Report

of

Test No. 806-2

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 to

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

April 22, 2010

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 806-2

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; L.P. Sandoval, B.S., Tech.; Y. Nakahama, Tech.

Study Schedule:

Test Beginning: 3-16-10, 1150 hrs.

Test Ending: 3-26-10, 1030 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.	3133G	3134G	3135G
Description	DPSC-G088	DPSC-G095	DPSC-G096
Collection Date	2/24/10	2/22/10	2/25/10
Receipt Date	3/4/10	3/4/10	3/4/10

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: 3-12-10

Water Quality:
pH: 6.9
conductivity: 130 µmhos/cm
hardness: 43mg/L as $CaCO_3$
alkalinity: 30 mg/L as $CaCO_3$.
total chlorine: < 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 ± 0.01 mg
Source: NAS cultures, originally obtained from EPA, Duluth, MN.
Acclimation: Holding conditions for the two weeks prior to testing averaged: Temperature, 20.6 ± 1.7°C; dissolved oxygen, 8.5 ± 0.2 mg/L; pH, 7.1 ± 0.3; conductivity, 147 ± 8 µmhos/cm; hardness, 47 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.: 999-2709
Reference Toxicant and Source: Potassium Chloride (KCl), Fisher Lot #073280.
Test Date: 3-16-10.
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 6.99 g/L. This result is within the laboratory's control chart warning limits (3.42 – 7.56 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 0.3 mg/L for all day 0 and day 10 measurements. Interstitial bulk sediment values for ammonia-N ranged from <0.5 to 1.7 mg/L.

The test met the survival and weight acceptability criteria specified in the test protocol with 97.5% mean control survival (≥70% required) and a control individual mean ash-free dry weight of 0.88 mg per larvae (0.48 mg required). The reference toxicant (positive control) EC50 result was within the laboratory's control chart limits (6.99 g/L; control chart mean ± 2 S.D. = 5.49 ± 2.07). It is concluded, therefore, that the test has developed fully acceptable data for use in making management decisions.

STUDY APPROVAL

Gerald Crissouri 4-20-10
Project Manager/Study Director Date

Julie R. Jiang 4-20-10
Quality Assurance Unit Date

Linda K. Nemeth 4/6/10
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 ± 0.3	22.0	23.2	44
Dissolved oxygen (mg/L)	5.9 ± 1.0	4.1	7.4	44
Conductivity (µmhos/cm)	149 ± 17	136	191	8
pH	6.6 ± 0.1	6.5	6.7	8
Hardness (mg/L as $CaCO_3$)	36 ± 4	34	43	8
Alkalinity (mg/L as $CaCO_3$)	29 ± 4	20	30	8
Total ammonia (mg/L)	---	<0.1	0.3	8

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	6.9	0.9	6.7	1.7
3133G	DPSC-G088	6.9	<0.5	6.5	1.0
3134G	DPSC-G095	7.1	<0.5	6.4	1.3
3135G	DPSC-G096	6.8	<0.5	6.6	0.7

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	2.5 ± 4.6	0.88 ± 0.09
3133G	DPSC-G088	1.3 ± 3.5	0.79 ± 0.08
3134G	DPSC-G095	2.5 ± 7.1	1.08 ± 0.09
3135G	DPSC-G096	3.8 ± 7.4	0.94 ± 0.06

*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 1-25 -GJI

Test No. 806-2 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. [illegible] 2. Lydia P. Sandoval LPS
3. Yves Nakagawa YN 4. D.S. Caldwell DSC
5. 6.
7. 8.

Study Schedule:

Test Beginning: 3-16-10 1150 Test Ending: 3-26-10 1030

TEST MATERIAL

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

NAS Sample No.:	3136G	3133G	3134G	3135G	
Description:	Control	DPSC-G088	DPSC-G095	DPSC-G096	
Collection Date:	3/10/10	2/24/10	2/22/10	2/25/10	
Receipt Date:	3/10/10	3/4/10	3/4/10	3/4/10	
_____:					
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 correct date
4) error found in measurement; measurement repeated

Test No. 806-2 Client Hart Crowser Investigator

SEDIMENT DESCRIPTIONS -- SUPPLEMENTAL NOTES

Sample No.	Description
3136G	FINE BLACK MUD
3133G	GRAY SAND w/ ROCKS AND WOODY DEBRIS
3134G	DARK BROWN GRAVEL & SAND w/ PLANT DEBRIS AND PAINT CHIPS
3135G	GRAY SAND w/ MEDIUM SIZED ROCKS (SOME RED)

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-2 Client Hart Crowser Investigator

TEST WATER

Source: Dechlorinated municipal tap water

Date of Collection: 3-12-10

pH 6.9

Cond (umhos/cm2) 130

Hardness (mg/L) 43

Alkalinity (mg/L) 30

Total Chlorine (mg/L) <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)	DO (mg/L)	pH	Cond. umhos/cm	Hardness (mg/L)	Alkalinity (mg/L)	Feeding	Water changes
3-3-10	20.7	8.4	7.1	153	43	30	Animals fed Tetra Fin	
3-5-10	21.2	8.5	7.0	151	–	–	and *Selenastrum*	
3-8-10	19.9	8.7	7.2	148	–	–	Details recorded on	
3-10-10	17.7	8.6	6.5	130	51	30	Chironomid culture	
3-12-10	22.0	8.3	7.3	148	–	–	data sheets	
3-15-10	22.3	8.7	7.4	149	–	–		
Mean	20.7	8.5	7.1	147	47	30		
S.D.	1.7	0.2	0.3	8	–	–		
(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 WQ 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
3-19-10	BEAKER #16	92	GJI

*To convert ft-candles to lux multipy by 10.76

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-2 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	36				
5	11	17	23	29	35				
4	10	16	22	28	34	40			
3	9	15	21	27	33	39			
2	8	14	20	26	32	38			
1	7	13	19	25	31	37			

Randomization chart:

Randomization chart:

Test No. 806-2 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 0 (3/16/10) GJ/YP

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond.* (umhos/cm)	pH*	Hardness* (mg/L)	Alkalinity* (mg/L)	NH3* (ppm)	Comments
3	22.7	7.2	145	6.7	43	30		Each beaker fed 1.5 ml
5	23.1	7.3	148	6.6	34	30		Tetra Fin suspension
9	22.7	7.4	136	6.6	34	20		Initials: GJ
33	22.8	7.4	191	6.7	34	30		
								Water changed in all
								beakers.
								Time: 0605
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1705
								Initials: LPS

*Water quality measurements to be taken.

