Proposed aquatic ecosystem protection guideline values for pesticides commonly used in the Great Barrier Reef catchment area: Part 1 – 2,4-D, Ametryn, Diuron, Glyphosate, Hexazinone, Imazapic, Imidacloprid, Isoxaflutole, Metolachlor, Metribuzin, Metsulfuron-methyl, Simazine and Tebuthiuron

Nouwen, A, Chambers, A, Chechlacz, M, Higgs, S, Blissett, J, Barrett, TG & Allen, HA

Proposed aquatic ecosystem protection guideline values for pesticides commonly used in the Great Barrier Reef catchment area: Part 1 – 2,4-D, Ametryn, Diuron, Glyphosate, Hexazinone, Imazapic, Imidacloprid, Isoxaflutole, Metolachlor, Metribuzin, Metsulfuron-methyl, Simazine and Tebuthiuron.

Water Quality and Investigations | Department of Science, Information Technology and Innovation

2017

Queensland
Government

Prepared by

O. C. King, R. A. Smith, R. M. Mann and M. St. J. Warne.
Water Quality and Investigations, Environmental Monitoring and Assessment Science,
Science Division, Department of Science, Information Technology and Innovation
PO Box 5078
Brisbane QLD 4001

Disclaimer

This document has been prepared with all due diligence and care, based on the best available information at the time of publication. The department holds no responsibility for any errors or omissions within this document. Any decisions made by other parties based on this document are solely the responsibility of those parties. Information contained in this document is from a number of sources and, as such, does not necessarily represent government or departmental policy.

If you need to access this document in a language other than English, please call the Translating and Interpreting Service (TIS National) on 131 450 and ask them to telephone Library Services on +61 7 3170 5725

Citation

Acknowledgements

Executive summary

The Australian and New Zealand Guideline for Fresh and Marine Water Quality, also referred to as the National Water Quality Guidelines (ANZECC and ARMCANZ 2000), provides toxicity-based default guideline values (formerly referred to as a trigger values) for numerous inorganic and organic chemicals of environmental concern, including guideline values for fifty individual pesticides. This number falls well short of the total number of pesticides used in Australia and under-represents many of the pesticides currently used in Great Barrier Reef catchments. The National Water Quality Guidelines are currently being revised as part of the National Water Quality Management Strategy (NWQMS).

Under several different funding arrangements, the Queensland Government Department of Science, Information Technology and Innovation (DSITI) have been engaged in the derivation of new or revised aquatic ecosystem protection guideline values for 27 pesticides commonly detected in the Great Barrier Reef catchments. All aquatic ecosystem protection guideline values have been derived using the revised method for deriving water quality guidelines for toxicants (Warne et al. 2015). In Australia, water quality guideline values are preferably derived using a species sensitivity distribution (SSD) approach. The intent is that all these aquatic ecosystem protection guideline values are ultimately endorsed as National default guideline values. Until such time that they have received endorsement by the Standing Committee for the Revision of the National Guidelines, these derived guideline values will be termed as proposed aquatic ecosystem protection guideline values. In the interest of brevity, in this report they are also referred to as Proposed Guideline Values (PGV).

This report is the first part of a two-part series that presents the Proposed Guideline Values for 27 pesticides commonly detected in both, freshwater catchments and marine waters of the Great Barrier Reef. The Proposed Guideline Values have been split across the two separate reports depending on the funding arrangements under which they were derived. The Proposed Guideline Values for glyphosate, metolachlor, metsulfuron-methyl, and simazine were derived under contract with the Commonwealth Government Department of the Environment (DoE) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO), and were derived for inclusion in the revised Australian and New Zealand Guidelines for Fresh and Marine Water Quality. At the time of writing, publication of the revised Australian and New Zealand Guidelines for Fresh and Marine Water Quality was still pending. The Proposed Guideline Values for 2,4-D, imazapic, isoxaflutole and metribuzin were derived as part of a project funded by the National Environmental Research Programme (NERP). The Proposed Guideline Values for ametryn, diuron, hexazinone, imidacloprid and tebuthiuron were derived as part of a research project funded by the Queensland Department of Science, Information Technology and Innovation (DSITI).

The 13 pesticides presented in Part 1 (this report) were selected based on the priorities of Commonwealth and State government departments and stakeholders, and are currently being reviewed for endorsement as National guideline values. The 14 pesticides included in Part 2 (King et al. 2017) are also detected regularly in catchments discharging to the GBR lagoon (Wallace et al. 2016). Currently, there are either, no, or only *low reliability* National guideline values in existence for these pesticides. As part of a project funded by the Queensland Department of Environment and Heritage Protection, the Queensland Department of Science, Information Technology and Innovation has derived Proposed Guideline Values for fresh and marine ecosystems for these 14 pesticides.

Background

Pesticides in the Great Barrier Reef

Pesticides pose a risk to freshwater ecosystems as well as inshore and coastal ecosystems of the Great Barrier Reef (GBR) (Waterhouse et al. 2017). Pesticides in the aquatic environment can cause direct and indirect effects that reduce the resilience of aquatic ecosystems to other stressors. Diffuse sources of pollution from agriculture are the largest contributors of pesticides to the GBR, and include cattle grazing and sugarcane cultivation as the dominant modified land uses (Brodie et al. 2013).

In an effort to protect the health and resilience of the GBR from poor water quality, the Reef Water Quality Protection Plan (Reef Plan) was established in 2003 in a joint collaboration by the Australian and Queensland governments (DPC 2013). In 2009, following the release of the Scientific Consensus Statement (Brodie et al. 2008), a comprehensive update of Reef Plan was undertaken. This addressed the elevated levels of pollutants leaving catchments adjacent to the GBR and entering the Reef, with a clear goal[1] and specific targets for reducing sediment, nutrient and pesticide loads (DPC 2013). The Reef Plan has since been updated in 2013 with the next version released in 2017.

The targets for pesticide reduction originally focused on the loads of five photosystem II herbicides. Since that time, water quality monitoring, by the Great Barrier Reef Catchment Loads Monitoring Program and the Marine Monitoring Program (as part of the Paddock to Reef Integrated Monitoring, Modelling and Reporting Program), has demonstrated that there are many different pesticides present in the catchments and the GBR lagoon (Wallace et al. 2016). Indeed, 56 pesticide residues (including seven herbicide metabolites) have been detected in the adjacent catchments, estuaries and wetlands and the GBR lagoon since 2009 (Devlin et al. 2015; Wallace et al. 2016).

In 2017, the Reef Plan pesticide targets will be re-evaluated to align closer with the National (ANZECC and ARMCANZ 2000), State (e.g. DEHP 2009) and GBR (e.g. GBRMPA 2010) water quality guidelines (WQG). In addition, regional Water Quality Improvement Plans prepared for GBR catchments, in alignment with the requirements of the Environmental Protection (Water) Policy 2009, rely on aquatic ecosystem protection guideline values to assess the potential hazard of pesticide contaminants in freshwater and estuarine ecosystems, and to set water quality objectives. Unfortunately, for the majority of the pesticides detected there are currently either, no guideline values (GV) available, or existing values are of *low reliability* (i.e. they were derived from ecotoxicity data using a limited number of species and taxanomic groups).

Water Quality Guidelines

Water quality guidelines (WQGs) are available at a National (ANZECC and ARMCANZ 2000), State (e.g. DEHP 2009) and regional (e.g. GBRMPA 2010) level. Water quality guidelines report Default Guideline Values (also referred to as criteria, standards, objectives, environmental protection guideline values or environmental thresholds in other jurisdictions) for toxicants. These being the scientific estimate of the maximum concentration of chemicals that can be present in aquatic ecosystems and still be considered as a low risk to the species within the ecosystem. The preferred

---

[1] Ensure that by 2020 the quality of water entering the reef from broadscale land use has no detrimental impact on the health and resilience of the Great Barrier Reef (DPC 2013).

method for deriving GVs for ecosystem protection (as opposed to GVs for drinking water or other environmental values) is through the use of species sensitivity distributions (SSD). These are cumulative frequency plots that facilitate an estimation of the concentrations at which toxic effects first occur in aquatic species that are representative of aquatic ecosystems. From SSDs, the percentage of species that are likely to be affected by a given concentration of a pesticide can be determined. The National WQGs (ANZECC and ARMCANZ 2000) provide four levels of environmental protection that should theoretically protect 99, 95, 90 and 80 per cent of species. The concentrations corresponding to these levels of protection are termed the PC99, PC95, PC90 and PC80, which are equivalent to the concentrations harmful to 1% (HC1), 5% (HC5), 10% (HC10) and 20% (HC20) of species in an ecosystem, respectively. The Queensland and GBR Marine Park adopt a similar approach for setting ecosystem protection levels (DEHP 2009; GBRMPA 2010).

The current National WQGs (ANZECC and ARMCANZ 2000) include freshwater and marine GVs[2] for 17 of the 49 pesticides detected in GBR catchments and lagoon in the last six years (Devlin et al. 2015), of which 10 are categorised as being of *low reliability*. The WQGs for the GBR Marine Park (GBRMPA 2010) report marine GVs for 11 pesticides - five of which are also categorised as being of *low reliability*. The Queensland WQGs (DEHP 2009) do not provide GVs for pesticides and defer to the National WQGs (ANZECC and ARMCANZ 2000) for freshwater and estuarine ecosystems and GBRMPA (2010) for waters in the marine zone and enclosed coastal waters.

The National WQGs (ANZECC and ARMCANZ 2000) are now under revision as part of the larger revision of the National Water Quality Management Strategy (NWQMS). One of the aims of the revision is to derive GVs for over 30 chemicals, including at least 18 pesticides. The revision also includes an update of the method for deriving GVs for chemicals. Most of the key principles for deriving GVs described in ANZECC and ARMCANZ (2000) and in Warne (2001) have been retained. However, significant improvements have been made in the derivation method in order to accommodate the most recent advances in ecotoxicology (Batley et al. 2014; Warne et al. 2015). The preferred method for GV derivation continues to be based on the use of SSDs of chronic toxicity data.

Scope of Report

This report is the first part to a two-part series that presents the proposed aquatic ecosystem protection guideline values (hereafter referred to as proposed guideline values (PGV)) for pesticides commonly detected in the GBR catchments. In total, PGVs for 27 pesticides were derived under different funding arrangements. The pesticide PGVs presented in each part have been grouped according to the source of funding. For all 27 pesticides, PGVs were derived for both freshwater and marine organisms (except where indicated below). These PGVs include 95% confidence intervals (95% CI) which are an indication of the level of certainty around the guideline.

This report, Part 1 of the two-part series, presents the freshwater and/or marine PGVs for 13 pesticides. These include; (i) PGVs for glyphosate (freshwater only), metolachlor (freshwater only), metsulfuron-methyl (freshwater only) and simazine that were funded through the Commonwealth Department of Environment (DoE) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO) for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality, (ii) PGVs for ametryn, diuron, hexazinone, imidacloprid and tebuthiuron that

---

[2] Guideline values are referred to as 'trigger values' in ANZECC and ARMCANZ (2000) and GBRMPA (2010). The term 'guideline value' will replace 'trigger value' in the revision of the National Guidelines.

were funded through the Queensland Department of Science, Information Technology and Innovation (DSITI), and lastly, (iii) PGVs for 2,4-D (marine only), imazapic, isoxaflutole and metribuzin that were funded through the National Environmental Research Programme (NERP).

Part 2 of the two-part series (King et al. 2017) presents the freshwater and/or marine PGVs for a further 14 pesticides commonly detected in the GBR catchments. These include; bromacil, chlorothalonil, fipronil (marine only), fluometuron, fluroxypyr, haloxyfop, MCPA (marine only), pendimethalin, prometryn, propazine, propiconazole, terbutryn, triclopyr, terbuthylazine, that were funded through the Queensland Department of Environment and Heritage Protection (DEHP).

References

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

Batley GE, van Dam, RA and Warne MStJ (2014). Technical rationale for changes to the revised method for deriving Australian and New Zealand Water Quality Guideline Values for toxicants, Prepared for the Countil of Australian Government's Standing Council on Environment and Water (SCEW), June 2014. Available from: http://www.environmetrics.net.au/docs/BATLEY%20ET%20AL_TECHNICAL_RATIONALE_GV_DERIVATION_METHOD_FINAL3.pdf. Accessed: April 2017.

Brodie J, Binney J, Fabricius K, Gordon I, Hoegh-Guldberg O, Hunter H, O'Reagain P, Pearson R, Quirk M, Thorburn P and Waterhouse J (2008). Synthesis of evidence to support the scientific consensus statement on water quality in the Great Barrier Reef. The State of Queensland (Department of Premier and Cabinet), Brisbane, 64pp. Available from: http://www.reefplan.qld.gov.au/resources/assets/reef-consensus.pdf.

Brodie J, Waterhouse J, Schaffelke B, Kroon F, Thorburn P, Rolfe J, Johnson J, Fabricius K, Lewis S, Devilin M, Warne MStJ and McKenzie L (2013). Scientific Consensus Statement: Land use impacts on the Great Barrier Reef water quality and ecosystem condition. Reef Water Quality Plan Secretariat, Queensland. http://www.reefplan.qld.gov.au/about/assets/scientific-consensus-statement-2013.pdf.

DEHP (2009). Queensland Water Quality Guidelines, Version 3. Department of Environment and Heritage Protection, Brisbane, Australia, 184pp. Available from: https://www.ehp.qld.gov.au/water/pdf/water-quality-guidelines.pdf.

Devlin M, Lewis S, Davis A, Smith R, Negri A, Thompson M and Poggio M (2015). Advancing our understanding of the source, management, transport and impacts of pesticides on the Great Barrier Reef 2011-2015. A report for the Queensland Department of Environment and Heritage Protection. Tropical Water & Aquatic Ecosystem Research (TropWATER) Publication, James Cook University, Cairns, 134pp. Available from: https://research.jcu.edu.au/tropwater/publications/PesticidesynthesisFinalversionAugust2015.pdf.

DPC (2013). Reef Water Quality Protection Plan (2013). Securing the health and resilience of the Great Barrier Reef World Heritage Area and adjacent catchments. The State of Queensland 2013, Department of the Premier and Cabinet. Reef Water Quality Protection Plan Secretariat 36pp. Available from: http://www.reefplan.qld.gov.au/resources/assets/reef-plan-2013.pdf.

GBRMPA (2010). Water Quality Guidelines for the Great Barrier Reef Marine Park. Great Barrier Reef Marine Park Authority, Townsville, 109pp. Available from: http://www.gbrmpa.gov.au/__data/assets/pdf_file/0017/4526/GBRMPA_WQualityGuidelinesGBRMP_RevEdition_2010.pdf.

King OC, Smith RA, Warne MStJ, Frangos JS and Mann R (2017). Proposed aquatic ecosystem protection guideline values for pesticides commonly used in the Great Barrier Reef catchment area: Part 2 - Bromacil, Chlorothalonil, Fipronil, Fluometuron, Fluroxypyr, Haloxyfop, MCPA, Pendimethalin, Prometryn, Propazine, Propiconazole, Terbutryn, Triclopyr and Terbuthylazine. Department of Science, Information Technology and Innovation. Brisbane, Queensland, Australia, 209pp.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Warne MStJ (2001). Derivation of the ANZECC and ARMCANZ Water Quality Guidelines for Toxicants. Australasian Journal of Ecotoxicology, 7, 123–136.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Waterhouse J, Brodie J, Tracey D, Smith R, Vandergragt M, Collier C, Petus C, Baird M, Kroon F, Mann R, Sutcliffe T, Waters D, Adame F (2017). Scientific Consensus Statement 2017: A synthesis of the science of land-based water quality impacts on the Great Barrier Reef, Chapter 3: The risk from anthropogenic pollutants to Great Barrier Reef coastal and marine ecosystems. State of Queensland, 2017.

Contents

List of tables

List of figures

Glossary, acronyms, abbreviations

Acute toxicity	An adverse effect that occurs as the result of a short-term exposure to a chemical relative to the organism's life span. Refer to Warne et al. (2015) for examples of acute exposures.
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	An adverse effect that occurs as the result of exposure to a chemical for a substantial portion of the organism's life span or an adverse sub-lethal effect on a sensitive early life stage. Refer to Warne et al. (2015) for examples of chronic exposures.
EC50 (Median effective concentration) / IC50 (Median inhibition concentration)	The concentration of a chemical in water that is estimated to produce a 50% effect on a sub-lethal endpoint. The EC50/IC50 is usually expressed as a time-dependent value (e.g. 24-hour or 96-hour EC50/IC50).
ECx	The concentration of a chemical in water that is estimated to produce an x% effect on a sub-lethal endpoint. The magnitude of x can vary from 1 to 100, however values between 5 and 50 are more typical. The ECx is usually expressed as a time-dependent value (e.g. 24-hour or 96-hour ECx).
Endpoint	A measurable biological effect including, but not limited to, lethality, immobility, growth inhibition, immunological responses, organ effects, developmental and reproductive effects, behavioural effects, biochemical changes, genotoxicity, etc.
Guideline value (GV)	A measurable quantity (e.g. concentration) or condition of an indicator for a specific environmental 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 environmental value. Guideline values for more than one indicator should be used simultaneously in a multiple lines of evidence approach.

LC50 (Median lethal concentration)	The concentration of a chemical in water that is estimated to kill 50% of the test organisms. The LC50 is usually expressed as a time-dependent value (e.g. 24-hour or 96-hour LC50).
LOEC (Lowest observed effect concentration) / LOEL (Lowest observed effect level)	The lowest concentration of a chemical used in a toxicity test that has a statistically significant (p≤0.05) adverse effect on the exposed population of test organisms compared to the controls. All higher concentrations should also cause statistically significant effects.
Mode of action	The means by which a chemical exerts its toxic effects. For example, triazine herbicides inhibit the photosystem II component of plants photosynthesis biochemical reaction.
NOEC (No observed effect concentration) / NOEL (No observed effect level)	The highest concentration of a toxicant used in a toxicity test that does not have a statistically significant (p>0.05) effect compared to the controls. The statistical significance is measured at the 95% confidence level.
Phototrophs	Organisms that photosynthesize as their main means of obtaining energy e.g. plants and algae.
Proposed aquatic ecosystem protection guideline value (PGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. site-specific). This term applies to those guideline values that have yet to be endorsed for inclusion in the Australian and New Zealand Water Quality Guidelines.
PSII	Photosystem II of the photosynthetic biochemical pathway.
Racemic mixture	A mixture containing two enantiomers (mirror image forms of a chemical) of a single chemical. For metolachlor the racemic mixture contains the r- and s-enantiomers of metolachlor.
Site-specific	Relating to something that is confined to, or valid for, a particular place. Site-specific trigger 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.
SSD	Species sensitivity distribution. A method that plots the cumulative frequency of species sensitivity and fits the best possible 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 concentration of chemical.

Summary of the data selection approach

The order of preference that was used to select ecotoxicity data to derive proposed aquatic ecosystem protection guideline values (PGVs) for individual pesticides is as follows;
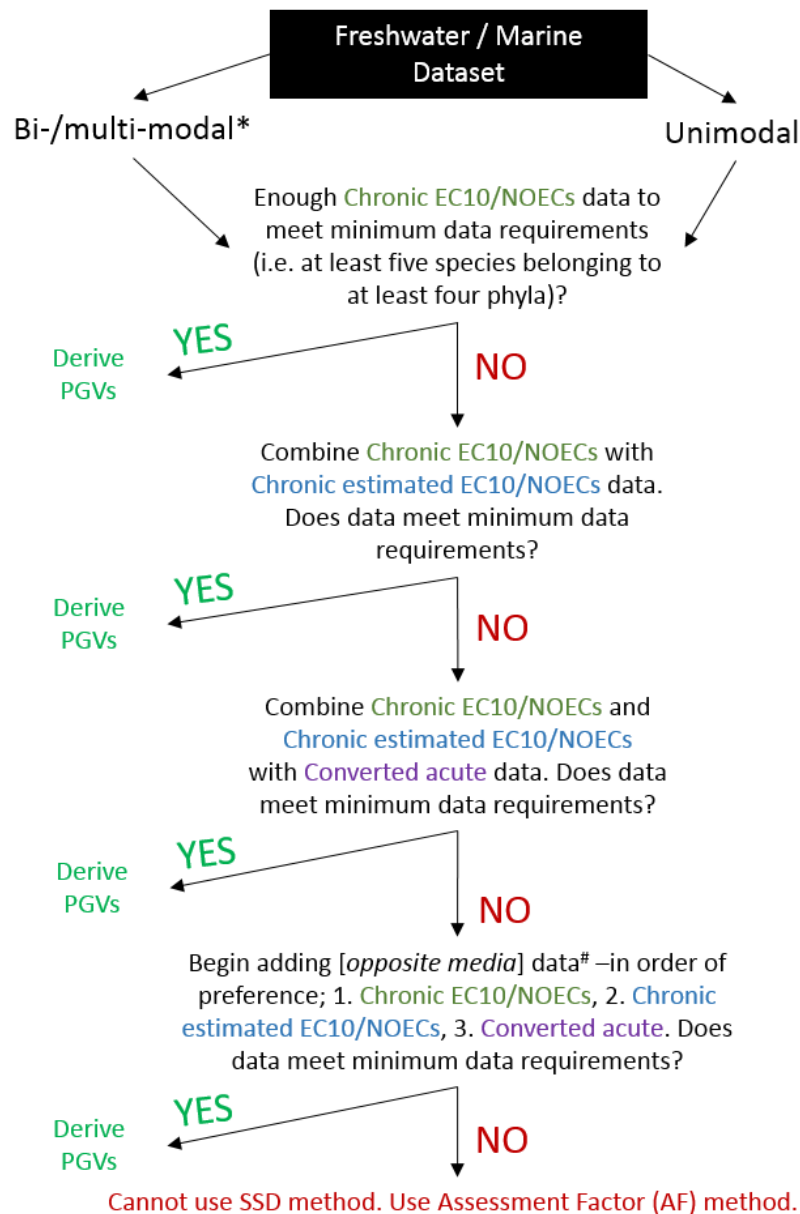
Chronic EC10/NOEC data = no conversions applied; Chronic estimated EC10/NOEC data = chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively; Converted acute = acute LC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 10 (Warne et al. 2015).

* If the dataset is statistically bi-/multi-modal, only use the most sensitive taxonomic subgroup for PGV derivation (Warne et al. 2015). For example, when calculating PGVs for a herbicide, the dataset may have a bimodal distribution with phototrophic species being more sensitive than non-phototrophic species. Therefore, only data for phototrophic species would be used to derivePGVs.

\# If there is evidence indicating that there is no difference between the sensitivity of freshwater and marine taxa (e.g. chemical, physiological or statistical evidence) then it is acceptable to bring in marine data (to a freshwater dataset) or freshwater data (into a marine dataset) to meet minimum data requirements (Warne et al. 2015).

1 2,4-Dichlorophenoxyacetic acid (2,4-D)

1.1 Introduction

2,4-dichlorophenoxyacetic acid, also known as 2,4-D is a herbicide ($C_8H_6Cl_2O_3$ and Figure 1) that at room temperature is in the form of a colourless powder with a slight phenolic odour. It is the active ingredient of a variety of commercial herbicide formulations and comes in a variety of chemical forms, with BCPC (2012) listing 14 forms. 2,4-D is also listed as a potential endocrine disrupting chemical (EDC) by the European Union, as there is 'more or less comprehensive evidence' of endocrine disrupting effects in exposed aquatic organisms (DEPA 2015). Endocrine disrupting effects were not considered in the derivation of the PGVs for 2,4-D.
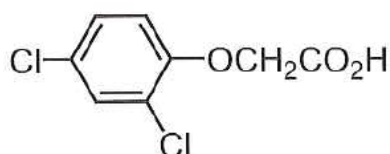
Figure 1 Structure of 2,4-D.

Physicochemical properties of 2,4-D that may affect its environmental fate and toxicity are presented in Table 1.

Table 1 Summary of selected physicochemical properties of 2,4-D.

Physicochemical property	Value
Molecular weight	221.0 amu[1]
Aqueous solubility	311 mg/L (pH 1), 20,031 mg/L (pH 5), 23,180 mg/L (pH 7), 34,196 mg/L (pH 9) @ temperature of 25 °C[1] 24.3 mg/L at 20 °C[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.58–2.83 (pH 1), 0.04–0.33 (pH 5), -0.75 (pH 7)[1] -0.82[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.78[1] 1.59[2]
Logarithm of the bioconcentration factor (log BCF)	1[2]
Half-life ($t_{1/2}$) in water	Stable between pH 5 – 9 @ temperature 20 °C[2] 7.7 days[2]
Half-life ($t_{1/2}$) in soil	Typical: 4.4 days (4.4 – 28.8 days in the lab (20 °C) and in the field, respectively)[2]

[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013).

2,4-D belongs to the phenoxyacetic group within the phenoxy family of herbicides, which also includes 2,4,5-T[3] and MCPA. 2,4-D is extensively used in agricultural, industrial and urban situations to control annual and perennial broad-leaved weeds in a variety of cereals (e.g. barley, wheat, corn, rye and oats) and sugar cane (University of Hertfordshire 2013). 2,4-D can be transported into aquatic environments as a result of direct application to aquatic situations to control invasive weeds, runoff from agricultural or urban land or vapour drift (Walters 1999). 2,4-D is also highly volatile and

---

[3] 2,4,5-T is no longer registered for use in Australia. It is listed under the Rottedam Convention because of the risk to human health from the 2,3,7,8 TCDD (Dioxin) contaminant, which forms during production (DEH 2004; DAFF 2006).

can enter waterways at some distance from its point of application as a consequence of wet deposition (Walters 1999).

2,4-D is generally applied as a liquid or a granular product, and is absorbed through the roots (acid and salt forms) and leaves (ester forms) of plants (Walters 1999). It is then translocated through the phloem to meristematic regions of plants (where cell division and growth occurs) where it exerts its toxicity (ANZECC and ARMCANZ 2000; BCPC 2012). 2,4-D acts by mimicking the plant hormone, auxin (indolylacetic acid, or IAA), which is responsible for promoting stem elongation and maintaining apical dominance in dicotyledons (also known as dicots). Indolylacetic acid systemic mobility and selective action mostly in dicots, whereas monocots are more resistant (Grossman 2003). The molecular mechanism explaining why monocots are more resistant than dicots is still uncertain; however, studies suggest it could be due to factors such as limited translocation in monocots or that accessory pathways associated with auxin transport metabolise excess synthetic IAA in monocots, giving them resistance to 2,4-D (Kelley and Riechers 2007; Song 2013). Following administration, 2,4-D acidifies the cell walls of plants, which causes cells to elongate in an uncontrolled and disorganised manner, ultimately leading to plant death (Walters 1999). 2,4-D also affects the metabolism of plants by affecting enzyme activity, respiration and cell division (Walters 1999).

2,4-D is moderately persistent in soils, with a relatively low log K_{oc} value (Table 1) which suggests it to be highly mobile in water (University of Hertfordshire 2013). Loss of 2,4-D via volatilisation is minimal due to its solubility in water (Table 1) and adsorption capabilities. Depending on the soil type, 2,4-D has potential to leach through the soil column to groundwater however it has short half-lives in both, aquatic environments and in soil.

1.2 Marine

1.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for 2,4-D in marine waters (Table 3) includes toxicity data for two marine species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine species that passed the screening and quality assurance processes are provided below.

Marine Chronic

There were marine chronic toxicity data for three microalgae species. The toxicity values for microalgae were 5-day NOEL (biomass yield, growth rate, area under the curve) values ranging from 130 to 4,700 µg/L, 5-day EC50 (biomass yield, growth rate, area under the curve) values ranging from 130 to 30,000 µg/L and a 10-day EC50 (biomass yield, growth rate, area under the curve) values of 50,000 and 75,000 µg/L.

Marine Acute

There were marine acute toxicity data for two crustaceans and one fish and one mollusc species. The toxicity values for the crustaceans were two 96-hour NOEL (mortality) values of 140 and 187,000 µg/L and a 96-hour LC50 value of 467,000 µg/L. The toxicity values for the single fish species were 96-hour NOEL, LOEL and EC50 (mortality) values of 240, 111,000 and 175,000 µg/L,

respectively. The toxicity values for the single mollusc species were a 96-hour NOEL (mortality, abnormal development value) of 30 µg/L, two 96-hour LOEL (mortality, abnormal development) values of 160 and 135,000 µg/L and two 96-hour EC50 (mortality, abnormal development) values ranging from 58,700 and 146,000 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

1.2.2   Factors affecting toxicity

Factors such as temperature, pH and water hardness have potential to modify the toxicity of 2,4-D (ANZECC and ARMCANZ 2000). However, no relationships have been developed to permit the calculation of temperature, pH or hardness specific PGVs. 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 2,4-D. However, any such effect would be relatively minor given the relatively low log K_{oc} value of 2,4-D (Table 1).

2,4-D comes in three broad forms – the acid, salt and ester, where the ester forms are reportedly more toxic to fish and aquatic species than the salt and acid forms (as they have very low solubility), and thus the latter are registered for use against aquatic weeds.

1.2.3   Guideline derivation

The derived PGVs for 2,4-D in marine waters are provided in Table 2. Details of how the PGVs were calculated and the toxicity data that were used are provided below. The ecotoxicity data for 2,4-D is different to that of most pesticides, as it quite common for the test compound to have a low proportion of the active ingredient. The relatively large proportion of additives in such test compounds may have a different toxicity to the active ingredient. Therefore, as with all the other pesticides that have GVs, the PGVs for 2,4-D are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for 2,4-D are low (Table 1) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for 2,4-D do not need to account for secondary poisoning.

Table 2 Proposed aquatic ecosystem protection guideline values (µg/L) for 2,4-D for the protection of marine ecosystems.

2,4-D proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)[3]	Criterion	Result
99%	1,000 (170 – 7,000)	Sample size	6
95%	2,500 (560 – 9,300)	Type of toxicity data	Chronic NOEL, chronic estimated NOEC/EC10 and converted acute values
90%	3,800 (980 – 11,000)	SSD model fit	Poor
80%	5,800 (1,900 – 14,000)	Reliability	Low

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. **[2]** See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability". **[3]** Values rounded to two significant figures.

1.2.4   Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for 2,4-D in marine environments was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was adopted from the freshwater GV which was based on acute toxicity data for 35 freshwater heterotrophic species that belonged to five taxonomic groups as well as an assessment factor (AF) of 10.2 (Warne 2001). Under the new method for deriving GVs (Warne et al. 2015) this value would be classified as having an unknown reliability.

To obtain toxicity data for 2,4-D to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now more 2,4-D toxicity data available that enable the calculation of PGVs in marine waters (see section 1.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of 2,4-D with marine phototrophic (e.g. plants and algae) species be conducted.

In total, there were marine toxicity data for six species (five phyla and six classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophya, Chordata and Mollusca. The six classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae incertae sedis (a group of diatoms), Bivalvia (a class of molluscs), Chlorophyceae (a major grouping of freshwater green algae), Mediophyceae (another algae grouping) and Malacostraca (a large grouping of crustaceans).

Based on the current understanding of the mode of action of 2,4-D, it would be expected that phototrophic species, particularly dicots, would be more sensitive than non-phototrophic species, as it mimics the IAA auxin (more so in dicot species) which is a plant growth hormone that exists in vascular plants as well as algal species. Therefore, the 2,4-D ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two sample t test to see if the toxic responses among different taxa were uni- or multi-modal. The t test indicated that the two groups did not have significantly different ($p = 0.300$, see section 1.2.7) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for 2,4-D in marine waters.

There were marine chronic no observed effect level (NOEL) data for only one species and chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) for another two species, which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). When the dataset was expanded to combine the chronic NOEL, chronic estimated NOEC and converted acute (acute EC50 toxicity data that had been converted to estimates of chronic NOEC/EC10 by dividing by 10) values of marine phototrophic and heterotrophic species, there were six species belonging to five phyla and six classes, that met the minimum data requirements to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 2) combined with the poor fit of the distribution to these toxicity data (Figure 2) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for 2,4-D in marine environments is provided in Table 3.

Table 3 Summary of the single toxicity value for each species that was used to derive the proposed aquatic ecosystem protection guideline values for 2,4-D in marine waters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Macroinvertebrate	*Crassostrea virginica*	Mollusca	Bivalvia	SPAT (juvenile)	4	Converted acute	Mortality, abnormal development	9,257.5	USEPA (2015b)
Microalgae	*Dunaliella tertiolecta*	Chlorophyta	Chlorophyceae	Not stated	10	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	15,000	USEPA (2015b)
Fish	*Menidia beryllina*	Chordata	Actinopterygii	Not stated	4	Converted acute	Mortality	17,500	USEPA (2015b)
Macroinvertebrate	*Penaeus duorarum*	Arthropoda	Malacostraca	Not stated	4	Converted acute	Mortality	46,700	USEPA (2015b)
Microalgae	*Phaeodactylum tricornutum**	Bacillariophyta	Bacillariophyta incertae sedis	Not stated	10	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	10,000	USEPA (2015b)
Microalgae	*Skeletonema costatum**	Bacillariophyta	Mediophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[2]	1,807.8	USEPA (2015b)

[1] **Chronic NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively; Converted acute = acute LC50 values that were converted to chronic NOEC/EC10 values by dividing by 10 (Warne et al. 2015).** [2] **AUC = area under the growth curve. *Species that originated from/are distributed in Australia and/or New Zealand.**

1.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the six marine phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 2.

Figure 2 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect level (NOEL), chronic estimated no observed effect concentration (NOEC) and converted acute values of marine phototrophic and heterotrophic species to 2,4-D. Black dashed lines indicate the 95% confidence intervals.

1.2.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for 2,4-D in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Arthropoda	Malacostraca	Prawn (*Penaeus duorarum*)	Not stated	4	Acute	LC50 (Mortality)	Natural or artificial filtered seawater	20 ± 3	23 ± 1.0	Not stated	467,000	USEPA (2015b)
											467,000	*GEOMETRIC MEAN*
											46,700[&]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyta incertae sedis	Marine diatom (*Phaeodactylum tricornutum*)	Not stated	10	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Not stated	Not stated	25	Not stated	50,000	USEPA (2015b)
											50,000	*GEOMETRIC MEAN*
											10,000[@]	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Marine diatom (*Skeletonema costatum*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	Natural or artificial filtered seawater	30 ± 5	20 ± 2.0	7.5 ± 0.1	3,750	USEPA (2015b)
Bacillariophyta	Mediophyceae	Marine diatom (*Skeletonema costatum*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	Natural or artificial filtered seawater	30 ± 5	20 ± 2.0	7.5 ± 0.1	780	USEPA (2015b)
Bacillariophyta	Mediophyceae	Marine diatom (*Skeletonema costatum*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	Natural or artificial filtered seawater	30 ± 5	20 ± 2.0	7.5 ± 0.1	2,020	USEPA (2015b)
											1,808	*GEOMETRIC MEAN*
											1,807.8	***VALUE USED IN SSD***

Chlorophyta	Chlorophyceae	Microalgae (*Dunaliella tertiolecta*)	Not stated	10	Chronic	EC50 (Body length, dry eight)	Synthetic saltwater or filtered natural saltwater	30 ± 5	20 ± 2.0	7.5 ± 0.1	75,000	USEPA (2015b)
											75,000	*GEOMETRIC MEAN*
											15,000[@]	***VALUE USED IN SSD***
Chordata	Actinopterygii	Inland Silverside (*Menidia beryllina*)	Not stated	4	Acute	LC50 (Mortality)	Surface/Ground or reconstituted water	20 ± 5	22 ± 2.0	>7.5 and <8.5	175,000	USEPA (2015b)
											175,000	*GEOMETRIC MEAN*
											17,500[&]	***VALUE USED IN SSD***
Mollusca	Bivalvia	Eastern Oyster (*Crassostrea virginica*)	SPAT (juvenile)	4	Acute	EC50 (Mortality, abnormal development)	Unfiltered natural or artificial (with food) seawater	>12	20 ± 5.0	Not stated	58,700	USEPA (2015b)
Mollusca	Bivalvia	Eastern Oyster (*Crassostrea virginica*)	SPAT (juvenile)	4	Acute	EC50 (Mortality, abnormal development)	Unfiltered natural or artificial (with food) seawater	>12	20 ± 5.0	Not stated	146,000	USEPA (2015b)
											92,575.4	*GEOMETRIC MEAN*
											9,257.5[&]	***VALUE USED IN SSD***

[@]Values were chronic EC/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 5 (Warne et al. 2015) [&] Values were acute EC50 values that were converted to chronic NOEC/EC10 values by 10 (Warne et al. 2015).

1.2.7   Distribution of sensitivities for aquatic species

Statistical analysis of the 2,4-D ecotoxicity data for freshwater and marine species indicated that there was no difference in the sensitivities of the two groups. The non-parametric Mann-Whitney test was used because, although the transformed 2,4-D freshwater and marine concentration data successfully met tests for normality (Anderson-Darling; $p = 0.234$), they were found to have unequal variances (Fisher's F-Test; $p = 0.046$). Results from the Mann-Whitney test indicated that the two groups were not significantly different ($p = 0.196$); therefore, the freshwater and the marine 2,4-D ecotoxicity data can be pooled for further analysis.

The toxicity data for 2,4-D to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the 2,4-D ecotoxicity data may be uni-modal (Figure 3).
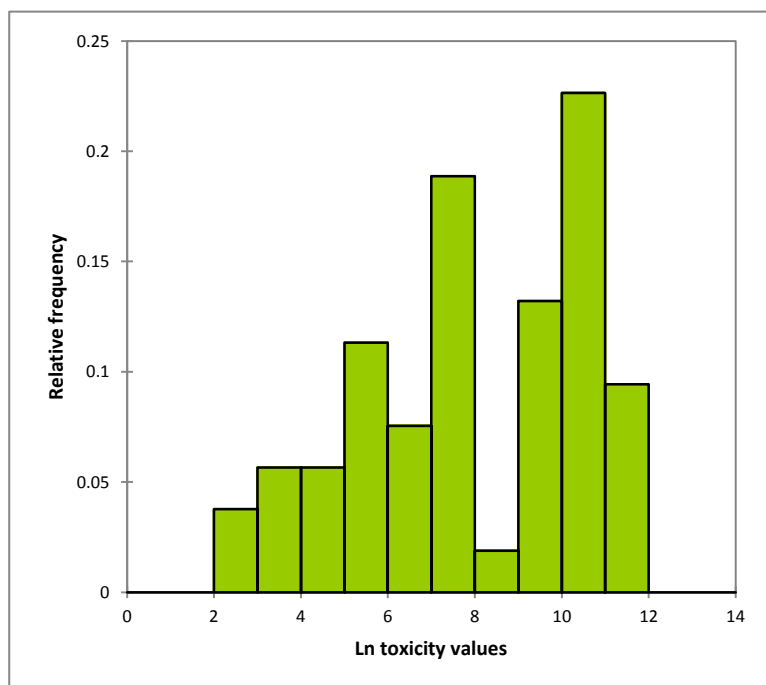
Figure 3 Histogram of the natural logarithm (ln) of all 2,4-D (freshwater and marine) toxicity data for phototrophic and non-phototrophic species ($n = 53$).

The 2,4-D ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the non-parametric Mann-Whitney test was used because the transformed 2,4-D concentration data had equal variances (Fisher's F-Test; $p = 0.087$) but did not follow a normal distribution (Anderson-Darling; $p = 0.013$). Results from the Mann-Whitney test indicated that the two groups were not significantly different ($p = 0.300$); therefore, it can be concluded that the distribution of the 2,4-D concentration data is uni-modal.

1.2.8 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A World Compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 1026–1028.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

DAFF (2006). Notice under section 69C of the agricultural and veterinary chemicals (administration) act 1992, Department of Agriculture, Fisheries and Forestry, Commonwealth of Australia, Gazette no. 4, 28pp. Available from: https://archive.apvma.gov.au/archive/gazette/2006/04/gazette_2006-04-04_page_28.pdf. Accessed: 11 August 2017.

DEH (2004). Natural dioxins program: Human health risk assessment of dioxins in Australia, Updated July, 2005. Prepared by the Office of Chemical Safety, Department of Environment and Heritage, Commonwealth of Australia, Technical Report no. 12, 224pp. Available from: https://www.environment.gov.au/system/files/pages/e66156a9-a7ac-4bc9-b256-5cf41daaaed1/files/report-12.pdf. Assessed: 11 August 2017.

DEPA (2015). The EU list of potential endocrine disruptors. Ministry of Environment and Food. Danish Environmental Protection Agency, København K, Denmark. Available from: http://eng.mst.dk/topics/chemicals/endocrine-disruptors/the-eu-list-of-potential-endocrine-disruptors/, Accessed: October–November, 2015.

Grossman K (2003). Mediation of herbicide effects by hormone interactions, *Journal of Plant Growth Regulation*, 22, 109–122.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Kelley KB and Riechers DE (2007). Recent developments in auxin biology and new opportunities for auxinic herbicide research, *Pesticide Biochemistry and Physiology*, 89, 1–11.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Song Y (2014). Insight into the mode of action of 2,4-Dichlorophenoxyacetic acid (2,4-D) as an herbicide, *Journal of Integrated Plant Biology*, 56(2), 106–113.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. United States Environmental Protection Agency, Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: May–September, 2015.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013.

Walters J (1999). Environmental fate of 2,4-dichlorophenoxyacetic acid. Department of Pesticide Regulations, Environmental Monitoring and Pest Management, Sacramento, California, CA 95814-3510. Available from: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/24-d.pdf , Accessed: November, 2015.

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

2 Ametryn

2.1 Introduction

Ametryn is a herbicide ($C_9H_{17}N_5S$ and Figure 4) that at room temperature is a white powder. It is the active ingredient of a variety of commercial herbicide formulations.
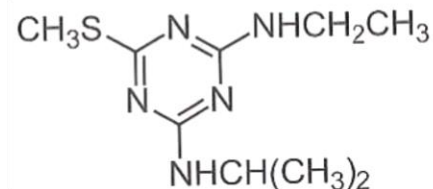
Figure 4 Structure of ametryn.

Physicochemical properties of ametryn that may affect its environmental fate and toxicity are presented in Table 4.

Table 4 Summary of selected physicochemical properties of ametryn.

Physicochemical property	Value
Molecular weight	227.3 amu[1]
Aqueous solubility	200 mg/L @ pH 7.1 and temperature 22 °C[1]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.63 @ pH 7 and temperature 20 °C[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.98 – 2.97[1], 2.5 [2]
Logarithm of the bioconcentration factor (log BCF)	1.52[2]
Half-life ($t_{1/2}$) in water	>1 week[3] Stable at normal aquatic pH values[4]
Half-life ($t_{1/2}$) in soil	11 – 280 days, median 62 days[1]

[1] **BCPC (2012).** [2] **Pesticide Properties Database (University of Hertfordshire 2013).** [3] **USEPA (1987).** [4] **USEPA (2013).**

Ametryn belongs to the methylthiotriazine group within the triazine family of herbicides, which also includes prometryn and terbutryn. Ametryn is extensively used in agriculture, forestry and grazing applications to control most annual grasses and broad-leaved weeds in a variety of crops such as pineapples, citrus, bananas, sugar cane, corn and potatoes (BCPC 2012, University of Hertfordshire 2013). However, it does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013). It is a selective, systemic herbicide (BCPC 2012) that exhibits moderate solubility in water (Table 4).

Ametryn is absorbed through the roots and leaves of plants. It is then translocated acropetally (i.e. movement upwards from the base of plants to the apex) in the xylem and accumulates in the apical meristems (BCPC 2012). Ametryn exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Triazine herbicides bind to the plastoquinone B (Q_B) protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (ATP, used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (NADPH, used in converting CO_2 to glucose), and therefore, prevents CO_2 fixation (Wilson et al. 2000).

In addition to its main mode of action, exposure to PSII inhibiting herbicides can lead to marked increases in the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen (OH⁻), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). Reactive oxygen species are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Reactive oxygen species are created during normal cellular functioning particularly in biochemical processes that involve the generation of energy (e.g. photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells). In phototrophs, ROS are formed when the absorbed light energy exceeds the ability to convert CO_2 to organic molecules, thus accumulating oxygen (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, prolonged exposure to elevated concentrations of ROS in plants, as a result of biotic (e.g. disease) and/or abiotic stressors (e.g. PSII inhibiting herbicides), can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

Information on the degradation of ametryn in water is limited. Loss from water is not expected as a consequence of hydrolysis due to the lack of appropriate functional groups, nor to volatilisation (Kim et al. 2016). Microbial degradation will contribute but binding to suspended solids and sediment is expected to be the major pathway for loss of ametryn from water (Kim et al. 2016). Ametryn has a low soil adsorption capacity and a moderate aqueous solubility (Table 4). Therefore, ametryn would be expected to have a high capacity to leach to groundwater and be transported in surface waters, although leaching studies indicate ametryn does not leach significantly (BCPC 2012). A USEPA report (USEPA 1987) of surface and groundwater samples in six states of the USA found ametryn in only three of over 1200 surface samples, but in approximately 4% of groundwater samples. A more recent USEPA report (USEPA 2013) concluded that because ametryn is highly persistent and relatively mobile, it may leach into aquatic systems after exaggerated rainfall, floods or from spray drift after application to control invasive weeds (USEPA 2013). Australian figures from 2011–15 show that ametryn has been detected in approximately 15.5% of surface water samples in catchments monitored as part of the Great Barrier Reef Catchment Loads Monitoring Program (based on data in Turner et al. 2013a, 2013b; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015).

In Australia, ametryn has been detected in estuarine and brackish waters in the Hervey Bay region (McMahon et al. 2005), coastal lagoons that are associated with seagrass beds and inshore coral reefs (Lewis et al. 2009), mangrove forests in the Mackay Whitsundays (Duke et al. 2005) and marine ecosystems including inshore Great Barrier Reef monitoring sites (Prange et al. 2009). Within some parts of Europe and South America (i.e. France and Brazil), detections of ametryn in marine ecosystems are still observable (Jacomini et al. 2011; Bocquene and France 2005) despite being banned in European Union member countries (EU Commission Regulation, 2010) in 2002.

2.2 Freshwater

2.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for ametryn in freshwaters (Table 6) includes toxicity data to five species (one freshwater and four marine) that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater and marine species that passed the screening and quality assurance processes are provided below and in section 2.3.1, respectively.

Freshwater Chronic

Typically, chronic toxicity values for microalgae and macrophytes to ametryn in freshwaters were lower than those for non-phototrophic species. Overall, species of microalgae had the lowest toxicity values consisting of 3- and 4-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 0.3 to 320 µg/L, 7-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 1.14 and 3.67 µg/L, respectively and two 10-day EC50 (biomass yield, growth rate, area under the growth curve) values of 10,000 µg/L. The toxicity values for the single macrophyte species were 7-day NOEL and EC50 (frond number, dry weight, frond area) values of 2 and 13 µg/L, respectively. The toxicity values for the single cladoceran species were markedly higher than the reported chronic toxicity values for phototrophic species, consisting of 21-day NOEL and LOEC (immobilisation) values of 240 and 320 µg/L, respectively. The least sensitive species, was the fish which had 35-day NOEL and LOEC (mortality) values of 700 and 1,400 µg/L.

Freshwater Acute

Similar to the chronic toxicity data for ametryn in freshwaters, the acute toxicity data indicated that phototrophic species – specifically macrophytes (no acute data for microalgal species was available), were the more sensitive organisms, followed by microinvertebrates and fish. The reported acute toxicity data of two macrophyte species consisted of two 3-day EC20 (growth rate) values of 12.27 and 27.5 µg/L, 4-day EC10 and EC50 (abundance) values of 1.09 and 6.74 µg/L, respectively and 6-day EC20 and EC50 values of 8.41 and 18.18 µg/L, respectively. The toxicity values for cladocerans consisted of 24- and 48-hour EC50 (immobilisation) values ranging from 28,000 to 73,000 µg/L, respectively and a 48-hour NOEL (immobilisation) value of 12,000 µg/L. The toxicity data for fish species consisted of 96-hour NOEL (mortality) values ranging from 700 to 9,000 µg/L, a 48-hour LOEL (mortality) value of 2,500 µg/L, a 48-hour LC50 value of 5,100 µg/L and 96-hour LC50 (mortality) values ranging from 3,200 to 16,000 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

2.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of ametryn. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of ametryn (Table 4).

2.2.3 Guideline derivation

The derived PGVs for ametryn in freshwaters are provided in Table 5. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for ametryn are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for ametryn are low (Table 4) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for ametryn do not need to account for secondary poisoning.

Table 5 Proposed aquatic ecosystem protection guideline values (μg/L) for ametryn for the protection of freshwater ecosystems.

Ametryn proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	0.074 (0.0021 − 1.1)	Sample size	17
95%	0.33 (0.040 − 1.8)	Type of toxicity data	Chronic EC10/NOEL and chronic estimated NOEC values (*freshwater and marine*)
90%	0.66 (0.14 − 2.5)	SSD model fit	Good
80%	1.4 (0.48 − 3.8)	Reliability	Moderate

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

2.2.4   Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for ametryn in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for ametryn to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more ametryn toxicity data available that enable the calculation of PGVs in freshwaters. However it was only possible to derive PGVs by using ecotoxicity data for a mixture of both freshwater and marine organisms (see section 2.2.6 and 2.3.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to freshwater ecosystems separately, it is recommended that additional chronic toxicity tests of ametryn with freshwater phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for ametryn in freshwaters, *Chloroccum* sp., *Neochloris* sp. and *Platymonas* sp. were included as no other toxicity data for these genera were used.

In total, there were freshwater toxicity data for 16 species (five phyla and six classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata and Tracheophyta. The six classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Liliopsida (monocots), and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of ametryn, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The ametryn ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p <0.0001, see section 2.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were freshwater chronic no observed effect level (NOEL) data for only two phototrophic species (that belonged to two phyla and two classes), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). When the dataset was expanded to include chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC/EC10 by dividing by 2.5 and 5, respectively) and converted acute (acute EC50/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 10) values, there were ten freshwater species belonging to three phyla and four classes, which was still insufficient data to use a SSD to derive a PGV. As no other ecotoxicity data for ametryn with freshwater phototrophic species were available, the chronic NOEL, chronic estimated NOEC and converted acute data for freshwater phototrophic species were combined with the available chronic 10% effect concentration (EC10) and chronic estimated NOEC data for marine phototrophic species to derive PGVs for ametryn in freshwaters. This dataset incorporated concentration data for 17 (eight freshwater and nine marine) phototrophic species belonging to five phyla and eight classes, which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 5) combined with the good fit of the distribution to these toxicity data (Figure 5) resulted in a moderate reliability set of PGVs. The combination of freshwater and marine ecotoxicity data reduces the reliability classification of PGVs as per Warne et al. (2015). A summary of the toxicity data (one value per species) used to calculate the PGVs for ametryn in freshwater environments is provided in Table 6.

Table 6 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for ametryn in freshwaters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Marine	Microalga	*Achnanthes brevipes**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	3.8	USEPA (2015b)
Fresh	Microalga	*Chlorella pyrenoidosa*[3*]	Chlorophyta	Trebouxiophyceae	Not stated	4	Chronic est. NOEC	Population (Abundance)	0.06	Ma et al. (2001); Ma et al. (2002).
Fresh	Microalga	*Chlorococcum* sp.	Chlorophyta	Chlorophyceae	Not stated	10	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	2,000	USEPA (2015b)
Marine	Microalga	*Dunaliella tertiolecta*	Chlorophyta	Chlorophyceae	Log growth phase	4	Chronic est. NOEC	Cell count	1.89	DeLorenzo et al. (2011)
Marine	Microalga	*Isochrysis galbana*	Haptophyta	Coccolithophyceae	Not stated	3	Chronic EC10	Population (Abundance)	1.31	Seery and Pradella (in prep.)
Fresh	Macrophyt	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	7	Chronic NOEL	Frond number, dry weight, frond area	2	USEPA (2015b)
Marine	Microalga	*Monochrysis lutheri*	Ochrophyta	Chrysophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	2.8	USEPA (2015b)
Marine	Microalga	*Navicula incerta*	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	19.4	USEPA (2015b)
Marine	Microalga	*Nitzschia closterium**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	12.4	USEPA (2015b)
Fresh	Microalga	*Neochloris* sp.	Chlorophyta	Chlorophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	7.2	USEPA (2015b)
Marine	Microalga	*Phaeodactylum tricornutum**	Bacillariophyta	Bacillariophyta incertae sedis	Not stated	10	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	6.32	USEPA (2015b)
Fresh	Microalga	*Platymonas* sp.	Chlorophyta	Chlorophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	4.8	USEPA (2015b)
Fresh	Microalga	*Scenedesmus quadricauda*	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Population (Abundance)	30	Ma et al. (2003)

Fresh	Microalga	*Selenastrum capricornutum*[4]	Chlorophyta	Chlorophyceae	Not stated	7	Chronic NOEL	Biomass yield, growth rate, AUC[2]	1.14	USEPA (2015b)
Fresh	Microalga	*Stauroneis amphoroides*	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	5.2	USEPA (2015b)
Marine	Microalga	*Thalassiosira fluviatilis**	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	11.6	USEPA (2015b)
Marine	Microalga	*Thalassiosira guillardii*	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	11	USEPA (2015b)

[1] Chronic NOEL/EC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] AUC = area under the growth curve. [3] This species has also been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*. [4] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. * Species that originated from/are distributed in Australia and/or New Zealand.

2.2.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 17 freshwater and marine phototrophic species that was used to derive the PGVs is presented in Figure 5.

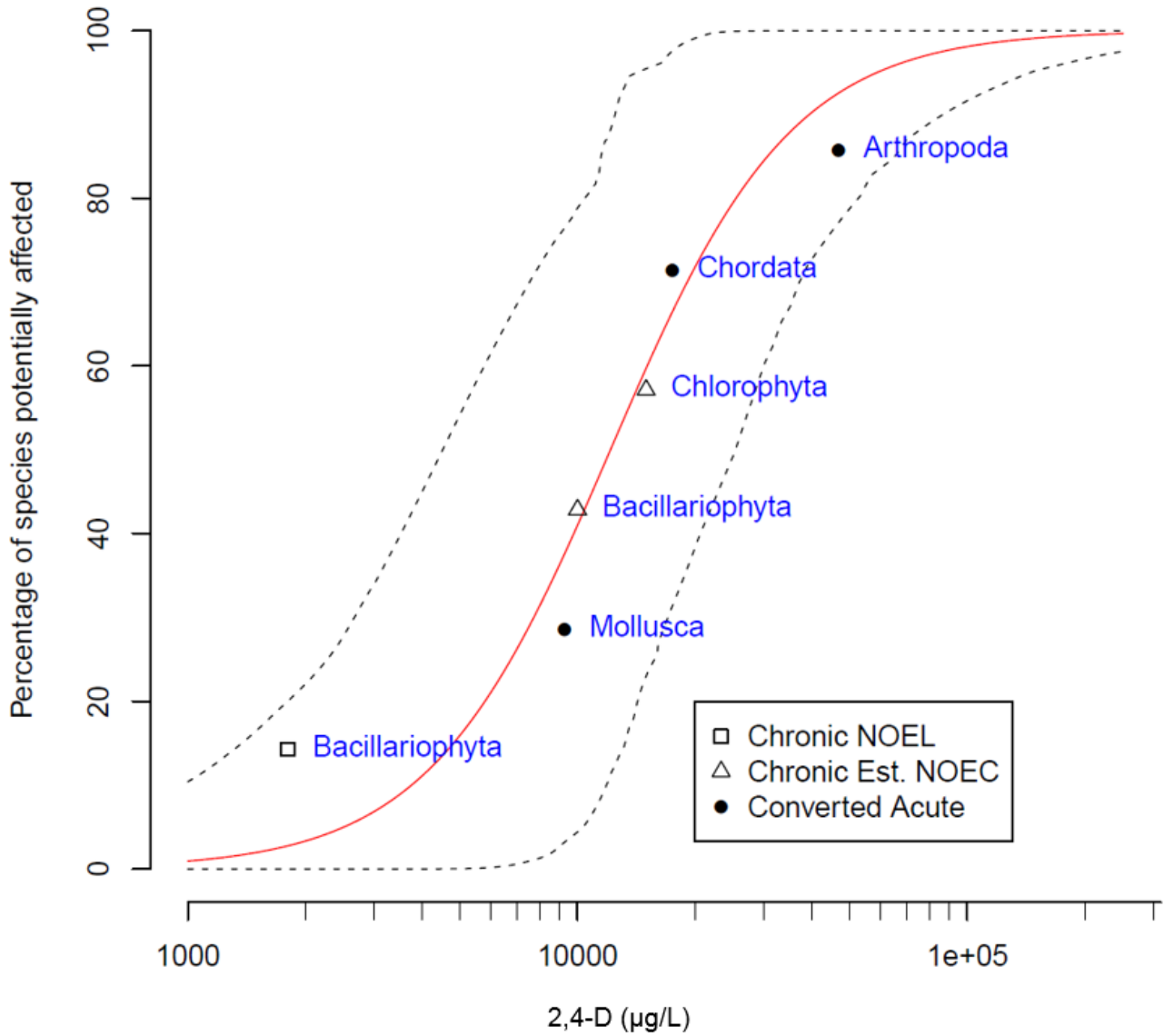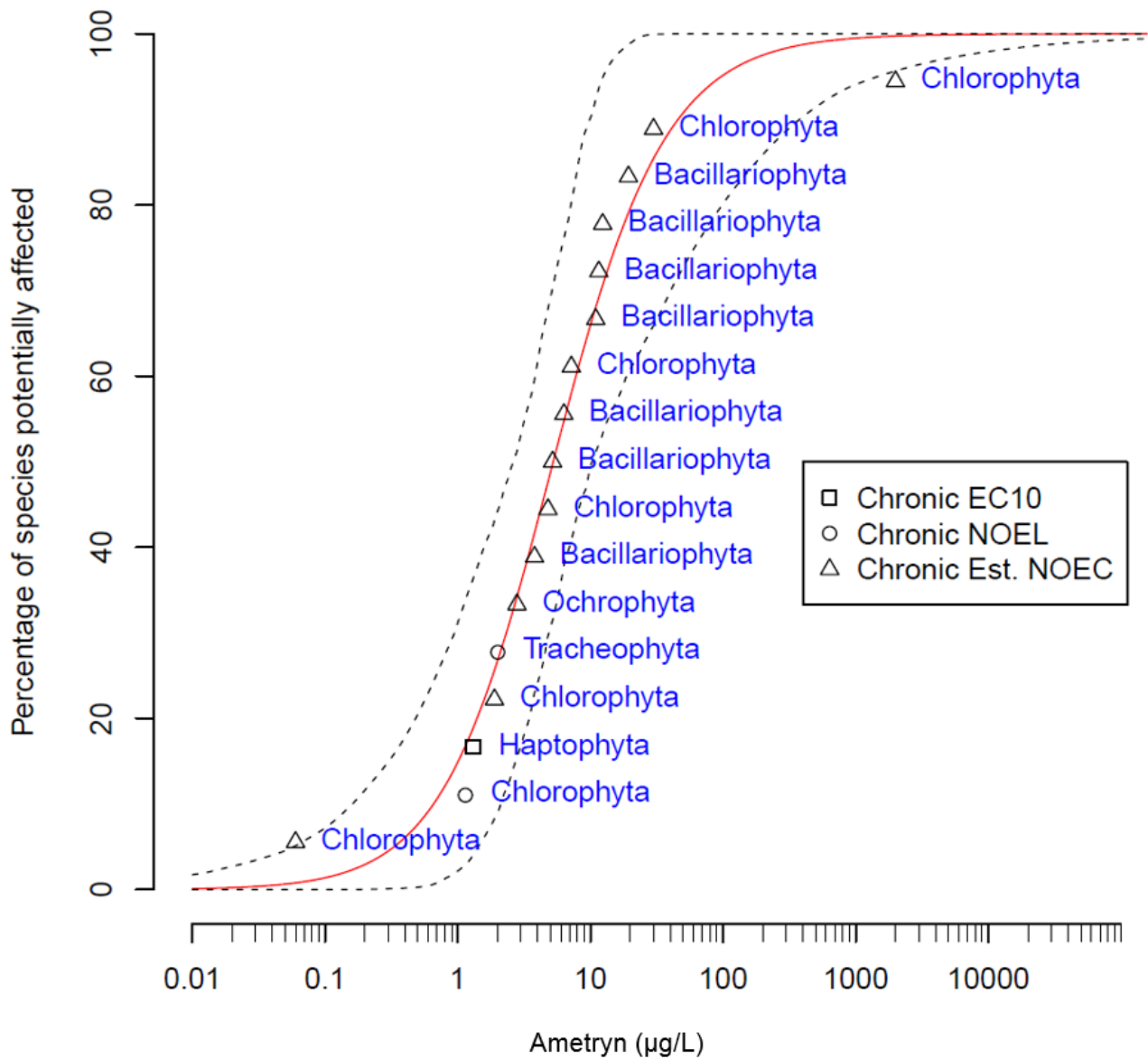

Figure 5 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated 10% effect concentration (EC10) and no observed level concentration (NOEL) data values of freshwater and marine phototrophic species to ametryn. Black dashed lines indicate the 95% confidence intervals.

2.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for ametryn in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Microalgae (*Stauroneis amphoroides*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	26	USEPA (2015b)
										26	*GEOMETRIC MEAN*
										5.2[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalgae (*Chlorococcum* sp.)	Not stated	10	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	2,000	USEPA (2015b)
										2,000	*GEOMETRIC MEAN*
										2,000[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalgae (*Neochloris* sp.)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	7.2	USEPA (2015b)
										7.2	*GEOMETRIC MEAN*
										7.2[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalgae (*Platymonas* sp.)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	4.8	USEPA (2015b)
										4.8	*GEOMETRIC*

										MEAN	
										4.8[@]	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae (*Selenastrum capricornutum[2]*)	Not stated	7	Chronic	NOEL (Biomass yield)	ASTM Type I water	24 ± 2	7.5 ± 0.1	1.14	USEPA (2015b)
										1.14	*GEOMETRIC MEAN*
										1.14	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae (*Selenastrum capricornutum[2]*)	Not stated	7	Chronic	EC50 (Biomass yield)	ASTM Type I water	24 ± 2	7.5 ± 0.1	3.67	USEPA (2015b)
										3.67	*GEOMETRIC MEAN*
Chlorophyta	Chlorophyceae	Microalgae (*Scenedesmus quadricauda*)	Not stated	4	Chronic	EC50 (Abundance)	HB-4 medium	Not stated	Not stated	150	Ma et al. (2003)
										150	*GEOMETRIC MEAN*
										30[@]	*VALUE USED IN SSD*
Chlorophyta	Trebouxiophyceae	Microalgae (*Chlorella pyrenoidosa[3]*)	Not stated	4	Chronic	EC50 (Abundance)	Liquid HB-4 medium	25	not stated	0.3	Ma et al. (2001)
Chlorophyta	Trebouxiophyceae	Microalgae (*Chlorella pyrenoidosa[3]*)	Not stated	4	Chronic	EC50 (Abundance)	Liquid HB-4 medium	25	not stated	0.3	Ma et al. (2002)
										0.3	*GEOMETRIC MEAN*
										0.06[@]	*VALUE USED IN SSD*
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	7	Chronic	NOEL (Frond	M-Hoagland's or 20X-AAP	25 ± 2	4.8-5.2 (M-Hoagland's)	2	USEPA (2015b)

					number, dry weight, frond area)	media. ASTM Type I water		and 7.5 ± 0.1 (20X-AAP)		
									2	*GEOMETRIC MEAN*
									2	***VALUE USED IN SSD***

[1] AUC = area under the growth curve. [2] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella capricornutum*. [3] This species has also been called *Chlorella vulgaris*. [@] Values were chronic LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).

