Part 1: Ranger – current operations

Chronic toxicity of uranium to larval purple-spotted gudgeon (*Mogurnda mogurnda*)

K Cheng, D Parry[1], A Hogan & R van Dam

Background

Toxicity tests to assess the effects of uranium (U) on freshwater species local to the Alligator Rivers Region have been employed since the late 1980s. From some of these data a high reliability site-specific water quality Limit for Magela Creek downstream of Ranger has been dervied, using the approach recommended by the Australian and New Zealand Water Quality Guidelines (WQGs; ANZECC/ARMCANZ 2000). The Limit of 6 µg L^{-1} U was derived using chronic toxicity no-observed-effect concentration (NOEC) data, ranging from 18–810 µg L^{-1}, for 5 species (Hogan et al 2005). However, two of the NOEC values, 400 and 810 µg L^{-1}, represent estimates for two fish species, the purple-spotted gudgeon, *Mogurnda mogurnda* and the chequered rainbowfish, *Melanotaenia splendida inornata*, respectively, based on mortality after only 7 d exposure (+ 7 d post-exposure for *M. mogurnda*; Holdway 1992). Although this endpoint satisfies the current WQGs criterion for a 'chronic' endpoint (ie >96 hour test duration), its appropriateness as an indicator of longer-term, sub-lethal chronic effects has been questioned.

In 2006-07 a 28 d larval growth chronic toxicity test protocol was developed for *M. mogurnda*. This test protocol was then used to assess the chronic toxicity of U[2], making comparisons to previous U toxicity data and discussing the implications for the uranium Limit for Magela Creek (see Cheng et al 2008a). The aims for 2007–08 were to:

1 Undertake a second uranium chronic toxicity test using larval *M. mogurnda*, focusing on concentrations within the range of approximately 500 to 3000 µg L^{-1}; and

2 Analyse whole body uranium content in larval *M. mogurnda* after 28 days exposure.

Chronic toxicity and uptake of uranium

The chronic toxicity test protocol was described in detail by Cheng (2008). Newly hatched (<10-h old) *M. mogurnda* larvae were exposed to various concentrations of U (control, 400, 800, 1200, 1600, 2000 and 2200 µg L^{-1} – measured concentrations) for 28-d. For each U concentration, 10 larvae were placed in each of four replicate test containers, each containing 500 mL of test solution. Test solutions were renewed daily, and larvae were fed live brine shrimp (*Artemia salina*) nauplii two times a day (morning and early evening), at a feeding rate of approximately 10–20, 20–30 and 30–40 nauplii per larva, through days 1–7, 8–25 and 26–28, respectively. Larval survival was monitored throughout and at the end of the test, while larval length and dry weight were measured at the end of the test.

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2 Project undertaken under Charles Darwin University Animal Ethics Approval Ref No. A06008.

At the end of the test, surviving larval *M. mogurnda* were euthanased and rinsed thoroughly in Milli-Q water, dried (72-h at 60°C; for dry weight and U analysis) and digested using AR grade HNO_3 followed by H_2O_2 at 135°C (for whole body U content). Uranium in larval whole body digests was measured using ICP-MS (Agilent 7500ce) The minimum biomass required for metal analysis was approximately 0.3 g in dry weight. In order to obtain this, replicate samples were pooled and, therefore, no statistical tests for significant differences between treatments could be performed on the data.

The results of the initial and second U toxicity tests are summarised in Table 1, while the effects of U exposure on larval *M. mogurnda* in the second test only are shown in Figure 1. Exposure to 1600, 2000 and 2200 µg L^{-1} uranium resulted in 100% larval mortality within the first 48-h of exposure. Significant mortality (~80%; ANOVA, $P < 0.05$) was observed in the group exposed to 1200 µg L^{-1}. Larvae exposed to 800 and 1200 µg L^{-1} U for 28-d exhibited significant 8% and 25% reductions in length, and 15% and 60% reductions in dry weight, respectively, relative to control larvae (ANOVA, $P \leq 0.05$). Based on larval length and dry weight, the lowest-observed-effect concentration (LOEC) and no-observed-effect concentration (NOEC) were 800 and 400 µg L^{-1} U, respectively (Figure 2). As can be seen in Table 1, larval *M. mogurnda* in test 2 were more sensitive to U than in test 1.

Table 1 Summary of uranium toxicity to *M. mogurnda* following 28-d exposure

Test	Endpoint	NOEC (µg L^{-1} U)	IC_{10}(µg L^{-1} U)	IC/LC_{50}(µg L^{-1} U)
1	Survival	1400	-	2150
(June 2007)	Dry Weight	880	862	>1400
	Length	880	1162	>1400
2	Survival	800	-	1060
(February 2008)	Dry Weight	400	652	1110
	Length	400	847	>1200

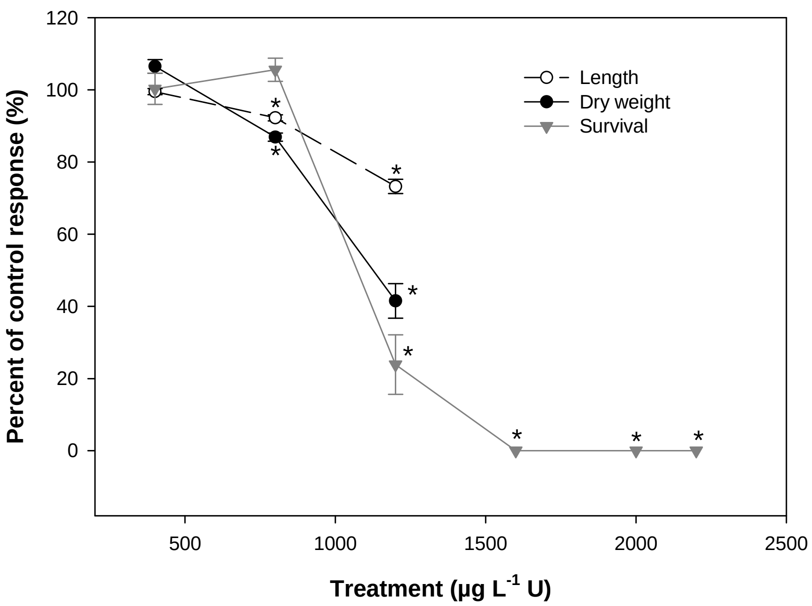

Figure 1 Uranium chronic toxicity test 2 (2007–08): Effect of U exposure over 28-d on mean (±SEM) dry weight (n = 4), length (n = 9–40) and survival (n = 4) of larval *M. mogurnda*, normalised against the control responses. Data points accompanied by an asterisk are significantly different from the control responses ($P \leq 0.05$). Mean control responses (±SEM) were as follows: dry weight – 1.70 (±0.03) mg; length – 11.2 (±0.1) mm; and survival – 95 (±3) %.

Whole body U concentrations for *M. mogurnda* in both tests are summarised as a pooled dataset in Figure 2 (data were pooled due to the similar response observed between tests). Uranium concentrations in control larvae were less than 0.05 µg g^{-1} for both toxicity tests. Results obtained from U exposed groups showed a dose dependent relationship, with whole body U concentration increasing with increasing exposure concentration. The similar whole body U concentrations for larval *M. mogurnda* at similar U exposure concentrations between the two tests suggest that the observed difference in toxicity may not have been due to a difference in U uptake (although it is acknowledged that the whole body concentrations may include U adsorbed to the outer surfaces of, as well as U taken up by, the larvae). Geochemical speciation modelling for U (see below) may shed further light on the bioavailability of U between the two tests.

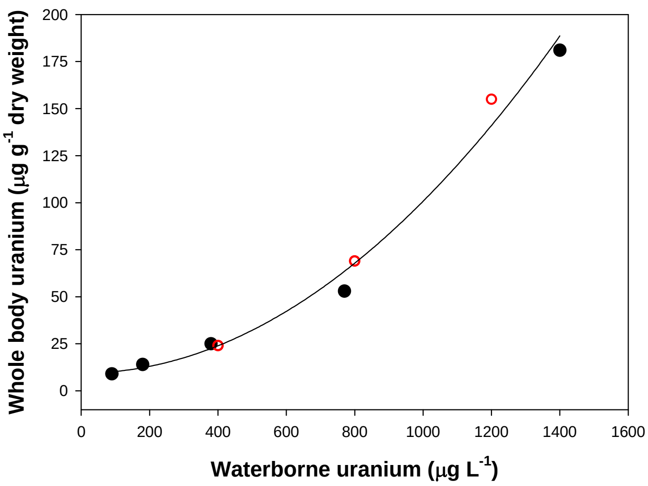

Figure 2 Whole body uranium concentrations for larval *M. mogurnda* following a 28-d exposure period. Data are pooled from tests 1 (closed circles) and 2 (open circles), and the associated relationship is described by a quadratic model ($R^2 = 0.983$, df = 7, $P < 0.001$).

