



Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Boron in freshwater

Technical brief

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Summary

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

Boron is widely distributed in the environment as a natural constituent of minerals, particularly in clay-rich sedimentary rocks, coal, shale, and some soils. The highest boron concentrations are found in marine sediments, and as a consequence, marine water has boron concentrations near 5 mg/L. By comparison, boron in freshwater is typically <0.5 mg/L, depending on the geochemical nature of the drainage catchment.

Since the last revision of the freshwater boron DGVs for toxicity in 2000 (ANZECC/ARMCANZ 2000), errors were identified in the derivation and new data have become available. The revised DGV for 95% species protection is approximately three times higher than the ANZECC/ARMCANZ (2000) value (changing from 370 μ g/L to 940 μ g/L). The current DGVs are of higher reliability than the previous values.

Very high reliability DGVs for (dissolved) boron in freshwater were derived from 22 chronic (longterm) toxicity data, comprising eight fish, two amphibians, three crustaceans, one bivalve, three macrophytes, one green microalga, three diatoms and one blue–green alga. Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values lists all chronic toxicity data used in the derivation. The DGVs for 99, 95, 90 and 80% species protection are 340 μ g/L, 940 μ g/L, 1 500 μ g/L and 2 500 μ g/L, respectively. The 95% species protection level for boron in freshwater (940 μ g/L) is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1 Introduction

Boron is ubiquitous in the environment, occurring as a trace element in igneous rocks and commonly found in sedimentary rocks derived from marine water (Mance et al. 1988, CCME 2009). Natural weathering of rocks is a major source of boron in the environment, and the amount released depends on the surrounding geology (CCME 2009). Boron is used in glass and ceramic production, fertilisers, pesticides, personal care products, household cleaning products, adhesives and flame retardants (CCME 2009, Soucek et al. 2011, Schoderboeck et al. 2011). Anthropogenic sources of boron in natural waters include sewage effluents, coal mining, coal combustion, oil exploration, boron mining and processing, copper smelters and agrochemicals (CCME 2009, Soucek et al. 2011, Scholerboeck et al. 2009, Soucek et al. 2011, Scholerboeck et al. 2010).

The current derivation of default guideline values (DGVs) for boron in freshwater corrects inconsistencies and erroneous data in, and adds new data published since, the ANZECC/ARMCANZ (2000) guideline value derivation. The DGVs are based on toxicity data for boron as either boric acid, H₃BO₃ (CAS 10043-35-3), or borax, Na₂B₄O₇10H₂O (CAS 1303-96-4), in freshwater. Boron in nature is predominantly found in one of these two forms (Howe 1998). Due to its high pKa value (9.24), boric acid is undissociated at the pH of most natural freshwaters (Parks & Edwards 2005). The proportion of borate anion B(OH)₄⁻ becomes significant at pH >7, while at pH 7–10 and boron concentrations greater than 270 mg/L, polyborates such as tetraborate (B₄O₇²⁻) are formed (Ezechi et al. 2012). Boron salts are highly soluble; the most soluble is borax (25.2 g/L), and the least soluble is boron trifluoride (2.4 g/L) (Kochkodan 2015). The main removal mechanism for boron is through adsorption onto suspended clays or sediments, and the extent to which this occurs is pH-dependent, with maximum adsorption observed between pH 7.5 and 9.0 (ANZECC/ARMCANZ 2000, WHO 1998).

Boron is an essential nutrient for higher plants (Eisler 1990), but its essentiality to other taxonomic groups (including microalgae) is species-specific. Marine water typically contains a background concentration of 4.5–5.1 mg/L, which plays an important role as a buffer in maintaining marine water pH (ANZECC/ARMCANZ 2000). The concentration of boron in freshwater depends on a number of factors, including proximity to marine water, inputs from industrial and municipal effluents and geology of the surrounding area (Butterwick 1989). In clean freshwater, the main sources of boron are atmospheric deposition (marine water spray/aerosols), runoff from the surrounding geology, marine water and groundwater, and magma intrusions (Arnorsson & Andresdottir 1995, Kot 2015). In geothermal waters, the concentration of boron increases with increasing temperature and can be as high as 72 mg/L (Arnorsson & Andresdottir 1995, Kot 2015). In New Zealand rivers with low or no geothermal influence, concentrations of boron range from <0.5 μ g/L to 410 μ g/L, with a geometric mean of 16 µg/L (Deely 1997). Australian fresh surface waters typically contain lower concentrations of dissolved boron (0.05–0.2 mg/L) than groundwater (0.05–2.9 mg/L), salt lake brines (1.8–19 mg/L) and volcanic maar lake surface water (0.9–5.6 mg/L) (Vengosh 1991, Hansen Bailey Environmental Consultants 2015). Water influenced by geothermal activity or groundwater intrusion will likely require site-specific guideline values for boron.

2 Aquatic toxicology

2.1 Mechanism of toxicity

Boric acid (the predominant form of boron in natural freshwater) is taken up by plant and animal cells by simple diffusion across the lipid bilayer of the plasma membrane (Dordas & Brown 2001, Uluisik et al. 2018). Active uptake via transporters and facilitated uptake via channels have also been reported for boron in plant and charophyte algal cells under boron limiting conditions (Tanaka & Fujiwara 2008). Once inside the cells, the mechanism of boron toxicity will vary according to species-specific boron essentiality, boron concentration, and cell type.

