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Toxicant default guideline values for aquatic ecosystem protection

Diuron in freshwater

Technical brief

October 2024

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Summary

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

Diuron (3-(3,4-dichlorophenyl)-1,1-dimethylurea, CAS no. 330-54-1) is a systemic urea herbicide, specifically a phenylurea herbicide. Other phenylurea herbicides include linuron, fluometuron and isoproturon. Diuron is a photosynthesis-inhibiting herbicide commonly used for the total control of weeds and mosses as well as selective control of germinating grass and broad-leaved weeds that occur in a variety of crops (University of Hertfordshire 2013). It is also used in urban and industrial environments (e.g., roadsides, railways, areas around industrial buildings), as well as for aquatic weed and algae control in flood mitigation channels and as a boat antifoulant.

The previous DGV for diuron in freshwater was a low reliability value (based on the ANZECC/ARMCANZ (2000) reliability scheme), as it was calculated using an assessment factor of 200 for one chronic toxicity value for the freshwater fish *Pimephales promelas* (ANZECC/ARMCANZ 2000). More data on diuron toxicity are now available, including data for phototrophic species, enabling the derivation of higher reliability DGVs.

The specificity of the mode of action of diuron and the distinct (albeit incomplete) separation in the sensitivity of different taxa groups indicate that the sensitivity of diuron is bimodal, with phototrophs (aquatic plants) being the more sensitive group. Therefore, only toxicity data for the most sensitive group of organisms (i.e., phototrophs) were used to derive the species sensitivity distribution (SSD) and DGVs for diuron in freshwater.

Very high reliability DGVs for diuron in freshwater were derived based on chronic 5% effect concentration (EC5), 10% effect concentration (EC10) and no observed effect level (NOEL) data for 16 freshwater phototrophic species from four phyla, with a good fit of the SSD to the toxicity data. Only toxicity data for technical grade material (or equivalent) with a purity greater than 80% were used to derive the DGVs (Warne et al. 2018). The DGVs are expressed in terms of the active ingredient; they relate to diuron only, and not any of its formulations or breakdown products. The DGVs for 99%, 95%, 90% and 80% species protection are 0.22 µg/L, 0.52 µg/L, 0.88 µg/L and 1.8 µg/L, respectively. The 95% species protection level for diuron in freshwater is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1 Introduction

Diuron is a herbicide ($C_9H_{10}Cl_2N_2O$; see Figure 1) that, at room temperature, exists in the form of odourless, colourless crystals. It is the active ingredient of a variety of commercial herbicide formulations. Major metabolites of diuron are the demethylated diuron compounds, N'-(3-chlorophenyl)-N,N-dimethylurea, N'-(3,4-dichlorophenyl)-N-methylurea, and 3,4-dichlorophenylurea (APVMA 2011). Physico-chemical properties of diuron that may affect its environmental fate and toxicity are in Table 1.

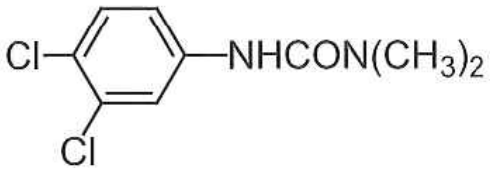

Figure 1 Structure of diuron

Table 1 Summary, selected physico-chemical properties of diuron

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

a BCPC (2012).
b University of Hertfordshire (2013).
c Peterson and Batley (1991).

Diuron belongs to the phenylurea group within the urea family of herbicides, which also includes linuron, fluometuron and isoproturon. In Australia, diuron has been one of the most heavily used herbicides, exceeded only by glyphosate, simazine, and atrazine (AATSE 2002). It is a pre-emergence, residual herbicide as well as a post-emergence knockdown (University of Hertfordshire 2013) that exhibits some solubility in water (Table 1). Diuron is extensively used in agriculture to control weeds in a variety of crops. In Australia, it is currently registered for application to 17 crops (APVMA 2020), which include: cereals (barley, lucerne, oats, rye, triticale); fruit (banana); vegetables (asparagus, potato); legumes (chickpea, faba bean, field pea, lentil, lupin, narbon bean, vetch); fibres (cotton); and sugar cane. Non-agricultural uses include application to pasture, fallow, channels and drains (APVMA 2020).

In New Zealand, diuron is registered for use on a range of crops, including grapes, kiwifruit, apples, asparagus, strawberries (grown in polyethylene) and bulb flowers, as well as for non-cropland areas such as roadsides, railways around farm buildings and irrigation and drainage ditches (ACVM 2020). Diuron is also used to control weeds and algae in and around waterbodies and is a component of marine antifouling paints (APVMA 2009).

Diuron can be transported to freshwater, estuarine, and marine environments via surface and/or sub-surface runoff from agricultural applications following heavy or persistent rain, as well as from antifouling paints (biocides) applied to marine vessels (APVMA 2009). Loss of diuron via volatilisation is minimal due to its solubility in water (Table 1) and low soil adsorption as indicated by its low log K_{OC} value (Table 1) (Field et al. 2003). Diuron is relatively mobile and has been found to leach to groundwater and be transported in surface water (Field et al. 2003, AVPMA 2011).

