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**GUIDELINES FOR
FRESH & MARINE
WATER QUALITY**

Toxicant default guideline values for aquatic ecosystem protection

Metolachlor in freshwater

Technical brief

June 2020

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New Zealand Government



Contents

Summary	iv
1 Introduction.....	1
2 Aquatic toxicology.....	2
2.1 Mechanism of toxicity	2
2.2 Toxicity	2
3 Factors affecting toxicity	4
4 Default guideline value derivation	4
4.1 Toxicity data used in derivation	4
4.2 Species sensitivity distribution	7
4.3 Default guideline values	7
4.4 Reliability classification	8
Glossary.....	9
Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values	11
Appendix B: Modality assessment for metolachlor toxicity to freshwater species	16
Appendix C: Rationale for selecting the final dataset for deriving the default guideline values for metolachlor in freshwater	18
References	20

Figures

Figure 1 Structure of metolachlor	1
Figure 2 Species sensitivity distribution, metolachlor in freshwater	7

Tables

Table 1 Summary of selected physico-chemical properties of metolachlor.....	1
Table 2 Summary of single toxicity values, all species used to derive default guideline values for metolachlor in freshwater.....	5
Table 3 Toxicant default guideline values, metolachlor in freshwater, very high reliability	8

Appendix tables

Table A 1 Summary, chronic toxicity data that passed the screening and quality assurance processes, metolachlor in freshwater.....	11
Table C 1 Protective concentration values, metolachlor in freshwater, chronic EC10/NOEC/NOEL data only	19

Appendix figures

Figure B 1 Histogram, all metolachlor toxicity data, phototrophic and heterotrophic species.....	16
Figure C 1 Species sensitivity distribution, metolachlor in freshwater, chronic EC10/NOEC/NOEL data only	19

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).

Metolachlor (2-Chloro-N-(2-ethyl-6-methylphenyl)-N-(2-methoxy-1-methylethyl)acetamide, CAS No. 51218-45-2) is a selective derivative of aniline and belongs to the chloroacetanilide group within the amide family of herbicides. Other chloroacetanilide herbicides include alachlor, acetochlor and propachlor. Metolachlor is commonly used in agriculture for broadleaf weed control in numerous different crops. It exerts toxicity by inhibiting the fatty acid elongation (FAE1)-synthase enzyme, which results in phytotoxic effects such as interferences of normal cell function. Non-agricultural uses of metolachlor include the application to urban and industrial situations (i.e. home garden and roadsides), as well as aquatic weed control in public waterways, most commonly through the use of commercial formulations (CCME 1999, ANZECC/ARMCANZ 2000). Metolachlor was initially a racemic mixture of two enantiomers (mirror image isomers), *S*-metolachlor and *R*-metolachlor. Increasingly, the racemic mixture is being replaced with forms that contain higher concentrations of *S*-metolachlor (Liu et al. 2006).

The previous Australian and New Zealand default guideline value (DGV) for metolachlor in freshwater environments was a low reliability value (using the ANZECC/ARMCANZ (2000) reliability scheme) as it was calculated by applying an assessment factor of 1 000 to one acute toxicity value for a freshwater fish, *Poecilia reticulata* (Warne 2001). More data on metolachlor toxicity to freshwater species are now available, which has enabled the derivation of improved DGVs compared to those in ANZECC/ARMCANZ (2000).

The available freshwater chronic toxicity data for metolachlor ranged from 1 µg/L for a microalga (4–7 day NOEC) to 310 000 µg/L for a diatom (96-hour EC50). The acute toxicity data ranged from 5.5 µg/L for a microalga (24-hour EC50) to 69 400 µg/L for a crustacean (24-hour LC50). A parametric two-sample *t* test on all available freshwater metolachlor toxicity data indicated that the dataset was unimodal.

Very high reliability DGVs for metolachlor in freshwater were derived based on chronic 10% effect concentration (EC10), no observed effect concentration (NOEC), no observed effect level (NOEL) and chronic estimated NOEC data for 21 freshwater species from six phyla and ten classes, with a good fit of the species sensitivity distribution (SSD) to the toxicity data. The DGVs derived here are expressed in terms of the active ingredient (metolachlor) rather than commercial formulations, and are based on toxicity data for the *S*- and *R*- enantiomers as well as for the racemic mixture. The DGVs for 99, 95, 90 and 80% species protection are 0.0084 µg/L, 0.46 µg/L, 2.6 µg/L and 15 µg/L, respectively. The 95% species protection level for metolachlor in freshwater (0.46 µg/L) is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1 Introduction

Metolachlor ($C_{15}H_{22}ClNO_2$ and Figure 1) is a herbicide that, at room temperature, is a colourless-to-light-tan liquid. It is the active ingredient of a variety of commercial herbicide formulations. Metolachlor is often mixed with other herbicides (e.g. alachlor as well as isomers *S*-metolachlor and *R*-metolachlor) to increase its efficacy (Liu et al. 2006). The physico-chemical properties of metolachlor that may affect its environmental fate and toxicity are presented in Table 1.

