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 LC₅₀ for *Daphnia magna* was 4.9 g/L and > 6.8 g/L Phoslock. The rainbow trout 96-hour LC₅₀ 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 Health 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™ and were also used for comparing biological responses in the Phoslock™ 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 (CaCO₃), lanthanum (La, total and
dissolved), aluminum (Al, total and dissolved), arsenic (As, total and dissolved) and reactive Silica (SiO₂).

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](image1)

![Hexagenia spp.](image2)

![Chironomus dilutus](image3)

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 LC50 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, CaCO3, La (total and dissolved), Al (total and dissolved), As (total and dissolved) and SiO2. 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 LC50 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 SiO₂ 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 from 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 LC₅₀ 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](image-url)  
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 $\geq 90\%$ 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 *Onchorhyncus mykiss* (rainbow trout)

### 3.2.6.1 Test Validity

A rainbow trout toxicity test is considered valid if survival in the control is \( \geq 90\% \) 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™.

<table>
<thead>
<tr>
<th>Phoslock Concentration (g/L)</th>
<th>% mortality</th>
<th>Daphnia magna (n=12)</th>
<th>Daphnia magna (n=12)</th>
<th>rainbow trout (n=10)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>site water</td>
<td>lab water</td>
<td>lab water</td>
<td></td>
</tr>
<tr>
<td>13.6</td>
<td>nt</td>
<td>nt</td>
<td>nt</td>
<td>10*</td>
</tr>
<tr>
<td>6.8</td>
<td>42</td>
<td>50*</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>33**</td>
<td>50</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>1.7</td>
<td>8*</td>
<td>25</td>
<td>0</td>
<td></td>
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<td>0</td>
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<td>0.10625</td>
<td>nt</td>
<td>0</td>
<td>nt</td>
<td></td>
</tr>
<tr>
<td>lab water</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td></td>
</tr>
<tr>
<td>Scanlon Pond water</td>
<td>0</td>
<td>nt</td>
<td>nt</td>
<td></td>
</tr>
</tbody>
</table>

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 LC50 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 LC50 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 LC50 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 LC50 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 LC50s 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 overlaying chironomid CAP dose sediment tests in this study were 340, 380 and 880 µg/L (Appendix 8). Khangarot (1991) determined the 24-hour EC₅₀ (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 LC₅₀s 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.
5.0 REFERENCES


6.0 APPENDICES
## 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

<table>
<thead>
<tr>
<th>Name of Samples</th>
<th>Treatment</th>
<th>ATU Sample No.</th>
<th>Sample Code</th>
<th>Test Codes</th>
<th>LIMS No.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Peche Island Control with control water</td>
<td>No Phoslock</td>
<td>n/a</td>
<td>CS-SW-NP</td>
<td>CTMOE, HXMOE</td>
<td>n/a</td>
</tr>
<tr>
<td>Peche Island Control with site water</td>
<td>No Phoslock</td>
<td>01080011</td>
<td>CTR-SW-NP</td>
<td>CTMOE, HXMOE</td>
<td>C160020-0002</td>
</tr>
<tr>
<td>Center of Scanlon Pond with water</td>
<td>Phoslock *</td>
<td>01080012</td>
<td>EST-SW-NP</td>
<td>CTMOE, HXMOE</td>
<td>C160020-0004</td>
</tr>
<tr>
<td>East site of Scanlon Pond with site water</td>
<td>No Phoslock</td>
<td>01080013</td>
<td>WST-SW-NP</td>
<td>CTMOE, HXMOE</td>
<td>C160020-0006</td>
</tr>
<tr>
<td>West site of Scanlon Pond with site water</td>
<td>Phoslock *</td>
<td>01080011</td>
<td>WST-SW-FRP</td>
<td>HXEC, CTMOE, HXMOE</td>
<td>C160020-0007</td>
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<tr>
<td>West site of Scanlon Pond with site water</td>
<td>Phoslock Pure Product</td>
<td>01080132</td>
<td>Phoslock - lab water</td>
<td>DMLC, RTLC</td>
<td>C160435-0001</td>
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<tr>
<td>Phoslock Pure Product 8 conc, series with lab water</td>
<td>01080135</td>
<td>Phoslock - site water</td>
<td>DMLC</td>
<td>C160435-0002</td>
<td></td>
</tr>
</tbody>
</table>

1. LIMS number which refers to requested toxicity test product codes  
2. 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**
  - Name of Samples: Center of Scanlon Pond, East site of Scanlon Pond, West site of Scanlon Pond  
  - Colour: black  
  - Odour: slight  
  - Soil Type: silt, humic  
  - Other: none

- **Water**
  - Name of Samples: Peche Island Control with control water, Peche Island Control with site water, Center of Scanlon Pond with control water  
  - Colour:  
  - Odour:  
  - Soil Type:  
  - Other:  

### Sediment Description:

<table>
<thead>
<tr>
<th>Name of Samples</th>
<th>Colour</th>
<th>Odour</th>
<th>Soil Type</th>
<th>Other</th>
</tr>
</thead>
<tbody>
<tr>
<td>Center of Scanlon Pond</td>
<td>black</td>
<td>slight</td>
<td>silt, humic</td>
<td>none</td>
</tr>
<tr>
<td>East site of Scanlon Pond</td>
<td>black</td>
<td>slight</td>
<td>silt, humic</td>
<td>none</td>
</tr>
<tr>
<td>West site of Scanlon Pond</td>
<td>dark brown/black</td>
<td>slight</td>
<td>silt, humic</td>
<td>some dried twigs</td>
</tr>
</tbody>
</table>
### Appendix 2:

#### SEDIMENT TOXICITY TEST REPORT

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

**TEST METHOD**

Based on:

**TEST SYSTEM:**

<table>
<thead>
<tr>
<th>Sediment volume:</th>
<th>100 mL</th>
<th>Test containers:</th>
<th>700 mL glass jars</th>
<th>Feeding:</th>
<th>2 mg NutraFin 3 times per week</th>
</tr>
</thead>
<tbody>
<tr>
<td>Water volume:</td>
<td>400 mL</td>
<td>Control water source:</td>
<td>dechlor, Toronto Tap</td>
<td>Test Option:</td>
<td>static, aerated</td>
</tr>
<tr>
<td>No. replicates:</td>
<td>3</td>
<td>Site water source:</td>
<td>Scanlon Pond water collected May.15.2008</td>
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<td></td>
</tr>
<tr>
<td>Orthophosphate of site water</td>
<td>0.0023 mg/L</td>
<td></td>
<td></td>
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<tr>
<td>FRP rate of 250 Phoslock :</td>
<td>1 FRP = 0.575 mg/L Phoslock</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>CAP rate =</td>
<td>3400 mg/L (0.25 kg/m²)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

**CULTURE INFORMATION:**

| Batch No.: | May 16 |
| Age Range at time 0: | 5 to 7 days |
| Site water source: | Scanlon Pond water collected May.15.2008 |
| FRP rate of 250 Phoslock : | 1 FRP = 0.575 mg/L Phoslock |
| CAP rate = | 3400 mg/L (0.25 kg/m²) |

