

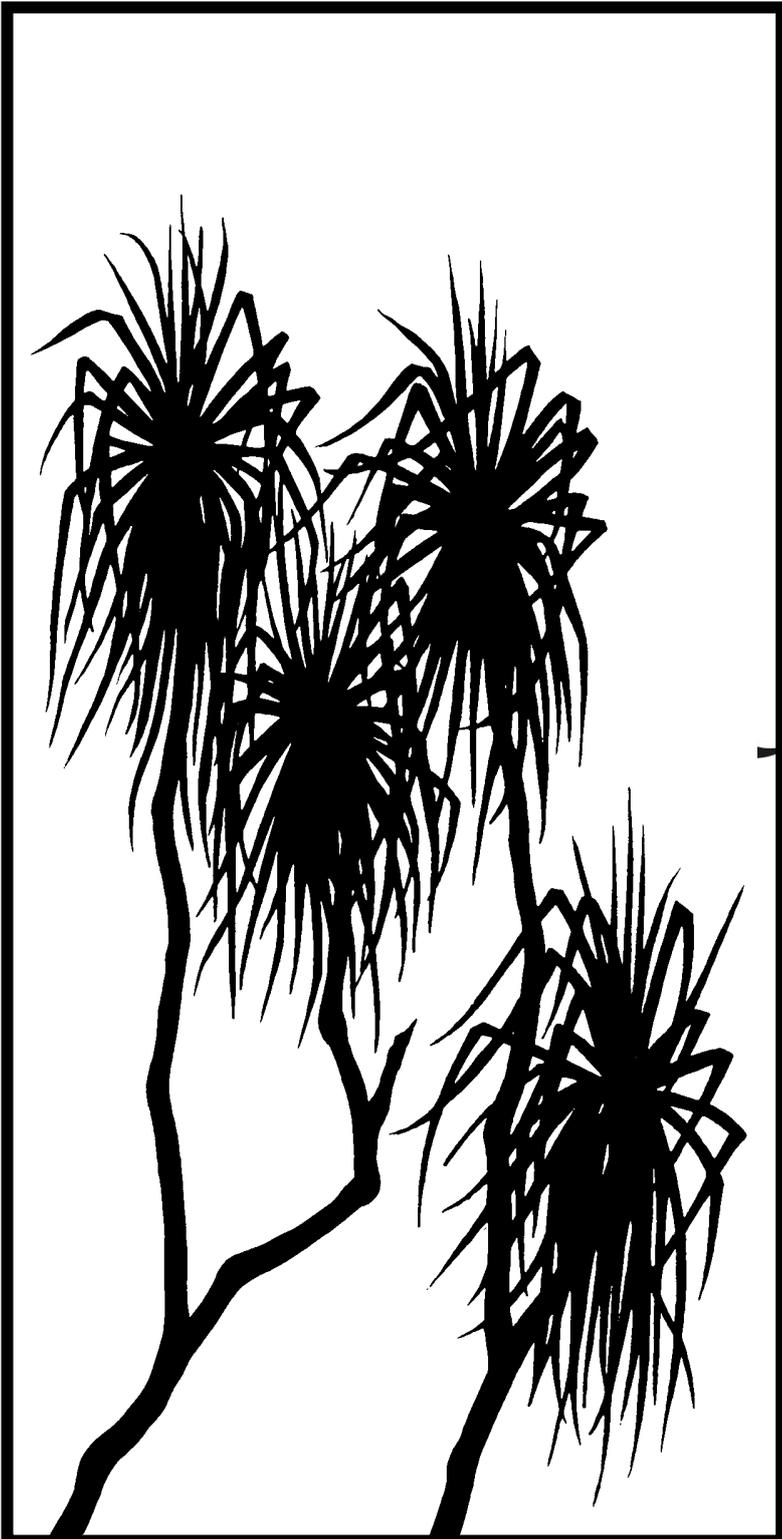


Australian Government

Department of Sustainability, Environment,
Water, Population and Communities
Supervising Scientist

*internal
report*

599



Ecotoxicological
assessment of distillate
product from a pilot-scale
brine concentrator

AJ Harford & RA van Dam

March 2012

(Release status – unrestricted)

This page has been left blank intentionally.

Ecotoxicological assessment of distillate product from a pilot-scale brine concentrator

AJ Harford & R A van Dam

Supervising Scientist Division
GPO Box 461, Darwin NT 0801

March 2012

Registry File SSD2011/0132

(Release status – unrestricted)



Australian Government

Department of Sustainability, Environment, Water, Population and Communities
Supervising Scientist

How to cite this report:

Harford AJ & van Dam RA 2012. Ecotoxicological assessment of distillate product from a pilot-scale brine concentrator. Internal Report 599, March, Supervising Scientist, Darwin.

Location of final PDF file in SSDX Sharepoint:

[Supervising Scientist Division > PublicationWork > Publications and Productions > Internal Reports \(IRs\) > Nos 500 to 599 > IR599 Distillate Ecotox \(AJH-RvD\)](#)

Location of all key data files for this report in SSDX Sharepoint:

[Supervising Scientist Division > SSDX > Ecotoxicology of the Alligator Rivers Region > Brine Concentrator Pilot Plant](#)

Authors of this report:

Andrew J Harford – Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin NT 0801, Australia

Rick A van Dam – Environmental Research Institute of the Supervising Scientist, GPO Box 461, Darwin NT 0801, Australia

The Supervising Scientist is part of the Australian Government Department of Sustainability, Environment, Water, Population and Communities.

© Commonwealth of Australia 2012

Supervising Scientist

Department of Sustainability, Environment, Water, Population and Communities

GPO Box 461, Darwin NT 0801 Australia

This work is copyright. Apart from any use as permitted under the Copyright Act 1968, no part may be reproduced by any process without prior written permission from the Supervising Scientist. Requests and enquiries concerning reproduction and rights should be addressed to Publications Enquiries, Supervising Scientist, GPO Box 461, Darwin NT 0801.

e-mail: publications_ssd@environment.gov.au

Internet: www.environment.gov.au/ssd (www.environment.gov.au/ssd/publications)

The views and opinions expressed in this report do not necessarily reflect those of the Commonwealth of Australia. While reasonable efforts have been made to ensure that the contents of this report are factually correct, some essential data rely on references cited and/or the data and/or information of other parties, and the Supervising Scientist and the Commonwealth of Australia do not accept responsibility for the accuracy, currency or completeness of the contents of this report, and shall not be liable for any loss or damage that may be occasioned directly or indirectly through the use of, or reliance on, the report. Readers should exercise their own skill and judgment with respect to their use of the material contained in this report.

Printed and bound in Darwin NT by Supervising Scientist Division

Contents

Tables	iv
Figures	v
Executive summary	vi
Recommendations	vii
1 Introduction	1
2 Methods	3
2.1 General laboratory procedures	3
2.2 Test waters	3
2.3 Test diluent	3
2.4 Toxicity test species and methods	4
2.5 Toxicity Identification Evaluation (TIE) tests	4
2.5.1 Graduated pH	6
2.5.2 EDTA addition	6
2.5.3 Ammonia stripping	6
2.5.4 Solid phase extraction (SPE) with Carbon 18 (C18)	7
2.5.5 Major ion additions	7
2.5.6 Effect of Mn in low major ion waters	8
2.6 Quality control	8
2.6.1 Chemistry	8
2.6.2 General water quality	8
2.6.3 Control responses	9
2.7 Statistics	9
3 Results and discussion	10
3.1 Quality control	10
3.2 Distillate chemistry	10
3.3 Toxicity test results	10
3.4 Toxicity identification evaluation (TIE) results	12
4 Conclusions and recommendations	16
5 Acknowledgments	17
6 References	17
Appendix A Measured water quality parameters for toxicity tests	19
Appendix B Chemical analyses	30
Appendix C Statistical summaries	41

Tables

Table 1	Details of toxicity tests for the five Australian tropical freshwater species used to assess the toxicity of pilot-scale brine concentrator distillate	5
Table 2	Toxicity Identification Evaluation toxicity tests using <i>H. viridissima</i>	6
Table 3	Composition of the process water before and after treatment with the brine concentrator	11
Table 4	Toxicity of the pilot brine concentrator distillate	12
Table 5	Results of toxicity identification evaluation toxicity tests using <i>H. viridissima</i>	13
Appendix A Measured Water Quality Parameters for Toxicity Tests		
Table A1	1193D Clad_RBCP_01 Cladoceran toxicity test	19
Table A2	1194B Hyd_RBCP_01 Hydra toxicity test	20
Table A3	1195G Alg_RBCP_01 Green alga toxicity test	20
Table A4	1205L Lem_RBCP_01 Duckweed toxicity test	20
Table A5	1206E Fry_RBCP_01 Fry toxicity test	21
Table A6	1207B Hyd_RBCP_02 Hydra toxicity test	21
Table A7	1208D Clad_RBCP_02 Cladoceran toxicity test	22
Table A9	1209G Alg_RBCP_02 Green alga toxicity test	22
Table A10	1210B Hyd_RBCP_03 Hydra graduate pH TIE	23
Table A11	1211B Hyd_RBCP_04 Hydra EDTA addition TIE	24
Table A12	1212B Hyd_RBCP_04 Hydra Ca addition TIE	25
Table A13	1213B Hyd_RBCP_06 Hydra NH ₃ stripped TIE	26
Table A14	1217B Hyd_RBCP_07 Hydra C18 Solid Phase Extraction TIE	26
Table A15	1220B Hyd_RBCP_08 Hydra major ion addition TIE	27
Table A16	1242B Hyd_RBCP_09 Modified SSW with Mn addition TIE	28
Table A17	pH measurements and adjustments for the graduate pH TIE	29
Appendix B Chemical Analyses		
Table B1	Total measured metals and major ions for Magela Creek Water (MCW), Synthetic Softwater (SSW) Procedural Blanks and Blanks	30
Table B2	Total and dissolved metals and major ions for the distillate	32
Table B3	Nitrate analysis of Blanks and QA/QC samples for the <i>Lemna</i> and Algae tests	34
Table B4	Alkalinity of Magela Creek Water, Distillate and the NaOH adjusted TIE samples	34
Table B5	Metal and major ion analyses of the pH adjusted TIE (1210B) samples	35

Table B6 Measured 'bioavailable' Manganese (Chelex assay) in EDTA TIE (1211B)	36
Table B7 Metal and major ion analyses of the Ca addition TIE (1212B) samples	37
Table B8 Metal and major ion analyses of the ammonia stripping TIE (1213B) samples	37
Table B9 Metal and major ion analyses of the major ion addition TIE (1220B) samples	38
Table B10 Organic compounds detected by GC-MS scan	39
Table B11 Measured calcium, sodium, potassium, magnesium and manganese concentrations in the Mn toxicity TIE (1242B)	40

Figures

Figure 1 Schematic diagram of the pilot Brine Concentrator	2
Figure 2 Concentration-response plots of the five species to the pilot brine concentrator distillate	11
Figure 3 Effect of manganese on <i>Hydra viridissima</i> in modified Synthetic Soft Water (SSW)	15

Executive summary

Steadily increasing process water inventory at the Ranger uranium mine has become a major operational issue for Energy Resources of Australia Ltd (ERA). Following an assessment of potential technology options ERA decided that brine concentration was the most viable technology to reduce the process water inventory. A brine concentrator produces large volumes of a purified water product (distillate) and a waste stream containing the salts present in the process water (brine concentrate). The distillate will be released into the environment via a yet to be determined method, while the brine concentrate will be returned to the tailings storage facility (TSF). Rio Tinto – Technology and Innovation (RT-TI, Bundoora, Victoria) were engaged by ERA to conduct trials on a pilot-scale brine concentrator plant. Two key aims of RT-TI trial were to (i) demonstrate that the distillate does not pose risks to operator health or the environment, and (ii) provide data to assist with designing water management and disposal systems. To assist with addressing the aquatic environment protection aspect, *eriss* undertook a comprehensive toxicity testing program of the pilot plant distillate. The aims of the toxicity test work were to: (i) detect and quantify any residual toxicity of the distillate and, (ii) in the event effects were observed, to identify the toxic constituent(s) of the distillate.

Initial toxicity screening of the distillate was conducted with a limited range of dilutions of the distillate using three aquatic species which had previously displayed sensitivity to treated process water permeate from the Ranger Treatment Water Plant. Specifically, *Chlorella* sp. (72-h cell division rate), *Hydra viridissima* (96-h population growth rate) and *Moinadaphnia macleayi* (3-brood reproduction) were exposed to Magela Creek water (MCW) control and three dilutions of the distillate (ie 0, 25, 50 and 100% distillate). Further testing was conducted on a second batch of distillate using the same concentration range and two additional species, *Lemna aequinoctialis* (96-h growth rate) and *Mogurnda mogurnda* (96-h larval survival). The toxicity of the second batch of distillate was also assessed using *Chlorella* sp., *H. viridissima* and *M. macleayi*, although only at 0 (MCW) and 100% distillate, in order to assess the inter-batch reproducibility of the test methods.

In order to identify the toxic constituents of the distillate, a range of Toxicity Identification Evaluation (TIE) tests were conducted using the sole sensitive species, *H. viridissima*. The TIE tests involved assessing the relative toxicity of distillate samples produced by specific physical and chemical manipulations to change its composition or the speciation of specific constituents of potential concern. The results enable conclusions about potential primary toxicants. Six TIE tests were conducted to identify the cause of adverse effects on *H. viridissima*.

The distillation process reduced all major ions, ammonia and metals to near detection limits. Some organic compounds that were not detected in the feed water were detected at low $\mu\text{g L}^{-1}$ concentrations in the distillate. The toxicity tests results showed that the distillate was of low toxicity to four of the five organisms tested. However, the population growth rate of *H. viridissima* was reduced by ~50% and 100% following exposure to undiluted (ie 100%) distillate samples from the first and second batch, respectively.

Initial chemical analysis of the distillate indicated that ammonia, manganese (Mn) and an organic component were potential candidate constituents for causing a toxic response. However, initial TIE results suggested none of these constituents were causing or contributing to the observed negative effect on *H. viridissima*. Specifically, pH manipulation (raising pH) and stripping to remove ammonia that was present indicated that ammonia was not causing the effect. Whilst the pH manipulation suggested Mn may be contributing to the effect, the effect of addition of Ethylenediamine tetraacetic acid (EDTA, a chelating agent) indicated

that this was unlikely. Removal of the organic component did not change the toxicity of the distillate, discounting organics as a cause of toxicity.

In light of the above negative findings, the issue of major ion deficiency was specifically investigated as a potential cause of the effect on *H. viridissima*. Firstly, Ca addition was investigated due to its importance for nematocyst function and other physiological processes in *H. viridissima*. The addition of 0.2 and 0.5 mg L⁻¹ Ca to the distillate resulted in a 61% and 66% recovery relative to the Synthetic Soft Water (SSW) control, suggesting Ca deficiency as a reason for the effect of distillate on *H. viridissima*. An additional test was conducted that involved the addition of sodium (Na), potassium (K) and Ca at concentrations that were 0, 50 and 100% that of SSW (SSW contains 0.5, 1.0 and 0.4 mg L⁻¹ of calcium, sodium and potassium, respectively). The results showed a 100% and 96% recovery of *H. viridissima* population growth rates with the addition of 50 and 100% major ions, respectively. This strongly indicates that the majority of the adverse effect from the distillate on *H. viridissima* was due to major ion deficiency issue rather than a chemical toxicity.

Despite the substantive removal of toxic effect by replacement of major cations, the concentrations of Mn in the distillate (130-230 µg L⁻¹) remained a concern as they were higher than the IC₁₀ of 60 µg L⁻¹ previously reported for *H. viridissima* in circumneutral pH (6.0–7.0) soft water. Additionally, the lack of major ions in the distillate had the potential to exacerbate Mn toxicity. Therefore, the effects of Mn in the presence of reduced concentrations of major ions were examined using modified SSW (ie pH ~6.0 with 0, 50 and 100% Na, K and Ca concentrations). Manganese concentrations of 250 µg L⁻¹ caused a 10–20% reduction in growth rate, independent of the major ion concentrations. Consequently, in addition to the recognised issue with deficiencies of major ions in the distillate, a potential for Mn toxicity was also identified.

Recommendations

1. Supplementation of the distillate with major ions (Ca, Na and K) may be required prior to its discharge to the off-site aquatic environment. This could be achieved actively by direct addition of relevant salts or passively by passing the distillate through a wetland system/watercourse and/or blending with mine site waters prior to discharge;
2. While the conditioning of the distillate through a wetland/watercourse is likely to improve water quality (by increasing major ion concentrations and, potentially, reducing dissolved Mn concentrations), the risk of exhausting of the system's capacity to sustainably contribute the required loading of salt may need to be considered if large volumes are to be flushed through the system;
3. Further site-specific data are needed to adequately assess the environmental risk of Mn in the distillate.
4. A baseline monitoring program for organic compounds in the TSF is needed to establish the likelihood of significant concentrations of sVOCs and VOCs entering the feed water, hence indicating the potential for transfer to the distillate. The distillate should also be monitored for organic compounds following the commissioning of the full-scale plant.
5. The effect of anti-scalant and anti-foaming agents that may be added to the concentrator's feed water needs to be assessed. This may be achieved with laboratory toxicity tests prior to the commissioning of the full-scale plant.
6. The distillate product from the full-scale plant will need to be assessed for toxicity and, if necessary, a TIE conducted to determine the cause(s) of any measured effects.

This page has been left blank intentionally.

Ecotoxicological assessment of distillate product from a pilot-scale brine concentrator

AJ Harford & RA van Dam

1 Introduction

Mine waters at Ranger uranium mine (Ranger) are segregated into four classes – process water, pond water, release waters and potable water – according to water quality. Process water includes all waters that have passed through or come into contact with the uranium (U) extraction circuit. It constitutes the poorest water quality on site, with key water quality characteristics typically as follows: pH: 3.7–4.0; electrical conductivity (EC): 22 000–27 000 $\mu\text{S}/\text{cm}$; sulfate (SO_4): 24 000–34 000 mg/L; U: 18–25 mg/L; and ammonia (NH_3): 780–950 mg/L N (ERA, Water Management Plans 2005–2011, unpublished).

A steadily increasing process water inventory at Ranger has become a major operational issue. Throughout the operation of the mine a number of process water treatment methods have been investigated including: passive and enhanced solar evaporation such as conventional and covered solar evaporation ponds; enhanced evaporation techniques such as sprinklers, misters, vortex generators, waste heat utilisation, high density sludge and membrane technology; alternative dam designs (ie fixed and floating storage facility covers); chemical precipitation; and thermal treatment (ERA, unpublished data). Currently, process water is stored in the Tailings Storage Facility (TSF) and Pit 1, and the primary method of process water inventory reduction has been through passive evaporation. During the 2009 and 2010 dry seasons, process water was also treated using a high density sludge–membrane ultra-filtration reverse osmosis (HDS-UF/RO) water treatment plant. However, the plant did not meet the treatment capacity required to meet the production demand. Thus, following an assessment of potential technology options, ERA decided that brine concentration was the most viable technology to reduce the process water inventory.

The planned full-scale brine concentration facility will consist of three brine concentrator units configured in a two stage process with the first two units feeding the third. The brine concentrator is a falling film evaporator, which means that heated process water is cascaded down a falling film tube bundle. The resultant vapour passes through a chevron separator and a vapour washer, which removes entrained water droplets which may further improve the vapour quality. The vapour is then compressed to heat the falling film tubes and condenses into a purified water product (distillate). The full-scale brine concentrator is forecast to produce 1.83 GL/annum of a distillate and a waste stream containing the salts present in the process water (brine concentrate). The distillate will be released into the environment via a yet to be determined method, while the brine concentrate will be managed on-site.