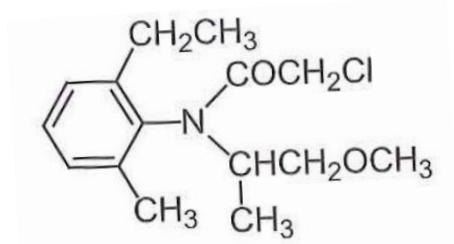


Figure 1 Structure of metolachlor

Table 1 Summary of selected physico-chemical properties of metolachlor

Physico-chemical property	Value
Molecular weight	283.8 amu ^a
Aqueous solubility	488 mg/L at temperature of 25 °C ^a 530 mg/L at temperature of 20 °C ^b
Logarithm of the octanol-water partition coefficient (log K_{ow})	2.9 at temperature 25 °C ^a 3.4 at pH 7 and temperature of 20 °C ^b
Logarithm of the organic carbon water partition coefficient (log K_{oc})	2.08–2.49 ^a 2.08 ^b
Logarithm of the bioconcentration factor (log BCF)	1.84 ^b
Half-life ($t_{1/2}$) in water	Stable at pH 7 and temperature 20 °C ^b >200 days (pH 1–9) at 20 °C ^{a, c}
Half-life ($t_{1/2}$) in soil	20 days (in field) ^a Typical: 90 days (15–21 days in the lab (20 °C) and the field, respectively) ^b

^a BCPC (2012).

^b University of Hertfordshire (2013).

^c CCME (1999).

Metolachlor is a derivative of aniline and belongs to the chloroacetanilide group within the amide family of herbicides. Other chloroacetanilide herbicides include alachlor, acetochlor and propachlor. Metolachlor is extensively used in agriculture. In Australia, it is approved for use on many crops, including barley, sweetcorn, soybeans, sorghum, cotton, sunflowers (Growcom Australia Pty Ltd 2019). In New Zealand, it is approved for use on maize, sweetcorn, asparagus, pumpkin and squash (New Zealand Food Safety 2019). The racemic (i.e. 1:1) mixture of metolachlor (containing *S*-metolachlor and *R*-metolachlor isomers) does not have regulatory approval to be used within the European Union; however, approval has been granted for *S*-metolachlor alone (University of

Hertfordshire 2013). Metolachlor is a selective pre-emergent and early post-emergent herbicide (CCME 1999, Liu & Xiong 2009) that does not affect established plants (Vallotton et al. 2008).

Metolachlor has a low binding affinity to soil particles; therefore, it has a high capacity to leach to groundwater and end up in surface water. The typical soil degradation (aerobic) half-life of metolachlor is 90 days; however, some field studies have reported much shorter half-lives (BCPC 2012, University of Hertfordshire 2013) (Table 1). The aqueous hydrolysis of metolachlor is slow, with a half-life of 100 days to >200 days at pH 1 to pH 9 and a temperature of 20 °C (University of Hertfordshire 2013) (Table 1).

Metolachlor has been frequently detected in surface waters of Europe (Balsiger et al. 2004, Konstantinou et al. 2006), North America (Battaglin et al. 2000, Gilliom et al. 2006) and Australia (AATSE 2002).

2 Aquatic toxicology

2.1 Mechanism of toxicity

Metolachlor exerts its toxicity following germination, where it inhibits the growth of susceptible weeds (Vallotton et al. 2008). It acts by interfering with cell division and cell enlargement of plants when absorbed by the hypocotyls in roots, seedling shoots and cotyledons (Böger et al. 2000). Metolachlor binds strongly and irreversibly (Götz & Böger 2004) to the fatty acid elongation (FAE1)-synthase enzyme, to inhibit the elongation of very long chain fatty acids (VLCFAs) in plants and algae (Böger 2003). Once the elongation process has been inhibited, the lack of VLCFAs (commonly C18 and C16) becomes phytotoxic, as they are no longer available to aid the maintenance of the rigidity and permeability of cell plasma membranes (Böger 2003, Vallotton et al. 2008).

2.2 Toxicity

A summary of the acceptable quality raw chronic and acute toxicity data for all freshwater species that passed the quality assurance and screening processes are provided in Section 2.2.1 and Section 2.2.2, respectively.

2.2.1 Freshwater chronic toxicity data

There were freshwater chronic toxicity data for one fish, one cladoceran, four macrophytes and 18 microalgae.

The toxicity data for fish consisted of:

- a 35-day LOEC (mortality) value of 1 600 µg/L.

The toxicity data for the single cladoceran species were:

- 21-day NOEC (length, longevity, broods per female, young per female) values ranging from 500 µg/L to 15 000 µg/L
- 21-day LOEC (immobilisation, length, longevity, broods per female, young per female) values ranging from 10 µg/L to 10 000 µg/L

- two 21-day EC10 (young per female) values of 100 µg/L and 500 µg/L
- a 21-day EC50 (immobilisation) value of 12 400 µg/L.

The toxicity values for macrophytes were:

- a 14-day NOEL (frond number) value of 8.4 µg/L
- 14-day EC50 (frond number, dry weight, frond area, wet weight) values ranging from 48 µg/L to 2 360 µg/L.

The toxicity values for microalgae were:

- 48-hour EC50 (chlorophyll-a content, cell density) values ranging from 2.3 µg/L to 241 µg/L
- two 72-hour NOEC (cell density) values of 25 µg/L and 30 µg/L
- a 72-hour LOEC (cell density) value of 77 µg/L
- 72-hour EC50 (cell density, chlorophyll-a content) values ranging from 44.3 µg/L to 177 µg/L
- 96-hour EC5 (cell density) values ranging from 5.38 µg/L to 5 960 µg/L
- 96-hour EC10 (cell density, chlorophyll-a content) values ranging from 27 µg/L to 112 000 µg/L
- two 96-hour NOEC (chlorophyll-a content) values of 1 µg/L and 38 µg/L
- 96-hour LOEC (chlorophyll-a content, chlorophyll-b content) values ranging from 1 µg/L to 75 µg/L
- 96-hour EC50 (cell density, chlorophyll-a content) values ranging from 68 µg/L to 310 000 µg/L
- 5-day EC50 (biomass yield, growth rate, area under the growth curve) values ranging from 10 µg/L to 1 200 µg/L
- 7-day NOEC (live cell density, chlorophyll-a content) values of 1 µg/L and 10 µg/L, respectively
- 7-day LOEC (live cell density, chlorophyll-a content) values of 10 µg/L and 100 µg/L, respectively.