2.3 Marine

2.3.1   Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for ametryn in marine waters (Table 8) includes toxicity data to four marine species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine species that passed the screening and quality assurance processes are provided below.

Marine Chronic

For the two types of organisms for which marine chronic toxicity data were available, microalgae was more sensitive to ametryn than crustaceans. The toxicity values for microalgae consisted of a 72-hour EC10 (abundance) value of 1.31 µg/L, 72-hour EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 14 to 97 µg/L, a 96-hour LOEC (cell count) value of 1.52 µg/L, two 96-hour EC50 (cell count) values of 1.4 and 3.2 µg/L and 10-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 2 to 10 µg/L. The toxicity values for crustaceans were 28-day NOEL and LOEC (mortality) values of 50 and 97 µg/L, respectively.

Marine Acute

The single toxicity value for the macroalga species was a 72-hour (biomass yield, growth rate, area under the growth curve) value of 36 µg/L. The single toxicity value for the cladoceran species was a 24-hour EC50 (immobilisation) value of 33,000 µg/L. The single toxicity value for the crustacean species was a 96-hour LC50 (mortality) value of 2,300 µg/L. The toxicity values for the single fish species were 96-hour NOEL and LC50 (mortality) values of 2,800 and 5,800 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

2.3.2   Factors affecting toxicity

No factors have been reported as modifying the toxicity of ametryn. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of ametryn (Table 4).

2.3.3   Guideline derivation

The derived PGVs for ametryn in marine waters are provided in Table 7. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have PVs, the PGVs for ametryn are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for ametryn are low (Table 4) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for ametryn do not need to account for secondary poisoning.

Table 7 Proposed aquatic ecosystem protection guideline values (µg/L) for ametryn for the protection of marine ecosystems.

Ametryn proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.10 (0.10 – 2.1)	Sample size	9
95%	0.61 (0.57 – 3.6)	Type of toxicity data	Chronic EC10 and chronic estimated NOEC values
90%	1.3 (1.1 – 4.7)	SSD model fit	Poor
80%	2.8 (1.5 – 6.3)	Reliability	Moderate

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

2.3.4   Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for ametryn in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for ametryn to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more ametryn toxicity data available that enable the calculation of PGVs in marine waters (see section 2.3.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of ametryn with phototrophic (e.g. plants and algae) marine species be conducted.

In total, there were marine toxicity data for 13 species (seven phyla and ten classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Haptophyta, Ochrophyta and Rhodophyta. The ten classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Bacillariophyta incertae sedis (a smaller grouping of green algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater/estuarine green algae), Chrysophyceae (a major grouping of freshwater/estuarine golden algae), Coccolithophyceae (a grouping of marine phytoplankton), Malacostraca (a larger grouping of crustaceans), Mediophyceae (another algae grouping) and Porphyridiophyceae (a class of red algae).

Based on the current understanding of the mode of action of ametryn, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The ametryn ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p <0.0001, see section 2.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were marine chronic 10% effect concentration (EC10) and chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC/EC10 by dividing by 2.5 and 5, respectively) values for nine phototrophic species (that belonged to four phyla

and six classes), which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 7) combined with the poor fit of the distribution to these toxicity data (Figure 6) resulted in a moderate reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for ametryn in marine environments is provided in Table 8.

Table 8 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for ametryn in marine waters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Microalga	*Achnanthes brevipes**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	3.8	USEPA (2015b)
Microalga	*Dunaliella tertiolecta*	Chlorophyta	Chlorophyceae	Log growth phase	4	Chronic est. NOEC	Cell count	1.89	DeLorenzo et al. (2011)
Microalga	*Isochrysis galbana*	Haptophyta	Coccolithophyceae	Not stated	3	Chronic EC10	Population (Abundance)	1.31	Seery and Pradella (in prep.)
Microalga	*Monochrysis lutheri*	Ochrophyta	Chrysophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	2.8	USEPA (2015b)
Microalga	*Navicula incerta*	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	19.4	USEPA (2015b)
Microalga	*Nitzschia closterium**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	12.4	USEPA (2015b)
Microalga	*Phaeodactylum tricornutum**	Bacillariophyta	Bacillariophyta incertae sedis	Not stated	10	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	6.32	USEPA (2015b)
Microalga	*Thalassiosira fluviatilis**	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	11.6	USEPA (2015b)
Microalga	*Thalassiosira guillardii*	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	11	USEPA (2015b)

[1] **Chronic NOEL/EC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] AUC = area under the growth curve.* Species that originated from/are distributed in Australia and/or New Zealand.**

2.3.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the nine marine phototrophic species that was used to derive the PGVs is presented in Figure 6.
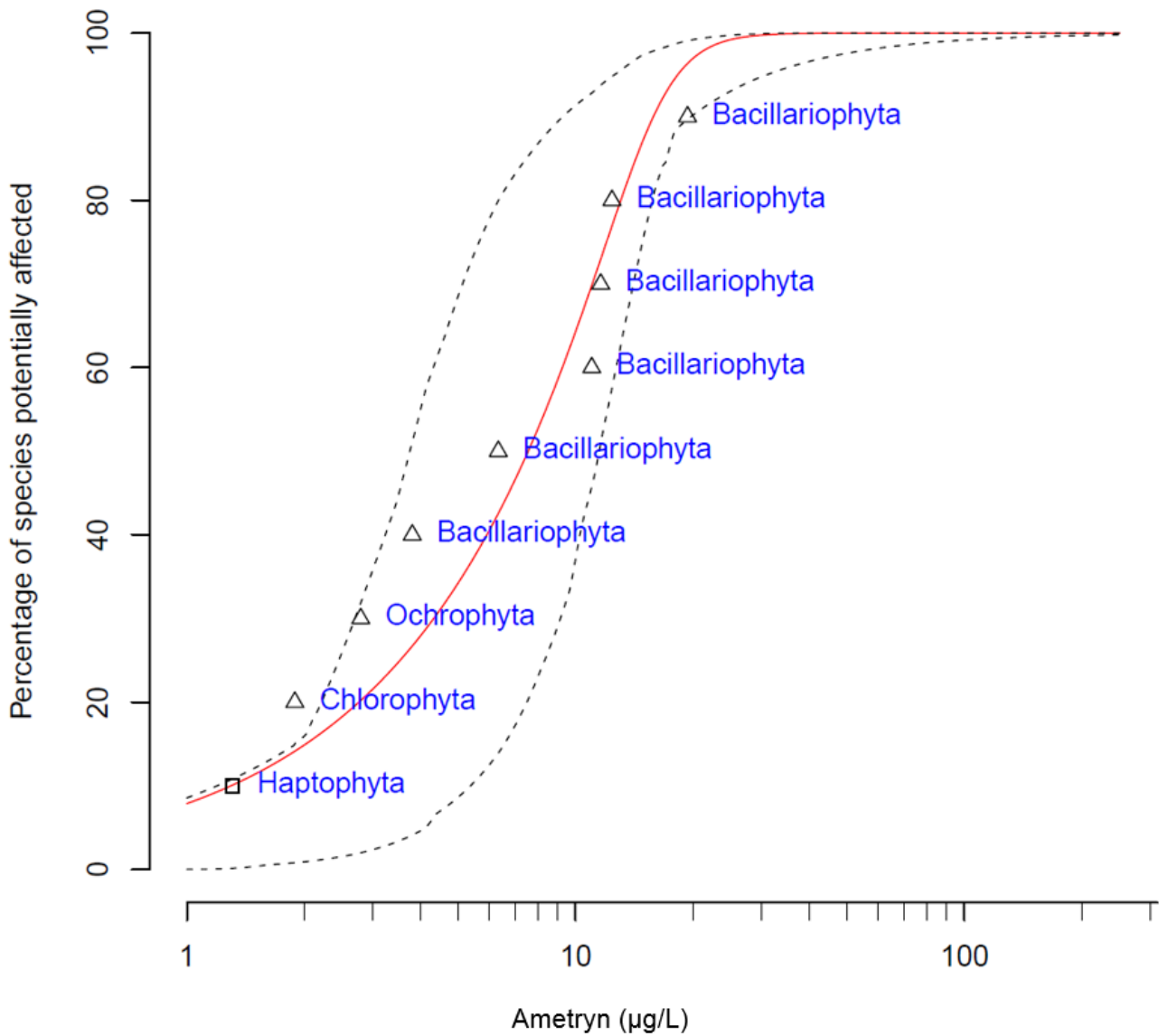
Figure 6 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic 10% effect concentration (EC10) and chronic estimated no observed effect concentration (NOEC) data values of marine phototrophic species to ametryn. Black dashed lines indicate the 95% confidence intervals.

2.3.6   Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for ametryn in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Microalgae (*Achnanthes brevipes*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	19	USEPA (2015b)
											19	*GEOMETRIC MEAN*
											3.8[@]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalgae (*Navicula incerta*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	97	USEPA (2015b)
											97	*GEOMETRIC MEAN*
											19.4[@]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalge (*Nitzschia closterium*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	62	USEPA (2015b)

											62	*GEOMETRIC MEAN*
											12.4[@]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyta incertae sedis	Microalgae (*Phaeodactylum tricornutum*)	Not stated	10	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	20	USEPA (2015b)
Bacillariophyta	Bacillariophyta incertae sedis	Microalgae (*Phaeodactylum tricornutum*)	Not stated	10	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	50	USEPA (2015b)
											31.6	*GEOMETRIC MEAN*
											6.32[@]	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalgae (*Thalassiosira fluviatilis*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	58	USEPA (2015b)
											58	*GEOMETRIC MEAN*
											11.6[@]	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalgae (*Thalassiosira*	Not stated	3	Chronic	EC50 (Biomass	ASTM Type I	30 ± 5	20 ± 2	8 ± 0.1	55	USEPA (2015b)

		guillardii)				yield, growth rate, AUC[1])	water with synthetic salt water or filtered natural salt water					
											55	*GEOMETRIC MEAN*
											11[@]	**VALUE USED IN SSD**
Chlorophyta	Chlorophyceae	Microalgae (*Dunaliella tertiolecta*)	Log growth phase	4	Chronic	LOEC (Cell count)	F/2 marine media	20	25	Not stated	3.8	DeLorenzo et al. (2011)
Chlorophyta	Chlorophyceae	Microalgae (*Dunaliella tertiolecta*)	Log growth phase	4	Chronic	EC50 (Cell count)	F/2 marine media	20	25	Not stated	7	DeLorenzo et al. (2011)
											5.16	*GEOMETRIC MEAN*
											1.89[@]	**VALUE USED IN SSD**
Haptophyta	Coccolithophyceae	Microalgae (*Isochrysis galbana*)	Not stated	3	Chronic	EC10 (Abundance)	Marine	31 ± 2	29 ± 1	8.2 ± 0.2	1.31	Seery and Pradella (in prep)
											1.31	*GEOMETRIC MEAN*
											1.31	**VALUE USED IN SSD**
Ochrophyta	Chrysophyceae	Microalgae (*Monochrysis lutheri*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water with synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8 ± 0.1	14	USEPA (2015b)
											14	*GEOMETRIC*

											MEAN
										2.8[@]	*VALUE USED IN SSD*

[1] **AUC = area under the growth curve.** [@] **Values were chronic LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).**

2.3.7 Distribution of sensitivities for aquatic species

Statistical analysis of the ametryn ecotoxicity data for freshwater and marine species indicated that there was no difference in the sensitivities of the two groups. The non-parametric Mann-Whitney test was used because, although the transformed ametryn freshwater and marine concentration data successfully met tests for normality (Anderson-Darling; p = 0.652), they were found to have unequal variances (Fisher's F-Test; p = 0.003). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.699); therefore, the freshwater and the marine ametryn ecotoxicity data can be pooled for further analysis.

The toxicity data for ametryn to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the ametryn ecotoxicity data may be bimodal (Figure 7).
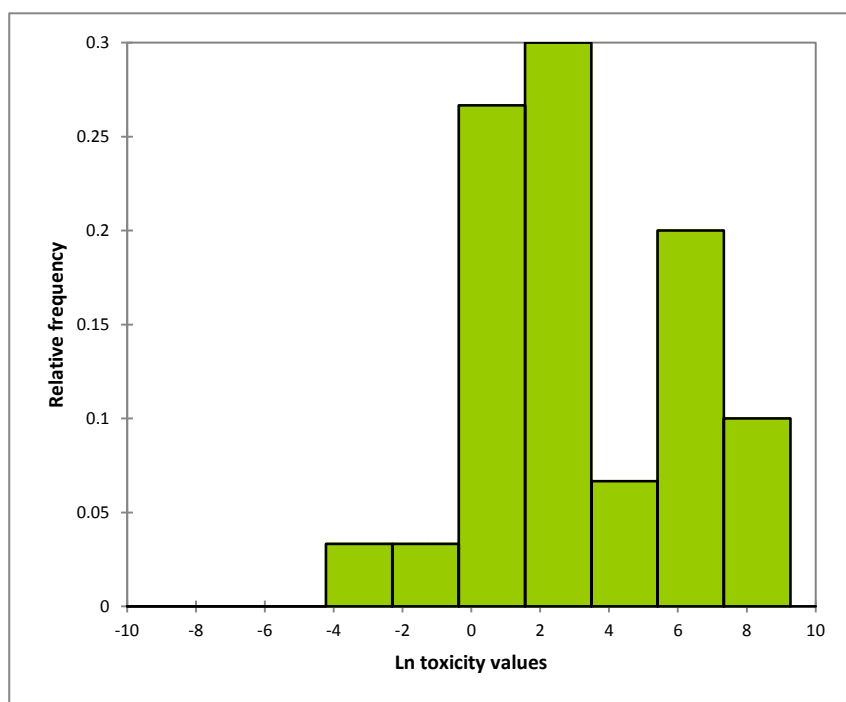
Figure 7 Histogram of the natural logarithm (ln) of all ametryn (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 30).

The ametryn ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample *t* test was used because the transformed ametryn concentration data had equal variances (Fisher's F-Test; p = 0.247) and followed a normal distribution (Anderson-Darling; p = 0.087). Results from the two-sample *t* test indicated that the two groups were significantly different (p <0.0001); therefore, it can be concluded that the distribution of the ametryn concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

2.3.8 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 1026–1028.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

Chen S, Yin C, Strasser RJ, Govinjee, Yang C and Qiang S (2012). Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*. Plant *Physiology and Biochemistry* 52, 38–51.

DeLorenzo ME, Danese LE and Baird TD (2011). Influence of increasing temperature and salinity on herbicide toxicity in estuarine phytoplankton, *Environmental Toxicology*, 28 (7), 359–371.

Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R, Thomson B and Warne MStJ (2015). *Total suspended solids, nutrients and pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef* – Great Barrier Reef Catchment Loads Monitoring Program 2013–2014. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 108pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2013-2014-gbr-catchment-loads-technical-report.pdf

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Kessler E and Huss V.A.R (1992). Comparative physiology and biochemistry and taxonomic assignment of the Chlorella (Chlorophyceae) strains of the culture collection of the University of Texas at Austin, *Journal of Phycology*, 28, 550–553.

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH (2016). PubChem Substance and Compound databases. Nucleic Acids Res. 2016 Jan 4; 44(D1):D1202-13. Epub 2015 Sep 22 [PubMed PMID: 26400175]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/13263. Downloaded: 13 May, 2016.

Ma J, Liang W, Xu L, Wang S, Wei Y and Lu J (2001). Acute toxicity of 33 herbicides to the green alga *Chlorella pyrenoidosa*, *Bulletin of Environmental Contamination and Toxicology*, 66 (4), 536–541.

Ma J, Lin F, Wang S and Xu L (2003). Toxicity of 21 Herbicides to the Green Alga *Scenedesmus quadricauda*, *Bulletin of Environmental Contamination and Toxicology*, 71 (3), 594–601.

Ma J, Xu L and Wang S (2002). A quick, simple, and accurate method of screening herbicide activity using green algae cell suspension cultures, *Weed Science*, 50 (5), 555–559.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Seery C and Pradella N (in prep). Toxicity of photosystem II inhibiting herbicides to four tropical alga.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Turner R, Huggins R, Wallace R, Smith R, Vardy S and Warne MStJ (2013a). Sediment, nutrient and pesticide loads: Great Barrier Reef Loads Monitoring 2009–2010. Water Sciences Technical Report, Volume 2012, Number 14. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0994. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2009-2010-gbr-catchment-loads-report.pdf.

Turner RDR, Huggins R, Wallace R, Smith RA, Vardy S and Warne MStJ (2013b). Total suspended solids, nutrient and pesticide loads for rivers that discharge to the Great Barrier Reef: Great Barrier Reef Loads Monitoring 2010–2011. Water Sciences Technical Report, Volume 2013, Number 1. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0996. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2010-2011-gbr-catchment-loads-report.pdf.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm. Accessed 13 May 2016.

USEPA (1987). Health Advisory Summary: Ametryn. United States Environmental Protection Agency, Washington, DC. Available from: http://nepis.epa.gov/Exe/ZyNET.exe/2000SP4C.txt?ZyActionD=ZyDocument&Client=EPA&Index=2011%20Thru%202015%7C1995%20Thru%201999%7C1981%20Thru%201985%7C2006%20Thru%202010%7C1991%20Thru%201994%7C1976%20Thru%201980%7C2000%20Thru%202005%7C1986%20Thru%201990%7CPrior%20to%201976%7CHardcopy%20Publications&Docs=&Query=ametryn&Time=&EndTime=&SearchMethod=2&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C86THRU90%5CTXT%5C00000014%5C2000SP4C.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=15&FuzzyDegree=0&ImageQuality=r85g16/r85g16/x150y150g16/i500&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x. Accessed: 13 May, 2016.

USEPA (2013). Problem formulation for the environmental fate, ecological risk, endangered species, and human health drink water exposure assessments in support of the registration review of ametryn. United States Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C. 7 March, 2013. Available from: https://www.regulations.gov/#!documentDetail;D=EPA-HQ-OPP-2013-0249-0002. Accessed: 17 May, 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/. Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/. Accessed: February–April, 2016.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and Warne MStJ. 2015. Total suspended solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-catchment-loads-report.pdf.

Wallace R, Huggins R, Smith RA, Turner RDR, Vardy S and Warne MStJ. 2014. Total suspended solids, nutrient and pesticide loads (2011–2012) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2011–2012. Department of Science, Information Technology, Innovation and the Arts. Brisbane, Queensland. 94pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2011-2012-gbr-catchment-loads-report.pdf.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology* 4, 93–144.

Wilson PC, Whitwell T and Klaine SJ (2000). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*, *Archives of Environmental Contamination and Toxicology* 39, 282–288.

3 Diuron

3.1 Introduction

Diuron is a herbicide ($C_9H_{10}Cl_2N_2O$ and Figure 8) that at room temperature is in the form of odourless, colourless crystals. It is the active ingredient of a variety of commercial herbicide formulations. Major metabolites of diuron are subsequentially demethylated diuron compounds, m-CPDMU, DCPMU and DCPU (APVMA 2011). The ecological effects of the minor metabolite 3,4-DCA are not well known.
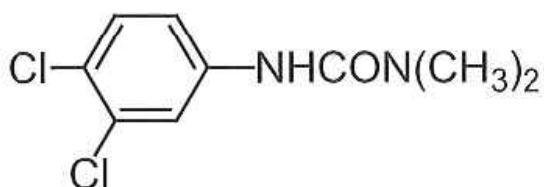
Figure 8 Structure of diuron.

Physicochemical properties of diuron that may affect its environmental fate and toxicity are presented in Table 9.

Table 9 Summary of selected physicochemical properties of diuron.

Physicochemical property	Value
Molecular weight	233.1 amu[1]
Aqueous solubility	37.4 mg/L @ temperature 25 °C[1] 35.6 mg/L @ temperature 20 °C[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.85 ± 0.03 @ temperature 25 °C[1] 2.87 @ pH 7 and temperature 20 °C[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.60[1], 2.91[2]
Logarithm of the bioconcentration factor (log BCF)	0.975[2]
Half-life ($t_{1/2}$) in water	175 days (lagoon prediction) with majority of diuron (90%) residing in sediment[3]
Half-life ($t_{1/2}$) in soil	90 – 180 days[1] 75.5 days[2]

[1] **BCPC (2012).** [2] **Pesticide Properties Database (University of Hertfordshire 2013).** [3] **Peterson and Batley (1991).**

Diuron belongs to the phenylurea group within the urea family of herbicides, which also includes linuron, fluometuron and isoproturon. Diuron is extensively used in agriculture and forestry applications for the control of broad-spectrum weeds as well as selective control of germinating grass and broad-leaved weeds in a variety of crops such as pineapples, bananas, asparagus, peas, cotton, sugarcane, wheat, barley oats, and ornamentals including tulips (BCPC 2012; University of Hertfordshire 2013). Diuron is also used to control weeds and algae in and around water bodies and is a component of marine antifouling paints (APVMA 2009). In Australia, diuron is one of the most heavily used herbicides, exceeded only by glyphosate, simazine and atrazine (AATSE 2002). It is a pre-emergence, residual herbicide as well as a post-emergence knockdown (University of Hertfordshire 2013) that exhibits some solubility in water (Table 9).

Diuron is absorbed principally through the roots of plants. It is then translocated acropetally (i.e. movement upwards from the base of plants to the apex) in the xylem and accumulates in the leaves (BCPC 2012). Diuron exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire

2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Photosynthesis inhibiting herbicides bind to the plastoquinone B (Q_B) protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (ATP, used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (NADPH, used in converting CO_2 to glucose), and therefore, prevents CO_2 fixation (Wilson et al. 2000).

In addition to its main mode of action, exposure to PSII inhibiting herbicides can lead to marked increases in the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen (OH^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). Reactive oxygen species are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Reactive oxygen species are created during normal cellular functioning particularly in biochemical processes that involve the generation of energy (e.g. photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells). In phototrophs, ROS are formed when the absorbed light energy exceeds the ability to convert CO_2 to organic molecules, thus accumulating oxygen (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, prolonged exposure to elevated concentrations of ROS in plants, as a result of biotic (e.g. disease) and/or abiotic stressors (e.g. PSII inhibiting herbicides), can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

Diuron ultimately ends up in aquatic environments as a result of surface and/or subsurface runoff from agricultural applications following heavy or persistent rain events, as well as from antifouling paints (biocides) applied to the hull of marine vessels (APVMA 2009). Loss of diuron via volatilisation is minimal due to its solubility in water (Table 9) and low soil adsorption characteristics as indicated by its low log K_{oc} value (Table 9) (Field et al. 2003). Diuron is relatively mobile and has been found to leach to groundwater and be transported in surface waters (Field et al. 2003; AVPMA 2011). A USEPA report (USEPA 1987) of surface and groundwater samples in six states of the USA did not detect diuron in any of eight surface water samples; however, it was detected in approximately 2.6% of groundwater samples in California and Georgia. Australian figures from 2011–15 show that diuron has been detected in approximately 66% of surface water samples in waterways that drain agricultural land and discharge to the Great Barrier Reef (based on data in Turner et al. 2013a, 2013b; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015).

In Australia, the APVMA suspended the registration of selected diuron products in late 2011 and enforced significant restrictions on the use of reaffirmed products. The main restriction prohibited the use of diuron during no-spray windows (from 5th December, 2011 to 31st March, 2012 onwards) for tropical crops including sugarcane, with restrictions being specific to the climatic and geographic conditions of each region. Other restrictions included the specification of maximum application rates for different times of the year. Diuron is currently registered for use in Australia and many other countries, however has been reviewed in the United States (draft 2003), Canada (2007), United Kingdom (2007) and Europe (2007 and 2008) (APVMA 2009). Current restraints on diuron use in Australia can be found at http://apvma.gov.au/node/12511.

3.2 Freshwater

3.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for diuron in freshwaters (Table 11) includes toxicity data to 16 freshwater species that either originated

from or are distributed within Australia and/or New Zealand. The lowest reported chronic toxicity value to freshwater species is for microalgae, *Fragilaria capucina var vaucheriae*, with a 96 hour EC05 of 0.069 µg/L. The lowest reported acute toxicity value to freshwater species is for macrophyte, *Lemna aequinoctialis*, with a 4 day EC10 of 2.79 µg/L.

3.2.2   Factors affecting toxicity

No factors have been reported as modifying the toxicity of diuron. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of diuron (Table 9).

3.2.3   Guideline derivation

The derived PGVs for diuron in freshwaters are provided in Table 10. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for diuron are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for diuron are low (Table 9) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for diuron do not need to account for secondary poisoning.

Table 10 Proposed aquatic ecosystem protection guideline values (µg/L) for diuron for the protection of freshwater ecosystems.

Diuron proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.080 (0.018 – 0.41)	Sample size	26
95%	0.23 (0.079 – 0.80)	Type of toxicity data	Chronic EC5/EC10/NOEC/NOEL and chronic estimated NOEC values
90%	0.42 (0.18 – 1.2)	SSD model fit	Good
80%	0.90 (0.43 – 2.3)	Reliability	Very High

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

3.2.4   Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for diuron in freshwater environments was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on a chronic toxicity value for a fish species (Warne 2001). This trigger value was calculated using the assessment factor (AF) method, dividing the lowest chronic toxicity value of 33.4 µg/L by an assessment factor of 200 (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this value would be classified as having an unknown reliability.

To obtain toxicity data for diuron to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al.

1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now more diuron toxicity data available that enable the calculation of PGVs in freshwaters (see section 3.2.6).

In total, there were toxicity data for 59 freshwater species (8 phyla and 14 classes) that passed the screening and quality assessment processes. The represented phyla were Annelida, Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria, Mollusca and Tracheophyta. The 14 classes were Actinopterygii (which accounts for approximately 99% of fish), Amphibia (tetrapod vertebrates), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Clitellata (a class of annelid worms), Cyanophyceae (a class of cyanobacteria), Fragilariophyceae (a grouping of pennate diatoms), Gastropoda (a grouping of molluscs), Insecta (invertebrates), Liliopsida (monocots), Malacostraca (a large grouping of crustaceans), Mediophyceae (another algae grouping) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of diuron, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The diuron ecotoxicity data for phototrophs and heterotrophs were then tested using the parametric two-sample t test to see if the toxic responses among different taxa were uni- or multi-modal. The t test indicated that the two groups had significantly different (p <0.0001, see section 3.3.8) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were freshwater chronic 5% effect concentration (EC5), 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data for 15 phototrophic species (that belonged to only three phyla and five classes), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). When the dataset was expanded to include chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively), there were data available for 26 phototrophic species (that belonged to four phyla and seven classes), which met the minimum data requirements to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 10) combined with the good fit of the distribution to these toxicity data (Figure 9) resulted in a very high reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for diuron in freshwater environments is provided in Table 11.

Table 11 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for diuron in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Microalgae	*Achnanthidium minutissimum**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	3.15	Larras et al. (2012)
Cyanobacteria	*Anabaena variabilis*	Cyanobacteria	Cyanophyceae	Not stated	12	Chronic est. NOEC	Chlorophyll-a	16	Singh et al. (2011)
Microalgae	*Chlorella pyrenoidosa[2]**	Chlorophyta	Trebouxiophyceae	Not stated	4	Chronic est. NOEC	Cell count	0.47	Ma et al. (2001); Ma et al. (2002)
Cyanobacteria	*Chroococcus minor**	Cyanobacteria	Cyanophyceae	<10 days	7	Chronic est. NOEC	Cell density	0.94	Bao et al. (2011)
Microalgae	*Craticula accomoda**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	261	Larras et al. (2012)
Microalgae	*Cyclotella meneghiniana**	Bacillariophyta	Mediophyceae	Exponential growth phase	4	Chronic EC5	Cell density	1.59	Larras et al. (2012)
Microalgae	*Cyclotella nana*	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	7.8	USEPA (2015b)
Microalgae	*Encyonema silesiacum**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	3.11	Larras et al. (2012)
Microalgae	*Eolimna minima**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	3007	Larras et al. (2012)
Microalgae	*Fragilaria capucina var vaucheriae**	Bacillariophyta	Fragilariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	0.069	Larras et al. (2012)
Microalgae	*Fragilaria rumpens**	Bacillariophyta	Fragilariophyceae	Exponential growth phase	4	Chronic EC10	Cell density	4.77	Larras et al. (2013)
Microalgae	*Fragilaria ulna[4]**	Bacillariophyta	Fragilariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	12.6	Larras et al. (2012)
Microalgae	*Gomphonema parvulum*	Bacillariophyta	Bacillariophyceae	Not stated	4	Chronic EC10	Chlorophyll-a	232	Larras et al. (2013)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	7	Chronic NOEL	Frond number, dry weight, frond area	2.49	USEPA (2015b)
Macrophyte	*Lemna minor**	Tracheophyta	Liliopsida	Not stated	7	Chronic est. NOEC	Total chlorophyll	3.16	Teisseire et al. (1999)
Macrophyte	*Lemna*	Tracheophyta	Liliopsida	Not stated	8	Chronic est.	Frond cover area	2.19	Grossmann

	paucicostata*					NOEC			et al. (1992)
Microalgae	*Mayamaea fossalis*	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC5	Cell density	74	Larras et al. (2012)
Microalgae	*Nitzschia palea**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	3	Chronic EC5	Cell density	106	Larras et al. (2012)
Microalgae	*Scenedesmus acutus**	Chlorophyta	Chlorophyceae	Not stated	8	Chronic est. NOEC	Cell count	2.66	Grossmann et al. (1992)
Microalgae	*Scenedesmus obliquus**	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Cell count	0.82	Ma (2002)
Microalgae	*Scenedesmus quadricauda*	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Cell count	0.54	Ma et al. (2003)
Microalga	*Scenedesmus subspicatus[5]**	Chlorophyta	Chlorophyceae	Not stated	3	Chronic NOEC	Cell count	10	Schafer et al. (1994)
Microalga	*Scenedesmus vacuolatus*	Chlorophyta	Chlorophyceae	Exponential growth phase	2	Chronic est. NOEC	Cell density	2.86	Copin and Chevre (2015)
Microalga	*Selenastrum capricornutum[6]*	Chlorophyta	Chlorophyceae	Not stated	4	Chronic NOEL	Biomass yield, growth rate, AUC[3]	0.44	USEPA (2015b)
Microalga	*Sellaphora minina*	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC10	Chlorophyll-a	1493.3	Larras et al. (2013)
Microalga	*Stauroneis amphoroides*	Bacillariophyta	Bacillariophyceae	Not stated	4	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	6.2	USEPA (2015b)

[1] Chronic NOEC/NOEL/EC5/EC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] This species has also been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*. [3] AUC = area under the growth curve. [4] This species has also been called *Ulnaria ulna*. [5] This species has also been called *Desmodesmus subspicatus*. [6] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.

3.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 26 freshwater phototrophic species that was used to derive the PGVs is presented in Figure 9.
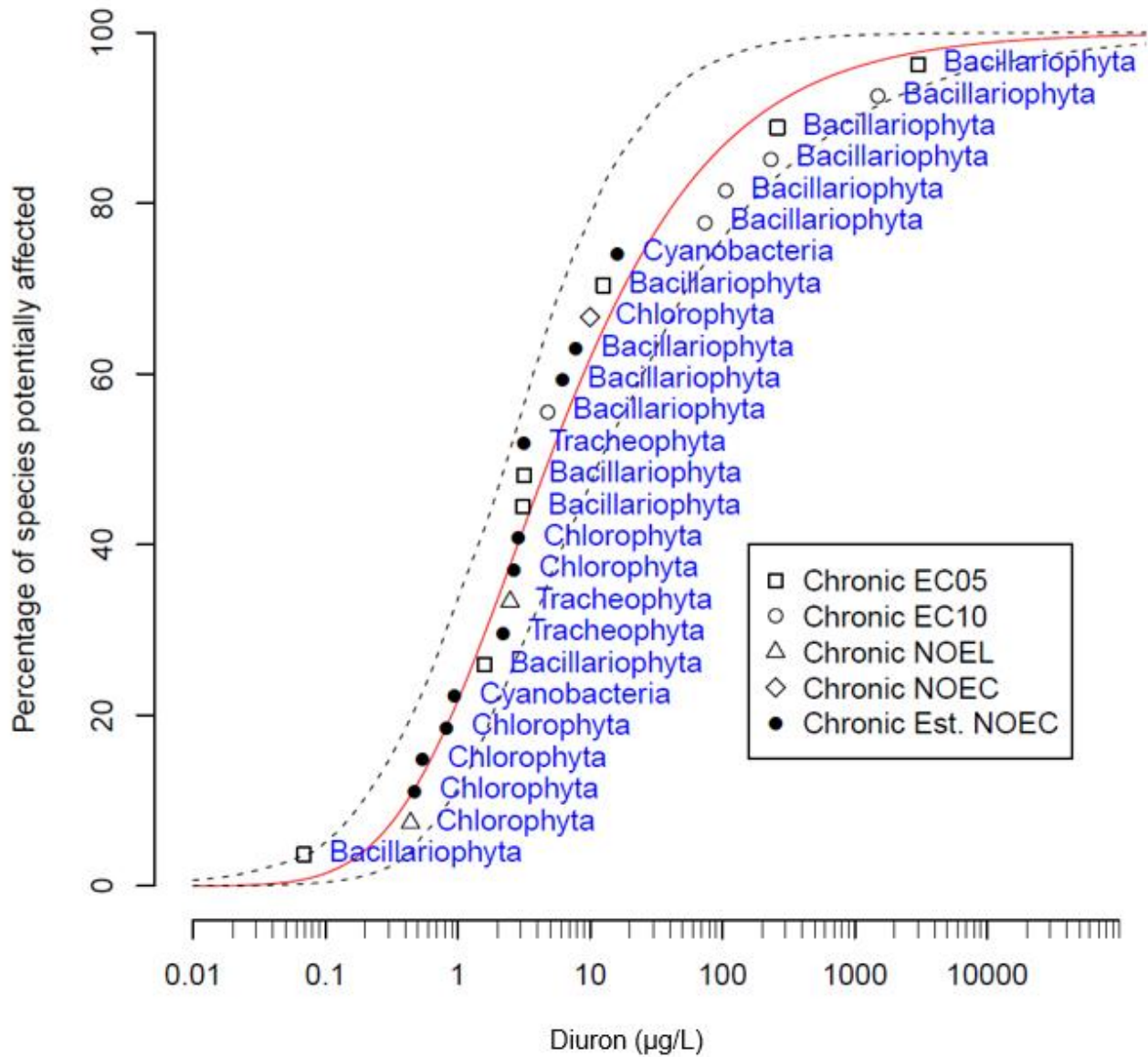
Figure 9 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic 5% effect concentration (EC5), 10% effect concentration (EC10), no observed effect concentration (NOEC), no observed effect level (NOEL) data and chronic estimated NOEC data values of freshwater phototrophic species to diuron. Black dashed lines indicate the 95% confidence intervals.

3.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for diuron in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Achnanthidium minutissimum)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	3.15	Larras et al. (2012)
										3.15	*GEOMETRIC MEAN*
										3.15	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Craticula accomoda)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	261	Larras et al. (2012)
										261	*GEOMETRIC MEAN*
										261	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalgae *(Cyclotella meneghiniana)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	1.59	Larras et al. (2012)
										1.59	*GEOMETRIC MEAN*
										1.59	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalgae *(Cyclotella nana)*	Not stated	3	Chronic	EC50 (Biomass yield, growth Rate, AUC[5])	ASTM Type 1 water	24 ± 2	7.5 ± 0.1	39	USEPA (2015b)
										39	*GEOMETRIC MEAN*
										7.8[@]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Encyonema*	Exponential growth	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	3.11	Larras et al. (2012)

		silesiacum)	phase								
										3.11	*GEOMETRIC MEAN*
										3.11	*VALUE USED IN SSD*
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Eolimna minima)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	3,007	Larras et al. (2012)
										3,007	*GEOMETRIC MEAN*
										3,007	*VALUE USED IN SSD*
Bacillariophyta	Fragilariophyc-eae	Microalgae *(Fragilaria capucina var vaucheriae)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	0.069	Larras et al. (2012)
										0.069	*GEOMETRIC MEAN*
										0.069	*VALUE USED IN SSD*
Bacillariophyta	Fragilariophyc-eae	Microalgae *(Fragilaria rumpens)*	Exponential growth phase	4	Chronic	EC10 (Growth rate/ chlorophyll *a* fluorescence)	DV culture medium	Not stated	Not stated	0.76	Larras et al. (2013)
Bacillariophyta	Fragilariophyc-eae	Microalgae *(Fragilaria rumpens)*	Exponential growth phase	4	Chronic	EC10 (Growth rate/ chlorophyll *a* fluorescence)	DV culture medium	Not stated	Not stated	30	Larras et al. (2013)
										4.77	*GEOMETRIC MEAN*
										4.77	*VALUE USED IN SSD*
Bacillariophyta	Fragilariophyc-eae	Microalgae *(Fragilaria ulna[1])*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	12.6	Larras et al. (2012)
										12.6	*GEOMETRIC MEAN*

										12.6	VALUE USED IN SSD
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Gomphonema parvulum)*	Exponential growth phase	4	Chronic	EC10 (Growth rate/ chlorophyll *a* fluorescence)	DV culture medium	Not stated	Not stated	53	Larras et al. (2013)
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Gomphonema parvulum)*	Exponential growth phase	4	Chronic	EC10 (Growth rate/ chlorophyll *a* fluorescence)	DV culture medium	Not stated	Not stated	1016	Larras et al. (2013)
										232.05	*GEOMETRIC MEAN*
										232.05	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Mayamaea fossalis)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	74	Larras et al. (2012)
										74	*GEOMETRIC MEAN*
										74	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Nitzschia palea)*	Exponential growth phase	4	Chronic	EC5 (Cell density)	DV culture medium	21 ± 2	Not stated	106	Larras et al. (2012)
										106	*GEOMETRIC MEAN*
										106	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Sellaphora minina)*	Exponential growth phase	4	Chronic	EC10 (Growth rate/ chlorophyll *a* fluorescence)	DV culture medium	Not stated	Not stated	693	Larras et al. (2013)
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Sellaphora minina)*	Exponential growth phase	4	Chronic	EC10 (Growth rate/ chlorophyll *a* fluorescence)	DV culture medium	Not stated	Not stated	3218	Larras et al. (2013)
										1493.34	*GEOMETRIC MEAN*

										1493.34	*VALUE USED IN SSD*
Bacillariophyta	Bacillariophyc-eae	Microalgae *(Stauroneis amphoroides)*	Not stated	3	Chronic	EC50 (Biomass yield, growth Rate, AUC[5])	ASTM Type 1 water	24 ± 2	7.5 ± 0.1	31	USEPA (2015b)
										31	*GEOMETRIC MEAN*
										6.2[@]	*VALUE USED IN SSD*
Chlorophyta	Trebouxiophyc-eae	Microalgae *(Chlorella pyrenoidosa[2])*	Not stated	4	Chronic	EC50 (Cell count)	HB-4 media	25	Not stated	2.3	Ma et al. (2002)
Chlorophyta	Trebouxiophyc-eae	Microalgae *(Chlorella pyrenoidosa[2])*	Not stated	4	Chronic	EC50 (Cell count)	HB-4 media	25	Not stated	1.3	Ma et al. (2001)
Chlorophyta	Trebouxiophyc-eae	Microalgae *(Chlorella vulgaris[2])*	Not stated	4	Chronic	EC50 (Cell count)	HB-4 media	25	Not stated	4.3	Ma et al. (2002)
										2.34	*GEOMETRIC MEAN*
										0.47[@]	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae *(Scenedesmus acutus)*	Not stated	8	Chronic	EC50 (Cell count)	Inorganic medium	23	Not stated	13.29	Grossmann et al. (1992)
										13.29	*GEOMETRIC MEAN*
										2.67[@]	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae *(Scenedesmus obliquus)*	Not stated	4	Chronic	EC50 (Cell count)	HB-4 media	25	Not stated	4.09	Ma (2002)
										4.09	*GEOMETRIC MEAN*
										0.82[@]	*VALUE USED IN*

											SSD
Chlorophyta	Chlorophyceae	Microalgae *(Scenedesmus quadricauda)*	Not stated	4	Chronic	EC50 (Cell count)	HB-4 media	Not stated	Not stated	2.7	Ma et al. (2003)
										2.7	*GEOMETRIC MEAN*
										0.54[@]	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae *(Scenedesmus vacuolatus)*	Exponential growth phase	2	Chronic	EC50 (Cell density)	Not stated	25	Not stated	14.3	Copin and Chevre (2015)
										14.3	*GEOMETRIC MEAN*
										2.86[@]	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae *(Scenedesmus subspicatus[3])*	Not stated	3	Chronic	NOEC (Cell count)	Inorganic medium containing sucrose	20 ± 2	Not stated	10	Schafer et al. (1994)
										10	*GEOMETRIC MEAN*
										10	*VALUE USED IN SSD*
Chlorophyta	Chlorophyceae	Microalgae *(Selenastrum capricornutum[4])*	Not stated	4	Chronic	NOEL (Biomass yield, Growth rate, AUC[5])	ASTM Type 1 water	24 ± 2	7.5 ± 0.1	0.44	USEPA (2015b)
										0.44	*GEOMETRIC MEAN*
										0.44	*VALUE USED IN SSD*
Cyanobacteria	Cyanophyceae	Cyanobacteria *(Anabaena variabilis)*	Not stated	12	Chronic	EC50 (Growth rate/ chlorophyll *a* fluorescence)	BG11 medium	25 ± 1	Not stated	80	Singh et al. (2011)
										80	*GEOMETRIC MEAN*

										16[@]	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Cyanobacteria *(Chroococcus minor)*	<10 days	7	Chronic	EC50 (Cell density)	MN medium without inoculants, 0.45 μm filtered	25 ± 1	8.1 - 8.4	4.7	Bao et al. (2011)
										4.7	*GEOMETRIC MEAN*
										0.94[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte *(Lemna gibba)*	Not stated	7	Chronic	NOEL (Frond number, dry weight, frond area)	M-Hoaglands or 20X-AAP nutrient media. ASTM type 1 water	25 ± 2	4.8 - 5.2 (M-Hoagl-ands) and 7.5 ± 0.1 20X-AAP).	2.49	USEPA (2015b)
										2.49	*GEOMETRIC MEAN*
										2.49	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte *(Lemna minor)*	Not stated	7	Chronic	LOEC (Total chlorophyll)	Mineral medium	25 ± 1	Not stated	5	Teisseire et al. (1999)
Tracheophyta	Liliopsida	Macrophyte *(Lemna minor)*	Not stated	7	Chronic	EC50 (Total chlorophyll)	Mineral medium	25 ± 1	Not stated	25	Teisseire et al. (1999)
										11.18	*GEOMETRIC MEAN*
										3.16[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte *(Lemna paucicostata)*	Not stated	8	Chronic	EC50 (Frond cover area)	Inorganic medium containing sucrose	25	Not stated	10.96	Grossmann et al. (1992)

										10.96	GEOMETRIC MEAN
										2.19[@]	VALUE USED IN SSD

[1] This species has also been called *Ulnaria ulna*. [2] This species has also been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*. [3] This species has also been called *Desmodesmus subspicatus*. [4] This species has been called *Raphidocelis subcapitata*, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*. [5] AUC = area under the growth curve. [@] Values were chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015).

3.3 Marine

3.3.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for diuron in marine waters (Table 13) includes toxicity data for nine marine species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine species that passed the screening and quality assurance processes are provided below.

Marine Chronic

There were marine chronic toxicity data for one coral, one crustacean, one fish, three macroalgae and 17 species of microalgae. The toxicity values for the corals consisted of 90-day NOEC (fecundity, size) values of 0.91 and 8.8 µg/L, respectively. The toxicity values for crustaceans were 28-day NOEL and LOEC (mortality) values of 270 and 560 µg/L, respectively. The toxicity value for the single fish species was a 38-day LOEC (mortality) value of 440 µg/L. The macroalgae toxicity values consisted of six 15-day EC10, LOEC and EC50 values ranging from 2.3 to 87.8 µg/L, a 7-day EC50 (length) value of 3.4 µg/L and 10-day NOEC and LOEC (biomass) values of 2.5 and 5 µg/L, respectively. The toxicity data for microalgae were 3-day EC10, NOEC, LOEC, EC50 and IC50 values for a variety of endpoints (biomass yield, growth rate, area under the curve; cell density; cell number and biomass) that ranged from 0.54 to 95 µg/L, 4-day LOEC and EC50 (cell density) values of 3.8 to 27 µg/L, respectively, and 10- and 14-day EC50 values that ranged from 10 to 76.9 µg/L.

Marine Acute

There were marine acute ecotoxicity data for three corals, seven crustaceans, one echinoid, four fish, four macroalgae, two molluscs and one polychaete. The six toxicity values for corals consisted of 24-hour LC10 and LC50 (mortality) values of 91 and 4,800 µg/L, respectively, 96-hour NOEC (fertilisation rate, survival) values both of 1,000 µg/L and 96-hour NOEC (survival) values of 100 and 1,000 µg/L (adult and larvae, respectively). The crustacean toxicity data consisted of 1-, 2- and 4-day EC/LC50 (mortality) values that ranged from 1,000 to 21,000 µg/L and a 4-day NOEL (mortality) value of 600 µg/L. The three toxicity values for echinoids were all for the same species and the 48-hour NOEC, LOEC and EC50 (fertilisation rate) values ranged from 500 to 5,090 µg/L. The 15 toxicity data for fish consisted of 36-hour to 6-day NOEC, NOEL, LOEC, LC10 and LC50 (mortality and hatching success) values ranging from 50 to 7,826 µg/L. The macroalgae toxicity data were 4-day NOEC and EC50 (biomass – fresh weight) values ranging from 1.3 to 20 µg/L, 2-day EC50 (germination) values of 4,650 and 6,290 µg/L, a 2-day EC50 (length) value of 6,750 µg/L and two 3-day NOEC (leaf length) values both of 87.8 µg/L. The mollusc toxicity data consisted of 24-hour LC10 and LC50 (mortality) values both of 1,000 µg/L, 48-hour LC10 and LC50 (mortality) values both of 1,000 µg/L, two 96-hour EC50 (mortality, abnormal development, growth) values of 1,800 and 4,800 µg/L and a 96-hour NOEL (mortality, abnormal development) value of 2,400 µg/L. The single toxicity value for a polychaete was a 48-hour LC50 (mortality) value of 16,000 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

3.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of diuron. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect

its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of diuron (Table 9).

3.3.3 Guideline derivation

The derived PGVs for diuron in marine waters are provided in Table 12. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for diuron are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for diuron are low (Table 9) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for diuron do not need to account for secondary poisoning.

Table 12 Proposed aquatic ecosystem protection guideline values (µg/L) for diuron for the protection of marine ecosystems.

Diuron proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.43 (0.12 – 0.82)	Sample size	20
95%	0.67 (0.45 – 1.2)	Type of toxicity data	Chronic NOEC/EC10 and chronic estimated NOEC values
90%	0.86 (0.61 – 1.4)	SSD model fit	Good
80%	1.2 (0.83 – 1.9)	Reliability	Very High

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

3.3.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for diuron in marine environments was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on one acute toxicity value for a marine mollusc species (Warne 2001). This trigger value was calculated using the assessment factor (AF) method, dividing the lowest acute toxicity value of 1,800 µg/L by an assessment factor of 1,000 (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this value would be classified as having an unknown reliability.

To obtain toxicity data for diuron to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more diuron toxicity data available enable the calculation of PGVs in marine waters (see section 3.3.6).

In total, there were toxicity data for 45 marine species (12 phyla and 20 classes) that passed the screening and quality assessment processes. The represented phyla were Annelida, Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cnidaria, Echinodermata, Haptophyta, Mollusca, Ochrophyta, Rhodophyta and Tracheophyta. The 20 classes were Actinopterygii (which accounts for approximately 99% of fish), Anthazoa (a class of cnidaria i.e. corals), Bacillariophyceae (diatoms; a major grouping of algae), Bacillariophyceae incertae sedis (a group of diatoms), Bivalvia (a class

of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of green algae), Chrysophyceae (a class of golden algae), Coccolithophyceae (a class of yellow algae), Echinodea (a class of echinoderms), Entognatha (a class of arthropods), Florideophyceae (a class or sub-class of red algae), Liliopsida (monocots), Malacostraca (a large grouping of crustaceans), Maxillopoda (a class of crustaceans), Mediophyceae (another algae grouping), Nephrophyceae (a class of green algae), Phaeophyceae (a class of brown algae), Polychaeta (a class of annelid worms) and Porphyridiophyceae (a class red algae).

Based on the current understanding of the mode of action of diuron, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The diuron ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p <0.0001, see section 3.3.8) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were marine chronic no observed effect concentration (NOEC) and 10% effect concentration (EC10) data available for seven phototrophic species (that belonged to five phyla and five classes), which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). However, the resulting protective concentration (PC) values were not recommended as the PGVs for diuron in marine waters due to the fit of the curve (refer to 3.3.7 for further explanation). Very high reliability PGVs were able to be derived by including chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) values in the derivation.

When the dataset was expanded to combine the chronic NOEC/EC10 and chronic estimated NOEC data values of marine phototrophic species, there were 20 species that belonged to six phyla and 11 classes, which met the minimum data requirements to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 12) combined with the good fit of the distribution to these toxicity data (Figure 12) resulted in a very high reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for diuron in marine environments is provided in Table 13.

Table 13 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for diuron in marine waters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Microalga	*Achnanthes brevipes**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	4.8	USEPA (2015b)
Microalga	*Amphora exigua*	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	6.2	USEPA (2015b)
Macroalga	*Ceramium tenuicorne*	Rhodophyta	Florideophyceae	Not stated	7	Chronic est. NOEC	Final length	0.68	Karlsson et al. (2006)
Microalga	*Chaetoceros gracilis*	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Cell number	7.2	Koutsaftis and Aoyama (2006)
Microalga	*Dunaliella tertiolecta*	Chlorophyta	Chlorophyceae	Log growth phase / Exponential growth phase	4	Chronic est. NOEC	Cell density	1.52	DeLorenzo et al. (2011); Gatidou and Thomaidis (2007)
Microalga	*Emiliania huxleyi*	Haptophyta	Coccolithophyceae	Exponential growth phase	3	Chronic NOEC	Mortality	0.54	Devilla et al. (2005)
Microalga	*Entomoneis punctulata**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic NOEC	Cell density	2	Stauber et al. (2008)
Microalga	*Isochrysis galbana*	Haptophyta	Coccolithophyceae	Not stated	3	Chronic EC10	Cell density	1.09	Seery et al. (in prep)
Microalga	*Monochrysis lutheri*	Ochrophyta	Chrysophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	3.6	USEPA (2015b)
Microalga	*Navicula forcipata*	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic est. NOEC	Cell density	5.4	Gatidou and Thomaidis (2007)
Microalga	*Navicula incerta*	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	18.6	USEPA (2015b)

					Chronic[1]				
Microalga	*Nephroselmis pyriformis**	Chlorophyta	Nephrophyceae	Not stated	3	Chronic EC10	Cell density	2.2	Magnusson et al. (2008)
Microalga	*Nitzschia closterium**	Bacillariophyta	Bacillariophyceae	Not stated	3	Chronic NOEC	Cell density	2	Stauber et al. (2008)
Microalga	*Phaeodactylum tricornutum**	Bacillariophyta	Bacillariophyta incertae sedis	Not stated	10	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	2	USEPA (2015b)
Microalga	*Porphyridium cruentum**	Rhodophyta	Porphyridiophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	4.8	USEPA (2015b)
Macroalga	*Saccharina japonica*	Ochrophyta	Phaeophyceae	Thalli	15	Chronic EC10	Fresh weight	2.3	Kumar et al. (2010)
Microalga	*Skeletonema costatum**	Bacillariophyta	Mediophyceae	<7 days old	4	Chronic est. NOEC	Cell density	1.18	Bao et al. (2011)
Microalga	*Thalassiosira fluviatilis**	Bacillariophyta	Mediophyceae	Not stated	3	Chronic est. NOEC	Biomass yield, growth rate, AUC[2]	19	USEPA (2015b)
Microalga	*Thalassiosira pseudonana**	Bacillariophyta	Mediophyceae	Not stated	4	Chronic est. NOEC	Cell density	0.86	Bao et al. (2011)
Macrophyte	*Zostera marina*	Tracheophyta	Liliopsida	Not stated	10	Chronic NOEC	Biomass (old and new growth)	2.5	Chesworth et al. (2004)

[1] Chronic NOEC/EC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] AUC = area under the growth curve.

3.3.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 20 marine phototrophic species that was used to derive the PGVs is presented in Figure 10.
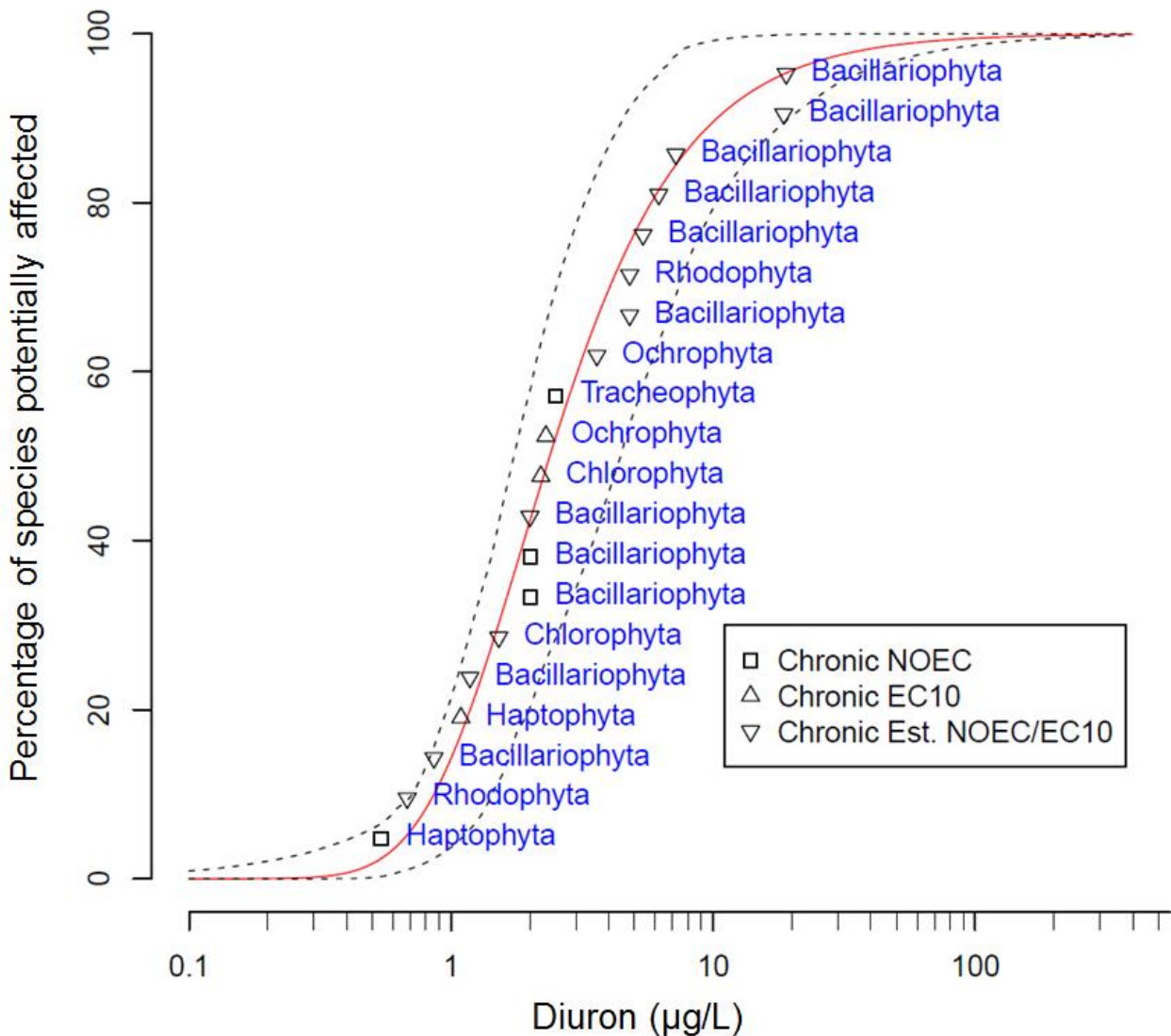
Figure 10 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic no observed effect concentration (NOEC), 10% effect concentration (EC10) and chronic estimated NOEC data values of marine phototrophic species to diuron. Black dashed lines indicate the 95% confidence intervals.

3.3.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for diuron in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Microalga (*Achnanthes brevipes*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	24	USEPA (2015b)
											24	*GEOMETRIC MEAN*
											4.8[@]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalga (*Amphora exigua*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	31	USEPA (2015b)
											31	*GEOMETRIC MEAN*
											6.2[@]	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalga (*Entomoneis punctulata*)	Not stated	3	Chronic	NOEC (Cell density)	Filtered seawater	30	21	8.1 – 8.4	2	Stauber et al. (2008)
											2	*GEOMETRIC MEAN*
											2	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalga (*Navicula forcipata*)	Exponential growth phase	4	Chronic	EC50 (Cell density)	F2 marine media	Not stated	20 ± 1	Not stated	27	Gatidou and Thomaidis (2007)
											27	*GEOMETRIC*

												MEAN
											5.4[@]	*VALUE USED IN SSD*
Bacillariophyta	Bacillariophyceae	Microalga (*Navicula incerta*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic salt water or filtered natural salt water	Not stated	20 ± 2	8.0 ± 0.1	93	USEPA (2015b)
											93	*GEOMETRIC MEAN*
											18.6[@]	*VALUE USED IN SSD*
Bacillariophyta	Bacillariophyceae	Microalga (*Nitzschia closterium*)	Not stated	3	Chronic	NOEC (Cell density)	Filtered seawater	30	21	8.1 – 8.4	2	Stauber et al. (2008)
											2	*GEOMETRIC MEAN*
											2	*VALUE USED IN SSD*
Bacillariophyta	Bacillariophyta incertae sedis	Microalga (*Phaeodactylum tricornutum*)	Not stated	10	Chronic	EC50 (Biomass yield, growth rate, AUC)	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	10	USEPA (2015b)
											10	*GEOMETRIC MEAN*
											2[@]	*VALUE USED IN SSD*
Bacillariophyta	Mediophyceae	Microalga (*Chaetoceros gracilis*)	Not stated	3	Chronic	IC50 (Cell number)	Provasoli medium	Not stated	25	Not stated	36	Koutsaftis and Aoyama (2006)
											36	*GEOMETRIC MEAN*
											7.2[@]	*VALUE USED IN SSD*

Bacillariophyta	Mediophyceae	Microalga (*Skeletonema costatum*)	<7 days old	4	Chronic	EC50 (Cell density)	Marine water	33 ± 0.5	25 ± 1	8.1 – 8.4	5.9	Bao et al. (2011)
											5.9	*GEOMETRIC MEAN*
											1.18[@]	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalga (*Thalassiosira fluviatilis*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic natural salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	95	USEPA (2015b)
											95	*GEOMETRIC MEAN*
											19[@]	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalga (*Thalassiosira pseudonana*)	Not stated	4	Chronic	EC50 (Cell density)	Marine water	33 ± 0.5	25 ± 1	8.1 – 8.4	4.3	Bao et al. (2011)
											4.3	*GEOMETRIC MEAN*
											0.86[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Dunaliella tertiolecta*)	Log growth phase	4	Chronic	EC50 (Cell density)	F2 marine media	20	25	Not stated	9.8	DeLorenzo et al. (2011)
Chlorophyta	Chlorophyceae	Microalga (*Dunaliella tertiolecta*)	Exponential growth phase	4	Chronic	EC50 (Cell density)	F2 marine media	Not stated	20 ± 1	Not stated	5.9	Gatidou and Thomaidis (2007)
											7.60	*GEOMETRIC MEAN*
											1.52[@]	***VALUE USED IN SSD***
Chlorophyta	Nephrophyceae	Microalga (*Nephroselmis pyriformis*)	Not stated	3	Chronic	EC10 (Cell density)	Filtered seawater	Not stated	24	Not stated	2.2	Magnusson et al. (2008)

											2.2	*GEOMETRIC MEAN*
											2.2	**VALUE USED IN SSD**
Haptophyta	Coccolithophyceae	Microalga (*Emiliania huxleyi*)	Exponential growth phase	3	Chronic	NOEC (Cell number)	Seawater	33	17	8.3 - 8.4	0.54	Devilla et al. (2005)
											0.54	*GEOMETRIC MEAN*
											0.54	***VALUE USED IN SSD***
Haptophyta	Coccolithophyceae	Microalga (*Isochrysis galbana*)	Not stated	3	Chronic	EC10 (Cell density)	0.45 mm filtered seawater, autoclaved and f/2 Guillard's Marine	31 ± 2	29 ± 1	8.2 ± 0.2	1.09	Seery et al. (in prep)
											1.09	*GEOMETRIC MEAN*
											1.09	***VALUE USED IN SSD***
Ochrophyta	Chrysophyceae	Microalga (*Monochrysis lutheri*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	18	USEPA (2015b)
											18	*GEOMETRIC MEAN*
											3.6[@]	***VALUE USED IN SSD***
Ochrophyta	Phaeophyceae	Macroalga (*Saccharina japonica*)	Thalli	15	Chronic	EC10 (Fresh weight)	Artificial seawater	Not stated	Not stated	8.4	2.3	Kumar et al. (2010)
											2.3	*GEOMETRIC MEAN*
											2.3	***VALUE USED***

												IN SSD
Rhodophyta	Florideophyceae	Macroalga (*Ceramium tenuicorne*)	Not stated	7	Chronic	EC50 (Final length)	Artificial seawater	5	22 ± 2	Not stated	3.4	Karlsson et al. (2006)
											3.4	*GEOMETRIC MEAN*
											0.68[@]	***VALUE USED IN SSD***
Rhodophyta	Porphyridiophyceae	Microalga (*Porphyridium cruentum*)	Not stated	3	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	24	USEPA (2015b)
											24	*GEOMETRIC MEAN*
											4.8[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophytye (*Zostera marina*)	Not stated	10	Chronic	NOEC (Biomass - old and new growth)	Seawater	Not stated	Not stated	Not stated	2.5	Chesworth et al. (2004)
											2.5	*GEOMETRIC MEAN*
											2.5	***VALUE USED IN SSD***

[1] AUC = area under the growth curve. [@] Values were chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015).

3.3.7 Rationale for the selected method for deriving the proposed aquatic ecosystem protection guideline values for diuron in marine waters

The preference of ecotoxicity data used to derive the protective concentration (PC)[4] values and/or PGVs for diuron to marine species is:

1. chronic NOEC/EC10 ecotoxicity data for phototrophs and heterotrophs;
2. chronic NOEC/EC10 and chronic estimated NOEC values for phototrophs and heterotrophs.

