Centre for Advanced Analytical Chemistry
Energy Technology

CANNING RIVER PHOSLOCK FIELD TRIAL – ECOTOXICITY TESTING FINAL REPORT

by J.L. Stauber and M.T. Binet

Report No: ET317R

Prepared for
CSIRO Land and Water and the WA Water and Rivers Commission

August, 2000

EXECUTIVE SUMMARY

The WA Water and Rivers Commission, in conjunction with CSIRO Land and Water, conducted a field trial of a lanthanum-modified clay (Phoslock) in the Canning River, WA. Phoslock has been shown in previous laboratory and field studies to reduce phosphate concentrations in natural waters and wastewaters (Douglas et al, 2000).

The aim of this project was to determine the toxicity of Canning River water samples collected prior to and after Phoslock application in January 2000 and April, 2000. Acute tests were conducted with the cladoceran *Ceriodaphnia dubia* (48-h survival) and juvenile eastern rainbow fish *Melanotaenia duboulayi* (96-h immobilisation). Chronic toxicity tests were carried out with *Ceriodaphnia dubia* (7-day reproduction) and the green alga *Selenastrum capricornutum* (72-h growth inhibition). In addition, the toxicity of lanthanum alone to each of the test species was determined.

In the first field trial in January, 2000, Canning River samples prior to Phoslock application were not toxic to any of the test species. Effects on the cladoceran and alga were detected within 24 h of Phoslock application, with no toxicity detectable after one week or more. The cladoceran was more sensitive to Phoslock than the freshwater alga. Only unfiltered samples inhibited growth of the alga, 24 h after application, and this effect was not solely due to phosphate limitation. Toxicity to the cladoceran was not only due to the presence of particles, as filtered samples were also toxic. Phoslock was not toxic to juvenile rainbow fish.

After application of Phoslock, substantial amounts of lanthanum were released from the Phoslock into surface and bottom waters. Total lanthanum concentrations of up to 15 mg/L were detectable in bottom waters one day after Phoslock application. Because there was a significant correlation between dissolved lanthanum concentrations and acute toxicity to *Ceriodaphnia*, the toxicity of lanthanum in laboratory water was investigated.

Lanthanum (added as lanthanum chloride) was toxic to each of the test species. Rainbow fish immobilisation, algal growth inhibition and cladoceran reproduction inhibition showed similar sensitivity to lanthanum, with EC50 values (i.e. the effective concentration to cause a 50% effect) of less than 0.6 mg La/L. The chronic lowest observable effect concentrations (LOEC) were 0.13 and 0.09 mg La/L for the alga and cladoceran respectively.

For the alga and the cladoceran, the absence or presence of toxicity in the Canning River samples was usually predictable from the measured total and dissolved lanthanum concentrations based on NOEC and LOEC values derived from the calibration bioassays with lanthanum alone. However, the magnitude of the effect was not predictable on the basis of the lanthanum concentration alone. Rainbow fish were much less sensitive to lanthanum in the Canning River samples than to lanthanum alone and toxicity was not predictable from

measured lanthanum concentrations in the river waters. Lanthanum is known to strongly bind to humic substances so it is possible that humic substances in the river waters ameliorated the toxicity of lanthanum.

Barriers in the Canning River were ineffective in preventing wash-through of Phoslock downstream and in preventing salinity intrusions upstream. Bottom waters were quite saline and had to be diluted to reduce salinity so that it did not cause toxicity. The higher ionic strength may have caused the release of lanthanum from the modified clay and resulted in Phoslock toxicity in bottom waters. Oxygenation had no effect on Phoslock toxicity.

After modifications to the Phoslock to reduce the lanthanum released in surface and bottom waters, a second trial was conducted in April, 2000. Total lanthanum concentrations in the river waters were substantially reduced, with a maximum concentration of 1.7 mg/L on the day of application. Although the lanthanum concentrations still exceeded the lowest observable effect concentrations for acute toxicity to the rainbow fish and chronic toxicity to the alga and cladoceran, no toxicity in either surface or bottom waters was detectable to any of the test species for up to 7 days after Phoslock application. Potential lanthanum toxicity was ameliorated completely by the river water, probably due to the presence of humic substances. Stimulation of algal growth in the Canning River water samples was frequently found in the laboratory bioassays.

The use of modified Phoslock, where lanthanum release is minimised, should pose minimal risk of acute or chronic toxicity to freshwater organisms.

TABLE OF CONTENTS

List of Tables:

List of Figures:

1 INTRODUCTION

The WA Water and Rivers Commission, in conjunction with CSIRO Land and Water, conducted a field trial of a modified clay (Phoslock) in the Canning River, WA. Phoslock has been shown in previous laboratory and field studies to reduce phosphate concentrations in natural and wastewaters (Douglas et al, 2000).

Preliminary toxicity tests using bacteria, microalgae, a crustacean and a fish showed that a freshwater leachate of the modified clay was of low toxicity to the cladoceran (*Ceriodaphnia dubia*) in acute tests, and was not toxic to bacteria (*Vibrio fischeri*), a freshwater green alga (*Selenastrum capricornutum*) or juvenile rainbow fish (Stauber, 1999; Vaughan, 1998; Lim 1999). Leachates of the modified clay were prepared under standard batch conditions (1 part clay:20 parts soft water) that are unlikely to reflect actual leaching of the clay in field applications.

To ensure that future field applications of the clay to freshwaters will not affect other aquatic species, an ecotoxicology monitoring program was instigated in conjunction with nutrient, water chemistry and biological monitoring. The aim of the study was to determine whether Phoslock application to the Canning River caused acute or chronic toxicity to species in the water column during and after Phoslock settling. The potential impact of Phoslock such as physical disturbances to sediment-dwelling organisms and sediment microbial communities was not included in this monitoring program.

This report outlines the results of acute and chronic toxicity tests with algae, juvenile fish and cladocerans, from two separate applications of Phoslock to the Canning River in January and April, 2000. Both events included monitoring prior to and immediately after Phoslock application.

In order to determine whether lanthanum released from the Phoslock was contributing to the toxicity of the Canning River samples, calibration bioassays with lanthanum chloride were also carried out with each of the test species. Very little data is available on lanthanum toxicity to aquatic species, although lanthanum has previously been shown to replace calcium in different cell functions, blocking uptake of calcium and competing for available binding sites (Das *et al*, 1988).

2 EXPERIMENTAL

2.1 Treatment Sites

Artificial weirs divided a portion of the Canning River into four sites, with four treatments (from downstream to upstream): oxygenation alone (WHA), Phoslock plus oxygenation (PO3), Phoslock alone (P3) and no treatment (control C2). Surface waters prior to Phoslock application, and surface and bottom waters after Phoslock application, were sampled for ecotoxicity testing using freshwater algae, invertebrates and fish. Acute tests were conducted with the cladoceran *Ceriodaphnia dubia* (48-h survival) and juvenile eastern rainbow fish *Melanotaenia duboulayi* (96-h immobilisation). Chronic toxicity tests were carried out with *Ceriodaphnia dubia* (7-day reproduction) and the green alga *Selenastrum capricornutum* (72-h growth inhibition).

2.2 First Phoslock Application (January, 2000)

Surface water samples from each of the four sites were sampled six weeks, three weeks, one week and one day before Phoslock application. In addition, samples from two drains Liege St and Cockram St were also tested on one occasion.

Phoslock was applied to site P3 over a few days from 5-7/1/00. Because Phoslock application was carried out over a few days, it was not possible to take 2- and 6-h post-application samples. Surface and bottom waters from site P3 and control sites C2 and WHA were sampled one day, one week, two weeks, three weeks and four weeks after Phoslock application. Phoslock plus oxygenation was applied to site PO3 approximately one week after application to P3. Surface and bottom waters from site PO3 and control sites C2 and WHA (with oxygenation) were sampled one day, one week, two weeks and three weeks after Phoslock application.

In addition, Phoslock was applied to Cockram St drain and sampled within 24 h for toxicity testing. Liege St drain (without Phoslock application) was also re-sampled at the same time.

2.3 Second Phoslock Application (April, 2000)

Modified Phoslock was applied to the Canning River for a second time on 20/4/00. Surface and bottom waters from site P3 and a control site (C2) were sampled one day before application, on the day of application and 1, 3 and 7 days after application.

2.4 Sample Collection and Storage

Surface and bottom waters were collected in Nalgene low density polyethylene bottles (1-4 L) which had been pre-washed in nitric acid and Milli-Q water and individually bagged. The samples were collected by staff from the WA Water and Rivers Commission. Water physicochemical parameters including salinity, temperature, dissolved oxygen and pH were recorded. The samples were immediately cooled to 4^{o}C and sent by overnight air courier to the CSIRO

Centre for Advanced Analytical Chemistry (CAAC) in Sydney. On arrival, the samples were immediately sub-sampled for chemical analysis and a 500 mL aliquot for the algal bioassays was filtered through an acid-washed 0.45 µm membrane filter. Physicochemical parameters including pH, conductivity and DO were measured on each sample.

Samples were sent by courier to the participating laboratories (Sinclair Knight Mertz for the chronic *Ceriodaphnia* bioassays, University of Technology, Sydney, for the fish bioassays and GM Laboratories for limited chemical analyses). All samples were stored at 4^{o}C in the dark prior to carrying out the bioassays.

2.5 Chemical Analyses

Hardness, alkalinity and orthophosphate in each water sample was determined using standard methods (APHA, 1991) at GM Laboratories. Calcium, magnesium, copper, zinc and lanthanum in unfiltered and filtered samples were determined by inductively coupled plasma atomic emission spectroscopy (ICPAES) at CSIRO CAAC.

