



Toxicant default guideline values for aquatic ecosystem protection

Iron in marine water

Technical brief

September 2025

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Contact

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

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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 <u>Australian and New Zealand Guidelines for Fresh and Marine Water Quality</u> website (ANZG 2018).

Iron, in the form of iron(II) (Fe²⁺) and iron(III) (Fe³⁺), is an essential element for marine biota. It is present at very low dissolved concentrations in the world's oceans due to its low solubility (0.01– 0.03 μ g/L). Iron(III) oxyhydroxide inorganic/organic colloids and precipitates are the dominant forms of iron under oxic conditions.

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 from 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 marine water. Chronic toxicity appears to occur in the range of hundreds to thousands of micrograms per litre. There is some indication that some marine species, particularly echinoderms, are more sensitive than this, although the reliability of the studies is unclear.

Moderate reliability DGVs for iron in marine water were derived based on chronic toxicity data for 16 species from 6 taxonomic groups, comprising one microalga, 2 cnidaria, one echinoderm, 10 bivalve molluscs, one gastropod mollusc and one crustacean. Toxicity values ranged from 724 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 410 µg/L, 540 µg/L, 640 µg/L and 810 µg/L, respectively. The 95% species-protection level for iron in marine water (540 µg/L) is recommended when assessing ecosystems that are slightly to moderately disturbed. 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.

Further toxicity testing using potentially sensitive life stages and species, such as sea urchins and oysters, is recommended to further improve 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 of 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).

Natural sources of iron include the 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). Catchment erosion and associated flood plumes introduce significant amounts of iron into coastal waters. In open oceans, major inputs of iron include atmospheric deposition, continental shelf sediments, hydrothermal vents and rivers (Worsfold et al. 2014). Anthropogenic releases of iron are mainly due to burning of fossil fuels, acid mine drainage, industrial waste discharges, and corrosion of iron and steel. Despite the high mineral abundance and biological demand for iron, the concentration of dissolved iron in non-contaminated seawater is very low (0.017–0.022 μ g/L in open oceans, increasing to 0.20–0.60 μ g/L in coastal waters; Kuma et al. 1998). The low dissolved concentrations are due to the very low solubility of iron in marine water (0.01–0.03 μ g/L) (Liu and Millero 2002).

Iron exists in 2 oxidation states – iron(II) (Fe²⁺) and iron(III) (Fe³⁺). Iron(III) dominates under oxic conditions. Under anaerobic conditions, iron(II) dominates and has higher water solubility. At the pH of seawater, iron(II) rapidly oxidises to form iron(III). Thus, iron(II) is expected to be present in negligible concentrations (Stumm and Morgan 1996). Iron(III) rapidly hydrolyses to form amorphous iron(III) hydroxide precipitates and colloidal iron oxyhydroxides, which remain in suspension or flocculate and deposit over time. Worsfold et al. (2014) operationally defined size-fractionated speciation of iron into categories of truly soluble, dissolved, colloidal and total iron according to the pore size used to filter the seawater. Truly soluble iron is defined by 0.02- μ m pore size, dissolved iron is defined by 0.2- μ m (or also commonly 0.45- μ m) pore size, and the colloidal fraction is between 0.02- μ m and 0.2- μ m (or 0.45- μ m) pore size. Gledhill and Buck (2012) reported that colloidal (inorganic or organic) iron in estuarine and coastal waters comprised between 30% and 91% of the dissolved iron pool. Some 99% of the dissolved iron is complexed with organic iron-binding ligands of varying binding strengths in oceanic waters (Gledhill and Buck 2012).

The solubility of iron(III) oxyhydroxide in seawater is dependent on a number of physicochemical parameters. In seawater, iron solubility increases as pH and temperature decrease and as salinity (when salinity is greater than 18 ppt) and dissolved organic matter increase (Liu and Millero 2002). In addition, the speciation of iron in seawater is influenced by water-quality parameters such as pH, redox potential (Eh), dissolved oxygen, carbon dioxide, sulfur species, natural organic matter, photoreduction of organic iron complexes and microbial activity (Norman et al. 2014).

