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Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Glyphosate in freshwater

Technical brief

July 2021

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Contents

Figures

Tables

Appendix tables

Appendix figures

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).

Glyphosate (N-(phosphonomethyl) glycine, CAS No. 1071-83-6) is a common non-selective, systemic organophosphorus herbicide. Other organophosphorus herbicides include bensulide, fosamine and glufosinate. Glyphosate exerts its toxicity to plants by binding to and inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, which prevents the formation of a range of hormones, vitamins and other essential plant metabolites through an inhibition of aromatic amino acid synthesis (APVMA 2014, Myers et al. 2016). Non-agricultural uses include the application of glyphosate to urban and industrial situations (i.e. home gardens and roadsides/golf courses) as well as aquatic weed control in public waterways, most commonly through the use of commercial formulations (CCME 2012, ANZECC/ARMCANZ 2000;). Glyphosate is the most widely used herbicide in Australia and is prevalent in aquatic ecosystems. There are concerns about the potential for some glyphosate formulations to exhibit higher toxicity in comparison to the parent compound (ANZECC/ARMCANZ 2000, AATSE 2002).

The previous Australian and New Zealand default guideline values (DGVs) for glyphosate in freshwater environments were based on acute toxicity data for 18 freshwater species consisting of fish, amphibians, crustaceans and other invertebrates (Warne 2001). More data on glyphosate chronic toxicity to freshwater species are now available, which has enabled the derivation of improved DGVs compared to those in ANZECC/ARMCANZ (2000).

The available chronic toxicity data for glyphosate ranged from 100 µg/L for the green algae *Chlorella vulgaris* (also called *Chlorella pyrenoidosa*) (72-hour growth inhibition LOEC), *Scenedesmus acutus* (72-h growth inhibition LOEC) and *Scenedesmus subspicatus* (72-h growth inhibition NOEC), and for the mollusc *Pseudosuccinea columella* (12-d reproductive impairment IC7), to 1 080 000 µg/L for *C. vulgaris* (96-h LC50). Acute toxicity values ranged from 500 µg/L for the freshwater macrophyte, *Lemna gibba* (2–5 d growth rate NOEC) to 830 800 µg/L for the freshwater fish *Lepomis macrochirus* (96-h LC50). An assessment of the modality of the available freshwater glyphosate chronic toxicity dataset indicated that the dataset was unimodal. Hence, all acceptable chronic toxicity data were considered in the derivation of the DGVs.

Very high reliability DGVs for glyphosate in freshwater were derived based on chronic negligible effect (e.g. NOEC, EC10) data for 15 freshwater species that belonged to six phyla and eight classes, with a good (visual) fit of the species sensitivity distribution (SSD) to the toxicity data. The DGVs are expressed in terms of the active ingredient (glyphosate) rather than commercial formulations, and do not relate to any of the breakdown products of glyphosate. The available literature indicates that commercial formulations of glyphosate are more toxic than the active ingredient alone (ANZECC/ARMCANZ 2000, AATSE 2002). Therefore, the actual levels of protection provided in freshwater ecosystems for the DGVs may be lower than specified in this technical brief. The DGVs for 99, 95, 90 and 80% species protection are 180 µg/L, 320 µg/L, 460 µg/L and 760 µg/L, respectively.

The 95% species protection level for glyphosate in freshwater (320 µg/L) is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1 Introduction

Glyphosate is a herbicide ($C_3H_8NO_5P$ and Figure 1) that, as a free acid at room temperature, is an odourless white crystal. It is the active ingredient of a variety of commercial herbicide formulations. Glyphosate often occurs in formulations with various surfactants and adjuvants (e.g. the surfactant polyethoxylated tallow amine, which is used in a number of commercial glyphosate-based products) to increase its efficacy. Glyphosate also has various salt forms, including isopropylamine, trimesium, diphenylamine and mono-ammonium, which are also regularly used in herbicide formulations, with the isopropylamine salt being the most commonly used form (ANZECC/ARMCANZ 2000). Physico-chemical properties of glyphosate that may affect its environmental fate and toxicity are presented in Table 1.

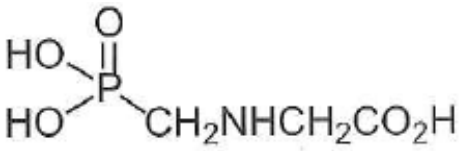

Figure 1 Structure of glyphosate

Table 1 Summary of selected physico-chemical properties of glyphosate

Physico-chemical property	Value
Molecular weight	169.1 amu [a, b]
Aqueous solubility	10 500 mg/L at pH 1.9 and temperature of 20 °C [a]
Logarithm of the octanol-water partition coefficient (log K_{ow})	-3.2 [a] -3.2 at pH 7 and temperature 20 °C [c]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	4.45 [b] 3.15 [c]
Logarithm of the bioconcentration factor (log BCF)	0.5 [c]
Half-life ($t_{1/2}$) in water	9.9 days [c] Hydrolysis: stable at pH 5–8 at temperature 25 °C [c] 33 days (pH 5), 77 days (pH 9) [c]
Half-life ($t_{1/2}$) in soil	74.5 days [c]

a BCPC (2012).
b CCME (2012).
c University of Hertfordshire (2013).