Little is known about the mechanism of boron toxicity to aquatic organisms; however, literature on terrestrial plants and animals may provide some insights. For the fungus *Saprolegnia* (a common causative agent for fish fungal infections), boron was shown to reduce metabolism through inhibition of mitochondrial function (Ali et al. 2014). Mechanisms of boron toxicity to microalgae and aquatic plants are not known, but are likely to be similar to those observed in terrestrial plants, that is through changes in cell wall structure, or by effects on metabolism, through binding to sugars in metabolically active important nucleotides (Uluisik et al. 2018).

Boric acid and borate salts are used as insecticides in terrestrial environments, acting as a stomach poison or as an abrasive dust to insect exoskeletons (Harper et al. 2012). However, for aquatic insects, where the primary routes of exposure are via gill uptake or cutaneous transfer, the mechanism of boron toxicity is likely to be different (Soucek et al. 2011).

In terrestrial animals, the primary uptake pathways are across digestive and pulmonary tissues, with little or no absorption of boron occurring across skin (Agency for Toxic Substances and Disease Registry 2010). For mature fish and amphibians, likely exposure routes will be via gills and ingestion, while for early life stages (e.g. embryos) boron passively diffuses across membranes from the water column (Dordas & Brown 2001). The diet-borne toxicity of boron to aquatic organisms is unknown (DeForest & Meyer 2015); however, in terrestrial animals, boron interferes with many metabolites, alters mineral and energy metabolism, and plays crucial roles in bone metabolism by interacting with minerals and vitamins (such as calcium, magnesium and vitamin D), and hormones that regulate bone formation (Uluisik et al. 2018). There is also some evidence that oral boron exposure in terrestrial animals alters gene expression, cell division and/or cell maturation rates (Uluisik et al. 2018).

2.2 Boron essentiality and toxicity

Boron is an essential element for a range of species; however, essentiality is not the same across all species within a taxonomic group. The essentiality of boron is known for higher plants; however, this cannot be generalised for microalgae, as increasing evidence has shown that microalgal growth is unaffected by the absence of boron for a range of species, including *Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Chlorella vannielli*, *Chlorella emersonii*, *Chlorella protothecoides* and *Haematococcus pluvialis* (Bowen et al. 1965, McBride et al. 1971, Fernandez et al. 1984, Fabregas et al. 2000). Although an early study found that boron was required for *C. vulgaris* growth (McIlrath & Skok 1958),

these results could not be reproduced in two later studies with the same strain of alga (Bowen et al. 1965, McBride et al. 1971). For cyanobacteria, non-heterocystous species are also non-reliant on boron for growth, while heterocystous species (e.g. *Nodularia, Chlorogloeopsis* and *Nostoc*) have reduced growth and nitrogenase activity in the absence of boron (Bollina et al. 1990). However, within the *Nostoc* genus, there is evidence that at least one species (*Nostoc punctiforme*) does not rely on boron for growth (Wilkinson 1985), suggesting that blue–green algae, like microalgae, are species-specific in their requirement for boron.

Boron has also been shown to be essential at low concentrations for some fish and amphibians, with characteristic U-shaped dose-response curves (Fort et al. 1999, Loewengart 2001). Therefore, although the current DGV provides a maximum concentration of boron to protect a percentage of species in the environment, for many species there will also be a minimum boron concentration required for normal growth, development and reproduction. Using a weight of evidence approach, Loewengart (2001) estimated this to be $30 \mu g/L$ for fish. Minimum requirements for other taxa are not known, but macrophyte growth media often contain between 0.5 mg/L and 2 mg/L as a micronutrient (Davis et al. 2002, Gur et al. 2016).

There is a narrow gap between the concentrations of boron that are essential and those that are toxic. Reported chronic toxicity values range from 0.6 mg/L to 27 mg/L for microalgae and blue– green algae, 1.4 mg/L to 20 mg/L for macrophytes, 2.4 mg/L to 29 mg/L for crustaceans, 1.8 mg/L to 102 mg/L for fish and 15 mg/L to 56 mg/L for amphibians (Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values). Additional information on boron toxicity is presented in Section 4.1, while the acute toxicity of boron has been reviewed elsewhere (Howe 1998, CCME 2009).

There is no evidence of boron biomagnification in aquatic food chains; however, boron is known to accumulate in microalgae, macrophytes, amphibians and fish (Saiki et al. 1993, Dordas & Brown 2001, Emiroglu et al. 2010, Adhikari & Mohanty 2012, Gur et al. 2016, Benzer 2017).

3 Factors affecting toxicity

The factors affecting boron toxicity are not yet well understood for all aquatic organisms. There is some evidence that pH, dissolved organic carbon (DOC) and chloride content can influence boron toxicity, but these effects appear to be species-specific, and targeted studies were limited to four invertebrate species (Maier & Knight 1991, Dethloff et al. 2009, Soucek et al. 2011). Additional information from older studies on fish and amphibians also suggests that pH is more influential than hardness on boron toxicity, but effects were species-specific (Birge & Black 1977, Black et al. 1993).

