Australian Government
Department of Climate Change, Energy, the Environment and Water

Australian & New Zealand
GUIDELINES FOR FRESH & MARINE WATER QUALITY

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

February 2026

Draft default guideline values (DGVs) for ammonia 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 ammonia in freshwater were received via public submission.

Responses to comments and any associated edits to the draft DGV technical brief are outlined in this report, 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 Committee.

The default guideline values for ammonia 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.

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: Ammonia in freshwater

### Submission A:

| Comment | Response | Action taken |
|---|---|---|
| Only a fleeting mention is made of ammonia guideline revisions in the New Zealand context. The 'draft ammonia guidelines' state, "a revised guideline value … was proposed as an indicative value in New Zealand", yet that derivation (NIWA, 2014) is the sole basis for the ammonia toxicity attribute states, which have informed the New Zealand National Policy Statement for Freshwater Management (NPSFM) since they were proposed in 2013, coming into force in 2017, and being updated in 2020. It is suggested that the 'draft ammonia guidelines' better detail why they should not only supersede the ANZECC (2000) ammonia guidelines but also the NPSFM (2020) attribute states, | Noted. The suggestion to include more context around the NZ ammonia toxicity attribute is welcomed, and the technical brief has been updated with the following relevant context for NZ:<br>"In the New Zealand National Policy Statement for Freshwater Management 2024 (NPS-FM), numeric attribute states for total ammonia of 0.24 mg N/L and 0.40 mg N/L (at pH 8 and 20°C) have been incorporated as "national bottom line" values, with the former being relevant to an annual median concentration and the latter being relevant to an annual 95$^{th}$ percentile concentration. The process of incorporating new information for the NPS-FM involves independent consideration and regulatory changes, and is not formally linked to the development of DGVs under ANZG (2018)."<br><br>Once the ammonia DGVs have been published, they will automatically supersede the ANZECC/ARMCANZ (2000) DGVs, as they are based on improved data and knowledge. However, it will be a decision for NZ as to whether the new DGVs should be reflected in its National Policy Statement for Freshwater Management 2020 attribute, as this is separate to the ANZG Guidelines. | NZ-relevant text added to the technical brief. |
| While the case for the phasing out of NOEC data (Warne & van Dam, 2008) is potent, the use of NOECs prevails in the 'draft ammonia guidelines' – being necessary still due to data sufficiency issues. Important, then, is the consistency of data selection according to ANZECC's (2018) hierarchy. The basis for this submission is a review of the data selected for use in the 'draft ammonia guidelines'.<br>Querying why the following data used in the ANZECC/ARMCANZ (2000) ammonia GVs and the USEPA | The reviewer queried why data for five freshwater species were not included in the final dataset used to derive the DGVs. The five species (and taxonomic group) include:<br>a) *Ceriodaphnia acanthina* (cladoceran)<br>b) *Catostomus commersoni* (fish)<br>c) *Lepomis macrochirus* (fish)<br>d) *Oncorhynchus nerka* (fish)<br>e) *Coloburiscus humeralis* (insect) | |

| Comment | Response | Action taken |
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| (2013) ammonia water quality criteria, were not also included for the draft ANZG DGVs: | Consistency of data selection according to Warne et al. (2018) was applied; chronic NEC, EC/IC/LCx where x≤10, BEC10, EC/IC/LC15–20, and NOEC, followed by estimated values by dividing LC/IC/EC50 values by 5.<br>A new review of toxicity data published since 2016 identified additional toxicity values that were incorporated into the final DGV dataset for ammonia in freshwater. In summary:<br>• 15 new species (including one species identified by the public reviewers) were incorporated into the final dataset.<br>• new toxicity data were added to one existing species in the dataset and the final toxicity value for this species was changed.<br>• new toxicity data were added to three species but the final toxicity value for each of the three species did not change because more sensitive alternative toxicity values were available.<br>• two species were removed from the dataset because sufficient EC/IC/LCx where x≤10, EC/IC/LC15–20 and NOEC values were available without incorporating estimated values from LC50s.<br>Further details on the changes to the final dataset for ammonia in freshwater can be found in Appendix C. For changes to the derived DGVs and incorporation of new supporting information, refer to the revised technical brief submitted separately. | |
| *Ceriodaphnia acanthina* (Mount, 1982), noting that while ANZECC's methodology states, "preference should be given to ecotoxicity data published in peer-reviewed papers" (Warne et al., 2018), there is no preclusion stated, and neither has it prevented such use in recently published ANZECC draft guidelines. | Refer to Attachment A for response. | Toxicity data for the cladoceran *Ceriodaphnia acanthina* from Mount (1982) were not added to the final DGV dataset.<br>A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the current DGV was added to the technical brief's supporting information data entry (Excel) file. |

| Comment | Response | Action taken |
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| *Catostomus commersoni* (Reinbold & Pescitelli, 1982) | Refer to Attachment A for response. | Toxicity data for *C. commersoni* were not added to the final DGV dataset.<br>The NOEC values for two fish species, *Ictalurus punctatus* and *Lepomis cyanellus*, and the cladoceran, *Daphnia magna* were added to the dataset for these three species already listed in the DGV dataset. The toxicity values used to derive the DGV for each of these three species did not change because alternative and more sensitive toxicity values were available. |
| *Lepomis macrochirus* (Smith et al., 1984; Diamond et al., 1993) | Refer to Attachment A for response. | Toxicity data for *Lepomis macrochirus* reported in Smith et al. (1984) and Diamond et al. (1993) were not added to the final DGV dataset.<br>A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file. |
| *Oncorhynchus nerka* (Rankin, 1979); noting that ANZECC's methodology states, "as a general rule, toxicity data published prior to 1980 should not be included". That cut-off date is arbitrary; data should be assessed on merit alone. Regardless, ANZECC's rule has not prevented the use of pre-1980 data in the 'draft ammonia guidelines', other recently published ANZECC draft guidelines (hyperlink as above), or | Refer to Attachment A for response. | Toxicity data for *O. nerka* from Rankin (1979) passed the quality criteria screening and were added to the data entry (Excel) file. However, it was not added to the final DGV dataset because |

| Comment | Response | Action taken |
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| in recently promulgated ANZECC guidelines, such as that for boron | | sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated toxicity values from LC50s.<br>A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file. |
| *Coloburiscus humeralis* (Hickey et al., 1999), which finds narrative comment in the 'draft ammonia guidelines' but no place in the summarised toxicity data that passed the screening and quality assessment (Table B1). | Refer to Attachment A for response. | The toxicity value for *C. humeralis* (NOEC of 0.159 mg N/L at test pH and temperature) was added to the final DGV dataset. |
| Of the data that is presented in Table B1, it is recommended that further consideration is given to the following: | See responses below. | |
| *Carassius carpio*<br>ANZECC takes the geometric mean of two normalised LC50s (78 mg/L and 85 mg/L, derived from Hasan & McIntosh, 1986) and divide by five to obtain an estimated NOEC of 16 mg/L ammoniacal nitrogen. According to the hierarchy (Warne, 2018), a normalised NOEC (calculable from zero mortality data in Hasan & McIntosh, 1986 – refer their Table 2) takes preference | Refer to Attachment A for response. | Toxicity data for *C. carpio* from Hasan & MacIntosh (1986) was removed from the final DGV dataset because sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated toxicity values from LC50s.<br>A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added |

| Comment | Response | Action taken |
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| | | to the supporting information presented in the data entry (Excel) file. |
| *Oncorhynchus clarki*<br>ANZECC takes the geometric mean of three normalised LC50s (60 mg/L, 40 mg/L and 39 mg/L, derived from Thurston et al,1978) and divide by five to obtain an estimated NOEC of 9.6 mg/L ammoniacal nitrogen. According to the hierarchy, a normalised NOEC (Thurston et al., 1978 – _refer the narrative on page 364, which provides a mortality NOEC of 12.5 mg/L ammoniacal nitrogen at pH = 7.87) takes preference. | Refer to Attachment A for response. | Toxicity data for *O. clarkii* from Thurston et al. (1978) was removed from the final DGV dataset because sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated toxicity values from LC50s.<br>A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file. |
| *Villosa iris* and *Lampsilis fasciola*<br>Given the consideration of Warne and van Dam (2008), against "statistical significance methods" versus the preferred "confidence interval-based methods", the case for using a growth IC10 of < 0.4 mg/L, derived from data that included a control (ammoniacal nitrogen = 0.08 mg/L) with a mean length of 1.52 mm (standard deviation = 0.11 mm) and a low effect (ammoniacal nitrogen = 0.40 mg/L) mean length of 1.32 mm (standard deviation = 0.05 mm), for *Villosa iris*, is questionable. A similar case can be applied to the use of data for *Lampsilis fasciola*, in which the mean low effect (mortality = 85%) is associated with a large 95% confidence interval (standard deviation = 15%); it is the uncertainty around the resultant IC10 < 0.13 mg/L that weakens confidence in the overall species sensitivity distribution in the 'draft ammonia guidelines', and probably | Refer to Attachment A for response. | The final toxicity value for *V. iris* was not changed.<br>The final toxicity value for *L. fasciola* was not changed.<br>The toxicity value for *L. siliquoidea* was also re-assessed and not changed.<br>Text was added to the technical brief to (i) acknowledge that it was not uncommon for toxicity tests to have measurable concentrations of ammonia in the control treatments, and (ii) no attempt was made to adjust |

| Comment | Response | Action taken |
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| unnecessarily since there is other more robust data for bivalves, which are reasonably well represented. | | the derived toxicity values to account for ammonia in the control treatments. |

