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Toxicant default guideline values for aquatic ecosystem protection

Diuron in marine water

Technical brief

November 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 (i.e. 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 marine water was a low reliability, indicative interim working level (based on the ANZECC/ARMCANZ (2000) reliability scheme) of 1.8 µg/L, calculated using an assessment factor of 1 000 applied to a chronic toxicity value for a marine mollusc (ANZECC/ARMCANZ 2000). More data on diuron toxicity are now available, including data for phototrophs, enabling the derivation of higher reliability DGVs.

The specificity of the mode of action of diuron and the distinct (albeit incomplete) separation in sensitivity of different taxa groups indicate that the sensitivity of diuron is bimodal, with phototrophs (aquatic plants) the more sensitive group. Therefore, as recommended by Warne et al. (2018), 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 marine water. The lowest reported chronic toxicity value for marine species (microalga) is 0.54 µg/L (3-d NOEC).

High reliability DGVs for diuron in marine water were derived based on chronic 10% effect concentration (EC10), no effect concentration (NEC) and no observed effect concentration (NOEC) data for 12 marine phototrophs from seven phyla, with a good fit of the SSD to the toxicity data. The DGVs are expressed in terms of the active ingredient; they relate to dissolved diuron only, and not any of its formulations or breakdown products. 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 for 99%, 95%, 90% and 80% species protection are 0.27 µg/L, 0.59 µg/L, 0.83 µg/L and 1.2 µg/L, respectively. The 95% species protection level for diuron in marine water is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1 Introduction

Diuron (CAS no. 330-54-1) is a herbicide ($C_9H_{10}Cl_2N_2O$; see Figure 1) that, at room temperature, is in the form of odourless, colourless crystals. It is the active ingredient of a variety of commercial herbicide formulations. Major metabolites of diuron are 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.

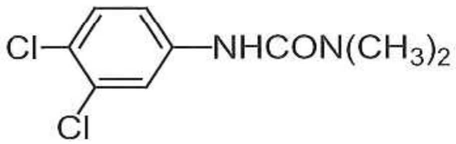


Figure 1 Structure of diuron

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 25°C [a] 35.6 mg/L at 20°C [b]
Logarithm of the octanol-water partition coefficient (log K_{OW})	2.85 ± 0.03 at 25°C [a] 2.87 at pH 7, 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 soil ($t_{1/2}$)	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. Diuron has been registered for use in Australia for over 30 years and is extensively used. 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 approved 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 water bodies and is a component of marine antifouling paints (APVMA 2009).

Diuron can be transported to marine environments by surface and/or subsurface 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 and low soil adsorption (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 sediments in a range of countries, including Australia (Konstantinou and 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. 2012, 2013; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015). After atrazine (91% of samples), diuron was the most frequently detected pesticide in flood plume water (89% of samples) in the Great Barrier Reef lagoon between 2016/2017 and 2018/2019 (Grant et al. 2018; Gallen et al. 2019; Thai et al. 2020). Outside of the flood plumes, diuron was the most frequently detected pesticide in the lagoon, occurring in 96% of samples, followed by atrazine (88% of samples), during the same period (Grant et al. 2018; Gallen et al. 2019; Thai et al. 2020). Diuron has also been detected in the Sydney estuary, which includes Sydney Harbour, Middle Harbour and Port Jackson (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 of runoff into watercourses. The APVMA deregistered selected products where the risk was unmanageable and modified 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 mechanism of toxicity, 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 a number of 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, as a result of 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 toxicity data for 51 marine species that passed the screening and quality assessment processes. These consisted of 32 phototrophs and 19 heterotrophs. The phototrophs consisted of 13 diatoms, three green algae, three haptophyte algae, three brown algae, three red algae, three macrophytes, one cryptomonad algae, one dinoflagellate and one cyanobacterium (blue–green algae). The 19 heterotrophs consisted of five fish, six crustaceans, three corals, two bivalves, one insect and two annelid worms.

The majority of phototrophs were more sensitive than the heterotrophs (Appendix B). This, combined with diuron’s mechanism of toxicity, indicated that the toxicity data were bimodal, with phototrophs the more sensitive. Thirteen marine heterotrophs had sensitivities within the range of phototrophs (Appendix B).

