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Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Fipronil in freshwater

Technical brief

July 2023

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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).

Fipronil is a pyrazole insecticide applied to many agricultural crops, and is used in seed dressings and as an acaricide (ESFA 2006, APVMA 2010, Bonmatin et al. 2015).

Fipronil has low to moderate solubility in water and will adsorb to sediment particles (WHO 1997, Demcheck & Skrobialowski 2003, EFSA 2006). Fipronil is non-volatile, and not readily biodegradable in water, with a slow hydrolysis half-life (EFSA 2006). The most significant degradation pathway for fipronil in the environment is photolysis (EFSA 2006).

With its widespread use, fipronil has been detected in surface water in low concentrations (microgram per litre range) (Demcheck & Skrobialowski 2003, Gunasekara & Troung 2007). Fipronil's mode of action is on the central nervous system, acting specifically on the gamma-aminobutyric acid (GABA) receptors and the glutamate-gated chloride (GluCl) channels of some invertebrates by disrupting the passage of chloride ions, resulting in central nervous system toxicity (uncontrolled central nervous system firing) and subsequent death (WHO 1997, Gunasekara & Troung 2007). Based on a review of the aquatic toxicology, and consistent with its mode of action, fipronil is highly toxic to aquatic invertebrates, with lower toxicity to fish, frogs and algae. Fipronil is not expected to bioaccumulate in aquatic organisms (EFSA 2006).

Fipronil is manufactured and used as a 1:1 mixture (i.e. racemate) of its R and S enantiomers (Overmyer et al. 2007, Wilson et al. 2008). Fipronil has a number of metabolites and degradation products with differing physical and chemical properties, environmental persistence and ecotoxicological effects. In the preparation of the fipronil default guideline values (DGVs), data on the ecotoxicological effects of the racemate as well as each of the enantiomers of fipronil have been considered. The effects of metabolites and degradation products of fipronil have not been considered.

Moderate reliability DGVs were derived based on acute (converted to chronic) toxicity values for 13 species from one taxonomic group (insects), with a good fit of the species sensitivity distribution to the toxicity data. The decision to use only insects in the DGV was made following a modality assessment in accordance with the Warne et al. (2018) derivation method. The modality assessment (Appendix B: Modality assessment for fipronil) was performed on the total permissible data (34 species from seven taxonomic groups: cyanobacteria, green algae, diatoms, insects, crustaceans, fish, frogs). The DGVs for 99, 95, 90 and 80% species protection are 0.013 µg/L, 0.018 µg/L, 0.022 µg/L and 0.029 µg/L, respectively. The 95% species protection level for fipronil is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems.

1 Introduction

Fipronil (CASRN 120068-37-3 and molecular formula $C_{12}H_4Cl_2F_6N_4OS$) is a chiral chemical (i.e. it consists of two enantiomers), and it is also known as (±)-5-amino-1-(2,6-dichloro-α,α,α-trifluoro-p-tolyl)-4-trifluoromethylsulfinylpyrazole-3-carbonitrile. It is a pyrazole insecticide, used globally for control of thrips, borers, weevils, locusts, cockroaches, moths, caterpillars, butterflies, and other pests in:

- agricultural crops (e.g. bananas, brassicas, cotton, potatoes, grapes, sugarcane and mushrooms)
- seed dressings (e.g. on rice, canola, sorghum and cotton)
- acaricides (e.g. in a spray formulation to control fleas, lice and ticks in pets, or in a concentrated form for spot-on application) (EFSA 2006, Gunasekara & Troung 2007, Jackson et al. 2009).

Domestic insecticide uses include control of ants, beetles, cockroaches, termites, and other insects. In Australia and New Zealand, fipronil is registered for use as a single active constituent, or combined with other actives, in a large number of products (approximately 150 for Australia and 30 for New Zealand) for the control of agricultural and domestic pests (APVMA 2019, ACVM 2019, Growcom 2019).

Fipronil has been used as a broad-spectrum insecticide in Australia since 1994, and as a veterinary chemical since 1995 (APVMA 2011). It was nominated for review by the Australian Pesticides and Veterinary Medicines Authority (APVMA) in 2003. At the commencement of the APVMA review, there were four active constituent approvals and 29 registered products containing fipronil as the active constituent (APVMA 2011). The review, including an assessment of the ecotoxicological effects of fipronil (particular to aquatic and terrestrial insects) and photodegradation products, is ongoing. The Phase 2 Environmental Assessment Report on fipronil was released in 2010 (APVMA 2010).

Fipronil is a white powder with a mouldy odour (Jackson et al. 2009) and low to moderate water solubility, ranging from 2.4 mg/L at pH 9 and 20°C (Jackson et al. 2009) to 3.78 mg/L at pH 6.58 and 20°C (EFSA 2006). Degradation in water occurs predominantly via photolysis (with a reported half-life of 3.6 h) (EFSA 2006). Hydrolysis is a less significant degradation process for fipronil in the environment, with reported half-lives up to 28 days at pH 5, 7, and 9 (EFSA 2006). Fipronil will adsorb to sediment particles (average log K_{oc} of 2.9 (Demcheck & Skrobialowski 2003)), and it has a degradation half-life ranging from 16.4 days to 119.6 days (EFSA 2006). Fipronil is not considered volatile based on a vapour pressure of $2x10^{-6}$ Pa at 25°C and a Henry's Law Constant of $2.31\ 10^{-4}$ Pa m^3 mol^{-1} at 25°C (EFSA 2006). Based on log K_{ow} values ranging from 3.5 to 4 (EFSA 2006), and a bioconcentration factor (BCF) of 321 L/kg (logBCF 2.5), fipronil is considered to present low bioaccumulation potential in fish (EFSA 2006).

