# 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

**King, O, Smith, R, Warne, M, Frangos, J & Mann, R**



**Original citation:**
King, O, Smith, R, Warne, M, Frangos, J & 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, Australia

Publisher: Department of Science, Information Technology and Innovation

Department of Science, Information Technology and Innovation

# 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.

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

2017

Queensland Government

**Prepared by**

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



**Disclaimer**



**Citation**

O. C. King, R. A. Smith, M. St. J. Warne, J. S. Frangos and R. M. Mann. 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.

August 2017

**Acknowledgements**

The authors gratefully acknowledge the funding provided by the Queensland Government Department of Environment and Hertiage Protection (DEHP). The authors thank Hannah Allan for assistance in quality checking the literature. The authors would also like to acknowledge valuable comments provided by the reviewers (Andrew Negri and Melanie Shaw) and discussions held with numerous colleagues during the derivation of the revised proposed aquatic ecosystem protection guideline values.

# 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 second 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 13 pesticides presented in Part 1 (King et al. 2017) 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 (this report) 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.

The 14 pesticides presented in Part 2 (this report), constitute a suite of chemicals for which there are comparatively few toxicity data. As a consequence, several of the Proposed Guideline Values presented in this report are categorised as being of *low reliability* (Warne et al. 2015). It is anticipated that some of the Proposed Guideline Values presented here will change when more toxicity data become available. Hence, the adoption of the Proposed Guideline Values as part of a risk assessment process needs to take into consideration the reliability rating and the pending availability of new data.

# 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 second part of a two-part series that presents the PGVs 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.

Part 1 of the two-part series (King et al. 2017) presents the freshwater and/or marine PGVs for a total of 13 pesticides. These include; (i) PGVs for glyphosate (freshwater only), metolachlor (freshwater only), metsulfuron-methyl (freshwater only) and simazine that were funded through the Australian 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 were funded through the Queensland Department of Science, Information

[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.

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).

This report, Part 2 of the two-part series presents the freshwater and/or marine PGVs for a further 14 pesticides that are 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, Mann R and Warne MStJ (2017). 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, Tebuthiuron. Department of Science, Information Technology and Innovation. Brisbane, Queensland, Australia, 294pp.

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

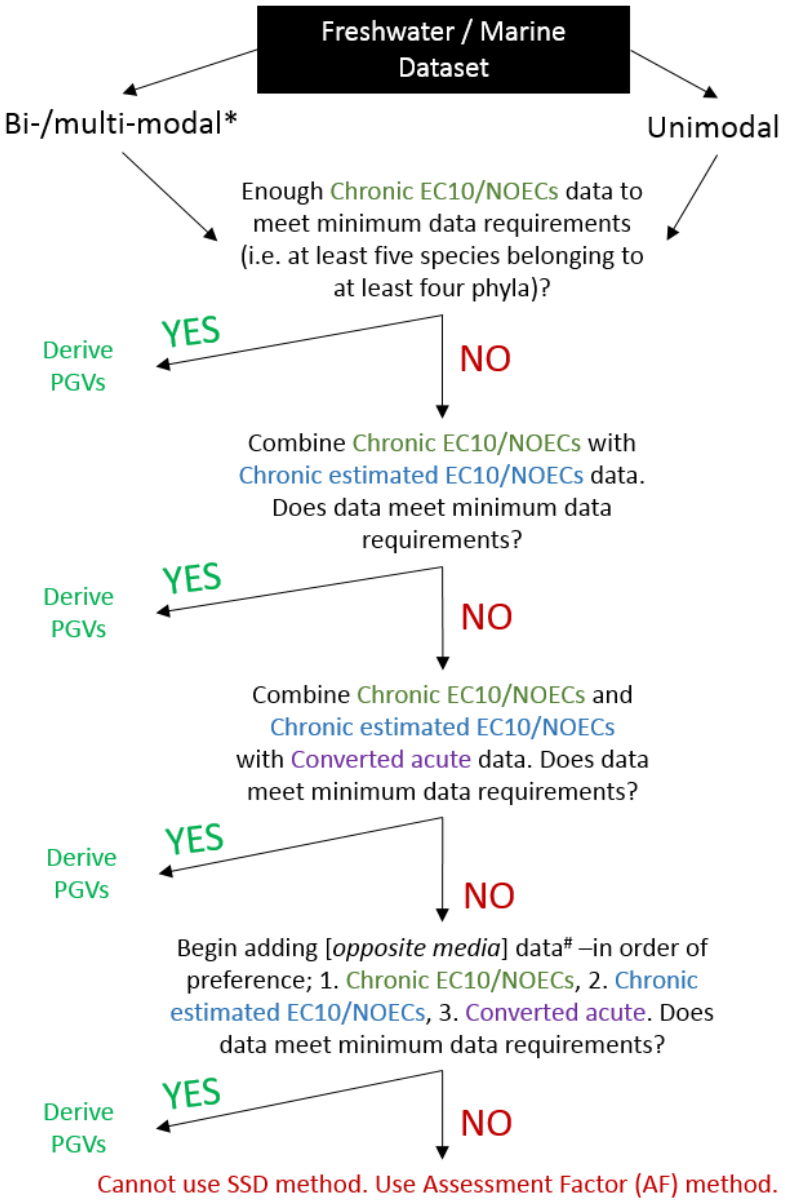
| | |
|---|---|
| **Acute toxicity** | An adverse effect that occurs as the result of a short-term exposure to a chemical relative to the organism's life span. Refer to Warne et al. (2015) for examples of acute exposures. |
| **ANZECC** | Australian and New Zealand Environment and Conservation Council. |
| **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 ($p \leq 0.05$) 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 toestimates 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).

## 1.1 Introduction

Bromacil ($C_9H_{13}BrN_2O_2$ and Figure 1) at room temperature is in the form of a white to tan crystalline solid. It is the active ingredient of a variety of commercial herbicide formulations.

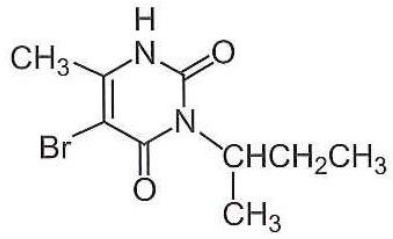



Figure 1 Structure of bromacil.

Physicochemical properties of bromacil that may affect its environmental fate and toxicity are presented in Table 1.

**Table 1 Summary of selected physicochemical properties of bromacil.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 261.1 amu[1] |
| Aqueous solubility | 807 mg/L @ pH 5 and temperature 25 °C[1]<br>700 mg/L @ pH7 and temperature 25 °C[1]<br>1287 mg/L @ pH 9 and temperature 25 °C[1] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 1.88 @ pH 5[1]<br>1.88 @ pH 7 and temperature 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 1.51[2] |
| Logarithm of the bioconcentration factor (log BCF) | 0.45[2] |
| Half-life ($t_{1/2}$) in water | Stable except under strongly acidic conditions and elevated temperatures[1]<br>Stable @ pH 7 and temperature 20 °C[2] |
| Half-life ($t_{1/2}$) in soil | Average 60 days[2] |

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

Bromacil belongs to the uracil group of herbicides, which also includes benzfendizone and terbacil. Bromacil is extensively used in agricultural, industrial and urban situations to control a wide variety of annual and perennial weeds, grasses and brushes – selectively in citrus and pineapple plantations and non-selectively on non-crop areas such as roadsides, rights-of-way, railways and pavements (BCPC 2012; University of Hertfordshire 2013). However, it does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013).

Bromacil is generally absorbed through the roots of plants, with slight absorption through leaves and stems. Bromacil 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. Uracil 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).

Bromacil is a broad-spectrum, systemic herbicide that may ultimately end up in aquatic ecosystems as a result of spray drift and surface run-off (USEPA 1996). Bromacil has a moderate capacity to leach to groundwater due to its weak soil sorption ability as indicated by its low log $K_{oc}$ value and relatively high solubility in water (Table 1). Bromacil is relatively persistent in water (Table 1) being stable at a pH of 7 and a temperature of 20 ºC, only being hydrolysed by acids and elevated temperatures (BCPC 2012; University of Hertfordshire 2013).

## 1.2 Freshwater

### 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 bromacil in freshwaters (Table 3) 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 two fish, one cladoceran, two macrophytes and three microalgae. The toxicity values for the fish species were two 64-day NOEC (mortality) values of 29,000 and 29,100 µg/L, 64-day NOEC (wet weight, length) values of 500 µg/L, 64-day LOEC (wet weight, length, standard length) values ranging from 1,000 to 1,060 µg/L and 90-day NOEL and LOEC (mortality) values of 3,000 and 7,200 µg/L, respectively. The toxicity values for the single cladoceran consisted of 21-day NOEL and LOEC (body length, dry weight) values of 8,200 and 21,000 µg/L, respectively. The toxicity values for the two macrophytes were 13-day NOEC (fresh weight, new leaf production biomass, length increase of leaves) values ranging from 20 to 36 µg/L, 13-day LOAEC (fresh weight, new leaf production biomass, length increase of leaves) values ranging from 36 to 54 µg/L, 13-day EC50 (fresh weight, new leaf production biomass, length increase of leaves) values ranging from 32 to 43 µg/L and 14-day NOEL and EC50 (frond number, dry weight, frond area) values of 17 and 45 µg/L, respectively. The toxicity values for the microalgae species were 72-hour NOEC and EC50 (cell density) values of 45 and 97 µg/L, respectively, 5-day NOEL and EC50 (biomass yield, growth rate and AUC) values ranging from 1.1 to 11.2 µg/L and 6.8 to 69.9 µg/L, respectively.

*Freshwater Acute*

There were freshwater acute toxicity data for four fish, one crustacean, one cladoceran and one microalga. The toxicity values for the fish species were a 24-hour LC50 (mortality) value of 185,000 µg/L, 48-hour LC50 (mortality) values ranging from 71,000 to 183,000 µg/L, two 96-hour NOEL (mortality) values of 71,000 and 16,900, 96-hour EC50/LC50 (mortality, immobilisation) values ranging from 36,000 to 186,000 µg/L, 5-day NOEC (hatchability, mortality, abnormal development, number of hatched embryos) values ranging from 12,000 to 29,100 µg/L, 5-day LOEC (abnormal development) value of 29,100 µg/L and a 168-hour LC50 (mortality) value of 167,000 µg/L. The single toxicity value for the crustacean was a 24-hour LC50 (mortality) value of 71,160 µg/L. The toxicity values for the single cladoceran consisted of 48-hour NOEL and EC50 (body length, dry weight) values of 83,000 and 121,000 µg/L, respectively. The single toxicity value for the microalga was a 24-hour NOEC (cell density) value of 24 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

No factors have been reported as modifying the toxicity of bromacil. 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 bromacil (Table 1).

### 1.2.3 Guideline derivation

The derived PGVs for bromacil in freshwaters are provided in Table 2. 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 PGVs, the PGVs for bromacil are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for bromacil 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 bromacil do not need to account for secondary poisoning.

**Table 2 Proposed aquatic ecosystem protection guideline values (µg/L) for bromacil for the protection of freshwater ecosystems.**

| Bromacil proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 1.6 (0.41 – 10) |
| 95% | 3.6 (1.3 – 14) |
| 90% | 5.2 (1.9 – 15) |
| 80% | 7.7 (2.8 – 19) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 5 |
| Type of toxicity data | Chronic NOEC/NOEL values |
| SSD model fit | Poor |
| 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".**

### 1.2.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for bromacil 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 fish species (Warne 2001). This trigger value was calculated using the assessment factor (AF) method, dividing the lowest acute toxicity value of 182,000 µg/L by an assessment factor of 1000 (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 bromacil 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. More data on bromacil toxicity are now available, including data for phototrophic species (species that photosynthesise, e.g. plants and algae) but the reliability remains low, using the scheme of Warne et al. (2015). Further chronic toxicity testing of bromacil with additional phototrophic freshwater species would result in a larger database to enable the calculation of moderate to high reliability PGVs.

In total, there were toxicity data for 10 freshwater 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 bromacil, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The bromacil 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 1.3.6) 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 for five phototrophic species (that belonged to four phyla and four 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 a PGV (Warne et al. 2015). The number of species and taxa 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 bromacil in freshwater environments is provided in Table 3.

**Table 3 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for bromacil 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] | 11.2 | USEPA (2015b) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Total frond number, dry weight, frond area | 17 | USEPA (2015b) |
| Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 3.39 | USEPA (2015b) |
| Microalga | *Scenedesmus subspicatus** | Chlorophyta | Chlorophyceae | Exponential growth phase | 3 | Chronic NOEC | Cell density | 45 | Call et al. (1987) |
| Macrophyte | *Vallisneria americana* | Tracheophyta | Liliopsida | Not stated | 13 | Chronic NOEC | Fresh weight | 20 | Wilson and Wilson (2010) |

**[1] Chronic NOEC/NOEL = no conversions applied (Warne et al. 2015). [2] AUC = area under the growth curve. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 1.2.5 Species sensitivity distribution

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

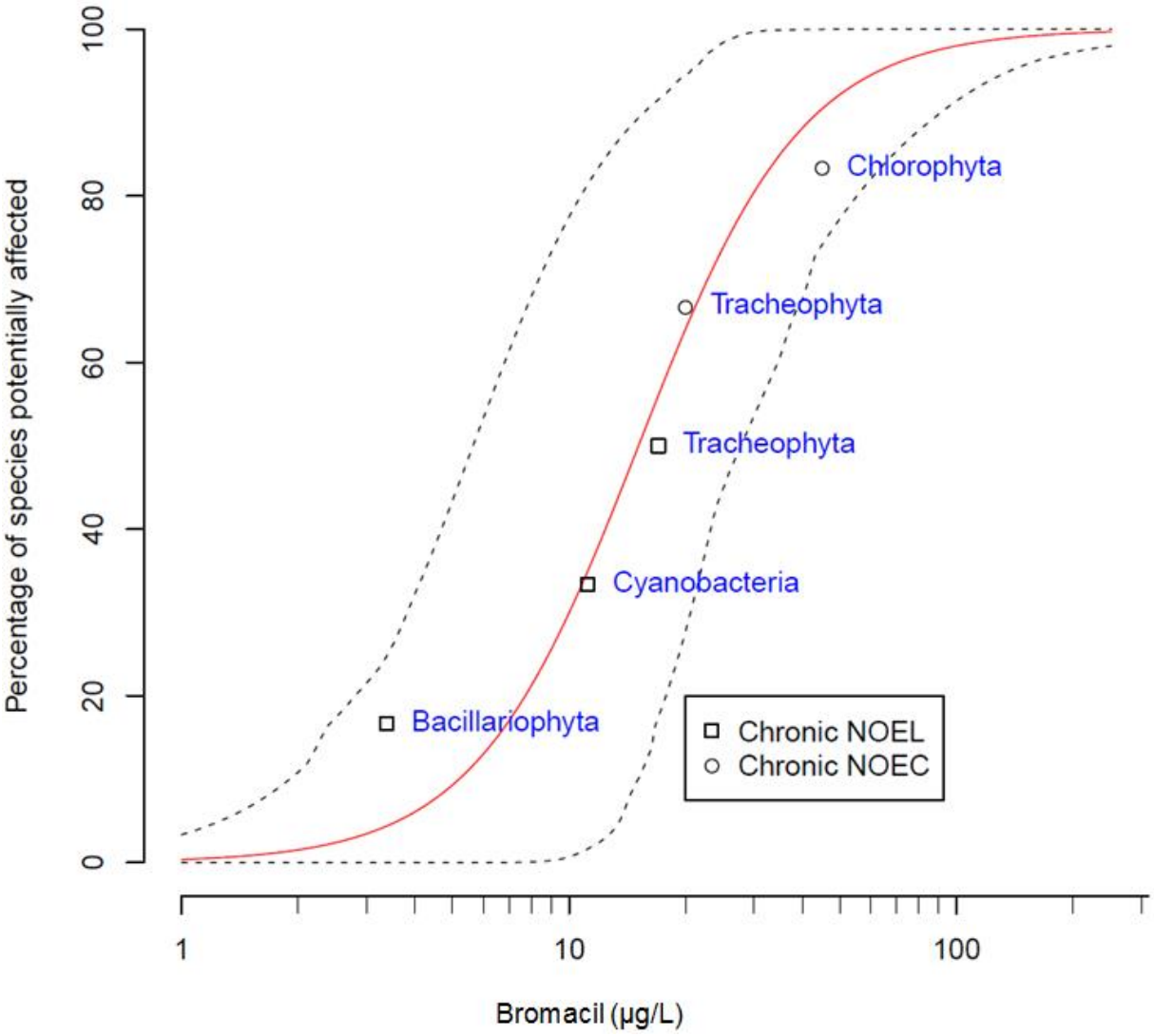



Figure 2 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic species to bromacil. Black dashed lines indicate the 95% confidence intervals.

## 1.3 Marine

### 1.3.1 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 bromacil in marine waters (Table 5) includes toxicity data for four species (two 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 1.2.1, respectively.

*Marine Chronic*

There were marine chronic toxicity data for one macrophyte and one microalga. The single toxicity value for the macrophyte was a 24-hour EC50 (germination inhibition) value of 6,880 µg/L. The toxicity values for the single microalga species were 5-day NOEL and EC50 (biomass yield, growth rate, AUC) values of 5.5 and 12.1 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for one fish, two crustaceans and one mollusc. The toxicity values for the fish were 96-hour NOEL and LC50 (mortality) values of 55,100 and 162,800 µg/L, respectively. The toxicity values for the crustaceans were a 48-hour LC50 (mortality) value of 1,000 µg/L and 48-hour NOEL and LC50 (mortality) values of 67,000 and 112,900 µg/L. The single toxicity value for the mollusc was a 48-day EC50 (mortality, abnormal development) value of 130,000 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.

### 1.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of bromacil. 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 bromacil (Table 1).

### 1.3.3 Guideline derivation

The derived PGVs for bromacil in marine waters are provided in Table 4. 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 PGVs, the PGVs for bromacil are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for bromacil 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 bromacil do not need to account for secondary poisoning.

**Table 4 Proposed aquatic ecosystem protection guideline values (µg/L) for bromacil for the protection of marine ecosystems.**

| Bromacil proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.23 (0.030 – 4.2) |
| 95% | 1.1 (0.36 – 7.1) |
| 90% | 2.2 (0.98 – 10) |
| 80% | 4.8 (2.5 – 15) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 7 |
| Type of toxicity data | Chronic NOEC/NOEL and chronic estimated NOEC 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 roposed aquatic ecosystem protection guideline value "reliability".**

### 1.3.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for bromacil 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 one 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 182,000 µg/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 bromacil 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 bromacil 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. 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 bromacil with marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

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

Based on the current understanding of the mode of action of bromacil, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The bromacil 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 1.3.6) 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 level (NOEL) ($n$ = 1) 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) ($n$ = 1) data for only two phototrophic 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). As no other ecotoxicity data for bromacil to marine phototrophic species was available, the chronic NOEL and chronic estimated NOEC values for marine phototrophic species (see section 1.2) to derive PGVs for bromacil in marine waters. This dataset included concentration data for seven phototrophic marine and freshwater species belonging 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 used to derive the PGVs (Table 4) combined with the good fit of the distribution to these toxicity data (Figure 3) 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 bromacil in marine environments is provided in Table 5.

**Table 5 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for bromacil 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 NOEL | Biomass yield, growth rate, AUC[2] | 11.2 | USEPA (2015b) |
| Marine | Macrophyte | *Hormosira banksii** | Ochrophyta | Phaeophyceae | Gamete | 2 | Chronic est. NOEC | Germination inhibition | 1,376 | Seery et al. (2006) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 17 | USEPA (2015b) |
| Fresh | Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 3.39 | USEPA (2015b) |
| Fresh | Microalga | *Scenedesmus subspicatus** | Chlorophyta | Chlorophyceae | Exponential growth phase | 3 | Chronic NOEC | Cell density | 45 | Schafer et al. (1994) |
| Marine | Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 5.5 | USEPA (2015b) |
| Fresh | Macrophyte | *Vallisneria americana* | Tracheophyta | Liliopsida | Not stated | 13 | Chronic NOEC | Fresh weight | 20 | Wilson and Wilson (2010) |

**[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] AUC = area under the growth curve. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 1.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the seven phototrophic marine and freshwater species that was used to derive the PGVs is presented in Figure 3.

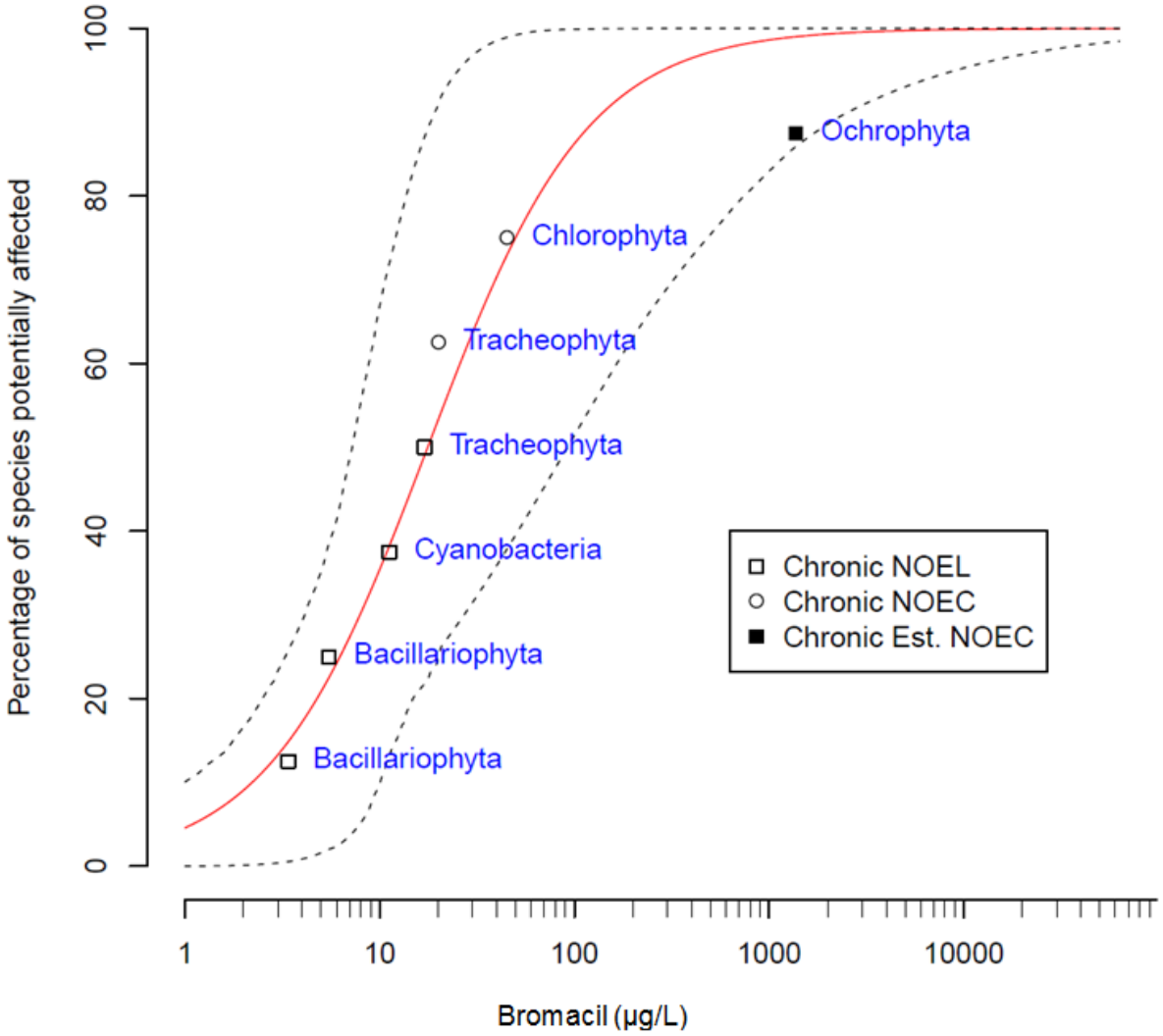



Figure 3 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC), no observed effect level (NOEL) data values of marine and freshwater phototrophic species to bromacil. Black dashed lines indicate the 95% confidence intervals.

### 1.3.6 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 [-0.691 and 5.03 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 bromacil.

The toxicity data for bromacil 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 bromacil ecotoxicity data may be bimodal (Figure 4).

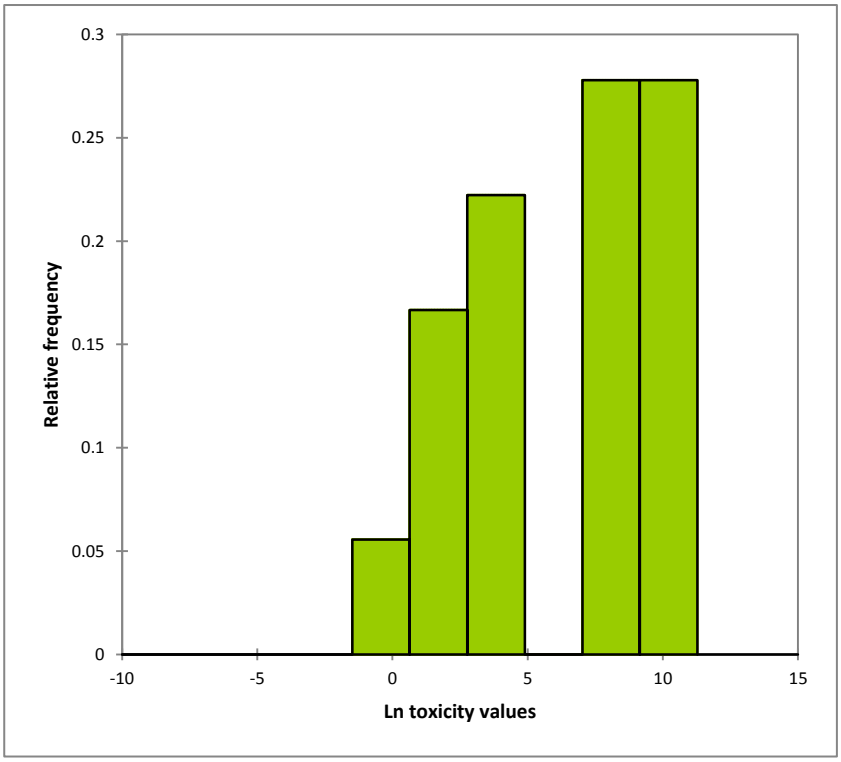



Figure 4 Histogram of the natural logarithm (ln) of all bromacil (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 18).

The bromacil 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 bromacil concentration data had equal variances (Fisher's F-Test; p = 0.362) and followed a normal distribution (Anderson-Darling; p = 0.376). Results from the two-sample *t* test indicated that the two groups were significantly different (p <0.0001); therefore, it was concluded that the distribution of the bromacil concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

### 1.3.7 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.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 125–127.

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/.

Call, DJ, Brooke LT, Kent RJ, Knuth ML, Poirier SH, Huot JM, and Lima AR (1987). Bromacil and diuron herbicides: toxicity, uptake, and elimination in freshwater fish. *Archives of Environmental Contamination and Toxicology* 16, 607–613.

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.

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.

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.

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

Seery CR, Gunthorpe L and Ralph PJ (2006). Herbicide impact on *Hormosira banksii* gametes measured by fluorescence and germination bioassays. *Environmental Pollution*, 140, 43–51.

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 (1996). R.E.D. Facts: Bromacil. United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances (7508W). Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/reregistration/fs_PC-012301_1-Aug-96.pdf , Accessed: March, 2017.

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 (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 (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.

# 2 Chlorothalonil

## 2.1 Introduction

Chlorothalonil is a fungicide ($C_8Cl_4N_2$ and Figure 5) that at room temperature is in the form of colourless, odourless crystals with a slightly pungent odour. It is the active ingredient of a variety of commercial fungicide formulations.

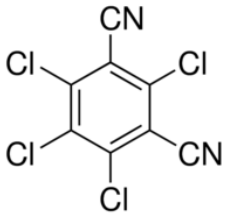



Figure 5 Structure of chlorothalonil.

Physicochemical properties of chlorothalonil that may affect its environmental fate and toxicity are presented in Table 6.

**Table 6 Summary of selected physicochemical properties of chlorothalonil.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 265.9 amu[1] |
| Aqueous solubility | 0.81 mg/L @ temperature 20–25 °C[1,2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 2.92 @ temperature 25 °C[1]<br>2.94 @ pH 7 and temperature 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 2.93–3.85[1,2] |
| Logarithm of the bioconcentration factor (log BCF) | 2[2] |
| Half-life ($t_{1/2}$) in water | Thermally stable at ambient temperatures[1]<br>Stable @ pH 5–7 and ambient temperatures[2]<br>16–38 days @ pH 9 and temperature 20–22 °C[2] |
| Half-life ($t_{1/2}$) in soil | 0.3–21 days @ temperature 20–24 °C [1]<br>22 days<br>(9.2–44 days in the lab (20 °C) and in field, respectively)[2] |

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

Chlorothalonil belongs to the aromatic group of fungicides, which also includes biphenyl, chloroneb and hexachlorobenzene. Chlorothalonil is extensively used in agricultural situations for the control of many fungal diseases in a variety of cereals, fruits and vegetables (e.g. wheat, pome fruit, stone fruit, citrus, bush and cane fruit, cranberries, strawberries, maize, potatoes) and other crops (e.g. soya beans, peanuts, almonds, tobacco, oil palms, rubber, coffee, tea) (BCPC 2012; University of Hertfordshire 2013). Non-agricultural uses include the application of chlorothalonil to ornamentals, turfs and remedial wood preservatives (i.e. protection of dry paint films/latex paints/other coatings from mildew and the protection of wood from mould, sap stain and decay) (BCPC 2012).

Chlorothalonil is a broad-spectrum, non-systemic, foliar fungicide (BCPC 2012). Following application, chlorothalonil is readily absorbed by plant tissues (rather than being translocated systemically), providing protective action when applied to leaves (BCPC 2012). Chlorothalonil exerts

its toxicity by binding to and depleting glutathione, a nonenzymatic antioxidant present in animals, plants, fungi and some bacteria. In fungi, glutathione activates the glyceraldehyde-3-phosphate dehydrogenase enzyme which allows fungal cells to obtain energy to infect plants (Syngenta Group 2003; Cox 1997). Therefore, when chlorothalonil binds to glutathione, the activation of glyceraldehyde-3-phosphate dehydrogenase is prevented, which disrupts glycosis and energy production in fungal cells, in turn interfering with cell survival and health (BCPC 2012; Zhao et al. 2011).

Chlorothalonil may ultimately end up in aquatic environments as a result of spray drift, runoff and via slow release into waterways where it is used as an additive of antifouling paints and wood protectants (CCME 1999; Sakkas et al. 2002). Chlorothalonil has low solubility in water and high soil adsorption ability as indicated by its log $K_{oc}$ value (Table 6) (BCPC 2012; University of Hertfordshire 2013). As a result, chlorothalonil tends to remain bound to soil particles, meaning the potential to leach into groundwater is negligible (Wu et al. 2002). Chlorothalonil reportedly persists in water, being stable at pHs ranging from pH 5 to pH 7 (under ambient temperatures) and having a half-life ($t_{1/2}$) of up to 38 days in more alkaline environments (pH 9) and a temperature of between 20 and 22 ºC (University of Hertfordshire 2013).

## 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 chlorothalonil in freshwaters (Table 8) includes toxicity data for six 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, two molluscs, one macrophyte, one fungus and nine microalgae. The toxicity values for the single fish species were 168-day NOEL and LOEC (mortality) values of 3 and 6.5 µg/L, respectively. The toxicity values for the cladocerans were 7- to 8-day NOEC and LOEC (offspring production) values of 55 and 100 µg/L, respectively, two 7- to 8-day IC25 (offspring production) values of 51.3 and 66.4 µg/L and 21-day NOEL and LOEC (immobilization) values of 39 and 79 µg/L, respectively. The toxicity values for the molluscs were two 48-hour EC50 (embryonic development, ability to attach to host) values of 0.97 and 40 µg/L, respectively. The toxicity values for the single macrophyte species were 14-day NOEL and EC50 (growth rate, frond area, dry weight) values of 290 and 630 µg/L, respectively. The toxicity values for the single fungus species consisted of a 7- to 14-day LOEC (zoospore concentration) value of 0.018 µg/L and an 8-day LOEC (cell density, area under the curve) value of 0.00018 µg/L. The toxicity values for the microalgae consisted of a 48-hour EC50 (cell count) value of 260 µg/L, two 72-hour LOEC (cell density, cell count) values both of 1 µg/L, 72-hour EC50 (cell density, cell count) values ranging from 7 to 270 µg/L, 96-hour NOEC (cell count) values ranging from 0.2 to 50 µg/L, 96-hour LOEC (cell count) values ranging from 0.5 to 100 µg/L, 96-hour EC50 (cell count) values ranging from 2 to 385 µg/L, two 5-day NOEL (biomass yield, growth rate, area under the curve) values of 3.9 and 50 µg/L and

two 5-day EC50 (biomass yield, growth rate, area under the curve) values ranging from 14 and 190 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for eight fish, four amphibians, two crustaceans, one cladoceran and one mollusc. The toxicity values for the fish were 24-hour LC50 (mortality) values ranging from 23.7 to 126 µg/L, 48-hour LC50 (mortality) values ranging from 18.2 to 116 µg/L, 96-hour NOEL (mortality) values ranging from 0.37 to 250 µg/L and 96-hour LC50 (mortality) values ranging from 0.076 to 430 µg/L. The toxicity values for the amphibians consisted of two 72-hour LOEC (mortality) values both of 172 µg/L, 96-hour NOEC (mortality, tail to length ratio, snout to vent length) values ranging from 1.76 to 34.6 µg/L, 96-hour LOEC (mortality, tail to length ratio, snout to vent length) values ranging from 5.9 to 36.4 µg/L and 96-hour LC50 (mortality) values ranging from 8.2 to 42.4 µg/L. The toxicity values for the crustaceans were two 4-day LC50 (mortality) values of 12 and 16 µg/L and two 7-day LC50 (mortality) values of 3.6 and 10.9 µg/L. The toxicity values for the single cladoceran species were two 48-hour NOEL (immobilisation) values of 6.8 and 31.6 µg/L, two 48-hour LOEC (immobilisation) values of 0.014 and 14 µg/L, 48-hour EC50 (immobilization, mortality) values ranging from 0.028 to 75 µg/L. The toxicity values for the single mollusc species were a 24-hour EC50 (ability to attach to host) value of 90 µg/L and a 96-hour EC50 (survival) value of 280 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 chlorothalonil. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the relatively high $K_{oc}$ value of chlorothalonil. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 2.2.3 Guideline derivation

The derived PGVs for chlorothalonil in freshwaters 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 PGVs, the PGVs for chlorothalonil are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for chlorothalonil are low (Table 6) 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 chlorothalonil do not need to account for secondary poisoning.

**Table 7 Proposed aquatic ecosystem protection guideline values (µg/L) for chlorothalonil for the protection of freshwater ecosystems.**

| Chlorothalonil proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.24 (0.13 – 0.84) |
| 95% | 0.48 (0.28 – 1.5) |
| 90% | 0.74 (0.42 – 2.2) |
| 80% | 1.3 (0.69 – 3.9) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 12 |
| Type of toxicity data | Chronic NOEC/NOEL values |
| SSD model fit | Good |
| 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".**

### 2.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for chlorothalonil in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for chlorothalonil 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 chlorothalonil toxicity data available that enable the calculation of PGVs in freshwaters; however, toxicity data for the target species was available for only one species of fungus. Despite this, Maltby et al. (2009) states that there is no evidence to suggest that GVs derived using non-fungal species pose a risk to aquatic fungi. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of chlorothalonil with freshwater species (particularly fungi) be conducted.

In total, there were toxicity data for 28 freshwater species (seven phyla and ten classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata, Chytridiomycota, Cyanobacteria, Mollusca and Tracheophyta. The ten classes were Actinopterygii (which accounts for approximately 99% of fish), Amphibia (tetrapod vertebrates), Bivalvia (a class of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Chytridiomycetes (a class of fungi), Cyanophyceae (a class of cyanobacteria), 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 chlorothalonil, a multi-site inhibitor of various enzymes, particularly glyceraldehyde-3-phosphate dehydrogenase in fungi, it would be expected that heterotrophic species (particularly fungi) would be more sensitive than phototrophic species, as the glyceraldehyde-3-phosphate dehydrogenase enzyme is critical to glycosis and energy production in fungal cells. Notwithstanding the acknowledged lack of fungi toxicity data in the database, the chlorothalonil ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two sample *t* test to see if 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 ($p = 0.399$,

see section 2.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for chlorothalonil in freshwater.

There were freshwater chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for 12 species (that belonged 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 7) combined with the good fit of the distribution to these toxicity data (Figure 6) resulted in a high reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for chlorothalonil in freshwater environments is provided in Table 8.

**Table 8 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 chlorothalonil 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 | 4 | Chronic NOEC | Cell count | 1 | Ma et al. (2011) |
| Macroinvertebrate | *Ceriodaphnia dubia** | Arthropoda | Branchiopoda | Neonate | 7-8 | Chronic NOEC | Offspring production | 55 | Phyu et al. 2013 |
| Microalga | *Chlorella pyrenoidosa*[2]* | Chlorophyta | Trebouxiophyceae | Not stated | 4 | Chronic NOEC | Cell count | 0.63 | Ma et al. (2011) |
| Macroinvertebrate | *Daphnia magna* | Arthropoda | Branchiopoda | Life cycle | 21 | Chronic NOEL | Immobilisation | 39 | USEPA (2015b) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 290 | USEPA (2015b) |
| Cyanobacteria | *Microcystis aeruginosa** | Cyanobacteria | Cyanophyceae | Not stated | 4 | Chronic NOEC | Cell count | 50 | Ma et al. (2011) |
| Cyanobacteria | *Microcystis flos-aquae** | Cyanobacteria | Cyanophyceae | Not stated | 4 | Chronic NOEC | Cell count | 2 | Ma et al. (2011) |
| Microalga | *Navicula pelliculosa** | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[3] | 3.9 | USEPA (2015b) |
| Fish | *Pimephales promelas* | Chordata | Actinopterygii | Early life stage | 168 | Chronic NOEL | Mortality | 3 | USEPA (2015b) |
| Microalga | *Scenedesmus obliquus** | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEC | Cell count | 0.5 | Ma et al. (2011) |
| Microalga | *Scenedesmus quadricauda* | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEC | Cell count | 5 | Ma et al. (2011) |
| Microalga | *Selenastrum capricornutum*[4] | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEC | Cell count | 20 | Ma et al. (2011) |

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

### 2.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 12 freshwater, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 6.

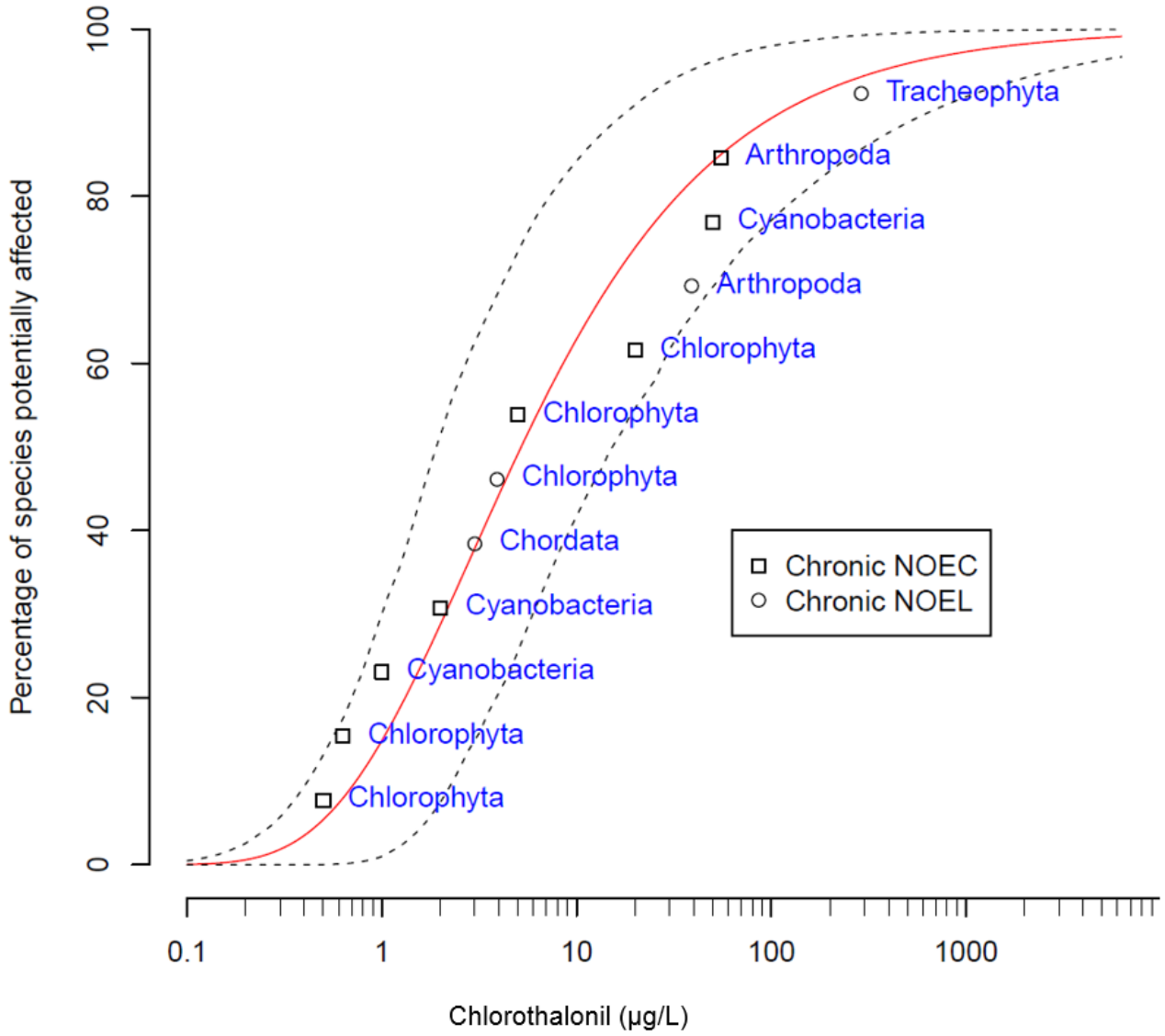



Figure 6 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016) of the sensitivity of sensitivity of chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to chlorothalonil. Black dashed lines indicate the 95% confidence intervals.

