Australian Government
Department of the Environment and Heritage
Supervising Scientist

internal report 412

Chronic toxicity of uranium to the tropical green alga *Chlorella* sp. for the derivation of a site specific Trigger Value for Magela Creek

AC Hogan, RA van Dam, SJ Markich, C McCullough & C Camilleri

December 2003

Chronic toxicity of uranium to the tropical green alga *Chlorella* sp. for the derivation of a site specific Trigger Value for Magela Creek

AC Hogan, RA van Dam, SJ Markich[1], C McCullough[2] & C Camilleri

Ecological Risk Assessment, Environmental Research Institute of the Supervising Scientist
GPO Box 461, Darwin NT 0801

1 Environment Division, Australian Nuclear Science and Technology Organisation, Private Mail Bag 1, Menai, NSW

2 Ecosystem Protection, Environmental Research Institute of the Supervising Scientist. Current address: Post-Doctoral Research Fellow, School of Natural Sciences, Edith Cowan University, 100 Joondalup Drive, Joondalup WA 6027

December 2003

Registry File SG2000/0093

Australian Government
Department of the Environment and Heritage
Supervising Scientist

Contents

Chronic toxicity of uranium to the tropical green alga *Chlorella* sp. for the derivation of a site specific Trigger Value for Magela Creek

AC Hogan, RA van Dam, SJ Markich, C McCullough & C Camilleri

Introduction

Uranium mining in the Magela Creek catchment of Kakadu National Park has occurred for over twenty years (Johnston & Needham 1999). Due to a very high wet season rainfall in the region, controlled releases of water are an essential part of the water management program of the ERA Ranger Mine. The revised Australian and New Zealand Guidelines for Fresh and Marine Water Quality recommend a receiving water Trigger Value (TV) for uranium (U) of 0.5 µg/L (ANZECC & ARMCANZ 2000a) for the protection of downstream aquatic life. The TV was considered to be of low reliability, due to an inadequate toxicity database and the subsequent derivation of the recommended value using the less preferred 'safety factor' approach. Given that the Magela Creek catchment is considered to be of high conservational and ecological value (Gardner et al 2002), a low reliability TV was considered inadequate and a site-specific assessment was considered essential. In order to derive a high reliability, site specific TV for U, chronic toxicity data for at least five local species from at least four taxonomic groups was required. However, appropriate data were limited to four local species from three taxonomic groups, namely the cladoceran, *Moinodaphnia macleayi;* the green hydra, *Hydra viridissima*; the purple spotted gudgeon, *Mogurnda mogurnda* and the chequered rainbowfish, *Melanotaenia splendida inornata*. The recent development of a unicellular algal toxicity test using the locally isolated *Chlorella* sp., enabled the inclusion of toxicity data for a primary producer, thus fulfilling the requirements for a high reliability site-specific TV. This report describes a series of experiments undertaken to refine the test protocol, determine the toxicity of U to this species of alga and subsequently derive a new TV for U in Magela Creek.

Materials and methods

Test organism

The green unicellular freshwater algae, *Chlorella* sp., was isolated from Georgetown Billabong within the Magela Creek Catchment of the Alligator Rivers Region in the Northern Territory of Australia (Padovan 1992). This isolate could not be identified and is possibly a new species of *Chlorella* (Franklin *et. al.* 2000). An axenic culture of the isolate was maintained at the ***eriss*** ecotoxicology laboratory in MBL medium (Stein 1973; Appendix 1) at 29 ±1°C on a 12:12 h day/night cycle (Philips TL 40 W cool white fluorescent lighting; 100-150 µmol m^{-2} s^{-1}). Tests were conducted using exponentially growing cells from a four to five day old culture.

General laboratory procedures

All equipment used that came in contact with test organisms, media, control water or test solutions was made of chemically inert materials (eg teflon, glass or polyethylene). All plastic and glassware was washed by soaking in 5% concentrated nitric acid for 24 h before undergoing a detergent wash (Gallay Clean A powder, Gallay Scientific, Burwood, Australia) and two reverse osmosis (RO) water rinses in a laboratory dishwasher, followed by a hand rinse in Milli-Q reagent grade water (Millipore, North Ryde, Australia; 18 MΩ/cm). Glassware used in the toxicity tests was silanised with 2% dimethyldichlorosilane in 1,1,1-trichloroethane (Coatasil, AJAX, Seven Hills, Australia,) to reduce U adsorption to the glass. All reagents used were analytical grade and stock solutions were made up in Milli-Q water.

Instrument optimisation: Coulter calibration

An automatic particle counter, Coulter Multisizer II, was used for counting algal cells. This method is faster and more precise than manual enumeration using a microscope and haemocytometer (Stauber et al 1994). However, a problem associated with electronic particle enumeration, is that at high cell densities, counts may be underestimated due to two or more cells being counted as one. Thus, a coulter calibration experiment was undertaken to determine the range of cell densities over which accurate cell counts are obtained.

A series of cell suspensions containing 10, 25, 50, 75, 100 and 200 x 10^4 cells/mL of *Chlorella* sp. was prepared by diluting a concentrated suspension of known cell density (manually determined) with filtered Magela creek water and 4% formaldehyde to arrest cell growth. A property of the coulter counter is that the cells must be suspended in an electrolyte solution. All cell suspensions were therefore diluted in Isoton (Coulter Electronics Pty Ltd) at a ratio of 1:4 before counting. The cell density of each suspension was counted both manually and with the coulter counter, and a linear regression was fitted to evaluate the coefficient of determination (r^2) between the two methods. Microscope counts were chosen as the standard variable in the regression as it is generally accepted that algal cells can be identified against other similar sized particles and that this method is highly accurate. Counts were also compared to theoretical counts (calculated by dividing the initial manually determined cell density by the dilution factor) to confirm the accuracy of both methods. In this case, theoretical counts (not prone to instrumental error) were used as the standard variable and therefore used as a basis for determining the accuracy of the other two counting methods. For each linear regression a t-test was performed to determine if the slope of the line was significantly different ($p \leq 0.05$) from unity and the y-intercept from zero ($p \leq 0.05$).

Toxicity test method

A detailed description of the methods for toxicity testing with *Chlorella* sp. is given by Riethmuller et al (2003). In brief, the chronic growth test involved the exposure of a standard number of algal cells (2-4 x 10^4 cells/mL) to several concentrations of U over a three day (72 h) period. Algal growth was measured by counting the cells daily and calculating the cell division rate (growth rate). The growth rates of algae exposed to U were compared to that of a control (background U ~ 0.1 µg/L). A sample was considered toxic when a significantly different ($p \leq 0.05$) concentration-dependent inhibition of algal growth was observed.

All previous U toxicity studies using *Chlorella* sp., e.g. Franklin et al (1998, 2000), have been conducted in a synthetic water with an inorganic chemical composition similar to that of sandy braided streams in tropical Northern Australia during the wet season (Appendix 2; Markich et al 2000). This enabled the assessment of a maximum risk scenario with respect to

the toxicity of metals as the water lacked the organic chelating agents present in natural water that can reduce the toxicity of metals to freshwater biota (Franklin et al 1998). However, to determine the site specific toxicity of U to *Chlorella* sp., natural Magela Creek water was used as a diluent, unless stated otherwise.

Statistical analysis

Toxicity test data were tested for normality (Shapiro-Wilk's Test) and homogeneity of variance (Bartlett's Test). All data for this study met these assumptions. Therefore the data did not require transformation prior to the calculation of a no-observed-effect concentration (NOEC) and a lowest-observed-effect concentration (LOEC) using a one-tailed Dunnett's test (Dunnett, 1955, 1964). Linear interpolation was used to calculate the concentrations at which there was a 50% reduction in algal growth compared to the controls (ie IC50) and associated 95% confidence intervals, for each experiment. Additional IC points (IC05, 15, 20, 25, 30 and 40) are presented in Appendix 3 in case of future application of these data.

Reference toxicity test in synthetic water

Since a *Chlorella* sp. toxicity test had not been conducted at ***eriss*** for approximately three years prior to the commencement of this study, a reference test using U in synthetic water was undertaken to assess if any change in the sensitivity of the algae had occurred. Test concentrations of 5, 10, 20, 40, 80, 120 and 320 µg/L U were chosen to encompass the range tested by Franklin et al (1998), thus allowing a direct comparison of sensitivity. In order to enable this comparison, test data from these historical tests that were originally analysed using the Trimmed Spearman-Karber method (Hamilton et al. 1977) were re-analysed to calculate IC50 and IC25 values. Significant differences between the two tests were determined using a standard error of the difference technique (Sprague, 1990) that compares IC50 values and their confidence limits.

Growth of *Chlorella* sp. in natural creek water compared to synthetic water

In order to provide site-specific information on the toxicity of U to *Chlorella* sp., all tests were conducted using natural Magela Creek water as the test diluent. To ensure that algal growth in creek water would be similar, if not better than in synthetic water, a test using three control replicates of each diluent (creek water and synthetic water) was set up and algal growth rates compared.

Effect of HEPES buffer on algal growth and toxicity of U

Test diluent, whether natural creek or synthetic water, requires the addition of nutrients and HEPES buffer to enable sufficient algal growth and pH stability, respectively, over the three day test period (Franklin et al 1998). The use of buffers in a test solution, although necessary, is not ideal, as no buffer is truly inert and unexpected side reactions may cause effects that are unrelated to buffering (Ferguson et al 1980). To investigate whether the HEPES buffer used in this test affected the toxicity of U to *Chlorella* sp. the algae were exposed to U (0, 50, 100, 200, 400, 600 and 800 µg/L U) with 2 mM HEPES and without HEPES. Each treatment consisted of three replicates. Test solution pH was measured daily to observe any changes over the three day period.