In total, there were chronic NOEC/EC10 data for seven phototrophic marine species (five phyla and five classes) that passed the screening and quality assessment processes. The represented phyla were Bacillariophyta, Chlorophyta, Haptophyta, Ochrophyta and Tracheoyphyta. The represented classes were Bacillariophyceae (a major grouping of diatoms), Coccolithophyceae (a grouping of marine phytoplankton), Liliopsida (monocots), Nephrophyceae (an algae grouping) and Phaeophyceae (a brown marine algae grouping). These data met the minimum data requirements of the SSD method (Warne et al. 2015). The resulting SSD and PC values using only this data are presented in Figure 11 and Table 14, respectively.
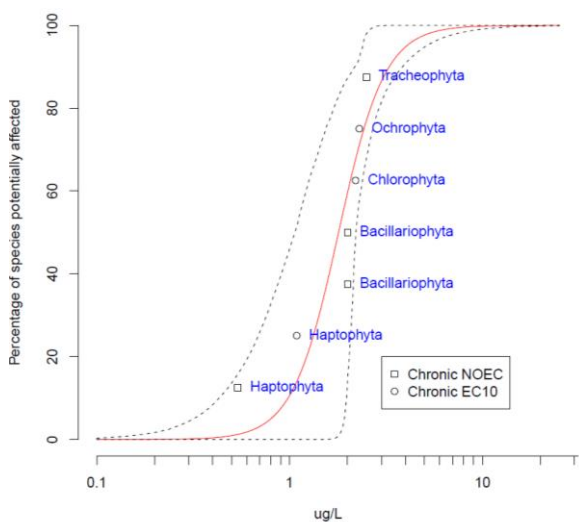
Figure 11 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of the chronic no observed effect concentration (NOEC) and 10% effect concentration (EC10) data values of marine phototrophic species to diuron.

---

[4] The values generated from a SSD are termed protective concentration (PC) values (as they are the concentrations that provide specific levels of protection e.g. PC99, PC95, PC90 and PC80 aim to protect 99, 95, 90 and 80 percent of species, respectively). Those PC values considered the most appropriate to use for ecosystem protection are adopted as the proposed PGVs.

Table 14 Protective concentration values (μg/L) of diuron for the protection of marine ecosystems generated from the species sensitivity distribution in Figure 11.

Diuron protective concentration values (marine)[1]		Reliability classification[2]	
Percent species protection	Concentration (μg/L)	Criterion	Result
99%	0.51	Sample size	7
95%	0.8	Type of toxicity data	Chronic NOEC/EC10 data
90%	0.98	SSD model fit	Poor
80%	1.2	Reliability	Low

[1] **Protective concentration values were derived using the Burrlioz 2.0 (2016) software.**

[2] **See Warne et al. (2015) for definitions of protective concentration value "reliability".**

The resulting PC values were considered to be of *low reliability* (Table 14) according to the methods of Warne et al. (2015) because the dataset consisted of chronic NOEC/EC10 values for seven phototrophic species and the cumulative distribution had a poor fit to the data (Figure 11). However, due to the fit and shape of the distribution model with the data (and the associated confidence intervals), there was some level of uncertainty in the estimation of the PC99 and PC95 values.

In response, the ecotoxicity dataset was expanded to also include the chronic estimated NOEC data (estimated from chronic LOEC and EC/LC50 data[5]), resulting in a total of 20 phototrophic species from six phyla (Table 12). Expanding the dataset markedly improved the fit of the distribution model to the ecotoxicity data (Figure 10), which subsequently improved the reliability classification of the SSD model fit to *good* and calculated very high reliability PC values (Table 12), according to Warne et al. (2015) (see section 3.3.4). Statistical methods, including the SSD methods, become more accurate and reliable as the amount of data available to analyse increases. All these factors combined led to the recommendation that the PC values derived using both chronic and chronic estimated ecotoxicity data (Table 12) be adopted as the PGVs for diuron in marine waters.

---

[5] chronic LOEC and EC/LC50 data were converted to chronic estimated NOEC data using the methods stated in Warne et al. (2015)

3.3.8 Distribution of sensitivities for aquatic species

Statistical analysis of the diuron ecotoxicity data for freshwater and marine species indicated that there was no difference in the sensitivities of the two groups. The non-parametric Mann-Whitney test was used because the transformed diuron freshwater and marine concentration data failed tests for normality (Anderson-Darling; p = 0.000) and had unequal variances (Fisher's F-Test; p = 0.030). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.565); therefore, the freshwater and the marine diuron ecotoxicity data can be pooled for further analysis.

The toxicity data for diuron to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the diuron ecotoxicity data may be bimodal (Figure **12**).
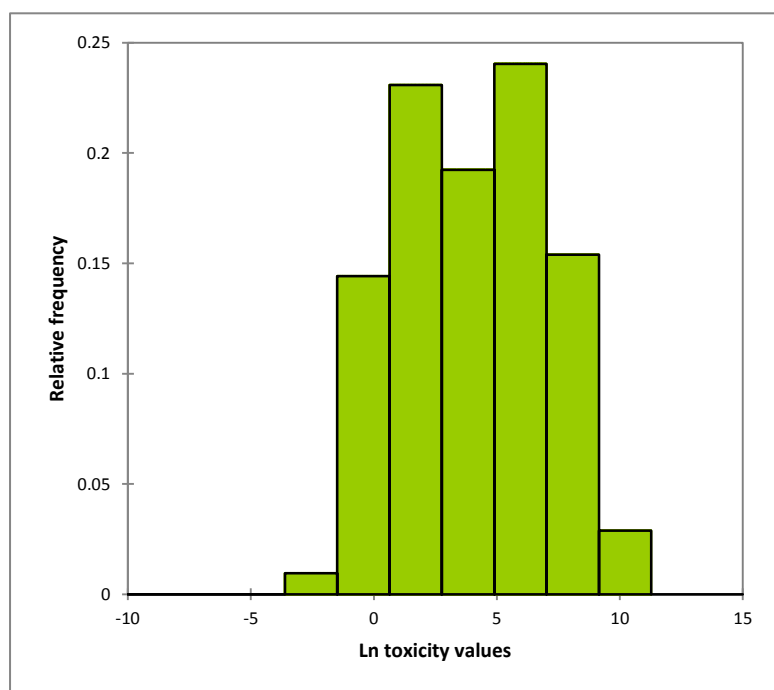
Figure 12 Histogram of the natural logarithm (ln) of all diuron (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 103).

The diuron ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the non-parametric Mann-Whitney test was used because the transformed diuron toxicity data had equal variances (Fisher's F-Test; p = 0.148) but did not follow a normal distribution (Anderson-Darling; p = 0.001). Results from the Mann-Whitney test indicated that the two groups were significantly different (p <0.0001); therefore, it can be concluded that the distribution of the diuron concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

3.3.9 References

AATSE (2002). *Pesticide use in Australia.* A review undertaken by the Australian Academy of Technological Sciences and Engineering. Australian Academy of Technological Sciences and Engineering, Parkville, Victoria, Australia, 309pp.

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

APVMA (2009). *Diuron Chemical Review,* Australian Pesticide and Veterinary Medicine Authority. Last updated 13 October, 2015. Available from: http://apvma.gov.au/node/12511. Accessed: 27 June 2016.

APVMA (2011). *Diuron Environmental Assessment report,* Australian Pesticide and Veterinary Medicine Authority. Last updated 1 July 2011. Available from: http://apvma.gov.au/node/15386. Accessed 27 June 2016.

Bao VWW, Leung KMY, Qiu JW and Lam MHW (2011). Acute toxicities of five commonly used antifouling booster biocides to selected subtropical and cosmopolitan marine species, *Marine Pollution Bulletin*, 62, 1147–1151.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 397–398.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

Chen S, Yin C, Strasser RJ, Govinjee, Yang C and Qiang S (2012). Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, 52, 38–51.

Chesworth JC, Donkin ME and Brown MT (2004). The interactive effects of the antifouling herbicides irgarol 1051 and diuron on the zeagrass *Zostera marina* (L.), *Aquatic Toxicology*, 66, 293–305.

Copin PJ and Chevre N (2015). Modelling the effects of pulse exposure of several PSII inhibitors on two algae, *Chemosphere*, 137, 70–77.

DeLorenzo ME, Danese LE and Baird TD (2011). Influence of increasing temperature and salinity on herbicide toxicity in estuarine phytoplankton, *Wiley Periodicals*, 359–371.

Devilla RA, Brown MT, Donkin M, Tarran GA, Aiken J and Readman JW (2005). Impact of antifouling booster biocides on single microalgal species and on a natural marine phytoplankton community, *Marine Ecology Progress Series*, 286, 1–12.

Field JA, Reed RL, Sawyer TE, Griffith SM and Wigington PJ (2003). Diuron occurrence and distribution in soil and surface and groundwater associated with grass seed production, *Journal of Environmental Quality*, 32, 171–179.

Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R, Thomson B and Warne MStJ (2015). Total suspended solids, nutrients and pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads

Monitoring Program 2013–2014. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 108pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2013-2014-gbr-catchment-loads-technical-report.pdf.

Gatidou G and Thomaidis NS (2007). Evaluation of single and joint toxic effects of two antifouling biocides, their main metabolites and copper using phytoplankton bioassays, *Aquatic Toxicology*, 85, 184–191.

Grossmann K, Berghaus R and Retzlaff G (1992). Heterotrophic plant cell suspension cultures for monitoring biological activity in agrochemical research. Comparison with screens using algae, germinating seeds and whole plants, *Pesticide Science*, 35, 283–289.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

Karlsson J, Breitholtz M and Eklund B (2006). A practical ranking system to compare toxicity of anti-fouling paints, *Marine Pollution Bulletin*, 52, 1661–1667

Koutsaftis A and Aoyama I (2006). The interactive effects of binary mixtures of three antifouling biocides and three heavy metals against the marine algae *Chaetoceros gracilis*, *Environmental Toxicology*, 21, 432–439.

Koutsaftis A and Aoyama I (2007). Toxicity of four antifouling biocides and their mixtures on the Brine Shrimp *Artemia salina*, *Science of the Total Environment*, 387, 166–174.

Kumar KS, Choo K, Yea SS, Seo Y and Han T (2010). Effects of the phenylurea herbicide diuron on the physiology of *Saccharina japonica* Aresch, *Toxicology and Environmental Health Scien*ces, 2, 188–199.

Larras F, Bouchez A, Rimet F and Montuelle B (2012). Using bioassays and species sensitivity distributions to assess herbicide toxicity towards benthic diatoms, *PLoS One*, 7, 1–9.

Larras F, Montuelle B and Bouchez A (2013). Assessment of toxicity thresholds in aquatic environments: Does benthic growth of diatoms affect their exposure and sensitivity to herbicides?, *Science of the Total Environment*, 463–464, 469–477.

Ma J (2002). Differential Sensitivity to 30 Herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*, *Bulletin of Environmental Contamination and Toxicology*, 68, 275–281.

Ma J, Liang W, Xu L, Wang S, Wei Y and Lu J (2001). Acute toxicity of 33 herbicides to the green alga *Chlorella pyrenoidosa*, *Bulletin of Environmental Contamination and Toxicology*, 66, 536–541.

Ma J, Lin F, Wang S and Xu L (2003). Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda*, *Bulletin of Environmental Contamination and Toxicology*, 71, 594–601.

Ma J, Xu L and Wang S (2002). A quick, simple, and accurate method of screening herbicide activity using green algae cell suspension cultures, *Weed Science*, 50, 555–559.

Magnusson M, Heimann K and Negri AP (2008). Comparative effects of herbicides on photosynthesis and growth of tropical estuarine microalgae, *Marine Pollution Bulle*tin, 56, 1545–1552.

Schafer H, Hettler H, Fritsche U, Pitzen G, Roderer G, and Wenzel A (1994). Biotests using unicellular algae and ciliates for predicting long-term effects of toxicants, *Ecotoxicology and Environmental Safety*, 27, 64–81.

Seery C and Pradella N (in prep). Toxicity of photosystem II inhibiting herbicides to four tropical alga.

Singh S, Datta P and Tirkey A (2011). Response of multiple herbicide resistant strain of diazotrophic cyanobacterium, *Anabaena variabilis*, exposed to atrazine and DCMU, *Indian Journal of Experimental Biology*, 49, 298–303.

Stauber JL, Binet MT, Bao VWW, Boge J, Zhang AQ, Leung KMY and Adams MS (2008). Comparison of the QwikLite algal bioluminescence test with marine algal growth rate inhibition bioassays, *Environmental Toxicology*, 23, 617–625.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Teisseire H, Couderchet M and Vernet G (1999). Phytotoxicity of diuron alone and in combination with copper or folpet on duckweed, *Environmental Pollution*, 106, 39–45.

Tsunemasa N and Okamura H (2011). Effects of organotin alternative antifoulants on oyster embryo, *Archives of Environmental Contamination and Toxicology*, 61, 128–134.

Turner R, Huggins R, Wallace R, Smith R, Vardy S and Warne MStJ (2013a). Sediment, nutrient and pesticide loads: Great Barrier Reef Loads Monitoring 2009–2010. Water Sciences Technical Report, Volume 2012, Number 14. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0994. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2009-2010-gbr-catchment-loads-report.pdf.

Turner RDR, Huggins R, Wallace R, Smith RA, Vardy S and Warne MStJ (2013b). Total suspended solids, nutrient and pesticide loads for rivers that discharge to the Great Barrier Reef: Great Barrier Reef Loads Monitoring 2010–2011. Water Sciences Technical Report, Volume 2013, Number 1. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0996. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2010-2011-gbr-catchment-loads-report.pdf.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm Accessed: 13 May 2016.

USEPA (1987). Health Advisory Summary: Diuron. United States Environmental Protection Agency, Office of Drinking Water. Washington, DC. Available from: http://nepis.epa.gov/Exe/ZyNET.exe/9100I9M9.txt?ZyActionD=ZyDocument&Client=EPA&Index=1995%20Thru%201999%7C1976%20Thru%201980%7C2006%20Thru%202010%7C1991%20Thru%201994%7CHardcopy%20Publications%7C2000%20Thru%202005%7C1986%20Thru%201990%7C2011%20Thru%202015%7C1981%20Thru%201985%7CPrior%20to%201976&Docs=&Query=DIURON%20&Time=&EndTime=&SearchMethod=2&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C86THRU90%5CTXT%5C00000021%5C9100I9M9.txt&User=ANONYMOUS&Password=anonymous&SortMethod=-%7Ch&MaximumDocuments=15&FuzzyDegree=0&ImageQuality=r85g16/r85g16/x150y150g16/i500&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1&SeekPage=x#. Accessed: 27 June, 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: February–April, 2016.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and Warne MStJ. 2015. Total suspended solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp.Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-catchment-loads-report.pdf.

Wallace R, Huggins R, Smith RA, Turner RDR, Vardy S and Warne MStJ. 2014. Total suspended solids, nutrient and pesticide loads (2011–2012) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2011–2012. Department of Science, Information Technology, Innovation and the Arts. Brisbane, Queensland. 94p. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2011-2012-gbr-catchment-loads-report.pdf.

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Canberra, Australia.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

Wilson PC, Whitwell T and Klaine SJ (2000). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*, *Archives of Environmental Contamination and Toxicology,* 39, 282–288.

4 Glyphosate

4.1 Introduction

Glyphosate is a herbicide ($C_3H_8NO_5P$ and Figure 13) which 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 the commercial product, Roundup® Herbicide) 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 and ARMCANZ 2000).
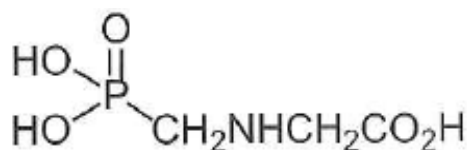
Figure 13 Structure of glyphosate.

Physicochemical properties of glyphosate that may affect its environmental fate and toxicity are presented in Table 15.

Table 15 Summary of selected physicochemical properties of glyphosate.

Physicochemical property	Value
Molecular weight	169.1 amu[1,2]
Aqueous solubility	10,500 mg/L @ pH 1.9 and temperature 20 °C[1]
Logarithm of the octanol-water partition coefficient (log K_{ow})	-3.2[1] -3.2 @ pH 7 and temperature 20 °C[3]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	4.45[2] 3.15[3]
Logarithm of the bioconcentration factor (log BCF)	0.5[3]
Half-life ($t_{1/2}$) in water	9.9 days[3] Hydrolysis: stable @ pH 5–8 and temperature 25 °C[3] 33 days (pH 5), 77 days (pH 9)[3]
Half-life ($t_{1/2}$) in soil	74.5 days[3]

[1] **BCPC (2012).** [2] **CCME (1999).** [3] **Pesticide Properties Database (University of Hertfordshire 2013).**

Glyphosate belongs to the organophosphorus group of herbicides, which also includes bensulide, fosamine and glufosinate. Glyphosate is extensively used in agriculture, forestry, industrial and urban situations. In agriculture it is predominantly used to control weeds and grasses in commercial crops that are genetically modified to resist its effects (BCPC 2012; University of Hertfordshire 2013). It is a broad spectrum (non-selective) systemic herbicide with high activity on virtually all plants. In Australia, glyphosate is the most heavily used herbicide, closely followed by simazine and atrazine (AATSE 2002). It is also widely used internationally.

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) as well as lignins, alkaloids, flavonoids, benzoic acids and plant hormones (Plant and Soil Sciences eLibrary 2015). 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 and Bidwell 1999). Some commercial formulations have been reported to be 3 to 42 times more toxic than the active ingredient - glyphosate (Folmar et al. 1979). Therefore, alternate replacements of less toxic formulations are encouraged (e.g. Roundup Biactive®) for use near waterways (AATSE 2002).

Glyphosate binds strongly to soil particles (Table 15) 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 microorganisms (AATSE 2002) that biodegrade it to aminomethylphosphonic acid (AMPA) and glyoxylate and, ultimately, to carbon dioxide (Schuette 1998).

4.2 Freshwater

4.2.1   Aquatic Toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for glyphosate in freshwaters (Table 17) includes toxicity data for four freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

The review of the literature revealed one published study (Bidwell and Gorrie 1995) that determined the toxicity of glyphosate to two Australasian frog species (*Crinia insignifera* and *Litoria moorei*). As these toxicity tests were carried out using high concentrations of glyphosate acid with low pHs (<3.0), it is more likely that mortality amongst the tadpoles was as a result of low pH levels of the higher exposure concentrations rather than the glyphosate acid exposure itself. Tadpoles have reportedly been unaffected by high concentrations (NOEC of >340 mg/L) of other forms of glyphosate such as glyphosate IPA (Mann and 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) was not included in the derivation of the PGVs for glyphosate and are not included in this report. A summary of the high and moderate quality raw toxicity data for freshwater species is provided below.

Freshwater Chronic

There are freshwater chronic toxicity data for one fish, two amphibians, one cladoceran, one crustacean, two molluscs, two macrophytes and 16 microalgae. The toxicity values for fish consisted

of 21-day LOEC (hatching success, number of eggs per female, mortality) values all of 10,000 µg/L. The toxicity data for amphibian were 26- and 57-day NOEC (metamorphosis, mortality) values all of 6.9 µg/L. The toxicity values for cladocerans consisted of 12-, 24-, 36- and 55-day NOEC (growth) values ranging from 450 to 4,050 µg/L, 12-, 24-, 36- and 55-day LOEC (growth) values ranging from 1,350 to 4,050 µg/L, 21- and 55-day NOEC/NOEL (immobilisation) values ranging from 450 to 50,000 µg/L, 21- and 55-day LOEC (immobilisation) values ranging from 1,350 to 96,000 µg/L, 55-day NOEC (fecundity, abortion rate) values ranging from 150 to 450 µg/L and 55-day LOEC (fecundity, abortion rate) ranging from 450 and 1,350 µg/L. The single toxicity value for the crustacean was a 50-day NOEC (growth rate) value of 22,500 µg/L. The toxicity values for molluscs consisted of 9- and 12-day NOEC (embryo length) values both of 10,000 µg/L, 12-day NOEC, LOEC, IC7 and IC66 (egg hatching success) values of 1,000, 10,000, 100 and 10,000 µg/L, respectively and 21-day NOEC and LOEC (shell length) values of 12,500 and 25,000 µg/L, respectively. The toxicity values for macrophytes consisted of 7- and 10-day NOEL, IC10 and EC10 (frond number, dry weight, frond area, chlorophyll-a content) values ranging from 940 to 14,100 µg/L, 7- and 10-day IC25 (frond growth) values ranging from 7,300 to 16,200 µg/L, two 7-day LOEC (frond growth) values both of 500 µg/L, 7- and 10-day EC50 and IC50 (frond growth, frond number, dry weight, frond area, chlorophyll-a content) values ranging from 18,300 to 46,900 µg/L, 14-day NOEL and LOEC (frond number, dry weight, frond area) values of 1,400 and 1,800 µg/L, respectively and 14-day EC50 (frond number, dry weight, frond area) values of 14,400 and 21,500 µg/L, respectively. The toxicity values for microalgae were 2-day EC10 and EC50 (chlorophyll-a) values of 92,500 and 270,000 µg/L, respectively, 3-day NOEC/EC10 and LOEC (cell density) values ranging from 100 to 3,000 µg/L and 100 to 1,560 µg/L, respectively, 3-day EC50 (cell density) values ranging from 24,500 to 41,700 µg/L, 4- and 5-day NOEL (biomass yield, growth rate, area under the growth curve) values ranging from 270 to 19,100 µg/L, 4-day EC50 (biomass yield, growth rate, area under the growth curve, cell density, mortality) values ranging from 390 to 1,082,050 µg/L, 5-day EC50 (biomass, growth rate, area under curve) values ranging from 15,000 to 170,000 µg/L and 21-day EC50 (total chlorophyll) values ranging from 4,100 to 598,400 µg/L.

Freshwater Acute

There are freshwater acute toxicity data for ten fish, three cladocerans, two crustaceans, one mollusc, one cnidarian, one insect and two macrophytes. The toxicity data for fish consisted of 24-hour NOEL and LC50 (mortality) values of 81,000 and 84,900 µg/L, respectively, 48-hour LC50 (mortality) values ranging from 13,000 to 645,000 µg/L, 72-hour LC50 (mortality) values ranging from 94,000 to 117,000 µg/L, 96-hour NOEL (mortality) values ranging from 2,200 µg/L to 180,000 µg/L, a 96-hour LOEL (mortality) value of 100,000 µg/L, 96-hour LC50 (mortality) values ranging from 1,300 to 830,800 µg/L and a 10-day LOEC (cumulative eggs laid per female) of 10,000 µg/L. The toxicity values for cladocerans consisted of 48-day NOEL (immobilisation, mortality) values ranging from 1,900 to 560,000 µg/L, a 48-hour LOEL (immobilisation, mortality) value of 60,000 µg/L, 48-hour LC50 and EC50 (immobilisation, mortality) values ranging from 5,300 to 869,000 µg/L, two 6-day NOEC (growth) values of 1,350 and 4,050 µg/L and a 6-day LOEC (growth) value of 4,050 µg/L. The toxicity data for crustaceans consisted of a 48-hour NOEL (mortality) value of 5,400 µg/L, two 48-hour LC50 (mortality) values of 42,000 and 62,000 µg/L and a 96-hour LC50 (mortality) value of 7,000 µg/L. The toxicity values for the single mollusc species consisted of 1-, 3- and 6-day NOEC (embryo length) values all of 10,000 µg/L. The toxicity values for the single cnidarian species consisted of 96-hour LC1, LC5, LC10, LC15, LC50 and LC85 (mortality) values of 14,800, 15,700, 16,200, 16,600, 18,200 and 20,000 µg/L, respectively. The toxicity values for the single insect species were 48-hour LC50 and EC50 (mortality) values of 13,000 and 55,000 µg/L, respectively. The toxicity values for macrophytes were 2-day IC25 and IC50 (growth rate) values of 151,000 and 33,100 µg/L, respectively, 2- to 5-day NOEC and LOEC (growth rate) values of 500 and 1,000 µg/L,

respectively, 2-day NOEL and EC50 (frond number, dry weight, frond area) values of 16,910 and 2,000 µg/L, respectively, and 5-day IC25 and IC50 (growth rate) values of 11,400 and 22,600 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

4.2.2   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 and ARMCANZ 2000). However, no relationships have been developed to permit the calculation of temperature, pH or water hardness specific PGVs.

No factors have been reported to modify the toxicity of glyphosate, however 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 particles (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.

4.2.3   Guideline derivation

The derived PGVs for glyphosate in freshwaters are provided in Table 16. Details of how the PGVs were calculated and the toxicity data that were used are provided below. Some of the data that were used to generate the previous PGV (ANZECC and ARMCANZ 2000) for glyphosate were omitted from the current derivation process as the toxicity tests used commercial formulations. As with all the other pesticides that have GVs, the PGVs for glyphosate are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for glyphosate are low (Table 15) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for glyphosate do not need to account for secondary poisoning.

Table 16 Proposed aquatic ecosystem protection guideline values (µg/L) for glyphosate for the protection of freshwater ecosystems.

Glyphosate proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)[3]	Criterion	Result
99%	140 (88 – 580)	Sample size	11
95%	250 (160 – 820)	Type of toxicity data	Chronic NOEC/NOEL/EC10 values
90%	340 (220 – 990)	SSD model fit	Poor
80%	530 (320 – 1,300)	Reliability	Moderate

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability". [3] Values rounded to two significant figures.

4.2.4   Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for glyphosate in freshwater environments was a moderate reliability value (using the ANZECC and 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 PGVs (Warne et al. 2015) this trigger value would be classified as having low reliability.

To obtain toxicity data for glyphosate to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now considerably more glyphosate toxicity data available that enable the calculation of PGVs in freshwaters (see section 4.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of glyphosate to phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were freshwater toxicity data for 39 species (eight different phyla and 13 classes) that passed the screening and quality assessment 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), Amphibia (tetrapod vertebrates), 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), Malacostraca (a large grouping of crustaceans) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of glyphosate, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as the EPSP enzyme is normally located within chloroplasts of plants and algae. The glyphosate ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups

did not have significantly different (p = 0.589, see section 104) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for glyphosate in freshwater.

There were freshwater chronic no observed effect concentration (NOEC), no observed effect level (NOEL) and 10% effect concentration (EC10) data available for 11 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 SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 16) combined with the poor fit of the distribution to these toxicity data (Figure 14) resulted in a moderate reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for glyphosate in freshwater environments is provided in Table 17.

Table 17 Summary of the single toxicity value for each phototrophic and heterotrophc species that was used to derive the proposed aquatic ecosystem protection guideline values for glyphosate in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[2]	12,000	USEPA (2015b)
Macroinvertebrate	*Cherax quadricarinatus**	Arthropoda	Malacostraca	Advanced juveniles	50	Chronic NOEC	Weight gain	22,500	Frontera et al. (2011)
Microalga	*Chlorella saccharophila*	Chlorophyta	Trebouxiophyceae	Exponential growth phase	3	Chronic NOEC/EC10	Cell density	1,081.7	Vendrell et al. (2009)
Cladoceran	*Daphnia magna*	Arthropoda	Branchiopoda	Not stated	21	Chronic NOEC	Immobilisation	259.8	Cuhra et al. (2013)
Macroinvertebrate	*Lampsilis siliquoidea*	Mollusca	Bivalvia	Juveniles	21	Chronic NOEC	Shell length	12,500	Bringolf et al. (2007)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	7–10	Chronic NOEL	Frond number, dry weight, frond area	1,400	USEPA (2015b)
Macrophyte	*Lemna minor**	Tracheophyta	Liliopsida	Not stated	7	Chronic EC10	Chlorophyll a content	3,780	Cedergreen and Streibig (2005)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	4	Chronic NOEL	Biomass yield, growth rate, AUC[2]	1,800	USEPA (2015b)
Macroinvertebrate	*Pseudosuccinea columella*	Mollusca	Gastropoda	Embryo	12	Chronic NOEC	Hatching success	316.2	Tate et al. (1997)
Microalga	*Scenedesmus subspicatus*[3]*	Chlorophyta	Chlorophyta	Exponential growth phase	3	Chronic NOEC/EC10	Cell density	400	Vendrell et al. (2009)
Microalga	*Selenastrum capricornutum*[4]	Chlorophyta	Chlorophyta	Not stated	2	Chronic NOEL	Chlorophyll a content	1,400	USEPA (2015b)

[1] **Chronic NOEC/NOEL = no conversions applied (Warne et al. 2015).** [2] **AUC = area under the growth curve.** [3] **This species has also been called *Desmodesmus subspicatus*.** [4] **This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. *Species that originated from/are distributed in Australia and/or New Zealand.**

4.2.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 11 freshwater, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 14.
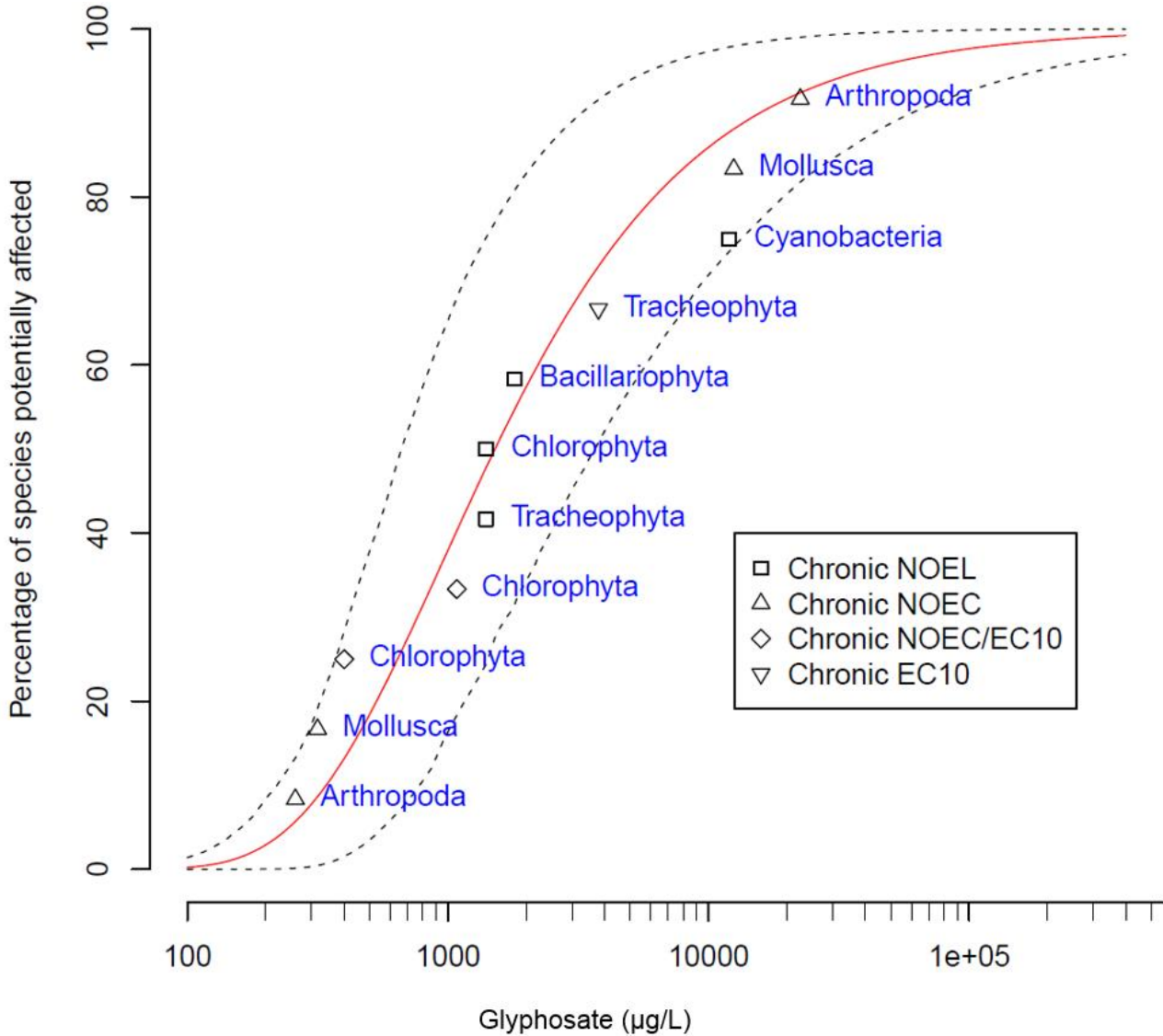
Figure 14 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to glyphosate. Black dashed lines indicate the 95% confidence intervals.

4.2.6   Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for glyphosate in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Arthropoda	Malacostraca	Australian Red Claw Crayfish (*Cherax quadricarinatus*)	Advanced juvenile	50	Chronic	NOEC (Growth rate)	Dechlorinated filtered tap water	27 ± 1	8.0 ± 0.5	22,500	Frontera et al. (2011)
										22,500	*GEOMETRIC MEAN*
										22,500	***VALUE USED IN SSD***
Arthropoda	Branchiopoda	Cladoceran (*Daphnia magna*)	Juvenile	55	Chronic	NOEC (Fecundity)	Aachener Daphnien Medium (adam)	27 ± 2	7.5 ± 0.7	450	Cuhra et al. (2013)
Arthropoda	Branchiopoda	Cladoceran (*Daphnia magna*)	Juvenile	55	Chronic	NOEC (Fecundity)	Aachener Daphnien Medium (adam)	27 ± 2	7.5 ± 0.7	150	Cuhra et al. (2013)
										259.8	*GEOMETRIC MEAN*
										259.8	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalga (*Navicula pelliculosa*)	Not stated	5	Chronic	NOEL (Biomass, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	1,800	USEPA (2015b)
										1,800	*GEOMETRIC MEAN*
										1,800	***VALUE USED IN SSD***
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella*	Exponential growth	3	Chronic	NOEC (Cell density)	ASTM medium	24 ± 2	Not stated	390	Vendrell et al. (2009)

		saccharophila)	phase								
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella saccharophila*)	Exponential growth phase	3	Chronic	EC10 (Cell density)	ASTM medium	24 ± 2	Not stated	3,000	Vendrell et al. (2009)
										1,081.7	*GEOMETRIC MEAN*
										1,081.7	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[1]*)	Not stated	4	Chronic	NOEL (Biomass, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	1,400	USEPA (2015b)
										1,400	*GEOMETRIC MEAN*
										1,400	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus subspicatus*)	Exponential growth phase	3	Chronic	NOEC (Cell density)	ASTM medium	24 ± 2	Not stated	100	Vendrell et al. (2009)
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus subspicatus*)	Exponential growth phase	3	Chronic	EC10 (Cell density)	ASTM medium	24 ± 2	Not stated	1,600	Vendrell et al. (2009)
										400	*GEOMETRIC MEAN*
										400	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Microalga (*Anabaena flos-aquae*)	Not stated	5	Chronic	NOEL (Biomass, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	12,000	USEPA (2015b)
										12,000	*GEOMETRIC MEAN*
										12,000	***VALUE USED IN SSD***

										pH		
Mollusca	Bivalvia	Fatmucket Clam (*Lampsilis siliquoidea*)	Juvenile	21	Chronic	NOEC (Growth)	Reconstituted hard water	21.1 ± 0.7	8.22–8.76	12,500	Bringolf et al. (2007)
										12,500	*GEOMETRIC MEAN*
										12,500	**VALUE USED IN SSD**
Mollusca	Gastropoda	Ribbed Fluke Snail (*Pseudosuccinea columella*)	Embryo	12	Chronic	NOEC (Hatching success)	Artificial spring water	25 ± 2	6.5–8.5	1,000	Tate et al. (1997)
Mollusca	Gastropoda	Ribbed Fluke Snail (*Pseudosuccinea columella*)	Embryo	12	Chronic	IC7 (Hatching success)	Artificial spring water	25 ± 2	6.5–8.5	100	Tate et al. (1997)
										316.2	*GEOMETRIC MEAN*
										316.2	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not-stated	14	Chronic	NOEL (Frond number, dry weight, frond area)	M-Hoagland's/20X-AAP nutrient media/ASTM Type I	25 ±2	(4.8-5.2 for M-Hoagland's / 7.5 ± 0.1 for 20X-AAP)	1,400	USEPA (2015b)
										1,400	*GEOMETRIC MEAN*
										1,400	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Lemna minor)*	Not stated	7	Chronic	EC10 (Chlorophyll*a*)	K' medium	24	5	3,780	Cedergreen and Streibig (2005)
										3,780	*GEOMETRIC MEAN*
										3,780	**VALUE USED IN**

											SSD

[1] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [2] AUC = area under the growth curve. Note: Table strictly excludes data that originated from the use of formulations (i.e. Roundup®).

4.2.7 Distribution of sensitivities for aquatic species

The toxicity data for glyphosate to all freshwater species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the glyphosate ecotoxicity data may be unimodal (Figure 15).
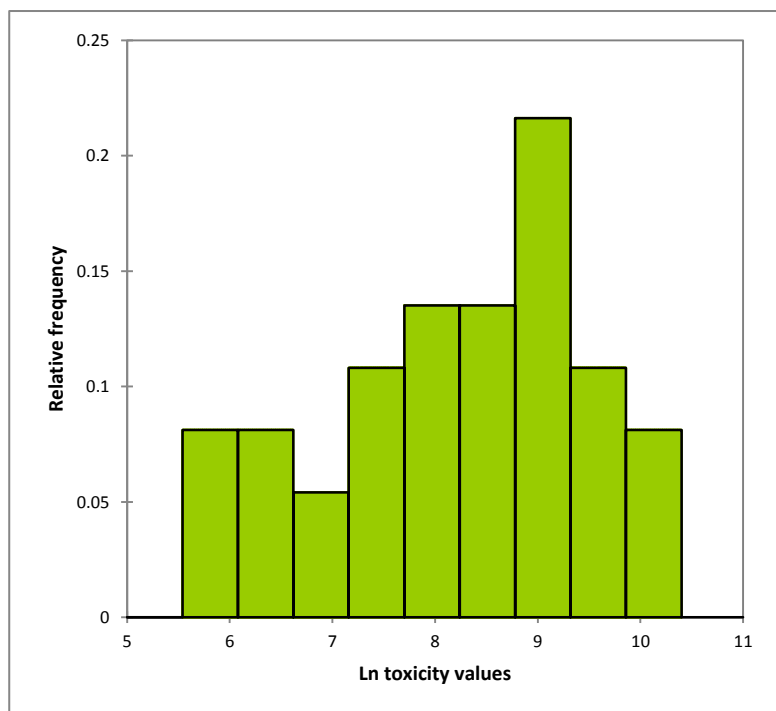
Figure 15 Histogram of the natural logarithm (ln) of all glyphosate freshwater toxicity data for phototrophic and non-phototrophic species (n = 37).

The glyphosate ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample t test was used because the transformed glyphosate concentration data had equal variances (Fisher's F-Test; p = 0.695) and followed a normal distribution (Anderson-Darling; p = 0.157). Results from the two-sample t test indicated that the two groups were not significantly different (p = 0.930); therefore, it can be concluded that the distribution of the glyphosate concentration data is uni-modal.

4.2.8   References

AATSE (2002). Pesticide use in Australia. A review undertaken by the Australian Academy of Technological Sciences and Engineering. Australian Academy of Technological Sciences and Engineering, Parkville, Victoria, Australia, 309pp.

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

APVMA (2014). Glyphosate. Australian Pesticide and Veterinary Medicine Authority. Last updated 1 July, 2014. Available from: http://apvma.gov.au/node/12556. Accessed: 27 June 2016.

BCPC (2012). A world compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 586–591.

Bidwell JR and Gorrie JR (1995). Acute toxicity of a herbicide to selected frog species. Final Report. Department of Environmental Protection, Perth, WA. Technical Series 79.

Bringolf RB, Cope WG, Mosher S, Barnhart MC, and Shea D (2007). Acute and chronic toxicity of glyphosate compounds to glochidia and juveniles of *Lampsilis siliquoidea* (Unionidae), *Environmental Toxicology and Chemistry*, 26 (10), 2094–2100.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

CCME (1999). Canadian water quality guidelines for the protection of aquatic life – Glyphosate. Canadian Council of Ministers of the Environment. Available from: http://ceqg-rcqe.ccme.ca/download/en/182. Downloaded: 9 September, 2015.

Cedergreen N and Streibig JC (2005). The toxicity of herbicides to non-target aquatic plants and algae: Assessment of predictive factors and hazard, *Pesticide Management Science*, 61(12), 1152–1160.

Cuhra M, Traavik T and Bohn T (2013). Clone- and age-dependent toxicity of a glyphosate commercial formulation and its active ingredient in *Daphnia magna*, *Ecotoxicology*, 22, 251–262.

Feng JC, Thompson DG and Reynolds PE (1990). Fate of glyphosate in a Canadian forest watershed. 1. Aquatic residues and off target deposit assessment, *Journal of Agriculture Food Chemistry*, 38, 1110–1118.

Folmar LC, Sanders HO and Julin AM (1979). Toxicity of the herbicides glyphosate and several of its formulations to fish and aquatic invertebrates. *Archives of Environmental Contamination and Toxicology* 8, 269–278.

Freda J (1986). The influence of acidic pond water on amphibians: a review. *Water, Air and Soil Pollution*, 30, 439–450.

Frontera JL, Vatnick I, Chaulet A and Rodriguez EM (2011). Effects of glyphosate and polyoxyethylenamine on growth and energetic reserves in the freshwater crayfish *Cherax quadricarinatus* (Decapoda, Parastacidae), *Archives of Environmental Contamination and Toxicology*, 61 (4), 590–598.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Mann RM and Bidwell JR (1999). The toxicity of glyphosate and several glyphosate formulations to four species of southwestern Australian frogs, *Archives of Environmental Contamination and Toxicology*, 36, 193–199.

Newton M, Homer LM, Cowell JE, White DE and Cole EC (1994). Dissipation of glyphosate and aminomethylphosphonic acid in North American forests, *Journal of Agriculture Food Chemistry*, 42, 1795–1802.

Plant and Soil Sciences eLibrary (2015). Inhibitors of aromatic amino acid biosynthesis. Available from; http://passel.unl.edu/pages/informationmodule.php?idinformationmodule=959117477&topicorder=4&maxto=7. Downloaded: 15 September, 2015.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Schönbrunn E, Eschenburg S, Shuttleworth WA, Schloss JV, Amrheim N, Evans JNS and Kabsch W (2001). Interacation of the hyrbicide glyphosate with its target enzyme 5-enolpyruvylshikimate 3-phosphate synthase in atomic detail, *Proceedings National Academy of Sciences of the United States of America*, 98(4), 1376–1380.

Schuette J (1998). Environmental fate of glyphosate. Department of Pesticide Regulation, Environmental Monitoring and Pesticide Management, Sacramento, California, CA 95824-5624. Available from: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/glyphos.pdf Accessed: 16 September 2015.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ water quality guideline database for toxicants. Supplied as part of a CD-rom in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Tate TM, Spurlock JO, Christian FA (1997). Effect of glyphosate on the development of *Pseudosuccinea columella* snails, *Archives of Environmental Contamination and Toxicology*, 33, 286–289.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm. Accessed: May 2015.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: May–September, 2015.

Vendrell E, Ferraz DGB, Sabater C and Carrasco JM (2009). Effect of glyphosate on growth of four freshwater species of phytoplankton: A microplate bioassay, *Bulletin of Environmental Contamination and Toxicology*, 82, 538–542.

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Canberra, Australia.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

5 Hexazinone

5.1 Introduction

Hexazinone is a herbicide ($C_{12}H_{20}N_4O_2$ and Figure 16) that at room temperature is in the form of colourless, odourless crystals. It is the active ingredient of a variety of commercial herbicide formulations.
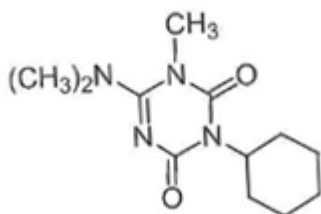
Figure 16 Structure of hexazinone.

Physicochemical properties of hexazinone that may affect its environmental fate and toxicity are presented in Table 18.

Table 18 Summary of selected physicochemical properties of hexazinone.

Physicochemical property	Value
Molecular weight	252.3 amu[1]
Aqueous solubility	29.8 g/L @ pH 7 and temperature 25°C[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	1.17 @ pH 7 and temperature 25 °C[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.72[2] –2.79[3]
Logarithm of the bioconcentration factor (log BCF)	0.85[2]
Half-life ($t_{1/2}$) in water	≥ 56 days (pH 7) @ 20°C[2,3]
Half-life ($t_{1/2}$) in soil	90 days[4]

[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3.] Ganapathy (1996). [4] Tu et al. (2001).

Hexazinone is absorbed through the roots and leaves (foliage) of plants following soil absorption and direct foliar application, respectively (Ganapathy 1996). It is then translocated acropetally (i.e. movement upwards from the base of plants to the apex). Hexazinone exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Triazinone herbicides bind to the plastoquinone B (Q_B) protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (ATP, used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (NADPH, used in converting CO_2 to glucose), and therefore, prevents CO_2 fixation (Wilson et al. 2000).

In addition to its main mode of action, exposure to PSII inhibiting herbicides can lead to marked increases in the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen (OH⁻), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). Reactive oxygen species are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Reactive oxygen species are created during normal cellular functioning particularly in biochemical processes that involve the

generation of energy (e.g. photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells). In phototrophs, ROS are formed when the absorbed light energy exceeds the ability to convert CO_2 to organic molecules, thus accumulating oxygen (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, prolonged exposure to elevated concentrations of ROS in plants, as a result of biotic (e.g. disease) and/or abiotic stressors (e.g. PSII inhibiting herbicides), can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

Hexazinone ultimately ends up in aquatic environments as a result of vapour drift, surface and/or subsurface runoff following application to control invasive weeds (Tu et al. 2001). Hexazinone has low soil adsorption characteristics as indicated by its low log K_{oc} value (Table 18) and thus, it has a high capacity to leach to groundwater and to be transported in surface waters (Tu et al. 2001). The aqueous hydrolysis of hexazinone can range from several days to more than nine months (Tu et al. 2001), with a half-life of 56 days at pH 7 and a temperature of 20 ºC (University of Hertfordshire 2013) (Table 18). This indicates hexazinone is persistent and highly mobile in surface, sub-surface and ground waters.

5.2 Freshwater

5.2.1   Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for hexazinone in freshwaters (Table 20) includes toxicity data for two freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, one cladoceran, one macrophyte and three microalgae. The toxicity values for the single fish species were 39-day NOEL and LOEC (mortality) values of 17,000 and 35,500 µg/L. The toxicity values for the single cladoceran species were two 21-day NOEL (immobilisation) values of 20,000 and 29,000 µg/L, two 21-day LOEC (immobilisation) values of 50,000 and 81,000 µg/L and a 21-day EC50 (immobilisation) value of 33,100 µg/L. The toxicity values for the single macrophyte species were a 7-day EC50 (growth) value of 72 µg/L and 14-day LOEC and EC50 (abundance) values of 26 and 37.4 µg/L, respectively. The toxicity values for microalgae consisted of a 96-hour EC50 (abundance) value of 24.5 µg/L, 5-day NOEC (abundance) values ranging from 3.5 to 150 µg/L and 5-day EC50 (abundance) values ranging from 6.8 to 210 µg/L.

Freshwater Acute

There were freshwater acute toxicity data for 10 fish, one cladoceran, one crustacean and two macrophytes. The toxicity values for the fish species consisted of 48-hour LC50 (mortality) values ranging from 75,000 to 974,000 µg/L, 72-hour LC50 (mortality) values ranging from 271,000 to 927,000 µg/L, 96-hour NOEL (mortality) values ranging from 148,000 to 370,000 µg/L, 96-hour LC50 (mortality) values ranging from 100,000 to 925,000 µg/L. The single toxicity value for the cladoceran was a 48-hour (immobilisation) value of 161,600 µg/L. The toxicity values for the single crustacean

species were 48-, 72- and 96-hour LC50 (mortality) values of 579,300, 46,200 and 19,500 µg/L, respectively. The toxicity values for macrophytes were 4-day EC10 and EC50 (abundance) values of 10.8 and 37.8 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

5.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of hexazinone. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of hexazinone (Table 18).

5.2.3 Guideline derivation

The derived PGVs for hexazinone in freshwaters are provided in Table 19. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for hexazinone are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for hexazinone are low (Table 18) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for hexazinone do not need to account for secondary poisoning.

Table 19 Proposed aquatic ecosystem protection guideline values (µg/L) for hexazinone for the protection of freshwater ecosystems.

Hexazinone proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.31 (0.11 – 3.0)	Sample size	5
95%	1.1 (0.61 – 5.2)	Type of toxicity data	Chronic NOEC and chronic estimated NOEC values
90%	1.9 (1.1 – 6.8)	SSD model fit	Poor
80%	3.4 (2.0 – 14)	Reliability	Low

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

5.2.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for hexazinone in freshwater environments was a low reliability value as it was based on an acute toxicity value for a fish species (Warne 2001). This trigger value was calculated using the assessment factor (AF) method, dividing the lowest acute toxicity value of 75 mg/L by an assessment factor of 1,000 (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as having an unknown reliability.

To obtain toxicity data for hexazinone to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now more hexazinone toxicity data available that enable the calculation of PGVs in freshwaters (see section 5.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of hexazinone with phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were freshwater toxicity data for 18 species (six phyla and seven classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The seven classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Liliopsida (monocots) and Malacostraca (a larger grouping of crustaceans).

Based on the current understanding of the mode of action of hexazinone, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The hexazinone ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample t test to see if the toxic responses among different taxa were uni- or multi-modal. The t test indicated that the two groups had significantly different (p <0.0001, see section 5.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were chronic no observed effect concentration (NOEC) data available for three freshwater phototrophic species (that belonged to three phyla and three classes) which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). When the dataset was expanded to combine the chronic NOEC values with the chronic estimated NOEC (chronic LOEC/EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) values, there were data available for five phototrophic species (that belonged to four phyla and four classes), which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 19) combined with the poor fit of the distribution to these toxicity data (Figure 17) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for hexazinone in freshwater environments is provided in Table 20.

Table 20 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for hexazinone in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flosaquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	150	USEPA (2015b)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Population (Abundance)	8.82	USEPA (2015b)
Macrophyte	*Lemna minor**	Tracheophyta	Liliopsida	Not stated	7	Chronic est. NOEC	Population (Growth)	14.4	Peterson et al. (1997)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	3.5	USEPA (2015b)
Microalga	*Pseudokirchneriella subcapitata*[2]	Chlorophyta	Chlorophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	4	USEPA (2015b)

[1] **Chronic NOEC = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015).** [2] **This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*. * Species that originated from/are distributed in Australia and/or New Zealand.**

5.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the five freshwater phototrophic species that was used to derive the PGVs is presented in Figure 17.
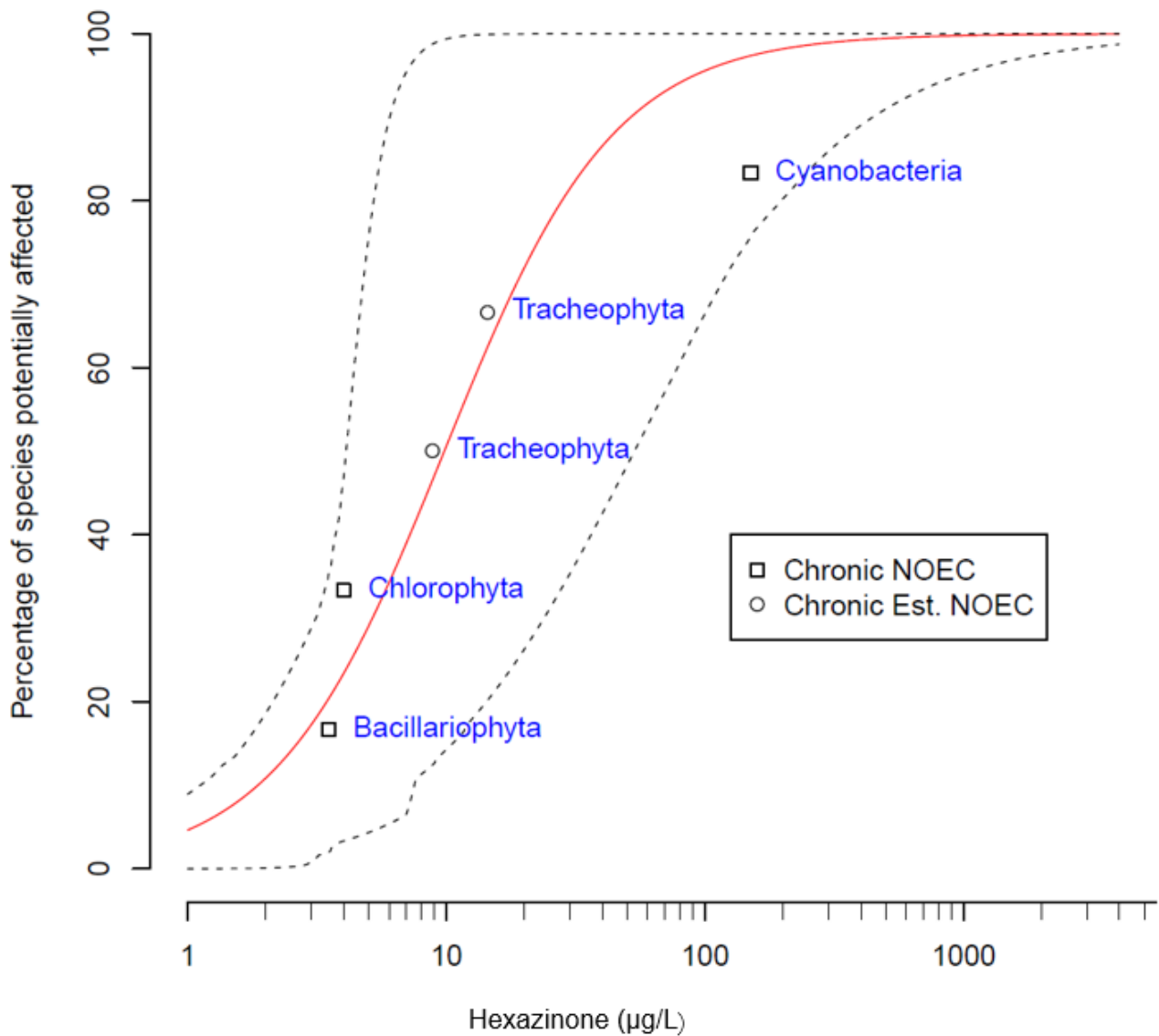
Figure 17 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC) data values of freshwater phototrophic species to hexazinone. Black dashed lines indicate the 95% confidence intervals.

5.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for hexazinone in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Microalga (*Navicula pelliculosa*)	Not stated	5	Chronic	NOEC (Abundance)	ASTM Type I water	24 ± 2	7.5 ± 0.1	3.5	USEPA (2015b)
										3.5	*GEOMETRIC MEAN*
										3.5	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Pseudokirchneriella subcapitata*[1])	Not stated	5	Chronic	NOEC (Abundance)	ASTM Type I water	24 ± 2	7.5 ± 0.1	4	USEPA (2015b)
										4	*GEOMETRIC MEAN*
										4	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Microalga (*Anabaena flosaquae*)	Not stated	5	Chronic	NOEC (Abundance)	ASTM Type I water	24 ± 2	7.5 ± 0.1	150	USEPA (2015b)
										150	*GEOMETRIC MEAN*
										150	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	14	Chronic	LOEC (Abundance)	Glass-distilled, deionized water, or ASTM Type I water	25 ± 2	4.8 and 5.2 for M-Hoagland's medium, 7.5 ± 0.1 for 20X-AAP medium	26	USEPA (2015b)
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	14	Chronic	EC50 (Abundance)	Glass-distilled,	25 ± 2	4.8–5.2 for M-	37.4	USEPA (2015b)

							deionized water, or ASTM Type I water		Hoagland's medium, 7.5 ± 0.1 for 20X-AAP medium		
										31.18	*GEOMETRIC MEAN*
										8.82[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte (*Lemna minor*)	Not stated	7	Chronic	EC50 (Growth)	ASTM Type I water	25	8.07	72	Peterson et al. (1997)
										72	*GEOMETRIC MEAN*
										14.4[@]	***VALUE USED IN SSD***

[1] **Previously this species has been called *Rhaphidocelis subcapitata* and *Selenastrum caprincornutum*. [@] Values were chronic LOEC/EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).**

5.3 Marine

5.3.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for hexazinone in marine waters (Table 22) includes toxicity data for four species (two marine and two freshwater) that either originated from or are distributed within Australia and/or New Zealand.

The review of the literature revealed three publications that contained hexazinone toxicity data for Australasian marine phototrophs (Jones and Kerswell 2003, Negri et al. 2011 and Flores et al. 2013). However, as these studies only measured effects based on fluorescence, they were not included in the derivation of the hexazinone PGVs and are not included in this report. A summary of the high and moderate quality raw toxicity data for all marine species that passed the screening and quality assurance processes are provided below.

Marine Chronic

There were marine chronic toxicity data for one fish, one crustacean, one mollusc and three microalgae. The single toxicity value for the fish species was a 21-day NOEC (weight) value of 79.8 µg/L. The single toxicity value for the crustacean species was a 96-hour LC50 (mortality) value of of 78,000 µg/L. The toxicity values for the single mollusc species were 48-hour NOEI and LOEC (mortality, abnormal development) values of 320,000 and 560,000 µg/L, respectively. The microalgae toxicity data consisted of 3-day EC10 (abundance, growth rate) values ranging from 3.8 to 19.34 µg/L, 3-day EC50 (abundance, growth rate) values ranging from 8.4 to 27.71 µg/L, 5-day NOEC and EC50 (abundance) values of 4.1 to 12 µg/L, respectively.

Marine Acute

There were no marine acute toxicity data available in the literature.

5.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of hexazinone. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of hexazinone (Table 18).

5.3.3 Guideline derivation

The derived PGVs for hexazinone in marine waters are provided in Table 21. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for hexazinone are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for hexazinone are low (Table 18) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for hexazinone do not need to account for secondary poisoning.

Table 21 Proposed aquatic ecosystem protection guideline values (µg/L) for hexazinone for the protection of marine ecosystems.

Hexazinone proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	1.8 (1.2 – 3.1)	Sample size	8
95%	2.5 (1.9 – 4.0)	Type of toxicity data	Chronic NOEC/EC10 and chronic estimated NOEC values (*freshwater and marine*)
90%	3.1 (2.5 – 4.9)	SSD model fit	Poor
80%	4.0 (3.4 – 7.0)	Reliability	Low

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

5.3.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for hexazinone in marine environments was the adopted freshwater PGV, which was of low reliability (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on acute toxicity data for a fish species (Warne 2001). This trigger value was calculated using the assessment factor (AF) method, dividing the lowest acute toxicity value of 75 mg/L by an assessment factor of 1,000 (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as having an 'unknown' reliability.

To obtain toxicity data for hexazinone to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now more hexazinone toxicity data available that enable the calculation of PGVs in marine waters. However it was only possible to derive PGVs by using ecotoxicity data for a mixture of both marine and freshwater organisms (see section 5.3.6 and 5.2.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to marine ecosystems separately, it is recommended that additional chronic toxicity tests of hexazinone to marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were marine toxicity data for six species (six phyla and six classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Haptophyta and Mollusca. The six classes were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Coccolithophyceae (a grouping of microalgae), Malacostraca (a larger grouping of crustaceans), Mediophyceae (an algae grouping) and Nephrophyceae (another algae grouping).

Based on the current understand of the mode of action of hexazinone, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The hexazinone ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different ($p < 0.0001$, see section 5.3.7) sensitivities. Therefore, as

recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were chronic 10% effect concentration (EC10) and no observed effect concentration (NOEC) data available for three marine phototrophic species (that belonged to three phyla and three classes) which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). As no other ecotoxicity data for hexazinone to marine phototrophic species were available, the chronic NOEC/EC10 data for marine phototrophic species were combined with the available chronic no observed effect concentration (NOEC)/NOEL and chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) data values for freshwater phototrophic species to derive PGVs for hexazinone in marine waters. This dataset incorporated concentration data for eight (three marine and five freshwater) phototrophic species belonging to five phyla and seven classes, which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 21) combined with the poor fit of the distribution to these toxicity data (Figure 18) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for hexazinone in marine environments is provided in Table 22.

Table 22 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for hexazinone in marine waters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Fresh	Cyanobacteria	*Anabaena flosaquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	150	USEPA (2015b)
Marine	Microalga	*Isochrysis galbana*	Haptophyta	Coccolithophyceae	Not stated	3	Chronic EC10	Population (Abundance)	19.34	Seery et al (2014)
Fresh	Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Population (Abundance)	8.82	USEPA (2015b)
Fresh	Macrophyte	*Lemna minor**	Tracheophyta	Liliopsida	Not stated	7	Chronic est. NOEC	Population (Growth)	14.4	Peterson et al. (1997)
Fresh	Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	3.5	USEPA (2015b)
Marine	Microalga	*Nephroselmis pyriformis*	Chlorophyta	Nephrophyceae	Not stated	3	Chronic EC10	Population (Abundance)	3.8	Magnusson et al (2008)
Fresh	Microalga	*Pseudokirchneriella subcapitata*[2]	Chlorophyta	Chlorophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	4	USEPA (2015b)
Marine	Microalga	*Skeletonema costatum*	Bacillariophyta	Mediophyceae	Not stated	5	Chronic NOEC	Population (Abundance)	4.1	USEPA (2015b)

[1] **Chronic NOEC/EC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*. * Species that originated from/are distributed in Australia and/or New Zealand.**

5.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the eight marine and freshwater phototrophic species that was used to derive the PGVs is presented in Figure 18.

Figure 18 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC) and 10% effect concentration (EC10) data values of marine and freshwater phototrophic species to hexazinone. Black dashed lines indicate the 95% confidence intervals.

5.3.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for hexazinone in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Haptophyta	Coccolithophyceae	Microalga (*Isochrysis galbana*)	Not stated	3	Chronic	EC10 (Abundance)	0.45 mm filtered and autoclaved seawater	31 ± 2	29 ± 1	8.2 ± 0.2	19.34	Seery et al (2014)
											19.34	*GEOMETRIC MEAN*
											1.34	***VALUE USED IN SSD***
Chlorophyta	Nephrophyceae	Microalga (*Nephroselmis pyriformis*)	Not stated	3	Chronic	EC10 (Abundance)	0.45 mm filtered and autoclaved seawater	35	Not stated	Not stated	3.8	Magnusson et al (2008)
											3.8	*GEOMETRIC MEAN*
											3.8	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalga (*Skeletonema costatum*)	Not stated	5	Chronic	NOEC (Abundance)	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 1	8.0 ± 0.1	4.1	USEPA (2015b)
											4.1	*GEOMETRIC MEAN*
											4.1	***VALUE USED IN SSD***

5.3.7 Distribution of sensitivities for aquatic species

Statistical analysis of the hexazinone ecotoxicity data for freshwater and marine species indicated that there was no difference in the sensitivities of the two groups. The parametric two-sample *t* test was used because the transformed hexazinone freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.628) and followed a normal distribution (Anderson-Darling; p = 0.075). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.678); therefore, the freshwater and the marine hexazinone ecotoxicity data can be pooled for further analysis.

