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FRESH & MARINE
WATER QUALITY**

Toxicant default guideline values for aquatic ecosystem protection

Ametryn in freshwater

Technical brief

October 2024

DRAFT

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Contact
Australian Government Department of Climate Change, Energy, the Environment and Water
GPO Box 3090 Canberra ACT 2601
General enquiries: 1800 920 528
Email waterquality@dcceew.gov.au

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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).

Ametryn (N2-ethyl-N4-isopropyl-6-methylthio-1,3,5-triazine-2,4-diamine; CAS No. 834-12-8) is a selective, systemic triazine herbicide or, more specifically, a methylthiotriazine herbicide. Other methylthiotriazine herbicides include prometryn, terbutryn and simetryn. Ametryn is a photosynthesis-inhibiting herbicide that is used in Australia to control most annual grasses and broad-leaved weeds in a variety of crops, such as pineapples and sugarcane, and areas including roadsides, drains, railway lines and footpaths (APVMA 2020). Ametryn is not registered for use in New Zealand (ACVM 2021).

Previously, no Australian and New Zealand DGVs existed for ametryn in freshwater or marine environments. Since the publishing of the ANZECC/ARMCANZ (2000) guidelines, more data on ametryn toxicity to freshwater species have become available, including data for phototrophic species (e.g. plants, algae), and have been used to derive the DGVs for Australia and New Zealand.

The available data indicate that ametryn is more toxic to phototrophic species than to heterotrophic species. The lowest reported toxicity values to freshwater species are 1.09 µg/L (acute, freshwater macrophyte, 4-d EC10) and 0.3 µg/L (chronic, freshwater microalga, 4-d EC50).

The ametryn DGVs for freshwater were derived based on chronic NOEL data and chronic EC50 data (converted to negligible effect concentrations) for eight phototrophic species from three phyla and four classes, with a good fit of the species sensitivity distribution (SSD) to the toxicity data.

The DGVs are expressed in terms of the dissolved active ingredient (ametryn) and relate to ametryn only—not its breakdown products. Only toxicity data for ametryn 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 0.017 µg/L, 0.10 µg/L, 0.24 µg/L and 0.67 µg/L, respectively. The 95% protection DGV of 0.10 µg/L is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1   Introduction

Ametryn (CAS No. 843-12-8, chemical formula $C_9H_{17}N_5S$, molecular mass 227.33 g/mol) is a selective, systemic herbicide that is a white powder at 25°C (Figure 1). Ametryn is the active ingredient of a variety of commercial herbicide formulations. The physico-chemical properties of ametryn that may affect its environmental fate and toxicity are presented in Table 1.
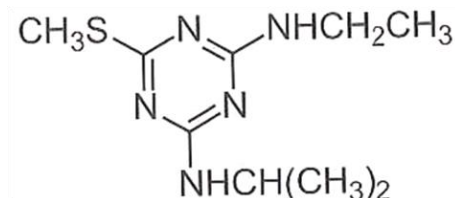
Figure 1 Structure of ametryn

Table 1 Summary, selected physico-chemical properties of ametryn

Physico-chemical property	Value
Molecular weight	227.3 amu [a]
Aqueous solubility	200 mg/L at pH 7.1 and 22°C [a]
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.63 at pH 7 and 20°C [b]
Logarithm of the organic carbon water partition coefficient (log K_{oc})	1.98–2.97 [a] 2.5 [b]
Logarithm of the bioconcentration factor (log BCF)	1.52 [b]
Half-life in water ($t_{1/2}$)	>1 week [c] Stable in aqueous solutions under natural sunlight [d]
Half-life in soil ($t_{1/2}$)	11–280 days, median 62 days [a]

a BCPC (2012).
b Pesticide Properties Database (University of Hertfordshire 2013).
c USEPA (1987).
d USEPA (2013).

Ametryn belongs to the methylthiotriazine group within the triazine family of herbicides, which also includes prometryn, terbutryn and simetryn. Ametryn is extensively used in Australian agriculture, forestry and grazing applications to control most annual grasses and broad-leaved weeds in a variety of crops, such as pineapples and sugarcane (APVMA 2020). Ametryn is also approved for use on commercial and industrial land, right-of-way areas, roadsides, railway lines, footpaths and drains (APVMA 2020). Ametryn is not approved for use in New Zealand (ACVM 2021).