## Submission B:

| Comment | Response | Action taken |
|---|---|---|
| No issues with the factual basis on which DGVs are quantified and updated to reflect the best available knowledge.<br>However, concerns that the more stringent draft DGVs (compared to existing GVs) will present problems with compliance of operations if they are applied as defaults rather than tailored to the local conditions. … it is important that regulation remains focused on achieving outcomes appropriate for the specific locations in which it applies. | Noted. The ANZG (2018) Guidelines (and in fact the ANZECC/ARMCANZ 2000 Guidelines) contain comprehensive guidance on the need to tailor guideline values to local conditions, and that site-specific guideline values are preferred.<br>As noted by the additional comments in this submission, this information is not explicit in the default guideline value technical briefs. This issue is addressed in the subsequent comments. | |
| The current ANZG G'lines website carries a risk that users will access information selectively rather than taking time to absorb the full context – simply put, they may jump straight to DGVs without having to take due regard of the framework as a whole. This relates particularly to the ANZG Guidelines emphasis on deriving site-specific GVs. This preference should be clearly stated in DGV technical briefs, for example, as follows (existing text in black, proposed new text in red):<br><br>"The default guideline values (DGVs) and associated information in this technical brief should only be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (www.waterquality.gov.au/anz-guidelines). In particular, it should be noted that use of locally relevant site-specific guideline values (SSGVs) will be | The DGV technical briefs clearly state in two locations (Executive summary and Section 4.3 Default guideline values) that the DGVs should be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (www.waterquality.gov.au/anz-guidelines). While there is some merit in adding more specific information related to how DGVs should be applied, it is difficult to determine what information should be included and what should be excluded. Moreover, if national guidance changes, then any such specific guidance captured in a technical brief will be outdated and incorrect. Consequently, it is preferred that key guidance on the application of DGVs is only retained on the ANZG website. All users are encouraged to use the ANZG Guidelines website and understand the process of the water quality management framework (or at least the elements that are relevant to them). However, the ANZG Guidelines provides guidance only, and it is up to each jurisdiction to decide if and how the DGVs and associated guidance are implemented. | No changes made to DGV technical brief. |

| Comment | Response | Action taken |
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| preferable where these are available or may be derived in accordance with the guidelines."<br><br>The following key principles from the ANZG Guidelines could also be included in DGV technical briefs. For example:<br>"The Water Quality Guidelines aim to facilitate the productive and sustainable use of water resources while still maintaining the biological communities and ecological processes that the resource supports, consistent with the principles of ecologically sustainable development."<br>and<br>"Planning and management of water quality should be coordinated with management of other stressors, such as water quantity and habitat quality (for aquatic ecosystems). This will require close alignment of water quality planning with other planning initiatives that deal with the management of water resources and ecosystem health."<br>and<br>"The Water Quality Guidelines' DGVs have no formal legal status, and their use is not mandatory, unless specified as being so by the relevant state, territory or local jurisdictional authority." | | |

## Submission C:

| Comment | Response | Action taken |
|---|---|---|
| There are no allowances made for the proposed changes in climate that will cause variations to the concentration of toxicants identified in various water bodies and potential bioaccumulation levels in species, or increased dissipation rates of some toxicants from elevated temperatures. | DGV documents typically do not have the capability to also factor in the effects of climate change. These effects may be varied and not always easy to predict, and may also be different across geographical regions. However, for ammonia at least, the DGVs do account for temperature and pH, two physico- | No changes made to DGV technical brief. |

| Comment | Response | Action taken |
|---|---|---|
| | chemical variables in waterbodies that may change as a result of climate change. | |
| While the current methodology utilised to form the basis for the DGVs is not currently regulated, varying factors used in the calculations could potentially increase the probability that these DGV's are not at the highest accuracy level. This is especially the case for toxicants such as those that do become highly mobile within an environment, but have limited conclusive studies to confirm the concentration in aquatic environments, due to the mobility within soils, and difficulties to extract or analyse as a singular toxicant. Using a variety of modality assessments for the adoption in the assessment of slightly to moderate disturbed ecosystems, has the potential to form inaccuracies in the final DGV's for both copper and ammonia.<br>It is recommended that any inconsistencies in the data sets are only used in accordance with the Australian and New Zealand Guidelines for Fresh and Marine Water Quality, and not to set the basis for a regulatory framework, and only intended for the application for water quality guidelines for slightly-to-moderately disturbed ecosystems as outlined in the toxicant default guideline technical briefs. | The DGV derivation method represents a transparent and technically robust approach to deriving DGVs. However, it is not possible to prescribe a specific set of rules that can always be followed; for example, data selection decisions will often require an element of professional judgement, and this judgement can differ between experts. The requirement to document the basis of all such decisions and the comprehensive technical review process should ensure that the most reliable DGVs are derived, albeit there is still always an element of uncertainty associated with DGVs. This is why DGVs are promoted as a starting point for a water quality assessment and should be used alongside other lines of evidence in a weight of evidence process.<br>The ANZG website provides guidance on how toxicant DGVs should be applied, but it also acknowledges that the DGVs are not mandatory and that it is up to local jurisdictions to determine if and how they will be implemented. | No changes made to DGV technical brief. |
| Currently there is no immediate pathway that provides the agricultural sector with a comprehensive toxicant list to provide reference to the changes to toxicant values and what impacts these changes could impose to chemical application rates and residue levels on farm. A cohesive and informed reporting framework is needed in Australia to provide a comprehensive list of all ANZG toxicants, and what these changes will mean to on farm use of pesticides, herbicides, and fungicides.<br>The ability for farmers to obtain clear and concise data on toxicant guidelines is paramount, to ensure the continued efficiency of production of food, fibre and foliage and best management practices (BMP's), whilst also maintaining the | Please note that the ANZ Guidelines include Livestock Drinking Water and Irrigation Guidelines documents that are currently under review. Draft Livestock Drinking Water guidelines are available on: https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/primary-industries/stock-water-guidance. The draft Irrigation Water guidelines are available on: https://www.waterquality.gov.au/anz-guidelines/guideline-values/default/primary-industries/irrigation-guidelines (see Chapter 4.2 of Vol 1 and Chapter 9.2 of Vol 3). The revisions to these documents will address public comments as well as include updated PFASs information. | No changes made to DGV technical brief. |

| Comment | Response | Action taken |
|---|---|---|
| level of water quality that is not only fit for human consumption, but also livestock and irrigation to ensure the continued provision of world class agricultural products for domestic and export markets.<br>The development of a single national point of reference, which includes an environmental monitoring platform, and performance measures to help farms identify water quality issues will help assist the agricultural sector to balance chemical application and environmental protection that will help support sustainable agriculture into the future. | Until these updated guidelines are released, the ANZECC 2000 Guidelines still remain the authoritative national reference on livestock and irrigation water quality.<br>If you would like to receive updates on the progress of these guidelines (and haven't done so already), please subscribe to our mailing list here: https://www.waterquality.gov.au/anz-guidelines/about/subscribe.<br>Additional information for Queensland can be found on the Queensland Government's water monitoring website: WMIP: Queensland Government. | |

# **Attachment A** Ammonia in freshwater DGV's author responses to technical public comments



Aquatic Ecotox

# Response to technical public comments for the draft ammonia in freshwater default guideline values: Responses to specific data inclusions and omissions

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Prepared for the Commonwealth Department of Climate Change, Energy, the Environment and Water

January 2025

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# Background

The draft ammonia in freshwater default guideline values (DGVs) were released in September 2023 by the Department of Climate Change, Energy, the Environment, and Water (DCCEEW) for public consultation. Three submissions were received during the 3-month consultation period, one of which raised several queries related to the inclusion or omission of specific data from the derivation of the DGVs, along with decisions on why certain data values were selected over other possible values.

An initial review of the public comments (Appendix A) identified that some of the technical comments could be readily addressed, while other comments required:

(i) additional data to be reviewed for potential inclusion in the derivation, and/or

(ii) existing data used in the derivation to be re-assessed for their suitability in the derivation of the DGVs.

The original literature searches carried out to source toxicity data for inclusion in the derivation of the DGVs for ammonia in freshwater were undertaken in 2015. The outcomes of addressing the technical comments received during public consultation is likely to result in the re-derivation of the DGVs, and so it was also recommended that recent literature (post 2015) be sourced to identify any further toxicity data, and relevant supporting information, that could strengthen the re-derived DGVs.

The overarching project to revise and update the DGVs for ammonia in freshwater consists of two main tasks.

1. To address the specific technical comments received during the public comment phase of the draft ammonia in freshwater DGVs (summarised in Appendix A).
2. Identify and assess new sources of toxicity data and supporting information and incorporate relevant new information into the revised DGVs to further strengthen the DGVs and technical brief.

This report addresses Task 1 by responding to reviewer comments related to the inclusion or omission of toxicity values for specific freshwater species, Task 2 by summarising the changes to the final DGV dataset and technical brief.

# Approach

The technical comments received from public consultation were provided by DCCEEW and are shown in Appendix A. References of interest for each specific comment were sourced, where possible, and reviewed in detailed with particular focus on the criteria for data selection and data quality assessment described in Warne et al. (2018).