## 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 chlorothalonil in marine waters (Table 10) includes toxicity data for three 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 crustacean, one mollusc, one echinoderm, one ascidian, one microinvertebrate and five microalgae. The toxicity values for the single crustacean species were 28-day NOEL and LOEC (mortality) values of 0.83 and 1.2 µg/L, respectively. The toxicity values for the single mollusc species were 48-hour EC10 and EC50 (embryonic development) values of 4.5 and 8.8 µg/L, respectively. The toxicity values for the single echinoderm species were 48-hour NOEC and LOEC (embryonic development) values of 3.98 and 6.12 µg/L, respectively, a 48-hour EC10 (embryonic development) value of 4.3 µg/L and two 48-hour EC50 values both of 6.6 µg/L. The toxicity values for the single ascidian species were 48-hour EC10 and EC50 (embryonic development) values of 12 and 33 µg/L, respectively, and 48-hour EC10 and EC50 (larvae settlement success) values of 28.7 and 42 µg/L. The toxicity values for the single microinvertebrate species were 16-day NOEC and EC20 (mature to adult, sex ratio, first and second brood size) values all of 23.5 µg/L. The toxicity values for the microalgae consisted of 96-hour NOEC and LOEC (cell density) values of 33 and 100 µg/L, respectively, 96-hour EC50 (cell density) values ranging from 4.4 to 390 µg/L, a 7-day EC50 (cell density) value of 150 µg/L and 14-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 5.9 and 13 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for three fish, three crustaceans, one mollusc, one echinoderm, two microinvertebrates and one polychaete. The toxicity values for the fish species were a 48-day LC50 (mortality) value of 32 µg/L, a 96-hour NOEL (mortality) value of 20 µg/L and two 96-hour LC50 (mortality) values of 32 and 110 µg/L. The toxicity values for the crustaceans were 24-, 48- and 96-hour LC50 (mortality) values ranging from 67 to 734.9 µg/L, two 96-hour NOEC (mortality) values of 75 and 125 µg/L and 96-hour LOEC (mortality) values ranging from 31.3 to 250 µg/L. The single toxicity value for the mollusc species was a 96-hour EC50 (mortality, abnormal development) value of 26 µg/L. The toxicity values for the single echinoderm species were a 48-hour EC10 (length) value of 0.5 µg/L and 48-hour EC50, NOEC and LOEC (maximum dimension) values of 3.98, 6.12 and 7.76 µg/L, respectively. The toxicity values for the microinvertebrates were 24-hour LC10 and LC50 (mortality) values of 121.8 and 167.8 µg/L, respectively, a 96-hour LC10 (mortality) value of 69.5 µg/L, 96-hour LC50 (mortality) values ranging from 26.72 to 90.6 µg/L and a 16-day NOEC (survival) of 23.5 µg/L. The single toxicity value for the polychaete species was a 48-hour LC50 (mortality) value of 12 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.

### 2.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of chlorothalonil. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the relatively high $K_{oc}$ value of chlorothalonil. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 2.3.3 Guideline derivation

The derived PGVs for chlorothalonil in marine waters are provided in Table 9. 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 PGVs, the PGVs for chlorothalonil are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for chlorothalonil are low (Table 6) 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 chlorothalonil do not need to account for secondary poisoning.

**Table 9 Proposed aquatic ecosystem protection guideline values (µg/L) for chlorothalonil for the protection of marine ecosystems.**

| Chlorothalonil proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.34 (0.061 – 2.9) |
| 95% | 1.0 (0.26 – 5.2) |
| 90% | 1.7 (0.51 – 7.0) |
| 80% | 2.9 (1.0 – 9.8) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 7 |
| Type of toxicity data | Chronic NOEC/NOEL/EC10 values |
| 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".**

### 2.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for chlorothalonil in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for chlorothalonil 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 chlorothalonil toxicity data available that enable the calculation of PGVs in marine waters; however, no toxicity data are available for the target species, fungi. Despite this, Maltby et al. (2009) states that there is no evidence to suggest that the PGVs derived using non-fungal species pose a risk to aquatic fungi. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of chlorothalonil with marine species (particularly fungi) be conducted.

In total, there were toxicity data for 19 marine species (eight phyla and ten classes) that passed the screening and quality assessment processes. The represented phyla were Annelida, Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria, Echinodermata and Mollusca. The ten classes were Actinopterygii (which accounts for approximately 99% of fish), Ascidiacea (invertebrate filter feeders), Bivalvia (a grouping of molluscs), Chlorophyceae (a major grouping of green algae), Cyanophyceae (a class of cyanobacteria), Echinoidea (a class of urchins), Malacostraca (a large grouping of crustaceans), Maxillopoda (another large grouping of crustaceans), Mediophyceae (a grouping of marine diatoms) and Polychaeta (a class of annelid worms).

Based on the current understanding of the mode of action of chlorothalonil, a multi-site inhibitor of various enzymes, particularly glyceraldehyde-3-phosphate dehydrogenase in fungi, it would be expected that heterotrophic species (particularly fungi) would be more sensitive than phototrophic species, as the glyceraldehyde-3-phosphate dehydrogenase enzyme is critical to glycosis and energy production in fungal cells. Notwithstanding the acknowledged absence of fungi toxicity data in the database, the chlorothalonil ecotoxicity data for phototrophs and heterotrophs were tested using the parametric two sample *t* test to see if 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 ($p = 0.399$, see section 2.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for chlorothalonil in marine water.

There were marine chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for seven species (that belonged to six 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 9) combined with the good fit of the distribution to these toxicity data (Figure 7) resulted in a moderate reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for chlorothalonil in marine environments is provided in Table 10.

**Table 10 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 chlorothalonil 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 | *Americamysis bahia* | Arthropoda | Malacostraca | Life cycle | 28 | Chronic NOEL | Mortality | 0.83 | USEPA (2015b) |
| Microinvertebrate | *Amphiascus tenuiremis* | Arthropoda | Maxillopoda | Stage 1 juvenile copepodite | 16 | Chronic NOEC | Mature to adult, sex ratio, 1st and 2nd brood size | 23.5 | Bejarano et al. 2005 |
| Macroinvertebrate | *Ciona intestinalis** | Chordata | Ascidiacea | Embryo / Larvae | 2 | Chronic EC10 | Embryonic development | 12 | Bellas (2006) |
| Microalga | *Dunaliella tertiolecta* | Chlorophyta | Chlorophyceae | Logarithmic growth phase | 4 | Chronic NOEC | Cell density | 33 | DeLorenzo and Serrano (2003) |
| Macroinvertebrate | *Mytilus edulis** | Mollusca | Bivalvia | Embryo | 2 | Chronic EC10 | Embryonic development | 4.5 | Bellas (2006) |
| Macroinvertebrate | *Paracentrotus lividus* | Echinodermata | Echinoidea | Embryo | 2 | Chronic NOEC/ EC10 | Embryonic development | 4.14 | Bellas (2006); Bellas (2008) |
| Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 14 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 5.9 | USEPA (2015b) |

**[1] Chronic NOEC/NOEL/EC10 = no conversions applied (Warne et al. 2015). [2] AUC = area under the growth curve. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 2.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the seven marine, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 7.

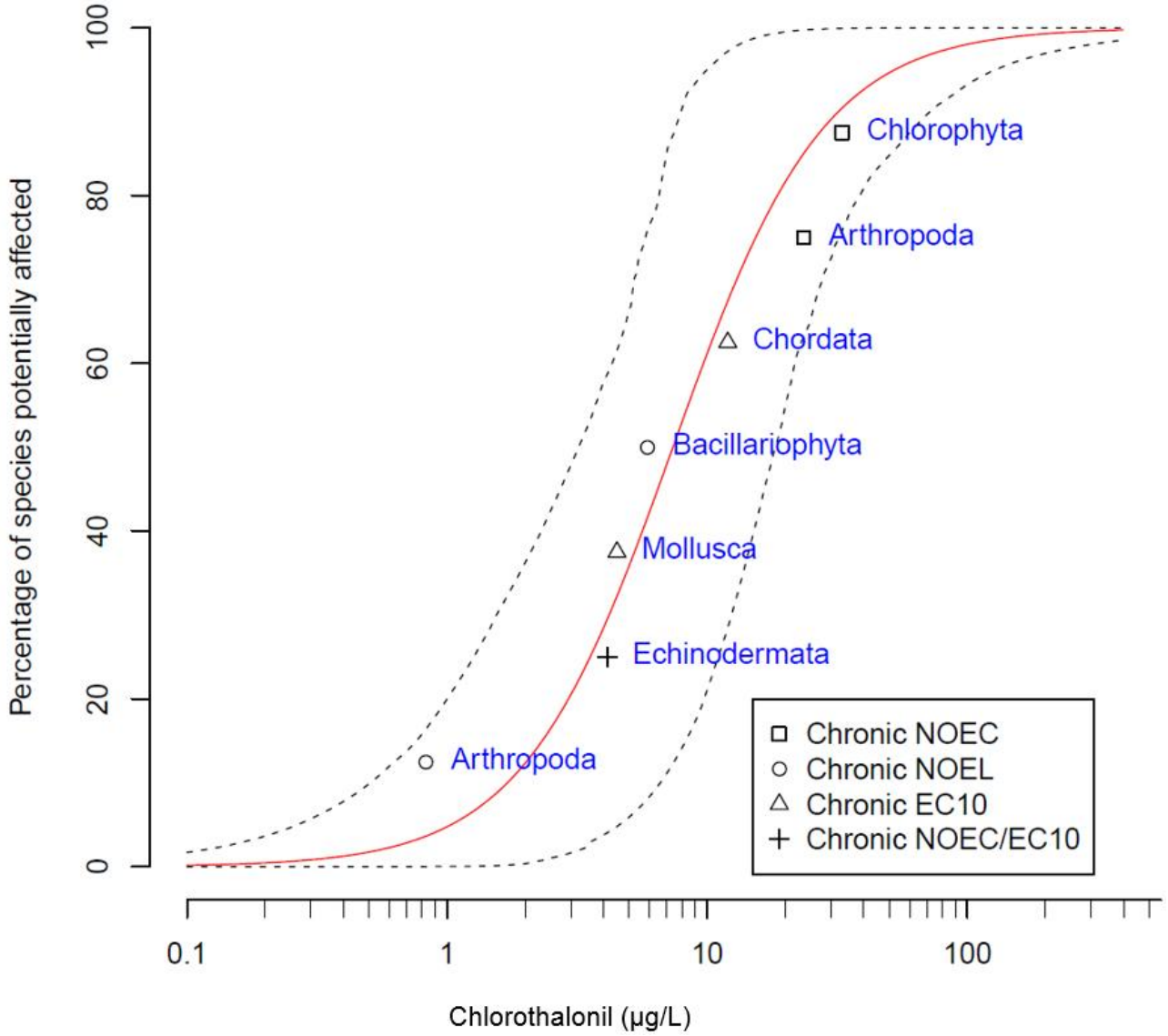



Figure 7 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016,) of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of marine phototrophic and heterotrophic species to chlorothalonil. Black dashed lines indicate the 95% confidence intervals.

### 2.3.6 Distribution of sensitivities for aquatic species

Statistical analysis of the chlorothalonil 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 chlorothalonil freshwater and marine concentration data failed tests for normality (Anderson-Darling; p = 0.008) and had unequal variances (Fisher's F-Test; p = 0.004). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.067); therefore, the freshwater and the marine chlorothalonil ecotoxicity data can be pooled for further analysis.

The toxicity data for chlorothalonil 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 chlorothalonil ecotoxicity data may be unimodal (Figure 8).

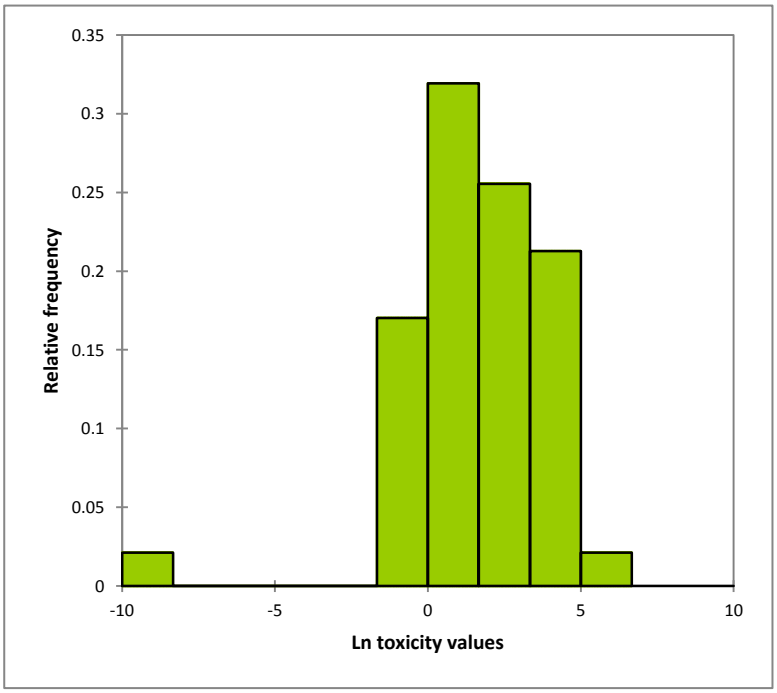



Figure 8 Histogram of the natural logarithm (ln) of all chlorothalonil (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 47).

The chlorothalonil 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 chlorothalonil concentration data had equal variances (Fisher's F-test; p = 0.456) but did not follow a normal distribution (Anderson-Darling; p = 0.003). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.399); therefore, it was concluded that the distribution of the chlorothalonil concentration data is unimodal.

### 2.3.7 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.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 194–196.

Bejarano AC, Chandler GT and Decho AW (2005). Influence of natural Dissolved Organic Matter (DOM) on acute and chronic toxicity of the pesticides chlorothalonil, chlorpyrifos and fipronil on the meiobenthic estuarine copepod *Amphiascus tenuiremis*, *Journal of Experimental Marine Biology and Ecology*, 321, 43–57.

Bellas J (2008) Prediction and assessment of mixture toxicity of compounds in antifouling paints using the sea-urchin embryo-larval bioassay, *Aquatic Toxicology*, 88, 308–315.

Bellas J(2006). Comparative toxicity of alternative antifouling biocides on embryos and larvae of marine invertebrates, *Science of the Total Environment*, 367, 573–585.

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 – Chlorothalonil, (Canadian Council of Ministers of the Environment). Available from: http://cegg-rcge.ccme.ca/download/en/163. Downloaded: 26 April, 2017.

Cox C (1997). Fungicide fact sheet: Chlorothalonil, *Journal of Pesticide Reform – Winter 1997*, 17, 14–20.

DeLorenzo ME and L. Serrano (2003) Individual and mixture toxicity of three pesticides; atrazine, chlorpyrifos and chlorothalonil to the marine phytoplankton species *Dunaliella tertiolecta*, *Journal of Environmental Science and Health, Part B*, 38, 529–538.

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.

Ma J, Tong S, Wang P and Chen J (2011). Differential toxicity of agricultural fungicides toward three cyanobacterial and five green algal species, Asian Journal of Chemistry, 23, 533–536.

Maltby L, Brock TCM and van der Brink PJ (2009). Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action and exposure regime, *Environmental Science and Technology*, 43, 7556–7563.

Phyu YL, Palmer CG, Warne M St.J, Dowse R, Mueller S, Chapman J, Hose GC and Lim RP (2013). Assessing the chronic toxicity of atrazine, permethrin and chlorothalonil to the cladoceran *Ceriodaphnia* cf. *dubia* in laboratory and natural river water, Archives of Environmental Contamination and Toxicology, 64, 419–426.

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.

Sakkas VA, Lambropoulou DA, Albanis TA (2002). Study of chlorothalonil photodegradation in natural waters and in the presence of humic substances, *Chemosphere*, 48, 939–945.

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.

Syngenta Group (2003). Chlorothalonil: The active ingredient in Daconil®, Syngenta Crop Protection, Syngenta Australia, New South Wales, Australia. Available from: http://www.syngentacropprotection.com/env_stewardship/futuretopics/daconil%20july17.pdf. Accessed: 27 April 2017.

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.

Wu L, Liu G, Yates MV, Green RL, Pacheco P, Gan J and Yates SR (2002). Environmental fate of metalaxyle and chlorothalonil applied to bentgrass putting green under southern California climatic conditions, Pesticide Management Science, 58, 335–342.

Zhao HM, Ruan HH and LI HT (2011). Progress in the research of GSH in cells, *Chinese Science Bulletin*, 56, 3057–3063.

# 3 Fipronil

## 3.1 Introduction

Fipronil is an insecticide ($C_{12}H_4Cl_2F_6N_4OS$ and Figure 9) that at room temperature is in the form of a white solid. It is the active ingredient of a variety of commercial insecticide formulations as well as some commercial herbicide formulations.

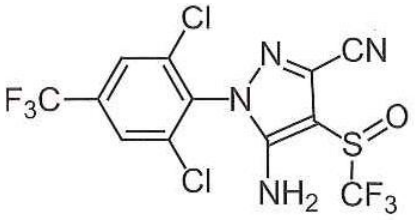



Figure 9 Structure of fipronil.

Physicochemical properties of fipronil that may affect its environmental fate and toxicity are presented in Table 11.

**Table 11 Summary of selected physicochemical properties of fipronil.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 437.2 amu[1] |
| Aqueous solubility | 1.9 mg/L @ pH 5 and temperature 20 °C/25 °C[1,3]<br>2.4 mg/L @ pH 9 and temperature 20 °C/25 °C[1,3]<br>3.78 mg/L @ temperature of 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 4.0 (shake flask method)[1]<br>3.75 @ pH 7 and temperature of 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 2.63 (Speyer 2.2) –3.09 (sandy loam)[1] |
| Logarithm of the bioconcentration factor (log BCF) | 2.51[2] |
| Half-life ($t_{1/2}$) in water | 54 days<br>Stable @ pH 5–7 and temperature 20 °C[2]<br>125 hours (5.2 days)[3] |
| Half-life ($t_{1/2}$) in soil | 68 days<br>(65 – 142 days in field and the lab (20 °C), respectively)[2]<br>438 hours (18.25 days)[3] |

**[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] Gunasekara et al. 2007.**

Fipronil belongs to the phenylpyrazole group within the pyrazole family of insecticides, which also includes acetoprole and flufiprole. Fipronil is extensively used on domestic pets for the rapid treatment of fleas and larvae, ticks and chewing lice as well as in selected agricultural applications for the control of a wide range of insect pests in a variety of crops such as cotton, potatoes, maize and rice (BCPC 2012; University of Hertfordshire 2013). It may ultimately end up in aquatic environments as a result of runoff, accumulation in arable soils and soil water and as a result of uptake by non-target plants via their roots or dust deposition on leaves (Bonmatin et al. 2015). Fipronil is one of the most heavily used insecticides worldwide, alongside neonicotinoids such as imidacloprid and clothianidin (Bonmatin et al. 2015).

Fipronil is a chiral molecule and occurs in two mirror-image forms known as the *R*- and *S*+ enantiomers (mirror image isomers). Fipronil is produced as a racemic mixture, i.e. it is produced

with equal amounts of the *R*- and *S*+ enantiomers. Following application as a racemic mixture, biological processes within the environment can alter the ratio of enantiomers resulting in the enrichment of one enantiomer whilst the other is transformed (Baird et al. 2013). Therefore, environmental concentrations are often comprised of mixtures of each enantiomer. Konwick et al. (2005) states that the *S*+ enantiomer is generally more toxic than the *R*- enantiomer or a 50:50 racemic mixture, however this trend is not distinctly recognisable in the present dataset due to the limited ecotoxicity data available for fipronil. Therefore, the PGVs for fipronil were derived using toxicity data for both enantiomers as well as the racemic mixture and are expressed in terms of the concentration of the active ingredient.

Fipronil is absorbed through the leaves of plants following foliar application. It is then translocated acropetally (i.e. movement upwards from the base of plants to the apex) in the xylem and accumulates in the plant tissues (Bonmatin et al. 2015). Fipronil exerts specific toxicity by binding to the γ-aminobutyric acid (GABA) receptors and glutamate-gated chloride channels in nerve cells, having a stronger affinity for receptors in insects and other arthropods than for receptors invertebrates (Baird et al. 2013, Konwick et al. 2005, Simon-Delso et al. 2015). Blocking these inhibiting receptors results in neuronal hyperexcitation, which paralyses and kills the organism (Simon-Delso et al. 2015). Specificity to invertebrates occurs predominantly because glutamate receptors are insect specific and do not occur in vertebrates (Simon-Delso et al. 2015). Its systemic properties make it most effective at controlling insects and arthropods with piercing/sucking mouthparts such as stem borers, leaf miners, plant hoppers, and weevils (BCPC 2012). Fipronil is also used to control rootworms, wireworms, termites and thrips following application to soils and seeds (BCPC 2012) and widely used in Australia for locust control (APVMA 2012).

Fipronil is a broad spectrum insecticide with systemic properties that has low to moderate solubility in water and high soil adsorption characteristics as indicated by its log $K_{oc}$ value (Table 11) (BCPC 2012; University of Hertfordshire 2013). It has a low potential for volatisation with variable persistence in soils, waterways and non-target plants (Table 11).

## 3.2 Marine

### 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 fipronil in marine waters (Table 13) includes toxicity data for six freshwater species that either originated from or are distributed within Australia and/or New Zealand. The dataset used in the guideline derivation process did not include any toxicity data for fipronil to Australian and/or New Zealand marine species. 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.

*Marine Chronic*

There were marine chronic toxicity data for three crustaceans, one molluscs, one fish and one microalga species. The toxicity values for crustaceans were 12-day NOEL and NOEC (mature to adult, fecundity) values of 0.16 and 0.5 µg/L, respectively, 12-day LOEL and LOEC (mature to adult, fecundity) values of 0.25 and 0.22 µg/L, respectively, 17-day NOEL and LOEL (egg production; egg extrusion time) values ranging from 0.16 to 0.22 µg/L, a 21-day NOEL value (mortality) of 0.42 µg/L, 28-day NOEC and LC50 (mortality) values of 150 and 357 µg/L, respectively, and 28-day LOEC (mortality) values ranging from 0.005 to 355 µg/L. 32-day NOEC and LOEC (survival) values of 0.25 to 0.5 µg/L, respectively, 45-day NOEC and LOEC (survival; body weight; body length) values ranging from 0.0979 to 0.143 µg/L. The toxicity values for the single mollusc species were 28-day NOEC and LOEC (shell length) values of 0.355 and 5 µg/L, respectively. The toxicity data for the single fish species consisted of 32-day NOEL and LOEC (mortality) values ranging from 0.24 to 1.6 µg/L and a 110-day LOEC (mortality) value of 0.85 µg/L. The toxicity data for the single microalga species were 96-hour NOEC, LOEC and EC50 (cellular bio-volume) values of 250, 500 and 631.2 µg/L.

*Marine Acute*

There were marine acute toxicity data for three crustaceans, one mollusc and one fish species. The toxicity values for crustaceans were 96-hour NOEC/NOEL, LOEC/LOEL and LC50 (mortality) values ranging from 0.031 to 32 µg/L, 96-hour EC50/LC50 (mortality; abnormal development) values ranging between 177 to 770 µg/L and 7-day NOEC and LOEC (survival) values of 0.15 and 0.355 µg/L, respectively. The toxicity values for the single mollusc species were 96-hour LC50 (mortality) values ranging from 117 to 208 µg/L. The toxicity values for fish were 96-hour LOEL and LC50 (mortality) values of 110 and 130 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.

*Freshwater Chronic*

There were freshwater chronic toxicity data for two cladocerans, four fish and five species of microalgae. The toxicity values for the cladocerans were 8-day NOEC, LOEC and LC50 values for a variety of endpoints (mortality, number of broods per female, time taken to release brood, brood size; fecundity) that ranged from 2 to 270 µg/L and 21-day NOEL and LOEC (immobilisation) values of 9.6 to 41 µg/L and 19.5 to 100 µg/L, respectively. The toxicity data for fish consisted of 7-day LC50 (mortality) values of 208 to 365 µg/L, 28-day NOEC and LOEC (mortality) values of 10 and 30 µg/L, respectively, three 60-day LOEC (survival; weight gain; average weight) values all of 42.8 µg/L and 90-day NOEL and LOEC (mortality) values of 6.6 and 15 µg/L, respectively. The toxicity data for microalgae were 72-hour EC50 (cell count) values ranging between 290 to 1,500 µg/L and 5-day NOEL and EC50 (biomass yield, growth rate and area under the curve) values ranging from 7.5 to 170 µg/L and 76 to 140 µg/L, respectively.

*Freshwater Acute*

There were freshwater acute toxicity data for five cladocerans, six crustaceans, 28 insects, seven fish, one amphibian and one macrophyte. The toxicity values for the cladocerans were a 24-hour LC50 (mortality) value of 33.3 µg/L, two 48-hour NOEL (immobilisation) values of 22 and 52 µg/L, two 48-hour (immobilisation) values of 19 and 34 µg/L, 48-hour EC50/LC50 (mortality, immobilisation) values ranging from 3.45 to 190 µg/L and a 96-hour LC50 (mortality) value of 143.4 µg/L. The toxicity values for the crustaceans consisted of a 48-hour LC50 (mortality) value of 437.2 µg/L, a 96-hour NOEC (mortality) value of 0.25 µg/L, 48-hour LOEC (mortality) values ranging from 0.13 to 32 µg/L and 96-hour LC50 (mortality) values ranging from 0.32 to 163.5 µg/L. The toxicity data for insects consisted of 24-hour LC50 (mortality) values ranging from 0.35 to 100 µg/L, a single 48-hour LOEC (mortality) value of 2.19 µg/L, 48-hour LC50 (mortality) values ranging from 0.105 to 646.3 µg/L, a 96-hour NOEL (mortality) value of 0.14 µg/L and 96 hour LC50 values ranging from 0.113 to 2.11 µg/L. The toxicity data for fish were 24-hour LC10, NOEC and LOEC (mortality) values of 305.6, 300 and 350 µg/L, respectively, 24-hour LC50 values of 220.4 and 398.29 µg/L, 96-hour NOEL (mortality) values ranging from 6.7 to 89 µg/L, 96-hour LC50 (mortality) values ranging from 20 to 448.5 µg/L, 5-day NOEC and LOEC (mortality) values of 1,000 and 5,000 µg/L and a single 5-day NOEC (body length) value of 161.75 µg/L. The toxicity data for the one amphibian species consisted of LC50 (mortality) values ranging from 850 to 1,140 µg/L. The single value for a macrophyte was a 5-day NOEL (growth rate, frond area, dry weight) of 100 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.

### 3.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of fipronil. 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 fipronil (Table 11).

### 3.2.3 Guideline derivation

The derived PGVs for fipronil 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 PGVs, the PGVs for fipronil are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for fipronil are low (Table 11) 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 fipronil do not need to account for secondary poisoning.

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

| Fipronil proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.0034 ($3.3 \times 10^{-8}$ – 0.012) |
| 95% | 0.0089 ($3.0 \times 10^{-5}$ – 0.025) |
| 90% | 0.016 (0.00058 – 0.040) |
| 80% | 0.033 (0.011 – 0.078) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 28 |
| Type of toxicity data | Chronic NOEC/NOEL, chronic estimated NOEC 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".**

### 3.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for fipronil in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for fipronil 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 fipronil 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. 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 fipronil 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 is 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 fipronil in marine waters, *Hexagenia* sp. and *Hydropsyche* sp. were included as no other toxicity data for these genera were used.

In total, there were toxicity data for eight marine species (four phyla and five classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata and Mollusca. The five classes were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Chlorophyceae (a major grouping of green algae), Malacostraca (a large grouping of crustaceans) and Maxillopoda (another large grouping of crustaceans).

Based on the current understanding of the mode of action of fipronil, it would be expected that arthropods (insects and crustaceans) would be more sensitive than other organisms as it is a GABA-

and glutamate-gated chloride channel antagonist, and glutamate receptors are insect specific. The fipronil 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 3.2.6) 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, chronic estimated and converted acute data) meet both requirements (Warne et al. 2015).

There were marine chronic no observed effect concentration (NOEC), no observed effect level (NOEL), 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) and converted acute (acute EC50/LC50 values that were converted to chronic NOEC values by dividing by 10) data values available for eight species (three arthropods belonging to one phylum and five non-arthropods belonging to three phyla). Despite meeting 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, this marine dataset did not meet the requirements for the modified criterion that applies when using the most sensitive group of organisms (in this case, arthropods). As no other ecotoxicity data for fipronil to marine arthropod species were available, the three chronic and chronic estimated NOEC values for marine arthropod species were combined with the available chronic NOEC and converted acute values for freshwater arthropod species to derive PGVs for fipronil in marine waters.

There were chronic NOEC/NOEL, chronic estimated NOEC and converted acute data available for 28 marine and freshwater arthropod species belonging to one phylum (Arthropoda) and four classes (Branchiopoda, Insecta, Malacostraca and Maxillopoda) (Table 3). The entire marine and freshwater dataset for fipronil (that included chronic NOEC/NOEL, chronic estimated and converted acute converted acute data) consisted of 45 arthropod ($n = 28$) and non-arthropod ($n = 17$) species that belonged to five phyla and nine 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/NOEL, chronic estimated NOEC and converted acute data values for the 28 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 12) combined with the good fit of the distribution to these toxicity data (Figure 10) 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 13.

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

| Media | Taxonomic group | Species | Class | Life stage | Duration (days) | Type[1] | Toxicity endpoint | Toxicity value (µg/L) | Reference |
|---|---|---|---|---|---|---|---|---|---|
| Fresh | Micro | *Acanthocyclops robustus* | Maxillopoda | Not stated | 2 | Converted acute | Mortality | 8.49 | Chaton et al. (2002) |
| Fresh | Macro | *Aedes aegypti** | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.32 | Ali et al. (1998) |
| Fresh | Macro | *Aedes albopictus HAmAal strain** | Insecta | First and fourth instar larvae | 2 | Converted acute | Mortality | 1.36 | Ali et al. (1998) |
| Fresh | Macro | *Aedes taeniorhynchus* | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.043 | Ali et al. (1998) |
| Marine | Macro | *Americamysis bahia* | Malacostraca | <24 hour | 28 | Chronic est. NOEC | Mortality | 0.0034 | USEPA (2015b) |
| Marine | Micro | *Amphiascus tenuiremis* | Maxillopoda | Life cycle / Nauplii stage I | 12–17 | Chronic NOEL | Mature to adult, egg production | 0.16 | Chandler et al. (2004) |
| Fresh | Macro | *Anopheles quadrimaculatus* | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.043 | Ali et al. (1998) |
| Fresh | Macro | *Baetis tricaudatus* | Insecta | Not stated | 2 | Converted acute | Mortality | 0.011 | Weston and Lydy (2013) |
| Fresh | Macro | *Ceriodaphnia dubia** | Branchiopoda | Neonate (<24 hour) | 8 | Chronic NOEC | Fecundity, brood size | 10 | Wilson et al. (2008) |
| Fresh | Macro | *Chaoborus crystallinus* | Insecta | Larvae | 2 | Converted acute | Mortality | 64.63 | Chaton et al. (2002) |
| Fresh | Macro | *Chironomus annularius* | Insecta | Larvae | 2 | Converted acute | Mortality | 0.24 | Chaton et al. (2002) |
| Fresh | Macro | *Chironomus crassicaudatus* | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.042 | Ali et al. (1998) |
| Fresh | Macro | *Culex nigripalpus* | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.087 | Ali et al. (1998) |
| Fresh | Macro | *Culex quinqefasciatus** | Insecta | First and fourth instar | 2 | Converted acute | Mortality | 0.58 | Ali et al. (1998) |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | larvae | | | | | |
| Fresh | Macro | *Daphnia magna* | Branchiopoda | Life cycle | 21 | Chronic NOEL | Immobilisation | 19.84 | USEPA (2015b) |
| Fresh | Micro | *Diaptomus castor* | Branchiopoda | Not stated | 2 | Converted acute | Mortality | 0.35 | Chaton et al. (2002) |
| Fresh | Macro | *Diphetor hageni* | Insecta | Not stated | 2 | Converted acute | Mortality | 0.035 | Weston and Lydy (2013) |
| Fresh | Macro | *Glyptotendipes paripes* | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.042 | Ali et al. (1998) |
| Fresh | Macro | *Hexagenia sp.* | Insecta | Nymph | 4 | Converted acute | Immobilisation | 0.044 | USEPA (2015b) |
| Fresh | Macro | *Hyalella azteca* | Insecta | Not stated | 4 | Converted acute | Mortality | 0.17 | Weston and Lydy (2013) |
| Fresh | Macro | *Hydropsyche sp.* | Malacostraca | Not stated | 4 | Converted acute | Mortality | 0.21 | Weston and Lydy (2013) |
| Fresh | Macro | *Isoperla quinquepunctata* | Insecta | Not stated | 4 | Converted acute | Mortality | 0.011 | Weston and Lydy (2013) |
| Marine | Macro | *Palaemonetes pugio* | Malacostraca | Adult | 45 | Chronic NOEC | Survival, weight, length | 0.098 | Volz et al. 2003 |
| Fresh | Macro | *Polypedilum nubiferum** | Insecta | Fourth instar larvae | 2 | Converted acute | Mortality | 0.15 | Stevens et al. (2011) |
| Fresh | Macro | *Procambarus clarkii* | Malacostraca | Adult | 4 | Converted acute | Mortality | 6.98 | Schlenk et al. (2001); |
| Fresh | Macro | *Procambarus zonangulus* | Malacostraca | Not stated | 4 | Converted acute | Mortality | 1.95 | Overmyer et al. (2007) |
| Fresh | Macro | *Simocephalus elizabethae** | Branchiopoda | Neonate | 2 | Converted acute | Mortality | 1.25 | Stevens et al. (2011) |
| Fresh | Macro | *Simulium vittatum* | Insecta | Fifth instar larvae | 2 | Converted acute | Mortality | 0.04 | Overmyer et al. (2005); Overmyer et al. (2007) |

**[1] Macro = macroinvertebrate; Micro = Microinvertebrate. [2.] Chronic NOEC/NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC and LC50 values that were converted to chronic NOEC values by dividing by 2.5 and 5, respectively; Converted acute = acute EC50/LC50 values that were converted to chronic NOEC values by dividing by 10 (Warne et al. 2015). * Species that originated from/is distributed in Australia and/or New Zealand.**

### 3.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 28 marine and freshwater, arthropod species that was used to derive the PGVs is presented in Figure 10.

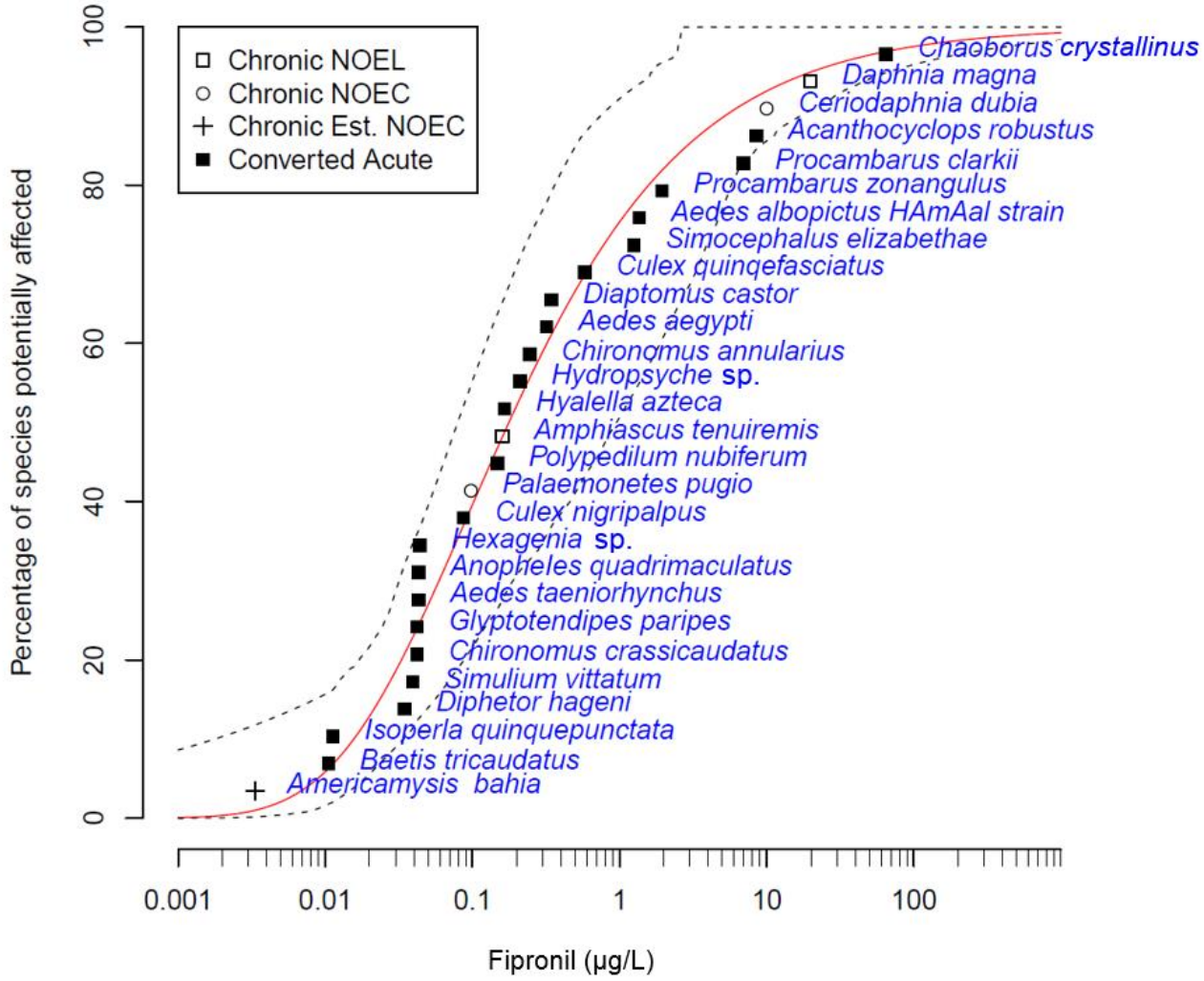



Figure 10 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016) of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC), no observed effect level (NOEL) and converted acute data values of marine and freshwater arthropod species to fipronil. Black dashed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

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

Statistical analysis of the fipronil 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 fipronil freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.959) and followed a normal distribution (Anderson-Darling; p = 0.120). Results from the two-sample *t* test test indicated that the two groups were not significantly different (p = 0.155); therefore, the freshwater and the marine fipronil ecotoxicity data can be pooled for further analysis.

The toxicity data for fipronil 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 the data to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the fipronil ecotoxicity data may be bimodal (Figure 11).

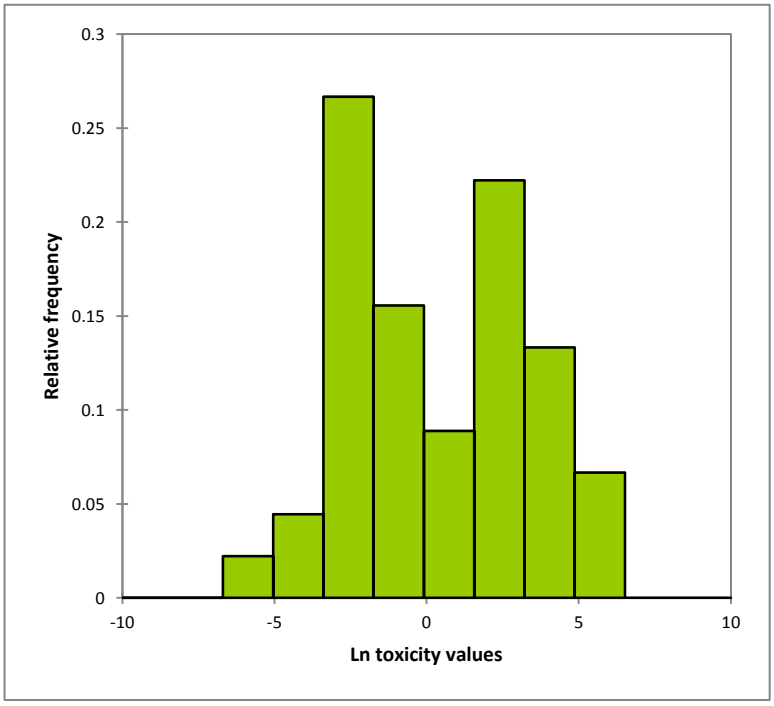



Figure 11 Histogram of the natural logarithm (ln) of all fipronil (freshwater and marine) toxicity data for arthropods and non-arthropods (*n* = 45).

The fipronil 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 fipronil concentration data had equal variances (Fisher's F-Test; p = 0.467) and followed a normal distribution (Anderson-Darling; p = 0.120). 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 fipronil concentration data is bi- or multi-modal, with arthropod species being the most sensitive group.

### 3.2.7 Rationale for the selected method for deriving the proposed aquatic ecosystem protection guideline values for fipronil in marine waters

The preference of ecotoxicity data used to derive the protective concentration (PC)[3] values and/or PGVs for fipronil to marine species is:

1. chronic NOEC/EC10 ecotoxicity data for arthropods;
2. chronic NOEC/EC10 and chronic estimated NOEC values for arthropods;
3. a combination of chronic, chronic estimated and converted acute ecotoxicity data for arthropods.

