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# Toxicity of Ranger mine RP2 and Pit 3 waters to native freshwater species: 2007 wet season

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**Australian Government**
**Department of the Environment, Water, Heritage and the Arts**
**Supervising Scientist**

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# Executive summary

Following extreme rainfall at ERA Ranger Mine in late February/early March 2007, a number of water management options were explored to reduce the volume of pond water being stored in Retention Pond 2 (RP2) and Pit 3. One possible option involved the short-term direct release of untreated Pond Water from RP2 and/or Pit 3 to Magela Creek. To provide information on the potential biological effects of such a strategy, the toxicities of RP2 water and Pit 3 water were assessed separately, using five and three native freshwater species, respectively. A key aim was to determine the maximum dilution of Pond Water that could be released whilst providing sufficient protection of the downstream aquatic ecosystems. It was also intended that the data would inform decisions about possible water management options for future wet seasons.

The toxicity of RP2 water (containing 1870 μg/L U – dissolved) varied markedly between the species tested, with $IC_{10}$ and $IC_{50}$ estimates in the range 0.6 – >100% and 1.8 – >100% RP2 water, respectively. Based on $IC_{50}$/$LC_{50}$ values, the order of sensitivity of the five test species was:

*Moinodaphnia macleayi* >> *Hydra viridissima* > *Chlorella* sp. > *Lemna aequinoctialis* >> *Mogurnda mogurnda*

Based on a species sensitivity distribution using species' $IC_{10}$ (or assumed equivalent) data, the dilution of RP2 water predicted to protect 99% of species was estimated to be 0.33% RP2 water (or 1 part RP2 water in ~300 parts Magela Creek water). At this dilution, the U concentration would have been approximately 6 μg/L U, the same as the current U Limit for Magela Creek, while the Mg concentration would have been approximately 0.5 mg/L, well below the recently proposed Limit for Magela Creek of 4.6 mg/L, and approximately equal to natural background concentrations.

The toxicity of Pit 3 water (~1620 μg/L U – dissolved) was assessed using *M. macleayi*, *H. viridissima* and *L. aequinoctialis*. The resultant $IC_{10}$ and $IC_{50}$ estimates were in the range 0.41 – 34% and 1.35 – >100% Pit 3 water, respectively. Based on $IC_{50}$ values, the order of sensitivity of the three test species was the same as that for RP2 water.

There was no significant difference between the toxicities of Pit 3 water and RP2 water to *M. macleayi* and *H. viridissima*. The general similarity in toxicity of the two water types to *M. macleayi* and *H. viridissima* was not unexpected given their similar uranium concentrations and the fact that RP2 receives water pumped from Pit 3. In contrast, the toxicity of Pit 3 water to *L. aequinoctialis* was significantly less than that of RP2 water. The difference in toxicity of the two water types to *L. aequinoctialis* may have been due to differences in (i) test organism responses between the tests, (ii) important physico-chemical variables that influence metal toxicity (eg. pH), and/or (iii) concentrations of, and interactions between, potentially toxic metals other than uranium (eg. Co, Cu, Mn, Ni, Pb and Zn).

At the time of completing the RP2 water and Pit 3 water toxicity assessments, flow in Magela Creek was insufficient for the direct release of Pond Water at acceptable/protective dilutions (ie. at least 1 in 300) to have significantly reduced the on-site water inventory. Consequently, this option was not progressed further. However, notwithstanding temporal variations in Pond Water composition/quality and how they may affect toxicity, the knowledge gained from the study will form a key part of the knowledge base required for the future evaluation of water management options at the Ranger Mine.

The results from the current study should not be used as the primary basis for determining protective dilutions of Pond Water that should be applied during future wet seasons. Any direct releases of Pond Water in the future will require new pre-release toxicity testing studies as soon as practicable prior to the intended time of discharge, recognising, however, the likely impracticality of running a full test suite at the time of release, given the likely narrow time window within which a significant volume of water could be released. Consequently, strategies for obtaining more timely toxicity information for Pond Waters may need to be considered, but would need to be rigorously tested and discussed and agreed by all stakeholders before being implemented.

# Toxicity of Ranger mine RP2 and Pit 3 waters to native freshwater species: 2007 wet season

**A Hogan, R van Dam, M Houston & N Lee**

## 1 Introduction

Extreme rainfall (840 mm over 5 days) at Jabiru East in late February/early March 2007 resulted in a major increase in the water inventory and associated on-site water management issues for the Energy Resources of Australia Ltd (ERA) Ranger Mine (Klessa & Puhalovich 2007). Consequently, a number of options to reduce the volume of pond waters stored on-site were proposed for urgent consideration and assessment. Given that flow in Magela Creek remained high for some time after the event (total discharge during March at station G8210009 was 535 GL, almost 50% greater than the mean annual discharge of 366 GL), the short-term direct release of untreated Pond Water from Retention Pond 2 (RP2) and/or Pit 3 to the creek was one of the water reduction initiatives that could have been implemented, in the event that approval was given by the Supervising Authority. Pond Water is derived from rainfall that falls on the active mine-site catchments, and from managed stockpile seepage, and typically has a uranium concentration between 2000–5000 μg/L.

In order to meet the primary objective of maintaining the natural biological diversity of aquatic ecosystems in the Alligator Rivers Region (as set out in the Environmental Requirements of the Commonwealth for the Operation of Ranger), the Ranger Authorisation states that ecotoxicity testing be undertaken on Pond Water prior to any discharge off-site. Site-specific toxicity of mine waters to a range of local aquatic organisms is undertaken to estimate dilution rates that provide environmental protection downstream of the mine. Using such data, ERA would determine the rate of release of Pond Water based on the existing flow regime in the creek. In addition, the data would possibly assist decisions about water management options for future wet seasons.

Earth Water Life (EWL) Sciences co-ordinated the ecotoxicity testing of RP2 and Pit 3 waters through the Supervising Scientist Division (SSD) of the Commonwealth Department of Environment and Water Resources (DEW). With the exception of a five or six year period from the mid 1990s to 2000 (when the Ranger Environmental Laboratory undertook its own pre-release toxicity testing), the SSD ecotoxicology laboratory has undertaken independent pre-release toxicity testing for Ranger since the late 1980s (van Dam 2004).

The current site-specific approach utilised by SSD, where at least five different local species are tested in receiving waters under local conditions, is fully consistent with the philosophy and approach recommended by the *Australian and New Zealand Guidelines for Fresh and Marine Water Quality* (WQGs; ANZECC/ARMCANZ 2000). Whereas three species were previously considered sufficient to calculate a protective discharge dilution, data for at least five species are now preferred as this provides a greater representation of the natural ecosystem and allows for the utilisation of risk-based, probablistic approaches using species sensitivity distributions, rather than the 'safety factor' approach that had been traditionally

used. The benefits of the probability distribution approach are outlined in Section 8.3.3.3 of the WQGs and in Section 3.9 below.

# 2 Aims

The aims of this study were as follows:

- To determine the site-specific toxicity of RP2 water to five local aquatic organisms in order to estimate a protective dilution rate for release into Magela Creek; and
- To determine the site-specific toxicity of Pit 3 water to three local species, including a discussion of the organisms' responses relative to those in RP2 water.

# 3 Materials and methods

## 3.1 Diluent water collection

Natural Magela Creek water (NMCW) was collected by SSD staff on 16 March 2007 from upstream of Georgetown Billabong (near the creekside monitoring pontoon, latitude 12° 40' 28'' longitude 132° 55' 22''). The water was collected in 20 L acid-washed plastic gerry cans and placed in storage at 4 ± 1°C within 1 h of collection. The water was then transported to Darwin in an air-conditioned vehicle. At the laboratory, the water was stored at 4 ± 1°C and filtered through Whatman #42 filter paper (2.5 μm pore size) within 4 d of collection.

## 3.2 Test sample water collection

A 22 L sample of RP2 water was collected from the Thompson B pump by EWLS and SSD staff on 25 March 2007. The outlet was flushed for 10 min prior to rinsing the acid-washed gerry container three times and collecting the sample. The sample bottle was filled and secured for transport by taping and signing the lids and containing it within a heavy duty plastic bag. Following overnight refridgeration in an ERA sample refrigerator, the sample was sent to Darwin on an early morning flight. SSD staff collected the sample from the airport and transported it to the laboratory where it was immediately filtered through Whatman #42 paper for testing.

The Pit 3 sample was collected on 16 April 2007 as described above, except that a grab sample was taken from the edge of a recently disturbed area of the pit. As no central pontoon-based pump was available, this was the only location in the pit from which a grab sample could be collected.

## 3.3 General laboratory procedures

All equipment in contact with test organisms, growth 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% v/v nitric acid ($HNO_3$) for 24 h before undergoing a detergent (Gallay Clean A non-phosphate powder, Gallay Scientific, Melbourne, Australia) wash and two rinses in a laboratory dishwasher with high purity Elix (Millipore, Molsheim, France) water. All reagents used were analytical grade and stock solutions were made up in high purity Milli Q (Millipore) water.

## 3.4 Toxicity test methods

Toxicity tests using the five species routinely tested at SSD (*Chlorella* sp, *Lemna aequinoctialis*, *Moinodaphnia macleayi*, *Hydra viridissima*, *Mogurnda mogurnda*) were used to determine the site-specific toxicity of RP2 water and to subsequently derive a protective dilution. The test procedures are summarised in Table 1 and described in greater detail below (for full test protocols refer to Riethmuller et al 2003). The dates that each of the tests were initiated, and the dilutions of of RP2 water tested, are summarised in Table 2.

**Table 1** Tropical freshwater species used to assess the toxicity of RP2 and Pit 3 water

| Test organism | Test duration and endpoint | Acute/chronic | Test protocol ID |
|---|---|---|---|
| *Chlorella* sp (unicellular alga) | 72 h cell division rate | Chronic | BTT G |
| *Lemna aequinoctialis* (duckweed) | 96 h plant growth | Chronic | BTT L |
| *Moinodaphnia macleayi* (cladoceran) | 3 brood (5–6 d) reproduction | Chronic | BTT D |
| *Hydra viridissima* (green hydra) | 96 h population growth | Chronic | BTT B |
| *Mogurnda mogurnda* (fish) | 96 h survival | Acute | BTT E |

**Table 2** Details of the toxicity tests undertaken to assess the toxicity of Ranger RP2 and Pit 3 water

| Sample tested | Test organism | Test code | Date initiated | Concentrations tested (% of water type)[a] |
|---|---|---|---|---|
| RP2 | *Chlorella* sp (unicellular alga) | 813G | 26/03/2007 | 0, 0.3, 1, 3, 10, 30% |
| | *Lemna aequinoctialis* (duckweed) | 812L | 26/03/2007 | 0, 0.3, 1, 3, 10, 30, 60% |
| | *Hydra viridissima* (green hydra) | 811B | 26/03/2007 | 0, 0.3, 1, 3, 10, 30% |
| | *Moinodaphnia macleayi* (cladoceran) | 815D | 27/03/2007 | 0, 0.3, 1, 3, 10, 30% |
| | *Mogurnda mogurnda* (fish) | 814E | 01/04/2007[b] | 6.25, 12.5, 25, 50, 100% |
| Pit 3 | *Lemna aequinocticalis* (duckweed) | 817L | 17/04/2007 | 0, 3.125, 6.25, 12.5, 25, 50, 100% |
| | *Hydra viridissima* (green hydra) | 819B | 17/04/2007 | 0, 0.75, 2.2, 6.7, 20, 60% |
| | *Moinodaphnia macleayi* (cladoceran) | 816D | 20/04/2007 | 0, 0.25, 0.75, 2.2, 6.7, 20% |

a The concentrations chosen for testing were based on contaminant concentrations in both the RP2 and Pit 3 waters in the month prior to testing (data provided by ERA & EWLS). Samples were diluted in natural Magela Creek water (NMCW).

b Note that the fish test was delayed by almost one week due to the lack of one day old larvae.

As the aim of testing the Pit 3 water was to gain an understanding of its relative toxicity compared to the RP2 water and to try to identify the primary toxicant (by comparing to single toxicant data for each species tested) rather than to derive a protective dilution, it was agreed that it would be sufficient to only test only the three species that were the most sensitive to RP2 water. Relative sensitivity was determined based on $IC_{10}$ estimates, as these were the values being used to derive the protective dilutions. The details of these tests are outlined in Table 2.

### 3.4.1 *Chlorella* sp 72 h algal growth test

*Chlorella* is a genus of simple green unicellular algae that has been shown to be fast growing under laboratory conditions. This has made it an ideal experimental organism for use in research on photosynthesis, nitrate reduction, physiology and biochemistry and has led to more practical uses in agri- and aquaculture (Wu et al 2001). The species maintained at the ***eriss*** ecotoxicology laboratory, *Chlorella* sp, was originally isolated from Kakadu National Park and has been used in assessing the toxicity of environmental contaminants over the past

eight years. During this period it has been shown to be a sensitive and reliable test organism. Being a primary producer and highly ubiquitous across tropical freshwaters, this genus is also of high ecological significance. The algal growth test measures growth of a standard number of algal cells exposed to a range of toxicant concentrations over 72 h under standard conditions. Effects of the toxicant are measured by comparing growth rate in the different toxicant treatments to that in a control.

Exponentially growing *Chlorella* sp cells were rinsed in diluent water (NMCW) three times using centrifugation. The cells were resuspended in 15 mL of diluent and the cell concentration was quantified by microscope using a haemocytometer. A calculated volume that resulted a final cell density of 3–4 × $10^4$ cells/mL was then dispensed into triplicate silanised 250 mL borosilicate glass Erlenmeyer flasks containing 50 mL of test solution. To support plant growth, $NaNO_3$ and $KH_2PO_4$ were added to achieve final concentrations of 14.5 mg $NO_3$/L and 0.14 mg $PO_4$/L, respectively. The flasks were randomly placed in an environmental cabinet and incubated at 29 ± 1°C on a 12:12 h light/dark cycle at 100–150 µmol photons PAR/$m^2$/s. After 48 h, a 5 mL sample of each solution was collected, diluted in an electrolyte solution (Isoton II) and enumerated using an automatic particle counter (Coulter Multisizer 3, Beckman Coulter, Miami, USA). This was repeated at 72 h, after which the water parameters (pH, EC and DO) of the solutions were measured and the test was terminated.

Cell densities were calculated from the counts by accounting for the dilution factor applied during sample preparation and the volume of sample drawn up into the particle counter. The growth rate was determined from these cell densities using linear regression. A regression line was plotted for $\log_{10}$ cell density vs time (h) to determine the slope of the line for each flask, which is equivalent to the cell division rate per hour (µ) for each treatment. Daily doubling times were calculated by multiplying this value µ × 24 × 3.32 (constant) and were statistically compared to establish differences between the control and treatment groups (see 'Statistical analysis').

### 3.4.2 *Lemna aequinoctialis* 96 h plant growth test

*Lemna aequinoctialis* is a small aquatic flowering macrophyte commonly known as duckweed. Duckweeds are ecologically relevant test organisms in that they are primary producers and a source of food for water fowl, fish and small invertebrates. Moreover, by floating in mats on the surface of still waters they provide habitat for a multitude of small organisms. Unlike many other of the evolutionary more complex plants, their small size and fast growth rates make them ideal for testing in the laboratory (Riethmuller et al 2003). The *L. aequinoctialis* plant growth test measures the growth rate of a standard number of vegetatively reproducing plants exposed to a range of concentrations of a toxicant for 96 h under controlled conditions. Effects of the toxicant are measured by comparing growth in the different toxicant treatments to that in a control.

Vegetatively reproducing plants (each with three fronds) were carefully removed from stock cultures using a plastic sterile inoculating loop and randomly placed into silanised 250 mL borosilicate glass Erlenmeyer flasks containing 100 mL of water sample. A total of four plants (ie 12 fronds) was added to each flask. To support plant growth, $NaNO_3$ and $KH_2PO_4$ were added to achieve final concentrations of 3 mg $NO_3$/L and 0.3 mg $PO_4$/L, respectively. The flasks were incubated for 96 h at 29 ± 1°C on a 12:12 h light/dark cycle at 100–150 µmol photons PAR/$m^2$/s.

At the completion of the test (96 h) the number of fronds in each test container was counted. The mean increase in numbers of fronds of *L. aequinoctialis* plants exposed to each treatment water were statistically compared (see 'Statistical analysis').

#### 3.4.3 *Hydra viridissima* 96 h population growth test

*Hydra viridissima* is a freshwater cnidarian (ie, in the same Phylum as the jellyfish) commonly known as 'green' hydra because of its green colouration resulting from the presence of a symbiotic green alga in the gastrodermal cells of the animal. Although the precise distribution of this species has not been mapped, it has been collected from a variety of aquatic habitats across northern Australia, including billabongs within the Magela Creek catchment. The *H. viridissima* population growth test measures the population growth rate of a standard number of asexually reproducing hydroids exposed to a range of concentrations of a toxicant for 96 h under controlled conditions. Effects of the toxicant are measured by comparing population growth in the different toxicant treatments to that in a clean water control.

Suitable test hydra, each bearing a newly-tentacled bud, were selected from the culture bowls using a dissecting microscope and transferred to three plastic petri dishes. Test hydra were transferred from the holding dishes into experimental petri dishes containing 30 mL of water sample using a Pasteur pipette, sequentially adding one animal to each treatment replicate until each contained ten hydroids. The test containers were then randomly placed in an environmental cabinet for 96 h at a temperature of 27 ± 1°C with a 12 h light:12 h dark photoperiod.

A fresh water sample (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, tentacle contraction and clubbing, discolouration) and the number of hydra were recorded daily for 96 h. Each hydra was fed 3–4 one 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 sample renewed with fresh treatment water.

At the completion of the test (96 h) the number of hydroids in each test container was counted and the growth rate (k) was calculated using the formula

$$\textbf{Growth rate (k)} = \frac{\ln(n_4) - \ln(n_0)}{T}$$

where $n_4$ = number of hydra at the end of the 4 d period

$n_0$ = number of hydra at the start of the 4 d period

$T$ = test duration (4 d)

The mean growth rates of *H. viridissima* exposed to each treatment water were statistically compared (see 'Statistical analysis').

