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Toxicity of Djalkmara Billabong water to local aquatic organisms: Pre-release biological testing for the 2002–2003 Wet season

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Toxicity of Djalkmara Billabong water to local aquatic organisms: Pre-release biological testing for the 2002-2003 wet season

Alicia Hogan

Introduction

Djalkmara Billabong is a natural water body located on the Ranger Uranium Mine site. It plays an important role in mine water management as it is used as a reservoir for low-level contaminated water and natural run-off prior to release into Magela Creek. During the wet season each year, toxicity tests using local aquatic species are undertaken on billabong water due for release. The results of these tests allow for the calculation of safe dilution levels to ensure the protection of downstream ecosystems.

Tests protocols using the local species *Moinodaphnia macleayi* (Cladoceran), *Hydra viridissima* (green hydra) and *Mogurnda mogurnda* (purple spotted gudgeon), were developed at ***eriss*** for this purpose, and have been used in recent years to assess release water toxicity (Allison et al 1991, McBride et al 1991, Markich & Camilleri 1997). An embryo mortality test using *Melanotaenia splendida inornata* was developed and used routinely from 1996–1999 by Ranger environmental staff.

Safe dilution concentrations are calculated by taking the No-Observed-Effect-Concentration (NOEC) of the most sensitive species tested and dividing this by a safety factor of 10, as recommended in the ANZECC/ARMCANZ Water Quality Guidelines (2000).

The relative sensitivities of the species used in pre-release testing to Ranger mine release water has only been determined in more recent years since significant toxic responses have been observed in testing. From 1996–1999, RP4 water exhibited no toxic effect on all species tested, except for a 1996 *M. splendida inornata* test where a NOEC of 1% and and a Lowest-Observed-Effect-Concentration (LOEC) of 3.2% were obtained (Appendix 1). During the 2000–2001 and 2001–2002 wet seasons, *M. macleayi* was found to be more sensitive than *H. viridissima* and *M. mogurnda* to both Djalkmara Billabong and Retention Pond #1 water (ERA 2001, ***eriss*** 2002 unpubl.; summarised in Appendix 1).

The sensitivity of each species to single mine contaminants has been tested also. *M. macleayi* was the most sensitive of the three species to uranium, with NOECs ranging from 8–31 μg/L U and LOECs from 20– 49 μg/L U (Semaan et al 2001). *H. viridissima* has been shown to be the next most sensitive, giving LOECs of 160 and 194 μg/L U (Allison and Holdway 1988) while *M. mogurnda* was relatively far less sensitive with Median Lethal Concentrations (LC50) of 1265–1665 μg/L U (Bywater et al 1991) and 1790–3750 μg/L U (Holdway 1992).

For magnesium, *H. viridissima* has been shown to be the most sensitive (NOEC = 2.2 mg/L Mg) followed by *M. macleayi* (10.2 mg/L Mg) and *M. mogurnda* (25.2 mg/L Mg) (McCullough et al 2003). Further laboratory work with *H. viridissima* has shown that Ca will ameliorate the toxicity of Mg as long as the Mg:Ca ratio remains at 10:1 or below (McCullough et al 2003). Mg:Ca ratios at the 009 sampling point downstream of Ranger mine have not exceeded a ratio of 3.6:1 over a 20 year period (ERA, unpubl. data) indicating that Mg toxicity in Ranger release water is likely to be ameliorated by Ca. Further work with other species to verify this finding is still underway (C. McCullough pers. comm.).

Pre-release toxicity tests had been undertaken by the Environmental Division at Ranger since the mid 1990s (van Dam et al 2002). In 2001, Energy Resources of Australia outsourced many of their laboratory based activities. The ecotoxicology laboratory at ***eriss*** has since been committed to conducting the annual pre-release toxicity testing on Djalkmara Billabong water. It is likely that this will continue until the 2003–2004 wet season, after which time it is expected that Djalkmara Billabong will be subsumed by Pit #3 (P. Waggitt pers. comm.).

This seasons' pre-release testing took place on two separate occasions. The first suite of tests were undertaken one week before Christmas to ensure that Ranger environmental staff had toxicological information in case of high rainfall, and a need to release, over the holiday break. These results indicated a high level of toxicity, resulting in Ranger environmental staff deciding to pump Djalkmara water to Retention Pond #2 (RP2) to allow dilution of the billabong water with natural run-off. The second suite of tests took place in early February after the billabong had been partially emptied and re-filled with rainwater. Throughout this report these will be referred to as the December and February runs.

Problems associated with culturing the gudgeons in the new Darwin laboratory, and the limited time-frame prior to Christmas, resulted in no fry being available for testing in the December run. The lower sensitivity of this species to past mine water samples, U and Mg, compared to the Cladocera and hydra, meant that a NOEC value from this test would not be used in the calculation of a dilution rate. Accordingly, the Cladocera and hydra were considered sufficient.

Further and more consequential problems arose in February due to the poor health of the cladoceran cultures. Cladoceran tests undertaken in January for another project had failed due to poor control survival. Because of the high sensitivity of this species, a decision was made to sacrifice the less sensitive gudgeon test and conduct two cladoceran tests, along with the hydra, to give a greater chance of a valid cladoceran test result. The outcomes and recommendations from this are described here.

Methods and materials

Diluent water collection

Magela Creek water was collected by ***eriss*** staff on the 16/12/02 and the 05/02/03 at the pump outlet of the upstream creekside monitoring station near Georgetown Billabong (latitude 12° 40' 28'' longitude 132° 55' 52''). 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 within the following 24 h. At the laboratory, the water was stored at 4°C for approximately 48 h prior to filtering through Whatman #42 filter paper immediately prior to testing.

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 the 16/12/02 and the 6/02/03. The sample was taken from the bank of the billabong as close as possible to the pump outlet to Magela Creek. An acid-washed plastic barrel was used to collect and store the sample, which was then transported to Darwin in an air-conditioned vehicle within 5 h. Once at the laboratory, the sample was stored at 4°C for approximately 24 h prior to filtering through Whatman # 42 filter paper.

General laboratory procedures

All equipment in contact with test organisms, media, control water or test solutions were made of chemically inert materials (eg. Teflon, glass or polyethylene). All plastic and glassware were washed by soaking in 5% nitric acid 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 method

The effect of Djalkmara Billabong water on two local aquatic species (the green hydra, *H. viridissima*; the Cladoceran, *M. macleayi*) was assessed in the laboratory using the standard protocols developed by ***eriss*** for the pre-release toxicity testing of retention pond water at Ranger Uranium Mine. These protocols are described in detail by Riethmuller et al (2003).

The green hydra test is a population growth test that is conducted over 96 h. Suitable test hydra, each bearing a newly tentacled bud, were selected from the culture bowls using a dissecting microscope and transferred to 3 plastic petri dishes. One hundred and fifty individuals were required to start the test with 30 hydra in each treatment (10 hydra x 3 replicates). The treatments consisted of 0 (control), 0.3, 1.0, 3.2, 10 & 32% Djalkmara Billabong water. Test hydra were transferred from the holding dishes into the experimental dishes using a Pasteur pipette and added one to each treatment (in ascending order). This process was repeated until each dish contained ten hydroids. The test containers were then randomly placed in an environmental cabinet under the conditions described above for culturing.

Fresh test solution was dispensed each day and allowed to warm for at least three hours in the environmental cabinet. The hydra were counted and their appearance (eg: rigidity, clubbing, colouration) noted. Each were 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.