**RESULTS:**

Date Test Initiated: May.21.2008  
Initiated by: T. Watson - Leung, J. Jassi  
Date Test Terminated: June.04.2008  
Terminated by: T. Watson - Leung, J. Jassi, A. Sharma  

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

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Percentage Survival (n = 10 per replicate)</th>
<th>Mean Survival per Sediment (%)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Survival of control</th>
<th>Fisher Exact Test</th>
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</thead>
<tbody>
<tr>
<td>CS-LW-FRP</td>
<td>100</td>
<td>90</td>
<td>80</td>
<td>90</td>
<td>14</td>
<td>18</td>
</tr>
<tr>
<td>CS-SW-FRP</td>
<td>90</td>
<td>100</td>
<td>90</td>
<td>90</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CTR-SW-FRP</td>
<td>50</td>
<td>100</td>
<td>90</td>
<td>80</td>
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<td>0</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>100**</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>WST-SW-FRP</td>
<td>100</td>
<td>100</td>
<td>80</td>
<td>93</td>
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<td>12</td>
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<tr>
<td>CTR-SW-CAP</td>
<td>90</td>
<td>100</td>
<td>90</td>
<td>90</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>EST-SW-CAP</td>
<td>90**</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

* 11 amphipods found. ** 12 amphipods found

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

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Dry weight per organism (mg)</th>
<th>Mean Dry Weight per sediment (mg)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Growth of control</th>
<th>&gt;60% Difference of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-LW-FRP</td>
<td>0.40</td>
<td>0.33</td>
<td>0.29</td>
<td>0.33</td>
<td>0.05</td>
<td>18</td>
</tr>
<tr>
<td>CS-SW-FRP</td>
<td>0.37</td>
<td>0.38</td>
<td>0.50</td>
<td>0.41</td>
<td>0.07</td>
<td>17</td>
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<tr>
<td>CTR-SW-FRP</td>
<td>0.64</td>
<td>0.60</td>
<td>0.50</td>
<td>0.58</td>
<td>0.07</td>
<td>13</td>
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<td>EST-SW-FRP</td>
<td>0.43</td>
<td>0.48</td>
<td>0.54</td>
<td>0.49</td>
<td>0.06</td>
<td>11</td>
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<tr>
<td>WST-SW-FRP</td>
<td>0.40</td>
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<td>0.37</td>
<td>0.42</td>
<td>0.07</td>
<td>18</td>
</tr>
<tr>
<td>CTR-SW-CAP</td>
<td>n/a</td>
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<td>0.21</td>
<td>0.31</td>
<td>0.14</td>
<td>44</td>
</tr>
<tr>
<td>EST-SW-CAP</td>
<td>1.14</td>
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<td>0.36</td>
<td>0.61</td>
<td>0.46</td>
<td>74</td>
</tr>
<tr>
<td>WST-SW-CAP</td>
<td>0.46</td>
<td>0.39</td>
<td>0.36</td>
<td>0.40</td>
<td>0.05</td>
<td>13</td>
</tr>
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## SEDIMENT TOXICITY TEST REPORT (continued)

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

Parameters measured at Day 0, 7 & 14

### Day 0

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO</th>
<th>Conductivity</th>
<th>Temperature °C</th>
<th>Ammonia NH₃ + NH₄ mg/L</th>
<th>Unionized Ammonia NH₃ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-FRP</td>
<td>A-C*</td>
<td>8.0</td>
<td>8.2</td>
<td>315</td>
<td>23.1</td>
<td>3.5</td>
<td>0.2</td>
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<tr>
<td>CS-SW-FRP</td>
<td>A-C*</td>
<td>8.3</td>
<td>8.3</td>
<td>455</td>
<td>23.1</td>
<td>3.6</td>
<td>0.3</td>
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<td>A-C*</td>
<td>8.2</td>
<td>8.1</td>
<td>483</td>
<td>23.3</td>
<td>3.1</td>
<td>0.2</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>A-C*</td>
<td>8.1</td>
<td>7.9</td>
<td>486</td>
<td>23.7</td>
<td>4.2</td>
<td>0.3</td>
</tr>
<tr>
<td>WST-SW-FRP</td>
<td>A-C*</td>
<td>8.0</td>
<td>6.9**</td>
<td>494</td>
<td>23.7</td>
<td>3.3</td>
<td>0.2</td>
</tr>
<tr>
<td>CTR-SW-CAP</td>
<td>A-C*</td>
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<td>23.7</td>
<td>3.1</td>
<td>0.3</td>
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<td>A-C*</td>
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<td>8.0</td>
<td>480</td>
<td>23.3</td>
<td>4.2</td>
<td>0.3</td>
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<td>WST-SW-CAP</td>
<td>A-C*</td>
<td>8.2</td>
<td>8.0</td>
<td>488</td>
<td>23.6</td>
<td>3.5</td>
<td>0.3</td>
</tr>
</tbody>
</table>

* A: 40ml B: 40ml C: 20ml
** aeration increased before animals were added

### Day 7

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

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO</th>
<th>Conductivity</th>
<th>Temperature °C</th>
<th>Ammonia NH₃ + NH₄ mg/L</th>
<th>Unionized Ammonia NH₃ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-FRP</td>
<td>A-C*</td>
<td>8.3</td>
<td>8.1</td>
<td>494</td>
<td>22.8</td>
<td>&lt; 0.8</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>CS-SW-FRP</td>
<td>A-C*</td>
<td>8.2</td>
<td>8.2</td>
<td>557</td>
<td>22.9</td>
<td>&lt; 0.8</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>CTR-SW-FRP</td>
<td>A-C*</td>
<td>8.1</td>
<td>7.1</td>
<td>545</td>
<td>22.7</td>
<td>5.5</td>
<td>0.3</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>A-C*</td>
<td>8.1</td>
<td>7.1</td>
<td>548</td>
<td>23.1</td>
<td>1.3</td>
<td>0.1</td>
</tr>
<tr>
<td>WST-SW-FRP</td>
<td>A-C*</td>
<td>8.3</td>
<td>7.8</td>
<td>565</td>
<td>23.1</td>
<td>1.7</td>
<td>0.1</td>
</tr>
<tr>
<td>CTR-SW-CAP</td>
<td>A-C*</td>
<td>8.2</td>
<td>7.1</td>
<td>542</td>
<td>22.8</td>
<td>5.7</td>
<td>0.4</td>
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<tr>
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<td>A-C*</td>
<td>8.2</td>
<td>7.6</td>
<td>544</td>
<td>23.1</td>
<td>&lt; 0.8</td>
<td>&lt; 0.1</td>
</tr>
<tr>
<td>WST-SW-CAP</td>
<td>A-C*</td>
<td>8.3</td>
<td>7.5</td>
<td>544</td>
<td>23.1</td>
<td>&lt; 0.8</td>
<td>&lt; 0.1</td>
</tr>
</tbody>
</table>

