Report

of

Test No. 806-4

Assessment of Freshwater Sediments as part of the Downtown Portland Sediment Characterization Project, using a 10-day Midge, *Chironomus dilutus*, Sediment Bioassay.

Submitted to

Hart Crowser, Inc.,
8910 SW Gemini Drive
Beaverton, OR 97008

Submitted by

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

May 18, 2010

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 806-4

Title: Toxicity of freshwater sediments using a 10-day midge, *Chironomus dilutus* (formerly *C. tentans*), sediment bioassay as part of the Downtown Portland Sediment Characterization Project.

Protocol No.: NAS-XXX-CT4b, April 7, 1998. Revision 1 (10-28-03). Based on ASTM 2001 (Standard test methods for measuring the toxicity of sediment-associated contaminants with fresh water invertebrates, E1706-00), Am. Soc. Test. Mat., Phila., PA, and EPA Method 100.2 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-99/064).

STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 8910 SW Gemini Drive, Beaverton, OR 97008

Sponsor's Study Monitor: Mr. Rick Ernst

Testing Laboratory: Northwestern Aquatic Sciences, P.O. Box 1437, Newport, OR 97365

Test Location: Newport laboratory

Laboratory's Study Personnel: G.J. Irissarri, B.S., Proj. Man./Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, PhD, Sr. Toxicologist; G.A. Buhler, B.S., Aq. Toxicologist; S.J. Gage, B.A., Sr. Tech.; L.P. Sandoval, B.S., Tech.; Y. Nakahama, Tech.

Study Schedule:

Test Beginning: 4-2-10, 1045 hrs.

Test Ending: 4-12-10, 1000 hrs.

Disposition of Study Records: All specimens, raw data, reports and other study records are stored according to Good Laboratory Practice regulations at Northwestern Aquatic Sciences, 3814 Yaquina Bay Rd., Newport, OR 97365.

Good Laboratory Practices: The test was conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

Statement of Quality Assurance: The test data were reviewed by the Quality Assurance Unit to assure that the study was performed in accordance with the protocol and standard operating procedures. This report is an accurate reflection of the raw data.

TEST MATERIAL

Test Sediments: Freshwater test sediments collected as part the Downtown Portland Sediment Characterization project. Details are as follows:

NAS Sample No.	3160G	3161G
Description	DPSC-G093	DPSC-G094
Collection Date	3/25/10	3/25/10
Receipt Date	3/27/10	3/27/10

Control Sediment: The negative control sediment (NAS#3136G) was collected on 3-10-10 from an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR.

Treatments: Homogenized at test set up by mixing using stainless steel implements.

Storage: All test and control sediments were stored at 4°C in the dark in sealed containers until used.

TEST WATER

Source: Dechlorinated municipal tap water.

Date of Preparation: 3-30-10, 4-9-10

Water Quality:
pH: 7.0, 7.1
conductivity: 137, 150 µmhos/cm
hardness: 43, 43 mg/L as $CaCO_3$
alkalinity: 30, 30 mg/L as $CaCO_3$.
total chlorine: < 0.02, <0.02 mg/L
Pretreatment: Dechlorinated and aerated ≥24 hr.

TEST ORGANISMS

Species: *Chironomus dilutus* (formerly *C. tentans*), midge.
Size: 3rd instar, mean initial wt: 0.17 ± 0.02 mg
Source: NAS cultures, originally obtained from EPA, Duluth, MN.
Acclimation: Holding conditions for the two weeks prior to testing averaged: Temperature, 22.0 ± 0.4°C; dissolved oxygen, 8.3 ± 0.3 mg/L; pH, 7.2 ± 0.4; conductivity, 146 ± 2 µmhos/cm; hardness, 43 mg/L as $CaCO_3$; and alkalinity, 30 mg/L as $CaCO_3$. Photoperiod was 16:8, L:D. Half of the water in culture tanks was replaced twice weekly with dechlorinated municipal tap water during holding. Animals were fed Tetra Fin suspension and *Selenastrum*.

TEST PROCEDURES AND CONDITIONS

The following is an abbreviated statement of the test procedures and a statement of the test conditions actually employed. See the test protocol (Appendix I) for a more detailed description of the test procedures used in this study.

Test Chambers: 300 ml high-form glass beakers
Test Volumes: 100 ml sediment layer; 175 ml test water.
Replicates/Treatment: 8 (plus two additional WQ beakers)
Organisms/Treatment: 80
Water Volume Changes: 2 water volumes per day
Aeration: None.
Feeding: Animals were fed 1.5 ml of Tetra Fin suspension (1.5 ml contains 6 mg dry solids) per beaker daily.
Effects Criteria: 1) survival after 10 days, and 2) average individual biomass (based on ash-free dry weight) after 10 days. Death is defined as no visible movement or response to tactile stimulation. Missing organisms were considered to be dead.
Water Quality and Other Test Conditions: The temperature, dissolved oxygen, conductivity, pH, hardness, alkalinity and ammonia-nitrogen were measured in the overlying water of one replicate test container per treatment on days 0 and 10 of the test. Temperature and dissolved oxygen were measured daily in the overlying water of one replicate test container per treatment. Hardness and alkalinity were measured with titrimetric methods. Total ammonia and pH were measured in the pore water from the bulk sediment prior to test initiation and an additional replicate beaker on day 10. Interstitial water samples were obtained by centrifugation. Ammonia-N was measured using Hach reagents based on the salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric method; samples were not distilled prior to analysis. The photoperiod was 16:8, L:D.

DATA ANALYSIS METHODS

Survival and individual biomass were calculated for each replicate as follows:

percent survival = 100 x (number surviving/initial number tested)
average individual ash-free dry wt. = (ash-free dry wt.)/number weighed,
where:
ash-free dry wt. = dry weight of organisms recovered on day 10 – ashed dry weight, in mg

Means and standard deviations for the biological endpoints described above, and for water quality data, were computed using Microsoft Excel 2000.

PROTOCOL DEVIATIONS

None

REFERENCE TOXICANT TEST

The reference toxicant test is a multi-concentration toxicity test using potassium chloride, to evaluate the performance of the test organisms used in the sediment toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-2713
Reference Toxicant and Source: Potassium Chloride (KCl), Fisher Lot #073280.
Test Date: 4-2-10.
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 4.19 g/L. This result is within the laboratory's control chart warning limits (3.42 – 7.75 g/L).

TEST RESULTS

Observations of water quality in the overlying water throughout the test are summarized in Table 1. A detailed tabulation of the water quality results by sample and test day can be found in Appendix II. Interstitial ammonia and pH measurements are listed in Table 2. The means and standard deviations of percent mortality and growth (ash-free dry wt.) of midges exposed for 10 days to sediments are summarized in Table 3. Detailed data organized by sample and replicate, and summary statistics for these observations, are given in Appendix II.

All water quality observations of overlying water temperature and dissolved oxygen were within the protocol specified ranges. Ammonia-N in the overlying water ranged between <0.1 and 0.2 mg/L for all day 0 and day 10 measurements. Interstitial bulk sediment values for ammonia-N ranged from 0.8 to 2.6 mg/L.

The test met the mortality and weight acceptability criteria specified in the test protocol with 5.0% mean control mortality (≤30% required) and a control individual mean ash-free dry weight of 0.83 mg per larvae (0.48 mg required). The reference toxicant (positive control) EC50 result was within the laboratory's control chart limits (4.19 g/L; control chart mean ± 2 S.D. = 5.58 ± 2.17). It is concluded, therefore, that the test has developed fully acceptable data for use in making management decisions.

STUDY APPROVAL

Gerald Chrisafi 5-11-10
Project Manager/Study Director Date

Julie R. Frost 5-11-10
Quality Assurance Unit Date

Linda K. Nemeth 5/5/10
Assistant Laboratory Director Date

Table 1. Summary of water quality conditions during tests of the midge, *Chironomus dilutus*, exposed to freshwater sediments.

Water Quality Parameter	Mean ± S.D.	Minimum	Maximum	N
Temperature (°C)	22.6 ± 0.2	22.2	23.2	33
Dissolved oxygen (mg/L)	5.9 ± 0.7	4.8	7.3	33
Conductivity (µmhos/cm)	156 ± 14	141	182	6
pH	6.6 ± 0.2	6.4	6.8	6
Hardness (mg/L as $CaCO_3$)	47 ± 4	43	51	6
Alkalinity (mg/L as $CaCO_3$)	32 ± 4	30	40	6
Total ammonia (mg/L)	---	<0.1	0.2	6

Table 2. Interstitial ammonia-N and pH in test sediments porewater on day 0 and at test termination.

NAS Sample No.	Sample Description	Day 0		Day 10	
		pH	Ammonia (mg/L)	pH	Ammonia (mg/L)
3136G	Control	6.7	2.6	7.0	2.4
3160G	DPSC-G093	6.8	0.8	6.6	1.3
3161G	DPSC-G094	6.6	0.9	6.6	1.3

Table 3. Mortality and growth results of *Chironomus dilutus* 10-day sediment toxicity test.

NAS Sample No.	Sample Description	Percent mortality (Mean ± SD)	Average ash-free dry wt/midge (mg)* (Mean ± SD)
3136G	Control	5.0 ± 5.3	0.83 ± 0.07
3160G	DPSC-G093	5.0 ± 5.3	0.82 ± 0.07
3161G	DPSC-G094	2.5 ± 4.6	0.82 ± 0.04

*Pupae were not included in the sample to estimate ash-free dry weight (as per EPA/600/R-99/064, p. 59, section 12.3.8.2)

APPENDIX I

PROTOCOL

TEST PROTOCOL

FRESHWATER MIDGE, *CHIRONOMUS TENTANS*, 10-DAY SEDIMENT TOXICITY TEST

1. INTRODUCTION

1.1 Purpose of Study: The purpose of this study is to characterize the toxicity of freshwater sediments based on midge survival and growth using the midge, *Chironomus tentans*.

