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Report prepared for ERA Ranger Mine

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**Rick van Dam, Alicia Hogan and Suthidha Nou**

Environmental Research Institute of the Supervising Scientist (***eriss***)
GPO Box 461, Darwin NT 0801

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# Pre-release biological toxicity testing of Djalkmara Billabong water: 2003–2004 Wet season

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## Introduction

Djalkmara Billabong is a modified natural water body located on the Ranger Uranium Mine site (operated by Energy Resources of Australia Pty Ltd; ERA). It plays an important role in mine water management as it is used as a reservoir for natural run-off from non-mineralised stockpiles and natural woodland areas on the mine site prior to controlled release to Magela Creek during the wet season. As part of its supervisory and monitoring role, the Supervising Scientist Division (SSD) of the Commonwealth Department of the Environment and Heritage (DEH) undertakes toxicity testing of Djalkmara Billabong water prior to its release (ie. pre-release toxicity testing) using local freshwater species. The outcomes are used to advise ERA of the conditions under which this water may be released to Magela Creek without causing harm to the downstream aquatic environment. The approach applied by SSD is fully consistent with the philosophy and approach recommended by the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* (ANZECC & ARMCANZ 2000).

A discussion of the history of the annual pre-release toxicity testing program, including the relative sensitivities of the various test organisms to Ranger retention pond waters and their primary contaminants has been detailed previously (Hogan 2003). Test protocols using the local species *Moinodaphnia macleayi* (cladoceran), *Hydra viridissima* (green hydra) and *Mogurnda mogurnda* (purple spotted gudgeon), which were developed at ***eriss***, have been used over a number of years for the primary purpose of pre-release toxicity testing (Allison et al 1991, McBride et al 1991, Markich & Camilleri 1997). 'Safe' dilutions of mine water for release are calculated by applying a safety factor of 10 (ie. dividing by 10) to the lowest No-Observed-Effect-Concentration (NOEC) of the three sensitive species tested, as recommended by ANZECC & ARMCANZ (2000).

The pre-release toxicity testing program for the 2003-04 wet season was agreed to by SSD and ERA as specified in the Letter of Agreement provided in Appendix A. Pre-release toxicity testing took place on two separate occasions. The first round of tests was undertaken one week before Christmas (late December 2003) to ensure that Ranger environmental staff had appropriate toxicological information and advice over the holiday break in the event that extremely high rainfall necessitated the release Djalkmara Billabong water during this period. The second round of tests took place in mid January 2004, after the billabong had received a large amount of rainfall and associated runoff from the designated areas on the mine site. Throughout this report the two testing rounds are referred to as the December and January testing rounds.

## Aims

The aim of the pre release toxicity testing program was to assess the toxicity of Djalkmara Billabong water to three local freshwater species and, based on the results, calculate a minimum 'safe' dilution level required for controlled release to Magela Creek.

## Materials and methods

### Diluent water collection

Magela Creek water was collected by ***eriss*** staff on 15 December 2003 from Bowerbird Billabong (latitude 12° 46' 15'' longitude 133° 02' 20'') and 8 January 2004 from upstream of Georgetown Billabong (at the pump outlet of the upstream creekside monitoring station; latitude 12° 40' 28'' longitude 132° 55' 52''), for the December and January testing rounds, respectively. The water was collected in 20 L acid-washed plastic gerry cans and placed in storage at 4°C within 1 h of collection. The water was transported to Darwin in an air-conditioned vehicle on the same day as collection. At the laboratory, the water was stored at 4°C and filtered through Whatman #42 filter paper (10 µm pore size) immediately prior to testing.

### Test sample water collection

A 20 L grab sample of Djalkmara Billabong water was taken by ***eriss*** and Ranger Environmental Division staff on the mornings of 15 December 2003 and 8 January 2004, for the December and January testing rounds, respectively. The sample was taken from the bank of the billabong as close as possible to the pump outlet to Magela Creek. The sampling site was the same as that used by ERA for its ongoing chemical monitoring program (John Milsom, ERA, pers comm.). An acid-washed plastic barrel was used to collect and store the sample, which was then transported to Darwin in an air-conditioned vehicle on the same day as collection. At the laboratory, the sample was stored at 4°C and filtered through Whatman # 42 filter paper (10 µm pore size) immediately prior to testing.

### General laboratory procedures

All equipment in contact with test organisms, media, control water or test solutions was made of chemically inert materials (eg. Teflon, glass or polyethylene). All plastic and glassware were washed by soaking in 5% nitric acid ($HNO_3$) for 24 h before undergoing a detergent (Gallay Clean A non-phosphate powder, Gallay Scientific, Burwood, Victoria, Australia) wash and two Elix water (Millipore, Molsheim, France) rinses in a laboratory dishwasher. All reagents used were analytical grade and stock solutions were made up in Milli-Q (Millipore) high purity water.

### Toxicity test methods

#### December 2003

The effect of Djalkmara Billabong water on three local aquatic species (green hydra, *H. viridissima*; cladoceran, *M. macleayi*; purple-spotted gudgeon, *M. mogurnda*) was assessed in the laboratory using the standard protocols developed by ***eriss*** for the pre-release toxicity testing of retention pond waters at Ranger Uranium Mine. A uranium concentration in Djalkmara Billabong water of approximately 4,200 µg/L was reported to ***eriss*** on

1 December 2003 (J. Milsom, ERA, pers comm). Based on the assumption that uranium is the primary toxicant[1], the test dilutions of Djalkmara Billabong water were determined such that they covered the critical range of dilutions over which effects in the test species were expected to become evident. Consequently, for all tests, the organisms were exposed to the following treatment dilutions: 0 (control; Magela creek water only), 0.03, 0.1, 0.3, 1.0, 3.2 and 10% Djalkmara Billabong water (diluted with Magela Creek water).

Testing commenced on 16 (hydra) and 17 (cladoceran, gudgeon larvae) December, within 48 h of sample collection. The test protocols are described in detail by Riethmuller et al (2003).

### H. viridissima *96 h population growth test*

Suitable test hydra, each bearing a newly tentacled bud, were selected from the culture bowls using a dissecting microscope and randomly transferred to 3 plastic petri dishes. Two hundred and ten (210) individuals were required to start the test, with 30 hydra per treatment (ie. 10 hydra × 3 replicates per treatment). Test hydra were transferred from the holding dishes into experimental petri dishes containing 30 mL of test solution using a Pasteur pipette, sequentially adding one animal to each treatment replicate (in ascending order). This process was repeated until each dish contained ten hydroids. The test containers were then randomly placed in an environmental cabinet at a temperature of 27 ± 1°C with a 12 h light:12 h dark photoperiod.

Fresh test solution (30 mL per replicate) was dispensed each day and allowed to warm for at least three hours in the environmental cabinet. Observations on the general appearance of the hydra (eg. rigidity, clubbing, colouration) and the number of hydra were recorded daily for 96 h. Each hydra was fed 3-4 day old artemia shrimp and returned to the incubator for 4 h to allow digestion. After this time the test containers were cleaned and the solution renewed.

After the 96 h test period, the number of hydra produced in each Djalkmara water treatment was compared to the control treatment to determine the No-Observed-Effect-Concentration (NOEC), Lowest-Observed-Effect-Concentration (LOEC) and median inhibition concentration ($IC_{50}$) as described below, in *Statistical analysis*.

### M. macleayi *3-brood (5-6 d) reproduction test*

Suitable *M. macleayi* neonates (ie. <6 h-old) were collected and randomly transferred to 3 crytallising dishes. Seventy individuals were required to start the test, with 10 individual animal replicates per treatment. Individual neonates were transferred from the crytallising dishes into 45 mL test vials containing 30 mL of test solution using a Pasteur pipette, sequentially adding one animal to each treatment replicate (in ascending order). This process was repeated until each test vial contained one neonate. The test containers were then randomly placed in an environmental cabinet at a temperature of 27 ± 1°C with a 12 h light:12 h dark photoperiod.

Fresh test solution (30 mL per replicate) was dispensed each day and allowed to warm for at least three hours in the environmental cabinet. Following observations and neonate counts, each individual adult cladocera was transferred to fresh solution using a Pasteur pipette and microscope. Cladocerans were fed daily with the unicellular green alga, *Chlorella* sp. (at a cell density of $2 \times 10^5$ cells $mL^{-1}$), as well as 1 µL of fermented food and vitamins (FFV) per mL of test solution.

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[1] This assumption was based on results of previous pre-release testing programs, particularly those of the 2002-03 Wet season, and is further discussed below, in the *Results and Discussion*.

Observations on the health of the female, the number of neonates produced and the number of surviving neonates were recorded daily until the time at which the control treatments produced their third brood (144 h). The mean number of neonates produced per adult cladoceran in each Djalkmara water treatment was compared to the controls to determine the NOEC, LOEC and $IC_{50}$ as described below, in *Statistical analysis*.

M. mogurnda *96 h larval survival test*

Suitable *M. mogurnda* larvae (ie. sac-fry, <10 h-old) were collected from hatching aquaria and randomly transferred to 3 petri dishes. Two hundred and ten (210) individuals were required to start the test, with 30 larvae per treatment (ie. 10 larvae × 3 replicates per treatment). Test larvae were transferred from the holding dishes into experimental petri dishes containing 30 mL of test solution using a Pasteur pipette, sequentially adding one animal to each treatment replicate (in ascending order). This process was repeated until each dish contained ten larvae. The test containers were then randomly placed in an environmental cabinet at a temperature of 27 ± 1°C with a 12 h light:12 h dark photoperiod.

Problems associated with maintaining productive breeding stock of *M. mogurnda* resulted in the need to commence the fish survival test with larvae that had hatched prematurely and were showing signs of stress (eg. fungal infection), and which would normally not have been used for testing purposes. The test was set up using larvae that appeared the most healthy and developed.

Fresh test solution (30 mL per replicate) was dispensed each day and allowed to warm for at least three hours in the environmental cabinet. Following observations and larval survival counts, the 24 h old test solution was carefully decanted and replaced with fresh solution. Larvae were not fed prior to or during the 96 h test duration.

Observations on the general appearance of the live larvae and the number of live and dead larvae were recorded daily for 96 h. The mean percent survival of larvae in each Djalkmara water treatment was then compared to the controls to determine the NOEC, LOEC and $IC_{50}$ as described below, in *Statistical analysis*.

### January 2004

Due to ongoing difficulties associated with maintaining productive breeding stock of *M. mogurnda*, no viable gudgeon larvae were available to be tested. Therefore, the effect of Djalkmara Billabong water on two local aquatic species (green hydra, *H. viridissima*; cladoceran, *M. macleayi*) was assessed in the laboratory using the relevant protocols described above. The reported concentration of uranium in Djalkmara Billabong water on 5 January 2004 was approximately 590 µg/L (J. Milsom, ERA, pers comm). Based on the results of the December testing round and the subsequent rainfall-derived dilution of Djalkmara billabong water since this time, the test dilutions of Djalkmara Billabong water were determined such that they covered the critical range of dilutions over which effects in the test species were expected to become evident. Consequently, for all tests, the organisms were exposed to the following treatment dilutions: 0 (control; Magela Creek water only), 0.3, 1.0, 3.2, 10 and 32% Djalkmara Billabong water (diluted with Magela Creek water).

Testing commenced on 10 January, within 48 h of sample collection.