Final day counts were used to calculate the relative population growth rate (K) using the following formula:

$$K = \frac{\ln(n_4) - \ln(n_0)}{T}$$

Where n_4 = number of hydra at the end of the 4 day test period

n_0 = number of hydra at the start of the test period ($n_0 = 10$)

T = length of test period in days (T = 4)

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. A linear interpolation method was undertaken to calculate the EC50 (the concentration that gave a 50% response in the hydra population growth). The statistical package ToxCalc™ (Tidepool Scientific Software, McKinleyville, California, USA) was used to undertake these analyses.

The *M. macleayi* tests involved exposing ten replicates per treatment, each containing one female Cladocera, to concentrations of 0 (control), 0.3, 1, 3.2, 10 and 32% Djalkmara

Billabong water. Fresh test solutions were dispensed daily and allowed to warm up in the environmental cabinet. Each individual female cladocera was then transferred to fresh solution using a Pasteur pipette and microscope. Observations on the health of the female and the number of neonates produced were recorded until the time at which the control treatments produced their third brood (144 h). The number of neonates produced in each Djalkmara water treatment was then compared to the controls to determine the NOEC, LOEC and EC50 as described above.

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 Djalkmara Billabong water and the 0, 0.3, 1, 3.2, 10 and 32% dilutions of the sample (diluted with Magela Creek water) were taken. 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 (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.

Filtered Magela Creek water samples (2 x 200 mL) were taken for each batch of water into acid-washed amber glass bottles. One sample was acidified with 10% HNO_3 for total organic carbon analysis and the other left unacidified for alkalinity analysis at the Australian Government Analytical Laboratories (Pymble, NSW), within 48 h of collection.

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 when more than 30 healthy hydroids remained in each control dish at the end of the test period. 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).

Determining a safe dilution ratio

The safe dilution ratio was determined by identifying the NOEC of the most sensitive species and dividing this by a safety factor of 10 as recommended by ANZECC and ARMCANZ (2000).

Results and Discussion

December 2002 *M. macleayi* test

Quality assurance

Good adult survival (90%) was observed in the control Cladocera. In addition, all were female and had produced three broods indicating acceptability of the test.

Control pH varied up to 0.91 units from the initial day measurement, while the sample treatment solutions varied up to 1.65 units (Appendix 6). This level of variability was not observed in the *H. viridissima* test conducted in parallel, indicating that it was an artefact of this particular test organism or procedure (for example the addition of food in this test may influence the level of pH drift). The low buffering capacity of Magela Creek water makes it difficult to obtain a stable pH throughout a test. It has been noted that Magela Creek water will often change up to 0.5 pH units after collection even without the addition of test organisms and their food supplements that would further contribute to pH change (C. leGras pers. comm.).

Conductivity generally remained within 10% of the initial day samples and, on the few occasions it exceeded this, it was by less than 1.5 µS/cm. Dissolved oxygen remained above 97.2% in all treatments throughout the test (Appendix 6).

Toxicity test

The Djalkmara Billabong water sample was found to be highly toxic to *M. macleayi* giving a NOEC of <0.3%, a LOEC of 0.3% and an EC50 of 1.6% (95% confidence limits 0.9–3.0%) Djalkmara water (Table 1, Appendix 10)). As 0.3% was the lowest concentration tested, the use of a lower concentration range would have almost certainly produced a lower LOEC (note the sudden drop in reproduction from the control to 0.3% Djalkmara water in Figure 1).

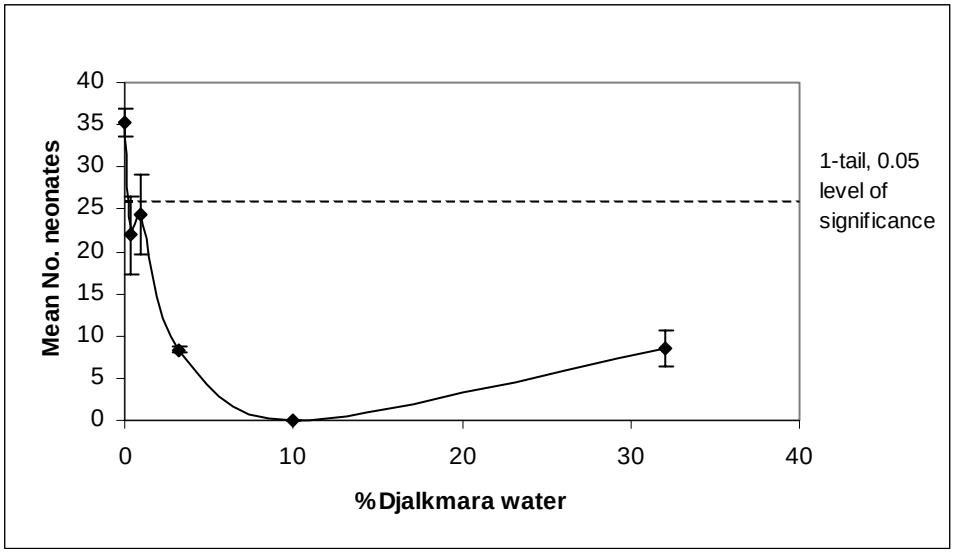

Figure 1 Toxicity of Djalkmara Billabong water to *M. macleayi* in December 2002. Vertical bars are the standard error of the mean.

Table 1 Results of the December 2002 pre-release toxicity tests

Test	Date	Mine water source	NOEC (%)	LOEC (%)	EC50 (95% confidence limits)
M. macleayi reproduction	18/12/02	Djalkmara	<0.3	0.3	1.6 (0.9 – 3.0)
H. viridissima population growth	17/12/02	Djalkmara	3.2	10	9.7

December 2002 *H. viridissima* test

Quality assurance

Good control growth (mean = 32 hydroids) and reproducibility (% co-efficient of variation = 6.9) was observed over the 96 h test period (Appendix 11).

Test solution pH in all treatments remained within 0.53 units of the initial day measurements (Appendix 7). All but one conductivity reading remained within 10% of the initial day measurements. This unusual reading was more than double the seven others taken for that treatment. As it was an isolated reading, it was most likely the result of contamination during the measurement process rather than in the test container, which would have been detected on subsequent days. Dissolved oxygen concentrations remained higher than 87.6% throughout the test (Appendix 7).

Toxicity test

Whilst *H. viridissima* was less sensitive than *M. macleayi*, it still showed a significant reduction in population growth, giving a NOEC of 3.2%, a LOEC of 10% and an EC50 of 9.7% Djalkmara water (Table 1, Figure 2, Appendix 11). This pattern of sensitivity corresponds to previous pre-release and uranium tests using these species (Appendix 1, van Dam et al 2000).