* A: 40ml B: 40ml C: 20ml
** aeration increased before animals were added

### Day 14

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO</th>
<th>Conductivity</th>
<th>Temperature °C</th>
<th>Ammonia NH₃ + NH₄ mg/L</th>
<th>Unionized Ammonia NH₃ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-FRP</td>
<td>C</td>
<td>8.5</td>
<td>8.1</td>
<td>476</td>
<td>22.8</td>
<td>&lt; 1.0</td>
<td>&lt; 0.1</td>
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<tr>
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<td>C</td>
<td>8.6</td>
<td>8.3</td>
<td>521</td>
<td>22.7</td>
<td>&lt; 1.0</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>CTR-SW-FRP</td>
<td>C</td>
<td>8.6</td>
<td>8.3</td>
<td>638</td>
<td>22.7</td>
<td>&lt; 1.0</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>C</td>
<td>8.6</td>
<td>7.9</td>
<td>634</td>
<td>22.7</td>
<td>&lt; 1.0</td>
<td>&lt; 0.2</td>
</tr>
<tr>
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<td>8.0</td>
<td>588</td>
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<td>&lt; 1.0</td>
<td>&lt; 0.2</td>
</tr>
<tr>
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<td>C</td>
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<td>7.0</td>
<td>786</td>
<td>22.7</td>
<td>&lt; 1.0</td>
<td>&lt; 0.1</td>
</tr>
<tr>
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<td>8.0</td>
<td>675</td>
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<td>&lt; 1.0</td>
<td>&lt; 0.2</td>
</tr>
<tr>
<td>WST-SW-CAP</td>
<td>C</td>
<td>8.6</td>
<td>7.8</td>
<td>630</td>
<td>22.7</td>
<td>&lt; 1.0</td>
<td>&lt; 0.2</td>
</tr>
</tbody>
</table>

* A: 20ml B: 40ml C: 40ml
## 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 | 1.1        | 0.1 | 1.0        | 0.1 | 1.1        | 0.1 | 1.1        | 0.1 | 1.0        | 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       | n/a | 8.4       | n/a | 8.4        | n/a | 8.4        | n/a | 8.5        | n/a | 8.5        | n/a | 8.5        | n/a | 8.5        | n/a |
| Total Phosphorous                | mg/L  | 0.073     | n/a | 0.080     | 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   | ND         | 1   | ND         | 1   | ND         | 1   | ND         | 1   |
| Total Arsenic                    | ug/L  | 3         | 1   | 3         | 1   | ND         | 1   | ND         | 1   | ND         | 1   | ND         | 1   | ND         | 1   | ND         | 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:


**TEST SYSTEM:**
- Sediment volume: 325 mL
- Water volume: 1300 mL
- No. animals/replicate: 10
- No. replicates: 3
- 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

**CULTURE INFORMATION:**
- Batch No.: 07-LP-08,09
- 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

**RESULTS:**
- Date Test Initiated: May.22.2008
- Date Test Terminated: June.12.2008

1) **Survival Effects** (≥ 80% required in the control)
   - CS-CW-NP
   - CS-SW-NP
   - CTR-SW-NP
   - EST-SW-NP
   - WST-SW-NP
   - CTR-SW-FRP
   - EST-SW-FRP
   - WST-SW-FRP

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

#### Results Table

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Percentage Survival</th>
<th>Mean Survival per sediment (%)</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Survival of control</th>
<th>Fisher Exact Test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>n/a</td>
</tr>
<tr>
<td>CS-SW-NP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100 not signif.</td>
</tr>
<tr>
<td>CTR-SW-NP</td>
<td>100 80 90</td>
<td>90</td>
<td>10</td>
<td>9</td>
<td>90 not signif.</td>
</tr>
<tr>
<td>EST-SW-NP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100 not signif.</td>
</tr>
<tr>
<td>WST-SW-NP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100 not signif.</td>
</tr>
<tr>
<td>CTR-SW-FRP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100 not signif.</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100 not signif.</td>
</tr>
<tr>
<td>WST-SW-FRP</td>
<td>100 100 100</td>
<td>100</td>
<td>0</td>
<td>0</td>
<td>100 not signif.</td>
</tr>
</tbody>
</table>

#### Wet Weight Table

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Wet weight per organism (mg)</th>
<th>Mean Wet Weight per sediment (mg)</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Weight of control</th>
<th>&gt;60% Difference of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>23.90 28.90 25.90</td>
<td>26.23 2.52</td>
<td>10</td>
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<td>n/a</td>
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<td>22.60 24.40 27.30</td>
<td>24.77 2.37</td>
<td>10</td>
<td>94</td>
<td>no</td>
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<tr>
<td>CTR-SW-NP</td>
<td>48.80 54.70 45.50</td>
<td>49.67 4.66</td>
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<td>189</td>
<td>no</td>
</tr>
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<td>37.60 46.38 37.67</td>
<td>40.55 5.05</td>
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<td>WST-SW-NP</td>
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<td>51.80 3.68</td>
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<td>50.10 45.90 47.40</td>
<td>47.80 2.13</td>
<td>4</td>
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<td>no</td>
</tr>
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<td>49.60 0.50</td>
<td>1</td>
<td>189</td>
<td>no</td>
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### SEDIMENT TOXICITY TEST REPORT

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

Parameters measured at Day 0, 9 & 21

#### DAY 0

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO mg/L</th>
<th>Conductivity µS/cm</th>
<th>Temperature °C</th>
<th>Ammonia NH₃ + NH₄ mg/L</th>
<th>Unionized Ammonia NH₃ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>A</td>
<td>8.4</td>
<td>8.7</td>
<td>317</td>
<td>22.3</td>
<td>2.5</td>
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<tr>
<td>CS-SW-NP</td>
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<td>8.5</td>
<td>452</td>
<td>22.3</td>
<td>2.2</td>
<td>0.3</td>
</tr>
<tr>
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<td>A</td>
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<td>8.5</td>
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<td>22.2</td>
<td>1.9</td>
<td>0.2</td>
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<td>8.4</td>
<td>8.4</td>
<td>485</td>
<td>22.0</td>
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</tr>
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<td>WST-SW-NP</td>
<td>A</td>
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<td>8.5</td>
<td>458</td>
<td>22.2</td>
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<td>A</td>
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<td>8.5</td>
<td>463</td>
<td>21.9</td>
<td>1.9</td>
<td>0.2</td>
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<tr>
<td>EST-SW-FRP</td>
<td>A</td>
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<td>8.5</td>
<td>469</td>
<td>21.9</td>
<td>3.1</td>
<td>0.4</td>
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<tr>
<td>WST-SW-FRP</td>
<td>A</td>
<td>8.5</td>
<td>8.5</td>
<td>459</td>
<td>22.3</td>
<td>2.3</td>
<td>0.3</td>
</tr>
</tbody>
</table>

