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### Response to public submissions on draft default guideline values for iron in freshwater

September 2025

Draft default guideline values (DGVs) for iron in freshwater were published on the Water Quality Guidelines website for a 3-month public consultation period. During this period, comments for the draft DGVs for iron in freshwater were received via public submission.

Responses to comments and any associated edits to the draft DGV technical brief are outlined in Attachment A, de-identified for public record. The responses and revisions have been approved by the original peer reviewers and the jurisdictional technical and policy oversight groups, and noted by the National Water Reform Committee.

The default guideline values for iron in freshwater are now published as final. For additional information on the publication process, please refer to the [pathway for toxicant default guideline value publication](https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/draft-dgvs).

The Water Quality Guidelines Improvement Program thanks all submissions for their valuable contribution to the development of default guideline values for the protection of aquatic ecosystems.

### Response to public submissions on draft default guideline values

**Toxicant: Iron in freshwater**

| **Submitter** | **Comment** | **Response** | **Action taken** |
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| **1.** | 1. The draft Australian & New Zealand guideline for iron has made notable advancement in describing freshwater iron toxicity for aquatic life and has captured the available toxicity data, except for the green algae, *Pseudokirchneriella subcapitata*, study by the Chilean Mining and Metallurgy Research Center (CIMM 11). | The data for *Pseudokirchneriella subcapitata* were not publicly available at the time the draft iron DGVs technical brief was drafted. However, some data from the study has since been published (Arbildua et al. 2017) and subsequently updated (Cardwell et al. 2023). As noted in the revised technical brief, the paper by Cardwell et al. (2023) updated previously published data (CIMM 1011, OSU 2013, Arbildua et al. 2017) by Cardwell’s co-authors. This was confirmed in writing by Cardwell. | The technical brief has been appropriately updated to include data for *Pseudokirchneriella subcapitata.* |
|  | 1. Although a detailed discussion is provided on toxicity modifying factors affecting iron toxicity to aquatic species, no specific conclusions could be drawn for *Ceriodaphnia dubia* and *Pimephales promelas* that could have assisted in deriving the site-specific guidelines for total iron. It would be helpful for the jurisdiction to apply the multiple linear regression (MLR) for evaluating the role of DOC, pH and hardness on iron toxicity, as one or more of these parameters have been shown to have important influence on iron bioavailability. Sufficient toxicity data are available for a range of pH, DOC and hardness values for running MLRs for both *C. dubia* and *P. promelas*. Also, consider MLR analysis for *P. subcapitata*. Subsequently, you can combine individual MLR models into a pooled MLR model, incorporating all toxicity endpoints for *C. dubia*, *P. promelas* and P*. subcapitata*. | The iron MLR information was not publicly available at the time the draft iron DGVs technical brief was drafted. We note that the Canadian guideline for iron in freshwater has since been published (ECCC 2024), while the MLR models that were used in the Canadian guideline were published in Brix et al. (2023).  Current policy for ANZG DGVs is that any bioavailability models that have been developed elsewhere (e.g. Nth America, Europe) need to be validated for Aust/NZ water quality types and species before they can be adopted here. Such validation work would take several years to complete and publish, and would require significant funding. In correspondence with the primary author of the Brix et al. (2023) paper (Kevin Brix, pers comm, 28 March 2024), Brix indicated strong support for such validation work, and noted that there are certain water types in Aust/NZ that he believes are not captured by the current MLRs and which would need to be assessed and the models potentially refined for Aust/NZ conditions (as was required and done for the draft nickel freshwater DGVs). Moreover, he believes that the current MLR for invertebrates may not be broadly applicable for this broad taxa grouping, and that validation with additional invertebrates would be valuable.  Several Australian and New Zealand metals toxicity experts were consulted on this issue, with the majority in strong favour of progressing the iron freshwater DGVs in the absence of locally validated MLRs, with a view to update the DGVs if the validation work can be completed. The general view is that the updated DGVs will be markedly better than the ANZECC/ARMCANZ (2000) GV (which was based on an interim Canadian value), especially given that the refined pH 2 extraction method has been validated/developed and incorporated into guidance accompanying the new DGV (see response to comment 5).  The preferred approach was to proceed with the finalising the derivation without the MLRs (i.e. so, no ability to correct for toxicity modifying factors), and update the DGVs if/once the necessary validation work is undertaken and published. Finally, it is worth noting that Aust/NZ now has DGVs for iron in freshwater that have a *Very high* reliability classification, compared to ANZECC/ARMCANZ (2000), in which the 1987 Canadian guideline value was recommended as an interim indicative working level. The decision to proceed with the DGVs based on the current knowledge is consistent with the precautionary principle. | The DGVs have not been updated to incorporate toxicity modifying factors. Justification for this has been included in the technical brief. |