Water hardness has been shown to have no effect on acute boron toxicity to three crustaceans (*Daphnia magna, Ceriodaphnia dubia* and *Hyalella azteca*), except when hardness was elevated above 500 mg CaCO₃/L (Maier & Knight 1991, Dethloff et al. 2009, Soucek et al. 2011). Acute toxicity of boron to *D. magna* was unchanged by varying sulfate concentrations (10–325 mg/L) (Maier & Knight 1991). Acute toxicity of boron to *C. dubia* was unaffected by changes in alkalinity, or sodium and chloride concentrations, but decreased with increasing DOC (Dethloff et al. 2009). Similarly, Soucek et al. (2011) showed that boron toxicity to *C. dubia* was unchanged by chloride, but for *H. azteca*, chloride reduced boron toxicity.

The effect of pH on boron toxicity is not consistent, although studies that looked at pH-related changes to boron toxicity were limited to two species (*C. dubia* and *H. azteca*). For *C. dubia* (Dethloff et al. 2009), a decrease by 1 pH unit (from pH 8.1 to 7.1, and from 8.4 to 7.4) caused a significant 1.6-fold increase in acute toxicity; however, the authors used CO₂ to alter pH and suggested that this may be a confounding factor. In a later study with the same species, Soucek et al. (2011) used mixtures of borax and boric acid to achieve target pH ranges of 6.5, 7.5 and 8.5 and again found a relationship of increasing toxicity (decreasing 48-h LC50 values) with decreasing pH. However, the same effect was not observed with the amphipod *H. azteca* in 96-h exposures. In older literature, studies on the toxicity of borax and boric acid to fish and amphibian embryo survival resulted in test solutions with varying pH values (Birge & Black 1977, Black et al. 1993). These studies showed that, for channel catfish (*Ictalurus punctatus*), within the same level of hardness (either 50 mg/L CaCO₃ or 200 mg/L CaCO₃), boron was more toxic at pH 7.5–7.6 than at 8.2–8.5. However, for the leopard frog (*Rana pipiens*), the inverse was found (higher boron toxicity at pH 8.3–8.4, than at 7.7, regardless of the hardness), while for rainbow trout (*Oncorhynchus mykiss*) and goldfish (*Carassius auratus*), there were no discernible patterns of toxicity.

While there was no information available on the effect of water quality parameters on boron toxicity to macrophytes, boron accumulation by the aquatic duckweed *Lemna minor* has been shown to be pH-dependent such that higher concentrations of boron are accumulated at lower pH (Frick 1985).

It is clear that the factors affecting boron toxicity to aquatic organisms are species-specific and the direction and magnitude of the effects are variable and generally not well quantified. Therefore, it was not possible to quantitatively account for any toxicity modifying factors in the current DGV derivation.

4 Default guideline value derivation

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

4.1 Toxicity data used in derivation

Since 2000, numerous significant publications concerning the aquatic toxicity of boron have been published, and these were quality assessed for use in the DGV derivation. These publications include a critical review of the ANZECC/ARMCANZ (2000) GV by Kingett Mitchell Ltd (2004), new guideline value derivations by Dyer (2001), Schoderboeck et al. (2011) and the Canadian Council of Ministers of the Environment (CCME 2009), as well as some targeted studies on the effects of water quality parameters on boron toxicity (Soucek et al. 2011).

In addition, considerable data, not previously used for boron DGV derivation, were found for boron toxicity to freshwater phytoplankton, including diatoms and blue–green algae (Wilkinson 1985). These data passed quality assessment for inclusion; however, only data for the diatoms and one of the blue–green algal species, *N. punctiforme*, were used, as the other blue–green algal species are nuisance/bloom forming species, ecologically advantaged to dominate in disturbed environments, and so their conservation is not targeted with these DGVs. *N. punctiforme* can form important symbiotic relationships with plants and fungi in aquatic systems (Meeks et al. 2001). For one diatom, *Navicula* sp. 1, an IC10 was re-derived using reported raw data (mean responses at each boron

concentration). This was done because the reported NOEC of <1 mg/L was of low reliability, and the concentration—response data conformed to the assumptions of linear interpolation, enabling a more reliable IC10 value to be calculated (Appendix B: Re-analysed toxicity data for *Navicula* sp. 1).

Additional chronic toxicity data published since 2000 for microalgae, macrophytes, a bivalve, an amphipod and two fish that passed the quality assessment were also used in the DGV derivation.

Another significant change since ANZECC/ARMCANZ (2000) is the use of updated toxicity values for several species of fish. Early reports of toxicity to rainbow trout and zebrafish at very low concentrations of boron (Birge & Black 1977, Black et al. 1993) were possibly due to boron deficiency rather than toxicity, and so some of these values were not used for the current DGV derivation. However, more recent fish toxicity estimates, obtained from less sensitive responses, have been included (Rowe et al. 1998). Where older data were included, LC/EC10 values revised by Dyer (2001) were used in place of the older NOEC values.

