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Australian & New Zealand

GUIDELINES FOR  
FRESH & MARINE  
WATER QUALITY

# Toxicant default guideline values for aquatic ecosystem protection

## Picloram in freshwater

Technical brief

May 2023

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This publication is available at [waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants](http://waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants).

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### Acknowledgements

These default guideline values (DGVs) were derived by Naomi Cooper, Kirsten Broadgate, Clare Papaleo and Carolyn Brumley of Golder Associates, Melbourne, Australia. The DGVs were peer reviewed by two anonymous reviewers and by two contracted technical advisors, Dr Rick van Dam and Alicia Hogan. The DGVs were also reviewed and approved by jurisdictional technical and policy oversight groups and a National Water Reform Committee prior to being published.

New Zealand Government



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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](http://www.waterquality.gov.au/anz-guidelines)).

Picloram (C<sub>6</sub>H<sub>3</sub>Cl<sub>13</sub>N<sub>2</sub>O<sub>2</sub>) is a pyridine herbicide used since the 1960s for the control of annual and perennial broadleaf weeds in winter cereal and linseed crops, as well as for the control of noxious, woody and herbaceous weeds in rights-of-way, forestry, pastures and non-crops areas (APVMA 2015). Picloram is used as a herbicide in a number of forms, including salts and an isooctyl ester (Cox 1998, APVMA 2015, 2020a).

Picloram is a systemic herbicide that mimics the plant growth hormone auxin, causing uncontrolled and disorganised growth, leading to plant death (Tu et al. 2001). Picloram is dicotyledon-selective, and it has low or no activity towards monocotyledons (Cox 1998, FWPRDC 2006, EFSA 2009).

Picloram does not bind strongly to soil. This, combined with its high solubility and low rate of degradation in soil, may result in runoff to surface water and leaching to groundwater (USEPA 1995, Tu et al. 2001, EFSA 2009, APVMA 2015, NCBI 2020). Picloram has a low potential to bioaccumulate (USEPA 1995, EFSA 2009, FAO 2012, NCBI 2020).

As picloram is dicotyledon-selective, it may be more toxic to dicotyledon aquatic plants than to other aquatic receptors. However, this could not be determined because the only data available for an aquatic dicot (water milfoil (*Myriophyllum sibiricum*) reported by Forsyth et al. 1997) did not pass the quality assessment. Therefore, both plant and animal data were considered in the default guideline value (DGV) derivation. It is possible that the DGVs may be under-protective for aquatic dicotyledons.

Low reliability DGVs for picloram in freshwater were derived based on chronic NOEC and acute LC50 (converted to chronic) data for 12 species from four taxonomic groups, with a poor fit of the species sensitivity distribution to the toxicity data. The DGVs derived here are expressed in terms of the active ingredient (picloram) rather than commercial formulations. The DGVs for 99%, 95%, 90% and 80% species protection are 6.3 µg/L, 87 µg/L, 270 µg/L and 850 µg/L, respectively. The 95% species protection level is typically recommended for assessing slightly-to-moderately disturbed ecosystems. However, and as recommended in ANZG (2018), low reliability DGVs are not adequate for assessing water quality but can be used as interim values until more reliable values are derived. If used as interim values, they should always be used in conjunction with other lines of evidence.

# 1 Introduction

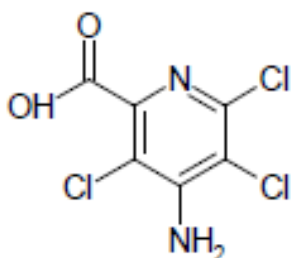
Picloram (C<sub>6</sub>H<sub>3</sub>Cl<sub>3</sub>N<sub>2</sub>O<sub>2</sub>, CASRN 1918-02-1) is a pyridine herbicide used for the control of annual and perennial broadleaf weeds in agricultural (crop and non-crop), forestry, commercial and industrial settings (ACVM 2020, APVMA 2020a). In Australia, it is approved for use on a range of crops, including (but not limited to) cereal, pasture, linseed, linola and sugarcane (APVMA 2015, 2020a). In New Zealand, it is approved for use for a range of agricultural purposes, including (but not limited to) pasture, amenity turf and fodder brassicas (ACVM 2020). Picloram is a selective, systemic and post-emergence herbicide with foliar and soil activity (FWPRDC 2006). It is used in the following forms: potassium salt, hexyloxypropylamine salt, triethanolamine salt, triisopropanolamine salt, diethanolamine salt, and isooctyl ester (Cox 1998, APVMA 2015, 2020a). Picloram acid is only used to manufacture other forms of picloram, whereas the amine salts, potassium salt and ester derivatives of picloram are produced as commercial herbicides (USEPA 1995, APVMA 2015).

In this technical brief, the term 'picloram' refers to the various forms of picloram; where data on specific forms of picloram are available, these are specifically described.

