Determination of HSNO Ecotoxic Thresholds for Granular Phoslock™ (Eureka 1 Formulation) Phase 1: Acute Toxicity

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Executive Summary

NIWA was contracted by Primaxa Ltd to undertake toxicity testing of Phoslock™ to determine if the compound would trigger HSNO ecotoxic thresholds (ERMA 2001), and subsequently require classification as a hazardous substance. Following negotiations with ERMA, a suite of three acute toxicity tests were used in phase 1 of the assessment. The species and reference methods used were:

- Algae – 72h test (*Pseudokirchneriella subcapitata*) — Environment Canada (1992) & USEPA (1987a)
- Crustacea – 48h test (*Daphnia magna)* — OECD (1984)
- Fish fry– 96h test (*Oncorhynchus mykiss*) — OECD (1992)

The findings were:

- Testing was undertaken using the granular 'Eureka 1' Phoslock™ formulation, supplied by Primaxa Ltd.
- Test organisms were exposed to solutions prepared from a filtered (40 μm) 50 g L^{-1} elutriate of Phoslock™ (NICNAS 2001).
- Fish were the most sensitive species (LC_{50} = 4350 mg Phoslock™ L^{-1})[1], 11.5 and 3.4 times more sensitive than the crustacea and algae respectively.
- Based on the most sensitive species (fish), the acute LC_{50} of Phoslock™ is markedly higher (44x), (i.e. less toxic) than the ERMA (2001) acute ecotoxic threshold of 100 mg L^{-1}.
- The algal NOEC and estimated fish chronic NOEC are 6,250 and 435 times higher, respectively, than the ERMA (2001) chronic ecotoxic threshold of 1 mg L^{-1}.
- The granular 'Eureka 1' formulation of Phoslock™ tested in this study did not trigger HSNO ecotoxic thresholds (ERMA 2001), and is therefore considered non-toxic for the purposes of the Hazardous Substances and New Organisms (HSNO) Act 1996.
- The **HSNO category** for Phoslock™ is '**Not Classified**' (i.e. not hazardous) (Table A).

[1] Based on 50 g L^{-1} (i.e. 50,000 mg L^{-1}) Phoslock™ elutriate.

Table A: Summary of HSNO classifications and toxicity results from a filtered (40 μm) 50 g Phoslock™ L^{-1} elutriate.

Test Organism and Endpoint	LC_{50} or EC_{50} % Elutriate	LC_{50} or EC_{50} mg Phoslock™ L^{-1}	ERMA (2001) HSNO Threshold mg L^{-1}	Safety Factor	**HSNO classification**
Fish fry - acute 96 h LC_{50}	8.7	4,350	100	44x	**Not classified**
Alga - acute 72 h EC_{50}	30.0	15,000	100	150x	**Not classified**
Crustacean - acute 48 h LC_{50}	>100	>50,000	100	>500x	**Not classified**

- Addition of phosphorus to 100% Phoslock™ elutriate resulted in significantly reduced mortality to fish. A dose of 2,500 μg L^{-1} P resulted in no mortality after 72 hours exposure to 100% elutriate.

- A risk-based assessment shows that for a normal phosphorus-related application (200 parts of Phoslock™: 1 part of phosphorus), and a high concentration of phosphorus likely to be found in New Zealand receiving waters, a low risk of adverse effects for aquatic biota exists. For an aerial dosing sediment capping scenario (200 g m^{-2} application rate) there is a greater risk, but there remains at least a 20 times factor of safety against adverse effects.

- The results of this study indicate that additional chronic toxicity testing would probably not trigger the ERMA (2001) ecotoxic thresholds and not require amendment of the '**Not Classified**' HSNO classification determined from the acute toxicity test results.

1. Introduction

Management options for remediation of eutrophic lakes can include addition of flocculants to precipitate phosphates, and remove them from the water column. Phoslock™ is one such compound that is under investigation for use in decreasing the concentration of soluble phosphate in water bodies (NICNAS 2001). Currently, Phoslock™ is unclassified by the Environmental Risk Management Authority NZ (ERMA) and therefore unable to be commercially used in New Zealand.

The Hazardous Substances and New Organisms (HSNO) Act 1996 reformed legislation relating to management of hazardous substances in New Zealand, and provides for a series of regulations to manage the risks associated with potentially hazardous substances. The hazardous substances part of the HSNO Act came into effect on July 2001 (ERMA 2001).

ERMA (2001) outlines procedures and guidelines for assessment of substances to comply with the HNSO Act. Aquatic biota toxicity tests are used to assess aquatic ecotoxic effects of a substance when it comes in contact with the aquatic environment. There are four basic elements to consider when determining aquatic effects (ERMA 2001):

- Acute aquatic ecotoxicity
- Potential for, or actual bioaccumulation
- Degradation (biotic or abiotic (e.g. hydrolysis) for organic chemicals
- Chronic aquatic ecotoxicity

ERMA (S. Scobie email 20/7/04) specified the required base set of data for aquatic toxicity as:

- *Fish acute toxicity test (96 hour LC_{50})*
- *Daphnia acute test (48 hour EC_{50})*
- *Algal growth inhibition (72 hour or 96 hour EC_{50})*
- *Fish, early life stage toxicity test (approx. 60 day NOEC*)
- *Daphnia reproduction toxicity test (21 day NOEC)*

The threshold criteria for classification as 'hazardous' are (ERMA 2001):

3(1) A substance with ecotoxic properties is not hazardous for the purposes of the Act unless –

(a) The substance is ecotoxic to aquatic organisms because –

(i) data for the substance indicates that the fish LC_{50} is 100 milligrams or less of the substance per litre of water over a 96-hour exposure period, as a result of exposure to the substance; or

(ii) data for the substance indicates that the crustacean EC_{50} is 100 milligrams or less of the substance per litre of water over a 48-hour exposure period, as a result of exposure to the substance; or

(iii) data for the substance indicates that the algal or other aquatic plant EC_{50} is 100 milligrams or less of the substance per litre of water over a 72-hour or 96-hour exposure period, as a result of exposure to the substance; or

(iv) data for the substance indicates that the chronic fish NOEC, or chronic crustacean NOEC, or algal or other aquatic plant NOEC, is 1 milligram or less of the substance per litre of water, as a result of exposure to the substance; or

(v) in the absence of the NOEC data prescribed in subparagraph (iv) data for the substance indicates that it is not rapidly degradable and is bioaccumulative.

Once a substance triggers the threshold it is classified into one of four categories (Categories 9.1A – 9.1D), based on the results of toxicity testing.

We have not undertaken discussions with ERMA as to the suitability of this suite of tests and their relevance to the type of product and its proposed use. Such risk assessment considerations are particularly relevant to decisions as to whether chronic tests are required. Subsequent discussions between Primaxa Ltd and ERMA resulted in ERMA agreeing to the assessment being undertaken in two phases. Phase 1 would use the three acute toxicity tests (fish 96h LC_{50}, *Daphnia* 48h EC_{50} and algal[2] 72h EC_{50}) for assessment and classification. Phase 2 may be undertaken using the two chronic tests (fish early life stage and *Daphnia* reproduction tests) if the results of

[2] The 72 h algal growth test is usually regarded as a chronic assessment (i.e. long-term effects relative to life stage or life cycle of the organism). However, ERMA (2001) classifies the test EC_{50} as acute for the purpose of assessing ecotoxic effects and classification.

phase one tests indicated chronic ecotoxic effects may be significant and affect classification.

Primaxa Ltd contracted NIWA to undertake toxicity testing of Phoslock™ to determine if acute toxicity test results would trigger ERMA (2001) ecotoxic thresholds, and subsequently require classification as a hazardous substance. NIWA also conducted a risk-based analysis of the potential for environmental effects based on the toxicity test results and expected application rates in New Zealand.

2. Methods and Materials

2.1 General Methods

Summaries of the test conditions are provided in Appendix. The toxicity tests were conducted according to the following reference methods:

- Algae – 72h test (*Pseudokirchneriella subcapitata*)[3] — Environment Canada (1992)& USEPA (1987a)
- Crustacea – 48h test (*Daphnia magna*) — OECD (1984)
- Fish – 96h test (*Oncorhynchus mykiss* - fry) — OECD (1992)

2.2 Test Material Preparation

All testing was undertaken on an "as supplied" basis, using a Primaxa Ltd supplied sample of the 'Eureka 1' granular formulation (Ian Dorset, Primaxa Ltd, email 4 November 2004) of Phoslock™. Details of organism source and exposure conditions are provided in Appendix 7.1.

A 70 g sample was received at NIWA, Hamilton on 28/8/04. This sample was used for the algal toxicity test, but was insufficient for the *Daphnia* and fish tests. An additional 1 kg sample of the same formulation was received at NIWA, Hamilton on 17/9/04. Each sample was assigned a unique laboratory identification number and stored in the Ecotoxicology laboratory chemical store until the test initiations.