Following two independent U chronic exposure tests, *M. mogurnda* did not appear to be more sensitive than that previously reported by Holdway (1992) following shorter (ie 7 and 14-d) exposure periods. This indicates that the one to two week period post-hatch provides the most sensitive time window for assessment of the toxic effects of U on *M. mogurnda*, and that longer exposure periods will not necessarily result in a more sensitive response. Consequently, the historical 7-d fish toxicity test results used for the derivation of the current U Limit of 6 µg L^{-1} appear to be reasonably representative of U concentrations that will not result in longer-term chronic effects. This provides assurance that the current uranium Limit is sufficiently conservative to ensure protection from chronic toxicity effects of U.

The results of this project have been published as an Internal Report (Cheng 2008; CDU Honours project) and were presented at the SETAC 5th World Congress in Sydney, 3–7 August 2008 (Cheng et al 2008b).

Steps for completion

At present, a manuscript detailing the results of this study is being drafted for peer-reviewed publication. The manuscript will include an assessment of U speciation and bioavailability based on geochemical speciation modelling.

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Influence of dissolved organic carbon on the toxicity of uranium

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Background

Mining represents one of the threats to the quality and biodiversity of freshwater ecosystems in northern Australia. Uranium (U), aluminium (Al) and arsenic (As) are priority metals of ecotoxicological concern for the region's mining industry for which insufficient toxicity data exist (Markich & Camilleri 1997). Uranium is a metal of concern for the Magela Creek system adjacent to the Ranger mine, whilst Al and As are of general concern for mining in the broader northern Australian region (Markich & Camilleri 1997, Lloyd et al 2002).

Few existing studies of the toxicity of these metals have incorporated dissolved organic carbon (DOC) as a variable (see toxicity reviews for U, Al and As by Sheppard et al 2005, Gensemer & Playle 1999 and Gomez-Caminero et al 2001, respectively), despite humic substances being recognised as playing a major role in metal speciation (Tipping 2002). The few tropical freshwater toxicity studies that have investigated the influence of DOC on metal speciation and bioavailability have demonstrated it can be a key determinant of metal toxicity (Markich et al 2000; Hogan et al 2005). It is essential to build upon these studies in order to gain a comprehensive understanding of the role of DOC in metal bioavailability and toxicity in tropical freshwater ecosystems.

The objective of this study is to quantify the influence of DOC on the toxicity of U, Al and As to four freshwater species, under fixed conditions of pH, water hardness and alkalinity.

Methods

The selected tropical species, northern trout gudgeon, *Mogurnda mogurnda*, green hydra, *Hydra viridissima*, the unicellular alga, *Chlorella* sp and a unicellular flagellate, *Euglena gracilis*, were chosen to cover a range of trophic levels. Laboratory toxicity testing is being conducted using a synthetic soft water (characteristic of sandy braided streams in tropical Australia) and a Suwannee River Fulvic Acid Standard I (SRFA) produced by the International Humic Substances Society (IHSS). Tests will also be conducted using natural waters with a range of DOC concentrations. SRFA was selected as the organic carbon source for this study as it is a well characterised reference material (Cabaniss & Shuman 1988) and the only biologically relevant standard for this study. This summary focuses on the toxicity of U to the first three aforementioned species.

The test organisms were exposed to a range of U (0 to 14, 1.1 and 1.5 mg/L, for gudgeon, hydra and alga, respectively) and DOC concentrations (0, 1, 5, 10 and 20 mg/L) in combination. The synthetic water used was of very low ionic strength (low hardness and alkalinity) and was slightly acidic (pH 6). Test systems were static with no test solution

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renewals for *Chlorella* sp and 24-h renewals for *M. mogurnda* and *H. viridissima*. Test temperatures were maintained at 27±1°C for *M. mogurnda* and *H. viridissima* and 28±1°C for *Chlorella* sp. Three tests were run for each species in order to fully characterise the concentration-response relationships.

The test durations and endpoints for the tests were as follows: *M. mogurnda* 96-h sac-fry survival; *H. viridissima* 96-h population growth rate; *Chlorella* sp 72-h population growth rate. For all tests, water parameters (pH, DO, EC) were monitored daily. Water samples were taken for analyses of DOC, alkalinity, hardness and a standard suite of metals and major ions. For each species, response data from three tests were pooled, and concentration–response relationships were determined (using regression analyses). Physicochemical variables were input into the HARPHRQ geochemical computer speciation model to determine the effect of DOC on U speciation, which was related back to U toxicity.

Progress

The U toxicity testing was completed for all three species. U toxicity to all species was reduced substantially with increasing SRFA. Concentration-response relationships, and associated linear regressions of toxicity (expressed as IC/LC_{50}) against DOC concentration, are shown in Figure 1. Toxicity summary data and fitted regression equations are shown in Table 1.

Table 1 Summary of selected uranium toxicity testing results for three local freshwater species

Species	DOC[a] (mg/L)	IC_{50}[b] (95%CL)[c] (μg/L)	Reduction in toxicity with 20× DOC increase	Regression equation, r^2 & *P* value for IC_{50} v DOC plots in Fig 1
Mogurnda mogurnda[d]	0 20	1550 (1057–1961) 7200 (5907–8903)	4.6×	IC_{50}=287[DOC]+1548, r^2=98%, *P*<0.0001
Hydra viridissima (green hydra)	0 20	65 (8–85) 470 (404–512)	7×	IC_{50}=19[DOC]+85, r^2=91%, *P*<0.0001
Chlorella sp (unicellular alga)	0 20	38 (22–69) 656 (454–954)	17×	IC_{50}=30[DOC]+86, r^2=92%, *P*<0.0001

a DOC: dissolved organic carbon

b IC_{50}: concentration that results in a 50% inhibition of response relative to the control response using dose-response relationships determined by ToxCalc

c 95% CL: 95% confidence limits determined by ToxCalc

d For *M. mogurnda*, the toxicity estimates relate to concentrations that affect survival, compared to sub-lethal endpoints, such as growth and reproduction, for the other species

The extent to which DOC ameliorated U toxicity differed for each species. DOC appeared to result in a more gradual (but higher overall) reduction in U toxicity for *Chlorella* sp and *H. viridissima* than *M. mogurnda*. For *M. mogurnda*, there was an apparent increase in the threshold U concentration (the point at which survival dropped from 100%) with increasing DOC. However, the slope of the response curve from 100% to 0% survival was similar for all DOC concentrations. This may indicate a similar toxic response is occurring across DOC treatments once cation-binding sites on the DOC are saturated with UO_2^{2+} or $UO_2(OH)^+$, the most toxic forms of U in solution around pH 6.