The toxicity data for hexazinone to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the hexazinone ecotoxicity data may be bimodal (Figure 19).
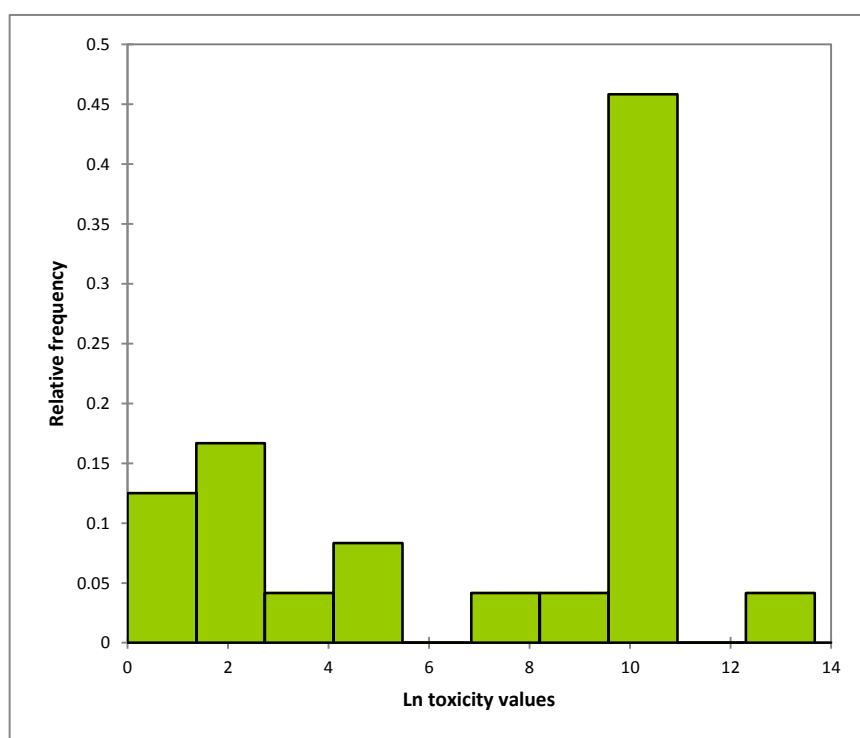
Figure 19 Histogram of the natural logarithm (ln) of all hexazinone (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 31).

The hexazinone ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample *t* test was used because the transformed hexazinone concentration data had equal variances (Fisher's F-Test; p = 0.288) and followed a normal distribution (Anderson-Darling; p <0.0001). Results from the two-sample *t* test indicated that the two groups were significantly different (p <0.0001), therefore it can be concluded that the distribution of the hexazinone concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

5.3.8   References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 613–614.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

Chen S, Yin C, Strasser RJ, Govinjee, Yang C and Qiang S (2012). Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*. *Plant Physiology and Biochemistry*, 52, 38–51.

Ganapathy C (1996). Environmental fate of hexazinone, Department of Pesticide Regulation, Environmental Monitoring and Pesticide Management Branch, Sacramento, California, CA 95814-5624. Available from: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/hxzinone.pdf , Accessed: 08 February, 2016.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Magnusson M, Heimann K and Negri AP (2008). Comparative effects of herbicides on photosynthesis and growth of tropical estuarine microalgae, *Marine Pollution Bulletin*, 56, 1545–1552.

Peterson HG, Boutin C, Freemark KE and Martin PA (1997). Toxicity of hexazinone and diquat to green algae, diatoms, cyanobacteria and duckweed, *Aquatic Toxicology*, 39, 111–134.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Seery C and Pradella N (in prep). Toxicity of photosystem II inhibiting herbicides to four tropical alga.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Tu M, Hurd C and Randall JM (2001). Weed Control Methods Handbook: Tools and Techniques for Use in Natural Areas. The Nature Conservancy, Virginia, United States of America, 135–141pp.

Available from: http://www.invasive.org/gist/products/handbook/methods-handbook.pdf. Accessed: 04 February 2016.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/. Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. United States Environmental Protection Agency, Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/. Accessed: May–September, 2015.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Canberra, Australia.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Wilson PC, Whitwell T and Klaine SJ (2000). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*, *Archives of Environmental Contamination and Toxicology* 39, 282–288.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

6 Imazapic

6.1 Introduction

Imazapic is a herbicide (C14H17N3O3 and Figure 20) that is in the form of an off-white to tan, odourless powder. It is the active ingredient of a variety of commercial herbicide formulations. Imazapic is often mixed with other herbicides including diquat and glyphosate (National Centre for Biotechnology Information, 2014) and additives such as surfactants to increase its efficacy.
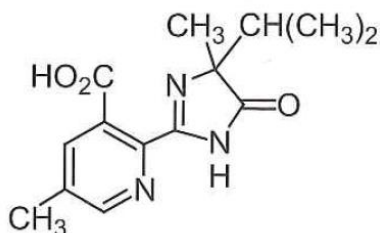
Figure 20 Structure of imazapic

Physicochemical properties of imazapic that may affect its environmental fate and toxicity are presented in Table 23.

Table 23 Summary of selected physicochemical properties of imazapic.

Physicochemical property	Value
Molecular weight	275.3 amu[1]
Aqueous solubility	2150 mg/L @ temperature 25 ºC[1] 2230 mg/L @ temperature 20 ºC[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	0.393 @ pH 4, 5, 6 and temperature 25 ºC[1] 2.47 @ pH 7 and temperature 20 ºC[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.14[2]
Logarithm of the bioconcentration factor (log BCF)	3[3] or low[2]
Half-life ($t_{1/2}$) in water	< 8 hours[1] < 8 hours[4]
Half-life ($t_{1/2}$) in soil	31 – 410 days depending on soil and climatic conditions[1] Typical: 120 days, In field: 232 days[2]

[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] National Centre for Biotechnology Information (2014). [4] Tu et al. (2001).

Imazapic belongs to the imidazolinone group of herbicides, which also includes imazaquin, imazapyr, and imazethapyr. Imazapic is extensively used in agricultural and industrial applications. In agriculture, it is used for the control of annual and perennial broadleaf grasses and some broad-leaved weeds in peanut crops, rangeland and non-cropped areas (University of Hertfordshire 2013). However, it does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013). It is a selective herbicide and can be used for pre- and post-emergent control (Tu et al. 2001).

Imazapic is mainly absorbed through the roots and shoots of plants, and is transported to the vascular tissues where it exerts its toxicity (Tu et al. 2001). Imazapic acts by inhibiting the acetohydroxy acid synthase (AHAS), which is an enzyme responsible for catalysing the formation of

three branched-chain aliphatic amino acids (leucine, valine and isoleucine) within target plants (Tu et al. 2001). Plants ultimately face a slow death due to inhibition of protein synthesis and cell growth as a result of the limited amino acids available to the plant (Tu et al. 2001).

Imazapic binds weakly to soil particles and has little adsorption to suspended soils, however has high aqueous solubility (Table 23) which would suggest that imazapic is moderately mobile. It is readily metabolised by soil microorganisms, with some capacity to leach to groundwater and end up in surface waters (Tu et al. 2001).

Imazapic is persistent in soils with half-lives ranging from 31 to 410 days depending on the soil type and climatic conditions it is exposed to (BCPC 2012; Tu et al. 2001) (Table 23). However, it is rapidly degraded by sunlight in the aquatic environment with aqueous hydrolysis ($t_{1/2}$) occurring at approximately 7.2 hours (University of Hertfordshire 2013).

Imazapic is persistent in soils with half-lives ranging from 31 to 410 days depending on the soil type and climatic conditions it is exposed to (BCPC 2012; Tu et al. 2001) (Table 23). However, it is rapidly degraded by sunlight in the aquatic environment with aqueous hydrolysis ($t_{1/2}$) occurring at approximately 7.2 hours (University of Hertfordshire 2013).

6.2 Freshwater

6.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for imazapic in freshwaters (Table 25) includes toxicity data for three freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, one cladoceran, one macrophyte and five microalgae. The toxicity values for the fish were 32-day NOEL and LOEC (mortality) values of 96,000 and >96,000 µg/L. The toxicity values for the cladoceran were 21-day NOEL and LOEC (length and dry weight) values of 96,000 and >96,000 µg/L. The toxicity values for the macrophyte were 14-day NOEL and EC50 (frond number, frond size, dry weight) values of 2.58 and 4.23 µg/L. The toxicity values for the microalgae consisted of 3-day EC10 and EC50 (cell density) values all of >1,100 µg/L, 5-day EC50 (biomass yield, growth rate, area under the curve) values ranging from 46.4 to 67.4 µg/L.

Freshwater Acute

There was a freshwater acute toxicity datum for only one cladoceran which was a 48-day EC50 (length and dry weight) value of >100,000 µg/L.

6.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of imazapic. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect

its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of imazapic (Table 23).

6.2.3 Guideline derivation

The PGVs for imazapic in freshwaters are provided in Table 24. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for imazapic are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for imazapic are low (Table 23) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for imazapic do not need to account for secondary poisoning.

Table 24 Proposed aquatic ecosystem protection guideline values (µg/L) for imazapic for the protection of freshwater and marine ecosystems.

Imazapic proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.036 (0.014 – 8.1)	Sample size	6
95%	0.41 (0.20 – 8.9)	Type of toxicity data	Chronic EC10/NOEL and chronic estimated NOEC values
90%	1.2 (0.66 – 22)	SSD model fit	Poor
80%	4.0 (1.6 – 60)	Reliability	Very low

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

6.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for imazapic in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for imazapic to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more imazapic toxicity data available that enable the calculation of PGVs in freshwaters (see section 6.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of imazapic to freshwater phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were freshwater toxicity data for 10 species (six phyla and six classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata, Cyanobacteria, Mollusca and Tracheophyta. The seven classes represented were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria) and Liliopsida (monocots).

Based on the current understanding of the mode of action of imazapic, an AHAS-inhibiting herbicide, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The imazapic ecotoxicity data for phototrophs and heterotrophs were then tested using the non-parametric Mann-Whitney test to see if the toxic responses among different taxa were uni- or multi-modal. The Mann-Whitney test indicated that the two groups had significantly different (p = 0.001, see section 6.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

At the time of searching the literature, papers determining the toxicity of imazapic to aquatic organisms were few in number and those that were available, did not contain data that were suitable for use. The freshwater data presented in section 6.3.6 were all extracted from either, the Office of the Pesticide Program (USEPA 2015b) or Stone (2016). The data extracted from the Office of the Pesticide Program (USEPA 2015b) are derived from reports from commercial laboratories for the purpose of product registration. The USEPA (2015b) follows strict quality assurance and quality check procedures within their organisation to ensure only high quality ecotoxicology data is reported and used. It was assumed that the toxicity data in the unpublished studies were the equivalent of either high or acceptable quality and were therefore considered usable in the derivation of PGVs for imazapic.

There were freshwater chronic 10% effect concentration (EC10), no observed effect level (NOEL) and chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) data for six phototrophic species, which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 24) combined with the poor fit of the distribution to these toxicity data (Figure 21) resulted in a low reliability set of PGVs. However, as the majority (five out of six) of the toxicity values used in the SSD are presented as 'greater than' values (see section 6.3.6), the reliability rating of the PGVs was reduced to very low reliability. The methods of Warne et al. (2015) clearly state that 'greater than, >' toxicity values can be used provided that, 1) there are no available normal (not '>' or '<') values for the same combination of species, measure and endpoint; and 2) they are used in the following manner, e.g. > 50 µg/L would be changed to 50 µg/L in all subsequent calculations. The reasons such data are acceptable for use is that they provide environmental protective estimate of the toxicity. A summary of the toxicity data (one value per species) used to calculate the PGVs for imazapic in freshwater environments is provided in Table 25.

Table 25 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for imazapic in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	13.48	USEPA (2015b)
Macrophyte	*Lemna gibba*	Traecheophyta	Liliopsida	Not stated	14	Chronic NOEC	Frond number, frond size, dry weight	2.58	USEPA (2015b)
Microalga	*Monoraphidium arcuatum**	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic EC10	Cell density	1,100	Stone (2016)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	9.28	USEPA (2015b)
Microalga	*Pediastrum duplex meyen**	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic EC10	Cell density	1,100	Stone (2016)
Microalga	*Selenastrum capricornutum*[2]	Chlorophyta	Chlorophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	10.46	USEPA (2015b)

[1] **Chronic NOEC/NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC values that were converted to chronic NOEC values by dividing by 5 (Warne et al. 2015).** [2] **This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.** [3] **AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.**

6.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the six freshwater phototrophic species that was used to derive the PGVs is presented in Figure 21.

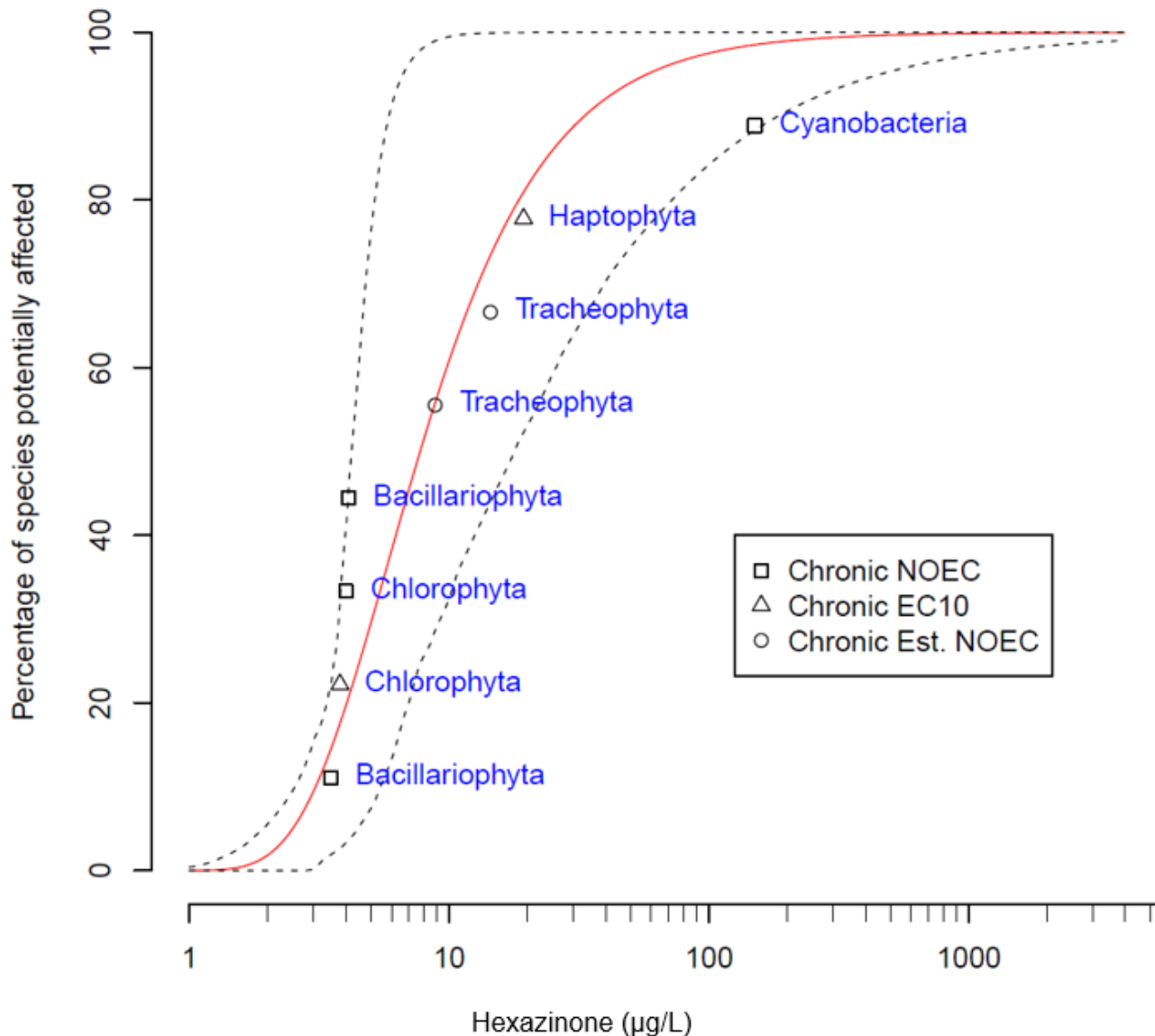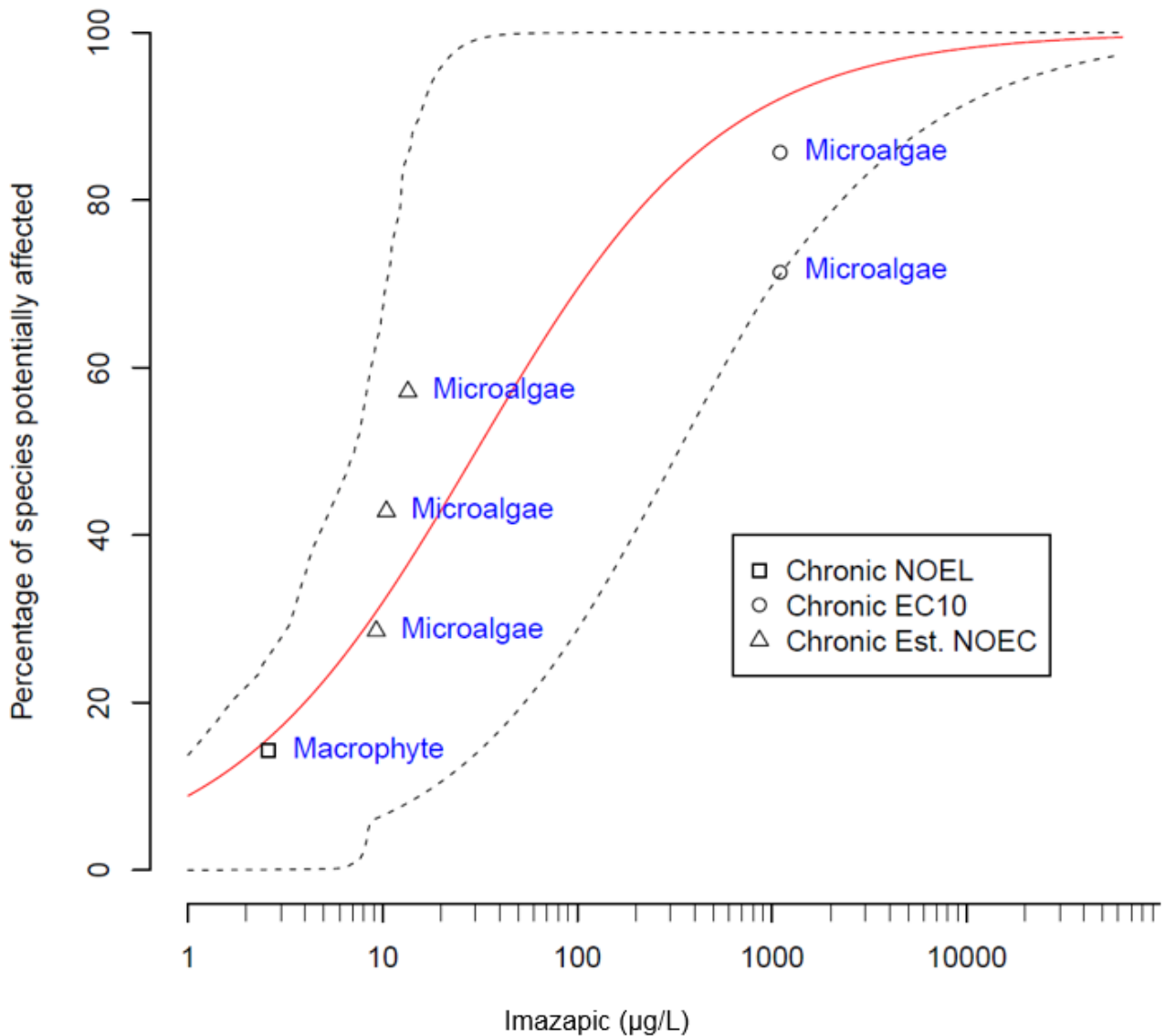Figure 21 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic 10% effect concentration (EC10), no observed effect level (NOEL) and chronic estimated no observed effect concentration (NOEC) values of freshwater phototrophic species to imazapic. Black dashed lines indicate the 95% confidence intervals.

6.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for imazapic in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Diatom (*Navicula pelliculosa*)	Not stated	5	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	>46.4	USEPA (2015b)
										46.4[2]	*GEOMETRIC MEAN*
										9.28[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Monoraphidium arcuatum*)	Exponential growth phase	3	Chronic	EC10 (Cell density)	0.45 µm filtered synthetic softwater	27 ± 2	7.5 ± 2	>1,100	Stone (2016)
										1,100[2]	*GEOMETRIC MEAN*
										1,100	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Pediastrum duplex meyen*)	Exponential growth phase	3	Chronic	EC10 (Cell density)	0.45 µm filtered synthetic softwater	27 ± 2	7.5 ± 2	>1,100	Stone (2016)
										1,100[2]	*GEOMETRIC MEAN*
										1,100	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[3]*)	Not stated	5	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	>52.3	USEPA (2015b)
										52.3[2]	*GEOMETRIC MEAN*
										10.46[@]	***VALUE USED***

											IN SSD
Cyanobacteria	Cyanophyceae	Microalga (*Anabaena flos-aquae*)	Not stated	5	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2	7.5 ± 0.1	>67.4	USEPA (2015b)
										67.4[2]	*GEOMETRIC MEAN*
										13.48[@]	*VALUE USED IN SSD*
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	14	Chronic	NOEL (Frond number, frond size, dry weight)	M - Hoagland's or 20X-AAP nutrient media/ASTM Type I water	25 ± 2	(4.8-5.2 for M-Hoagland's / 7.5 ± 0.1 for 20X-AAP)	2.58	USEPA (2015b)
										2.58	*GEOMETRIC MEAN*
										2.58	*VALUE USED IN SSD*

[1] AUC = area under the growth curve. [2] In calculating the Geometric mean censored (< or >) values were treated as absolute values (e.g. > 320 μg/L became 320 μg/L). [3] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [@] Values were chronic LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).

6.3 Marine

6.3.1   Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for imazapic in marine waters (Table 27) includes toxicity data to four species (three freshwater and one marine) that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine and freshwater species that passed the screening and quality assurance processes are provided below and in section 6.2.1, respectively.

Marine Chronic

There was a marine chronic toxicity datum for only one microalga which was a 5-day EC50 (biomass yield, growth rate, area under the curve) of >45 µg/L.

Marine Acute

There were marine acute toxicity data for one fish and two macroinvertebrates. The toxicity data of the fish consisted of 96-hour NOEL and LC50 (mortality) values of 987,000 and >987,000 µg/L. The toxicity data for the macroinvertebrates were 96-hour NOEL and EC/LC50 (mortality, abnormal development) values ranging from 99,200 to 97,700 µg/L and > 99,200 and >99,700 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

6.3.2   Factors affecting toxicity

No factors have been reported as modifying the toxicity of imazapic. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of imazapic (Table 23).

6.3.3   Guideline derivation

The PGVs for imazapic in marine waters are provided in Table 26. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for imazapic are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for imazapic are low (Table 23) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for imazapic do not need to account for secondary poisoning.

Table 26 Proposed aquatic ecosystem protection guideline values (µg/L) for imazapic for the protection of marine ecosystems.

Imazapic proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.049 (0.015 – 3.6)	Sample size	7
95%	0.44 (0.25 – 8.0)	Type of toxicity data	Chronic EC10/NOEL and chronic estimated NOEC values (*freshwater and marine*)
90%	1.2 (0.71 – 17)	SSD model fit	Poor
80%	3.6 (1.9 – 53)	Reliability	Very low

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

6.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for imazapic in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for imazapic to marine and freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more imazapic toxicity data available that enable the calculation of PGVs in marine waters. However, it was only possible to derive PGVs by using ecotoxicity data for a mixture of both marine and freshwater organisms (see section 6.3.6 and 6.2.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to marine ecosystems separately, it is recommended that additional chronic toxicity tests of imazapic with marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were marine toxicity data for four species (four phyla and four classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chordata and Mollusca. The four classes represented were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Malacostraca (a larger grouping of crustaceans) and Mediophyceae (an algae grouping).

Based on the current understanding of the mode of action of imazapic, an AHAS-inhibiting herbicide, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The imazapic ecotoxicity data for phototrophs and heterotrophs were tested using the non-parametric Mann-Whitney test to see if the toxic responses among different taxa were were uni- or multi-modal. The Mann-Whitney test indicated that the two groups had significantly different (p = 0.001, see section 6.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

At the time of searching the literature, papers determining the toxicity of imazapic to aquatic organisms were few in number and those that were available, did not contain data that were suitable for use. The marine and freshwater data presented in section 6.3.6 and 6.2.6, respectively, were all extracted from either, the Office of the Pesticide Program (USEPA 2015b) or Stone (2016). The data extracted from the Office of the Pesticide Program (USEPA 2015b) are derived from reports from

commercial laboratories for the purpose of product registration. The USEPA (2015b) follows strict quality assurance and quality check procedures within their organisation to ensure only high quality ecotoxicology data is reported and used. It was assumed that the toxicity data in the unpublished studies were the equivalent of either high or acceptable quality and were therefore considered usable in the derivation of PGVs for imazapic.

There were marine chronic estimated no observed effect concentration (NOEC) (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) data available for only one phototrophic species (that belonged to one phylum and one class) which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). As no other ecotoxicity data for imazapic for marine phototrophic species were available, the single chronic estimated NOEC value for marine phototrophic species was combined with the available chronic 10% effect concentration (EC10), chronic no observed effect level (NOEL) and chronic estimated NOEC values for freshwater phototrophic species (see section 6.2) to derive PGVs for imazapic in marine waters. This dataset incorporated concentration data for seven (one marine and six freshwater) phototrophic species belonging to four phyla and five classes, which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 26) combined with the poor fit of the distribution to these toxicity data (Figure 22) resulted in a low reliability set of PGVs. However, as the majority (six out of seven) of the toxicity values used in the SSD are presented as 'greater than' values (see section 6.3.6 and 6.2.6), the reliability rating of the PGVs was reduced to very low reliability. The methods of Warne et al. (2015) clearly state that 'greater than, >' toxicity values can be used provided that, 1) there are no available normal (not '>' or '<') values for the same combination of species, measure and endpoint; and 2) they are used in the following manner, e.g. > 50 µg/L would be changed to 50 µg/L in all subsequent calculations. The reasons such data are acceptable for use is that they provide environmental protective estimate of the toxicity. A summary of the toxicity data (one value per species) used to calculate the PGVs for imazapic in marine environments is provided in Table 27.

Table 27 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for imazapic in marine waters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Fresh	Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	13.48	USEPA (2015b)
Fresh	Macrophyte	*Lemna gibba*	Traecheophyta	Liliopsida	Not stated	14	Chronic NOEC	Frond number, frond size, dry weight	2.58	USEPA (2015b)
Fresh	Microalga	*Monoraphidium arcuatum**	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic EC10	Cell density	1,100	Stone (2016)
Fresh	Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	9.28	USEPA (2015b)
Fresh	Microalga	*Pediastrum duplex meyen**	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic EC10	Cell density	1,100	Stone (2016)
Fresh	Microalga	*Selenastrum capricornutum*[2]	Chlorophyta	Chlorophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	10.46	USEPA (2015b)
Marine	Microalga	*Skeletonema costatum**	Bacillariophyta	Mediophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	9	USEPA (2015b)

[1] Chronic NOEC/NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC values that were converted to chronic NOEC values by dividing by 5 (Warne et al. 2015). [2] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [3] AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.

6.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the seven marine and freshwater, phototrophic species that was used to derive the PGVs is presented in Figure 22.
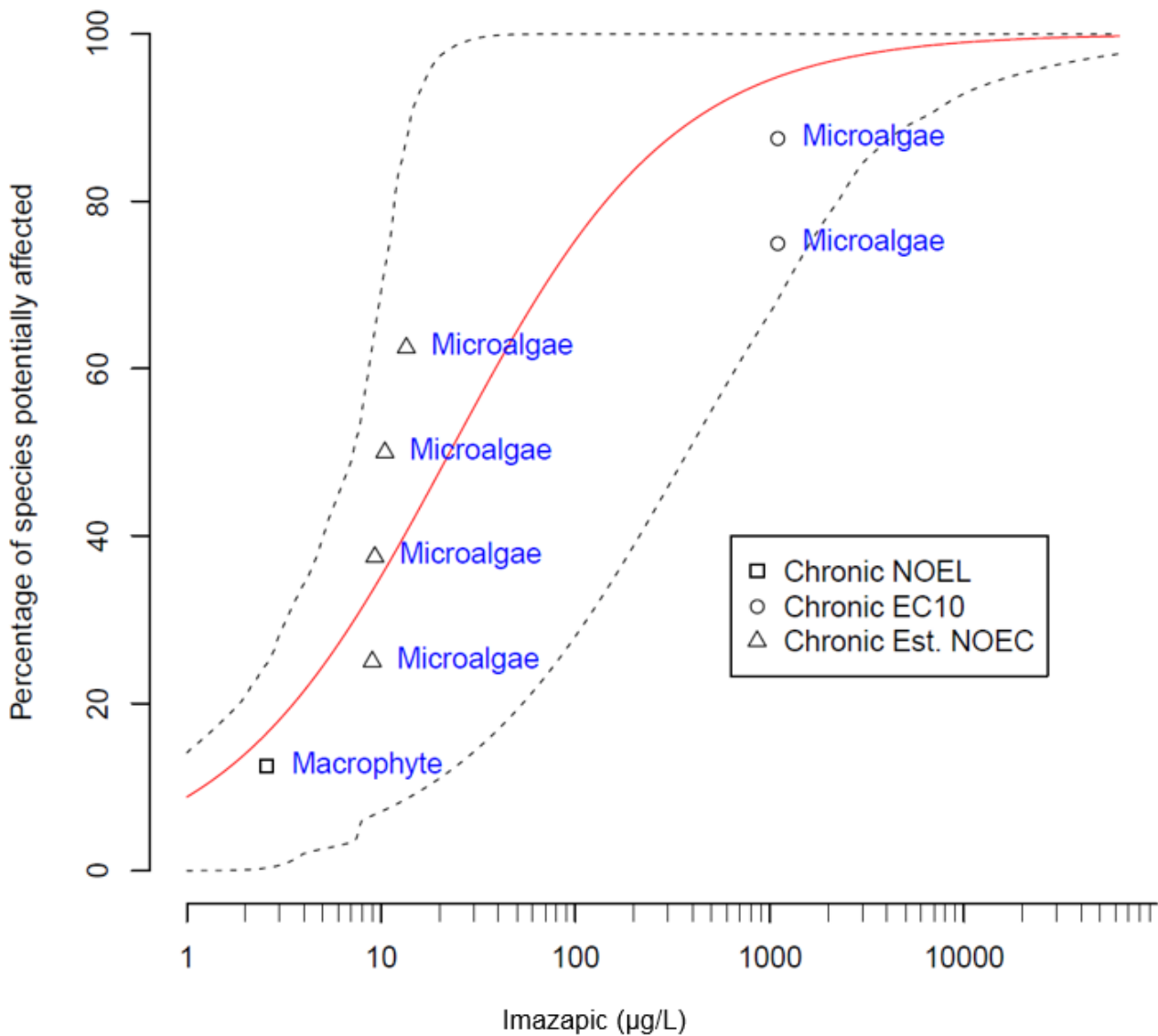
Figure 22 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic 10% effect concentration (EC10), no observed effect level (NOEL) and chronic estimated no observed effect concentration (NOEC) values of marine and freshwater phototrophic species to imazapic. Black dashed lines indicate the 95% confidence intervals.

6.3.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for imazapic in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Mediophyceae	Microalga (*Skeletonema costatum*)	Not stated	5	Chronic	EC50 (Biomass yield, growth rate, AUC[1])	Synthetic salt water or Filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	>45	USEPA (2015b)
											45[2]	*GEOMETRIC MEAN*
											9[@]	***VALUE USED IN SSD***

[1] AUC = area under the growth curve. [2] In calculating the Geometric mean censored (< or >) values were treated as absolute values (e.g. > 320 µg/L became 320 µg/L). [@] Values were chronic LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).

6.3.7   Distribution of sensitivities for aquatic species

The transformed ecotoxicity data for marine species ($n = 1$) fell within the lower and upper 95% confidence intervals [-1.467 and 8.844 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater species ($n = 6$). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for imazapic.

The toxicity data for imazapic to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the imazapic ecotoxicity data may be bimodal (Figure 23).
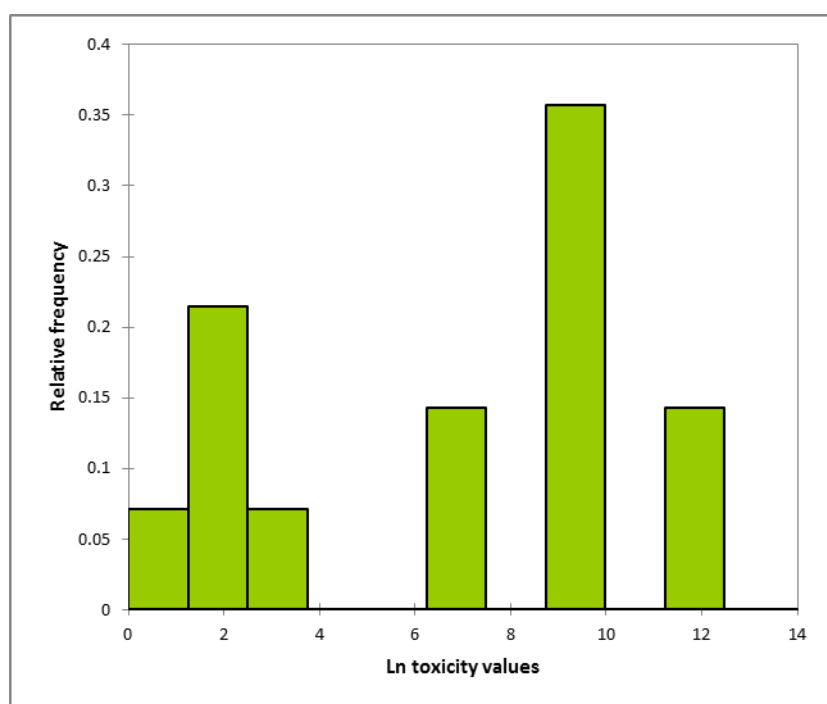
Figure 23 Histogram of the natural logarithmic (ln) of all imazapic (freshwater and marine) toxicity data for phototrophic and non-phototrophic species ($n = 14$).

The imazapic ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the non-parametric Mann-Whitney test was used because the transformed imazapic concentration data had equal variances (Fisher's F-Test; p = 0.0072) but did not follow a normal distribution (Anderson-Darling; p = 0.013). Results from the Mann-Whitney test indicated that the two groups were significantly different (p = 0.002); therefore, it was concluded that the distribution of the imazapic concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

6.3.8 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A world compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 630–631.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

National Center for Biotechnology Information (2014). PubChem Compound Database; Imazapic CID=91770, Available from: https://pubchem.ncbi.nlm.nih.gov/compound/91770, Accessed 21 November, 2015.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Stone S (2016). Development and application of a multispecies microalgal toxicity test to assess the toxicity of herbicides in Northern Queensland catchments. University of Wollongong, Australia.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Tu M, Hurd C and Randall JM (2001). Weed Control Methods Handbook: Tools and Techniques for Use in Natural Areas. The Nature Conservancy, Virginia, United States of America, 135–141pp. Available from: http://www.invasive.org/gist/products/handbook/methods-handbook.pdf. Accessed: 26–28 October 2015.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: February–April, 2016.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm Accessed 13 May 2016.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, Australasian Journal of Ecotoxicology, 4, 93–144.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

7 Imidacloprid

7.1 Introduction

Imidacloprid in an insecticide ($C_9H_{10}ClN_5O_2$ and Figure 24) that at room temperature is in the form of colourless crystals with a weak characteristic odour. It is the synthetic active ingredient of a variety of commercial insecticide formulations.
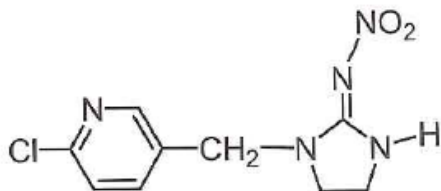
Figure 24 Structure of imidacloprid.

Physicochemical properties of imidacloprid that may affect its environmental fate and toxicity are presented in Table 28.

Table 28 Summary of selected physicochemical properties of imidacloprid.

Physicochemical property	Value
Molecular weight	255.7 amu[1]
Aqueous solubility	0.61 g/L @ temperature 20 °C[1,3]
Logarithm of the octanol-water partition coefficient (log K_{ow})	0.57 @ pH 7 and temperature 20 °C[1,3]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.3–2.4[2]
Logarithm of the bioconcentration factor (log BCF)	-0.21[3]
Half-life ($t_{1/2}$) in water	30 days[3,4]
Half-life ($t_{1/2}$) in soil	191 days[3] 130–160 days[4]

[1] BCPC (2012). [2] CCME (1999). [3] Pesticide Properties Database (University of Hertfordshire 2013). [4] Tišler et al. (2009).

Imidacloprid belongs to both, the nitroguanidine and the pyridylmethylamine groups within the neonicotinoid family of insecticides. Other nitroguanidine insecticides include clothianidin and thiamethoxam, and other pyridylmethylamine insecticides include acetamiprid and thiacloprid. Imidacloprid is commonly used on domestic pets for the rapid treatment of fleas as well as on lawns/turfs and in selected agricultural applications to control sucking, soil and some biting insects (BCPC 2012). Imidacloprid is used in a variety of crops such as rice, cereals, maize, potatoes and sugar beet (University of Hertfordshire 2013). Products containing imidacloprid are licensed for use on over 140 crops in 120 countries (Jeschke et al. 2011). In Australia, imidacloprid is registered for use on a variety of land uses and has become the most commonly-applied insecticide for canegrub control in the Great Barrier Reef catchment area (APVMA 2014; Davis et al. 2008). Neonicotinoids such as imidacloprid are the most heavily used insecticides worldwide (Bonmatin et al. 2015). Estimates of the total amount of imidacloprid manufactured globally include 5,450 tonnes in 2008 (Pollack 2011) and 20,800 tonnes in 2015 (CCM International 2016).

Imidacloprid is a residual insecticide used for seed treatment, and foliar and soil applications (BCPC 2012). It is absorbed through the leaves of plants following foliar application, and by the roots following soil application and then translocated acropetally (i.e. movement upwards from the base of the plants to the apex) because of its high mobility in the xylem (Chauhan et al. 2013, Elbert 2008).

This systemic action makes imidacloprid most effective at controlling insects with piercing/sucking mouthparts, such as rice-, leaf- and plant hoppers, aphids, thrips and whiteflies that feed within the vascular system of plants (BCPC 2012, University of Hertfordshire 2013). Imidacloprid also has translaminar activity (i.e. it penetrates the leaf tissues and forms a reservoir of active ingredient within the leaf) which provides pronounced residual protection against certain other foliar-feeding insects and mites that don't otherwise feed within vascular tissues of the plant (Chauhan et al. 2013, Elbert 2008).

Imidacloprid interferes with normal neurotransmission through the nicotinic acetylcholine receptor (nAChR) within an organism (Buckingham et al. 1997; Suchail et al. 2000). Specifically, imidacloprid competes with acetylcholine (ACh) (a neurotransmitter) at the α-subunit of the nACh receptor (nAChR) (Tomizwa et al. 1995). The agonistic action of imidacloprid activates the sodium ion channel in much the same way that Ach does; however, this activation appears to be irreversible and ultimately inhibits normal neurotransmission (Tomizwa et al. 1995). The toxicity of neonectinoids to bees is hotly debated in the literature and these insecticides have been implicated in the collapse of bee colonies. As a result of these concerns and the importance of bees to agriculture, the European Commission restricted the use of clothiadin, thiamethoxam and imidacloprid within the European Union in December 2013 (EC 2013). The European Commission is currently revisiting these restrictions. Meanwhile the USEPA has completed a preliminary assessment of the potential harmful effects of imidacloprid insecticides to pollinators (USEPA and California Dept. of Pesticide Regulation 2016) as the first step in reviewing the registration of neonectinoids in the USA. Such potential restrictions could have a major impact on the volumes of imidacloprid applied globally.

Imidacloprid is a broad spectrum, synthetic nitromethylene derivative that exhibits very high solubility in water (Table 28). Information on the fate of imidacloprid in soils is variable. It has a medium to high soil adsorption ability as indicated by its log K_{oc} value (Table 28) which would suggest a moderate potential to leach in soil. However, imidacloprid has also been reported as being relatively immobile in soils (BCPC 2012, Krohn and Hellpointner 2002, University of Hertfordshire 2013) indicating a low potential to leach into groundwater.

Imidacloprid may ultimately end up in aquatic environments as a result of spray drift or via run-off after application (Tišler et al. 2009). In aquatic systems, imidacloprid is unlikely to bioaccumulate as indicated by the very low log K_{ow} value (Table 28). Imidicloprid is non-volatile and highly persistent in soils with a half-life ($t_{1/2}$) of 191 days (Table 28) and thus it retains its biological effectiveness in soil long after application. One study (Masters et al. 2014) detected imidacloprid in leachate approximately 2.5 years after the time of application.

Imidacloprid has been detected in groundwaters, wetlands, creeks and rivers, estuaries, flood plumes and marine environments. Australian figures from 2011–15 show that imidacloprid has been detected in approximately 50% of surface water samples in waterways that drain agricultural land and discharge to the Great Barrier Reef (based on data in Turner et al. 2013a, 2013b; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015). Imidacloprid is also present in marine waters, with figures from 2011-2014 indicating that imidacloprid has been detected in approximately 3% of marine samples (maximum concentration 0.09 µg/L) in the Wet Tropics region - off the coast of northern Queensland, Australia (O'Brien et al. 2015).

7.2 Freshwater

7.2.1   Aquatic Toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for imidacloprid in freshwaters (Table 30) includes toxicity data for three freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, four insects, two cladocerans, one crustacean and one microalga. The single toxicity value for the fish species was a 98-day LOEC (mortality) value of 1,200 µg/L. The toxicity values for the insects were two 10-day EC25 (dry weight, survival) values of 2.08 and 3.12 µg/L, respectively, 10-day EC50/LC50 (head capsule width, dry mass, survival) values ranging from 1.04 to 16.6 µg/L, two 14-day NOEC (dry weight, survival) values of 1.17 and 3.57 µg/L, respectively, and a 14-day LOEC (dry weight, survival) value of 3.67 µg/L. The toxicity values for crustaceans consisted of 7-day EC20 and EC50 (mortality) values of 27,600 and 40,170 µg/L, respectively, a 8-day NOEC (offspring per female) value of 19.15 µg/L, two 8-day LOEC (growth rate, final number of individuals) values of 0.282 and 170.4 µg/L, respectively, 9-day EC13.3 and EC50 (mortality) values of 27,600 and 37,360 µg/L, respectively, 15-day EC10 and EC50 (mortality) values of 27.6 and 34.76 µg/L, respectively, 21-day NOEC/NOEL/EC10 (immobilisation, days to first brood, broods per adult, brood size, neonates per adult, cumulative no. offspring, body length, mortality) values ranging from 1,250 µg/L to 20,000 µg/L, 21-day LOEC (immobilisation, days to first brood, broods per adult, brood size, neonates per adult, cumulative no. offspring, body length, mortality) values ranging from 2,500 to 40,000 µg/L, and 21-day EC50 (cumulative no. offspring, mortality) values ranging from 5,500 and 10,000 µg/L. The toxicity values for the crustaceans were two 10- and 28-day NOEC (survival, dry weight) values of 3.44 and 11.95 µg/L, 10- and 28-day LOEC/EC25 (survival, dry weight) values ranging from 2.31 to 11.95 µg/L and 10- and 28-day EC50 (survival, dry weight) values ranging from 7.01 and 10.31 µg/L. The toxicity values for the single microalga species were a 4-day NOEL (biomass yield, growth rate, area under the curve) value of 10,000 µg/L and 3-day IC10 and IC50 (cell count) values of 106,000 and 389,000 µg/L, respectively.

Freshwater Acute

There were freshwater acute toxicity data for three fish, three amphibians, seven insects, five cladocerans, seven crustaceans, two annelid worms and one nematode. The toxicity values for the fish consisted of 96-hour NOEL (mortality) values ranging from 25,000 to 52,100 µg/L, a 96-hour LC10 (mortality) of 201,000 µg/L and two 96-hour LC50 (mortality) values of 241,000 and 229,100 µg/L. The toxicity values for the amphibian species were 1-, 2-, 3- and 4-day LC50 values ranging from 82,000 to 269,000 µg/L. The toxicity values for the insects were 1-, 2- and 4-day EC50/LC50 (mortality, immobilisation) values ranging from 0.65 to 45 µg/L, 4-day NOEC, LC25 and LOEC (survival) values of 1.03, 2.46 and 4.39 µg/L, respectively. The toxicity data for the cladocerans were a 24-hour EC10 and EC50 (immobilization) values ranging from 11,822 to 97,900 µg/L, 48-hour NOEL/EC10 (mortality, immobilisation) values of 22,500 and 42,000 µg/L, 48-hour EC50/LC50 (mortality, immobilisation) values ranging from 2.07 to 97,000 µg/L and 5-day EC20

and EC50 (mortality) values of 27,600 and 51,880 µg/L, respectively. The toxicity values for crustaceans consisted of 24-, 48- and 96-hour NOEC/NOEL/EC10 (mortality, moulted individuals) values ranging from 0.35 to 582 µg/L, 24- and 96-hour LOEC/LC25 (immobilisation, moulted individuals) values ranging from 15.73 to 255.6 µg/L, 24-, 48-, 72- and 96-hour EC50/LC50 (immobilization, mortality) values ranging from 3 to 8,760 µg/L. The toxicity values for the annelid worms consisted of a 1-day LC50 (mortality) value of 320 µg/L and a 96-hour EC50 (immobilisation) value of 6.2 µg/L, respectively. The toxicity values for the nematode consisted of 24-hour LOEC and LC50 (mortality) values of 40 and 1,580 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

7.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of imidacloprid. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of imidacloprid (Table 28).

7.2.3 Guideline derivation

The PGVs for imidacloprid in freshwaters are provided in Table 29. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for imidacloprid are expressed in terms of the concentration of the active ingredient.

One study (Tišler et al. 2009) compared the acute and chronic toxicity of the active ingredient and the commercial formulation Confidor SL 200 and found that the formulation was approximately 1.7 times more toxic with respect to its acute toxicity but half as toxic with respect to its chronic toxicity. It should be noted that Tišler et al. (2009) also found the commercial formulation (Confidor SL 200) was more toxic than the active ingredient to the alga *Desmodesmus subspicatus* and the fish *Danio rerio*. The increase in toxicity was approximately 20-fold when IC10 values of *D. subspicatus* were compared, approximately 3-fold when IC50 values were compared and 0.5-fold when IC90 values were compared. Therefore, Tišler et al. (2009) recommended that further toxicity testing of other commercial formulations of imidacloprid be conducted. Despite the increased toxicity of the commercial formulation of imidacloprid tested to algae and fish, the resulting toxicity value are still considerably larger than any of the available toxicity data for imidacloprid to arthropods. Therefore, they are unlikely to affect the following PGV derivation or decrease the validity of the values. The extremely limited amount of data available for the chronic toxicity of commercial formulations of imidacloprid to arthropod species indicates that the PGVs, based on the active ingredient concentrations, will provide adequate environmental protection. In conclusion, the recommendation by Tišler et al. (2009) is supported, but the focus should be on comparing the chronic toxicity of the active ingredient and commercial formulations to arthropods.

Measured log BCF values for imidacloprid are low (Table 28) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for imidacloprid do not need to account for secondary poisoning.

Table 29 Proposed aquatic ecosystem protection guideline values (µg/L) for imidacloprid for the protection of freshwater ecosystems.

Imidacloprid proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	0.025 (0.010 – 0.11)	Sample size	21
95%	0.074 (0.032 – 0.24)	Type of toxicity data	Chronic NOEC/LC10/EC20 and converted acute values
90%	0.14 (0.063 – 0.43)	SSD model fit	Good
80%	0.34 (0.15 – 1.0)	Reliability	Moderate

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

7.2.4   Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for imidacloprid in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for imidacloprid to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more imidacloprid toxicity data available that enable the calculation of PGVs in freshwaters (see section 7.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of imidacloprid with freshwater arthropod species (particularly crustaceans) be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for imidacloprid in freshwaters, *Tipula* sp. were included as no other toxicity data for these genera were used.

In total, there were toxicity data for 31 freshwater species (six phyla and ten classes) that passed the screening and quality assessment processes. The represented phyla were Annelida, Arthropoda, Chlorophyta, Chordata, Mollusca and Nematoda. The ten classes were Actinopterygii (which accounts for approximately 99% of fish), Adenophorea (a class of nematodes), Amphibia (tetrapod vertebrates), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of green algae), Clitellata (a class of annelid worms), Gastropoda (a grouping of molluscs), Insecta (invertebrates), Malacostraca (a large grouping of crustaceans) and Ostracoda (another grouping of crustaceans).

Based on the current understanding of the mode of action of imidacloprid, a neonicotinoid that binds to nicotinic acetylcholine receptors (nAChRs) of cells, it would be expected that arthropods (insects and crustaceans) would be more sensitive than other organisms. The imidacloprid ecotoxicity data for arthropods and non-arthropods (including phototrophs) were tested using the parametric two-sample t test to see if the toxic responses among different taxa were uni- or multi-modal. The t test indicated that the two groups had significantly different ($p < 0.0001$, see section 7.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, arthropods) were used in calculating the PGVs. In cases like these where the SSD uses the most sensitive species from a single phylum, the requirement for data representing at least four taxonomic groups is offset by the need to obtain a good fit of the SSD and reliable PGVs. This is acceptable provided that this criterion (i.e. at least five species belonging to at least four phyla) is still met for the entire dataset for the chemical (the more and less sensitive groups combined), and only if all the data of the same type as those used to derive the PGVs (in this case, chronic and converted acute data) meet both requirements (Warne et al. 2015).

There were freshwater chronic no observed effect concentration (NOEC), 10% lethal concentration (LC10) and 20% effect concentration (EC20) and converted acute (acute EC50/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 10) data available for 21 freshwater arthropod species belonging to one phylum (Arthropoda) and four classes (Branchiopoda, Insecta, Malacostraca and Ostracoda) (Table 30). The entire freshwater dataset for imidacloprid (that included chronic NOEC/LC10/EC20 data plus converted acute data) consisted of 31 arthropod ($n = 21$) and non-arthropod ($n = 10$) species that belonged to six phyla and ten classes, which successfully met the modified criterion that applies when using the most sensitive group of organisms to derive PGVs (i.e. at least five species belonging to at least four phyla). Therefore as per Warne et al. (2015), it was acceptable to derive PGVs using the chronic NOEC/LC10/EC20 and converted acute data values for the 21 freshwater arthropod species despite belonging to only one phylum (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 29) combined with the good fit of the distribution to these toxicity data (Figure 25) resulted in a moderate reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for imidacloprid in freshwater environments is provided in Table 30.

Table 30 Summary of the single toxicity value for each arthropod species that were used to derive the proposed aquatic ecosystem protection guideline values for imidacloprid in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group[1]	Species	Class	Life stage	Duration (day)	Type[2]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Macro	*Aedes aegypti**	Insecta	First instar larvae	2	Converted acute	Mortality	4.5	Song et al. (1997)
Macro	*Baetis rhodani*	Insecta	Larvae	2	Converted acute	Mortality	0.85	Beketov and Liess (2008)
Micro	*Ceriodaphnia dubia**	Branchiopoda	Third filial generation (<24 hour)	8	Chronic NOEC	Offspring per female	19.15	Chen et al. (2010)
Micro	*Ceriodaphnia reticulata**	Branchiopoda	Neonates (<24 hour)	2	Converted acute	Immobilisation	555.3	Hayasaka et al. (2012)
Macro	*Cheumatopsyche brevilineata*	Insecta	Fifth instar larvae (<24 hour)	2	Converted acute	Immobilisation	0.49	Yokoyama et al. (2009)
Macro	*Chironomus dilutus*	Insecta	Second instar larvae	40	Chronic EC20	Adult emergence	0.06	Cavallaro et al. (2016)
Macro	*Chironomus tentans*	Insecta	Larvae	28	Chronic NOEC	Survival, dry weight	1.14	Stoughton et al. (2008)
Micro	*Cypretta seurati*	Ostracoda	Not stated	2	Converted acute	Immobilisation	1.6	Sanchez-Bayo and Goka (2006)
Micro	*Cypridopsis vidua*	Ostracoda	Not stated	2	Converted acute	Immobilisation	0.3	Sanchez-Bayo and Goka (2006)
Macro	*Daphnia magna*	Branchiopoda	<24 hour	21	Chronic NOEC	Neonates per adult	1,250	Jemec et al. 2007
Macro	*Daphnia pulex*	Branchiopoda	Neonates (<24 hour)	2	Converted acute	Immobilisation	3,687.2	Hayasaka et al. (2012)
Macro	*Epeorus longimanus*	Insecta	Late instar	4	Converted acute	Mortality	0.065	Alexander et al. (2007)
Macro	*Gammarus pulex*	Malacostraca	Adult	1	Converted acute	Immobilisation	10.33	Ashauer et al. (2011)
Macro	*Gammarus roeseli*	Malacostraca	Adult	4	Converted acute	Immobilisation	0.52	Bottger et al.

								(2012)
Macro	*Hyalella azteca*	Malacostraca	Juvenile	28	Chronic NOEC	Mortality	3.44	Stoughton et al. (2008)
Micro	*Ilyocypris dentifera*	Ostracoda	Not stated	2	Converted acute	Immobilisation	0.3	Sanchez-Bayo and Goka (2006)
Micro	*Moina macrocopa*	Branchiopoda	Neonates (<24 hour)	2	Converted acute	Immobilisation	4,527.1	Hayasaka et al. (2012)
Macro	*Pteronarcys dorsata*	Insecta	Not stated	14	Chronic NOEC/LC10	Mortality	18.2	Kreutzweiser et al. (2008)
Macro	*Simulium latigonium*	Insecta	Larvae	4	Converted acute	Mortality	0.37	Beketov and Liess (2008)
Macro	*Simulium vittatum*	Insecta	Larvae	2	Converted acute	Mortality	0.81	Overmyer et al. (2005)
Macro	*Tipula sp.*	Insecta	Not stated	14	Chronic LC10	Mortality	16.2	Kreutzweiser et al. (2008)

[1] Macro = macroinvertebrate, Micro = microinvertebrate. [2] Chronic NOEC/LC10/EC20 = no conversions applied; Converted acute = acute EC50/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 10 (Warne et al. 2015). * Species that originated from/are distributed in Australia and/or New Zealand.

7.2.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 21 freshwater arthropod species that was used to derive the PGVs is presented in Figure 25.
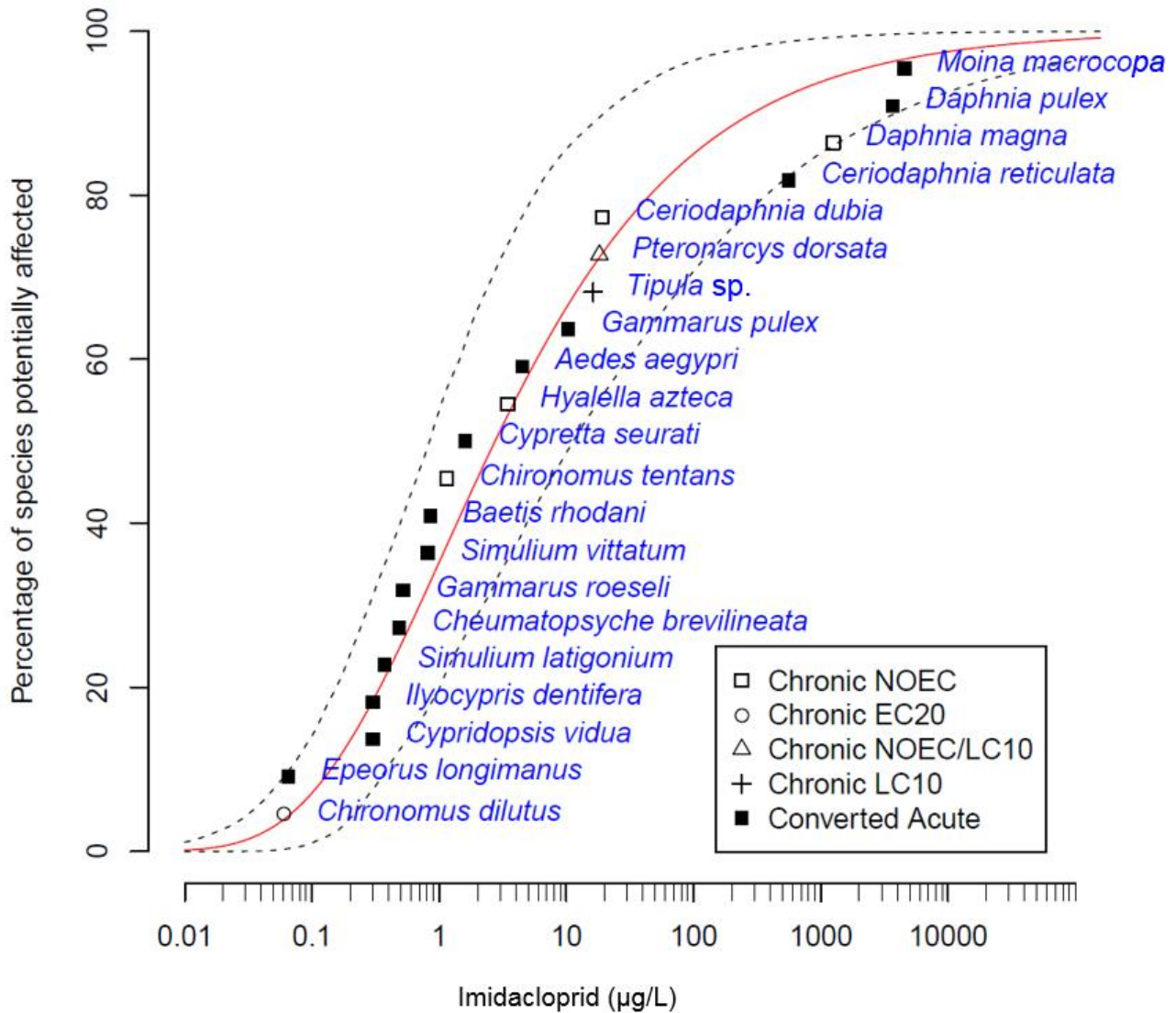
Figure 25 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC), 10% lethal concentration (LC10), 20% effect concentration (EC20) and converted acute data values of freshwater arthropod species to imidacloprid. Black dashed lines indicate the 95% confidence intervals.

7.2.6 **Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for imidacloprid in freshwaters.**

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Arthropoda	Branchiopoda	Cladoceran *(Ceriodaphnia dubia)*	Third filial generation (F3) (<24 hour)	8	Chronic	NOEC (Offspring per female)	Reconstituted dilution water	25 ± 0.1	7.4–7.8	19.15	Chen et al. (2010)
										19.15	*GEOMETRIC MEAN*
										19.15	***VALUE USED IN SSD***
Arthropoda	Branchiopoda	Cladoceran *(Ceriodaphnia reticulata)*	Neonates (<24 hours)	2	Acute	EC50 (Immobilisation)	DTW and distilled water	22 ± 1	7.92–7.84	5,552.9	Hayasaka et al. (2012)
										5,552.9	*GEOMETRIC MEAN*
										555[&]	***VALUE USED IN SSD***
Arthropoda	Branchiopoda	Cladoceran *(Daphnia magna)*	<24 hours old	21	Chronic	NOEC (Neonates per adult)	Modified M4 media	22 ± 1	Not stated	1,250	Jemec et al. 2007
										1,250	*GEOMETRIC MEAN*
										1,250	***VALUE USED IN SSD***
Arthropoda	Branchiopoda	Cladoceran *(Daphnia pulex)*	Neonates (<24 hours)	2	Acute	EC50 (Immobilisation)	DTW and distilled water	22 ± 1	7.92–7.84	36,872	Hayasaka et al. (2012)
										36,872	*GEOMETRIC MEAN*
										3,687[&]	***VALUE***

											USED IN SSD
Arthropoda	Branchiopoda	Cladoceran *(Moina macrocopa)*	Neonates (<24 hours)	2	Acute	EC50 (Immobilisation)	DTW and distilled water	22 ± 1	7.92–7.84	45,271	Hayasaka et al. (2012)
										45,271	*GEOMETRIC MEAN*
										4,527[&]	**VALUE USED IN SSD**
Arthropoda	Insecta	Yellow Fever Mosquito *(Aedes aegypri)*	First instar larvae	2	Acute	LC50 (Mortality)	M4 culture medium and pond water	20	Not stated	45	Song et al. (1997)
										45	*GEOMETRIC MEAN*
										4.5[&]	**VALUE USED IN SSD**
Arthropoda	Insecta	Mayfly *(Baetis rhodani)*	Larvae	2	Acute	LC50 (Mortality)	M7 medium and stream water	15 ± 2	7.4	8.49	Beketov and Liess (2008)
										8.49	*GEOMETRIC MEAN*
										0.85[&]	**VALUE USED IN SSD**
Arthropoda	Insecta	Cassidfly *(Cheumatopsyche brevilineata)*	5th instar larvae (<24hour)	2	Acute	EC50 (Immobilisation)	Dechlorinated tap water	20	7.55 ± 0.1	4.85	Yokoyama et al. (2009)
										4.85	*GEOMETRIC MEAN*
										0.49[&]	**VALUE USED IN SSD**
Arthropoda	Insecta	Midge *(Chironomus dilutus)*	Second instar larvae	40	Chronic	EC20 (Adult emergence)	Carbon-filtered, biofiltered City of Saskatoon	23 ± 1	8.2 ± 0.3	0.06	Cavallaro et al. (2016)

							municipal water				
										0.06	*GEOMETRIC MEAN*
										0.06	***VALUE USED IN SSD***
Arthropoda	Insecta	Midge *(Chironomus tentans)*	Larvae	28	Chronic	NOEC (Survival)	Carbon-filtered Saskatoon municipal water	23 ± 1	8.18 ± 0.21	1.14	Stoughton et al. (2008)
Arthropoda	Insecta	Midge *(Chironomus tentans)*	Larvae	28	Chronic	NOEC (Dry weight)	Carbon-filtered Saskatoon municipal water	23 ± 1	8.18 ± 0.21	1.14	Stoughton et al. (2008)
										1.14	*GEOMETRIC MEAN*
										1.14	***VALUE USED IN SSD***
Arthropoda	Insecta	Mayfly *(Epeorus longimanus)*	Late instar	4	Acute	EC50 (Mortality)	Dechlorinated ground water	20 ± 1	8.1	0.65	Alexander et al. (2007)
										0.65	*GEOMETRIC MEAN*
										0.065[&]	***VALUE USED IN SSD***
Arthropoda	Insecta	Stonefly *(Pteronarcys dorsata)*	Not stated	14	Chronic	NOEC (Mortality)	Stream water	20 ± 3	Not stated	24	Kreutzweiser et al. (2008)
Arthropoda	Insecta	Stonefly *(Pteronarcys dorsata)*	Not stated	14	Chronic	NOEC (Mortality)	Stream water	20 ± 3	Not stated	12	Kreutzweiser et al. (2008)
Arthropoda	Insecta	Stonefly *(Pteronarcys dorsata)*	Not stated	14	Chronic	LC10 (Mortality)	Stream water	20 ± 3	Not stated	20.8	Kreutzweiser et al. (2008)

										18.16	*GEOMETRIC MEAN*
										18.2	***VALUE USED IN SSD***
Arthropoda	Insecta	Blackfly *(Simulium latigonium)*	Larvae	4	Acute	LC50 (Mortality)	M7 medium and stream water	15 ± 2	7.4	3.73	Beketov and Liess (2008)
										3.73	*GEOMETRIC MEAN*
										0.37[&]	***VALUE USED IN SSD***
Arthropoda	Insecta	Blackfly *(Simulium vittatum)*	Larvae	2	Acute	LC50 (Mortality)	Moderately-hard reconstituted water	19.9–20.2	7.3–7.7	6.75	Overmyer et al. (2005)
Arthropoda	Insecta	Blackfly *(Simulium vittatum)*	Larvae	2	Acute	LC50 (Mortality)	Moderately-hard reconstituted water	19.9–20.2	7.3–7.7	8.25	Overmyer et al. (2005)
Arthropoda	Insecta	Blackfly *(Simulium vittatum)*	Larvae	2	Acute	LC50 (Mortality)	Moderately-hard reconstituted water	19.9–20.2	7.3–7.7	9.54	Overmyer et al. (2005)
										8.1	*GEOMETRIC MEAN*
										0.81[&]	***VALUE USED IN SSD***
Arthropoda	Insecta	Cranefly *(Tipula* sp.*)*	Not stated	14	Chronic	LC10 (Mortality)	Stream water	20 ± 3	Not stated	16.2	Kreutzweiser et al. (2008)
										16.2	*GEOMETRIC MEAN*
										16.2	***VALUE USED IN SSD***
Arthropoda	Malacostraca	Amphipod	Adult	1	Acute	LC50	Aerated pond	13	Not	103.29	Ashauer et

		(Gammarus pulex)				(Immobilisation)	water		stated		al. (2011)
										103.29	*GEOMETRIC MEAN*
										10.33[&]	***VALUE USED IN SSD***
Arthropoda	Malacostraca	Amphipod *(Gammarus roeseli)*	9mm Adult	4	Acute	EC50 (Immobilisation)	Stream water	12	7.6–7.8	1.9	Bottger et al. (2012)
Arthropoda	Malacostraca	Amphipod *(Gammarus roeseli)*	6mm Adult	4	Acute	EC50 (Immobilisation)	Artificial water	17	7.6–7.8	14.2	Bottger et al. (2012)
										5.19	*GEOMETRIC MEAN*
										0.52[&]	***VALUE USED IN SSD***
Arthropoda	Malacostraca	Amphipod *(Hyalella azteca)*	Juvenile	28	Chronic	NOEC (Mortality)	Carbon-filtered Saskatoon municipal water	23 ± 1	8.18 ± 0.21	3.44	Stoughton et al. (2008)
										3.44	*GEOMETRIC MEAN*
										3.44	***VALUE USED IN SSD***
Arthropoda	Ostracoda	Ostracod *(Cypretta seurati)*	Not stated	2	Acute	EC50 (Immobilisation)	Drinking tap water	22 ± 1	7.83 ± 0.44	16	Sanchez-Bayo and Goka (2006)
										16	*GEOMETRIC MEAN*
										1.6[&]	***VALUE USED IN SSD***
Arthropoda	Ostracoda	Ostracod *(Cypridopsis*	Not stated	2	Acute	EC50 (Immobilisation)	Drinking tap water	22 ± 1	7.83 ± 0.44	3	Sanchez-Bayo and

		vidua)									Goka (2006)
										3	*GEOMETRIC MEAN*
										0.3[&]	***VALUE USED IN SSD***
Arthropoda	Ostracoda	Ostracod *(Ilyocypris dentifera)*	Not stated	2	Acute	EC50 (Immobilisation)	Drinking tap water	22 ± 1	7.83 ± 0.44	3	Sanchez-Bayo and Goka (2006)
										3	*GEOMETRIC MEAN*
										0.3[&]	***VALUE USED IN SSD***

[&] **Values were acute LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 5 and 10, respectively (Warne et al. 2015).**

7.3 Marine

7.3.1 Aquatic Toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for imidacloprid in marine waters (Table 32) includes toxicity data four four species (one marine and three freshwater) that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine and freshwater species that passed the screening and quality assurance processes are provided below and in section 7.2.1, respectively.

