Chronic exposure of midge larvae to Phoslock

NIWA Client Report: AUS2004-005
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Prepared for

ECOWISE Environmental Pty Ltd

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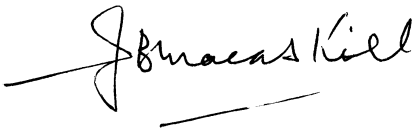

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Executive Summary

NIWA was contracted by ECOWISE Environmental Pty Ltd to conduct a series of toxicity tests examining the effects of applying a granular formulation of Phoslock to freshwater on sediment-dwelling freshwater invertebrates and a fish species. The tests were conducted in a manner designed to simulate the application of granular Phoslock to freshwaters and utilised a range of exposure concentrations in order to encompass potential application scenarios.

The mode of action of this formulation of Phoslock is that granules are added to water, the granules swell and disintegrate as they fall rapidly through the water column, and the product settles on the sediment. The proposed field dosing rate of the compound is 200:1 on a phosphorus mass basis. Thus scenarios were developed for this series of toxicity tests that simulated a lake application. The baseline scenario assumed: (i) a nominal water depth of 2m; and (ii) a phosphorus concentration of 0.2 mg/L; and was equivalent to a Phoslock dose of 40 mg/L in water 2 m deep. Phoslock loadings 0.5X, 5X, or 10X of the baseline scenario were also tested.

This report summarizes the results of 1 chronic sediment toxicity (38 d) test on the freshwater midge larvae *Chironomus zealandicus.* The sample of granular Phoslock manufactured in Australia and known as the Eureka formulation, was provided to NIWA by ECOWISE Environmental Pty Ltd in late April or early May 2004. There is now a second granular formulation of Phoslock available that is manufactured in China and is referred to as the China formulation. We did not test the China formulation.

Results are summarised below. Midge survival, emergence and adult sex ratios appeared to be unaffected by 38 d exposure to even the highest Phoslock application rate (400 mg/L).

Table E1: Summary of Phoslock toxicity testing results (mg Phoslock/L).

Test Species	Test Duration (d)	NOEC[1] (mg/L)	LOEC[2] (mg/L)	LC_{50} [3] (mg/L)	TEC [4] (mg/L)
Chironomus zealandicus	38	400	>400	>400	---

[1]NOEC: The sample concentration causing No Observable Effect relative to the controls
[2]LOEC: The sample concentration causing the Lowest Observable Effect relative to the controls
[3]LC_{50}: The concentration lethal to 50% of the test organisms by the end of the test.
[4] TEC: The Threshold Effect Concentration, is the geometric mean of NOEC and LOEC.

Chemical monitoring of the overlying water showed pH, dissolved oxygen (DO), and conductivity did not change markedly even with addition of high concentrations of Phoslock. Dissolved lanthanum (La) concentrations were relatively low at the end of the toxicity test (7 µg/L), but sediment concentrations were higher at 1830 mg/kg dry weight and corresponded to 30 mg La/g Phoslock added.

1. Introduction

NIWA was contracted by ECOWISE Environmental Pty Ltd to conduct a series of toxicity tests examining the effects of applying the granular formulation of Phoslock to freshwater on sediment-dwelling freshwater invertebrates and a fish species. The results of the 4-d test on rainbow trout and the 10-d survival tests on amphipods, oligochaete worms and midge larvae were reported in a previous document (Clearwater & Hickey, 2004). This report summarizes the results of the chronic midge toxicity test which tested the effect of Phoslock on midge emergence after approximately 30-d exposure.

The test was conducted in a manner designed to simulate the application of granular Phoslock to freshwaters and utilised a range of exposure concentrations in order to encompass potential application scenarios. It is our understanding that granular Phoslock consists primarily of lanthanum-modified bentonite clay, and that lanthanum (La) can leach from the product. The sample of granular Phoslock was provided to NIWA by ECOWISE Environmental Pty Ltd. ECOWISE has informed NIWA that this batch of Phoslock was manufactured in Australia and is known as the "Eureka formulation". A second granular formulation of Phoslock has recently been manufactured in China and will be known as the "China formulation"; NIWA did not test the China formulation.

The mode of action of this formulation of Phoslock is that granules are added to water, the granules swell and disintegrate as they fall rapidly through the water column, and the product settles on the sediment. The toxicity tests therefore need to examine the potential toxicity of Phoslock when it settles on the bed, along with the potential effects of dissolved compounds in the water column. The tests undertaken address both potential exposure routes. The proposed field dosing rate of the compound is 200:1 on a phosphorus mass basis (Gary Bennison, ECOWISE, *Pers. comm.*). Thus scenarios were developed for this series of toxicity tests that simulated a lake application. The baseline scenario assumed: (i) a nominal water depth of 2m; and (ii) a phosphorus concentration to 0.2 mg/L (g/m^3). These depth and phosphorus conditions were used to calculate the baseline Phoslock dose for the tests which was 40 mg Phoslock/L. We tested Phoslock loadings 0.5X, 5X and 10X of the baseline scenario.

The background and basis for the exposure scenarios is provided in the quotation for this study (Appendix 1). The deviations from this quote were: (i) an additional sediment concentration was included for each species; (ii) a chronic (c.30d) chironomid test was also included in the suite of tests; (iii) a higher maximum Phoslock loading (5 mg/L equivalent to 5 g/m^3 P) was included for some tests (requested by G. Bennison, ECOWISE).

2. Methods and Materials

2.1. Test Organisms

The midge (chironomid) species used in this test is a native New Zealand species commonly found in eutrophic freshwaters. Test organisms were obtained by culturing larvae from egg masses. The egg masses were obtained from the NIWA maturation troughs at the Ruakura research station. The ponds receive secondary/tertiary-treated domestic wastewater. The midge larvae were cultured at 20°C in Fernhollow spring water with either pre-sieved (< 500 μm) mud or shredded paper towels as substrate and fed on a mixture of Tetramin and cerophyll until approximately the 2nd instar. Prior to testing the larvae were sorted into groups of 5 in ~8mL Fernhollow spring water using disposable plastic pipettes.