ERMA (S. Scobie pers comm) directed that test solutions be prepared using the method prescribed in NICNAS (2001). This method uses the USEPA Toxic Characteristic Leachate Procedure (TCLP) to provide the material for toxicity testing (USEPA 1992). This procedure uses a standardised approach to extract chemical contaminants from 50 g of material in 1 L (i.e., 50,000 mg L^{-1}) of synthetic dilution water. The leachate procedure minimises potential experimental artefacts of adverse effect on species of high concentrations of particulate solids, which in this case could be derived from the bentonite in the Phoslock™.

In this method 50 g L^{-1} of Phoslock was mixed gently (4 rpm) with a phosphorus-free synthetic soft water[4] (hardness 32 mg L^{-1} $CaCO_3$) for 18 hours on a rotary tumbler in a

[3] Formerly *Selenastrum capricornutum.*

plastic container in complete darkness at 15°C. The solution was allowed to stand for 1 hour, then the supernatant liquid was siphoned off and filtered through a 40 μm nylon mesh filter before use in the toxicity tests. Test solutions were prepared as percent by volume solution of the filtered supernatant, using either *Daphnia* culture water for the crustacea test, or de-chlorinated Hamilton City tap water for the fish tests.

2.3 Algal test

In the algal growth test, the test solution was further filtered through a 0.45 μm membrane filter before the test solutions were prepared. Algal cell growth was determined using the microplate method (Environment Canada 1992, USEPA 1987a) and cell growth determined by flow cytometry[5] (Hall & Cummings 2003). A summary of the test methods and conditions is included in Appendix 7.1. Performance of the flow cytometer was investigated using TruCount beads[6] and showed that there was no interference from clay particles to the instrument operation and detection of algal cells. No EDTA was used in the algal growth media for this test.

2.4 Crustacea

Test organisms (24 h old juveniles) were obtained from our laboratory culture. Dilutions for the *Daphnia* test were prepared using our standard laboratory *Daphnia* culture water (NIWA 1995), adapted to moderate hardness (40 - 50 mg L^{-1} $CaCO_3$). A summary of the test methods and conditions is included in Appendix 7.1.

2.5 Fish

Fish fry were obtained from the Fish and Game (NZ) hatchery, Rotorua. Dilutions for the fish test were prepared using dechlorinated Hamilton City Council tap water (30 - 40 mg L^{-1} $CaCO_3$). The mean weight length of the fish was 0.39 g (±SD 0.13) and 36.5 mm (±SD 3.51) mm respectively.

Two fish tests were undertaken: the first test was a standard toxicity test conducted in accordance with OECD (1992), as required by ERMA for HSNO classification. This

[4] 48 mg L^{-1} $NaHCO_3$, 30 mg L^{-1} $CaSO_4.2H_2O$, 30 mg L^{-1} $MgSO_4$, 2 mg L^{-1} KCl (NICNAS 2001).

[5] Flow cytometry measures fluorescence and scatter of cells from a laser beam, see Hall & Cummings (2003) for details of instrument operation and features.

[6] 10 μm fluorescent beads similar to algal cells.

test used 5 control replicates and 3 replicates for each of the five elutriate dilutions. A summary of the test methods and conditions is included in Appendix 7.1.

The second test investigated the mitigating effects of phosphorus on the toxicity detected by the fish in the first test. Four containers of 3 L of 100% elutriate were dosed with a different P concentration (20, 100, 500, 2500 µg L^{-1} P). One replicate of each concentration and a control (no elutriate and no P) was used. A 1,000 mg L^{-1} P solution was prepared from K_2HPO_4, and aliquots of this solution were added to 100% elutriate to make 3 L of the test solution. To overcome the lowering of the pH due to the addition of the P solution, the pH of each container was adjusted to pH 7.5 by addition of 30 mL of 2,480 mg L^{-1} $NaHCO_3$ solution. Further details of the testing methodology are included in Appendix 7.1.

2.6 Water Samples

Selected water samples from the fish and daphnia toxicity tests were acidified and analysed for total lanthanum. Samples from the fish mortality mitigation by phosphorus additions were filtered, not acidified, and analysed for dissolved lanthanum.

Samples from the test solutions were analysed by Hill Laboratories Ltd for total and dissolved lanthanum, using a boiling nitric acid digestion (APHA 1998a) and ICP MS analysis (APHA 1998b).

2.7 Data Analysis

Test results were analysed using Toxcalc^{TM} software (Tidepool 1994). Algal test results were analysed by linear interpolation (ICp) combined with bootstrapping to derive EC values. Fish and *Daphnia* results were analysed using the Probit or Trimmed Spearman-Karber methods to derive EC or LC values. Hypothesis testing of the algal, *Daphnia* and fish test results was undertaken using ANOVA and USEPA approved methods (Tidepool 1994).

2.8 Reference Toxicant

Reference toxicant tests with zinc sulphate were concurrently undertaken for each species. The EC_{50} and LC_{50} results were compared to results from previous exposures to zinc to determine organism health and test acceptability.

3. Results

All original data and project notes are maintained at NIWA, Hamilton in a confidential project file. A summary of the data observations and statistical analyses is included in the Appendix.

3.1 Assessment of Ecotoxic Thresholds

The toxicity results are summarised in Table 1. Control survival for the fish and immobility for the crustacea controls were greater than 90%, and within the test acceptability criteria. These results showed that the fish (rainbow trout) were the most sensitive species (96 h LC_{50} = 8.7 % elutriate) being 11.5 times more sensitive that the crustacea (48 h EC_{50} > 100% elutriate), and 3.4 times more sensitive than the algae (72 h EC_{50} = 30 % elutriate). Toxicity to the fish occurred within 48 hours, and no further mortality was recorded after this time.

Table 1: Summary of crustacean, fish and algal acute toxicity test results for Phoslock^{TM} elutriate (50 g Phoslock^{TM} L^{-1}, 40 μm filtered).

Test organism	End-point	Phoslock Elutriate (%)					Control
		LC_{50} or EC_{50} [a] (95% CI)	EC_{20}	NOEC[b]	LOEC[b]	TEC[b]	% Survival
Crustacea	24 h immobility	>100	70.5	50.0	100	70.7	98.0
	48 h immobility	>100	74.6	50.0	100	70.7	94.0
Rainbow trout fry	24 h survival	16.6(20.1-13.8)[c]	-	<6.25	6.25	<6.25	100
	48 h survival	8.7(11.8-5.3)	3.4	<6.25	6.25	<6.25	100
	72 h survival	8.7(11.8-5.3)	3.4	<6.25	6.25	<6.25	100
	96 h survival	8.7(11.8-5.3)	3.4	<6.25	6.25	<6.25	100
Algae	72 h cell growth	30.0(31.4 28.3)	18.9	12.5	25.0	17.7	

[a] The lower the LC_{50} or EC_{50} the greater the toxicity, indicating that a higher dilution was required to cause a 50% effect on the test organisms. LC = lethal concentration & EC = effective concentration (see glossary)

[b] NOEC = No observed effect concentration; LOEC = Lowest observed effect concentration; TEC = threshold effect concentration (Geometric mean of NOEC and LOEC)

[c] Analysed using the Trimmed Spearman-Karber method, which calculates only EC_{50} values

Although the 48 h crustacea immobility EC_{50} value was >100%, there was some effect on mobility of the organism in the highest test concentration (33% and 37% immobile after 24 h and 48 h exposure respectively). At this concentration, organisms became trapped in a white gelatinous precipitate, which formed during the test, and resulted in

immobility and ultimately mortality. There was little change in crustacea immobility after 24 hours exposure.

Based on the weight of material used in the extraction procedure (50 g L^{-1}), and assuming a linear relationship between the mass of Phoslock™ added and the elutriate composition, the 96 h trout LC_{50} is equivalent to 4,350 mg Phoslock™ L^{-1} (Table 2). The crustacean (*Daphnia*) and algal EC_{50} values indicate lower sensitivity. The trout LC_{50} value is markedly (44 times) higher than the ERMA ecotoxic threshold of 100 mg L^{-1} for acute effects.

Although ERMA (2001) consider the 72 h algal EC_{50} an acute value, section 3(1)(iv) allows use of the NOEC as a chronic value. The algal 72 h NOEC value (12.5%, equivalent to 6,250 mg Phoslock™ L^{-1}) is 6,250 times higher than the ERMA (2001) ecotoxic threshold of 1 mg L^{-1} for chronic effects.

Therefore, the **HSNO category** for Phoslock™ would be '**Not Classified**', i.e. not hazardous (ERMA 2001; summary table Part VII, page 9).

Table 2: Summary of toxicity test results and equivalent Phoslock concentrations based on exposure to 50 g L^{-1} of Phoslock™ (40μm filtered) elutriate, expressed in terms of mg Phoslock™ L^{-1}.