The low solubility of iron in marine water has obvious implications for toxicity testing conducted with iron concentrations that exceed the limit of solubility (i.e. $> 0.03 \,\mu g/L$). Very few toxicity-test data identify the form of iron present in test solutions, which means that test organisms are likely exposed to a combination of dissolved, colloidal and precipitated iron. This confounds the interpretation of toxicity data, as these forms of iron likely have different modes of toxic action and, hence, different

effect levels. Therefore, the derived iron DGVs are based on potentially bioavailable iron that includes dissolved, colloidal and precipitated forms of iron.

There were no ANZECC and ARMCANZ (2000) default guideline values (DGVs) for iron in marine water. However, an interim indicative working level of 300 μ g/L for iron in freshwater was recommended, based on a Canadian water-quality guideline value for long-term exposure to total iron (CCREM 1987). 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 marine species (as per Warne et al. 2018). 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 A. 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

The mechanism of toxicity of iron is unclear. However, the current knowledge is described in the iron in freshwater DGVs technical brief (ANZG 2025).

2.2 Toxicity

Data on the toxicity of iron to marine species are limited but available for a range of taxonomic groups, including algae, rotifers, echinoderms, crustaceans, bivalve molluscs, gastropod molluscs, corals and fish. Data for bivalve molluscs dominate the available data.

High toxicity of iron has been reported for sea urchins (*Sphaerechinus granularis*, *Paracentrotus lividus*, *Psammechinus microtuberculatus*) – 72-hour embryo development NOECs (see 'Glossary and acronyms' for definitions) were $0.56-56~\mu g/L$ (Pagano et al. 1996). However, these data were based on nominal (i.e. not measured) concentration data, and tests were conducted using a concentration range – each treatment nominally increased in concentration by an order of magnitude, so their reliability is questionable. In contrast to the results of Pagano et al. (1996), Doyle (1999, as cited by Markich et al. 2002) reported a much higher NOEC (72-hour larval development) of 2,000 $\mu g/L$ for the sea urchin *Heliocidaris tuberculata*, which was also based on nominal concentrations. The mudskipper *Periophthalmus waltoni* also showed apparent high sensitivity to iron – a 96-hour LC50 was $6.5~\mu g/L$ (Bu-Olayan and Thomas 2008), but the data were confounded by pseudo-replication and also had questionable reliability.

Bivalves appear to be a relatively sensitive taxonomic group. Toxicity levels are generally in the range of hundreds of micrograms per litre. Wilson and Hyne (1997) reported 48-hour larval development NOECs of 100 μ g/L and 150 μ g/L for the oyster *Saccostrea glomerata* (formerly *Saccostrea commercialis*). However, the pH of the test water was only 6.5, as the results were from studies of acid leachates mixed with seawater. Markich (2021) determined no-effect concentrations (NECs) for 48-hour embryo larval development of 10 Australian bivalve species, comprising 2 oysters, one mussel, 2 cockles, one scallop and 4 clams. The NECs spanned a narrow range of 724–1,270 μ g/L. Kadar et al. (2010) showed no effects on the 48-hour larval development of the mussel *Mytilus galloprovincialis* up to a total iron concentration of 800 μ g/L but did not test any higher concentrations.

There are limited data on the chronic toxicity of iron to other taxa. The crab *Cancer anthonyi* was relatively sensitive to iron exposure – a 7-day NOEC for embryo hatching was 1,000 μ g/L. However, in contrast, the 7-day NOEC for mortality was 100,000 μ g/L (Macdonald et al. 1988). Corals have variable sensitivity to iron – EC10s/NOECs ranged from 2,750 μ g/L (*Platygyra daedalea*) to 18,700 μ g/L (*Acropora spathulata*), based on fertilisation (Leigh-Smith et al. 2018). The microalga *Isochrysis galbana* was insensitive to iron exposure – a 96-hour LOEC and NOEC (growth rate) were 75,000 μ g/L and 50,000 μ g/L, respectively (Keller et al. 2012). Rotifers also appear insensitive to iron exposure. Han et al. (2022) reported 7-day EC50 values for the rotifers *Brachionus plicatilis* and *Brachionus rotundiformus* of 59 mg/L and 55 mg/L total iron, respectively, in test water with a salinity of 17‰.

