Phoslock™ Toxicity Testing with Three Sediment Dwelling Organisms (*Hyalella azteca, Hexagenia spp.* and *Chironomus dilutus*) and Two Water Column Dwelling Organisms (Rainbow Trout and *Daphnia magna*)

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Technical Memorandum

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Executive Summary

A series of toxicity tests with sediment and water column dwelling organisms were performed to assess the toxicity of Phoslock™ granules. Phoslock™ is a phosphorous binding clay that may be applied in the Lake Simcoe watershed in an attempt to reduce the phosphorous levels that currently lead to problematic algal bloom formation. Standard water only 96-hour toxicity tests were performed with rainbow trout and 48-hour toxicity tests were performed with *Daphnia magna*. Standard sediment toxicity tests were performed using *Chironomus dilutus* (10-day exposure), *Hexagenia spp.* (21-day exposure) and *Hyalella azteca* (14-day exposure) in sediment and water collected from the Lake Simcoe watershed. Two application rates were assessed in the sediment test; the filtered reactive phosphorous (FRP) rate dictates the amount of Phoslock required to remove the FRP from the water column and the capping rate (3.4 mg/L) delivers enough Phoslock to create a 1 mm thick layer on the sediment surface. Nutrient and metal concentrations were monitored in the sediment and water for all tests. The 48-hour LC_{50} for *Daphnia magna* was 4.9 g/L and > 6.8 g/L Phoslock. The rainbow trout 96-hour LC_{50} was>13.6 g/L. No significant survival or growth impacts were observed in any of the sediment toxicity test species for either of the dose rates. It should be noted that application rates used in 2008 field trials in the Lake Simcoe watershed were 0.02 and 0.05 g/L.

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1.0 INTRODUCTION

Phoslock™ is a modified bentonite clay product developed and produced by Australia's CSIRO (Commonwealth Scientific and Industrial Research Organization) to permanently bind phosphorous in systems where phosphorous levels lead to problematic algal bloom formation. Phosphorous levels have been recognized as a long-term problem in the Lake Simcoe region of Ontario. As part of the Ontario government's commitment to protecting Lake Simcoe, Phoslock™ addition to the lake is being examined as a phosphorous reduction strategy.

Field trials were to be conducted in the Scanlon Pond Reservoir and the Cane Parkway (aquatic ecosystems in the Lake Simcoe watershed) in July 2008. Since this product had never been used in a Canadian ecosystem, a thorough review, as well as laboratory testing was necessary prior to application. The review of available data on ecological and toxicological impacts of Phoslock™ revealed that the majority of research has been conducted on Australian or New Zealand ecosystems and biota (Moore and Chiswell 2006; Ecotox 2006a, 2006b; National Occupational Heath and Safety Commission 2001). In addition, little information was available on the impact of this product on sediment dwelling invertebrates (Clearwater 2004; Clearwater and Hickey 2004). Phoslock™ settles out of the water column onto the sediment surface. Due to direct exposure to this Phoslock™ layer, sediment dwelling animals are potentially most sensitive to contaminants associated with the sediment.

Laboratory toxicity testing was performed to test for effects of Phoslock™ prior to field trial applications in the Lake Simcoe watershed. Phoslock™ application rates varied across the tests performed but in most cases were much greater than that applied in the Scanlon Pond Reservoir (20 mg/L) and the Cane Parkway (50 mg/L) in July 2008. Excessively high application rates of Phoslock™ (up to 13600 mg/L) were tested to examine the worst-case scenario of a pulse of Phoslock™ entering the ecosystem due to equipment malfunction or human error.

Toxicity testing was performed with five different lab-reared aquatic species to evaluate potential impacts on various components of the aquatic ecosystem. Standard Environment Canada (EC) procedures were used to assess toxicity of Phoslock™ to rainbow trout (EC 2000a) and *Daphnia magna* (freshwater crustacean)(EC 2000b). These two species test for toxicity due to contaminants in the water column. In addition, a battery of sediment toxicity tests was performed to examine the effect of Phoslock™ on the sediment surface. The whole-sediment tests performed were the *Chironomus dilutus* (midge) 10-day exposure, survival and growth test (EC 1997a), *Hyalella azteca* (juvenile amphipod) 14-day exposure, survival and growth test (EC 1997b) and the *Hexagenia* spp. (mayfly nymph) 21-day exposure, survival and growth test (Bedard *et al.* 1993)(Figure A).

All five organisms are important sources of food for fish, waterfowl and larger invertebrates. Rainbow trout and *Daphnia magna* are exposed to chemicals in the water

column and on food particles. Sediment dwelling organisms represent three different exposure routes. The midge lives at the sediment-water interface, constructs tubes and feeds on detritus on the sediment surface. The amphipod is an epibenthic, sediment-burrowing, freshwater crustacean that selectively ingests bacteria and algae that adhere to sediment particles. The mayfly builds burrows in soft sediments and feeds on detritus, organic matter and sediment particles. All species tested vary in their sensitivity to environmental contaminants and are ecologically relevant and important in the Lake Simcoe watershed.

2.0 MATERIALS AND METHODS

2.1 Sediment and Water Collection and Storage

Sediment samples were collected from three sites across a transect of Scanlon Pond reservoir (East (EST), Centre (CTR) and West (WST)). Approximately 10 L of sediment per site was collected, composited, placed into a plastic pail lined with a food-grade polyethylene bag, and transported to the Aquatic Toxicology Unit (ATU) at the MOE Laboratory Services Branch (LaSB) in Etobicoke, Ontario.

Previously collected sediment from Peche Island (near the head of the Detroit River) was used as the control sediment (CS). The control sediment is relatively uncontaminated sediment, known to support organism survival and growth in the laboratory and provides a measure of organism health and test system integrity. The control sediment provides a basis for comparing the biological responses in the treatments (ASTM 2000). The Scanlon Pond sediments acted as reference sediments when tested without the addition of Phoslock^{TM} and were also used for comparing biological responses in the Phoslock^{TM} treatments.

Once sediment samples were received at the testing facility, sediment was thoroughly homogenized with a stainless steel spoon and any large indigenous organisms or debris were removed. Sediments were stored at approximately 4 °C (± 3 °C) without headspace in the dark until testing.

Site water was collected on three separate occasions (May 15, May 20 and May 26, 2008) from Scanlon Pond as close to the bottom of the water body as could be obtained without colloidal material. This water was stored at approximately 4 °C (± 3 °C) until it was used for toxicity testing.

2.2 Physical/ Chemical Analysis of Sediment and Scanlon Pond Water

Sub-samples of homogenized Scanlon Pond sediments (CTR, WST and EST) were sent to Maxxam Analytics Inc. where pore water extracted and analyzed for total Kjeldahl nitrogen (TKN), total phosphorus (TP), orthophosphate (also known as filtered reactive phosphorous (FRP)), pH, alkalinity ($CaCO_3$), lanthanum (La, total and

dissolved), aluminum (Al, total and dissolved), arsenic (As, total and dissolved) and reactive Silica (SiO_2).

In addition, Maxxam Analytics Inc. performed a weak acid extraction on sub-samples of the three Scanlon Pond sediments to determine the leachable quantity of La (total and dissolved), Al (total and dissolved), and As (total and dissolved).

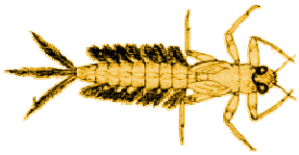
The site water collected from Scanlon Pond was analyzed at the MOE LaSB for FRP. This allowed for the calculation of the amount of Phoslock™ necessary to achieve the 250 parts Phoslock™ to 1 part FRP dosing rate. This dosing rate was decided by the Phoslock Steering Committee and was based on previous methods used in the application of Phoslock™. Application rates have since been refined and are now formulated based on a phosphorous mass balance for the system (E. Edmunds, personal communication March 20, 2009).

2.3 Biological Tests

2.3.1 Sediment Study Design

Based on consultation with Dave Lembke of the Lake Simcoe Region Conservation Authority (personal communication May 16, 2008) the initial study design for sediment toxicity tests consisted of assessing survival and growth impacts due to exposure to a 250:1 Phoslock™: FRP application rate. Sediment from three sample sites across a transect of Scanlon Pond were tested with three species (*Hyalella azteca, Hexagenia spp.,* and *Chironomus dilutus*). Test endpoints were survival and growth.

Hyalella azteca



Hexagenia spp.

Chironomus dilutus

Figure A: Pictures of the three species used in sediment testing.

In most cases three lab replicates were set up for each treatment. Four treatments were to be assessed (coding given in italics):

1) Control sediment with lab dilution water (Toronto tap water) (CS-CW-NP)
2) Control sediment with Scanlon Pond water (CS-SW-NP)
3) Scanlon Pond sediment with site water (EST-SW-NP; WST-SW-NP and CTR-SW-NP)
4) Scanlon Pond sediment with Scanlon Pond water and addition of the FRP application rate of Phoslock™ (EST-SW-FRP; WST-SW-FRP and CTR-SW-FRP)

Three additional treatments were added for the reasons explained below:

5) Scanlon Pond sediment with Scanlon Pond water and addition of the capping application rate of Phoslock™ (EST-SW-CAP; WST-SW-CAP and CTR-SW-CAP)

6) Control sediment with lab water and addition of FRP application rate of Phoslock™ (CS-CW-FRP)

7) Control sediment with lab water and addition of capping application rate of Phoslock™ (CS-CW-CAP)

Treatment 1 allowed for the assessment of culture health. If the organisms in this treatment did not meet their respective health criteria it is suspected that the organisms were not healthy and survival or growth results from all treatments are suspect. Treatment 2 was included to assess impacts due to exposure to Scanlon Pond water. Treatment 3 was included to assess impacts caused by Scanlon Pond sediment. Treatment 4 assessed impacts caused by exposure to the FRP application rate of Phoslock™.

Scanlon Pond water was collected early in the spring and the FRP concentrations were low in relation to what can be expected in late summer conditions. Using the measured FRP, the amount of Phoslock™ required to achieve the 250:1 exposure Phoslock™: FRP application rate was so small (<2 mg/L, refer to section 3.1) it seemed unlikely that assessment at this concentration would fairly represent potential toxicity from environmentally relevant applications of Phoslock™. A presentation given by Dr. David Garman (Chairman, Phoslock Limited) stated that sediment capping (i.e., the application of 3.4 g Phoslock™/L to provide a 1-3 mm thick cap on the sediment) is a potential use of this product in Canada (Garman 2008). Lab application of 3.4 g/L in the toxicity test vessels used in this study confirmed that this dose created a 1mm cap of Phoslock on the sediment™. The capping dose of 3.4 g/L is 170 times greater than the 20 mg/L applied in the Scanlon Pond Reservoir and 68 times greater than the 50 mg/L applied to Cane Parkway in the July 2008 field trials. Additional test treatments (treatment 5 and 7) were added to assess a 3.4 g/L capping (CAP) application rate with Scanlon Pond sediment.

Due to experimenter error the FRP dose (0.58 mg/L; refer to section 3.1) was applied to all vessels for the *H. azteca* test. Therefore for this species treatment 1 was not assessed. Based on the above-mentioned concerns about the protectiveness of the 250:1 Phoslock™: FRP application rate, it was decided to add additional Phoslock™ to half of the Scanlon Pond sediments vessels on day 7 to bring them up to the CAP dose (treatment 5). Therefore the WST-SW-CAP, EST-SW-CAP and CTR-SW-CAP exposure was a 7-day FRP and 7-day CAP dose exposure. The organisms in the WST-SW-FRP, EST-SW-FRP and CTR-SW-FRP vessels were exposed to the FRP dose for 14 days.

Due to limited staff availability and to allow for assessment of both CAP and FRP application rates, the decision was made to modify the test design for C. *dilutus* to only

test one vessel (i.e. no replication) for the Scanlon Pond sediment sites with Scanlon Pond water (treatment 3). The survival and growth criteria in control sediment were assessed in vessels with Scanlon Pond water (treatment 2) and survival and growth in control sediment with lab water (treatment 1) was not assessed.

Additional testing was performed in December 2008 to assess the impact of the CAP application rate on *Hexagenia spp.* This testing was done using control sediment and laboratory water for both the control and the addition of the CAP application rate of Phoslock™ (treatment 7).

2.3.2 Sediment Toxicity Test Methods

The organisms used for testing were cultured in-house at the MOE ATU. Summaries of the test methods are given in Appendix 13-1, 13-2 and 13-3 for the chironomid, amphipod and mayfly test, respectively. MOE modifications to the EC standard methods for the chironomid and amphipod test are identified in appendix 13-1 and 13-2, respectively.

For each test organism, a sediment sample was placed into three replicate containers for each treatment in a 1:4 ratio of sediment: water. Vessels were then aerated overnight before test organisms were introduced. Up to twenty-four hours after the introduction of organisms, Phoslock™ was applied to the test vessels. The dry Phoslock™ product was ground to a fine texture using a mortar and pestle. Based on water volume in the test vessels (400 mL in the *H. azteca* and *C. dilutus* tests and 1.3 L in the *Hexagenia spp.* test) Phoslock™ required per jar to get the desired application rate was weighed out into plastic plates. A small amount of lab (*Hexagenia spp.* CAP dose only) or Scanlon Pond water was added to each plastic plate of Phoslock™ and blended to make a slurry (Figure B). The slurry was added to the test vessels by dipping the plastic plate below the water surface (Figure B).

Figure B: A capping dose (3.4 g/L) of Phoslock™ is made into a slurry (left) and added to a *C. dilutus* test vessel. A 1 mm layer of Phoslock™ formed on the sediment surface.

During testing, vessels were maintained at 23 ± 2°C. Vessels were continuously aerated and exposed to a 16:8 hour light:dark photoperiod. At the start, middle and end of each test, sub-samples of overlying water were collected from the test vessels and monitored for pH, conductivity, ammonia and dissolved oxygen using specific ion meters in the ATU laboratory. Temperature and evidence of organism death were monitored daily.

2.3.3 Assessment of Impacts

The Fisher's exact test (SYSTAT. 2004) at the 5% significance level was used to assess for survival impacts. Growth of an organism was considered to be impaired when there was a >60% reduction in size. Impacts were assessed in terms of exposure to Scanlon Pond water (SW), FRP dose of Phoslock™ (FRP) and/or capping dose of Phoslock™ (CAP).