Information on the degradation of ametryn in water is limited. Decomposition of ametryn due to hydrolysis is not expected due to the lack of appropriate functional groups; loss due to volatilisation is also not expected to occur (PubChem 2021). Microbial degradation will contribute to the decomposition of ametryn in water, but binding to suspended solids and sediment is expected to be the major pathway for such decomposition (PubChem 2021). Ametryn has a low soil adsorption capacity (K_{oc}) and a moderate aqueous solubility (Table 1), suggesting it has a high potential to leach to groundwater and be transported in surface water; however, leaching studies have indicated that

ametryn does not leach significantly (BCPC 2012). Supporting this finding, a study of surface water and groundwater samples in six US states found ametryn in only 0.2% of surface water samples, compared to approximately 4% of groundwater samples (USEPA 1987). A more recent assessment of ametryn by USEPA (2013) concluded that because ametryn is highly persistent and relatively mobile, it may leach into aquatic systems after elevated rainfall, floods or from spray drift after application to control weeds.

Australian data from 2011 to 2015 show that ametryn was detected in approximately 15.5% of surface water samples in catchments monitored as part of the Great Barrier Reef Catchment Loads Monitoring Program (based on data in Turner et al. 2012, 2013; Wallace et al. 2014, 2015, 2016; Garzon-Garcia et al. 2015).

2 Aquatic toxicology

2.1 Mechanisms of toxicity

Ametryn is absorbed through the roots and leaves of plants. It is then translocated acropetally (i.e. movement upwards from base of plant to apex) in the xylem and accumulates in the apical meristems (BCPC 2012). Ametryn exerts its toxicity in aquatic plants 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), therefore preventing 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), 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 (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).

2.2 Relative toxicity

There were toxicity data for 15 freshwater species that passed the screening and quality assessment processes. These consisted of nine phototrophic species and six heterotrophic species. The phototrophic species consisted of one diatom, six green algae and two macrophytes. The heterotrophs consisted of four fish and two crustaceans.

The available evidence indicates that phototrophic species are more sensitive to ametryn than heterotrophic species (Appendix B: Modality assessment for ametryn). However, four heterotrophic species had sensitivities within the range of the phototrophic species.

Toxicity values for the three types of phototrophic species varied markedly, although it is not possible to discern whether there are differences in the sensitivities of the three groups to ametryn.

- A 72-h EC50 of 26 µg/L was reported for the diatom *Stauroneis amphoroides* (USEPA 2015).

- Green algae toxicity values ranged from a 96-h EC50 of 0.3 µg/L for *Chlorella pyrenoidosa* (Ma et el. 2001, 2002) to a 10-d EC50 of 10 000 µg/L for *Chlorococcum* sp. (USEPA 2015).

- Macrophyte toxicity values ranged from an acute 96-h EC10 of 1.09 µg/L for *Lemna aequinoctialis* (Seery and Pradella 2014) to a chronic 7-d NOEL and EC50 of 2 µg/L and 13 µg/L, respectively, for *L. gibba* (USEPA 2015).

Toxicity values for heterotrophic species ranged from 240 µg/L to 73 000 µg/L. Fish toxicity values ranged from a 96-h NOEL (mortality) of 700 µg/L for *Oncorhynchus mykiss* to a 96-h LC50 (mortality) of 16 000 µg/L for *Pimephales promelas* (USEPA 2015). Crustacean toxicity values ranged from a 21-d NOEL (immobilisation) of 240 µg/L (USEPA 2015) to a 24-h EC50 (immobilisation) of 73 000 µg/L (Marchini et al. 1988) for *Daphnia magna*.

3   Factors affecting toxicity

There are no studies on factors affecting the toxicity of ametryn. However, such information is available for other PSII herbicides, including atrazine, diuron and terbuthylazine, which are discussed below. This information provides insights on factors that may affect the toxicity of ametryn.

The potential effect of particulate matter (e.g. natural black carbon, combusted black carbon, and suspended solids) and dissolved organic matter on toxicity is not clear. Knauer et al. (2007) found that the addition of natural black carbon and combusted black carbon could reduce or completely remove the impact of diuron on photosynthesis. In contrast, a comprehensive review by Knauer et al. (2017) reported that suspended solids did not significantly affect the toxicity and bioavailability of atrazine and terbuthylazine (PSII herbicides) to aquatic organisms in 13 out of 16 datasets. The review included a series of studies on the effect of suspended solids on the toxicity of atrazine to Australian freshwater heterotrophs (i.e. cladocerans *Ceriodaphnia* cf. *dubia* and *Daphnia carinata*, shrimp *Paratya australiensis*, midge *Chironomus tepperi*, and fish *Melanotaenia fluviatilis* (Phyu et al. 2004; 2005a, b; 2006; 2008, 2013)).

