GemChem, Inc.
An Environmental Management Company
Dedicated to Providing Superior Service
Since 1980

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November 2, 2010

Dr. Barry Scheetz, Center Director
Center for Dirt & Gravel Roads
The Larson Institute
201 Transportation Research Building
The Pennsylvania State University
University Park, PA 16802

RE: Environmental Performance Test Report
Roadbond EN 1™ – Patented Road Base Stabilizer
C.S.S. Technology, Inc.,
P.O. Box 549
Tolar, Texas 76476

Dear Dr. Scheetz:

On behalf of C.S.S. Technology, Inc. (CSS) of Tolar, Texas, GemChem, Inc. (GCI) is pleased to present this letter report to document the performance of an aquatic bio-toxicity evaluation on CSS's, patented road bond stabilizer product, Roadbond EN 1™. This evaluation is being submitted for consideration and approval by Penn State University's Center for Dirt & Gravel Roads. The purpose of the testing program is to determine if the Roadbond EN 1.™ soil stabilizer is safe to use, and is an environmentally friendly road bonding application on dirt and gravel roadways in the Commonwealth of Pennsylvania.

General Product Information

The Roadbond EN 1™ soil stabilizer is a patented stabilizer liquid solution, that when mixed with and diluted in water and appropriately applied to dirt and gravel roadways, bonds to the roadway surface and subsurface to produce a stronger, more stable and less erosive roadway. According to manufacturer specifications, Roadbond EN 1™ has three main uses:

- Replaces lime stabilization
- Reduces Portland cement and flyash
- Reinforces strength of base material and recycled in-place material

The Roadbond EN 1™ soil stabilizer is a blend of benign chemicals that promote a sustainable environment. The primary material is recovered from the ore smelting industry and is recycled to prevent pollution. It is also removed from crude oil in the refining process to prevent pollution and to make cleaner diesel fuel and gasoline. The supplier, as part of their prevention and recovery effort, captures the material to prevent release into the atmosphere.

A secondary ingredient comes from the waste material of citrus fruit and it is a rapidly renewable resource. The interaction of these compounds in the soil:

- Increases strength and strength improves over time
- Reduces permeability
- Controls shrink and swell
- Increases dry weight

In addition the above referenced attributes, the Roadbond EN 1™ soil stabilizer product is easy to use, cost efficient, accepted as a "go green" product and acknowledged as being environmentally harmless by the U.S. Forest Service when administered to a dirt and gravel roadway in accordance with proper manufacturer preparation and application specifications.

A copy of CSS's product brochure for the Roadbond EN 1™ soil stabilizer along with the instructions for installation and treatment on crushed stone and subgrade soils and a copy of the Material Safety Data Sheets for the product are provided in **Attachment A**.

Test Preparations

To assure that Roadbond EN 1™ soil stabilizer product is environmentally friendly in its final bonded state, a 28-Day Chronic Toxicity Evaluation of the Roadbond Leachate on Rainbow Trout (Oncorhynchus mykiss) was performed by the EnviroScience, Inc. (ES) laboratory, in Stow, Ohio. The ES laboratory is nationally accredited for its studies in bioassay and aquatic toxicological testing.

Prior to implementing the toxicity evaluation on the Roadbond EN 1™ soil stabilizer product, a test Study Plan was derived by the ES laboratory. The Study Plan was initially submitted to GCI for review. The Study Plan outlined the test methods to be employed, sample collection procedures, sample handling and storage, toxicity testing procedures, quality assurance procedures, and the guidelines for data analyses and reporting. The Study Plan was designed in accordance with testing procedures described in the OECD Guideline for testing chemicals; Fish, Juvenile Growth Test (OECD 215), OPPTS 850.1075; Fish Acute Toxicity Test, Freshwater and Marine (EPA 717-C-118) and the Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (EPA-821-R-02-013). These guidelines were adhered to during a 28 chronic test utilizing rainbow trout (Oncorhynchus mykiss). In addition, a simulated leachate was proposed for design in accordance with manufacturer's product preparatory and application procedures provided by CSS. Testing guidelines for the proposed leachate were conducted in accordance with the Evaluation of Dredged Material Proposed for Discharge in Waters of the U.S. (EPA-823-B-98-004). GCI evaluated each of these test procedures and acknowledged that the overall ES Study Plan was acceptable to perform the evaluation. A copy of the ES Study Plan is provided in **Attachment B.**

Test Program

The rainbow trout toxicity testing program performed by ES was performed for 28 days from September 23, 2010 through October 21, 2010. To perform the test, GCI shipped approximately 120 pounds of a gravel/dirt mix obtained from a local property (41° 22' 20.57" N latitude and 75° 18' 56.71" W longitude) in Wayne County, Pennsylvania, near active Marcellus Shale drilling operations. These soils are classified as the Walcksville and Long Run Members (undivided) of the Catskill Formation. The Catskill Formation is Devonian age and consists of sequences of sandstone, siltstone and mudstone. The dirt/gravel mix collected for sampling was hand dug from the above referenced location, from the ground surface down to shallow bedrock (approximately 3-feet below the ground surface). The aggregate mix consisted of a combination loose overburden and weathered bedrock aggregates of sandstone and siltstone.

Upon receipt, the ES laboratory used this soil mix as base material to apply the Roadbond EN 1™ soil stabilizer to, for the leachate in the toxicity test. The manufacturer's suggested sample preparation was conducted according to American Society for Testing Materials (ASTM) standard D698. The manufacturer's suggested application rates were also applied by ES to determine the necessary volumes of the Roadbond EN 1™ soil stabilizer for the sediment. Copies of the Roadbond EN 1™ Soil Stabilizer Procedures for Sample Preparation, ASTM Standard D698 and the Roadbond EN 1™ Soil Stabilizer Application Rates, are provided in **Attachment C**.

To perform the test, two gallons of the dirt/gravel mix were used to make eight gallons of run-off that was distributed among the lab replicates to formulate the desired concentrations. The proper application rate for two gallons of sediment was calculated by ES. An application rate of 0.0057 gallons per square yard at a

depth of six inches per manufacturer's specifications was calculated by the ES lab to properly prepare the simulated run-off. This ratio was utilized to determine the necessary amount of 1.25 milliliters of Roadbond EN 1™ soil stabilizer to treat two gallons of dirt/gravel mix. A 200:1 ratio of working solution of the Roadbond EN 1™ soil stabilizer was produced by ES in accordance with manufacturer's specifications. ES then dispersed 12.5 milliliters of working Roadbond EN 1™ solution over two gallons of sediment to obtain a concentrated leachate. The working solution was applied with a sterile spray bottle and manually mixed it in. The container of treated sediment was then allowed to set overnight for 12 hours tin accordance with manufacturer's specifications so enough time would be allotted for proper drying and bonding to take place. The following concentrated leachate or stock solutions were used by ES in the toxicity tests:

- One half the application rate
- One times the application rate
- Two times the application rate
- Four times the application rate

A sediment control was also produced by the ES laboratory that contained two liters of run-off in culture water. This volume was used to mimic the one time the application rate level for the test. It should be noted that the sediment control was not treated with the Roadbond EN 1™ solution. However, it was prepared in the same manner as the stock leachate for the test.

The test organisms of fingerling rainbow trout (Oncorhynchus mykiss) were purchased from Freshwater Farms of Ohio, Inc. The fingerling rainbow trout were initially 2-inches to 4-inches in length at the time they were purchased. The fish were initially held in untreated culture water at 14° celsius by the ES laboratory for a two week acclimation period. During this acclimation period and prior to the implementation of the toxicity test, the majority of the trout died as a result of overcrowding in their respective tanks.

Therefore, more rainbow trout were purchased for the test and the two week acclimation period was repeated at the ES laboratory. During this period very few mortalities and no disease was observed in the second group of organisms. The ES lab prepared the test vessels, and only seven fingerling rainbow trout were placed in each of the test vessels to adhere to mass loading recommendations of the methods followed and eliminate overcrowding. Prior to placing them in their respective tanks, the ES laboratory pre-weighed each test replicate containing seven fish and determined an average weight per fish. During the test, the rainbow trout were fed twice a day with a mixture of AquaMax trout chow, once at the beginning and again at the end of the work day.

The toxicity test was officially commenced on September 23, 2010 after the first group of test trout were exposed to the test solution. The test was performed by exposing a group of test fish to the Roadbond EN 1™ solution and comparing the results to a control group of fish that were not exposed. Accordingly, the mortality rates and growth of the exposed test fish exposed versus the non-exposed test fish were compared to determine whether or not the Roadbond EN 1™ solution leachate at one-half, one time, two times and four times the concentration was toxic to the fish.

During the test, testing solution temperatures were measured daily, water quality was sampled for chemical analyses and each test vessel was observed by laboratory personnel to determine the number of surviving fish in each vessel. Temperature, observations, and other water chemistry parameters, including conductivity, dissolved oxygen (DO), and pH were measured daily at each vessel. Test solutions were renewed weekly following leachate preparation procedures and the test vessels were siphoned to remove dirty water and debris prior to renewal proceedings.

Test vessels were checked daily, and if necessary, were siphoned cleaned when excess food and waste was observed in the vessels. However, during this cleaning process, no new water was added to the vessels to assure that the administered concentration of the Roadbond EN 1™ solution was not diluted. During this process, general water quality parameters (temperature, DO, pH, and conductivity) were monitored at test initiation, test renewal and daily in between renewals. Other chemistry parameters, including, alkalinity,

hardness, and residual chlorine were measured in the culture water and in the highest concentration (4 times the application rate), at the initiation of test and during each renewal process. This information was recorded daily on bench sheets (daily logs) by the laboratory technicians performing the test.

Dead fish were removed by lab personnel as soon as they were observed and recorded on the log sheets daily by the lab. Also, at the conclusion of the test, the no observable effect concentration (NOEC) and inhibition concentration (IC_{25}) was calculated from the survival and growth data using the ToxStat version 3.5 program. It should be noted that the NOEC end point represents the highest concentration of product that would not be expected to show significant impairment in the growth of the test organisms. The inhibition concentration is simply an estimate of the concentration of product that would cause a 25% reduction in measured growth response. Copies of the laboratory bench sheets and some photographs of the lab proceedings are provided in **Attachment D**.