In addition, an investigation was carried out to find the lowest concentration of HEPES buffer that would maintain test solution pH (< ± 0.5 units) without reducing U toxicity to *Chlorella*

sp. Algae were exposed to either a control (< 0.1 μg/L U) or 200 μg/L U in a diluent containing 0, 0.5, 1 or 2 mM HEPES. There were three replicates per treatment. Algal growth rates in the 200 μg/L U treatment were expressed as a percentage of the control for each HEPES concentration. Test solution pH was also measured daily to assess the effectiveness of each buffer concentration at maintaining pH over three days.

A further experiment was then undertaken to determine if the observed differences in the toxicity of U were due to the addition of HEPES buffer or a direct effect of pH, which increases throughout the test in the unbuffered solution. To uncouple these potential effects, *Chlorella* sp. were exposed to 0.1, 5, 10, 25, 50, 80, 125 and 300 μg/L U in synthetic water for 48 h. The pH of the test waters was maintained at pH 6.5 ± 0.2 using either 1 mM HEPES buffer or manual adjustment with 0.05 M H_2SO_4 or NaOH. Two replicates were used per treatment.

First rangefinder test in natural creek water

Due to the greater complexing capacity of natural creek water compared to synthetic water, it was expected that the toxicity of U to *Chlorella* sp. would be significantly lower in this diluent. A concentration range of 0, 12.5, 25, 50, 100, 250, and 500 μg/L U was therefore chosen for the first rangefinder test based on the results of the reference toxicant test. There were three replicates for each treatment concentration.

Definitive U toxicity tests

Once the test protocol was refined, four definitive tests were undertaken in natural creek water with 1 mM HEPES buffer (see the results section for the justification of a reduction of buffer concentration), using the following U concentrations.

1st definitive - 0.1, 50, 100, 150, 175, 200, 250 and 300 μg/L U.
2nd definitive - 0.1, 100, 130, 170, 220, 290, 370 and 480 μg/L U.
3rd definitive - 0.1, 100, 120, 145, 170, 210, 250 and 300 μg/L U.
4th definitive - 0.1, 75, 125, 175, 225, 275 and 325 μg/L U.

There were three replicates for each concentration. Final (72 h) IC25, IC50, NOEC and LOEC values based on cell division (growth) rate were calculated for each test.

A comprehensive suite of physico-chemical analyses was undertaken on waters from each test to calculate the speciation of U in the test solutions using the speciation modelling program, HARPHRQ (Brown et al 1991). This enabled an estimation of the proportion of U available to the algae as the free uranyl ion UO_2^{2+} which is the U species considered to be most responsible for eliciting a toxic response to aquatic organisms (Markich et al 2000). These results were used to explain differences in algal sensitivity observed between tests conducted with natural creek water (with varying organic matter levels) and synthetic water (no organic matter).

Derivation of a site specific trigger value for U

A statistical extrapolation technique recommended by ANZECC and ARMACANZ (2000a) was used to calculate a high reliability, site specific U trigger value for Magela Creek. Chronic NOEC data from at least five local species from four taxonomic groups are required to derive a trigger value. In cases where multiple NOEC values based on the same endpoint for a single species exist, the geometric mean of the NOEC values is accepted as the NOEC value to be used for the trigger value derivation (ANZECC & ARMCANZ 2000a). The NOEC data obtained for *Chlorella* sp. in this study were added to the already existing data for

four other local species. These data were analysed using the BurrliOZ software (Campbell et al 2000) which uses a maximum likelihood method to determine which of the family of Burr Type III statistical distributions best fit the data (Shao 2000). The best fitting distribution is then used to extrapolate a trigger value that will protect a specified percentage of species with either 50 or 95% confidence. Considering the ecological and conservational importance of Kakadu National Park, the trigger value was calculated using the 1st percentile (protection of 99% of species) following the recommendations of Warne (2001).

Although the guidelines specify that a high reliability trigger value can be calculated with a minimum of five species, the maximum likelihood method used in BurrliOZ to determine the best fitting distribution can be unreliable when a dataset with less than 8 data values is used (ANZECC & ARMCANZ 2000b). In order to check the reliability of the method, Minitab statistical software (Version 13.1; Minitab Inc, State College, PA, USA) was used to compare the fit of the data to four common statistical distributions (Weibull, log-normal base 10, log-logistic and logistic) using an adjusted Anderson-Darling goodness-of-fit statistic (Stephens 1974). A smaller Anderson-Darling statistic indicates the better fit of the data.

To support this, predicted NOECs were calculated for each distribution using the cumulative frequency (cf) for each actual NOEC. The formulas used to calculate cf in the BurrliOz and Minitab programs are expressed below (ANZECC & ARMCANZ 2000b).

BurrliOz: $$cf = \frac{rank - 0.5}{n}$$

Minitab: $$cf = \frac{rank - 0.375}{n + 0.25}$$

Predicted NOECs were compared to the actual NOECs for each of the five different distributions using correlation analysis and the one showing the greater agreement was used to derive the TV.

Results and discussion

Instrument optimisation: Coulter calibration

Actual counts of algal cells using the Coulter counter showed excellent agreement with the theoretical counts with an r^2 value of 0.993 and a slope of 1.054 (not significantly different from unity; Figure 1a). In addition, there was no significant difference between the y-intercept and zero. This analysis indicated that the Coulter counter was accurate for counting algal cells, as predicted using theoretical counts.

Microscope counts also showed good agreement with theoretical counts ($r^2 = 0.996$, slope = 0.914, Figure 1b). However, while the regression line was shown to pass through the origin (y-intercept was not significantly different from zero) the slope of the line was significantly ($p \leq 0.05$) different from unity. This indicated the incorporation of a small amount of error, either with the microscope counting method or the dilution of the cell suspension used in the calibration. More opportunities for error arise during microscope counting than the Coulter counting method because of a greater number of steps.

As expected from the above results, a positive linear relationship ($r^2 = 0.998$, slope = 1.154) was found between the microscope accounts and Coulter counts (Figure 1c). However, a t-test indicated that the slope was significantly ($p \leq 0.05$) different from unity and the y-intercept was significantly ($p \leq 0.05$) different from zero. Accordingly, direct comparisons should not

be made between counts conducted using these two methods unless corrected using the regression equation. In terms of this study, where the intention was to replace the older and more labour intensive microscope count method with coulter counting, this was not considered important. Overall, this trial indicated that the Coulter counting method would be accurate for counting algal cells over the density range tested.

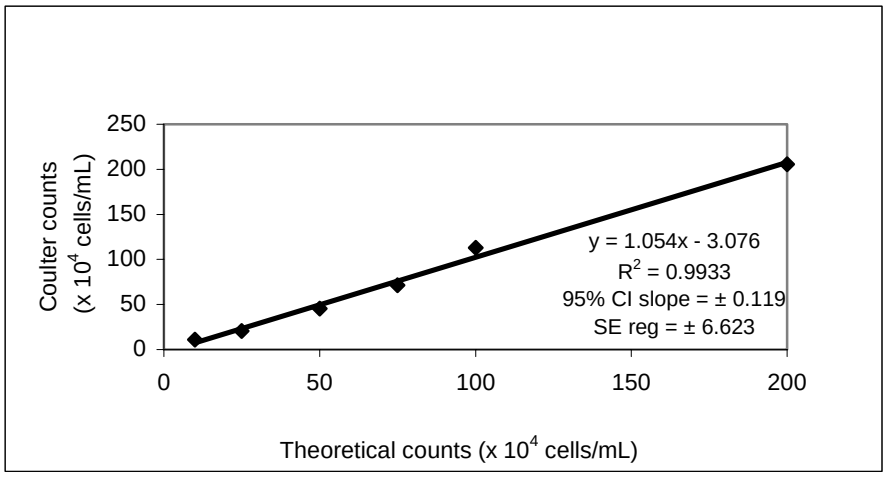

Figure 1a Coulter counts vs Theoretical counts of *Chlorella* sp. cell density

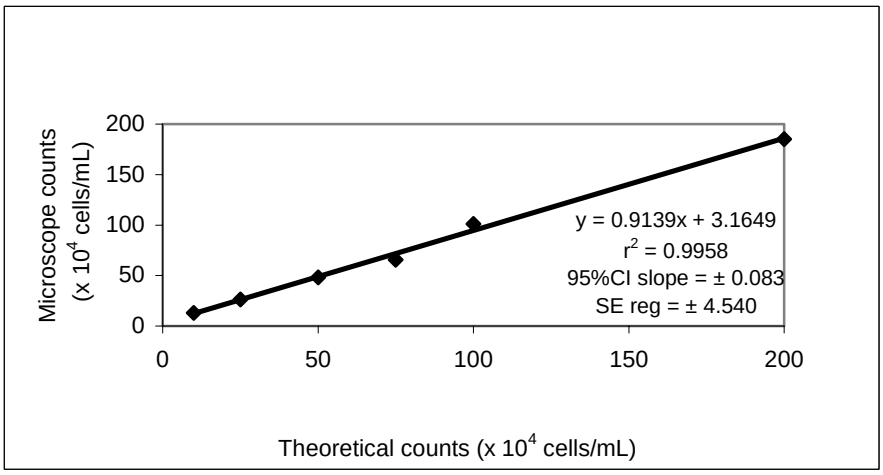

Figure 1b Microscope counts vs. theoretical counts of *Chlorella* sp. cell density