1.2 Referenced Method: This protocol is based on EPA Method 100.2 (EPA/600/R-99/064) and ASTM Method E 1706-00 (ASTM 2001).

1.3 Summary of Method: A summary of test conditions for the midge 10-day sediment toxicity test is tabulated below. The 10-day sediment toxicity test with *Chironomus tentans* is conducted at 23°C with a 16L:8D photoperiod at an illuminance of about100-1000 lux. Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten second to third-instar midges are used in each replicate (all organisms must be third instar or younger and at least 50% of the larvae must be third instar). The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing. Midges in each test chamber are fed 1.5 mL of a 4 g/L fish food flakes suspension daily. Each chamber receives two volume additions per day of overlying water. Overlying water can be culture water, well water, surface water, site water, or reconstituted water. Test endpoints include survival and/or growth.

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

_____
_____
_____
_____

2.2 Sponsor's Study Monitor:

_____

2.3 Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road, P.O. Box 1437
Newport, OR 97365.

2.4 Test Location: _____

2.5 Laboratory's Personnel to be Assigned to the Study:
Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Testing Schedule: Tests are to begin within 14 days of sample collection. Eight week holding times may apply in some circumstances. Reference toxicant test to be run concurrently.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

The test materials are freshwater sediments. The control, reference, and test sediments are placed in solvent cleaned 1 L glass jars fitted with PTFE-lined screw caps. At the laboratory the samples are stored at 4°C in the dark. The original sealed containers may be stored for up to 14 days prior to testing. Eight week holding times may apply in some circumstances. If jars are not full when received or if sediment is removed for testing, headspaces should be filled with nitrogen to retard deterioration. A negative control sediment is collected from a clean site. In addition, a reference sediment, a clean sediment with physical characteristics similar to the test sediments, may be employed as a comparison station.

4. TEST WATER

Test water (overlying water) at NAS is normally *C. tentans* culture water, which is moderately hard synthetic water at a hardness of 80-100 mg/L as $CaCO_3$ and alkalinity of 60-70 mg/L as $CaCO_3$. Dilution water is prepared from Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water or site water depending on the study design.

5. TEST ORGANISMS

5.1 Species: midge, *Chironomus tentans.*

5.2 Source: Cultured at NAS (Originally obtained from U.S. EPA Environmental Research Lab, Duluth, MN) or purchased from a reputable commercial supplier.

5.3 Age: Third instar or younger larvae (at least 50% of the larvae must be in the third instar at the start of the test). Third instar is normally 9 to 11 days after hatching; head capsule widths range from 0.33 to 0.45 mm; or length ranges from 4-6 mm; or dry weight ranges 0.08 to 0.23 mg/individual.

5.4 Acclimation and Pretest Observation: Cultures are maintained at 23 ± 1°C under a 16:8 L:D photoperiod. The culture water is moderately hard synthetic water. Midge are fed finely ground Tetrafin flakes in suspension (10g Tetrafin in 100 mL Milli-Q water). Mortality during the 48-hr prior to testing should not be excessive.

6. DESCRIPTION OF TEST SYSTEM

6.1 Test Chambers and Environmental Control: Test chambers used in the toxicity test are 300-mL high-form lipless glass beakers (Pyrex® 1040 or equivalent). Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by placement in a temperature-controlled room. Aeration is not empolyed unless dissolved oxygen drops below 2.5 mg/L. The test is conducted under an illuminance of 100 to 1000 lux with a 16L:8D photoperiod.

6.2 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of midge larvae to test, control, and reference sediments. The sediments are placed on the bottom of the test containers and are overlain with test water. The test exposure is

for 10 days. The renewal of overlying water consists of two volume additions per day, either continuous or intermittent. Each treatment consists of eight replicate test containers, each containing 10 organisms. Test chamber positions are completely randomized. Test organisms are randomly distributed to the test chambers. Blind testing is normally used.

7.2 Setup of Test Containers: Sediments are homogenized and placed in test chambers on the day before addition of test organisms. Sediment (100 ml) is placed into each of eight replicate beakers. After addition of the sediment, 175 ml of test water is gently added to each beaker in a manner to prevent resuspension. The overlying water is replaced twice daily. The test begins when midges are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Effect Criterion: The acute effect criterion used in the midge bioassay is mortality, defined as the lack of movement of body or appendages on response to tactile stimulation. The optional chronic effect criterion is growth which is determined by using dry weight measurements.

7.4 Test Conditions: No aeration is employed unless dissoved oxygen falls below 2.5 mg/L. The test temperature employed is 23°C (range of ± 1°C). A 16:8, L:D photoperiod is used. Illumination is supplied by daylight fluorescent lamps at 100-1000 lux. The overlying water is replaced twice daily.

7.5 Beginning the Test: The test is begun by adding the organisms to the equilibrated test containers as previously described. Three extra replicates of midge larvae should be counted out and randomly selected for drying to determine initial average weight and instar data.

7.6 Feeding: Midge larvae are fed 1.5 mL daily per test chamber (1.5 mL contains 6.0 mg of dry solids). A feeding may be skipped if there is a build up of excess food. However, all beakers must be treated similarly.

7.7 Test Duration, Type and Frequency of Observations, and Methods: The duration of the acute toxicity test is 10 days. The type and frequency of observations to be made are summarized as follows:

Type Of Observation	Times Of Observation
Biological Data	
Survival, growth	Day 10
Physical And Chemical Data	
Hardness, alkalinity, ammonia-N, conductivity, pH, dissolved oxygen, and temperature	Beginning and end of test in overlying water of one replicate beaker from each treatment.
Dissolved oxygen, temperature	Daily in overlying water of one replicate beaker from each treatment.

Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH is measured using a pH probe and a properly calibrated meter with scale divisions of 0.1 pH units. Temperature is measured with a calibrated mercury thermometer or telethermometer. Conductivity is measured with a conductivity meter. Hardness and alkalinity are measured using titrometric methods. Ammonia-nitrogen is measured using the salicylate colerimetric method (Clin. Chim. Acta 14:403, 1996).

7.8 Growth Measurement: Growth is measured as ash-free dry weight (AFDW) of animals in a test replicate at the end of the test on day 10. Pooled animals from each test replicate are rinsed with deionized water, gently blotted and placed into tared aluminum weigh pans. The pans are dried at 60-90°C to constant weight. The dried organisms are placed into a dessicator and weighed as soon as possible to the nearest 0.01 mg (desirable to use 0.001 mg). The total weight of the dried midge in each pan is divided by the number of midge weighed to obtain an average dry weight per midge. The dried larvae in the pan are then ashed at 550°C for two hours. The pan with the ashed larvae is then reweighed and the tissue mass of the larvae is determined as the difference between the weight

of the dried larvae plus pan and the weight of the ashed larvae plus pan. Pupae or adult organisms are not included in the sample to estimate AFDW.

8. CRITERIA OF TEST ACCEPTANCE:

The test results are acceptable if the minimum survival of organisms in the control treatment at the end of the test is at least 70% and the average ash-free dry weight of *C. tentans* in the surviving controls is at least 0.48 mg.

9. DATA ANALYSIS

The endpoints of the toxicity test are survival and growth. Survival is obtained as a direct count of living organisms in each test container at the end of the test. Average midge ash-free dry weight, also measured at the end of the test, may be used to compare growth between treatment sediments and the control or reference sediment. Ordinarily the following data analysis is performed. Due to special requirements, alternative methods may be used. The means and standard deviations are calculated for each treatment level. Identification of toxic sediments is established by statistical comparison of test endpoints between test and control or reference sediments. Between treatment comparisons may be made using a Student's t-test or Wilcoxon's Two-Sample test, where each treatment is compared to the control or the reference sediment. An arcsine-square root transformation of proportional data, and tests for normality and heterogeneity of variances, are performed prior to statistical comparisons.

10. REPORTING

The final report of the test results must include all of the following standard information at a minimum: name and identification of the test; the investigator and laboratory; date and time of test beginning and end; information on the test material; information on the source and quality of the overlying/test water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including feeding, if any, and water quality; definition of the effect criteria and other observations; responses, if any, in the control treatment; tabulation and statistical analysis of measured responses and a summary table of endpoints; a description of the statistical methods used; any unusual information about the test or deviations from procedures; reference toxicant testing information.

11. STUDY DESIGN ALTERATION

Amendments made to the protocol must be approved by the sponsor and study director and should include a description of the change, the reason for the change, the date the change took effect and the dated signatures of the study director and sponsor. Any deviations in the protocol must be described and recorded in the study raw data.

12. REFERENCE TOXICANT

The reference toxicant test is a standard multi-concentration toxicity test using a specified chemical toxicant to evaluate the performance of test organisms used in the study. Reference toxicant tests are 96-hour, water only exposures, not 10-day sediment exposures. The reference toxicant test is run concurrently. Performance is evaluated by comparing the results of the reference toxicant test with historical results (e.g., control charts) obtained at the laboratory.

13. REFERENCED GUIDELINES

ASTM. 2001. Standard Test Methods for Measuring the Toxicity of Sediment-associated Contaminants with Fresh water Invertebrates. ASTM Standard Method No. E 1706-00. Am. Soc. Test. Mat., Philadelphia, PA.