The seven types of marine phototrophs showed overlapping ranges of sensitivity to diuron. Toxicity values for diatoms ranged from 1.5 µg/L (72-h NEC, growth rate) for *Chaetoceros muelleri* (Negri et al. 2020) to 95 µg/L (72-h EC50, growth rate/biomass yield/area under the curve) for *Thalassiosira fluviatilis* (USEPA 2015). Toxicity values for green algae ranged from 1.6 µg/L (72-h EC10, growth rate) for *Tetraselmis* sp. (Negri et al. 2020) to 20 µg/L (10-d EC50, growth rate/biomass yield/area under the curve) for *Dunaliella tertiolecta* (USEPA 2015). The toxicity values for haptophyte algae ranged from 0.54 µg/L (3-d NOEC, abundance) for *Emiliania huxleyi* (Devilla et al. 2005) to 10 µg/L (10-d EC50, growth rate/biomass yield/ area under the curve) for *Isochrysis galbana* (USEPA 2015). Toxicity values for brown algae ranged from 2.3 µg/L (15-d EC10, fresh weight) for *Saccharina japonica* (Kumar et al. 2010) to 4 650 µg/L and 6 290 µg/L (2-d EC50, germination) for an Australian and New Zealand species of *Hormosira banksii* (Myers et al. 2006; Seery et al. 2006). Toxicity values for red algae ranged from 1.3 µg/L (4-d NOEC, growth) to 20 µg/L (4-d EC50, growth) for *Gracilaria tenuistipitata* (Haglund et al. 1996). Toxicity values for macrophytes ranged from 2.5 µg/L (10-d NOEC, biomass) for *Zostera marina* (Chesworth et al. 2004) to 87.8 µg/L (3-d NOEC, leaf length) for *Halodule uninervis* (Flores et al. 2013). For the cryptomonad *Rhodomonas salina*, toxicity values ranged from 1.7 µg/L (72-h NEC, growth rate) to 6.3 µg/L (72-h EC50, growth rate) (Negri et al. 2020). For the dinoflagellate *Cladocopium goreaui*, toxicity values ranged from 2.5 µg/L (14-d EC10, growth rate) to 4.5 µg/L (14-d EC50, growth rate) (Negri et al. 2020). Finally, the cyanobacterium *Chroococcus minor* had a single toxicity value of 4.7 µg/L (7-d EC50, cell density) (Bao et al. 2011).

For heterotrophs, reported toxicity values ranged from 1 µg/L to 21 000 µg/L. Fish toxicity values ranged from 50 µg/L (36-h NOEC, hatching success) for *Pagrus auratus* (Gagnon and Rawson 2009) to 7 826 µg/L (4-d LC50, mortality) for *Psetta maxima* (Mhadhbi and Beiras 2012). Crustacean toxicity values ranged from 270 µg/L (28-d NOEL, mortality) for *Americamysis bahia* (USEPA 2015) to 21 000 µg/L (24-h LC50, mortality) for *Balanus amphitrite* (Bao et al. 2011). Coral toxicity values ranged from 1 µg/L (4-d NOEC, abundance) for *Pocillopora damicornis* (Negri et al. 2005) to 4 800 µg/L (24-h LC50, mortality) for *Acropora tumida* (Bao et al. 2011). Bivalve toxicity values ranged from >1 000 µg/L (24–48-h LC10/50, mortality) for *Crassostrea gigas* (Tsunemasa and Okamura 2011) to 4 800 µg/L (96-h EC50, mortality/abnormal development) for *Crassostrea virginica* (USEPA 2015). The insect *Aedes aegypti* had a toxicity value of 1 200 µg/L (96-h LC50, mortality) (Knapek and Lakota 1974). Annelid toxicity values ranged from 1.8 µg/L (10-d NOEC, reduced weight) for *Lumbriculus variegatus* (Nebeker and Schuytema 1998) to 16 000 µg/L (48-h LC50, mortality) for *Hydroides elegans* (Bao et al. 2011).