Rio Tinto – Technology and Innovation (RT-TI, Bundoora, Victoria) was engaged by ERA to conduct trials on a pilot-scale brine concentrator plant (Figure 1; ERA, unpublished data). Two key aims of the RT-TI trial were to (i) demonstrate that the distillate does not pose risks to operator health or the environment, and (ii) provide data to assist with designing water management and disposal systems. To assist with addressing the aquatic environment protection aspect, the Environmental Research Institute of the Supervising Scientist (*eriss*) undertook a comprehensive toxicity testing program of the pilot plant distillate.

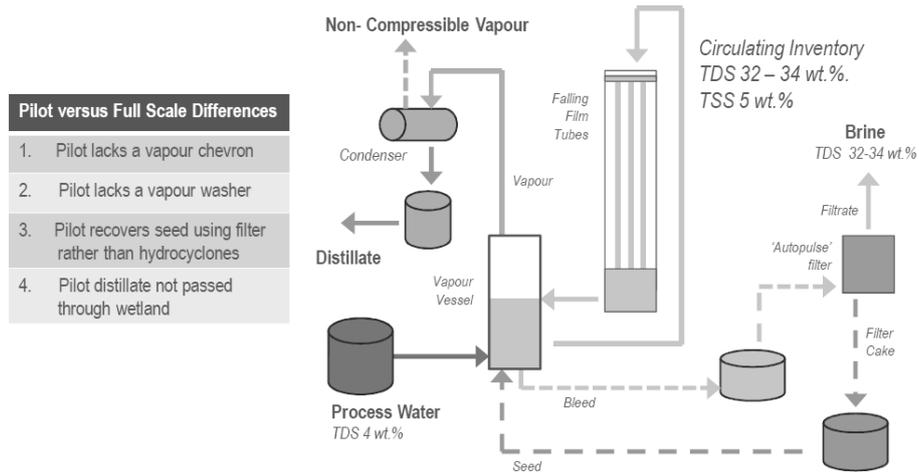


Figure 1 Schematic diagram of the pilot Brine Concentrator (ERA, unpublished)

There are numerous examples of mining operations that have used passive and/or active water treatments to improve water quality prior to environmental release (Masarczyk et al 1989, Driussi & Jansz 2006, Allen 2008, Butler et al 2011). However, a key consideration in choosing an appropriate treatment process is the extent to which the water quality is actually improved. The residual toxicity of the treated water can be assessed using traditional ecotoxicological protocols, while Toxicity Identification Evaluation (TIE) may be able to identify the toxic constituents of the water. Toxicity Identification Evaluations involve specific manipulations of a whole effluent in order to change the amount and/or speciation/bioavailability of potential toxic constituents, eg pH adjustment. The subsequent level of toxicity of the manipulated waters relative to the unmanipulated waters can then provide information on the likely toxic constituents. There are three phases that may be included in a TIE Phase I involves manipulations of the effluent that only enables broad screening of the toxic constituents. Phase II involves manipulations that specifically identify the toxicants of interest that have been indicated in Phase I. Phase III involves the reintroduction of the toxicants to confirm the toxicity of the suspected contaminants in the effluent (Mirenda & Hall 1992).

Toxicity Identification Evaluations have well-established USEPA protocols (Norberg-King et al 1991, Durhan et al 1993, Mount & Norberg-King 1993) and have been used to identify toxic constituents in a wide range of industrial, urban and mining effluents (Sauer et al 1997, Tietge et al 1997, Deanovic et al 1999, Neculita et al 2008). The information obtained from a TIE can inform the management strategy for waste water disposal and/or identify improvements in water treatment processes.

The hypothesis of the present study was that the distillate contained residual toxicity due to constituents that were not removed by the pilot-scale brine concentration. Consequently, the objectives of the toxicity test work were to: (i) detect and quantify any residual toxicity of the distillate and, (ii) in the event effects were observed, to identify the toxic constituent(s) of the distillate using TIE methods.

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 (eg Teflon, glass or polyethylene). All plastics and glassware were washed by soaking in 5% (v/v) HNO₃ for 24 h before being washed with a non-phosphate detergent (Gallay Clean A powder, Gallay Scientific, Burwood, Australia) 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).

2.2 Test waters

Distillate waters were produced by a pilot-scale brine concentrator, which used a falling film evaporator process. The pilot-scale plant did not have a chevron or vapour washer, which is planned to be included in the full-scale facility and is predicted to further improve distillate water quality. Two separate batches of distillate were collected from the brine concentrator for toxicity testing. The first batch was a 20 L composite sample collected from 11–17 July 2011, and was used for the initial screening toxicity tests involving three species (see section 2.4). The second batch was a 20 L grab sample collected on 10 August 2011, and was used for the remainder of the toxicity and TIE tests. This sample was collected as a grab because the pilot-plant project was due to be terminated. Furthermore, an attempt to create scale within the brine concentrator had been initiated and there was a concern that the water quality of the distillate would degrade. Both batch samples were collected in acid-washed high-density polyethylene containers and immediately air-freighted at 4°C to the *eriss* laboratory.

On receipt of samples, the distillate was immediately sub-sampled for physico-chemical analyses. Specifically, pH, Dissolved Oxygen (DO), Electrical Conductivity (EC) and Dissolved Organic Carbon (DOC) were measured in-house. Additional sub-samples were sent to Envirolab Services (Envirolab; Chatswood, NSW) for measurement of alkalinity (APHA2320B), total and filtered (< 0.45 µm) metals (Inductively Coupled Plasma – Mass Spectrometry (ICP-MS) full scan), nitrate, phosphate, ammonia (Colourimetric methods, EPA 353.2, EPA 365.1, EPA 350.1), and volatile and semi-volatile organic analyses (Gas Chromatography – Mass Spectrometry (GC-MS) scan).

2.3 Test diluent

Natural Magela Creek water (MCW) was used as the control treatment and for dilution of the distillate samples in all 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 water was collected in 20 L acid-washed plastic containers and placed in storage at 4 ± 1°C within 1 h of collection. The water was then transported to the laboratory in an air-conditioned vehicle. At the laboratory, it was stored at 4 ± 1°C prior to filtration through 3.0 µm pore size (Sartopure PP2 depth filter MidiCaps, Sartorius, Göttingen, Germany) within 3 days of collection. Throughout the testing period, the MCW had a pH of 6.2–6.8 units, an EC of 15–20 µS cm⁻¹ and DO of > 90% saturation.

Diluent water was sub-sampled for physico-chemical analyses. Specifically pH, DO, EC and DOC were measured in-house. Additional sub-samples were sent to Envirolab (Chatswood, NSW) for alkalinity (APHA2320B), a limited metal and major ion suite (totals only; Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, SO₄ (analysed as S and converted)), nitrate, phosphate and ammonia (Colourimetric methods, EPA 353.2, EPA 365.1, EPA 350.1).

2.4 Toxicity test species and methods

The toxicity of the distillate was assessed using five Australian tropical freshwater species: the unicellular green alga (*Chlorella* sp); the duckweed (*Lemna aequinoctialis*); the green hydra (*Hydra viridissima*); the cladoceran (*Moinodaphnia macleayi*); 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). 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.

Initial toxicity screening of the distillate was conducted with a limited range of dilutions of the distillate using three aquatic species which had previously displayed sensitivity to treated process water permeate from the Ranger Water Treatment Plant (van Dam et al 2011). Specifically, *Chlorella* sp (72-h cell division rate), *H. viridissima* (96-h population growth rate) and *M. macleayi* (3-brood reproduction) were exposed to MCW control and three dilutions of the distillate (ie 0, 25, 50 and 100% distillate).

Further testing was conducted on the second batch of distillate using the same concentration range (0, 25, 50 and 100% distillate) and two different species, *L. aequinoctialis* (96-h growth rate) and *M. mogurnda* (96-h larval survival). The toxicity of the second batch of distillate was also assessed using *Chlorella* sp, *H. viridissima* and *M. macleayi*, although only at 0 (MCW control) and 100% distillate, in order to assess the inter-batch reproducibility of the test methods.

2.5 Toxicity Identification Evaluation (TIE) tests

In order to identify the toxic constituents of the distillate, a range of Phase I TIE toxicity tests were conducted using the sole sensitive species, *H. viridissima*. A Phase II TIE test involving ammonia stripping was conducted, as ammonia was a toxicant of interest. No other Phase II or Phase III toxicity tests were deemed necessary due to the results returned by the Phase I TIE. However, it should be noted that the TIE tests involving the addition of major ions (see below) would be classified as Phase II TIEs except that they are not standard USEPA methods, which focus on complex effluents containing organic and/or inorganic toxicants. The major ion TIEs were required due to the purity of the distillate and to specifically identify if the adverse effects were due to a lack of essential ions.

All TIE tests used the standard *H. viridissima* protocol described in section 2.4, except that the tests involved assessing the relative toxicity of distillate samples that had undergone specific physical and chemical manipulations to change their composition or the speciation of specific constituents of potential concern. The results enabled conclusions about potential primary toxicants. Six TIE tests were conducted to identify the cause of adverse effects on *H. viridissima* (Table 2).

Table 1 Details of toxicity tests for the five Australian tropical freshwater species used to assess the toxicity of pilot-scale brine concentrator distillate. Full details of the methods are provided in Riethmuller et al (2003).

Species (common name)	Test duration and endpoint	Control response acceptability criterion	Temperature, light intensity, photoperiod	Feeding/ nutrition	No. replicates (Individuals per replicate)	Test volume (mL)	Static/daily renewals
<i>Chlorella</i> 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
<i>Lemna aequinoctialis</i> (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
<i>Hydra viridissima</i> (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 <i>Artemia</i> nauplii day ⁻¹	3 (10)	30	Daily renewals
<i>Moinodaphnia macleayi</i> (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 <i>Chlorella</i> sp. d ⁻¹	10 (1)	30	Daily renewals
<i>Mogurnda mogurnda</i> (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 CV: Percent co-efficient of variation

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

All TIE tests included a control water (MCW or Synthetic Soft water, SSW) and distillate that were treated as described below, as well as untreated control water (MCW and/or SSW) and distillate.

Table 2 Toxicity Identification Evaluation toxicity tests using *H. viridissima*

TIE test	Test solution manipulation	Reason for manipulation
Graduated pH	MCW and Distillate adjusted to pH (nominal) 5.5 and 7.5	Differentially alters speciation and toxicity of chemicals
EDTA ^a addition	0, 2.8, 5.5 and 11.0 mg/L EDTA added to MCW and distillate	EDTA binding reduces cationic metal bioavailability and toxicity
C18 Solid Phase Extraction (SPE)	MCW and distillate post-C18 column water tested. Eluate of distillate tested in MCW	Tests for toxicity of organic compounds
Major ion addition	0, 50 and 100% proportions (compared to SSW ^b) of sodium, calcium and potassium added to SSW and distillate	Reintroduction of essential elements

^a Ethylenediaminetetraacetic acid

^b Synthetic Soft Water contains 0.5, 1.0 and 0.4 mg L⁻¹ of calcium, sodium and potassium, respectively.

2.5.1 Graduated pH

Changing the pH of an effluent can change the speciation of toxicants, which subsequently can change their bioavailability and toxicity. For example, decreasing the pH can increase the proportion of toxic free metal ions, while increasing the pH can increase the proportion of toxic ammonia ions. The pH of the distillate and MCW was decreased from 6.5 to pH 5.5 using 1 M HCl and increased to pH 8.0 using 1 M NaOH. The pH adjustments were made daily (see Table A17 for pH measurements and adjustments).

Subsamples of the treatments were sent to Envirolab for measurement of a limited metal and major ion suite (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, SO₄ - analysed as S and converted; Table B5). The controls (pH 6.5) and HCl treated solutions (pH 5.5) were also analysed for Cl⁻ (Table B5), while the controls (pH 6.5) and NaOH treated solutions (pH 8.0) were analysed for alkalinity (Table B4).

2.5.2 EDTA addition

Ethylenediamine Tetraacetic acid (EDTA) is a strong chelator of divalent cations such as Mn, which was the key elevated metal in the distillate. Hence, the addition of EDTA may reduce the bioavailability and toxicity of these cations.

Concentrations of 0, 2.8, 5.5 and 11 mg L⁻¹ EDTA (BDH, Kilsyth, NSW, Australia) were added to both MCW and the distillate. These concentrations were based on a calculation of a 1:1 molar ratio of all major divalent cations and a Mn concentration of 230 µg L⁻¹, which was the concentration measured in the first batch of the distillate.

Sub-samples of the treatments were sent to Envirolab for chemical analysis of the 'bioavailable' Mn fraction using the Chelex assay (Table B6). The Chelex-100 resin will not react with Mn that is complexed with EDTA, but has a high affinity for free Mn²⁺, which is considered the toxic chemical species (Haraldsson et al 1993).

2.5.3 Ammonia stripping

The unprotonated ammonia ion (NH₃) is relatively volatile compared to the protonated ammonium ion (NH₄⁺). The proportion of ammonia ions increases with increasing pH.

Consequently, raising the pH of the distillate combined with vigorous aeration effectively removes the ammonia from the effluent.

The day prior to the TIE test, 1 L of both the MCW and distillate were increased to pH 11 using 10 M NaOH. The pH was checked and re-adjusted throughout the day as the pH had the tendency to drift towards pH 9. The samples were left aerating overnight while covered at room temperature. The pH of the MCW and distillate were readjusted to pH 6.5 before initiation of the TIE test.

Ammonia content was assessed using a colourimetric NH₃ test kit (Merck, Darmstadt, Germany). After ~23 h of pH and aeration treatment, the distillate contained 0 mg L⁻¹ ammonia. Sub-samples of the treatments were sent for the measurement of total metal and major ion concentrations for a limited suite (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, K and SO₄ - analysed as S and converted; Table B8).

2.5.4 Solid phase extraction (SPE) with Carbon 18 (C18)-based resin

Non-polar (lipophilic) organic constituents can be removed from an effluent using solid phase extraction (SPE) columns containing Carbon 18 (C18) moieties. However, these columns will also remove some metals and surfactants. In Phase I TIE testing, the waters are passed through the column at ambient pH and the column is then eluted using a small volume of methanol. The eluate is also added to the reference water and tested for toxicity. Hence, if an organic chemical is the cause of toxicity the filtrate should exhibit less toxicity but the toxicity should be transferred to the eluate-spiked water.

Both MCW and the distillate were treated by SPE by drawing 1 L through 1000 mg of C18 resin (Restek, Bellefonte, PA, USA) using a vacuum pump at a flow rate of ~5 mL min⁻¹. The fraction collected after passing through the column was designated 'filtrate'. The column was kept wet and the fraction retained on the C18 resin was eluted using 3 mL of pure methanol (BDH, Kilsyth, NSW, Australia). The eluate was added to MCW and tested for toxicity.

A subsample of the distillate filtrate was sent to Envirolab and analysed for volatile and semi-volatile organics via a GC/MS scan (Table B10). The GC/MS scanning method used in this project is not a definitive method of organic compound identification. The retention peaks and mass spectra of the detected compounds are matched to a library, and estimates of concentrations are inferred from the closest surrogate compounds. Thus, all detected compounds are reported with a match quality score, and concentrations are considered estimates.

2.5.5 Major ion additions

Calcium is an essential element that is necessary for a variety of fundamental physiological processes in *Hydra* (Gitter et al 1994, Kawaii et al 1999, Zaluzniak et al 2006). It is also a well-known ameliorator of metal and major ion toxicity (Markich & Jeffree 1994, van Dam et al 2010). Hence, the very low concentration of Ca in the distillate (Table 3) was initially targeted as a cause of the adverse effects of the distillate. In the first major ion addition TIE, SSW and distillate were prepared with nominal concentrations of 0.0, 0.2, 0.5 mg L⁻¹ Ca, which is equivalent to 0, 50 and 100% of the standard SSW Ca concentrations. An untreated MCW control was included as a QA/QC control because *H. viridissima* growth rates are known to be slightly lower in SSW.

However, it was also noted that the distillate contained concentrations of sodium (Na) and potassium (K) that were below detection limits (Table 3). Thus, it was hypothesised that the addition of the Ca, Na and K up to concentrations that were consistent with those in Magela Creek would improve the condition of the distillate for *H. viridissima*. Thus, in a second

major ion TIE, SSW and distillate were prepared with nominal concentrations of 0.0, 0.2 and 0.5 mg L⁻¹ Ca, 0.0, 0.5 and 1.0 mg L⁻¹ Na and 0.0, 0.2 and 0.4 mg L⁻¹ K, which is equivalent to 0, 50 and 100% of the standard SSW concentrations. An untreated MCW control was included as a QA/QC control for reasons described above. Magnesium was measured in the distillate at environmentally relevant concentrations so was not considered in the TIE

Sub-samples of the treatments were sent for the measurement of total metal and major ion concentrations for a limited suite (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, K and SO₄ - analysed as S and converted; Table B9)

2.5.6 Effect of Mn in low major ion waters

The concentrations of Mn in the distillate (130–230 µg L⁻¹) were identified as a potential concern as they were higher than the IC₁₀ of 60 µg L⁻¹ previously reported for *H. viridissima* in circumneutral pH (6.0–7.0) soft waters (Harford et al 2009). Additionally, the lack of major ions in the distillate had the potential to exacerbate Mn toxicity. Therefore, the effects of Mn in the presence of reduced concentrations of major ions were examined using modified SSW (ie pH 6.0 with 0, 50 and 100% Na, K and Ca concentrations).

Three types of SSW were prepared with Ca, Na and K at concentrations equivalent to 0, 50 and 100% of the standard SSW concentrations (as described above). These waters were spiked with manganese to concentrations of 0, 130 and 230 µg L⁻¹ Mn to produce 9 treatments (3 Mn concentrations × 3 major ion concentrations).

Sub-samples of the treatments were sent for the measurement of total metal and major ion concentrations for a limited suite (Al, Cd, Co, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, K and SO₄ - analysed as S and converted). Additionally, dissolved (0.1 µm filtered) and total Mn concentrations were measured in all treatments (Table B11).

2.6 Quality control

2.6.1 Chemistry

For each test, blanks and procedural blanks (ie 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, Cr, Cu, Fe, Mn, Ni, Pb, Se, U, Zn, Ca, Mg, Na, SO₄ – 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 2 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.

2.6.2 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 (ie 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 (ie 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 (ie 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 (ie 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 (ie 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.

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.

2.7 Statistics

For the toxicity tests, linear interpolation or non-linear regression (2-parameter log-logistic) analysis were used to determine point estimates of Inhibitory Concentrations (ICs) that reduced endpoint responses by 10% and 50% (ie IC10 and IC50) relative to the control responses (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. All statistical analyses for the full dilution toxicity tests were undertaken using CETIS™ (V 5.0.23, TidePool Scientific).