Glyphosate belongs to the organophosphorus group of herbicides, which also includes bensulide, fosamine and glufosinate. In Australia and New Zealand, glyphosate is extensively used either on its own or in combination with various other herbicides for control of annual and perennial grasses and broadleaf weeds in agriculture (e.g. barley, beans, citrus fruit, pastures, peas, stone fruit, vineyards), forestry, industrial, urban and other situations (e.g. national parks, bushland reserves, waterways, drains, roadsides) (ACVM 2020, APVMA 2020). It is a broad spectrum (non-selective) systemic herbicide with high activity on virtually all plants. In Australia, glyphosate has historically been the

most widely used herbicide, closely followed by simazine and atrazine (AATSE 2002). It is also widely used internationally.

Glyphosate and glyphosate salts in commercial formulations are often used in conjunction with various surfactants to increase efficacy. Several different kinds of surfactants are used depending on the intended use of the product. Where a product is registered for use near waterways, relatively benign surfactants are used in the formulation. However, for those products that include label restrictions with respect to usage near waterways, the surfactants employed (i.e. polyethoxylated tallow amine (POEA)) may be largely responsible for the aquatic toxicity among non-target organisms (Mann & Bidwell 1999). Some commercial formulations have been reported to be three to 42 times more toxic than the active ingredient—glyphosate (Folmar et al. 1979). Therefore, use of less toxic formulations (e.g. Roundup Biactive®) has been encouraged for use near waterways (AATSE 2002). The extent to which commercial formulations differ in their toxicity to the active ingredient will vary depending on the other chemicals added to the formulations. If there are concerns that the glyphosate default guideline values (DGVs) may be under-protective or over-protective due to differences in overall formulation toxicity, a formulation modified DGV could be derived using the methods in Warne et al. (2018).

Glyphosate binds strongly to soil particles (Table 1) and often remains in the top layer of soil; therefore, it does not have a high capacity to leach to groundwater. It is susceptible to off-site transport bound to soil particles (Schuette 1998). It is a post-emergence knockdown herbicide as it does not retain its biological effectiveness in soil after application (Franz et al. 1997 cited in Schuette 1998). Glyphosate is readily metabolised by soil micro-organisms (AATSE 2002) that biodegrade the carbon from glyphosate to aminomethylphosphonic acid (AMPA) and glyoxylate and, ultimately, to carbon dioxide (Schuette 1998).

The previous Australian and New Zealand DGV for glyphosate in freshwater environments was a moderate reliability value (using the ANZECC/ARMCANZ 2000 reliability scheme) as it was based on acute toxicity data for 18 phototrophic and heterotrophic species (Warne 2001). Under the new method for deriving DGVs (Warne et al. 2018), the ANZECC/ARMCANZ (2000) DGV would be classified as having low reliability. More data on glyphosate chronic toxicity to freshwater species are now available, which has enabled the derivation of improved DGVs compared to the ANZECC/ARMCANZ (2000) DGVs. This technical brief provides revised DGVs for glyphosate in freshwater that supersede the ANZECC/ARMCANZ (2000) DGVs.

2 Aquatic toxicology

2.1 Mechanisms of toxicity

Glyphosate is absorbed through plant foliage and stems rather than roots and is translocated in the phloem to growing points within the organism (AATSE 2002, APVMA 2014). Glyphosate acts by binding to and inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, which is responsible for catalysing chemical reactions within plants and algae. The binding of glyphosate to EPSP blocks the shikimate pathway and ultimately results in plant death from a lack of aromatic amino acids, such as tryptophan, phenylalanine and tyrosine (Schönbrunn et al. 2001, APVMA 2014,

Myers et al. 2016) as well as lignins, alkaloids, flavonoids, benzoic acids and plant hormones (Plant and Soil Sciences eLibrary 2015).

2.2 Toxicity

There is a significant body of literature on the toxicity of glyphosate to freshwater species. A literature search identified acceptable quality toxicity data for 36 freshwater species comprising 19 phototrophic species and 17 heterotrophic species (see Section 4.1 for details and associated links to supporting information). As glyphosate has a specific mode of action that targets plants and algae, it would be expected that phototrophic species would be more sensitive than heterotrophic species. An assessment of the relative sensitivity of freshwater phototrophic and heterotrophic species indicated that, although there is a general trend for phototrophic species to be more sensitive, there is a large overlap in sensitivities between the two groups (Appendix B). A summary of the toxicity of glyphosate to freshwater phototrophic species and heterotrophic species is provided below.