The "control" to which impacts were compared was dependent on study design for each sediment organism. For *H. azteca* impacts were assessed through comparison to treatment 6 (CS-CW-FRP). Treatment 1 (CS-CW-NP) was used to assess for significant impacts due to *Hexagenia spp.* CAP and FRP exposures. *Chironomus dilutus* impacts were determined based on comparison to organisms in treatment 3 (CS-SW-NP) and 4 (CTR-SW-NP, WST-SW-NP and EST-SW-NP) combined.

2.3.4 Water Toxicity Test Design

The organisms used for testing were cultured in-house at the MOE ATU. Two test species, rainbow trout (*Onchorykis mykiss*) and *Daphnia magna* (Figure C) were used to determine a LC_{50} for exposure to Phoslock™. For both species tests were run under static non-renewal conditions. Refer to Appendix 13-4 and 13-5 for summaries of the *D. magna* and rainbow trout methods (respectively). The stock concentration of Phoslock™ was prepared by grinding pure product Phoslock™ granules with a mortar and pestle.

For the trout test a 30 g/L Phoslock™ stock solution was prepared with lab water (dechlorinated Toronto tap water, hardness 128 mg/L). The highest test concentration of Phoslock™ was 13.6 g/L. For the daphnia test the highest concentration tested was 6.8 g Phoslock™ /L in both lab water and Scanlon Pond water (collected May 26, 2008). Half-by-half dilutions were performed.

Rainbow trout (*Onchorykis mykiss*)

Daphnia magna

Figure C: Pictures of the two species used in water-only toxicity testing

2.3.4.1 *Daphnia magna* 48-hour Acute Lethality Test Design

The *D. magna* culture used in testing met culture health criteria. Conductivity, pH, DO, hardness and temperature were measured in each test concentration upon test initiation and termination. Twelve daphnia neonates less than 24 hours old were transferred into a glass beaker containing 200 mL of test solution (one beaker for each test concentration) using a pipette. Daphnia were not fed during the test, and temperature and light intensity was monitored daily. After 48 (± 2) hours the organisms were observed and mortality, immobility and/or other impairments were recorded.

2.3.4.2 Rainbow trout 96-hour Acute Lethality Test Design

The rainbow trout culture used in testing met the culture health criteria.

Conductivity, DO, pH, hardness and temperature were measured in each concentration upon test initiation and termination. All pails were aerated through silica air stones at a rate of 6.5 ± 1 ml/min/L. Ten juvenile rainbow trout were transferred each test concentration using a net. Trout were not fed during the test and temperature was monitored daily. Mortalities were removed daily. After 96 (± 2) hours the organisms were observed for mortality, immobility and/or other impairments were recorded.

2.3.4.3 Assessment of Impacts

The effect measured for *Daphnia magna* and rainbow trout was mortality. When possible, mortality data was reported as an LC50 calculated by Probit analysis.

2.4 Chemical Analysis of Water

Overlying water from each of the sediment vessels was collected from all replicates upon time of test termination. Replicates from each treatment were pooled and sub-samples were taken. These were sent to Maxxam Analytics Inc. (with the exception of the December 2008 assessment of CAP application rates on *Hexagenia spp.)*. Overlying water from the *Hexagenia* spp. (FRP experiment only), *H. azteca* and *C. dilutus* test vessels was analyzed for TKN, TP, FRP, pH, $CaCO_3$, La (total and dissolved), Al (total and dissolved), As (total and dissolved) and SiO_2. In addition, water overlying the *C. dilutus* test vessels was analyzed for a full suite of dissolved metals.

A sample of each concentration used in the dilution series *D. magna* was analyzed by Maxxam Analytics Inc. for total and dissolved La.

3.0 RESULTS AND DISCUSSION

3.1 Physical/ Chemical Analysis of Sediment and Site Water and Dose Rate Calculations

The results of the chemical analysis of Scanlon Pond sediment pore water, sediment and water are presented in Appendix 12 A, B and C, respectively. Chemistry of the three Scanlon Pond sites (WST, EST and CTR) was similar and there were no concentrations that could impact the survival of aquatic organisms. In addition, the Scanlon Pond water sampled on various dates within the month of May 2008 were similar.

The application rates recommended by Phoslock Water Solutions have been refined over time and with experience (E. Edmunds, Phoslock Water Solutions, Sydney, Australia, personal communication March 20, 2009). The FRP Phoslock™ dose rate of 250 Phoslock™ to 1 FRP used in this study is no longer used. The dose rates are now formulated based on a phosphorous mass balance for the system. Included in this mass

balance is the contribution of FRP/total phosphorous (TP) from the sediment, water column, algal biomass and inflows (E. Edmunds, personal communication March 20, 2009). This is the dosing technique that was used in the Lake Simcoe watershed.

Based on FRP of May 15 Scanlon Pond water used in the amphipod test (0.0023 mg/L) the FRP Phoslock™ dose rate of 250 Phoslock™ to 1 FRP was calculated to be 0.58 mg/L Phoslock™ (refer to section 3.2.1).

The average FRP in May 15 and May 20 Scanlon Pond water used in the FRP mayfly test (0.0023 and 0.0013 mg/L, respectively) and the FRP dose rate was calculated to be 0.45 mg/L Phoslock™ (refer to section 3.2.2).

The May 26 Scanlon Pond water used in the chironomid test had 0.0074 mg/L FRP and the FRP dose rate was calculated to be 1.85 mg/L Phoslock™ (refer to section 3.2.4).

3.2 Biological Tests

3.2.1 *Hyalella azteca* (Amphipod) Survival and Growth Test

3.2.1.1 Test Validity Based on *H. azteca* Exposed to Control Sediment

Results from reference toxicant testing confirmed the integrity of the test system, good organism health as well as technician proficiency (Appendix 2). *Hyalella azteca* average survival following exposure to control (Peche Island) sediments with lab water (90% CS-CW-FRP) and Scanlon Pond water (93% CS-SW-FRP) was 92% (Figure 1, Appendix 2). A toxicity test is considered acceptable if *H. azteca* survival is ≥ 80% and dry weight is >0.1 mg in control sediments. It should be noted that there was no true control for *H. azteca* due to the accidental addition of the FRP dose of Phoslock™ to all test vessels.

3.2.1.2 Survival and Growth

There were no significant survival effects observed among amphipods exposed to any of the test sediments when compared to amphipod survival in the CS-CW-FRP or CS-SW-FRP treatments ($p > 0.05$, Figure 1, Appendix 2).

There was no significant reduction in growth of amphipods exposed to the Scanlon Pond FRP (0.58 mg/L Phoslock™) and CAP (3.4 g/L Phoslock™) treatments in comparison to the amphipods exposed to the CS-CW-FRP or CS-SW-FRP treatments (Figure 1, Appendix 2).

There was no significant difference observed in survival when the FRP and CAP treatments for each of the three Scanlon Pond sediments (CTR, EST and WST) were

compared. The growth observed in the CTR-SW-CAP treatment (0.31±0.14 mg) was only 53% of that measured in the CTR-SW-FRP treatment (0.58±0.07 mg). This may indicate a significant growth reduction in the CAP exposed organisms however there was high variability in the weight measurements for the CTR-SW-CAP treatment (CV 44%).

With the exception of CTR-SW-CAP all amphipods exposed to Scanlon Pond sediments met the test acceptability criteria for growth and survival. The average survival in the CTR-SW-CAP treatment was 77% (±6%).

3.2.1.3 Overlying Water Quality

For the duration of the test, pH and dissolved oxygen in the all vessels were at acceptable levels (Appendix 2). For all vessels total ammonia levels throughout the duration of the test were less than the published LC_{50} for total ammonia (35.2 mg/L) or unionized ammonia (5.38 mg/L) (Ankley *et al*. 1995). Vessel temperature monitored daily was maintained within the range of 23 ± 2°C.

Results of chemical analysis of water overlying the sediment in *H. azteca* test vessels upon test termination are presented in Appendix 3. All treatments had similar overlying water chemistry with some exceptions. The SiO_2 was higher in Scanlon Pond sediment CAP treatments (37 ±5 mg/L, n=3) than in FRP treatments (20 ± 6 mg/L, n=3). It would be expected that La in overlying water should be found at higher concentrations in CAP treatments than in FRP treatments. This pattern was observed in water overlying chironomid sediment tests (Appendix 8). Total La was elevated in the WST-SW-FRP (585 ppb) treatment above the total La measured in the CTR-SW-CAP (246 ppb and WST-SW-CAP (133 ppb) vessels. The validity of the WST-SW-FRP result is questionable. Total Al concentrations in the water overlying hyalella tests did not follow any apparent pattern.

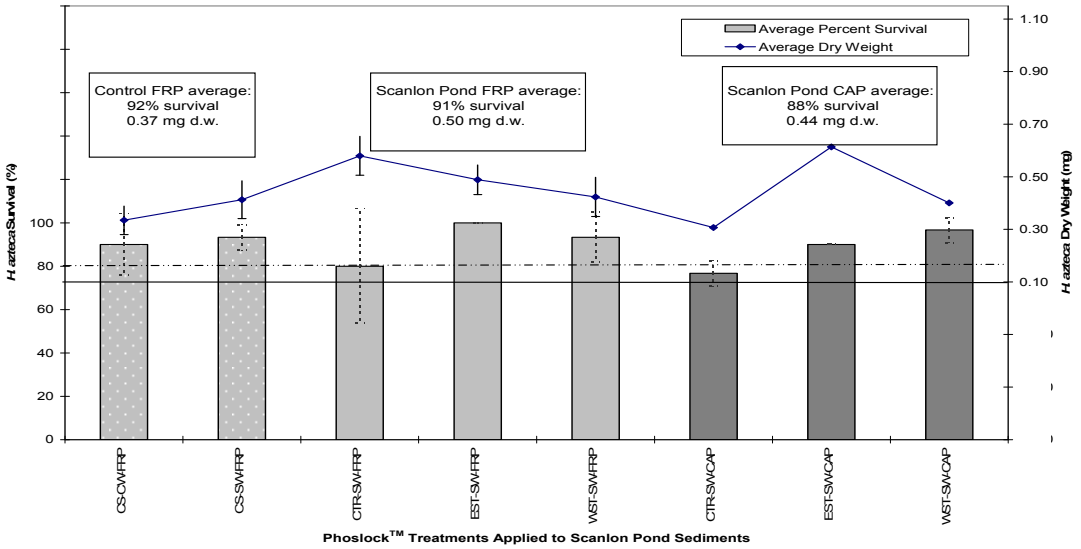


Figure 1. Average percent survival and average dry weight (mg) (± standard deviation) of *Hyalella azteca* after a 14 day exposure to Scanlon Pond sediments (CTR, EST and WST) and Peche Island control sediment (CS) with lab control water (CW) and Scanlon pond water (SW). The Phoslock™ application rates of 250 Phoslock™:1 FRP (FRP) and 3.4 g/L Phoslock™ (CAP) were assessed. Control survival criterion of 80% is indicated by the dashed line and the control dry weight criterion of 0.1 mg is indicated by the solid line.

3.2.2 *Hexagenia* spp. (Mayfly) Survival and Growth Test with FRP application rate of Phoslock™

3.2.2.1 Test Validity Based on *Hexagenia spp.* Exposed to Control Sediment

Hexagenia spp. average survival following exposure to control sediment (CS-CW-NP and CS-SW-NP) was 100% (Appendix 4). A toxicity test is considered acceptable if *Hexagenia* spp. survival ≥ 80% in control sediments. Results from reference toxicant testing confirmed the integrity of the test system, good organism health as well as technician proficiency (Appendix 4). Average individual *Hexagenia* spp. wet weight following exposure to control sediment was 25.5 mg (average of lab control water (CS-CW-NP) and site water (CS-SW-NP) exposures), which is more than twice the initial (4.29 ± 1.89 mg) wet weight (Appendix 4). Measurements of pH, DO, conductivity and temperature in waters overlying control sediment at test initiation, mid-way, and at test termination (Appendix 4) were all within acceptable test limits.

3.2.2.2 Survival and Growth

Survival was ≥ 80% in all test vessels. Therefore there was no significant reduction in survival observed among *Hexagenia* spp. exposed to control or Scanlon Pond sediments with Scanlon Pond water and no addition of Phoslock™, or Scanlon Pond sediments with the FRP dose addition of 0.45 mg/L Phoslock™ when compared to mayfly survival in the control sediment (CS-CW-NP) ($p > 0.05$, Figure 2, Appendix 4).

Hexagenia spp. grew much better in Scanlon Pond sediment than control sediment (155 to 197 times greater); independent of exposure to Phoslock™ (Figure 2, Appendix 4). This growth can be attributed to the sediment and not the Scanlon Pond water since *Hexagenia* spp. exposed to the CS-SW-NP treatment were only 94% as large as those in the CS-CW-NP treatment.

Comparison between the mayflies exposed to the control sediment with lab water (CS-CW-NP) and Scanlon Pond water (CS-SW-NP) revealed no significant difference in survival or growth. Nor was there a significant difference in survival or growth when the FRP and CAP treatments for each of the three Scanlon Pond sediments (CTR, EST and WST) were compared. All mayflies exposed to Scanlon Pond sediments met the test acceptability criteria for growth and survival.

3.2.2.3 Overlying Water Quality

Test solutions were monitored for dissolved oxygen, pH, conductivity, and ammonia at the beginning, middle (day 10), and end of testing (Appendix 4). For the duration of the test, pH and dissolved oxygen in the all exposure vessels were at acceptable levels. Unionized ammonia measured in water overlying all test sediments throughout the test was less than the published LC_{50} for mayflies (1.80 – 5.88 mg/L) (Thurston *et al*. 1984). Temperature monitored daily during the test was maintained within 23 ± 2°C.

Results from chemical analysis of water overlying *Hexagenia* spp. test vessels upon test termination are presented in Appendix 5. All treatments had similar chemistry. There was no apparent impact of the FRP treatment on any of the chemical parameters measured.