#### 3.4.4 *Moinodaphnia macleayi* 3 brood (5–6 d) reproduction test

*Moinodaphnia macleayi* is a freshwater microcrustacean, commonly known as a water flea or cladoceran. The cladocera are ecologically relevant test organisms as they are filter feeding primary consumers and a source of food for water fowl, fish and other invertebrate predators. The *M. macleayi* reproduction test measures the number of offspring produced by parthenogenetically-reproducing female test cladocerans exposed to a range of concentrations of a toxicant over three reproductive broods (~6 d). Effects of the toxicant are measured by comparing neonate production in the different toxicant treatments to that in a control.

Suitable *M. macleayi* neonates (ie <6 h old) were collected and randomly transferred to two crystallising dishes. Individual neonates were transferred from the crystallising dishes into 45 mL test vials containing 30 mL of water sample using a Pasteur pipette, sequentially adding one animal to each treatment replicate. There were 10 replicate vials for each of the five treatment waters. The test containers were then randomly placed in an environmental cabinet and incubated at a temperature of 27 ± 1°C with a 12-h light:12-h dark photoperiod.

Fresh water sample (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 treatment water using a Pasteur pipette and microscope. Cladocerans were fed daily with the unicellular green alga, *Chlorella* sp ($2 \times 10^5$ cells $mL^{-1}$), as well as 1 µL of fermented food and vitamins (FFV) per mL of test solution.

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 NMCW control treatments produced their third brood (~6-d). The mean numbers of offspring per adult exposed to each treatment water were statistically compared (see 'Statistical analysis').

#### 3.4.5 *Mogurnda mogurnda* 96 h larval survival test

Gudgeons are distributed worldwide, mostly in tropical and subtropical latitudes of the Indo-Pacific (Allen et al 2002). The majority inhabit brackish estuaries or inland fresh waters while the remainder dwell in coastal seas. *M. mogurnda* is widely distributed across northern Australia, from the Mitchell River in WA to Mossman (Qld), occurring in rivers, creeks and billabongs, in quiet or slow-flowing sections among vegetation or rocks (Allen et al 2002). *M. mogurnda* has been collected from a wide variety of habitats within the Alligator Rivers Region, including floodplain billabongs, lowland shallow backflow billabongs, lowland sandy creeks, channel billabongs and escarpment mainchannel waterbodies (Bishop et al 2001).

Whilst fish are typically the least sensitive taxa used in ecotoxicity testing at SSD, they represent a wide range of species that inhabit northern waters, many of which have high social, cultural and economic value. In addition, the approach recommended by ANZECC/ARMCANZ (2000) is to test as many species from as wide as practicable range of taxonomic groups and trophic levels rather than only targeting sensitive taxa.

The *M. mogurnda* test involves assessing the survival of day old fish fry exposed to a arrange of toxicant concentrations over 96 h. Effects of the toxicant are measured by comparing fish fry survival in the different toxicant treatments to that in a clean water control.

Ten healthy sac fry were systematically transferred into plastic petri dishes containing 30 mL of test solution using a wide-mouth Pasteur pipette. The dishes were randomly assigned a position within an environmental cabinet and incubated for 96 h at a temperature of 27 ± 1°C with a 12 h light:12 h dark photoperiod. The dishes were removed from the cabinet daily at the test start time and observations on the fishes appearance, behaviour and mortality were recorded until the test was terminated at 96 h. At 96 h the mean number of fish surviving in each treatment were statistically compared (see 'Statistical analysis').

### 3.5 Water quality parameters

With the exception of the plant tests (which are static non-renewal tests), all treatment waters were replaced every 24 h with fresh sample. A 70 mL sub-sample of replacement water sample was collected at the time of dispensing, and the 24 h old water samples from each treatment replicate were pooled when the samples were changed. The pH, electrical conductivity (EC) and dissolved oxygen (DO) of both the fresh and 24 h old water samples

were then measured using WTW brand water parameter meters (Weilheim, Germany). For the plant tests, pH, EC and DO were measured at the beginning and end of the tests.

## 3.6 Water chemistry

Comprehensive sampling was undertaken to determine the chemical composition of the RP2, Pit 3 and Magela Creek waters and that filtration through 2.5 μm Whatman paper (to remove plankton from the samples) did not alter the chemical composition of the water. Samples of the test solutions were also taken to ensure that the dilutions used for the tests were accurate, that no chemical contaminants were introduced to the test solutions during preparation and the nutrient additions to the solutions used in the plants tests were accurate. In order to meet all of these requirements, the following water chemistry sampling and analysis regime was adopted:

- Metal suite (Al, Ca, Cd, Co, Cr, Cu, Fe, Mg, Mn, Na, Ni, Pb, $SO_4$, Se, U & Zn) measured on 0.45 μm filtered water and on acid-digested samples of RP2, Pit 3 and Magela Creek waters after filtration through 2.5 μm paper.
- Metal suite (as above) on unfiltered acid digested RP2 water that had not been filtered through 2.5 μm paper. This provides the total (particulate plus dissolved) metal concentrations in the original unprocessed water sample.
- $NO_3$, $NO_2$, $PO_4$ and $NH_3$ only measured on 0.45 μm filtered water and total N and P only measured on acid digested samples of RP2, Pit 3 and Magela Creek waters after filtration through 2.5 μm paper.
- Metal suite (as above) on unfiltered and undigested procedural blanks (Milli-Q water that had been subjected to the test solution preparation method) and the Magela Creek water control in each toxicity test to check for contaminants.
- Mg only on unfiltered, undigested samples of each of the treatment solutions used in the toxicity test to determine the accuracy of the dilutions undertaken.
- $NO_3$ and $PO_4$ on unfiltered, undigested samples of the Magela Creek control water used in the plant toxicity tests.
- Appropriate Milli-Q blanks were submitted for all of the sample manipulations and analytical methods undertaken.

### 3.6.1 Metals

Samples to be analysed for dissolved metals were filtered through a 0.45 μm regenerated cellulose filter (Sartorius) using a 50 mL Terumo syringe (Elkton) directly into a 70 mL high density polyethylene (HDPE) sample bottle (Azlon). Prior to filtering the water samples, the syringes and filters were rinsed twice with Milli-Q water. The filtered sample was acidified to 0.7% $HNO_3$ by adding 10 μL of 69% Aristar $HNO_3$ (BDH) for every mL of sample.

Samples for total metal analysis were dispensed directly into 70 mL HDPE sample bottles and acidified to approximately 3.5% $HNO_3$ by adding 50 μL of 69% Aristar $HNO_3$ for every mL of sample.

The samples for both soluble and total metals analysis were stored at 4 ± 1°C until until sent to the Northern Territory Environmental Laboratories (NTEL, Berrimah, Northern Territory, Australia) for analysis.

### 3.6.2 Nutrients

Samples to be analysed for soluble nutrients ($NO_2$, $NO_3$, $NH_3$, $PO_4$) were filtered through a 0.45 μm PVDF filter (Millex-HV, Millipore) using a 50 mL Terumo syringe. Prior to filtering the sample for analysis, syringes and filters were rinsed with two aliquots of MilliQ water. Sample bottles (70 mL HDPE) were squeezed to remove air, then capped and frozen, and sent for analysis at NTEL. Samples for total N and P analyses were dispensed directly into bottles and frozen with no air space.

### 3.6.3 Dissolved organic carbon and alkalinity

As is standard for each batch of diluent water collected, samples (500 mL) of both filtered (2.5 μm) and unfiltered Magela Creek water, together with a blank, were taken into separate 500 mL HDPE (Azlon) plastic bottles and sent to the Australian Water Quality Centre (Bolivar, South Australia) within one week for analysis of dissolved organic carbon and alkalinity.

## 3.7 Quality assurance

### 3.7.1 Water quality

For each test, data were considered acceptable if: the recorded temperature of the incubator remained within the prescribed limits (see test descriptions, above); the recorded pH was within ± 0.5 unit of values at test commencement (ie Day 0); the EC for each test solution was within 10% of the values at test commencement; and the DO concentration was greater than 70% throughout the test.

For each test, Milli Q water and procedural blanks were analysed as described above. Chemistry data for the blanks were initially assessed by searching for analyte concentrations higher than detection limits. If any of these concentrations were greater than 2 μg/L, duplicate blank samples were re-analysed and/or the control water concentrations were compared to those in tests without blank contamination to determine if the contamination was limited to the one sample bottle or experienced throughout the test. Consequences of any contamination were then investigated and addressed on a case-by-case basis.

### 3.7.2 Control responses

Tests were considered valid if the control organisms met the following criteria.

Chlorella *sp algal growth test*

- The growth rate of the control algae was within the range of 1.4 ± 0.3 doublings/day.
- There was < 20% variability (co-efficient of variation, CV < 20%) in the control growth rate.

L. aequinoctialis *plant growth test*

- The average increase in frond number in any control flask at test conclusion was at least four times that at test start (ie increase of 48 fronds/flask).
- There was < 20% variability (CV < 20%) in the control growth rate.

H. viridissima *population growth test*

- Healthy hydroid numbers in the control averaged 30 or more (ie $k > 0.27$ day$^{-1}$) over the test period.
- There was < 20% variability (CV <20%) in the control growth rate.

M. macleayi *3 brood reproduction test*

- 80% or more of the cladocera were alive, were female and produced three broods at the end of the test period.
- Reproduction in the control averaged 30 or more neonates surviving per female over the test period.
- There was < 20% variability (CV <20%) in control neonate production.

Mogurnda mogurnda *survival test*

- The mean mortality of the combined control did not exceed 20%.
- There was < 20% variability (CV <20%) in control survival.
- The presence of fungus on the sac fry did not exceed 20% in the control animals.

### 3.7.3 Reference toxicant testing

Reference toxicity testing provides a means of monitoring the response, over time, of a test species to a standard toxicant. An ongoing reference toxicity testing program is in place in the ***eriss*** ecotoxicology laboratory to enable the detection of any changes in the health or sensitivity of the organisms routinely used in testing. Control charts exhibiting upper and lower 'warning' (ie ± two standard deviations) and 99% confidence limits (ie. ± three standard deviations) based on the tests' IC/EC/$LC_{50}$ values (ie. values resulting in a 50% response in the measured endpoint relative to the control response) have been developed for four of the five test species assessed in the present study, namely *Chlorella* sp, *H. viridissima*, *M macleayi* and *M. mogurnda*.

Test results that lie within the upper and lower warning limits are considered acceptable and require no additional investigation. Results from reference toxicity tests conducted as close as practicable prior to and following the present study were used to determine the acceptability of the performance of the species during the testing program. At the time of the present study, a reference toxicity test protocol for *L. aequinoctialis* was under development, and hence, performance of this species was based primarily on the response of plants in the control treatment.

## 3.8 Statistical analysis

The estimates that are used to determine the toxicity of waters to the five species used at SSD are:

- the no-observed-effect concentration (NOEC);
- the lowest-observed-effect concentration (LOEC);
- the concentration resulting in a 10% inhibition of response compared to the control organisms ($IC_{10}$), and, for *M. mogurnda*, the concentration resulting in 5% mortality ($LC_{05}$); and
- the concentration resulting in a 50% inhibition of response compared to the control organisms ($IC_{50}$), and, for *M. mogurnda*, the concentration resulting in 50% mortality ($LC_{50}$).

The NOEC and LOEC from each test were determined using one-way analysis of variance (ANOVA) and Dunnett's *post hoc* test (except for the *M. mogurnda* test, where there was no mortality at any concentration – see Section 4.1.3), using the statistical software Toxcalc V.5.0.23F (Tidepool Scientific Software). The NOEC represents the highest test concentration at which the response of the organisms was *not* statistically different from the

response of the control organisms ($\alpha$ = 0.05), while the LOEC represents the lowest test concentration at which the response of the organisms was statistically different from the response of the control organisms ($\alpha$ = 0.05). Because the statistical test compares the various test concentrations to the control treatment, the NOEC and LOEC values can only be one of the concentrations/dilutions that were tested.

The $IC_{10}$ and $IC_{50}$ were determined for all species except the fish (*M. mogurnda*), using linear interpolation (Toxcalc V.5.0.23F, Tidepool Scientific Software), which involves fitting straight lines between each successive concentration (and 'smoothing' or averaging where non-monotonic responses exist) then interpolating the relevant 'effect' or 'inhibition' size of interest (associated confidence intervals are calculated using a process known as bootstrapping). The $IC_{10}$ (or $LC_{05}$, in the case where the response being measured is lethality) was selected as it is considered to be a reasonable estimate of an acceptable effect size (ie. one that is unlikely to be ecologically relevant and, statistically difficult to detect), and therefore can be thought of as an 'acceptable' concentration (or dilution) that is unlikely to result in significant population-level effects. Therefore, such values can be used to give an indication of species *protection*. At present, the use of 'low-effect' IC values (eg. $IC_{10}$) for characterising acceptable levels of contaminants is considered a more robust approach than the use of NOECs (van der Hoeven 1997; OECD 1998).

Finally, analysis of covariance (ANCOVA; $\alpha$ = 0.05) was used to compare the toxicity of RP2 and Pit 3 water to each of the three species assessed for both waters (ie *M. macleayi*, *H. viridissima*, *L. aequinoctialis*). The toxicity raw data for each species were expressed as the 'percent of control response', and transformed (arcsine or [natural] log) to ensure the data were normally distributed, before being analysed (NB: for the *M. macleayi* test, the RP2/Pit 3 water dilutions had to be $\log_{10}$ transformed in order to ensure the dataset was normally distributed).

## 3.9 Determining a protective dilution for discharge

In order to predict the dilution of RP2 water required to protect a given percentage of species, a species sensitivity distribution was constructed using the toxicity data for the five different species tested. A species sensitivity distribution is a statistical distribution describing the variation among a set of species in the toxicity of a certain compound or mixture. The rationale behind, and assumptions of, species sensitivity distributions are discussed in detail in ANZECC/ARMCANZ (2000) and Posthuma et al (2002). Such distributions are commonly used for probabilistic risk assessment (US EPA 1998) and are also used to derive toxicant trigger values in Australia and New Zealand (ANZECC/ARMCANZ 2000).

For the reasons outlined in Section 3.8, $IC_{10}$ toxicity values (or $LC_{05}$ values for *M. mogurnda*[1]) were used as the input data to construct the species sensitivity distribution rather than the hypothesis testing estimates (eg NOEC; ANZECC/ARMCANZ 2000). A distribution based on $IC_{10}/LC_{05}$ data was constructed based on the Burr Type III family of distributions, using the BurrliOZ© software developed for ANZECC/ARMCANZ (2000). The Burr Type III family of distributions provides greater flexibility in the range of shapes to be fitted, for example, with some of the more traditionally applied models such as the lognormal

[1] According to ANZECC/ARMCANZ (2000), the standard approach for incorporating acute toxicity data with chronic toxicity data for deriving trigger values is to use the estimate that results from dividing the acute $LC_{50}$ by an acute-to-chronic ratio of 10. However, we have found that the $LC_{05}$, without application of an acute-to-chronic ratio, represents a meaningful and sufficiently conservative value for incorporation with chronic data.

and loglogistic distributions (ANZECC/ARMCANZ 2000; Shao 2000). The concentration of sample that would be protective of a specified percent of species, in this case 99% due to the high conservation value of the receiving catchment, was extrapolated from this model and recommended as the 'protective' dilution rate.

# 4 Results and discussion

## 4.1 RP2 testing

### 4.1.1 Water chemistry

The physical and chemical characteristics of RP2 water are provided in Appendix 1. The pH of the sample collected for ecotoxicological testing was near neutral at 6.83. EC was high compared to NMCW at 1205 μS $cm^{-1}$, and DO was 92.6% saturation.

Concentrations of $NO_3$ and $PO_4$ in RP2 water were around half that which is added to the two plant tests to support growth. Thus, it is possible that plant responses to the lower dilutions of RP2 water may have been influenced by the nutrient concentrations present in the test water.

Filtered concentrations of the major inorganic contaminants of potential toxicological concern were 38 μg/L Al, 12 μg/L Co, 14 μg/L Cu, 159 mg/L Mg, 397 μg/L Mn, 40 μg/L Ni, 3.9 μg/L Pb, 355 mg/L $SO_4$, 14 μg/L Se, 1870 μg/L U and 32 μg/L Zn. Concentrations of these metals in the associated Milli-Q blanks (for both filtered and total metals) were mostly below detection limits, and all were below 0.5 μg/L. The occurrence of some reverse trends when comparing total and filtered metal concentrations, most obviously for Ni, was noted (see Appendix 1). This was discussed with the analytical laboratory and has been attributed to the filtered sample being run as a 1:10 dilution, which can lead to a greater degree of error for trace concentrations, while the total digested sample was analysed without dilution.

Total metal concentrations in the RP2 sample prior to filtration through 2.5 μm paper were similar (within 10%) to that post filtration with the exception of Al and Pb. Al was reduced from 57 to 27 μg/L and Pb from 9.6 to 6.9 μg/L. Al is relatively insoluble at pH 6 to 8 (Gensemer & Playle 1999), while Pb is known to strongly adsorb to suspended matter (ANZECC/ARMCANZ 2000).

Given the concentration of U in RP2 water (1870 μg/L) and the known site-specific toxicity of U to the species used in testing (Table 3), it was expected that U would be the major contaminant contributing to any observed toxicity. Toxicity due to Mg (present at 159 mg/L) was considered less likely given the Mg:Ca (mass) ratio was ~7:1, which is sufficiently low to prevent Mg toxicity to all the species, except *M. macleayi* (ie $IC_{50}$ ~137 mg/L at Mg:Ca 9:1), at the Mg concentration present in the RP2 water sample (van Dam et al 2006a).

There are few site-specific toxicity data for the other metals measured in the RP2 water, making it difficult to gauge whether they were likely to contribute to the overall toxicity of the sample. Whilst the concentrations of potentially toxic metals such as Co, Cu, Ni, Pb, Se and Zn exceeded ANZECC/ARMCANZ (2000) 99% species protection trigger values (non-hardness corrected), they were present in very low concentrations relative to U, and were not considered further unless the observed toxicity could not be mostly explained by U.