One of the modes of action of ametryn in phototrophs is to increase the formation of ROS (see Section 2.1). Given that the formation of ROS is dependent on light intensity, increased turbidity (e.g. from increased suspended solids) may decrease ametryn toxicity. Elevated light intensity was found to interact additively or synergistically with diuron to damage the PSII of the seagrass *Halophila ovalis*, while lower light generally resulted in impacts that were sub-additive (Wilkinson et al. 2015). King et al. (2022a) found that the chronic toxicity of diuron to the marine diatom *Phaeodactylum tricornutum* under low light conditions varied depending on the stressor intensity and endpoint measured; they reported a mild inhibition of photosynthesis but a major inhibition of growth (i.e. cell density). King et al. (2022b) found that diuron and reduced light resulted in additive, antagonistic or

synergistic interactions, depending on the stressor intensity, exposure period and the measured biological response.

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

This information indicates that the combined effects of PSII herbicides and other stressors may be species-specific and difficult to predict.

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 ametryn toxicity to freshwater organisms, a search of the scientific literature was conducted. In addition, the following databases were searched: USEPA (2015) ECOTOX Knowledgebase; Australasian Ecotoxicology Database (Warne et al. 1998); and ANZECC/ARMCANZ (2000) toxicant database (Sunderam et al. 2000). There are now sufficient ametryn toxicity data available to derive DGVs for freshwater (Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values). To derive higher reliability DGVs in the future, additional chronic toxicity tests of ametryn with freshwater phototrophic species should be conducted.

There were freshwater toxicity data for 15 species from five phyla that passed the screening and quality assessment processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata and Tracheophyta. Chronic toxicity data were available for 10 of the 15 species, comprising eight phototrophic and two heterotrophic species; acute toxicity data were available for seven species, comprising one phototrophic and six heterotrophic species.

Based on the mode of action of ametryn (Section 2) and the results of the modality assessment in Appendix B: Modality assessment for ametryn (i.e. toxicity data distribution is likely bimodal), it was concluded that phototrophs were more sensitive than heterotrophs to ametryn. Therefore, as recommended by Warne et al. (2018), only phototroph toxicity data were used to calculate the DGVs.

Normally, species classified only to genus (e.g. *Chlorella* sp.) are not used in the DGV derivation process, as species specificity is required. The use of such data in DGV 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 data available, these data can be included in the DGV derivation. In deriving the DGVs for ametryn in

freshwater, *Chlorococcum* sp., *Neochloris* sp. and *Platymonas* sp. were included as no other toxicity data for these genera were used.

There were insufficient chronic negligible effect values (e.g. EC10, NOEC/NOEL) for phototrophs to derive DGVs for ametryn in freshwater. Therefore, these data were combined with estimated chronic negligible effect data (chronic EC50 toxicity data converted to negligible effect estimates by dividing by 5) for phototrophic species. Toxicity data based on both measured and nominal concentrations were used because ametryn is water soluble, has low partition coefficients (K_{oc} and K_{ow}) (Table 1), and has a low vapour pressure (2.74 x 10^{-6} mm Hg at 25°C (PubChem 2021)); therefore, nominal concentrations were not expected to differ markedly to measured concentrations. This resulted in a final dataset of toxicity values for eight phototrophic species belonging to three phyla (Bacillariophyta, Chlorophyta and Tracheophyta) (Table 2). Although the phototroph-only dataset did not meet the standard requirement for data from at least four taxonomic groups, it was still acceptable to use because the full dataset of chronic toxicity values for phototrophs and heterotrophs (10 species from five phyla) met both the number of species and taxonomic group requirements (i.e. at least five species from at least four taxonomic groups (Warne et al. 2018)).