There were marine chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for six species (three arthropods belonging to one phylum and five non-arthropods belonging to three phyla) 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 PCs using the SSD method, nor the modified criterion that applies when using the most sensitive group of organisms to derive PCs. As no other ecotoxicity data for fipronil to marine species were available, the three chronic and chronic estimated NOEC values for marine arthropod species were combined with the available chronic NOEC/NOEL values for freshwater arthropod species to derive PCs for fipronil in marine waters.

There were chronic NOEC/NOEL and chronic estimated NOEC data available for five marine and freshwater arthropod species belonging to one phylum (Arthropoda) and three classes (Branchiopoda, Malacostraca and Maxillopoda). The entire marine and freshwater dataset for fipronil (that included chronic NOEC/NOEL and chronic estimated data) consisted of 17 arthropod ($n$ = 5) and non-arthropod ($n$ = 12) species that belonged to five phyla and seven classes, which successfully meets the modified criterion that applies when using the most sensitive group of organisms to derive PCs (i.e. at least five species belonging to at least four phyla). Therefore, as per Warne et al. (2015), it was acceptable to derive PCs using the chronic NOEC/NOEL and chronic estimated NOEC data values for the 17 marine and freshwater arthropod species despite belonging to only one phylum (Warne et al. 2015). The resulting SSD and PC values using only this data are presented in Figure 12 and Table 14, respectively.

[3] 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.

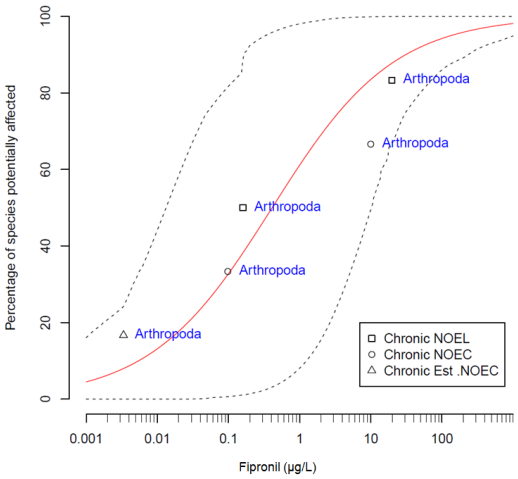



Figure 12 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016) of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of marine and freshwater arthropod species to fipronil.

Table 14 Protective concentration values (µg/L) of fipronil for the protection of marine ecosystems generated from the species sensitivity distribution in Figure 12.

| **Fipronil protective concentration values (marine)[1]** | | | **Reliability classification[2]** | |
|---|---|---|---|---|
| **Percent species protection** | **Concentration (µg/L)** | | **Criterion** | **Result** |
| 99% | 0.000049 | | Sample size | 5 |
| 95% | 0.0013 | | Type of toxicity data | Chronic NOEC/NOEL and chronic estimated NOEC values *(freshwater and marine)* |
| 90% | 0.0055 | | SSD model fit | Poor |
| 80% | 0.027 | | *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 used consisted of chronic NOEC/NOEL and chronic estimated NOEC values for five species and had a poor fit to the data (Figure 12). 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 NOEC/NOEL, chronic estimated NOEC (estimated from chronic LOEC and EC/LC50 data[4]) and converted acute data (estimated from acute EC50/LC50 data[5]), resulting in a total of 28 arthropod species from the one phylum (Table 13). 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 to *good* and calculated moderate reliability PC values (Table 13), according to Warne et al. (2015). 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 the chronic, chronic estimated and converted acute ecotoxicity (Table 12) data be adopted as the PGVs for fipronil in marine waters.

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

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

### 3.2.8 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

Ali A, Nayar JK and Gu WD (1998). Toxicity of a phenyl pyrazole insecticide, fipronil, to mosquito and chironomid midge larvae in the laboratory, *Journal of American Mosquito Control Association*, 14, 216–218.

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 (2012). Fipronil – Review Scope Document, Part 2: Environmental Considerations Summary. Australian Pesticides and Veterinary Medicines Authority, Kensington, Australian Capital Territory, Australia, 54pp.

Baird S, Garrison A, Jones J, Avants J, Bringolf R and Black M (2013). Enantioselective toxicity and bioaccumulation of fipronil in fathead minnows (*Pimephales promelas*) following water and sediment exposures, *Environmental Toxicology and Chemistry*, 32, 222–227.

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

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.

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/.

Chandler GT, Cary TL, Volz DC, Walse SS, Ferry JL and Klosterhaus SL (2004). Fipronil effects on estuarine copepod (*Amphiascus tenuiremis*) development, fertility and reproduction: A rapid life-cycle assay in 96-well microplate format, *Environmental Toxicology and Chemistry*, 23, 117–124.

Chaton PF, Ravanel P, Tissut M and Meyran JC (2002). Toxicity and bioaccumulation of fipronil in the nontarget arthropodan fauna associated with subalpine mosquito breeding sites, *Ecotoxicology and Environmental Safety*, 52, 8–12.

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.

Gunasekara AS, Truong T, Goh KS, Spurlock F and Tjeerdema RS (2007). Environmental fate and toxicology of fipronil, *Journal of Pesticide Science*, 32, 189–199.

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.

Konwick BJ, Fisk AT, Garrisson AW, Avants JK and Black MC (2005). Acute enantioselective toxicity of fipronil and its desulfinyl photoproduct to *Ceridaphnia dubia*, *Environmental Toxicology and Chemistry*, 24, 2350–2355.

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

Overmyer JP, Rouse DR, Avants JK, Garrison AW, DeLorenzo ME, Chung KW, Key PB, Wilson WA and Black MC (2007). Toxicity of fipronil and its enantiomers to marine and freshwater non-targets, *Journal of Environmental Science and Health: Part B, Pesticides, Food and Contaminants, and Agricultural Wastes*, 42, 471–480.

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.

Schlenk D, Huggett DB, Allgood J, Bennett E, Rimoldi J, Beeler AB, Block D, Holder AW, Hovinga R and Bed P (2001). Toxicity of fipronil and its degradation products to *Procambarus* sp.: field and laboratory studies, *Archives of Environmental Contamination and Toxicology*, 41, 325–332.

Simon-Delso N, Amaral-Rogers V, Belzunces LP, Bonmatin JM, Chagnon M, Downs C, Furlan L, Gibbons DW, Giorio C, Girolami V, Goulson D, Kreutzweiser DP, Krupke CH, Liess M, Long E, McField M, Mineau P, Mitchell EAD, Morrissey CA, Noome DA, Pisa L, Settele J, Stark JD, Tapparo A, Van Dyck H, Van Praagh J, Van der Sluijs JP, Whitehorn PR and Wiemers M (2015). Systemic insecticides (neonicotinoids and fipronil): trends, uses, mode of action and metabolites, Environmental Science and Pollution Research, 22, 5–34.

Stevens MM, Burdett AS, Mudford EM, Helliwell S and Doran G (2011) The acute toxicity of fipronil to two non-target invertebrates associated with mosquito breeding sites in Australia, *Acta Tropica*, 117, 125–130.

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.

Volz DC, Wirth EF, Fulton MH, Scott GI, Strozier E, Block DS, Ferry JL, Walse SS and Chandler GT (2003). Effects of fipronil and chlorpyrifos on endocrine-related endpoints in female grass shrimp (*Palaemonetes pugio*), *Bulletin of Environmental Contamination and Toxicology*, 71, 497–503.

Warne MStJ (2000). Description of how each toxicant trigger value was derived. Supplied as part of a 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.

Weston DP and Lydy MJ (2013). Toxicity of the insecticide fipronil and its degradates to benthic macroinvertebrates of urban streams, *Environmental Science and Technology*, 48(2), 1290–1297.

Wilson WA, Konwick BJ, Garrison AW, Avants JK and Black MC (2008). Enantioselective chronic toxicity of fipronil to *Ceriodaphnia dubia*, *Archives of Environmental Contamination and Toxicology*, 54(1), 36–43.

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

# 4 Fluometuron

## 4.1 Introduction

Fluometuron ($C_{10}H_{11}F_3N_20$ and Figure 13) at room temperature is in the form of white crystals. It is the active ingredient of a variety of commercial herbicide formulations.

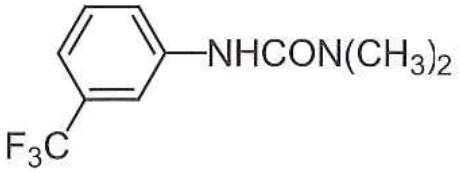



Figure 13 Structure of fluometuron.

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

**Table 15 Summary of selected physicochemical properties of fluometuron.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 232.2 amu[1] |
| Aqueous solubility | 110 mg/L @ temperature of 20 °C[1] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 2.38[1]<br>2.28 @ temperature 20 °C and pH 7[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 1.49–2.07 (8 soil types)[1] |
| Logarithm of the bioconcentration factor (log BCF) | 1.61[2] |
| Half-life ($t_{1/2}$) in water | Stable @ pH 5–9 and temperature 25 °C[2] |
| Half-life ($t_{1/2}$) in soil | Average: 30 days[1]<br>Range: 10–100 days[1] |

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

Fluometuron belongs to the phenylurea group within the urea family of herbicides, which also includes diuron, linuron and isoproturon. Fluometuron is extensively used in agricultural situations to control annual broad-leaved weeds and grasses – especially in cotton and sugarcane plantations (BCPC 2012). Non-agricultural uses include the application of fluometuron to railroads and industrial sites for weed control (US National Library of Medicine 2002). It is a selective pre-emergent and early-post emergent herbicide that may also have some effect on established plants (USEPA 2005).

Fluometuron is absorbed principally through the roots of plants, with some absorption via foliage. 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). Fluometuron 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 (O2-) and hydrogen peroxide (H2O2) (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 CO2 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).

Fluometuron reportedly also inhibits carotenoid biosynthesis and causes bleaching in treated plants; however, information regarding the molecular mode of this inhibition is limited (Hock and Elstner 2004; BCPC 2012).

Fluometuron is a selective, systemic herbicide which may ultimately end up in aquatic ecosystems as a result of spray drift and surface run-off (USEPA 2005). Fluometuron is readily mobile and has the capacity to leach to groundwater because of its moderately-weak soil sorption ability as indicated by its low log $K_{oc}$ and its solubility in water (Table 15). Fluometuron reportedly persists in water, being stable at a pHs ranging from pH 5 to pH 9 and a temperature of 25 ºC (Table 15) (USEPA 2005).

## 4.2 Freshwater and Marine

### 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 fluometuron in fresh and marine waters (Table 17) includes toxicity data for three species (one freshwater and two marine) that either originated from or are distributed within Australia and/or New Zealand.

One published study (Mowbray 1978) determined the toxicity of fluometuron to freshwater fish *Gambusia affinis* and *Hypseleotris galii* (Mowbray 1978; as cited in Warne et al. 1998). This document is a Ph.D thesis which was unattainable by various libraries (including the University of Sydney) and thus, was unable to be put through the standard ecotoxicity data quality checking procedures (Warne et al. 2015). Therefore, the fish toxicity data reported by Mowbray (1978) were not included in the guideline derivation process for fluometuron and are not included in this report. 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 four microalgae. The toxicity values for the single fish species were 34-day NOEC and LOEC (mortality) values of 3,100 and 3,640 µg/L, respectively. The toxicity values for the single cladoceran species were 21-day NOEC and LOEC (growth of total body length and dry weight) values of 1,730 and 2,520 µg/L, respectively. The toxicity values for the single macrophyte species were two 14-day NOEC (frond number, dry weight, frond area) values of 115 and 310 µg/L., two 14-day EC50 (frond

number, dry weight, frond area) values of 220 and 590 µg/L. The toxicity values for the microalgae consisted of 48-hour NOEC, LOEC and IC50 (chlorophyll-a content) values of 23.2, 232.2 and 534.1 µg/L, respectively, 96-hour IC50 (cell number, cell density) values ranging from 557.3 to 766.3 µg/L, 5-day NOEC (biomass yield, growth rate, area under the growth curve) values ranging from 70 to 220 µg/L and 5-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 30 to 306 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for nine fish, one cladoceran and three microalgae. The toxicity values for the fish consisted of 48-hour LC50 (mortality) values ranging from 48,000 to 200,000 µg/L, 96-hour NOEC (mortality) values ranging from 4,300 to 25,000 µg/L and 96-hour LC50 (mortality) values ranging from 640 to 170,000 µg/L. The toxicity values for the single cladoceran species were a 48-hour LOEL and EC50 (growth of total body length and dry weight) values of 1,800 and 1,980 µg/L, respectively. The toxicity values for the microalgae were two 24-hour NOEC (cell density) values of 23.2 and 232.2 µg/L, 24-hour LOEC (cell density) values ranging from 23.2 to 2,322.1 µg/L and 24-hour IC50 (cell density) values ranging from 278.7 to 2,322.1 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.

*Marine Chronic*

There were marine chronic toxicity data for two microalgae which were two 5-day NOEC (biomass yield, growth rate, area under the growth curve) values of 107 and 410 µg/L and two 5-day EC50 (biomass yield, growth rate, area under the growth curve) values of 310 and 620 µg/L.

*Marine Acute*

There were marine acute toxicity data for one fish, one crustacean and one mollusc. The toxicity values for the single fish species were two 96-hour NOEC (mortality) values of 17,000 and 18,100 µg/L and two 96-hour LC50 (mortality) values of 48,000 and 55,300 µg/L. The toxicity values for the single crustacean species were two 96-hour NOEC (mortality) values of 1,600 and 2,100 µg/L and two 96-hour LC50 (mortality) values of 3,800 and 6,800 µg/L. The toxicity values for the single mollusc species consisted of 96-hour NOEC (mortality, abnormal development) values of 4,350 and 9,100 µg/L, a 96-hour LOEL (mortality, abnormal development) value of 2,500 µg/L and 96-hour EC50 (mortality, abnormal development) values ranging from 6,530 to 22,000 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.

### 4.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of fluometuron. 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 fluometuron (Table 15).

### 4.2.3 Guideline derivation

The derived PGVs for fluometuron in fresh and marine waters are provided in Table 16. 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 PGVs, the PGVs for fluometuron are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for fluometuron 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 fluometuron do not need to account for secondary poisoning.

**Table 16 Proposed aquatic ecosystem protection guideline values (µg/L) for fluometuron for the protection of freshwater and marine ecosystems.**

| Fluometuron proposed aquatic ecosystem protection guideline values (fresh and marine waters)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 20 (3.3 – 84) |
| 95% | 40 (10 – 100) |
| 90% | 55 (17 – 120) |
| 80% | 77 (30 – 140) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 7 |
| Type of toxicity data | Chronic NOECs and chronic estimated NOEC values (*freshwater and marine*) |
| SSD model fit | Poor |
| 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.**

### 4.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for fluometuron in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for fluometuron to freshwater and 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 database (Sunderam et al. 2000) were searched. There are now more fluometuron toxicity data available that enable the calculation of PGVs in fresh and marine waters. However it was only possible to derive PGVs by using ecotoxicity data for a mixture of both freshwater and marine organisms. In order to derive higher reliability PGVs in the future that are of greater relevance to freshwater and marine ecosystems separately, it is recommended that additional chronic toxicity tests of fluometuron with freshwater and 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 fluometuron in fresh and marine waters, *Chlorococcum sp.*, *Lyngbya sp.* and *Perca sp.* were included as no other toxicity data for these genera were used.

In total, there were toxicity data for 20 freshwater and marine species (seven phyla and nine classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria, Mollusca and Tracheophyta. The nine classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Bivalvia (a grouping of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), 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 fluometuron, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The fluometuron 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 4.2.6) 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 ecotoxicity data available for only six freshwater phototrophic species (that belonged to three phyla and four classes) and two 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 for either media type (Warne et al. 2015). In cases like these, the Assessment Factor (AF) method would have to be used to derive PGVs for each ecosystem separately. However, it was deemed preferable to combine the ecotoxicity data for the freshwater phototrophic species with the marine phototrophic species to derive PGVs using the SSD method (and thus using the data for all the available phototrophic species) rather than deriving PGVs for freshwater and marine ecosystems separately using the single lowest value in each ecosystem.

When combining the freshwater and marine datasets, there were chronic no observed effect concentration (NOEC) 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 available for seven (five freshwater and two marine) phototrophic species that belonged to four phyla and five classes, which met the minimum data requirements 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 16) combined with the poor fit of the distribution to these toxicity data (Figure 14) resulted in a low 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 fluometuron in freshwater and marine environments is provided in Table 17.

**Table 17 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for fluometuron in fresh and 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 NOEC | Biomass yield, growth rate, AUC[2] | 124.1 | USEPA (2015b) |
| Fresh | Cyanobacteria | *Anabaena variabilis* | Cyanobacteria | Cyanophyceae | Not stated | 2 | Chronic NOEC | Growth (chlorophyll-a) | 23.2 | Hawxby et al. (1977) |
| Fresh | Microalga | *Chlorella pyrenoidosa*[3]* | Chlorophyta | Trebouxiophyceae | Logarithmic growth phase | 4 | Chronic est. NOEC | Cell number | 123.9 | Blythe et al. (1979) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEC | Frond number, dry weight, frond area | 188.8 | USEPA (2015b) |
| Marine | Marine diatom | *Nitzschia palea** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEC | Biomass yield, growth rate, AUC[2] | 107 | USEPA (2015b) |
| Fresh | Microalga | *Selenastrum capricornutum*[4] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEC | Biomass yield, growth rate, AUC[2] | 180 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema costatum** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEC | Biomass yield, growth rate, AUC[2] | 410 | USEPA (2015b) |

**[1] Chronic NOEC/NOEL = no conversions applied; Chronic est. NOEC = chronic LOEC and IC50 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 been also been called *Chlorella vulgaris*. [4] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 4.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the seven freshwater and marine phototrophic species that was used to derive the PGVs is presented in Figure 14.

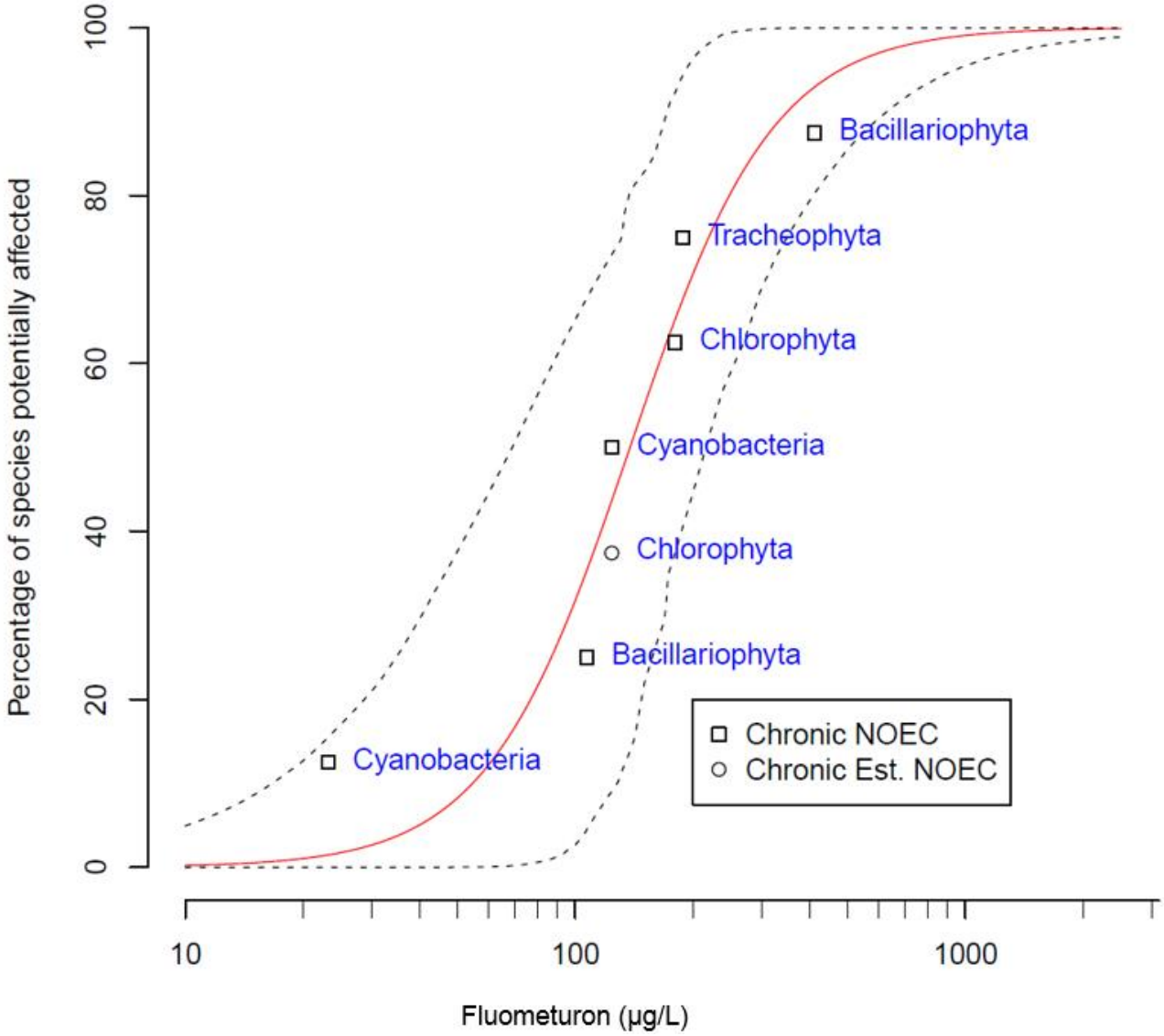



Figure 14 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC) data values of freshwater and marine phototrophic species to fluometuron. Black dashed lines indicate the 95% confidence intervals.

### 4.2.6 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 [2.584 and 6.265 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 fluometuron.

The toxicity data for fluometuron 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 fluometuron ecotoxicity data may be bimodal (Figure 15).

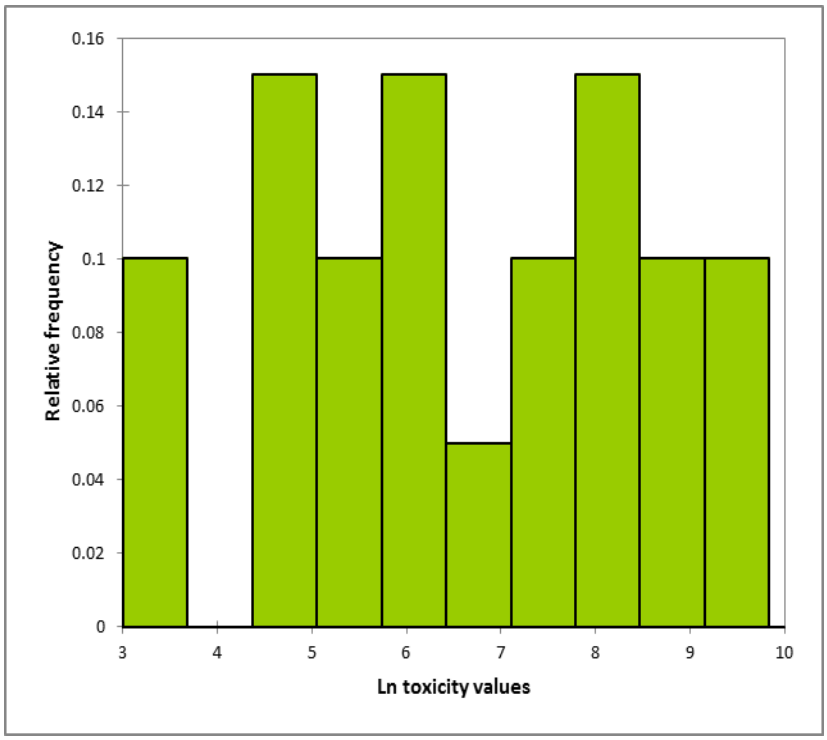



Figure 15 Histogram of the natural logarithm (ln) of all fluometuron (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 20).

The fluometuron 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 fluometuron concentration data had equal variances (Fisher's F-Test; p = 0.793) and followed a normal distribution (Anderson-Darling; p = 0.182). Results from the two-sample *t* test indicated that the two groups were significantly different (p <0.0001); therefore, it was concluded that the distribution of the fluometuron concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

### 4.2.7 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.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 525–526.

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/.

Blythe TO, Grooms SM and Frans RE (1979). Determination and characterization of the effects of fluometuron and MSMA on *Chlorella*. *Weed Science*, 27, pp. 294–99.

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.

Hawxby K, Tubea B, Ownby J and Basler E (1977). Effects of various classes of herbicides on four species of algae. *Pesticide Biochemistry and Physiology*, 7, pp. 203–9.

Hock B and Elstner EF (2004). Plant Toxicology, Forth Edition. United States of America, CRC Press Online, 664pp. Accessed: March, 2017.

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.

Mowbray DL (1978). The ecological effects of pesticides on non-target organisms: a study of the environmental impact of pesticides on wildlife in the Namoi River Valley cotton growing area, 1972-1976. Thesis, Ph.D, University of Sydney, Australia.

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 (1996). Reregistration Eligibility Decision (RED) for Fluometuron. United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances (7508C).

Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/fluometuron_red.pdf. Accessed: March, 2017.

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.

US National Library of Medicine (2002). TOXNET: Toxicology Data Network, National Institutes of Health and Human Services. Maryland. November 8, 2002. Available from: https://toxnet.nlm.nih.gov/cgi-bin/sis/search2/r?dbs+hsdb:@term+@DOCNO+1721. Accessed: March 22, 2017.

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 Fluroxypyr

## 5.1 Introduction

Fluroxypyr is a herbicide (C7H5Cl2FN2O3 and Figure 16) that at room temperature is an odourless, white crystalline solid. Fluroxypyr is generally applied as an ester, such as fluroxypyr-meptyl or 2-butoxy-1-methylethyl, which are active ingredients of a variety of commercial herbicide formulations (BCPC 2012).

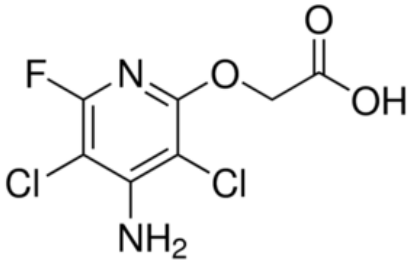



Figure 16 Structure of fluroxypyr.

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

**Table 18 Summary of selected physicochemical properties of fluroxypyr.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 255.0 amu[1] |
| Aqueous solubility | 5,700 mg/L @ pH 5 and temperature 20 °C[1]<br>7,300 mg/L @ pH 9.2 and temperature 20 °C[1]<br>6,500 mg/L @ temperature of 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | -1.24 (unstated pH)[1]<br>0.04 @ pH 7 and temperature 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | Average: 1.83 (1.71–1.91)[3] |
| Logarithm of the bioconcentration factor (log BCF) | 1.79 |
| Half-life ($t_{1/2}$) in water | 185 days @ pH 9 and temperature 20 °C[1]<br>223 days @ pH 7 and temperature 20 °C[2]<br>Stable @ pH 4–7[2] |
| Half-life ($t_{1/2}$) in soil | 5–9 days @ temperature 23 °C (laboratory studies) [1]<br>13.1 – 51 days in the lab (20 °C) and field, respectively[2] |

**[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] USEPA 2007.**

Fluroxypyr belongs to the pyridine group of herbicides, which also includes haloxydine, thiazopyr and triclopyr. Fluroxypyr is extensively used in uncultivated areas (non-crop land such as grassland and pastures), plantation crops (i.e. rubber and oil palm), agricultural (i.e. cereals, maize, sorghum, sugarcane and orchards – apple only) and forestry (i.e. coniferous forests) situations for the control of broad-leaved, woody and herbaceous weeds (BCPC 2012; University of Hertfordshire 2013; APVMA 2014). Fluroxypyr is a systemic herbicide and is generally applied after weeds emerge (i.e. it is a post-emergent herbicide) (BCPC 2012).

Fluroxypyr is applied as an ester (fluroxypyr-meptyl or 2-butoxy-1-methylethyl) and is absorbed mainly through the foliage of plants where it is hydrolysed to the parent acid which is the active form (BCPC 2012). 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). Fluroxypyr acts by mimicking the plant hormone, auxin (indolylacetic acid), which is responsible for promoting stem elongation and maintaining apical dominance in dicots. Similar to triclopyr, fluroxypyr acidifies the cell walls of plants which causes cells to elongate in an uncontrolled and disorganised manner (approximately 1,000 times natural levels), ultimately leading to plant death (Ganapathy 1997; Tu et al. 2001).

Fluroxypyr may ultimately end up in aquatic environments as a result of aerial/spray drift, runoff following rainfall events and residue leaching (USEPA 1998; WSDOT 2006). Fluroxypyr binds weakly to soil particles as indicated by its low log $K_{oc}$ value (Table 18) and has high aqueous solubility (Table 18) which would suggest great potential to leach into groundwater and end up in surface waters (WSDOT 2006). Fluroxypyr is relatively persistent in water, being stable at a pH ranging from pH 4 to pH 7 and a half-life ranging from 185 to 223 days at pH 7 to pH 9 and a temperature of 20 ºC (BCPC 2012; University of Hertfordshire 2013). Fluroxypyr is quite mobile in soils; however, not as persistent as it is in water (Table 18) with a half-life ranging from 5 to 51 days in both field and laboratory studies (USEPA 1998; BCPC 2012; University of Hertfordshire 2013).

## 5.2 Freshwater and Marine

### 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 fluroxypyr in fresh and marine waters (Table 20) includes toxicity data for 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 freshwater and marine species that passed the screening and quality assurance processes are provided below.

*Freshwater Chronic*

There were freshwater chronic toxicity data for two macrophyte and four microalgae. The toxicity values for the macrophytes were 11-day EC50 (frond number, dry weight, frond area) values ranging from 7,700 to 103,400 µg/L, two 14-day NOEL (frond number, dry weight, frond area) values of 3,200 and 3,500 µg/L and a 14-day EC50 (frond number, dry weight, frond area) value of 5,800 µg/L. The toxicity values for the microalgae consisted of two 48-hour LOEC (chlorophyll content, cell count) values of 500 and 750 µg/L, 96-hour NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 830 and 1,400 µg/L, respectively, 5-day NOEL (biomass yield, growth rate, area under the growth curve) values ranging from 190 to 3,400 µg/L and 5-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 1,100 to 4,600 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for two fish which consisted of a 96-hour NOEL (mortality) value of 7,280 µg/L and two 96-hour LC50 (mortality) values of 14,300 and 40,000 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.

*Marine Chronic*

There were marine chronic toxicity data for one microalga which consisted of 96-hour NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 1,200 and 7,000 µg/L,

respectively, 5-day NOEL (biomass yield, growth rate, area under the growth curve) values ranging between 179 to 3,000 µg/L and a 5-day EC50 (biomass yield, growth rate, area under the growth curve) value of 292 µg/L.

*Marine Acute*

There were marine acute toxicity data for one crustacean and one mollusc. The single toxicity value for the crustacean species was a 96-hour LC50 (mortality) value of 120,000 µg/L. The toxicity values for the single mollusc species were two 96-hour NOEL (mortality) values of 790 and 16,000 µg/L and a 96-hour EC50 (mortality) value of 51,000 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.

### 5.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of fluroxypyr. 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 fluroxypyr (Table 18).

### 5.2.3 Guideline derivation

The derived PGVs for fluroxypyr in fresh and marine waters 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 PGVs, the PGVs for fluroxypyr are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for fluroxypyr 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 fluroxypyr do not need to account for secondary poisoning.

**Table 19 Proposed aquatic ecosystem protection guideline values (µg/L) for fluroxypyr for the protection of freshwater and marine ecosystems.**

| Fluroxypyr proposed aquatic ecosystem protection guideline values (freshwater and marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 87 (29 – 480) |
| 95% | 200 (82 – 670) |
| 90% | 290 (130 – 790) |
| 80% | 440 (200 – 980) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 7 |
| Type of toxicity data | Chronic NOEL and chronic estimated NOEC 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". [3] Values rounded to two significant figures.**

### 5.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for fluroxypyr in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for fluroxypyr to freshwater and 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 fluroxypyr toxicity data available that enable the calculation of PGVs in fresh and marine waters. However, it was only possible to derive PGVs by using ecotoxicity data for a mixture of both freshwater and marine organisms. In order to derive higher reliability PGVs in the future that are of greater relevance to freshwater and marine ecosystems separately, it is recommended that additional chronic toxicity tests of fluroxypyr with freshwater and marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were toxicity data for 11 freshwater and marine species (six phyla and seven classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata, Cyanobacteria, Mollusca and Tracheophyta. The seven classes were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Liliopsida (monocots), Malacostraca (a large grouping of crustaceans) and Mediophyceae (another algae grouping).

Based on the current understanding of the mode of action of fluroxypyr, it would be expected that phototrophic species would be more sensitive than non-phototrophic species as it mimics auxin, which is a plant growth hormone that exists in vascular plants as well as algal species. The fluroxypyr 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 ($p = 0.019$, see section 5.2.6) 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) 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 available for seven (six freshwater and one marine) phototrophic species that belonged to only three phyla and four classes. This dataset 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 for either media type (Warne et al. 2015). In cases like these where the SSD uses the most sensitive species, 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).

The entire freshwater and marine dataset for fluroxypyr (that included chronic data) consisted of 11 phototrophic ($n$ = 7) and heterotrophic ($n$ = 4) species that belonged to six phyla and seven classes, which 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 NOEL and chronic estimated NOEC data values for the seven freshwater and 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 19) combined with the good fit of the distribution to these toxicity data (Figure 17) resulted in a set of moderate reliability PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for fluroxypyr in freshwater and marine environments is provided in Table 20.

**Table 20 Summary of the single toxicity value for each phototrophic species that were used to derive the proposed aquatic ecosystem protection guideline values for fluroxypyr in fresh and 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 NOEL | Biomass yield, growth rate, AUC[2] | 360 | USEPA (2015b) |
| Fresh | Microalga | *Chlamydomonas reinhardtii** | Chlorophyta | Chlorophyceae | Exponential growth phase | 2 | Chronic est. NOEC | Chlorophyll content | 200 | Zhang et al. (2011) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 3,346.64 | USEPA (2015b) |
| Fresh | Macrophyte | *Lemna minor** | Tracheophyta | Liliopsida | Not stated | 11 | Chronic est. NOEC | Frond number, Dry weight, Frond area | 1,540 | USEPA (2015b) |
| Fresh | Microalga | *Navicula pelliculosa** | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 779.74 | USEPA (2015b) |
| Fresh | Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 938 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema costatum** | Chlorophyta | Mediophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 1,172.28 | 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 (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/is distributed in Australia and/or New Zealand.**

### 5.2.5 Species sensitivity distribution

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

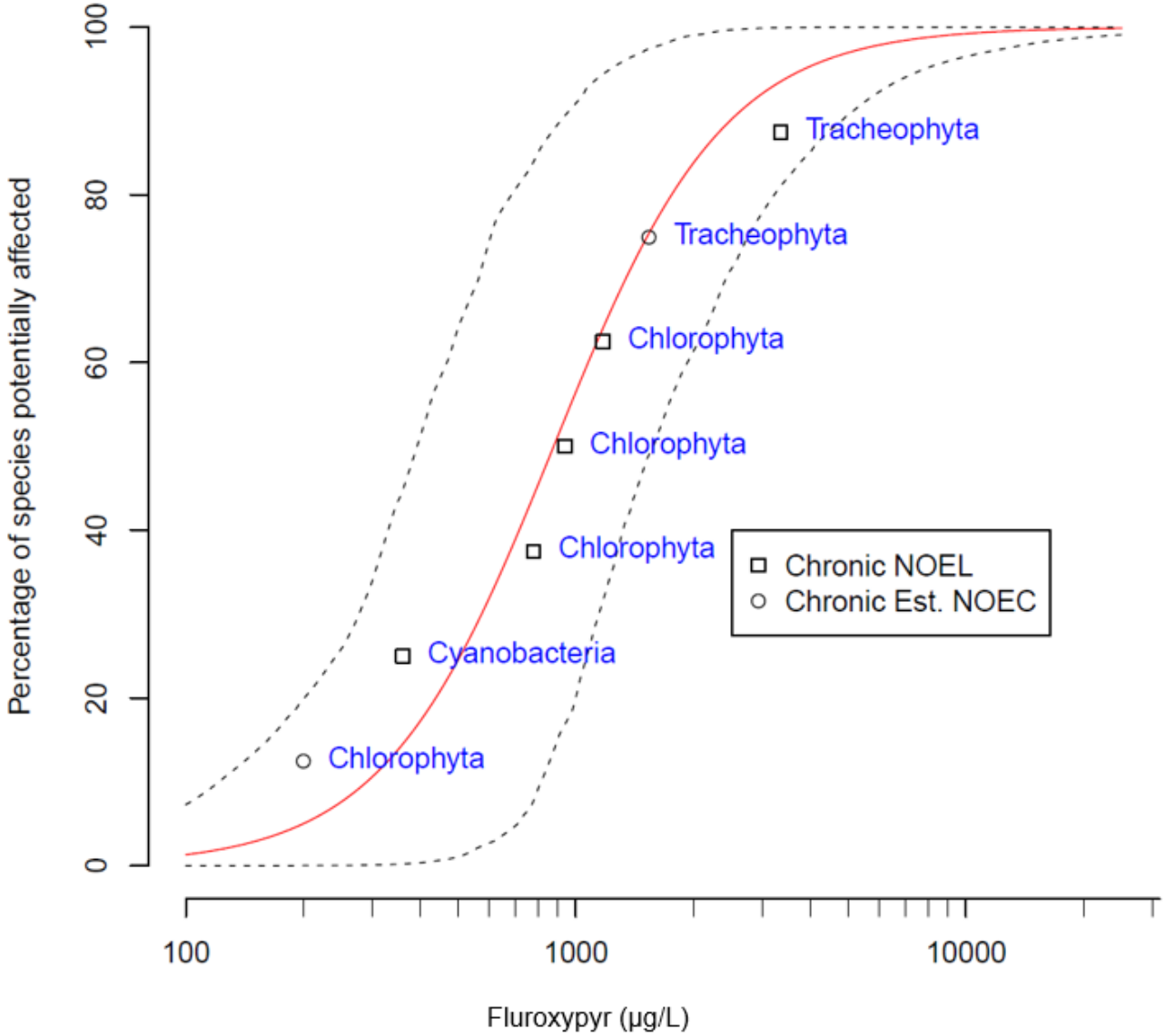



Figure 17 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016,) of the sensitivity of chronic no observed effect level (NOEL) and chronic estimated no observed effect concentration (NOEC) data values of freshwater and marine phototrophic species to fluroxypyr. Black dashed lines indicate the 95% confidence intervals.

### 5.2.6 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 [4.718 and 8.663 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 fluroxypyr.

The toxicity data for fluroxypyr 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 fluroxypyr ecotoxicity data may be bimodal (Figure 18).

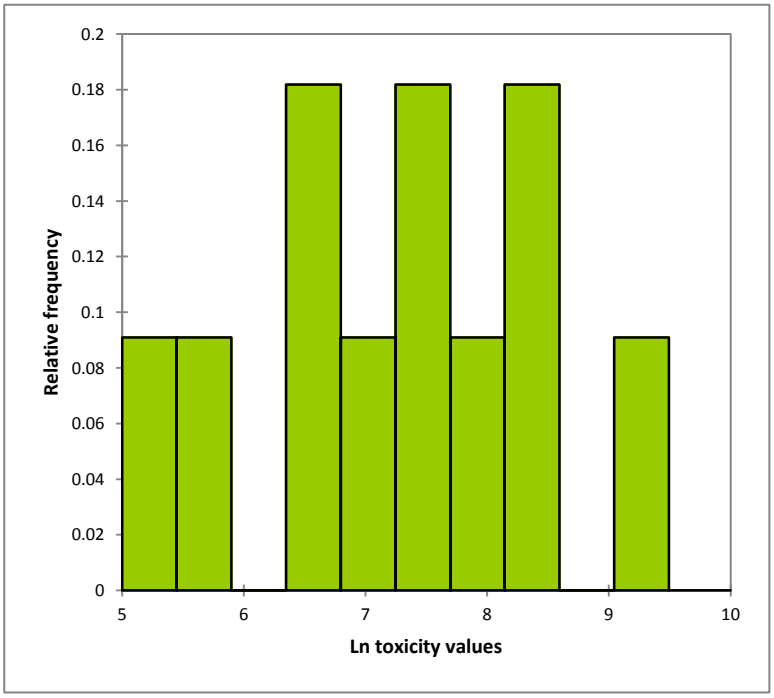



Figure 18 Histogram of the natural logarithm (ln) of all fluroxypyr (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 11).

The fluroxypyr 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 fluroxypyr concentration data had equal variances (Fisher's F-Test; p = 0.999) and followed a normal distribution (Anderson-Darling; p = 0.874). Results from the two-sample *t* test indicated that the two groups were significantly different (p = 0.019); therefore, it was concluded that the distribution of the fluroxypyr concentration data was bi- or multi-modal, with phototrophic species being the most sensitive group.

### 5.2.7 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.

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: 13 April 2017.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 545–547.

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/.

Ganapathy C (1997). Environmental fate of triclopyr, Department of Pesticide Regulation Environmental Monitoring and Pesticide Management Branch, Sacramento, CA 95814-5624. Available from: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/triclopyr.pdf, Accessed 11 April 2017.

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.

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.

Tu M, Hurd C, Robinson R and Randall JM (2001). Weed Control Methods Handbook: Triclopyr. The Nature Conservancy, Virginia, United States of America, 135–141. Available from: https://www.invasive.org/gist/products/handbook/methods-handbook.pdf, Accessed: 11 April 2017.

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 (1998). EPA Pesticide Fact Sheet: Fluroxypyr, United States Environmental Protection Agency, Prevention, Office of Prevention, Pesticides and Toxic Substances (7501C), Washington DC. Available from: https://www3.epa.gov/pesticides/chem_search/reg_actions/registration/fs_PC-128959_30-Sep-98.pdf, Accessed: 13 April 2017.