With the main exception of U, the inorganic composition of the RP2 water was similar to that reported by ERA for the weeks leading up to the ecotoxicological testing (Appendix 2). In a sample collected by ERA on 7 March 2007, total (2770 μg/L) and filtered (2360 μg/L) values for U were substantially higher than those measured in the sample collected on 25 March 2007 for toxicity testing (total – 2130 μg/L ; filtered – 1870 μg/L). This highlights the need

for a good understanding of the spatial and temporal variation in the chemical composition of on-site waterbodies being sampled for pre-release testing. It is critical that the sample taken for toxicity testing is either representative of the water body as a whole or else represents the upper concentration limit of the water that is to be released, to make a reliable prediction of the likely ecological effects downstream of the mine.

**Table 3** Existing site-specific toxicity data for U (μg/L) in natural Magela Creek water to the five tropical freshwater species tested. Data selected were as representative as possible of the tests and endpoints used for the present study.

| Species | Endpoint | $IC_{10}$ | $IC/LC_{50}$ | NOEC | LOEC | Reference |
|---|---|---|---|---|---|---|
| *Chlorella* sp. | 72 h cell division rate | - | 176 (160–190) | 117 | 153 | Hogan et al (2005) |
| *Lemna aequinoctialis* | 96 h plant growth | 187 (130–228) | 504 (449–572) | 216 | 247 | van Dam et al (2006b) |
| *Hydra viridissima* | 96 h population growth | ~130–140[a] | ~180–>340[a] | 160 | 190 | ARRRI (1988); Hyne et al (1992) |
| *Moinodaphnia macleayi* | 3 brood reproduction | 24 (2.0–27) | 67 (49–83) | 18 | 54 | SSD unpubl. data (March 2007) |
| | | 2.6 (1.7–9.9) | 16 (14–19) | 10 | 17 | SSD unpubl. data (July 2007) |
| *Mogurnda mogurnda* | Mortality (96 h exposure, 6-d old fish, unfed) | - | 1570 (1215–1840) | - | - | Holdway (1992) |
| | | - | | - | - | |
| | Mortality (7 d exposure, 1-d old fish, unfed) | - | 1590 (1520–1660) | - | - | Holdway (1992) |
| | Mortality (96 h exposure, 1-d old fish, fed) | | 2206 (2166–2248) | | | Cheng unpubl. data (April 2007) |

a $IC_{10}$ and $IC_{50}$ values for *H. viridissima* estimated from data reported by ARRRI (1988) or Hyne et al (1992) using non-linear regression.

The physical and chemical characteristics of the Magela Creek diluent water (Appendix 1) were within the range typically observed in the creek (Klessa 2000, Iles 2004, SSD unpublished data).

### 4.1.2 Quality assurance

*Water quality*

General water parameters, pH, EC and DO were generally within the range considered acceptable during toxicity testing, and are provided in Appendix 3. The only exceptions were pH measurements in the lower treatments in the *Chlorella* sp and *L. aequinoctialis* tests, which varied slightly more than the criterion (± 0.5 units from initial test solution pH), with a maximum variation of 0.6 and 0.7 units, respectively. The increase in pH over the test duration was likely due to utilisation of carbon dioxide by the test plants. This may have had consequences for the speciation, bioavailability and toxicity of uranium and other metals in the RP2 water. One pH measurement in the *M. mogurnda* test appeared to be erroneous as it was approximately 1.1 units higher than that measured on 'new' waters on subsequent days and 0.9 units higher than the same water sample measured the following day from the fish test containers (24 h old water). The fact that the measured pH in the 24 h old water was well within the acceptable range indicates that the higher measurement is likely to be due to a contaminant in the water parameter vial and that the water the fish were exposed to was of acceptable quality.

Variation in EC measurements was higher than that specified in the criteria (± 10% of initial EC) in many of the low EC (<25 μS $cm^{-1}$) treatments. This is commonly found to be the case as the test organisms themselves may contribute 2–5 μS $cm^{-1}$ of salt to the test solution. As such, where an increase in EC was < 5 μS $cm^{-1}$ or within 10% of the initial EC it was considered acceptable.

Metal concentrations were below detection limits in the majority of Milli-Q blanks and procedural blanks sampled at the start of each toxicity test. In the few samples where low level contaminantion was observed, the highest concentrations of each metal measured were: 1.2 μg/L Al and 0.35 μg/L Cu for the *H. viridissima* test; 1.1 mg/L Na, 0.04 μg/L Pb, 0.182 μg/L U and 1.1 μg/L Zn for the *Chlorella* sp test; and 0.71 μg/L Ni for the *M. mogurnda* test. This degree of contamination in toxicity testing, where various manipulations of the test sample occur during test set-up, is considered acceptable and mostly unavoidable. Regardless, such low level contamination is highly unlikely to affect the test organisms.

Measured Mg concentrations in each of the solutions prepared for testing were within 10% of the expected concentration based on that in the undiluted RP2 water, indicating that the dilutions undertaken for testing were accurate.

Measured $NO_3$ and $PO_4$ concentrations were within 13% of the nominal concentrations spiked into the test solutions for the *Chlorella* sp and *L. aequinoctialis* tests, which is within the range normally measured for the plant tests.

*Control responses*

All test organism control responses were considered acceptable as:

- Growth in the *Chlorella* sp. control was 1.44 doublings $day^{-1}$ and the CV was 3%;
- The mean increase in frond number in the *L. aequinoctialis* control was 59 and the CV was 2%;
- The mean number of *H. viridissima* in the control was 32.3 (which equates to a mean growth rate (k) of 0.29 $day^{-1}$ and the CV was 17%;
- The control *M. macleayi* produced a mean number of 39.6 neonates each and the CV was 4%;
- There were no mortalities or fungus infected fish in the *M. mogurnda* test controls and as such there was no variability in the control response.

*Reference toxicity testing*

Table 4 shows the results of reference toxicity tests conducted as close as practicable to the RP2 toxicity testing, in comparison with the long-term running mean toxicity value (as at September 2007). Reference toxicant data cannot be provided at present for *L. aequinoctialis* due to ongoing reference toxicity test development. For the remaining four species, all reference toxicity test results (ie $IC/EC/LC_{50}$ estimates) were within the acceptable range based on the reference toxicant control charts (ie within 2 standard deviations of the running mean $IC/EC/LC_{50}$).

It should be noted that, at present, there is a 'watching brief' over the reference toxicant testing program for *M. macleayi*. After the first two tests of the reference toxicity testing program for this species (mean $EC_{50}$ ± SEM = 158 ± 36 μg/L U), which only commenced in July 2006, there appeared to be an increase in sensitivity of *M. macleayi* to U. The subsequent seven reference toxicity tests have yielded a mean $EC_{50}$ ± SEM of 46 ± 7 μg/L U. At present, it is difficult to know whether there has been an unexplainable increase in sensitivity of the *M. macleayi*

laboratory stock, or whether in fact the first two toxicity results were anomalous. There exist no acute toxicity data in synthetic Magela Creek water prior to July 2006 that can be used as a benchmark, while comparisons between recent and past U chronic toxicity (3 brood reproduction) tests in natural Magela Creek water are inconclusive at this stage. Consequently, the response of *M. macleayi* to U will continue to be monitored through the ongoing reference (acute) toxicity testing program as well as additional chronic toxicity testing.

**Table 4** Results of reference toxicity tests for four species used to assess the toxicity of RP2 water

| Species | Date of test | Uranium toxicity (µg/L) | | Result acceptable? (Yes/No) |
|---|---|---|---|---|
| | | Test $IC/EC/LC_{50}$ | Running mean $IC/EC/LC_{50}$ (±2 SD)[a] | |
| *Chlorella* sp (unicellular alga) | 19/06/07 | 53 | 52 (±31) | Yes |
| *Hydra viridissima* (green hydra) | 29/01/07<br>04/06/07 | 77<br>109 | 95 (±36) | Yes<br>Yes |
| *Moinodaphnia macleayi* (cladoceran) | 20/02/07<br>08/05/07 | 47<br>48 | 71 (±109) | Yes[b]<br>Yes[b] |
| *Mogurnda mogurnda* (fish) | 12/01/07<br>25/06/07 | 1690<br>1570 | 1670 (±578) | Yes<br>Yes |

a Running mean $IC/EC/LC_{50}$ as at September 2007.

b See text for further discussion of reference toxicity testing results for *M. macleayi*.

### 4.1.3 Toxicity tests

The toxicity estimates for each species, calculated using point estimation ($IC_{10}$s and $IC_{50}$s) and hypothesis testing (NOECs and LOECs) are presented in Table 5, while the concentration-response plots for each species are shown in Figure 1 and again, combined, in Figure 2. Raw data and statistical summaries are provided at Appendix 5. Only one of the four species, the fish, *M. mogurnda*, did not respond to the sample. Of the remaining four species, the cladoceran, *M. macleayi*, was found to be the most sensitive with a 10% effect on neonate production ($IC_{10}$) at 0.6% RP2 water and a 50% reduction ($IC_{50}$) at 1.8% RP2 water. The unicellular alga, *Chlorella* sp, the duckweed, *L. aequinoctialis* and the hydra, *H. viridissima* were also found to be highly sensitive to the RP2 water with $IC_{10}$ values of 7.5, 2.1 and 3.5% RP2 water, and $IC_{50}$ values of 22.4, >60 and 6.9% RP2 water, respectively.

Based on $IC_{50}/LC_{50}$ values, the order of sensitivity of the five test species was:

*M. macleayi* >> *H. viridissima* > *Chlorella* sp > *L. aequinoctialis* >> *M. mogurnda*

**Table 5** Summary of toxicity of RP2 water to the five tropical freshwater species tested

| Species | RP2 water toxicity (% RP2 water) | | | |
|---|---|---|---|---|
| | $IC_{10}$ (95%CL) | $IC_{50}$ (95%CL) | NOEC | LOEC |
| *Chlorella* sp (unicellular alga) | 7.5<br>(0–16) | 22<br>(18–26) | 10 | NR[a] |
| *Lemna aequinoctialis* (duckweed) | 2.1<br>(0.4–13) | >60<br>(not calculable) | 3 | 10 |
| *Hydra viridissima* (green hydra) | 3.5<br>(0–4.3) | 6.9<br>(5.0–8.9) | 3 | NR |
| *Moinodaphnia macleayi* (cladoceran) | 0.6<br>(0.2–0.8) | 1.8<br>(1.8–1.9) | 0.3 | 1.0 |
| *Mogurnda mogurnda* (fish) | >100 | >100 | ≥100 | >100 |

a NR: Not reported, due to LOEC being greater than the $IC_{50}$ value.

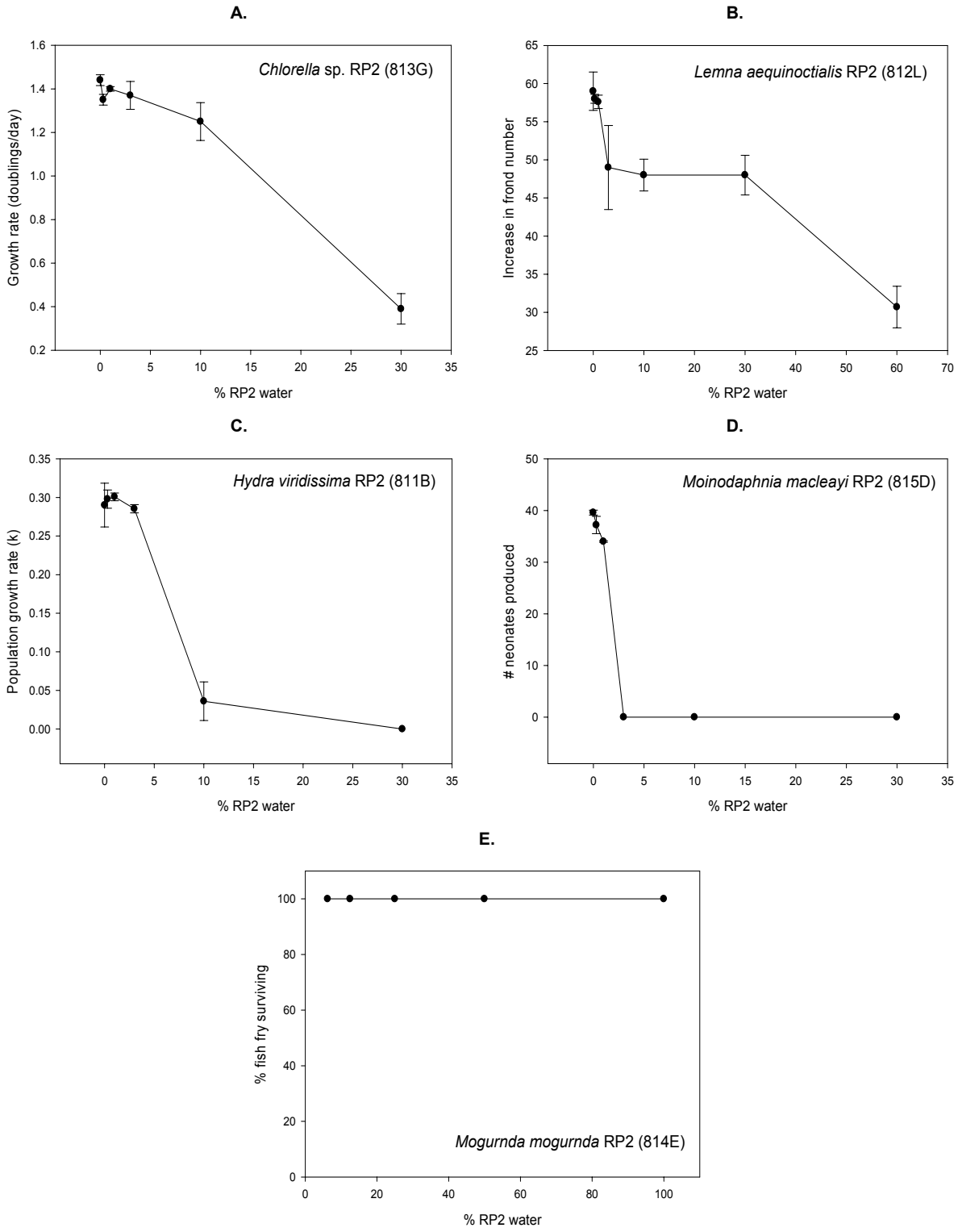


**Figure 1** Concentration-response plots showing the toxicity of RP2 water to the five tropical freshwater species tested. Data points represent the mean ± standard error (n = 3, except for *M. macleayi* where n = 10).

The responses of three of the four species that were affected by RP2 water generally increased in magnitude with increasing concentration (Figure 1). In contrast, *L. aequinoctialis* displayed an unusual response, where a 20% effect on plant growth was observed at only 3% RP2 water, after which the effect plateaued, with growth remaining quite stable up to 30% RP2 water. Such a plateau effect has not been previously observed in U only tests with

*L. aequinoctialis*, and was also not observed for Pit 3 water (see below), and suggests that U may not have been the only toxicant eliciting an effect, and/or that the toxicity of U, and/or other metals, was affected by interactions with other constituents (eg nutrients, major ions/conductivity) in the sample.

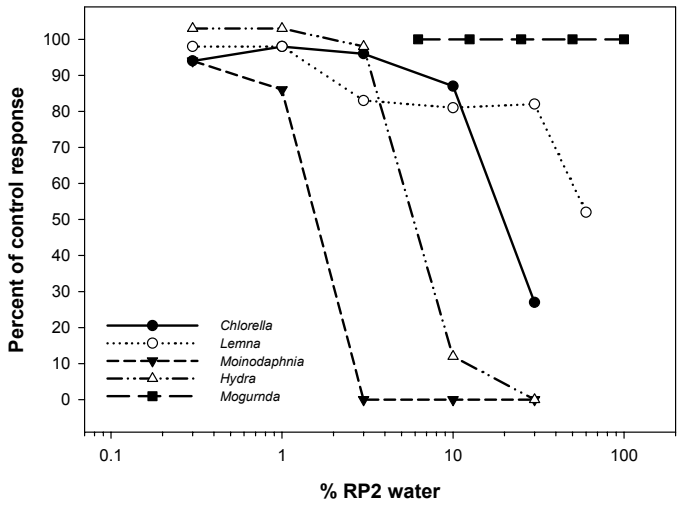


**Figure 2** Combined concentration-response plot for each species, presented as a % of the control response with regard to RP2 water concentration. Standard error bars removed to aid visual interpretation.

Table 6 provides a comparison for each species of the previously reported $IC_{50}$ values for uranium alone (based on values from Table 3) with the U concentrations corresponding to the RP2 water $IC_{50}$ values. As can be seen, the toxicity of RP2 water was somewhat different to what would be predicted based on the toxicity of U. RP2 water appeared to be slightly more toxic to *M. macleayi* (although see below) and *H. viridissima*, and less toxic to *Chlorella* sp and *L. aequinoctialis*, than would be predicted based on the U toxicity data. The lack of a toxic effect of RP2 water to *M. mogurnda* was consistent with recent U toxicity data for this species, which observed almost 100% survival at 1400 μg/L, and an $LC_{50}$ of 2206 μg/L (K Cheng, unpublished data).

**Table 6** Comparison of historical U $IC_{50}$ values (μg/L) with the concentrations of U (μg/L) corresponding to the $IC_{50}$ values of RP2 water

| Species | U $IC_{50}$ (95%CL)[a] | [U] at RP2 water $IC_{50}$ (95%CL)[b] | Comments |
|---|---|---|---|
| *Chlorella* sp. (unicellular alga) | 176 (160–190) | 419 (346–496) | RP2 water less toxic than would be predicted based on U concentration/toxicity alone. |
| *Lemna aequinoctialis* (duckweed) | 504 (449–572) | >1120 | RP2 water less toxic than would be predicted based on U concentration/toxicity alone.[f] |
| *Hydra viridissima* (green hydra) | 180–>340 (-)[c] | 129 (101–153) | RP2 water more toxic than would be predicted based on U concentration/toxicity alone. |
| *Moinodaphnia macleayi* (cladoceran) | 67 (49–83)[d] | 34.4 (33.8–34.8) | RP2 water more toxic than would be predicted based on U concentration/toxicity alone.[f] |
| *Mogurnda mogurnda* (fish) | 2206 (2166–2248)[e] | >1870 | Lack of RP2 water toxicity consistent with U concentration/toxicity |

a Based on values reported and explained in Table 3.

b Based on the values reported in Table 5.

c 95% CLs could not be calculated for these data

d Used $IC_{50}$ value from toxicity test done during the same month as the RP2 water toxicity testing.

e Used 96-h $LC_{50}$ value from 28 day larval growth toxicity test done during April 2007, the month after the RP2 water toxicity testing.

f See text for additional discussion.