U.S. EPA. 2000. Section 12, Test Method 100.2, *Chironomus tentans* 10-d Survival and Gowth Test for Sediments, pp. 55-62. In: Methods for Measuring the Toxicity and Bioaccumulation of Sediment-associated Contaminants with Freshwater Invertebrates (Second Edition). EPA/600/R-99/064.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

14. APPROVALS

_____ for _____
Name Date

_____ for **Northwestern Aquatic Sciences**
Name Date

Appendix A
Test Conditions Summary

1. Test type	whole sediment toxicity test with renewal of overlying water
2. Test duration	10 days
3. Temperature	23 ± 1°C
4. Light quality	daylight flourescent light
5. Illuminance	100-1000 lux
6. Photoperiod	16L:8D
7. Test chamber size	300-mL high-form lipless beakers (Pyrex® 1040 or equivalent)
8. Sediment volume	100 mL
9. Overlying water volume	175 mL
10. Renewal overlying water	2 volume additions/day (continuous or intermittent)
11. Age of test organisms	2nd to 3rd instar or younger larvae (≥ 50% of organisms must be 3rd instar)
12. Organisms per test chamber	10
13. Replicates per treatment	8 recommended for routine (depends on design)
14. Organisms per treatment	80
15. Feeding regime	Fish food flakes, fed 1.5 mL chamber (1.5 mL contains 6.0 mg of dry solids) daily on days 0 - 9.
16. Aeration	None, unless DO falls below 2.5 mg/L.
17. Overlying (test) water	**Culture water**, well water, surface water, site water or reconstituted water
18. Water quality	Hardness, alkalinity, conductivity, pH, ammonia-N beginning and end; temperature and DO daily
19. Endpoints	Survival and growth (dry weight)
20. Test acceptability criteria	Minimum control survival of 70%; mean weight of surviving control organisms 0.48 mg AFDW
21. Sample holding	≤14 days at 4°C in the dark Longer under certain conditions
22. Sample volume required	1L (800 mL per sediment)
23. Reference toxicant	Concurrent testing required

APPENDIX II

RAW DATA

TEST DESCRIPTION, MONITORING, AND RESULTS BENCHSHEETS

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

REVIEWED PAGES 1-24 -GJI

Test No. 806-4 Client Hart Crowser Investigator

STUDY MANAGEMENT

Client: Hart Crowser, Inc., 8910 SW Gemini Drive, Beaverton, OR 97008

Client's Study Monitor: Mr. Rick Ernst

Testing Laboratory: Northwestern Aquatic Sciences

Test Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Man./Study Dir. G.J. Irissarri GJI

QA Officer L.K. Nemeth

1. GABrin GB 2. Susan Gage SG
3. Yves Nakahama YN 4. Lidia P. Sandoval LPS
5. 6. R.S. Caldwell RSC
7. 8.

Study Schedule:

Test Beginning: 4-2-10 1045 Test Ending: 4-12-10 1000

TEST MATERIAL

General description (see sample logbook/chain-of-custody for details):

NAS Sample No.:	3136G	3160G	3161G		
Description:	Control	DPSC-G093	DPSC-G094		
Collection Date:	3/10/10	3/25/10	3/25/10		
Receipt Date:	3/10/10	3/27/10	3/27/10		
:					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
:					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
:					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
:					
NAS Sample No.:					
Description:					
Collection Date:					
Receipt Date:					
:					

Error codes: 1) correction of handwriting error
2) written in wrong location; entry deleted
3) wrong date deleted, replaced with corrrect date
4) error found in measurement; measurement repeated

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

SEDIMENT DESCRIPTIONS -- SUPPLEMENTAL NOTES

Sample No.	Description
3136G	FINE BLACK MUD
3160G	BROWN SAND w/ LARGE ROCKS AND CLAMS
3161G	BROWN FINE SAND w/ ROCKS AND PLANT MATERIAL

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 804-6 Client HART CROWSER Investigator _____

Test conducted in (circle one): room 1 room 2 trailer water bath other: ROOM #3

Randomization chart: TOP SHELF

6	12	18	24	30					
5	11	17	23	29					
4	10	16	22	28					
3	9	15	21	27					
2	8	14	20	26					
1	7	13	19	25					

Randomization chart:

Randomization chart:

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

TEST WATER

Source: Dechlorinated municipal tap water

Date of Collection: 3-30-10, 4-9-10

pH 7.0, 7.1

Cond (umhos/cm2) 137, 150

Hardness (mg/L) 43, 43

Alkalinity (mg/L) 30, 30

Total Chlorine (mg/L) <0.02, <0.02

Treatments: Dechlorinated, aerated

TEST ORGANISMS

Species: *Chironomus dilutus* Age: 3rd instar

Source: NAS cultures Date received: N/A

Acclimation Data:

Date	Temp. (deg.C)	pH	DO (mg/L)	Cond. umhos/cm	Hardness (mg/L)	Alkalinity (mg/L)	Feeding	Water changes
3-24-10	22.2	7.0	7.9	145	43	30	Animals fed Tetra Fin	–
3-26-10	22.6	7.8	8.2	147	–	–	and *Selenastrum*	YES
3-29-10	21.9	7.3	8.3	147	–	–	Details recorded on	YES
3-31-10	21.7	7.4	8.6	143	43	30	Chironomid culture	–
4-1-10	21.7	6.8	8.5	147	–	–	data sheets	YES
4-2-10	21.9	7.1	8.0	146	–	–		
Mean	22.0	7.2	8.3	146	43	30		
S.D.	0.4	0.4	0.3	2	–	–		
(N)	6	6	6	6	2	2		

Photoperiod during acclimation: 16:8, L:D

TEST PROCEDURES AND CONDITIONS

Test chambers: 300 ml glass beakers

Test volumes: 100 ml of test sediment; 275 ml total volume

Replicates/treatment: (8) 8 + 2 EXTRA REPLICATES Organisms/treatment: (80) 80 (10/REP)

Test water changes: Twice daily

Aeration: only if DO falls below 2.5 mg/L

Beaker placement: Total randomization

Feeding: everyday beginning with day zero

Photoperiod: 16:8, L:D

Test temperature (°C): 23 ± 1

Control Sediment:

Source: From an area approximately one mile east of the Hwy. 101 bridge at Beaver Creek, approx. 8 miles south of Newport, OR.

Date collected: 3/10/10

Sieved through 0.5 -mm screen

Storage: darkness at 4°C, in sealed containers NAS# 3136G

MISCELLANEOUS NOTES

Light intensity:

Date	Location	Light Intensity (ft-candles*)	Initials
4-5-10	BEAKER #16	84	GJL

*To convert ft-candles to lux multipy by 10.76

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 0 (4/2/10) GJ/YN

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond.* (umhos/cm)	pH*	Hardness* (mg/L)	Alkalinity* (mg/L)	NH3* (ppm)	Comments
11	22.5	7.3	182	6.8	51	30		Each beaker fed 1.5 ml
19	22.5	7.0	141	6.7	51	30		Tetra Fin suspension
20	22.4	7.1	144	6.7	51	30		Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1645
								Initials: YN

*Water quality measurements to be taken.

Day 1 (4/3/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	23.0	6.9						Each beaker fed 1.5 ml
19	22.9	6.6						Tetra Fin suspension
20	22.7	6.2						Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1645
								Initials: [illegible]

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 2 (4/4/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	23.1	6.7						Each beaker fed 1.5 ml
19	22.9	6.6						Tetra Fin suspension
20	22.8	6.4						Initials: GJ
								Water changed in all
								beakers.
								Time: 0615
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1645
								Initials: [illegible]

*Water quality measurements to be taken.

Day 3 (4/5/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	22.8	6.4						Each beaker fed 1.5 ml
19	22.7	6.3						Tetra Fin suspension
20	22.5	6.3						Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1640
								Initials: [illegible]

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 4 (4/6/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	22.6	6.3						Each beaker fed 1.5 ml
19	22.4	5.9						Tetra Fin suspension
20	22.3	6.2						Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1650
								Initials: LA

*Water quality measurements to be taken.

Day 5 (4/7/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	22.7	5.6						Each beaker fed 1.5 ml
19	22.5	5.6						Tetra Fin suspension
20	22.4	5.5						Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1650
								Initials: LA

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 6 (4 / 8 /10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	22.6	5.3						Each beaker fed 1.5 ml
19	22.4	5.3						Tetra Fin suspension
20	22.2	5.1						Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1645
								Initials: YN

*Water quality measurements to be taken.

Day 7 (4 / 9 / 10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	22.7	5.6						Each beaker fed 1.5 ml
19	22.6	5.4						Tetra Fin suspension
20	22.4	5.1						Initials: GB
								Water changed in all
								beakers.
								Time: 0610
								Initials: GB
								Water changed in all
								beakers.
								Time: 1645
								Initials: UPS

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 8 (4/10/10) GB

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	22.6	5.5						Each beaker fed 1.5 ml
19	22.4	5.4						Tetra Fin suspension
20	22.3	5.4						Initials: GB
								Water changed in all
								beakers.
								Time: 0610
								Initials: GB
								Water changed in all
								beakers.
								Time: 1715
								Initials: [illegible]

*Water quality measurements to be taken.

Day 9 (4/11/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
11	23.2	5.1						Each beaker fed 1.5 ml
19	22.9	4.8						Tetra Fin suspension
20	22.7	4.9						Initials: GJ
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1720
								Initials: [illegible]

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 10 (4 /12 /10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond.* (umhos/cm)	pH*	Hardness* (mg/L)	Alkalinity* (mg/L)	NH3* (ppm)	Comments
11	23.0	5.4	158	6.4	43	40		Each beaker fed 1.5 ml
19	22.9	5.3	155	6.4	43	30		Tetra Fin suspension
20	22.7	5.5	156	6.4	43	30		Initials: —
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: —
								Initials: —

*Water quality measurements to be taken.

