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Toxicant default guideline values for aquatic ecosystem protection

Mancozeb in freshwater

Technical brief

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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).

Mancozeb (manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salt) is a broad-spectrum fungicide used on food and ornamental crops such as fruit, tobacco, turf, and vegetables. Application is aimed at preventing crop damage in the field and protecting harvested crops from deteriorating during storage or transport (USEPA 2005a, 2005b, DOW 2010). Mancozeb belongs to the dithiocarbamate group of fungicides and to the class of compounds known as ethylene bisdithiocarbamates (EBDCs), which share the common metabolite and environmental degradate ethylenethiourea (ETU) (USEPA 2005a, 2005b). In water, mancozeb rapidly hydrolyses to the 'mancozeb complex', which includes ETU (USEPA 2005c, Ruhman et al. 2011). Mobility of the mancozeb complex is limited because of its strong tendency to adsorb to soil and sediment. ETU is more mobile than the mancozeb complex, and has high water solubility. In mammals, ETU affects the thyroid and can cause developmental toxicity and carcinomas (EC 2005, USEPA 2005d, Gullino et al. 2010). ETU may also be a potential endocrine disruptor, but it appears to have lower acute toxicity than mancozeb to aquatic organisms (WHO 1998, USEPA 2005d, Ruhman et al. 2011).

In acute toxicity studies on aquatic organisms, mancozeb has been two to three orders of magnitude more toxic than ETU (WHO 1998, Ruhman et al. 2011). In longer-term studies on fish, mancozeb exposure has produced physical abnormalities such as spinal curvature and notochord distortion, as well as haematological changes (Atamanalp & Yanik 2003, Tilton et al. 2006, Ruhman et al. 2011). In aquatic invertebrates, mancozeb exposure can affect growth and time to first brood (WHO 1998, Ruhman et al. 2011).

Given its rapid hydrolysis, concentrations of mancozeb in water are either reported as nominal concentrations, or are based on the concentration of the indicator chemical—carbon disulfide (CS_2). CS_2 is released from dithiocarbamate compounds during chemical analysis, specifically following acid digestion. A factor is then applied to convert the CS_2 concentration to an equivalent mancozeb concentration.

Moderate reliability default guideline values (DGVs) for mancozeb in freshwater were derived based on chronic toxicity values for eight species from four taxonomic groups, with a poor fit of the species sensitivity distribution to the toxicity data. The DGVs for 99%, 95%, 90% and 80% species protection are 0.1 µg/L, 1.2 µg/L, 3.6 µg/L, and 11 µg/L, respectively. The 95% species protection level for mancozeb is recommended for adoption in the assessment of slightly-to-moderately disturbed ecosystems. Given that current routine chemical analyses cannot discriminate between the various dithiocarbamate or EBDC fungicides, it is recommended that the concentration of ETU (which represents the sum of the EBDC fungicides) in a water sample is measured and compared with the ETU concentration equivalent to the relevant mancozeb DGV (i.e. the mancozeb equivalent ETU DGV). The mancozeb equivalent ETU DGVs for 99%, 95%, 90% and 80% species protection are 0.008 µg/L, 0.09 µg/L, 0.27 µg/L and 0.83 µg/L, respectively. Given the analytical challenges associated with applying the mancozeb DGVs, they should be used with caution and always in conjunction with other lines of evidence.

1   Introduction

Mancozeb (manganese ethylenebis (dithiocarbamate) polymeric complex with zinc salt, CASRN 8018-01-7) (USEPA 2005a) is a broad-spectrum fungicide used on food and ornamental crops such as fruit, tobacco, turf and vegetables to prevent crop damage in the field and to protect harvested crops from deteriorating during storage or transport (USEPA 2005a, 2005b, DOW 2010). Globally, mancozeb has been used since the 1940s (USEPA 2005a), and has been registered for use in over 100 countries, on over 70 different crops, to treat more than 400 diseases (USEPA 2005b, Leader et al. 2009, Gullino et al. 2010). In 2005, an estimated 2.5 million kilograms of mancozeb were used annually in the United States, with the greatest use on potatoes and apples (USEPA 2005a, 2005b). In Australia and New Zealand, mancozeb is registered for use as a single active constituent, or combined with other actives, in a large number of products for the control of fungal and bacterial diseases such as leaf spot, leaf speckle, botrytis, blue mould, brown spot, rust, blights, bacterial specks and downy mildew (APVMA 2015, ACVM 2019).

