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GUIDELINES FOR  
FRESH & MARINE  
WATER QUALITY

# Toxicant default guideline values for aquatic ecosystem protection

## Nitrate in freshwater

Technical brief

May 2024

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## Summary

Nitrate ( $\text{NO}_3^-$ ) occurs naturally in the environment and is produced and consumed through the nitrogen cycle. It is also anthropogenically produced as a fertiliser for agricultural use in the form of nitrate salts, such as ammonium, sodium, potassium, calcium and magnesium nitrate. The major anthropogenic sources of nitrate to surface waters are agricultural and urban runoff, municipal and industrial wastewaters and groundwater inputs. Nitrate concentration is an important indicator of nutrient enrichment of surface waters from agricultural sources and an indicator of the overall health of an ecosystem. As such, it is an important component of the management of freshwaters, requiring robust guideline values to support environmental planning and management.

Since the freshwater nitrate default guideline values (DGVs) for toxicity were derived and published in 2000 (ANZECC and ARMCANZ 2000), errors in the derivation were identified and new data have become available. For the DGVs reported here, chronic toxicity data were available for 36 species from 8 taxonomic groups. These comprised 3 microalgae, one cnidarian, 2 bivalve molluscs, one gastropod mollusc, 3 insects, 9 crustaceans, 13 fish and 5 amphibians. In general, fish and invertebrates show wide ranges in sensitivity to nitrate, with reported negligible-to-low effect (e.g.  $\leq \text{EC}_{25}$ ,  $\text{NOEC}$ ; see 'Glossary and acronyms' for definitions) values of 1–700 mg/L  $\text{NO}_3^-$ -N (nitrate-nitrogen) and 1–350 mg/L  $\text{NO}_3^-$ -N, respectively. The most sensitive species are the gastropod *Potamopyrgus antipodarum* and the rainbow trout *Oncorhynchus mykiss*, both with negligible-effect values of approximately 1 mg/L  $\text{NO}_3^-$ -N. The toxicity of nitrate to freshwater species decreases as water hardness increases, although it remains unclear whether this effect is due to hardness alone or whether other major ion variables such as ionic strength, chloride and alkalinity also play a role.

Data for potassium nitrate were excluded from this derivation of the nitrate DGVs due to potential toxicity introduced by potassium. Elevated potassium is not normally found in contaminated surface waters, and the toxicity of potassium nitrate is significantly higher than sodium nitrate to both fish and macroinvertebrates.

There was sufficient published chronic toxicity data available for use in DGV derivation, while the ANZECC and ARMCANZ (2000) derivation was based on acute data only.

Three sets of DGVs were derived for a range of water hardness, because the toxicity of nitrate depends on hardness, and there was data available for sufficient species at various water-hardness values. This is better than having a single set of DGVs across a very wide hardness range that would likely be under-protective for soft waters and over-protective for hard waters.

High reliability DGVs for nitrate in soft, moderately hard and hard freshwaters were derived based on chronic toxicity data for 14, 11 and 12 species belonging to 5, 5 and 6 taxonomic groups, respectively. The species sensitivity distributions (SSDs) were good fits to the toxicity data. The DGVs (as mg/L  $\text{NO}_3^-$ -N) for 99%, 95%, 90% and 80% species-protection levels are (respectively):

- soft waters (< 30 mg/L as  $\text{CaCO}_3$ ) – 0.64 mg/L, 1.1 mg/L, 1.5 mg/L and 2.3 mg/L
- moderately hard waters (30–150 mg/L as  $\text{CaCO}_3$ ) – 1.0 mg/L, 2.6 mg/L, 4.2 mg/L and 7.1 mg/L
- hard waters (> 150 mg/L as  $\text{CaCO}_3$ ) – 18 mg/L, 29 mg/L, 38 mg/L and 56 mg/L.

The 95% species-protection level DGVs for nitrate should be used when assessing ecosystems that are slightly to moderately disturbed. However, given the uncertainties associated with the key

factors that affect nitrate toxicity, users should consider other major ion variables in their local waters to help determine the appropriateness of the relevant hardness-based nitrate DGV for their waters. These could include variables such as electrical conductivity, alkalinity and chloride concentration. Further information regarding this is provided in this technical brief. Finally, it must be emphasised that the DGVs protect aquatic ecosystems against the toxicity of nitrate. They do not necessarily protect aquatic ecosystems against the negative effects of eutrophication that stem from the stimulatory effects that nitrate can have on plant and algal growth.

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# 1 Introduction

Nitrate occurs naturally in the environment and is produced and consumed through the nitrogen cycle. Nitrate represents the final product in the process of nitrification, which converts ammonia to nitrite and then nitrate. As a result, nitrate tends to be present in aquatic ecosystems at higher concentrations than ammonia and nitrite under oxic conditions (Camargo et al. 2005). Under hypoxic conditions (e.g. in the hypolimnion or remnant pools during post-flow phases), the lack of oxygen will limit the rate of nitrification, and ammonia concentrations will tend to be higher than nitrate (Harris 2001; US EPA 2023b).

Nitrate is highly soluble in freshwater, and its speciation does not change with pH. Most (> 99.5%) nitrate is present in its ionic form ( $\text{NO}_3^-$ ) (Richards et al. 2010). It is highly mobile, non-reactive and readily leaches from soil to groundwater, where it can discharge to environmentally sensitive surface waters (Adelana et al. 2020; Singh et al. 2022).

In addition to contaminated groundwater, major anthropogenic sources of nitrate to surface waters include runoff from agricultural and urban areas (from the use of fertilisers and production of animal waste), and discharges from municipal and industrial activities (including sewage effluent) (Camargo et al. 2005; Singh et al. 2022). Nitrate concentration is an important indicator of nutrient enrichment to surface waters from agricultural sources and an indicator of the overall health of an ecosystem. As such, it is an important component of the management of freshwaters, requiring robust guideline values to support environmental planning and management.

Besides being directly toxic, nitrate can affect aquatic ecosystems through its role as an essential nutrient for plants, where above-background concentrations and loads can cause excessive algal and plant growth (e.g. algal blooms). Concentrations of nitrate that result in this excessive growth are typically lower than those that are toxic to aquatic biota. Guideline values to protect aquatic ecosystems from the nutrient-related effects of nitrate may be derived by jurisdictions at catchment, basin or physiographic levels (see [Australia's inland waters](#) or [Jurisdictional information](#) in ANZG 2018 for further information). The current document focuses on nitrate as a direct toxicant.

Concentrations of nitrate in relatively undisturbed Australian and New Zealand freshwater systems are typically < 0.1 mg/L as  $\text{NO}_3^-$ -N (ANZECC and ARMCANZ 2000; Whitehead et al. 2021). However, concentrations are higher in disturbed systems, particularly those associated with agricultural or urban development. For example, Whitehead et al. (2021) reported the median concentrations of nitrate (as  $\text{NO}_3^-$ -N) in New Zealand waterways for pastoral, urban and exotic forest areas to be 0.37 mg/L, 0.69 mg/L and 0.24 mg/L, respectively, while maximum concentrations were 13 mg/L, 5.1 mg/L and 0.96 mg/L, respectively.

The ANZECC and ARMCANZ (2000) DGVs for nitrate were found to be erroneous (Hickey 2002) and were subsequently withdrawn. As a result, a revised derivation was reported by Hickey and Martin (2009), with a subsequent update by Hickey (2013). The 95% species-protection guideline value for nitrate (as  $\text{NO}_3^-$ -N) was 2.4 mg/L, based on chronic toxicity derived by Hickey (2013). The guideline values reported by Hickey (2013) formed the basis for the standards legislated as part of New Zealand's *National Policy Statement for Freshwater Management 2014* (MfE 2014, 2015). However, these values were never formalised as DGVs for Australia. Since the Hickey (2013) report, additional data have been published on the chronic toxicity of nitrate to freshwater species. These have been incorporated into the DGVs reported here. Therefore, the updated DGVs for nitrate in freshwater reported here build upon and supersede the guideline values derived by Hickey (2013).

It should be noted that all nitrate concentrations are reported in this document as nitrate-nitrogen ( $\text{NO}_3^-$ -N) rather than the nitrate ion ( $\text{NO}_3^-$ ) that is the basis for guidance derived by some other jurisdictions (e.g. CCME 2012). This difference in reporting convention makes no difference to the toxic sensitivity. A nitrate-ion concentration can be converted to nitrate-nitrogen by multiplying by 0.23 (or nitrate-nitrogen converted to nitrate ion by multiplying by 4.43).

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## 2 Aquatic toxicology

### 2.1 Mechanism of toxicity

The toxicity of nitrate to freshwater biota has been relatively well studied, despite nitrate being better known as a nutrient that can stimulate algal and plant growth than as a toxicant. However, the underlying mechanisms of toxicity are less well understood. Toxic effects in animals are likely due to the conversion of oxygen-carrying pigments (e.g. haemoglobin, hemocyanin) to forms that cannot carry oxygen (e.g. methemoglobin) and osmoregulatory effects due to the higher salinity associated with elevated nitrate levels (Camargo et al. 2005; CCME 2012). This toxic action is likely due to the reduction of nitrate to nitrite in the blood (Guillette and Edwards 2005) and a metabolic pathway involving production of nitric oxide (Hannas et al. 2010).

### 2.2 Toxicity

Nitrate is less toxic than ammonia and nitrite, the other 2 forms of nitrogen involved in nitrification. This is primarily due to the lower permeability of the gills to nitrate, which reduces its uptake by organisms with gills (Camargo et al. 2005). The available data for chronic nitrate toxicity concentrations span more than 3 orders of magnitude, from 0.88 mg/L (31-day growth EC10) for the New Zealand mudsnail *Potamopyrgus antipodarum* (Hickey et al. 2016) to 1,700 mg/L (3-day cell yield EC10) for the green alga *Oocystis solitaria* (van Dam et al. 2022). In general, invertebrates and fish span a similarly wide range of sensitivities to chronic exposure of nitrate, with no-effect or low-effect concentrations ranging from < 5 mg/L to > 500 mg/L. The highest toxicities (lowest toxic concentrations) have been consistently reported for fish. However, this does not necessarily imply that fish are the most sensitive taxonomic group, as numerous fish species show low sensitivity to nitrate. There is enough overlap in the sensitivity of invertebrates to make it difficult to identify differences between groups. In a review of nitrate toxicity to aquatic organisms, Camargo et al. (2005) found that nitrate toxicity decreased with increasing body size (and water salinity). Nitrate toxicity data reported by Camargo et al. (2005) and Hickey et al. (2016) demonstrate acute-to-chronic ratios typically well above 10 (i.e. acute toxicity is much lower than chronic toxicity).