Diuron has been commonly detected in estuarine and marine water and sediment in countries including Australia, Italy, Japan, the Netherlands, Portugal, Spain, Sweden, and the UK (Konstantinou & Albanis 2004, Ali et al. 2014, Ansanelli et al. 2017). This is due to sources associated with agricultural land use and, to a lesser extent, urban use, and its use as a component of antifouling paints (AVPMA 2011). For example, diuron was detected in approximately 66% of surface water samples collected between 2011 and 2015 in waterways that drained agricultural land and discharged to the Great Barrier Reef (based on data in Turner et al. 2013a, 2013b, Wallace et al. 2014, 2015, 2016, Garzon-Garcia et al. 2015). Related to this, diuron has been widely detected throughout the Great Barrier Reef and was the most frequently detected pesticide in these waters between 2010 and 2015 (Kennedy et al. 2011, Bentley et al. 2012, Gallen et al. 2013, 2014, 2016). It has also been detected in the Sydney estuary (Birch et al. 2015).

The Australian Pesticides and Veterinary Medicines Authority (APVMA) finalised the chemical review of diuron, including an environmental assessment, in November 2012. The review identified that a principal concern was the risk associated with diuron runoff to waterways. The APVMA deregistered selected products where the risk was unmanageable and varied the approved label instructions to remove or amend uses where the risk of runoff could not be managed. Current restraints on diuron use in Australia are on the APVMA website.

2 Aquatic toxicology

2.1 Mechanisms of toxicity

Diuron is absorbed principally through the roots of plants. It is then translocated acropetally (i.e., movement upwards from the base of the plant to the apex) in the xylem and accumulates in the leaves (BCPC 2012). Diuron exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Photosynthesis inhibiting herbicides bind to the plastoquinone B protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (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 increase the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen ($^1O_2$), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). ROS are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). ROS are created during normal cellular functions, particularly in biochemical processes that involve the generation of energy (e.g., photosynthesis in chloroplasts and the Krebs cycle in the mitochondria of cells) and are involved in cellular processes (Chen et al. 2012). 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). Prolonged exposure to elevated concentrations of ROS in plants, resulting from biotic (e.g., disease) and/or abiotic (e.g., PSII inhibiting herbicides) stressors, can cause irreversible cell damage and ultimately lead to cell death (apoptosis) (Vass 2011).

2.2 Relative toxicity

There were acute or chronic toxicity data for 58 freshwater species that passed the screening and quality assessment processes. These consisted of 27 phototrophic species and 31 heterotrophic species. The 27 phototrophic species consisted of 15 diatoms, six green algae, four macrophytes and two cyanobacteria (blue–green algae). The 31 heterotrophic species consisted of 14 fish, nine crustaceans, four amphibians, two insects, one gastropod and one ciliate.

The toxicity data indicated that the phototrophs are generally more sensitive than the heterotrophs; only eight (of the 31) heterotrophic species had sensitivities within the range of the phototrophic species. This finding is consistent with diuron's primary mode of action on the PSII complex.

The following discussion of the relative sensitivity of phototrophs and heterotrophs is based on chronic toxicity data. There did not appear to be any difference in the sensitivity of the four types of freshwater phototrophs. Chronic toxicity values for diatoms ranged from 0.069 µg/L (96 h EC50, population growth) for *Fragilaria capucina* var. *vaucheriae* to 4 236 µg/L (96 h EC50, population growth) for *Eolimna minima* (Larras et al. 2012). Toxicity values for green algae ranged from 0.44 µg/L (96 h EC50, cell yield) for *Pseudokirchneriella subcapitata* (USEPA 2015b) to 46.3 µg/L (72 h, population growth) for *Desmodesmus subspicatus* (Masojidek et al. 2011). The toxicity values for macrophytes ranged from 2.49 µg/L (7 d NOEL, frond number) for *Lemna gibba* (USEPA 2015b) to 28.3 µg/L (7 d EC50, frond number) for *Lemna minor* (Gatidou et al. 2015), while the toxicity values for blue–green algae ranged from 1.14 µg/L (72 h NOEL, biomass yield) for *Synechococcus leopoliensis* (USEPA 2015b) to 80 µg/L (12 d EC50, population growth) for *Anabaena variabilis* (Singh et al. 2011).

Chronic toxicity values for heterotrophic species ranged from 3.3 µg/L (10 d LC50) for *Chironomus tentans* (Nebeker & Schuytema 1998) to 22 200 µg/L (14 d LC50) for *Rana aurora* (Schuytema & Nebeker 1998). Fish toxicity values ranged from 26.4 µg/L (60 d NOEC, mortality) for *Pimephales promelas* (USEPA 2015b) to 5 900 µg/L (21 d LC50) for *Oncorhynchus mykiss* (Okamura et al. 2002). The amphibians had toxicity values that ranged from 7 600 µg/L for *Rana aurora* (14 d NOEC, wet weight) and *R. catesbeianato* (10 d and 21 d NOEC, dry weight) to 22 200 µg/L for *R. aurora* (14 d LC50, mortality) (Schuytema & Nebeker 1998). Crustacean toxicity data ranged from 4 µg/L (7 d NOAEL, mortality) for *Daphnia pulex* (Nebeker & Schuytema 1998) to 560 µg/L (28 d LOEC, mortality)

for *Americamysis bahia* (USEPA 2015b). The toxicity data for insects ranged from 1.9 µg/L (10 d NOAEL, mortality) to 7.10 µg/L (10 d LOAEL, growth) for *Chironomus tentans* (Nebeker & Schuytema 1998).