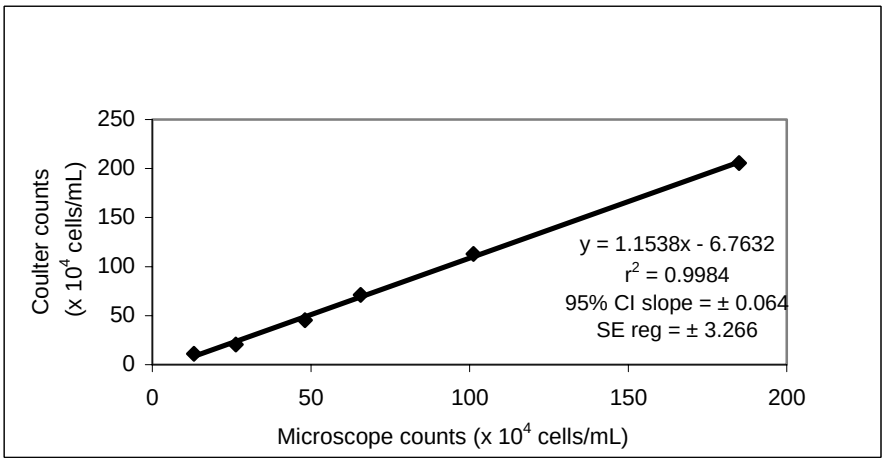

Figure 1c Coulter counts vs. microscope counts of *Chlorella* sp. cell density

Reference toxicity test in synthetic water

The results of the reference U toxicity test is summarised in table 1. Good algal growth and reproducibility were observed in the controls (1.50 doublings/day, percent coefficient of variation (CV) =10.2%) and the test solution pH remained stable over 72 h (range 6.4-6.6) indicating that the test was acceptable. The sensitivity of *Chlorella* sp. to U in the reference test was not significantly different to that reported by Franklin et al (1998) according to Sprague's (1990) standard error of the difference technique (table 1). Higher NOEC and LOEC values were observed in the reference tests (table 1), giving concentration response relationships with a steeper slope than observed by Franklin et al (1998).

Table 1 Toxicity of U to *Chlorella* sp. in synthetic water over 72 h

Test	Mean control cell division rate (doublings/day)	U Toxicity (µg/L)		
		72 h IC50[1] (95% CL)	NOEC[2]	LOEC[3]
Test 1 (this study)	1.50	74 (48–103)	38	70
Test 1 (Franklin et al 1998)	1.62	54 (41–74)	<18	18
Test 2 (Franklin et al 1998)	1.56	63 (50–80)	9	18
Test 3 (Franklin et al 1998)	1.62	67 (48–78)	8	17

[1] IC50, concentration where there is a 50% inhibition of cell division (growth).

[2] NOEC, no observed effect concentration.

[3] LOEC, lowest observed effect concentration.

Growth of *Chlorella* sp. in natural creek water compared to synthetic water

Chlorella sp. exhibited better growth in natural creek water (growth = 1.33 doublings/day) than in synthetic water (growth = 0.91 doublings/day). Growth in both water types was lower than observed in other tests completed for this study (range = 1.50-1.82). These data are useful as they represent the only direct comparison undertaken for the two water types.

Nutrient levels in Magela Creek water have historically been very low with NO_3 concentrations measured over a 19 year period being at, or below, analytical detection levels (0.05-0.1 mg/L NO_3) (Klessa 2000). Considering that relatively high concentrations of nitrate (14.5 mg NO_3/L) and phosphate (0.14 mg PO_4/L) are added to both the synthetic and natural creek water for a test, the amount contributed by the creek water would have a negligible effect on algal growth rates. Instead, it may be that natural organic matter, such as tannins and humic substances found in the natural creek water act as growth stimulants and promote algal growth. The synthetic water is based only on the inorganic components of Magela Creek water, and therefore lacks the organic matter found in the natural creek water. These results support the use of natural creek water for site-specific studies such as this, with synthetic water being of more value for mechanistic studies of organism response to varying organic levels (eg as in a well-defined chemical medium that can be interpreted through speciation calculations).

Comparison of U toxicity to *Chlorella* sp. in creek water with and without HEPES buffer

The control treatments containing 2 mM HEPES fulfilled all the test acceptability criteria with good growth (1.63 doublings/day), reproducibility (CV = 0.7%) and pH stability (6.3–6.5). Furthermore, good growth and reproducibility (1.82 doublings/day, CV = 0.32%) were also

observed in the unbuffered control treatments, however the test solution pH was very unstable, drifting from 6.1 to 7.5 after 72 h. This instability emphasises the necessity of having a buffered test solution for freshwater algal testing as even small changes in pH can significantly influence the toxicity of U to aquatic biota (Franklin et al 2000, Markich et al 2000).

An IC50 (and 95% confidence limit, CL) of 167 (94-208) μg/L U was obtained when the algae was tested in unbuffered creek water compared to 196 (168-209) μg/L U when 2 mM HEPES was added. Based on the standard error of the difference method described by Sprague (1990), to test for significant differences between IC50 values, these results are not significantly different ($p > 0.05$). However, by comparing the concentration response curves of the two tests (Figures 2a & 2b), it can be seen that algal growth (doublings/day) at 140 μg/L U was significantly lower ($p \leq 0.05$) in the unbuffered test resulting in different NOEC and LOEC values (buffered test NOEC = 136 μg/L U, LOEC = 272 μg/L U; unbuffered test NOEC = 55 μg/L U, LOEC = 147 μg/L U). This finding is important for applications such as trigger value derivation where NOEC and LOEC data are used in order to protect aquatic biota against toxicants.

It is not possible from these data to determine from these two tests the extent to which this difference in toxicity is due to an interaction between the U and HEPES buffer or whether or not it is a direct pH effect on the speciation of U. However, if the former is true it is possible that a lower concentration of HEPES buffer would maintain a stable pH and not affect U toxicity. To test for this, three concentrations of HEPES buffer were chosen and the effectiveness of each at maintaining test solution pH was assessed by taking daily pH readings of each treatment. The 2 mM concentration recommended in the current test protocol gave the best control, with pH drifting only 0.14 units in three days (Figure 3). The pH drift in the 1 mM treatment was also small at 0.28 units, however further reduction of the buffer concentration to 0.5 mM increased pH drift to 0.80 units. As expected, pH control in the unbuffered solution was poor, with a drift of 1.5 observed over 72 h.

A significant ($p \leq 0.05$) inhibition of algal growth was observed in the 2 mM HEPES treatment, while the 1 and 0.5 mM concentrations had no significant effect (Figure 4a). This indicated that the buffer was exerting a slight toxic effect on the algae at the concentration used previously for testing, although algal growth in the HEPES buffer was still within the control acceptability range of 1.4 ± 0.3 doublings/day. Considering that pH control was good in 1 mM HEPES, it was decided that the concentration of HEPES be reduced to 1 mM for future testing.

A comparison of the sensitivity of *Chlorella* sp. to U in an unbuffered test solution and three different buffer concentrations (0.5, 1 and 2 mM HEPES) is given in Figure 4b. *Chlorella* sp. growth in 200 μg/L U was expressed as a percentage of the control (ie 0.1 μg/L U and either 0, 0.5, 1 or 2 mM HEPES) to adjust for differences in control growth according to buffer concentration, and thus, enable a direct comparison of sensitivity. Algal growth at 200 μg/L U was significantly ($p \leq 0.05$) higher in all treatments containing HEPES compared to the treatment without HEPES, suggesting that even low HEPES concentrations (ie down to 0.5 mM HEPES) interfered with U bioavailability to *Chlorella* sp. This posed a problem, as there was no option to further reduce buffer concentration without seriously sacrificing pH control in the test.

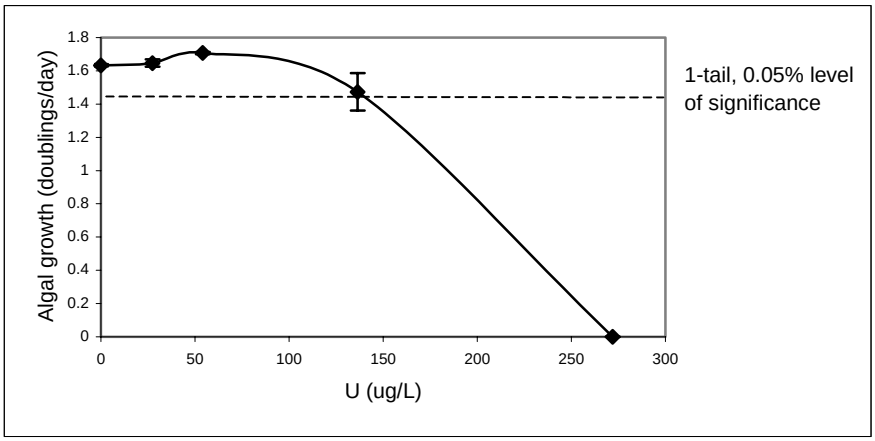

Figure 2a Response of *Chlorella* sp. to U in the presence of 2 mM HEPES buffer. Verticle bars represent the standard error of the mean.

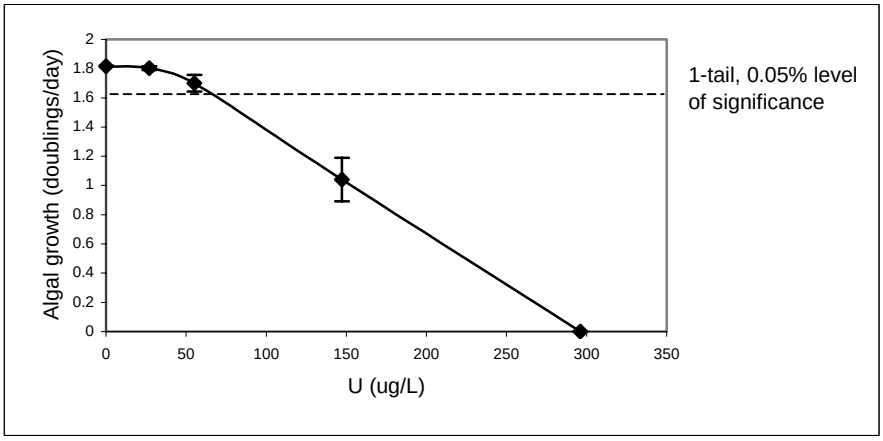

Figure 2b Response of *Chlorella* sp. to U without HEPES buffer. Verticle bars represent the standard error of the mean.