There did not appear to be any difference in the sensitivity of the four types of freshwater phototrophic organisms. Toxicity values for green algae ranged from 100 µg/L for the green algae *Chlorella vulgaris* (also called *Chlorella pyrenoidosa*) (72-h LOEC, growth inhibition), *Scenedesmus acutus* (72-h LOEC, growth inhibition) and *Scenedesmus subspicatus* (72-h NOEC, growth inhibition) (Vendrell et al. 2009), to approximately 1 080 000 µg/L for *C. pyrenoidosa* (96-hour LC50) (Anton et al. 1993). Toxicity values for blue-green algae ranged from 2 900 µg/L (21-d EC50, population growth) for *Anabaena catenula* to 598 400 µg/L (21-d EC50, population growth) for *Nostoc punctiforme* (Lipok et al. 2010). Toxicity values for macrophytes ranged from 500 µg/L (2–5-d NOEC, frond number) for *Lemna gibba* (Sobrero et al. 2007) to 46 900 µg/L (7-d EC50, growth rate) for *Lemna minor* (Cedergreen and Streibig 2005). Toxicity values for a diatom *Navicula pelliculosa* ranged from 1 800 µg/L (5-d NOEL, growth rate) to 38 600 µg/L (4-d EC50, growth rate) for *Navicula pelliculosa* (USEPA 2015b).

Toxicity values for heterotrophic species ranged from 100 to 830 800 µg/L. Fish toxicity values ranged from 10 000 µg/L (10–21-d LOEC, reproduction/mortality) for *Danio rerio* (Uren Webster et al. 2014) to 830 800 µg/L (96-h LC50, mortality) for *Lepomis macrochirus* (USEPA 2015b). Toxicity values for crustaceans ranged from 450 µg/L (36-d NOEC, growth, and 55-d NOEC, reproduction) (Cuhra et al. 2013) to 780 000 µg/L (48-h EC50, immobilisation) (USEPA 2015b), with both values being for *Daphnia magna*. Toxicity values for molluscs ranged from 100 µg/L (12-d IC7, reproduction) for *Pseudosuccinea columella* (Tate et al. 1997) to 25 000 µg/L (21-d LOEC, growth) for *Lampsilis siliquoidea* (Bringolf et al. 2007). Other toxicity values for heterotrophic species include a 48-h LC50 of 13 000 µg/L for the insect *Chironomus plumosus* (USEPA 2015b), and a 96-h LC5 and LC50 of 15 700 µg/L and 18 200 µg/L, respectively, for the cnidarian *Hydra attenuata* (Demetrio et al. 2012). The data suggest there might be some differences in the sensitivity of the various types of heterotrophs, but the dataset is too small to make a definitive conclusion about this.

3 Factors affecting toxicity

Factors such as temperature, pH (in formulations such as Roundup® only) and increased water hardness have been reported as modifying the toxicity of glyphosate (ANZECC/ARMCANZ 2000).

However, no relationships have been developed to permit the calculation of temperature–, pH– or water hardness–specific DGVs.

Various surfactants and adjuvants used in combination with glyphosate in commercial formulations are known to significantly increase the toxicity of the herbicide to target and non-target organisms (Folmar et al. 1979). Removal of glyphosate from the water column occurs mainly by binding to sediment and suspended solids, as well as via microbial degradation. The rate of biodegradation in water bodies appears to be positively related to the concentration of suspended solids (Feng et al. 1990, Newton et al. 1994). Thus, as with many organic chemicals, it might be expected that dissolved and particulate organic matter and suspended solids would affect the bioavailability and toxicity of glyphosate.

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. Although some decisions on data selection/manipulation may reflect the Warne et al. (2015) method rather than the Warne et al. (2018) method, these were found to have no material effect on the final DGVs.

4.1 Toxicity data used in derivation

As with all the other pesticides that have DGVs, the DGVs for glyphosate were based on data from experiments using technical or higher grades of glyphosate or with a minimum purity of 80% active ingredient (Warne et al. 2018). Consequently, some of the data that were used to generate the ANZECC/ARMCANZ (2000) DGVs for glyphosate were omitted from the current derivation process as the toxicity tests used commercial formulations.

To obtain toxicity data for glyphosate to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the ECOTOXicology Database System (USEPA 2015a), Office of Pesticide Program database (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC/ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now considerably more glyphosate chronic toxicity data available, including for phototrophic species (species that photosynthesise, e.g. plants and algae), to enable the calculation of DGVs in freshwater based on chronic toxicity alone.

In total, there were freshwater toxicity data for 36 species (eight different phyla and 13 classes) that passed the quality assessment and screening processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cnidaria, Cyanobacteria, Mollusca and Tracheophyta. The 13 classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Bivalvia (a grouping of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Gastropoda (another grouping of molluscs), Hydrozoa (a diverse group of cnidarians), Insecta (invertebrates), Liliopsida (monocots), Magnoliopsida (dicots), Malacostraca (a large grouping of crustaceans) and Trebouxiophyceae (another grouping of green algae). Chronic toxicity data were

available for 26 of the 36 species, comprising 19 phototrophs and seven heterotrophs, while acute toxicity data were available for 15 species, comprising one phototroph and 14 heterotrophs.