Day _____(/ /)

Beaker No.	Temp. (deg.C)	DO (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
								Each beaker fed 1.5 ml
								Tetra Fin suspension
								Initials:
								Water changed in all
								beakers.
								Time:
								Initials:
								Water changed in all
								beakers.
								Time:
								Initials:

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

DAY 10 TEST TERMINATION SHEET

Beaker No.	Number of survivors	Initials
1	10	GJL
2	9	GJL
3	9	GJL
4	9	GJL
5	9	GJL
6	9	GJL
7	10	GJL
8	10	GJL
9	9	GJL
10	10	GJL
11		
12	10	GJL
13	10	GJL
14	9	GJL
15	10	GJL
16	10	GJL
17	10	GJL
18	10	GJL
19		
20		
21	10	GJL
22	10	GJL
23		
24	10	GJL
25	9	GJL
26		
27	10	GJL
28		
29	9	GJL
30	9	GJL
31		
32		
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45		

Beaker No.	Number of survivors	Initials
46		
47		
48		
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CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

Tare: Date 4-1-10 Oven temp (C.) 62 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.010 100mg: 100.016

Final #1 Date 4-5-10 Oven temp (C.) 63 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.010 100mg: 100.015

Final: #2 Date 4-6-10 Oven temp (C.) 63 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.011 100mg: 100.017

Equip. used: Oven: BLUE M #1 Balance: SARTORIUS M3P

(Dry overnight at 60-90 degrees C)

Pan #	Tare wt. (mg)	Total wt. (mg) 1	Total wt. (mg) 2	#weighed	
1	56.52	58.38	58.37	10	
2	55.52	57.33	57.31	10	
3	57.45	58.78	58.77	10	
4	56.10	57.65	57.64	10	
5	53.91	55.69	55.68	10	

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-4 Client Hart Crowser Investigator

WEIGHING DATA SHEET

Tare: Date 4-6-10 Oven temp (C.) 550 Drying time (hr.) 2 Initials JTZF
Standard Weights: 10 mg: 10.011 100mg: 100.017

Final #1: Date 4-13-10 Oven temp (C.) 64 Drying time (hr.) 25 Initials GJL
Standard Weights: 10 mg: 10.011 100mg: 100.017

Final #2: Date 4-14-10 Oven temp (C.) 65 Drying time (hr.) 23 Initials GJL
Standard Weights: 10 mg: 10.008 100mg: 100.015

Final #3: Date 4-15-10 Oven temp (C.) 550 Drying time (hr.) 2 Initials GJL
Standard Weights: 10 mg: 10.008 100mg: 100.015

Equip. used: Oven BLUE M #1, FISHER ISOTEMP MUFFLE FURNACE Balance Sartorius M3P

(Dry overnight at 60-90 degrees C) **(Final ashing is at 550 degrees C for 2 hours)**

Bkr. #	Pan #	Tare wt. (mg)	Dry total wt. (mg) 1	Dry total wt. (mg) 2	no. weighed	put into pans-initials	Ash weight (mg)	Comments
1	1	90.13	101.94	101.80	10	GJL	93.207 GJL 4-15-10	
2	2	87.16	95.92	95.82	9	GJL	88.73	
3	3	86.07	96.39	96.30	9	GJL	88.92	
4	4	87.31	98.36	98.27	9	GJL	90.42	
5	5	89.31	98.12	98.05	9	GJL	91.72	
6	6	84.96	94.95	94.88	9	GJL	87.92	
7	7	86.64	97.73	97.64	10	GJL	89.63	
8	8	90.18	101.22	101.14	10	GJL	91.98	
9	9	92.37	101.90	101.82	9	GJL	93.87	
10	10	85.96	98.29	98.22	10	GJL	90.12	
11	11	87.67	—	—	—	—	—	
12	12	90.01	101.28	101.20	10	GJL	92.99	
13	13	91.70	104.89	104.80	10	GJL	96.11	
14	14	89.75	99.41	99.33	9	GJL	91.53	
15	15	90.10	100.27	100.19	10	GJL	92.01	
16	16	82.69	95.08	95.01	10	GJL	86.47	
17	17	65.16	73.99	73.94	10	GJL	66.88	
18	18	80.04	92.01	91.94	10	GJL	83.63	
19	19	81.05	—	—	—	—	—	
20	20	96.59	—	—	—	—	—	
21	21	89.08	102.22	102.15	10	GJL	93.12	
22	22	78.57	89.26	89.20	10	GJL	81.35	
23	23	86.83	—	—	—	—	—	
24	24	84.73	94.26	94.21	10	GJL	86.61	
25	25	87.84	97.47	97.43	9	GJL	89.53	
26	26	84.10	—	—	—	—	—	
27	27	89.09	99.97	99.93	10	GJL	92.71	
28	28	89.82	—	—	—	—	—	
29	29	86.96	98.14	98.10	9	GJL	90.18	
30	30	90.03	101.03	101.00	9	GJL	93.33	
31	31							
32	32							
33	33							

TEST DATA ANALYSIS RECORDS

Data entry verified against laboratory bench sheets 4-20-10 JHF

Test number: 806-4

Freshwater Sediment Test
10-day Chironomus dilutus

Endpoints Data Entry and Calculations File

BKR=beaker number
INIT=initial number
SURV=number survivors
MORT=number dead=INIT-SURV
PSURV=%survival=100(SURV/INIT)
PMORT=%mortality=100(MORT/INIT)

TARE WT= ashed weight of pan used for that replicate at test termination (mg), or dry weight of pan if ash-free dry weight is not an endpoint
WT COUNT= number of test organisms weighed at test end
DRY WT= TARE WT + dry weight of test organisms recovered at test termination (mg)
TWT=total biomass=DRY WT-TARE WT
WT=average individual biomass=TWT/WT COUNT

ASHED DRY WT= weight of ashed pan + weight of ashed test organisms recovered at test termination
TAFDW=DRY WT - ASHED DRY WT= total ash-free organism weight for given replicate
AFDW=average individual ash-free biomass=TAFDW/WT COUNT

INITIAL WEIGHT				
pan #	tare wt (mg)	final wt (mg)	wt count	avg. wt/ organism
1	56.52	58.37	10	0.18
2	55.52	57.31	10	0.18
3	57.45	58.77	10	0.13
4	56.10	57.64	10	0.15
5	53.91	55.68	10	0.18
			avg.	0.17
			sd	0.02

INDEX	BKR	NAS SMPL	CLIENT DESCRIP	REPL		INIT	SURV	MORT	PSURV	PMORT	TARE WT (mg)	WT COUNT	DRY WT (mg)	ASHED DRY WT (mg)	TWT (mg)	WT (mg)	TAFDW (mg)	AFDW (mg)		SURV	MORT	PSURV	PMORT	WT	AFDW
1	25	3136G	Control	1		10	9	1	90.0	10.0	87.84	9	97.43	89.53	9.59	1.07	7.90	0.88							
2	17	3136G	Control	2		10	10	0	100.0	0.0	65.16	10	73.94	66.88	8.78	0.88	7.06	0.71							
3	9	3136G	Control	3		10	9	1	90.0	10.0	92.37	9	101.82	93.87	9.45	1.05	7.95	0.88							
4	24	3136G	Control	4		10	10	0	100.0	0.0	84.73	10	94.21	86.61	9.48	0.95	7.60	0.76							
5	2	3136G	Control	5		10	9	1	90.0	10.0	87.16	9	95.82	88.73	8.66	0.96	7.09	0.79	Mean	9.5	0.5	95.0	5.0	1.01	0.83
6	15	3136G	Control	6		10	10	0	100.0	0.0	90.10	10	100.19	92.01	10.09	1.01	8.18	0.82	SD	0.5	0.5	5.3	5.3	0.07	0.07
7	8	3136G	Control	7		10	10	0	100.0	0.0	90.18	10	101.14	91.98	10.96	1.10	9.16	0.92	n	8	8	8	8	8	8
8	14	3136G	Control	8		10	9	1	90.0	10.0	89.75	9	99.33	91.53	9.58	1.06	7.80	0.87							
9	11	3136G	Control	9	wq replicate																				
10	26	3136G	Control	10	day 0 replicate																				
11	29	3160G	DPSC-G093	1		10	9	1	90.0	10.0	86.96	9	98.10	90.18	11.14	1.24	7.92	0.88							
12	13	3160G	DPSC-G093	2		10	10	0	100.0	0.0	91.70	10	104.80	96.11	13.10	1.31	8.89	0.87							
13	30	3160G	DPSC-G093	3		10	9	1	90.0	10.0	90.03	9	101.00	93.33	10.97	1.22	7.67	0.85							
14	27	3160G	DPSC-G093	4		10	10	0	100.0	0.0	89.09	10	99.93	92.71	10.84	1.08	7.22	0.72							
15	1	3160G	DPSC-G093	5		10	10	0	100.0	0.0	90.13	10	101.80	93.21	11.67	1.17	8.59	0.86	Mean	9.5	0.5	95.0	5.0	1.18	0.82
16	10	3160G	DPSC-G093	6		10	10	0	100.0	0.0	85.96	10	98.22	90.12	12.26	1.23	8.10	0.81	SD	0.5	0.5	5.3	5.3	0.11	0.07
17	5	3160G	DPSC-G093	7		10	9	1	90.0	10.0	89.31	9	98.05	91.72	8.74	0.97	8.33	0.70	n	8	8	8	8	8	8
18	4	3160G	DPSC-G093	8		10	9	1	90.0	10.0	87.31	9	98.27	90.42	10.96	1.22	7.85	0.87							
19	19	3160G	DPSC-G093	9	wq replicate																				
20	28	3160G	DPSC-G093	10	day 0 replicate																				
21	7	3161G	DPSC-G094	1		10	10	0	100.0	0.0	88.64	10	97.64	89.63	11.00	1.10	8.01	0.80							
22	3	3161G	DPSC-G094	2		10	9	1	90.0	10.0	88.07	9	96.30	88.92	10.23	1.14	7.38	0.82							
23	6	3161G	DPSC-G094	3		10	9	1	90.0	10.0	87.96	9	94.88	87.92	6.92	0.77	6.96	0.77							
24	22	3161G	DPSC-G094	4		10	10	0	100.0	0.0	78.57	10	89.20	81.35	10.63	1.06	7.85	0.79							
25	18	3161G	DPSC-G094	5		10	10	0	100.0	0.0	80.04	10	91.94	83.63	11.90	1.19	8.31	0.83	Mean	9.8	0.3	97.5	2.5	1.11	0.82
26	16	3161G	DPSC-G094	6		10	10	0	100.0	0.0	82.69	10	95.01	86.47	12.32	1.23	8.54	0.85	SD	0.5	0.5	4.6	4.6	0.16	0.04
27	21	3161G	DPSC-G094	7		10	10	0	100.0	0.0	89.08	10	102.15	93.12	13.07	1.31	9.03	0.90	n	8	8	8	8	8	8
28	12	3161G	DPSC-G094	8		10	10	0	100.0	0.0	90.01	10	101.20	92.99	11.19	1.12	8.21	0.82							
29	20	3161G	DPSC-G094	9	wq replicate																				
30	23	3161G	DPSC-G094	10	day 0 replicate																				