Picloram may be formulated in a variety of concentrated products (emulsifiable, suspension, soluble or liquid) and applied using ground or aerial methods to control weeds such as blackberry (*Rubus fruticosus*), hawthorn (*Crataegus monogyna*), lantana (*Lantana camera*), and prickly pear (*Opuntia stricta*) (ACVM 2020, APVMA 2020a).

Picloram is registered for use as a single active constituent, although it is more often formulated in combination with other actives (e.g. clopyralid, triclopyr, MCPA and 2,4-D) in a large number of products (ACVM 2020, APVMA 2015, 2020a). Picloram is currently on the Australian Pesticides and Veterinary Medicines Authority's list of chemicals nominated and prioritised for reconsideration, in part due to its potential environmental risk to non-target organisms (APVMA 2020b).

The IUPAC chemical name of picloram is 4-amino-3,5,6-trichloropicolinic acid, and it is a colourless-to-white-to-tan solid (powder or crystals) with a chlorine-like odour (NCBI 2020). The molecular weights of some of the forms of picloram are: 241.46 g/mol for the acid; 279.6 g/mol for the potassium salt, 364.68 g/mol for the isooctyl ester; and 432.8 g/mol for the triisopropanolamine salt (NCBI 2020). The structure of picloram is shown in Figure 1.



**Figure 1 Structure of picloram**

The vapour pressures of picloram and its isooctyl ester (at 25°C) are in the order of 6.0x10<sup>-16</sup> mm Hg to 6.0x10<sup>-10</sup> mm Hg (EFSA 2009, NCBI 2020) and 3.0x10<sup>-7</sup> mm Hg to 1.9x10<sup>-7</sup> mm Hg (Hamilton 1980), respectively, indicating that volatile losses are not an important fate mechanism. Long-range

atmospheric transport is also unlikely given an estimated atmospheric half-life of 12.5 hours (EFSA 2009).

The solubility of picloram in water varies in its different forms. For the acid, solubility is reported to range from 430 mg/L to 560 mg/L (USEPA 1995, NCBI 2020) but is also known to be pH-dependent (Cheung & Biggar 1974). Solubility of the potassium salt is much higher: 740 000 mg/L at 20°C (USEPA 1995). The isooctyl ester derivative has low solubility in water: 0.23 mg/L (USEPA 1995, NCBI 2020).

The acid dissociation constant (pKa) of picloram is 2.3 for acid and approximately 2 for picloram salts, indicating that picloram acid and salts will readily dissociate into the anionic form in the environment (USEPA 1995, NCBI 2020). The isooctyl ester form of picloram is expected to degrade rapidly in the environment (reported aerobic half-life of 2 days) to the same anion as the acid and salts (USEPA 1995). Therefore, the environmental fate of the various forms of picloram are expected to be similar (USEPA 1995).

Picloram (as the anion) is not readily degraded in the environment, and it has an estimated half-life in aerobic soils ranging from a month to several years, depending on soil type, organic content, and climatic and microbiological conditions (USEPA 1995, Tu et al. 2001, EFSA 2009, Assis et al. 2011). This wide-ranging half-life may also be due to dose-dependent degradation: the higher the application rate of picloram, the slower its degradation in soil (EFSA 2009). Degradation in soil is primarily by microbial metabolism, but can also occur by sunlight (Tu et al. 2001). Carbon dioxide is the major degradate in aerobic soils (Tu et al. 2001, EFSA 2009). Under anaerobic conditions, picloram acid is stable, with over 90% remaining in soil and water after 300 days (USEPA 1995). Picloram can also be released from the roots of treated plants into the soil (Tu et al. 2001).

Picloram does not bind strongly to soil ( $K_{oc}$  values of 0.026–100 L/kg). This property (combined with its low volatility, high solubility and high persistence) means picloram has a high potential to leach to groundwater and enter surface water (USEPA 1995, EFSA 2009, Tu et al. 2001, APVMA 2015, NCBI 2020). Once picloram has reached groundwater, it is unlikely to degrade, even over several years (USEPA 1995). Groundwater monitoring data from the United States indicated picloram contamination across 10 states at concentrations up to 30 µg/L. USEPA (1995) stated that *'eventual contamination of groundwater [by picloram] is virtually certain in areas where residues persist in the overlying soil'*. In a study in Texas, picloram was detected at a concentration of 184 µg/L in a pond down-gradient of treated pastures 2 weeks after soil application (Haas et al. 1971). Surface water concentrations were detected 6 months after treatment, and concentrations were negatively correlated with the interval between soil treatment and first rainfall (Haas et al. 1971). In Australia, picloram has been detected in surface water at a concentration of 25 µg/L, 20 minutes after spraying blackberries on adjacent land, although the detected concentrations did not persist beyond 1 hour of spraying (Moore et al. 2010).