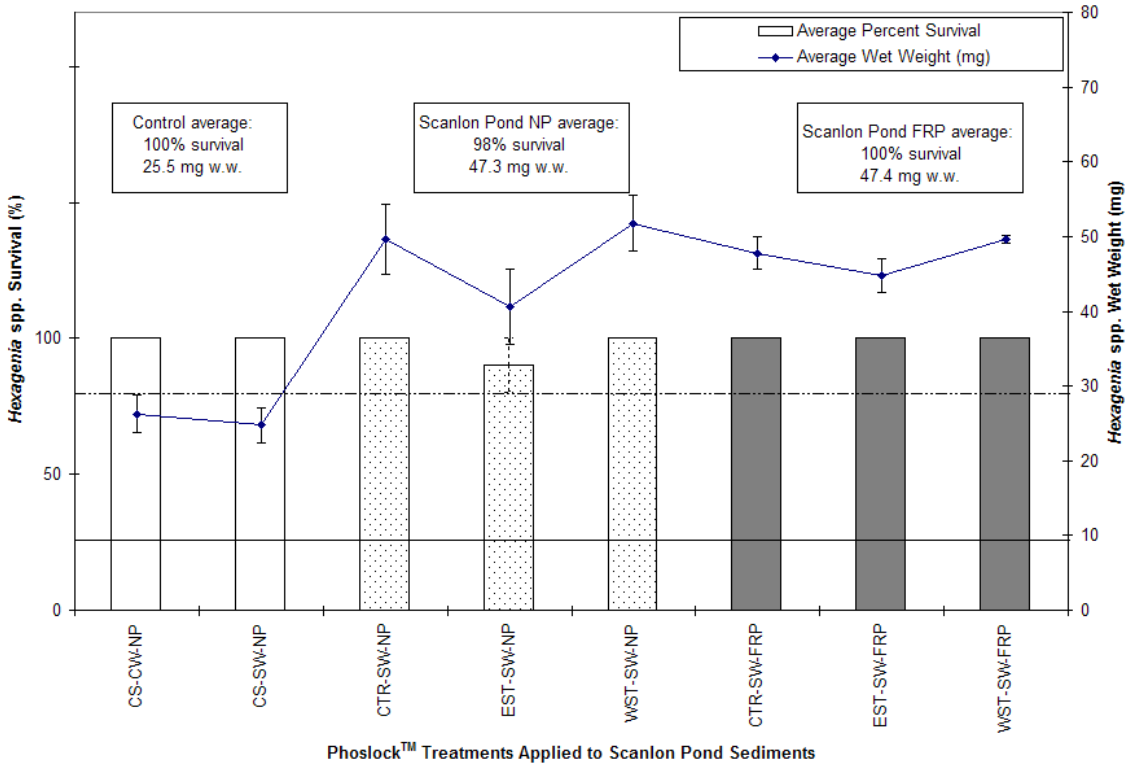


Figure 2. Average percent survival and average wet weight (mg) (± standard deviation) of *Hexagenia* spp. after a 21-day exposure to Scanlon Pond sediments (CTR, EST and WST) and Peche Island control sediment (CS) with lab control (CW) and Scanlon pond water (SW). The Phoslock™ application rate of 0.45 mg/L (250 Phoslock™:1 FRP) (FRP) was compared to exposures without the addition of Phoslock™ (NP). Control survival criterion of 80% is indicated by the dashed line and the control dry weight criterion of 2x the initial wet weight (8.58 mg) is indicated by the solid line.

3.2.3 *Hexagenia* spp. (Mayfly) Survival and Growth Test with CAP application rate of Phoslock™

3.2.3.1 Test Validity Based on *Hexagenia spp.* Exposed to Control Sediment

Hexagenia spp. average survival following exposure to control (CS) sediment was 97% (± 6%)(Figure 3, Appendix 6). A toxicity test is considered acceptable if *Hexagenia* spp. survival ≥ 80% in control sediments. Results from reference toxicant testing confirmed the integrity of the test system, good organism health as well as technician proficiency (Appendix 6). At test termination, *Hexagenia* spp. exposed to control sediment should be ≥ 2 times their initial weight. Average individual *Hexagenia* spp. wet weight following exposure to control sediment was 16.7 mg (CS-CW-NP), which is more than twice the initial (6.6 mg ± 1.3 mg) wet weight (Appendix 6). Measurements of pH, DO, conductivity ammonia and temperature in water overlying control sediment at test initiation, mid-way, and at test termination (Appendix 6) were within acceptable limits.

3.2.3.2 Toxicity

Average mayfly survival was 93% (± 6%) in the three replicates exposed to the CS-CW-CAP treatment. There was no significant reduction in survival when compared to mayfly survival in the control sediment (CS-CW-NP) ($p > 0.05$, Figure 3, Appendix 6).

Hexagenia spp. growth in the CS-CW-CAP treatment was 84% of that seen in the untreated control (CS-CW-NP)(Figure 3, Appendix 6). A reduction in growth is considered biologically significant only when growth is <60% of that seen in control organisms.

3.2.3.3 Overlying Water Quality

It was noted that initial pH was depressed in the high Phoslock™ concentrations and shifted to a pH similar to that in the low Phoslock™ concentrations of the *D. magna* (Appendix 9) and rainbow trout (Appendix 10) tests. Based on these observations, it was decided to measure pH, DO, conductivity and ammonia 1 hour after the addition of Phoslock™ (Appendix 6). This was in addition to the beginning (prior to the addition of the mayflies), middle and end of test measurements standard in all of the sediment tests. Ammonia and pH appeared to be altered by the addition of Phoslock™. In the time between the initial measurements and 1 hour after the addition of Phoslock™ the pH decreased from 8.1 to 7.8 and total ammonia increased from 1.6 to 6.5 mg/L. It should be noted that there were also changes in the CS-CW-NP treatment where the pH changed from 8.1 to 9.0 and total ammonia increased form 1.6 to 2.4 mg/L. Despite this alteration in chemistry post Phoslock™ addition, the unionized ammonia in water overlying all test sediments was less than the published LC_{50} for mayflies (1.80 – 5.88 mg/L) (Thurston *et al.* 1984) for the test duration. Measured conductivity, pH, and DO were at acceptable levels for organism health. Temperature monitored daily was maintained within 23 ± 2°C.

Water overlying the *Hexagenia* spp. test vessels after the CAP exposure was not analyzed. It is assumed that overlying water chemistry would be similar to that seen with the capping dose for *C. dilutus* exposures (Appendix 8).

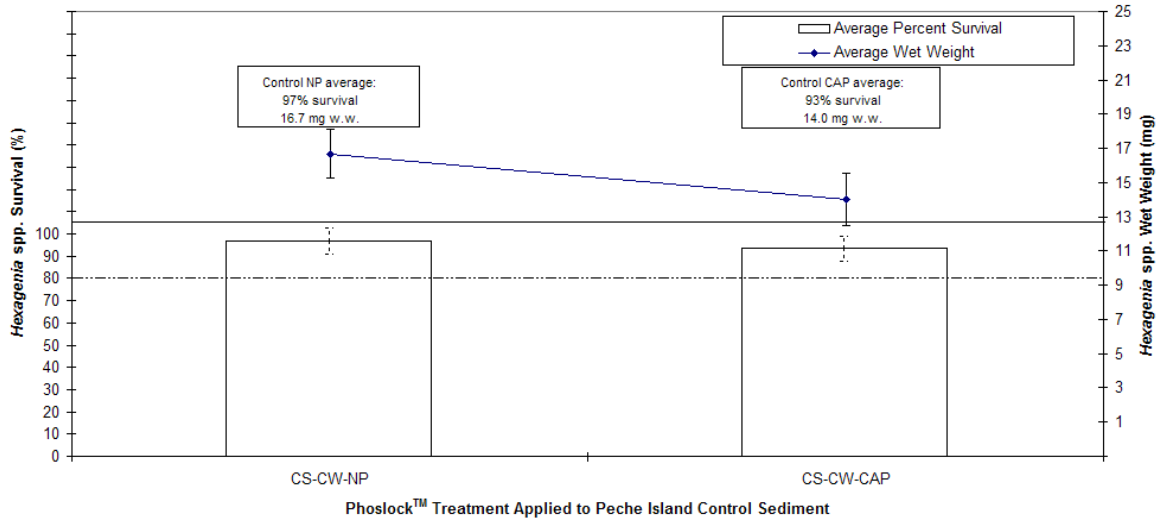


Figure 3. Average percent survival and average wet weight (mg) (± standard deviation) of *Hexagenia* spp. after a 21-day exposure to Peche Island control sediment (CS) with lab control (CW) water. The Phoslock™ application rate of 3.4 g/L Phoslock™ (CAP) was compared to exposures without Phoslock™ (NP). Control survival criterion of 80% is indicated by the dashed line and control dry weight criterion of 2x the initial wet weight (13.2 mg) is indicated by the solid line.

3.2.4 *Chironomus dilutus* (Midge) Survival and Growth Test

3.2.4.1 Test Validity Based on *C. dilutus* Exposed to Control Sediment

Results from reference toxicant testing confirmed the integrity of the test system, good organism health as well as technician proficiency (Appendix 7). The control for the *C. dilutus* test included a single lab replicate of control (CS-SW-NP), Scanlon Pond east (EST-SW-NP), west (WST-SW-NP) and centre (CTR-SW-NP) sediments with Scanlon Pond water (collectively referred to as NP treatment). In contrast, the amphipod and mayfly test control was the Peche Island sediment with lab water (CS-CW-NP) exposure. Average survival of the NP treatment was 80% (± 24%)(Appendix 7, Figure 4). The high variability in the NP treatment survival should be noted (CV = 31%) and survival in the single replicate of CS-SW-NP exposure was only 50%, however a toxicity test is considered acceptable if *C. dilutus* average control survival is ≥ 70%.

The average individual midge wet weight following exposure to the NP treatment was 11.50 mg (Figure 4). The minimum acceptable wet weight of *C. dilutus* exposed to control sediments is 0.6 mg (EC 1997a). When the dry weight criterion is multiplied by a dry to wet weight conversion factor of 8.36 mg (Watson-Leung, unpublished data) the

acceptable criterion is 5.0 mg wet weight. Therefore the wet weight of midges in the control (NP) was over 2 times the minimum acceptable value. It should be noted that the feeding rate used followed Bedard *et al.* (1992) and was less than the amount recommended by Environment Canada (EC 1997a). The lower feeding rate did reduce survival or growth in the control (NP) to below test validity criteria and was consistent across all sites so growth data are still comparable. Measurements of pH, DO, conductivity, ammonia and temperature in the NP treatment were not at levels which may cause toxicity (Appendix 7).

3.2.4.2 Survival and Growth

It should be noted that survival in one replicate of the WST-SW-FRP exposure was only 30% and the CV for this treatment was 52%. However, the average survival across the six Phoslock™ exposure treatments was greater than or equal to the Environment Canada 70% survival criterion (EC 1997a) and was ≥88% of the average survival in the NP treatments. Therefore there was no significant reduction in survival observed among *C. dilutus* exposed to either the 1.85 mg/L FRP or 3.4 g/L CAP Phoslock™ dosing rates ($p > 0.05$, Figure 4, Appendix 7).

Growth of *C. dilutus* exposed to the FRP dose of Phoslock™ was not impaired relative to the growth seen in the organisms not dosed with Phoslock™ (NP treatments) however growth did appear to be reduced in the CAP treatment (Figure 4, Appendix 7). Midges exposed to the capping dose of Phoslock™ were 74-82% of the weight of those not exposed to Phoslock™. The coefficient of variation is tight across all observations for the CAP dose treatment (7.6% CV) however, this is not considered to be a biologically significant reduction in growth.

There was no significant difference observed in survival and growth between the three Scanlon Pond sediments (CTR, EST and WST) within each Phoslock™ dose (FRP and CAP). When all average wet weights of all the Scanlon Pond sediments for the FRP dose were pooled the CV was 13%. Similarly the CV for the pooled CAP dose average wet weights was 8%. This highlights the high degree of similarity independent of Scanlon Pond sediment.

Since there was no control sediment with lab water exposure for *C. dilutus* comparison between lab water and Scanlon Pond water treatments was not possible. However, average survival in the Scanlon Pond water met test validity criteria in all treatments so there does not appear to be an impact due to exposure to Scanlon Pond water.

3.2.4.3 Overlying Water Quality

For the duration of the test, pH and dissolved oxygen in the all exposure vessels were at acceptable levels (Appendix 7). Unionized ammonia measured in water overlying all test sediments was less than the published LC_{50} for mayflies (1.80 – 5.88 mg/L) (Thurston et al. 1984). Temperature during the test was maintained within 23 ± 2°C.

Results from chemical analysis of water overlying *C. dilutus* test vessels upon test termination are presented in Appendix 8. As seen in the CAP treatment after 14 days exposure of the *H. azteca,* the reactive silica was elevated in the CAP treatments (average 28 mg/L) when compared to the FRP treatments (average = 13 mg/L). Orthophosphate, TKN and TP were all lower in the CAP treatments (0.02, 1.3 and 0.12 mg/L, respectively) than in the FRP treatments (0.05, 1.9 and 0.25 mg/L, respectively). Orthophosphate was also measured in the NP treatment and was higher seen in both the FRP and CAP treatments (average 0.09 mg/L). Dissolved Al also followed this pattern (FRP average = 11 µg/L; not detected in CAP) however total Al did not follow any pattern, possibly due to variation in the amount of particulate matter in the samples. Dissolved silicon and dissolved sodium were elevated in the CAP dose (average 13 and 33 mg/L, respectively) compared to the FRP dose (average 6 and 23 mg/L, respectively). The additional metals measured were similar between treatments.

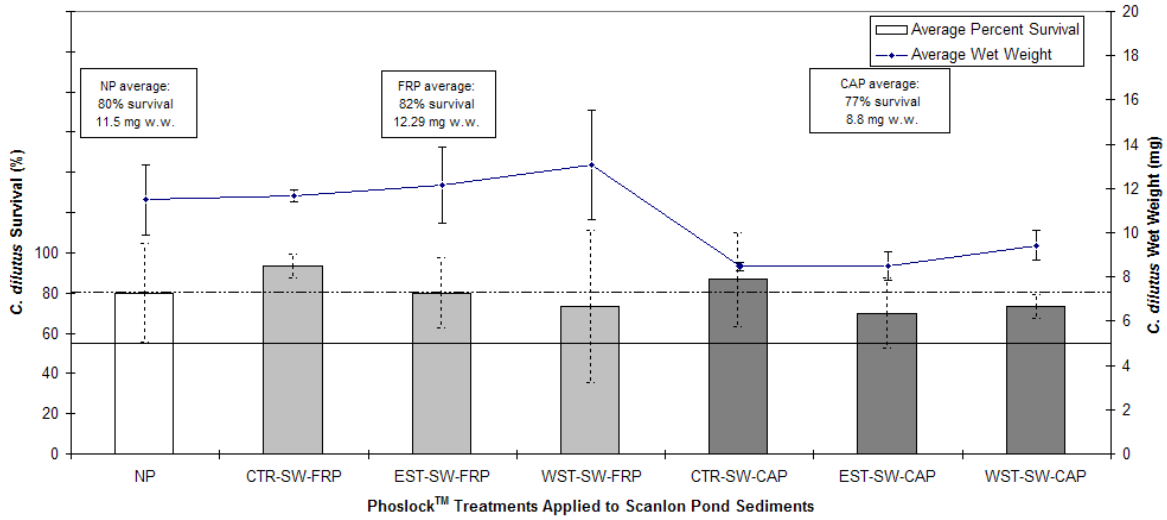


Figure 4. Average percent survival and average wet weight (mg) (± standard deviation) of *Chironomus dilutus.* after a 10 day exposure to Scanlon Pond sediments (CTR, EST and WST) and Peche Island control sediment (part of the NP treatment). All tests were run with Scanlon Pond water as the overlying water. The Phoslock™ application rates of 250 Phoslock™:1 FRP (FRP) and 3.4 g/L Phoslock™ (CAP) were compared to exposures without the addition of Phoslock™ (NP). Control survival criterion of 80% is indicated by the dashed line and the control dry weight criterion of 5.0 mg is indicated by the solid line.