Day 1 (3/17/10) GB

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	23.0	7.0						Each beaker fed 1.5 ml
5	23.0	7.2						Tetra Fin suspension
9	23.0	7.1						Initials: GB
33	22.9	7.0						
								Water changed in all
								beakers.
								Time: 0600
								Initials: GB
								Water changed in all
								beakers.
								Time: 1635
								Initials: YP

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-2 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 2 (3/18/10) GB

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.4	6.8						Each beaker fed 1.5 ml
5	22.4	6.4						Tetra Fin suspension
9	22.4	6.6						Initials: GB
33	22.2	6.6						
								Water changed in all
								beakers.
								Time: 0600
								Initials: GB
								Water changed in all
								beakers.
								Time: 1705
								Initials: GB

*Water quality measurements to be taken.

Day 3 (3/19/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.4	6.7						Each beaker fed 1.5 ml
5	22.4	6.6						Tetra Fin suspension
9	22.2	6.6						Initials: GJ
33	22.0	6.6						
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1635
								Initials: YN

*Water quality measurements to be taken.

Test No. 806-2 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 4 (3/20/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	23.2	6.8						Each beaker fed 1.5 ml
5	22.9	6.6						Tetra Fin suspension
9	22.7	6.6						Initials: GJ
33	22.6	6.5						
								Water changed in all
								beakers.
								Time: 0615
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1745
								Initials: UPS

*Water quality measurements to be taken.

Day 5 (3/21/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.9	5.9						Each beaker fed 1.5 ml
5	22.9	5.8						Tetra Fin suspension
9	22.6	6.2						Initials: GJ
33	22.5	6.2						
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1735
								Initials: UPS

*Water quality measurements to be taken.

Test No. 806-2 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 6 (3/22/10) GJL

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.8	5.5						Each beaker fed 1.5 ml
5	22.8	5.3						Tetra Fin suspension
9	22.5	5.2						Initials: GB
33	22.4	5.4						
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJL
								Water changed in all
								beakers.
								Time: 1630
								Initials: YN

*Water quality measurements to be taken.

Day 7 (3/23/10) GJL

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.6	5.4						Each beaker fed 1.5 ml
5	22.6	5.0						Tetra Fin suspension
9	22.4	5.0						Initials: GB
33	22.3	4.8						
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJL
								Water changed in all
								beakers.
								Time: 1635
								Initials: YN

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-2 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 8 (3/24/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.6	5.0						Each beaker fed 1.5 ml
5	22.6	4.4						Tetra Fin suspension
9	22.4	4.6						Initials: GB
33	22.2	4.8						
								Water changed in all
								beakers.
								Time: 0605
								Initials: GB
								Water changed in all
								beakers.
								Time: 1645
								Initials: LPS

*Water quality measurements to be taken.

Day 9 (3/25/10) GJ

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	NH3 (ppm)	Comments
3	22.6	4.6						Each beaker fed 1.5 ml
5	22.7	4.1						Tetra Fin suspension
9	22.4	4.2						Initials: GJ
33	22.3	4.2						
								Water changed in all
								beakers.
								Time: 0610
								Initials: GJ
								Water changed in all
								beakers.
								Time: 1650
								Initials: LPS

*Water quality measurements to be taken.

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-2 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 10 (3/26/10) 651

Beaker No.	Temp.* (deg.C)	DO* (ppm)	Cond.* (umhos/cm)	pH*	Hardness* (mg/L)	Alkalinity* (mg/L)	NH3* (ppm)	Comments
3	22.5	6.2	138	6.6	34	30		Each beaker fed 1.5 ml
5	22.6	5.9	143	6.5	43	30		Tetra Fin suspension
9	22.3	5.4	145	6.6	34	30		Initials: —
33	22.1	5.2	149	6.6	34	30		
								Water changed in all
								beakers.
								Time: 0610
								Initials: BJS
								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. 806-2 Client Hart Crowser Investigator

DAY 10 TEST TERMINATION SHEET

Beaker No.	Number of survivors	Initials
1		
2	10	GJL
3		
4	10	GJL
5		
6	10	GJL
7	8	GJL
8	10	GJL
9		
10	10	GJL
11	10	GJL
12	9	GJL
13	10	GJL
14	10	GJL
15	10	GJL
16	10	GJL
17		
18	9	GJL
19	10	GJL
20	10	GJL
21	10	GJL
22	10	GJL
23	10	GJL
24	10	GJL
25	10	GJL
26	10	GJL
27		
28	10	GJL
29	9	GJL
30		
31	10	GJL
32	10	GJL
33		
34	10	GJL
35	8	GJL
36	10	GJL
37	10	GJL
38	10	GJL
39	10	GJL
40	9	GJL
41		
42		
43		
44		
45		

Beaker No.	Number of survivors	Initials
46		
47		
48		
49		
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CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 806-2 Client Hart Crowser Investigator

Tare: Date 3-16-10 Oven temp (C.) 62 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.009 100mg: 100.017

Final #1 Date 3-19-10 Oven temp (C.) 63 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.009 100mg: 100.011

Final: #2 Date 3-22-10 Oven temp (C.) 64 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.011 100mg: 100.018

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	51.98	53.49	53.48	10	
2	55.38	57.04	57.04	10	
3	56.58	58.25	58.24	10	
4	56.95	58.63	58.63	10	
5	58.70	60.50	60.50	10	

Test No. 806-2 Client Hart Crowser Investigator

WEIGHING DATA SHEET

Tare:	Date 3-23-10	Oven temp (C.) 550	Drying time (hr.) 2	Initials GJL
	Standard Weights:	10 mg: 10.008	100mg: 100.018	
Final #1:	Date 3-29-10	Oven temp (C.) 62	Drying time (hr.) 24	Initials GJL
	Standard Weights:	10 mg: 10.009	100mg: 100.016	
Final #2:	Date 3-30-10	Oven temp (C.) 64	Drying time (hr.) 24	Initials GJL
	Standard Weights:	10 mg: 10.011	100mg: 100.012	
Final #3:	Date 3-31-10	Oven temp (C.) 550	Drying time (hr.) 2	Initials GJL
	Standard Weights:	10 mg: 10.010	100mg: 100.017	