A summary of the toxicity data (one value per species) used to calculate the DGVs for ametryn in freshwater is provided in Table 2. Further details of the water quality parameters for each species used to calculate the DGVs are 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 ametryn in freshwater

Taxonomic group	Species	Life stage	Duration (hours)	Toxicity measure (endpoint) [a]	Reported toxicity value (µg/L)	Final toxicity value (µg/L)
Green alga	*Chlorella pyrenoidosa* [b, c]	–	96	EC50 (abundance)	0.3	0.06 [d]
	Chlorococcum sp.	–	240	EC50 (biomass yield, growth rate, AUC [e])	10 000	2 000 [d]
	Neochloris sp.	–	72	EC50 (biomass yield, growth rate, AUC [e])	36	7.2 [d]
	Platymonas sp.	–	72	EC50 (biomass yield, growth rate, AUC [e])	24	4.8 [d]
	Scenedesmus quadricauda	–	96	EC50 (abundance)	150	30 [d]
	Selenastrum capricornutum [f]	–	168	NOEL (biomass yield, growth rate)	1.14	1.14
Macrophyte	*Lemna gibba*	–	168	NOEL (frond number, dry weight, frond area)	2	2
Diatom	*Stauroneis amphoroides*	–	72	EC50 (biomass yield, growth rate, AUC [e])	26	5.2 [d]

– : Not stated / no data.

a The measure of toxicity being estimated/determined. EC50: 50% effect concentration. NOEL: no observed effect level.

b Species also known as *Chlorella vulgaris*.

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

d Chronic EC50 converted to chronic negligible effect estimate by dividing by 5 (Warne et al. 2018).

e AUC: area under the growth curve.

f Species also known as *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.

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) and the Integrated Taxonomic Information System (ITIS 2017). The dataset used in the DGVs derivation for ametryn in freshwater (Table 2) includes toxicity data for one freshwater species that either originated from, or is distributed in, Australia and/or New Zealand.

4.2   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the eight chronic freshwater species 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).
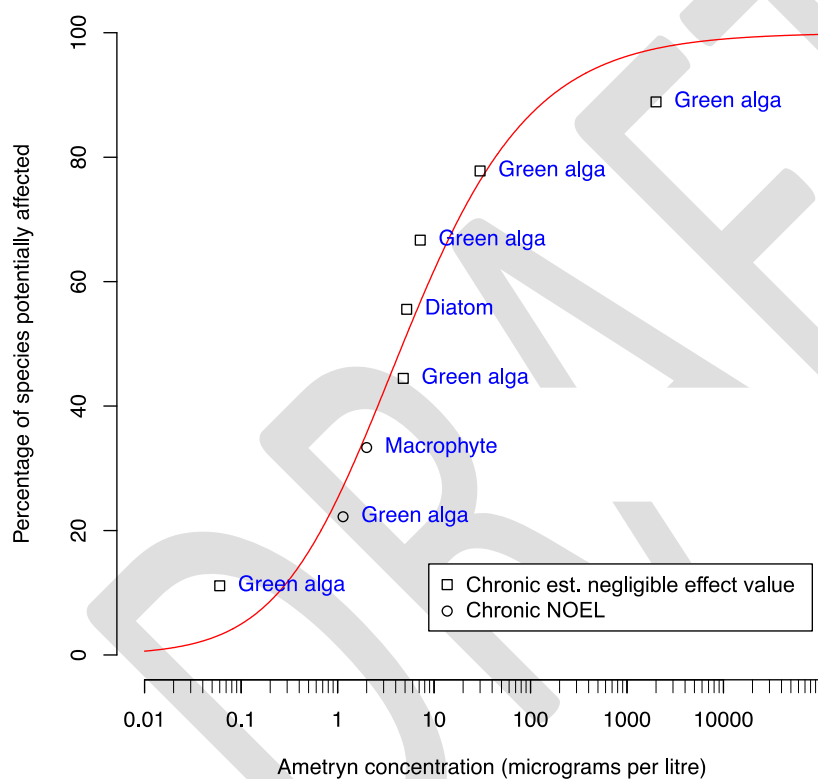
Figure 2 Species sensitivity distribution, ametryn in freshwater

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 ametryn DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 3. The ametryn DGVs are expressed in terms of the concentration of the active ingredient. The DGVs relate to dissolved ametryn only, and not its breakdown products. ANZG (2018) provides guidance on what to do if the DGVs are under-protective due to formulation-related factors. The 95% species protection DGV is recommended for application to slightly-to-moderately disturbed ecosystems.

Measured log BCF values for ametryn are low (**Error! Reference source not found.**) and below the threshold at which secondary poisoning must be considered (i.e. threshold log BCF = 4 (Warne et al. 2018)). Therefore, the ametryn DGVs do not need to account for secondary poisoning.