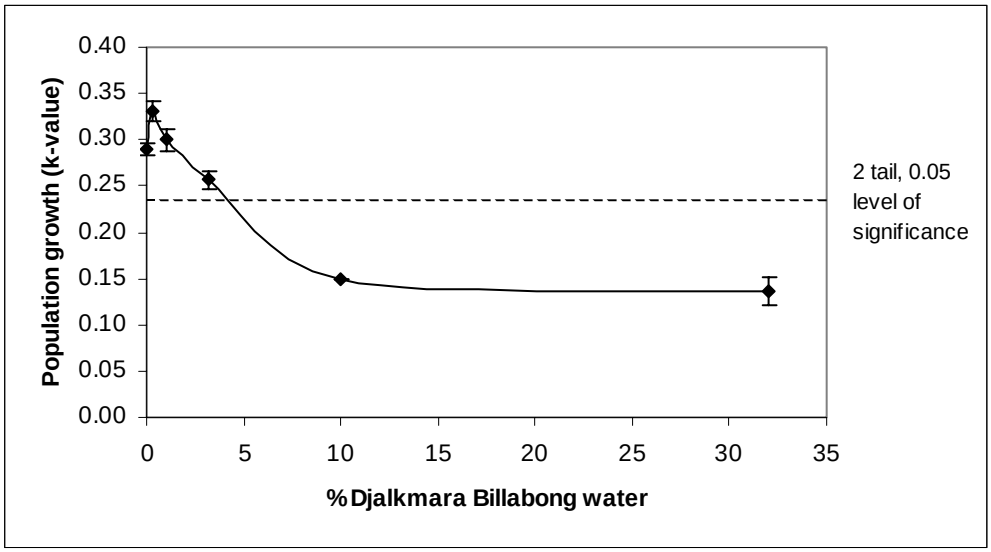

Figure 2 Toxicity of Djalkmara Billabong water to *H. viridissima* in December 2002. Vertical bars are the standard error of the mean.

February 2003 *M. macleayi* tests

Quality assurance

Higher than acceptable control mortality was observed in both the Cladoceran tests undertaken on the second Djalkmara sample (1st Cladoceran test = 50% control mortality, 2nd test = 70% control mortality). Hence, these tests were considered invalid.

Test solution pH drifted 0.76 units from the initial day pH in the controls and up to 1.18 units in the Djalkmara water treatments. A similar level of pH drift was observed in the December *M. macleayi* tests and is likely to be due to the low buffering capacity of the diluent along with the addition of Cladoceran food and metabolites. Electrical conductivity remained within 10% of the initial day measurements except for one reading where the sample was contaminated with KCl from the pH probe. Dissolved oxygen concentration remained above 92.4% throughout the tests. These indicate that the high mortality rate observed in the tests was not a result of poor water quality, rather that there may have been a problem with the health of the cultures.

February 2003 *H. viridissima* test

Quality assurance

Good control growth (mean = 44 hydroids) and reproducibility (% co-efficient of variation = 8.3) was observed in the February *H. viridissima* test (Appendix 12).

Test solution pH remained within 0.5 units of the initial day measurement in the control and all treatments. Conductivity generally remained within 10% of the initial day measurements and where this was exceeded, it was by less than 2.5 µS/cm. Dissolved oxygen concentrations remained higher than 86.4% throughout the test. (Appendix 9).

Toxicity test

As expected, the toxicity of Djalkmara Billabong water to *H. viridissima* was lower at this time than in December. Population growth was significantly reduced at only the highest concentration of Djalkmara water tested, giving a NOEC of 10% and a LOEC of 32% (Figure 3, Table 2, Appendix 12). This indicated that the actions taken by Ranger Environmental staff throughout January to dilute and, consequently, lower the toxicity of the water had been successful.

Table 2 Results of the February 2003 pre-release toxicity tests

Test	Date	Mine water source	NOEC (%)	LOEC (%)
M. macleayi reproduction	09/02/03	Djalkmara	Invalid*	Invalid*
M. macleayi reproduction	09/02/03	Djalkmara	Invalid*	Invalid*
H. viridissima population growth	10/02/03	Djalkmara	≥32	>32

* Test invalid due to higher than acceptable control mortality

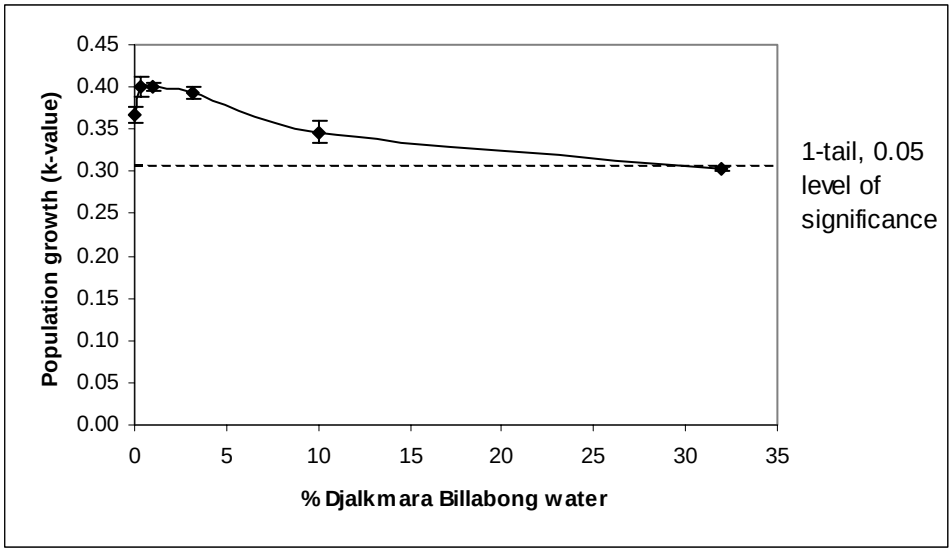

Figure 3 Toxicity of Djalkmara Billabong water to *H. viridissima* in February 2003. Vertical bars are the standard error of the mean.

Recommendations for the release of Djalkmara Billabong water into Magela Creek (February 2003)

The occurrence of the two invalid *M. macleayi* reproduction tests in February meant that it was necessary to base the final recommended dilution concentration on an assessment of both toxicity and chemistry data, rather than using the NOEC/safety factor approach normally undertaken.

A dilution of 1 part Djalkmara Billabong water to 300 parts Magela Creek water was recommended based on the following considerations:

- The undiluted Djalkmara Billabong water contained 431 μg/L U (Appendix 3 & 4). To maintain creek concentrations lower than the action level of 1.4 μg/L U, a dilution of 1:310 is required. A dilution of 1:300 would maintain the creek concentration well below the 99% trigger value of 5.5 μg/L U.
- Electrical conductivity in the sample was 650 μS/cm (Appendix 5) that would be diluted to 2.2 μS/cm. This is lower than natural creek levels.
- Manganese concentration would be reduced to 0.12 μg/L (Appendix 3 & 4) that is also below natural creek concentrations.
- A NOEC of 10% was obtained for the February *H. viridissima* test. Using the recommended approach and dividing by a safety factor of 10, a dilution of 1% was obtained. Note however, that *M. macleayi* has been shown in the past to be more sensitive to Djalkmara Billabong water (Appendix 1).

Actions to investigate the occurrence of invalid *M. macleayi* reproduction tests

The high control mortality observed in the February *M. macleayi* tests was not an isolated case. Out of the eight tests conducted for the derivation of a Mg trigger value, six tests were invalid. Four were due to high control mortality, while the remaining two were due to the presence of male Cladocera throughout the treatments and controls. These observations

indicate that the health of the laboratory Cladoceran culture was not optimal and, as such, actions need to be undertaken to investigate why.

Invalid tests have also occured in the old laboratory in Jabiru and in the new Darwin laboratory. Cladoceran cultures have continued to be maintained in Magela Creek water since the relocation and a valid trial test was conducted in the first few months of moving into the new laboratory, indicating that the new laboratory is unlikely to be the cause.

Actions completed

Handling and feeding techniques of different operators have been compared with reference to protocols (eg, the method for determining the density of the *Chlorella* sp. used for feeding).