In water, picloram is stable at pH 5, 7 and 9, with the hydrolytical half-life estimated to be greater than 1 year (EFSA 2009). Picloram is susceptible to direct photolysis in clear, shallow water, with a photolytic half-life in freshwater estimated to be 2 days (Hall et al. 1968, EFSA 2009), resulting in the degradates carbon dioxide (CO<sub>2</sub>), oxamic acid, and 3-oxo-β-alanine (EFSA 2009). However, picloram may persist in turbid water and in sediment (USEPA 1995, EFSA 2009). Biological degradation in surface water is likely to be slow under both aerobic and anaerobic conditions (USEPA 1995).

Picloram has a low potential to bioconcentrate, with reported log  $K_{ow}$  values ranging from -2.21 at pH 9 to 0.63 at pH 3 (USEPA 1995, EFSA 2009, FAO 2012, NCBI 2020). Bioconcentration factors of 0.11–31 in fish have been reported (USEPA 1995, NCBI 2020), which are consistent with the low  $K_{ow}$  values. Therefore, bioaccumulation and secondary poisoning are unlikely to be a concern for picloram (USEPA 1995).

## 2 Aquatic toxicology

### 2.1 Mechanism of toxicity

Picloram is a systemic herbicide that is absorbed through the foliage and roots of susceptible plants (i.e. cotyledons) and accumulates in meristematic tissues (growth zones) (APVMA 2015). It has an 'auxinic' or synthetic growth hormone mode of action, mimicking the plant growth hormone indole acetic acid (Tu et al. 2001). When picloram is administered at a sufficiently high dose, it causes uncontrolled and disorganised growth in the susceptible plants, leading to plant death (Tu et al. 2001). Picloram is dicotyledon-selective, and it has low or no activity towards grasses and other terrestrial monocotyledons (Cox 1998, Tu et al. 2001, FWPRDC 2006).

### 2.2 Toxicity

A literature review (and associated data quality assessment) of the effects of picloram on freshwater organisms identified 55 toxicity values for 15 species consisting of 29 acute values for 12 species from three taxonomic groups (crustacean, insect and fish) and 26 chronic values for 5 species from four taxonomic groups (microalga, crustacean, macrophyte and fish).

Some toxicity studies assessed formulations containing picloram as the active ingredient. These formulations include a carrier solvent and, in some cases, other proprietary ingredients for which the combined toxicity is not well understood. Studies using formulations are typically not used to derive guideline values.

Chronic studies for picloram have typically reported effects on growth, reproduction and mortality endpoints. Based on the limited available data, fish appear to be the most sensitive taxonomic group, with a chronic NOEC of 550  $\mu\text{g/L}$  for rainbow trout (*Oncorhynchus mykiss*, 60 day growth (Mayes et al. 1987)). Algae and crustaceans were less sensitive, with chronic NOECs reported as 8 000  $\mu\text{g/L}$  for the green alga *Raphidocelis subcapitata* (4 day growth (USEPA OPP 2019)), 10 000  $\mu\text{g/L}$  for the green alga *Chlorella vulgaris* (4 day growth (Garten 1990)) and 11 800  $\mu\text{g/L}$  for the crustacean (waterflea) *Daphnia magna* (21 day reproduction (Gerish et al. 1985)).

The available acute aquatic toxicity data for picloram typically report effects on survival, predominantly for fish, with some studies for crustaceans and insects. Of the data where purity was greater than 80%, fish were more sensitive. Data presented in USEPA OPP (2019) reported 96 h LC50s of 1 400  $\mu\text{g/L}$  for channel catfish (*Ictalurus punctatus*), 1 500  $\mu\text{g/L}$  and 3 500  $\mu\text{g/L}$  for cutthroat trout (*Oncorhynchus clarkii*), and 1 900  $\mu\text{g/L}$  and 2 400–2 900  $\mu\text{g/L}$  for lake trout (*Salvelinus namaycush*). Other fish species were less sensitive, with 96 h LC50s ranging between 13 500  $\mu\text{g/L}$  and 44 500  $\mu\text{g/L}$  for bluegill (*Lepomis macrochirus*) and 55 300  $\mu\text{g/L}$  for fathead minnow (*Pimephales promelas*). Of the remaining data, crustaceans were more sensitive than insects, although only within

a factor of 2–3. The 96 h LC50s for the crustaceans *Gammarus pseudolimnaeus* and *Gammarus fasciatus* were 16 500 µg/L and 27 000 µg/L, respectively, while a 96 h LC50 of 48 000 µg/L was reported for the stonefly *Pteronarcys californica* (USEPA OPP 2019).

## 3 Factors affecting toxicity

No abiotic factors affecting the toxicity of picloram were identified.

## 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

A summary of the toxicity data (one value per species) and conversions used to calculate the DGVs for picloram in freshwater is provided in Table 1. Further details on the data that passed the screening and quality assurance schemes, including those used to derive the single species values 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.

Only data for picloram where the purity was greater than 80% was used to calculate the DGVs. Results from studies using formulations where the purity was not known, or where the purity was less than 80%, were excluded. For example, the following toxicity data were excluded from the DGV derivation because the studies used formulations.