#### DAY 9

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO mg/L</th>
<th>Conductivity µS/cm</th>
<th>Temperature °C</th>
<th>Ammonia NH₃ + NH₄ mg/L</th>
<th>Unionized Ammonia NH₃ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>A-C*</td>
<td>8.2</td>
<td>8.0</td>
<td>461</td>
<td>22.1</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CS-SW-NP</td>
<td>A-C*</td>
<td>8.4</td>
<td>8.4</td>
<td>467</td>
<td>22.2</td>
<td>1.3</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CTR-SW-NP</td>
<td>A-C*</td>
<td>8.2</td>
<td>7.2</td>
<td>587</td>
<td>22.5</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>EST-SW-NP</td>
<td>A-C*</td>
<td>8.4</td>
<td>8.0</td>
<td>636</td>
<td>22.6</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>WST-SW-NP</td>
<td>A-C*</td>
<td>8.1</td>
<td>7.7</td>
<td>485</td>
<td>22.6</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CTR-SW-FRP</td>
<td>A-C*</td>
<td>8.4</td>
<td>8.3</td>
<td>605</td>
<td>22.3</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>A-C*</td>
<td>8.4</td>
<td>8.2</td>
<td>671</td>
<td>22.5</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>WST-SW-FRP</td>
<td>A-C*</td>
<td>8.3</td>
<td>8.3</td>
<td>485</td>
<td>22.6</td>
<td>&lt;0.8</td>
<td>&lt;0.1</td>
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</tbody>
</table>

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

#### DAY 21

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO mg/L</th>
<th>Conductivity µS/cm</th>
<th>Temperature °C</th>
<th>Ammonia NH₃ + NH₄ mg/L</th>
<th>Unionized Ammonia NH₃ mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>A</td>
<td>8.4</td>
<td>8.6</td>
<td>526</td>
<td>22.1</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CS-SW-NP</td>
<td>A</td>
<td>8.4</td>
<td>8.4</td>
<td>531</td>
<td>22.6</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CTR-SW-NP</td>
<td>A</td>
<td>8.2</td>
<td>8.2</td>
<td>843</td>
<td>22.4</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>EST-SW-NP</td>
<td>A</td>
<td>8.2</td>
<td>8.5</td>
<td>979</td>
<td>21.9</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>WST-SW-NP</td>
<td>A</td>
<td>8.3</td>
<td>8.4</td>
<td>673</td>
<td>22.1</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>CTR-SW-FRP</td>
<td>A</td>
<td>8.1</td>
<td>8.5</td>
<td>832</td>
<td>22.2</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>EST-SW-FRP</td>
<td>A</td>
<td>8.3</td>
<td>8.3</td>
<td>1032</td>
<td>22.2</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>WST-SW-FRP</td>
<td>A</td>
<td>8.3</td>
<td>8.5</td>
<td>637</td>
<td>22.1</td>
<td>&lt;0.9</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
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                                         |       | 8.2      | 8.2 | 8.1      | 8.1 | 8.1    | 8.1 | 8.1    | 8.1 | 8.1    | 8.1 | 8.0     | 8.1 | 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 | 13     | 0.5 | 11      | 0.5 | 12      | 0.5 |         |     |
| Alkalinity (total as CaCO3) mg/L           |       | 105      | 1   | 105      | 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       |     | <3     |     | <2     |     | <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   | 2       | 1   | 1       | 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:


and


**TEST SYSTEM:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Sediment volume:</td>
<td>325 mL</td>
</tr>
<tr>
<td>Water volume:</td>
<td>1300 mL</td>
</tr>
<tr>
<td>No. animals/replicate:</td>
<td>10</td>
</tr>
<tr>
<td>No. replicates:</td>
<td>3</td>
</tr>
<tr>
<td>Test containers:</td>
<td>1.8 L glass jars</td>
</tr>
<tr>
<td>Control water source:</td>
<td>dechlor. Toronto Tap</td>
</tr>
<tr>
<td>Site water source:</td>
<td>dechlor. Toronto Tap</td>
</tr>
<tr>
<td>Feeding:</td>
<td>none</td>
</tr>
<tr>
<td>Test Option:</td>
<td>static, aerated</td>
</tr>
</tbody>
</table>

**CULTURE INFORMATION:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Batch No.:</td>
<td>08-PI-62</td>
</tr>
<tr>
<td>Average wet weight (mg ± s.d.):</td>
<td>6.8 ± 1.3 mg</td>
</tr>
<tr>
<td>ATU Sample No.:</td>
<td>01080237</td>
</tr>
<tr>
<td>Reference test date:</td>
<td>Nov.27.2008</td>
</tr>
<tr>
<td>Historical Mean (g/L):</td>
<td>2.1</td>
</tr>
<tr>
<td>Historical Warning Limits (g/L):</td>
<td>1.2 - 3.0</td>
</tr>
<tr>
<td>96-hr LC50 (g/L KC1):</td>
<td>2.60</td>
</tr>
<tr>
<td>95% confidence limits (g/L):</td>
<td>0.71 - 1.12</td>
</tr>
</tbody>
</table>

**RESULTS:**

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Date Test Initiated:</td>
<td>Nov.27.2008</td>
</tr>
<tr>
<td>Date Test Terminated:</td>
<td>Dec.18.2008</td>
</tr>
<tr>
<td>Initiated by:</td>
<td>T. Watson - Leung, L. Simmie</td>
</tr>
<tr>
<td>Terminated by:</td>
<td>T. Watson - Leung, L. Simmie</td>
</tr>
</tbody>
</table>

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

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

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Percentage Survival (n = 10 per replicate)</th>
<th>Mean Survival per sediment (%)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Survival of control</th>
<th>Fisher Exact Test*</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>100</td>
<td>90</td>
<td>90</td>
<td>97</td>
<td>6</td>
<td>6</td>
</tr>
<tr>
<td>CS-CW-CAP</td>
<td>90</td>
<td>100</td>
<td>100</td>
<td>93</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Wet weight per organism (mg)</th>
<th>Mean Wet Weight per sediment (mg)</th>
<th>Standard Deviation</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Weight of control</th>
<th>&gt;60% Difference of Control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-CW-NP</td>
<td>15.05</td>
<td>17.29</td>
<td>17.99</td>
<td>16.88</td>
<td>1.42</td>
<td>9</td>
</tr>
<tr>
<td>CS-CW-CAP</td>
<td>14.52</td>
<td>12.27</td>
<td>15.26</td>
<td>14.02</td>
<td>1.56</td>
<td>11</td>
</tr>
</tbody>
</table>
## SEDIMENT TOXICITY TEST REPORT