Although there is a large number of published data on boron toxicity, not all data met the preferred requirements and associated acceptability criteria for the derivation of DGVs. In the first instance, this requires chronic NEC, NOEC or EC10 data rather than values converted from other acute or chronic measures. No converted values, including acute toxicity values, were used in the DGV derivation, as the minimum data requirements were met with chronic EC/LC10 and NOEC data alone. Some toxicity data used by other jurisdictions to derive boron guideline values (e.g. CCME, 2009) were not included for various reasons (e.g. source reference was not available, data did not pass quality assessment, test conditions were sub-optimal, source references could not be accessed or were not in English and data quality could not be determined).

Chronic toxicity data for 22 species from eight taxonomic groups (one blue–green alga, one green microalga, three diatoms, three macrophytes, three crustaceans (two cladocerans and one amphipod), one bivalve, two amphibians and eight fish) passed the quality assessment and screening processes (Warne et al. 2018) and were used to derive the DGVs. The dataset comprised seven chronic LC10s, one chronic EC10, two chronic IC10s and 12 chronic NOECs. A summary of the toxicity data (one value per species) used to calculate the DGVs for boron in freshwater is provided in Table 1. Further details of the water quality parameters for each single species value used to calculate the DGVs are presented in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. The single species values represent the most sensitive life stage, toxicity test duration and endpoint measured based on recommendations by Warne et al. (2018). Details of the <u>data quality assessment</u> and the <u>data that passed the quality assessment</u> are provided as supporting information.

The chronic toxicity values for boron ranged from 0.6 mg/L to 102 mg/L. The most sensitive species was the diatom *Navicula* sp. 1, with an IC10 of 0.6 mg/L, similar to a second species of the same genus *Navicula* sp. 2, which had a NOEC of 1.0 mg/L. Other microalgae and diatoms were less sensitive, with NOECs of 2.8 mg/L (*Pseudokirchneriella subcapitata*) and 10 mg/L (*Cyclotella* sp.). For *P. subcapitata*, there were three separate studies available with toxicity data for boron. The toxicity values from these studies ranged from a NOEC of 2.8 mg/L to a NEC of 27 mg/L, varying with endpoint, duration and test medium used. Boron was least toxic to *P. subcapitata* when tested in algal growth medium with added NaHCO₃, suggesting that carbonate addition may have influenced boron toxicity. Therefore, although NECs are preferred to NOECs or EC10s (Warne et al. 2018), in this

instance, a reliable NOEC of 2.8 mg/L was the most sensitive toxicity value for *P. subcapitata* and was used for the derivation.

Taxonomic group (phylum)	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Toxicity value (mg/L)
Amphibian (Chordata)	Anaxyrus fowleri	Embryo	7.5	LC10 (Mortality and development)	41 ª
	Rana pipiens	Embryo	7.5	LC10 (Mortality and development)	29 ª
Fish	Carassius auratus	Embryo	7	LC10 (Mortality)	17 ª
(Chordata)	Danio rerio	Embryo	34	NOEC (Biomass)	1.8 ^b
	Ictalurus punctatus	Embryo	9	LC10 (Mortality)	14 ª
	Melanotaenia splendida	Embryo	12	LC10 (Mortality)	102
	Micropteris salmoides	Embryo	11	LC10 (Mortality)	6.0
	Oncorhynchus mykiss	Embryo	28	LC10 (Mortality)	6.2 ^{a, b}
	Pimephales promelas	Embryo	32	NOEC (Mortality)	11 ª
	Cirrhinus mrigala	Juvenile	52	NOEC (Growth rate)	4
Bivalve (Mollusca)	Lampsilis siliquoidea	Juvenile	21	NOEC (Biomass)	10 ^b
Macrocrustacean	Hyalella azteca	Juvenile	42	NOEC (Reproduction)	6.6 ^b
(Arthropoda)	Daphnia magna	Neonate	14	NOEC (Reproduction)	2.4 ^{a, b}
Microcrustacean (Arthropoda)	Ceriodaphnia dubia	Neonate	7	NOEC (Reproduction)	5.6 ^{b, c}
Macrophyte (Charophyta)	Egeria densa	Apical stem cutting	28	NOEC (Biomass)	6.1
	Lemna disperma	NR	7	EC10 (Growth)	1.4 ^b
	Potamogeton ochreatus	Apical stem cutting	30	IC10 (Shoot growth)	4.9 ^b
Green microalga (Chlorophyta)	Pseudokirchneriella subcapitata	NR	4	NOEC (Growth)	2.8 ^{b, c}
Diatom	<i>Cyclotella</i> sp.	NR	4–14	NOEC (Biomass)	10 °
(Bacillariophyta)	Navicula sp. 1	NR	4–12	IC10 (Growth)	0.6 c, d
	Navicula sp. 2	NR	4–16	NOEC (Biomass)	1.0 °
Blue–green alga (Cyanobacteria)	Nostoc punctiforme sp. 2	NR	6–26	NOEC (Growth)	10 °

Table 1 Summary of single chronic toxicity values, all species used to derive default guideline
values for boron in freshwater, toxicity values expressed in mg/L

NR = not reported.

a Geometric mean of toxicity values with the same test conditions and endpoint for a single species.

b The minimum toxicity value from different endpoints from a single species was used.

c Nominal boron concentration with partial validation. All other listed boron concentrations are measured.

d IC10 value was derived from raw data presented in the study, since NOEC value was unreliable.