### Statistical analysis

All test data were analysed for normality (Shapiro Wilk's Test) and homogeneity of variance (Bartlett's Test) and transformed if required so that a Dunnett's Test (Dunnett 1955, Dunnett 1964) or Bonferoni Adjusted T-test could be used to determine the NOEC and LOEC ($\alpha$ = 0.05). For *H. viridissima* and *M. macleayi*, a linear interpolation method was used to calculate the $IC_{50}$ (the concentration that gave a 50% inhibition in hydra population growth or cladoceran reproduction). Due to the problems with the *M. mogurnda* tests (see *Results and Discussion*), no statistical analyses were required. The statistical package ToxCalc™ Version 5.0.23F (Tidepool Scientific Software, McKinleyville, California, USA) was used to undertake all statistical analyses.

## Water quality parameters

Throughout each test, the solutions were replaced every 24 h with fresh test solution. A 70 mL sample of fresh test solution was collected at the time of dispensing, and the old test solutions from each treatment replicate were pooled when the solutions were changed. The pH, electrical conductivity (EC) and dissolved oxygen (DO) of both the fresh and old test solution samples were then measured using WTW brand water parameter meters (Weilheim, Germany).

## Water chemistry

Sub-samples (60 mL) of unfiltered and filtered (10 µm) Djalkmara Billabong water, the control (Magela Creek water) and treatment dilutions were collected for chemical analysis. Each sub-sample was collected in an acid-washed plastic bottle and acidified with 1% $HNO_3$ (BDH Aristar, Poole, UK). Samples and blanks were analysed at the Northern Territory Environmental Laboratories (NTEL, Berrimah, NT) for Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, Se, $SO_4$, U and Zn using ICPMS or ICPOES and Cl by spectrophotometry. A separate suite of chemical anlyses was undertaken for the the December *H. viridissima* test, as it was commenced on a different day using a separate set of test solutions than the *M. macleayi* and *M. mogurnda* tests. Only the one set of test solutions was used and therefore analysed for the January testing round.

Filtered Magela Creek water samples (3 × 200 mL) were collected in acid-washed amber glass bottles for each batch of water and sent to the Australian Government Analytical Laboratories (AGAL, Pymble, NSW) within 48 h of collection. One sample was acidified with 10% $HNO_3$ for total organic carbon (TOC) analysis and the other two left unacidified for dissolved organic carbon (DOC) and alkalinity analysis. In addition, an unacidified sub-sample of each of the Djalkmara Billabong water samples collected was sent to AGAL for DOC analysis.

## Quality assurance

Test data were considered acceptable if: the recorded temperature of the incubator remained within the prescribed limits; the recorded pH was within ± 0.5 unit of Day 1 values; the conductivity for each test solution was within 10% of the values obtained on Day 1; and the dissolved oxygen concentration was greater than 70% throughout the test.

For the hydra test, the control population growth was considered acceptable if more than 30 healthy hydroids remained in each control dish at the end of the test duration. For an acceptable cladoceran test, 80% or more of the test control cladocera are required to be alive and female, and to have produced three broods at the end of the test period. Reproduction in the control should have averaged 30 or more neonates surviving per female over the test period and no more than 20% of parental cladocerans should have been reported missing in any treatment (except if all other cladocerans are dead in that group). The gudgeon test was considered acceptable if the mean survival of the combined control was greater than 80% and the presence of fungus on the larvae does not exceed 20% at the end of the test duration.

## Determining a 'safe' dilution ratio

The 'safe' dilution ratio for Djalkmara Billabong water was determined by identifying the lowest NOEC for the three species and dividing this by a safety factor of 10 as recommended by ANZECC & ARMCANZ (2000).

# Results and discussion

## December 2003

### Chemistry

The pH, conductivity and dissolved organic carbon (DOC) concentration of the Djalkmara Billabong water were 7.87, 1946 µS/cm and 22 mg/L, respectively. The pH, conductivity, alkalinity and dissolved organic carbon (DOC) concentration of the Magela Creek water diluent were 6.29, 16 µS/cm, <5 mg/L (as $CaCO_3$) and 7 mg/L, respectively. The chemistry data for diluent (Magela Creek) water, Djalkmara Billabong water and test solutions used for the *H. viridissima* and *M. macleayi* tests are provided at Appendices 2 and 3, respectively. In general, the concentrations of the key contaminants between the two sets of test solutions showed good agreement. Of particular note was that the U concentration in the undiluted Djalkmara billabong sample was approximately 1,400 µg/L, 3 times lower than that reported by ERA only 2 weeks earlier on 1 December (ie. 4,200 µg/L).

To check the accuracy of the original U analysis, a sub-sample of the Djalkmara Billabong water sample collected on 15 December was sent for analysis, whilst a 5% $HNO_3$ rinsate from the original barrel used to collect the water sample was also analysed to ascertain the extent of loss of uranium from solution due to adsorption to the walls of the sample barrel. The U concentrations in the second sub-sample and $HNO_3$ rinsate were 1,250 and 90 µg/L, respectively, confirming that the original analysis was correct and also that there had been little loss of uranium due to adsorption.

More recent correspondence with ERA (John Milsom) revealed that the U concentration reported to ***eriss*** on 1 December of 4,200 µg/L was a measurement from a sample collected on 11 November, approximately 1 month prior to the December testing round. A subsequent comparison of U concentration in Djalkmara Billabong with rainfall recorded at the Jabiru airport from mid November to mid January suggested that the difference in the values could possibly be explained by dilution (Figure 1). Indeed, a regression analysis of U concentration in Djalkmara Billabong versus cumulative rainfall over the period shown in Figure 1 resulted in a strong negative exponential relationship ($r^2 = 0.94$, n = 9, $P < 0.05$), indicating that the discrepancy between the two U values could be attributed to dilution through rainfall/run-off in the period 11 November to 15 December.

The concentrations of the primary contaminants in Djalkmara Billabong water differed markedly to the the December 2002 pre-release toxicity testing round (Hogan 2003). With the exception of U, which was about half the concentration in December 2003 compared to December 2002 (2,750 µg/L), the primary contaminants, Mg, $SO_4$, and Mn were substantially higher in December 2003 compared to December 2002. In particular, Mn was 912 µg/L in December 2003 compared to only 27 µg/L in December 2002.

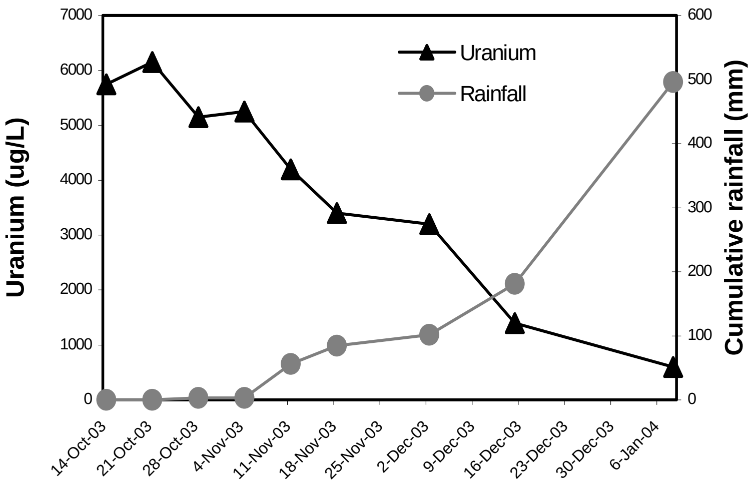


**Figure 1** Comparison of uranium concentration in Djalkmara Billabong with cumulative rainfall. Data supplied by J. Milsom and J. Kepui (ERA) and R. McAllister (oss).

## Quality assurance

*H. viridissima*

Physico-chemical data for the *H. viridissima* test are provided at Appendix 5. The pH, conductivity and DO remained within the prescribed limits. Good control growth (mean = 46 hydroids) and reproducibility (co-efficient of variation [CV] = 6.5%) were observed over the 96 h test period (Appendix 9), indicating test acceptability.

*M. macleayi*

Physico-chemical data for the *M. macleayi* test are provided at Appendix 6. With the exception of the control pH at the commencement of testing, the pH remained within the prescribed limits. Inspection of the pH values over the remainder of the test for the control and other treatments suggested the day 1 value represented a measurement error. Whilst some variability also was observed for conductivity and DO, in general they remained within the prescribed limits. Such varability is not uncommon in *M. macleayi* tests and can probably be attributed to the addition of food in the form of algae and FFV. Good control adult survival (100%), reproduction (mean = 36 offspring per adult) and reproducibility (CV = 5.1%) were observed over the 144 h (6 d) test period (Appendix 10), indicating test acceptability.

*M. mogurnda*

By day 3 of the test, control survival had dropped to 60%, well below the test acceptability criterion of 80%. Consequently, the test was terminated and no results were acquired for *M. mogurnda* larval survival. The excessive mortality was observed throughout all treatments and was due to the initial poor health and development of the larvae.

## Toxicity tests

The toxicity of the Djalkmara Billabong water sample tested in December 2003 is summarised in Table 1, with the concentration-response curves for *H. viridissima* and *M. macleayi* shown in Figures 2 and 3, respectively. The raw data and ToxCalc statistics reports are provided at Appendices 9 (*H. viridissima*) and 10 (*M. macleayi*). The order of sensitivity of the test species was *H. viridissima* > *M. macleayi*, which was opposite to the responses observed for the previous two years' pre-release toxicity testing programs (***eriss*** unpublished data 2002, Hogan 2003).

**Table 1** Summary results of the December 2003 pre-release toxicity tests for Djalkmara Billabong water

| Test | Date | Toxicity (% Djalkmara Billabong water) | | |
|---|---|---|---|---|
| | | NOEC | LOEC | $IC_{50}$ |
| *H. viridissima* population growth | 16-12-03 | 1 | 3 | >10 |
| *M. macleayi* reproduction | 17-12-03 | ≥10 | >10 | NC[1] |
| *M. mogurnda* survival | 17-12-03 | NR[2] | NR | NR |

[1] NC: Not calculable due to insufficient concentration-response relationship over the dilution range tested.

[2] NR: No result – test invalid due to higher than acceptable control mortality.

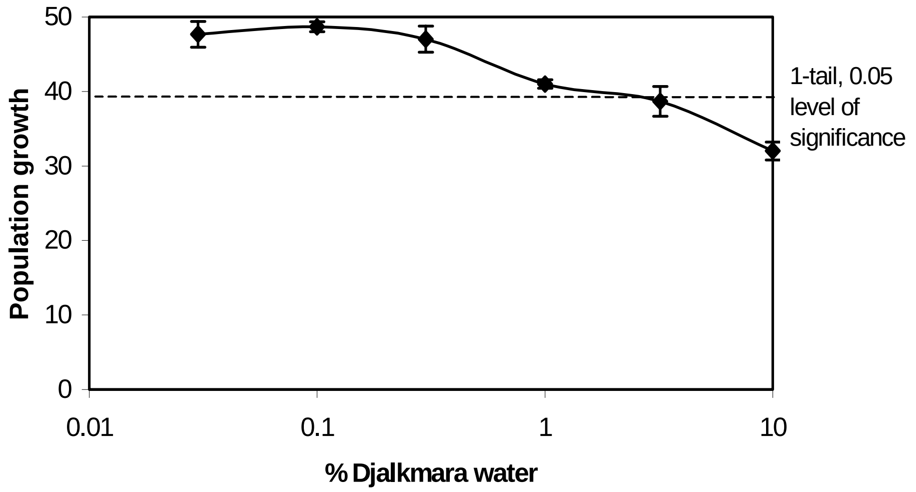


**Figure 2** Toxicity of Djalkmara Billabong water to *H. viridissima* in December 2003. Vertical bars represent the standard error of the mean (SEM). Control mean (±SEM) = 46 ± 2 hydroids.