Table 3 Default guideline values, ametryn in freshwater, high reliability

Level of species protection (%)	DGV for ametryn in freshwater (µg/L) [a]
99	0.017
95	0.10
90	0.24
80	0.67

[a] Default guideline values were derived using the Burrlioz 2.0 software and are reported to two significant figures.

4.4   Reliability classification

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

- sample size—8 (good)

- type of toxicity data—chronic

- SSD model fit—good (Burr type III).

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 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 allocated to it by the American Chemical Society.
chronic toxicity	A lethal or sublethal adverse effect that occurs after exposure to a chemical for a period of time that is a substantial portion of the organism's life span or an adverse effect on a sensitive early life stage.
default guideline value (DGV)	A guideline value recommended for generic application in the absence of a more specific guideline value (e.g. 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).
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.
mode of action	The means by which a chemical exerts its toxic effects.
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.
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' 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, ametryn in freshwater

Taxonomic group	Species	Life stage	Exposure duration (hours)	Toxicity measure (test endpoint) [a]	Test medium	Temp. (°C)	pH	Concentration (μg/L)	Reference
Diatom	*Stauroneis amphoroides*	–	72	EC50 (biomass yield, growth rate, AUC [c])	ASTM Type I water	24 ± 2	7.5 ± 0.1	26	USEPA (2015)
	–							**5.2 [b]**	**Value used in SSD**
Green alga	*Chlorococcum* sp.	–	240	EC50 (biomass yield, growth rate, AUC [c])	ASTM Type I water	24 ± 2	7.5 ± 0.1	10 000	USEPA (2015)
	–							**2 000 [b]**	**Value used in SSD**
	Neochloris sp.	–	72	EC50 (biomass yield, growth rate, AUC [c])	ASTM Type I water	24 ± 2	7.5 ± 0.1	36	USEPA (2015)
	–							**7.2 [b]**	**Value used in SSD**
	Platymonas sp.	–	72	EC50 (biomass yield, growth rate, AUC [c])	ASTM Type I water	24 ± 2	7.5 ± 0.1	24	USEPA (2015)
	–							**4.8 [b]**	**Value used in SSD**
	Selenastrum capricornutum [d]	–	168	NOEL (biomass yield, growth rate)	ASTM Type I water	24 ± 2	7.5 ± 0.1	1.14	USEPA (2015)
	–							**1.14**	**Value used in SSD**
	Scenedesmus quadricauda	–	96	EC50 (abundance)	HB-4 medium	–	–	150	Ma et al. (2003)
	–							**30 [b]**	**Value used in SSD**

Taxonomic group	Species	Life stage	Exposure duration (hours)	Toxicity measure (test endpoint) [a]	Test medium	Temp. (°C)	pH	Concentration (µg/L)	Reference
	Chlorella pyrenoidosa [e, f]	–	96	EC50 (abundance)	Liquid HB-4 medium	25	–	0.3	Ma et al. (2001)
	Chlorella pyrenoidosa [e, f]	–	96	EC50 (abundance)	Liquid HB-4 medium	25	–	0.3	Ma et al. (2002)
	–							*0.3*	*Geometric mean*
	–							**0.06 [b]**	**Value used in SSD**
Macrophyte	*Lemna gibba*	–	168	NOEL (frond number, dry weight, frond area)	M-Hoagland's or 20X-AAP media. ASTM Type I water	25 ± 2	4.8–5.2 (M-Hoagland's) and 7.5 ± 0.1 (20X-AAP)	2	USEPA (2015)
	–							**2**	**Value used in SSD**

– : No data / not stated.

a The measure of toxicity being estimated/determined. EC50: 50% effect concentration. NOEL: no observed effect level.

b EC50 values converted to negligible effect estimates by dividing by 5 (Warne et al. 2018).

c AUC = area under the growth curve.

d Species also known as *Raphidocelis subcapitata* and *Pseudokirchneriella subcapitata*.

e Species also known as *Chlorella vulgaris*.

f Species that originated from, or is distributed in, Australia and/or New Zealand.

Appendix B: Modality assessment for ametryn

A modality assessment was undertaken for ametryn 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?
Ametryn exerts its toxicity in aquatic plants (including aquatic macrophytes and algae) by inhibiting electron transport in the photosystem II (PSII) complex (University of Hertfordshire 2013), a key process in photosynthesis that occurs in the thylakoid membranes of chloroplasts.

In addition to its main mode of action, exposure to PSII inhibiting herbicides can increase the formation of reactive oxygen species (ROS) (Halliwell 1991). 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).