Whilst *L. aequinoctialis* appeared to be less sensitive to RP2 water than would be predicted based on the U $IC_{50}$ data, the initial 20% effect observed at as low as 3% RP2 water was greater than would be predicted based on U toxicity. As expressed above, this response was unusual and difficult to explain.

The range of reported toxicity values for U in natural Magela Creek water to *M. macleayi* makes it difficult to be conclusive about comparisons with whole-of-effluent RP2 water toxicity. For example, U toxicity tests conducted in March and July 2007 resulted in $IC_{50}$ values of 67 and 16 µg/L, respectively (see Table 3). Further highlighting the large range in laboratory test values, Semaan et al (2001) reported a U NOEC range for *M. macleayi* of 8–46 µg/L, while the reference toxicity testing program for this species has also yielded variable results (see Section 4.1.2). A U $IC_{50}$ value of 67 µg/L was chosen for the comparison with RP2 water toxicity because the toxicity test from which the $IC_{50}$ was derived was conducted during the same month as the RP2 water toxicity testing. However, if the U $IC_{50}$ value of 16 µg/L (from the test conducted in July 2007) was compared to the RP2 water toxicity, the outcome of the comparison would have been that RP2 water was less toxic to *M. macleayi* than would be predicted based on the U toxicity data. Consequently, the above comparisons of RP2 water toxicity with U toxicity should be considered with caution.

The fact that RP2 water toxicity did not necessarily correlate to reported U toxicity is not unusual or unexpected, and was potentially due to several factors, including contributions to toxicity from other potentially toxic metals (eg copper, lead, nickel, zinc) and/or, most likely, the range of physico-chemical interactions that can occur in, and influence the toxicity of, complex mixtures, but which are not accounted for in single toxicant toxicity testing. The ability to undertake assessments that integrate such factors to give an understanding of how whole organisms respond to a complex sample is the key benefit of direct toxicity assessment using whole effluent (van Dam & Chapman 2001, Warne 2003).

### 4.1.4 Recommendations for release

The species sensitivity distribution for RP2 water for all five species tested is shown in Figure 3. Note that $IC_{10}$ data from the alga, duckweed, hydra and cladoceran toxicity tests (ie the chronic tests) were used for the species sensitivity dataset. For *M. mogurnda*, however, no corresponding toxicity estimate (ie. $LC_{05}$) could be calculated from the acute test because RP2 water had no effect on larval survival. Therefore, a conservative toxicity estimate for *M. mogurnda*, of half that of the highest concentration tested, being 50% RP2 water, was used for the species sensitivity dataset. The implications of this are briefly discussed below.

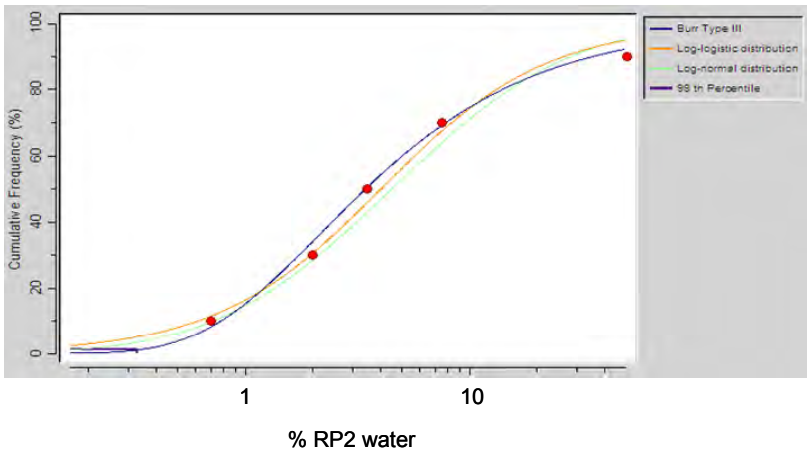


**Figure 3** Species sensivity distribution (from BurrliOZ©) based on $IC_{10}/LC_{05}$ values (assuming a value of 50% for *M. mogurnda*). A concentration of 0.33% RP2 water (approximately a 1 in 300 dilution) was predicted to protect 99% of species with 50% confidence. This equates to approximately 6 µg/L U.

With regard to species protection, ANZECC/ARMCANZ (2000) recommend a 99% level of species protection for areas of high conservation value. Based on the species sensitivity distribution, a protective dilution of 0.33% (1 part RP2 water to ~300 parts Magela Creek water) would be expected to ensure the appropriate level of protection for the downstream aquatic ecosystem. Uranium concentration at this dilution would equate to 6 μg/L, which is the same as the current site-specific trigger value of 6 μg/L for U in Magela Creek.

To gauge the implications of using the conservative toxicity estimate of 50% RP2 water for *M. mogurnda*, the protective dilution was recalculated substituting the 50% estimate with a value of 100% RP2 water (ie. the highest concentration tested and at which no effect on larval survival was recorded). The resultant 99% species protection dilution of 0.3% RP2 water (or 1 in 330) was very similar to the dilution of 0.33% RP2 water derived from the dataset incorporating the toxicity estimate of 50% for *M. mogurnda*. Consequently, the exact value of the *M. mogurnda* toxicity estimate was not critical to the estimation of the protective dilution.

## 4.2 Pit 3 testing

### 4.2.1 Water chemistry

The physical and chemical characteristics of the Pit 3 water are provided in Appendix 1. The pH of the sample collected for ecotoxicological testing was 7.66 units. EC was much higher than NMCW at 893 μS $cm^{-1}$, and DO was at 92.6% saturation.

As with the RP2 sample, nutrient concentrations (1.4 mg/L $NO_3$ and 0.02 mg/L $PO_4$) were sufficient to potentially have some positive influence on *L. aequinoctialis* growth at higher concentrations, although it was expected that the toxicity of the sample would overide any beneficial effects of the nutrients.

Filtered concentrations of the major inorganic contaminants of toxicological concern were 12 μg/L Al, 2.3 μg/L Cu, 107 mg/L Mg, 33 μg/L Mn, 4.7 μg/L Ni, 0.09 μg/L Pb, 445 mg/L $SO_4$, 8.4 μg/L Se, 1620 μg/L U and 2.8 μg/L Zn. Concentrations of these metals were mostly below detection limits, but all were at or below 0.6 μg/L in the associated Milli-Q blanks (for both filtered and total metals).

As expected from Pit 3 water chemistry data provided by EWLS prior to the commencement of the toxicity testing (from 28/03/07), the Pit 3 water quality was generally better than the RP2 water collected for toxicity testing. However, the U concentration measured in the sample collected on 16/04/07 for the toxicity testing (1620 μg/L) was somewhat higher than expected based on the late March measurement (1300 μg/L), and in fact was reasonably similar to the U concentration in the RP2 water that was tested (1890 μg/L).

### 4.2.2 Quality assurance

*Water quality*

The general water parameters, pH, EC and DO were generally within the range considered acceptable during toxicity testing, and are given in Appendix 4. The only exceptions were that pH measurements in some of the *L. aequinoctialis* treatments, varied unacceptably, by almost 1 unit, and this may have had consequences for the speciation, bioavailability and toxicity of uranium and other metals in the Pit 3 water. There was more than 10% variation in EC of the lower Pit 3 water concentrations in the *H. viridissima* and *M. macleayi* tests, the significance of which was described in Section 4.1.2 for the RP2 tests. Finally, pH in the control treatment water over the first 24-h of the *M. maclaeyi* test increased by over 1 unit (ie 6.02–7.09). However, when compared to the other pH measurements recorded during the test, the 24-h result of 7.09 appears highly anomalous and was almost certainly an erroneous measurement.

Metal concentrations were below detection limits in the majority of Milli-Q blanks and procedural blanks sampled at the start of each toxicity test. In the few samples where low level contaminantion was observed, the highest concentrations of each metal measured were: 0.57 µg/L Cu for the *H. viridissima* test and 0.2 µg/L Al, 0.13 µg/L Ni, 0.11 µg/L Pb and 0.037 µg/L U for the *L. aequinoctialis* test.

While it was intended that Mg concentrations would be used to determine the accuracy of the dilutions undertaken for testing (as per the RP2 water toxicity testing), analyses were mistakenly requested for U, rather than Mg in these samples. The U measurements confirmed that the dilutions were accurate.

The nutrient concentrations measured in the *L. aequinoctialis* test waters were within 6% of the nominal concentrations, indicating accurate addition of nutrients at the start of the test.

*Control responses*

Growth in the control treatment of the *L. aequinoctialis* test (increase of 41 fronds) was slightly lower than the minimum acceptability criterion (increase of 48 fronds). This is an uncommon response for *L. aequinoctialis* tests conducted in Magela Creek water, though has occurred previously when a proportion of the plants selected to initiate the test possess a third frond which is less than half grown. Discussions with laboratory staff indicated that this was the most likely cause for this apparent reduction in growth, and the criteria for choosing suitable plants for test initiation have been tightened. Given the likely reason for the sub-optimal performance of the control organisms, the data were still considered useful and the test was accepted as valid.

The *H. viridissima* control response was acceptable with a mean final number of hydroids of 39.7, which equates to a growth rate (k-value) of 0.34.

Lower than acceptable neonate numbers were produced by the control organisms in the *M. macleayi* test, where final mean neonate numbers (per adult) were 24.3, compared to the minimum acceptability criterion of 30 offspring per adult. The *M. macleayi* laboratory cultures periodically experience periods of lower than normal neonate numbers for reasons difficult to identify, but always recover after a week or two. Until recently, the reference toxicity testing program undertaken in the laboratory had not yet determined whether the sensitivity of *M. macleayi* is altered during these periods. For the purposes of this study, a reference toxicity test was undertaken as soon as practicable after this test was completed. The $EC_{50}$ (immobilisation) of this test (48 µg/L) was well within the acceptable range for test acceptability (see Section 4.1.2), and hence, there was no indication of altered sensitivity (but see discussion on *M. macleayi* sensitivity to U in Section 4.1.2). Moreover, a chronic (3-brood reproduction) U toxicity test conducted in February 2007, where control neonate numbers were again low (mean of 21.8 offspring per adult), gave no indication of altered sensitivity beyond the typically broad range of sensitivity to U that is observed for *M. macleayi* (as described in Section 4.1.3). Consequently, the data were still considered useful and the test was accepted as valid.

*Reference toxicity testing*

Reference toxicity testing results and issues described for RP2 water in Section 4.1.2 are applicable also to the Pit 3 water toxicity testing.

### 4.2.3 Toxicity tests

The toxicity estimates for each species, calculated using point estimation ($IC_{10}$s and $IC_{50}$s) and hypothesis testing (NOECs and LOECs), are presented in Table 7, while the concentration-response plots for each species are shown in Figure 4 and again, combined, in

Figure 5. Raw data and statistical summaries are provided at Appendix 6. All three species responded to Pit 3 water. *M. macleayi* and *H. viridissima* were highly sensitive to Pit 3 water, while *L. aequinoctialis* was moderately sensitive. Based on $IC_{50}$ values, the order of sensitivity of the three test species was:

*M. macleayi* >> *H. viridissima* >> *L. aequinoctialis*.

Table 8 provides a comparison for each species of the previously reported $IC_{50}$ values for uranium alone (based on values from Table 3) with the U concentrations corresponding to the Pit 3 water $IC_{50}$ values. Pit 3 water appeared to be more toxic to *M. macleayi* and less toxic to *L. aequinoctialis*, than would be predicted based on the U toxicity data. The toxicity of Pit 3 water to *H. viridissima* was similar to that which would be predicted based on U toxicity.

The order of sensitivity of the species to Pit 3 water was the same as for RP2 water. A comparison of the toxicity of RP2 water and Pit 3 water to the three species is shown in Figure 6. For *M. macleayi* and *H. viridissima*, the toxicity of Pit 3 water was not significantly different to that of RP2 water (based on ANCOVA; *M.macleayi*, df = 1, $F = 0.29$, $P = 0.620$; *H. viridissima*, df = 1, $F = 0.90$, $P = 0.373$). However, the toxicity of Pit 3 water to *L. aequinoctialis* was significantly lower than that of RP2 water (df = 1, $F = 7.22$, $P = 0.023$; see Figure 6A). Whereas RP2 water resulted in an initial 20% inhibition in plant growth from 3% to 30% RP2 water, Pit 3 water resulted in stimulatory growth responses (up to 20% above the control response) over this concentration range, before toxic effects were first observed. There were no differences in measured nutrients (ie. N and P) concentrations between RP2 water and Pit 3 water that might have explained this difference in response (see Appendix 1). A noticeable difference between RP2 and Pit 3 water was the pH (see Appendix 1). pH is known to influence the bioavailability and toxicity of U (at both high and low pH) to various species (eg *Chlorella* sp – Franklin et al 2000; freshwater mussel, *Velesunio angasi* – Markich et al 1996), however, the influence of pH on the toxicity of U to *L. aequinoctialis* is unknown. Overall, RP2 water contained higher concentrations of numerous potentially toxic metals (eg Co, Cu, Mn, Ni, Pb and Zn – see Appendix 1), which may to some degree have contributed to its higher toxicity to *L. aequinoctialis*.

**Table 7** Summary of toxicity of Pit 3 water to the three tropical freshwater species tested

| | Pit 3 water toxicity (% Pit 3 water) | | | |
|---|---|---|---|---|
| **Species** | **$IC_{10}$** | **$IC_{50}$** | **NOEC** | **LOEC** |
| *Lemna aequinoctialis* (duckweed) | 34 (31–38) | >100 | 25 | 50 |
| *Hydra viridissima* (green hydra) | 2.9 (0.9–5.3) | 11 (10–12) | 2.2 | 6.7 |
| *Moinodaphnia macleayi* (cladoceran) | 0.41 (0.20–0.63) | 1.35 (1.31–1.38) | <0.25 | ≤0.25 |

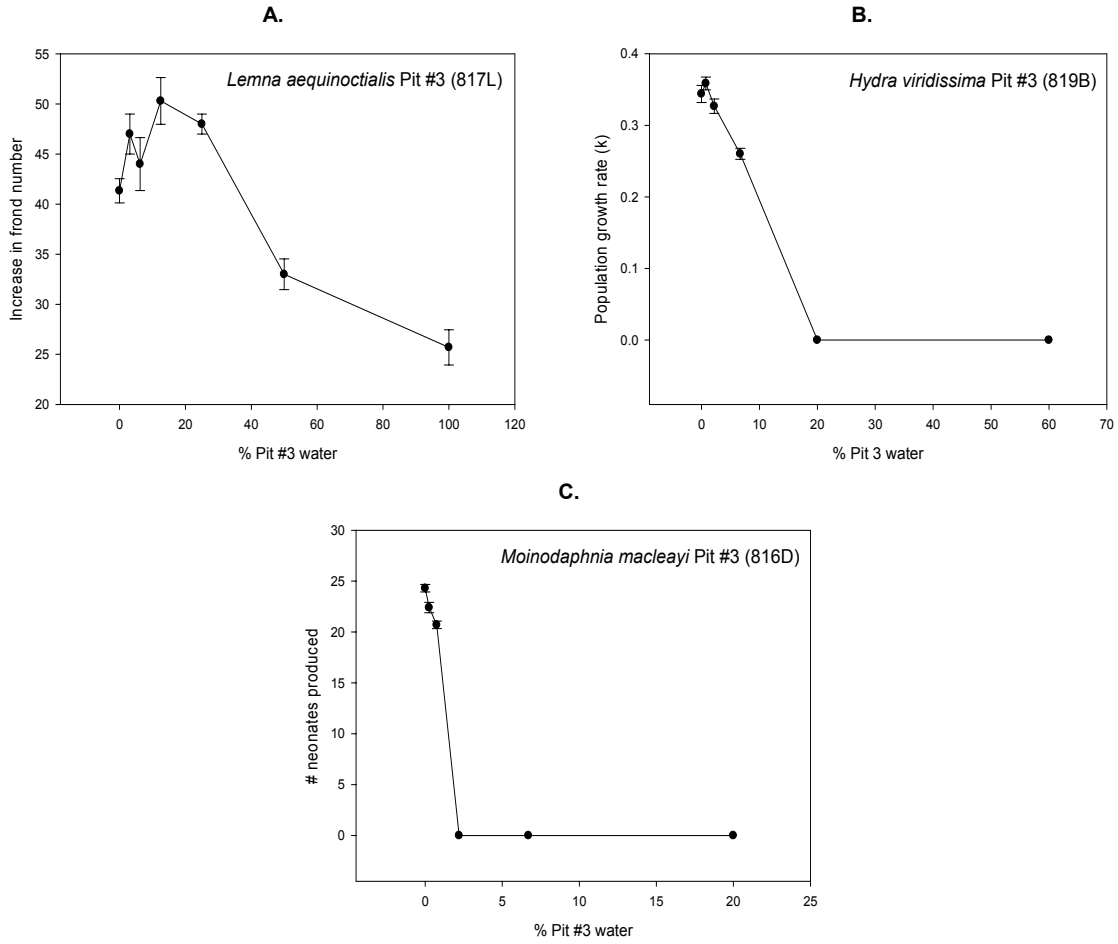


**Figure 4** Concentration response plots showing the toxicity of Pit 3 water to the three tropical freshwater species tested. Data points represent the mean ± SEM (n = 3, except for *M. macleayi* where n = 10).

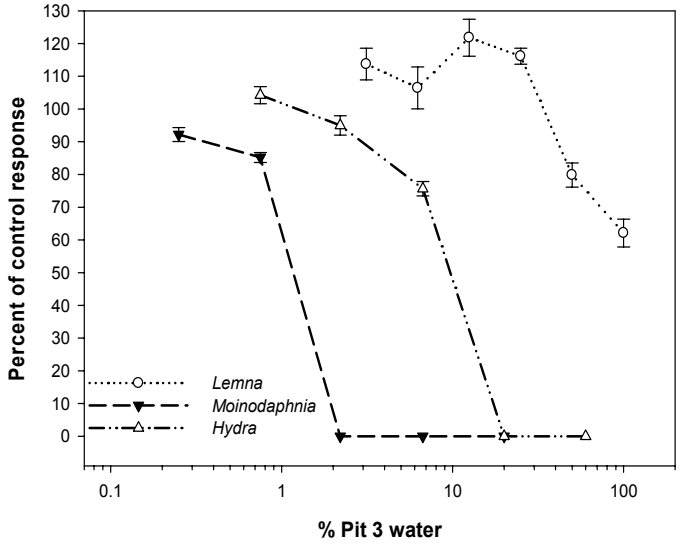


**Figure 5** Combined concentration response plot for each species presented as a percent of the control response with regard to Pit 3 water concentration. Data points represent the mean (±SEM) of three replicates for *L. aequinoctialis* and *H. viridissima*, and 10 replicates for *M. macleayi*.