Unsurprisingly, acute toxicity is relatively low. Frias-Espericueta et al. (2003) reported a 96-hour LC50 for the shrimp *Litopenaeus vannamei* of approximately 45,000 μ g/L, while Francesconi and Edmonds (1995) reported 24-hour LC50s for the sea trumpeter *Therapon humeralis* of 11,800 μ g/L and 16,700 μ g/L. Toxicity to the fish involves gill clogging. The experiments were undertaken with iron(II) additions but this oxidised within 10 minutes to iron(III) precipitates.

3 Factors affecting toxicity

Factors that influence the speciation of iron in seawater (as described in section 1) will also influence bioavailability and toxicity. It remains unclear what forms of iron in marine water are responsible for toxicity to aquatic biota, as there is a paradox between the essential biological requirements for iron, the low concentrations of bioavailable iron, and what forms are bioavailable. Organisms have evolved mechanisms such as iron-specific membrane-transport proteins and the production of iron-binding organic ligands (siderophores) to meet their iron requirements. These mechanisms are likely to be adversely affected when iron concentrations exceed requirements. In terms of toxicity modifying factors, dissolved organic carbon reduces iron toxicity to freshwater species (Cardwell et al. 2023) and would likely have a similar effect in marine waters.

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 marine water 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 B, Table B1. 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 to derive default guideline values for iron in marine water

Taxonomic group	Species	Life stage	Duration (h)	Toxicity measure (test endpoint)	Final toxicity value (µg/L)
Microalga	Isochrysis galbana	Not applicable	96	NOEC (growth rate inhibition)	50,000
Cnidaria (coral)	Acropora spathulata	Gametes	5.5	EC10 (fertilisation)	18,700
Cnidaria (coral)	Platygyra daedalea	Gametes	5.5	NOEC (fertilisation)	2,750
Echinoderm (sea urchin)	Heliocidaris tuberculata	Embryo/larva	72	NOEC (larval development)	2,000
Mollusc (bivalve)	Anadara trapezia	Embryo	48	NEC (abnormalities)	935
Mollusc (bivalve)	Barnea australasiae	Embryo	48	NEC (abnormalities)	893
Mollusc (bivalve)	Fulvia tenuicostata	Embryo	48	NEC (abnormalities)	806
Mollusc (bivalve)	Hiatula alba	Embryo	48	NEC (abnormalities)	810
Mollusc (bivalve)	Irus crenatus	Embryo	48	NEC (abnormalities)	1,020
Mollusc (bivalve)	Magallana gigas	Embryo	48	NEC (abnormalities)	724
Mollusc (bivalve)	Saccostrea glomerata	Embryo	48	NEC (abnormalities)	738
Mollusc (bivalve)	Scaeochlamys livida	Embryo	48	NEC (abnormalities)	1,270
Mollusc (bivalve)	Spisula trigonella	Embryo	48	NEC (abnormalities)	948
Mollusc (bivalve)	Xenostrobus securis	Embryo	48	NEC (abnormalities)	896
Mollusc (gastropod)	Haliotis rubra	Embryo-larval (1 hour post fertilisation)	48	EC10 (normal development)	4,360
Crustacean (crab)	Cancer anthonyi	Embryo	168	NOEC (hatching)	1,000

There were chronic toxicity data for 16 marine species from 6 taxonomic groups (cnidarians, echinoderms, crustaceans, microalgae, bivalve molluscs and gastropod molluscs) and acute toxicity data for 2 marine species from 2 taxonomic groups (crustaceans and fish) that passed the quality

assessment (Appendix B, Table B1) (Warne et al. 2018). Best professional judgement was used in combination with data-quality assessment scores of 50% or higher to consider the inclusion of important representative taxonomic groups. Decisions relating to the inclusion or exclusion of specific data are detailed in the following paragraphs.