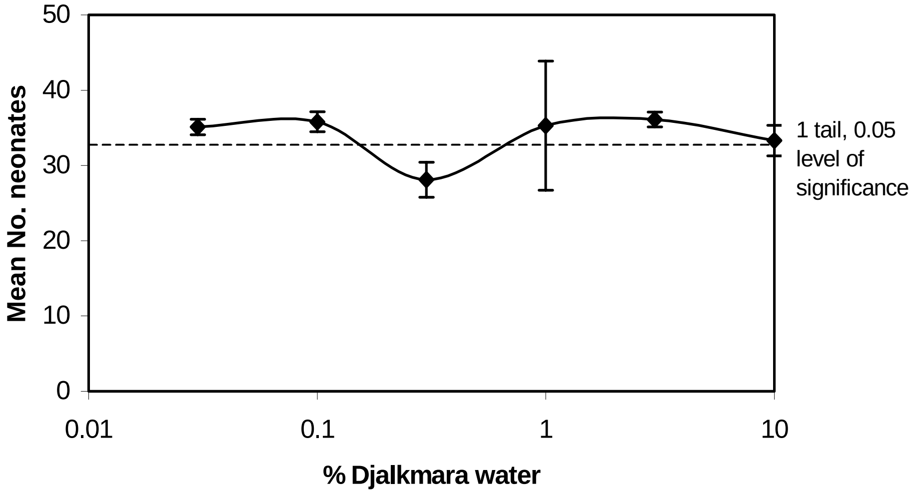


**Figure 3** Toxicity of Djalkmara Billabong water to *M. macleayi* in December 2003. Vertical bars represent the standard error of the mean (SEM). Control mean (±SEM) = 36 ± 1 neonates.

Djalkmara Billabong water was highly toxic to *H. viridissima*, with significant inhibition of population growth being observed at dilutions of 3% and above ($P < 0.05$; Figure 2). The significant effects at 3% and 10% Djalkmara Billabong water corresponded to a 16% and 30% inhibition of population growth, respectively (Figure 2). The U concentration of 45 µg/L in the 3% Djalkmara dilution was substantially lower than that reported to be toxic to *H. viridissima* (ie. ~200 µg/L; ARRRI 1988, Hyne et al 1992), suggesting that other contaminants may have contributed to the toxicity of the Djalkmara billabong sample. However, from the chemistry data at Appendix 2, it is unclear which other contaminants might have contributed to toxicity. For example, while the Mg concentration was greater than that reported to be toxic to *H. viridissima*, the Mg:Ca ratio of approximately 7:1 was within the range shown for *H. viridissima* to be protective of Mg toxicity (≤9:1; McCullough et al 2004).

In general, the sample was not as toxic to *M. macleayi* as was anticipated based on the expected concentration of uranium, with no significant effect observed up to the highest Djalkmara Billabong water dilution of 10% ($P > 0.05$; Figure 3). This was largely due to the actual concentration of uranium in the Djalkmara Billabong water sample being approximately only one third of what was expected based on the concentration reported by ERA of 4,200 µg/L. However, even when accounting for this discrepancy, the U concentration of 110 µg/L in the 10% Djalkmara dilution would have been expected to result in adverse effects based on the known toxicity of U to the cladoceran (ie. LOECs ~20-50 µg/L; Semaan et al 2001) and results of previous pre-release toxicity testing programs (eg. Hogan 2003). Consequently, a retrospective U reference test was undertaken for *M. macleayi* to determine whether the sensitivity of the culture to U had changed over time. The NOEC, LOEC and $IC_{50}$ of U to *M. macelayi* were 36.4, 72.5 and >144 µg/L U respectively, which compared reasonably well with previous data (Semaan et al 2001), suggesting that the response of *M. macleayi* was not due to a change in sensitivity to U.

## January 2004

### Chemistry

The pH, conductivity and dissolved organic carbon (DOC) concentration of the Djalkmara Billabong water were 7.59, 1074 µS/cm and 15 mg/L, respectively. The pH, conductivity, alkalinity and dissolved organic carbon (DOC) concentration of the Magela Creek water diluent were 6.21, 14 µS/cm, <5 mg/L (as $CaCO_3$) and 6 mg/L, respectively. The chemistry data for diluent (Magela Creek) water, Djalkmara Billabong water and test solutions used for the *H. viridissima* and *M. macleayi* tests are provided at Appendix 4. The substantial reduction in the concentrations of the key contaminants (ie. U, Mg, $SO_4$, Mn) compared to the previous sample is evident, and due to dilution from the high rainfall (~300 mm; James Kepui, ERA, pers comm.) at the mine site during this period (see Figure 1). Of particular note, U and Mn had decreased to around 600 and 260 µg/L, respectively.

### Quality assurance

*H. viridissima*

Physico-chemical data for the *H. viridissima* test are provided at Appendix 7. In general, the pH, conductivity and DO remained within the prescribed limits. Good control growth (mean = 38 hydroids) and reproducibility (CV = 9.1%) were observed over the 96 h test period (Appendix 11), indicating test acceptability.

*M. macleayi*

Physico-chemical data for the *M. macleayi* test are provided in Appendix 8. In general, the pH, conductivity and DO remained within the prescribed limits. Good control adult survival (100%), reproduction (mean = 40 offspring per adult) and reproducibility (CV = 5.4%) were observed over the 144 h (6 d) test period (Appendix 10), indicating test acceptability.

### Toxicity tests

The toxicity of the Djalkmara Billabong water sample tested in January 2004 is summarised in Table 2, with the concentration-response curves for *H. viridissima* and *M. macleayi* shown in Figures 4 and 5, respectively. The raw data and ToxCalc statistics reports are provided at Appendices 11 (*H. viridissima*) and 12 (*M. macleayi*). As expected, the toxicity of Djalkmara Billabong water to both species was lower at this time than in December.

Djalkmara Billabong water was not toxic to *H. viridissima* up to a concentration of 32% (Figure 4). The response of *H. viridissima* in this testing round was more characteristic of that observed in previous pre-release toxicity testing programs (eg. Hogan 2003) and based on its known sensitivity to U (ARRRI 1988, Hyne et al 1992).

Djalkmara Billabong water was moderately toxic to *M. macleayi*. Although the LOEC was calculated to be the highest concentration tested (ie. 32% Djalkmara Billabong water; $P < 0.05$), suggesting low toxicity, it represented an 80% reduction in offspring numbers (Figure 5). As has been observed in previous pre-release toxicity testing programs (***eriss*** unpublished data 2002, Hogan 2003), and is known to occur for several key contaminants including U (Semaan et al 2001) and Mg (McCullough et al, unpublished data), the reduction in the number of offspring per adult was primarily due to adult mortality during the experiment rather than an impairment of reproductive function *per se*. Compared to the December testing round, where a Djalkmara Billabong dilution containing 110 µg/L U (ie. the 10% dilution) caused no toxicity, a dilution containing 170 µg/L U in this testing round resulted in 100% adult mortality, and consequently, an 80% reduction in offspring numbers. As for *H. viridissima*, the response observed for *M. macleayi* in this testing round was more characteristic of what would be expected based upon existing knowledge.

## Recommendations for the release of Djalkmara Billabong water into Magela Creek

### December 2003

The December testing round was undertaken at a time when water level in Djalkmara Billabong was still very low and the major contaminants were highly concentrated. The need to release the water to Magela Creek before January was highly unlikely unless the mine site experienced the type of extreme rainfall that would normally be associated with a cyclonic event. Nevertheless, the testing was undertaken to ensure the availability of the necessary information in the (highly unlikely) event that release was required over the Christmas/New Year period, and prior to another round of testing of Djalkmara Billabong water more representative of that which would be released (ie. more dilute than the December sample).

**Table 2** Summary results of the January 2004 pre-release toxicity tests for Djalkmara Billabong water

| Test | Date | Toxicity (% Djalkmara Billabong water) | | |
|---|---|---|---|---|
| | | NOEC | LOEC | $IC_{50}$ |
| *H. viridissima* population growth | 10-01-04 | ≥32 | >32 | NC[1] |
| *M. macleayi* reproduction | 10-01-04 | 10 | 32 | 22 (19-28)[2] |

[1] NC: Not calculable due to insufficient concentration-response relationship over the dilution range tested.

[2] Values in parenthese represent 95% confidence limits (CLs).

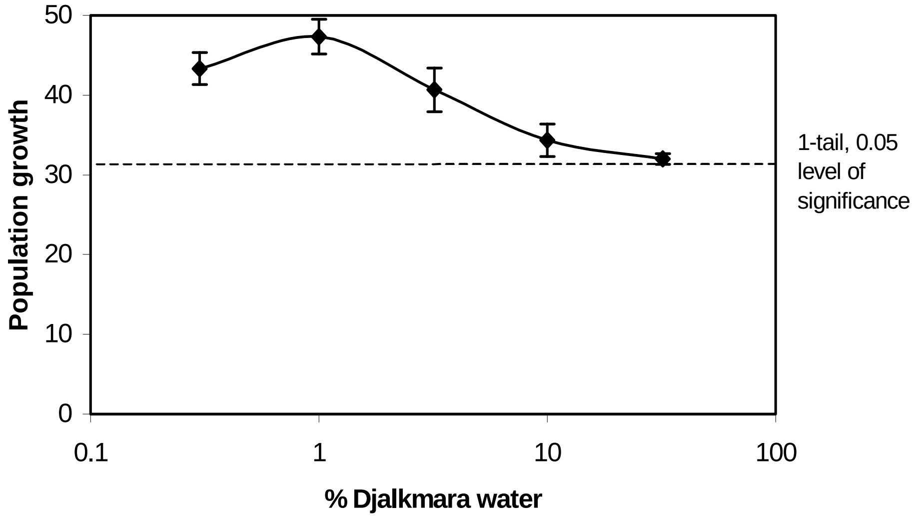


**Figure 4** Toxicity of Djalkmara Billabong water to *H. viridissima* in January 2004. Vertical bars represent the standard error of the mean (SEM). Control mean (±SEM) = 38 ± 2 hydroids.

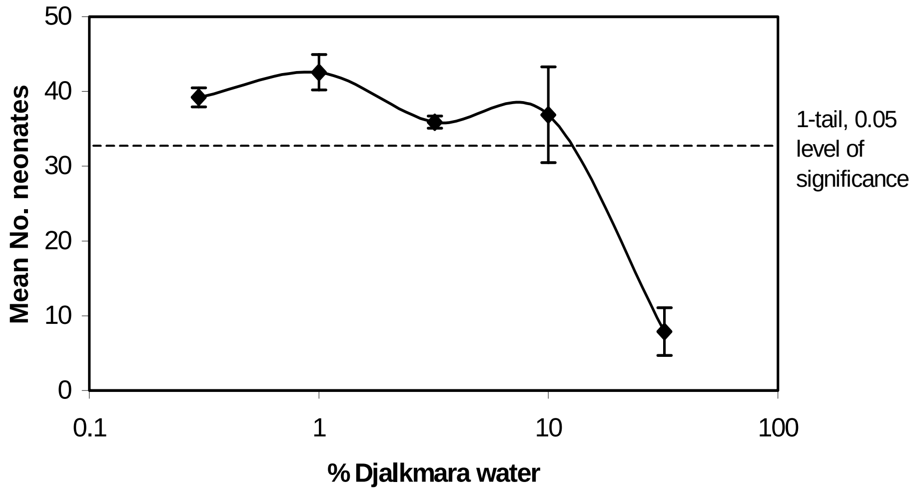


**Figure 5** Toxicity of Djalkmara Billabong water to *M. macleayi* in January 2004. Vertical bars represent the standard error of the mean (SEM). Control mean (±SEM) = 40 ± 1 neonates.