Organism	LC_{50} or EC_{50} % Elutriate	LC_{50} or EC_{50} mg Phoslock L^{-1}	NOEC % Elutriate	NOEC mg Phoslock L^{-1}
Alga	30	15,000	12.5	6,250
Crustacean	>100	>50,000	50.0	25,000
Fish fry	8.7	4,350	<6.25	<3,125

3.2 Mitigation of Phoslock™ Toxicity by Phosphorus

There was 100% and 37% mortality for the fish and *Daphnia* respectively in the highest test concentration (100% elutriate) at the conclusion of the tests. Rainbow trout were chosen to investigate the effect of phosphorus on fish survival, as they were more sensitive to Phoslock™ exposure than *Daphnia* (Table 1). As there was no change in mortality after 48 h in the standard toxicity test, a 72 h survival end point was used for this part of the study. Addition of phosphorus to the 100% elutriate solutions significantly mitigated fish mortality, with the highest P dose (2,500 μg L^{-1} P) resulting in 0% mortality (i.e. 100% survival) after 72 h exposure (Table 3, Figure 1).

Table 3: Fish (rainbow trout fry) survival in 100% elutriate (50 g Phoslock™ L^{-1}, 40 µm filtered) and control solutions after addition of phosphorus.

Phosphorus	Survival (%) after			
µg L^{-1} P	24 h	48 h	72 h	96 h
Control	100	100	100	100
0[a]	0 [a]	0 [a]	0 [a]	0[a]
20	30	0	0	
100	50	20	10	
500	40	20	20	
2,500	100	100	100	

[a] Results from acute toxicity test.

3.3 Lanthanum (La) Analyses

Total La results for the acute toxicity tests indicate a significant reduction of total La concentrations in the test solutions over time. After 96 hours, total La in the test solutions was 1-2% of the concentration at the test initiation (Figure 2). The high initial total La concentrations for day 1 elutriate solution could possibly be due to particle-associated material passed through the 40 µm filter and turbulence caused by the fish fry in the test container. The results indicate that La is settling from the test solutions during the exposure procedures. In all the test solutions, a white precipitate forming during the test was noted in the test containers. We would expect some variability in the total-La because of the large filter size (40 µm) used in the TCLP procedure as directed by NICNAS (2001).

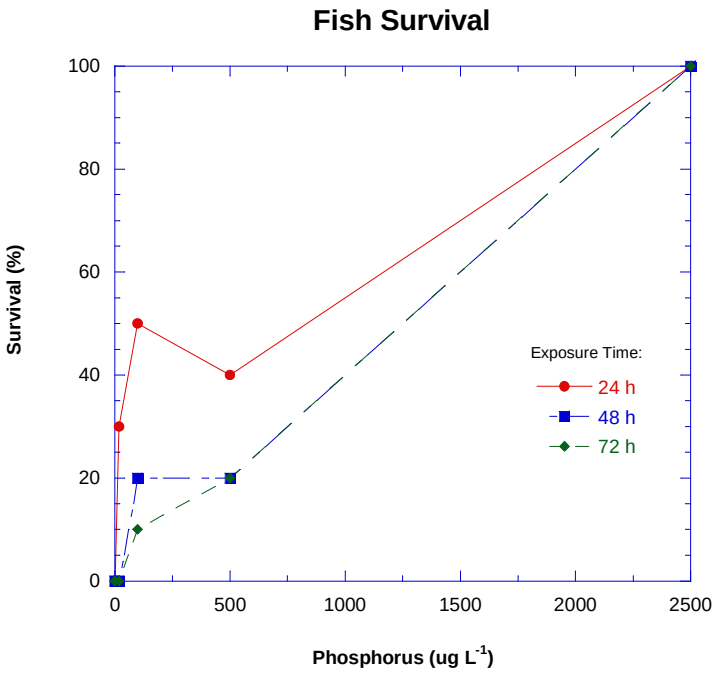

Figure 1: Mitigation of fish fry survival in 100% Phoslock™ elutriate after addition of phosphorus.

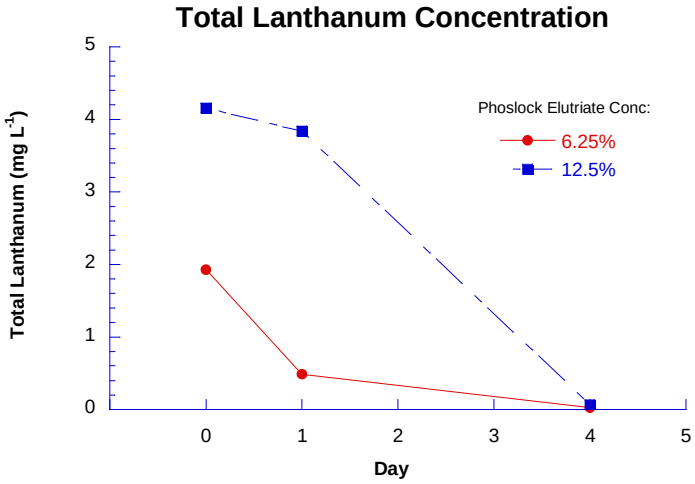

Figure 2: Total lanthanum concentrations in 6.25% and 12.5% Phoslock™ elutriate solutions during acute toxicity tests.

Dissolved and total La concentrations were analysed from the phosphorus mitigation experiment for the 500 and 2,500 μg L^{-1} P treatments bounding the survival effects threshold after 1 and 3 days exposure. The results show total La concentrations were markedly lower than the initial acute toxicity testing trial. Also dissolved La concentrations were reduced by at least 3.6 times by a 5-fold increase in phosphorus (Table 4). Combined with the fish survival results, the decrease in La concentrations suggests that phosphorus reduced the bioavailability of La, and decreased mortality. The rapid onset of mortality in the acute fish test suggests that the initial high La concentrations were the cause of fish mortality. However, we do not know the rate of reaction between La (from Phoslock™) and phosphorus.

Table 4: Lanthanum concentrations in 100% Phoslock™ elutriate solutions after addition of phosphorus.

Phosphorus μg L^{-1} P	Lanthanum Concentration (mg L^{-1})					
	Day 1			Day 3		
	Dissolved	Total	Survival (%)	Dissolved	Total	Survival (%)
500	na[a]	na	40	0.0018	0.34	20
2,500	< 0.0005	0.17	100	< 0.0005	0.27	100

[a] Not analysed.

3.4 Reference Toxicant

The algal, *Daphnia* and fish fry reference toxicant EC_{50} results for zinc sulphate were within the expected range for the reference toxicant (Table 5). The fish and *Daphnia* used in this suite of toxicity tests would be ranked in the most sensitive 25%ile of the test organisms used by USEPA for the ambient water quality criteria for zinc (USEPA 1987a). The algae are approximately 9 times more sensitive to zinc than the most sensitive species used by the USEPA. By using these three species that exhibit such sensitivity, the results from this suite of toxicity tests provide a moderate degree of confidence in assessing the toxic hazard of the sample. However, care must be taken when extrapolating these results for protection of organisms present in a particular receiving water environment.

Table 5: Summary of reference toxicant (zinc sulphate) EC_{50} results.

Organism	Species	EC_{50} (95%CI) mg L^{-1} Zn
Algae	*P. subcapitata*	0.011(0.012-0.008)
Daphnia	*D. magna*	1.1 (1.3-0.8)[a]
Fish fry	*O. mykiss*	0.43 (0.50-0.37)

[a] 48 h survival endpoint

4. Conclusions and discussion

NIWA was contracted by Primaxa Ltd to undertake toxicity testing of Phoslock™ to determine if the compound would trigger ERMA (2001) ecotoxic thresholds, and subsequently require classification as a hazardous substance. Following negotiations with ERMA, a suite of three acute toxicity tests (freshwater algae, *Daphnia*, and freshwater fish fry) were used in phase 1 of the assessment. Toxicity tests were undertaken in accordance with internationally recognised reference methods.

Primaxa Ltd supplied 2 samples of the granulated 'Eureka 1' formulation of Phoslock™ for this assessment. An elutriation procedure (USEPA 1992), previously used in Australia for assessment of the ecotoxic effects of Phoslock™ on aquatic biota (NICNAS 2001) was used in this study. The procedure is used to assess the mobility of both organic and inorganic analytes present in liquid, solid and multiphasic wastes, where the solid material is the dominant factor determining the pH of the extract. In this assessment the extraction procedure did not result in physiologically unacceptable pH values. The pH of 100% elutriate was 7.8 prior to the fish toxicity test initiation. The elutriate is prepared by gently tumbling the specified weight of substance in a phosphorus-free synthetic soft water (50 g Phoslock™ L^{-1}) for 18 hours. The mixture was allowed to settle, decanted and filtered (40 μm), and used to make up solutions for the toxicity tests. The elutriate was further filtered through a 0.45 μm membrane filter for the algal test, to prevent interference of clay particles in the cell counting procedure. No EDTA was used in the media for the algal test.