2.2. General Methods

A chronic sediment toxicity test of midge larvae (*Chironomus zealandicus*) was conducted using a sample of granular Phoslock supplied to NIWA by ECOWISE Environmental Pty Ltd (May 2004) and according to the Organization for Economic Cooperation and Development draft protocol 219 "Sediment-water chironomid toxicity test using spiked water" (OECD 2001). The protocol was developed for the midge species *Chironomus riparius* and *Chironomus tentans*, but can be used for "other well documented chironomid species". NIWA has recently developed protocols for rearing and toxicity testing using the native New Zealand species *Chironomus zealandicus*, therefore we used this species in the toxicity tests of Phoslock (Table 1).

The protocol was slightly modified because a) this was not a standard test of sediment toxicity, rather we were testing the toxicity of a substance that settles on the sediment surface and b) if necessary, methods were adapted to native New Zealand species (e.g. test duration) .

Table 1: Test species, test duration and protocols followed to examine the effect of granular Phoslock on midge larvae emergence.

Test Organism	Test Species	Test Duration (d)	Protocol
Midge Larvae (chironomid or bloodworm)	*Chironomus zealandicus*	38	OECD (2001) modified

A summary of the test method is included in Appendix B, but in brief, 20 days prior to commencing the Phoslock exposure, control sediments were prepared by mixing ~50% sieved (<250 µm) surficial mud from Fernhollow spring and ~50% sieved (<500 µm) sand from the Waikato River. Equal amounts (~114 g wet weight) of sediment were added to acid-washed 500 mL glass jars, and overlaid with ~408 mL. The test start was delayed because midge larvae were unexpectedly unavailable, therefore the overlying water in the jars was gently aerated until the day the test started.

After 20 days the overlying water was removed by suction, replaced by pouring gently onto plastic covers laid on top of the sediment to minimise resuspension of fine particles, and gently aerated. The test organisms were added after approximately 2 h aeration and allowed to settle in the new environment for approximately 7 h prior to the addition of Phoslock. Phoslock was added as a slurry[1] by pre-weighing 4 separate lots of pellets (one for each treatment) adding 260 mL freshwater to each aliquot of pellets, mixing thoroughly with a trimmed 10 mL pipette tip and gently adding 20 mL slurry to the water surface of each jar. Control jars were treated in a similar manner by addition of 20 mL freshwater. Gentle aeration commenced approximately 1 h after addition of Phoslock. All testing was undertaken at 20°C. All tests were modified from the standard protocol to include feeding during tests in order to prevent starvation as a result of the addition of the inorganic Phoslock layer. Larvae were fed 1 h after being transferred to the jars and every second day thereafter. We consider that normal lake processes would result in continued deposition of organic food material in the natural situation. Larvae responded strongly to feeding by emerging from burrows almost immediately, and approximate numbers visible were recorded on each feeding occasion.

Water quality, aeration and animal behaviour were monitored daily throughout the exposures. Prior to midge emergence the hard plastic jar cover was weighed down so that it was flush with the jar openings, preventing the escape of adult midges. During the period of midge emergence, adult midges were counted and sexed every second day then removed from the jars. Numbers of emergence cases and visible pupae and larvae were also recorded.

Once control emergence was 50-70% (as stipulated in the protocol) (i.e., after 38 d in this test) water samples were removed, and all sediments were sieved to retrieve any remaining test organisms. Sediment samples were taken from the sixth replicate (or eleventh replicate in the case of the controls) of each treatment which was used to

[1] Note that preliminary trials found that addition of Phoslock granules to sediment test jars did not result in an even distribution of material over the sediment. Thus a slurry was used which resulted in an even sediment distribution.

measure water quality, but not behavioural responses of the organisms (i.e., these sediments were not sieved at the end of the exposure).

2.3. Application rates

After discussion with the client we chose to simulate application of the product to water 2 m deep, and for a phosphorus concentration of 0.2 mg/L. The water depth was chosen on the basis that many of the water bodies likely to be treated with Phoslock will be at least 2 m deep, and the density of sediment-dwelling invertebrates is likely to be highest at 2 m depth and shallower (non-light limited). Granular Phoslock will be applied to the water surface and will sink rapidly through the water column forming a layer on the sediment surface whose thickness will depend on the application rate. These toxicity tests were aimed at examining the effect of Phoslock on sediment-dwelling organisms, therefore although the toxicity testing containers were not 2 m deep we applied Phoslock at concentrations that would simulate the quantity of product that would accumulate on the corresponding sediment surface area at the bottom of a 2 m deep water column.

We were informed that Phoslock is sometimes used in Australia to treat phosphorus concentrations as high as 5 mg/L (G. Bennison, ECOWISE, *Pers. Comm.* 11/5/04), therefore this concentration was used as our maximum treatment in two initial toxicity tests performed on amphipods and worms (Clearwater & Hickey, 2004). A lower concentration of 2 mg/L was used as the maximum application rate in the other tests (10 d midge larvae, rainbow trout). Preliminary tests showed that granular Phoslock sinks rapidly through the water column and it is our understanding that Phoslock application rates are currently set at 200 parts Phoslock:1 part phosphorus, however in the future application rates may be targeted at producing a 1 mm layer of Phoslock on the sediment surface. With this in mind, we have reported our testing concentrations in terms of Phoslock concentrations (mg/L) applied to a 2 m water column but have also provided measurements of the depth of the Phoslock layer these application rates produced on the sediment in the various tests (Table 2).