USEPA (2007). Drinking Water Assessment for Fluroxypyr New Uses on Pome Fruit and Millet. Report dated 3 October 2007, United States Environmental Protection Agency, Office of Prevention, Pesticides, and Toxic Substances. Office of Pesticide Programs. Washington, D.C. Docket No. HQ-OPP-2007-0114. [E-Docket 02]. Available from: https://www.regulations.gov/document?D=EPA-HQ-OPP-2007-0114-0006, Accessed: 13 April 2017.

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.

WSDOT (2006). Roadside vegetation management herbicide fact sheet: Fluroxypyr, Developed by Oregon State University and Intertox, Inc., Washington State Department of Transportation's Integrated Management program, Washing State Department of Transport (WSDOT), Washington. Available from: https://www.wsdot.wa.gov/NR/rdonlyres/767D2219-C458-4CCA-A5DC-3F185580580F/0/fluroxypyr.pdf, Accessed: 13 April 2017.

Zhang S, Qiu CB, Zhou Y, Jin ZP and Yang H (2011) Bioaccumulation and degradation of pesticide fluroxypyr are associated with toxic tolerance in green alga *Chlamydomonas reinhardtii*, *Ecotoxicology*, 20, 337–347.

# 6 Haloxyfop

## 6.1 Introduction

Haloxyfop is a herbicide ($C_{15}H_{11}ClF_3NO_4$ and Figure 19) that at room temperature is in the form of colourless crystals. Haloxyfop (CAS RN 69806-34-4) is a racemic mixture (i.e. it is produced with equal amounts of two enantiomers – mirror image isomers), with the r-isomer being the herbicidal active compound. Haloxyfop-r is commercially produced and known as haloxyfop-P (CAS RN 95977-29-0). Other forms of haloxyfop are haloxyfop-methyl (CAS RN 69806-40-2) and haloxyfop-p-methyl (CAS RN 72619-32-0). Haloxyfop, haloxyfop-P and their methyl esters are all active ingredients of a variety of commercial herbicide formulations.

In Australia, registration is granted for commercial products which contain haloxyfop-r as a methyl ester only (APVMA 2016). When applied to plants, the methyl ester is rapidly hydrolysed to the haloxyfop-r (acid) which has herbicidal activity.

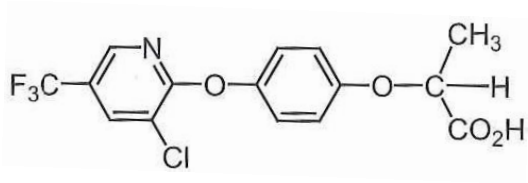



Figure 19 Structure of haloxyfop.

Physicochemical properties of haloxyfop that may affect its environmental fate and toxicity are presented in Table 21

**Table 21 Summary of selected physicochemical properties of haloxyfop.**

| Physicochemical property | Value |
|---|---|
| CAS Registration number | 69806-34-4[1] |
| Molecular weight | 361.7 amu[1] |
| Aqueous solubility | 43.4 mg/L @ pH 2.6 and temperature 25 °C[1]<br>1.590 mg/L @ pH 6 and temperature 20 °C[1]<br>6.980 mg/L @ pH 9 and temperature 20 °C[1] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 4.2[3] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 1.88[2] |
| Logarithm of the bioconcentration factor (log BCF) | (not available) |
| Half-life ($t_{1/2}$) in water | 78 days @ pH 5[1]<br>73 days @ pH 7[1]<br>51 days @ pH 9[1]<br>Stable @ pH 7 and temperature 20 °C[2] |
| Half-life ($t_{1/2}$) in soil | Typical: 9 days (several soils)[1,2]<br>28 days (sandy loam), 38 days (heavy clay) and 92 days (clay loam)[4] |

**[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] National Centre for Biotechnology Information (2016). [4] Rao (2000).**

Haloxyfop belongs to the aryloxyphenoxypropionic group within the phenoxy family of herbicides, which also includes haloxyfop-r, chlorazifop and fenthiaprop. Haloxyfop is extensively used in agricultural situations to control annual and perennial grass weeds amongst a variety of broad-leaved crops such as sugar beet, leafy vegetables, vines, sunflowers and strawberries (BCPC 2012).

Haloxyfop is a selective herbicide and is typically applied after weeds emerge (i.e. it is a post-emergent herbicide). However, it does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013).

Haloxyfop is mainly absorbed through the foliage and to a lesser extent through roots of plants where it is hydrolysed before being translocated to meristematic tissues where it exerts its toxicity. Haloxyfop targets and inhibits acetyl-CoA carboxylase (ACCase), the enzyme responsible for the biosynthesis of fatty acids (BCPC 2012).

Haloxyfop and other ACCase inhibiting herbicides bind to and inhibit the eukaryotic form of the enzyme rather than the prokaryotic form (Purdue University n.d.). Monocots express eukaryotic ACCase in the cytoplasm and chloroplasts whereas dicots express prokaryotic ACCase in their chloroplasts. Therefore, monotocs (i.e. grasses) are susceptible to ACCase inhibitors such as haloxyfop whereas dicots (i.e. broad-leaved plants) are resistant (Iowa State University 2017). As a result, respiration and the synthesis of lipids within exposed monocots is inhibited (Cho et al. 1988). The ACCase within plants is essential for cell membrane formation; therefore, when inhibited, tissue growth is impossible and symptoms of necrosis occur (Purdue University n.d.). Affected plants typically die within one to two weeks.

Haloxyfop is moderately persistent in soils; however, has a low affinity for binding to soil particles (Table 21). It is relatively mobile and has the ability to leach from soils into groundwater and end up in surface waters. The half-life ($t_{1/2}$) of haloxyfop in water ranges from 51 (or stable) to 78 days at pH 5 through to pH 9.

## 6.2 Freshwater and Marine

### 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 haloxyfop in fresh and marine waters (Table 23) includes toxicity data for one freshwater species that either originated from or is distributed within Australia and/or New Zealand. Neither, the dataset used in the guideline derivation process nor the review of the literature revealed any toxicity data for haloxyfop to Australian and/or New Zealand marine species (e.g. Warne et al. 1998). 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 only one microalga which were 96-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 5,000 and 106,000 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity values for one fish and one cladoceran. The toxicity values for the single fish species were 96-hour LOEC and LC50 (mortality) values of 328,000 and 548,000 µg/L, respectively. The toxicity values for the single cladoceran species were 48-hour LOEC and EC50 (immobilisation) values of 35,000 and 96,700 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.

*Marine Chronic*

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

*Marine Acute*

There were marine acute toxicity values for one fish, one crustacean and one mollusc. The toxicity values for the single fish species were 96-hour NOEL and LC50 (mortality) values of 196,000 and 383,000 µg/L, respectively. The toxicity values for the single crustacean species were 96-hour NOEL and LC50 (mortality) values of 191,000 and 572,000 µg/L, respectively. The single value for a mollusc was a 96-hour EC50 (mortality, abnormal development) value of 33,000 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.

### 6.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of haloxyfop. 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 haloxyfop (Table 21).

### 6.2.3 Guideline derivation

The derived PGVs for haloxyfop in fresh and marine waters are provided in Table 22. 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 PGVs, the PGVs for haloxyfop are expressed in terms of the concentration of the active ingredient.

**Table 22 Proposed aquatic ecosystem protection guideline values (µg/L) for haloxyfop for the protection of freshwater and marine ecosystems.**

| Haloxyfop proposed aquatic ecosystem protection guideline values (fresh and marine waters)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 590 (250 – 13,000) |
| 95% | 2,000 (950 – 21,000) |
| 90% | 3,400 (1,600 – 26,000) |
| 80% | 6,100 (2,700 – 33,000) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 6 |
| Type of toxicity data | Chronic NOEL and converted acute values (*freshwater and marine*) |
| SSD model fit | Poor |
| 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.**

### 6.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value or a TV) existed for haloxyfop in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for haloxyfop to freshwater and 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 database (Sunderam et al. 2000) were searched. There are now more haloxyfop toxicity data available that enable the calculation of PGVs in fresh and marine waters. However it was only possible to derive PGVs by using ecotoxicity data for a mixture of both freshwater and marine organisms. In order to derive higher reliability PGVs in the future that are of greater relevance to freshwater and marine ecosystems separately, it is recommended that additional chronic toxicity tests of haloxyfop with freshwater and marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were toxicity data for six freshwater and marine species (belonging to four phyla and five classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata and Mollusca. The five classes were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae) and Malacostraca (a large grouping of crustaceans).

Based on the current understanding of the mode of action of haloxyfop, an ACCase-inhibiting herbicide, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as the enzyme is present as eukaryotic and prokaryotic forms within cells of plants and algae. Due to the small sample size, 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 haloxyfop 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 haloxyfop 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 ecotoxicity data available for only three freshwater species (that belonged to three phyla and three classes) and three marine 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 for either media type (Warne et al. 2015). In cases like these, the Assessment Factor (AF) method would need to be used to derive PGVs for each ecosystem separately. However, it was deemed preferable to combine the ecotoxicity data for the freshwater phototrophic species with the marine phototrophic species to derive PGVs using the SSD method (and thus using the data for all the available phototrophic species) rather than deriving PGVs for freshwater and marine ecosystems separately using the single lowest value in each ecosystem.

When combining the freshwater and marine datasets, there were chronic 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) ecotoxicity data available for six (three freshwater and three marine) species that belonged to four phyla and five classes, which met the minimum data requirements 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 22) combined with the poor fit of the distribution to these toxicity data (Figure 20) resulted in a low 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 haloxyfop in freshwater and marine environments is provided in Table 23.

Despite the small sample size for haloxyfop ecotoxicity data, the low representation of phototrophic species (*n* = 1) and the low reliability rating of the PGVs, our confidence in these values are supported with additional phototrophic toxicity data for commercial formulations of haloxyfop-r (CAS RN 69806-34-4). Although the ecotoxicity values using these formulas failed the screening and quality assessment processes becayse they contained a low percentage (10.8%) of active ingredient, six different sources (provided in section 6.2.6) contained 96-hour EC50 (cell density) values ranging from 1,076 to 251,574 µg/L. These ecotoxicity values for haloxyfop-r all fall within the range of the toxicity values for haloxyfop in Table 23.

**Table 23 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 haloxyfop in fresh and 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 | *Crassostrea virginica* | Mollusca | Bivalvia | SPAT | 96 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 3,300 | USEPA (2015b) |
| Fresh | Macroinvertebrate | *Daphnia magna* | Arthropoda | Branchiopoda | <24 hours old | 48 | Converted acute | Immobilisation | 9,670 | USEPA (2015b) |
| Fresh | Fish | *Lepomis macrochirus* | Chordata | Actinopterygii | Not stated | 96 | Converted acute | Mortality | 54,800 | USEPA (2015b) |
| Marine | Fish | *Menidia menidia* | Chordata | Actinopterygii | Not stated | 96 | Converted acute | Mortality | 38,300 | USEPA (2015b) |
| Marine | Macroinvertebrate | *Penaeus duorarum* | Arthropoda | Malacostraca | Not stated | 96 | Converted acute | Mortality | 57,200 | USEPA (2015b) |
| Fresh | Microalga | *Scenedesmus subspicatus** | Chlorophyta | Chlorophyceae | Not stated | 96 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 5,000 | USEPA (2015b) |

**[1] Chronic NOEL = 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).[2] AUC = area under the growth curve. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 6.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the six freshwater and marine, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 20.

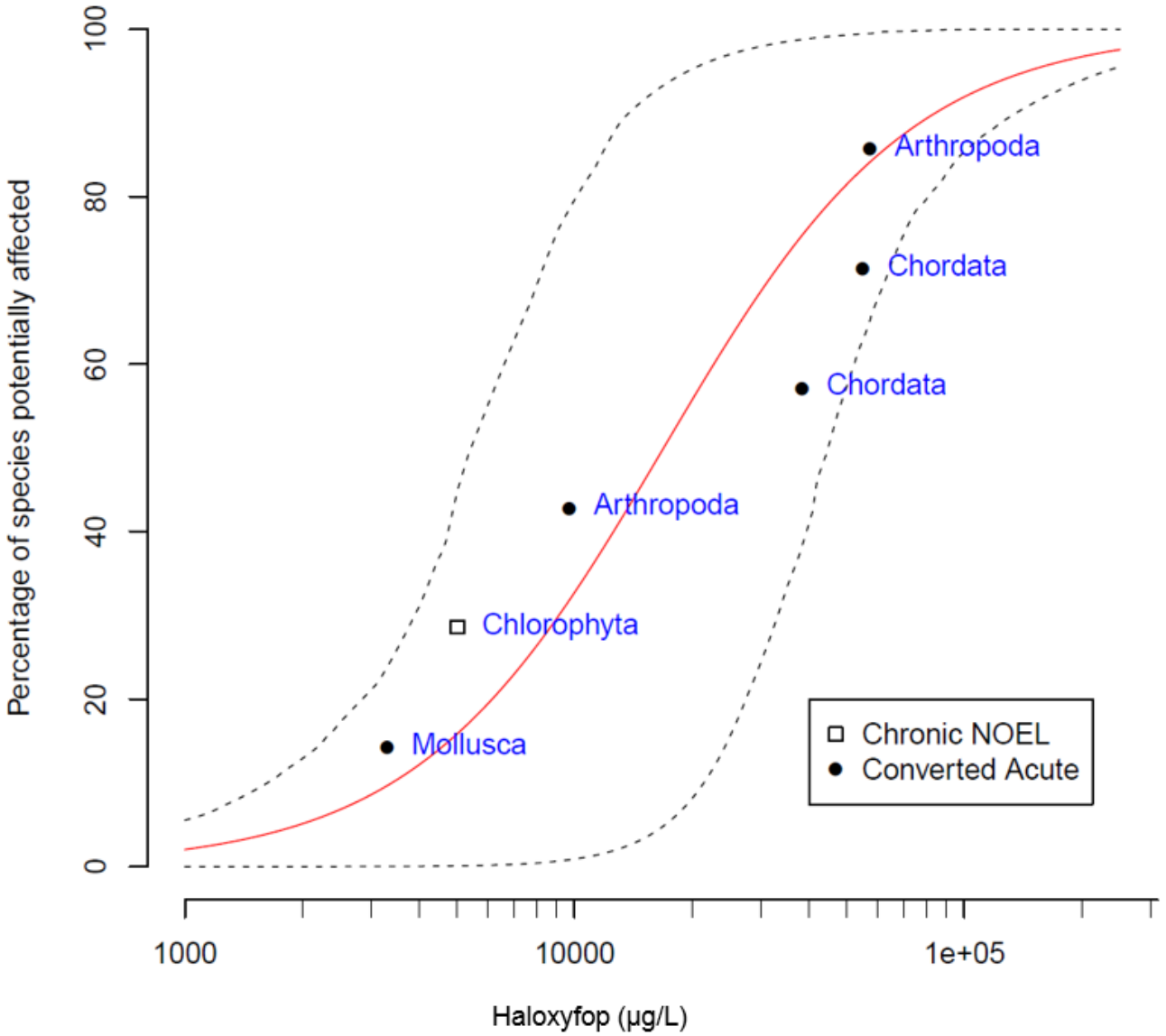



Figure 20 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect level (NOEL) and converted acute data values of freshwater and marine (phototrophic and heterotrophic) species to haloxyfop. Black dashed lines indicate the 95% confidence intervals.

### 6.2.6 Toxicity values for species which failed the screening and quality assessment processes

Additional phototrophic toxicity data using haloxyfop-r (CAS RN 69806-34-4) product formulas is provided in Table 24 below.

**Table 24 Summary of the toxicity data generated from commercial formulations (10.8% active ingredient) of haloxyfop-r (CAS RN 69806-34-4) that were not used in the derivation of the haloxyfop proposed aquatic ecosystem protection guideline values as they failed the screening and quality assessment processes. Data are arranged in alphabetical order of the test species and references are listed below.**

| Taxonomic group | Species | Phyla | Class | Duration (days) | Type (acute/ chronic)[1] | Toxicity endpoint | Toxicity value (µg/L) | Reference |
|---|---|---|---|---|---|---|---|---|
| Microalga | *Chlorella pyrenoidosa* | Chlorophyta | Trebouxiophyceae | 4 | Chronic EC50 | Cell density | 5,340 | Ma et al. (2001) |
| Microalga | *Chlorella pyrenoidosa* | Chlorophyta | Chlorophyceae | 4 | Chronic EC50 | Cell density | 5,348 | Ma et al (2002a) |
| Microalga | *Chlorella vulgaris* | Chlorophyta | Trebouxiophyceae | 4 | Chronic EC50 | Cell density | 109,594 | Ma et al. (2002b) |
| Microalga | *Raphidocelis subcapitata* | Chlorophyta | Chlorophyceae | 4 | Chronic EC50 | Cell density | 1,075.6 | Ma et al. (2006) |
| Microalga | *Scenedesmus obliquus* | Chlorophyta | Chlorophyceae | 4 | Chronic EC50 | Cell density | 251,574 | Ma (2002) |
| Microalga | *Scenedesmus quadricauda* | Chlorophyta | Chlorophyceae | 4 | Chronic EC50 | Cell density | 62,800 | Ma et al. (2004) |

### 6.2.7 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.

APVMA (2016). Public Chemical Registration Information System – PUBCRIS. Australian Pesticides and Veterinary Medicine Authority. [http://apvma.gov.au/] Canberra: Commonwealth of Australia; 2016 October 14. Accessed: 12 October 2016. Updated: September 2012. Available from: https://portal.apvma.gov.au/pubcris.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 598–601.

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/.

Cho HY, Widholm JM and Slife FW (1988). Haloxyfop inhibition of the pyruvate and the α-ketoglutarate dehydrogenase complexes of corn (*Zea mays* L.) and soybean (*Glycine max* [L.] Merr.), *Plant Physiology*, 87, 334–340.

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.

Iowa State University (2017). Herbicide metabolism in plants: Plant metabolism and biochemical interactions of herbicides, Developed by the Department of Agronomy (DoA), Iowa State University, 2007. Available from: https://masters.agron.iastate.edu/classes/533/lesson04/4.2.1.html. Accessed: 24 March 2017.

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

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 and Xu L (2004). Acute toxicity assessment of 20 herbicides to the green alga *Scenedesmus quadricau*da (Turp.) Breb, *Bulletin of Environmental Contamination and Toxicology*, 72, 1164–1171.

Ma J, Wang S, Wang P, Ma L, Chen X and Xu R (2006). Toxicity assessment of 40 herbicides to the green alga *Raphidocelis subcapitata*, *Ecotoxicology and Environmental Safety*, 63, 456–462.

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.

National Center for Biotechnology Information (2016). PubChem Compound Database; CID=50895. Developed by the U.S. National Library of Medicine, Bethesda USA. Available from: https://pubchem.ncbi.nlm.nih.gov/compound/haloxyfop#section=Top, Accessed: September 2016.

Purdue University (n.d.). Acetyl CoA Carboxylase (ACCase) Inhibitors: Lipid Biosynthesis Inhibitors, Developed by Purdue Agriculture, Purdue University, Indiana. Available from: https://www.btny.purdue.edu/WeedScience/MOA/index.html. Accessed: 24 March 2017.

Rao VS (2000). Principles of Weed Science, 2nd edn, Taylor & Francis, Oxfordshire, United Kingdom.

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.

# 7 Monochlorophenoxyacetic acid (MCPA)

## 7.1 Introduction

Monochlorophenoxyacetic acid, or MCPA is a herbicide ($C_9H_9ClO_3$ and Figure 21) that at room temperature is in the form of off-white crystals with a mild phenolic odour. MCPA-acid is the parent compound; however, MCPA is formulated into various esters, salts and amine derivatives (CCME, 1999). It is the active ingredient of a variety of commercial herbicide formulations and comes in a variety of chemical forms, with BCPC (2012) listing 7 forms. MCPA is often used in tank-mixes with other active ingredients (i.e. 2,4-D) to improve and broaden its spectrum efficacy (CCME 1999).

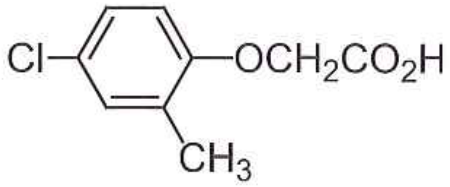



Figure 21 Structure of MCPA.

Physicochemical properties of MCPA that may affect its environmental fate and toxicity are presented in Table 25.

**Table 25 Summary of selected physicochemical properties of MCPA.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 200.6 amu[1] |
| Aqueous solubility | 0.395 g/L @ pH 1 and temperature 25 °C[1]<br>26.2 g/L @ pH 5 and temperature 25 °C[1]<br>293.9 g/L @ pH 7 and temperature 25 °C[1,2]<br>320.1 g/L @ pH 9 and temperature 25 °C[1] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 2.75 (pH 1), 0.59 (pH 5), -0.71 (pH 7) @ temperature 20 °C[1]<br>-0.81 @ pH 7 and temperature 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 1.05 – 1.65[3] |
| Logarithm of the bioconcentration factor (log BCF) | 0[2] |
| Half-life ($t_{1/2}$) in water | 13.5 days[2] |
| Half-life ($t_{1/2}$) in soil | <7 days after initial lag phase[1]<br>Typical: 24 days<br>(24 – 25 days in the lab (20 °C) and field, respectively)[2] |

**[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] Hiller et al. 2006.**

MCPA belongs to the phenoxyacetic group within the phenoxy class of herbicides, which also includes 2,4-D and 2,4,5-T[6]. MCPA is extensively used in agricultural situations to control annual and perennial broad-leaved weeds in a variety of cereals (e.g. wheat, rye and oats) and other crops (e.g. asparagus, rice, peas, potatoes and lindseed) (University of Hertfordshire 2013). MCPA is also used in forestry for the control of woody-weeds, as well as in industrial and urban situation (e.g. grasslands, turf, roadsides and embankments) (BCPC 2012). MCPA may ultimately end up in aquatic environments as a result of spray drift, surface runoff and/or leaching (CCME 1999).

[6] 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 (DAFF 2006; DEH 2004).

MCPA is generally applied as a liquid or an emulsifiable concentrate product, and is absorbed through the roots (acid and salt forms) and leaves (ester forms) of plants (CCME 1999; BCPC 2012). 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). MCPA acts by mimicking the plant hormone, auxin (indolylacetic acid), which is responsible for promoting stem elongation and maintaining apical dominance in dicots (BCPC 2012). Following administration, MCPA acidifies the cell walls of plants, which causes cells to elongate in an uncontrolled and disorganised manner, ultimately leading to plant death (ANZECC and ARMCANZ 2000; Walters 1999). MCPA, like other phenoxy herbicides, also affects the metabolism of plants by affecting enzyme activity, respiration and cell division (Walters 1999).

MCPA is a selective, systemic herbicide which has a low affinity for binding to most soils (Table 25) and has high aqueous solubility reaching up to 320.1 g/L at pH 9 and a temperature of 25 ºC (BCPC 2012; University of Hertfordshire 2013). MCPA reportedly does not accumulate in the environment due to rapid metabolic-, bio- and photolysis-degradation rates (CCME 1999; University of Hertfordshire 2013).

## 7.2 Marine

### 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 MCPA in marine waters (Table 27) includes toxicity data for one marine 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 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 one microalga. The toxicity values for the single mollusc species consisted of a 48-hour EC50 (mortality, abnormal development) value of 155,000 µg/L, a 48-hour TLm[7] (normal larval development) value of 15,620 µg/L, a 12-day TLm (survival) value of 31,300 µg/L and 12-day NOEC and LOEC (mean length) values of 250 and 500 µg/L, respectively. The toxicity values for the single microalga species were 5-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 11,000 and 32,000 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for two fish, two crustaceans, one mollusc and one macrophyte. The toxicity values for the fish were 96-hour NOEL and EC50 (mortality) values of 4,100 and 179,000 µg/L, respectively. The toxicity values for the crustaceans were a 96-hour NOEL (mortality) values of 40 µg/L, and two 96-hour LC50 (mortality) values of 200 and 236,000 µg/L. The single toxicity value for the mollusc was a 96-hour NOEL (mortality, abnormal development) value of 2,800 µg/L. The toxicity values for the single macrophyte species were two 3-day NOEC (length, weight) values both of 2,006 µg/L. As stated in Warne et al. (2015), acute EC10/NOEC and LOEC

[7]The concentration that would cause an approximate 50-percent reduction in the number of eggs developing into normal straight-hinge larvae.

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 MCPA. However, trends may be similar to that of 2,4-D for which factors such as temperature, pH and water hardness have been reported as modifying toxicity (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 MCPA. However, any such effect would be relatively minor given the relatively low log $K_{oc}$ value of MCPA (Table 25).

MCPA comes in three broad forms – the acids, salts and esters, 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).

### 7.2.3 Guideline derivation

The derived PGVs for MCPA 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. The ecotoxicity data for MCPA 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 significantly alter the overall toxicity of the formulation compared to the active ingredient. Therefore, as with all the other pesticides that have PGVs, the PGVs for MCPA are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for MCPA are low (Table 25) 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 MCPA do not need to account for secondary poisoning.

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

| MCPA proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 1.0 (0.018 – 9,500) |
| 95% | 17 (0.51 – 12,000) |
| 90% | 60 (2.3 – 13,000) |
| 80% | 240 (12 – 15,000) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 5 |
| Type of toxicity data | Chronic NOEC/NOEL and converted acute values |
| SSD model fit | Poor |
| 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.**

### 7.2.4 Toxicity data used in derivation

The previous Australian and New Zealand GV (formerly referred to as a trigger value) for MCPA in marine environments was a low reliability value (using the ANZECC and ARMCANZ 2000 reliability scheme) as it was adopted from the freshwater PGV value which was based on one acute toxicity value for a fish species (Warne 2001). This value was calculated using the assessment factor (AF) method, dividing the lowest acute toxicity value for *Cyprinus carpio* of 1,440 µg/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 MCPA 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 MCPA toxicity data available that enable the calculation of PGVs in marine waters. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of MCPA with phototrophic (e.g. plants and algae) marine species be conducted.

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

Based on the current understanding of the mode of action of MCPA, 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 MCPA 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 ($p = 0.545$, see section 7.2.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for MCPA in marine waters.

There were marine chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) toxicity data for only two marine phototrophic and heterotrophic 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 include all the marine toxicity data, there were chronic NOEC/NOEL and converted acute (acute LC50 toxicity data that had been converted to estimates of chronic NOEC/EC10 by dividing by 10) values for five marine phototrophic and heterotrophic species (that belonged to four phyla and four classes), which met the minimum data requirements 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 26) combined with the poor fit of the distribution to these toxicity data (Figure 22) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for MCPA in marine environments is provided in Table 27.

**Table 27 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 MCPA 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 | *Americamysis bahia* | Arthropoda | Malacostraca | <24 hours old | 4 | Converted acute | Mortality | 20 | USEPA (2015b) |
| Macroinvertebrate | *Crassostrea virginica* | Mollusca | Bivalvia | 2 day old larvae | 12 | Chronic NOEC | Mean length | 250 | David and Hidu (1969) |
| Fish | *Menidia menidia* | Chordata | Actinopterygii | Not stated | 4 | Converted acute | Mortality | 17,900 | USEPA (2015b) |
| Macroinvertebrate | *Penaeus duorarum* | Arthropoda | Malacostraca | Juvenile | 4 | Converted acute | Mortality | 23,600 | USEPA (2015b) |
| Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 11,000 | USEPA (2015b) |

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

### 7.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the five marine phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 22.

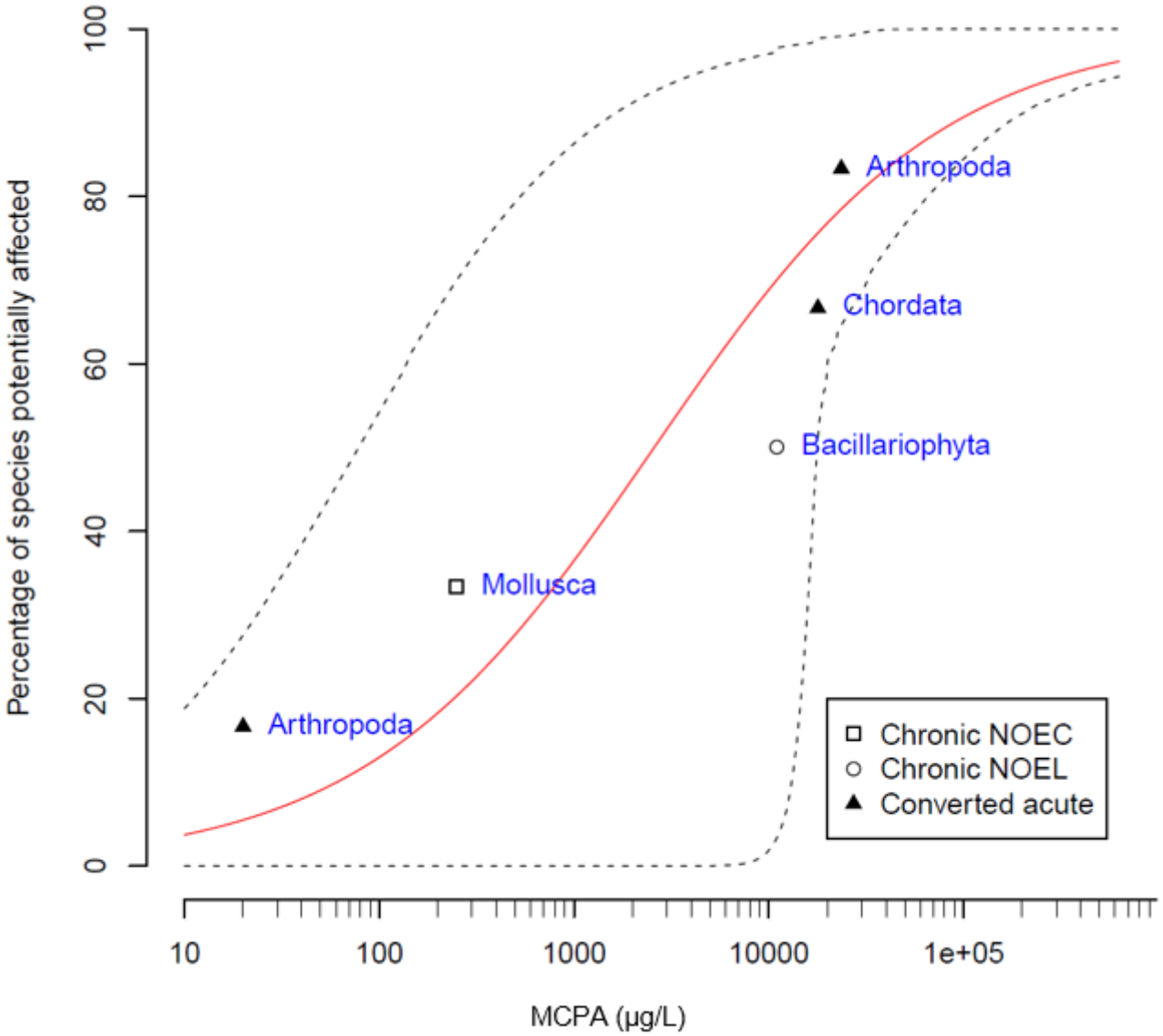



Figure 22 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC), no observed effect level (NOEL) and converted acute data values of marine phototrophic and heterotrophic species to MCPA. Black dashed lines indicate the 95% confidence intervals.

### 7.2.6 Distribution of sensitivities for aquatic species

Statistical analysis of the MCPA 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 MCPA freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.827) and followed a normal distribution (Anderson-Darling; p = 0.527). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.948); therefore, the freshwater and the marine MCPA ecotoxicity data can be pooled for further analysis.

The toxicity data for MCPA 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 MCPA ecotoxicity data may be unimodal (Figure 23).

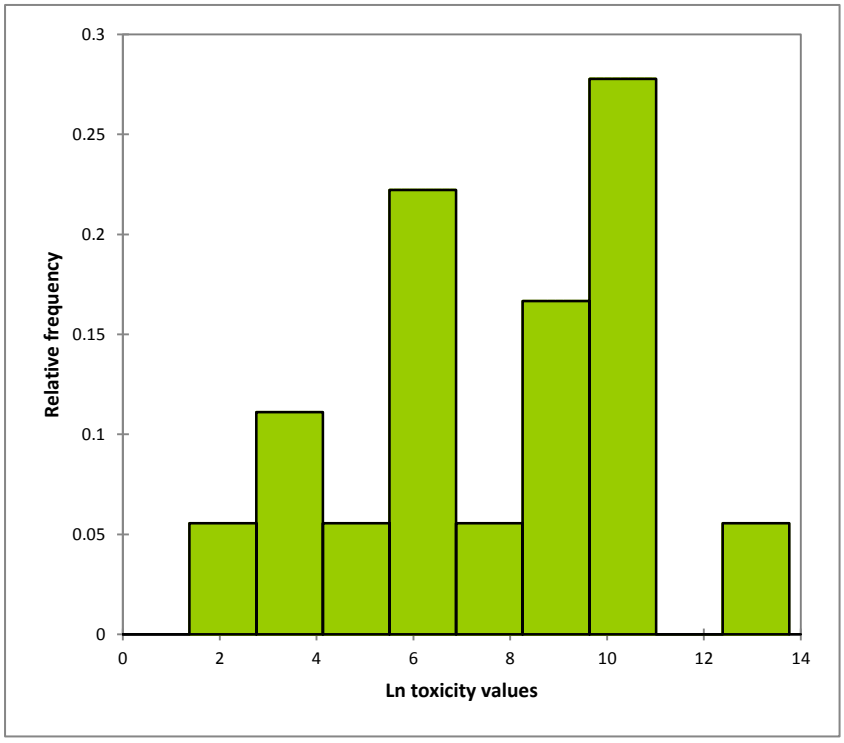



Figure 23 Histogram of the natural logarithm (ln) of all MCPA (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 18).

The MCPA 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 MCPA concentration data had equal variances (Fisher's F-Test; p = 0.725) and followed a normal distribution (Anderson-Darling; p = 0.056). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.545); therefore, it was concluded that the distribution of the MCPA concentration data was uni-modal.

### 7.2.7 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.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 705–709.

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 – MCPA. Canadian Council of Ministers of the Environment. Available from: http://ceqg-rcqe.ccme.ca/download/en/190. Downloaded: 27 March, 2017.

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.

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.

Hiller E, Khun M, Zemanova L, Jurkovic L and Bartal M (2006). Laboratory study of retention and release of weak acid herbicide MCPA by soils and sediments and leaching potential of MCPA, *Plant Soil and Environment*, 52, 550–558.

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

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: 27 March 2017.

USEPA (2015a). ECOTOX User Guide: ECOTOXicology Database System. Version 4.0. United States Environmental Protection Agency. Available from: http://cfpub.epa.gov/ecotox/, Accessed: August–December, 2016.

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: August–December, 2016.

Walters J (1999). Environmental fate of 2,4-dichlorophenoxyacetic acid. Department of Pesticide Regulation, 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, 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.

# 8 Pendimethalin

## 8.1 Introduction

Pendimethalin is a herbicide ($C_{13}H_{19}N_3O_4$ and Figure 24) that at room temperature is in the form of orange-yellow crystals. It is the active ingredient of a variety of commercial herbicide formulations.

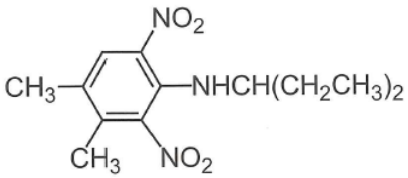



Figure 24 Structure of pendimethalin.

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

**Table 28 Summary of selected physicochemical properties of pendimethalin.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 281.3 amu[1] |
| Aqueous solubility | 0.33 mg/L @ pH 7 and temperature of 20 °C[1] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 5.2[1]<br>5.4 @ pH 7 and temperature of 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 4.243[2] |
| Logarithm of the bioconcentration factor (log BCF) | 3.71[2] |
| Half-life ($t_{1/2}$) in water | <21 days[1]<br>Stable @ pH 4–9[2] |
| Half-life ($t_{1/2}$) in soil | 3–4 months [1]<br>Typical: 182.3 days<br>(100.6–182.3 days in the field and the lab (20 °C), respectively)[2] |

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

Pendimethalin belongs to the dinitroaniline group of herbicides, which also includes benfluralin, isopropalin and trifluralin. Pendimethalin is extensively used in agricultural situations to control most annual grasses and common broad-leaved weeds in a variety of cereals, fruits, vegetables (e.g. wheat, stone fruit, berry fruit, citrus, lettuce, onions, beans, carrots) and other crops (e.g. rice, soya, peanuts, tulips, cotton) (BCPC 2012; University of Hertfordshire 2013). It is also used for the control of suckers/lateral shoots in tobacco (BCPC 2012). Non-agricultural uses include the application of pendimethalin to commercial and industrial situations such as paths, lawns, golf course turfs and Christmas tree plantations (USEPA 1997). Pendimethalin is generally applied as a pre-emergence or early post-emergence herbicide (BCPC 2012).

Pendimethalin is absorbed through the roots and emerging shoots of plants with little to no translocation occurring acropetally (i.e. movement upwards from the base of plants to the apex) (BCPC 2012; Appleby and Valverde 1989). Pendimethalin exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting the assembly of microtubules, a process of plant cell division responsible for chromosome separation and cell wall formation (BCPC 2012; Strandberg and Scott-Fordsmand 2004). Pendimethalin also exerts toxicity on non-target organisms,

by targeting the branchial epithelium in fish and aquatic invertebrates, which can affect gill function resulting in mortality (Abd-Algadir et al. 2011).

Pendimethalin is a selective herbicide which may ultimately end up in surface waters as a result of spray drift, leaching and runoff through irrigation or following heavy rainfall (Strandberg and Scott-Fordsmand 2004). Pendimethalin is essentially immobile and has low capacity to leach to groundwater due to its very high soil sorption characteristics as indicated by its high log $K_{oc}$ value and relatively low solubility in water (Table 28) (USEPA 1997). Pendimethalin is moderately persistent in aerobic soils; however, is less persistent in aquatic environments (Table 28) with an aqueous half-life of less than 21 days (USEPA 1997; BCPC 2012).

## 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 pendimethalin 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, one cladoceran, three macrophytes and three microalgae. The toxicity values for the single fish species were 288-day NOEL and LOEC (mortality) values of 6.3 and 9.8 µg/L, respectively. The toxicity values for the single cladoceran were 21-day NOEL and LOEC (immobilisation) values of 14 and 17 µg/L, respectively. The toxicity values for macrophytes were two 7-day EC10 (frond area) values of 36 and 90 µg/L, two 7-day EC50 (frond area) values of 177.2 and 634 µg/L and 14-day NOEL and EC50 (frond number, dry weight, frond area) values of 5.6 and 12.5 µg/L, respectively. The toxicity values for the microalgae consisted of a 48-hour EC50 (cell growth) value of 26 µg/L, a 72-hour NOEC (cell count) value of 6 µg/L, 72-hour EC50 (cell number, cell count, cell size, area under the growth curve) values ranging from 14 to 25 µg/L, two 120-hour NOEL (biomass yield, growth rate, area under the growth curve) values of 3 and 3.2 µg/L, two 120-hour EC50 (biomass yield, growth rate, area under the growth curve) values of 5.4 and 6.7 µg/L, 16-day NOEC and LOEC (chlorophyll content) values of 250 and 500 µg/L, respectively, and 16-day LOEC (cell count, dry weight, chlorophyll-a content) values all of 150 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for three fish, one cladoceran, one crustacean, one macrophyte and two microalgae. The toxicity values for the fish consisted of 96-hour NOEL (mortality) values ranging from 75 to 320 µg/L and 96-hour LC50 (mortality) values ranging from 138 to 418 µg/L. The toxicity values for the single cladoceran were 48-hour NOEL and EC50 (immobilisation) values of 160 and 280 µg/L, respectively. The single toxicity value for the crustacean was a 96-hour LC50 (mortality) value of 208 µg/L. The toxicity values for the single macrophyte were 4-day EC10 and EC50 (frond area) values of 27 and 85.2 µg/L, respectively. The toxicity values for microalgae were a 24-hour NOEC (cell count) value of 0.68 µg/L and two 24-hour EC50 (cell number, cell counts) values of 2.4 and 900 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.

### 8.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of pendimethalin. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the high $K_{oc}$ value of pendimethalin. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 8.2.3 Guideline derivation

The derived PGVs for pendimethalin 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 PGVs, the PGVs for pendimethalin are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for pendimethalin 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 pendimethalin do not need to account for secondary poisoning.

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

| Pendimethalin proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 1.3 (0.83 – 3.3) |
| 95% | 2.1 (1.5 – 5.3) |
| 90% | 2.9 (2.1 – 7.4) |
| 80% | 4.5 (3.0 – 12) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 8 |
| Type of toxicity data | Chronic NOEC/NOEL/EC10 values |
| SSD model fit | Poor |
| 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".**

### 8.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for pendimethalin in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for pendimethalin 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 pendimethalin toxicity data available that enable the calculation of PGVs in freshwaters. In order to derive higher reliability PGVs in the future, it is recommended that additional

chronic toxicity tests of pendimethalin with phototrophic and heterotrophic freshwater species be conducted.

In total, there were toxicity data for 15 freshwater species (five phyla and six classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The six classes were Actinopterygii (which accounts for approximately 99% of fish), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Liliopsida (monocots) and Malacostraca (a large grouping of crustaceans).

Based on the current understanding of the mode of action of pendimethalin, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as it is a selective herbicide that inhibits the assembly of microtubules, a process essential for plant cell division and elongation (BCPC 2012). However, pendimethalin also targets the branchial epithelium in fish and aquatic invertebrates, which can affect gill function resulting in mortality (Abd-Algadir et al. 2011). The pendimethalin 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 did not have significantly different ($p = 0.348$, see section 8.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for pendimethalin in freshwaters.