Test Results

The overall laboratory findings for the rainbow trout (Oncorhynchus mykiss) toxicity test revealed that there were no significant differences in the mortality and growth for the fish exposed to the Roadbond EN 1™ solution leachate at one-half times, one time, two times and/or four times the normal rate of application, and those that were not exposed.

Based on the overall results of the toxicity tests, the ES laboratory concluded that the Roadbond EN 1™ soil stabilizer product should not impair the survival or growth of rainbow trout that live in waterways near roads where Roadbond EN 1™ soil stabilizer is applied. The statistical analyses and growth and survival data tabulated for the test is also documented on the bench sheets in **Attachment D**. A copy of the ES Laboratory's detailed Final Report, entitled "28 Day Chronic Toxicity Test Evaluating Roadbond Leachate Utilizing Rainbow Trout (Oncorhynchus mykiss), dated October 28, 2010, has been provided in **Attachment E**.

Conclusions and Recommendations

In conclusion, the ES laboratory 28-day toxicity test evaluation of the Roadbond EN 1™ soil stabilizer product on the rainbow trout (Oncorhynchus mykiss) has been adequately performed in accordance with all applicable ASTM standards, EPA testing procedures, and manufacturer's specifications. The results of the toxicity test ultimately revealed that there were no significant differences in the mortality and growth of the fish exposed to the Roadbond EN 1™ solution leachate from those that were not exposed. This includes fish that were exposed from one-half times the normal application concentration rate up to four times the normal application concentration rate. Therefore, GCI concludes that Roadbond EN 1™ soil stabilizer product is environmentally safe to fish that live in the waters of Pennsylvania when it is applied to dirt and gravel roadway surfaces according to manufacturer's specifications.

Based on the overall findings and conclusions, as presented herewith, GCI on behalf of CSS respectfully requests and recommends an approval by The Penn State Center for Dirt and Gravel Roads Committee for the future use and application of this product on dirt and gravel roadways within the Commonwealth of Pennsylvania.

Respectfully,

Alan R. Hirschfeld, P.G.
Project Scientist/Geologist

ATTACHMENT B

LABORATORY TOXICITY TEST STUDY PLAN

28 DAY CHRONIC TOXICITY TEST EVALUATING ROADBOND LEACHATE UTILIZING RAINBOW TROUT (*Oncorhynchus mykiss*)

STUDY PLAN

Submitted to:

GemChem, Inc.
PO Box 384
53 N Cedar St.
Lititz, PA 17543

Prepared by:

EnviroScience, Inc.
3781 Darrow Rd., Stow, OH 44224
800-940-4025 FAX: 330-688-3858

August 13, 2010

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1.0 INTRODUCTION

GemChem, Inc. seeks to evaluate leachate/simulated run-off toxicity as a result from application of Roadbond Stabilizer product to gravel roads. The following work plan outlines procedures described in OECD Guideline for the testing of Chemicals; Fish, Juvenile Growth Test (OECD 215), OPPTS 850.1075; Fish Acute Toxicity Test, Freshwater and Marine (EPA 712-C-96-118), & Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (EPA-821-R-02-013) to complete a 28 day chronic test utilizing rainbow trout, *Oncorhynchus mykiss*. Simulated leachate will be prepared in accordance with documents from C.S.S. Technology, Inc. and Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. (EPA-823-B-98-004).

2.0 TEST METHOD

The leachate preparation, test waters, toxicity test type, and test organism are defined in sections 2.1 through 2.3.

2.1 Leachate Preparation

Simulated leachate will be prepared in accordance with documents from C.S.S. Technology, Inc. and Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. (EPA-823-B-98-004). Gravel will be obtained local property near active Marcellus Shale drilling operations in Wayne County, PA by GemChem, Inc. and shipped to EnviroScience, Inc. (ES) for preparation of the leachate. Roadbond will also be provided to ES. Roadbond will be diluted as specified by the manufacturer and applied to the gravel at recommended application rates. This mixture will be allowed to bond for a 12 hour period, once bonded 1 liter of gravel will be added to 4 liters of culture water to make the leachate. This elutriate preparation procedure species a 30 minute mixing period and a one hour settling period before use in the test. The resulting elutriate/leachate will be used as the 100% concentration during the test. If deemed acceptable,

the Roadbond product will be applied at a higher than acceptable application rate to the gravel in order to obtain a concentrated stock solution of leachate which will be used to dose each aquaria.

2.2 Test Waters

In accordance with the OECD methodology any water may be used that shows suitable long-term survival and growth. Fish at the ES lab are cultured in de-chlorinated and filtered tap water; due to the volumes needed this will be the test water also. The ES synthesizes fresh water in accordance with USEPA protocols. Upon client request this may also be used as the dilution and test water. This synthetic moderately hard reconstituted water (MHRW) is prepared with ulta-pure deionized water provided by a Millipore® MilliQ plus UV water treatment system, with the addition of reagent grade salts (Appendix A). The alkalinity and hardness of this MHRW fall between 60-70 mg/l as $CaCO_3$ and 80-100 mg/l as $CaCO_3$, respectively.

2.3 Test Organism

OECD guidance recommends rainbow trout (*Oncorhynchus mykiss*) for this test procedure. The Rainbow Trout is a sensitive species stocked into lakes and streams in Pennsylvania around the primary locations that Roadbond will be used. Rainbow Trout prefer cold water habitats hence, the test will be performed at 14°C. Fingerling Rainbow Trout 2 inches to 4 inches in length of a known age will be purchased from Freshwater Farms of Ohio, Inc. and transported to the ES facility.

3.0 SAMPLE COLLECTION

Gravel to prepare the leachate will be collected from an approved quarry site in Pennsylvania and Roadbond will be provided by the manufacturer.

3.1 Test Schedule

Once fingerling Rainbow Trout are receive from the supplier, Freshwater Farms of Ohio, Inc. they will need 2 weeks to acclimate to the laboratory and for potential disease and mortality to be observed. Leachate will be prepared on September 13 and the test will begin on September 14 and last for 28 days.

4.0 SAMPLE HANDLING AND STORAGE

Upon receipt at EnviroScience, the gravel and Roadbond will be assigned tracking numbers and stored in a dark cool place until ready for use in the test.

5.0 TOXICITY TEST PROCEDURES

Toxicity test procedures including sample preparation and testing conditions are described in sections 5.1 through 5.4.

5.1 Test Organisms - Source and Acclimation Procedures

Oncorhynchus mykiss test specimens will be obtained from a commercial supplier yet to be determined.

Oncorhynchus mykiss test specimens will be given a two week acclimation period once they arrive at the ES facility during which any signs of mortality, disease and/or stress will be observed. Test specimens will be fingerling trout ranging from 2 to 4 inches in length and weighting between 1 and 5 grams. The acclimation conditions will mimic testing conditions with the temperature (14±1 °C), photoperiod (16h light/ 8h dark), and culture water that will be required for 28 day toxicity tests.

5.2 Toxicity Test Type

Test procedures have been determined from OECD Guideline for the testing of Chemicals; Fish, Juvenile Growth Test (OECD 215), OPPTS 850.1075; Fish Acute Toxicity Test, Freshwater and Marine (EPA 712-C-96-118), & Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (EPA-821-R-02-013) and have been modified to ES facility and client requests as needed. The test will be a 28 day, semi-static, renewal definitive toxicity test with *Oncorhynchus mykiss* at 14 °C.

Test conditions are summarized in the Table 5.1.

Table 5.1 Summary of toxicity test conditions for chronic testing with *Oncorhynchus mykiss*

1. Source and age of test organisms:	Freshwater Farms of Ohio, Inc.
2. Test type and duration:	Semi-static, renewal, 28 days
3. Photoperiod:	16 hours light / 8 hours dark
4. Light quality:	wide spectrum fluorescent light, 50-100fc
5. Test solution temperatures:	14±1 °C
6. Feeding regime:	Trout chow daily
7. Size of test vessel:	5 gallon aquaria
8. Volume of test solutions:	5 gallons
9. No. of test organisms per vessel:	10
10. No. of vessels per solution:	2
11. Total no. of organisms per solution:	20
12. Test concentrations:	10, 18, 32, 56, & 100
13. Renewal:	Once a week
14. Dilution/primary control waters:	culture water
17. Endpoints:	mortality - no movement with gentle prodding (LC_{50}); survival and growth NOEC; IC_{25}

5.3 Preparation of Test Solutions

The leachate will prepared following a number of different procedures. The Roadbond concentrate will be diluted with water using a ratio of 200:1 as per manufacturer guidance. Using a pipette 5 ml of concentrate will be added to 1000 ml liter of culture water to make the working solution. Once the suggested application rate is determined per manufacturer guidelines, it will be applied to the provided sediment and allowed to adhere for 12 hours. Another option is to add the working solution of Roadbond at a higher than recommended application rate (i.e. 2x, 5x or 10x) to make a concentrated leachate or stock solution which would them be dosed into the test vessels to create the same values of leachate as the previous procedure would. This second option would use less sediment and would allow for better renewal options of the test volumes.

Once the working solution of Roadbond is applied to the sediment a 12 hour mixing period will be observed. After 12 hours the leachate will be made using procedures for elutriate preparation outlined in the USEPA dredged material manuals. One liter of treated sediment will be added to 4 liters of culture water. If larger containers can be located, larger amounts of sediment and water will be mixed simultaneously following the 1 liter sediment to 4 liters of water ratio. The mixture will be stirred rapidly for 30 minutes using a magnetic stirring apparatus. A one hour settling period will follow the mixing period. Leachate will be siphoned off the top after the settling period and stored until all the leachate is made. The leachate will be placed into an incubator to acclimate to the testing temperature 14°C.

Once the leachate is 14°C ± 1°C, it will be added to the aquaria to achieve the desired concentrations of 10% leachate, 18% leachate, 32% leachate, 56% leachate and 100% leachate. Actual stocking volumes of the leachate will depend on the way the leachate was prepared (i.e. a concentrated stock solution was made). If the working solution was concentrated 10 times the rate of application one/tenth of the required volume will be needed to make the test

concentrations. ES would use 0.5 gallons of stock solution to create the 100% level instead of 5 gallons of un-concentrated leachate.