Mancozeb belongs to the dithiocarbamate grouping of fungicides, specifically to the class of ethylene bisdithiocarbamate (EBDC) compounds. Related compounds in the dithiocarbamate grouping include maneb, metiram, nabam, propineb, thiram, zineb and ziram (USEPA 2005b, FRAC 2019), while the EBDC class is limited to mancozeb, maneb, metiram, nabam and zineb (Lentza-Rizos 1990). The EBDCs share the common degradation product ethylenethiourea (ETU) (Lentza-Rizos 1990, USEPA 2005a, 2005b, López-Fernández et al. 2016). Mancozeb itself is not a fungicide; rather, it is a pro-fungicide. When mancozeb is exposed to water it rapidly breaks down to release ETU, ethyleneurea (EU) and ethylene bisisothiocyanate sulfide (EBIS) (López-Fernández et al. 2016). EBIS, in turn, is converted via photolysis into ethylene bisisothiocyanate (EBI) (Xu 2000, Gullino et al. 2010). The nature and abundance of the degradation products of mancozeb are largely dependent on temperature, light and pH (López-Fernández et al. 2016). López-Fernández et al. (2016) found that, under 'typical' water quality conditions of pH 8, 25°C and the presence of light, the per cent conversion of mancozeb to ETU was 17.5% over 72 hours, but that under warmer conditions (e.g. 30°C) this could increase to 40–60%. Notably, ETU is used in the production of rubber and also has other industrial uses; hence, it can enter the environment via multiple sources (National Toxicology Program 2016). However, the dominant source of ETU, particularly in agricultural areas and large areas of manicured green space, is via the use of EBDC fungicides.

The hydrolytic decomposition of mancozeb appears to involve the initial formation of polymer fragments, monomeric species and EBDC ligands in association with metal ions (USEPA 2005c). The final product of the hydrolytic decomposition of mancozeb is the multi-species complex termed the 'mancozeb complex', which consists of transient species, degradates (including ETU) and other unidentified materials (USEPA 2005c, Ruhman et al. 2011). The mancozeb complex has a molecular formula $(C_4H_6MnN_2S_4)x(Zn)y$, and contains 20% manganese and 2.5% zinc. The molecular weights of the polymer and monomer are 541 and 271.3, respectively (EC 2005). The chemical structure of mancozeb is usually represented by one unit of the polymer: a monomeric Mn^{2+} and Zn^{2+} EBDC. The structure of the polymer is shown in Figure 1.
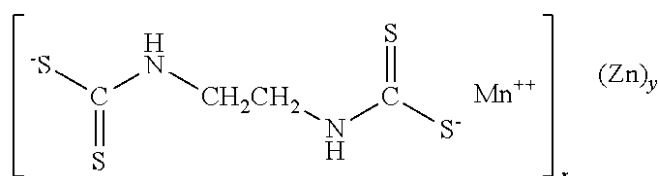
x:y = 1:0.091

Figure 1 Structure of mancozeb

Mancozeb has low water solubility (ranging from 2 mg/L to 20 mg/L) (USEPA 2005c, EC 2005, NCBI 2020) but hydrolyses rapidly in water (reported half-lives of 0.7 days at pH 7 and 1.4 days at pH 9) (USEPA 2005c). In contrast to the rapid hydrolysis, mancozeb is photolytically stable and is not expected to volatilise from water and/or dry/moist soil surfaces based on a low vapour pressure (1.33×10^{-10} atm) and a calculated Henry's Law constant of 5.5×10^{-9} atm m^3/mol (USEPA 2005c, EC 2005, NCBI 2020).

Mancozeb has a low potential to bioaccumulate in aquatic organisms, with a log K_{ow} of 1.33 (USEPA 2005c, EC 2005, NCBI 2020). A bioconcentration factor (BCF) of 4 L/kg (log BCF 0.6) has been estimated for fish (NCBI 2020). No measured BCF data, and few accumulation studies, were found during the preparation of this default guideline value (DGV). Regulatory registration, assessment and review documents on mancozeb have typically been silent on, or waived the need for, bioaccumulation studies on mancozeb (USEPA 2005a, EC 2005). A study on the uptake of mancozeb by soft-shell clams (*Mya arenaria*) reported rapid (<24 h) initial accumulation of manganese in the mantle, which decreased over the duration of the exposure (Pariseau et al. 2009).

