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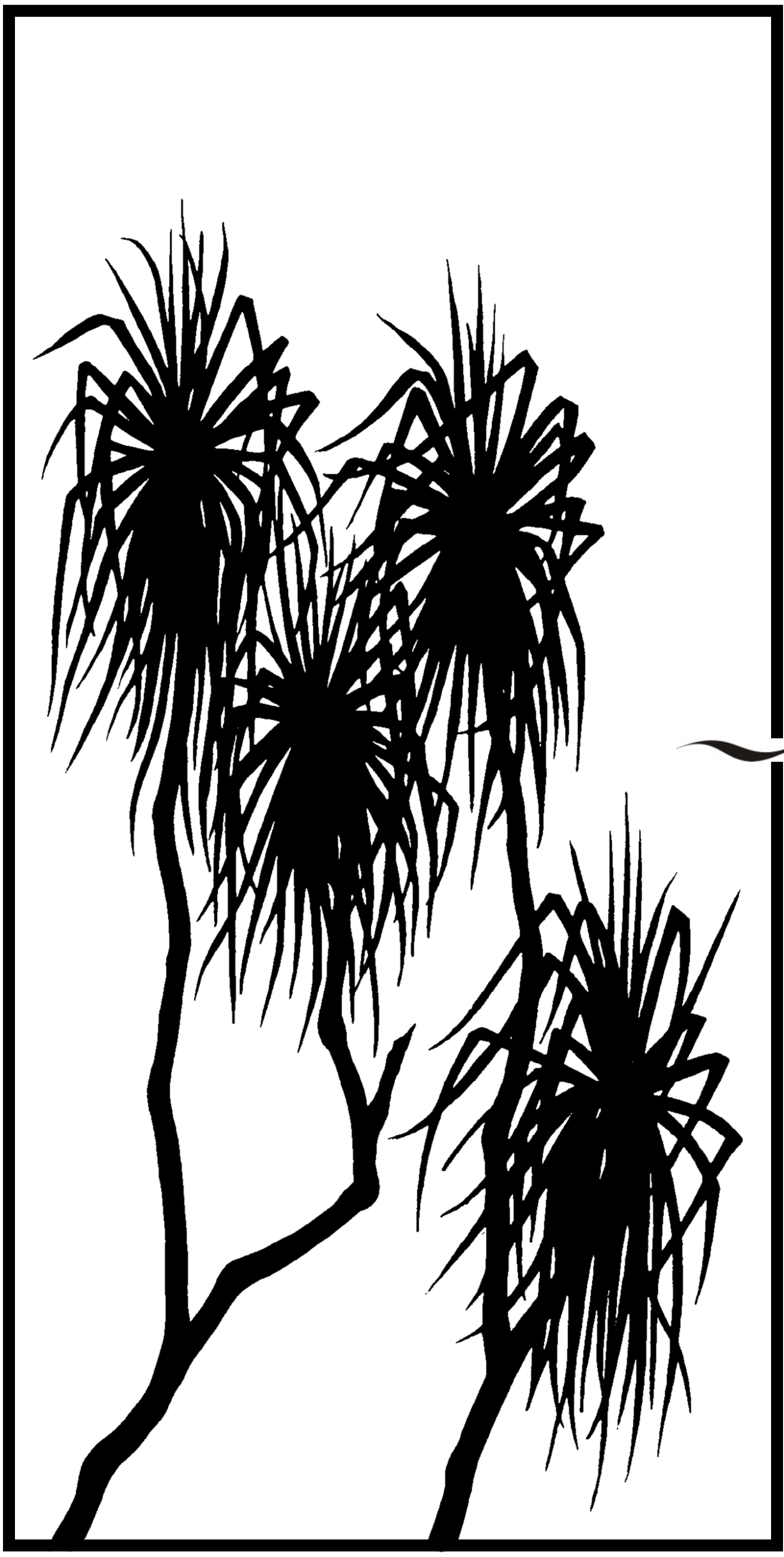

Ecotoxicological assessment of manganese

AJ Harford, MA Trenfield, KL Cheng, & RA van Dam

April 2014

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Executive summary

Manganese (Mn) is a ubiquitous element in the earth's mantle and a key contaminant of Ranger mine process water. Manganese toxicity is dependent on pH and water hardness, which is consistent with what is known for other metals. However, Mn aquatic chemistry is also a complex function of the pH and redox micro-environment with Mn primarily existing as soluble Mn(II) and insoluble Mn(IV) oxidation states. The risks of Mn toxicity to aquatic biota of Magela Creek have been considered low to date. However, groundwater modeling of Pit 1 and Pit 3 closures has found that elevated concentrations of Mn may reach Magela Creek and indicated that Mn will be a key contaminant of concern. Additionally, the likelihood of higher Mn concentrations being released to Magela Creek may increase following the commissioning of the brine concentrator plant. Insufficient Mn toxicity data existed for local species in local natural waters to be able to (i) conclude with high confidence that no adverse effects would be expected given the current water quality and (ii) predict at what Mn concentrations adverse effects would be expected to occur. A site-specific assessment of Mn is of particular pertinence given the low water hardness and relatively low pH of natural waters of the Alligator Rivers Region, which could potentially result in higher than expected (i.e. from existing literature) Mn toxicity. The aims of this study were to:

1. Assess the toxicity of manganese (Mn) in Magela Creek water (pH ~6–6.5) to six tropical freshwater species.
2. Derive a site-specific Trigger Value (TV) for Mn in Magela Creek.
3. Recommend Limit, Focus and Action Trigger Values, which can be incorporated into the Water Quality Objective (WQO) for Magela Creek.

The TVs derived in this project were incorporated into the water quality trigger framework for Magela Creek that has been described by Iles (2004). The framework consists of a hierarchy of TVs (Focus, Action and Limits) and exceedance of these TVs initiate increasingly strict reporting and investigation actions by the mine's operator.

The six local freshwater species tested in this study had a broad range of sensitivities to Mn in the soft surface waters of Ngarradj and Magela Creeks. For three of the species, Mn toxicity was higher than many of the species reported in the literature, which was probably due to the low concentration of Ca^{2+} in the natural waters. The low pH may have decreased to the toxicity of Mn to *Chlorella* sp., but increased the potential for Mn^{2+} to remain dissolved and, hence bioavailable. A loss of Mn was observed on the final day of a number of the *H. viridissima* toxicity tests but the Mn could not be recovered from the test system. This observation may be a result of the previously reported complex speciation of Mn. We accounted for such issues through extensive analysis of Mn (0.1 µm filtered and total) at the start and end of the tests. Toxicity estimates were adjusted using the measured Mn concentrations. The Species Sensitivity Distribution (SSD), which used the three international toxicity estimates derived under relevant physico-chemical conditions, produced a 99% TV that can be implemented in Magela Creek

It is recommended that a 99% protection TV of 75 µg L^{-1} Mn be applied at MG009. The Focus and Action TVs should be 35 and 45 µg L^{-1}, respectively. These TVs are rounded out from the calculated 99% TV of 73 µg L^{-1} and the 95^{th} and 80^{th} confidence intervals of 33 and 46 µg L^{-1}, respectively.

1 Introduction

Manganese (Mn) is a ubiquitous element in the earth's mantle and is present in most rocks and soil types (Homoncik et al. 2010). Trace amounts are an essential element for organisms and human-health because it is a constituent in a number of important enzymes and co-factors. It is considered less of an environmental hazard than many other metals and evidence from the literature suggests that the acute and chronic toxicity of Mn to many freshwater biota was low (i.e. in the mg L^{-1} range). This was reflected in the relatively high 95% protection trigger value (TV) reported by ANZECC and ARMCANZ (2000) of 1900 µg L^{-1}. However, recent studies have reported particularly sensitive species, e.g. *Hyalella azteca* with an IC10 of 96 µg L^{-1} Mn (IMnI 2009, cited Peters 2010). A review of Mn toxicity in freshwaters by the Environment Agency (UK) recommended a Predicted No Effect Concentration (PNEC) of 62 - 123 µg L^{-1} (Peters et al. 2010), which was based on a Species Sensitivity Distribution (SSD) of 12 toxicity estimates. The calculated Hazardous Concentration predicted to effect 5% of species (HC5; equivalent to a 95% TV) was 246 µg L^{-1}. The aforementioned PNECs were derived by applying 2–4 Application Factors (AF; aka Safety Factor) to the HC5. The use of an AF is mandatory for the derivation of an Environmental Quality Standard (EQS) under Annex VIII of the Water Framework Directive of the European Commission (EC) but this led to an EQS that was too stringent for many waterways, although it was considered relevant to conditions of high bioavailability, i.e. low pH, hardness, alkalinity and Dissolved Organic Carbon (DOC). This issue was addressed by the EC through the development of a Biotic Ligand Model (BLM) for Mn, which allowed for the adjustment of the EQS under different physico-chemical conditions (Peters et al. 2011).

The Mn BLM reported by Peters et al. 2011 describes its toxicity as a function of water quality. They found that increasing H^+ ions, or low pH, ameliorates the toxicity of Mn to algae. Additionally, Ca^{2+} cations ameliorate the toxicity of Mn to fish and invertebrates while Mg^{2+} cations ameliorate the toxicity to only invertebrates but not to the extent of Ca^{2+}. This is because these ions compete with Mn for binding sites on/in organisms, noting that the nature of these binding sites is likely to differ across taxa. The dependence of Mn toxicity on pH and water hardness is also consistent with what is known for other metals (Peters et al. 2011). However, Mn aquatic chemistry is also a complex function of the pH and redox micro-environment with Mn primarily existing as soluble Mn(II) and insoluble Mn(IV) oxidation states. Increasing pH and redox of a solution generally results in particulate formation due to the oxidation of Mn(II) to form Mn(III)/Mn(IV) oxyhydroxide precipitates. These reactions are slow in the absence of a catalyst (Chiswell & Mokhtar 1986) but many aquatic bacteria use Mn(II) as a terminal electron acceptor during respiration, which results in the production of insoluble Mn(IV) oxides in the environment (Horsburgh et al. 2002). Richardson et al. (1988) also showed that microalgae can form micro-environments of high pH and high O_2, which promotes the formation of insoluble MnO_2 colloids. Hence, compared to other metals Mn can be a problematic metal in toxicity tests and detailed chemical analyses are essential to determine accurate exposure measurements.

Due to observations at Ranger in the early 2000s of increasing concentrations of (Mn) in a shallow groundwater bore adjacent to Magela Creek greater attention was paid to (Mn) as a contaminant of potential ecotoxicological concern, (MC20; up to 50 000 µg L^{-1}; ERA 2002). Additionally, concentration 'spikes' have been observed in early wet season surface water in lower Corridor Creek (GC2; 700–800 μg L^{-1}) and Coonjimba Billabong (1300 μg L^{-1} in December 2002/January 2003) (van Dam 2004). Since then, Mn concentrations in bore MC20, which is in a local depression and acts as a collection point for surface drainage, have consistently been measured at 40 000–50 000 μg L^{-1} during the dry season (ERA 2008), with much lower values (100-1000 μg L^{-1}; based on limited data) in the wet season following flushing of the shallow groundwater system. This appeared to be a localised effect, with dry season Mn concentrations in nearby shallow groundwater bores over the same time period being at least two orders of magnitude lower than in bore MC20. Four more occurrences of Mn above 800 μg L^{-1} (with a maximum of 1690 μg L^{-1} in November 2004) have been measured at GC2, while Coonjimba Billabong has experienced one additional spike above 800 μg/L, in December 2007 (ERA 2008). Two of the measured spikes exceeded the ANZECC/ARMCANZ (2000) 99% species protection trigger of 1200 μg L^{-1}, and were above concentrations reported in the literature to cause chronic toxicity to some species. The current site-specific guideline for Mn in Magela Creek downstream of Ranger is 26 μg L^{-1} (based on upstream reference site data; Iles 2004). This value was derived from statistical analysis of water quality data from the upstream reference site data, and applicable only when flow in Magela Creek is greater than 5 cumecs. It is approximately two orders of magnitude more conservative than the ANZECC/ARMCANZ (2000) trigger value.

Notwithstanding these high concentrations, Mn concentrations in Magela Creek downstream of the mine have remained between 3 and 15 μg L^{-1} (5^{th} and 95^{th} percentile, n = 557). Even during periods of low flow in the creek the maximum concentration measured was 50 μg L^{-1}. The current site-specific guideline for Mn in Magela Creek of 26 μg L^{-1} has been exceeded in less than 2% of the Magela Creek water samples collected since 1980 (Harford et al. 2009). The majority of exceedances have occurred during early wet season flows or end of wet season recessional flows, often when flow is less than 5 cumecs. These periods are considered to be atypical of the season as a whole given the increased contributions from shallow groundwater at these times. Consequently, the risks of Mn toxicity to aquatic biota have been considered low to date. However, groundwater modeling of Pit 1 and 3 closures has found that elevated concentrations of Mn may reach Magela Creek and indicated that Mn will be a key contaminant of concern (reported at ARRTC 31). Additionally, the likelihood of higher Mn concentrations being released to Magela Creek may increase following the commissioning of the brine concentrator plant. The pilot-scale brine concentrator plant tested in 2011 produced two distillate waters containing Mn at concentrations of 130 and 240 μg L^{-1} (Harford et al. 2013), which is residual from the 1400 mg L^{-1} Mn in the untreated process water. The full-scale brine concentrator has produced typically cleaner distillates due to additional vapor scrubbing facilities. The median Mn concentration was 1.0 μg L^{-1} (n=61, ARRTC31) but a maximum concentration of 110 μg L^{-1} was reported. Such Mn concentrations are higher than those currently measured in mine waters discharged from Ranger (RP1 had 0.2 to 63 μg L^{-1} during 2011–2012), and the addition of distillate to such waters may eventually result in higher Mn concentrations in Magela Creek than have previously been measured.

Insufficient Mn toxicity data existed for local species in local natural waters to be able to (i) conclude with high confidence that no adverse effects would be expected given the current water quality and (ii) predict at what Mn concentrations adverse effects would be expected to occur. A site-specific assessment of Mn is of particular pertinence given the low water hardness and relatively low pH of natural waters of the Alligator Rivers Region, which could potentially result in higher than expected (i.e. from existing literature) Mn toxicity. The aims of this study were to:

1. Assess the toxicity of manganese (Mn) in Magela Creek water (pH ~6–6.5) to six tropical freshwater species.
2. Derive a site-specific Trigger Value (TV) for Mn in Magela Creek.
3. Recommend Limit, Focus and Action Trigger Values, which can be incorporated into the Water Quality Objective (WQO) for Magela Creek.

The TVs derived in this project were incorporated into the water quality trigger framework for Magela Creek that has been described by Iles (2004). The framework consists of a hierarchy of TVs (Focus, Action and Limits) and exceedance of these TVs initiate increasingly strict reporting and investigation actions by the mine's operator.

2 Methods

2.1 General laboratory procedures

All equipment which test organisms or media came in contact with, or were exposed to, was made of chemically inert materials (e.g. Teflon, glass or polyethylene). All plastics and glassware were washed by soaking in 5% (v/v) HNO_3 for 24 h before being washed with a non-phosphate detergent (Dr. Weigert, neodisher® LaboClean FLA, Hamburg, Germany) in a laboratory dishwasher operated with reverse osmosis/deionised water (Elix, Millipore, Molshiem, France). All reagents used were analytical grade and stock solutions were made up in high purity water (18 MΩ, Milli-Q Element, Millipore, Molshiem, France).

Glassware used in the toxicity tests was silanised with 2% dimethyldichlorosilane in 1,1,1-trichloroethane (Coatasil, AJAX, Seven Hills, Australia) to reduce Mn adsorption to the glass. All reagents used were analytical grade and stock solutions were made up in Milli-Q water.

2.2 Test diluents

A low pH diluent water (Ngarradj Creek Water, NCW) was chosen for preliminary toxicity tests because the bioavailability of Mn was likely to be higher at a lower pH. NCW was collected from near the Ngarradj Creek Upstream gauging station (NCUS: 0275473; 8616847; WGS84, Zone 53).

Natural Magela Creek water (MCW) was used as the control treatment and for dissolution media in all other tests, and was obtained from Bowerbird Billabong (latitude 12° 46' 15", longitude 133° 02' 20"). This natural water has been extensively characterised and has been used as a diluent in toxicity testing for over 20 years in the ***eriss*** ecotoxicology laboratory.

The natural waters were collected in 20 L acid-washed plastic containers and transported 2.5 h to the laboratory at ambient temperature. At the laboratory, they were filtered within 3 days of collection (2.5 µm, Filter paper no 42, Whatman or 3.0 µm, Sartopure PP2 depth filter MidiCaps, Sartorius). The waters were stored at 4 ± 1°C prior to filtration and up to 1 month following collection. For the *A. cumingi* tests, the NMCW diluent water was as per that described above, with the exception that given the high volumes of water required for a single toxicity test, it was not pre-filtered. This had the potential to introduce coarse particulates and wild zooplankton into the test. However, both the diluent and test solutions were visibly free of coarse particulates, whereas wild zooplankton were not observed in the test (possibly because the waters were stored at 4°C after collection). Even if they were present in low numbers, they were considered unlikely to adversely affect the snails' reproduction or affect the toxicity of Mn.

Diluent waters were sub-sampled for physico-chemical analyses. Specifically pH, DO, EC and DOC were measured in-house. Additional sub-samples were sent to an environmental chemistry laboratory (Envirolab, Chatswood, NSW) for measurement of alkalinity (APHA2320B), and a limited metal and major ion suite (totals only; Al, Cd, Co, C, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, SO_4 (analysed as S and converted)) by ICP-MS and ICP-AES.

2.3 Toxicity Tests

The toxicity of Mn was assessed using six Australian tropical freshwater species: the unicellular green alga (*Chlorella* sp.); the duckweed (*Lemna aequinoctialis*); the green hydra (*Hydra viridissima*); the cladoceran (*Moinodaphnia macleayi*); the aquatic snail (*Amerianna cumingi*) and the Northern trout gudgeon (*Mogurnda mogurnda*). All the organisms were isolated from soft surface waters in Kakadu National Park and have been cultured continuously at the Environmental Research Institute of the Supervising Scientist over many years (10–25 years depending on the species). The test methods are described in detail by Riethmuller et al. (2003) and, for *A. cumingi* only, Houston et al. (2007). Key details of each test are provided in Table 1. For the *L. aequinoctialis* and *Chlorella* sp. tests, nutrients (nitrate and phosphate) were added at the minimum concentrations that would sustain acceptable growth (see Table 1). The MCW used in the *Chlorella* sp. tests also had 1 mM HEPES buffer added to maintain a stable pH.

The natural water diluents were spiked with Mn using a stock solution of 52.5 mg L^{-1} manganese sulfate ($MnSO_4.H_2O$, Sigma-Aldrich). Concentrations of dissolved Mn (0.1 µm filtered) were measured before and after the test exposure through ICP-MS analysis (see QC section below).

2.3.1 Ngarradj Creek Water Study

Preliminary experiments were undertaken using Ngarrdj Creek Water (NCW) and *Chlorella* sp. and *H. viridissima*. For *M. macleayi* a modified chronic toxicity tests and an acute test were conducted (Table 2), in order to determine the influence of the algal food source on Mn toxicity. A Magela Creek Water (MCW) quality control group was included for each test conducted in NCW (i.e. organisms were maintained in the standard natural MCW; pH – 6.8, EC – 16 µS/cm, DO – 97.5% saturation).

With the exception of one of the *M. macleayi* tests (see below), all experiments were conducted in accordance with the standardised ***eriss*** ecotoxicological protocols described in Riethmuller et al. (2003). Two of the *M. macleayi* chronic toxicity tests were conducted simultaneously with one of the tests excluding the algal component of the cladocerans' food (Table 1). This was done to determine if the presence of actively photosynthesising algae would result in oxidation of the manganese and production of insoluble manganese oxyhydroxides (MnO, Richardson et al. 1988), thereby reducing the bioavailability and toxicity of Mn.

2.3.2 Magela Creek Water Study

At least two valid toxicity tests were completed for each species and for most of the toxicity tests a modified design was used (Table 2). Specifically, the concentration range was increased by reducing treatment replication from 3 replicates to 2 replicates. The design has the advantage of being able to better characterise the concentration-response relationships and derive toxicity estimates with increased accuracy. Due to logistical reasons, the modified design was not used for the snail toxicity tests.

Table 1 Details of toxicity tests for the six Australian tropical freshwater species used to assess the toxicity of manganese. Full details of the methods are provided in Riethmuller et al. (2003) and Houston et al. (2007).

Species (common name)	**Test duration and endpoint**	**Control response acceptability criterion**	**Temperature, light intensity, photoperiod**	**Feeding/ nutrition**	**No. replicates (Individuals per replicate)[a]**	**Test volume (mL)**	**Static/daily renewals**
Chlorella sp. (unicellular green alga)	72-h population growth rate	1.4 ± 0.3 doublings day^{-1}; % CV[a] <20%	29 ± 1°C 100-150 μmol m^{-2} sec^{-1} 12:12h	14.5 mg L^{-1} NO_3 0.14 mg L^{-1} PO_4	3 (3×10^{4} cells ml^{-1})	50	Static
Lemna aequinoctialis (tropical duckweed)	96-h growth rate	Mean surface area growth rate (k, mm^{2} day $^{-1}$) ≥0.40; % CV <20%	29 ± 1°C 100-150 μmol m^{-2} sec^{-1} 12:12h	3 mg L^{-1} NO_3 0.3 mg L^{-1} PO_4	3 (4 with 3 fronds)	100	Static
Hydra viridissima (green hydra)	72-h population growth rate	Mean population growth rate (k, day $^{-1}$) ≥0.27; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	3-4 *Artemia* nauplii day^{-1}	3 (10)	30	Daily renewals
Moinodaphnia macleayi (cladoceran)	3-brood (120-144 h) reproduction	Mean adult survival ≥80%; mean neonates per adult ≥30; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	30 μl FFV[b] and 6 × 10^{6} cells of Chlorella sp. d^{-1}	10 (1)	30	Daily renewals
Amerianna cumingi	96-h reproduction	Mean eggs per snail pair ≥100; %CV<30%	30°C; 30 - 100 mmol m^{-2} sec^{-1}; 12:12h	2 cm^{2} lettuce disc per snail per day	3 (12)	1750	Daily renewals
Mogurnda mogurnda (Northern trout gudgeon)	96-h survival	Mean larval survival ≥80%; % CV <20%	27 ± 1°C 30-100 μmol m^{-2} sec^{-1} 12:12h	Nil	3 (10)	30	Daily renewals

[a] Replication was reduced for modified tests in order to increase the number of treatments. See Table 2

[b] CV: Percent co-efficient of variation

[c] FFV: fermented food with vitamins. Represents an organic and bacterial suspension prepared by method described in Riethmuller *et al* (2003)

Table 2 Details of the manganese concentration-response tests conducted

Test ID	Date	Species name	Endpoint	Mn concentration range tested (μg L⁻¹)[a]	Comments
Ngarradj Creek Water					
933D	31/05/08	*M. macleayi*	Reproduction	4.2 - 1870	Modified design – no algae, 30 μL FFV only
934D 937D	31/05/08 20/06/08	*M. macleayi*	Reproduction	4.2 – 1840 4.6 – 15300	As per protocol
938I	20/06/08	*M. macleayi*	Survival	4.6 – 15100	No food
936B	16/06/08	*H. viridissima*	Population growth	5.2 – 19150	As per protocol
939G	24/06/08	*Chlorella* sp.	Population growth	4.5 – 59 300	As per protocol
Magela Creek Water					
1278G 1294G	30/04/12 28/08/12	*Chlorella sp.*	Population growth	4.0 – 480000 3.0– 135000	Modified design[b]
1276L 1279L 1297L	30/04/12 23/04/12 10/09/12	*L. aequinoctialis*	Surface area growth rate	3.0 – 44000 2.0 – 19000 0.3 – 39000	Modified design[b]
1290B 1277B 1310B 1318B 1379B 1381B	30/07/12 30/05/12 19/11/12 11/02/2013 21/01/14 28/01/14	*H. viridissima*	Population growth	0.6 – 755 0.3 – 840 1.8 – 1950 5.0 - 1750 3.0 – 2300 3.0 – 2300	Modified design[b] 1290B and 1277B not used in toxicity estimate due to Mn loss 1379B and 1381 tests conducted with pH 5.2 MCW
1299D 1345D	14/09/12 1/08/13	*M. macleayi*	Reproduction	3.0 – 1150 2.0 – 4700	Modified design for first test only[b]
1275S 1307S 1335S	23/04/12 29/10/12 29/04/13	*A. cumingi*	Reproduction	1.8 – 33500 2.0 – 10500 2.0 – 29500	As per protocol
1284E 1293E 1300E	14/06/12 23/08/12 20/09/12	*M. mogurnda*	Survival	2.0 – 46500 4.0 – 295000 4.0 - 360000	Modified design[b]

[a] Concentration range is based on the mean of start and end Mn values
[b] A modified design of less replicates and more treatments was used were indicated

2.3.3 Fate of manganese in the *H. viridissima* test

Due to observed losses of Mn in the *H. viridissima* toxicity tests, an experiment was conducted to assess the fate of Mn in the hydra test system. Three Mn concentrations in MCW (background, 250 and 600 μg L^{-1}) were assessed. An additional treatment was included for each Mn concentration, whereby the test petri dishes were pre-inoculated with a solution of 250 μg Mn L^{-1} for 24 h prior to the test commencement, i.e. 'primed'. This treatment was incorporated to see if Mn binding sites on the petri dishes could be saturated prior to the experiment, thereby reducing this source of Mn loss during the test. Measurements of Mn were made on the following components of the test system:

1. Test solutions from the test petri dishes at test commencement and every 24 h just prior to test solution renewal, until the end of the test (96 h) (total and 0.1 μm filtered Mn)
2. Test solutions from the 5 L test solution storage bottles at the commencement and end of the test (total and 0.1 μm filtered Mn)
3. Hydra tissue at the end of the test (total Mn in all hydra)
4. The surface of the test petri dishes, following rinsing with 5% HNO_3 (total Mn).

2.4 Quality Control

2.4.1 Manganese chemistry

Water samples (total and 0.1 μm filtered) for chemical analyses were collected and analysed both before and after exposure to track the status of the added Mn. Filtration through 0.1 μm membranes, rather than the conventional 0.45 μm filtration, was used specifically for this work to provide increased ability to identify Mn oxides in colloidal form.