A leachate will also be prepared from the sediment without addition of Roadbond to evaluate any contaminants that may be present in the soil that could cause adverse effects to the test speciments. This will be tested at a 100% level with no culture water added.

5.4 Test Initiation, Conduct, and Termination

Test specimens will be acclimated to the appropriate temperature and water for 2 weeks prior to the test initiation. Water quality and mortality will be observed daily. If greater than 10% mortality is observed the entire batch of organisms will be discarded and new O.mykiss will be ordered. If between 5% and 10% mortality is observed an additional 7 day holding period is required. Test specimens will be held in an environmental chamber set to operate at 14 °C.

Once the acceptability of the batch has been determined, collective weight of each aquaria must be determined. Fish will be exposed to a mild anaesthetic solution, 10 specimens will be chosen at random, blotted dry and weighed to determine initial weight of each tank. This will be done carefully to minimize stress and damage to the organisms. After initial weight is determined the batch of fish will be placed in one aquarium. This will continue until all aquaria at all concentrations have 10 fish in them. Test initiation time is recorded on the test's bench sheet when the first organism has been exposed to a test solution.

Fish will be fed trout chow twice daily at a rate of 4% of the tank rate. This rate may be recalculated on Day 14. Fish will not be fed within 24 hours of test termination. Any extra food or other debris will be siphoned daily when mortality data is observed and recorded.

Water will be renewed weekly in accordance with preparation procedures listed in Section 5.3.

Tests will be terminated at 28 days ±1 hour. The date and time of test termination, solution temperatures, and the numbers of dead and affected specimens will be recorded on the bench sheet. Total tank weights will be determined again by the same method listed above.

6.0 PHYSICAL AND CHEMICAL PARAMETERS

Various physical and chemical parameters will be monitored at test initiation, during conduct, and at test termination. Table 6.1 summarizes the physical-chemical measurements that will be included with this study.

6.1 Physical and Chemical Analysis Associated with Initiation of WET Tests

The culture water will be characterized by the following water quality parameters: temperature, dissolved oxygen concentration (DO), pH, conductivity, alkalinity, hardness (EDTA method), and total residual chlorine (TRC). These parameters will also be determined for the highest leachate concentration and the secondary control of sediment water. Temperature, DO, pH, and conductivity will be measured and recorded on the bench sheets at the time of test initiation for each test solution (initial or 0-hour chemistry). Sub-samples for analysis of "0-hour" chemistry (except temperature) will be collected shortly before solutions are dispensed into test vessels.

Temperature, DO, pH, alkalinity, hardness, and TRC will be measured at EnviroScience's laboratory following written SOPs. Samples containing a detectable level of TRC (MDL 0.02 mg/l) will be de-chlorinated with sodium thiosulfate prior to use.

6.2 Physical and Chemical Analysis During Conduct and at Termination of WET Tests

Physical and chemical data typically collected after test initiation includes: temperature, DO, and pH. These parameters will be monitored daily from subsample of water collected from each test

vessel. The entire test will be aerated according to USEPA procedures to maintain a level of 60% oxygen saturation throughout the test.

At test termination immediately after temperature data and the number of observed mortalities and behavioral effects have been recorded, the contents of each replicate will be sub-sampled for analysis of DO, pH, and conductivity.

Table 6.1. Test methods for physical - chemical analysis
ES = EnviroScience, Inc. Ecotoxicology laboratory

Parameter	Method	frequency; test levels	Lab	Comments
temperature	EPA 170.1	daily; all levels	ES	digital; calibrated monthly with NBS certified thermometer
dissolved oxygen	APHA 4500-G	daily; all levels	ES	YSI 5100 meter
pH	APHA 4500-H	daily; all levels	ES	Orion 920A meter
conductivity	APHA 2510-B	weekly; all levels	ES	Orion 160 meter
alkalinity	APHA 2320-B	once, prior to test initiation; controls	ES	titrimetric, pH 4.5
hardness	APHA 2340-C	once, prior to test initiation; controls	ES	titrimetric, EDTA
total residual chlorine	APHA 4500-Cl D	once, prior to test initiation; controls	ES	amperometric, HACH Auto CAT 9000 meter

7.0 QUALITY ASSURANCE

This section describes quality assurance and quality control elements (QA/QC) of the Rainbow Trout study.

7.1 Dilution Water Acceptability

- Survival in control group must be 90% or greater at test termination.
- The mean weight must have increased by 50% of their mean initial weight over 28 days.
- The dissolved oxygen must have been at least 60% saturations throughout the test.
- The water temperature must not differ by more than ± 1°C.

7.2 Physical and Chemical Data Collected in EnviroScience's Laboratory

Accuracy and precision of routine physical and chemical measurements is monitored with duplicate and spiked samples (10%) where applicable to the method. Endpoints and control limits are plotted on control charts which are displayed in the laboratory. QC procedures are described in EnviroScience's written SOPs for analysis of physical-chemical parameters, which are available upon request.

8.0 DATA ANALYSIS

No Observable Effect Level (NOEC/NOEL) and Lowest Observable Effect Level (LOEC/LOEL) will be determined by statistical analysis using average growth data determined from the tanks' weights at test termination. Survival and mortality endpoints may also be determined depending on the outcome of the test. The computer program CT-TOX 1.1 will be used to derive these values using statistical analyses to test for normal distribution, heterogeneous variance and ANOVA followed by Dunnett's or Williams's tests will be performed.

9.0 REPORTING

Deliverables will be presented in the form of a final written report detailing toxicity test methods and results, and chemical-specific data. Elements of the written report will include:

- Test substance
  - Physical nature and relevant physical-chemical properties of the test substance
- Test species
  - Scientific name, size, supplier, acclimation procedures
- Test conditions
  - Test procedures
  - Method of preparation of solutions and frequency of renewal
  - Test concentrations
  - Control water characteristics
  - Water quality data
  - Detailed information on feeding
- Results
  - Evidence that controls met acceptability criteria
  - Statistical analyses
  - Tabulated data on fish weights and growth
  - Survival and affected data including descriptions of any adverse effects observed.

10.0 REFERENCES

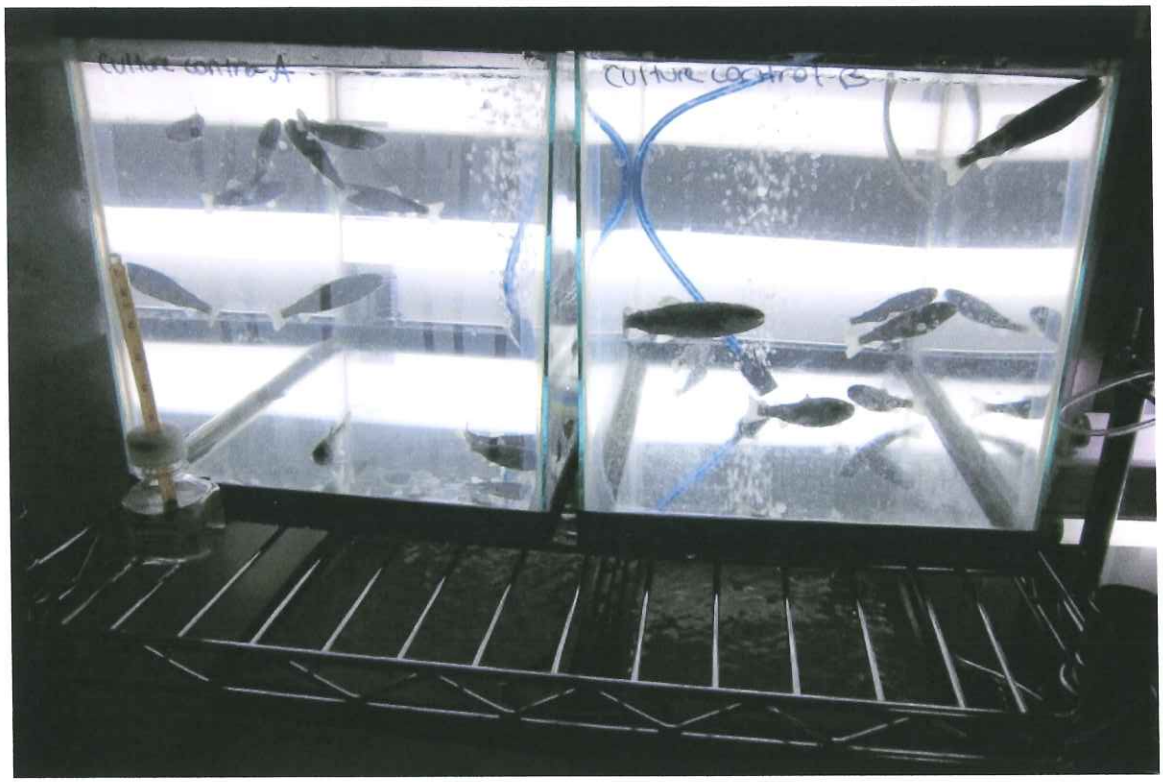
OECD/OCDE. 2000. *OECD Guideline for the testing of chemicals, Fish, Juvenile Growth Test.* OECD 215.

U.S. EPA. 1996. Ecological Effects Test Guidelines. *OPPTS 850-1075 Fish Acute Toxicity Test, Freshwater and Marine.* EPA-712-C-96-118.

