An Australian Government Initiative

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Ammonia in freshwater

Technical brief

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The default guideline values (DGVs) and associated information in this technical brief should be used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (www.waterquality.gov.au/anz-guidelines).

Ammonia is ubiquitous in the aquatic environment and plays a significant role in the nitrogen cycle. It is formed through animal waste, bushfires, nitrogen fixation reactions, and the decomposition of animals and plants. Ammonia is also a common industrial chemical (e.g. fertiliser) that enters the environment via municipal, industry and agricultural processes.

The chemical forms of ammonia in surface water comprise un-ionised ammonia (NH_3) and ionised ammonia or ammonium ion (NH_4^+). Their sum is termed *total ammonia* and is commonly reported on the basis of nitrogen (N) as total ammonia N (also referred to as total ammonia nitrogen or TAN). The proportions of NH_3 and NH_4^+ in total ammonia primarily depend on pH and temperature and, to a lesser extent, ionic strength. Essentially, as pH and temperature increase, the proportion of NH_3 increases and the proportion of NH_4^+ decreases. Although NH_3 and NH_4^+ can both be toxic to biota, NH_3 readily diffuses across cell membranes, and its higher bioavailability relative to NH_4^+ makes it more toxic. In addition to pH and temperature affecting the chemical equilibrium of ammonia, both factors affect the toxicity of ammonia (temperature for invertebrates only), with toxicity being higher at higher pH and temperature. Joint-toxicity models characterising these toxicity relationships were developed by USEPA (1999, 2013) and were used in the DGV derivation.

Chronic toxicity data for 27 species from nine taxonomic groups passed the quality assessment criteria for the derivation of DGVs for ammonia in freshwater. Only studies that reported ammonia concentrations, pH and temperature were used. Bivalves were generally the taxonomic group most sensitive to ammonia.

Very high reliability DGVs for ammonia in freshwater were derived, with a good fit of the species sensitivity distribution (SSD) to the dataset. Due to the influence of pH and temperature on ammonia toxicity, joint-toxicity models were used to convert toxicity values to, and derive DGVs at, a standard pH and temperature (USEPA 1999, 2013). DGVs for 99%, 95%, 90% and 80% species protection are provided for freshwater at the standard pH (7 and 8 for Australia and New Zealand, respectively) and temperature (20°C) as well as a range of other pH values (6–9) and temperatures (10–30°C) (Appendix D: Ammonia freshwater default guideline values at different pH and temperature). Where site-specific pH and temperature data are not available to calculate DGVs, conservative pH and temperature data (i.e. which reflect an increased proportion of NH_3) should be used.

The 95% species protection DGVs for ammonia at pH 7 and 20°C and pH 8 and 20°C, which are considered typical conditions for Australia and New Zealand, respectively, are 0.79 mg N/L and 0.33 mg N/L, respectively. These DGVs should be used in the absence of the ability to select a DGV most relevant to the local pH and temperature conditions.

The 95% species protection DGVs (adjusted for site-specific pH and temperature) are recommended for application to slightly-to-moderately disturbed ecosystems. However, where bivalve species are present and are considered an important component of the aquatic ecosystem, the 99% DGVs or site-specific guideline values may be more appropriate. Site-specific guideline values or local

validation studies should also be considered for water with low electrical conductivity (e.g. <50 µS/cm) or that is outside the pH (6–9) and temperature (10–30°C) ranges used to calculate the DGVs in Appendix D: Ammonia freshwater default guideline values at different pH and temperature.

1 Introduction

1.1 Background information

Ammonia (CAS 7664-41-7) is a colourless alkaline gas that is highly soluble in water. Total ammonia (also reported as total ammonia nitrogen or TAN) is the sum of un-ionised ammonia (NH_3) and ionised ammonia or ammonium ion (NH_4^+), and is referred to in this technical brief as either ammonia or total ammonia. Un-ionised ammonia (NH_3) and NH_4^+ salts are highly soluble in water. The proportions of NH_3 and NH_4^+ in total ammonia are affected by a variety of environmental parameters, including pH, temperature and ionic strength.

Ammonia is an important component of the nitrogen cycle and is a significant source of available nitrogen (e.g. nitrate, nitrite) in the environment via microbial processes such as nitrification (Environment Canada 2001). Ammonia is released by the decomposition of organic waste matter from animals and plants. Other natural sources of ammonia include nitrogen fixation reactions, bushfires, lightning and volcanic activity (Environment Canada 2010, USEPA 2013).

Ammonia is a common component used in the synthesis of nitrogen-containing organic and inorganic chemicals, for the manufacture of fertilisers, or as a fertiliser itself by direct injection into soil. Ammonia is also used in applications such as refrigeration, pulp and paper production, mining, food processing, refining, and animal husbandry. Anthropogenic sources of ammonia enter the environment via municipal, industrial and agricultural processes.

Ammonia is a non-persistent and non-accumulative toxicant, with the toxicity of ammonia predominantly attributed to NH_3. Due to its neutral form, NH_3 can more readily cross epithelial membranes of aquatic organisms compared to NH_4^+. However, NH_4^+ can also contribute to the overall toxicity of ammonia. The formation of NH_3 is favoured at increased pH and temperature values (Emerson et al. 1975). This means that although the concentration of total ammonia may remain constant in a water body, the proportions of NH_3 and NH_4^+ fluctuate with temperature and pH. For example, for the same concentration of total ammonia and:

- a pH of 7 and temperature of 20°C, 0.394% of the total ammonia is NH_3
- a pH of 8 and temperature of 20°C, the proportion of NH_3 is 10-fold higher, at 3.81%.

To allow for comparison of total ammonia concentrations, concentrations of NH_3 are converted to equivalent total ammonia concentrations based on nominal pH and temperature (e.g. pH 7 and 20°C) (Appendix A: Relationship between un-ionised ammonia and total ammonia, based on Emerson et al. 1975). Therefore, wherever possible and unless otherwise stated (i.e. by virtue of different units being clearly reported), ammonia toxicity values reported in this document are reported as total ammonia nitrogen.

Ammonia guideline values for 20 international jurisdictions range from 0.2 mg N/L to 42 mg N/L for total ammonia (normalised to pH 7 and 20°C) and from 0.96 µg NH_3/L to 115 µg NH_3/L for NH_3 (Wang & Leung 2015). Differences in guideline values can be attributed to a range of factors, including:

- the use of different selection criteria for toxicity values (and inclusion of recent toxicity data)

- whether toxicity and guideline values were derived for total ammonia or NH_3
- whether toxicity values were normalised to a standard pH and temperature using joint-toxicity models (USEPA 2013)
- different approaches for deriving guideline values.

USEPA (2013) derived a chronic ambient water quality criterion of 1.9 mg N/L and an acute criterion of 17 mg N/L for total ammonia (at pH 7 and 20°C). These criteria were derived from fish toxicity data normalised to pH 7 and invertebrate toxicity data normalised to pH 7 and 20°C by applying a joint-toxicity model (USEPA 2013). The Canadian guideline value for total ammonia is 3.96 mg N/L (at pH 7 and 20°C) (reported in CCME (2010) as 4.82 mg ammonia/L). A revised guideline value of 0.24 mg N/L for total ammonia (at pH 8 and 20°C) was proposed as an indicative value in New Zealand (Hickey 2014).

The ANZECC/ARMCANZ (2000) default guideline values (DGVs) for total ammonia in freshwater are 2.18 mg N/L (at pH 7) and 0.9 mg N/L (at pH 8). At the time, these values were considered to be of high reliability because they were derived from chronic toxicity values for 17 species that also measured pH and temperature. The ammonia in freshwater DGVs reported in this technical brief supersede the ANZECC/ARMCANZ (2000) DGVs.

1.2 NH_3 and NH_4^+ equilibrium

The equilibrium of NH_3 and NH_4^+ in total ammonia is influenced by pH and temperature. As pH and temperature increase, the proportion of NH_3 increases and the proportion of NH_4^+ decreases.

Emerson et al. (1975) examined the ammonia–water equilibrium (equation 1 in Table 1) and derived pKa values at different temperatures (equation 2 in Table 1). As a result, an algorithm calculating the percentage of NH_3 in a solution as a function of temperature and pH was developed (equation 3 in Table 1). Appendix A: Relationship between un-ionised ammonia and total ammonia shows the percentage of NH_3 in total ammonia for the pH range 6.5–9.5 and temperature range 10–30°C. For pH and temperature values not listed in Appendix D: Ammonia freshwater default guideline values at different pH and temperature, NH_3 can be calculated using equation 2 and equation 3 in Table 1.

Table 1 Equations for calculating NH_3 (from Emerson 1975)

Equation		Notes
1	$Ka = \frac{[NH3][H+]}{[NH4+]}$	Acid dissociation constant (Ka) for ammonia.
2	$pKa = 0.09018 + \frac{2729.92}{T}$	Deriving pKa values for ammonia at different temperatures.
3	$f = 1 \div \left[10^{(pKa-pH)} + 1\right]$	Fraction of NH_3 in a solution as a function of temperature and pH.

T = temperature in Kelvin (K) and $T(K) = 273.15 + T(°C)$.
pKa = negative log of the dissociation constant Ka.
f = fraction of total ammonia that is NH_3 (multiply by 100 to obtain percentage of NH_3).

Ionic strength also influences the ammonia equilibrium; however, the influence is less than that of pH and temperature (Emerson et al. 1975, CCME 2010). As the ionic strength increases, the fraction of un-unionised ammonia decreases.

1.3 Joint-toxicity models

Although NH_3 is more toxic than NH_4^+, there are mechanisms by which NH_4^+ is transported across cell membranes and it is also present in the environment in much higher concentrations than NH_3; therefore, NH_4^+ is also an important contributor to the toxicity of ammonia, especially at lower pH (USEPA 1999). As already described in Section 1, the relative contribution of NH_3 and NH_4^+ to ammonia toxicity is primarily influenced by pH and temperature. To account for the joint toxicity of NH_3 and NH_4^+ and also the effect of pH and temperature on ammonia toxicity, USEPA (1999) developed joint-toxicity models to describe acute toxicity and chronic toxicity of total ammonia based on water pH and temperature. These algorithms can be used to normalise toxicity data to a standard pH and temperature. This is described in Section 3.

2 Aquatic toxicology

The toxicity of ammonia is largely dependent on its chemical speciation, which is influenced by pH, temperature and ionic strength (see Section 1). The data presented here are reported as: total ammonia nitrogen (mg N/L) normalised to a pH of 7 and temperature of 20°C (invertebrates only) (USEPA 2013); or total ammonia nitrogen (mg N/L) at the toxicity test pH and temperature; or un-ionised ammonia nitrogen (µg N/L). For total ammonia nitrogen (mg N/L), the acronym TAN is used by some jurisdictions.

2.1 Mechanism of toxicity

Numerous studies have investigated the toxicity of ammonia and its mode of toxic action to freshwater organisms, particularly sensitive fish species and bivalves (USEPA 1985, 1999, 2013). As an endogenously produced toxicant, organisms have developed strategies to excrete ammonia, mainly via passive diffusion of NH_3. High external concentrations of NH_3 reduce or reverse diffusion gradients, causing a build-up of ammonia in internal tissues and blood.

The mechanisms of toxicity in fish may include:

- proliferation of gill tissues, increased ventilation rates and damage to the gill epithelium
- reduction in blood oxygen-carrying capacity due to progressive acidosis
- uncoupling oxidative phosphorylation causing inhibition of production and depletion of adenosine triphosphate in the brain
- disruption of osmoregulatory and circulatory activity disrupting normal metabolic functioning of the liver and kidneys (USEPA 2013).