2.6 Acute Toxicity Tests

2.6.1 Ceriodaphnia dubia 48-h immobilisation bioassays

The starter cultures of *Ceriodaphnia dubia* were obtained from the NSW EPA/UTS Centre for Ecotoxicology, Gore Hill, NSW. The cladoceran was originally isolated from Lake Parramatta (CET,1997).

Mass cultures were maintained in 1.4 L of synthetic soft water (USEPA, 1993) at 25 $\pm$ 1°C under ambient laboratory light levels on a light:dark cycle of 16 h light: 8 h dark. The cultures were fed every second day with YCT (yeast-Cerophyll-trout chow) and the alga *Selenastrum capricornutum* (USEPA, 1993). Twenty-four hours prior to test commencement, adults were isolated from the mass cultures and the resulting neonates (<24 h old) were used in the toxicity tests.

The 48-h cladoceran immobilisation test was carried out according to USEPA (1993) protocols, with minor modifications summarised in Table 1. Most toxicity tests were screening tests with unfiltered, undiluted samples only (100%). Selected samples were also tested at a range of concentrations after dilution with synthetic soft water. All samples were tested in quadruplicate, together with synthetic soft water controls and three concentrations of the reference toxicant, copper. An additional set of controls, matched to the same conductivity of the sample, was also included in each test. Five neonates, less than 24-h old, were added to each 30 mL polycarbonate vial containing 15 mL of sample. Four replicate containers were used for each sample and control, giving a total of 20 organisms per sample. The numbers of immobile *Ceriodaphnia* were recorded at 24 and 48 h. Water quality parameters including pH, dissolved oxygen and conductivity were measured at the beginning and end of the test.

For water samples with conductivities higher than 2000 µS, it was necessary to dilute the sample with synthetic soft water to distinguish real toxicity from toxicity caused by high conductivity. Any unfiltered sample that was found to

be toxic was re-tested after filtration (0.45µm) to determine whether toxicity was associated with particulate matter.

Quality assurance

Reference toxicant tests with copper (1-10 µg/L) were run concurrently with the water samples to ensure that each batch of cladocerans was healthy and responding to a known toxicant in a reproducible way. Toxicity tests were acceptable if the EC50 for copper fell within the acceptable range of 4.1 ± 2.1 µg/L and if there was at least 90% survival in controls.

Statistical analyses

Toxicity data was arcsine transformed, if necessary, prior to calculation of toxicity endpoints, using ToxCalc Version 5.0.14 (Tidepool Software). After testing the data for normality and homogeneity of variance, Dunnett's Multiple Comparison Test was used to determine which water sample was significantly different to the controls.

2.6.2 Juvenile eastern rainbow fish 96-h imbalance bioassays

Acute tests with juvenile eastern rainbow fish *Melanotaenia duboulayi* were carried out at the University of Technology, Sydney.

Juvenile fish ranging in age from 5-11 weeks (10-20 mm) were acclimated to the test conditions and were not fed for 24 hours prior to the tests.

Treated Sydney tapwater was used as the control water. Tapwater was passed through sand and carbon filters and stored in epoxy-lined concrete tanks. Prior to use in the bioassay, the stored water was filtered through 5 µm and carbon filter cartridges and finally UV sterilised. The conductivity of this water was approximately 190 µS/cm and the pH was around 7.5.

All glassware and test containers used in the test were initially washed in detergent, rinsed with acetone and finally rinsed in reverse osmosis water. The test samples were brought up to the test temperature before test commencement.

Undiluted samples were tested, together with controls in treated tapwater, each in quadruplicate. The conductivity of all controls was adjusted to 1000 µS/cm, except the first control (test date 17/11/99). River water samples (500 mL) were dispensed into each 1 L glass container. Five fish were randomly selected and placed in each container. The fish were not fed over the test period of 96 h (OECD 1987). Temperature, pH, conductivity and dissolved oxygen of the test solutions were measured at the commencement and at 24-h intervals. The test solutions were not renewed throughout the test period but were gently aerated to maintain the dissolved oxygen levels above 60% to meet OECD requirements for fish toxicity tests (OECD 1987). The number of imbalanced fish fry was noted at 24-h intervals over the 96-h test period.

A reference toxicant test using phenol was run concurrently with each suite of tests to determine whether test sensitivity varied with age and batch of fish. The test method was based on the Up-and-Down Procedure, originally developed for estimating the acute oral toxicity of materials to rats (ASTM, 1997). This approach was used to meet one of the guiding principles of the

Animal Care and Ethics Committee, which requires the reduction of animals used in tests. In this procedure, a single fish was exposed for 24 h to a concentration of phenol that approximated the EC50 value as estimated by the Centre for Ecotoxicology. If this fish remained balanced, then the next fish was exposed to a concentration of phenol 1.3 times higher than the previous concentration; but if the fish was imbalanced, then the next fish was exposed to a concentration 1.3 times lower than the previous concentration. Thus the exposure concentration of phenol was adjusted up or down depending upon the outcome for the previous fish. The tests were repeated until four fish had been exposed after reversal of the outcome for the initial exposure. Exposures were conducted in 250 mL beakers using a test volume of 200 mL without aeration. A control fish was used for each exposure.

The Steels Many One Rank method was used to determine significant differences between the toxicity of river water samples from Phoslock treated and reference sites and those of the diluent water controls. The EC50 values for the reference toxicant test were determined using the maximum likelihood method using the Basic Computer Programme given in ASTM-E1163-90 (1997). The EC50 value of the fish exposed to lanthanum was determined using the Trimmed Spearman-Karber method (Hamilton *et al*., 1977).

Table 1 Summary of Test Conditions for the *Ceriodaphnia dubia* Acute Toxicity Test

1	**Test type**	**Static non-renewal**
2	Test duration	48 h
3	Temperature	25 ± 1°C
4	Light quality	Ambient laboratory illumination
5	Light intensity	10-20 μmol photons s^{-1} m^{-2}
6	Photoperiod	16 h light, 8 h darkness
7	Test chamber size	30 mL
8	Test solution volume	15 mL
9	Age of test organisms	Less than 24-h old
10	No. test organisms per test chamber	5
11	No. replicate chambers per sample/control	4
12	No. organisms per sample/control	20
13	Feeding regime	Fed YCT and *Selenastrum* while holding prior to the test; newly-released young had food available a minimum of 2 h prior to use in a test
14	Test chamber aeration	None
15	Dilution water	Synthetic soft water
16	Endpoint	Immobility
17	Test acceptability	90% or greater survival in controls

2.7 Chronic Toxicity Tests

2.7.1 *Selenastrum capricornutum* growth inhibition bioassay

Stock Cultures

The freshwater green alga, *Selenastrum capricornutum* Printz, was imported from the American Type Culture Collection (ATCC 22662). This alga was renamed *Raphidocellus subcapitata,* however, the species is commonly known as *Selenastrum capricornutum* in the literature (Environment Canada, 1992). Stock algal cultures were maintained axenically in EPA medium (USEPA, 1994) at 24 ± 2° C under continuous “cool white” fluorescent light with an intensity of 4000 ± 10% lux (light quantal flux approximately 60 to 80 $\mu E/m^2.s$). Cells in exponential phase growth were used in the algal bioassays, after washing to remove culture medium.

Algal bioassay

The algal bioassay was based on the OECD Test Guideline 201(1984) and the USEPA protocol (USEPA, 1994) and is summarised in Table 2. Most tests

were screening tests carried out using undiluted riverwater (100%). Six replicates of each sample were prepared in 20 mL silanised glass scintillation vials containing 6 mL of algal culture medium (without EDTA). Six replicate controls in algal culture medium were also prepared. Of these six replicates, four were used for determining algal growth over 72 h while the remaining two replicates were used for measuring test water physicochemical parameters at the beginning and end of the test.

For some water samples, full definitive tests at a range of dilutions were carried out. Water samples were diluted with Milli-Q water and then spiked with algal nutrient medium, so that the added nutrients in each vial were the same as the controls.

Each vial was inoculated with 1-2 x 10^4 cells/mL of a *Selenastrum* suspension. Vials were incubated at 24 ± 2^o C under continuous light at 4000 ± 10% lux on an orbital shaker (100 rpm). Cell density in each treatment was determined daily for 3 days by counting cells using a Coulter Multisizer IIE particle analyser with 70 µm aperture. For unfiltered samples, cells were either counted manually in a hemacytometer using phase contrast microscopy, or counted in a Bio-Rad Bryte flow cytometer with xenon excitation at 488 nm. Non-algal particles and dead cells were excluded from the analysis by setting an acquisition threshold on chlorophyll *a* fluorescence (FL3). Data were collected and displayed in one-dimensional histograms based on cell number versus light scatter. Cell counts were obtained directly from histograms as the area under the curve. The flow cytometer was operated at a constant flow rate of 20 µL/min and a constant pressure of 0.7 Bar. Samples were accumulated for a pre-set time of 120 sec.

The pH in each test vial was determined at the beginning and end of the test.

Quality assurance

One concentration of the copper reference toxicant (8 µg/L) was included in each bioassay to ensure that each batch of algae was responding to a known toxicant in a reproducible way. The bioassay was acceptable if copper caused a significant reduction in algal growth over 72 h.

For the *Selenastrum* bioassay the test was acceptable if the final cell densities in the controls were greater than 2 x 10^5 cells/mL, with variability of less than 20%.

Statistical analyses

The bioassay endpoint, cell yield after 72 h, was calculated using the following equation:

$$I = \frac{Rc - R}{Rc} x100$$

where:

I is the percentage inhibition of algal growth for each test-concentration replicate

Rc is the mean cell yield for the control; and

R is the cell yield for each test-concentration replicate.

After testing the data for normality and homogeneity of variance using Toxcalc Version 5.0.14 (Tidepool Software), Dunnett's Multiple Comparison Test was used to determine which treatment concentrations were significantly different to the controls. For the full definitive tests, the 72-h EC50 value (the effective concentration of water sample which gave a 50% reduction in cell division rate compared to the controls) was calculated.