Due to the concentrated nature of the water in Djalkmara Billabong in December, it was acknowledged that the results would most likely overestimate the toxicity of water that might need to be released to Magela Creek prior to the second testing round, it having been greatly diluted by rainfall. Consequently, basing a 'safe' dilution rate on the toxicity of the 'whole effluent' would be inappropriately stringent. An alternative approach was agreed, based on the assumption that U represents the primary toxicant in Djalkmara Billabong water (see below for further discussion), whereby ERA could adjust for a 'safe' dilution of Djalkmara Billabong water based on the concentration of U at the 'safe' Djalkmara Billabong water dilution derived from the toxicity testing.

Based on the application of a 10× safety factor to the lowest NOEC of 1% Djalkmara Billabong water (for *H. viridissima*), a 'safe' dilution of 0.1%, or 1 in 1,000 was calculated. This corresponded to a U concentration of 1.3 μg/L, from which it was expected that ERA could back-calculate a 'safe' dilution depending on the concentration of U in Djalkmara Billabong water just prior to any need to release. However, in the context of natural background concentrations (which are typically in the order of 0.05 μg/L) and an over-arching goal of trying to minimise perturbations of mine site derived contaminants in the off-site environment, the Office of the Supervising Scientist (OSS) strongly encouraged ERA to set in place a management regime for the release of Djalkmara Billabong water that set 0.2 μg/L (ie. the current Focus level) as a target for water quality in Magela Creek (see Appendix 13).

#### January 2004

Based on the application of a 10× safety factor to the lowest NOEC of 10% Djalkmara Billabong water (for *M. macleayi*), a 'safe' dilution of 1%, or 1 in 100 was calculated. However, this corresponded to a U concentration of approximately 6 μg/L, slightly exceeding the current Limit of 5.8 μg/L for U in Magela Creek downstream of Ranger (ie. at MG0009). As with the December testing round, the OSS strongly encouraged ERA to set in place a management regime for the release of Djalkmara Billabong water that sets 0.2 μg/L (ie. the current Focus level) as a target for water quality in Magela (see Appendix 14).

### Temporal changes in toxicity of Djalkmara Billabong water

The toxicity of the December 2003 Djalkmara Billabong water sample was highly unusual in comparison to previous years, with *H. viridissima* exhibiting greater sensitivity than expected and *M. macleayi* much lower sensitivity than expected. There were some marked differences in the composition of the Djalkmara Billabong water collected on 15 December 2003 compared to the corresponding sampling date for the 2002 pre-release toxicity testing program (ie. 1.5 to 3-fold increases or decreases in contaminants such as U, Mg, $SO_4$ and Cr), and none more so than the 30-fold increase from 2002 to 2003 in the concentration of Mn, from around 30 to 900 μg/L. Mn concentrations of around 900 μg/L are briefly observed periodically in Djalkmara Billabong (as reported on ERA's LIMS database), although the ecotoxicological implications of this are unknown. Further, it cannot be determined whether or not any of the differences in Djalkmara Billabong water chemistry in the December 2003 sample resulted in or contributed to the unusual and constrasting responses observed for *H. viridissima* and *M. macleayi*.

In contrast, the toxicity of Djalkmara Billabong water sample collected in January 2004 did not appear to be markedly different to similar periods in previous years, with significant effects generally only being observed, if at all, at the highest dilution of 32% (ERA 2001, ***eriss*** unpublished data 2002, Hogan 2003). It is likely that the dilution of Djalkmara Billabong water due to Wet season rainfall during the months of December and January 'smooths out' any unusual chemistry that could have the potential for causing similarly unusual toxicity.

# 4 Summary and conclusions

The toxicity of Djalkmara Billabong water to three local freshwater species was assessed on two occasions over the 2003-04 Wet season.

In mid December 2003, Djalkmara Billabong water was found to be highly toxic to the green hydra, *H. viridissima*, with a LOEC and NOEC of 3.2% and 1% dilution, respectively, but non-toxic to the cladoceran, *M. macleayi*, up to the highest dilution tested, of 10%. A larval survival test using the purple-spotted gudgeon, *M. mogurnda*, failed due to excessive control mortality. By dividing the lowest NOEC (1%; *H. viridissima*) by a safety factor of 10, a 'safe' dilution of 0.1% (or 1 in 1,000) was calculated for release of Djalkmara Billabong water to Magela Creek. However, as this would have resulted in a U concentration of 1.3 µg/L in Magela Creek, substantially above background concentrations of *circa* 0.05 µg/L, ERA were strongly encouraged to set in place a management regime for the release of Djalkmara Billabong water that set 0.2 µg/L U as a target for water quality in Magela Creek.

In mid January 2004, Djalkmara Billabong water was found to be non-toxic to *H. viridissima* with a LOEC of >32% and moderately toxic to *M. macleayi*, with a LOEC and NOEC of 32% and 10%, respectively. *M. mogurnda* larvae were unavailable for testing during this round. By dividing the lowest NOEC (10%; *M. macleayi* ) by a safety factor of 10, a 'safe' dilution of 1% (or 1 in 100) was calculated for release of Djalkmara Billabong water to Magela Creek. However, this would have resulted in a U concentration of approximately 6 µg/L in Magela Creek, thus, exceeding the current Limit (5.8 µg/L). Consequently, ERA were again strongly encouraged to set in place a management regime for the release of Djalkmara Billabong water that set 0.2 µg/L U as a target for water quality in Magela Creek.

The December 2003 Djalkmara Billabong water sample was quite different in composition and toxicity to previous years. For this sample, *H. viridissima* exhibited greater than previously reported sensitivity and *M. macleayi* exhibited less than previously reported sensitivity. However, following dilution from rainfall and runoff, the January 2004 Djalkmara Billabong water sample exhibited comparable toxicity to previous years.

# 5 References


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## Appendix 1 Letter of Agreement between SSD and ERA for 2003-04 pre-release toxicity testing

Australian Government

Department of the Environment and Heritage

Supervising Scientist

**file ref:** SG2003/0180 19 November 2003

**doc name:**

Mr Chris Leiner
Manager, Environment, Safety and Health
ERA Ranger Mine
Locked Bag 1
Jabiru NT 0886
**cc:**

Dear Chris

**Re: Pre-Release Toxicity Testing of Djalkmara Billabong Water**

SSD will be undertaking pre-release toxicity testing of Djalkmara Billabong water in the 2003/04 wet season as has been the case in previous wet seasons to determine minimum dilution criteria for the release of this water to Magela Creek. We have prepared the attached document outlining the testing regime for your comment and to ensure that our advice meets ERA's operational needs. I hope to establish, by an exchange of letters, your agreement that the program outlined in the attachment meets ERA's needs, and also ensure that coordination between ERA and SSD required to implement the program is effective.

Please respond with comments on the attached (if any) by the end of November. This will allow us sufficient time to address your comments prior to the commencement of the program on 8 December.

Regards

Alex Zapantis
Assistant Secretary
OSS

**Appendix 1 (cont.)**

# Pre-release Toxicity Testing of Djalkmara Billabong Water

## Introduction

The Supervising Scientist Division (SSD) undertakes pre-release toxicity testing of Djalkmara Billabong Water and advises ERA of the conditions under which this water may be released to Magela Creek without causing harm to the environment. The rationale applied by SSD is based upon the ANZECC Water Quality Guidelines (ANZECC & ARMCANZ 2000). The purpose of this document is to briefly describe the toxicity testing process and the key components of the associated reporting and communication regime to ensure advice provided by SSD meets ERA's operational needs.

## Scope

SSD will:

- collect Djalkmara Billabong water and assess its toxicity in December 2003 using three local aquatic species;
- calculate and advise ERA of the minimum 'safe' dilution of Djalkmara Billabong water into Magela Creek;
- determine, in consultation with ERA considering relevant factors such as the results of the toxicity testing completed in December 2003, whether a second round of toxicity testing is necessary in January/February 2004 and if so, undertake a second round of testing;
- Provide a final report on the toxicity testing to ERA incorporating all relevant information, including background/context, aims, sampling and test details, QA/QC, data analysis, results and raw data, and discussion and recommendations.

## Description of the Service

### Water collection

Sufficient Djalkmara Billabong water (test sample) and Magela Creek water (from upstream of Ranger; for dilution water) for toxicity testing and chemical analysis will be collected by SSD staff, or by ERA staff accompanied by SSD staff on or as close as possible to 8 December 2003.

### Toxicity testing

SSD will conduct pre-release toxicity tests on Djalkmara Billabong water using the following toxicity test protocols:

- **BTT-B:** Green hydra (*Hydra viridissima*) 96 h population growth;
- **BTT-D:** Cladoceran (*Moinodaphnia macleayi*) 3-brood reproduction; and

- **BTT-E:** Purple-spotted gudgeon (*Mogurnda mogurnda*) 96 h sac-fry survival;

Details of these toxicity test protocols are provided in Riethmuller et al. (2003).

5-6 different dilutions of Djalkmara Billabong water (diluted in Magela Creek water) plus a Control treatment (ie. Magela Ck water only) will be assessed for toxicity. The dilutions will be determined such that they cover the critical range of dilutions over which effects in the test species are expected to become evident. The concentration of uranium in Djalkmara Billabong water (as advised by ERA), assumed to be the primary toxicant, will be used to target the critical range of dilutions.

Where possible, toxicity tests will be initiated within 48 h of collection of the Djalkmara Billabong water sample.

The following analytes will be measured for each of the specified dilutions:

- **Control & 100% Djalkmara Billabong water:** Al, Ca, Cd, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, Se, $SO_4$, U, Zn and Cl; and
- **All other dilutions:** Mg, Mn, $SO_4$ and U.

## Reporting

The test results will be expressed as follows:

- **Green hydra & cladoceran:** lowest-observed-effect concentration (LOEC), no-observed-effect concentration (NOEC) and median growth (for green hydra) and median reproductive (for cladoceran) inhibition concentration ($IC_{50}$); and
- **Purple-spotted gudgeon:** LOEC, NOEC and median lethal concentration ($LC_{50}$).

Using the results of the toxicity tests, a minimum dilution for release of Djalkmara Billabong water into Magela Creek will be calculated by applying a safety factor of 10 to the NOEC of the most sensitive species.

The summary results and recommended dilution will be reported to ERA by E-mail as soon as practicable following completion of the toxicity tests (ie. approximately 1 week after test commencement).

A final report will be provided that includes background/contextual information, project aims, sampling, test and statistical details, QA/QC, toxicity and chemistry results (including raw data), and discussion and recommendations in the context of previous years' results and the implications for water releases to Magela Creek.

In the event that no additional pre-release toxicity testing is necessary in January/February 2004, the final draft report will be provided to ERA for comment by the end of January 2004. If additional toxicity testing is undertaken, the final draft report will be provided to ERA on a date agreed between ERA and SSD.

## Communication

The Primary SSD contact is Rick van Dam. The Secondary SSD contact is Alex Zapantis. The Primary ERA contact is John Milsom. The Secondary ERA contact is Chris Leiner. In order to ensure appropriate coordination between SSD and ERA, SSD will liaise with ERA;

- at least one week prior to the agreed week of the testing program to arrange/confirm sample collection details and discuss/clarify any other relevant details;

- as soon as practicable during the toxicity testing to discuss any issues or problems that arise that may affect the outcomes of the project;
- as soon as practicable following completion of the toxicity tests to communicate the recommended minimum dilution for release of Djalkmara Billabong water into Magela Creek; and
- during the final reporting stage to clarify or discuss relevant project/reporting details.

