	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	74.6	8.0	4.0	15,474	Cardwell et al. (2023)
Fish (Chordata)	*Pimephales promelas*	Larva	7	EC10 (growth, biomass)	Reconstituted water	25	245	7.9	0.3	973	Cardwell et al. (2023)
Fish (Chordata)	*Prospium williamsoni*	Egg	78	EC10 (biomass)	Dechlorinated tap water	7–10	44	7.5	—	868	Cadmus et al. (2018a)
Fish (Chordata)	*Salmo trutta*	Egg	79	EC20 (biomass)	Dechlorinated tap water	7–10	44	7.5	—	> 5,000[a]	Cadmus et al. (2018a)
Fish (Chordata)	*Salvelinus fontinalis*	Eggs and post hatch	90	NOEC (survival)	Well water	11.6	170	7.9	1.6	> 10,280[a]	Smith and Sykora (1976)

Toxicity values represented total measured or nominal iron at pH 6–9. NR = not reported.

[a] Toxic concentrations expressed as greater than (>) were used as the actual value.

[b] Dissolved organic carbon (DOC) was not added, therefore the treatment was nominal 0 mg/L DOC.

Table C2. Summary of mesocosm chronic toxicity data from Cadmus et al. (2018a, 2018b) and Kotalik et al. (2019) that were used to validate the default guideline values for iron in freshwater

Taxonomic group (phylum)	Genus, sub-family or tribe	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper-ature (°C)	Water hardness (mg/L CaCO$_3$)	pH	DOC (mg/L)	Concentration (µg/L)	Reference
Insect (Arthropod)	*Baetis* spp.	Nymph	10	EC10 (abundance)	Dechlorinated tap water	11.8	34	7.2	2.5	3,905[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Brachycentrus* spp.	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	5,690[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Capnia* spp.	Nymph	10	EC10 (abundance)	River water	11.8	34	7.2	2.5	2,200[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Cinygmula* spp.	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	930[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Epeorus* spp.	Nymph	10	EC20 (abundance)	River water	11.8	35	7.2	2.5	335	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Ephemerella* spp.	Nymph	10	E10 (abundance)	River water	11.8	35	7.2	2.5	> 14,073[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Heterlimnius* spp.	Nymph	10	EC10 (abundance)	Dechlorinated tap water	11.8	44	7.2	2.5	747[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Micrasema* spp.	Nymph	10	EC20 (abundance)	River water	11.8	35	7.2	2.5	356	Cadmus et al. (2018a, b)
Insect (Arthropod)	Orthocladiinae (sub-family)	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	776[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Prostoia* spp.	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	1,176[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Rhithrogena* spp.	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	> 14,073[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Sweltsa* spp.	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	> 14,073[a]	Cadmus et al. (2018a, b)
Insect (Arthropod)	*Taenionema* spp.	Nymph	10	EC10 (abundance)	River water	11.8	35	7.2	2.5	1,626[a]	Cadmus et al. (2018a, b)

Taxonomic group (phylum)	Genus, sub-family or tribe	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper-ature (°C)	Water hardness (mg/L CaCO$_3$)	pH	DOC (mg/L)	Concentration (μg/L)	Reference
Insect (Arthropod)	Tanytarsini (tribe)	Nymph	10	EC20 (abundance)	River water	11.8	34	7.2	2.5	234	Cadmus et al. (2018a, b)
Phytoplankton	—	—	14	EC20 (chlorophyll-*a* biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	0.4	Kotalik et al. (2019)
Heterokonto-phyta	Diatoms	—	14	EC20 (colonisation biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	40	Kotalik et al. (2019)
Chlorophyta	Green algae	—	14	EC20 (colonisation biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	261	Kotalik et al. (2019)
Insecta	Baetidae	Larva	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	312	Kotalik et al. (2019)
Insecta	Baetidae	Larva	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	422	Kotalik et al. (2019)
Insecta	Chironomidae	Larva	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	15	Kotalik et al. (2019)
Insecta	Simuliidae	Larva	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	16	Kotalik et al. (2019)
Insecta	Chironomidae	Adult	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	795	Kotalik et al. (2019)
Insecta	Baetidae	Adult	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	1	Kotalik et al. (2019)
Insecta	Chironomidae	Larva	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	298	Kotalik et al. (2019)
Insecta	Simuliidae	Adult	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	393	Kotalik et al. (2019)
Insecta	Simuliidae	Adult	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	414	Kotalik et al. (2019)

Taxonomic group (phylum)	Genus, sub-family or tribe	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper-ature (°C)	Water hardness (mg/L CaCO₃)	pH	DOC (mg/L)	Concentration (µg/L)	Reference
Insecta	Baetidae	Adult	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	11	Kotalik et al. (2019)
Total macroin-vertebrate		Larva	14	EC20 (biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	368	Kotalik et al. (2019)
Total macroin-vertebrate		Larva	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	824	Kotalik et al. (2019)
Total macroin-vertebrate		Adult	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	694	Kotalik et al. (2019)
Cyanobacteria	Blue-green algae	—	14	EC20 (colonisation biomass)	River water	14.3–14.9	31	7.4	2.5–3.0	1,935	Kotalik et al. (2019)
Insecta	Chironomidae	Adult	14	EC20 (abundance)	River water	14.3–14.9	31	7.4	2.5–3.0	1,009	Kotalik et al. (2019)

[a] Values recalculated by Brix et al. (2023).