3 Factors affecting toxicity

The following discussion of the factors affecting the toxicity of diuron is based on freshwater studies and should be cautiously applied to marine environments. 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 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 a chemical rather than the total or particle-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. This was attributed to the biofilms being thicker and more able to accumulate diuron at lower flow compared to higher flow (Chaumet et al. 2019).

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) 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

Scientific literature was searched to obtain data for diuron toxicity to marine organisms. In addition, the following databases were searched: ECOTOX Knowledgebase (USEPA 2015); an Australasian pesticide toxicity data compilation (Warne et al. 1998); and ANZECC/ARMCANZ (2000) and Sunderam et al. (2000) toxicant databases. Compared to the ANZECC/ARMCANZ (2000) DGVs, there are now more toxicity data available, including data for phototrophs, which enable the derivation of higher reliability DGVs for diuron in marine water. All 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 51 marine species from 14 phyla and 23 classes that passed the screening and quality assessment processes. The phyla were Annelida, Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cnidaria, Cryptophyta, Cyanobacteria, Dinoflagellate, Haptophyta, Mollusca, Ochrophyta, Rhodophyta and Tracheophyta. The 23 classes were Actinopterygii (which accounts for approximately 99% of fish), Anthozoa (cnidaria i.e. corals), Bacillariophyceae (diatom), Bivalvia (mollusc), Branchiopoda (crustacean), Chlorophyceae (green alga), Chrysophyceae (golden alga), Clitellata (annelid worm), Coccolithphycea (yellow alga), Cyptophyceae (cryptomonad), Cyanophyceae (blue–green alga), Dinophyceae (dinoflagellate), Florideophyceae (red alga), Fragilariophyceae (microalga), Insecta (invertebrate), Liliopsida (monocot), Malacostraca (crustacean), Maxillopoda (crustacean), Mediophyceae (alga), Nephrophyceae (green alga), Phaeophyceae (brown alga), Polychaeta (annelid worm) and Porphyridiophyceae (red alga). Chronic toxicity data were available for 29 of the 51 species, comprising 27 phototrophs and two heterotrophs; acute toxicity data were available for 24 species, comprising five phototrophs and 19 heterotrophs.

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). The majority of the lines of evidence supported the conclusion that the distribution of toxicity data is bimodal, with phototrophs generally more sensitive than heterotrophs (Appendix B). 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 NEC, NOEC and EC10 data for 12 phototrophs from seven 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 species sensitivity distribution (SSD) to derive a DGV (Warne et al. 2018). A summary of the toxicity data (one value per species) used to calculate the DGVs for diuron in marine water is in Table 2. Further details of the water quality parameters for each single species value used to calculate the DGVs are presented in Appendix A. 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 the default guideline values for diuron in marine water

Taxonomic group	Species	Life stage	Duration (days)	Toxicity measure [a] (endpoint)	Reported toxicity value (µg/L)	Final toxicity value (µg/L) [b]
Diatom	*Chaetoceros muelleri*	Exponential growth phase	3	NEC (specific growth rate)	1.47	1.5
	Entomoneis punctulata [c]	–	3	NOEC (cell density)	2	2
	Nitzschia closterium [c]	–	3	NOEC (cell density)	2	2
Green alga	*Nephroselmis pyriformis* [c]	–	3	EC10 (cell density)	2.2	2.2
	Tetraselmis sp.	Exponential growth phase	3	EC10 (specific growth rate)	1.64	1.64
Cryptomonad	*Rhodomonas salina*	Exponential growth phase	3	NEC (specific growth rate)	1.68	1.7
Dinoflagellate	*Cladocopium goreaui*	Exponential growth phase	14	EC10 (specific growth rate)	2.54	2.5
Golden alga	*Emiliania huxleyi*	Exponential growth phase	3	NOEC (cell density)	0.54	0.54
	Isochrysis galbana	–	3	EC10 (cell density)	1.09	1.09
	Tisochrysis lutea	Exponential growth phase	3	EC10 (specific growth rate)	0.6	0.6
Brown alga	*Saccharina japonica*	Thalli	15	EC10 (fresh weight)	2.3	2.3
Macrophyte	*Zostera marina*	–	10	NOEC (biomass – old and new growth))	2.5	2.5

a The measure of toxicity being estimated/determined: EC10: 10% effect concentration; NEC: no effect concentration; NOEC: no observed effect concentration.
b Chronic NOEC/EC10 values. All values are reported to a maximum of three significant figures.
c Species that originated from or are distributed in Australia and/or New Zealand.
–: No data available/not stated.