In bivalves, which are known to be sensitive to ammonia, toxic effects of ammonia to freshwater (and marine) species result in:

- reduced opening of valves for respiration and feeding
- impaired secretion of the byssus (i.e. anchoring filaments in bivalves)
- reduced ciliary action
- depletion of lipid and carbohydrate stores leading to metabolic alteration as well as mortality.

Overall, these effects may decrease bivalve populations due to reduced feeding, fecundity and survival (USEPA 2013).

Studies on the mechanism of ammonia toxicity to aquatic plants or algae are rare, with most studies focussed on terrestrial plants. Mechanisms of ammonia toxicity in plants include:

- damaged chloroplast ultrastructure
- deficiency of mineral cations
- disruptions in hormonal homeostasis and photosynthesis
- inhibition of the enzyme GDP-mannose pyrophosphorylase
- oxidative stress
- uncoupling of photophosphorylation (Bittsánszky et al. 2015, Esteban et al. 2016).

In one of the few studies for aquatic plants, Bittsánszky et al. (2015) reported oxidative stress responses in leaf tissues of the aquatic plant *Myriophyllum mattogrossense* in response to ammonia exposure; however, results for ammonia-related oxidative stress responses in terrestrial plants are equivocal.

2.2 Chronic toxicity

2.2.1 Vertebrates

Chronic toxicity of ammonia to fish varies with species, life stage (fertilised eggs, embryos, larvae/fry and juvenile), measured endpoint (hatching, biomass, development, growth and mortality), test duration and pH (Burkhalter & Kaya 1977, Colt & Tchobanoglous 1978, Thurston et al. 1978, Swigert & Spacie 1983, Thurston & Russo 1983, McCormick et al. 1984, Broderius et al. 1985, Thurston et al. 1986, Hasan & MacIntosh 1986, Mayes et al. 1986, Meyer & Hansen 2002, Harrahy et al. 2004, Fairchild et al. 2005, Aldelman et al. 2009, Brinckman et al. 2009, Yang et al. 2010). Chronic toxicity values representing LC1, EC10, EC20 and NOEC values for 12 fish species ranged from 5.2 mg N/L to 64 mg N/L for total ammonia (at pH 7). The most sensitive endpoint was larval biomass (wet weight) of *Ictalurus punctatus* over 31 d, with a total ammonia NOEC of 5.2 mg N/L (normalised to pH 7) (Swigert & Spacie 1983). Amphibians exhibited similar sensitivity to fish, with embryo biomass being a more sensitive endpoint than embryo and tadpole mortality (Schuytemer & Nebeker 1999a, 1999b). USEPA (1999, 2013) concluded that the chronic toxicity of ammonia to vertebrates is influenced by pH but not by temperature (see Section 3).

2.2.2 Invertebrates

Bivalves appear to be highly sensitive to ammonia, with IC10 or NOEC values for seven species ranging from <0.43 mg N/L to 6.0 mg N/L for total ammonia (at pH 7 and 20°C) (Sparks & Sandusky 1981, Hickey & Martin 1999, Wang et al. 2007, 2011). Reproduction of a North American isolate of the cladoceran *Ceriodaphnia dubia* was approximately 2–3 times more sensitive than the Australian strain despite a slightly longer test duration of 10 days (compared to 7 days for the North American isolate), but this is expected to be within the normal variation observed within replicate experiments (Nimmo et al. 1989, Manning et al. 1996). A macroinvertebrate community response to chronic ammonia exposure was investigated by Hickey et al. (1999) using artificial stream mesocosms containing New Zealand field-collected macroinvertebrates. After 29 days of exposure to 0 mg N/L, 0.9 mg N/L, 2.3 mg N/L or 6.3 mg N/L of total ammonia (at pH 8.3–8.4 and 16°C), there was a significant reduction in the abundance of two species of mayfly *Deleatidium* sp. and *Coloburiscus humeralis* at 2.3 mg N/L and 6.3 mg N/L, respectively. USEPA (1999, 2013) found that the toxicity of ammonia to invertebrates is influenced by pH and temperature (see Section 3).

2.2.3 Algae

Ammonia is used by algae and aquatic plants as a nutrient source, and freshwater algae and plants are generally insensitive to ammonia toxicity. The green microalgae *Chlorella vulgaris* and *Pseudokirchneriella subcapitata* were found to have low sensitivity to ammonia, with 3 d EC10 growth rate values of 714 mg N/L and 622 mg N/L for total ammonia (at pH 6.6 and 25°C), respectively (Wang & Leung 2015).

2.2.4 Site-specific sensitivity to ammonia

A comprehensive study of the chronic toxicity of ammonia to endemic species in the very low ionic strength waters of a northern Australian stream showed that these native species are among the most sensitive to ammonia (Kleinhenz et al. 2019, Mooney et al. 2018, 2019). These species included a cnidarian (*Hydra viridissima*), a microalga (*Chlorella* sp.), a cladoceran (*Moinodaphnia macleayi*), a gastropod (*Amerianna cumingi*), two bivalves (*Velesunio* sp. and *V. angasi*), and a fish (*Mogurnda mogurnda*).

A unique characteristic of these species compared to other species for which ammonia toxicity has been assessed is that they live in water of low pH (~6), electrical conductivity (<20 µS/cm) and hardness (~3 mg/L $CaCO_3$) at high temperature (27–30°C). The authors concluded that the high sensitivity to ammonia was largely due to the low conductivity/ionic strength of the natural water.

2.3 Acute toxicity

No acute data were required to derive the ammonia DGVs as there were sufficient chronic data; however, a brief summary of acute toxicity data is provided here. Comprehensive reviews of acute ammonia toxicity to freshwater species have been published by USEPA (2013) and Wang and Leung (2015).

Species relevant to North America were used to derive the US acute total ammonia criterion of 17 mg N/L (at pH 7 and 20°C) based on genus mean acute total ammonia values from 23 mg N/L to 2 515 mg N/L (at pH 7 and 20°C) (USEPA 2013). The larval (glochidia) and juvenile life stages of freshwater bivalves and some non-pulmonate snails were the most sensitive taxonomic groups and life stages. Acute ammonia toxicity to temperate and tropical species was compared by Wang and Leung (2015) and included new (i.e. post-USEPA 2013) toxicity data for crustaceans, insects, molluscs and fish. Acute IC50/EC50 values ranged from 0.095 mg N/L to 2.63 mg N/L (un-ionised ammonia, pH 7.5, 25°C; corresponding to a range of 3.9 mg N/L to 963 mg N/L total ammonia), with the most sensitive species being a freshwater snail (*Sulcospira hainanesis*). Tropical species appeared to be more sensitive than temperate species, prompting Wang and Leung (2015) to suggest that a factor of four be applied to surrogate temperate data (or water quality guideline values) to protect tropical species.

Acute ammonia toxicity has also been assessed for a range of New Zealand freshwater invertebrate and fish species. Clearwater et al. (2014) found the glochidia of the mussel *Echyridella menziesii* to be as sensitive as North American mussels, with a geometric mean 48-h EC50 of 14 mg N/L for total ammonia (at pH 7.8 and 20°C). A suite of New Zealand macroinvertebrate species (mayflies, caddisflies, snails, shrimp, worms,, clams) ranged in sensitivity to ammonia, with 48 h or 96 h EC50 values of 0.18 mg NH_3/L to >0.80 mg NH_3/L (at pH 7.6 or 8.2 and 15°C) (corresponding to a range of 8.8 mg N/L to 35 mg N/L total ammonia) (Hickey & Vickers 1994). The most sensitive macroinvertebrate species were the amphipod *Paracalliope fluviatilis*, snail *Potamopyrgus antipodarum*, mayfly *Deleatidium* sp., and stonefly *Pycnocentria evecta*. Among the least sensitive macroinvertebrates were the shrimp *Paratya curvirostris*, stonefly *Zelandobius furcillatus*, and mayfly *Zephlebia dentata* (Hickey & Vickers 1994, Richardson 1997).

Some New Zealand fish species (i.e. *Gobiomorphus huttoni, Gobiomorphus cotidianus* (juvenile), *Retropinna retropinna*, *Galaxias huttoni*, *Galaxias maculatus*, *Anguilla dieffenbachii* and *Anguilla australis*), were less sensitive to acute ammonia exposure than macroinvertebrates, with 96 h LC50 values of 0.75–2.35 mg NH_3/L (at pH 7.5 or 8.1 and 15°C) (corresponding to a range of 19–226 mg N/L for total ammonia) (Richardson 1997).

The acute toxicity of ammonia has been investigated for several Australian species. A 48 h LC50 of 49 mg N/L for total ammonia (at pH 7.0–7.8 and 25°C) was reported for an Australian strain of *C. dubia* (Manning et al. 1996). The tropical mussels *Velesunio* sp. and *V. angasi* were the most sensitive to acute ammonia exposure compared to other freshwater species following a 24 h exposure, based on normalisation of total ammonia toxicity values to pH 7 and 20°C (Kleinhenz et al. 2018). These tests were carried out in site-specific water with low pH (~6), low conductivity (~20 µS/cm) and low hardness (3 mg/L $CaCO_3$) at 27°C, and resulted in 24 h EC50 values of 6.8–14 mg N/L total ammonia (at the site-specific pH and temperature) and 8.3–17 mg N/L total ammonia (at pH 7 and 20°C).

3 Factors affecting toxicity

Ammonia toxicity is influenced by the proportions of NH_3 and NH_4^+, which are influenced by pH, temperature, ionic strength and dissolved oxygen. The effect of these parameters has predominantly been studied on fish; however, ammonia toxicity to fish can also decrease after acclimation to ammonia concentrations (Camargo & Alonso 2006).

Studies investigating the influence of pH on the effects of ammonia on the mortality, length and weight of the fish *Micropterus dolomieue* found that as pH increased (from 6.6 to 8.7), toxicity to NH_3 decreased (Broderius et al. 1985). This suggests that factors other than the proportion of NH_3 influenced the toxicity either directly or indirectly. Similarly, the toxicity of ammonia to the amphipod *Hyalella azteca* at different pH values was also observed to be unrelated to NH_3 concentrations, suggesting that total ammonia concentrations provided a better comparison of ammonia toxicity (Borgmann 1994).

A study with the cnidarian *H. viridissima* found that the toxicity of ammonia increased from 9.6 mg N/L to 1.3 mg N/L (based on 96 h EC50) as pH was increased from 6.0 to 8.1 (at 27°C) when expressed as the total ammonia concentration (Mooney et al. 2018). In contrast, when expressed as un-ionised ammonia, the toxicity decreased from 0.01 to 0.12 µg NH_3/L (based on 96 h EC50) when pH was increased from 6.0 to 8.1 (at 27°C). These results support that ammonia toxicity follows a joint-toxicity model incorporating un-ionised and ionised ammonia. Mooney et al. (2018) compared the experimentally derived EC50 value at each pH to the predicted EC50 values calculated from the joint-toxicity model reported in USEPA (1999). The modelled EC50 values were slightly underestimated (more conservative) at low pH and overestimated (less conservative) at higher pH when compared to the experimentally derived EC50 values, suggesting that the USEPA (1999) joint-toxicity model may not be appropriate for this species.

Ionic strength has been shown to affect toxicity to the amphipod *H. azteca*, with chronic toxicity (6 week and 10 week reproduction) increasing in low ionic strength water (Borgmann 1994). The most sensitive species to ammonia were tropical species from northern Australia isolated from, and tested in, very low ionic strength soft waters (Mooney et al. 2018, 2019, Kleinhenz et al. 2019). Mooney et al. (2019) suggested that the relatively high sensitivity was likely due to the very low ionic strength of the test waters, providing further evidence on the effect of ionic strength on ammonia toxicity. The use of different ammonium salts in toxicity tests (e.g. ammonium chloride, ammonium sulfate, ammonium nitrate) has been shown to have negligible influence on the toxicity of ammonia to rainbow trout (Thurston & Russo 1983).