Chronic toxicity values for ammonia toxicity to freshwater species were used to derive the DGVs. Due to the availability of sufficient data, only chronic toxicity data from toxicity tests with measured concentrations of ammonia, pH and temperature were considered to derive the DGVs. This also ensured that toxicity values could be normalised to a standard pH and temperature (as described in USEPA, 2013) prior to incorporation into the species sensitivity distribution (SSD). Toxicity values

with a high (≥80%) or acceptable (50-79%) quality assessment score were added to the data entry Excel file for consideration and prioritisation of the final toxicity value for each individual species.

Where ambiguity on whether toxicity values should be included, or omitted, a brief justification was noted in the data entry file. Selection of the final toxicity value for individual species generally fell into two main categories.

(i) Factors within individual studies/publications. For example, evidence of reasonable concentration-response relationships, variability of the measured test endpoints (e.g. mortality, length) within and between treatments, and robust statistical methods to derive toxicity values (e.g. NOEC, LC50).

(ii) The influence of including, or excluding, specific data values on the SSD and resulting DGVs. For example, data may be excluded if it was the most sensitive data value in the SSD and has a high level of uncertainty; or unreliable toxicity value within a taxonomic group that is already well represented.

A literature review was also undertaken using Web of Science to source published ammonia toxicity data from 2016 to March 2024. The studies were screened for chronic toxicity values from toxicity tests suitable to derive DGVs. Supporting literature on the effects of ammonia in freshwater were also sourced and reviewed for use as supporting information that could be presented in the technical brief.

# Detailed Responses

## Technical comment 1

*General comment*

While the case for the phasing out of NOEC data (Warne & van Dam, 2008) is potent, the use of NOECs prevails in the 'draft ammonia guidelines' – being necessary still due to data sufficiency issues. Important, then, is the consistency of data selection according to ANZECC's (2018) hierarchy. The basis for this submission is a review of the data selected for use in the 'draft ammonia guidelines'.

Querying why the following data used in the ANZECC/ARMCANZ (2000) ammonia guideline values (GVs) and the USEPA (2013) ammonia water quality criteria, were not also included for the draft ANZG DGVs:

*General response*

Comments 1a to 1e query why data for five freshwater species were not included in the final dataset used to derive the DGVs. The five species (and taxonomic group) include:

f) *Ceriodaphnia acanthina* (cladoceran)

g) *Catostomus commersoni* (fish)

h) *Lepomis macrochirus* (fish)

i) *Oncorhynchus nerka* (fish)

j) *Coloburiscus humeralis* (insect)

Consistency of data selection according to Warne et al. (2018) was applied; chronic NEC, EC/IC/LCx where x≤10, BEC10, EC/IC/LC15–20, and NOEC, followed by estimated values by dividing LC/IC/EC50 values by 5.

A new review of toxicity data published since 2016 identified additional toxicity values that were incorporated into the final DGV dataset for ammonia in freshwater. In summary:

- 15 new species (including one species identified by the public reviewers) were incorporated into the final dataset.
- new toxicity data were added to one existing species in the dataset and the final toxicity value for this species was changed.
- new toxicity data were added to three species but the final toxicity value for each of the three species did not change because more sensitive alternative toxicity values were available.
- two species were removed from the dataset because sufficient EC/IC/LCx where x≤10, EC/IC/LC15–20 and NOEC values were available without incorporating estimated values from LC50s.

Further details on the changes to the final dataset for ammonia in freshwater can be found in Appendix C. For changes to the derived DGVs and incorporation of new supporting information, refer to the revised technical brief submitted separately.

## Technical comment 1a

### *Comment*

*Ceriodaphnia acanthina* (Mount, 1982), noting that while ANZECC's methodology states, "preference should be given to ecotoxicity data published in peer-reviewed papers" (Warne et al., 2018), there is no preclusion stated, and neither has it prevented such use in recently published ANZECC draft guidelines.

**Reference of interest:** Mount, D.I. (1982). Ammonia toxicity tests with *Ceriodaphnia acanthina* and *Simocephalus vetulus*. USEPA Duluth, MN (Letter to R. C. Russo USEPA Duluth, MN).

### *Response*

References provided by the Commonwealth at the start of the project (which predominantly included references in ANZECC/ARMCANZ 2000) did not include Mount (1982). At the time of scoring the ammonia toxicity data, neither a literature search, Google search or request via the CSIRO Library service could source the relevant document. A recent search (March 2024) also could not locate a publicly available source of the document.

Data from Mount (1982) were included in the USEPA (2013) water quality criteria for ammonia to generate a genus mean toxicity value for *Ceriodaphnia* sp. (Figure B1). However, the document could not be sourced and sighted to confirm the data reliability, resulting in a decision to exclude the reference from the current DGVs.

Inclusion of data for *C. acanthina*, if it were publicly available, would have a negligible effect on the final DGVs because the data point would sit near the top of the SSD (4$^{th}$ least sensitive species out of 27). *C. acanthina* is also slightly less sensitive than the two other cladoceran species represented in

the DGV dataset: *Ceriodaphnia dubia* (Mimmo et al., 1998[1]) and *Daphnia magna* (Gersich & Hopkins, 1986). Hence, *C. acanthina* is adequately protected by considering toxicity data for other species within this taxonomic group. Consequently, this species was not added to the dataset.

### *Outcomes and actions*

- Toxicity data for the cladoceran *Ceriodaphnia acanthina* from Mount (1982) was not considered further.
- A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the current DGV has been added to the technical briefs' supporting information data entry (Excel) file.

## Technical comment 1b

### *Comment*

*Catostomus commersoni* (Reinbold & Pescitelli, 1982)

**Reference of interest:** Reinbold, K.A. & Pescitelli, S.M. (1982). Effects of exposure to ammonia on sensitive life stages of aquatic organisms. Project Report, Contract No. 68-01-5832, Illinois Natural History Survey, Champaign, IL.

### *Response*

The report by Reinbold & Pescitelli (1982) could not be sourced at the time of scoring the toxicity data; however, it has since been sourced and is available publicly online.

Chronic toxicity data for *C. commersoni* (white sucker) was measured as growth (length) from the embryo life stage after 29 days development. The reported NOEC of 0.040 mg N/L (as un-ionised ammonia at the test pH and temperature) passed the data quality assessment criteria (72.5%). The study provided detailed measurements of relevant water quality parameters (pH, temperature, dissolved oxygen, and ammonia concentrations) throughout the test duration. However, there are some aspects of the study that were not ideal, and these are described below.

(i) The statistical derivation of the NOEC value was carried out using the Student-Newman-Keul's test which is not commonly used to derive NOEC values, and the level of significance (e.g. $p \leq 0.05$) was not reported.

(ii) The concentrations of ammonia in the control treatments were not measured.

(iii) The concentration-response relationship was minimal with a decrease in fish length from 15.6 and 14.8 mm in the two control treatments to 14.0 and 14.4 mm in the highest ammonia treatment (Figure B2). This equated to no more than a 10% decrease in fish growth over the five concentrations of ammonia tested (0.023 to 0.197 mg N/L) with the reported LOEC of 0.058 mg N/L reducing growth by only 3.3%. The study does not report the variability of fish measurements within treatments, and so it is difficult to assess the significance of these very small changes in growth.

A NOEC value of 0.040 mg N/L (un-ionised ammonia at test pH and temperature) equates to a normalised total ammonia toxicity value of 2.3 mg N/L (pH 7, 20°C). This value would make *C. commersoni* the most sensitive fish species out of 15 already included in the revised SSD. With fish

[1] Manning et al. (1996) was also considered for this species (refer to technical brief supporting information, data entry (Excel) file).

species well represented in the DGV, inclusion of an unreliable and possibly overly conservative NOEC was not warranted.

The study by Reinbold & Pescitelli (1982) also presented data for two other fish species, *Ictalurus punctatus* and *Lepomis cyanellus*, and the cladoceran, *Daphnia magna*. Toxicity data for these three species passed the data quality assessment criteria and provided reasonable NOEC estimates based on evidence of concentration-response relationships and reasonable percent effects at NOEC and LOEC concentrations. The NOEC values were added to the dataset for these three species that were already listed in the ammonia toxicity dataset. Despite the addition of these toxicity values, the final toxicity values for each of these three species used to derive the DGV did not change because alternative and more sensitive toxicity values were available.

*Outcomes and actions*

- Toxicity data for *C. commersoni* was not added to the final DGV dataset.
- The NOEC values for two fish species, *Ictalurus punctatus* and *Lepomis cyanellus*, and the cladoceran, *Daphnia magna* were added to the dataset for these three species already listed in the DGV dataset. The toxicity values used to derive the DGV for each of these three species did not change because alternative and more sensitive toxicity values were available.

## Technical comment 1c

*Comment*

*Lepomis macrochirus* (Smith et al., 1984; Diamond et al., 1993)

**Reference of interest:** Smith, W.E., Roush, T.H & Fiandt, J.T. (1984). Toxicity of ammonia to early life stages of bluegill (*Lepomis macrochirus*). Internal Report 600/X-84-175. Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, Duluth, MN.

**Reference of interest:** Diamond, J., Mackler D., Rasnake W., & Gruber, D. (1993). Derivation of site-specific ammonia criteria for an effluent-dominated headwater stream. *Environmental Toxicology and Chemistry*, 12(4): 649-658.

*Response*

The USEPA (2013) chronic criterion includes a 30-d ELS biomass value from Smith et al. (1984); however, the report could not be sourced at the time of scoring the toxicity data, or more recently (March 2024). ANZECC/ARMCANZ (2000) provides a range of chronic toxicity values for nine fish species, but *L. macrochirus* was not specifically mentioned (Section 8.3.7). The current DGV incorporates toxicity data for 15 fish species, and so the taxonomic group is already well represented. Consequently, the data for this species from Smith et al. (1984) was not considered further.