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 DGV derivation for diuron in marine water (Table 2) includes toxicity data for three marine 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 12 chronic toxicity values reported in Table 2 is shown in Figure 2. The SSD was plotted using the Burrlioz 2.0 software. The model provided a good fit to the data (Figure 2).

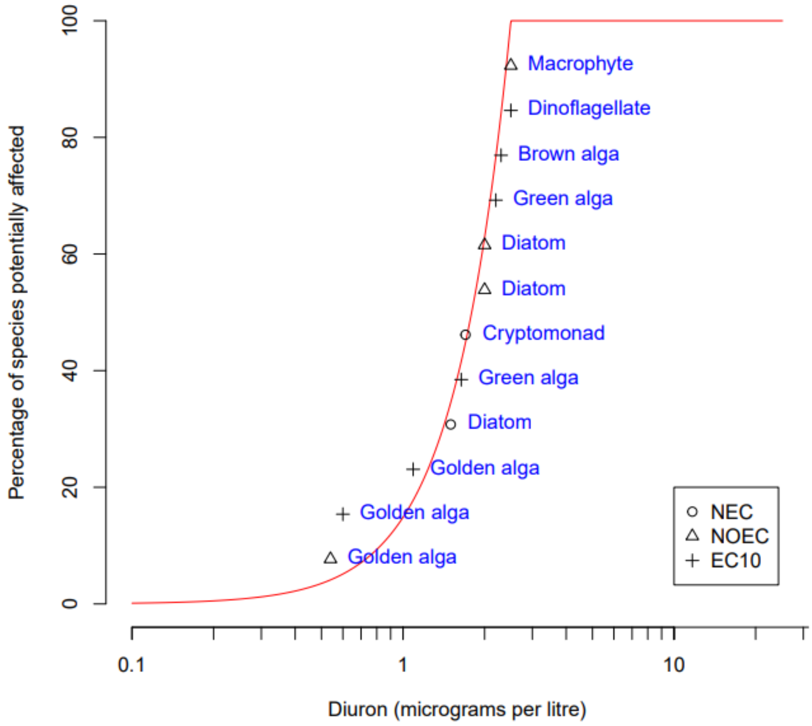


Figure 2 Species sensitivity distribution, diuron in marine water

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 diuron in marine water are provided 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 of 0.59 µg/L is recommended for application to slightly-to-moderately disturbed ecosystems.

Table 3 Default guideline values, diuron in marine water, high reliability

Level of species protection (%)	DGV for diuron in marine water (µg/L) [a]
99	0.27
95	0.59
90	0.83
80	1.2

a The DGVs were derived using Burrlioz 2.0 software and rounded to two significant figures.

4.4 Reliability classification

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

- sample size—12 (good)
- type of toxicity data—chronic
- SSD model fit—good (inverse Pareto).

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.
bimodality	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.
chronic toxicity	A lethal or sublethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
cryptomonads (cryptophytes)	A group of algae common to freshwater, brackish and marine environments, distinguished by the presence of characteristic membrane-bound structures, which consist of two connected spiral ribbons held under tension.
default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. a site-specific guideline value) in the Australian and New Zealand Guidelines for Fresh and Marine Water Quality.
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.
haptophytes	A large group of predominantly, but not exclusively, marine algae that have calcium carbonate scales on their surface and a flagellum-like (lash or whip-like) structure used for feeding and/or for attachment to external surfaces.
heterotrophs	Plants and animals that are dependent on organic matter for food.
LC50 (median lethal concentration)	The concentration of a substance in water or sediment that is estimated to be lethal to 50% of a group of test organisms, relative to the control response, under specified conditions.
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms under specified conditions.
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.
NEC (no effect concentration)	The highest concentration of a material used in a toxicity test that has no effect on the exposed population of test organisms as compared with the controls.
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.