Test Number: 806-4

Freshwater Sediment Test
10-day Chironomus dilutus

Data entry verified against laboratory bench sheet 4-20-10 JMF

Water Quality Data

BKR	NAS SMPL	CLIENT DESCRIP	DAY	Interstitial water pH	Interstitial water NH3
23	3161G	DPSC-G094	0	6.6	0.9
26	3136G	Control	0	6.7	2.6
28	3160G	DPSC-G093	0	6.8	0.8
11	3136G	Control	10	7.0	2.4
19	3160G	DPSC-G093	10	6.6	1.3
20	3161G	DPSC-G094	10	6.6	1.3

BKR	NAS SMPL	CLIENT DESCRIP	REPL	DAY	Overlying water TEMP	DO	COND	pH	NH3	HARD	ALK	Interstitial water pH	Interstitial water NH3
11	3136G	Control	9	0	22.5	7.3	182	6.8	0.2	51	30		
19	3160G	DPSC-G093	9	0	22.5	7.0	141	6.7	0.1	51	30		
20	3161G	DPSC-G094	9	0	22.4	7.1	144	6.7	<0.1	51	30		
11	3136G	Control	9	1	23.0	6.9							
19	3160G	DPSC-G093	9	1	22.9	6.6							
20	3161G	DPSC-G094	9	1	22.7	6.2							
11	3136G	Control	9	2	23.1	6.7							
19	3160G	DPSC-G093	9	2	22.9	6.6							
20	3161G	DPSC-G094	9	2	22.8	6.4							
11	3136G	Control	9	3	22.8	6.4							
19	3160G	DPSC-G093	9	3	22.7	6.3							
20	3161G	DPSC-G094	9	3	22.5	6.3							
11	3136G	Control	9	4	22.6	6.3							
19	3160G	DPSC-G093	9	4	22.4	5.9							
20	3161G	DPSC-G094	9	4	22.3	6.2							
11	3136G	Control	9	5	22.7	5.6							
19	3160G	DPSC-G093	9	5	22.5	5.6							
20	3161G	DPSC-G094	9	5	22.4	5.5							
11	3136G	Control	9	6	22.6	5.3							
19	3160G	DPSC-G093	9	6	22.4	5.3							
20	3161G	DPSC-G094	9	6	22.2	5.1							
11	3136G	Control	9	7	22.7	5.6							
19	3160G	DPSC-G093	9	7	22.6	5.4							
20	3161G	DPSC-G094	9	7	22.4	5.1							
11	3136G	Control	9	8	22.6	5.5							
19	3160G	DPSC-G093	9	8	22.4	5.4							
20	3161G	DPSC-G094	9	8	22.3	5.4							
11	3136G	Control	9	9	23.2	5.1							
19	3160G	DPSC-G093	9	9	22.9	4.8							
20	3161G	DPSC-G094	9	9	22.7	4.9							
11	3136G	Control	9	10	23.0	5.4	158	6.4	0.2	43	40		
19	3160G	DPSC-G093	9	10	22.9	5.3	155	6.4	0.1	43	30		
20	3161G	DPSC-G094	9	10	22.7	5.5	156	6.4	0.1	43	30		
				Mean	22.6	5.9	156	6.6	--	47	32	6.7	1.6
				SD	0.2	0.7	14	0.2	--	4	4	0.2	0.8
				n	33	33	6	6	6	6	6	6	6
				Min	22.2	4.8	141	6.4	<0.1	43	30	6.6	0.8
				Max	23.2	7.3	182	6.8	0.2	51	40	7.0	2.6

Freshwater Sediment Test
10-day Chironomus dilutus

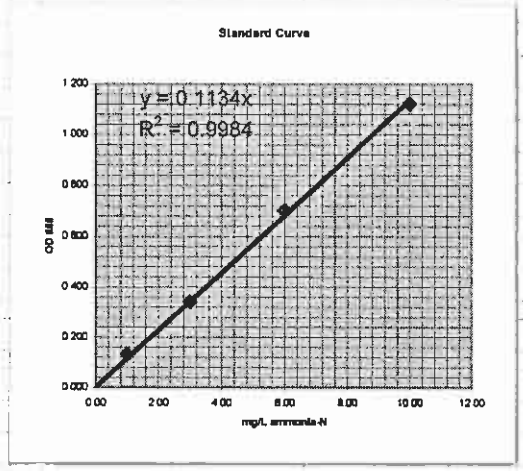
25	3136G	Control	1	
17	3136G	Control	2	
9	3136G	Control	3	
24	3136G	Control	4	
2	3136G	Control	5	
15	3136G	Control	6	
8	3136G	Control	7	
14	3136G	Control	8	
11	3136G	Control	9	wq replicate
26	3136G	Control	10	day 0 replicate
29	3160G	DPSC-G093	1	
13	3160G	DPSC-G093	2	
30	3160G	DPSC-G093	3	
27	3160G	DPSC-G093	4	
1	3160G	DPSC-G093	5	
10	3160G	DPSC-G093	6	
5	3160G	DPSC-G093	7	
4	3160G	DPSC-G093	8	
19	3160G	DPSC-G093	9	wq replicate
28	3160G	DPSC-G093	10	day 0 replicate
7	3161G	DPSC-G094	1	
3	3161G	DPSC-G094	2	
6	3161G	DPSC-G094	3	
22	3161G	DPSC-G094	4	
18	3161G	DPSC-G094	5	
16	3161G	DPSC-G094	6	
21	3161G	DPSC-G094	7	
12	3161G	DPSC-G094	8	
20	3161G	DPSC-G094	9	wq replicate
23	3161G	DPSC-G094	10	day 0 replicate

AMMONIA EXPOSURE BENCHSHEETS AND ANALYSIS

Total Ammonia-N in Sediment Pore Water: Computation Worksheet
Salicylate Method (SOP #5492)

Result

	Sample description	Dilution factor	OD655	NH3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	-----		
	1.0 mg/L NH3-N Std.	-----	0.132	1.00		
	3.0 mg/L NH3-N Std.	-----	0.340	3.00		
	6.0 mg/L NH3-N Std.	-----	0.700	6.00		
	10.0 mg/L NH3-N Std.	-----	1.120	10.00		
	3.0 mg/L spike	-----	0.341	3.01		
	3.0 mg/L spike dupl.	-----	0.357	3.15		
	5.0 mg/L 2nd source		0.572	5.04		
1.	23	5	0.021	0.93		
2.	26	5	0.058	2.56		
3.	28	5	0.019	0.84		



Reporting limit (mg/L) =	0.5
Recovery (%) =	102.6
Precision (RPD) =	-4.58
2nd source (%) =	100.9
Sample volume (ml):	0.10
Dilution factor	5

Sample Set Description:

Test No.: 806-4
Test Day: 0
Species: *Chironomus*

Sediment porewaters

Analyst: RSC
Date analysed: 4/2/2010

Total Ammonia-N in Sediment Pore Water: Computation Worksheet
Salicylate Method (SOP #5492)

Result

	Sample description	Dilution factor	OD655	NH3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	-----		
	1.0 mg/L NH3-N Std.	-----	.132	1.00		
	3.0 mg/L NH3-N Std.	-----	.340	3.00		
	6.0 mg/L NH3-N Std.	-----	.700	6.00		
	10.0 mg/L NH3-N Std.	-----	1.12	10.00		
	3.0 mg/L spike	----	.341			
	3.0 mg/L spike dupl.	-----	.357			
	5.0 mg/L 2nd source		.572			
1.	23	5	.021		6.6	
2.	26	5	.058		6.7	
3.	28	5	.019		6.8	
4.						
5.						
6.						
7.						
8.						
9.						
10.						
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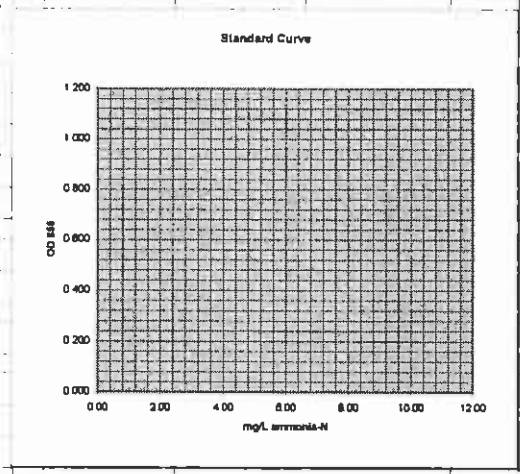

Reporting limit (mg/L) =	0.5
Recovery (%) =	#VALUE!
Precision (RPD) =	#VALUE!
2nd source (%) =	#VALUE!
Sample volume (ml):	0.10
Dilution factor	5

Sample Set Description:
Test No.: 806-4
Test Day: 0
Species: *Chironomus*

Sediment porewaters

Analyst:	RSC
Date analysed:	4/2/2010

Total Ammonia-N in Sediment Pore Water: Computation Worksheet
Salicylate Method (SOP #5492)