New Cladoceran stock were collected from Bowerbird Billabong in November 2002. Both old and new stock have been maintained in the laboratory to enable a comparison of health over time. To date, the new stock have shown similar survival patterns as the old stock, with periodic culture crashes, however this is yet to be quantified.

Historical culturing data including notes on the survival of individual Cladocera, their reproductive success and appearance, have been collated to enable a time analysis to be undertaken to identify possible patterns indicative of a cause.

Actions to be undertaken

Cladoceran cultures are to be continued until individuals have had their third brood (in addition to normal conditions where cultures are restarted when they have their second brood). This greatly increases the maintenance load and will commence when a new research assistant is appointed.

A time analysis of the historical culturing data is to be undertaken. Data on cultures maintained until their third brood will be included after 2–3 months of data have been collected.

A review of culturing techniques in other ecotoxicology laboratories.

The use of acute toxicity testing for any future pre-release toxicity testing (see following section) should be considered.

Observations regarding the use of *M. macleayi* for pre-release and mine contaminant toxicity testing

During recent toxicity tests with *M. macleayi*, reduction in reproduction with increasing toxicant concentration appears to be caused by increased adult mortality rather than a reduction in neonate production. This trend is illustrated in the graphs presented below. Figure 4 shows the results from the December 2002 test presented above. Note that the number of neonates produced follows the number of surviving adults in each treatment.

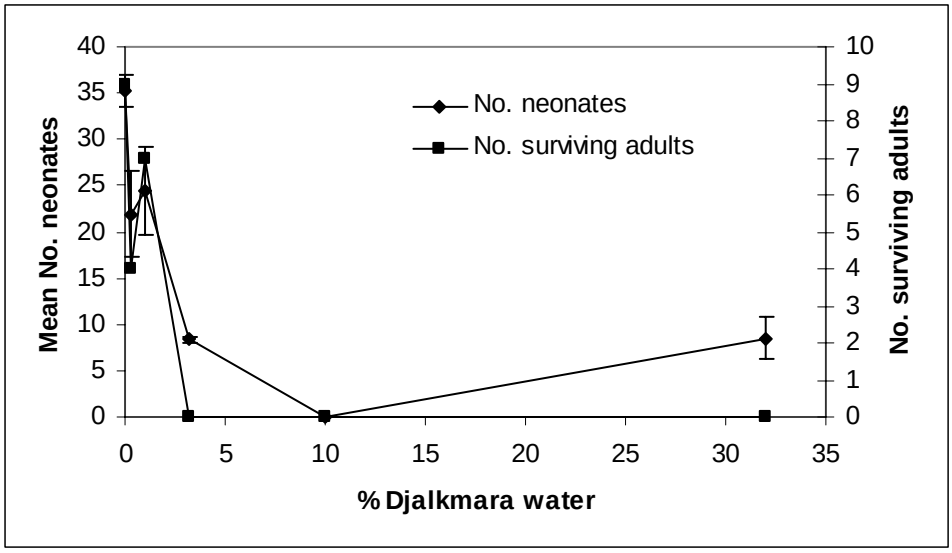

Figure 4 The relationship between neonate production and adult survival in Djalkmara Billabong water, December 2002. Vertical bars are the standard error of the mean.

This same pattern was observed in the two pre-release tests from the 2001–2002 Wet season (Figures 5 & 6).

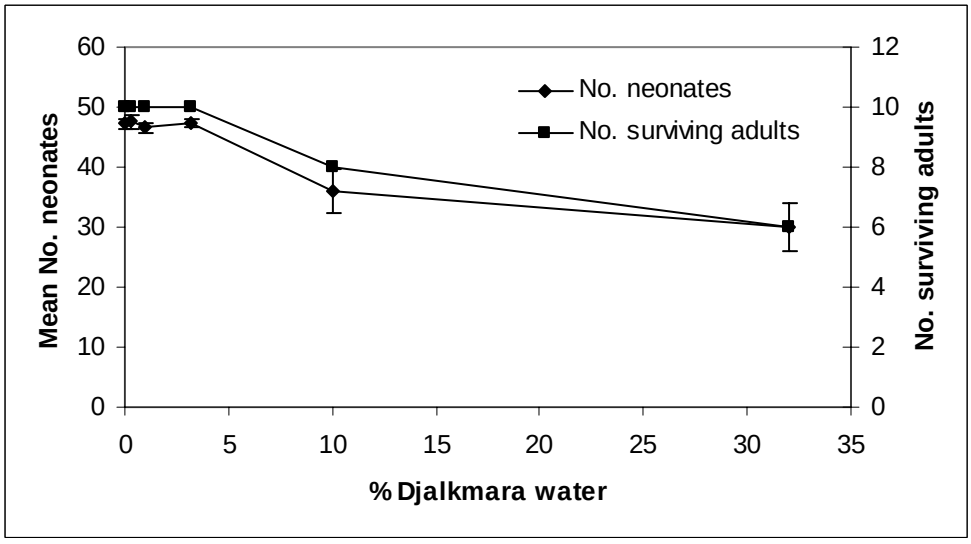

Figure 5 The relationship between neonate production and adult survival in Djalkmara Billabong water, December 2001. Vertical bars are the standard error of the mean.

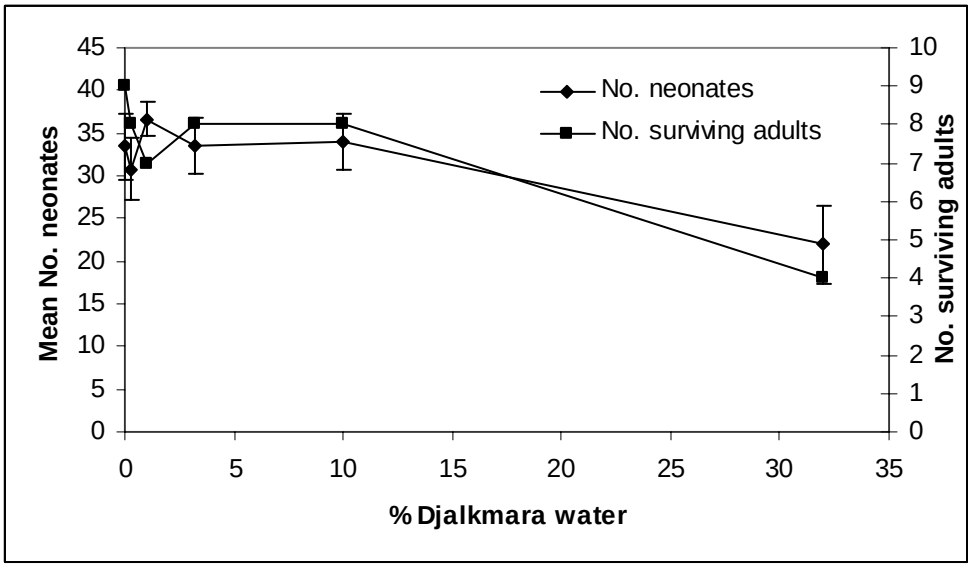

Figure 6 The relationship between neonate production and adult survival in Djalkmara Billabong water, January 2002. Vertical bars are the standard error of the mean.

This pattern also holds true for the single mine contaminants $MgSO_4$ (Figure 7) and an anonymous mine effluent. (Figure 8).