3 Factors affecting toxicity

Black carbon and suspended solids have been reported to modify the toxicity of diuron, while water flow rate has been reported to affect the accumulation of diuron. The addition of 50 mg/L of natural black carbon to 5 µg/L of diuron reduced the inhibition of photosynthesis in a green alga from 55% to 40% (Knauer et al. 2007). The addition of the same concentration of combusted black carbon to 5 µg/L of diuron caused a complete recovery of photosynthesis (Knauer et al. 2007). It is expected that dissolved and particulate organic matter and suspended solids would also affect the bioavailability and toxicity of diuron, as particle-bound forms may be less bioavailable to aquatic phototrophs. Davis et al. (2012) found that approximately 33% of the diuron that discharges to the Great Barrier Reef from tropical rivers was transported in a particle-bound form, although it should be noted that DGVs typically relate only to the dissolved fraction of chemical rather than the total or particulate-bound fractions. Chaumet et al. (2019) found that reduced flow rate in artificial stream channels increased the concentrations of diuron in the tissue of freshwater biofilms, indirectly leading to greater toxicity.

As noted in Section 2.1, one of the modes of action of diuron is to increase the formation of ROS. Given that the formation of ROS is dependent on the presence of light, it is plausible that increased turbidity (e.g., from increased suspended solids) could decrease diuron toxicity. However, the information on this potential toxicity modifying factor for PSII herbicides is contradictory. A review by Knauer et al. (2017) concluded that the presence of suspended solids did not significantly decrease the toxicity of a range of pesticides, including atrazine (a PSII herbicide, like diuron), to freshwater species. Wilkinson et al. (2015) examined the combined effects of diuron and light intensity to the seagrass *Halophila ovalis* and found that the interaction was sub-additive (antagonistic) at low light intensity, additive at saturating light intensity and additive or synergistic at elevated light intensity.

Wilkinson et al. (2017) also found that water temperatures greater or less than the thermal optimum for *H. ovalis* exerted sub-additive effects when combined with diuron. However, these sub-additive effects were still greater than the effect of each stressor alone.

4 Default guideline value derivation

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

4.1 Toxicity data used in derivation

To obtain data for diuron toxicity to freshwater organisms, a search of the scientific literature was conducted. In addition, the following databases were searched: USEPA (2015a) ECOTOX Knowledgebase; Office of Pesticide Programs Database (USEPA 2015b); Australasian Ecotoxicology Database (Warne et al. 1998); and ANZECC/ARMCANZ (2000) and Sunderam et al. (2000) toxicant

databases. Compared to the ANZECC/ARMCANZ (2000) DGV, there are now more diuron toxicity data available, including data for phototrophic species, which enabled the derivation of higher reliability DGVs for diuron in freshwater. The toxicity data used to calculate the DGVs were determined from experiments using technical or higher grade diuron with a minimum purity of 80% active ingredient (Warne et al. 2018).

There were toxicity data for 58 freshwater species (eight phyla and 13 classes) that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Ciliophora, Cyanophyta, Mollusca and Tracheophyta. The 13 classes were Actinopterygii (which accounts for approximately 99% of fish), Amphibia (tetrapod vertebrates), Bacillariophyceae (a major grouping of diatoms), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of cyanobacteria), Fragilariophyceae (a grouping of pennate diatoms), Gastropoda (a grouping of molluscs), Insecta (invertebrates), Liliopsida (monocots), Malacostraca (a large grouping of crustaceans), Mediophyceae (another algae grouping) and Oligohymenophorea (a large class of ciliates). Chronic toxicity data were available for 37 of the 58 species, comprising 27 phototrophic species and 10 heterotrophic species; acute toxicity data were available for 27 species, comprising three phototrophic species and 24 heterotrophic species.

A modality assessment of the diuron toxicity data (to both marine and freshwater species) was undertaken according to the weight of evidence approach described by Warne et al. (2018). Most lines of evidence supported the conclusion that the distribution of toxicity data is bimodal, with phototrophs generally more sensitive than heterotrophs (Appendix B: Modality assessment for diuron). Therefore, as recommended by Warne et al. (2018), only the ecotoxicity data for the more sensitive group of organisms (i.e., phototrophs) were used to calculate the DGVs.