Marine Chronic

There were no marine chronic toxicity data available in the literature.

Marine Acute

There were marine acute toxicity data for one fish, one insect, one cladoceran, three crustaceans and one mollusc. The toxicity values for the fish species consisted of NOEL and LC50 (mortality) values of 58,200 and 163,000 µg/L, respectively. The single toxicity value for the insect was a 48-hour LC50 (mortality) value of 13 µg/L. The toxicity values for the single cladoceran species were 24- and 48-hour EC50/LC50 (immobilization, mortality) values ranging from 2,209 to 161,950 µg/L. The toxicity values for the crustaceans were two 24-hour NOEC/NOEL (mortality) values of 32 and 100 µg/L, a 24-hour LOEC (mortality) value of 200 µg/L, 24- and 96-hour EC50/LC50 (mortality) values ranging from 10.04 to 1,112 µg/L. The single toxicity value for the mollusc species was a 96-hour NOEL (mortality, abnormal development) value of 145,000 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

7.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of imidacloprid. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of imidacloprid (Table 28).

7.3.3 Guideline derivation

The derived PGVs for imidacloprid in marine waters are provided in Table 31. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for imidacloprid are expressed in terms of the concentration of the active ingredient.

One study (Tišler et al. 2009) compared the acute and chronic toxicity of the active ingredient and the commercial formulation Confidor SL 200 and found that the formulation was approximately 1.7 times more toxic with respect to its acute toxicity but half as toxic with respect to its chronic toxicity. It should be noted that Tišler et al. (2009) also found the commercial formulation (Confidor SL 200) was more toxic than the active ingredient to the alga *Desmodesmus subspicatus* and the fish *Danio rerio*. The increase in toxicity was approximately 20-fold when IC10 values of *D. subspicatus* were

compared, approximately 3-fold when IC50 values were compared and 0.5-fold when IC90 values were compared. Therefore, Tišler et al. (2009) recommended that further toxicity testing of other commercial formulations of imidacloprid be conducted. Despite the increased toxicity of the commercial formulation of imidacloprid tested to algae and fish, the resulting toxicity value are still considerably larger than any of the available toxicity data for imidacloprid to arthropods. Therefore, they are unlikely to affect the following PGV derivation or decrease the validity of the values. The extremely limited amount of data available for the chronic toxicity of commercial formulations of imidacloprid to arthropod species indicates that the PGVs, based on the active ingredient concentrations, will provide adequate environmental protection. In conclusion, the recommendation by Tišler et al. (2009) is supported, but the focus should be on comparing the chronic toxicity of the active ingredient and commercial formulations to arthropods.

Measured log BCF values for imidacloprid are low (Table 28) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for imidacloprid do not need to account for secondary poisoning.

Table 31 Proposed aquatic ecosystem protection guideline values (µg/L) for imidacloprid for the protection of marine ecosystems.

Imidacloprid proposed aquatic ecosystem protection guideline values (marine)[1]	
Species protection	Concentration (µg/L) (95% CI)
99%	0.034 (0.013 – 0.13)
95%	0.099 (0.042 – 0.30)
90%	0.19 (0.087 – 0.55)
80%	0.45 (0.21 – 1.3)

Reliability classification[2]	
Criterion	Result
Sample size	26
Type of toxicity data	Chronic NOEC/LC10/EC20 and converted acute values (freshwater and marine)
SSD model fit	Good
Reliability	Moderate

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

7.3.4   Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for imidacloprid in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for imidacloprid to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more imidacloprid toxicity data available that enable the calculation of PGVs in marine waters. However it was only possible to derive PGVs by using ecotoxicity data for a mixture of both marine and freshwater organisms (see section 7.3.6 and 7.2.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to marine ecosystems separately, it is recommended that additional chronic toxicity tests of imidacloprid with marine arthropod species (particularly crustaceans) be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is

usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for imidacloprid in marine waters, *Tipula* sp. were included as no other toxicity data for these genera were used.

In total, there were toxicity data for six marine species (two phyla and four classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda and Chordata. The four classes were Actinopterygii (which accounts for approximately 99% of fish), Branchiopoda (a grouping of crustaceans), Insecta (invertebrates) and Malacostraca (a large grouping of crustaceans).

Based on the current understanding of the mode of action of imidacloprid, a neonicotinoid that binds to nicotinic acetylcholine receptors (nAChRs) of cells, it would be expected that arthropods (insects and crustaceans) would be more sensitive than other organisms. The imidacloprid ecotoxicity data for arthropods and non-arthropods (including phototrophs) were tested using the parametric two-sample t test to see if the toxic responses among different taxa were uni- or multi-modal. The t test indicated that the two groups had significantly different (p <0.0001, see section 7.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, arthropods) were used in calculating the PGVs. In cases like these where the SSD uses the most sensitive species from a single phylum, the requirement for data representing at least four taxonomic groups is offset by the need to obtain a good fit of the SSD and reliable PGVs. This is acceptable provided that this criterion (i.e. at least five species belonging to at least four phyla) is still met for the entire dataset for the chemical (the more and less sensitive groups combined), and only if all the data of the same type as those used to derive the PGVs (in this case, chronic and converted acute data) meet both requirements (Warne et al. 2015).

There were marine converted acute (acute EC50/LC50 values that were converted to chronic NOEC values by dividing by 10) data available for only six species (five arthropods belonging to one phylum and one non-arthropod belonging to one phylum), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) that usually apply when deriving PGVs using the SSD method, nor the modified criterion that applies when using the most sensitive group of organisms to derive PGVs. As no other ecotoxicity data for imidacloprid to marine species were available, the five converted acute values for marine arthropod species were combined with the available chronic no observed effect concentration (NOEC), 10% lethal concentration (LC10) and 20% effect concentration (EC20) and converted acute values for freshwater arthropod species to derive PGVs for imidacloprid in marine waters.

There were chronic NOEC/LC10/EC20 and converted acute data available for 26 marine and freshwater arthropod species belonging to one phylum (Arthropoda) and four classes (Branchiopoda, Insecta, Malacostraca and Ostracoda) (Table 32). The entire marine and freshwater dataset for imidacloprid (that included chronic NOEC/LC10/EC20 data plus converted acute data) consisted of 37 arthropod ($n = 26$) and non-arthropod ($n = 11$) species that belonged to six phyla and ten classes, which successfully meets the modified criterion that applies when using the most sensitive group of organisms to derive PGVs (i.e. at least five species belonging to at least four phyla). Therefore, as per Warne et al. (2015), it was acceptable to derive PGVs using the chronic NOEC/LC10/EC20 and converted acute data values for the 26 marine and freshwater arthropod species despite belonging to only one phylum (Warne et al. 2015). The number of species and taxa

in the toxicity data used to derive the PGVs (Table 31) combined with the good fit of the distribution to these toxicity data (Figure 26) resulted in a moderate reliability set of PGVs. The combination of freshwater and marine ecotoxicity data reduces the reliability classification of PGVs as per Warne et al. (2015). A summary of the toxicity data (one value per species) used to calculate the PGVs for imidacloprid in freshwater environments is provided in Table 32.

Table 32 Summary of the single toxicity value for each arthropod species that were used to derive the proposed aquatic ecosystem protection guideline values for imidacloprid in marine waters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group[1]	Species	Class	Life stage	Duration (days)	Type[2]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Fresh	Macro	*Aedes aegypti**	Insecta	First instar larvae	2	Converted acute	Mortality	4.5	Song et al. (1997)
Marine	Macro	*Aedes taeniorhynchus*	Insecta	First instar larvae	2	Converted acute	Mortality	1.3	Song et al. (1997)
Marine	Macro	*Americamysis bahia*	Malacostraca	Juvenile	4	Converted acute	Mortality	6.11	USEPA (2015b)
Fresh	Macro	*Baetis rhodani*	Insecta	Larvae	2	Converted acute	Mortality	0.85	Beketov and Liess (2008)
Marine	Macro	*Callinectes sapidus*	Malacostraca	Megalopae / Juvenile	1	Converted acute	Mortality	10.57	Osterberg (2010)
Fresh	Micro	*Ceriodaphnia dubia**	Branchiopoda	Third filial generation (<24 hour)	8	Chronic NOEC	Offspring per female	19.15	Chen et al. (2010)
Fresh	Micro	*Ceriodaphnia reticulata**	Branchiopoda	Neonates (<24 hour)	2	Converted acute	Immobilisation	555.3	Hayasaka et al. (2012)
Fresh	Macro	*Cheumatopsyche brevilineata*	Insecta	Fifth instar larvae (<24 hour)	2	Converted acute	Immobilisation	0.49	Yokoyama et al. (2009)
Fresh	Macro	*Chironomus dilutus*	Insecta	Second instar larvae	40	Chronic EC20	Adult emergence	0.06	Cavallaro et al. (2016)
Fresh	Macro	*Chironomus tentans*	Insecta	Larvae	28	Chronic NOEC	Survival, dry weight	1.14	Stoughton et al. (2008)
Marine	Micro	*Chydorus sphaericus**	Branchiopoda	Not stated	2	Converted acute	Immobilisation	220.9	Sanchez-Bayo and Goka (2006)
Fresh	Micro	*Cypretta seurati*	Ostracoda	Not stated	2	Converted acute	Immobilisation	1.6	Sanchez-Bayo and Goka (2006)
Fresh	Micro	*Cypridopsis vidua*	Ostracoda	Not stated	2	Converted acute	Immobilisation	0.3	Sanchez-Bayo and Goka (2006)
Fresh	Macro	*Daphnia magna*	Branchiopoda	<24 hour	21	Chronic NOEC	Neonates per adult	1,250	Jemec et al. 2007
Fresh	Macro	*Daphnia pulex*	Branchiopoda	Neonates (<24 hour)	2	Converted acute	Immobilisation	3,687.2	Hayasaka et al. (2012)

Fresh	Macro	*Epeorus longimanus*	Insecta	Late instar	4	Converted acute	Mortality	0.065	Alexander et al. (2007)
Fresh	Macro	*Gammarus pulex*	Malacostraca	Adult	1	Converted acute	Immobilisation	10.33	Ashauer et al. (2011)
Fresh	Macro	*Gammarus roeseli*	Malacostraca	Adult	4	Converted acute	Immobilisation	0.52	Bottger et al. (2012)
Fresh	Macro	*Hyalella azteca*	Malacostraca	Juvenile	28	Chronic NOEC	Mortality	3.44	Stoughton et al. (2008)
Fresh	Micro	*Ilyocypris dentifera*	Ostracoda	Not stated	2	Converted acute	Immobilisation	0.3	Sanchez-Bayo and Goka (2006)
Fresh	Micro	*Moina macrocopa*	Branchiopoda	Neonates (<24 hour)	2	Converted acute	Immobilisation	4,527.1	Hayasaka et al. (2012)
Marine	Macro	*Palaemonetes pugio*	Malacostraca	Larvae / Adult	4	Converted acute	Mortality	41.714	Key et al. (2007)
Fresh	Macro	*Pteronarcys dorsata*	Insecta	Not stated	14	Chronic NOEC/LC10	Mortality	18.2	Kreutzweiser et al. (2008)
Fresh	Macro	*Simulium latigonium*	Insecta	Larvae	4	Converted acute	Mortality	0.37	Beketov and Liess (2008)
Fresh	Macro	*Simulium vittatum*	Insecta	Larvae	2	Converted acute	Mortality	0.81	Overmyer et al. (2005)
Fresh	Macro	*Tipula sp.*	Insecta	Not stated	14	Chronic LC10	Mortality	16.2	Kreutzweiser et al. (2008)

[1] **Macro = macroinvertebrate, Micro = microinvertebrate. [2] Chronic NOEC/LC10/EC20 = no conversions applied; Converted acute = acute EC50/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 10 (Warne et al. 2015). * Species that originated from/are distributed in Australia and/or New Zealand.**

7.3.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 26 marine and freshwater, arthropod species that was used to derive the PGVs is presented in Figure 26.
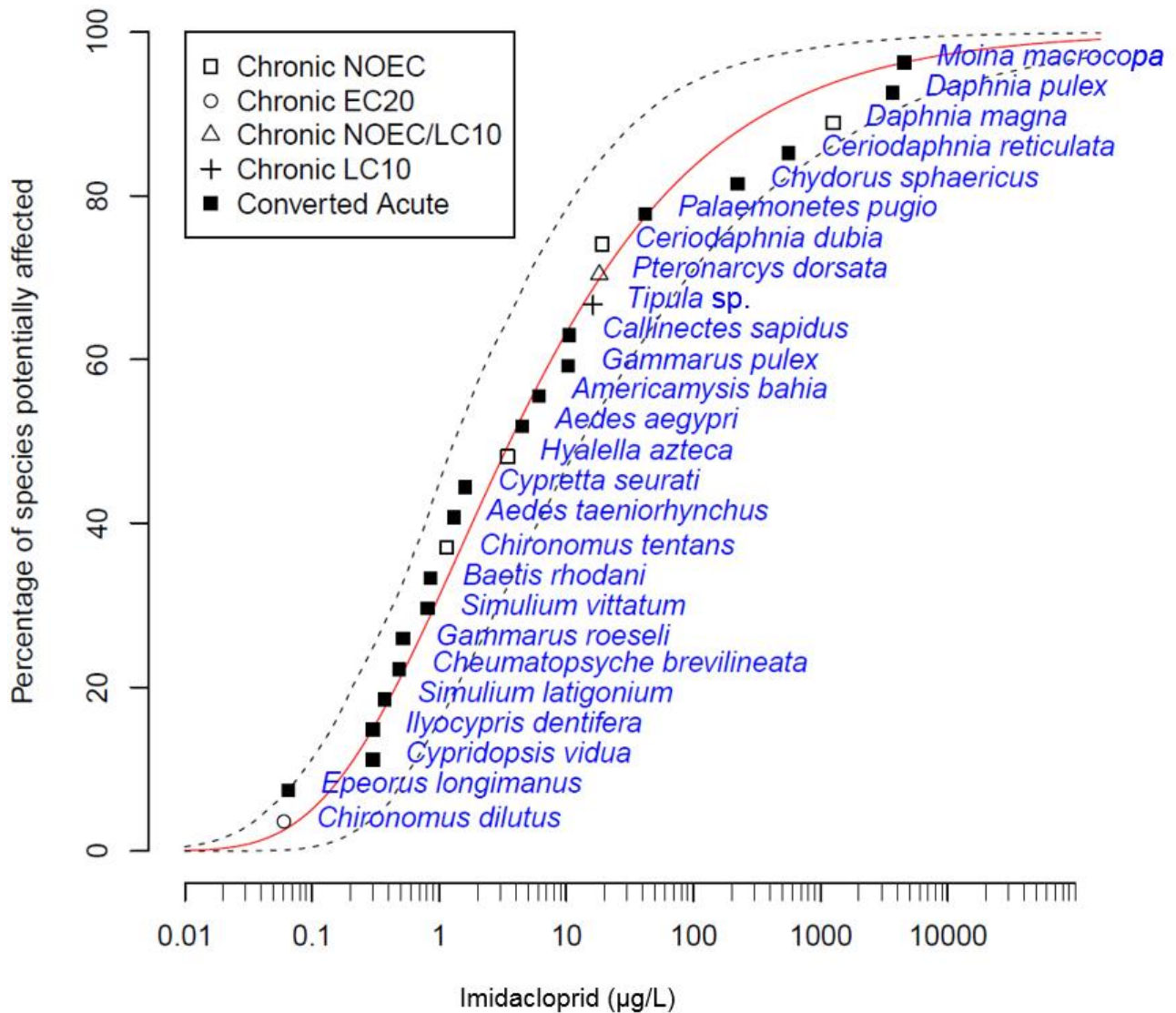
Figure 26 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC), 10% lethal concentration (LC10), 20% effect concentration (EC20) and converted acute data values of marine and freshwater arthropod species to imidacloprid. Black dashed lines indicate the 95% confidence intervals.

7.3.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for imidacloprid in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Arthropoda	Insecta	Black Salt Marsh Mosquito (*Aedes taeniorhynchus*)	First instar larvae	2	Acute	LC50 (Mortality)	Artificial sea water (ASW)	Not stated	27	8	13	Song et al. (1997)
											13	*GEOMETRIC MEAN*
											1.3[&]	***VALUE USED IN SSD***
Arthropoda	Malacostraca	Shrimp (*Americamysis bahia*)	Juvenile	4	Acute	LC50 (Mortality)	Natural or artificial filtered seawater	20 ± 3	25 ± 2	Not stated	37.7	USEPA (2015b)
Arthropoda	Malacostraca	Shrimp (*Americamysis bahia*)	Not stated	4	Acute	EC50 (Mortality)	Natural or artificial filtered seawater	20 ± 3	25 ± 2	Not stated	38	USEPA (2015b)
Arthropoda	Malacostraca	Shrimp (*Americamysis bahia*)	Not stated	4	Acute	EC50 (Mortality)	Natural or artificial filtered seawater	20 ± 3	25 ± 2	Not stated	159	USEPA (2015b)
											61.07	*GEOMETRIC MEAN*
											6.11[&]	***VALUE USED IN SSD***
Arthropoda	Malacostraca	Chesapeake Blue Crab (*Callinectes sapidus*)	Megalopae	1	Acute	LC50 (Mortality)	Aged sea water (ASW)	35	25	Not stated	10.04	Osterberg (2010)
Arthropoda	Malacostraca	Chesapeake Blue Crab (*Callinectes sapidus*)	Juvenile	1	Acute	LC50 (Mortality)	Aged sea water (ASW)	35	25	Not stated	1,112	Osterberg (2010)

										105.66	*GEOMETRIC MEAN*
										10.57[&]	***VALUE USED IN SSD***
Arthropoda	Branchiopoda	Cladoceran *(Chydorus sphaericus)*	Not stated	2	Acute	EC50 (Immobilisation)	Not stated	Not stated	22 ± 1	7.83 ± 0.44	2,209	Sanchez-Bayo and Goka (2006)
										2,209	*GEOMETRIC MEAN*
										220.9[&]	***VALUE USED IN SSD***
Arthropoda	Malacostraca	Shrimp *(Palaemonetes pugio)*	Larvae	4	Acute	LOEC (Mortality)	Salt water	20	25	Not stated	200	Key et al. (2007)
Arthropoda	Malacostraca	Shrimp *(Palaemonetes pugio)*	Larvae	4	Acute	LC50 (Mortality)	Salt water	20	25	Not stated	308.8	Key et al. (2007)
Arthropoda	Malacostraca	*Shrimp (Palaemonetes pugio)*	Adult	4	Acute	LC50 (Mortality)	Salt water	20	25	Not stated	563.5	Key et al. (2007)
										417.14	*GEOMETRIC MEAN*
										41.71[&]	***VALUE USED IN SSD***

[&] Values were acute LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 5 and 10, respectively (Warne et al. 2015).

7.3.7   Distribution of sensitivities for aquatic species: Arthropods vs. non-Arthropods

Statistical analysis of the imidacloprid ecotoxicity data for freshwater and marine species indicated that there was no difference in the sensitivities of the two groups. The parametric two-sample *t* test was used because the transformed imidacloprid freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.292) and followed a normal distribution (Anderson-Darling; p = 0.103). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.493); therefore, the freshwater and the marine imidacloprid ecotoxicity data can be pooled for further analysis.

The toxicity data for imidacloprid to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the imidacloprid ecotoxicity data may be bimodal (Figure 27).
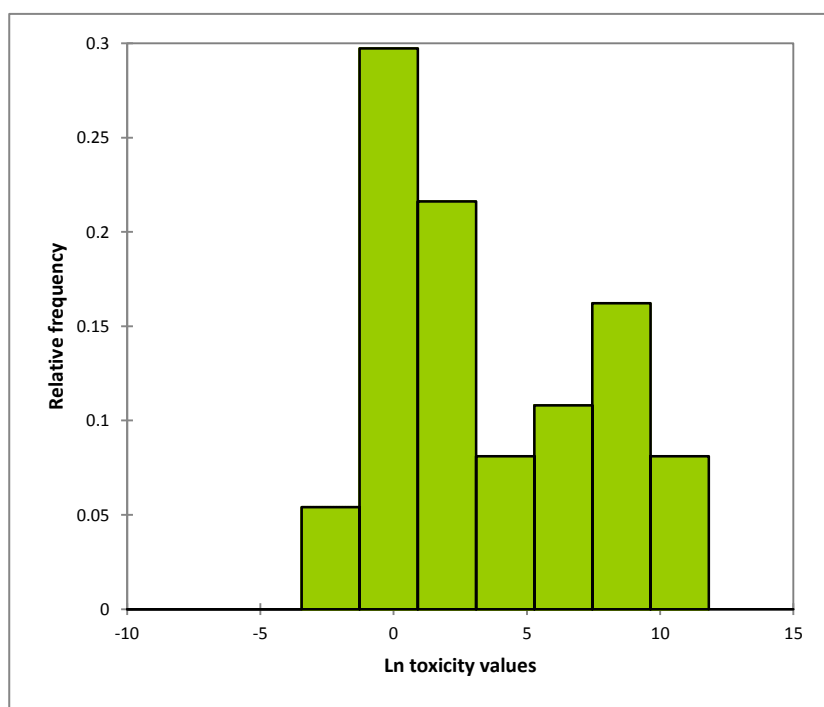
Figure 27 Histogram of the natural logarithm (ln) of all imidacloprid (freshwater and marine) toxicity data for arthropods and non-arthropods (*n* = 37).

The imidacloprid ecotoxicity data for arthropods and non-arthropods were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample *t* test was used because the transformed imidacloprid concentration data had equal variances (Fisher's F-Test; p = 0.655) and followed a normal distribution (Anderson-Darling; p = 0.103). Results from the two-sample *t* test indicated that the two groups were significantly different (p <0.0001); therefore, it can be concluded that the distribution of the arthropod vs. non-arthropod concentration data for imidacloprid is bi- or multi-modal, with arthropod species being the most sensitive group.

7.3.8 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

Alexander AC, Culp JM, Liber K and Cessna AJ (2007). Effects of insecticide exposure on feeding inhibition in mayflies and oligochaetes, *Environmental Toxicology and Chemistry*, 26, 1726–1732.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

APVMA (2014). Public chemical registration information system (PubCris), Australian Pesticides and Veterinary Medicines Authority, Australian Government. Available from: https://portal.apvma.gov.au/pubcris, Accessed: 16 August 2017.

Ashauer R, Hintermeister A, Potthoff E and Escher BI (2011). Acute toxicity of organic chemicals to *Gammarus pulex* correlates with sensitivity of *Daphnia magna* across most modes of action, *Aquatic Toxicology*, 103, 38–45.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 460–462.

Beketov MA and Liess M (2008). Potential of 11 pesticides to initiate downstream drift of stream macroinvertebrates, *Archives of Environmental Contamination and Toxicology*, 55, 247–253.

Bonmatin JM, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke C, Liess M, Long E, Marzaro M, Mitchell EAD, Noome DA, Simon-Delso N and Tapparo A (2015). Environmental fate and exposure; neonicotinoids and fipronil, *Environmental Science and Pollution Research International*, 22, 35–67.

Bottger R, Schaller J and Mohr S (2012). Closer to reality – the influence of toxicity test modifications on the sensitivity of *Gammarus roeseli* to the insecticide imidacloprid, *Ecotoxicology and Environmental Safety*, 81, 49–54.

Buckingham SD, Lapied B, Le Corronc H, Grolleau F and Sattelle DB (1997). Imidacloprid actions on insect neuronal acetylocholine receptors, *The Journal of Experimental Biology*, 200, 2685–2692.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

Cavallaro MC, Morrissey CA, Headley JV, Peru KM and Liber K (2017). Comparative chronic toxicity of imidacloprid, clothianidin and thiamethoxam to *Chironomus dilutus* and estimation of toxic equivalency factors, *Environmental Toxicology and Chemistry*, 36, 372–382.

CCM International Ltd (2016). Accessible at: https://www.researchandmarkets.com/reports/2646434/production_and_market_of_imidacloprid_in_china#pos-2. Accessed 25/11/2016.

Chauhan SS, Agrawal S and Srivastava A (2013). Effect of imidacloprid insecticide residue on biochemical parameters in potatoes and its estimation by HPLC, *Asian Journal of Pharmaceutical and Clinical Research*, 6, 114–117.

Chen XD, Culbert E, Hebert V and Start JD (2010). Mixture effects of the nonylphenyl polyethoxylate, R-11 and the insecticide, imidacloprid on population growth rate and other parameters of the crustacean, *Ceriodaphnia dubia*, *Ecotoxicology and Environmental Safety*, 73, 132–137.

Davis AM, Lewis SE, Bainbridge Z, Brodie JE and Shannon E (2008). Pesticide residues in waterways of the lower Burdekin region: challenges in ecotoxicological interpretation of monitoring data, *Australasian Journal of Ecotoxicology*, 14, 89–108.

Devlin M, Lewis S, Davis A, Smith R, Negri A, Thompson M and Poggio M (2015). Advancing our understanding of the source, management, transport and impacts of pesticides on the Great Barrier Reef 2011-2015. A report for the Queensland Department of Environment and Heritage Protection. Tropical Water & Aquatic Ecosystem Research (TropWATER) Publication, James Cook University, Cairns, 134 pp.

EC (2013) Commission implementing regulation (EU) No 485/2103. Official Journal of the European Union, European Commission. L 139/12–L139/14. Available from: http://eur-lex.europa.eu/LexUriServ/LexUriServ.do?uri=OJ:L:2013:139:0012:0026:EN:PDF. Accessed: 25/11/2016.

Elbert A, Haas M, Springer B, Thielert W and Nauen R (2008). Mini Review: Applied aspects of neonicotinoid uses in crop protection, *Pesticide Management Science*, 64, 1099–1105.

Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R, Thomson B and Warne MStJ (2015). *Total suspended solids, nutrients and pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef* – Great Barrier Reef Catchment Loads Monitoring Program 2013–2014. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 108pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2013-2014-gbr-catchment-loads-technical-report.pdf

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

Hayasaka D, Korenaga T, Suzuki K, Sanchez-Bayo F and Goka K (2012). Differences in susceptibility of five cladoceran species to two systemic insecticides, imidacloprid and fipronil, *Ecotoxicology*, 21, 421–427.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Jemec A, Tišler T, Drobne D, Sepcic K, Fournier D and Trebse P (2007). Comparative toxicity of imidacloprid, of its commercial liquid formulation and of diazinon to a non-target arthropod, the microcrustacean *Daphnia magna*, *Chemosphere*, 68, 1408–1418.

Jeschke P, Nauen R, Schindler M, Elbert A (2011) Overview of the status and global strategy for neonicotinoids. *Journal of Agriculture and Food Chemistry,* 59, 2897–2908.

Kessler E and Huss V.A.R (1992). Comparative physiology and biochemistry and taxonomic assignment of the Chlorella (Chlorophyceae) strains of the culture collection of the University of Texas at Austin*, Journal of Phycology*, 28, 550–553.

Key P, Chung K, Siewicki T and Fulton M (2007). Toxicity of three pesticides individually and in mixture to larval grass shrimp (*Palaemonetes pugio*), *Ecotoxicology and Environmental Safety*, 68, 272–277.

Kreutzweiser DP, Good KP, Chartrand DT, Scarr TA and Thompson DG (2008). Toxicity of the systemic insecticide, imidacloprid, to forest stream insects and microbial communities, *Bulletin of Environmental Contamination and Toxicology*, 80, 211–214.

Krohn J and Hellpointner E (2002). Environmental fate of imidacloprid. *Pflanzenschutz-Nachrichten Bayer*, 55, 1–25.

Masters B, Mortimore C, Armour J and Silburn DM (2014). Pesticides in groundwater of the Tully-Murray and Johnstone catchments: 2012/2013 Report, Wet Tropics Region. Queensland Department of Natural Resources and Mines.

O'Brien D, Nash M, Di Bella L and Brodie J (2015). Reef Plan Herbert Water Quality Monitoring Program (HWQMP) Final report for monitoring undertaken between 2011 and 2014. Centre for Tropical Water and Aquatic Ecosystem Research Publication, James Cook University, Townsville, Australia pp. 167. Available from: https://research.jcu.edu.au/tropwater/publications/1518ReefplanHerbertwaterqualitymonitoringprogram.pdf.

Osterberg JS (2010). Ecotoxicology of natural and anthropogenic extreme environments, PhD Thesis, Duke University, Raleigh, North Carolina, 178 pp.

Overmyer JP, Mason BN and Armbrust KL (2005). Acute toxicity of imidacloprid and fipronil to nontarget aquatic insect, *Simulium vittatum* Zetterstedt cytospecies IS-7, *Bulletin of Environmental Contamination and Toxicology*, 74, 872–879.

Pollack P (2011). Fine chemicals: the industry and the business – 2nd edition. John Wiley & Sons, New Jersey, USA.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Sanchez-Bayo F and Goka K (2006). Influence of light in acute toxicity bioassays of imidacloprid and zinc pyrithione to zooplankton crustaceans, *Aquatic Toxicology*, 78, 262–271.

Song MY, Stark JD and Brown JJ (1997). Comparative toxicity of four insecticides, including imidacloprid and tebufenozide, to four aquatic arthropods, *Environmental Toxicology and Chemistry*, 16(, 2494–2500.

Stoughton SJ, Liber K, Culp J and Cessna A (2008). Acute and chronic toxicity of imidacloprid to the aquatic invertebrates *Chironomus tentans* and *Hyalella Azteca* under constant- and pulse-exposure conditions, *Archives of Environmental Contamination and Toxicology*, 54, 662–673.

Suchail S, Guez D and Bekzunces LP (2000). Characteristics of imidacloprid toxicity in two *Apis mellifera subspecies*, *Environmental Toxicology and Chemistry*, 19(7), 1901–1905.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Tišler T, Jemec A, Mozetič B and Trebše P (2009). Hazard identification of imidacloprid to aquatic environment, *Chemosphere*, 76, 907–914.

Tomizwa M, Otsuka H and Miyamoto T (1995). Pharmacological effects of imidacloprid and its related compounds on the nicotinic acetylcholine receptor with its ion channel from the *Torpedo* electric organ, *Journal of Pesticide Science*, 20, 49–56.

Turner R, Huggins R, Wallace R, Smith R, Vardy S and Warne MStJ (2013a). *Sediment, nutrient and pesticide loads: Great Barrier Reef Loads Monitoring 2009–2010*. Water Sciences Technical Report, Volume 2012, Number 14. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0994. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2009-2010-gbr-catchment-loads-report.pdf

Turner RDR, Huggins R, Wallace R, Smith RA, Vardy S and Warne MStJ (2013b). *Total suspended solids, nutrient and pesticide loads for rivers that discharge to the Great Barrier Reef: Great Barrier Reef Loads Monitoring 2010–2011.* Water Sciences Technical Report, Volume 2013, Number 1. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0996. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2010-2011-gbr-catchment-loads-report.pdf

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm. Accessed 13 May 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/. Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/. Accessed: February–April, 2016.

USEPA (2016). Preliminary pollinator assessment to support the registration review of imidacloprid. United States Environmental Protection Agency and California Dept. of Pesticide Regulation, Office of Pesticide Programs, Washington, DC, USA. 305p. Available from: file:///C:/Users/ac2458/Downloads/EPA-HQ-OPP-2008-0844-0140.pdf. Accessed: 25/11/2016.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and Warne MStJ. 2015. *Total suspended solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef* – Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-catchment-loads-report.pdf

Wallace R, Huggins R, Smith RA, Turner RDR, Vardy S and Warne MStJ. 2014. *Total suspended solids, nutrient and pesticide loads (2011–2012) for rivers that discharge to the Great Barrier Reef* – Great Barrier Reef Catchment Loads Monitoring Program 2011–2012. Department of Science, Information Technology, Innovation and the Arts. Brisbane, Queensland. 94p. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2011-2012-gbr-catchment-loads-report.pdf

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for

toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, Australasian Journal of Ecotoxicology, 4, 93–144.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

Yokoyama A, Ohtsu K, Iwafune T, Nagai T, Ishihara S, Kobara Y, Horio T and Endo S (2009). A useful new insecticide bioassay using first-instar larvae of a net-spinning caddisfly, *Cheumatopsyche brevilineata* (Trichoptera: Hydropsychidae), *Journal of Pesticide Science*, 34, 13–20.

8 Isoxaflutole

8.1 Introduction

Isoxaflutole is a herbicide ($C_{15}H_{12}F_3NO_4S$ and Figure 28) that at room temperature is an off-white or pale-yellow solid. It is the active ingredient of a variety of commercial herbicide formulations. Isoxaflutole is often used in tank-mixes with other active ingredients (e.g. atrazine and paraquat) to improve and broaden its spectrum efficacy.
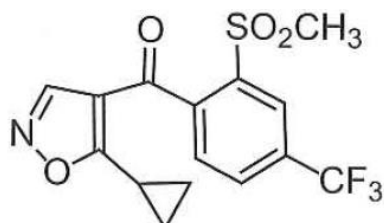
Figure 28 Structure of isoxaflutole

Physicochemical properties of isoxaflutole that may affect its environmental fate and toxicity are presented in Table 33.

Table 33 Summary of selected physicochemical properties of isoxaflutole.

Physicochemical property	Value
Molecular weight	359.3 amu[1]
Aqueous solubility	6.2 mg/L @ pH 5.5 and temperature of 20 ºC[1]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.34[1]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.05 for isoxaflutole[1] 2.04 for diketonitrile[1] 2.34 for isoxaflutole[2]
Logarithm of the bioconcentration factor (log BCF)	1.04[2]
Half-life ($t_{1/2}$) in water	11 days for isoxaflutole @ pH 4-5 and temperature 25 ºC[2] 3.2 hours for isoxaflutole @ pH 9 and temperature 25 ºC[2]
Half-life ($t_{1/2}$) in soil	1.3–2.3 days for isoxaflutole in the field and in the lab (20 ºC), respectively)[1] 11.5–45 days for dikenitrile in the field and in the lab (20 ºC), respectively)[1] Typical: 0.9 days for isoxaflutole* (0.9–1.3 days in the lab (20 ºC) and the field, respectively)[2]

[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). * In modelling exercises it should be noted that the degradation rate of this active substance is very fast and the relevant metabolite should be modelled instead (see text below).

Isoxaflutole belongs to the cyclopropylisoxazole family of herbicides, which also includes isoxachlortole. Isoxaflutole is extensively used to control broadleaf weeds and suppress annual grasses – especially amongst corn, maize and sugar cane. It is often used in tank-mixes (with paraquat and atrazine) to improve efficacy and broaden the weed-control spectrum, and is generally applied before weeds emerge (i.e. it is a pre-emergent herbicide) (BCPC 2012).

Isoxaflutole is a systemic soil applied herbicide that is mainly absorbed through the roots and leaves of plants. It is then translocated in the xylem and phloem where it exerts its toxicity (Kaur et al. 2004). Once in plants, isoxaflutole is rapidly converted to the diketonitrile metabolite, which is actually the active species (Pallett et al. 2001 and references therein). Diketonitrile is a very potent inhibitor of

the 4-hydroxyphenylpyruvate dioxygenase (4-HPPD) enzyme responsible for the desaturation process in carotenoid biosynthesis (Kaur et al. 2004). Once carotenoid biosynthesis is inhibited within plants (including algae), new growth is prevented (BCPC 2012).

Isoxaflutole ultimately ends up in aquatic environments as a result of surface and/or subsurface runoff from agricultural applications following high rainfall (BCPC 2012). Isoxaflutole and its metabolites are highly mobile in soil however rapid degradation in both soil and water dramatically reduces the potential for isoxaflutole to be transported to either ground or surface water (Table 33).

8.2 Freshwater

8.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for isoxaflutole in freshwaters (Table 35) includes toxicity data for two freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater and marine species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, one cladoceran, one macrophyte and three microalgae. The single toxicity value for the fish was a 28-day NOEC (mortality) value of 80 µg/L. The single toxicity value for the cladoceran was a 21-day NOEC (total body length, dry weight) value of 350 µg/L. The toxicity data for the macrophyte consisted of a 14-day NOEC (frond number, dry weight, frond area) value of 1.1 µg/L. The toxicity data for the microalgae consisted of 5-day NOEL (biomass yield, growth rate, area under the growth curve) values of 3.1 and 8.6 µg/L, 5-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 140 to 380 µg/L and 120-day NOEC and EC50 (biomass yield, growth rate, area under the growth rate) values of 16 and 120 µg/L, respectively.

Freshwater Acute

There were freshwater acute toxicity data for two fish, one cladoceran and one macrophyte. The toxicity data for the fish consisted of two 96-hour NOEL (mortality) values ranging from 1,700 to 4,500 µg/L. The toxicity data for the single cladoceran species was a 48-hour NOEL (total body length and dry weight) value of 1,500 µg/L. The toxicity data for the single macrophyte species were 3-day NOEC and LOEC (frond number, dry weight, frond area) values of 610 and 8 µg/L, respectively, and 6-day EC10 and EC50 (frond number, dry weight, frond area) values of 0.4 and 21.9 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

8.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of isoxaflutole. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of isoxaflutole (Table 33).

8.2.3 Guideline derivation

The derived PGVs for isoxaflutole in freshwaters are provided in Table 34. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for isoxaflutole are expressed in terms of the concentration of the active ingredient. There were ecotoxicology data available for commercial formulations which contain isoxaflutole as the active ingredient and for two degradation products (RPA 202248 and RPA 203328). The formulations were 7 to 67 times less toxic than isoxaflutole (the active ingredient) alone (USEPA 2015b). The RPA 202248 degradation product was 18 times less toxic to rainbow trout, 40 times less toxic to cladocerans and 1,800 times less toxic to mysids than isoxaflutole alone. The RPA 203328 degradation product was 12 times less toxic to sheepshead minnow and 15 to 2,000 times less toxic to duckweed than isoxaflutole alone (USEPA 2015b). Therefore, the PGVs derived using the isoxaflutole ecotoxicity data will provide adequate protection to organisms exposed to commercial herbicide formulations that contain isoxaflutole and the two key isoxaflutole degradation products.

Measured log BCF values for isoxaflutole are low (Table 33) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for isoxaflutole do not need to account for secondary poisoning.

Table 34 Proposed aquatic ecosystem protection guideline values (μg/L) for isoxaflutole for the protection of freshwater ecosystems.

Isoxaflutole proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	0.068 (0.012 – 2.1)	Sample size	6
95%	0.46 (0.12 – 6.8)	Type of toxicity data	Chronic NOEC/NOEL values
90%	1.1 (0.34 – 12)	SSD model fit	Poor
80%	2.8 (0.87 – 23)	Reliability	Low

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

8.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for isoxaflutole in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for isoxaflutole to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more isoxaflutole toxicity data available that enable the calculation of PGVs in freshwaters (see section 8.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of isoxaflutole with freshwater phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were freshwater toxicity data for six species (six phyla and six classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The six classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria) and Liliopsida (monocots).

Based on the current understanding of the mode of action of isoxaflutole, a 4-HPPD-inhibiting herbicide, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The isoxaflutole ecotoxicity data for phototrophs and heterotrophs were then tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups did not have significantly different ($p = 0.089$, see section 8.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for isoxaflutole in freshwater.

There were chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for six freshwater species (that belonged to six phyla and six classes) which meets the minimum data requirements (i.e., at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 34) combined with the poor fit of the distribution to these toxicity data (Figure 29) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for isoxaflutole in freshwater environments is provided in Table 35.

Table 35 Summary of the single toxicity value for each phototrophic and heterotrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for isoxaflutole in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[3]	8.6	USEPA (2015b)
Cladoceran	*Daphnia magna*	Arthropoda	Branchiopoda	Not stated	21	Chronic NOEC	Total body length, dry weight	350	ECHA (2013)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic NOEL	Frond number, dry weight, frond area	1.1	USEPA (2015b)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[3]	3.1	USEPA (2015b)
Fish	*Oncorhynchus mykiss**	Chordata	Actinopterygii	Not stated	5	Chronic NOEC	Mortality	80	ECHA (2013)
Microalga	*Selenastrum capricornutum*[2]	Chlorophyta	Chlorophyceae	Not stated	5	Chronic NOEC	Biomass yield, growth rate, AUC[3]	16	ECHA (2013)

[1] **Chronic NOEC/NOEL = no conversions applied (Warne et al. 2015).** [2] **This species is also been called *Raphiodocelis subcapitata* and *Pseudokirchneriella subcapitata*.** [3] **AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.**

8.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the six freshwater phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 29.

Figure 29 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic no observed effect level (NOEL) and no observed effect concentration (NOEC) data values of freshwater phototrophic and heterotrophic species to isoxaflutole. Black dashed lines indicate the 95% confidence intervals.

8.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for isoxaflutole in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
Arthropoda	Branchiopoda	Cladoceran (*Daphnia magna*)	Not stated	21	Chronic	NOEL (Body length, dry weight)	Freshwater	*	*	350	ECHA (2013)
										350	*GEOMETRIC MEAN*
										350	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Diatom (*Navicula pelliculosa*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	3.1	USEPA (2015b)
										3.1	*GEOMETRIC MEAN*
										3.1	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[1]*)	Not stated	5	Chronic	NOEC (Biomass yield, growth rate, AUC[2])	Freshwater	*	*	16	ECHA (2013)
										16	*GEOMETRIC MEAN*
										16	***VALUE USED IN SSD***
Chordata	Actinopterygii	Rainbow Trout (*Oncorhynchus mykiss*)	Not stated	28	Chronic	NOEC (Mortality)	Freshwater	*	*	80	ECHA (2013)
										80	*GEOMETRIC MEAN*
										80	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Microalga (*Anabaena flos-aquae*)	Not stated	5	Chronic	NOEC (Biomass yield, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	8.6	USEPA (2015b)

										8.6	*GEOMETRIC MEAN*
										8.6	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	14	Chronic	NOEL (Frond number, dry weight, frond area)	M-Hoagland's or 20X-AAP nutrient media/ASTM Type I water	25 ±2	(4.8-5.2 M-Hoagland's / 7.5 ± 0.1 20X-AAP)	1.1	USEPA (2015b)
										1.1	*GEOMETRIC MEAN*
										1.1	***VALUE USED IN SSD***

* Data were obtained from ECHA (2013), with methods originating from unpublished studies by Bettencourt (1993a) and Bettencourt (1993b). The unpublished studies were unattainable; therefore, detail of media, temperature and pH for those entries were unavailable. It is important to note that ECHA (2013) follows strict quality assurance and quality check procedures within their organisation to ensure only high quality ecotoxicology data are reported. It was assumed the data were the equivalent of either high or acceptable quality and were, therefore useable in the derivation of proposed aquatic ecosystem protection guideline values for isoxaflutole. [1] This species is also called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [2] AUC = area under the growth curve.

8.3 Marine

8.3.1   Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for isoxaflutole in marine waters (Table 37) includes toxicity data for three species (one marine and two freshwater) that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine and freshwater species that passed the screening and quality assurance processes are provided below and in section 8.2.1, respectively.

Marine Chronic

There were marine chronic toxicity data for one macroinvertebrate and one microalga. The toxicity data for the macroinvertebrate were 28-day NOEL and LOEC (mortality) values of 1 and 2 µg/L. The toxicity data for the single microalga species were 14-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 2.2 and 110 µg/L.

Marine Acute

There were marine acute toxicity data for one fish, one macroinvertebrate and one mollusc. The toxicity data for the single fish species was a 96-hour NOEL (mortality) value of 6,400 µg/L. The toxicity data for the macroinvertebrates were 96-hour NOEL and LC50 (mortality) values of 5.1 and 17.8 µg/L, respectively. The toxicity data for the single mollusc species were 96-hour NOEL and EC50 (mortality, abnormal development) values of 980 and 3,300 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

8.3.2   Factors affecting toxicity

No factors have been reported as modifying the toxicity of isoxaflutole. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of isoxaflutole (Table 33).

8.3.3   Guideline derivation

The derived PGVs for isoxaflutole in marine waters are provided in Table 36. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for isoxaflutole are expressed in terms of the concentration of the active ingredient. There were ecotoxicology data available for commercial formulations which contain isoxaflutole as the active ingredient and for two degradation products (RPA 202248 and RPA 203328). The formulations were 7 to 67 times less toxic than isoxaflutole (the active ingredient) alone (USEPA 2015b). The RPA 202248 degradation product was 18 times less toxic to rainbow trout, 40 times less toxic to cladocerans and 1,800 times less toxic to mysids than isoxaflutole alone. The RPA 203328 degradation product was 12 times less toxic to sheepshead minnow and 15 to 2,000 times less toxic to duckweed than isoxaflutole alone (USEPA 2015b). Therefore, the PGVs derived using the isoxaflutole ecotoxicity data will provide adequate protection to organisms exposed to

commercial herbicide formulations that contain isoxaflutole and the two key isoxaflutole degradation products.

Measured log BCF values for isoxaflutole are low (Table 33) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for isoxaflutole do not need to account for secondary poisoning.

Table 36 Proposed aquatic ecosystem protection guideline values (μg/L) for isoxaflutole for the protection of marine ecosystems.

Isoxaflutole proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	0.33 (0.18 – 1.4)	Sample size	9
95%	0.69 (0.42 – 2.8)	Type of toxicity data	Chronic NOEC/NOEL and converted acute values *(freshwater and marine)*
90%	1.1 (0.68 – 4.4)	SSD model fit	Poor
80%	2.0 (1.1 – 8.5)	Reliability	Moderate

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

8.3.4   Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for isoxaflutole in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for isoxaflutole to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more isoxaflutole toxicity data available that enable the calculation of PGVs in marine waters. However it was only possible to derive PGVs by using ecotoxicity data for a mixture of both marine and freshwater organisms (see section8.3.6 and 8.2.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to marine ecosystems separately, it is recommended that additional chronic toxicity tests of isoxaflutole with marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were marine toxicity data for three species (three phyla and three classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta and Mollusca. The three classes represented were Bivalvia (a grouping of molluscs), Malacostraca (a larger grouping of crustaceans) and Mediophyceae (another algae grouping).

Based on the current understanding of the mode of action of isoxaflutole, a 4-HPPD-inhibiting herbicide, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The isoxaflutole ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups did not have significantly different ($p = 0.089$, see section 8.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for isoxaflutole in freshwater.

There were marine chronic no observed effect concentration (NOEC), no observed effect level (NOEL) and converted acute (acute EC50/LC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 10) data available for only three species (that belonged to three phyla and three classes), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). As no other ecotoxicity data for isoxaflutole to marine species were available, the chronic NOEC/NOEL and converted acute values for marine species were combined with the available chronic NOEC/NOEL values for freshwater species to derive PGVs for isoxaflutole in marine waters. This dataset incorporated concentration data for nine (three marine and six freshwater) phototrophic and heterotrophic species belonging to seven 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 SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 36) combined with the poor fit of the distribution to these toxicity data (Figure 30) resulted in a moderate reliability set of PGVs. The combination of freshwater and marine ecotoxicity data reduces the reliability classification of PGVs as per Warne et al. (2015). A summary of the toxicity data (one value per species) used to calculate the PGVs for isoxaflutole in marine environments is provided in Table 37.

Table 37 Summary of the single toxicity value for each phototrophic and heterotrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for isoxaflutole in marine waters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Marine	Macroinvertebrate	*Americamysis bahia*	Arthropoda	Arthropoda	Not stated	28	Chronic NOEL	Mortality	1	USEPA (2015b)
Fresh	Microalga	*Anabaena flos-aquae*	Cyanobacteria	Cyanobacteria	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[3]	8.6	USEPA (2015b)
Marine	Macroinvertebrate	*Crassostrea virginica*	Mollusca	Mollusca	Embryo / Larvae	4	Converted acute	Mortality, abnormal development	330	USEPA (2015b)
Fresh	Cladoceran	*Daphnia magna*	Arthropoda	Arthropoda	Not stated	21	Chronic NOEC	Total body length, dry weight	350	ECHA (2013)
Fresh	Macrophyte	*Lemna gibba*	Tracheophyta	Tracheophyta	Not stated	14	Chronic NOEL	Frond number, dry weight, frond area	1.1	USEPA (2015b)
Fresh	Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyta	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[3]	3.1	USEPA (2015b)
Fresh	Fish	*Oncorhynchus mykiss**	Chordata	Chordata	Not stated	5	Chronic NOEC	Mortality	80	ECHA (2013)
Fresh	Microalga	*Selenastrum capricornutum*[2]	Chlorophyta	Chlorophyta	Not stated	5	Chronic NOEC	Biomass yield, growth rate, AUC[3]	16	ECHA (2013)
Marine	Microalga	*Skeletonema costatum**	Bacillariophyta	Bacillariophyta	Not stated	14	Chronic NOEL	Biomass yield, growth rate, AUC[3]	2.2	USEPA (2015b)

[1] Chronic NOEC/NOEL = no conversions applied; Converted acute = acute LC50 values that were converted to chronic NOEC values by dividing by 10 (Warne et al. 2015). [2] This species is also been called *Raphiodocelis subcapitata* and *Pseudokirchneriella subcapitata*. [3] AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.

8.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the nine marine and freshwater, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 30.
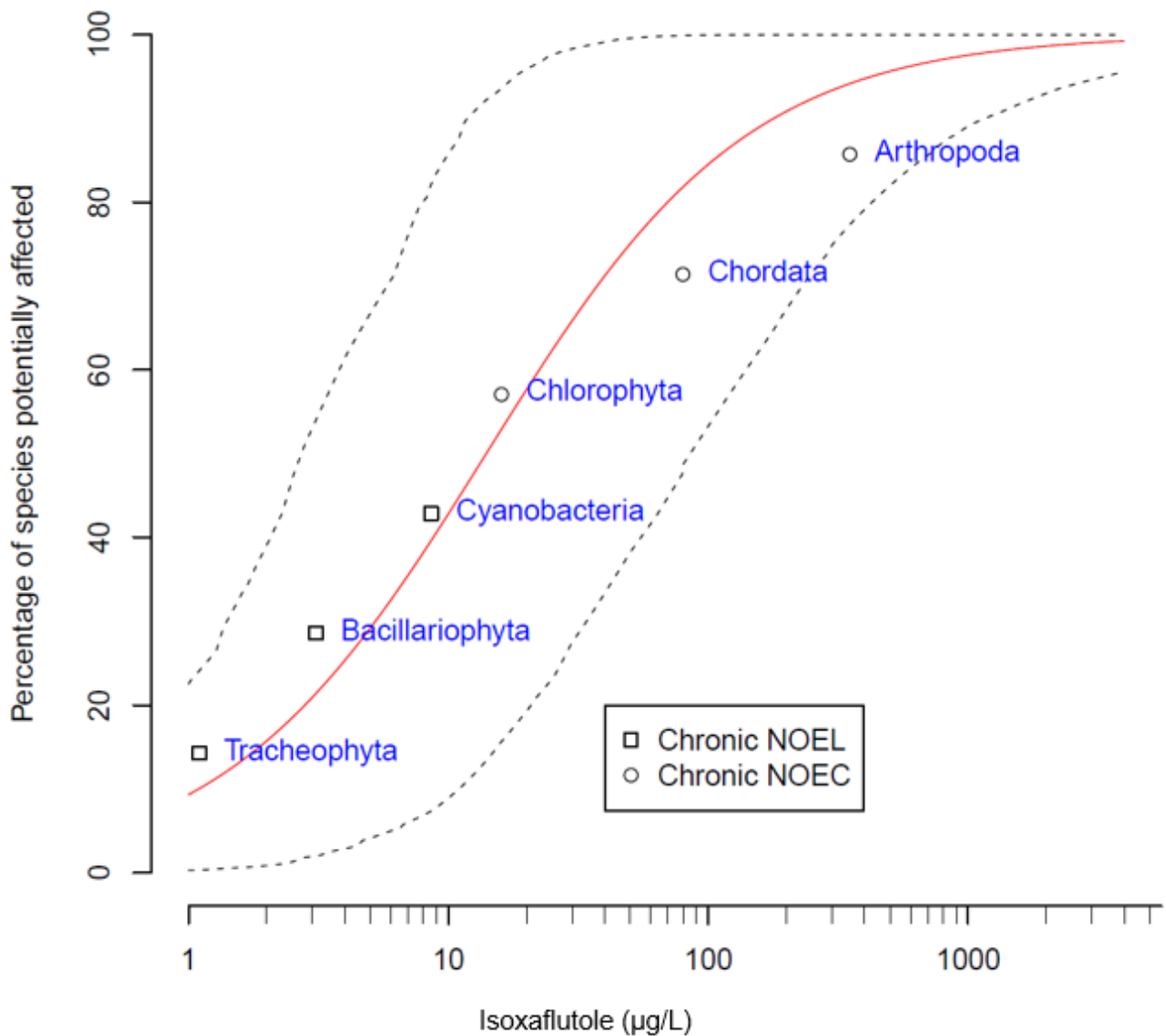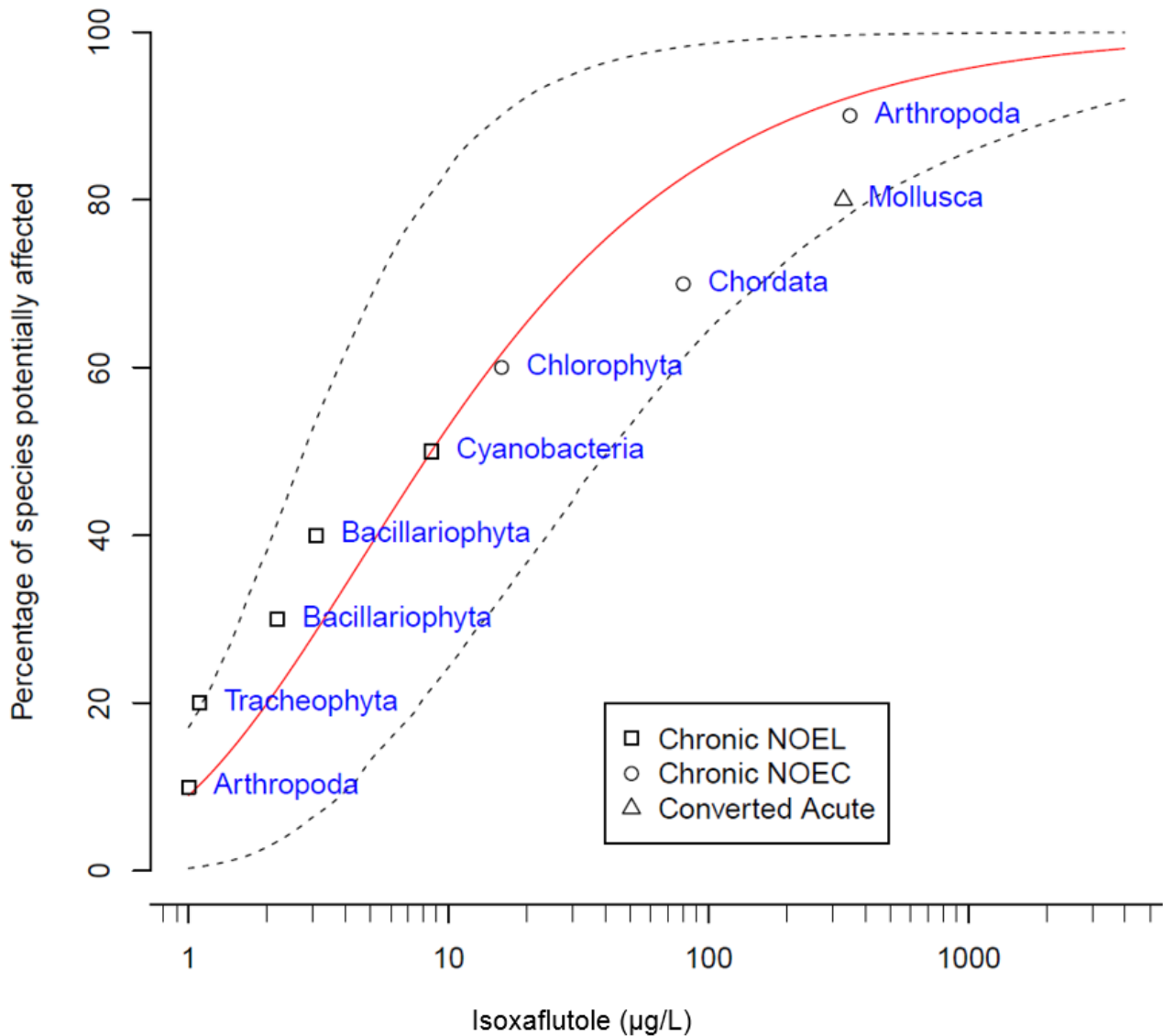

Figure 30 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic no observed effect level (NOEL), no observed effect concentration (NOEC) and converted acute data values of marine and freshwater species to isoxaflutole. Black dashed lines indicate the 95% confidence intervals.

8.3.6 Summary details of all marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for isoxaflutole in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp (°C)	pH	Concentration (μg/L)	Reference
Arthropoda	Malacostraca	Shrimp (*Americamysis bahia*)	Not stated	28	Chronic	NOEL (Mortality)	Natural or artificial seawater	20 ± 3	25 ± 2	Not stated	1	USEPA (2015b)
											1	*GEOMETRIC MEAN*
											1	***VALUE USED IN SSD***
Bacillariophyta	Mediophyceae	Microalga (*Skeletonema costatum*)	Not stated	14	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	Synthetic salt water or Filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	2.2	USEPA (2015b)
											2.2	*GEOMETRIC MEAN*
											2.2	***VALUE USED IN SSD***
Mollusca	Bivalvia	Eastern Oyster (*Crassostrea virginica*)	Embryo / Larve	4	Acute	EC50 (Mortality/ abnormal development)	Unfiltered natural or Artificial (with food added) seawater	> 12	20 ± 5	Not stated	3,300	USEPA (2015b)
											3,300	*GEOMETRIC MEAN*
											330[@]	***VALUE USED IN SSD***

[1] This species is also called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [3] AUC = area under the growth curve. [@] Values were acute EC/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 10 (Warne et al. 2015).

8.3.7 Distribution of sensitivities for aquatic species

The transformed ecotoxicity data for marine species ($n = 3$) fell within the lower and upper 95% confidence intervals [-1.412 and 6.875 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater species ($n = 6$). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for isoxaflutole.

The toxicity data for isoxaflutole to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the isoxaflutole ecotoxicity data may not be unimodal (Figure 31).
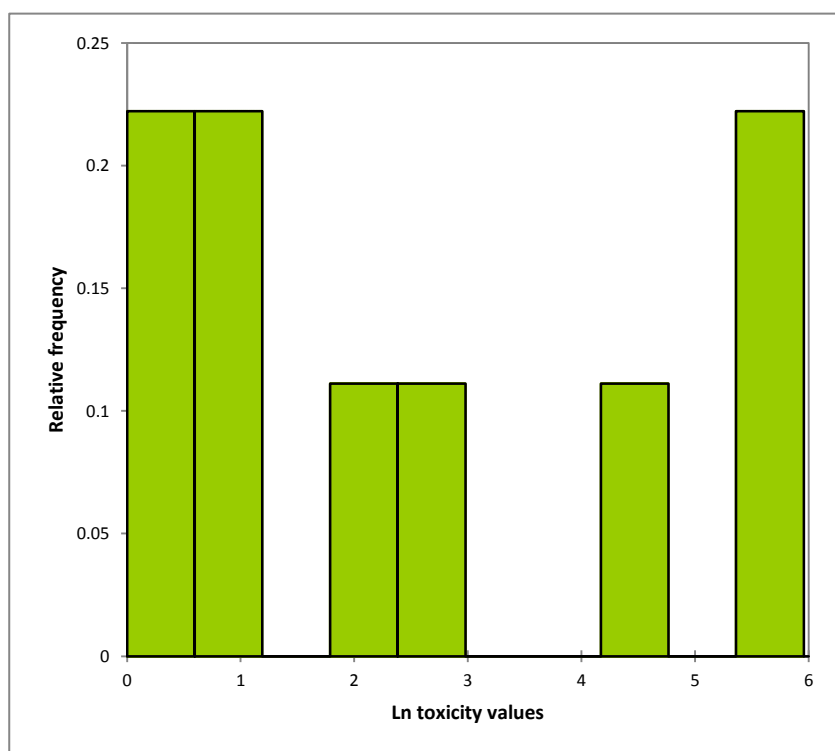
Figure 31 Histogram of the natural logarithmic (ln) of all isoxaflutole (freshwater and marine) toxicity data for phototrophic and non-phototrophic species ($n = 9$).