ERA will liaise with SSD:

- at least one week prior to the agreed week of the testing program to confirm the concentration of uranium in Djalkmara Billabong water and to confirm or notify of any necessary delays or changes to the sample collection details;
- as necessary during the final reporting stage to clarify or discuss relevant project/reporting details; and
- following receipt of the final draft report to provide comments for report completion.

## References

ANZECC & ARMCANZ 2000. *Australian and New Zealand guidelines for fresh and marine water quality*. National Water Quality Management Strategy Paper No. 4, Australian and New Zealand Environment and Conservation Council and Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

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.

## Appendix 2  Water chemistry results for Djalkmara Billabong water diluted with Magela Creek water in the December 2003 *H. viridissima* test

| % Djalkmara Billabong Water | Element | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Al (µg/L) | Ca (mg/L) | Cl (mg/L) | Cd (µg/L) | Cr (µg/L) | Cu (µg/L) | Fe (µg/L) | Mg (mg/L) | Mn (µg/L) | Na (mg/L) | Ni (µg/L) | Pb (µg/L) | Se (µg/L) | $SO_4$ (mg/L) | U (µg/L) | Zn (µg/L) | DOC[2] (mg/L) |
| Blank | <0.1 | <0.1 | N/A[1] | <0.02 | <0.1 | N/A | <20 | <0.1 | 0.07 | 0.3 | <0.01 | <0.01 | <0.2 | <0.1 | <0.001 | <0.1 | N/A |
| 0 | 171 | 0.2 | 2.9 | 0.36 | 0.7 | 1.13 | 180 | 0.7 | 9.89 | 0.3 | 0.31 | 0.09 | <0.2 | 0.6 | 0.059 | 2.2 | 7 |
| 0.03 | N/A | 0.2 | N/A | N/A | N/A | 0.76 | N/A | 1 | 7.55 | N/A | N/A | N/A | N/A | 1.5 | 0.936 | 1.2 | N/A |
| 0.1 | N/A | 0.2 | N/A | N/A | N/A | 0.36 | N/A | 1.1 | 6.36 | N/A | N/A | N/A | N/A | 1.9 | 1.3 | 0.6 | N/A |
| 0.3 | N/A | 0.3 | N/A | N/A | N/A | 0.55 | N/A | 1.7 | 8.73 | N/A | N/A | N/A | N/A | 4 | 3.9 | 1 | N/A |
| 1.0 | N/A | 0.6 | N/A | N/A | N/A | 0.46 | N/A | 3.7 | 13.4 | N/A | N/A | N/A | N/A | 11.6 | 11.6 | 1 | N/A |
| 3.0 | N/A | 1.3 | N/A | N/A | N/A | 0.84 | N/A | 9.2 | 37.6 | NA | N/A | N/A | N/A | 32.7 | 45.2 | 1.3 | N/A |
| 10 | N/A | 3.9 | N/A | N/A | N/A | 0.6 | N/A | 29.8 | 84.3 | N/A | N/A | N/A | N/A | 107 | 121 | 1.3 | N/A |
| 100 | 10.9 | 37.2 | 10.4 | <0.02 | 3.9 | 0.49 | 220 | 287 | 912 | 22.1 | 0.36 | 0.06 | 4.6 | 1050 | 1350 | 6.0 | 22 |

[1] N/A: Not analysed

[2] DOC: dissolved organic carbon

## Appendix 3 Water chemistry results for Djalkmara Billabong water diluted with Magela Creek water in the December 2003 *M. macleayi* test

| % Djalkmara Billabong Water | Element | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Al (µg/L) | Ca (mg/L) | Cl (mg/L) | Cd (µg/L) | Cr (µg/L) | Cu (µg/L) | Fe (µg/L) | Mg (mg/L) | Mn (µg/L) | Na (mg/L) | Ni (µg/L) | Pb (µg/L) | Se (µg/L) | $SO_4$ (mg/L) | U (µg/L) | Zn (µg/L) | DOC[2] (mg/L) |
| Blank | N/A[1] | <0.1 | N/A | <0.02 | 0.1 | <0.01 | <20 | <0.1 | 0.01 | 0.3 | <0.01 | <0.01 | 0.4 | <0.1 | <0.001 | <0.1 | N/A |
| 0 | 96.4 | <0.1 | 4.1 | 0.04 | 0.1 | 0.24 | 80 | 0.3 | 6.26 | <0.1 | 0.22 | 0.05 | <0.2 | 0.4 | 0.049 | 3.2 | 7 |
| 0.03 | N/A | 0.2 | N/A | N/A | N/A | 0.31 | N/A | 0.8 | 6.32 | N/A | N/A | N/A | N/A | 1.1 | 0.409 | 0.9 | N/A |
| 0.1 | N/A | 0.2 | N/A | N/A | N/A | 1.62 | N/A | 1.1 | 8.08 | N/A | N/A | N/A | N/A | 1.8 | 1.46 | 1.7 | N/A |
| 0.3 | N/A | 0.3 | N/A | N/A | N/A | 0.22 | N/A | 1.6 | 8.26 | N/A | N/A | N/A | N/A | 3.8 | 3.67 | 0.7 | N/A |
| 1.0 | N/A | 0.6 | N/A | N/A | N/A | 0.49 | N/A | 3.7 | 22.7 | N/A | N/A | N/A | N/A | 11.1 | 18 | 0.7 | N/A |
| 3.0 | N/A | 1.3 | N/A | N/A | N/A | 0.87 | N/A | 9.1 | 41.8 | N/A | N/A | N/A | N/A | 32.2 | 43.2 | 1.7 | N/A |
| 10 | N/A | 3.9 | N/A | N/A | N/A | 0.25 | N/A | 29.2 | 74.7 | N/A | N/A | N/A | N/A | 105 | 110 | 12.9 | N/A |
| 100 | 10.9 | 37.2 | 10.4 | <0.02 | 3.9 | 0.49 | 220 | 287 | 912 | 22.1 | 0.36 | 0.06 | 4.6 | 1050 | 1350 | 6.0 | 22 |

[1] N/A: Not analysed

[2] DOC: dissolved organic carbon

## Appendix 4 Water chemistry results for Djalkmara Billabong water diluted with Magela Creek water in the January 2004 *H. viridissima* and *M. macleayi* tests

| % Djalkmara Billabong Water | Element | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | Al (µg/L) | Ca (mg/L) | Cl (mg/L) | Cd (µg/L) | Cr (µg/L) | Cu (µg/L) | Fe (µg/L) | Mg (mg/L) | Mn (µg/L) | Na (mg/L) | Ni (µg/L) | Pb (µg/L) | Se (µg/L) | $SO_4$ (mg/L) | U (µg/L) | Zn (µg/L) | DOC[2] (mg/L) |
| Blank | 2.9 | <0.1 | 2.1 | 0.04 | <0.1 | <0.01 | <20 | <0.1 | <0.01 | N/A* | <0.01 | 0.01 | <0.2 | <0.1 | <0.001 | <0.1 | N/A |
| 0 | 141 | 0.3 | 4.1 | 0.02 | 0.3 | 0.38 | 340 | 0.5 | 12.7 | 1.5 | 0.14 | 0.07 | <0.2 | 0.4 | 0.241 | 3.2 | 7 |
| 0.3 | N/A[1] | 0.4 | N/A* | N/A | N/A | 0.92 | N/A | 0.8 | 12.8 | N/A | N/A | N/A | N/A | <0.1 | 1.62 | 3.7 | N/A |
| 1.0 | N/A | 0.5 | N/A | N/A | N/A | 0.48 | N/A | 1.8 | 14.7 | N/A | N/A | N/A | N/A | 2.2 | 5.42 | 2.3 | N/A |
| 3.2 | N/A | 0.9 | N/A | N/A | N/A | 0.38 | N/A | 4.5 | 19.9 | N/A | N/A | N/A | N/A | 12.8 | 17 | 2.6 | N/A |
| 10 | N/A | 2 | N/A | N/A | N/A | 0.5 | N/A | 13.2 | 35.8 | N/A | N/A | N/A | N/A | 45.8 | 50.6 | 2 | N/A |
| 32 | N/A | 5.5 | N/A | N/A | N/A | 0.86 | N/A | 41.1 | 83.1 | N/A | N/A | N/A | N/A | 163 | 171 | 2.6 | N/A |
| 100 | 33 | 16.8 | 7.1 | <0.02 | 0.5 | 1.5 | 240 | 131 | 258 | 12.8 | 0.37 | 0.11 | 2.2 | 533 | 595 | 1.3 | 15 |

[1] N/A: Not analysed

[2] DOC: dissolved organic carbon

# Appendix 5 Physico-chemical data for the December 2003 *H. viridissima* test

| | | Djalkmara Billabong water concentration (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.03 | | 0.1 | | 0.3 | | 1.0 | | 3.0 | | 10.0 | |
| Day | 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 |
| 1 | pH | 6.06 | 6.47 | 6.55 | 6.62 | 6.40 | 6.47 | 6.44 | 6.42 | 6.56 | 6.56 | 6.89 | 6.79 | 7.31 | 7.21 |
| | Conductivity[1] | 16 | 19 | 18 | 23 | 19 | 23 | 26 | 30 | 46 | 49 | 104 | 108 | 280 | 293 |
| | DO[2] | 104.5 | 119.1 | 122.7 | 133.5 | 124.0 | 127.3 | 126.3 | 129.6 | 124.2 | 125.2 | 126.5 | 123.8 | 122.8 | 123.5 |
| 2 | pH | 6.39 | 6.59 | 6.54 | 6.70 | 6.40 | 6.51 | 6.43 | 6.55 | 6.56 | 6.50 | 6.90 | 6.79 | 7.31 | 7.36 |
| | Conductivity | 16 | 24 | 19 | 23 | 19 | 23 | 26 | 30 | 46 | 51 | 102 | 110 | 276 | 296 |
| | DO | 122.4 | 96.6 | 132.1 | 97.9 | 136.5 | 96.2 | 144.5 | 97.2 | 139.9 | 97.8 | 139.2 | 99.2 | 138.4 | 100.3 |
| 3 | pH | 6.08 | 6.48 | 6.50 | 6.57 | 6.37 | 6.46 | 6.38 | 6.48 | 6.49 | 6.48 | 7.07 | 6.60 | 7.36 | 6.94 |
| | Conductivity | 17 | 21 | 19 | 23 | 20 | 23 | 27 | 32 | 47 | 52 | 104 | 110 | 282 | 293 |
| | DO | 116.7 | 84.9 | 119.1 | 84.2 | 119.5 | 84.5 | 117.1 | 80.9 | 117.2 | 80.3 | 116.2 | 81.5 | 115.2 | 81.6 |
| 4 | pH | 6.25 | 7.12 | 6.46 | 6.96 | 6.36 | 6.77 | 6.40 | 6.73 | 6.51 | 6.29 | 6.83 | 7.06 | 7.21 | 6.89 |
| | Conductivity | 16 | 17 | 19 | 20 | 19 | 21 | 26 | 28 | 46 | 48 | 103 | 107 | 279 | 286 |
| | DO | 109.6 | 93.5 | 110.4 | 94.5 | 111.4 | 94.7 | 109.4 | 95.4 | 107.5 | 95.2 | 106.4 | 96.4 | 104.3 | 94.5 |

[1] Conductivity units are in µS/cm.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation.