Of the available chronic toxicity data, there were negligible effect (i.e., EC5, EC10, NOEC and NOEL) data available for 16 phototrophic species from four phyla and six classes, which met the minimum data requirements (i.e., at least five species belonging to at least four phyla) to use a species sensitivity distribution (SSD) to derive DGVs (Warne et al. 2018). A summary of the toxicity data (one value per species) used to calculate the DGVs for diuron in freshwater is in Table 2. Further details of the water quality parameters for each species used to calculate the DGVs are presented in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

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

Taxonomic group	Species	Life stage	Duration (day)	Toxicity measure [a] (endpoint)	Reported toxicity value (µg/L)	Final toxicity value (µg/L) [b]
Diatom	*Achnanthidium minutissimum* [c]	Planktonic/Benthic mode of growth	4	EC5/EC10 (growth rate)	10.28	10.3

Taxonomic group	Species	Life stage	Duration (day)	Toxicity measure [a] (endpoint)	Reported toxicity value (µg/L)	Final toxicity value (µg/L) [b]
	Craticula accomoda [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	314.46	315
	Cyclotella meneghiniana [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	4.90	4.90
	Encyonema silesiacum [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	10.37	10.4
	Eolimna minima [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	1885.75	1886
	Fragilaria capucina var. *vaucheriae* [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	0.542	0.54
	Fragilaria rumpens [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	7.43	7.43
	Fragilaria ulna [c, d]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	17.59	17.6
	Gomphonema parvulum	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	365.13	365
	Mayamaea fossalis	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	86.47	86.5
	Navicula pelliculosa [c]	–	3	NOEL (biomass yield, growth rate, AUC [e])	9.17	9.17
	Nitzschia palea [c]	Planktonic mode of growth/Benthic mode of growth	4	EC5/EC10 (growth rate)	199.12	199

Taxonomic group	Species	Life stage	Duration (day)	Toxicity measure [a] (endpoint)	Reported toxicity value (µg/L)	Final toxicity value (µg/L) [b]
Green alga	*Scenedesmus subspicatus* [c, f]	–	3	NOEC (biomass yield, growth rate, AUC [e])	2.3	2.30
	Selenastrum capricornutum [g]	–	4	NOEL (biomass yield, growth rate, AUC [e])	0.44	0.44
Macrophyte	*Lemna gibba*	–	7	NOEL (frond number, dry weight, frond area)	2.49	2.49
Cyanobacteria	*Synechococcus leopoliensis* [c]	–	3	NOEL (biomass yield, growth rate, AUC [e])	1.14	1.14

a The measure of toxicity being estimated/determined: EC5: 5% effect concentration; EC10: 10% effect concentration; NOEC: no observed effect concentration; NOEL: no observed effect level.
b Chronic NOEC/NOEL/EC5/EC10 values = no conversions applied (Warne et al. 2018). Values are reported to a maximum of three significant figures.
c Species that originated from, or are distributed in, Australia and/or New Zealand.
d This species has also been called *Ulnaria ulna*.
e AUC = area under the growth curve.
f This species has also been called *Desmodesmus subspicatus*.
g This species has also been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.
–: no data available / not stated.

To identify species that were regionally relevant to Australia and New Zealand ecosystems, a search of Algaebase (Guiry & Guiry 2017), Atlas of Living Australia (ALA 2017), Catalogue of Life (Roskov et al. 2017), Integrated Taxonomic Information System (ITIS 2017) and the World Register of Marine Species (WoRMS 2017) was conducted. The dataset used in the DGV derivation for diuron in freshwater (Table 2) includes toxicity data for 12 freshwater species that either originated from, or are distributed in, Australia and/or New Zealand.

4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 16 freshwater diuron chronic toxicity values reported in Table 2 is shown in Figure 2. The SSD was plotted using Burrlioz 2.0 software. The model provided a good fit to the data (Figure 2).