4.1 ERMA (2001) Classification

Some classification methods base compliance of mixtures on the concentration of the 'active ingredient' (e.g., Zucker 1985). ERMA (2001) defines 'mixture' as a substance that is a combination of two or more chemical substances that have not reacted to form other chemical entities at the time of classification. The Phoslock™ assessed in this study is a mixture of individual substances, but will be treated commercially as a single entity. As no toxicity testing was undertaken on the individual components of Phoslock™, comparison with the ERMA (2001) ecotoxic threshold values has been undertaken using EC_{50} or LC_{50} values expressed as a concentration (mg L^{-1}) of Phoslock™ used to prepare the elutriate.

The toxicity results are summarised in Table 6 together with the LC_{50} and EC_{50} and results for the 3 test species. The results showed that the rainbow trout were the most sensitive species, being 11.5 times more sensitive than *Daphnia*, and 3.4 times more

sensitive than algae. The Phoslock™ concentration at the trout LC_{50} was equivalent to 4,350 mg Phoslock™ L^{-1}.

The ERMA (2001) ecotoxic threshold for acute effects is 100 mg L^{-1}, and if LC_{50} or EC_{50} values are greater than the threshold, they are not required to be classified. For chronic effects, if the chronic NOEC is greater 1 mg L^{-1}, classification is also not required. When these two conditions are met, environmental persistence and bioaccumulation do not require consideration.

Based on the most sensitive species (fish, 96 h LC_{50} = 4350 mg Phoslock™ L^{-1}), the acute LC_{50} of Phoslock™ is markedly higher (44 times) than the (ERMA 2001) ecotoxic threshold. Based on these acute values (Table 6), the **HSNO category** for Phoslock™ would be '**Not Classified**', i.e. not hazardous (ERMA 2001; summary table Part VII, page 9).

Estimations of potential chronic effects thresholds may also be determined. The algal NOEC (72 h NOEC = 6,250 mg Phoslock™ L^{-1}) is markedly higher (6,250 times) than the ERMA (2001) ecotoxic threshold for chronic effects of 1 mg L^{-1}. An estimate of a chronic NOEC can also be made by applying a 10 times application factor (AF) to an acute LC_{50} or EC_{50} (ANZECC 2000). Using the fish LC_{50}, this procedure gives an estimated chronic NOEC of 435 mg Phoslock™ L^{-1}, which is 435 times higher than the ERMA (2001) ecotoxic threshold.

Table 6: Summary of HSNO classification and toxicity results expressed in terms of equivalent Phoslock™ concentrations based on exposure to a filtered (40 μm) 50 g L^{-1} elutriate.

Test Organism	Endpoint	LC_{50} or EC_{50} mg Phoslock™ L^{-1}	ERMA (2001) Threshold mg L^{-1}	Safety Factor	**HSNO classification**
Acute classification					
Fish fry	acute 96 h LC_{50}	4,350	100	44x	**Not classified**
Alga	acute 96 h EC_{50}	15,000	100	150x	**Not classified**
Crustacean	acute 48 h EC_{50}	>50,000	100	500x	**Not classified**
Chronic classification					
Alga	chronic 72 h NOEC	6,250	1	6,250x	**Not classified**
Fish fry	chronic 96 h NOEC[a]	435	1	435x[a]	**Not classified**

[a] Estimated after application of 10x safety factor to acute LC_{50} (ANZECC 2000).

The results of the acute toxicity tests are dissimilar to those reported in NICNAS (2001), which reported growth stimulation at all concentrations above 6.25% elutriate for the same species of algae. However, 'Milli-Q' water was used to prepare the elutriate in NICNAS (2001), which may account for the different response to this study. The fish test was undertaken using the eastern rainbow fish (*M. duboulayi*), with a sublethal endpoint (imbalance, 96 h EC_{50} > 100% elutriate), which was markedly less sensitive than the rainbow trout fry mortality endpoint we employed. The same synthetic soft water was used for both fish tests. NICNAS (2001) used *C. dubia* in an invertebrate immobilisation test (48 h EC_{50} = 49% elutriate), which was at least 2 times more sensitive than *D. magna* in this study. The differences in sensitivity for the fish and invertebrates between this study and NICNAS (2001) may be due to the different physiology and metabolism of the two species used in the respective studies. In addition, NICNAS (2001) does not identify the formulation used in their study, which may be significantly different than the granulated 'Eureka 1' formulation used in this study. In fact, it was noted by NICNAS (2001) that modifications to the production processes may reduce the amount of free La released by Phoslock™, and hence reduce toxicity.

4.2 Mitigation of Phoslock™ Toxicity by Phosphorus

Phoslock™ is a La modified clay that has been developed to remove filterable reactive phosphorus (FRP) from water. The mechanism of FRP removal involves the reaction of phosphate anions with La, leading to formation of a single species of lanthanum phosphate, or Rabdophane, which is highly insoluble (Haghsereht 2004).

$$La^{3+} + PO_4^{3-} \longrightarrow LaPO_4$$

The majority of the lanthanum is reportedly strongly bound to the clay matrix, and is therefore likely to be minimally released into the water column. Significant release of free lanthanum from the clay and its associated toxicity appears to occur for 1-2 days after application (NICNAS 2001). Reaction with phosphate to the insoluble Rabdophane and precipitation would probably result in lanthanum not being bioavailable to aquatic biota.

In 100% Phoslock™ elutriate there was no survival of fish fry after 24 hours exposure. However, addition of 4 different phosphorus concentrations to separate 100% elutriate solutions significantly mitigated fish mortality, with the highest P dose (2,500 μg L^{-1} P) resulting in 0% mortality (i.e. 100% survival) after 72 h exposure (Table 3, Figure 1). Reduction of fish mortality and the dissolved lanthanum concentrations for the

highest phosphorus dose indicates that when phosphorus combines with free lanthanum in the water column, it is not toxic to the fish.

Dissolved reactive phosphorus (DRP) concentrations in the lakes of the Rotorua region can be between 80–500 μg L^{-1} (Max Gibbs, NIWA, pers comm August 2004). The standard method of application is based on 200 parts of Phoslock™: 1 part of phosphorus (Ian Dorset, Primaxa Ltd, pers comm. September 2004). At 500 μg DRP L^{-1}, an application of 100 mg Phoslock™ L^{-1} would be required, which is equivalent to 0.2 % elutriate used for this assessment (assuming a linear relationship between the mass of Phoslock™ added and the elutriate composition). From the fish dose-response (Appendix 7.2) less than 5% mortality would be expected, and 500 μg DRP L^{-1} would probably only slightly improve fish survival in this case. The high concentrations of phosphorus (>500 μg L^{-1}) used to demonstrate mitigation of mortality would be unlikely to occur in New Zealand lakes.

4.3 Risk-Based Assessment Relative to Environmental Application

A risk-based assessment of potential environmental effects was also undertaken. A hazard quotient (Q) can be calculated from the ratio of estimated environmental concentration/effect concentration of the most sensitive species (fish, 96 h LC_{50} = 4,350 mg Phoslock™ L^{-1}). Calculated values of Q below 1 indicate low environmental risk, while values greater than 1 indicate high risk.

Assessment was based on the anticipated environmental concentrations that would result from use at label rates. Two application scenarios may be used for this product: (i) based on measured phosphorus concentrations in the receiving water – the normally recommended approach; and (ii) on an aerial basis to achieve a uniform coating on the sediments of the receiving water. These scenarios are addressed below to establish potential maximum exposure concentrations to be assessed for potential toxicological effects.

(i) *Normal phosphorus-related application.* The standard method of application is based on 200 parts of Phoslock™: 1 part of phosphorus (Ian Dorset, Primaxa Ltd, pers comm. September 2004). A high level of phosphorus in New Zealand lakes would be 0.500 mg L^{-1}, requiring application of 100 mg L^{-1} of Phoslock™. Applying a 10x uncertainty factor would give a predicted maximum dose of 1,000 mg L^{-1}. For this scenario, Q is 0.23, approximately a further 4-fold safety factor.

(ii) *Aerial dosing for sediment capping*. The objective of the aerial dosing approach is to minimise phosphorus exchange from lake or pond sediments to the overlying waters. This is achieved by capping the sediments with an approximate 1 mm thickness of Phoslock™, based on a predicted application rate[7] of 200 g m^{-2} (Ian Dorset, Primaxa Ltd, pers comm. September 2004). Assuming this was in a depth of 1 metre, then the predicted maximum would be 200 mg L^{-1}, and applying a 10x uncertainty factor would give a predicted maximum dose of 2,000 mg L^{-1}. For this scenario, Q is 0.46, approximately a further 2-fold safety factor.

