An Australian Government Initiative

Australian & New Zealand GUIDELINES FOR FRESH & MARINE WATER QUALITY

Toxicant default guideline values for aquatic ecosystem protection

Paraquat in freshwater

Technical brief

August 2024

Water Quality Guidelines is a joint initiative of the Australian and New Zealand governments, in partnership with the Australian states and territories.

Cataloguing data
This publication (and any material sourced from it) should be attributed as: ANZG (2024) *Toxicant default guideline values for aquatic ecosystem protection: paraquat in freshwater,* Australian and New Zealand Guidelines for Fresh and Marine Water Quality, CC BY 4.0. Australian and New Zealand governments and Australian state and territory governments, Canberra, ACT, Australia.

This publication is available at waterquality.gov.au/anz-guidelines/guideline-values/default/water-quality-toxicants/toxicants.

Contact
Australian Government Department of Climate Change, Energy, the Environment and Water
GPO Box 858 Canberra ACT 2601
General enquiries: 1800 920 528
Email waterquality@dcceew.gov.au

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 2 anonymous reviewers and by contracted technical advisors Dr Rick van Dam and Dr Melanie Trenfield.

Contents

Figures

Tables

Appendix Figures

Appendix Tables

Summary

Paraquat (1,1'-dimethyl-4,4'-bipyridinium dichloride; CASRN 1910-42-5) is a broad-spectrum, non-selective contact herbicide. It is commonly used to control weeds for a range of agricultural and non-agricultural purposes (Eisler 1990; NZ MPI 2023; APVMA n.d.). Paraquat may be present in soil following direct application and in surface waters following run-off and accidental release.

Paraquat acts by inhibiting the photosynthetic process in plants, diverting electrons from photosystem I. This results in the production of highly reactive free radicals that generate superoxides, causing lipid peroxidation, membrane damage and rapid death (APVMA 2016).

Moderate reliability default guideline values (DGVs) for paraquat (as the paraquat cation) in freshwater were derived based on 6 acute (converted to chronic) and 4 chronic toxicity values for 10 species from 6 taxonomic groups. There was a good fit of the distribution to the toxicity data. The DGVs for 99%, 95%, 90% and 80% species protection are 0.32 µg/L, 1.2 µg/L, 2.2 µg/L and 4.2 µg/L, respectively. The 95% species-protection level for paraquat (1.2 µg/L) is recommended for adoption in the assessment of slightly to moderately disturbed ecosystems.

1 Introduction

Paraquat is a non-selective contact herbicide belonging to the bipyridinium class of compounds (APVMA 2016). It is one of the most used non-selective contact herbicides and acts by disrupting the photosynthetic process in plants. Application to terrestrial weeds can result in paraquat run-off into surface waters.

In Australia and New Zealand, paraquat has been used extensively to control a wide range of grasses and broad-leaf weeds for agricultural (e.g. in lucerne crops, orchards and vineyards and to desiccate seed crops prior to harvest) and non-agricultural (e.g. alongside roads and paths, around buildings) purposes (NZ MPI 2023; APVMA n.d.). Formulations of paraquat have been available in Australia since the early 1960s. Paraquat was listed as a high-priority chemical for review under Australia's Chemical Review Program, administered by the Australian Pesticides and Veterinary Medicines Authority (APVMA), due to human health and environmental concerns (APVMA 2016). Paraquat was also recently reassessed on a similar basis in New Zealand, with its use now restricted to horticulture, agriculture and some biosecurity purposes. Certain paraquat-based substances can no longer be sold or used in New Zealand (NZ EPA 2020).

Paraquat is most often supplied in the form of a cationic salt, typically paraquat dichloride (1,1'-dimethyl-4,4'-bipyridinium dichloride; $C_{12}H_{14}Cl_2N_2$; CASRN 1910-42-5), which has a molecular weight of 257.2 g/mol and a water solubility of approximately 600 g/L at 20 °C (NCBI 2023). Figure 1 shows its chemical structure. In surface waters, paraquat dichloride will dissociate to the paraquat cation (1,1'-dimethyl-4,4'-bipyridinium; $C_{12}H_{14}N_2$; CASRN 4685-14-7), which has a molecular weight of 186.3 g/mol (APVMA 2016; Sartori and Vidrio 2018). APVMA (2016) reports a log K_{ow} of −4.5 at 20 °C for paraquat, indicating a low potential for bioaccumulation. Paraquat has a half-life of 16 months in soil (Rao and Davidson 1980) and 23 weeks in water (US EPA 1988).

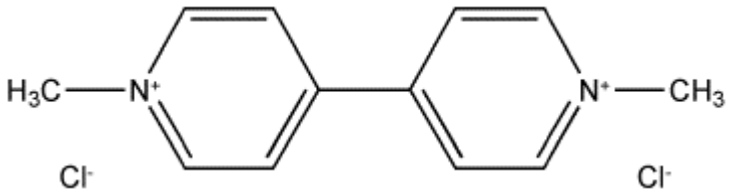

Figure 1 Chemical structure of paraquat dichloride

Paraquat dichloride is the active ingredient in all 132 paraquat products listed for use in Australia (Growcom Australia Pty Ltd n.d.). Paraquat dichloride is also the predominant form of paraquat used in New Zealand (NZ MPI 2023). Therefore, in preparation of this technical brief, only data based on the dichloride salt (CASR # 1910-42-5) were used.

Paraquat applied to terrestrial and aquatic plants is absorbed, while residues photodegrade over time (Zaranyika and Nyoni 2013). Paraquat strongly adsorbs to soil as well as suspended and benthic sediments in water, particularly clay particles (Zaranyika and Nyoni 2013; Huang et al. 2019). This

binding reduces the mobility of the herbicide through leaching. Although paraquat is generally persistent in the environment, it disappears rapidly from the water column due to its strong adsorption to sediment particles (Huang et al. 2019; University of Hertfordshire 2023). This may have implications for the levels of paraquat exposure for sediment-ingesting biota in the hyporheic zone and other sub-surface zones, although such exposures are outside the scope of the current document.

Photodegradation occurs in surface soils and in surface waters, while microbial breakdown also contributes to small amounts of degradation (Eisler 1990; Huang et al. 2019). In aqueous solution, photodegradation is slow under natural environmental conditions without a catalyst (Huang et al. 2019; Moctezuma et al. 1999). Physico-chemical and microbial breakdown products of paraquat include monoquat, monopyridone and unsaturated aminoaldehyde, and associated breakdown products thereof (Zaranyika and Nyoni 2013; Huang et al. 2019). These are typically less toxic than paraquat (Moctezuma et al. 1999).