Lemna disperma was the most sensitive macrophyte (EC10 1.4 mg/L), while *Egeria densa* was the least sensitive macrophyte (NOEC of 6.1 mg/L). Of the crustaceans, *D. magna* was best represented in the literature, with 18 published NOEC values (ranging from 2.4 mg/L to 29 mg/L) for six different endpoints from six different publications. The final NOEC of 2.4 mg/L used in the DGV derivation was lower than that for *C. dubia* (NOEC 5.6 mg/L) and for the amphipod *H. azteca* (NOEC 6.6 mg/L).

For the sediment-dwelling bivalve *Lampsilis siliquodea*, boron toxicity assessments in both wateronly and whole-sediment exposure, with analyses of overlying water boron concentrations, showed that the route of boron exposure was through the water column, rather than the sediment. Therefore, the water-only chronic NOEC (10 mg/L) was considered appropriate in the DGV derivation.

Fish sensitivity to boron ranged from the least sensitive species in the dataset (*Melanotaenia splendida*, LC10 102 mg/L) to the third most sensitive species in the dataset (*Danio rerio*, NOEC 1.8 mg/L).

Amphibians (*Rana pipiens* and *Anaxyrus fowleri*) were generally the least sensitive taxonomic group, with LC10 values of 29 mg/L and 41 mg/L, respectively, although data were available for only two species.

Although pH, chloride and DOC are known to influence boron speciation and/or toxicity, these relationships are not well defined, and are species-specific. Therefore, no toxicity values were modified based on these factors. However, a toxicity estimate for one species of duckweed (*Spirodella polyrrhiza*) was removed from the dataset due to the low pH of test solutions (pH range of 5.2–5.8). This pH range was below that of most natural freshwaters, and it has been shown to increase boron uptake in aquatic plants (Frick 1985). The hardness and pH of waters used in remaining toxicity tests ranged from 9.3 mg CaCO₃/L to 250 mg CaCO₃/L and pH 6.8 to 10, respectively. Measured chloride concentrations were only reported for three studies (Hooftman et al. 2000a, Soucek et al. 2011, Acqua Della Vita 2014). For a further nine studies, the concentration of chloride was calculated from provided recipes; however, for the remaining 13 studies, chloride concentrations were not known. Combined available measured and estimated chloride concentrations ranged from 4 mg/L to 121 mg/L.

Although sufficient chronic toxicity data were available with measured concentrations of boron (16 species, from seven taxonomic groups), additional chronic toxicity data with nominal boron concentrations for a cladoceran, three diatoms, a green alga and a blue–green alga were also included in the DGV derivation. These data were from studies where boron concentrations were not reported for test solutions, but methods to validate boron concentrations were included in the study, (e.g. measurement of boron in stock solutions (Hickey & Macaskill 1988)). Two of these additional nominal toxicity values were for the most sensitive species in the dataset (*Navicula* sp. 1 and *Navicula* sp. 2). Evidence from toxicity tests with measured boron concentrations (16 species) showed that measured concentrations of boron were usually within 5–10% (and always within 20%) of nominal concentrations. The one exception to this was for *L. disperma*, where nominal concentrations were half those measured (Acqua Della Vita 2014). However, this was likely due to error in the preparation of boron solutions, rather than loss of boron in test solutions, based on the

minor losses noted for other tests. Therefore, it was assumed that dissolved concentrations of boron were likely to be within 5–10% of nominal boron concentrations for the studies where measured concentrations were not reported. Although there is a preference for measured concentrations to be used in high reliability DGVs, chronic boron toxicity data for 16 species with measured values were combined with data for six species with nominal values for the current DGV derivation.

Bimodal or multimodal toxicity was determined to be unlikely for boron. Although boron may have specific modes of actions in plants, these are species-specific, and macrophyte toxicity values were spread evenly across the species sensitivity distribution curve. The data were not indicative of bimodality based on histogram statistics, a bimodality coefficient value of 0.27, and the even spread of taxa (from toxicity studies spanning four decades) in the species sensitivity distribution (Warne et al. 2018). Therefore, all the toxicity data in the final dataset (i.e. from 22 species) were used for the derivation.

4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) based on the 22 freshwater boron chronic toxicity data (Table 1) is presented in Figure 1. The SSD was plotted using the Burrlioz 2.0 software and is presented in mg/L to improve the visual appearance of the plot. The fit of the model was considered to be good.



Figure 1 Species sensitivity distribution, boron in freshwater, toxicity values expressed in mg/L

4.3 Default guideline values

It is important that the DGVs (Table 2) and associated information in this technical brief are used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality <u>website</u> (ANZG 2018).

The DGVs for 99, 95, 90 and 80% species protection are shown in Table 2. The DGVs apply to dissolved boron. The 95% species protection DGV for boron in freshwater (940 μ g/L) is recommended for application to slightly-to-moderate disturbed ecosystems.