2.4.2 Quality Control Chemistry

For each test, blanks and procedural blanks (i.e. ultra-pure water that has been exposed to all components of the test system) were also analysed for a limited metal and major ion suite (Al, Cd, Co, C, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, SO_4 - analysed as S and converted). Chemistry data for the blanks and procedural blanks were initially assessed by searching for analyte concentrations higher than detection limits. Where these concentrations were greater than 1 μg L^{-1} and above background levels of MCW, duplicate procedural blank samples were re-analysed and/or the control water concentrations were compared to those in tests without blank contamination, to determine if the contamination was limited to the one sample bottle or experienced throughout the test. The likelihood that contamination may have confounded the toxicity test results was investigated and discussed on a case-by-case basis.

2.4.3 General water quality

For each test, data were considered acceptable if: the recorded temperature of the incubator remained within the prescribed limits (see test descriptions, above); the recorded pH was within ± 1 unit of values at test commencement (i.e. Day 0); the EC for each test solution was within 10% (or 5 μS cm^{-1} for samples with low conductivity) of the values at test commencement; and the DO concentration was greater than 70% throughout the test (see Appendix A for data). The occurrence of any significant water quality changes were investigated and discussed on a case-by-case basis.

2.6.3 Control responses

Tests were considered valid if the organisms in the QC treatment (i.e. those in the MCW or SSW control) met the following criteria:

Chlorella sp. cell division rate test

- The algal growth rate is within the range 1.4 ± 0.3 doublings day^{-1}; and
- There is <20% variability (i.e. co-efficient of variation, CV <20%) in growth rate.

L. aequinoctialis plant growth test

- The average increase in frond number in any flask at test conclusion is at least four times that at test start (i.e. a total of 60 fronds/flask or specific growth rate (k) > 0.4 day^{-1}); and
- There is <20% variability (CV < 20%) in growth rate.

M. macleayi 3-brood reproduction test

- 80% or more of the cladocera are alive and female, and have produced three broods at the end of the test period;
- Reproduction in the control averages 30 or more live neonates per female over the test period; and

H. viridissima population growth test

- More than 30 healthy hydroids (i.e. specific growth rate specific growth rate (k) > 0.27 day^{-1}) remain in each dish at the end of the test period; and
- There is <20% variability (CV <20%) in growth rate.

A. *cumingi* reproduction test

- More than 100 eggs per snail pair
- There is <30% variability (CV<30%) in mean egg production

M. mogurnda larval fish survival test

- The mean mortality or presence of fungus on the fish does not exceed 20%; and
- There is <20% variability (CV <20%) in survival.

2.5 Toxicity estimate calculations

For the NCW toxicity tests, linear interpolation analyses were used to determine point estimates of Inhibitory Concentrations (ICs) that reduced endpoint responses by 10% and 50% (i.e. IC10 and IC50) relative to the control responses (ToxCalc version 5.0.23F, Tidepool Scientific Software; Appendix C). Non-linear regression could not be used due to an insufficient number of data points for the NCW tests. For the MCW toxicity tests, the individual tests were pooled and the raw data analysed. Two valid hydra tests, where significant loss of Mn was measured, were not used in the calculation of the toxicity estimate because a reliable exposure concentration could not be estimated (Table 2). Non-linear regression (3-parameter log-logistic) analyses were used to determine point estimates of Inhibitory Concentrations (ICs) that reduced endpoint responses by 10% and 50% (i.e. IC10 and IC50) relative to the control responses (CETIS version 1.8.7.4, Tidepool Scientific Software; Appendix C). Because the *M. mogurnda* test represents an acute exposure and measures lethality, a more conservative 5% effect/lethal concentration was estimated instead of a 10% effect/lethal concentration.

2.6 Trigger Value Derivation

A site-specific 99% protection Trigger Value (TV) was derived using the Species Sensitivity Distribution (SSD) method (BurrilOz 2.0, CSIRO). In order to improve the fit of the distribution three extra toxicity estimates from international studies in physico-chemical conditions closely related to Magela Creek were added to the local species dataset. Specifically, toxicity estimates from the temperate, northern hemisphere species, *Pseudokirchneriella subcapitata* (alga)*, Ceriodaphnia dubia* (cladoceran) and *Pimephales promelas* (fish) were added to the SSD. These toxicity tests were conducted at 25°C in a natural soft water (Hardness = 12 mg L^{-1} CaCO3, Ca = 4 mg L^{-1}) with a pH of 6.7. The Dissolved Organic Carbon (DOC) was 12 mg/L, which is four times higher than MCW (typically <3 mg L^{-1}). However, DOC has been reported to have less of an influence on Mn toxicity compared to other physico-chemical parameters (Peters et al. 2011). Focus and Action TVs were calculated using the lower 95 and 80% confidence intervals of the site-specific 99% protection TV.

3 Results

3.1 Ngarradj Creek Water Study

3.1.1 Chemistry

Prior to filtering, the NCW had a pH of 5.3, an electrical conductivity (EC) of 13 μS cm^{-1} and a dissolved oxygen (DO) content of 86%. Following filtration, the water had a pH of 5.6, an EC of 12 μS cm^{-1} and a DO content of 75%. For the testing, the pH was higher again, but remained 6.0–7.0 for all tests. Metal analysis of filtered NCW indicated that it contained some aluminium (3.0 μg L^{-1}), zinc (2.0 μg L^{-1}), nickel (1.6 μg L^{-1}) and manganese (3.8 μg L^{-1}). All other metals analysed were at concentrations <1 μg L^{-1}.

The results of Mn analyses for the toxicity tests are reported in Appendix B (Table B1). The total concentration of Mn did not change during the course of the experiments, indicating that there was no loss to the test system (e.g. walls of the test vials). At the commencement of the tests, ~92% of the total Mn was present in the <0.1 μm fraction (i.e. dissolved or very fine colloidal fraction), compared to approximately 86-92% by the end of the tests. Furthermore, tests that did not receive daily water renewal and were conducted over longer time periods (i.e. 72-h algae test and 48-h acute flea tests) did not show markedly larger losses of Mn. To account for the change in soluble (i.e. bioavailable) Mn, the calculation of toxicity estimates used an average of the start and end of test filtered concentrations. Analysis of the test solutions from the initial two cladoceran tests (i.e. 933D and 943D) indicated that significant concentrations of oxidised Mn forms (i.e. insoluble forms) were not being formed in the presence of photosynthetic organisms (i.e. the algal food source).

3.1.2 Toxicity

The initial chronic toxicity experiment with *M. macleayi* demonstrated that excluding the algal food from the test significantly reduced their reproductive health (Figure 1a). Exposure of *M. macleayi* to Mn with and without the algal food in the test system resulted in a similar concentration-response. Excluding the algal food resulted in a significant reduction in neonate numbers of ~40% at 1840 μg L^{-1} Mn and while there was a similar reduction in the test with algae, the larger variation in the control response resulted in no statistically significant effects (Figure 1a). In order to further understand the affect of algae, a 6-d chronic test with algal food and a 48-h acute test without food were conducted at higher concentrations. Both these studies resulted in 100% lethality to *M. macleayi* within 48 h at concentrations ≥1845 μg L^{-1} Mn (Figure 1a and b). A Mn concentration of 870 μg L^{-1} Mn resulted in a statistically significant reduction in the number of neonates (i.e. 13%) in the chronic test, while in the acute test no significant effects were observed at 770 μg L^{-1} Mn (Figure 1). The results of the tests indicate a dramatic threshold response for *M. macleayi* survival at between 1000–2000 μg L^{-1} Mn and showed that the presence of algae did not markedly alter the toxicity.

Of the three species tested, *H. viridissima* was the most sensitive to Mn exposure but the lowest concentration of Mn tested resulted in a significant reduction of population growth rate. An IC10 of 60 (30 – 330) μg L^{-1} and an IC50 of 770 (590 – 940) μg L^{-1} were determined but it should be noted that only a limited number of concentrations were tested and these tests were not repeated. Manganese only inhibited the growth rate of *Chlorella* sp by 13.5% over the concentration range that was tested (Figure 1, Table 3). An IC10 of 5100 μg L^{-1} was calculated, while the IC50 could not be determined but was >59 300 μg L^{-1}. However, due to low intra-treatment variability in the control and treatment groups in a statistically significant inhibition of growth rate was detected in the intermediate treatments of 1860 μg L^{-1} and 5960 μg L^{-1} Mn. The results demonstrate that *Chlorella* sp. is tolerant to Mn exposure, especially in comparison to *H. viridissima*

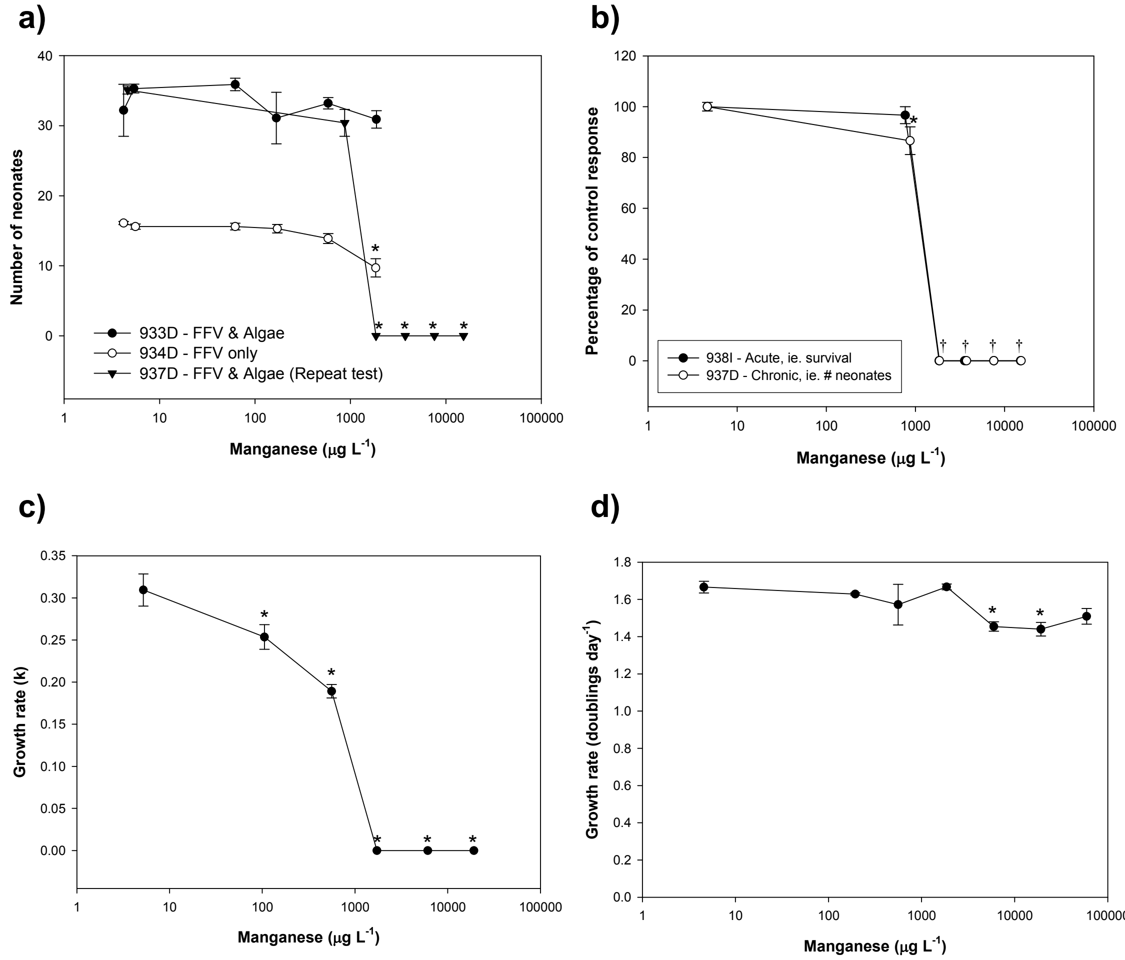

Figure 1 Effect of Manganese on a) the reproduction of *M. macleayi* over six days b) the survival and reproduction of *M. macleayi* over 48 h c) the population growth rate of *H. viridissima* over 96 h and d) the growth rate of *Chlorella* sp. over 72 h. * and † denote significantly different from the NCW control ($p<0.05$)

Table 3 Summary of the Mn toxicity estimates to three local freshwater species in Ngarradj Creek Water

Test ID and Date	Species name	Endpoint	Control performance			Toxicity (µg L^{-1})	
			Creek water	mean	%CV[2]	IC10[3]	IC50[4]
933D 31/05/08	*M. macleayi*	# neonates	Magela	35.4	6.2	1750 (nc)[5]	>1870 (nc)
			Ngarradj	32.2	36.4		
934D 31/05/08	*M. macleayi*	# neonates	Magela	13.6	8.6	410 (nc)	>1840 (nc)
			Ngarradj	16.1	4.6		
936B 16/06/08	*H. viridissima*	Population growth rate	Magela	0.3	5.8	60 (30-330)	770 (590-940)
			Ngarradj	0.3	10.6		
937D 20/06/08	*M. macleayi*	# neonates	Magela	27	43	650 (360-920)	1290 (1200-1340)
			Ngarradj	35.1	5.3		
938I 20/06/08	*M. macleayi*	Survival	Magela	100	0	880 (730-880)	1310 (1230-1310)
			Ngarradj	100	0		
939G 24/06/08	*Chlorella* sp.	Growth rate	Magela	1.8	3.3	5100 (nc)	<59300 (nc)
			Ngarradj	1.7	3.3		

[1] Control growth rate in doublings day^{-1}

[2] %CV: percent co-efficient of variation

[3] IC_{10}: the concentration that results in a 10% reduction in growth rate relative to the controls

[4] IC_{50}: the concentration that results in a 50% reduction in growth rate relative to the controls

[5] nc = not calculable

3.2 Magela Creek Water

3.2.1 Chemistry

Physicochemical parameters of the control MCW were maintained within the following ranges across all tests: pH 5.7-7.1, DO 80–119%, and EC (of controls only) 15-47 µs cm^{-1} (higher EC occurs in the algae test due to the addition of nutrients; see Appendix A).

With the exception of three tests there was little difference between the 0.1 µm filtered Mn concentrations measured before and after the tests, indicating negligible loss (including precipitation) of Mn from the test systems. An unexpected observation during the study was the loss of a significant proportion of Mn from the test solutions during some of the hydra tests and a snail test, especially at Mn concentrations below 230 µg L^{-1}. This loss of Mn from the test waters was not observed for any of the other toxicity tests and also did not occur in the NCW toxicity tests. Potential sources of Mn loss included adsorption to the test solution bottles and/or the test containers, precipitation and/or adsorption/absorption by the test animals. Experiments aimed to determine the fate of Mn in the test system were unable to definitively identify the cause of the loss (see section 3.2.3). The toxicity estimates reported in Table 4 were based on Mn concentrations calculated by averaging the before and after test 0.1 µm filtered Mn concentrations in the test solutions.

3.2.2 Toxicity

Manganese toxicity varied markedly between the six local tropical freshwater species assessed (Figure 2 and Table 4). Concentration-response relationships were established for all species (Figure 2). Toxicity to the fish, *Mogurnda mogurnda*, duckweed, *Lemna* *aequinoctialis*, and green alga, *Chlorella* sp., was low, with IC10 values all above 2000 µg L^{-1} (Table 4). The aquatic snail, *Amerianna cumingi*, the cladoceran, *Moinodaphnia macleayi*, and the hydra, *H. viridissima* were markedly more sensitive, with IC10 values lower than 610 µg L^{-1} for these three species. The hydra was the most sensitive species that was tested with an IC10 of 140 µg L^{-1} (Table 4).

A noteworthy loss of Mn was observed in two out of four *H. viridissima* toxicity tests. Due to the chemistry sampling design, the loss Mn of ~250 µg L^{-1} was measured in only half of the treatments in the first *H. viridissima* toxicity test (1277B). Hence, because Mn was not measured in all treatments this test was omitted from the derivation of the toxicity estimate. A similar Mn loss was seen in one other *H. viridissima* toxicity test (1290B). For this test, the concentration of Mn was measured in all treatments at the end of the test and therefore an average Mn concentration could be used for the toxicity estimate. Interestingly, a loss of Mn was not observed in the following two *H. viridissima* toxicity tests and the concentrations of Mn at the end of the test were within 10% of the starting concentrations. The fate and rate of the Mn loss in the test system was specifically examined (see section 3.2.3). The toxicity estimates reported in Table 2 for *H. viridissima* were based on Mn concentrations calculated by averaging the before and after test 0.1 µm filtered Mn concentrations in the test solutions. The IC10 for *H. viridissima* was 2 times higher in MCW compare to NCW at 140 (100 – 180) µg L^{-1} compared to 60 (30 – 330) µg L^{-1}.

Typically, Mn no/low effect toxicity estimates (e.g. EC/IC10s, no-observed-effect-concentrations) for freshwater species are > 1000 µg L^{-1}. It is noteworthy that three of the species tested in the present study had IC10s < 1000 µg L^{-1}. The order of sensitivity of the six species to Mn was:

H. viridissima > A. cumingi >M. macleayi >> L. aequinoctialis > Chlorella sp. >> M. mogurnda

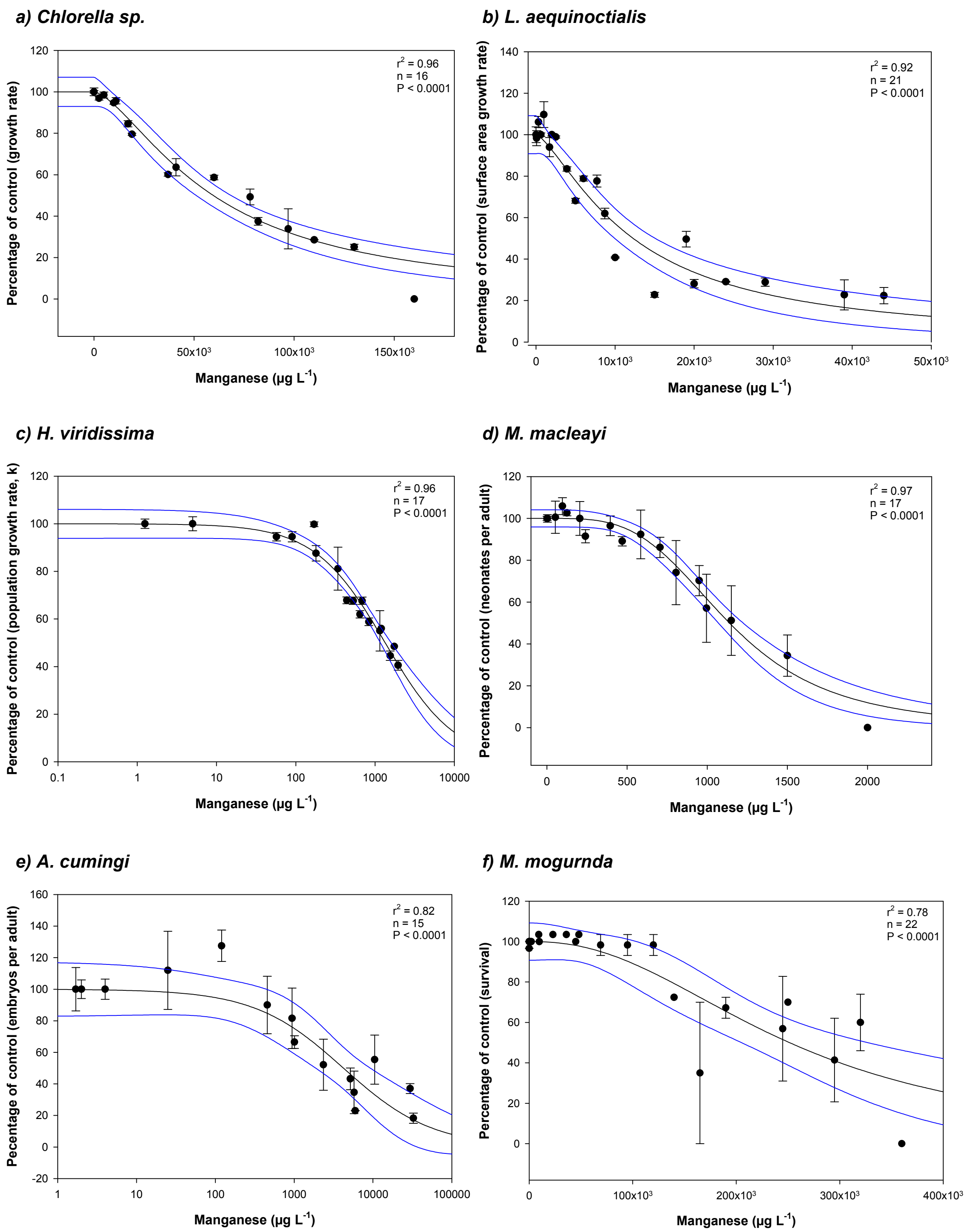

Figure 2 Manganese concentration-response relationships for the six tested species. Data points represent the mean ± standard error of 2-3 replicates, except for *M. macleayi* (*n* = 5-10 replicates). 3-parameter logistic models were used to determine toxicity estimates for all species.

Table 4 Summary of the Mn toxicity estimates to three local freshwater species in Magela Creek Water[a]

Species	IC10 (µg L^{-1})[b]	IC50 (µg L^{-1})c
Chlorella sp.	12 × 10^3 (10 – 14 × 10^3)	60 × 10^3 (55 – 70 × 10^3)
L. aequinoctialis	2200 (910 - 3400)	11 × 10^3 (9 – 13 × 10^3)
H. viridissima	140 (100 - 180)	1380 (1200 - 1560)
M. macleayi	610 (500 - 690)	1100 (1030 - 1170)
A. cumingi	340 (830 - 920)	5660 (2830 - 12660)
M. mogurnda [d]	80 × 10^3 (40 – 110 × 10^3)	240 × 10^3 (200 – 320 × 10^3)

[a] Statistical analyses are in appendix C; nc = not calculable
[b] IC_{10}: the concentration that results in a 10% reduction in growth rate relative to the controls
[c] IC_{50}: the concentration that results in a 50% reduction in growth rate relative to the controls
[d] Toxicity estimates for *M. mogurnda* are LC05 and LC50, that is the concentration that results in 10 and 50% reduction in the survival of the fish

3.2.3 Fate of manganese in the *H. viridissima* toxicity test

An unexpected observation was the loss of a significant proportion of Mn from the test solutions during the *H. viridissima* tests, especially below 230 µg L^{-1} (Figure 3). Total Mn loss (from beginning of test to end of test) in the Mn fate tests was similar in magnitude compared to that observed in the toxicity tests (Figure 3). The measured concentration of dissolved Mn at the start of the test (Day 0) was 60 and 40% lower than expected in the 250 and 600 µg L^{-1} treatments, respectively. This appeared to be erroneous because water samples taken from the same bottle on following days were all within 10% of the nominal concentrations, which indicates that the correct concentration of Mn was added. It may have been due to the Mn not being fully dissolved but there were no signs of precipitates and this did not occur in any of the other toxicity test.

Observed Mn loss, when measured on a day by day basis, was greatest on day 4. This is counter to the hypothesis that Mn is adsorbing to the test dishes where a decrease in daily loss over the test period is normally observed, e.g. (Hogan et al. 2010). Additionally, the similarity in Mn concentrations from solutions taken from primed and unprimed plates also provides evidence that the adsorption of Mn to plates is not the primary issue. The higher loss of Mn on day 4 coincided with the appearance of a floating precipitate on the last day of the test (presumably a form of Mn-oxyhydroxide, although this was not characterised), particularly in the 600 µg L^{-1} treatment. Despite extensive sampling of the test solutions, petri dishes and hydra tissues, a large proportion of the Mn was unrecovered in the treatments ≤250 µg L^{-1} (Figure 4).

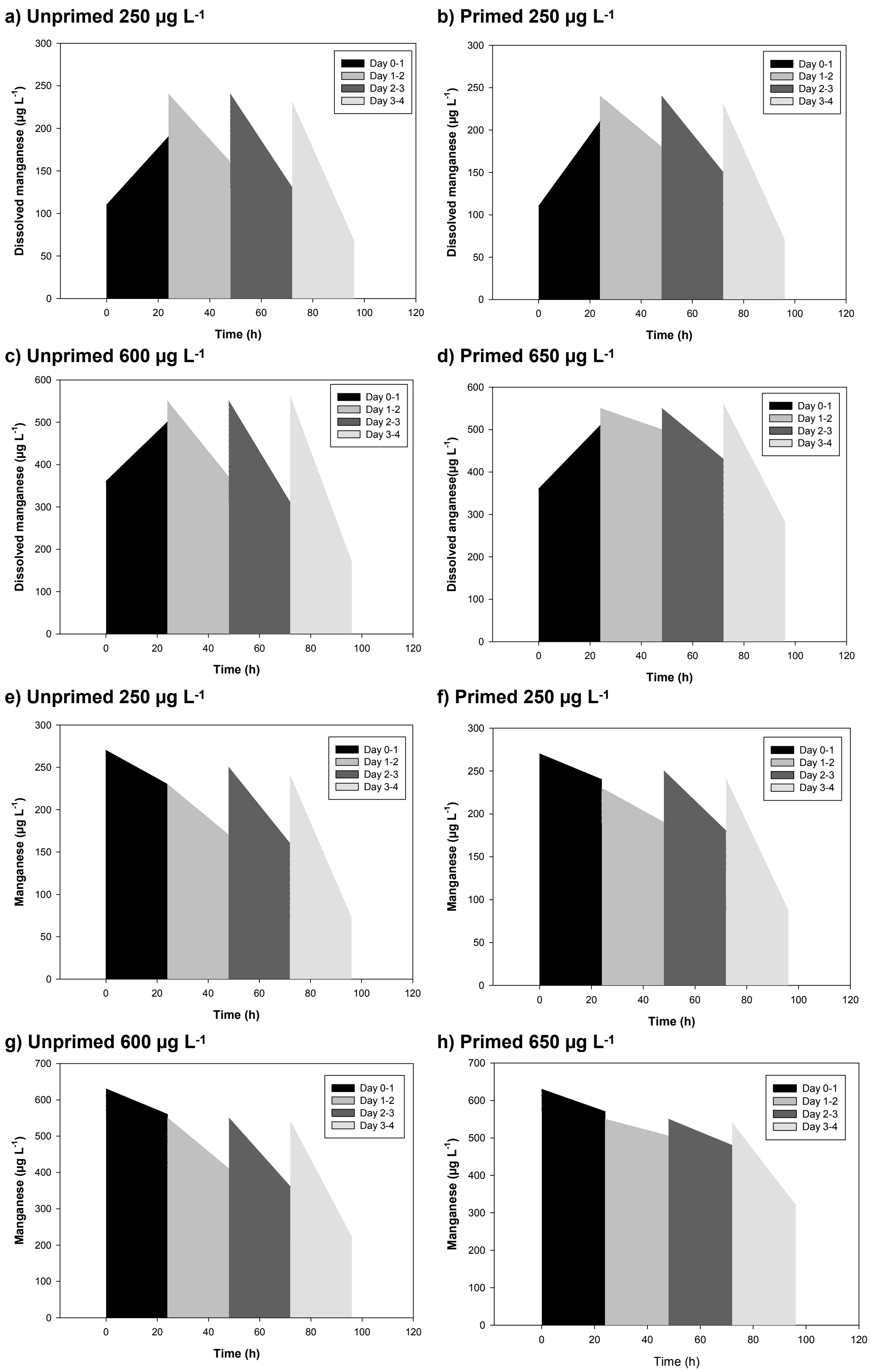

Figure 3 Loss of dissolved (0.1 µm) (a-d) and total manganese (e-g) from the test solutions during the 96-h exposure.