2 Aquatic toxicology

2.1 Mechanism of toxicity

Paraquat acts by inhibiting photosynthesis (specifically photosystem I). Photosystem I transfers energy from sunlight (captured by chlorophyll in chloroplasts) into a flow of electrons that drives photosynthesis. Paraquat interferes with the activation of photosystem I, diverting the flow of electrons. This results in the production of highly reactive oxygen species (ROS), including superoxide, leading to lipid peroxidation, membrane damage and other oxidative stress-related effects. Plants die rapidly after treatment and exposure to light (APVMA 2016).

Paraquat metabolism in animals is similar and results in the production of the superoxide anion and other ROS, and consequent peroxidation of membrane lipids, sulfhydryl groups, proteins and DNA. This leads to membrane damage and cell death (Eisler 1990; APVMA 2016).

2.2 Toxicity

A literature review on the effects of paraquat on freshwater organisms identified many toxicity studies using formulations that contain paraquat as the active ingredient together with other ingredients. The combined toxicity of these ingredients may not be well understood. The toxicity of paraquat when present in a formulation may be different from paraquat alone. However, only studies that assessed the individual toxicity of paraquat (as paraquat dichloride) are summarised below.

Most of the acceptable data described acute and chronic growth and mortality effects, with a limited number of reproduction and immobilisation studies.

Chronic toxicity data were available for cyanobacteria, green algae, macrophytes, protozoa and crustaceans. Toxicity estimates ranged from 2.5 µg/L to 88,323 µg/L. Effect concentrations for the cyanobacterium *Oscillatoria* cf. *chalybea* ranged from 12 to 14 µg/L (4-day growth IC50s [see 'Glossary and acronyms' for definitions]) (Schrader et al. 1997). For the green alga *Rhapidocelis subcapitata*, effect concentrations ranged from 114 µg/L (4-day growth NOEC) to 559 µg/L (4-day growth LC50) (Fairchild et al. 1997; Schrader et al. 1997). For macrophytes, the lowest reported effect concentration was 2.5 µg/L for *Lemna gibba* (28-d growth LOEC) (Mohammad et al. 2010), while the highest reported effect concentration was 159 µg/L for *L. paucicostata* (7-day growth EC50) (Michel et al. 2004). For the protozoans *Paramecium caudatum* and *P. trichium*, IC50s (5-day growth inhibition) of 697 µg/L and 88,323 µg/L, respectively, have been reported (Miyoshi et al. 2003). For the crustacean *Daphnia magna*, a LOEC (21-day reproduction) of 100 µg/L was reported, which corresponded to an approximate 30% reduction relative to the control response (Ha and Choi 2009).

Acute studies were available for seven species, including a macrophyte, several macroinvertebrates (including 3 crustaceans and an insect), a fish and a frog. The acute LC50/EC50 data ranged from 51 µg/L for the macrophyte *Lemna minor* (4-day growth EC50) (Fairchild et al. 1997) to 1,325 mg/L for the insect *Chironomous riparius* (1-day LC50) (Ha and Choi 2008). Reported 4–5-day EC50s for the zooplanktonic crustaceans *Mesocyclops* sp. and *Mesocyclops aspericornis* were 152 µg/L and 207 µg/L, respectively (Leboulanger et al. 2011), and approximately 1,130 µg/L for *D. magna* (Barata

et al 2005; Ha and Choi 2009). A 24-hour LC50 of 84 µg/L was reported for the fish *Oncorhynchus mykiss* (Martinez-Tabche et al. 2004), and a 5-day LC50 of 138 µg/L was reported for the frog *Xenopus laevis* (Vismara et al. 2000).

The production of ROS associated with the photochemical behaviour of paraquat suggests the potential for genotoxic effects. APVMA (2016) reported that the weight of evidence in the mammalian toxicology literature indicates that paraquat does not pose a genotoxic hazard. Numerous studies have assessed the genotoxicity of paraquat to aquatic organisms via *in-vivo* exposures (compared with *in-vitro* exposure, for which the resulting data are not admissible for the derivation of DGVs) (e.g. Vismara et al. 2001; Martinez-Tabche et al. 2004; Mantecca et al. 2006; Prado et al. 2009; Lacaze et al. 2010). While a number of these studies have reported genotoxic effects of paraquat at exposure concentrations similar to, or even lower than, effects on other endpoints, their link to the manifestation of effects at the whole organism level was not demonstrated and remains unclear.

3 Factors affecting toxicity

A study by Parker (1966) into the toxicity of paraquat using waters of varying hardness indicated that aquatic toxicity may be affected by an increase in cations – in particular, calcium ions. The study found that paraquat toxicity decreased with increased concentration of cations and hypothesised that this may have been due to calcium or other ions interfering with paraquat uptake. Additional studies into the effects of dissolved cations on paraquat toxicity were not found during preparation of the current DGVs. Further investigation into the comparative toxicity of paraquat with varying concentrations of cations would be required to determine if or how to incorporate water hardness into the DGV derivation.

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

Table 1 provides a summary of the toxicity data (one value per species) and conversions used to calculate the DGVs for paraquat in freshwaters. Further details on the data that passed the quality screening and were used to calculate the DGVs are presented in Appendix A. Although some genotoxicity effects data from *in-vivo* exposures were considered in the current DGV derivation (e.g. Vismara et al. 2001; Martinez-Tabche et al. 2004), genotoxicity data were not included in the derivation dataset because the ecological relevance of endpoints such as those measured by the comet assay and micronucleus assay was not demonstrated. If such ecological relevance can be demonstrated in the future, then genotoxicity endpoints should be considered for inclusion in subsequent revisions of the paraquat DGVs.

Paraquat is used almost exclusively as a dichloride salt (WHO 1984). However, the dimethylsulfate form is occasionally used in other countries (Ma 2002; Ma et al. 2002, 2003). Five species of microalgae exposed to pesticide formulations (with < 70% active ingredient) of the dimethylsulfate form were very sensitive, with EC50 values ranging from 0.0013 µg/L for *Scenedesmus quadricauda* to 22.5 µg/L for *Scenedesmus obliquus* (Ma 2002; Ma et al. 2003). Most, if not all, products used in Australia and New Zealand use paraquat dichloride. The percent active ingredient used in the studies identified for paraquat dimethylsulfate was below the minimum requirement for use in derivation of a DGV (purity of < 80% active ingredient), so the DGVs were derived using toxicity data based on paraquat dichloride as the test substance.