Based on the current understanding of the mode of action of glyphosate (see Section 2.1), it is expected that phototrophic species would be more sensitive than non-phototrophic species, as the EPSP enzyme is normally found within chloroplasts of plants and algae. However, a modality assessment of the glyphosate toxicity data, undertaken according to the approach described by Warne et al. (2018), concluded that the dataset was unimodal, with no apparent difference between the sensitivity of phototrophic and non-phototrophic species (see Appendix B: Modality assessment for glyphosate toxicity to freshwater species for details). Therefore, as recommended by Warne et al. (2018), the data for both phototrophs and heterotrophs were combined to calculate the DGVs for glyphosate in freshwater.

Of the 26 species for which there were acceptable chronic toxicity data, there were negligible effect (e.g. NOEC,EC10) data available for 15 species (that belonged to six phyla and nine classes), which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a species sensitivity distribution (SSD) to derive DGVs (Warne et al. 2018). For two species, *Ceriodaphnia dubia* and *Hyalella azteca*, pH values of <5 were reported for glyphosate test concentrations of ≥250 mg/L (SEC 2007). Although the toxicity of glyphosate is known to be affected by pH, the *C. dubia* and *H. azteca* data were included in the final dataset as the toxicity values (65 and 19 mg/L, respectively) were markedly lower than 250 mg/L, and it was assumed that the pH was within the acceptable range of 6 to 9 (Warne et al. 2018). Moreover, the inclusion of these data is consistent with their inclusion in the Canadian water quality guideline for glyphosate (CCME 2102).

A summary of the toxicity data (one value per species) used to calculate the DGVs for glyphosate in freshwater is provided in Table 2. Further details on the data that passed the quality assessment and screening process and were used to derive 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. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of AlgaeBase (Guiry & Guiry 2017), Atlas of Living Australia (ALA 2017), Catalogue of Life (Roskov et al. 2017), Integrated Taxonomic Information System (ITIS 2017) and the World Register of Marine Species (WoRMS 2017) was conducted. The dataset used in the DGV derivation process for glyphosate in freshwater includes toxicity data for six freshwater species that either originated from or are distributed within Australia and/or New Zealand. There was one published study (Bidwell & Gorrie 1995) that determined the toxicity of glyphosate to two Australasian frog species. As these toxicity tests were undertaken using high concentrations of glyphosate acid with low pH (<3.0), it is more likely that mortality amongst the tadpoles was a result of low pH levels of the higher exposure concentrations rather than the glyphosate acid exposure. Tadpoles have reportedly been unaffected by high concentrations (NOEC of >340 mg/L) of other forms of glyphosate, such as glyphosate IPA (Mann & Bidwell 1999), and it is well documented that amphibian larvae are intolerant to acid environments (Freda 1986). Therefore, the amphibian toxicity data reported by Bidwell and Gorrie (1995) were not included in the derivation of the DGVs for glyphosate.

Table 2 Summary of single chronic toxicity values, all species used to derive default guideline values for glyphosate in freshwater

Taxonomic group (Phylum)	Species	Life stage	Duration (d)	Toxicity measure [a]	Test endpoint	Final toxicity value (µg/L)
Blue–green alga (Cyanobacteria)	*Anabaena flosaquae*	Not stated	5	NOEL	Biomass yield, growth rate, AUC [b]	12 000
Crustacean (Arthropoda)	*Ceriodaphnia dubia* [c]	<24-hour old	7	NOEC	Survival	65 000
Crustacean (Arthropoda)	*Cherax quadricarinatus* [c]	Advanced juvenile	50	NOEC	Growth	22 500
Green alga (Chlorophyta)	*Chlorella saccharophila*	Exponential growth phase	3	NOEC/EC10	Cell density	1 082 [d]
Crustacean (Arthropoda)	*Daphnia magna*	Neonate	21	NOEC	Reproduction	450
Amphipod (Arthropoda)	*Hyalella azteca*	Juvenile	14	NOEC, EC10	Survival	19 145 [d]
Bivalve (Mollusca)	*Lampsilis siliquoidea*	Juvenile	21	NOEC	Shell length	12 500
Macrophyte (Tracheophyta)	*Lemna gibba*	Not stated	14	NOEL	Frond number, growth rate, mortality	1 400
Macrophyte (Tracheophyta)	*Lemna minor* [c]	Not stated	7	EC10	Chlorophyll-a content	3 780
Diatom (Bacillariophyta)	*Navicula pelliculosa* [c]	Not stated	5	NOEL	Biomass yield, growth rate, AUC [b]	1 800
Gastropod (Mollusca)	*Pseudosuccinea columella*	Embryo	12	NOEC, IC7	Hatching success	316 [d]
Green alga (Chlorophyta)	*Scenedesmus acutus* [c]	Not stated	4	NOEC	Chlorophyl a content	2 000
Green alga (Chlorophyta)	*Scenedesmus quadricauda*	Not stated	4	NOEC	Chlorophyl a content	770
Green alga (Chlorophyta)	*Scenedesmus subspicatus* [c, f]	Exponential growth phase	3	NOEC, EC10	Cell density	400 [d]
Green alga (Chlorophyta)	*Selenastrum capricornutum* [g]	Not stated	5	NOEL	Chlorophyll-a content	10 000

a The measure of toxicity being estimated/determined: EC10: 10% effect concentration; IC7: 7% inhibition concentration; NOEC: No observed effect concentration; NOEL: No observed effect level.