Table 2 Summary of Toxicity Test Conditions for the Freshwater Algal *Selenastrum capricornutum* Growth Inhibition Test

1	Test type:	Static
2	Temperature:	24 ± 2°C
3	Light quality:	"Cool white" fluorescent lighting
4	Light intensity:	4000 ± 10% lux (150 µmol photons s^{-1} m^{-2}
5	Photoperiod:	Continuous illumination
6	Test chamber size:	20 mL
7	Test solution volume:	6 mL
8	Renewal of test solutions:	None
9	Age of test organisms:	4 - 7 days (in exponential phase of growth)
10	Initial cell density:	10,000 cells/mL
11	No. replicate chambers /concentration:	3
12	Shaking rate:	100 rpm
13	Dilution water:	Algal culture medium (without EDTA)
14	pH range:	5 - 10
15	Test duration:	72 h
16	Effect measured:	Growth (cell yield) inhibition
17	Test acceptability:	At least $2x10^5$ cells/mL in the controls after 72 h, with variability in the controls not to exceed 20%.

2.7.2 *Ceriodaphnia dubia* reproduction bioassay

These tests were carried out by the Sinclair Knight Mertz Ecotoxicology Laboratory, Sydney. The method (SOP#2-2) was based on the standard method (USEPA, 1994) which determines survival and reproduction of the daphnid over 7 days (three broods) in a static renewal test. A summary of the test method is given in Table 3.

All tests were screening tests carried out using undiluted riverwater (100%). If the conductivity in the riverwater samples was above the tolerable range for *Ceriodaphnia* reproduction, samples were diluted in soft water prior to testing. Controls in synthetic soft water were also prepared. One neonate, less than 24-h old, was added to each of 10 replicates, giving a total of 10 organisms per sample. Survival and reproduction (the mean number of young per adult female) was determined over 7 days.

Quality assurance

Reference toxicant tests with potassium chloride were run concurrently with the water samples to ensure that each batch of cladocerans were healthy and responding to a known toxicant in a reproducible way. Toxicity tests were acceptable if the EC50 fell within the range of 140-264 mg/L.

For test acceptability, the survival of control organisms had to exceed 90% over 7 days, with a mean number of young per female in the controls of greater than 15.

Statistical analyses

Statistical analysis of the test data was carried out using ToxStat software. After checking the data for normality and homogeneous variance, Boneferroni t tests were used to determine whether immobilisation and inhibition of reproduction over 7 days in each sample was significantly different ($p<0.05$) to the controls.

Table 3. Summary of Test Conditions for the *Ceriodaphnia dubia* Chronic Toxicity Test

1	Test type:	Static non-renewal
2	Test duration:	Usually 7 days (until 60% of surviving control organisms have three broods)
3	Temperature:	25 ± 1°C
4	Light quality:	Ambient laboratory illumination
5	Light intensity:	10-20 µmol photons s^{-1} m^{-2} (ambient laboratory levels)
6	Photoperiod:	16 h light, 8 h darkness
7	Test chamber size:	30 mL min
8	Test solution volume	15 mL min
8	Renewal of test solution:	daily
9	Age of test organisms:	Less than 24-h old
10	No. organisms per test chamber:	1
11	No. replicate chambers per concentration:	10
13	Feeding regime:	Daily feeding with algal suspension
14	Test chamber aeration:	None
15	Control/Dilution water:	Synthetic soft water
16	Endpoint:	Survival (immobilisation) and reproduction
17	Test acceptability criterion:	90% or greater survival in controls and a mean of >15 young per surviving female in controls

2.8 Lanthanum Calibration Bioassays

In order to determine whether lanthanum released from the Phoslock was contributing to the toxicity of the Canning River samples, calibration bioassays with lanthanum chloride were carried out with each of the test species. The lanthanum chloride was supplied by CSIRO Land and Water so that the batch of lanthanum salt used in the bioassays was the same as that used to manufacture the Phoslock.

Range finding tests were carried out with each test species except fish to determine the appropriate concentration range for each of the definitive tests. For the definitive tests, five concentrations of lanthanum were used. Total and dissolved lanthanum concentrations were measured on Day 0 of each test using ICPAES so that toxicity indices could be calculated based on measured rather than nominal concentrations.

3 RESULTS

3.1 Lanthanum Calibration Bioassays

The lanthanum chloride calibration bioassays were confounded by the poor solubility of lanthanum in the test media. At concentrations above about 1 mg/L, lanthanum tended to precipitate, probably as a carbonate ($La_2(CO_3)_3$) or phosphate complex. Calculation of toxicity indices such as EC50 values was therefore of limited use when the solubility limits were exceeded.

3.1.1 Effect of lanthanum on algal growth

A summary of the effects of lanthanum on growth of *Selenastrum capricornutum* is given in Tables 4 and 5, with raw data in Appendix A.

Quality assurance

Selenastrum control cell yield over 72 h (no EDTA) was 3.8 $x10^5$ cells/mL, with coefficients of variation of <20% in controls, indicating test acceptability. The reference toxicant copper (8 µg/L) gave a 26% inhibition of algal growth over a 72-h exposure.

Toxicity test

Total lanthanum concentrations on Day 0 were close to nominal concentrations (Table 4). However, total lanthanum concentrations by Day 3 and dissolved lanthanum concentrations were much lower. The algal test medium contained 15 mg $NaHCO_3$/L and 570 µg PO_4/L. Consequently lanthanum precipitated as a carbonate or phosphate complex over the course of the bioassay, leaving little lanthanum or phosphate in solution (Table 4). Phosphate concentrations in the test medium decreased from 170 µg P/L to <10 µg P/L in lanthanum treatments of greater than 1.2 mg/L. Although measured total lanthanum concentrations on Day 0 were used to calculate toxicity results, the EC50 values should be interpreted with caution due to the losses of lanthanum over the course of the bioassay.

Lanthanum inhibited algal growth (Table 5 and Figure 1). Although dissolved lanthanum concentrations were below detection limits (<0.01 mg/L), significant growth inhibition of the alga was observed even at the lowest concentrations tested. It is likely that growth inhibition was due to phosphate limitation as the lanthanum phosphate complex precipitated. Although a 72-h EC50 of 0.45 mg total La/L (95% confidence limits (CL) of 0.1-2.4 mg/L) can be derived, it is of limited use due to the confounding effects of lanthanum phosphate/carbonate precipitation.

Selenastrum capricornutum was more sensitive to lanthanum chloride than *Chlorella vulgaris.* Den Dooren de Jong (1965) found a LOEC value of 10 mg/L for *C. vulgaris* after a 90-120 day exposure to lanthanum (as lanthanum chloride), with a NOEC of 4.1 mg/L. Lanthanum has also been shown to inhibit phototaxis by blocking calcium transport in the green alga *Chlamydomonas reinhardtii* and the blue-green alga *Phormidium uncinatum* (Nultsch, 1979; Hader, 1982).

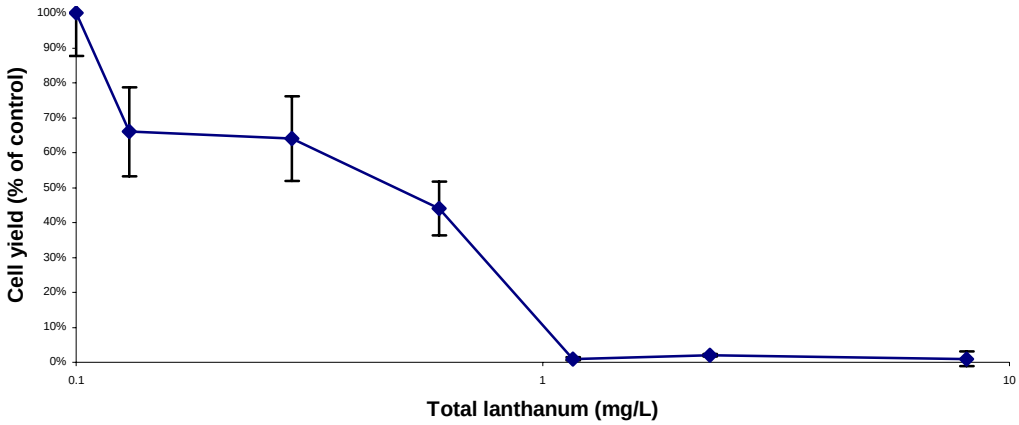


Figure 1. The Effect of Lanthanum on the Growth of *Selenastrum capricornutum* .

Table 4 Measured Lanthanum and Phosphate Concentrations Throughout the Algal Bioassay

Nominal Lanthanum (mg/L)	Measured Phosphate (µg P/L)		Measured Lanthanum (mg/L)			
	Day 0	Day 3	Day 0		Day 3	
			Unfiltered	Filtered	Unfiltered	Filtered
0	166	170	<0.01	<0.01	<0.01	<0.01
0.15	130	131	0.13	<0.01	0.06	<0.01
0.30	96	96	0.29	<0.01	0.15	<0.01
0.61	34	32	0.60	<0.01	0.48	<0.01
1.2	<10	<10	1.2	0.15	0.56	0.07
2.3	<10	<10	2.3	1	1.2	0.34
8.1	<10	<10	8.1	6.3	3.5	3.5

Table 5 The Effect of Lanthanum on the Growth of *Selenastrum capricornutum*

Total Lanthanum (mg/L)	Cell Yield (x10^4 cells/mL)	% Inhibition	CV (%)
<0.01 (Control)	38	0	12
0.13	25	33	19
0.29	24	36	19
0.60	17	56	18
1.2[a]	0.47	99	33
2.3[a]	0.93	98	16
8.1[a]	0.60	99	88

[a]Lanthanum precipitation observed

3.1.2 Acute effects of lanthanum on *Ceriodaphnia dubia*

A summary of the acute toxicity data for lanthanum to *Ceriodaphnia dubia* is given in Tables 6 and 7, with raw data in Appendix A.