The wide use of fipronil has resulted in environmental releases (e.g. via spray or dust drift, and runoff from land into waterways) and exposure of non-target species, such as aquatic organisms (Bonmatin et al. 2015). Once released to water, the majority of fipronil will degrade via photolysis. However, given the average log K_{oc}, fipronil has the potential to bind to sediments and may present an ongoing source of release to overlying waters. The presence of fipronil (and its sulfone and sulfide degradates) has been reported in surface water in the low microgram per litre (µg/L) range in the United States (Demcheck & Skrobialowski 2003, Gunasekara & Troung 2007).

Fipronil is manufactured and used as a 1:1 mixture (i.e. racemate) of its R and S enantiomers (Overmyer et al. 2007, Wilson et al. 2008). Biological processes in organisms or the environment can alter the enantiomeric fractions of fipronil, resulting in enrichment in one enantiomer over the other, where one is selectively biotransformed. Notably, exposures of fipronil in the environment may be from mixtures enriched in either enantiomer (i.e. non-racemic), even though fipronil is applied as a racemate (Overmyer et al. 2007).

Fipronil has a number of metabolites and degradation products with differing physical and chemical properties, environmental persistence and ecotoxicological effects (ESFA 2006, Gunasekara & Troung 2007, APVMA 2010, Bonmatin et al. 2015). Metabolites and degradation products include: fipronil-desulfinyl (or MB46513, produced following photolysis of fipronil), fipronil-sulfide (MB45950, following reduction of fipronil), fipronil-sulfone (MB46136, following oxidation of fipronil), and fipronil-amide (following hydrolysis of fipronil) (Gunasekara & Troung 2007). The toxicity of fipronil metabolites varies depending on the species, life-stage and duration of exposure. For example, fipronil-desulfinyl is considered to present greater toxicity than the parent compound for some organisms (WHO 1997). Similarly, the ecotoxicology of the R and S enantiomers, and racemate, of fipronil indicates some evidence of enantiomer-specific toxicity. However, insufficient data were available to support intra-species or inter-species comparisons to confirm whether the racemate or either of the enantiomers is more toxic.

In the preparation of the fipronil default guideline values (DGVs), data on the ecotoxicological effects of the racemate as well as each of the enantiomers have been considered. The form of fipronil that was the most toxic for each organism was used to derive the DGVs. In the future, if enantiomer-specific DGVs are to be derived, a routine analytical method that can distinguish the enantiomers would be needed. The ecotoxicological effects of metabolites and degradation products of fipronil have not been considered in the derivation of the fipronil DGVs.

2 Aquatic toxicology

2.1 Mechanism of toxicity

Fipronil's mode of action is on the central nervous system, acting specifically on the gamma-aminobutyric acid (GABA) receptors and the glutamate-gated chloride (GluCl) channels by disrupting the passage of chloride ions, resulting in central nervous system toxicity (uncontrolled central nervous system firing) and subsequent death (WHO 1997, Gunaskara & Troung 2007). The GluCl channel mechanism of toxicity is only applicable to invertebrate protosome phyla such Mollusca, Nematoda and Arthropoda that have this neurochemical pathway (Wolstenholme 2012). Review of the aquatic toxicology indicates that fipronil is highly toxic to aquatic invertebrates, with lower toxicity to fish, frogs and algae.

2.2 Toxicity

A literature review of the effects of fipronil on freshwater organisms indicated that extensive research has been undertaken, with the majority of the studies representing acute duration exposures. A total of 139 toxicity values for 47 species, representing acute and chronic exposure

durations, were identified in the literature review. A limited number of the studies reviewed were based on enantiomer-specific toxicity, with the majority of studies performed using the racemate. Some toxicity studies assessed formulations containing fipronil as the active ingredient with other ingredients (e.g. a carrier solvent), for which the combined toxicity is not well understood. Accordingly, such studies are not used in the current DGV derivation and are not discussed further.

The review identified data of acceptable quality (i.e. the studies passed quality assessment, were not assessed using a formulation, and the test substance was of >80% purity) for 61 acute toxicity values for 29 species from four taxonomic groups (crustaceans, insects, fish, and a frog), and 43 chronic toxicity values for 11 species from five taxonomic groups (cyanobacteria, green algae, diatoms, crustaceans, and fish).

Review of the acceptable chronic toxicity studies indicated the majority of effects reported (27 values) were for cladocerans, with toxicity values ranging from 2 µg/L (LOEC) to 270 µg/L (LOEC) for survival and reproductive endpoints (i.e. brood size, number of neonates per female, time to first brood). There were fewer effects reported for fish (10 values), with toxicity values ranging from 0.24 µg/L (NOEC) to 365 µg/L (LC50) for survival and growth endpoints. Microalgae and cyanobacteria were the least sensitive species, with toxicity values ranging from 14 µg/L (NOEC) to 1 500 µg/L (EC50). No acceptable chronic toxicity studies on insects were available.