Level of species protection (%)	DGV for boron in freshwater (µg/L) $^{\rm a}$
99	340
95	940
90	1 500
80	2 500

Table 2 Toxicant default guideline values, boron in freshwater, very high reliability

a Default guideline values were derived using the Burrlioz 2.0 software, and based on data from toxicity tests conducted for a pH range of 6.8–10 and hardness range of 9.3–250 mg CaCO₃/L. They are reported as μ g/L and have been rounded to two significant figures.

4.4 Reliability classification

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

- Sample size—22 (preferred)
- Type of toxicity data—chronic
- SSD model fit—good (Burr Type III model).

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.
ANZECC	Australian and New Zealand Environment and Conservation Council
ARMCANZ	Agricultural and Resource Management Council of Australia and New Zealand
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. Formerly known as 'trigger values'.
DOC	Dissolved organic carbon.
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.
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.
endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, 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 to be a low risk of unacceptable effects occurring to that community value. Guideline values for more than one indicator should be used simultaneously in a multiple lines of evidence approach. (Also refer to default guideline value and site-specific guideline value.)
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 under specified conditions.
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.
lowest observed effect concentration (LOEC)	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.
no effect concentration (NEC)	Parametric or Bayesian estimate of the highest concentration of a chemical below which no effect occurs.
no observed effect concentration (NOEC)	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 guideline value	A guideline value that is relevant to the specific location or conditions that are the focus of a given assessment or issue.
Species (biological)	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
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: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values

Table A 1 Summary, chronic toxicity data that passed the screening and quality assurance processes, boron in freshwater

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO ₃ /L)	рН	Final concentration (mg/L) ^a	Reference
Amphibian (Chordata)	Anaxyrus fowleri	Embryo	7.5	LC10 (Mortality & development)	Reconstituted water	24	57	7.6	55	Dyer (2001), Birge & Black (1977)
		Embryo	7.5	LC10 (Mortality & development)	Reconstituted water	24	108	7.6	30	Dyer (2001), Birge & Black (1977)
-									41 ^b	VALUE USED IN SSD
Amphibian (Chordata)	Rana pipiens	Embryo	7.5	LC10 (Mortality & development)	Reconstituted water	25	53	7.7	48	Dyer (2001), Birge & Black (1977)
		Embryo	7.5	LC10 (Mortality & development)	Reconstituted water	25	212	7.7	56	Dyer (2001), Birge & Black (1977)
		Embryo	7.5	LC10 (Mortality & development)	Reconstituted water	25	46	8.3	18	Dyer (2001), Birge & Black (1977)
		Embryo	7.5	LC10 (Mortality & development)	Reconstituted water	25	203	8.4	15	Dyer (2001), Birge & Black (1977)
-									29 ^b	VALUE USED IN SSD

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ª	Reference
Fish (Chordata)	Carassius auratus	Embryo	7	LC10 (Mortality)	Reconstituted water	25	54	7.9	16	Dyer (2001), Birge & Black (1977)
		Embryo	7	LC10 (Mortality)	Reconstituted water	25	208	7.6	15	Dyer (2001), Birge & Black (1977)
		Embryo	7	LC10 (Mortality)	Reconstituted water	25	46	7.5	20	Dyer (2001) Birge & Black (1977)
		Embryo	7	LC10 (Mortality)	Reconstituted water	25	195	8.1	16	Dyer (2001), Birge & Black (1977)
-									17 ^b	VALUE USED IN SSD
Fish (Chordata)	Danio rerio	Embryo	34	NOEC (Mortality)	DSWL-E medium	24–26	212	7.2–8.0	5.6	Hooftman et al. (2000a)
		Embryo	34	NOEC (Biomass, length)	DSWL-E medium	24–26	212	7.2–8.0	5.6	Hooftman et al. (2000a)
		Embryo	34	NOEC (Biomass, dry weight)	DSWL-E medium	24–26	212	7.2–8.0	1.8	Hooftman et al. (2000a)
-									1.8	VALUE USED IN SSD
Fish (Chordata)	Ictalurus punctatus	Embryo	9	LC10 (Mortality & development)	Reconstituted water	25	52	7.5	5	Dyer (2001), Birge & Black (1977)
		Embryo	9	LC10 (Mortality & development)	Reconstituted water	29	47	8.5	33	Dyer (2001) Birge & Black (1977)

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ª	Reference
		Embryo	9	LC10 (Mortality & development)	Reconstituted water	29	195	8.2	16	Dyer (2001), Birge & Black (1977)
-									14 ^b	VALUE USED IN SSD
Fish (Chordata)	Melanotaenia splendida	Embryo	12	LC10 (Mortality)	Condamine River water	25	81	6.9–7.5	102	Acqua Della Vita (2014)
-									102	VALUE USED IN SSD
Fish (Chordata)	Micropteris salmoides	Embryo	11	LC10 (Mortality)	Reconstituted water	20	204	7.5	6.0	Dyer (2001), Birge & Black (1977)
-									6.0	VALUE USED IN SSD
Fish (Chordata)	Oncorhynchus mykiss	Embryo	28	LC10 (Mortality)	Reconstituted water	14	54	7.7	2.0	Dyer (2001), Birge & Black (1977)
		Embryo	28	LC10 (Mortality)	Reconstituted water	14	49	7.9	8.0	Dyer (2001) Birge & Black (1977)
		Embryo	28	LC10 (Mortality)	Reconstituted water	13	191	7.8	15	Dyer (2001), Birge & Black (1977)
		-							6.2 ^b	
		Embryo	32	LC10 (Mortality)	Reconstituted water	13	197	7.4	30	Dyer (2001), Black et al. (1993)
		Embryo	42	NOEC (Mortality)	ASTM ultrapure grade	13	NR	NR	86	Rowe et al. (1998)