The literature review identified data that was of acceptable quality (i.e. the studies that passed quality assessment, did not use a formulation as the test substance, and used paraquat dichloride as the test substance at > 80% purity) for a total of 10 species, consisting of 15 chronic toxicity values for 5 species from 3 taxa, and 8 acute toxicity values for 6 species from 4 taxa.

As noted in section 2.2, many data on the effects of paraquat on freshwater organisms are based on paraquat formulations. The combined toxicity for these formulations is not well understood, may be different to that of the active ingredient alone, and may use the active ingredient with less than 80% purity. Accordingly, such studies are typically not appropriate for inclusion in the derivation of DGVs and so were not used for the derivation of the paraquat DGVs. These studies included numerous species of fish and green algae, in addition to macrophytes, crustaceans and amphibians.

Toxicity data on the effects of paraquat on *C. riparius* (24-hour LC50 of 1,325,000 µg/L) (Ha and Choi 2008), *D. magna* (10 µg/L 21-day reproduction NOEC, 100 µg/L 21-day reproduction LOEC, 1,126 µg/L 1-day immobilisation EC50) (Ha and Choi 2009), *P. caudatum* (697 µg/L 5-day growth IC50, 1008 µg/L 2-day growth IC50) (Miyoshi et al. 2003) and *P. trichium* (50,925 µg/L 2-day growth IC50, 88,323 µg/L 5-day growth IC50) (Miyoshi et al. 2003) were excluded because they were derived from experiments with 10-fold differences between the test concentrations. Additionally, a study by Kuster et al. (2007) on the effects of paraquat on *L. minor* was excluded because the endpoint measured (fluorescence)

is non-standard and of unknown ecological relevance. Toxicity values representing acute NOECs or LOECs were excluded from the DGV derivation because they are unacceptable for the derivation of DGVs (Warne et al. 2018). These included: 5-day NOEC of 62.5 µg/L for survival, 5-day LOEC of 62.5 µg/L for survival and 5-day LOEC of 125 µg/L for growth for the amphibian *X. laevis* (Vismara et al. 2000); 4-day NOEC of 114 µg/L for growth and 4-day LOEC of 227 µg/L for growth for the green alga *R. subcapitata* (Fairchild et al. 1997); 2-day LOEC of 155 µg/L for survival for the crustacean *M. aspericornis* (Leboulanger et al. 2011) and 2-day LOEC of 49 µg/L for survival for the crustacean *Mesocyclops* sp. (Leboulanger et al. 2011).

Where only one toxicity value was available for a species, that value was used for the calculation of 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). In total, 10 species from 6 taxonomic groups (cyanobacteria, green algae, macrophytes, crustaceans, fish, amphibians) were considered for the SSD (Table 1). These species were one cyanobacterium (*Oscillatoria cf. chalybea*), one green alga (*R. subcapitata*), 3 macrophytes (*L. paucicostata*, *L. minor* and *L. gibba*), 3 crustaceans (*D. magna*, *M. aspericornis* and *Mesocyclops* sp.), one fish (*O. mykiss*) and one amphibian (*X. laevis*). The toxicity data for the 10 species are based on 4 chronic exposures (one NOEC, one LOEC, one EC50, one IC50) and 6 acute exposures (4 EC50s, 2 LC50s). The chronic LOEC, IC50 and EC50 were converted to chronic negligible-effect estimates (e.g. NOECs, EC10s) by dividing by default factors of 2.5, 5 and 5, respectively. The acute EC50s and LC50s were converted to chronic negligible-effect estimates based on a default acute-to-chronic ratio of 10 (Warne et al. 2018).

Table 1 Summary of chronic and estimated chronic toxicity data values used to derive the default guideline values for paraquat in freshwater. Estimated chronic values are reported to no more than 3 significant figures.

Taxonomic group	Species	Life stage	Duration (h)	Type (acute/ chronic)	Toxicity measure[a] (test endpoint)	Toxicity value (µg/L)	Estimated chronic value (µg/L)
Cyanobacterium	*Oscillatoria cf. chalybea*	—	96	Chronic	IC50 (growth)	13[b]	2.6[c]
Green alga	*Raphidocelis subcapitata*[d]	—	96	Chronic	NOEC (growth)	114	114[e]
Macrophyte	*Lemna minor*	—	96	Acute	EC50 (growth)	51	5.1[f]
	Lemna gibba	—	672	Chronic	LOEC (growth)	2.5	1[g]
	Lemna paucicostata	—	168	Chronic	EC50 (growth)	159	31.8[c]
Crustacean	*Mesocyclops* sp.	Nauplii	48	Acute	EC50 (mortality)	152	15.2[f]
	Mesocyclops aspericornis	Nauplii	48	Acute	EC50 (mortality)	207	20.7[f]
	Daphnia magna	Neonates	48	Acute	EC50 (immobilisation)	1,250	125[f]
Fish	*Oncorhynchus mykiss*	Juveniles	24	Acute	LC50 (mortality)	84	8.4[f]
Amphibian	*Xenopus laevis*	Embryo	120	Acute	LC50 (mortality)	138	13.8[f]

[a] The measure of toxicity being estimated/determined. IC50/EC50 = median effect concentration. NOEC = no-observed-effect concentration. LOEC = lowest-observed-effect concentration. LC50 = median lethal concentration.
[b] Geometric mean of 4 values (see Appendix A).
[c] Chronic IC50, EC50, LC50 values were converted to chronic NOEC/EC10 values by dividing by 5.
[d] Formerly *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*.
[e] Actual chronic NOEC/EC10.
[f] Acute EC50 and LC50 values were converted to chronic NOEC/EC10 values by dividing by 10.
[g] Chronic LOEC values were converted to chronic NOEC/EC10 values by dividing by 2.5.

Modality checks were performed according to the method stipulated in Warne et al. (2018), with the details of the assessment provided in Appendix B. The weight-of-evidence assessment concluded that the dataset did not exhibit bimodality or multimodality and so supported use of the data for 10 species for derivation of the DGVs.

4.2 Species sensitivity distribution

Figure 2 shows the cumulative frequency (species sensitivity) distribution of the 10 chronic and estimated chronic paraquat freshwater toxicity data reported in Table 1. The model was judged to provide a good (visual) fit to the data.