# Appendix 6 Physico-chemical data for the December 2003 *M. macleayi* test

| | | Djalkmara Billabong water concentration (%) | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.03 | | 0.1 | | 0.3 | | 1.0 | | 3.0 | | 10.0 | |
| Day | 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 |
| 1 | pH | 4.94 | 7.14 | 6.30 | 6.78 | 6.39 | 6.96 | 6.43 | 7.04 | 6.55 | 6.94 | 6.82 | 7.31 | 7.11 | 7.61 |
| | Conductivity[1] | 25 | 20 | 18 | 19 | 22 | 24 | 27 | 30 | 48 | 49 | 105 | 116 | 279 | 297 |
| | DO[2] | 129.6 | 103.5 | 124.8 | 103.4 | 127.3 | 102.6 | 126.7 | 103.6 | 122.0 | 104.0 | 120.0 | 103.2 | 121.4 | 105.6 |
| 2 | pH | 6.62 | 7.30 | 6.34 | 7.07 | 6.44 | 7.17 | 6.47 | 7.29 | 6.61 | 7.32 | 6.84 | 7.44 | 7.16 | 7.68 |
| | Conductivity | 23 | 23 | 20 | 21 | 26 | 25 | 35 | 31 | 55 | 51 | 112 | 106 | 288 | 284 |
| | DO | 105.2 | 97.9 | 113.9 | 97.8 | 115.2 | 96.0 | 115.7 | 98.7 | 113.9 | 98.8 | 111.0 | 99.3 | 113.4 | 98.2 |
| 3 | pH | 6.02 | 6.69 | 6.31 | 6.76 | 6.36 | 6.77 | 6.44 | 6.76 | 6.53 | 6.93 | 6.77 | 7.13 | 7.10 | 7.57 |
| | Conductivity | 18 | 20 | 19 | 21 | 22 | 23 | 27 | 29 | 47 | 52 | 103 | 108 | 278 | 288 |
| | DO | 103.5 | 88.8 | 107.3 | 91.7 | 109.5 | 91.0 | 105.2 | 91.6 | 110.0 | 93.3 | 106.7 | 93.2 | 109.9 | 95.4 |
| 4 | pH | 6.22 | 7.61 | 6.31 | 6.87 | 6.39 | 7.03 | 6.46 | 7.10 | 6.58 | 7.09 | 6.80 | 7.24 | 7.20 | 7.61 |
| | Conductivity | 18 | 22 | 19 | 21 | 22 | 25 | 28 | 31 | 48 | 52 | 105 | 108 | 280 | 289 |
| | DO | 102.4 | 99.3 | 105.8 | 101.2 | 108.5 | 97.5 | 108.0 | 102.8 | 108.2 | 103.9 | 105.9 | 103.9 | 109.8 | 103.5 |
| 5 | pH | 6.05 | 6.83 | 6.31 | 6.95 | 6.28 | 6.79 | 6.41 | 6.62 | 6.46 | 6.75 | 6.75 | 6.61 | 7.12 | 7.09 |
| | Conductivity | 18 | 23 | 19 | 23 | 22 | 24 | 27 | 28 | 47 | 49 | 103 | 106 | 277 | 285 |
| | DO | 132.2 | 87.6 | 109.1 | 87.8 | 113.5 | 88.6 | 111.7 | 87.3 | 111.6 | 89.3 | 112.0 | 87.6 | 117.6 | 88.6 |
| 6 | pH | 6.08 | 6.35 | 6.50 | 6.70 | 6.43 | 6.78 | 6.46 | 6.88 | 6.53 | 6.87 | 6.87 | 7.12 | 7.19 | 7.58 |
| | Conductivity | 28 | 20 | 23 | 21 | 22 | 23 | 28 | 30 | 47 | 48 | 103 | 106 | 274 | 287 |
| | DO | 98.3 | 96.9 | 90.5 | 97.7 | 94.4 | 94.4 | 93.7 | 98.2 | 93.0 | 97.5 | 94.1 | 99.6 | 95.3 | 97.7 |

[1] Conductivity units are in µS/cm.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation.

# Appendix 7 Physico-chemical data for the January 2004 *H. viridissima* test

| | | Djalkmara Billabong water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.3 | | 1.0 | | 3.2 | | 10 | | 32 | |
| Day | 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 |
| 1 | pH | 5.93 | 6.09 | 6.28 | 6.93 | 6.31 | 6.86 | 6.48 | 6.85 | 6.77 | 7.12 | 7.28 | 7.60 |
| | Conductivity[1] | 14 | 21 | 18 | 25 | 29 | 34 | 60 | 65 | 149 | 156 | 401 | 410 |
| | DO[2] | 123.9 | 131.2 | 120.5 | 135.3 | 125.1 | 135.4 | 121.1 | 137.3 | 120.4 | 136.4 | 120.2 | 137.3 |
| 2 | pH | 6.50 | 6.40 | 6.56 | 6.92 | 6.40 | 8.87 | 6.56 | 7.20 | 6.82 | 7.02 | 7.45 | 7.16 |
| | Conductivity | 18 | 28 | 22 | 28 | 31 | 58 | 63 | 69 | 152 | 159 | 406 | 411 |
| | DO | 144.8 | 127.1 | 146.6 | 121.7 | 156.1 | 122.9 | 155.1 | 130.3 | 151.8 | 128.2 | 154.3 | 132.9 |
| 3 | pH | 6.57 | 6.65 | 6.52 | 6.62 | 6.43 | 6.64 | 6.52 | 6.74 | 6.92 | 7.00 | 7.40 | 7.41 |
| | Conductivity | 18 | 19 | 19 | 24 | 31 | 35 | 60 | 66 | 149 | 158 | 399 | 417 |
| | DO | 151.7 | 89.3 | 148.8 | 89.6 | 147.8 | 89.2 | 148.4 | 90.9 | 142.9 | 90.3 | 147.8 | 89.3 |
| 4 | pH | 5.97 | 6.57 | 6.27 | 6.92 | 6.30 | 6.58 | 6.46 | 6.99 | 6.79 | 7.20 | 7.25 | 7.66 |
| | Conductivity | 14 | 30 | 18 | 53 | 29 | 43 | 60 | 74 | 149 | 161 | 400 | 413 |
| | DO | 111.1 | 97.8 | 109.7 | 100.1 | 105.7 | 105.2 | 108.3 | 106.1 | 108.6 | 105.9 | 108.8 | 106.0 |

[1] Conductivity units are in µS/cm.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation.

# Appendix 8 Physico-chemical data for the *M. macleayi* January 2004 test

| | | Djalkmara Billabong water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.3 | | 1.0 | | 3.2 | | 10 | | 32 | |
| Day | 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 |
| 1 | pH | 6.25 | 6.52 | 6.55 | 7.20 | 6.59 | 7.08 | 6.74 | 7.48 | 6.96 | 7.61 | 7.47 | 7.95 |
| | Conductivity[1] | 16 | 20 | 21 | 29 | 31 | 36 | 62 | 71 | 150 | 162 | 402 | 419 |
| | DO[2] | 107.2 | 135.4 | 113.4 | 140.4 | 121.4 | 144.9 | 122.4 | 143.5 | 128.9 | 142.2 | 134.2 | 144.4 |
| 2 | pH | 6.57 | 7.18 | 6.70 | 7.08 | 6.65 | 6.66 | 6.77 | 7.11 | 6.98 | 7.01 | 7.47 | 7.69 |
| | Conductivity | 17 | 22 | 22 | 23 | 32 | 33 | 66 | 65 | 154 | 156 | 408 | 409 |
| | DO | 154.5 | 141.1 | 149.6 | 141.8 | 153.8 | 142.7 | 152.7 | 143.1 | 144.9 | 142.1 | 155.4 | 143 |
| 3 | pH | 6.31 | 6.36 | 6.57 | 6.56 | 6.77 | 6.67 | 6.73 | 7.06 | 7.09 | 7.31 | 7.42 | 7.86 |
| | Conductivity | 20 | 17 | 25 | 20 | 40 | 32 | 67 | 64 | 165 | 157 | 402 | 411 |
| | DO | 152.3 | 92.7 | 147.9 | 98.3 | 149.2 | 99.2 | 148.1 | 99.9 | 151.7 | 96.6 | 151.8 | 98.8 |
| 4 | pH | 6.50 | 6.89 | 6.51 | 7.06 | 6.53 | 7.07 | 6.62 | 7.09 | 6.87 | 7.26 | 7.30 | 7.87 |
| | Conductivity | 16 | 21 | 21 | 29 | 31 | 39 | 62 | 70 | 149 | 159 | 402 | 421 |
| | DO | 110.9 | 107.8 | 110.8 | 105.3 | 107.4 | 105.7 | 111.0 | 103.6 | 109.4 | 107.2 | 109.6 | 107.2 |
| 5 | pH | 6.43 | 7.02 | 6.52 | N/A[3] | 6.61 | 7.08 | 6.64 | 7.10 | 7.05 | 7.29 | 7.55 | N/A |
| | Conductivity | 25 | 26 | 24 | N/A | 35 | 38 | 66 | 74 | 153 | 161 | 402 | N/A |
| | DO | 113.2 | 93.6 | 106.3 | N/A | 106.5 | 96.9 | 108.3 | 99.1 | 110.1 | 99.0 | 110.5 | N/A |
| 6 | pH | 6.38 | 6.46 | 6.57 | 6.54 | 6.32 | 6.69 | 6.52 | 6.73 | 6.98 | 7.12 | 7.44 | 7.68 |
| | Conductivity | 22 | 28 | 33 | 23 | 38 | 33 | 65 | 65 | 155 | 157 | 408 | 425 |
| | DO | 109.6 | 96.5 | 101.0 | 93.6 | 106.0 | 96.3 | 103.4 | 96.8 | 110.6 | 99.7 | 104.7 | 100.3 |

[1] Conductivity units are in µS/cm.

[2] DO: Dissolved oxygen. Measurements are expressed as percent saturation.