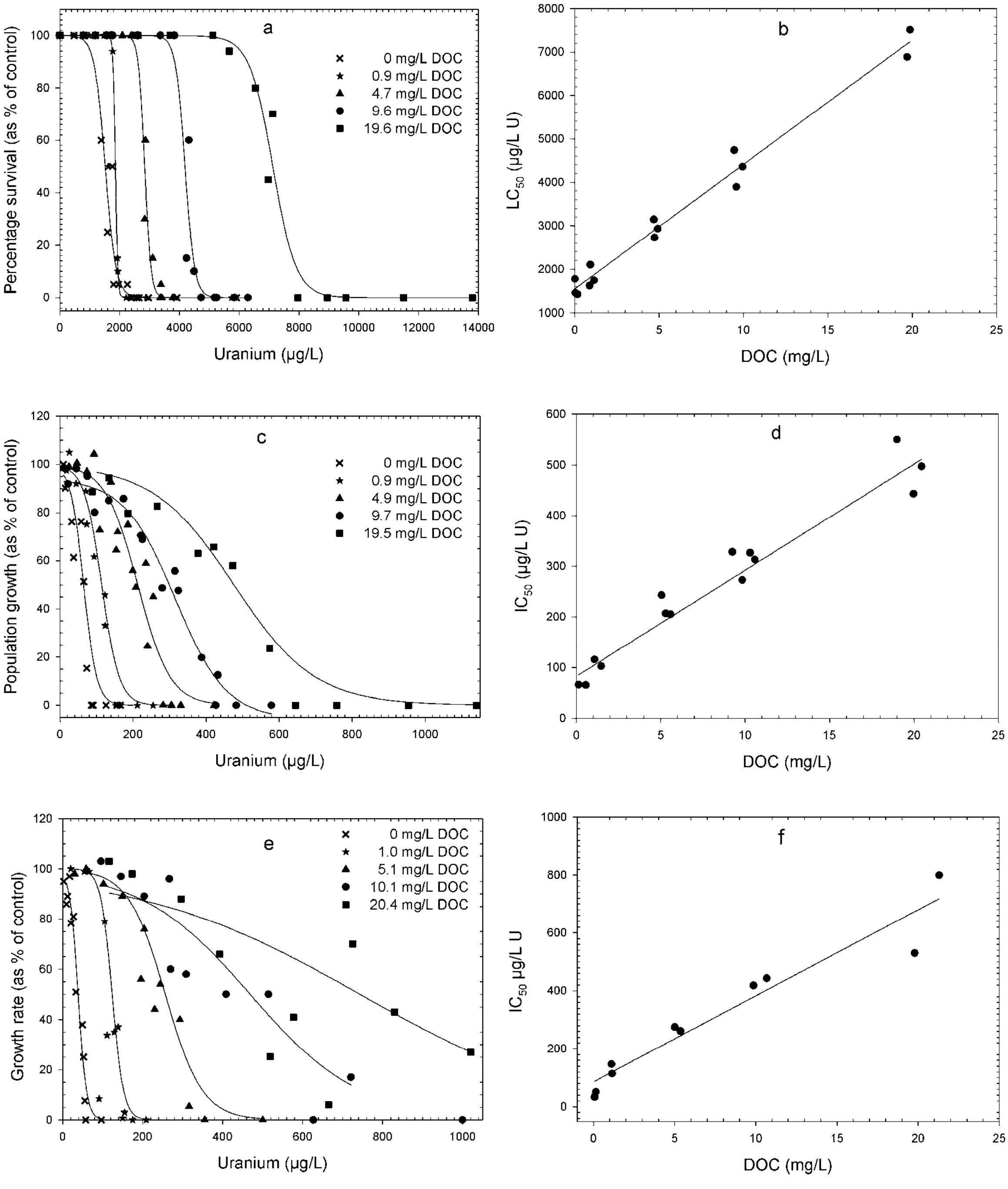

Figure 1 Effect of DOC on U toxicity to *Mogurnda mogurnda* (a & b), *Hydra viridissima* (c & d) and *Chlorella* sp (e & f). Left plots represent the concentration-response relationships, with curve fits based on a sigmoidal, 3-parameter model. Right plots represent the linear regressions of U toxicity (expressed as the IC/LC_{50}) against DOC concentration.

The decrease in toxicity of U in the presence of SRFA for all three species was shown to be due to a reduction in the free uranyl ion concentration due to its being bound by the fulvic acid (Figure 2). Differences in the proportions of U species between the three test species are most likely due to small pH and other physico-chemical differences in the test diluent waters.

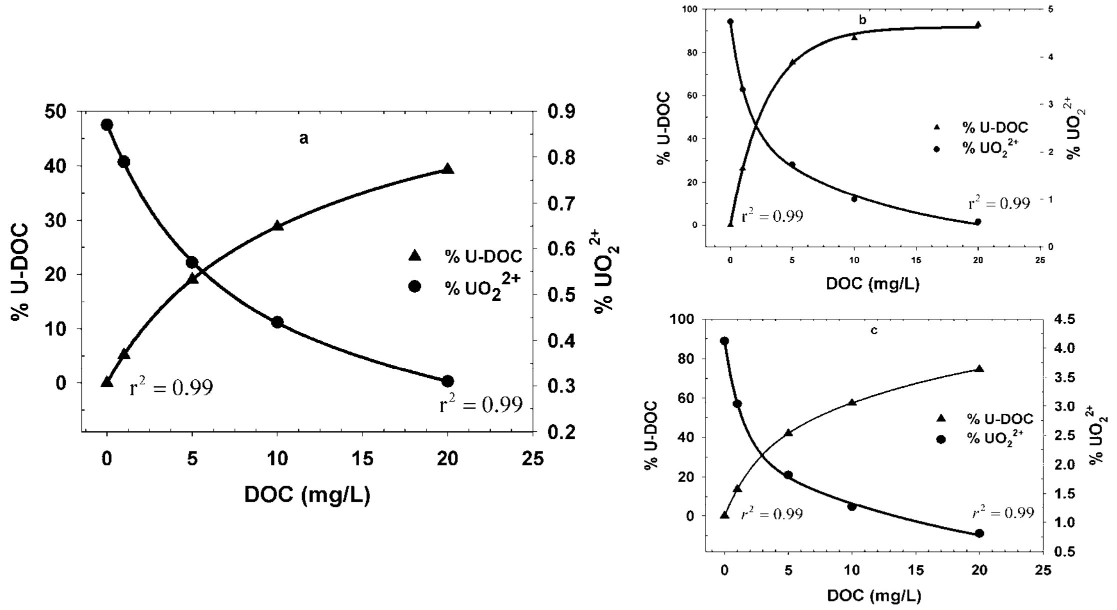

Figure 2 Percentage of U bound to DOC and UO_2^{2+} in solution at increasing DOC concentrations calculated using the HARPHRQ speciation model: (a) *Mogurnda mogurnda*, (b) *Hydra viridissima* and (c) *Chlorella* sp

Concentration-response plots were produced recently based on the concentration of the two uranyl species (UO_2^{2+} and UO_2OH^+) suggested by Markich et al (2000) to be most responsible for inducing toxicity (Figure 3). If U toxicity could be explained entirely by the concentrations of the above two U species, the separate DOC plots in each of Figure 3a–c would be expected to converge into one concentration-response relationship. Substantial convergence was observed for all three species, suggesting that U toxicity was likely to be due largely to UO_2^{2+} and UO_2OH^+. However, for *M. mogurnda* and *Chlorella* sp in particular, other U complex species may also be contributing to toxicity. In this context it must be noted that the SRFA represents a distribution of organic species, not a single organic chemical reagent. Hence it is not unreasonable to expect that there would be a distribution of toxicity across the range of U-organic species. Stepwise multiple regression will be used (as per Markich et al 2000) to identify the U species most likely contributing to the observed toxic responses.

The next phase of the work for U will involve comparison of the above U toxicity results obtained using SRFA in SSW to that in natural waters with natural DOC. This will be done using DOC-rich fresh water from billabongs. Attentuation of U toxicity will be especially important in natural billabongs, such as Georgetown Billabong on the Ranger lease, where the setting of water body specific surface water closure criteria for U may require consideration of the DOC concentrations, which can be higher than in Magela Creek.

The next stage of the project involving the effect of SRFA on toxicity will be undertaken for Al and As, using the same suite of test organisms as for U. The effect on As toxicity will be especially interesting given that As occurs as an oxyanion with very different chemistry, compared with the cationic forms of Al and U.