3.2.5 Toxicity Tests with *Daphnia magna*

3.2.5.1 Test Validity

A *D. magna* toxicity test is considered valid if survival in the control is ≥90% and survival in lab and Scanlon Pond water (0 g/L) controls was 100% (Appendix 9 and 10). Results from reference toxicant testing confirmed the integrity of the test system, good organism health as well as technician proficiency (Appendix 9 and 10).

3.2.5.2 Toxicity

The results for the dilution series prepared using lab water are presented in Appendix 9 and Table 1. In the two highest test concentrations (3.4 g/L and 6.8 g/L) 50% of the daphnia were dead. Twenty-five percent were dead in the 1.7 g/L concentration. The 48-hour LC_{50} was calculated to be 4.9 g/L Phoslock™ for daphnia in this test.

The results for the dilution series prepared with Scanlon Pond water are found in Appendix 10 and Table 1. Similar to the lab water dilutions, there was mortality in the 1.7, 3.4 and 6.8 g/L (8, 22 and 42% mortality, respectively) concentrations with Scanlon Pond water. The 48-hour LC_{50} of Phoslock™ for daphnia could not be calculated from this test since there was only 42% mortality in the highest test concentration.

3.2.5.3 Water Quality

Dissolved oxygen, pH and conductivity were at acceptable levels for daphnid health (Appendix 9 & 10). Temperature was monitored daily and was maintained at 20 ± 2°C.

The results of the total and dissolved La concentrations in the dilution series of Phoslock™ used in the daphnia toxicity tests with lab water are presented in Appendix 11. Dissolved and total La both increased with increasing concentrations of Phoslock™ until the 6.8 g/L concentration was reached. It is not clear why the concentration of total and dissolved La was lower (194.4 mg/L total, 14 mg/L dissolved) in the 6.8 g/L Phoslock™ concentration than it was in the 3.4 g/L concentration (239.4 mg/L total, 63.3 mg/L dissolved).

3.2.6 Toxicity Tests with *Onchorykis mykiss* (rainbow trout)

3.2.6.1 Test Validity

A rainbow trout toxicity test is considered valid if survival in the control is ≥90% and survival in the control (0 g/L) treatment was 100% (Appendix 10). Results from reference toxicant testing confirmed the integrity of the test system, good organism health as well as technician proficiency (Appendix 10).

3.2.6.2 Toxicity

In the highest test concentration (13.6 g/L) only nine organisms were recovered upon test termination (Table 1 and Appendix 10). It is unlikely that a tenth trout died and decayed during the test. It is probable that due to experimenter error only nine organisms were added at test initiation and the turbidity of the water in this test vessel made it difficult to realize this error until the test was terminated. One of the nine organisms recovered alive was trapped in the Phoslock™ product. The LC_{50} for rainbow trout exposed to Phoslock™ is greater than 13.6 g/L. The Phoslock™ did not settle out of the water column to the same degree as was seen in the daphnia test vessels, likely due to continual agitation by fish movement.

3.2.6.3 Water Quality

Test solutions were monitored for dissolved oxygen, pH, conductivity, and temperature at the beginning and at the end of testing (Appendix 10). Dissolved oxygen, pH and conductivity were all at acceptable levels for rainbow trout health. Temperature was monitored daily in one vessel throughout the test and was maintained at 15 ± 1°C. It was noted that there was an initial lowering of pH in the highest concentrations of Phoslock and buffering of this pH occurred over the 96-hour test duration (e.g. 3.4 g/L time 0 pH was 7.1 and 96 hour pH was 8.1) (Appendix 10).

The Phoslock™ solutions used in the trout toxicity test were not sent for chemical analysis. It can be assumed the results of total and dissolved La concentrations will be similar to results from the daphnia test where concentrations overlap (Appendix 11).

Table 1: Summary of the response of trout and daphnia after exposure to a concentration series of Phoslock™.

Phoslock Concentration (g/L)	% mortality		
	Daphnia magna (n=12) site water	*Daphnia magna* (n=12) lab water	rainbow trout (n=10) lab water
13.6	nt	nt	10*
6.8	42	50*	0
3.4	33**	50	0
1.7	8*	25	0
0.85	0	0	0
0.425	0	0	0
0.2125	0	0	nt
0.10625	nt	0	nt
lab water	0	0	0
Scanlon Pond water	0	nt	nt

nt = not tested.

* Asterix quantity equals the number of organisms found alive but impaired or immobile due to being stuck in the Phoslock.

4.0 INTERPRETATIONS AND CONCLUSIONS

Lanthanum ions (La^{3+}) are the active components (5% by weight) in Phoslock™ (Yasseri and Nowak 2008). There is evidence that lanthanum and lanthanum salts in water can be toxic to many aquatic species. For example, Barry and Meehan (2000) found the 48-hour acute EC_{50} (lethality) for *Daphnia carinata* exposed to lanthanum chloride (total) to be 1.2 mg La/L (in ASTM hardwater). Birge *et al.* (1979) found lanthanum to be highly toxic to rainbow trout (28-day LC_{50} 20 µg/L). Unfortunately, no short-term rainbow trout LC_{50} data is available at this time.

It has been reported that in the presence of FRP Phoslock™ has no detectable toxic effect (Yasseri and Nowak 2008) due to the binding of the free Lanthanum ions with FRP. Toxicological testing with Phoslock™ (Yasseri and Nowak 2008) determined the 48-hour EC_{50} for *D. magna* and the 96-hour EC_{50} for rainbow trout fry to be 103 and 150 mg La/L, respectively. The total La concentration measured in the 6.8 and 3.4 g/L Phoslock™ solutions made with lab dilution water were 194.4 and 239.4 mg/L La, respectively (note: it is unclear why the La concentration was higher in the 3.4 g/L concentration). The *D. magna* LC_{50} was 4.94 g/L and the trout LC_{50} was greater than 13.6 g/L (Appendix 10 and 11). Based on these results, the La released into solution from Phoslock™ appears to be less toxic to daphnids than was seen in the testing with La alone, described above (Barry and Meehan 2000).

In the present study, the highest concentration of Phoslock™ tested in the water exposures using rainbow trout and *Daphnia* was 13.6 g/L and 6.8 g/L, respectively. Mortalities seen in daphnia (1.7 g/L and greater) appeared to be caused by physical entrapment in Phoslock™ and some of the organisms physically trapped in the Phoslock™ slurry were alive upon test termination. The 48-hour daphnia LC_{50} calculated in this study was greater than 6.8 g/L (42% mortality in 6.8 g/L) when the dilutions were made with Scanlon Pond water and was 4.94 g/L when dilutions were made with lab water.

Comparison of our study with a previous study on toxicity of Phoslock™ to *D. magna* (Martin and Hickey 2004) is difficult. The Martin and Hickey (2004) *D. magna* test was conducted following the OECD (1984) method while this study followed the EC (2000a) method. These methods are very similar however different formulations of Phoslock™ were used and Martin and Hickey (2004) prepared test solutions using a modified USEPA Toxicity Characteristic Leachate Procedure (TCLP)(USEPA 1992) and 50 g/L of Phoslock™. This TCLP technique was performed with reagent water instead of acid and was chosen in order to remove the impact of high concentrations of particulate solids on the test species. The organisms were exposed to supernatant that had been filtered through a 40 um nylon mesh. The Martin and Hickey (2004) 48-hour LC_{50} for *D. magna* was >50 g/L of Phoslock™ (average survival 63% in the 50 g/L exposure). In our study the 48-hour *D. magna* LC_{50} was much lower (4.94-<6.8 g/L). This may be due to changes in the formulation of the Phoslock™ or because our test design did not remove the potential confounding effect of toxicity due to particulate solids. We feel our study design tested the formulation as it would be applied and may be a better predictor of toxicity in the field. Ecotox (2008) performed 48-hour toxicity tests with locally collected *Ceriodaphnia dubia*. *C. dubia* is a common and ecologically relevant Canadian toxicity test species. The granules of Phoslock™ were the more recent formulation and were added to field collected water in the same way as was done in our study. The concentrations tested were much lower than in our study and the 48-hour LC_{50} was >50 mg/L.

Martin and Hickey (TCLP procedure)(2004) and Clearwater and Hickey (2004) conducted Phoslock™ 4-day toxicity tests with rainbow trout. The LC_{50}s were 4350 mg/L and 200 mg/L, respectively. These are much lower than was seen in our study, where only 10% of the exposed trout were found dead in 13600 mg/L of Phoslock™. In the Clearwater and Hickey (2004) study the fish were pre-treated for disease and were fed which may have led to increased ingestion of the Phoslock™ granules. The difference may also be due to the difference in Phoslock™ formulations between studies.

The impact of not only the free La ions but also the potential physical smothering effect was of importance in the sediment toxicity testing of the Phoslock™ product. Sediment toxicity tests revealed no biologically or statistically significant growth or survival impairment in the midge, mayfly or amphipod with Phoslock™. Both CAP (3.4 g/L) and FRP (<0.001 g/L) application rates of Phoslock™ were tested for the three sediment dwelling invertebrates. In previous toxicology and field studies with Phoslock™ the highest concentrations of La were detected immediately after the addition

of Phoslock™ and decline over time (McIntosh 2007; Martin and Hickey 2004). In our sediment toxicity tests concentrations of La in the overlying water were not measured until the end of the tests and based on reductions shown by Martin and Hickey (2004) could have been less than 1% of the concentration at test initiation. Total La concentrations measured in water overlying chironomid CAP dose sediment tests in this study were 340, 380 and 880 µg/L (Appendix 8). Khangarot (1991) determined the 24-hour EC_{50} (intoxication) of La hydroxide to *Tubifex tubifex* to be 33500 (24450-36390) µg/L. Unfortunately, there were no published studies on the chronic effects of La on benthic invertebrates at the time of this study.

Previous toxicity testing (10-day) was performed in New Zealand on the freshwater amphipod *Phreatogammarus helmsii*, the worm *Lumbriculus variegatus* and the midge larvae *Polypedilum parvidum* (Clearwater and Hickey 2004). The resulting LC_{50}s were 33 mg/L, >1000 mg/L and >400 mg/L, respectively. The authors hypothesized that physical smothering of the sediment or chemical changes in the pore water were responsible for reduced amphipod survival. Again, comparison of this study with ours is difficult since a different formulation of Phoslock™ and a TCLP preparation procedure were used. The experimental design used in this study portrayed a real-life application scenario that included the possibility of physical and chemical impairment as opposed to looking at chemical toxicity alone. *H. azteca* in our study were not impaired by concentrations of Phoslock™ as high as 3400 mg/L.

It is important to remember that the actual application rates used in the Lake Simcoe region in 2008 were only 0.05 g/L (Cane Parkway) and 0.02 g/L (Scanlon Pond reservoir), much less than the concentrations assessed in this toxicology study. Toxicity tests should always be compared with field results (EC 2008). Additional Phoslock™ application field trials and thorough chemical and benthic community monitoring is being conducted by the Lake Simcoe Region Conservation Authority and the Phoslock™ Steering Committee.

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6.0 APPENDICES

Appendix 1:

Ontario

Ministry of the Environment
Aquatic Toxicology Unit
Physical Chemistry and Litigation Section
Laboratory Services Branch

125 Resources Road
Etobicoke, ON
M9P 3V6
Phone: 416-235-6363
FAX: 416-235-5744

TOXICITY TEST REPORT

Sample Information

Submitted by: Lake Simcoe Conservation Authority (Mike Walters) and MOE Standards Development Branch
Location: Scanlon Pond, Lake Simcoe Basin
No. of Samples: 7

Name of Samples	Treatment	ATU Sample No.	Sample Code	Test Codes	LIMS No[1].
Peche Island Control with control water	No Phoslock	n/a	CS-CW-NP	CTMOE, HXMOE	n/a
Peche Island Control with site water	No Phoslock	n/a	CS-SW-NP	CTMOE, HXMOE	C160020-0001
Center of Scanlon Pond with site water	No Phoslock	01080011	CTR-SW-NP	CTMOE, HXMOE	C160020-0002
Center of Scanlon Pond with site water	Phoslock *	01080011	CTR-SW-FRP and/or CTR-SW-CAP	HYEC, CTMOE, HXMOE	C160020-0003
East site of Scanlon Pond with site water	No Phoslock	01080012	EST-SW-NP	CTMOE, HXMOE	C160020-0004
East site of Scanlon Pond with site water	Phoslock *	01080012	EST-SW-FRP and/or EST-SW-CAP	HYEC, CTMOE, HXMOE	C160020-0005
West site of Scanlon Pond with site water	No Phoslock	01080013	WST-SW-NP	CTMOE, HXMOE	C160020-0006
West site of Scanlon Pond with site water	Phoslock *	01080013	WST-SW-FRP and/or WST-SW-CAP	HYEC, CTMOE, HXMOE	C160020-0007
Phoslock Pure Product	8 conc. series with lab water	01080132	Phoslock - lab water	DMLC, RTLC	C160435-0001
Phoslock Pure Product	8 conc. series with site water	01080134	Phoslock - site water	DMLC	C160435-0002

[1]LIMS number which refers to requested toxicity test product codes

* Phoslock applied based on 250 Phoslock : 1 Filtered Reactive Phosphorous (FRP (i.e. Orthophosphate)) or in a capping dose (CAP) of 3.4 g/L

Unless otherwise noted all of the above samples were:

Sediment
Sampled by: Rob Wilson & Dave Lembke
Sample Method: Ekman
Date Sampled: May.15.2008
Date Received: May.16.2008
Received by: T. Watson - Leung
Condition on Receipt: seals intact, bags in tact
Storage: 4°C in laboratory cooler
Tests performed: 14-day *Hyalella azteca* growth and survival test (SOP HA2)
10-day *Chironomid dilutus* growth and survival test (SOP CT2)
21-day *Hexagenia sp.* growth and survival test (SOP HX2)

Water
Sampled by: Rob Wilson & Dave Lembke
Sample Method: grab
Date Sampled: May 15, May 20 and May 26
Water Chem. Submission codes: C159649-0001, C159649-0002, C159829-0001, C159829-0002
Received by: T. Watson - Leung
Condition on Receipt: seals intact, bags in tact
Storage: 4°C in laboratory cooler
Tests performed: 14-day *Hyalella azteca* growth and survival test (SOP HA2)
10-day *Chironomid dilutus* growth and survival test (SOP CT2)
21-day *Hexagenia sp.* growth and survival test (SOP HX2)
48 hour *Daphnia magna* LC_{50}

Sediment Description:

Name of Samples	colour	odour	soil type	Other
Center of Scanlon Pond	black	slight	silt, humic	none
East site of Scanlon Pond	black	slight	silt, humic	none
West site of Scanlon Pond	dark brown/black	slight	silt, humic	some dried twigs

Appendix 2:

SEDIMENT TOXICITY TEST REPORT

Hyalella azteca: 14-day survival and growth Test; Results Summary

TEST METHOD ***Based on:***

Environment Canada. Biological Test Method: Test for Survival and Growth in Sediment Using the Freshwater Amphipod Hyalella azteca. EPS 1/RM/33. December 1997.
and
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and
Borgmann, U, K.M. Ralph and W.P. Norwood. 1989. Toxicity Test Procedures for *Hyalella azteca* , and Chronic Toxicity of Cadmium and Pentachlorophenol to H. *azteca* , *Gammarus fasciatus* , and *Daphnia magna* . Arch. Environ. Contam. Toxicol., 18: 756-764.