The joint-toxicity models introduced in Section 1 have attempted to address some of these toxicity modifying factors (e.g. USEPA 2013) to better describe the toxicity of ammonia to freshwater organisms and to derive more reliable guideline values for ammonia. USEPA (1999) provides a detailed review and assessment of the relationships between acute and chronic toxicity to freshwater invertebrates and vertebrates with changes in water pH and temperature. USEPA (1999) concluded that the acute and chronic toxicity of ammonia to vertebrates and invertebrates is dependent on pH, while for invertebrates the toxicity of ammonia is also dependent on temperature. This resulted in the development of joint-toxicity models to normalise toxicity values to pH 8, with an additional normalisation of temperature for invertebrates.

For chronic toxicity, the joint-toxicity model for normalisation of pH was derived from studies using the smallmouth bass *Micropterus dolomieu* (Broderius et al. 1985) and the cladoceran *C. dubia* (Johnson 1995). The relationship between toxicity and temperature for invertebrates was based on an acute toxicity study with seven invertebrate species (Arthur et al. 1987) with the data converted to chronic toxicity values. The lack of a relationship between toxicity and temperature for vertebrates was based on the acute-to-chronic ratio of toxicity data (after pH normalisation) of the fathead minnow *Pimephales promelas* at various temperatures (DeGraeve et al. 1987). Both the pH and temperature relationships for chronic toxicity were consistent with the recommendations for acute toxicity relationships for vertebrates and invertebrates derived from larger datasets.

In 2013, new toxicity data were evaluated and the pH and temperature relationships remained relevant, with USEPA (2013) re-deriving the ammonia water quality criteria for acute and chronic exposure durations. Chronic toxicity values for all species were first normalised to pH 7 using equation 1 (Table 2), with invertebrate toxicity values further normalised to a temperature of 20°C using equation 2 (Table 2). USEPA (2013) provides similar equations to convert acute toxicity values.

Table 2 Equations for normalising toxicity values to pH and temperature (from USEPA 2013)

Equation		Notes
1	$CVt,7 = \frac{CVt}{\left(\frac{0.0278}{1+10^{7.688-pH}} + \frac{1.1994}{1+10^{pH-7.688}}\right)}$	Chronic toxicity relationship to pH (applied to vertebrate and invertebrate data).
2	$log(CVt,7,20) = log(CVt,7) - [-0.028(Tt - 20°C)]$	Chronic toxicity relationship to temperature (applied to invertebrate data only).

$CV_{t,7}$ = chronic toxicity value normalised to pH 7.
CV_t = chronic toxicity value at the test pH and temperature.
pH = the pH of the toxicity test waters.
$CV_{t,7,20}$ = chronic toxicity value normalised to pH 7 and 20°C.
T_t = the temperature of the test waters (°C).

The joint-toxicity model presented in USEPA (1999) normalised chronic toxicity values to pH 8 instead of pH 7 as in USEPA (2013). The equations differ only in their constants. Normalisation of toxicity values to pH 7 using equation 1 (Table 2) and to pH 8 using the equation in USEPA (1999) resulted in a negligible (<0.2%) difference in toxicity values when recalculated over a pH range of 6–9. In the absence of a normalisation equation to pH 7.5, which is more reflective of average Australian and New Zealand waters (Stauber et al. 2021), the more recent normalisation equation for pH 7 (USEPA 2013) was selected to standardise toxicity values and derive the DGVs in this technical brief.

Normalisation of ammonia toxicity values for conductivity does not exist; however, the most sensitive group of species to ammonia were tropical species from northern Australia isolated from, and tested in, very low ionic strength soft waters (Mooney et al. 2018, 2019, Kleinhenz et al. 2019). The conductivity of toxicity test media was not commonly reported for the toxicity studies that passed the quality assurance screening criteria. Where conductivity was reported, it ranged from 75 µS/cm to 784 µS/cm for vertebrates and invertebrates and was 13 000 µS/cm (at the EC10 values) for the two insensitive microalgal species *C. vulgaris* and *P. subcapitata*, (see Section 2.2.3). The high conductivity in the algal species tests may have contributed to the low sensitivity of these two species. Because of the apparent influence of conductivity on ammonia toxicity, freshwater with very low conductivity (e.g. <50 µS/cm), and outside the ranges of pH (6–9) and temperature (10–30°C) accounted for in the DGVs derivation, should be subjected to site-specific assessments to either derive site-specific guideline values or to validate the use of the DGVs.

4 Default guideline value derivation

The DGVs for ammonia in freshwater were derived using the method for deriving guideline values in Australia and New Zealand (Warne et al. 2018) and using Burrlioz 2.0 software.

4.1 Toxicity data used in derivation

Due to the availability of sufficient data, only chronic toxicity data with measured ammonia concentrations, pH and temperature were used to derive the DGVs. For the majority of the chronic ammonia toxicity studies, total ammonia was measured multiple times throughout the duration of the toxicity tests. A number of studies also regulated or, at a minimum, monitored the pH throughout the duration of the toxicity test. Toxicity data were generally reported as either total ammonia (mg N/L) at the test pH and temperature and/or un-ionised ammonia (µg/L of NH_3 or N).

The toxicity tests used ammonium chloride, with the exception of the test for *X. laevis*, which used ammonium nitrate. All toxicity data were generated from laboratory-based single-species toxicity tests, with the exception of the toxicity value for the insect *Deleatidium* sp., which was obtained from a mesocosm study. Normalisation of toxicity values to pH 7, and also 20°C for invertebrates, (described in Section 3) is also presented in Appendix B: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. Toxicity data from the tropical site-specific studies with low pH, conductivity and hardness waters (Mooney et al. 2018, 2019, Kleinhenz et al. 2019) were not included in the DGV derivation. The unique high sensitivity of these endemic species, likely due to the very low ionic strength of the waters, would provide an overly conservative assessment of ammonia toxicity under more typical water quality conditions.

Chronic toxicity data for 27 species from nine taxonomic groups (microalga, flatworm, cladoceran, insect, amphipod, gastropod, bivalves, fish and amphibian) passed the quality assessment criteria (Warne et al. 2018). A summary of the toxicity data (one value per species) used to calculate the DGVs for ammonia in freshwater is in Table 3 with additional details provided in Appendix B: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. These species represent the most sensitive life stage, toxicity test duration and endpoint measured based on Warne et al. (2018). Sufficient chronic toxicity data were available to derive the DGVs. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

The toxicity values included five IC/EC10 values, one EC20 value, three LC1 values, 16 NOEC values and two estimated chronic LC10 values (from chronic LC50 values). Uncertainty in LC1 values can be high due to a lack of data at the lower end of the concentration–response curve to generate reliable estimates. The LC1 values for the fish species *P. promelas*, *P. lucius* and *X. texanus* were cross-checked with available NOEC and EC20 values for the same species from the same study (Fairchild et al. 2005); the NOEC and EC20 values were approximately two times higher than the LC1 and, consequently, the LC1 values were considered to be a reasonable estimate of a 'negligible effect concentration' and were included in the final dataset over the less preferred NOEC and EC20 values.

Toxicity values for the bivalves *L. fasciola, L. siliquoidea* and *V. iris* were reported as 'less than' IC10 values, as reported by Wang et al. (2007). As bivalves were the most sensitive taxon, it was considered important to include such values even if they were not definitive. The IC10 values were included in the DGV derivation without further modification because the percentage effect relative to the control at the reported IC10 values was 13–17%, which is within the upper limit of 20% effect (EC20) accepted for inclusion in guideline value derivations (Warne et al. 2018).

The inclusion of microalgal and aquatic plant data in the derivation of water quality guideline values for ammonia is inconsistent between international jurisdictions due to the role ammonia plays as a nutrient for aquatic photosynthetic organisms and their lack of sensitivity to ammonia compared to vertebrates and invertebrates (CCME 2010, USEPA 2013). Canada (CCME 2010), the UK (Environment Agency 2007), Mooney et al. (2019) and Wang & Leung (2015) included microalgae/macrophytes in the derivation of their guideline values for ammonia. In contrast, USEPA (2013) eliminated microalgae/macrophytes from its guideline value derivation for ammonia, although this did not affect the final guideline value, which was derived from the most sensitive 5th percentile of the mean genus toxicity values. Assessment of the dataset in Table 3 confirmed that there was no evidence to suggest that the microalgal data resulted in a bimodal distribution, and that there was insufficient evidence to justify the removal of the microalgal data from the final dataset (Appendix C: Modality assessment for ammonia). Therefore, the microalgal toxicity data were used to derive the DGVs for ammonia. Despite microalgae/macrophytes not being considered in the derivation of the joint-toxicity models and ammonia guideline values by USEPA (2013), there is no evidence to suggest that the joint-toxicity models should not be applied to microalgae. Therefore, the joint-toxicity models were also applied to the microalgal data.

Table 3 Summary of single chronic toxicity values, all species used to derive the default guideline values for total ammonia in freshwater

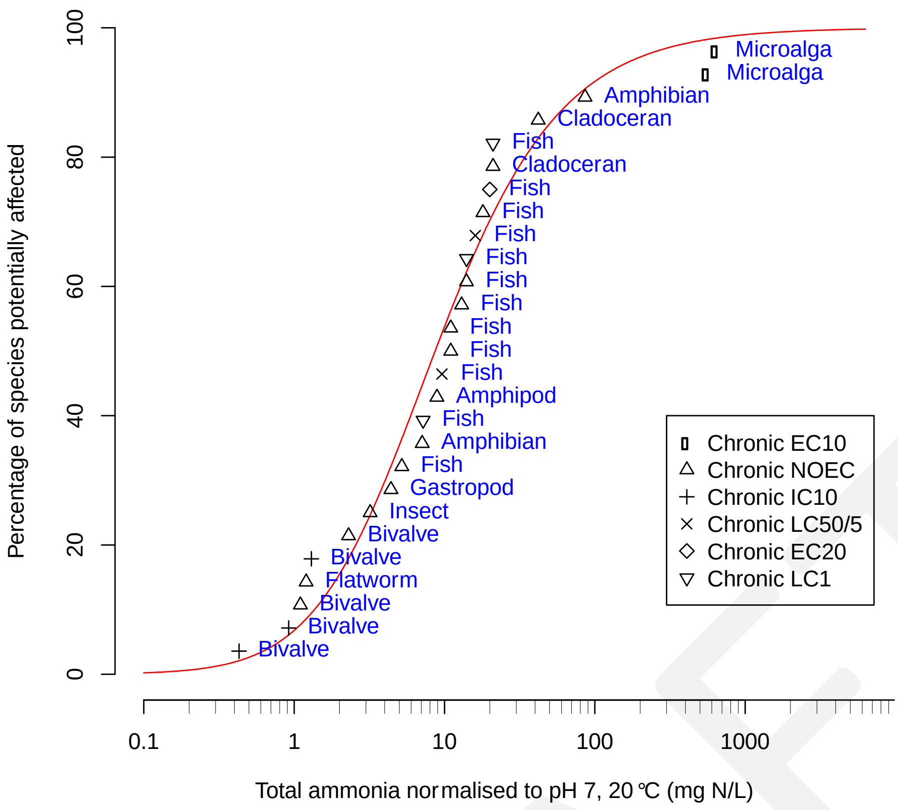
Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (endpoint) [a]	Toxicity value (mg N/L) (pH 7, 20°C)	Final toxicity value (mg N/L) (pH 7, 20°C)
Microalga	*Chlorella vulgaris*	Exponential growth (<72 h old)	3	EC10 (growth rate)	640	640
	Pseudokirchneriella subcapitata	Exponential growth (<72 h old)	3	EC10 (growth rate)	560	560
Flatworm	*Polycelis felina*	–	30	NOEC (mortality)	1.2	1.2
Cladoceran	*Ceriodaphnia dubia*	Neonate (<24 h)	7	NOEC (reproduction)	42	42
	Daphnia magna	Neonate	21	NOEC (reproduction and mortality)	21	21
Insect	*Deleatidium* sp.	Juvenile	29	NOEC (mortality)	3.2	3.2
Amphipod	*Hyalella azteca*	Young (<1 week)	70	NOEC (reproduction)	8.9	8.9
Gastropod	*Potamopyrgus antipodarum*	–	40	NOEC (immobility)	4.4	4.4
Bivalve	*Lampsilis fasciola*	Juvenile	28	IC10 (mortality)	<0.43	0.43
	Lampsilis siliquoidea	Juvenile (8 weeks)	28	IC10 (mortality)	0.92 [e]	0.92
	Musculium transversum	–	42	NOEC (mortality)	2.3	2.3
	Sphaerium novaezelandiae	Young	60	NOEC (growth–length)	1.1	1.1
	Villosa iris	Juvenile	28	IC10 (growth–shell length)	<1.3	1.3