Phosphorus concentrations were not changed between the different treatments. The measured background P concentrations were 0.05 mg P/L in the filtered dilution water, which is ~25% of the P concentration assumed in the baseline scenario (0.2 mg/L). If the presence of P affects either the leaching of La from Phoslock, or the toxicity of La then the toxicity of Phoslock to aquatic organisms might be different (possibly lower) than measured in the current tests.

Table 2: Phoslock application rates (mg/L) appropriate for waters 2 m deep used in the toxicity test, mass of Phoslock added to each replicate, water volume in each replicate, and the depth of the Phoslock layer measured on the sediment surface.

Test Species	Test Duration (d)	Corresponding Phosphorus Concentration (mg/L)	Application Rate (mg/L)	Nominal Scenario	Mass Phoslock added to each replicate (g)	Water volume in each replicate (mL)	Depth of Phoslock on sediment surface (mm)
Midge (*C. zealandicaus*)	38	0.0	0	Control	0	407±8	0
		0.1	20	0.5X	0.24	407±8	<0.4
		0.2	40	1.0X	0.48	407±8	1.0
		1.0	200	5.0X	2.38	407±8	2-4[1]
		2.0	400	10.0X	4.76	407±8	4-8[1]

[1]Bioturbation created a light flocculated layer of Phoslock on the sediment surface mixed in with sediment

2.4. Chemical analyses of water and sediment

Composite water samples were taken of each treatment at the beginning and end of each toxicity test. A selection of subsamples were filtered (0.45 µm), all samples were acidified and stored (4°C). One filtered water sample of the highest application rate treatment from the end of the Phoslock exposure was analysed for dissolved lanthanum (La), calcium (Ca) and phosphorus (P) (Table 9). Sediment samples were taken from the chemistry replicates after thoroughly mixing the sediment. The samples were dried at 60°C until a constant dry weight was achieved and a single sediment sample was submitted to Hill Laboratories for analysis of total recoverable La, P and Ca.

2.5. Reference toxicity test

A reference toxicity test was conducted using the reference toxicant zinc (Zn) added as $ZnSO_4.7H_2O$. The midge larvae were exposed for 96 h in a static non-renewal "water only" (i.e., no sediment) exposure. Test methods are summarised in Appendix B but briefly, test solutions were prepared by diluting a $ZnSO_4.7H_2O$ solution in Fernhollow spring water. Test organisms were counted out in groups of 5 and added to ~30 mL test solution in plastic containers (3 replicates/concentration). The containers were covered, placed in the dark at 20°C, and checked daily. After 48 and 96 hours exposure, numbers of live and dead organisms were recorded.

The reference toxicant results would normally be compared to the mean EC_{50} values obtained from the NIWA Ecotoxicology Laboratory quality control programme to determine whether the toxicity testing organisms had similar toxicant sensitivity to previous tests, however, NIWA has no prior official quality control data on this species. Previous research on this species guided the choice of Zn concentrations. In addition, the results can be compared to published values for a variety of freshwater species to determine the sensitivity of the species in comparison to others (USEPA 1987)

3. Results and Discussion

3.1. General toxicity results

Statistical results, data observations, original raw data and project notes are maintained at NIWA in a confidential project file.

The dosing system was successful in generating an even sediment distribution of Phoslock and a range of sediment thickness from <0.4 mm to >5mm (Table 2). The slurry material settled rapidly (<24h).

Average control survival was good (>89%) and within acceptable limits for test validity (Appendix B). Chironomid toxicity tests are notorious for poor control survival and high variability, therefore the results of this trial were of a high standard. Results are summarised below for organism survival and midge emergence (Table 3). Even the highest Phoslock application rate (400 mg/L, or 10X the baseline scenario) had no significant effect on midge survival or emergence (Figure 1A-B, Appendix C) or the sex ratio (Figure 2). This result agrees with the result of the 10 d sediment toxicity test of the chironomid *Polypedilum parvidum* in which even the highest Phoslcok application rate (400 mg/L) had no effect on larval survival (10 day NOEC = 400 mg/L, LOEC = >400 mg/L, EC_{50} = >400 mg/L) (Clearwater and Hickey, 2004).

Table 3: Summary of the toxicity testing results for Phoslock effects on midge larval survival and emergence after 38 d exposure.

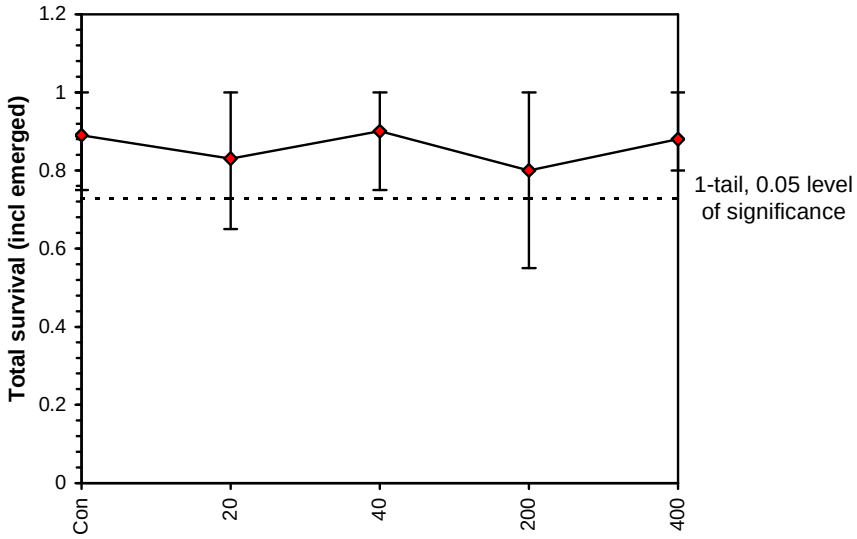
Test Species	Endpoint	Test Duration (d)	NOEC[1] (mg/L)	LOEC[2] (mg/L)	LC_{50} [3] (mg/L)	TEC [4] (mg/L)
Midge	Survival	38	400	>400	>400	---
	Emergence	38	400	>400	>400	---
	Sex ratio	38	400	>400	>400	---

[1]NOEC: The highest sample concentration causing No Observed Effect relative to the controls.