TEST SYSTEM:

Sediment volume: 100 mL
Water volume: 400 mL
No. animals/replicate: 10
No. replicates: 3

Test containers: 700 mL glass jars
Control water source: dechlor. Toronto Tap
Site water source: Scanlon Pond water collected May.15.2008
Orthophosphate of site water = 0.0023 mg/L
FRP rate of 250 Phoslock : 1 FRP = 0.575 mg/L Phoslock
CAP rate = 3400 mg/L (0.25 kg/m^2)

Feeding: 2 mg NutraFin 3 times per week
Test Option: static, aerated

CULTURE INFORMATION:

Batch No.: May 16
Age Range at time 0: 5 to 7 days

QA/QC DATA (a reference toxicant test was performed)**:**

Reference test date: May.22.2008
ATU Sample No.: 01080119
96-hr LC50 (mg/L KCl): 308 mg/L
95% confidence limits (mg/L): 258 - 367

Historical Mean (mg/L): 280
Historical Warning Limits (mg/L): 221 - 340

RESULTS:

Date Test Initiated: May.21.2008
Date Test Terminated: June.04.2008
Statistical Software: SYSTAT Statistics, Inc. 2004. SYSTAT® 11 Statistics I. Richmond, CA. Version 11.0

Initiated by: T. Watson - Leung, J. Jassi
Terminated by: T. Watson - Leung, J. Jassi, A. Sharma

1) Survival Effects (≥ 80% required in the control)

Sediment	Percentage Survival (n = 10 per replicate)			Mean Survival per Sediment (%)	Standard Deviation	Coefficient of Variation (%)	Percent Survival of control	Fisher Exact Test
	rep. A	rep. B	rep. C					
CS-CW-FRP	100	100*	80	90	14	16	n/a	n/a
CS-SW-FRP	90	100	90	93	6	6	104	Not significant
CTR-SW-FRP	50	100	90	80	26	33	89	Not significant
EST-SW-FRP	100**	100	100	100	0	0	111	Not significant
WST-SW-FRP	100	100	80	93	12	12	104	Not significant
CTR-SW-CAP	80	80	70	77	6	8	85	Not significant
EST-SW-CAP	90	90	90	90	0	0	100	Not significant
WST-SW-CAP	90	100	100	97	6	6	107	Not significant

* 11 amphipods found; ** 12 amphipods found

2) Growth Effects (dry weight measured, ≥ 0.1 mg required in the control)

Sediment	Dry weight per organism (mg)			Mean Dry Weight per sediment (mg)	Standard Deviation	Coefficient of Variation (%)	Percent Growth of control	>60% Difference of Control
	rep. A	rep. B	rep. C					
CS-CW-FRP	0.40	0.32	0.29	0.33	0.05	16	n/a	n/a
CS-SW-FRP	0.37	0.38	0.50	0.41	0.07	17	123	no
CTR-SW-FRP	0.64	0.60	0.50	0.58	0.07	13	173	no
EST-SW-FRP	0.43	0.49	0.54	0.49	0.06	11	146	no
WST-SW-FRP	0.40	0.51	0.37	0.42	0.07	18	127	no
CTR-SW-CAP	n/a	0.40	0.21	0.31	0.14	44	92	no
EST-SW-CAP	1.14	0.34	0.36	0.61	0.46	74	183	no
WST-SW-CAP	0.46	0.39	0.36	0.40	0.05	13	120	no

SEDIMENT TOXICITY TEST REPORT (continued)

Hyalella azteca: 14-day survival and growth Test; Results Summary

Parameters measured at Day 0, 7 & 14

DAY 0						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-FRP	A-C*	8.0	8.2	315	23.1	3.5	0.2
CS-SW-FRP	A-C*	8.3	8.3	455	23.1	3.6	0.3
CTR-SW-FRP	A-C*	8.2	8.1	483	23.3	3.1	0.2
EST-SW-FRP	A-C*	8.1	7.9	486	23.7	4.2	0.3
WST-SW-FRP	A-C*	8.0	6.9**	494	23.7	3.3	0.2
CTR-SW-CAP	A-C*	8.3	8.1	522	23.7	3.1	0.3
EST-SW-CAP	A-C*	8.2	8.0	480	23.3	4.2	0.3
WST-SW-CAP	A-C*	8.2	8.0	488	23.6	3.5	0.3

* A:40ml B:40ml C:20ml

** aeration increased before animals were added

DAY 7						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-FRP	A-C*	8.3	8.1	494	22.8	< 0.8	<0.1
CS-SW-FRP	A-C*	8.3	8.1	464	22.7	< 0.8	<0.1
CTR-SW-FRP	A-C*	8.4	7.8	557	22.9	< 0.8	<0.1
EST-SW-FRP	A-C*	8.1	7.1	545	22.7	5.5	0.3
WST-SW-FRP	A-C*	8.3	7.8	548	23.1	1.3	0.1
CTR-SW-CAP	A-C*	8.2	7.1	565	23.1	1.7	0.1
EST-SW-CAP	A-C*	8.2	7.6	542	22.8	5.7	0.4
WST-SW-CAP	A-C*	8.2	7.5	544	23.1	< 0.8	<0.1

* A:20ml B:40ml C:40ml

Day 7 (May 27th) after Day 7 parameters were measured Phoslock was added to NP replicates top bring them from a 250:1 Phoslock:FRP dose up to a 1mm thick (3.4 g/L) capping dose.

DAY 14						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-FRP	C	8.5	8.1	476	22.8	< 1.0	< 0.1
CS-SW-FRP	C	8.6	8.3	521	22.7	< 1.0	<0.2
CTR-SW-FRP	C	8.6	8.3	638	22.7	< 1.0	<0.2
EST-SW-FRP	C	8.6	7.9	634	22.7	< 1.0	<0.2
WST-SW-FRP	C	8.6	8.0	588	22.7	< 1.0	<0.2
CTR-SW-CAP	C	8.5	7.0	786	22.9	< 1.0	< 0.1
EST-SW-CAP	C	8.6	8.0	675	22.7	< 1.0	<0.2
WST-SW-CAP	C	8.6	7.8	630	22.7	< 1.0	<0.2

Appendix 3:

Overlying Water in *Hyalella azteca* Test Vessels at Test Termination

Parameter	units	CS-CW-FRP	RDL	CS-SW-FRP	RDL	CTR-SW-FRP	RDL	EST-SW-FRP	RDL	WST-SW-FRP	RDL	CTR-SW-CAP	RDL	EST-SW-CAP	RDL	WST-SW-CAP	RDL
Total Kjeldahl Nitrogen (TKN)	mg/L	0.8	n/a	0.9	0.1	0.9	0.1	0.9	0.1	1.1	n/a	1.0	0.1	1.1	0.1	1	0.1
Orthophosphate (P)	mg/L	0.05	n/a	0.05	0.01	0.03	0.01	0.04	0.01	0.06	n/a	0.02	0.01	0.01	0.01	0.05	0.01
pH	pH	8.4		8.4		8.4		8.4		8.5		8.5		8.5		8.5	
Total Phosphorous	mg/L	0.073	n/a	0.080	0.002	0.072	0.002	0.076	0.002	0.110	n/a	0.040	0.002	0.062	0.002	0.120	0.002
Reactive Silica (SiO2)	mg/L	17	n/a	18	0.5	17	0.5	16	0.5	26	n/a	40	1	39	1	31	1
Alkalinity (total as CaCO3)	mg/L	145	n/a	156	1	201	1	186	1	203	n/a	207	1	187	1	208	1
*Lanthanum (total)	ppb	4		3		2		<2		585		240		1790		133	
*Lanthanum (dissolved)	ppb	<2		<2		<2		<2		3		4		7		3	
Total Aluminum	ug/L	3000	5	1400	5	100	5	110	5	130	5	37	5	2300	5	120	5
Dissolved Arsenic	ug/L	2	1	2	1	ND	1	ND	1	1	1	ND	1	ND	1	ND	1
Total Arsenic	ug/L	3	1	3	1	ND	1	ND	1	1	1	1	1	1	1	1	1

* Lanthanum results are questionable. Possible mix up of labels or samples.

RDL = Reportable Detection Limit

Appendix 4:

SEDIMENT TOXICITY TEST REPORT

Hexagenia spp: 21-day survival and growth Test; Results Summary

TEST METHOD *Based on:*

Bedard D, A Hayton & D Persaud. 1992. *Ontario Ministry of the Environment Laboratory Sediment Biological Testing Protocol,* Ontario Ministry of the Environment, Toronto, ON. 23 p.

and

American Society for Testing and Materials. 2000. *Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with* Freshwater Invertebrates. E 1706-00. Annual Book of ASTM Standards, Vol 11.05. pp 1109-1223.

TEST SYSTEM:

Sediment volume: 325 mL
Water volume: 1300 mL
No. animals/replicate: 10
No. replicates: 3

Test containers: 1.8 L glass jars
Control water source: dechlor. Toronto Tap
Site water source: Scanlon Pond water collected May.15 & May 20.2008
Orthophosphate of site water = 0.0023 mg/L and 0.0013 mg/L, respectively. Average = 0.0018 mg/L
FRP rate of 250 Phoslock : 1 FRP = 0.450 mg/L Phoslock

Feeding: none
Test Option: static, aerated

CULTURE INFORMATION:

Batch No.: 07-LP-08,09
Average wet weight (mg ± s.d.): 4.29 ± 1.89 mg

QA/QC DATA (a reference toxicant test was performed)**:**

Reference test date: May.16.2008
ATU Sample No.: 01080108
96-hr LC50 (g/L KCl): 1.54
95% confidence limits (g/L): 1.21 - 1.96

Historical Mean (g/L): 2.2
Historical Warning Limits (g/L): 1.3 - 3.1

RESULTS:

Date Test Initiated: May.22.2008
Date Test Terminated: June.12.2008
Statistical Software: SYSTAT Statistics, Inc. 2004. SYSTAT® 11 Statistics I. Richmond, CA. Version 11.0

Initiated by: T. Watson - Leung, J. Jassi, M. Appleton
Terminated by: T. Watson - Leung, J. Jassi, R. Chong-Kit

1) Survival Effects (≥ 80% required in the control)

* significantly different than survival in the control sediment using Fisher's Exact test (α = 0.05)

Sediment	Percentage Survival (n = 10 per replicate)			Mean Survival per sediment (%)	Standard Deviation	Coefficient of Variation (%)	Percent Survival of control	Fisher Exact Test*
	rep. A	rep. B	rep. C					
CS-CW-NP	100	100	100	100	0	0	n/a	n/a
CS-SW-NP	100	100	100	100	0	0	100	not signif.
CTR-SW-NP	100	100	100	100	0	0	100	not signif.
EST-SW-NP	100	80	90	90	10	11	90	not signif.
WST-SW-NP	100	100	100	100	0	0	100	not signif.
CTR-SW-FRP	100	100	100	100	0	0	100	not signif.
EST-SW-FRP	100	100	100	100	0	0	100	not signif.
WST-SW-FRP	100	100	100	100	0	0	100	not signif.