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	—	—	—	—	—	—	
2	2	86.94	102.14	102.01	10	LPS	91.02	
3	3	—	—	—	—	—	—	
4	4	84.01	97.18	97.06	10	LPS	87.21	
5	5	—	—	—	—	—	—	
6	6	90.38	102.14	102.04	10	LPS	94.11	
7	7	80.24	91.21	91.12	8	LPS	83.59	
8	8	86.02	98.33	98.21	10	LPS	89.13	
9	9	—	—	—	—	—	—	
10	10	88.96	104.26	104.13	10	LPS	93.83	
11	11	86.52	99.16	99.03	10	LPS	89.66	
12	12	88.46	101.09	100.99	9	LPS	92.91	
13	13	92.66	106.86	106.77	10	LPS	97.92	
14	14	83.43	96.97	96.86	10	LPS	88.05	
15	15	83.04	96.76	96.65	10	LPS	88.40	
16	16	85.33	100.63	100.50	10	LPS	90.09	
17	17	—	—	—	—	—	—	
18	18	88.64	100.39	100.28	9	LPS	91.98	
19	19	89.48	104.14	104.03	10	LPS	95.25	
20	20	82.39	99.25	99.12	10	LPS	88.67	
21	21	77.97	93.94	93.80	10	LPS	83.79	
22	22	191.87	208.16	208.00	10	LPS	196.68	
23	23	89.78	100.99	100.88	10	LPS	92.41	
24	24	93.29	108.15	108.02	10	LPS	98.85	
25	25	82.58	94.00	93.88	10	LPS	85.53	
26	26	89.40	105.66	105.50	10	LPS	94.64	
27	27	—	—	—	—	—	—	
28	28	89.29	105.53	105.37	10	LPS	94.76	
29	29	95.10	105.54	105.41	9	LPS	97.43	
30	30	—	—	—	—	—	—	
31	31	81.03	92.47	92.35	10	LPS	85.55	
32	32	88.95	103.57	103.41	10	LPS	94.46	
33	33	—	—	—	—	—	—	

Test No. 806-2 Client Hart Crowser Investigator

WEIGHING DATA SHEET

See page _____ for information on drying times and temperatures, standard weights,etc.

Bkr. #	Pan #	Tare wt. (mg)	Dry total wt. (mg) 1	Dry total wt. (mg) 2	no. weighed	put into pans-initials	Ash weight (mg)	
34	34	88.01	101.58	101.42	10	LPS	93.56	
35	35	85.92	101.44	101.25	8	LPS	91.46	
36	36	84.33	93.92	93.80	10	LPS	86.98	
37	37	93.02	109.14	108.97	10	LPS	99.66	
38	38	87.25	102.08	101.91	10	LPS	92.62	
39	39	93.12	104.32	104.19	10	LPS	97.20	
40	40	96.58	109.05	108.89	9	LPS	101.72	
41	41							
42	42							
43	43							
44	44							
45	45							
46	46							
47	47							
48	48							
49	49							
50	50							
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75	75							
76	76							

TEST DATA ANALYSIS RECORDS

data entry verified against laboratory bench sheets 4-1-10 JRF

Test number: 806-2

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	51.98	53.49	10	0.15
2	55.38	57.04	10	0.17
3	56.58	58.25	10	0.17
4	56.95	58.63	10	0.17
5	58.7	60.5	10	0.18
			avg.	0.17

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	10	0	100.0	0.0	82.58	10	93.88	85.53	5.24	0.52	8.35	0.83							
2	18	3136G	Control	2	10	9	1	90.0	10.0	88.64	9	100.28	91.98	11.64	1.29	8.30	0.92							
3	4	3136G	Control	3	10	10	0	100.0	0.0	84.01	10	97.06	87.21	13.05	1.31	9.85	0.99							
4	8	3136G	Control	4	10	10	0	100.0	0.0	86.02	10	98.21	89.13	12.19	1.22	9.08	0.91							
5	11	3136G	Control	5	10	10	0	100.0	0.0	86.52	10	99.03	89.66	12.51	1.25	9.37	0.94	Mean	9.8	0.3	97.5	2.5	1.10	0.88
6	36	3136G	Control	6	10	10	0	100.0	0.0	84.33	10	93.80	86.98	9.47	0.95	8.82	0.68	SD	0.5	0.5	4.6	4.6	0.26	0.09
7	29	3136G	Control	7	10	9	1	90.0	10.0	95.10	9	105.41	97.43	10.31	1.15	7.98	0.89	n	8	8	8	8	8	8
8	23	3136G	Control	8	10	10	0	100.0	0.0	89.78	10	100.88	92.41	11.10	1.11	8.47	0.85							
9	33	3136G	Control	9	wq replicate																			
10	17	3136G	Control	10	day 0 replicate																			
11	40	3133G	DPSC-G088	1	10	9	1	90.0	10.0	96.58	9	108.89	101.72	12.31	1.37	7.17	0.80							
12	39	3133G	DPSC-G088	2	10	10	0	100.0	0.0	93.12	10	104.19	97.20	11.07	1.11	6.99	0.70							
13	31	3133G	DPSC-G088	3	10	10	0	100.0	0.0	81.03	10	92.35	85.55	11.32	1.13	6.80	0.68							
14	15	3133G	DPSC-G088	4	10	10	0	100.0	0.0	83.04	10	96.65	88.40	13.61	1.36	8.25	0.83							
15	6	3133G	DPSC-G088	5	10	10	0	100.0	0.0	90.38	10	102.04	94.11	11.66	1.17	7.93	0.79	Mean	9.9	0.1	98.8	1.3	1.30	0.79
16	32	3133G	DPSC-G088	6	10	10	0	100.0	0.0	88.95	10	103.41	94.46	14.46	1.45	8.95	0.90	SD	0.4	0.4	3.5	3.5	0.14	0.08
17	19	3133G	DPSC-G088	7	10	10	0	100.0	0.0	89.48	10	104.03	95.25	14.55	1.46	8.78	0.88	n	8	8	8	8	8	8
18	34	3133G	DPSC-G088	8	10	10	0	100.0	0.0	88.01	10	101.42	93.56	13.41	1.34	7.86	0.79							
19	5	3133G	DPSC-G088	9	wq replicate																			
20	27	3133G	DPSC-G088	10	day 0 replicate																			
21	10	3134G	DPSC-G095	1	10	10	0	100.0	0.0	88.96	10	104.13	93.83	15.17	1.52	10.30	1.03							
22	38	3134G	DPSC-G095	2	10	10	0	100.0	0.0	87.25	10	101.91	92.62	14.66	1.47	9.29	0.93							
23	16	3134G	DPSC-G095	3	10	10	0	100.0	0.0	85.33	10	100.50	90.09	15.17	1.52	10.41	1.04							
24	26	3134G	DPSC-G095	4	10	10	0	100.0	0.0	89.40	10	105.50	94.64	16.10	1.61	10.86	1.09							
25	28	3134G	DPSC-G095	5	10	10	0	100.0	0.0	89.29	10	105.37	94.76	16.08	1.61	10.61	1.06	Mean	9.8	0.3	97.5	2.5	1.59	1.08
26	35	3134G	DPSC-G095	6	10	8	2	80.0	20.0	85.92	8	101.25	91.46	15.33	1.92	9.79	1.22	SD	0.7	0.7	7.1	7.1	0.14	0.09
27	2	3134G	DPSC-G095	7	10	10	0	100.0	0.0	86.94	10	102.01	91.02	15.07	1.51	10.99	1.10	n	8	8	8	8	8	8
28	22	3134G	DPSC-G095	8	10	10	0	100.0	0.0	191.87	10	208.00	196.68	16.13	1.61	11.32	1.13							
29	9	3134G	DPSC-G095	9	wq replicate																			
30	1	3134G	DPSC-G095	10	day 0 replicate																			
31	37	3135G	DPSC-G096	1	10	10	0	100.0	0.0	93.02	10	108.97	99.66	15.95	1.60	9.31	0.93							
32	13	3135G	DPSC-G096	2	10	10	0	100.0	0.0	92.68	10	106.77	97.92	14.11	1.41	8.85	0.88							
33	24	3135G	DPSC-G096	3	10	10	0	100.0	0.0	93.29	10	108.02	98.85	14.73	1.47	9.17	0.92							
34	12	3135G	DPSC-G096	4	10	9	1	90.0	10.0	88.46	9	100.99	92.91	12.53	1.39	8.08	0.90							
35	7	3135G	DPSC-G096	5	10	8	2	80.0	20.0	80.24	8	91.12	83.59	10.88	1.36	7.53	0.94	Mean	9.6	0.4	96.3	3.8	1.48	0.94
36	14	3135G	DPSC-G096	6	10	10	0	100.0	0.0	83.43	10	96.86	88.05	13.43	1.34	8.81	0.88	SD	0.7	0.7	7.4	7.4	0.12	0.06
37	20	3135G	DPSC-G096	7	10	10	0	100.0	0.0	82.39	10	99.12	88.67	16.73	1.67	10.45	1.05	n	8	8	8	8	8	8
38	21	3135G	DPSC-G096	8	10	10	0	100.0	0.0	77.97	10	93.80	83.79	15.83	1.58	10.01	1.00							
39	3	3135G	DPSC-G096	9	wq replicate																			
40	30	3135G	DPSC-G096	10	day 0 replicate																			