|  | 1. The National Guidelines and Standard Office of Environment and Climate Change Canada developed a draft freshwater quality guideline for iron last year (2019). In response to public review comments, we hope to have additional toxicity data soon for improving the existing MLR models and developing a pooled MLR model, similar to what we did for lead. We hope our iron guidelines work would be helpful and please let us know if you have any questions. | Thank you for providing the links. They were useful.  See response to comment 2. | See action taken for comment 2. |
| **2.** | 1. The MCA supports the inclusion of iron as part of the process for new and revised toxicant DVGs to ensure that allowable values are accurate, reliable and appropriately protective of our aquatic ecosystems. However, the establishment of guideline values at this time is premature. It is therefore recommended that this assessment process for iron be paused.   The science related to iron toxicity in fresh and marine waters is still building. Significant international research is currently underway in the US, Canada and Europe to better understand the toxicity of bioavailable forms of iron, with expanded species toxicity testing across ranges of pH, DOC and water hardness using the Multiple Linear Regression (MLR) Model for Bioavailability. This work is expected to conclude by mid-2021.  Given that extensive detailed research is due to become available in the near-term which directly informs the current gaps in data, the MCA considers it would be appropriate to delay the finalisation of this DVG until findings from this research are released.  Guideline values inform regulatory values set for industry. Accordingly, finalising a DVG for iron in fresh and marine water prior to accessing the full suite of data may cause significant unnecessary impact to industry.  This is of particular concern where these values are translated to regulatory compliance thresholds, resulting in unnecessary non-compliances and affecting industry operation. Accordingly, DGVs should be evidence based to allow an informed assessment of actual environmental risk. | See response to comment 2.  Also, since the draft iron freshwater DGVs were derived, more toxicity data have been published. These data were assessed for quality and included in the DGVs where of acceptable quality.  In addition to single species data that were included in the DGVs derivation, some mesocosm and field data were used to help validate the DGVs.  Also, and as already noted in response to comment 2, the new DGVs are a marked improvement to the interim indicative working level that was recommended in ANZECC/ARMCANZ (2000). | Where new data were of acceptable quality and met the requirements of the Warne et al. (2018) derivation method, they were included in the DGVs derivation or to help validate the DGVs |
|  | 1. While the guiding process for the formulation of the current DGVs for iron is clear, the derived values are immature as they are currently based on incomplete data. Both the freshwater and marine water technical briefs acknowledge the complexity of deriving defensible guideline values for iron. This complexity arises as iron can exist in soluble, colloidal, particulate, adsorbed and mineral forms in environmental waters and toxicity may occur as a result of chemical or physical stresses. Mineral forms of iron are not toxic.   Given this complexity, the recommendation of the 95 per cent protection level for freshwater (700 µg/L) and marine water (180 µg/L), for slight to moderately disturbed ecosystems cannot be justified.  As iron is a sparingly soluble metal, filtration at 0.45 µm is not recommended to provide a full understanding of possible iron toxicity as it excludes precipitated and mineralised forms. In Appendix C (freshwater) and Appendix B (marine water), sample treatment by acid extraction at pH 2 is ‘considered adequate at this stage’. However, recent research has found that extraction at a pH of 4 is best suited to derivation of the toxic fraction for aluminium [1]. This may also be the case for iron.  It has also been suggested that use of a reducing agent (hydroxylamine hydrochloride) may be preferred to acid extraction to avoid dissolving suspended solids. Additional research is needed to confirm that extraction of iron at pH 2 is not overly conservative or under protective. Studies that correlate toxicity with pH 2 extraction are needed.  As referred to above, research is currently underway in the US, Canada and Europe to better understand the toxicity of bioavailable forms of iron, with expanded species toxicity testing across ranges of pH, DOC and water hardness using the Multiple Linear Regression (MLR) Model for Bioavailability. This work is expected to conclude mid-2021.  The MCA requests additional information on the proposed development of algorithm/s for incorporation of pH & DOC to assess bioavailability.  Once evidence-based values are drafted, their application should also be considered in the context of the Australian environment (e.g. major iron mineralised provinces) and recommend any necessary adjustments in their application.  [1] Rodriguez PH,  Arbildua JJ, Villavicencio G, Urrestarazu P, Opazo M, Cardwell AS, Stubblefield W, Nordheim E & Adams W 2019 *Determination of bioavailable aluminum in natural waters in the presence of suspended solids* Environmental Toxicology and Chemistry, 38, 1668–1681. | The USEPA (1991) pH 2 extraction method has recently been refined and validated for iron in freshwater and marine water (Balsamo-Crespo et al. 2023, ANZG 2025). It measures potentially bioavailable iron (i.e. dissolved, colloidal and precipitated iron). Additional research to correlate iron toxicity with the pH 2 extraction measurement was completed as part of a PhD project at Southern Cross University (by Balsamo-Crespo). Although the research is yet to be published, a short summary of the findings has been included in Appendix B, while explicitly acknowledging that the work is currently unpublished but expected to be published in 2025. The results were positive in that the pH 2 method was a better predictor of toxicity than either a standard 0.45 um filtered or total recoverable iron method. This further supports the use of the pH 2 method.  With regards to the recently-developed MLRs for iron toxicity, see response to comment 2. | The DGV technical brief (particularly Appendix B) has been updated to reflect the refined and validated pH 2 method (ANZG 2025) and how this should be used in conjunction with the iron freshwater DGVs.  Information on the fraction of iron recovered by the pH 2 method, is included.  Justification has been provided in the paper by Balsamo Crespo et al. for the use of pH 2 rather than pH 4 extractions and this is included in the discussions. |
|  | 1. **Overall recommendations**   The MCA recommends that proposed changes to the toxicant default guideline values for iron in fresh and marine waters (as contained in the Technical Briefs) should be postponed, pending the results of further research, to be completed in 2021.  The MCA would welcome the opportunity to work with the Australian Government to help complete guideline values for iron that are accurate, reliable and appropriately protective of our aquatic ecosystems. | See response to comments 4 and 5. | Refer to action taken for comments 4 and 5. |
| **3.** | 1. **Factors that influence the bioavailability of Iron:**   The role of DOC and pH (and potentially hardness) have been shown to influence iron bioavailability and toxicity. Given the high variability of iron complexes depending on the geology and land use it is recommended that further studies are undertaken characterising the bioavailability of iron in tandem with DOC and pH (and hardness) to decrease uncertainty and improve model reliability.  Further, given the local variability of inland waters in Northern Australia taking into account geology and land use and variability in modifying factors such as pH and Dissolved Organic Carbon (DOC), it is proposed that an assessment is undertaken to investigate the feasibility of applying a formula to calculate site specific guideline values where users can enter site specific DOC and pH factors to the guideline equation to output a site specific guideline value protective, this approach would be consistent with the proposed iron toxicity guidelines for Canada (Draft Federal Environmental Quality Guidelines, May 2019).  It is proposed that an assessment is undertaken to determine the value of establishing a guideline value for dissolved (<0.45µm filtered) iron as a secondary line of evidence when total iron GV is exceeded. This may be considered in situations where baseline monitoring has measured dissolved iron as per previous recommendations. Or in situations where for example, total iron DGV may be exceeded due to environmental factors such as first flush events where high load of suspended materials and high flow conditions exist. In these cases, the dissolved iron guideline may be referred to as an indicator for aquatic organisms. | See response to comment 2.  Regarding the suggestion to assess the value of “…establishing a guideline value for dissolved (<0.45µm filtered) iron as a secondary line of evidence when total iron GV is exceeded.”, this is unlikely to work given the low solubility of iron. Moreover, the recently validated pH 2 extraction method for iron (ANZG 2025) (see response to comment 5) should negate the need for different DGVs based on different fractions. | Refer to action taken for comments 2 and 5. |
|  | 1. **Proposed analytical methodology**   It is considered that further evidence should be presented and compared to other analytical methods, to justify that the proposed methodology for total iron is the most reliable and suitable for determination of bioavailable iron in complex freshwater systems and that the methodology can readily measure the bioavailable fraction in iron. This is to ensure that the analytical method provides a measure of the bioavailable fraction not a default analytical method that provides a conservative estimate of bioavailable Iron. | See response to comment 5. | Refer to action taken for comment 5. |
|  | 1. In conclusion, it is recommended that further research and / or validation of above-mentioned limitations is conducted, specifically:  * Investigation to characterise the bioavailability of iron in tandem with modifying factors (for example DOC and pH (and hardness)) to decrease uncertainty and improve model reliability, including investigation into the application of site-specific formula; and * Research and/or further presentation of evidence for proposed analytical methodology to ensure the methodology can readily measure the bioavailable fraction of iron.   Accordingly, it is recommended to defer the adoption of toxicant DGV for iron until such research/ validation has been completed. | See responses to comments 2 and 5. | Refer to actions taken for comments 2 and 5 |