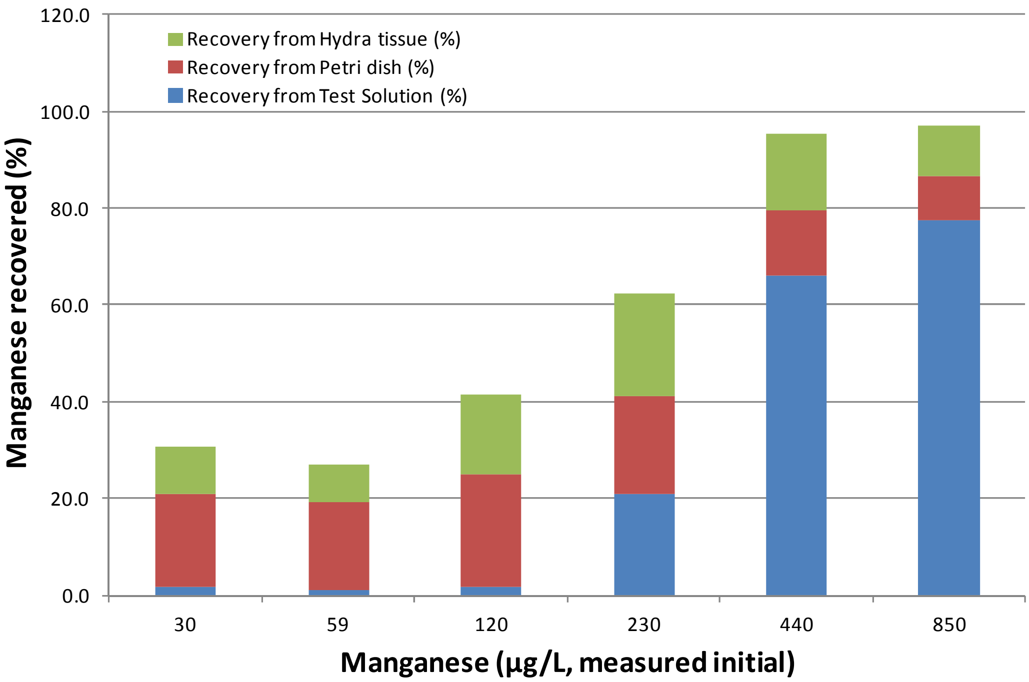

Figure 4 Percentage recovery of Mn from test solutions, petri dishes and hydra at the end of a *Hydra viridissima* Mn toxicity test. Samples from each replicate were pooled for chemical analysis.

3.2.4 *H. viridissima* toxicity tests conducted in pH 5.2 Magela Creek Water

In January 2014, the pH of MCW was pH 5.1. Consequently, in order to estimate the effect of pH on the toxicity of Mn to *H. viridissima* two additional toxicity tests were conducted. The results showed similar IC10s, with overlapping confidence intervals, of 140 (100 – 180) µg L^{-1} and 200 (80 – 270) µg L^{-1} for the MCW at a starting pH 5.9 compared to that with a pH 5.1 (Figure 5). However, there were different IC50s of 1380 (1200 – 1570) and 800 (610 – 1040) µg L^{-1} Mn for the MCW with a pH of 5.9 and 5.1, respectively. The confidence intervals of the IC50 toxicity estimates did not overlap, which indicates that the concentration-response relationship may have been significantly different at the lower pH. However, it should be noted that these tests did not meet the minimum QC criterion for growth.

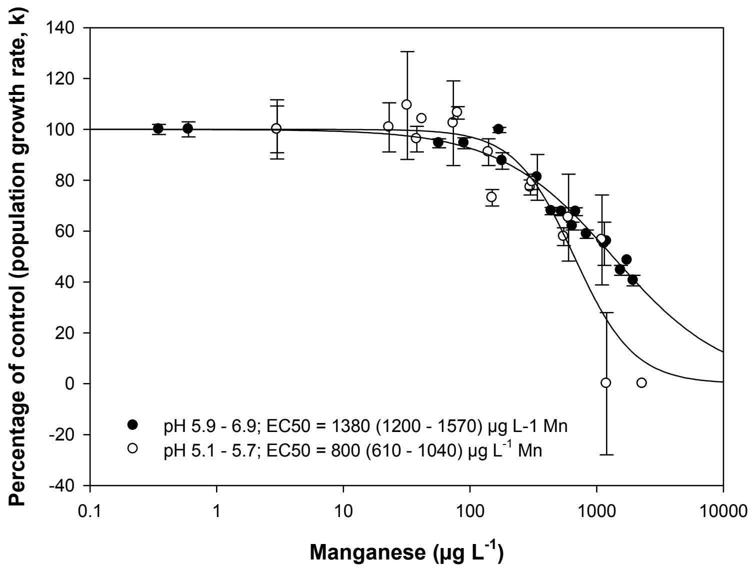

Figure 5 Comparison of Mn toxicity to hydra at two different pH concentrations. The

3.3 Derivation of a Trigger Value for Magela Creek

The toxicity estimates from the Magela Creek Water study (Table 2) were used to construct a SSD and derive a 99% Protection TV (Figure 5). The 99% TV derived from the SSD was 4.1 (0.7 – 182) µg L^{-1} Mn, which is below the 50th percentile of the concentrations measured at the Magela Creek Upstream Monitoring site (MCUS; Figure 6). International data from toxicity tests conducted in a natural water (Pinelands, New Jersey, USA) with a similar physico-chemistry to MCW (i.e. temperature = 24–25°C, pH = 6.7, alkalinity = 8 mg L^{-1}, hardness = 12 mg L^{-1} and DOC = 12 mg L^{-1}) were combined with the site-specific data in order to improve the SSD. This approach produced a 99% TV of 73 (33 -466) µg L^{-1} Mn (Figure 7). The 95th and 80th confidence limits of this TV were 33 and 46 µg L^{-1}, respectively.

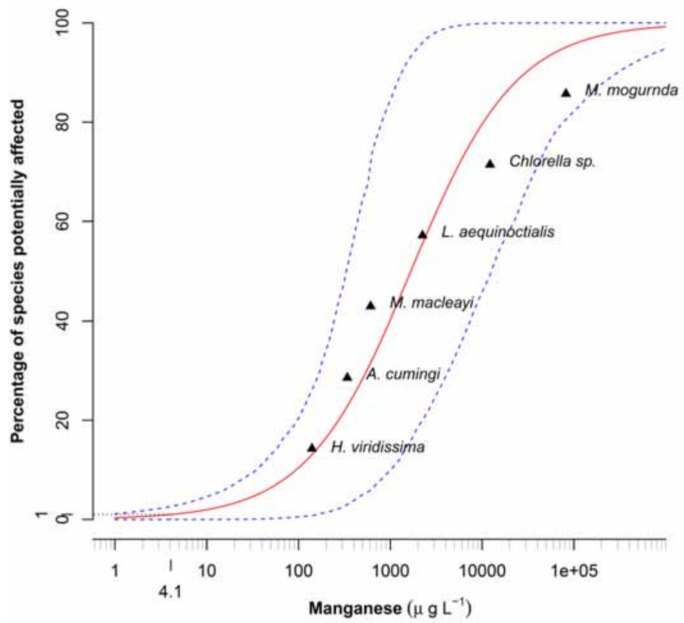

Figure 6 Species Sensitivity Distribution of manganese toxicity estimates for the six local species.

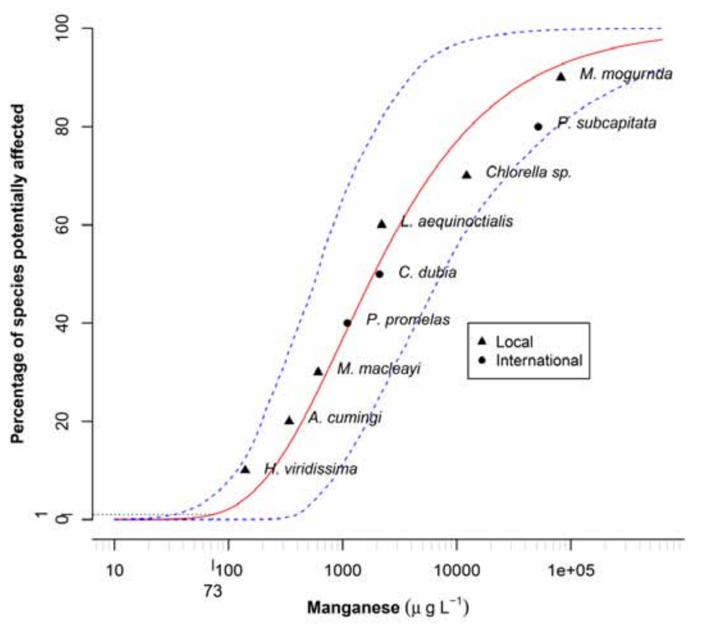

Figure 7 Species Sensitivity Distribution of manganese toxicity estimates for the six local species and including 3 toxicity estimates from international datasets (*P. subcapitata*, *C. dubia* and *P. promelas*).

4 Discussion

The results from the pilot study using Ngarradj Creek Water found that Mn toxicity was relatively high to *H. viridissima* and *M. macleayi* compared to values reported in the literature and those toxicity estimates used in the ANZEEC and ARMCANZ (2000) default TV. Conversely, the green alga, *Chlorella* sp. was extremely tolerant to Mn and was only effected by 10–15% by concentrations up to 50 000 μg L^{-1}. The Mn appeared acutely toxic to *M. macleayi* as there were similar concentration-response relationships for the chronic and acute endpoints. Removal of the algal food from the *M. macleayi* test system aimed to determine if the algae were creating microenvironments that produced Mn oxides, which would reduce the bioavailable Mn^{2+} (Richardson et al. 1988). This test did produce a slightly more sensitive response compared to the test with algal food but organism in this test did not reproduce optimally due to their need for an algal food source. Hence, it was impossible to determine if the higher sensitivity was due to Mn bioavailability or because the organisms were stressed. The hydra, *H. viridissima*, was clearly the most sensitive species with a significant reduction in population growth rate at the lowest concentration tested (106 μg L^{-1}), with a resultant IC_{10} value of 60 μg L^{-1}. This result warranted further investigation because the concentration-response was not comprehensively characterised during the preliminary studies but it was the one of the most sensitive toxicity estimates reported in the literature at that time. Additionally, further characterisation of *M. macleayi's* strong threshold response was needed strengthen confidence in the toxicity estimates obtained by this study.

The results of the comprehensive study using MCW as the diluent found that three of the tropical species were more sensitive to Mn than most of species in the international literature (Figure 7). Namely, *M. macleayi*, *A. cumingi* and *H. viridissima* showed a relatively high sensitivity to Mn and only one other international species, the amphipod, *Hyalella azteca,* was more sensitive to Mn exposure (Peters et al. 2010). However, the toxicity estimates for *H. azteca* varied markedly when different container materials were used. Toxicity tests performed in glass resulted in high sensitivity to Mn (i.e. EC25 = ~100 μg L^{-1}) (Norwood et al. 2007, Peters et al. 2010), while toxicity tests performed in high density polyethylene resulted in a markedly different EC25 of 7000 μg L^{-1}. This difference was not explained but concurring toxicity estimates in glass were consistently were derived by two different research groups. Hence, the values were considered reliable by Peters et al. (2010) and used for the European EQS. One other study has reported a more sensitive Mn toxicity estimate than *H. azteca*. Fargašová (1997) reported 43% mortality of the midge larva, *Chironomus plumosus*, at 55 μg L^{-1} Mn. However, this was the only concentration tested and many details of the test method (e.g. physico-chemistry of diluent water, chemical analysis of the test chemical) were not described, making it difficult to establish the quality of the data. Hence, this result was not used by ANZECC and ARMCANZ (2000) in the derivation of the default TV or the more recent European EQS (Peters et al. 2010).

Strong concentration-response relationships with r^2 values >0.9 were established for all species except *M. mogurnda*. The concentration-response relationship for *M. mogurnda* may have been better characterised with further toxicity testing but the fit of the logistic model was reasonable (r^2 = 0.78) and there was clearly no effect at concentrations up to ~100 000 μg L^{-1}. The IC10 of 80 000 μg L^{-1} appeared accurate and further testing was unlikely to produce a toxicity estimate that would affect the TV because the IC10 was 100 times higher than the most sensitive species. Overall, despite the extremely broad range of toxicity estimates, the values obtained in these tests were what would be expected in the local soft waters.

The higher toxicity found in three species of this study is possibly due to the low hardness and ionic strength of the soft waters of Magela and Ngarradj Creeks. Research involving the development of a Biotic Ligand Model (BLM) for Mn has reported that there is competition between Mn and cations in solution, primarily H^+ and Ca^{2+} (Peters et al. 2011). Other studies have also specifically demonstrated the amelioration of Mn toxicity by increasing water hardness (Lasier et al. 2000). The present study assessed Mn under conditions of extremely low water hardness (i.e. ~5 mg L^{-1} as $CaCO_3$) and, thus, Mn was expected to be of higher toxicity. However, it should be noted that Mn discharged from the mine could be associated with Mg^{2+} and Ca^{2+} concentrations that are higher than typical Magela Creek concentrations. Higher Mg^{2+} and Ca^{2+} ameliorates Mn toxicity in exotic species but the ability of these major ions to ameliorate toxicity in local species was not studied. Conversely, Mg has a higher toxicity in the soft waters of Magela Creek compared to its toxicity in harder waters and the combined toxic effects of Mn and Mg in extremely soft waters of Magela creek is also unknown.

The low pH (i.e. pH <6.5) of MCW might be expected to reduce the toxicity if competition between H^+ and Mn^{2+} ions was significant. We found that *Chlorella* sp. had a similar insensitive response to Mn in both NCW and MCW (Figure 9). There might have been a difference in response at a concentration of 60 000 μg L^{-1} Mn, which resulted in a 50% effect in MCW but only a 10% effect in NCW. However, the start-of-test pH for NCW and MCW were similar at 5.9 and 6.2, respectively while the end-of test pH was 6.4 for both waters. Algae have been found to be particularly tolerant to Mn exposure in studies at low pH conducted by other researchers (Peters et al. 2010; Peters et al. 2011). Hence, the lower pH of NCW and MCW may have reduced the alga's sensitivity to Mn compared to other studies. Past studies have also reported that *Chlorella* sp. uptake and sensitivity to U is reduced at lower pH, with the hypothesis being that U also competes with H^+ ions (Franklin et al. 2000). Nevertheless, these observations do not further inform the role that pH play in determining Mn toxicity. Further studies would need to be initiated if the influence of toxicity modifying factors, such as pH and hardness, needs to be understood in the context of Magela Creek.

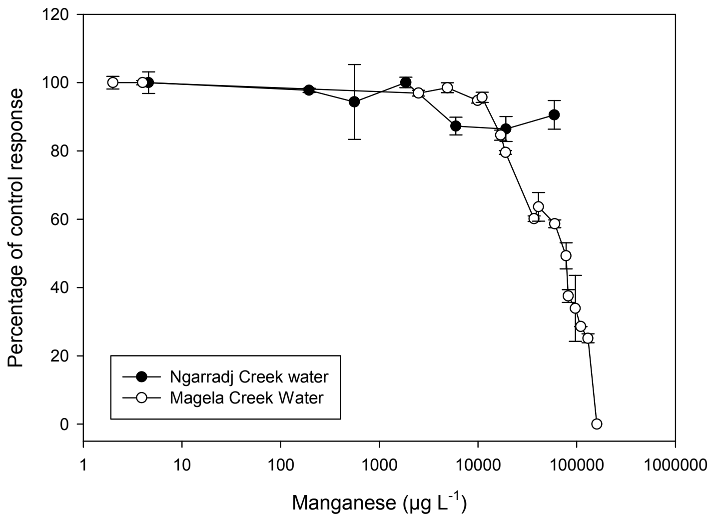

Figure 9 Comparison of the Mn toxicity to algae in Ngarradj Creek Water and Magela Creek Water.

Hydra viridissima was less sensitive in MCW compared with the preliminary Mn toxicity testing undertaken using NCW, which might be due to reduced bioavailability for the metal (Harford et al. 2009). Two hydra toxicity tests conducted at a low pH of 5.1 (Figure 8) indicated that decreasing pH may increase the toxicity of Mn to *H. viridissima*. However, the hydra in these tests did not meet the minimum acceptable growth rates, which indicates that the organisms may have been stressed at pH 5.1. The loss of Mn from the first two of the MCW hydra tests had not occurred in the hydra toxicity test conducted in NCW and was an unexpected occurrence. Manganese loss was also observed in one of the *A. cumingi* toxicity tests but this is a complex test system containing both glass and plastic. Hence, the potential for losing metals in this system was greater compared to the *H. viridissima* toxicity test, which is conducted in only plastic dishes.

The potential sources of Mn loss included adsorption to the test solution bottles and/or the test containers, precipitation and/or adsorption/absorption by the test animals. However, attempts to recover the Mn from the *H. viridissima* test were unproductive. The unrecoverable Mn may have been bound to the petri dishes and the 5% HNO_3 acid-extraction may have been insufficient extract the bound Mn. However, pre-inoculating the test dishes with Mn, with the aim of reducing Mn binding to the dishes, only slightly reduced Mn loss. Compared with no Mn pre-inoculation, the Mn loss in the 600 $\mu g\ L^{-1}$ treatment was reduced by ~20% but no reductions were noted in the 250 $\mu g\ L^{-1}$ treatment. Measured Mn in the hydra tissues at the end of the tests showed a good relationship between nominal Mn concentration and hydra tissue concentrations (Figure 10) but the amount recovered did not account for the missing proportion of Mn. This suggests that the Mn was not bound to the dishes or absorbed/adsorbed by the hydra and hence, the fate of some of the Mn is unknown. Although there was some difference in the pH of the test diluents between the NCW and MCW studies (pH 6.2 for NCW compared to pH 6.5 for the MCW) and even though Mn speciation is pH-dependent, the kinetics of Mn speciation are extremely slow and such pH differences are considered unlikely to result in significant speciation changes over the 96-h time course of a hydra experiment (Barry Chiswell, University of Queensland, pers. Comm.). Furthermore, extensive chemical analysis of the 'old' waters (i.e. those used to expose the hydra for 24 h) showed that the loss was only measurable between 72 –96 h, or the last day of the test. This time also coincided with the appearance of a white floating precipitate, which was suspected to be an oxy-hydroxide of Mn. A speciation change due to an increase in pH was not responsible for the precipitation as the pH of test solution on day 4 was not higher than on the previous 3 days. The sudden loss of the Mn on the final day suggests that the reaction may be biologically catalysed. Indeed, *H. viridissima* contain a symbiotic *Chlorella* sp. that may be producing Mn oxidising microenvironments described by Richardson et al. (1988). Additionally, Mn-oxidising bacteria are well-known, reported to be ubiquitous in freshwater environments and are also credited for the majority of Mn oxidation (Tebo et al. 2005, Anderson et al. 2009). The intermittent appearance of Mn-oxidising bacteria would also explain why the loss of Mn was not experienced in the final two hydra toxicity tests. Manganese oxidising microorganism would need time to grow and might preferably proliferate in a Mn rich culture medium. However, this does not explain why the loss occurred only in the hydra test when tests on other species used the same water, which indicates that the hydra played a role. Further, experiments would be needed to determine if the loss was due to Mn oxidising bacteria and if hydra also participated in removing Mn from the system. Ultimately, the Mn losses were accounted for by averaging the start and end of test Mn concentrations in order to derive the toxicity estimates.

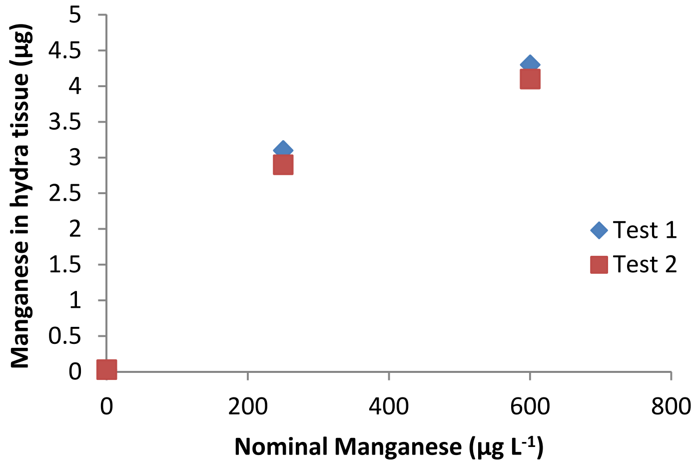

Figure 10 Amount of manganese measured in hydra tissues

The Species Sensitivity Distribution (SSD) using the six local species produced a 99% Trigger value of 4.1 (0.7 – 182) µg/L, which has been exceeded at least 50% of the time at the downstream monitoring site. Hence, it was a TV that could not be implemented. It is noteworthy that implementation of the European EQS for Mn was also problematic due to the same reason, i.e. it was too often exceeded. The European's solution to this issue was to recommend that the EQS was useful only in situations where Mn was of highest bioavailability and then developed a BLM to predict Mn toxicity for waters with other physico-chemical conditions (Peters et al. 2010; Peters et al. 2011). The low site-specific TV produced by this study was a result of the wide range of toxicity estimates used in the SSD. Ironically, it is the high toxicity estimates in the SSD that push the lower-end of the log-logistic model to lower concentrations. Including the extra international data to site-specific is a method recommended by ANZECC and ARMCANZ (2000) provided that the toxicity tests were conducted under relevant physico-chemical conditions. Additionally, researchers have recommended the inclusion of extra samples to SSDs in order to increase the reliability of the TV (Newman et al. 2000) and the European Commission and Australia are now recommending that a minimum of 8 toxicity estimates are needed for an "high reliability" TV (European Commission 2011; Batley et al. 2013). In this case, three toxicity estimates were identified as being conducted in natural water with sufficiently low hardness (12 mg L^{-1} $CaCO_3$, Ca = 4 mg L^{-1}) and a temperature similar to that used for the site specific species, i.e. 25°C compared to 27–29°C. The inclusion of these additional toxicity estimates produced a TV of 73 (33 – 466) µg L^{-1}, which has not been exceeded in the creek and can be implemented as a guideline value for the Ranger mine. The 95th and 80th confidence intervals of the statistical distribution were 33 and 46 µg L, which form the basis of the Focus and Action TVs.

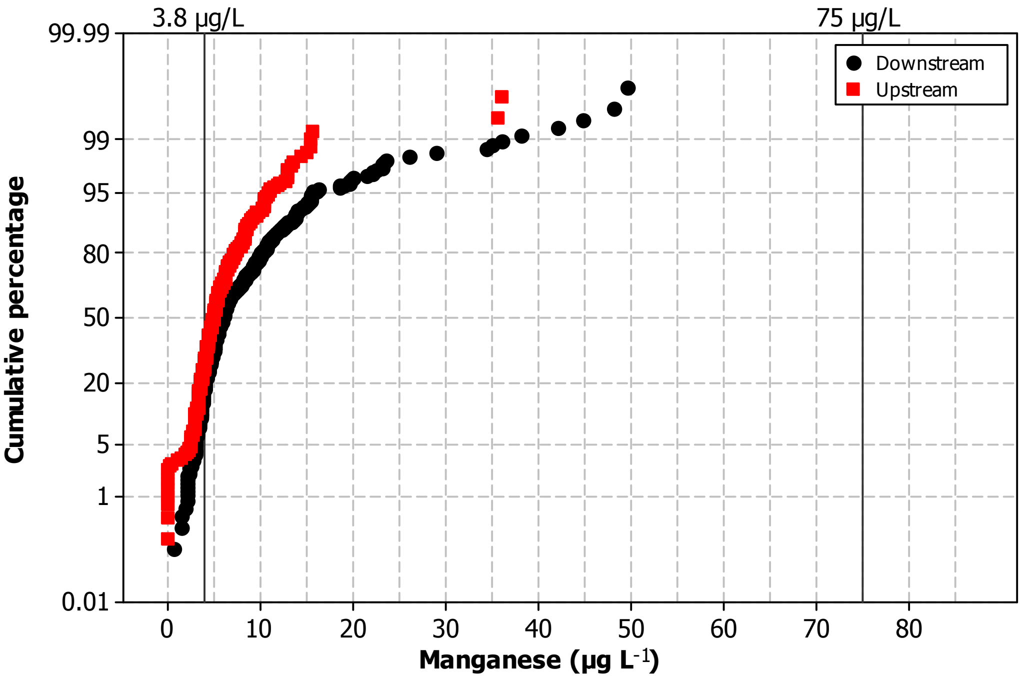

Figure 6 Comparison of environmental Mn chemistry (0.45 µm filtered) with the calculated 99% Trigger Values.