Result

	Sample description	Dilution factor	OD655	NH3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	----	-----		
	1.0 mg/L NH3-N Std.	-----	0.141	1.00		
	3.0 mg/L NH3-N Std.	-----	0.347	3.00		
	6.0 mg/L NH3-N Std.	-----	0.705	6.00		
	10.0 mg/L NH3-N Std.	-----	1.200	10.00		
	3.0 mg/L spike	-----	0.349	2.93		
	3.0 mg/L spike dupl.	-----	0.351	2.94		
	5.0 mg/L 2nd source		0.558	4.68		
1.	11	5	0.058	2.43	7.0	
2.	19	5	0.030	1.26	6.6	
3.	20	5	0.031	1.30	6.6	
4.						
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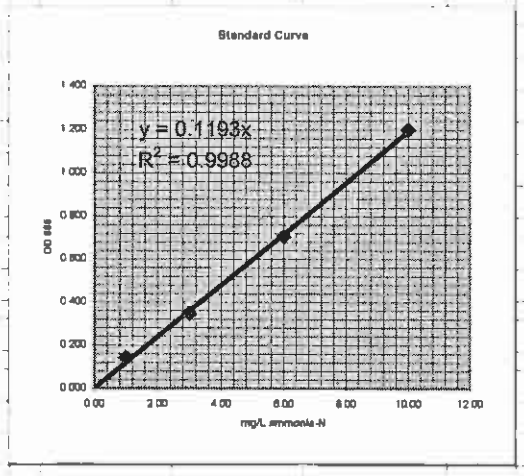

Reporting limit (mg/L) =	0.5
Recovery (%) =	97.8
Precision (RPD) =	-0.57
2nd source (%) =	93.5
Sample volume (ml):	0.10
Dilution factor	5

Sample Set Description:

Test No.: 806-4
Test Day: 10
Species: *Chironomus*

Sediment porewaters

Analyst: RSC
Date analysed: 4/12/2010

Total Ammonia-N in Sediment Pore Water: Computation Worksheet
Salicylate Method (SOP #5492)

Result

	Sample description	Dilution factor	OD655	NH3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	----		
	1.0 mg/L NH3-N Std.	-----	.141	1.00		
	3.0 mg/L NH3-N Std.	-----	.347	3.00		
	6.0 mg/L NH3-N Std.	-----	.708	6.00		
	10.0 mg/L NH3-N Std.	----	1.20	10.00		
	3.0 mg/L spike	-----	.349			
	3.0 mg/L spike dupl.	-----	.351			
	5.0 mg/L 2nd source		.558			
1.	11	5	.058			
2.	19	5	.030			
3.	20	5	.031			
4.						
5.						
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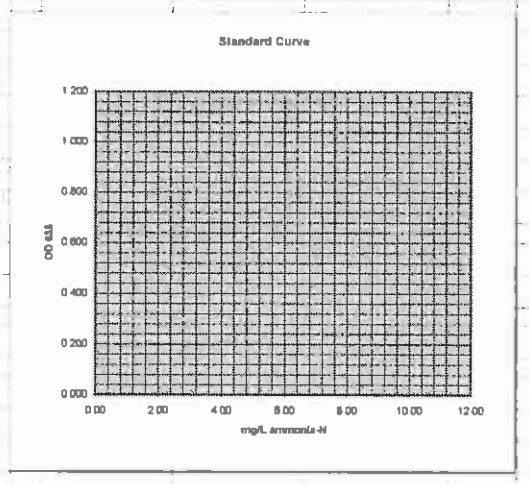


Reporting limit (mg/L) = 0.5

Recovery (%) = #VALUE!
Precision (RPD) = #VALUE!
2nd source (%) = #VALUE!

Sample volume (ml): 0.10
Dilution factor 5

Sample Set Description:
Test No.: 806-4
Test Day: 10
Species: *Chironomus*

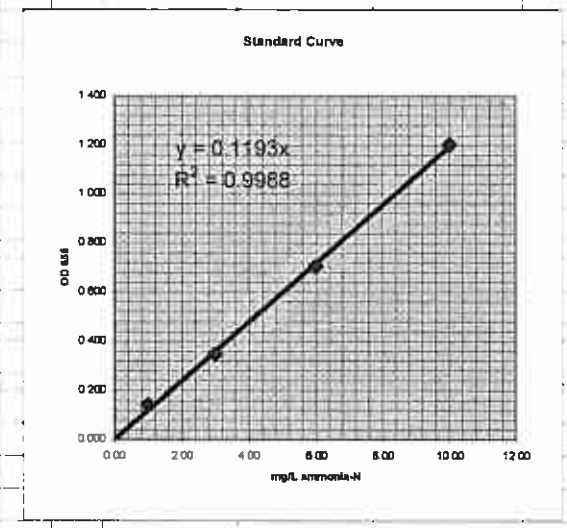
Sediment porewaters

Analyst: RSC
Date analysed: 4/12/2010

Total Ammonia-N in Water: Computation Worksheet
Salicylate Method (SOP #5492)

Result

	Sample description	Dilution factor	OD_{655}	NH_3-N (mg/L)
	Blank	-----	-----	-----
	1.0 mg/L NH_3-N Std.	-----	0.141	1.00
	3.0 mg/L NH_3-N Std.	-----	0.347	3.00
	6.0 mg/L NH_3-N Std.	-----	0.705	6.00
	10.0 mg/L NH_3-N Std.	-----	1.200	10.00
	3.0 mg/L spike	-----	0.349	2.93
	3.0 mg/L spike dupl.	-----	0.351	2.94
	5.0 mg/L 2nd source	-----	0.558	4.68
1.	Day 0 (4-2-10)	######		
2.	11	1	0.020	0.17
3.	19	1	0.013	0.11
4.	20	1	0.010	ND
5.	Day 10 (4-12-10)	######		
6.	11	1	0.028	0.23
7.	19	1	0.013	0.11
8.	20	1	0.015	0.13



Reporting limit (mg/L) =	0.1
Recovery (%) =	97.8
Precision (RPD) =	-0.57
2nd source (%) =	93.5
Sample volume (ml):	0.50
Dilution factor	1

Sample Set Description:

Test No.: 806-4
Test Day: Days 0 & 10
Species: *Chironomus*

Overlying water

Analyst: RSC
Date analysed: 4/12/2010

Total Ammonia-N in Water: Computation Worksheet
Salicylate Method (SOP #5492)

Result

	Sample description	Dilution factor	OD655	NH3-N (mg/L)
	Blank	-----	-----	-----
	1.0 mg/L NH3-N Std.	-----	.141	1.00
	3.0 mg/L NH3-N Std.	-----	.347	3.00
	6.0 mg/L NH3-N Std.	-----	.705	6.00
	10.0 mg/L NH3-N Std.	-----	1.20	10.00
	3.0 mg/L spike	-----	.349	
	3.0 mg/L spike dupl.	-----	.351	
	5.0 mg/L 2nd source	-----	.558	
1.	Day 0 (4-2-10)	######		
2.	11	1	.020	
3.	19	1	.013	
4.	20	1	.010	
5.	Day 10 (4-12-10)	######		
6.	11	1	.028	
7.	19	1	.013	
8.	20	1	.015	
9.				
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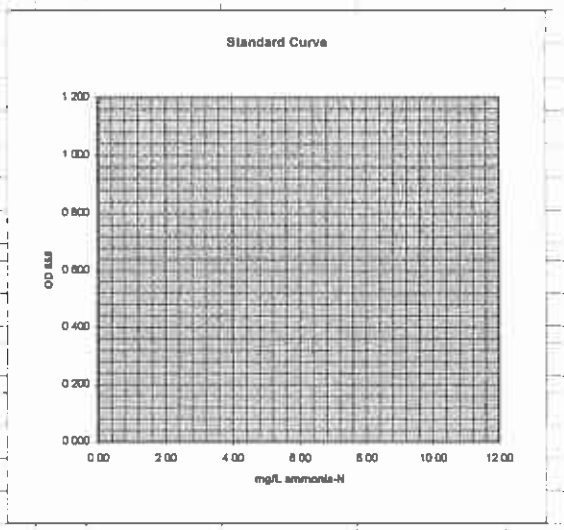


Reporting limit (mg/L) =	0.1
Recovery (%) =	#VALUE!
Precision (RPD) =	#VALUE!
2nd source (%) =	#VALUE!
Sample volume (ml):	0.50
Dilution factor	1

Sample Set Description:

Test No.: 806-4
Test Day: Days 0 & 10
Species: *Chironomus*

Overlying water

Analyst: RSC
Date analysed: 4/12/2010

CHAIN-OF-CUSTODY RECORDS

GSI Water Solutions, Inc.

Lab submittal Columbia Analytical Services, 1317 South 13th Ave., Kelso, WA 98626

CHAIN OF CUSTODY / LABORATORY ANALYSIS REQUEST FORM

Date March 25, 2010 Page 1 of 1

Project Name: DPSC Phase II
Project Number: 0205.008
Project Manager: Kevin Parrett
Company/Address:
GSI Water Solutions
55 SW Yamhill Street
Portland, OR
Phone: (503) 239-8799
Fax: (503) 239-8940

Sampler's Signature: Erin Carroll for JN

Analysis Requested

NAS #	Sample I.D.	Date	Time	LAB I.D.	Sample Matrix	Number of Containers	Biossay	REMARKS
31606	DPSC-G093	3/25/2010	11 41		Sediment	3		
31616	DPSC-G094	3/25/2010	12 35		Sediment	3		2.5 L provided. Insufficient volume for 3 L

Relinquished By	Received By
Erin Carroll	Gary Butler
Signature: Erin Carroll	Signature: GARY Butler
Printed Name: GSI Water Solutions	Printed Name: NAS
Firm: GC 3-26-10 10:30	Firm: 3-27-10 0910
Date/Time	Date/Time

Relinquished By	Received By
Signature	Signature
Printed Name	Printed Name
Firm	Firm
Date/Time	Date/Time

TURNAROUND REQUIREMENTS

Send Reports to: Attn: Erin Carroll, GSI Water Solutions, 55 SW Yamhill St, Suite 400, Portland, OR 97201

Send Reports to:

REPORT REQUIREMENTS

1. Standard Report
2. Report includes DQO Info
3. Report includes All Raw Data

INVOICE INFORMATION

Attn: Rick Lower III, Hart Crowser, Inc., 8410 SW Cherry Drive, Hillsboro, OR 97123

(NDI INQ#/TRACK#)

SAMPLE RECEIPT

Temp=18°C

Special Instructions/Comments:

Per Final Sampling and Analysis Plan (February 2010)

13473

053888_P1

11046537

29 of 100

Next Day Air® Early A.M.®

Shipping Document

See instructions on back. Visit UPS.com® or call 1-800-PICK-UPS® (800-742-5877) for additional information and UPS Tariff/Terms and Conditions.