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)
Fish	*Carassius carpio*	Fry (larvae)	7	LC50 (mortality)	82 [b]	16 [c]
	Deltistes luxatus	Larvae (late stage)	30	NOEC (mortality)	14	14
	Esox lucius	Fertilised eggs	52	EC20 (biomass–weight)	20	20
	Ictalurus punctatus	Embryo	30	NOEC (biomass–weight)	5.2	5.2
	Lepomis cyanellus	Embryo	44	NOEC (biomass–weight)	11	11
	Micropterus dolomieue	Embryo	32	NOEC (biomass–length)	13 [b]	13
	Notropis topeka	Juvenile [d]	30	NOEC (growth rate)	18	18
	Oncorhynchus clarkii	Fry (1 g and 3 g)	29	LC50 (mortality)	48 [b]	9.6 [c]
	Oncorhynchus mykiss	Embryo	35	NOEC (growth–length)	11	11
	Pimephales promelas	Young (4 d post hatch)	28	LC1 (mortality)	7.2	7.2
	Ptychocheilus lucius	Young (8 d post hatch)	28	LC1 (mortality)	21	21
	Xyrauchen texanus	Young (9 d post hatch)	28	LC1 (mortality)	14	14
Amphibian	*Pseudacris regilla*	Embryo	10	NOEC (deformities)	7.1	7.1
	Xenopus laevis	Tadpole	10	NOEC (biomass–length and weight)	86 [b]	86

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.

4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of ammonia toxicity data for the 27 freshwater species normalised to pH 7 and 20°C that were used to derive the DGVs is presented in Figure 1. The model was judged to provide a good fit to the data.



Note: values in SSD are for total ammonia (mg N/L) normalised to pH 7 and 20°C where applicable (i.e. temperature applies only to invertebrates).

Figure 1 Species sensitivity distribution, total ammonia in freshwater

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 website (ANZG 2018).

The total ammonia DGVs for each species protection level are not a single value but rather a range of values over various pHs and temperatures, reflecting the influence of pH and temperature on ammonia toxicity. The DGVs are expressed as total ammonia concentration (mg N/L) and are applicable to temperate and tropical ecosystems. Freshwaters in Australia and New Zealand generally have pH values of around 7 and 8, respectively. Therefore, the ammonia DGVs for 99%, 95%, 90% and 80% species protection at pH 7 and pH 8 at 20°C are provided in Table 4.

DGVs for waters at different pH (6–9) and temperature (10–30°C) were calculated using joint-toxicity normalisation equations (USEPA 2013) and are given in Appendix D: Ammonia freshwater default guideline values at different pH and temperature. SSDs were plotted using toxicity values normalised to pH 7, with invertebrate toxicity values further normalised to a range of temperatures (10–30°C) (using equation 2 in Table 2). Values for 99%, 95%, 90% and 80% species protection from each SSD at each temperature were then derived for different pH values (6–9) (using equation 1 in Table 2). Where local pH and temperature data are not available, it is recommended that DGVs that represent a regionally-relevant high bioavailability condition (i.e. higher pH, higher temperature) are selected, in liaison with the local jurisdiction.

The total ammonia concentration (as mg N/L) measured in the receiving water can be compared to the DGV that matches the pH and temperature of the receiving water (Appendix D: Ammonia freshwater default guideline values at different pH and temperature). If the ammonia concentration is reported as un-ionised ammonia, it must be converted to total ammonia (Appendix A: Relationship between un-ionised ammonia and total ammonia) before comparison with the relevant DGV.

Generally, the 95% DGV (adjusted for local pH and temperature) is recommended for application to slightly-to-moderately disturbed ecosystems. However, there are some exceptions. Bivalves were the most sensitive taxa, and the SSD indicates that not all bivalves may be protected by the 95% species protection DGV (Table 3, Figure 1). Therefore the 99% species protection DGV or a site-specific guideline value may be required where bivalve species are present and considered to be an important component of the aquatic ecosystem. Site-specific guideline values or validation of the DGVs for local conditions may also be needed for water that has low conductivity (e.g. <50 µS/cm; see Mooney et al. 2019) or is outside the pH and temperature ranges for which the DGVs are provided.

Table 4 Default guideline values, total ammonia in freshwater, very high reliability

Level of species protection (%)	DVG for total ammonia in freshwater (mg N/L) [a, b]	
	pH: 7 **Temperature: 20°C**	**pH: 8** **Temperature: 20°C**
99	0.26	0.11
95	0.79	0.33
90	1.4	0.58
80	2.6	1.1

a See Appendix D: Ammonia freshwater default guideline values at different pH and temperature for ammonia DGVs applicable to a range of pH and temperatures.
b The DGVs were derived using the Burrlioz 2.0 software and are reported to two significant figures. They are not applicable to low ionic strength waters (e.g. <50 µS/cm).

4.4 Reliability classification

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

- sample size—27 (preferred)
- type of toxicity data—chronic EC/IC10, LC1, EC20, NOEC and estimated chronic LC50 from chronic LC50
- SSD model fit—good.

Glossary

Term	Definition
acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short exposure period to a chemical relative to the organism's life span.
acute-to-chronic ratio	The species mean acute value (LC/EC50) divided by the chronic value (e.g. NOEC) for the same species.
bioaccumulation	The process by which chemical substances are accumulated by aquatic organisms by all routes of exposures (dietary and the ambient environment).
chronic toxicity	A lethal or sublethal 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 effect on a sensitive early life stage.
default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. site-specific) in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality.
DOC	Dissolved organic carbon.
EC50 (median effective concentration)	The concentration of a substance in water or sediment that is estimated to produce a 50% change in the response being measured or a certain effect in 50% of the test organisms relative to the control response, under specified conditions.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
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.
ionised ammonia	Ammonium ion, NH_4^+.
LC50 (median lethal concentration)	The concentration of a substance in water or sediment that is estimated to be lethal to 50% of a group of 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 under specified conditions.
LOEC (lowest observed effect concentration)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
macrophyte	A member of the macroscopic plant life of an area, especially of a body of water; large aquatic plant.
mesocosm	An artificial system containing complex and self-sustaining populations or communities set in natural environmental conditions.
NOEC (no observed effect concentration)	The highest concentration of a material used in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
site-specific	Relating to something that is confined to, or valid for, a particular place. Site-specific guideline values are relevant to the location or conditions that are the focus of a given assessment.
species	A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group.

Term	Definition
SSD (species sensitivity distribution)	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.
TAN	Total ammonia expressed as total nitrogen.
total ammonia	The sum of un-ionised ammonia (NH_3) and ammonium ion (NH_4^+).
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.
un-ionised ammonia	NH_3.

Appendix A: Relationship between un-ionised ammonia and total ammonia

Table A 1 Percentage of un-ionised ammonia (NH_3) at different pH and temperatures (based on Emerson et al. 1975)