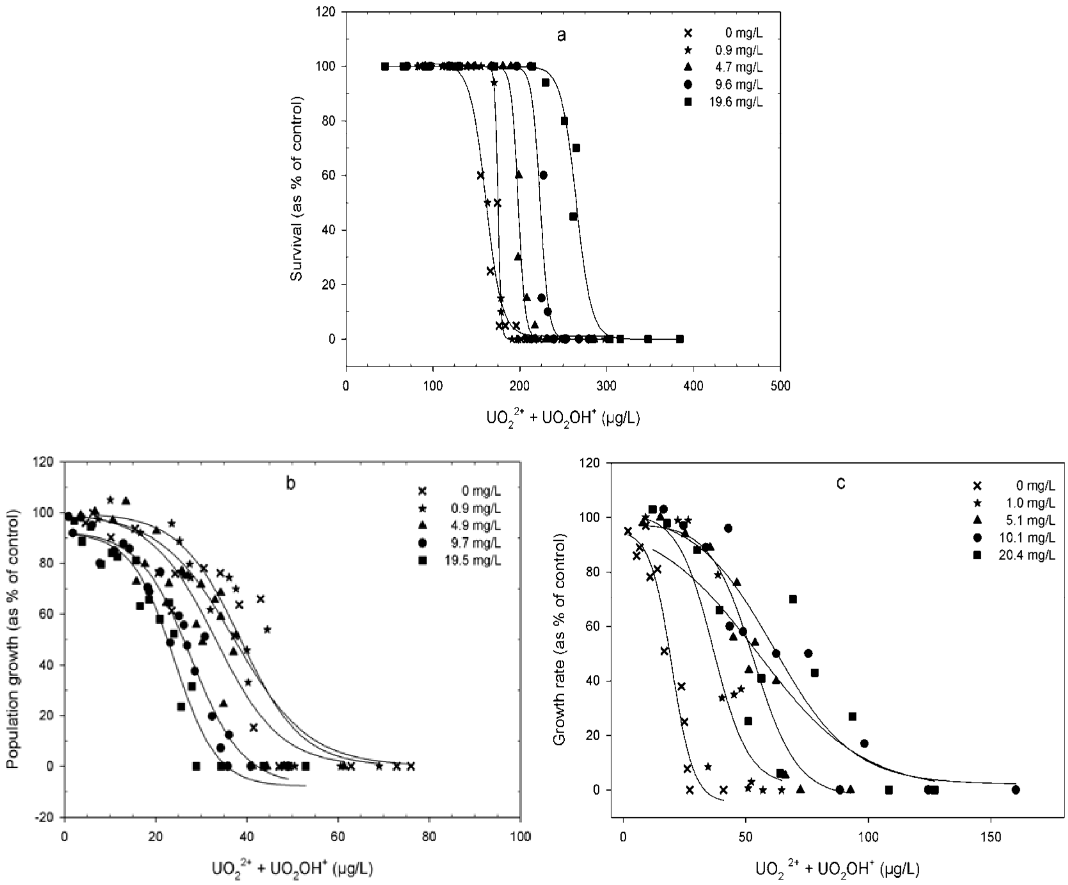

Figure 3 Concentration response plots based on the concentration of the two U species, UO_2^{2+} and UO_2OH^+: (a) *Mogurnda mogurnda*, (b) *Hydra viridissima*, (c) *Chlorella* sp

In addition, short-term (~3 minute) exposures of unicellular *Euglena gracilis* to all three metals will be used to assess cellular responses to each metal and DOC combination. The wildtype plant strain and mutant animal strain of this species will hopefully enable sites of intracellular damage to be identified and for this information to aid in assessment of potential toxic mechanisms in other unicellular or multicellular organisms. The use of *Euglena* as a test species is beneficial not only because it is a simple, rapid test system but also because small volumes of test solution are required (which lowers the demand for costly fulvic acid standard and the volume of natural water required to be collected).

The results of this study were presented at the 5th SETAC World Congress in Sydney, 3–7 August 2008 (Houston et al 2008a) and the 14th Meeting of International Humic Substances Society in Moscow, 13–19 September 2008 (Houston et al 2008b). This project is funded by an ARC Linkage grant (LP 0562507).

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Preliminary assessment of the toxicity of manganese to three tropical freshwater species

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Background

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

Evidence from the literature suggests the acute and chronic toxicity of Mn to freshwater biota is low (ie in the mg L^{-1} range), and this is reflected in the relatively high trigger value reported above. The current site-specific guideline for Mn in Magela Creek downstream of Ranger is 26 µg L^{-1}. This value was derived from statistical analysis of water quality data from the upstream reference site data, and applicable only when flow in Magela Creek is greater than 5 cumecs. It is approximately two orders of magnitude more conservative than the ANZECC/ARMCANZ (2000) trigger value. Since 1980, this local guideline has been exceeded less than 2% of the time. The majority of exceedances have occurred during early wet season flows or end of wet season recessional flows, often when flow is less than 5 cumecs. These periods are considered to be atypical of the season as a whole given the increased contributions from shallow groundwater at these times. Based on the very low frequency of exceedance of the local guideline for Mn, and the existing ANZECC/ARMCANZ (2000) 99% species protection trigger value of 1200 µg L^{-1}, it was perceived that Mn posed a low toxicity risk to aquatic biota in Magela Creek.

However, insufficient Mn toxicity data exist for local species in Magela Creek water to be able to (i) conclude with high confidence that no adverse effects would be expected given the current water quality and (ii) predict at what Mn concentrations adverse effects would be expected to occur. This is particularly important given that the low water hardness and relatively low pH of Magela Creek water could potentially result in higher than expected (ie from existing literature) Mn toxicity.

A previous, albeit limited, study by ***eriss*** (unpublished data, 1993) on the toxicity of Mn to *Hydra viridissima* (population growth) reported relatively low NOEC/LOEC values of 20/200 $\mu g\ L^{-1}$ and 180/630 $\mu g\ L^{-1}$ in synthetic and natural water, respectively, compared to the existing literature. The apparent sensitivity of *H. viridissima* to Mn and paucity of data for other local species provided sufficient basis to conduct a pilot site-specific ecotoxicological assessment, since it is possible that in the future higher concentrations of Mn could occur in the Creek as a result of inputs from the mine site.

The aim of this study was to determine the toxicity of manganese (Mn) to three generally sensitive tropical freshwater species, the green alga, *Chlorella* sp, the green hydra, *Hydra viridissima*, and the cladoceran, *Moinodaphnia macleayi*. Specifically, the study set out to assess whether Mn may represent a significant environmental hazard downstream of Ranger, and hence warrant a further, more detailed risk assessment.

Methods

Mn is a difficult metal to work with, because its chemistry in water is a complex function of pH and redox microenvironment. The kinetics of Mn(II) oxidation increases at higher pH. Thus, a lower pH diluent water (Ngarradj Creek Water, NCW) was chosen to reduce the probability of particulate formation as a result of the oxidation of Mn(II) in solution to form Mn(III)/Mn(IV) oxyhydroxide precipitates. NCW was collected from near the Ngarradj Creek Upstream gauging station (NCUS: 0275473; 8616847; WGS84, Zone 53) and was filtered (2.5 μm) upon arrival at the ***eriss*** laboratories. All manganese treatments were diluted in NCW. In addition, a Magela Creek Water (MCW) quality control group was included for each test (ie organisms were cultured in the standard natural MCW; pH – 6.77, EC – 16 μS/cm, DO – 97.5% saturation).

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

One experiment was undertaken for *Chlorella* sp and *H. viridissima*, while for *M. macleayi* three chronic toxicity tests and an acute test were conducted (Table 1).

Table 1 Details of the Mn toxicity tests conducted

Test ID and Date	Species name	Endpoint	Test Duration	Feeding/ nutrition	Acute/ Chronic	Static/ daily renewals
933D 31/05/08	*Moinodaphnia macleayi*	Reproduction	3 broods 120–144 h	30 μl FFV only	Chronic	Daily renewals
934D 31/05/08	*Moinodaphnia macleayi*	Reproduction	3 broods 120–144 h	30 μl FFV + 6 x 10^6 cells of *Chlorella* sp	Chronic	Daily renewals
936B 16/06/08	*Hydra viridissima*	Population growth	72 h	30–40 artemia nauplii	Chronic	Daily renewals
938I 20/06/08	*Moinodaphnia macleayi*	Survival	48 h	No food	Acute	Static
937D 20/06/08	*Moinodaphnia macleayi*	Reproduction	3 broods 120–144 h	30 μl FFV + 6 x 10^6 cells of *Chlorella* sp	Chronic	Daily renewals
939G 24/06/08	*Chlorella* sp.	Population growth	72 h	14.5 mg/L NO_3 0.14 mg/L PO_4	Chronic	Static

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

Results and discussion

Chemistry

Prior to filtering, the NCW had a pH of 5.29, an electrical conductivity (EC) of 13 µS/cm and a dissolved oxygen (DO) content of 85.5%. Following filtration, the water had a pH of 5.58, an EC of 12 µS/cm and a DO content of 74.9%. For the testing, the pH was slightly higher again, but remained between 6.0–7.0 for all tests. Metal analysis of filtered NCW indicated that it contained some aluminium (3.0 µg L^{-1}), zinc (2.0 µg L^{-1}), nickel (1.6 µg L^{-1}) and manganese (3.8 µg L^{-1}). All other metals analysed were at concentrations <1 µg L^{-1}.

The results of Mn analyses for the toxicity tests are reported in Table 2. The total concentration of Mn did not change during the course of the experiments, indicating that there was no loss to the test system (eg walls of the test vials). At the commencement of the tests, ~92% of the total Mn was present in the <0.1 µm fraction (ie dissolved or very fine colloidal fraction), compared to approximately 86–92% by the end of the tests. Furthermore, tests that did not receive daily water renewal and were conducted over longer time periods (ie 72-h algae test and 48-h acute flea tests) did not show markedly larger losses of Mn. To account for the change in soluble (ie bioavailable) Mn, the calculation of toxicity estimates used an average of the start and end of test filtered concentrations. Analysis of the test solutions from the initial two cladoceran tests (ie 933D and 943D) indicated that significant concentrations of oxidised Mn forms (ie insoluble forms) were not being formed in the presence of photosynthetic organisms (ie the algal food source), which was probably due to the pH of the solutions being below 7.0 units (Richardson et al 1988).

Toxicity

The concentration-response relationships for the three species are shown in Figure 1 and the toxicity estimates and control responses are summarised in Table 3.