Chronic NOEC values for the fish *Cyprinodon variegatus*, *Oncorhynchus mykiss* and *Oryzias latipes* were reported to be 0.24 µg/L (32-d growth), 6.6 µg/L (90-d growth) and 10 µg/L (28-d growth), respectively (Sun et al. 2014, USEPA OPP 2019). The chronic NOECs available for cladocerans ranged from 8 µg/L for *Ceriodaphnia dubia* reproduction (F1 generation neonates exposed for 8 days) (Wilson et al. 2008) to 90 µg/L for *Ceriodaphnia dubia* survival (F1 generation neonates exposed for 8 days) (Wilson et al. 2008). Notably, however, Wilson et al. (2008) reported significant effects on *C. dubia* average brood size and the number of neonates per female at the lowest concentration tested of 2 µg/L, thus resulting in NOECs of <2 µg/L for these two endpoints. Chronic NOECs for non-animal species ranged from 14 µg/L for *Raphidocelis subcapitata* (5-d growth) (USEPA OPP 2019) to 170 µg/L for the cyanobacteria *Anabaena flos-aquae* (5-d growth) (USEPA OPP 2019).

The majority of available aquatic toxicity studies for fipronil represent acute exposures assessing survival. Of the acceptable data (based on fipronil of >80% purity), insects are the most sensitive, followed by crustaceans, fish and frogs. Acceptable acute values (LC50s) for insects ranged from 0.153 µg/L for the caddisfly *Cheumatopsyche brevilineata* (2-d exposure) (Shan et al. 2003) to 646 µg/L for the midge *Chaoborus crystallinus* (2-d exposure) (Chaton et al. 2002). Other acute values included an EC50 of 162 µg/L for growth in the zebrafish *Danio rerio* (5-d exposure) (Stehr et al. 2006) and an LC50 of 1 140 µg/L for the frog *Xenopus laevis* (4-d exposure) (Overmyer et al. 2007). It is worth noting that the most sensitive acute toxicity responses for insects are more sensitive than chronic responses for other taxa.

3 Factors affecting toxicity

To date, there is no evidence of abiotic factors affecting the toxicity of fipronil to freshwater aquatic organisms.

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 fipronil 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 B: . Details of the data quality assessment and the data that passed the quality assessment are provided as supporting information.

Results from toxicity testing using insecticide formulations containing fipronil as the active ingredient were excluded from the DGV derivation because the toxicity of the carrier solvent (and other ingredients where stated) was not known (Section 2). Results from studies where the fipronil purity was not known or was <80% were excluded.

Where only one value was available for a species, that value was included in the final dataset for the derivation of the DGVs. For species with more than one value available, the data selected for the final dataset were in accordance with Warne et al. (2018). Overall, 34 species from seven taxonomic groups were considered for the final dataset. These species included: two algae, one diatom, one cyanobacterium, seven crustaceans, 14 insects, eight fish and one frog. Of the toxicity data available for these 34 species, eight were chronic NOEC values, one was a chronic LOEC value, two were chronic EC50/LC50 values, and 23 were acute EC50/LC50 values that were converted to chronic negligible effect (e.g. NOEC/EC10) values using the default acute-to-chronic ratio of 10. The chronic LOEC was converted using the default chronic NOEC to LOEC ratio of 2.5, and the chronic EC50s/LC50s were converted using the default ratio of 5.

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 fipronil. Although there were chronic studies for 11 of the 34 species available, the weight of evidence assessment concluded that acute toxicity to insects was greater than both acute and chronic toxicity to other taxa; therefore, only acute insect data for 13 species were used to derive the DGVs. A toxicity value for the midge *C. crystallinus* was excluded from the insect dataset as the (unconverted) acute LC50 (646 µg/L) was approximately two to three orders of magnitude higher than for the other insects (0.15–8.1 µg/L). According to Chaton et al. (2002), the insensitivity of Chaoboridae larvae to fipronil may be due to their large biomass and also lower integumental uptake of fipronil when compared to many other insect larvae.

Where only the data for the most sensitive taxonomic group from a bimodal dataset are used in a species sensitivity distribution (SSD) to derive DGVs, the usual minimum data requirements specified by Warne et al. (2018) do not apply. In such situations, the data for the most sensitive group of organisms must still meet the minimum requirement for species (i.e. toxicity data for at least five species), but the minimum requirement for the number of taxonomic groups (i.e. at least four

taxonomic groups) can be relaxed. However, the full bimodal dataset (in this case, all chronic and acute—converted to chronic—toxicity data) must still meet the minimum data requirements for at least five species belonging to at least four taxonomic groups. For fipronil toxicity to freshwater species, the entire dataset of 34 species from seven taxonomic groups met the minimum data requirements; therefore, it was acceptable to derive DGVs using the converted acute data for the 13 insect species even though they belong to one taxonomic group.

Table 1 Summary of converted acute-to-chronic values, all species used to derive default guideline values for fipronil in freshwater

Taxonomic group	Species	Life stage	Duration (h)	Type (acute/ chronic)	Toxicity measure [a]	Reported toxicity value (μg/L)	Final toxicity value (μg/L) [b]
Insecta	*Cheumatopsyche brevilineata*	Larvae	48	Acute	LC50	0.153	0.015
	Simulium vittatum	Larvae	48	Acute	LC50	0.23 [c]	0.023
	Culex quinqefasciatus	Larvae	24	Acute	LC50	0.35	0.035
	Chironomus crassicaudatus	Larvae	48	Acute	LC50	0.42	0.042
	Glyptotendipes paripes	Larvae	48	Acute	LC50	0.42	0.042
	Aedes taeniorhynchus	Larvae	48	Acute	LC50	0.43	0.043
	Anopheles quadrimaculatus	Larvae	48	Acute	LC50	0.43	0.043
	Hexagenia sp.	Nymph	96	Acute	LC50	0.44	0.044
	Culex nigripalpus	Larvae	48	Acute	LC50	0.87	0.087
	Polypedilum nubiferum	Larvae	48	Acute	LC50	1.0	0.1
	Aedes aegypti	Larvae	24	Acute	LC50	1.2	0.12
	Chironomus annularius	Larvae	48	Acute	LC50	2.45	0.245
	Aedes albopictus	Larvae	48	Acute	LC50	8.1	0.81

Note: Final toxicity values are reported to no more than three significant figures.
a The measure of toxicity being estimated/determined: LC50: median lethal concentration.
b A default conversion from acute LC50 to chronic negligible effect (NOEC/EC10) concentration (i.e. acute LC50/10 = chronic negligible effect concentration) was applied to all values.
c Value is the geometric mean of LC50 values of 0.19 μg/L and 0.29 μg/L for the same species, life stage, endpoint and duration of exposure.