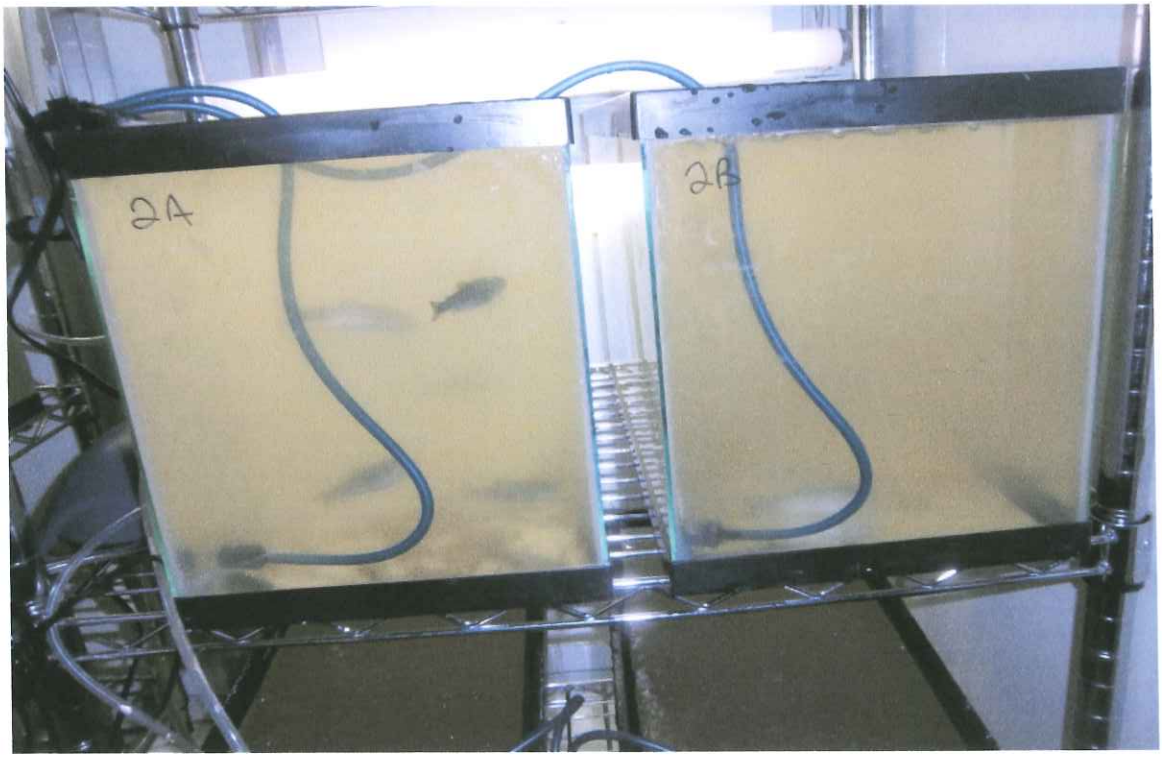
U.S. EPA. 1998. *Evaluation of Dredge Material Proposed For Discharge in Waters of the U.S. – Inland Testing Manual.* EPA-823-B-98-004.

ATTACHMENT D

PHOTOGRAPHS AND LABORATORY DAILY LOG BENCH SHEETS



Photograph of Rainbow Trout in Vessels– Untreated Control Group



Photograph of Rainbow Trout in Vessels – Water Treated with Roadbond EN 1™ Leachate

Photograph of Laboratory Procedures and Measurements for Roadbond EN 1™ Leachate Solutions

Photograph of Laboratory Preparations for Roadbond EN 1™ Leachate to be Placed in the Tanks

Photograph of Laboratory Soil/Gravel Mix Handling Preparations to Produce Leachate

Photograph of Soil/Gravel Mix Preparations in Laboratory Handled Beakers

Rainbow Trout Mortality Tally*																														total fish
Day	initial "n"	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
Concentration																									-					
Culture Control A	7																													7
Culture Control B	7	1 jumper						1																						5
Sediment Control A	7					1		2					1 jumper						3											3
Sediment Control B	7																													7
0.5x A	7							1																						6
0.5x B	7					3 jumpers																								4
1.0x A	7											1																		6
1.0x B	7											1								2		3								4
2.0x A	7													1													1 jumper			5
2.0x B	7												1 jumper									1								5
4.0x A	7														1					2										5
4.0x B	7										1	2			3															4

*accumulative mortality data for the 28 day time period

Mortality Percentage*

Concentration	% mortality	mean mortality per concentration:
Culture Control A	0%	
Culture Control B	17%	9%
Sediment Control A	50%	
Sediment Control B	0%	25%
0.5x A	14%	
0.5x B	0%	7%
1.0x A	14%	
1.0x B	43%	29%
2.0x A	17%	
2.0x B	17%	17%
4.0x A	29%	
4.0x B	43%	36%

GV ✓

*jumpers were removed from initial "n" for mortality calculations

Title: GemChem Rainbow Trout Survival; ToxStat 3.5:
File: gemcsur Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro - Wilk's Test for Normality

D = 0.2892
W = 0.9916
Critical W = 0.8050 (alpha = 0.01 , N = 12)
W = 0.8590 (alpha = 0.05 , N = 12)

Data PASS normality test (alpha = 0.01). Continue analysis.

Bartlett's Test for Homogeneity of Variance

These two tests can not be performed because at least one group has zero variance.
Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Summary Statistics on Transformed Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Culture	2	1.1458	1.3807	1.2632
2	Sediment Contro	2	0.7854	1.3807	1.0830
3	0.5	2	1.1873	1.3807	1.2840
4	1.0	2	0.8556	1.1873	1.0215
5	2.0	2	1.1458	1.1458	1.1458
6	4.0	2	0.8556	1.0021	0.9289

Summary Statistics on Transformed Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Culture	0.0276	0.1661	0.1174	13.1468
2	Sediment Contro	0.1772	0.4209	0.2976	38.8652
3	0.5	0.0187	0.1367	0.0967	10.6494
4	1.0	0.0550	0.2345	0.1658	22.9598
5	2.0	0.0000	0.0000	0.0000	0.0000
6	4.0	0.0107	0.1036	0.0732	11.1517

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	TRANS T STAT	SIG 0.05
1	Culture	1.2632	0.9150		
2	Sediment Contro	1.0830	0.7500	0.8208	
3	0.5	1.2840	0.9300	-0.0945	
4	1.0	1.0215	0.7150	1.1013	
5	2.0	1.1458	0.8300	0.5349	
6	4.0	0.9289	0.6400	1.5230	

Dunnett critical value = 2.8300 (1 Tailed, alpha = 0.05, df = 5,6)

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Culture	2			
2	Sediment Contro	2	0.5499	60.5	0.1650
3	0.5	2	0.5499	60.5	-0.0150
4	1.0	2	0.5499	60.5	0.2000
5	2.0	2	0.5499	60.5	0.0850
6	4.0	2	0.5499	60.5	0.2750

Gem Chem Rainbow Trout initial weights per fish:

sample	weight replicate (grams)	number fish	weight fish (grams)	mean weight (grams)	
Culture Water Control					
a	30.21	7	4.31571		
b	35.33	6	5.88833	5.102	Culture Water Control
	65.54			1.112	s.d.
				21.800	C.V.
Sediment Control					
a	32.61	7	4.65857		
b	34.19	7	4.88429	4.771	Sediment Control
	66.80			0.16	s.d.
				3.400	C.V.
0.5 application rate					
a	21.91	7	3.13000		
b	32.52	7	4.64571	3.888	0.5 application rate
	54.43			1.072	s.d.
				27.600	C.V.
1 application rate					
a	37.18	7	5.31143		
b	34.91	7	4.98714	5.149	1 application rate
	72.09			0.229	s.d.
				4.400	C.V.
2 application rate					
a	38.11	7	5.44429		
b	23.79	7	3.39857	4.421	2 application rate
	61.90			1.447	s.d.
				32.700	C.V.
4 application rate					
a	34.57	7	4.93857		
b	34.93	7	4.99000	4.964	4 application rate
	69.50			0.036	s.d.
				0.700	C.V.

O. mykiss

EnviroScience Inc. ~~*Pimephales promelas*~~ **Random Weight Data: pg. _ of _**

Project ID:	GenQuan
Permit No.:	

Start Date:	092310

	Toxicity Test Replicate Fish Weights
Control A	30.21
Control B	41.21 - 5.88 (1 jumped) 35.33
Sed A	32.61
Sed B	34.19
0.5A	21.91
0.5B	32.52
1A	37.18
1B	34.91
2A	38.11
2B	23.79
4A	34.57
4B	34.93
Initials	CW

S Weight calibrations	S weight	Date: 092310
	200g	100.00
	20g	20.00
	10g	10.00
	1g	1.00
	Initials	CW

Gem Chem Rainbow Trout final weights per fish:

sample	weight replicate (grams)	number fish	weight fish (grams)	mean weight (grams)	
Culture Water Control					
a	59.25	7	8.46429		
b	45.36	5	9.07200	8.768	Culture Water Control
	104.61			0.43	s.d.
				4.900	C.V.
Sediment Control					
a	27.75	3	9.25000		
b	53.46	7	7.63714	8.444	Sediment Control
	81.21			1.14	s.d.
				13.500	C.V.
0.5 application rate					
a	43.95	6	7.32500		
b	33.25	4	8.31250	7.819	0.5 application rate
	77.20			0.698	s.d.
				8.900	C.V.
1 application rate					
a	50.03	6	8.33833		
b	35.54	4	8.88500	8.612	1 application rate
	85.57			0.387	s.d.
				4.500	C.V.
2 application rate					
a	37.50	5	7.50000		
b	40.45	5	8.09000	7.795	2 application rate
	77.95			0.417	s.d.
				5.300	C.V.
4 application rate					
a	41.25	5	8.25000		
b	45.14	4	11.28500	9.768	4 application rate
	86.39			2.146	s.d.
				22.000	C.V.

GJV

O. mykiss

EnviroScience Inc. ~~*Pimephales promelas*~~ **Random Weight Data: pg. _ of _**

Project ID:	GENCHEM
Permit No.:	

Start Date:	10 092310

	Toxicity Test Replicate Fish Weights
Control A	59.25
Control B	~~45.30 54.36~~ CW 45.36
Sed A	27.75
Sed B	49.91 + 3.52
0.5A	43.95
0.5B	33.25
1A	50.03
1B	35.54
2A	37.75
2B	40.45
4A	41.25
4B	45.14
Initials	CW/MW

S Weight calibrations	S weight	Date: 10/21/10
	1200g	100.00
	20g	20.00
	10g	10.00
	1g	1.00
	Initials	CW

Title: GemChem Rainbow Trout final weights; ToxStat 3.5:
File: rt092310 Transform: NO TRANSFORMATION

Shapiro - Wilk's Test for Normality

D = 6.9020
W = 0.9772
Critical W = 0.8050 (alpha = 0.01 , N = 12)
W = 0.8590 (alpha = 0.05 , N = 12)

Data PASS normality test (alpha = 0.01). Continue analysis.