As the dithiocarbamates, including mancozeb, are not stable, they are difficult to extract or analyse directly. Consequently, chemical analyses are typically based on the concentration of the hydrolysis product—carbon disulfide (CS$_2$)—or, for EBDCs, the degradate—ETU. Consequently, analysis of CS$_2$ or ETU will measure the collective concentration of dithiocarbamates and EBDCs, respectively, rather than just mancozeb. This has implications for the assessment of environmental monitoring data against the mancozeb DGVs, which are addressed in Section 4.3.

Mancozeb may be released to the environment through its use as a fungicide and subsequent releases to soil, and runoff and spray drift from land to surface water (Ruhman et al. 2011). Given the rapid hydrolysis of mancozeb, concentrations in aquatic environments are very low, and the main stressor is expected to be the mancozeb complex, including ETU and other degradates, as well as manganese and zinc (USEPA 2005c, Lopez-Fernandez et al. 2016). A study on mancozeb use in banana plantations did not find detectable concentrations of ETU in groundwater, but reported surface water concentrations up to 22.5 µg/L (Geissen et al. 2010). Another study monitored mancozeb spray drift residue over surface water (using drift card analysis). The drift cards reported concentrations of mancozeb between 0.5 µg/cm^2 and 4.0 µg/cm^2, although these concentrations were not used to predict a surface water concentration (Mortensen et al. 1998). A pesticide risk assessment for agricultural lands of the Great Barrier Reef catchment area estimated that mancozeb was of '*very low*' risk of mobility to groundwater for six crops assessed (beans, tomatoes, mangoes, melons, cucumbers and capsicums), and a '*very low*' to '*medium*' risk of mobility to surface water (Lewis & Glendenning 2009). Concentrations of the mancozeb complex in surface water near

agricultural fields have been estimated by USEPA (2005a) to range from 2.2 µg/L to 7.3 µg/L (60 day average) to 4.3 µg/L to 16.7 µg/L (21 day average), with peak concentrations up to 210.8 µg/L.

The mancozeb complex has a high potential for adsorption to soil and sediment (reported log K_{oc} values in the range of 2.93 L/kg to 3.21 L/kg), followed by limited biotic degradation (USEPA 2005c, 2005d, Ruhman et al. 2011). Estimated half-lives for the mancozeb complex in river water/sediment are 111 days and in pond/water sediment are 391 days (USEPA 2005c). As a result, there can be slow and continuous release of transient species and degradates of mancozeb, including ETU, at low concentrations (Ruhman et al. 2011). Mobility of the mancozeb complex is expected to be limited because of its strong potential to adsorb. The degradate ETU is more mobile, with high water solubility (20 000 mg/L) and lower potential to adsorb (reported log K_{oc} values range from 1.53 L/kg to 2.93 L/kg) (USEPA 2005d). ETU does not readily hydrolyse or photolyse in direct sunlight. However, indirect photolysis is considered to be a major degradation pathway in natural waters (USEPA 2005d). ETU is volatile (reported vapour pressures of $1.28\text{x}10^{-3}$ atm and $6.59\text{x}10^{-6}$ atm) (USEPA 2005d) and is not expected to bioaccumulate, with a log K_{ow} value of -0.66 (NCBI 2020).

2 Aquatic toxicology

2.1 Mechanism of toxicity

Mancozeb acts as a contact fungicide (non-systemic) that disrupts cellular metabolism at several sites in the fungal cell (USEPA 2005a, FRAC 2019). The mode of action of mancozeb is via the mancozeb complex and associated degradates, and it involves interference with enzymes containing sulfhydryl groups (Gullino et al. 2010). This disruption of enzymatic processes is thought to inhibit or interfere with multiple biochemical processes within the fungal cell cytoplasm and mitochondria (FRAC 2019).

2.2 Toxicity

A literature review (and associated data quality assessment) of the effects of mancozeb on freshwater organisms identified toxicity data for 20 species, consisting of 31 chronic toxicity values for 14 species, and 17 acute toxicity values for 10 species.

The toxicity of mancozeb to aquatic organisms is thought to be due to the chemical species produced by the hydrolysis of mancozeb: the mancozeb complex (Ruhman et al. 2011). In the environment, the mancozeb complex will change over time, as more ETU and ETU degradates are formed. Thus, the toxicity of mancozeb may also change over time.

Effects on fish following short-term exposure to mancozeb include effects on thyroid hormones reported in the zebrafish (*Danio rerio*) (Thienpont et al. 2011). In longer-term studies on fish, physical abnormalities have been reported, including spinal curvature in fathead minnow (*Pimephales promelas*) (Ruhman et al. 2011) and notochord distortion in zebrafish (Tilton et al. 2006), as well as haematological changes in rainbow trout (Atamanalp & Yanik 2003). In invertebrates, mancozeb is acutely toxic and affects growth and time to first brood (WHO 1998, Ruhman et al. 2011).