2) Growth Effects (wet weight measured; 2x initial weight required in control)

Sediment	Wet weight per organism (mg)			Mean Wet Weight per sediment (mg)	Standard Deviation	Coefficient of Variation (%)	Percent Weight of control	>60% Difference of Control
	rep. A	rep. B	rep. C					
CS-CW-NP	23.90	28.90	25.90	26.23	2.52	10	n/a	n/a
CS-SW-NP	22.60	24.40	27.30	24.77	2.37	10	94	no
CTR-SW-NP	48.80	54.70	45.50	49.67	4.66	9	189	no
EST-SW-NP	37.60	46.38	37.67	40.55	5.05	12	155	no
WST-SW-NP	54.80	52.90	47.70	51.80	3.68	7	197	no
CTR-SW-FRP	50.10	45.90	47.40	47.80	2.13	4	182	no
EST-SW-FRP	42.20	46.30	45.70	44.73	2.21	5	171	no
WST-SW-FRP	49.10	50.10	49.60	49.60	0.50	1	189	no

SEDIMENT TOXICITY TEST REPORT

Hexagenia spp: 21-day survival and growth Test; Results Summary

Parameters measured at Day 0, 9 & 21

DAY 0						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	A	8.4	8.7	317	22.3	2.5	0.3
CS-SW-NP	A	8.5	8.5	452	22.3	2.2	0.3
CTR-SW-NP	A	8.4	8.5	461	22.2	1.9	0.2
EST-SW-NP	A	8.4	8.4	485	22.0	3.3	0.3
WST-SW-NP	A	8.5	8.5	458	22.2	2.2	0.3
CTR-SW-FRP	A	8.4	8.5	463	21.9	1.9	0.2
EST-SW-FRP	A	8.5	8.5	469	21.9	3.1	0.4
WST-SW-FRP	A	8.5	8.5	459	22.3	2.3	0.3

DAY 9						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	A-C*	8.2	8.0	461	22.1	<0.8	<0.1
CS-SW-NP	A-C*	8.4	8.4	467	22.2	1.3	<0.1
CTR-SW-NP	A-C*	8.2	7.2	587	22.5	<0.8	<0.1
EST-SW-NP	A-C*	8.4	8.0	636	22.6	<0.8	<0.1
WST-SW-NP	A-C*	8.1	7.7	485	22.6	<0.8	<0.1
CTR-SW-FRP	A-C*	8.4	8.3	605	22.3	<0.8	<0.1
EST-SW-FRP	A-C*	8.4	8.2	671	22.5	<0.8	<0.1
WST-SW-FRP	A-C*	8.3	8.3	485	22.6	<0.8	<0.1

* A:40ml B:40ml C:20ml

DAY 21						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	A	8.4	8.6	526	22.1	<0.9	<0.1
CS-SW-NP	A	8.4	8.4	531	22.6	<0.9	<0.1
CTR-SW-NP	A	8.2	8.2	843	22.4	<0.9	<0.1
EST-SW-NP	A	8.2	8.5	979	21.9	<0.9	<0.1
WST-SW-NP	A	8.3	8.4	673	22.1	<0.9	<0.1
CTR-SW-FRP	A	8.1	8.5	832	22.2	<0.9	<0.1
EST-SW-FRP	A	8.3	8.3	1032	22.2	<0.9	<0.1
WST-SW-FRP	A	8.3	8.5	637	22.1	<0.9	<0.1

Appendix 5:

Overlying Water in *Hexagenia spp.* Test Vessels at Test Termination (FRP application rate = 0.45 mg/L)

Parameter Name	units	CS-CW-NP	RDL	CS-SW-NP	RDL	CTR-NP	RDL	EST-NP	RDL	WST-NP	RDL	CTR-FRP	RDL	EST-FRP	RDL	WST-FRP	RDL
Total Kjeldahl Nitrogen (TKN)	mg/L	2	1	2.5	0.1	2.1	0.5	2.1	0.5	2.1	0.5	1.7	0.1	3	1	2.1	0.5
Orthophosphate (P)	mg/L	0.02	0.01	0.02	0.01	0.02	0.01	0.02	0.01	0.04	0.01	0.03	0.01	0.03	0.01	0.05	0.01
pH	pH	8.2		8.2		8.1		8.2		8.1		8.1		8.0		8.2	
Total Phosphorous	mg/L	0.18	0.01	0.17	0.01	0.24	0.01	0.21	0.01	0.24	0.01	0.18	0.004	0.28	0.01	0.29	0.004
Reactive Silica (SiO2)	mg/L	17	0.5	17	0.5	13	0.5	12	0.5	12	0.5	13	0.5	11	0.5	12	0.5
Alkalinity (total as CaCO3)	mg/L	105	1	106	1	105	1	125	1	103	1	102	1	101	1	102	1
Lanthanum (total)	ppb	9		10		8		5		9		6		5		7	
Lanthanum (dissolved)	ppb	<2		<2		<2		<3		<2		<2		<2		<3	
Dissolved Aluminum	ug/L	ND	5	ND	5	8	5	11	5	12	5	9	5	12	5	11	5
Total Aluminum	ug/L	5000	5	5600	5	2700	5	3200	5	3100	5	2200	5	3000	5	2700	5
Dissolved Arsenic	ug/L	2	1	2	1	ND	1	ND	1	ND	1	ND	1	ND	1	ND	1
Total Arsenic	ug/L	3	1	3	1	1	1	2	1	1	1	1	1	2	1	1	1

RDL = Reportable Detection Limit

Appendix 6:

SEDIMENT TOXICITY TEST REPORT

Hexagenia spp: 21-day survival and growth Test; Results Summary

TEST METHOD ***Based on:***

Bedard D, A Hayton & D Persaud. 1992. *Ontario Ministry of the Environment Laboratory Sediment Biological Testing Protocol,* Ontario Ministry of the Environment, Toronto, ON. 23 p.

and

American Society for Testing and Materials. 2000. *Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with* Freshwater Invertebrates. E 1706-00. Annual Book of ASTM Standards, Vol 11.05. pp 1109-1223.

TEST SYSTEM:

Sediment volume:	325 mL	**Test containers:**	1.8 L glass jars	**Feeding:**	none
Water volume:	1300 mL	**Control water source:**	dechlor. Toronto Tap		
No. animals/replicate:	10	**Site water source:**	dechlor. Toronto Tap	**Test Option:**	static, aerated
No. replicates:	3				

CULTURE INFORMATION:

QA/QC DATA (a reference toxicant test was performed)**:**

Batch No.:	08-PI-62	**Reference test date:** Nov.27.2008	**Historical Mean (g/L):** 2.1
Average wet weight (mg ± s.d.):	6.6 ± 1.3 mg	**ATU Sample No.:** 01080237	**Historical Warning Limits (g/L):** 1.2 - 3.0
		96-hr LC50 (g/L KCl): 2.60	
		95% confidence limits (g/L): 0.71 - 1.12	

RESULTS:

Date Test Initiated:	Nov.27.2008	**Initiated by:**	T. Watson - Leung, L. Simmie
Date Test Terminated:	Dec.18.2008	**Terminated by:**	T. Watson - Leung, L. Simmie
Statistical Software:	SYSTAT Statistics, Inc. 2004. SYSTAT® 11 Statistics I. Richmond, CA. Version 11.0		

1) Survival Effects (? 80% required in the control)

* significantly different than survival in the control sediment using Fisher's Exact test ($\alpha = 0.05$)

Sediment	Percentage Survival (n = 10 per replicate)			Mean Survival per sediment (%)	Standard Deviation	Coefficient of Variation (%)	Percent Survival of control	Fisher Exact Test*
	rep. A	rep. B	rep. C					
CS-CW-NP	100	90	100	97	6	6	n/a	n/a
CS-CW-CAP	90	100	90	93	6	6	0.97	not signif.

2) Growth Effects (wet weight measured; 2x initial weight required in control)

Sediment	Wet weight per organism (mg)			Mean Wet Weight per sediment (mg)	Standard Deviation	Coefficient of Variation (%)	Percent Weight of control	>60% Difference of Control
	rep. A	rep. B	rep. C					
CS-CW-NP	15.05	17.29	17.69	16.68	1.42	9	n/a	n/a
CS-CW-CAP	14.52	12.27	15.26	14.02	1.56	11	84	no

SEDIMENT TOXICITY TEST REPORT

Hexagenia spp: 21-day survival and growth Test; Results Summary

Parameters measured at Day 0, 11 & 21

DAY 0 - before phoslock						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	B	8.1	7.9	351	22.3	1.6	0.1
CS-CW-CAP	B	8.1	8.0	336	22.2	1.6	0.1

DAY 0 - 1 hour after phoslock						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	B	9.0	7.8	394	22.3	2.4	0.8
CS-CW-CAP	B	7.8	8.1	404	22.3	6.5	0.2

DAY 11						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	A	8.2	8.1	460	22.5	<0.6	<0.1
CS-CW-CAP	A	8.2	8.2	395	22.3	<0.6	<0.1

DAY 21						Ammonia	Unionized Ammonia
Sample Name	Replicate	pH	DO	Conductivity	Temperature	$NH_3 + NH_4$	NH_3
			mg/L	µS/cm	°C	mg/L	mg/L
CS-CW-NP	C	8.2	8.1	468	21.8	<0.1	<0.1
CS-CW-CAP	C	8.3	8.3	545	21.6	<0.1	<0.1

Appendix 7:

SEDIMENT TOXICITY TEST REPORT

Chironomus dilutus* (formerly *tentans*): *10-day survival and growth Test; Results Summary

TEST METHOD: (modified based on the following 2 methods)

Environment Canada. Biological Test Method: Test for Survival and Growth in Sediment Using the Larvae of Freshwater Midges (*Chironomus tentans* or *Chironomus riparius*). EPS 1/RM/32. December 1997.

and

Bedard, D., A. Hayton and D. Persaud. 1992. Ontario Ministry of the Environment Laboratory Sediment Biological Testing Protocol, Ontario Ministry of the Environment, Water Resources Branch, ISBN 0-7729-9924-4, Toronto, Ontario. 23 p.

TEST SYSTEM:

Sediment volume: 100 mL
Water volume: 400 mL
No. animals/replicate: 10
No. replicates: 3

Test containers: 700 mL glass jars
Site water source: Scanlon Pond water collected May.26.2008
Orthophosphate of site water = 0.0074 mg/L
FRP rate of 250 Phoslock : 1 FRP = 1.85 mg/L Phoslock
CAP rate = 3400 mg/L (0.25 kg/m^2)

Feeding: 1.5 mL (6.0 mg dw) of 3:2 ceral leaves (CerophII®) : fish food flakes (Spirulina®); daily

Test Option: static, aerated

CULTURE INFORMATION:

Tank No./source: ECT 08-45
Date of Hatch out: May.19.2008
Mean Head Capsule Width at t=0: 0.36 ± 0.02

QA/QC DATA (a KCl reference toxicant test was performed)**:**

Reference test date: May.26.2008
ATU Sample No.: 01080123
96-hr LC50 (g/L KCl): 5.0
95% confidence limits: 3.89 - 6.43 g/L

Historical Mean: 4.8 g/L
Historical Warning Limits (g/L KCl): 2.7 - 7.1

RESULTS:

Date Test Initiated: May.30.2008
Date Test Terminated: Jun.09.2008
Statistical Software: SYSTAT Statistics, Inc. 2004. SYSTAT® 11 Statistics I. Richmond, CAersion 11.0

Initiated by: T. Watson-Leung, J. Jassi
Terminated by: T. Watson-Leung, J. Jassi

1) Survival Effects (≥ 70% required in the control)

* significantly different than survival in the control sediment using Fisher's Exact test (α = 0.05)

Sediment	Percentage Survival (n = 10 per replicate)			Mean Survival per sediment (%)	Standard Deviation	Coefficient of Variation (%)	Percent Survival of control	Fisher Exact Test
	rep. A	rep. B	rep. C					
CS-SW-NP	50	n/a	n/a					
CTR-SW-NP	70	n/a	n/a	80	24	31	n/a	n/a
EST-SW-NP	100	n/a	n/a					
WST-SW-NP	100	n/a	n/a					
CTR-SW-FRP	90	100	90	93	6	6	117	not signif.
EST-SW-FRP	90	90	60	80	17	22	100	not signif.
WST-SW-FRP	30	90	100	73	38	52	92	not signif.
CTR-SW-CAP	100	60	100	87	23	27	108	not signif.
EST-SW-CAP	80	80	50	70	17	25	88	not signif.
WST-SW-CAP	70	80	70	73	6	8	92	not signif.

2) Growth Effects (wet weight measured; ≥ 5.0 mg per organism (average) required in control)

Sediment	Wet weight per organism (mg)			Mean Wet Weight per sediment (mg)	Standard Deviation	Coefficient of Variation (%)	Percent Weight of control	>60% Difference of Control
	rep. A	rep. B	rep. C					
CS-SW-NP	12.59	n/a	n/a					
CTR-SW-NP	12.62	n/a	n/a	11.50	2	14	n/a	n/a
EST-SW-NP	9.24	n/a	n/a					
WST-SW-NP	11.57	n/a	n/a					
CTR-SW-FRP	11.75	11.36	11.91	11.67	0.28	2	101	no
EST-SW-FRP	11.47	10.87	14.11	12.15	1.72	14	106	no
WST-SW-FRP	15.79	10.95	12.44	13.06	2.48	19	114	no
CTR-SW-CAP	8.26	8.53	8.65	8.48	0.20	2	74	no
EST-SW-CAP	8.95	7.75	8.79	8.50	0.65	8	74	no
WST-SW-CAP	9.86	8.64	9.77	9.42	0.68	7	82	no

SEDIMENT TOXICITY TEST REPORT

Chironomus dilutus (formerly tentans): 10-day survival and growth Test; Results Summary

Parameters measured at Day 0, 5 & 10

DAY 0 Sample Name	Replicate	pH	DO mg/L	Conductivity µS/cm	Temperature °C	Ammonia $NH_3 + NH_4$ mg/L	Unionized Ammonia NH_3 mg/L
CS-SW-NP	A						
CTR-SW-NP	A	8.4	8.4	442	21.6	3.2	0.3
EST-SW-NP	A						
WST-SW-NP	A						
CTR-SW-FRP	A-C*	8.4	8.4	543	22.0	3.5	0.4
EST-SW-FRP	A-C*	8.3	8.5	535	21.6	5.0	0.4
WST-SW-FRP	A-C*	8.3	8.5	530	21.8	4.0	0.3
CTR-SW-CAP	A-C*	8.3	8.4	544	22.0	3.5	0.3
EST-SW-CAP	A-C*	8.3	8.5	533	21.7	5.2	0.4
WST-SW-CAP	A-C*	8.3	8.4	537	21.5	3.9	0.3

* A:40ml B:40ml C:20ml

DAY 5 Sample Name	Replicate	pH	DO mg/L	Conductivity µS/cm	Temperature °C	Ammonia $NH_3 + NH_4$ mg/L	Unionized Ammonia NH_3 mg/L
CS-SW-NP	B						
CTR-SW-NP	B	8.9	7.9	520	23.3	<1.0	<0.3
EST-SW-NP	B						
WST-SW-NP	B						
CTR-SW-FRP	A-C*	9.1	7.9	648	23.1	<1.0	<0.4
EST-SW-FRP	A-C*	8.9	7.1	584	23.2	<1.0	<0.3
WST-SW-FRP	A-C*	8.9	7.4	585	23.4	<1.0	<0.3
CTR-SW-CAP	A-C*	9.0	7.2	678	23.1	<1.0	<0.3
EST-SW-CAP	A-C*	8.9	7.9	623	23.0	<1.0	<0.3
WST-SW-CAP	A-C*	8.7	7.2	610	23.2	<1.0	<0.2