Bartlett's Test for Homogeneity of Variance

Calculated B1 statistic = 3.6771 (p-value = 0.5968)
Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

Critical B = 15.0863 (alpha = 0.01, df = 5)
= 11.0705 (alpha = 0.05, df = 5)

Summary Statistics on Data TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	Culture water	2	8.4643	9.0720	8.7681
2	Sediment Contro	2	7.6371	9.2500	8.4436
3	0.5	2	7.3250	8.3125	7.8187
4	1	2	8.3383	8.8850	8.6117
5	2	2	7.5000	8.0900	7.7950
6	4	2	8.2500	11.2850	9.7675

Summary Statistics on Data TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM	C.V. %
1	Culture water	0.1847	0.4297	0.3039	4.9009
2	Sediment Contro	1.3007	1.1405	0.8064	13.5069
3	0.5	0.4876	0.6983	0.4938	8.9307
4	1	0.1494	0.3866	0.2733	4.4887
5	2	0.1740	0.4172	0.2950	5.3521
6	4	4.6056	2.1461	1.5175	21.9715

Dunnett's Test - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG 0.05
1	Culture water	8.7681	8.7681		
2	Sediment Contro	8.4436	8.4436	0.3026	
3	0.5	7.8187	7.8187	0.8852	
4	1	8.6117	8.6117	0.1459	
5	2	7.7950	7.7950	0.9073	
6	4	9.7675	9.7675	-0.9318	

Dunnett critical value = 2.8300 (1 Tailed, alpha = 0.05, df = 5,6)

Title: GemChem Rainbow Trout final weights; ToxStat 3.5:
File: rt092310 Transform: NO TRANSFORMATION

Dunnett's Test - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	MIN SIG DIFF (IN ORIG. UNITS)	% OF CONTROL	DIFFERENCE FROM CONTROL
1	Culture water	2			
2	Sediment Contro	2	3.0353	34.6	0.3246
3	0.5	2	3.0353	34.6	0.9494
4	1	2	3.0353	34.6	0.1565
5	2	2	3.0353	34.6	0.9731
6	4	2	3.0353	34.6	-0.9994

GRP	IDENTIFICATION	MEAN	SMOOTHED MEAN	CONCENTRATION
1	Culture water	8.7681	8.7681	0.0000
2	Sediment Contro	8.4436	8.4873	0.0000
3	0.5	7.8187	8.4873	0.5000
4	1	8.6117	8.4873	1.0000
5	2	7.7950	8.4873	2.0000
6	4	9.7675	8.4873	4.0000

ICp estimate with p = 25 is > 4.0000

CHRONIC *Rainbow Trout* SURVIVAL DATA: pg. 1 of 1

Permit No.: CHEMCHEM

TEST TEMPERATURE (C)

DAY	Culture Control		Sediment Control		0.5		1		2		4		DATE/ TIME	TECH INITIALS
	NEW	OLD	NEW	OLD	NEW	OLD	NEW	OLD	NEW	OLD	NEW	OLD		
0	*													
1		*												
2		*												
3		12.3		12.3		11.7		12.3		12.5		12.9	092610 0745	JA
4		14.9		13.8		13.5		13.6		13.9		13.6	092710 0805	JA
5		13.4		13.1		12.7		13.1		13.4		13.1	092810 0905	JM
6		12.5		12.1		12.0		13.1		13.5		12.8	092910 0740	TM
7	16.1	12.9	16.5	12.8	16.3	12.4	16.5	12.8	16.6	13.3	16.7	12.6	093010 0755	TM
8		12.3		12.2		11.9		12.3		12.8		12.1	100110 0805	KH
9		12.3		12.3		11.9		12.3		12.9		11.9	100210 0805	KH
10		13.0		12.5		12.0		12.3		12.4		12.2	100310 0715	JA
11		13.0		12.6		12.3		12.8		12.6		12.7	100410 0800	JA
12		13.2		12.8		12.5		13.0		13.2		12.7	100510 0710	JM
13		13.5		13.2		12.8		13.0		13.3		12.6	100610 0830	TM
14	16.2	13.9	15.9	13.4	15.4	12.8	16.4	13.5	16.4	13.7	16.4	12.8	100710 0810	TM
15		13.5		12.4		11.9		13.1		12.8		12.4	100810 0830	JA
16		12.4		12.4		12.2		13.1		12.9		12.4	100910 0800	MW
17		13.2		12.8		12.0		13.2		13.1		12.3	101010 0700	JA
18		13.1		12.4		12.1		12.9		12.8		12.1	101110 0810	TM
19		13.7		12.7		12.1		12.8		12.7		12.1	101210 0750	TM
20		13.1		12.7		12.1		10.9		12.7		12.2	101310 0815	TM
21	18.0	13.1	17.3	12.7	17.2	12.2	17.9	13.2	17.6	12.9	17.5	12.4	101410 0800	TM
22		12.4		12.8		12.1		12.3		13.0		12.8	101510 0915	JA
23		12.7		12.4		11.9		12.8		12.6		11.9	101610 0805	MW
24		13.4		12.5		11.9		13.4		12.8		12.3	101710 0740	TM
25		13.3		12.6		12.2		13.4		13.0		12.4	101810 0715	TM
26		13.4		12.6		12.1		13.3		12.9		12.4	101910 0815	TM
27		13.4		12.7		12.0		13.0		12.7		12.3	102010 0710	TM
28		13.2		12.5		12.1		13.2		12.6		12.3	102110 0735	TM

* temperature was erroneously not recorded for the first couple days of the test -W

EnviroScience Inc. Chronic Toxicity - Daily Chemistry Bench Sheet for ☑ *O.mykiss* 28-day

Project ID:	E	E	M	C	Date:	09	23	10	

Conductivity µmhos/cm

level	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Control	499	495	516	520	529	553	569	589	570	599	603	617	652	651
Sed C	498	501	513	519	526	539	552	570	551	563	569	580	603	607
0.5	497	503	511	518	524	539	560	572	551	569	572	602	612	636
1	498	501	520	527	537	547	565	582	569	600	599	628	662	664
2	498	498	512	518	523	535	542	556	549	568	581	583	614	607
4	494	499	515	522	529	542	558	576	551	577	588	587	621	627

Dissolved Oxygen mg/l

level	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Control	8.6	8.6	8.6	7.3	7.3	6.3	6.2	5.6	6.7	6.2	6.2	7.5	4.8	7.0
Sed C	8.6	8.6	8.6	8.0	7.5	6.6	6.8	6.2	7.5	7.5	7.3	7.1	5.6	7.0
0.5	8.6	8.6	8.6	8.3	7.6	7.0	6.3	6.4	7.6	7.4	7.0	5.9	6.0	7.1
1	8.6	8.6	8.6	7.9	7.5	7.1	5.1	4.8	5.7	5.9	6.5	6.3	6.4	6.5
2	8.6	8.6	8.6	7.9	7.9	7.7	7.0	7.0	7.6	7.5	7.1	7.2	5.5	7.5
4	8.6	8.6	8.6	8.0	7.8	6.7	6.7	6.2	7.1	6.7	7.2	6.8	5.6	7.1

pH s.u.

level	0	1	2	3	4	5	6	7	8	9	10	11	12	13
Contr	7.8	7.4	7.6	7.6	7.6	7.7	7.7	7.7	7.7	7.7	7.8	7.9	7.7	7.8
Sed C	7.8	7.5	7.7	7.6	7.7	7.7	7.7	7.8	7.8	7.8	7.9	8.0	7.7	7.8
0.5	7.8	7.5	7.7	7.6	7.7	7.7	7.7	7.8	7.8	7.8	7.9	8.0	7.7	7.8
1	7.8	7.5	7.7	7.6	7.7	7.7	7.6	7.6	7.6	7.8	7.9	7.9	7.8	7.8
2	7.6	7.5	7.7	7.6	7.7	7.7	7.7	7.8	7.8	7.8	7.9	7.9	7.8	7.8
4	7.7	7.5	7.7	7.6	7.8	7.7	7.7	7.7	7.7	7.8	7.8	7.9	7.7	7.9

please initial ~~and enter "I" and "F" in the appropriate instrument box.~~

day«➔	0	1	2	3	4	5	6	7	8	9	10	11	12	13
TECH	MW	KH	KH	CW	CW	KT	CNV	KH	KB/CNV	KB/CNV	JS	JS/KT	JS/KT	CNV/KT
DO-YSI	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
pH -920	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓		✓	✓
pH-2Star												✓		
Cond.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

EnviroScience Inc. Chronic Toxicity - Daily Chemistry Bench Sheet for ☑ *O.mykiss* 28-day

Project ID:	G	E	M	C	Date:	09	23	10	

Conductivity µmhos/cm

level	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Control	654	610	604	604/631	631	662	684	673	595	641	659	649	652	670	722
Sed C	619	587	592	614/619	615	634	648	666	595	613	621	631	652	682	684
0.5	650	597	600	630/637	626	661	678	674	606	648	630	679	657	665	718
1	684	587	581	596	638	612	666	635	603	619	632	621	662	639	649
2	620	598	598	606	611	629	642	689	593	610	622	665	650	694	672
4	628	599	595	619	623	639	662	673	609	610	651	672	695	684	731