5 Recommendations

It is recommended that a 99% protection TV of 75 µg L^{-1} Mn be applied at MG009. The Focus and Action TVs should be 35 and 45 µg L^{-1}, respectively. These TVs are rounded out from the calculated 99% TV of 73 µg L^{-1} and the 95th and 80th confidence intervals of 33 and 46 µg L^{-1}, respectively.

6 Conclusions

The six local freshwater species tested in this study had a broad range of sensitivities to Mn in the soft surface waters of Ngarradj and Magela Creeks. For three of the species, Mn toxicity was higher than many of the species reported in the literature, which was probably due to the low concentration of Ca^{2+} in the natural waters. The low pH may have decreased to the toxicity of Mn to *Chlorella* sp. but increased the potential for Mn^{2+} to remain dissolved and, hence bioavailable. A loss of Mn was observed on the final day of a number of the *H. viridissima* toxicity tests but the Mn could not be recovered from the test system. This observation may be a result of the previously reported complex speciation of Mn. We accounted for such issues through extensive analysis of Mn (0.1 µm filtered and total) at the start and end of the tests. Toxicity estimates were adjusted using the average of measured Mn concentrations taken at the start and end of the toxicity tests. The Species Sensitivity Distribution, which used the three international toxicity estimates derived under relevant physico-chemical conditions, produced a 99% TV that can be implemented in Magela Creek

7 Acknowledgements

Approval for the ethical use of *M. mogurnda* was granted through the Charles Darwin University's Animal Ethics Committee. The authors would like to thank Alicia Hogan and Tom Mooney for their critical review of the report and Claire Costello and Ceiwen Pease for their technical expertise in conducting the toxicity tests.

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Appendix A Water quality measurements for toxicity tests

Ngarradj Creek Water

Table A1 936B *H. viridissima*

Treatment (µg L^{-1} Mn)		MCW		NCW (0)		200		660		2000		6600		20000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	6.6	7.0	6.2	6.4	6.1	6.4	6.1	6.4	6.1	6.3	6.2	6.3	5.8	6.2
	EC (µS cm^{-1})	17	17	12	13	13	14	16	17	21	22	42	41	97	92
	DO (%)	101	94	100	95	97	98	100	95	95	93	101	95	102	93
Day 1	pH	6.7	6.9	6.1	6.4	6.0	6.4	6.0	6.4	6.0	6.3	6.0	6.2	NM	NM
	EC (µS cm^{-1})	16	17	12	14	13	14	16	17	22	22	42	43	NM	NM
	DO (%)	109	95	105	97	107	98	106	93	102	93	108	96	NM	NM
Day 2	pH	6.7	6.9	6.2	6.6	6.1	6.4	6.1	6.3	6.1	6.3	NM	NM	NM	NM
	EC (µS cm^{-1})	16	18	12	14	13	14	16	17	22	22	NM	NM	NM	NM
	DO (%)	116	95	114	93	114	94	113	96	113	97	NM	NM	NM	NM
Day 3	pH	6.5	6.9	6.3	6.4	6.0	6.4	6.0	6.3	6.0	6.3	NM	NM	NM	NM
	EC (µS cm^{-1})	16	17	13	13	13	14	16	16	21	22	NM	NM	NM	NM
	DO (%)	118	95	115	95	119	96	117	96	113	95	NM	NM	NM	NM

[a] NM = Not measured due to complete mortality in the treatment

Table A2 937D *M. macleayi*

Treatment (µg L^{-1} Mn)		MCW		NCW (0)		1000		2000		4000		8000		16000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	6.8	7.0	6.2	6.4	6.2	6.5	6.1	6.2	6.1	6.4	6.2	6.3	6.1	6.3
	EC (µS cm^{-1})	21	26	17	16	20	19	24	23	32	31	49	48	32	81
	DO (%)	100	94	106	91	103	93	102	89	104.9	95.5	102.3	93.5	99.0	93.6
Day 1	pH	6.8	7.4	6.4	7.0	6.4	6.8	6.4	6.6	NM [a]	NM	NM	NM	NM	NM
	EC (µS cm^{-1})	44	27	18	13	25	18	27	22	NM	NM	NM	NM	NM	NM
	DO (%)	103	100	102	97	94	96	106	99	NM	NM	NM	NM	NM	NM
Day 2	pH	6.7	7.3	6.1	6.5	6.2	6.7	6.1	NM	NM	NM	NM	NM	NM	NM
	EC (µS cm^{-1})	21	26	14	13	18	27	23	NM	NM	NM	NM	NM	NM	NM
	DO (%)	106	24	108	25	103	24	103	NM	NM	NM	NM	NM	NM	NM
Day 3	pH	6.8	7.2	6.1	6.5	6.3	6.3	NM	NM	NM	NM	NM	NM	NM	NM
	EC (µS cm^{-1})	17	16	14	13	21	18	NM	NM	NM	NM	NM	NM	NM	NM
	DO (%)	109	102	111	98	109	99	NM	NM	NM	NM	NM	NM	NM	NM
Day 4	pH	6.7	7.6	6.3	6.4	6.3	6.3	NM	NM	NM	NM	NM	NM	NM	NM
	EC (µS cm^{-1})	17	16	14	14	18	18	NM	NM	NM	NM	NM	NM	NM	NM
	DO (%)	106	99	110	92	113	95	NM	NM	NM	NM	NM	NM	NM	NM
Day 5	pH	6.7	6.8	6.4	6.4	6.3	6.5	NM	NM	NM	NM	NM	NM	NM	NM
	EC (µS cm^{-1})	17	18	14	16	19	18	NM	NM	NM	NM	NM	NM	NM	NM
	DO (%)	107	96	105	90	109	97	NM	NM	NM	NM	NM	NM	NM	NM

[a] NM = Not measured due to complete mortality in the treatment

Table A3 938I *M. macleayi* (acute)

Treatment (µg L^{-1} Mn)	MCW		0 (NCW)		1000		2000		4000		8000		16000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.6	6.9	5.6	6.2	5.8	6.0	5.7	6.0	5.7	5.9	5.7	5.9	5.6	5.9
EC (µS cm^{-1})	14	14	12	12	14	18	20	19	28	29	46	46	78	79
DO (%)	97	94	96	93	108	93	104	92	99.6	94.7	96.7	94.2	100.9	91.6

Table A4 939G *Chlorella* sp.

Treatment (µg L^{-1} Mn)	MCW		(NCW) 0		200		660		2000		6660		20000		66000	
Parameter	**0 h**	**72 h**	**0 h**	**72 h**	**0 h**	**72 h**	**0 h**	**72 h**	**0h**	**72 h**	**0h**	**72 h**	**0 h**	**72 h**	**0 h**	**72 h**
pH	6.3	6.7	5.9	6.5	5.9	6.3	5.9	6.5	6.0	6.5	5.93	6.42	5.9	6.4	NM	6.4
EC (µS cm^{-1})	44	42	43	41	44	42	46	44	52	50	72	71	126	126	NM	287
DO (%)	109	97	112	93	110	93	109	94	109	96.5	112	93	112	92	NM	89

[a] NM = Not measured due to complete mortality in the treatment

Magela Creek Water

Table A5 1275S *A. cumingi*

Treatment (µg L^{-1} Mn)	0		8x10³		4x10⁴		2x10⁵		1x10⁶		5x10⁶	
Parameter	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0h**	**24 h**
pH	6.5	6.9	6.4	6.9	6.4	7.1	5.8	7.0	3.7	4.9	3.1	3.5
EC (µS cm^{-1})	16	37	44	66	149	176	560	582	2150	1950	7630	6900
DO (%)	100	83	106	80	106	83	108	83	108	87	103	89
pH	6.5	7.1	6.5	7.0	6.5	7.0	5.8	7.1	NM	NM	NM	NM
EC (µS cm^{-1})	17	36	47	69	151	177	562	602	NM	NM	NM	NM
DO (%)	96	85	106	84	102.5	82	104	80	NM	NM	NM	NM
pH	6.5	6.9	6.6	6.9	6.5	7.0	5.9	6.8	NM	NM	NM	NM
EC (µS cm^{-1})	16	35	45	60	150	168	566	580	NM	NM	NM	NM
DO (%)	92	85	94	86	95	83	93	89	NM	NM	NM	NM
pH	6.3	7.0	6.3	7.1	6.4	7.0	NM	NM	NM	NM	NM	NM
EC (µS cm^{-1})	17	36	46	60	150	170	NM	NM	NM	NM	NM	NM
DO (%)	99	87	105	83	102	84	NM	NM	NM	NM	NM	NM

[a] NM = Not measured due to complete mortality in the treatment

Table A6 1276L *L. aequinoctialis*

Treatment (µg L^{-1} Mn)	0		80		400		2000		10 000		50 000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.2	6.5	6.1	6.6	6.1	6.4	6.1	6.4	6.1	6.5	6.0	6.1
EC (µS cm^{-1})	19	17	20	15	22	16	27	23	58	56	192	195
DO (%)	107	95	106	81	108	90	108	97	107	89	109	87

Table A7 1277B *H. viridissima*

Treatment (µg L^{-1} Mn)		0		31		63		125		250		500		1000	
Parameter		**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**
Day 0	pH	6.5	6.7	6.5	6.7	6.4	6.7	6.5	6.6	6.5	6.6	6.5	6.7	6.5	6.7
	EC (µS cm^{-1})	18	18	17	18	17	18	17	18	17	18	19	20	21	21
	DO (%)	104	88	106	91	112	88	114	91	107	86	103	90	104	86
Day 1	pH	6.7	6.7	6.6	6.7	6.5	6.8	6.4	6.7	6.5	6.8	6.5	6.7	6.6	6.6
	EC (µS cm^{-1})	17	20	17	18	17	18	17	18	17	18	19	20	20	22
	DO (%)	103	90	108	91	113	93	105	93	110	89	101	91	106	91
Day 2	pH	6.5	6.7	6.5	6.8	6.5	6.8	6.4	6.8	6.4	6.7	6.5	6.7	6.4	6.6
	EC (µS cm^{-1})	16	18	17	18	17	18	17	18	17	18	18	19	20	21
	DO (%)	106	94	105	94	109	95	109	94	109	94	109	94	108	92
Day 3	pH	6.6	6.9	6.5	6.8	6.5	6.8	6.5	6.9	6.5	6.8	6.5	6.8	6.5	6.6
	EC (µS cm^{-1})	17	17	17	17	17	17	17	17	17	17	19	17	20	20
	DO (%)	104	96	108	92	114	93	114	93	113	89	111	91	110	92

Table A8 1278G *Chlorella* sp.

Treatment (µg L^{-1} Mn)	0		31250		62500		125000		250000		500000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.4	6.6	6.2	6.5	6.3	6.5	6.2	6.4	6.3	6.5	6.3	6.6
EC (µS cm^{-1})	47	45	236	236	273	275	461	466	793	795	1330	1344
DO (%)	116	97	113	92	106	93	110	89	104	91.4	103.8	90

Table A9 1292G *Chlorella* sp.

Treatment (µg L^{-1} Mn)	0		2500		5000		10000		20000		40000		80000		160000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.1	6.5	6.2	6.5	6.2	6.5	6.2	6.5	6.2	6.5	6.2	6.4	6.2	6.3	6.0	6.1
EC (µS cm^{-1})	46.0	43.0	57.0	54.0	67.0	64.0	87.0	85.0	122	121	186	187	326	328	558	564
DO (%)	108.4	90.2	109.1	93.1	104.4	92.5	105.1	90.0	106	95.1	100.6	94	101.9	95.3	103.9	94

Table 10 1294G *Chlorella* sp.

Treatment (µg L^{-1} Mn)	0		10000		20000		40000		60000		80000		100000		120000		140000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.2	6.6	6.2	6.6	6.3	6.5	6.2	6.4	6.2	6.4	6.2	6.4	6.2	6.4	6.2	6.4	6.2	6.4
EC (µS cm^{-1})	47.0	43.0	90.0	85.0	127.0	123.0	198.0	198.0	271	268	330	332	387	396	449	454	509	514
DO (%)	104.2	97.4	108.9	93.8	106.6	93.0	108.9	94.6	101.9	90.4	99.8	91.6	102.2	91.7	98.5	91.4	97.7	92

Table A11 1276L *L. aequinoctialis*

Treatment (µg L^{-1} Mn)	0		80		400		2000		10000		50000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.2	6.5	6.1	6.6	6.1	6.4	6.1	6.4	6.1	6.5	6.0	6.1
EC (µS cm^{-1})	19	17	20	15	22	16	27	23	58	56	192	195
DO (%)	107	95	106	81	108	90	108	97	107	89	109	87

Table A12 1279L *L. aequinoctialis*

Treatment (µg L^{-1} Mn)	0		1000		4000		6000		8000		20000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.5	7.1	6.4	6.9	6.3	6.5	6.3	6.7	6.1	6.5	6.0	6.2
EC (µS cm^{-1})	24	23	29	25	42	40	50	49	59	60	106	106
DO (%)	102	92	99	93	104	93	103	94	103	90	96	0

Table A13 1297L *L. aequinoctialis*

Treatment (µg L^{-1} Mn)	0		2500		5000		10000		15000		20000		25000		30000		40000	
Parameter	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**	**0h**	**72h**
pH	6.5	7.0	6.3	6.8	6.5	6.5	6.4	6.7	6.4	6.7	6.4	6.8	6.61	6.81	6.5	6.9	6.5	6.9
EC (µS cm^{-1})	23.0	19.0	33.0	28.0	44.0	41.0	65.0	64.0	84.0	82.0	104.0	104.0	123	123	141.0	140.0	174	177
DO (%)	96.1	88.6	92.2	88.8	88.1	89.0	97.9	87.2	97.2	89.0	97.2	90.0	99.9	89.8	95.5	85.1	95.5	87.2

Table A14 1277B *H. viridissima*

Treatment (µg L^{-1} Mn)		0		31		63		125		250		500		1000	
Parameter		**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**	**0h**	**24h**
Day 0	pH	6.5	6.7	6.5	6.7	6.4	6.7	6.5	6.6	6.5	6.6	6.5	6.7	6.5	6.7
	EC (µS cm^{-1})	18	18	17	18	17	18	17	18	17	18	19	20	21	21
	DO (%)	104	88	106	91	112	88	114	91	107	86	103	90	104	86
Day 1	pH	6.7	6.7	6.6	6.7	6.5	6.8	6.4	6.7	6.5	6.8	6.5	6.7	6.6	6.6
	EC (µS cm^{-1})	17	20	17	18	17	18	17	18	17	18	19	20	20	22
	DO (%)	103	90	108	91	113	93	105	93	110	89	101	91	106	91
Day 2	pH	6.5	6.7	6.5	6.8	6.5	6.8	6.4	6.8	6.4	6.7	6.5	6.7	6.4	6.6
	EC (µS cm^{-1})	16	18	17	18	17	18	17	18	17	18	18	19	20	21
	DO (%)	106	94	105	94	109	95	109	94	109	94	109	94	108	92
Day 3	pH	6.6	6.9	6.5	6.8	6.5	6.8	6.5	6.9	6.5	6.8	6.5	6.8	6.5	6.6
	EC (µS cm^{-1})	17	17	17	17	17	17	17	17	17	17	19	17	20	20
	DO (%)	104	96	108	92	114	93	114	93	113	89	111	91	110	92

Table A15 1290B *H. viridissima*

Treatment (µg L^{-1} Mn)		0		31.25		62.5		125		250		500		1000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	6.6	6.5	6.5	6.6	6.4	6.7	6.4	6.6	6.3	6.6	6.4	6.6	6.4	6.6
	EC (µS cm^{-1})	15.0	16.0	14.0	15	14.0	15.0	15.0	16.0	16.0	16.0	16.0	17.0	18.0	19.0
	DO (%)	109.3	92.8	111.5	95	118.9	94.9	113.1	96.3	117.3	94.1	109.2	96.0	109.4	94.6
Day 1	pH	6.4	6.4	6.4	6.6	6.4	6.6	6.4	6.6	6.3	6.6	6.3	6.6	6.3	6.5
	EC (µS cm^{-1})	14.0	16.0	14.0	15	14.0	15.0	14.0	15.0	15.0	15.0	15.0	16.0	17.0	18.0
	DO (%)	107.4	94.3	113.8	98	108.1	95.6	117.9	96.5	119.3	96.7	112.0	92.8	108.9	92.7
Day 2	pH	6.4	6.5	6.5	6.7	6.3	6.6	6.4	6.6	6.4	6.6	6.4	6.6	6.4	6.5
	EC (µS cm^{-1})	14.0	15.0	14.0	15	14.0	15.0	14.0	15.0	15.0	15.0	16.0	16.0	17.0	18.0
	DO (%)	108.9	93.9	114.6	94	115.5	93.4	117.8	93.1	115.8	96.0	110.9	94.9	114.1	94.9
Day 3	pH	6.3	6.4	6.4	6.5	6.5	6.5	6.7	6.5	6.6	6.5	6.5	6.5	6.4	6.5
	EC (µS cm^{-1})	14.0	15.0	14.0	15	14.0	15.0	14.0	15.0	15.0	15.0	16.0	16.0	17.0	18.0
	DO (%)	103.9	92.8	107.3	95	105.0	93.7	110.1	93.9	108.6	92.5	105.1	92.9	106.5	91.8

Table A16 1310B *H. viridissima*

Treatment (μg L^{-1} Mn)		0		250		500		750		1000		1250		1750		2000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0h**	**24 h**	**0h**	**24 h**	**0h**	**24 h**
Day 0	pH	6.6	6.9	6.4	6.8	6.4	6.8	6.5	6.8	6.4	6.8	6.5	6.8	6.4	6.7	6.4	6.7
	EC (μS cm^{-1})	19	19	20	20	20	20	21	21	22	23	24	22	25	25	28	26
	DO (%)	100.5	89.4	103.9	91.1	103.7	92.9	100.4	93.4	101.1	88.7	99.1	92.9	103.4	92.3	102.3	90.8
Day 1	pH	6.4	6.7	6.4	6.8	6.3	6.7	6.4	6.7	6.4	6.6	6.3	6.7	6.4	6.7	6.4	6.7
	EC (μS cm^{-1})	17	19	18	19	20	20	20	21	21	23	22	22	24	25	26	26
	DO (%)	110.9	88.2	110.3	90.4	107.8	92.5	112.9	90.5	114.1	93.6	114.2	90.6	112.5	89.9	112	98.8
Day 2	pH	6.4	6.7	6.5	6.7	6.4	6.8	6.4	6.7	6.4	6.7	6.4	6.7	6.4	6.7	6.4	6.6
	EC (μS cm^{-1})	17	19	18	20	20	20	20	21	22	23	23	22	24	25	26	27
	DO (%)	104.2	90.8	112.4	91.4	108	91	112.5	86.7	112	92	111.1	93.8	106.1	92.6	99.7	89.5
Day 3	pH	6.4	7.0	6.4	6.9	6.5	7.0	6.5	6.9	6.5	6.8	6.5	6.9	6.5	6.8	6.6	6.8
	EC (μS cm^{-1})	18	18	19	18	19	20	21	20	22	23	22	21	24	23	26	26
	DO (%)	113.1	84.2	118.5	89.5	118.6	87.3	112.8	89.4	101.2	91	114.5	88	112.3	91	108.5	90.7

Table A17 1318B *H. viridissima*

Treatment (µg L^{-1} Mn)		0		50		100		200		400		600		800		1400		2000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0h**	**24 h**	**0h**	**24 h**	**0h**	**24 h**	**0h**	**24 h**
Day 0	pH	6.1	6.4	6.0	6.4	6.0	6.4	6.0	6.4	6.0	6.4	6.0	6.5	5.9	6.4	6.0	6.4	5.9	6.4
	EC (µS cm^{-1})	15	16	15	16	15	16	16	16	17	17	17	18	18	20	21	22	24	24
	DO (%)	107.3	93.7	109.1	93.3	108.8	93.7	109	92.3	107.3	93.6	103.4	92.6	107.8	92.2	109	93	105	91.2
Day 1	pH	6.1	6.4	6.1	6.5	6.1	6.4	6.1	6.4	6.1	6.4	6.1	6.4	6.0	6.4	6.1	6.4	6.1	6.4
	EC (µS cm^{-1})	16	16	15	16	15	16	16	16	17	18	17	18	18	19	21	22	23	24
	DO (%)	111.4	89	114.3	92	111.7	94.3	114.4	92.9	110.8	90.6	110.4	93.1	112.2	94.3	110.5	93.3	110.8	93.8
Day 2	pH	6.2	6.4	6.2	6.4	6.1	6.4	6.1	6.4	6.2	6.4	6.1	6.4	6.1	6.4	6.1	6.4	6.2	6.4
	EC (µS cm^{-1})	15	17	15	16	15	16	15	16	16	17	17	18	18	19	21	22	23	24
	DO (%)	109	93.4	113.8	94.7	110.6	93.1	103	92.7	110.1	89.9	108.1	92.7	112.9	93.1	112.2	92.7	113.6	92.8
Day 3	pH	6.2	6.6	6.2	6.6	6.2	6.5	6.2	6.5	6.2	6.5	6.1	6.5	6.1	6.5	6.1	6.5	6.2	6.5
	EC (µS cm^{-1})	16	15	15	15	15	15	16	16	16	16	17	17	18	18	21	21	23	24
	DO (%)	106.6	89.9	115	92.7	112.7	93	115	92.3	116.2	92.1	116.7	92.7	118.8	93.8	115.1	91.8	114.6	92.2

Table A18 1299D *M. macleayi*

Treatment (µg L^{-1} Mn)		0		50		100		200		400		600		800		1000		1200	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	6.3	6.6	6.4	6.6	6.5	6.6	6.4	6.7	6.4	6.6	6.4	6.6	6.4	6.6	6.4	6.67	6.4	6.6
	EC (µS cm^{-1})	17.0	19.0	19.0	18.0	18.0	19.0	18.0	18.0	18.0	20.0	20.0	21.0	20.0	21.0	22	22	23.0	23.0
	DO (%)	95.5	88.1	99.5	90.0	98.1	91.3	100.3	91.9	97.5	90.2	94.8	91.8	96.8	89.6	96.1	89.7	98.1	87.6
Day 1	pH	6.5	6.5	6.5	6.6	6.5	6.6	6.5	6.7	6.5	6.7	6.5	6.7	6.5	6.6	6.4	6.7	6.4	6.6
	EC (µS cm^{-1})	17.0	18.0	19.0	19.0	19.0	18.0	19.0	19.0	20.0	20.0	21.0	21.0	22.0	22.0	22	22	23.0	23.0
	DO (%)	102.8	90.9	99.8	92.1	100.7	89.7	101.6	89.8	105.6	90.2	99.9	89.4	102.6	90.7	98.3	90.2	100.3	90.0
Day 2	pH	6.6	6.5	6.5	6.6	6.5	6.6	6.5	6.6	6.6	6.6	6.6	6.6	6.6	6.6	6.5	6.5	6.5	6.5
	EC (µS cm^{-1})	19.0	19.0	18.0	19.0	19.0	19.0	19.0	19.0	20.0	20.0	21.0	21.0	22.0	22.0	22	23	23.0	23.0
	DO (%)	104.5	91.6	102.9	92.1	106.3	88.1	101.7	90.9	101.3	94.6	102.0	93.9	99.9	94.9	103.9	94.1	102.6	97.0
Day 3	pH	6.5	6.6	6.5	6.6	6.5	6.8	6.5	6.6	6.5	6.6	6.5	6.7	6.5	6.6	6.5	6.6	6.5	6.6
	EC (µS cm^{-1})	18.0	19.0	19.0	19.0	19.0	18.0	19.0	19.0	20.0	20.0	21.0	21.0	22.0	21.0	22	22	23.0	23.0
	DO (%)	98.9	89.0	100.5	90.2	97.4	90.2	100.2	89.8	101.4	91.0	100.5	89.8	98.5	90.4	102.1	91.2	99.6	88.7
Day 4	pH	6.5	6.7	6.4	6.7	6.5	6.7	6.5	6.7	6.4	6.7	6.4	6.7	6.5	6.6	6.4	6.6	6.4	6.6
	EC (µS cm^{-1})	18.0	18.0	19.0	19.0	19.0	19.0	19.0	19.0	20.0	19.0	21.0	20.0	22.0	22.0	22	22	23.0	23.0
	DO (%)	101.8	93.3	106.0	92.5	103.9	93.7	105.8	93.8	104.9	94.2	104.3	97.8	106.4	96.8	106.2	94.9	104.5	94.4
Day 5	pH	6.3	6.6	6.5	6.6	6.4	6.7	6.4	6.6	6.5	6.6	6.4	6.6	6.4	6.6	6.5	6.7	6.4	6.7
	EC (µS cm^{-1})	18.0	18.0	19.0	18.0	19.0	18.0	19.0	19.0	20.0	20.0	21.0	21.0	21.0	22.0	21	22	23.0	22.0
	DO (%)	107.5	90.9	109.3	88.4	106.3	91.0	115.9	87.7	104.8	88.9	108.6	90.1	111.5	84.1	113.1	86.9	101.6	86.7