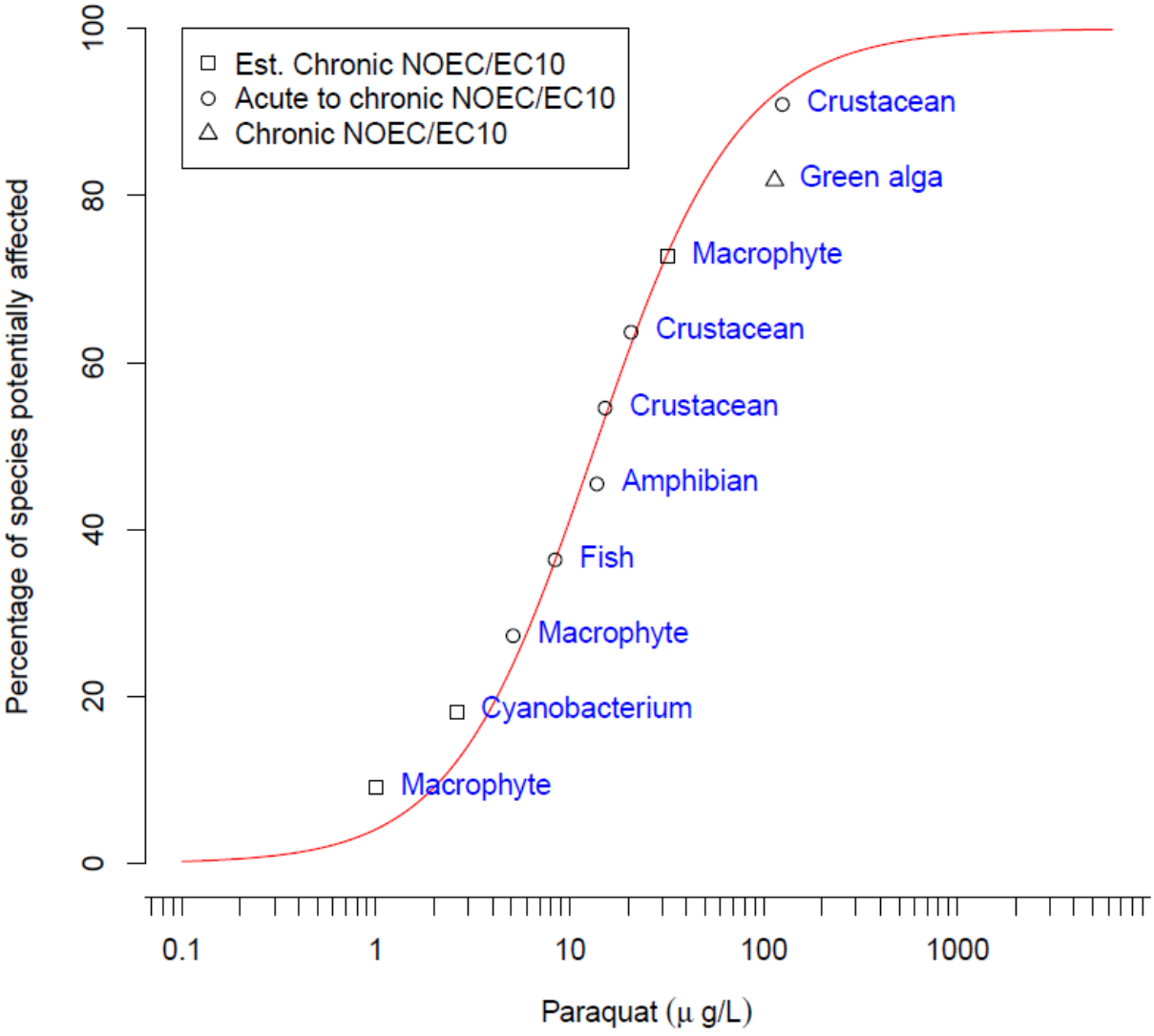

Figure 2 Cumulative frequency distribution (from Burrlioz 2.0) for paraquat freshwater chronic toxicity (chronic NOEC = no-observed-effect concentration; estimated chronic toxicity data)

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* (ANZG 2018).

Table 2 shows the paraquat freshwater DGVs for 99%, 95%, 90% and 80% species protection. The DGVs relate to paraquat (as the paraquat cation) only and not to any of its breakdown products. In situations where paraquat environmental concentrations are approaching the relevant DGV, users are advised to review the available literature on the toxicity of paraquat formulations to determine

whether a formulation-corrected guideline value can and should be derived or whether a formulation-specific guideline value should be derived based on the toxicity of the formulation predominantly used in the area (see Warne et al. 2018).

Table 2 Toxicant default guideline values for paraquat in freshwater, with moderate reliability

Level of species protection (%)	DGV for paraquat in fresh water (µg/L)
99	0.32
95	1.2
90	2.2
80	4.2

The DGVs were compared to the converted chronic and converted acute toxicity data that passed the quality assessment and were compiled from the literature review (i.e. 26 chronic values for 12 species). The theoretical protection offered by the DGVs for 99%, 95%, 90% and 80% species protection is considered to be adequate. Therefore, the 95% species-protection DGV of 1.2 µg/L paraquat is recommended for application to slightly to moderately disturbed ecosystems.

4.4 Reliability classification

The paraquat freshwater DGVs have a moderate reliability classification (Warne et al. 2018) based on the outcomes for the following 3 criteria.

- sample size – 10 (good)
- type of toxicity data – chronic and converted acute
- SSD model fit – good (Burr type III).