Temp. (°C)	pH																														
	6.5	**6.6**	**6.7**	**6.8**	**6.9**	**7.0**	**7.1**	**7.2**	**7.3**	**7.4**	**7.5**	**7.6**	**7.7**	**7.8**	**7.9**	**8.0**	**8.1**	**8.2**	**8.3**	**8.4**	**8.5**	**8.6**	**8.7**	**8.8**	**8.9**	**9.0**	**9.1**	**9.2**	**9.3**	**9.4**	**9.5**
10	0.0587	0.0738	0.0929	0.117	0.147	0.185	0.233	0.293	0.369	0.464	0.584	0.734	0.922	1.16	1.45	1.82	2.28	2.86	3.57	4.45	5.54	6.88	8.51	10.5	12.8	15.7	18.9	22.7	27.0	31.8	37.0
11	0.0634	0.0798	0.100	0.126	0.159	0.200	0.252	0.317	0.399	0.502	0.631	0.793	0.996	1.25	1.57	1.97	2.46	3.08	3.85	4.80	5.97	7.40	9.14	11.2	13.7	16.7	20.2	24.1	28.6	33.5	38.8
12	0.0685	0.0863	0.109	0.137	0.172	0.216	0.272	0.343	0.431	0.542	0.681	0.856	1.08	1.35	1.69	2.12	2.66	3.32	4.15	5.17	6.42	7.95	9.80	12.0	14.7	17.8	21.4	25.6	30.2	35.3	40.7
13	0.0740	0.0932	0.117	0.148	0.186	0.234	0.294	0.370	0.465	0.585	0.735	0.924	1.16	1.46	1.83	2.29	2.86	3.58	4.46	5.56	6.90	8.53	10.5	12.9	15.7	19.0	22.8	27.1	31.8	37.0	42.5
14	0.0799	0.101	0.127	0.159	0.200	0.252	0.317	0.399	0.502	0.631	0.793	0.997	1.25	1.57	1.97	2.47	3.09	3.85	4.80	5.97	7.40	9.15	11.2	13.8	16.7	20.2	24.1	28.6	33.5	38.8	44.4
15	0.0862	0.108	0.137	0.172	0.216	0.272	0.342	0.431	0.541	0.681	0.855	1.07	1.35	1.69	2.12	2.66	3.32	4.14	5.16	6.41	7.94	9.80	12.0	14.7	17.8	21.4	25.6	30.2	35.2	40.7	46.3
16	0.0929	0.117	0.147	0.185	0.233	0.293	0.369	0.464	0.584	0.734	0.922	1.16	1.45	1.82	2.28	2.86	3.57	4.45	5.54	6.88	8.51	10.5	12.8	15.7	18.9	22.7	27.0	31.8	37.0	42.5	48.2
17	0.100	0.126	0.159	0.200	0.251	0.316	0.398	0.500	0.629	0.790	0.993	1.25	1.56	1.96	2.46	3.07	3.84	4.79	5.95	7.38	9.11	11.2	13.7	16.7	20.1	24.1	28.5	33.4	38.8	44.3	50.1
18	0.108	0.136	0.171	0.215	0.271	0.340	0.428	0.538	0.677	0.851	1.07	1.34	1.68	2.11	2.64	3.30	4.12	5.14	6.38	7.90	9.75	12.0	14.6	17.7	21.3	25.5	30.1	35.1	40.5	46.2	51.9
19	0.116	0.146	0.184	0.232	0.291	0.366	0.461	0.580	0.728	0.915	1.15	1.44	1.81	2.27	2.84	3.55	4.43	5.51	6.84	8.46	10.4	12.8	15.6	18.8	22.6	26.9	31.6	36.8	42.3	48.0	53.8
20	0.125	0.157	0.198	0.249	0.313	0.394	0.496	0.623	0.784	0.984	1.24	1.55	1.95	2.44	3.05	3.81	4.75	5.90	7.32	9.04	11.1	13.6	16.6	20.0	23.9	28.4	33.3	38.5	44.1	49.9	55.6
21	0.134	0.169	0.213	0.268	0.337	0.424	0.533	0.670	0.842	1.06	1.33	1.67	2.09	2.62	3.27	4.08	5.09	6.32	7.83	9.66	11.9	14.5	17.6	21.2	25.3	29.9	34.9	40.3	45.9	51.7	57.4
22	0.145	0.182	0.229	0.288	0.362	0.456	0.573	0.720	0.905	1.14	1.43	1.79	2.24	2.81	3.51	4.38	5.45	6.76	8.37	10.3	12.6	15.4	18.7	22.4	26.7	31.4	36.6	42.0	47.7	53.5	59.1
23	0.155	0.195	0.246	0.309	0.389	0.489	0.615	0.773	0.972	1.22	1.53	1.92	2.41	3.01	3.76	4.69	5.83	7.23	8.94	11.0	13.5	16.4	19.8	23.7	28.1	33.0	38.2	43.8	49.5	55.3	60.9
24	0.167	0.210	0.264	0.332	0.418	0.525	0.661	0.830	1.04	1.31	1.64	2.06	2.58	3.23	4.03	5.02	6.24	7.72	9.53	11.7	14.3	17.4	20.9	25.0	29.6	34.6	39.9	45.6	51.3	57.0	62.6
25	0.179	0.225	0.283	0.356	0.448	0.564	0.709	0.891	1.12	1.40	1.76	2.21	2.76	3.45	4.31	5.37	6.66	8.25	10.2	12.5	15.2	18.4	22.1	26.3	31.1	36.2	41.7	47.3	53.1	58.8	64.2
26	0.192	0.242	0.304	0.382	0.481	0.605	0.760	0.955	1.20	1.51	1.89	2.36	2.96	3.70	4.61	5.74	7.11	8.79	10.8	13.3	16.1	19.5	23.4	27.7	32.6	37.8	43.4	49.1	54.8	60.4	65.8
27	0.206	0.259	0.326	0.410	0.516	0.648	0.815	1.02	1.29	1.61	2.02	2.53	3.17	3.95	4.93	6.13	7.59	9.37	11.5	14.1	17.1	20.6	24.6	29.2	34.1	39.5	45.1	50.8	56.6	62.1	67.4
28	0.221	0.278	0.349	0.439	0.553	0.695	0.873	1.10	1.38	1.73	2.16	2.71	3.39	4.23	5.26	6.54	8.09	9.98	12.2	14.9	18.1	21.8	26.0	30.6	35.7	41.2	46.8	52.6	58.3	63.7	68.9
29	0.236	0.298	0.374	0.471	0.592	0.744	0.935	1.17	1.47	1.85	2.32	2.90	3.62	4.52	5.62	6.97	8.62	10.6	13.0	15.8	19.2	23.0	27.3	32.1	37.3	42.8	48.5	54.3	59.9	65.3	70.3
30	0.253	0.319	0.401	0.504	0.634	0.796	1.00	1.26	1.58	1.98	2.48	3.10	3.87	4.82	5.99	7.43	9.18	11.3	13.8	16.8	20.2	24.2	28.7	33.6	38.9	44.5	50.3	56.0	61.6	66.8	71.7

These values differ slightly to those reported in ANZECC/ARMCANZ (2000) Table 8.3.6. This is due to the use of a temperature conversion of 273.15 instead of the 273.2 used in Emerson et al. (1975) and ANZECC/ARMCANZ (2000).

To convert ammonia concentrations from NH_3 to N, multiply the concentration by 0.8227 (i.e. 14.01÷17.03).

Total ammonia (mg N/L) = un-ionised ammonia (µg N/L) ÷ (% un-ionised ÷ 100) ÷ 1000.

Example: for an un-ionised ammonia concentration of 70 µg N/L, at a pH of 7.5 and temperature of 21°C, the total ammonia concentration (mg N/L) = 70 ÷ (1.33÷100) ÷ 1000 = 5.3 mg N/L.

Appendix B: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table B 1 Summary, chronic toxicity data that passed the screening and quality assessment processes, total ammonia in freshwater

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium (conductivity)	Temp. (°C)	pH	Reported concentration (mg N/L)	Normalised concentration (mg N/L) (pH 7, 20°C)	Reference
Microalga	*Chlorella vulgaris*	<72 h	3	EC10 (growth rate)	Bolds Basal Medium (12 900 µS/cm at EC10)	25	6.6	714 [a]	640	Wang & Leung (2015)
	–								**640**	**Value used in SSD**
	Pseudokirchneriella subcapitata	<72 h	3	EC10 (growth rate)	Bolds Basal Medium (13 000 µS/cm at EC10)	25	6.6	622 [a]	560	Wang & Leung (2015)
	–								**560**	**Value used in SSD**
Flatworm	*Polycelis felina*	–	30	NOEC (mortality)	Bottled water, no chlorine (734 µS/cm)	15	8.1 ± 0.3	0.6 [a]	1.2	Alonso & Camargo (2011)
	–								**1.2**	**Value used in SSD**
Cladoceran	*Ceriodaphnia dubia*	Neonate (<24 h)	7	NOEC (reproduction)	St Vrain River water	25	8.0	13 [a]	42	Nimmo et al. (1989)
	–								**42**	**Value used in SSD**
	Daphnia magna	Neonate	21	NOEC (reproduction, and mortality)	Tittabawassee River water, filtered (380–440 µS/cm)	20	8.3–8.6	4.2 [a]	21	Gersich & Hopkins (1986)
	–								**21**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium (conductivity)	Temp. (°C)	pH	Reported concentration (mg N/L)	Normalised concentration (mg N/L) (pH 7, 20°C)	Reference
Insect	*Deleatidium* sp.	Juvenile	29	NOEC (mortality)	Filtered reservoir water and sediment (mesocosm)	16	8.4	0.95	3.2	Hickey et al. (1999)
	–								**3.2**	**Value used in SSD**
Amphipod	*Hyalella azteca*	Young (<1 week)	70	NOEC (reproduction)	Dechlorinated tap water	25	8.0 ± 0.4	2.5	8.9	Borgmann (1994)
	–								**8.9**	**Value used in SSD**
Gastropod	*Potamopyrgus antipodarum*	–	40	NOEC (immobility) [b]	Bottled water, no chlorine	15	8.1 ± 0.3	2.1	4.4	Alonso & Camargo (2009)
	–								**4.4**	**Value used in SSD**
Bivalve	*Lampsilis fasciola*	Juvenile	28	IC10 (mortality)	ASTM hard water	20	8.2	<0.13	<0.43	Wang et al. (2007)
	–								**0.43**	**Value used in SSD**
	Lampsilis siliquoidea	Juvenile (8 weeks)	28	IC10 (mortality)	ASTM hard water (160–180 mg/L hardness)	20	8.3	0.54	2.0	Wang et al. (2011)
		Juvenile (8 weeks)	28	IC10 (mortality)	ASTM hard water	20	8.2 ± 0.1	<0.13	<0.43	Wang et al. (2007)
	–								**0.92**	**Geometric mean; value used in SSD**
	Musculium transversum	–	42	NOEC (mortality)	Well water (unchlorinated)	22	7.6–8.1	1.1	2.3	Sparks & Sandusky (1981)
	–								**2.3**	**Value used in SSD**
	Sphaerium novaezelandiae	Young	60	NOEC (growth–length)	Fern Hollow spring water	20	5.8–8.2 [c]	0.65	1.1	Hickey & Martin (1999)
	–								**1.1**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium (conductivity)	Temp. (°C)	pH	Reported concentration (mg N/L)	Normalised concentration (mg N/L) (pH 7, 20°C)	Reference
	Villosa iris	Juvenile	28	IC10 (growth–shell length)	ASTM hard water	20	8.2 ± 0.1	<0.4	<1.3	Wang et al. (2007)
	–								**1.3**	**Value used in SSD**
Fish	*Carassius carpio*	Fry (larvae)	7	LC50 (mortality)	Synthetic soft water	28	7.7	46 [a]	78	Hasan & MacIntosh (1986)
		Fry (larvae)	7	LC50 (mortality)	Synthetic soft water	28	7.7	51 [a]	85	Hasan & MacIntosh (1986)
									82	*Geometric mean*
	–								**16 [d]**	**Value used in SSD**
	Deltistes luxatus	Larvae (late stage)	30	NOEC (mortality)	20% well water (162 µS/cm)	22	9.5 ± 0.1	0.64	14	Meyer & Hansen (2002)
	–								**14**	**Value used in SSD**
	Esox lucius	Fertilised eggs	52	EC20 (biomass–weight)	Dechlorinated tap water	8.6	7.6	13	20	Harrahy et al. (2004)
	–								**20**	**Value used in SSD**
	Ictalurus punctatus	Embryo	30	NOEC (biomass-weight)	Treated well water (660 µS/cm)	24–28	7.5–8.1	2.9	5.2	Swigert & Spacie (1983)
	–								**5.2**	**Value used in SSD**
	Lepomis cyanellus	Embryo	44	NOEC (biomass–weight)	Lake Superior water	22	7.9 ± 0.1	181 [a]	11	McCormick et al. (1984)
	–								**11**	**Value used in SSD**
	Micropterus dolomieue	Embryo	32	NOEC (biomass–length)	Lake Superior water (filtered)	22	6.6 ± 0.1	8.3	7.4	Broderius et al. (1985)
		Embryo	32	NOEC (biomass–length)	Lake Superior water (filtered)	22	7.3 ± 0.1	18	21	Broderius et al. (1985)

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium (conductivity)	Temp. (°C)	pH	Reported concentration (mg N/L)	Normalised concentration (mg N/L) (pH 7, 20°C)	Reference
		Embryo	32	NOEC (biomass–length)	Lake Superior water (filtered)	22	7.8 ± 0.1	6.7	13	Broderius et al. (1985)
		Embryo	32	NOEC (biomass–length)	Lake Superior water (filtered)	22	8.7 ± 0.1	1.8	13	Broderius et al. (1985)
	–								**13**	**Geometric mean; value used in SSD**
	Notropis topeka	Adult	30	NOEC (survival)	Well water	24	7.9	8.1	18	Aldelman et al. (2009)
		Juvenile	30	NOEC (growth rate)	Well water	12	8.1	6.7	18	Aldelman et al. (2009)
	–								**18 [e]**	**Value used in SSD**
	Oncorhynchus clarkii	Fry (1 g)	29	LC50 (mortality)	Spring water (328 µS/cm)	13	7.8	31	58	Thurston et al. (1978)
		Fry (1 g)	29	LC50 (mortality)	Spring water (328 µS/cm)	13	7.8	32	60	Thurston et al. (1978)
		Fry (3 g)	29	LC50 (mortality)	Spring water (328 µS/cm)	12	7.8	22	40	Thurston et al. (1978)
		Fry (3 g)	29	LC50 (mortality)	Spring water (328 µS/cm)	12	7.8	21	39	Thurston et al. (1978)
	–								*48*	*Geometric mean*
	–								**9.6 [d]**	**Value used in SSD**
	Oncorhynchus mykiss	Embryo	35	NOEC (growth–length)	Dechlorinated tap water (202−262 µS/cm)	10–12	7.4–7.6	7.9 [a]	11	Burkhalter & Kaya (1977)
	–								**11**	**Value used in SSD**
	Pimephales promelas	Young (4 d post hatch)	28	LC1 (mortality)	Well water	20	8.2	2.0 [a]	7.2	Fairchild et al. (2005)