Quality assurance

Control survival was 90% after a 48-h exposure to lanthanum chloride in synthetic soft water, indicating test acceptability. The reference toxicant copper gave a 48-h EC50 of 4.2 µg/L, within the normal range of 4.1 ± 2.1 µg/L, indicating the cladocerans were responding to a known reference toxicant in a reproducible way. Water quality characteristics were within acceptable limits for the test.

Toxicity test

Total measured lanthanum concentrations were similar to nominal concentrations over the 2-day test (Table 6). Concentrations of lanthanum in filtered samples were much lower and decreased to below detection limits after 2 days. The synthetic soft water contained 48 mg $NaHCO_3$/L so it is likely that the lanthanum precipitated as a lanthanum carbonate complex over the course of the bioassay.

Lanthanum toxicity was not observed at concentrations below its solubility limit in soft water. It appeared that particulate lanthanum was toxic (see Figure 2) as the 48-h EC50 i.e. the effective concentration of lanthanum to cause immobilisation in 50% of the cladocerans, was 5.0 mg/L (95% CL of 3 -8 mg/L), well above the solubility limit for lanthanum in soft water containing carbonate. There was no effect at 2.6 mg/L total lanthanum. It is possible that precipitation of carbonate (as lanthanum carbonate) may have contributed to the toxic effects observed. *Ceriodaphnia dubia* does not survive in low ionic strength water such as Milli-Q water.

Peterson et al. (1971) found that the 3-day LC50 for lanthanum chloride to *Daphnia magna* was 2.8 mg/L. *Daphnia carinata* was more sensitive, with a 48-h EC50 of 0.043 mg La/L in dechlorinated Melbourne tapwater (pH 7.5-7.8,

22 mg CaCO3/L) and 1.2 mg/L in hardwater (160 mg/L) (Barry and Meehan, 1997). Chronic NOEC values were 0.1-0.2 mg La/L, higher than acute values.

Table 6 Measured Lanthanum Concentrations Throughout the *Ceriodaphnia* Bioassay

Nominal Lanthanum (mg/L)	Measured Lanthanum (mg/L)			
	Day 0		Day 2	
	Unfiltered	Filtered	Unfiltered	Filtered
<0.01	<0.01	<0.01	<0.01	<0.01
0.3	0.29	0.16	0.30	<0.01
0.8	0.88	0.26	0.92	<0.01
2.5	2.6	0.12	2.6	<0.01
7.6	7.6	0.35	7.6	<0.01
23	23	6.1	23	0.67

Table 7 Acute Toxicity of Lanthanum to *Ceriodaphnia dubia*

Total La (mg/L)[a]	48-h Survival (% of Control)	CV (%)
<0.01 (Control)	100	11
0.29	89	24
0.88[b]	72	21
2.6[b]	72	21
7.6[bc]	56	35
23[bc]	6	42

[a] Lanthanum concentrations measured on Day 0
[b] Lanthanum precipitation observed
[c] Significantly different to the control (alpha=0.05)

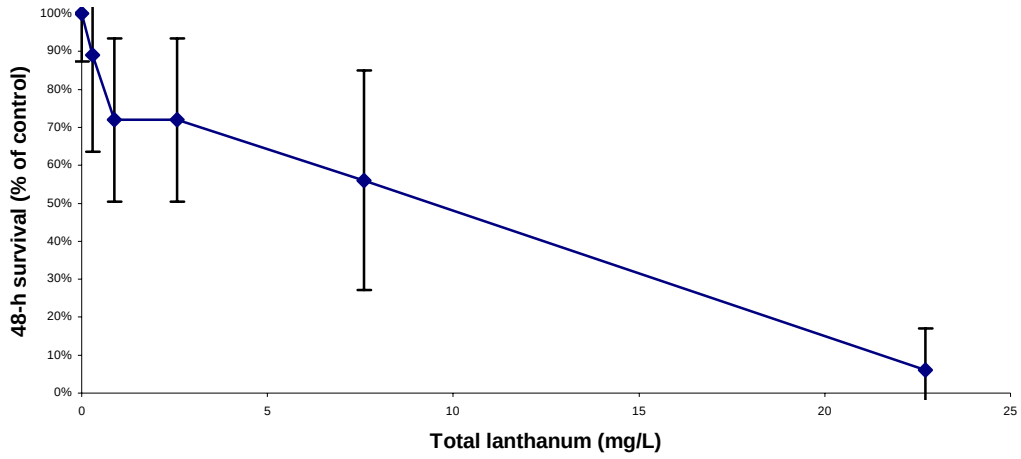


Figure 2. Acute toxicity of lanthanum to *Ceriodaphnia dubia*.

3.1.3 Chronic effects of lanthanum on *Ceriodaphnia dubia* reproduction

A summary of the chronic toxicity data for lanthanum to *Ceriodaphnia dubia* is given in Table 8, with raw data in Appendix A.

Quality assurance

Control survival over 7 days was 90%. Control reproduction was 16.5 young produced per adult, within test acceptability limits of >15 young per surviving adult. The reference toxicant potassium chloride gave a 7-day EC50 of 225 mg/L within the range of 140-264 mg/L, indicating test acceptability. All measured water quality parameters were within acceptable limits for the test.

Toxicity test

Measured lanthanum concentrations on Day 0 were close to nominal concentrations and were used to calculate toxicity indices. Lanthanum was acutely toxic to the cladocerans over the 7 day test with a 7-day EC50 of 0.51 (0.41-0.62 mg/L). Reproduction was significantly inhibited compared to controls (see Figure 3), with the LOEC at 0.09 mg La/L and no effect (NOEC) at 0.05 mg/L. The 7 day EC50 for reproduction impairment was 0.43 (0.39-0.47) mg La/L.

Table 8. Chronic Toxicity of Lanthanum to *Ceriodaphnia dubia*

Total La (mg/L)[a]	48-h Survival	Mean Young Produced	
	(% of Control)	Mean ± SD	% of Control
Control	100	16.5 ± 2.1	100
0.02	100	15.4 ± 1.4	93
0.05	90	12.9 ± 5.1	78
0.09	100	11.7 ± 1.5	71[b]
0.17	80	10.3 ± 5.5	62[b]
0.34	100	11.6 ± 1.9	70[b]
0.62	30	2.4 ± 4.1	15[b]

[a] Lanthanum concentrations measured on Day 0
[b] Significantly different to the control ($p<0.05$)

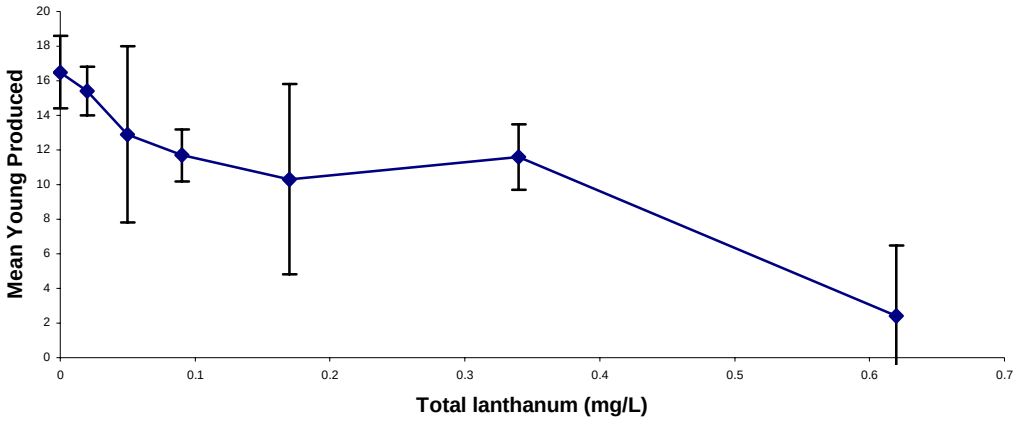


Figure 3. Chronic toxicity of lanthanum to *Ceriodaphnia dubia*.

3.1.4 Acute effects of lanthanum on rainbow fish

Control survival over 96 h was 90%, indicating test acceptability. All water quality parameters were within test acceptability limits. Temperature ranged between 23.0 and 24.5^{o}C. Conductivity and pH ranged between 997 and 1064µS/cm and 6.50 and 8.10, respectively. Dissolved oxygen saturation was well above 60%.

Measured concentrations of total lanthanum were 75-100% of nominal concentrations on Day 0. Lanthanum concentrations in filtered samples were similar to unfiltered samples at concentrations up to 1.2 mg La/L (Table 9). At higher concentrations, dissolved lanthanum was 32-68% of the total lanthanum. Lanthanum was toxic to fish at all concentrations tested. Complete imbalance of fish was observed at all total lanthanum concentrations above 0.6 mg/L. It was not possible to repeat the test at lower lanthanum concentrations as no additional rainbow fish were available.

Toxicity of lanthanum chloride to freshwater fish has been reported to range from 2 mg/L for Coho salmon (1-day exposure) to 60 mg/L for a 7-day exposure to goldfish. Rainbow trout were more sensitive, with an LC50 of 0.02 mg/L for a 28-day exposure (Birge, 1978; Peterson et al., 1974).