Both scenarios indicate a low environmental risk for biota, with the normal application scenario (i) having the highest protection (at least 30-fold) and therefore less risk to aquatic biota. The aerial capping scenario (ii), may present a greater potential for adverse environmental effects, due to greater application rates.

Although the EC_{50} or LC_{50} values of the three toxicity tests used in this study are considered acute values for classification, chronic sensitivity must also be considered. The algal NOEC value can be used as a chronic value, and combined with the estimated chronic fish NOEC, indicate that chronic adverse effects from Phoslock™ at 'label' application rates would be unlikely to occur in the receiving water environment. Therefore, further testing using the chronic *Daphnia* 21day reproduction test and the 60 day early life stage fish test may not be necessary.

[7] Note that this application rate is based on lake area rather than water volume, and will not change with water depth.

5. Glossary

Acute toxicity	Is a discernible adverse effect (lethal or sublethal) induced in the test organisms within a short period of exposure to a test material.
Chronic toxicity	Implies long-term effects that are related to changes in metabolism, growth, reproduction, or ability to survive. In this test, chronic toxicity is a discernible adverse effect (lethal or sublethal) induced in the test organism during a significant and sensitive part of the life-cycle.
EC_{50}	Is the median effective concentration (i.e., the concentration of material in water that is estimated to produce a specifically quantified effect to 50% of the test organisms). The EC_{50} and its 95% confidence limits are usually derived by statistical analysis of a quantal, "all or nothing", response (such as death, fertilization, germination, or development) in several test concentrations, after a fixed period of exposure.
End point	The adverse biological response in question that is measured. May vary with the level of biological organisation examined, but may include biochemical markers, mortality or reproduction. End points are used in toxicity tests as criteria for effects.
IC_{50}	Is the median inhibition concentration, i.e., the concentration estimated to cause a 50 % reduction in growth compared to a control. The exposure time must be specified, e.g., "IC_{50} (72 h)", for a growth rate derived IC_{50} and a test duration of 72 h.
Lethal	Means causing death by direct action. Death of fish is defined as the cessation of all visible signs of movement or other activity.
LC_{50}, LC_{20}	The lethal toxicant concentration resulting in a 50% or 20% mortality (respectively) at a specific time of exposure.
LOEC	Lowest observed effect concentration. The lowest concentration tested causing a statistically measurable effect to the test system.
NOEC	No observed effect concentration. The highest concentration tested causing no statistically measurable effect to the test system.
Toxicity test	Is a method to determine the effect of a material on a group of selected organisms under defined conditions. An aquatic toxicity test usually measures either (a) the proportions of organisms affected (quantal) as measured by EC_{50}, or (b) the degree of effect shown (graded or quantitative) after exposure to specific concentrations of whole effluents or receiving water as measured by an IC_{50}.
Toxicity	Is the inherent potential or capacity of a material to cause adverse effects on living organisms.

6. References

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7. Appendix

7.1 Summary of Test Conditions

Summary of test conditions for algal bioassay

Project Name: Phoslock™ Ecotoxic Effects	**Project Number: PXL05201**
Test Initiation: 3/9/04	
Test Protocol:	Hall & Golding (1998)
Reference Method:	Environment Canada (1992) & USEPA (1987a)[1]
Test Material:	Phoslock™ granules, 'Eureka 1' formulation
Test Organisms:	*Pseudokirchneriella subcapitata*[2]
Source:	University of Texas, USA
Organisms/Container:	10,000 mL^{-1}
Test Concentrations:	Control, 0.2, 0.4, 0.8, 1.56, 3.13, 6.25, 12.5, 25.0, 50.0, 100.0%
Replicates:	10 for controls, 5 for dilutions
Reference Toxicant:	Zinc Sulphate
Test Duration:	72 hours
Sample pre treatment:	0.45µm filtration
Dilution and Control Water:	UVNP
Test Chambers:	96 well polystyrene microplates
Lighting:	Continuous overhead lighting
Temperature:	24 ± 1℃
Aeration:	Nil.
Chemical Data:	Temperature, pH
Effect Measured:	Growth inhibition relative to controls.
Test Acceptability:	Control CV < 20%, 16x increase in cell growth for controls

[1] No EDTA added to media

[2] Formerly *Selenastrum capricornutum*

Summary of test conditions for freshwater fish bioassay

Project Name: Phoslock™ Ecotoxic Effects	**Project Number: PXL05201**
Test Initiation: 22/9/04	
Reference Method:	OECD (1992)
Test Organisms:	*Oncorhynchus mykiss* fry
Test Material:	Phoslock™ granules, 'Eureka 1' formulation
Mean weight (±SD):	0.39 (±0.13) g
Mean length (±SD):	36.5 (±3.51) mm
Source:	Fish and Game NZ hatchery, Rotorua
Organisms/Container:	10
Test Concentrations:	Control, 6.25, 12.5, 25.0, 50.0, 100%
Stock Holding Period:	19 days
Mortality During Holding	< 2%
Feeding During Holding	2% of wet body weight daily
Replicates:	5 for controls, 3 for treatments, except 1 for 50% and 100%
Reference Toxicant:	Zinc sulphate
Test Duration:	96 hours (observations every 24 h)
Dilution Water:	Hamilton City Council dechlorinated & aerated
Test Chambers:	4 L polythene lined plastic containers (4 L test volume)
Lighting:	16:8 light :dark photoperiod
Temperature:	17 ± 1℃
Aeration:	Moderate aeration at > 100 bubbles/min
Chemical Data:	pH, temperature, dissolved oxygen, total La
Effect Measured:	Survival
Test Acceptability:	Mean control mortality no greater than 10%

Summary of test conditions for freshwater fish bioassay (mortality mitigation by P additions)

Project Name: Phoslock™ Ecotoxic Effects	**Project Number: PXL05201**
Test Initiation: 5/10/04	
Reference Method:	Adapted from OECD (1992),
Test Organisms:	*Oncorhynchus mykiss* fry
Test Material:	Phoslock™ granules, 'Eureka 1' formulation
Mean weight (±SD):	0.39 (±0.13) g
Mean length (±SD):	36.5 (±3.51) mm
Source:	Fish and Game NZ hatchery, Rotorua
Organisms/Container:	10
Test Concentrations:	Control, 100%
Pretreatment:	P additions (20, 100, 500, 2500 µg L^{-1})
Stock Holding Period:	33 days
Mortality During Holding	< 2%
Feeding During Holding	2% of wet body weight daily
Replicates:	1
Reference Toxicant:	None
Test Duration:	72 hours (observations every 24 h)
Dilution Water:	Hamilton City Council dechlorinated & aerated
Test Chambers:	4 L polythene lined plastic containers (3 L test volume)
Lighting:	16:8 light :dark photoperiod
Temperature:	17 ± 1℃
Aeration:	Moderate aeration at > 100 bubbles/min
Chemical Data:	pH, temperature, dissolved oxygen, total and dissolved La
Effect Measured:	Survival
Test Acceptability:	Mean control mortality no greater than 10%

Summary of test conditions for cladoceran water bioassay.

Project Name: Phoslock™ Ecotoxic Effects	**Project Number: PXL05201**
Test Initiation: 24/9/04	
Reference Method:	OECD (1984)
Test Organisms:	*Daphnia magna* < 24 h old juveniles
Source:	Laboratory culture
Organisms/Container:	10
Test Concentrations:	Control, 6.25, 12.5, 25.0, 50.0, 100%
Replicates:	5 for controls, 3 for treatments
Reference Toxicant:	Zinc sulphate
Test Duration:	48 hours (observations at 24 h)
Dilution Water:	Laboratory culture 'soft' hardness
Test Chambers:	50 mL polystyrene beakers
Lighting:	16:8 light :dark photoperiod
Temperature:	20 ± 1℃
Aeration:	Nil
Chemical Data:	pH, temperature, dissolved oxygen
Effect Measured:	Immobility
Test Acceptability:	Mean control mortality no greater than 10%

7.2 Summary of Statistical Analyses

Phytoplankton Test-Algal cells/mL

Start Date:	3/09/2004		Test ID:	2390/KF1		Sample ID:	PS-Phoslock			
End Date:	6/09/2004		Lab ID:	KM-Karen McCluskie		Sample Type:	PHOSLOCK			
Sample Date:	2/09/2004		Protocol:	EPA 1987		Test Species:	MP2-Minutocellus polymorphus			
Comments:	PXL05201 with UV Nanopure water									

Conc-%	1	2	3	4	5	6	7	8	9	10
Control Plate	1327015	1497382	1610969	1727370	1525791	1296104	1769101	1613008	1072714	1123739
Sample Control	1899928	1390515	1728758	1757792	1619615	1822346	1677928	1616010	1312164	1699007
0.2	1714930	1717414	1840738	1515412	1532304					
0.4	1621343	1650202	1528205	1732495	1644772					
0.8	1923188	2021386	1768392	1950125	1708745					
1.56	2118325	1800217	2220792	2059648	2092054					
3.13	1986466	2190413	2057771	1993968	2107977					
6.25	2030135	2071324	2384456	1942075	1876020					
12.5	2013549	1773473	1930899	2283845	1878765					
25	1080738	1238943	1195275	1067027	1126015					
50	101283	102021	89545.2	161068	92853.3					
100	20576.1	19916	21072.1	29919.4	37673.8					