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ª	Reference
-									6.2	VALUE USED IN SSD
Fish (Chordata)	Pimephales promelas	Embryo	32	NOEC (Mortality)	USEPA synthetic water	25	91	8	11	Soucek et al. (2011)
-									11	VALUE USED IN SSD
Fish (Chordata)	Cirrhinus mrigala	Juvenile	52	NOEC (Growth rate)	Diluted pond water	23–29	108-120	7.3–7.5	4	Adhikiri & Mohanty (2012)
-									4	VALUE USED IN SSD
Bivalve (Mollusca)	Lampsilis siliquoidea	Juvenile	21	NOEC (Biomass, length)	USEPA moderately hardwater	19–22	89–108	6.8–7.9	10	Hall et al. (2014)
		Juvenile	21	NOEC (Mortality)	USEPA moderately hardwater	19–22	89–108	6.8–7.9	32	Hall et al. (2014)
-									10	VALUE USED IN SSD
Macrocrustacean (Arthropoda)	Hyalella azteca	Juvenile	42	NOEC (Mortality)	USEPA synthetic water	22	106	8.1	26	Soucek et al. (2011)
		Juvenile	42	NOEC (Reproduction)	USEPA synthetic water	22	106	8.1	6.6	Soucek et al. (2011)
-									6.6	VALUE USED
Macrocrustacean (Arthropoda)	Daphnia magna	Neonate	21	NOEC (Reproduction, no. of young)	DSWL-E medium	19–21	212	7.2–8.0	10	Hooftman et al. (2000b)

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ª	Reference
		Neonate	21	NOEC (Reproduction, no. of young)	Lake Huron water, filtered, sterilised, hardness adjusted	20–21	148	8.1 (dilu- ent)	6.4	Gersich (1984)
		Neonate	21	NOEC (Reproduction, no. of young)	Carbon filtered well water	19	166	7.1–8.7	6.0	Lewis & Valentine (1981)
		-							7.3 ^b	
		Neonate	14	NOEC (Reproduction, no. of young)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.4	Gersich & Milazzo (1990)
		Neonate	14	NOEC (Reproduction, no. of young)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.5	Gersich & Milazzo (1990)
		Neonate	14	NOEC (Reproduction, no. of young)	HMSO, British standard synthetic water	20	250	7.9	18 °	Hickey (1989)
		Neonate	14	NOEC (Reproduction, no. of young)	EPA soft water	22	44	7.5–8.5	18	Hickey & Macaskill (1988)
		-							6.6 ^b	
		Neonate	14	NOEC (Reproduction, brood size)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.4	Gersich & Milazzo (1990)

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L)ª	Reference
		Neonate	14	NOEC (Reproduction, brood size)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.5	Gersich & Milazzo (1990)
		-							2.4 ^b	
		Neonate	14	NOEC (Mortality)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.4	Gersich & Milazzo (1990)
		Neonate	14	NOEC (Mortality)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.5	Gersich & Milazzo (1990)
		-							2.4 ^b	
		Neonate	21	NOEC (Biomass, length)	Lake Huron water, filtered, sterilised, hardness adjusted	20–21	148	8.1 (dilu- ent)	6.4	Gersich & Milazzo (1990)
		Neonate	21	NOEC (Biomass, length)	Carbon filtered well water	19	166	7.1–8.7	27	Lewis & Valentine (1981)
		_							13 ^b	
		Neonate	14	NOEC (Biomass, dry weight)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.4	Gersich & Milazzo (1990)

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ^a	Reference
		Neonate	14	NOEC (Biomass, dry weight)	Lake Huron water, filtered, sterilised, hardness adjusted	24	170	7.3–8.2	2.5	Gersich & Milazzo (1990)
		-							2.4 ^b	
		Neonate	21	NOEC (Reproduction, no. of broods)	Lake Huron water, filtered, sterilised, hardness adjusted	20–21	148	8.1 (dilu- ent)	6.4	Gersich (1984)
		Neonate	21	NOEC (Reproduction, brood size)	Lake Huron water, filtered, sterilised, hardness adjusted	20–21	148	8.1 (dilu- ent)	6.4	Gersich (1984)
		Neonate	21	NOEC (Mortality)	Lake Huron water, filtered, sterilised, hardness adjusted	20–21	148	8.1 (dilu- ent)	29	Gersich (1984)
-									2.4	VALUE USED IN SSD
Microcrustacean (Arthropoda)	Ceriodaphnia dubia	Neonate	14	NOEC (Reproduction, number of young)	HMSO, British standard synthetic water	20	250	7.9	10 °	Hickey (1989)
		Neonate	7	NOEC (Reproduction, number of young)	EPA synthetic soft water	25	44	7.5–8.5	5.6 °	Hickey & Macaskill (1988)
-									5.6	VALUE USED