Long-term (126–146 days) chronic sensitivity studies of early life stages of lake trout (*Salvelinus namaycush*) and lake whitefish (*Coregonus clupeaformis*) yielded some of the lowest toxicity estimates (McGurk et al. 2006). Lake trout was the most sensitive species, with a NOEC of 1.6 mg/L and a LOEC of 6.25 mg/L for both growth and development endpoints. Growth showed a progressive concentration response, with a 12% reduction in wet weight at the LOEC and a 22% reduction at 25 mg/L. The delayed-development endpoint (based on the number of days taken for 90% or more of the alevins to reach the swim-up fry stage) had a comparable sensitivity.

Other highly sensitive fish species include rainbow trout (*Oncorhynchus mykiss*), chinook salmon (*Oncorhynchus tshawytscha*) and cutthroat trout (*Oncorhynchus clarki*) (Kincheloe et al. 1979). Kincheloe et al. (1979) measured mortality of eggs and fry of these species after a 30-day exposure period (post-first feed). The NOEC values (based on nominal concentrations) ranged from < 1.1 mg/L to 2.3 mg/L (these data are further discussed in section 4.1). However, a subsequent study by Nautilus Environmental (2011a) on nitrate toxicity (and the influence of water hardness – see section 3) to *O. mykiss* (7-day embryo-larval tests) was unable to reproduce the same high sensitivity.

High sensitivity has also been reported for several invertebrate species, particularly from New Zealand. As noted above, the New Zealand mudsnail (*P. antipodarum*) exhibited 10% effects for a range of endpoints (i.e. growth, morbidity, mortality) after exposure for 30–40 days to concentrations of around 1–3 mg/L (Hickey et al. 2016). The New Zealand freshwater crayfish *Paranephrops planifrons* was similarly sensitive, with a 60-day EC10 of just over 2 mg/L for growth (Hickey et al. 2016). Both species were tested at low hardness (< 15 mg/L CaCO<sub>3</sub>) and relatively high pH (7.8–8.2). The tropical cladoceran *Ceriodaphnia silvestrii* was also sensitive to nitrate exposure, with significant effects on reproduction after 7 days' exposure to 2.2 mg/L (Sueitt et al. 2015).

Very few nitrate toxicity data exist for freshwater plants, presumably because nitrate is considered more a beneficial nutrient for plants than a toxicant. Of the limited data available, the results are quite divergent. The EC50 (14-day growth rate) for the charophyte *Chara globularis* (14-day growth), was 5.6 mg/L (Lambert and Davy 2010). In contrast, the EC50 (3-day cell yield) for the green alga *Raphidocelis subcapitata* (formerly *Pseudokirchneriella subcapitata*) was 470 mg/L (Nautilus Environmental 2011b), while EC50 values (3-day cell yield) for the green algae *Chlorella* sp. and *O. solitaria* were both > 1,600 mg/L (van Dam et al. 2022). The high sensitivity of *C. globularis* to nitrate in the laboratory was consistent with field and mesocosm studies, which have shown effects on charophytes above concentrations of 1–2 mg/L (Barker et al. 2008, Lambert and Davy 2010).

The acute toxicity of nitrate has been reviewed elsewhere (CCME 2012) and is not discussed here given that the DGVs reported in this document relate to chronic exposure.

### 3 Factors affecting toxicity

Factors affecting the toxicity of nitrate have been reviewed in detail by van Dam et al. (2022). Water hardness has been identified as a key modifier of both acute and chronic nitrate toxicity (CCME 2012; Baker et al. 2017; van Dam et al. 2022). Effects are species-dependent, with chronic toxicity for some species being reduced up to 4-fold across the range of 12–90 mg/L CaCO<sub>3</sub> (Baker et al. 2017). A hardness-related response was also found for chronic growth in juvenile inanga (or common jollytail, *Galaxias maculatus*) but not for acute exposures (Hickey et al. 2013). In a study of rainbow trout, the IC<sub>10</sub> of 95 mg/L for the most sensitive endpoint (growth [weight]) increased to 335 mg/L when hardness increased from 10 mg/L to 176 mg/L CaCO<sub>3</sub> (Nautilus Environmental 2011a). It is important to note that all the published studies on the effect of hardness on nitrate toxicity are confounded by the presence of several other ions at elevated concentrations (e.g. bicarbonate, chloride), so it is not possible to fully discern which variable was the main toxicity modifier. Nevertheless, as hardness is often correlated with other major ion variables, any ameliorative effects associated with one or more of them would likely be captured by focusing on hardness as the critical variable (van Dam et al. 2022). Regardless, further studies to characterise the effects of true hardness (i.e. calcium and magnesium) and other factors on nitrate toxicity are desirable.

Rescan (2012) used the data from Nautilus Environmental (2011b) (which was subsequently published by Baker et al. 2017) on the effect of hardness on nitrate toxicity to 4 species to develop an algorithm that could be used to adjust a nitrate site-specific guideline value for a diamond mine in Canada based on water hardness (up to ~160 mg/L CaCO<sub>3</sub>). van Dam et al. (2022) considered using the algorithm for site-specific guideline values for nitrate associated with iron-ore mine discharges in the Pilbara region of north-western Australia. However, they concluded that the algorithm had too many limitations to be adopted, including the fact that it was not published in the peer-reviewed literature and has not been validated for Australian species and water-quality conditions. For the reasons detailed by van Dam et al. (2022), the Rescan (2012) hardness algorithm was also not adopted for the current derivation of DGVs.

Chloride has also been shown to modify nitrate toxicity, although not to the extent that it is known to modify nitrite toxicity (Harris and Coley 1991; Jensen 1996; Camargo and Alonso 2006). Chloride reduced chronic toxicity of nitrate to *Hyalella azteca*, depending on the endpoint measured (Soucek and Dickinson 2016), while the effect of chloride was less clear for the cladoceran *Ceriodaphnia dubia*. Soucek and Dickinson (2016) concluded that chloride-dependent nitrate sensitivity is not universal among freshwater crustaceans and is not as strong as the effect of hardness. Moreover, van Dam et al. (2022) suggested that any effect of chloride on nitrate toxicity might be integrated in effects associated with hardness or ionic strength. However, more research is required on the effects of chloride on nitrate toxicity to freshwater species to understand its importance relative to hardness and if it needs to be incorporated as a factor that modifies nitrate toxicity.

van Dam et al. (2022) found no strong evidence that other physicochemical variables, such as pH, temperature and dissolved oxygen, modified nitrate toxicity. While no significant effects of temperature were seen, increased toxicity was seen in one instance at low pH (pH 4) and low oxygen, but whether these were co-stressor effects was not determined. Effectively, physicochemical variables may primarily act as additional stressors alongside nitrate, rather than by directly affecting nitrate toxicity (van Dam et al. 2022).

As noted above, a hardness correction for the nitrate DGVs was not included in the current derivation. However, there were sufficient chronic toxicity data for nitrate across a range of hardness to partition the data and derive DGVs for 3 different hardness categories. The details are provided in section 4.1. Although

further understanding of factors that modify toxicity and their mechanisms is required, particularly for hardness and chloride, the derivation of nitrate toxicity DGVs for 3 different hardness categories represents a significant improvement from the ANZECC and ARMCANZ (2000) DGVs.

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## 4 Default guideline value derivation

### 4.1 Toxicity data used in derivation

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

Nitrate chronic toxicity data were obtained from the scientific literature by conducting searches using the US EPA ECOTOX database (US EPA 2023a), the Australasian Ecotoxicology Database (Markich et al. 2002; Langdon et al. 2009) and the Google Scholar search engine. A recent literature review of post-2016 nitrate toxicity data conducted by van Dam et al. (2022) was also used to support the literature search. Data from tests using potassium nitrate were omitted from the dataset due to the possibility that potassium, which is known to have a relatively high toxicity as a major ion (Mount et al. 1997), was contributing to toxicity.

In total, chronic toxicity data were available for 36 species from 8 taxonomic groups. These comprised 3 microalgae, one cnidarian, 2 bivalve molluscs, one gastropod mollusc, 3 insects, 9 crustaceans, 13 fish and 5 amphibians. All data passed the quality-assessment process prescribed by Warne et al. (2018), although not all subsequently passed the data-screening process (see below). All toxicity data were generated from laboratory-based single-species toxicity tests.

In selecting the final dataset, preference was given to EC/IC10 values over EC/IC20 values over NOEC values. NOEC values were considered for species for which there were no EC/IC10 or EC/IC20 data available or based on professional judgement that the NOEC represented a more appropriate negligible-effect concentration than an available EC/IC10 or EC/IC20. EC/IC25, EC/IC50 and LOEC values were not included in the dataset. There were 32 species for which EC/IC10, EC/IC20 or NOEC values were available. A modality assessment for this dataset based on a single value per species (as selected according to Warne et al. (2018) [Appendix A]) concluded that the toxicity of nitrate to freshwater species exhibits a unimodal relationship (excluding any effect of hardness) and, therefore, the full dataset could be considered for the derivation of the DGVs.

All but 6 studies in the available dataset reported hardness concentrations (i.e. for the species *Bufo americanus*, *Gammarus pseudolimnaeus*, *Astacus astacus*, *Sander lucioperca*, *Macrobrachium rosenbergii* and *Pomacea paludosa*). Given that the final dataset was split based on different water-hardness categories, toxicity values for which the accompanying hardness was not reported were not used in the derivation of the DGVs.

A NOEC of  $\geq 1,600$  mg/L for *Chlorella* was used in the derivation. This met the criteria for inclusion specified by Warne et al. (2018) that (i) the value was not too far outside the existing data range or (ii) did not have an overly large influence on the final DGVs. For the amphibian *Rana aurora*, a 16-day LOEC of  $\leq 29.1$  mg/L for length was reported. However, this value was difficult to justify for inclusion in the derivation, because the effect size at this concentration was only 3% and there was no subsequent reduction in length for the following 4 test concentrations (i.e. up to 472 mg/L). Instead, a NOEC of 117 mg/L for weight, which represented a 10% effect size, was used in the final dataset. For the amphibian *Pseudacris regilla*, the lowest available value was a 10-day LOEC of  $\leq 30$  mg/L (i.e. effect at lowest tested concentration) based on tadpole wet weight (Schuytema and Nebeker 1999a). However, the corresponding LOEC for tadpole length was over 8 times higher (259 mg/L). Therefore, given that wet weight is known to be a potentially unreliable indicator of growth, the wet-weight LOEC was not used. Instead, the next lowest value, a 10-day NOEC of 56.7 mg/L for embryos (Schuytema and Nebeker 1999b), was selected for this species.