- 96 h LC50 acute data for three amphibian species (Johnson 1976, Lajmanovich et al. 2013):
  - *Adelotus brevis* (tucked frog) (96 h LC50s of 95 000–154 000 µg/L)
  - *Limnodynastes peronii* (striped marsh frog) (96 h LC50 of 105 000 µg/L)
  - *Rhinella arenarum* (beaked toad) (48 h LC50 of 25 µg/L; 48 h NOEC of 19.5 µg/L; and 48 h LOEC of 39 µg/L).
- Two chronic macrophyte tests (Forsyth et al. 1997):
  - *Potamogeton pectinatus* (pondweed) (growth NOEC of 100 µg/L)
  - *Myriophyllum sibiricum* (water milfoil) (growth NOEC of 100 µg/L).
- Acute studies for three fish (Fairchild et al. 2007, Botelho et al. 2012):
  - *Ctenopharyngodon idella* (grass carp) (96 h LC50 of 4 000 µg/L)
  - *Salvelinus confluentus* (bull trout) (96 h LC50 of 24 000 µg/L)
  - *Oncorhynchus mykiss* (rainbow trout) (96 h LC50 of 41 000 µg/L).
- Acute studies for two algae (Tubea et al. 1981):
  - *Chlorella pyrenoidosa* (green alga) (no effects up to 2 400 µg/L)
  - *Lyngbya birgii* (blue–green alga) (no effects up to 2 400 µg/L).
- Acute study for a crustacean (Sanders 1969):
  - *Gammarus lacustris* (96 h LC50 of 27 000 µg/L).



Additionally, some studies were excluded due to other factors, for example (toxicity data not shown):

- only one test concentration was used:
  - *Lemna minor* (duckweed) chronic growth (Peterson et al. 1994)
  - *Oncorhynchus clarkii* (fish) acute survival (Woodward 1982)
- the endpoints were acute NOECs:
  - *Danio rerio* (zebrafish) acute survival (Stehr et al. 2009)
  - *Daphnia magna* (water flea) acute survival (Gersich et al. 1985)
- effects were reported to be greater than the highest exposure concentration:
  - *Pteronarcella* sp. (insect) acute survival (USEPA OPP 2019).

Where only one toxicity value was available for a species, that value was used in the final dataset to be used for the species sensitivity distribution (SSD). For species with more than one toxicity value available, the data selected for the SSD was in accordance with Warne et al. (2018).

Picloram toxicity data for 12 species from four taxonomic groups were considered for the SSD. These species included: two microalgae (*C. vulgaris* and *R. subcapitata*), three crustaceans (*D. magna*, *G. pseudolimnaeus* and *G. fasciatus*), one insect (*P. californica*), and six fish (*I. punctatus*, *O. mykiss*, *S. namaycush*, *O. clarkii*, *L. macrochirus* and *P. promelas*). Of the toxicity data used for the 12 species, four were chronic NOEC values, and eight were from acute exposures. The eight acute exposures were all LC50 values that were converted to chronic negligible effect values using a default acute-to-chronic ratio of 10 (Warne et al. 2018).

Modality checks were performed according to the four questions stipulated in Warne et al. (2018), with the details of the assessment provided in Appendix B: Modality assessment for Picloram. The weight of evidence assessment concluded that the dataset did not exhibit bimodality or multimodality and, hence, supported use of the data for the 12 species for the DGV derivation.

**Table 1 Summary of single toxicity values, all species used to derive default guideline values for picloram in freshwater**

Taxonomic group	Species	Life stage	Duration (h)	Type (acute/chronic)	Toxicity measure <sup>a</sup>	Toxicity value (µg/L)	Estimated chronic value (µg/L)
Microalga	<i>Rhapidocelis subcapitata</i>	–	96	Chronic	NOEC	8 944 <sup>c</sup>	8 944 <sup>b</sup>
	<i>Chlorella vulgaris</i>	–	96	Chronic	NOEC	10 000	10 000 <sup>b</sup>
Crustacean	<i>Gammarus pseudolimnaeus</i>	Juvenile	96	Acute	LC50	16 500	1 650 <sup>d</sup>
	<i>Gammarus fasciatus</i>	Adult	96	Acute	LC50	27 000	2 700 <sup>d</sup>
	<i>Daphnia magna</i>	Neonate	504	Chronic	NOEC	11 800	11 800 <sup>b</sup>
Insect	<i>Pteronarcys californica</i>	YC-2	240	Acute	LC50	48 000	4 800 <sup>d</sup>
Fish	<i>Ictalurus punctatus</i>	Juvenile	96	Acute	LC50	1 400	140 <sup>d</sup>
	<i>Oncorhynchus clarkii</i>	Juvenile	96	Acute	LC50	2 290 <sup>c</sup>	229 <sup>d</sup>
	<i>Salvelinus namaycush</i>	Juvenile	96	Acute	LC50	2 360 <sup>c</sup>	236 <sup>d</sup>
	<i>Oncorhynchus mykiss</i>	Embryo	1 440	Chronic	NOEC	550	550 <sup>b</sup>

Taxonomic group	Species	Life stage	Duration (h)	Type (acute/chronic)	Toxicity measure <sup>a</sup>	Toxicity value (µg/L)	Estimated chronic value (µg/L)
	<i>Lepomis macrochirus</i>	Juvenile	96	Acute	LC50	22 260 <sup>c</sup>	2 226 <sup>d</sup>
	<i>Pimephales promelas</i>	Juvenile	96	Acute	LC50	55 300 <sup>c</sup>	5 530 <sup>d</sup>

Note: Values are reported to no more than three significant figures.