For the two major ion addition TIEs and the Mn toxicity test, two-way Analysis of Variance (ANOVA) and Tukey's post hoc tests ($\alpha = 0.05$) were performed using water type and major ion or Mn concentration as the two factors (Appendix C). Prior to ANOVA, the assumptions of normality and homoscedasticity were tested (SigmaPlot 11.0, Systat software, Germany). The failure of normality was not considered to be consequential to the analyses because sample sizes were the same across groups and the datasets had equal variances (Zar 1984). For the remaining TIEs, ANOVA results did not prove informative and are not reported. Rather, as recommended by the USEPA protocols (Norberg-King et al 1991), judgements on significance of the improvements or reductions in the *H. viridissima* population growth rate were made based on experience and knowledge of the speciation of the key chemicals of concern.

3 Results and discussion

3.1 Quality control

The quality of the toxicity tests and TIEs were assessed based on criteria for control performance, water quality measurements and chemical analyses of blank and procedural blank samples. All TIE and toxicity tests met the criteria for control performance except for the first *M. macleayi* test, which produced an average of 25 neonates per adult (see caption of Figure 2). However, this result was considered valid because the number of neonates produced was only marginally below the criterion of 30 neonates adult⁻¹, adult survival was 100% and the second cladoceran toxicity test, which was a valid test, produced a similar result. Electrical Conductivity increases of greater than 5 $\mu\text{S cm}^{-1}$ were measured in the MCW controls of the ‘confirmatory’ toxicity tests conducted with *Chlorella* sp, *M. macleayi* and *H. viridissima* using the second batch of distillate. However, the increases were inconsequential to the performance of controls and they were all similar to the results of the toxicity tests using the first distillate batch. Furthermore, the results of these tests could not be used to derive toxicity estimates because they consisted of only MCW control and 100% distillate treatments. In the duckweed toxicity test, the pH of the 100% distillate water was measured at 4.5 at the end of the 96-h test, which appeared anomalously low and may have been due to an erroneous reading or sample preparation. However, the growth rate of the group was the same as the control and therefore the pH change was inconsequential. Chemical analyses of the blank and procedural blank samples showed that all tests were free from confounding metal contaminants (Table B1). Hence, all tests reported here were of acceptable quality.

3.2 Distillate chemistry

The compositions (selected components) of the distillate and the process water feed are presented in Table 2 (see Appendix B for detailed results). The distillation process reduced all major ions, ammonia and trace metals to near detection limits. Some organic compounds that were not detected in the feed water were detected at low $\mu\text{g L}^{-1}$ concentrations in the distillate. In this context, it is important to note that the sub-sampling of the second distillate batch for organic compounds was not ideal, in that plastic was used instead of glass, and some of the detected compounds are known to leach from plastics (Table 3). The decane, which had a 70% match quality score and was estimated at 2 $\mu\text{g L}^{-1}$, may have been misidentified nonane because they are aliphatic hydrocarbons with 10 and 9 carbons, respectively. Nonane is also a major component of Shellsol, which was identified as an organic chemical of interest due to its use in the process circuit and occasional disposal in the TSF.

3.3 Toxicity test results

The toxicity test results showed that the distillate was of low toxicity to four of the five organisms tested (Table 4; Figure 2). However, the population growth rate of *H. viridissima* was reduced by ~50% following exposure to an undiluted (100%) sample of the first batch of distillate (Figure 2). The second batch of distillate was found to be higher in toxicity to *H. viridissima*, with a full toxic effect observed following exposure to 100% distillate (Table 4). In contrast, the second batch of distillate appeared to be of lower toxicity to *M. macleayi* and *Chlorella* sp. A toxicity estimate for *M. mogurnda* could not be calculated due to the limits in the dataset, eg a low number of treatments and variation within treatments. This resulted in concentration-response models not being able to significantly fit the dataset (see Appendix C).

Table 3 Composition of the process water before and after treatment with the brine concentrator

Analyte	Detection limit	Process water (feed) ^a	First distillate batch	Second distillate batch
pH	0.1	4.1 – 4.5	5.8	6.7
Electrical Conductivity ($\mu\text{S cm}^{-1}$)	1.0	20 900 – 29 700	17	12
DOC (mg L^{-1})	0.1	<1 – 6	0.6	NM ^c
Calcium (mg L^{-1})	0.1	300 – 341	0.11	<0.1
Magnesium (mg L^{-1})	0.1	3607 – 4123	0.6	0.4
Sodium (mg L^{-1})	0.1	73 - 107	<0.1	<0.1
Potassium (mg L^{-1})	0.1	67 - 115	<0.1	<0.1
Biocarbonate ($\text{mg L}^{-1} \text{CaCO}_3$)	1.0	<1	7	6
Ammonia ($\text{mg L}^{-1} \text{N}$)	5.0×10^{-3}	550 – 756	0.7	0.8
Manganese (mg L^{-1})	1.0×10^{-4}	1367 – 1551	0.23	0.13
Uranium ($\mu\text{g L}^{-1}$)	1.0×10^{-3}	9600 – 25 300	1.1	1.5
Decane ($\mu\text{g L}^{-1}$)	1.0	Not detected	NM ^c	2 ^d
Phenol, 3,5-bis (1,1-dimethylethyl) ($\mu\text{g L}^{-1}$) ^b	1.0	Not detected	NM ^c	6 ^d
Phenol, 2,4-bis (1,1-dimethylethyl) ($\mu\text{g L}^{-1}$) ^b	1.0	Not detected	NM ^c	12 ^d
1,2-Benzenedicarboxylic acid, buty ($\mu\text{g L}^{-1}$) ^b	1.0	Not detected	NM ^c	10 ^d

^a Value ranges based on numerous composite samples of the feed taken from 10 July – 9 August 2011 (data provided by ERA).

^b Known to leach from plastics

^c NM = Not measured

^d Not a definitive measurement. Concentration was estimated from the closest matching surrogate compound.

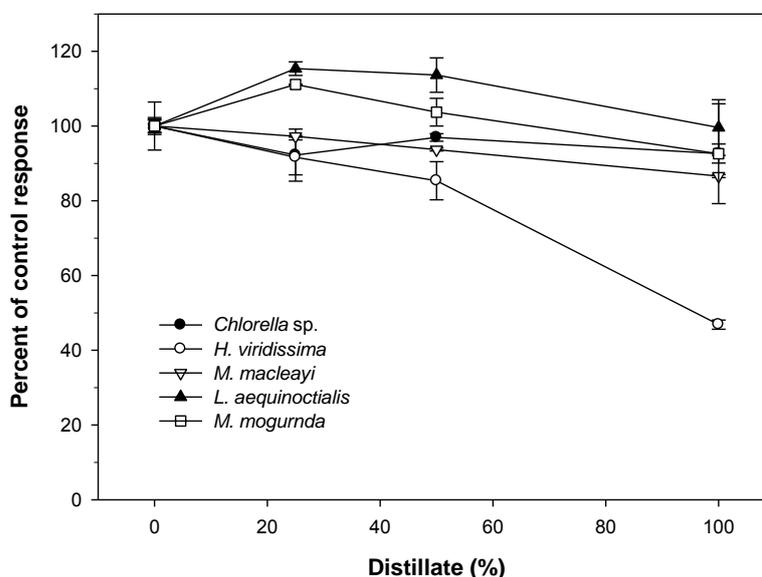


Figure 2 Concentration-response plots for the toxicity of the pilot brine concentrator distillate to five freshwater species (distillate batch 1 for *Chlorella* sp., *Hydra. viridissima* and *Moinodaphnia macleayi*; distillate batch 2 for *Lemna aequinoctialis* and *Morgurnda mogurnda*). Magela Creek Water control responses were; *Chlorella* sp. = 1.7 ± 0.3 doubling day⁻¹; *H. viridissima* = 0.35 ± 0.01 day⁻¹; *M. macleayi* = 25 ± 0.5 neonates adult⁻¹; *L. aequinoctialis* = 0.35 ± 0.01 day⁻¹; *M. mogurnda* = $90 \pm 5\%$ survival.

Table 4 Toxicity of the pilot brine concentrator distillate

Species	Endpoint	IC ₁₀ or IC ₅ ^a (95% confidence limits)	Percentage effect relative to the control (± CV% ^b) following exposure to 100% distillate	
			1 st batch	2 nd batch
<i>Chlorella</i> sp. (unicellular alga)	72-h cell division rate	79 (N.C.) ^c	11 ± 2	0 ± 0
<i>Lemna aequinoctialis</i> (duckweed)	96-h growth rate	>100 (N.C.) ^d	N.T. ^e	0 ± 0
<i>Hydra viridissima</i> (green hydra)	96-h population growth rate	30 (N.C. – 77)	53 ± 5	100 ± 0
<i>Moinodaphnia macleayi</i> (cladoceran)	3-brood (6-d) reproduction	72 (50 – 100)	13 ± 6	6 ± 13
<i>Mogurnda mogurnda</i> (fish)	96-h survival	NC ^b	N.T.	7 ± 25

^a Inhibitory Concentrations (IC) are expressed as percentage of distillate that causes 10% or 5% effect, ie IC₁₀ for all species except for IC₅ for *M. mogurnda*

^b Percentage Coefficient of Variation

^c NC = Not calculable

^d derived from test conducted on the 2nd batch

^e N.T. = Not tested

3.4 Toxicity identification evaluation (TIE) results

Chemical analysis of the distillate (Table 3) indicated that ammonia, manganese (Mn) and an organic component were potential candidate constituents for causing a toxic response. However, initial TIE results suggested none of these constituents were causing or contributing to the observed negative effect on *H. viridissima* (Table 5).

Increasing the pH of the distillate to 7.5 decreased its toxicity and improved the growth of the *H. viridissima* relative to pH 6.5 control. This indicated that the toxicity was not due to ammonia, as the higher pH would have resulted in greater toxicity due to a higher proportion of toxic ammonia (NH₃) ions (Table 5). This was confirmed by the result of the ammonia stripping experiment, where ammonia in the distillate was reduced to below detection, but the distillate's toxicity was unchanged (Table 5).

The improved *H. viridissima* growth rate at pH 7.5 may have been due to one or more of several reasons, including: (i) the addition of sodium ions in the form of sodium hydroxide, which was used to increase the pH; (ii) an improved physiological/metabolical function of the *H. viridissima* at pH 7.5; and (iii) reduced toxicity of Mn (or metals in general) due to a reduction in the proportion of bioavailable free Mn ions compared with the lower pHs of 5.5 and 6.5.

However, the TIE based on the addition of EDTA to the distillate indicated that toxicity due to Mn was unlikely. Only the lowest concentration of EDTA (2.8 mg L⁻¹) provided informative results, as the higher EDTA concentrations resulted in no growth of the *H. viridissima* in MCW (Table 5). This was probably due to the binding of essential cations, such as Ca²⁺, due to excess EDTA being added to the treatment. The calculation of EDTA needed for complete binding of all the Mn was based on the first batch of distillate, which contained 230 µg L⁻¹ Mn, while the second batch contained only half the concentration, ie 130 µg L⁻¹ Mn. Nevertheless, the addition of 2.8 mg L⁻¹ EDTA resulted in a *H. viridissima* population growth of 0.17 day⁻¹ in the MCW control but no growth in the distillate control. Thus, if the toxic effect of the distillate was due to Mn then it would have been expected that growth in the 2.8 mg L⁻¹ EDTA treated distillate would be similar to the MCW control. Furthermore, the results from the Chelex assay also

supported the conclusion that Mn was not the toxic component (Table B6). The fraction that was able to bind to the Chelex-100 resin was effectively reduced to below detection limits, but the toxicity of the distillate was not reduced. This indicated that Mn was not toxic in the second batch, which had a concentration of 110 $\mu\text{g L}^{-1}$.

Treatment of the distillate using a C18 SPE column did not change the toxicity of the distillate (Table 5). The organic content of the distillate was low ($\sim 1\text{-}4 \text{ mg L}^{-1}$ TOC) but a GC-MS scan of the second batch estimated decane (70% match quality) at 2.0 $\mu\text{g L}^{-1}$, phthalate (86% match quality) at 9.9 $\mu\text{g L}^{-1}$ and two bis-phenols ($>90\%$ match quality) at 5.8 and 12.0 $\mu\text{g L}^{-1}$. The phthalate and bis-phenols are known to leach from plastic and were probably present because the sample was held in polyethylene containers. However, the decane was unlikely to have leached from the plastic and may have been concentrated from the feedwater by the brine concentrator. Two chemicals that closely matched methyl ethyl ketone (MEK; 86% match quality) and phenol, 2-(1,1-dimethylethyl)-4-methyl- (97% match quality) were estimated in a blank sample at concentrations of 5.0 and 1.5 $\mu\text{g L}^{-1}$, respectively.

Table 5 Results of toxicity identification evaluation toxicity tests using *H. viridissima*

TIE test	Treatment ^a	Control growth rate (mean day ⁻¹ \pm SE)	Distillate growth rate (mean day ⁻¹ \pm SE)	Distillate compared to control (mean % \pm SE)
TIEs with Magela Creek Water as the control water				
Graduated pH	Daily pH adjusted to ~ 5.5	0.34 \pm 0.02	0.00 \pm 0.00	0.0
	pH unadjusted (~ 6.5)	0.33 \pm 0.00	0.04 \pm 0.02	11 \pm 6
	Daily pH adjusted to ~ 7.5	0.34 \pm 0.01	0.16 \pm 0.03	50 \pm 1
EDTA addition	0 mg L ⁻¹ EDTA added	0.31 \pm 0.02	0.04 \pm 0.04	12 \pm 12
	2.5 mg L ⁻¹ EDTA added	0.17 \pm 0.01	0.00 \pm 0.00	0.0
	5.0 mg L ⁻¹ EDTA added	0.00 \pm 0.00	0.00 \pm 0.00	0.0
	10 mg L ⁻¹ EDTA added	0.00 \pm 0.00	0.00 \pm 0.00	0.0
Ammonia stripping	Unadjusted	0.32 \pm 0.02	0.00 \pm 0.00	0.0
	pH increased and aerated 'NH ₃ stripped'	0.16 \pm 0.01	0.00 \pm 0.00	0.0
C18 SPE ^b	Untreated	0.28 \pm 0.01	0.14 \pm 0.02	50 \pm 6
	Filtrate 'Organic stripped'	0.29 \pm 0.01	0.16 \pm 0.00	59 \pm 1
	Eluate added to MCW	0.27 \pm 0.00	0.27 \pm 0.01	94 \pm 3
TIEs with Synthetic Soft Water as the control water				
Calcium addition	0.0 mg L ⁻¹ Ca added	0.14 \pm 0.00	0.00 \pm 0.00	0.0
	0.2 mg L ⁻¹ Ca added	0.27 \pm 0.01	0.16 \pm 0.00	61 \pm 2
	0.5 mg L ⁻¹ Ca added	0.28 \pm 0.00	0.18 \pm 0.01	66 \pm 5
Major ion addition	0.0 mg L ⁻¹ Ca, Na and K added	0.04 \pm 0.06	0.13 \pm 0.01	353 \pm 23 ^c
	0.2, 0.5 and 0.2 mg L ⁻¹ Ca, Na and K added	0.29 \pm 0.02	0.29 \pm 0.01	100 \pm 4
	0.5, 1.0 and 0.4 mg L ⁻¹ Ca, Na and K added	0.34 \pm 0.01	0.32 \pm 0.02	96 \pm 5

^a Controls were either MCW or SSW that were treated the same as the distillate. ^b Solid Phase Extraction. ^c Growth rate in the distillate was three times higher compared to SSW with no major ions.

These chemicals were probably contaminants from the sampling method and highlight the sensitivity of the analytical method. No VOCs and sVOCs were detected in the batch of distillate used for the TIE studies, but two sVOCs that matched 2-Furancarboxaldehyde, 5-methyl- (93% match quality) and 2,5-Heptadien-4-one, 2,6-dimethyl- (92% match quality) were estimated at $<1.5 \mu\text{g L}^{-1}$ (Table B10). The source of these compounds was unclear but they may have been acquired during the SPE treatment. Importantly, their presence in the filtrate did not change the toxicity of the water. Furthermore, no toxicity was observed in the MCW containing the eluate (Table 5). Hence, all results suggested that the toxicity of the distillate was not due to the presence of trace amounts of the organic compounds.

If organic chemicals are present in the feed water, there is a risk that these chemicals may be concentrated by the brine concentrator. A single sample taken on 27 July 2011 directly from the solvent extraction line (representing the most concentrated source of organic compounds that may contribute to the process water) contained naphthalene at $1000 \mu\text{g L}^{-1}$. No VOCs or sVOCs were detected in two samples of process water taken at the Ranger mine site on 8 and 10 August 2011. However, it should be noted that organic compounds that may be present at concentrations below detection limits in the process water could be concentrated by the brine concentrator. It is worth noting that the issue of organic compounds being concentrated could not be adequately assessed during this study. Of primary concern was that the process waters used to feed the pilot-scale plant were transported long distances (Darwin to Melbourne) and stored for long storage durations, which would have resulted in the evaporation of sVOCs and VOCs. Additionally, the process water used to produce the two batches of distillate tested in this study were obtained during the period of 8 to 24 June 2011 whilst the Ranger processing plant was not operating until 15 June 2011. This meant that the probability of organics being in process water was lower because the primary source of organics in the TSF is waste from the extraction circuit used during the processing of the ore. Regardless, the presence of organic chemicals in the TSF, the temporal variation and, ultimately, their ability to enter the feed water for the brine concentrator is unclear and needs to be better understood, eg through the monitoring of process water and distillate.

In addition to the issue of organic chemicals in the TSF process water, the fate and environmental risk of anti-scalant and anti-foaming chemicals was not adequately assessed during this project. Scale inhibiting organic chemicals (eg. amino-trimethylene phosphonic acid; ATMP), will be routinely added to the feed water to manage scaling of the brine concentrator units, while foam inhibiting chemicals (eg. silicon emulsions), will be added only when needed if foaming appears in the units. However, the distillates tested during this study did not contain such chemicals. Furthermore, the ability of chemical additives to report to the distillate is currently unclear, although analyses of two distillate samples that were produced from feed water dosed with anti-scalant (ATMP) and anti-foam (silicon emulsion) showed VOCs and sVOCs at below detection limits. However, another distillate sample that was also produced from this feed water with the chemical additives contained ethanol (95% match quality) and octanal (82% match quality) estimated at 5.0 and $1.0 \mu\text{g L}^{-1}$, respectively (Table B10). This indicates that the ability of chemical additives to report to the distillate warrants further investigation

In light of the above negative findings, the issue of major ion deficiency was specifically investigated as a potential cause of the effect on *H. viridissima*. Firstly, Ca addition was investigated due to its importance for nematocyst function and other physiological processes in *Hydra* (Gitter et al 1994, Kawaii et al 1999, Zaluzniak et al 2006). The addition of 0.2 and 0.5 mg L^{-1} Ca to the distillate resulted in a significant ($P < 0.001$) 61% and 66% recovery relative to the SSW control, respectively, suggesting Ca deficiency as a major reason for the

effect of distillate on *H. viridissima* (Table 5). Subsequently, it was thought that full recovery of *H. viridissima* might be achieved through the addition of sodium (Na) and potassium (K), as well as Ca. The results showed a significant ($P < 0.001$) 100% and 96% recovery of *H. viridissima* population growth rates with the addition of 50 and 100% major ions, respectively (Table 5). Furthermore, there was no statistical difference ($P = 0.293$) between the SSW and distillate water types if the major ions were at the same concentrations. This strongly indicated that the majority of the adverse effect from the distillate on *H. viridissima* was due to major ion deficiency issue rather than a chemical toxicity.