b AUC = area under the growth curve.

c Species that originated from/are distributed in Australia and/or New Zealand.

d Based on a geometric mean (see Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values).

e This species has also been called *Scenedesmus obliquus*.

f This species has also been called *Desmodesmus subspicatus*.

g This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.

4.2 Species sensitivity distribution

The SSD of the 15 freshwater glyphosate chronic toxicity values reported in Table 2 is presented in Figure 2. The SSD was plotted using the Burrlioz 2.0 software. The model was judged to provide a good fit to the data (Figure 2).

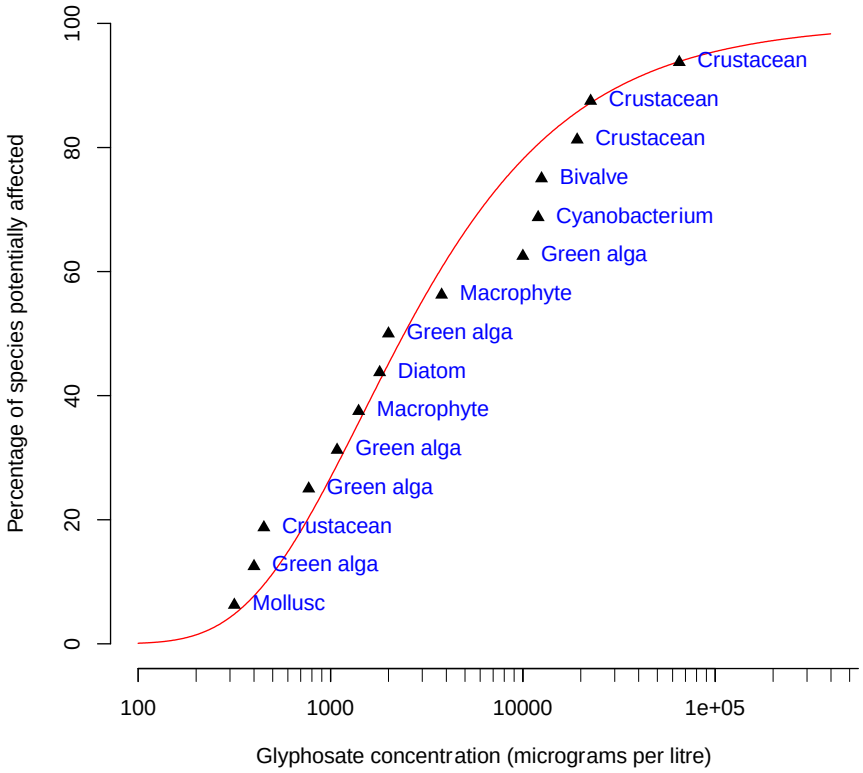

Figure 2 Species sensitivity distribution, glyphosate in freshwater

4.3 Default guideline values

It is important that the DGVs (Table 3) 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 DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 3. The DGVs are expressed in terms of the active ingredient (glyphosate) rather than commercial formulations, and do not relate to any of the breakdown products of glyphosate. The 95% species protection DGV of 320 µg/L glyphosate is recommended for application for slightly-to-moderately disturbed ecosystems. However, as the available literature indicates that commercial formulations containing glyphosate can be more toxic than glyphosate alone, the actual levels of protection provided by the DGVs for freshwater ecosystems may be lower than specified in Table 3. ANZG (2018; see Accounting for local conditions) provides guidance on what to do if the DGVs are under-protective due to formulation-related factors.

The DGVs are considerably lower than the ANZECC/ARMCANZ (2000) DGVs and the most recent international guideline value (at the 95% species protection level) for glyphosate (CCME 2012). The ANZECC/ARMCANZ (2000) DGV for glyphosate of 1 220 μg/L for 95% species protection was derived using acute toxicity data ranging from 100 μg/L to 641 000 μg/L, with a safety factor of 10 applied to convert it to a chronic guideline value. Thus, the ANZECC/ARMCANZ (2000) value had a high degree of uncertainty in its ability to protect against chronic effects. In Canada, the guideline value of 800 μg/L for 95% species protection was derived using chronic toxicity data ranging from 1 090 μg/L to 150 000 μg/L (CCME 2012). Notably, the chronic toxicity dataset used to derive the current DGVs contained five values that were lower than the lowest value in the Canadian dataset, three of which were published after the Canadian derivation was undertaken. Thus, given the current DGVs are based on the most up-to-date international chronic toxicity dataset, they represent the most reliable of currently available guideline values for glyphosate in freshwater.