**a** The measure of toxicity being estimated/determined: LC50: median lethal concentration; NOEC: no observed effect concentration.

**b** Actual chronic NOEC.

**c** Geometric mean

**d** Default conversion from acute LC50 to chronic negligible effect value = (acute LC50 ÷ 10).

## 4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 12 chronic and acute (converted to chronic) picloram toxicity data reported in Table 1 is shown in Figure 2. The model was judged to provide a poor fit to the data (Figure 2).

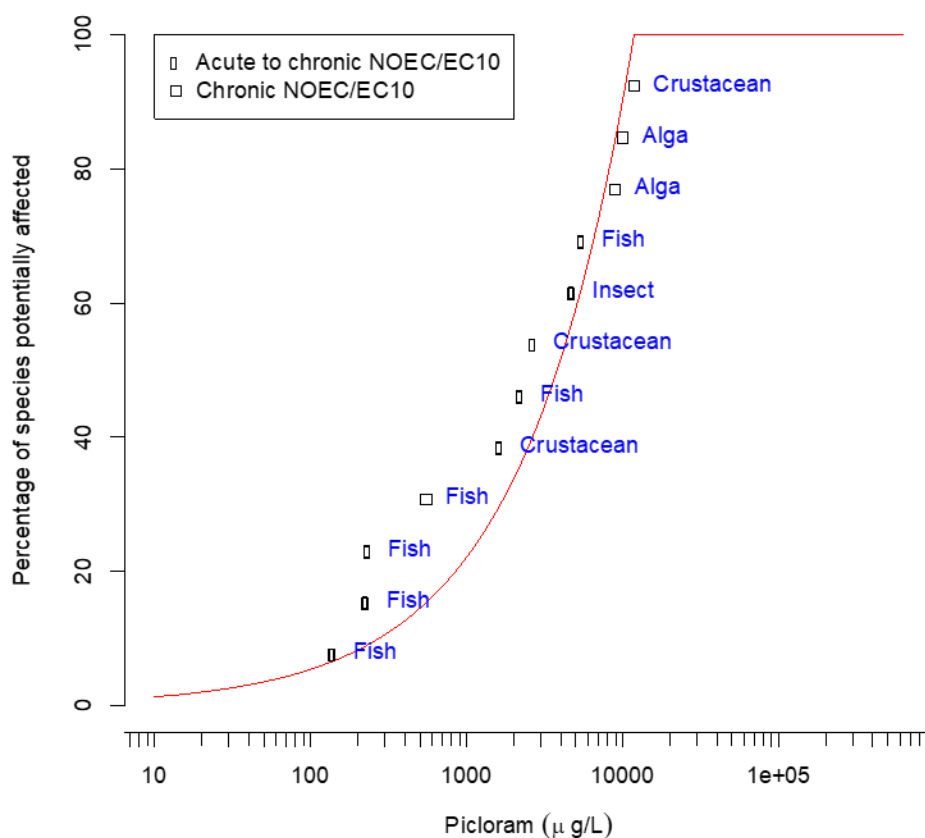


Figure 2 Species sensitivity distribution, picloram in freshwater

## 4.3 Default guideline values

It is important that the DGVs (Table 2) 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 picloram DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 2. The DGVs are expressed in terms of the active ingredient (picloram) rather than commercial formulations, and do not relate to any of the breakdown products of picloram. The 95% species protection DGV is typically recommended for application to slightly-to-moderately disturbed ecosystems. However, for picloram, also see the additional guidance in Section 4.4, on application of the DGVs.

**Table 2 Toxicant default guideline values, picloram in freshwater, low reliability**

Level of species protection (%)	DGV for picloram in freshwater ( $\mu\text{g/L}$ ) <sup>a</sup>
99	6.3
95	87
90	270
80	850

<sup>a</sup> The DGVs were derived using the Burrlioz 2.0 software, and have been rounded to two significant figures.

The DGVs were compared to the acute (converted to chronic) and chronic toxicity data that passed the quality assessment compiled from the literature review (i.e. 25 acute values from 10 species and 25 chronic values for four species). The 80% species protection DGV was higher than 30% of the toxicity values, indicating that the protection of this DGV may be inadequate. This is reflective of the poor fit in the SSD (Figure 2). Consequently, the 80% species protection DGV for picloram is not recommended for application to highly disturbed ecosystems, and a higher species protection level should be applied.