The chronic toxicity data from Diamond et al (1993) were not used because of uncertainties in the reliability of the data. The USEPA (2013) noted that “such factors as dilution water and test duration varied between tests at different temperatures and possibly confounded the results (see Appendix 1 of the 1999 update), raising doubts about the temperature comparisons for the vertebrates and invertebrates.”. This statement raises doubts about the applicability of normalising toxicity data from this study to a standardised pH and temperature, which is an essential step when integrating toxicity values for ammonia.

Recent re-review of the publication confirmed a lack of specific information and/or clarity for the data presented. For example, it was unclear if the NOEC value for survival and growth of *L. macrochirus* was normalised to a standard pH or represented the test pH. While the manuscript describes extensive measuring of pH and temperature within the toxicity tests, the measurements are presented as a range of values for all test species, and across all acute and chronic tests (pH 7.14-8.21, 18.4-20.8°C) making the specific test conditions for *L. macrochirus* difficult to confirm. Fish are currently well represented in the DGV and inclusion of a potentially compromised toxicity value may not be warranted. Consequently, the data for this species from Diamond et al. (1993) was not considered further.

Diamond et al. (1993) also reported chronic toxicity to *Rana pipiens* (frog), *Crangonyx* spp. (amphipod), and *Procamberus clarkii* (crayfish). However, these species were also not quoted in ANZECC/ARMCANZ (2000). Because of the reasons provided above for *L. macrochirus*, data for these species from Diamond et al. (1993) were also not considered further.

### *Outcomes and actions*

- Toxicity data for *Lepomis macrochirus* reported in Smith et al. (1984) and Diamond et al. (1993) was not considered further.
- A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file.

## Technical comment 1d

### *Comment*

*Oncorhynchus nerka* (Rankin, 1979); noting that ANZECC's methodology states, "as a general rule, toxicity data published prior to 1980 should not be included". That cut-off date is arbitrary; data should be assessed on merit alone. Regardless, ANZECC's rule has not prevented the use of pre-1980 data in the 'draft ammonia guidelines', other recently published ANZECC draft guidelines (hyperlink as above), or in recently promulgated ANZECC guidelines, such as that for boron.

**Reference of interest:** Rankin, D. (1979). The influence of unionized ammonia on the long-term survival of sockeye salmon eggs. Fisheries and Marine Service Technical Report No. 912. Pacific Biological Station, Nanaimo, B.C., Canada.

### *Response*

The report by Rankin (1979) could not be sourced at the time of scoring the toxicity data; however, it has since been sourced and is available publicly online.

A chronic toxicity value for *O. nerka* (sockeye salmon) was measured as the median tolerance limit (TLm) after monitoring the mortality of *O. nerka* embryos over 66 days in treatment tanks consisting of four concentrations of ammonia, a control and a pH-buffer control. After applying a regression analysis (n=3, consisting of two ammonia treatments and a control) to the mortality data, a 62-d TLm toxicity value of 0.11 mg N/L as un-ionised ammonia (at test pH and temperature) was reported. This toxicity value passed the quality assessment criteria.

The TLm (representing the LC50) of 0.11 mg N/L (test pH and temperature) as un-ionised ammonia would equate to a normalised total ammonia value of 11.3 mg N/L (pH 7, 20°C). Applying a factor of

5 to convert the LC50 to an estimated NOEC/LC10 derives an estimated normalised total ammonia value of 2.3 mg N/L (pH 7, 20°C). This would make *O. nerka* the most sensitive fish species in the DGV dataset (n=15).

Following a review of published toxicity data up to March 2024, sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated no- or low-effect values from LC50 values. Therefore, the TLm toxicity value reported by Rankin (1979), and hence *O. nerka*, was not included in the final DGV dataset.

### *Outcomes and actions*

- Toxicity data for *O. nerka* from Rankin (1979) passed the quality criteria screening and was added to the data entry (Excel) file. However, it was not added to the final DGV dataset because sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated toxicity values from LC50s.
- A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file.

## Technical comment 1e

### *Comment*

*Coloburiscus humeralis* (Hickey et al., 1999), which finds narrative comment in the 'draft ammonia guidelines' but no place in the summarised toxicity data that passed the screening and quality assessment (Table B1).

**Reference of interest:** Hickey, C.W., Golding, L.A., Martin, M.L. and Croker, G.F. (1999). Chronic toxicity of ammonia to New Zealand freshwater invertebrates: a mesocosm study. *Archives of Environmental Contamination and Toxicology*, 37:338–351.

### *Response*

The study by Hickey et al. (1999) assessed invertebrates in mesocosms after exposure to ammonia for 29 days. Toxicity was measured as the number of mayflies in three concentrations of ammonia and a control. Numbers of adult *Deleatidium* sp., juvenile *Deleatidium* sp., and *Coloburiscus humeralis* (life stage not quoted) were reported (reproduced in Figure B3). There was a high variability in ammonia concentrations within treatments over the 29-day exposure; however, the authors noted that this should not influence the results.

Results for juvenile *Deleatidium* sp. (from the same study) were used to derive the current DGVs, with a NOEC value of 0.066 mg N/L (un-ionised ammonia at test pH and temperature). The results showed a clear concentration-response relationship (Figure B3) providing confidence in relating ammonia concentration with toxicity to this species' life stage.

In contrast, the concentration-response relationship for *C. humeralis* was not as clear, especially at the low and medium ammonia treatment concentrations (Figure 3B) and so results for this species were originally omitted from the DGV. Upon re-evaluation, the high ammonia treatment concentration significantly reduced the abundance of *C. humeralis* and a NOEC of 0.159 mg N/L (un-ionised ammonia at test pH and temperature) was considered appropriate for inclusion in the

revised final DGV dataset. This NOEC value was equivalent to 7.2 mg N/L when normalised to pH 7 and 20°C.

Results for adult *Deleatidium* sp. did not show a concentration-response relationship and were ultimately not considered further (Figure B3). The lack of a concentration-response relationship was also acknowledged by the authors (and EC50, EC25 values were not reported).

*Outcomes and actions*

- The toxicity value for *C. humeralis* (NOEC of 0.159 mg N/L at test pH and temperature) was added to the final DGV dataset.

## Technical comment 2

*General comment*

Of the data that is presented in Table B1 of the Ammonia in Freshwater Technical Brief, it is recommended that further consideration is given to the final data value selected for the following species (taxonomic group):

a) *Carassius carpio* (fish)

b) *Oncorhynchus clarki* (fish)

c) *Villosa iris* and *Lampsilis fasciola* (bivalves)

*General response*

Comments 2a to 2c query the selection of the toxicity value (e.g. NOEC, LC50) used in the derivation of the DGVs over other potential toxicity values reported by, or inferred, by the original publication. Specific responses are provided below for each species or taxonomic group.

### Technical comment 2a

*Comment*

*Carassius carpio*[2]: ANZECC/ARMCANZ (2000) took the geometric mean of two normalised LC50s (78 mg/L and 85 mg/L, derived from Hasan & McIntosh, 1986) and divided it by five to obtain an estimated NOEC of 16 mg/L ammoniacal nitrogen. According to the hierarchy (Warne, 2018), a normalised NOEC (calculable from zero mortality data in Hasan & McIntosh, 1986 – refer their Table 2) takes preference.

**Reference of interest:** Hasan, MR & MacIntosh, DJ 1986. Acute toxicity of ammonia to common carp fry. *Aquaculture*, 54, 97–107.

*Response*

While NOEC values are preferred over LC50 values (Warne et al., 2018) a NOEC value was not quoted in Hasan & MacIntosh (1986). The NOEC is defined as the concentration tested where the response

[2] Hasan & MacIntosh (1986) refer to the fish species as *Cyprinus carpio* (common carp) which is used throughout this report and in the DGV documents.

is statistically indistinguishable from the control response (Warne et al., 2018). A summary of the ammonia toxicity to *Cyprinus carpio* was provided in the publication (and reproduced in Figure B4) but the lack of mortality data for individual replicates prevented the analysis of an independent statistically derived NOEC. Even if the data for each replicate was provided, hypothesis testing with only two replicates would not be ideal.

Although applying a default ratio of 5 to the LC50 values is also not ideal, this approach was used in the DGV derivation. Assuming an observational NOEC of 0.43 and 1.0 mg/L (for Tests 1 and 2, respectively) at the test pH was used instead, the values are only slightly higher (less sensitive) than the toxicity value of 0.34 mg N/L for *C. carpio* derived by dividing the LC50 by 5. Hence, the LC50 divided by 5 provided a reasonable estimate of the toxicity value for this species.

Following a review of published toxicity data up to March 2024, sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated no- or low-effect values from LC50 values. Therefore, the LC50 toxicity value reported by Hasan & MacIntosh (1986), and hence *C. carpio*, was no longer included in the final DGV dataset.

### *Outcome and actions*

- Toxicity data for *C. carpio* from Hasan & MacIntosh (1986) was removed from the final DGV dataset because sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated toxicity values from LC50s.
- A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file.

## Technical comment 2b

### *Comment*

*Oncorhynchus clarkii:* ANZECC/ARMCANZ (2000) took the geometric mean of three normalised LC50s (60 mg/L, 40 mg/L and 39 mg/L, derived from Thurston et al,1978) and divided by five to obtain an estimated NOEC of 9.6 mg/L ammoniacal nitrogen. According to the hierarchy, a normalised NOEC (Thurston et al., 1978 – _refer the narrative on page 364, which provides a mortality NOEC of 12.5 mg/L ammoniacal nitrogen at pH = 7.87) takes preference.