There were freshwater chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for eight 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 PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 29) combined with the poor 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 pendimethalin in freshwater environments is provided in Table 30.

**Table 30 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 pendimethalin 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] | 98 | USEPA (2015b) |
| Macroinvertebrate | *Daphnia magna* | Arthropoda | Branchiopoda | Life cycle | 21 | Chronic NOEL | Immobilisation | 14 | USEPA (2015b) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Total frond number, Growth rate, Mortality | 5.6 | USEPA (2015b) |
| Macrophyte | *Lemna minor** | Tracheophyta | Liliopsida | Not stated | 7 | Chronic EC10 | Frond area | 56.93 | Cedergreen and Streibig (2005); Cedergreen et al. (2005) |
| Microalga | *Navicula pelliculosa** | Chlorophyta | Chlorophyacae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 3.2 | USEPA (2015b) |
| Fish | *Pimephales promelas* | Chordata | Actinopterygii | Life cycle | 288 | Chronic NOEL | Mortality | 6.3 | USEPA (2015b) |
| Microalga | *Protosiphon botryoides** | Chlorophyta | Chlorophyacae | Not stated | 16 | Chronic NOEC | Chlorophyll content | 250 | Shabana et al. (2001) |
| Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEC | Biomass yield, growth rate, AUC[2] | 3 | USEPA (2015b) |

**[1] Chronic NOEC/NOEL/EC10 = 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 subcapita*. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 8.2.5 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 25.

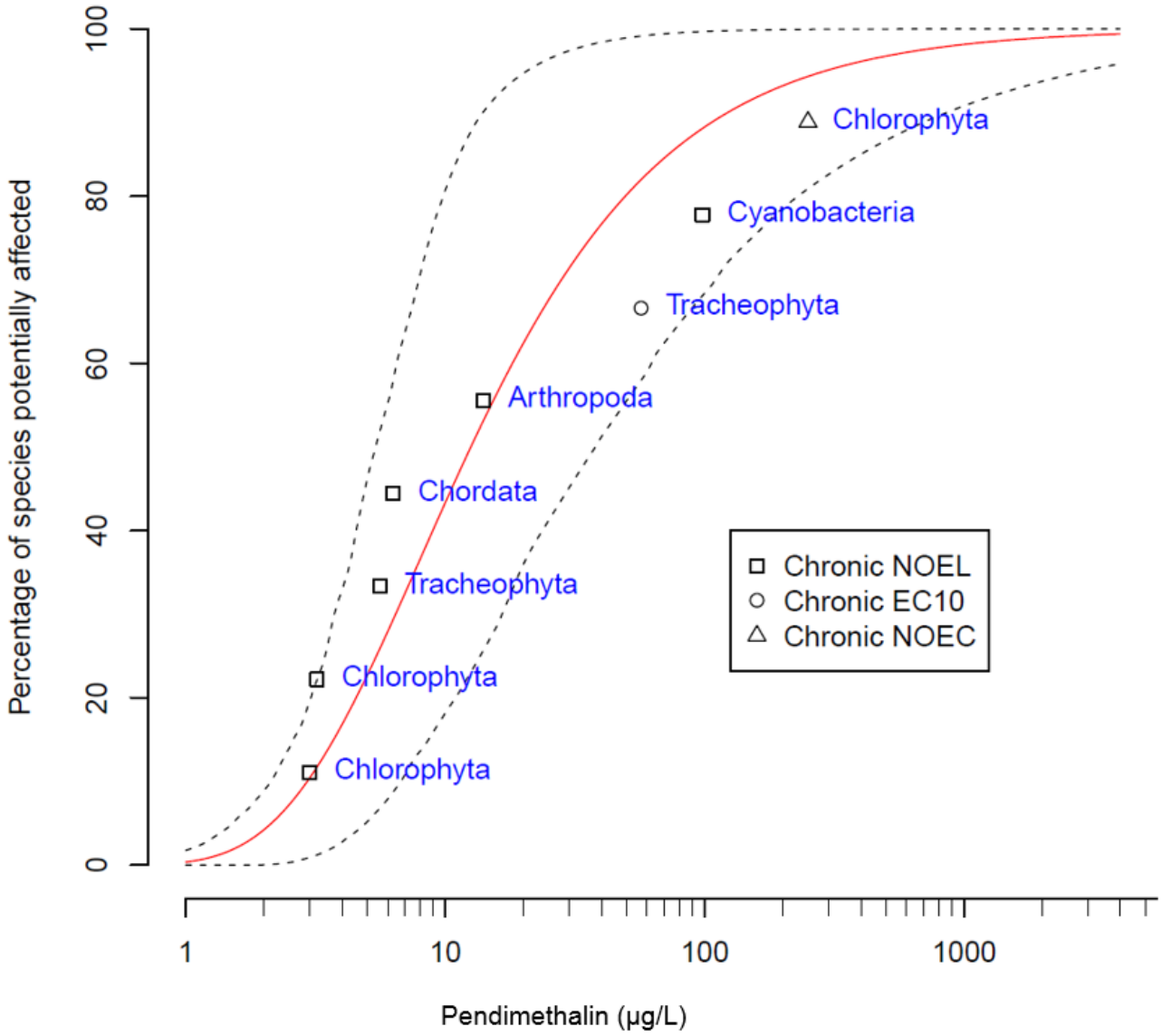



Figure 25 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to pendimethalin. Black dashed lines indicate the 95% confidence intervals.

## 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 pendimethalin in marine waters (Table 32) includes toxicity data for 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 8.2.1, respectively.

*Marine Chronic*

There were marine chronic toxicity data for only one microalga species which consisted of 120-hour NOEL and EC50 (biomass yield, growth rate, area under the curve) values of 0.7 and 5.2 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for one fish, one crustacean and one mollusc. The toxicity values for the single fish species were 96-hour NOEL and LC50 (mortality) values of 200 and 710 µg/L, respectively. The toxicity values for the single crustacean species were 96-hour LOEL and LC50 (mortality) values of 1,000 and 1,600 µg/L, respectively. The toxicity values for the single mollusc species were 48-hour NOEL and EC50 (mortality) values of 60 and 210 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 pendimethalin. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the high $K_{oc}$ value of pendimethalin. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 8.3.3 Guideline derivation

The derived PGVs for pendimethalin 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 PGVs, the PGVs for pendimethalin are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for pendimethalin 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 pendimethalin do not need to account for secondary poisoning.

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

| Pendimethalin proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.24 (0.0022 – 2.0) |
| 95% | 0.97 (0.12 – 4.1) |
| 90% | 1.9 (0.61 – 7.8) |
| 80% | 4.1 (1.5 – 19) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 12 |
| Type of toxicity data | Chronic NOEC/NOEL/EC10 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".**

### 8.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for pendimethalin in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for pendimethalin 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 pendimethalin 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 both marine and freshwater organisms. 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 pendimethalin with marine phototrophic and heterotrophic species be conducted.

In total, there were toxicity data for four marine species (four phyla and four classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Chlorophyta, Chordata and Mollusca. The four 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 freshwater green algae), and Malacostraca (a larger grouping of crustaceans).

Based on the current understanding of the mode of action of pendimethalin, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as it is a selective herbicide that inhibits the assembly of microtubules, a process essential for cell division within plants (BCPC 2012). However, pendimethalin also targets the branchial epithelium in fish and aquatic invertebrates, which can affect gill function resulting in mortality (Abd-Algadir et al. 2011). The pendimethalin 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 did not have significantly different ($p = 0.348$, see section 8.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for pendimethalin in marine waters.

There were chronic no observed effect level (NOEL) and converted acute data available for only four marine species (that belonged to four 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). As no other ecotoxicity data for pendimethalin to marine species were available, the chronic NOEL and converted acute data for marine species were combined with the available chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and NOEL data values for freshwater species to derive PGVs for pendimethalin in marine waters. This dataset incorporated concentration data for 12 (four marine and eight freshwater) species belonging to six 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 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 pendimethalin in marine environments is provided in Table 32.

**Table 32 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 pendimethalin 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 | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 98 | USEPA (2015b) |
| Marine | Macroinvertebrate | *Crassostrea virginica* | Mollusca | Bivalvia | Embryo / Larvae | 2 | Converted acute | Mortality, abnormal development | 21 | USEPA (2015b) |
| Marine | Fish | *Cyprinodon variegatus* | Chordata | Actinopterygii | Not stated | 4 | Converted acute | Mortality | 71 | USEPA (2015b) |
| Fresh | Macroinvertebrate | *Daphnia magna* | Arthropoda | Branchiopoda | Life cycle | 21 | Chronic NOEL | Immobilisation | 14 | USEPA (2015b) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Total frond number, Growth rate, Mortality | 5.6 | USEPA (2015b) |
| Fresh | Macrophyte | *Lemna minor** | Tracheophyta | Liliopsida | Not stated | 7 | Chronic EC10 | Frond area | 56.93 | Cedergreen and Streibig (2005); Cedergreen et al. (2005) |
| Fresh | Microalga | *Navicula pelliculosa** | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 3.2 | USEPA (2015b) |
| Marine | Macroinvertebrate | *Penaeus duorarum* | Arthropoda | Malacostraca | Not stated | 4 | Converted acute | Mortality | 160 | USEPA (2015b) |
| Fresh | Fish | *Pimephales promelas* | Chordata | Actinopterygii | Life cycle | 288 | Chronic NOEL | Mortality | 6.3 | USEPA (2015b) |
| Fresh | Microalga | *Protosiphon botryoides** | Chlorophyta | Chlorophyceae | Not stated | 16 | Chronic NOEC | Chlorophyll content | 250 | Shabana et al. (2001) |
| Fresh | Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEC | Biomass yield, growth rate, AUC[2] | 3 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema costatum** | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 0.7 | USEPA (2015b) |

**[1] Chronic NOEC/NOEL/EC10 = no conversions applied; Converted acute = acute EC50/LC50 values that were converted to chronic NOEC values by dividing by 10 (Warne et al. 2015).**
**[2] AUC = area under the growth curve. [3] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapita*. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 8.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 12 marine and freshwater species that was used to derive the PGVs is presented in Figure 26.

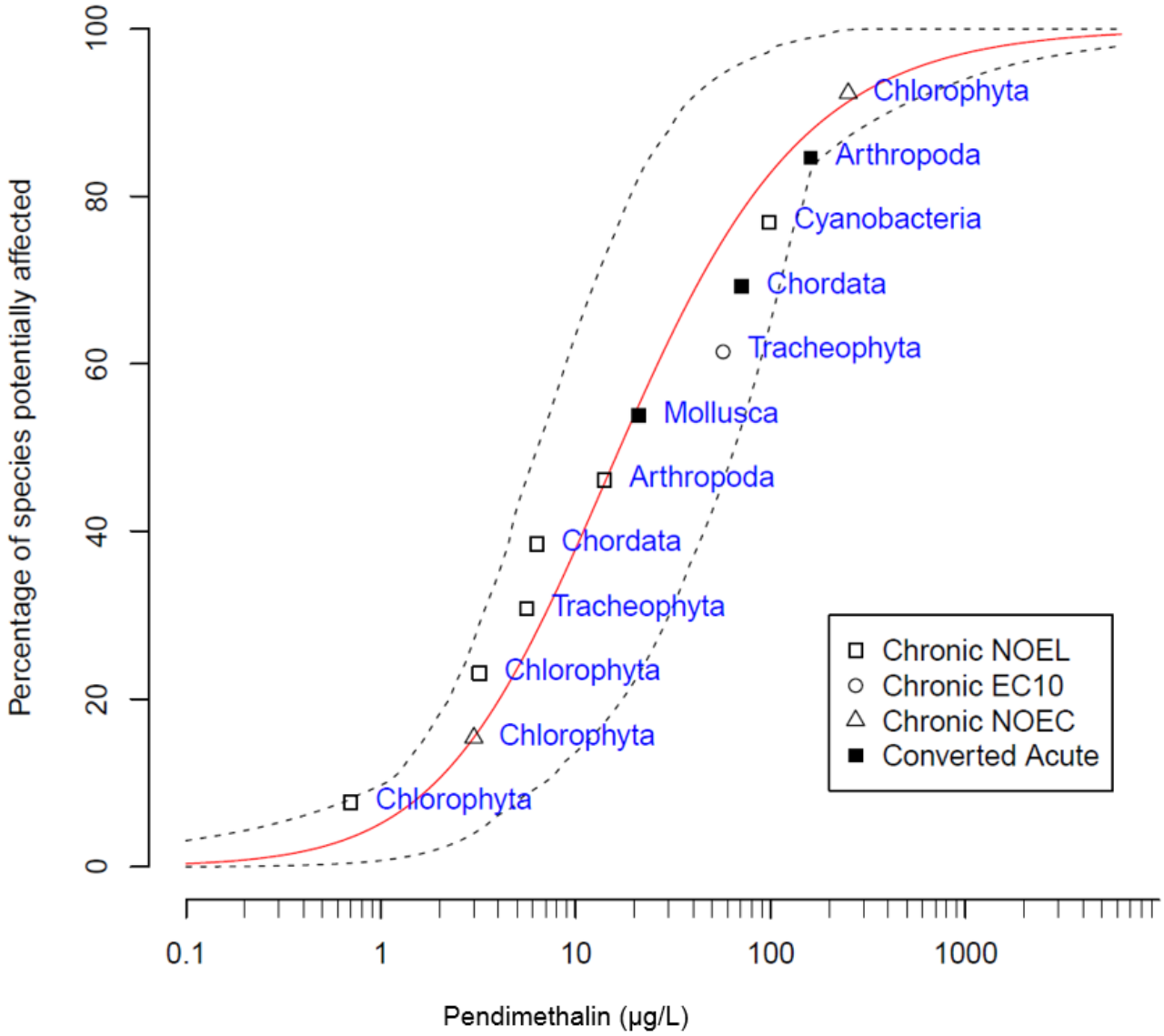



Figure 26 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016,) of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC), no observed effect level (NOEL) and converted acute data values of marine and freshwater, phototrophic and heterotrophic species to pendimethalin. Black dashed lines indicate the 95% confidence intervals.

### 8.3.6 Distribution of sensitivities for aquatic species

Statistical analysis of the pendimethalin 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 pendimethalin freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.348) and followed a normal distribution (Anderson-Darling; p = 0.738). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.756); therefore, the freshwater and the marine pendimethalin ecotoxicity data can be pooled for further analysis.

The toxicity data for pendimethalin 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 pendimethalin ecotoxicity data may be unimodal (Figure 27).

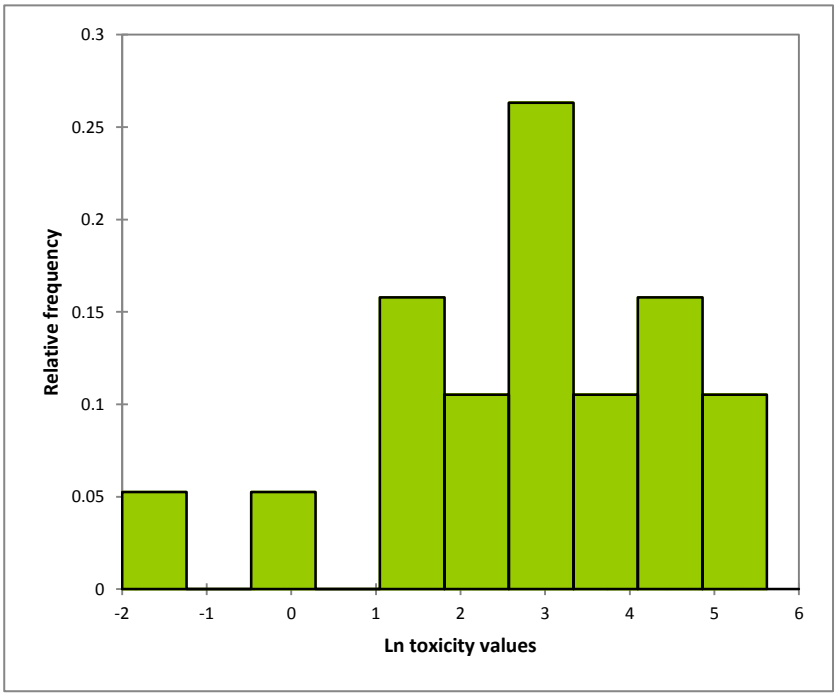



Figure 27 Histogram of the natural logarithm (ln) of all pendimethalin (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 19).

The pendimethalin 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 pendimethalin concentration data successfully met tests for normality (Anderson-Darling; p = 0.738), the data were found to have unequal variances (Fisher's F-Test; p = 0.023). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.348); therefore. it can be concluded that the distribution of the pendimethalin concentration data is uni-modal.

### 8.3.7 References


Abd-Algadir MI, Idris OF and Elkhier MKS (2011). Effect of pendimethalin herbicide on fish (*Tilapia nilotica*) skeletal muscles, gills and its influence on human, *World Journal of Life Science and Medical Research*, 1, 5–10.

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.

Appleby AP and Velverde BR (1989). Behaviour of dinitroaniline herbicides in plants, *Weed Technology*, 3, 198–206.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 862–863.

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/. Last accessed: December 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.

Cedergreen N, Andersen L, Olesen CF, Spliid HH and Streibig JC (2005). Does the effect of herbicide pulse exposure on aquatic plants depend on KOW or mode of action?, *Aquatic Toxicology*, 71, 261–271.

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.

Monteiro SM, Mancera JM, Fontainhas-Fernandes A and Sousa M (2005). Copper induced alterations of biochemical parameters in the gill and plasma of *Oreochromis niloticus*, *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 141, 375–383.

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.

Shabana EF, Battah MG, Kobbia IA and Eladel HM (2001). Effect of pendimethalin on growth and photosynthetic activity of *Protosiphon botryoides* in different nutrient states, *Ecotoxicology and Environmental Safety*, 49, 106–110.

Strandberg M and Scott-Fordsmand J (2004). Effects of pendimethalin at lower trophic levels – a review, *Ecotoxicology and Environmental Safety*, 57, 190–201.

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, 2017.

USEPA (1997). Reregistration Eligibility Decision (RED) for Pendimethalin. United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances (7508W). Available from: https://nepis.epa.gov/Exe/ZyPDF.cgi/20000MQZ.PDF?Dockey=20000MQZ.PDF. Accessed: March, 2017.

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, 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 Prometryn

## 9.1 Introduction

Prometryn is a herbicide ($C_{10}H_{19}N_5S$ and Figure 28) that at room temperature is in the form of a white powder. It is the active ingredient of a variety of commercial herbicide formulations.

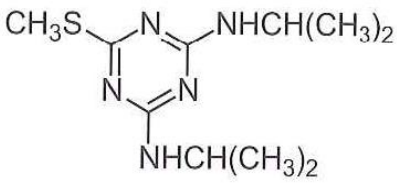



Figure 28 Structure of prometryn.

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

**Table 33 Summary of selected physicochemical properties of prometryn.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 241.4 amu[1] |
| Aqueous solubility | 33 mg/L @ pH 6.7 and temperature 22 °C[1]<br>33 mg/L @ temperature 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 3.1 @ temperature of 25 °C, unionised[1]<br>3.34[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | Average 2.42 (2.05–2.69) [1]<br>2.6[2] |
| Logarithm of the bioconcentration factor (log BCF) | 1.93[2] |
| Half-life ($t_{1/2}$) in water | 5.3 – 10.9 days[1]<br>56 days[2]<br>270 days[3] |
| Half-life ($t_{1/2}$) in soil | 14 – 158 days[1]<br>Typical: 41 days[2]<br>(41 days in the lab @ 20 °C)[2] |

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

Prometryn belongs to the methylthiotriazine group within the triazine family of herbicides, which also includes ametryn, prometryn and terbutryn. Prometryn can be used in agricultural, permanent pasture (as in grazing) and forestry to control annual grasses and broad-leaved weeds as well as grasses (University of Hertfordshire 2013). Prometryn is applied as a pre-emergence herbicide to a variety of crops such as cotton, sunflowers, peanuts, potatoes, carrots, peas and beans, however it can also be applied at post-emergence in cotton, potatoes, carrots, celery and leek (BCPC 2012). Prometryn does not have regulatory approval to be used within the European Union (University of Hertfordshire 2013).

Prometryn is generally 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). Prometryn exerts its toxicity in aquatic plants (including 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 (O2-) and hydrogen peroxide (H2O2) (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 CO2 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).

Prometryn a selective, systemic herbicide that may ultimately end up in aquatic ecosystems as a result of volatilization and leaching via preferential flow pathways (USEPA 1996). Prometryn has low to medium mobility in soil (BCPC 2012) and has the potential to leach to groundwater and move offsite to surface waters (USEPA 1996). Information on the environmental fate of prometryn in water is contradictory; however, reports have suggested it to be relatively persistent in water (USEPA 1996). The half-lives of prometryn in water reportedly range between 5 and 270 days (Table 33).

## 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 prometryn in freshwaters (Table 35) 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 were freshwater chronic toxicity data for one fish, one cladoceran, four macrophytes and six microalgae. The toxicity values for the single fish species were two 32-day NOEL (mortality) values of 0.62 and 0.802 µg/L and two 32-day LOEC (mortality) values of 1.2 and 1.39 µg/L. The toxicity values for the single cladoceran species were 21-day NOEL and LOEC (body length, dry weight) values of 1 and 2 µg/L, respectively. The toxicity values for the macrophytes were 7- and 8-day EC50 (frond count, frond cover/area) values ranging from 13 to 84.5 µg/L and 14-day NOEL and EC50 (total frond number, growth rate, mortality) values of 4 and 11.8 µg/L, respectively. The toxicity values for the microalgae consisted of 48-hour NOEC and IC50 (chlorophyll-a concentration) values of 241.4 and 724.1 µg/L, respectively, a 96-hour NOEL (biomass yield, growth rate, area under the curve) value of 8 µg/L, two 96-hour EC50 (cell counts, chlorophyll-a content) values of 15.94 and

21 µg/L, two 5-day NOEL (biomass yield, growth rate, area under the curve) values of 0.3 and 20.2 µg/L, two 5-day EC50 (biomass yield, growth rate, area under the curve) values of 1 and 40.1 µg/L, 6-, 7-, 8- and 14-day NOEC/EC10 (average cell number, area under the growth curve, cell density) values ranging from 6.9 to 34.8 µg/L, 6-, 7-, 8- and 14-day LOEC (area under the growth curve) values ranging from 10.3 to 33.8 µg/L and 6-, 7- and 8-day EC50 (average cell number, cell density) values ranging from 17.7 to 31.5 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for three fish, one crustacean, one cladoceran, one macrophyte, four microalgae and one bacteria. The toxicity values for the fish were two 96-hour NOEL (mortality) values of 560 and 5,600 µg/L, a 96-hour LOEL (mortality) value of 560 µg/L and 96-hour LC50 (mortality) values ranging from 2,900 to 10,000 µg/L. The toxicity values for the single crustacean species were 24-, 48-, 72- and 96-hour LC50 (mortality) values of 95,800, 37,600, 17,600 and 14,400 µg/L, respectively. The toxicity values for the single cladoceran species were 48-hour LOEL and EC50 (body length, dry weight) values of 10,000 and 18,590 µg/L, respectively. The toxicity values for the single macrophyte species were 3-day EC20 and EC50 (frond count) values of 35.7 and 69.9 µg/L, respectively and 6-day EC20 and EC50 (frond count) values of 28.5 and 53.8 µg/L, respectively. The toxicity values for microalgae consisted of 24-day NOEC/EC10 (cell density, cell number) values ranging from 0.82 to 2,400 µg/L, a 24-hour LOEC (cell density) value of 241.4 µg/L, 24-hour EC50/IC50 (cell count, cell number, cell density) ranging from 12.5 to 4,300 µg/L. The toxicity values for the single bacteria species were LOEC and IC50 (cell density) values of 0.1 and 1.3 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 prometryn. 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 prometryn (Table 33).

### 9.2.3 Guideline derivation

The derived PGVs for prometryn 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 PGVs, the PGVs for prometryn are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for prometryn 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 prometryn do not need to account for secondary poisoning.

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

| Prometryn proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.094 (0.0040 – 3.1) |
| 95% | 0.49 (0.044 – 4.9) |
| 90% | 1.0 (0.12 – 6.6) |
| 80% | 2.3 (0.41 – 9.4) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 7 |
| Type of toxicity data | Chronic NOEC/NOEL values |
| SSD model fit | Poor |
| 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".**

### 9.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for prometryn in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for prometryn 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 prometryn toxicity data available that enable the calculation of PGVs in freshwaters. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of prometryn 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 prometryn in freshwaters, *Chlorococcum sp.* and *Lyngbya sp.*, were included as no other toxicity data for these genera were used.

In total, there were toxicity data for 22 freshwater species (eight phyla and ten classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Ciliophora, Cryptophyta, 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), Cryptophyceae (an algae grouping), Cyanophyceae (a class of cyanobacteria), Liliopsida (monocots), malacostraca (a large grouping of crustaceans), Prostomatea (a grouping of protozoans) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of prometryn, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The prometryn ecotoxicity data for phototrophs and heterotrophs were therefore 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 9.3.6) 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 for seven phototrophic species (that belonged to six 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 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 prometryn in freshwater environments is provided in Table 35.

**Table 35 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for prometryn 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] | 20.2 | USEPA (2015b) |
| Cyanobacteria | *Anabaena variabilis* | Cyanobacteria | Cyanophyceae | Not stated | 2 | Chronic NOEC | Chlorophyll-a concentration | 241.4 | Hawxby et al. (1977) |
| Microalga | *Cryptomonas sp.* | Cryptophyta | Cryptophyceae | >23 days old | 7 | Chronic NOEC | AUC[2] | 7.4 | Liebig et al. (2008) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | 7-11 days old | 14 | Chronic NOEL | Frond number, dry weight, frond area | 4 | USEPA (2015b) |
| Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 0.3 | USEPA (2015b) |
| Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | 6-8 days | 4 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 8 | USEPA (2015b) |
| Microalga | *Urotricha furcata* | Ciliophora | Prostomatea | Not stated | 8 | Chronic NOEC | Cell number, AUC[2], cell density | 15.2 | Liebig et al. (2008) |

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

### 9.2.5 Species sensitivity distribution

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

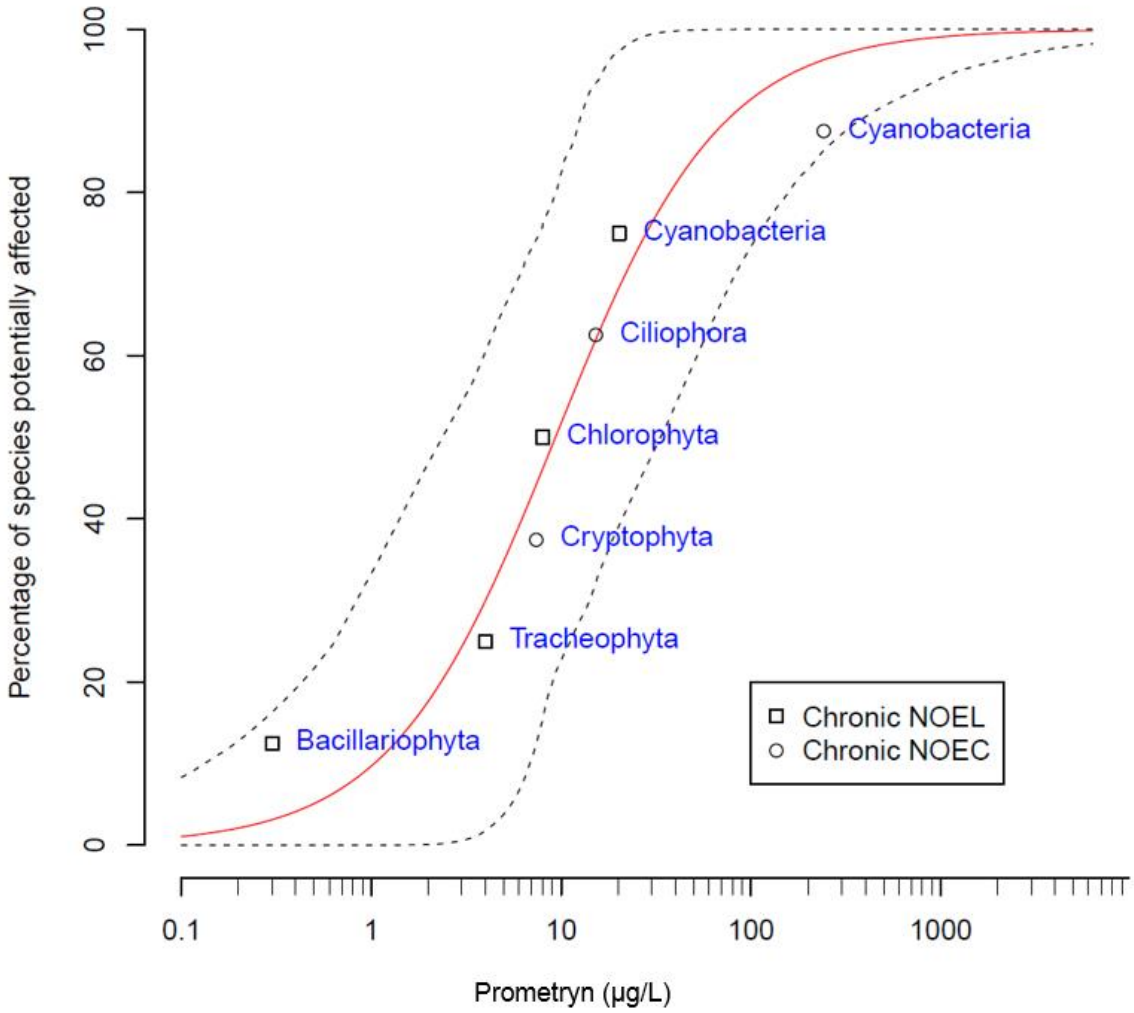



Figure 29 Cumulative frequency distribution generated using BurrliOz 2.0 (2016) of the sensitivity of chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic species to prometryn. Black dashed lines indicate the 95% confidence intervals.

## 9.3 Marine

### 9.3.1 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 prometryn in marine waters (Table 36) 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 9.2.1, respectively.

*Marine Chronic*

There were marine chronic toxicity data for two microalgae which consisted of a 96-hour EC50 (chlorophyll-a content) value of 53 µg/L and 5-day NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 2.22 and 7.6 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for one fish, one crustacean and one mollusc. The toxicity values for the single fish species were 96-hour NOEL and LC50 (mortality) values of 880 and 5,100 µg/L, respectively. The toxicity values for the single crustacean species were 96-hour LOEL and LC50 (mortality) values of 650 and 2,320 µg/L, respectively. The toxicity values for the single mollusc species were NOEL and EC50 (mortality, abnormal development) values of 16,000 and 21,000 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.

### 9.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of prometryn. 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 prometryn (Table 33).

### 9.3.3 Guideline derivation

The derived PGVs for prometryn 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 PGVs, the PGVs for prometryn are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for prometryn 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 prometryn do not need to account for secondary poisoning.

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

| Prometryn proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.11 (0.0030 – 3.2) |
| 95% | 0.52 (0.053 – 4.6) |
| 90% | 1.1 (0.18 – 5.6) |
| 80% | 2.2 (0.48 – 7.9) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 9 |
| Type of toxicity data | Chronic NOEC/NOEL and chronic estimated NOEC values (*freshwater and marine*) |
| SSD model fit | Poor |
| 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".**

### 9.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for prometryn in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for prometryn 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 prometryn 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. 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 prometryn with phototrophic (e.g. plants and algae) marine water 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 prometryn in marine waters, *Chlorococcum sp.* and *Lyngbya sp.*, were included as no other toxicity data for these genera were used.

In total, there were toxicity data for 5 marine species (five phyla and five classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata and Mollusca. The five classes were Actinopterygii (which accounts for approximately 99% of fish), Bivalvia (a grouping of molluscs), Chlorophyceae (a major grouping of freshwater green algae), Malacostraca (a large grouping of crustaceans) and Mediophyceae (another algae grouping).

Based on the current understanding of the mode of action of prometryn, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The prometryn ecotoxicity data for phototrophs and heterotrophs were therefore 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 9.3.6) 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 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 only two phototrophic 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). As no other ecotoxicity data for prometryn to marine phototrophic species was available, the chronic NOEL and chronic estimated NOEC values for marine phototrophic species were combined with the available chronic NOEC/NOEL data for freshwater phototrophic species (see section 9.2) to derive PGVs for prometryn in marine waters. This dataset incorporated concentration data for nine phototrophic species belonging to six 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 36) combined with the poor fit of the distribution to these toxicity data (Figure 30) resulted in a low 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 prometryn in marine environments is provided in Table 37.

**Table 37 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for prometryn 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 NOEL | Biomass yield, growth rate, AUC[3] | 20.2 | USEPA (2015b) |
| Fresh | Cyanobacteria | *Anabaena variabilis* | Cyanobacteria | Cyanophyceae | Not stated | 2 | Chronic NOEC | Chlorophyll-a concentration | 241.4 | Hawxby et al. (1977) |
| Fresh | Microalga | *Cryptomonas sp.* | Cryptophyta | Cryptophyceae | >23 days old | 7 | Chronic NOEC | AUC[3] | 7.4 | Liebig et al. (2008) |
| Marine | Microalga | *Dunaliella tertiolecta* | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic est. NOEC | Chlorophyll-a concentration | 10.6 | Gaggi et al. (1995) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | 7-11 days old | 14 | Chronic NOEL | Frond number, dry weight, frond area | 4 | USEPA (2015b) |
| Fresh | Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[3] | 0.3 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[3] | 2.22 | USEPA (2015b) |
| Fresh | Microalga | *Selenastrum capricornutum*[2] | Chlorophyta | Chlorophyceae | 6-8 days | 4 | Chronic NOEL | Biomass yield, growth rate, AUC[3] | 8 | USEPA (2015b) |
| Fresh | Microalga | *Urotricha furcata* | Ciliophora | Prostomatea | Not stated | 8 | Chronic NOEC | Cell number, AUC[3], cell density | 15.2 | Liebig et al. (2008) |

**[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 *Raphidocelis subcapitata* and is currently called *Pseudokirchneriella subcapitata*. [3]AUC = area under the growth curve. *Species that originated from/is distributed in Australia and/or New Zealand.**

### 9.3.5 Species sensitivity distribution

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

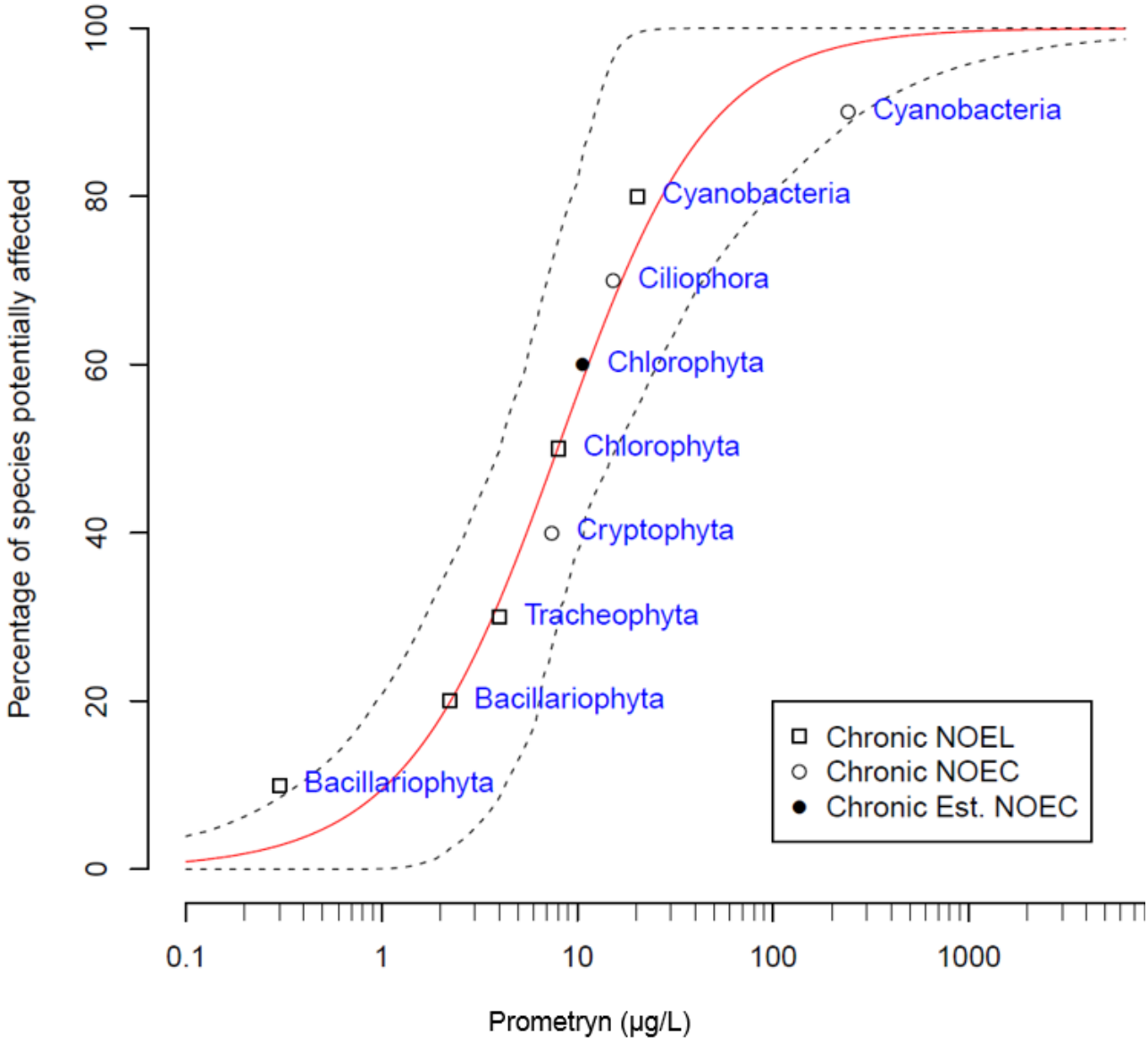



Figure 30 Cumulative frequency distribution generated using Burrlioz 2.0 (2016) of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of marine and freshwater phototrophic species to prometryn. Black dashed lines indicate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

### 9.3.6 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 [-0.833 and 5.314 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater phototrophic species (*n* = 16). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for prometryn.

The toxicity data for prometryn 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 prometryn ecotoxicity data may be bimodal (Figure 31).

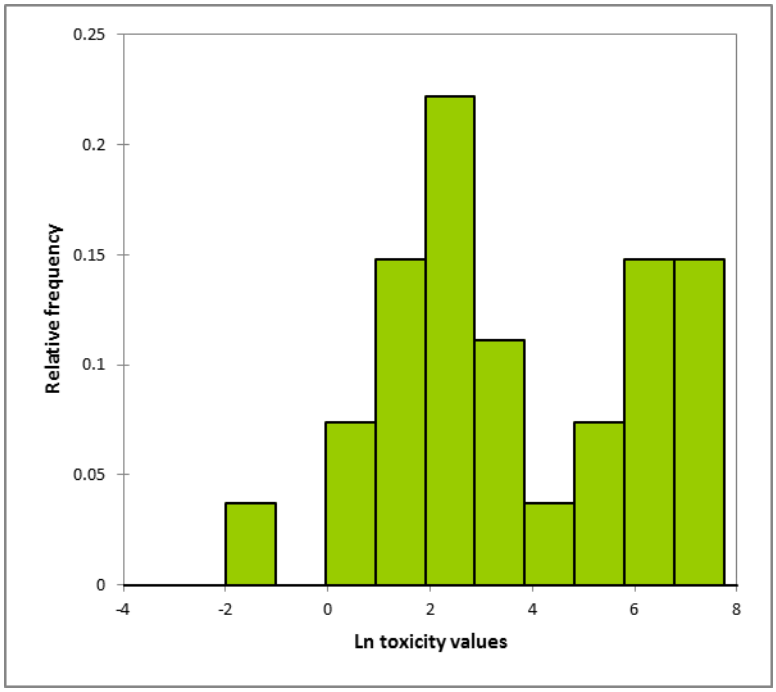



Figure 31 Histogram of the natural logarithm (ln) of all prometryn (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 32).

The prometryn 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 prometryn concentration data successfully met tests for normality (Anderson-Darling; p = 0.071), the data were found to have unequal variances (Fisher's F-Test; p = 0.029). 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 prometryn concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

### 9.3.7 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.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 929–930.

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 Biochemis*try 52, 38–51.

Gaggi C, Sbrilli G, El Naby AMH, Bucci M, Duccini M, and Bacci E (1995). Toxicity and hazard ranking of s-triazine herbicides using microtox, two green algal species and a marine crustacean. *Environmental Toxicology and Chemistry*, 14, 1065-1069.

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.

Hawxby K, Tubea B, Ownby J and Basler E (1977). Effects of various classes of herbicides on four species of algae. *Pesticide Biochemistry and Physiology*, 7, 203–209.

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.

Liebig M, Schmidt G, Bontje D, Kooi BW, Streck G, Traunspurger W and Knacker, T (2008). Direct and indirect effects of pollutants on algae and algivorous ciliates in an aquatic indoor microcosm. Aquatic Toxicology, 88, 102–110.

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 (1996). Reregistration Eligibility Decision (RED) for Prometryn. United States Environmental Protection Agency, Prevention, Pesticides and Toxic Substances (7508W). Available from: https://archive.epa.gov/pesticides/reregistration/web/pdf/0467.pdf. Accessed: March, 2017.

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.

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.

# 10 Propazine

## 10.1 Introduction

Propazine is a herbicide ($C_9H_{16}ClN_5$ and Figure 32) that at room temperature is in the form of a white powder. It is the active ingredient of a variety of commercial herbicide formulations.

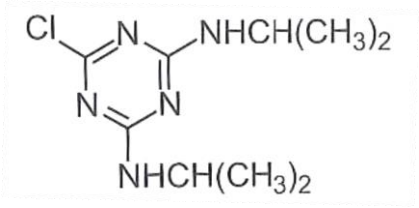



Figure 32 Structure of propazine.