Despite the substantive removal of adverse effect by replacement of major cations, the concentrations of Mn in the distillate (130-230 $\mu\text{g L}^{-1}$) remained a concern as they were higher than the IC_{10} of 60 $\mu\text{g L}^{-1}$ previously reported for *H. viridissima* in circumneutral pH (6.0–7.0) soft waters (Harford et al 2009). Additionally, the lack of major ions in the distillate had the potential to exacerbate Mn toxicity (Peters et al 2011). Therefore, the effects of Mn in the presence of reduced concentrations of major ions were examined using modified SSW (ie with 0, 50 and 100% of the major ion concentrations in unmodified SSW).

Chemical analyses of the test solutions measured Mn concentrations at 10, 130 and 250 $\mu\text{g L}^{-1}$, close to the nominal concentrations of 0, 130 and 230 $\mu\text{g L}^{-1}$ (Table B8). The measured concentrations of K in the no Ca, Na and K group were higher than expected and similar to the half concentrations of Ca, Na and K group (Table B8). Nevertheless, in all SSW types, Mn reduced the growth rate of *H. viridissima* relative to the relevant SSW type control. The effect was most noticeable in the SSW with half the Na, K and Ca concentrations where growth rate was reduced by 9 and 20% in the 130 and 250 $\mu\text{g L}^{-1}$ treatments (Figure 3).

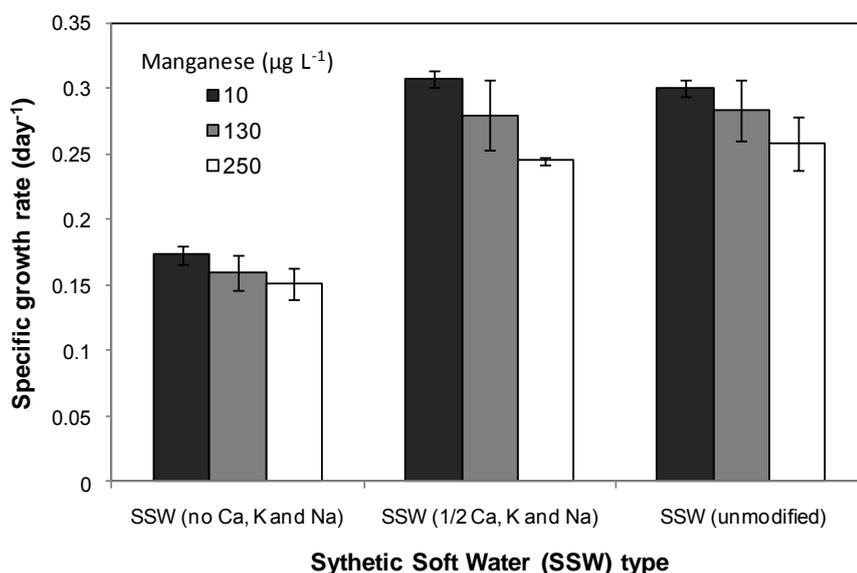


Figure 3 Effect of manganese on *Hydra viridissima* in modified Synthetic Soft Water (SSW). Data represent the mean \pm se ($n = 3$).

A two-way ANOVA of the results showed that the growth rates of *H. viridissima* in the 250 $\mu\text{g L}^{-1}$ Mn treatments were statistically lower than the controls but there was no interaction between major ion concentration and Mn toxicity ($P = 0.76$). Thus, Mn caused a similar reduction in the growth rate of *H. viridissima* regardless of the SSW type. Consequently, despite the recognised issue with deficiencies of major ions in the distillate, a specific toxic response to Mn was identified. It is clear that further investigation of Mn

toxicity is warranted to better understand the potential for toxicity under various physico-chemical conditions relevant to the catchments on the mine site to which distillate will be discharged, as well as Magela Creek itself. It is noteworthy that a current draft recommended environmental quality standard for Mn in the European Union is 62–123 $\mu\text{g L}^{-1}$ (Peters et al 2010). Consequently, it is recommended that further site-specific data are needed to adequately assess the environmental risk of Mn in the distillate.

4 Conclusions and recommendations

Whilst the undiluted distillate was generally of low toxicity, it did cause a 50–100% reduction in population growth rate of *H. viridissima*. Application of a Toxicity Identification Evaluation (TIE) procedure demonstrated that a lack of major ions was the primary factor causing the reduced rate of growth for the *H. viridissima*. Additional toxicity tests indicated that the highest concentrations of Mn ($> 200 \mu\text{g L}^{-1}$) that were measured in the distillate may also have had a negative impact on *H. viridissima* growth rate.

Given the results of the study, it is concluded that brine concentrator distillate should not be directly discharged to Magela Creek. The following recommendations have been made to ‘condition’ the water along catchment flow lines such that the issues identified above can be remediated by the time the water is discharged:

- 1 Supplementation of the distillate with major ions (Ca, Na and K) may be required prior to its discharge to the off-site aquatic environment. This could be achieved actively by direct addition of relevant salts or passively by passing the distillate through a wetland system/watercourse and/or blending with mine site waters prior to discharge;
- 2 While the conditioning of the distillate through a wetland/watercourse is likely to improve water quality (by increasing major ion concentrations and, potentially, reducing dissolved Mn concentrations), the risk of exhausting of the system’s capacity to sustainably contribute the required loading of salt may need to be considered if large volumes are to be flushed through the system;
- 3 Further site-specific data are needed to adequately assess the environmental risk of Mn in the distillate. Concentrations of Mn measured in the distillate inhibited the growth of *H. viridissima* and were above a previously reported IC_{10} of $60 \mu\text{g L}^{-1}$. Concentrations were also detected above a draft freshwater guideline of 62–123 $\mu\text{g L}^{-1}$, which is being recommended in Europe.

The issue of organic compounds could not be adequately assessed during this pilot study. The presence of organic chemicals in the TSF and their ability to enter the feed water for the brine concentrator, and for VOCs and sVOCs to report to the distillate, is unclear. Furthermore, the effects of the distillate produced from the full-scale brine concentrator will need to be specifically assessed as part of the risk assessment needed to be completed prior to release of the distillate. Accordingly, it is recommended that:

- 1 A baseline monitoring program for organic compounds in the TSF is needed to establish the likelihood of significant concentrations of sVOCs and VOCs entering the feed water, hence indicating the potential for transfer to the distillate. The distillate should also be monitored for organic compounds following the commissioning of the full-scale plant.

- 2 The effect of anti-scalant and anti-foaming agents that may be added to the concentrator's feed water needs to be assessed. This may be achieved with laboratory toxicity tests prior to the commissioning of the full-scale plant.
- 3 The distillate product from the full-scale plant will need to be assessed for toxicity and, if necessary, a TIE conducted to determine the cause(s) of any measured effects.

5 Acknowledgments

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 Kim Cheng, Claire Costello and Melanie Trenfield for their flawless execution of the toxicity and TIE tests. We would also like to thank the RT-TI team of Anna Zonneveld, Nicole Jarvie and Mark Coghill, and the ERA team of Shelly Iles, Nicole Jacobsen and Greg Sinclair, for their cooperation and provision of data and the distillate waters. We would also like to thank the Envirolab team, David Springer, Giovanni Agosti and Nancy Zhang, for their expert advice concerning organic analyses and for performing the chelex assay.

6 References

- Allen EW 2008. Process water treatment in Canada's oil sands industry: I. Target pollutants and treatment objectives. *Journal of Environmental Engineering and Science* 7 (2), 123-138.
- Butler BA, Smith ME, Reisman DJ & Lazorchak JM 2011. Metal removal efficiency and ecotoxicological assessment of field-scale passive treatment biochemical reactors. *Environmental Toxicology and Chemistry* 30 (2), 385-392.
- Deanovic L, Connor VM, Knight AW & Maier KJ 1999. The Use of Bioassays and Toxicity Identification Evaluation (TIE) Procedures to Assess Recovery and Effectiveness of Remedial Activities in a Mine Drainage-Impacted Stream System. *Archives of Environmental Contamination and Toxicology* 36 (1), 21-27.
- Driussi C & Jansz J 2006. Pollution minimisation practices in the Australian mining and mineral processing industries. *Journal of Cleaner Production* 14 (8), 673-681.
- Durhan EJ, Norberg-King TJ & Burkhard LP 1993. Methods for aquatic toxicity identification evaluations: Phase 2 toxicity identification procedures for samples exhibiting acute and chronic toxicity. EPA/600/R-92/080 Office of Research and Development, USEPA, Washington DC, USA.
- Gitter AH, Oliver D & Thurm U 1994. Calcium-and voltage-dependence of nematocyst discharge in *Hydra vulgaris*. *Journal of Comparative Physiology A: Neuroethology, Sensory, Neural, and Behavioral Physiology* 175 (1), 115-122.
- Haraldsson C, Lyvén B, Pollak M & Skoog A 1993. Multi-element speciation of trace metals in fresh water adapted to plasma source mass spectrometry. *Analytica Chimica Acta* 284 (2), 327-336.
- Harford AJ, Hogan AC, Cheng K, Costello C, Houston M & van Dam RA 2009. Preliminary assessment of the toxicity of manganese to three tropical freshwater species. In *eriss research summary 2007-2008*. Supervising Scientist Report 200, Supervising Scientist, Darwin NT, 12-19.
- Kawaii S, Yamashita K, Nakai M, Takahashi M & Fusetani N 1999. Calcium dependence of settlement and nematocyst discharge in actinulae of the hydroid *Tubularia mesembryanthemum*. *The Biological Bulletin* 196 (1), 45-51.

- Markich SJ & Jeffree RA 1994. Absorption of divalent trace metals as analogues of calcium by Australian freshwater bivalves: An explanation of how water hardness reduces metal toxicity. *Aquatic toxicology* 29 (3–4), 257–290.
- Masarczyk J, Hansson C-H, Solomon R & Hallmans B 1989. Advances mine drainage water treatment, engineering for zero discharge. *Desalination* 75 (0), 259–287.
- Mirenda R & Hall W 1992. The application of effluent characterization procedures in toxicity identification evaluations. *Water Science & Technology* 25 (3), 39–44.
- Mount DI & Norberg-King TJ 1993. Methods for aquatic toxicity identification evaluations: Phase 3 toxicity confirmation. Procedures for samples exhibiting acute and chronic toxicity. EPA/600/R-92/081, Office of Research and Development, USEPA, Washington DC, USA.
- Neculita C-M, Vigneault B & Zagury GJ 2008. Toxicity and metal speciation in acid mine drainage treated by passive bioreactors. *Environmental Toxicology and Chemistry* 27 (8), 1659–1667.
- Norberg-King TJ, Mount D, Durhan E, Ankley GT & Burkhard L 1991. Methods for aquatic toxicity identification evaluations. Phase 1. Toxicity characterization procedures, Environmental Research Lab, US Environmental Protection Agency Duluth, Minnesota, United States.
- Peters A, Crane M, Maycock D, Merrington G, Simpson P, Sorokin N & Atkinson C 2010. Proposed EQS for Water Framework Directive Annex VIII substances: manganese (total dissolved). Bristol, United Kingdom: Environment Agency.
- Peters A, Lofts S, Merrington G, Brown B, Stubblefield W & Harlow K 2011. Development of biotic ligand models for chronic manganese toxicity to fish, invertebrates, and algae. *Environmental Toxicology and Chemistry* 30 (11), 2407–2415.
- Riethmuller N, Camilleri C, Franklin N, Hogan AC, King A, Koch A, Markich SJ, Turley C & van Dam R 2003. *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.
- Sauer TC, Costa HJ, Brown JS & Ward TJ 1997. Toxicity identification evaluations of produced-water effluents. *Environmental Toxicology and Chemistry* 16 (10), 2020–2028.
- Tietge JE, Hockett JR & Evans JM 1997. Major ion toxicity of six produced waters to three freshwater species: Application of ion toxicity models and tie procedures. *Environmental Toxicology and Chemistry* 16 (10), 2002–2008.
- van Dam RA, Hogan AC, Harford AJ, Cheng KC & Costello C 2011. Toxicity testing of Ranger process water permeate. In *eriss research summary 2009–2010*. Supervising Scientist Report 202, Supervising Scientist, Darwin NT, 28–31.
- van Dam RA, Hogan AC, McCullough CD, Houston MA, Humphrey CL & Harford AJ 2010. Aquatic toxicity of magnesium sulfate, and the influence of calcium, in very low ionic concentration water. *Environmental Toxicology and Chemistry* 29 (2), 410–421.
- Zalizniak L, Kefford BJ & Nuggeoda D 2006. Is all salinity the same? I. The effect of ionic compositions on the salinity tolerance of five species of freshwater invertebrates. *Marine and freshwater research* 57 (1), 75–82.
- Zar JH 1984. *Biostatistical analysis*. 2nd edn, Prentice-Hall Inc, Englewood Cliffs, New Jersey, USA.

Appendix A Measured water quality parameters for toxicity tests

Table A1 1193D Clad_RBCP_01 Cladoceran toxicity test

Treatment		0%		25%		50%		100%	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Day 0	pH	6.5	6.5	6.5	6.5	6.5	6.6	6.5	6.6
	EC ($\mu\text{S cm}^{-1}$)	21.0	18.0	18.0	18.0	19.0	19.0	19.0	20.0
	DO (%)	109	91	101	94	114	91	107	92
	Temp ($^{\circ}\text{C}$)	25.0	24.7	24.9	25.3	24.7	25.1	24.7	24.6
Day 1	pH	6.6	6.3	6.3	6.4	6.4	5.2 ^a	5.7	6.3
	EC ($\mu\text{S cm}^{-1}$)	17.0	20.0	17.0	20.0	18.0	26.0	23.0	21.0
	DO (%)	106	96	107	93	111	91	111	90
	Temp ($^{\circ}\text{C}$)	27.3	26.5	27.3	26.7	27.1	26.2	25.3	25.7
Day 2	pH	6.5	6.8	6.5	6.9	6.5	6.4	6.1	6.4
	EC ($\mu\text{S cm}^{-1}$)	17.0	18.0	20.0	20.0	19.0	23.0	22.0	20.0
	DO (%)	110	90	12	90	114	91	115	89
	Temp ($^{\circ}\text{C}$)	26.6	23.3	26.9	23.3	27.3	23.1	26.8	23.0
Day 3	pH	6.7	6.6	6.8	6.8	6.7	6.7	6.6	6.7
	EC ($\mu\text{S cm}^{-1}$)	21.0	19.0	21.0	19.0	20.0	20.0	21.0	21.0
	DO (%)	111	92	112	89	114	90	115	94
	Temp ($^{\circ}\text{C}$)	24.2	24.3	23.8	23.9	23.7	23.8	23.6	23.9
Day 4	pH	6.7	6.9	6.7	6.9	6.6	6.9	6.5	6.8
	EC ($\mu\text{S cm}^{-1}$)	22.2	23.0	19.0	25.0	19.0	20.0	20.0	21.0
	DO (%)	118	88	120	86	121	90	110	90
	Temp ($^{\circ}\text{C}$)	26.1	22.9	24.9	23.5	24.5	24.9	24.3	25.0

^a Likely erroneous measurement

Table A2 1194B Hyd_RBCP_01 Hydra toxicity test

Treatment		0%		25%		50%		100%	
Parameter		0 h	24 h						
Day 0	pH	6.7	6.5	6.7	6.6	6.7	6.5	6.5	6.3
	EC ($\mu\text{S cm}^{-1}$)	19	18	14	18	18	19	18	19
	DO (%)	114	92	114	91	110	93	106	90
	Temp ($^{\circ}\text{C}$)	26	24.1	25.3	24.6	24.7	24.5	23.9	24.2
Day 1	pH	6.3	6.6	6.4	6.8	6.5	6.7	6.5	6.8
	EC ($\mu\text{S cm}^{-1}$)	19	16	17	17	16	17	18	18
	DO (%)	113	90	121	91	116	92	116	92
	Temp ($^{\circ}\text{C}$)	24.9	24.8	25.9	24.7	25.2	24.8	25.2	25.2
Day 2	pH	6.7	6.7	6.6	6.7	6.6	6.7	6.6	6.7
	EC ($\mu\text{S cm}^{-1}$)	21	15	19	16	16	16	17	18
	DO (%)	116	92	113	92	110	91	111	92
	Temp ($^{\circ}\text{C}$)	24.9	24.2	24.7	25	24.7	25.5	24.6	25.5
Day 3	pH	6.7	6.8	6.7	6.8	6.6	6.9	6.6	6.8
	EC ($\mu\text{S cm}^{-1}$)	14	16	15	15	16	15	17	17
	DO (%)	110	95	120	92	114	92	113	91
	Temp ($^{\circ}\text{C}$)	25	27.3	25.3	27	25.1	27	24.9	26.5

Table A3 1195G Alg_RBCP_01 Green alga toxicity test

Treatment		0%		25%		50%		100%	
Parameter		0 h	24 h						
pH		6.4	6.9	6.3	6.2	6.2	6.3	6.1	6.0
EC ($\mu\text{S cm}^{-1}$)		42	40	44	44	45	41	48	46
DO (%)		112	94	112	94	109	94	104	93
Temp ($^{\circ}\text{C}$)		24.9	23.1	25.2	23.5	25.2	23.7	25.2	24

Table A4 1205L Lem_RBCP_01 Duckweed toxicity test

Treatment		0%		25%		50%		100%	
Parameter		0h	72h	0h	72h	0h	72h	0h	72h
pH		6.4	6.9	6.4	6.9	6.3	6.5	6.3	4.5
EC ($\mu\text{S cm}^{-1}$)		21	16	21	12	21	12	20	19
DO (%)		102	90	109	92	106	94	106	96
Temp ($^{\circ}\text{C}$)		24.5	24.6	24	24.7	23	22.9	23	23.4

Table A5 1206E Fry_RBCP_01 Fry toxicity test

Treatment		0 %		25 %		50 %		100 %	
Parameter		0 h	24 h	0 h	24 h	0 h	24 h	0 h	24 h
Day 0	pH	6.2	6.6	6.3	6.6	6.2	6.7	6.3	6.7
	EC ($\mu\text{S cm}^{-1}$)	15	21	14	19	13	19	12	17
	DO (%)	116	92	106	93	115	94	112	93
	Temp ($^{\circ}\text{C}$)	24.0	25.6	24.1	24.1	24.0	24.4	24.1	24.4
Day 1	pH	6.3	6.7	6.3	6.7	6.2	6.7	6.2	6.6
	EC ($\mu\text{S cm}^{-1}$)	15	19	14	19	14	15	13	15
	DO (%)	101	96	107	100	101	92	106	96
	Temp ($^{\circ}\text{C}$)	26.5	23.8	26.0	24.1	25.9	23.3	25.8	23.6
Day 2	pH	6.4	6.5	6.5	6.6	6.4	6.6	6.5	6.7
	EC ($\mu\text{S cm}^{-1}$)	14	17	14	17	14	16	12	15
	DO (%)	118	90	115	89	118	88	115	87
	Temp ($^{\circ}\text{C}$)	23.5	22.9	23.5	22.7	23.8	22.9	23.9	23
Day 3	pH	6.4	6.9	6.4	6.9	6.4	6.8	6.4	7.0
	EC ($\mu\text{S cm}^{-1}$)	14	20	14	17	13	17	12	16
	DO (%)	117	89	118	91	115	89	117	92
	Temp ($^{\circ}\text{C}$)	23.0	24.9	23.3	24.3	23.1	23.5	22.8	22.8