**Reference of interest:** Thurston, R, Russo, R and Smith, C. (1978). Acute toxicity of ammonia and nitrite to Cutthroat trout fry. *Transactions of the American Fisheries Society*, 107, 361-368.

### *Response*

As highlighted by the reviewer, narrative in Thurston et al. (1978) (reproduced in Figure B5) states that 'no mortalities were observed among 1-g trout exposed to 12.5 mg/L $NH_3$-N (0.22 mg/L $NH_3$) for 36 days'. The statement follows a description identifying 'the highest concentrations in each test below which no mortalities occurred'.

The publication doesn't quote a statistical test, or level of significance (e.g. $p \leq 0.05$), to identify the highest concentration tested with no significant effect on fish mortality. The toxicity data for each ammonia treatment (and replicate) was not presented and so an independent statistical analysis of the data could not be carried out.

For this reason, the statistically derived LC50 values reported in Thurston et al. (1978) were quality assessed for suitability. This included four 29-d LC50 values and two 36-d LC50 values (Figure B6). Ultimately, the geometric mean of 9.6 mg N/L from four 29-d LC50 values (total ammonia, normalised to pH 7) was used as the final toxicity value for *O. clarkii*. This value is lower than the observational NOEC of 23.5 mg N/L (as total ammonia normalised to pH 7), providing protection for this species.

Following a review of published toxicity data up to March 2024, sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated no- or low-effect values from LC50 values. Therefore, the LC50 toxicity value reported by Thurston et al. (1978), and hence *O. clarkii*, was no longer included in the final DGV dataset.

### *Outcome and actions*

- Toxicity data for *O. clarkii* from Thurston et al. (1978) was removed from the final DGV dataset because sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs without the addition of estimated toxicity values from LC50s.
- A note acknowledging the inclusion of this species in the ANZECC/ARMCANZ (2000) GV for ammonia and omission from the revised DGV has been added to the supporting information presented in the data entry (Excel) file.

## Technical comment 2c

### *Comment*

*Villosa iris* and *Lampsilis fasciola:* Given the consideration of Warne and van Dam (2008), against "statistical significance methods" versus the preferred "confidence interval-based methods", the case for using a growth IC10 of < 0.4 mg/L, derived from data that included a control (ammoniacal nitrogen = 0.08 mg/L) with a mean length of 1.52 mm (standard deviation = 0.11 mm) and a low effect (ammoniacal nitrogen = 0.40 mg/L) mean length of 1.32 mm (standard deviation = 0.05 mm), for *Villosa iris*, is questionable. A similar case can be applied to the use of data for *Lampsilis fasciola*, in which the mean low effect (mortality = 85%) is associated with a large 95% confidence interval (standard deviation = 15%); it is the uncertainty around the resultant IC10 < 0.13 mg/L that weakens confidence in the overall species sensitivity distribution in the 'draft ammonia guidelines', and probably unnecessarily since there is other more robust data for bivalves, which are reasonably well represented.

**Reference of interest:** Wang, N., Ingersoll, C.G., Greer, I.E., Hardesty, D.K., Ivey, C.D., Kunz, J.L., Brumbaugh, W.G., Dwyer, E.J., Roberts, A.D., Augspurger, T., Kane, C.M., Neves, R.J. & Barnhart, M.C. (2007). Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry*, 26, 2048–2056.

### *Response*

The study by Wang et al. 2007 describes the chronic toxicity of ammonia to three mussel species: *V. iris*, *L. fasciola*, and *Lampsilis siliquoidea*. The reviewer's concerns are noted and the DGV authors also recall scrutinising over these values for a long time. Ultimately, evidence of a concentration-response relationship and robust statistical methods were prioritised when selecting the values.

Concentrations of ammonia above the detection limit were common within control treatments for most species reporting ammonia toxicity. A study by the same authors (Wang et al., 2011) with *L. siliquoidea* also reported concentrations (0.06 mg N/L) in the control treatment similar to those reported in Wang et al. (2007) (0.04 and 0.08 mg N/L). The influence of these low concentrations of ammonia in the control treatments on individual toxicity values was not investigated further.

The chronic toxicity values considered for *V. iris* and *L. fasciola* are summarised in Table 1, with the original data extracted from Wang et al. (2007) shown in Figure B7.

**Table 1.** Summary of toxicity values for *Villosa iris* and *Lampsilis fasciola* from Wang et al. (2007). The toxicity value used in the current DGV for each species is in bold.

| Mussel species | Endpoint | IC10 | NOEC |
|---|---|---|---|
| *V. iris* | Survival | 0.89 (0.84-0.92) | 0.81 |
| | Growth (length) | **<0.4** | <0.4 |
| *L. fasciola* | Survival | **<0.13** | 0.44 |
| | Growth (length) | NA[a] | NA |

[a] Not applicable. Growth (measured as shell length) did not show a concentration-response relationship; hence, the values reported for this endpoint were not considered.

The most sensitive endpoint for each species was selected, and there were no obvious reasons for prioritising NOEC values over the available IC10 values. Growth of *V. iris* clearly decreased with increasing ammonia concentration with the variability within treatments ≤7.2%. Survival of *L. fasciola* also decreased with increasing ammonia concentrations, although the survival decreased in only small increments at low concentrations and resulted in an IC10 value about four times lower than the NOEC. The NOEC of 0.44 mg N/L caused a 27% decrease in *L. fasciola* survival, which can be considered high for a 'no-effect' concentration. Survival at the lowest ammonia concentrations was 83, 77 and 73% of the control (where all mussels survived) with reasonable variability (<20%) within treatments (15, 5.8 and 15%, respectively). With consistently more than 10-20% effect at the lower concentrations, the IC10 of <0.13% was selected as a reasonable toxicity value.

Upon inspection of the toxicity data for the two mussel species, re-assessment of the third mussel species in Wang et al. (2007) indicated that the IC10 value of <0.13 mg N/L for *L. siliquoidea* in the current DGV may be overly conservative. The concentration-response relationship for survival of *L. siliquoidea* was clearly interrupted (Figure B7). While this toxicity value was ultimately averaged with another IC10 value from Wang et al. (2011), further consideration of the selected toxicity value from Wang et al. (2007) was warranted.

A substantial investment in developing toxicity tests with mussels has occurred over the past few years because of their loss in freshwater ecosystems and high sensitivity to a wide range of contaminants. While most of these studies have investigated acute toxicity (Raimondo et al., 2016; Wang et al., 2017; Popp et al., 2018; Salerno et al., 2020; Wang et al., 2021), some studies investigated chronic toxicity (Archambault et al., 2017; Wang et al., 2023). However, despite mussel

species consistently reported as the most sensitive taxonomic group to ammonia (USEPA, 2013: Wang et al., 2018; current DGV), additional chronic ammonia toxicity values were not available for comparison to the values reported for the three species in Wang et al. (2007).

*Outcome and actions*

- The final toxicity value for *V. iris* was not changed.
- The final toxicity value for *L. fasciola* was not changed.
- The toxicity value for *L. siliquoidea* was also re-assessed and not changed.
- Text was added to the technical brief to (i) acknowledge that it was not uncommon for toxicity tests to have measurable concentrations of ammonia in the control treatments, and (ii) no attempt was made to adjust the derived toxicity values to account for ammonia in the control treatments.

# Outcomes and Actions

A summary of the outcomes and actions completed, or underway, for each freshwater species is provided in Table 2.

**Table 2.** Summary of outcomes and actions in response to addressing technical comments on the draft ammonia in freshwater default guideline values following public consultation (September 2023).

| Comment, species | Outcome | Actions |
|---|---|---|
| 1 General | | |
| 1a *Ceriodaphnia acanthina* | • No change – species not added to DGV dataset | • Note added to DGV supporting information acknowledging why this species (and associated reference) was not considered in the DGV dataset. |
| 1b *Catostomus commersoni* | • No change – species not added to DGV dataset | • Note added to DGV supporting information acknowledging why this species (and associated reference) was not considered in the DGV dataset.<br>• The NOEC values for two fish and one cladoceran species were added to the data compilation; however, the final value toxicity value for these three species did not change because more sensitive alternative toxicity values were available. |
| 1c *Lepomis macrochirus* | • No change – species not added to DGV dataset | • Note added to DGV supporting information acknowledging why this species (and associated references) was not considered in the DGV dataset. |
| 1d *Oncorhynchus nerka* | • No change – species not added to DGV dataset | • Sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs and so estimated toxicity values from a TLm (i.e. LC50) value were not added to the final DGV dataset.<br>• Note added to DGV supporting information acknowledging why this species (and associated reference) was not considered in the DGV dataset. |
| 1e *Coloburiscus humeralis* | • Species added to the DGV dataset | • Toxicity data (NOEC) for this species was added to the DGV dataset. |
| 2a *Carassius carpio* | • Species removed from DGV dataset | • Potential NOEC reported were not statistically derived; hence, the LC50 value was assessed.<br>• Sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs and so this estimated toxicity value from LC50s was not added to the final DGV dataset. |

| | | |
|---|---|---|
| | | • Note added to DGV supporting information acknowledging why this species (and associated reference) was not considered in the DGV dataset. |
| 2b *Oncorhynchus clarkii* | • Species removed from DGV dataset | • Potential NOEC reported were not statistically derived; hence, the LC50 value was assessed.<br>• Sufficient IC/EC10, LC1, EC/LC20, and NOEC values were available to derive the DGVs and so an estimated toxicity value from LC50s was not added to the final dataset.<br>• Note added to DGV supporting information acknowledging why this species (and associated reference) was not considered in the DGV dataset. |
| 2c *Villosa iris* | • No change – toxicity value retained | • NA |
| 2c *Lampsilis fasciola* | • No change – toxicity value retained | • NA<br>• A general statement was added to the technical brief to acknowledge that ammonia concentrations in the control treatments were not uncommon. |

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# **Appendix A** – Technical public comments.