Table A19 1345D *M. macleayi*

Treatment (µg L^{-1} Mn)		0		125		250		500		750		1000		1500		2000		3000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**
Day 0	pH	6.5	6.9	6.6	6.9	6.6	6.9	6.6	6.9	6.5	6.9	6.5	6.9	6.5	6.8	6.5	6.9	6.5	6.8
	EC (µS cm^{-1})	18.0	18.0	18.0	20.0	18.0	20.0	20.0	21.0	21.0	22.0	22.0	23.0	24.0	25.0	26	27	38.0	39.0
	DO (%)	100.8	91.1	110.2	92.4	108.0	92.3	105.8	92.3	106.6	91.1	107.9	93.3	102.0	89.9	101.3	90.3	104.9	90.1
Day 1	pH	6.7	7.0	6.7	6.9	6.7	6.9	6.6	6.9	6.6	6.9	6.6	6.8	6.7	6.8	6.7	6.9	6.6	NM
	EC (µS cm^{-1})	18.0	18.0	18.0	18.0	19.0	19.0	20.0	21.0	21.0	21.0	22.0	22.0	25.0	25.0	25	27	39.0	NM
	DO (%)	106.1	91.2	107.1	90.6	103.4	94.1	98.1	92.2	103.2	91.1	100.0	92.4	100.3	90.6	98.8	91.3	99.0	NM
Day 2	pH	6.8	7.0	6.6	6.9	6.6	6.9	6.6	6.9	6.6	6.9	6.6	6.9	6.6	6.9	6.6	6.9	NM [a]	NM
	EC (µS cm^{-1})	18.0	19.0	19.0	19.0	19.0	20.0	20.0	21.0	21.0	22.0	22.0	23.0	24.0	27.0	27	28	NM	NM
	DO (%)	110.4	93.3	109.2	92.4	108.0	92.0	107.7	91.8	106.2	91.6	109.6	90.5	107.8	90.6	104.6	90.6	NM	NM
Day 3	pH	6.7	6.8	6.7	6.9	6.7	6.8	6.6	6.8	6.6	6.8	6.7	6.8	6.6	6.8	6.7	NM	NM	NM
	EC (µS cm^{-1})	18.0	19.0	18.0	19.0	19.0	20.0	20.0	21.0	21.0	22.0	22.0	23.0	25.0	26.0	27	NM	NM	NM
	DO (%)	109.6	88.3	109.9	84.3	111.5	89.3	108.2	87.8	104.3	87.7	104.3	90.0	106.7	91.3	103.2	NM	NM	NM
Day 4	pH	6.6	6.9	6.6	6.8	6.7	6.8	6.6	6.8	6.7	6.8	6.7	6.9	6.7	6.9	NM	NM	NM	NM
	EC (µS cm^{-1})	18.0	18.0	19.0	19.0	19.0	20.0	20.0	20.0	21.0	22.0	23.0	24.0	25.0	25.0	NM	NM	NM	NM
	DO (%)	103.5	91.1	106.4	90.5	110.2	91.3	107.6	90.2	103.2	89.4	105.4	89.3	103.5	89.4	NM	NM	NM	NM
Day 5	pH	6.6	6.9	6.7	6.9	6.7	6.8	6.6	6.8	6.8	6.8	6.7	6.8	6.7	6.8	NM	NM	NM	NM
	EC (µS cm^{-1})	19.0	19.0	20.0	19.0	20.0	20.0	21.0	21.0	23.0	22.0	23.0	23.0	25.0	25.0	NM	NM	NM	NM
	DO (%)	105.7	86.6	98.8	85.7	102.7	89.5	100.2	90.2	99.7	87.3	101.0	89.3	99.8	88.1	NM	NM	NM	NM

[a] NM = Not measured due to complete mortality in the treatment

Table A20 1275S *A. cumingi*

Treatment (µg L^{-1} Mn)		0		8000		40000		200000		1000000		5000000	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0h	24 h
Day 0	pH	6.5	6.9	6.4	6.9	6.4	7.1	5.8	7.0	3.7	4.9	3.1	3.5
	EC (µS cm^{-1})	16	37	44	66	149	176	560	582	2150	1950	7630	6900
	DO (%)	100	83	106	80	106	83	108	83	108	87	103	89
Day 1	pH	6.5	7.1	6.5	7.0	6.5	7.0	5.8	7.1	NM [a]	NM	NM	NM
	EC (µS cm^{-1})	17	36	47	69	151	177	562	602	NM	NM	NM	NM
	DO (%)	96	85	106	84	103	82	104	80	NM	NM	NM	NM
Day 2	pH	6.5	6.9	6.6	6.9	6.5	7.0	5.9	6.8	NM	NM	NM	NM
	EC (µS cm^{-1})	16	35	45	60	150	168	566	580	NM	NM	NM	NM
	DO (%)	91.8	85	94.2	85.5	95.4	83	92.6	89	NM	NM	NM	NM
Day 3	pH	6.3	7.0	6.3	7.1	6.4	7.1	NM	NM	NM	NM	NM	NM
	EC (µS cm^{-1})	17	36	46	60	150	170	NM	NM	NM	NM	NM	NM
	DO (%)	99	87	105	83	102	84	NM	NM	NM	NM	NM	NM

[a] NM = Not measured due to complete mortality in the treatment

Table A21 1307S *A. cumingi*

Treatment (µg L^{-1} Mn)		0		625		1250		2500		5000		10000	
Parameter		**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0 h**	**24 h**	**0h**	**24 h**
Day 0	pH	6.7	6.9	6.7	6.9	6.6	6.8	6.5	6.9	6.6	6.9	6.5	7.0
	EC (µS cm^{-1})	16	28	22	33	24	36	29	38	39	48	58	75
	DO (%)	91.3	85.3	92.9	84.6	97	80	94.9	85	92.8	87.4	92.6	83.4
Day 1	pH	6.6	7.1	6.6	7.0	6.5	7.0	6.5	7.0	6.5	7.0	6.4	6.9
	EC (µS cm^{-1})	17	27	20	34	23	37	25	40	38	48	58	73
	DO (%)	93.1	90	91.9	83.7	93.1	84.5	92.1	83.3	93.6	84.7	91.7	78
Day 2	pH	6.5	7.0	6.5	7.0	6.5	7.0	6.4	6.9	6.4	6.9	6.4	6.9
	EC (µS cm^{-1})	17	26	20	29	23	31	29	36	39	48	58	70
	DO (%)	99	86.9	96.5	89.2	100.2	88.1	99.3	89	97.6	85.8	96.4	90.2
Day 3	pH	6.5	7.0	6.6	7.0	6.5	7.1	6.4	7.0	6.6	7.0	5.7	7.0
	EC (µS cm^{-1})	17	28	22	29	23	32	29	37	38	49	58	68
	DO (%)	105.7	87.4	96.3	88.6	105.2	90.4	110.5	91.6	92.3	93.4	64.2	85.6

Table A22 1284E *M. mogurnda*

Treatment (µg L^{-1} Mn)		0		80		400		2000		10000		50000	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0h	24 h
Day 0	pH	6.5	6.8	6.5	6.7	6.5	6.7	6.5	6.6	6.6	6.6	6.4	6.5
	EC (µS cm^{-1})	18.0	21.0	18.0	21.0	20.0	22.0	27.0	29.0	60.0	62.0	205.0	205.0
	DO (%)	100.1	88.3	104.4	89.9	101.3	88.8	101.0	93.4	101.9	91.7	102.4	90.5
Day 1	pH	6.8	7.2	6.8	7.2	6.7	7.1	6.6	7.0	6.5	6.8	6.5	6.8
	EC (µS cm^{-1})	18.0	20.0	18.0	92.9	19.0	22.0	26.0	29.0	58.0	62.0	205.0	211.0
	DO (%)	98.0	91.2	99.8	24.5	98.1	92.3	98.7	92.2	100.3	91.2	97.4	90.7
Day 2	pH	7.0	7.1	7.0	7.1	7.0	7.1	6.9	6.9	6.9	6.8	6.8	6.9
	EC (µS cm^{-1})	18.0	22.0	18.0	20.0	19.0	22.0	26.0	29.0	59.0	63.0	204.0	211.0
	DO (%)	106.1	90.1	107.8	94.0	111.2	93.3	110.9	92.9	108.9	91.0	100.2	89.6
Day 3	pH	6.8	7.1	6.8	7.1	6.9	7.0	6.9	7.0	6.8	6.8	6.7	6.8
	EC (µS cm^{-1})	18.0	20.0	18.0	21.0	19.0	22.0	26.0	29.0	59.0	64.0	201.0	214.0
	DO (%)	105.9	23.1	109.0	89.5	112.8	84.5	111.1	91.3	112.6	92.0	111.2	87.6

Table A23 1293E *M. mogurnda*

Treatment (μg L^{-1} Mn)		0		12500		25000		50000		100000		150000		200000		250000		300000	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0h	24 h	0h	24 h	0h	24 h	0h	24 h
Day 0	pH	6.4	6.5	6.3	6.6	6.3	6.7	6.3	6.7	6.3	6.6	6.3	6.6	6.3	6.7	6.3	6.7	6.4	6.7
	EC (μS cm^{-1})	15	17	65	67	113	116	202	206	360	363	501	507	636	643	751	764	888	893
	DO (%)	100.2	94.6	103.1	98.3	102	93.8	98	97.9	102.1	92.5	97.5	93.9	94.3	93.3	95.4	90.1	97.7	88.8
Day 1	pH	6.4	6.5	6.3	6.7	6.6	6.6	6.6	6.6	6.7	6.5	6.5	6.7	6.6	6.6	6.5	6.6	6.65	6.7
	EC (μS cm^{-1})	15	17	64	68	112	118	201	209	360	374	500	516	635	654	760	784	884	908
	DO (%)	102.9	102.2	107.3	98.8	110.9	98.5	108.6	98.5	107.3	95.3	104	96.8	103.5	96	108.6	95.1	105.8	92.5
Day 2	pH	6.2	6.7	6.2	6.7	6.6	6.8	6.6	6.7	6.6	6.7	6.5	6.7	6.7	6.7	6.6	6.7	6.6	6.8
	EC (μS cm^{-1})	15	17	64	68	112	117	203	211	360	372	500	516	632	652	761	782	886	906
	DO (%)	118.1	95.9	117.3	97.2	122	97	119.2	99.7	116.9	98.7	116.7	97.7	114	96.2	116.3	96.2	116.4	95.3
Day 3	pH	6.3	6.7	6.3	6.7	6.4	6.7	6.3	6.8	6.4	6.8	6.4	6.8	6.4	6.8	6.4	6.8	6.5	6.8
	EC (μS cm^{-1})	15	18	64	69	112	118	203	211	360	367	501	513	633	655	762	788	884	910
	DO (%)	124.1	89.7	120.8	91.6	123.8	92.6	126.5	90.6	124	92	123.8	92.6	121.6	92.5	125.7	93.9	127.2	91.8

Table A24 1300E *M. mogurnda*

Treatment (µg L^{-1} Mn)		0		37500		75000		125000		175000		275000		350000		400000	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0h	24 h	0h	24 h	0h	24 h
Day 0	pH	6.5	6.9	6.4	6.6	6.4	6.6	6.3	6.6	6.3	6.7	6.3	6.7	6.3	6.7	6.3	6.8
	EC (µS cm^{-1})	15	18	162	167	280	287	433	441	585	593	819	827	990	1004	1107	1125
	DO (%)	98.9	87.3	105.7	93.4	105.2	90.1	103.9	91.3	101.7	90.9	100.4	90.5	101.8	92.8	98.6	87.1
Day 1	pH	6.3	7.0	6.4	6.7	6.6	6.7	6.4	6.8	6.4	6.8	6.4	6.8	6.5	6.8	6.4	7.0
	EC (µS cm^{-1})	15	18	161	165	280	288	432	444	581	599	814	830	980	1014	1100	1122
	DO (%)	102	93.5	105.2	93	99.8	93.4	106.6	91.8	107.5	95.2	104.3	95.1	103.2	93.9	103	91.4
Day 2	pH	6.4	7.1	6.4	6.8	6.4	6.8	6.5	6.9	6.4	6.9	6.5	7.0	6.5	7.0	6.6	7.0
	EC (µS cm^{-1})	15	18	160	166	280	289	432	444	583	599	813	838	986	1012	1103	1129
	DO (%)	100.1	94.5	113.5	95.8	111.2	91.9	109.9	96.8	111	95.7	106	93.3	110	92.4	110.4	91.2
Day 3	pH	6.5	6.9	6.4	6.6	6.5	6.7	6.5	6.9	6.6	6.9	6.5	6.9	6.6	6.9	NM	NM
	EC (µS cm^{-1})	15	19	160	166	280	293	433	456	582	603	808	835	985	1023	NM	NM
	DO (%)	101.2	89.2	113.6	90.7	109.6	92.7	114.4	90.3	114.7	89.3	113.8	93.4	114.8	92.2	NM	NM

Table A25 1379B *H. viridissima*

Treatment (µg L^{-1})		0		20		40		80		160		320		640		1200		2560	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0h	24 h	0h	24 h	0h	24 h	0h	24 h
Day 0	pH	5.1	5.7	5.2	5.6	5.2	5..5	5.2	5.6	5.1	5.6	5.2	5.6	5.2	5.7	5.3	5.6	5.0	5.6
	EC (µS cm^{-1})	10	11	11	11	10	10	10	11	11	11	11	12	13	14	16	16	21	22
	DO (%)	105	88	107	91	106	91	106	92	106	92	104	92	104	90	103	92	101	92
Day 1	pH	5.2	5.5	5.1	5.5	5.1	5.5	5.1	5.4	5.1	5.4	5.1	5.4	5.1	5.4	5.3	5.3	4.9	5.3
	EC (µS cm^{-1})	10	11	10	11	10	10	11	11	11	11	12	11	13	13	15	16	27	21
	DO (%)	103	94	107	92	104	96	105	95	105	93	103	93	103	95	99	92	100	94
Day 2	pH	5.2	5.7	5.2	5.7	5.1	5.6	5.2	5.6	5.1	5.5	5.1	5.6	5.1	5.5	5.2	5.5	5.0	5.4
	EC (µS cm^{-1})	10	11	10	11	10	10	10	11	11	11	11	12	13	13	15	16	22	22
	DO (%)	110	88	114	91	106	95	101	91	110	93	109	91	104	93	103	90	104	90
Day 3	pH	5.2	5.6	5.2	5.5	5.2	5.5	5.2	5.4	5.2	5.3	5.2	5.4	5.2	5.3	5.2	5.3	5.1	NM
	EC (µS cm^{-1})	10	10	10	13	10	12	10	11	11	11	11	13	13	11	15	16	21	NM
	DO (%)	11	92	110	96	111	92	110	93	108	95	116	94	109	95	113	93	110	NM

Table A26 1381B *H. viridissima*

Treatment (µg L^{-1})		0		20		40		80		160		320		640		1200		2560	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h	0h	24 h	0h	24 h	0h	24 h	0h	24 h
Day 0	pH	5.4	5.7	5.4	5.7	5.4	5.6	5.2	5.6	5.3	5.6	5.3	5.6	5.2	5.6	5.2	5.5	5.2	5.4
	EC (µS cm^{-1})	12	11	10	11	11	11	10	11	10	11	12	13	13	14	15	16	21	21
	DO (%)	106	93	99	93	105	94	106	90	106	90	102	94	102	93	103	92	105	91
Day 1	pH	5.5	5.8	5.5	5.9	5.5	5.6	5.3	5.6	5.3	5.6	5.3	5.5	5.2	5.5	5.3	5.5	5.3	5.3
	EC (µS cm^{-1})	11	11	10	11	10	11	10	11	10	11	11	12	13	13	15	16	21	22
	DO (%)	106	90	107	88	105	91	101	91	104	93	105	86	106	92	104	82	104	94
Day 2	pH	5.2	5.7	5.2	5.6	5.2	5.6	5.2	5.5	5.2	5.5	5.3	5.5	5.2	5.5	5.2	5.4	NM	NM
	EC (µS cm^{-1})	10	11	10	10	10	11	10	11	10	11	11	12	13	13	15	16	NM	NM
	DO (%)	101	93	105	94	103	93	103	94	104	93	102	92	106	93	100	92	NM	NM
Day 3	pH	5.3	5.4	5.3	5.4	5.3	5.4	5.1	5.3	5.1	5.3	5.2	5.3	5.2	5.3	5.1	5.3	NM	NM
	EC (µS cm^{-1})	10	10	10	10	10	10	10	10	10	11	11	12	12	13	15	16	NM	NM
	DO (%)	106	89	105	95	106	94	104	94	104	94	102	93	99	94	99	95	NM	NM

Table A27 Summary for control water

Species	Test	pH		EC (µS/cm)		DO (%)		DOC (mg/L)	Alkalinity (mg/L CaCO3)
		new	old	new	old	new	old		
Chlorella sp.	1278G	6.4	6.6	47	45	116	97	2.48	8
	1292G	6.1	6.5	46	43	108.4	90.2	1.8	7
	1294G	6.2	6.6	47	43	104.2	97.4	1.8	7
Lemna aequinoctialis	1276L	6.2	6.5	19	17	107	95	2.48	8
	1279L	6.5	7.1	24	23	102	92	2.48	8
	1297L	6.5	7.0	23	19	96	89	2.22	5
Hydra viridissima	1277B	6.6	6.8	17	18	104	92	2.6	7
	1290B	6.4	6.5	14	16	107	94	1.8	7
	1310B	6.4	6.8	18	19	107	88	2.7	8
	1318B	6.1	6.5	16	16	109	92	3.97	6
Moinodaphnia macleayi	1299D	6.4	6.6	18	19	102	90.6	2.22	5
	1345D	6.7	6.9	18	19	106	90.3	2.3	NM
Amerianna cumingi	1275S	6.5	7.0	17	36	97	85	2.48	8
	1307S	6.6	7.0	17	27	97	87.4	2.68	8
Mogurnda mogurnda	1284E	6.8	7.0	18	21	103	89.9	2.26	7
	1293E	6.4	6.6	15	17	111	95.6	1.8	7
	1300E	6.4	70	15	18	101	91	2.22	5
Average		**6.4**	**6.8**	**23**	**24**	**105**	**92**	**2.37**	**6.9**

Appendix B Chemical analyses

Table B1 Measured and predicted[1] Mn concentrations in the Ngarradj Creek Water tests

Test number/Code	Nominal Mn (µg L^{-1})	Start of test (µg L^{-1})		End of test (µg L^{-1})	
		Total Mn	0.1 µm filtered Mn	Total Mn	0.1 µm filtered Mn
Initial chronic cladoceran tests					
934D/933D Pro Blank[2]	0	0.3	NA[3]	NA	NA
934D/933D A	0 (NCW)	5.3	3.8	***5.3***	***4.6***
934D/933D B	20	7.2	6.5	NA	4.7[4]/4.3[5]
934D/933D C	63	70	60	***70***	***60***
934D/933D D	200	210	190	NA	150/150
934D/933D E	630	660	600	***660***	***570***
934D/933D F	2000	2040	1940	NA	1740/1800
Chronic hydra test					
936B Pro Blank	0	<0.01	NA	NA	NA
936B B	0 (NCW)	6.3	5.0	***6.3***	***5.5***
936B C	200	200	140	170	70
936B D	666	670	540	***670***	***580***
936B E	2000	2070	1800	2170	1650
936B F	6660	6600	6470	***6590***	***5700***
936B G	20,000	22,100	19200	21700	19100
Repeat chronic cladoceran test					
937D Pro Blank	0	0.06	NA	NA	NA
937D B	0 (NCW)	5.1	4.9	***5.1***	***4.4***
937D C	1000	1030	950	1010	800
937D D	2000	2080	1910	***2080***	***1800***
937D E	4000	4160	3800	4080	3700
937D F	8000	8380	7900	***8380***	***7250***
937D G	16000	16500	15500	16300	1510
Acute cladoceran test					
938I Pro Blank	0	0.06	NA	NA	NA
938I B	0 (NCW)	5.1	4.9	***5.1***	***4.4***
938I C	1000	1030	950	1010	590
938I D	2000	2080	1910	***2080***	***1800***
938I E	4000	4160	3800	4050	3570
938I F	8000	8380	7900	***8380***	***7250***
938I G	16000	16500	15500	16400	14700
Chlorella test					
939G Pro Blank	0	0.02	NA	NA	NA
939G B	0 (NCW)	5.2	4.7	***5.2***	***4.5***
939G C	200	220	200	***220***	***190***
939G D	1000	720	660	650	460
939G E	2000	2090	1910	***2090***	***1810***
939G F	8000	7180	6320	7030	5600
939G G	20000	21400	19800	***21400***	***18520***
939G H	66000	68300	***62500***	69000	59300

1 Predicted concentrations (shown in bold italics) were determine based on regression equations derived from the measured Mn concentrations, ie End of test total Mn = 1 x start of test total Mn (r^2 = 0.99); End of test filtered Mn = 0.87 x end of test Total Mn (r^2 = 0.99) .

2 Pro Blank=Procedural Blank 3 NA = Not Analysed

4 Measured Mn at the end of test 933D and 5 Measured Mn at the end of test 934D

Table B2 Measured manganese concentrations in the Magela Creek water tests

Test number/Code	Nominal Mn (μg/L)	Start of Test (μg L-1)		End of Test (μg L-1)	
		Total Mn	0.1 μm Filtered Mn	Total Mn	0.1 μm Filtered Mn
1st Sub-chronic snail test					
1275S Pro Blank	0	0.055	0.1	NM	NM
1275S Blank	0	<0.01	N.M	0.3	0.5
1275S A	0 (MCW)	3.1	2	0.38	0.5
1275S B	8000	6500	6400	5600	5500
1275S C	40000	32000	33000	29000	32000
2nd Sub-chronic snail test					
1307S Pro Blank	0	NM	2	NM	NM
1307S Blank	0	NM	NM	NM	<0.01
1307S A	0	NM	4	NM	<0.01
1307S B	625	NM	560	NM	350
1307S C	1250	NM	1200	NM	820
1307S D	2500	NM	2800	NM	1900
1307S E	5000	NM	5500	NM	4800
1307S F	10000	NM	11000	NM	10000
1st Chronic Lemna test					
1276L Pro Blank	0	0.23	NM	NM	NM
1276L Blank	0	0.3	0.1	1.2	0.4
1276L A	0	3.3	3	1.3	0.4
1276L B	80	NM	65	NM	NM
1276L C	400	320	310	140	130
1276L D	2000	NM	1700	NM	NM
1276L E	10000	8700	8700	8100	8300
1276L F	50000	NM	44000	NM	NM
2nd Chronic Lemna test					
1279L Pro Blank	0	0.31	0.05	NM	NM
1279L Blank	0	<0.01	NM	0.22	0.2
1279L A	0	1.8	2	3.4	3
1279L B	1000	NM	980	836	836
1279L C	4000	3900	3900	3400	3500
1279L D	6000	NM	6000	5334	5334
1279L E	8000	7700	7700	7200	7300
1279L F	20000	NM	19000	17928	17928

NM = Not measured

Table B2 continued Measured manganese concentrations in the Magela Creek water tests

Test number/Code	Nominal Mn (μg/L)	Start of test (μg L^{-1})		End of test (μg L^{-1})	
		Total Mn	0.1 μm filtered Mn	Total Mn	0.1 μm filtered Mn
3rd chronic Lemna test					
1297L Pro Blank	0	NM	<0.01	NM	NM
1297L Blank	0	NM	NM	NM	<0.01
1297L A	0	NM	6	NM	0.2
1297L B	2500	NM	2500	NM	1900
1297L C	5000	NM	5000	NM	4500
1297L D	10000	NM	10000	NM	9700
1297L E	15000	NM	15000	NM	14000
1297L F	20000	NM	20000	NM	19000
1297L G	25000	NM	24000	NM	24000
1297L H	30000	NM	29000	NM	28000
1297L I	40000	NM	39000	NM	39000
1st chronic hydra test					
1277B Pro Blank	0	0.14	0.3	NM	NM
1277B Blank	0	0.27	NM	0.042	0.06
1277B A	0	2.4	2	0.55	0.3
1277B B	31	32	31	NM	NM
1277B C	63	60	61	2.7	0.9
1277B D	125	120	120	NM	NM
1277B E	250	240	240	85	76
1277B F	500	476.0742	480	NM	NM
1277B G	1000	950	960	720	690
2nd chronic hydra test					
1290B Pro Blank	0	NM	<0.01	NM	NM
1290B Blank	0	NM	NM	<0.01	0.05
1290B A	0	NM	1	2.4	0.2
1290B B	31.25	NM	30	NM	0.5
1290B C	62.5	NM	59	NM	0.6
1290B D	125	NM	120	NM	2
1290B E	250	NM	230	85	48
1290B F	500	NM	440	NM	290
1290B G	1000	NM	850	780	660

NM = Not measured

Table B2 continued Measured manganese concentrations in the Magela Creek water tests

Test number/Code	Nominal Mn (μg/L)	Start of test (μg L-1)		End of test (μg L-1)	
		Total Mn	0.1 μm filtered Mn	Total Mn	0.1 μm filtered Mn
3rd chronic hydra test					
1310B Pro Blank	0	NM	<0.01	NM	NM
1310B Blank	0	NM	NM	0.02	NM
1310B A	0	NM	2	NM	0.5
1310B B	250	NM	230	NM	130
1310B C	500	NM	490	NM	390
1310B D	750	NM	710	NM	580
1310B E	1000	NM	890	830	780
1310B F	1250	NM	1200	NM	1100
1310B G	1750	NM	1600	NM	1500
1310B H	2000	NM	2000	NM	1900
4th chronic hydra test					
1318B Pro Blank	0	NM	0.01	NM	NM
1318B Blank	0	NM	NM	NM	<0.01
1318B A	0	NM	7	NM	5
1318B B	50	NM	62	NM	58
1318B C	100	NM	98	NM	96
1318B D	200	NM	180	NM	180
1318B E	400	NM	340	NM	340
1318B F	600	NM	540	NM	520
1318B G	800	NM	700	NM	710
1318B H	1400	NM	1200	NM	1200
1318B I	2000	NM	1700	NM	1700
1st Chronic algae test					
1278G Pro Blank	0	<0.010	NM	NM	NM
1278G Blank	0	<0.010	<0.01	<0.01	1
1278G A	0	0.43	2	3.5	6
128GL B	31250	NM	49000	NM	NM
1278G C	62500	59000	61000	60000	59000
1278G D	125000	NM	120000	NM	NM
1278G E	250000	230000	230000	230000	240000
1278G F	500000	NM	480000	NM	NM

NM = Not measured

Table B2 continued Measured manganese concentrations in the Magela Creek Water tests

Test number/Code	Nominal Mn (μg/L)	Start of test (μg L^{-1})		End of test (μg L^{-1})	
		Total Mn	0.1 μm filtered Mn	Total Mn	0.1 μm filtered Mn
2nd chronic algae test					
1292G Pro Blank	0	NM	0.02	NM	NM
1292G Blank	0	NM	<0.01	NM	0.4
1292G A	0	NM	4	NM	5
1292G B	2500	NM	2500	NM	2400
1292G C	5000	NM	4900	NM	4900
1292G D	10000	NM	11000	NM	10000
1292G E	20000	NM	19000	NM	19000
1292G F	40000	NM	37000	NM	38000
1292G G	80000	NM	82000	NM	83000
1292G H	160000	NM	160000	NM	160000
3rd chronic algae test					
1294G Pro Blank	0	NM	0.1	NM	NM
1294G Blank	0	NM	<0.01	NM	0.01
1294G A	0	NM	2	NM	4
1294G B	10000	NM	9900	NM	8900
1294G C	20000	NM	17000	NM	17000
1294G D	40000	NM	41000	NM	36000
1294G E	60000	NM	60000	NM	54000
1294G F	80000	NM	78000	NM	74000
1294G G	100000	NM	97000	NM	94000
1294G H	120000	NM	110000	NM	120000
1294G I	140000	NM	130000	NM	140000
1st fish test					
1284E Pro Blank	0	NM	<0.01	NM	NM
1284E Blank	0	0.89	NM	NM	NM
1284E A	0	NM	2	NM	2
1284E B	80	NM	99	NM	75
1284E C	400	NM	380	NM	390
1284E D	2000	NM	2000	NM	2000
1284E E	10000	NM	9800	NM	9700
1284E F	50000	NM	45000	NM	47000