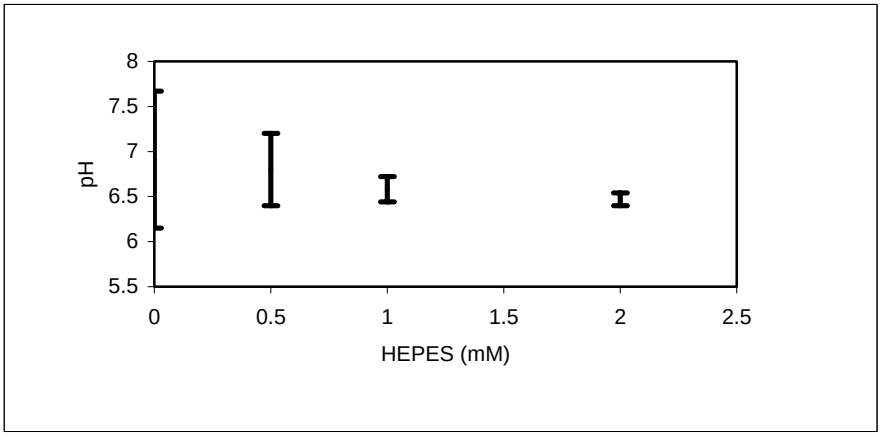

Figure 3 pH range in the test solutions as a function of HEPES buffer concentration

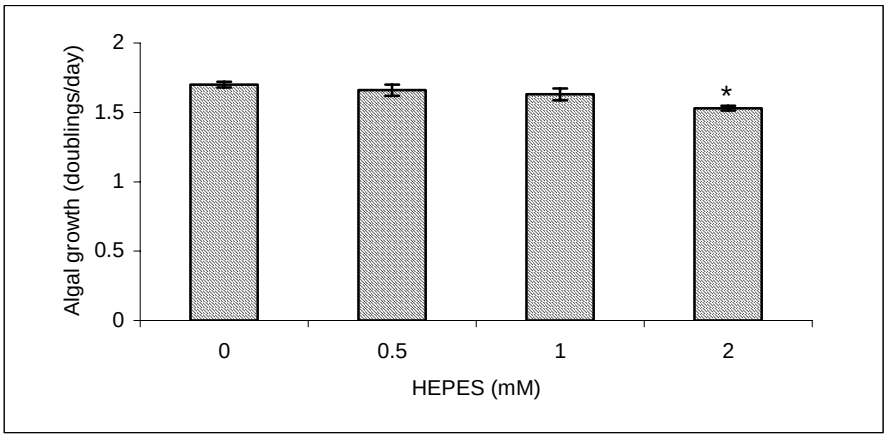

Figure 4a Mean growth of *Chlorella* sp. in four different HEPES buffer concentrations. *indicates a significant ($p \leq 0.05$) difference from 0 mM HEPES. Verticle bars indicate standard error of the mean.

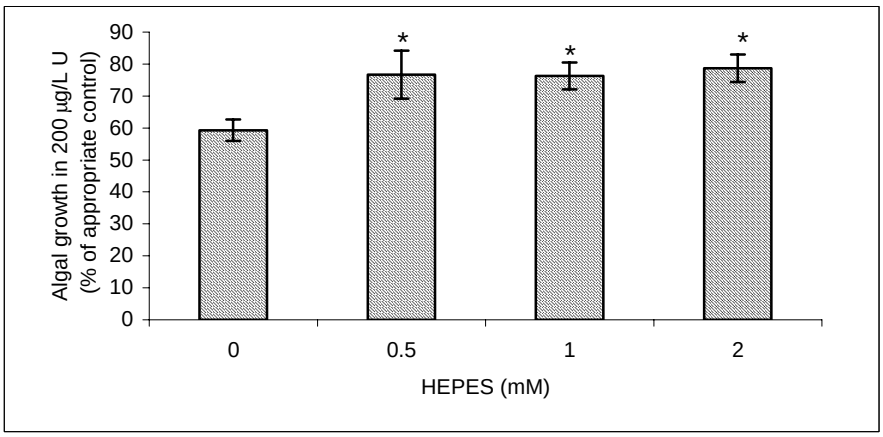

Figure 4b *Chlorella* sp. growth rates in 200 µg/L U as a percentage of the control at four different HEPES concentrations. * indicates a significant ($p \leq 0.05$) difference from 0 mM HEPES. Verticle bars indicate standard error of the mean.

A further experiment where pH was adjusted manually in the absence of HEPES buffer was therefore undertaken to determine if the difference in toxicity observed was due to an interaction with HEPES or a direct pH effect. Results of these tests showed that there was no significant ($p > 0.05$) difference in the growth rates (doublings/day) of *Chlorella* sp. or the toxicity (48 h EC50) of U in synthetic water at pH 6.5, with and without the addition of 1 mM HEPES buffer. The EC50 value (and 95% confidence interval) for U was 50 (45-55) µg/L without HEPES buffer and 53 (47-59) µg/L with 1 mM HEPES[1]. In addition, the concentration-response curves for the two tests were analogous (Figure 5). These results indicate that 1 mM HEPES is suitable for maintaining pH within the desired limits of the test, whilst not affecting the toxicity of U to *Chlorella* sp. This was important, as small pH changes are known to influence the toxicity of U to this species (Franklin et al 2000). This work aimed to minimise the variability of the response of *Chlorella* sp. to U over successive tests, by maintaining pH, water hardness and alkalinity, relatively constant.

[1] Note that IC50s, NOECs and LOECs were not calculated for these tests that were conducted externally.

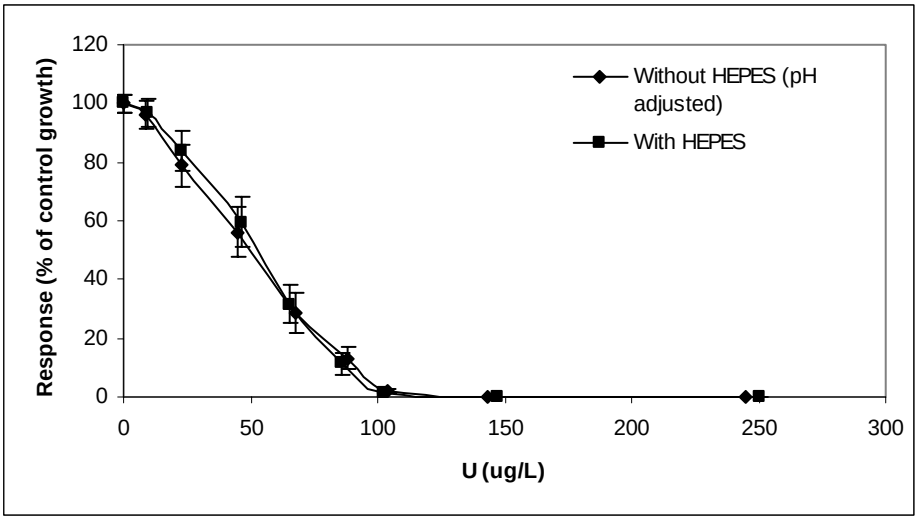

Figure 5 Comparative response of *Chlorella* sp. to U over 48 h in test diluent containing 1 mM HEPES and without HEPES.

First rangefinder test in creek water

Good growth and reproducibility were observed in the controls (1.43 doublings/day, CV = 3.3%) and test solution pH remained stable over 72 h (range 6.47–6.82) indicating acceptability of the test. The toxicity of U to *Chlorella* sp. was substantially lower in natural creek water (IC50 of 144 (133-161) μg/L) compared to synthetic water (IC50 of 74 (48-103) μg/L). The NOEC and LOEC concentrations for the rangefinder test were 84 and 194 μg/L U, respectively. This information provided a good basis for the definitive tests.

Definitive tests

Chlorella sp. was found to be highly sensitive to U with IC50 values for the four tests ranging from 137 to 238 μg/L U, NOECs from 72 to 157 μg/L U and LOECs from 120 to 187 μg/L U (table 2).

It was, however, observed that it was difficult to obtain the level of reproducibility across tests in natural creek water that was obtained for this species in synthetic water (table 1). Figures 6a–d illustrate the different responses of *Chlorella* sp. in the four tests using natural creek water collected at different times. These variable results could be attributed to the levels of dissolved organic matter (DOM; measured as dissolved organic carbon – DOC) in Magela Creek water, as higher DOC was associated with lower U toxicity (see table 2). Supporting this, geochemical speciation modelling (HARPHRQ) indicated that the variability in U toxicity in Magela Creek water was largely explained by differences in the percentage of uranium bound to DOC (ie there was a strong negative relationship between % U-DOC and uranium toxicity; r = 0.988, n = 4, P = 0.012; figure 6). Not surprisingly, the increase in U-DOC was associated with a decrease in the percentage of the free uranyl ion (figure 7; UO_2^{2+}; r = 0.977, n = 4, P = 0.023). Thus, as DOC concentration increases, less U is present as UO_2^{2+}, and toxicity decreases.