Dissolved Oxygen mg/l

level	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Control	8.1	7.8	8.6	7.3	7.4	7.4	7.6	7.5	7.6	7.2	7.5	7.4	5.8	8.0	6.4
Sed C	7.7	7.6	8.6	7.4	7.4	8.3	8.3	7.7	7.7	7.3	8.0	7.7	4.8	7.6	5.6
0.5	8.0	7.1	8.1	6.8	7.4	7.9	7.9	7.7	7.8	7.3	8.5	7.4	5.1	8.3	5.7
1	7.7	7.4	8.6	6.3	7.6	8.4	8.4	8.3	7.9	7.6	8.0	8.1	4.5	8.4	6.7
2	7.5	8.0	8.6	6.3	7.3	6.9	7.0	7.3	7.2	7.1	6.1	7.7	4.3	7.2	5.4
4	7.2	7.0	8.1	6.5	7.0	6.8	7.7	7.4	7.3	6.5	7.5	6.7	4.1 KT	7.3	4.9

pH s.u.

level	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
Contr	7.7	8.0	8.0	7.9	7.9	7.8	7.9	7.9	7.8	7.9	7.5	7.4	7.8	7.8	7.9
Sed C	7.6	7.9	7.9	7.7	7.9	7.8	8.0	7.8	7.9	7.9	7.9	7.5	7.8	7.7	7.8
0.5	7.7	7.9	7.9	7.7	8.0	7.8	7.9	7.8	7.9	7.8	7.6	7.6	7.8	7.8	7.8
1	7.7	7.9	7.9	7.8	8.0	7.8	8.0	7.9	7.9	7.9	7.5	7.7	7.8	7.8	7.9
2	7.6	8.0	7.9	7.8	7.9	7.6	7.8	7.8	7.7	7.8	7.3	7.6	7.7	7.6	7.7
4	7.5	7.9	7.9	7.7	8.0	7.7	7.9	7.8	7.8	7.8	7.5	7.5	7.8	7.7	7.8

please initial and enter "I" and "F" in the appropriate instrument box.

day«→	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28
TECH	KH/JS	KB/CNV	KB	MW	JS/KT	JS	JS/KT	JS	KB/CNV	KB	KT	JS	CNV/KT	JS	CNV
DO-YSI	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓
pH -920	✓		✓	✓		✓		✓	✓	✓	✓	✓		✓	✓
pH-2Star		✓			✓		✓						✓		
Cond.	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓	✓

CHRONIC BIOASSAY:
INITIAL WATER QUALITY CHECKS (DO, pH, conductivity); CHLORINE, ALKALINITY, HARDNESS DATA.

Project ID: GEM CHEM Permit No.: _____
Test Date: 092310

Sample Type: →	EFFLUENT					
EnviroScience No.:→	092310 Culture H2O	4X 092310	093010 Culture water	092010 4X	100710 Culture H2O	100710 4X
D. Oxygen (mg/l-%sat) **>4 & <100%?**	8.6	8.6	8.6	8.6	6.8	6.9
pH (s.u) **6-9?**	7.8	7.7	7.9	8.1	7.8	7.8
Conductivity (µmhos/cm)	499	494	540	530	627	585
Alkalinity (mg/l $CaCO_3$) **MDL = 20 mg/l**	(4.5) 90	(4.6) 92	(5.6) 112	(5.6) 112	(6.2) 124	(6.0) 120
Hardness (mg/l $CaCO_3$) **MDL = 5 mg/l**	(3.6) 144	(3.5) 140	(3.7) 148	(3.9) 156	(4.1) 164	(4.1) 164
TRC_I (mg/l) **<0.02?**	—	—	—	—	—	—
TRC_A (mg/l) <0.02?	—	—	—	—	—	—
Tech Initials : →	MW	MW	KH	KH	JS+CW	JS+CW

Methods/Instrumentation :
DO: APHA (1998) 4500-O G, YSI 5100; pH: APHA (1998) 4500-H B, Orion 920A/SA250; Conductivity: APHA (1998) 2510-B, Orion 160; Hardness: APHA (1998) 2340-C; Alkalinity: APHA (1998) 2320-B; Chlorine: APHA (1998) 4500-Cl D, ampero Wallace & Tiernan

Sample Type: effluent (outfall # if more than one), upstream, downstream (NF, FF), lab water, etc.

EnviroScience No.: Tracking number from C-O-C (client code+date received+type/outfall/unique #)

CW ✓

Dechlorination procedure:
TRC_I = total residual chlorine, initial value measured prior to dilution or use of sample.
TRC_A = TRC value measured after dechlorination.

Sodium thiosulfate is used to reduce Total Residual Chlorine by dosing with 6.7 mg $Na_2S_2O_3$ per mg TRC.
A 6.7 mg/ml $Na_2S_2O_3$ solution is used; dose mls = X mg/l * liters in sample container being treated.

Comments: Describe dechlorination/pH-adjustments including lot numbers, concentration, volumes of sodium thiosulfate or acid/base solutions and volume of sample treated, preparation of blanks; problems associated with data collection, etc. Initial all entries along with date/time/sample #. Attach additional pages if necessary.

CHRONIC BIOASSAY:
INITIAL WATER QUALITY CHECKS (DO, pH, conductivity); CHLORINE, ALKALINITY, HARDNESS

Project ID: GEM Chem
Permit No.: [illegible]
Test Date: 101410

Sample Type: →	EFFLUENT			UPSTREAM		
EnviroScience No.:→	101416 Culture H_2O	101416 4x				
D. Oxygen (mg/l-%sat) >4 & <100%?	8.6	8.6				
pH (s.u) 6-9?	8.1	8.0				
Conductivity (µmhos/cm)	575	584				
Alkalinity (mg/l $CaCO_3$) MDL = 20 mg/l	(6.0) 120	(6.6) 132				
Hardness (mg/l $CaCO_3$) MDL = 5 mg/l	(4.7) 198	(4.5) 180				
TRC_I (mg/l) <0.02?						
TRC_A (mg/l) <0.02?						
Tech Initials : →	JS/KT	JS/KT				

Methods/Instrumentation/MDL:
DO: APHA (1998) 4500-O G, YSI 5100, MDL not determined; pH: APHA (1998) 4500-H B, Orion 920A/SA250, MDL= 0.043 s.u.; Conductivity: APHA (1998) 2510-B, Orion 160. MDL=2.566 µmhos/cm; Hardness: APHA (1998) 2340-C, MDL=4.752 mg/L; Alkalinity: APHA (1998) 2320-B, MDL=1.054 mg/L; Chlorine: APHA (1998) 4500-Cl D, ampero HACH AutoCAT 9000, MDL=0.015 mg/L.

Sample Type: effluent (outfall # if more than one), upstream, downstream (NF, FF), lab water, etc.

EnviroScience No.: Tracking number from C-O-C (client code+date received+type/outfall/unique #)

CW

Dechlorination procedure:
TRC_I = total residual chlorine, initial value measured prior to dilution or use of sample.
TRC_A = TRC value measured after dechlorination.

Sodium thiosulfate is used to reduce Total Residual Chlorine by dosing with 6.7 mg $Na_2S_2O_3$ per mg TRC.
A 6.7 mg/ml $Na_2S_2O_3$ solution is used; dose mls = X mg/l * liters in sample container being treated.

Comments: Describe dechlorination/pH-adjustments including lot numbers, concentration, volumes of sodium thiosulfate or acid/base solutions and volume of sample treated, preparation of blanks; problems associated with data collection, etc. Initial all entries along with date/time/sample #. Attach additional pages if necessary.

Toxicity Test Daily Comment Sheet
Client: GemChem
Test dates: 092310 – 102110

Day -1 Date 092210 Initials CW + JA

prepared sediment; determined application rate + sprayed product on soil/gravel; put water in coolers + refrigerators

Day 0 Date 092310 Initials CW

1 liter treated soil to 5 liters of water; mixed + let settle; poured through filter + distributed to levels set up tanks, siphoned old tanks + placed trout in coolers to hold; set up aeration of all levels to maintain DO test initiated @ 1045

Day 1 Date 092410 Initials MW/CW

1 fish jumped out of control B; body recovered + weighed tanks siphoned out to remove debris fed am + pm

Day 2 Date 092510 Initials KH

chemistry grabbed at 0835 and fed
↳ from B tanks
fed pm @ 1525

Day 3 Date 092610 Initials JA

Sampled Chemistry + fed 0720; Took Temps.
Feed 1505 pm. - TM

Day 4 Date 092710 Initials JA

Sampled Chemistry + fed @ 0805', Took Temps.
Feed 1455 PM - TM

Day 5 Date 092810 Initials KH

Sampled chemistry and fed at 0820
siphoned out debris - 1615 - CW
Fed 1630 - CW
sediment control A had one dead fish, body removed
2 jumpers found on bottom of incubator

Day 6 Date 092910 Initials TM

Sampled chemistry and fed at 0740

ENVIRO SCIENCE INCORPORATED

Toxicity Test Daily Comment Sheet
Client: GemChem
Test dates: 092310 – 102110

Day 7 Date 093010 Initials TM

Sampled chemistry and fed at 0755.
one dead control B - removed
one dead 5A - removed
renewed water prepared 24 hrs water renewal for each tank - CV

Day 8 Date 100110 Initials KH - CV

Sampled chemistry and fed at 0810
Siphoned debris + fed 1530
sed control A - one dead removed

Day 9 Date 100210 Initials KH / JA

Sampled chemistry and fed at 0810
fed @ 1540

Day 10 Date 100310 Initials JA

Sampled chemistry took temps + fed @ 0715
Removed 1 dead fish from 4B.

PM feeding 1622 TM

Toxicity Test Daily Comment Sheet
Client: ExxonChem
Test dates: 092310 - 102110

Day 11 Date 100410 Initials JA
Sampled Chemistry; took temps + fed @ 0800
1 dead fish each from 1A, 1B, + 4B

Day 12 Date 100510 Initials TM
Sampled Chemistry; took temps + fed @ 0746
found a dead dried up fish on the bottom of the incubator on the left side. Discarded.
trout cow @ 1700 - CW

Day 13 Date 100610 Initials TM
Sampled chemistry; took temp + fed @ 0830. Found 1 dead + removed from 2A.
Fed @ 1630; prepared sediment for renewal. prepped one gallon of sediment + sprayed w/ 6.5mL Roundup covered w/ foil + let set overnight

Day 14 Date 100710 Initials TM
Sampled chemistry; took temps + fed @ 0810.
4A 1 dead removed @ renewal 4B 1 dead
fed @ 1710. -MW

7 fish	Control A	5 fish	3A	5 fish in	2A
5 fish	4 B	4 "	5B	6	2B
4 "	sed A	6	1A	5	4A
7 "	sed B	4	1B	4	4B

ENVIRO SCIENCE INCORPORATED

Toxicity Test Daily Comment Sheet
Client: GenOn Chem
Test dates: 092310-102110

Day 15 Date 100810 Initials JA

Took temps, Sampled Chemistry + fed @ 0830
fed @ 1610

Day 16 Date 100910 Initials MW

Took Temperatures, Sampled Chemistry and feed trout chow at 0800.
fed in afternoon 1525.