In contrast, ETU is less acutely toxic than mancozeb, with LC50 values in fish reported two to three orders of magnitude higher than for mancozeb (WHO 1998, Ruhman et al. 2011). In mammals, the

thyroid is a target organ for ETU, where decreases in thyroxine (T4) and increases in thyroid-stimulating hormone (TSH) have been observed in rodents (EC 2005, USEPA 2005d, Gullino et al. 2010). Developmental toxicity and effects on the liver have also been observed, and, at high exposure concentrations, ETU has been linked with thyroid adenomas and carcinomas in rats (USEPA 2005d, Gullino et al. 2010). Based on the available effects data in mammals, ETU may be an endocrine disruptor (USEPA 2005d).

Acute toxicity data for mancozeb are predominantly based on survival, whereas most of the chronic toxicity data are based on growth and reproduction endpoints. Chronic toxicity values ranged from 1.35 µg/L (for fathead minnow) to 382 113 µg/L (for red-eyed tree frog). Acute toxicity values ranged from 200 µg/L (for northern leopard frog) to 24 000 µg/L (for carp). Chronic effects in fish, frogs and cladocerans were among the most sensitive effects reported and included: a NOEC of 1.35 µg/L for the fathead minnow *P. promelas* (215 d reproduction) (USEPA OPP 2019); a NOEC of 7 µg/L for the cladoceran *Daphnia magna* (21 d reproduction) (USEPA OPP 2019); a chronic LOEC of 16 µg/L for the northern leopard frog *Rana pipiens* (49 d growth) (Shenoy et al. 2009); and an LC50 of 23 µg/L for the green frog *Rana clamitans* (13 d survival) (Harris et al. 1998). The least sensitive species was the red-eyed tree frog *Agalychnis callidryas*, with an LC50 of 382 113 µg/L (8 d tadpole survival) (Ghose et al. 2014). However, as the concentration was greater than two times the solubility (2–20 mg/L) of mancozeb, the study was excluded from the current DGVs derivation.

Toxicity studies that used formulations containing mancozeb as the active ingredient included a carrier solvent and, in some cases, other proprietary ingredients, for which the combined toxicity is not well understood. Accordingly, such studies are typically not appropriate to include in the derivation of guideline values for active ingredients. These studies included fish, frogs, a toad, invertebrates and plants (green algae and cyanobacteria).

3   Factors affecting toxicity

Physico-chemical variables such as light, temperature and pH affect the conversion of mancozeb to ETU and other degradation products (López-Fernández et al. 2016, 2017). López-Fernández et al. (2017) found that mancozeb degradation was fast under all the conditions they studied, but was faster at lower pH, higher temperature and in the presence of light. As mancozeb is thought to be more toxic to aquatic biota than ETU, it could be hypothesised that the above-mentioned environmental conditions would reduce the toxicity of mancozeb to freshwater biota, although no studies have assessed this. Moreover, other environmental and physico-chemical factors may limit the degradation of mancozeb (e.g. via adsorption to sediment (USEPA 2005c, 2005d, Ruhman et al. 2011)).

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 mancozeb in freshwater is provided in Table 1. Further details on the data that passed the screening and quality assurance schemes, including those used to derive the single species values used to calculate the DGVs, are presented in Appendix A: Toxicity data that passed the screening and quality assessment and were used to derive the default guideline values. Details of the data quality assessment and all the data that passed the quality assessment are provided as supporting information.

Only data for mancozeb were used. Results from toxicity testing using insecticide formulations containing mancozeb as the active ingredient were excluded from the DGV because the toxicity of the carrier solvent (and other ingredients where stated) was not known. Results from studies where the purity was not known or was less than 80% were excluded. For example, chronic toxicity data for two frog species, the green frog (mortality LC50 of 200 µg/L after 13 d exposure) and the northern leopard frog (growth LOEC of 16 µg/L after 49 d exposure), were excluded from the DGV derivation because the test chemicals used were formulations (Harris et al. 1998, Shenoy et al. 2009). Acute toxicity data for five species were excluded because of testing using formulations.