TRACKING NUMBER A561 448 929 2

1 SHIPMENT FROM

Ship to Account No.: 71WOE1-GEI Water Solutions

Reference Number: 0205.008.004

Name: Erin Carroll Telephone: 503-239-8790

Company: SS SW Yamhill, Suite 400

Street Address: Portland, OR 97204

City and State: Zip Code:

2 EXTREMELY URGENT DELIVERY TO

Name: Gerald Irassarri Telephone: 541-265-7225

Company: Northwestern Aquatic Sciences

Street Address: 3814 Yaquina Bay Rd. Dept./Flr.: Residential Delivery ☐

City and State: Newport, OR Zip Code: 97365

3 Weight: Enter "LTR" if Letter: 052 | Dimensional Weight: If Applicable | Large Package ☐

4 Shipper Release ☐

5 Next Day Air Freight Rate

6 Additional Services

- ☐ Saturday Pickup — See instructions
- ☒ Saturday Delivery — See instructions
- ☐ Declared Value for Carriage — Contents are automatically protected up to $100. For declared value over $100, see instructions. $ Amount
- ☐ C.O.D. — If C.O.D., enter amount to be collected and attach completed UPS C.O.D. tag to package. $ Amount
- ☐ Verbal Confirmation Of Delivery — See instructions

7 Additional Handling Charges

- ☒ An Additional Handling Charge applies for certain items. See instructions

Total Charges $

8 Method of Payment

- Bill Shipper's Account Number — In Section 1
- Bill Receiver
- Bill Third Party
- Credit Card — American Express, Diner's Club, MasterCard, Visa
- Record Account No. in Section 9
- Check

9 Receiver's/Third Party's UPS Acct. No. or Major Credit Card No. Expiration Date

Third Party's Company Name

Street Address

City and State Zip Code

All shipments are subject to the terms contained in the UPS Tariff/Terms and Conditions of Service available at UPS.com and local UPS offices.

10 Shipper's Signature: X Erin Carroll Date of Shipment: 3/26/10

020191126 1/07 S

UPS COPY

This form not needed with UPS Internet Shipping at UPS.com

DPSC Phase II 3-25-2010
Custody Seal
Erin Carroll

1 of 1

DPSC Phase II 3-25-10
Custody Seal
Erin Carroll

1 of 1

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS- _____

ACUTE TOXICITY TEST (ALL SPECIES)

REVIEWED PAGES 1-7 GJI

Test No. 999-2713 Client: QC Test Investigator _____

Test Type (rangefinding/definitive) _____ Test Length (hr) 96

Species *Chironomus dilutus*

STUDY MANAGEMENT

Client: QC test

Client's Study Monitor: QC test

Testing Laboratory: Northwestern Aquatic Sciences

Test Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Man./Study Dir. G.J. Irissarri GJI

QA Officer L. K. Nemeth

1. M.S. Redmond MSR 2. _____

3. _____ 4. _____

Study Schedule:

Test Beginning: 4-2-10 1030 Test Ending: 4-6-10 1050

TEST MATERIAL

Description: Potassium Chloride Crystals - Lot No.: FISHER 073280

NAS Sample No.				
Date of Collection:				
Date of Receipt:				
Temperature (deg C):				
Dissolved oxygen (mg/L):				
pH:				
Conductivity (umhos/cm):				
Hardness (mg/L):				
Alkalinity (mg/L):				
Salinity (ppt):				
Total chlorine (mg/L):				
Total ammonia-N (mg/L):				
_____:				

DILUTION WATER

Description: Moderately hard synthetic water

Date of Preparation/Collection: 3-30-10

Water Quality: Cond. (umhos/cm): 268 Salinity (ppt) [redacted] pH 7.8

Hardness (mg/L as $CaCO_3$): 94 Alkalinity (mg/L as $CaCO_3$): 70

Treatments: Aerated ≥ 24 hrs

TEST LOCATION

Test conducted in (circle one): room 1 room 2 trailer water bath other: Room # 3

Randomization chart:

∅	2.5	20	5	20	5	10	∅	5	1.25
10	10	1.25	1.25	1.25	10	∅	2.5	10	20
5	5	2.5	20	10	∅	5	20	2.5	∅
2.5	20	∅	10	5	1.25	2.5	20	1.25	10
20	∅	10	2.5	∅	2.5	20	1.25	∅	2.5
1.25	1.25	5	∅	2.5	20	1.25	5	20	5

Error codes: 1) Correction of handwriting error
2) Written in wrong location; entry deleted
3) Wrong date deleted; replaced with correct date
4) Error found in measurement; measurement repeated

Revised 12-5-01

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-2713 Client QC Test Investigator _____

TEST ORGANISMS

Species: *Chironomus dilutus* Age: 3rd instar

Source: NAS cultures Date received: N/A

Acclimation Data:

Date	Temp. (deg.C)	DO (mg/L)	pH	Cond. umhos/cm	Hardness (mg/L)	Alkalinity (mg/L)	Feeding	Water changes
3-24-10	22.2	7.9	7.0	145	43	30	Animals fed Tetra Fin	–
3-26-10	22.6	8.2	7.8	147	–	–	and *Selenastrum*	YES
3-29-10	21.9	8.3	7.3	147	–	–	Details recorded on	YES
3-31-10	21.7	8.6	7.4	147	43	30	Chironomid culture	–
4-1-10	21.7	8.5	6.8	147	–	–	data sheets	YES
4-2-10	21.8	8.0	7.1	146	–	–		– ~~YES~~ 6J1 4-5-10
Mean	22.0	8.3	7.2	146	43	30		
S.D.	0.4	0.3	0.4	2	–	–		
(N)	6	6	6	6	2	2		

Photoperiod during acclimation: 16:8, L:D

TEST PROCEDURES AND CONDITIONS

Test concentrations (50% series recommended): 20, 10, 5, 2.5, 1.25, 0 g/L

Test chamber: 30 ml plastic cups Test volume: 20 ml

Replicates/treatment: 10 Organisms/treatment: 10 (1/rep)

Test water changes: None Aeration during test: None

Feeding: 0.25 ml Prime Tropical Flakes (4g/L) suspension per cup on days 0 and 2

Duration: 24-hr, 48-hr, 96-hr Test temperature (deg.C): 23 ± 1

Beaker placement: Stratified randomization Photoperiod: 16:8, L:D

MISCELLANEOUS NOTES

Test solution preparation:

Working stock: Dissolve 10g KCl crystals in dilution water and dilute to 500 mL.
Final conc.: 20 g/L.

	Test concentration (g/L)	KCl working stock (ml/200ml)	ml of dilution water per 200 ml
	20	200	0
4-2-10 6J1	10	100	100
	5	50	150
	2.5	25	175
	1.25	12.5	187.5
	0	0	0

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-2713 Client QC Test

DAILY RECORD SHEET

Day 0 (4 / 2 /10) GJI Temp Beaker (°C): 22.4

Conc. (g/L)	Temp. (deg.C)	pH	Cond. (umhos/cm)	DO (ppm)	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20	22.0	7.9	14230	9.0	86	70	
2. 10	22.2	7.9	11570	8.9			
3. 5	22.2	7.9	7230	9.0			
4. 2.5	22.1	7.9	4010	9.1			
5. 1.25	22.2	7.9	2160	9.0			
6. 0	22.2	7.8	264	9.0	94	70	

All animals fed 0.25 ml Tetra Fin suspension. Initials: GJI

Day 1 (4 /3 /10) GJI Temp Beaker (°C): 23.0

Conc. (g/L)	Temp. (deg.C)	pH	Cond. (umhos/cm)	DO (ppm)	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20							
2. 10							
3. 5							
4. 2.5							
5. 1.25							
6. 0							

Day 2 (4 / 4 /10) GJI Temp Beaker (°C): 22.9

Conc. (g/L)	Temp. (deg.C)	pH	Cond. (umhos/cm)	DO (ppm)	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20							
2. 10							
3. 5							
4. 2.5							
5. 1.25							
6. 0							

All animals fed 0.25 ml Tetra Fin suspension. Initials: GJI

Day 3 (4 /5 /10) GJI Temp Beaker (°C): 22.7

Conc. (g/L)	Temp. (deg.C)	pH	Cond. (umhos/cm)	DO (ppm)	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20							
2. 10							
3. 5							
4. 2.5							
5. 1.25							
6. 0							

Day 4 (4 /6 /10) GJI Temp Beaker (°C): 22.5

Conc. (g/L)	Temp. (deg.C)	pH	Cond. (umhos/cm)	DO (ppm)	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20	–	–	–	–	–	–	
2. 10	–	–	–	–			
3. 5	23.1	7.9	7930	7.1			
4. 2.5	23.0	7.9	4440	7.1			
5. 1.25	22.9	7.9	2490	7.1			
6. 0	22.7	7.9	315	7.2	94	80	
Mean	22.5	7.9		8.3	91	73	
SD	0.4	0		1.0	5	6	
n	10	10		10	3	3	

Revised 12-5-01

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-2713 Client QC Test Investigator _____

DAILY RECORD SHEET - Survivors

Day 0 (4 / 2 / 10) GSL

Conc. (g/L)	Survivors in Replicate: 1	2	3	4	5	6	7	8	9	10	Total
1. 20	1	1	1	1	1	1	1	1	1	1	10
2. 10	1	1	1	1	1	1	1	1	1	1	10
3. 5	1	1	1	1	1	1	1	1	1	1	10
4. 2.5	1	1	1	1	1	1	1	1	1	1	10
5. 1.25	1	1	1	1	1	1	1	1	1	1	10
6. 0	1	1	1	1	1	1	1	1	1	1	10