Glossary and acronyms

Term	Definition
Acute toxicity	A lethal or adverse sub-lethal effect that occurs as the result of a short (relative to the organism's life span) exposure period to a chemical.
Acute-to-chronic ratio	The species' mean acute value (LC50/EC50) divided by the chronic value (NOEC) for the same species.
APHA	American Public Health Association
APVMA	Australian Pesticides and Veterinary Medicines Authority
ASTM	American Society for Testing and Materials
BC	Bimodality coefficient
BCF (bioconcentration factor)	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 the basis of wet weight, dry weight or lipid weight.
CASRN	Chemical Abstracts Service Registry Number
Chronic toxicity	A lethal or sub-lethal 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.
Endpoint	The specific response of an organism that is measured in a toxicity test (e.g. mortality, growth, a particular biomarker).
Formulation-corrected guideline value	A guideline value derived by adjusting a default guideline value by the difference in toxicity between a specific formulation of the toxicant (e.g. a pesticide formulation) and the toxicant alone (i.e. the active ingredient of the formulation).
Formulation-specific guideline value	A guideline value derived using toxicity data for a specific formulation of the toxicant (e.g. a pesticide formulation) rather than just the toxicant alone (i.e. the active ingredient of the formulation).
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.)
ICx	The concentration of a substance in water or sediment that is estimated to produce an x% inhibition in the response being measured relative to the control (unexposed) response, under specified conditions.
K_{ow}	The ratio of a chemical's solubility in *n*-octanol divided by its solubility in water. It is a measure of the preference for a substance to dissolve in an organic solvent or water and it is used as a measure of lipophilicity and movement of a substance across a cell membrane. It is usually expressed in the logarithmic form (log K_{ow}). It can be used to estimate environmental fate and transport of a chemical.

Term	Definition
LCx	The concentration of a substance in water or sediment that is estimated to be lethal to x% of a group of test organisms, relative to the control response, under specified conditions.
LOEC (lowest-observed-effect concentration)	The lowest concentration of a material used in a toxicity test that has a statistically significant ($p \leq 0.05$) adverse effect on the exposed population of test organisms as compared with the controls. All higher concentrations should also cause statistically significant effects.
NOEC (no-observed-effect concentration)	The highest concentration of a material used in a toxicity test that has no statistically significant ($p > 0.05$) adverse effect on the exposed population of test organisms as compared with the controls. The statistical significance is measured at the 95% confidence interval.
Paraquat	1,1'-dimethyl-4,4'-bipyridinium dichloride
ROC	Reactive oxygen species
Species sensitivity distribution (SSD)	A method that plots the cumulative frequency of species' sensitivity 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.
Sub-lethal	Involving an adverse effect below the level that causes death.
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 A1 Summary of the toxicity data that passed the screening and quality assurance processes for paraquat in freshwater

Phyla/Division	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure[a] (test endpoint)	Test medium	Temp. (°C)	Salinity (µS/cm)	pH	Conc (µg/L)	Reference
Cyanophyta	*Oscillatoria cf. chalybea*	—	96	Chronic	IC50 (growth)	Filtered de-ionised water	26 ± 1	—	—	13.2	Schrader et al. 1997
	Oscillatoria cf. chalybea	—	96	Chronic	IC50 (growth)	Filtered de-ionised water	26 ± 1	—	—	14	Schrader et al. 1997
	Oscillatoria cf. chalybea	—	96	Chronic	IC50 (growth)	Filtered de-ionised water	26 ± 1	—	—	12.9	Schrader et al. 1997
	Oscillatoria cf. chalybea	—	96	Chronic	IC50 (growth)	Filtered de-ionised water	26 ± 1	—	—	12	Schrader et al. 1997
										13[b]	**Geometric mean**
Chlorophyta	*Raphidocelis subcapitata*[c]	—	96	Chronic	NOEC (growth)	ASTM growth media	25	—	—	114[d]	Fairchild et al. 1997
Macrophyte	*Lemna minor*	—	96	Acute	EC50 (growth)	APHA nutrient-enriched water	25	—	—	51[e]	Fairchild et al. 1997
	Lemna gibba	—	672	Chronic	LOEC (growth)	Growth medium	—	—	—	2.5[f]	Mohammad et al. 2010
	Lemna paucicostata	—	168	Chronic	EC50 (growth)	Hoagland media	—	—	—	159[b]	Michel et al. 2004

Phyla/Division	Species	Life stage	Exposure duration (h)	Test type	Toxicity measure[a] (test endpoint)	Test medium	Temp. (°C)	Salinity (µS/cm)	pH	Conc (µg/L)	Reference
Arthropoda	*Mesocyclops* sp.	Nauplii	48	Acute	LC50 (mortality)	Filtered tap water	25	—	—	152[e]	Leboulanger et al. 2011
	Mesocyclops aspericornis	Nauplii	48	Acute	LC50 (mortality)	Filtered tap water	25	—	—	207[e]	Leboulanger et al. 2011
	Daphnia magna	Neonates	48	Acute	EC50 (immobilisation)	M4 media	20 ± 1	—	—	1,250[e]	US EPA 2023
Chordata (fish)	*Oncorhynchus mykiss*	Juvenile	24	Acute	LC50 (mortality)	Reconstituted water	9 ± 1	—	—	84[e]	Martinez-Tabche et al. 2004
Chordata (amphibian)	*Xenopus laevis*	Embryo	120	Acute	LC50 (mortality)	FETAX solution	23 ± 0.5	—	—	138[e]	Vismara et al. 2000

[a] The measure of toxicity being estimated/determined. IC50/EC50 = median effect concentration. NOEC = no-observed-effect concentration. LOEC = lowest-observed-effect concentration. LC50 = median lethal concentration.

[b] Value included in the dataset to derive the default guideline values, after application of a default chronic EC50/LC50 to NOEC/EC10 conversion factor of 5.

[c] Formerly *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*.

[d] Value included in the dataset to derive the default guideline values, as reported.

[e] Value included in the dataset to derive the default guideline values, after application of a default acute-to-chronic conversion factor of 10.

[f] Value included in the dataset to derive the default guideline values, after application of a default chronic LOEC to NOEC/EC10 conversion factor of 2.5.

Appendix B: modality assessment for paraquat

A modality assessment was undertaken for paraquat according to the 4 questions stipulated in Warne et al. (2018). These questions and their answers are listed below.

1) **Is there a specific mode of action that could result in taxa-specific sensitivity?**

Paraquat acts by inhibiting photosynthesis (specifically photosystem I). This generates superoxide, leading to lipid peroxidation and membrane damage, and results in rapid plant death after treatment and exposure to light (APVMA 2016). In animals, paraquat metabolism also results in the production of the superoxide anion and other highly reactive free radicals, with consequent peroxidation of membrane lipids, sulfhydryl groups, proteins and DNA, leading to membrane damage and cell death (Eisler 1990; APVMA 2016). This mode of action does not suggest taxa-specific sensitivity.

2) **Does the dataset suggest bimodality?**

The recommended lines of evidence in evaluating whether the dataset is bimodal or multimodal are visual representation of the data, calculation of the bimodality coefficient (BC), and consideration of the range in the effect concentrations. These are discussed below.