The purity of mancozeb in the studies used to calculate the DGVs was greater than 80%. Where only one toxicity value was available for a species, that value was used to calculate 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). Overall, mancozeb toxicity data for eight species from four taxonomic groups were considered for the SSD. These species included: five microalgae (*Chlorella pyrenoidosa*, *Chlorella vulgaris*, *Rhapidocelis subcapitata*, *Scenedesmus quadricauda* and *Scenedesmus obliquus*), one crustacean (*D. magna*), one insect (*Chironomus dilutus*), and one fish (*P. promelas*). Of the toxicity data used for the eight species, seven were NOEC values, and one was a LOEC value. All data were from chronic exposures. The 10 d LOEC for *C. dilutus* was re-classified as chronic (rather than acute as recommended for macroinvertebrates in Warne et al. (2018)) after review of the species' life cycle. As *C. dilutus* completes its life cycle in 23–30 d (Benoit et al. 1997), a 10 d test duration represents ≥33% of this species' life span, which is sufficient to classify the test as a chronic exposure. The chronic LOEC value was converted to a negligible effect (e.g. EC10, NOEC) equivalent using the default value of 2.5.

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 mancozeb. The weight of evidence assessment concluded that the dataset did not exhibit bimodality or multimodality and, hence, supported use of the data for eight species for the DGV derivation.

Table 1 Summary of single chronic toxicity values, all species used to derive the default guideline values for mancozeb in freshwater

Taxonomic group	Species	Life stage	Duration (h)	Toxicity measure [a]	Toxicity value (µg/L)	Estimated chronic value (µg/L)
Microalga	*Chlorella pyrenoidosa*	–	96	NOEC	20	20 [b]
	Chlorella vulgaris	–	96	NOEC	100	100 [b]
	Scenedesmus quadricauda	–	96	NOEC	100	100 [b]
	Rhapidocelis subcapitata [d]	–	96	NOEC	100	100 [b]
	Scenedesmus obliquus	–	96	NOEC	500	500 [b]
Crustacean	*Daphnia magna*	Neonate	504	NOEC	7	7 [b]
Insect	*Chironomus dilutus*	Larvae	240	LOEC	5 250	2 100 [c]
Fish	*Pimephales promelas*	Larvae	5 160	NOEC	1.35	1.35 [b]

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

[a] The measure of toxicity being estimated/determined: NOEC: no observed effect concentration; LOEC: lowest observed effect concentration.

[b] Actual chronic NOEC.

[c] Default conversion from chronic LOEC to chronic negligible effect concentration (i.e. chronic LOEC ÷ 2.5).

[d] Formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*.

4.2   Species sensitivity distribution

The cumulative frequency (species sensitivity) distribution (SSD) of the eight chronic mancozeb toxicity values reported in Table 1, is shown in Figure 2. The model was judged to provide a poor fit to the data (Figure 2).
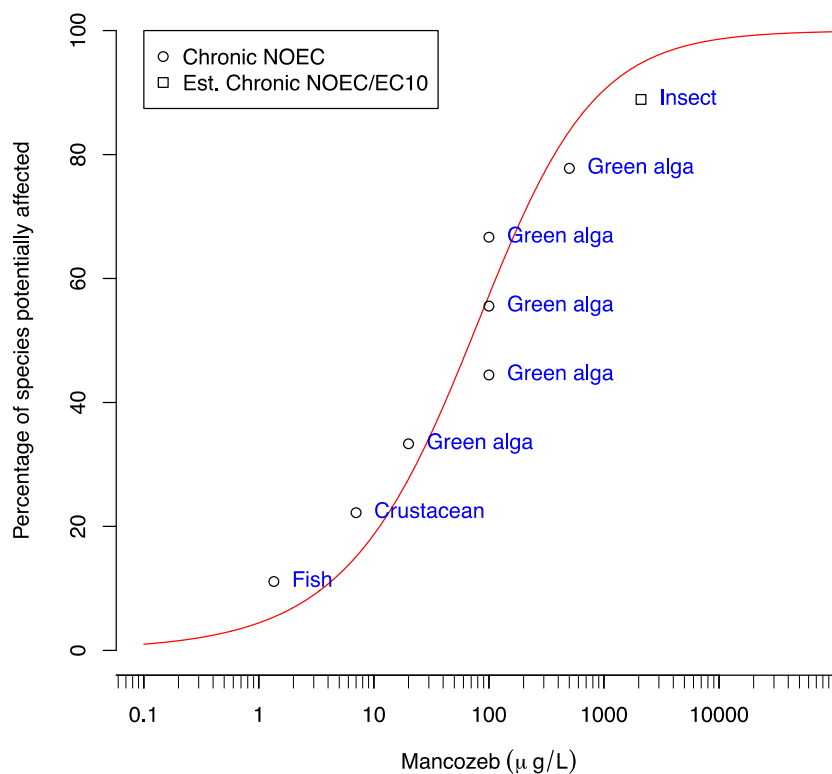
Figure 2 Species sensitivity distribution, mancozeb in freshwater