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium (conductivity)	Temp. (°C)	pH	Reported concentration (mg N/L)	Normalised concentration (mg N/L) (pH 7, 20°C)	Reference
	–								**7.2**	**Value used in SSD**
	Ptychocheilus lucius	Young (8 d post hatch)	28	LC1 (mortality)	Well water	20	8.2	5.9 [a]	21	Fairchild et al. (2005)
	–								**21**	**Value used in SSD**
	Xyrauchen texanus	Young (9 d post hatch)	28	LC1 (mortality)	Well water	20	8.2	3.9 [a]	14	Fairchild et al. (2005)
	–								**14**	**Value used in SSD**
Amphibian	*Pseudacris regilla*	Embryo	10	NOEC (deformities)	Well water (196 µS/cm)	22	7.0-7.6	6.1	7.1	Schuytema & Nebeker (1999a)
	–								**7.1**	**Value used in SSD**
	Xenopus laevis	Tadpole	10	NOEC (biomass–length and weight)	Well water (75 µS/cm)	22	6.7–7.6	49	54	Schuytema & Nebeker (1999b)
		Tadpole	10	NOEC (biomass–length and weight)	Well water (75 µS/cm)	22	6.7–7.6	100	110	Schuytema & Nebeker (1999b)
		Tadpole	10	NOEC (biomass–length and weight)	Well water (75 µS/cm)	22	6.7–7.6	100	110	Schuytema & Nebeker (1999b)
	–								**86**	**Geometric mean; value used in SSD**

a Toxicity value published as un-ionised ammonia; toxicity value reported here has been converted to total ammonia (N) at the test pH and temperature.

b Behavioural endpoint immobility – defined as no movement after 6 minutes observation. Mortality assessed separately in the same animals, but no significant effect on mortality found.

c pH range across all treatments, pH at NOEC concentration was 7.7.

d LC50 ÷ 5.

e Two endpoints gave the same response; the toxicity value using the earlier life stage (juvenile) is reported in Table 3.

Appendix C: Modality assessment for ammonia

The incorporation of plants and algae in the derivation of water quality guidelines for ammonia is inconsistent between jurisdictions due to the role ammonia plays as a nutrient for aquatic photosynthetic organisms and their general lack of sensitivity to ammonia (CCME 2010, USEPA 2013).

The acceptable dataset for chronic ammonia toxicity, incorporating data for two microalgae and 25 vertebrate and invertebrate species, was assessed for bimodality according to the four questions in Warne et al. (2018).

Is there a specific mode of action that could result in taxa-specific sensitivity?
Ammonia can be used by plants/algae as a nutrient source for nitrogen, but it is not an essential nutrient. Ammonia is also an endogenous toxicant excreted from vertebrates and invertebrates. Numerous toxic modes of action have been proposed for fish, invertebrates, plants and algae (Section 2.1). Apart from plants and algae being capable of using ammonia as a nutrient source, evidence about its mode of action does not suggest that it could have taxa-specific sensitivity. Although bivalves have been demonstrated to be particularly sensitive to ammonia, it is not clear whether this is due to a specific mode of action targeting only this taxon.

Does the dataset suggest bimodality?
Visual observation of the log transformed dataset does not indicate signs of bimodality (Figure C 1). The statistical test deriving the bimodality coefficient (BC) also indicated that the dataset was not bimodal, with the calculated BC of 0.319 for the full dataset less than the indicative bimodality threshold value of 0.555 (representing a uniform distribution).

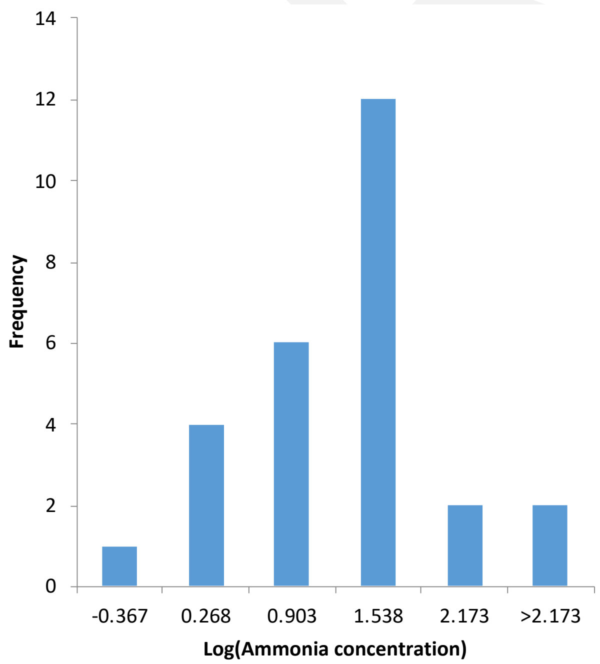
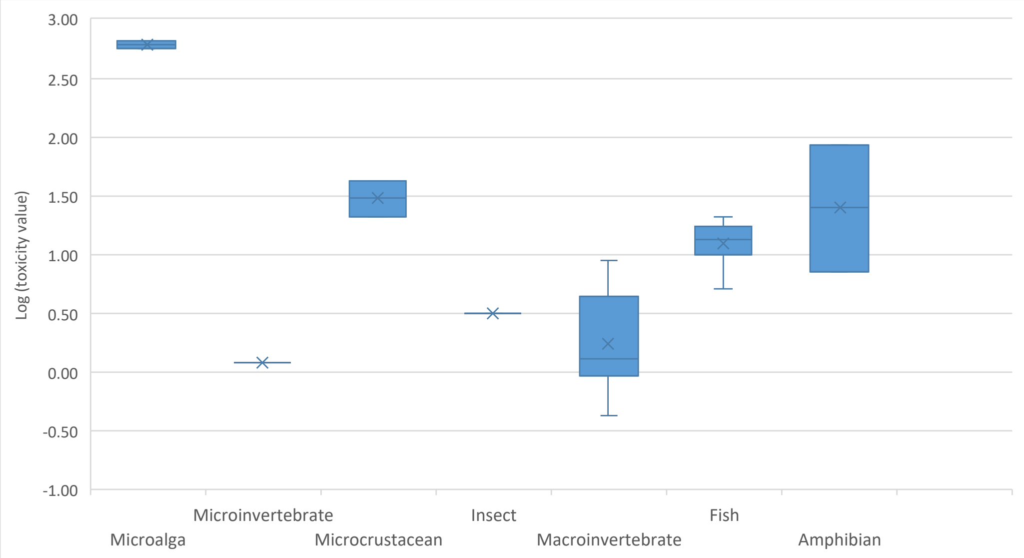


Figure C 1 Histogram of log transformed data, ammonia freshwater toxicity

Do the data show taxa-specific sensitivity (i.e. through distinct grouping of different taxa types)?
Green microalgae may be considered to have a different taxa-specific sensitivity, with the microalgae (n=2) about 7× less sensitive than the next most sensitive species (amphibian). However, the sample size for microalgae is relatively small, making it difficult to draw conclusions. Also, bivalves—specifically mussels—appear to be a particularly sensitive taxon, although this is not well captured in Figure C 2 as they are incorporated with other macroinvertebrates. It is unclear why mussels are highly sensitive to ammonia relative to other taxa.



Note: "×" denotes the mean.

Figure C 2 Box plot, comparison of ammonia toxicity to taxonomic groups

Is it likely that indications of bimodality or multimodality or distinct clustering of taxa groups are *not* due to artefacts of data selection, small size, test procedures, or other reasons unrelated to a specific mode of action?
The species sensitivity distribution (SSD) for the full dataset incorporates 27 chronic toxicity values including two toxicity values that were converted from an LC50 divided by 5. The fit of the data to the curve was good. Bivalves were generally the most sensitive taxon. The two green microalgal species are chlorophytes and the least sensitive species (Figure C 2). This is a relatively small sample size and does not include other classes of microalgae (e.g. diatoms, dinoflagellates) or macrophytes (e.g. duckweed). Chronic ammonia toxicity to species from (and tested in) waters with very low conductivity (15–20 µS/cm), hardness (~5 mg/L $CaCO_3$) and pH (6) showed that a green microalga was the least sensitive species (out of eight species) followed by a cladoceran, a snail and an aquatic macrophyte (Mooney et al. 2019). Even if ammonia exhibits taxa-specific toxicity, the majority of the available evidence indicates that the final chronic toxicity dataset is not bimodal.

Overall assessment
There was no conclusive evidence to remove taxonomic groups from the ammonia DGV dataset due to bimodality.

Appendix D: Ammonia freshwater default guideline values at different pH and temperature

The DGVs provided in this appendix for different pH and temperature are not applicable to water that has low ionic strength (e.g. <50 µS/cm) or that is outside the pH (6–9) and temperature (10–30°C) ranges. For such water, site-specific guideline values or validation of the DGVs for local conditions may need to be considered. Where local pH and temperature data are not available, DGVs that represent a regionally-relevant high bioavailability condition (i.e. higher pH, higher temperature) should be used, in liaison with the local jurisdiction.

Table D 1 Freshwater 99% species protection guideline values for total ammonia (mg N/L) at different pH and temperature