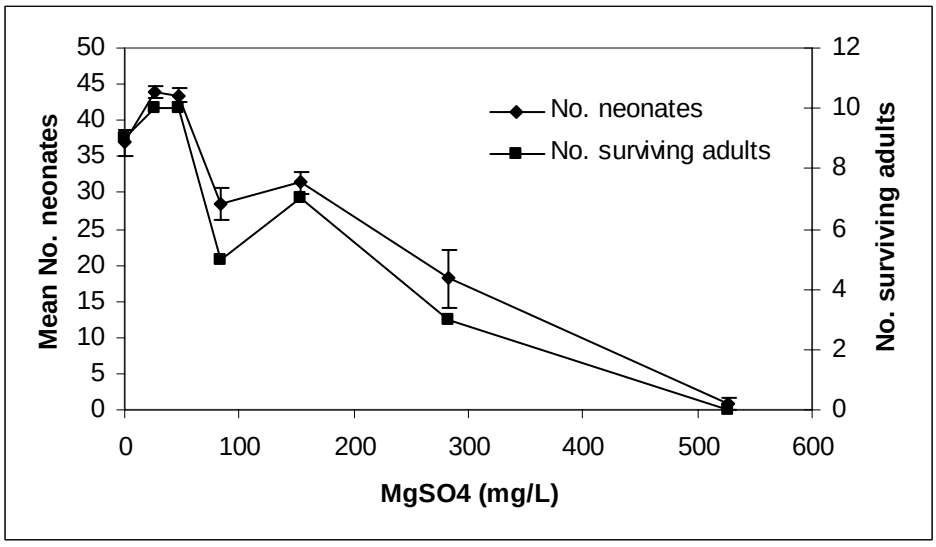

Figure 7 The relationship between neonate production and adult survival when exposed to $MgSO_4$. Vertical bars are the standard error of the mean.

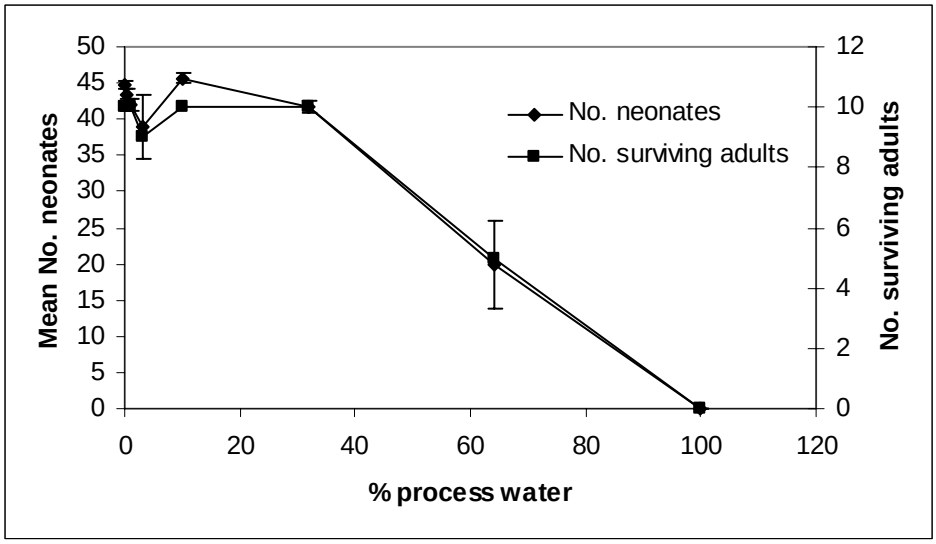

Figure 8 The relationship between neonate production and adult survival when exposed to an effluent tested 'in confidence'. Vertical bars are the standard error of the mean.

All of these results indicate that the chronic reproduction endpoint is no more sensitive than acute survival over a 6 day period and suggests that it would be equally as relevant, or in fact more appropriate, to run longer term (4–6 day) acute tests.

One of the acceptability criteria from the reproduction test protocol states that 'no more than 20% of parental cladocerans should be reported missing in any treatment (except if all other cladocerans are dead in that group)'. In all the tests presented above, this criterion was not met due to the deaths of adult fleas across numerous treatments. Furthermore, the experimental design used in an acute test would greatly reduce the variability that is frequently observed when one or more adult cladocera die in the reproduction test. As each replicate consists of individual fleas, the loss of one adult equals the loss of an entire replicate. If this occurs early in the test then no neonates are recorded for that individual, which leads to large variability in the final results when neonate counts of zero are analysed with counts over 35 within the same treatment. This problem would be reduced in an acute test design where each replicate has ten individuals.

4 Conclusions

Djalkmara Billabong water was found to be highly toxic to *M. macleayi* (NOEC < 0.3%) and *H. viridissima* (NOEC = 3.2%) in December 2002. As such, this water was not released and the billabong was partially emptied (by pumping water to RP2) and allowed to dilute with rainfall run-off.

A second run of tests conducted in February 2003 indicated that Djalkmara water toxicity had been reduced (NOEC for *H. viridissima* = 10%). The invalidity of the *M. macleayi* tests conducted at this time complicated the methodology for recommending a safe dilution rate. Consequently, a recommended rate of one part Djalkmara water to 300 parts Magela Creekwater was based on an assessment of water chemistry and the *H. viridissima test* results.

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Appendix 1 Results of pre-release toxicity tests 1996–2002

Test	Date	Mine water source	NOEC (%)	LOEC (%)	Reference
H. viridissima population growth	05/01/96	RP4	≥32	>32	ERA 1996
M. splendida inornata embryo mortality	18/01/96	RP4	1.0	3.2	ERA 1996
M. macleayi adult mortality	20/01/96	RP4	≥32	>32	ERA 1996
M. macleayi reproduction	20/01/96	RP4	≥32	>32	ERA 1996
H. viridissima population growth	21/01/96	RP4	≥32	>32	ERA 1996
H. viridissima population growth	14/01/97	RP4	≥32	>32	ERA 1997
M. splendida inornata embryo mortality	14/01/97	RP4	≥32	>32	ERA 1997
M. macleayi reproduction	14/01/97	RP4	≥32	>32	ERA 1997
H. viridissima population growth	15/01/98	RP4	≥32	>32	ERA 1998
M. splendida inornata embryo mortality	05/01/98	RP4	≥32	>32	ERA 1998
M. macleayi reproduction	20/01/98	RP4	≥32	>32	ERA 1998
H. viridissima population growth	11/12/98	RP4	3.2	10	ERA 1999
M. splendida inornata embryo mortality	12/12/98	RP4	3.2	10	ERA 1999
M. macleayi reproduction	18/12/98	RP4	3.2	10	ERA 1999
H. viridissima population growth	11/11/99	Djalkmara	≥32	>32	ERA 2000
M. splendida inornata embryo mortality	12/11/99	Djalkmara	≥32	>32	ERA 2000
M. macleayi reproduction	13/11/99	Djalkmara	≥32	>32	ERA 2000
H. viridissima population growth	04/12/00	Djalkmara	≥32	>32	ERA 2001

M. macleayi reproduction	07/12/00	Djalkmara	3.2	10	ERA 2001
M. mogurnda sac-fry survival	12/12/00	Djalkmara	≥32	>32	ERA 2001
M. macleayi reproduction	10/12/00	RP1	10	32	ERA 2001
H. viridissima population growth	11/12/00	RP1	≥32	>32	ERA 2001
M. mogurnda sac-fry survival	10/01/01	RP1	≥32	>32	ERA 2001
M. macleayi reproduction	18/12/01	Djalkmara	3.2	10	***eriss*** unpublished data
H. viridissima population growth	14/01/02	Djalkmara	≥32	>32	***eriss*** unpublished data
M. macleayi reproduction	15/01/02	Djalkmara	10	32	***eriss*** unpublished data