2.2.2 Freshwater acute toxicity data

There were freshwater acute toxicity data for six fish, two cladocerans, two insects, one macrophyte and two microalgae.

The fish toxicity data consisted of:

- 96-hour LOEL (mortality) values ranging from 2 100 µg/L to 6 500 µg/L
- 96-hour LC50 (mortality) values ranging from 3 900 µg/L to 10 000 µg/L.

The toxicity values for the cladoceran species were:

- two 24-hour LC50 (mortality) values of 51 200 µg/L and 69 400 µg/L
- 48-hour EC50 (immobilisation) values ranging from 22 300 µg/L to 26 000 µg/L
- a 48-hour LC50 (mortality) value of 1 950 µg/L.

The toxicity values for insects consisted of:

- two 48-hour LC50 (immobilisation) values of 3 800 µg/L and 4 400 µg/L
- 72-hour NOEC, EC58 and LOEC (immobilisation) values of 100 µg/L, 1 000 µg/L and 1 000 µg/L, respectively.

The toxicity values for the macrophyte were:

- 96-hour NOEC and EC50 (frond number) values of 187 µg/L and 343 µg/L, respectively
- a 96-hour EC50 (wet weight) value of 360 µg/L.

The toxicity values for microalgae were:

- a 24-hour NOEC (cell density) value of 120 µg/L
- 24-hour EC50 (cell density) values ranging from 5.5 µg/L to 341 µg/L.

As stated in Warne et al. (2018), acute EC10/NOEC and LOEC values should not be converted to chronic EC10/NOEC values and have not been used to derive default guideline values (DGVs).

3 Factors affecting toxicity

No factors have been reported as modifying the toxicity of metolachlor. As with many organic chemicals, it might be expected that dissolved and particulate organic matter and suspended solids would affect its bioavailability and toxicity. However, any such effect would be relatively minor given the relatively low log K_{oc} value of metolachlor (Table 1).

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. Although some decisions on data selection/manipulation may reflect the Warne et al. (2015) method rather than the Warne et al. (2018) method, these were found to have no material effect on the final DGVs.

4.1 Toxicity data used in derivation

The previous Australian and New Zealand DGV for metolachlor in freshwater environments was a low reliability value (using the ANZECC/ARMCANZ (2000) reliability scheme) as it was based on one acute toxicity value for a freshwater fish species, *Poecilia reticulata* (Warne 2001). This value was calculated using the assessment factor method, dividing the lowest acute toxicity value of 20 µg/L by an assessment factor of 1 000 (Warne 2001). Under the new method for deriving DGVs (Warne et al. 2018), this value would be classified as having an 'unknown' reliability.

To obtain toxicity data for metolachlor to freshwater organisms, an extensive search of the scientific literature was conducted. In addition, the ECOTOXicology Database System (USEPA 2015a), Office of Pesticide Programs database (USEPA 2015b), the Australasian Ecotoxicology Database (Warne et al. 1998) and the ANZECC/ARMCANZ (2000) toxicant databases (Sunderam et al. 2000) were searched. There are now considerably more metolachlor chronic toxicity data available to enable the derivation of DGVs in freshwater based on chronic toxicity.

There were freshwater toxicity data for 30 species (six phyla and 11 classes) that passed the quality assessment and screening processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta, Chordata, Cyanobacteria and Tracheophyta. The 11 classes were Actinopterygii (which accounts for approximately 99% of fish), Bacillariophyceae (diatoms; a major grouping of algae),

Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Cyanophyceae (a class of Cyanobacteria), Fragilariophyceae (a grouping of pennate diatoms), Insecta (invertebrates), Liliopsida (monocots), Magnoliopsida (dicots), Mediophyceae (another algae grouping) and Trebouxiophyceae (another grouping of green algae). Chronic toxicity data were available for 21 of the 30 species, comprising 19 phototrophs and two heterotrophs, while acute toxicity data only were available for nine species, comprising one phototroph and eight heterotrophs.

Based on the current understanding of the mode of action of metolachlor, it is expected that phototrophic species would be more sensitive than non-phototrophic (i.e. heterotrophic) species, as metolachlor binds to and interferes with the FAE1-synthase enzyme that is part of the normal metabolism of plants and algae. However, a modality assessment of the metolachlor freshwater toxicity data, undertaken according to the approach described by Warne et al. (2015), concluded that the data were unimodal, with no significant difference between the sensitivity of phototrophic and heterotrophic species (see Appendix B: Modality assessment for metolachlor toxicity to freshwater species for details). Therefore, as recommended by Warne et al. (2018), the data for both phototrophs and heterotrophs were combined to calculate the DGVs for metolachlor in freshwater.