- The histogram of the raw effect-concentration SSD data (Figure B1, left) could be interpreted as positively right-skewed, typical of concentration-based data (Warne et al. 2018). The log-transformed histogram does not show a discernible distribution (Figure B1, right).
- Data that span large ranges (> 4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018). The paraquat data span 2 orders of magnitude.
- A BC > 0.555 indicates the data does not follow a typical normal distribution and may be bimodal. The BC of the log-transformed data is 0.253, indicating that the dataset is not bimodal.

Based on the lines of evidence described above, the distribution of the log-transformed data does not indicate a bimodal distribution.

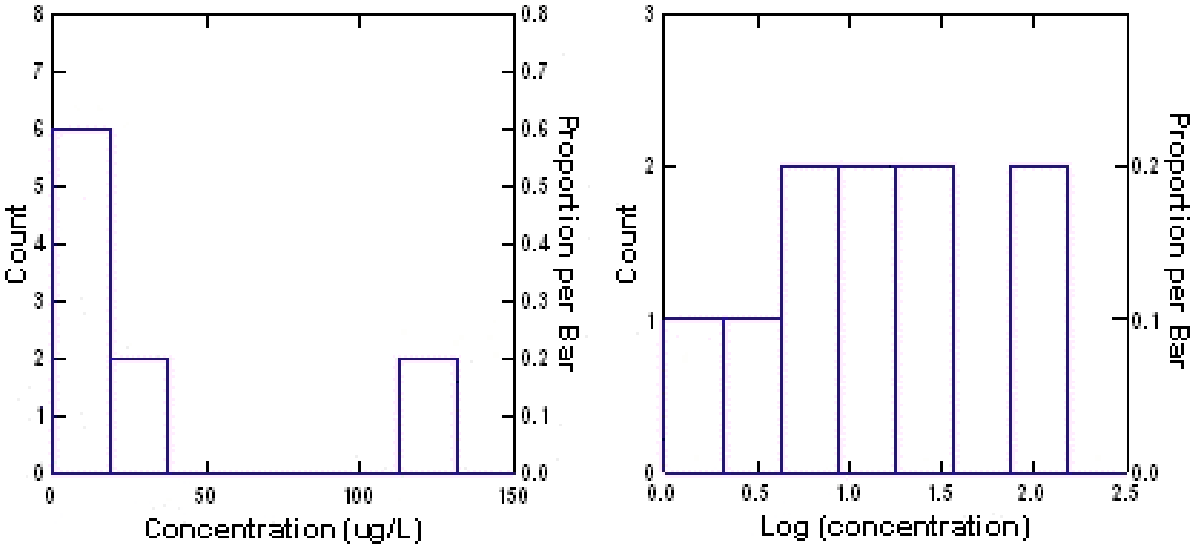

Figure B1 Histogram of raw data (left) and log-transformed data (right)

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

As shown in Figure B2 (data grouped by phylum or clade), data do not appear to show taxa-specific sensitivity. When grouped by phylum or clade, there is a slight trend for Arthropoda (n = 3) and Chlorophyta (n = 1) to be less sensitive to paraquat. However, the sample sizes for other phyla/clades are small, with n = 2 for Chordata, n = 1 for Cyanophyta and n = 3 for Tracheophyta. The trend may be attributable to real differences in the response of these organisms or may be an artefact of the sample size.

Heterotrophs and autotrophs do not appear to have different sensitivities to paraquat (Figure B3).

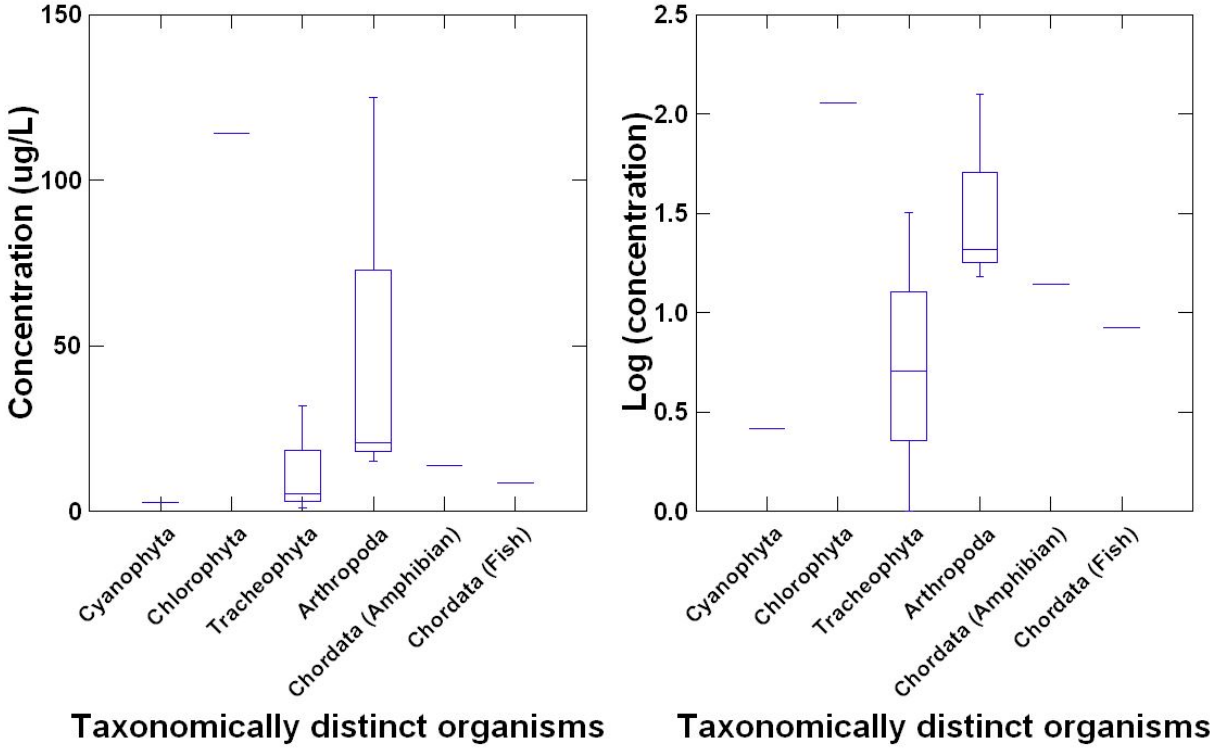

Figure B2 Boxplots of raw (left) and log-transformed (right) data for paraquat toxicity, grouped by phylum or clade