Table 9 Acute Toxicity of Lanthanum to Rainbow Fish

Nominal Total Lanthanum	Measured Lanthanum (Day 0) (mg La /L)		% Mortality after 96 h
(mg La/L)	Unfiltered	Filtered	
0 (Control)	<0.01	<0.01	10
0.69	0.6	0.6	100
1.4	1.2	1.1	100
2.8	2.4	1.3	100
5.5	4.1	1.6	100
11	9.7	3.1	100
22	19	9.2	100
44	40	27	100

3.1.5 Summary of lanthanum toxicity to test species

A summary of the effects of lanthanum on each of the test species is given in Table 11. The rainbow fish *Melanotaenia duboulayi* was the most sensitive to lanthanum chloride, followed by the cladoceran and the alga, which showed similar sensitivity to lanthanum in the chronic bioassays. Lanthanum was not acutely toxic to the cladoceran after short term exposures (48 h) at concentrations below its solubility limit, but was toxic after longer exposure times (7 days).

Table 10 Effect of Lanthanum on Each of the Test Species

Test Species	Test Endpoint	EC50 (mg La/L)	LOEC (mg/L)	NOEC (mg/L)
Selenastrum capricornutum	72-h Growth Inhibition	0.45[a]	0.13	<0.13
Ceriodaphnia dubia	48-h Immobilisation	5.0[b]	-	2.6[a]
	7-day Immobilisation	0.51	-	-
	7-day Reproduction inhibition	0.43	0.09	0.05
Melanotaenia duboulayi	96-h Immobilisation	<0.6	<0.6	<0.6

[a] Lanthanum toxicity partly due to phosphate limitation in the test
[b] Toxicity only observed at concentrations exceeding lanthanum solubility limits

3.2 First Phoslock Application (January, 2000)

A summary of the analytical and toxicity testing results for each sample at each site is given in Appendix B and Table 11.

3.2.1 Algal growth inhibition bioassays

Quality assurance

Selenastrum control cell yields over 72 h (no EDTA) were 3 ± 1.3 x 10^5 cells/mL, with coefficients of variation of <20% in controls, indicating test acceptability. The reference toxicant copper (8 µg/L) gave a mean inhibition of algal growth of 40 ± 28% over a 72-h exposure.

Pre-application

Filtered surface water samples from each of the four sites were tested for chronic toxicity to the alga six weeks, three weeks, one week and one day before Phoslock application. In addition, samples from two drains Liege St and Cockram St were also tested on one occasion.

No inhibition of algal growth was observed at any of the four river sites on any of the four sampling occasions. Algal growth at all sites was significantly stimulated compared to media controls (79-163% stimulation).

Water from Cockram St drain (sampled once only) inhibited algal growth by 44% compared to media controls. Liege St drain water was not toxic, significantly stimulating algal growth (62% stimulation). Algal growth stimulation in the water samples (compared to media controls) was probably due to additional nutrients and trace elements that are not included in the basic algal growth medium used for the controls.

Post Phoslock application

WHA and C2: At both these control sites, neither filtered nor unfiltered samples inhibited algal growth. Significant stimulation of algal growth was observed. Lanthanum concentrations were less than 0.2 mg/L at the upstream site C2. Some wash-through of lanthanum from sites P3 and PO3 to control site WHA was found, with concentrations of lanthanum in unfiltered samples of up to 3 mg/L at site WHA, one day after Phoslock application to site PO3. Total lanthanum concentrations, and occasionally dissolved lanthanum concentrations, exceeded the algal LOEC of 0.13 mg La/L, and yet no inhibition of algal growth was detectable at WHA.

Table 11 Summary of Ecotoxicity Testing of Canning River Samples Prior to and After First Phoslock Application (January, 2000).

	Control Site C2			Site P3			Site PO3			Control Site WHA		
	Alga	**Clad-Acute**	**Clad-Chronic**	**Alga**	**Clad-Acute**	**Clad-Chronic**	**Alga**	**Clad-Acute**	**Clad-Chronic**	**Alga**	**Clad-Acute**	**Clad-Chronic**
Pre-Application												
- 6 weeks	NT[b]	NT	-	NT	NT	-	NT	NT	-	NT	NT	-
-3 weeks	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
-1 week	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
-1 day	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT
Post Application[a]												
+1 day	NT	NT	NT	TOX^{USBc}	TOX^{SB}	TOX^{SB}	NT	TOX^{S}	TOX^{S}	NT	TOX^{S}	TOX^{S}
+1 week	NT	NT	-	NT	NT	-	NT	TOX^{SBd}	TOX^{SBd}	NT	NT	TOX^{SBd}
+2 weeks	NT	NT	-	NT	NT	-	NT	NT	-	NT	NT	-
+3 weeks	NT	NT	-	NT	NT	-	NT	NT	-	NT	NT	-
+4 weeks	NT	NT	-	NT	NT	-	NT	NT	-	NT	NT	-

[a] Refers to application of Phoslock to site P3
[b] NT Not toxic
[c] TOX- toxic
[d] Note that Phoslock application to site PO3 was one week later than site P3, so this result refers to 1 day after Phoslock application to site PO3
[U] Unfiltered only (Filtered samples were not toxic)
[S] surface water
[B] bottom water

PO3: No effect of Phoslock was found at site PO3 after Phoslock application, despite total lanthanum concentrations of up to 4 mg/L and dissolved concentrations of up to 1 mg La/L. Again, growth was stimulated in all samples at site PO3.

P3: Filtered samples from site P3 did not inhibit algal growth. However, unfiltered samples collected one day after Phoslock application reduced algal growth by 61% and 52% in surface and bottom waters respectively. Lanthanum concentrations in these unfiltered samples were 11-15 mg/L, well above the algal LOEC of 0.13 mg La/L. It is also possible that growth inhibition was due to removal of phosphate from solution. In the unfiltered surface water, the phosphate concentration was <0.05 mg/L, however, in the bottom water, the phosphate concentration was 0.06 mg/L which was similar to other samples showing no inhibition, suggesting that phosphate was not limiting algal growth. Whatever the cause of the growth inhibition one day after Phoslock application, toxicity did not persist. Samples taken one week post application (unfiltered) did not inhibit algal growth and in fact significantly stimulated algal growth. These samples contained less than 1 mg La/L and 0.04 mg phosphate/L.

Drains: Samples were also collected from Liege St drain (control) and Cockram St drain (after Phoslock application). The filtered Leige St drain sample stimulated algal growth by 66%. Application of Phoslock did not reduce the previously observed growth inhibition in the Cockram St drain, with 75% inhibition compared to 44% inhibition prior to Phoslock application. It is difficult to compare two sampling events because the water quality in the drain would be highly dependent on flows. The unfiltered Cockram St drain water contained 10 mg La/L, while the filtered sample used for the toxicity test contained only 0.81 mg La/L. It is likely that toxicity of the drain water was due to other compounds e.g concentrations of dissolved copper and zinc were 23 and 104 µg/L respectively.

Summary

Filtered Canning River samples were not toxic to algal growth prior to or after application of Phoslock. Growth inhibition was only detected one day after Phoslock application in unfiltered surface and bottom samples from site P3. No inhibition was detectable one week later or at any other site.

3.2.2 Acute *Ceriodaphnia* bioassays

A summary of the acute toxicity data for *Ceriodaphnia dubia*, pre- and post-Phoslock application is given in Appendix C.

Quality assurance

In all tests, survival in conductivity-matched controls was greater than 90% indicating test acceptability. The reference toxicant copper gave a mean 48-h EC50 of 4.5 ± 1.5 µg/L, within the normal range of 4.1 ± 2.1 µg/L, indicating the cladocerans were responding to a known reference toxicant in a reproducible way. Water quality characteristics were within acceptable limits for each test.

Pre-application

Unfiltered surface water samples from each of the four sites were tested for acute toxicity to the cladoceran six weeks, three weeks, one week and one day before Phoslock application. In addition, samples from two drains Liege St and Cockram St were also tested on one occasion.

No toxicity to *Ceriodaphnia* survival was observed at any of the four river sites on any of the four sampling occasions. Neither Cockram St drain nor Liege St drain (sampled once only) had any effect on *Ceriodaphnia* survival.

Post Phoslock application

Some bottom water samples collected from both control and Phoslock sites were toxic due to the high conductivity of the samples. These samples were diluted to bring the conductivity below 2000 µS and within the tolerable range for this freshwater cladoceran. Controls matched to the same conductivity were also included in each experiment.

WHA: At control site WHA, toxicity was detected in surface waters only, one day after Phoslock application to site P3 further upstream. *Ceriodaphnia* survival over 48 h was only 18% of the conductivity-matched control. Toxicity may be due to wash through from the upstream site, however the total lanthanum concentration in unfiltered WHA water was only 1.6 mg/L, below the NOEC (2.6 mg/L) for lanthanum derived from the calibration bioassays. The following week (1 day after Phoslock application to site PO3) no significant toxicity was detected in the surface or bottom waters at site WHA, with all lanthanum concentrations below the no effect value for the cladoceran. No toxicity was detectable one, two or three weeks after Phoslock application.

PO3: At site PO3, toxicity was detected in surface waters only, one day after Phoslock application to site P3 further upstream. *Ceriodaphnia* survival over 48 h was only 29% of the conductivity-matched control. Similar to site WHA, toxicity was probably due to wash through of Phoslock from upstream, as the lanthanum concentration in the surface PO3 water (3.1 mg/L) exceeded the NOEC of 2.6 mg La/L. No toxicity was detectable in the bottom waters after a 1:4 dilution to reduce conductivity. Toxicity was not always predictable from the measured lanthanum concentrations. One day after Phoslock application to site PO3, acute toxicity was detectable, with 50% survival in undiluted surface water (1.64 mg La/L) and 47% survival in bottom waters diluted 1:4 (0.41 mg La/L). To determine if toxicity was associated with the particles in the unfiltered sample, the toxicity of the filtered surface water was also tested. *Ceriodaphnia* survival was 56% of the control, similar to the unfiltered sample, suggesting that toxicity was due to the dissolved/colloidal component. No toxicity was detectable one, two or three weeks after Phoslock application.