Of the 21 freshwater species for which there were acceptable chronic toxicity data, there were chronic EC10, NOEC or NOEL data for 13 species belonging to four 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 DGVs (Warne et al. 2018). However, because the resulting fit of the model to this dataset was poor, the effect of adding the chronic values based on LOECs and EC50s (converted to estimated chronic NOEC/EC10 equivalents) for the additional eight species was assessed. The fit of the model to the chronic toxicity dataset for 21 species (belonging to six phyla and 10 classes) was good; hence, this dataset was used to derive the DGVs (refer to Appendix C: Rationale for selecting the final dataset for deriving the default guideline values for metolachlor in freshwater for further details of this assessment). A summary of the toxicity data (one value per species) used to calculate the DGVs for metolachlor in freshwater is provided in Table 2. Further details on the data that passed the quality assessment and screening process and were used to derive the DGVs are presented in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. Details of the [data quality assessment](#) and the [data that passed the quality assessment](#) are provided as supporting information.

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

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

Taxonomic group (Phylum)	Species	Life stage	Duration (d)	Toxicity measure ^a	Test endpoint	Final toxicity value (µg/L)
Diatom (Bacillariophyta)	<i>Achnanthes minutissimum</i> ^d	Exponential growth phase	4	Chronic EC10	Cell density	6 528

Taxonomic group (Phylum)	Species	Life stage	Duration (d)	Toxicity measure ^a	Test endpoint	Final toxicity value (µg/L)
Blue-green alga (Cyanobacteria)	<i>Anabaena flosaquae</i>	Not stated	5	Chronic EC50	Biomass yield, growth rate, AUC ^c	240
Macrophyte (Tracheophyta)	<i>Ceratophyllum demersum</i> ^d	Not stated	14	Chronic EC50	Wet weight	14
Green alga (Chlorophyta)	<i>Chlamydomonas reinhardtii</i> ^d	Not stated	4	Chronic EC50	Chlorophyll-a content	228
Green alga (Chlorophyta)	<i>Chlorella pyrenoidosa</i> ^d	Exponential growth phase	4	Chronic NOEC	Chlorophyll-a content	1
Diatom (Bacillariophyta)	<i>Craticula accomoda</i> ^d	Exponential growth phase	4	Chronic EC10	Chlorophyll-a content	4 016
Diatom (Bacillariophyta)	<i>Cyclotella meneghiniana</i> ^d	Exponential growth phase	4	Chronic EC10	Cell density	925
Macroinvertebrate (Arthropoda)	<i>Daphnia magna</i>	<24 hour old	21	Chronic EC10	Young per female	224
Macrophyte (Tracheophyta)	<i>Elodea canadensis</i> ^d	Not stated	14	Chronic EC50	Wet weight	471
Diatom (Bacillariophyta)	<i>Encyonema silesiacum</i> ^d	Exponential growth phase	4	Chronic EC10	Chlorophyll-a content	1 048
Diatom (Bacillariophyta)	<i>Fragilaria capucina</i> var <i>vaucheriae</i> ^d	Not stated	4	Chronic EC10	Chlorophyll-a content	90
Diatom (Bacillariophyta)	<i>Gomphonema gracile</i> ^d	Exponential growth phase	7	Chronic NOEC	Live cell density	1
Diatom (Bacillariophyta)	<i>Gomphonema parvulum</i>	Exponential growth phase	4	Chronic EC10	Chlorophyll-a content	6 384
Macrophyte (Tracheophyta)	<i>Lemna gibba</i>	Stage 3 (3 fronds/plants)	14	Chronic NOEL	Frond number	8.4
Diatom (Bacillariophyta)	<i>Mayamaea fossalis</i>	Exponential growth phase	4	Chronic EC10	Chlorophyll-a content	863
Macrophyte (Tracheophyta)	<i>Najas</i> sp.	Not stated	14	Chronic EC50	Wet weight	48.4
Diatom (Bacillariophyta)	<i>Navicula pelliculosa</i> ^d	Not stated	5	Chronic EC50	Biomass yield, growth rate, AUC ^c	76
Fish (Chordata)	<i>Pimephales promelas</i>	Early life stage	35	Chronic LOEC	Mortality	640
Green alga (Chlorophyta)	<i>Pseudokirchneriella subcapitata</i> ^b	Not stated	3	Chronic NOEC	Cell density	27.4
Green alga (Chlorophyta)	<i>Scenedesmus vacuolatus</i>	Exponential growth phase	2	Chronic EC50	Cell density	0.53
Diatom (Bacillariophyta)	<i>Ulnaria ulna</i> ^d	Exponential growth phase	4	Chronic EC10	Chlorophyll-a content	27

a Chronic NOEC/NOEL/EC10 = no conversions applied to toxicity values; chronic LOEC = chronic LOEC value that was converted to chronic NOEC/EC10-equivalent value by dividing by 2.5; chronic EC50 = chronic EC50 value that was converted to chronic NOEC/EC10-equivalent value by dividing by 5 (Warne et al. 2018).

b This species has also been called *Raphidocelis subcapitata* and *Selenastrum capricornutum*.

c AUC = area under the growth curve.

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

4.2 Species sensitivity distribution

The SSD of the 21 freshwater metolachlor chronic toxicity data reported in Table 2 is presented in Figure 2. The SSD was plotted using the BurrIoz 2.0 software. The model was judged to provide a good fit to the data (Figure 2).