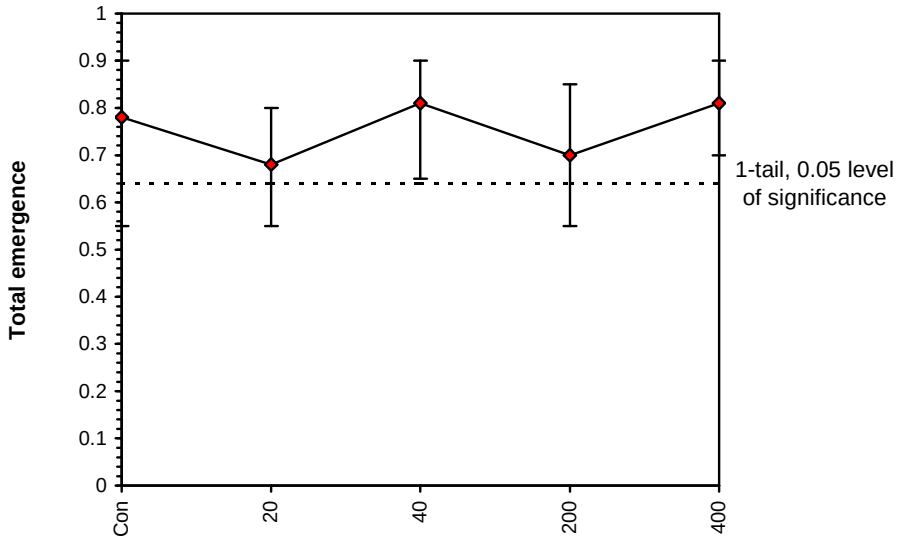
[2]LOEC: The sample concentration causing the Lowest Observed Effect relative to the controls.

[3]EC_{50}: The concentration lethal to 50% of the test organisms by the end of the test.

[4] TEC: The Threshold Effect Concentration, is the geometric mean of NOEC and LOEC.



A)



B)

Phoslock application rate (mg Phoslock/L)

Figure 1: **A)** Midge larvae survival after 38 d exposure to different Phoslock application rates. **B)** Midge emergence after 38 d exposure to different Phoslock application rates.

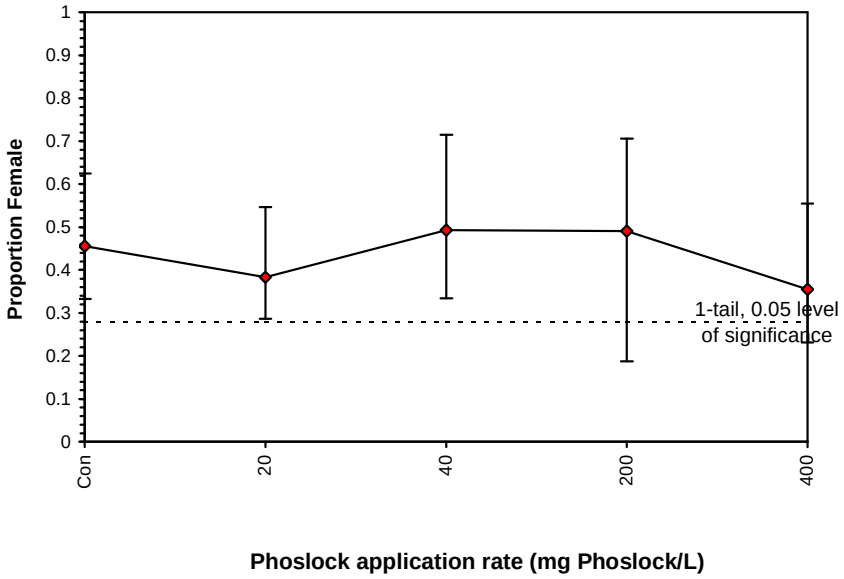


Figure 2: Midge sex ratio after 38 d exposure to different Phoslock application rates**.**

3.2. Reference toxicity test results and test sensitivity

The reference toxicity test showed that the midge species *C. zealandicus* is relatively insensitive to Zn (96 h LC_{50} 81 mg Zn/L), but is more sensitive than the other native New Zealand midge *Polypedilum parvidum* which was used for the 10 d survival sediment toxicity test (Clearwater & Hickey, 2004). *C. zealandicus* would rank between the 34th and 35th least sensitive freshwater species listed by the USEPA (1987). Although we have not conducted official reference toxicant tests with this species before *C. zealandicus* was chosen for the test because it is a common New Zealand native chironomid species found in eutrophic waters, that feeds and burrows in the first 1-5 mm of sediment during its larval stage and will therefore interact significantly with Phoslock settled on the sediment surface. The midges were observed to have made burrows through the layer of Phoslock in the highest application rate treatments and appeared to be feeding normally. The physiologically demanding processes of morphogenesis into pupae and emergence from pupae into flying adults do not appear to have been affected by the presence of Phoslock on or in the sediment surface.