#### 4.4 Reliability classification

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

- Sample size—12 (good)
- Type of toxicity data—combined chronic and acute (converted to chronic) data
- SSD model fit—poor (Inverse Pareto model).

As recommended in [ANZG \(2018\)](#), low reliability DGVs are typically not adequate to assess water quality, but can be used as interim values until more reliable values are derived. If used as interim values, they must be used in conjunction with other lines of evidence.

# Glossary

Term	Definition
2,4-D	2,4-dichlorophenoxyacetic acid.
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.
acute-to-chronic ratio (ACR)	The species mean acute value (LC/EC50) divided by the chronic value (e.g. NOEC or EC10) for the same species.
CAS RN	Chemical Abstracts Service Registry 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.
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. Formerly known as 'trigger values'.
dicotyledon	A flowering plant with an embryo that bears two cotyledons (seed leaves). Dicotyledons constitute the larger of the two great divisions of flowering plants (angiosperms).
endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
guideline value (GV)	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. (Also refer to default guideline value and site-specific guideline value.)
IUPAC	International Union of Pure and Applied Chemistry.
K <sub>ow</sub>	The ratio of a chemical's solubilities in n-octanol and water at equilibrium. The logarithm of K <sub>ow</sub> (or P <sub>ow</sub> ) is used as an indication of a chemical's propensity for bioconcentration by aquatic organisms.
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.
macrophyte	A member of the macroscopic plant life of an area, especially of a body of water; large aquatic plant
no observed effect concentration (NOEC)	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.
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.
species sensitivity distribution (SSD)	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, toxicity data that passed the screening and quality assurance processes, picloram in freshwater**

Taxonomic group	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure <sup>a</sup> (test endpoint)	Test medium	Temp. (°C)	Salinity (‰)	pH	Concentration (µg/L)	Reference
Microalga	<i>Rhaphidocelis subcapitata</i>	–	96	Chronic	NOEC (Growth)	–	–	–	–	8 000	USEPA OPP 2019
		–	96	Chronic	NOEC (Growth)	Growth medium	24–26	–	–	10 000	Garten 1990
	–									8 944 <sup>b</sup>	<b>Geometric mean</b>
	<i>Chlorella vulgaris</i>	–	96	Chronic	NOEC (Growth)	Growth medium	24–26	–	–	10 000 <sup>b</sup>	Garten 1990
Crustacean	<i>Gammarus pseudolimnaeus</i>	Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	16 500 <sup>c</sup>	USEPA OPP 2019
	<i>Gammarus fasciatus</i>	Adult	96	Acute	LC50 (Survival)	–	–	–	–	27 000 <sup>c</sup>	USEPA OPP 2019
	<i>Daphnia magna</i>	Neonate	504	Chronic	NOEC (Reproduction)	Treated tap water	20–20.9	–	7.2–8.2	11 800 <sup>b</sup>	USEPA OPP 2019
Insect	<i>Pteronarcys californica</i>	YC-2	240	Acute	LC50 (Survival)	–	–	–	–	48 000 <sup>c</sup>	USEPA OPP 2019
Fish	<i>Ictalurus punctatus</i>	Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	1 400 <sup>c</sup>	USEPA OPP 2019
		Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	1 500	USEPA OPP 2019
	<i>Oncorhynchus clarkii</i>	Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	3 500	USEPA OPP 2019
	–									2 290 <sup>c</sup>	<b>Geometric mean</b>

Toxicant default guideline values for aquatic ecosystem protection: Picloram in freshwater

Taxonomic group	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure <sup>a</sup> (test endpoint)	Test medium	Temp. (°C)	Salinity (‰)	pH	Concentration (µg/L)	Reference
–	<i>Salvelinus namaycush</i>	Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	1 900	USEPA OPP 2019
		Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	2 400	USEPA OPP 2019
		Fry	96	Acute	LC50 (Survival)	–	–	–	–	2 900	USEPA OPP 2019
	–	–	–	–	–	–	–	–	–	2 360 <sup>c</sup>	<b>Geometric mean</b>
–	<i>Oncorhynchus mykiss</i>	Embryo	1 440	Chronic	NOEC (Growth)	Treated lake water	11.8–12.5	–	7.8–8.2	550 <sup>b</sup>	Mayes et al. 1987
–	<i>Lepomis macrochirus</i>	Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	13 500	USEPA OPP 2019
		Juvenile	96	Acute	LC50 (Survival)	Reconstituted water	23–25	–	7.2	14 500	Mayes & Oliver 1985
		Juvenile	96	Acute	LC50 (Survival)	Reconstituted water	21–23	–	7.9	19 400	Mayes & Oliver 1985
		Fry– Juvenile	96	Acute	LC50 (Survival)	Reconstituted water	21–23	–	7.4–8.1	21 900	Mayes & Oliver 1985
		Juvenile	96	Acute	LC50 (Survival)	Reconstituted water	21–23	–	7.4–8.1	32 900	Mayes & Oliver 1985
		Juvenile	96	Acute	LC50 (Survival)	Treated lake water	17–17.4	–	7.4–8.1	44 500	Mayes & Dill 1984
–	–	–	–	–	–	–	–	–	22 260 <sup>c</sup>	<b>Geometric mean</b>	
–	<i>Pimephales promelas</i>	Juvenile	96	Acute	LC50 (Survival)	Treated lake water	17–17.4	–	7.2–8.0	55 300	Mayes & Dill 1984
		Juvenile	96	Acute	LC50 (Survival)	–	–	–	–	55 300	USEPA OPP 2019
–	–	–	–	–	–	–	–	–	–	55 300 <sup>c</sup>	<b>Geometric mean</b>