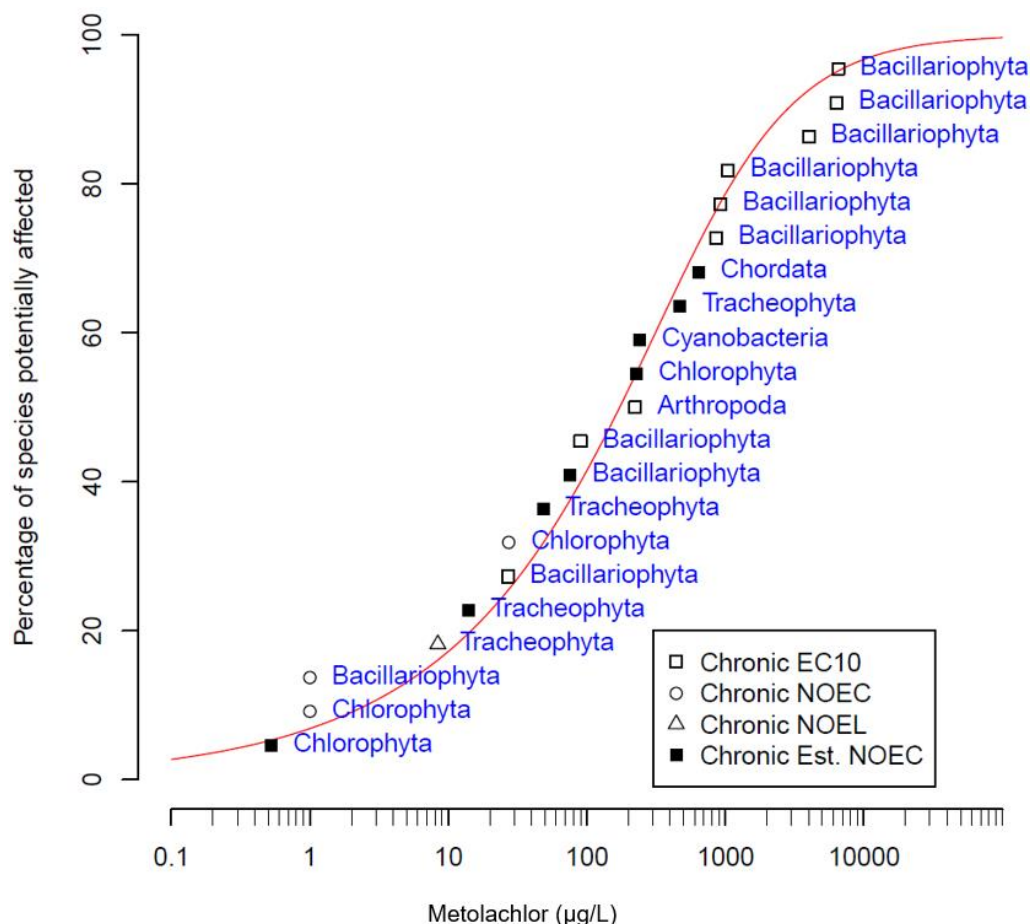


Figure 2 Species sensitivity distribution, metolachlor in freshwater

4.3 Default guideline values

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

The DGVs for 99, 95, 90 and 80% species protection are shown in Table 3. The DGVs for metolachlor are expressed in terms of the concentration of the active ingredient. The 95% species protection DGV of 0.46 µg/L metolachlor is recommended for application to slightly-to-moderately disturbed ecosystems.

Measured log BCF values for metolachlor 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 metolachlor do not need to account for effects due to long-term bioaccumulation or secondary poisoning.

Table 3 Toxicant default guideline values, metolachlor in freshwater, very high reliability

Level of species protection (%)	DGV for metolachlor in freshwater (µg/L) ^a
99	0.0084
95	0.46
90	2.6
80	15

^a The DGVs were derived using the BurrIioz 2.0 software and have been reported to two significant figures.

4.4 Reliability classification

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

- Sample size—21 (preferred)
- Type of toxicity data—chronic EC10/NOEC/NOEL and chronic estimated NOEC values
- SSD model fit—good (Burr Type III model).

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.
ANZECC	Australian and New Zealand Environment and Conservation Council.
ARMCANZ	Agricultural and Resource Management Council of Australia and New Zealand.
assessment factor (AF)	A unitless number applied to the lowest toxicity figure for a chemical to derive a concentration that should not cause adverse environmental effects. The size of the AF varies with the type of data. Also called 'application factor' or 'safety factor'.
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.
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 (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.)
heterotroph	An organism that cannot produce its own food (e.g. by photosynthesis), relying instead on the intake of nutrition from other sources of organic carbon, mainly plant or animal matter.
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.
lowest observed effect concentration (LOEC) or lowest observed effect level (LOEL)	The lowest concentration of a material used in a toxicity test that has a statistically significant adverse effect on the exposed population of test organisms as compared with the controls. Also sometimes referred to as a lowest observed effect level.
no observed effect concentration (NOEC) or no observed effect level (NOEL)	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. Also sometimes referred to as a no observed effect level.
phototroph	An organism that photosynthesizes as its main means of obtaining energy e.g. plants and algae.
site-specific guideline value	A guideline value that is relevant to the specific location or conditions that are the focus of a given assessment or issue.

Term	Definition
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, chronic toxicity data that passed the screening and quality assurance processes, metolachlor in freshwater

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Crustacean (Arthropoda)	<i>Daphnia magna</i>	<24 hour juveniles (neonates)	21	EC10 (Young per female)	Elendt M4 or M7	18-22 ± 2	Not stated	100	Liu et al. (2006)
		<24 hour juveniles (neonates)	21	EC10 (Young per female)	Elendt M4 or M7	18-22 ± 2	Not stated	500	Liu et al. (2006)
		–						224	VALUE USED IN SSD (GEOMETRIC MEAN)
Diatom (Bacillariophyta)	<i>Craticula accomoda</i>	Exponential growth phase	4	EC10 (Chlorophyll-a content)	DMSO dissolved in DV ° medium	Not stated	Not stated	4 016	Larras et al. (2012)
								4 016	VALUE USED IN SSD
Diatom (Bacillariophyta)	<i>Achnantheidium minutissimum</i>	Exponential growth phase	4	EC10 (Chlorophyll-a content)	DMSO dissolved in DV medium	Not stated	Not stated	6 528	Larras et al. (2012)
								6 528	VALUE USED IN SSD