data entry verified against laboratory bench sheets 4-1-10 JMF

Water Quality Data

BKR	NAS SMPL	CLIENT DESCRIP	DAY	Interstitial water pH	Interstitial water NH3
1	3134G	DPSC-G095	0	7.1	<0.5
17	3136G	Control	0	6.9	0.9
27	3133G	DPSC-G088	0	6.9	<0.5
30	3135G	DPSC-G096	0	6.8	<0.5
3	3135G	DPSC-G096	10	6.6	0.7
5	3133G	DPSC-G088	10	6.5	1.0
9	3134G	DPSC-G095	10	6.4	1.3
33	3136G	Control	10	6.7	1.7

BKR	NAS SMPL	CLIENT DESCRIP	REPL	DAY	Overlying water TEMP	DO	COND	pH	NH3	HARD	ALK
3	3135G	DPSC-G096	9	0	22.7	7.2	145	6.7	<0.1	43	30
5	3133G	DPSC-G088	9	0	23.1	7.3	148	6.6	0.1	34	30
9	3134G	DPSC-G095	9	0	22.7	7.4	136	6.6	<0.1	34	20
33	3136G	Control	9	0	22.8	7.4	191	6.7	0.2	34	30
3	3135G	DPSC-G096	9	1	23.0	7.0					
5	3133G	DPSC-G088	9	1	23.0	7.2					
9	3134G	DPSC-G095	9	1	23.0	7.1					
33	3136G	Control	9	1	22.9	7.0					
3	3135G	DPSC-G096	9	2	22.4	6.8					
5	3133G	DPSC-G088	9	2	22.4	6.6					
9	3134G	DPSC-G095	9	2	22.4	6.6					
33	3136G	Control	9	2	22.2	6.6					
3	3135G	DPSC-G096	9	3	22.4	6.7					
5	3133G	DPSC-G088	9	3	22.4	6.6					
9	3134G	DPSC-G095	9	3	22.2	6.6					
33	3136G	Control	9	3	22.0	6.6					
3	3135G	DPSC-G096	9	4	23.2	6.8					
5	3133G	DPSC-G088	9	4	22.9	6.6					
9	3134G	DPSC-G095	9	4	22.7	6.6					
33	3136G	Control	9	4	22.6	6.5					
3	3135G	DPSC-G096	9	5	22.9	5.9					
5	3133G	DPSC-G088	9	5	22.9	5.8					
9	3134G	DPSC-G095	9	5	22.6	6.2					
33	3136G	Control	9	5	22.5	6.2					
3	3135G	DPSC-G096	9	6	22.8	5.5					
5	3133G	DPSC-G088	9	6	22.8	5.3					
9	3134G	DPSC-G095	9	6	22.5	5.2					
33	3136G	Control	9	6	22.4	5.4					
3	3135G	DPSC-G096	9	7	22.6	5.4					
5	3133G	DPSC-G088	9	7	22.6	5.0					
9	3134G	DPSC-G095	9	7	22.4	5.0					
33	3136G	Control	9	7	22.3	4.8					
3	3135G	DPSC-G096	9	8	22.6	5.0					
5	3133G	DPSC-G088	9	8	22.6	4.4					
9	3134G	DPSC-G095	9	8	22.4	4.6					
33	3136G	Control	9	8	22.2	4.8					
3	3135G	DPSC-G096	9	9	22.6	4.6					
5	3133G	DPSC-G088	9	9	22.7	4.1					
9	3134G	DPSC-G095	9	9	22.4	4.2					
33	3136G	Control	9	9	22.3	4.2					
3	3135G	DPSC-G096	9	10	22.5	6.2	138	6.6	0.2	34	30
5	3133G	DPSC-G088	9	10	22.6	5.9	143	6.5	0.1	43	30
9	3134G	DPSC-G095	9	10	22.3	5.6	145	6.6	0.2	34	30
33	3136G	Control	9	10	22.1	5.2	149	6.6	0.3	34	30
				Mean	22.6	5.9	149	6.6	—	36	29
				SD	0.3	1.0	17	0.1	—	4	4
				n	44	44	8	8	8	8	8
				Min	22.0	4.1	136	6.5	<0.1	34	20
				Max	23.2	7.4	191	6.7	0.3	43	30