**Table A1.** Requirements to address technical public comments on the draft ammonia in freshwater DGVs

| Technical comment | Requirement to address |
|---|---|
| 1. While the case for the phasing out of NOEC data (Warne & van Dam, 2008) is potent, the use of NOECs prevails in the 'draft ammonia guidelines' – being necessary still due to data sufficiency issues. Important, then, is the consistency of data selection according to ANZECC's (2018) hierarchy. The basis for this submission is a review of the data selected for use in the 'draft ammonia guidelines'.<br>Querying why the following data used in the ANZECC/ARMCANZ (2000) ammonia GVs and the USEPA (2013) ammonia water quality criteria, were not also included for the draft ANZG DGVs: | Provide any general comments if necessary. |
| a) *Ceriodaphnia acanthina* (Mount, 1982), noting that while ANZECC's methodology states, "preference should be given to ecotoxicity data published in peer-reviewed papers" (Warne et al., 2018), there is no preclusion stated, and neither has it prevented such use in recently published ANZECC draft guidelines. | Based on discussions with technical manager, draft response to justify non-inclusion of data from this publication. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference**: Mount DI (1982) Ammonia toxicity tests with *Ceriodaphnia acanthina* and *Simocephalus vetulus*. USEPA Duluth, MN (Letter to R. C. Russo USEPA Duluth, MN). |
| b) *Catostomus commersoni* (Reinbold & Pescitelli, 1982) | Quality assess and determine the acceptability of the data from this publication to be included in the derivation. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference:** Reinbold, K.A. and S.M. Pescitelli. 1982a. Effects of exposure to ammonia on sensitive life stages of aquatic organisms. Project Report, Contract No. 68-01-5832, Illinois Natural History Survey, Champaign, IL. |
| c) *Lepomis macrochirus* (Smith et al., 1984; Diamond et al., 1993) | Based on discussions with technical manager, draft response to justify non-inclusion of data from these publications. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference:** Smith, W.E., T.H. Roush and J.T. Fiandt. 1984. Toxicity of ammonia to early life stages of bluegill (*Lepomis macrochirus*). Internal Report 600/X-84-175. Environmental Research Laboratory-Duluth, U.S. Environmental Protection Agency, Duluth, MN.<br>**Reference:** Diamond, J., Mackler D., Rasnake W., and Gruber, D. (1993). Derivation of site-specific ammonia criteria for an effluent-dominated headwater stream. *Environmental Toxicology and Chemistry*, 12(4): 649-658. |
| d) *Oncorhynchus nerka* (Rankin, 1979); noting that ANZECC's methodology states, "as a general rule, toxicity data published prior to 1980 should not be included". That cut-off date is arbitrary; data should be assessed on merit alone. Regardless, ANZECC's rule has not prevented the use of pre- | Quality assess and determine the acceptability of the data from this publication to be included in the derivation. If necessary, update the DGVs technical brief accordingly to reflect decision making. |

| Technical comment | Requirement to address |
|---|---|
| 1980 data in the 'draft ammonia guidelines', other recently published ANZECC draft guidelines (hyperlink as above), or in recently promulgated ANZECC guidelines, such as that for boron. | **Reference:** Rankin, D. (1979). The influence of unionized ammonia on the long-term survival of sockeye salmon eggs. Fisheries and Marine Service Technical Report No. 912. Pacific Biological Station, Nanaimo, B.C., Canada. |
| e) *Coloburiscus humeralis* (Hickey et al., 1999), which finds narrative comment in the 'draft ammonia guidelines' but no place in the summarised toxicity data that passed the screening and quality assessment (Table B1). | Re-consider the acceptability of the data from this publication. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference:** Hickey, C.W., Golding, L.A., Martin, M.L. and Croker, G.F. (1999) Chronic toxicity of ammonia to New Zealand freshwater invertebrates: a mesocosm study. *Archives of Environmental Contamination and Toxicology*, 37:338–351. |
| 2. Of the data that is presented in Table B1, it is recommended that further consideration is given to the following: | |
| *a) Carassius carpio*<br>ANZECC takes the geometric mean of two normalised LC50s (78 mg/L and 85 mg/L, derived from Hasan & McIntosh, 1986) and divide by five to obtain an estimated NOEC of 16 mg/L ammoniacal nitrogen. According to the hierarchy (Warne, 2018), a normalised NOEC (calculable from zero mortality data in Hasan & McIntosh, 1986 – refer their Table 2) takes preference | Based on discussions with technical manager, draft response to justify the approach that was originally taken. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference:** Hasan, MR & MacIntosh, DJ 1986. Acute toxicity of ammonia to common carp fry. *Aquaculture*, 54, 97–107. |
| *b) Oncorhynchus clarki*<br>ANZECC takes the geometric mean of three normalised LC50s (60 mg/L, 40 mg/L and 39 mg/L, derived from Thurston et al,1978) and divide by five to obtain an estimated NOEC of 9.6 mg/L ammoniacal nitrogen. According to the hierarchy, a normalised NOEC (Thurston et al., 1978 – _refer the narrative on page 364, which provides a mortality NOEC of 12.5 mg/L ammoniacal nitrogen at pH = 7.87) takes preference. | Based on discussions with technical manager, draft response to justify the approach that was originally taken. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference:** Thurston, R, Russo, R and Smith, C. (1978). Acute toxicity of ammonia and nitrite to Cutthroat trout fry. *Transactions of the American Fisheries Society*, 107, 361-368. |
| *c) Villosa iris* and *Lampsilis fasciola*<br>Given the consideration of Warne and van Dam (2008), against "statistical significance methods" versus the preferred "confidence interval-based methods", the case for using a growth IC10 of < 0.4 mg/L, derived from data that included a control (ammoniacal nitrogen = 0.08 mg/L) with a mean length of 1.52 mm (standard deviation = 0.11 mm) and a low effect (ammoniacal nitrogen = 0.40 mg/L) mean length of 1.32 mm (standard deviation = 0.05 mm), for *Villosa iris*, is questionable. A similar case can be applied to the use of data for *Lampsilis fasciola*, in which the mean low effect (mortality = 85%) is associated with a large 95% confidence interval (standard deviation = 15%); it is the uncertainty around the resultant IC10 < 0.13 mg/L that weakens | Based on discussions with technical manager, re-consider the data selection decisions for these species. If necessary, update the DGVs technical brief accordingly to reflect decision making.<br>**Reference:** Wang, N, Ingersoll, CG, Greer, IE, Hardesty, DK, Ivey, CD, Kunz, JL, Brumbaugh, WG, Dwyer, EJ, Roberts, AD, Augspurger, T, Kane, CM, Neves, RJ & Barnhart, MC 2007. Chronic toxicity of copper and ammonia to juvenile freshwater mussels (Unionidae). *Environmental Toxicology and Chemistry*, 26, 2048–2056. |

| Technical comment | Requirement to address |
| --- | --- |
| confidence in the overall species sensitivity distribution in the 'draft ammonia guidelines', and probably unnecessarily since there is other more robust data for bivalves, which are reasonably well represented. | |

# Appendix B – Supporting information reproduced from references of interest.

## Supporting information related to Comment 1a

**Appendix B. Chronic Toxicity of Ammonia to Aquatic Animals.**

| Appendix B. Chronic Toxicity of Ammonia to Aquatic Animals | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| **Species** | **Test and Effect** | **pH** | **Temp. (°C)** | **Total Ammonia (mg TAN/L)** | **Chronic value[a] Adjusted to pH 7 (all *organisms*) and 20°C (*invertebrates*) (mg TAN/L)** | **SMCV (mg TAN/L)** | **GMCV (mg TAN/L)** | **Reference** |
| Stonefly, *Pteronarcella badia* | 30-d Juv Survival | 8.04 | 12.1 | 133.8 | **207.0** | | | Thurston et al. 1984b |
| Stonefly, *Pteronarcella badia* | 24-d Juv Survival | 7.81 | 13.2 | 21.66 | **26.27** | 73.74 | 73.74 | Thurston et al. 1984b |
| | | | | | | | | |
| Water flea, *Ceriodaphnia acanthina* | 7-d LC Reproduction | 7.15 | 24.5 | 44.90 | **64.10** | 64.10 | | Mount 1982 |
| | | | | | | | | |
| Water flea, *Ceriodaphnia dubia* | 7-d LC Reproduction | 7.80 | 25.0 | 15.20 | **38.96** | | | Nimmo et al. 1989 |
| Water flea, *Ceriodaphnia dubia* | 7-d LC Reproduction | 8.57 | 26.0 | 5.800 | **52.15** | 45.08 | 53.75 | Willingham 1987 |
| | | | | | | | | |
| Water flea, *Daphnia magna* | 21-d LC Reproduction | 8.45 | 19.8 | 7.370 | **36.27** | | | Gersich et al. 1985 |
| Water flea, *Daphnia magna* | 21-d LC Reproduction | 7.92 | 20.1 | 21.70 | **47.40** | 41.46 | 41.46 | Reinbold and Pescitelli 1982a |

**Figure B1.** Extracted from USEPA (2013) water quality criteria showing the toxicity of cladoceran *Ceriodaphnia acanthina* compared to *Ceriodaphnia dubia* and *Daphnia magna*.