The initial chronic toxicity experiment with *M. macleayi* demonstrated that excluding the algal food from the test significantly reduced their reproductive health (Figure 1a). Exposure of *M. macleayi* in the presence of the algal food resulted in no observed toxicity to concentrations up to 1840 µg L^{-1} Mn, while excluding the algal food resulted in a significant reduction in neonate numbers of ~40% at the highest concentration (Figure 1a). Consequently, a 6-d chronic test with algal food and a 48 h acute test without food were conducted at higher concentrations. Both these studies resulted in 100% lethality to *M. macleayi* within 48 h at concentrations ≥1845 µg L^{-1} Mn (Figure 1a & b). A Mn concentration of 870 µg L^{-1} Mn resulted in a statistically significant reduction in the number of neonates (ie 13%) in the chronic test, while in the acute test no significant effects were observed at 770 µg L^{-1} Mn (Table 3). The initial (ie 933D and 934D) and subsequent cladoceran tests (ie 937D and 938I) were somewhat contrasting in terms of the measured responses at around 1800–1900 µg L^{-1}. Nevertheless, the results of the second set of tests indicate a dramatic threshold response for survival at between

1000–2000 µg L^{-1} Mn. This concentration is quite low compared to other studies reported in the literature with the exception of one previous freshwater study (see below).

Table 2 Measured and predicted[1] Mn concentrations in the tests

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

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

2 PB=Procedural Blank

3 NA = not analysed

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

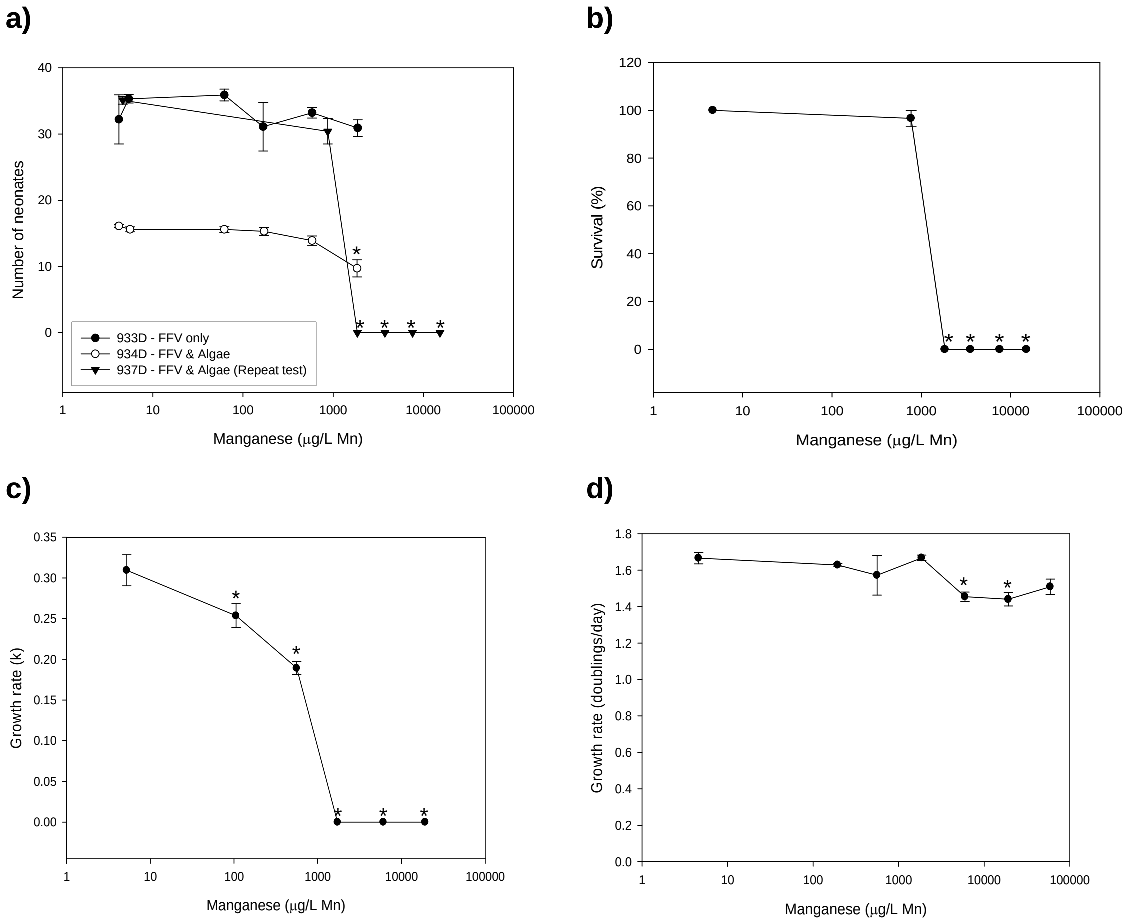

Figure 1 Effect of manganese on a) the reproduction of *M. macleayi* over 6 days; b) the survival of *M. macleayi* over 48 h; c) the population growth rate of *H. viridissima* over 96 h; and d) the growth rate of *Chlorella* sp over 72 h. * denote significantly different from the NCW control (p<0.05).

Of the three species tested, *H. viridissima* was the most sensitive to Mn exposure. The lowest concentration of Mn tested resulted in a significant reduction of population growth rate (ie a NOEC of <106 µg L^{-1} and a LOEC of 106 µg L^{-1}; Figure 1, Table 3). An IC_{10} of 60 µg L^{-1} and an IC_{50} of 770 µg L^{-1} were determined from the concentration-response relationship. Reanalysis of data from the previous *H. viridissima* study conducted at ***eriss*** (described above), based on population growth rate over 96 h as the test endpoint, yielded a NOEC and LOEC of 180 and 600 µg L^{-1}, respectively, an IC_{10} of 270 µg L^{-1} and an IC_{50} of 990 µg L^{-1}.

The IC_{50} values, which are the more reliable toxicity estimate for comparison purposes, demonstrate that the two studies found similar responses of *H. viridissima* to Mn. Only one other study has reported higher toxicity of Mn to a freshwater organism. Fargašová (1997) reported 43% mortality of the midge larva, *Chironomus plumosus*, at 55 µg L^{-1} Mn. However, this was the only concentration tested and many details of the test method (eg. physico-chemistry of diluent water, chemical analysis of the test chemical) were not described, making it difficult to establish the quality of the data. Hence this result was not used by ANZECC/ARMCANZ (2000) in the derivation of the default water quality trigger value for Mn.

Table 3 Summary of the Mn toxicity estimates to three local freshwater species

Test ID and Date	Species name	Endpoint	Control performance			Toxicity (μg L-1)			
			Creek water	mean	%CV[2]	IC_{10}[3]	IC_{50}[4]	NOEC[5]	LOEC[6]
933D 31/05/08	*M. macleayi*	# neonates	Magela	35.4	6.2	1750	>1870	1870	>1870
			Ngarradj	32.2	36.4				
934D 31/05/08	*M. macleayi*	# neonates	Magela	13.6	8.6	410	>1840	580	1840
			Ngarradj	16.1	4.6				
936B 16/06/08	*H. viridissima*	Population growth rate	Magela	0.3	5.8	60 (30–330)	770 (590-940)	<106	106
			Ngarradj	0.3	10.6				
937D 20/06/08	*M. macleayi*	# neonates	Magela	27	43	650 (360–920)	1290 (1200–1340)	<870	870
			Ngarradj	35.1	5.3				
938I 20/06/08	*M. macleayi*	Survival	Magela	100	0	880 (730–880)	1310 (1230–1310)	770	1850
			Ngarradj	100	0				
939G 24/06/08	*Chlorella* sp	Growth rate	Magela	1.8	3.3	5100	<59300	1860	5960
			Ngarradj	1.7	3.3				

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

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

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

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

[5] NOEC: highest concentration tested where growth was not significantly different from the control growth rate

[6] LOEC: lowest concentration tested where growth was significantly different from the control growth rate

Manganese had very little effect on the growth rate of *Chlorella* sp (Figure 1, Table 3) over the concentration range that was tested. An EC_{10} of 5100 μg L^{-1} was calculated, while the EC_{50} could not be determined but was > 59 300 μg L^{-1}. However, due to very low intra-treatment variability in the control and treatment groups in a statistically significant inhibition of growth rate was detected in the intermediate treatments of 1860 μg L^{-1} and 5960 μg L^{-1} Mn. The results demonstrate that *Chlorella* sp is very tolerant to Mn exposure.