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 mancozeb DGVs for 99%, 95%, 90% and 80% species protection are shown in Table 2. The 95% species protection DGV of 1.2 µg/L is recommended for application to slightly-to-moderately disturbed ecosystems. However, given that current routine chemical analyses cannot discriminate between the various dithiocarbamate or EBDC fungicides, it is recommended that the concentration of ETU (which represents the sum of the EBDC fungicides) in the sample is measured and compared with the ETU concentration equivalent to the relevant mancozeb DGV (i.e. the mancozeb equivalent ETU DGV, see below). Thus, if the mancozeb equivalent ETU DGV is exceeded, further investigation would be required, which could include a more complex analysis specific to mancozeb (if available), as well as an assessment of the EBDC fungicides used in the catchment.

A mancozeb equivalent ETU concentration can be calculated by:

- accounting for the 2:1 molar ratio between ETU and mancozeb, and

- accounting for the compounds' respective molecular weights of 541 and 102, and

- assuming a conversion factor for mancozeb to ETU of 20% (based on López-Fernández et al. (2016)). Although the conversion of mancozeb may be higher than 20% under certain water quality conditions (Section 1), assuming a 20% conversion should be conservative for an initial assessment of mancozeb in freshwater environments.

This entails dividing the mancozeb DGVs by $(541/(102*2))/0.2 = 13.25$. Thus, the equivalent ETU concentrations for the 99%, 95%, 90% and 80% mancozeb DGVs are 0.008 µg/L, 0.09 µg/L, 0.27 µg/L and 0.83 µg/L, respectively. Analytical detection limits for ETU should allow reliable measurement of these concentrations, bar the 99% species protection value.

Carbon disulfide (CS_2) could also be used as an indicator compound for mancozeb, although it represents the sum of all the dithiocarbamate compounds and is reported as total dithiocarbamates. Also, analytical detection limits may not reliably measure CS_2 concentrations equivalent to the 99%, 95% and 90% species protection DGVs for mancozeb.

Given the analytical challenges, there is significant uncertainty associated with applying the mancozeb DGVs; as such, they should be used with caution and always in conjunction with other lines of evidence.

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

Level of species protection (%)	DGV for mancozeb in freshwater (µg/L)
99	0.1
95	1.2
90	3.6
80	11

The DGVs were compared to the raw chronic toxicity data compiled from the literature review (i.e. 31 chronic toxicity values for 14 species). This check confirmed that the theoretical protection offered by the DGVs is expected to be adequate.

4.4 Reliability classification

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

- Sample size—eight (good)

- Type of toxicity data—chronic data

- SSD model fit—poor (Burr Type III model).

Notwithstanding the moderate reliability classification, the analytical challenges associated with applying the mancozeb DGVs (Section 4.3) mean that they should always be used in conjunction with other lines of evidence.

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.
atm	Atmosphere.
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.
CS_2	Carbon disulfide.
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'.
EBDC	Ethylene bisdithiocarbamate.
EBIS	Ethylene bisisothiocyanate sulfide.
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.
ETU	Ethylenethiourea.
EU	Ethyleneurea.
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}	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.
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.

Term	Definition
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.
T4	Thyroxine.
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, mancozeb in freshwater

Taxonomic group	Species	Life stage	Exposure duration (h)	Toxicity measure [a] (test endpoint)	Test medium	Temperature (°C)	Salinity (‰)	pH	Concentration (µg/L) [b]	Reference
Microalgae	*Chlorella pyrenoidosa*	–	96	NOEC (Growth)	HB4 medium	25	–	–	20	Ma et al. 2007
	Chlorella vulgaris	–	96	NOEC (Growth)	HB4 medium	25	–	–	100	Ma et al. 2007
	Scenedesmus quadricauda	–	96	NOEC (Growth)	HB4 medium	25	–	–	100	Ma et al. 2007
	Rhapidocelis subcapitata [d]	–	96	NOEC (Growth)	HB4 medium	25	–	–	100	Ma et al. 2007
	Scenedesmus obliquus	–	96	NOEC (Growth)	HB4 medium	25	–	–	500	Ma et al. 2007
Crustacean	*Daphnia magna*	Neonate	504	NOEC (Reproduction)	–	–	–	–	7	USEPA OPP 2019
Insect	*Chironomus dilutus*	Larvae	240	LOEC (Growth)	–	–	–	–	5 250 [c]	USEPA OPP 2019
Fish	*Pimephales promelas*	Larvae	5 160	NOEC (Reproduction)	–	–	–	–	1.35	USEPA OPP 2019

a The measure of toxicity being estimated/determined: NOEC: no observed effect concentration; LOEC: lowest observed effect concentration.

b Value was used as is for the DGV derivation, unless otherwise specified.

c Value was divided by default conversion factor of 2.5 to estimate the chronic negligible effect (NOEC/EC10) value for use in the DGV derivation.

d Formerly known as *Selenastrum capricornutum* and *Pseudokirchneriella subcapitata*.