## Supporting information related to Comment 1b

Table 6. Hatchability, larval survival, development, and growth data for eggs and larvae of white sucker exposed to ammonia for 1 month.

| | Mean un-ionized ammonia (mg/L) | % hatch | % survival | % swimming up 72 h after hatch | Mean total length (mm) | Mean weight (mg) |
|---|---|---|---|---|---|---|
| A | Control | 96.5 | 65.4 | 65.0 | 15.6 | 8.7 |
| B | Control | 90.5 | 68.5 | 77.5 | 14.8 | 10.7 |
| A | 0.023 | 93.0 | 60.8 | 82.5 | 14.5 | 10.2 |
| B | 0.023 | 93.5 | 62.0 | 76.9 | 15.3 | 12.5 |
| A | 0.040 | 92.5 | 73.0 | 67.4 | 15.3 | 11.2 |
| B | 0.034 | 85.5 | 73.7 | 72.7 | 14.6 | 9.4 |
| A | 0.068 | 95.5 | 65.4 | 55.6[b] | 14.1[a] | 14.7 |
| B | 0.058 | 82.0 | 67.7 | 64.9[b] | 14.7[a] | 10.4 |
| A | 0.101 | 94.0 | 61.7 | 45.0[b] | 14.3[a] | 10.8 |
| B | 0.101 | 94.0 | 68.1 | 55.0[b] | 14.6[a] | 11.0 |
| A | 0.176 | 91.5 | 56.3 | 40.9[a,b] | 14.0[a] | 10.5 |
| B | 0.197 | 90.5 | 64.1 | 15.8[a,b] | 14.4[a] | 10.7 |

[a]Values significantly lower than controls (Student-Newman-Keul's test, $P = 0.05$).
[b]Values significantly lower than controls (2 x 2 contingency table using number swimming up rather than percentage, $P = 0.05$).

**Figure B2.** Extract from Reinbold & Pescitelli (1982) showing the toxicity of ammonia to the fish *Catostomus commersoni.*

## Supporting information related to Comment 1e

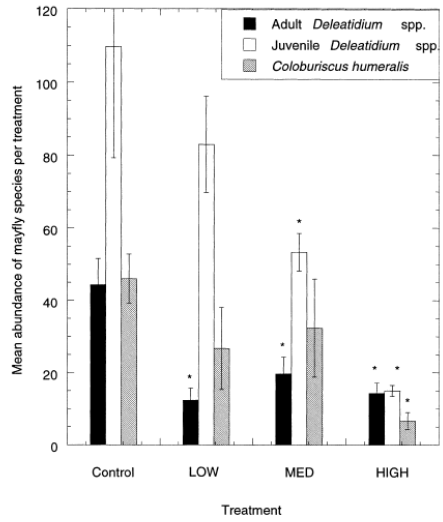



Fig. 4. Mean abundance of mayfly species per treatment (0.17 $m^2$) in the control, LOW, MED, and HIGH treatments (with ±SE). * = significantly different from control ($p < 0.05$)

**Figure B3.** Extract from Hickey et al. (1999) showing the mean abundance of mayflies in three ammonia treatments and a control mesocosm.

## Supporting information related to Comment 2a

TABLE 2

The percentage mortality of common carp fry after 168 h exposure to different concentrations of $NH_3$

| | Trial 1 | | Trial 2 | |
|---|---|---|---|---|
| Solution no. | $NH_3$ Conc. (mg $l^{-1}$ $NH_3$-N) Mean (range) | Mortality (%) | $NH_3$ Conc. (mg $l^{-1}$ $NH_3$-N) Mean (range) | Mortality (%) |
| Control | 0.00[1] (0.00[1]—0.00[2]) | 3.1 | 0.00[2] (0.00[2]—0.00[3]) | 0 |
| 1 | 0.19 (0.17—0.21) | 0 | 0.50 (0.49—0.52) | 0 |
| 2 | 0.23 (0.22—0.25) | 0 | 0.84 (0.78—0.90) | 0 |
| 3 | 0.43 (0.43—0.44) | 3.1 | 1.00 (0.94—1.05) | 0 |
| 4 | 0.69 (0.58—0.74) | 6.3 | 1.56 (1.47—1.64) | 15.6 |
| 5 | 1.39 (1.27—1.48) | 12.5 | 2.13 (2.12—2.13) | 81.3 |
| 6 | 2.54 (2.47—2.62) | 100 | 2.42 (2.35—2.49) | 100 |
| 7 | 4.80 (4.62—4.98) | 100 | 2.62 (2.60—2.64) | 100 |

**Figure B4.** Extracted from Hasan & MacIntosh (1986).

## Supporting information related to Comment 2b

for the 1-g fish and 0.36 for the 3-g fish. The highest concentrations in each test at and below which no mortalities occurred in 5 days were 15.5 and 12.5 mg/liter $NH_3$-N (0.28 and 0.22 mg/liter $NH_3$) for 1-g trout and 24.6 and 21.4 mg/liter $NH_3$-N (0.42 and 0.34 mg/liter $NH_3$) for 3-g trout. No mortalities were observed among 1-g trout exposed to 12.5 mg/liter $NH_3$-N (0.22 mg/liter $NH_3$) for 36 days.

**Figure B5.** Narrative in Thurston et al. (1978) indicating the highest concentrations tested with no observed mortalities for the fish *Oncorhynchus clarkii*.

TABLE 2.—*Acute toxicity of ammonia to cutthroat trout* (Salmo clarki).

| Test no. | Mean fish wt. (g) | No. fish per tank | Concentration range tested (mg/liter $NH_3$-N) | LC50 (95% confidence limits) (mg/liter $NH_3$-N) | | | LC50 (95% confidence limits) (mg/liter un-ionized $NH_3$) | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | 4-day | 29-day | 36-day | 4-day | 29-day | 36-day |
| 178 | 1.0 | 20 | 15.5–55.5 | 43.6 (40.5–46.9) | 30.8 (28.2–33.8) | 30.8 (28.2–33.8) | 0.80 (0.74–0.86) | 0.56 (0.52–0.62) | 0.56 (0.52–0.62) |
| 179 | 1.0 | 20 | 12.5–52.5 | 37.8 (35.7–40.0) | 32.2 (29.0–35.7) | 32.2 (29.0–35.7) | 0.66 (0.62–0.70) | 0.56 (0.51–0.62) | 0.56 (0.51–0.62) |
| 192 | 3.3 | 10 | 18.7–66.5 | 36.7 (33.0–40.8) | 21.6 (19.4–24.0) | | 0.62 (0.56–0.69) | 0.37 (0.33–0.41) | |
| 193 | 3.4 | 10 | 21.4–65.9 | 32.4 (29.2–36.9) | 21.4 | | 0.52 (0.47–0.59) | 0.34 | |

**Figure B6.** Extract from Thurston et al. (1978) showing six chronic LC50 values (four 29-d and two 36-d exposures) for the fish *Oncorhynchus clarkii*.

## Supporting information related to Comment 2c

Table 8. Mean survival and growth (shell length) of juveniles of three mussel species at the end of 28-d ammonia toxicity tests ($n$ = 4, except for test with wavy-rayed lampmussel, $n$ = 3). Chronic value (ChV) and 10% and 25% inhibition concentrations (IC10, IC25 with 95% confidence interval [CI]) are presented for each endpoint. Standard deviation is in parenthesis. An asterisk (*) indicates a significant reduction relative to control. Concentrations above the concentration causing significant reduction in survival were excluded from the hypothesis test for growth effect

| Rainbow mussel | | | Fatmucket | | | Wavy-rayed lampmussel | | |
|---|---|---|---|---|---|---|---|---|
| Mean concn. | Survival (%) | Length (mm) | Mean concn. | Survival (%) | Length (mm) | Mean concn. | Survival (%) | Length (mm) |
| 0.08 | 100 (0) | 1.52 (0.11) | 0.04 | 98 (5.0) | 0.95 (0.18) | 0.04 | 100 (0) | 0.72 (0.03) |
| 0.40 | 98 (5.0) | 1.32 (0.05)* | 0.13 | 83 (15) | 0.84 (0.07) | 0.13 | 83 (15) | 0.77 (0.04) |
| 0.81 | 98 (5.0) | 1.10 (0.08)* | 0.28 | 88 (10) | 0.95 (0.11) | 0.34 | 77 (5.8) | 0.74 (0.04) |
| 1.67 | 15 (24)* | — | 0.49 | 18 (21)* | — | 0.44 | 73 (15) | 0.72 (0.09) |
| 3.45 | 0* | — | 1.00 | 45 (39)* | — | 1.02 | 30 (36)* | — |
| 7.56 | 0* | — | 1.99 | 13 (25)* | — | 1.98 | 0* | — |
| ChV | 1.16 | <0.4 | ChV | 0.37 | 0.37 | ChV | 0.67 | 0.67 |
| IC10 (CI) | 0.89 (0.84-0.92) | <0.4 | IC10 (CI) | <0.13 | 0.32 (0.28–1.1) | IC10 (CI) | <0.13 | 0.48 (0.40–0.50) |
| IC25 (CI) | 1.0 (0.99–1.1) | 0.73 (0.56–0.84) | IC25 (CI) | 0.32 (0.30–0.36) | 0.44 (0.31–1.4) | IC25 (CI) | 0.39 (0.23–0.55) | 0.57 (0.52–0.59) |

**Figure B7.** Extract from Wang et al. (2007) showing the survival and growth (length) of *Villosa iris* (rainbow mussel), *Lampsilis fasciola* (wavy-rayed lampmussel) and *Lampsilis siliquoidea* (fatmucket) at each ammonia concentration tested (and control). Toxicity values reported include the IC10, IC25 and chronic value (ChV, mean of NOEC and LOEC).