Temp. (°C)	pH																													
	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
10	0.87	0.87	0.86	0.85	0.85	0.83	0.82	0.81	0.79	0.77	0.74	0.71	0.67	0.64	0.59	0.55	0.50	0.45	0.40	0.35	0.30	0.26	0.22	0.19	0.16	0.14	0.12	0.097	0.083	0.071	0.061
11	0.81	0.81	0.80	0.80	0.79	0.78	0.77	0.75	0.73	0.71	0.69	0.66	0.63	0.59	0.55	0.51	0.46	0.42	0.37	0.33	0.28	0.24	0.21	0.18	0.15	0.13	0.11	0.091	0.077	0.066	0.057
12	0.73	0.73	0.72	0.72	0.71	0.70	0.69	0.68	0.66	0.64	0.62	0.59	0.57	0.53	0.50	0.46	0.42	0.38	0.33	0.29	0.26	0.22	0.19	0.16	0.14	0.11	0.096	0.082	0.069	0.059	0.051
13	0.65	0.64	0.64	0.63	0.63	0.62	0.61	0.60	0.59	0.57	0.55	0.53	0.50	0.47	0.44	0.41	0.37	0.33	0.30	0.26	0.23	0.20	0.17	0.14	0.12	0.10	0.086	0.072	0.062	0.053	0.045
14	0.61	0.61	0.60	0.60	0.59	0.59	0.58	0.57	0.55	0.54	0.52	0.50	0.47	0.45	0.42	0.38	0.35	0.31	0.28	0.25	0.21	0.18	0.16	0.13	0.11	0.096	0.081	0.068	0.058	0.050	0.043
15	0.54	0.54	0.53	0.53	0.53	0.52	0.51	0.50	0.49	0.48	0.46	0.44	0.42	0.40	0.37	0.34	0.31	0.28	0.25	0.22	0.19	0.16	0.14	0.12	0.10	0.085	0.072	0.061	0.051	0.044	0.038
16	0.48	0.48	0.48	0.47	0.47	0.46	0.46	0.45	0.44	0.42	0.41	0.39	0.37	0.35	0.33	0.30	0.28	0.25	0.22	0.19	0.17	0.15	0.12	0.11	0.089	0.076	0.064	0.054	0.046	0.039	0.034
17	0.44	0.43	0.43	0.43	0.42	0.42	0.41	0.40	0.39	0.38	0.37	0.35	0.34	0.32	0.30	0.27	0.25	0.22	0.20	0.18	0.15	0.13	0.11	0.10	0.081	0.068	0.058	0.049	0.041	0.035	0.030
18	0.39	0.39	0.38	0.38	0.38	0.37	0.37	0.36	0.35	0.34	0.33	0.32	0.30	0.28	0.26	0.24	0.22	0.20	0.18	0.16	0.14	0.12	0.10	0.085	0.072	0.061	0.051	0.043	0.037	0.032	0.027
19	0.35	0.35	0.35	0.35	0.34	0.34	0.33	0.33	0.32	0.31	0.30	0.29	0.27	0.26	0.24	0.22	0.20	0.18	0.16	0.14	0.12	0.11	0.091	0.077	0.065	0.055	0.047	0.039	0.034	0.029	0.025
20	0.31	0.30	0.30	0.30	0.30	0.29	0.29	0.28	0.28	0.27	0.26	0.25	0.24	0.22	0.21	0.19	0.17	0.16	0.14	0.12	0.11	0.092	0.079	0.067	0.057	0.048	0.040	0.034	0.029	0.025	0.021
21	0.27	0.27	0.27	0.27	0.26	0.26	0.26	0.25	0.24	0.24	0.23	0.22	0.21	0.20	0.18	0.17	0.15	0.14	0.12	0.11	0.095	0.082	0.070	0.059	0.050	0.042	0.036	0.030	0.026	0.022	0.019
22	0.26	0.26	0.26	0.25	0.25	0.25	0.24	0.24	0.23	0.23	0.22	0.21	0.20	0.19	0.18	0.16	0.15	0.13	0.12	0.10	0.091	0.078	0.067	0.057	0.048	0.041	0.034	0.029	0.025	0.021	0.018
23	0.22	0.22	0.22	0.22	0.22	0.21	0.21	0.21	0.20	0.20	0.19	0.18	0.17	0.16	0.15	0.14	0.13	0.12	0.10	0.090	0.078	0.067	0.058	0.049	0.041	0.035	0.030	0.025	0.021	0.018	0.016
24	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.14	0.14	0.13	0.12	0.11	0.10	0.091	0.081	0.071	0.062	0.053	0.046	0.039	0.033	0.028	0.023	0.020	0.017	0.014	0.012
25	0.18	0.18	0.17	0.17	0.17	0.17	0.17	0.16	0.16	0.16	0.15	0.14	0.14	0.13	0.12	0.11	0.10	0.091	0.081	0.071	0.062	0.053	0.046	0.039	0.033	0.028	0.023	0.020	0.017	0.014	0.012
26	0.15	0.15	0.15	0.15	0.15	0.15	0.14	0.14	0.14	0.13	0.13	0.12	0.12	0.11	0.10	0.10	0.087	0.079	0.070	0.062	0.054	0.046	0.039	0.034	0.028	0.024	0.020	0.017	0.015	0.012	0.011
27	0.13	0.13	0.13	0.13	0.13	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.10	0.095	0.088	0.081	0.074	0.067	0.059	0.052	0.045	0.039	0.033	0.028	0.024	0.020	0.017	0.014	0.012	0.011	0.009
28	0.12	0.12	0.12	0.12	0.11	0.11	0.11	0.11	0.11	0.10	0.10	0.10	0.091	0.086	0.080	0.074	0.067	0.061	0.054	0.047	0.041	0.035	0.030	0.026	0.022	0.018	0.016	0.013	0.011	0.010	0.008
29	0.10	0.10	0.10	0.10	0.10	0.10	0.10	0.095	0.093	0.090	0.09	0.08	0.08	0.075	0.070	0.064	0.059	0.053	0.047	0.041	0.036	0.031	0.026	0.022	0.019	0.016	0.014	0.011	0.010	0.008	0.007
30	0.088	0.088	0.087	0.087	0.086	0.085	0.083	0.082	0.080	0.078	0.08	0.072	0.068	0.064	0.060	0.055	0.050	0.045	0.040	0.036	0.031	0.027	0.023	0.019	0.016	0.014	0.012	0.010	0.008	0.007	0.006

Table D 2 Freshwater 95% species protection guideline values for total ammonia (mg N/L) at different pH and temperature

Temp. (°C)	pH																														
	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
10	1.8	1.8	1.7	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.5	1.4	1.4	1.3	1.2	1.1	1.0	0.91	0.81	0.71	0.62	0.53	0.46	0.39	0.33	0.28	0.23	0.20	0.17	0.14	0.12
11	1.8	1.8	1.7	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.5	1.4	1.4	1.3	1.2	1.1	1.0	0.91	0.81	0.71	0.62	0.53	0.46	0.39	0.33	0.28	0.23	0.20	0.17	0.14	0.12
12	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.4	1.4	1.3	1.3	1.2	1.1	1.0	0.94	0.85	0.75	0.66	0.58	0.50	0.42	0.36	0.31	0.26	0.22	0.18	0.16	0.13	0.12
13	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.3	1.3	1.2	1.2	1.1	1.0	0.96	0.87	0.79	0.70	0.62	0.54	0.46	0.39	0.34	0.28	0.24	0.20	0.17	0.15	0.12	0.11
14	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.2	1.2	1.2	1.1	1.0	0.96	0.89	0.81	0.73	0.65	0.57	0.49	0.43	0.36	0.31	0.26	0.22	0.19	0.16	0.13	0.11	0.099
15	1.3	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.0	0.95	0.88	0.81	0.74	0.67	0.59	0.52	0.45	0.39	0.33	0.28	0.24	0.20	0.17	0.14	0.12	0.11	0.090
16	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.0	1.0	0.96	0.91	0.86	0.80	0.74	0.67	0.61	0.54	0.47	0.41	0.35	0.30	0.26	0.22	0.18	0.16	0.13	0.11	0.096	0.082
17	1.2	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.0	0.98	0.94	0.89	0.84	0.78	0.72	0.66	0.59	0.53	0.46	0.40	0.35	0.30	0.25	0.21	0.18	0.15	0.13	0.11	0.094	0.081
18	1.1	1.1	1.1	1.1	1.1	1.0	1.0	1.0	0.98	0.95	0.92	0.88	0.84	0.79	0.74	0.68	0.62	0.56	0.50	0.44	0.38	0.33	0.28	0.24	0.20	0.17	0.14	0.12	0.10	0.088	0.076
19	1.0	1.0	1.0	0.99	0.98	0.97	0.96	0.94	0.92	0.89	0.86	0.82	0.78	0.74	0.69	0.64	0.58	0.52	0.46	0.41	0.35	0.31	0.26	0.22	0.19	0.16	0.13	0.11	0.096	0.082	0.071
20	0.93	0.92	0.92	0.91	0.90	0.89	0.88	0.86	0.84	0.82	0.79	0.76	0.72	0.68	0.63	0.58	0.53	0.48	0.43	0.37	0.33	0.28	0.24	0.20	0.17	0.15	0.12	0.10	0.088	0.075	0.065
21	0.86	0.85	0.85	0.84	0.83	0.82	0.81	0.80	0.78	0.76	0.73	0.70	0.67	0.63	0.58	0.54	0.49	0.44	0.39	0.35	0.30	0.26	0.22	0.19	0.16	0.13	0.11	0.096	0.082	0.070	0.060
22	0.82	0.82	0.81	0.81	0.80	0.79	0.78	0.76	0.75	0.72	0.7	0.67	0.64	0.60	0.56	0.52	0.47	0.42	0.38	0.33	0.29	0.25	0.21	0.18	0.15	0.13	0.11	0.092	0.078	0.067	0.058
23	0.75	0.75	0.74	0.74	0.73	0.72	0.71	0.70	0.68	0.66	0.64	0.61	0.58	0.55	0.51	0.47	0.43	0.39	0.34	0.30	0.26	0.23	0.19	0.17	0.14	0.12	0.10	0.084	0.072	0.061	0.053
24	0.66	0.66	0.65	0.65	0.64	0.63	0.62	0.61	0.60	0.58	0.56	0.54	0.51	0.48	0.45	0.41	0.38	0.34	0.30	0.27	0.23	0.20	0.17	0.14	0.12	0.10	0.087	0.074	0.063	0.054	0.046
25	0.65	0.64	0.64	0.63	0.63	0.62	0.61	0.60	0.59	0.57	0.55	0.53	0.50	0.47	0.44	0.41	0.37	0.33	0.30	0.26	0.23	0.20	0.17	0.14	0.12	0.10	0.086	0.072	0.062	0.053	0.045
26	0.60	0.60	0.59	0.59	0.58	0.58	0.57	0.56	0.54	0.53	0.51	0.49	0.47	0.44	0.41	0.38	0.34	0.31	0.27	0.24	0.21	0.18	0.15	0.13	0.11	0.094	0.079	0.067	0.057	0.049	0.042
27	0.55	0.55	0.55	0.54	0.54	0.53	0.52	0.51	0.50	0.49	0.47	0.45	0.43	0.40	0.38	0.35	0.32	0.28	0.25	0.22	0.19	0.17	0.14	0.12	0.10	0.087	0.073	0.062	0.053	0.045	0.039
28	0.52	0.51	0.51	0.51	0.50	0.50	0.49	0.48	0.47	0.46	0.44	0.42	0.40	0.38	0.35	0.32	0.30	0.27	0.24	0.21	0.18	0.16	0.13	0.11	0.10	0.081	0.068	0.058	0.049	0.042	0.036
29	0.47	0.47	0.47	0.46	0.46	0.45	0.44	0.44	0.43	0.41	0.40	0.38	0.36	0.34	0.32	0.30	0.27	0.24	0.22	0.19	0.16	0.14	0.12	0.10	0.087	0.074	0.062	0.053	0.045	0.038	0.033
30	0.44	0.43	0.43	0.43	0.42	0.42	0.41	0.40	0.39	0.38	0.37	0.35	0.34	0.32	0.30	0.27	0.25	0.22	0.20	0.18	0.15	0.13	0.11	0.10	0.081	0.068	0.058	0.049	0.041	0.035	0.030

Table D 3 Freshwater 90% species protection guideline values for total ammonia (mg N/L) at different pH and temperature