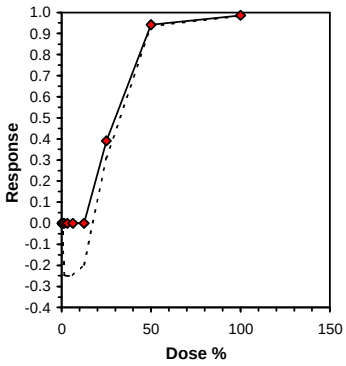
			Transform: Untransformed					Rank	1-Tailed	Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Sum	Critical	Mean	N-Mean
Control Plate	1456319	0.8813	1456319	1072714	1769101	16.607	10				
Sample Control	1652406	1.0000	1652406	1312164	1899928	10.987	10			1873596	1.0000
0.2	1664160	1.0071	1664160	1515412	1840738	8.289	5	40.00	19.00	1873596	1.0000
0.4	1635404	0.9897	1635404	1528205	1732495	4.475	5	36.00	19.00	1873596	1.0000
0.8	1874367	1.1343	1874367	1708745	2021386	6.977	5	59.00	19.00	1873596	1.0000
1.56	2058207	1.2456	2058207	1800217	2220792	7.595	5	63.00	19.00	1873596	1.0000
3.13	2067319	1.2511	2067319	1986466	2190413	4.105	5	65.00	19.00	1873596	1.0000
6.25	2060802	1.2472	2060802	1876020	2384456	9.523	5	64.00	19.00	1873596	1.0000
12.5	1976106	1.1959	1976106	1773473	2283845	9.756	5	62.00	19.00	1873596	1.0000
*25	1141600	0.6909	1141600	1067027	1238943	6.476	5	15.00	19.00	1141600	0.6093
*50	109354	0.0662	109354	89545.2	161068	26.887	5	15.00	19.00	109354	0.0584
*100	25831.5	0.0156	25831.5	19916	37673.8	30.125	5	15.00	19.00	25831.5	0.0138

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Kolmogorov D Test indicates normal distribution (p > 0.01)	0.81249	1.035	-0.0275	1.10378
Bartlett's Test indicates unequal variances (p = 1.51E-04)	34.5234	23.2093		
The control means are not significantly different (p = 0.06)	2.05044	2.10092		

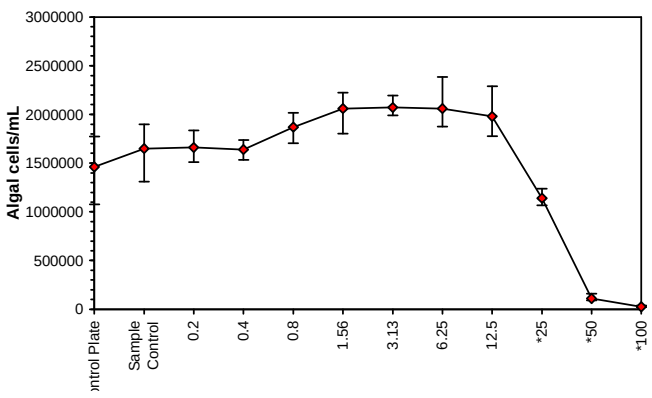
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Wilcoxon Rank Sum Test	12.5	25	17.6777	8

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	14.100	0.151	13.547	14.316	-3.8267
IC10	15.699	0.187	15.174	16.132	-0.9987
IC15	17.299	0.243	16.704	17.948	0.0384
IC20	18.899	0.309	18.163	19.764	0.3314
IC25	20.499	0.379	19.608	21.580	0.4211
IC40	25.422	0.716	23.835	27.439	0.4807
IC50	29.960	0.669	28.101	31.599	0.0609



Dose-Response Plot



Acute 48h Survival Test-48 h Immobility

Start Date:	24/09/2004	Test ID:	2390/KF1dm	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	26/09/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	24/09/2004	Protocol:	OECD202-Daphnia sp., Acut	Test Species:	DM-Daphnia magna
Comments:	Phoslock elutriate				

Conc-%	1	2	3	4	5
D-Control	0.9000	0.9000	1.0000	1.0000	0.9000
6.25	0.9000	0.9000	1.0000		
12.5	1.0000	1.0000	0.7000		
25	0.9000	1.0000	0.9000		
50	0.9000	0.9000	0.8000		
100	0.7000	0.5000	0.7000		

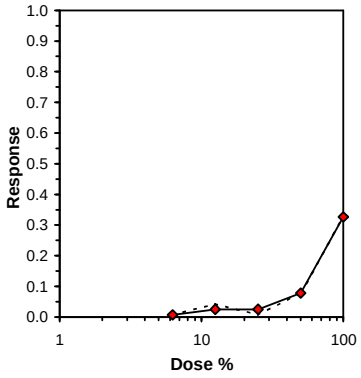
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
D-Control	0.9400	1.0000	0.9400	0.9000	1.0000	5.827	5				0.9400	1.0000
6.25	0.9333	0.9929	0.9333	0.9000	1.0000	6.186	3	0.099	2.624	0.1765	0.9333	0.9929
12.5	0.9000	0.9574	0.9000	0.7000	1.0000	19.245	3	0.595	2.624	0.1765	0.9167	0.9752
25	0.9333	0.9929	0.9333	0.9000	1.0000	6.186	3	0.099	2.624	0.1765	0.9167	0.9752
50	0.8667	0.9220	0.8667	0.8000	0.9000	6.662	3	1.091	2.624	0.1765	0.8667	0.9220
*100	0.6333	0.6738	0.6333	0.5000	0.7000	18.232	3	4.561	2.624	0.1765	0.6333	0.6738

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.89248	0.868	-0.8667	0.6384
Bartlett's Test indicates equal variances (p = 0.37)	5.3628	15.0863		

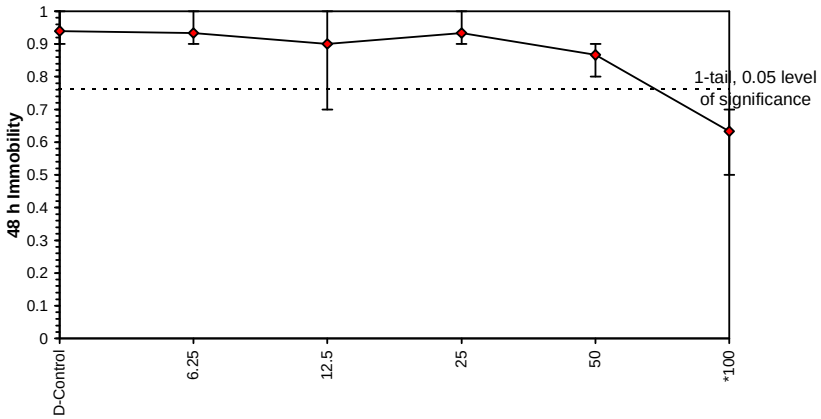
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	50	100	70.7107	2	0.17646	0.18772	0.04377	0.00848	0.00682	5, 14

Log-Logit Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05	36.030	16.090	0.000	72.419	0.0950
IC10	54.621	13.820	0.000	75.319	-1.4521
IC15	64.718	7.907	33.941	88.677	-1.2597
IC20	74.563	7.674	41.965	104.243	0.0856
IC25	84.432				
IC40	>100				
IC50	>100				



Dose-Response Plot



Acute Fish Test-24 h survival

Start Date:	22/09/2004	Test ID:	2390/KF2t	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	26/09/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	22/09/2004	Protocol:	OECD203-Fish Acute Toxicit	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phoslock elutriate				

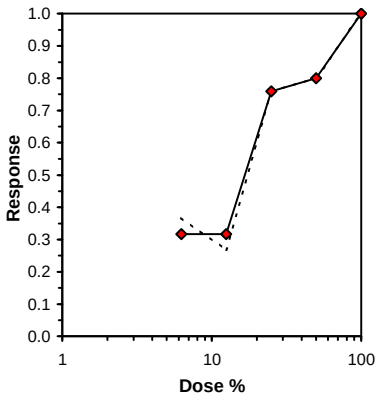
Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	0.9000	0.4000	0.6000		
12.5	0.9000	0.5000	0.8000		
25	0.5000	0.1111	0.1000		
50	0.2000				
100	0.0000				