3.3. Water quality

The water quality data suggest that factors other than the presence of Phoslock did not cause mortality (Tables 4-8). Dissolved oxygen was >6.5 mg/L throughout all of the tests, and was usually >7.9 mg/L. The pH was usually around 8.2, varying between a minimum of 7.8 and a maximum of 8.4 over all four toxicity tests. Un-ionised ammonia concentrations in chemistry replicates of the controls and the highest application rate (400 mg/L) were undetectable (<0.02 mg/L) at the start and end of the test and were less than the chronic ANZECC (2000) guideline value (1.2 mg/L, pH 7.8, page 8.3-161). Hardness was 123-202 mg/L as $CaCO_3$ in composite samples from the controls and highest application rate taken at the start and end of the exposure.

Table 4: Dissolved oxygen concentrations (mg/L) measured in chemistry replicates throughout the toxicity test (n = 39).

Treatment	Average	Std Dev	Maximum	Minimum
Control	8.08	0.55	9.07	6.72
20	7.98	0.63	9.25	6.58
40	8.00	0.59	9.22	6.63
200	8.09	0.58	9.26	6.86
400	8.09	0.56	9.21	6.88

Table 5: The pH measured in chemistry replicates throughout the toxicity test (n = 13).

Treatment	Average	Std Dev	Maximum	Minimum
Control	8.15	0.13	8.28	7.76
20	8.22	0.08	8.36	8.06
40	8.23	0.08	8.39	8.10
200	8.23	0.08	8.34	8.10
400	8.16	0.12	8.3	7.91

Table 6: Temperature (^{o}C) measured in chemistry replicates throughout the toxicity test (n = 15).

Treatment	Average	Std Dev	Maximum	Minimum
Control	19.7	0.7	20.7	18.5
20	19.7	0.6	20.6	18.6
40	19.7	0.6	20.4	18.5
200	19.7	0.6	20.3	18.5
400	19.7	0.6	20.4	18.4

Table 7: Conductivity (µS/cm) measured in chemistry replicates throughout the toxicity test (n = 7).

Treatment	Average	Std Dev	Maximum	Minimum
Control	480	54	536	372
20	483	48	528	383
40	496	55	556	389
200	554	54	596	441
400	571	58	622	452

Table 8: Hardness (mg/L as $CaCO_3$) and ammonia (total NH_3-N mg/L) measured in composite samples taken from the controls and highest application rate at the start and end of the Phoslock exposure.

Treatment	Hardness –Start[1] (mg/L as $CaCO_3$)	Hardness-End (mg/L as $CaCO_3$)	Ammonia –Start (mg NH_3-N /L)	Ammonia – End (mg NH_3-N /L)
Control	138	186	<0.02	<0.02
400	123	202	<0.02	<0.02

[1] Prior to addition of Phoslock

3.4. Lanthanum concentrations in water and sediment

Dissolved La concentrations were relatively low (7 µg/L) by the end of the 38d midge exposure Table 9. In the previous 10 d midge test of this granular formulation of Phoslock dissolved La concentrations in the highest treatment (400 mg/L) were 140 µg/L 10 d after application of the Phoslock (Clearwater & Hickey, 2004).

Table 9: Concentration of dissolved lanthanum (La), calcium (Ca) or phosphorus (P) in selected samples taken from the end of the chronic midge toxicity test. The water sample was a composite of all replicates in the treatment (not including the chemistry replicates).

Media	Test Concentration (mg Phoslock/L)	Sampling Time (d)	Dissolved La (µg/L)	Dissolved Ca (mg/L)	Dissolved P (mg/L)
Water	400	38	7.7	45.1	0.05

Table 10: Total lanthanum (La), calcium (Ca) and phosphorus (P) measured in a selected sediment sample taken at the end of the 38 d midge test from the chemistry replicate after thorough mixing, but not sieving to remove organisms. Lanthanum concentrations converted to (µg La/cm^2 sediment using, jar area (59.45 cm^2), actual weights of sediment, and dry:wet weight sediment ratios.

Media	Test Concentration (mg Phoslock/L)	Total La (mg/kg dry weight)	Total La (µg La/cm^2 sediment)	Total Ca (mg/kg dry weight)	Total P (mg/kg dry weight)
Sediment	400	1830	2437	18000	218

It is likely that La concentrations were similar at 10 d in the chronic midge exposure, because the tests were run simultaneously under identical conditions, except that the sediment was placed in the jars for the chronic test 20 d earlier than for the acute test. The sediment was prepared early because the chronic test did not begin as planned because test organisms were unexpectedly not available. Water hardness was much higher in the chronic midge toxicity test (123-202 mg /L as $CaCO_3$), than in the acute midge test (73-76 mg/L as $CaCO_3$), possibly because the sediment chemistry was different between the two tests due to different equilibration times. Accordingly dissolved Ca concentrations were higher at the end of the chronic midge test (45 mg/L) than at the end of the 10 d midge test (9 mg/L). Dissolved phosphorus concentrations (0.05 mg/L) were also slightly higher at the end of the chronic midge tests than in all of NIWA's previous Phoslock tests where dissolved phosphorus was below the detection limit (<0.02 mg/L).

The La concentrations in the sediment at the end of the chronic midge test (1830 mg/kg dry weight) were comparable to the sediment concentration (1990 mg/kg dry weight) measured at the end of the worm test in the 1000 mg/L treatment , but were much lower than measured at the end of the 10 d midge test in the 400 mg/L treatment (5120 mg/kg dry weight). This latter value is markedly higher than concentrations measured in the other tests and may represent difficulties associated with sub-sampling of this test, including the uneven layer of Phoslock over the sediment, and only measuring one sample. The La dose in the chronic midge test was 30 mg La/g Phoslock which compares well with the values calculated for the previous worm and amphipod tests (25 and 33 mg La/g Phoslock), but is much lower than the 86 mg La/g Phoslock measured in the 10 d midge test. These data also suggest the sample taken from the 10 d midge test is an anomaly compared to the others, rather than representing a real trend.