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

**Table 38 Summary of selected physicochemical properties of propazine.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 229.7 amu[1] |
| Aqueous solubility | 5.0 mg/L @ temperature of 20 °C[1]<br>8.6 mg/L @ temperature of 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 3.01[1]<br>3.95 @ pH 7 and temperature 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 1.81–2.43 (8 soil types)[1]<br>2.19[2] |
| Logarithm of the bioconcentration factor (log BCF) | 1.79[2] |
| Half-life ($t_{1/2}$) in water | 83 days @ pH 7 and temperature of 20 °C[2] |
| Half-life ($t_{1/2}$) in soil | 80–100 days[1]<br>Typical: 131 days<br>(135 days in the lab @ temperature 20 °C)[2] |

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

Propazine 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 shoots and leaves (BCPC 2012). Propazine exerts its toxicity in aquatic plants (including 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).

Propazine is a selective, systemic herbicide which may ultimately end up in aquatic ecosystems as a result of leaching and runoff through irrigation or following heavy rainfall (Worthing 1983 cited in Cornell University 1993). Propazine is moderately mobile in soils and has weak sorption ability as indicated by its low log $K_{oc}$ value (Table 38) (University of Hertfordshire 2013). Compared to other triazine herbicides, propazine reportedly has the greatest potential for leaching into groundwater as it binds weakly to soil particles and has the potential to come unbound given the right soil temperature, pH and moisture conditions (Cornell University 1993). Propazine is relatively persistent in water and soil (Table 38) under normal environmental conditions, only being hydrolysed by acids and alkalis at elevated temperatures (University of Hertfordshire 2013).

## 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 propazine in freshwaters (Table 40) 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 were freshwater chronic toxicity data for one fish, one cladoceran, one macrophyte and three microalgae. The toxicity data for the fish were 36-day NOEL and LOEC (mortality) values of 1,340 and 2,590 µg/L, respectively. The toxicity values for the single cladoceran species were 21-day NOEL and LOEC (immobilisation) values of 47 and 91 µg/L. The toxicity values for the single macrophyte species were 14-day NOEL and EC50 (frond number, dry weight, frond area) values of 22 and 100 µg/L. The toxicity values for microalgae consisted of 5-day NOEL (biomass yield, growth rate, area under the growth curve) values ranging from 6.5 to 68 µg/L, respectively, and 5-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 25 to 180 µg/L, respectively.

*Freshwater Acute*

There were freshwater acute toxicity data for one fish, one cladoceran and one microalga. The single toxicity value for the fish species was a 96-hour NOEL (mortality) value of 4,500 µg/L. The single toxicity value for the cladoceran species was a 48-hour NOEL (immobilization) value of 5,320 µg/L. The toxicity values for the single microalga species were 24-hour NOEC and EC50 (cell count) values of 6.5 and 71.1 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.

### 10.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of propazine. 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 propazine (Table 38).

### 10.2.3 Guideline derivation

The derived PGVs for propazine 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 PGVs, the PGVs for propazine are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for propazine 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 propazine do not need to account for secondary poisoning.

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

| Propazine proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 1.3 (0.59 – 6.5) |
| 95% | 3.1 (1.9 – 9.7) |
| 90% | 4.5 (3.2 – 12) |
| 80% | 6.8 (6.8 – 18) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 5 |
| Type of toxicity data | Chronic NOELs and a converted acute value |
| SSD model fit | Poor |
| 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".**

### 10.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for propazine in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for propazine 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 propazine toxicity data available that enable the calculation of PGVs in freshwaters. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of propazine with phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were toxicity data for six freshwater species (five phyla and five classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Cyanobacteria and Tracheophyta. The five classes were 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 propazine, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The propazine ecotoxicity data for phototrophs and heterotrophs were therefore 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 ($p = 0.011$, see section 10.3.6) 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 available for four phototrophic species (that belonged to four 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). Therefore, the dataset was expanded to include the available converted acute (acute EC50/LC50 toxicity data that had been converted to estimates of chronic NOEC/EC10 by dividing by 10) data for freshwater phototrophic species to derive PGVs for propazine in freshwaters. This dataset included concentration data for five phototrophic freshwater species belonging to four phyla and four classes, which met the minimum data requirements to use a SSD to PGVs (Warne et al. 2015). The number of species and taxa used to derive the PGVs (Table 39) combined with the poor fit of the distribution to these toxicity data (Figure 33) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for propazine in freshwater environments is provided in Table 40.

**Table 40 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for propazine 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] | 68 | USEPA (2015b) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 22 | USEPA (2015b) |
| Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 6.5 | USEPA (2015b) |
| Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | Autospores | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 12 | USEPA (2015b) |
| Microalga | *Scenedesmus vacuolatus* | Chlorophyta | Chlorophyceae | Not stated | 1 | Converted acute | Cell count | 7.11 | Faust et al. (2001) |

**[1] Chronic NOEL = no conversions applied; Converted Acute = acute EC50 value that was converted to chronic NOEC/NOEL/EC10 values by dividing by 10 (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/is distributed in Australia and/or New Zealand.**

### 10.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the five phototrophic freshwater species that was used to derive the PGVs is presented in Figure 33.

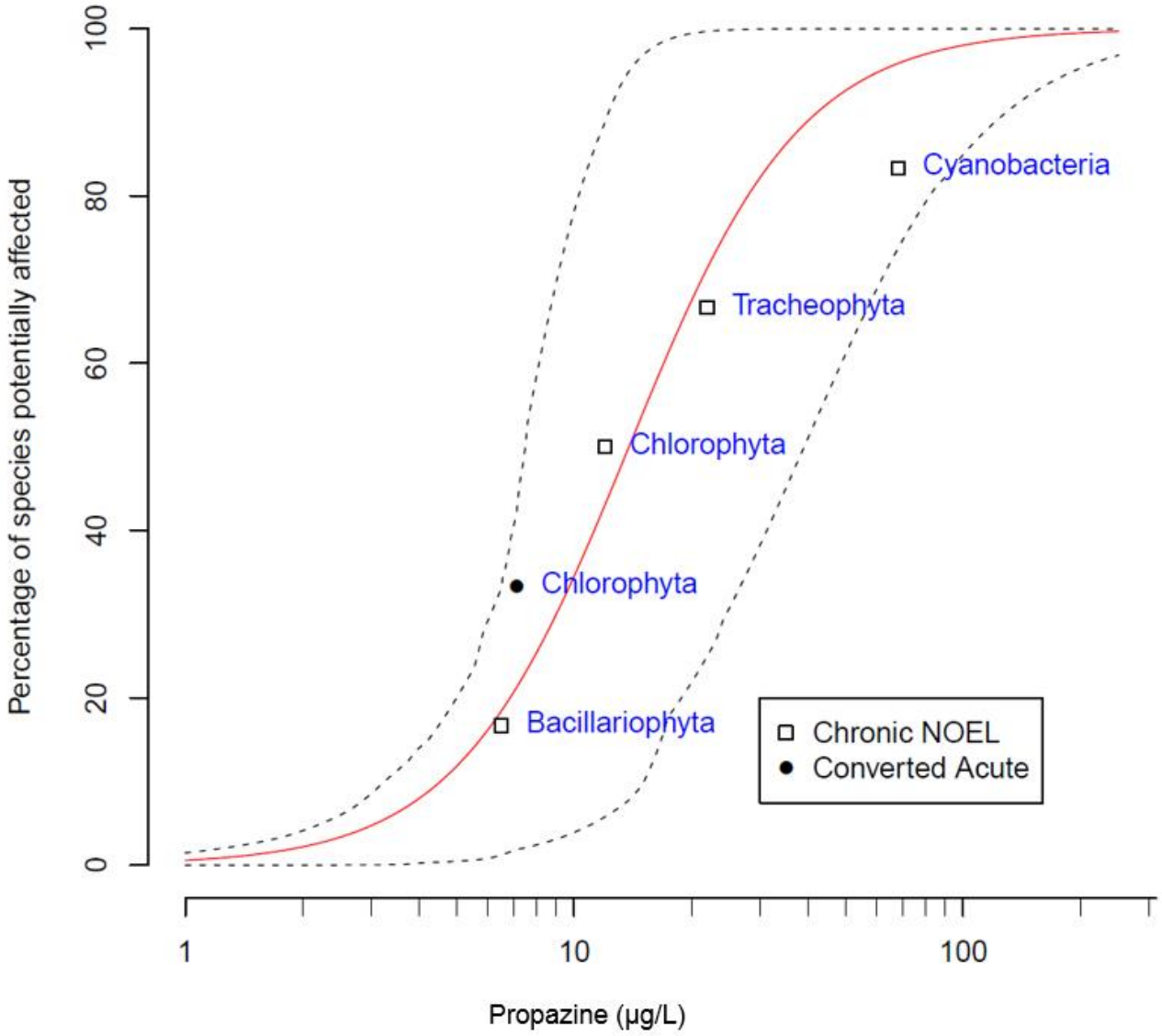



Figure 33 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect level (NOEL) and converted acute data values of freshwater phototrophic species to propazine. Black dashed lines indicate the 95% confidence intervals.

## 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 propazine in marine waters (Table 42) 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 10.2.1, respectively.

*Marine Chronic*

There were marine chronic toxicity data for one fish, one crustacean and one microalga. The toxicity values for the single fish species were 36-day NOEL and LOEC (mortality) values of 1,340 and 2,590 µg/L, respectively. The toxicity values for the single crustacean species were 28-day NOEL and LOEC (mortality) values of 269 and 706 µg/L. The toxicity values for the single microalga species were NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 17 and 25 µg/L.

*Marine Acute*

The single toxicity value for a mollusc species was a 96-hour NOEL (mortality, abnormal development) value of 3,720 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 propazine. 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 propazine (Table 38).

### 10.3.3 Guideline derivation

The derived PGVs for propazine in marine waters are provided in Table 41. 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 PGVs, the PGVs for propazine are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for propazine 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 propazine do not need to account for secondary poisoning.

**Table 41 Proposed aquatic ecosystem protection guideline values (µg/L) for propazine for the protection of marine ecosystems.**

| Propazine proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 2.2 (0.92 – 10) |
| 95% | 4.6 (2.4 – 14) |
| 90% | 6.4 (3.4 – 16) |
| 80% | 9.2 (5.5 – 22) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 5 |
| Type of toxicity data | Chronic NOEL values *(freshwater and marine)* |
| SSD model fit | Poor |
| 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".**

### 10.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for propazine in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for propazine 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 propazine 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. 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 propazine with marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

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

Based on the current understanding of the mode of action of propazine, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The propazine ecotoxicity data for phototrophs and heterotrophs were therefore 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 ($p = 0.011$, see section 10.3.6) 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 level (NOEL) 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 propazine to marine phototrophic species was available, the chronic NOEL value for the marine phototrophic species was combined with the

chronic NOEL values for freshwater phototrophic species (see section 10.2) to derive PGVs for propazine in marine waters. This dataset incorporated concentration data for five 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 41) combined with the poor fit of the distribution to these toxicity data (Figure 34) resulted in a low 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 propazine in marine environments is provided in Table 42.

**Table 42 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for propazine 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 NOEL | Biomass yield, growth rate, AUC[2] | 68 | USEPA (2015b) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 22 | USEPA (2015b) |
| Fresh | Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 6.5 | USEPA (2015b) |
| Fresh | Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 12 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 17 | USEPA (2015b) |

**[1] Chronic 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/is distributed in Australia and/or New Zealand.**

### 10.3.5 Species sensitivity distributions

The cumulative frequency (species sensitivity) distribution (SSD) of the five phototrophic marine and freshwater species that was used to derive the PGVs is presented in Figure 34.

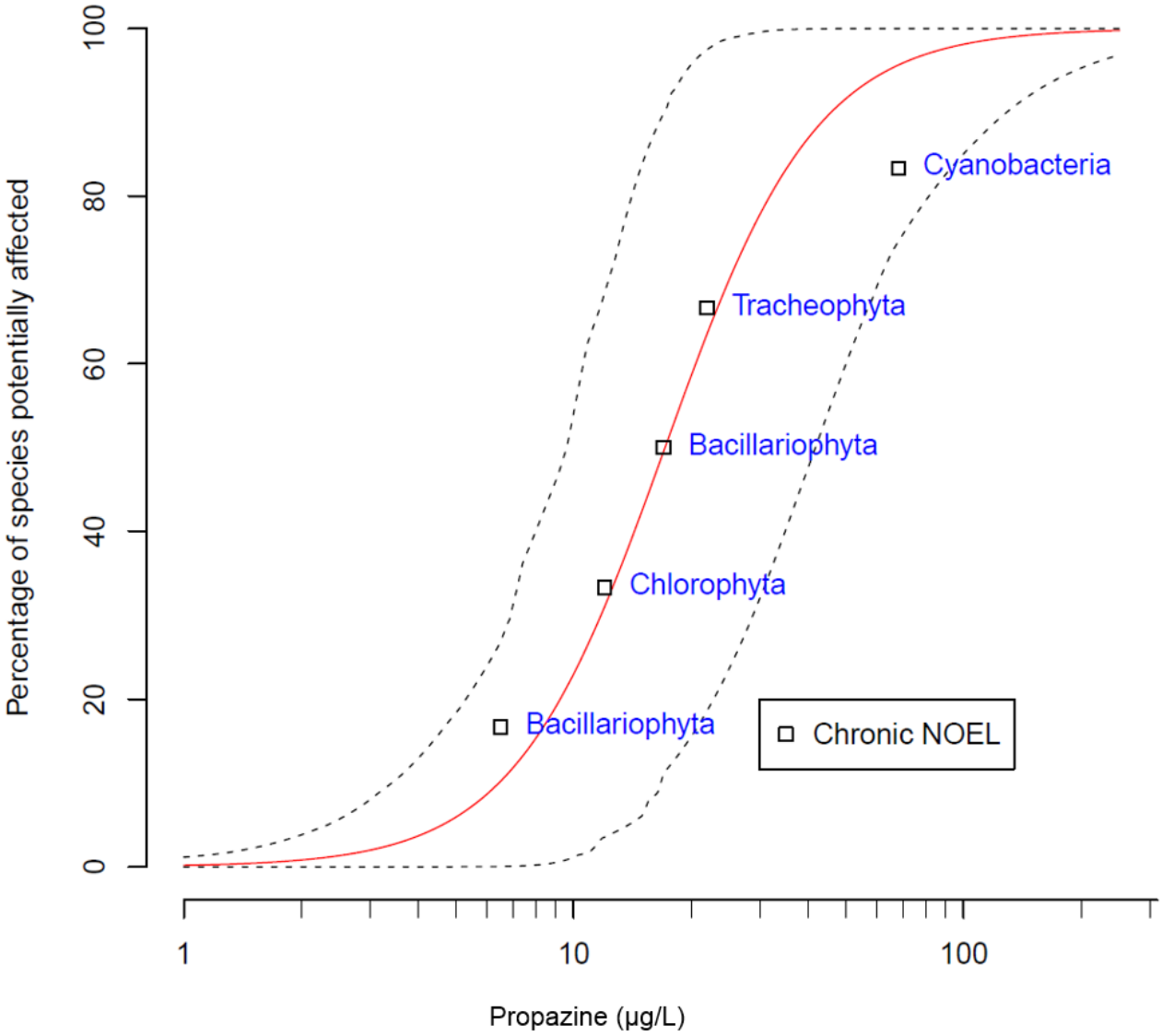



Figure 34 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic no observed effect level (NOEL) data values of marine and freshwater phototrophic species to propazine. Black dashed lines indicate the 95% confidence intervals.

### 10.3.6 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 [0.832 and 4.620 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater phototrophic species (*n* = 5). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for propazine.

The toxicity data for propazine 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 propazine ecotoxicity data may be bimodal (Figure 35).

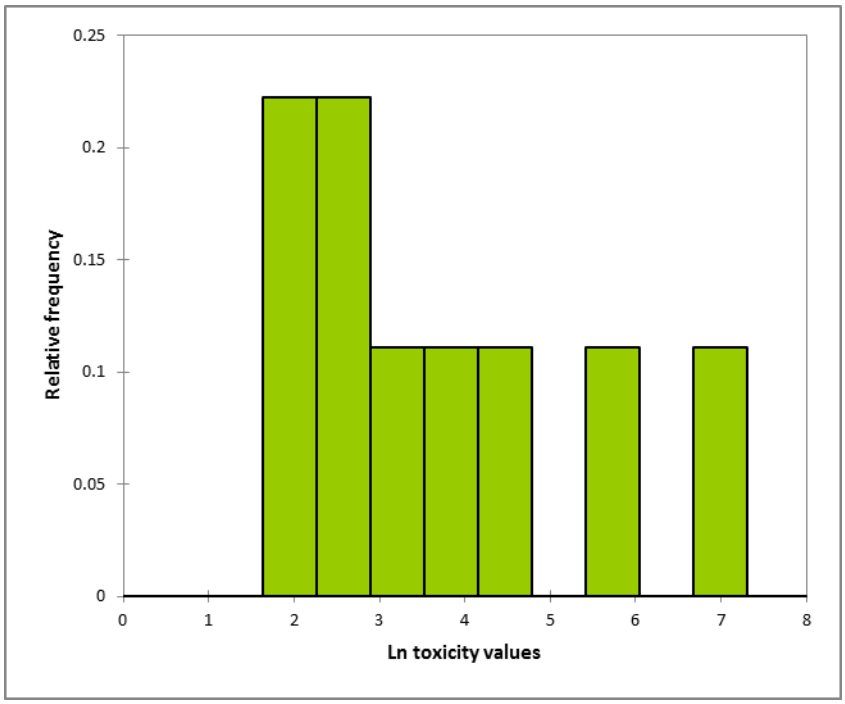



Figure 35 Histogram of the natural logarithm (ln) of all propazine (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 9).

The propazine 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 propazine concentration data had equal variances (Fisher's F-Test; p = 0.203) and followed a normal distribution (Anderson-Darling; p = 0.05). Results from the two-sample *t* test indicated that the two groups were significantly different (p = 0.011); therefore, it was concluded that the distribution of the propazine concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

### 10.3.7 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.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 939–941.

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 Biochemis*try 52, 38–51.

Cornell University (1993). EXTOXNET (Extension Toxicology Network): Propazine, Developed by the Pesticide Management Education Program (PMEP), Cornell University, 1993. Available from: http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/propazine-ext.html#20.

Faust M, Altenburger R, Backhaus T, Blanck H, Boedeker W, Gramatica P, Hamer V, Scholze M, Vighi M and Grimme LH (2001). Predicting the joint algal toxicity of multi-component s-triazine mixtures at low-effect concentrations of individual toxicants, Aquatic Toxicology, 56, 13–32.

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.

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.

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 Propiconazole

## 11.1 Introduction

Propiconazole is a fungicide ($C_{15}H_{17}Cl_2N_3O_2$ and Figure 36) that at room temperature is in the form of a yellow, odourless, viscous liquid. It is the active ingredient of a variety of commercial fungicide formulations.

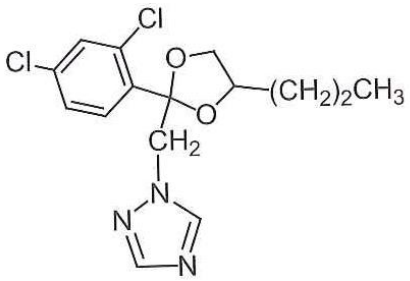



Figure 36 Structure of propiconazole.

Physicochemical properties of propiconazole that may affect its environmental fate and toxicity are presented in Table 43.

**Table 43 Summary of selected physicochemical properties of propiconazole.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 342.2 amu[1] |
| Aqueous solubility | 100 mg/L @ temperature of 20 °C[1]<br>150 mg/L @ temperature of 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 3.72 @ pH 6.6 and temperature 25 °C[1]<br>3.72 @ pH 7 and temperature of 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 950 mL/g (ads)[1]<br>3.04[2] |
| Logarithm of the bioconcentration factor (log BCF) | 2.06[2] |
| Half-life ($t_{1/2}$) in water | Stable up to 100 °C; hydrolysed slowly in acidic and alkaline media.<br>53.5 days @ pH 7 and temperature 20 °C[2] |
| Half-life ($t_{1/2}$) in soil | 15 days @ temperature 16 °C, 5 days @ temperature 29 °C, can vary greatly with microbial activity and moisture content of soil[1]<br>Typical: 214 days<br>(90 – 214 days in the lab @ temperature 20 °C and in the field, respectively)[2] |

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

Propiconazole belongs to the triazole group within the conazole family of fungicides, which also includes azaconazole, hexaconazole and myclobutanil. Propiconazole is extensively used in agricultural situations for the control of diseases amongst a variety of crops such as mushrooms, corn, peanuts, almonds, oats and some fruits (University of Hertfordshire 2013). Non-agricultural uses include the application of propiconazole to turf and remedial wood preservatives (i.e. anti-sap stain, wood joinery and remedial wood treatment) (PMRA 2011).

Propiconazole is both, a systemic and foliar fungicide (University of Hertfordshire 2013; BCPC 2012). Following application, systemic translocation of propiconazole is transported acropetally (i.e. movement upwards from the roots to the foliage or from lower leaves to upper leaves) in the xylem

(BCPC 2012; Cornell University 1997). Propiconazole is also readily absorbed by plant tissues, providing protective and curative action when applied to leaves (BCPC 2012). Propiconazole exerts its toxicity by binding to and inhibiting the 14-α-demethylase enzyme which is present in the plasma (cell) membrane of target organisms (BCPC 2012; AgChemAccess 2015). The 14-α-demethylase enzyme plays an essential role in the biosynthesis of steroids in eukaryotes – specifically, ergosterol for fungi (AgChemAccess 2015). The ergosterol biosynthesis pathway is fungal-specific and is required for the generation and stabilization of fungal plasma (cell) membranes (Sanglard 2002). Therefore, when the 14-α-demethylase enzyme is inhibited, ergosterol is no longer produced within cell walls which effectively slows or stops the growth of fungus (Cornell University 1997). As a result, propiconazole effectively prevents further infection and/or invasion of host fungal-tissues amongst plants (Cornell University 1997).

In addition to preventing the growth of fungi, propiconazole has been suggested to also target a variety of other taxa such as algae, cladocerans and bivalves (Ochoa-Acuña et al. 2009; Bringolf et al. 2007).

## 11.2 Freshwater

### 11.2.1 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 propiconazole in freshwaters (Table 45) includes toxicity data for five 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 eight microalgae. The toxicity data for the single fish species were 21-day NOEC and LOEC (fecundity) values of 53 and 563 µg/L, respectively. The toxicity values for the single cladoceran species were 8-day NOEC and LOEC (length) values of 500 and 1,000 µg/L, respectively and 21-day NOEL and LOEC (immobilisation) values of 310 and 690 µg/L. The toxicity values for the single macrophyte species were 14-day LOEL and EC50 (frond number, dry weight, frond area) values of 2,590 and 9,020 µg/L, respectively. The toxicity data for microalgae consisted of 72-hour IC10 and IC50 (cell density) values of 6.8 and 390 µg/L, 96-hour NOEC (cell density) values ranging between 50 and 5,000 µg/L, 96-hour LOEC (cell density) values ranging between 100 and 10,000 µg/L, 96-hour EC50 (cell density) values ranging between 1,290 and 27,970 µg/L, a 9-day NOEL (biomass yield, growth rate, area under the growth curve) value of 511 µg/L, two 9-day EC50 (biomass yield, growth rate, area under the growth curve) values of 716 and 1,500 µg/L, two 11-day NOEL (biomass yield, growth rate, area under the growth curve) values of 51 and 2,940 µg/L and 11-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 93 to 13,580 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for five fish, two crustaceans, one mollusc, one cladoceran and two microalgae. The toxicity data for the fish were 96-hour NOEL and NOEC (mortality) values of 320 and 2,000 µg/L, respectively, a 96-hour LOEC (mortality) value of 4,000 µg/L and 96-hour

LC50 (mortality) values ranging between 850 and 5,700 µg/L. The toxicity values for the crustaceans consisted of 24-, 48- and 72-hour LC50 (mortality) values of 11,805.9, 7699.5 and 6707.1 µg/L, respectively, a 96-hour NOEL (mortality) value of 1,600 µg/L, a 96-hour LC5 (mortality) value of 3,384 µg/L and 96-hour LC50 (mortality) values ranging from 4,703 to 49,000 µg/L. The toxicity values for the single mollusc species were 24-, 48- and 96-hour EC50 (ability to attach to host, survival) values of 20.8, 19.2 and 10 µg/L, respectively. The toxicity values for the single cladoceran were 24-hour LC10 and LC50 (mortality) values of 4,300 and 9,500 µg/L, respectively, 48-hour NOEL and LOEL (immobilisation) values of 560 and 3,700 µg/L, respectively, two 48-hour LC10 (mortality) values of 630 and 1,200 µg/L, 48-hour LC50/EC50 (mortality, immobilisation) ranging between 4,800 and 11,499.8 µg/L, 72-hour LC10 and LC50 (mortality) values of 530 and 6,800 µg/L, respectively, 96-hour NOEC and LOEC (length) values of 500 and 1,000 µg/l, respectively and 96-hour LC10 and LC50 (mortality) values of 2.7 and 180 µg/L. The toxicity values for the microalgae were 24-hour EC50 (cell division, cell volume) values ranging from 171.1 and 34,562.2 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.

### 11.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of propiconazole. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the relatively high $K_{oc}$ value of propiconazole. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 11.2.3 Guideline derivation

The derived PGVs for propiconazole in freshwaters are provided in Table 44. 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 PGVs, the PGVs for propiconazole are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for propiconazole are low (Table 43) 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 propiconazole do not need to account for secondary poisoning.

**Table 44 Proposed aquatic ecosystem protection guideline values (µg/L) for propiconazole for the protection of freshwater ecosystems.**

| Propiconazole proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 3.7 (0.018 – 32) |
| 95% | 10 (0.90 – 52) |
| 90% | 18 (3.5 – 81) |
| 80% | 35 (9.6 – 190) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 10 |
| Type of toxicity data | Chronic NOEC/NOEL/IC10 values |
| SSD model fit | Poor |
| 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.**

### 11.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for propiconazole in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for propiconazole 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 propiconazole toxicity data available that enable the calculation of PGVs in freshwaters; however, no toxicity data are available for the target species, fungi. Despite this, Maltby et al. (2009) states that there is no evidence to suggest that the PGVs derived using non-fungal species pose a risk to aquatic fungi. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of propiconazole with freshwater species (particularly fungi) be conducted.

In total, there were toxicity data for 21 freshwater species (seven phyla and eight classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria, Mollusca and Tracheophyta. The eight classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Bivalvia (a class of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Liliopsida (monocots) and Malacostraca (a large grouping of crustaceans).

Based on the current understanding of the mode of action of propiconazole, a sterol demethylation (ergosterol biosynthesis) inhibitor, it would be expected that heterotrophic species, particularly fungi, would be more sensitive than phototrophic species, as the ergosterol biosynthesis pathway is fungal-specific and is required for generation of a major constituent of the fungal plasma membrane. Notwithstanding the acknowledged absence of fungi toxicity data in the database, the propiconazole 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 did not have significantly different ($p = 0.248$, see section

11.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for propiconazole in freshwater.

There were freshwater chronic 10% inhibition concentration (IC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for ten 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 PGVs (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 44) combined with the poor fit of the distribution to these toxicity data (Figure 37) resulted in a moderate reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for propiconazole in freshwater environments is provided in Table 45.

**Table 45 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 propiconazole 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 | *Anabaena flos-aquae* | Cyanobacteria | Cyanophyceae | Not stated | 11 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 2,940 | USEPA (2015b) |
| Microalga | *Chlorella pyrenoidosa*[3]* | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEC | Cell density | 100 | Ma et al. (2008) |
| Macroinvertebrate | *Daphnia magna* | Arthropoda | Branchiopoda | Life cycle | 21 | Chronic NOEL | Immobilisation | 310 | USEPA (2015b) |
| Microalga | *Microcystis aeruginosa** | Cyanobacteria | Cyanophyceae | Not stated | 4 | Chronic NOEC | Cell density | 2,000 | Ma et al. (2008) |
| Microalga | *Microcystis flos-aquae** | Cyanobacteria | Cyanophyceae | Not stated | 4 | Chronic NOEC | Cell density | 2,000 | Ma et al. (2008) |
| Microalga | *Navicula seminulum** | Bacillariophyta | Bacillariophycidae | Not stated | 11 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 51 | USEPA (2015b) |
| Fish | *Pimephales promelas* | Chordata | Actinopterygii | 5-6 months | 21 | Chronic NOEC | Fecundity | 53 | Skolness et al. (2013) |
| Microalga | *Pseudokirchneriella subcapitata*[4] | Chlorophyta | Chlorophyceae | Not stated | 3 | Chronic IC10 | Cell density | 6.8 | Ochoa-Acuña et al. (2009) |
| Microalga | *Scenedesmus obliquus** | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEC | Cell density | 50 | Ma et al. (2008) |
| Microalga | *Scenedesmus quadricauda* | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEC | Cell density | 100 | Ma et al. (2008) |

**[1] Chronic NOEC/NOEL/IC10 = no conversions applied (Warne et al. 2015). [2] AUC = area under the growth curve. [3] This species has been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*. [4] This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 11.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the ten freshwater, phototrophic and heterotrophic freshwater species that was used to derive the PGVs is presented in Figure 37.

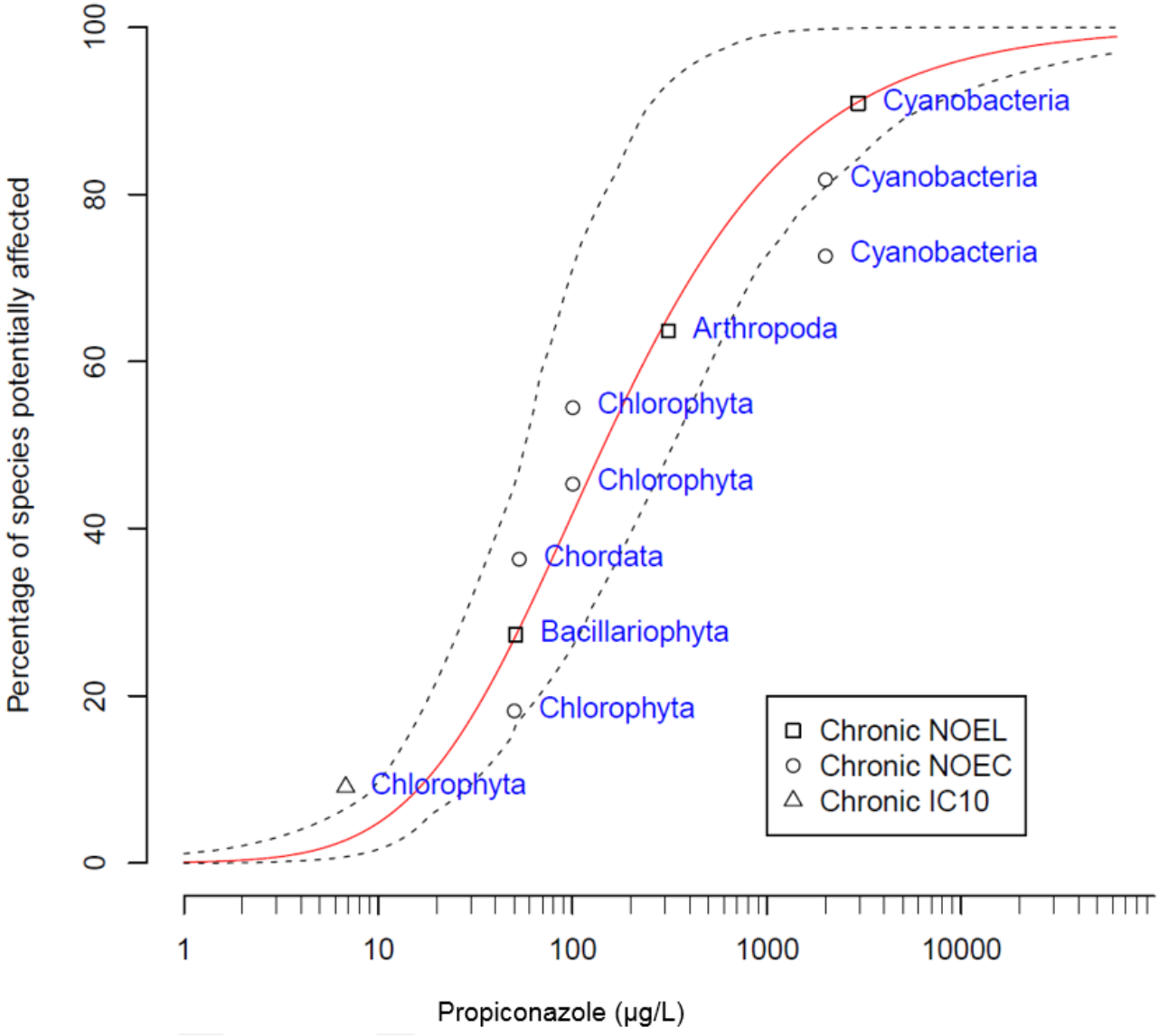



Figure 37 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic 10% inhibition concentration (IC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to propiconazole. Black dashed lines indicate the 95% confidence intervals.

## 11.3 Marine

### 11.3.1 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 propiconazole in marine waters (Table 47) includes toxicity data for one marine 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 marine species that passed the screening and quality assurance processes are provided below.

*Marine Chronic*

There were marine chronic toxicity data for one fish and two microalgae. The toxicity data for the single fish species were 100-day NOEL and LOEC (mortality) values of 150 and 290 µg/L, respectively. The toxicity data for the microalgae were a 96-hour NOEC (cell count) value of 375 µg/L, two 96-hour LOEC (cell count, cell volume) values of 750 and 2,330 µg/L, respectively, a 96-hour EC50 (cell count) value of 2,330 µg/L and 11-day LOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 18 and 21 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for one crustacean and one mollusc. The toxicity data for the single crustacean species were 96-hour NOEL and LC50 (mortality) values of 158 and 510 µg/L, respectively. The toxicity data for the single mollusc species were 48- and 96- EC50 (mortality, abnormal development) values of 3,400 and 1,700 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.

### 11.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of propiconazole. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the relatively high $K_{oc}$ value of propiconazole. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 11.3.3 Guideline derivation

The derived PGVs for propiconazole in marine waters are provided in Table 46. 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 PGVs, the PGVs for propiconazole are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for propiconazole are low (Table 43) 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 propiconazole do not need to account for secondary poisoning.

**Table 46 Proposed aquatic ecosystem protection guideline values (µg/L) for propiconazole for the protection of marine ecosystems.**

| Propiconazole proposed aquatic exosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 2.1 (0.19 – 83) |
| 95% | 8.2 (1.1 – 120) |
| 90% | 15 (2.1 – 130) |
| 80% | 30 (4.3 – 150) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 5 |
| Type of toxicity data | Chronic NOEC/NOEL, chronic estimated NOEC and converted acute values |
| SSD model fit | Poor |
| 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.**

### 11.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for propiconazole in marine or freshwater environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for propiconazole 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 propiconazole toxicity data available that enable the calculation of PGVs in marine waters; however, no toxicity data are available for the target species, fungi. Despite this, Maltby et al. (2009) states that there is no evidence to suggest that the PGVs derived using non-fungal species pose a risk to aquatic fungi. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of propiconazole with marine species (particularly fungi) be conducted.

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

Based on the current understanding of the mode of action of propiconazole, a sterol demethylation (ergosterol biosynthesis) inhibitor, it would be expected that heterotrophic species, particularly fungi, would be more sensitive than phototrophic species, as the ergosterol biosynthesis pathway is fungal-specific and is required for generation of a major constituent of the fungal plasma membrane. Notwithstanding the acknowledged absence of fungi toxicity data in the database, the propiconazole 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 did not have significantly different ($p = 0.248$, see section 11.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for propiconazole in marine water.

There were chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data for two 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 by dividing by 2.5 and 5, respectively) and converted acute (acute EC50/LC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 10) values, there were five species belonging to four phyla and four 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 46) combined with the poor fit of the distribution to these toxicity data (Figure 38) resulted in a low reliability set of PGVs. A summary of the toxicity data (one value per species) used to calculate the PGVs for propiconazole in marine environments is provided in Table 47.

**Table 47 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 propiconazole 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 | *Americamysis bahia* | Arthropoda | Malacostraca | Not stated | 4 | Converted acute | Mortality | 51 | USEPA (2015b) |
| Macroinvertebrate | *Crassostrea virginica* | Arthropoda | Malacostraca | SPAT | 4 | Converted acute | Cell density | 170 | USEPA (2015b) |
| Fish | *Cyprinodon variegatus* | Chordata | Actinopterygii | Early life stage | 100 | Chronic NOEL | Mortality | 150 | USEPA (2015b) |
| Microalga | *Dunaliella tertiolecta* | Chlorophyta | Chlorophyceae | Logarithmic growth phase | 4 | Chronic NOEC | Cell count/density | 375 | Baird and DeLorenzo (2010) |
| Microalga | *Skeletonema costatum** | Chlorophyta | Chlorophyacae | Not stated | 11 | Chronic est. NOEC | Biomass yield, growth rate, AUC[2] | 5.5 | USEPA (2015b) |

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

### 11.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the five marine, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 38.

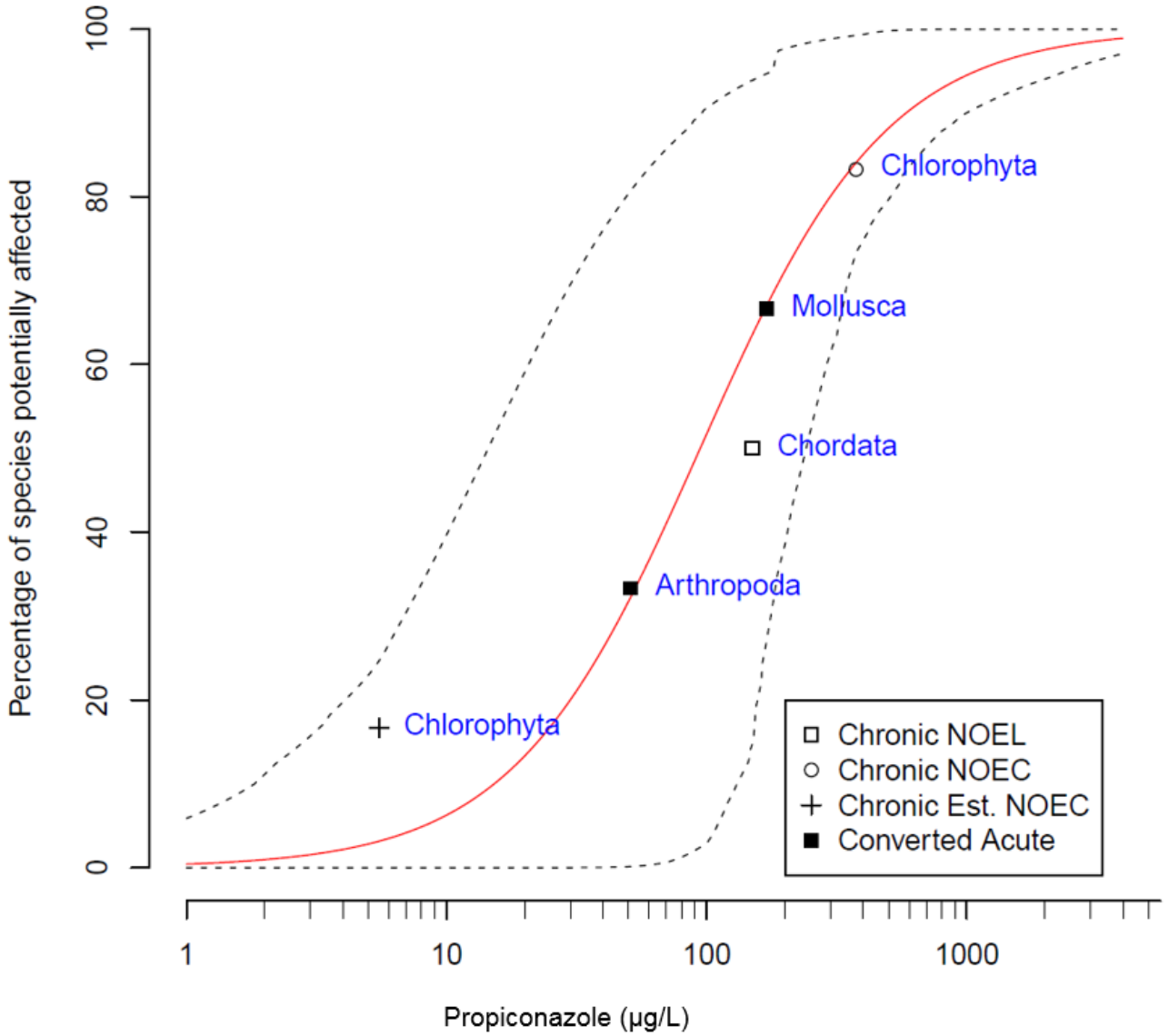



Figure 38 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated no observed effect concentration (NOEC) and no observed effect level (NOEL) with converted acute data values of marine phototrophic and heterotrophic species to propiconazole. Black dashed lines indicate the 95% confidence intervals.

### 11.3.6 Distribution of sensitivities for aquatic species

Statistical analysis of the propiconazole 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 propiconazole freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.989) and followed a normal distribution (Anderson-Darling; p = 0.887). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.191); therefore, the freshwater and the marine propiconazole ecotoxicity data can be pooled for further analysis.

The toxicity data for propiconazole 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 propiconazole ecotoxicity data may be unimodal (Figure 39).