**Table 8** Comparison of historical U $IC_{50}$ values (μg/L) with the concentrations of U (μg/L) corresponding to the $IC_{50}$ values of Pit 3 water

| Species | U $IC_{50}$ (95%CL)[a] | [U] at Pit 3 water $IC_{50}$ (95%CL)[b] | Comments |
|---|---|---|---|
| *Lemna aequinoctialis* (duckweed) | 504 (449-572) | >1620 | RP2 water less toxic than would be predicted based on U concentration/toxicity alone. |
| *Hydra viridissima* (green hydra) | 180 – >340 (-)[c] | 178 (162-194) | RP2 water toxicity similar to that which would be predicted based on U concentration/ toxicity alone. |
| *Moinodaphnia macleayi* (cladoceran) | 67 (49-83)[d] | 21.9 (21.2-22.4) | RP2 water more toxic than would be predicted based on U concentration/toxicity alone. |

[a] Based on values reported and explained in Table 3.

[b] Based on the values reported in Table 7.

[c] 95% CLs could not be calculated for these data

[d] Used $IC_{50}$ value from toxicity test done during the same month as the RP2 water toxicity testing.

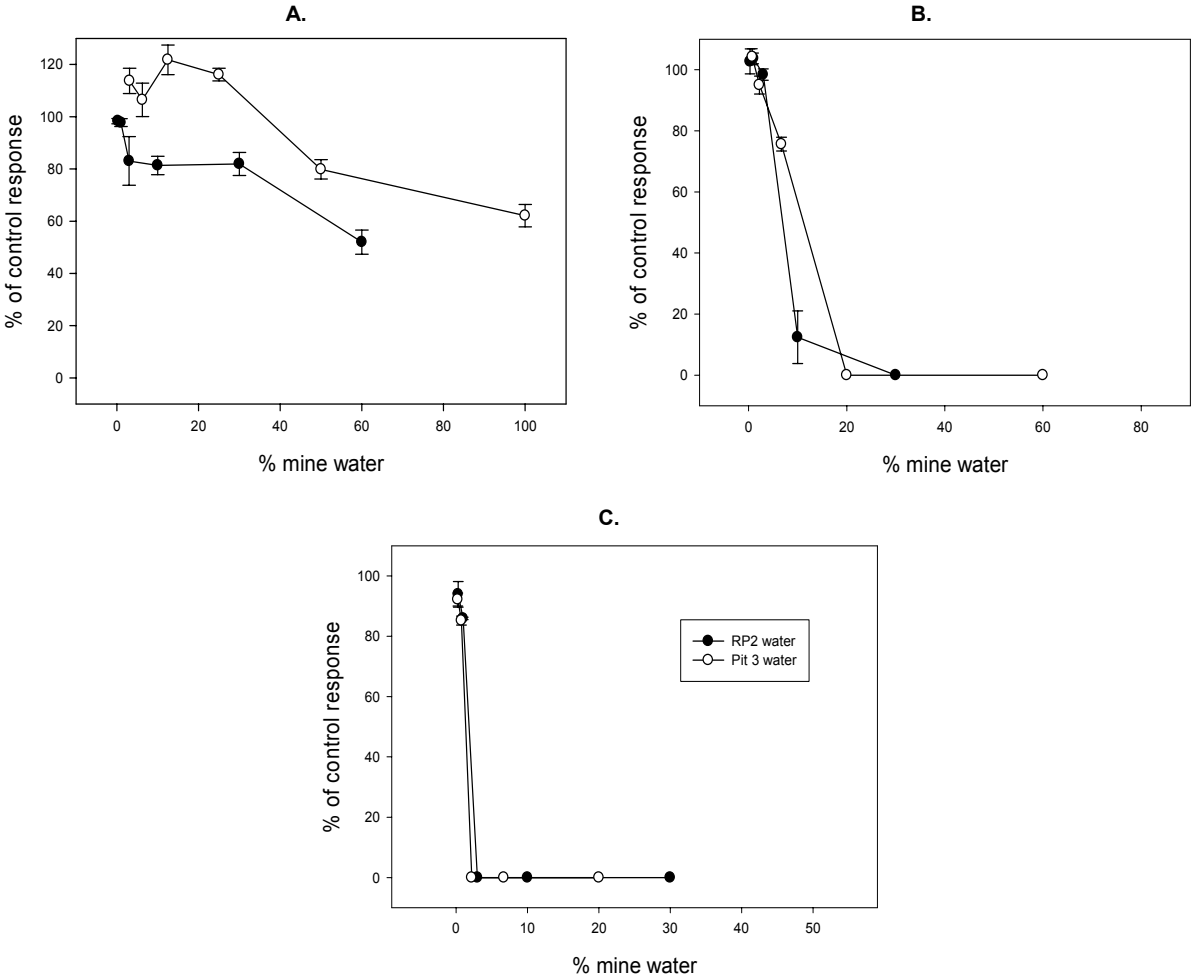


**Figure 6** Comparison of the toxicity of RP2 water and Pit 3 water to to A. *Lemna aequinoctialis*, B. *Hydra viridissima* and C. *Moinodaphnia macleayi*. Data points represent the mean ± standard error (n = 3, except for *M. macleayi* where n = 10).

# 5 Summary and conclusions

The toxicity of RP2 water (1870 µg/L U – dissolved) from ERA Ranger Mine, collected on 25th March 2007, was assessed using five freshwater species native to Magela Creek. The toxicity of RP2 water spanned a wide range, with $IC_{10}$ and $IC_{50}$ estimates in the range 0.6 – >100% and 1.8 – >100% RP2 water, respectively. Based on $IC_{50}$/$LC_{50}$ values, the order of sensitivity of the five test species was:

*M. macleayi* >> *H. viridissima* > *Chlorella* sp. > *L. aequinoctialis* >> *M. mogurnda*

Based on a species sensitivity distribution using species' $IC_{10}$ (or assumed equivalent) data, the dilution of RP2 water that would be expected to protect 99% of species was estimated to be 0.33% RP2 water (or 1 part RP2 water in ~300 parts Magela Creek water). At this dilution, the U concentration would have been approximately 6 µg/L U, the same as the current U Limit for Magela Creek, while the Mg concentration would have been approximately 0.5 mg/L, well below the current interim Limit for Magela Creek of 4 mg/L, and approximately equal to natural background concentrations.

The toxicity of Pit 3 water (~1620 µg/L U – dissolved), collected on 16 April 2007, was assessed using three native freshwater species that were found to be sensitive to RP2 water. The resultant $IC_{10}$ and $IC_{50}$ estimates were in the range 0.41 – 34% and 1.35 – >100% Pit 3 water, respectively. Based on $IC_{50}$ values, the order of sensitivity of the three test species was:

*M. macleayi* >> *H. viridissima* >> *L. aequinoctialis*.

While there was no significant difference between the toxicities of Pit 3 water and RP2 water to *M. macleayi* and *H. viridissima*, the toxicity of Pit 3 water to *L. aequinoctialis* was significantly less than that of RP2 water. The similarity in toxicity of the two water types to *M. macleayi* and *H. viridissima* was not unexpected given their similar uranium concentrations and the fact that RP2 receives water pumped from Pit 3. The difference in toxicity of the two water types to *L. aequinctialis* may have been due to differences in (i) variability of test organism responses between the tests, (ii) important physico-chemical variables that influence metal bioavailablity and toxicity (eg pH), and/or (iii) concentrations of, or interactions between, potentially toxic metals other than uranium (eg Co, Cu, Mn, Ni, Pb and Zn).

At the time of completion of the RP2 water and Pit 3 water toxicity assessments, flow in Magela Creek was insufficient for the direct release of Pond Water at acceptable/protective dilutions (ie at least 1 in 300) to have significantly contributed to reduction of the on-site water inventory. Consequently, this option was not progressed further. However, notwithstanding temporal variations in Pond Water composition/quality and how they may affect toxicity, the knowledge gained from the study will form a key part of the knowledge base required for the future evaluation of water management options at the Ranger mine.

It should be noted that the results of the current study cannot be used as the primary basis for determining protective dilutions of Pond Water discharge during future wet seasons. Consequently, potential releases of Pond Water in the future will require new pre-release toxicity testing studies as soon as practicable prior to the intended time of discharge.

However, a toxicity testing program utilising the full suite of five test species typically requires at least one week's lead notice and takes a further two weeks to complete. As periods of very high rainfall and associated Magela Creek flow/discharge (such as that which occurred in late February/early March 2007) are difficult to forecast, and typically occur over a time scale of days rather than weeks, a full (ie five species) testing program is unlikely to be

able to provide timely results for informing water management options. Rapid testing strategies to overcome this limitation may need to be considered, and could include:

- the testing of only a limited number of species that are known, through extensive toxicity testing experience, to be the most sensitive to Ranger Pond Water;
- routine periodic toxicity testing of Pond Water throughout the wet season, such that its toxicity at any given time is reasonably well known; or
- the application of technologies for 'in-line' continuous biomonitoring of Pond Water toxicity.

However, any such strategy for obtaining more timely information on Pond Water toxicity for direct discharge options should be rigorously tested and would need to be discussed and agreed by all stakeholders.

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# Appendix 1 Physical and chemical characteristics of the RP2, Pit 3 and Magela Creek waters used in the toxicity testing

| Parameter/analyte | Magela Creek | RP2 | Pit 3 |
|---|---|---|---|
| pH | 6.43 | 6.83 | 7.66 |
| Electrical conductivity (μS $cm^{-1}$) | 10 | 1205 | 893 |
| Dissolved oxygen (% saturation) | 96.3 | 92.6 | 93.1 |
| Dissolved organic carbon (mg/L) | 2.2 | NM[1] | NM |
| Alkalinity (mg/L as $CaCO_3$) | 5 | NM | NM |
| Total N (mg/L) | NM | 1.59 | 1.96 |
| $NO_3$_N (mg/L) | <0.005[2] | 1.32 | 1.4 |
| $NO_2$_N (mg/L) | NM | <0.005 | 0.02 |
| $NH_3$_N (mg/L) | <0.005[2] | <0.005 | <0.005 |
| Total P (mg/L) | NM | 0.02 | 0.01 |
| $PO_4$_P (mg/L) | (TRP) <0.005[2] | 0.01 | 0.01 |
| Al, total (μg/L) | 116 | 26.6 | 26.3 |
| Al, filtered[3] (μg/L) | 42.6 | 37.9 | 12.1 |
| Ca, total (mg/L) | 0.2 | 23.4 | 20.1 |
| Ca filtered (mg/L) | 0.2 | 23.8 | 18.7 |
| Cd total (μg/L) | <0.02 | <0.2 | <0.2 |
| Cd, filtered (μg/L) | <0.02 | <0.2 | <0.2 |
| Co, total (μg/L) | 0.07 | 12.9 | 0.49 |
| Co, filtered (μg/L) | 0.02 | 12 | 0.21 |
| Cr, total (μg/L) | 0.4 | <2 | <2 |
| Cr, filtered (μg/L) | 0.2 | <2 | <2 |
| Cu, total (μg/L) | 1.01 | 12.7 | 2.35 |
| Cu, filtered (μg/L) | 1.70 | 14 | 2.26 |
| Fe, total (mg/L) | 200 | <200 | <20 |
| Fe, filtered (μg/L) | 80 | <200 | 60 |
| Mg, total (mg/L) | 0.4 | 155 | 115 |
| Mg, filtered (mg/L) | 0.3 | 159 | 107 |
| Mn, total (μg/L) | 2.29 | 435 | 41.2 |
| Mn, filtered (μg/L) | 0.58 | 397 | 32.6 |
| Na, total (mg/L) | 1.1 | 8.8 | 9.7 |
| Na, filtered (mg/L) | 1.0 | 8.4 | 8.3 |
| Ni, total (μg/L) | 0.54 | 23.3 | 5.26 |
| Ni, filtered (μg/L) | 0.28 | 39.5 | 4.7 |
| Pb, total (μg/L) | 0.1 | 6.92 | 0.74 |
| Pb, filtered (μg/L) | 0.04 | 3.91 | 0.09 |
| $SO_4$, total (mg/L) | 0.5 | 782 | 453 |
| $SO_4$, filtered (mg/L) | 0.5 | 655 | 445 |
| Se, total (μg/L) | <0.2 | 14.4 | 8.6 |
| Se, filtered (μg/L) | <0.2 | 14.4 | 8.4 |

| Parameter/analyte | Magela Creek | RP2 | Pit 3 |
|---|---|---|---|
| U, total (μg/L) | 0.058 | 2130 | 1760 |
| U, filtered (μg/L) | 0.083 | 1870 | 1620 |
| Zn, total (μg/L) | 1.1 | 33.6 | 3.7 |
| Zn, filtered (μg/L) | 0.8 | 32.1 | 2.8 |

[1] NM = not measured.

[2] Taken from routine SSD statuatory water quality monitoring data (12/4/07 & 19/4/07).

[3] 'filtered' refers to the 0.45 μm filtered fraction.

# Appendix 2 Water chemistry data provided by ERA (RP2) and EWLS (Pit 3) just prior to the commencement of the ecotoxicological testing

| Parameter/analyte | RP2 | | Pit 3 |
|---|---|---|---|
| | 04/03/2007 | 07/03/2007 | 28/03/2007 |
| pH | 6.30 | 5.95 | 7.38 |
| Electrical conductivity (μS $cm^{-1}$) | 1030 | 1071 | 854 |
| Dissolved oxygen (% saturation) | NP[1] | NP | NP |
| Dissolved organic carbon (mg/L) | NP | NP | NP |
| Alkalinity (mg/L as $CaCO_3$) | <1 | NP | <1 |
| Turbidity (NTU) | NP | 2 | 4 |
| Total N (mg/L) | NP | NP | NP |
| $NO_3$_N (mg/L) | NP | 1.24 | NP |
| $NO_2$_N (mg/L) | NP | 0.01 | NP |
| $NH_3$_N (mg/L) | NP | 0.0095 | NP |
| Total P (mg/L) | NP | NP | NP |
| $PO_4$_P (mg/L) | NP | NP | NP |
| Al, total (μg/L) | NP | 324 | NP |
| Al, filtered (μg/L) | 107 | 98.1 | 26.8 |
| Ca, total (mg/L) | NP | NP | NP |
| Ca filtered (mg/L) | 22.6 | 23.0 | 16.8 |
| Cd total (μg/L) | NP | NP | NP |
| Cd, filtered (μg/L) | NP | NP | NP |
| Co, total (μg/L) | NP | NP | NP |
| Co, filtered (μg/L) | NP | NP | NP |
| Cr, total (μg/L) | NP | NP | NP |
| Cr, filtered (μg/L) | NP | NP | NP |
| Cu, total (μg/L) | NP | 32.0 | NP |
| Cu, filtered (μg/L) | 24.0 | 28.5 | 2.4 |
| Fe, total (mg/L) | NP | 220 | NP |
| Fe, filtered (μg/L) | <200 | 80 | <20 |
| Mg, total (mg/L) | NP | NP | NP |
| Mg, filtered (mg/L) | 136 | 136 | 101 |
| Mn, total (μg/L) | NP | 447 | NP |
| Mn, filtered (μg/L) | 414 | 445 | 87.5 |
| Na, total (mg/L) | NP | NP | NP |
| Na, filtered (mg/L) | 7.6 | NP | 7.2 |
| Ni, total (μg/L) | NP | NP | NP |
| Ni, filtered (μg/L) | NP | NP | NP |
| Pb, total (μg/L) | NP | 34.2 | NP |
| Pb, filtered (μg/L) | 21.5 | 26.0 | 0.55 |
| $SO_4$, total (mg/L) | NP | NP | NP |

| Parameter/analyte | RP2 | | Pit 3 |
|---|---|---|---|
| | 04/03/2007 | 07/03/2007 | 28/03/2007 |
| $SO_4$, filtered (mg/L) | 600 | 574 | 418 |
| Se, total (μg/L) | NP | NP | NP |
| Se, filtered (μg/L) | NP | NP | NP |
| U, total (μg/L) | NP | 2770 | NP |
| U, filtered (μg/L) | 2400 | 2360 | 1300 |
| Zn, total (μg/L) | NP | 45.2 | NP |
| Zn, filtered (μg/L) | 78.5 | 45.5 | 1.5 |

1 NP: Not provided

# Appendix 3 Physicochemical data for the RP2 ecotoxicological tests

## 3.1 *Chlorella* sp test (813G)

| | | RP2 water concentration (%) | | | | | |
|---|---|---|---|---|---|---|---|
| Day | Parameter | 0 | 0.3 | 1 | 3 | 10 | 30 |
| 1 | pH | 5.67 | 5.69 | 5.68 | 5.70 | 5.81 | 5.98 |
| | Conductivity[1] | 42 | 48 | 60 | 89 | 196 | 462 |
| | DO[2] | 96.0 | 91.6 | 94.9 | 95.2 | 94.9 | 94.5 |
| 3 | pH | 6.21 | 6.22 | 6.23 | 6.27 | 6.27 | 6.15 |
| | Conductivity | 41 | 47 | 60 | 90 | 200 | 473 |
| | DO | 98.6 | 99.1 | 97.6 | 99.0 | 97.9 | 97.5 |

1 Conductivity units are in μS/cm.

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

## 3.2 *Lemna aequinoctialis* test (812L)

| | | RP2 water concentration (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day | Parameter | 0 | 0.3 | 1 | 3 | 10 | 30 | 60 |
| 1 | pH | 6.20 | 6.14 | 6.18 | 6.13 | 6.25 | 6.63 | 6.71 |
| | Conductivity[1] | 15 | 21 | 33 | 66 | 171 | 442 | 784 |
| | DO[2] | 94.9 | 103.0 | 100.9 | 102.0 | 98.7 | 100.4 | 96.3 |
| 4 | pH | 6.73 | 6.81 | 6.89 | 6.79 | 6.82 | 6.86 | 6.93 |
| | Conductivity | 10 | 16 | 28 | 61 | 168 | 439 | 784 |
| | DO | 96.9 | 94.5 | 96.5 | 95.3 | 94.7 | 95.4 | 92.4 |