P3: At site P3, *Ceriodaphnia* survival was reduced (0-12% of the controls) in both surface and bottom waters sampled one day after Phoslock application. Total lanthanum concentrations were 11-15 mg/L, well above the no effect level of 2.6 mg/L derived from the cladoceran calibration bioassays. Filtered surface water was less toxic but still reduced *Ceriodaphnia* survival over 48 h (69% of control). No toxicity was detectable one, two, three or four weeks after Phoslock application.

C2: At control site C2, no toxicity was detectable at any sampling time. Total lanthanum concentrations were always less than 0.2 mg/L.

Liege St drain (control) was not toxic, however Cockram St drain after Phoslock application was acutely toxic with survival of 44% of the control (10 mg La/L).

Summary

Phoslock was acutely toxic to *Ceriodaphnia* one day after application. No toxicity was detectable after one or more weeks, post application. Some toxicity was associated with particulates, however in another sample, toxicity was due to the dissolved/colloidal fraction. The observed toxicity may be due partly to lanthanum as there was a general trend of increasing toxicity with increasing dissolved lanthanum concentrations (Figure 4a). This correlation was highly significant ($p<0.05$) with $r^2= 0.65$. With two exceptions, acute toxicity to the cladocerans was never observed in Canning River samples containing less than 2.6 mg La/L, the cladoceran NOEC. Concentrations of lanthanum in river samples above this NOEC were always toxic, with one exception at site PO3.

3.2.3 Chronic *Ceriodaphnia* bioassays

A summary of the chronic toxicity data for *Ceriodaphnia dubia*, pre- and post-Phoslock application is given in Appendix D.

Quality assurance

Control survival over 7 days was always greater than 90%, with greater than 15 young produced per female in controls. The 7-day EC50 for the reference toxicant potassium chloride was always within the normal range of 140-264 mg/L, indicating test acceptability. Water quality parameters including pH, dissolved oxygen and temperature were within acceptable limits for all tests.

Pre-application

Unfiltered surface water samples from each of the four sites were tested for chronic toxicity to the cladoceran three weeks, one week and one day before Phoslock application. No toxic effect on *Ceriodaphnia* survival or reproduction over three broods (7 days) was observed at any of the four river sites on any of the three sampling occasions. The mean number of young produced was significantly higher in most river water samples compared to controls.

Post Phoslock application

Surface and bottom samples from sites WHA, PO3, C2 and P3 were tested one day after Phoslock application to site P3. Samples from WHA, PO3 and C2 were tested one week later, one day after application of Phoslock to site PO3.

WHA: At site WHA**,** *Ceriodaphnia* survival and mean number of young produced per female were significantly lower than controls in surface water collected one day after Phoslock application to site P3. This is in agreement with the acute *Ceriodaphnia* tests over 48 h and due to wash through of the Phoslock from upstream sites. The bottom waters were too saline and had to be diluted 1:10, and no toxicity was detectable at this dilution. One day after application of Phoslock at site PO3, *Ceriodaphnia* survival and reproduction were affected. The bottom waters were toxic, reducing the number of young produced to <1 per female even after dilution to 20% (1:5). In agreement with the lanthanum chronic calibration bioassay, total or dissolved lanthanum concentrations in the water samples of greater than 0.09 mg/L (the cladoceran LOEC) were toxic. The higher chloride concentrations in the bottom waters may lead to the formation of soluble LaCl complexes, which have previously been shown to be more toxic to aquatic organisms.

PO3: At site PO3**,** surface water collected one day after Phoslock application to site P3 upstream, killed all the test cladocerans over 7 days (no young produced). Bottom water diluted 1:10 was not toxic. Both undiluted surface water and bottom water diluted 1:10,

sampled one day after Phoslock application to the site PO3, significantly reduced *Ceriodaphnia* survival and reproduction. Lanthanum concentrations in these samples were above the cladoceran LOEC value predicted from the lanthanum calibration bioassay.

P3: At site P3, one day after Phoslock application, surface and bottom waters were toxic, with no *Ceriodaphnia* surviving over 7 days. Total and dissolved lanthanum concentrations in these samples were above the cladoceran LOEC value predicted from the lanthanum calibration bioassay.

C2: At site C2, no chronic toxicity to *Ceriodaphnia* was ever detected. A significant increase in the mean number of young produced compared to controls was found in three of four samples tested. Although total lanthanum concentrations exceeded the cladoceran LOEC on two occasions, the dissolved lanthanum concentrations were below the LOEC and no toxicity was detectable.

Summary

Phoslock caused chronic toxicity to *Ceriodaphnia dubia* in samples collected one day after application. Both survival over 7 days and mean number of young produced per female were significantly reduced. Saline bottom waters may have contributed to lanthanum release from Phoslock during settling. Although chronic toxicity was predictable from a comparison of the lanthanum concentrations in the water samples with the concentrations known to cause an effect from the lanthanum calibration bioassay, the magnitude of the toxic effect was not predictable. However, there was a highly significant correlation ($p<0.05$) between dissolved lanthanum concentrations and toxicity to *Ceriodaphnia* reproduction (Figure 4b).

3.2.4 Juvenile rainbow fish imbalance bioassay

A summary of the acute toxicity tests with juvenile rainbow fish are given in Appendix E.

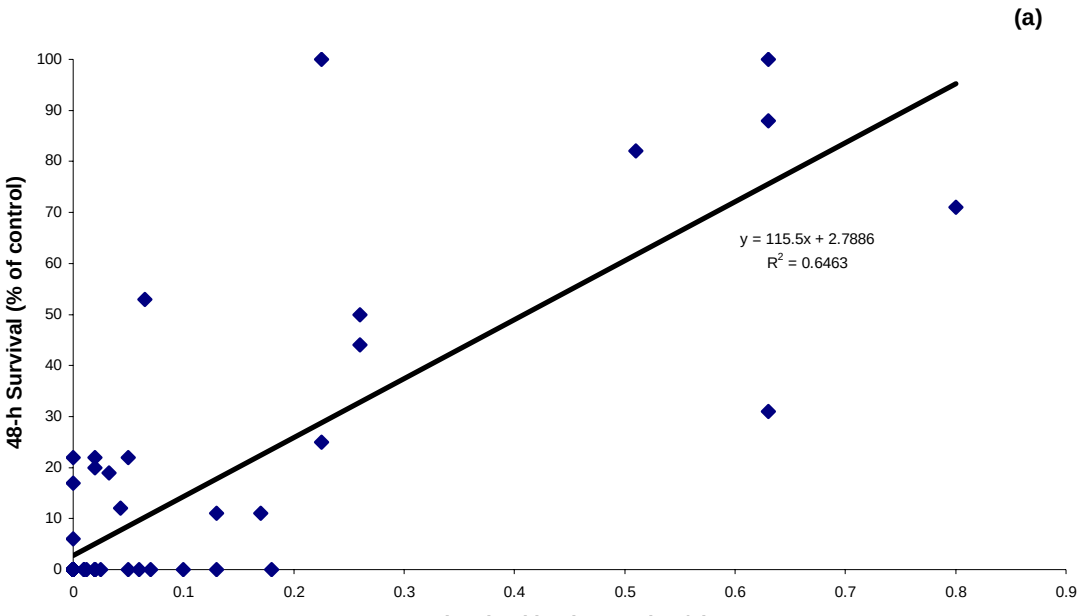
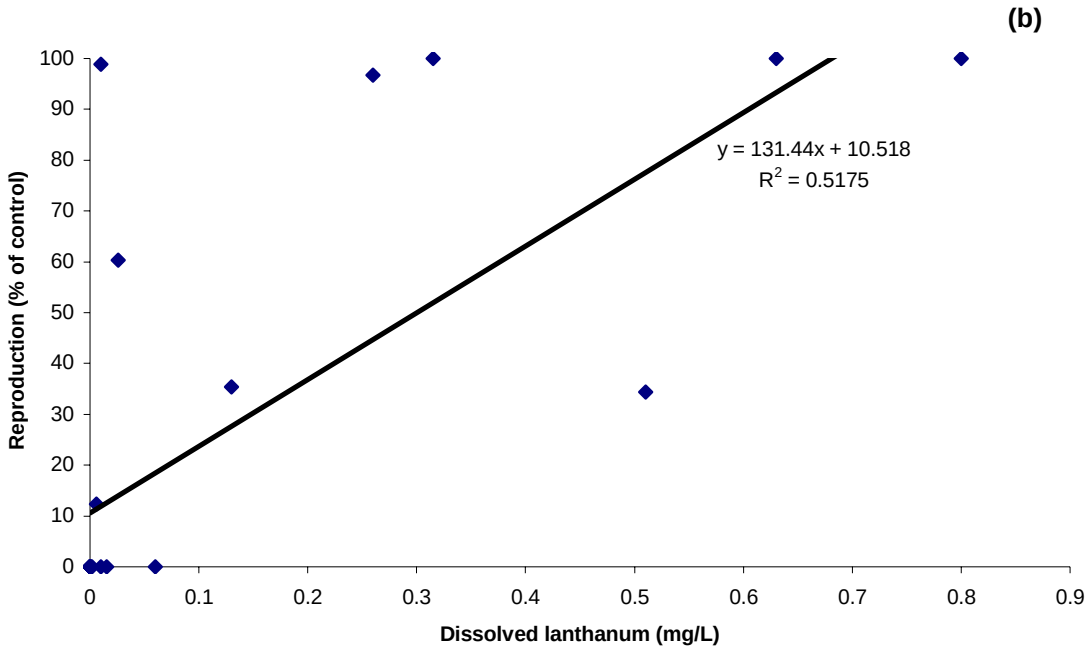


Figure 4. Correlations for dissolved lanthanum found in Canning River samples with (a) acute *Ceriodaphnia dubia* toxicity and (b) chronic *Ceriodaphnia dubia* toxicity.