Table A6 1207B Hyd_RBCP_02 Hydra toxicity test

Treatment		0%		100%	
Parameter		0 h	24 h	0 h	24 h
Day 0	pH	6.2	6.7	6.3	6.6
	EC ($\mu\text{S cm}^{-1}$)	15	15	12	13
	DO (%)	109	101	105	101
	Temp ($^{\circ}\text{C}$)	26	23.4	26	23.9
Day 1	pH	6.5	6.2	6.5	6.4
	EC ($\mu\text{S cm}^{-1}$)	16	16	13	13
	DO (%)	120	94	118	92
	Temp ($^{\circ}\text{C}$)	25	23.3	25	23.3
Day 2	pH	6.1	6.7	6.2	6.8
	EC ($\mu\text{S cm}^{-1}$)	14	15	12	13
	DO (%)	118	96	107	97
	Temp ($^{\circ}\text{C}$)	23.1	24.1	23.4	24.4
Day 3	pH	6.4	6.8	6.4	6.6
	EC ($\mu\text{S cm}^{-1}$)	33	17	12	13
	DO (%)	119	93	118	96
	Temp ($^{\circ}\text{C}$)	23.7	25.9	23.4	26

Table A7 1208D Clad_RBCP_02 Cladoceran toxicity test

Treatment		0%		100%	
Parameter		0 h	24 h	0 h	24 h
Day 0	pH	5.9	6.3	6.2	6.4
	EC ($\mu\text{S cm}^{-1}$)	21.0	21.0	18.0	16.0
	DO (%)	90	103	89	101
	Temp ($^{\circ}\text{C}$)	24.2	23.9	24.1	24.0
Day 1	pH	4.3	5.9	6.2	6.0
	EC ($\mu\text{S cm}^{-1}$)	34.0	20.0	18.0	25.0
	DO (%)	102	101	102	108
	Temp ($^{\circ}\text{C}$)	25.3	23.9	24.9	23.9
Day 2	pH	6.2	6.4	6.3	6.2
	EC ($\mu\text{S cm}^{-1}$)	19.0	33.0	17.0	15.0
	DO (%)	92	126	88	126
	Temp ($^{\circ}\text{C}$)	24.3	24.3	23.4	24.0
Day 3	pH	6.2	6.2	6.2	6.2
	EC ($\mu\text{S cm}^{-1}$)	25.0	20.0	16.0	15.0
	DO (%)	93	121	93	123
	Temp ($^{\circ}\text{C}$)	23.7	24.3	23.8	24.4
Day 4	pH	6.5	6.0	6.2	6.2
	EC ($\mu\text{S cm}^{-1}$)	25.0	18.0	17.0	15.0
	DO (%)	95	106	94	103
	Temp ($^{\circ}\text{C}$)	23.9	24.1	23.3	23.9

Table A9 1209G Alg_RBCP_02 Green alga toxicity test

Treatment		0 %		100%	
Parameter		0h	72 h	0h	72 h
pH		6.4	6.5	6.2	6.0
EC ($\mu\text{S cm}^{-1}$)		50	42	43	39
DO (%)		100	93	102	100
Temp ($^{\circ}\text{C}$)		24	23.2	23.8	23.5

Table A10 1210B Hyd_RBCP_03 Hydra graduate pH TIE

Treatment		MCW pH 5.5		MCW pH 6.4		MCW pH 8.0		Distillate pH 5.5		Distillate pH 6.4		Distillate pH 8.0	
Parameter		0h	24 h	0h	24 h	0h	24 h	0h	24 h	0h	24 h	0h	24 h
Day 0	pH	5.5	6.0	6.2	6.3	7.9	6.9	5.5	6.0	6.2	6.2	7.8	6.7
	EC ($\mu\text{S cm}^{-1}$)	20	18	16	17	22	23	19	15	19	13	24	18
	DO (%)	105	96	107	96	105	93	105	96	103	96	101	96
	Temp ($^{\circ}\text{C}$)	24.5	24.6	23.8	25.6	25.4	23.6	24.2	23.6	23.8	23.7	23.5	23.3
Day 1	pH	5.7	6.2	6.2	6.4	7.0	6.9	6.1	6.3	6.2	6.4	7.4	6.8
	EC ($\mu\text{S cm}^{-1}$)	18	19	16	17	24	25	16	15	18	14	19	21
	DO (%)	99	92	95	96	96	93	97	95	96	94	96	95
	Temp ($^{\circ}\text{C}$)	25	24.1	24.6	24.3	24.4	24	24.4	23.6	24.2	23.3	23.8	23.1
Day 2	pH	5.7	5.8	6.4	6.4	7.8	7.0	5.6	5.8	6.3	6.2	7.6	7.0
	EC ($\mu\text{S cm}^{-1}$)	19	20	16	17	26	28	15	15	14	14	21	22
	DO (%)	94	95	95	97	93	94	95	96	91	93	92	96
	Temp ($^{\circ}\text{C}$)	24	0	23.7	0	24.1	0	23.9	0	23.8	0	23.9	0
Day 3	pH	5.6	5.8	6.3	6.3	7.5	6.9	5.6	6.3	6.2	6.5	7.5	7.1
	EC ($\mu\text{S cm}^{-1}$)	19	21	16	22	29	29	16	16	14	14	24	24
	DO (%)	95	92	97	93	94	94	95	95	96	96	95	96
	Temp ($^{\circ}\text{C}$)	0	24.8	0	25.5	0	25.6	0	25.7	0	25.5	0	25.7

Table A11 1211B Hyd_RBCP_04 Hydra EDTA addition TIE

Treatment		MCW 0 mg L ⁻¹ EDTA		MCW 2.8 mg L ⁻¹ EDTA		MCW 5.5 mg L ⁻¹ EDTA		MCW 11 mg L ⁻¹ EDTA		Distillate 0 mg L ⁻¹ EDTA		Distillate 2.8 mg L ⁻¹ EDTA		Distillate 5.5 mg L ⁻¹ EDTA		Distillate 11 mg L ⁻¹ EDTA	
		0 h	24 h	0h	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	6.2	6.3	6.4	6.4	6.3	6.5	6.4	6.4	6.3	6.3	6.4	6.4	6.5	6.4	6.4	6.5
	EC (μS cm ⁻¹)	15	16	16	17	17	18	20	21	12	13	15	16	16	16	19	19
	DO (%)	101	91	100	90	102	94	98	96	100	93	100	94	99	92	100	90
	Temp (°C)	25.7	0	25.9	0	25.6	0	25.4	0	25.3	0	25.5	0	25.4	0	25.4	0
Day 1	pH	6.5	6.6	6.5	6.6	6.5	6.6	6.4	6.6	6.4	6.4	6.5	6.4	6.6	6.5	6.6	6.6
	EC (μS cm ⁻¹)	15	16	16	17	18	19	20	21	13	13	15	16	15	16	19	20
	DO (%)	97	94	98	96	97	96	96	93	92	94	94	97	94	95	96	96
	Temp (°C)	23.2	24.9	23.2	25	23	25.1	22.8	25	23.1	25	23	24.8	23	24.9	23	24.9
Day 2	pH	6.2	6.7	6.4	6.7	6.5	6.7	6.5	6.8	6.4	6.6	6.4	6.6	6.4	6.6	6.5	6.6
	EC (μS cm ⁻¹)	15	17	16	18	18	19	20	21	12	15	15	16	15	16	19	21
	DO (%)	98	90	96	91	95	90	94	91	94	89	92	91	94	91	92	90
	Temp (°C)	24.8	24.3	24.8	23.6	24.2	25.3	24.5	25	24.3	25.1	24.3	25.2	24.2	24.6	24.3	24.8
Day 3	pH	6.6	6.7	6.6	6.8	6.7	6.9	6.6	6.9	6.6	6.6	6.6	6.7	6.6	6.7	6.6	6.6
	EC (μS cm ⁻¹)	15	16	16	18	18	19	20	20	12	13	15	15	15	16	19	20
	DO (%)	94	93	96	95	97	94	93	95	88	96	90	94	91	93	92	95
	Temp (°C)	23.6	24.5	23.4	24.6	23.5	25.3	23.9	25.2	22.5	25.1	22.4	25.6	22	25.9	21.9	26

Table A12 1212B Hyd_RBCP_04 Hydra Ca addition TIE

Treatment		MCW		SSW no Ca		SSW 0.2 mg L ⁻¹ Ca		SSW 0.5 mg L ⁻¹ Ca		Distillate no Ca		Distillate 0.2 mg L ⁻¹ Ca		Distillate 0.5 mg L ⁻¹ Ca	
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	6.4	6.4	6.4	6.4	6.4	6.3	6.3	6.2	6.3	6.2	6.4	6.2	6.4	6.2
	EC (μS cm ⁻¹)	15	14	19	18	14	14	19	18	13	12	14	14	16	15
	DO (%)	94	111	94	86	95	90	93	92	95	109	95	107	92	109
	Temp (°C)	24.7	24.8	25	25.2	24.9	25.4	24.7	25.4	24.7	24.9	24.3	24.5	23.8	24.4
Day 1	pH	6.7	6.5	6.5	6.3	6.4	6.3	6.5	6.3	6.5	6.3	6.5	6.3	6.5	6.3
	EC (μS cm ⁻¹)	18	14	19	18	14	14	19	18	13	12	14	13	16	15
	DO (%)	93	100	94	101	97	101	96	99	94	113	95	117	93	115
	Temp (°C)	24	23.2	24.3	22.5	23	22.1	22.8	22.3	22.6	22.4	22.5	22.5	22.3	22.4
Day 2	pH	6.7	6.6	6.6	6.4	6.5	6.3	6.4	6.3	6.6	6.4	6.5	6.3	6.5	6.3
	EC (μS cm ⁻¹)	15	16	19	18	14	14	19	18	13	12	14	13	16	14
	DO (%)	93	118	92	107	92	111	97	109	95	118	95	118	97	122
	Temp (°C)	25.2	22.7	25.1	22.8	24.5	23	24.4	22.8	24	22.8	24.5	22.6	24.3	22.5
Day 3	pH	6.5	6.4	6.7	6.3	6.5	6.3	6.5	6.3	6.7	6.3	6.6	6.3	6.6	6.3
	EC (μS cm ⁻¹)	15	14	18	18	14	13	19	18	13	12	14	13	15	15
	DO (%)	96	118	96	111	96	113	94	113	95	119	94	122	92	122
	Temp (°C)	26.2	20.8	26.1	21.9	25.7	20.9	25.1	21.1	25.6	21.4	26.1	21.2	25.3	21.2

Table A13 1213B Hyd_RBCP_06 Hydra NH₃ stripped TIE

Treatment		MCW		Distillate		MCW - NH ₃ stripped		Distillate - NH ₃ stripped	
		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	6.8	7.7	7.1	7.7
	EC ($\mu\text{S cm}^{-1}$)	15	15	12	13	1086	1069	1132	1111
	DO (%)	115	97	113	98	101	95	101	96
	Temp ($^{\circ}\text{C}$)	25	25.1	24.7	24.6	24.9	24.2	24.5	24.2
Day 1	pH	6.4	6.3	6.4	6.6	6.6	7.8	6.7	7.8
	EC ($\mu\text{S cm}^{-1}$)	14	15	12	13	1095	1109	1136	1107
	DO (%)	116	100	118	95	114	94	111	94
	Temp ($^{\circ}\text{C}$)	23.8	24.8	23.5	24.3	23.4	24.1	23.6	24.3
Day 2	pH	6.3	6.4	6.3	6.6	6.8	7.9	6.8	7.8
	EC ($\mu\text{S cm}^{-1}$)	14	15	12	13	1084	1104	1139	1162
	DO (%)	118	96	119	94	115	94	115	94
	Temp ($^{\circ}\text{C}$)	21.2	26.2	21	26.2	20.9	23.9	21.2	24.6
Day 3	pH	6.3	6.5	6.5	6.8	6.9	8.1	7.0	N.M.
	EC ($\mu\text{S cm}^{-1}$)	16	14	12	12	1074	1115	1138	N.M.
	DO (%)	114	91	118	94	116	96	12	N.M.
	Temp ($^{\circ}\text{C}$)	24.3	26.5	24.9	26.9	24.4	26.5	23.9	N.M.

N.M. = Not measured due to end of treatment

Table A14 1217B Hyd_RBCP_07 Hydra C18 Solid Phase Extraction TIE

Treatment		MCW		Distillate		MCW filtrate		Distillate filtrate		MCW + methanol		MCW with elaute	
		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	6.6	6.5	6.6	6.6	6.6	6.6	6.7	6.5	6.7	6.4	6.6
	EC ($\mu\text{S cm}^{-1}$)	15	16	12	15	16	18	13	15	14	16	14	16
	DO (%)	118	97	116	95	111	95	111	93	110	94	113	94
	Temp ($^{\circ}\text{C}$)	23.4	23.9	23.2	23.6	23.1	23.6	23	23.3	23	23.4	23	23.3
Day 1	pH	6.7	6.8	6.7	6.7	6.7	7.0	6.7	7.0	6.6	6.8	6.5	6.9
	EC ($\mu\text{S cm}^{-1}$)	15	16	13	14	16	17	14	15	14	16	15	16
	DO (%)	119	93	114	93	114	85	112	91	114	92	114	94
	Temp ($^{\circ}\text{C}$)	23.2	23.9	23.2	23.9	23.1	23.9	23	24	23	23.8	22.9	23.7
Day 2	pH	6.8	6.8	6.6	6.7	6.7	6.7	6.7	6.6	6.6	6.6	6.6	6.6
	EC ($\mu\text{S cm}^{-1}$)	15	16	13	13	16	17	14	14	14	16	15	16
	DO (%)	115	95	120	96	119	94	114	94	115	99	115	98
	Temp ($^{\circ}\text{C}$)	23.3	23.9	23.2	23.9	23.1	24.3	23.2	24.5	23.2	23.9	23.1	23.8
Day 3	pH	6.7	6.9	6.6	7.0	6.5	6.8	6.6	6.8	6.5	6.8	6.4	6.7
	EC ($\mu\text{S cm}^{-1}$)	15	15	13	13	16	17	14	13	15	15	15	15
	DO (%)	111	90	115	91	112	95	114	92	113	91	114	93
	Temp ($^{\circ}\text{C}$)	23.1	23.2	22.8	23	22.4	23.4	22.4	23.3	22.4	23.1	22.4	23.2

Table A15 1220B Hyd_RBCP_08 Hydra major ion addition TIE

Treatment		MCW		SSW no major ions		SSW 50% major ions		SSW 100% major ions ^a		Distillate no major ions ^a		Distillate 50% major ions		Distillate 100% major ions	
		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	6.7	6.9	6.5	6.5	6.4	6.5	6.4	6.5	6.5	6.6	6.5	6.5	6.6
	EC ($\mu\text{S cm}^{-1}$)	19	16	10	11	13	14	16	16	13	14	17	18	21	22
	DO (%)	115	95	81	95	78	96	82	95	117	97	116	95	117	95
	Temp ($^{\circ}\text{C}$)	23.7	24.3	24.3	24.7	24	24.2	24	24.2	23.7	24	23.6	24.2	23.6	23.9
Day 1	pH	6.8	6.7	6.7	6.6	6.5	6.5	6.5	6.5	6.5	6.5	6.5	6.6	6.6	6.7
	EC ($\mu\text{S cm}^{-1}$)	21	16	10	11	13	16	16	17	13	14	17	18	21	22
	DO (%)	103	95	97	90	107	99	107	96	115	97	119	98	114	96
	Temp ($^{\circ}\text{C}$)	23.4	25.1	23.6	25.5	23.4	25.9	23.5	25.6	23.4	27.7	23.4	27.3	23.1	25.1
Day 2	pH	6.5	6.5	6.3	6.3	6.1	6.1	6.2	6.2	6.3	6.4	6.4	6.4	6.5	6.5
	EC ($\mu\text{S cm}^{-1}$)	15	16	10	12	13	14	16	17	13	14	17	18	21	23
	DO (%)	109	93	111	96	112	95	112	97	111	95	110	100	114	96
	Temp ($^{\circ}\text{C}$)	24.2	23.8	23.8	23.9	23.8	24	23.7	24.6	23.6	24.5	23.5	24.3	23.3	24.2
Day 3	pH	6.2	6.8	6.1	6.7	6.1	6.3	6.2	6.2	6.3	6.3	6.3	6.5	6.4	6.6
	EC ($\mu\text{S cm}^{-1}$)	17	15	11	11	13	13	16	16	14	13	17	17	22	21
	DO (%)	116	95	116	95	111	94	115	95	119	96	118	91	120	96
	Temp ($^{\circ}\text{C}$)	23.7	24.7	23.7	24.6	23.8	25.1	23.6	25.2	23.6	25.2	23.4	25.3	23.3	24.9

^a These treatments represent unmodified SSW and distillate

Table A16 1242B Hyd_RBCP_09 Modified SSW with Mn addition TIE

Treatment	SSW 0% major ions; 0 µg L ⁻¹ Mn		SSW 0% major ions; 130 µg L ⁻¹ Mn		SSW 0% major ions; 230 µg L ⁻¹ Mn		SSW 50% major ions; 0 µg L ⁻¹ Mn		SSW 50% major ions; 130 µg L ⁻¹ Mn		SSW 50% major ions; 230 µg L ⁻¹ Mn		SSW 100% major ions; 0 µg L ⁻¹ Mn		SSW 100% major ions; 130 µg L ⁻¹ Mn		SSW 100% major ions; 230 µg L ⁻¹ Mn		
	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	6.2	6.1	6.1	6.0	6.1	6.1	6.0	6.1	5.9	6.1	6.0	6.1	6.0	6.1	6.0	6.1	6.5	6.1
	EC (µS cm ⁻¹)	19	11	11	11	11	12	13	13	13	14	13	14	17	18	18	19	23	19
	DO (%)	99	92	89	92	100	99	98	94	99	94	99	94	98	94	101	93	98	93
	Temp (°C)	25.2	24.2	24.6	24.4	24.2	24.1	23.1	24.1	24	24.2	24.1	24	23.7	24	23.5	24.2	23.3	24
Day 1	pH	5.8	6.1	5.9	6.0	6.0	6.2	6.0	6.1	6.0	6.3	6.0	6.2	6.0	6.2	6.0	6.2	6.0	6.2
	EC (µS cm ⁻¹)	16	10	12	11	12	12	12	14	13	15	13	14	17	18	17	18	18	19
	DO (%)	106	92	104	94	109	94	105	93	105	93	107	95	108	92	107	94	101	92
	Temp (°C)	23.2	25.6	23.5	25.8	23.6	25.1	23.5	25.3	23.2	25.3	23.5	25.1	23.3	25.1	23.3	25.2	23.3	25
Day 2	pH	5.8	6.0	6.0	6.1	6.0	6.1	6.0	6.2	6.0	6.3	6.0	6.2	6.0	6.2	6.1	6.2	6.1	6.2
	EC (µS cm ⁻¹)	16	11	11	11	11	12	12	13	13	13	13	14	17	18	18	19	18	19
	DO (%)	102	96	109	94	115	93	112	93	114	95	107	94	110	94	110	94	112	94
	Temp (°C)	25.8	24.9	26.2	25.3	25.7	25	25.4	25	25.4	25	25.5	24.9	25.4	24.8	25.4	25.1	25.4	24.9
Day 3	pH	6.1	6.1	6.0	6.2	6.1	6.2	6.0	6.3	6.0	6.4	6.0	6.3	6.0	6.3	6.0	6.3	6.0	6.3
	EC (µS cm ⁻¹)	13	12	11	11	11	11	12	13	13	13	13	13	13	17	18	18	18	19
	DO (%)	116	92	115	95	117	93	110	95	112	94	113	93	111	90	108	87	110	93
	Temp (°C)	24.2	27.1	24.1	27.7	23.9	27.1	23.7	26.9	23.6	26.6	23.8	26.1	23.6	26.1	23.6	25.2	23.8	25.2