Table 2 Toxicity of U to *Chlorella* sp. in Magela Creek water with 1 mM HEPES buffer over 72 h and corresponding dissolved organic carbon concentrations

Test	Mean control cell division rate (doublings/day)	U Toxicity (µg/L)			Dissolved organic carbon (mg/L)
		IC50[1] (95% CL)	NOEC[2]	LOEC[3]	
1st definitive	1.60	177 (148-210)	150	179	4.1
2nd definitive	1.48	166 (157-173)	109	136	3.4
3rd definitive	1.48	238 (233-241)	157	187	8.1
4th definitive	1.55	137 (122-150)	72	120	2.6

[1] IC50, concentration where there is a 50% inhibition of cell division (growth).

[2] NOEC, no observed effect concentration.

[3] LOEC, lowest observed effect concentration

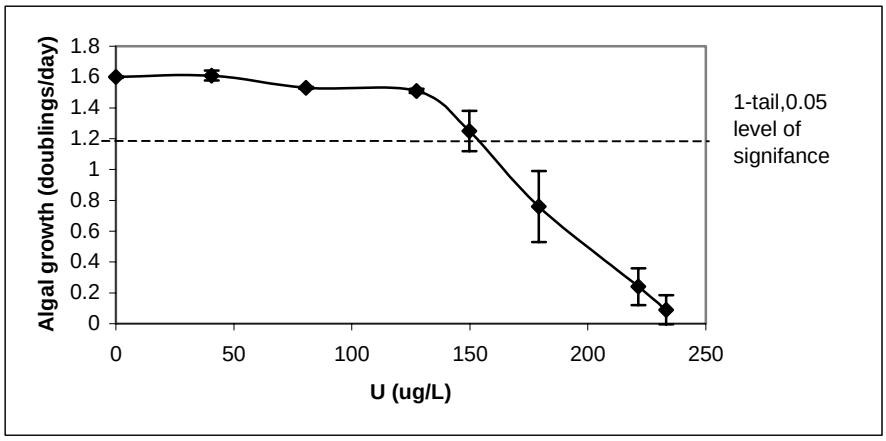

Figure 6a Concentration-response curve for definitive test 1. Verticle bars represent the standard error of the mean.

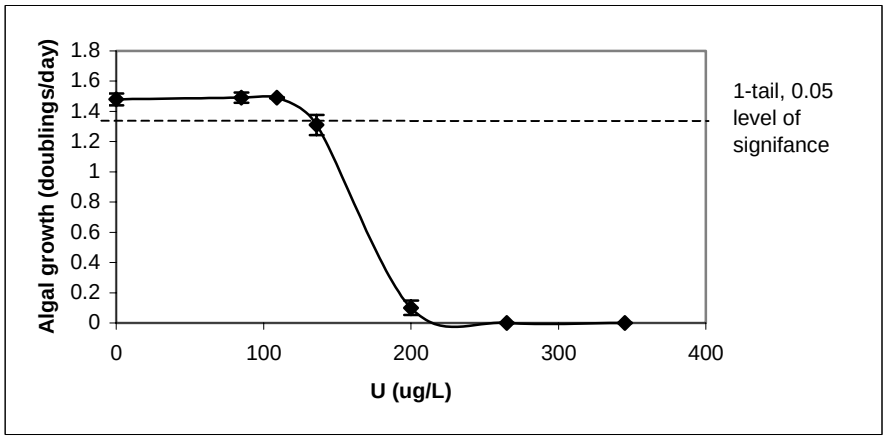

Figure 6b Concentration-response curve for definitive test 2. Verticle bars represent the standard error of the mean.

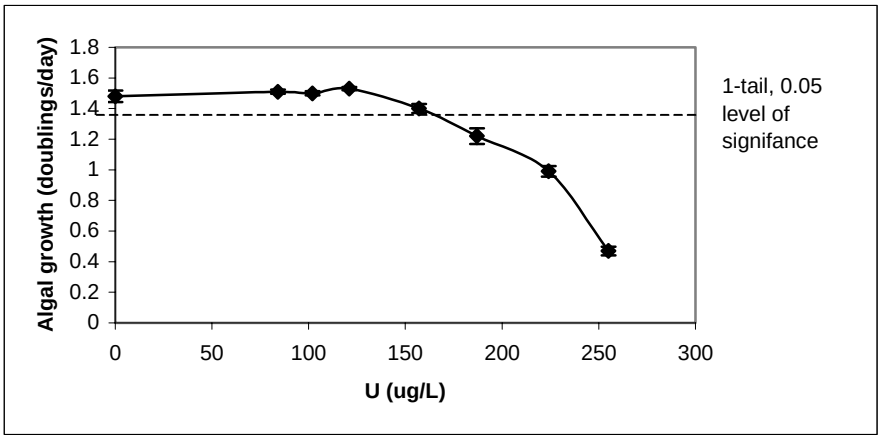

Figure 6c Concentration-response curve for definitive test 3. Verticle bars represent the standard error of the mean.

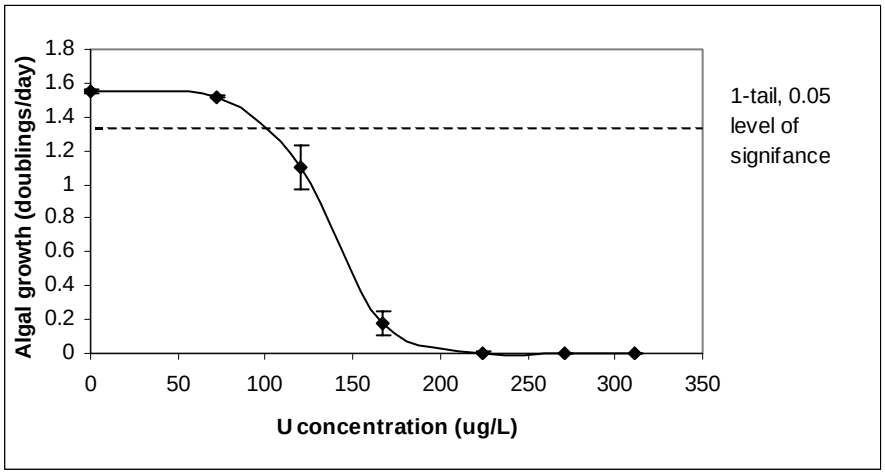

Figure 6d Concentration-response curve for definitive test 4. Verticle bars represent the standard error of the mean.

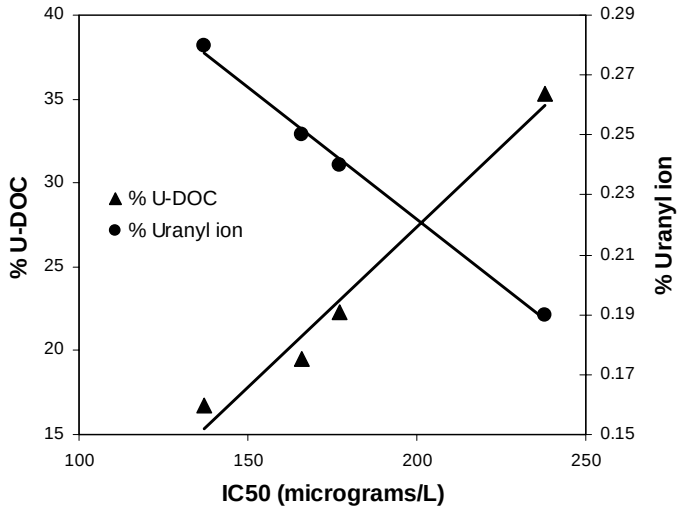

Figure 7 Relationship between the concentration of U bound to dissolved organic carbon, the concentration of the free U ion and toxicity to *Chlorella* sp. in the four definitive tests.

When comparing the toxicity of U in Magela Creek water to tests undertaken in synthetic softwater, the mean IC50 of the four definitive tests in Magela Creek water was four times higher (ie less toxic) than the mean IC50 of those tests conducted in synthetic water (table 3). This corresponded to a four-fold decrease in the proportion of U (as % uranyl ion, UO_2^{2+}) available to the algae (as calculated using the HARPHRQ speciation model), most likely due to the presence of DOM. Supporting these results, Markich et al (1996) observed that the toxicity of uranium to *Velesunio angasi* was substantially ameliorated in synthetic Magela Creek water with increasing concentration of DOC, in the form of a synthetic fulvic acid. These results indicate that DOM in Magela Creek water (or its synthetic equivalent) is a major determinant of the bioavailability and toxicity of uranium to aquatic biota.

Table 3 Toxicity of U to *Chlorella* sp. over 72 h, according to test water type and the % available uranyl ion, as calculated using the HARPHRQ speciation model

Diluent	Mean U toxicity (IC50[1]) (µg/L)	% uranyl ion	Reference
Natural creek water	180	0.25	This study
Synthetic water	44	1	Franklin et al 2000

[1] IC50, concentration where there is a 50% inhibition of cell division (growth).

In comparison to the historical chronic toxicity data obtained for the four other local species tested (table 4), *Chlorella* sp. was found to be the second most sensitive species to U after the cladoceran *Moinodaphnia macleayi*. The green hydra, *Hydra viridissima,* was the next most sensitive species, followed by the purple spotted gudgeon, *Mogurnda mogurnda,* and the chequered rainbowfish, *Melanotaenia splendida inornata*.

Table 4 Summary of chronic toxicity of uranium in Magela Creek water to local species

Species	Test endpoint	NOEC (µg L^{-1})	Reference
Moinodaphnia macleayi	Reproduction (3 brood)	18 [1]	***eriss*** unpubl, Semaan (1999)
Chlorella sp.	Cell division rate (72 h)	117[1]	This study
Hydra viridissima	Population growth (96 h)	183[1]	Hyne et al (1992)[2] ; ARRRI 1988
Mogurnda mogurnda	Mortality (7 d exposure / 7 d post-exposure)	400	Holdway (1992)
Melanotaenia splendida inornata	Mortality (7 d)	810	Holdway (1992)

[1] Toxicity values represent geometric means from ≥ 2 tests.