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

Parameters measured at Day 0, 11 & 21

<table>
<thead>
<tr>
<th>DAY 0 - before phoslock</th>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature (°C)</th>
<th>Ammonia (NH₃ + NH₄) (mg/L)</th>
<th>Unionized Ammonia (NH₃) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS-CW-NP</td>
<td>B</td>
<td>8.1</td>
<td>7.9</td>
<td>351</td>
<td>22.3</td>
<td>1.6</td>
<td>0.1</td>
</tr>
<tr>
<td></td>
<td>CS-CW-CAP</td>
<td>B</td>
<td>8.1</td>
<td>8.0</td>
<td>336</td>
<td>22.2</td>
<td>1.6</td>
<td>0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAY 0 - 1 hour after phoslock</th>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature (°C)</th>
<th>Ammonia (NH₃ + NH₄) (mg/L)</th>
<th>Unionized Ammonia (NH₃) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS-CW-NP</td>
<td>B</td>
<td>9.0</td>
<td>7.8</td>
<td>394</td>
<td>22.3</td>
<td>2.4</td>
<td>0.8</td>
</tr>
<tr>
<td></td>
<td>CS-CW-CAP</td>
<td>B</td>
<td>7.8</td>
<td>8.1</td>
<td>404</td>
<td>22.3</td>
<td>6.5</td>
<td>0.2</td>
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</table>

<table>
<thead>
<tr>
<th>DAY 11</th>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature (°C)</th>
<th>Ammonia (NH₃ + NH₄) (mg/L)</th>
<th>Unionized Ammonia (NH₃) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS-CW-NP</td>
<td>A</td>
<td>8.2</td>
<td>8.1</td>
<td>460</td>
<td>22.5</td>
<td>&lt;0.6</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>CS-CW-CAP</td>
<td>A</td>
<td>8.2</td>
<td>8.2</td>
<td>395</td>
<td>22.3</td>
<td>&lt;0.6</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>DAY 21</th>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO (mg/L)</th>
<th>Conductivity (µS/cm)</th>
<th>Temperature (°C)</th>
<th>Ammonia (NH₃ + NH₄) (mg/L)</th>
<th>Unionized Ammonia (NH₃) (mg/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>CS-CW-NP</td>
<td>C</td>
<td>8.2</td>
<td>8.1</td>
<td>468</td>
<td>21.8</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td></td>
<td>CS-CW-CAP</td>
<td>C</td>
<td>8.3</td>
<td>8.3</td>
<td>545</td>
<td>21.6</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
</tbody>
</table>
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)


and


**TEST SYSTEM:**

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

**TEST CONTAINERS:**

- 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/m²)

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

- Tank No./source: ECT 08-45
- Reference test date: May 26, 2008
- Mean Head Capsule 96-hr LC50 (g/L KCl): 5.0
- 95% confidence limits: 3.89 - 6.43 g/L
- Warning Limits (g/L KCl): 2.7 - 7.1

**RESULTS:**

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Percentage Survival</th>
<th>Mean Survival per sediment (%)</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Survival of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-SW-NP</td>
<td>50%</td>
<td>80 ± 24</td>
<td>n/a</td>
<td>117 ± 6</td>
</tr>
<tr>
<td>CTR-SW-NP</td>
<td>70%</td>
<td>80 ± 24</td>
<td>n/a</td>
<td>100 ± 6</td>
</tr>
<tr>
<td>EST-SW-NP</td>
<td>100%</td>
<td>80 ± 24</td>
<td>n/a</td>
<td>87 ± 23</td>
</tr>
<tr>
<td>WST-SW-NP</td>
<td>100%</td>
<td>80 ± 24</td>
<td>n/a</td>
<td>70 ± 17</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Sediment</th>
<th>Wet weight per organism (mg)</th>
<th>Mean Wet Weight per sediment (mg)</th>
<th>Coefficient of Variation (%)</th>
<th>Percent Weight of control</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-SW-NP</td>
<td>12.59</td>
<td>11.50</td>
<td>14</td>
<td>n/a</td>
</tr>
<tr>
<td>CTR-SW-NP</td>
<td>12.62</td>
<td>11.35</td>
<td>2</td>
<td>n/a</td>
</tr>
<tr>
<td>EST-SW-NP</td>
<td>9.24</td>
<td>11.35</td>
<td>14</td>
<td>106 ± 7</td>
</tr>
<tr>
<td>WST-SW-NP</td>
<td>11.57</td>
<td>11.35</td>
<td>2</td>
<td>100 ± 6</td>
</tr>
</tbody>
</table>

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

### 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

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO mg/L</th>
<th>Conductivity µS/cm</th>
<th>Temperature °C</th>
<th>Ammonia NH3 + NH4 mg/L</th>
<th>Unionized Ammonia NH3 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-SW-NP</td>
<td>A</td>
<td>8.4</td>
<td>8.4</td>
<td>442</td>
<td>21.6</td>
<td>3.2</td>
<td>0.3</td>
</tr>
<tr>
<td>CTR-SW-NP</td>
<td>A</td>
<td>8.3</td>
<td>8.5</td>
<td>535</td>
<td>21.6</td>
<td>5.0</td>
<td>0.4</td>
</tr>
<tr>
<td>EST-SW-NP</td>
<td>A</td>
<td>8.3</td>
<td>8.5</td>
<td>530</td>
<td>21.8</td>
<td>4.0</td>
<td>0.3</td>
</tr>
<tr>
<td>WST-SW-NP</td>
<td>A</td>
<td>8.3</td>
<td>8.4</td>
<td>544</td>
<td>22.0</td>
<td>3.5</td>
<td>0.4</td>
</tr>
</tbody>
</table>

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

#### DAY 5

<table>
<thead>
<tr>
<th>Sample Name</th>
<th>Replicate</th>
<th>pH</th>
<th>DO mg/L</th>
<th>Conductivity µS/cm</th>
<th>Temperature °C</th>
<th>Ammonia NH3 + NH4 mg/L</th>
<th>Unionized Ammonia NH3 mg/L</th>
</tr>
</thead>
<tbody>
<tr>
<td>CS-SW-NP</td>
<td>B</td>
<td>8.9</td>
<td>7.9</td>
<td>520</td>
<td>23.3</td>
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<td>7.8</td>
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* A:20ml B:40ml C:40ml