These modes of action suggest that ametryn is more toxic to phototrophs than to heterotrophs.

Does the dataset suggest bimodality?
Modality was assessed using a dataset that combined all ametryn freshwater and marine data that passed the screening and quality assessment schemes (n = 27). 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 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. Calculation of the bimodality coefficient (BC) on log-transformed data yielded a value of 0.38, which—being below the indicative BC threshold for bimodality of 0.55—suggested the dataset does not exhibit bimodality. However, a frequency histogram indicated that the dataset may not be unimodal (Figure B 2).
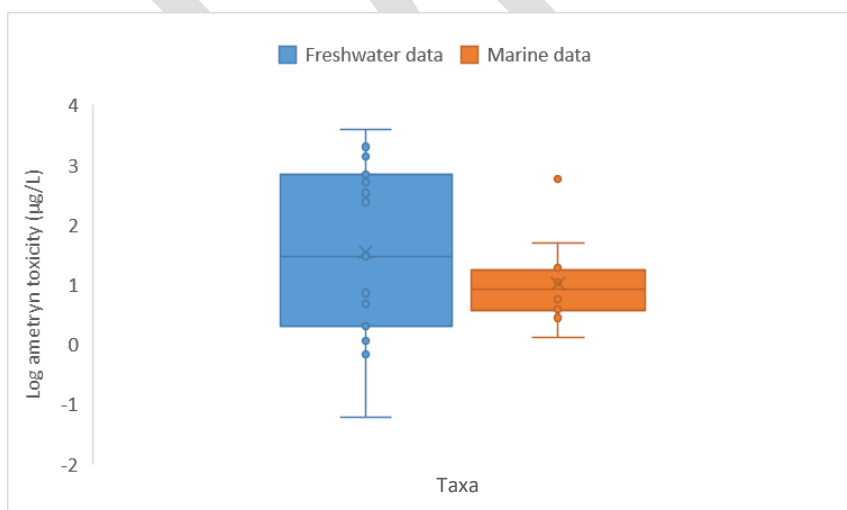
Note: 'x' denotes the mean; circles represent the individual toxicity values.

Figure B 1 Box plot, comparison of freshwater and marine species sensitivities to ametryn
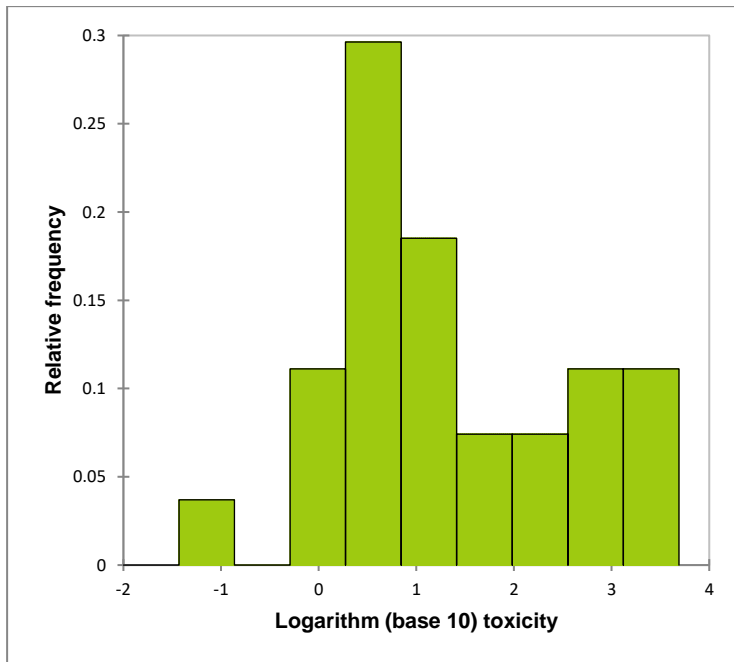
Figure B 2 Histogram, freshwater and marine species dataset

**Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?**

The relative sensitivity of different taxa groups to ametryn was compared using box and whisker plots (Figure B 3) and a species sensitivity distribution (SSD) (Figure B 4). These analyses indicated that there is a marked, but not complete, separation in the sensitivities of phototrophic and heterotrophic species to ametryn. Also, the inclusion of toxicity data for heterotrophs resulted in an offset of datapoints at the top of the SSD (Figure B 4). This pattern is typically seen among chemicals with a specific mode of action and is a strong visual indication of bimodality.