The isoxaflutole ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample *t* test was used because the transformed isoxaflutole concentration data had equal variances (Fisher's F-Test; p = 0.099) and followed a normal distribution (Anderson-Darling; p = 0.306). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.089); therefore, it can be concluded that the distribution of the isoxaflutole concentration data is uni-modal.

Visually, the histogram looks as though the isoxaflutole ecotoxicity data is bi- or multi-modal, with just one heterotrophic species (*Americamysis bahia* = 1 µg/L) having a lower concentration than other heterotrophic species (ranging from 80 – 350 µg/L). This data point was thought to be skewing

the dataset to result in a statistically not significant result, and thus was examined further (see below section 8.3.8).

8.3.8 Distribution of sensitivities for aquatic species omitting outlier

The same transformed, freshwater and marine data for isoxaflutole including both, the phototrophic and non-phototrophic species that passed the screening and quality assessment schemes was used, however omitting the heterotrophic outlier concentration of 1 µg/L for *Americamysis bahia*. Visual examination of the histogram of transformed freshwater and marine data with the one heterotrophic outlier concentration omitted indicated that the distribution may be bimodal (Figure 32).
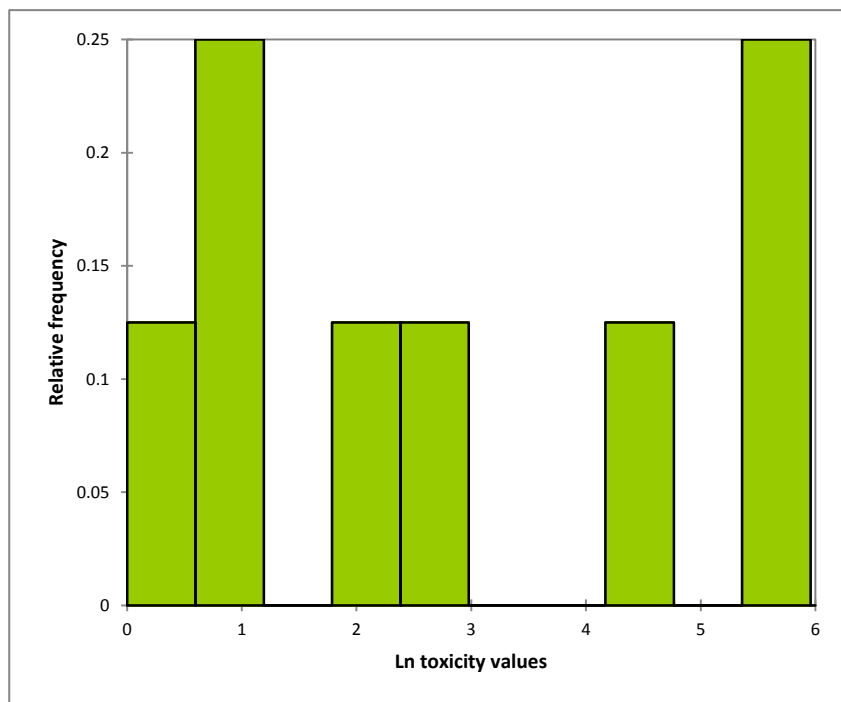
Figure 32 Histogram of the natural logarithmic (ln) of all isoxaflutole (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 8).

The isoxaflutole ecotoxicity data for phototrophic and non-phototrophic species were tested again with the one heterotrophic data point excluded to see if it was skewing the data and if, in fact, the phototrophs and heterotrophs are from different populations. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample *t* test was used because the transformed isoxaflutole concentration data had equal variances (Fisher's F-Test; p = 0.824) and followed a normal distribution (Anderson-Darling; p = 0.463). Results from the two-sample *t* test indicated that the two groups were significantly different (p = 0.002); therefore, it can be concluded that the distribution of the isoxaflutole concentration, when excluding the outlier data point, is bi- or multi-modal, with phototrophic species being the most sensitive group.

There is insufficient ecotoxicity data for isoxaflutole to determine whether phototrophic and heterotrophic species have different sensitivities when including the heterotrophic outlier value (*Americamysis bahia* = 1 µg/L), and thus, it is difficult to demonstrate that there may actually be a bi- or multi-modal response. In order to qualify the modality of isoxaflutole ecotoxicity data and increase the reliability of PGVs, additional toxicity testing of both, phototrophic and heterotrophic species is needed.

8.3.9 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A world compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 677–678.

Bettencourt M (1993a). RPA 201772 - Acute toxicity to Bluegill sunfish *(Lepomis macrochirus)* under flow-through conditions. Unpublished study performed by Springborn Laboratories, Inc. (Report No. 93-8-4886, Study # 10566.0493.6283.105 and submitted by Rhone Poulenc Ag Company, North Carolina. (MRID# 43573235), November, 1993.

Bettencourt M (1993b). RPA 201772 - Acute toxicity to Rainbow trout *(Oncorhynchus mykiss)* under flow-through conditions. Unpublished study performed by Springborn Laboratories, Inc. (Report No. 93-8-4860, Study # 10566.0493.6284.108 and submitted by Rhone Poulenc Ag Company, North Carolina. (MRID# 43573236), December, 1993.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

ECHA (2013). Committee for risk assessment: RAC, Annex 1 background document to the opinion proposing harmonized classification and labelling at EU level of Isoxaflutole. An agency of the European Union, European Chemicals Agency. March 8, 2013. Available from: http://echa.europa.eu/documents/10162/13579/isoxaflutole_annex_1_bd_en.pdf , Accessed: October, 2015. Methods available from: http://www3.epa.gov/pesticides/chem_search/cleared_reviews/csr_PC-123000_29-Jun-95_020.pdf. Accessed: October 2015.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Kaur H, Inderjit and Bhowmik PC (2004). Phytotoxicity of isoxaflutole to *Phalaris minor* Retz, *Plant and Soil*, 258, 161–168.

Pallett KE, Cramp SM, Little JP, Veerasekaran P, Crudace AJ and Slater AE (2001). Isoxaflutole: the background to its discovery and the basis of its herbicidal properties, *Pest Management Science*, 57, 133–142.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm Accessed 13 May 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: February–April, 2016.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

9 Metolachlor

9.1 Introduction

Metolachlor ($C_{15}H_{22}ClNO_2$ and Figure 34) is a herbicide that at room temperature is a colourless to light tan liquid. It is the active ingredient of a variety of commercial herbicide formulations. Metolachlor is often mixed with other herbicides (e.g. alachlor as well as isomers *S*-metolachlor and *R*-metolachlor) to increase its efficacy (Liu et al. 2006).
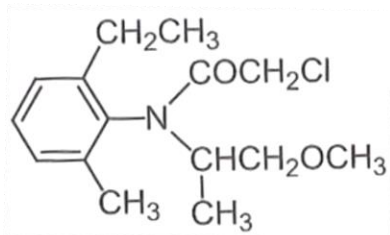
Figure 33 Structure of metolachlor

Physicochemical properties of metribuzin that may affect its environmental fate and toxicity are presented in Table 38.

Table 38 Summary of selected physicochemical properties of metolachlor.

Physicochemical property	Value
Molecular weight	283.8 amu[1]
Aqueous solubility	488 mg/L @ temperature of 25 °C[1] 530 mg/L @ temperature of 20 °C[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.9 @ temperature 25 °C[1] 3.4 @ pH 7 and temperature of 20°C[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.08–2.49[1] 2.08[2]
Logarithm of the bioconcentration factor (log BCF)	1.84[2]
Half-life ($t_{1/2}$) in water	Stable @ pH 7 and temperature 20 °C[2] >200 days (pH 1–9) @ 20 °C [1, 3]
Half-life ($t_{1/2}$) in soil	20 days (in field)[1] Typical: 90 days (15–21 days in the lab (20 °C) and the field, respectively)[2]

[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] CCME (1999).

Metolachlor is a derivative of aniline and belongs to the chloroacetanilide group within the amide family of herbicides. Other chloroacetanilide herbicides include alachlor, acetochlor and propachlor. Metolachlor is extensively used in agriculture (i.e. corn, soybeans, sorghum, potatoes, cotton, sunflowers), forestry, and along roadsides to control annual and perennial broadleaf weeds and grasses. The racemic mixture of metolachlor (containing (1*S*)- and (1*R*)- isomers) does not have regulatory approval to be used within the European Union; however, approval has been granted for *S*-metolachor alone (University of Hertfordshire 2013). Metolachlor is a selective pre-emergent and early-post emergent herbicide (Liu and Xiong 2009; CCME 1999) that does not affect established plants (Vallotton et al. 2008).

Metolachlor exerts its toxicity following germination, where it inhibits the growth of susceptible weeds (Vallotton et al. 2008). It acts by interfering with cell division and cell enlargement of plants when

absorbed by the hypocotyls in roots, seedling shoots and cotyledons (Böger et al. 2000). Metolachlor binds strongly and irreversibly (Gotz and Böger 2004) to the fatty-acid elongation (FAE1)-synthase enzyme to inhibit the elongation of very long chain fatty acids (VLCFA) in plants and algae (Böger 2003). Once inhibited, the lack of VLCFAs (commonly C18 and C16) becomes phytotoxic, as they are no longer available to aid in the maintenance of the rigidity and permeability of cell plasma membranes (Vallotton et al. 2008; Böger 2003).

Metolachlor has a low binding affinity to soil particles and therefore has a high capacity to leach to groundwater and end up in surface waters. The typical soil degradation (aerobic) half-life of metolachlor is 90 days, however some field studies have reported much shorter half-lives (BCPC 2012; University of Hertfordshire 2013) (Table 38). The aqueous hydrolysis of metolachlor is slow, with a half-life of 100 to greater than 200 days at pH 1 to pH 9 and a temperature of 20 ⁰C (University of Hertfordshire 2013) (Table 38).

Metolachlor has been frequently detected in surface waters of Europe (Balsiger et al. 2004; Konstantinou et al. 2006), North America (Battaglin et al. 2000; Gilliom et al. 2006) and Australia (AATSE 2002 and references therein).

9.2 Freshwater

9.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for metolachlor in freshwaters (Table 40) includes toxicity data to 13 freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, one cladoceran, four macrophytes and 18 microalgae. The toxicity data for fish consisted of a 26-day LOEC (mortality, hatching, growth) value of 2,200 µg/L and a 35-day LOEC (mortality) value of 1,600 µg/L. The toxicity data for the single cladoceran species were 21-day NOEC (length, longevity, broods per female, young per female) values ranging from 500 to 15,000 µg/L, 21-day LOEC (immobilisation, length, longevity, broods per female, young per female) values ranging from 10 to 10,000 µg/L, two 21-day EC10 (young per female) values of 100 and 500 µg/L and a 21-day EC50 (immobilisation) value of 12,400 µg/L. The toxicity values for macrophytes were a 14-day NOEL (frond number) value of 8.4 µg/L and 14-day EC50 (frond number, dry weight, frond area, wet weight) values ranging from 48 to 2,355 µg/L. The toxicity values for microalgae were two 48-hour NOEC (chlorophyll-a content) values both of 200 µg/L, a 48-hour LOEC (cell density) value of 50 µg/L, 48-hour EC50 (chlorophyll-a content cell density) values ranging from 2.3 to 5165.2 µg/L, two 72-hour NOEC (cell density) values of 25 and 30 µg/L, a 72-hour LOEC (cell density) value of 77 µg/L, 72-hour EC50 (cell density, chlorophyll-a content) values ranging from 44.3 to 177 µg/L, 96-hour EC5 (cell density) values ranging from 5.38 to 5,957 µg/L, 96-hour EC10 (cell density, chlorophyll-a content) values ranging from 27 to 111,666 µg/L, two 96-hour NOEC (chlorophyll-a content) values of 1 and 38 µg/L, respectively, 96-hour LOEC (chlorophyll-a content, chlorophyll-b content) values ranging from 1 to 75 µg/L, 96-hour EC50 (cell density, chlorophyll-a content) values ranging from 68 to 37,567 µg/L, 5-day EC50

(biomass yield, growth rate, area under the growth curve) values ranging from 10 to 1,200 µg/L, 7-day NOEC (live cell density, chlorophyll-a content) values of 1 and 10 µg/L, respectively and 7-day LOEC (live cell density, chlorophyll-a content) values of 10 and 100 µg/L, respectively.

Freshwater Acute

There were freshwater acute toxicity data for seven fish, one cladoceran, two insects, one crustacean, one microinvertebrate, one macrophyte and three microalgae. The fish toxicity data consisted of 96-hour LOEL (mortality) values ranging from 2,100 to 6,500 µg/L and 96-hour LC50 (mortality) values ranging from 3,900 to 10,000 µg/L. The toxicity values for the single cladoceran were two 24-hour LC50 (mortality) values of 51,200 and 69,400 µg/L, 48-hour EC50 (immobilisation) values ranging from 22,300 to 26,000 µg/L. The toxicity values for insects consisted of two 48-hour LC50 (immobilisation) values of 3,800 and 4,400 µg/L and 72-hour NOEC, EC58 and LOEC (immobilisation) values of 100, 1000 and 1000 µg/L, respectively. The single toxicity value for the crustacean species was a 96-hour LC50 (mortality) value of 4,900 µg/L. The single toxicity value for the microinvertebrate species was a 48-hour LC50 (mortality) value of 1,950 µg/L. The toxicity values for macrophytes were 96-hour NOEC and EC50 (frond number) values of 187 and 343 µg/L, respectively, and a 96-hour EC50 (wet weight) value of 360 µg/L. The toxicity values for microalgae were two 4-hour NOEC (chlorophyll-a content) values of 200 µg/L, 24-hour NOEC (chlorophyll-a content, cell density) values ranging from 119.8 to 200 µg/L and 24-hour EC50 (cell density) values ranging from 5.5 to 341 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

9.2.2   Factors affecting toxicity

No factors have been reported as modifying the toxicity of metolachlor. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of metolachlor (Table 38).

9.2.3   Guideline derivation

The derived PGVs for metolachlor in freshwaters are provided in Table 39. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for metolachlor are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for metolachlor are low (Table 38) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for metolachlor do not need to account for secondary poisoning.

Table 39 Proposed aquatic ecosystem protection guideline values (μg/L) for metolachlor for the protection of freshwater ecosystems.

Metolachlor proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	0.016 (0.000070 – 4.3)	Sample size	25
95%	0.71 (0.043 – 12)	Type of toxicity data	Chronic EC10/NOEC/NOEL and chronic estimated NOEC values
90%	3.7 (0.66 – 27)	SSD model fit	Good
80%	19 (4.7 – 76)	Reliability	Very high

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

9.2.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for metolachlor in freshwater environments was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on one acute toxicity value for a freshwater fish species, *Poecilia reticulata* (Warne 2001). This value was calculated using the assessment factor (AF) method, dividing the lowest acute toxicity value of 20 μg/L by an assessment factor of 1000 (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this value would be classified as having an 'unknown' reliability.

To obtain toxicity data for metolachlor to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now considerably more metolachlor toxicity data available that enable the calculation of PGVs in freshwaters (see section 9.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of metolachlor with phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were freshwater toxicity data for 35 species (six phyla and 12 classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The 12 classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Fragilariophyceae (a grouping of pennate diatoms), Insecta (invertebrates), Liliopsida (monocots), Magnoliopsida (dicots), Malacostraca (a larger grouping of crustaceans), Mediophyceae (another algae grouping) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of metolachlor, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as metolachlor binds to and interferes with the FAE1-synthase enzyme which is part of the normal metabolism of plants and algae. The metolachlor ecotoxicity data for phototrophs and heterotrophs were then tested using the non-parametric Mann-Whitney test to see if the toxic responses among different taxa were uni-

or multi-modal. The Mann-Whitney test indicated that the two groups did not have significantly different (p = 0.092, see section 9.2.7) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for metolachlor in freshwater.

There were freshwater chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data for 14 species (that belonged to four phyla and seven classes) which meets the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). However, because the resulting fit of the curve was poor, the protective concentration (PC) values were not recommended as the PGVs for metolachlor in freshwaters (refer to section 9.2.8 for further explanation). Very high reliability PGVs were derived by including chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) values in the derivation.

When the dataset was expanded to combine chronic EC10/NOEC/NOEL data with the chronic estimated NOEC values of freshwater phototrophic and heterotrophic species, there were 25 species belonging to six phyla and ten classes, which met the minimum data requirements to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 39) combined with the good fit of the distribution to these toxicity data (Figure 34) resulted in a very high reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for metolachlor in freshwater environments is provided in Table 40.

Table 40 Summary of the single toxicity value for each phototrophic and heterotrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for metolachlor in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Microalga	*Achnanthidium minutissimum**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC10	Cell density	6,528	Larras et al. (2012)
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	240	USEPA (2015b)
Macrophyte	*Ceratophyllum demersum**	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	14	Fairchild et al. (1998)
Microalga	*Chlamydomanas - Strain CC125*	Chlorophyta	Chlorophyceae	Late logarithmic growth phase	2	Chronic est. NOEC	Chlorophyll content	595.4	Fischer et al. (2012)
Microalga	*Chlamydomanas reinhardi**	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll *a* content	227.6	Fairchild et al. (1998)
Microalga	*Chlorella kessleri*	Chlorophyta	Trebouxiophyceae	Stationary growth phase	2	Chronic NOEC	Chlorophyll *a* content	200	Spoljaric et al. (2012)
Microalga	*Chlorella pyrenoidosa**	Chlorophyta	Trebouxiophyceae	Exponential growth phase	4	Chronic NOEC	Chlorophyll *a* content	1	Liu and Xiong (2009)
Microalga	*Craticula accomoda**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC10	Chlorophyll *a* content	4,016	Larras et al. (2012)
Microalga	*Cyclotella meneghiniana**	Bacillariophyta	Mediophyceae	Exponential growth phase	4	Chronic EC10	Cell density	925	Larras et al. (2012)
Fish	*Cyprinodon variegatus*	Chordata	Actinopterygii	Early life stage	26	Chronic est. NOEC	Mortality	880	USEPA (2015b)
Macroinvertebrate	*Daphnia magna*	Arthropoda	Branchiopoda	<24 hour old	21	Chronic EC10	Young per female	223.6	Liu et al. (2006)
Macrophyte	*Elodea canadensis**	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Wet weight	471	Fairchild et al. (1998)
Microalgae	*Encyonema silesiacum**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC10	Chlorophyll *a* content	1,047.9	Larras et al. (2012); Larras et al. (2013)
Microalga	*Fragilaria capucina var vaucheriae**	Bacillariophyta	Fragilariophyceae	Not stated	4	Chronic EC10	Chlorophyll *a* content	90	Larras et al. (2013)

Microalga	*Gomphonema gracile**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	7	Chronic NOEC	Live cell density	1	Coquillé et al. (2015)
Microalga	*Gomphonema parvulum*	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC10	Chlorophyll *a* content	6,384.2	Larras et al. (2012); Larras et al. (2013)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Stage 3 (3 fronds/plant)	14	Chronic NOEL	Frond number	8.4	USEPA (1982)
Microalga	*Mayamaea fossalis*	Bacillariophyta	Bacillariophyceae	Exponential growth phase	4	Chronic EC10	Chlorophyll *a* content	862.6	Larras et al. (2012); Larras et al. (2013)
Macrophyte	*Najas* sp.	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Wet weight	48.4	Fairchild et al. (1998)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[3]	76	USEPA (2015b)
Fish	*Pimephales promelas*	Chordata	Actinopterygii	Early life stage	35	Chronic est. NOEC	Mortality	640	USEPA (2015b)
Microalga	*Pseudokirchneriella subcapitata*[2]	Chlorophyta	Chlorophyceae	Not stated	3	Chronic NOEC	Cell density	27.4	Perez et al. (2011); Sbrilli et al. (2005)
Microalga	*Scenedesmus obliquus**	Chlorophyta	Chlorophyceae	Exponential growth phase	4	Chronic est. NOEC	Cell density	31.2	Bian et al. (2009)
Microalga	*Scenedesmus vacuolatus*	Chlorophyta	Chlorophyceae	Exponential growth phase	2	Chronic est. NOEC	Cell density	0.53	Vallotton et al. (2008)
Microalga	*Ulnaria ulna**	Bacillariophyta	Fragilariophyceae	Exponential growth phase	4	Chronic EC10	Chlorophyll *a* content	27	Larras et al. (2013)

[1] Chronic NOEC/NOEL/EC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*. [3] AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.

9.2.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 25 freshwater phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 34.
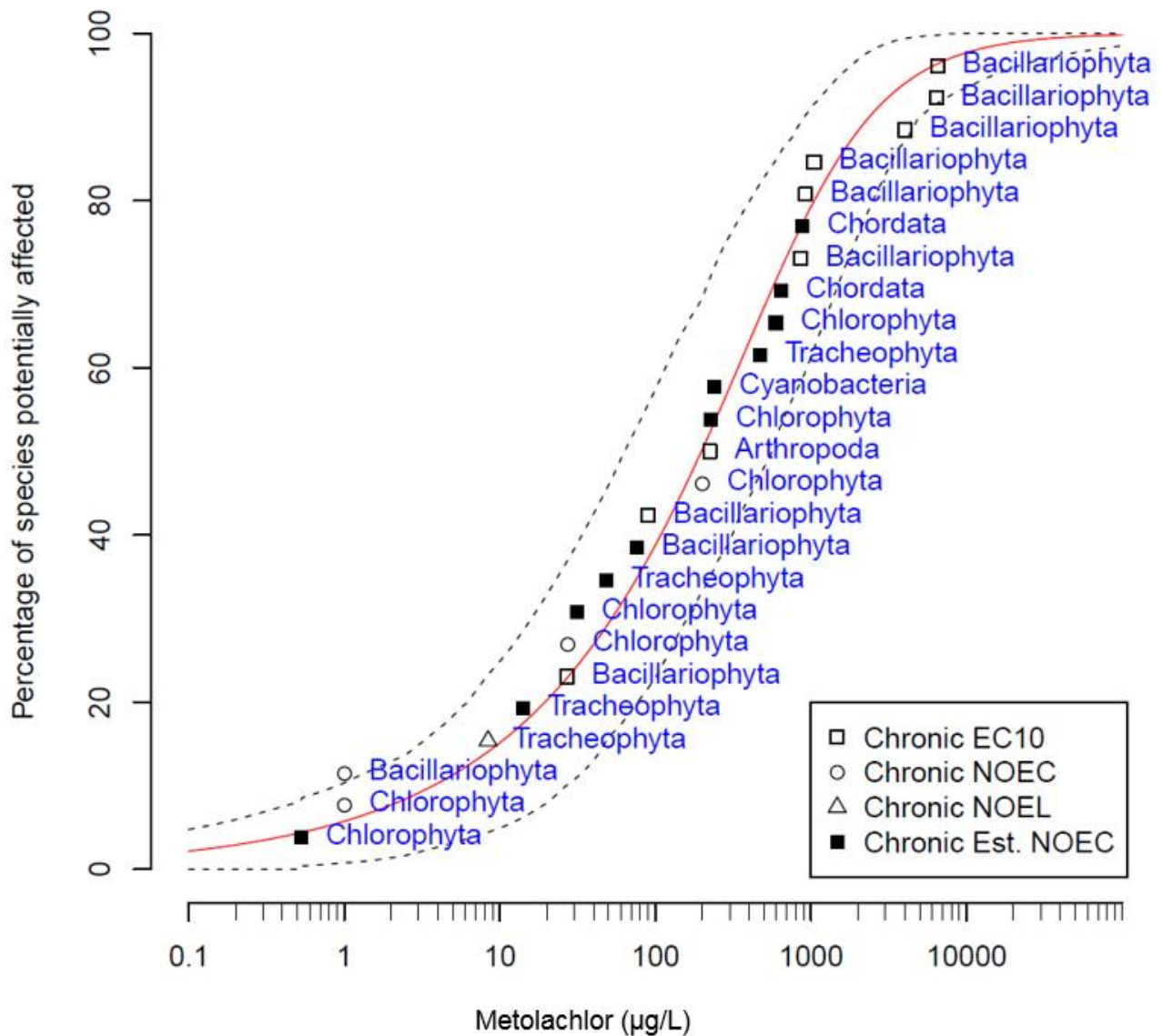
Figure 34 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to metolachlor. Black dashed lines indicate the 95% confidence intervals.

9.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for metolachlor in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration	Test type	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
Arthropoda	Branchiopoda	Cladoceran (*Daphnia magna*)	< 24 hour juveniles (neonates)	21	Chronic	EC10 (Young per female)	Elendt M4 or M7	18-22 ± 2	Not stated	100	Liu et al (2006)
Arthropoda	Branchiopoda	Cladoceran (*Daphnia magna*)	< 24 hour juveniles (neonates)	21	Chronic	EC10 (Young per female)	Elendt M4 or M7	18-22 ± 2	Not stated	500	Liu et al (2006)
										223.6	*GEOMETRIC MEAN*
										223.6	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyceae	Diatom (*Craticula accomoda*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	DMSO dissolved in DV growth media	Not stated	Not stated	4,016	Larras et al (2012)
										4,016	*GEOMETRIC MEAN*
										4,016	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyceae	Freshwater Diatom (*Achnanthidium minutissimum*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	DMSO dissolved in DV growth media	Not stated	Not stated	6,528	Larras et al (2012)
										6,528	*GEOMETRIC MEAN*
										6,528	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyceae	Freshwater Diatom	Not stated	5	Chronic	EC50 (Biomass	ASTM Type I water	24 ± 2	7.5 ± 0.1	380	USEPA (2015b)

		(*Navicula pelliculosa*)				yield, growth rate, AUC)					
										380	*GEOMETRIC MEAN*
										76[@]	**VALUE USED IN SSD**
Bacillariophyta	Mediophyceae	Microalga (*Cyclotella meneghiniana*)	Exponential growth phase	4	Chronic	EC10 (Cell density)	DMSO dissolved in DV growth media	Not stated	Not stated	925	Larras et al (2012)
										925	*GEOMETRIC MEAN*
										925	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyceae	Microalga (*Encyonema silesiacum*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	DMSO dissolved in DV growth media	Not stated	Not stated	432	Larras et al (2012)
Bacillariophyta	Bacillariophyceae	Microalga (*Encyonema silesiacum*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	Diatom medium + Vitamines (DV) Media	21 ± 2	Not stated	2,542	Larras et al (2013)
										1,048	*GEOMETRIC MEAN*
										1,048	**VALUE USED IN SSD**
Bacillariophyta	Fragilariophyceae	Microalga (*Fragilaria capucina var vaucheriae*)	Not stated	4	Chronic	EC10 (Chlorophyll *a* content)	Diatom medium + Vitamines (DV) media	21 ± 2	Not stated	90	Larras et al (2013)
										90	GEOMETRIC MEAN
										90	**VALUE USED IN SSD**

Bacillariophyta	Bacillariophyceae	Microalga (*Gomphonema gracile*)	Exponential growth phase	7	Chronic	NOEC (Live cell density)	Dauta medium	20	Not stated	1	Coquillé et al (2015)
										1	*GEOMETRIC MEAN*
										1	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyceae	Microalga (*Gomphonema parvulum*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	DMSO dissolved in DV growth media	Not stated	Not stated	365	Larras et al (2012)
Bacillariophyta	Bacillariophyceae	Microalga (*Gomphonema parvulum*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	Diatom medium + Vitamines (DV) Media	21 ± 2	Not stated	111,666	Larras et al (2013)
										6,384	*GEOMETRIC MEAN*
										6,384	**VALUE USED IN SSD**
Bacillariophyta	Bacillariophyceae	Microalga (*Mayamaea fossalis*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	DMSO dissolved in DV growth media	Not stated	Not stated	979	Larras et al (2012)
Bacillariophyta	Bacillariophyceae	Microalgae (*Mayamaea fossalis*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	Diatom medium + Vitamines (DV) Media	21 ± 2	Not stated	760	Larras et al (2013)
										862.6	*GEOMETRIC MEAN*
										862.6	**VALUE USED IN SSD**
Bacillariophyta	Fragilariophyceae	Microalga (*Ulnaria ulna*)	Exponential growth phase	4	Chronic	EC10 (Chlorophyll *a* content)	Diatom medium + Vitamines (DV) Media	21 ± 2	Not stated	27	Larras et al (2013)
										27	*GEOMETRI*

										C MEAN
									27	**VALUE USED IN SSD**
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC125*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	2,497	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC1373*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	3,519	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC2290*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	5,165	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC2342*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	2,668	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC2343*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	2,299	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC2344*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	3,235	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC2931*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	1,419	Fischer et al (2012)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas - Strain CC2935*)	Late logarithmic phase	2	Chronic	EC50 (Chlorophyll content)	Talaquil media	25	Not stated	4,825	Fischer et al (2012)
									2,977	*GEOMETRIC MEAN*
									595.4[@]	**VALUE USED IN**

											SSD
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomanas reinhardi*)	Not stated	4	Chronic	EC50 (Chlorophyll-*a* content)	ASTM medium	25	Not stated	1,138	Fairchild et al (1998)
										1,138	*GEOMETRIC MEAN*
										227.6[@]	**VALUE USED IN SSD**
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella kessleri*)	Stationary phase	2	Chronic	NOEC (Chlorophyll *a* content)	Bold's basal medium (BBM)	25	Not stated	200	Spoljaric et al (2011)
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella kessleri*)	Stationary phase	2	Chronic	NOEC (Chlorophyll *b* content)	Bold's basal medium (BBM)	25	Not stated	200	Spoljaric et al (2011)
										200	*GEOMETRIC MEAN*
										200	**VALUE USED IN SSD**
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella pyrenoidosa[2]*)	Exponential growth phase	4	Chronic	NOEC (Chlorophyll *a* content)	HB-4 medium	25	Not stated	1	Liu and Xiong (2009)
										1	*GEOMETRIC MEAN*
										1	**VALUE USED IN SSD**
Chlorophyta	Chlorophyceae	Microalga (*Pseudokirchneriella subcapitata[3]*)	Exponential growth phase	3	Chronic	NOEC (Cell density)	Marine biological laboratory (MBL) medium	21 ± 2	Not stated	25	Perez et al (2011)
Chlorophyta	Chlorophyceae	Microalga (*Pseudokirchneriella subcapitata[3]*)	Exponential growth phase	3	Chronic	NOEC (Cell density)	ASTM Type I water	24 ± 2	6.5–8.5	30	Sbrilli et al (2005)
										27.4	*GEOMETRI*

											C MEAN
										27.4	**VALUE USED IN SSD**
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus obliquus*)	Exponential growth phase	4	Chronic	EC50 (Cell density)	OECD TG 201 or AAP medium and Deionised water	24 ± 0.5	7.5-8.1	156	Bian et al (2009)
										156	*GEOMETRIC MEAN*
										31.2[@]	**VALUE USED IN SSD**
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus vacuolatus*)	Exponential growth phase	2	Chronic	EC50 (Cell density)	Sterile inorganic medium	25	Not stated	2.3	Vallotton et al (2008)
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus vacuolatus*)	Exponential growth phase	2	Chronic	EC50 (Cell density)	Sterile inorganic medium	25	Not stated	3	Vallotton et al (2008)
										2.6	*GEOMETRIC MEAN*
										0.52[@]	**VALUE USED IN SSD**
Chordata	Actinopterygii	Sheepshead Minnow (*Cyprinodon variegatus*)	Early life	26	Chronic	LOEC (Mortality, hatching, growth)	Clean surface or Ground water/Reconstituted water	22 ± 2	>6.0 and <8.0	2,200	USEPA (2015b)
										2,200	*GEOMETRIC MEAN*
										880[@]	**VALUE USED IN SSD**
Chordata	Actinopterygii	Fathead Minnow (*Pimephales promelas*)	Early life	35	Chronic	LOEC (Mortality)	Deionized water	25 ± 2	Not stated	1,600	USEPA (2015b)
										1,600	*GEOMETRI*

											C MEAN
										640[@]	**VALUE USED IN SSD**
Cyanobacteria	Cyanophyceae	Cyanobacteria (*Anabaena flos-aquae*)	Not stated	5	Chronic	EC50 (Growth, growth rate)	ASTM Type I water	24 ± 2	7.5 ± 0.1	1,200	USEPA (2015b)
										1,200	*GEOMETRIC MEAN*
										240[@]	**VALUE USED IN SSD**
Tracheophyta	Magnoliopsida	Macrophyte (*Ceratophyllum demersum*)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM medium with sediment layer	25	7.2	70	Fairchild et al (1998)
										70	*GEOMETRIC MEAN*
										14[@]	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM medium with sediment layer	25	7.2	2,355	Fairchild et al (1998)
										2,355	*GEOMETRIC MEAN*
										471[@]	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Stage 3 - 3 fronds/plant	14	Chronic	NOEL (Frond number)	M-Hoagland's or 20X-AAP with deionized water/ASTM Type I water	25 ± 2	4.8-5.2 (Hoagland's)/ 7.5 ± 0.1 (20X-AAP)	8.4	USEPA (2015b)
										8.4	*GEOMETRIC MEAN*
										8.4	**VALUE**

											USED IN SSD
Tracheophyta	Liliopsida	Macrophyte (*Najas* sp.)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM medium	25	Not stated	242	Fairchild et al (1998)
										242	*GEOMETRIC MEAN*
										48.4[@]	**VALUE USED IN SSD**

[1] **AUC = area under the growth curve.** [2] **This species has also called** *Chlorella vulgaris* **and** *Chlorella pyrenoidosa*. [3] **This species has been called** *Raphidocelis subcapitata*, *Pseudokirchneriella subcapitata* **and** *Selenastrum capricornutum*. [@] **Values were chronic LOEC andEC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).**

9.2.7 Distribution of sensitivities for aquatic species

The toxicity data for metolachlor to all freshwater species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the metolachlor ecotoxicity data may be bimodal (Figure 35).
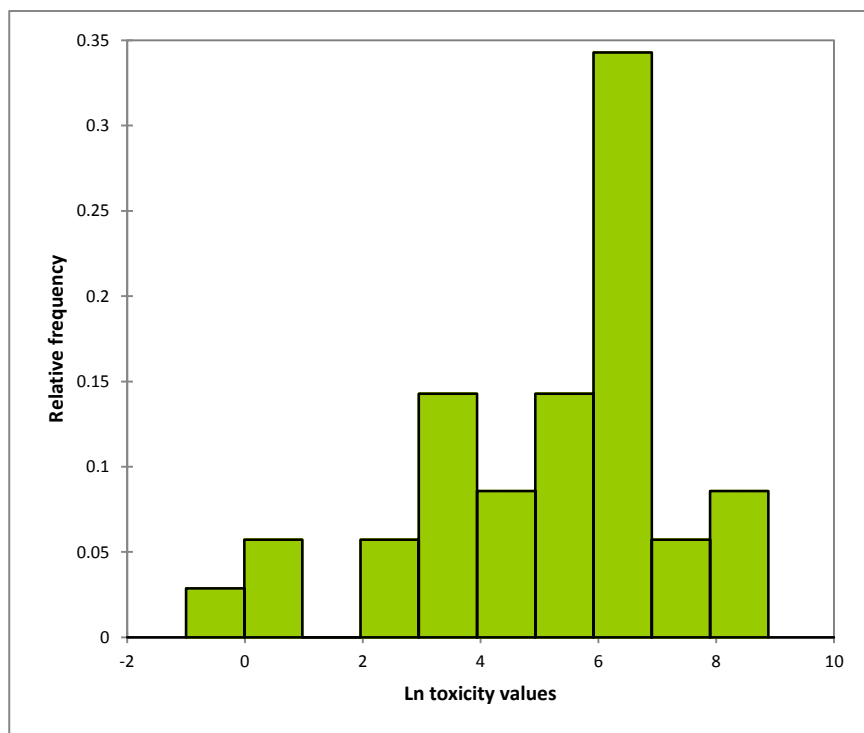
Figure 35 Histogram of the natural logarithm (ln) of all metolachlor (freshwater) toxicity data for phototrophic and non-phototrophic species ($n = 35$).

The metolachlor ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences, (i.e. p-value ≤ 0.05) between the two groups, the non-parametric Mann-Whitney test was used because the transformed metolachlor concentration data failed tests for normality (Anderson-Darling; p = 0.016) and had unequal variances (Fisher's F-Test; p <0.0001). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.099); therefore it can be concluded that the distribution of the metolachlor concentration data is uni-modal.

9.2.8 Rationale for the selected method for deriving the proposed aquatic ecosystem protection guideline values for metolachlor in freshwaters

The preference of ecotoxicity data used to derive the protective concentration (PC)[6] values and/or PGVs for metolachlor to freshwater species is:

1. chronic NOEC/EC10 ecotoxicity data for phototrophs and heterotrophs;
2. chronic NOEC/EC10 and chronic estimated NOEC values for phototrophs and heterotrophs.

In total, there were chronic EC10/NOEC/NOEL data for 14 phototrophic and heterotrophic freshwater species (four phyla and seven classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta and Tracheoyphyta. The represented classes were Bacillariophyceae (a major grouping of green algae diatoms), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Fragilariophyceae (a grouping of pennate diatoms), Liliopsida (monocots), Mediophyceae (another algae grouping) and Trebouxiophyceae (another grouping of green algae). These data met the minimum data requirements of the SSD method (Warne et al. 2015). The resulting SSD and PC values using only this data are presented in Figure 36 and Table 41, respectively.
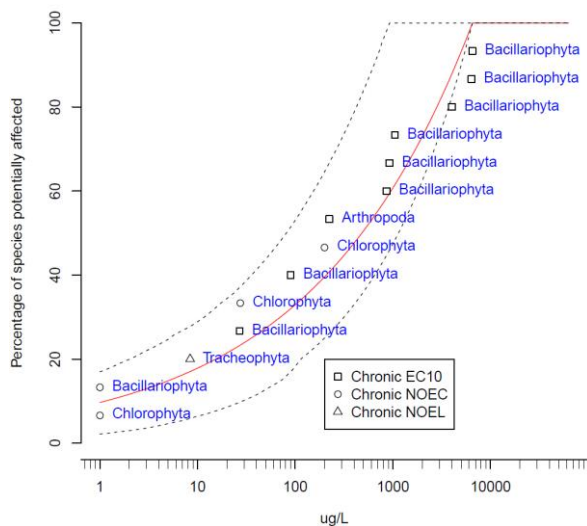
Figure 36 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to metolachlor.

---

[6] The values generated from a SSD are termed protective concentration (PC) values (as they are the concentrations that provide specific levels of protection e.g. PC99, PC95, PC90 and PC80 aim to protect 99, 95, 90 and 80 percent of species, respectively). Those PC values considered the most appropriate to use for ecosystem protection are adopted as the PGVs.

Table 41 Protective concentration values (µg/L) of metolachlor for the protection of freshwater ecosystems generated from the species sensitivity distribution in Figure 38.

Metolachlor protective concentration values (freshwater)[1]		Reliability classification[2]	
Percent species protection	Concentration (µg/L)	Criterion	Result
99%	0.0002	Sample size	14
95%	0.084	Type of toxicity data	Chronic EC10/NOEC/NOEL data
90%	1.1	SSD model fit	Poor
80%	15	*Reliability*	Moderate

[1] **Protective concentration values were derived using the Burrlioz 2.0 (2016) software.**

[2] **See Warne et al. (2015) for definitions of protective concentration value "reliability".**

The resulting PC values were considered to be of moderate reliability (Table 41) according to the methods of Warne et al. (2015) because the dataset consisted of chronic EC10/NOEC/NOEL values for 14 species and the cumulative distribution had a poor fit to the data (Figure 36). However, due to the fit and shape of the distribution model with the data (and the associated confidence intervals), there was a high level of uncertainty in the estimation of the PC99 and PC95 values. That is, the calculated PC99 and PC95 were ~77 000 and ~70 times less than the lowest chronic NOEC/EC10 ecotoxicity value of 1 µg/L (respectively), which suggested the calculated PC values might be highly conservative. The overly conservative estimations of the PC99 and PC95 values occur because the fitted curve sits relatively high on the y-axis where $x = 1$ µg/L (i.e. the lowest toxicity value). The lowest toxicity value would be equivalent to a PC90.

In response, the ecotoxicity dataset was expanded to also include the chronic estimated NOEC data (estimated from chronic LOEC and EC/LC50 data[7]), resulting in a total of 25 species from six phyla (Table 39). Expanding the dataset markedly improved the fit of the distribution model to the ecotoxicity data (Figure 34), which subsequently generated PC99 and PC95 estimations (Table 39) much closer to the lowest ecotoxicity value of this expanded dataset (0.53 µg/L); ~30 and ~1 times, respectively. Additionally, expanding the dataset improved the reliability classification of the SSD model fit to *good* and calculated *very high reliability* PC values (Table 39), according to Warne et al. (2015) (see section 9.2.4). Statistical methods, including the SSD methods, become more accurate and reliable as the amount of data available to analyse increases. All these factors combined led to the recommendation that the PC values derived using both chronic and chronic estimated ecotoxicity data be adopted as the PGVs for metolachlor in freshwaters.

---

[7] chronic LOEC and EC/LC50 data were converted to chronic estimated NOEC data using the methods stated in Warne et al. (2015).

9.2.9 References

AATSE (2002). Pesticide use in Australia. A review undertaken by the Australian Academy of Technological Sciences and Engineering. Australian Academy of Technological Sciences and Engineering, Parkville, Victoria, Australia, 309pp.

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

Balsiger C, Niederhauser P, Jäggi O and Meier W (2004). Pestizide in Fliessgewässern des Kantons Zürich – Auswertung der Untersuchungen von 1999 bis 2003. Amt für Abfall, Wasser, Energie und Luft (AWEL), Zurich, Switzerland.

Battaglin WA, Furlong ET, Burkhardt MR and Peter CJ (2000). Occurrence of sulfonylurea, sulfonamide, imidazolinone, and other herbicides in rivers, reservoirs and ground water in the Midwestern United States, 1998, *Science of the Total Environment*, 248, 123–133.

BCPC (2012). A world compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 772–775.

Bian H, Chen J, Cai X, Liu P, Wang Y, Huang L, Qiao X and Hao C (2009). Dechlorination of chloroacetanilide herbicides by plant growth regulator sodium bisulfite, *Water Research*, 43, 3566–3574.

Böger P (2003). Mode of action for chloroacetamides and functionally related compounds, *Journal of Pesticide Science*, 28, 324–329.

Böger P, Matthes B and Schmalfuß J (2000). Towards the primary target of chloroacetamides –new findings pave the way, *Pest Management Science*, 56, 497–508.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

CCME (1999). Canadian Water Quality Guidelines for the protection of aquatic life – Metolachlor. Canadian Council of Ministers of the Environment. Available from: http://www.ccme.ca/en/search.html?keywords=metolachlor&submit=&search=. Accessed: 9 September, 2015.

Coquillé N, Jan G, Moreira A and Morin S (2015). Use of diatom motility features as endpoints of metolachlor toxicity, *Aquatic Toxicology*, 158, 202–210.

Fairchild JF, Ruessler DS and Carlson AR (1998). Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor, and metolachlor, *Environmental Toxicology and Chemistry*, 17, 1830–1834.

Fischer BB, Roffler S and Eggen RIL (2012). Multiple stressor effects of predation by rotifers and herbicide pollution on different *Chlamydomonas* strains and potential impacts on population dynamics, *Environmental Toxicology and Chemistry*, 31, 2832–2840.

Gilliom RJ, Barbash JE, Crawford CG, Hamilton PA, Martin JD, Nakagaki N, Nowell LH, Scott JC, Stackelberg PE, Thelin GP and Wolock DM (2006). The quality of our nation's waters-Pesticides

in the nation's streams and ground water, 1992–2001. U.S. Geological Survey Circular 1291, USA, 172pp.

Götz T and Böger P (2004). The very-long-chain fatty acid synthase is inhibited by chloroacetamides, *Z. Naturforsch (C),* 59, 549–553.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Konstantinou IK, Hela DG and Albanis TA (2006). The status of pesticide pollution in surface waters (rivers and lakes) of Greece. Part I. Review on occurrence and levels, *Environmental Pollution,* 141, 555–570.

Larras F, Bouchez A, Rimet F and Montuelle B (2012). Using bioassays and species sensitivity distributions to assess herbicide toxicity towards benthic diatoms, PLoS One, 7, 1–9.

Larras F, Montuelle B and Bouchez A (2013). Assessment of toxicity thresholds in aquatic environments: Does benthic growth of diatoms affect their exposure and sensitivity to herbicides?, *Science of the Total Environment*, 469–477.

Liu H and Xiong M (2009). Comparative toxicity of racemic metolachlor and S-metolachlor to *Chlorella pyrenoidosa*, *Aquatic Toxicology*, 93, 100–106.

Liu H, Ye W, Zhan X and Liu W (2006). A comparative study of rac- and s-metolachlor toxicity to *Daphnia magna*, *Ecotoxicology and Environmental Safety*, 63, 451–455.

Perez J, Domingues I, Soares AMVM and Loureiro S (2011). Growth rate of *Pseudokirchneriella subcapitata* exposed to herbicides found in surface waters in the Alqueva Reservoir (Portugal): A Bottom-up approach using binary mixtures, *Ecotoxicology*, 20, 1167–1175.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Sbrilli G, Bimbi B, Cioni F, Pagliai L, Luchi F and Lanciotti E (2005). Surface and ground waters characterization in Tuscany (Italy) by using algal bioassay and pesticide determinations: Comparative evaluation of the results and hazard assessment of the pesticides impact on primary product, *Chemosphere*, 58, 571–578.

Spoljarich D, Cipak A, Horvatic J, Andrisic L, Waeg G, Zarkovic N and Jaganjac M (2011). Endogenous 4-hydroxy-2-nonenal in microalga *Chlorella kessleri* acts as a bioactive indicator of pollution with common herbicides and growth regulating factor of hormesis, *Aquatic Toxicology*, 105, 552–558.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ water quality guideline database for toxicants. Supplied as part of a CD-rom in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm. Accessed: May 2015.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: May–September, 2015.

Vallotton N, Moser D, Eggen RIL, Junghans M and Chèvre N (2008). S-metolachlor pulse exposure on the alga *Scenedesmus vacuolatus*: Effects during exposure and the subsequent recovery, *Chemosphere*, 73, 395–400.

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Canberra, Australia.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

10 Metribuzin

10.1 Introduction

Metribuzin is a herbicide ($C_8H_{14}N_4OS$ and Figure 37) that at room temperature is in the form of white crystals with a weak characteristic sulphurous odour. It is the active ingredient of a variety of commercial herbicide formulations.
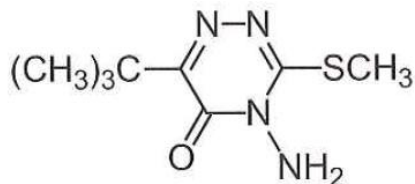
Figure 37 Structure of metribuzin

Physicochemical properties of metribuzin that may affect its environmental fate and toxicity are presented in Table 42.

Table 42 Summary of selected physicochemical properties of metribuzin.

Physicochemical property	Value
Molecular weight	214.3 amu[1]
Aqueous solubility	1165 mg/L @ temperature 20 ºC[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	1.6 @ pH 5.6 and temperature 20 ºC[1] 1.65 @ pH 7 and temperature 20 ºC[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.98[4]
Logarithm of the bioconcentration factor (log BCF)	1[2]
Half-life ($t_{1/2}$) in water	Stable @ pH 7 and temperature 20 ºC[2] 7 days[1], 2.5 to 7.5 days[3]
Half-life ($t_{1/2}$) in soil	11.5 days (19 – 11.5 days in field and the lab (20 ºC), respectively)[2]

[1] **BCPC (2012).** [2] **Pesticide Properties Database (University of Hertfordshire 2013).** [3] **CCME (1999).** [4] **Kim and Feagley (1998).**

Metribuzin belongs to the triazinone group of herbicides, which also includes amibuzin, hexazinone and trifludimoxazin. Metribuzin is extensively used in agriculture (e.g., soybeans; potatoes; barley, wheat; asparagus; sugarcane; tomatoes; peas; lentils) to control broadleaf weeds and annual grasses (CCME 1999). It is a selective, systemic, pre- and post-emergent herbicide (CCME 1999) which is highly soluble in water (Table 42).

Metribuzin in mainly absorbed through the roots of plants and to a lesser extent by leaves. Following absorption by roots, it is translocated in the xylem to the leaves where it exerts its toxicity. Metribuzin exerts its toxicity in plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Triazinone herbicides bind to the plastoquinone B (Q_B) protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (ATP, used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (NADPH, used in converting CO_2 to glucose), and therefore, prevents CO_2 fixation (Wilson et al. 2000).

In addition to its main mode of action, exposure to PSII inhibiting herbicides can lead to marked increases in the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen (OH^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). Reactive oxygen species are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Reactive oxygen species are created during normal cellular functioning particularly in biochemical processes that involve the generation of energy (e.g. photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells). In phototrophs, ROS are formed when the absorbed light energy exceeds the ability to convert CO_2 to organic molecules, thus accumulating oxygen (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, prolonged exposure to elevated concentrations of ROS in plants, as a result of biotic (e.g. disease) and/or abiotic stressors (e.g. PSII inhibiting herbicides), can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

Metribuzin can also exert biochemical effects in other non-target organisms. It is known to cause endocrine disrupting effects (Mnif et al. 2011). For example, metribuzin reportedly interferes with the normal thyroxine function in vertebrates (Porter et al. 1993). Metribuzin is classed as a potential endocrine disrupting chemical (EDC) by the European Union, as there is 'more or less comprehensive evidence' of endocrine disrupting effects in exposed aquatic organisms (DEPA 2015). Endocrine disrupting effects were not considered in the derivation of the PGVs for metribuzin.

Metribuzin ultimately ends up in aquatic environments as a result of accidental discharge, runoff, vapour drift, rainfall or direct application to watercourses to control aquatic plants and algae (CCME 1999). Metribuzin has low soil adsorption characteristics and thus has a high capacity to leach to groundwater and end up in surface waters (Kim and Feagley 1998). Australian figures from 2011–15 show that metribuzin has been detected in approximately 14.5% of surface water samples in waterways that drain agricultural land and discharge to the Great Barrier Reef (based on data in Turner et al. 2013a, 2013b; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015). Metribuzin is also present in marine waters, with figures from 2011–14 indicating that metribuzin has been detected in approximately 3% of marine samples (maximum concentration 4 µg/L) in the Wet Tropics region - off the coast of northern Queensland, Australia (O'Brien et al. 2015).

10.2 Freshwater

10.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for metribuzin in freshwaters (Table 44) includes toxicity data for ten freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, two cladocerans, nine macrophytes and eight microalgae. The toxicity value for the single fish species was a 95-day LOEC (mortality) value of 3,000 µg/L. The toxicity values for the cladocerans were 2-day NOEC and LC50 (fecundity) values of 6,250 and 8,840 µg/L, respectively, a 21-day NOEL (body length, dry weight) value of 1,290 µg/L

and two 21-day LOEC (body length, dry weight) values of 320 and 2,620 µg/L. The toxicity values for the macrophytes consisted of an 8-day EC50 (frond area) value of 45 µg/L, a 14-day NOEL (frond number, dry weight, frond area) value of 18 µg/L, 14-day EC50 (frond number, dry weight, frond area, wet weight) values ranging from 14 to 90 µg/L, two 28-day NOEC (stem length) values of 10 and 32 µg/L, 28-day LOEC (stem length) values ranging from 10 to 100 µg/L and 28-day IC50 (stem length) values ranging from 16 to 64 µg/L. The toxicity values for the microalgae consisted of 3-day EC50 (cell density) values ranging from 22.5 to 180 µg/L, 4-day NOEL and LOEC (chlorophyll content) values of 19 and 38 µg/L, respectively, 4-day EC50 (chlorophyll content) ranging from 23 to 152 µg/L, 5-day NOEL (biomass yield, growth rate, area under the curve) values ranging from 2.33 to 8.9 µg/L, a 5-day LOEL (biomass yield, growth rate, area under the curve) value of 9.7 µg/L, 5-day EC50 (biomass yield, growth rate, area under the curve) values ranging from 8.09 to 119 µg/L and a 14-day EC50 (wet weight) value of 100 µg/L.

Freshwater Acute

There were freshwater acute toxicity data for four fish, one crustacean, two cladocerans, one insect, one macrophyte and two microalgae. The toxicity values for the fish were a 96-hour LOEL (mortality) value of 32,000 and 96-hour LC50 values ranging from 42,000 to 92,000 µg/L. The toxicity values for the crustacean were 48-, 72- and 96-hour LC50 (mortality) values of 206,300 µg/L, 58,700 µg/L and 30,600 µg/L, respectively. The cladoceran toxicity data consisted of 48-hour NOEC and LC50 (mortality) values of 25,000 and 35,360 µg/L, respectively, and 48-hour NOEL and EC50 (body length, dry weight) values ranging from 1,000 to 75,000 µg/L and 4,180 to 98,500 µg/L, respectively. The toxicity values for the insect were 24- and 48-hour EC50 (immobilisation) values of 175,000 and 43,500 µg/L, respectively. The toxicity values for the macrophytes consisted a 96-hour EC36 (frond count) value of 19 and two 96-hour EC50 (frond count) values of 36 to 37 µg/L. The microalgae toxicity data consisted of 14-hour EC25 and EC50 (cell volume) values of 7.5 and 14.8 µg/L, respectively a 24-hour EC25 (cell number) value of 7.3 µg/L and two 24-hour EC50 (cell number) values of 11.1 and 25.7 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

10.2.2   Factors affecting toxicity

No factors have been reported as modifying the toxicity of metribuzin. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of metribuzin (Table 42).

10.2.3   Guideline derivation

The derived PGVs for metribuzin in freshwaters are provided in Table 43. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for metribuzin are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for metribuzin are low (Table 42) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for metribuzin do not need to account for secondary poisoning.

Table 43 Proposed aquatic ecosystem protection guideline values (µg/L) for metribuzin for the protection of freshwater ecosystems.

Metribuzin proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	2.0 (1.7 – 2.6)	Sample size	18
95%	2.6 (2.2 – 3.3)	Type of toxicity data	Chronic NOEC/NOEL and chronic estimated NOEC values
90%	3.1 (2.7 – 4.0)	SSD model fit	Good
80%	3.9 (3.3 – 5.2)	Reliability	Very high

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline values "reliability".**

10.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for metribuzin in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for metribuzin to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more metribuzin toxicity data available that enable the calculation of PGVs in freshwaters (see section 10.2.6).

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for metribuzin in freshwaters, *Microcystic* sp. and *Najas* sp. were included as no other toxicity data for these genera were used.

In total, there were freshwater toxicity data for 27 species (six phyla and ten classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The ten classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Insecta (invertebrates), Liliopsida (monocots), Magnoliopsida (dicots), Malacostraca (a larger grouping of crustaceans) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of metribuzin, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The metribuzin ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test

to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p <0.0001, see section 10.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for five freshwater phototrophic species (that belonged to three phyla and four classes), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). When the dataset was expanded to combine the chronic NOEC/NOEL data with the chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively), there were data available for 18 freshwater phototrophic species (that belonged to four phyla and six classes), which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 43) combined with the good fit of the distribution to these toxicity data (Figure 38) resulted in a very high reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for metribuzin in freshwater environments is provided in Table 44.

Table 44 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for metribuzin in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[4]	3.6	USEPA (2015b)
Macrophyte	*Ceratophyllum demersum**	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	2.8	Fairchild et al (1998)
Microalga	*Chlamydomonas reinhardtii**	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll content	4.6	Fairchild et al (1998)
Microalga	*Chlorella kessleri*	Chlorophyta	Trebouxiophyceae	Exponential growth phase	3	Chronic est. NOEC	Cell density	6.2	Pavlic et al (2006)
Microalga	*Chlorella vulgaris*[2]*	Chlorophyta	Trebouxiophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll content	6.2	Fairchild et al (1998)
Microalga	*Desmodesmus subspicatus**	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic est. NOEC	Cell density	33.4	Pavlic et al (2006)
Macrophyte	*Egeria densa**	Tracheophyta	Liliopsida	Not stated	28	Chronic NOEC	Stem length	32	Forney and Davis (1981)
Macrophyte	*Elodea canadensis**	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	4.2	Fairchild et al (1998)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic NOEL	Frond number, dry weight, frond area	18	USEPA (2015b)
Macrophyte	*Lemna paucicostata**	Tracheophyta	Liliopsida	Not stated	8	Chronic est. NOEC	Frond area	9	Grossman et al (1992)
Macrophyte	*Lemna perpusilla**	Tracheophyta	Liliopsida	Not stated	28	Chronic est. NOEC	Stem length	3.58	Forney and Davis (1981)
Cyanobacteria	*Microcystis* sp.	Cyanobacteria	Cyanophyceae	Not stated	14	Chronic NOEC	Wet weight	20	Fairchild et al. (1998)
Macrophyte	*Myriophyllum heterophyllum*	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	3.4	Fairchild et al (1998)
Macrophyte	*Myriophyllum*	Tracheophyta	Magnoliopsida	Not stated	28	Chronic	Stem length	10	Forney and

						NOEC			Davis (1981)
	spicatum								
Macrophyte	*Najas* sp.	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Wet weight	3.8	Fairchild et al (1998)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[4]	8.9	USEPA (2015b)
Microalga	*Scenedesmus quadricauda**	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll content	30.4	Fairchild et al (1998)
Microalga	*Selenastrum capricornutum*[3]	Chlorophyta	Chlorophyta	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[4]	3.1	USEPA (2015b)

[1] **Chronic NOEC/NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] This species has also been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*. [3] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [4] AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.**

10.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 18 phototrophic freshwater species that was used to derive the PGVs is presented in Figure 38.
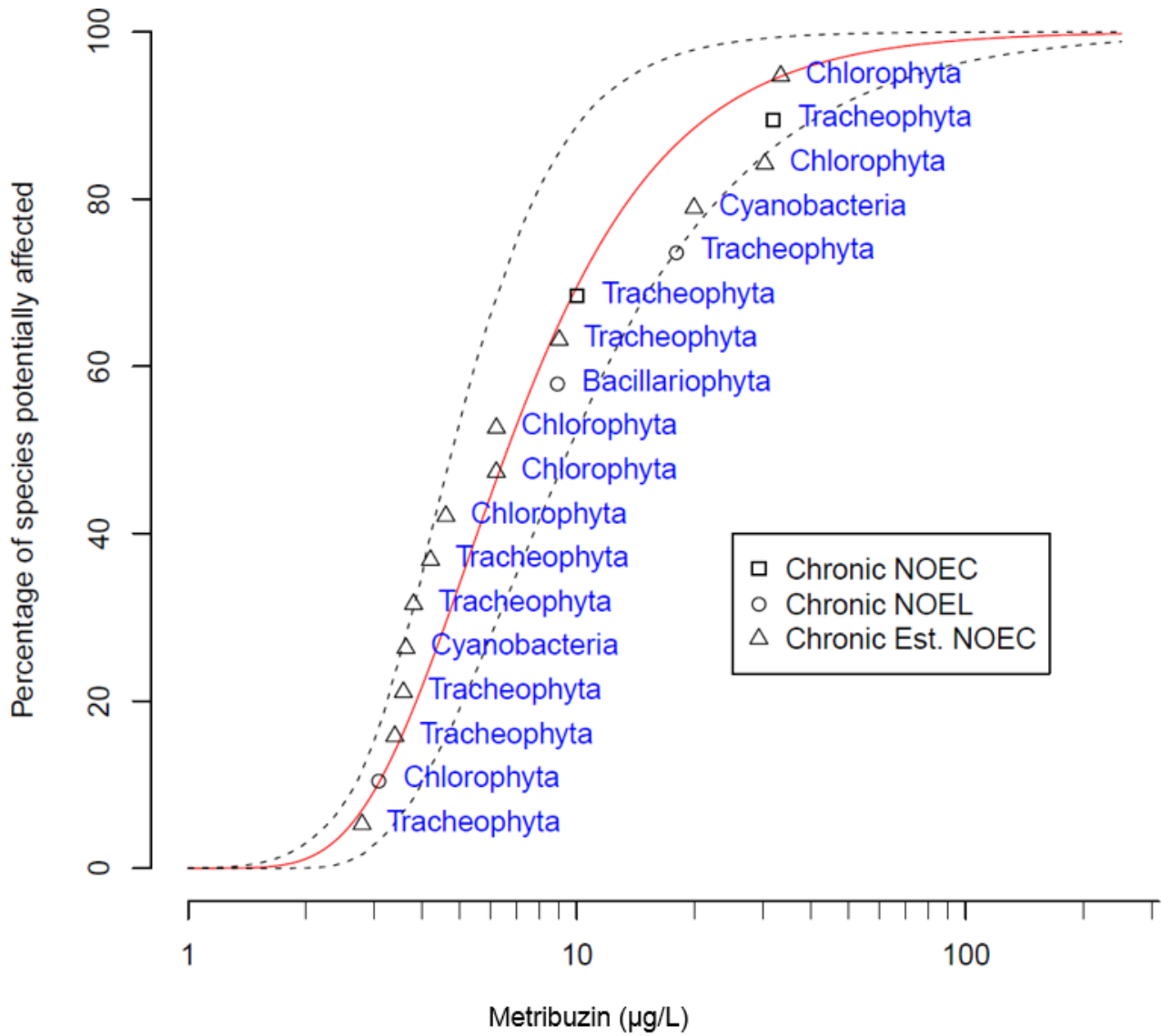
Figure 38 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect level (NOEL) and chronic estimated no observed effect concentration (NOEC) data values of freshwater phototrophic species to metribuzin. Black dashed lines indicate the 95% confidence intervals.