#### DAY 10

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<th>Replicate</th>
<th>pH</th>
<th>DO mg/L</th>
<th>Conductivity µS/cm</th>
<th>Temperature °C</th>
<th>Ammonia NH3 + NH4 mg/L</th>
<th>Unionized Ammonia NH3 mg/L</th>
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Appendix 8:
Overlying Water in *Chironomus dilutus* Test Vessels at Test Termination

| Parameter Name                      | units | RDL CS-NP | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | RDL | 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 (PO₄)                | 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                                  |       | 8.4      | 8.3 | 8.1 | 8.1 | 8.3 | 8.2 | 8.2 | 8.4 | 8.3 | 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 | 157 | 1 |
| Lanthanum (total)                   | ppb   | <2       | <2 | <3 | 880 | 1 | 340 |
| Dissolved Aluminum                  | ug/L  | 14       | 1 | 11 | 9 | ND | ND | ND |
| Total Aluminum                      | ug/L  | 780      | 3300 | 880 | 3100 | 940 | 500 |
| Dissolved Arsenic                   | ug/L  | 1        | ND | ND | ND | 1 | 2 | 1 | 1 |
| Total Arsenic                       | ug/L  | 1        | ND | 2 | 1 | ND | ND |
| Dissolved Antimony                  | ug/L  | 0.5      | ND | ND | ND | ND | ND |
| Dissolved Barium                    | ug/L  | 40       | 41 | 43 | 53 | 49 | 55 |
| Dissolved Beryllium                 | ug/L  | 0.5      | ND | ND | ND | ND | ND |
| Dissolved Bismuth                   | ug/L  | 1        | ND | ND | ND | ND | ND |
| Dissolved Boron                     | ug/L  | 41       | 49 | 36 | 44 | 49 | 35 |
| Dissolved Cadmium                   | ug/L  | 0.1      | ND | ND | ND | ND | ND |
| Dissolved Calcium                   | ug/L  | 84000    | 77000 | 64000 | 75000 | 76000 | 64000 |
| Dissolved Chromium                  | ug/L  | 5        | ND | ND | ND | ND | ND |
| Dissolved Cobalt                    | ug/L  | 0.7      | ND | ND | ND | 0.6 | ND |
| Dissolved Copper                    | ug/L  | 2        | 2 | 2 | 2 | 2 | 3 |
| Dissolved Iron                      | ug/L  | 100      | ND | ND | ND | ND | ND |
| Dissolved Lead                      | ug/L  | 0.5      | ND | ND | ND | ND | ND |
| Dissolved Lithium                   | ug/L  | 5        | ND | ND | ND | ND | ND |
| Dissolved Magnesium                 | ug/L  | 13000    | 11000 | 11000 | 12000 | 10000 | 11000 |
| Dissolved Manganese                 | ug/L  | 2        | ND | ND | ND | ND | ND |
| Dissolved Molybdenum                | ug/L  | 1        | ND | ND | ND | ND | ND |
| Dissolved Nickel                    | ug/L  | 1        | ND | ND | ND | ND | ND |
| Dissolved Phosphorous               | ug/L  | 100      | ND | ND | ND | ND | ND |
| Dissolved Potassium                 | ug/L  | 8600    | 7700 | 6000 | 7800 | 7500 | 6200 |
| Dissolved Selenium                  | ug/L  | 2        | ND | ND | ND | ND | ND |
| Dissolved Silicon                   | ug/L  | 64000   | 6100 | 6000 | 14000 | 12000 | 14000 |
| Dissolved Silver                    | ug/L  | 100      | ND | ND | ND | ND | ND |
| Dissolved Sodium                    | ug/L  | 240000 | 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 |
| Dissolved Thallium                  | ug/L  | 0.05     | ND | ND | ND | ND | ND |
| Dissolved Thorium                   | ug/L  | 1        | ND | ND | ND | ND | ND |
| Dissolved Tin                       | ug/L  | 1        | ND | ND | ND | ND | ND |
| Dissolved Titanium                  | ug/L  | 5        | ND | ND | ND | ND | ND |
| Dissolved Tungsten                  | ug/L  | 1        | 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 |
| Dissolved Zinc                      | ug/L  | 5        | ND | ND | ND | ND | ND |
| Dissolved Zirconium                 | ug/L  | 1        | ND | ND | ND | ND | ND |

RDL = Reportable Detection Limit
Appendix 9:

AQUATIC TOXICITY TEST REPORT

Acute toxicity to Rainbow trout and Daphnia magna

Sample Information

Submitted by: Bannie, Central
Industry: Phoslock
IMIS Code: 143
Location: Lake Simcoe
Sample (CP): 1
Sample Name: Phoslock Pure Product (Dry chemical)
Date Sampled: 18-JUL-2008
Sample Method: grab
Date Received: 18-JUN-08 12:00 AM
Condition on Receipt: sealed
Composited: No
Storage: Cooler
Notes:

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

Test Results Summary

2) Daphnia magna: 48-Hour Acute Lethality Test


Date of Test: 17-JUN-06 02:45 PM
Test Type: LC50
LC50 (%): 4.94
95% confidence limits: 10.10, 3.06
Calculation Method: Probit

Reported by:
Report Date:
**Daphnia magna Toxicity Test Data and Related QA/QC Data**

**Time Sample No:** 900800112  
**Sample Number:** 010003.212  
**Sample Name:** Phosphorus Dose Product  
**Sample Initial Parameters:**  
Temp (degrees C): 26.6  
Cond. (μmol/L): 401  
D.O. (mg/L): 112  
Hardness: 102 (mg/L as CaCO3)

**Initiated by:**  
A. Sharma

### TEST SYSTEM

- **Test Volume:** 200 ml  
- **Aeration Rate (ml/min):** 25-50  
- **pH:** 7.3  
- **Light Intensity (lx):** 518  
- **Teal containers:** Deskers  
- **No. of Daphnia/Replicates:** 12  
- **No. of Animals/Replicates:** 12

### CULTURE INFORMATION

- **Brood jar no.:** May 20 D.C  
- **Avg. no. nauplius/brood:** 25

### Observation at: 17-JUN-08 14:05:00

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<th>pH</th>
<th>D.O. (mg/L)</th>
<th>Cond. (μmol/L)</th>
<th>Temperature (°C)</th>
<th>Mortality</th>
<th>Impairment</th>
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### Control

- **Concentration %:** 8.3  
- **pH:** 8.1  
- **D.O. (mg/L):** 308  
- **Cond. (μmol/L):** 20.3  
- **Temperature (°C):** 0  
- **Mortality:** 0  
- **Impairment:** 0

### Observation at: 19-JUN-08 15:00:00

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<th>Temperature (°C)</th>
<th>Mortality</th>
<th>Impairment</th>
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<td>0.6</td>
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<td>20.3</td>
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<td>0</td>
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### Control

- **Concentration %:** 8.3  
- **pH:** 8.3  
- **D.O. (mg/L):** 584  
- **Cond. (μmol/L):** 20.3  
- **Temperature (°C):** 0  
- **Mortality:** 0  
- **Impairment:** 0

### Testing Notes:

**GA/QC DATA**  
Reference Test Date: 11-JUN-08

A reference 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 NaCl):** 5.51  
- **Historical Mean:** 5.70

25% confidence limits: 5.12, 5.69  
Historical warning limits: 6.26, 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. 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:

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

AQUATIC TOXICITY TEST REPORT
Acute toxicity to Rainbow trout and Daphnia magna

Sample Information
Submitted by: Barrie, Central
Industry: Phoslock
IMS Code: 143
Location: Lake Simcoe
Sample (CP): 1
Sample Name: Phoslock Pure Product (Dry chemical)
Date Sampled: 16-JUN-2005
Sample Method: grab
Date Received: 16-JUN-06 12:00 AM
Condition on Receipt: sealed
Composited: No
Storage: Cooler
Notes: Lake Simcoe Study