Appendix B: Modality assessment for mancozeb

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

Is there a specific mode of action that could result in taxa-specific sensitivity?
As discussed in Section 1 and Section 2, the 'mancozeb complex' acts as a contact fungicide (non-systemic) that disrupts cellular metabolism at several sites in the fungal cell. The mode of action of mancozeb is interference with enzymes containing sulfhydryl groups. This disruption of enzymatic processes is thought to inhibit or interfere with multiple biochemical processes within the fungal cell cytoplasm and mitochondria. Taxa-specific sensitivity to mancozeb is not expected because the metabolic systems that mancozeb affects are present in animals and plants. Therefore, both plant and animal data were considered in the DGV derivation.

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

- The histogram of the raw effect concentration SSD data and the log transformed histogram generally follow a normal to slightly right-skewed distribution (Figure B 1).

- Data that span large ranges (>4 orders of magnitude) indicate potential for underlying bimodality or multimodality (Warne et al. 2018); the mancozeb data span three orders of magnitude.

- When the BC is greater than 0.555, it indicates that the data do not follow a normal distribution and may be bimodal. The BC of the log transformed data is 0.22, which does not support an assertion of bimodality.

Based on these lines of evidence, the log transformed dataset is generally in accordance with a unimodal normal distribution.
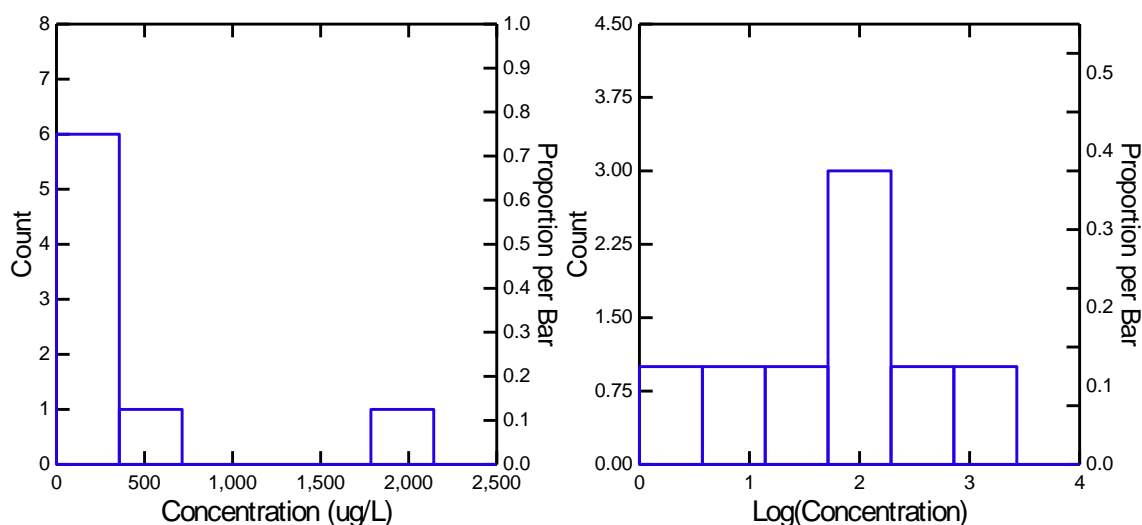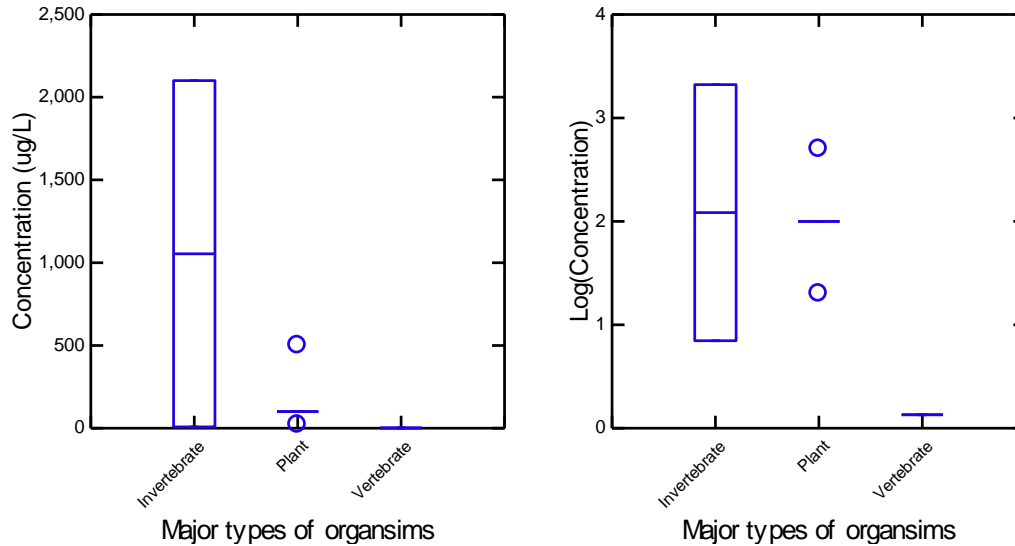