[2] Publication presented nominal concentration, therefore, measured concentrations from eriss records were used for TV derivation.

Deriving a site-specific guideline trigger value for U

With uranium toxicity data now available for a fifth local species from a fourth taxonomic group, a site-specific guideline trigger value for U in Magela Creek was derived using the statistical extrapolation methodology recommended by ANZECC & ARMCANZ (2000a). A geometric mean of 117 µg/L U was calculated from the NOEC values of the four definitive tests presented in table 2. This value was added to the existing U toxicity dataset from four other local species (18, 183, 400, 810 µg/L U) and analysed using the BurrliOZ software.

Figure 8 shows the resulting distribution fit which gave a trigger value of 0.54 µg/L U for the protection of 99% of species. However, given the warning by ANZECC and ARMCANZ (2000b) regarding the use of small datasets, care was taken to ensure that the BurrliOZ program had selected the most appropriate distribution for the U data.

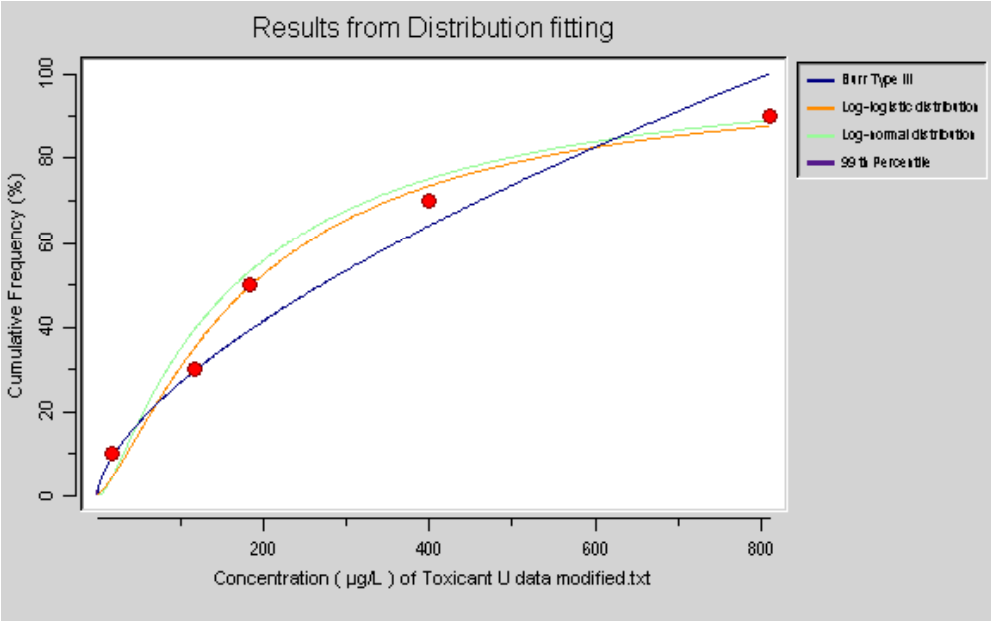

Figure 8 BurrliOZ result plot for trigger value derivation using the geometric means of the NOECs from five local species

Visual examination of figure 8 indicated that the overlaying log-logistic and log-normal distributions may have better described the distribution of the data. To verify this, the NOEC data were entered into Minitab (Version 13.1) and fitted to four common statistical distributions, Weibull, lognormal (base 10), log-logistic and logistic, using maximum likelihood (ML) estimation. Goodness-of-fit testing, using the Anderson-Darling statistic indicated that the log-logistic distribution provided the better fit (table 5).

To further verify the choice of the log-logistic as the better fitting distribution, and for direct comparison with the Burr Type III distribution, predicted NOEC values were calculated for each of the distributions based on their respective cumulative frequency plots and compared with the actual NOEC values using correlation analysis (table 6). All five distributions described the data set quite well, with correlation coefficients (r) greater than 0.9. However, two distinct groupings were evident; the log-logistic, lognormal and Weibull distributions had similarly high r values of >0.99 and gave reasonably similar 99% TVs for U of 5.9, 8.2 and 2.8 µg/L, respectively, while the Burr Type III and logistic distributions had lower r values in the order of 0.94 – 0.97 and gave much lower TVs (table 6). Based on the goodness-of-fit testing (ie both the Anderson–Darling test and correlation analysis), the log-logistic distribution was found to be an equal or better fit than the other distributions (albeit not by a large margin). Finally, to complete the evaluation, the use of the ML method of distribution fitting was compared to the other commonly used method for fitting distributions, least squares (LS) estimation. For the log-logistic distribution, the ML method described the data set better than LS estimation based on both the Anderson-Darling statistic (LS, AD = 2.094) and correlation analysis (LS, r = 0.986). Thus, on the basis of the above evaluation, the log-logistic distribution (maximum likelihood estimation) was chosen as the model to use for the derivation of the U TV.

Therefore, the site specific trigger value for U in Magela Creek predicted to protect 99% of species was 6 µg/L, rounded up from 5.9 µg/L (figure 9, appendix 4). This value supersedes all other interim U trigger values previously derived for Magela Creek, however, is to be reviewed when more site-specific toxicity data become available.

Table 5 Anderson-Darling goodness of fit test to determine the distribution that best fits the U NOEC data (lowest value = best fit)

Distribution	Anderson-Darling Index
Log-logistic	2.046
Weibull	2.120
Lognormal base 10	2.180
Logistic	2.374

Table 6 Actual NOEC data and predicted NOEC data for each distribution, and corresponding Pearson correlation co-efficients and 99% TVs for U

Actual NOECs (µg/L U)	BurrliOZ predicted NOECs (Burr Type III)	Minitab predicted NOECs			
		Weibull	Lognormal base 10	Log-logistic	Logistic
18	21.0	37.2	36.3	41.8	-55.5
117	120	110	87.3	102	137
150	270	209	166	186	266
400	460	356	315	339	395
810	685	652	757	827	587
r (actual vs predicted)	**0.974**	**0.997**	**0.995**	**0.994**	**0.943**
P value	**0.005**	**0.000**	**0.000**	**0.001**	**0.016**
99% Trigger Value (µg/L U)	**0.54**	**2.8**	**8.2**	**5.9**	**-475**

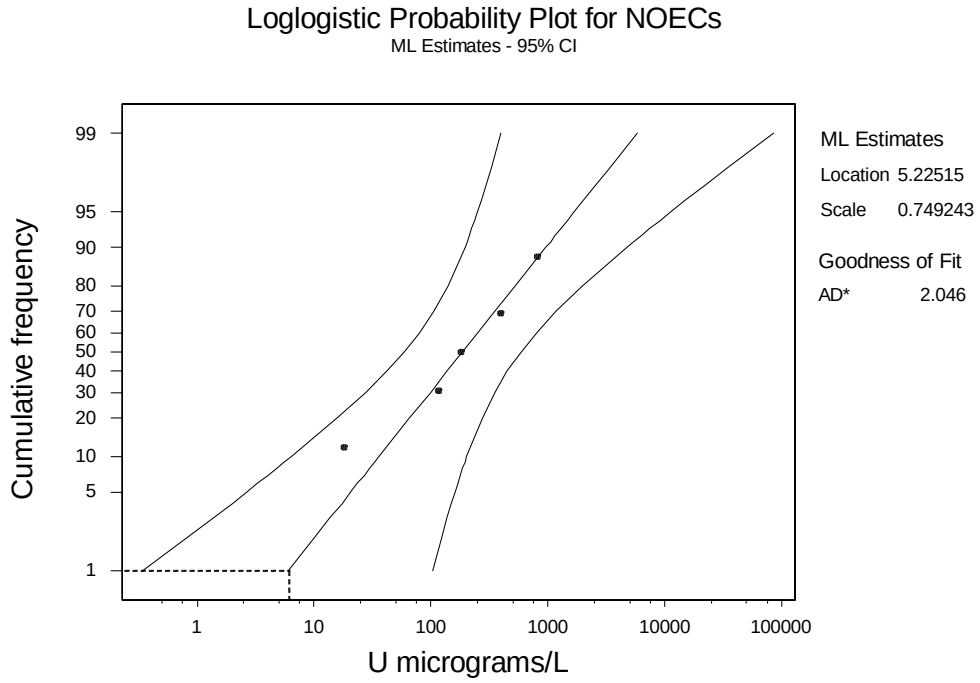

Figure 9 Log-logistic probability plot using Minitab to calculate the U trigger value at the 99% level

The fact that all of the distributions described the data reasonably to very well, yet in some cases yielded vastly different 99% TVs, highlights the model dependency associated with fitting distributions to small data sets, especially to extrapolation to the tails of those distributions. The large uncertainty surrounding the distributions and associated implications for TVs was identified and discussed in brief by van Dam (2002), and is intended to be analysed and discussed in greater detail in a separate paper.

Conclusions

Chlorella sp. was found to be the second most sensitive species to U out of the five local species tested in natural Magela creek water. The toxicity of U to *Chlorella* sp. was shown to be approximately four times lower when tested in natural Magela Creek water compared to synthetic water that contained no organic matter. Repeated tests in natural creek water also showed that the toxicity of U to *Chlorella* sp. decreased as the concentration of dissolved organic matter in the natural Magela Creek water increased, over the sampling periods. These observations were supported with calculations from the HARPHRQ speciation model, which estimated the free (and bioavailable) uranyl ion (UO_2^{2+}) to be four times higher in the synthetic water than in natural Magela Creek water, and more available in the Magela Creek water with lower dissolved organic carbon concentrations. Finally, the geometric mean NOEC value from four definitive tests conducted in natural Magela Creek water was added to the suite of data using other local species to derive a high reliability TV to protect 99% of species. Following an assessment of the goodness-of-fit to the data set of various statistical distributions, a TV of 6 μg/L for U in Magela Creek was derived. This value supersedes all other interim U trigger values previously derived for Magela Creek, however, is to be reviewed when more site-specific toxicity data becomes available.