10.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for metribuzin in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Diatom (*Navicula pelliculosa*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2.0	7.5 ± 0.1	8.9	USEPA (2015b)
										8.9	*GEOMETRIC MEAN*
										8.9	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomonas reinhardtii*)	Not stated	4	Chronic	EC50 (Chlorophyll content)	ASTM medium	25	Not stated	23	Fairchild et al (1998)
										23	*GEOMETRIC MEAN*
										4.6@	***VALUE USED IN SSD***
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella kessleri*)	Exponential growth phase	3	Chronic	EC50 (Cell density)	Freshwater	23 ± 2.0	Not stated	26	Pavlic et al (2006)
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella kessleri*)	Exponential growth phase	3	Chronic	EC50 (Cell density)	Freshwater	23 ± 2.0	Not stated	37	Pavlic et al (2006)
										31.0	*GEOMETRIC MEAN*
										6.2@	***VALUE USED IN SSD***
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella vulgaris[2]*)	Not stated	4	Chronic	EC50 (Chlorophyll)	ASTM medium	25	Not stated	31	Fairchild et al (1998)

										31	*GEOMETRIC MEAN*
										6.2[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Desmodesmus subspicatus[3]*)	Exponential growth phase	3	Chronic	EC50 (Cell density)	Freshwater	23 ± 2.0	Not stated	155	Pavlic et al (2006)
Chlorophyta	Chlorophyceae	Microalga (*Desmodesmus subspicatus[3]*)	Exponential growth phase	3	Chronic	EC50 (Cell density)	Freshwater	23 ± 2.0	Not stated	180	Pavlic et al (2006)
										167	*GEOMETRIC MEAN*
										33.4[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus quadricauda*)	Not stated	4	Chronic	EC50 (Chlorophyll content)	ASTM medium	25	Not stated	152	Fairchild et al (1998)
										152	*GEOMETRIC MEAN*
										30.4[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[4]*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2.0	7.5 ± 0.1	4.1	USEPA (2015b)
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[4]*)	Not stated	5	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	ASTM Type I water	24 ± 2.0	7.5 ± 0.1	2.33	USEPA (2015b)
										3.1	*GEOMETRIC MEAN*
										3.1	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Microalga	Not stated	5	Chronic	LOEL	ASTM Type	24 ±	7.5 ± 0.1	9.7	USEPA

		(*Anabaena flos-aquae*)				(Biomass yield, growth rate, AUC[1])	I water	2.0			(2015b)
										9.7	*GEOMETRIC MEAN*
										3.6[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Duckweed (*Lemna gibba*)	Not stated	14	Chronic	NOEL (Frond number, dry weight, frond area)	M-Hoagland's or 20X-AAP with deionized water/ASTM Type I water	25 ± 2	4.8-5.2 (Hoagland's)/7.5 ± 0.1 (20X-AAP)	18	USEPA (2015b)
										18	*GEOMETRIC MEAN*
										18	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Microalga (*Microcystis* sp.)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM medium	25	Not stated	100	Fairchild et al. (1998)
										100	*GEOMETRIC MEAN*
										20[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Duckweed (*Egeria densa*)	Not stated	28	Chronic	NOEC (Stem length)	Nutrient solution	20 – 30	Not stated	32	Forney and Davis (1981)
										32	*GEOMETRIC MEAN*
										32	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Duckweed (*Lemna gibba*)	Not stated	14	Chronic	NOEL (Frond number, dry weight, frond	M-Hoagland's or 20X-AAP with	25 ± 2	4.8-5.2 (Hoagland's)/7.5 ± 0.1 (20X-AAP)	18	USEPA (2015b)

						area)	deionized water/ASTM Type I water				
										18	*GEOMETRIC MEAN*
										18	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Duckweed (*Lemna paucicostata*)	Not stated	8	Chronic	EC50 (Frond area)	Inorganic medium containing sucrose	25	Not stated	45	Grossman et al (1992)
										45	*GEOMETRIC MEAN*
										9[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Duckweed (*Lemna perpusilla*)	Not stated	28	Chronic	LOEC (Stem length)	Nutrient solution	27	Not stated	10	Forney and Davis (1981)
Tracheophyta	Liliopsida	Duckweed (*Lemna perpusilla*)	Not stated	28	Chronic	LC50 (Stem length)	Nutrient solution	27	Not stated	16	Forney and Davis (1981)
										12.65	*GEOMETRIC MEAN*
										3.58[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Duckweed (*Najas* sp.)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM medium	25	Not stated	19	Fairchild et al (1998)
										19	*GEOMETRIC MEAN*
										19[@]	***VALUE USED IN SSD***
Tracheophyta	Magnoliopsida	Macrophyte (*Ceratophyllum demersum*)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM medium	25	Not stated	14	Fairchild et al (1998)

										14	*GEOMETRIC MEAN*
										2.8[@]	***VALUE USED IN SSD***
Tracheophyta	Magnoliopsida	Macrophyte (*Elodea canadensis*)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM with sediment layer	25	Not stated	21	Fairchild et al (1998)
										21	*GEOMETRIC MEAN*
										4.2[@]	***VALUE USED IN SSD***
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum heterophyllum*)	Not stated	14	Chronic	EC50 (Wet weight)	ASTM + Nutrient-enriched water (NEW) medium	25	Not stated	17	Fairchild et al (1998)
										17	*GEOMETRIC MEAN*
										3.4[@]	***VALUE USED IN SSD***
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	28	Chronic	NOEC (Stem length)	Nutrient solution	27	Not stated	10	Forney and Davis (1981)
										10	*GEOMETRIC MEAN*
										10	***VALUE USED IN SSD***

[1] AUC = area under the growth curve. [2] This species has also been called *Chlorella pyrenoidosa*. [3] This species has also been called *Scenedesmus subspicatus*. [4] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella capricornutum*. [@] Values were chronic LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2015).

10.3 Marine

10.3.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for metribuzin in marine waters (Table 46) includes toxicity data for 11 species (one marine and ten freshwater) that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine and freshwater species that passed the screening and quality assurance processes are provided below and in section 10.2.1, respectively.

Marine Chronic

There were marine chronic toxicity data for only one microalga species which consisted of 5-day NOEL and EC50 (biomass yield, growth rate, area under the curve) values of 5.8 and 8.8 µg/L, respectively.

Marine Acute

There were marine acute toxicity data for one fish, two crustaceans and a mollusc species. The toxicity values for the single fish species were 96-hour NOEL and LC50 (mortality) values of 60,000 and 85,000 µg/L, respectively. The toxicity values for the crustaceans consisted of a 96-hour NOEL (mortality) value of 65,000 µg/L and a 96-hour LC50 values of 48,270 µg/L. The mollusc toxicity data consisted of a 96-hour LOEL (mortality, abnormal development) value of 33,000 µg/L and 96-hour EC50 (mortality, abnormal development) values ranging from 40,700 to 49,800 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

10.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of metribuzin. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of metribuzin (Table 42).

10.3.3 Guideline derivation

The derived PGVs for metribuzin in marine waters are provided in Table 45. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for metribuzin are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for metribuzin are low (Table 42) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for metribuzin do not need to account for secondary poisoning.

Table 45 Proposed aquatic ecosystem protection guideline values (μg/L) for metribuzin for the protection of freshwater ecosystems.

Metribuzin proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	2.0 (1.7 – 2.7)	Sample size	19
95%	2.7 (2.3 – 3.5)	Type of toxicity data	Chronic NOEC/NOEL and chronic estimated NOEC values *(freshwater and marine)*
90%	3.1 (2.7 – 4.1)	SSD model fit	Good
80%	3.9 (3.3 – 5.3)	Reliability	Moderate

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

10.3.4   Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for metribuzin in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for metribuzin to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant database (Sunderam et al. 2000) were searched. There are now more metribuzin toxicity data available that enable the calculation of PGVs in marine waters. However, it was only possible to derive PGVs by using ecotoxicity data for a mixture of both marine and freshwater organisms (see section 10.3.6 and 10.2.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to marine ecosystems separately, it is recommended that additional chronic toxicity tests of metribuzin with marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for metribuzin in freshwaters, *Microcystic* sp. and *Najas* sp. were included as no other toxicity data for these genera were used.

In total, there were marine toxicity data for four species (four phyla and four classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chordata and Mollusca. The four classes were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Malacostraca (a larger grouping of crustaceans) and Mediophyceae (another algae grouping).

Based on the current understanding of the mode of action of metribuzin, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The metribuzin ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p <0.0001, see section 10.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were chronic no observed effect level (NOEL) data available for only one marine phototrophic species (that belonged to one phylum and one class), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). As no other ecotoxicity data for metribuzin to marine phototrophic species were available, the chronic NOEL data for marine phototrophic species were combined with the available chronic no observed effect concentration (NOEC)/NOEL and chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) data values for freshwater phototrophic species (see section 10.2) to derive PGVs for metribuzin in marine waters. This dataset incorporated concentration data for 19 (one marine and 18 freshwater) phototrophic species belonging to four phyla and seven classes that met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 45) combined with the good fit of the distribution to these toxicity data (Figure 39) resulted in a moderate reliability set of PGVs. The combination of freshwater and marine ecotoxicity data reduces the reliability classification of PGVs as per Warne et al. (2015). A summary of the toxicity data (one value per species) used to calculate the PGVs for metribuzin in marine environments is provided in Table 46.

Table 46 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for metribuzin in freshwaters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Fresh	Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic est. NOEC	Biomass yield, growth rate, AUC[4]	3.6	USEPA (2015b)
Fresh	Macrophyte	*Ceratophyllum demersum**	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	2.8	Fairchild et al (1998)
Fresh	Microalga	*Chlamydomonas reinhardtii**	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll content	4.6	Fairchild et al (1998)
Fresh	Microalga	*Chlorella kessleri*	Chlorophyta	Trebouxiophyceae	Exponential growth phase	3	Chronic est. NOEC	Cell density	6.2	Pavlic et al (2006)
Fresh	Microalga	*Chlorella vulgaris*[2]*	Chlorophyta	Trebouxiophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll content	6.2	Fairchild et al (1998)
Fresh	Microalga	*Desmodesmus subspicatus**	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic est. NOEC	Cell density	33.4	Pavlic et al (2006)
Fresh	Macrophyte	*Egeria densa**	Tracheophyta	Liliopsida	Not stated	28	Chronic NOEC	Stem length	32	Forney and Davis (1981)
Fresh	Macrophyte	*Elodea canadensis**	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	4.2	Fairchild et al (1998)
Fresh	Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic NOEL	Frond number, dry weight, frond area	18	USEPA (2015b)
Fresh	Macrophyte	*Lemna paucicostata**	Tracheophyta	Liliopsida	Not stated	8	Chronic est. NOEC	Frond area	9	Grossman et al (1992)
Fresh	Macrophyte	*Lemna perpusilla**	Tracheophyta	Liliopsida	Not stated	28	Chronic est. NOEC	Stem length	3.58	Forney and Davis (1981)
Fresh	Cyanobacteria	*Microcystis* sp.	Cyanobacteria	Cyanophyceae	Not stated	14	Chronic NOEC	Wet weight	20	Fairchild et al. (1998)
Fresh	Macrophyte	*Myriophyllum heterophyllum*	Tracheophyta	Magnoliopsida	Not stated	14	Chronic est. NOEC	Wet weight	3.4	Fairchild et al (1998)
Fresh	Macrophyte	*Myriophyllum*	Tracheophyta	Magnoliopsida	Not stated	28	Chronic	Stem length	10	Forney and

		spicatum					NOEC		Davis (1981)
Fresh	Macrophyte	*Najas* sp.	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Wet weight	3.8	Fairchild et al (1998)
Fresh	Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[4]	8.9	USEPA (2015b)
Fresh	Microalga	*Scenedesmus quadricauda**	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Chlorophyll content	30.4	Fairchild et al (1998)
Fresh	Microalga	*Selenastrum capricornutum*[3]	Chlorophyta	Chlorophyta	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[4]	3.1	USEPA (2015b)
Marine	Microalga	*Skeletonema costatum**	Bacillariophyta	Mediophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[3]	5.8	USEPA (2015b)

[1] **Chronic NOEC/NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). [2] This species has also been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*. [3] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [4] AUC = area under the growth curve. * Species that originated from/are distributed in Australia and/or New Zealand.**

10.3.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 19 marine and freshwater, phototrophic species that was used to derive the PGVs is presented in Figure 39.

Figure 39 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC), no observed effect level (NOEL) and chronic estimated no observed effect concentration (NOEC) data values of marine and freshwater phototrophic species to metribuzin. Black dashed lines indicate the 95% confidence intervals.

10.3.6   Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for metribuzin in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Mediophyceae	Marine Diatom (*Skeletonema costatum*)	Not stated	5	Chronic	NOEL (Biomass yield, Growth rate, AUC[2])	Synthetic saltwater or filtered natural saltwater	30 ± 5	20 ± 2.0	7.5 ± 0.1	5.8	USEPA (2015b)
											5.8	*GEOMETRIC MEAN*
											5.8	***VALUE USED IN SSD***

10.3.7   Distribution of sensitivities for aquatic species

The transformed ecotoxicity data for marine phototrophic species (n = 1) fell within the lower and upper 95% confidence intervals [0.029 and 3.704 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater phototrophic species (n = 21). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for metribuzin.

The toxicity data for metribuzin to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the metribuzin ecotoxicity data may be bimodal (Figure 40).
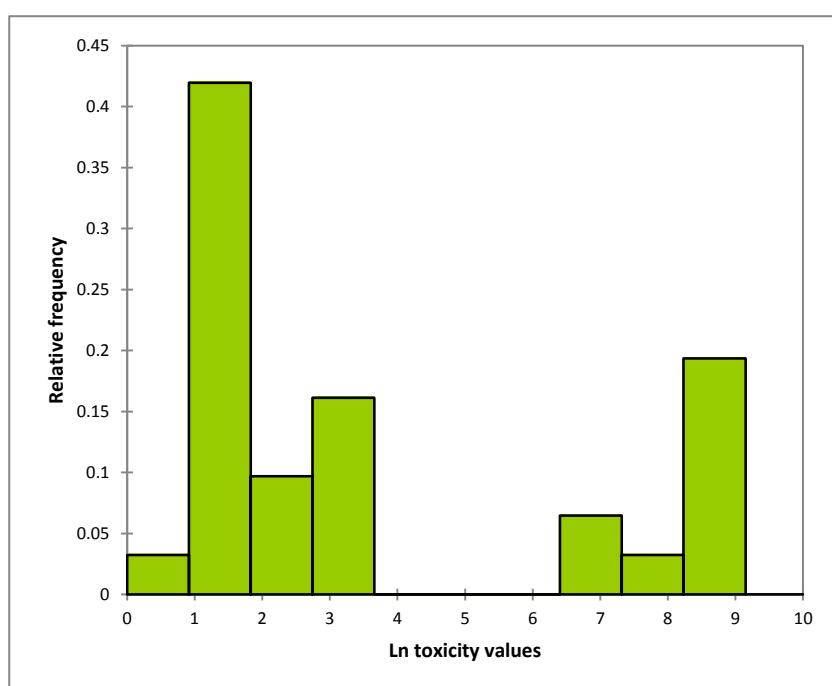
Figure 40 Histogram of the natural logarithm (ln) of all metribuzin (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (n = 31).

The metribuzin ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample t test was used because the transformed metribuzin concentration data had equal variances (Fisher's F-Test; p = 0.497) and followed a normal distribution (Anderson-Darling; p = 0.060). Results from the two-sample t test indicated that the two groups were significantly different (p <0.0001); therefore, it can be concluded that the distribution of the metribuzin concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

10.3.8 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 1026–1028.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

CCME (1999). Canadian Water Quality Guidelines for the protection of aquatic life – Metribuzin, Canadian Council of Ministers of the Environment. Accessed: 9 September, 2015.

Chen S, Yin C, Strasser RJ, Govinjee, Yang C and Qiang S (2012). Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*. Plant *Physiology and Biochemistry* 52, 38–51.

DEPA (2015). The EU list of potential endocrine disruptors. Ministry of Environment and Food, The Danish Environmental Protection Agency, København K, Denmark. Available from: http://eng.mst.dk/topics/chemicals/endocrine-disruptors/the-eu-list-of-potential-endocrine-disruptors/ , Accessed: October–November, 2015.

Fairchild JF, Ruessler DS and Carlson AR (1998). Comparative sensitivity of five species of macrophytes and six species of algae to atrazine, metribuzin, alachlor and metolachlor, *Environmental Toxicology and Chemistry*, 17, 1830–1834.

Forney DR and Davis DE (1981). Effects of low concentrations of herbicides on submersed aquatic plants, Weed Science, 29, 677–685.

Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R, Thomson B and Warne MStJ (2015). *Total suspended solids, nutrients and pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef* – Great Barrier Reef Catchment Loads Monitoring Program 2013–2014. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 108pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2013-2014-gbr-catchment-loads-technical-report.pdf

Grossmann K, Berghaus R and Retzlaff G (1992). Heterotrophic plant cell suspension cultures for monitoring biological activity in agrochemical research. Comparison with screens using algae, germinating seeds and whole plants, *Pesticide Science*, 35, 283–289.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Kessler E and Huss V.A.R (1992). Comparative physiology and biochemistry and taxonomic assignment of the Chlorella (Chlorophyceae) strains of the culture collection of the University of Texas at Austin*, Journal of Phycology*, 28, 550–553.

Kim S, Thiessen PA, Bolton EE, Chen J, Fu G, Gindulyte A, Han L, He J, He S, Shoemaker BA, Wang J, Yu B, Zhang J, Bryant SH (2016). PubChem Substance and Compound databases. Nucleic Acids Res. 2016 Jan 4; 44(D1):D1202-13. Epub 2015 Sep 22 [PubMed PMID: 26400175]. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/13263. Downloaded: 13 May, 2016.

Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O and Roig B (2011). Effect of endocrine disruptor pesticides: A review, *International Journal of Environmental Research and Public Health,* 8, 2265–2303.

Pavlic Z, Stjepanovic B, Horvatic J, Persic V, Puntaric D and Culig J (2006). Comparative sensitivity of green algae to herbicides using erlenmeyer flask and microplate growth-inhibition assays, *Bulletin of Environmental Contamination and Toxicology*, 76, 883–890.

Porter WP, Green SM, Debbink NL and Carlson I (1993). Groundwater pesticides: Interactive effects of low concentrations of carbamates aldicarb and methomyl and the triazine metribuzin on thyroxine and somatotropin levels in white rats, *Journal of Toxicology and Environmental Health*, 40, 15pp.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Turner R, Huggins R, Wallace R, Smith R, Vardy S and Warne MStJ (2013a). *Sediment, nutrient and pesticide loads: Great Barrier Reef Loads Monitoring 2009–2010*. Water Sciences Technical Report, Volume 2012, Number 14. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0994. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2009-2010-gbr-catchment-loads-report.pdf

Turner RDR, Huggins R, Wallace R, Smith RA, Vardy S and Warne MStJ (2013b). *Total suspended solids, nutrient and pesticide loads for rivers that discharge to the Great Barrier Reef: Great Barrier Reef Loads Monitoring 2010–2011.* Water Sciences Technical Report, Volume 2013, Number 1. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0996. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2010-2011-gbr-catchment-loads-report.pdf.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm Accessed 13 May 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs.

Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: February–April, 2016.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and Warne MStJ. 2015. Total suspended solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-catchment-loads-report.pdf

Wallace R, Huggins R, Smith RA, Turner RDR, Vardy S and Warne MStJ. 2014. Total suspended solids, nutrient and pesticide loads (2011–2012) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2011–2012. Department of Science, Information Technology, Innovation and the Arts. Brisbane, Queensland. 94p. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2011-2012-gbr-catchment-loads-report.pdf

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

Wilson PC, Whitwell T and Klaine SJ (2000). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*, *Archives of Environmental Contamination and Toxicology* 39, 282–288.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

11   Metsulfuron-methyl

11.1 Introduction

Metsulfuron-methyl is a herbicide ($C_{14}H_{15}N_5O_6S$ and Figure 41) that at room temperature is a white to pale-yellow solid with a characteristic ester-like odour. It is the active ingredient of a variety of commercial herbicide formulations. Metsulfuron-methyl is often mixed with other herbicides (e.g. terbutryn and glyphosate formulations) to increase its efficacy.
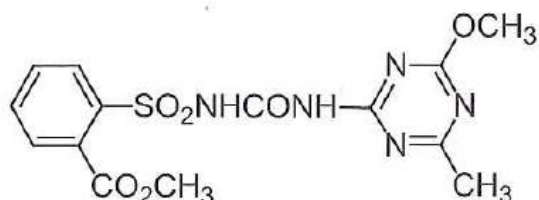
Figure 41 Structure of metsulfuron-methyl

Physicochemical properties of metsulfuron-methyl that may affect its environmental fate and toxicity are presented in Table 47.

Table 47 Summary of selected physicochemical properties of metsulfuron-methyl.

Physicochemical property	Value
Molecular weight	381.4 amu[1]
Aqueous solubility	548 mg/L (pH 5), 2,790 mg/L (pH 7), 213,000 mg/L (pH 9) @ temperature 25 ºC[1] 2,790 mg/L @ temperature 20 ºC[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	-1.87 @ pH 7 and temperature 20 ºC[1,2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.54[3]
Logarithm of the bioconcentration factor (log BCF)	1[3]
Half-life ($t_{1/2}$) in water	22 days @ pH 5 – 9 and temperature 25 ºC[2]
Half-life ($t_{1/2}$) in soil	Typical: 10 days (13.3 – 102.4 days in field and the lab (20 ºC), respectively)[2]

[1] **BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] Barcelo and Henion 2003.**

Metsulfuron-methyl belongs to the triazinylsulfonylurea group that sits within the sulfonylurea group of the urea family of herbicides, which also includes iodosulfuron, ethametsulfuron, thifensulfuron and their methylated forms. Metsulfuron-methyl is extensively used in agriculture, particularly in cereals and forestry to control broadleaf weeds and some annual grasses. Metsulfuron-methyl is a selective residual herbicide, and it retains its biological effectiveness in soil, with reported half-lives of a month to a year (CDC 2013); recommended exclusion times for some crops being up to 22 months (Cornell University 1993). The higher the soil moisture content and temperature and the lower the acidity the more rapidly metsulfuron-methyl is degraded in soil (Smith 1986). It is a systemic herbicide and can be applied before and after weeds emerge (i.e. it is a pre- and post-emergent herbicide).

Metsulfuron-methyl is mainly absorbed through the roots and foliage of plants and is transported to the leaves and shoots where it exerts its toxicity. Metsulfuron-methyl binds to, and inhibits, the acetolactate synthase (ALS) enzyme, which is responsible for catalysing the formation of amino acids. As a result, the biosynthesis of amino acid branches within sensitive plants is inhibited (FAO

UN 2015). Exposed plants typically die within two to four weeks due to cessation of cell division and growth processes.

Metsulfuron-methyl has a low affinity for binding to soil particles (Table 47) therefore it has a high capacity to leach to groundwater and end up in surface waters. The aqueous hydrolysis of metsulfuron-methyl is relatively fast with a half-life of 22 days at pH 5 through to pH 9 and a temperature of 25°C (University of Hertfordshire 2013) (Table 47).

11.2 Freshwater

11.2.1   Aquatic toxicicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for metsulfuron-methyl in freshwaters (Table 49) includes toxicity data for four freshwater species that either originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There were freshwater chronic toxicity data for one fish, 12 macrophytes and six microalgae. The toxicity values for the single fish species were 90-day NOEL and LOEC values of 4,500 and 8,000 µg/L. The toxicity values for macrophytes consisted of EC10 (frond count, frond area) values ranging of 0.16 and 0.37 µg/L, 7-day EC50 (frond count, frond number, dry weight, frond area) values ranging from 0.06 to 0.79 µg/L, two 8-day NOEC (dry weight) values of 10 to 20 µg/L, an 8-day NOEC (shoot length) of 0.054 µg/L, two 8-day LOEC (shoot length) values of 0.1 and 0.2 µg/L, 14-day NOEC (root occurrence, dry weight, frond count, frond number, frond area) values ranging from 0.16 to 20 µg/L, a 14-day LOEC (chlorophyll-a content) value of 2.7 µg/L, 14-day EC50 (leaf area, fresh weight: dry weight, frond number, dry weight, frond area, chlorophyll-a content, total shoot length) values ranging from 0.1 to 26.7 µg/L and 42-day NOEC, LOEC and EC50 (frond count) values of 0.1, 0.5 and 0.99 µg/L, respectively. The toxicity values for microalgae were a 48-hour EC10 (chlorophyll-a content) value of 292 µg/L, two 48-hour EC50 (chlorophyll-a content) values of 677 and 1,934 µg/L, a 72-hour IC50 (cell density) value of 611.8 µg/L, two 96-hour NOEL (biomass yield, growth rate, area under the growth curve) values of 14.5 and 92,800 µg/L, 96-hour EC50 (cell density, biomass, growth rate, area under the growth curve) values ranging from 26 to 14,556.4 µg/L, two 5-day NOEL (biomass, growth rate, area under the curve) values of 10 and 95.4 µg/L, a 5-day EC50 (biomass, growth rate, area under the curve) value of 285.6 µg/L and 6-day EC20 and EC50 (chlorophyll-a content) values of 68.6 and 1,563.5 µg/L, respectively.

Freshwater Acute

There were freshwater acute toxicity data for only one microalga species which were 24-hour NOEC, EC5 and EC50 (cell density) values of 165.1, 64.8 and 1,163.2 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

11.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of metsulfuron-methyl. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the low log K_{oc} value of metsulfuron-methyl (Table 47).

11.2.3 Guideline derivation

The derived PGVs for metsulfuron-methyl in freshwaters are provided in Table 48. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for metsulfuron-methyl are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for metsulfuron-methyl are low (Table 47) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for metsulfuron-methyl do not need to account for secondary poisoning.

Table 48 Proposed aquatic ecosystem protection guideline values (μg/L) for metsulfuron-methyl for the protection of freshwater ecosystems.

Metsulfuron- proposed aquatic ecosystem protection guideline values values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	0.0047 (0.0012 – 0.12)	Sample size	8
95%	0.025 (0.0074 – 0.62)	Type of toxicity data	Chronic EC10/NOEC/NOEL values
90%	0.069 (0.02 – 2.0)	SSD model fit	Poor
80%	0.28 (0.062 – 8.7)	Reliability	Moderate

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

11.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for metsulfuron-methyl, however there was an environmental concern level (ECL[8]) for metsulfuron-methyl in freshwater environments which was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on acute toxicity data for two freshwater fish species, one invertebrate and one microalga only (ANZECC and ARMCANZ 2000). Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as having an 'unknown' reliability.

To obtain toxicity data for metsulfuron-methyl to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA

---

[8] ECLs are not to substitute for water quality guidelines but instead stand as working levels which are derived for chemicals where there is no trigger value. ECLs should only be used until more data can be obtained or the guidelines can be independently derived.

2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now considerably more metsulfuron-methyl toxicity data available that enable the calculation of PGVs in freshwaters (see section 11.2.6). In order to derive higher reliability PGVs, it is recommended that additional chronic toxicity tests of metsulfuron-methyl with phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were freshwater toxicity data for 20 species (five phyla and seven classes) that passed the screening and quality assessment processes. The represented phyla were Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The seven classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Liliopsida (monocots), Magnoliopsida (dicots) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of metsulfuron-methyl, an ALS-inhibiting herbicide, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. Due to the small sample size of heterotrophic species, it was not possible to ascertain distinctions in sensitivity between different groups of species, e.g. between phototrophic and heterotrophic species. Therefore, both phototrophic and heterotrophic species were used to calculate the metsulfuron-methyl PGVs, as recommended in Warne et al. (2015). However, by combining phototrophic and heterotrophic species to derive PGVs for a herbicide that is expected to be more sensitive to phototrophs, it is possible that the PGVs for metsulfuron-methyl may not provide adequate protection to phototrophic species. In addition to this, phototrophs are at the bottom of most aquatic food webs and thus, the PGVs may not provide sufficient protection to non-phototrophic species (as a result of potential indirect effects).

There were freshwater chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data for eight species (seven phototrophic and one heterotrophic) that belonged to five phyla and six classes, which met the minimum data requirements to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 48) combined with the poor fit of the distribution to these toxicity data (Figure 42) resulted in a moderate reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for metsulfuron-methyl in freshwater environments is provided in Table 49.

Table 49 Summary of the single toxicity value for each phototrophic and heterotrophic species that were used to derive the proposed aquatic ecosystem protection guideline values for metsulfuron-methyl in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[2]	95.4	USEPA (2015b)
Macrophyte	*Elodea canadensis**	Tracheophyta	Liliopsida	Apical shoot (19.5 cm)	8	Chronic NOEC	Shoot length	0.054	Wendt-Rasch et al. (2003)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	7	Chronic EC10	Frond count	0.21	Rosenkrantz et al. (2012)
Macrophyte	*Lemna minor**	Tracheophyta	Liliopsida	Exponential growth phase	42	Chronic NOEC	Frond count	0.1	Boxall et al. (2013)
Macrophyte	*Myriophyllum spicatum*	Tracheophyta	Magnoliopsida	Not stated	14	Chronic NOEC	Dry weight, root occurrence	20	Wendt-Rasch et al. (2003)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	4	Chronic NOEL	Biomass yield, growth rate, AUC[2]	92,800	USEPA (2015b)
Fish	*Oncorhynchus mykiss**	Chordata	Actinopterygii	Early life	90	Chronic NOEL	Mortality	4,500	USEPA (2015b)
Microalga	*Pseudokirchneriella subcapitata*[3]	Chlorophyta	Chlorophyceae	Not stated	5	Chronic NOEL	Biomass yield, growth rate, AUC[2]	10	USEPA (2015b)

[1] **Chronic EC10/NOEC/NOEL = no conversions applied (Warne et al. 2015).** [2] **AUC = area under the growth curve.** [3] **This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*.**

11.2.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the eight freshwater phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 42.
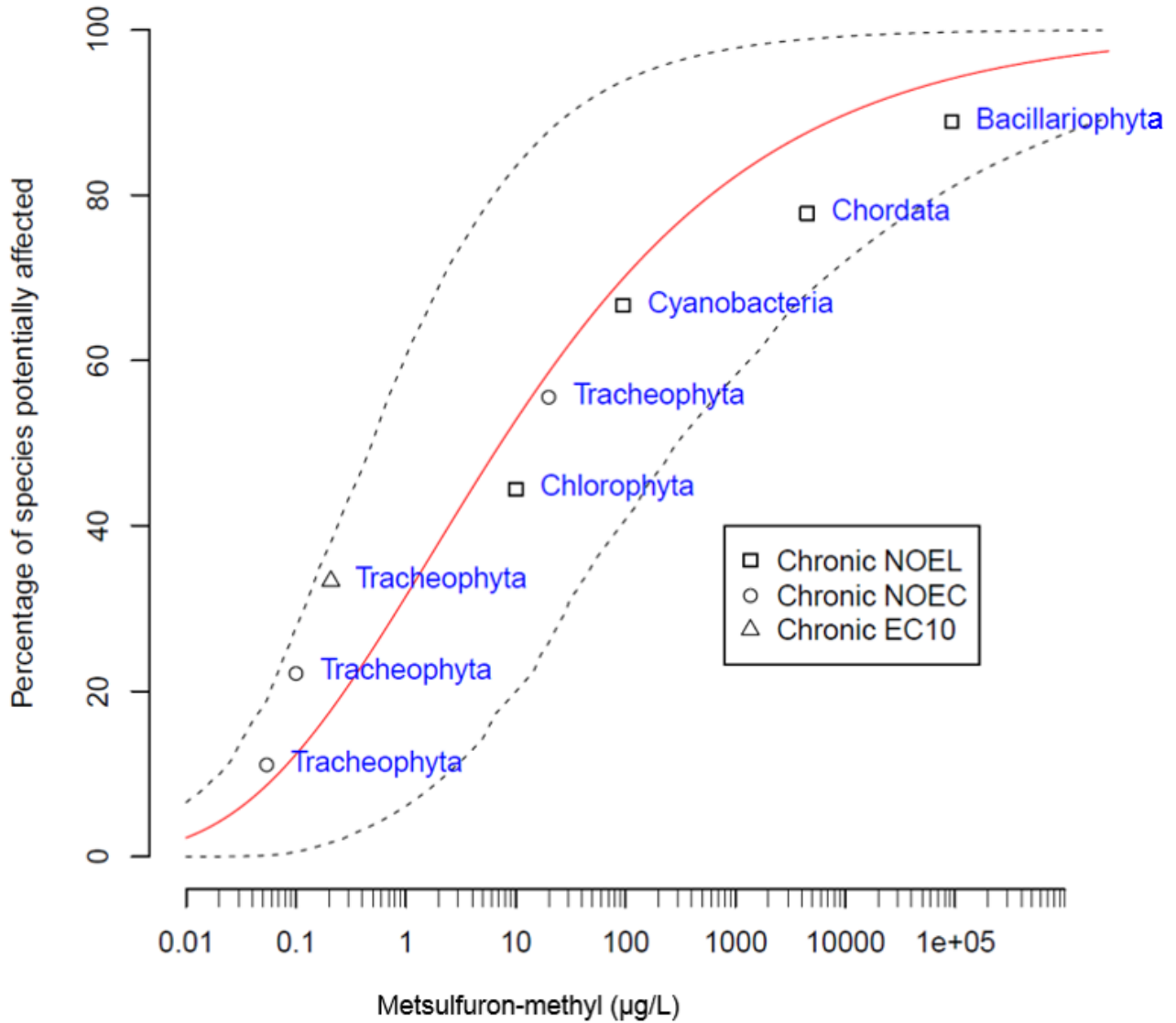
Figure 42 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to metsulfuron-methyl. Black dashed lines indicate the 95% confidence intervals.

11.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for metsulfuron-methyl in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Bacillariophyceae	Microalga (*Navicula pelliculosa*)	Not stated	4	Chronic	NOEL (Biomass, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	92,800	USEPA (2015b)
										92,800	*GEOMETRIC MEAN*
										92,800	**VALUE USED IN SSD**
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[1]*)	Not stated	5	Chronic	NOEL (Biomass, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	10	USEPA (2015b)
										10	*GEOMETRIC MEAN*
										10	**VALUE USED IN SSD**
Chordata	Actinopterygii	Rainbow Trout (*Oncorhynchus mykiss*)	Early life	90	Chronic	NOEL (Mortality)	Dilution water	12 ± 2	Not stated	4,500	USEPA (2015b)
										4,500	*GEOMETRIC MEAN*
										4,500	**VALUE USED IN SSD**
Cyanobacteria	Cyanophyceae	Microalga (*Anabaena flos-aquae*)	Not stated	5	Chronic	NOEL (Biomass, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	95.4	USEPA (2015b)
										95.4	*GEOMETRIC MEAN*
										95.4	**VALUE USED IN**

											SSD
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Apical shoot (19.5 cm)	8	Chronic	NOEC (Shoot length)	Filtered freshwater	22 ± 2	Not stated	0.054	Wendt-Rasch et al. (2003)
										0.054	*GEOMETRIC MEAN*
										0.054	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	7	Chronic	EC10 (Frond count)	Algae assay procedure (AAP) medium	24 ± 2	7.5	0.27	Rosenkrantz et al. (2012)
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	7	Chronic	EC10 (Frond count)	Algae assay procedure (AAP) medium	24 ± 2	7.5	0.16	Rosenkrantz et al. (2012)
										0.21	*GEOMETRIC MEAN*
										0.21	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Lemna minor*)	Exponential growth phase	42	Chronic	NOEC (Frond count)	Swedish standard (SIS)	20 ± 1	6.5 ± 0.2	0.1	Boxall et al. (2013)
										0.1	*GEOMETRIC MEAN*
										0.1	**VALUE USED IN SSD**
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	14	Chronic	NOEC (Dry weight)	Filtered freshwater	22 ± 2	Not stated	20	Wendt-Rasch et al. (2003)
										20	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	14	Chronic	NOEC (Root occurrence)	Filtered freshwater	22 ± 2	Not stated	20	Wendt-Rasch et al. (2003)
										20	*GEOMETRIC MEAN*

										20	VALUE USED IN SSD

[1] This species has been called Raphidocelis subcapitata, Pseudokirchneriella subcapitata and Selenastrum capricornutum. [2] AUC = area under the growth curve.

11.2.7 References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

Barcelö D and Hennion MC (2003). Pesticides and their degradation products: characteristics, usage and environmental behaviour. In: Trace determination of pesticides and their degradation products in water. Techniques and Instrumentation in Analytical Chemistry, Amsterdam, Elsevier Science B, 19, 1–89pp.

BCPC (2012). A world compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 782–783.

Boxall AB, Fogg LA, Ashauer R, Bowles T, Sinclair CJ, Colyer A and Brain RA (2013). Effects of repeated pulsed herbicide exposures on the growth of aquatic macrophytes, *Environmental Toxicology and Chemistry*, 32, 193–200.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

CDC (2013). Biomonitoring Summary: Substituted Urea Herbicides. Centers for Disease Control and Prevention. Available from: http://www.cdc.gov/biomonitoring/SulfonylureaHerbicides_BiomonitoringSummary.html . Accessed: 9 November, 2015.

Cornell University (1993). EXTOXNET (Extension Toxicology Network): Metsulfuon-methyl, Developed by the Pesticide Management Education Program (PMEP), Cornell University, 1993. Available from: http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/metsulfuron-methyl-ext.html. Accessed: 9 November, 2015.

FAO UN (2015). FAO Specifications and evaluations for plant protection products: Metsulfuron-methyl, methyl 2-(4-methoxy-6-methyl-1,3,5-triazin-2-ylcarbamoylsulfamoyl)benzoate, Food and Agriculture Organisation of the United Nations, Rome, Italy, 38. Available from: http://www.fao.org/fileadmin/templates/agphome/documents/Pests_Pesticides/Specs/Metsulfuron-methyl_2011.pdf. Accessed: 12 September, 2015.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Rosenkrantz RT, Cedergreen N, Baun A and Kusk KO (2012). Influence of pH, light cycle, and temperature on ecotoxicity of four sulfonylurea herbicides towards *Lemna gibba*, *Ecotoxicology*, 22, 33–41.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Smith AE (1986). Persistence of the herbicides [14c] chlorsulfuron and [14C] metsulfuron-methyl in prairie soils under laboratory conditions, *Bulletin of Environmental Contamination and Toxicology*, 37, 698–704.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm Accessed 13 May 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/, Accessed: February–April, 2016.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology*, 4, 93–144.

Wendt-Rasch L, Pirzadeh P and Woin P (2003). Effects of metsulfuron methyl and cypermethrin exposure on freshwater model ecosystems, *Aquatic Toxicology*, 63, 243–256.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

12 Simazine

12.1 Introduction

Simazine is a triazine herbicide ($C_7H_{12}ClN_5$ and Figure 45) that at room temperature is a white powder. It is the active ingredient of a variety of commercial herbicide formulations. Simazine is often mixed with other herbicides (e.g. ametryn, atrazine, diuron, metolachlor and paraquat) to increase its efficacy.
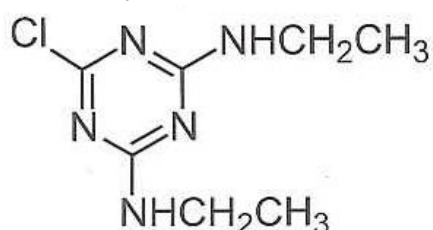
Figure 43 Structure of simazine

Physicochemical properties of simazine that may affect its environmental fate and toxicity are presented in Table 50.

Table 50 Summary of selected physicochemical properties of simazine.

Physicochemical property	Value
Molecular weight	201.7 amu[1]
Aqueous solubility	6.2 mg/L @ pH 7 and temperature 22 ºC[1] 5 mg/L @ temperature 20 ºC[2]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.1[1] 2.3 @ pH 7 and temperature 20 ºC[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.20[1] 2.14 @ temperature 25 ºC[2]
Logarithm of the bioconcentration factor (log BCF)	2.34[2] <2.0[3]
Half-life ($t_{1/2}$) in water	Freshwater: 8.8 days (pH 1), 96 days (pH 5), 3.7 days (pH 13)[1] Marine: 579 ± 294 days (dark, at temperature 25 ºC) 96 days @ pH 7 and temperature 20 ºC[2]
Half-life ($t_{1/2}$) in soil	90 days (field)[2] Typical: 60 days[2]

[1] **BCPC (2012).** [2] **Pesticide Properties Database (University of Hertfordshire 2013).** [3] **CCME (1999).** [4] **Mercurio et al. (2015).**

Simazine belongs to the chlorotriazine group within the triazine family of herbicides, which also includes atrazine, propazine and terbuthylazine. Simazine is extensively used in agriculture, forestry and in urban situations to control broadleaf weeds and grasses and to control macrophytes in still or slow flowing waterways. In Australia, simazine is one of the most heavily used herbicides, exceeded only by glyphosate (AATSE 2002). It is used as both a knockdown and residual herbicide and it can retain its biological effectiveness in soil for a year after application. However, it does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013). Simazine is generally applied before weeds emerge (i.e. it is a pre-emergent herbicide).

Simazine is mainly absorbed through the roots of plants and transported to the leaves, where it exerts its toxicity. Simazine exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of

Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Triazine herbicides bind to the plastoquinone B (Q_B) protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (ATP, used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (NADPH, used in converting CO_2 to glucose), and therefore, prevents CO_2 fixation (Wilson et al. 2000a).

In addition to its main mode of action, exposure to PSII inhibiting herbicides can lead to marked increases in the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen (OH^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). Reactive oxygen species are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Reactive oxygen species are created during normal cellular functioning particularly in biochemical processes that involve the generation of energy (e.g. photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells). In phototrophs, ROS are formed when the absorbed light energy exceeds the ability to convert CO_2 to organic molecules, thus accumulating oxygen (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, prolonged exposure to elevated concentrations of ROS in plants, as a result of biotic (e.g. disease) and/or abiotic stressors (e.g. PSII inhibiting herbicides), can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

While simazine predominantly targets the PSII complex it can also exert biochemical effects in other non-target organisms. It is also known to cause endocrine disrupting effects (Mnif et al. 2011), for example, concentrations of 1 to 2 µg/L can lead to inhibition of the endocrine mediated olfactory response of male Atlantic salmon (*Salmo salar* L.) to the female priming pheromone, prostaglandin (Moore and Lower 2001).

Simazine has poor to moderate soil binding characteristics due to its low log K_{oc} value but has a low leaching potential because of its low aqueous solubility (Table 50). Nonetheless, it is frequently detected in surface and ground waters throughout Europe (Oropressa et al. 2008 and references therein), Northern America (Stone et al. 2014) and Eastern Australia (e.g. Devlin et al. 2015; Wallace et al. 2015, 2016).

Although simazine is used in terrestrial applications, its presence in marine habitats demonstrates its mobility and long half-life in aquatic environments (Table 50).Simazine has been detected frequently in Australian estuarine, coastal and marine ecosystems, including the Great Barrier Reef (Shaw and Müller 2005), seagrass communities in Hervey Bay (McMahon et al. 2005), and mangrove forest in the Mackay Whitsundays (Duke et al. 2005). Within Europe, detections of simazine in marine ecosystems are still observable, but well below the levels from one to two decades earlier (Mai et al. 2013), i.e. before it was banned in European Union member countries (EU Commission Regulation 2010).

Due to its widespread detection at elevated concentrations and its broad range of adverse effects, simazine has been included in the EU Priority Pollutants List and the equivalent USEPA list (Stara et al. 2012).

12.1.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for simazine in freshwaters (Table 52) includes toxicity data to seven freshwater species that either

originated from or are distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

Toxicity values for fish were 90-day NOEC, EC2.71, EC2.52 and EC6.99 (mortality, height, length, weight) values all of 45 µg/L, a 28-day LC50 (mortality) value of 2,500 µg/L, a 120-day LOEC (mortality) value of 2,500 µg/L and two 1-year LOEL (mortality) values both of 2,500 µg/L. A 21-day LOEC (mortality) value of 2,500 µg/L was determined for a crustacean. Typically toxicity values for macrophytes and microalgae are lower than those for non-phototrophic species. For example, 7- to 84-day NOEC (growth) values range from 58 to 8,470 µg/L and a 14-day EC50 (biomass yield) of 140 µg/L have been determined. Microalgae are more sensitive still with 3- to 6-day EC/LC50 (growth and cell density) values of 36 to 2,174 µg/L being reported.

Freshwater Acute

For the seven types of organisms for which acute toxicity data were available, sensitivity to simazine decreased in the following order – microalgae, insects, fish, crustaceans, molluscs, annelids and amphibians. As with the chronic toxicity data for simazine, algae are the most sensitive type of organism with 48- to 96-hour EC50 (growth and population growth) values of 160 to 320 µg/L and as low as 2.24 µg/L for photosynthesis inhibition. The 48- to 96-hour LC50 for insects ranged from 1,900 to 3,580 µg/L and a 96-hour EC50 (mortality) of 1,900 µg/L was reported. Fish 24- to 96-hour LC50 values range from 3,000 to 1,100,000 µg/L; while a 120-day LOEC (mortality) value of 2,500 µg/L was also reported. Toxicity values for crustaceans are similar to those for fish, having 1- to 4-day LC50 values ranging from 1,100 to 270,000 µg/L and a 2-day NOEC (mortality) value of 40,000 µg/L. Molluscs had 4-day LC50 values ranging from 98,600 to 228,000 µg/L. Annelid 4-day LC50 values ranged from 1,090,000 to 1,897,000 µg/L while equivalent toxicity data for amphibians (4-day LC50) had a value of 1,780,000 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

12.1.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of simazine. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of simazine (Table 50).

12.1.3 Guideline derivation

The derived PGVs for simazine in freshwaters are provided in Table 51. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for simazine are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for simazine are low (Table 50) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for simazine do not need to account for secondary poisoning.

Table 51 Proposed aquatic ecosystem protection guideline values (µg/L) for simazine for the protection of freshwater ecosystems.

Simazine proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	3.2 (0.93 – 24)	Sample size	17
95%	10 (4.9 – 31)	Type of toxicity data	Chronic NOEC/NOAEC and chronic estimated NOEC values
90%	17 (9.3 – 37)	SSD model fit	Good
80%	29 (16 – 47)	Reliability	High

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".

12.1.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for simazine in freshwater environments was a moderate reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on acute toxicity values for 12 phototrophic and heterotrophic species (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as having a moderate reliability.

To obtain toxicity data for simazine to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now more simazine toxicity data available that enable the calculation of PGVs in freshwaters (see section 12.1.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of simazine with phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were freshwater toxicity data for 43 species (eight phyla and 12 classes) that passed the screening and quality assessment processes. The represented phyla were Annelida, Arthropoda, Chlorophyta, Chordata, Cyanobacteria, Mollusca, Ochrophyta and Tracheophyta. The 12 classes were Actinopterygii (which accounts for approximately 99% of fish), Amphibia (tetrapod vertebrates), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of green algae), Clitellata (a class of annelid worms), Cyanophyceae (a class of cyanobacteria), Gastropoda (another grouping of molluscs), Liliopsida (monocots), Magnoliopsida (dicots), Malacostraca (a large grouping of crustaceans) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of simazine, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The simazine ecotoxicity data for phototrophs and heterotrophs were tested using the non-parametric Mann-Whitney test to see if the toxic responses among different taxa were uni- or multi-modal. The Mann-Whitney test indicated that the two groups had significantly different (p <0.0001, see section 12.2.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were freshwater chronic no observed effect concentration (NOEC) and no observed adverse effect concentration (NOAEC) data for nine phototroph species (that belonged to two phyla and three classes), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). When the dataset was expanded to combine the chronic NOEC/NOAEC data with the chronic estimated NOEC data (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) for freshwater phototrophic species, there were 17 species belonging to four phyla and six classes, which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 51) combined with the good fit of the distribution to these toxicity data (Figure 44) resulted in a high reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for simazine in freshwater is provided in Table 52.

Table 52 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for simazine in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Macrophyte	*Acorus gramineus*	Tracheophyta	Liliopsida	Not stated	7	Chronic NOEC	Fresh weight	100	Wilson et al. (2000a)
Cyanobacteria	*Anabaena flosaquae*	Cyanobacteria	Cyanophyceae	Not stated	5	Chronic est. NOEC	Cell density	7.2	USEPA (2015b)
Microalga	*Chlamydomonas geitleri*	Chlorophyta	Chlorophyceae	Exponential growth phase	3	Chronic est. NOEC	Chlorophyll *a* content	171	Kamaya et al. (2004)
Microalga	*Chlorella vulgaris*[2]*	Chlorophyta	Trebouxiophyceae	Not stated	4	Chronic est. NOEC	Growth rate	84.4	Ma et al. (2002)
Macrophyte	*Elodea canadensis**	Tracheophyta	Liliopsida	Not stated	28–84	Chronic NOEC	Dry weight, fresh weight number of shoots, length	83	Vervliet-Scheebaum et al. (2010)
Macrophyte	*Glyceria maxima**	Tracheophyta	Liliopsida	Not stated	28–84	Chronic NOEC	Dry weight, fresh weight number of shoots, length	83	Vervliet-Scheebaum et al. (2010)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic est. NOEC	Biomass yield	28	USEPA (2015b)
Macrophyte	*Myriophyllum aquaticum**	Tracheophyta	Magnoliopsida	2 weeks old	7	Chronic est. NOEC	Fresh weight	20	Knuteson et al. (2002)
Macrophyte	*Myriophyllum spicatum*	Tracheophyta	Magnoliopsida	Not stated	28–84	Chronic NOEC	Dry weight, fresh weight number of shoots, length	83	Vervliet-Scheebaum et al. (2010)
Microalga	*Navicula pelliculosa**	Ochrophyta	Bacillariophyceae	Not stated	5	Chronic est. NOEC	Cell density	18	USEPA (2015b)
Macrophyte	*Persicaria amphibia*	Tracheophyta	Magnoliopsida	Not stated	28–84	Chronic NOEC	Dry weight, fresh weight number of shoots, length	83	Vervliet-Scheebaum et al. (2010)
Macrophyte	*Pontederia cordata*	Tracheophyta	Liliopsida	Not stated	7	Chronic NOEC	Fresh weight	100	Wilson et al. (2000a)
Microalga	*Pseudokirchneriella subcapitata*[3]	Chlorophyta	Chlorophyceae	Not stated	3	Chronic NOEC	Growth rate	32	Perez et al. (2011)

Microalga	*Scenedesmus obliquus**	Chlorophyta	Chlorophyceae	Exponential growth phase	4–6	Chronic est. NOEC	Growth rate	51.4	Ma (2002)
Microalga	*Scenedesmus quadricauda*	Chlorophyta	Chlorophyceae	Not stated	4	Chronic est. NOEC	Abundance	30	Ma et al. (2003)
Macrophyte	*Typha latifolia**	Tracheophyta	Liliopsida	Not stated	7	Chronic NOEC	Fresh weight	300	Wilson et al. (2000a)
Macrophyte	*Vallisneria americana*	Tracheophyta	Liliopsida	Not stated	13 days	Chronic NOAEC	Fresh weight and length	58	Wilson and Wilson (2010)

[1] Chronic NOEC/NOAEC = no conversions applied; Chronic est. NOEC = chronic EC50/LOEC values that were converted to chronic NOEC values by dividing by 5 (Warne et al. 2015). [2] This species has also been called *Chlorella pyrenoidosa*. [3] This species has also been called *Raphidocelis subcapitata* and *Selenastrum caprincornutum*. * Species that originated from/are distributed in Australia and/or New Zealand.

12.1.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 17 freshwater phototrophic species that was used to derive the PGVs is presented in Figure 44.
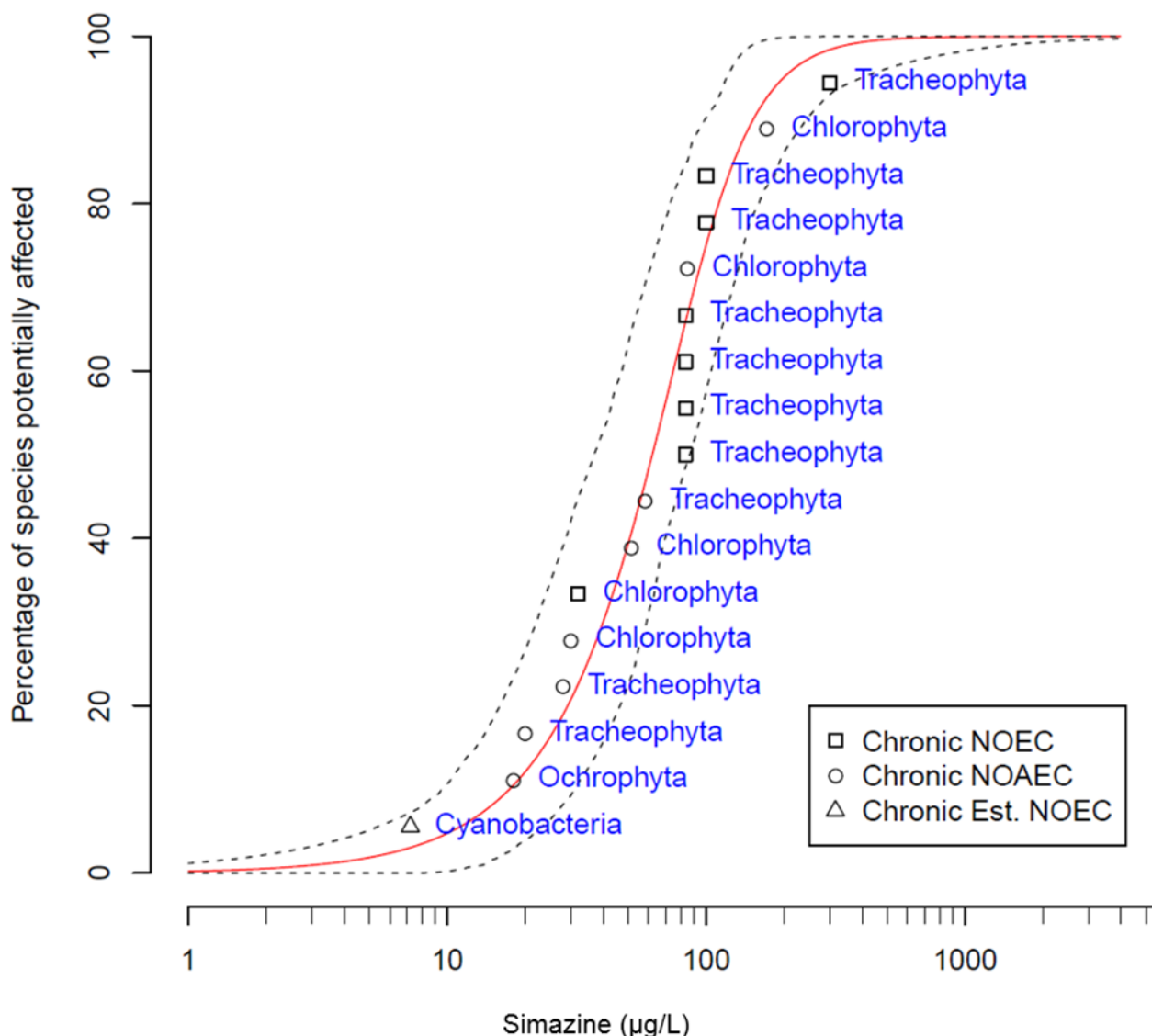
Figure 44 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of no observed effect concentration (NOEC), no observed adverse effect concentration (NOAEC) and chronic estimated no observed effect concentration (NOEC) data values of freshwater phototrophic species to simazine. Black dashed lines indicate the 95% confidence intervals.

12.1.6   Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for simazine in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomonas geitleri*)	Exponential growth phase	3	Chronic	EC50 (Growth rate)	Freshwater	23	7.8	1,032	Francois and Robinson (1990)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomonas geitleri*)	Exponential growth phase	3	Chronic	EC50 (Growth rate)	Freshwater	23	7.8	812	Francois and Robinson (1990)
Chlorophyta	Chlorophyceae	Microalga (*Chlamydomonas geitleri*)	Exponential growth phase	3	Chronic	EC50 (Growth rate)	Freshwater	23	7.8	746	Francois and Robinson (1990)
										855.5	*GEOMETRIC MEAN*
										171[@]	***VALUE USED IN SSD***
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella vulgaris*[1])	Not stated	4	Chronic	EC50 (Abundance)	Liquid HB-4 medium	25	Not stated	2,173	Ma et al. (2002b)
Chlorophyta	Trebouxiophyceae	Microalga (*Chlorella vulgaris*[1])	Not stated	4	Chronic	EC50 (Abundance)	Liquid HB-4 medium	25	Not stated	82	Ma et al. (2002a)
										82	*GEOMETRIC MEAN*
										84.4[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Pseudokirchneriella subcapitata*[2])	Exponential growth phase	3	Chronic	NOEC (Growth rate)	Marine Biological Laboratory (MBL) medium	24 ± 2	Not stated	32	Perez et al. (2011)
										32	*GEOMETRIC MEAN*
										32[@]	***VALUE***

											USED IN SSD
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus obliquus*)	Not stated	4	Chronic	EC50 (Growth rate)	Liquid HB-4 medium	25	not stated	257	Ma (2002)
										257	*GEOMETRIC MEAN*
										51.4[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Scenedesmus quadricauda*)	Not stated	4	Chronic	EC50 (Abundance)	Liquid HB-4 medium	Not stated	Not stated	150	Ma et al. (2003)
										150	*GEOMETRIC MEAN*
										30[@]	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Microalga (*Anabaena flosaquae*)	Not stated	5	Chronic	EC50 (Cell density)	Algal nutrient medium	20 - 24 ± 2	Not stated	36	USEPA (2015b)
										36	*GEOMETRIC MEAN*
										7.2[@]	***VALUE USED IN SSD***
Ochrophyta	Bacillariophyceae	Freshwater Diatom (*Navicula pelliculosa*)	Not stated	5	Chronic	EC50 (Cell density)	Algal nutrient medium	20 - 24 ± 2	Not stated	90	USEPA (2015b)
										90	*GEOMETRIC MEAN*
										18[@]	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte (*Acorus gramineus*)	Not stated	7	Chronic	NOEC (Fresh weight)	Hoagslands Nutrient Solution	25 ± 2	Not stated	100	Wilson et al. (2000b)
										100	*GEOMETRIC MEAN*
										100	***VALUE USED IN SSD***

Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	28	Chronic	NOEC (Dry weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	28	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	28	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	56	Chronic	NOEC (Dry weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	56	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	56	Chronic	NOEC (Length)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	84	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Elodea canadensis*)	Not stated	84	Chronic	NOEC (Length)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)

										83	*GEOMETRIC MEAN*
										83	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	28	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	56	Chronic	NOEC (Dry weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	56	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	56	Chronic	NOEC (Length)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	56	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	84	Chronic	NOEC (Dry weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	84	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)

										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	84	Chronic	NOEC (Length)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Liliopsida	Macrophyte (*Glyceria maxima*)	Not stated	84	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
										83	**VALUE USED IN SSD**
Tracheophyta	Liliopsida	Macrophyte (*Lemna gibba*)	Not stated	14	Chronic	EC50 (Biomass yield)	20X-AAP medium	25 ± 2	7.5 ± 0.1	140	USEPA (2015b)
										140	*GEOMETRIC MEAN*
										28[@]	**VALUE USED IN SSD**
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum aquaticum*)	2 weeks old	7	Chronic	LOEC (Fresh weight)	Hoagslands nutrient solution	24 ± 4	Not stated	50	Knuteson et al. (2002)
										50	*GEOMETRIC MEAN*
										20[&]	**VALUE USED IN SSD**
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	28	Chronic	NOEC (Dry weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	28	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC*

											MEAN
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	28	Chronic	NOEC (Length)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	28	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	56	Chronic	NOEC (Dry weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	56	Chronic	NOEC (Fresh weight)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Myriophyllum spicatum*)	Not stated	56	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
										83	***VALUE USED IN SSD***
Tracheophyta	Magnoliopsida	Macrophyte (*Persicaria amphibia*)	Not stated	84	Chronic	NOEC (Length)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC MEAN*
Tracheophyta	Magnoliopsida	Macrophyte (*Persicaria amphibia*)	Not stated	84	Chronic	NOEC (Number of shoots)	Aged tap water	15.0-22.7 ± 0.2	7.5-8.5	83	Vervliet-Scheebaum et al. (2010)
										83	*GEOMETRIC*

											MEAN
										83	*VALUE USED IN SSD*
Tracheophyta	Liliopsida	Macrophyte (*Pontederia cordata*)	Not stated	7	Chronic	NOEC (Fresh weight)	Hoaglands Nutrient Solution	25 ± 2	Not stated	100	Wilson et al. (2000b)
										100	*GEOMETRIC MEAN*
										100	*VALUE USED IN SSD*
Tracheophyta	Liliopsida	Macrophyte (*Typha latifolia*)	Not stated	7	Chronic	NOEC (Fresh weight)	Hoaglands Aqueous Nutrient Media	25 ± 2	Not stated	300	Wilson et al. (2000a)
										300	*GEOMETRIC MEAN*
										300	*VALUE USED IN SSD*
Tracheophyta	Liliopsida	Macrophyte (*Vallisneria americana*)	Not stated	13	Chronic	NOAEC (Length)	Reconstituted very hard water	25	8.2 ± 0.2	58	Wilson and Wilson (2010)
										58	*GEOMETRIC MEAN*
										58	*VALUE USED IN SSD*

[1] This species has also been called *Chlorella pyrenoidosa*. [2] This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*. [@] Values were chronic EC/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 5 (Warne et al. 2015). [&] Values were chronic LOEC values that were converted to chronic NOEC/EC10 values by dividing by 2.5 (Warne et al. 2015).

12.2 Marine

12.2.1 Aquatic toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for simazine in marine waters (Table 54) includes toxicity data for three marine species that either originated from or are distributed within Australia and/or New Zealand. There were some additional studies that contained toxicity data for simazine to Australasian marine species, however they measured photosynthetic inhibition, which is currently not accepted as an ecologically relevant endpoint (Warne et al. 2015), and thus were not included in the PGV derivation for simazine in marine ecosystems. A summary of the high and moderate quality raw toxicity data for all marine species that passed the screening and quality assurance processes are provided below.

Marine Chronic

There were marine chronic toxicity data for one mollusc and six microalgae. The toxicity value for the single mollusc species was a 7-day NOEL (mortality, abnormal development) value of 1,000 µg/L. The toxicity values for microalgae consisted of two 72-hour IC10 (growth) values of 100 and 310 µg/L, a 72-hour IC50 (growth) value of 580 µg/L, 5-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 250 and 600 µg/L, respectively, and 10-day EC50 (cell density) values ranging from 500 to 5,000 µg/L.

Marine Acute

There were marine acute toxicity data for three fish and one crustacean and one mollusc. The toxicity values for fish consisted of a 72-hour LC10 and LC50 (mortality) values of 2,360 and 4,190 µg/L, respectively, two 72-hour NOEC (mortality, dry weight) values of 2,250 and 4,500 µg/L and 96-hour NOEL and LOEL (mortality) values of 4,300 and 1,000 µg/L, respectively for different species. The toxicity values for the single crustacean species were 96-hour NOEL and LC50 (mortality) values of 75,000 and 113,000 µg/L. The toxicity values for the single mollusc species was a 96-hour NOEL of 3,700 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

12.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of simazine. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of simazine (Table 50).

12.2.3 Guideline derivation

The derived PGVs for simazine in marine waters are provided in Table 53. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for simazine are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for simazine are low (Table 50) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for simazine do not need to account for secondary poisoning.

Table 53 Proposed aquatic ecosystem protection guideline values (µg/L) for simazine for the protection of marine ecosystems.

Simazine proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)[3]	Criterion	Result
99%	28 (12 – 130)	Sample size	6
95%	63 (36 – 190)	Type of toxicity data	Chronic NOEL/IC10 and chronic estimated NOEC values
90%	89 (53 – 240)	SSD model fit	Poor
80%	130 (77 – 290)	Reliability	Low

[1] Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability". [3] Values rounded to two significant figures.

12.2.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for simazine in marine environments was a low reliability value as it was the adopted freshwater TV (using the ANZECC and ARMCANZ 2000 reliability scheme) based on acute and chronic toxicity values for 12 phototrophic and heterotrophic species (Warne 2001). Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as having a very low reliability.

To obtain toxicity data for simazine to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now more simazine toxicity data available that enable the calculation of PGVs in marine waters (see section 12.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of simazine with phototrophic (e.g. plants and algae) marine species be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for simazine in marine waters, *Chlorococcum* sp. was included as no other toxicity data for these genera were used.