1 Conductivity units are in μS/cm.

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

## 3.3 *Hydra viridissima* test (811B)

| | | RP2 water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.3 | | 1 | | 3 | | 10 | | 30 | |
| 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 | 6.18 | 6.43 | 6.45 | 6.37 | 6.22 | 6.36 | 6.18 | 6.39 | 6.30 | 6.50 | 6.45 | All dead |
| | Conductivity[1] | 9 | 10 | 16 | 17 | 25 | 27 | 59 | 61 | 166 | 167 | 454 | |
| | DO[2] | 96.2 | 96.3 | 97.2 | 97.2 | 101.0 | 98.1 | 99.8 | 98.0 | 99.7 | 97.6 | 99.9 | |
| 2 | pH | 6.08 | 6.41 | 6.57 | 6.55 | 6.29 | 6.45 | 6.31 | 6.37 | 6.37 | 6.43 | All dead | All dead |
| | Conductivity | 9 | 11 | 15 | 19 | 25 | 26 | 57 | 53 | 160 | 171 | | |
| | DO | 109.9 | 98.5 | 106.2 | 98.3 | 105.1 | 98.1 | 112.0 | 99.6 | 106.3 | 98.8 | | |
| 3 | pH | 6.03 | 6.37 | 6.32 | 6.45 | 6.11 | 6.47 | 6.23 | 6.35 | 6.31 | 6.42 | All dead | All dead |
| | Conductivity | 8 | 11 | 16 | 22 | 26 | 26 | 59 | 63 | 167 | 175 | | |
| | DO | 106.2 | 91.9 | 107.5 | 93.7 | 116.6 | 91.4 | 113.0 | 92.7 | 113.2 | 91.6 | | |
| 4 | pH | 6.02 | 6.45 | 6.22 | 6.62 | 6.09 | 6.51 | 6.10 | 6.45 | 6.25 | 6.53 | All dead | All dead |
| | Conductivity | 9 | 9 | 15 | 17 | 25 | 27 | 58 | 62 | 167 | 177 | | |
| | DO | 110.7 | 98.3 | 118.8 | 98.0 | 120.9 | 96.6 | 118.0 | 100.5 | 116.4 | 104.9 | | |

1 Conductivity units are in μS/cm.

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

## 3.4 *Moinodaphnia macleayi* test (815D)

| | | RP2 water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.3 | | 1 | | 3 | | 10 | | 30 | |
| 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 | 6.08 | 6.25 | 6.57 | 6.56 | 6.29 | 6.48 | 6.31 | 6.49 | 6.37 | 6.70 | 6.58 | 6.59 |
| | Conductivity[1] | 9 | 11 | 15 | 18 | 25 | 27 | 57 | 61 | 160 | 169 | 436 | 456 |
| | DO[2] | 109.9 | 94.9 | 106.2 | 94.0 | 105.1 | 96.3 | 112.0 | 97.3 | 106.3 | 96.4 | 108.4 | 95.0 |
| 2 | pH | 6.03 | 6.43 | 6.32 | 6.57 | 6.11 | 6.39 | 6.23 | 6.47 | 6.31 | 6.50 | All dead | All dead |
| | Conductivity | 8 | 11 | 16 | 19 | 26 | 28 | 59 | 62 | 167 | 171 | | |
| | DO | 106.2 | 96.3 | 107.5 | 97.6 | 116.6 | 96.0 | 113.0 | 97.9 | 113.2 | 96.1 | | |
| 3 | pH | 6.02 | 6.32 | 6.22 | 6.63 | 6.09 | 6.39 | All dead | All dead | All dead | All dead | All dead | All dead |
| | Conductivity | 9 | 11 | 15 | 18 | 25 | 28 | | | | | | |
| | DO | 110.7 | 95.9 | 118.8 | 98.3 | 120.9 | 94.5 | | | | | | |
| 4 | pH | 6.26 | 6.44 | 6.21 | 6.56 | 6.03 | 6.48 | All dead | All dead | All dead | All dead | All dead | All dead |
| | Conductivity | 9 | 11 | 15 | 22 | 25 | 27 | | | | | | |
| | DO | 111.4 | 95.5 | 115.4 | 97.1 | 119.6 | 95.1 | | | | | | |
| 5 | pH | 6.21 | 6.24 | 6.49 | 6.52 | 6.25 | 6.48 | All dead | All dead | All dead | All dead | All dead | All dead |
| | Conductivity | 8 | 11 | 14 | 18 | 25 | 28 | | | | | | |
| | DO | 107.4 | 94.0 | 108.8 | 96.3 | 106.5 | 94.9 | | | | | | |

1 Conductivity units are in µS/cm.

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

## 3.5 *Mogurnda mogurnda* test (814E)

| | | RP2 water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 6.25 | | 12.5 | | 25 | | 50 | | 100 | |
| 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 | 7.36 | 6.50 | 6.45 | 6.56 | 6.81 | 6.54 | 6.64 | 6.72 | 6.64 | 6.86 | 6.80 | 7.12 |
| | Conductivity[1] | 14 | 10 | 111 | 120 | 209 | 207 | 369 | 376 | 669 | 674 | 1204 | 1205 |
| | DO[2] | 104.7 | 96.7 | 107.1 | 95.4 | 105.2 | 95.0 | 110.9 | 94.9 | 111.0 | 96.8 | 112.5 | 95.5 |
| 2 | pH | 6.22 | 6.55 | 6.34 | 6.49 | 6.53 | 6.50 | 6.44 | 6.68 | 6.64 | 6.78 | 6.82 | 7.04 |
| | Conductivity | 9 | 13 | 109 | 115 | 203 | 212 | 367 | 385 | 668 | 712 | 1191 | 1227 |
| | DO | 101.8 | 92.9 | 105.7 | 92.5 | 101.7 | 92.2 | 115.1 | 92.0 | 116.7 | 94.2 | 118.9 | 93.6 |
| 3 | pH | 6.16 | 6.78 | 6.25 | 6.65 | 6.33 | 6.63 | 6.71 | 6.69 | 6.76 | 6.99 | 6.88 | 7.14 |
| | Conductivity | 9 | 11 | 109 | 115 | 202 | 210 | 368 | 385 | 673 | 685 | 1168 | 1226 |
| | DO | 106.3 | 95.9 | 107.1 | 98.2 | 104.6 | 97.8 | 101.2 | 99.3 | 107.5 | 98.5 | 107.6 | 97.7 |
| 4 | pH | 6.19 | 6.54 | 6.30 | 6.64 | 6.31 | 6.61 | 6.55 | 6.72 | 6.59 | 6.81 | 6.89 | 7.02 |
| | Conductivity | 8 | 11 | 107 | 116 | 200 | 212 | 355 | 383 | 634 | 674 | 1182 | 1243 |
| | DO | 103.8 | 97.4 | 109.1 | 99.8 | 106.8 | 95.5 | 106.2 | 97.1 | 118.3 | 97.6 | 118.7 | 96.3 |

1 Conductivity units are in µS/cm.

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

# Appendix 4 Physicochemical data for the Pit 3 ecotoxicological tests

## 4.1 *Lemna aequinoctialis* test (817L)

| | | Pit 3 water concentration (%) | | | | | | |
|---|---|---|---|---|---|---|---|---|
| Day | Parameter | 0 | 3.25 | 6.25 | 12.5 | 25 | 50 | 100 |
| 1 | pH | 6.35 | 6.48 | 6.49 | 6.86 | 7.03 | 7.31 | 7.64 |
| | Conductivity[1] | 16 | 53 | 86 | 152 | 274 | 491 | 887 |
| | DO[2] | 97.3 | 96.4 | 103.1 | 102.1 | 104.2 | 103.3 | 98.8 |
| 4 | pH | 6.67 | 6.99 | 7.10 | 7.43 | 7.72 | 8.27 | 8.26 |
| | Conductivity | 13 | 49 | 85 | 152 | 278 | 492 | 897 |
| | DO | 96.0 | 98.4 | 98.8 | 98.1 | 99.9 | 100.6 | 98.7 |

1 Conductivity units are in μS/cm.

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

## 4.2 *Hydra viridissima* test (819B)

| | | Pit 3 water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.75 | | 2.2 | | 6.7 | | 20 | | 60 | |
| 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 | 6.32 | 6.35 | 6.27 | 6.39 | 6.29 | 6.52 | 6.57 | 6.65 | 6.93 | 6.92 | 7.42 | 7.53 |
| | Conductivity[1] | 10 | 10 | 18 | 18 | 35 | 34 | 86 | 83 | 222 | 218 | 571 | 563 |
| | DO[2] | 98.1 | 92.4 | 980 | 92.5 | 100.8 | 91.7 | 98.8 | 92.9 | 97.8 | 92.4 | 95.1 | 92.0 |
| 2 | pH | 6.82 | 6.41 | 6.29 | 6.45 | 6.24 | 6.48 | 6.49 | 6.75 | 6.86 | 6.91 | All dead | All dead |
| | Conductivity | 9 | 11 | 17 | 19 | 35 | 37 | 86 | 89 | 220 | 226 | | |
| | DO | 104.8 | 97.9 | 100.8 | 98.0 | 103.0 | 96.2 | 99.7 | 95.8 | 101.9 | 93.4 | | |
| 3 | pH | 6.02 | 6.51 | 6.11 | 6.37 | 6.18 | 6.46 | 6.46 | 6.69 | All dead | All dead | All dead | All dead |
| | Conductivity | 9 | 11 | 17 | 20 | 35 | 38 | 86 | 89 | | | | |
| | DO | 107.4 | 93.9 | 108.6 | 93.0 | 108.9 | 93.2 | 107.0 | 91.4 | | | | |
| 4 | pH | 6.11 | 6.68 | 6.29 | 6.48 | 6.26 | 6.55 | 6.64 | 6.71 | All dead | All dead | All dead | All dead |
| | Conductivity | 9 | 10 | 18 | 19 | 34 | 38 | 84 | 91 | | | | |
| | DO | 108.8 | 99.2 | 111.7 | 98.6 | 115.5 | 97.0 | 112.8 | 97.1 | | | | |

1 Conductivity units are in μS/cm.

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

## 4.3 *Moinodaphnia macleayi* test (816D)

| | | Pit 3 water concentration (%) | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | | 0 | | 0.25 | | 0.75 | | 2.2 | | 6.7 | | 20 | |
| 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 | 6.02 | 7.09 | 6.32 | 6.53 | 6.11 | 6.50 | 6.18 | 6.50 | 6.46 | 6.65 | 6.80 | 6.90 |
| | Conductivity[1] | 9 | 11 | 11 | 14 | 17 | 20 | 35 | 38 | 86 | 89 | 219 | 225 |
| | DO[2] | 107.4 | 90.8 | 105.3 | 93.7 | 108.6 | 91.9 | 108.9 | 92.3 | 107.0 | 91.9 | 105.7 | 93.2 |
| 2 | pH | 6.11 | 6.46 | 6.36 | 6.44 | 6.29 | 6.42 | 6.26 | 6.49 | 6.64 | 6.68 | 6.85 | 7.00 |
| | Conductivity | 9 | 12 | 12 | 14 | 18 | 20 | 34 | 37 | 84 | 89 | 216 | 224 |
| | DO | 108.8 | 95.2 | 109.9 | 97.8 | 111.7 | 96.0 | 115.5 | 97.4 | 112.8 | 93.7 | 111.6 | 98.0 |
| 3 | pH | 6.19 | 6.48 | 6.13 | 6.44 | 6.14 | 6.47 | 6.35 | 6.49 | All dead | All dead | All dead | All dead |
| | Conductivity | 9 | 12 | 11 | 14 | 17 | 21 | 35 | 38 | | | | |
| | DO | 115.3 | 110.8 | 113.1 | 97.2 | 115.2 | 96.1 | 109.5 | 97.2 | | | | |
| 4 | pH | 5.97 | 6.41 | 6.04 | 6.41 | 6.09 | 6.46 | All dead | All dead | All dead | All dead | All dead | All dead |
| | Conductivity | 8 | 12 | 11 | 14 | 17 | 21 | | | | | | |
| | DO | 108.5 | 95.8 | 113.6 | 96.9 | 114.3 | 94.4 | | | | | | |
| 5 | pH | 5.96 | 6.39 | 6.10 | 6.49 | 6.16 | 6.43 | All dead | All dead | All dead | All dead | All dead | All dead |
| | Conductivity | 8 | 12 | 11 | 14 | 18 | 20 | | | | | | |
| | DO | 106.9 | 97.0 | 106.5 | 94.7 | 109.4 | 95.9 | | | | | | |
| 6 | pH | 6.16 | 6.45 | 6.19 | 6.44 | 6.20 | 6.43 | All dead | All dead | All dead | All dead | All dead | All dead |
| | Conductivity | 9 | 12 | 12 | 14 | 17 | 20 | | | | | | |
| | DO | 109.0 | 95.5 | 105.4 | 98.1 | 111.4 | 97.0 | | | | | | |

1 Conductivity units are in μS/cm.

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

3 NM: Not measured

# Appendix 5 ToxCalc statistical summaries for the RP2 ecotoxicological tests

## 5.1 *Chlorella* sp test (813G)

**Algal Growth Inhibition Test-Growth rate**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 26/03/2007 | Test ID: | 813G | Sample ID: | RP2 |
| End Date: | 29/03/2007 | Lab ID: | ERISS-eriss ecotoxicology la | Sample Type: | MRP-Mine retention pond |
| Sample Date: | | Protocol: | BTT G-eriss tropical freshwa | Test Species: | CH-Chlorella sp. |
| Comments: | | | | | |

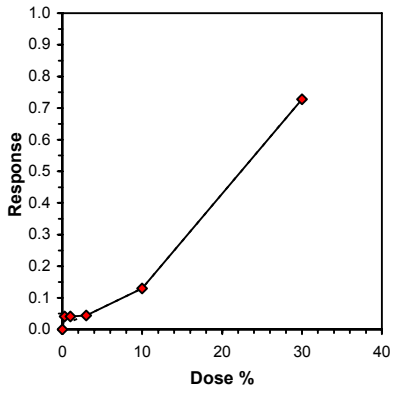
| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| B-Control | 1.4383 | 1.3913 | 1.4779 |
| 0.3 | 1.3379 | 1.3996 | 1.3185 |
| 1 | 1.3875 | 1.3935 | 1.4216 |
| 3 | 1.4305 | 1.4429 | 1.2446 |
| 10 | 1.2851 | 1.3777 | 1.0835 |
| 30 | 0.4955 | 0.4189 | 0.2588 |

| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| B-Control | 1.4358 | 1.0000 | 1.4358 | 1.3913 | 1.4779 | 3.018 | 3 | | | | 1.4358 | 1.0000 |
| 0.3 | 1.3520 | 0.9416 | 1.3520 | 1.3185 | 1.3996 | 3.131 | 3 | 1.087 | 2.500 | 0.1929 | 1.3764 | 0.9586 |
| 1 | 1.4009 | 0.9756 | 1.4009 | 1.3875 | 1.4216 | 1.302 | 3 | 0.453 | 2.500 | 0.1929 | 1.3764 | 0.9586 |
| 3 | 1.3727 | 0.9560 | 1.3727 | 1.2446 | 1.4429 | 8.094 | 3 | 0.819 | 2.500 | 0.1929 | 1.3727 | 0.9560 |
| 10 | 1.2488 | 0.8697 | 1.2488 | 1.0835 | 1.3777 | 12.046 | 3 | 2.425 | 2.500 | 0.1929 | 1.2488 | 0.8697 |
| *30 | 0.3911 | 0.2724 | 0.3911 | 0.2588 | 0.4955 | 30.891 | 3 | 13.542 | 2.500 | 0.1929 | 0.3911 | 0.2724 |

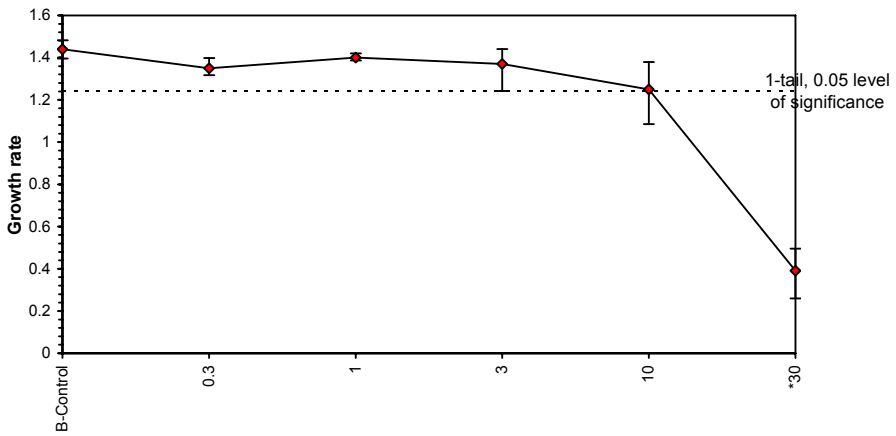
| Auxiliary Tests | | | | | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | | | | | 0.9435 | | 0.858 | | -0.6584 | 0.11984 |
| Bartlett's Test indicates equal variances (p = 0.16) | | | | | 7.88556 | | 15.0863 | | | |
| **Hypothesis Test (1-tail, 0.05)** | **NOEC** | **LOEC** | **ChV** | **TU** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Dunnett's Test | 10 | 30 | 17.3205 | 10 | 0.19287 | 0.13433 | 0.48336 | 0.00893 | 8.1E-08 | 5, 12 |
| Treatments vs B-Control | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 3.487 | 2.698 | 0.000 | 17.627 | 0.9122 |
| IC10 | 7.543 | 2.620 | 0.000 | 16.250 | -0.1599 |
| IC15 | 10.660 | 1.764 | 2.684 | 15.905 | -0.5994 |
| IC20 | 12.334 | 1.484 | 5.277 | 17.158 | -0.5505 |
| IC25 | 14.008 | 1.379 | 7.168 | 18.529 | -0.6088 |
| IC40 | 19.030 | 1.060 | 14.004 | 23.067 | -0.2470 |
| IC50 | 22.379 | 0.979 | 18.083 | 26.473 | 0.0175 |