4.2 Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the 13 converted acute fipronil toxicity data reported in Table 1 is shown in Figure 1. The SSD was plotted using the Burrlioz 2.0 software. The model was judged to provide a good fit to the data.

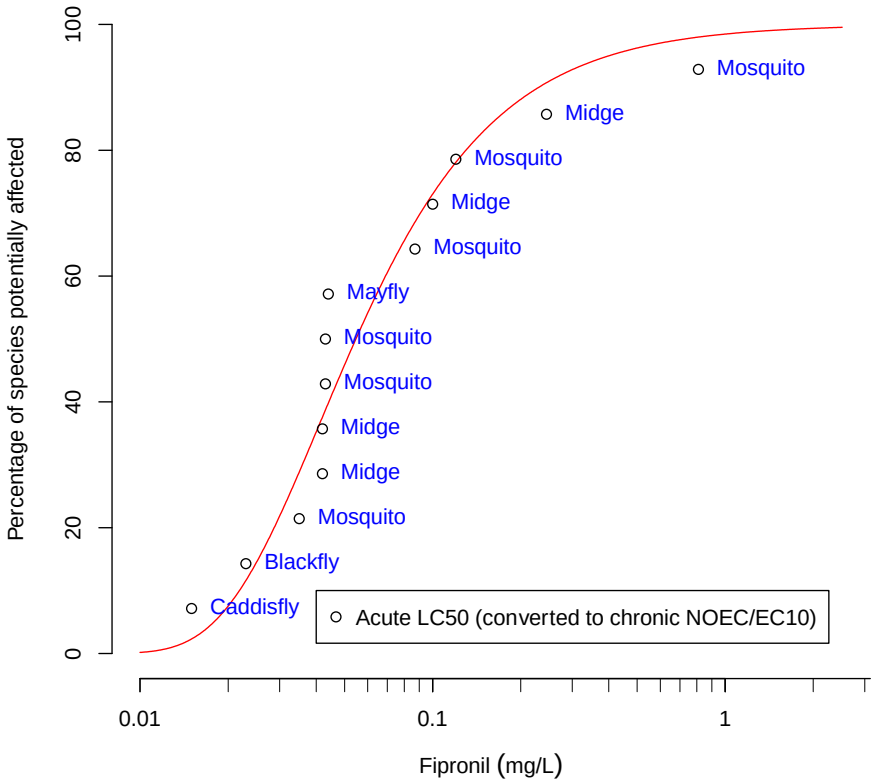


Figure 1 Species sensitivity distribution, fipronil in freshwater

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 fipronil DGVs for 99, 95, 90 and 80% species protection are shown in Table 2. The DGVs relate to fipronil only, and not any of its breakdown products. The 95% species protection DGV of 0.018 µg/L is recommended for application to slightly-to-moderately disturbed ecosystems.

Table 2 Toxicant default guideline values, fipronil in freshwater, moderate reliability

Level of species protection (%)	DGV for fipronil in freshwater (µg/L) [a]
99	0.013
95	0.018
90	0.022
80	0.029

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

The DGVs were compared to the raw chronic toxicity data and converted acute data for all permissible species effects compiled from the literature review. This check confirmed that the theoretical protection offered by the DGVs is expected to be adequate.

4.4 Reliability classification

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

- sample size—13 (good)
- type of toxicity data—converted acute data
- SSD model fit—good (Inverse Weibul model).

Glossary

Term	Definition
acaricide	A substance poisonous to mites or ticks.
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.
bioconcentration factor (BCF)	The ratio of the concentration of a contaminant in an organism to its concentration in the ambient water (or sediment) at a steady state. It can be expressed on a wet weight, dry weight or lipid weight basis.
CASRN	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'.
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.
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.
enantiomer	Chiral molecules that are mirror images of each other; that is, they are not superimposable on one another.
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.)
K_{OW} (or P_{OW})	The ratio of a chemical's solubilities in n-octanol and water at equilibrium. The logarithm of K_{OW} (or P_{OW}) 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.
lowest observed effect concentration (LOEC)	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.
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.
racemate (or racemic)	A racemate or racemic mixture contains equal proportions of left and right enantiomers of a chiral substance (such as fipronil).