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ª	Reference
Macrophyte (Charophyta)	Egeria densa	Apical stem cutting	28	NOEC (Biomass, dry weight)	Modified Ruakura nutrient solution	19–21	23	7.7–10	6.1	Thompson (1987)
-									6.1	VALUE USED IN SSD
Macrophyte (Charophyta)	Lemna disperma	NR	7	EC10 (Growth)	Condamine River water	25	81	7.0–7.2	1.4	Acqua Della Vita (2014)
-									1.4	VALUE USED IN SSD
Macrophyte (Charophyta)	Potamogeton ochreatus	Apical stem cutting	30	IC10 (Growth)	Dechlorinated tap water	22	NR	6.8–7.7	4.9	Golder Associates (2010)
			30	NOEC (Biomass, weight)	Dechlorinated tap water	22	NR	6.8–7.7	7.5	Golder Associates (2010)
			30	NOEC (Reproduction)	Dechlorinated tap water	22	NR	6.8–7.7	20	Golder Associates (2010)
-									4.9	VALUE USED IN SSD
Green Microalgae (Chlorophyta)	Pseudokirchneriella subcapitata	Exponentially growing	3	NEC (Growth)	Modified OECD growth medium with additional NaHCO ₃	23	24	7.5–8.3	27	Hanstveit & Oldersma (2000)
		NR	3	IC10 (Growth)	Condamine River Water	25	81	7.7–8.1	12	Acqua Della Vita (2014)
		-							13 ^b	
		NR	4	NOEC (Growth)	Algal growth medium without EDTA	24	9.3	7.5–8.5	2.8 °	Hickey and Macaskill (1988)

Taxonomic Group (phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temper- ature (°C)	Water hardness (mg CaCO₃/L)	рН	Final concentration (mg/L) ª	Reference
		NR	8	NOEC (Growth)	Algal growth medium without EDTA	24	9.3	7.5–8.5	11 °	Hickey & Macaskill (1988)
-									2.8	VALUE USED IN SSD
Diatom (Bacillariophyta)	<i>Cyclotella</i> sp.	NR	4–14	NOEC (Biomass, maximum yield)	1/10 Woods Hole MBL medium	20	NR	NR	10 °	Wilkinson (1985)
-									10	VALUE USED IN SSD
Diatom (Bacillariophyta)	<i>Navicula</i> sp. 1	NR	4–12	IC10 (Maximum growth rate) ^d	1/10 Woods Hole MBL medium	20	NR	NR	0.6 ^{c, d}	Wilkinson (1985)
-									0.6	VALUE USED IN SSD
Diatom (Bacillariophyta)	Navicula sp. 2	NR	4–16	NOEC (Biomass, maximum yield)	1/10 Woods Hole MBL medium	20	NR	NR	1.0 °	Wilkinson (1985)
-									1.0	VALUE USED IN SSD
Blue–green alga (Cyanobacteria)	Nostoc punctiforme sp. 2	NR	6–26	NOEC (Maximum growth rate)	1/10 Woods Hole MBL medium	20	NR	NR	10 °	Wilkinson (1985)
-									10	VALUE USED IN SSD

a Toxicity value for boron after any groupings, expressed in mg/L.

b Geometric mean.

c Toxicity value based on nominal boron concentrations. All other toxicity values were derived using measured boron concentrations.

d IC10 value derived using raw data in original study. Concentration response curve in Appendix B: Re-analysed toxicity data for Navicula sp. 1

The data analysis shown in Figure B 1 is based on the reported growth rate data (average of three replicates for each treatment), normalised to the percent of control growth, for *Navicula* sp. 1, from Wilkinson (1985). The estimated boron IC10 for *Navicula* sp. 1 is approximately 0.6 mg/L.

Appendix B: Re-analysed toxicity data for *Navicula* sp. 1

The data analysis shown in Figure B 1 is based on the reported growth rate data (average of three replicates for each treatment), normalised to the percent of control growth, for *Navicula* sp. 1, from Wilkinson (1985). The estimated boron IC10 for *Navicula* sp. 1 is approximately 0.6 mg/L.

	Algal growth test-%Control										
Start Date:		Test ID: Wilk data	Sample ID:	boron							
End Date:		Lab ID:	Sample Type:								
Sample Date:		Protocol:	Test Species :	Navicula s p 1							
Comments :	recalculation of K	10 value from Wilkinson (1985)									
Conc-mg/L	1										
Control	100.00										
1	83.00										
3	72.00										
10	50.00										
30	0.00										

		_		Transform: Untransformed				lsot	onic
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
Control	100.00	1.0000	100.00	100.00	100.00	0.000	1	100.00	1.0000
1	83.00	0.8300	83.00	83.00	83.00	0.000	1	83.00	0.8300
3	72.00	0.7200	72.00	72.00	72.00	0.000	1	72.00	0.7200
10	50.00	0.5000	50.00	50.00	50.00	0.000	1	50.00	0.5000
30	0.00	0.0000	0.00	0.00	0.00	0.000	1	0.00	0.0000



Figure B 1 Boron toxicity data analysis and concentration–response curve, *Navicula* sp. 1, reported raw data

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