[3] N/a: Not analysed; samples were accidently discarded before parameters were measured

# Appendix 9 Toxcalc statistical summary for the December 2003 *H. viridissima* test

**Hydra population growth**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 16/12/2003 | Test ID: | 641B | Sample ID: | Djalkmara water |
| End Date: | 20/12/2003 | Lab ID: | eriss-ecotoxicology | Sample Type: | Mine water |
| Sample Date: | | Protocol: | BTT B | Test Species: | *Hydra viridissima* |
| Comments: | Analysed using no. of hydra | | | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| Control | 4.6E+07 | 4.3E+07 | 4.9E+07 |
| 0.03 | 4.7E+07 | 4.7E+07 | 4.9E+07 |
| 0.1 | 4.6E+07 | 4.8E+07 | 5.2E+07 |
| 0.3 | 4.7E+07 | 4.6E+07 | 4.8E+07 |
| 1 | 4.3E+07 | 4.3E+07 | 3.7E+07 |
| 3 | 4E+07 | 3.6E+07 | 4E+07 |
| 10 | 2.9E+07 | 2.8E+07 | 3.9E+07 |

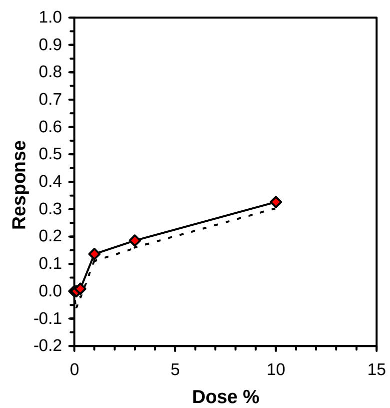
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control | 4.6E+07 | 1.0000 | 4.6E+07 | 4.3E+07 | 4.9E+07 | 6.522 | 3 | | | | 4.7E+07 | 1.0000 |
| 0.03 | 4.8E+07 | 1.0362 | 4.8E+07 | 4.7E+07 | 4.9E+07 | 2.422 | 3 | -0.624 | 2.530 | 6761709 | 4.7E+07 | 1.0000 |
| 0.1 | 4.9E+07 | 1.0580 | 4.9E+07 | 4.6E+07 | 5.2E+07 | 6.278 | 3 | -0.998 | 2.530 | 6761709 | 4.7E+07 | 1.0000 |
| 0.3 | 4.7E+07 | 1.0217 | 4.7E+07 | 4.6E+07 | 4.8E+07 | 2.128 | 3 | -0.374 | 2.530 | 6761709 | 4.7E+07 | 0.9906 |
| 1 | 4.1E+07 | 0.8913 | 4.1E+07 | 3.7E+07 | 4.3E+07 | 8.449 | 3 | 1.871 | 2.530 | 6761709 | 4.1E+07 | 0.8642 |
| *3 | 3.9E+07 | 0.8406 | 3.9E+07 | 3.6E+07 | 4E+07 | 5.973 | 3 | 2.744 | 2.530 | 6761709 | 3.9E+07 | 0.8150 |
| *10 | 3.2E+07 | 0.6957 | 3.2E+07 | 2.8E+07 | 3.9E+07 | 19.009 | 3 | 5.238 | 2.530 | 6761709 | 3.2E+07 | 0.6745 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.9505 | 0.873 | 0.56323 | 0.6035 |
| Bartlett's Test indicates equal variances (p = 0.33) | 6.90941 | 16.8119 | | |

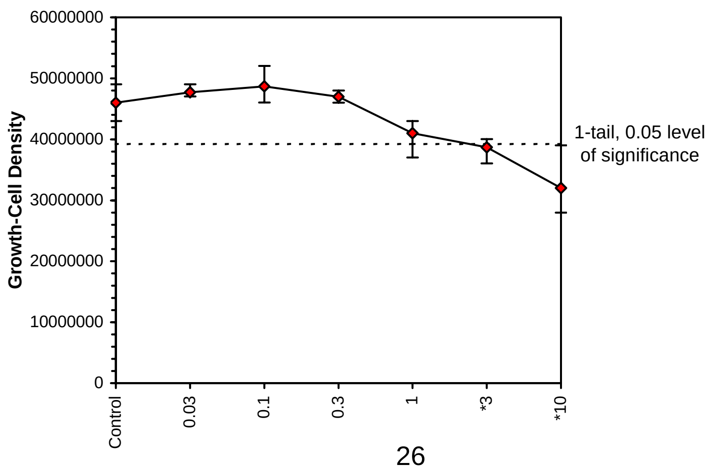
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 1 | 3 | 1.73205 | 100 | 6761709 | 0.14699 | 1.1E+14 | 1.1E+13 | 1.8E-04 | 6, 14 |
| Treatments vs Control | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 0.5249 | 0.1048 | 0.1548 | 0.9510 | -0.0020 |
| IC10 | 0.8017 | 0.2287 | 0.3413 | 2.1991 | 1.3942 |
| IC15 | 1.5762 | 0.7818 | 0.0000 | 5.2263 | 0.6150 |
| IC20 | 3.7467 | | | | |
| IC25 | 6.2375 | | | | |
| IC40 | >10 | | | | |
| IC50 | >10 | | | | |




**Dose-Response Plot**



# Appendix 10 Toxcalc statistical summary for the December 2003 *M. macleayi* test

**Moinodaphnia reproduction test**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 17/12/2003 | Test ID: | 642D | Sample ID: | Djalkmara water |
| End Date: | 23/12/2003 | Lab ID: | eriss-ecotoxicology | Sample Type: | Mine water |
| Sample Date: | | Protocol: | BTT D | Test Species: | *Moinodaphnia macleayi* |
| Comments: | | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 35.000 | 35.000 | 37.000 | 34.000 | 40.000 | 35.000 | 35.000 | 36.000 | 37.000 | 34.000 |
| 0.03 | 38.000 | 35.000 | 32.000 | 37.000 | 35.000 | 35.000 | 37.000 | 37.000 | 34.000 | 31.000 |
| 0.1 | 33.000 | 38.000 | 26.000 | 38.000 | 38.000 | 38.000 | 40.000 | 34.000 | 37.000 | 36.000 |
| 0.3 | 34.000 | 36.000 | 34.000 | 36.000 | 36.000 | 33.000 | 0.000 | 0.000 | 36.000 | 36.000 |
| 1 | 35.000 | 35.000 | 36.000 | 37.000 | 37.000 | 33.000 | 37.000 | 36.000 | 35.000 | 32.000 |
| 3.2 | 40.000 | 29.000 | 38.000 | 38.000 | 40.000 | 36.000 | 32.000 | 35.000 | 35.000 | 38.000 |
| 10 | 36.000 | 36.000 | 34.000 | 34.000 | 24.000 | 35.000 | 34.000 | 34.000 | 33.000 | 33.000 |

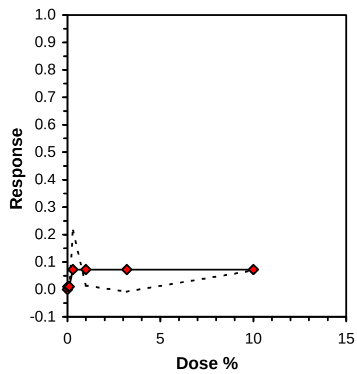
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control | 35.800 | 1.0000 | 35.800 | 34.000 | 40.000 | 5.066 | 10 | | | | 35.800 | 1.0000 |
| 0.03 | 35.100 | 0.9804 | 35.100 | 31.000 | 38.000 | 6.504 | 10 | 0.251 | 2.347 | 6.546 | 35.450 | 0.9902 |
| 0.1 | 35.800 | 1.0000 | 35.800 | 26.000 | 40.000 | 11.235 | 10 | 0.000 | 2.347 | 6.546 | 35.450 | 0.9902 |
| *0.3 | 28.100 | 0.7849 | 28.100 | 0.000 | 36.000 | 52.850 | 10 | 2.761 | 2.347 | 6.546 | 33.200 | 0.9274 |
| 1 | 35.300 | 0.9860 | 35.300 | 32.000 | 37.000 | 4.824 | 10 | 0.179 | 2.347 | 6.546 | 33.200 | 0.9274 |
| 3.2 | 36.100 | 1.0084 | 36.100 | 29.000 | 40.000 | 9.724 | 10 | -0.108 | 2.347 | 6.546 | 33.200 | 0.9274 |
| 10 | 33.300 | 0.9302 | 33.300 | 24.000 | 36.000 | 10.311 | 10 | 0.896 | 2.347 | 6.546 | 33.200 | 0.9274 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | 1.91809 | 1.035 | -3.0534 | 13.0534 |
| Bartlett's Test indicates unequal variances (p = 6.41E-14) | 73.9192 | 16.8119 | | |

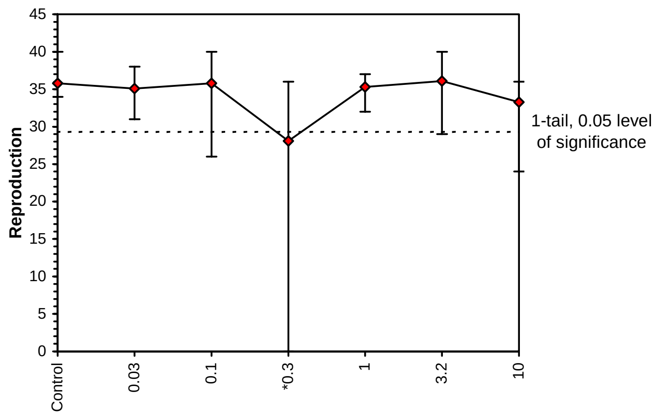
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 10 | >10 | | 10 | 6.546 | 0.18285 | 81.281 | 38.8905 | 0.06682 | 6, 63 |
| Treatments vs Control | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL | Skew |
|---|---|---|---|---|
| IC05 | 0.2280 | | | |
| IC10 | >10 | | | |
| IC15 | >10 | | | |
| IC20 | >10 | | | |
| IC25 | >10 | | | |
| IC40 | >10 | | | |
| IC50 | >10 | | | |




**Dose-Response Plot**



# Appendix 11 Toxcalc statistical summary for the January 2004 *H. viridissima* test

**Hydra population growth test**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 10/01/2004 | Test ID: | 644B | Sample ID: | DJALKMARA |
| End Date: | 14/01/2004 | Lab ID: | ERISS ECOT | Sample Type: | MINE WATER |
| Sample Date: | | Protocol: | BTT B | Test Species: | *Hydra viridissima* |
| Comments: | | | | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| Control | 3.6E+07 | 4.2E+07 | 3.6E+07 |
| 0.3 | 3.9E+07 | 4.6E+07 | 4.5E+07 |
| 1 | 4.9E+07 | 5.1E+07 | 4.2E+07 |
| 3.2 | 4.1E+07 | 4.4E+07 | 3.7E+07 |
| 10 | 3.5E+07 | 3.5E+07 | 3.3E+07 |
| 32 | 3.2E+07 | 3.1E+07 | 3.3E+07 |

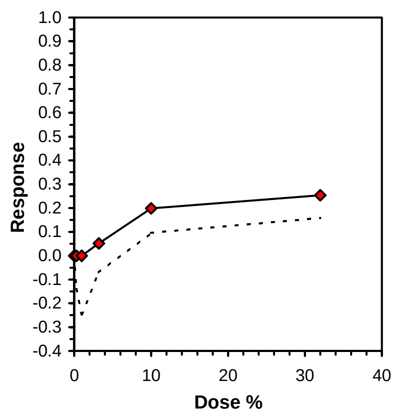
| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control | 3.8E+07 | 1.0000 | 3.8E+07 | 3.6E+07 | 4.2E+07 | 9.116 | 3 | | | | 4.3E+07 | 1.0000 |
| 0.3 | 4.3E+07 | 1.1404 | 4.3E+07 | 3.9E+07 | 4.6E+07 | 8.737 | 3 | -2.010 | 2.500 | 6631854 | 4.3E+07 | 1.0000 |
| 1 | 4.7E+07 | 1.2456 | 4.7E+07 | 4.2E+07 | 5.1E+07 | 9.984 | 3 | -3.518 | 2.500 | 6631854 | 4.3E+07 | 1.0000 |
| 3.2 | 4.1E+07 | 1.0702 | 4.1E+07 | 3.7E+07 | 4.4E+07 | 8.636 | 3 | -1.005 | 2.500 | 6631854 | 4.1E+07 | 0.9482 |
| 10 | 3.4E+07 | 0.9035 | 3.4E+07 | 3.3E+07 | 3.5E+07 | 3.363 | 3 | 1.382 | 2.500 | 6631854 | 3.4E+07 | 0.8005 |
| 32 | 3.2E+07 | 0.8421 | 3.2E+07 | 3.1E+07 | 3.3E+07 | 3.125 | 3 | 2.262 | 2.500 | 6631854 | 3.2E+07 | 0.7461 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.96268 | 0.858 | -0.3955 | -0.5834 |
| Bartlett's Test indicates equal variances (p = 0.40) | 5.16784 | 15.0863 | | |