Term	Definition
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, fipronil in freshwater

Taxonomic group	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L) [b]	Reference
Insecta	*Cheumatopsyche brevilineata*	Larvae	48	Acute	LC50 (Survival)	Dechlorinated tap water	20	–	0.153	Yokoyama et al. (2009)
	Simulium vittatum	Larvae	48	Acute	LC50 (Survival)	Moderately hard reconstituted water	20	7.1–7.8	0.19	Overmyer et al. (2005)
		Larvae	48	Acute	LC50 (Survival)	Moderately hard reconstituted water	20	7.1–7.8	0.29	Overmyer et al. (2005)
	–								**0.23**	**Geometric mean (value used in SSD)**
	Culex quinqefasciatus	Larvae	24	Acute	LC50 (Survival)	Tap water	24–28	–	0.35	Ali et al. (1998)
	Chironomus crassicaudatus	Larvae	48	Acute	LC50 (Survival)	Tap water	24–28	–	0.423	Ali et al. (1998)
	Glyptotendipes paripes	Larvae	48	Acute	LC50 (Survival)	Tap water	24–28	–	0.423	Ali et al. (1998)
	Aedes taeniorhynchus	Larvae	48	Acute	LC50 (Survival)	Tap water	24–28	–	0.434	Ali et al. (1998)
	Anopheles quadrimaculatus	Larvae	48	Acute	LC50 (Survival)	Tap water	24–28	–	0.435	Ali et al. (1998)
	Hexagenia sp.	Nymph	96	Acute	LC50 (Survival)	–	–	–	0.44	USEPA OPP (2019)

Taxonomic group	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	pH	Concentration (µg/L) [b]	Reference
	Culex nigripalpus	Larvae	48	Acute	LC50 (Survival)	Tap water	24–28	–	0.87	Ali et al. (1998)
	Polypedilum nubiferum	Larvae	48	Acute	LC50 (Survival)	Martin's rearing solution	24–26	–	1.0	Stevens et al. (2011)
	Aedes aegypti	Larvae	24	Acute	LC50 (Survival)	Deionised water	–	–	1.2	Prigdeon et al. (2014)
	Chironomus annularius	Larvae	48	Acute	LC50 (Survival)	Dechlorinated tap water	–	–	2.45	Chaton et al. (2002)
	Aedes albopictus	Larvae	48	Acute	LC50 (Survival)	Tap water	24–28	–	8.1	Ali et al. (1998)

a The measure of toxicity being estimated/determined: LC50: the lethal concentration for 50% of the test organisms.
b All values were divided by the default acute-to-chronic ratio of 10 to estimate the chronic negligible effect (NOEC/EC10) value for use in DGV derivation.

Appendix B: Modality assessment for fipronil

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

Is there a specific mode of action that could result in taxa-specific sensitivity?
As discussed in Section 2, the mode of action for fipronil is on the central nervous system, disrupting the passage of chloride ions, resulting in central nervous system toxicity and subsequent death. Based on a review of the aquatic toxicology of fipronil, and consistent with its mode of action, fipronil is highly toxic to a range of aquatic invertebrates, with lower toxicity to fish, frogs and algae.

Does the dataset suggest bimodality?
The modality assessment was undertaken on the lowest toxicity value for each species (or the appropriate geometric mean of the lowest values) that passed the screening and quality assessment stipulated in Warne et al. (2018). Table B 1 summarises the data considered for the SSD.

The data were subject to visual assessment, calculation of the bimodality coefficient (BC), and consideration of the range in the effect concentrations. These factors 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 raw effect concentration SSD data (Figure B 1) could be interpreted as positively right skewed, typical of concentration-based data (Warne et al. 2018). The log transformed histogram appears to be distinctly bimodal (Figure B 1).
- Data that span large ranges (>4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018); the fipronil data span five 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 for the log transformed data is 0.545, which is marginally less than the 0.555 cutoff stipulated in Warne et al. (2018)

Based on these lines of evidence, the distribution of the log transformed dataset appears to have a bimodal distribution.

Table B 1 Lowest toxicity value (or appropriate geometric mean), each species that passed the screening and quality assessment stipulated in Warne et al. (2018)

Concentration (µg/L)	Toxicity estimate	Species	Major type of organism	Taxonomic group
0.015	LC50 converted to chronic NOEC/EC10	*Cheumatopsyche brevilineata*	Invertebrate	Insect
0.023	LC50 converted to chronic NOEC/EC10 (Geometric mean of two values)	*Simulium vittatum*	Invertebrate	Insect
0.035	LC50 converted to chronic NOEC/EC10	*Culex quinqefasciatus*	Invertebrate	Insect
0.042	LC50 converted to chronic NOEC/EC10	*Chironomus crassicaudatus*	Invertebrate	Insect
0.042	LC50 converted to chronic NOEC/EC10	*Glyptotendipes paripes*	Invertebrate	Insect
0.043	LC50 converted to chronic NOEC/EC10	*Aedes taeniorhynchus*	Invertebrate	Insect
0.043	LC50 converted to chronic NOEC/EC10	*Anopheles quadrimaculatus*	Invertebrate	Insect
0.044	LC50 converted to chronic NOEC/EC10	*Hexagenia sp.*	Invertebrate	Insect
0.087	LC50 converted to chronic NOEC/EC10	*Culex nigripalpus*	Invertebrate	Insect
0.1	LC50 converted to chronic NOEC/EC10	*Polypedilum nubiferum*	Invertebrate	Insect
0.12	LC50 converted to chronic NOEC/EC10	*Aedes aegypti*	Invertebrate	Insect
0.24	NOEC	*Cyprinodon variegatus*	Vertebrate	Fish
0.245	LC50 converted to chronic NOEC/EC10	*Chironomus annularius*	Invertebrate	Insect
0.345	LC50 converted to chronic NOEC/EC10	*Diaptomus castor*	Invertebrate	Crustacean
0.81	LC50 converted to chronic NOEC/EC10	*Aedes albopictus*	Invertebrate	Insect
1.113	LC50 converted to chronic NOEC/EC10	*Simocephalus elizabethae*	Invertebrate	Crustacean
1.95	LC50 converted to chronic NOEC/EC10	*Procambarus zonangulus*	Invertebrate	Crustacean
3.5	LC50 converted to chronic NOEC/EC10	*Lepomis macrochirus*	Vertebrate	Fish
4.23	LC50 converted to chronic NOEC/EC10	*Procambarus clarkii*	Invertebrate	Crustacean
6.6	NOEC	*Oncorhynchus mykiss*	Vertebrate	Fish