References

Angel BM, Apte SC, Batley GE and Golding LA (2016) '[Geochemical controls on aluminium concentrations in coastal waters](#)', *Environmental Chemistry*, 13:111–118, doi:10.1071/EN15029.

ANZECC and ARMCANZ (Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand) (2000) *[Australian and New Zealand Guidelines for Fresh and Marine Water Quality, volume 1, the guidelines](#)*, ANZECC and ARMCANZ.

ANZG (Australian and New Zealand Guidelines) (2018) *[Australian and New Zealand Guidelines for Fresh and Marine Water Quality](#)*, Australian and New Zealand governments and Australian state and territory governments.

ANZG (2025) *Optimisation of the US EPA (1991) pH 2 extraction method for measuring potentially bioavailable iron (iron III)*, Australian and New Zealand guidelines for fresh and marine water quality. CC BY 4.0. Australian and New Zealand Governments and Australian state and territory governments.

Arbildua JJ, Villavicencio G, Urrestarazu P, Opazo M, Brix KV, Adams WJ and Rodriguez PH (2017) '[Effect of Fe (III) on *Pseudokirchneriella subcapitata* at circumneutral pH in standard laboratory tests is explained by nutrient sequestration](#)', *Environmental Toxicology and Chemistry*, 36:952–958, doi:10.1002/etc.3609.

Balsamo Crespo E, Reichelt-Brushett A, Smith REW, Rose AL and Batley GE (2023) '[Improving the measurement of iron(III) bioavailability in freshwater samples: methods and performance](#)', *Environmental Toxicology and Chemistry,* 42:303–316, doi:10.1002/etc.5530.

Batty LC and Younger PL (2002) '[Critical role of macrophytes in achieving low iron concentrations in mine water treatment wetlands](#)', *Environmental Science and Technology*, 36:3997–4002, doi:10.1021/es020033+.

Bermingham S, Maltby L and Cooke RC (1996) '[Effects of a coal mine effluent on aquatic hyphomycetes. II. Laboratory toxicity experiments](#)', *Journal of Applied Ecology*, 33:1322–1328, doi:10.2307/2404773.

Biesinger KE and Christensen GM (1972) '[Effects of various metals on survival, growth, reproduction, and metabolism of *Daphnia magna*](#)', *Journal of the Fisheries Research Board of Canada*, 29:1691–1700, doi:10.1139/f72-269.

Birge WJ, Black JA, Westerman AG, Short TM, Taylor SB, Bruser DM and Wallingford ED (1985) *[Recommendations on numerical values for regulating iron and chloride concentrations for the purpose of protecting warm water species of aquatic life in the Commonwealth of Kentucky](#)* [PDF 664 KB], Memorandum of Agreement No. 5429, Kentucky Natural Resources and Environmental Protection Cabinet.

Brix KV, Tear LM, DeForest DK and Adams WJ (2023) '[Development of multiple linear regression models for predicting chronic iron toxicity to aquatic organisms](#)', *Environmental Toxicology and Chemistry*, 42:1386–1400, doi:10.1002/etc.5623.

Bury NR, Boyle D and Cooper CA (2011) 'Iron', in Wood CM (ed) *Homeostasis and toxicology of essential metals. Vol 31: fish physiology*, Academic Press..

Cadmus P, Brinkman SF and May MK (2018a) 'Chronic toxicity of ferric iron for North American aquatic organisms: derivation of a chronic water quality criterion using single species and mesocosm data', *Archives of Environmental Contamination and Toxicology*, 74:605–618, doi:10.1007/s00244-018-0505-2.

Cadmus P, Guasch H, Herdrich AT, Bonet B, Urrea G and Clements WH (2018b) 'Structural and functional responses of periphyton and macroinvertebrate communities to ferric Fe, Cu, and Zn in stream mesocosms', *Environmental Toxicology and Chemistry,* 37:1320–1329, doi:10.1002/etc.4070.

Cardwell AS, Rodriguez PH, Stubblefield WA, DeForest DK and Adams WJ (2023) 'Chronic toxicity of iron to aquatic organisms under variable pH, hardness, and dissolved organic carbon conditions', *Environmental Toxicology and Chemistry*, 42:1371–1385, doi:10.1002/etc.5627.