* A:20ml B:40ml C:40ml

DAY 10 Sample Name	Replicate	pH	DO mg/L	Conductivity µS/cm	Temperature °C	Ammonia $NH_3 + NH_4$ mg/L	Unionized Ammonia NH_3 mg/L
CS-SW-NP	A	8.5	8.3	484	23.2	<1.1	<0.2
CTR-SW-NP	A	8.5	8.2	569	23.2	<1.1	<0.2
EST-SW-NP	A	8.3	7.8	537	23.1	<1.1	<0.1
WST-SW-NP	A	8.3	8.0	481	23.4	<1.1	<0.1
CTR-SW-FRP	A	8.5	8.1	608	23.0	<1.1	<0.1
EST-SW-FRP	A	8.1	6.9	568	22.9	<1.1	<0.1
EST-SW-FRP	C	8.3	7.3	574	23.2	<1.1	<0.1
WST-SW-FRP	A	8.2	7.7	549	23.0	<1.1	<0.1
CTR-SW-CAP	A	8.4	7.8	633	23.0	<1.1	<0.1
CTR-SW-CAP	B	8.5	8.3	646	23.1	<1.1	<0.1
EST-SW-CAP	A	8.4	8.1	633	23.0	<1.1	<0.1
WST-SW-CAP	A	8.2	7.6	569	22.9	<1.1	<0.1

Appendix 8:

Overlying Water in *Chironomus dilutus* Test Vessels at Test Termination

Parameter Name	units	RDL	CS-NP	RDL	CTR-NP	RDL	EST-NP	RDL	WST-NP	RDL	CTR-FRP	RDL	EST-FRP	RDL	WST-FRP	RDL	CTR-CAP	RDL	EST-CAP	RDL	WST-CAP	RDL
Total Kjeldahl Nitrogen (TKN)	mg/L										1.8	0.2	2.2	0.1	1.8	0.1	1.1	0.1	1.5	0.1	1.3	0.2
Orthophosphate (P)	mg/L		0.06	0.01	0.08	0.01	0.12	0.01	0.09	0.01	0.06	0.01	0.04	0.01	0.05	0.01	0.01	0.01	0.02	0.01	0.03	0.01
pH	pH		8.4		8.3		8.1		8.1		8.3		8.2		8.2		8.4		8.3		8.2	
Total Phosphorous	mg/L										0.26	0.002	0.26	0.002	0.22	0.002	0.076	0.002	0.17	0.002	0.12	0.002
Reactive Silica (SiO2)	mg/L										14	0.5	13	0.5	13	0.5	29	1	26	1	29	1
Alkalinity (total as CaCO3)	mg/L		179	1	155	1	122	1	123	1	172	1	138	1	139	1	172	1	143	1	137	1
Lanthanum (total)	ppb										<2		<2		<3		880		380		340	
Dissolved Aluminum	ug/L	5									14		11		9		ND		ND		ND	
Total Aluminum	ug/L	5									780		3300		880		3100		940		500	
Dissolved Arsenic	ug/L	1									1		ND		ND		1		1		ND	
Total Arsenic	ug/L	1									1		2		1		1		1		ND	
Dissolved Antimony	ug/L	0.5									ND		ND		ND		ND		ND		ND	
Dissolved Barium	ug/L	5									40		41		43		53		49		55	
Dissolved Beryllium	ug/L	0.5									ND		ND		ND		ND		ND		ND	
Dissolved Bismuth	ug/L	1									ND		ND		ND		ND		ND		ND	
Dissolved Boron	ug/L	10									41		49		36		44		49		35	
Dissolved Cadmium	ug/L	0.1									ND		ND		ND		ND		ND		ND	
Dissolved Calcium	ug/L	200									84000		77000		64000		75000		76000		64000	
Dissolved Chromium	ug/L	5									ND		ND		ND		ND		ND		ND	
Dissolved Cobalt	ug/L	0.5									ND		0.7		ND		ND		0.6		ND	
Dissolved Copper	ug/L	1									2		2		2		2		2		3	
Dissolved Iron	ug/L	100									ND		ND		ND		ND		ND		ND	
Dissolved Lead	ug/L	0.5									ND		ND		ND		ND		ND		ND	
Dissolved Lithium	ug/L	5									ND		ND		ND		ND		ND		ND	
Dissolved Magnesium	ug/L	50									13000		11000		11000		12000		10000		11000	
Dissolved Manganese	ug/L	2									ND		ND		ND		ND		ND		ND	
Dissolved Molybdenum	ug/L	1									ND		1		ND		ND		ND		ND	
Dissolved Nickel	ug/L	1									ND		ND		ND		ND		ND		ND	
Dissolved Phosphorous	ug/L	100									ND		ND		ND		ND		ND		ND	
Dissolved Potassium	ug/L	200									8600		7700		6000		7800		7500		6200	
Dissolved Selenium	ug/L	2									ND		ND		ND		ND		ND		ND	
Dissolved Silicon	ug/L	50									6400		6100		6000		14000		12000		14000	
Dissolved Silver	ug/L	0.1									ND		ND		ND		ND		ND		ND	
Dissolved Sodium	ug/L	100									24000		22000		22000		33000		33000		34000	
Dissolved Strontium	ug/L	1									230		190		190		220		210		200	
Dissolved Tellurium	ug/L	1									ND		ND		ND		ND		ND		ND	
Dissolved Thallium	ug/L	0.05									ND		ND		ND		ND		ND		ND	
Dissolved Thorium	ug/L	1									ND		ND		ND		ND		ND		ND	
Dissolved Tin	ug/L	1									ND		ND		ND		ND		ND		ND	
Dissolved Titanium	ug/L	5									ND		ND		ND		ND		ND		ND	
Dissolved Tungsten	ug/L	1									ND		ND		ND		ND		ND		ND	
Dissolved Uranium	ug/L	0.1									1.0		0.8		0.6		0.5		0.7		0.4	
Dissolved Vanadium	ug/L	1									3		2		2		2		2		2	
Dissolved Zinc	ug/L	5									ND		ND		ND		ND		ND		ND	
Dissolved Zirconium	ug/L	1									ND		ND		ND		ND		ND		ND	

RDL = Reportable Detection Limit

Appendix9:

Ontario Ministry of Environment

Aquatic Toxicology Unit
Physical Standards and Litigation Services Section
Laboratory Services Branch

125 Resources Road
Etobicoke, ON
M9P 3V6
Phone: 416-235-5792
FAX: 416-235-5744

AQUATIC TOXICITY TEST REPORT

Acute toxicity to Rainbow trout and Daphnia magna

Sample Information

Submitted by:	Barrie, Central
Industry:	Phoslock
IMIS Code:	143
Location:	Lake Simcoe
Sample (CP):	1
Sample Name:	Phoslock Pure Product (Dry chemical)
Date Sampled:	16-JUN-2008
Sample Method:	grab
Date Received:	16-JUN-08 12:00 AM
Condition on Receipt:	sealed
Composited:	No
Storage:	Cooler
Notes:	

Sample Number	01080132
Lims Sample No	C160435-0001
Field Sample Number :	
Legal Seal?:	N
Legal Seals:	
Sampled by:	Mike Walters
Sample Appearance:	Colour: Tan
	Odour: None
	Turbidity: Heavy

Test Results Summary

2) Daphnia magna: 48-Hour Acute Lethality Test

Environment Canada. 1990. Biological Test Method: Acute Lethality Test Using Daphnia spp. EPS 1/RM/11. with May 1996 amendments.

Date of Test:	17-JUN-08 02:45 PM	**LC50 (%):**	4.94
Test Type	LC50	**95% confidence limits:**	18.18, 3.06
		Calculation Method:	Probit

Reported by:

Report Date:

Aquatic Toxicology

Daphnia magna Toxicity Test Data and Related QA/QC Data

Lims Sample No:	C160435-0001				
Sample Number:	01080132	**Sample Initial Parameters:**			
Sample Name:	Phoslock Pure Product	**temp (degrees C):**	20.9	**pH:**	6.8
Date Test Initiated:	17-JUN-08	**Cond. (uS/cm):**	401	**D.O. (mg/L):**	9
Time Test Initiated:	02:45 PM	**hardness:**	192	(mg/L as CaCO3)	
Initiated by:	A. Sharma				

TEST SYSTEM

test volume:	200 ml	**aeration rate*(ml/min/L):**	25-50	**dil. water source:**	Dechlorinated Toronto Tap
no. Animals/conc.:	12	**pre-aeration time (min):**	0	**dil. water hardness:**	128 (mg/L as CaCO3)
no. Replicates:	1	**pH Adjusted:**	No		
no. daphnia/replicate:	12	**light intensity (lux):**	518	*refers to before testing from oil-free compressed air.	
		test containers:	Beakers		

CULTURE INFORMATION

brood jar no.:	May 26 B,C	**avg. no. neonates/brood:**	25
days to first brood:	9	**% adult mortality:**	0

Observation at:	17-JUN-08 14:45:00	0.00 hrs.		D. magna		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
6.8	6.8	9	401	20.9	0	0
3.4	7.3	9	348	20.6	0	0
1.7	7.6	9	322	20.4	0	0
.85	7.9	9.1	314	20.2	0	0
.425	8.1	9	310	20.2	0	0
Control	8.3	9.1	308	20.2	0	0

Observation at:	19-JUN-08 15:00:00	48.25 hrs.		D. magna		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
6.8	8	9.5	397	20.9	6	1
3.4	8.2	9.5	335	20.9	6	0
1.7	8.3	9.5	306	20.9	3	0
.85	8.3	9.6	316	20.8	0	0
.425	8.3	9.6	308	20.5	0	0
Control	8.2	9.5	294	20.8	0	0

Testing Notes:

QA/QC DATA **Reference Test Date:** 16-JUN-08

A reference toxicant test was performed using the same culture of daphnids under the same test conditions as described above using Sodium Chloride (NaCl) as the reference toxicant.

48-hour LC50 (g/l NaCl):	5.51	**Historical Mean:**	5.70
95% confidence limits:	5.10 , 5.69	**Historical warning limits:**	5.26 , 6.17

The reference test indicates that the test system, technical performance and organism response are within established limits.

Data Verified by:

Sample No: 01080132 Page 2

NOTE: Concentrations are in g/L, not % as shown. Concentrations 0.213 and 0.106 g/L were excluded from this data sheet. Survival was 100% at these concentrations. Dilution water was dechlorinated tap water.

Appendix 10:

Ontario Ministry of Environment

Aquatic Toxicology Unit
Physical Standards and Litigation Services Section
Laboratory Services Branch

125 Resources Road
Etobicoke, ON
M9P 3V6
Phone: 416-235-5792
FAX: 416-235-5744

AQUATIC TOXICITY TEST REPORT

Acute toxicity to Rainbow trout and Daphnia magna

Sample Information

Submitted by:	Barrie, Central
Industry:	Phoslock
IMIS Code:	143
Location:	Lake Simcoe
Sample (CP):	1
Sample Name:	Phoslock Pure Product (Dry chemical)
Date Sampled:	16-JUN-2008
Sample Method:	grab
Date Received:	16-JUN-08 12:00 AM
Condition on Receipt:	sealed
Composited:	No
Storage:	Cooler
Notes:	Lake Simcoe Study

Sample Number	01080134
Lims Sample No	C160435-0002
Field Sample Number :	
Legal Seal?:	N
Legal Seals:	
Sampled by:	Mike Walters
Sample Appearance:	Colour: White
	Odour: None
	Turbidity: Heavy

Test Results Summary

1) Rainbow Trout: 96-Hour Acute Lethality Test

Environment Canada. 1990. Biological Test Method: Acute Lethality Test Using Rainbow Trout. EPS 1/RM/9. with May 1996 amendments.

Date of Test:	03-JUL-08 11:59 AM	**LC50 (%):**	> 100
Test Type	LC50	**95% confidence limits:**	,
		Calculation Method:	

2) Daphnia magna: 48-Hour Acute Lethality Test

Environment Canada. 1990. Biological Test Method: Acute Lethality Test Using Daphnia spp. EPS 1/RM/11. with May 1996 amendments.

Date of Test:	02-JUL-08 03:15 PM	**LC50 (%):**	> 100
Test Type	LC50	**95% confidence limits:**	,
		Calculation Method:	

Reported by:

Report Date:

Aquatic Toxicology

Rainbow Trout Toxicity Test Data and Related QA/QC Data

Lims Sample No:	C160435-0002				
Sample Number:	01080134	**Sample Initial Parameters:**			
Sample Name:	Phoslock Pure Product	**temp (degrees C):**	15.3	**pH:**	7.1
Date Test Initiated:	03-JUL-08	**Cond. (uS/cm):**	422	**D.O. (mg/L):**	10
Time Test Initiated:	11:59 AM				
Initiated by:	R. Chong-Kit, A. Sharma				

TEST SYSTEM

test volume:	10 litres	**aeration rate*(ml/min/L):**	6.5 ± 1	**dil. water source:**	Dechlorinated Toronto Tap
no. Animals/conc.:	10	**pre-aeration time (min):**	0	**dil. water hardness:**	128 (mg/L as CaCO3)
no. Replicates:	1	**pH Adjusted:**	No	**min solution depth (cm):**	15
		light intensity (lux):	316	*refers to before and during testing from oil-free compressed air.	
		test containers:	lined pails		

CULTURE INFORMATION

stock no.:	RBT-08-06-(1,2)	**mean weight ± SD (g):**	0.53 ± 0.14	**mean length ± SD (mm):**	42.50±3.72
% mortality (week prior):	0	**weight range (g):**	0.30 to 0.70	**length range (mm):**	33.00 to 47.00
		load density (g/L):	0.53		

CONTROL DATA

Observation at:	03-JUL-08 11:59:00		0.00 hrs.	trout		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
13.6	7.1	10	422	15.3	0	0
6.8	7.5	10	317	15.2	0	0
3.4	7.8	9.9	285	15.2	0	0
1.7	7.9	9.9	268	15.9	0	0
.85	8.1	10	261	15.2	0	0
.425	8.3	10	259	15.2	0	0
Control	8.3	10	254	15.1	0	0

Observation at:	04-JUL-08 10:35:00		22.60 hrs.	trout		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
13.6				14.6	0	0
6.8				14.4	0	0
3.4				14.5	0	0
1.7				14.5	0	0
.85				14.6	0	0
.425				14.5	0	0
Control				14.7	0	0

Observation at:	05-JUL-08 14:16:00		50.28 hrs.	trout		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
13.6				14.5	0	0
6.8				14.5	0	0
3.4				14.5	0	0
1.7				14.5	0	0
.85				14.5	0	0
.425				14.5	0	0
Control				14.5	0	0

NOTE: Concentrations are in g/L, not % as shown.