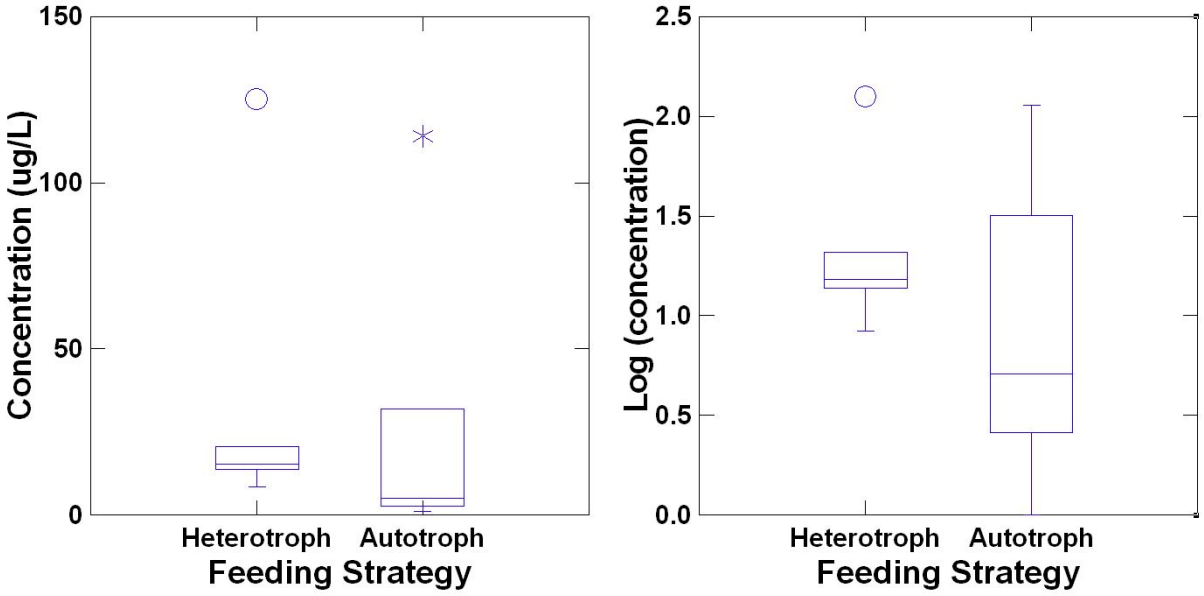

Figure B3 Boxplots of raw (left) and log-transformed (right) data for paraquat toxicity, grouped by feeding strategy as defined in Table 6 of Warne et al. (2018); n = 5 for both heterotrophs and autotrophs

4) 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?

Based on outcomes of questions 1–3, the data are unlikely to be bimodal or multimodal. When grouped by phylum or clade, there is a slight trend for Chordata, Cyanophyta, and Tracheophyta to be more sensitive to paraquat than other taxonomic divisions (Arthropoda, Chlorophyta). However, this may be attributable to small differences in sample groups. The small sample size prevents discerning any trends in the data and whether such trends are artefacts of data selection, test procedures, or other reasons unrelated to a specific mode of action. The weight of evidence supports use of all 10 species identified in preparation of the SSD.

References

ANZG (Australian and New Zealand Guidelines) (2018) *Australian and New Zealand Guidelines for Fresh and Marine Water Quality*, Australian and New Zealand governments and Australian state and territory governments.

APVMA (Australian Pesticides and Veterinary Medicines Authority) (2016) *Review of the mammalian toxicology and metabolism/toxicokinetics of paraquat summary report*, Australian Pesticides and Veterinary Medicines Authority Australia, Chemical Review Program, Department of Health, Canberra.

APVMA (Australian Pesticides and Veterinary Medicines Authority) (n.d.) *Public chemical registration information system search*, APVMA website, accessed 12 November 2019.

Barata C, Varo I, Navarro JC, Arun S and Porte C (2005) 'Antioxidant enzyme activities and lipid peroxidation in the freshwater cladoceran *Daphnia magna* exposed to redox cycling compounds', *Comparative Biochemistry and Physiology Part C: Toxicology and Pharmacology*, 140:175–86, doi:10.1016/j.cca.2005.01.013.

Eisler R (1990) *Paraquat hazards to fish, wildlife and invertebrates: a synoptic review*, US Fish and Wildlife Service Biological Report 85(1.22), Laurel.

Fairchild JG, Ruessler DS, Haverland PS and Carlson AR (1997) 'Comparative sensitivity of *Selenastrum capricornutum* and *Lemna minor* to sixteen herbicides', *Archives of Environmental Contamination and Toxicology,* 32:353–357, doi:10.1007/s002449900196.

Growcom Australia Pty Ltd (n.d.) *Infopest*, Growcom website, accessed 27 April 2020.

Ha MH and Choi J (2008) 'Effects of environmental contaminants on haemoglobin of larvae of aquatic midge, *Chironomus riparius* (Diptera: Chironomidae): a potential biomarker for ecotoxicity monitoring', *Chemosphere*, 71:1928–1936, doi:10.1016/j.chemosphere.2008.01.018.

Ha M-H and Choi J (2009) 'Effects of environmental contaminants on haemoglobin gene expression in *Daphnia magna*: a potential biomarker for freshwater quality monitoring', *Archives of Environmental Contamination and Toxicology*, 57:330–337, doi:10.1007/s00244-007-9079-0.

Huang Y, Bhatt P and Chen S (2019) 'Paraquat degradation from contaminated environments: current achievements and perspectives', *Frontiers in Microbiology*, 10:1754, doi:10.3389/fmicb.2019.01754.

Kuster A, Pohl K and Altenburger R (2007) 'A fluorescence-based bioassay for aquatic macrophytes and its suitability for effect analysis of non-photosystem II inhibitors', *Environmental Science and Pollution Research*, 14:377–383, doi:10.1065/espr2007.04.410.

Lacaze E, Geffard O, Bony S and Devaux A (2010) 'Genotoxicity assessment in the amphipod *Gammarus fossarum* by use of the alkaline Comet assay', *Mutation Research*, 700:32–38, doi:10.1016/j.mrgentox.2010.04.025.

Leboulanger C, Schwartz C, Somville P, Diallo AO and Pagano M (2011) 'Sensitivity of two mesocyclops (Crustacea, Copepoda, Cyclopidae), from tropical and temperate origins, to the

herbicides, diuron and paraquat, and the insecticides temephos and fenitrothion', *Bulletin of Environmental Contamination and Toxicology*, 87:487–493, doi:10.1007/s00128-011-0406-9.