The results from this pilot investigation indicate that, compared to values reported in the scientific literature, Mn toxicity was higher to two of the three local species tested. The higher toxicity may be due to the lower pH and ionic strength of the NCW diluent. Although, the green alga, *Chlorella* sp was extremely tolerant to Mn, concentrations of 1000–2000 μg L^{-1} appeared to be acutely toxic to the cladoceran, *M. macleayi*. Further characterisation of this latter species' strong threshold response would strengthen confidence in the toxicity estimates obtained by this study. The hydra, *H. viridissima*, was clearly the most sensitive species with a significant reduction in population growth rate at the lowest concentration tested (106 μg L^{-1}), with a resultant IC_{10} value of 60 μg L^{-1}. This is one of the most sensitive responses reported in the literature and warrants further investigation.

Comparison of *H. viridissima* toxicity data with environmental concentrations

Figure 2 presents a comparison of the *H. viridissima* IC_{10} value with concentrations of Mn recorded at the Magela creek downstream (ie MG009) and upstream (ie MCUS) monitoring points. Although recorded Mn concentrations at MG009 have not exceeded the IC_{10} concentration, they have approached it on several occasions, whereas Mn upstream of Ranger remains mostly below 25 µg L^{-1}. The highest concentrations of Mn occur at MG009 during the late wet season/early dry season recessional flow period, and appear to be at least partially due to the surface expression of mine-impacted groundwaters. This period of the annual seasonal cycle coincides with the time of year when the maximum number of aquatic biota have recolonised the creek channels and, therefore, can be potentially exposed to contaminants. Although pH in Magela Creek is typically about one unit higher than Ngarradj Creek, which may reduce the toxicity of Mn, the results still suggest that a closer look at Mn toxicity in Magela Creek water is warranted.

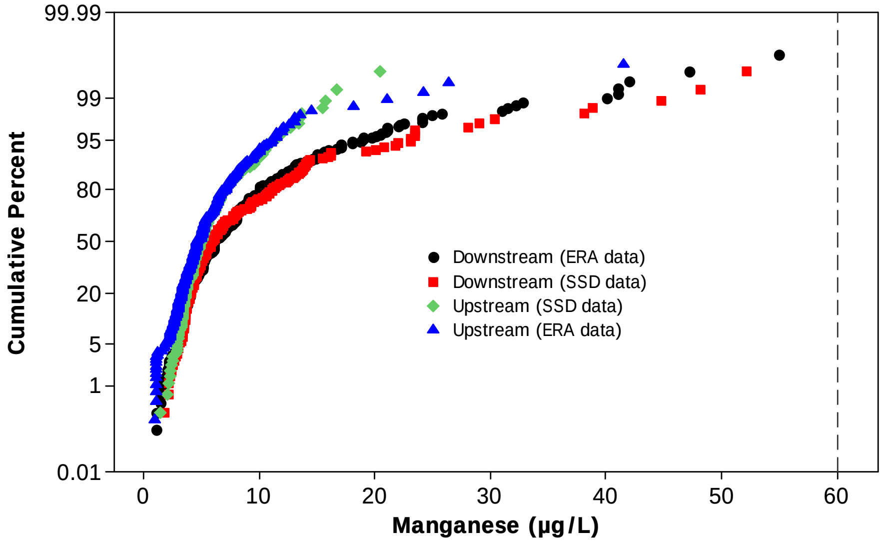

Figure 2 Cumulative frequency distributions for filtered (<0.45 µm) manganese concentrations at the Magela Creek downstream monitoring point (MG009) based on ERA data (June 1980 to June 2008) and SSD data (November 2000 to June 2008) and at the upstream monitoring point (MCUS) based on ERA data (December 1993 to June 2008) and SSD data (November 2000 to June 2008). The reference line represents the manganese IC_{10} value for *Hydra viridissima*.

Recommendations for further work

Based on the results of this study, the following additional studies are recommended:

i Assess the toxicity of Mn in Magela Creek water (pH ~6–6.5) to *H. viridissima* and *M. macleayi* to determine if it is similar to that observed in Ngarradj water;

ii Assess the toxicity of Mn in Magela Creek water to four additional species from the standard suite of test species and derive a site-specific water quality trigger value/Limit.

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Screening level ecotoxicological assessment of treated pond water from Ranger uranium mine to five local freshwater species

A Hogan, R van Dam, A Harford & C Costello

Background

Ecotoxicological testing of Ranger mine treated pond water from a trial portable treatment unit (that treated water using microfiltration and reverse osmosis alone) was requested by Energy Resources of Australia Ltd (ERA) in July 2007. This followed an ecotoxicological assessment in late 2005 on samples of treated pond water from a permanent treatment plant. In the 2005 study, treated pond water permeate was found to be non toxic to four of the five species tested. The affected species, the water flea, *Moinodaphnia macleayi*, was found to respond to the 67 and 100% concentrations of permeate, resulting in ~ 25 and 50% reductions in reproduction, respectively (van Dam et al 2007). There was some uncertainty regarding the cause of this toxicity, as uranium (U), at 4 µg L^{-1}, was the only contaminant measured above detection limits and historical data indicated that *M. macleayi* is only sensitive to concentrations above 18 µg/L U (***eriss*** unpublished data; Semaan et al 2001). As such, two hypotheses were proposed to explain the effect on *M. macleayi*:

3 Enhanced U toxicity due to a lack of dissolved organic carbon in the treated water; and

4 Nutrient/essential ion deficiency due to the minerally deficient nature of the treated water.

An experiment was undertaken during 2006-2007 to address these hypotheses, where the toxicity of U to *M. macleayi* was tested concurrently in (i) 'synthetic' Magela Creek water (SMCW), which simulates the inorganic composition of the creek water but contains no DOC; and (ii) natural Magela Creek water (van Dam et al 2008). The results indicated that not only was *M. macleayi* reproduction impaired in SMCW but that sensitivity to U was at least three fold higher than the response of the water fleas in NMCW. As such, further testing was required to further elucidate whether either of the above factors, or a combination of both (ie. enhanced sensitivity of *M. macleayi* as a result of the stress also imposed by nutrient/essential ion deficiency) was the primary contributor to the observed toxicity. As such, this research summary not only describes the screening level testing undertaken on treated pond waters from the portable treatment unit, but also presents the results of further exploratory testing to determine the cause of observed toxicity to *M. macleayi*.

Methods

A similar approach to that undertaken in late 2005 was used in this study. In late July 2007, five local freshwater species, a unicellular alga (*Chlorella* sp), macrophyte (duckweed; *Lemna aequinoctialis*), cnidarian (*Hydra viridissima*), crustacean (water flea, *Moinodaphnia macleayi*) and a fish species (*Mogurnda mogurnda*) were exposed to treated pond water permeate and a natural Magela Creek water (NMCW) control. Physical and chemical parameters of the water indicated that the expected toxicity would be very low (U = 7.7 µg L^{-1}, pH = 6.43, EC = 19 µS cm^{-1}). Therefore, it was considered unnecessary to expose the organisms to a typical series of 5–6 dilutions. Instead, a much reduced testing regime was employed where all species except *M. macleayi* were exposed only to 100% treated pond water, with *M. macleayi* exposed to 25, 50 and 100% treated pond water permeate (in addition to the NMCW control). Testing methods followed those described by Riethmuller et al (2003).

A further experiment was undertaken in order to investigate the cause of the observed toxicity to *M. macleayi*, and more specifically, to understand the role of dissolved organic carbon on the response of the water fleas in NMCW. To do this, the acute toxicity (48-h survival) of U to *M. macleayi* was assessed in three water types: (i) SMCW alone; (ii) SMCW + 3.8 mg L^{-1} DOC (as IHSS Suwannee River Fulvic Acid Standard 1); and (iii) natural Magela Creek water alone (which had a measured DOC concentration of 3.7 mg L^{-1}). Acute toxicity was assessed because the test animals are not fed during the 48-h survival test, and hence there is no additional organic component to potentially confound the results. The main aim of the experiment was to see whether the addition of DOC to SMCW would change the sensitivity of *M. macleayi* to U compared to in SMCW alone, and how this would compare to NMCW with a similar natural DOC concentration.

Results and discussion

The effects of treated pond water permeate on the five species tested are reported in Table 1. A full report on the study is provided by Harford et al (2008). The permeate had no significant effect (P>0.05) on *Chlorella* sp, *L. aequinoctialis* and *M. mogurnda*. However, exposure to 100% permeate resulted in statistically significant reductions in the growth of *H. viridissima* (12% reduction relative to control growth; P<0.05) and the reproductive success of *M. macleayi* (53% reduction relative to control reproduction; P<0.05). *M. macleayi* was unaffected by exposure to 25 or 50% permeate (P>0.05). The IC_{10} and IC_{50} (95% confidence limits; CLs) of pond water permeate to *M. macleayi* were 54 (19–63)% and 97 (CLs not calculable)%, respectively.