Sueitt et al. (2015) reported nitrate chronic toxicity data for the cladoceran *C. silvestrii* and the midge *Chironomus xanthus* from which IC10 data could be calculated. However, the tests used calcium nitrate rather than sodium nitrate, resulting in hardness increasing with increasing nitrate concentration. Consequently, the data for these 2 species were not used.

Egg sensitivity data reported for 4 fish species (*Oncorhynchus kisutch*, *O. mykiss*, *O. tshawytscha* and *O. clarki*) by Kincheloe et al. (1979) were compromised by mortalities associated with *Saprolegnia* fungal infestations and so were not included for consideration. However, there was no indication that the accompanying fry-survival data were also compromised by fungal infestation, with good control survival (> 95%) for all species and a partial concentration response observed for 3 species. Although neither the stock solutions nor the exposure solution concentrations were analytically confirmed in this study, the data were included in the dataset due to the apparent sensitivity of at least 3 of these fish species. A NOEC value of  $\geq 4.5$  mg/L for *O. kisutch* (Hickey et al. 2013) was not used in the final dataset because it is a '>' value and is at the very sensitive end of the SSD. Although its inclusion made little difference to the DGVs (data not shown), it was more defensible to exclude than include this value.

Where studies for individual species have demonstrated a significant dependence of toxicity on a physicochemical variable, as is the case for nitrate toxicity and hardness, Warne et al. (2018) recommend selecting the toxicity data for the species that correspond to the most toxic set of conditions (i.e. typically the lowest hardness). Doing so results in DGVs that are appropriate for more toxic conditions (i.e. lower hardness) and potentially over-conservative for less toxic conditions (i.e. higher hardness). The nitrate chronic toxicity dataset included data across a hardness range of approximately 6–380 mg/L CaCO<sub>3</sub>. Given the relatively large number of species for which suitable data were available, the ability to partition the data across, and derive DGVs for, several hardness categories were explored (see Appendix B). This assessment concluded that the data could be partitioned according to the following 3 hardness ranges based on the hardness values reported for the studies:

- reported values of 6–28 mg/L CaCO<sub>3</sub> represent a 'soft' water category of < 30 mg/L CaCO<sub>3</sub>
- reported values of 39–125 mg/L CaCO<sub>3</sub> represent a 'moderately hard' water category of 30–150 mg/L CaCO<sub>3</sub>
- reported values of 156–380 mg/L CaCO<sub>3</sub> represent a 'hard' water category of > 150 mg/L CaCO<sub>3</sub>.

Each of these categories had a sufficient sample size (i.e. > 10 species) to enable robust DGVs to be derived. The dataset was not further screened for other toxicity-modifying factors such as chloride, primarily because of a lack of data and evidence of strong ameliorative effects on nitrate toxicity.

The final datasets for each of the 3 hardness categories comprised the following data (Table 1):

- Soft water (< 30 mg/L CaCO<sub>3</sub>) – 14 species from 5 taxonomic groups, comprising 8 fish, 2 molluscs (one gastropod and one bivalve), 2 amphibians, one arthropod and one microalga. The dataset comprised 5 EC/IC10, one LC10 and 8 NOEC values.
- Moderately hard water (30–150 mg/L CaCO<sub>3</sub>) – 11 species from 5 taxonomic groups, comprising one amphibian, 5 fish, 2 insects, 2 crustaceans (one cladoceran and one amphipod) and 1 bivalve mollusc. The dataset comprised of 6 EC/IC10, 2 EC20 and 3 NOEC values.
- Hard water (> 150 mg/L CaCO<sub>3</sub>) – 12 species from 6 taxonomic groups, comprising 3 fish, 4 crustaceans (3 cladocerans and one amphipod), one insect, 2 microalgae, one amphibian and one cnidarian. The dataset comprised 8 EC/IC10, one EC20 and 3 NOEC values.

**Table 1 Summary of chronic toxicity data values for nitrate in freshwaters for 3 hardness ranges; values are reported to no more than 3 significant figures**

Taxonomic group	Species (common name)	Life stage	Duration (days)	Toxicity measure (test endpoint)	Reported toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	Final toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)
<b>Soft water (&lt; 30 mg/L CaCO<sub>3</sub>)</b>						
Microalga	<i>Raphidocelis subcapitata</i> (green alga)	Exponential growth	3	IC10 (growth)	247	<b>247</b>
Mollusc (bivalve)	<i>Sphaerium novaezelandiae</i> (fingernail clam) <sup>a</sup>	Juvenile	60	LC10 (mortality)	8.6	<b>8.6</b>
Mollusc (gastropod)	<i>Potamopyrgus antipodarum</i> (New Zealand mudsnail) <sup>a</sup>	Juvenile	40	EC10 (growth – length)	0.88, 2.3	<b>1.4<sup>b</sup></b>
Crustacean	<i>Paranephrops planifrons</i> (Koura, freshwater crayfish) <sup>a</sup>	Juvenile	60	EC10 (growth – length)	2.2	<b>2.2</b>
Fish	<i>Coregonus clupeaformis</i> (lake whitefish)	Embryo, alevin, fry	126	NOEC (development)	6.3	<b>6.3</b>
Fish	<i>Galaxias maculatus</i> (inanga [NZ]; common jollytail [Australia]) <sup>a</sup>	Juvenile	40	EC10 (mortality)	2.0	<b>2.0</b>
Fish	<i>Gobiomorphus cotidianus</i> (common bully) <sup>a</sup>	Juvenile	21	EC10 (growth – weight)	22.5	<b>22.5</b>
Fish	<i>Oncorhynchus mykiss</i> (rainbow trout) <sup>a</sup>	Fry	30	NOEC (mortality)	1.1, ≥ 4.5	<b>2.2<sup>b</sup></b>
Fish	<i>Oncorhynchus tshawytscha</i> (chinook salmon)	Fry	30	NOEC (mortality)	2.3	<b>2.3</b>
Fish	<i>Pimephales promelas</i> (fathead minnow)	Embryo, larvae	7	IC10 (growth – weight)	<b>52</b>	<b>52</b>
Fish	<i>Salmo clarki</i> (Lahontan cutthroat)	Fry	30	NOEC (mortality)	4.5	<b>4.5</b>
Fish	<i>Salvelinus namaycush</i> (lake trout)	Embryo, alevin, fry	146	NOEC (growth – weight)	1.6	<b>1.6</b>
Amphibian	<i>Rana aurora</i> (Pacific treefrog)	Embryo, larvae	16	NOEC (growth – weight)	117	<b>117</b>
Amphibian	<i>Xenopus laevis</i> (African clawed frog)	Tadpole	10	NOEC (growth – weight)	24.8	<b>24.8</b>
<b>Moderately hard water (30–150 mg/L CaCO<sub>3</sub>)</b>						
Mollusc (bivalve)	<i>Lampsilis siliquoidea</i> (fatmucket clam)	Juvenile	28	EC20 (weight)	17	<b>17</b>
Insect	<i>Chironomus dilutus</i> (midge)	Larvae	10	IC10 (growth – weight)	5.8	<b>5.8</b>
Insect	<i>Deleatidium</i> sp. (mayfly) <sup>a</sup>	Larvae	20	NOEC (mortality)	20.3	<b>20.3</b>
Crustacean	<i>Ceriodaphnia dubia</i> (cladoceran) <sup>a</sup>	Neonates	7	IC10 (reproduction)	1.94	<b>19.4</b>
Crustacean	<i>Hyalella azteca</i> (amphipod)	Juvenile	42	EC20 (growth – weight)	11	<b>11</b>

Taxonomic group	Species (common name)	Life stage	Duration (days)	Toxicity measure (test endpoint)	Reported toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	Final toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)
Fish	<i>Danio rerio</i> (zebrafish)	Juvenile	29	NOEC (mortality and growth – weight)	200	<b>200</b>
Fish	<i>Galaxias maculatus</i> (inanga [NZ]; common jollytail [Australia]) <sup>a</sup>	Juvenile	40	LC10 (mortality)	26.6	<b>26.6</b>
Fish	<i>Gobiomorphus cotidianus</i> (common bully) <sup>a</sup>	Juvenile	21	LC10 (mortality)	24.9	<b>24.9</b>
Fish	<i>Oncorhynchus mykiss</i> (rainbow trout) <sup>a</sup>	Fry	42	EC10 (yolk development)	120	<b>120</b>
Fish	<i>Pimephales promelas</i> (fathead minnow)	Embryo, larvae	7	IC10 (growth – weight)	6.6	<b>6.6</b>
Amphibian	<i>Pseudacris regilla</i> (Pacific treefrog)	Embryo	10	NOEC (length)	56.7	<b>56.7</b>
<b>Hard water (&gt; 150 mg/L CaCO<sub>3</sub>)</b>						
Microalga	<i>Chlorella</i> sp. (green alga) <sup>a</sup>	Exponential growth	3	NOEC (growth)	≥ 1,600	<b>1,600</b>
Microalga	<i>Oocystis solitaria</i> (green alga) <sup>a</sup>	Exponential growth	3	IC10 (growth)	1,700	<b>1,700</b>
Cnidarian	<i>Hydra viridissima</i> (green hydra) <sup>a</sup>	Adult	4	IC10 (population growth)	220	<b>220</b>
Insect	<i>Chironomus dilutus</i> (midge)	Larvae	10	IC10 (growth – weight)	120	<b>120</b>
Crustacean	<i>Ceriodaphnia dubia</i> (cladoceran) <sup>a</sup>	Neonates	7	IC10 (reproduction)	28.5	<b>28.5</b>
Crustacean	<i>Daphnia magna</i> (cladoceran)	Neonates	7	NOEC (reproduction)	358	<b>358</b>
Crustacean	<i>Hyalella azteca</i> (amphipod)	Juvenile	14	IC10 (growth – weight)	102	<b>102</b>
Crustacean	<i>Simocephalus heilongjiangensis</i> (cladoceran) <sup>a</sup>	Neonates	13	IC10 (3-brood reproduction)	45	<b>45</b>
Fish	<i>Notropis topeka</i> (Topeka shiner)	Juvenile	30	NOEC (growth)	268	<b>268</b>
Fish	<i>Oncorhynchus mykiss</i> (rainbow trout) <sup>a</sup>	Fry	42	IC10 (growth)	335	<b>335</b>
Fish	<i>Pimephales promelas</i> (fathead minnow)	Embryo, larvae	32	EC10 (growth – weight)	46.7	<b>46.7</b>
Amphibian	<i>Hyla versicolor</i> (gray treefrog)	Juvenile	52	EC20 (metamorphosis)	47	<b>47</b>

<sup>a</sup> Species known to occur in Australasian temperate or tropical freshwaters.