Conc-%	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	0	50	50	5			0	50
*6.25	0.6333	0.6333	11	19	30	3	0.0000	0.0500	11	30
*12.5	0.7333	0.7333	8	22	30	3	0.0002	0.0500	8	30
*25	0.2414	0.2414	22	7	29	3	0.0000	0.0500	22	29
*50	0.2000	0.2000	8	2	10	1	0.0000	0.0500	8	10
100	0.0000	0.0000	10	0	10	1			10	10

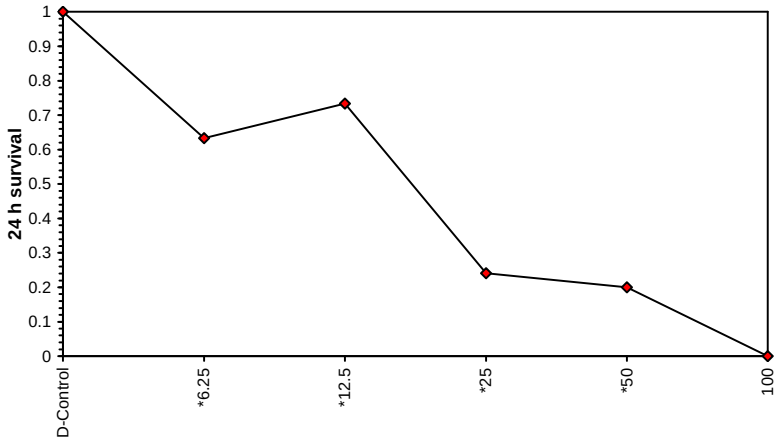
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	<6.25	6.25		

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%			
10.0%			
20.0%			
Auto-31.7%	16.664	13.821	20.092



Dose-Response Plot



Acute Fish Test-48 h survival

Start Date:	22/09/2004	Test ID:	2390/KF2t	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	26/09/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	22/09/2004	Protocol:	OECD203-Fish Acute Toxicit	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phoslock elutriate				

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	0.5000	0.4000	0.6000		
12.5	0.4000	0.5000	0.8000		
25	0.2000	0.2222	0.0000		
50	0.0000				
100	0.0000				

			Transform: Untransformed					1-Tailed			Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	5				0	50
*6.25	0.5000	0.5000	0.5000	0.4000	0.6000	20.000	3	5.857	2.466	0.2105	15	30
*12.5	0.5667	0.5667	0.5667	0.4000	0.8000	36.735	3	5.076	2.466	0.2105	13	30
*25	0.1407	0.1407	0.1407	0.0000	0.2222	86.962	3	10.066	2.466	0.2105	25	29
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	1				10	10
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	1				10	10

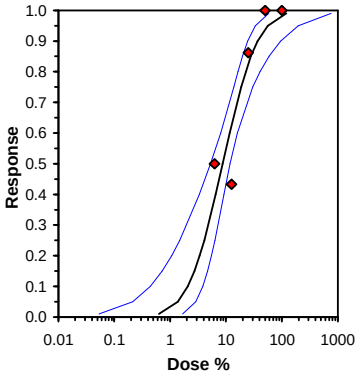
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93416	0.825	0.47804	1.03891
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	<6.25	6.25			0.2105	0.2105	0.48752	0.01366	1.2E-05	3, 10

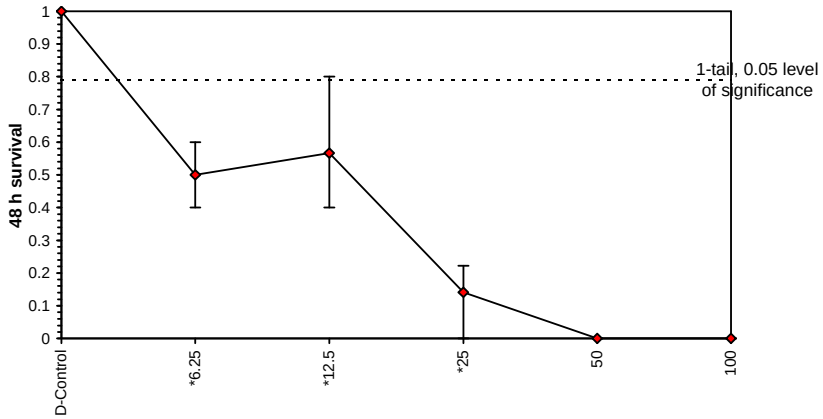
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.04421	0.46659	1.1297	2.95873	0	7.50393	7.81472	0.06	0.94154	0.48919	4
Intercept	3.07528	0.53696	2.02284	4.12773							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.6361	0.05357	1.67012
EC05	3.355	1.37058	0.21253	2.86991
EC10	3.718	2.06361	0.4415	3.84365
EC15	3.964	2.71979	0.72118	4.69302
EC20	4.158	3.38715	1.06271	5.51279
EC25	4.326	4.08878	1.47846	6.34469
EC40	4.747	6.57068	3.33451	9.21121
EC50	5.000	8.74065	5.29369	11.8435
EC60	5.253	11.6273	8.03476	15.9277
EC75	5.674	18.6851	13.81	30.3431
EC80	5.842	22.5555	16.3991	40.9134
EC85	6.036	28.09	19.7079	58.9297
EC90	6.282	37.022	24.476	94.6357
EC95	6.645	55.742	33.2287	193.939
EC99	7.326	120.105	57.7322	760.932



Dose-Response Plot



Acute Fish Test-72 h survival

Start Date:	22/09/2004	Test ID:	2390/KF2t	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	26/09/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	22/09/2004	Protocol:	OECD203-Fish Acute Toxicit	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phoslock elutriate				

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	0.5000	0.4000	0.6000		
12.5	0.4000	0.5000	0.8000		
25	0.2000	0.2222	0.0000		
50	0.0000				
100	0.0000				

			Transform: Untransformed						1-Tailed		Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	5				0	50
*6.25	0.5000	0.5000	0.5000	0.4000	0.6000	20.000	3	5.857	2.466	0.2105	15	30
*12.5	0.5667	0.5667	0.5667	0.4000	0.8000	36.735	3	5.076	2.466	0.2105	13	30
*25	0.1407	0.1407	0.1407	0.0000	0.2222	86.962	3	10.066	2.466	0.2105	25	29
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	1				10	10
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	1				10	10

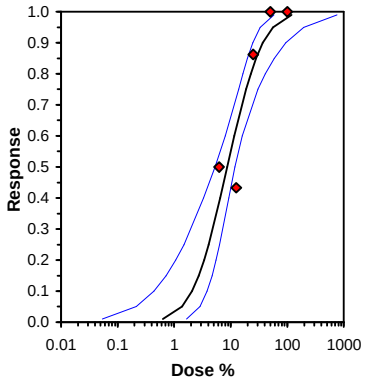
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93416	0.825	0.47804	1.03891
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	<6.25	6.25			0.2105	0.2105	0.48752	0.01366	1.2E-05	3, 10

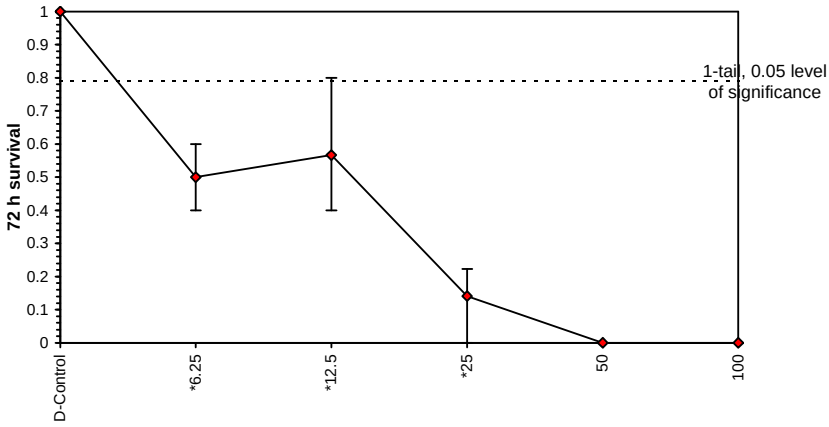
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.04421	0.46659	1.1297	2.95873	0	7.50393	7.81472	0.06	0.94154	0.48919	4
Intercept	3.07528	0.53696	2.02284	4.12773							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.6361	0.05357	1.67012
EC05	3.355	1.37058	0.21253	2.86991
EC10	3.718	2.06361	0.4415	3.84365
EC15	3.964	2.71979	0.72118	4.69302
EC20	4.158	3.38715	1.06271	5.51279
EC25	4.326	4.08878	1.47846	6.34469
EC40	4.747	6.57068	3.33451	9.21121
EC50	5.000	8.74065	5.29369	11.8435
EC60	5.253	11.6273	8.03476	15.9277
EC75	5.674	18.6851	13.81	30.3431
EC80	5.842	22.5555	16.3991	40.9134
EC85	6.036	28.09	19.7079	58.9297
EC90	6.282	37.022	24.476	94.6357
EC95	6.645	55.742	33.2287	193.939
EC99	7.326	120.105	57.7322	760.932