Ma J (2002) 'Differential sensitivity to 30 herbicides among populations of two green algae *Scenedesmus obliquus* and *Chlorella pyrenoidosa*', *Bulletin of Environmental Contamination and Toxicology*, 68:275–281, doi:10.1007/s001280249.

Ma J, Xu L and Wang S (2002) 'A quick, simple and accurate method of screening herbicide activity using green algae cell suspension cultures', *Weed Science*, 50:555–559, doi:10.1614/0043-1745(2002)050[0555:AQSAAM]2.0.CO;2.

Ma J, Xu L, Wang S, Zheng R, Jin S, Huang S and Huang Y (2002) 'Toxicity of 40 herbicides to the green alga *Chlorella vulgaris*', *Ecotoxicology and Environmental Safety*, 51:128–132, doi:10.1006/eesa.2001.2113.

Ma J, Lin F, Wang S and Xu L (2003) 'Toxicity of 21 herbicides to the green alga *Scenedesmus quadricauda*', *Bulletin of Environmental Contamination and Toxicology*, 71:594–601, doi:10.1007/s00128-003-8521-x.

Mantecca P, Vailati G and Bacchetta R (2006) 'Histological changes and micronucleus induction in the zebra mussel *Dreissena polymorpha* after paraquat exposure', *Histology and Histopathology*, 21:829840, doi:10.14670/hh-21.829.

Martinez-Tabche, L, Madrigal-Bujaidar E and Negrete T (2004) 'Genotoxicity and lipoperoxidation produced by paraquat and 2,4-dichlorophenoxyacetic acid in the gills of rainbow trout (*Oncorhynchus mykiss*)', *Bulletin of Environmental Contamination and Toxicology*, 73:146–152, doi:10.1007/s00128-004-0406-0.

Michel A, Johnson RD, Duke SO and Scheffler BE (2004) 'Dose-response relationships between herbicides with different modes of action and growth of *Lemna paucicostata*: an improved ecotoxicological method', *Environmental Toxicology and Chemistry*, 23:1074–1079, doi:10.1897/03-256.

Miyoshi N, Kawano T, Tanaka M, Kadono T, Kosaka T, Kunimoto M, Takahashi T and Hosoya H (2003) 'Use of *Paramecium* species in bioassays for environmental risk management: determination of IC50 values for water pollution', *Journal of Health Science*, 49:429–435, doi:10.1248/jhs.49.429.

Moctezuma E, Leyva E, Monreal E, Villegas N and Infante D (1999) 'Photocatalytic degradation of the herbicide paraquat', *Chemosphere*, 39:511–517, doi:10.1016/S0045-6535(98)00599-2.

Mohammad M, Itoh K and Kuyama K (2010) 'Effects of herbicides on *Lemna gibba* and recovery from damage after prolonged exposure', *Archives of Environmental Contamination and Toxicology*, 58:605–612, doi:10.1007/s00244-010-9466-9.

NCBI (National Center for Biotechnology Information) (2023) *Paraquat dichloride*, NCBI website, accessed 16 January 2023.

NZ EPA (New Zealand Environmental Protection Authority) (2020) *Reassessment of paraquat*, NZ EPA website, accessed 15 January 2020.

NZ MPI (New Zealand Ministry for Primary Industries) (2023) *Agricultural compounds and veterinary medicines register*, NZ MPI website, accessed 15 January 2023.

Parker C (1966) 'Influence of water hardness on the phytotoxicity of paraquat', *Nature*, 5069:1465–1466, doi:10.1038/2121465a0.

Prado R, García R, Rioboo C, Herrero C, Albade J and Cid A (2009) 'Comparison of the sensitivity of different toxicity test endpoints in a microalga exposed to the herbicide paraquat', *Environment International*, 35:240–247, doi:10.1016/j.envint.2008.06.012.

Rao PS and Davidson JM (1980) 'Estimation of pesticide retention and transformation parameters required in nonpoint source pollution models', in Overcash MR and Davidson JM (eds) *Environmental impact of nonpoint source pollution*, Ann Arbor Science, Ann Arbor.

Sartori F and Vidrio E (2018) 'Environmental fate and ecotoxicology of paraquat: a California perspective', *Toxicological and Environmental Chemistry*, 100:479–517, doi:10.1080/02772248.2018.1460369.

Schrader KK, De Regt MQ, Tucker CS and Duke SO (1997) 'A rapid bioassay for selective biocides', *Weed Technology* 11:767–774.

University of Hertfordshire (2023) *The pesticide properties database: paraquat*, Agriculture and Environment Research Unit, University of Hertfordshire, accessed 30 January 2024.

US EPA (United States Environmental Protection Agency) (1988) *Paraquat health advisory*, Office of Drinking Water, US EPA, Washington, DC, accessed 30 January 2024.

US EPA (2023) *ECOTOX knowledgebase. Version 4.0*, US EPA website, accessed 1 February 2024.

Vismara C, Battista V, Vailati G and Bacchetta R (2000) 'Paraquat induced embryotoxicity on *Xenopus laevis* development', *Aquatic Toxicology*, 49:171–179, doi:10.1016/s0166-445x(99)00080-6.

Vismara C, Bacchetta R, Cacciatore B, Vailati G and Fascio U (2001) 'Paraquat embryotoxicity in the *Xenopus laevis* cleavage phase', *Aquatic Toxicology*, 55:85–93, doi:10.1016/s0166-445x(01)00153-9.

Warne MStJ, Batley GE, van Dam RA, Chapman JC, Fox DR, Hickey CW and Stauber JL (2018) *Revised method for deriving Australian and New Zealand water quality guideline values for toxicants*, report prepared for the revision of the Australian and New Zealand Guidelines for Fresh and Marine Water Quality, Australian and New Zealand governments and Australian state and territory governments.

WHO (World Health Organization) (1984) *Paraquat and diquat*, Environmental Health Criteria 39, International Programme on Chemical Safety website, accessed 22 July 2015.

Zaranyika MF and Nyoni S (2013) 'Degradation of paraquat in the aquatic environment: a proposed enzymatic kinetic model that takes into account adsorption/desorption of the herbicide by colloidal and sediment particles', *International Journal of Research in Chemistry and Environment*, 3:26–35.