Table 1 Effect of pond water permeate on five local freshwater species

Test organism	Test duration & endpoint (metric)	% permeate	Mean ± SEM
Chlorella sp (unicellular alga)	72 h cell division rate (doublings/day)	0	1.41 ± 0.02
		100	1.19 ± 0.11
Lemna aequinoctialis (duckweed)	96 h plant growth (growth rate)	0	0.492 ± 0.005
		100	0.482 ± 0.013
Moinodaphnia macleayi (cladoceran)	3 brood (5-6 d) reproduction (number of neonates)	0	35.7 ± 3.1
		25	35.4 ± 1.8
		50	33.5 ± 3.0
		100	16.7 ± 2.9*
Hydra viridissima (green hydra)	96 h population growth (growth rate)	0	0.402 ± 0.005
		100	0.355 ± 0.002*
Mogurnda mogurnda (fish)	96 h survival (percentage surviving)	0	100 ± 0
		100	100 ± 0

* Significantly different from the control (P < 0.05)

The results of the current study were generally consistent with those from the 2005 study where pond water permeate exhibited no or low ‘toxicity’ to the five species assessed, as follows:

- 100% pond water permeate had no adverse effects on the green alga, *Chlorella* sp, the duckweed, *Lemna aequinoctialis*, and the purple-spotted gudgeon, *Mogurnda mogurnda*;
- A 12% reduction in growth rate of the green hydra, *Hydra viridissima*, was observed at 100% pond water permeate; and
- A 53% reduction in reproduction of the cladoceran, *Moinodaphnia macleayi*, occurred at 100% pond water permeate.

The minor response of *H. viridissima* was most most likely attributable to the nutrient/mineral deficient nature of the pond water permeate. The U concentration was at least five-fold lower than that known to exhibit low toxic effects to *H. viridissima* in organic-deficient synthetic Magela Creek water (ie >40 µg L^{-1}), which is considered a reasonable analogue of pond water permeate. Hence, U toxicity is highly unlikely to have contributed to the response of *H. viridissima*.

The larger response of *M. macleayi* also was most likely attributable to the nutrient/mineral deficient nature of the pond water permeate. This was supported by existing data that showed an approximate 50% reduction in reproduction of *M. macleayi* in synthetic Magela Creek water compared to natural Magela Creek water. Uranium is unlikely to have been a causative factor for the following reasons. Firstly, the pond water permeate U concentration was lower than that previously reported to exhibit low toxic effects to *M. macleayi* in synthetic Magela Creek water (ie ~11 µg/L, for reproductive test endpoint with feeding). Secondly, because of an unexpected and unacceptable increase (from ~6.4 to ~8.2) in the pH of the test water during the test, the the bioavailability of U would have been greatly reduced owing to changes in solution speciation.

More broadly, however, the range of concentrations reported for pond water permeate from Ranger’s existing on-site water treatment plant overlap with those known to be toxic to *M. macleayi* in SMCW. Consequently, it is important that the cause(s) of adverse effects exhibited by *M. macleayi* exposed to pond water permeate are further elucidated.

The results of the 48 h acute flea experiment are shown in Figure 1. Control survival was >90% in all three water types. The addition of 3.8 mg L^{-1} DOC to SMCW resulted in an 8–9 fold reduction in the toxicity of U to *M. macleayi* (based on EC_{50} values). Uranium was approximately 4 times less toxic in NMCW (3.7 mg L^{-1} DOC) compared to SMCW + DOC. Notwithstanding the potential influence of a nutrient/mineral deficiency, the difference in toxicity of U between NMCW and SMCW + DOC could be attributed to a difference in the DOC constitutents between the two water types. There is some concern that extraction procedures may result in chemical and structural alterations of fulvic acids which may alter these standards from being representative of humic substances in their natural state (Aiken & Malcolm 1981). Moreover, the difference in the ability of NMCW DOC to complex U may be due to it containing a mixture of humic substances (both humic and fulvic acids). Fulvic acids are known to have greater complexing ability than humic acids (Tipping 2002). In addition, natural waters also contain other constituents that may combine with each other in simultaneous and competitive reactions. The amelioration of metal toxicity by DOC is well documented for many metals (Tipping 2002), and the effect of DOC on U toxicity is under continued investigation in our laboratory (see summary above).

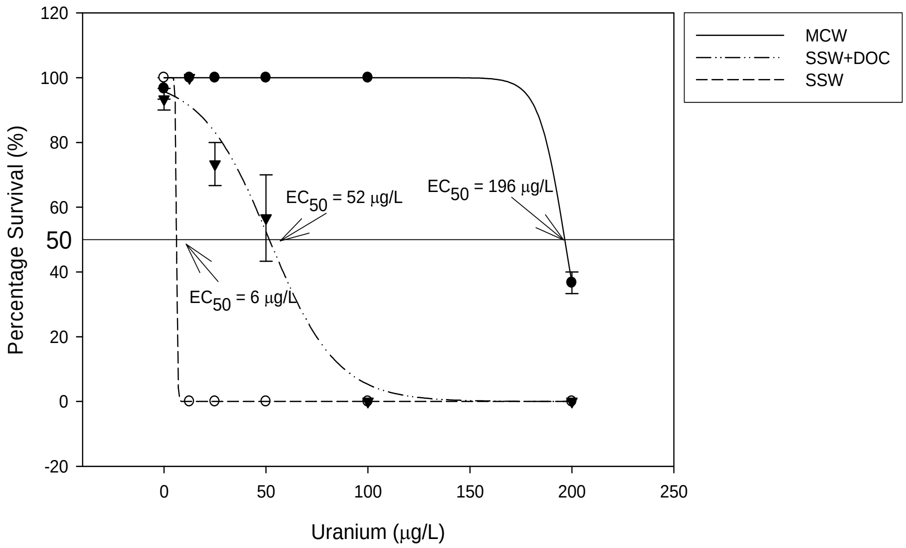

Figure 1 Acute toxicity of uranium to *Moinodaphnia macleayi* (48-h survival) in three water types: (i) synthetic Magela Creek water (SMCW) only; (ii) SMCW + 3.8 mg/L DOC (as IHSS Suwannee River Fulvic Acid Standard 1); and (iii) natural Magela Creek water (NMCW; containing 3.7 mg/L natural DOC)

Steps to completion

The issue of poor pH control in low ionic strength test solutions such as the treated pond waters needs to be addressed. Laboratory tests have shown that the food provided to the water fleas is primarily responsible for the increase in pH that occurs with the use of poorly buffered water (***eriss*** unpublished data). Laboratory staff are currently investigating the viability of reducing the food ration for this test protocol with the aim of reducing pH drift during testing.

As the potential for U to be toxic in treated pond water is not yet fully understood, additional acute and chronic tests using *M. macleayi* could be undertaken where a high quality treated pond water sample (ie one with starting U concentration < 1 µg L^{-1}) is tested after it has been spiked with additional U at various increasing concentrations. Such a study would be relevant and informative to the overall issue and associated management of treated pond waters, but is not of high priority at present.

Although the testing of treated pond water has been completed, the treatment plant is yet to be commissioned for mine process water, at which time the process water permeate containing ammonium ion will require ecotoxicological assessment. This work has been delayed due to delays in commissioning the plant, with the latest information indicating that testing is unlikely to be required until 2009.

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Development of a reference toxicity testing program for routine toxicity test species

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Background

Over the past four years, and in response to recommendations by van Dam (2004) and also by Dr Jenny Stauber at ARRTC's 14th meeting, the ***eriss*** ecotoxicology laboratory has been implementing a program of reference toxicant testing, using uranium, for its routine testing species. Since 2004–05, reference toxicant control charts have been developed for four of the five routine testing species. The aims for 2007–08 were to:

1 continue with the established reference toxicity testing programs for *Moinodaphnia macleayi*, *Chlorella* sp, *Hydra viridissima* and *Mogurnda mogurnda*; and

2 continue to investigate problems with the *Lemna aequinoctialis* reference toxicity test.

Progress

In total, 20 reference toxicants tests (*Chlorella* – 5; *Hydra* – 4; *Moinodaphnia* – 4; and *Mogurnda* – 7) were completed during 2007–08, 18 of which provided valid results. The results of these tests are summarised in Table 1, with the control charts presented in Figure 1.