**a** The measure of toxicity being estimated/determined: NOEC: no observed effect concentration; LC50: median lethal concentration.

**b** Value, as reported, included in the dataset to derive the DGVs.

**c** Value, after application of a default acute-to-chronic conversion factor of 10, included in the dataset to derive the DGVs.

## Appendix B: Modality assessment for Picloram

A modality assessment was undertaken for picloram according to the four questions stipulated in Warne et al. (2018). These questions and their answers are listed below. It is important to note that the small sample size of the dataset makes it difficult to draw conclusions about modality:

### Is there a specific mode of action that could result in taxa-specific sensitivity?

As discussed in Section 1, the mode of action for picloram is as a synthetic growth hormone or auxinic, mimicking the plant growth hormone indole acetic acid and causing uncontrolled and disorganised growth leading to plant death. Picloram is dicotyledon-selective. Therefore, it could be more toxic to dicotyledon aquatic plants than to other aquatic receptors. However, this could not be determined as there were no data for aquatic dicots that passed the quality assessment.

### Does the dataset suggest bimodality?

Visual representation of the data, calculation of the bimodality coefficient (BC), and consideration of the range in the effect concentrations are recommended lines of evidence for evaluating whether bimodality or multimodality of the dataset is apparent. This is discussed as follows.

- The histogram of the log transformed data (Figure B 1) is not normal, indicating that the data may be multimodal.
- Data that span large ranges (>4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018); the picloram data span three orders of magnitude.
- When the BC is greater than 0.555, it indicates that the data do not follow a normal distribution and may be bimodal; the BC of the log transformed data is 0.449, which does not support an assertion of bimodality.

Based on these lines of evidence, the histogram (Figure B 1) suggests the dataset may be multimodal; however, the span of the data and BC do not support this.

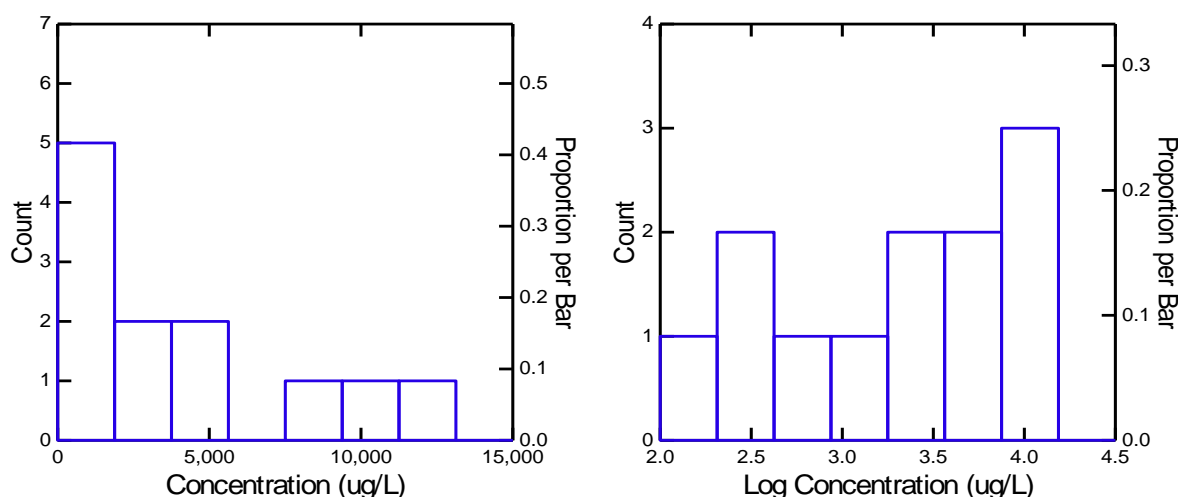
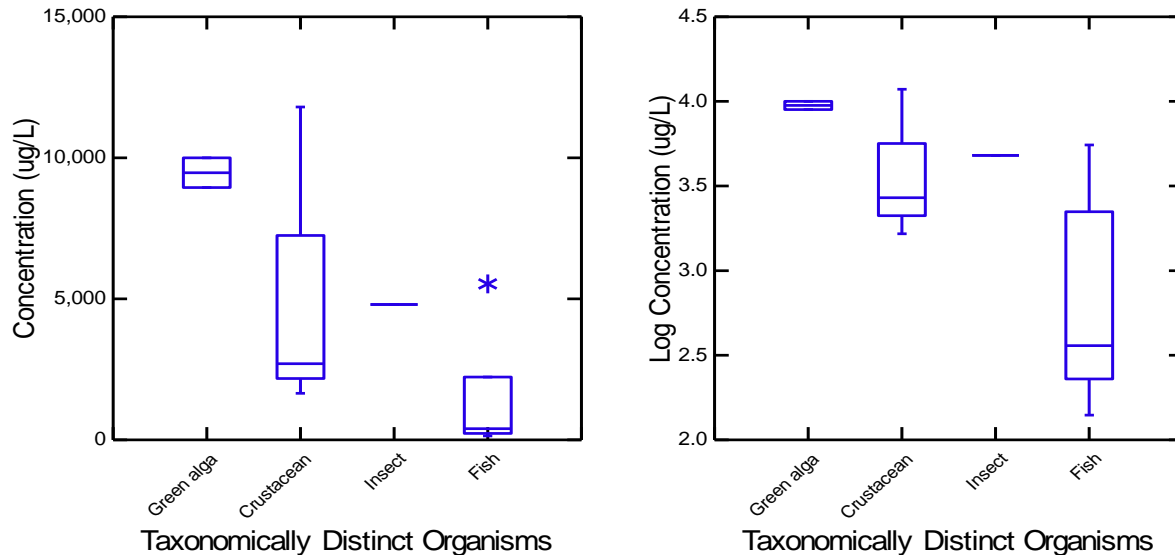