<sup>b</sup> Geometric mean.

Twelve of the 28 species represented across the 3 datasets are known to occur in Australasian temperate or tropical freshwaters (Table 1). Appendix C presents further details on the data that passed the quality assessment and screening process and were used to derive the DGVs. Details of the data-quality assessment and the data that passed the quality assessment are provided as supporting information.



# 4.2 Species sensitivity distribution

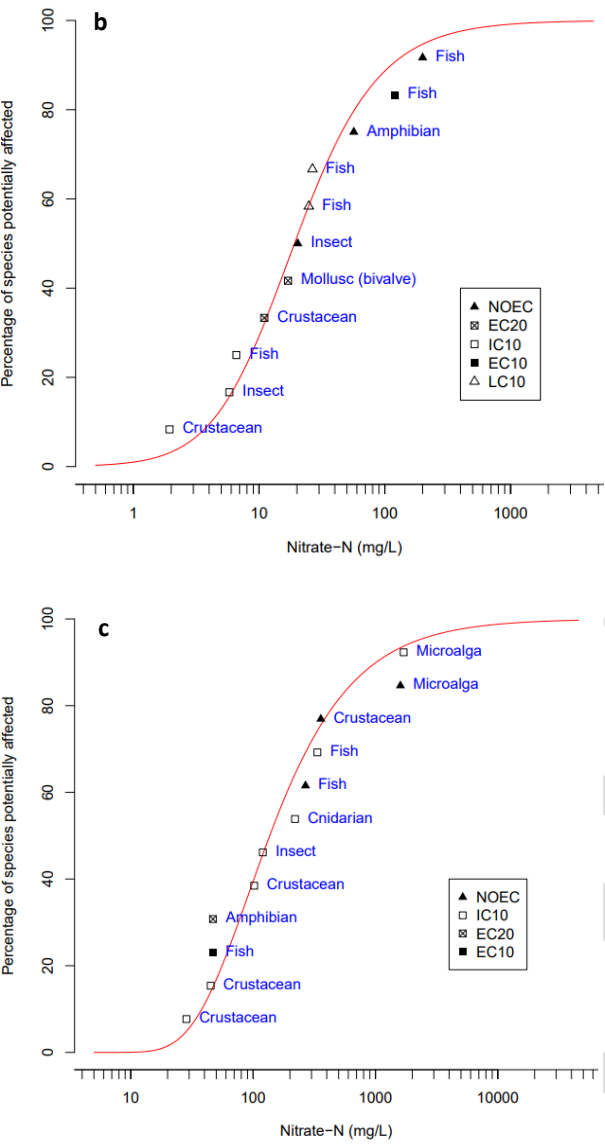
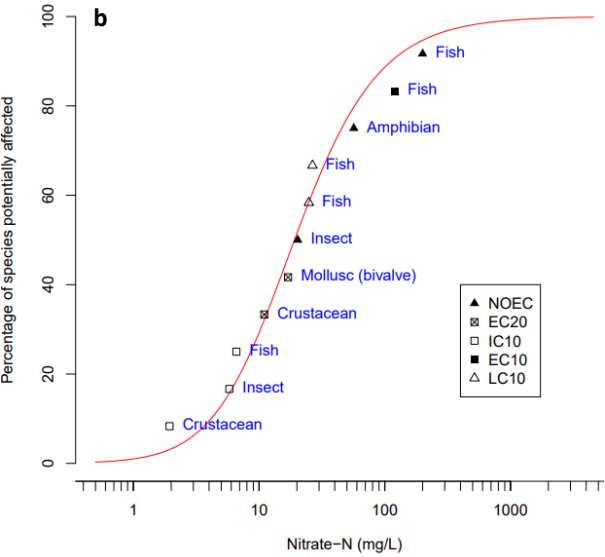


Figure 1 shows the cumulative frequency (species sensitivity) distributions for nitrate toxicity values for the 3 hardness categories reported in Table 1. All 3 models provide a good fit to the data (



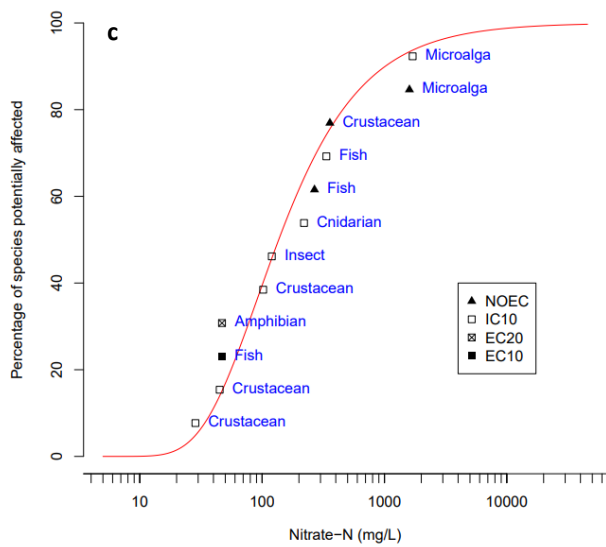
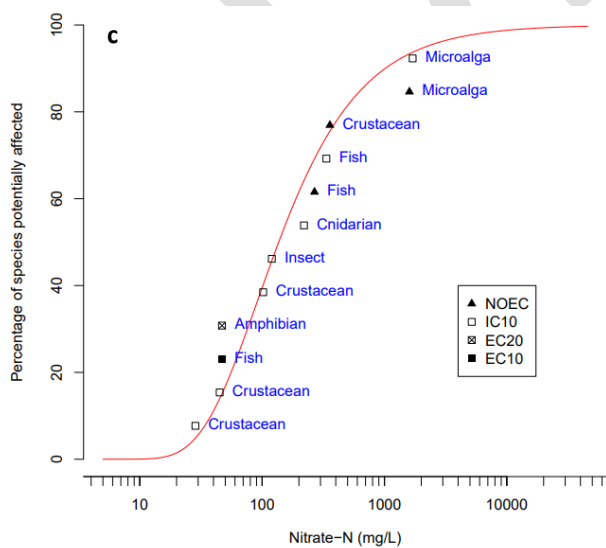
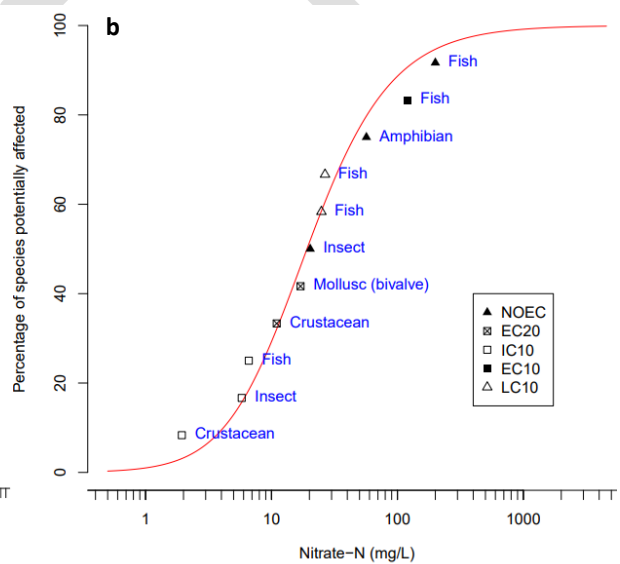
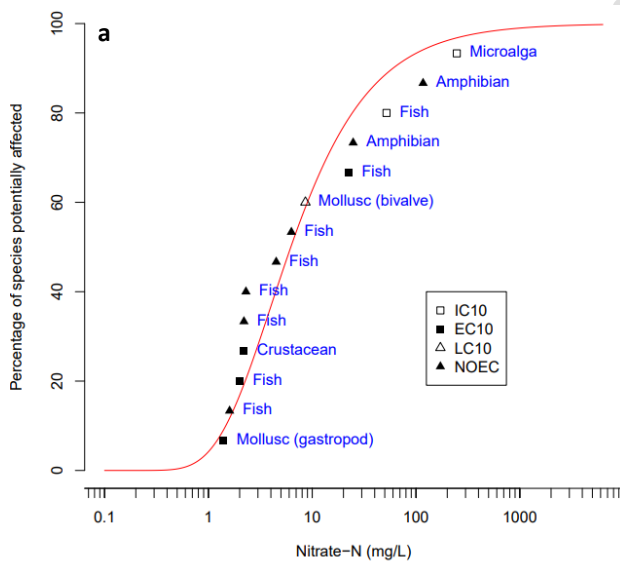


Figure 1).



**Figure 1 Species sensitivity distributions (from Burrlioz) of chronic toxicity data for nitrate ( $\text{NO}_3^-$ -N) in freshwater. (a) Soft water (< 30 mg/L  $\text{CaCO}_3$ ), (b) moderately hard water (30–150 mg/L  $\text{CaCO}_3$ ) and (c) hard water (> 150 mg/L  $\text{CaCO}_3$ )**

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### 4.3 Default guideline values

It is important that the DGVs and associated information in this technical brief are used in accordance with the detailed guidance provided in the [Australian and New Zealand Guidelines for Fresh and Marine Water Quality](#) (ANZG 2018).

Table 2 shows the DGVs for nitrate toxicity in freshwater for each of the 3 hardness categories. The DGVs relate to dissolved nitrate, operationally defined as the < 0.45 µm-filtered measurement. The 95% species-protection DGVs are recommended for assessing ecosystems that are slightly to moderately disturbed.

**Table 2 Toxicant default guideline values for nitrate in freshwater, with high reliability**

Level of species protection (%)	DGV for nitrate in freshwater (mg/L NO <sub>3</sub> <sup>-</sup> -N) <sup>a,b</sup>		
	Soft water (< 30 mg/L CaCO <sub>3</sub> )	Moderately hard water (30–150 mg/L CaCO <sub>3</sub> )	Hard water (> 150 mg/L CaCO <sub>3</sub> )
99	0.64	1.0	18
95	1.1	2.6	29
90	1.5	4.2	38
80	2.3	7.1	56

<sup>a</sup> The DGVs were derived using the Burrlioz 2.0 software and rounded to 2 significant figures.

<sup>b</sup> The DGVs are expressed as nitrate-nitrogen (NO<sub>3</sub><sup>-</sup>-N). They can be converted to the nitrate ion (NO<sub>3</sub><sup>-</sup>) by multiplying by 4.43.