Test number: 806-2

Freshwater Sediment Test
10-Day Chironomus dilutus

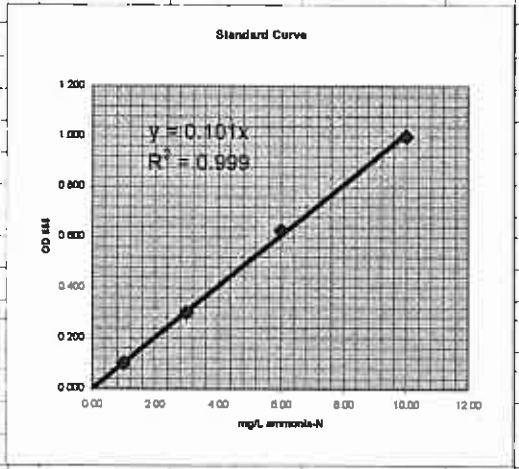
BKR	NAS SMPL	CLIENT DESCRIP	REPL	
25	3136G	Contol	1	
18	3136G	Contol	2	
4	3136G	Contol	3	
8	3136G	Contol	4	
11	3136G	Contol	5	
36	3136G	Contol	6	
29	3136G	Contol	7	
23	3136G	Contol	8	
33	3136G	Contol	9	wq replicate
17	3136G	Contol	10	day 0 replicate
40	3133G	DPSC-G088	1	
39	3133G	DPSC-G088	2	
31	3133G	DPSC-G088	3	
15	3133G	DPSC-G088	4	
6	3133G	DPSC-G088	5	
32	3133G	DPSC-G088	6	
19	3133G	DPSC-G088	7	
34	3133G	DPSC-G088	8	
5	3133G	DPSC-G088	9	wq replicate
27	3133G	DPSC-G088	10	day 0 replicate
10	3134G	DPSC-G095	1	
38	3134G	DPSC-G095	2	
16	3134G	DPSC-G095	3	
26	3134G	DPSC-G095	4	
28	3134G	DPSC-G095	5	
35	3134G	DPSC-G095	6	
2	3134G	DPSC-G095	7	
22	3134G	DPSC-G095	8	
9	3134G	DPSC-G095	9	wq replicate
1	3134G	DPSC-G095	10	day 0 replicate
37	3135G	DPSC-G096	1	
13	3135G	DPSC-G096	2	
24	3135G	DPSC-G096	3	
12	3135G	DPSC-G096	4	
7	3135G	DPSC-G096	5	
14	3135G	DPSC-G096	6	
20	3135G	DPSC-G096	7	
21	3135G	DPSC-G096	8	
3	3135G	DPSC-G096	9	wq replicate
30	3135G	DPSC-G096	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	NH_3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	-----		
	1.0 mg/L NH_3-N Std.	-----	0.101	1.00		
	3.0 mg/L NH_3-N Std.	-----	0.300	3.00		
	6.0 mg/L NH_3-N Std.	-----	0.625	6.00		
	10.0 mg/L NH_3-N Std.	-----	1.000	10.00		
	3.0 mg/L spike	-----	0.272	2.69		
	3.0 mg/L spike dupl.	-----	0.282	2.79		
	5.0 mg/L 2nd source		0.483	4.78		
1.	1	5	0.007	ND		
2.	17	5	0.019	0.94		
3.	27	5	0.007	ND		
4.	30	5	0.002	ND		



Reporting limit (mg/L) =	0.5
Recovery (%) =	91.4
Precision (RPD) =	-3.61
2nd source (%) =	95.6
Sample volume (ml):	0.10
Dilution factor	5

Sample Set Description:

Test No.: 806-2
Test Day: 0
Species: *Chironomus*

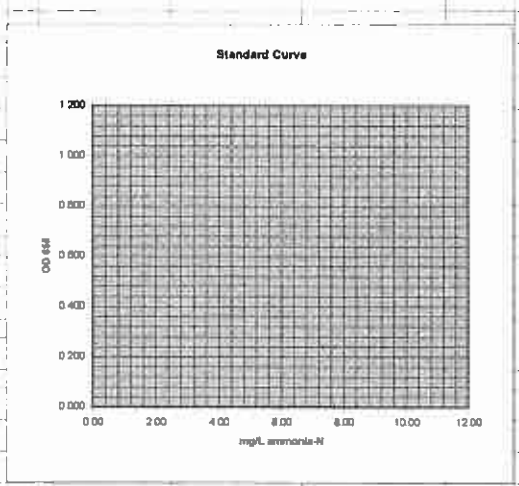
Sediment porewaters

Analyst: RSC
Date analysed: 3/16/2010

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

Result

	Sample description	Dilution factor	OD655	NH_3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	-----		
	1.0 mg/L NH_3-N Std.	-----	.101	1.00		
	3.0 mg/L NH_3-N Std.	-----	.300	3.00		
	6.0 mg/L NH_3-N Std.	-----	.625	6.00		
	10.0 mg/L NH_3-N Std.	-----	1.00	10.00		
	3.0 mg/L spike	-----	.272			
	3.0 mg/L spike dupl.	-----	.282			
	5.0 mg/L 2nd source	-	.483			
1.	1	5	.007		7.1	
2.	17	5	.019		6.7	
3.	27	5	.007		6.9	
4.	30	5	.002		6.8	



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.: 806-2
Test Day: 0
Species: *Chironomus*

Sediment porewaters

Analyst:	RSC
Date analysed:	3/16/2010

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

Result

	Sample description	Dilution factor	OD655	NH_3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	-----		
	1.0 mg/L NH_3-N Std.	-----	0.129	1.00		
	3.0 mg/L NH_3-N Std.	-----	0.301	3.00		
	6.0 mg/L NH_3-N Std.	-----	0.608	6.00		
	10.0 mg/L NH_3-N Std.	-----	1.080	10.00		
	3.0 mg/L spike	-----	0.326	3.07		
	3.0 mg/L spike dupl.	-----	0.330	3.11		
	5.0 mg/L 2nd source		0.542	5.11		
1.	3	5	0.014	0.66		
2.	5	5	0.022	1.04		
3.	9	5	0.028	1.32		
4.	33	5	0.035	1.65		
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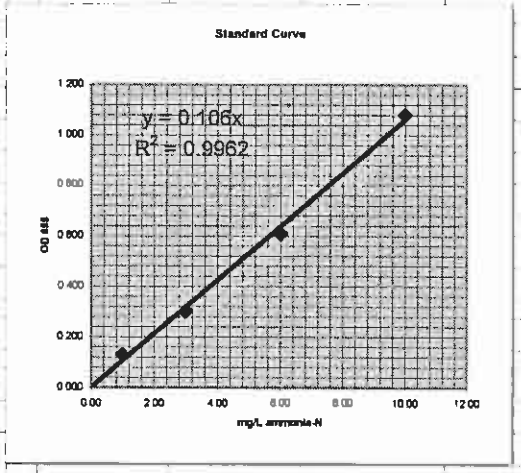