The available data emphasise the need to determine the form of iron and the mechanism of iron toxicity to marine biota, especially for potentially sensitive taxa and endpoints, such as sea urchin embryo development (Pagano et al. 1996). The apparent very high sensitivity of 3 sea urchin species (S. granularis, Paracentrotus lividus, Psammechinus microtuberculatus) came from only one publication (Pagano et al. 1996) that was not of acceptable quality, and the data were not used in the current derivation. However, a toxicity value for another sea urchin, H. tuberculata, which indicated lower sensitivity to iron (Doyle 1999, as cited by Markich et al. 2002), was included in the derivation. This value was sourced from the Australasian Ecotoxicology Database (Markich et al. 2002) and was deemed to be of acceptable quality. Nevertheless, further testing of echinoderm species is desirable, as negligible-effect values could be markedly below the lowest currently accepted data. The mudskipper (Periophthalmus waltoni) data referred to in section 2.2 (Bu-Olayan and Thomas 2008) also suggested very high sensitivity relative to other species, but the data did not pass the quality assessment and, consequently, the toxicity value was not used in the current derivation. Notably, the toxicity values for the sea urchins and mudskipper currently represent outliers in the overall iron toxicity dataset (for both freshwater and marine species), and the veracity of instances of such high iron aquatic toxicity would need to be confirmed before such data could be incorporated into the derivation of DGVs.

As noted in section 2.2, Han et al. (2022) reported iron toxicity values for the rotifers *B. plicatilis* and *B. rotundiformus*. However, the salinity of the test solutions (17‰) was well below the acceptable lower limit of 25‰ (Warne et al. 2018), so the data were excluded from the derivation. In contrast, the toxicity values for the corals *A. spathulata* and *Playgyra daedalea* were obtained in seawater with salinities of 37‰ to 39‰, which is slightly above the acceptable upper limit of 36‰ (Warne et al. 2018). The high salinity reflected the naturally high background salinity of the tropical reef flat where the corals were collected (Leigh-Smith et al. 2018). The influence of high salinity (> 36‰) on iron solubility and, hence, toxicity is unknown, but it is likely to be minimal compared to other stronger influences, such as organic complexation. Consequently, the coral data were included in the derivation.

The toxicity value (NOEC \geq 800 µg/L) for the mussel M. galloprovincialis from Kadar et al. (2010) was excluded from the derivation because the study used only 3 concentrations, increasing by an order of magnitude (i.e. 8 µg/L, 80 µg/L and 800 µg/L), and showed no effects at any of the concentrations. Moreover, there were numerous other bivalves represented in the dataset, as discussed in the following paragraph.

The inclusion of the iron toxicity data for 10 species of bivalve mollusc published by Markich (2021) resulted in 10 of the 16 species in the chronic toxicity dataset being bivalves (i.e. 63% of the species). Appendix C presents an assessment of the effect of inclusion of the Markich (2021) bivalve species on the SSD and associated protective concentration values. This assessment provided no strong argument to exclude the Markich (2021) bivalve data on the basis that they resulted in an over-representation of this taxonomic group or overly biased the DGVs. Consequently, the Markich (2021)

data were included in the final dataset used to derive the DGVs. As there were sufficient chronic toxicity data (n = 16) to derive the DGVs using the SSD method, the available acute toxicity data were not used in the derivation.

Speciation of iron is important. Organically complexed iron likely represents a significant proportion of the background dissolved iron concentrations (Gledhill and Buck 2012), while colloidal and particulate iron species dominate at the higher test concentrations. At the DGV concentrations, iron is in these latter forms. The toxic mechanisms of these forms and the truly dissolved forms are species dependent.