As noted in section 3, it is not clear whether the reported effects of hardness on nitrate toxicity are due to water hardness alone or if they represent an effect associated with ionic strength, chloride or alkalinity (or a combination of these). Consequently, the DGVs are based on the assumption that hardness and these other variables are closely correlated. This was assessed for New Zealand freshwaters, as described in Appendix D. No such analysis could be undertaken for Australian freshwaters. For the New Zealand data, the relationships between hardness and ionic strength or alkalinity were good, while the relationship between hardness and chloride was poor. This means that if chloride modifies nitrate toxicity more than hardness, the high-hardness DGVs might be under-protective in waters with high hardness but low chloride concentrations. However, given the apparent lack of high-hardness/high-chloride freshwaters in New Zealand (see Appendix D), the high-hardness DGVs for nitrate could potentially not be used for New Zealand freshwaters to minimise the risk of applying under-protective DGVs. In such cases, the intermediate DGV of 2.6 mg/L could be applied to high-hardness/low-chloride waters. Otherwise, the hardness-based DGVs appear to be generally applicable to many New Zealand water types, as described in Appendix D.

Further research on the key factors that modify nitrate toxicity (e.g. hardness, chloride, alkalinity) would be informative and might enable further refinement of the DGVs. Until such knowledge exists, the following guidance should be followed for both Australia and New Zealand.

- Where the relationship between hardness and these other variables is good, the DGVs in Table 2 can be used.
- Where the relationship between hardness and one or more of these other variables is poor, the selection of the appropriate DGV should err on the side of caution to ensure protection of the aquatic ecosystem (i.e. adopt a conservative DGV relative to the water hardness), or site-specific guideline values should be derived.

Finally, it must be emphasised that the DGVs do not necessarily protect aquatic ecosystems against the negative effects of eutrophication that stem from the stimulatory effects that nitrate can have on plant and algal growth. Guideline values to protect against excessive plant and algal growth may be derived by jurisdictions at catchment, basin or physiographic levels (see [Australia's inland waters](#) or [Jurisdictional information](#) in ANZG 2018 for further information).

#### 4.4 Reliability classification

The DGVs for nitrate in freshwater for each of the 3 hardness categories have a high reliability classification (Warne et al. 2018) based on the outcomes for the following 3 criteria for each hardness dataset.

- soft water (< 30 mg/L CaCO<sub>3</sub>)
  - sample size – 14 (good)
  - type of toxicity data – chronic EC10, IC10, NOEC
  - SSD model fit – good (inverse Weibull)
- moderately hard water (30–150 mg/L CaCO<sub>3</sub>)
  - sample size – 11 (good)
  - type of toxicity data – chronic EC10, IC10, EC20, NOEC
  - SSD model fit – good (Burr Type III)
- hard water (> 150 mg/L CaCO<sub>3</sub>)
  - sample size – 12 (good)
  - type of toxicity data – chronic EC10, IC10, EC20, NOEC
  - SSD model fit – good (inverse Weibull).

# Glossary and acronyms

Term	Definition
Acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short (relative to the organism's life span) exposure to a chemical. Refer to Warne et al. (2018) for examples of acute exposures.
Acute-to-chronic ratio	The species' mean acute value (LC50/EC50) divided by the chronic value (NOEC) for the same species.
Chronic toxicity	A lethal or sub-lethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse sub-lethal effect on a sensitive early life stage. Refer to Warne et al. (2018) for examples of chronic exposures.
Default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. a site-specific value) in the <i>Australian and New Zealand Guidelines for Fresh and Marine Water Quality</i> . Formerly known as 'trigger values'.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
Endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, reproduction, a particular biomarker).
Guideline value	A measurable quantity (e.g. concentration) or condition of an indicator for a specific community value below which (or above which, in the case of stressors such as pH, dissolved oxygen and many biodiversity responses) there is considered a low risk of unacceptable effects occurring to that community value. Guideline values for more than one indicator should be used simultaneously in a multiple lines of evidence approach.
Hypolimnion	The lower layer of water in a stratified waterbody, typically cooler than the water above and relatively stagnant.
ICx	The concentration of a substance in water or sediment that is estimated to produce an x% inhibition of the response being measured in test organisms relative to the control response, under specified conditions.
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms relative to the control response, under specified conditions.
LOEC (lowest-observed-effect concentration)	The lowest concentration of a chemical used in a toxicity test that has a statistically significant ( $p \leq 0.05$ ) adverse effect on the exposed population of test organisms as compared with the controls. All higher concentrations should also cause statistically significant effects.
NEC (no-effect concentration)	The maximum concentration of a toxicant that causes no adverse effect in a target organism.
NO <sub>3</sub> <sup>-</sup> -N (nitrate-nitrogen)	The nitrogen portion of the total nitrate in a sample.
NOEC (no-observed-effect concentration)	The highest concentration of a toxicant used in a toxicity test that does not have a statistically significant ( $p > 0.05$ ) effect on the exposed population of test animals as compared to the controls. The statistical significance is measured at the 95% confidence level.
Species sensitivity distribution (SSD)	A method that plots the cumulative frequency of species' sensitivities to a toxicant and fits a statistical distribution to the data. From the distribution, the concentration that should theoretically protect a selected percentage of species can be determined.
Toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
Toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.

# Appendix A: modality assessment for nitrate

A modality assessment was undertaken for nitrate toxicity to freshwater biota according to the 4 questions stipulated in Warne et al (2018), as follows.

## 1. Is there a specific mode of action that could result in taxa-specific sensitivity?

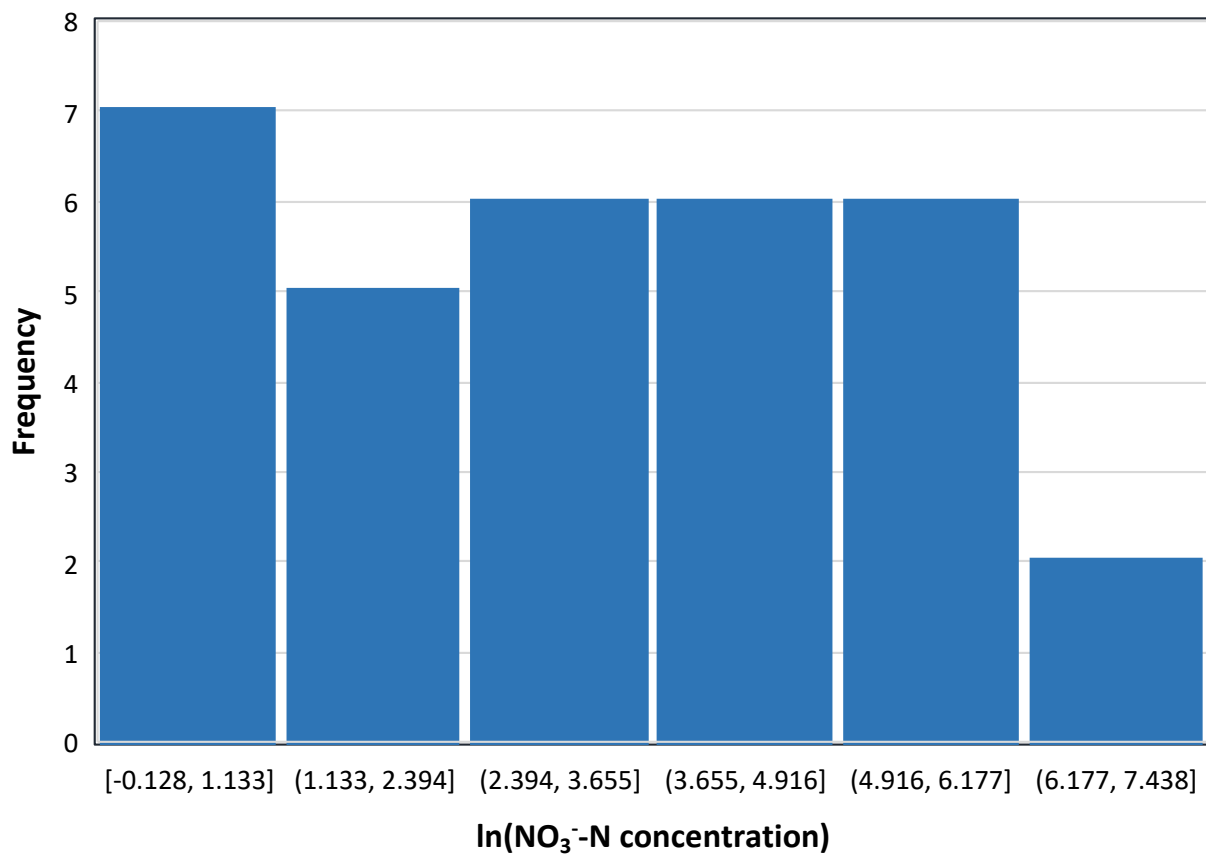
As discussed in section 2.1, nitrate has numerous potential mechanisms of toxicity, including the stimulation of reactive oxygen species, that are applicable to a range of taxonomic groups. Although there may be a mechanism of toxicity specific to plants and algae, there is otherwise little evidence to suggest that the toxicity of nitrate would target one taxonomic group more than another.

## 2. Does the dataset suggest bimodality?

Visual representation of the data, calculation of the bimodality coefficient (BC), and consideration of the range in the effect concentrations are recommended lines of evidence for evaluating whether bimodality or multimodality of the dataset is apparent. For the nitrate dataset:

- The histogram of the natural-log-transformed toxicity data (Figure A1) appears to be right-skewed but does not suggest a bimodal distribution, although the small sample size hinders the ability to make a definitive conclusion.
- Datasets that span large ranges (i.e. > 4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018). The nitrate dataset spans < 3 orders of magnitude.
- When the BC is > 0.555, it indicates that the data do not follow a typical normal distribution and may be bimodal. The BC for the log-transformed data is 0.439, which does not support a conclusion of bimodality.

Based on the lines of evidence described above, the distribution of the log-transformed dataset is likely to be unimodal.