Reporting limit (mg/L) = 0.5

Recovery (%) = 103.0
Precision (RPD) = -1.22
2nd source (%) = 102.1

Sample volume (ml): 0.10
Dilution factor 5

Sample Set Description:
Test No.: 806-2
Test Day: 10
Species: *Chironomus*

Sediment porewaters

Analyst: RSC
Date analysed: 3/26/2010

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

Result

	Sample description	Dilution factor	OD655	NH_3-N (mg/L)	pH	Salinity (ppt)
	Blank	-----	-----	-----		
	1.0 mg/L NH_3-N Std.	-----	.129	1.00		
	3.0 mg/L NH_3-N Std.	——	.301	3.00		
	6.0 mg/L NH_3-N Std.	——	.608	6.00		
	10.0 mg/L NH_3-N Std.	——	1.08	10.00		
	3.0 mg/L spike	-----	.326			
	3.0 mg/L spike dupl.	-----	.330			
	5.0 mg/L 2nd source		.542			
1.	3	5	.014		6.6	
2.	5	5	.022		6.5	
3.	9	5	.028		6.4	
4.	33	5	.035		6.7	
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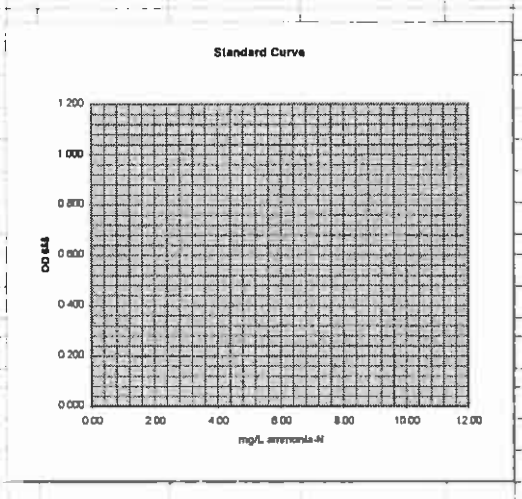
36						



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.: 806-2
Test Day: 10
Species: *Chironomus*

Sediment porewaters

Analyst: RSC
Date analysed: 3/26/2010

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

Result

	Sample description	Dilution factor	OD655	NH_3-N (mg/L)
	Blank	-----	-----	-----
	1.0 mg/L NH_3-N Std.	-----	0.129	1.00
	3.0 mg/L NH_3-N Std.	-----	0.301	3.00
	6.0 mg/L NH_3-N Std.	-----	0.608	6.00
	10.0 mg/L NH_3-N Std.	-----	1.080	10.00
	3.0 mg/L spike	-----	0.326	3.07
	3.0 mg/L spike dupl.	-----	0.330	3.11
	5.0 mg/L 2nd source	-----	0.542	5.11
1.	Day 0 (3-16-10)	######		
2.	3	1	0.004	ND
3.	5	1	0.013	0.12
4.	9	1	0.005	ND
5.	33	1	0.016	0.15
6.	Day 10 (3-26-10)	######		
7.	3	1	0.021	0.20
8.	5	1	0.012	0.11
9.	9	1	0.021	0.20
10.	33	1	0.030	0.28
11.				
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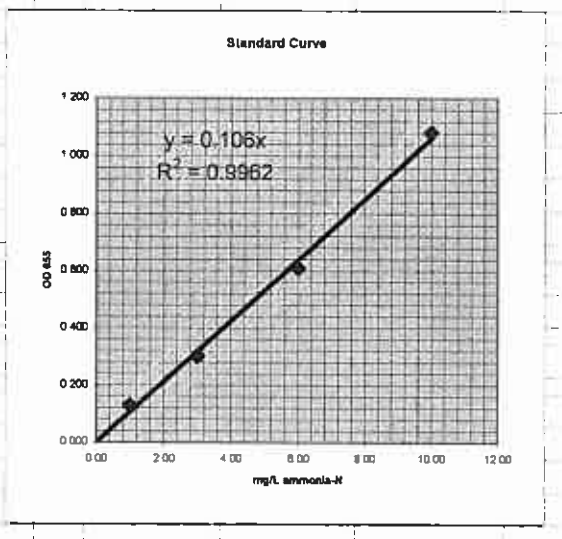

Reporting limit (mg/L) =	0.1
Recovery (%) =	103.0
Precision (RPD) =	-1.22
2nd source (%) =	102.1
Sample volume (ml):	0.50
Dilution factor	1

Sample Set Description:

Test No.: 806-2
Test Day: Days 0 & 10
Species: *Chironomus*

Overlying water

Analyst: RSC RSC
Date analysed: 3/26/2010

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

Result

	Sample description	Dilution factor	OD655	NH_3-N (mg/L)
	Blank	----	-----	-----
	1.0 mg/L NH_3-N Std.	----	.129	1.00
	3.0 mg/L NH_3-N Std.	----	.301	3.00
	6.0 mg/L NH_3-N Std.	-----	.608	6.00
	10.0 mg/L NH_3-N Std.	-----	1.08	10.00
	3.0 mg/L spike	-----	.326	
	3.0 mg/L spike dupl.	----	.330	
	5.0 mg/L 2nd source	-----	.542	
1.	Day 0 (3-16-10)	######		
2.	3	1	.004	
3.	5	1	.013	
4.	9	1	.005	
5.	33	1	.016	
6.	Day 10 (3-26-10)	######		
7.	3	1	.021	
8.	5	1	.012	
9.	9	1	.021	
10.	33	1	.030	
11.				
12.				
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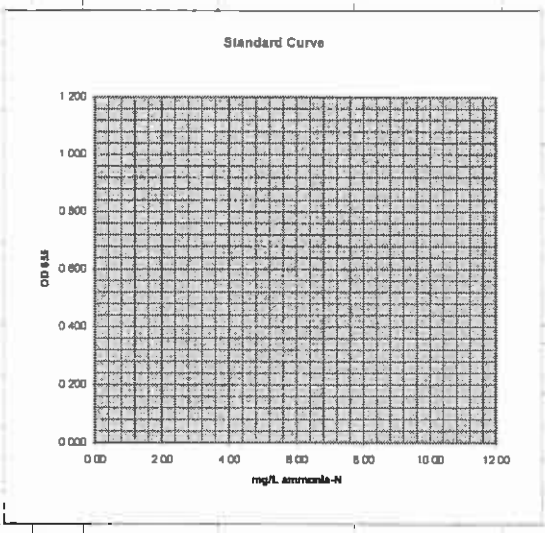


Reporting limit (mg/L) =	0.1
Recovery (%) =	#VALUE!
Precision (RPD) =	#VALUE!
2nd source (%) =	#VALUE!
Sample volume (ml):	0.50
Dilution factor	1

Sample Set Description:

Test No.: 806-2
Test Day: Days 0 & 10
Species: *Chironomus*

Overlying water

Analyst: RSC
Date analysed: 3/26/2010

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 3, 2010 Page 1 of 1

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

Sampler's Signature: Erin Carroll

Analysis Requested

Sample I.D.	Date	Time	LAB I.D.	Sample Matrix	Number of Containers[1]	Bioassay														REMARKS
DPSC-G088	2/24/2010			Sediment	3	X			NAS Ha		31336									Bioassay
DPSC-G096	2/25/2010			Sediment	2	X					31356									Bioassay
DPSC-G095	2/22/2010			Sediment	3	X					31346									Bioassay

Relinquished By	Received By
Erin Carroll	Julie Fiore
Signature: Erin Carroll	Signature: JULIE FIORE
Printed Name: GSI Water Solutions	Printed Name: NAS
Firm: 3-3-10 10:30	Firm: 3-4-10 1010
Date/Time	Date/Time

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

TURNAROUND REQUIREMENTS

___ 24 hr ___ 48 hr ___ 5 day

x Standard (10 working days)

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

Email Reports to: ecarroll@gsiwatersolutions.com, kparrett@gsiwatersolutions.com, rick.ernst@hartcrowser.com

REPORT REQUIREMENTS

___ I Routine Report

X II Report (includes DUP, MS MSD, as required, may be charged as samples)

___ III Data Validation Report (includes All Raw Data)

___ RWQCB (MDLs/PQLs/TRACE)

INVOICE INFORMATION

PO # ___

Bill to: Attn: Rick Ernst RG, Hart Crowser, Inc, 8910 SW Gemini Drive, Beaverton, OR 97008-7123

SAMPLE RECEIPT

Shipping VIA ___
Shipping # ___
Condition ___
Lab No ___

Special Instructions/Comments:

Per Final Sampling and Analysis Plan (February 2010) - outlined below

Custody Seal

Date 3-3-2010 Project DPSC - Phase II

Signature Erin Cassell Container# 1 of 1

Custody Seal

Date 3-3-2010 Project DPSC - Phase II

Signature Erin Cassell Container# 1 of 1

Custody Seal

Date 3-3-2010 Project DPSC Phase II

Signature Erin Cassell Container# 1 of 1

Receipt Temp = 1°C

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. 999-2709 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. GABritter GB 2. Lidia F. Sandoval LFS

3. M.S. Redmond MSR 4.

Study Schedule:

Test Beginning: 3-16-10 1040 Test Ending: 3-20-10 0910

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: 3-9-10

Water Quality: Cond. (umhos/cm): 275 Salinity (ppt) [redacted] pH 7.7

Hardness (mg/L as $CaCO_3$): 103 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	ϕ	10	5	1.25	2.5	20	1.25	10
20	ϕ	10	2.5	ϕ	2.5	20	1.25	ϕ	2.5
ϕ	2.5	20	5	20	5	10	ϕ	5	1.25
1.25	1.25	5	ϕ	2.5	20	1.25	5	20	5
10	10	1.25	1.25	1.25	10	ϕ	2.5	10	20
5	5	2.5	20	10	ϕ	5	10	2.5	ϕ

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. 999-2709 Client QC Test Investigator _____

TEST ORGANISMS

Species: *Chironomus dilutus* Age: 3rd instar

Source: NAS cultures Date received: _____

Acclimation Data:

Date	Temp. (deg.C)	DO (mg/L)	pH	Cond. umhos/cm	Hardness (mg/L)	Alkalinity (mg/L)	Feeding	Water changes
3-3-10	20.7	8.4	7.1	153	43	30	Animals fed Tetra Fin	
3-5-10	21.2	8.5	7.0	151	–	–	and *Selenastrum*	
3-8-10	19.9	8.7	7.2	148	–	–	Details recorded on	
3-10-10	17.7	8.6	6.5	130	51	30	Chironomid culture	
3-12-10	22.0	8.3	7.3	148	–	–	data sheets	
3-15-10	22.3	8.7	7.4	149	–	–		
Mean	20.96	8.5	7.1	147	47	30		
S.D.	1.7	0.2	0.3	8	–	–		
(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.

3-16-10
6 x 2

Test concentration (g/L)	KCl working stock (ml/200ml)	ml of dilution water per 200 ml
20	200	0
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. 999-2709 Client QC Test

DAILY RECORD SHEET

Day 0 (3 /16 /10) GJL Temp Beaker (°C):

Conc. (g/L)	Temp. (deg.C)	DO (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20	22.6	8.9	14380	7.9	86	70	
2. 10	22.7	8.9	11680	79			
3. 5	22.8	8.8	7310	7.9			
4. 2.5	22.8	8.8	3960	7.9			
5. 1.25	22.8	8.7	2150	7.8			
6. 0	23.0	8.7	260	7.6	86	70	
All animals fed 0.25 ml Tetra Fin suspension. Initials: GJL							

Day 1 (3 /17 /10) GB Temp Beaker (°C): 23.3

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

Day 2 (3 /18 /10) GB Temp Beaker (°C): 22.8

Conc. (g/L)	Temp. (deg.C)	DO (ppm)	Cond. (umhos/cm)	pH	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: GB							

Day 3 (3 /19 /10) GJL Temp Beaker (°C): 22.7

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

Day 4 (3 /20 /10) GJL Temp Beaker (°C): 23.2

Conc. (g/L)	Temp. (deg.C)	DO (ppm)	Cond. (umhos/cm)	pH	Hardness (mg/L)	Alkalinity (mg/L)	Comments
1. 20	—	—	—	—	—	—	
2. 10	—	—	—	—			
3. 5	23.5	7.4	7480	7.6			
4. 2.5	23.5	7.7	4240	7.6			
5. 1.25	23.6	7.6	2280	7.4			
6. 0	23.6	7.6	291	7.2	86	80	
Mean	23.1	7.3		7.7	86	73	
SD	0.4	0.6		0.2	Ø	6	
n	10	10		10	3	3	

Revised 12-5-01

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-2709 Client QC Test Investigator _____

DAILY RECORD SHEET - Survivors

Day 0 (3 /16 /10) 6SJ

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 (3 /17/10) 6SJ

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 2 (3 /18/10) 6SJ

Conc. (g/L)	Survivors in Replicate: 1	2	3	4	5	6	7	8	9	10	Total
1. 20	1	0	1	1	0	0	0	0	1	1	5
2. 10	0	1	0	1	1	1	1	0	1	1	7
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 3 (3 /19/10) 6SJ

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	∅ (5D)
2. 10	0	0	0	0	0	0	0	0	0	0	∅ (7D)
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 4 (3 /20/10) 6SJ