# Appendix C – Summary of changes to the final dataset and technical brief

**Table C3 Summary of single chronic toxicity values, all species used to derive the default guideline values for total ammonia in freshwater (Dec 2024).**

| Taxonomic group | Species | Life stage | Duration (d) | Toxicity measure (endpoint) [a] | Toxicity value (mg N/L) (pH 7, 20°C) | Final toxicity value (mg N/L) (pH 7, 20°C) | Dec 2024 Comments/notes |
|---|---|---|---|---|---|---|---|
| Microalga | *Chlorella vulgaris* | Exponential growth (<120 h old) | 3 | EC10 (growth rate) | 165 [b] | 165 | Value changed from 640 following inclusion of a 72-h IC10 of 42 mg N/L to give a geometric mean of 165 |
| | *Pseudokirchneriella subcapitata* | Exponential growth (<72 h old) | 3 | EC10 (growth rate) | 560 | 560 | No changes |
| Flatworm | *Polycelis felina* | – | 30 | NOEC (mortality) | 1.2 | 1.2 | No changes |
| Cladoceran | *Ceriodaphnia dubia* | Neonate (<24 h) | 7 | NOEC (reproduction) | 42 | 42 | No changes |
| | *Daphnia magna* | Neonate | 21 | NOEC (reproduction and mortality) | 21 | 21 | Value not changed. Two new studies provided extra data for the reproduction endpoint. |
| Insect | *Chironomus riparius* | Larvae (1st instar) | 21 | NOEC (development-emergence ratio) | 29 [b] | 29 | New species; 2 studies with values of 25 and 35 mg N/L |
| | *Coloburiscus humeralis* | Mixed | 29 | NOEC (abundance) | 7.2 | 7.2 | New species: 1 study (added in response to reviewer comments) |
| | *Deleatidium sp.* | Juvenile | 29 | NOEC (abundance) | 3.2 | 3.2 | No changes to value<br>Endpoint redefined from mortality to abundance to better reflect the endpoint measured) |
| Amphipod | *Hyalella azteca* | Young (<1 week) | 70 | NOEC (reproduction) | 8.9 | 8.9 | No changes |
| Shrimp | *Neocardidina denticulata sinensis* | Adult | 21 | NOEC (survival) | 19 | 19 | New species |
| Gastropod | *Bellamya aeruginosa* | – | 26 | NOEC (survival) | 48 | 48 | New species |
| | *Fluminicola sp.* | Adult (6 months) | 28 | EC20 | 8.8 | 8.8 | New species |

| Taxonomic group | Species | Life stage | Duration (d) | Toxicity measure (endpoint) [a] | Toxicity value (mg N/L) (pH 7, 20°C) | Final toxicity value (mg N/L) (pH 7, 20°C) | Dec 2024 Comments/notes |
|---|---|---|---|---|---|---|---|
| | | | | (growth-shell diameter) | | | |
| | *Fontigens aldrichi* | Mixed | 28 | LC20 (survival) | 2.8 | 2.8 | New species |
| | *Lymnaea stagnalis* | Young (<1 week) | 28 | EC20 (growth-shell diameter) | 31 | 31 | New species |
| | *Physa gyrina* | Young (2-3 weeks) | 28 | EC20 (growth-shell diameter) | 38 | 38 | New species |
| | *Potamopyrgus antipodarum* | – | 40 | NOEC (immobility) | 4.4 | 4.4 | No changes |
| | *Pyrgulopis robusta* | Mixed | 28 | LC20 (survival) | 14 | 14 | New species |
| | *Taylorconcha serpenticola* | Mixed | 28 | LC20 (survival) | 12 | 12 | New species |
| Bivalve | *Lampsilis fasciola* | Juvenile | 28 | IC10 (mortality) | <0.43 | 0.43 | No changes<br>Value was re-assessed following reviewer comments |
| | *Lampsilis siliquoidea* | Juvenile (8 weeks) | 28 | IC10 (mortality) | 0.92 [e] | 0.92 | No change<br>Value was re-assessed |
| | *Musculium transversum* | – | 42 | NOEC (mortality) | 2.3 | 2.3 | No changes |
| | *Sphaerium novaezelandiae* | Young | 60 | NOEC (growth–length) | 1.1 | 1.1 | No changes |
| | *Villosa iris* | Juvenile | 28 | IC10 (growth–shell length) | <1.3 | 1.3 | No changes<br>Value was re-assessed following reviewer comments |

| Taxonomic group | Species | Life stage | Duration (d) | Toxicity measure (endpoint) [a] | Toxicity value (mg N/L) (pH 7, 20°C) | Final toxicity value (mg N/L) (pH 7, 20°C) | Dec 2024 Comments/notes |
|---|---|---|---|---|---|---|---|
| Fish | *Bidyanus bidyanus* | Juvenile | 39 | EC10 (growth rate) | 4.8 | 4.8 | New species |
| | *Carassius carpio* | Fry (larvae) | 7 | LC50 (mortality) | 82 [b] | 16 [c] | Species to be removed; estimated NOECs/LC10s. The value was also re-assessed following reviewer comments; however, a statistically derived NOEC could not be derived and used in place of the LC50 value. |
| | *Deltistes luxatus* | Larvae (late stage) | 30 | NOEC (mortality) | 14 | 14 | No changes |
| | *Esox lucius* | Fertilised eggs | 52 | EC20 (biomass–weight) | 20 | 20 | No changes |
| | *Ictalurus punctatus* | Embryo | 30 | NOEC (biomass–weight) | 5.2 | 5.2 | Data not changed. A new data value was available (5.5 mg N/L), but it was no more sensitive than the original value. |
| | *Lepomis cyanellus* | Embryo [f] | 44 | NOEC (biomass–weight) | 11 | 11 | Data not changed. A new data value was available, but it was the same as that reported here (refer to footnote f). |
| | *Lota lota* | Juvenile | 60 | EC10 (growth rate) | 3.2 | 3.2 | New species |
| | *Megalobrama amblycephala* | Juvenile | 63 | NOEC (growth rate) | <10 | 10 | New species |
| | *Micropterus dolomieue* | Embryo | 32 | NOEC (biomass–length) | 13 [b] | 13 | No changes |
| | *Notropis topeka* | Juvenile [d] | 30 | NOEC (growth rate) | 18 | 18 | No changes |
| | *Oncorhynchus clarkii* | Fry (1 g and 3 g) | 29 | LC50 (mortality) | 48 [b] | 9.6 [c] | Species to be removed; estimated NOECs/LC10s. The value was also re-assessed following reviewer comments; however, a |

| Taxonomic group | Species | Life stage | Duration (d) | Toxicity measure (endpoint) [a] | Toxicity value (mg N/L) (pH 7, 20°C) | Final toxicity value (mg N/L) (pH 7, 20°C) | Dec 2024 Comments/notes |
|---|---|---|---|---|---|---|---|
| | | | | | | | statistically derived NOEC could not be derived and used in place of the LC50 value. |
| | *Oncorhynchus mykiss* | Embryo | 35 | NOEC (growth–length) | 11 | 11 | No changes |
| | *Oreochromis niloticus* | Juvenile | 75 | NOEC (growth rate) | 3.8 | 3.8 | New species |
| | *Pelteobagrus fulvidraco* | Juvenile | 56 | NOEC (survival) | <4.1 | 4.1 | New species |
| | *Pimephales promelas* | Young (4 d post hatch) | 28 | LC1 (mortality) | 7.2 | 7.2 | No changes |
| | *Ptychocheilus lucius* | Young (8 d post hatch) | 28 | LC1 (mortality) | 21 | 21 | No changes |
| | *Xyrauchen texanus* | Young (9 d post hatch) | 28 | LC1 (mortality) | 14 | 14 | No changes |
| Amphibian | *Pseudacris regilla* | Embryo | 10 | NOEC (deformities) | 7.1 | 7.1 | No changes |
| | *Xenopus laevis* | Tadpole | 10 | NOEC (biomass–length and weight) | 86 [b] | 86 | No changes |

Orange = 15 new species (including 1 new species identified by the public reviewer)

Green = 1 existing species with updated toxicity values after incorporating new toxicity data

Blue = 3 existing species with new toxicity values, however the original values did not change (i.e. new data were no more sensitive than original)

Grey = 2 species to be removed to eliminate estimated toxicity values in the dataset

**Note: toxicity values are reported as total ammonia (mg N/L) normalised to pH 7 and 20°C where applicable (i.e. temperature applies to invertebrate data). Toxicity values are reported to two significant figures.**

–: no data / not reported.

**a** As described in Warne et al. (2018).

**b** Geometric mean.

**c** Chronic LC50 ÷ 5.

**d** Adult survival 30-d NOEC resulted in the same NOEC of 18 mg N/L.

**e** Geometric mean of two toxicity values; one is a 'less than' value.

**f** Embryo survival 31-d NOEC resulted in the same NOEC of 11 mg N/L.