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

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Diatom (Bacillariophyta)	<i>Navicula pelliculosa</i>	Not stated	5	EC50 (Biomass yield, growth rate, AUC ^a)	ASTM Type I water	24 ± 2	7.5 ± 0.1	380	USEPA (2015a)
–								76 ^d	VALUE USED IN SSD
Diatom (Bacillariophyta)	<i>Cyclotella meneghiniana</i>	Exponential growth phase	4	EC10 (Cell density)	DMSO dissolved in DV medium	Not stated	Not stated	925	Larras et al. (2012)
–								925	VALUE USED IN SSD
Diatom (Bacillariophyta)	<i>Encyonema silesiacum</i>	Exponential growth phase	4	EC10 (Chlorophyll-a content)	DMSO dissolved in DV medium	Not stated	Not stated	432	Larras et al. (2012)
		Exponential growth phase	4	EC10 (Chlorophyll-a content)	DV medium	21 ± 2	Not stated	2 542	Larras et al (2013)
–								1 048	VALUE USED IN SSD (GEOMETRIC MEAN)
Diatom (Bacillariophyta)	<i>Fragilaria capucina</i> var <i>vaucheriae</i>	Not stated	4	EC10 (Chlorophyll-a content)	DV medium	21 ± 2	Not stated	90	Larras et al. (2013)
–								90	VALUE USED IN SSD
Diatom (Bacillariophyta)	<i>Gomphonema gracile</i>	Exponential growth phase	7	NOEC (Live cell density)	Dauta medium	20	Not stated	1	Coquillé et al. (2015)
–								1	VALUE USED IN SSD

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

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Diatom (Bacillariophyta)	<i>Gomphonema parvulum</i>	Exponential growth phase	4	EC10 (Chlorophyll-a content)	DMSO dissolved in DV medium	Not stated	Not stated	365	Larras et al. (2012)
		Exponential growth phase	4	EC10 (Chlorophyll-a content)	DV medium	21 ± 2	Not stated	111 666	Larras et al. (2013)
–								6 384	VALUE USED IN SSD (GEOMETRIC MEAN)
Diatom (Bacillariophyta)	<i>Mayamaea fossalis</i>	Exponential growth phase	4	EC10 (Chlorophyll-a content)	DMSO dissolved in DV medium	Not stated	Not stated	979	Larras et al. (2012)
		Exponential growth phase	4	EC10 (Chlorophyll-a content)	DV medium	21 ± 2	Not stated	760	Larras et al. (2013)
–								863	VALUE USED IN SSD (GEOMETRIC MEAN)
Diatom (Bacillariophyta)	<i>Ulnaria ulna</i>	Exponential growth phase	4	EC10 (Chlorophyll-a content)	DV medium	21 ± 2	Not stated	27	Larras et al. (2013)
–								27	VALUE USED IN SSD
Green alga (Chlorophyta)	<i>Chlamydomonas reinhardtii</i>	Not stated	4	EC50 (Chlorophyll-a content)	ASTM medium	25	Not stated	1 138	Fairchild et al. (1998)
–								228^d	VALUE USED IN SSD

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

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Green alga (Chlorophyta)	<i>Chlorella pyrenoidosa</i> ^b	Exponential growth phase	4	NOEC (Chlorophyll-a content)	HB-4 medium	25	Not stated	1	Liu and Xiong (2009)
–								1	VALUE USED IN SSD
Green alga (Chlorophyta)	<i>Pseudokirchneriella subcapitata</i> ^c	Exponential growth phase	3	NOEC (Cell density)	Marine biological laboratory medium	21 ± 2	Not stated	25	Perez et al. (2011)
		Exponential growth phase	3	NOEC (Cell density)	ASTM Type I water	24 ± 2	6.5–8.5	30	Sbrilli et al. (2005)
–								27.4	VALUE USED IN SSD (GEOMETRIC MEAN)
Green alga (Chlorophyta)	<i>Scenedesmus vacuolatus</i>	Exponential growth phase	2	EC50 (Cell density)	Sterile inorganic medium	25	Not stated	2.3	Vallotton et al. (2008)
		Exponential growth phase	2	EC50 (Cell density)	Sterile inorganic medium	25	Not stated	3	Vallotton et al. (2008)
–								0.53^d	VALUE USED IN SSD (GEOMETRIC MEAN)
Fish (Chordata)	<i>Pimephales promelas</i>	Early life	35	LOEC (Mortality)	Deionised water	25 ± 2	Not stated	1 600	USEPA (2015b)
–								640^d	VALUE USED IN SSD