Temp. (°C)	pH																														
	6.0	**6.1**	**6.2**	**6.3**	**6.4**	**6.5**	**6.6**	**6.7**	**6.8**	**6.9**	**7.0**	**7.1**	**7.2**	**7.3**	**7.4**	**7.5**	**7.6**	**7.7**	**7.8**	**7.9**	**8.0**	**8.1**	**8.2**	**8.3**	**8.4**	**8.5**	**8.6**	**8.7**	**8.8**	**8.9**	**9.0**
10	2.7	2.7	2.7	2.7	2.6	2.6	2.6	2.5	2.4	2.4	2.3	2.2	2.1	2.0	1.8	1.7	1.5	1.4	1.2	1.1	0.95	0.82	0.70	0.59	0.50	0.42	0.36	0.30	0.26	0.22	0.19
11	2.6	2.6	2.6	2.5	2.5	2.5	2.4	2.4	2.3	2.3	2.2	2.1	2.0	1.9	1.8	1.6	1.5	1.3	1.2	1.0	0.91	0.78	0.67	0.57	0.48	0.41	0.34	0.29	0.25	0.21	0.18
12	2.5	2.5	2.4	2.4	2.4	2.4	2.3	2.3	2.2	2.2	2.1	2.0	1.9	1.8	1.7	1.6	1.4	1.3	1.1	0.99	0.86	0.75	0.64	0.54	0.46	0.39	0.33	0.28	0.23	0.20	0.17
13	2.4	2.3	2.3	2.3	2.3	2.3	2.2	2.2	2.1	2.1	2	1.9	1.8	1.7	1.6	1.5	1.3	1.2	1.1	0.95	0.82	0.71	0.61	0.52	0.44	0.37	0.31	0.26	0.22	0.19	0.16
14	2.2	2.2	2.2	2.2	2.2	2.1	2.1	2.1	2.0	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.0	0.90	0.78	0.67	0.58	0.49	0.41	0.35	0.30	0.25	0.21	0.18	0.16
15	2.1	2.1	2.1	2.1	2.1	2.0	2.0	2.0	1.9	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	1.0	0.85	0.74	0.64	0.55	0.46	0.39	0.33	0.28	0.24	0.20	0.17	0.15
16	2.0	2.0	2.0	2.0	1.9	1.9	1.9	1.9	1.8	1.8	1.7	1.6	1.6	1.5	1.4	1.3	1.1	1.0	0.92	0.80	0.70	0.60	0.52	0.44	0.37	0.31	0.26	0.22	0.19	0.16	0.14
17	1.9	1.9	1.9	1.8	1.8	1.8	1.8	1.7	1.7	1.7	1.6	1.5	1.5	1.4	1.3	1.2	1.1	1.0	0.86	0.76	0.66	0.57	0.49	0.41	0.35	0.29	0.25	0.21	0.18	0.15	0.13
18	1.8	1.8	1.7	1.7	1.7	1.7	1.7	1.6	1.6	1.6	1.5	1.4	1.4	1.3	1.2	1.1	1.0	0.91	0.8	0.71	0.62	0.53	0.46	0.39	0.33	0.28	0.23	0.20	0.17	0.14	0.12
19	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.4	1.4	1.3	1.3	1.2	1.1	1.0	0.94	0.85	0.75	0.66	0.58	0.50	0.42	0.36	0.31	0.26	0.22	0.18	0.16	0.13	0.12
20	1.6	1.6	1.6	1.6	1.6	1.6	1.6	1.5	1.5	1.4	1.4	1.3	1.3	1.2	1.1	1.0	0.94	0.85	0.75	0.66	0.58	0.50	0.42	0.36	0.31	0.26	0.22	0.18	0.16	0.13	0.12
21	1.5	1.5	1.5	1.5	1.5	1.5	1.4	1.4	1.4	1.3	1.3	1.2	1.2	1.1	1.0	0.96	0.87	0.79	0.70	0.62	0.54	0.46	0.39	0.34	0.28	0.24	0.20	0.17	0.15	0.12	0.11
22	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.2	1.2	1.2	1.1	1.0	0.96	0.89	0.81	0.73	0.65	0.57	0.49	0.43	0.36	0.31	0.26	0.22	0.19	0.16	0.13	0.11	0.099
23	1.4	1.4	1.4	1.4	1.4	1.4	1.3	1.3	1.3	1.2	1.2	1.2	1.1	1.0	0.96	0.89	0.81	0.73	0.65	0.57	0.49	0.43	0.36	0.31	0.26	0.22	0.19	0.16	0.13	0.11	0.099
24	1.3	1.3	1.3	1.3	1.3	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.0	0.95	0.88	0.81	0.74	0.67	0.59	0.52	0.45	0.39	0.33	0.28	0.24	0.20	0.17	0.14	0.12	0.105	0.090
25	1.2	1.2	1.2	1.2	1.1	1.1	1.1	1.1	1.1	1.0	1.0	0.96	0.91	0.86	0.80	0.74	0.67	0.61	0.54	0.47	0.41	0.35	0.30	0.26	0.22	0.18	0.16	0.13	0.11	0.096	0.082
26	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.1	1.0	1.0	0.97	0.93	0.88	0.83	0.78	0.72	0.65	0.59	0.52	0.46	0.40	0.34	0.29	0.25	0.21	0.18	0.15	0.13	0.11	0.093	0.080
27	1.1	1.1	1.1	1.0	1.0	1.0	1.0	0.99	0.97	0.94	0.91	0.87	0.83	0.78	0.73	0.67	0.61	0.55	0.49	0.43	0.37	0.32	0.28	0.23	0.20	0.17	0.14	0.12	0.10	0.087	0.075
28	1.0	1.0	1.0	0.99	0.98	0.97	0.96	0.94	0.92	0.89	0.86	0.82	0.78	0.74	0.69	0.64	0.58	0.52	0.46	0.41	0.35	0.31	0.26	0.22	0.19	0.16	0.13	0.11	0.096	0.082	0.071
29	0.96	0.96	0.95	0.95	0.94	0.92	0.91	0.89	0.87	0.85	0.82	0.79	0.75	0.70	0.66	0.61	0.55	0.50	0.44	0.39	0.34	0.29	0.25	0.21	0.18	0.15	0.13	0.11	0.092	0.078	0.067
30	0.91	0.90	0.90	0.89	0.88	0.87	0.86	0.84	0.82	0.80	0.77	0.74	0.70	0.66	0.62	0.57	0.52	0.47	0.41	0.36	0.32	0.27	0.23	0.20	0.17	0.14	0.12	0.10	0.086	0.074	0.063

Table D 4 Freshwater 80% species protection guideline values for total ammonia (mg N/L) at different pH and temperature

Temp. (°C)	pH																													
	6.0	6.1	6.2	6.3	6.4	6.5	6.6	6.7	6.8	6.9	7.0	7.1	7.2	7.3	7.4	7.5	7.6	7.7	7.8	7.9	8.0	8.1	8.2	8.3	8.4	8.5	8.6	8.7	8.8	8.9	9.0
10	4.4	4.3	4.3	4.3	4.2	4.2	4.1	4.0	3.9	3.8	3.7	3.5	3.4	3.2	3.0	2.7	2.5	2.2	2.0	1.8	1.5	1.3	1.1	0.95	0.81	0.68	0.58	0.49	0.41	0.35	0.30
11	4.2	4.2	4.2	4.2	4.1	4.1	4.0	3.9	3.8	3.7	3.6	3.5	3.3	3.1	2.9	2.7	2.4	2.2	1.9	1.7	1.5	1.3	1.1	0.93	0.79	0.66	0.56	0.47	0.40	0.34	0.30
12	4.1	4.1	4.1	4.0	4.0	3.9	3.9	3.8	3.7	3.6	3.5	3.4	3.2	3.0	2.8	2.6	2.4	2.1	1.9	1.7	1.4	1.2	1.1	0.90	0.76	0.65	0.54	0.46	0.39	0.33	0.29
13	3.9	3.9	3.8	3.8	3.8	3.7	3.7	3.6	3.5	3.4	3.3	3.2	3.0	2.8	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.0	0.85	0.72	0.61	0.51	0.43	0.37	0.32	0.27
14	3.9	3.9	3.8	3.8	3.8	3.7	3.7	3.6	3.5	3.4	3.3	3.2	3.0	2.8	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.0	0.85	0.72	0.61	0.51	0.43	0.37	0.32	0.27
15	3.6	3.6	3.6	3.6	3.5	3.5	3.4	3.4	3.3	3.2	3.1	3.0	2.8	2.7	2.5	2.3	2.1	1.9	1.7	1.5	1.3	1.1	0.94	0.80	0.68	0.57	0.48	0.41	0.35	0.30	0.26
16	3.5	3.5	3.5	3.5	3.4	3.4	3.3	3.3	3.2	3.1	3	2.9	2.7	2.6	2.4	2.2	2.0	1.8	1.6	1.4	1.2	1.1	0.91	0.77	0.65	0.55	0.47	0.39	0.34	0.29	0.25
17	3.4	3.4	3.4	3.3	3.3	3.3	3.2	3.2	3.1	3.0	2.9	2.8	2.6	2.5	2.3	2.1	2.0	1.8	1.6	1.4	1.2	1.0	0.88	0.75	0.63	0.53	0.45	0.38	0.32	0.28	0.24
18	3.3	3.3	3.3	3.2	3.2	3.2	3.1	3.1	3.0	2.9	2.8	2.7	2.6	2.4	2.2	2.1	1.9	1.7	1.5	1.3	1.2	0.99	0.85	0.72	0.61	0.52	0.44	0.37	0.31	0.27	0.23
19	3.2	3.2	3.1	3.1	3.1	3.0	3.0	2.9	2.9	2.8	2.7	2.6	2.5	2.3	2.2	2.0	1.8	1.6	1.5	1.3	1.1	0.96	0.82	0.70	0.59	0.50	0.42	0.36	0.30	0.26	0.22
20	3.1	3.0	3.0	3.0	3.0	2.9	2.9	2.8	2.8	2.7	2.6	2.5	2.4	2.2	2.1	1.9	1.7	1.6	1.4	1.2	1.1	0.92	0.79	0.67	0.57	0.48	0.40	0.34	0.29	0.25	0.21
21	2.9	2.9	2.9	2.9	2.9	2.8	2.8	2.7	2.7	2.6	2.5	2.4	2.3	2.1	2.0	1.8	1.7	1.5	1.3	1.2	1.0	0.89	0.76	0.64	0.55	0.46	0.39	0.33	0.28	0.24	0.21
22	2.8	2.8	2.8	2.8	2.7	2.7	2.7	2.6	2.6	2.5	2.4	2.3	2.2	2.1	1.9	1.8	1.6	1.5	1.3	1.1	0.99	0.85	0.73	0.62	0.52	0.44	0.37	0.32	0.27	0.23	0.20
23	2.7	2.7	2.7	2.7	2.6	2.6	2.6	2.5	2.4	2.4	2.3	2.2	2.1	2.0	1.8	1.7	1.5	1.4	1.2	1.1	0.95	0.82	0.70	0.59	0.50	0.42	0.36	0.30	0.26	0.22	0.19
24	2.6	2.6	2.6	2.5	2.5	2.5	2.4	2.4	2.3	2.3	2.2	2.1	2.0	1.9	1.8	1.6	1.5	1.3	1.2	1.0	0.91	0.78	0.67	0.57	0.48	0.41	0.34	0.29	0.25	0.21	0.18
25	2.5	2.5	2.4	2.4	2.4	2.4	2.3	2.3	2.2	2.2	2.1	2.0	1.9	1.8	1.7	1.6	1.4	1.3	1.1	0.99	0.86	0.75	0.64	0.54	0.46	0.39	0.33	0.28	0.23	0.20	0.17
26	2.4	2.3	2.3	2.3	2.3	2.3	2.2	2.2	2.1	2.1	2	1.9	1.8	1.7	1.6	1.5	1.3	1.2	1.1	0.95	0.82	0.71	0.61	0.52	0.44	0.37	0.31	0.26	0.22	0.19	0.16
27	2.2	2.2	2.2	2.2	2.2	2.1	2.1	2.1	2.0	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.0	0.90	0.78	0.67	0.58	0.49	0.41	0.35	0.30	0.25	0.21	0.18	0.16
28	2.2	2.2	2.2	2.2	2.2	2.1	2.1	2.1	2.0	2.0	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.0	0.90	0.78	0.67	0.58	0.49	0.41	0.35	0.30	0.25	0.21	0.18	0.16
29	2.1	2.1	2.1	2.1	2.1	2.0	2.0	2.0	1.9	1.9	1.8	1.7	1.6	1.5	1.4	1.3	1.2	1.1	0.97	0.85	0.74	0.64	0.55	0.46	0.39	0.33	0.28	0.24	0.20	0.17	0.15
30	2.0	2.0	2.0	2.0	1.9	1.9	1.9	1.9	1.8	1.8	1.7	1.6	1.6	1.5	1.4	1.3	1.1	1.0	0.92	0.80	0.70	0.60	0.52	0.44	0.37	0.31	0.26	0.22	0.19	0.16	0.14

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