3.5. General discussion

3.5.1. Dosing system approach

The slurry dosing was required to create an even distribution in the sediment toxicity test jars. Our observations indicated that the Phoslock pellets disintegrated rapidly when added to water, however, a depth of 1-2 m would have been required to give even coverage in the testing jars. Our application method meant that the organisms were exposed to relatively high turbidity for <12 h after application of the Phoslock, but for the majority of the exposure experienced only low levels of suspended material in the water column (visual assessment) in the highest concentrations (200 and 400 mg/L), and leachate from the Phoslock. For most of the exposure period the sediment-dwelling organisms were exposed primarily to the deposited Phoslock layer. There

was no obvious behavioural response of the midges to the application of Phoslock. The midges had burrowed into the sediment at the time of Phoslock application and remained out of sight.

The use of the standard sediment testing approach to test Phoslock did not include replacement of the overlying water, hence excessive leaching of Phoslock might have occurred that might not be representative of Phoslock used in a natural situation. However even under this scenario any La that may have leached from the Phoslock had no observable adverse effect on the midge development and emergence.

3.5.2. Scaling to in-lake situations

The baseline scenario tested represented a nominal lake depth of 2 m and a moderately high phosphorus concentration of 0.2 mg P/L (0.2 g/m^3) and a 200:1 Phoslock:P application rate. The highest organism exposures were up to 10X this baseline scenario. Extrapolation of these results to lake applications may be satisfied by a range of water depth and phosphorus combinations. For example, assuming an effects threshold at 0.5X the baseline scenario corresponds to a 1 m deep lake with 0.2 mg P/L, or a 2 m deep lake with 0.1 mg P/L.

In practice a wide range of factors should be considered in determining an appropriate lake application rate. These include considering whether thermal stratification and deoxygenation is occurring in the water body which will mean no living organisms are present in sediments and thus allow higher dosing; and adjusting application rates relative to areas of differing lake depth. Notably, the toxicity data have indicated worms and chironomid larvae may be particularly tolerant of high sediment applications, even during midge larval morphogenesis into pupae and emergence into adults. Thus, high application rates may not result in extreme effects on all sediment-dwelling species but only sensitive species. To date, the toxicity data generated by NIWA indicate that the amphipod *Phreatogammarus helmsii* is the species most sensitive to Phoslock applications with an 10d LC_{50} of 33 mg/L. These conclusions apply only to the Eureka granular formulation (manufactured in Australia) of Phoslock tested by NIWA in June – July 2004. We have been informed that a different granular formulation of Phoslock is now available (July 2004) and is manufactured in China (China formulation). NIWA did not test the China formulation of Phoslock.

4. References

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Appendix A

Quotation brief for toxicity testing programme

NIWA
Australia

12 March 2004

Dr Garry Bennison
ECOWISE Environmental Pty Ltd
PO Box 673
Spring Hill
Queensland
Australia 4004

Dear Garry

Ecotoxicity testing of Phoslock

Based on discussions with yourself and Jamie Corfield of NIWA Australia, we have prepared the following quote to undertake toxicity testing of the granule form of Phoslock on aquatic biota.

Our understanding of the mode of action of this compound is that granules are added to water, rapid dispersion occurs and the product then slowly settles on the bed. The toxicity tests therefore need to examine the potential toxicity of the flocculant when it settles on the bed, along with the potential effects of dissolved compounds in the water column. The tests we propose address both potential exposure routes. Results will be reported on the basis of chemically analysed lanthanum (La) concentrations, since this is the key toxicity component. The analytical detection limits for La are: 0.2 mg m^{-3} for water and 0.2 mg kg^{-1} dry wt for sediments. The water detection limit is well below known species sensitivity to La.

We propose that a risk assessment approach be taken to determining the dose at which no effects would be observed on the test organisms. The tests proposed attempt to mimic the exposure route most likely to occur in the field. The proposed dosing rate of the compound is 200:1 on a phosphorus basis. We would expose test animals (in 5 replicate tests) to 3 doses. For sediment dwelling invertebrates, these doses would assume the proposed dosing rate represented minimum exposure and undertake to establish whether effects would or would not occur at progressively higher dose levels (e.g., 5X, 10X). The dosed rate will assume a nominal water depth of 2m and a phosphorus concentration to 200 mg m^{-3} to obtain the sediment dose. We anticipate that this will result in an approximate 1 mm layer thickness of Phoslock on sediments at the recommended dose. As fish are mobile, tests undertaken in beakers are likely to represent the worst case scenario for exposure, as they would be representative of only the shallow regions of the anticipated field environment (e.g., a lake). The proposed dosing rate would therefore represent the highest exposure rate, with concentrations higher and lower than the recommended dose. Our test regime would involve exposing fish to this range of concentrations to enable the calculation of a dose-

response. All sediment tests would be conducted using established laboratory protocols. For the trout tests we would use sand sediment in the tanks with the trout in place during the dosing procedure. This would simulate both water column and ongoing exposure from sediments. Supporting chemical analyses will include lanthanum concentrations of the dosed sediments, their overlying water and total and dissolved lanthanum in the trout exposures.

-2-

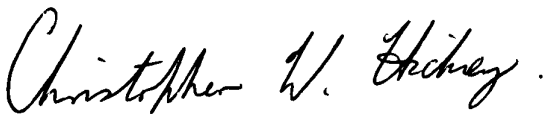
We propose the following standard test species be used to examine toxicity.

Species	**Duration/Effect measured**	**Test type**
Sediment Amphipod (*Chaetocorophium cf lucasi* or similar species)	10d survival	Acute
Oligochaete (*Lumbriculus variegatus*)	10d survival	Acute
Chironomid (*Chironomus sp*)	10d survival	Acute
Water column Rainbow trout (*Oncorhynchus mykiss*)	96hr survival	Acute

Analytical quality control tests using reference toxicants (zinc) are included in these tests. Pore water testing will include pH and ammonia following completion of experiments.