Quality assurance

Immobilisation of fish over 96 h in controls was always less than 10% indicating test acceptability. Temperature of the sample solutions ranged between 22.1 to 24.9^{o}C. Conductivity of the sample solutions ranged between 465 and 11,470µS/cm. Overall, the pH values for the sample solutions ranged between 6.76 and 8.53. The percentage saturation of dissolved oxygen was maintained well above 60% in all sample solutions, which met the requirements recommended for fish toxicity tests (OECD 1987) except for the sample from Cochram Drain, which was 56% saturation. This, however, increased to 94% with gentle aeration over the 96-hour test period.

Limited testing with the reference toxicant phenol (due to animal ethics constraints) showed that the EC50 values were similar for different batches of fish. The EC50 values for phenol ranged from 25-57 mg/L.

Pre-application

No toxicity to juvenile rainbow fish was observed in any Canning River sample prior to Phoslock application.

Post Phoslock application

No toxicity to juvenile rainbow fish was observed in any Canning River sample after application of Phoslock. Although the calibration bioassay with lanthanum alone suggested that fish immobilisation would occur at concentrations of lanthanum of less than 0.6 mg/L, no toxicity was observed in Canning River samples containing up to 15 mg total La/L. Clearly, other factors in the river water, such as humic substances, were ameliorating the potential toxic effect. Lanthanum is known to strongly bind to humic substances (Clark and Chopin, 1996; Nanny and Minear, 1994).

3.2.5 Summary of first Phoslock application results

- Canning River samples prior to Phoslock application were not toxic to any of the test species.
- Samples from Cockram St drain were toxic to algal growth pre- and post-application, and were toxic to *Ceriodaphnia*, post-application only.
- Phoslock application did not cause any acute toxicity to juvenile rainbow fish.
- Toxicity to algal growth and cladoceran survival and reproduction was only detected within 24 h of Phoslock application, with no toxicity detectable after one week or more.
- The cladoceran was more sensitive to Phoslock than the freshwater alga. Only unfiltered samples were toxic to the alga, 24 h after application and this toxicity was not solely due to phosphate limitation. Toxicity to the cladoceran was not solely due to the presence of particles, as filtered samples were also toxic. There was a weak correlation between dissolved lanthanum concentrations and acute toxicity to *Ceriodaphnia.*

- For the alga and the cladoceran, the absence or presence of toxicity in the Canning River samples was usually predictable from the measured total and dissolved lanthanum concentrations based on NOEC and LOEC values derived from the calibration bioassays with lanthanum alone. However, the magnitude of the toxic effect was not predictable on the basis of lanthanum concentration alone. Rainbow fish were much less sensitive to lanthanum in the Canning River samples than to lanthanum alone and toxicity was not predictable from measured lanthanum concentrations in the river waters.

- Oxygenation had little effect on Phoslock toxicity

- Barriers in the Canning River were ineffective in preventing wash-through of Phoslock downstream and in preventing salinity intrusions upstream.

- Bottom waters were quite saline and had to be diluted to remove salinity as the cause of toxicity. The higher ionic strength may have caused the release of lanthanum from the modified clay and resulted in Phoslock toxicity in bottom waters.

3.3 Second Phoslock Application (April, 2000)

A summary of the analytical and toxicity testing results for each sample at each site is given in Appendix F and Table 12.

3.3.1 Algal growth inhibition bioassays

Quality assurance

Selenastrum control cell yields over 72 h (no EDTA) were 6 ± 3.4 x 10^5 cells/mL, indicating test acceptability. However, for two tests, the coefficients of variation in the controls were >20%, which did not meet test acceptability criteria. These tests were repeated so that test acceptability criteria were met for either filtered or unfiltered samples. The reference toxicant copper (8 µg/L) gave a mean inhibition of algal growth of 41 ± 10% over a 72-h exposure.

Pre-application

Algal cell growth in unfiltered samples was variable due to the presence and growth of natural algae in the water samples, which sometimes interfered with the flow cytometer counts of *Selenastrum capricornutum*. For this reason all samples were also filtered through a 0.45 µm membrane filter and re-tested for toxicity. No toxicity of either filtered or unfiltered samples was found at site C2 (control) or site P3, prior to Phoslock application. Significant stimulation of algal growth was found in filtered bottom waters from site C2 and in filtered surface waters from site P3.

Post Phoslock application

Although total lanthanum concentrations exceeded the algal LOEC of 0.13 mg/L on the day of application, no toxicity to algal growth was observed at site C2 or P3 after application of Phoslock (Day 0, 1, 3 or 7). Slightly reduced growth (22% inhibition) was found in unfiltered surface water only at the control site (C2) on one occasion but this was not related to Phoslock application. After filtering the sample, no growth inhibition was detectable. Algal growth in both

filtered and unfiltered samples was often stimulated compared to controls (65-450%) at both C2 and P3.

3.3.2 Acute *Ceriodaphnia* bioassays

A summary of the acute toxicity data for *Ceriodaphnia dubia*, pre- and post-Phoslock application is given in Appendix G.

Quality assurance

In all tests, survival in conductivity-matched controls was greater than 90% indicating test acceptability. The reference toxicant copper gave a mean 48-h EC50 within the normal range of 4.1 ± 2.1 µg/L, indicating the cladocerans were responding to a known reference toxicant in a reproducible way. Water quality characteristics were within acceptable limits for each test.

Pre-application

No acute toxicity to cladocerans was detected in unfiltered surface or bottom waters from Site C2 or P3 prior to Phoslock application.

Post Phoslock application

No acute toxicity to cladocerans was detected in unfiltered surface or bottom waters from Site C2 or P3, 0-7 days after Phoslock application. Total and dissolved lanthanum concentrations at C2 were always below detection limits (<0.01 mg/L). At site P3, total lanthanum concentrations ranged from 0.02 mg/L prior to Phoslock application to 1.7 mg/L on the day of application. This is below the NOEC for lanthanum of 2.6 mg/L and therefore no toxicity was detectable. One day later, lanthanum concentrations were down to background levels (<0.01 mg/L).

Table 12 Summary of Ecotoxicity Testing of Canning River Samples Prior to and After Second Phoslock Application (April, 2000).

Day	Site C2				Site P3			
	Alga	Acute-Clad	Chronic - Clad	Fish	Alga	Acute-Clad	Chronic - Clad	Fish
Pre-Application								
-1 day	NT	NT	NT	NT	NT	NT	NT	NT
Post Application[a]								
0 day	NT	NT	NT	NT	NT	NT	NT	NT
+1 day	NT	NT	NT	NT	NT	NT	NT	NT
+3 days	NT	NT	NT	NT	NT	NT	NT	NT
+7 days	TOX^{US}	NT	NT	NT	NT	NT	NT	NT

[a] *NT Not toxic*
[b] *TOX- toxic*
[U] *Unfiltered*
[S] *surface water*

3.3.3 Chronic *Ceriodaphnia* bioassays

A summary of the chronic toxicity data for *Ceriodaphnia dubia*, pre- and post-Phoslock application is given in Appendix H.

Quality assurance

Control survival over 7 days was always 100%, with greater than 15 young produced per female in controls. Mean young produced in each experiment was 19.3 ± 7.9, 19.1 ± 4.7 and 17.5 ± 5.7, indicating test acceptability. The 7-day EC50 for the reference toxicant potassium chloride was always within the normal range of 140-264 mg/L, indicating test acceptability (Appendix E). Water quality parameters including pH, dissolved oxygen and temperature were within acceptable limits for all tests.

Pre-application

No chronic toxicity to cladocerans was detected in unfiltered surface or bottom waters from Site C2 or P3 prior to Phoslock application.

Post Phoslock application

No chronic toxicity to cladoceran reproduction was detectable in surface or bottom waters at sites C2 or P3, 0, 1, 3 or 7 days after Phoslock application. Lanthanum concentrations did exceed the LOEC of 0.09 mg/L for chronic toxicity, however, the natural waters ameliorated potential lanthanum toxicity. Mean young produced ranged from 81-149% of controls.

3.3.4 Juvenile rainbow fish imbalance bioassay

A summary of the acute toxicity tests with juvenile rainbow fish are given in Appendix I.

Quality assurance

Immobilisation of fish over 96 h in controls was always less than 10% indicating test acceptability. The age of the fish used for testing ranged from 3-6 weeks. Limited testing with the reference toxicant phenol (due to animal ethics constraints) showed that the EC50 values were similar for different batches of fish (36-52 mg/L).

Pre-application

No toxicity to juvenile rainbow fish was observed in any Canning River sample prior to Phoslock application, with 100% survival in all samples.

Post Phoslock application

No toxicity to juvenile rainbow fish was observed in any Canning River sample after application of Phoslock. Although the calibration bioassay with lanthanum alone suggested that fish immobilisation would occur at concentrations of lanthanum of less than 0.6 mg/L, no toxicity was observed in Canning River

samples containing up to 1.7 mg total La/L. Clearly, other factors in the river water were ameliorating the potential toxic effect.

3.3.5 Summary

Modifications to Phoslock to reduce lanthanum released into surface and bottom waters were successful in removing toxicity. No acute toxicity to juvenile rainbow fish or cladoceran immobilisation was detectable prior to or up to 7 days after Phoslock application. Similarly, no chronic toxicity to algal growth or cladoceran reproduction was found. Although lanthanum concentrations in surface and bottom waters sometimes exceeded the LOEC values derived from lanthanum calibration bioassays, toxicity was never observed in the Canning River waters. It is possible that humic substances in the river waters ameliorated any potential toxicity from lanthanum.

4 CONCLUSIONS

The use of modified Phoslock, where lanthanum release is minimised, should pose minimal risk of acute or chronic toxicity to freshwater organisms.