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 single 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. The bimodality coefficient (Freeman and Dale 2013) calculated on the log-10transformed values used in the SSD was 0.697, which was above the threshold of 0.555, indicating that the data may exhibit bimodality. This was attributed to the predominance of bivalve toxicity values within a very narrow range of iron concentrations (i.e. 700–1,300 μg/L) at the sensitive end of the SSD. While this could be interpreted as bivalves being more sensitive than other taxa, this observation may equally be an artefact of the unusually high proportion of bivalves in the dataset (all from a single study by Markich 2021) relative to other taxonomic groups. Moreover, there is other evidence, albeit from less reliable studies (i.e. Pagano et al. 1996; Bu-Olayan and Thomas 2008), to suggest that species from other taxonomic groups could be more sensitive than bivalves. Therefore, the distribution of bivalve data at the sensitive end of the SSD was not taken as evidence that (i) bivalves are more sensitive than other taxa and (ii) as a result, the dataset is bimodal. Overall, the dataset was assumed to be unimodal.

4.2 Species sensitivity distribution

The cumulative frequency SSD of the 16 chronic toxicity values from 6 taxonomic groups for iron in marine water reported in **Error! Reference source not found.** is shown in **Error! Reference source not found.** The SSD was plotted using the Burrlioz 2.0 software, and the model was judged to provide a poor fit to the dataset (**Error! Reference source not found.**).

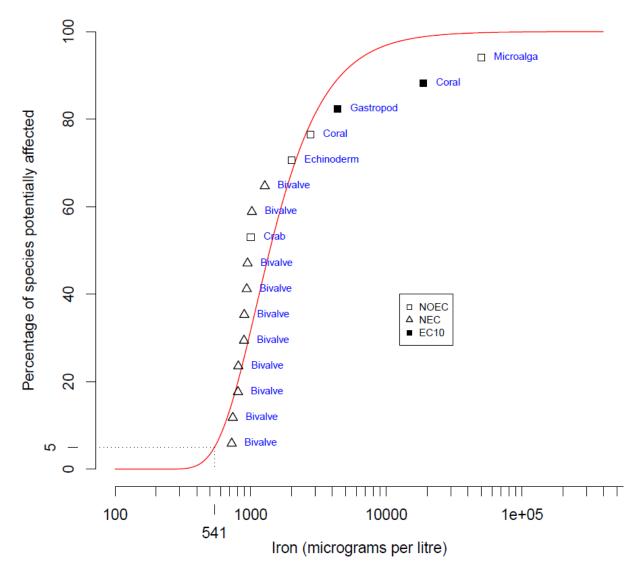


Figure 1. Species sensitivity distribution for iron in marine water

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 <u>Australian and New Zealand Guidelines for</u> 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 (540 μ g/L) is recommended for assessing ecosystems that are slightly to moderately disturbed.

Table 2. Toxicant default guideline values for iron in marine water with moderate reliability

Level of species protection (%)	DGV for iron in marine water $(\mu g/L)^{a,b}$
99	410
95	540
90	640
80	810

^a The default guideline value (DGVs) were derived using the Burrlioz 2.0 software and reported to 2 significant figures.

When applying the DGVs for iron to an unfiltered water sample, it is recommended to distinguish the iron associated with mineralised iron 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 (non-bioavailable) 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 guideline values to metals of low solubility, such as iron(III), are presented in Appendix A.

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/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 DGVs for iron in marine water have a moderate reliability classification (Warne et al. 2018) based on the outcomes for the following 3 criteria:

- Sample size 16 (preferred)
- Type of toxicity data chronic (EC10s, NOECs, NECs)
- SSD model fit poor (inverse Weibull).

^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 factions of iron should not be compared with the DGVs. See text and Appendix A for further details.