NM = Not measured

Table B2 continued Measured manganese concentrations in the Magela Creek water tests

Test number/Code	Nominal Mn (μg/L)	Start of test (μg L^{-1})		End of test (μg L^{-1})	
		Total Mn	0.1 μm filtered Mn	Total Mn	0.1 μm filtered Mn
2nd fish test					
1293E Pro Blank	0	NM	0.8	NM	NM
1293E Blank	0	NM	<0.01	NM	<0.01
1293E A	0	NM	4	NM	7
1293E B	12500	NM	9300	NM	13000
1293E C	25000	NM	23000	NM	23000
1293E D	50000	NM	47000	NM	49000
1293E E	100000	NM	93000	NM	97000
1293E F	150000	NM	140000	NM	140000
1293E G	200000	NM	190000	NM	190000
1293E H	250000	NM	240000	NM	250000
1293E I	300000	NM	290000	NM	300000
3rd fish test					
1300E Pro Blank	0	NM	<0.01	NM	NM
1300E Blank	0	NM	<0.01	NM	<0.01
1300E A	0	NM	3	NM	5
1300E B	37500	NM	36000	NM	37000
1300E C	75000	NM	69000	NM	73000
1300E D	125000	NM	120000	NM	120000
1300E E	175000	NM	160000	NM	170000
1300E F	275000	NM	250000	NM	250000
1300E G	350000	NM	310000	NM	330000
1300E H	400000	NM	360000	NM	360000
1st cladoceran test					
1299D Pro Blank	0	NM	<0.01	NM	NM
1299D Blank	0	NM	NM	NM	<0.01
1299D A	0	NM	3	NM	3
1299D B	50	NM	54	NM	51
1299D C	100	NM	100	NM	93
1299D D	200	NM	210	NM	200
1299D E	400	NM	400	NM	390
1299D F	600	NM	590	NM	580
1299D G	800	NM	790	NM	820
1299D H	1000	NM	1000	NM	990
1299D I	1200	NM	1200	NM	1100

NM = Not measured

Table B2 continued Measured manganese concentrations in the Magela Creek water tests

2nd cladoceran test					
1345D Pro Blank	0	NM	<0.01	NM	NM
1345D Blank	0	NM	NM	NM	0.01
1345D A	0	NM	1	NM	3
1345D B	125	NM	120	NM	130
1345D C	250	NM	240	NM	240
1345D D	500	NM	480	NM	460
1345D E	750	NM	710	NM	700
1345D F	1000	NM	950	NM	950
1345D G	1500	NM	1500	NM	1500
1345D H	2000	NM	2000	NM	NM
1345D I	3000	NM	4800	NM	NM

NM = Not measured

Table B3 Measured elements in the Blank and Procedural Blank (Pro Blank) samples

Test code/Sample	Date Sampled	Al µg/L	Cd µg/L	Co µg/L	Cr µg/L	Cu µg/L	Fe µg/L	Mn µg/L	Ni µg/L	Pb µg/L	Se µg/L	U µg/L	Zn µg/L	Ca mg/L	Mg mg/L	Na mg/L	SO_4 mg/L
1275S Pro Blank	24/04/2012	0.9	<0.02	<0.01	<0.1	0.026	<1	0.055	0.15	0.23	<0.2	<0.001	3.7	<0.1	<0.1	<0.1	<0.5
1275S Blank	24/04/2012	1.4	<0.02	<0.01	<0.1	<0.01	<1	<0.01	0.14	0.051	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1275S Pro. Blank	24/04/2012	0.2	<0.02	<0.01	<0.1	0.02	<1	0.1	0.07	0.07	<0.2	0.002	4	<0.1	<0.1	<0.1	<0.5
1276L Pro Blank	23/04/2012	1.1	<0.02	<0.01	<0.1	0.036	<1	0.23	0.2	0.011	<0.2	0.0032	<0.1	<0.1	<0.1	<0.1	<0.5
1276L Blank	23/04/2012	0.12	<0.02	<0.01	<0.1	0.013	<1	0.3	0.19	<0.01	<0.2	0.0023	<0.1	<0.1	<0.1	<0.1	<0.5
1276L Blank	23/04/2012	3.0	<0.02	<0.01	<0.1	0.03	<1	0.1	0.09	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1277B Pro Blank	1/05/2012	<0.1	<0.02	<0.01	<0.1	<0.01	<1	0.14	0.15	0.043	<0.2	0.006	0.58	<0.1	<0.1	<0.1	<0.5
1277B Blank	1/05/2012	<0.1	<0.02	<0.01	<0.1	<0.01	<1	0.27	0.13	<0.01	<0.2	0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1277B Pro Blank	1/05/2012	0.9	<0.02	<0.01	<0.1	<0.01	<1	0.3	0.05	0.02	<0.2	0.006	0.5	<0.1	<0.1	<0.1	<0.5
1278G Pro Blank	30/07/2012	2.2	<0.02	<0.01	<0.1	0.11	<1	<0.000	0.26	0.068	0.36	0.0044	0.46	<0.1	<0.1	<0.1	<0.5
1278G Blank	30/07/2012	1.7	<0.02	<0.01	<0.1	0.053	<1	<0.000	0.21	<0.01	<0.2	<0.001	0.18	<0.1	<0.1	<0.1	<0.5
1278G Pro Blank	30/07/2012	2.0	<0.02	<0.01	<0.1	0.01	<1	<0.01	0.1	0.04	<0.000	0.03	<0.000	<0.1	<0.1	<0.1	<0.5
1279L Pro Blank	30/04/2012	<0.1	0.082	<0.01	<0.1	0.053	<1	0.31	0.18	0.057	<0.2	0.014	<0.1	<0.1	<0.1	<0.1	<0.5
1279L Pro Blank	30/04/2012	<0.1	<0.02	<0.01	<0.1	<0.01	<1	0.05	0.06	<0.01	0.3	0.02	<0.1	<0.1	<0.1	<0.1	<0.5
1279L Blank	30/04/2012	<0.1	<0.02	<0.01	<0.1	<0.01	<1	<0.01	0.13	<0.01	<0.2	0.01	<0.1	<0.1	<0.1	<0.1	<0.5
1283E Pro Blank	14/06/2012	0.14	<0.02	<0.01	<0.1	<0.01	<1	1.8	<0.01	0.039	<0.2	0.024	<0.1	<0.1	<0.1	<0.1	<0.5
1283E Pro Blank	14/06/2012	0.5	<0.02	<0.01	<0.1	<0.01	<1	<0.01	<0.01	0.1	<0.2	0.01	0.4	<0.1	<0.1	<0.1	<0.5
1283E Blank	14/06/2012	<0.1	<0.02	<0.01	<0.1	<0.01	<1	1.2	<0.01	<0.01	<0.2	0.002	<0.1	<0.1	<0.1	<0.1	<0.5
1290B Pro Blank	30/07/2012	0.3	<0.02	<0.01	<0.1	0.06	<1	<0.01	0.1	0.01	<0.2	0.1	<0.1	<0.1	<0.1	<0.1	<0.5
1290B Blank	30/07/2012	<0.1	<0.02	<0.01	<0.1	0.073	<1	<0.01	0.12	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1292G Pro Blank	13/08/2012	0.9	<0.02	<0.01	<0.1	0.1	<1	0.02	0.1	0.05	<0.2	0.01	0.7	0.4	<0.1	<0.1	<0.5

Table B3 continued Measured elements in the Blank (Totals) and Procedural Blank (Pro Blank, 0.1 µm filtered) samples

Test code/Sample	Date Sampled	Al µg/L	Cd µg/L	Co µg/L	Cr µg/L	Cu µg/L	Fe µg/L	Mn µg/L	Ni µg/L	Pb µg/L	Se µg/L	U µg/L	Zn µg/L	Ca mg/L	Mg mg/L	Na mg/L	SO_4 mg/L
1292G Blank Totals	13/08/2012	<0.1	<0.02	<0.01	<0.1	0.06	<1	<0.01	0.23	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1293E Pro Blank	23/08/2012	<0.1	<0.02	0.08	<0.1	0.2	1	0.8	0.9	0.2	<0.2	0.001	3	<0.1	<0.1	<0.1	<0.5
1293E Blank	23/08/2012	<0.1	<0.02	<0.01	<0.1	0.054	<1	<0.01	0.2	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1294G Pro Blank	28/08/2012	0.7	<0.02	<0.01	<0.1	0.09	<1	0.1	0.2	0.05	0.2	0.02	0.5	<0.1	<0.1	<0.1	<0.5
1294G Blank Totals	28/08/2012	<0.1	<0.02	<0.01	<0.1	0.04	<1	<0.01	0.22	0.012	<0.2	0.002	<0.1	<0.1	<0.1	<0.1	<0.5
1297L Pro Blank	10/09/2012	2	<0.02	<0.01	<0.1	0.06	<1	<0.01	0.1	0.08	<0.2	0.007	<0.1	<0.1	<0.1	<0.1	<0.5
1297L Blank	10/09/2012	<0.1	<0.02	<0.01	<0.1	0.054	<1	<0.01	0.2	<0.01	<0.2	0.004	<0.1	<0.1	<0.1	<0.1	<0.5
1299D Pro Blank	14/09/2012	0.6	<0.02	<0.01	<0.1	0.1	<1	<0.01	0.3	0.05	<0.2	0.003	0.4	<0.1	<0.1	<0.1	<0.5
1299D Blank	14/09/2012	<0.1	<0.02	<0.01	<0.1	0.093	<1	0.51	0.18	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1300E Pro Blank	20/09/2012	0.2	<0.02	<0.01	<0.1	0.09	<1	<0.01	0.2	0.06	<0.2	0.006	0.2	<0.1	<0.1	<0.1	<0.5
1300E Blank	20/09/2012	0.15	<0.02	<0.01	<0.1	0.073	<1	<0.01	0.19	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1307S Pro Blank	29/10/2012	0.7	0.06	<0.01	<0.1	0.08	<1	2	0.02	0.3	<0.2	0.004	0.2	<0.1	<0.1	<0.1	<0.5
1307S Blank	29/10/2012	0.15	<0.02	<0.01	<0.1	0.065	<1	0.04	0.083	0.018	<0.2	0.005	<0.1	<0.1	<0.1	<0.1	<0.5
1310B Pro Blank	19/11/2012	0.7	<0.02	<0.01	<0.1	0.06	<1	<0.01	0.05	0.03	<0.2	0.006	<0.1	<0.1	<0.1	<0.1	<0.5
1310B Blank	19/11/2012	0.6	<0.02	<0.01	<0.1	0.057	<1	0.032	0.05	<0.01	<0.2	0.001	<0.1	<0.1	<0.1	<0.1	<0.5
1318B Pro Blank	11/02/2013	0.1	<0.02	<0.01	<0.1	0.1	<1	<0.01	0.04	0.04	<0.2	0.002	0.2	<0.1	0.2	1.2	<0.5
1345D Blank	1/08/2013	<0.1	<0.02	<0.01	<0.1	0.01	<1	0.01	0.01	0.01	<0.2	0.002	<0.1	<0.1	<0.1	<0.1	<0.5
1345D Pro Blank	1/08/2013	0.1	<0.02	<0.01	<0.1	0.02	<1	0.01	<0.01	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5

Appendix C Statistical Summaries

Ngarradj Creek Water

Moinodaphnia macleayi 933D

Cladoceran Reproduction Test-Total neonates

Start Date:	31/05/2008	Test ID: 933D		Sample ID:	ST-Spiked Toxicant
End Date:	5/06/2008	Lab ID:	ERISS-eriss ecotoxicology	Sample Type:	MNSO4-Manganese sulfate
Sample Date		Protocol	BTT D-eriss tropical freshw	Test Species:	MOMA-Moinodaphnia macleayi
Comments:					

Conc-ug/L	1	2	3	4	5	6	7	8	9	10
MCW	36.000	35.000	39.000	36.000	36.000	36.000	35.000	36.000	35.000	30.000
4.2	37.000	38.000	31.000	33.000	37.000	42.000	35.000	36.000	0.000	33.000
5.4	35.000	31.000	34.000	38.000	35.000	36.000	35.000	35.000	38.000	36.000
62.08	38.000	39.000	33.000	32.000	36.000	40.000	34.000	34.000	37.000	
167.5	0.000	38.000	37.000	34.000	42.000	29.000	29.000	32.000	35.000	35.000
583.8	33.000	31.000	34.000	35.000	33.000	30.000	33.000	33.000	39.000	31.000
1870	33.000	31.000	29.000	29.000	29.000	35.000	32.000	34.000	35.000	22.000

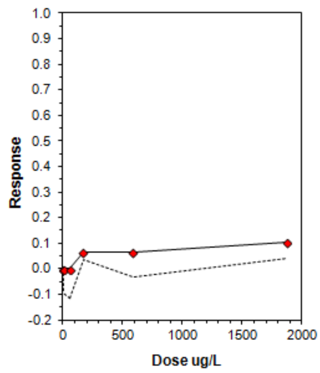
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
MCW	35.400	1.0994	35.400	30.000	39.000	6.274	10				
4.2	32.200	1.0000	32.200	0.000	42.000	36.418	10			34.463	1.0000
5.4	35.300	1.0963	35.300	31.000	38.000	5.674	10	106.50	74.00	34.463	1.0000
62.08	35.889	1.1146	35.889	32.000	40.000	7.812	9	97.00	61.00	34.463	1.0000
167.5	31.100	0.9658	31.100	0.000	42.000	37.389	10	97.50	74.00	32.150	0.9329
583.8	33.200	1.0311	33.200	30.000	39.000	7.620	10	88.50	74.00	32.150	0.9329
1870	30.900	0.9596	30.900	22.000	35.000	12.714	10	78.50	74.00	30.900	0.8966

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.9924	1.035	-3.307	14.274
Bartlett's Test indicates unequal variances (p = 3.00E-09)	48.36	15.086		
The control means are not significantly different (p = 0.41)	0.8479	2.1009		

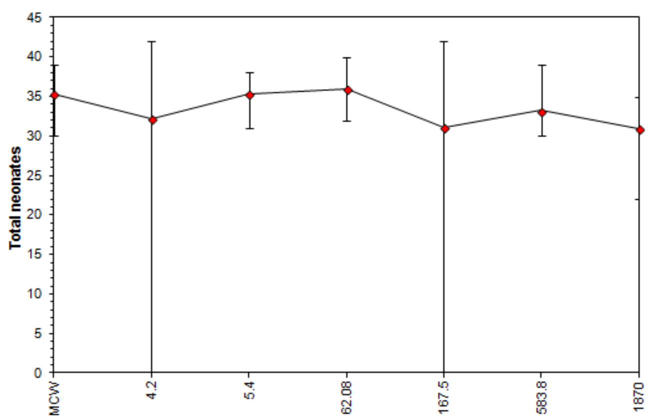
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	1870	>1870		
Treatments vs 4.2				

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL	Skew
IC05	140.62			
IC10	1750			
IC15	>1870			
IC20	>1870			
IC25	>1870			
IC40	>1870			
IC50	>1870			



Dose-Response Plot



Moinodaphnia macleayi 934D

Cladoceran Reproduction Test-Total neonates

Start Date:	31/05/2008	Test ID: 934D		Sample ID:	ST-Spiked Toxicant
End Date:	5/06/2008	Lab ID:	ERISS-eriss ecotoxicology	Sample Type:	MNSO4-Manganese sulfate
Sample Date		Protocol	BTT D-eriss tropical freshv	Test Species:	MOMA-Moinodaphnia macleayi
Comments:					

Conc-ug/L	1	2	3	4	5	6	7	8	9	10
MCW	15.000	13.000	14.000	13.000	14.000	15.000	14.000	14.000	11.000	13.000
4.2	16.000	17.000	16.000	16.000	16.000	15.000	15.000	17.000	17.000	16.000
5.59	13.000	17.000	15.000	15.000	16.000	16.000	17.000	16.000	16.000	15.000
62.08	16.000	18.000	14.000	16.000	16.000	17.000	16.000	14.000	16.000	13.000
171.5	15.000	17.000	15.000	15.000	20.000	14.000	14.000	14.000	15.000	14.000
583.8	11.000	12.000	13.000	12.000	12.000	15.000	17.000	14.000	16.000	17.000
1840	6.000	11.000	10.000	10.000	12.000	11.000	6.000	7.000	19.000	5.000

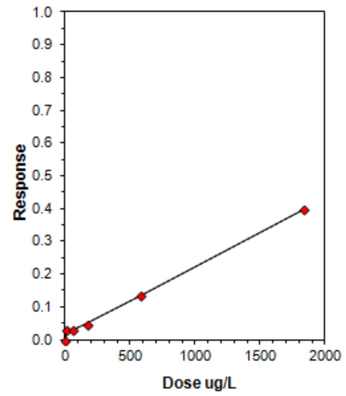
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
MCW	13.600	0.8447	13.600	11.000	15.000	8.631	10				
4.2	16.100	1.0000	16.100	15.000	17.000	4.583	10			16.100	1.0000
5.59	15.600	0.9689	15.600	13.000	17.000	7.524	10	93.00	75.00	15.600	0.9689
62.08	15.600	0.9689	15.600	13.000	18.000	9.651	10	96.00	75.00	15.600	0.9689
171.5	15.300	0.9503	15.300	14.000	20.000	12.344	10	77.50	75.00	15.300	0.9503
583.8	13.900	0.8634	13.900	11.000	17.000	16.069	10	77.50	75.00	13.900	0.8634
*1840	9.700	0.6025	9.700	5.000	19.000	42.381	10	65.00	75.00	9.700	0.6025

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.1146	1.035	1.2739	5.4748
Bartlett's Test indicates unequal variances (p = 2.44E-05)	28.885	15.086		
The control means are significantly different (p = 2.09E-05)	5.7021	2.1009		

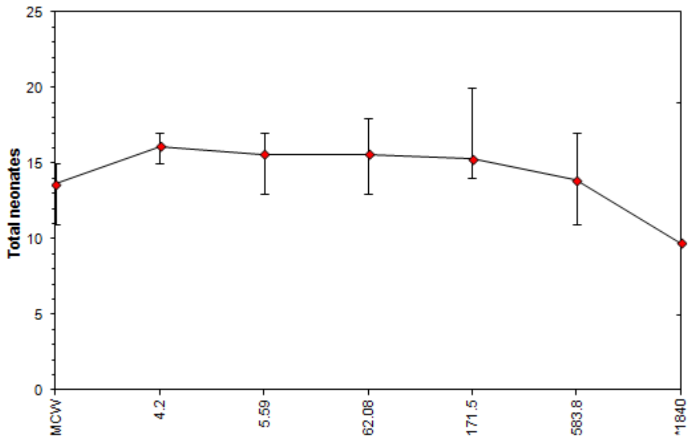
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	583.8	1840	1036.4	
Treatments vs 4.2				

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL		Skew
IC05	172.97	127.71	5.2955	579.84	1.4012
IC10	410.05	165.48	153.91	782.07	0.4590
IC15	648.11	173.87	394.66	984.93	0.3986
IC20	888.88				
IC25	1129.6				
IC40	>1840				
IC50	>1840				



Dose-Response Plot



Hydra viridissima 936B

Green Hydra Population Growth Test-Population growth rate (k					
Start Date:	16/06/2008	Test ID: 936B		Sample ID:	ST-Spiked Toxicant
End Date:	20/06/2008	Lab ID: ERISS-eriss ecotoxicology		Sample Type:	MNSO4-Manganese sulfate
Sample Date		Protocol BTT B-eriss tropical freshv		Test Species:	HV-Hydra viridissima
Comments:					

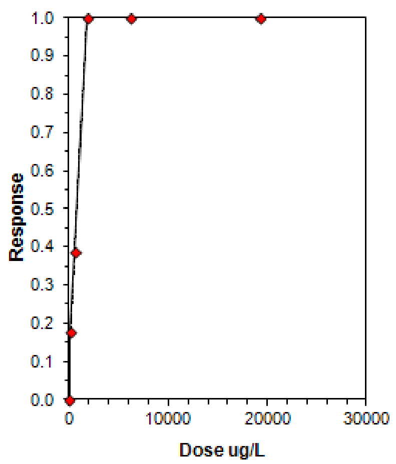
Conc-ug/L	1	2	3
MCW	0.3132	0.3132	0.2829
5.22	0.3132	0.3402	0.2747
105.55	0.2829	0.2389	0.2389
559.8	0.1971	0.1733	0.1971
1725	0.0000	0.0000	0.0000
6086	0.0000	0.0000	0.0000
19150	0.0000	0.0000	0.0000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	0.3031	0.9797	0.3031	0.2829	0.3132	5.780	3					
5.22	0.3094	1.0000	0.3094	0.2747	0.3402	10.655	3				0.3094	1.0000
*105.55	0.2535	0.8195	0.2535	0.2389	0.2829	10.013	3	2.702	2.340	0.0483	0.2535	0.8195
*559.8	0.1892	0.6115	0.1892	0.1733	0.1971	7.272	3	5.818	2.340	0.0483	0.1892	0.6115
1725	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000
6086	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000
19150	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	3				0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9339	0.764	0.0547	-0.779
Bartlett's Test indicates equal variances (p = 0.57)	1.1169	9.2103		
The control means are not significantly different (p = 0.79)	0.2916	2.7764		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<105.55	105.55			0.0483	0.1563	0.0109	0.0006	0.0034	2, 6
Treatments vs 5.22										

Linear Interpolation (200 Resamples)					
Point	ug/L	SD	95% CL(Exp)		Skew
IC05	33.02	16.36	17.33	108.07	3.1331
IC10	60.82	31.20	29.44	219.32	2.3423
IC15	88.62	53.21	41.55	342.46	1.6396
IC20	148.22	81.36	34.58	461.77	0.8689
IC25	257.38	101.84	0.00	551.36	0.2147
IC40	581.69	75.04	327.71	786.93	0.1494
IC50	772.24	61.58	592.75	943.28	0.1504



Moinodaphnia macleayi 937D

Cladoceran Reproduction Test-Total neonates

Start Date: 20/06/2008	Test ID: 937D	Sample ID:	ST-Spiked Toxicant
End Date: 26/06/2008	Lab ID: ERISS-eriss ecotoxicology	Sample Type:	MNSO4-Manganese sulfate
Sample Date	Protocol BTT D-eriss tropical freshv	Test Species:	MOMA-Moinodaphnia macleayi
Comments:			

Conc-ug/L	1	2	3	4	5	6	7	8	9	10	SE
MCW	40.000	33.000	31.000	36.000	30.000	20.000	0.000	17.000	32.000	32.000	3.7102
4.635	35.000	36.000	36.000	34.000	38.000	33.000	34.000	34.000	38.000	33.000	0.5859
870	32.000	32.000	34.000	33.000	31.000	31.000	29.000	14.000	36.000	32.000	1.916
1855	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
3750	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
7575.5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0
15300	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0

			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
MCW	27.100	0.7721	27.100	0.000	40.000	43.294	10				
4.635	35.100	1.0000	35.100	33.000	38.000	5.279	10			35.100	1.0000
*870	30.400	0.8661	30.400	14.000	36.000	19.931	10	66.50	82.00	30.400	0.8661
1855	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000	0.0000
3750	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000	0.0000
7575.5	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000	0.0000
15300	0.000	0.0000	0.000	0.000	0.000	0.000	10			0.000	0.0000

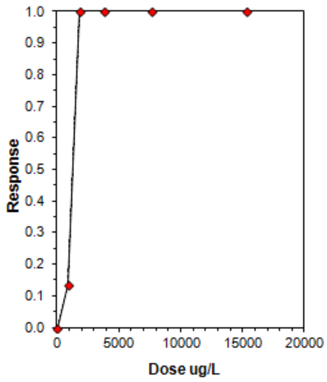
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.6926	0.868	-2.915	11.194
F-Test indicates unequal variances (p = 1.61E-03)	10.693	6.5411		
The control means are significantly different (p = 0.05)	2.1298	2.1009		

Hypothesis Test (1-tail, 0.05)

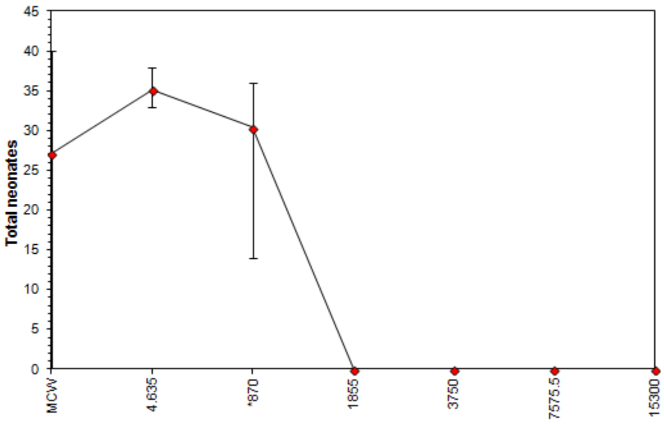
Wilcoxon Two-Sample Test indicates significant differences
Treatments vs 4.635

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL		Skew
IC05	327.77	156.79	185.3	805.63	1.2002
IC10	650.9	181.74	365.96	917.89	-0.1850
IC15	888.31	122.31	546.62	969.95	-1.1986
IC20	945.17	72.112	727.28	1022	-1.6889
IC25	1002	52.632	883.46	1074.1	-0.8710
IC40	1172.6	41.042	1077.9	1230.3	-0.7131
IC50	1286.4	34.201	1207.4	1334.4	-0.7131