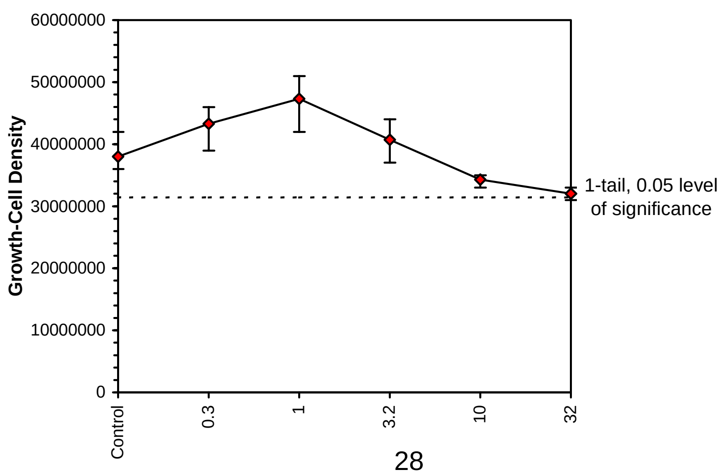
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test | 32 | >32 | | 3.125 | 6631854 | 0.17452 | 9.7E+13 | 1.1E+13 | 8.5E-04 | 5, 12 |
| Treatments vs Control | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 3.123 | 1.055 | 0.578 | 7.345 | 0.5120 |
| IC10 | 5.419 | 1.333 | 0.000 | 9.242 | -0.1538 |
| IC15 | 7.721 | 1.285 | 2.852 | 12.140 | 0.7800 |
| IC20 | 10.210 | | | | |
| IC25 | 30.429 | | | | |
| IC40 | >32 | | | | |
| IC50 | >32 | | | | |




**Dose-Response Plot**



# Appendix 12 Toxcalc statistical summary for the January 2004 *M. macleayi* test

**Moinodaphnia reproduction test**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 10/01/2004 | Test ID: | 645D | Sample ID: | Djalkmara water |
| End Date: | 16/01/2004 | Lab ID: | eriss-ecotoxicology | Sample Type: | Mine water |
| Sample Date: | | Protocol: | BTT D | Test Species: | *Moinodaphnia macleayi* |
| Comments: | | | | | |

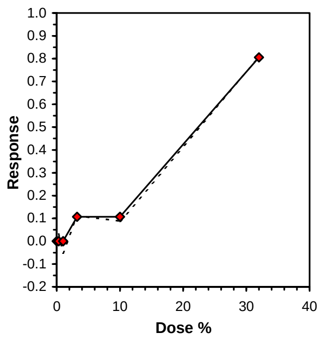
| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 42.000 | 43.000 | 41.000 | 40.000 | 40.000 | 36.000 | 40.000 | 39.000 | 43.000 | |
| 0.3 | 38.000 | 44.000 | 40.000 | 38.000 | 29.000 | 40.000 | 42.000 | 41.000 | 42.000 | 38.000 |
| 1 | 42.000 | 42.000 | 42.000 | 41.000 | 42.000 | 44.000 | 44.000 | 45.000 | 41.000 | |
| 3.2 | 42.000 | 25.000 | 42.000 | 8.000 | 41.000 | 42.000 | 41.000 | 40.000 | 41.000 | 37.000 |
| 10 | 41.000 | 25.000 | 35.000 | 37.000 | 42.000 | 39.000 | 41.000 | 35.000 | | |
| 32 | 9.000 | 40.000 | 0.000 | 0.000 | 22.000 | 0.000 | 0.000 | 0.000 | 0.000 | |

| | | | Transform: Untransformed | | | | | | 1-Tailed | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control | 40.444 | 1.0000 | 40.444 | 36.000 | 43.000 | 5.404 | 9 | | | | 40.733 | 1.0000 |
| 0.3 | 39.200 | 0.9692 | 39.200 | 29.000 | 44.000 | 10.470 | 10 | 0.339 | 2.293 | 8.420 | 40.733 | 1.0000 |
| 1 | 42.556 | 1.0522 | 42.556 | 41.000 | 45.000 | 3.346 | 9 | -0.560 | 2.293 | 8.639 | 40.733 | 1.0000 |
| 3.2 | 35.900 | 0.8876 | 35.900 | 8.000 | 42.000 | 30.864 | 10 | 1.238 | 2.293 | 8.420 | 36.388 | 0.8933 |
| 10 | 36.875 | 0.9117 | 36.875 | 25.000 | 42.000 | 14.955 | 8 | 0.919 | 2.293 | 8.905 | 36.388 | 0.8933 |
| *32 | 7.889 | 0.1951 | 7.889 | 0.000 | 40.000 | 179.540 | 9 | 8.643 | 2.293 | 8.639 | 7.889 | 0.1937 |

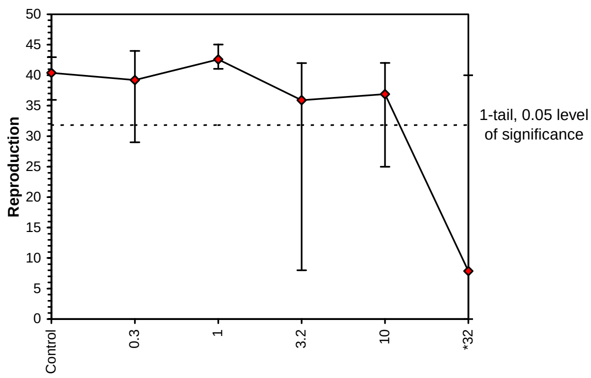
| Auxiliary Tests | | | | | Statistic | | Critical | | | Skew | Kurt |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | | | | | 1.52457 | | 1.035 | | | 0.38184 | 7.83564 |
| Bartlett's Test indicates unequal variances (p = 3.28E-09) | | | | | 48.1695 | | 15.0863 | | | | |
| **Hypothesis Test (1-tail, 0.05)** | **NOEC** | **LOEC** | **ChV** | **TU** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** | |
| Dunnett's Test | 10 | 32 | 17.8885 | 10 | 8.63915 | 0.21361 | 1508.13 | 63.8512 | 5.3E-12 | 5, 49 | |
| Treatments vs Control | | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05 | 2.031 | 2.357 | 1.431 | 10.219 | 2.1186 |
| IC10 | 3.062 | 3.690 | 2.050 | 11.772 | 0.1666 |
| IC15 | 11.362 | 3.295 | 2.614 | 13.252 | -1.3315 |
| IC20 | 12.934 | 2.416 | 3.154 | 15.558 | -2.3017 |
| IC25 | 14.506 | 1.760 | 11.500 | 18.213 | -0.6888 |
| IC40 | 19.223 | | | | |
| IC50 | 22.368 | | | | |




**Dose-Response Plot**



# Appendix 13 Interim reporting of December 2003 pre-release toxicity testing results by OSS to ERA

| | |
|---|---|
| **From:** | Alex Zapantis |
| **To:** | Chris.Leiner@era.riotinto.com; john.millsom@era.riotinto.com |
| **Date:** | 12/24/03 11:09am |
| **Subject:** | Djalkmara Toxicity Testing |

Hello Chris

We have completed the Toxicity Testing of Djalkmara Billabong water. The full report of the tests is planned for completion in March 2004, however a summary is presented below.

Djalkmara Billabong water and Magela Creek water (the diluent) was collected on 15 December 2003. A sample of the Djalkmara water was sent to NTEL for chemical (ICPMS) analysis. Of note was the uranium concentration of 1350ppb and the manganese concentration of 912ppb. Previous analyses of samples of Djalkmara water taken by ERA had returned uranium concentrations of approximately 4200ppb and a similar concentration was expected. The manganese concentration is significantly higher than last year. Also, Ca, Mg and SO4 concentrations are higher this year than in the Dec 2002 sample and the U conc is approximately half that of last year, indicating a different chemical composition for Djalkmara Billabong water this year.

Gudgeons, hydra, and cladocera were exposed to a range of dilutions of Djalkmara Billabong water in Magela Creek water (control (0%), 0.03%, 0.1%, 0.3%, 1%, 3%, 10%). The Gudgeon test commenced on 17 December but was declared invalid due to an unacceptable number of deaths in the control. The Cladoceran test commenced on 17 December and was completed on 23 December however no response at any dilution up to and including the minimum dilution of 10% Djalkmara water in Magela water was observed. The hydra test commenced on 16 December, was completed on 20 December with a NOEC of 1% Djalkmara water in Magela water observed.

Based upon the NOEC observed for hydra, the dilution required for release of Djalkmara Billabong water is 0.1% or 1 in 1000 (ie, NOEC/10). However, the release of Djalkmara water at a dilution of 1:1000 will result in a uranium concentration at 009 of approximately 1ppb given a concentration of uranium in Djalkmara water of 1350ppb. You will recall that there was considerable discussion between OSS and ERA in February 2003 when release at the minimum dilution required by the toxicity tests would have resulted in a similar downstream uranium concentration. As we did in February 2003, we strongly encourage ERA to set in place a managment regime for the release of Djalkmara water that sets 0.2ppb as a target for water quality in Magela. To ERA's credit, this target was achieved during the 2002/03 wet season.

ERISS will undertake another round of toxicity tests on Djalkmara Billabong water after the new year with results expected by 19 January.

Please do not hesitate to call me if you have any questions or concerns.

Merry Christmas

Alex Zapantis
Assistant Secretary
Office of the Supervising Scientist
Ph: int+ 61 8 8920 1102
Fax: int+ 61 8 8920 1190
email: alex.zapantis@deh.gov.au

**CC:** Alan.Hughes@nt.gov.au

# Appendix 14 Interim reporting of January 2004 pre-release toxicity testing results by OSS to ERA

**From:** Alex Zapantis
**To:** Chris.Leiner@era.riotinto.com; john.milsom@era.riotinto.com
**Date:** 1/16/04 3:50pm
**Subject:** Pre-release Tox testing results

Hello Chris

We have completed the 2nd Round pre-release toxicity testing for Djalkmara billabong water.
Summarised results as follows:

Hydra: LOEC 32%; NOEC 10%.
Cladoceran: LOEC 32%; NOEC 10%
Gudgeon: No test - larvae hatched early and were not fully developed

Thus, lowest NOEC was 10%, divided by a safety factor of 10, arrives at a minimum dilution of 1% Djalkmara billabong water (ie 1 in 100).

Major water chemistry for Djalkmara water (collected 8th Jan) was as follows:
U - 595 micrograms/L (this corresponds well with ERA's 5th Jan measurement of 586 micrograms/L).
Mn - 258 micrograms/L
Mg - 131 mg/L
SO4 - 533 mg/L

However, the release of Djalkmara water at a dilution of 1:100 will result in a uranium concentration at 009 of approximately 6ppb, exceeding the limit, given a concentration of uranium in Djalkmara water of 595ppb. You will recall that there was considerable discussion between OSS and ERA in February 2003 when release at the minimum dilution required by the toxicity tests would have resulted in a uranium concentration of approximately 1ppb. As we did in February 2003, we strongly encourage ERA to set in place a managment regime for the release of Djalkmara water that sets 0.2ppb as a target for water quality in Magela. To ERA's credit, this target was achieved during the 2002/03 wet season.

Please do not hesitate to call if you want to discuss.

regards

Alex Zapantis
Assistant Secretary
Office of the Supervising Scientist
Ph: int+ 61 8 8920 1102
Fax: int+ 61 8 8920 1190
email: alex.zapantis@deh.gov.au

**CC:** Alan.Hughes@nt.gov.au; VanDam, Rick; Waggitt, Peter