[Section deleted]

Yours sincerely



Christopher W. Hickey (Dr)
Senior Research Scientist

Appendix B

Summary of toxicity test protocol

Summary of test conditions for chronic midge bioassay

Project Name: Phoslock **Test Initiation:** 1/6/04	**Project Number:** NAU04927/2384
Test Protocol:	OECD 2001 (modified)
Test Material:	Phoslock
Test Organisms:	*Chironomus zealandicus* – laboratory reared
Source:	Fernhollow Farm, Hamilton
Organisms/Container:	20 per replicate
Test Concentrations:	Control, 20, 40, 200, 400 mg Phoslock/L
Replicates:	11 (included a chemistry replicate)
Feeding:	Fed 0.8 mg Cerophyll/organism every 2 days
Reference Toxicant:	Zinc sulphate
Reference Toxicant Concentrations:	0, 5, 10, 100, 500, 1000 mg Zn/L[1]
Test Duration:	38 d
Sample Pre-treatment:	Freshwater added to pellets to make a slurry
Dilution Water	Fernhollow Spring Water
Test Chambers:	500 mL glass jars
Lighting:	16L:8D
Temperature:	20 ± 1 ^{o}C
Salinity:	0 ppt
Aeration:	Aerated
Chemical Data:	Initial and final hardness and ammonia in control and highest concentration. Final conductivity in all treatments Dissolved oxygen daily, temperature & pH weekly, all treatments. Water and sediment samples beginning and end.
Behavioural Data:	Presence/absence, live/dead, & behaviour noted daily, emergence (including sex) recorded every second day.
Effect Measured:	Emergence, survival
Test Acceptability:	Minimum average control survival 80%, minimum control survival in each replicate 75%, LC_{50} for reference toxicant within ±2S.D. of NIWA long term average[1].

[1]NIWA has no previous reference toxicity data on this species.

Appendix C

Response data statistics for tests

96 h Midge Larval Survival-Total survival (incl emer

Start Date:	1/06/2004	Test ID:	2384/JZczs	Sample ID:	PS-Phoslock
End Date:	9/07/2004	Lab ID:	SC-Sue Clearwater	Sample Type:	STDFW-Standard Freshwater Sediment
Sample Date:	1/06/2004	Protocol:	OECD 2001 -Draft (revised fr	Test Species:	CZ-Chironomus zealandicus
Comments:	28 Midge survival data from emergence test -35d long				

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
Con	1.0000	1.0000	0.8000	0.9500	0.9000	0.9000	0.9500	0.7500	0.9000	0.7500
20	0.8000	1.0000	0.6500	0.8500	0.8500					
40	0.8500	0.9500	0.9500	0.7500	1.0000					
200	0.9000	0.6000	0.9500	1.0000	0.5500					
400	0.8500	0.8000	0.9000	1.0000	0.8500					

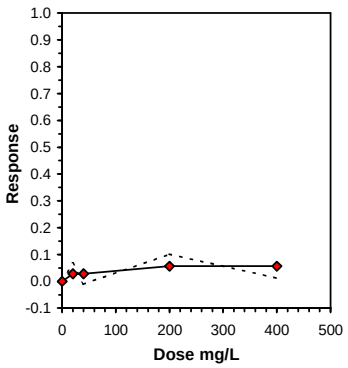
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Con	0.8900	1.0000	0.8900	0.7500	1.0000	10.527	10				0.8900	1.0000
20	0.8300	0.9326	0.8300	0.6500	1.0000	15.120	5	0.889	2.385	0.1610	0.8650	0.9719
40	0.9000	1.0112	0.9000	0.7500	1.0000	11.111	5	-0.148	2.385	0.1610	0.8650	0.9719
200	0.8000	0.8989	0.8000	0.5500	1.0000	26.146	5	1.333	2.385	0.1610	0.8400	0.9438
400	0.8800	0.9888	0.8800	0.8000	1.0000	8.617	5	0.148	2.385	0.1610	0.8400	0.9438

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.96788	0.9	-0.4035	-0.465
Bartlett's Test indicates equal variances (p = 0.23)	5.63127	13.2767		

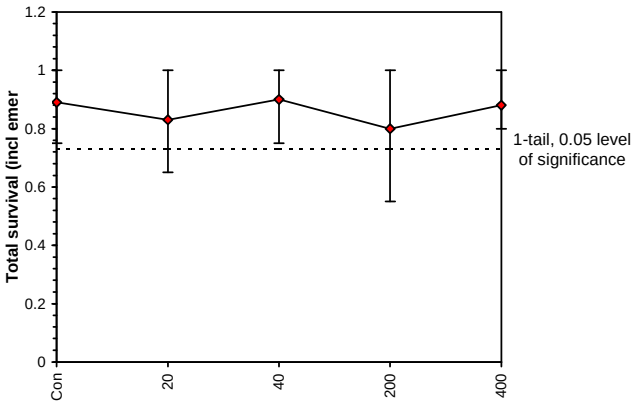
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	400	>400			0.16103	0.18093	0.01019	0.0152	0.61874	4, 25

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	164.80			
IC10	>400			
IC15	>400			
IC20	>400			
IC25	>400			
IC40	>400			
IC50	>400			



Dose-Response Plot



Midge Emergence ~28d-Total emergence

Start Date:	1/06/2004	Test ID:	2384/JZ1cz	Sample ID:	PS-Phoslock
End Date:	9/07/2004	Lab ID:	SC-Sue Clearwater	Sample Type:	STDFW-Standard Freshwater Sediment
Sample Date:	1/06/2004	Protocol:	OECD 2001 -Draft (revised fr	Test Species:	CZ-Chironomus zealandicus
Comments:	28 Midge emergence test				