Concentration (µg/L)	Toxicity estimate	Species	Major type of organism	Taxonomic group
8	NOEC	*Ceriodaphnia dubia*	Invertebrate	Crustacean
8.49	LC50 converted to chronic NOEC/EC10	*Acanthocyclops robustus*	Invertebrate	Crustacean
9.6	NOEC	*Daphnia magna*	Invertebrate	Crustacean
10	NOEC	*Oryzias latipes*	Vertebrate	Fish
16.2	EC50 converted to chronic NOEC/EC10	*Danio rerio*	Vertebrate	Fish
17.12	LOEC converted to chronic NOEC/EC10	*Cyprinus carpio*	Vertebrate	Fish
41.6	LC50 converted to chronic NOEC/EC10	*Pimephales promelas*	Vertebrate	Fish
44.3	NOEC (Geometric mean of two values)	*Raphidocelis subcapitata*	Plant	Green alga
56	LC50 converted to chronic NOEC/EC10	*Ictalurus punctatus*	Vertebrate	Fish
58	EC50 converted to chronic NOEC/EC10	*Scenedesmus obliquus*	Plant	Green alga
64.6	LC50 converted to chronic NOEC/EC10	*Chaoborus crystallinus*	Invertebrate	Insect
85	LC50 converted to chronic NOEC/EC10	*Xenopus laevis*	Vertebrate	Frog
120	NOEC	*Navicula pelliculosa*	Plant	Diatom
170	NOEC	*Anabaena flos-aquae*	Other	Blue–green alga (cyanobacteria)

Note: The groups used to define ‘major type of organism’ and ‘taxonomic group’ were obtained from Table 6 of Warne et al. (2018).

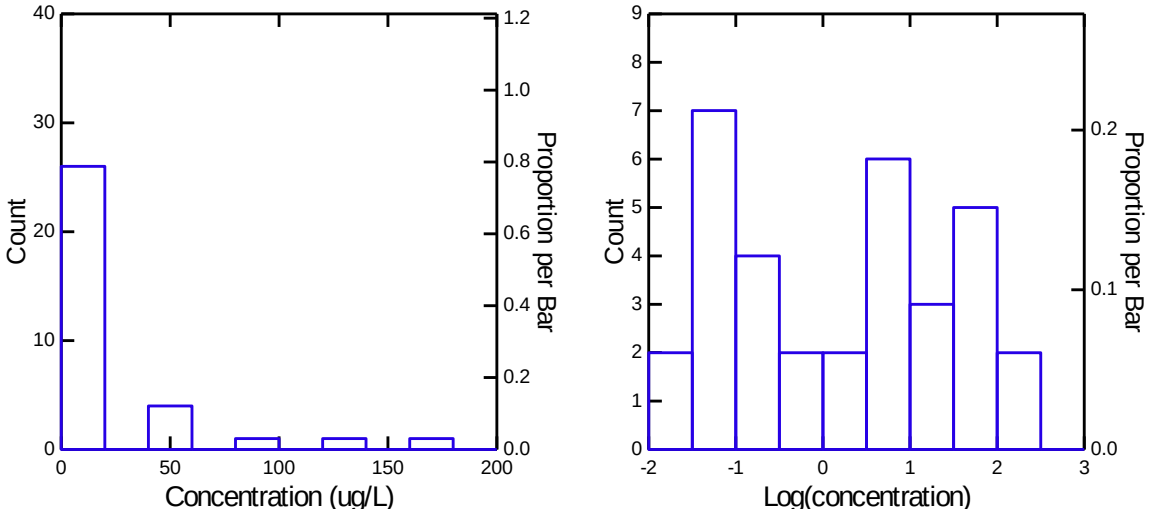
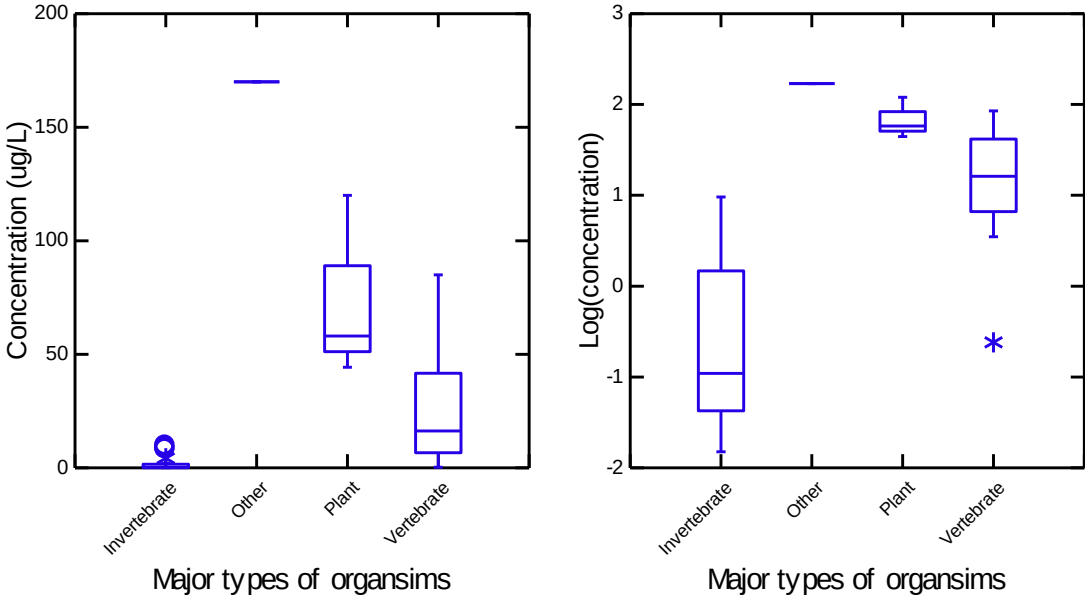