Measured log BCF values for glyphosate are low (Table 1) and are below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4 (Warne et al. 2018)). Therefore, the DGVs for glyphosate do not need to account for secondary poisoning.

Table 3 Toxicant default guideline values, glyphosate in freshwater, very high reliability

Level of species protection (%)	DGV for glyphosate in freshwater (μg/L) [a]
99	180
95	320
90	460
80	760

a The DGVs were derived using the Burrlioz 2.0 software. They have been rounded to two significant figures.

4.4 Reliability classification

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

- Sample size—15 (preferred)
- Type of toxicity data—chronic NOEC/NOEL/EC10 values
- SSD model fit—good (Inverse Weibull 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
bimodal	When the distribution of the sensitivity of species to a toxicant has two modes. This typically occurs with chemicals with specific modes of action. For example, herbicides are designed to affect plants at low concentrations but most animals are only affected at high concentrations.
CAS no. (Chemical Abstracts Service number)	Each chemical has a unique identifying number that is allocated to it by the American Chemical Society.
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. a site-specific guideline value) in the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*. Formerly known as 'trigger values'.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
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.
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, relative to the control response, under specified conditions.
lowest observed effect concentration (LOEC) or lowest observed effect level (LOEL)	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. Also sometimes referred to as a lowest observed effect level (LOEL).

Term	Definition
no observed effect concentration (NOEC) or no observed effect level (NOEL)	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. Also sometimes referred to as a no observed effect level (NOEL).
Phototrophs	Organisms that photosynthesize as their main means of obtaining energy, for example plants and algae.
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.
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, glyphosate in freshwater

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Crustacean (Arthropoda)	*Ceriodaphnia dubia*	<24-hour old	7	NOEC (Survival)	Dilution water	24–25	4.7–8.2	65 000	SEC (2007)
–								65 000	**VALUE USED IN SSD**
Crustacean (Arthropoda)	*Cherax quadricarinatus*	Advanced juvenile	50	NOEC (Growth)	Dechlorinated filtered tap water	27 ± 1	8.0 ± 0.5	22 500	Frontera et al. (2011)
–								**22 500**	**VALUE USED IN SSD**
Crustacean (Arthropoda)	*Daphnia magna*	Neonates	55	NOEC (Fecundity)	Aachener Daphnien Medium (adam)	27 ± 2	7.5 ± 0.7	450	Cuhra et al. (2013)
–								**450**	**VALUE USED IN SSD**
Crustacean (Arthropoda	*Hyalella azteca*	Juvenile	14	EC10 (Survival)	Dilution water	22–23	3.5–7.9	53 900	SEC (2007)
		Juvenile	14	NOEC (Survival)	Dilution water	22–23	3.5–7.9	6 800	SEC (2007)

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
–								**19 145**	**VALUE USED IN SSD (GEOMTERIC MEAN)**
Diatom (Bacillariophyta)	*Navicula pelliculosa*	Not stated	5	NOEL (Biomass, growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	1 800	USEPA (2015b)
–								**1 800**	**VALUE USED IN SSD**
Green alga (Chlorophyta)	*Chlorella saccharophila*	Exponential growth phase	3	NOEC (Cell density)	ASTM medium	24 ± 2	Not stated	390	Vendrell et al. (2009)
		Exponential growth phase	3	EC10 (Cell density)	ASTM medium	24 ± 2	Not stated	3 000	Vendrell et al. (2009)
–								**1 082**	**VALUE USED IN SSD (GEOMETRIC MEAN)**
Green alga (Chlorophyta)	*Selenastrum capricornutum* [a]	Not stated	5	NOEL (Biomass, growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	10 000	USEPA (2015b)
–								**10 000**	**VALUE USED IN SSD**
Green alga (Chlorophyta)	*Scenedesmus acutus* [c]	Not stated	4	NOEC (Chlorophyl a content)	Modified Detmer's nutrient medium	22 ± 2	7.5	2 000	Saenz et al. (1997)
–								**2 000**	**VALUE USED IN SSD**

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Green alga (Chlorophyta)	*Scenedesmus quadricauda*	Not stated	4	NOEC (Chlorophyl a content)	Modified Detmer's nutrient medium	22 ± 2	7.5	770	Saenz et al. (1997)
–								**770**	**VALUE USED IN SSD**
Green alga (Chlorophyta)	*Scenedesmus subspicatus* [d]	Exponential growth phase	3	NOEC (Cell density)	ASTM medium	24 ± 2	Not stated	100	Vendrell et al. (2009)
		Exponential growth phase	3	EC10 (Cell density)	ASTM medium	24 ± 2	Not stated	1 600	Vendrell et al. (2009)
–								**400**	**VALUE USED IN SSD (GEOMETRIC MEAN)**
Blue–green alga (Cyanobacteria)	*Anabaena flosaquae*	Not stated	5	NOEL (Biomass, growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	12 000	USEPA (2015b)
–								**12 000**	**VALUE USED IN SSD**
Bivalve (Mollusca)	*Lampsilis siliquoidea*	Juvenile	21	NOEC (Growth)	Reconstituted hard water	21.1 ± 0.7	8.22–8.76	12 500	Bringolf et al. (2007)
–								**12 500**	**VALUE USED IN SSD**
Gastropod (Mollusca)	*Pseudosuccinea columella*	Embryo	12	NOEC (Hatching success)	Artificial spring water	25 ± 2	6.5–8.5	1 000	Tate et al. (1997)
		Embryo	12	IC7 (Hatching success)	Artificial spring water	25 ± 2	6.5–8.5	100	Tate et al. (1997)