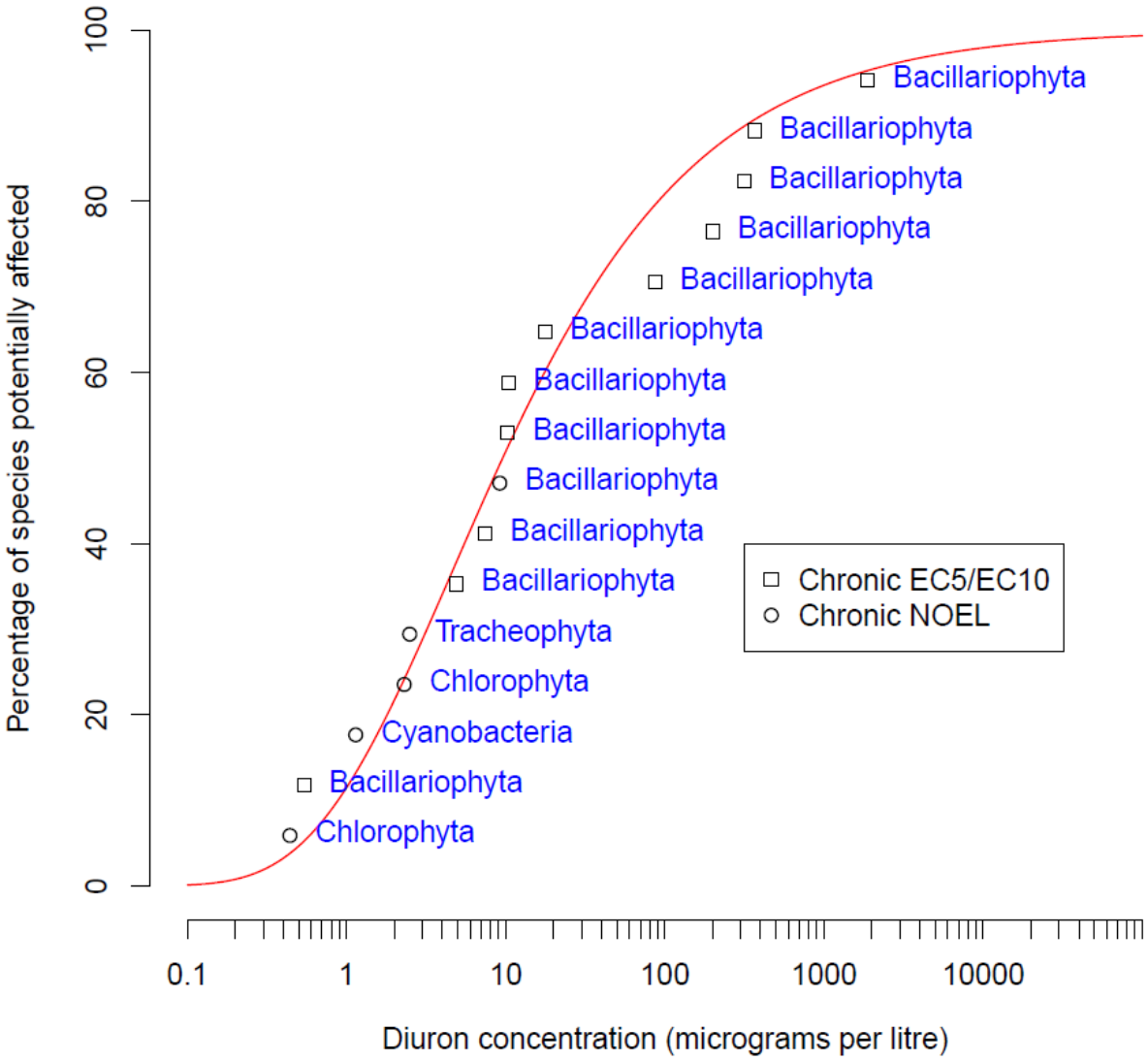

Figure 2 Species sensitivity distribution, diuron in freshwater

4.3 Default guideline values

It is important that the DGVs (Table 3) and associated information in this technical brief are used in accordance with the detailed guidance provided in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality website (ANZG 2018).

The DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 3. As with other pesticides, the diuron DGVs apply to the concentration of the active ingredient. The DGVs relate to dissolved diuron only, and not its breakdown products.

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

The 95% species protection DGV is recommended for application to slightly-to-moderately disturbed ecosystems.

Table 3 Default guideline values, diuron in freshwater, very high reliability

Level of species protection (%)	DGV for diuron in freshwater (µg/L) [a]
99	0.22
95	0.52
90	0.88
80	1.8

a The DGVs were derived using Burrlioz 2.0 software.

4.4 Reliability classification

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

- Sample size—16 (preferred)
- type of toxicity data—chronic
- SSD model fit—good (Inverse Weibull).

Glossary

Term	Definition
acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short exposure period to a chemical relative to the organism's life span.
bimodal	When the distribution of the sensitivity of species to a toxicant has two modes. This typically occurs with chemicals with specific mechanisms 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.
chronic toxicity	A lethal or sublethal adverse effect that occurs after exposure to a chemical for a period that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. site-specific), in the Australian and New Zealand Water Quality Guidelines.
EC50 (median effective concentration)	The concentration of a substance in water or sediment that is estimated to produce a 50% change in the response being measured or a certain effect in 50% of the test organisms relative to the control response, under specified conditions.
ECx	The concentration of a substance in water or sediment that is estimated to produce an x% change in the response being measured or a certain effect in x% of the test organisms, under specified conditions.
endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
guideline value	A measurable quantity (e.g. concentration) or condition of an indicator for a specific community value below which (or above which, in the case of stressors such as pH, dissolved oxygen and many biodiversity responses) there is considered to be a low risk of unacceptable effects occurring to that community value. Guideline values for more than one indicator should be used simultaneously in a multiple lines of evidence approach.
LC50 (median lethal concentration)	The concentration of a substance in water or sediment that is estimated to be lethal to 50% of a group of test organisms, relative to the control response, under specified conditions.
LOEC (lowest observed effect concentration)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
log BCF	Logarithm of the bioconcentration factor.
NOEC (no observed effect concentration)	The highest concentration of a material used in a toxicity test that has no statistically significant adverse effect on the exposed population of test organisms as compared with the controls.
NOEL (no observed effect level)	Synonymous with NOEC.
phototrophs	Organisms that photosynthesise as their main means of obtaining energy (e.g. plants and algae).
PSII	Photosystem II of the photosynthetic biochemical pathway.
ROS (reactive oxygen species)	Highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA).
site-specific guideline value	A guideline value that is relevant to the specific location or conditions that are the focus of a given assessment or issue.