Figure B 1 Histogram, raw (left) and log transformed (right) data

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Do data show taxa-specific sensitivity (i.e. through distinct groupings of different taxa types)?
The potential for taxa-specific sensitivity in the data was examined using box plots of the SSD data grouped by major types of organisms (e.g. plants, vertebrates) (Figure B 2).

As shown in Figure B 2, the available data suggest there might be a difference between vertebrates (n=1), invertebrates (n=2) and plants (n=5), with vertebrates more sensitive than invertebrates and plants. However, the sample size is too small to conclude this with confidence.
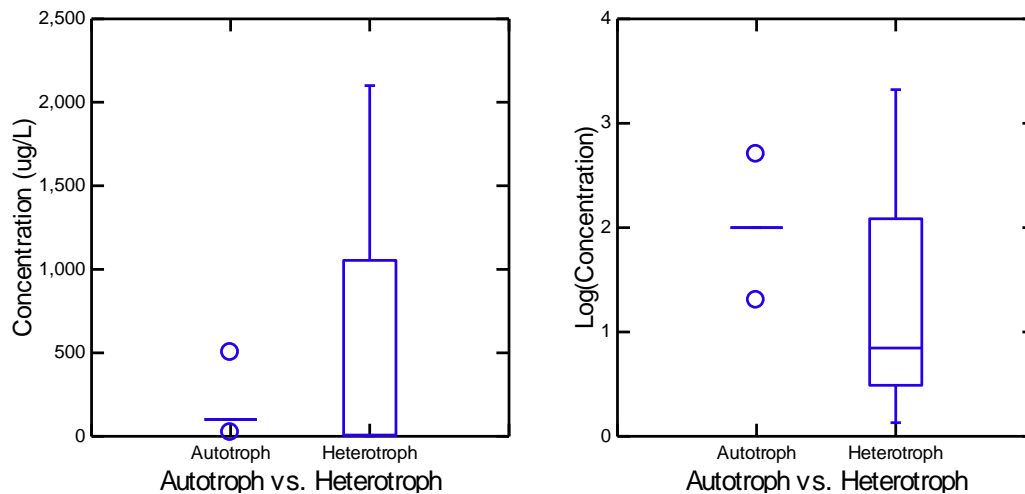

Note: an open circle represents an outlying value >3x the interquartile range.

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

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

As shown in Figure B 3, the autotroph (n=5) and heterotroph (n=3) groupings appear to differ in sensitivity. The median for each grouping indicates difference in effect for animals and plants, with animals more sensitive, and the span in the animal data sensitivity is large compared to plants. However, the sample size is too small to conclude this with confidence.

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

Figure B 3 Box plots, raw (left) and log transformed (right) data grouped by autotrophic and heterotrophic organisms

Is it likely that indications of bimodality or multimodality or distinct clustering of taxa groups are not due to artefacts of data selection, small sample size, test procedures, or other reasons unrelated to a specific mode of action?

The data appear to not show signs of bimodality or multimodality. The suggestion of bimodality from the comparison of major types of organisms in Figure B 2 is potentially due to artefacts associated with the small sample size of the dataset. Thus, on the basis of the available evidence, the dataset appears to be unimodal.

The weight of evidence supports use of the eight species identified in preparation of the SSD.

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