**Dose-Response Plot**



## 5.2 *Lemna aequinoctialis* test (812L)

**Lemna Growth Inhibition-Incr. in biomass**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 26/03/2007 | Test ID: | 812L | Sample ID: | RP2 |
| End Date: | 30/03/2007 | Lab ID: | ERISS-eriss ecotoxicology la | Sample Type: | MRP-Mine retention pond |
| Sample Date: | | Protocol: | BTT L-eriss tropical freshwate | Test Species: | LAE-Lemna aequinoctialis |
| Comments: | | | | | |

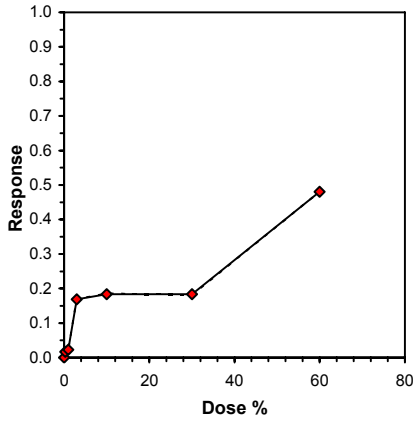
| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| B-Control | 62.000 | 54.000 | 61.000 |
| 0.3 | 58.000 | 59.000 | 57.000 |
| 1 | 56.000 | 58.000 | 59.000 |
| 3 | 50.000 | 39.000 | 58.000 |
| 10 | 44.000 | 51.000 | 49.000 |
| 30 | 48.000 | 53.000 | 44.000 |
| 60 | 36.000 | 27.000 | 29.000 |

| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| B-Control | 59.000 | 1.0000 | 59.000 | 54.000 | 62.000 | 7.388 | 3 | | | | 59.000 | 1.0000 |
| 0.3 | 58.000 | 0.9831 | 58.000 | 57.000 | 59.000 | 1.724 | 3 | 0.249 | 2.530 | 10.150 | 58.000 | 0.9831 |
| 1 | 57.667 | 0.9774 | 57.667 | 56.000 | 59.000 | 2.649 | 3 | 0.332 | 2.530 | 10.150 | 57.667 | 0.9774 |
| 3 | 49.000 | 0.8305 | 49.000 | 39.000 | 58.000 | 19.468 | 3 | 2.493 | 2.530 | 10.150 | 49.000 | 0.8305 |
| *10 | 48.000 | 0.8136 | 48.000 | 44.000 | 51.000 | 7.512 | 3 | 2.742 | 2.530 | 10.150 | 48.167 | 0.8164 |
| *30 | 48.333 | 0.8192 | 48.333 | 44.000 | 53.000 | 9.329 | 3 | 2.659 | 2.530 | 10.150 | 48.167 | 0.8164 |
| *60 | 30.667 | 0.5198 | 30.667 | 27.000 | 36.000 | 15.410 | 3 | 7.062 | 2.530 | 10.150 | 30.667 | 0.5198 |

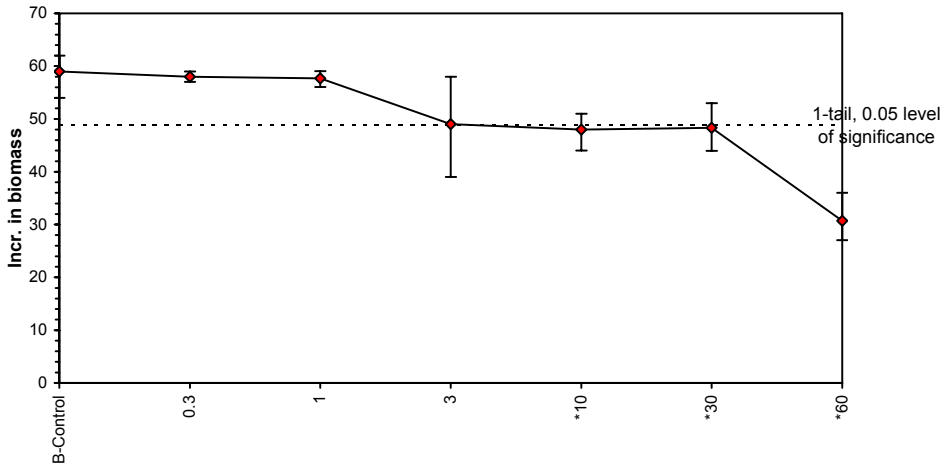
| Auxiliary Tests | | | | | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | | | | | 0.97699 | | 0.873 | | -0.2229 | 1.09316 |
| Bartlett's Test indicates equal variances (p = 0.18) | | | | | 8.90741 | | 16.8119 | | | |
| **Hypothesis Test (1-tail, 0.05)** | **NOEC** | **LOEC** | **ChV** | **TU** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Dunnett's Test | 3 | 10 | 5.47723 | 33.3333 | 10.1501 | 0.17204 | 292.635 | 24.1429 | 7.6E-05 | 6, 14 |
| Treatments vs B-Control | | | | | | | | | | |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 1.373 | 1.031 | 0.000 | 7.948 | 1.7378 |
| IC10 | 2.054 | 1.567 | 0.375 | 12.506 | 2.1370 |
| IC15 | 2.735 | 8.214 | 0.991 | 64.985 | 2.4292 |
| IC20 | 31.657 | 13.686 | 0.000 | 41.612 | -0.0107 |
| IC25 | 36.714 | 6.960 | 0.000 | 46.068 | -2.8591 |
| IC40 | 51.886 | | | | |
| IC50 | >60 | | | | |




**Dose-Response Plot**



## 5.3 *Hydra viridissima* test (811B)

| Green Hydra Population Growth Test-Population growth rate (k | | | |
|---|---|---|---|
| Start Date: 26/03/2007 | Test ID: 811B | Sample ID: | RUM-Ranger Uranium Mine |
| End Date: 30/03/2007 | Lab ID: ERISS-eriss ecotoxicology la | Sample Type: | MRP-Mine retention pond |
| Sample Date: | Protocol: BTT B-eriss tropical freshwat | Test Species: | HV-Hydra viridissima |
| Comments: 1st RP2 test with hydra | | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| B-Control | 0.2574 | 0.3466 | 0.2662 |
| 0.3 | 0.3132 | 0.3059 | 0.2747 |
| 1 | 0.3059 | 0.2908 | 0.3059 |
| 3 | 0.2908 | 0.2908 | 0.2747 |
| 10 | 0.0841 | 0.0238 | 0.0000 |
| 30 | 0.0000 | 0.0000 | 0.0000 |

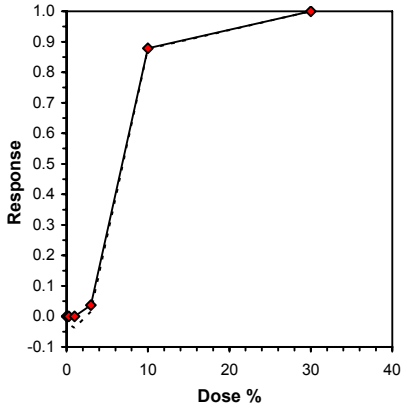
| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| B-Control | 0.2901 | 1.0000 | 0.2901 | 0.2574 | 0.3466 | 16.944 | 3 | | | | 0.2963 | 1.0000 |
| 0.3 | 0.2979 | 1.0272 | 0.2979 | 0.2747 | 0.3132 | 6.874 | 3 | -0.309 | 2.470 | 0.0630 | 0.2963 | 1.0000 |
| 1 | 0.3009 | 1.0374 | 0.3009 | 0.2908 | 0.3059 | 2.908 | 3 | -0.425 | 2.470 | 0.0630 | 0.2963 | 1.0000 |
| 3 | 0.2854 | 0.9840 | 0.2854 | 0.2747 | 0.2908 | 3.264 | 3 | 0.182 | 2.470 | 0.0630 | 0.2854 | 0.9633 |
| *10 | 0.0360 | 0.1241 | 0.0360 | 0.0000 | 0.0841 | 120.494 | 3 | 9.963 | 2.470 | 0.0630 | 0.0360 | 0.1214 |
| 30 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.000 | 3 | | | | 0.0000 | 0.0000 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.91414 | 0.835 | 0.82921 | 0.55613 |
| Bartlett's Test indicates equal variances (p = 0.13) | 7.07687 | 13.2767 | | |

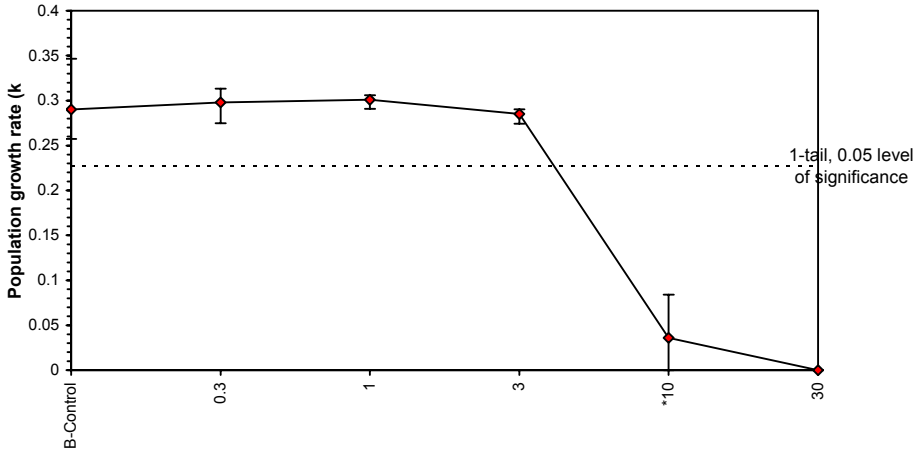
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test Treatments vs B-Control | 3 | 10 | 5.47723 | 33.3333 | 0.06299 | 0.21717 | 0.03992 | 0.00098 | 3.6E-06 | 4, 10 |

Linear Interpolation (200 Resamples)

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 3.1104 | 1.2762 | 0.0000 | 3.8268 | -0.9629 |
| IC10 | 3.5261 | 0.8845 | 0.0000 | 4.3177 | -2.3783 |
| IC15 | 3.9419 | 0.6891 | 0.0000 | 4.8086 | -3.1259 |
| IC20 | 4.3577 | 0.3933 | 2.2076 | 5.3301 | -0.8129 |
| IC25 | 4.7734 | 0.3891 | 2.6517 | 5.9265 | -0.6359 |
| IC40 | 6.0207 | 0.4032 | 4.0303 | 7.7156 | -0.0045 |
| IC50 | 6.8522 | 0.4330 | 4.9809 | 8.9085 | 0.3414 |




Dose-Response Plot



## 5.4 *Moinodaphnia macleayi* test (815D)

**Cladoceran Reproduction Test-Total neonates**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 27/03/2007 | Test ID: | 815D | Sample ID: | RP2 |
| End Date: | 1/04/2007 | Lab ID: | ERISS-eriss ecotoxicology la | Sample Type: | MRP-Mine retention pond |
| Sample Date: | | Protocol: | BTT D-eriss tropical freshwat | Test Species: | MOMA-Moinodaphnia macleayi |
| Comments: | | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| Control | 39.000 | 40.000 | 40.000 | 39.000 | 40.000 | 37.000 | 43.000 | 39.000 | 39.000 | 40.000 |
| 0.3 | 37.000 | 38.000 | 39.000 | 43.000 | 41.000 | 41.000 | 34.000 | 24.000 | 36.000 | 39.000 |
| 1 | 34.000 | 34.000 | 34.000 | 34.000 | 34.000 | 34.000 | 33.000 | 34.000 | 35.000 | 34.000 |
| 3 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 10 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 30 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

| | | | Transform: Untransformed | | | | | Rank | 1-Tailed | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Mean | N-Mean |
| Control | 39.600 | 1.0000 | 39.600 | 37.000 | 43.000 | 3.802 | 10 | | | 39.600 | 1.0000 |
| 0.3 | 37.200 | 0.9394 | 37.200 | 24.000 | 43.000 | 14.326 | 10 | 90.00 | 75.00 | 37.200 | 0.9394 |
| *1 | 34.000 | 0.8586 | 34.000 | 33.000 | 35.000 | 1.386 | 10 | 55.00 | 75.00 | 34.000 | 0.8586 |
| *3 | 0.000 | 0.0000 | 0.000 | 0.000 | 0.000 | 0.000 | 10 | 55.00 | 75.00 | 0.000 | 0.0000 |
| *10 | 0.000 | 0.0000 | 0.000 | 0.000 | 0.000 | 0.000 | 10 | 55.00 | 75.00 | 0.000 | 0.0000 |
| *30 | 0.000 | 0.0000 | 0.000 | 0.000 | 0.000 | 0.000 | 10 | 55.00 | 75.00 | 0.000 | 0.0000 |

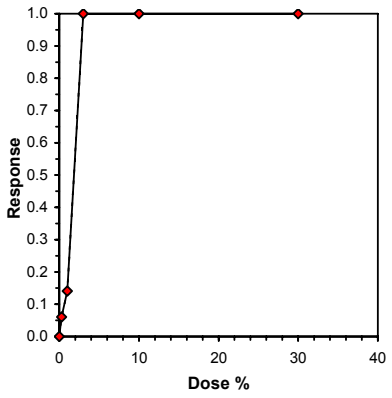
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | 2.61523 | 1.035 | -3.4223 | 24.0711 |
| Equality of variance cannot be confirmed | | | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | 0.3 | 1 | 0.54772 | 333.333 |
| Treatments vs Control | | | | |

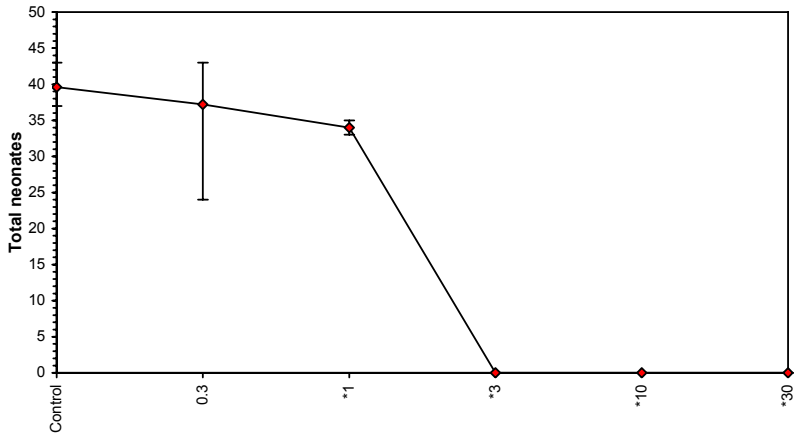
**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05* | 0.2475 | 0.1353 | 0.1086 | 0.5539 | 0.6628 |
| IC10 | 0.6413 | 0.1786 | 0.2172 | 0.8367 | -0.8012 |
| IC15 | 1.0200 | 0.0816 | 0.9046 | 1.0550 | -7.0228 |
| IC20 | 1.1365 | 0.0200 | 1.0922 | 1.1694 | -0.2534 |
| IC25 | 1.2529 | 0.0188 | 1.2115 | 1.2838 | -0.2534 |
| IC40 | 1.6024 | 0.0150 | 1.5692 | 1.6271 | -0.2534 |
| IC50 | 1.8353 | 0.0125 | 1.8076 | 1.8559 | -0.2534 |

* indicates IC estimate less than the lowest concentration




**Dose-Response Plot**



## 5.5 *Mogurnda mogurnda* test (814E)

**Gudgeon Sac Fry Survival Test-96 Hr Survival**

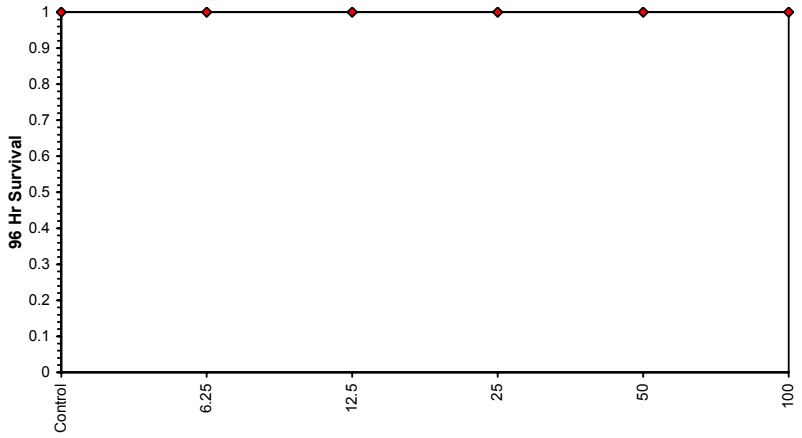
| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 1/04/2007 | Test ID: | 814E | Sample ID: | RUM-Ranger Uranium Mine |
| End Date: | 5/04/2007 | Lab ID: | ERISS-eriss ecotoxicology la | Sample Type: | RP2 |
| Sample Date: | | Protocol: | BTT E-eriss tropical freshwat | Test Species: | MMO-Mogurnda mogurnda |
| Comments: | | | | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| Control | 1.0000 | 1.0000 | 1.0000 |
| 6.25 | 1.0000 | 1.0000 | 1.0000 |
| 12.5 | 1.0000 | 1.0000 | 1.0000 |
| 25 | 1.0000 | 1.0000 | 1.0000 |
| 50 | 1.0000 | 1.0000 | 1.0000 |
| 100 | 1.0000 | 1.0000 | 1.0000 |

| | | | Transform: Arcsin Square Root | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | | |
| Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 3 | 0 | 30 |
| 6.25 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 3 | 0 | 30 |
| 12.5 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 3 | 0 | 30 |
| 25 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 3 | 0 | 30 |
| 50 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 3 | 0 | 30 |
| 100 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 3 | 0 | 30 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 1 | 0.858 | | |
| Equality of variance cannot be confirmed | | | | |