Appendix 2. Water chemistry results for Djalkmara Billabong water diluted with Magela Creek water in the December 2002 *M. macleayi* and *H. viridissima* 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)
Blank	0.6	<0.1	<0.1	<0.02	0.2	0.23	<20	<0.1	0.07	<0.1	<0.01	<0.01	<0.2	<0.1	0.019	1.2
0	83.0	0.3	2.3	<0.02	0.3	1.04	240	0.6	18.9	1.3	0.39	0.08	<0.2	0.8	0.036	3.9
0.3	84.6	0.3	1.9	<0.02	0.3	1.14	260	1.2	18.9	1.4	0.33	0.08	<0.2	2.6	8.22	2.7
1.0	90.0	0.5	2.0	<0.02	0.3	5.62	260	2.4	19.8	1.4	0.36	0.36	<0.2	7.9	26.3	4.3
3.2	94.4	0.9	2.2	<0.02	0.4	1.13	360	6.4	18.5	1.7	0.36	0.07	<0.2	21.6	77.9	2.7
10	132.0	2.2	2.7	<0.02	0.4	1.02	260	17.9	18.3	2.4	0.29	0.07	0.4	63.9	238	3.5
32	86.0	6.6	3.4	<0.02	0.6	1.58	220	56.5	20.4	4.7	0.47	0.08	1.0	203	806	2.8
100	18.6	20.8	N/A*	<0.02	1.4	1.31	100	181	26.8	12.5	0.32	0.11	3.0	698	2750	2.1

*Not analysed

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

% Djalkmara Billabong Water	Element													
	Al (µg/L)	Ca (mg/L)	Cd (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)
Blank	0.9	<0.1	<0.02	0.22	<20	<0.1	0.08	<0.1	<0.01	<0.01	<0.2	<0.1	0.008	0.3
0	93.2	0.3	<0.02	7.18	220	0.5	5.8	1.3	0.27	0.09	<0.2	0.5	0.228	2
0.3	136	0.4	<0.02	4.96	260	0.7	9.73	1.3	1.07	0.17	<0.2	1.1	1.35	4.9
1.0	114	0.4	<0.02	4.33	240	1.3	10.1	1.4	0.47	0.08	0.2	3.1	4.28	5.8
3.2	104	0.6	<0.02	7.36	220	3.0	6.61	1.4	0.22	0.1	<0.2	9.1	13.2	3.4
10	159	1.3	<0.02	4.57	260	8.6	10.7	1.8	0.23	0.06	0.2	28.3	40.1	5.4
32	118	3.1	<0.02	4.6	220	24.7	14.3	3.0	0.38	0.06	0.6	85.5	133	12.8
100	32.7	9.2	<0.02	1.87	140	79.8	37.3	7.0	0.42	0.1	1.6	279	431	35.0

Appendix 4. Water chemistry results for Djalkmara Billabong water diluted with Magela Creek water in the February 2003 *H. vridissima* test

% Djalkmara Billabong Water	Element													
	Al (μg/L)	Ca (mg/L)	Cd (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)
Blank	0.9	<0.1	<0.02	<0.1	<20	<0.1	0.07	<0.1	<0.01	<0.01	<0.2	0.2	0.024	9.4
0	119	0.4	<0.02	3.48	240	0.5	9.35	1.3	0.41	0.09	<0.2	0.4	0.055	8.4
0.3	114	0.4	<0.02	3.48	240	0.7	9.67	1.3	0.24	0.1	<0.2	1.1	1.32	6.8
1.0	101	0.5	<0.02	5.2	220	1.3	8.35	1.3	0.38	0.12	<0.2	2.8	4.31	6.0
3.2	105	0.6	<0.02	6.17	240	2.9	7.84	1.4	0.28	0.14	<0.2	7.5	12.6	6.8
10	114	1.2	<0.02	5.99	240	8.3	9.83	1.8	0.26	0.14	0.2	23.5	39.2	9.4
32	91.5	3.1	<0.02	3.31	200	25.0	17.7	3.0	0.33	0.09	0.4	71.8	126	15.9
100	32.7	9.2	<0.02	1.87	140	79.8	37.3	7.0	0.42	0.1	1.6	279	431	35.0

Appendix 5. Physico-chemical data for the undiluted Djalkmara Billabong samples

Sampling date	pH	DO[1]	EC[2]
16/12/02	8.08	87.0	1308
06/02/03	7.48	102.0	650

[1] Conductivity units are in μS/cm.

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

Appendix 6. Physico-chemical data for the December 2002 *M. macleayi* 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	6.29	6.93	6.43	6.99	6.28	7.29	6.69	7.75	7.33	8.44	7.70	8.67
	Conductivity[1]	17.9	17.7	23.6	23.8	33.4	36.7	72.6	76.2	183.7	189.4	494	501
	DO[2]	97.6	97.2	102.6	99.4	101.9	99.7	100.0	97.4	100.9	102.2	101.1	103.7
2	pH	6.55	6.78	6.89	6.92	6.93	7.20	7.18	7.95	7.56	8.62	8.10	8.64
	Conductivity	17.8	18.8	23.8	23.7	36.4	36.5	76.5	77.4	186.7	188.9	495	502
	DO	112.0	98.6	112.4	99.9	100.0	99.9	116.6	102.4	113.8	103.3	112.9	103.6
3	pH	6.31	7.12	6.38	6.93	6.52	7.01	6.78	7.44	7.19	8.06	7.75	8.45
	Conductivity	14.6	17.8	20.6	23.7	33.7	36.8	72.5	76.2	184.1	191.6	495	504
	DO	111.0	106.8	112.8	104.6	112.2	103.0	109.2	105.8	113.5	105.9	110.5	105.9
4	pH	6.29	6.57	6.24	6.86	6.41	7.30	6.88	7.80	7.26	8.54	7.79	8.61
	Conductivity	18.0	17.7	24.1	24.3	36.8	36.7	75.4	75.2	186.6	188.1	496	500
	DO	115.7	99.5	122.5	100.8	117.5	102.0	124.3	104.6	116.7	107.0	120.8	105.9
5	pH	6.00	7.20	6.38	7.08	6.52	7.19	6.72	8.34	6.98	8.86	7.63	8.76
	Conductivity	20.0	17.9	24.8	23.8	37.2	36.9	75.4	77.0	186.1	190.6	495	509
	DO	118	99.6	120.0	100.7	119.1	99.6	118.8	104.1	119.8	106.8	120.3	106.9
6	pH	6.07	6.61	6.46	6.91	6.63	7.42	7.05	All dead	7.14	All dead	8.02	8.73
	Conductivity	14.8	17.6	20.4	23.7	33.4	36.3	72.3		183.1		496	507
	DO	119.0	102.3	115.4	101.9	117.2	104.7	116.8		115.6		114.5	104.9

[1] Conductivity units are in µS/cm.