Glossary and acronyms

Term	Definition
Acute toxicity	A lethal or adverse sub-lethal effect that occurs 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.
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 <i>Australian and New Zealand Guidelines for Fresh and Marine Water Quality</i> . Formerly known as 'trigger values'.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
Endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
Guideline value (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'.)
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 material used in a toxicity test that has a statistically significant ($p \le 0.05$) adverse effect on the exposed population of test organisms as compared with the controls. All higher concentrations should also cause statistically significant effects.
No-effect concentration) (NEC)	The maximum concentration of a toxicant that causes no adverse effect in a target organism, based on a threshold parameter in a concentration–response model.
No-observed-effect concentration (NOEC)	The highest concentration of a toxicant used in a toxicity test that does not have a statistically significant ($p \le 0.05$) adverse effect on the exposed population of test organisms as compared with the controls.
Site-specific	Relating to something that is confined to, or valid for, a particular place. Site-specific guideline values are relevant to the specific 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 specific level of stimulus (or concentration of chemical) for a specified test period.
Toxicity value	A value defining the concentration of a toxicant that represents an estimate of its toxicity to a species.

Appendix A: 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 μ g/L; Liu and Millero 2002), iron(III) in freshwater (solubility < 0.05 μ g/L; Phippen et al. 2008), chromium(III) in freshwater (solubility < 5 μ g/L; Rai et al. 1989) and aluminium in marine water (solubility ~500 μ g/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 *Ceriodaphnia 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 for 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 doseresponse relationship for 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 exposures 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 guideline value, 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 B: toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table B1. Summary of toxicity data that passed the screening and quality assurance processes for iron in marine water

Taxonomic group	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	Salinity (‰)	рН	Concentratio n (μg/L) ^a	Reference
Microalga	Isochrysis galbana	Not applicable	96	Chronic	NOEC (growth rate inhibition)	F/2 media without trace metals and without EDTA	20	34	8.1–8.3	50,000	Keller et al. (2012)
Cnidaria (coral)	Acropora spathulata	Gametes	5.5	Chronic	EC10 (fertilisation)	Seawater	24–25	37–39	7.9–8.5	26,000	Leigh-Smith et al. (2018)
Cnidaria (coral)	Acropora spathulata	Gametes	5.5	Chronic	EC10 (fertilisation)	Seawater	24–25	37–39	7.9–8.5	12,000	Leigh-Smith et al. (2018)
Cnidaria (coral)	Acropora spathulata	Gametes	5.5	Chronic	EC10 (fertilisation)	Seawater	24–25	37–39 ^b	7.9–8.5	21,000	Leigh-Smith et al. (2018)
Cnidaria (coral)	Platygyra daedalea	Gametes	5.5	Chronic	NOEC (fertilisation)	Seawater	24–25	37–39 ^b	7.9–8.5	2,750	Leigh-Smith et al. (2018)
Echinoderm (sea urchin)	Heliocidaris tuberculata	Embryo/larva	72	Chronic	NOEC (larval development)	Filtered seawater	18 ± 2	34±2	_	2,000	Doyle (1999) as cited by Markich et al. (2002)
Mollusc (bivalve)	Anadara trapezia	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	935	Markich (2021)
Mollusc (bivalve)	Barnea australasiae	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	893	Markich (2021)