**Dose-Response Plot**



# Appendix 6 ToxCalc statistical summaries for the Pit 3 ecotoxicological tests

## 6.1 *Lemna aequinoctialis* test (817L)

**Lemna Growth Inhibition-Incr. in biomass**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 17/04/2007 | Test ID: | 817L | Sample ID: | PIT # 3 RA |
| End Date: | 21/04/2007 | Lab ID: | ERISS-eriss ecotoxicology la | Sample Type: | MRP-Mine retention pond |
| Sample Date: | | Protocol: | BTT L-eriss tropical freshwat | Test Species: | LAE-Lemna aequinoctialis |
| Comments: | | | | | |

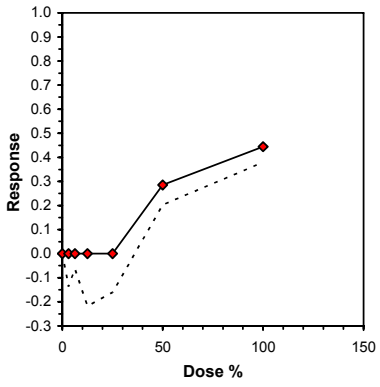
| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| Control | 43.000 | 42.000 | 39.000 |
| 3.125 | 45.000 | 51.000 | 45.000 |
| 6.25 | 39.000 | 48.000 | 45.000 |
| 12.5 | 51.000 | 46.000 | 54.000 |
| 25 | 46.000 | 49.000 | 49.000 |
| 50 | 34.000 | 30.000 | 35.000 |
| 100 | 23.000 | 29.000 | 25.000 |

| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control | 41.333 | 1.0000 | 41.333 | 39.000 | 43.000 | 5.036 | 3 | | | | 46.133 | 1.0000 |
| 3.125 | 47.000 | 1.1371 | 47.000 | 45.000 | 51.000 | 7.370 | 3 | -2.149 | 2.530 | 6.671 | 46.133 | 1.0000 |
| 6.25 | 44.000 | 1.0645 | 44.000 | 39.000 | 48.000 | 10.415 | 3 | -1.011 | 2.530 | 6.671 | 46.133 | 1.0000 |
| 12.5 | 50.333 | 1.2177 | 50.333 | 46.000 | 54.000 | 8.029 | 3 | -3.413 | 2.530 | 6.671 | 46.133 | 1.0000 |
| 25 | 48.000 | 1.1613 | 48.000 | 46.000 | 49.000 | 3.608 | 3 | -2.528 | 2.530 | 6.671 | 46.133 | 1.0000 |
| *50 | 33.000 | 0.7984 | 33.000 | 30.000 | 35.000 | 8.017 | 3 | 3.160 | 2.530 | 6.671 | 33.000 | 0.7153 |
| *100 | 25.667 | 0.6210 | 25.667 | 23.000 | 29.000 | 11.903 | 3 | 5.942 | 2.530 | 6.671 | 25.667 | 0.5564 |

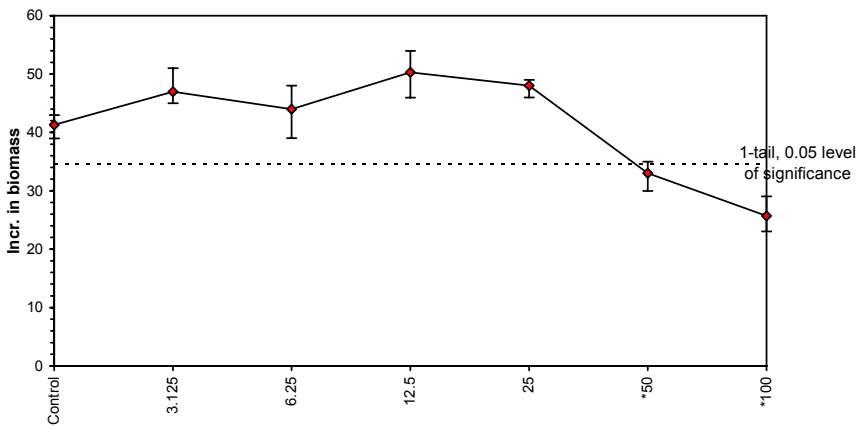
| Auxiliary Tests | | | | | Statistic | | Critical | | Skew | Kurt |
|---|---|---|---|---|---|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | | | | | 0.94629 | | 0.873 | | -0.1619 | -0.9341 |
| Bartlett's Test indicates equal variances (p = 0.89) | | | | | 2.25919 | | 16.8119 | | | |
| **Hypothesis Test (1-tail, 0.05)** | **NOEC** | **LOEC** | **ChV** | **TU** | **MSDu** | **MSDp** | **MSB** | **MSE** | **F-Prob** | **df** |
| Dunnett's Test<br>Treatments vs Control | 25 | 50 | 35.3553 | 4 | 6.67094 | 0.16139 | 239.778 | 10.4286 | 1.7E-06 | 6, 14 |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 29.391 | 0.547 | 26.928 | 31.089 | -0.8581 |
| IC10 | 33.782 | 0.962 | 30.036 | 37.178 | -0.0734 |
| IC15 | 38.173 | 1.403 | 32.560 | 43.267 | 0.0212 |
| IC20 | 42.563 | 1.852 | 35.080 | 49.356 | 0.0380 |
| IC25 | 46.954 | 2.579 | 37.600 | 58.138 | 0.5099 |
| IC40 | 86.273 | | | | |
| IC50 | >100 | | | | |




**Dose-Response Plot**



## 6.2 *Hydra viridissima* test (819B)

**Green Hydra Population Growth Test-Population growth rate (k**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 17/04/2007 | Test ID: | 819B | Sample ID: | PIT # 3 RA |
| End Date: | 21/04/2007 | Lab ID: | ERISS-eriss ecotoxicology la | Sample Type: | MRP-Mine retention pond |
| Sample Date: | | Protocol: | BTT B-eriss tropical freshwat | Test Species: | HV-Hydra viridissima |
| Comments: | | | | | |

| Conc-% | 1 | 2 | 3 |
|---|---|---|---|
| Control | 0.3588 | 0.3202 | 0.3527 |
| 0.75 | 0.3527 | 0.3466 | 0.3760 |
| 2.2 | 0.3132 | 0.3466 | 0.3202 |
| 6.7 | 0.2747 | 0.2483 | 0.2574 |
| 20 | 0.0000 | 0.0000 | 0.0000 |
| 60 | 0.0000 | 0.0000 | 0.0000 |

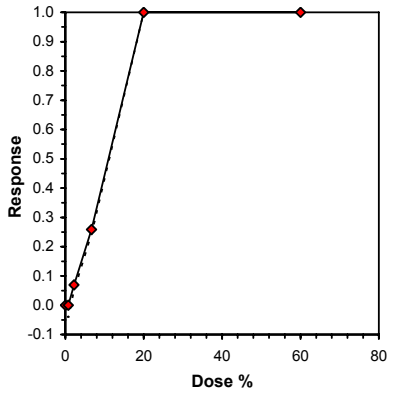
| | | | Transform: Untransformed | | | | | 1-Tailed | | | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | t-Stat | Critical | MSD | Mean | N-Mean |
| Control | 0.3439 | 1.0000 | 0.3439 | 0.3202 | 0.3588 | 6.028 | 3 | | | | 0.3512 | 1.0000 |
| 0.75 | 0.3584 | 1.0422 | 0.3584 | 0.3466 | 0.3760 | 4.332 | 3 | -1.280 | 2.500 | 0.0284 | 0.3512 | 1.0000 |
| 2.2 | 0.3267 | 0.9498 | 0.3267 | 0.3132 | 0.3466 | 5.387 | 3 | 1.520 | 2.500 | 0.0284 | 0.3267 | 0.9302 |
| *6.7 | 0.2601 | 0.7564 | 0.2601 | 0.2483 | 0.2747 | 5.143 | 3 | 7.382 | 2.500 | 0.0284 | 0.2601 | 0.7407 |
| *20 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.000 | 3 | 30.300 | 2.500 | 0.0284 | 0.0000 | 0.0000 |
| *60 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.000 | 3 | 30.300 | 2.500 | 0.0284 | 0.0000 | 0.0000 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Shapiro-Wilk's Test indicates normal distribution (p > 0.01) | 0.94822 | 0.858 | 0.03428 | -0.2724 |
| Equality of variance cannot be confirmed | | | | |

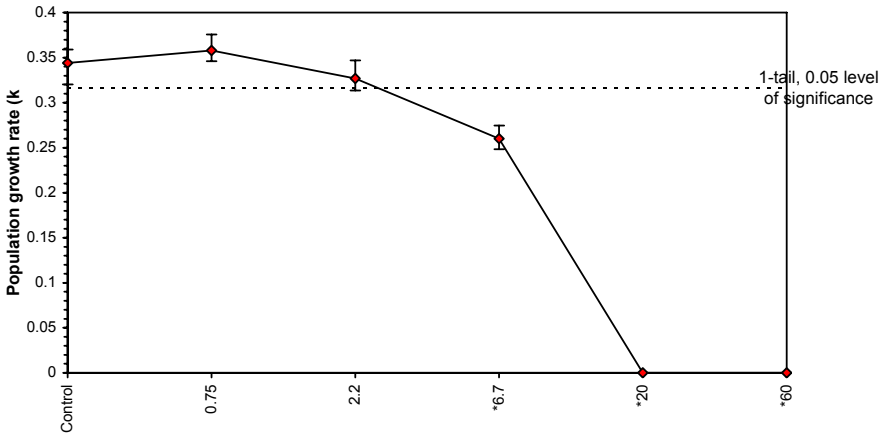
| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU | MSDu | MSDp | MSB | MSE | F-Prob | df |
|---|---|---|---|---|---|---|---|---|---|---|
| Dunnett's Test<br>Treatments vs Control | 2.2 | 6.7 | 3.83927 | 45.4545 | 0.02838 | 0.08251 | 0.08649 | 0.00019 | 3.4E-13 | 5, 12 |

**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL(Exp) | | Skew |
|---|---|---|---|---|---|
| IC05 | 1.789 | 0.447 | 0.805 | 4.109 | 1.0377 |
| IC10 | 2.917 | 0.557 | 0.867 | 5.030 | 0.2834 |
| IC15 | 4.104 | 0.519 | 1.984 | 6.419 | 0.1689 |
| IC20 | 5.292 | 0.500 | 3.627 | 7.717 | 0.4089 |
| IC25 | 6.479 | 0.462 | 4.718 | 8.362 | 0.0330 |
| IC40 | 9.227 | 0.330 | 7.969 | 10.642 | 0.1065 |
| IC50 | 11.022 | 0.275 | 9.974 | 12.202 | 0.1065 |




**Dose-Response Plot**



## 6.3 *M. macleayi* test (816D)

**Cladoceran Reproduction Test-Total neonates**

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 19/04/2007 | Test ID: | 816D | Sample ID: | RUM-Ranger Uranium Mine |
| End Date: | 25/04/2007 | Lab ID: | ERISS-eriss ecotoxicology lal | Sample Type: | PIT 3 |
| Sample Date: | | Protocol: | BTT D-eriss tropical freshwat | Test Species: | MOMA-Moinodaphnia macleayi |
| Comments: | 1st reproductive test with Pit 3 water | | | | |

| Conc-% | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 |
|---|---|---|---|---|---|---|---|---|---|---|
| MCW | 24.000 | 27.000 | 25.000 | 24.000 | 23.000 | 24.000 | 24.000 | 25.000 | 23.000 | 24.000 |
| 0.25 | 22.000 | 24.000 | 23.000 | 23.000 | 23.000 | 18.000 | 22.000 | 23.000 | 23.000 | 23.000 |
| 0.75 | 20.000 | 19.000 | 21.000 | 20.000 | 21.000 | 20.000 | 22.000 | 23.000 | 20.000 | 21.000 |
| 2.2 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 6.7 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |
| 20 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 | 0.000 |

| | | | Transform: Untransformed | | | | | Rank | 1-Tailed | Isotonic | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| Conc-% | Mean | N-Mean | Mean | Min | Max | CV% | N | Sum | Critical | Mean | N-Mean |
| MCW | 24.300 | 1.0000 | 24.300 | 23.000 | 27.000 | 4.772 | 10 | | | 24.300 | 1.0000 |
| *0.25 | 22.400 | 0.9218 | 22.400 | 18.000 | 24.000 | 7.351 | 10 | 65.50 | 75.00 | 22.400 | 0.9218 |
| *0.75 | 20.700 | 0.8519 | 20.700 | 19.000 | 23.000 | 5.601 | 10 | 56.00 | 75.00 | 20.700 | 0.8519 |
| *2.2 | 0.000 | 0.0000 | 0.000 | 0.000 | 0.000 | 0.000 | 10 | 55.00 | 75.00 | 0.000 | 0.0000 |
| *6.7 | 0.000 | 0.0000 | 0.000 | 0.000 | 0.000 | 0.000 | 10 | 55.00 | 75.00 | 0.000 | 0.0000 |
| *20 | 0.000 | 0.0000 | 0.000 | 0.000 | 0.000 | 0.000 | 10 | 55.00 | 75.00 | 0.000 | 0.0000 |

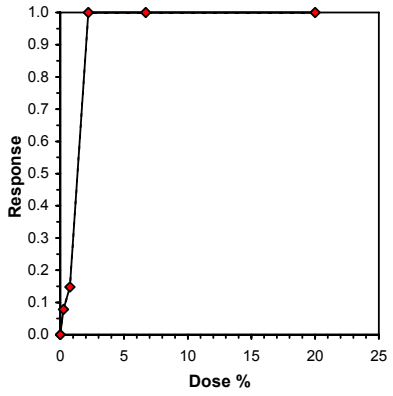
| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Kolmogorov D Test indicates non-normal distribution (p <= 0.01) | 1.96143 | 1.035 | -1.3115 | 10.1634 |
| Equality of variance cannot be confirmed | | | | |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Steel's Many-One Rank Test | <0.25 | 0.25 | | |
| Treatments vs MCW | | | | |

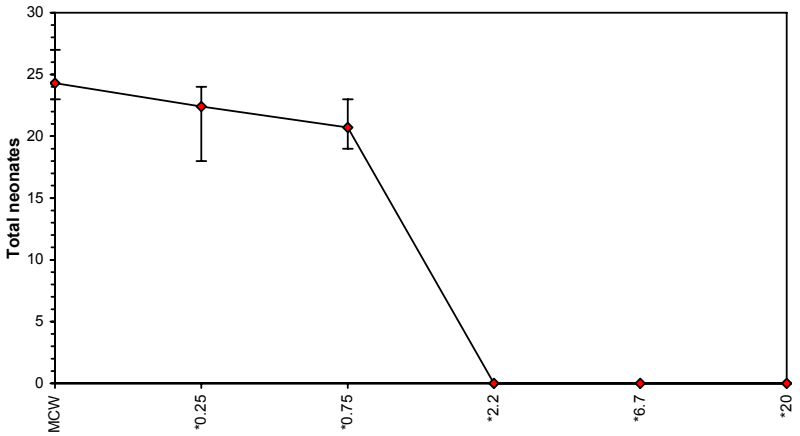
**Linear Interpolation (200 Resamples)**

| Point | % | SD | 95% CL | | Skew |
|---|---|---|---|---|---|
| IC05* | 0.1599 | 0.0585 | 0.1038 | 0.3164 | 1.1985 |
| IC10 | 0.4059 | 0.1262 | 0.2077 | 0.6809 | 0.2199 |
| IC15 | 0.7532 | 0.0715 | 0.5416 | 0.8144 | -1.2223 |
| IC20 | 0.8383 | 0.0279 | 0.7854 | 0.8959 | 0.0384 |
| IC25 | 0.9234 | 0.0261 | 0.8738 | 0.9774 | 0.0584 |
| IC40 | 1.1787 | 0.0209 | 1.1390 | 1.2219 | 0.0584 |
| IC50 | 1.3489 | 0.0174 | 1.3158 | 1.3849 | 0.0584 |

* indicates IC estimate less than the lowest concentration




**Dose-Response Plot**



# Appendix 7 Interim reporting of the RP2 results to EWL Sciences

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**From:** Jones, David Richard
**Sent:** Thursday, 5 April 2007 5:42 PM
**To:** Klessa, David (EWLS); 'Jacobsen, Nicole (EWLS)'
**Cc:** Zapantis, Alex (ERA)
**Subject:** RP2 tox test results [SEC=UNCLASSIFIED]
**Importance:** High

David and Nicole,
The RP2 tox test results for five species have just been finalised. Based on our now preferred IC10 point distribution fitting approach the dilution required for 99.5% protection is approxinately a 1 in 300 (0.35% to be precise) dilution of RP2 water. The details of the RP2 work will be provided next week.

Testing of Pit 3 water will start next Monday/Tuesday

Regards
David

**Dr David Jones**
Acting Supervising Scientist
Supervising Scientist Division
Department of the Environment and Water Resources

Postal address: GPO Box 461, Darwin NT 0801
t: +61 8 8920 1104; f: +61 8 8920 1190; m: 0418 835239
david.richard.jones@environment.gov.au
www.environment.gov.au/ssd

# Appendix 8 Interim reporting of the Pit 3 results to EWL Sciences

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**From:** Hogan, Alicia
**Sent:** Friday, 27 April 2007 5:33 PM
**To:** 'Jacobsen, Nicole (EWLS)'
**Cc:** Van Dam, Rick; JONES, David; HOUSTON, Melanie; LEE, Nichole
**Subject:** RE: Pit 3 ecotox testing [SEC=UNCLASSIFIED]

Hi Nicole,

The results for the Pit 3 ecotox testing are below. We haven't yet had time to fully interpret the data, however, we will provide a full discussion of the results and how they compare to U-only and RP2 tests in the final report.

NOEC = No observed effect concentration
LOEC = Lowest observed effect concentration
IC10 = Concentration that resulted in a 10% reduction of the test endpoint
IC50 = Concentration that resulted in a 50% reduction of the test endpoint

***Hydra:***
NOEC = 2.2%
LOEC = 6.7%
IC10 = 2.9%
IC50 = 11.0%

***Cladocera:***
NOEC <0.25%
LOEC < or = 0.25%
IC10 = 0.4%
IC50 = 1.3%

***Lemna:***
NOEC = 25%
LOEC = 50%
IC10 = 34%
IC50 > 100%

Cheers,
Alicia

Alicia Hogan
Ecotoxicology Section
Ecological Sciences Group
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