Acknowledgements

The authors would like to thank Amy Koch for technical assistance and Natasha Franklin for providing raw data from her 1998 study. Thanks also to Ann Webb, Dr Peter Bayliss and Dr Max Finlayson for comments on the draft.

References

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

ANZECC & ARMCANZ 2000b. Derivation of toxicant trigger values: Explanatory documentation. 'TOX – Read Me' file provided with the BurrliOZ software. Australian and New Zealand Environment and Conservation Council & Agriculture and Resource Management Council of Australia and New Zealand, Canberra.

ARRRI 1988. *Alligator Rivers Region Research Institute: Annual research summary 1987-1988.* Supervising Scientist for the Alligator Rivers Region, AGPS, Canberra.

Brown PL, Haworth A, Sharland SM, Tweed CJ 1991. HARPHRQ: an Extended Version of the Geochemical Code PHREEQE. NSS/R 188. UK Atomic Energy Authority, Oxford.

Campbell E, Palmer MJ, Shao Q, Warne MStJ & Wilson D 2000. *BurrliOZ: A computer program for calculating toxicant trigger values for the ANZECC and ARMCANZ water quality guidelines.* Perth WA.

Dunnett CW 1955. Multiple comparison procedure for comparing several treatments with a control. *Journal of the American Statistical Association* 50, 1096–1121.

Dunnett CW 1964. New table for multiple comparisons with a control. *Biometrics* 20, 482.

Ferguson WJ, Braunschweiger KI, Braunschweiger WR, Smith JR, McCormick JJ, Wasman CC, Jarvis NP, Bell DH, Good NE 1980. Hydrogen ion buffers for biological research. *Analytical Biochemistry* 104, 300–310.

Franklin N, Stauber J, Markich S & Lim R 1998. *A new tropical algal test to assess the toxicity of metals in freshwaters*. Supervising Scientist Report 133, Supervising Scientist, Canberra.

Franklin NM, Stauber JL, Markich SJ and Lim RP 2000. pH-dependent toxicity of copper and uranium to a tropical freshwater alga (Chlorella sp.). *Aquatic Toxicology* 48, 275–289.

Gardner S, Finlayson CM & Pidgeon RWJ (eds) 2002. *Description and literature review of the flora and vertebrate fauna of Magela Creek, Alligator Rivers Region, northern Australia*. Scientist Report 169, Supervising Scientist, Darwin NT.

Hamilton MA, Russo RC & Thurston RV 1977. Trimmed Spearman-Karber method for estimating median lethal concentratrions in toxicity bioassays. *Environmental Science and Technology* 11, 714–719.

Holdway DA 1992. Uranium toxicity to two species of Australian tropical fish. *The Science of the Total Environment* 125, 137–158.

Hyne RV, Rippon GD and Ellender G 1992. pH dependent uranium toxicity to freshwater hydra. *The Science of the Total Environment* 125, 159–173.

Johnston A & Needham S 1999. *Protection of the environment near the Ranger uranium mine*. Supervising Scientist Report 139, Supervising Scientist, Canberra.

Klessa DA 2000. *The chemistry of Magela Creek: A baseline for assessing change downstream of Ranger*. Supervising Scientist Report 151, Supervising Scientist, Darwin.

Markich SJ, Brown PL and Jeffree RA 1996. The use of geochemical speciation modelling to predict the impact of uranium to freshwater biota. *Radiochimica Acta* 74, 321–326.

Markich SJ, Brown PL, Jeffree RA and Lim RP 2000. Valve movement responses of *Velesunio angasi* (Bivalvia: Hyriidae) to manganese and uranium: An exception to the free ion activity model. *Aquatic Toxicology* 51, 155–175.

Padovan A 1992. *Isolation and culture of five species of freshwater algae from the Alligator Rivers Region, Northern Territory.* Technical Memorandum 37, Supervising Scientist for the Alligator Rivers Region, Canberra.

Riethmuller N, Camilleri C, Franklin N, Hogan AC, King A, Koch A, Markich SJ, Turley C & van Dam R 2003. *Ecotoxicological testing protocols for Australian tropical freshwater ecosystems*. Supervising Scientist Report 173, Supervising Scientist, Darwin NT.

Semaan M, Holdway DA and van Dam RA 2001. Comparative sensitivity of three populations of the Cladoceran Moinodaphnia macleayi to acute and chronic uranium exposure. *Environmental Toxicology* 16, 365–376.

Shao Q 2000. Estimation for hazardous concentrations based on NOEC toxicity data: an alternative approach. *Environmetrics* 11, 583–595.

Sprague JB 1990. Aquatic toxicology. In *Methods for fish biology*, eds Schreck CB & Moyle PB, American Fisheries Society, Maryland, 491–527.

Stauber JL, Tsai J, Vaughan GT, Peterson SM, Brockbank CI 1994. *Algae as indicators of toxicity of the effluent from bleached eucalypt kraft pulp mills*. National Pulp Mills Research Program Technical Report No. 3. CSIRO Canberra.

Stein JR 1973. *Handbook of phycological methods*. *Culture methods and growth measurements*. Cambridge University Press, Cambridge.

Stephens MA 1974. EDF statistics for goodness of fit and some comparisons. *Journal of the American Statistical Association* 69, 730–737.

Supervising Scientist 1998. Protection of the environment near the Ranger Uranium Mine. Supervising Scientist, Darwin.

van Dam RA 2002. Derivation of a site-specific water quality trigger value for uranium in Magela Creek In Environmental Research Institute of the Supervising Scientist research summary 1995–2000, eds J Rovis-Hermann, KG Evans, AL Webb & RWJ Pidgeon. Supervising Scientist Report 166, Supervising Scientist, Darwin NT, pp. 171-176.

van Dam RA 2002. Derivation of a site-specific water quality trigger value for uranium in Magela Creek. In *Environmental Research Institute of the Supervising Scientist research summary 1995–2000*, eds Rovis-Hermann J, Evans KG, Webb AL & Pidgeon RWJ, Supervising Scientist Report 166, Supervising Scientist, Darwin NT, 171–176.

Warne MS 2001. Derivation of the Australian and New Zealand water quality guidelines for toxicants. *Australasian Journal of Ecotoxicology* 7, 123–136.

Appendices

Appendix 1 MBL growth media for culturing *Chlorella* sp.

MBL is prepared each month for the maintenance of starter cultures, and the mass culturing and harvesting of *Chlorella sp*.

Required volumes:

1 L for Starter Cultures

4-6 L for harvesting algae (eg for cladoceran food)

Working Solution Preparation:

1. Add 1 mL of each stock solution (described below) per litre of deionised (Milli Q) water, except Tris Buffer - add 5 mL/L.

Note: these ingredients are stored at 4°C and require replacing every 18-24 months.

	Ingredient	Stock Solution	Media Solution
1	Tris Buffer	100 g/L	5 mL/L
2	$NaNO_3$	85.24 g/L	1 mL/L
3	$CaCl_2.2H_2O$	36.76 g/L	1 mL/L
4	$MgSO_4.7\ H_2O$	36.97 g/L	1 mL/L
5	$NaHCO_3$	12.6 g/L	1 mL/L
6	K_2HPO_4	8.72 g/L	1 mL/L
7	Na_2EDTA	4.36 g/L	1 mL/L
8	$FeCl_3.6H_2O$	0.727 g/L	1 mL/L
9	**Vitamins** Cyanocobalamin (Vitamin B12) Thiamine hydrochloride (Vitamin B1) d-Biotin (Vitamin H)	*See below*	1 mL/L
10	Trace metals	In 1 L add:	1 mL/L
	$CoCl_2.6H_2O$	10 mg/L	
	$CuSO_4.5H_2O$	9 mg/L	
	$Na_2SiO_3.5H_2O$	7 mg/L	
	$MnCl_2.4H_2O$	180 mg/L	
	$ZnSO_4.7H_2O$	22 mg/L	

2. Adjust media to pH 7.1-7.3 using 10% HCl or 1 M NaOH.
3. Pour MBL media into: 4-6 x 2 L flasks, such that there is 1 L per flask for the Harvest Culture **OR** 10 x 250 mL flasks, such that there is 100 mL per flask for Starter cultures.
4. Use a bung to plug the top of the each flask (Refer to Section B, Part 4 to construct bungs). Cover the bung and mouth of flask with aluminium foil. Record the date the media is autoclaved and media type on a strip of autoclave tape and place on aluminium foil.
5. Autoclave at 121^{o}C for 20 min.
6. Allow the media to cool to room temperature before inoculating.
7. Media may be stored at room temperature while not in use.

Appendix 2 Synthetic water preparation

1. Prepare the stock solutions (described below) in 1 L volumetric flasks with deionised (Milli-Q) water. Transfer to clean 1 L plastic bottles and store at 4°C until required.

Note: these ingredients are stored at 4°C, and require replacing every 18-24 months.