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
–								**316**	**VALUE USED IN SSD (GEOMETRIC MEAN)**
Macrophyte (Tracheophyta)	*Lemna gibba*	Not stated	14	NOEL (Frond number, growth rate, mortality)	M-Hoagland's/20X-AAP nutrient media/ASTM Type I	25 ±2	4.8–5.2 / 7.5 ± 0.1	1 400	USEPA (2015b)
–								**1 400**	**VALUE USED IN SSD**
Macrophyte (Tracheophyta)	*Lemna minor*	Not stated	7	EC10 (Chlorophyll-a)	K' medium	24	5	3 780	Cedergreen and Streibig (2005)
–								**3 780**	**VALUE USED IN SSD**

a This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.
b AUC = area under the growth curve.
c This species has also been called *Scenedesmus obliquus*.
d This species has also been called *Desmodesmus subspicatus*.
Note: Table strictly excludes data that originated from the use of formulations (e.g. Roundup).

Appendix B: Modality assessment for glyphosate toxicity to freshwater species

A modality assessment was undertaken for glyphosate according to the weight of evidence approach specified in Warne et al. (2018).

Is there a specific mode of action that could result in taxa-specific sensitivity?
Glyphosate acts by binding to and inhibiting the enzyme 5-enolpyruvylshikimate 3-phosphate (EPSP) synthase, which blocks the shikimate pathway and ultimately results in plant death from a lack of aromatic amino acids, such as tryptophan, phenylalanine and tyrosine (Schönbrunn et al. 2001, APVMA 2014, Myers et al. 2016) as well as lignins, alkaloids, flavonoids, benzoic acids and plant hormones (Plant and Soil Sciences eLibrary 2015). The shikimate pathway is present in bacteria, archaea, fungi, algae, some protozoans, and plants—but not in animals. Therefore, it might be expected that plants and micro-organisms are more sensitive to glyphosate than animals.

Does the dataset suggest bimodality?
Modality was assessed using a freshwater toxicity dataset for which all data had passed the quality assessment and screening processes (n = 36). All data that were not chronic negligible effect values (e.g. EC10, NOEC) were first converted to this type of data using the methods recommended by Warne et al. (2018). Calculation of the bimodality coefficient (BC) on log-transformed data yielded a value of 0.46, which, being below the indicative threshold BC for bimodality of 0.55, suggested the dataset does not exhibit bimodality. Additionally, a frequency histogram of the data suggested that the distribution of toxicity data was unimodal (Figure B 1).

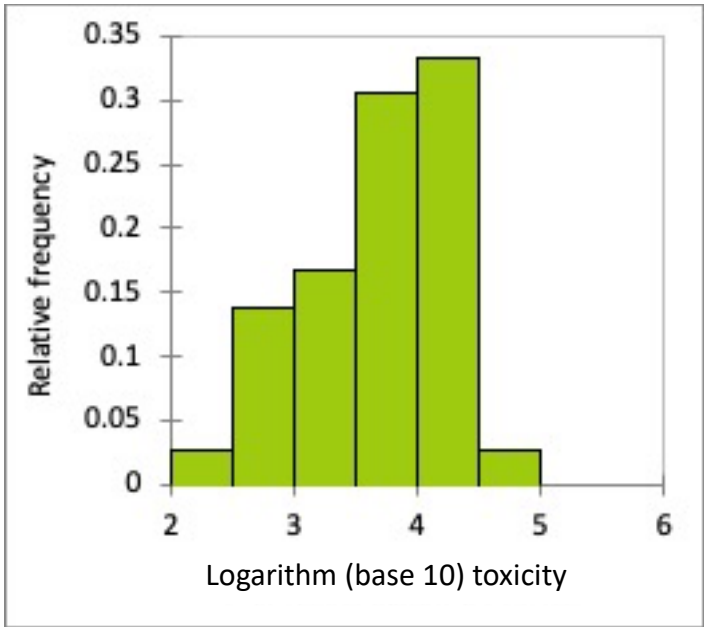

Figure B 1 Frequency histogram, log-transformed glyphosate ecotoxicity data, freshwater species

Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?
The relative sensitivity of different taxa groups to glyphosate was compared using box and whisker plots (Figure B 2) and a species sensitivity distribution (plotted using the Burrlioz 2.0 software) (Figure B 3). Although these graphical analyses indicate a general trend for phototrophs to be more sensitive than heterotrophs (as would be expected given the mode of action), there is almost a complete overlap in the toxicity values of phototrophs and heterotrophs, indicating that there is no clear separation between these groups.