In total, there were toxicity data for 10 marine species (six phyla and eight classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda,

Bacillariophyta, Chlorophyta, Chordata, Haptophyta and Mollusca. The eight classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Bivalvia (a grouping of molluscs), Chlorophyceae (a major grouping of green microalgae), Malacostraca (a larger grouping of crustaceans), Mediophyceae (an algae grouping), Ostracoda (another grouping of crustaceans) and Prymnesiophyceae (a grouping of haptophyta).

Based on the current understanding of the mode of action of simazine, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The simazine ecotoxicity data for phototrophs and heterotrophs were tested using the non-parametric Mann-Whitney test to see if the toxic responses among different taxa were uni- or multi-modal. The Mann-Whitney test indicated that the two groups had significantly different (p <0.0001, see section 12.2.7) sensitivities. As recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) should be used to calculate the PGVs. However, there were insufficient data for marine phototrophic species alone (see below) to be used to calculate PGVs.

In such cases, it is acceptable to combine the marine phototrophic data with freshwater phototrophic data to create a larger dataset, provided that statistical analysis shows no difference in the sensitivity of simazine in the two media types (Warne et al. 2015). The simazine ecotoxicity data for freshwater and marine species were tested using the non-parametric Mann-Whitney test to see if they had similar sensitivities. This indicated that the freshwater and marine datasets had significantly different (p = 0.002, Attachment B) sensitivities. As a result, the freshwater and marine phototrophic data could not be combined to use a SSD to derive PGVs (Warne et al. 2015).

As the dataset has a more sensitive grouping of species from a single phylum, the requirement for data representing at least four taxonomic groups is offset by the need to obtain a good fit of the SSD and reliable PGVs. This is acceptable provided that this criterion (i.e. at least five species belonging to at least four phyla) is still met for the entire dataset for the chemical (the more and less sensitive groups combined), and only if all the data of the same type as those used to derive the PGVs (in this case, chronic data) meet both requirements (Warne et al. 2015).

There were marine 10% inhibition concentration (IC10), no observed effect level (NOEL) and chronic estimated NOEC (chronic LOEC and EC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 2.5 and 5, respectively) data available for six phototrophic species (that belonged to three phyla and four classes), which did not meet the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive a PGV (Warne et al. 2015). No other ecotoxicity data for simazine to marine phototrophic species were available, and the addition of marine phototrophic species to the dataset was not allowed.

The marine dataset for simazine (that included chronic data) consisted of seven phototrophic (n = 6) and heterotrophic (n = 1) marine species that belonged to four phyla and six classes, that successfully met the minimum data requirements (i.e. at least five species belonging to at least four phyla). Therefore as per Warne et al. (2015), it was acceptable to derive PGVs using the chronic IC10/NOEL and chronic estimated NOEC data values for the six marine phototrophic species despite belonging to only three phyla (Warne et al. 2015). The number of species and taxa used to derive the PGVs (Table 53) combined with the poor fit of the distribution to these toxicity data (Figure 45) resulted in a set of low reliability PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for simazine in marine water is provided in Table 54.

Table 54 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for simazine in marine waters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type [1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Microalga	*Ceratoneis closterium**	Bacillariophyta	Bacillariophyceae	Exponential growth phase	3	Chronic IC10	Cell size	310	Hook et al. (2014)
Microalga	*Chlorococcum* sp.	Chlorophyta	Chlorophyceae	Not stated	10	Chronic est. NOEC	Cell density	400	USEPA (2015b)
Microalga	*Dunaliella tertiolecta*	Chlorophyta	Chlorophyceae	Not stated	10	Chronic est. NOEC	Cell density	1,000	USEPA (2015b)
Microalga	*Isochrysis galbana*	Haptophyta	Prymnesiophyceae	Not stated	10	Chronic est. NOEC	Cell density	100	USEPA (2015b)
Microalga	*Phaeodactylum tricornutum**	Bacillariophyta	Bacillariophyta incertae sedis	Exponential growth phase	3	Chronic IC10	Cell size	100	Osborn and Hook (2013)
Microalga	*Skeletonema costatum**	Bacillariophyta	Mediophyceae	Not stated	5	Chronic NOEL	Cell density	250	USEPA (2015b)

[1] Chronic IC10 = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively (Warne et al. 2015). * Species that originated from/are distributed in Australia and/or New Zealand.

12.2.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the six marine phototrophic species that was used to derive the PGVs is presented in Figure 45.
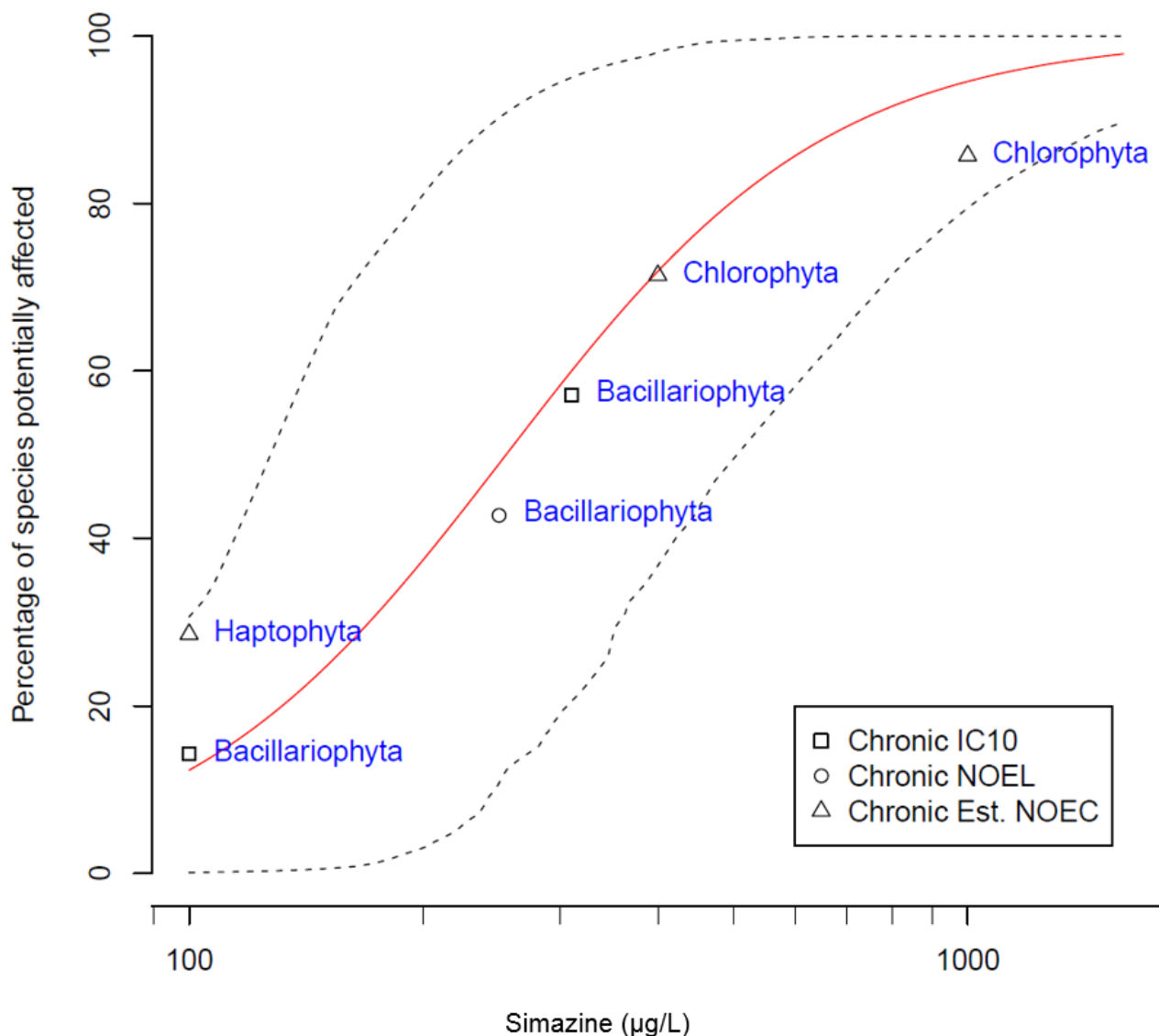

Figure 45 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic and chronic estimated 10% inhibition concentration (IC10) data and no observed effect concentration (NOEC) data values of marine phototrophic species to simazine. Black dashed lines indicate the 95% confidence intervals.

12.2.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for simazine in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Fragilariophyceae	Diatom *(Ceratoneis closterium)*	Exponential growth phase	3	Chronic	IC10 (Growth rate)	Filtered (0.45 µm) seawater	35 ± 2	21 ± 2	8.2 ± 0.1	310	Hook et al. (2014)
											310	*GEOMETRIC MEAN*
											310	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Diatom *(Phaeodactylum tricornutum)*	Exponential growth phase	3	Chronic	IC10 (Growth rate)	Filtered (0.45 µm) seawater	35 ± 2	21 ± 2	8.2 ± 0.1	100	Osborn and Hook (2013)
											100	*GEOMETRIC MEAN*
											100	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga *(Chlorococcum sp.)*	Not stated	10	Chronic	EC50 (Cell density)	Algal nutrient medium	30 ± 5	20 - 24 ± 2	Same as media	2,000	USEPA (2015b)
											2,000	*GEOMETRIC MEAN*
											400[@]	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga *(Dunaliella tertiolecta)*	Not stated	10	Chronic	EC50 (Cell density)	Algal nutrient medium	30 ± 5	20 - 24 ± 2	Same as media	5,000	USEPA (2015b)
											5,000	*GEOMETRIC MEAN[@]*

											1,000[@]	**_VALUE USED IN SSD_**
Haptophyta	Prymnesiophyceae	Microalga _(Isochrysis galbana)_	not stated	10	Chronic	EC50 (Cell density)	Algal nutrient medium	30 ± 5	20 - 24 ± 2	Same as media	500	USEPA (2015b)
											_500_	_GEOMETRIC MEAN_
											100[@]	**_VALUE USED IN SSD_**
Ochrophyta	Coscinodiscophyceae	Microalga _(Skeletonema costatum)_	Not stated	5	Chronic	NOEL (Cell density)	Algal nutrient medium	30 ± 5	20 - 24 ± 2	Same as media	250	USEPA (2015b)
											_250_	_GEOMETRIC MEAN_
											250	**_VALUE USED IN SSD_**

[@] **Values were chronic EC/LC50 values that were converted to chronic NOEC/EC10 values by dividing by 5 (Warne et al. 2015).**

12.2.7 Distribution of sensitivities for aquatic species

Statistical analysis of the simazine ecotoxicity data for freshwater and marine species indicated that there was a difference in the sensitivities of the two groups. The non-parametric Mann-Whitney test was used because the transformed simazine freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.768) but did not follow a normal distribution (Anderson-Darling; p = 0.002). Results from the Mann-Whitney test indicated that the two groups were significantly different (p = 0.002); therefore, it was concluded that the simazine freshwater data is statistically different to the simazine marine data. Despite a significantly different result, there is a level of uncertainty due to the small sample size of the marine dataset. To confirm this result, more ecotoxicity data for marine phototrophic species to simazine is needed.

The toxicity data for simazine to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was first applied to the data to normalise the data. Visual examination of the histogram of transformed data indicated that the distribution of the simazine ecotoxicity data may be bimodal (Figure 46).
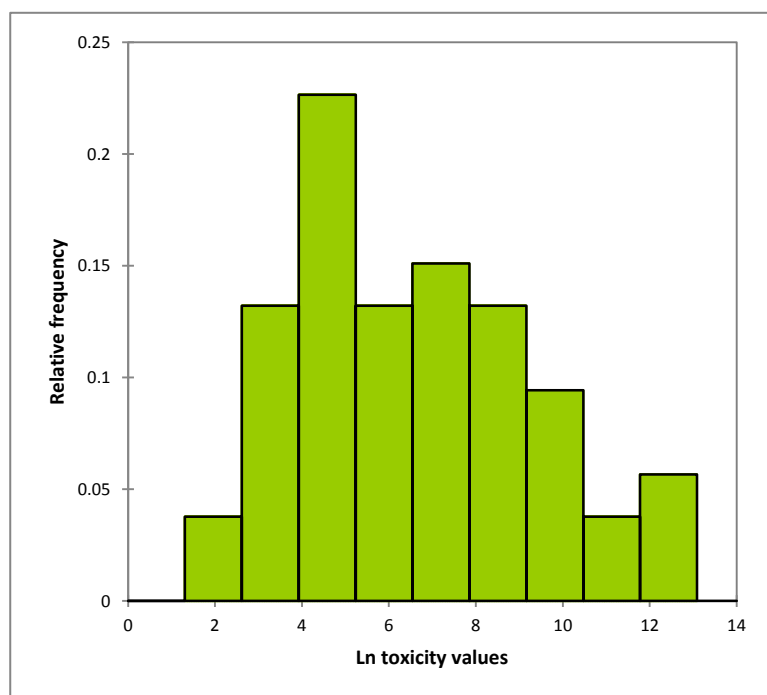
Figure 46 Histogram of the natural logarithm (ln) of all simazine (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (n = 53).

The simazine ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the non-parametric Mann-Whitney test was used because although the transformed simazine concentration data successfully met tests for normality (Anderson-Darling; p =0.095); the data were found to have unequal variances (Fisher's F-Test; p = 0.011). Results from the Mann-Whitney test indicated that the two groups were significantly different (p <0.0001); therefore, it was concluded that the distribution of the simazine concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

12.2.8   References

AATSE (2002). Pesticide use in Australia. A review undertaken by the Australian Academy of Technological Sciences and Engineering. Australian Academy of Technological Sciences and Engineering, Parkville, Victoria, Australia, 309pp.

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

APVMA (2009). Simazine. Australian Pesticide and Veterinary Medicine Authority. Last updated 14 January, 2009. Available from: http://archive.apvma.gov.au/archive/our_view/2009/2009-02-19_simazine.php. Downloaded on: 2 August, 2015.

BCPC (2012). A world compendium. The Pesticide Manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 1026–1028.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

CCME (1999). Canadian water quality guidelines for the protection of aquatic life – Simazine. Canadian Council of Ministers of the Environment.Available from: http://ceqg-rcqe.ccme.ca/download/en/127. Downloaded: 24 June, 2015.

Chen S, Yin C, Strasser RJ, Govinjee, Yang C and Qiang S (2012). Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*. *Plant Physiology and Biochemi*stry 52, 38–51.

Devlin M, Lewis S, Davis A, Smith RA, Negri A, Thompson M and Poggio M (2015). Advancing our understanding of the source, management, transport and impacts of pesticides on the Great Barrier Reef 2011 – 2015. A Centre for Tropical water and Aquatic Ecosystem Research Report (Report No. 15/14) for the Department of Environment and Heritage Protection, 126pp.

Duke NC, Bell AM, Pederson DK, Roelfsema CM and Nash SB (2005). Herbicides implicated as the cause of severe mangrove dieback in the Mackay region, NE Australia: Consequences for marine plant habitats of the GBR World Heritage Area, *Marine Pollution Bulletin* 51, 308–324.

EU Commission Regulation (2010). No. 196/2010 of 9 March 2010 – Amending Annex 1 to Regulation (EC) No 689/2008 for the European Parliament and of the Council concerning the export and import of dangerous chemicals. Available from: file:///C:/Users/warnem/Downloads/JOL_2010_060_R_0005_01.en.pdfa1a.pdf. Downloaded: 24 June 2015.

Francois DL and Robinson GGC (1990). Indices of triazine toxicity in *Chlamydomonas geitleri* Ettl, *Aquatic Toxicology*, 16, 205–228.

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

Hook SE, Osborn HL, Gissi F, Moncuquet P, Twine NA, Wilkins MR and Adams MS (2014). RNA-seq analysis of the toxicant-induced transcriptome of the marine diatom, *Ceratoneis closterium*, *Marine Genomics* 9, 45–53.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Kessler E and Huss V.A.R (1992). Comparative physiology and biochemistry and taxonomic assignment of the *Chlorella* (Chlorophyceae) strains of the culture collection of the University of Texas at Austin, *Journal of Phycology*, 28, 550–553

Knuteson SL, Whitwell T and Klaine SJ (2002). Influence of plant age and size on simazine toxicity and uptake, *Journal of Environmental Quality*, 31, 2096–2103.

Ma J, Lin F, Wang S and Xu L (2003). Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda*, *Bulletin of Environmental Contamination and Toxicology*, 71, 594–601.

Ma J, Xu L and Wang S (2002a). A quick, simple, and accurate method of screening herbicide activity using green algae cell suspension cultures, *Weed Science*, 50, 555–559.

Ma J, Xu L, Wang S, Zheng R, Jin S, Huang S and Huang Y (2002b). Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*, *Ecotoxicology and Environmental Safety*, 51, 128–132.

Mai C, Theobald N, Lammel G and Hühnerfuss H (2013). Spatial, seasonal and vertical distributions of currently-used pesticides in the marine boundary layer of the North Sea, *Atmospheric Environment* 75, 92–102.

McMahon K, Nash SB, Eaglesham G, Müller JF, Duke NC and Winderlich S (2005). Herbicide contamination and the potential impact to seagrass meadows in Hervey Bay, Queensland, Australia, *Marine Pollution Bulletin* 51, 325–334.

Mercurio P, Mueller JF, Eaglesham G, Flores F and Negri AP (2015). Herbicide persistence in seawater simulation experiments, *PloS one*, 10, e0136391.

Mnif W, Hassine AIH, Bouaziz A, Bartegi A, Thomas O and Roig B (2011). Effect of endocrine disruptor pesticides: A review. *International Journal of Environmental Research and Public Health* 8, 2265–2303.

Moore A and Lower N (2001). The impact of two pesticides on olfactory-mediated endocrine function in mature male Atlantic salmon (*Salmo salar* L.) parr. Comparative Biochemistry and Physiology Part B: *Biochemistry and Molecular Biology* 129, 269–276.

Oropesa AL, García Cambero JP and Soler F (2008). Effect of long-term exposure to simazine on brain and muscle acetylcholinesterase activity of common carp (*Cyprinus carpio*), *Environmental Toxicology* 23, 285–293.

Osborn HL and Hook SE (2013). Using transcriptomic profiles in the diatom *Phaeodactylum tricornutum* to identify and prioritize stressors, Aquatic Toxicology, 138/139, 12–25.

Perez J, Domingues I, Soares AMVM and Loureiro S (2011). Growth rate *of Pseudokirchneriella subcapitata* exposed to herbicides found in surface waters in the Alqueva Reservoir (Portugal): A bottom-up approach using binary mixtures, *Ecotoxicology*, 20, 1167–1175.

Piletska EV, Turner NW, Turner APF and Piletsky SA (2005). Controlled release of the herbicide simazine from computationally designed molecularly imprinted polymers, *Journal of Controlled Release,* 108, 132–139.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life,

30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Shaw M and Müller JF (2005). Preliminary evaluation of the occurrence of herbicides and PAHs in the Wet Tropics region of the Great Barrier Reef, Australia, using passive samplers, *Marine Pollution Bulletin,* 51, 876–881.

Stara A, Machova J and Velisek J (2012). Effect of chronic exposure to simazine on oxidative stress and antioxidant response in common carp (*Cyprinus carpio* L.). *Environmental Toxicology and Pharmacology,* 33, 334–343.

Stone WW, Gilliom RJ and Martin JD (2014). An overview comparing results from two decades of monitoring for pesticides in the Nation's streams and Rivers, 1992–2001 and 2002–2011: U.S. Geological Survey Scientific Investigations Report 2014–5154, 23p., Available from: http://dx.doi.org/10.3133/sir20145154. Downloaded: 24 June 2015.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ Water Quality Guideline Database for Toxicants. Supplied as part of a CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm. Accessed 13 May 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/. Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/. Accessed: February–April, 2016.

Vervliet-Scheebaum M, Straus A, Tremp H, Hamer M, Maund SJ, Wagner E and Schulz R (2010). A microcosm system to evaluate the toxicity of the triazine herbicide simazine on aquatic macrophytes, *Environmental Pollution*, 158, 615–623.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and Warne MStJ. 2015. Total suspended solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-catchment-loads-report.pdf.

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Canberra, Australia.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for

toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology*, 4, 93–144.

Wilson PC and Wilson SB (2010). Toxicity of the herbicides bromacil and simazine to the aquatic macrophyte, *Vallisneria americana* Michx, *Environmental Toxicology and Chemistry* 29, 201–211.

Wilson PC, Whitwell T and Klaine SJ (2000a). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*. *Archives of Environmental Contamination and Toxicology* 39, 282–288.

Wilson PC, Whitwell T and Klaine SJ (2000b). Phytotoxicity, uptake, and distribution of 14C-Simazine in *Acorus gramenius* and *Pontederia cordata*. *Weed Science*, 48, 701–709.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.

13 Tebuthiuron

13.1 Introduction

Tebuthiuron is a urea herbicide ($C_9H_{16}N_4OS$ and Figure 47) that at room temperature is in the form of a white to buff, odourless solid. It is the active ingredient of a variety of commercial herbicide formulations.
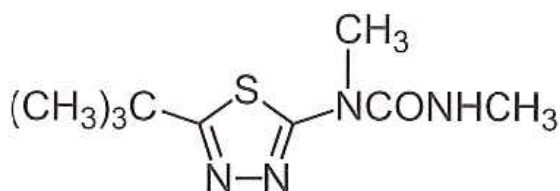

Figure 47 Structure of tebuthiuron

Physicochemical properties of tebuthiuron that may affect its environmental fate and toxicity are presented in Table 55.

Table 55 Summary of selected physicochemical properties of tebuthiuron.

Physicochemical property	Value
Molecular weight	228.3 amu[1]
Aqueous solubility	2.5 g/L @ temperature 20 ºC[1]
Logarithm of the octanol-water partition coefficient (log K_{ow})	1.82 @ temperature 20 ºC[1] 1.79 @ pH 7 and temperature 20 ºC[2]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.9 –2.1[2]
Logarithm of the bioconcentration factor (log BCF)	0.41[2]
Half-life ($t_{1/2}$) in water	>64 days @ pH 3, 6, 9 and temperature 25 ºC[1] 64 days @ pH 7 and temperature 20 ºC[2] Marine: 944, 1,474, 1,766 and 3,300 days (light with sediment, dark with sediment, dark no sediment and light no sediment, respectively)[3]
Half-life ($t_{1/2}$) in soil	360 days @ temperature 20 ºC[2] Typical: 400 days[2]

[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] Mercurio et al. (2016).

Tebuthiuron belongs to the thiadiazolylurea group within the urea family of herbicides, which also includes buthiuron, ethidimuron and thiazafluron. Tebuthiuron can be sprayed or applied as granules. Tebuthiuron can be used in agricultural, permanent pasture (as in grazing), forestry and industrial situations (roads, railway lines and rights of way) to control herbaceous and woody plants, annual and perennial broadleaf weeds as well as grasses (CCME 1999, University of Hertfordshire 2013). It is also used for the total control of vegetation in non-crop areas (CCME 1999). In Northern Australia, it has been extensively used to control the invasive weed, *Mimosa pigra* (Van Dam et al. 2004). However, it does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013). Tebuthiuron is a non-selective, systemic, soil applied herbicide (CCME 1999, BCPS 2012) that is highly soluble in water (Table 55).

Tebuthiuron is mainly absorbed through the roots of plants and is translocated to the target sites in the stems and leaves (Steinert and Stritzke 1977). Tebuthiuron exerts its toxicity in plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid

membranes of chloroplasts. Urea herbicides bind to the plastoquinone B (Q_B) protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (ATP, used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (NADPH, used in converting CO_2 to glucose), and therefore, prevents CO_2 fixation (Wilson et al. 2000).

In addition to its main mode of action, exposure to PSII inhibiting herbicides can lead to marked increases in the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen (OH^-), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). Reactive oxygen species are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). Reactive oxygen species are created during normal cellular functioning particularly in biochemical processes that involve the generation of energy (e.g. photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells). In phototrophs, ROS are formed when the absorbed light energy exceeds the ability to convert CO_2 to organic molecules, thus accumulating oxygen (Chen et al. 2012). Normal concentrations of ROS are involved in a number of cellular processes (Chen et al. 2012). However, prolonged exposure to elevated concentrations of ROS in plants, as a result of biotic (e.g. disease) and/or abiotic stressors (e.g. PSII inhibiting herbicides), can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

Tebuthiuron ultimately ends up in aquatic environments as a result of aerial or direct contamination from spray drift, surface and/or subsurface runoff from treated and soil leaching (CCME 1999). Tebuthiuron has high water solubility (Table 55) and low soil adsorption characteristics as indicated by its low log K_{oc} value (Table 55) and thus has a high capacity to leach to groundwater and to be transported in surface waters (University of Hertfordshire 2011, BCPC 2012). The aqueous hydrolysis of tebuthiuron is relatively fast with a half-life of greater than 64 days at pH values between pH 3 and pH 9 and a temperature of 20 ºC (University of Hertfordshire 2013) (Table 55). In marine environments, it has been reported that the simultaneous effects of sediment and light rapidly degrade tebuthiuron (Mercurio et al. 2016).

Australian figures from 2011–15 show that tebuthiuron has been detected in approximately 15.7% of surface water samples in waterways that drain agricultural land and discharge to the Great Barrier Reef (based on data in Turner et al. 2013a, 2013b; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015). Tebuthiuron is also present in marine waters, with figures from 2011–14 indicating that tebuthiuron has been detected in approximately 3% of marine samples (maximum concentration 0.04 µg/L) in the Wet Tropics region - off the coast of northern Queensland, Australia (O'Brien et al. 2015).

13.2 Freshwater

13.2.1   Aquatic Toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for tebuthiuron in freshwaters (Table 57) includes toxicity data for one freshwater species that either originated from or is distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all freshwater species that passed the screening and quality assurance processes are provided below.

Freshwater Chronic

There are freshwater chronic toxicity data for two fish, one cladoceran, one macrophyte and five microalgae. The toxicity data for the fish were a 7-day NOEC (mortality) of 90,000 µg/L, 28-day NOEC (mortality, total length, average weight) values ranging from 9,300 to 76,000 µg/L, a 28-day LOEC (total length) value of 7,200 µg/L, a 33-day NOEC (percent embryo hatch) value of 76,000 µg/L and 45-day NOEC and LOEC (mortality) values of 26,000 and 52,000 µg/L, respectively. The toxicity values for the single cladoceran species were 21-day NOEC (mortality, length, young per adult, brood size, days to first brood) values ranging from 21,800 µg/L to 90,200 µg/L and 21-day LOEC (length, young per adult, brood size, days to first brood) values ranging from 17,680 to 36,080 µg/L. The toxicity values for the single macrophyte were 14-day NOEL and EC50 (frond number, dry weight, frond area) values of 66 and 135 µg/L, respectively. The toxicity values for microalgae were 72-hour NOEC, LOEC and IC50 (cell density) values of 100, 190 and 281 µg/L, 96-hour EC50/IC50 (cell count, growth rate) values ranging from 80 to 102 µg/L, two 5-day NOEC (cell counts, growth rate) values of 10 and 50 µg/L, two 6-day NOEC (cell counts, growth rate) values both of 50 µg/L, two 7-day NOEL (biomass yield, growth rate, area under the growth curve) values of 56 and 310 µg/L, a 7-day LOEC (biomass yield, growth rate, area under the growth curve) value of 620 µg/L, a 7-day EC25 (biomass yield, growth rate, area under the growth curve) value of 4,060 µg/L, 7-day EC50 (biomass yield, growth rate, area under the growth curve) values of 193 and 213 µg/L, 14-day NOEC/NOEL (general population change, biomass, cell density, cell counts, biomass yield, growth rate, area under the growth curve) values ranging from 13 to 79 µg/L, 14-day LOEC (general population change, biomass, cell density) values ranging from 79 to 168 µg/L and two 14-day EC50 (general population change, biomass yield, growth rate, area under the growth curve) values of 50 and 307 µg/L.

Freshwater Acute

There are freshwater acute toxicity data for five fish, one amphibian, two crustacean, one cnidarian and one macrophyte. The toxicity values for the fish were 96-hour BEC10, MDEC, NOEC and LOEC (mortality) values of 108,000, 133,000, 200,000 and 225,000 µg/L, respectively and 96-hour LC50 (mortality) values ranging from 106,000 to 291,000 µg/L. The toxicity values for the single amphibian species were 48- and 72-hour LC50 (mortality) values of 332,000 and 316,000 µg/L. The toxicity values for the crustacean species were a 48-hour EC50 (immobilisation) value of 297,000 µg/L, 6-day BEC10, MDEC, NOEC, LOEC and EC50 (brood size) values of 17,400, 41,800, 20,000, 40,000 and 134,000 µg/L, respectively. The toxicity values for the cnidarian species were 96-hour BEC10, MDEC, NOEC, LOEC and EC50 (hydroid growth) values of 40,600, 53,200, 50,000, 75,000 and 150,000 µg/L, respectively. The toxicity values for the single macrophyte species were 96-hour EC10/NOEC (frond count) values ranging from 47 to 61 µg/L, two 96-hour IC20 (frond count) values of 53 and 109 µg/L and 96-hour IC50/EC50 (frond count) values ranging from 144 to 297 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

13.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of tebuthiuron. As with many organic chemicals it might be expected that any factor that influences the degradation of the active ingredient (e.g. light), or the complexation/adsorption of tebuthiuron to dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of tebuthiuron (Table 55).

13.2.3 Guideline derivation

The derived PGVs for tebuthiuron in freshwaters are provided in Table 56. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other

pesticides that have GVs, the PGVs for tebuthiuron are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for tebuthiuron are low (Table 55) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for tebuthiuron do not need to account for secondary poisoning.

Table 56 Proposed aquatic ecosystem protection guideline values (µg/L) for tebuthiuron for the protection of freshwater ecosystems.

Tebuthiuron proposed aquatic ecosystem protection guideline values (freshwater)[1]		Reliability classification[2]	
Species protection	Concentration (µg/L) (95% CI)	Criterion	Result
99%	4.8 (0.98 – 48)	Sample size	5
95%	13 (4.2 – 57)	Type of toxicity data	Chronic NOEC/NOEL values
90%	19 (6.7 – 72)	SSD model fit	Poor
80%	31 (10 – 92)	Reliability	Low

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software. [2] See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline values "reliability".**

13.2.4   Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for tebuthiuron in freshwater environments was a high reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was based on chronic toxicity value for six species (Warne 2001). The freshwater data for tebuthiuron was distinctly bimodal (phototrophic species were more sensitive); however, there were insufficient data in each mode to use the species sensitivity distribution (SSD) method for a restricted dataset. Therefore, this TV was calculated using chronic freshwater data for six phototrophic and heterotrophic species. Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as having a low to very low reliability.

To obtain toxicity data for tebuthiuron to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ WQG toxicant databases (Sunderam et al. 2000) were searched. There are now more tebuthiuron toxicity data available that enable the calculation of PGVs in fresh waters (see section 13.2.6). In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of tebuthiuron with phototrophic (e.g. plants and algae) freshwater species be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when

there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for tebuthiuron in freshwaters, *Chlorella* sp. was included as no other toxicity data for these genera were used.

In total, there were freshwater toxicity data for 14 species (seven phyla and eight classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cnidaria, Cyanobacteria and Traceophyta. The eight classes were Actinopterygii (which accounts for approximately 99% of fish), Amphibia (tetrapod vertebrates), Bacillariophyceae (diatoms; a major grouping of algae), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Hydrozoa (a diverse group of cnidarians), Liliopsida (monocots).

Based on the current understanding of the mode of action of tebuthiuron, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The tebuthiuron ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p <0.0001, see section 13.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were freshwater chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data value for five species (that belonged to four phyla and four classes), which met the minimum data requirements (i.e. at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 56) combined with the poor fit of the distribution to these toxicity data (Figure 48) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for tebuthiuron in freshwaters is provided in Table 57.

Table 57 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for tebuthiuron in freshwaters. Data are arranged in alphabetical order of the test species.

Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	7	Chronic NOEL	Biomass yield, growth rate, AUC[2]	310	USEPA (2015b)
Microalga	*Chlorella* sp.	Chlorophyta	Chlorophyceae	4-5 days old	3	Chronic NOEC	Cell density	100	Van Dam et al. (2004)
Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic NOEL	Frond number, frond size, dry weight	66	USEPA (2015b)
Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	7	Chronic NOEL	Biomass yield, growth rate, AUC[2]	56	USEPA (2015b)
Microalga	*Selenastrum capricornutum[3]*	Chlorophyta	Chlorophyceae	Not stated	14	Chronic NOEL	Biomass yield, growth rate, AUC[2]	13	USEPA (2015b)

[1.] **Chronic NOEC/NOEL = no conversions applied (Warne et al. 2015).** [2.] **AUC = area under the growth curve.** [3.] **This species has also been called** *Raphidocelis subcapitata* **and** *Pseudokirchneriella subcapitata*. *** Species that originated from/are distributed in Australia and/or New Zealand.**

13.2.5　Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the five freshwater phototrophic species that was used to derive the PGVs is presented in Figure 48.
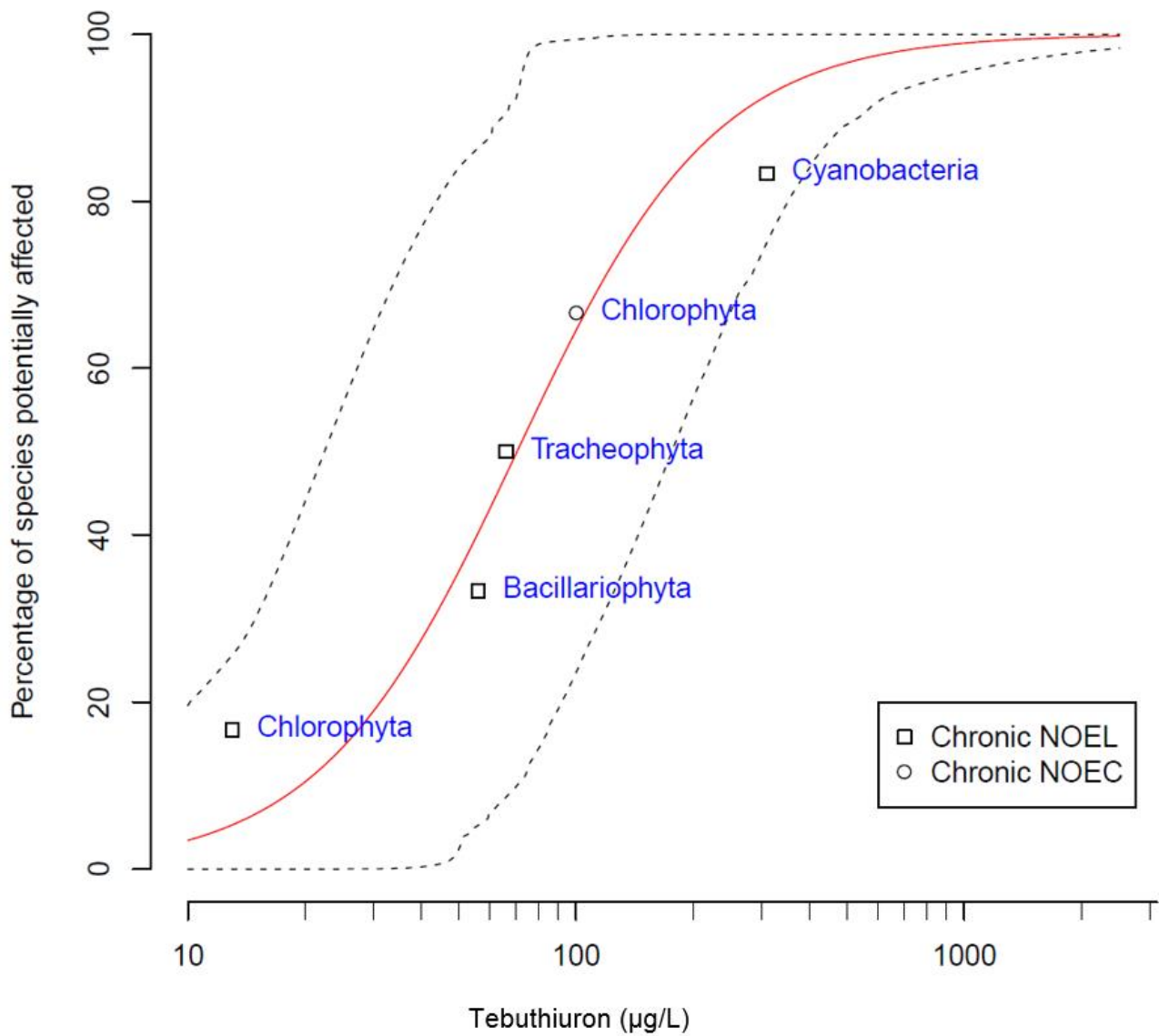

Figure 48 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic species to tebuthiuron. Black dashed lines indicate the 95% confidence intervals.

13.2.6 Summary details of freshwater toxicity data used to derive proposed aquatic ecosystem protection guideline values for tebuthiuron in freshwaters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
Chlorophyta	Chlorophyceae	Microalga (*Chlorella* sp.)	4–5 days old	3	Chronic	NOEC (Cell density)	Modified MBL medium, synthetic soft water	27 ± 1	6.5	100	Van Dam et al. (2004)
										100	*GEOMETRIC MEAN*
										100	***VALUE USED IN SSD***
Chlorophyta	Chlorophyceae	Microalga (*Selenastrum capricornutum[1]*)	Not stated	14	Chronic	NOEL (Biomass yield, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	13	USEPA (2015b)
										13	*GEOMETRIC MEAN*
										13	***VALUE USED IN SSD***
Bacillariophyta	Bacillariophyceae	Microalga (*Navicula pelliculosa*)	Not stated	7	Chronic	NOEL (Biomass yield, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	56	USEPA (2015b)
										56	*GEOMETRIC MEAN*
										56	***VALUE USED IN SSD***
Cyanobacteria	Cyanophyceae	Cyanobacteria (*Anabaena flos-aquae*)	Not stated	7	Chronic	NOEL (Biomass yield, growth rate, AUC[2])	ASTM Type I water	24 ± 2	7.5 ± 0.1	310	USEPA (2015b)
										310	*GEOMETRIC MEAN*
										310	***VALUE USED IN SSD***
Tracheophyta	Liliopsida	Macrophyte	Not	14	Chronic	NOEL	M-Hoagland's	25 ± 2	4.8-5.2 (M-	66	USEPA

		(*Lemna gibba*)	stated			(Frond number, frond size, dry weight)	or 20X-AAP nutrient media. ASTM Type I water.		Hoagland's) and 7.5 ± 0.1 (20x-AAP)		(2015b)
										66	*GEOMETRIC MEAN*
										66	***VALUE USED IN SSD***

[1] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. [2] AUC = area under the growth curve.

13.3 Marine

13.3.1   Aquatic Toxicology

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry and 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 guideline derivation process for tebuthiuron in marine waters (Table 59) includes toxicity data for two species (one marine and one freshwater) that either originated from or is distributed within Australia and/or New Zealand. A summary of the high and moderate quality raw toxicity data for all marine and freshwater species that passed the screening and quality assurance processes are provided below and in section 13.2.1, respectively.

Marine Chronic

There were marine chronic toxicity data for two species of microalgae, which were 3-day EC10 and EC50 (cell density) values of 29.97 and 64.39 µg/L, respectively, and 7-day NOEL and EC50 (biomass, growth rate, area under the curve) values of 36 and 60 µg/L, respectively.

Marine Acute

There were marine acute toxicity data for one crustaceans and one mollusc. The toxicity values for the single crustacean species were a 48-hour LC50 (mortality) value of 84,000 µg/L and 96-hour LC50 and EC50 (mortality) values of 48,000 to 62,000 µg/L, respectively. The single toxicity value for the mollusc species was a 48-hour NOEL (mortality) value of 180,000 µg/L, respectively. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive PGVs.

13.3.2   Factors affecting toxicity

No No factors have been reported as modifying the toxicity of tebuthiuron. As with many organic chemicals it might be expected that any factor that influences the degradation of the active ingredient (e.g. light), or the complexation/adsorption of tebuthiuron to dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of tebuthiuron (Table 55).

13.3.3   Guideline derivation

The derived PGVs for tebuthiuron in marine waters are provided in Table 58. Details of how the PGVs were calculated and the toxicity data that were used are provided below. As with all the other pesticides that have GVs, the PGVs for tebuthiuron are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for tebuthiuron are low (Table 55) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4, Warne et al. 2015). Therefore, the PGVs for tebuthiuron do not need to account for secondary poisoning.

Table 58 Proposed aquatic ecosystem protection guideline values (μg/L) for tebuthiuron for the protection of marine ecosystems.

Tebuthiuron proposed aquatic ecosystem protection guideline values (marine)[1]		Reliability classification[2]	
Species protection	Concentration (μg/L) (95% CI)	Criterion	Result
99%	4.7 (1.6 – 23)	Sample size	7
95%	11 (5.1 – 34)	Type of toxicity data	Chronic NOEC/NOEL/EC10 values (*freshwater and marine*)
90%	17 (8.2 – 42)	SSD model fit	Good
80%	26 (14 – 55)	Reliability	Moderate

[1] **Proposed aquatic ecosystem protection guideline values were derived using the Burrlioz 2.0 (2016) software.** [2] **See Warne et al. (2015) for definitions of proposed aquatic ecosystem protection guideline value "reliability".**

13.3.4   Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for tebuthiuron in marine environments was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was adopted from the freshwater GV, which was based on chronic toxicity data for six species. The data that were used to derive the freshwater PGV for tebuthiuron were distinctly bimodal (phototrophic species were more sensitive) however there were insufficient data in each mode to use the species sensitivity distribution (SSD) method. As a result, the previous freshwater, and, therefore marine PGVs were calculated using chronic freshwater data for six phototrophic and heterotrophic species. Under the new method for deriving PGVs (Warne et al. 2015) this trigger value would be classified as very low reliability.

To obtain toxicity data for tebuthiuron to marine organisms, an extensive search of the scientific literature was conducted. In addition, the databases of the USEPA ECOTOX (USEPA 2015a), Office of the Pesticide Program (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC and ARMCANZ WQG toxicant databases (Sunderam et al. 2000) were searched. There are now more tebuthiuron toxicity data available that enable the calculation of PGVs for marine waters. However, it was only possible to derive PGVs by using ecotoxicity data for both marine and freshwater organisms (see section 13.3.6 and 13.2.6, respectively). In order to derive higher reliability PGVs in the future that are of greater relevance to marine ecosystems separately, it is recommended that additional chronic toxicity tests of tebuthiuron with phototrophic (e.g. plants and algae) marine species be conducted.

Normally, species classified only to the level of genus (e.g. *Chlorella* sp.) are not used in the PGV derivation process as species specificity is required. The use of such data in PGV derivations is usually avoided as the ambiguity at the genus level could result in more than one toxicity value being assigned to a single species. However, visual identification and classification of species within a genus, particularly for microalgae, can be difficult for some genera due to their lack of characteristic morphological features (Kessler and Huss 1992). Nonetheless, when there are no other data for species belonging to the same genus (i.e. there is no chance of duplicating a species) and/or when there are limited amounts of toxicity data available, then such data could be included in the derivation of PGVs. In deriving the PGVs for tebuthiuron in marine waters, *Chlorella* sp. was included as no other toxicity data for these genera were used.

In total, there were marine toxicity data for three species (three phyla and three classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta and Haptophyta. The three classes were Coccolithophyceae (a class of yellow algae), Malacostraca (a large grouping of crustaceans) and Mediophyceae (another algae grouping).

Based on the current understanding of the mode of action of tebuthiuron, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The tebuthiuron ecotoxicity data for phototrophs and heterotrophs were then tested using the parametric two-sample *t* test to see if the toxic responses among different taxa were uni- or multi-modal. The *t* test indicated that the two groups had significantly different (p = <0.0001, see section 13.3.7) sensitivities. Therefore, as recommended by Warne et al. (2015), only the ecotoxicity data for the more sensitive group of organisms (in this case, phototrophs) were used in calculating the PGVs.

There were marine 10% effect concentration (EC10) and chronic no observed effect concentration (NOEC) data available for only two phototrophic species (that belonged to two phyla and two classes), which did not meet the minimum data requirements (i.e., at least five species belonging to four phyla) to use a SSD to derive a PGV (Warne et al. 2015). As no other ecotoxicity data for tebuthiuron to marine phototrophic species were available, the chronic EC10/NOEC values for the marine phototrophic species were combined with the available chronic NOEC/no observed effect level (NOEL) values for freshwater phototrophic species to derive PGVs for tebuthiuron in marine waters. This dataset incorporated concentration data for seven (two marine and five freshwater) phototrophic species belonging to five phyla and six classes, which met the minimum data requirements (i.e., at least five species belonging to at least four phyla) to use a SSD to derive PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 58) combined with the good fit of the distribution to these toxicity data (Figure 49) resulted in a moderate reliability set of PGVs. The combination of freshwater and marine ecotoxicity data reduces the reliability classification of PGVs as per Warne et al. (2015). A summary of the toxicity data (one value per species) used to calculate the PGVs for tebuthiuron in marine waters is provided in Table 59.

Table 59 Summary of the single toxicity values for each phototrophic species that were used to derive the proposed aquatic ecosystem protection guideline values for tebuthiuron in marine waters. Data are arranged in alphabetical order of the test species.

Media	Taxonomic group	Species	Phyla	Class	Life stage	Duration (days)	Type[1]	Toxicity endpoint	Toxicity value (µg/L)	Reference
Fresh	Cyanobacteria	*Anabaena flos-aquae*	Cyanobacteria	Cyanophyceae	Not stated	7	Chronic NOEL	Biomass yield, growth rate, AUC[2]	310	USEPA (2015b)
Fresh	Microalga	*Chlorella* sp.	Chlorophyta	Chlorophyceae	4-5 days old	3	Chronic NOEC	Cell density	100	Van Dam et al. (2004)
Marine	Microalga	*Isochrysis galbana*	Haptophyta	Coccolithophyceae	Not stated	3	Chronic EC10	Cell density	29.9	Seery and Pradella (2014)
Fresh	Macrophyte	*Lemna gibba*	Tracheophyta	Liliopsida	Not stated	14	Chronic NOEL	Frond number, frond size, dry weight	66	USEPA (2015b)
Fresh	Microalga	*Navicula pelliculosa**	Bacillariophyta	Bacillariophyceae	Not stated	7	Chronic NOEL	Biomass yield, growth rate, AUC[2]	56	USEPA (2015b)
Fresh	Microalga	*Selenastrum capricornutum*[3]	Chlorophyta	Chlorophyceae	Not stated	14	Chronic NOEL	Biomass yield, growth rate, AUC[2]	13	USEPA (2015b)
Marine	Microalga	*Skeletonema costatum**	Bacillariophyta	Mediophyceae	Not stated	7	Chronic NOEL	Biomass yield, growth rate, AUC[2]	36	USEPA (2015b)

[1] **Chronic NOEC/NOEL = no conversions applied (Warne et al. 2015).** [2] **AUC = area under the growth curve.** [3] **This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. * Species that originated from/are distributed in Australia and/or New Zealand.**

13.3.5   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the seven marine and freshwater phototrophic species that was used to derive the PGVs is presented in Figure 49.
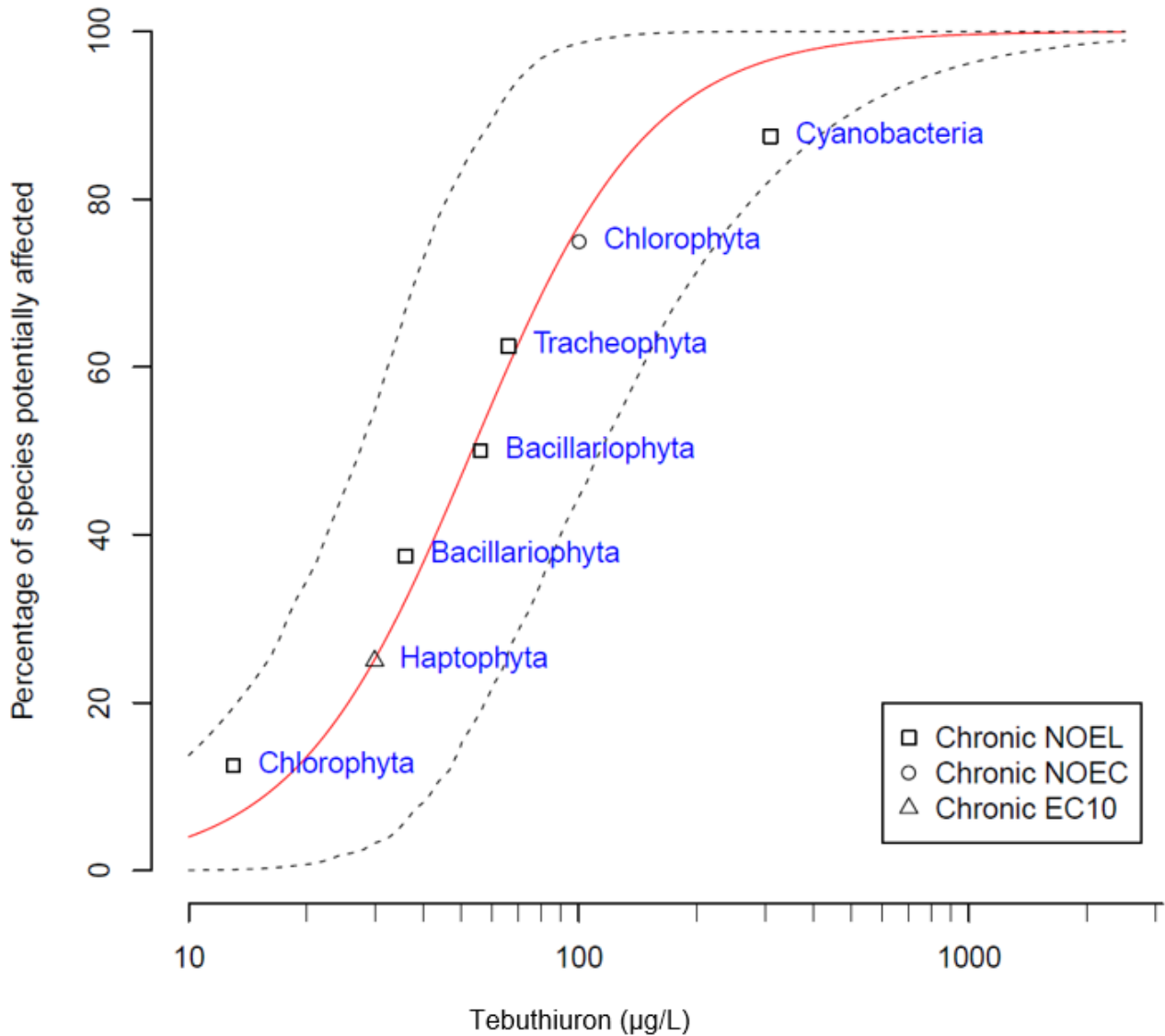

Figure 49 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC), no observed effect level (NOEL) and 10% effect concentration (EC10) data values of marine and freshwater phototrophs to tebuthiuron. Black dashed lines indicate the 95% confidence intervals.

13.3.6 Summary details of marine toxicity data used to derive proposed aquatic ecosystem protection guideline values for tebuthiuron in marine waters.

Phyla	Class	Species	Life stage	Exposure duration (days)	Test type	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Bacillariophyta	Mediophyceae	Microalga (*Skeletonema costatum*)	Not stated	7	Chronic	NOEL (Biomass yield, growth rate, AUC[1])	Synthetic salt water or filtered natural salt water	30 ± 5	20 ± 2	8.0 ± 0.1	36	USEPA (2015b)
											36	*GEOMETRIC MEAN*
											36	***VALUE USED IN SSD***
Haptophyta	Coccolithophyceae	Microalga (*Isochrysis galbana)*	Not stated	3	Chronic	EC10 (Cell density)	F/2 Guillard's Marine, filtered seawater	31 ± 2	29 ± 1	8.2 ± 0.2	29.97	Seery et al.
											29.97	*GEOMETRIC MEAN*
											29.9	***VALUE USED IN SSD***

[1] **AUC = area under the growth curve.**

13.3.7   Distribution of sensitivities for aquatic species

The transformed ecotoxicity data for marine phototrophic species (n = 2) fell within the lower and upper 95% confidence intervals [1.821 and 6.240 ln(μg/L), respectively] of the transformed ecotoxicity data for freshwater phototrophic species (n = 6). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for tebuthiuron.

The toxicity data for tebuthiuron to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2015). A natural logarithmic (ln) transformation was then applied to normalise the data. Visual examination of the histogram of transformed data indicated that the distribution of the tebuthiuron ecotoxicity data may be bimodal (Figure 50).
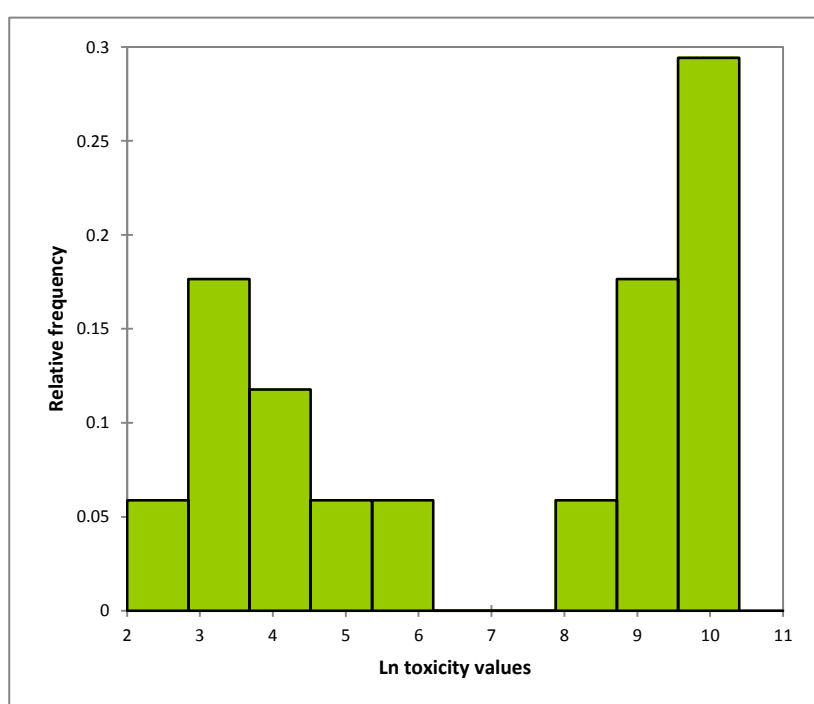

Figure 50 Histogram of the natural logarithm (ln) of all tebuthiuron (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (n = 17).

The tebuthiuron ecotoxicity data for phototrophic and non-phototrophic species were tested to see if they came from the same population. To test for significant differences (i.e. p-value ≤ 0.05) between the two groups, the parametric two-sample t test was used because the transformed tebuthiuron concentration data had equal variances (Fisher's F-Test; p = 0.150) and followed a normal distribution (Anderson-Darling; p = 0.881). Results from the two-sample t test indicated that the two groups were significantly different (p <0.0001); therefore, it can be concluded that the distribution of the tebuthiuron concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

13.3.8　References

ALA (2017). Atlas of Living Australia. Developed by the National Research Infrastructure for Australia (NCRIS) and the Commonwealth Scientific and Industrial Research Organisation (CSIRO). Available from: https://www.ala.org.au/, Accessed: May 2017.

ANZECC and ARMCANZ (2000). Australian and New Zealand Guidelines for fresh and marine water quality/aquatic ecosystems – Rationale and background information (Chapter 8). Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra, Australian Capital Territory, Australia, 678pp.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 1069–1071.

Burrlioz 2.0 (2016). Statistical software package to generate trigger values for local conditions within Australia. CSIRO (http://www.csiro.au). [Online] Available from: https://research.csiro.au/software/burrlioz/.

Camilleri C, Markich S, van Dam R, Pfeifle V (1998). Toxicity of the Herbicide Tebuthiuron to Australian Tropical Freshwater Organisms: Towards an Ecological Risk Assessment. Supervising Scientist Report 131. Supervising Scientist, Canberra, Australia. 49pp.

CCME (1999). Canadian water quality guidelines for the protection of aquatic life – Tebuthiuron, Canadian Council of Ministers of the Environment. Available from: http://www.ccme.ca/files/ceqg/en/backup/213.pdf. Downloaded: 8 April, 2016.

Chen S, Yin C, Strasser RJ, Govinjee, Yang C and Qiang S (2012). Reactive oxygen species from chloroplasts contribute to 3-acetyl-5-isopropyltetramic acid-induced leaf necrosis of *Arabidopsis thaliana*. *Plant Physiology and Biochemi*stry 52, 38–51.

Garzon-Garcia A, Wallace R, Huggins R, Turner RDR, Smith RA, Orr D, Ferguson B, Gardiner R, Thomson B and Warne MStJ (2015). *Total suspended solids, nutrients and pesticide loads (2013–2014) for rivers that discharge to the Great Barrier Reef* – Great Barrier Reef Catchment Loads Monitoring Program 2013–2014. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 108pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2013-2014-gbr-catchment-loads-technical-report.pdf

Guiry MD and Guiry GM (2017). AlgaeBase. World-wide electronic publication, National University of Ireland, Galway. Available from: http://www.algaebase.org. Accessed: May 2017.

Halliwell B (1991). Oxygen radicals: Their formation in plant tissues and their role in herbicide damage. In: Baker NR and Percival MP (Eds.), Herbicides, Elsevier Science, Amsterdam, 87–129.

ITIS (2017). Integrated Taxonomic Information System. Available from: https://www.itis.gov/, Accessed: May 2017.

Kessler E and Huss V.A.R (1992). Comparative physiology and biochemistry and taxonomic assignment of the *Chlorella* (Chlorophyceae) strains of the culture collection of the University of Texas at Austin, *Journal of Phycology*, 28, 550–553.

Mercurio P, Mueller JF, Eaglesham G, O'Brien J, Flores F and Negri AP (2016). Degradation of herbicides in the Tropical Marine Environment: Influence of Light and Sediment, PLoS ONE 11. doi:10.1371/journal.

O'Brien D, Nash M, Di Bella L and Brodie J (2015). Reef Plan Herbert Water Quality Monitoring Program (HWQMP) Final report for monitoring undertaken between 2011 and 2014. Centre for

Tropical Water and Aquatic Ecosystem Research Publication, James Cook University, Townsville, Australia pp. 167. Available from: https://research.jcu.edu.au/tropwater/publications/1518ReefplanHerbertwaterqualitymonitoringprogram.pdf.

Pfeifle V (1996). The effect of the herbicide tebuthiuron on two tropical non-target freshwater organisms, Internal report 218, Environmental Research Institute of the Supervising Scientist, Darwin, Northern Territory, Australia. 55pp.

Roskov Y, Abucay L, Orrell T, Nicolson D, Bailly N, Kirk PM, Bourgoin T, DeWalt RE, Decock W, De Wever A, Nieukerken E, Zarucchi J, Penev L, eds. (2017). Species 2000 & ITIS Catalogue of Life, 30th April 2017. Digital resource available from: www.catalogueoflife.org/col. Species 2000: Naturalis, Leiden, the Netherlands. ISSN 2405-8858. Accessed: May 2017.

Seery C and Pradella N (in prep.). Toxicity of photosystem II inhibiting herbicides to four tropical alga.

Steinert WG and Stritzke JF (1977). Uptake and phytotoxicity of tebuthiuron. *Weed Science*, 25, 390–395.

Sunderam RIM, Warne MStJ, Chapman JC, Pablo F, Hawkins J, Rose RM and Patra RW (2000). The ANZECC and ARMCANZ water quality guideline database for toxicants. Supplied as part of a CD-rom in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality.

Turner R, Huggins R, Wallace R, Smith R, Vardy S and Warne MStJ (2013a). *Sediment, nutrient and pesticide loads: Great Barrier Reef Loads Monitoring 2009–2010.* Water Sciences Technical Report, Volume 2012, Number 14. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0994. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2009-2010-gbr-catchment-loads-report.pdf

Turner RDR, Huggins R, Wallace R, Smith RA, Vardy S and Warne MStJ (2013b). *Total suspended solids, nutrient and pesticide loads for rivers that discharge to the Great Barrier Reef: Great Barrier Reef Loads Monitoring 2010–2011.* Water Sciences Technical Report, Volume 2013, Number 1. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, Australia. 53p. ISSN 1834-3910. ISBN 978-1-7423-0996. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2010-2011-gbr-catchment-loads-report.pdf

University of Hertfordshire (2013). The Pesticide Properties Data Base (PPDB). Developed by the Agriculture & Environment Research Unit (AERU), University of Hertfordshire, 2006–2013. Available from: http://sitem.herts.ac.uk/aeru/ppdb/en/Reports/27.htm. Accessed 13 May 2016.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/. Accessed: May–September, 2015.

USEPA (2015b). Office of Pesticide Programs Database. Office of Prevention, Pesticides, and Toxic Substances, United States Environmental Protection Agency, Office of Pesticide Programs. Washington, D.C. January 23, 2004. Available from: http://www.ipmcenters.org/ecotox/. Accessed: February–April, 2016.

Van Dam RA, Camilleri C, Turley C, Binet MT and Stauber JL (2004). Chronic toxicity of the herbicide tebuthiuron to the tropical green alga *Chlorella* sp. and the duckweed *Lemna aequinoctialis*, *Australasian Journal of Ecotoxicology*, 10, 97–104.

Wallace R, Huggins R, King O, Gardiner R, Thomson B, Orr DN, Ferguson B, Taylor C, Smith RA, Warne MStJ, Turner RDR, Mann RM (2016). Total suspended solids, nutrient and pesticide loads (2014–2015) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program. Department of Science, Information Technology and Innovation. Brisbane, 111pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2014-2015-gbr-catchment-loads-technical-report.pdf.

Wallace R, Huggins R, Smith RA, Turner R, Garzon-Garcia A and Warne MStJ. 2015. Total suspended solids, nutrients and pesticide loads (2012–2013) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2012–2013. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 99pp. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2012-2013-gbr-catchment-loads-report.pdf

Wallace R, Huggins R, Smith RA, Turner RDR, Vardy S and Warne MStJ. 2014. Total suspended solids, nutrient and pesticide loads (2011–2012) for rivers that discharge to the Great Barrier Reef – Great Barrier Reef Catchment Loads Monitoring Program 2011–2012. Department of Science, Information Technology, Innovation and the Arts. Brisbane, Queensland. 94p. Available from: http://www.reefplan.qld.gov.au/measuring-success/paddock-to-reef/assets/2011-2012-gbr-catchment-loads-report.pdf

Warne MStJ (2001). Description of how each toxicant trigger value was derived. CD-ROM in the ANZECC and ARMCANZ (2000) Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Canberra, Australia.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2015). Revised Method for Deriving Australian and New Zealand water quality guideline values for toxicants. Updated May, 2017. Department of Science, Information Technology, Innovation and the Arts, Brisbane, Queensland, 48pp.

Warne MStJ, Westbury A-M and Sunderam R (1998). A compilation of toxicity data for chemicals to Australasian aquatic species. Part 1: Pesticides, *Australasian Journal of Ecotoxicology,* 4, 93–144.

Wilson PC, Whitwell T and Klaine SJ (2000). Metalaxyl and simazine toxicity to and uptake by *Typha latifolia*, *Archives of Environmental Contamination and Toxicology* 39, 282–288.

WoRMS Editorial Board (2017). World Register of Marine Species. Available from: http://www.marinespecies.org at VLIZ, Accessed: May 2017. doi:10.14284/170.