Figure B 1 Histogram, raw (left) and log transformed (right) data

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

Given its mode of action, it is anticipated that fipronil will show taxa-specific sensitivity . Taxa-specific sensitivity is considered likely to account for the bimodality identified in the data. Therefore, box plots (Figure B 2) of the data in Table B 1 were created to visualise the difference between effect concentrations in different major types of organism.

Figure B 2 shows there is a general trend for invertebrates to be more sensitive to fipronil than other taxonomic groups. Of the nine data points available for vertebrates, only one (the sheepshead minnow (*Cyprinodon variegatus*) fell within the interquartile range of the invertebrate data. This data point is a statistical outlier for vertebrates; therefore, it likely reflects a single species with unusual sensitivity rather than a true overlapping distribution between invertebrates and vertebrates. As invertebrates were the most sensitive type of organism, further investigations focused exclusively on invertebrate data.



Note: An asterisk represents an outlying value >1.5x the interquartile range. An open circle represents an outlying value >3x the interquartile range.

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

The invertebrate dataset consisted of 21 species, 14 of which were insects and seven of which were crustaceans, as shown in Table B 1. The invertebrate dataset was subject to a modality assessment, the results of which are as follows.

- The histogram of the raw effect concentration SSD data (Figure B 3) could be interpreted as positively right skewed, typical of concentration-based data (Warne et al. 2018). The log transformed histogram appears to be distinctly bimodal (Figure B 3).
- The invertebrate data span three orders of magnitude, which does not indicate bimodality.
- The BC for the log transformed data is 0.556, which is marginally greater than the 0.555 cutoff stipulated in Warne et al. (2018), indicating bimodality.
- The available invertebrate data were visually assessed using box plots, with data grouped by 'organisms considered to be taxonomically different' as defined in Table 6 of Warne et al. (2018). As shown in Figure B 4, insects were more sensitive to fipronil than crustaceans.

Based on this information, the invertebrate dataset was identified as bimodal, with the trend driven by the greater sensitivity of insects to fipronil than of crustaceans to fipronil. As insects were the most sensitive taxonomic group based on the available dataset, insect data were used to derive the DGVs.

Review of the box plots of insect data presented in Figure B 4 indicated there was an outlying data point. The *Chaoborus crystallinus* effect concentration (LC50) was two or more orders of magnitude higher than the other insect data. *Chaoborus* spp. larvae are considerably larger than other insect larvae, with evidence of reduced bioaccumulation of fipronil following uptake from water compared to other insects (Chaton et al. 2002). Calculation of a BC of 0.66 for the insect data indicated the data may be bimodal. Therefore, the toxicity value for *C. crystallinus* was considered an outlier and was removed from the dataset.

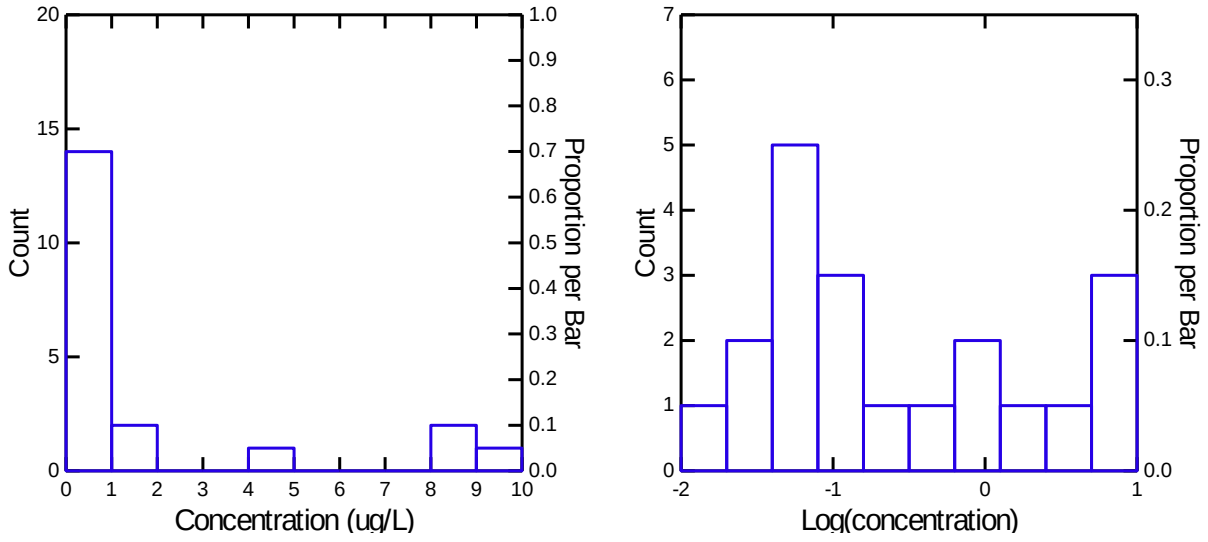
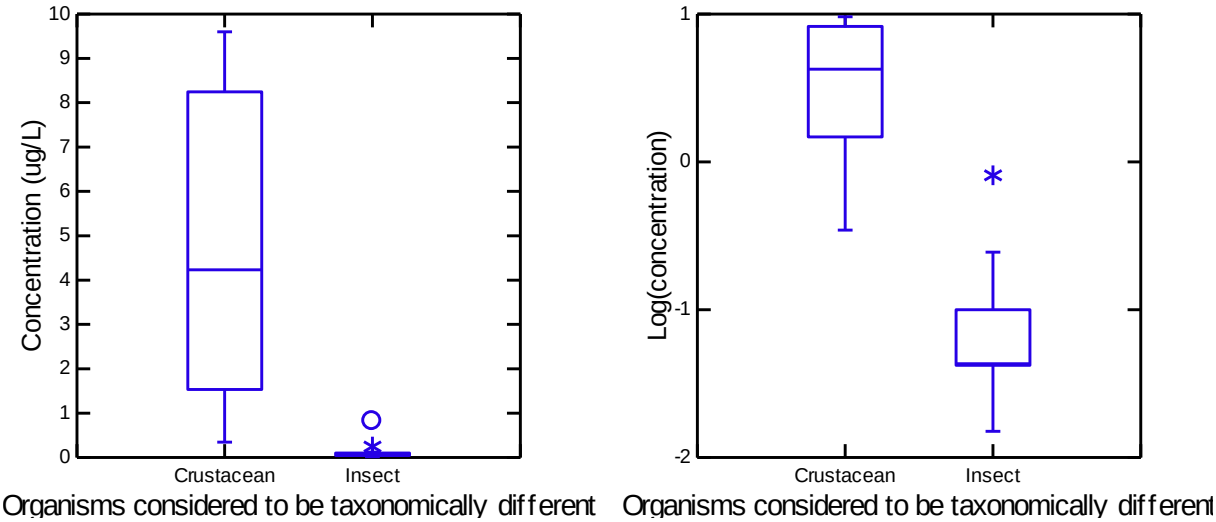