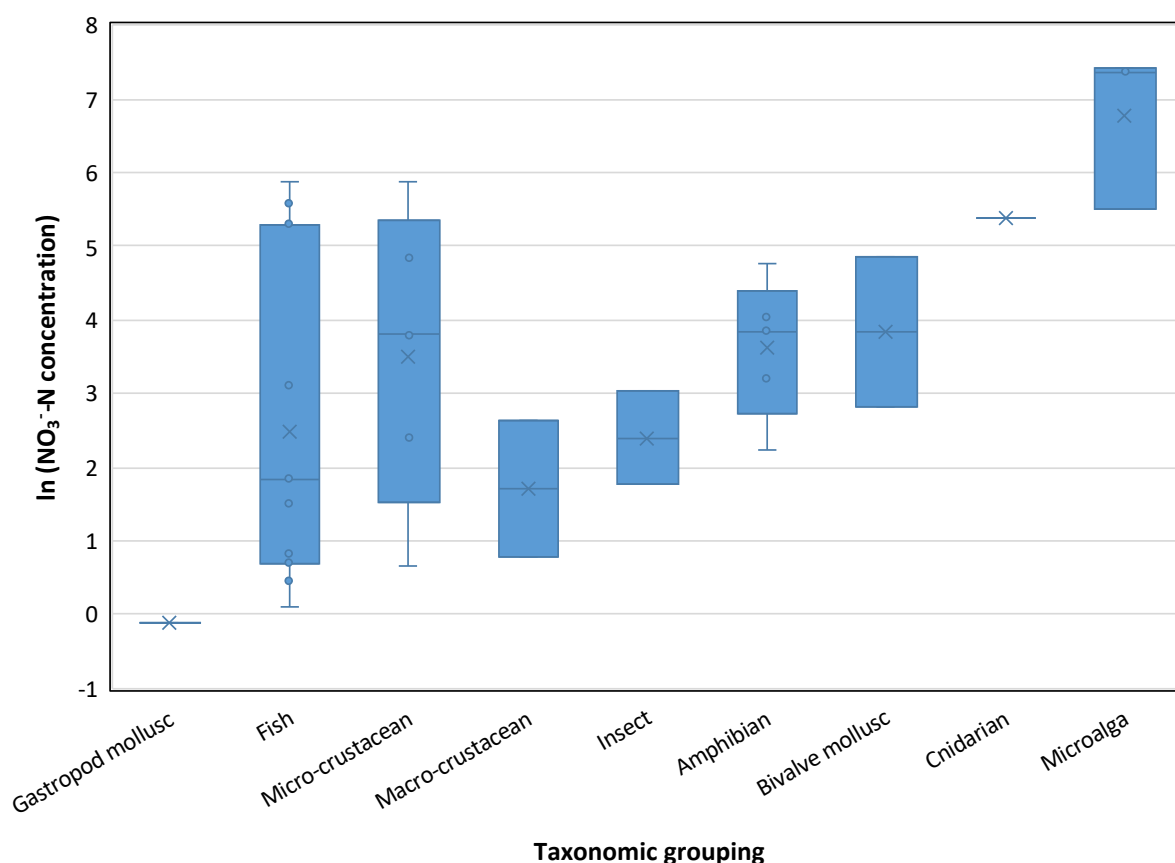


**Figure A1 Histogram of natural-log-transformed data for nitrate freshwater toxicity**

### **3. Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?**

Figure A2 suggests that there is no single taxonomic group that is the most sensitive to nitrate. The sample sizes for most of the taxonomic groups are very small ( $n = 1-3$ ), making it difficult to draw any strong conclusions, except that microalgae are likely to be less sensitive than most other species. This is not an unexpected observation.





**Figure A2 Box plot of natural-log-transformed data for nitrate freshwater toxicity by taxonomic group**

**4. Is it likely that indications of bimodality or multimodality or distinct clustering of taxa groups are not due to artefacts of data selection, small sample size, test procedures or other reasons unrelated to a specific mode of action?**

Overall, sample sizes are small and hamper the ability to make definitive conclusions. However, none of the factors assessed (mode of action, indications of bimodality of dataset, taxa-specific sensitivity), provided strong evidence of bimodality. Based on the available evidence, the dataset is unimodal, which supports the use of the full dataset identified in the preparation of the DGV derivation.

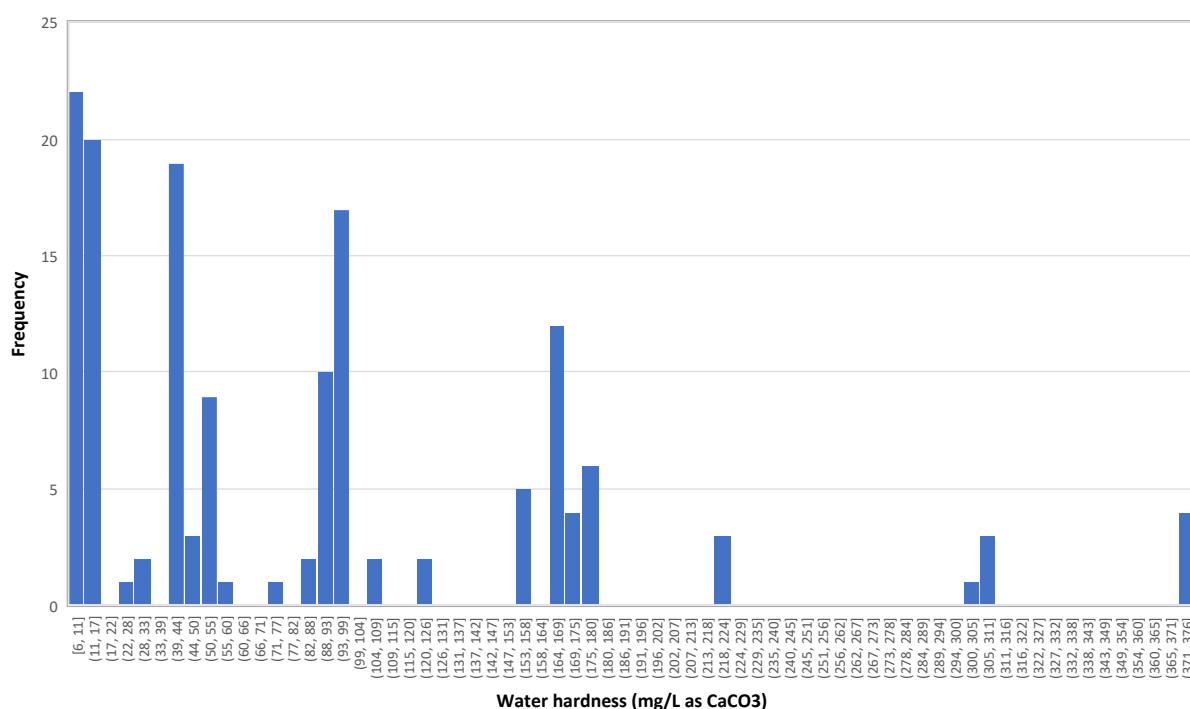
# Appendix B: assessment of appropriate hardness categories for nitrate toxicity data and default guideline values

The nitrate freshwater toxicity dataset was assessed to determine if it could be split into multiple water-hardness categories and, if so, the appropriate number of categories. The following 2 key criteria were applied when considering the appropriate hardness categories.

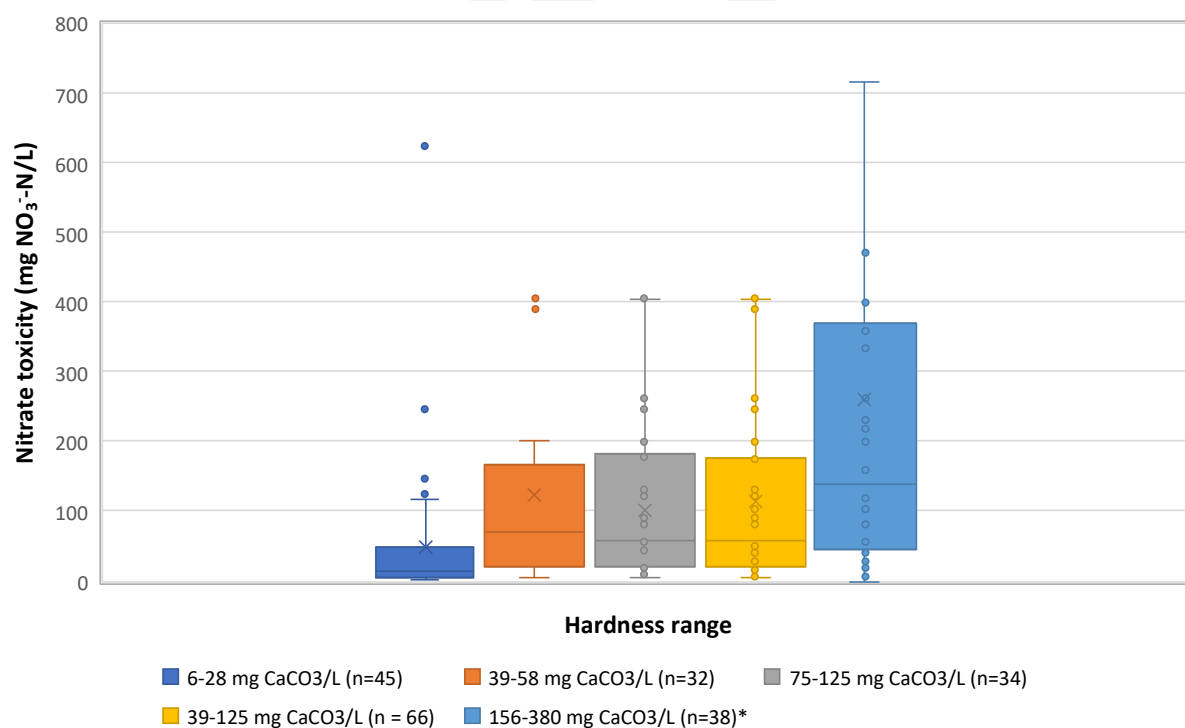
- There are apparent differences in nitrate toxicity between the categories.
- The number of species and taxonomic groups represented in each category exceed the minimum requirements and enable the derivation of robust DGVs that minimise the additional uncertainty associated with the use of smaller datasets. Thus, a preferred default requirement was set of at least 10 species from at least 4 taxonomic groups for each category.

The chronic toxicity dataset that was assessed included IC/LC10, IC/LC20 and NOEC values and excluded LOEC, IC25 and LC50 values as well as values for which the corresponding water hardness was not reported. The available dataset comprised 149 toxicity values across 35 hardness levels (ranging from 6 to 376 mg/L CaCO<sub>3</sub>) for 28 species from 8 taxonomic groups. For 9 of the 28 species, toxicity values were available for at least 2 different hardness levels. Where hardness values associated with a single toxicity value were reported as a range (e.g. 107–140 mg/L CaCO<sub>3</sub>), the mid-point of the 2 values was used.