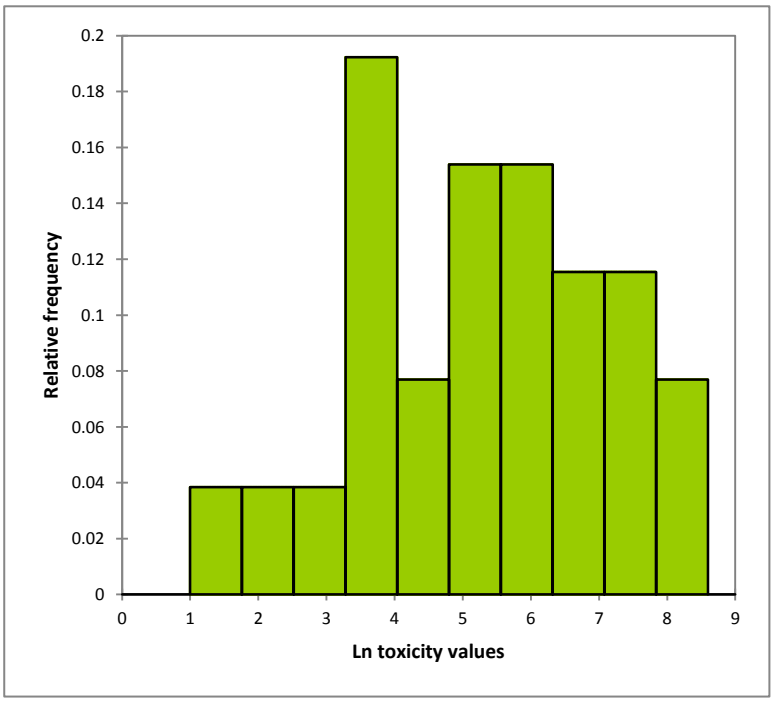



Figure 39 Histogram of the natural logarithm (ln) of all propiconazole (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 26).

The propiconazole 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 propiconazole concentration data successfully met tests for normality (Anderson-Darling; p = 0.887), the data were found to have unequal variances (Fisher's F-Test; p = 0.047). Results from the Mann-Whitney test indicated that the two groups were not significantly different (p = 0.248); therefore, it can be concluded that the distribution of the propiconazole concentration data is uni-modal.

### 11.3.7 References


AgChemAccess (2015). Propiconazole. AgChemAccess Limited, Norfolk, UK. Available from: http://www.agchemaccess.com/Propiconazole . Accessed: November, 2016.

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.

Baird TD and DeLorenzo ME (2010). Descriptive and mechanistic toxicity of conazole fungicides using the model test alga *Dunaliella tertiolecta* (Chlorophyceae), Environmental Toxicology, 25, 213–220.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 942–944.

Bringolf RB, Cope GW, Eads CB, Lazaro PR, Barnhart CM and Shea D (2007). Acute and chronic toxicity of technical-grade pesticides to glochidia and juveniles of freshwater mussels (Unionidae), *Environmental Toxicology and Chemistry*, 26, 2086–2093.

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/.

Cornell University (1997). EXTOXNET (Extension Toxicology Network): Propiconazole, Developed by the Pesticide Management Education Program (PMEP), Cornell University, 1997. Available from: http://pmep.cce.cornell.edu/profiles/extoxnet/metiram-propoxur/propiconazole-ext.html, Accessed 7 April 2017.

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.

Ma J, Chen J, Wang P and Tong S (2008). Comparative sensitivity of eight freshwater phytoplankton species to isoprocarb, propargite, flumetralin and propiconazol, Polish Journal of Environmental Studies, 17, 525–529.

Maltby L, Brock TCM and van der Brink PJ (2009). Fungicide risk assessment for aquatic ecosystems: importance of interspecific variation, toxic mode of action and exposure regime, *Environmental Science and Technology*, 43, 7556–7563.

Ochoa-Acuña HG, Bialkowski W, Yale G and Hahn L (2009). Toxicity of soybean rust fungicides to freshwater algae and *Daphnia magna*, *Ecotoxicology*, 18, 440–446.

PMRA (2011). Proposed re-evaluation decision: Propiconazole, Health Canada Pesticide Management Regulatory Agency, Ontario, Canada, PRVD2011-02. Available from: http://pesticidetruths.com/wp-content/uploads/2013/06/Health-Canada-propiconazole-2011-02-04-Proposed-Re-Evaluation-Decision-PRVD2011-02.pdf, Accessed: 6 April 2017.

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.

Sanglard S (2002). Resistance of human fungal pathogens to antifungal drugs, *Current Opinion in Microbiology*, 5, 379–385.

Skolness SY, Blanksma CA, Cavallin JE, Churchill JJ, Durhan EJ, Jensen KM, Johnson RD, Kahl MD, Mak EA (2013). Propiconazole inhibits steroidogenesis and reproduction in the fathead minnow (*Pimephales promelas*), Toxicological Sciences, 132, 284–297.

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.

# 12 Terbuthylazine

## 12.1 Introduction

Terbuthylazine is a triazine herbicide ($C_9H_{16}ClN_5$ and Figure 40) that at room temperature is in the form of a colourless and rancid smelling powder. It is the active ingredient of a variety of commercial herbicide formulations.

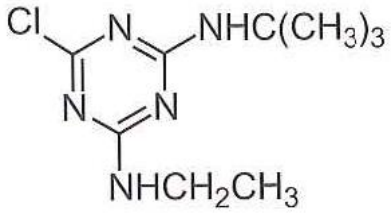



Figure 40 Structure of terbuthylazine.

Physicochemical properties of terbuthylazine that may affect its environmental fate and toxicity are presented in Table 48.

**Table 48 Summary of selected physicochemical properties of terbuthylazine.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 229.7 amu[1] |
| Aqueous solubility | 9 mg/L @ pH 7.4 and temperature 25 °C[1]<br>6.6 mg/L @ temperature 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 3.4 @ temperature of 25 °C [1]<br>3.4 @ pH 7 and temperature 20 °C[2]<br>3.01[3] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 2.43–2.76 (range of soil orders)[3] |
| Logarithm of the bioconcentration factor (log BCF) | 1.53[2] |
| Half-life ($t_{1/2}$) in water | Stable @ pH 7–9 and temperature 20°C; 73 days @ pH 5 and temperature 50 °C[2] |
| Half-life ($t_{1/2}$) in soil | 6.5–149 days[1]<br>33–73 days (water-sediments)[1]<br>Typical 75.1 days<br>(22.4–353 days in the field and in the lab @ 20 °C, respectively)[2] |

**[1] BCPC (2012). [2] Pesticide Properties Database (University of Hertfordshire 2013). [3] Rolando and Watt (2012).**

Terbuthylazine belongs to the chlorotriazine group within the triazine class of herbicides, which also includes atrazine, propazine and simazine. Terbuthylazine is extensively used in agricultural, forestry and industrial situations for pre- and post-emergence control of grasses and broad-leaved weeds in a variety of crops such as maize, sorghum, vines, fruit trees, citrus, potatoes, beans and plantation crops (coffee, cocoa, oil palm) as well as in tree nurseries and new plantings (BCPC 2012; University of Hertfordshire 2013). Non-agricultural uses include the application of terbuthylazine to swimming pools, roads, railways and industrial sites for the control of slime-forming algae, fungi and bacteria (NRAAVC 2001; University of Hertfordshire 2013). The mode of action of terbuthylazine to fungi and bacteria is unknown.

Terbuthylazine 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 shoots and leaves (BCPC 2012). Terbuthylazine exerts its toxicity in aquatic plants (including 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).

Terbuthylazine is a broad-spectrum herbicide which may ultimately end up in aquatic environments as a result of offsite movement via leaching and run-off following rainfall events (BCPC 2012; Bailie 2016). Terbuthylazine is persistent and soils and has a moderate affinity for binding to soil particles as indicated by its log $K_{oc}$ value (Table 48). Terbuthylazine is slightly mobile and has little ability to leach from soils to groundwater and end up in surface waters (Bailie 2016). Terbuthylazine reportedly persists in water, being stable at a pH ranging from pH 7 to pH 9 and a temperature of 20 ºC (Table 48) (BCPC 2012).

## 12.2 Freshwater

### 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 terbuthylazine in freshwaters (Table 50) includes toxicity data for eight 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 11 macrophytes and seven microalgae. The toxicity values for the macrophytes consisted of 7-day EC10/NOEC (relative frond area, frond area, frond count) values ranging from 35 to 110 µg/L, 7-day EC50 (frond area, frond count, leaf surface area) values ranging from 105 to 230 µg/L, 14-day EC10/NOEL (frond number, dry weight, frond area, relative frond area) values ranging from 2 to 1,500 µg/L, 14-day EC50 (frond number, dry weight, frond area, relative frond area) values ranging from 16 to 305 µg/L and 21- and 28-day NOEC (relative frond area) values both of 42 µg/L. The toxicity values for the microalgae were 48-hour

EC10/NOEC (chlorophyll-a concentration, cell density) values ranging from 5 to 1,000 µg/L, 48-hour EC50 (chlorophyll-a concentration, cell density, chlorophyll density) values ranging from 20 to 1,033.7 µg/L, a 72-hour NOEC (cell density) value of 2 µg/L, 72-hour EC50 (cell density, cell count, area under the growth curve) values ranging from 9 to 36 µg/L, 5-day NOEL (biomass yield, growth rate, area under the growth curve) values ranging from 0.6 to 17 µg/L, 5-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 3.2 to 99 µg/L and two 6-day IC50 (cell counts) values of 72.4 and 150.2 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for six fish, one cladoceran one macrophyte and five microalgae. The toxicity values for the fish were 48-hour LC50 (mortality) values ranging from 8,000 to 90,000 µg/L, two 96-hour LOEL (mortality) values of 1,900 and 5,600 µg/L and 96-hour LC50 (mortality) values of 3,400 to 9,000 µg/L. The toxicity values for the single cladoceran species were 48-hour LOEL and LC50 (immobilisation) values of 10,000 and 21,200 µg/L and 96-hour LOEL and LC50 (immobilisation) values of 9,800 and 50,900 µg/L. The toxicity values for the single macrophyte species were 4- to 7-day EC10 (frond area) values ranging from 5.3 to 44.8 µg/L and 4- to 7-day EC50 (frond area) values ranging from 32.4 to 182.8 µg/L. The toxicity values for the microalgae consisted of a 4-hour NOEC (chlorophyll-a content) value of 1,000 µg/L, 24-hour NOEC (chlorophyll-a content, cell density, cell number) values ranging from 2.2 to 22.9 µg/L, a 24-hour LOEC (cell density) value of 229.7 µg/L, a EC62 (cell density) value of 22.9 µg/L and 24-hour EC50 (cell density and cell number) values ranging from 15.9 to 666.2 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 terbuthylazine. 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 moderate given the log $K_{oc}$ value of terbuthylazine (Table 53).

### 12.2.3 Guideline derivation

The derived PGVs for terbuthylazine in freshwaters are provided in Table 49. 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 PGVs, the PGVs for terbuthylazine are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for terbuthylazine are low (Table 48) 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 terbuthylazine do not need to account for secondary poisoning.

**Table 49 Proposed aquatic ecosystem protection guideline values (µg/L) for terbuthylazine for the protection of freshwater ecosystems.**

| Terbuthylazine proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.43 (0.035 – 2.7) |
| 95% | 1.2 (0.37 – 4.2) |
| 90% | 2.0 (0.84 – 5.8) |
| 80% | 3.8 (1.6 – 9.1) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 16 |
| Type of toxicity data | Chronic NOEC/NOEL/EC10 data values |
| SSD model fit | Good |
| 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".**

### 12.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for terbuthylazine in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for terbuthylazine 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 considerably more terbuthylazine toxicity data available that enable the calculation of PGVs in freshwaters.

In total, there were toxicity data for 28 freshwater species (six phyla and eight classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The eight 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), Magnoliopsida (dicots) and Trebouxiophyceae (another grouping of green algae).

Based on the current understanding of the mode of action of terbuthylazine, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The terbuthylazine ecotoxicity data for phototrophs and heterotrophs were therefore 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 12.3.6) 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 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for 16 phototrophic species (that belonged to four phyla and six 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 a PGV (Warne et al. 2015). The number of species and taxa used to derive the PGVs (Table 49) combined with the good fit of the distribution to these toxicity data (Figure 41) 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 terbuthylazine in freshwater environments is provided in Table 50.

**Table 50 Summary of the single toxicity value for each phototrophic species that was used to derive the proposed aquatic ecosystem protection guideline values for terbuthylazine 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] | 17 | USEPA (2015b) |
| Cyanobacteria | *Anabaena variabilis* | Cyanobacteria | Cyanophyceae | Not stated | 2 | Chronic NOEC | Chlorophyll-a concentration | 229.71 | Hawxby et al. (1977) |
| Macrophyte | *Callitriche platycarpa* | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 27.49 | Cedergreen et al. (2004) |
| Macrophyte | *Ceratophyllum demersum** | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 4 | Cedergreen et al. (2004) |
| Macrophyte | *Ceratophyllum submersum** | Tracheophyta | Liliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 4 | Cedergreen et al. (2004) |
| Microalga | *Chlorella kessleri* | Chlorophyta | Trebouxiophyceae | Stationary growth phase | 2 | Chronic NOEC | Cell density | 5 | Spoljaric et al. (2011) |
| Macrophyte | *Elodea canadensis** | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 41.57 | Cedergreen et al. (2004) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 2.1 | USEPA (2015b) |
| Macrophyte | *Lemna minor** | Tracheophyta | Liliopsida | Not stated | 14 | Chronic EC10 | Biomass (dry weight) | 16.06 | Cedergreen et al. (2004) |
| Macrophyte | *Lemna trisulca** | Tracheophyta | Liliopsida | 3-5 leaf stage | 14 | Chronic EC10 | Biomass (dry weight) | 38 | Cedergreen et al. (2004) |
| Macrophyte | *Myriophyllum spicatum* | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 20 | Cedergreen et al. (2004) |
| Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 5.6 | USEPA (2015b) |
| Macrophyte | *Potamogeton crispus** | Tracheophyta | Liliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 37.23 | Cedergreen et al. (2004) |
| Microalga | *Selenastrum capricornutum*[2] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 0.6 | Sbrilli et al. (2005); Cedergreen and |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | Streibig (2005) |
| Macrophyte | *Sparganium emersum* | Tracheophyta | Liliopsida | Basal shoot meristem | 14 | Chronic NOEC | Biomass (dry weight) | 1,500 | Cedergreen et al. (2004) |
| Macrophyte | *Spirodela polyrrhiza** | Tracheophyta | Liliopsida | Not stated | 14 | Chronic EC10 | Biomass (dry weight) | 9.8 | Cedergreen et al. (2004) |

**[1] Chronic NOEC/NOEL/EC10 = 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/is distributed in Australia and/or New Zealand.**

### 12.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 16 phototrophic freshwater species that was used to derive the PGVs is presented in Figure 41.

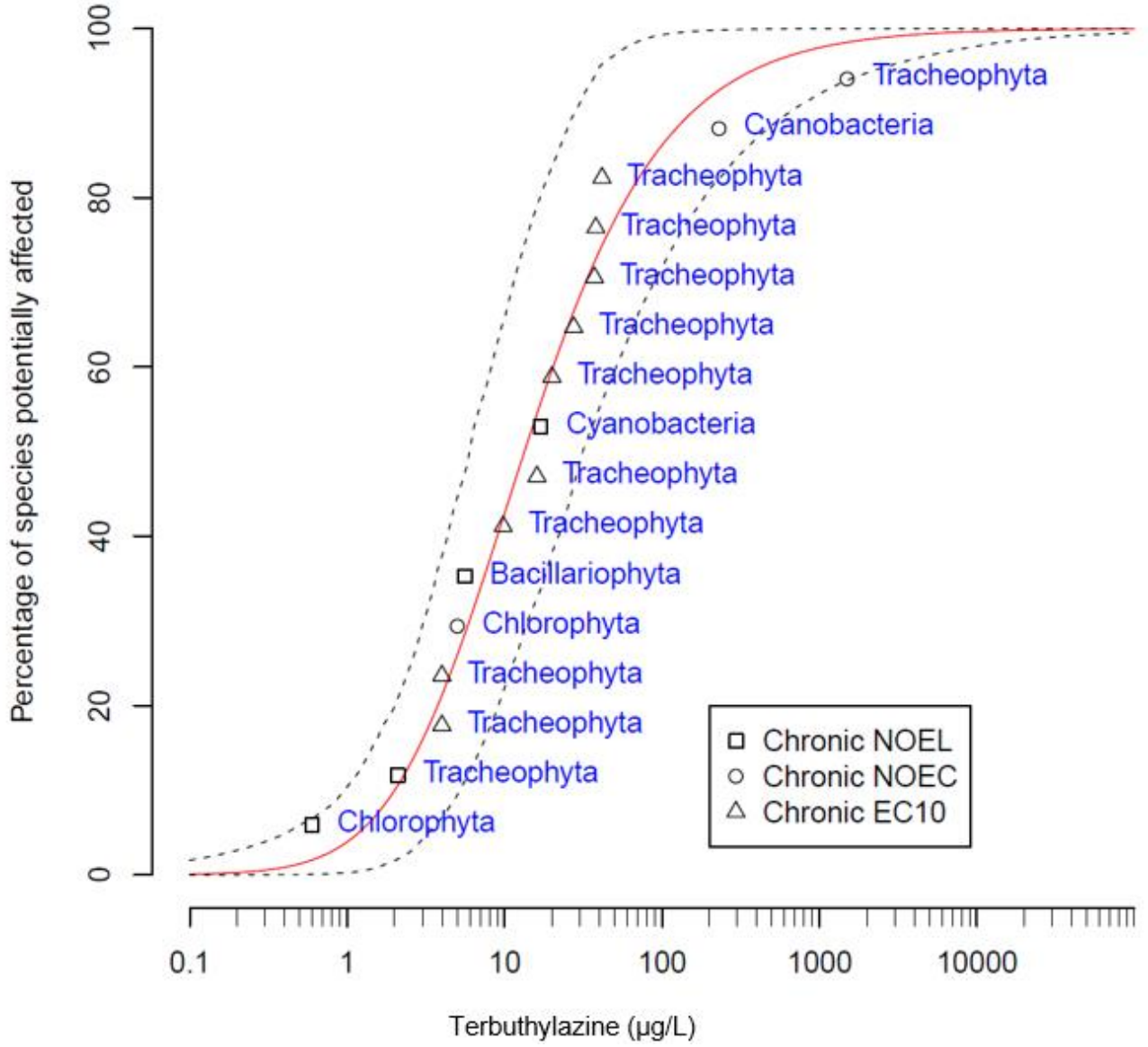



Figure 41 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic species to terbuthylazine. Black dashed lines indicate the 95% confidence intervals.

## 12.3 Marine

### 12.3.1 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 terbuthylazine in marine waters (Table 52) includes toxicity data for nine species (one marine and eight 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 12.2.1, respectively.

*Marine Chronic*

There were marine chronic toxicity data for two microalgae which were >24-hour NOEC and LOEC (cell count) values of 1 and 5 µg/L, respectively and 5-day LOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 9 and 31 µg/L, respectively.

*Marine Acute*

There were marine acute toxicity data for one fish and two crustaceans. The single toxicity value for the fish species was a 96-hour NOEC (mortality) value of 16.2 µg/L. The toxicity values for the crustaceans were a 48-hour LC50 (mortality) value of 2,517 µg/L and 96-hour LOEL and LC50 (mortality) values of 13 and 109 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.

### 12.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of terbuthylazine. 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 moderate given the log $K_{oc}$ value of terbuthylazine (Table 53).

### 12.3.3 Guideline derivation

The derived PGVs for terbuthylazine in marine waters 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 PGVs, the PGVs for terbuthylazine are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for terbuthylazine are low (Table 48) 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 terbuthylazine do not need to account for secondary poisoning.

**Table 51 Proposed aquatic ecosystem protection guideline values (µg/L) for terbuthylazine for the protection of marine ecosystems.**

| Terbuthylazine proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.40 (0.031 – 1.9) |
| 95% | 0.97 (0.36 – 3.0) |
| 90% | 1.6 (0.77 – 4.1) |
| 80% | 2.8 (1.4 – 6.9) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 18 |
| Type of toxicity data | Chronic NOEC/NOEL/EC10 and a chronic estimated NOEC value (*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".**

### 12.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for terbuthylazine in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for terbuthylazine 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 considerably more terbuthylazine 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. 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 terbuthylazine with marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

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

Based on the current understanding of the mode of action of terbuthylazine, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The terbuthylazine ecotoxicity data for phototrophs and heterotrophs were therefore 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 12.3.6) 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 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 only two 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 terbuthylazine to marine phototrophic species were available, the chronic NOEC and chronic estimated NOEC values for the marine phototrophic species were combined with the chronic NOEC, 10% effect concentration (EC10) and no observed effect level (NOEL) values for freshwater phototrophic species (see section 12.2) to derive PGVs for terbuthylazine in marine waters. This dataset incorporated concentration data for 18 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 51) combined with the good fit of the distribution to these toxicity data (Figure 42) 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 terbuthylazine in marine environments 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 terbuthylazine 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 | Microalga | *Anabaena flos-aquae* | Cyanobacteria | Cyanophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 17 | USEPA (2015b) |
| Fresh | Microalga | *Anabaena variabilis* | Cyanobacteria | Cyanophyceae | Not stated | 2 | Chronic NOEC | Chlorophyll-a concentration | 229.71 | Hawxby et al. (1977) |
| Fresh | Macrophyte | *Callitriche platycarpa* | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 27.49 | Cedergreen et al. (2004) |
| Fresh | Macrophyte | *Ceratophyllum demersum** | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 4 | Cedergreen et al. (2004) |
| Fresh | Macrophyte | *Ceratophyllum submersum** | Tracheophyta | Liliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 4 | Cedergreen et al. (2004) |
| Fresh | Microalga | *Chlorella kessleri* | Chlorophyta | Trebouxiophyceae | Stationary growth phase | 2 | Chronic NOEC | Cell density | 5 | Spoljaric et al. (2011) |
| Fresh | Macrophyte | *Elodea canadensis** | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 41.57 | Cedergreen et al. (2004) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 2.1 | USEPA (2015b) |
| Fresh | Macrophyte | *Lemna minor** | Tracheophyta | Liliopsida | Not stated | 14 | Chronic EC10 | Biomass (dry weight) | 16.06 | Cedergreen et al. (2004) |
| Fresh | Macrophyte | *Lemna trisulca** | Tracheophyta | Liliopsida | 3-5 leaf stage | 14 | Chronic EC10 | Biomass (dry weight) | 38 | Cedergreen et al. (2004) |
| Fresh | Macrophyte | *Myriophyllum spicatum* | Tracheophyta | Magnoliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 20 | Cedergreen et al. (2004) |
| Fresh | Microalga | *Navicula pelliculosa** | Bacillariophyta | Bacillariophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 5.6 | USEPA (2015b) |
| Fresh | Macrophyte | *Potamogeton crispus** | Tracheophyta | Liliopsida | Apical shoots | 14 | Chronic EC10 | Biomass (dry weight) | 37.23 | Cedergreen et al. (2004) |
| Fresh | Microalga | *Selenastrum capricornutum*[3] | Chlorophyta | Chlorophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 0.6 | Sbrilli et al. (2005); Cedergreen and Streibig |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | (2005) |
| Marine | Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 5 | Chronic est. NOEC | Biomass yield, growth rate, AUC[2] | 4.72 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema marinoi* | Bacillariophyta | Mediophyceae | Not stated | > 24 | Chronic NOEC | Cell count | 1 | Fiori and Pistocchi (2014) |
| Fresh | Macrophyte | *Sparganium emersum* | Tracheophyta | Liliopsida | Basal shoot meristem | 14 | Chronic NOEC | Biomass (dry weight) | 1,500 | Cedergreen et al. (2004) |
| Fresh | Macrophyte | *Spirodela polyrrhiza** | Tracheophyta | Liliopsida | Not stated | 14 | Chronic EC10 | Biomass (dry weight) | 9.80 | Cedergreen et al. (2004) |

**[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] AUC = area under the growth curve. [3] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*. *Species that originated from/is distributed in Australia and/or New Zealand.**

### 12.3.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 18 phototrophic marine and freshwater species that was used to derive the PGVs is presented in Figure 42.

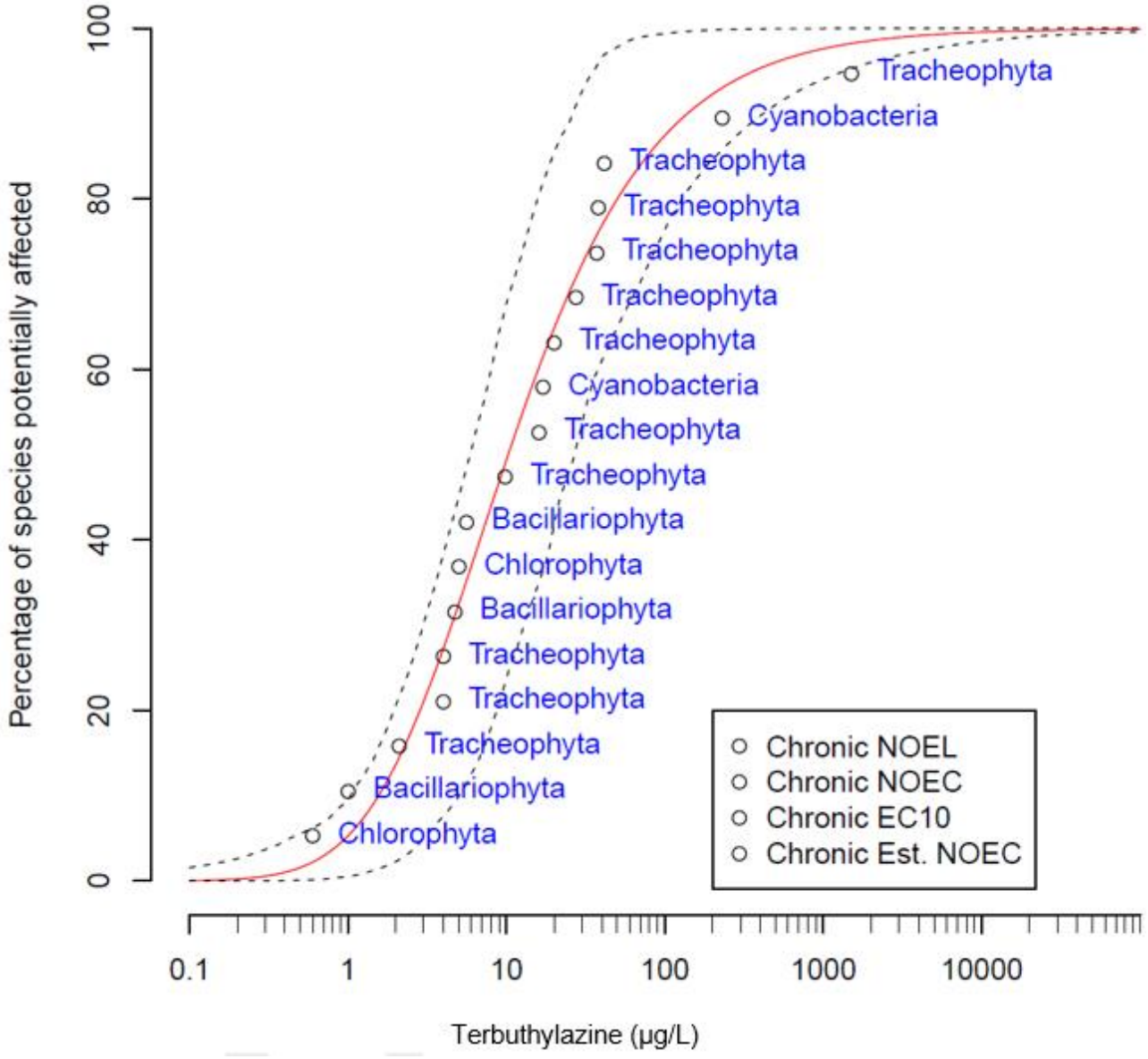



Figure 42 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated 10% effect concentration (EC10), no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of marine and freshwater phototrophic species to terbuthylazine. Black dashed lines indicate the 95% confidence intervals.

### 12.3.6 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 [-0.908 and 6.059 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater phototrophic species (*n* = 22). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for terbuthylazine.

The toxicity data for terbuthylazine 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 terbuthylazine ecotoxicity data may be bimodal (Figure 43).

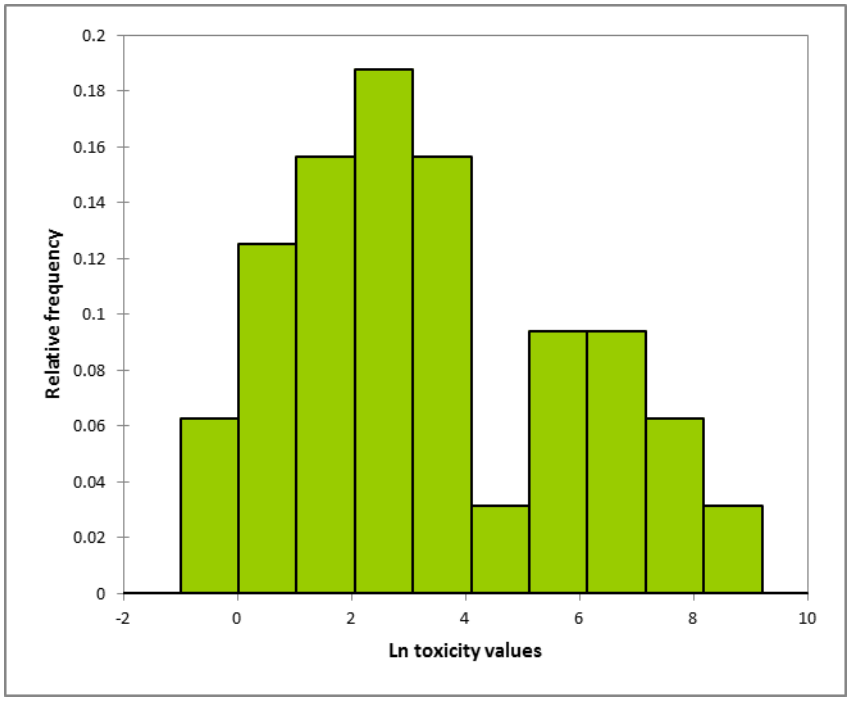



Figure 43 Histogram of the natural logarithm (ln) of all terbuthylazine (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 32).

The terbuthylazine 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 terbuthylazine concentration data had equal variances (Fisher's F-Test; p = 0.702) and followed a normal distribution (Anderson-Darling; p = 0.334). Results from the two-sample *t* test indicated that the two groups were significantly different (p <0.0001); therefore, it was concluded that the distribution of the terbuthylazine concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

### 12.3.7 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.

Bailie BR (2016). Herbicide concentrations in waterways following aerial application in a steepland planted forest in New Zealand, *New Zealand Journal of Forestry Science*, 46, 1–10.

BCPC (British Crop Production Council) (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), BCPC, Alton, United Kingdom, 939–941.

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/.

Cedergreen N, Spliid NH and Streibig JC (2004). Species-specific sensitivity of aquatic macrophytes towards two herbicide. *Ecotoxicology and Environmental Safety*, 58, 314–323.

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

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 Biochemis*try 52, 38–51.

Fiori E, and Pistocchi R (2014). *Skeletonema marinoi* (Bacillariophyceae) sensitivity to herbicides and effects of temperature increase on cellular responses to terbuthylazine exposure. *Aquatic Toxicology*,147, 112-120

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.

Hawxby K, Tubea B, Ownby J and Basler E (1977). Effects of various classes of herbicides on four species of algae. *Pesticide Biochemistry and Physiology*, 7, 203–209.

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

NRAAVC (2001). Evaluation of the active terbuthylazine in the product SwimCare® T swimming pool algaecide, Public Release Summary, National Registration Authority for Agricultural and Veterinary Chemicals, Canberra, Australia, 29pp. Available from: http://apvma.gov.au/sites/default/files/publication/14061-prs-terbuthylazine.pdf, Accessed 12 April 2017.

Rolando CA and Watt MS (2012). Perspectives on Forest Stewardship Council certification and vegetation management in New Zealand, *New Zealand Journal of Forestry*, 54, 37–40.

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.

Spoljaric 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 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.

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

# 13 Terbutryn

## 13.1 Introduction

Terbutryn is a herbicide ($C_{10}H_{19}N_5S$ and Figure 44) that at room temperature is in the form of a white powder. It is the active ingredient of a variety of commercial herbicide formulations.

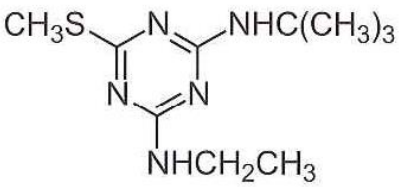



Figure 44 Structure of terbutryn.

Physicochemical properties of terbutryn that may affect its environmental fate and toxicity are presented in Table 53.

**Table 53 Summary of selected physicochemical properties of terbutryn.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 241.4 amu[1] |
| Aqueous solubility | 22 mg/L @ pH 6.8 and temperature 22 °C[1]<br>25 mg/L @ temperature of 20 °C[2] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 3.65 @ temperature 25 °C (unionized)[1]<br>3.66 @ pH 7 and temperature 20 °C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 2.59–2.78[1]<br>3.39[2] |
| Logarithm of the bioconcentration factor (log BCF) | 1.86[2] |
| Half-life ($t_{1/2}$) in water | Stable @ pH 5–7 and temperature 20 °C[2] |
| Half-life ($t_{1/2}$) in soil | 15.4–84 days in the lab @ temperature 20–25 °C[1]<br>9–47 days in the field[1]<br>Typical: 74 days<br>52–74 days (in the field and in the lab @ temperature 20 °C)[2] |

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

Terbutryn belongs to the methylthiotriazine group within the triazine class of herbicides, which also includes ametryn and prometryn. Terbutryn is extensively used in agricultural and forestry situations for pre- and post- emergent control of some grasses and autumn-germinating broad-leaved weeds in a variety of crops such as winter cereals, maize, sugar cane, beans, potatoes, cotton, peanuts and sunflowers (BCPC 2012). Terbutryn is also used to control submerged vascular plants and free-floating weeds and algae in and around water bodies such as reservoirs and fish ponds (BCPC 2012; Cornell University 1995). Terbutryn is most commonly used in urban and industrial situations, for the application to the outside of houses and other buildings to protect dry film coatings from discolouration and destruction by algae (i.e. used as an algaecide) (Entec 2011). However, it does not have regulatory approval to be used within the European Union (BCPC 2012).

Terbutryn is generally absorbed through the roots of plants, with some absorption through foliage. 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). Terbutryn exerts its toxicity in aquatic plants (including 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).

Terbutryn 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 guidelines for terbutryn.

Terbutryn is a selective herbicide which may ultimately end up in aquatic ecosystems as a result of run-off from urban and industrial applications following rainfall, ultimately ending up in urban stormwater drains leading to the surface water of nearby catchments (Burkhardt et al. 2011). Terbutryn also ends up in aquatic ecosystems as a result of direct application to watercourses for the control of submerged and free-floating weeds and algae (Cornell University 1995). Terbutryn has little mobility in soils and low capacity to leach in agricultural soils to groundwater due to its high soil sorption ability as indicated by its relatively high log $K_{oc}$ value (Table 53) (BCPC 2012; USEPA 1986). Terbutryn reportedly has the potential to come unbound from soil particles given the right soil temperature, pH and moisture conditions (USEPA 1986). Information on the aqueous hydrolysis of terbutryn is vague, with relatively short half-lives ranging from 9 to 84 days (Table 53) (BCPC 2012; University of Hertfordshire 2013).

## 13.2 Freshwater and Marine

### 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 terbutryn in fresh and marine waters (Table 55) includes toxicity data for 14 freshwater species that either originated from or are distributed within Australia and/or New Zealand. The dataset used in the guideline derivation process did not include any toxicity data for terbutryn to Australian and/or New Zealand marine species. 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 and 19 microalgae. The single toxicity value for the fish species was a 35-day NOEL (mortality) of 1,000 µg/L. The microalgae toxicity data consisted of 48-, 72- and 96-hour NOEC (algal cell viability) values all of 120.7 µg/L, a 96-hour NOEC (chlorophyll content) value of 3.02 µg/L, 48-, 72- and 96-hour LOEC (chlorophyll content) values ranging from 3.02 to 6.04 µg/L, two 72-hour EC50 (area under the growth curve, cell count) values of 2 and 3.3 µg/L, respectively, 96-hour NOEC and LOEC (cell size) values of 24.14 and 60.04 µg/L, respectively, 96-hour EC5 (cell count) values ranging from 0.7 to 1,450 µg/L, 96-hour EC10 (biomass) values ranging from 0.015 to 1,699 µg/L, 96-hour EC50 (biomass, cell count) values ranging from 0.1 to 3,133 µg/L and a 14-hour EC53 (biomass) value of 1,000 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for three fish, one crustacean, one cladoceran and two microalgae. The toxicity data for the fish were a 24-hour LC50 (mortality) value of 10,000 µg/L, a 48-hour LOEL (mortality) value of 2,800 µg/L, two 48-hour LC50 (mortality) values of 3,500 and 8,900 µg/L, two 96-hour NOEL (mortality) values of 1,000 to 3,200 µg/L and 96-hour LC50 (mortality) values ranging from 820 to 5,800 µg/L. The toxicity data for the single crustacean species were 24-, 48-, 72- and 96-hour LC50 (mortality) values of 259,100, 71,600, 22,500 and 13,900 µg/L, respectively. The toxicity data for the single cladoceran species were 48-hour NOEL and EC50 (body length, dry weight) values of 560 and 2,660 µg/L, respectively. The toxicity data for the microalgae consisted of 24-hour NOEC (algal cell viability, cell count) values ranging from 1.6 to 181 µg/L, two 24-hour LOEC (cell count, dry weight) values of 6.03 µg/L and a 24-hour EC50 (cell count) value of 7.81 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.

*Marine Chronic*

There were marine chronic toxicity data for only one microalga which was a 96-hour EC50 (biomass) value of 3.1 µg/L.

*Marine Acute*

There were marine acute toxicity data for one fish, two crustaceans and one mollusc. The toxicity data for the single fish species were 96-hour LOEL and LC50 (mortality) values of 540 and 1,500 µg/L. The toxicity data for the crustaceans were a 24-hour EC50 (immobilisation) value of 22,000 µg/L and 96-hour NOEL and LC50 (mortality) values of 250 and 740 µg/L. The toxicity data for the single mollusc species were 48-hour NOEL and EC50 (mortality, abnormal development) values of 1,500 and 5,800 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.

### 13.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of terbutryn. As with many organic chemicals it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. The capacity for this may be higher than most pesticides due to the high $K_{oc}$ value of terbutryn. However, any such effect would be dependent on a variety of environmental and physicochemical conditions.

### 13.2.3 Guideline derivation

The derived PGVs for terbutryn in fresh and marine waters are provided in Table 54. 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 PGVs, the PGVs for terbutryn are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for terbutryn are low (Table 53) 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 terbutryn do not need to account for secondary poisoning.

**Table 54 Proposed aquatic ecosystem protection guideline values (µg/L) for terbutryn for the protection of freshwater and marine ecosystems.**

| Terbutryn proposed aquatic ecosystem protection guideline values (freshwater and marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.079 (0.00031 – 0.55) |
| 95% | 0.26 (0.032 – 1.2) |
| 90% | 0.51 (0.18 – 2.0) |
| 80% | 1.2 (0.43 – 5.7) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 19 |
| Type of toxicity data | Chronic EC5/EC10/NOEC and chronic estimated NOEC 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".**

### 13.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for terbutryn in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for terbutryn to freshwater and 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 terbutryn toxicity data available that enable the calculation of PGVs in fresh and marine waters. However, it was only possible to derive PGVs by using ecotoxicity data for a mixture of both freshwater and marine organisms. In order to derive higher reliability PGVs in the future that are of greater relevance to freshwater and marine ecosystems separately, it is recommended that additional chronic toxicity tests of terbutryn with freshwater and marine phototrophic species (species that photosynthesise, e.g. plants and algae) be conducted.

In total, there were toxicity data for 29 freshwater and marine (six phyla and nine classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Mollusca and Tracheophyta. The nine classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae), Bivalvia (a grouping of molluscs), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), 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 terbutryn, it would be expected that phototrophic species would be more sensitive than non-phototrophic species. The terbutryn 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 13.2.6) 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 5% effect concentration (EC5), 10% effect concentration (EC10), no observed effect concentration (NOEC) 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 available for 19 (18 freshwater and one marine) phototrophic species that belonged to only three phyla and five classes. This dataset 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 for either media type (Warne et al. 2015). In cases like these where the SSD uses the most sensitive species, 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).

The entire freshwater and marine dataset for terbutryn (that included chronic data) consisted of 20 phototrophic ($n = 19$) and heterotrophic ($n = 1$) species that belonged to four phyla and six classes, which 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 EC5/EC10/NOEC and chronic estimated NOEC data values for the 19 freshwater and marine phototrophic species despite belonging to only three phyla (Warne et al. 2015). The number of species and taxa in the toxicity data used to derive the PGVs (Table 54) combined with the good fit of the distribution to these toxicity data (Figure 45) 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 terbutryn in freshwater and marine environments is provided in Table 55.