Taxonomic group	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	Salinity (‰)	рН	Concentratio n (μg/L)ª	Reference
Mollusc (bivalve)	Fulvia tenuicostata	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	806	Markich (2021)
Mollusc (bivalve)	Hiatula alba	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	810	Markich (2021)
Mollusc (bivalve)	Irus crenatus	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	1,020	Markich (2021)
Mollusc (bivalve)	Magallana gigas ^b	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	724	Markich (2021)
Mollusc (bivalve)	Saccostrea glomerata	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	738	Markich (2021)
Mollusc (bivalve)	Scaeochlamys livida	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	1,270	Markich (2021)
Mollusc (bivalve)	Spisula trigonella	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	948	Markich (2021)
Mollusc (bivalve)	Xenostrobus securis	Embryo	48	Chronic	NEC (abnormalities)	Seawater	21	30	7.9	896	Markich (2021)
Mollusc (gastropod)	Haliotis rubra	Embryo-larval (1 hour post fertilisation)	48	Chronic	EC10 (normal development)	Seawater	20	Not stated	7.3	4,360	Gorski and Nugegoda (2006)
Mollusc (gastropod)	Haliotis rubra	Embryo-larval (1 hour post fertilisation)	48	Chronic	NOEC (normal development)	Seawater	20	Not stated	7.3	1,280	Gorski and Nugegoda (2006)
Crustacean (crab)	Cancer anthonyi	Embryo	168	Chronic	NOEC (mortality)	Seawater	20	34	7.8	100,000	Macdonald et al. (1988)
Crustacean (crab)	Cancer anthonyi	Embryo	168	Chronic	NOEC (hatching)	Seawater	20	34	7.8	1,000	Macdonald et al. (1988)

^a The data reported here went through a further selection process, including calculation of geometric means and selection of the lowest value where necessary, to have one value for each species as shown in **Error! Reference source not found.**.

^b Geometric mean.

Appendix C: comparison of iron toxicity datasets with and without Markich (2021) bivalve data

The inclusion of the iron toxicity data for 10 bivalve species published by Markich (2021) resulted in almost two-thirds of the dataset (i.e. 10 of the 16 species) being bivalves, thus potentially resulting in an unacceptable bias towards this taxonomic group. Consequently, an assessment of the effect of inclusion of the Markich (2021) bivalve species on the SSD and associated protective-concentration (PC) values was undertaken. While there were other bivalve data from other studies, these were excluded as not being of appropriate quality. Species sensitivity distributions were constructed for the following 3 datasets: (a) full dataset with all the Markich (2021) bivalve data (n = 16), (b) dataset without the Markich (2021) bivalve data (n = 6) and (c) dataset with only the lowest value from each of the bivalve groupings in Markich (2021) (i.e. oyster, mussel, cockle, clam, scallop) (n = 11). The SSDs are shown in Figure C1, Figure C2 and Figure C3 and the derived PC values in Table C1.

Overall, the PC95 and PC90 values did not differ much between the 3 datasets, but there were more noticeable differences in the PC99 and PC80 values (Table C1). The SSD including all of the Markich (2021) data (Figure C1) had a steep slope at the lower end of the concentration range due to the bivalve data covering a relatively narrow concentration range. The steep slope for the full dataset resulted in a markedly narrower range of PC values compared to the dataset without the bivalve data (Table C1). There was very little difference between the 2 datasets that used all or a subset of the bivalve data. The fits of all 3 SSDs were poor. Therefore, combined with the sample sizes, the PC values for datasets (a) and (c) would be assigned as moderate reliability, while those for dataset (b) would be assigned as low reliability.

Overall, the results did not provide any compelling argument to exclude the Markich (2021) bivalve data or only include a subset of the data on the basis that including all the bivalve data would result in an over-representation of this taxonomic group or greatly influence the PC values. Moreover, excluding the bivalve data would result in DGVs that were of lower reliability than if the bivalve data are included. Consequently, the Markich (2021) bivalve data were included as individual species values in the final dataset used to derive the DGVs.

Figure C1. Species sensitivity distribution for the dataset with (n = 16) the Markich (2021) bivalve species data