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

Taxonomic group (Phylum)	Species	Life stage	Exposure duration (d)	Toxicity measure (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L)	Reference
Blue-green alga (Cyanobacteria)	<i>Anabaena flosaquae</i>	Not stated	5	EC50 (Growth, growth rate)	ASTM Type I water	24 ± 2	7.5 ± 0.1	1 200	USEPA (2015a)
–								240^d	VALUE USED IN SSD
Macrophyte (Tracheophyta)	<i>Ceratophyllum demersum</i>	Not stated	14	EC50 (Wet weight)	ASTM medium with sediment layer	25	7.2	70	Fairchild et al. (1998)
–								14^d	VALUE USED IN SSD
Macrophyte (Tracheophyta)	<i>Elodea canadensis</i>	Not stated	14	EC50 (Wet weight)	ASTM medium with sediment layer	25	7.2	2 355	Fairchild et al. (1998)
–								471^d	VALUE USED IN SSD
Macrophyte (Tracheophyta)	<i>Lemna gibba</i>	Stage 3 (3 fronds/plants)	14	NOEL (Frond number)	M-Hoagland's or 20X-AAP with deionised water/ASTM Type I water	25 ± 2	4.8-5.2 (Hoagland's)/7.5 ± 0.1 (20X-AAP)	8.4	USEPA (2015a)
–								8.4	VALUE USED IN SSD
Tracheophyta	<i>Najas</i> sp.	Not stated	14	EC50 (Wet weight)	ASTM medium	25	Not stated	242	Fairchild et al. (1998)
–								48.4^d	VALUE USED IN SSD

a AUC = area under the growth curve.

b This species has also been called *Chlorella vulgaris* and *Chlorella pyrenoidosa*.

c This species has also been called *Raphidocelis subcapitata*, *Pseudokirchneriella subcapitata* and *Selenastrum capricornutum*.

d Values were chronic LOEC and EC50 values that were converted to chronic NOEC/EC10 values by dividing by 2.5 and 5, respectively (Warne et al. 2018).

e DV: 'Diatom medium + vitamins'.

Appendix B: Modality assessment for metolachlor toxicity to freshwater species

A modality assessment was undertaken for metolachlor according to the weight of evidence approach specified in Warne et al. (2015).

Statistical analysis of the metolachlor ecotoxicity data for freshwater and marine species indicated that there was no difference in the sensitivities of the two groups. The parametric two-sample *t* test was used because the transformed metolachlor freshwater and marine concentration data had equal variances (Fisher's F-Test, $p = 0.911$) and followed a normal distribution (Anderson–Darling, $p = 0.524$). Results from the two-sample *t* test indicated that the two groups were not significantly different ($p = 0.896$); therefore, the freshwater and the marine metolachlor ecotoxicity data can be pooled for further analysis.

The toxicity data for metolachlor to all freshwater and marine species that passed the screening and quality assessment schemes were combined to create a larger dataset ($n = 33$) to determine the modality of the data. All data that were not chronic NOEC or EC10 values were first converted to this type of data using the methods recommended by Warne et al. (2018). A natural logarithmic (Ln) transformation was then applied to normalise the data. Visual examination of the histogram of the transformed data indicated that the distribution of the metolachlor ecotoxicity data may be bimodal (Figure B 1).

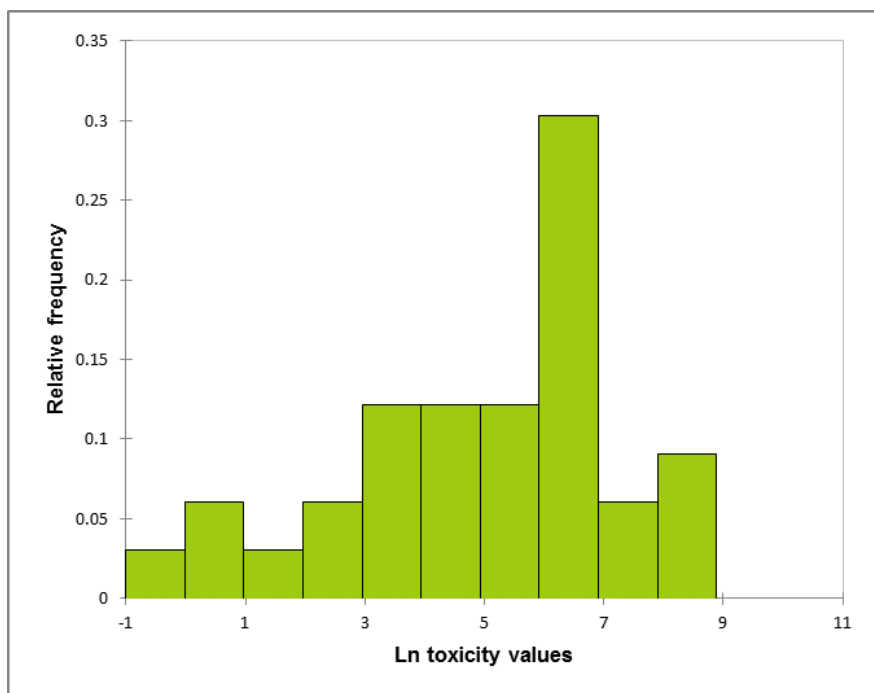


Figure B 1 Histogram, all metolachlor toxicity data, phototrophic and heterotrophic species

The metolachlor ecotoxicity data for phototrophic and heterotrophic species were tested to see if they came from the same population. To test for significant differences, (i.e. $p\text{-value} \leq 0.05$) between the two groups, the non-parametric Mann–Whitney test was used because the transformed metolachlor concentration data failed tests for normality (Anderson–Darling, $p = 0.067$) and had unequal variances (Fisher’s F-Test, $p = <0.0001$). Results from the Mann–Whitney test indicated that the two groups were not significantly different ($p = 0.118$). In addition, the three species that appeared to form a more sensitive group were from taxa that are well represented across the SSD (i.e. all data were for plant species, with the exception of two points), and there was only a $7 \mu\text{g/L}$ difference between the two groups, which is small compared to errors associated with toxicity testing and chemical analysis. Therefore, it was concluded that the distribution of the metolachlor concentration data is unimodal.