CCREM (Canadian Council of Resource and Environment Ministers) (1987) *Canadian water quality guidelines*, CCREM, Inland Water Directorate, Environment Canada, Ottawa.

Chen PJ, Su CH, Tseng CY, Tan SW and Cheng CH (2011) 'Toxicity assessments of nanoscale zerovalent iron and its oxidation products in medaka (*Oryzias latipes*) fish', *Marine Pollution Bulletin*, 63:339–346, doi:10.1016/j.marpolbul.2011.02.045.

Chester R and Jickells T (2012) 'The transport of material to the Oceans: the fluvial pathway', in Chester R and Jickells T (eds) *Marine geochemistry*, John Wiley and Sons, Ltd, doi:10.1002/9781118349083.ch3.

CIMM (Chilean Mining and Metallurgy Research Center) (2011) '*Iron (III) sulfate hydrate:* Ceriodaphnia dubia *survival and reproduction test at pH 6.3*', CIMM.

Crane M, Kwok KWH, Wells C, Whitehouse P and Lui GCS (2007) 'Use of field data to support European Water Framework Directive quality standards for dissolved metals', *Environmental Science and Technology*, 41(14):5014–5021, doi:10.1021/es0629460.

Dalzell D and Macfarlane N (1999) 'The toxicity of iron to brown trout and effects on the gills: a comparison of two grades of iron sulphate', *Journal of Fish Biology*, 55:301–315, doi:10.1111/j.1095-8649.1999.tb00680.x.

ECCC (Environment and Climate Change Canada) (2024) *Federal environmental quality guidelines – iron*, National Guidelines and Standards Office, ECCC, Gatineau.

EPRI (Electric Power Research Institute) (2004) *Water quality criteria development for iron* [PDF 1.78 MB], EPRI, Palo Alto.

Freeman JB and Dale R (2013) 'Assessing bimodality to detect the presence of a dual cognitive process', *Behavior Research Methods*, 45:83–97, doi:10.3758/s13428-012-0225-x.

Gerhardt A (1992) 'Effects of subacute doses of iron (Fe) on *Leptophlebia marginata* (Insecta: Ephemeroptera)', *Freshwater Biology*, 27:79–84, doi:10.1111/j.1365-2427.1992.tb00524.x.

Haese RR (2006) 'The biogeochemistry of iron', in Schulz HD and Zabel M (eds) *Marine geochemistry*, 2nd edn, Springer Verlag, Berlin, Heidelberg.

Hernández-Flores S, Santos-Medrano GE, Rubio-Franchini I and Rico-Martínez R (2020) 'Evaluation of bioconcentration and toxicity of five metals in the freshwater rotifer *Euchlanis dilatata* Ehrenberg, 1832', *Environmental Science and Pollution Research*, 7:14058–14069, doi:10.1007/s11356-020-07958-3.

Johnson I, Sorokin N, Atkinson C, Rule K and Hope S-J (2007) *Proposed EQS for Water Framework Directive Annex VIII substances: iron (total dissolved)* [PDF 396 KB], Science Report: SC040038/SR9, SNIFFER Report: WFD52(ix).

Kotalik C, Cadmus P and Clements W (2019) 'Indirect effects of iron oxide on stream benthic communities: capturing ecological complexity with controlled mesocosm experiments', *Environmental Science and Technology,* 53:11532–11540, doi:10.1021/acs.est.9b04236.

Linton TK, Pacheco MAW, McIntyre DO, Clement WH and Goodrich-Mahoney J (2007) 'Development of bioassessment-based benchmarks for iron', *Environmental Toxicology and Chemistry*, 26:1291–1298, doi:10.1897/06-431.1.

Liu X and Millero FJ (2002) 'The solubility of iron in seawater', *Marine Chemistry,* 77:43–54, doi:10.1016/S0304-4203(01)00074-3.

Markich SJ, Brown PR, Batley GE, Apte SC and Stauber JL (2001) 'Incorporation of metal speciation and bioavailability into water quality guidelines for protecting aquatic ecosystems', *Australasian Journal of Ecotoxicology* 7:109–122.

Norman L, Cabanes DJE, Blanco-Ameijeiras S, Moisset SAM and Hassler CS (2014) 'Iron biogeochemistry in aquatic systems: from source to bioavailability', *Chimia*, 68:764–771, doi:10.2533/chimia.2014.764.

OSU (Oregon State University) (2013) *Toxicity of iron to the fathead minnow,* Pimephales promelas*, under varying pH, hardness, and dissolved organic carbon (DOC) conditions*, prepared by OSU Aquatic Toxicology Laboratory, Albany.

Pehkonen S (1995) 'Determination of the oxidation states of iron in natural waters – a review', *The Analyst*, 120:2655–2663, doi:10.1039/AN9952002655.