	Ingredient	Stock Solution	Media Solution
1	$NaHCO_3$	72.34 g/L	1 mL/20L
2	$Al_2(SO_4)_3.6H_2O$	17.26 g/L	1 mL/20L
3	$MgSO_4.7H_2O$	121.52 g/L	1 mL/20L
4	$CaCl_2.2H_2O$	32.96 g/L	1 mL/20L
5	KCl	14.09 g/L	1 mL/20L
6	$FeCl_3.6H_2O$	10 g/L	1 mL/20L
7	Trace Element Solution	In 1 L add:	0.5 mL/20L
	$CuSO_4.5H_2O$	0.11	
	$ZnSO_4.7H_2O$	0.123	
	$Pb(NO_3)_2$	0.008	
	$MnSO_4.H_2O$	1.188	
	$UO_2SO_4.3H_2O$	0.007	

2. Fill a 5L volumetric flask with deionised water and pour this into a clean 25 L plastic barrel designated for synthetic water preparation.
3. Add the appropriate amount of the seven solutions (described below) to the partially filled 5 L volumetric flask. Make flask up to volume with deionised water and pour into the barrel.
4. Fill the 5 L flask twice more to make the volume in the barrel equal 20 L.
5. Aerate overnight to allow mixing and gaseous exchange.
6. Check pH after a minimum of 12 h aeration and adjust pH to 6.0 ± 0.15 using 0.05 M H_2SO_4 or NaOH.
7. The water can be stored at 4°C for up to two weeks if required. The pH is to be checked before use to ensure it remains within the required range.

Appendix 3 Additional IC points and 95% confidence limits for the *Chlorella* sp. testing undertaken in this study

eriss test code	Description	IC05 (95% CL)	IC10 (95% CL)	IC15 (95% CL)	IC20 (95% CL)	IC25 (95% CL)	IC40 (95% CL)
564G	Reference toxicity test in synthetic soft water	38 (0 - 46)	42 (0 – 53)	46 (29 – 61)	50 (35 – 70)	54 (39 – 81)	65 (45 – 95)
566G(a)	With HEPES buffer	90 (50 – 192)	127 (45 – 167)	142 (64 – 164)	150 (90 – 171)	157 (116 – 177)	180 (147 – 196)
566G(b)	Without HEPES buffer	48 (24 – 77)	64 (32 – 89)	77 (48 – 107)	90 (64 – 125)	103 (72 – 144)	141 (85 – 190)
568G	Rangefinder	90 (83 – 92)	96 (89 – 99)	102 (94 – 107)	108 (100 – 115)	114 (106 – 122)	132 (123 – 146)
569G	1st definitive	53 (34 – 190)	66 (37 – 225)	79 (35 – 234)	143 (0 – 172)	153 (0 – 173)	167 (144 – 201)
589G	2nd definitive	121 (103 – 147)	132 (110 – 147)	139 (120 – 148)	143 (130 – 152)	146 (135 – 155)	158 (149 – 166)
591G	3rd definitive	146 (121 – 173)	164 (150 – 179)	176 (159 – 202)	188 (166 – 208)	201 (170 – 215)	229 (223 – 234)
606G	4th definitive	78 (72 – 92)	87 (77 – 124)	96 (81 – 148)	105 (85 – 145)	114 (90 – 142)	129 (112 – 145)

Appendix 4 Minitab Session for Uranium trigger value derivation

———— 13/11/2003 10:27:41 AM ————————————————————

Welcome to Minitab, press F1 for help.

Distribution ID Plot

Variable: U NOECs

Goodness of Fit

Distribution	Anderson-Darling (adj)
Weibull	2.120
Lognormal base 10	2.180
Loglogistic	2.046
Logistic	2.374

Table of Percentiles

Distribution	Percent	Percentile	Standard Error	95% Normal CI Lower	Upper
Weibull	12	37.165	37.767	5.072	272.35
Lognormal base 10	12	36.284	27.268	8.318	158.28
Loglogistic	12	41.777	35.162	8.026	217.45
Logistic	12	-55.516	162.204	-373.430	262.40
Weibull	31	110.226	75.880	28.597	424.87
Lognormal base 10	31	87.287	53.467	26.276	289.96
Loglogistic	31	102.071	65.808	28.848	361.15
Logistic	31	136.703	129.016	-116.164	389.57
Weibull	50	208.516	112.207	72.626	598.67
Lognormal base 10	50	165.688	95.774	53.366	514.42
Loglogistic	50	185.890	109.072	58.858	587.09
Logistic	50	265.695	126.700	17.367	514.02
Weibull	69	356.072	165.734	143.003	886.61
Lognormal base 10	69	314.509	192.650	94.675	1044.79
Loglogistic	69	338.539	206.852	102.214	1121.26
Logistic	69	394.687	142.106	116.165	673.21
Weibull	88	652.390	308.877	257.931	1650.11
Lognormal base 10	88	756.591	568.593	173.447	3300.32
Loglogistic	88	827.136	642.291	180.550	3789.27
Logistic	88	586.907	187.476	219.460	954.35

Table of MTTF

Distribution	Mean	Standard Error	95% Normal CI Lower	Upper
Weibull	305.697	139.507	124.980	747.73
Lognormal base 10	382.005	299.147	82.318	1772.73
Loglogistic	617.323	825.373	44.920	8483.70
Logistic	265.695	126.700	17.367	514.02

ID Plot for U NOECs

Correlations: U NOECs, weibull

Pearson correlation of U NOECs and weibull = 0.997
P-Value = 0.000

Correlations: U NOECs, lognormal

Pearson correlation of U NOECs and lognormal = 0.995
P-Value = 0.000

Correlations: U NOECs, loglogistic

Pearson correlation of U NOECs and loglogistic = 0.994
P-Value = 0.001

Correlations: U NOECs, logistic

Pearson correlation of U NOECs and logistic = 0.943
P-Value = 0.016

Correlations: U NOECs, Burr

Pearson correlation of U NOECs and Burr = 0.974
P-Value = 0.005

Distribution ID Plot

Variable: U NOECs

Goodness of Fit

Distribution	Anderson-Darling (adj)
Weibull	2.120
Lognormal base 10	2.180
Loglogistic	2.046
Logistic	2.374

Table of Percentiles

Distribution	Percent	Percentile	Standard Error	95% Normal CI Lower	Upper
Weibull	1	2.776	5.275	0.07	115.067
Lognormal base 10	1	8.192	9.116	0.93	72.546
Loglogistic	1	5.943	8.658	0.34	103.288
Logistic	1	-475.111	289.694	-1042.90	92.678

Distribution Function Analysis

Loglogistic Dist. Parameter Estimates (ML)

Variable: U NOECs

Location 5.22515
Scale 0.749243

Goodness of Fit

Anderson-Darling (adjusted) = 2.046

Percentile Estimates

Percent	Percentile	95% CI Approximate Lower Limit	95% CI Approximate Upper Limit
1	5.94	0.342	103.3
2	10.07	0.821	123.4
3	13.75	1.370	137.9
4	17.18	1.971	149.8
5	20.47	2.615	160.3
6	23.66	3.297	169.7
7	26.77	4.012	178.6
8	29.82	4.759	186.9
9	32.84	5.535	194.9
10	35.83	6.339	202.6
20	65.79	15.757	274.7
30	98.53	27.538	352.5
40	137.19	41.785	450.4
50	185.89	58.858	587.1
60	251.88	79.464	798.4
70	350.72	105.074	1170.6
80	525.22	139.334	1979.9
90	964.31	195.049	4767.5
91	1052.19	203.426	5442.3
92	1158.71	212.809	6309.0
93	1291.05	223.489	7458.1
94	1460.78	235.904	9045.5
95	1687.92	250.747	11362.4
96	2010.80	269.218	15018.8
97	2513.91	293.647	21521.6
98	3432.60	329.547	35754.4
99	5813.95	395.949	85369.6

Prob Plot for U NOECs

Saving file as: X:\Uranium tox_TV info\Uranium TV_Nov 2003.MPJ

Distribution Function Analysis

Loglogistic Dist. Parameter Estimates (LS)

Variable: U NOECs

Location   5.11011
Scale   0.925362

Goodness of Fit

Anderson-Darling (adjusted) = 2.094
Pearson Correlation Coefficient = 0.976

Percentile Estimates

Percent	Percentile	95% CI Approximate Lower Limit	95% CI Approximate Upper Limit
1	2.4	0.027	206
2	4.5	0.093	221
3	6.6	0.191	231
4	8.8	0.319	240
5	10.9	0.477	247
6	13.0	0.663	254
7	15.1	0.878	261
8	17.3	1.120	267
9	19.5	1.390	273
10	21.7	1.689	279
20	45.9	6.290	336
30	75.6	14.174	404
40	113.9	25.896	501
50	165.7	41.848	656
60	241.1	61.990	938
70	362.9	85.860	1534
80	597.6	113.313	3152
90	1265.6	147.055	10893
91	1409.6	151.197	13141
92	1587.9	155.605	16204
93	1814.8	160.349	20540
94	2113.9	165.532	26994
95	2527.0	171.313	37274
96	3136.8	177.953	55292
97	4133.0	185.936	91868
98	6072.0	196.333	187791
99	11640.6	212.495	637679

Prob Plot for U NOECs

Saving file as: X:\Uranium tox_TV info\Uranium TV_Nov 2003.MPJ
* NOTE * Existing file replaced.

Distribution ID Plot

Variable: U NOECs

Goodness of Fit

Distribution	Anderson-Darling (adj)	Correlation Coefficient
Loglogistic	2.094	0.976

Table of Percentiles

Distribution	Percent	Percentile	Standard Error	95% Normal CI Lower	CI Upper
Loglogistic	12	26.22	32.15	2.371	289.92

Loglogistic	31	79.02	66.55	15.165	411.75
Loglogistic	50	165.69	116.33	41.848	656.01
Loglogistic	69	347.41	253.09	83.318	1448.59
Loglogistic	88	1047.15	1077.42	139.378	7867.20

Table of MTTF

Distribution	Mean	Standard Error	95% Normal CI Lower	Upper
Loglogistic	2073.14	12966.2	9.84E-03	4.37E+08

ID Plot for U NOECs

Correlations: U NOECs, loglog (LS)

Pearson correlation of U NOECs and loglog (LS) = 0.986
P-Value = 0.002