Appendix C: Rationale for selecting the final dataset for deriving the default guideline values for metolachlor in freshwater

The preference of ecotoxicity data used to derive the protective concentration (PC) values and/or DGVs of metolachlor to freshwater species is (Warne et al. 2018):

- 1) chronic NOEC/EC10 ecotoxicity data for phototrophs and heterotrophs
- 2) chronic NOEC/EC10 and chronic estimated NOEC values for phototrophs and heterotrophs.

In total, there were chronic EC10/NOEC/NOEL data for 12 phototrophic and one heterotrophic freshwater species (four phyla and seven classes) that passed the quality assessment and screening processes. The represented phyla were Arthropoda, Bacillariophyta, Chlorophyta and Tracheoophyta. The represented classes were Bacillariophyceae (a major grouping of green algae diatoms), Branchiopoda (a grouping of crustaceans), Chlorophyceae (a major grouping of freshwater green algae), Fragilariophyceae (a grouping of pennate diatoms), Liliopsida (monocots), Mediophyceae (another algae grouping) and Trebouxiophyceae (another grouping of green algae). These data met the minimum data requirements of the SSD method (Warne et al. 2018). The resulting SSD and PC values using only these data are presented in

Figure C 1 and Table C 1, respectively.

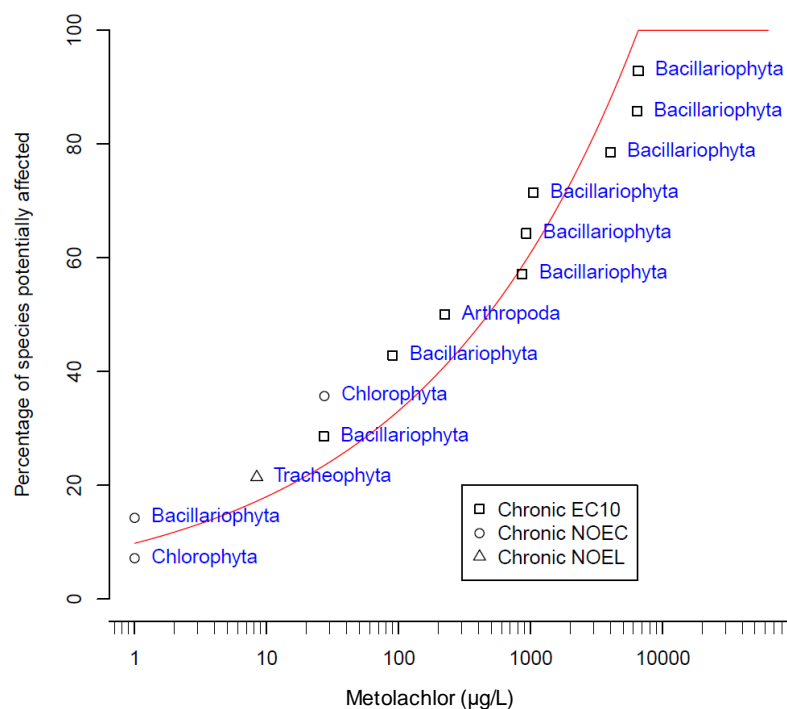


Figure C 1 Species sensitivity distribution, metolachlor in freshwater, chronic EC10/NOEC/NOEL data only**Table C 1 Protective concentration values, metolachlor in freshwater, chronic EC10/NOEC/NOEL data only**

Metolachlor protective concentration values ^a		Reliability classification ^b	
Percent species protection	Concentration (µg/L)	Criterion	Result
99%	0.00018	Sample size	13
95%	0.079	Type of toxicity data	Chronic EC10/NOEC/NOEL
90%	1.1	SSD model fit	Poor
80%	15	Reliability	Moderate

a Protective concentration values were derived using the BurrIioz 2.0 software.

b See Warne et al. (2018) for definitions of protective concentration value 'reliability'.

The resulting PC values were considered to be of *moderate reliability* (Table C 1) according to the method of Warne et al. (2018), because the dataset consisted of chronic EC10/NOEC/NOEL values for 13 species and the SSD had a poor fit to the data (Figure C 1). However, due to the fit and shape of the distribution model to the data, there was a high level of uncertainty in the estimation of the PC99 and PC95 values. That is, the estimated PC99 and PC95 were ~5 500 and ~13 times less than the lowest chronic EC10/NOEC/NOEL ecotoxicity value of 1 µg/L (respectively), which suggested the PC values might be overly conservative. The conservative estimates of the PC99 and PC95 values occur because the fitted model sits relatively high on the y-axis where $x = 1 \mu\text{g/L}$ (i.e. the lowest toxicity value). The lowest toxicity value would be equivalent to a PC90.

To try to improve the reliability of the DGVs, the ecotoxicity dataset was expanded to also include the chronic estimated NOEC data (estimated from chronic LOEC and EC50 data using the methods in Warne et al. (2018)), resulting in data for a total of 21 species from six phyla (Table 2). Expanding the dataset markedly improved the fit of the model to the ecotoxicity data (Figure 2), which subsequently generated PC99 and PC95 estimates (Table 3) much closer to the lowest ecotoxicity value of this expanded dataset (0.53 µg/L)—~60 times less than and similar to the lowest value, respectively. Additionally, the resulting PC values were considered to be of *very high reliability* according to the method of Warne et al. (2018) (see Section 4.4).

Statistical methods, including the SSD methods, become more accurate and reliable as the amount of (acceptable quality) data available to analyse increases. All these factors combined led to the recommendation that the PC values derived using both the chronic and chronic estimated ecotoxicity data be adopted as the DGVs for metolachlor in freshwater.

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