Day 17 Date 101010 Initials JA

Took temps; Sampled chemistry + fed @ 0700
Fed 1550 TM

Day 18 Date 101110 Initials TM

Took temps; sampled chemistry + fed @ 0810. 1 dead in sed. control A
removed dead fish.
Feed pm @ 1540 TM

ENVIRO SCIENCE INCORPORATED

Toxicity Test Daily Comment Sheet
Client: Gem Chem
Test dates: 092310 - 102110

Day 19 Date 101210 Initials TM

Took temps; sampled chemistry and fed at 0750. Found 1 dead fish in 4A and removed. Removed 1 dead fish in 1B.
fed at 1630

Day 20 Date 101310 Initials TM

Took temps; sampled chemistry and fed at 0815.
Fed pm at 1500.
prepared renewal sediment @ 1615 1 gallon sed + applied 6.5 mL Roadrunner - CW

Day 21 Date 101410 Initials TM

Took temps, sampled chemistry and fed at 0800. Found 1 dead in 1B and 1 dead in 2B.
renewed 2/5 water in each tank. siphoned debris + gave fresh H2O - CW
fed @ 1600 - CW

Day 22 Date 101510 Initials CW DVB

took temps, sampled chemistry + fed @ 0915
fed @ 1540

ENVIRO SCIENCE INCORPORATED

Toxicity Test Daily Comment Sheet
Client: Chem Chem
Test dates: 092310 - 102110

Day 23 Date 101610 Initials MW

- Sampled Chemistry from tank A at 0825 and took temperatures and fed trout chow.
Fed @ 1435

Day 24 Date 101710 Initials TM

Sampled chemistry, took temps, and fed at 0740.
Fed @ 1600 PM.

Day 25 Date 101810 Initials TM

Sampled chemistry, took temps and fed at 0715.
Fed @ 1540 PM.

Day 26 Date 101910 Initials TM

Sampled chemistry; took temps and fed at 0820.

Toxicity Test Daily Comment Sheet
Client: GenOn
Test dates: 092310 - 102110

Day 27 Date 10 20 10 Initials TM

Sampled chemistry; took temps and fed at 0710.
fed @ 1555

Day 28 Date 10 21 10 Initials TM

Sampled chemistry; took temps at 0735

scale calibrated + fish weighed per replicate
fish were pulled out w/ a net + put into a pretared cup + put on scale. -CW

1 fish body found under tanks after tear down

Day 28 Date _____ Initials CW

Final # fish per replicate (taken as they were weighed)

CA	7	SA	6	2A	5
CB	5	SB	4	2B	5
SA	3	1A	6	4A	5
SB	7	1B	4	4B	4

Day _____ Date _____ Initials _____

ATTACHMENT E

LABORATORY TOXICITY TEST EVALUATION
FINAL REPORT

28 DAY CHRONIC TOXICITY TEST EVALUATING ROADBOND LEACHATE UTILIZING RAINBOW TROUT (*Oncorhynchus mykiss*)

Final Report

Submitted to:

GemChem, Inc.
PO Box 384
53 N Cedar St.
Lititz, PA 17543

Prepared by:

EnviroScience, Inc.
3781 Darrow Rd., Stow, OH 44224
1-800-940-4025 FAX: 330-688-3858

October 28, 2010

0.0 EXECUTIVE SUMMARY

This report describes a 28 day chronic toxicity test utilizing Rainbow Trout (*Oncorhynchus mykiss*) to evaluate toxicity of simulated run-off of Roadbond EN1Soil stabilizer. Test methods followed the procedures described in OECD Guideline for the testing of Chemicals; Fish, Juvenile Growth Test (OECD 215), OPPTS 850.1075; Fish Acute Toxicity Test, Freshwater and Marine (EPA 712-C-96-118), & Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (EPA-821-R-02-013). Simulated leachate was prepared in accordance with documents from C.S.S. Technology, Inc. and Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. (EPA-823-B-98-004).

The test was conducted from September 23, 2010 through October 21, 2010. Mortality per replicate and concentration was determined at the end of the 28 day exposure period and statistically compared to survival of the control groups. Average weights were also determined per fish per replicate and a statistical analysis was run compared to a laboratory control group and a sediment control group. No statistically significant difference was observed in either mortality levels or growth of the Rainbow Trout (O.mykiss) in comparison to the control groups.

The results of this study indicate that Roadbond EN1 Soil Stabilizer does not adversely affect the growth or survival of Rainbow Trout (O.mykiss) at expected run-off levels when following manufacturer's guidelines when compared to the non-addition of Roadbond or normal runoff conditions involving sediment.

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1.0 INTRODUCTION

GemChem, Inc. sought to evaluate leachate/simulated run-off toxicity as a result from application of Roadbond EN1 Soil Stabilizer product to gravel roads. This report describes a 28 day chronic toxicity test utilizing Rainbow Trout (*Oncorhynchus mykiss*) to evaluate toxicity at simulated run-off levels of Roadbond EN1Soil stabilizer. Test methods followed the procedures described in OECD Guideline for the testing of Chemicals; Fish, Juvenile Growth Test (OECD 215), OPPTS 850.1075; Fish Acute Toxicity Test, Freshwater and Marine (EPA 712-C-96-118), & Short Term Methods for Estimating Chronic Toxicity of Effluents and Receiving Water to Freshwater Organisms (EPA-821-R-02-013). Simulated leachate was prepared in accordance with documents from C.S.S. Technology, Inc. and Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. (EPA-823-B-98-004).

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2.0 METHODS

The sections below provide information regarding the location of test laboratory, the sediment sampling site and describes procedures associated with all aspects of the toxicity study.

2.1 Facilities.

Toxicity Laboratory:	EnviroScience, Inc. 3781 Darrow Rd. Stow, OH 44224 330-688-0111	Primary investigator: Courtney Van Voorhis Laboratory Manager
Sampling Site:	Wayne County, PA	

2.2 Culture Laboratory Water

In accordance with the OECD methodology any water may be used that shows suitable long-term survival and growth. Fish at the EnviroScience lab are cultured in de-chlorinated and filtered tap water. EnviroScience synthesizes fresh water in accordance with USEPA protocols. The tap water is passed through three carbon filters and goes through reverse osmosis before any fish are exposed to it. This is referred to as culture water in this report. Culture water prior to initiation and the weekly renewal of the test was placed in refrigerators to acclimate it to the appropriate test temperature.

2.3 Leachate Preparation

Simulated leachate was prepared in accordance with documents from C.S.S. Technology, Inc. and Evaluation of Dredged Material Proposed For Discharge in Waters of the U.S. (EPA-823-B-98-004). A gravel/dirt mix was obtained from a local property near active Marcellus Shale drilling operations in Wayne County, PA by GemChem, Inc. and shipped to EnviroScience, Inc. for preparation of the leachate. Roadbond EN1 Soil Stabilizer was received at the same time as the soil. The soil and Roadbond EN1 were stored in a cool, dry, dark area of the laboratory until the test was initiated.

Manufacturer's suggested application rates were used by EnviroScience personnel to determine necessary volumes of Roadbond EN1 to be applied to the sediment. In order to initiate the test it was necessary to treat 2 gallons of sediment to make 8 gallons of run-off that was distributed among the replicates to create the desired concentrations. Two gallons of sediment was placed in a sterilized plastic container. Application rate for the size of the container (9.31 milliliters for 1.99 cubic feet) was calculated in accordance with the recommended manufacturer's application rate of 0.0057 gallons per square yard at a six inch dept and compared to the volume of sediment; 2 gallons (0.267 cubic feet) needed to prepare the simulated run-off. This ratio was utilized to determine the necessary amount of 1.25 milliliters of RoadBond EN1 Soil Stabilizer to treat 2 gallons of sediment.

The working solution of Roadbond EN1 Soil Stabilizer was created in accordance with manufacturer's directions to make a ratio of 200:1. A sterilized pipette was utilized to measure 5 milliliters of concentrated product into 1000 milliliters of water. Due to the volume of water required and the pre-determined test levels the product was concentrated to ten times the recommended application rate; therefore, 12.5 milliliters of working solution was dispersed over the 2 gallons of sediment with a small sterile spray bottle and manually stirred. The container was sealed with foil to keep out additional moisture and allowed to set for 12 hours.

After the sediment set overnight one liter was placed in a five liter sterilized plastic pitcher and filled with culture water in a 1 to 4 ratio, sediment to culture water. The pitchers were stirred for 30 minutes and allowed to settle for one hour. After one hour, the concentrated leachate was poured through a 60 micron nylon mesh filter to remove large debris that floated up from the soil. The concentrated leachate, or stock solution, was used to create concentrations of; half the application rate (.5x), one time the application rate (1x), 2 times the application rate (2x) and four times the application rate (4x) in five gallon aquaria. Stock solution was placed in refrigerators along with culture water to allow water to cool to test temperature.