Term	Definition
phototrophs	Organisms (e.g. plants and algae) that are dependent on photosynthesis for food.
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.
species (biological)	A group of organisms that resemble each other to a greater degree than members of other groups and that form a reproductively isolated group that will not produce viable offspring if bred with members of another group.
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 marine water

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Diatom	*Chaetoceros muelleri*	Exponential growth phase	3	NEC (specific growth rate)	Guillard's f/2 medium	34.6 ± 0.8	27.5 ± 0.4	8.24 ± 0.2	1.47	Negri et al. (2020)
	–								**1.5**	**Value used in SSD**
	Entomoneis punctulata	–	3	NOEC (cell density)	Filtered seawater	30	21	8.1–8.4	2	Stauber et al. (2008)
	–								**2**	**Value used in SSD**
	Nitzschia closterium	–	3	NOEC (cell density)	Filtered seawater	30	21	8.1–8.4	2	Stauber et al. (2008)
	–								**2**	**Value used in SSD**
Green alga	*Nephroselmis pyriformis*	–	3	EC10 (cell density)	Filtered seawater	–	24	–	2.2	Magnusson et al. (2008)
	–								**2.2**	**Value used in SSD**
	Tetraselmis sp.	Exponential growth phase	3	EC10 (specific growth rate)	EDTA-free Guillard's f/2 medium	32–33	27–29	8.1–8.2	1.64	Negri et al. (2020)
	–								**1.64**	**Value used in SSD**
Cryptomonad	*Rhodomonas salina*	Exponential growth phase	3	NEC (specific growth rate)	Guillard's f/2 medium	34.2 ± 0.6	26.0 ± 0.6	8.5 ± 0.4	1.68	Negri et al. (2020)
	–								**1.7**	**Value used in SSD**

Taxonomic group	Species	Life stage	Duration (d)	Toxicity measure (test endpoint)	Test medium	Salinity (‰)	Temp. (°C)	pH	Concentration (µg/L)	Reference
Dinoflagellate	*Cladocopium goreaui*	Exponential growth phase	14	EC10 (specific growth rate)	IMK nutrient media	32.5 ± 0.7	27 ± 0.6	7.8 ± 0.5	2.54	Negri et al. (2020)
	–								**2.5**	**Value used in SSD**
Golden alga	*Emiliania huxleyi*	Exponential growth phase	3	NOEC (cell density)	Seawater	33	17	8.3–8.4	0.54	Devilla et al. (2005)
	–								**0.54**	**Value used in SSD**
	Isochrysis galbana	–	3	EC10 (cell density)	0.45 mm filtered seawater, autoclaved and f/2 Guillard's Marine	31 ± 2	29 ± 1	8.2 ± 0.2	1.09	Seery and Pradella (2014)
	–								**1.09**	**Value used in SSD**
	Tisochrysis lutea	Exponential growth phase	3	EC10 (specific growth rate)	EDTA-free Guillard's f/2 medium	28–33	27–29	7.9–8.3	0.6	Negri et al. (2020)
	–								**0.6**	**Value used in SSD**
Brown alga	*Saccharina japonica*	Thalli	15	EC10 (fresh weight)	Artificial seawater	–	–	8.4	2.3	Kumar et al. (2010)
	–								**2.3**	**Value used in SSD**
Macrophyte	*Zostera marina*	–	10	NOEC (biomass – old and new growth)	Seawater	–	–	–	2.5	Chesworth et al. (2004)
	–								**2.5**	**Value used in SSD**

–: 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.