Day 1 (4 / 3 / 10) GSL

Conc. (g/L)	Survivors in Replicate: 1	2	3	4	5	6	7	8	9	10	Total
1. 20	0	0	0	0	0	0	0	0	0	0	Ø (10D)
2. 10	1	0	0	1	0	0	0	0	0	0	2 (8D)
3. 5	1	1	1	1	1	1	1	1	0	1	9 (1D)
4. 2.5	1	1	1	1	1	1	1	1	1	1	10
5. 1.25	1	1	1	1	1	1	1	1	1	1	10
6. 0	1	1	1	1	1	1	1	1	1	1	10

Day 2 (4 / 4 / 10) GSL

Conc. (g/L)	Survivors in Replicate: 1	2	3	4	5	6	7	8	9	10	Total
1. 20	0	0	0	0	0	0	0	0	0	0	Ø
2. 10	0	0	0	0	0	0	0	0	0	0	Ø (2D)
3. 5	0	1	1	1	0	1	1	1	0	1	7 (2D)
4. 2.5	0	1	1	1	1	1	1	1	1	1	9 (1D)
5. 1.25	1	1	1	1	1	1	1	1	1	1	10
6. 0	1	1	1	1	1	1	1	1	1	1	10

Day 3 (4 / 5 / 10) GSL

Conc. (g/L)	Survivors in Replicate: 1	2	3	4	5	6	7	8	9	10	Total
1. 20	0	0	0	0	0	0	0	0	0	0	Ø
2. 10	0	0	0	0	0	0	0	0	0	0	Ø
3. 5	0	1	1	1	0	1	1	1	0	1	7
4. 2.5	0	1	1	1	1	1	1	1	1	1	9
5. 1.25	1	0	1	1	1	1	1	1	1	1	9 (1D)
6. 0	1	1	1	1	1	1	1	1	1	1	10

Day 4 (4 / 6 / 10) GSL

Conc. (g/L)	Survivors in Replicate: 1	2	3	4	5	6	7	8	9	10	Total
1. 20	0	0	0	0	0	0	0	0	0	0	Ø
2. 10	0	0	0	0	0	0	0	0	0	0	Ø
3. 5	0	1	1	1	0	1	0	0	0	1	5 (2D)
4. 2.5	0	1	1	1	1	1	1	1	1	1	9
5. 1.25	1	0	1	1	1	1	1	1	1	1	9
6. 0	1	1	1	1	1	1	1	1	1	1	10

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	4/2/2010 10:30	Test ID:	999-2713	Sample ID:	REF-Ref Toxicant
End Date:	4/6/2010 10:50	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CT-Chironomus dilutus
Comments:					

Conc-gm/L	1
D-Control	1.0000
1.25	0.9000
2.5	0.9000
5	0.5000
10	0.0000
20	0.0000

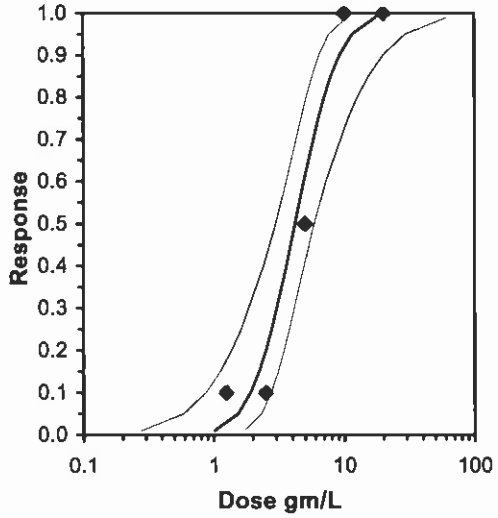
Conc-gm/L	Mean	N-Mean	Resp	Not Resp	Total	N	Fisher's Exact P	1-Tailed Critical	Number Resp	Total Number
D-Control	1.0000	1.0000	0	10	10	1			0	10
1.25	0.9000	0.9000	1	9	10	1	0.5000	0.0500	1	10
2.5	0.9000	0.9000	1	9	10	1	0.5000	0.0500	1	10
*5	0.5000	0.5000	5	5	10	1	0.0163	0.0500	5	10
*10	0.0000	0.0000	10	0	10	1	0.0000	0.0500	10	10
*20	0.0000	0.0000	10	0	10	1	0.0000	0.0500	10	10

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Fisher's Exact Test	2.5	5	3.53553	

Maximum Likelihood-Probit

Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	3.75244	0.87939	2.02884	5.47604	0	4.47911	7.81472	0.21	0.62253	0.26649	5
Intercept	2.66398	0.60622	1.47579	3.85218							
TSCR											

Point	Probits	gm/L	95% Fiducial Limits	
EC01	2.674	1.00595	0.27725	1.69074
EC05	3.355	1.52823	0.58661	2.3065
EC10	3.718	1.90989	0.86778	2.74352
EC15	3.964	2.21988	1.12394	3.1014
EC20	4.158	2.50176	1.37389	3.43514
EC25	4.326	2.77195	1.6247	3.76722
EC40	4.747	3.58936	2.41054	4.88838
EC50	5.000	4.19308	2.97554	5.87289
EC60	5.253	4.89833	3.58704	7.22469
EC75	5.674	6.34278	4.67445	10.6736
EC80	5.842	7.0278	5.13207	12.608
EC85	6.036	7.92019	5.68993	15.3967
EC90	6.282	9.20572	6.43781	19.924
EC95	6.645	11.5047	7.66397	29.4492
EC99	7.326	17.4779	10.4635	62.2594



ToxCalc v5.0.23

Reviewed by:_____

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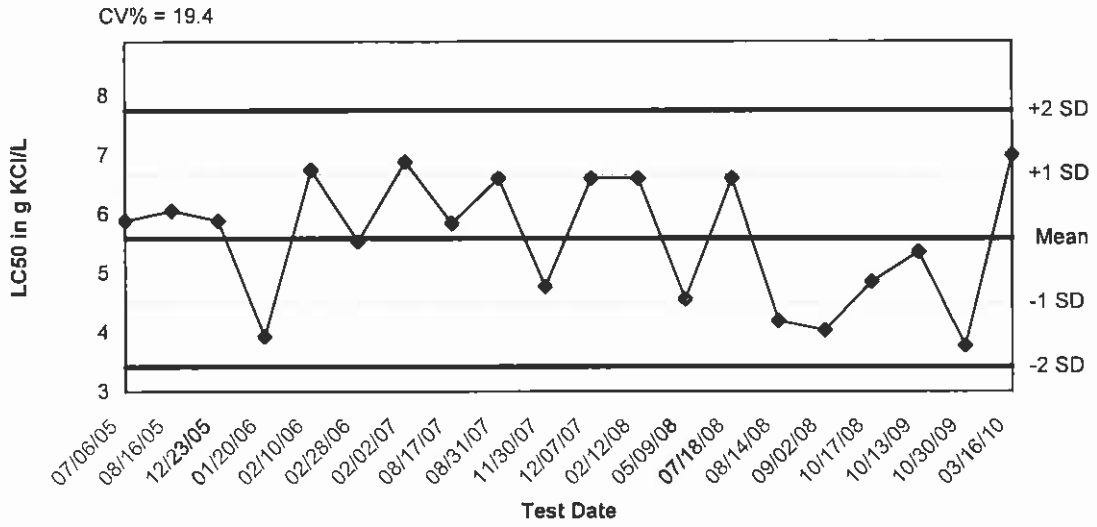
Test: AT-Acute 96-hr Toxicity Test	Test ID: 999-2713
Species: CT-Chironomus dilutus	Protocol: EPAF 91-EPA Freshwater
Sample ID: REF-Ref Toxicant	Sample Type: KCL-Potassium chloride
Start Date: 4/2/2010 10:30 End Date: 4/6/2010 10:50	Lab ID: ORNAS-Northwestern Aquatic Sciences

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	D-Control	10				10	
	2	1	1.250	10				9	
	3	1	2.500	10				9	
	4	1	5.000	10				5	
	5	1	10.000	10				0	
	6	1	20.000	10				0	

Comments: data verified against laboratory bench sheets 4-19-10 JLF

Midge, Chironomus dilutus, larvae (3rd instar) acute reference toxicant test



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
07/06/05	5.8900	5.5840	4.5009	3.4178	6.6671	7.7502
08/16/05	6.0600	5.5840	4.5009	3.4178	6.6671	7.7502
12/23/05	5.8900	5.5840	4.5009	3.4178	6.6671	7.7502
01/20/06	3.9300	5.5840	4.5009	3.4178	6.6671	7.7502
02/10/06	6.7400	5.5840	4.5009	3.4178	6.6671	7.7502
02/28/06	5.5300	5.5840	4.5009	3.4178	6.6671	7.7502
02/02/07	6.8800	5.5840	4.5009	3.4178	6.6671	7.7502
08/17/07	5.8400	5.5840	4.5009	3.4178	6.6671	7.7502
08/31/07	6.6000	5.5840	4.5009	3.4178	6.6671	7.7502
11/30/07	4.7700	5.5840	4.5009	3.4178	6.6671	7.7502
12/07/07	6.6000	5.5840	4.5009	3.4178	6.6671	7.7502
02/12/08	6.6000	5.5840	4.5009	3.4178	6.6671	7.7502
05/09/08	4.5600	5.5840	4.5009	3.4178	6.6671	7.7502
07/18/08	6.6000	5.5840	4.5009	3.4178	6.6671	7.7502
08/14/08	4.1900	5.5840	4.5009	3.4178	6.6671	7.7502
09/02/08	4.0300	5.5840	4.5009	3.4178	6.6671	7.7502
10/17/08	4.8500	5.5840	4.5009	3.4178	6.6671	7.7502
10/13/09	5.3600	5.5840	4.5009	3.4178	6.6671	7.7502
10/30/09	3.7700	5.5840	4.5009	3.4178	6.6671	7.7502
03/16/10	6.9900	5.5840	4.5009	3.4178	6.6671	7.7502

MSR
4-7-10