In the laboratory calibration bioassays, lanthanum (added as lanthanum chloride) was toxic to each of the test species. Rainbow fish immobilisation, algal growth inhibition and cladoceran reproduction inhibition showed similar sensitivity to lanthanum, with EC50 values (i.e. the effective concentration to cause a 50% effect) of less than 0.6 mg La/L. The chronic lowest observable effect concentrations (LOEC) were 0.13 and 0.09 mg La/L for the alga and cladoceran respectively.

In the first field trial in January, 2000, Canning River samples prior to Phoslock application were not toxic to any of the test species. After application of Phoslock, substantial amounts of lanthanum were released from the Phoslock into surface and bottom waters. Total lanthanum concentrations of up to 15 mg/L were detectable in bottom waters one day after Phoslock application. Toxicity to the cladoceran and algal growth inhibition were detected within 24 h of Phoslock application, with no toxicity detectable after one week or more. Phoslock was not toxic to juvenile rainbow fish.

The cladoceran was more sensitive to Phoslock than the freshwater alga. Only unfiltered samples caused growth inhibition to the alga, 24 h after application, and this inhibition was not solely due to phosphate limitation. Toxicity to the cladoceran was not only due to the presence of particles, as filtered samples were also toxic. There was a significant correlation between dissolved lanthanum concentrations and acute toxicity to *Ceriodaphnia*.

For the alga and the cladoceran, the absence or presence of toxicity in the Canning River samples was usually predictable from the measured total and dissolved lanthanum concentrations based on NOEC and LOEC values derived from the calibration bioassays with lanthanum alone. However, the magnitude of the effect was not predictable on the basis of lanthanum concentration alone. Rainbow fish were much less sensitive to lanthanum in the Canning River samples than to lanthanum alone and toxicity was not predictable from measured lanthanum concentrations in the river waters. Lanthanum is known to strongly bind to humic substances so it is possible that humic substances in the river waters ameliorated the toxicity of lanthanum.

Barriers in the Canning River were ineffective in preventing wash-through of Phoslock downstream and in preventing salinity intrusions upstream. Bottom waters were quite saline and had to be diluted to reduce salinity so that it did not cause toxicity. The higher ionic strength may have caused the release of lanthanum from the modified clay and resulted in Phoslock toxicity in bottom waters.

Oxygenation had no effect on Phoslock toxicity.

After modifications to the Phoslock to reduce the lanthanum released in surface and bottom waters, a second trial was conducted in April, 2000. Total lanthanum concentrations in the river waters were substantially reduced, with a maximum concentration of 1.7 mg/L on the day of application. Although the

lanthanum concentrations still exceeded the lowest observable effect concentrations for acute toxicity to the rainbow fish and chronic toxicity to the alga and cladoceran, no toxicity in either surface or bottom waters was detectable to any of the test species for up to 7 days after Phoslock application. Potential lanthanum toxicity was ameliorated completely by the river water.

[redacted]

5 FUTURE WORK

- Although no acute or chronic toxicity to freshwater aquatic species was observed using the modified Phoslock, potential physical effects on sediment-dwelling biota have not been investigated. Sediment toxicity tests using whole sediment or porewaters may be designed to help assess these effects.

- Potential physical effects such as gill irritation of fish may be determined by sprinkling Phoslock over the water surface of tanks containing fish.

- Lanthanum insolubility due to the formation of carbonate and phosphate complexes in the test media partly confounded the results of the lanthanum laboratory calibration bioassays. The fish bioassay should be repeated at lower lanthanum concentrations to enable determination of toxicity indices.

- Amelioration of lanthanum toxicity by humic substances could be investigated further by spiking lanthanum into waters containing varying dissolved organic carbon concentrations.

6 ACKNOWLEDGMENTS

The authors would like to thank Jackie Levy, Mathew Tiltman and Kazuyo Natoyama for carrying out the algal bioassays, Richard Lim and Chris Doyle from the University of Technology, Sydney for the fish bioassays and Rick Krassoi from Sinclair Knight-Mertz for the chronic cladoceran bioassays.

7 REFERENCES

APHA (1989) Standard methods for the examination of water and wastewater. 17th edition, American Public Health Association.

ASTM (1997) Standard test method for estimating acute oral toxicity in rats. Annual Book of ASTM Standards.

Barry, M.J. and Meehan, B. (1997) Toxicity of the rare earth element lanthanum to *Daphnia*. Poster P3 at the 4th Annual Conference for the Australian Society for Ecotoxicology, Brisbane, July, 1997.

Birge, W.J., Black, J.A. and Westerman, A.G. (1979) Evaluation of aquatic pollutants using fish and amphibian eggs as bioassay organisms. In: S.W. Nielsen, G. Migaki and D.G. Scarpelli (eds) Symp. Animals Monitors Environ. Pollut. 1977, Storrs, C.T. 12, 108-118.

CET (1997) Laboratory procedures manual. Centre for Ecotoxicology, NSW EPA/UTS, Sydney, Australia.

Clark, S.B. and Choppin, G.R. (1996). A comparison of dissociation kinetics of rare earth element complexes with synthetic polyelectrolytes and humic acid. In: Humic and Fulvic Acids, Isolation Structure and Environmental Role. ACS Symposium Series 651, Gaffney, J.S, Marley, M.A and Clark, S.B (eds) Chapter 13, pp 207-219.

Das, T., Sharma, A. and Talukder, G. (1988) Effects of lanthanum in cellular systems. A review. Biol. Trace Element Res. 18, 201-228.

Den Dooran de Jong, J.E. (1965) Tolerance of *Chlorella vulgaris* to metallic and non-metallic ions. Antonie Leeuwenhoek J. Microbiol. Serol 31, 301-313.

Douglas, G.B., Adeney, J. A. and Zappia, L.R. (2000). Sediment remediation project: Laboratory trials 1998/9. Confidential report prepared for Water and Rivers Commission. *CSIRO Land and Water* Report 6/00, 92pp.

Environment Canada (1992) Biological test method: growth inhibition test using the freshwater alga *Selenastrum capricornutum*. Environment Canada Environmental Protection Series Report EPS 1/RM/25, 41 pp.

Hader, D.-P. (1982) Gated fluxes involved in photophobic responses of the blue-green alga *Phormidium uncinatum*. Arch. Microbiol. 131, 77-80.

Hamilton, M.A., R.C. Russo, and R.V. Thurston (1977). Trimmed Spearman-Karber methods for estimating median lethal concentrations in toxicity bioassays. Environmental Science and Technology 11:714-719. Correction: Ibid 1978 12:417.

Lim, R.P. (1999) Toxicity of modified clay leachate to juveniles of the eastern rainbow fish *Melanotaenia duboulayi.* Insearch Ltd Report, Project No. E99/62/004, 5 pp.

Nultsch, W. (1979) Effect of external factors on phototaxis of *Chlamydomonas reinhardtii*. III Cations. Arch. Microbiol. 123, 93-99.

OECD (1984) Guideline for testing of chemicals. Alga, growth inhibition test. Test Guideline No. 201. Organisation for Economic Cooperation and Development, Paris, France.

OECD (1987) Guideline for testing of chemicals. Guideline No. 203. Organisation for Economic Cooperation and Development. Paris, France.

Nanny, M.A. and Minear, R.A. (1994). Use of lanthanide shift reagents with 31P NMR spectroscopy to analyse concentrated lake water samples. Environ. Sci. Technol. 28, 1521-1527.

Peterson, S.A., Sanville, W.D., Stay, F.S. and Powers, C.F. (1974) Nutrient inactivation as a lake restoration procedure. Laboratory investigations US EPA Report EPA-660/3-74-032, Corvallis, Oregon, 118 pp.

Stauber, J.L (1999) Further toxicity testing of modified clay leachates using freshwater and marine organisms. CSIRO Energy Technology Report CET/IR202R, 22 pp.

USEPA (1993) Methods for measuring the acute toxicity of effluents and receiving waters to freshwater and marine organisms. Fourth Edition. EPA/600/4-90/027F, Environmental Monitoring Systems Laboratory, Cincinnati, OH, US Environmental Protection Agency.

USEPA (1994) Short term methods for estimating the chronic toxicity of effluents and receiving water to freshwater organisms. 3rd edition, EPA-600-4-91-002. US Environmental Protection Agency.

Vaughan, G.T. (1998) Toxicity of clay leachates to freshwater and marine organisms. CSIRO Energy Technology Report, 23 pp.

APPENDIX A

Lanthanum Calibration Bioassays

APPENDIX B

Summary of Water Quality Characteristics and Toxicity Test Results for Canning River Samples - First Phoslock Application (January, 2000)

APPENDIX C

Summary of Acute Toxicity of Canning River Samples to *Ceriodaphnia dubia* - First Phoslock Application (January, 2000)

APPENDIX D

Summary of Chronic Toxicity of Canning River Samples to *Ceriodaphnia dubia* - First Phoslock Application (January, 2000)

APPENDIX E

Summary of Acute Toxicity of Canning River Samples to Juvenile Eastern Rainbow Fish - First Phoslock Application (January, 2000)

APPENDIX F

Summary of Water Quality Characteristics and Toxicity Test Results for Canning River Samples - Second Phoslock Application (April, 2000)

APPENDIX G

Summary of Chronic Toxicity of Canning River Samples to *Selenastrum capricornutum* - Second Phoslock Application (April, 2000)

APPENDIX H

Summary of Acute Toxicity of Canning River Samples to *Ceriodaphnia dubia* - Second Phoslock Application (April, 2000)

APPENDIX I

Summary of Chronic Toxicity of Canning River Samples to *Ceriodaphnia dubia* - Second Phoslock Application (April, 2000)

APPENDIX J

Summary of Acute Toxicity of Canning River Samples to Juvenile Eastern Rainbow Fish - Second Phoslock Application (April, 2000)