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	∅
2. 10	0	0	0	0	0	0	0	0	0	0	∅
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	0	1	9 (1D)
6. 0	1	1	1	1	1	1	1	1	1	1	10

Revised 12-5-01

Acute 96-hr Toxicity Test-96 Hr Survival

Start Date:	3/16/2010 10:40	Test ID:	999-2709	Sample ID:	REF-Ref Toxicant
End Date:	3/20/2010 09:10	Lab ID:	ORNAS-Northwestern Aquati	Sample Type:	KCL-Potassium chloride
Sample Date:		Protocol:	EPAF 91-EPA Freshwater	Test Species:	CT-Chironomus dilutus
Comments:					

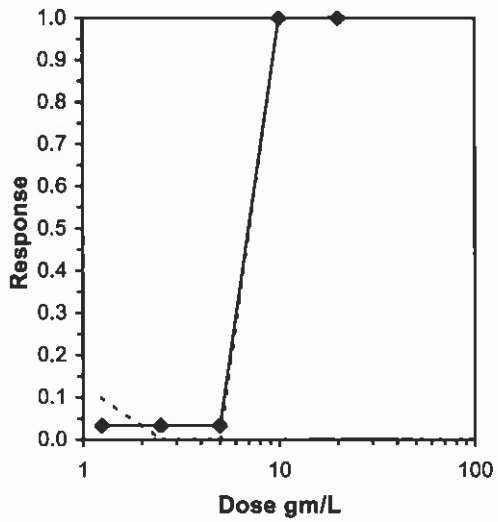
Conc-gm/L	1
D-Control	1.0000
1.25	0.9000
2.5	1.0000
5	1.0000
10	0.0000
20	0.0000

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	1.0000	1.0000	0	10	10	1	1.0000	0.0500	0	10
5	1.0000	1.0000	0	10	10	1	1.0000	0.0500	0	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	5	10	7.07107	

Trimmed Spearman-Karber

Trim Level	EC50	95% CL	
0.0%			
5.0%	6.9871	6.6990	7.2875
10.0%	6.9871	6.6990	7.2875
20.0%	6.9871	6.6990	7.2875
Auto-3.3%	6.9871	6.6990	7.2875



ToxCalc v5.0.23

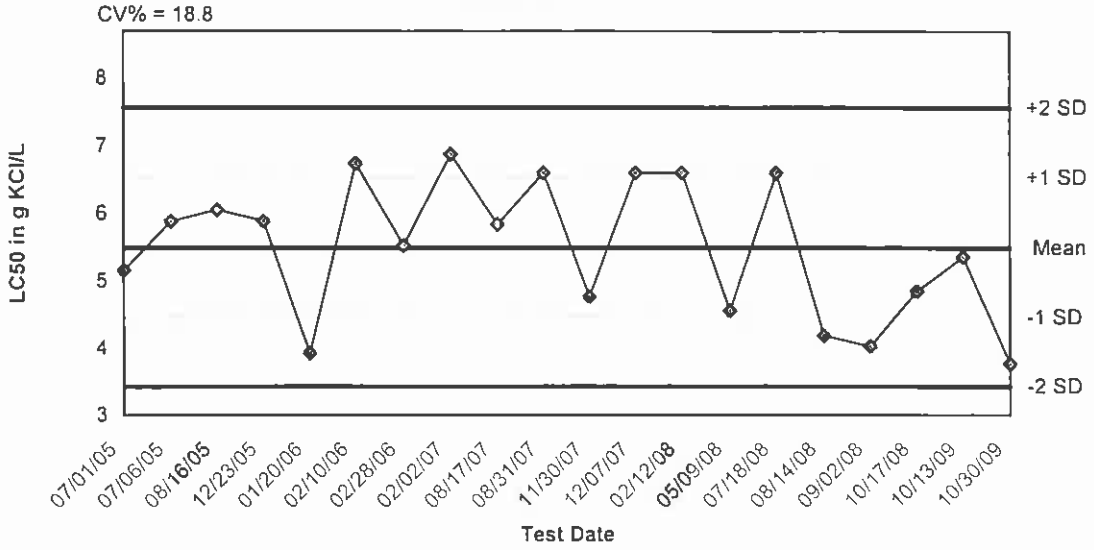
Reviewed by:_____

Test: AT-Acute 96-hr Toxicity Test	Test ID: 999-2709
Species: CT-Chironomus dilutus	Protocol: EPAF 91-EPA Freshwater
Sample ID: REF-Ref Toxicant	Sample Type: KCL-Potassium chloride
Start Date: 3/16/2010 10:40 End Date: 3/20/2010 09:1	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	10	10	10	
	2	1	1.250	10	10	10	10	9	
	3	1	2.500	10	10	10	10	10	
	4	1	5.000	10	10	10	10	10	
	5	1	10.000	10	10	7	0	0	
	6	1	20.000	10	10	5	0	0	

Comments:

Midge larvae, Chironomus dilutus, acute reference toxicant test - 3RD INSTAR



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
07/01/05	5.1600	5.4925	4.4582	3.4240	6.5268	7.5610
07/06/05	5.8900	5.4925	4.4582	3.4240	6.5268	7.5610
08/16/05	6.0600	5.4925	4.4582	3.4240	6.5268	7.5610
12/23/05	5.8900	5.4925	4.4582	3.4240	6.5268	7.5610
01/20/06	3.9300	5.4925	4.4582	3.4240	6.5268	7.5610
02/10/06	6.7400	5.4925	4.4582	3.4240	6.5268	7.5610
02/28/06	5.5300	5.4925	4.4582	3.4240	6.5268	7.5610
02/02/07	6.8800	5.4925	4.4582	3.4240	6.5268	7.5610
08/17/07	5.8400	5.4925	4.4582	3.4240	6.5268	7.5610
08/31/07	6.6000	5.4925	4.4582	3.4240	6.5268	7.5610
11/30/07	4.7700	5.4925	4.4582	3.4240	6.5268	7.5610
12/07/07	6.6000	5.4925	4.4582	3.4240	6.5268	7.5610
02/12/08	6.6000	5.4925	4.4582	3.4240	6.5268	7.5610
05/09/08	4.5600	5.4925	4.4582	3.4240	6.5268	7.5610
07/18/08	6.6000	5.4925	4.4582	3.4240	6.5268	7.5610
08/14/08	4.1900	5.4925	4.4582	3.4240	6.5268	7.5610
09/02/08	4.0300	5.4925	4.4582	3.4240	6.5268	7.5610
10/17/08	4.8500	5.4925	4.4582	3.4240	6.5268	7.5610
10/13/09	5.3600	5.4925	4.4582	3.4240	6.5268	7.5610
10/30/09	3.7700	5.4925	4.4582	3.4240	6.5268	7.5610

MSL
12-4-09