Figure B1 shows the spread of the data across the entire hardness range. Based on the spread, 4 hardness ranges were initially assessed: 0–28 mg/L, 39–58 mg/L, 75–125 mg/L and 156–380 mg/L CaCO<sub>3</sub>. Figure B2 presents a box plot of the data for these 4 ranges along with the range of 39–125 mg/L, which is a combination of the 2 mid-range categories. Table B1 presents the key descriptive statistics. There is a general trend of decreasing toxicity with increasing hardness. However, there is no apparent difference between the 39–58 mg/L and 75–125 mg/L CaCO<sub>3</sub> hardness ranges. Moreover, data were available for fewer than 10 species for each of these ranges. Therefore, another assessment was performed that combined the 2 middle ranges, resulting in just 3 hardness ranges (i.e. 6–28 mg/L, 39–125 mg/L and 156–380 mg/L CaCO<sub>3</sub>). The use of these 3 ranges met both of the key criteria above, in that there were apparent differences in toxicity between the ranges, and there were data available for more than 10 species (for at least 4 taxonomic groups) for each range (Figure B2 and Table B1). Thus, DGVs for nitrate toxicity were derived for each of these 3 hardness ranges, which were slightly rounded to form the following 3 hardness categories: < 30 mg/L CaCO<sub>3</sub> (i.e. soft water), 30–150 mg/L CaCO<sub>3</sub> (i.e. moderately hard water) and > 150mg/L CaCO<sub>3</sub> (i.e. hard water).



**Figure B1** Frequency histogram of the available nitrate toxicity data for freshwater species based on water hardness



**Figure B2** Box plot of available chronic toxicity data for nitrate at candidate hardness ranges; note that the 2 highest toxicity values of 1,600 mg/L and 1,700 mg/L are not shown on the plot. The range shown in yellow represents a combination of the data from the ranges coloured orange and grey.

**Table B1 Descriptive statistics for nitrate chronic toxicity dataset for candidate hardness ranges**

Parameter	Hardness range (mg/L CaCO <sub>3</sub> )				
	6–28 <sup>a</sup>	39–58	75–125	39–125 <sup>a</sup>	156–380 <sup>a</sup>
Sample size	45	32	34	66	38
10th percentile nitrate toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	2.1	10	15	11	24
Median nitrate toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	15	70	56	56	160
90th percentile nitrate toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	114	389	231	380	431
Number of species	14	9	8	11	12
Number of taxonomic groups	5	4	5	5	6

<sup>a</sup> Shaded columns represent the final hardness ranges for which DGVs were derived. These ranges were used to represent hardness categories of < 30 mg/L CaCO<sub>3</sub>, 30–150 mg/L CaCO<sub>3</sub> and > 150 mg/L CaCO<sub>3</sub>, respectively.

# Appendix C: full details of chronic toxicity data used to derive nitrate (freshwater) guideline values sorted into hardness ranges

**Table C1 Summary of chronic toxicity data that passed the screening and quality assurance processes and were used to derive default guideline values for nitrate in freshwaters for 3 hardness ranges**

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Toxicity measure	Endpoint	Test medium	Temperature (°C)	Hardness (mg/L CaCO <sub>3</sub> )	Chloride (mg/L)	pH	Toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	Final concentration (mg/L NO <sub>3</sub> <sup>-</sup> -N) <sup>a</sup>	Reference
Hardness < 30 mg/L CaCO <sub>3</sub>													
Chlorophyta (microalga)	<i>Raphidocelis subcapitata</i>	Exponential growth	3	IC10	Growth	Reconstituted water	24	~10	NR	6.9–7.5	247	247	Nautilus Environmental 2011b
Mollusca (bivalve mollusc)	<i>Sphaerium novaezelandiae</i>	Juvenile	60	LC10	Mortality	Spring water	20	14	6.5	7.8–8.2	8.6	8.6	Hickey et al. 2016
Mollusca (gastropod mollusc)	<i>Potamopyrgus antipodarum</i>	Juvenile	31	EC10	Growth (length)	Spring water	20	11	6.5	7.8–8.2	0.88	0.88	Hickey et al. 2016
		Juvenile	31	EC10	Growth (length)	Spring water	20	11	6.5	7.8–8.2	2.3	2.3	Hickey et al. 2016
													1.4
Arthropoda (crustacean)	<i>Paranephrops planifrons</i>	Juvenile	60	EC10	Growth (length)	Spring water	20	14	6.5	7.8–8.2	2.2	2.2	Hickey et al. 2016
Chordata (fish)	<i>Coregonus clupeaformis</i>	Embryo, alevin, fry	126	NOEC	Development	Tap water (dechlorinated)	7.5	10–16	NR <sup>b</sup>	6.0–7.4	6.3	6.3	McGurk et al. 2006
	<i>Galaxias maculatus</i>	Juvenile	40	EC10	Growth (weight)	Spring-fed river water	15	14	6.5	7.2–7.5	2.0	2.0	Hickey et al. 2013
	<i>Gobiomorphus cotidianus</i>	Juvenile	21	EC10	Growth (weight)	Spring water	15	11	6.5	7.8–8.2	22.5	22.5	Hickey et al. 2016
	<i>Oncorhynchus mykiss</i>	Egg, fry	30 (post-first feed)	NOEC	Larval mortality	Well water	10	8–10	2.3	6.2	1.1	1.1	Kincheloe et al. 1979

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Toxicity measure	Endpoint	Test medium	Temperature (°C)	Hardness (mg/L CaCO <sub>3</sub> )	Chloride (mg/L)	pH	Toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	Final concentration (mg/L NO <sub>3</sub> <sup>-</sup> -N) <sup>a</sup>	Reference
		Egg, fry	30 (post first feed)	NOEC	Larval mortality	Well water	10	8–10	2.3	6.2	≥ 4.5	4.5	Kincheloe et al. 1979
												2.2	Geometric mean used in SSD
	<i>Oncorhynchus tshawytscha</i>	Egg, fry	30 (post first feed)	NOEC	Larval mortality	Well water	10	8–10	2.3	6.2	2.3	2.3	Kincheloe et al. 1979
	<i>Pimephales promelas</i>	Larvae	7	IC10	Growth (weight)	Tap water (dechlorinated)	25	12	NR <sup>b</sup>	6.9–7.5	52	52	Nautilus Environmental 2011b
	<i>Salmo clarki</i>	Egg, fry	30 (post first feed)	NOEC	Larval mortality	Well water	13	6–9	5	7.6	4.5	4.5	Kincheloe et al. 1979
	<i>Salvelinus namaycush</i>	Embryo, alevin, fry	146	NOEC	Growth (weight)	Tap water (dechlorinated)	7.5	10–16	NR <sup>b</sup>	6.0–7.4	1.6	1.6	McGurk et al. 2006
Chordata (amphibian)	<i>Rana aurora</i>	Embryo, larvae	16	NOEC	Growth (length)	Deep well water	15	25.5	NR <sup>b</sup>	6.8	117	117	Schuytema and Nebeker 1999c
	<i>Xenopus laevis</i>	Tadpole	10	NOEC	Growth (weight)	Reconstituted water	22	21–36	NR <sup>b</sup>	7.0–7.6	24.8	24.8	Schuytema and Nebeker 1999b
<b>Hardness 30–150 mg/L CaCO<sub>3</sub></b>													
Mollusca (mollusc)	<i>Lampsilis siliquoidea</i>	Juvenile	28	EC20	Growth (weight)	Diluted well water	23	105	12	8.0	17	17	Wang et al. 2020
Arthropoda (insect)	<i>Chironomus dilutus</i>	Larvae	10	IC10	Growth (weight)	Reconstituted water	23	46	NR <sup>b</sup>	6.9–7.5	5.8	5.8	Nautilus Environmental 2011b
	<i>Deleatidium sp.</i>	Larvae	20	NOEC	Mortality	Tap water (dechlorinated)	15	40	NR <sup>b</sup>	7.5–7.8	20.3	20.3	Martin and Thompson 2012
Arthropoda (crustacean)	<i>Ceriodaphnia dubia</i>	Neonates	7	IC10	Reproduction	Reconstituted water	25	44	NR <sup>b</sup>	6.9–7.5	1.94	1.94	Nautilus Environmental 2011b
	<i>Hyalella azteca</i>	Juvenile	42	EC20	Biomass	Reconstituted water	22.9	94	10	8.0	11	11	Soucek and Dickinson 2016
Chordata (fish)	<i>Danio rerio</i>	Juvenile	29	NOEC	Mortality	Tap water (dechlorinated)	28	107–142	NR <sup>b</sup>	8.1–8.3	200	200	Learmonth and Carvalho 2015
	<i>Galaxias maculatus</i>	Juvenile	40	LC10	Mortality	Spring-fed river water	15	40	17.8	7.5–7.6	26.6	26.6	Hickey et al. 2013

Taxonomic group (phylum or clade)	Species	Life stage	Exposure duration (days)	Toxicity measure	Endpoint	Test medium	Temperature (°C)	Hardness (mg/L CaCO <sub>3</sub> )	Chloride (mg/L)	pH	Toxicity value (mg/L NO <sub>3</sub> <sup>-</sup> -N)	Final concentration (mg/L NO <sub>3</sub> <sup>-</sup> -N) <sup>a</sup>	Reference
	<i>Gobiomorphus cotidianus</i>	Juvenile	21	LC10	Mortality	Dechlorinated tap water (riverine)	15	39	17.8	7.8	24.9	24.9	Hickey et al. 2016
	<i>Oncorhynchus mykiss</i>	Embryo, alevin, fry	42	IC10	Yolk development	Spring-fed river water	14	39	17.8	7.6–7.7	120	120	Hickey et al. 2013
	<i>Pimephales promelas</i>	Larvae	7	IC10	Growth (weight)	Tap water (dechlorinated)	25	50	NR <sup>b</sup>	6.9–7.5	6.6	6.6	Nautilus Environmental 2011b
Chordata (amphibian)	<i>Pseudacris regilla</i>	Embryo	10	NOEC	Growth (length)	Well water	22	75	NR <sup>b</sup>	6.6–6.7	56.7	56.7	Schuytema and Nebeker 1999b
<b>Hardness &gt; 150 mg/L CaCO<sub>3</sub></b>													
Chlorophyta (microalga)	<i>Chlorella</i> sp.	Exponential growth	3	IC10	Cell yield	Creek water	25	376	77	7.9	> 1,600	1,600	van Dam et al. 2022
	<i>Oocystis solitaria</i>	Exponential growth	3	IC10	Cell yield	Creek water	25	376	77	7.9	1700	1,700	van Dam et al. 2022
Cnidaria (cnidarian)	<i>Hydra viridissima</i>		4	IC10	Population growth	Creek water	27	376	77	7.9	220	220	van Dam et al. 2022
Arthropoda (insect)	<i>Chironomus dilutus</i>	Larvae	10	IC10	Growth (weight)	Reconstituted water	23	172	NR <sup>b</sup>	6.9–7.5	120	120	Nautilus Environmental 2011b
Arthropoda (crustacean)	<i>Ceriodaphnia dubia</i>	Neonates	7	IC10	Reproduction	Reconstituted water	25	166	NR <sup>b</sup>	6.9–7.5	28.5	28.5	Nautilus Environmental 2011b
	<i>Daphnia magna</i>	Neonates	7	NOEC	Reproduction	Reconstituted water	25	156–172	NR <sup>b</sup>	7.5–8.6	358	358	Scott and Crunkilton 2000
	<i>Hyalella azteca</i>	Juvenile	14	IC10	Growth (weight)	Reconstituted water	23	172	NR <sup>b</sup>	6.9–7.5	102	102	Nautilus Environmental 2011b
	<i>Simocephalus heilongjiangensis</i>		11	IC10	Reproduction	Creek water	25	376	77	8.1	45	45	van Dam et al. 2022
Chordata (fish)	<i>Notropis topeka</i>	Juvenile	30	NOEC	Growth	Deep well	23.4	210–230	0.64–1.04	8.3	268	268	Adelman et al. 2009
	<i>Oncorhynchus mykiss</i>	Embryo, alevin, fry	37–40	EC10	Growth (weight)	Tap water (dechlorinated)	14	176	NR <sup>b</sup>	6.9–7.3	335	335	Nautilus Environmental 2011a
	<i>Pimephales promelas</i>	Embryo, larvae	32	EC10	Growth (biomass)	Dechlorinated Lake Michigan Water	25	132–180	NR <sup>b</sup>	8.0–8.3	46.7	46.7	US EPA 2010
Chordata (amphibian)	<i>Hyla versicolor</i>	Juvenile	52	EC20	Metamorphosis	Well water	12	300	12	8.0	47	47	Wang et al. 2020