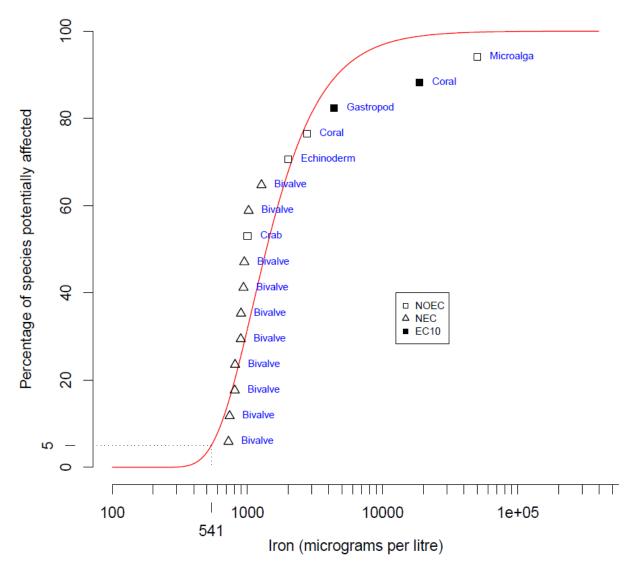


Figure C2. Species sensitivity distribution for the dataset (n = 6) without the Markich (2021) bivalve species data

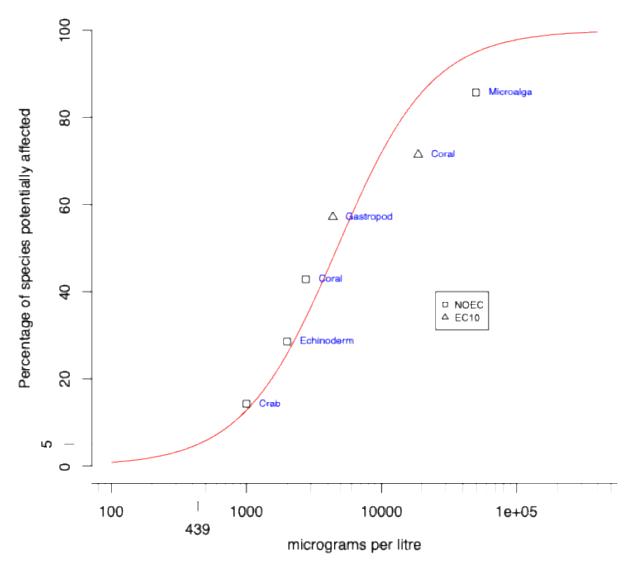


Figure C3. Species sensitivity distribution for the dataset (n = 11) with only the most sensitive value for each of the bivalve groupings

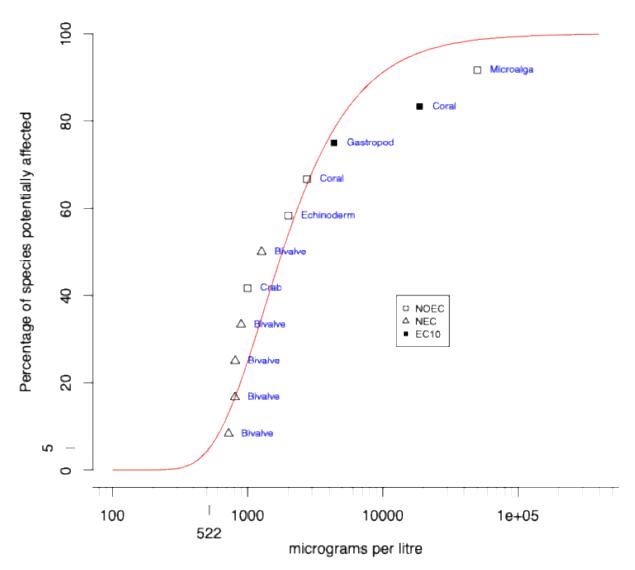


Table C1. Toxicant protective concentration values for iron in marine water

Percent species protection (%)	Toxicant protective concentration values (µg/L) for dataset including Markich (2021) bivalve data (n = 16)	Toxicant protective concentration values (µg/L) for dataset excluding Markich (2021) bivalve data (n = 6)	Toxicant protective concentration values (μg/L) for dataset including only most sensitive value for each bivalve grouping (n = 11)		
99	410	120	360		
95	540	440	520		
90	640	800	650		
80	810	1,500	890		

^a The protective concentration values were derived using the Burrlioz 2.0 software and rounded to 2 significant figures.

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