Observation at:	06-JUL-08 09:05:00		69.10 hrs.	trout		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
13.6				14.8	0	0
6.8				14.8	0	0
3.4				14.8	0	0
1.7				14.8	0	0
.85				14.8	0	0
.425				14.8	0	0
Control				14.8	0	0

Observation at:	07-JUL-08 11:47:00		95.80 hrs.	trout		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
13.6	8	9.9	447	14.7	1	1
6.8	8.1	10	325	14.9	0	0
3.4	8.2	10	286	14.8	0	0
1.7	8.2	10	271	14.8	0	0
.85	8.2	10	270	14.9	0	0
.425	8.3	9.9	266	14.8	0	0
Control	8.4	9.9	269	14.7	0	0

Testing Notes:

QA/QC DATA **Reference Test Date:** 20-JUN-08

A reference toxicant test was performed using the same culture of rainbow trout under the same test conditions as described above using Potassium Chloride (KCl) as the reference toxicant.

96-hour LC50 (g/l KCl): 3.50 **Historical Mean:** 3.05

95% confidence limits: 2.50 , 5.00 **Historical warning limits:** 1.93 , 4.82

The reference test indicates that the test system, technical performance and organism response are within established limits.

Data Verified by:

NOTE: Concentrations are in g/L, not % as shown. Dilution water was dechlorinated tap water.

Aquatic Toxicology

Daphnia magna Toxicity Test Data and Related QA/QC Data

Lims Sample No:	C160435-0002				
Sample Number:	01080134	**Sample Initial Parameters:**			
Sample Name:	Phoslock Pure Product	**temp (degrees C):**	19.4	**pH:**	8.4
Date Test Initiated:	02-JUL-08	**Cond. (uS/cm):**	536	**D.O. (mg/L):**	9
Time Test Initiated:	03:15 PM	**hardness:**	296	(mg/L as CaCO3)	
Initiated by:	A. Sharma				

TEST SYSTEM

test volume:	200 ml	**aeration rate*(ml/min/L):**	25-50	**dil. water source:**	Dechlorinated Toronto Tap
no. Animals/conc.:	12	**pre-aeration time (min):**	0	**dil. water hardness:**	128 (mg/L as CaCO3)
no. Replicates:	1	**pH Adjusted:**	No		
no. daphnia/replicate:	12	**light intensity (lux):**	468	*refers to before testing from oil-free compressed air.	
		test containers:	Beakers		

CULTURE INFORMATION

brood jar no.:	Jun 11 A,B,C	**avg. no. neonates/brood:**	25
days to first brood:	8	**% adult mortality:**	12

Observation at:	02-JUL-08 15:15:00	0.00 hrs.		D. magna		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
6.8	7.3	9.2	576	19.8	0	0
3.4	8	9.3	549	19.4	0	0
1.7	8.4	9.3	535	19.1	0	0
.85	8.5	9.3	527	19.1	0	0
.425	8.5	9.3	523	19.1	0	0
Control	8.5	9.3	287	19	0	0

Observation at:	04-JUL-08 14:01:00	46.77 hrs.		D. magna		
Concentration %	pH	D.O. (mg/L)	Cond. (umhos)	Temperature (C)	Mortality	Impairment
6.8	8.2	8.8	601	20.3	5	0
3.4	8.5	8.7	557	20.3	4	2
1.7	8.5	8.8	549	20.4	1	1
.85	8.5	8.8	541	20.4	0	0
.423	8.5	8.8	539	20.4	0	0
Control	8.3	9.1	306	20.3	0	0

Testing Notes:

QA/QC DATA **Reference Test Date:** 05-JUL-08

A reference toxicant test was performed using the same culture of daphnids under the same test conditions as described above using Sodium Chloride (NaCl) as the reference toxicant.

48-hour LC50 (g/l NaCl):	5.52	**Historical Mean:**	5.69
95% confidence limits:	4.87 , 6.24	**Historical warning limits:**	5.25 , 6.17

The reference test indicates that the test system, technical performance and organism response are within established limits.

Data Verified by:

NOTE: Concentrations are in g/L, not % as shown. Concentration 0.213 g/L was excluded from this data sheet. There was 100% survival in this concentration. Dilution water was Scanlon Pond water.

Appendix 11:

Lanthunum Concentration in Dilution Series used in *Daphnia magna* Testing

Dilution Concentration (g Phoslock/L)	Lanthanum (ppb)	
	Total	Dissolved
0 (site water)	2	<1
0 (lab water)	<1	2
0.2125	10260	6360
0.425	17910	11160
0.85	31950	17370
1.7	57600	31770
3.4	239400	63270
6.8	194400	14000

Appendix 12:

A) Analysis of Pore Water Extracted from Sediment Collected from Three Locations within Scanlon Pond

Parameter Name	Units	WST	RDL	EST	RDL	CTR	RDL
Lanthanum (total)	ppb	<5		<5		<5	
Lanthanum (dissolved)	ppb	<5		<6		<6	
Total Kjeldahl Nitrogen (TKN)	mg/L	16	0.5	20	0.5	14	0.5
Orthophosphate (FRP)	mg/L	ND	0.01	ND	0.01	ND	0.01
pH	pH	8.1		8.0		8.1	
Total Phosphorous	mg/L	0.042	0.002	0.022	0.002	0.13	0.002
Reactive Silica (SiO2)	mg/L	20	0.5	22	0.5	14	0.5
Alkalinity (total as CaCO3)	mg/L	361	1	273	1	339	1
Total Aluminum	ug/L	77	5	28	5	160	5
Total Arsenic	ug/L	3	1	3	1	3	1
Dissolved Aluminum	ug/L	6	5	5	5	5	5
Dissolved Arsenic	ug/L	3	1	2	1	2	1

B) Weak Acid Extraction on Sediment from Three Locations within Scanlon Pond

Parameter Name	Units	WST	RDL	EST	RDL	CTR	RDL
Lanthanum (total)	ppb	86		120		103	
Lanthanum (dissolved)	ppb	82		105		75	
Total Aluminum	ug/L	14000	5	24000	5	21000	5
Total Arsenic	ug/L	ND(1)	50	ND(1)	50	ND(1)	50
Dissolved Aluminum	ug/L	13000	5	23000	50	20000	5
Dissolved Arsenic	ug/L	ND(1)	5	ND(1)	5	ND(1)	5

C) Scanlon Pond Water

Parameter Name	units	*May 26	RDL	**May26A	Qual	**May26B	Qual	**May15	Qual	** May20	RDL
Total Kjeldahl Nitrogen (TKN)	mg/L	1.2	0.1	1.29		1.13					
Orthophosphate (FRP)	mg/L	0.02	0.01	0.0074	AIN	0.0036	AIN	0.0023	<T	0.0013	<T
pH	pH	8.3									
Total Phosphorous	mg/L	0.069	0.002	0.118		0.095					
Reactive Silica (SiO2)	mg/L	2.8	0.5								
Alkalinity (total as CaCO3)	mg/L	203	1								
Chlorophyll a	ug/L	24	0.5								
Nitrogen; ammonia+ammonium	mg/L			0.089		0.077		0.168		0.040	
Nitrogen; nitrate+nitrite	mg/L			0.111		0.117		0.666		0.396	
Nitrogen; nitrite	mg/L			0.015		0.015		0.022		0.018	
Total Aluminum	ug/L	ND	5								
Total Arsenic	ug/L	ND	1								
Dissolved Aluminum	ug/L	ND	5								
Dissolved Arsenic	ug/L	ND	1								

RDL = Reportable Detection Limit; ND = Not detected; AIN = Approx. result: Interference suspected; <T = A Measurable trace amount: Interpret with caution

* Maxxam Analytics Inc.; ** Ontario Ministry of the Environment Laboratory Services Branch

Appendix 13:

Summary of Toxicity Testing Methodologies

Appendix 13-1: (Note *Chironomus dilutus* was formerly known as *Chironomus tentans)*

***Chironomus tentans* Test for Survival and Growth in Sediment: Summary (from Bedard et al., 1992 and Environment Canada 1997; with in-lab refinement)**

Test Type and Duration	Static Non-Renewal, 10 days
Photoperiod, Light Intensity	16 hr light, 8 hr darkness; 500 - 1000 lux
Temperature	23°C ± 2°C
Dilution Water	Dechlorinated Toronto tap water
Renewal of Test Solutions	Replenish water loss due to evaporation as needed
Organism Age	>50% sub-sampled organisms head capsule width within 0.33-0.45 mm (10-12 days old; 2nd or 3rd instar)
Test Chambers and Sediment to Water Ratio*	7 cm diameter (>500 mL) glass vessels with 400 mL of dechlorinated water and 100 mL sediment
Organisms/Concentration	10 organisms per replicate . Minimum of 3 replicates.
Feeding Regime	Daily feed a 1.5 mL aliquot of 6 mg (d.w.) or 4 times during test feed 3.75 mL of 15 mg (d.w.) of a 3:2 mixture of Cerophyll®:Tetra Conditioning food®.
Test Solution Aeration	Aerate a minimum of 1 hour prior to adding organisms and gently aerate for the duration of the test.
Endpoints	Mortality, growth.
Time to Test Initiation	Test preferably within 2 weeks, must within 6 weeks of sample collection.
Sample Volume Required	5 to 10 L

* denotes a difference from Environment Canada (1997) method

Appendix 13-2:

***Hyalella azteca* Test for Survival and Growth in Sediment: Summary (from Bedard *et al.*, 1992 and Environment Canada 1997; with in-lab refinement)**	
Test Type and Duration	Static Non-Renewal, 14 days
Photoperiod; Light Intensity	16 hr light, 8 hr darkness; 500 - 1000 lux
Temperature	23°C ± 2°C
Dilution Water	Dechlorinated Toronto tap water
Renewal of Test Solutions	Replenish water loss due to evaporation as needed
Organism Age	2 to 9 days old
Test Chambers and Sediment to Water Ratio *	7 cm diameter (>500 mL) glass vessels with 400 mL of dechlorinated water and 100 mL sediment
Organisms/Concentration	10 organisms per replicate. Minimum of 3 replicates.
Feeding Regime *	3 times per week 2 mg of ground fish flake food (NutraFin®)
Test Solution Aeration	Aerate a minimum of 1 hour prior to adding organisms and gently aerate for the duration of the test.
Endpoints	Mortality, growth.
Time to Test Initiation	Test preferably within 2 weeks, must within 6 weeks of sample collection.
Sample Volume Required	5 to 10 L

* denotes differences from Environment Canada 1997

Appendix 13-3:

***Hexagenia* spp. Test for Survival and Growth in Sediment: Summary (from Bedard *et al.*, 1992; with in-lab refinement)**	
Test Type and Duration	Static Non-Renewal, 21 days
Photoperiod; Light Intensity	16 hr light, 8 hr darkness; 500 - 1000 lux
Temperature	23°C ± 2°C
Dilution Water	Dechlorinated Toronto tap water
Renewal of Test Solutions	Replenish water loss due to evaporation as needed
Organism Age	3 to 4 month old nymphs, average weight 5 mg.
Test Chambers	1.8 L glass vessels with 1300 mL of dechlorinated water and 325 mL sediment
Organisms/Concentration	10 organisms per replicate for a test density 0.08 nymphs per cm^2. Minimum of 3 replicates.
Feeding Regime	Animals are not fed during the test.
Test Solution Aeration	Aerate a minimum of 1 hour prior to adding organisms and gently aerate for the duration of the test.
Endpoints	Mortality, growth.
Time to Test Initiation	Test preferably within 2 weeks, must test within 6 weeks of sample collection.
Sample Volume Required	5 to 10 L

Appendix 13-4:

***Daphnia magna* Acute Lethality Test Summary (from Environment Canada, 1990, with May 1996 and December 2000 amendments)**	
Test Type and Duration	Static Non-Renewal, 48 hours
Photoperiod; Light Intensity	16 hr light, 8 hr darkness; 400 - 800 lux
Temperature	20°C ± 2°C
Dilution Water	Dechlorinated Toronto tap water; D.O. between 90 and 100% saturation, Total Residual Chlorine < 20 µg/L
Renewal of Test Solutions	No renewal during 48 hr tests
Organism Age	≤24 hr old
Test Chambers	Glass or clear plastic vessels large enough to ensure a minimum loading density of 1 daphnid/15 ml of solution
Organisms/Concentration	LC50 test - Minimum of 10 organisms/concentration Single Concentration Test - Minimum of 30 organisms/concentration
Feeding Regime	Organisms are not fed during the 48 hr test period
Test Solution Aeration	None - unless initial test solutions D.O. <40% or >100% air saturation, and then pre-aerate no more than 30 minutes. No aeration during test.
Concentration Range	Effluents: Normally 100%, 50%, 25%, 12.5%, 6.25% and control (100% dilution water), or as selected (but must include an undiluted (100%) rep, and all other concentrations must be at least 50% of preceding one. Receiving Waters: 100% receiving water and control Single Concentration Tests: 100% sample and control
Endpoints	Mortality, immobility
Time to Test Initiation	Test within 5 days of sample collection (5 days from end of sampling collection period), preferably within 3 days.
Sample: Volume Required	≥500 ml (or greater)

Appendix 13-5:

Rainbow Trout Acute Lethality Test Summary (Environment Canada, 1990, with May 1996 and December 2000 amendments)	
Test Type and Duration	Static Non-Renewal, 96 hour
Photoperiod	16 ± 1 hr light, 8 ± 1 hr darkness
Light Intensity	100 - 500 lux
Test Chambers	20 or 30 L buckets with plastic-liners or 10L stainless- steel containers and plastic covers
Test Solution Volume	2L/g fish
Test Solution Depth	15 cm minimum per bucket
Organism Age	Fish 30-120 days old, from swim-up (button-up) stage
Organism Loading Density	Not to exceed 0.5 g/L over 4 days
Organisms/Replicate	10 fish/ concentration/replicate or a total of 10 fish divided into 2 or more replicates if volume is limited
Organism Size	0.3 to 2.5 g
Replications/Concentration	1-2 replicates in single concentration tests 1 replicate/concentration in LC50 tests
Acclimation	Minimum 14 days in lab culture water at 15 ± 2°C
Aeration Rate	6.5 ± 1 ml/min/L
Organisms/Concentration	10 fish/concentration
Reference Toxicant	Potassium Chloride (KCl)
Concentration Series	logarithmic series; must include a 100% undiluted sample, and each successive dilution must be at least 50% of the preceding concentration (e.g. 100, 50, 25, 12.5, 6.25% and control (0%))
Feeding Regime	Fish are not fed for 16 hr before, nor during test
Test Solution Aeration	Pre-aerate all test solution for 30 minutes. If D.O. level is <70% or >100% air saturation, continue aeration for the lesser of 90 minutes and attaining 70% air saturation or 100% air saturation. Aerate throughout the 96-hr test period.
Dilution Water	Dechlorinated tap water
Endpoint	Mortality, 96 hr (%, LC_x, LT_x)
Exposure Temperature	15°C ± 1°C