Table 1 Summary of uranium reference toxicity test results for 2007–08

Species & endpoint	Test Code	IC_{50} (μg/L)	Valid test?	Comments
Chlorella sp	893G	61 (29, 77)	Yes	
(72-h cell division rate)	898G	NC[b]	No	Culture 10 days old at start of test[b]
	899G	37 (32, 42)	Yes	
	942G	34 (22, 70)	Yes	
	941G	35 (25, 45)	Yes	
Moinodaphnia macleayi	855I	50 (41, 62)	Yes	
(48-h immobilisation)	870I	14(12, 16)	Yes	
	912I	9 (83, 10)	Yes	
	929I	16 (14, 19)	Yes	
Hydra viridissima	834B	84 (73, 96)	Yes	
(96-h population growth)	879B	66 (89, 138)	Yes	
	886B	65 (56, 72)	Yes	
	942B	NC[a]	No	No effect at highest conc[b]
Mogurnda mogurnda	859E	1383 (NC)	Yes	
(96-h sac fry survival)	869E	1166 (1109, 1225)	Yes	
	872E	1777 (1609, 1962)	Yes	
	875E	1428 (1057, 1576)	Yes	
	880E	1458 (1338, 1590)	Yes	
	896E	1226 (1057, 1422)	Yes	
	928E	1262 (1131, 1366)	Yes	

[a] NC: Not calculable. See 'comments' column for reason

[b] See text for discussion

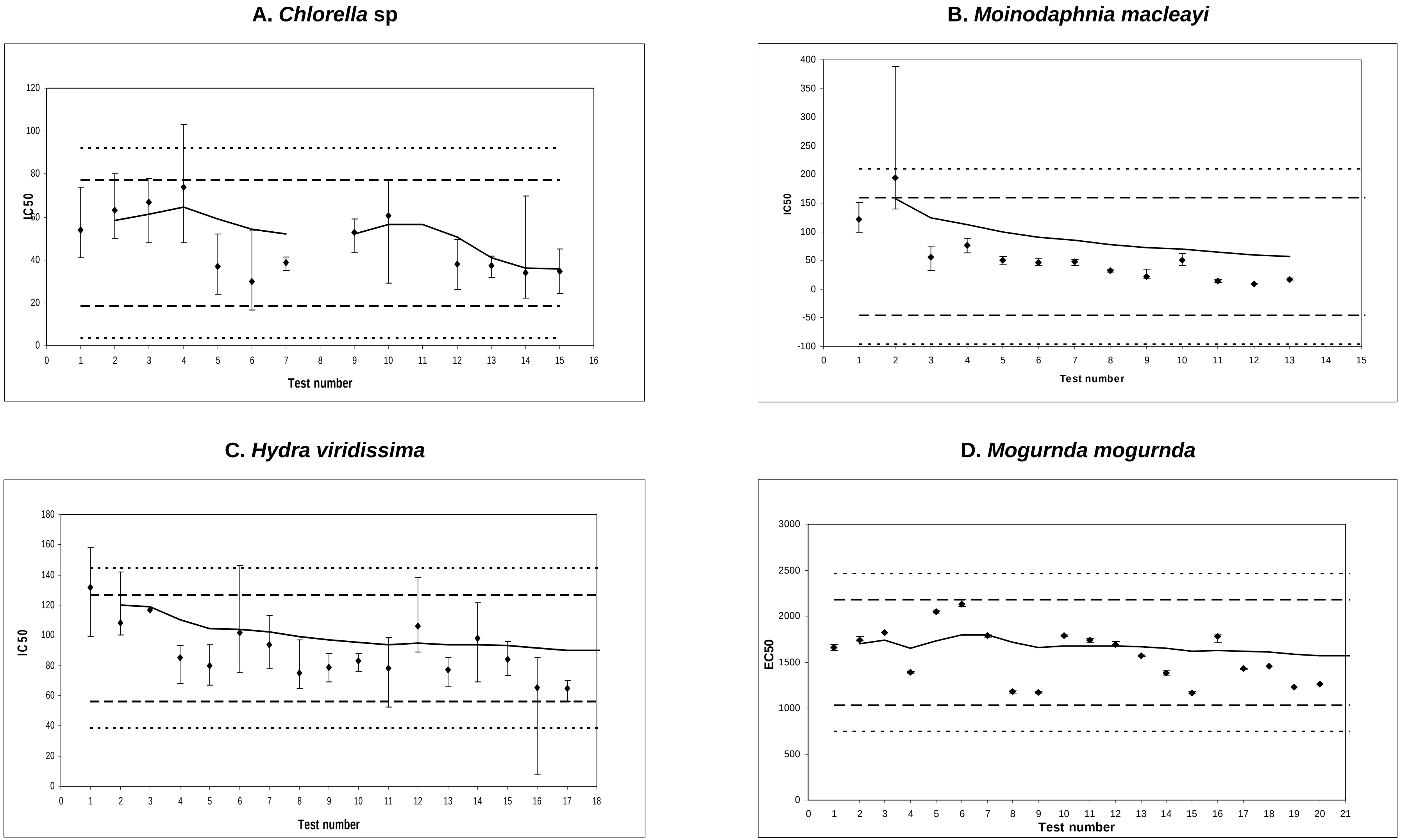

Figure 1 Reference toxicant control charts for A. *Chlorella* sp, B. *M. macleayi*, C. *H. viridissima* and D. *M. mogurnda*, as at September 2008. Data points represent IC_{50} or EC_{50} toxicity estimates. Reference lines represent the following: dotted lines – upper and lower 99% confidence limits; broken lines – upper and lower warning limits; unbroken line – running mean.

A brief summary of the major issues for this program is provided below.

Chlorella sp

Over the course of the year algal toxicity testing results were valid with the exception of one test, where a 10-d old culture was used inadvertently instead of a 4 to 5-d old culture.

H. viridissima

Three of four reference toxicity tests for *H. viridissima* were valid. Test 942B showed no effect at the highest concentration, therefore an IC_{50} could not be established. Hydra in this test appeared less sensitive than the previous three tests. Chemistry results for all tests were within acceptable ranges.

M. macleayi

M. macleayi reference toxicity testing was initiated for the first time in 2006–07. Few problems were observed with this test, with all tests being valid. The three tests conducted in 2007-08 were also valid. However, there has been a trend where the IC_{50} appears to be decreasing over time (See Figure 1B), indicating that the *M. macleayi* stock may be becoming more sensitive. This will be closely monitored during 2008-09 to determine if this is the case. If necessary, an investigation will be initiated to assess whether the current laboratory stock of *M. macleayi* differs in its sensitivity to U from 'wild' *M. macleayi*. If so, a new stock of *M. macleayi* will most likely be sourced.

M. mogurnda

All seven reference toxicity tests for *M. mogurnda* were valid. There are no problems associated with this protocol.

Reference toxicity test development for L. aequinoctialis

The development of the *L. aequinoctialis* reference toxicity test continues to be problematic. The challenge has been optimising the test medium so as to enable adequate control growth and also an effect to be observed at uranium concentrations that are not excessively high. Unfortunately, nutrients (eg PO_4) and trace elements added to the test medium to facilitate plant growth also interact with uranium, greatly reducing its bioavailability and toxicity. In 2006–07, numerous experiments were done, assessing different diluent water types and nutrient additions (eg SSW + NO_3 and PO_4; MilliQ water + various concentrations of CAAC medium). Despite some promising indications, consistent results could not be obtained.

A potential solution to this problem, which needs further discussion, is to lower the minimum acceptability criterion for control plant growth (eg. from a four-fold increase in frond numbers to a three-fold increase in frond numbers). A trial reference toxicity test (831 L) was conducted in 2007–08, using 0.5% CAAC medium as the diluent/control water. Due to insufficient control growth (ie increase in frond number of 41, compared to the minimum acceptability criterion of 48) the test was deemed invalid. However, if the minimum acceptability criterion for control plant growth was lowered as suggested above, the test would have been valid. The resultant IC_{50} (95% CLs) from the test (based on nominal U concentrations) was 1591 (894–2812) µg/L. However, reflecting the previously observed inconsistency in plant growth, a growth trial using 0.5% CAAC medium conducted in

September 2008 resulted in an increase in frond number of 33, which does not meet either the existing or proposed lower minimum acceptability criterion for growth.

Planned testing in 2008–09

The reference toxicity testing programs for *Chlorella* sp, *M. macleayi*, *H. viridissima* and *M. mogurnda* will continue in 2008–09, with the aim of completing at least four tests per species. Further testing will take place to try to resolve the issues of the *L. aequinoctialis* reference toxicity test protocol. This will involve optimisation of the test medium so as to enable adequate control growth and also a concentration-response relationship to be observed at uranium concentrations that are not exececssively high. In addition to the reference toxicity test protocol, there is a need to develop an additional endpoint, based on plant surface area or dry weight. The development of this endpoint will be done in conjunction with the reference toxicity test development work.

References

van Dam R 2004. *A review of the* ***eriss*** *Ecotoxicology Program*. Supervising Scientist Report 182, Supervising Scientist, Darwin NT.