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

Appendix 7. Physico-chemical data for the December 2002 *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	6.24	6.58	6.43	6.47	6.56	6.31	6.89	6.91	7.34	7.29	7.90	7.73
	Conductivity[1]	14.8	17.7	20.7	22.1	33.7	38.6	72.7	76.1	183.7	184.4	495	495
	DO[2]	95.3	92.0	92.5	91.5	94.7	89.1	96.1	90.7	97.3	89.5	97.7	91.0
2	pH	6.29	6.53	6.43	6.57	6.28	6.67	6.69	6.92	7.33	7.35	7.70	7.88
	Conductivity	14.7	17.4	20.4	22.9	33.4	35.5	72.6	77.1	183.7	189.9	494	508
	DO	97.6	88.9	102.6	89.8	101.9	98.3	100.0	88.7	100.9	90.2	101.2	87.6
3	pH	6.35	6.42	6.37	6.52	6.53	6.61	6.85	6.87	7.36	7.30	7.82	7.81
	Conductivity	15.2	37.7	21.3	22.9	33.6	36.2	74.2	78.0	185.0	190.2	495	507
	DO	113.8	88.3	112.0	88.4	109.4	90.0	113.2	91.1	112.9	90.6	111.2	89.9
4	pH	6.77	6.72	6.81	6.64	6.90	6.75	7.10	7.05	7.55	7.47	8.03	8.03
	Conductivity	17.5	15.4	23.5	21.5	35.9	35.0	74.4	76.8	186.2	189.3	496	512
	DO	119.2	100.5	113.1	100.5	116.7	102.5	117.5	101.6	119.7	100.7	118.2	100.2

[1] Conductivity units are in µS/cm.

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

Appendix 8. Physico-chemical data for both the *M. macleayi* February 2003 tests

		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	6.05	EF	6.35	EF	6.53	EF	6.70	EF	6.96	EF	7.42	EF
	Conductivity[1]	16.4	14.7	18.6	17.4	24.0	23.4	41.0	40.9	92.9	93.2	244	247
	DO[2]	112.2	100.3	109.4	104.4	110.3	102.6	108.2	103.1	111.5	106.6	109.5	105.9
2	pH	EF	6.66	EF	6.95	EF	7.08	EF	7.24	EF	7.78	EF	8.56
	Conductivity	16.2	14.7	14.7	17.3	23.7	23.1	40.9	40.5	92.7	92.9	244	248
	DO	112.6	99.2	115.2	101.1	116.5	102.6	113.2	102.5	114.3	122.7	111.7	111.3
3	pH	6.35	6.63	6.48	6.88	6.61	7.09	6.88	7.23	7.16	7.57	7.54	8.00
	Conductivity	15.6	14.7	17.9	17.2	23.8	23.3	41.0	40.5	92.3	93.2	243	249
	DO	116.6	100.3	114.6	101.9	114.9	101.0	114.1	101.5	108.3	101.3	119.2	101.6
4	pH	6.43	6.67	6.42	6.74	6.73	6.92	6.80	7.18	7.04	7.51	7.60	8.02
	Conductivity	13.6	14.8	17.5	17.0	23.4	22.9	40.7	40.8	93.1	93.5	246	250
	DO	117.1	96.8	120.7	99.0	118.8	99.7	118.9	99.6	114.7	101.1	114.8	100.7
5	pH	6.38	6.48	6.65	6.85	6.72	6.88	6.84	7.03	7.12	7.39	7.52	7.98
	Conductivity	67.6	14.7	18.3	19.3	23.9	23.7	40.7	41.1	92.5	93.7	243	248
	DO	96.7	93.5	115.3	96.5	114.9	97.9	115.7	97.4	116.2	99.8	115.1	99.6
6	PH	6.29	6.81	6.42	6.80	6.65	6.82	6.78	7.08	7.05	7.42	7.45	8.60
	Conductivity	15.6	17.2	18.3	17.9	23.8	23.2	40.8	40.4	93.4	92.7	245	251
	DO	118.5	110.1	118.7	99.6	92.4	99.1	115.5	99.7	117.2	101.6	115.2	101.5

[1] Conductivity units are in µS/cm.

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

EF. Equipment failure on day of measurement.

Appendix 9. Physico-chemical data for the February 2003 *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	EF	6.41	EF	6.55	EF	6.54	EF	6.76	EF	7.03	EF	7.46
	Conductivity[1]	12.4	15.2	14.7	17.2	20.5	22.1	37.3	38.8	89.6	91.1	240	241
	DO[2]	102.1	92.9	103.1	95.4	103.7	95.4	103.8	96.5	101.3	109.7	103.3	112.8
2	pH	6.07	6.31	6.34	6.36	6.36	6.43	6.69	6.58	7.06	6.93	7.57	7.34
	Conductivity	12.9	15.2	14.9	17.5	20.4	22.8	37.0	39.3	88.9	92.4	238	246
	DO	105.2	89.7	110.5	86.4	109.5	87.7	107.7	87.2	98.0	89.9	100.0	91.9
3	pH	5.97	6.58	6.21	6.62	6.33	6.63	6.52	6.75	6.98	7.10	7.39	7.54
	Conductivity	13.0	16.1	15.6	19.5	21.1	24.0	37.9	41.1	89.5	95.2	240	248
	DO	108.7	96.8	111.8	96.0	111.1	95.8	113.4	96.4	111.3	94.5	114.3	96.7
4	pH	6.05	6.57	6.28	6.62	6.36	6.71	6.63	6.87	7.01	7.20	7.41	7.65
	Conductivity	12.7	12.6	14.8	15.0	20.7	21.1	37.4	38.0	89.9	91.9	241	245
	DO	111.0	96.0	116.0	94.4	118.6	95.6	110.5	95.1	113.9	95.2	116.8	93.5

[1] Conductivity units are in µS/cm.

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

EF. Equipment failure on day of measurement.

Appendix 10 Toxcalc statistical summary for the December 2002 *M. macleayi* test

Ceriodaphnia Survival and Reproduction Test-Reproduction

Start Date:	18/12/2002	Test ID:	611D	Sample ID:	DJALKMARA
End Date:	24/12/2002	Lab ID:	ERISS	Sample Type:	RANGER MIN
Sample Dat		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CD-Ceriodaphnia dubia
Comments:					

Conc-%	1	2	3	4	5	6	7	8	9	10
Control	38.000	41.000	34.000	40.000	40.000	37.000	34.000	33.000	36.000	22.000
0.3	10.000	39.000	0.000	10.000	24.000	24.000	9.000	32.000	38.000	44.000
1	41.000	11.000	10.000	10.000	0.000	36.000	37.000	34.000	36.000	29.000
3.2	9.000	9.000	8.000	9.000	8.000	10.000	16.000	9.000	8.000	8.000
10	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000	0.000
32	10.000	10.000	0.000	0.000	20.000	8.000	10.000	2.000	18.000	9.000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	35.500	1.0000	35.500	22.000	41.000	15.500	10				35.500	1.0000
*0.3	23.000	0.6479	23.000	0.000	44.000	65.906	10	2.960	2.287	9.657	23.700	0.6676
*1	24.400	0.6873	24.400	0.000	41.000	61.192	10	2.628	2.287	9.657	23.700	0.6676
*3.2	9.400	0.2648	9.400	8.000	16.000	25.669	10	6.180	2.287	9.657	9.400	0.2648
*10	0.000	0.0000	0.000	0.000	0.000	0.000	10	8.406	2.287	9.657	4.350	0.1225
*32	8.700	0.2451	8.700	0.000	20.000	78.155	10	6.346	2.287	9.657	4.350	0.1225