Table A17 pH measurements and adjustments for the graduate pH TIE

Time	Measurement	Magela Creek Water			Distillate		
		pH 5.5	pH 6.6	pH 7.5	pH 5.5	pH 6.5	pH 7.5
- 24 h	Initial	6.4	6.4	6.4	6.4	6.4	6.4
	Adjusted	5.5	N.A.	8.1	5.5	N.A.	8.0
0 h	Initial	5.7	6.2	7.6	5.5	6.2	7.8
	Adjusted – start of test	5.5	N.A.	8.1	N.A.	N.A.	8.1
24 h	Initial	5.5	6.2	7.2	5.5	6.2	7.2
	Adjusted	N.A.	N.A.	8.1	N.A.	N.A.	8.1
48 h	Initial	5.8	6.2	7.1	5.7	6.1	7.2
	Adjusted	5.3	N.A.	8.1	5.4	N.A.	8.1
72 h	Initial	5.6	6.4	7.4	5.5	6.3	7.3
	Adjusted	N.A.	N.A.	8.1	N.A.	N.A.	8.1
96 h	Initial – end of test	5.8	6.3	6.9	6.2	6.5	7.1

Appendix B Chemical analyses

Table B1 Total measured metals and major ions for Magela Creek Water (MCW), Synthetic Softwater (SSW) Procedural Blanks and Blanks

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Ca	Mg	Na	SO ₄
Units	µg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹											
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.1	0.1	0.1	0.5
MCW	11	<0.02	0.033	0.13	0.16	50	1.3	0.37	0.12	<0.2	0.0083	0.5	0.1	0.8	1.0	<0.5
MCW (25/7/11)	11	<0.02	0.046	<0.1	0.15	61	1.5	0.07	0.01	<0.2	0.0072	<0.1	<0.5	0.9	1.1	<0.5
MCW	11	<0.02	0.048	<0.1	0.073	62	1.5	0.041	<0.01	<0.2	0.0082	<0.1	<0.5	0.9	1.1	<0.5
MCW (22/8/11)	14	<0.02	0.04	<0.1	0.1	55	1.0	0.3	0.02	<0.2	0.01	<0.1	0.2	0.9	0.2	1.0
MCW (05/09/11)	12	<0.02	0.04	<0.1	0.1	50	2.0	0.3	0.08	<0.2	0.006	<0.1	0.2	0.9	0.2	1.0
SSW (14/11/11)	17	0.033	0.018	<0.1	2.0	50	11	0.84	0.031	<0.2	0.056	4.2	0.5	0.6	1.1	3.0
1193D Pro blank	0.97	<0.02	<0.01	<0.1	<0.01	<1	0.03	0.19	<0.01	<0.2	<0.001	0.22	<0.5	<0.5	<0.5	<0.5
1194B Pro blank	0.8	<0.02	<0.01	<0.1	0.015	<1	0.014	<0.01	<0.01	0.25	0.0062	<0.1	<0.5	<0.5	<0.5	<0.5
1195G Pro blank	1.9	<0.02	<0.01	<0.1	<0.01	<1	<0.01	<0.01	0.028	<0.2	0.0025	<0.1	<0.5	<0.5	<0.5	<0.5
1205L Pro Blank	1.8	<0.02	<0.01	<0.1	0.11	<1	<0.01	0.74	0.06	<0.2	0.0063	0.67	<0.1	<0.1	<0.1	<0.5
1207B Pro Blank	0.88	<0.02	<0.01	<0.1	0.056	<1	<0.01	0.26	0.019	<0.2	0.0016	<0.1	<0.1	<0.1	<0.1	<0.5
1206E Pro Blank	0.2	<0.02	<0.01	<0.1	0.16	<1	<0.01	0.08	0.02	<0.2	0.02	0.1	<0.1	<0.1	<0.1	<0.1
1208D Pro Blank	1.3	<0.02	<0.01	<0.1	0.036	<1	<0.01	0.28	0.041	<0.2	0.0019	0.36	<0.1	<0.1	<0.1	<0.5
1209G Pro Blank	3.0	0.022	<0.01	<0.1	0.1	1.1	<0.01	0.29	0.33	<0.2	0.0043	4.4	<0.1	<0.1	0.1	<0.5
1210B Pro Blank	0.98	<0.02	<0.01	<0.1	0.077	<1	<0.01	0.27	0.018	<0.2	0.0024	<0.1	<0.1	<0.1	<0.1	<0.5
1212B Pro Blank	1.0	<0.02	<0.01	<0.1	0.03	<1	<0.01	0.2	<0.01	<0.2	0.01	<0.1	<0.1	<0.1	<0.1	<0.1
1213B Pro Blank	<0.1	<0.02	<0.01	<0.1	0.08	<1	<0.01	0.2	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.1
1220B Pro Blank	<0.1	<0.02	<0.01	<0.1	0.01	2.0	<0.01	0.2	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.1
1242B Pro Blank	<0.1	<0.02	0.011	<0.1	<0.01	<1	0.11	0.57	0.05	<0.2	0.051	<0.1	<0.1	<0.1	<0.1	<0.5

Table B1 (continued) Total measured metals and major ions for Magela Creek Water (MCW), Synthetic Softwater (SSW) Procedural Blanks and Blanks

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Ca	Mg	Na	SO ₄
Units	µg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹											
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.1	0.1	0.1	0.5
Blank (10/8/11)	1.5	<0.02	<0.01	<0.1	0.058	<1	<0.01	0.27	<0.01	<0.2	0.0022	<0.1	<0.1	<0.1	<0.1	<0.5
Blank (15/8/11)	0.72	<0.02	<0.01	<0.1	0.043	<1	<0.01	0.27	0.011	<0.2	0.003	<0.1	<0.1	<0.1	<0.1	<0.5
Blank (15/8/11)	0.97	<0.02	<0.01	<0.1	0.032	<1	<0.01	0.27	<0.01	<0.2	0.002	<0.1	<0.1	<0.1	<0.1	<0.5
Blank (17/8/11)	0.51	N.M.	N.M.	N.M.	<0.01	<1	0.016	N.M.	0.04	N.M.	0.002	<0.1	<0.5	<0.5	N.M.	N.M.
Blank (22/8/11)	<0.1	<0.02	<0.01	<0.1	<0.01	<1	<0.01	0.2	<0.01	<0.2	0.003	<0.1	<0.1	<0.1	<0.1	<0.1
Blank (05/09/11)	<0.1	<0.02	<0.01	<0.1	0.02	<1	<0.01	0.2	<0.01	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.1
Blank (14/11/11)	<0.1	<0.02	<0.01	<0.1	<0.01	<1	0.04	0.54	0.045	<0.2	<0.001	<0.1	<0.1	<0.1	<0.1	<0.5

Table B2 Total and dissolved metals and major ions for the distillate

Analyte	First distillate batch		Second distillate batch
	Totals ($\mu\text{g L}^{-1}$)	Dissolved ($\mu\text{g L}^{-1}$)	Totals ($\mu\text{g L}^{-1}$)
Calcium	0.11	N.M.	<0.1
Magnesium	0.6	N.M.	0.4
Sodium	<0.1	N.M.	<0.1
Potassium	<0.1	N.M.	<0.1
Sulfur, SO ₄	N.M.	N.M.	2
Aluminium	18	<0.02	23
Cadmium	<0.02	0.45	<0.02
Cobalt	0.49	0.13	0.26
Chromium	0.17	0.61	<0.1
Copper	1.2	<1	0.25
Iron	3.5	230	1.4
Manganese	250	0.73	120
Nickel	0.84	0.016	0.64
Lead	0.17	<0.2	0.22
Selenium	0.39	0.69	<0.2
Uranium	1.5	0.35	1.1
Zinc	0.19	<0.05	2
Silver	<0.05	0.1	<0.05
Arsenic	0.3	0.1	0.1
Gold	0.4	110	0.07
Boron	100	0.09	88
Barium	0.1	<0.05	0.06
Beryllium	<0.05	0.01	<0.05
Bismuth	0.2	1	0.02
Bromine	4	<0.01	<1
Cerium	0.02	0.02	<0.01
Caesium	0.03	0.01	<0.01
Dysprosium	0.05	<0.01	0.02
Erbium	0.03	<0.01	<0.01
Europium	<0.01	0.01	<0.01
Gallium	0.02	<0.01	<0.01
Gadolinium	0.03	<0.01	<0.01
Hafnium	0.2	<0.02	0.07
Mercury	<0.02	<0.01	<0.02
Holmium	0.01	<0.01	<0.01
Indium	<0.01	<0.01	<5
Lanthanum	0.01	0.3	<0.01
Lithium	0.3	<0.01	0.2
Lutetium	<0.01	0.07	<0.01

Table B2 (continued) Total and dissolved metals and major ions for the distillate

Analyte	First distillate batch		Second distillate batch
	Totals ($\mu\text{g L}^{-1}$)	Dissolved ($\mu\text{g L}^{-1}$)	Totals ($\mu\text{g L}^{-1}$)
Molybdenum	0.4	0.2	0.2
Niobium	0.5	<0.01	0.1
Neodymium	0.02	0.1	<0.01
Osmium	0.1	<0.05	<0.1
Palladium	<0.05	<0.01	<0.05
Praseodymium	<0.01	0.1	<0.01
Rubidium	0.1	0.03	0.07
Rhenium	0.03	0.8	<0.01
Antimony	3	<0.5	2
Scandium	<0.5	<0.01	<0.5
Samarium	0.01	<0.1	<0.01
Tin	0.2	0.8	0.2
Strontium	0.9	0.1	0.4
Tantalum	0.6	<0.01	0.2
Terbium	0.01	0.3	<0.01
Tellurium	0.7	<0.01	0.1
Thorium	0.1	<2	0.03
Titanium	<2	<0.01	<2
Thallium	0.02	<0.01	<0.01
Thulium	<0.01	0.06	<0.01
Vanadium	0.07	0.3	<0.05
Tungsten	0.9	0.03	0.3
Yttrium	0.2	<0.01	0.1
Ytterbium	0.02	<0.05	<0.01
Zirconium	0.1	1.4	0.08
Sulfur	8.1	N.M.	0.6

Table B3 Nitrate analysis of Blanks and QA/QC samples for the *Lemna* and Algae tests

Analyte	Nitrate as N	Phosphate as P
Unit	mg L ⁻¹	mg L ⁻¹
PQL	0.005	0.005
MCW	0.65	0.095
100% Distillate	0.71	0.097
1205L Pro blank Blank	<0.005	<0.005
1205L Blank	<0.005	<0.005
1209G Pro Blank	<0.005	<0.005
1209G A	3	0.048
1209G B	3.1	0.061

Table B4 Alkalinity of Magela Creek Water, Distillate and the NaOH adjusted TIE samples

Sample description	Hydroxide Alkalinity (OH ⁻) as CaCO ₃	Bicarbonate Alkalinity as CaCO ₃	Carbonate Alkalinity as CaCO ₃	Total Alkalinity as CaCO ₃
	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹
Blank	<1	<1	<1	<1
MCW	<1	10	<1	10
MCW	<1	5	<1	5
100% Distillate	<1	6	<1	6
1210B B	<1	7	<1	7
1210B C	<1	10	<1	10
1210B E	<1	4	<1	4
1210B F	<1	10	<1	10

Table B5 Metal and major ion analyses of the pH adjusted TIE (1210B) samples

Analyte	Ca	Mg	Na	SO ₄	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Cl	
Units	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	mg L ⁻¹	µg L ⁻¹	mg L ⁻¹												
PQL	0.1	0.1	0.1	0.5	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	1	
1210B A	totals	0.1	0.8	1.1	<0.5	15	<0.02	0.033	0.1	0.15	58	1.3	0.34	0.078	<0.2	0.11	0.17	3
	dissolved	0.1	0.9	1.1	N.M.	12	<0.02	0.032	<0.1	0.18	55	1.3	0.14	0.077	<0.2	0.1	0.45	N.M.
1210B B	totals	0.1	0.8	1	<0.5	13	<0.02	0.035	<0.1	0.13	59	1.3	0.34	0.078	<0.2	0.0096	<0.1	2
	dissolved	0.1	0.8	1.1	N.M.	12	<0.02	0.033	<0.1	0.19	56	1.2	0.13	0.067	<0.2	0.0083	0.27	N.M.
1210B C	totals	0.1	0.8	2.5	<0.5	13	<0.02	0.033	0.13	0.23	59	1.3	0.34	0.11	<0.2	0.016	1.6	N.M.
	dissolved	0.1	0.8	2.6	N.M.	12	<0.02	0.033	<0.1	0.14	56	1.2	0.14	0.084	<0.2	0.017	0.12	N.M.
1210B D	totals	<0.1	0.4	<0.1	2	19	<0.02	0.25	<0.1	0.22	1.3	130	0.65	0.11	<0.2	0.96	1.4	1
	dissolved	<0.1	0.4	<0.1	N.M.	13	<0.02	0.25	<0.1	0.2	<1	120	0.41	0.053	<0.2	0.87	1.6	N.M.
1210B E	totals	<0.1	0.4	<0.1	2	18	<0.02	0.25	<0.1	0.28	1.2	130	0.62	0.05	<0.2	0.94	1.4	<1
	dissolved	<0.1	0.4	<0.1	N.M.	14	<0.02	0.24	<0.1	0.27	<1	120	0.39	0.012	<0.2	0.92	1.3	N.M.
1210B F	totals	<0.1	0.3	1.3	2	22	<0.02	0.19	0.1	0.15	1.3	88	0.56	0.099	<0.2	0.3	0.31	N.M.
	dissolved	<0.1	0.3	1.3	N.M.	23	<0.02	0.16	<0.1	0.15	<1	72	0.58	0.067	<0.2	0.26	<0.1	N.M.

Table B6 Measured 'bioavailable' Manganese (Chelex assay) in EDTA TIE (1211B)

Sample	Manganese dissolved (before Chelex)	Manganese-dissolved (% retained)
	$\mu\text{g L}^{-1}$	%
	0.1	1
Blank	0.0016	N.M.
MCW 0 mg L ⁻¹ EDTA	1	93
MCW 2.8 mg L ⁻¹ EDTA	1	<1
MCW 5.5 mg L ⁻¹ EDTA	1	<1
MCW 11 mg L ⁻¹ EDTA	1	<1
DISTILLATE 0 mg L ⁻¹ EDTA	110	100
DISTILLATE 2.8 mg L ⁻¹ EDTA	110	<1
DISTILLATE 5.5 mg L ⁻¹ EDTA	110	<1
DISTILLATE 11 mg L ⁻¹ EDTA	110	<1

Table B7 Metal and major ion analyses of the Ca addition TIE (1212B) samples

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Ca	Mg	K	Na	SO ₄
Units	µg L ⁻¹	mg L ⁻¹															
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.1	0.1	0.1	0.1	0.5
SSW – no Ca	64	<0.02	0.01	<0.1	0.6	95	11	0.2	0.03	<0.2	0.3	0.2	<0.1	0.6	0.4	1.9	5
SSW – 0.25 mg L ⁻¹ Ca	69	<0.02	0.01	<0.1	0.6	97	10	0.2	0.04	<0.2	0.2	<0.1	0.2	0.6	0.4	0.9	3
SSW – 0.5 mg L ⁻¹ Ca ^a	62	<0.02	<0.01	<0.1	0.6	94	11	0.2	0.02	<0.2	0.2	0.2	0.4	0.6	0.4	1.5	4
Distillate – no Ca	17	<0.02	0.3	<0.1	0.2	<1	130	0.7	0.1	<0.2	0.8	1	<0.1	0.4	<0.1	<0.1	2
Distillate – 0.25 mg L ⁻¹ Ca	16	<0.02	0.3	<0.1	0.1	<1	130	0.6	0.1	<0.2	0.7	0.9	0.2	0.4	<0.1	<0.1	2
Distillate – 0.5 mg L ⁻¹ Ca	14	<0.02	0.3	<0.1	0.1	1	130	0.5	0.1	<0.2	0.7	0.8	0.4	0.4	<0.1	<0.1	2

^a Unmodified Synthetic Soft Water (SSW) contains 0.5, mg L⁻¹ of Ca

37

Table B8 Metal and major ion analyses of the ammonia stripping TIE (1213B) samples

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Ca	Mg	K	Na	SO ₄
Units	µg L ⁻¹	mg L ⁻¹															
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.1	0.1	0.1	0.1	0.5
MCW	11	<0.02	0.04	<0.1	0.1	52	1	0.4	0.1	<0.2	0.007	<0.1	0.2	0.9	0.2	1	<0.5
Distillate	13	<0.02	0.3	<0.1	0.3	<1	130	0.6	0.2	<0.2	0.7	1	<0.1	0.4	<0.1	<0.1	2
MCW – stripped	9	<0.02	0.04	0.3	9	38	6	1	0.5	<0.2	0.09	2	0.3	1	0.4	210	<0.5
Distillate - stripped	36	<0.02	0.2	0.2	1	4	95	1	0.2	<0.2	0.8	1	<0.1	0.4	0.9	210	2

Table B9 Metal and major ion analyses of the major ion addition TIE (1220B) samples

Analyte	Al	Cd	Co	Cr	Cu	Fe	Mn	Ni	Pb	Se	U	Zn	Ca	Mg	K	Na	SO ₄
Units	µg L ⁻¹	mg L ⁻¹															
PQL	0.1	0.02	0.01	0.1	0.01	1	0.01	0.01	0.01	0.2	0.001	0.1	0.1	0.1	0.1	0.1	0.5
SSW – no Ca, K, N	66	<0.02	<0.01	<0.1	0.7	94	12	0.3	0.05	<0.2	0.2	<0.1	<0.1	0.6	<0.1	0.6	2
SSW – 1/2 Ca, K, Na	67	<0.02	0.01	<0.1	0.6	96	11	0.2	0.04	<0.2	0.2	<0.1	0.2	0.6	0.2	0.7	3
SSW – unmodified ^a	69	<0.02	<0.01	<0.1	0.6	99	10	0.2	0.03	<0.2	0.1	<0.1	0.4	0.6	0.4	0.9	3
Distillate – unmodified	14	<0.02	0.3	<0.1	0.2	<1	130	0.6	0.1	<0.2	0.8	1	<0.1	0.4	0.1	<0.1	2
Distillate – 1/2 Ca, K, Na	13	<0.02	0.3	<0.1	0.2	<1	140	0.6	0.04	<0.2	0.7	1	0.2	0.4	0.3	0.5	2
Distillate – full Ca, K, Na	13	<0.02	0.2	<0.1	0.2	<1	140	0.6	0.05	<0.2	0.7	1	0.4	0.4	0.5	1	2

^a Unmodified Synthetic Soft Water (SSW) contains 0.5, 1.0 and 0.4 mg L⁻¹ of Ca, Na and K respectively

Table B10 Organic compounds detected by GC-MS scan

Sample	Sample date	Volatile Organic Compounds			Semi Volatile Organic Compounds		
		Best 75K NBS Library Match	Concentration ($\mu\text{g L}^{-1}$) ^a	Match Quality	Best 75K NBS Library Match	Concentration ($\mu\text{g L}^{-1}$) ^a	Match Quality
Blank ^b	07/09/11	Methyl Ethyl Ketone (MEK)	1.3	86%	no peaks detected	N/A	N/A
Process Water (feed water) ^b	07/09/11	no peaks detected	N/A	N/A	no peaks detected	N/A	N/A
Distillate ^c	12/08/11	Decane	2.0	70%	Phenol, 3,5-bis (1,1-dimethylethyl)	5.8	93%
					Phenol, 2,4-bis (1,1-dimethylethyl)	12.0	97%
					1,2-Benzenedicarboxylic acid, butyl (2-methylpropyl) ester ^d	9.9	86%
Blank ^a	08/09/11	Methyl Ethyl Ketone (MEK)	5.0	85%	Phenol, 2-(1,1-dimethylethyl)-4-methyl-	1.5	97%
Distillate ^{b e}	08/09/11	no peaks detected	N/A	N/A	no peaks detected	N/A	N/A
Distillate filtrate (SPE TIE)	20/09/11	no peaks detected	N/A	N/A	2-Furancarboxaldehyde, 5-methyl-	1.0	93%
					2,5-Heptadien-4-one, 2,6-dimethyl-	1.4	92%
Distillate ^{b f}	10/09/11	Ethanol	5.0	95%	no peaks detected	N/A	N/A
		Octanal	1.0	82%			

^a Concentration estimated from closest surrogate compound. ^b Sampled into glass bottles at RioTinto Technology and Innovation, Bundoora; ^c Sampled from a polyethylene container at ERISS, Darwin; ^d Also known as Phthalate; ^e Water used in the SPE TIE; ^f Sample not use in toxicity or TIE tests. Anti-scalant and anit-foam was added to the feed water for this sample.