Dose-Response Plot



Acute Fish Test-96 h survival

Start Date:	22/09/2004	Test ID:	2390/KF2t	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	26/09/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	22/09/2004	Protocol:	OECD203-Fish Acute Toxicity	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phoslock elutriate				

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	0.5000	0.4000	0.6000		
12.5	0.4000	0.5000	0.8000		
25	0.2000	0.2222	0.0000		
50	0.0000				
100	0.0000				

			Transform: Untransformed								Number	Total
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD	Resp	Number
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	5				0	50
*6.25	0.5000	0.5000	0.5000	0.4000	0.6000	20.000	3	5.857	2.466	0.2105	15	30
*12.5	0.5667	0.5667	0.5667	0.4000	0.8000	36.735	3	5.076	2.466	0.2105	13	30
*25	0.1407	0.1407	0.1407	0.0000	0.2222	86.962	3	10.066	2.466	0.2105	25	29
50	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	1				10	10
100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	1				10	10

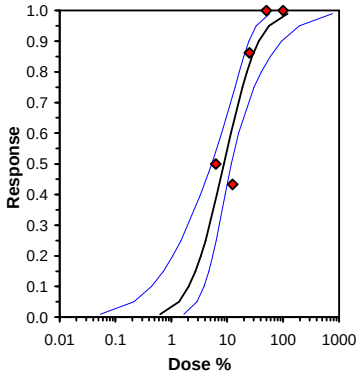
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93416	0.825	0.47804	1.03891
Equality of variance cannot be confirmed				

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	<6.25	6.25			0.2105	0.2105	0.48752	0.01366	1.2E-05	3, 10

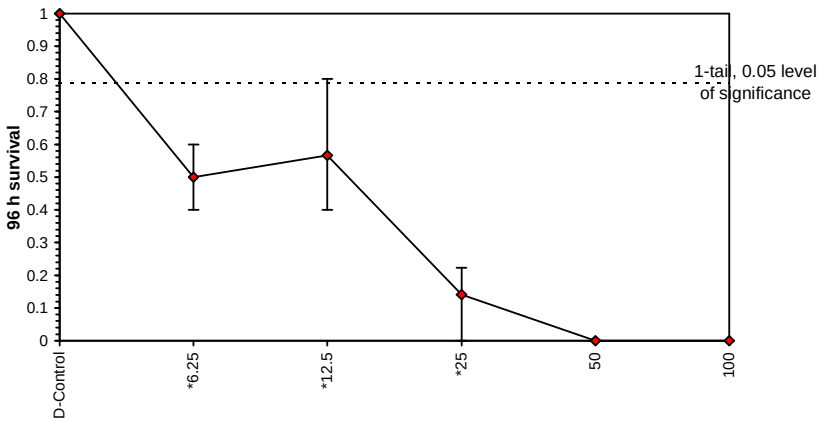
Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.04421	0.46659	1.1297	2.95873	0	7.50393	7.81472	0.06	0.94154	0.48919	4
Intercept	3.07528	0.53696	2.02284	4.12773							
TSCR											

Point	Probits	%	95% Fiducial Limits	
EC01	2.674	0.6361	0.05357	1.67012
EC05	3.355	1.37058	0.21253	2.86991
EC10	3.718	2.06361	0.4415	3.84365
EC15	3.964	2.71979	0.72118	4.69302
EC20	4.158	3.38715	1.06271	5.51279
EC25	4.326	4.08878	1.47846	6.34469
EC40	4.747	6.57068	3.33451	9.21121
EC50	5.000	8.74065	5.29369	11.8435
EC60	5.253	11.6273	8.03476	15.9277
EC75	5.674	18.6851	13.81	30.3431
EC80	5.842	22.5555	16.3991	40.9134
EC85	6.036	28.09	19.7079	58.9297
EC90	6.282	37.022	24.476	94.6357
EC95	6.645	55.742	33.2287	193.939
EC99	7.326	120.105	57.7322	760.932



Dose-Response Plot



Acute Fish Test-24 h survival

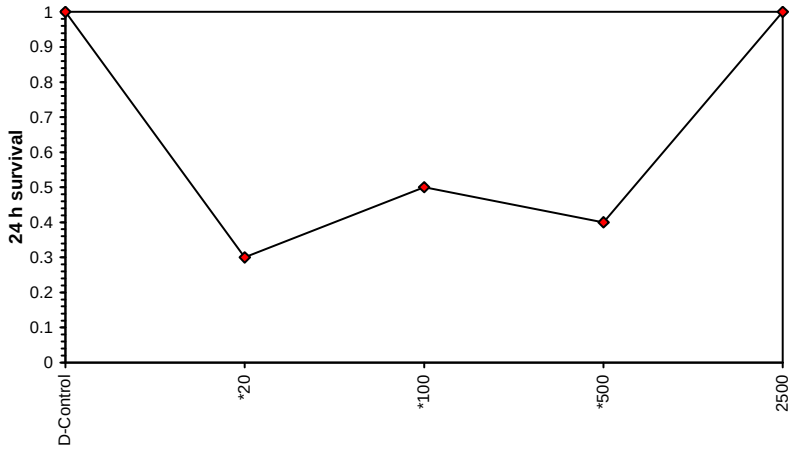
Start Date:	5/10/2004	Test ID:	2390/KF2f	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	6/10/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	5/10/2004	Protocol:	OECD203-Fish Acute Toxicity	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phosphorous additions to 100% elutriate				

P Conc-ppb	1
D-Control	1.0000
20	0.3000
100	0.5000
500	0.4000
2500	1.0000

P Conc-ppb	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	1		
*20	0.3000	0.3000	7	3	10	1	0.0015	0.0500
*100	0.5000	0.5000	5	5	10	1	0.0163	0.0500
*500	0.4000	0.4000	6	4	10	1	0.0054	0.0500
2500	1.0000	1.0000	0	10	10	1	1.0000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	<20	20		

Dose-Response Plot



Acute Fish Test-48 h survival

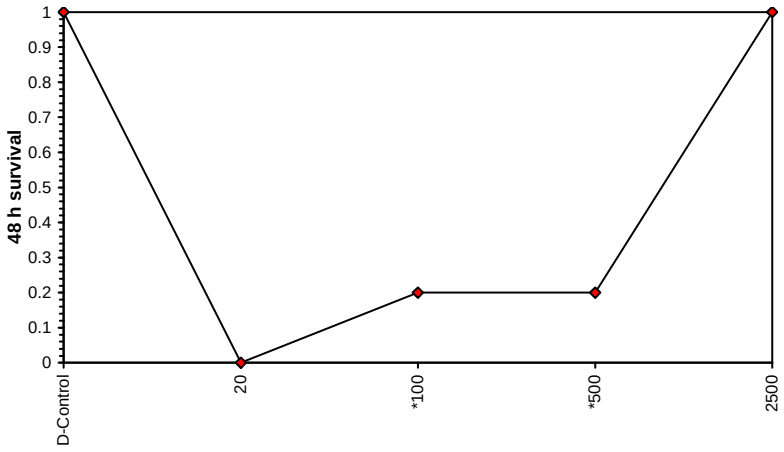
Start Date:	5/10/2004	Test ID:	2390/KF2f	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	6/10/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	5/10/2004	Protocol:	OECD203-Fish Acute Toxicit	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phosphorous additions to 100% elutriate				

P Conc-ppb	1
D-Control	1.0000
20	0.0000
100	0.2000
500	0.2000
2500	1.0000

P Conc-ppb	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	1		
20	0.0000	0.0000	10	0	10	1		
*100	0.2000	0.2000	8	2	10	1	0.0004	0.0500
*500	0.2000	0.2000	8	2	10	1	0.0004	0.0500
2500	1.0000	1.0000	0	10	10	1	1.0000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	<100	100		

Dose-Response Plot



Acute Fish Test-72 h survival

Start Date:	5/10/2004	Test ID:	2390/KF2f	Sample ID:	PNL-Primaxa NZ Ltd
End Date:	6/10/2004	Lab ID:	MLM	Sample Type:	CLAY -Clay leachate
Sample Date:	5/10/2004	Protocol:	OECD203-Fish Acute Toxicit	Test Species:	OM-Oncorhynchus mykiss
Comments:	Phosphorous additions to 100% elutriate				

P Conc-ppb	1
D-Control	1.0000
20	0.0000
100	0.1000
500	0.2000
2500	1.0000

P Conc-ppb	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical
D-Control	1.0000	1.0000	0	10	10	1		
20	0.0000	0.0000	10	0	10	1		
*100	0.1000	0.1000	9	1	10	1	0.0001	0.0500
*500	0.2000	0.2000	8	2	10	1	0.0004	0.0500
2500	1.0000	1.0000	0	10	10	1	1.0000	0.0500

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	<100	100		

Dose-Response Plot