Dose-Response Plot



***Moinodaphnia macleayi* 938I**

Cladoceran Immobilisation Test-Survival

Start Date:	20/06/2008	Test ID:	938I	Sample ID:	ST-Spiked Toxicant
End Date:	22/06/2008	Lab ID:	ERISS-eriss ecotoxicology	Sample Type:	MNSO4-Manganese sulfate
Sample Date		Protocol	BTT G-eriss tropical freshv	Test Species:	MOMA-Moinodaphnia macleayi
Comments:					

Conc-ug/L	1	2	3
MCW	1.0000	1.0000	1.0000
4.635	1.0000	1.0000	1.0000
767.5	1.0000	1.0000	0.9000
1845	0.0000	0.0000	0.0000
3560	0.0000	0.0000	0.0000
7575.6	0.0000	0.0000	0.0000
15100	0.0000	0.0000	0.0000

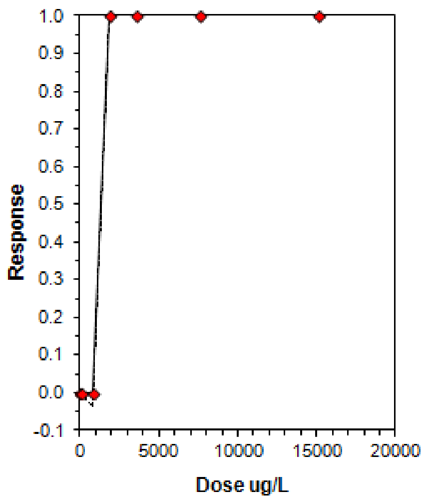
			Transform: Arcsin Square Root					1-Tailed			Isotonic	
Conc-ug/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	3				1.0115	1.0000
4.635	1.0000	1.0000	1.0472	1.0472	1.0472	0.000	3	0.000	2.530	0.0910	1.0115	1.0000
767.5	0.9667	0.9667	1.1145	1.0472	1.2490	10.457	3	-1.871	2.530	0.0910	1.0115	1.0000
*1845	0.0000	0.0000	0.0003	0.0003	0.0003	0.000	3	29.109	2.530	0.0910	0.0000	0.0000
*3560	0.0000	0.0000	0.0003	0.0003	0.0003	0.000	3	29.109	2.530	0.0910	0.0000	0.0000
*7575.6	0.0000	0.0000	0.0003	0.0003	0.0003	0.000	3	29.109	2.530	0.0910	0.0000	0.0000
*15100	0.0000	0.0000	0.0003	0.0003	0.0003	0.000	3	29.109	2.530	0.0910	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.4881	0.873	2.0179	10
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	767.5	1845	1190		0.0825	0.11	0.9816	0.0019	1.5E-15	6, 14
Treatments vs MCW										

Linear Interpolation (200 Resamples)

Point	ug/L	SD	95% CL(Exp)		Skew
IC05	821.38	111.24	297.72	821.38	-1.4106
IC10	875.25	28.708	727.77	875.25	-0.5210
IC15	929.13	27.113	789.84	929.13	-0.5210
IC20	983	25.518	851.9	983	-0.5210
IC25	1036.9	23.923	913.97	1036.9	-0.5210
IC40	1198.5	19.139	1100.2	1198.5	-0.5210
IC50	1306.3	15.949	1224.3	1306.3	-0.5210



***Chlorella* sp. 939G**

Algal Growth Inhibition Test-Growth rate					
Start Date:	24/06/2008	Test ID:	939G	Sample ID:	ST-Spiked Toxicant
End Date:	27/06/2008	Lab ID:	ERISS-eriss ecotoxicology	Sample Type:	MNSO4-Manganese sulfate
Sample Date		Protocol	BTT G-eriss tropical freshv	Test Species:	CH-Chlorella sp.
Comments:					

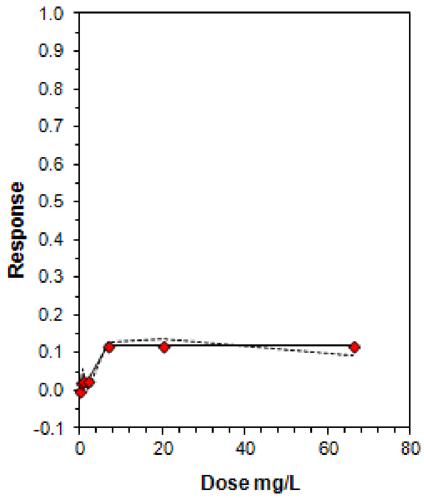
Conc-mg/L	1	2	3
MCW	1.8121	1.7235	1.8334
Ng Water	1.7022	1.6933	1.6033
0.2	1.6309	1.6166	1.6382
0.66	1.6248	1.3619	1.7295
2	1.6374	1.6762	1.6881
6.66	1.4457	1.5029	1.4148
20	1.3728	1.4987	1.4491
66	1.4782	1.4570	1.5916

			Transform: Untransformed						1-Tailed		Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
MCW	1.7897	1.0741	1.7897	1.7235	1.8334	3.258	3					
Ng Water	1.6662	1.0000	1.6662	1.6033	1.7022	3.283	3				1.6662	1.0000
0.2	1.6286	0.9774	1.6286	1.6166	1.6382	0.673	3	0.541	2.530	0.1762	1.6286	0.9774
0.66	1.5721	0.9435	1.5721	1.3619	1.7295	12.048	3	1.352	2.530	0.1762	1.6196	0.9720
2	1.6672	1.0006	1.6672	1.6374	1.6881	1.592	3	-0.014	2.530	0.1762	1.6196	0.9720
*6.66	1.4545	0.8729	1.4545	1.4148	1.5029	3.073	3	3.040	2.530	0.1762	1.4679	0.8809
*20	1.4402	0.8643	1.4402	1.3728	1.4987	4.404	3	3.245	2.530	0.1762	1.4679	0.8809
66	1.5089	0.9056	1.5089	1.4570	1.5916	4.796	3	2.259	2.530	0.1762	1.4679	0.8809

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9225	0.873	-0.774	3.4853
Bartlett's Test indicates equal variances (p = 0.04)	13.354	16.812		
The control means are not significantly different (p = 0.06)	2.6736	2.7764		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	2	6.66	3.6497		0.1762	0.1058	0.0278	0.0073	0.0181	6, 14
Treatments vs Ng Water										

Linear Interpolation (200 Resamples)					
Point	mg/L	SD	95% CL(Exp)		Skew
IC05	3.1267	1.3500	0.0000	5.2978	-0.5401
IC10	5.6847				
IC15	>66				
IC20	>66				
IC25	>66				
IC40	>66				
IC50	>66				



Magela Creek Water

Chlorella sp. 1292G, 1294G pooled

CETIS Analytical Report

Report Date: 11 Mar-14 14:42 (p 1 of 3)
Test Code: 1292G+1294G | 11-1498-2620

Algal Growth Inhibition Test — eriss ecotoxicology lab

Analysis ID:	01-4202-5137	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 14:42	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Batch ID:	21-0973-0439	**Test Type:**	Algal growth inhibition	**Analyst:**	Andrew J Harford
Start Date:	17 Jul-13 10:37	**Protocol:**	Alga eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	17 Jul-13 10:37	**Species:**	Chlorella sp.	**Brine:**	Not Applicable
Duration:	NA	**Source:**	In-House Culture	**Age:**	

Sample ID:	09-9000-3510	**Code:**	3B024136	**Client:**	Core project
Sample Date:	17 Jul-13 10:37	**Material:**	Manganese sulfate	**Project:**	Mn Toxicity
Receive Date:	17 Jul-13 10:37	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**	N/A		

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Logistic [Y=A/(1+exp(-C(X-D)))]	Log X	None	Normal [W=1]	Box-Cox [Y'=(Y

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
26	35.4	-62.8	-62.48	0.9815	No				Lack of Fit Not Tested

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC10	12200	9991	14410
IC50	60440	54550	67410

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	1.658	0.04242	1.575	1.741	39.08	<0.0001	Significant Parameter
C	-3.175	0.2862	-3.736	-2.614	-11.1	<0.0001	Significant Parameter
D	4.783	0.02896	4.726	4.84	165.2	<0.0001	Significant Parameter
Z	1.85						

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	3.901153	3.901153	1	798.4	<0.0001	Significant
Residual	0.063517	0.004886	13			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.683	2.586	1.0000	No Outliers Detected
Distribution	Shapiro-Wilk W Normality	0.9527	0.887	0.5338	Normal Distribution
	Anderson-Darling A2 Normality	0.3906	2.492	0.3858	Normal Distribution

000-428-181-1 CETIS™ v1.8.7.4 Analyst:________ QA:________

Algal Growth Inhibition Test | eriss ecotoxicology lab

Analysis ID:	01-4202-5137	Endpoint:	Growth rate (db/d)	CETIS Version:	CETISv1.8.7
Analyzed:	11 Mar-14 14:42	Analysis:	Nonlinear Regression	Official Results:	Yes

Growth rate (db/d) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
4	Pooled Controls	5	1.66	1.615	1.72	0.01911	0.04272	2.57%	0.0%
2450		2	1.595	1.58	1.61	0.015	0.02122	1.33%	3.93%
4900		2	1.62	1.595	1.644	0.0245	0.03465	2.14%	2.45%
9400		2	1.586	1.574	1.598	0.012	0.01697	1.07%	4.47%
10500		2	1.574	1.549	1.599	0.025	0.03536	2.25%	5.19%
17000		2	1.416	1.392	1.441	0.0245	0.03465	2.45%	14.7%
19000		2	1.308	1.299	1.316	0.008501	0.01202	0.92%	21.2%
37500		2	0.9893	0.9755	1.003	0.01375	0.01944	1.97%	40.4%
38500		2	1.065	0.995	1.135	0.07	0.09899	9.3%	35.9%
57000		2	0.9819	0.9628	1.001	0.0191	0.02701	2.75%	40.9%
76000		2	0.825	0.7611	0.889	0.06395	0.09044	11.0%	50.3%
82500		2	0.6168	0.5859	0.6477	0.0309	0.0437	7.08%	62.8%
95500		2	0.5675	0.4057	0.7292	0.1617	0.2287	40.3%	65.8%
115000		2	0.478	0.4772	0.4789	0.000852	0.001206	0.25%	71.2%
135000		2	0.4206	0.3987	0.4424	0.02185	0.0309	7.35%	74.7%
160000		2	0	0	0	0	0		100.0%

Growth rate (db/d) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
4	Pooled Controls	1.72	1.686	1.615	1.65	1.63
2450		1.58	1.61			
4900		1.595	1.644			
9400		1.598	1.574			
10500		1.599	1.549			
17000		1.441	1.392			
19000		1.299	1.316			
37500		1.003	0.9755			
38500		1.135	0.995			
57000		1.001	0.9628			
76000		0.889	0.7611			
82500		0.5859	0.6477			
95500		0.7292	0.4057			
115000		0.4789	0.4772			
135000		0.4424	0.3987			
160000		0	0			

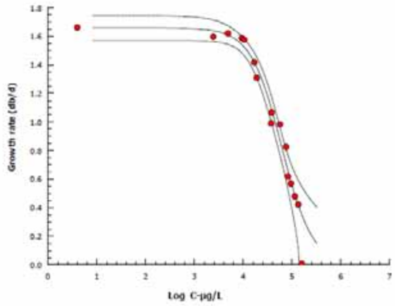
CETIS Analytical Report

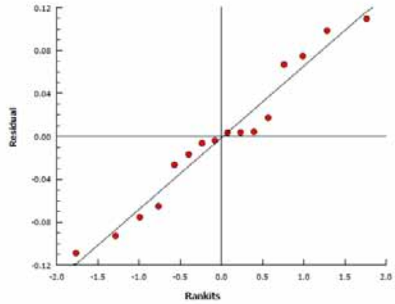
Report Date:	11 Mar-14 14:42 (p 3 of 3)
Test Code:	1292G+1294G | 11-1498-2620

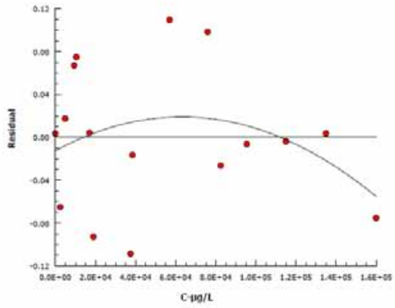
Algal Growth Inhibition Test | **eriss ecotoxicology lab**

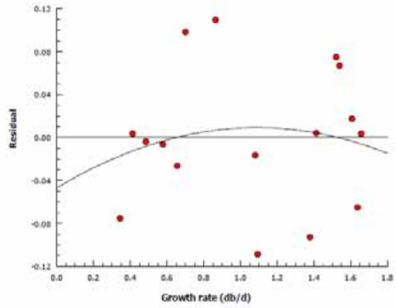
Analysis ID:	01-4202-5137	**Endpoint:**	Growth rate (db/d)	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 14:42	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Graphics 3P Logistic [Y=A/(1+exp(-C(X-D)))]









Lemna aequinoctialis 1276L, 1279L, 1297L pooled

CETIS Analytical Report

Report Date: 11 Mar-14 15:43 (p 1 of 3)
Test Code: 1276L 1279L 129 | 18-2568-0069

Lemna Growth Inhibition — **eriss ecotoxicology lab**

Analysis ID:	08-6415-9487	**Endpoint:**	Growth rate (surface area)	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 15:43	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes
Batch ID:	15-1914-8333	**Test Type:**	Lemna Growth	**Analyst:**	Andrew J Harford
Start Date:	11 Mar-14 15:32	**Protocol:**	Lemna eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	11 Mar-14 15:32	**Species:**	Lemna aequinoctialis	**Brine:**	Not Applicable
Duration:	NA	**Source:**	In-House Culture	**Age:**	
Sample ID:	18-9792-1516	**Code:**	711FFBEC	**Client:**	Internal Lab
Sample Date:	11 Mar-14 15:32	**Material:**	Manganese sulfate	**Project:**	Manganese toxicity
Receive Date:	11 Mar-14 15:32	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**	N/A		

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Logistic [Y=A/(1+exp(-C(X-D)))]	Log X	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
6	51.9	-96.19	-94.96	0.9161	No				Lack of Fit Not Tested

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC10	2239	914.5	3439
IC50	10970	9073	13250

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	0.4512	0.02165	0.4088	0.4937	20.84	<0.0001	Significant Parameter
C	-3.241	0.5272	-4.275	-2.208	-6.149	<0.0001	Significant Parameter
D	4.047	0.05623	3.936	4.157	71.97	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	0.367962	0.367962	1	198.6	<0.0001	Significant
Residual	0.02965	0.001853	16			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.097	2.681	0.5022	No Outliers Detected
Distribution	Shapiro-Wilk W Normality	0.9654	0.9007	0.6817	Normal Distribution
	Anderson-Darling A2 Normality	0.403	2.492	0.3613	Normal Distribution

Analysis ID:	08-6415-9487	Endpoint:	Growth rate (surface area)	CETIS Version:	CETISv1.8.7
Analyzed:	11 Mar-14 15:43	Analysis:	Nonlinear Regression	Official Results:	Yes

Growth rate (surface area) Summary

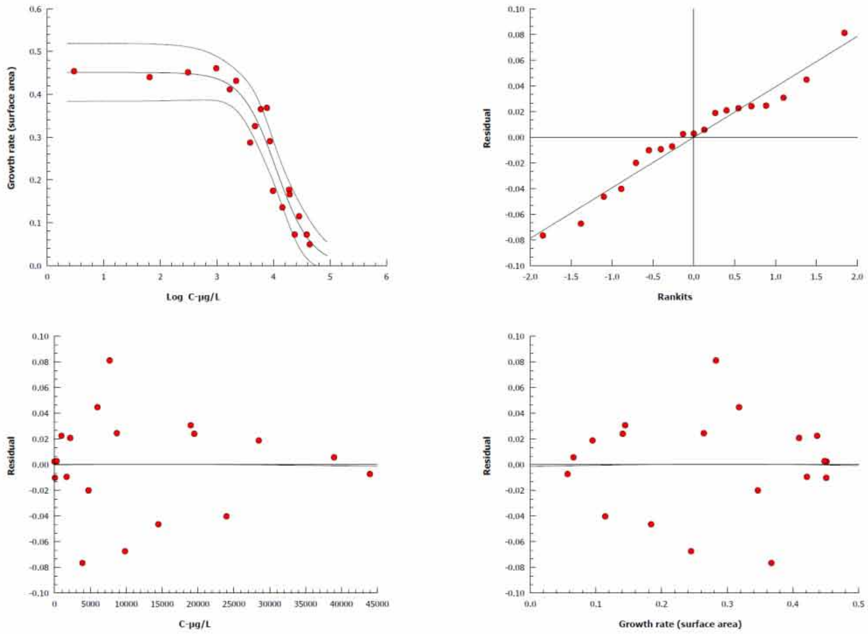
			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
3	Pooled Controls	9	0.4536	0.4146	0.4953	0.008462	0.02539	5.6%	0.0%
65		3	0.4401	0.4224	0.468	0.0141	0.02442	5.55%	2.99%
310		3	0.4511	0.4409	0.468	0.008489	0.0147	3.26%	0.56%
980		3	0.4605	0.4299	0.4835	0.0159	0.02754	5.98%	-1.5%
1700		3	0.4113	0.3895	0.4548	0.02175	0.03767	9.16%	9.34%
2200		2	0.4312	0.4146	0.4479	0.01669	0.02361	5.47%	4.94%
3900		3	0.2868	0.05579	0.4065	0.1155	0.2001	69.8%	36.8%
4750		2	0.3255	0.2529	0.3982	0.07263	0.1027	31.6%	28.2%
6000		3	0.365	0.3072	0.3982	0.029	0.05023	13.8%	19.6%
7700		3	0.368	0.3617	0.3806	0.006292	0.0109	2.96%	18.9%
8700		3	0.29	0.2291	0.3806	0.04619	0.08	27.6%	36.1%
9850		2	0.1742	0.1277	0.2206	0.04645	0.06568	37.7%	61.6%
14500		2	0.1353	0.08708	0.1835	0.04821	0.06818	50.4%	70.2%
19000		3	0.1767	0.1733	0.1835	0.003402	0.005892	3.33%	61.1%
19500		2	0.1661	0.08708	0.2452	0.07907	0.1118	67.3%	63.4%
24000		2	0.07192	0.07192	0.07192	0	0	0.0%	84.1%
28500		2	0.1145	0.1014	0.1277	0.01317	0.01863	16.3%	74.8%
39000		2	0.07192	0.07192	0.07192	0	0	0.0%	84.1%
44000		3	0.04924	0.02001	0.07192	0.01534	0.02657	54.0%	89.1%

Growth rate (surface area) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9
3	Pooled Controls	0.4953	0.4514	0.4774	0.4444	0.4647	0.4262	0.468	0.4146	0.4409
65		0.468	0.4299	0.4224						
310		0.4444	0.468	0.4409						
980		0.4835	0.4299	0.468						
1700		0.4548	0.3895	0.3895						
2200		0.4479	0.4146							
3900		0.05579	0.4065	0.3982						
4750		0.3982	0.2529							
6000		0.3072	0.3895	0.3982						
7700		0.3806	0.3617	0.3617						
8700		0.3806	0.2291	0.2604						
9850		0.2206	0.1277							
14500		0.1835	0.08708							
19000		0.1835	0.1733	0.1733						
19500		0.2452	0.08708							
24000		0.07192	0.07192							
28500		0.1277	0.1014							
39000		0.07192	0.07192							
44000		0.05579	0.02001	0.07192						

Analysis ID:	08-6415-9487	**Endpoint:**	Growth rate (surface area)	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 15:43	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Graphics 3P Logistic [Y=A/(1+exp(-C(X-D)))]



Hydra viridissima 1310B, 1318B pooled

CETIS Analytical Report

Report Date:	11 Mar-14 11:43 (p 1 of 3)
Test Code:	1310B 1318B pol | 10-9312-3220

Green Hydra Population Growth Test — **eriss ecotoxicology lab**

Analysis ID:	01-3038-6627	**Endpoint:**	Specific growth rate (96h)	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 11:42	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes
Batch ID:	03-3554-8728	**Test Type:**	Hydra population growth	**Analyst:**	Andrew J Harford
Start Date:	11 Mar-13	**Protocol:**	Hydra eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	15 Mar-13	**Species:**	Hydra viridissima	**Brine:**	Not Applicable
Duration:	96h	**Source:**	In-House Culture	**Age:**	
Sample ID:	07-4305-0061	**Code:**	2C4A0B4D	**Client:**	Internal Lab
Sample Date:	11 Mar-14 11:12	**Material:**	Manganese sulfate	**Project:**	Manganese toxicity
Receive Date:	11 Mar-14 11:12	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**	N/A		

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Log-Logistic EV [Y=A/(1+(X/D)^C)]	None	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
9	63.16	-118.3	-118	0.9611	No				Lack of Fit Not Tested

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC10	136.6	96.53	180.6
IC50	1375	1207	1567

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	0.3449	0.01147	0.3224	0.3674	30.08	<0.0001	Significant Parameter
C	0.9038	0.1064	0.6953	1.112	8.495	<0.0001	Significant Parameter
D	1273	117.1	1043	1502	10.87	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	0.062941	0.062941	1	373.1	<0.0001	Significant
Residual	0.002193	0.000169	13			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.78	2.586	0.0162	Outlier Detected
Distribution	Shapiro-Wilk W Normality	0.9004	0.887	0.0817	Normal Distribution
	Anderson-Darling A2 Normality	0.5428	2.492	0.1670	Normal Distribution

000-428-181-1 CETIS™ v1.8.7.4 Analyst:________ QA:________

Green Hydra Population Growth Test | **eriss ecotoxicology lab**

Analysis ID: 01-3038-6627 **Endpoint:** Specific growth rate (96h) **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-14 11:42 **Analysis:** Nonlinear Regression **Official Results:** Yes

Specific growth rate (96h) Summary

			Calculated Variate						
C-µg/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
5	Pooled Controls	7	0.3389	0.3132	0.3647	0.005855	0.01549	4.57%	0.0%
57		2	0.3131	0.3059	0.3202	0.007145	0.0101	3.23%	7.62%
90		2	0.3131	0.3059	0.3202	0.007145	0.0101	3.23%	7.62%
170		2	0.3304	0.3271	0.3338	0.003334	0.004714	1.43%	2.51%
180		2	0.302	0.2908	0.3132	0.0112	0.01584	5.25%	10.9%
340		2	0.2687	0.2389	0.2985	0.0298	0.04215	15.69%	20.72%
440		2	0.234	0.2291	0.2389	0.004903	0.006933	2.96%	30.96%
530		2	0.224	0.2189	0.2291	0.005103	0.007216	3.22%	33.92%
645		2	0.2135	0.2082	0.2189	0.00532	0.007524	3.52%	36.99%
685		2	0.224	0.2189	0.2291	0.005103	0.007216	3.22%	33.92%
835		2	0.2027	0.1971	0.2082	0.005557	0.007858	3.88%	40.2%
950		2	0.1897	0.1605	0.2189	0.0292	0.0413	21.77%	44.04%
1200		2	0.1855	0.1855	0.1855	0	0	0.0%	45.27%
1550		2	0.1537	0.1469	0.1605	0.006758	0.009558	6.22%	54.65%
1750		2	0.1605	0.1605	0.1605	0	0	0.0%	52.65%
1950		2	0.1398	0.1327	0.1469	0.007145	0.0101	7.23%	58.75%

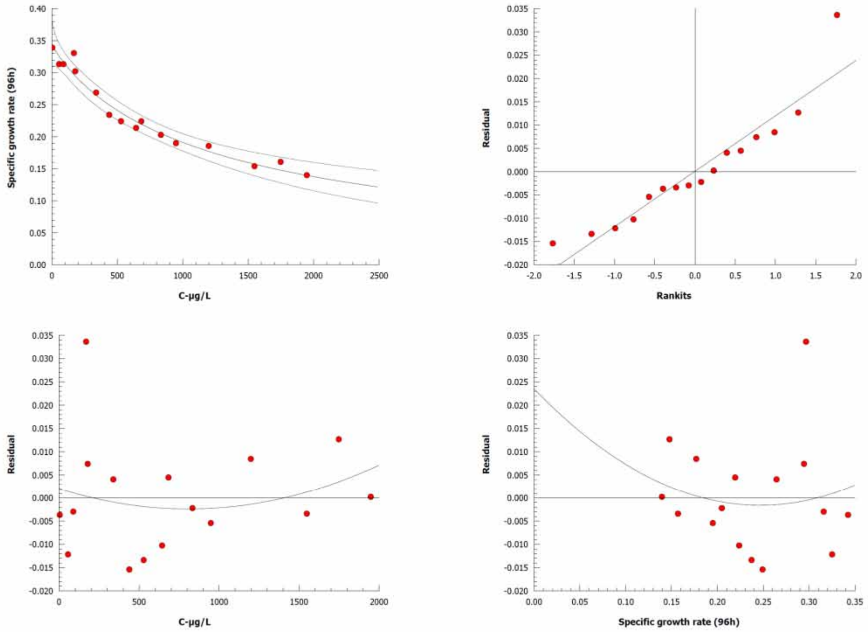
Specific growth rate (96h) Detail

C-µg/L	**Control Type**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Rep 6**	**Rep 7**
5	Pooled Controls	0.3402	0.3402	0.3647	0.3338	0.3466	0.3132	0.3338
57		0.3202	0.3059					
90		0.3202	0.3059					
170		0.3338	0.3271					
180		0.2908	0.3132					
340		0.2985	0.2389					
440		0.2291	0.2389					
530		0.2189	0.2291					
645		0.2082	0.2189					
685		0.2291	0.2189					
835		0.1971	0.2082					
950		0.1605	0.2189					
1200		0.1855	0.1855					
1550		0.1605	0.1469					
1750		0.1605	0.1605					
1950		0.1327	0.1469					

Green Hydra Population Growth Test | **eriss ecotoxicology lab**

Analysis ID:	01-3038-6627	**Endpoint:**	Specific growth rate (96h)	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 11:42	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Graphics 3P Log-Logistic EV [Y=A/(1+(X/D)^C)]