Term	Definition
SSD (species sensitivity distribution)	A method that plots the cumulative frequency of species' sensitivities to a toxicant and fits a statistical distribution to the data. From the distribution, the concentration that should theoretically protect a selected percentage of species can be determined.
toxicity	The inherent potential or capacity of a material to cause adverse effects in a living organism.
toxicity test	The means by which the toxicity of a chemical or other test material is determined. A toxicity test is used to measure the degree of response produced by exposure to a specific level of stimulus (or concentration of chemical) for a specified test period.

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

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

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
Diatom	*Achnanthidium minutissimum*	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	3.15	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	45	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	7.67	Larras et al. (2013)
	–							*10.28*	*Geometric mean*
	–							**10.23**	**Value used in SSD**
	Craticula accomoda	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	261	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	185	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	644	Larras et al. (2013)
	–							*314.46*	*Geometric mean*

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
	–							**314.46**	**Value used in SSD**
	Encyonema silesiacum	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	3.11	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	90	Larras et al. (2013)
		Planktonic mode of growth		EC10 (Growth rate)	DV culture medium	–	–	3.98	Larras et al. (2013)
	–							*10.37*	*Geometric mean*
	–							**10.37**	**Value used in SSD**
	Eolimna minima	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	3 007	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	693	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	3 218	Larras et al. (2013)
	–							*1 885.75*	*Geometric mean*
	–							**1 885.75**	**Value used in SSD**
	Gomphonema parvulum	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	904	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	53	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	1016	Larras et al. (2013)

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
	–							*365.13*	*Geometric mean*
	–							**365.13**	**Value used in SSD**
	Mayamaea fossalis	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	74	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	91	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	96	Larras et al. (2013)
	–							*86.47*	*Geometric mean*
	–							**86.47**	**Value used in SSD**
	Navicula pelliculosa	–	3	NOEL (Biomass yield, growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	9.17	USEPA (2015b)
	–							**9.17**	**Value used in SSD**
	Nitzschia palea	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	106	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	380	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	196	Larras et al. (2013)
	–							*199.12*	*Geometric mean*
	–							**199.12**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
	Fragilaria capucina var. *vaucheriae*	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	0.069	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	21	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	0.11	Larras et al. (2013)
	–							*0.54*	*Geometric mean*
	–							**0.54**	**Value used in SSD**
	Fragilaria rumpens	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	18	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	0.76	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	30	Larras et al. (2013)
	–							*7.43*	*Geometric mean*
	–							**7.43**	**Value used in SSD**
	Fragilaria ulna [a]	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	12.6	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	24	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	18	Larras et al. (2013)
	–							*17.59*	*Geometric mean*

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
	–							**17.59**	**Value used in SSD**
	Cyclotella meneghiniana	Exponential growth phase	4	EC5 (Growth rate)	DV culture medium	21 ± 2	–	1.59	Larras et al. (2012)
		Benthic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	27	Larras et al. (2013)
		Planktonic mode of growth	4	EC10 (Growth rate)	DV culture medium	–	–	2.74	Larras et al. (2013)
	–							*4.90*	*Geometric mean*
	–							**4.90**	**Value used in SSD**
Green alga	*Scenedesmus subspicatus* [c]	–	3	NOEC (Biomass yield, Growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	2.3	USEPA (2015b)
	–							**2.3**	**Value used in SSD**
	Selenastrum capricornutum [d]	–	4	NOEL (Biomass yield, Growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	0.44	USEPA (2015b)
	–							**0.44**	**Value used in SSD**
Cyanobacteria	*Synechococcus leopoliensis*	–	3	NOEL (Biomass yield, Growth rate, AUC [b])	ASTM Type I water	24 ± 2	7.5 ± 0.1	1.14	USEPA (2015b)
	–							**1.14**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (day)	Toxicity measure (test endpoint)	Test medium	Temp (°C)	pH	Concentration (µg/L)	Reference
Macrophyte	*Lemna gibba*	–	7	NOEL (Frond number, dry weight, frond area)	M-Hoaglands or 20X-AAP nutrient media. ASTM type I water	25 ± 2	4.8–5.2 (M-Hoagl-ands) and 7.5 ± 0.1 20X-AAP)	2.49	USEPA (2015b)
	–							**2.49**	**Value used in SSD**

a This species has also been called *Ulnaria ulna*.

b AUC = area under the growth curve.

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

d This species has been called *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.

–: no data available / not stated.

Appendix B: Modality assessment for diuron

A modality assessment was undertaken for diuron according to the four questions stipulated in Warne et al. (2018). These questions and their answers are listed below.