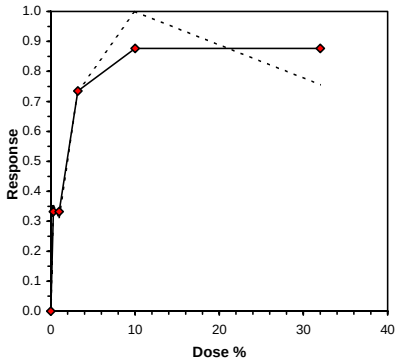
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates non-normal distribution (p <= 0.01)	1.7056383	1.035	-0.363137	0.7447808
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	<0.3	0.3			9.6568872	0.272025	1696.9867	89.174074	7.2E-11	5, 54
Treatments vs Control										

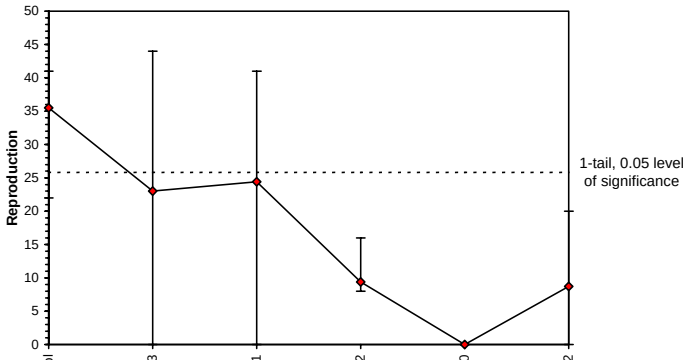
Linear Interpolation (200 Resamples)

Point	%	SD	95% CL		Skew
IC05*	0.0451	0.0340	0.0290	0.1385	5.6405
IC10*	0.0903	0.0585	0.0580	0.2769	3.9240
IC15*	0.1354	0.1298	0.0870	0.5417	5.0406
IC20*	0.1805	0.2309	0.1160	1.0487	3.0244
IC25*	0.2256	0.3547	0.1450	1.2655	1.7528
IC40	1.3692	0.5493	0.2320	1.9348	-0.4509
IC50	1.9154	0.4868	0.2900	2.3884	-1.2252

* indicates IC estimate less than the lowest concentration



Dose-Response Plot



Appendix 11. Toxcalc statistical summary for the December 2002 *H. viridissima* test

Hydra population growth					
Start Date:	17/12/2002	Test ID:	611B	Sample ID:	DJALKMARA
End Date:	21/12/2002	Lab ID:	ERISS	Sample Type:	RANGER MIN
Sample Dat		Protocol:	BTT - B	Test Species:	*Hydra viridissima*
Comments:					

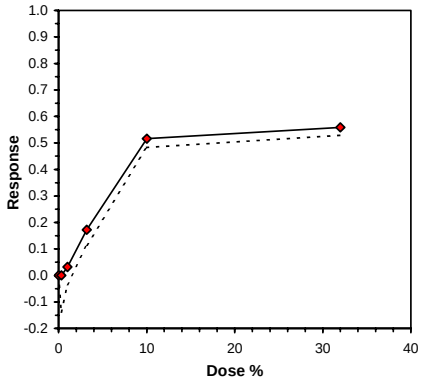
Conc-%	1	2	3
Control	310000	290000	270000
0.3	310000	370000	310000
1	260000	310000	330000
3.2	220000	270000	280000
10	150000	150000	150000
32	170000	160000	80000

			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Control	290000	1.0000	290000	270000	310000	6.897	3				310000	1.0000
0.3	330000	1.1379	330000	310000	370000	10.497	3	-1.504	2.500	66492.829	310000	1.0000
1	300000	1.0345	300000	260000	330000	12.019	3	-0.376	2.500	66492.829	300000	0.9677
3.2	256666.67	0.8851	256666.67	220000	280000	12.524	3	1.253	2.500	66492.829	256666.67	0.8280
*10	150000	0.5172	150000	150000	150000	0.000	3	5.264	2.500	66492.829	150000	0.4839
*32	136666.67	0.4713	136666.67	80000	170000	36.094	3	5.765	2.500	66492.829	136666.67	0.4409

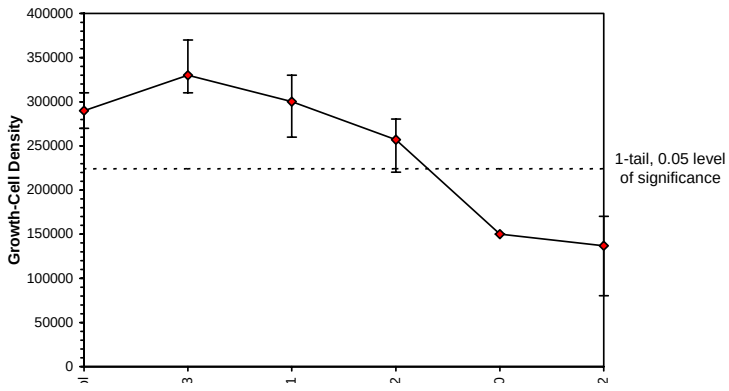
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9531551	0.858	-0.498581	-0.540963
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	3.2	10	5.6568542	31.25	66492.829	0.2292856	1.99E+10	1.061E+09	2.7E-05	5, 12
Treatments vs Control										

Linear Interpolation (200 Resamples)					
Point	%	SD	95% CL(Exp)		Skew
IC05	1.2792	0.4990	0.0000	3.3572	0.2963
IC10	2.0662	0.7180	0.0000	5.1988	0.1208
IC15	2.8531	0.8336	0.0000	6.0242	-0.2415
IC20	3.7525	0.8503	0.3241	6.7259	-0.0907
IC25	4.7406	0.9005	0.6379	7.3300	-0.4602
IC40	7.7050	0.6059	4.7691	9.3601	-1.1809
IC50	9.6813				



Dose-Response Plot



Appendix 12. Toxcalc statistical summary for the February 2003 *H. viridissima* test

Hydra population growth					
Start Date:	10/02/2003	Test ID:	620B	Sample ID:	DJALKMARA
End Date:	14/02/2003	Lab ID:	ERISS	Sample Type:	WHOLE EFFL
Sample Dat		Protocol:	BTT B	Test Species:	*Hydra viridissima*
Comments:	@nd run of pre-release tests				

Conc-%	1	2	3
Control	400000	360000	340000
0.3	440000	360000	400000
1	420000	390000	390000
3.2	410000	370000	400000
10	380000	360000	300000
32	300000	310000	300000

			Transform: Untransformed					1-Tailed		
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
Control	366666.67	1.0000	366666.67	340000	400000	8.332	3			
0.3	400000	1.0909	400000	360000	440000	10.000	3	-1.410	2.500	59121.657
1	400000	1.0909	400000	390000	420000	4.330	3	-1.410	2.500	59121.657
3.2	393333.33	1.0727	393333.33	370000	410000	5.292	3	-1.128	2.500	59121.657
10	346666.67	0.9455	346666.67	300000	380000	12.010	3	0.846	2.500	59121.657
*32	303333.33	0.8273	303333.33	300000	310000	1.903	3	2.678	2.500	59121.657

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.9734847	0.858	-0.211255	-0.402297
Bartlett's Test indicates equal variances (p = 0.33)	5.7538033	15.086317		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	10	32	17.888544	10	59121.657	0.1612409	4.397E+09	838888889	0.0088045	5, 12
Treatments vs Control										

Dose-Response Plot