Test vessels were set up in the environmental chamber, labeled with the concentration and replicate (A or B). Then culture water and stock leachate were combined to create 5 gallons of test solution per replicate. The highest concentration contained 3 gallons (12000 milliliter) of culture water and 2 gallons (8000 milliliters) of stock leachate to create four times the recommended application rate. The additional levels decreased in half the amount of stock leachate and were measured out as follows:

0.5x	1000 milliliters of stock solution and 19000 milliliters of culture water
1.0x	2000 milliliters of stock solution and 18000 milliliters of culture water
2.0x	4000 milliliters of stock solution and 16000 milliliters of culture water
4.0x	8000 milliliters of stock solution and 12000 milliliters of culture water

A sediment control was also utilized that contained two liters of sediment run-off in culture water. This volume was used to mimic that 1.0x application rate level. The sediment control was not treated with Roadbond EN1 but was prepared in the same manner as the stock leachate. This group was used to evaluate any contaminants that may be present in the soil that could cause adverse effects to the test specimens. After water was loaded into the environmental chambers aeration was added to each replicate to maintain the high oxygen level that Rainbow Trout (O.mykiss) require. The guidance documents indicate that oxygen levels for Rainbow Trout (O.mykiss) should not decrease below 60% saturation or 6.0 mg/L at the test temperature. Daily oxygen levels are noted on the attached bench sheets.

2.4 Toxicity test procedures.

A chronic toxicity test was conducted following aforementioned guidance documents. Test conditions are presented in table 2-1.

Table 2-1 Summary of toxicity test conditions for chronic testing with *Oncorhynchus mykiss*	
1. Source of test organisms:	Freshwater Farms of Ohio, Inc.
2. Test type and duration:	Static, renewal, 28 days
3. Photoperiod:	16 hours light / 8 hours dark
4. Light quality and intensity:	wide spectrum fluorescent light, 50-100fc
5. Test solution temperatures °C:	14±1 °C
6. Feeding regime:	Trout chow two times daily
7. Size of test vessel:	5 gallon aquaria
8. Volume of test solutions:	5 gallons
9. No. of test organisms per vessel:	7
10. No. of vessels per solution:	2
11. Total no. of organisms per solution:	14
12. Test concentrations:	0.5x, 1x, 2x, & 4x
13. Renewal:	Once a week; 09/30, 10/07, & 10/14
14. Dilution/primary control waters:	culture water
15. Secondary control:	culture water containing sediment without Roadbond EN1 added
16. Aeration:	constant; maintain DO >6.0 mg/L
17. Endpoints:	mortality - no movement with gentle prodding (LC_{50}); survival and growth NOEC; IC_{25}

The test was initiated September 23, 2010 at 1045 and was terminated on October 21, 2010 at 1030.

2.4.1 Test organisms.

Test organisms were purchased from Freshwater Farms of Ohio, Inc. and transported to the EnviroScience facility. Fingerling Rainbow trout (*Oncorhynchus mykiss*) 2 inches to 4 inches in length were held in culture water at 14°C for the recommended 2 week acclimation period. After the acclimation period but before the test could be initiated the majority of the organisms died due to overcrowding in the holding chambers. New fingerling Rainbow Trout (O.mykiss) were purchased and overcrowding was alleviated during the subsequent acclimation period. Minimal

mortality and no disease was observed in the second group of organisms, therefore, the test was initiated.

Trout were removed from holding tanks and placed in coolers with cool aerated water while the test chambers were set up. Due to the lateness of the season and the fact that Rainbow Trout (O.mykiss) spawn in the spring the organisms were larger than recommended by the method, therefore, ten organisms could not be added to each replicate as originally planned. Each test vessel was loaded with seven randomly selected specimens to follow the wet fish weight loading recommendations of the various methods followed. Before addition of the organisms to the water each replicate (7 fish together) was weighed on a balance (FY300) to determine average initial weight of the fish. This data including initial and final weights is listed in Table 3-2. To obtain an accurate weight, fish were caught with a dip net, excess water was blotted off and fish were placed in a pre-tarred plastic cup and weighed. This was done quickly to minimize the stress and air exposure of the organisms.

The test was initiated when the first organism was exposed to a test solution. Test solution temperatures were measured and recorded from random tanks each day. Rainbow Trout (O.mykiss) were fed a ground up mixture of AquaMax trout chow two times per day, once at the beginning of the work day and at the end of the work day.

2.4.2 Observations.

Solution temperatures were measured daily in a randomly selected replicate, water was sub-sampled for chemical analysis, and then each test vessel was viewed to determine numbers surviving. The test vessels became very cloudy with debris as the test proceeded and it became increasingly difficult to determine number of fish per replicate, therefore, the survival ('n' number) data was collected during the weekly renewal. Temperature, observations, time, and technicians' initials were recorded on the bench sheets. Sub-samples were taken to the chemistry laboratory where conductivity, DO, and pH were measured and recorded daily.

Test solution was renewed weekly following leachate preparation procedures with the exception of volumes prepared. The test vessels were siphoned to clean out debris and dirty water before renewal. Two gallons of water was removed from each tank to allow for the addition of two gallons of freshly prepared leachate. The test vessels received partial renewal of the test solution to minimize temperature fluctuations and stress on the test organisms. Volumes renewed were as follows:

0.5x	378.5 milliliters of stock solution and 7621.5 milliliters of culture water
1.0x	757.1 milliliters of stock solution and 7242.9 milliliters of culture water
2.0x	1514.2 milliliters of stock solution and 6785.8 milliliters of culture water
4.0x	3028.3 milliliters of stock solution and 4971.7 milliliters of culture water

All volumes were measured by sterilized graduated cylinders (for the smaller ones) or sterilized graduated pitcher and poured into a cleaned watering can to add to the aquaria in the environmental chamber. A watering can with a long neck was used due to clearance of the shelving in the environmental chamber.

Test vessels were checked daily and if needed were siphoned clean when they appeared to contain a lot of excess food and waste between renewal days. A minimal amount of water was removed during the cleaning process, but no new water was added so the concentration of Roadbond EN1 was not diluted. Dead fish were removed as soon as they were observed and recorded on the daily log sheet.

2.4.3 Physical and chemical measurements.

General water quality parameters (temperature, pH, dissolved oxygen (DO), and conductivity) were monitored at test initiation, test renewal and daily in between renewals. Alkalinity, hardness, and residual chlorine were measured in the culture water and highest concentration (4.0x) at test initiation and during each renewal process.

Table 2-2. Physical-chemical parameters.			
Parameter	Method	frequency; test levels	Comments
temperature	EPA 170.1	daily; all levels	digital; calibrated quarterly with NIST certified thermometer
dissolved oxygen	APHA 4500-G	daily; all levels	YSI 5100 meter
pH	APHA 4500-H	daily; all levels	Orion 920A meter
conductivity	APHA 2510-B	daily; all levels	Orion 160 meter
alkalinity	APHA 2320-B	3x, prior to test initiation and at renewal; control and highest concentration	titrimetric, pH 4.5
hardness	APHA 2340-C	3x, prior to test initiation and at renewal; control and highest concentration	titrimetric, EDTA
total residual chlorine	APHA 4500-Cl	once, prior to test initiation; control	amperometric, HACH Auto Cat 900 meter

2.5 Data analysis.

The no observable effect concentration (NOEC) and inhibition concentration (IC_{25}) were computed from survival and growth data using ToxStat version 3.5. The NOEC endpoint represents the highest concentration of product that would not be expected to show significant impairment in growth compared to the control organisms. The inhibition concentration is an estimate of the concentration of product that would cause a 25% reduction in the measured response (trout growth).

3.0 RESULTS

Results of Rainbow Trout (O.mykiss) mortality and growth in the concentrations of Roadbond EN1 indicate that there is no statistical significance between the applications of Roadbond EN1 and control groups. All statistical analyses, growth and survival data are included in the attached benchsheets.

The indication of the statistical analyses is that Roadbond EN1 Soil Stabilizer is not significantly toxic to Rainbow Trout (*O.mykiss*) at simulated run-off levels. Some mortality was observed at four times the application rate but it is not significantly different than the mortality observed in the control group over the 28-day test period. Growth was consistent throughout the concentrations and averaged between 2.06 and 6.30 grams per fish with the majority of the fish almost doubling the average initial weight. According to the results of this study, Roadbond EN1 Soil Stabilizer should not impair survival or growth of Rainbow Trout (O.mykiss) the live in streams near roads where Roadbond EN1 Soil Stabilizer will be applied.

Table 3-1. Chronic Endpoints for Mortality and Growth of *O.mykiss*.

Survival - 28-day LC_{50}	Survival - 28-day NOEC	Growth - 28-day IC_{25}	Growth - 28-day NOEC
>4.0x	>4.0x	>4.0x	>4.0x

Table 3-2. Initial and Final Weights per Replicate (average grams per fish)

Concentration	Initial 'n'	Initial weight	% mortality	Final 'n'	Final weight	Growth: final - initial
Culture Control A	7	4.32	0%	7	8.46	4.14
Culture Control B	6	5.89	17%	5	9.07	3.81
Sediment Control A	7	4.66	50%	3	9.25	4.59
Sediment Control B	7	4.88	0%	7	7.64	2.76
0.5x A	7	3.13	14%	6	7.33	4.20
0.5x B	7	4.65	0%	4	8.31	3.66
1.0x A	7	5.31	14%	6	8.34	3.03
1.0x B	7	4.99	43%	4	8.89	3.90
2.0x A	7	5.44	17%	5	7.50	2.06
2.0x B	7	3.40	17%	5	8.09	4.69
4.0x A	7	4.94	29%	5	8.25	3.31
4.0x B	7	4.99	43%	4	11.29	6.30

Water quality data for measurements of pH, DO, conductivity, alkalinity, hardness, and residual chlorine (TRC) are included in the attached benchsheets.

4.0 QUALITY ASSURANCE

The test met the requirements for test validation with regard to survival among specimens in the control groups. Control group survival was equal to or greater than 90% through the 28-day exposure period. A survival rate of 90% is required for test validation or no more than one organism died per test vessel in culture control.

5.0 REFERENCES

OECD/OCDE. 2000. *OECD Guideline for the testing of chemicals, Fish, Juvenile Growth Test.* OECD 215.

U.S. EPA. 1996. Ecological Effects Test Guidelines. *OPPTS 850-1075 Fish Acute Toxicity Test, Freshwater and Marine.* EPA-712-C-96-118.

U.S. EPA. 1998. *Evaluation of Dredge Material Proposed For Discharge in Waters of the U.S. – Inland Testing Manual.* EPA-823-B-98-004.