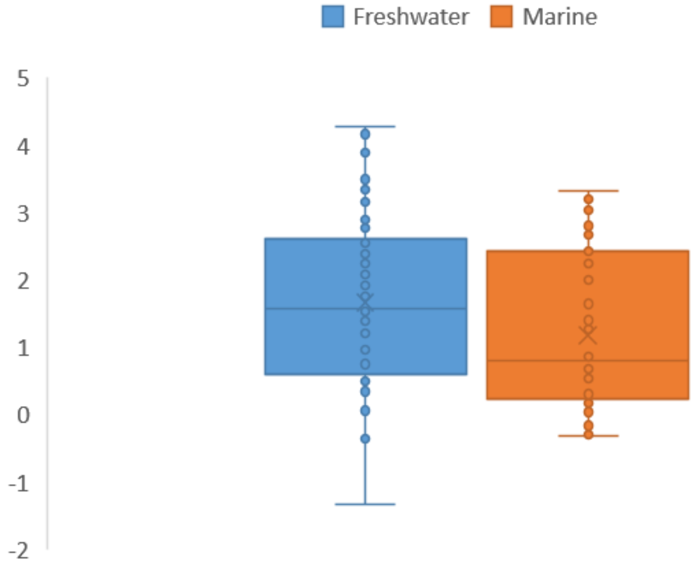
Is there a specific mode of action that could result in taxa-specific sensitivity?
Diuron exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis 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 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). Prolonged exposure to elevated concentrations of ROS in plants, as a result of 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 of diuron is the inhibition of electron transport in the PSII complex, diuron is expected to be 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 (n = 109). This was done to increase the sample size of the dataset being assessed.

All acute data (e.g. LC50) or chronic effect data (e.g. EC50) were converted to chronic negligible effect data (e.g. NEC, EC10, NOEC) 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.

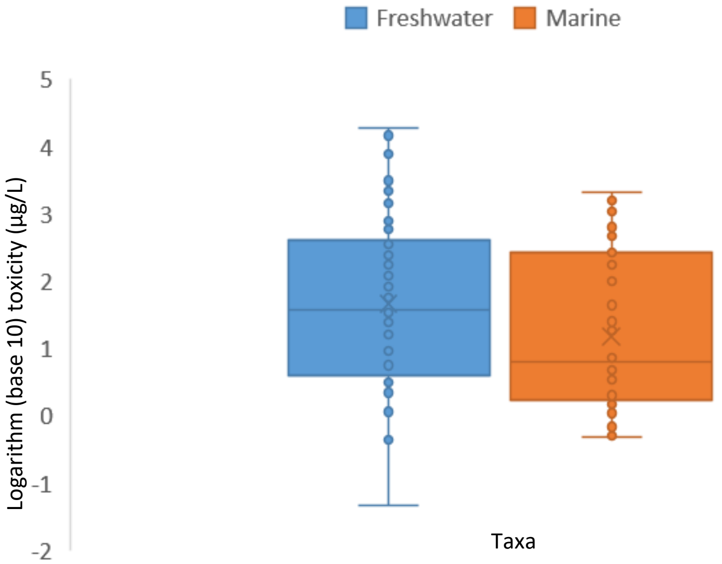


Figure B 1 Box plot, comparison of freshwater and marine species sensitivities to diuron

Calculation of the bimodality coefficient (BC) on log-transformed data yielded a value of 0.498. This is below the indicative threshold BC for bimodality of 0.55, suggesting the dataset does not exhibit bimodality. However, a frequency histogram provided no strong evidence that the dataset was either unimodal or bimodal (Figure B 2).