<sup>a</sup> Values in this column used in individual SSD for each hardness range.

<sup>b</sup> NR = not reported.

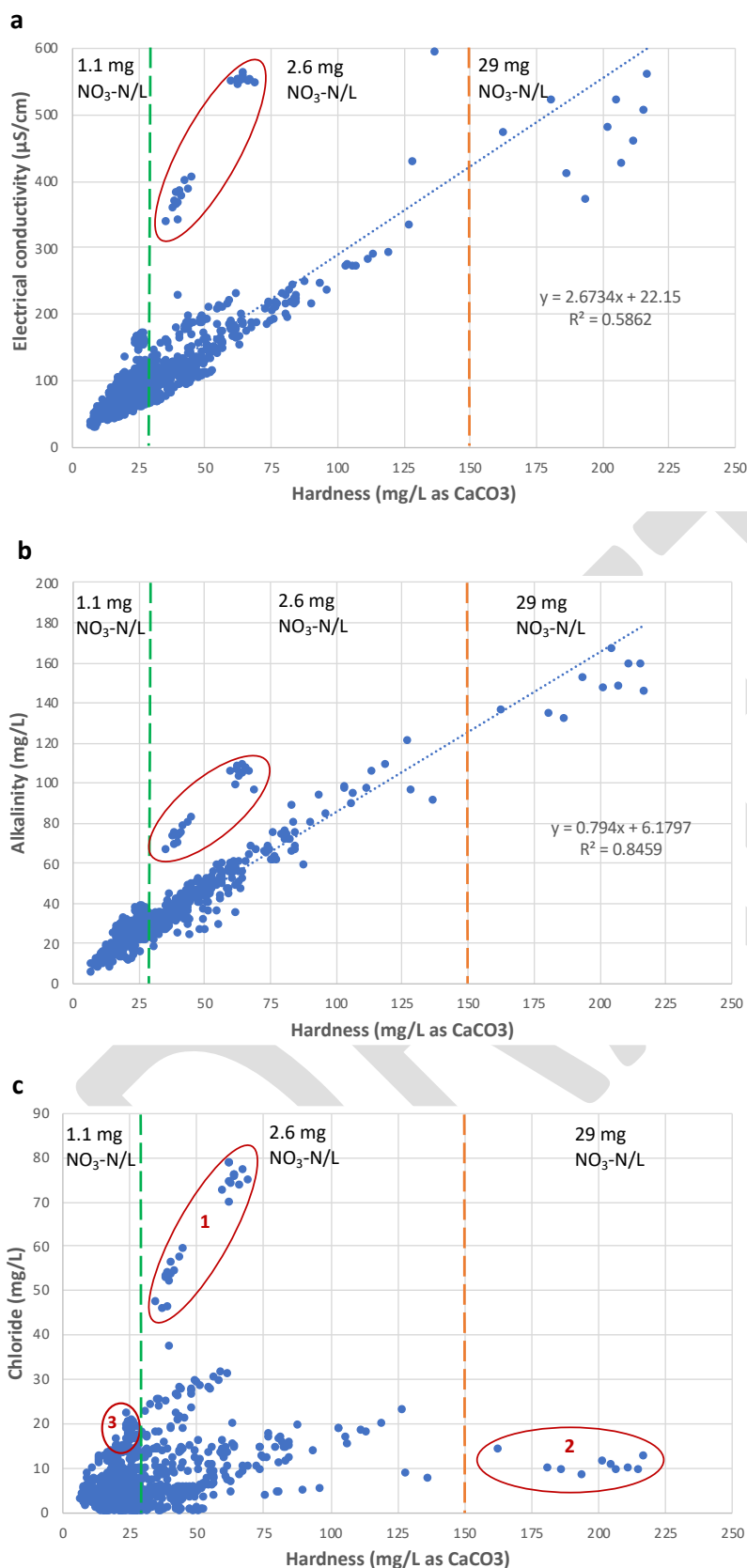
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## Appendix D: relationships between hardness and other major ion variables for Australian and New Zealand freshwaters

The studies that assessed the effect of hardness on nitrate toxicity all used the US EPA synthetic freshwater recipe (US EPA 2002) to prepare waters of different hardness. However, these water types also contain different concentrations of other major ions, including chloride and bicarbonate (the dominant contributor to alkalinity), making it impossible to fully conclude that hardness is the sole or key factor modifying nitrate toxicity. Hardness is often correlated with other major ions and water-quality characteristics that are derived from major ion concentrations and compositions, such as alkalinity and ionic strength (which is typically measured as electrical conductivity [EC]) and, in theory, hardness should represent a reasonable surrogate for these other variables. The extent to which this is the case for New Zealand freshwaters was assessed to ensure that the hardness-based nitrate DGVs can be validly applied. Unfortunately, no consolidated water-quality dataset for Australia was available to undertake the same analysis. It would be useful to undertake such an analysis in the future.

Freshwater data for New Zealand were supplied by the National Institute of Water and Atmospheric Research (NIWA), based on New Zealand's National Water Quality Monitoring Network (NIWA 2023). This dataset contained approximately monthly data for major ions and associated variables (e.g. EC, alkalinity) for 77 river sites for a 12-month period in 1989. In total, there were 943 values for each variable. Figure D1 shows the relationships for hardness with EC, alkalinity and chloride. Each plot is overlain with the nitrate DGVs for waters of soft, moderate and high hardness, to assist with the assessment.



**Figure D1 Relationships for (a) hardness and electrical conductivity, (b) hardness and alkalinity and (c) hardness and chloride for freshwater in New Zealand (based on data from 1989 for 77 freshwater sites around New Zealand). Green and orange dashed lines separate the hardness ranges for the 3 hardness-related DGVs**

**Hardness versus EC (Figure D1a):** There is a reasonably good positive relationship between hardness and EC, although there is also marked variability including several outlier sites. In particular, there are 2 sites for which EC is higher than hardness would otherwise suggest (circled). The DGVs would be protective (and potentially over-protective) for the 2 outlier sites if ionic strength is a stronger toxicity-modifying factor than hardness alone. Overall, the hardness-based nitrate DGVs would still be applicable if ionic strength was the key factor modifying nitrate toxicity.

**Hardness versus alkalinity (Figure D1b):** There is a strong positive relationship between hardness and alkalinity, albeit with 2 outlier sites (circled). However, the DGVs will be protective (and potentially over-protective) for these if alkalinity is a stronger toxicity-modifying factor than hardness. Overall, the hardness-based nitrate DGVs would still be applicable if alkalinity was the key factor modifying nitrate toxicity.

**Hardness versus chloride (Figure D1c):** There is a poor relationship between hardness and chloride. While the relationship is generally positive (i.e. chloride increases as hardness increases), the extent to which chloride increases as hardness increases varies greatly. This is of potential concern for the nitrate DGVs, given that chloride is the most likely alternative candidate to act as a significant modifier of nitrate toxicity. There are 2 main groups of outlier sites (circled and marked '1' and '2' in Figure D1c). Where chloride is a stronger toxicity-modifying factor than hardness, the following can be concluded.

- Waters with low hardness/low chloride will have a conservative soft-water DGV of 1.1 mg/L, which seems appropriate. There might be some cases where the soft-water DGV is overly conservative (i.e. over-protective) for low-hardness sites that have slightly higher chloride concentrations, as roughly denoted by the group marked as '3' in Figure D1c.
- Waters with intermediate hardness/intermediate chloride will have an intermediate DGV of 2.6 mg/L, which seems appropriate. However, the DGVs might be over-protective for the group marked as '1' in Figure D1c (lower hardness/higher chloride).
- The DGVs might not be protective for the outlier sites marked as '2' in Figure D1c (higher hardness/lower chloride). However, the dataset indicates that there are no (or very few) high-hardness/high-chloride waters in New Zealand, so the high-hardness DGV of 29 mg/L could potentially not be used for New Zealand waters, with the intermediate DGV of 2.6 mg/L being applied to waters with high hardness/low chloride (i.e. to minimise the risk of applying under-protective DGVs).

Overall, and notwithstanding the lack of a good relationship between hardness and chloride, the hardness-based DGVs appear to be generally applicable to many New Zealand water types. Where they are not applicable (e.g. the outlier sites indicated in Figure D1), it would be expected that conservative DGVs would be applied or that site-specific DGVs would need to be derived.

Without a similar analysis for Australia, it is difficult to conclude the applicability of the hardness-based nitrate DGVs. However, the same principles would apply as described above for New Zealand waters. Users should assess the relationships between hardness and EC, alkalinity and chloride, and determine if the relevant hardness-based nitrate DGV is appropriate or potentially over-protective or under-protective if these other 3 variables are stronger drivers of nitrate toxicity than hardness. As noted in sections 3 and 4.3, more research is needed to better understand the key factors that

modify nitrate toxicity. However, until such knowledge exists, the current hardness-based DGVs for nitrate are considered appropriate within the constraints described above.

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