Table B11 Measured calcium, sodium, potassium, magnesium and manganese concentrations in the Mn toxicity TIE (1242B)

Analyte	Calcium		Potassium		Sodium		Magnesium		Manganese		
	Nominal mg L ⁻¹	Measured mg L ⁻¹	Nominal µg L ⁻¹	Measured µg L ⁻¹	Measured µg L ⁻¹						
Sample	totals	totals	totals	totals	totals	totals	totals	totals	totals	dissolved (0.1 µm)	totals
SSW – No major ions 0 µg L ⁻¹ Mn	0	0.1	0.0	<0.1	0.0	0.4	0.6	0.6	0	17	17
SSW – No major ions 130 µg L ⁻¹ Mn	0	0.1	0.0	<0.1	0.0	0.5	0.6	0.6	130	140	140
SSW – No major ions 230 µg L ⁻¹ Mn	0	0.1	0.0	<0.1	0.0	0.5	0.6	0.6	230	250	260
SSW – 50% major ions 0 µg L ⁻¹ Mn	0.2	0.3	0.2	0.2	0.5	0.5	0.6	0.6	0	12	15
SSW – 50% major ions 130 µg L ⁻¹ Mn	0.2	0.3	0.2	0.3	0.5	0.8	0.6	0.6	130	130	130
SSW – 50% major ions 230 µg L ⁻¹ Mn	0.2	0.3	0.2	0.2	0.5	0.5	0.6	0.6	230	250	250
SSW – 100% major ions 0 µg L ⁻¹ Mn	0.5	0.5	0.4	0.5	1.0	1.1	0.6	0.6	0	10	11
SSW – 100% major ions 130 µg L ⁻¹ Mn	0.5	0.4	0.4	0.5	1.0	1.1	0.6	0.6	130	130	130
SSW – 100% major ions 230 µg L ⁻¹ Mn	0.5	0.4	0.4	0.5	1.0	1.1	0.6	0.6	230	250	250

Appendix C Statistical summaries

CETIS Analytical Report

Report Date: 10 Jan-12 15:54 (p 1 of 1)
 Test Code: 1193D | 12-6762-6525

Cladoceran Reproduction Test			eriss ecotoxicology lab		
Analysis ID:	16-3873-9099	Endpoint:	Total neonates	CETIS Version:	CETISv1.8.1
Analyzed:	10 Jan-12 15:51	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes
Batch ID:	05-2916-7653	Test Type:	Cladoceran reproduction	Analyst:	Andrew J Harford
Start Date:	27 Jan-12	Protocol:	Clad (chronic) eriss tropical freshwater	Diluent:	Magela Creek Water
Ending Date:	01 Aug-11	Species:	Moinodaphnia macleayi	Brine:	Brine distillate
Duration:	N/A	Source:	In-House Culture	Age:	<6 h
Sample ID:	05-8160-6031	Code:	22AA9A8F	Client:	Energy Resources of Australia - Enviro
Sample Date:	11 Jul-11	Material:	Ranger Brine Concentrator Distillate	Project:	Ranger Brine Concentrator Plant
Receive Date:	21 Jan-12	Source:	Ranger Brine Concentrator Plant		
Sample Age:	200d 0h	Station:	N/A		

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1.360E+09	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	39.83	13.11	62.77	2.51	1.593	7.625
IC10	71.87	47.6	N/A	1.391	N/A	2.101
IC15	>100	N/A	N/A	<1	N/A	N/A
IC20	>100	N/A	N/A	<1	N/A	N/A
IC25	>100	N/A	N/A	<1	N/A	N/A
IC40	>100	N/A	N/A	<1	N/A	N/A
IC50	>100	N/A	N/A	<1	N/A	N/A

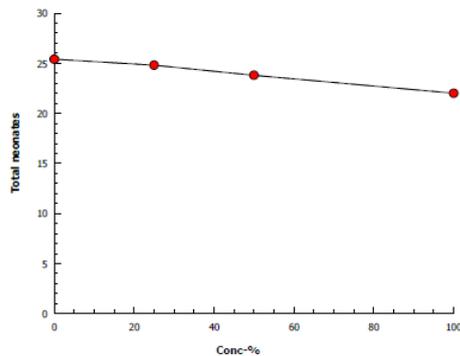
Total neonates Summary

Conc-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Magela Creek W	10	25.4	22	27	0.4761	1.506	5.93%	0.0%
25		10	24.8	24	26	0.2494	0.7888	3.18%	2.36%
50		10	23.8	22	25	0.3887	1.229	5.17%	6.3%
100		10	22	20	24	0.3944	1.247	5.67%	13.39%

Total neonates Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Magela Creek Wa	27	27	26	25	22	25	26	26	26	26
25		24	24	26	25	25	24	25	26	24	25
50		25	24	25	25	25	22	23	23	22	24
100		22	24	22	22	24	20	22	22	21	21

Graphics



CETIS Analytical Report

Report Date: 10 Jan-12 15:39 (p 1 of 2)
Test Code: 1194B | 12-0993-3878

Green Hydra Population Growth Test			eriss ecotoxicology lab		
Analysis ID: 11-0374-6964	Endpoint: Specific growth rate (96h)	CETIS Version: CETISv1.8.1			
Analyzed: 10 Jan-12 15:38	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			
Batch ID: 04-0677-5006	Test Type: Hydra population growth	Analyst: Andrew J Harford			
Start Date: 25 Jul-11 13:00	Protocol: Alga eriss tropical freshwater	Diluent: Magela Creek Water			
Ending Date: 01 Aug-11	Species: Hydra viridissima	Brine: Brine distillate			
Duration: 6d 11h	Source: In-House Culture	Age:			
Sample ID: 05-2878-0677	Code: 528780677	Client: Energy Resources of Australia - Enviro			
Sample Date: 14 Jul-11	Material: Ranger Brine Concentrator Distillate	Project: Ranger Brine Concentrator Plant			
Receive Date: 21 Jul-11 10:00	Source: Ranger Brine Concentrator Plant				
Sample Age: 11d 13h (20 °C)	Station:				
Batch Note: Verification data					
Sample Note: Composite from 7 sampling dates, ie. 11/072011 to 17/07/2011					

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	474363916	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	6.011	N/A	59.33	16.64	1.685	N/A
IC10	29.99	N/A	76.64	3.334	1.305	N/A
IC15	50.33	N/A	62.75	1.987	1.594	N/A
IC20	55.09	29.31	67.03	1.815	1.492	3.412
IC25	60.3	44.16	71.6	1.658	1.397	2.264
IC40	78.99	67.46	88.09	1.266	1.135	1.482
IC50	94.53	86.65	102.7	1.058	0.9738	1.154

Specific growth rate (96h) Summary			Calculated Variate							
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	
0	Magela Creek W	3	0.3507	0.34	0.359	0.005607	0.009712	2.77%	0.0%	
25		3	0.3213	0.298	0.353	0.01641	0.02843	8.85%	8.37%	
50		3	0.2993	0.266	0.327	0.01784	0.03089	10.32%	14.64%	
100		3	0.1643	0.16	0.173	0.004333	0.007506	4.57%	53.14%	

Specific growth rate (96h) Detail				
Conc-%	Control Type	Rep 1	Rep 2	Rep 3
0	Magela Creek Wa	0.359	0.353	0.34
25		0.353	0.313	0.298
50		0.327	0.305	0.266
100		0.16	0.173	0.16

CETIS Analytical Report

Report Date: 10 Jan-12 15:39 (p 2 of 2)
Test Code: 1194B | 12-0993-3878

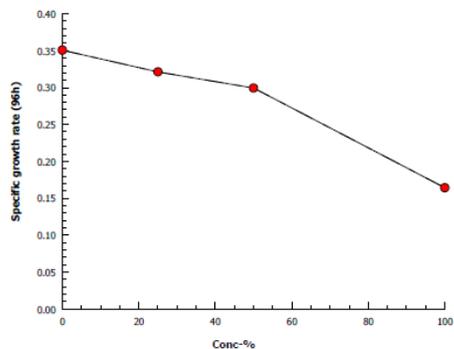
Green Hydra Population Growth Test

eriss ecotoxicology lab

Analysis ID: 11-0374-6964 Endpoint: Specific growth rate (96h)
Analyzed: 10 Jan-12 15:38 Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.1
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 10 Jan-12 16:00 (p 1 of 2)
Test Code: 1195G | 01-3033-2488

Algal Growth Inhibition Test			eriss ecotoxicology lab		
Analysis ID: 20-2559-3607	Endpoint: Growth rate (db/d)	CETIS Version: CETISv1.8.1			
Analyzed: 02 Aug-11 12:01	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			
Batch ID: 07-4604-6695	Test Type: Algal growth inhibition	Analyst:			
Start Date: 25 Jul-11	Protocol: Alga eriss tropical freshwater	Diluent: Magela Creek Water			
Ending Date: 29 Jul-11	Species: Chlorella sp.	Brine: Not Applicable			
Duration: 96h	Source: In-House Culture	Age: 4-5d			
Sample ID: 05-2878-0677	Code: 528780677	Client: Energy Resources of Australia - Enviro			
Sample Date: 14 Jul-11	Material: Ranger Brine Concentrator Distillate	Project: Ranger Brine Concentrator Plant			
Receive Date: 21 Jul-11 10:00	Source: Ranger Brine Concentrator Plant				
Sample Age: 11d 0h (20 °C)	Station: N/A				

Sample Note: Composite from 7 sampling dates, ie. 11/072011 to 17/072011

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	576399713	200	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	6.226	N/A	110.9	16.06	0.9018	N/A
IC10	78.82	N/A	N/A	1.269	N/A	N/A
IC15	>100	N/A	N/A	<1	N/A	N/A
IC20	>100	N/A	N/A	<1	N/A	N/A
IC25	>100	N/A	N/A	<1	N/A	N/A
IC40	>100	N/A	N/A	<1	N/A	N/A
IC50	>100	N/A	N/A	<1	N/A	N/A

Growth rate (db/d) Summary

Conc-%	Control Type	Count	Calculated Variate						
			Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Magela Creek W	3	1.736	1.674	1.78	0.03201	0.05545	3.19%	0.0%
25		3	1.529	1.392	1.602	0.06885	0.1192	7.8%	11.91%
50		3	1.657	1.64	1.67	0.009001	0.01559	0.94%	4.56%
100		3	1.546	1.511	1.571	0.01812	0.03138	2.03%	10.93%

Growth rate (db/d) Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3
0	Magela Creek Wa	1.674	1.755	1.78
25		1.595	1.392	1.602
50		1.64	1.662	1.67
100		1.511	1.557	1.571

CETIS Analytical Report

Report Date: 10 Jan-12 16:00 (p 2 of 2)
Test Code: 1195G | 01-3033-2488

Algal Growth Inhibition Test

eriss ecotoxicology lab

Analysis ID: 20-2559-3607

Endpoint: Growth rate (db/d)

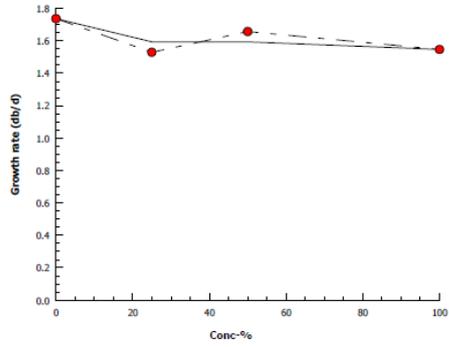
CETIS Version: CETISv1.8.1

Analyzed: 02 Aug-11 12:01

Analysis: Linear Interpolation (ICPIN)

Official Results: Yes

Graphics



000-428-181-1

CETIS™ v1.8.1.2

Analyst: _____ QA: _____

CETIS Analytical Report

Report Date: 10 Jan-12 16:05 (p 1 of 1)
Test Code: 1205L | 16-0995-0771

Lemna Growth Inhibition			eriss ecotoxicology lab		
Analysis ID: 05-2095-7097	Endpoint: Surface area	CETIS Version: CETISv1.8.1			
Analyzed: 10 Jan-12 16:04	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			
Batch ID: 14-6984-7174	Test Type: Lemna Growth	Analyst: Andrew J Harford			
Start Date: 15 Aug-11 11:50	Protocol: Lemna eriss tropical freshwater	Diluent: Laboratory Water			
Ending Date: 19 Aug-11 11:50	Species: Lemna aequinoctialis	Brine: Not Applicable			
Duration: 96h	Source: eriss ecotoxicology lab	Age: 4 d			
Sample ID: 21-4586-6685	Code: 528780677	Client: Energy Resources of Australia - Enviro			
Sample Date: 10 Aug-11 17:00	Material: Ranger Brine Concentrator Distillate	Project: Ranger Brine Concentrator Plant			
Receive Date: 12 Aug-11 09:00	Source: Ranger Brine Concentrator Plant				
Sample Age: 4d 19h	Station: Not Applicable				

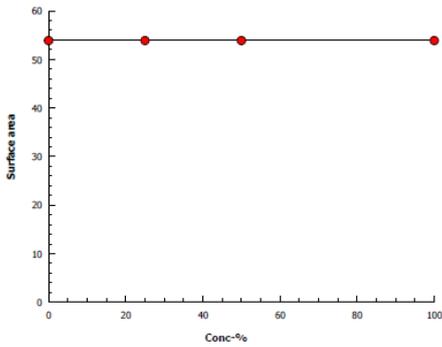
Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	169225357	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
IC5	>100	N/A	N/A	<1	N/A	N/A
IC10	>100	N/A	N/A	<1	N/A	N/A
IC15	>100	N/A	N/A	<1	N/A	N/A
IC20	>100	N/A	N/A	<1	N/A	N/A
IC25	>100	N/A	N/A	<1	N/A	N/A
IC40	>100	N/A	N/A	<1	N/A	N/A
IC50	>100	N/A	N/A	<1	N/A	N/A

Surface area Summary			Calculated Variate						
Conc-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Magela Creek W	3	53.87	53.87	53.87	0	0	0.0%	0.0%
25		3	53.87	53.87	53.87	0	0	0.0%	0.0%
50		3	53.87	53.87	53.87	0	0	0.0%	0.0%
100		3	53.87	53.87	53.87	0	0	0.0%	0.0%

Surface area Detail				
Conc-%	Control Type	Rep 1	Rep 2	Rep 3
0	Magela Creek Wa	53.87	53.87	53.87
25		53.87	53.87	53.87
50		53.87	53.87	53.87
100		53.87	53.87	53.87

Graphics



Gudgeon Sac Fry Survival Test

eriss ecotoxicology lab

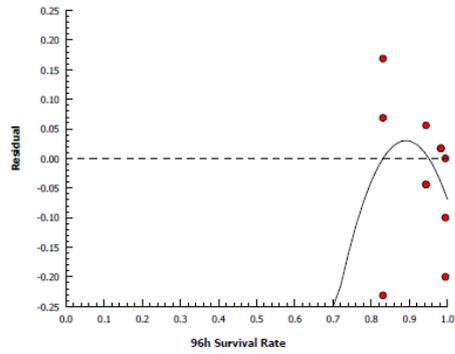
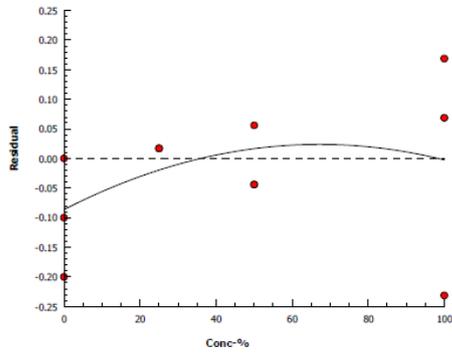
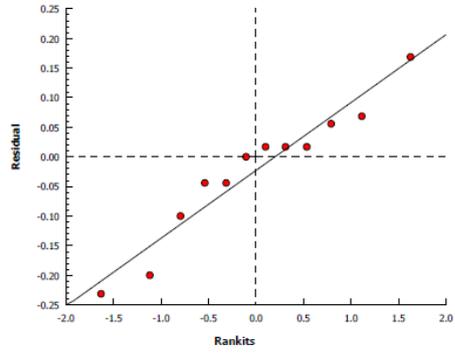
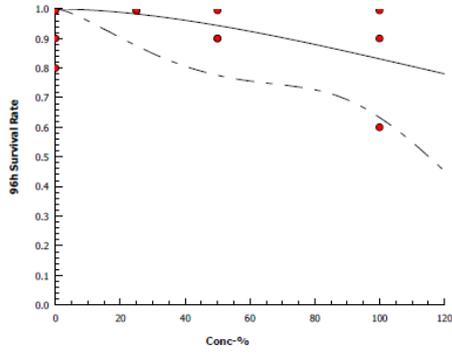
Analysis ID: 05-1464-0067
Analyzed: 10 Jan-12 16:16

Endpoint: 96h Survival Rate
Analysis: Nonlinear Regression

CETIS Version: CETISv1.8.1
Official Results: Yes

Graphics

2P Log-Logistic EV [Y=1/(1+(X/D)^C)]



CETIS Analytical Report

Report Date: 12 Jan-12 11:51 (p 1 of 1)
 Test Code: 1207B | 20-6778-7179

Green Hydra Population Growth Test			eriss ecotoxicology lab		
Analysis ID: 11-6512-5155	Endpoint: Specific growth rate (96h)	CETIS Version: CETISv1.8.1			
Analyzed: 12 Jan-12 11:50	Analysis: Parametric-Two Sample	Official Results: Yes			
Batch ID: 13-9857-0343	Test Type: Hydra population growth	Analyst: Andrew J Harford			
Start Date: 15 Aug-11	Protocol: Alga eriss tropical freshwater	Diluent: Magela Creek Water			
Ending Date: 19 Aug-11	Species: Hydra viridissima	Brine: Brine distillate			
Duration: 96h	Source: In-House Culture	Age:			
Sample ID: 13-5465-1553	Code: 528780677	Client: Energy Resources of Australia - Enviro			
Sample Date: 10 Aug-11 17:00	Material: Ranger Brine Concentrator Distillate	Project: Ranger Brine Concentrator Plant			
Receive Date: 11 Aug-11 09:00	Source: Ranger Brine Concentrator Plant				
Sample Age: 4d 7h (10 °C)	Station: Not Applicable				