Figure B 3 Histogram, raw (left) and log transformed (right) invertebrate data



Note: An asterisk represents an outlying value >1.5x the interquartile range. An open circle represents an outlying value >3x the interquartile range.

Figure B 4 Box plots, raw (left) and log transformed (right) invertebrate data grouped by 'organisms considered to be taxonomically different'

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?

It is unlikely that the modality of the dataset is an artefact of data selection, small sample size, test procedures, or other reasons unrelated to a specific mode of action. The weight of evidence supports the use of the 13 species identified in preparation of the SSD.

To ensure that the use of the insect-only data (minus *C. crystallinus*) did not introduce unexpected and unacceptable artefacts into the dataset, an assessment of insect-only data (minus *C. crystallinus*) was undertaken, with the following results.

- The histogram of the raw effect concentration SSD data (Figure B 5) could be interpreted as positively right skewed, typical of concentration-based data (Warne et al. 2018). The log transformed histogram appears to either have no discernible distribution or be trending towards a normal distribution (Figure B 5).
- Data that span large ranges (>4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018); the insect data (minus *C. crystallinus*) span two 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 for the log transformed data is 0.425, which is lower than the 0.555 cutoff stipulated in Warne et al. (2018).

Based on this information, the use of insect-only data is unlikely to introduce artefacts into the DGV derivation.

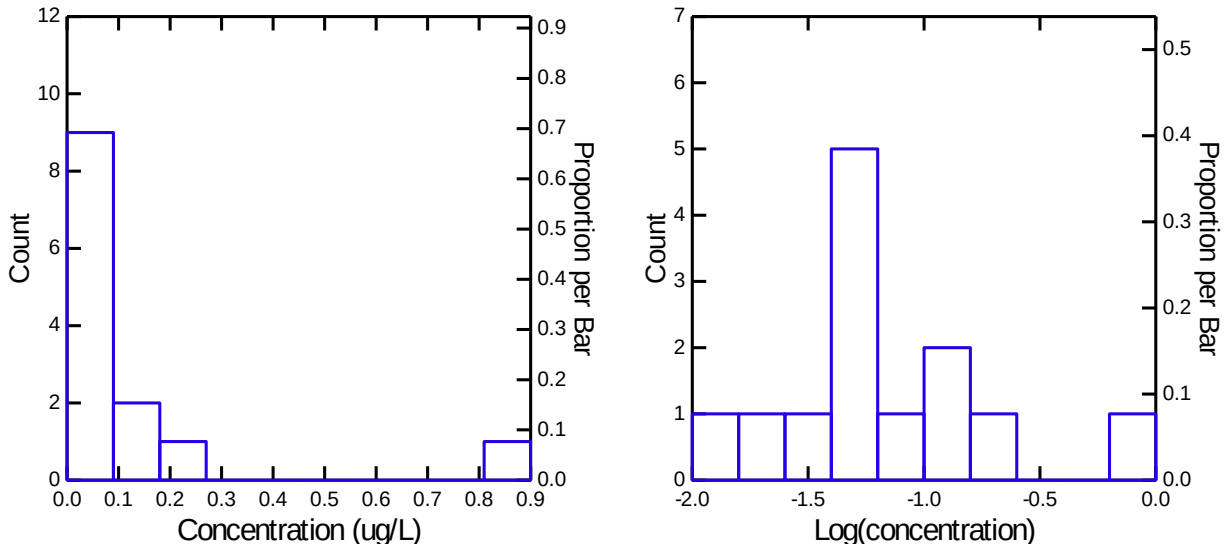


Figure B 5 Histogram, raw (left) and log transformed (right) insect-only data

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