Sample Number: 01080134
Lims Sample No: C100425-0002
Field Sample Number: 
Legal Seal?: N
Legal Seals:

Sampled by: Mike Waters
Sample Appearance: Colour: White
Odour: None
Turbidity: Heavy

Test Results Summary
1) Rainbow Trout: 96-Hour Acute Lethality Test

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

2) Daphnia magna: 48-Hour Acute Lethality Test

Date of Test: 02-JUL-08 03:15 PM
Test Type: LC50
LC50 (%): > 100
95% confidence limits: ,
Calculation Method:
Rainbow Trout Toxicity Test Data and Related QA/QC Data

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<th>C16020-0021</th>
<th>Sample Initial Parameters: temp (degree C):</th>
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<th>pH:</th>
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<td>Cond. (us/cm):</td>
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<tr>
<td>Initiated by:</td>
<td>R. Chong-05, A. Sharma</td>
<td></td>
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**TEST SYSTEM**
- Test volume: 10 litres
- Aeration rate (ml/min/L): 6.5 ± 1
- Water source: Decarbonated Toronto Tap
- Water hardness: 128 (mg/L as CaCO3)
- Solution depth (cm): 15

**CULTURE INFORMATION**
- Strain: BST-08-06-12
- Mean weight ± SD (g): 0.53 ± 0.14
- Mean length ± SD (mm): 42.5 ± 3.72
- Mortality (week prior): 0%
- Weight range (g): 0.39 to 0.70
- Length range (mm): 33.00 to 47.00
- Load density (g/L): 0.63

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<td>D.O. (mg/L)</td>
<td>Cond. (us/cm)</td>
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<td>13.0</td>
<td>7.1</td>
<td>10</td>
<td>332</td>
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<td>317</td>
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Sample No: 01600134

**NOTE:** Concentrations are in g/L, not % as shown.
### Observation a: 06-JUL-06 09:05:00

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<td></td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.4</td>
<td></td>
<td></td>
<td></td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.7</td>
<td></td>
<td></td>
<td></td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.65</td>
<td></td>
<td></td>
<td></td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.425</td>
<td></td>
<td></td>
<td></td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td></td>
<td></td>
<td></td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

### Observation b: 07-JUL-06 11:47:00

<table>
<thead>
<tr>
<th>Concentration (g/L)</th>
<th>pH</th>
<th>D.O. (mg/L)</th>
<th>Cond. (μmhos)</th>
<th>Temperature (°C)</th>
<th>Mortality</th>
<th>Impairment</th>
</tr>
</thead>
<tbody>
<tr>
<td>13.0</td>
<td>8</td>
<td>9.9</td>
<td>447</td>
<td>14.7</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>6.8</td>
<td>8.1</td>
<td>10</td>
<td>325</td>
<td>14.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>3.4</td>
<td>8.2</td>
<td>10</td>
<td>386</td>
<td>14.3</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>1.7</td>
<td>8.2</td>
<td>10</td>
<td>371</td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.65</td>
<td>8.2</td>
<td>10</td>
<td>370</td>
<td>14.9</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>.425</td>
<td>8.3</td>
<td>9.9</td>
<td>366</td>
<td>14.8</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Control</td>
<td>8.4</td>
<td>9.9</td>
<td>369</td>
<td>14.7</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

**Testing Notes:**

**QA/QC DATA**

Reference Test Date: 20-JUN-06

A reference bioassay test was performed using the same culture of rainbow trout under the same test conditions as described above using potassium chlorate (KClO3) as the reference toxicant.

96-hour LC50 (g/L KClO3): 3.50

5% confidence limits: 2.95, 5.00

Historical Mean: 3.65

Historical Warning limits: 1.93, 4.12

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

Data Verified by: [Signature]

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

<table>
<thead>
<tr>
<th>Dilution Concentration (g Phoslock/L)</th>
<th>Lanthanum (ppb)</th>
<th>Total</th>
<th>Dissolved</th>
</tr>
</thead>
<tbody>
<tr>
<td>0 (site water)</td>
<td>2</td>
<td>&lt;1</td>
<td></td>
</tr>
<tr>
<td>0 (lab water)</td>
<td>&lt;1</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>0.2125</td>
<td>10260</td>
<td>6360</td>
<td></td>
</tr>
<tr>
<td>0.425</td>
<td>17910</td>
<td>11160</td>
<td></td>
</tr>
<tr>
<td>0.85</td>
<td>31950</td>
<td>17370</td>
<td></td>
</tr>
<tr>
<td>1.7</td>
<td>57600</td>
<td>31770</td>
<td></td>
</tr>
<tr>
<td>3.4</td>
<td>239400</td>
<td>63270</td>
<td></td>
</tr>
<tr>
<td>6.8</td>
<td>194400</td>
<td>14000</td>
<td></td>
</tr>
</tbody>
</table>
### Appendix 12:

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

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

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

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

**C) Scanlon Pond Water**

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

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*)

<table>
<thead>
<tr>
<th><strong>Chironomus tentans Test for Survival and Growth in Sediment: Summary</strong> (from Bedard et al., 1992 and Environment Canada 1997; with in-lab refinement)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Test Type and Duration</td>
</tr>
<tr>
<td>Photoperiod, Light Intensity</td>
</tr>
<tr>
<td>Temperature</td>
</tr>
<tr>
<td>Dilution Water</td>
</tr>
<tr>
<td>Renewal of Test Solutions</td>
</tr>
<tr>
<td>Organism Age</td>
</tr>
<tr>
<td>Test Chambers and Sediment to Water Ratio*</td>
</tr>
<tr>
<td>Organisms/Concentration</td>
</tr>
<tr>
<td>Feeding Regime</td>
</tr>
<tr>
<td>Test Solution Aeration</td>
</tr>
<tr>
<td>Endpoints</td>
</tr>
<tr>
<td>Time to Test Initiation</td>
</tr>
<tr>
<td>Sample Volume Required</td>
</tr>
</tbody>
</table>