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Untransformed	0	C > T	Not Run	Sample fails specific growth rate (96h) endpoint	3.06%

Equal Variance t Two-Sample Test								
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Magela Creek Wate		100*	69.63	2.132	4	0.009872	<0.0001	Significant Effect

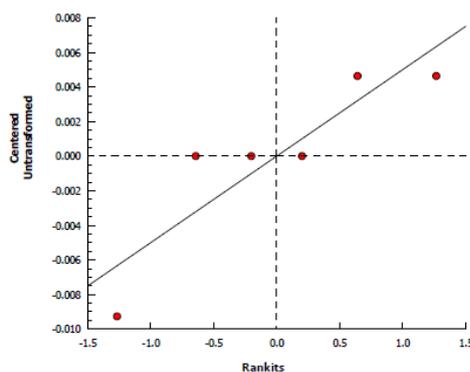
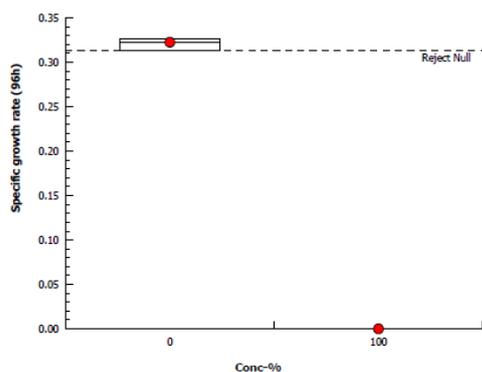
ANOVA Table							
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)	
Between	0.1559633	0.1559633	1	4849	<0.0001	Significant Effect	
Error	0.0001286672	3.216679E-05	4				
Total	0.156092	0.1559955	5				

Distributional Tests							
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)		
Variances	Mod Levene Equality of Variance	1	98.5	0.4226	Equal Variances		
Distribution	Shapiro-Wilk W Normality	0.8137	0.43	0.0778	Normal Distribution		

Specific growth rate (96h) Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Magela Creek W	3	0.3225	0.3194	0.3255	0.3132	0.3271	0.004631	0.008021	2.49%	0.0%
100		3	0	0	0	0	0	0	0		100.0%

Specific growth rate (96h) Detail					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	
0	Magela Creek W	0.3271	0.3132	0.3271	
100		0	0	0	

Graphics



000-428-181-1

CETIS™ v1.8.1.2

Analyst: _____ QA: _____

CETIS Analytical Report

Report Date: 12 Jan-12 11:43 (p 1 of 1)
 Test Code: 1208D | 05-9175-0772

Cladoceran Reproduction Test			eriss ecotoxicology lab		
Analysis ID:	21-3945-8834	Endpoint:	Total neonates	CETIS Version:	CETISv1.8.1
Analyzed:	12 Jan-12 11:43	Analysis:	Parametric-Two Sample	Official Results:	Yes
Batch ID:	06-3413-4192	Test Type:	Cladoceran reproduction	Analyst:	Andrew J Harford
Start Date:	14 Aug-11	Protocol:	Clad (chronic) eriss tropical freshwater	Diluent:	Magela Creek Water
Ending Date:	19 Aug-11	Species:	Moinodaphnia macleayi	Brine:	Brine distillate
Duration:	5d 0h	Source:	In-House Culture	Age:	<6 h
Sample ID:	13-5465-1553	Code:	528780677	Client:	Energy Resources of Australia - Enviro
Sample Date:	10 Aug-11 17:00	Material:	Ranger Brine Concentrator Distillate	Project:	Ranger Brine Concentrator Plant
Receive Date:	11 Aug-11 09:00	Source:	Ranger Brine Concentrator Plant		
Sample Age:	79h (10 °C)	Station:	Not Applicable		

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Untransformed	0	C > T	Not Run	Sample passes total neonates endpoint	9.38%

Equal Variance t Two-Sample Test								
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Magela Creek Wate		100	1.17	1.734	18	2.963	0.1285	Non-Significant Effect

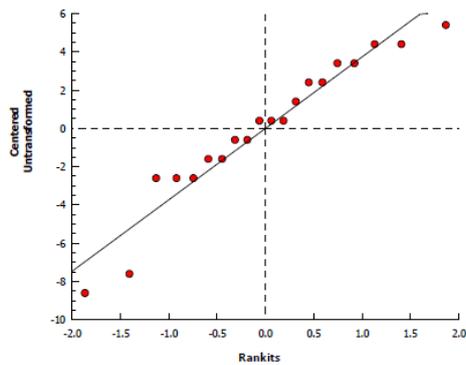
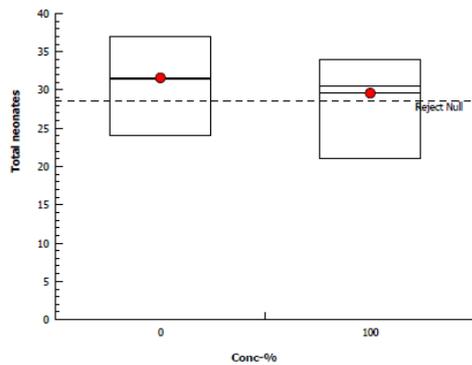
ANOVA Table							
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)	
Between	20	20	1	1.37	0.2571	Non-Significant Effect	
Error	262.8	14.6	18				
Total	282.8	34.6	19				

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Variance Ratio F	1.015	6.541	0.9823	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9337	0.866	0.1819	Normal Distribution	

Total neonates Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Magela Creek W	10	31.6	30.14	33.06	24	37	1.213	3.836	12.14%	0.0%
100		10	29.6	28.15	31.05	21	34	1.204	3.806	12.86%	6.33%

Total neonates Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	Magela Creek W	36	31	24	29	32	32	35	29	37	31
100		32	34	28	33	27	28	21	31	30	32

Graphics



000-428-181-1

CETIS™ v1.8.1.2

Analyst: _____ QA: _____

CETIS Analytical Report

Report Date: 12 Jan-12 11:56 (p 1 of 1)
Test Code: 1209G | 09-0423-3577

Algal Growth Inhibition Test			eriss ecotoxicology lab		
Analysis ID: 12-1303-0687	Endpoint: Growth rate (db/d)	CETIS Version: CETISv1.8.1			
Analyzed: 12 Jan-12 11:55	Analysis: Parametric-Two Sample	Official Results: Yes			
Batch ID: 09-6499-7729	Test Type: Algal growth inhibition	Analyst: Andrew J Harford			
Start Date: 15 Aug-11 11:45	Protocol: Alga eriss tropical freshwater	Diluent: Magela Creek Water			
Ending Date: 18 Aug-11 11:45	Species: Chlorella sp.	Brine: Not Applicable			
Duration: 72h	Source: In-House Culture	Age: 4d			
Sample ID: 13-5465-1553	Code: 528780677	Client: Energy Resources of Australia - Enviro			
Sample Date: 10 Aug-11 17:00	Material: Ranger Brine Concentrator Distillate	Project: Ranger Brine Concentrator Plant			
Receive Date: 11 Aug-11 09:00	Source: Ranger Brine Concentrator Plant				
Sample Age: 4d 19h (10 °C)	Station: Not Applicable				

Data Transform	Zeta	Alt Hyp	MC Trials	Test Result	PMSD
Untransformed	0	C > T	Not Run	Sample passes growth rate (db/d) endpoint	1.3%

Equal Variance t Two-Sample Test								
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)
Magela Creek Wate		100	0.3015	2.132	4	0.02357	0.3890	Non-Significant Effect

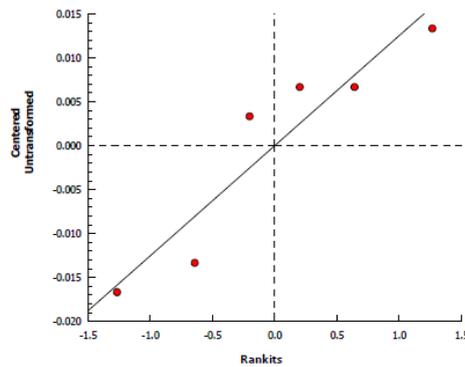
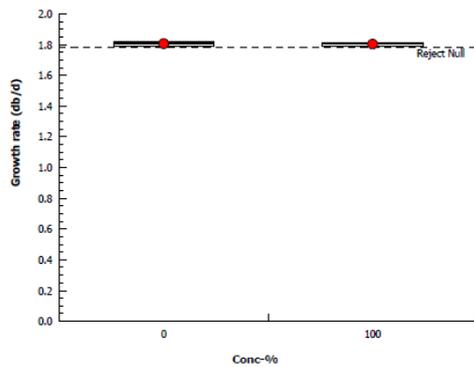
ANOVA Table							
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)	
Between	1.666703E-05	1.666703E-05	1	0.09091	0.7780	Non-Significant Effect	
Error	0.0007333351	0.0001833338	4				
Total	0.0007500021	0.0002000008	5				

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Variance Ratio F	1.75	199	0.7273	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.8658	0.43	0.2100	Normal Distribution	

Growth rate (db/d) Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Magela Creek W	3	1.807	1.801	1.812	1.79	1.82	0.008818	0.01527	0.85%	0.0%
100		3	1.803	1.799	1.808	1.79	1.81	0.006664	0.01154	0.64%	0.18%

Growth rate (db/d) Detail					
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	
0	Magela Creek W	1.82	1.79	1.81	
100		1.81	1.79	1.81	

Graphics



000-428-181-1

CETIS™ v1.8.1.2

Analyst: _____ QA: _____

1212B Calcium addition TIE

Two Way Analysis of Variance

Wednesday, January 11, 2012, 2:40:15 PM

Data source: Data 1 in 1212B two-way ANOVA

Balanced Design

Dependent Variable: Pop growth rate

Normality Test (Shapiro-Wilk) Passed (P = 0.187)

Equal Variance Test: Passed (P = 1.000)

Source of Variation	DF	SS	MS	F	P
Water type	1	0.0584	0.0584	429.777	<0.001
Ca (mg/L)	2	0.0950	0.0475	349.655	<0.001
Water type x Ca (mg/L)	2	0.00186	0.000928	6.829	0.010
Residual	12	0.00163	0.000136		
Total	17	0.157	0.00923		

Main effects cannot be properly interpreted if significant interaction is determined. This is because the size of a factor's effect depends upon the level of the other factor.

The effect of different levels of Water type depends on what level of Ca (mg/L) is present. There is a statistically significant interaction between Water type and Ca (mg/L). (P = 0.010)

Power of performed test with alpha = 0.0500: for Water type : 1.000

Power of performed test with alpha = 0.0500: for Ca (mg/L) : 1.000

Power of performed test with alpha = 0.0500: for Water type x Ca (mg/L) : 0.775

Least square means for Water type :

Group	Mean
SSW	0.230
Distillate	0.116

Std Err of LS Mean = 0.00389

Least square means for Ca (mg/L) :

Group	Mean
0.000	0.0711
0.250	0.217
0.500	0.232

Std Err of LS Mean = 0.00476

Least square means for Water type x Ca (mg/L) :

Group	Mean
SSW x 0.000	0.142
SSW x 0.250	0.269
SSW x 0.500	0.280
Distillate x 0.000	0.000
Distillate x 0.250	0.165
Distillate x 0.500	0.185

Std Err of LS Mean = 0.00673

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Ca (mg/L) within SSW**

Comparison	Diff of Means	p	q	P	P<0.05
0.500 vs. 0.000	0.138	3	20.496	<0.001	Yes
0.500 vs. 0.250	0.0112	3	1.666	0.488	No
0.250 vs. 0.000	0.127	3	18.830	<0.001	Yes

Comparisons for factor: **Ca (mg/L) within Distillate**

Comparison	Diff of Means	p	q	P	P<0.05
0.500 vs. 0.000	0.185	3	27.449	<0.001	Yes
0.500 vs. 0.250	0.0200	3	2.970	0.132	No
0.250 vs. 0.000	0.165	3	24.479	<0.001	Yes

Comparisons for factor: **Water type within 0**

Comparison	Diff of Means	p	q	P	P<0.05
SSW vs. Distillate	0.142	2	21.127	<0.001	Yes

Comparisons for factor: **Water type within 0.25**

Comparison	Diff of Means	p	q	P	P<0.05
SSW vs. Distillate	0.104	2	15.478	<0.001	Yes

Comparisons for factor: **Water type within 0.5**

Comparison	Diff of Means	p	q	P	P<0.05
SSW vs. Distillate	0.0954	2	14.175	<0.001	Yes

1220B Major ion addition TIE

Two Way Analysis of Variance

Wednesday, January 11, 2012, 12:10:31 PM

Data source: Data 1 in 1220B

Balanced Design

Dependent Variable: Pop growth rate

Normality Test (Shapiro-Wilk) Failed (P < 0.050)

Equal Variance Test: Passed (P = 0.430)

Source of Variation	DF	SS	MS	F	P
Water Type	1	0.00318	0.00318	1.209	0.293
Major Ion strength (%)	2	0.210	0.105	39.935	<0.001
Water Type x Major Ion str	2	0.0106	0.00531	2.017	0.176
Residual	12	0.0316	0.00263		
Total	17	0.256	0.0150		

The difference in the mean values among the different levels of Water Type is not great enough to exclude the possibility that the difference is just due to random sampling variability after allowing for the effects of differences in Major Ion strength (%). There is not a statistically significant difference (P = 0.293).

The difference in the mean values among the different levels of Major Ion strength (%) is greater than would be expected by chance after allowing for effects of differences in Water Type. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Water Type does not depend on what level of Major Ion strength (%) is present. There is not a statistically significant interaction between Water Type and Major Ion strength (%). (P = 0.176)

Power of performed test with alpha = 0.0500: for Water Type : 0.0677

Power of performed test with alpha = 0.0500: for Major Ion strength (%) : 1.000

Power of performed test with alpha = 0.0500: for Water Type x Major Ion str : 0.181

Least square means for Water Type :

Group	Mean
SSW	0.223
Distillate	0.250

Std Err of LS Mean = 0.0171

Least square means for Major Ion strength (%) :

Group	Mean
0.000	0.0849
50.000	0.293
100.000	0.331

Std Err of LS Mean = 0.0210

Least square means for Water Type x Major Ion str :

Group	Mean
SSW x 0.000	0.0375
SSW x 50.000	0.294
SSW x 100.000	0.338
Distillate x 0.000	0.132
Distillate x 50.000	0.293
Distillate x 100.000	0.323

Std Err of LS Mean = 0.0296

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: **Water Type**

Comparison	Diff of Means	p	q	P	P<0.050
Distillate vs. SSW	0.0266	2	1.555	0.293	No

Comparisons for factor: **Major Ion strength (%)**

Comparison	Diff of Means	p	q	P	P<0.050
100.000 vs. 0.000	0.246	3	11.727	<0.001	Yes
100.000 vs. 50.000	0.0373	3	1.782	0.443	No
50.000 vs. 0.000	0.208	3	9.945	<0.001	Yes

Comparisons for factor: **Major Ion strength (%) within SSW**

Comparison	Diff of Means	p	q	P	P<0.05
100.000 vs. 0.000	0.300	3	10.134	<0.001	Yes
100.000 vs. 50.000	0.0440	3	1.487	0.561	No
50.000 vs. 0.000	0.256	3	8.647	<0.001	Yes

Comparisons for factor: **Major Ion strength (%) within Distillate**

Comparison	Diff of Means	p	q	P	P<0.05
100.000 vs. 0.000	0.191	3	6.450	0.002	Yes
100.000 vs. 50.000	0.0306	3	1.033	0.751	No
50.000 vs. 0.000	0.161	3	5.417	0.006	Yes

Comparisons for factor: **Water Type within 0**

Comparison	Diff of Means	p	q	P	P<0.05
Distillate vs. SSW	0.0949	2	3.203	0.043	Yes

Comparisons for factor: **Water Type within 50**

Comparison	Diff of Means	p	q	P	P<0.05
SSW vs. Distillate	0.000814	2	0.0275	0.985	No

Comparisons for factor: **Water Type within 100**

Comparison	Diff of Means	p	q	P	P<0.05
SSW vs. Distillate	0.0143	2	0.481	0.740	No

1242B Effect of Mn in low major ion waters

Two Way Analysis of Variance

Monday, October 24, 2011, 3:03:44 PM

Data source: Data 1 in Notebook1

Balanced Design

Dependent Variable: Col 3

Normality Test (Shapiro-Wilk) Passed (P = 0.578)

Equal Variance Test: Passed (P = 0.271)

Source of Variation	DF	SS	MS	F	P
Diluent	2	0.0838	0.0419	58.816	<0.001
Mn	2	0.00813	0.00406	5.705	0.012
Diluent x Mn	4	0.00130	0.000326	0.457	0.766
Residual	18	0.0128	0.000712		
Total	26	0.106	0.00408		

The difference in the mean values among the different levels of Diluent is greater than would be expected by chance after allowing for effects of differences in Mn. There is a statistically significant difference (P = <0.001). To isolate which group(s) differ from the others use a multiple comparison procedure.

The difference in the mean values among the different levels of Mn is greater than would be expected by chance after allowing for effects of differences in Diluent. There is a statistically significant difference (P = 0.012). To isolate which group(s) differ from the others use a multiple comparison procedure.

The effect of different levels of Diluent does not depend on what level of Mn is present. There is not a statistically significant interaction between Diluent and Mn. (P = 0.766)

Power of performed test with alpha = 0.0500: for Diluent : 1.000

Power of performed test with alpha = 0.0500: for Mn : 0.717

Power of performed test with alpha = 0.0500: for Diluent x Mn : 0.0500

Least square means for Diluent :

Group	Mean
0.000	0.161
0.500	0.278
1.000	0.281

Std Err of LS Mean = 0.00890

Least square means for Mn :

Group	Mean
0.000	0.261
110.000	0.241
220.000	0.218

Std Err of LS Mean = 0.00890

Least square means for Diluent x Mn :

Group	Mean
0.000 x 0.000	0.173
0.000 x 110.000	0.160
0.000 x 220.000	0.151
0.500 x 0.000	0.308
0.500 x 110.000	0.280
0.500 x 220.000	0.245
1.000 x 0.000	0.301
1.000 x 110.000	0.284
1.000 x 220.000	0.259

Std Err of LS Mean = 0.0154

All Pairwise Multiple Comparison Procedures (Tukey Test):

Comparisons for factor: Diluent

Comparison	Diff of Means	p	q	P	P<0.050
1.000 vs. 0.000	0.120	3	13.459	<0.001	Yes
1.000 vs. 0.500	0.00318	3	0.357	0.966	No
0.500 vs. 0.000	0.117	3	13.101	<0.001	Yes

Comparisons for factor: Mn

Comparison	Diff of Means	p	q	P	P<0.050
0.000 vs. 220.000	0.0425	3	4.772	0.009	Yes
0.000 vs. 110.000	0.0196	3	2.204	0.289	No
110.000 vs. 220.000	0.0228	3	2.568	0.193	No