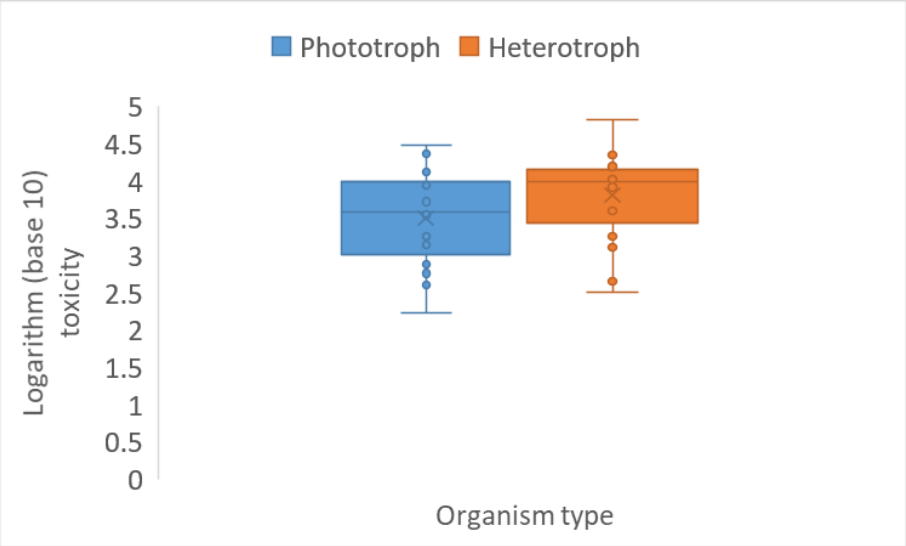

Figure B 2 Box and whisker plots, glyphosate toxicity, freshwater phototrophs and heterotrophs

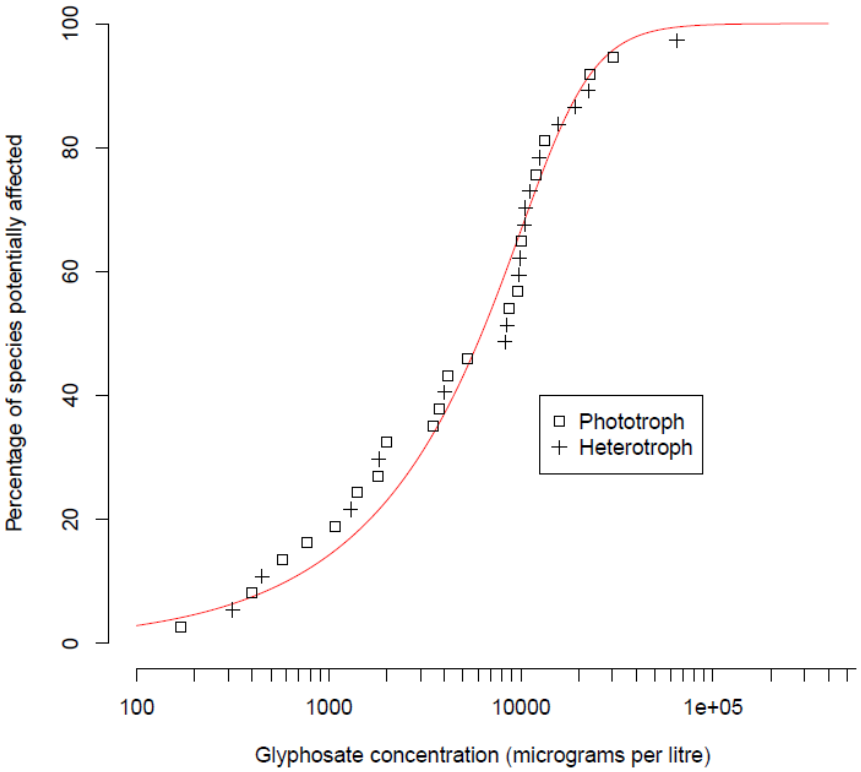

Figure B 3 Species sensitivity distribution, glyphosate toxicity, freshwater phototrophs and heterotrophs

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

Given that the sample sizes are quite high for both phototrophs (n = 19) and heterotrophs (n = 17), it is likely that the distributions are representative, although a bias cannot be ruled out. The factors in the weight of evidence were: a potentially specific mode of action; a bimodality coefficient that indicated the dataset was likely to be bimodal; and three graphical analyses that indicated the distribution of toxicity data is unimodal despite a general trend for phototrophs to be more sensitive than heterotrophs. Overall, the information indicated that the toxicity of glyphosate to freshwater species exhibits a unimodal relationship; therefore, all the available toxicity data were used in the DGV derivation.

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