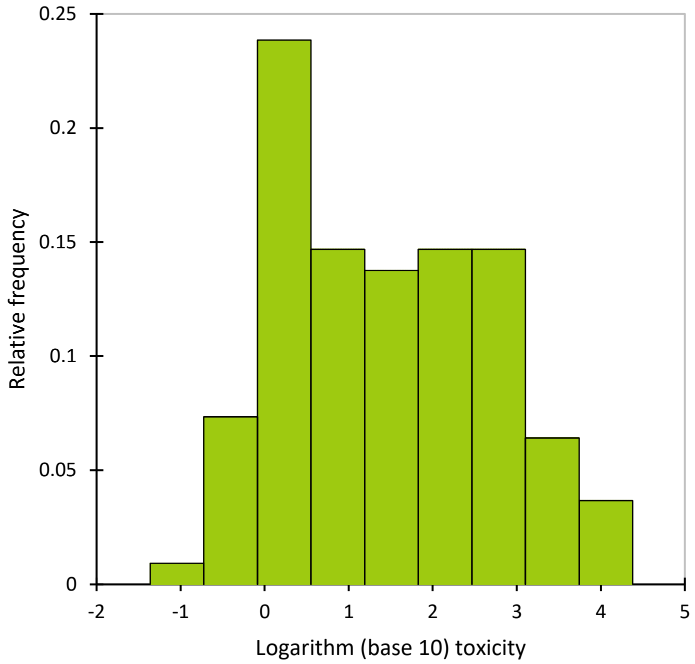


Figure B 2 Histogram of freshwater and marine species dataset

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 a distinct (albeit incomplete) separation in the sensitivity of phototrophs and heterotrophs to diuron.

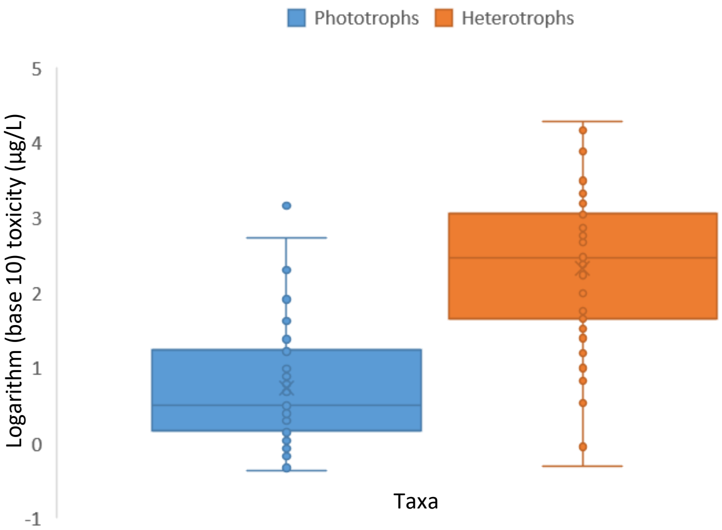


Figure B 3 Box plot, comparison of phototroph and heterotroph sensitivity to diuron

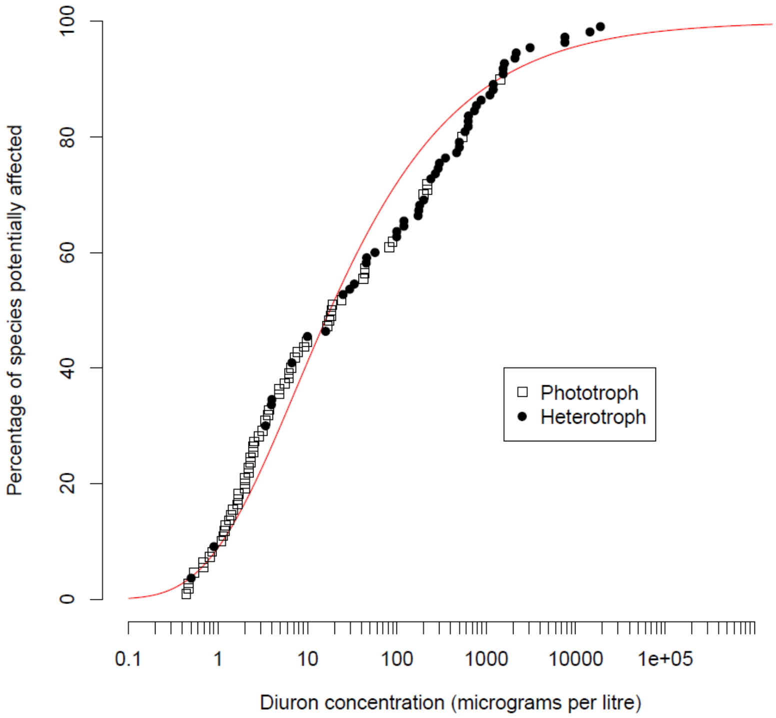


Figure B 4 Species sensitivity distribution, comparison of phototroph and heterotroph sensitivity to diuron

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 mechanism of toxicity of diuron and the distinct separation of sensitivity indicate 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. phototrophs) were used to derive the SSD and DGVs for diuron in marine water.

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