Peters A, Merrington G, Simpson P and Crane M (2011a) *Proposed quality standards for iron in freshwaters based on field evidence (for consultation)* [PDF 1,131 KB], Water Framework Directive – United Kingdom Technical Advisory Group (WFD-UKTAG), Edinburgh.

Peters A, Crane M and Adams W (2011b) 'Effects of iron on benthic macroinvertebrate communities in the field', *Bulletin of Environmental Contamination and Toxicology*, 86:591–595, doi:10.1007/s00128-011-0283-2.

Phippen BW, Horvath C, Nordin R and Nagpal N (2008) *Ambient water quality guidelines for iron* [PDF 250 KB], prepared for Science and Information Branch, Water Stewardship Division, Ministry of Environment, Province of British Columbia.

Rai D, Eary LE and Zachara JM (1989) 'Environmental chemistry of chromium', *Science of the Total Environment,* 86:15–23, doi:10.1016/0048-9697(89)90189-7.

Rodriguez PH, Arbildua JJ, Villavicencio G, Urrestarazu P, Opazo M, Cardwell AS, Stubblefield W, Nordheim E and Adams W (2019) 'Determination of bioavailable aluminum in natural waters in the presence of suspended solids', *Environmental Toxicology Chemistry*, 38:1668–1681, doi:10.1002/etc.4448.

Shuhaimi-Othman M, Nadzifah Y, Nur-Amalina R and Umirah NS (2012) 'Deriving freshwater quality criteria for iron, lead, nickel, and zinc for protection of aquatic life in Malaysia', *The Scientific World Journal*, Article ID 861576, doi:10.1100/2012/861576.

Smith EJ and Sykora JL (1976) 'Early developmental effects of lime-neutralized iron hydroxide suspensions on brook trout and coho salmon', *Transactions of the American Fisheries Society*, 105:308–312, doi:10.1577/1548-8659(1976)105%3C308:edeoli%3E2.0.co;2.

Smith EJ, Sykora JL and Shapiro MA (1973) 'Effect of lime neutralized iron hydroxide suspensions on survival, growth, and reproduction of the fathead minnow (*Pimephales promelas*)', *Journal of the Fisheries Research Board of Canada*, 30:1147–1153, doi:10.1139/f73-185.

Sunda WG and Huntsman SA (1998) 'Processes regulating cellular metal accumulation and physiological effects: phytoplankton as model systems'. *Science of the Total Environment*, 219:165–181.

Sykora JL, Smith EJ and Synak M (1972a) 'Effect of lime neutralized iron hydroxide suspensions on juvenile brook trout (*Salvelinus fontinalis*, Mitchill)', *Water Research*, 6:935–950, doi:10.1016/0043-1354(72)90045-0.

Sykora JL, Smith EJ, Shapiro MA and Synak N (1972b) 'Chronic effect of ferric hydroxide on certain species of aquatic animals', in *Proceedings of 4th Symposium on Coal Mine Drainage Research*, Mellon Institute, Pittsburgh.

Sykora JL, Smith EJ, Synak M and Shapiro MA (1975) 'Some observations on spawning of brook trout (*Salvelinus fontinalis* Mitchill) in lime neutralized iron hydroxide suspensions', *Water Research*, 9:451–458, doi:10.1016/0043-1354(75)90192-X.

Teien HC, Garmo ØA, Åtland Å and Salbu B (2008) 'Transformation of iron species in mixing zones and accumulation on fish gills', *Environmental Science and Technology*, 42:1780–1786, doi:10.1021/es071965m.

US EPA (United States Environmental Protection Agency) (1986) *Quality criteria for water* [PDF 13.7MB], US EPA Agency Report No. EPA 440/5-86-001, Springfield.

US EPA (1991) *Method 200.1 – determination of acid-soluble metals*, US EPA Report No. EPA/600/4-91/010, Cincinnati.

US EPA (1994) *Method 200.8: determination of trace elements in waters and wastes by inductively coupled plasma–mass spectrometry*, Revision 5.4, Cincinnati.

US EPA (2002) *Short-term methods for estimating the chronic toxicity of effluents and receiving waters to freshwater organisms* [PDF 4.7 MB], 4th edn, US EPA Report No. EPA-821-R-02-013, Office of Science and Technology, Washington DC.

Vuori KM (1995) '[Direct and indirect effects of iron on river ecosystems [PDF 3.3 MB]](#)', *Annales Zoologici Fennici*, 32:317–329.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2018) *Revised method for deriving Australian and New Zealand water quality guideline values for toxicants*, report prepared for the Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australian and New Zealand governments and Australian state and territory governments.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2024) *Revised method for deriving Australian and New Zealand water quality guideline values for toxicants – update of 2018 version*, prepared for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australian and New Zealand governments and Australian state and territory governments.