Moinodaphnia macleayi 1299D, 1345D pooled

CETIS Analytical Report

Report Date: 11 Mar-14 12:32 (p 1 of 3)
Test Code: 1299D 1345D | 01-4264-3105

Cladoceran Reproduction Test — eriss ecotoxicology lab

Analysis ID:	19-4213-0924	**Endpoint:**	Total neonates	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 12:31	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes
Batch ID:	06-6725-8279	**Test Type:**	Cladoceran reproduction	**Analyst:**	Andrew J Harford
Start Date:	01 Aug-13	**Protocol:**	Clad (chronic) eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	07 Aug-13	**Species:**	Moinodaphnia macleayi	**Brine:**	Not Applicable
Duration:	6d 0h	**Source:**	In-House Culture	**Age:**	
Sample ID:	13-3548-1664	**Code:**	4F99D540	**Client:**	Core project
Sample Date:	11 Mar-14 10:55	**Material:**	Manganese sulfate	**Project:**	Manganese toxicity
Receive Date:	11 Mar-14 10:55	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**			

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Logistic [Y=A/(1+exp(-C(X-D)))]	Log X	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
6	-17.47	42.95	43.26	0.9625	No				Lack of Fit Not Tested

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC10	609.8	504.3	686.3
IC50	1098	1032	1167

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	33.78	0.7473	32.31	35.24	45.2	<0.0001	Significant Parameter
C	-8.797	1.169	-11.09	-6.505	-7.524	<0.0001	Significant Parameter
D	3.043	0.01493	3.014	3.073	203.8	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	1555.165	1555.165	1	386.6	<0.0001	Significant
Residual	52.28909	4.022238	13			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.702	2.586	1.0000	No Outliers Detected
Distribution	Shapiro-Wilk W Normality	0.9643	0.887	0.7395	Normal Distribution
	Anderson-Darling A2 Normality	0.2674	2.492	0.7138	Normal Distribution

000-428-181-1 CETIS™ v1.8.7.4 Analyst:________ QA:________

Cladoceran Reproduction Test | **eriss ecotoxicology lab**

Analysis ID: 19-4213-0924 **Endpoint:** Total neonates **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-14 12:31 **Analysis:** Nonlinear Regression **Official Results:** Yes

Total neonates Summary

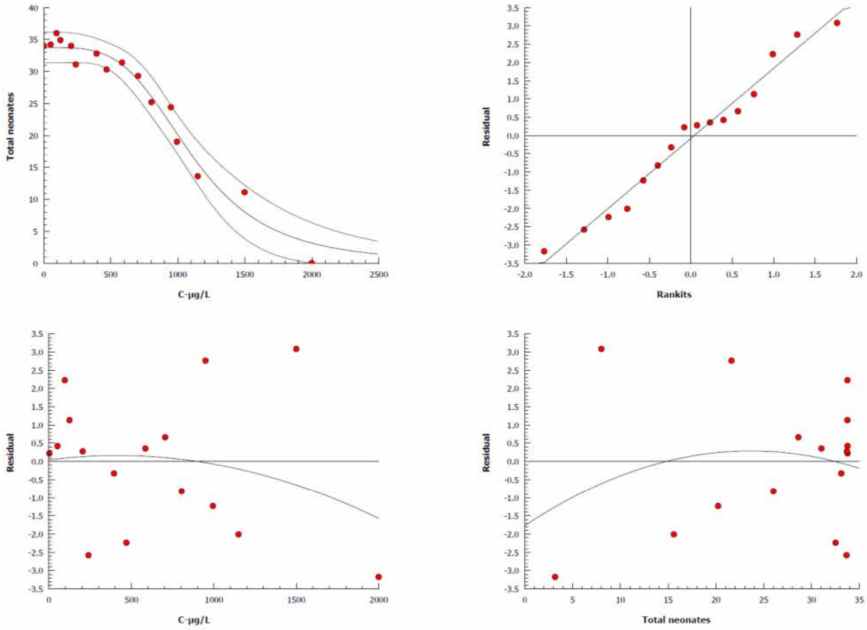
			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
3	Pooled Controls	20	34	31	37	0.3403	1.522	4.48%	0.0%
52.5		5	34.2	24	38	2.615	5.848	17.1%	-0.59%
96.5		5	36	33	40	1.378	3.082	8.56%	-5.88%
125		10	34.9	32	37	0.4819	1.524	4.37%	-2.65%
205		5	34	24	39	2.739	6.124	18.0%	0.0%
240		10	31.1	26	35	1.08	3.414	11.0%	8.53%
395		5	32.8	29	36	1.594	3.564	10.9%	3.53%
470		10	30.3	25	33	0.7608	2.406	7.94%	10.9%
585		5	31.4	16	37	3.945	8.82	28.1%	7.65%
705		10	29.3	19	35	1.647	5.208	17.8%	13.8%
805		5	25.2	7	34	5.21	11.65	46.2%	25.9%
950		10	24.4	8	33	2.459	7.777	31.9%	28.2%
995		5	19	0	28	5.404	12.08	63.6%	44.1%
1150		5	13.6	0	29	5.662	12.66	93.1%	60.0%
1500		10	11.1	0	25	3.526	11.15	100.0%	67.4%
2000		10	0	0	0	0	0		100.0%

Total neonates Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
3	Pooled Controls	34	35	34	33	34	35	33	33	36	37
		34	31	34	35	33	35	34	31	36	33
52.5		36	24	38	38	35					
96.5		40	33	38	33	36					
125		37	36	34	32	37	35	34	35	35	34
205		34	39	24	34	39					
240		29	35	27	28	26	35	32	34	31	34
395		29	34	29	36	36					
470		32	32	32	28	33	29	31	30	25	31
585		36	16	32	37	36					
705		19	34	35	31	32	29	27	32	32	22
805		34	32	20	7	33					
950		33	21	30	27	18	31	8	30	19	27
995		28	0	14	28	25					
1150		29	25	0	8	6					
1500		16	22	4	25	0	0	0	23	21	0
2000		0	0	0	0	0	0	0	0	0	0

Cladoceran Reproduction Test					**eriss ecotoxicology lab**
Analysis ID: 19-4213-0924		**Endpoint:** Total neonates		**CETIS Version:** CETISv1.8.7	
Analyzed: 11 Mar-14 12:31		**Analysis:** Nonlinear Regression		**Official Results:** Yes	

Graphics 3P Logistic [Y=A/(1+exp(-C(X-D)))]



Amerianna cumingi 1275S 1307S 1335S pooled

CETIS Analytical Report

Report Date: 13 Mar-14 11:29 (p 1 of 3)
Test Code: 1275S1307S1335S | 17-8869-9162

Gastropod Reproduction test — **eriss ecotoxicology lab**

Analysis ID:	11-4175-7523	**Endpoint:**	Number of embryos	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-14 11:29	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes
Batch ID:	16-1761-4921	**Test Type:**	Snail reproduction	**Analyst:**	Kim Cheng
Start Date:	16 May-13 11:12	**Protocol:**	Snail eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	16 May-13 11:12	**Species:**	Amerianna cumingi	**Brine:**	Not Applicable
Duration:	NA	**Source:**	In-House Culture	**Age:**	
Sample ID:	20-3317-3808	**Code:**	792FC530	**Client:**	Core project
Sample Date:	16 May-13 11:12	**Material:**	Manganese sulfate	**Project:**	Manganese toxicity
Receive Date:	16 May-13 11:12	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**	N/A		

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Logistic [Y=A/(1+exp(-C(X-D)))]	Log X	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
22	-48.31	105.3	104.3	0.7963	No				Lack of Fit Not Tested

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC5	217.6	33.05	645.5
IC10	343.2	80.45	927.3
IC15	512.2	155.9	1282
IC20	737.7	270.6	1721
IC25	1039	439.2	2269
IC36	2129	1091	4190
IC40	2760	1455	5395
IC50	5440	2748	11970

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	211.5	26.02	160.5	262.5	8.128	<0.0001	Significant Parameter
C	-1.427	0.4455	-2.3	-0.5539	-3.203	0.0094	Significant Parameter
D	3.284	0.2623	2.77	3.798	12.52	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	39539.18	39539.18	1	48.9	<0.0001	Significant
Residual	8085.325	808.5325	10			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.037	2.462	0.3409	No Outliers Detected
Distribution	Shapiro-Wilk W Normality	0.9298	0.8685	0.3388	Normal Distribution
	Anderson-Darling A2 Normality	0.4286	2.492	0.3150	Normal Distribution

000-428-181-1 CETIS™ v1.8.7.4 Analyst:________ QA:________

Gastropod Reproduction test — **eriss ecotoxicology lab**

Analysis ID:	11-4175-7523	**Endpoint:**	Number of embryos	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-14 11:29	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Number of embryos Summary

			Calculated Variate						
C-µg/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**
2	Pooled Controls	9	178.5	134	250.2	11.08	33.25	18.6%	0.0%
25		3	203.8	156	245.3	25.98	44.99	22.1%	-14.1%
120		3	232.1	213.7	249.8	10.45	18.09	7.8%	-30.0%
455		3	137.6	101.5	192.4	27.85	48.23	35.0%	22.9%
940		3	148.4	116	185.2	20.08	34.78	23.4%	16.9%
1010		3	101.7	94	113.7	6.088	10.54	10.4%	43.1%
2350		3	79.79	54.83	129.2	24.71	42.79	53.6%	55.3%
5150		3	66.06	46.17	82	10.53	18.24	27.6%	63.0%
5750		3	63	48	91.33	14.17	24.55	39.0%	64.7%
5950		3	46.28	46.17	46.5	0.1111	0.1924	0.42%	74.1%
10500		3	84.77	61	132.3	23.77	41.17	48.6%	52.5%
29500		3	67.44	62.17	73.5	3.295	5.707	8.46%	62.2%
32500		3	36.72	26.17	48.67	6.532	11.31	30.8%	79.4%

Number of embryos Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9
2	Pooled Controls	197	250.2	155	188.3	169.7	188	167.2	157.5	134
25		245.3	210	156						
120		232.8	213.7	249.8						
455		192.4	101.5	119						
940		116	144.2	185.2						
1010		97.33	94	113.7						
2350		129.2	54.83	55.33						
5150		82	70	46.17						
5750		49.67	91.33	48						
5950		46.17	46.17	46.5						
10500		61	61	132.3						
29500		73.5	62.17	66.67						
32500		48.67	35.33	26.17						

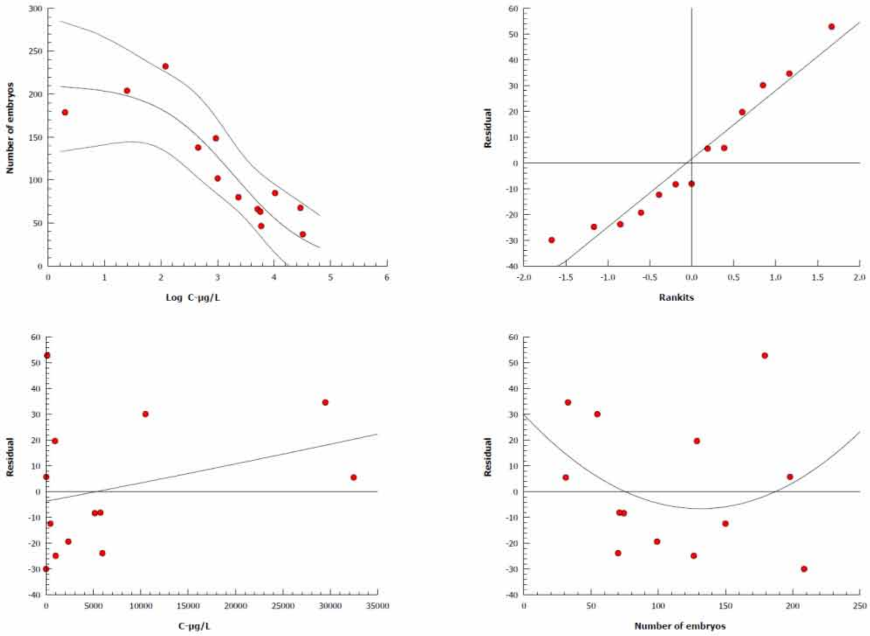
CETIS Analytical Report

Report Date: 13 Mar-14 11:29 (p 3 of 3)
Test Code: 1275S1307S1335S | 17-8869-9162

Gastropod Reproduction test **eriss ecotoxicology lab**

Analysis ID:	11-4175-7523	**Endpoint:**	Number of embryos	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-14 11:29	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Graphics 3P Logistic [Y=A/(1+exp(-C(X-D)))]



Mogurnda mogurnda 1293E, 1300E

CETIS Analytical Report

Report Date: 11 Mar-14 08:51 (p 1 of 4)
Test Code: 1284E1293E1300E | 07-7964-5421

Gudgeon Sac Fry Survival Test — **eriss ecotoxicology lab**

Analysis ID:	01-5996-0537	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 8:50	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes
Batch ID:	11-5140-9308	**Test Type:**	Survival (96h)	**Analyst:**	Kim Cheng
Start Date:	16 May-13 13:21	**Protocol:**	Gudgeon (acute) eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	16 May-13 13:21	**Species:**	Mogurnda mogurnda	**Brine:**	Not Applicable
Duration:	NA	**Source:**	eriss ecotoxicology lab	**Age:**	
Sample ID:	16-7010-2305	**Code:**	Pooled fry data	**Client:**	Core project
Sample Date:	16 May-13 13:21	**Material:**	Manganese sulfate	**Project:**	Manganese toxicity
Receive Date:	16 May-13 13:21	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**	N/A		

Linear Regression Options

Model Function	Threshold Option	Threshold	Optimized	Pooled	Het Corr	Weighted
Log-Normal [NED=A+B*log(X)]	Control Threshold	0.011111	No	Yes	Yes	Yes

Regression Summary

Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)
8	-130.2	265.1	266.5	2.387	0.288	0.8559				Lack of Fit Not Tested

Point Estimates

Level	mg/L	95% LCL	95% UCL
LC5	81.88	36.45	114.6
LC10	104.2	55.59	137
LC15	122.6	73.54	155.3
LC20	139.5	91.42	172.5
LC25	155.8	109.5	189.7
LC36	192.1	150.3	233.3
LC40	206	165	252.7
LC50	243.7	201.2	315.3

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
Threshold	0.01111	0.01048	-0.01082	0.03304	1.06	0.3023	Non-Significant Parameter
Slope	3.473	0.7387	1.927	5.019	4.701	0.0002	Significant Parameter
Intercept	-8.289	1.718	-11.88	-4.693	-4.824	0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	232.5159	232.5159	1	119.8	<0.0001	Significant
Residual	36.89065	1.941613	19			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Goodness-of-Fit	Pearson Chi-Sq GOF	36.89	30.14	0.0082	Significant Heterogenity
	Likelihood Ratio GOF	43.59	30.14	0.0011	Significant Heterogenity
Extreme Value	Grubbs Extreme Value	2.689	2.734	0.0617	No Outliers Detected
Distribution	Shapiro-Wilk W Normality	0.7995	0.9079	0.0006	Non-normal Distribution
	Anderson-Darling A2 Normality	2.195	2.492	<0.0001	Non-normal Distribution

000-428-181-1 CETIS™ v1.8.7.4 Analyst:________ QA:________

Gudgeon Sac Fry Survival Test — **eriss ecotoxicology lab**

Analysis ID: 01-5996-0537 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-14 8:50 **Analysis:** Linear Regression (MLE) **Official Results:** Yes

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-mg/L	**Control Type**	**Count**	**Mean**	**Min**	**Max**	**Std Err**	**Std Dev**	**CV%**	**%Effect**	**A**	**B**
0.0004	Magela Creek W	9	0.9889	0.9	1	0.01111	0.03333	3.37%	0.0%	89	90
0.087		3	1	1	1	0	0	0.0%	-1.12%	30	30
0.39		3	1	1	1	0	0	0.0%	-1.12%	30	30
2		3	1	1	1	0	0	0.0%	-1.12%	30	30
9.5		2	1	1	1	0	0	0.0%	-1.12%	20	20
9.75		3	1	1	1	0	0	0.0%	-1.12%	30	30
23		2	1	1	1	0	0	0.0%	-1.12%	20	20
36.5		2	1	1	1	0	0	0.0%	-1.12%	20	20
46		3	1	1	1	0	0	0.0%	-1.12%	30	30
48		2	1	1	1	0	0	0.0%	-1.12%	20	20
71		2	0.95	0.9	1	0.05	0.07071	7.44%	3.93%	19	20
95		2	0.95	0.9	1	0.05	0.07071	7.44%	3.93%	19	20
120		2	0.95	0.9	1	0.05	0.07071	7.44%	3.93%	19	20
140		2	0.7	0.7	0.7	0	0	0.0%	29.2%	14	20
165		2	0.35	0	0.7	0.35	0.495	141.0%	64.6%	7	20
190		2	0.65	0.6	0.7	0.05	0.07071	10.9%	34.3%	13	20
245		2	0.55	0.3	0.8	0.25	0.3536	64.3%	44.4%	11	20
250		2	0.7	0.7	0.7	0	0	0.0%	29.2%	14	20
295		2	0.4	0.2	0.6	0.2	0.2828	70.7%	59.6%	8	20
320		2	0.6	0.5	0.7	0.1	0.1414	23.6%	39.3%	12	20
360		2	0	0	0	0	0		100.0%	0	20

96h Survival Rate Detail

C-mg/L	**Control Type**	**Rep 1**	**Rep 2**	**Rep 3**	**Rep 4**	**Rep 5**	**Rep 6**	**Rep 7**	**Rep 8**	**Rep 9**
0.0004	Magela Creek Wa	1	1	1	0.9	1	1	1	1	1
0.087		1	1	1						
0.39		1	1	1						
2		1	1	1						
9.5		1	1							
9.75		1	1	1						
23		1	1							
36.5		1	1							
46		1	1	1						
48		1	1							
71		0.9	1							
95		1	0.9							
120		0.9	1							
140		0.7	0.7							
165		0	0.7							
190		0.6	0.7							
245		0.3	0.8							
250		0.7	0.7							
295		0.6	0.2							
320		0.5	0.7							
360		0	0							

Gudgeon Sac Fry Survival Test | **eriss ecotoxicology lab**

Analysis ID: 01-5996-0537 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-14 8:50 **Analysis:** Linear Regression (MLE) **Official Results:** Yes

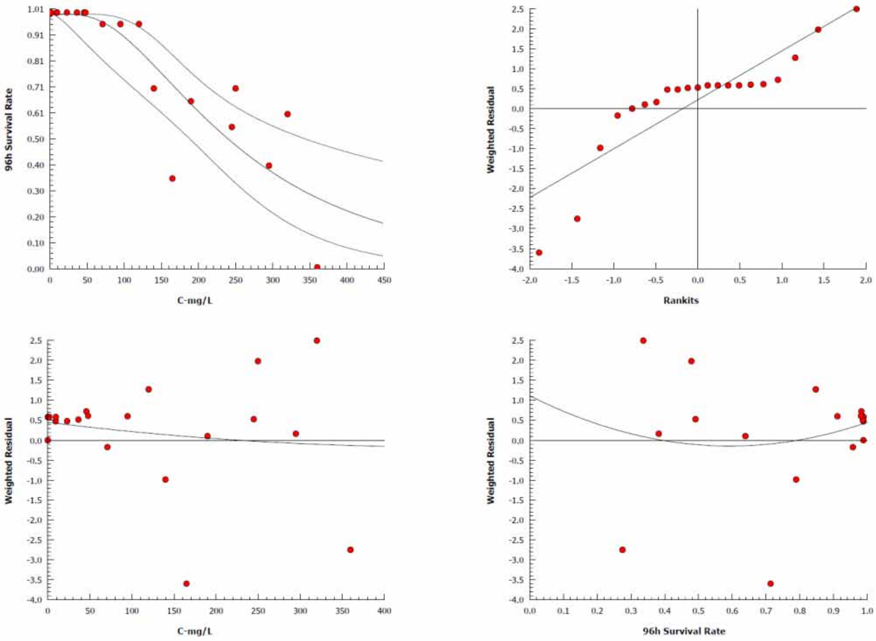
96h Survival Rate Binomials

C-mg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9
0.0004	Magela Creek	10/10	10/10	10/10	9/10	10/10	10/10	10/10	10/10	10/10
0.087		10/10	10/10	10/10						
0.39		10/10	10/10	10/10						
2		10/10	10/10	10/10						
9.5		10/10	10/10							
9.75		10/10	10/10	10/10						
23		10/10	10/10							
36.5		10/10	10/10							
46		10/10	10/10	10/10						
48		10/10	10/10							
71		9/10	10/10							
95		10/10	9/10							
120		9/10	10/10							
140		7/10	7/10							
165		0/10	7/10							
190		6/10	7/10							
245		3/10	8/10							
250		7/10	7/10							
295		6/10	2/10							
320		5/10	7/10							
360		0/10	0/10							

Gudgeon Sac Fry Survival Test — **eriss ecotoxicology lab**

Analysis ID:	01-5996-0537	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-14 8:50	**Analysis:**	Linear Regression (MLE)	**Official Results:**	Yes

Graphics Log-Normal [NED=A+B*log(X)]



Hydra viridissima 1379B 1381B (low pH tests)

CETIS Analytical Report

Report Date:	14 Mar-14 12:30 (p 1 of 3)		
Test Code:	1379B 1381B | 12-7931-8843		

Green Hydra Population Growth Test — **eriss ecotoxicology lab**

Analysis ID:	07-1710-3512	**Endpoint:**	Specific growth rate (96h)	**CETIS Version:**	CETISv1.8.7
Analyzed:	14 Mar-14 12:29	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes
Batch ID:	01-9605-0788	**Test Type:**	Hydra population growth	**Analyst:**	Andrew J Harford
Start Date:	20 Jan-14	**Protocol:**	Hydra eriss tropical freshwater	**Diluent:**	Magela Creek Water
Ending Date:	24 Jan-14	**Species:**	Hydra viridissima	**Brine:**	Not Applicable
Duration:	96h	**Source:**	In-House Culture	**Age:**	
Sample ID:	00-7239-8233	**Code:**	450B599	**Client:**	Internal Lab
Sample Date:	14 Mar-14 12:04	**Material:**	Manganese sulfate	**Project:**	Manganese toxicity
Receive Date:	14 Mar-14 12:04	**Source:**	Manganese Toxicity (MNTOXICITY)		
Sample Age:	NA	**Station:**	In House		

Non-Linear Regression Options

Model Function	X Transform	Y Transform	Weighting Function	PTBS Function
3P Logistic [Y=A/(1+exp(-C(X-D)))]	Log X	None	Normal [W=1]	Off [Y*=Y]

Regression Summary

Iters	Log LL	AICc	BIC	Adj R2	Optimize	F Stat	Critical	P-Value	Decision(α:5%)
7	54.92	-101.8	-101.5	0.8903	No				Lack of Fit Not Tested

Point Estimates

Level	µg/L	95% LCL	95% UCL
IC10	179.6	81.31	274.1
IC50	795.4	612.6	1037

Regression Parameters

Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)
A	0.218	0.01005	0.1983	0.2377	21.7	<0.0001	Significant Parameter
C	-3.192	0.732	-4.627	-1.757	-4.36	0.0008	Significant Parameter
D	2.873	0.06731	2.741	3.005	42.69	<0.0001	Significant Parameter

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Model	0.058462	0.058462	1	123.7	<0.0001	Significant
Residual	0.006144	0.000473	13			

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.13	2.586	0.3468	No Outliers Detected
Distribution	Shapiro-Wilk W Normality	0.9305	0.887	0.2481	Normal Distribution
	Anderson-Darling A2 Normality	0.6584	2.492	0.0860	Normal Distribution

000-428-181-1 CETIS™ v1.8.7.4 Analyst:________ QA:________

Green Hydra Population Growth Test — eriss ecotoxicology lab

Analysis ID:	07-1710-3512	Endpoint:	Specific growth rate (96h)	CETIS Version:	CETISv1.8.7
Analyzed:	14 Mar-14 12:29	Analysis:	Nonlinear Regression	Official Results:	Yes

Specific growth rate (96h) Summary

			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
3	Magela Creek W	6	0.2133	0.1855	0.2662	0.01425	0.03491	16.4%	0.0%
21.5		2	0.218	0.1971	0.2389	0.02088	0.02953	13.5%	-2.21%
30		2	0.2301	0.1855	0.2747	0.04458	0.06305	27.4%	-7.87%
36.5		2	0.208	0.1971	0.2189	0.01088	0.01538	7.4%	2.48%
40		2	0.2189	0.2189	0.2189	0	0	0.0%	-2.62%
70		2	0.2214	0.1855	0.2574	0.03596	0.05086	23.0%	-3.83%
80		2	0.224	0.2189	0.2291	0.005103	0.007216	3.22%	-5.01%
140		2	0.1969	0.1855	0.2082	0.01137	0.01608	8.17%	7.7%
150		2	0.1537	0.1469	0.1605	0.006758	0.009558	6.22%	27.9%
300		2	0.1669	0.1605	0.1733	0.006412	0.009067	5.43%	21.8%
310		2	0.1669	0.1605	0.1733	0.006412	0.009067	5.43%	21.8%
565		2	0.1251	0.1175	0.1327	0.007578	0.01072	8.57%	41.4%
625		2	0.1373	0.1014	0.1733	0.03596	0.05086	37.0%	35.6%
1100		2	0.1223	0.08412	0.1605	0.03817	0.05398	44.1%	42.7%
1200		2	0.05875	0	0.1175	0.05875	0.08309	141.0%	72.5%
2350		4	0	0	0	0	0		100.0%

Specific growth rate (96h) Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
3	Magela Creek Wa	0.2483	0.1855	0.1971	0.1855	0.2662	0.1971
21.5		0.1971	0.2389				
30		0.2747	0.1855				
36.5		0.1971	0.2189				
40		0.2189	0.2189				
70		0.1855	0.2574				
80		0.2189	0.2291				
140		0.2082	0.1855				
150		0.1469	0.1605				
300		0.1605	0.1733				
310		0.1733	0.1605				
565		0.1327	0.1175				
625		0.1733	0.1014				
1100		0.08412	0.1605				
1200		0.1175	0				
2350		0	0	0	0		

Green Hydra Population Growth Test — **eriss ecotoxicology lab**

Analysis ID:	07-1710-3512	**Endpoint:**	Specific growth rate (96h)	**CETIS Version:**	CETISv1.8.7
Analyzed:	14 Mar-14 12:29	**Analysis:**	Nonlinear Regression	**Official Results:**	Yes

Graphics 3P Logistic [Y=A/(1+exp(-C(X-D)))]