Figure B 1 Histogram, raw (left) and log transformed (right) data

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

The box plots (Figure B 2) suggest that fish may be more sensitive to picloram than other taxa groups. However, the taxa sample size is small (six fish, three crustaceans, two algae and one insect), making it difficult to assess possible trends. Moreover, there is a strong reliance on acute toxicity data that were converted to estimated chronic toxicity data using a default acute-to-chronic ratio.

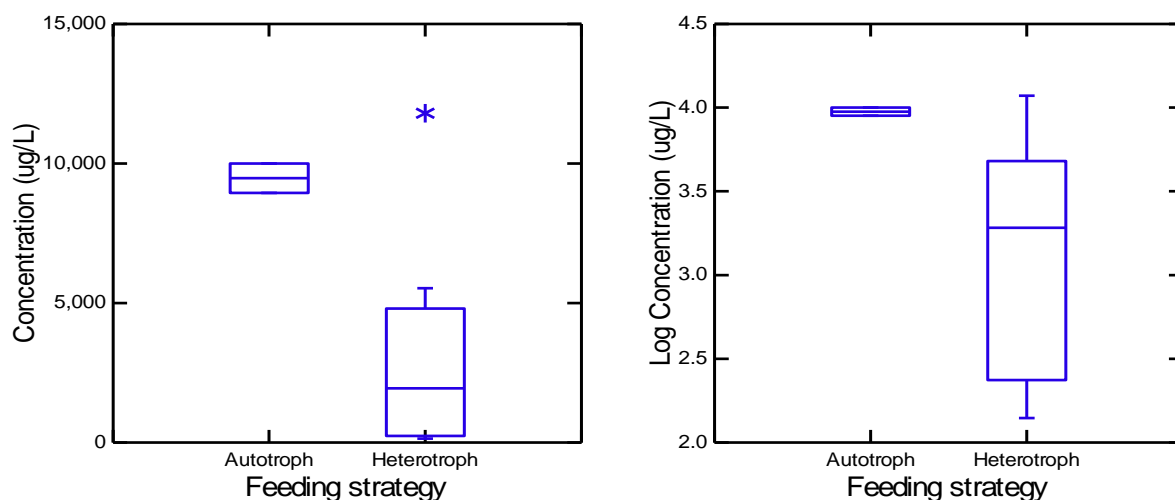


Note: an asterisk represents an outlying value >1.5x the interquartile range.

**Figure B 2 Box plots, raw (left) and log transformed (right) data grouped by major types of organisms**

The data were further examined using box plots of the SSD data to compare autotrophs and heterotrophs (Figure B 3)

As shown in Figure B 3, the autotroph (n=2) and heterotroph (n=10) groupings appear to differ in sensitivity. The median for each grouping indicates difference in effect for animals and plants, with animals more sensitive. However, this may be an artefact of the small sample size for autotrophs.



Note: an asterisk represents an outlying value >1.5x the interquartile range.

**Figure B 3 Box plots, raw (left) and log transformed (right) data grouped by autotrophs and heterotrophs**



**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?**

The data appear to show signs of bimodality or multimodality, with fish potentially being a more sensitive taxa group. However, this may be due to artefacts associated with the small sample size of the other taxa and a reliance on acute toxicity data converted to estimated chronic toxicity data. Thus, the weight of evidence supports use of the 12 species identified in derivation of the SSD.

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