**Table 55 Summary of the single toxicity value for each phototrophic species that were used to derive the proposed aquatic ecosystem protection guideline values for terbutryn in fresh and 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 | Microalga | *Achnanthidium minutissimum** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 35.51 | Larras et al. (2013) |
| Fresh | Microalga | *Chlorella vulgaris** | Chlorophyta | Trebouxiophyceae | Logarithmic growth phase | 4 | Chronic NOEC | Biomass (Chlorophyll-a fluorescence) | 3.02 | Rioboo et al. (2009) |
| Fresh | Microalga | *Craticula accomoda** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 1.87 | Larras et al. (2013) |
| Fresh | Microalga | *Cyclotella meneghiniana** | Bacillariophyta | Mediophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 5.07 | Larras et al. (2013) |
| Marine | Microalga | *Dunaliella tertiolecta* | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic est. NOEC | Biomass (Chlorophyll-a fluorescence) | 0.62 | Gaggi et al. (1995) |
| Fresh | Microalga | *Encyonema silesiacum** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 1.22 | Larras et al. (2013) |
| Fresh | Microalga | *Eolimna minima** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC5 | Cell count | 1,450 | Larras et al. (2012) |
| Fresh | Microalga | *Fistulifera saprophila** | Bacillariophyta | Bacillariophyceae | Not stated | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 67 | Larras et al. (2014) |
| Fresh | Microalga | *Fragilaria capucina var vaucheriae** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 5.62 | Larras et al. (2013) |
| Fresh | Microalga | *Fragilaria crotonensis** | Bacillariophyta | Bacillariophyceae | Not stated | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 2 | Larras et al. (2014) |
| Fresh | Microalga | *Fragilaria rumpens** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a | 0.12 | Larras et al. (2013) |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | fluorescence) | | |
| Fresh | Microalga | *Fragilaria ulna*[3]* | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 5.6 | Larras et al. (2013) |
| Fresh | Microalga | *Gomphonema clavatum** | Bacillariophyta | Bacillariophyceae | Not stated | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 122 | Larras et al. (2014) |
| Fresh | Microalga | *Gomphonema parvulum* | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 60.01 | Larras et al. (2013) |
| Fresh | Macrophyte | *Hydrilla verticillata** | Tracheophyta | Liliopsida | 1-2 weeks | 14 | Chronic est. NOEC | Biomass | 200 | Sutton et al. (1971) |
| Fresh | Microalga | *Mayamaea fossalis* | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 2.92 | Larras et al. (2013) |
| Fresh | Microalga | *Nitzschia palea** | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 94.95 | Larras et al. (2013) |
| Fresh | Microalga | *Selenastrum capricornutum*[2] | Chlorophyta | Chlorophyceae | Not stated | 3 | Chronic est. NOEC | Cell count (AUC[4]) | 0.4 | Okamura et al. (2000) |
| Fresh | Microalga | *Sellaphora minima* | Bacillariophyta | Bacillariophyceae | Exponential growth phase | 4 | Chronic EC10 | Biomass (Chlorophyll-a fluorescence) | 410.12 | Larras et al. (2013) |

**[1] Chronic EC5/EC10/NOEC = no conversions applied; Chronic est. NOEC = chronic LOEC and EC50/EC53 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 *Pseudokirchneriella subcapitata*. [3] This species has also been called *Ulnaria ulna*. [4] AUC = area under the growth curve. * Species that originated from/is distributed in Australia and/or New Zealand.**

### 13.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 19 freshwater and marine, phototrophic species that was used to derive the PGVs is presented in Figure 45.

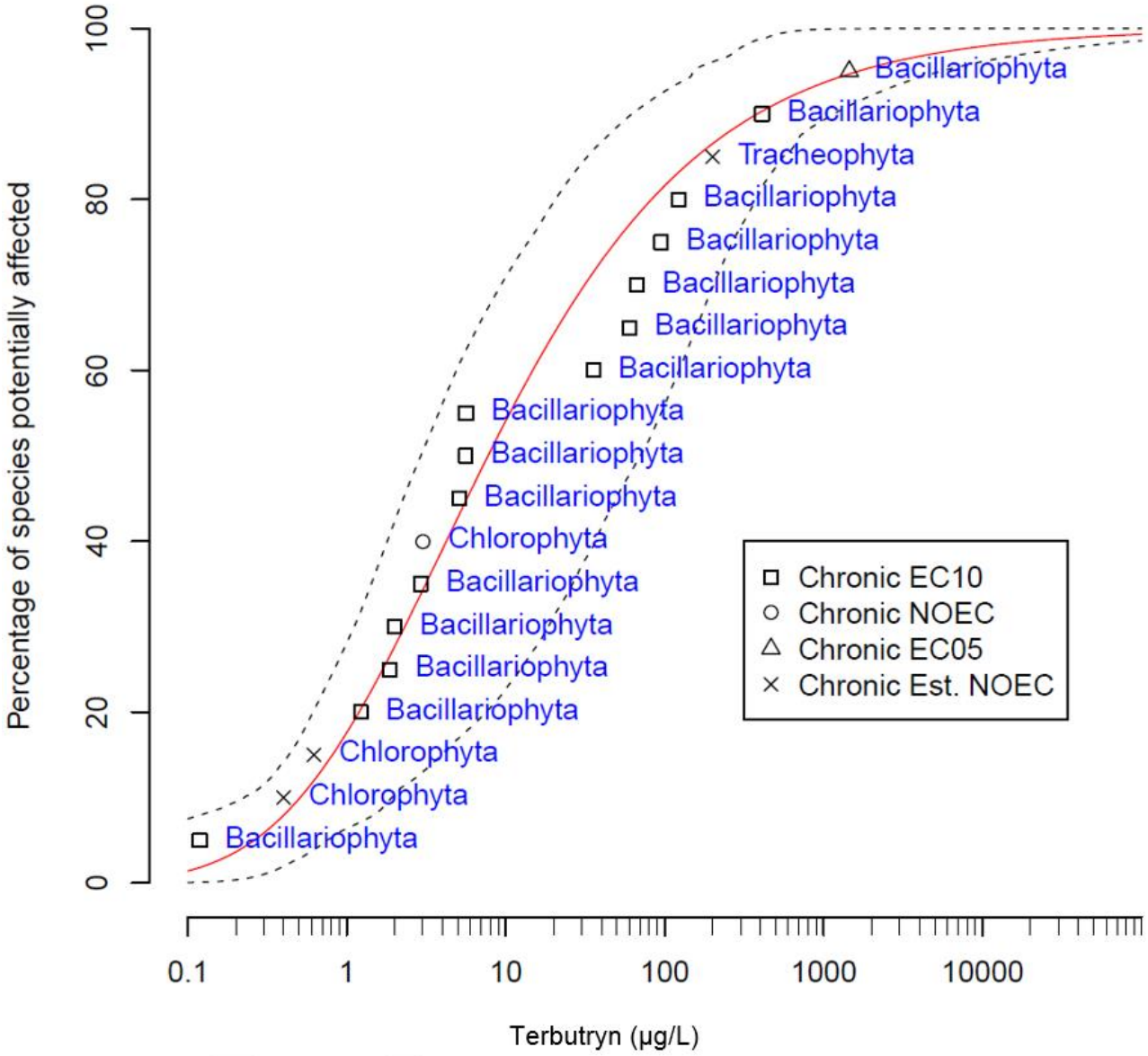



Figure 45 Cumulative frequency distribution, generated using Burrlioz 2.0 (2016), of the sensitivity of chronic and chronic estimated 5% effect concentration (EC5), 10% effect concentration (EC10) and no observed effect concentration (NOEC) data values of freshwater and marine phototrophic species to terbutryn. Black dashed lines indicate the 95% confidence intervals.

### 13.2.6 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 [-2.626 and 7.394 ln(µg/L), respectively] of the transformed ecotoxicity data for freshwater phototrophic species (*n* = 19). On this basis, it was determined that there was no difference in the sensitivities of freshwater and marine species for terbutryn.

The toxicity data for terbutryn 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 terbutryn ecotoxicity data may be bimodal (Figure 29).

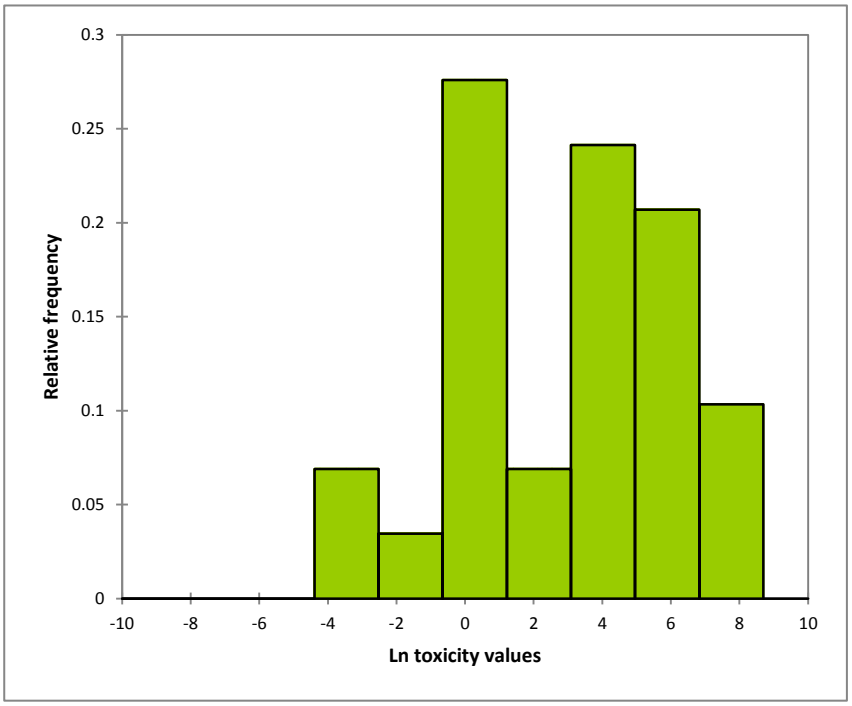



Figure 46 Histogram of the natural logarithm (ln) of all terbutryn (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 29).

The terbutryn 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 terbutryn concentration data successfully met tests for normality (Anderson-Darling; p = 0.258), the data 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 significantly different (p <0.0001); therefore, it was concluded that the distribution of the terbutryn concentration data is bi- or multi-modal, with phototrophic species being the most sensitive group.

### 13.2.7 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.

BCPC (British Crop Production Council) (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), BCPC, Alton, United Kingdom, 1085–1087.

Burkhardt M, Zuleeg S, Vonbank R, Schmid P, Hean S, Lamani X, Bester K and Boller M (2011). Leaching of additives from construction materials to urban storm water runoff, *Water Science and Technology*, 63, 1974–1982.

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/. Last accessed: December 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.

Cornell University (1995). EXTOXNET (Extension Toxicology Network): Terbutryn, Developed by the Pesticide Management Education Program (PMEP), Cornell University, 1995. Available from: http://pmep.cce.cornell.edu/profiles/extoxnet/pyrethrins-ziram/terbutryn-ext.html, Accessed: 10 April 2017.

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.

Entec (2011). Technical support for the impact assessment of the review of priority substances under directive 2000/60/EC – Substance assessment: Terbutryn, Developed by Entec UK Limited for European Commission (DG Environment), 2011. Available from: https://circabc.europa.eu/webdav/CircaBC/env/wfd/Library/framework_directive/thematic_documents/priority_substances/supporting_substances/substance_impacts/Terbutryn.pdf, Accessed: 10 April 2017.

Gaggi C, Sbrilli G, El Naby AMH, Bucci M, Duccini M and Bacci E (1995). Toxicity and hazard ranking of s-triazine herbicides using microtox, two green algal species and a marine crustacean, *Environmental Toxicology and Chemistry*, 14, 1065–1069.

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.

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, Keck F, Montuelle B, Rimet F and Bouchez A (2014). Linking diatom sensitivity to herbicides to phylogeny: a step forward for biomonitoring?, *Environmental Science and Technology*, 48, 1921–1930.

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.

Okamura H, Aoyama I, Liu D, Maguire RJ, Pacepavicius GD and Lau YL (2000). Fate and ecotoxicity of the new antifouling compound irgarol 1051 in the aquatic environment, *Weed Research*, 34, 3523–3530.

Rioboo C, O'Connor JE, Prado R, Herrero C and Cid A (2009). Cell proliferation alterations in Chlorella cells under stress conditions, *Aquatic Toxicology*, 94, 229–237.

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.

Sutton DL, Blackburn RD and Steward KK (1971). Influence of herbicides on the uptake of copper in Hydrilla, *Weed Research*, 11, 99–105.

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 (1986). EPA Pesticide Fact Sheet: Terbutryn, United States Environmental Protection Agency, Prevention, Office of Pesticides and Toxic Substances, Office of Pesticide Programs (TS788C), Washington DC. Available from: https://nepis.epa.gov/Exe/ZyNET.exe/91024KIW.txt?ZyActionD=ZyDocument&Client=EPA&Index=1986%20Thru%201990&Docs=&Query=&Time=&EndTime=&SearchMethod=1&TocRestrict=n&Toc=&TocEntry=&QField=&QFieldYear=&QFieldMonth=&QFieldDay=&UseQField=&IntQFieldOp=0&ExtQFieldOp=0&XmlQuery=&File=D%3A%5CZYFILES%5CINDEX%20DATA%5C86THRU90%5CTXT%5C00000034%5C91024KIW.txt&User=ANONYMOUS&Password=anonymous&SortMethod=h%7C-&MaximumDocuments=1&FuzzyDegree=0&ImageQuality=r75g8/r75g8/x150y150g16/i425&Display=hpfr&DefSeekPage=x&SearchBack=ZyActionL&Back=ZyActionS&BackDesc=Results%20page&MaximumPages=1&ZyEntry=1, Accessed: 10 April 2017.

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.

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.

# 14 Triclopyr

## 14.1 Introduction

Triclopyr is a herbicide ($C_7H_4Cl_3NO_3$ and Figure 47) which as a free acid at room temperature is in the form of a fluffy, colourless solid. Triclopyr is generally sold in commercial formulations as triclopyr butoxyethyl ester (TBEE) or triclopyr triethylamine salt (TEA), which are both derivatives of the parent compound, triclopyr acid (Tu et al. 2001). TBEE and TEA are both rapidly converted - within a few seconds to a few hours - to triclopyr acid once applied to soils and/or water (Ganapathy 1997). Triclopyr is the active ingredient of a variety of commercial herbicide formulations.

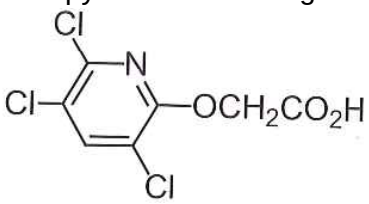



Figure 47 Structure of triclopyr.

Physicochemical properties of triclopyr that may affect its environmental fate and toxicity are presented in Table 56.

**Table 56 Summary of selected physicochemical properties of triclopyr.**

| Physicochemical property | Value |
|---|---|
| Molecular weight | 256.5 amu[1] |
| Aqueous solubility | 7.69 g/L @ pH 5 and temperature 20 °C[1]<br>8.10 g/L @ pH 7 and temperature 20 °C[1,2]<br>8.22 g/L @ pH 9 and temperature 20 °C[1] |
| Logarithm of the octanol-water partition coefficient (log $K_{ow}$) | 0.42 @ pH 5, -0.45 @ pH 7 and -0.96 @ pH 9[1]<br>4.62 @ pH 7 and temperature 20°C[2] |
| Logarithm of the organic carbon water partition coefficient (log $K_{oc}$) | 1.43[2] |
| Logarithm of the bioconcentration factor (log BCF) | -0.11[2] |
| Half-life ($t_{1/2}$) in water | 8.7 days @ pH 7 and temperature 20 °C[2] |
| Half-life ($t_{1/2}$) in soil | Typical: 39 days<br>(30–39 days in the field and in the lab @ 20 °C, respectively)[2]<br>Average: 30 days<br>(3.7–314 days)[3] |

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

Triclopyr belongs to the pyridine group of herbicides, which also includes fluroxypyr, haloxydine and thiazopyr. Triclopyr is extensively used in uncultivated areas (non-crop land such as grassland, rangelands, pastures and ornamental turf), plantation crops (i.e. rice fields), forestry (i.e. coniferous forests), and industrial situations to control perennial broad-leaved and woody weeds (BCPC 2012; University of Hertfordshire 2013; APVMA 2014). Triclopyr has little to no effect on grasses (Tu et al. 2001).

Triclopyr is rapidly absorbed through the roots and foliage of plants. 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). Triclopyr acts by mimicking the plant

hormone, auxin (indolylacetic acid), which is responsible for promoting stem elongation and maintaining apical dominance in dicots. Following administration, triclopyr acidifies the cell walls of plants which causes cells to elongate in an uncontrolled and disorganised manner (approximately 1,000× natural levels), ultimately leading to plant death (Ganapathy 1997; Tu et al. 2001).

Triclopyr is a selective, systemic herbicide which may ultimately end up in aquatic ecosystems as a result of aerial drift and inadvertent overspray (BCPC 2012; Tu et al. 2001). Triclopyr has weak soil sorption ability as indicated by its low log $K_{oc}$ value (Table 1) and has the potential to move offsite following the first heavy rainfall event (BCPC 2012; Ganapathy 1997). The degradation of triclopyr in water is relatively fast, with a half-life of 8.7 days at pH 7 and a temperature of 20 ºC (University of Hertfordshire 2013). Triclopyr is moderately mobile in soils, however Tu et al. (2001) suggests that triclopyr is only prone to lateral movement rather than vertical movement – generally remaining in the top 15 cm of soil. The degradation of triclopyr in soils is a little slower, with an average half-life of between 30 and 39 days (University of Hertfordshire 2013; Tu et al. 2001). Removal of triclopyr from soil is predominantly via microbial degradation; as temperature and moisture conditions in the soil increase, the rate of degradation also increases (Newton et al. 1990; Ganapathy 1997).

## 14.2 Freshwater

### 14.2.1 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 triclopyr in freshwaters (Table 58) 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 macrophytes and two microalgae. The toxicity values for the single fish species were 65-day NOEL and LOEC (mortality) values of 26 and 48 µg/L, respectively. The toxicity values for macrophytes were 14-day NOEC/NOEL (frond number, dry weight, frond area, fresh weight, shoot length, plant area, area under the growth curve) values ranging from 9.1 to 1,020 µg/L, two 14-day LOEC/LOEL (frond number, dry weight, frond area) values of 9.1 and 160 µg/L, 14-day IC25 (dry weight, fresh weight, shoot length, root length, root number, plant area, area under the growth curve) values ranging from 20.6 to 2,660 µg/L, 14-day EC50/IC50 (frond number, dry weight, frond area, fresh weight, shoot length, root length, root number, plant area, area under the growth curve) values ranging from 560 to 6,460 µg/L. The toxicity values for the microalgae were 96-hour NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 0.096 and 2.9 µg/L, respectively, two 5-day NOEL (biomass yield, growth rate, area under the growth curve) values of 353 and 7,000 µg/L and two 5-day EC50 (biomass yield, growth rate, area under the growth curve) values of 2,000 and 32,500 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for 10 fish, one cladoceran and two microalgae. The toxicity values for the fish consisted of a 24-hour LOEL (mortality) value of 1,300 µg/L, 24-hour LC50 (mortality) values ranging from 2,310 to 13,300 µg/L, 48-hour LC50 (mortality) values ranging from

7,500 to 9,600 µg/L, 72-hour LC50 (mortality) values ranging from 6,100 to 9,700 µg/L, two 96-hour NOEL (mortality) values of 280 and 290 µg/L, 96-hour LC50 (mortality) values ranging from 360 to 148,000 µg/L and a 5-day NOEC (length) value of 10,000 µg/L. The toxicity values for the single cladoceran were two 48-hour NOEL (body length, dry weight) values of 1,500 and 32,000 µg/L, a 48-hour LOEC (body length, dry weight) value of 700 µg/L and 48-hour EC50 (body length, dry weight) values ranging from 1,700 to 132,900 µg/L. The toxicity values for the microalgae were two 24-hour NOEL (biomass yield, growth rate, area under the growth curve) values of 2 and 520 µg/L and 24-hour EC50 (biomass yield, growth rate, area under the growth curve) values of 100 and 1,970 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.

### 14.2.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of triclopyr. 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 triclopyr (Table 56).

### 14.2.3 Guideline derivation

The derived PGVs for triclopyr in freshwaters are provided in Table 57. 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 PGVs, the PGVs for triclopyr are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for triclopyr are low (Table 56) 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 triclopyr do not need to account for secondary poisoning.

**Table 57 Proposed aquatic ecosystem protection guideline values (µg/L) for triclopyr for the protection of freshwater ecosystems.**

| Triclopyr proposed aquatic ecosystem protection guideline values (freshwater)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI)[3] |
| 99% | 1.6 (0.35 – 84) |
| 95% | 6.4 (1.6 – 130) |
| 90% | 12 (3.2 – 150) |
| 80% | 24 (6.9 – 180) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 5 |
| Type of toxicity data | Chronic NOEC/NOEL values |
| SSD model fit | Poor |
| 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.**

### 14.2.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for triclopyr in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity

data for triclopyr 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 triclopyr toxicity data available that enable the calculation of PGVs in freshwaters. In order to derive higher reliability PGVs in the future, it is recommended that additional chronic toxicity tests of triclopyr with phototrophic (e.g. plants and algae) freshwater species be conducted.

In total, there were toxicity data for 14 freshwater 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 Magnoliopsida (a grouping of flowering plants).

Based on the current understanding of the mode of action of triclopyr, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as it mimics auxin, which is a plant growth hormone that exists in vascular plants as well as algal species. The triclopyr 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 ($p = 0.067$, see section 14.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for triclopyr in freshwater.

There were freshwater chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data available for five species (that belonged 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 57) 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 triclopyr in freshwater environments is provided in Table 58.

**Table 58 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 triclopyr 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 | *Anabaena flos-aquae* | Cyanobacteria | Cyanophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 353 | USEPA (2015b) |
| Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 255.5 | USEPA (2015b) |
| Macrophyte | *Myriophyllum sibiricum* | Tracheophyta | Magnoliopsida | Not stated | 14 | Chronic NOEC | Dry weight, shoot length, plant area, AUC[2] | 9.1 | Roshon (1997) |
| Fish | *Oncorhynchus mykiss** | Chordata | Actinopterygii | Early life stage | 65 | Chronic NOEL | Mortality | 26 | USEPA (2015b) |
| Microalga | *Selenastrum capricornutum*[2] | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEL | Biomass yield, growth rate, AUC[2] | 117 | USEPA (2015b) |

**[1] Chronic NOEC/NOEL = no conversions applied (Warne et al. 2015). [2] This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.**

### 14.2.5 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the five freshwater, phototrophic and heterotrophic species that was used to derive the PGVs is presented in Figure 48.

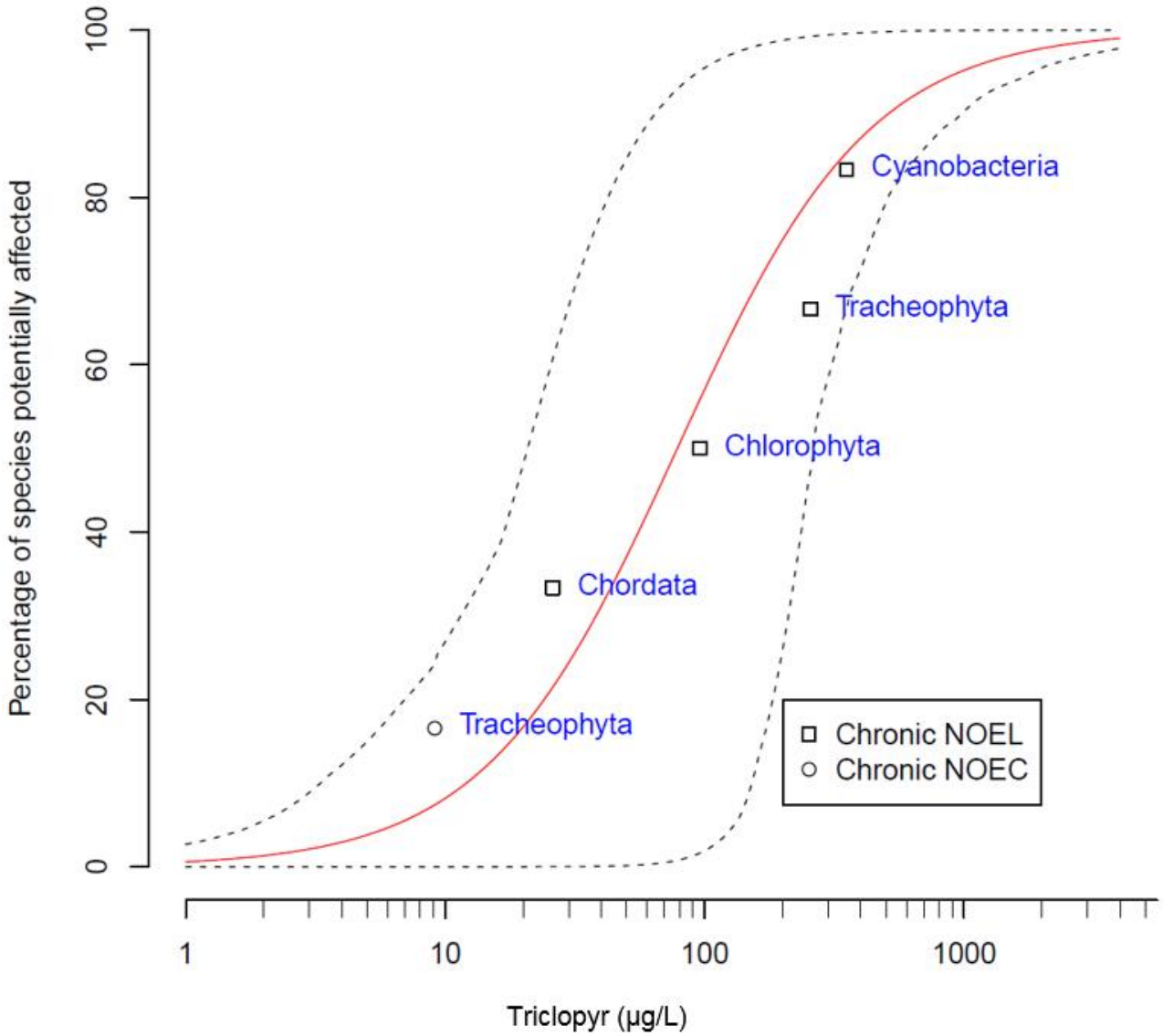



Figure 48 Cumulative frequency distribution, generated using BurrliOz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) data values of freshwater phototrophic and heterotrophic species to triclopyr. Black dashed lines indicate the 95% confidence intervals.

## 14.3 Marine

### 14.3.1 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 triclopyr in marine waters (Table 60) includes toxicity data to two species (one marine and one 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.

*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 crustacean, one mollusc and one microalga. The toxicity values for the single fish species were 96-hour NOEL and LC50 (mortality) values of 300 and 450 µg/L, respectively. The toxicity values for the single crustacean species were 96-hour NOEL and LC50 (mortality) values of 370 and 2,480 µg/L, respectively. The toxicity values for the single mollusc species were 96-hour LOEL and EC50 (mortality, abnormal development) values of 300 and 460 µg/L, respectively. The toxicity values for the single microalga species were 24-hour NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 210 and 1,170 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.

*Freshwater Chronic*

There were freshwater chronic toxicity data for one fish, two macrophytes and two microalgae. The toxicity values for the single fish species were 65-day NOEL and LOEC (mortality) values of 26 and 48 µg/L, respectively. The toxicity values for macrophytes were 14-day NOEC/NOEL (frond number, dry weight, frond area, fresh weight, shoot length, plant area, area under the growth curve) values ranging from 9.1 to 1,020 µg/L, two 14-day LOEC/LOEL (frond number, dry weight, frond area) values of 9.1 and 160 µg/L, 14-day IC25 (dry weight, fresh weight, shoot length, root length, root number, plant area, area under the growth curve) values ranging from 20.6 to 2,660 µg/L, 14-day EC50/IC50 (frond number, dry weight, frond area, fresh weight, shoot length, root length, root number, plant area, area under the growth curve) values ranging from 560 to 6,460 µg/L. The toxicity values for the microalgae were 96-hour NOEL and EC50 (biomass yield, growth rate, area under the growth curve) values of 0.096 and 2.9 µg/L, respectively, two 5-day NOEL (biomass yield, growth rate, area under the growth curve) values of 353 and 7,000 µg/L and two 5-day EC50 (biomass yield, growth rate, area under the growth curve) values of 2,000 and 32,500 µg/L.

*Freshwater Acute*

There were freshwater acute toxicity data for 10 fish, one cladoceran and two microalgae. The toxicity values for the fish consisted of a 24-hour LOEL (mortality) value of 1,300 µg/L, 24-hour LC50 (mortality) values ranging from 2,310 to 13,300 µg/L, 48-hour LC50 (mortality) values ranging from 7,500 to 9,600 µg/L, 72-hour LC50 (mortality) values ranging from 6,100 to 9,700 µg/L, two 96-hour NOEL (mortality) values of 280 and 290 µg/L, 96-hour LC50 (mortality) values ranging from 360 to

148,000 µg/L and a 5-day NOEC (length) value of 10,000 µg/L. The toxicity values for the single cladoceran were two 48-hour NOEL (body length, dry weight) values of 1,500 and 32,000 µg/L, a 48-hour LOEC (body length, dry weight) value of 700 µg/L and 48-hour EC50 (body length, dry weight) values ranging from 1,700 to 132,900 µg/L. The toxicity values for the microalgae were two 24-hour NOEL (biomass yield, growth rate, area under the growth curve) values of 2 and 520 µg/L and 24-hour EC50 (biomass yield, growth rate, area under the growth curve) values of 100 and 1,970 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.

### 14.3.2 Factors affecting toxicity

No factors have been reported as modifying the toxicity of triclopyr. 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 triclopyr (Table 56).

### 14.3.3 Guideline derivation

The derived PGVs for triclopyr in marine waters are provided in Table 59. 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 PGVs, the PGVs for triclopyr are expressed in terms of the concentration of the active ingredient.

Measured log BCF values for triclopyr are low (Table 56) 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 triclopyr do not need to account for secondary poisoning.

**Table 59 Proposed aquatic ecosystem protection guideline values (µg/L) for triclopyr for the protection of marine ecosystems.**

| Triclopyr proposed aquatic ecosystem protection guideline values (marine)[1] | |
|---|---|
| Species protection | Concentration (µg/L) (95% CI) |
| 99% | 0.36 (0.058 – 14) |
| 95% | 4.0 (1.2 – 33) |
| 90% | 11 (3.9 – 51) |
| 80% | 32 (12 – 88) |

| Reliability classification[2] | |
|---|---|
| Criterion | Result |
| Sample size | 6 |
| Type of toxicity data | Chronic NOEC/NOEL and converted acute values (*freshwater and marine*) |
| SSD model fit | Poor |
| 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".**

### 14.3.4 Toxicity data used in derivation

Previously, no Australian and New Zealand GV (formerly referred to as a trigger value) existed for triclopyr in freshwater or marine environments (ANZECC and ARMCANZ 2000). To obtain toxicity data for triclopyr 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 triclopyr 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. 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 triclopyr with phototrophic (e.g. plants and algae) marine species be conducted.

In total, there were toxicity data for four marine 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), Bacillariophyceae (diatoms; a major grouping of algae), Bivalvia (a grouping of molluscs) and Malacostraca (a large grouping of crustaceans).

Based on the current understanding of the mode of action of triclopyr, it would be expected that phototrophic species would be more sensitive than non-phototrophic species, as it mimics auxin, which is a plant growth hormone that exists in vascular plants as well as algal species. The triclopyr 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 ($p = 0.067$, see section 14.3.6) sensitivities. Therefore, as recommended by Warne et al. (2015), the data for both phototrophs and heterotrophs were combined to calculate the PGVs for triclopyr in marine water.

There were marine converted acute (acute EC50/LC50 toxicity data that had been converted to estimates of chronic NOEC by dividing by 10) data available for only four species (that belonged to only four species and four phyla), 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 triclopyr to marine species were available, the converted acute values for marine species were combined with the available chronic no observed effect concentration (NOEC) and no observed effect level (NOEL) values for freshwater species to derive PGVs for triclopyr in marine waters. This dataset incorporated concentration data for nine (four marine and five freshwater) phototrophic and heterotrophic species belonging to seven 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 59) combined with the poor fit of the distribution to these toxicity data (Figure 49) resulted in a low 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 triclopyr in marine environments is provided in Table 60.

**Table 60 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 triclopyr 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 | Microalga | *Anabaena flos-aquae* | Cyanobacteria | Cyanophyceae | Not stated | 5 | Chronic NOEL | Biomass yield, growth rate, AUC[3] | 353 | USEPA (2015b) |
| Marine | Macroinvertebrate | *Crassostrea virginica* | Mollusca | Magnoliopsida | SPAT | 4 | Converted acute | Mortality, abnormal development | 46 | USEPA (2015b) |
| Fresh | Macrophyte | *Lemna gibba* | Tracheophyta | Liliopsida | Not stated | 14 | Chronic NOEL | Frond number, dry weight, frond area | 255.5 | USEPA (2015b) |
| Marine | Fish | *Menidia beryllina* | Chrodata | Actinopterygii | Juvenile | 4 | Converted acute | Mortality | 46 | USEPA (2015b) |
| Fresh | Macrophyte | *Myriophyllum sibiricum* | Tracheophyta | Magnoliopsida | Not stated | 14 | Chronic NOEC | Dry weight, shoot length, plant area, AUC[3] | 9.1 | Roshon (1997) |
| Fresh | Fish | *Oncorhynchus mykiss** | Chordata | Actinopterygii | Early life stage | 65 | Chronic NOEL | Mortality | 26 | USEPA (2015b) |
| Marine | Macroinvertebrate | *Palaemonetes pugio* | Arthropoda | Malacostraca | Not stated | 4 | Converted acute | Mortality | 248 | USEPA (2015b) |
| Fresh | Microalga | *Selenastrum capricornutum*[2] | Chlorophyta | Chlorophyceae | Not stated | 4 | Chronic NOEL | Biomass yield, growth rate, AUC[3] | 117 | USEPA (2015b) |
| Marine | Microalga | *Skeletonema costatum** | Bacillariophyta | Mediophyceae | Not stated | 1 | Converted acute | Biomass yield, growth rate, AUC[23] | 117 | 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.**

### 14.3.5 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 49.

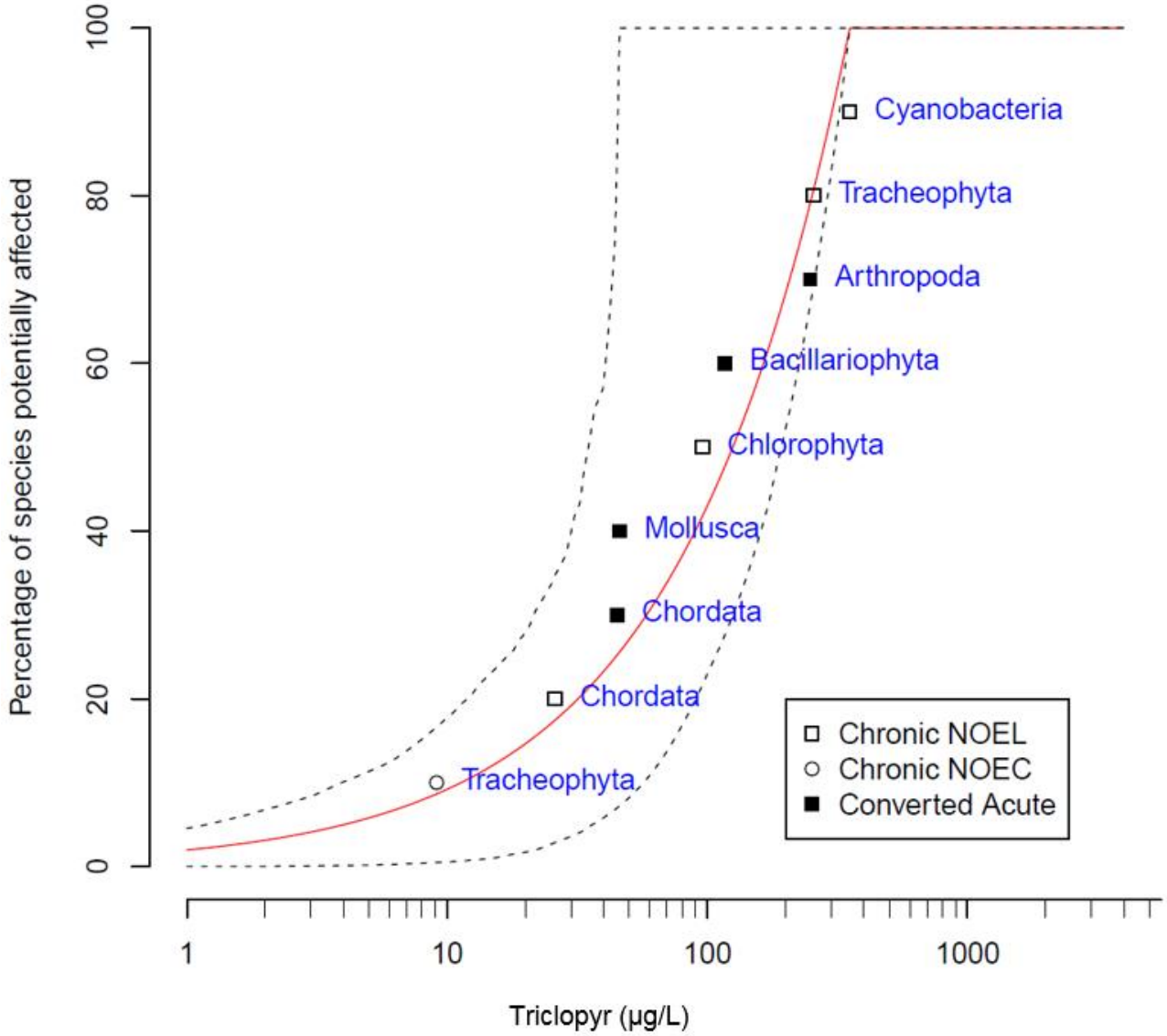



Figure 49 Cumulative frequency distribution, generated using BurrliOz 2.0 (2016), of the sensitivity of chronic no observed effect concentration (NOEC), no observed effect level (NOEL) and converted acute data values of marine and freshwater, phototrophic and heterotrophic species to triclopyr. Black dashed lines indicate the 95% confidence intervals.

### 14.3.6 Distribution of sensitivities for aquatic species

Statistical analysis of the triclopyr 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 triclopyr freshwater and marine concentration data had equal variances (Fisher's F-Test; p = 0.271) and followed a normal distribution (Anderson-Darling; p = 0.287). Results from the two-sample *t* test test indicated that the two groups were not significantly different (p = 0.344); therefore, the freshwater and the marine triclopyr ecotoxicity data can be pooled for further analysis.

The toxicity data for triclopyr 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 triclopyr ecotoxicity data may be unimodal (Figure 50).

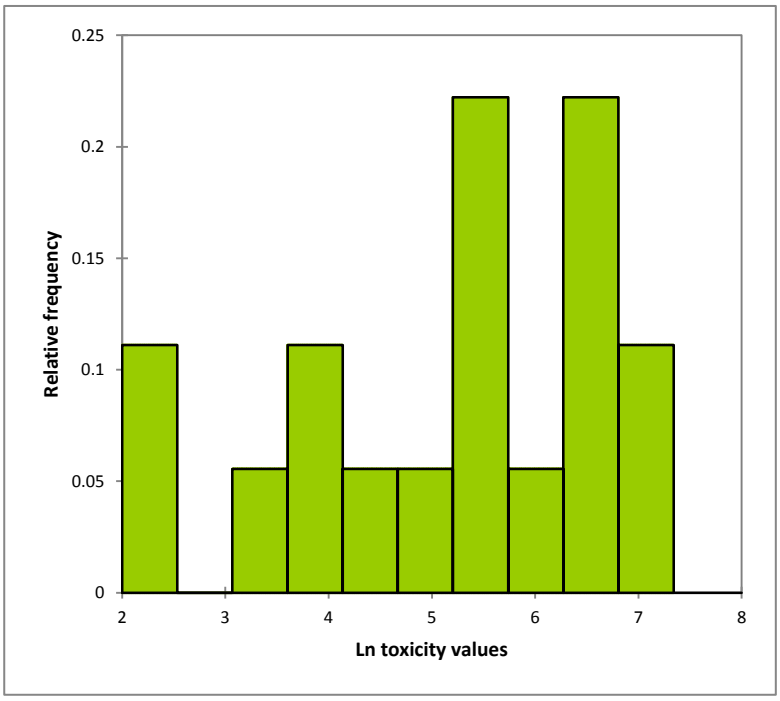



Figure 50 Histogram of the natural logarithm (ln) of all triclopyr (freshwater and marine) toxicity data for phototrophic and non-phototrophic species (*n* = 18).

The triclopyr 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 triclopyr concentration data had equal variances (Fisher's F-Test; p = 0.598) and followed a normal distribution (Anderson-Darling; p = 0.287). Results from the two-sample *t* test indicated that the two groups were not significantly different (p = 0.067), therefore it can be concluded that the distribution of the triclopyr concentration data is uni-modal.

### 14.3.7 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.

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: 11 April 2017.

BCPC (2012). A world compendium. The pesticide manual. Sixteenth Edition. MacBean (Ed), British Crop Production Council, Alton, United Kingdom, 1151–1153.

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/.

Ganapathy C (1997). Environmental fate of triclopyr, Department of Pesticide Regulation Environmental Monitoring and Pesticide Management Branch, Sacramento, CA 95814-5624. Available from: http://www.cdpr.ca.gov/docs/emon/pubs/fatememo/triclopyr.pdf, Accessed 11 April 2017.

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.

Newton M, Roberts F, Allen A, Kelpsas B, White D and Boyd P (1990). Deposition and dissipation of three herbicides in foliage, litter and soil of brushfields of southwest Oregon, *Journal of Agricultural and Food Chemistry*, 38, 574–583.

Roshon RD (1997). A toxicity test for the effects of chemicals on the non-target submersed aquatic macrophyte, *Myriophyllum sibiricum* Komarov, Ph.D.Thesis, University of Guelph, Ontario, Canada, 464 pp.

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.

Tu M, Hurd C, Robinson R and Randall JM (2001). Weed Control Methods Handbook: Triclopyr. The Nature Conservancy, Virginia, United States of America, 135–141. Available from: https://www.invasive.org/gist/products/handbook/methods-handbook.pdf, Accessed: 11 April 2017.

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.