Overall, the specificity of the mode of action of ametryn (to a protein only in phototrophs) and the separation in sensitivity indicate that the sensitivity of ametryn is bimodal, with phototrophic species being the more sensitive group.
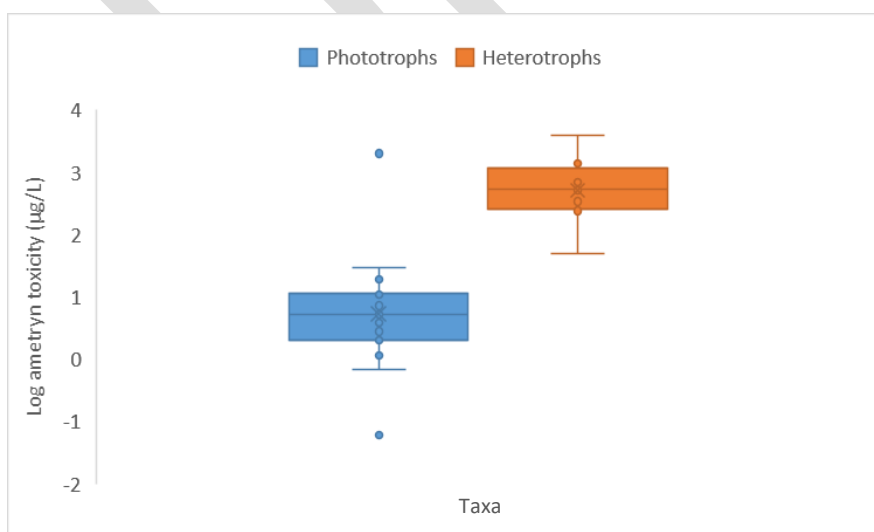
Note: 'x' denotes the mean; circles represent the individual toxicity values.

Figure B 3 Box plot, comparison of phototroph and heterotroph sensitivity to ametryn
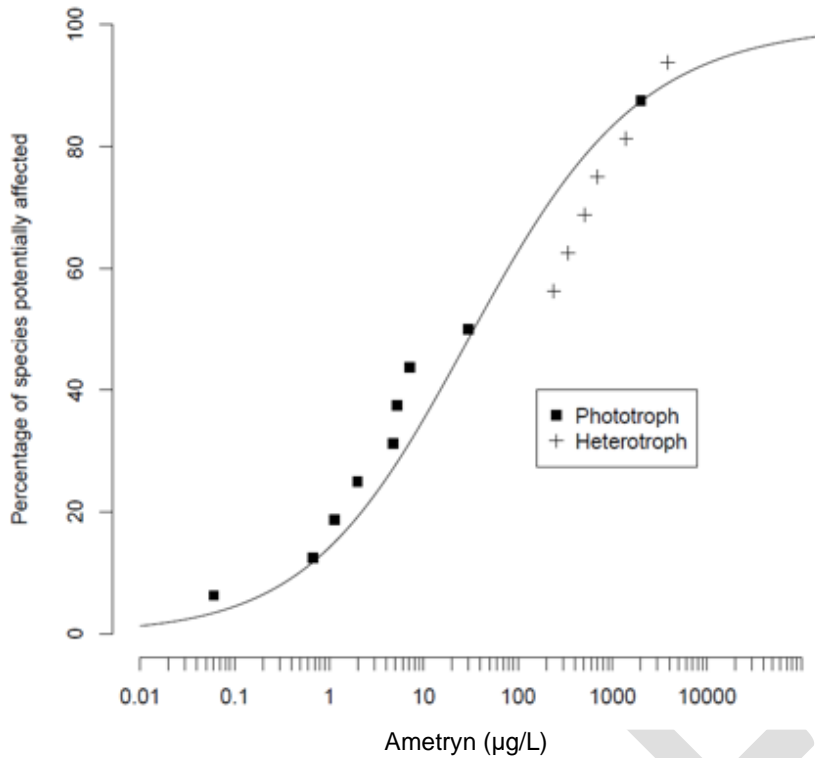
Figure B 4 Species sensitivity distribution, comparison of phototroph and heterotroph sensitivity to ametryn

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 19 phototrophs and 8 heterotrophs, the distributions are relatively representative.

Overall, the specificity of the mode of action of ametryn and the separation in sensitivity indicate that the toxicity of ametryn is bimodal, with phototrophs being the more sensitive group.

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