* 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)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Type and Duration</strong></td>
<td>Static Non-Renewal, 14 days</td>
</tr>
<tr>
<td><strong>Photoperiod; Light Intensity</strong></td>
<td>16 hr light, 8 hr darkness; 500 - 1000 lux</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>23°C ± 2°C</td>
</tr>
<tr>
<td><strong>Dilution Water</strong></td>
<td>Dechlorinated Toronto tap water</td>
</tr>
<tr>
<td><strong>Renewal of Test Solutions</strong></td>
<td>Replenish water loss due to evaporation as needed</td>
</tr>
<tr>
<td><strong>Organism Age</strong></td>
<td>2 to 9 days old</td>
</tr>
<tr>
<td><strong>Test Chambers and Sediment to Water Ratio</strong></td>
<td>7 cm diameter (&gt;500 mL) glass vessels with 400 mL of dechlorinated water and 100 mL sediment</td>
</tr>
<tr>
<td><strong>Organisms/Concentration</strong></td>
<td>10 organisms per replicate. Minimum of 3 replicates.</td>
</tr>
<tr>
<td><strong>Feeding Regime</strong></td>
<td>3 times per week 2 mg of ground fish flake food (NutraFin®)</td>
</tr>
<tr>
<td><strong>Test Solution Aeration</strong></td>
<td>Aerate a minimum of 1 hour prior to adding organisms and gently aerate for the duration of the test.</td>
</tr>
<tr>
<td><strong>Endpoints</strong></td>
<td>Mortality, growth.</td>
</tr>
<tr>
<td><strong>Time to Test Initiation</strong></td>
<td>Test preferably within 2 weeks, must within 6 weeks of sample collection.</td>
</tr>
<tr>
<td><strong>Sample Volume Required</strong></td>
<td>5 to 10 L</td>
</tr>
</tbody>
</table>

* 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)*

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Specification</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Test Type and Duration</strong></td>
<td>Static Non-Renewal, 21 days</td>
</tr>
<tr>
<td><strong>Photoperiod; Light Intensity</strong></td>
<td>16 hr light, 8 hr darkness; 500 - 1000 lux</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>23°C ± 2°C</td>
</tr>
<tr>
<td><strong>Dilution Water</strong></td>
<td>Dechlorinated Toronto tap water</td>
</tr>
<tr>
<td><strong>Renewal of Test Solutions</strong></td>
<td>Replenish water loss due to evaporation as needed</td>
</tr>
<tr>
<td><strong>Organism Age</strong></td>
<td>3 to 4 month old nymphs, average weight 5 mg</td>
</tr>
<tr>
<td><strong>Test Chambers</strong></td>
<td>1.8 L glass vessels with 1300 mL of dechlorinated water and 325 mL sediment</td>
</tr>
<tr>
<td><strong>Organisms/Concentration</strong></td>
<td>10 organisms per replicate for a test density 0.08 nymphs per cm². Minimum of 3 replicates.</td>
</tr>
<tr>
<td><strong>Feeding Regime</strong></td>
<td>Animals are not fed during the test.</td>
</tr>
<tr>
<td><strong>Test Solution Aeration</strong></td>
<td>Aerate a minimum of 1 hour prior to adding organisms and gently aerate for the duration of the test.</td>
</tr>
<tr>
<td><strong>Endpoints</strong></td>
<td>Mortality, growth.</td>
</tr>
<tr>
<td><strong>Time to Test Initiation</strong></td>
<td>Test preferably within 2 weeks, must test within 6 weeks of sample collection.</td>
</tr>
<tr>
<td><strong>Sample Volume Required</strong></td>
<td>5 to 10 L</td>
</tr>
<tr>
<td>Appendix 13-4:</td>
<td></td>
</tr>
<tr>
<td>----------------</td>
<td></td>
</tr>
<tr>
<td><strong>Daphnia magna Acute Lethality Test Summary</strong></td>
<td></td>
</tr>
<tr>
<td>(from Environment Canada, 1990, with May 1996 and December 2000 amendments)</td>
<td></td>
</tr>
<tr>
<td><strong>Test Type and Duration</strong></td>
<td>Static Non-Renewal, 48 hours</td>
</tr>
<tr>
<td><strong>Photoperiod; Light Intensity</strong></td>
<td>16 hr light, 8 hr darkness; 400 - 800 lux</td>
</tr>
<tr>
<td><strong>Temperature</strong></td>
<td>20°C ± 2°C</td>
</tr>
<tr>
<td><strong>Dilution Water</strong></td>
<td>Dechlorinated Toronto tap water; D.O. between 90 and 100% saturation, Total Residual Chlorine &lt; 20 μg/L</td>
</tr>
<tr>
<td><strong>Renewal of Test Solutions</strong></td>
<td>No renewal during 48 hr tests</td>
</tr>
<tr>
<td><strong>Organism Age</strong></td>
<td>≤24 hr old</td>
</tr>
<tr>
<td><strong>Test Chambers</strong></td>
<td>Glass or clear plastic vessels large enough to ensure a minimum loading density of 1 daphnid/15 ml of solution</td>
</tr>
</tbody>
</table>
| **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)*

<table>
<thead>
<tr>
<th>Test Type and Duration</th>
<th>Static Non-Renewal, 96 hour</th>
</tr>
</thead>
<tbody>
<tr>
<td>Photoperiod</td>
<td>16 ± 1 hr light, 8 ± 1 hr darkness</td>
</tr>
<tr>
<td>Light Intensity</td>
<td>100 - 500 lux</td>
</tr>
<tr>
<td>Test Chambers</td>
<td>20 or 30 L buckets with plastic-liners or 10L stainless-steel containers and plastic covers</td>
</tr>
<tr>
<td>Test Solution Volume</td>
<td>2L/g fish</td>
</tr>
<tr>
<td>Test Solution Depth</td>
<td>15 cm minimum per bucket</td>
</tr>
<tr>
<td>Organism Age</td>
<td>Fish 30-120 days old, from swim-up (button-up) stage</td>
</tr>
<tr>
<td>Organism Loading Density</td>
<td>Not to exceed 0.5 g/L over 4 days</td>
</tr>
<tr>
<td>Organisms/Replicate</td>
<td>10 fish/concentration/replicate or a total of 10 fish divided into 2 or more replicates if volume is limited</td>
</tr>
<tr>
<td>Organism Size</td>
<td>0.3 to 2.5 g</td>
</tr>
<tr>
<td>Replications/Concentration</td>
<td>1-2 replicates in single concentration tests</td>
</tr>
<tr>
<td></td>
<td>1 replicate/concentration in LC50 tests</td>
</tr>
<tr>
<td>Acclimation</td>
<td>Minimum 14 days in lab culture water at 15 ± 2°C</td>
</tr>
<tr>
<td>Aeration Rate</td>
<td>6.5 ± 1 ml/min/L</td>
</tr>
<tr>
<td>Organisms/Concentration</td>
<td>10 fish/concentration</td>
</tr>
<tr>
<td>Reference Toxicant</td>
<td>Potassium Chloride (KCl)</td>
</tr>
<tr>
<td>Concentration Series</td>
<td>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%))</td>
</tr>
<tr>
<td>Feeding Regime</td>
<td>Fish are not fed for 16 hr before, nor during test</td>
</tr>
<tr>
<td>Test Solution Aeration</td>
<td>Pre-aerate all test solution for 30 minutes. If D.O. level is &lt;70% or &gt;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.</td>
</tr>
<tr>
<td>Dilution Water</td>
<td>Dechlorinated tap water</td>
</tr>
<tr>
<td>Endpoint</td>
<td>Mortality, 96 hr (%, LC₅₀, LT₅₀)</td>
</tr>
<tr>
<td>Exposure Temperature</td>
<td>15°C ± 1°C</td>
</tr>
</tbody>
</table>