Is there a specific mode of action that could result in taxa-specific sensitivity?
Diuron exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts. Photosynthesis inhibiting herbicides bind to the plastoquinone B protein binding site on the D1 protein in PSII. This prevents the transport of electrons to synthesise adenosine triphosphate (used for cellular metabolism) and nicotinamide adenine dinucleotide phosphate (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 increase the formation of reactive oxygen species (ROS), including the synthesis of singlet oxygen ($^1O_2$), superoxide (O_2^-) and hydrogen peroxide (H_2O_2) (Halliwell 1991). ROS are highly reactive forms of oxygen that readily react with, and bind to, biomolecules including deoxyribonucleic acid (DNA) and ribonucleic acid (RNA). ROS are created during normal cellular functions, 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). Prolonged exposure to elevated concentrations of ROS in plants, due to biotic (e.g., disease) and/or abiotic (e.g., PSII inhibiting herbicides) stressors, can cause irreversible cell damage and ultimately lead to cell death (apoptosis).

Given the main mode of action is the inhibition of electron transport in the PSII complex, it is expected that diuron is more toxic to phototrophs than to heterotrophs.

Does the dataset suggest bimodality?
Modality was assessed using a dataset that combined all freshwater and marine data that passed the screening and quality assessment processes (*n* = 109). This was done to increase the sample size of the dataset being assessed.

All data that were not chronic negligible effect values (e.g., EC10, NOEC) were first converted to this type of data using the methods recommended by Warne et al. (2018). Box and whisker plots for the freshwater data and marine data indicated that there was no difference in the sensitivities of the two groups (Figure B 1). Therefore, the pooled dataset was retained for the modality assessment.

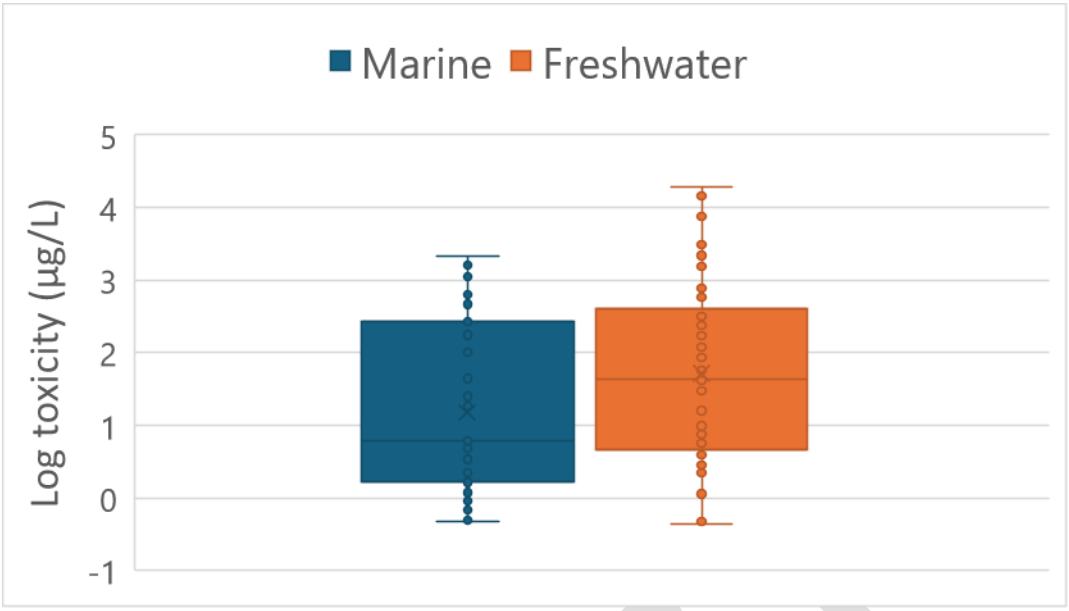

Figure B 1 Box plot, comparison of freshwater and marine species sensitivities to diuron

Calculation of the bimodality coefficient (BC) on log-transformed data yielded a value of 0.542. This is slightly below the indicative threshold BC for bimodality of 0.55, suggesting the dataset may not exhibit bimodality. A frequency histogram of the dataset provided no strong evidence that the dataset was either unimodal or bimodal and a kernal density plot indicated substantial overlap of the two datasets (Figure B 2).

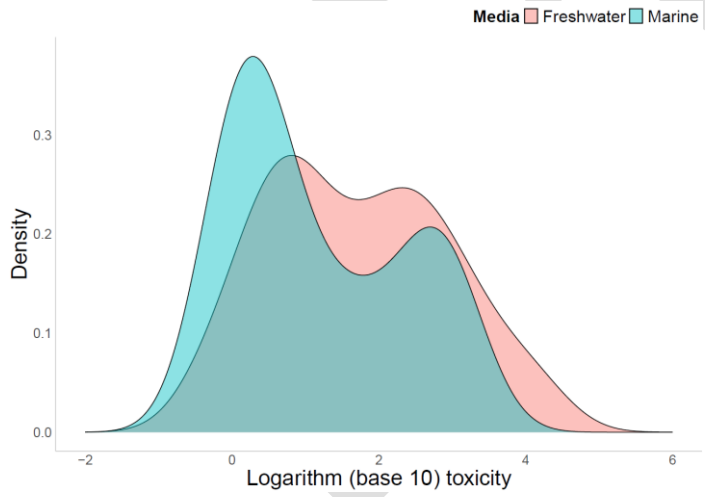

Figure B 2 Kernal density plot of the log-transformed toxicity data for heterotrophic (pink shaded area) and phototrophic (blue shaded area) freshwater and marine species exposed to diuron

Do data show taxa-specific sensitivity (i.e., through distinct groupings of different taxa types)?
The relative sensitivity of different taxa to diuron was compared using box and whisker plots (

Figure B 3) and a species sensitivity distribution (SSD) (

Figure B 4). These indicated that there is a distinct (albeit incomplete) separation in the sensitivity of phototrophs and heterotrophs to diuron.