Conc-mg/L	1	2	3	4	5	6	7	8	9	10
Con	0.8500	0.8000	0.5500	0.9000	0.6000	0.8500	0.8500	0.7500	0.9000	0.7500
20	0.7000	0.8000	0.5500	0.6500	0.7000					
40	0.7500	0.9000	0.8500	0.6500	0.9000					
200	0.7500	0.6000	0.8500	0.7500	0.5500					
400	0.8000	0.8000	0.7000	0.9000	0.8500					

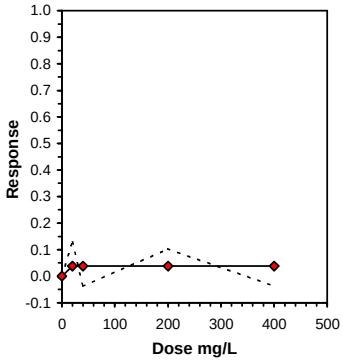
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-mg/L	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Con	0.7800	1.0000	0.7800	0.5500	0.9000	15.467	10				0.7800	1.0000
20	0.6800	0.8718	0.6800	0.5500	0.8000	13.357	5	1.686	2.385	0.1414	0.7500	0.9615
40	0.8100	1.0385	0.8100	0.6500	0.9000	13.382	5	-0.506	2.385	0.1414	0.7500	0.9615
200	0.7000	0.8974	0.7000	0.5500	0.8500	17.496	5	1.349	2.385	0.1414	0.7500	0.9615
400	0.8100	1.0385	0.8100	0.7000	0.9000	9.156	5	-0.506	2.385	0.1414	0.7500	0.9615

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.93788	0.9	-0.6592	-0.4399
Bartlett's Test indicates equal variances (p = 0.85)	1.36166	13.2767		

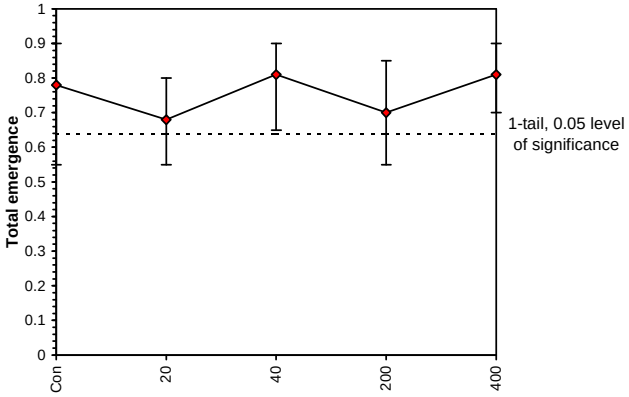
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	400	>400			0.1414	0.18128	0.01975	0.01172	0.18488	4, 25

Linear Interpolation (200 Resamples)

Point	mg/L	SD	95% CL(Exp)	Skew
IC05	>400			
IC10	>400			
IC15	>400			
IC20	>400			
IC25	>400			
IC40	>400			
IC50	>400			



Dose-Response Plot



Midge Sex Ratio Chronic-Proportion Female

Start Date:	1/06/2004	Test ID:	2384/JZ1cz	Sample ID:	PS-Phoslock
End Date:	9/07/2004	Lab ID:	SC-Sue Clearwater	Sample Type:	STDFW-Standard Freshwater Sedimen
Sample Date:	1/06/2004	Protocol:	OECD 2001 -Draft (revised fr	Test Species:	CZ-Chironomus zealandicus
Comments:	sex ratio data added				

Conc-%	1	2	3	4	5	6	7	8	9	10
Con	0.4706	0.4000	0.3333	0.3333	0.4167	0.6250	0.4118	0.6000	0.3889	0.5714
20	0.3846	0.4118	0.5455	0.2857	0.2857					
40	0.3333	0.4444	0.5000	0.7143	0.4706					
200	0.4286	0.6000	0.7059	0.5333	0.1875					
400	0.2308	0.2667	0.2857	0.5556	0.4375					

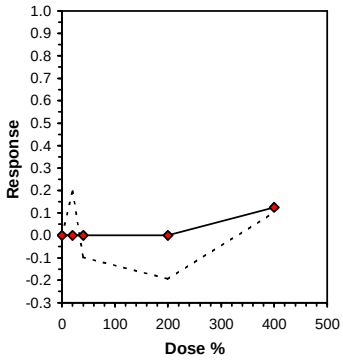
			Transform: Untransformed					1-Tailed			Isotonic	
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD	Mean	N-Mean
Con	0.4551	1.0000	0.4551	0.3333	0.6250	23.628	10				0.4898	1.0000
20	0.3827	0.8408	0.3827	0.2857	0.5455	28.071	5	0.977	2.385	0.1769	0.4898	1.0000
40	0.4925	1.0822	0.4925	0.3333	0.7143	28.237	5	-0.505	2.385	0.1769	0.4898	1.0000
200	0.4911	1.0790	0.4911	0.1875	0.7059	40.198	5	-0.485	2.385	0.1769	0.4898	1.0000
400	0.3552	0.7806	0.3552	0.2308	0.5556	38.563	5	1.346	2.385	0.1769	0.4286	0.8749

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95701	0.9	0.06107	-0.1745
Bartlett's Test indicates equal variances (p = 0.65)	2.46679	13.2767		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Bonferroni t Test	400	>400		0.25	0.17688	0.38866	0.02035	0.01834	0.37393	4, 25

Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)	Skew
IC05	279.97			
IC10	359.93			
IC15	>400			
IC20	>400			
IC25	>400			
IC40	>400			
IC50	>400			



Dose-Response Plot