**Additional improvements made while addressing public comments**

| **Issue** | **Response** | **Action taken** |
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| The original draft iron freshwater DGVs technical brief, which was drafted in 2020, was not as detailed as more recently drafted DGV technical briefs. | Additional information was added to the technical brief to make the level of detail more consistent with that in more recently drafted DGV technical briefs. This included, but was not limited to:   * The content of the Summary was improved. * Section 2.3 on general toxicity was expanded, including description of/summarising two mesocosm studies (Cadmus et al. 2018a, b; Kotalik et al. 2019) and a field study (Peters et al. 2011). * A modality assessment was added to Section 4.1. * In Section 4.3, the mesocosm and field studies were used to help validate the DGVS. * A second table was added to Appendix B with details of the mesocosm toxicity data reported by Cadmus et al. (2018a, b) and Kotalik et al. (2019). | As detailed in the “Response” column. |

### References

ANZECC/ARMCANZ 2000. Australian and New Zealand guidelines for fresh and marine water quality. Canberra (AU). National Water Quality Management Strategy Paper No 4.

ANZG (2025) [*Optimisation of the USEPA (1991) pH 2 extraction method for measuring potentially bioavailable iron (iron III)*](https://www.waterquality.gov.au/anz-guidelines/resources/publications/optimisation-us-epa-1991-ph-2-extraction-method-measuring-potentially-bioavailable-iron-2025), 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.

Balsamo Crespo E, Reichelt‐Brushett A, Smith RE, Rose AL & Batley GE 2023. Improving the measurement of iron (III) bioavailability in freshwater samples: Methods and performance. *Environmental Toxicology and Chemistry*, 42, 303-316.

Brix KV, Tear LM, DeForest DK & Adams WJ 2023. Development of multiple linear regression models for predicting chronic iron toxicity to aquatic organisms. *Environmental Toxicology and Chemistry*, 42(6), 1386–1400.

Cadmus P, Guasch H, Herdrich AT, Bonet B, Urrea G & Clements W H 2018a. 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.

Cadmus P, Brinkman SF & May MK 2018b. 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.

ECCC 2019. Federal Environmental Quality Guidelines – Iron. Canadian Environmental Protection Act, 1999, Environment and Climate Change Canada. Draft for public comment. <https://www.canada.ca/en/environment-climate-change/services/evaluating-existing-substances/federal-environmental-quality-guidelines-iron.html>.

Kotalik CJ, Cadmus P & Clements WH 2019. Indirect effects of iron oxide on stream benthic communities: capturing ecological complexity with controlled mesocosm experiments. *Environmental Science & Technology*, 53, 11532-11540.

Peters A, Crane M & Adams W 2011. Effects of iron on benthic macroinvertebrate communities in the field. *Bulletin of Environmental Contamination and Toxicology*, 86, 591-595.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW & Stauber JL 2018. Revised Method for Deriving Australian and New Zealand Water Quality Guideline Values for Toxicants – update of 2015 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, Canberra, 48 pp.