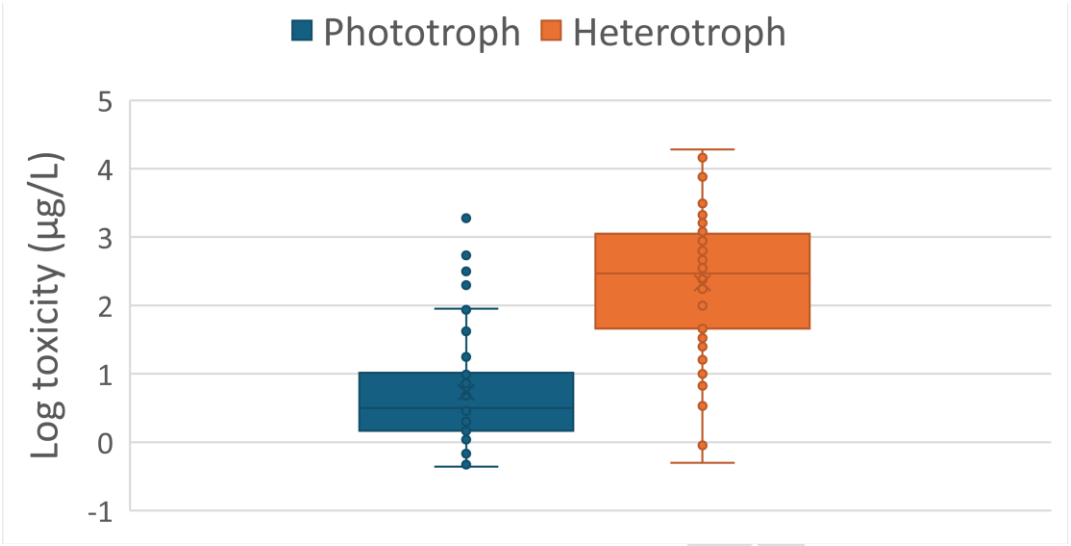

Figure B 3 Box plot, comparison of phototroph and heterotroph sensitivity to diuron

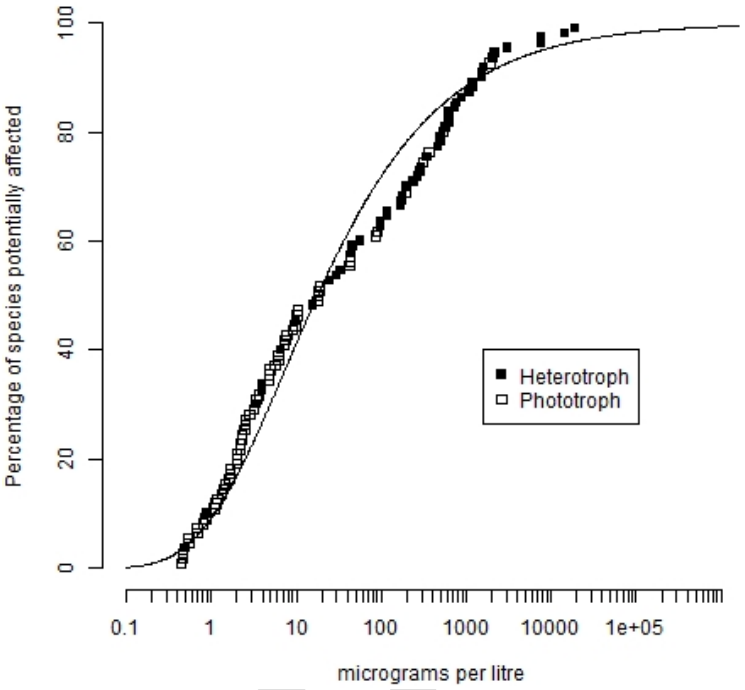

Figure B 4 Species sensitivity distribution, comparison of phototroph and heterotroph sensitivity to diuron

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

No. Given that there are ecotoxicity data for 59 phototrophs and 50 heterotrophs, it is likely that the distributions are representative. Overall, the specificity of the mode of action and the distinct separation of sensitivity indicates that the toxicity of diuron exhibits a bimodal relationship, with phototrophs being the more sensitive group. Therefore, as recommended by Warne et al. (2018), only toxicity data for the most sensitive group of organisms (i.e., aquatic plants) were used to derive the SSD and DGVs for diuron in freshwater.

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