# REPORT

# of

# TEST NO. 862-1 & 3

## Toxicity of Freshwater Sediments Using Sediment Bioassays as Part of the Remedial Investigation at the Jacobson Terminals in Seattle, Washington

**Submitted to**

**Hart Crowser, Inc.**
**1700 Westlake Ave. North, Suite 200**
**Seattle, WA 98109**

**Submitted by**

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

**March 19, 2015**

## EXECUTIVE SUMMARY OF SEDIMENT BIOASSAYS

Two freshwater sediment bioassays, a 28-day *Hyalella* amphipod survival and growth test and a 10-day *Chironomus dilutus* midge survival and growth test were conducted for Hart Crowser as part of the Remedial Investigation at the Jacobson Terminals property in Seattle, WA. Three test sediments were compared to the control sediment to assess sediment toxicity and to interpret organism response under the Washington State *Sediment Management Standards* (Chapter 173-204 WAC, Last Update: 2/25/13). The test and control sediments tested are listed in Table 1.

### TEST AND CONTROL SEDIMENT INFORMATION

The test sediments were provided to Northwestern Aquatic Sciences by Hart Crowser personnel. The negative control sediment was collected by NAS personnel from an area approximately one mile east of the Highway 101 bridge at Beaver Creek, approximately 8 miles south of Newport, Oregon. All sediments were stored at 4°C in the dark until used. Sample identification and collection information is as follows:

**Table 1. Sample identification and collection dates.**

| Sample description | Hart Crowser Sample Identification | NAS Sample Identification | Collection Date | Receipt Date |
|---|---|---|---|---|
| Beaver Creek Control* | Control | 5195G | 1-19-15 | 1-19-15 |
| Test sediment | JT-SS-06 | 5192G | 1-12-15 | 1-14-15 |
| Test sediment | JT-SS-08 | 5193G | 1-12-15 | 1-14-15 |
| Test sediment | JT-SS-10 | 5194G | 1-12-15 | 1-14-15 |

*Control used for data interpretation.

### BIOASSAY INTERPRETATION CRITERIA

Biological test interpretation for freshwater sediments, as presented in the *Sediment Management Standards*, (SMS) uses biological criteria to identify sediments that have no adverse effects on biological resources, and correspond to no significant health risk to humans. The SMS includes Sediment Cleanup Objectives (SCO) and Cleanup Screening Levels (CSL) biological criteria. The Sediment Cleanup Objectives establish a no adverse effects level, including no acute or chronic adverse effect, to the benthic community. The Cleanup Screening Levels establish a minor adverse effects level, including acute or chronic effects, to the benthic community. The SCO biological criteria are exceeded when one of the biological tests results is above the SCO for that bioassay endpoint. The CSL biological criteria is exceeded when 1) any two bioassay test results are above the SCO criteria; or 2) when one of the bioassay test results is above the CSL criteria.

#### Sediment Cleanup Objectives Criteria

When any one of the biological test results shows a test sediment response that exceeds the bioassay-specific response guidelines presented below, and that response is statistically different ($p \leq 0.05$) from the control, the test sediment is judged to have exceeded the SCO.

In accordance with the SMS, the bioassay-specific response guidelines for evaluating an exceedance under the SCO criteria are as follows:

**Amphipod 28-day Survival/Growth Bioassay.** Mean mortality in the test sediment is greater than 10 percent over the mean control and statistically different from the control ($p \leq 0.05$). For the growth endpoint, a mean reduction in the biomass that is greater than 25 percent of the control sediment response and statistically different from the control ($p \leq 0.05$).

**Midge 10-Day Survival/Growth Bioassay.** Mean mortality in the test sediment is 20 percent over the control mortality and statistically different from control ($p \leq 0.05$). For the growth endpoint, a mean reduction in biomass that is greater than 20 percent compared to the control and statistically different from the control ($p \leq 0.05$).

**Cleanup Screening Levels Criteria**

When any two biological test results show test sediment responses that are above the SCO listed above, OR when any one of the biological test results is above the Cleanup Screening Level criteria listed below, that sediment is judged to have exceeded the CSL.

In accordance with the SMS, the bioassay-specific response guidelines for evaluating an exceedance under the CSL criteria are as follows:

**Amphipod 28-day Survival/Growth Bioassay.** Mean mortality in the test sediment is greater than 25 percent over the mean control and statistically different from the control ($p \leq 0.05$). For the growth endpoint, a mean reduction in the biomass that is greater than 40 percent from the control and statistically different from the control ($p \leq 0.05$).

**Midge 10-Day Survival/Growth Bioassay.** Mean mortality in the test sediment is 30 percent over the control mortality and statistically different from the control ($p \leq 0.05$). For the growth endpoint, a mean reduction in biomass that is greater than 30 percent from the control sediment response and statistically different from the control ($p \leq 0.05$).

## RESULTS OF AMPHIPOD, *HYALELLA AZTECA* 28-DAY SURVIVAL TEST (862-1)

All water quality observations were within the protocol specified ranges (Table 1, Section A). The test met applicable test acceptability criteria. Although the reference toxicant (positive control) LC50 result was slightly outside the laboratory's control chart action limits (0.50 g/L; control chart mean ± 2 S.D. = 0.36 ± 0.12), a review of test conditions and procedures did not detect any unusual circumstances. (Section A).

The control sediment exhibited a mean mortality of 5.0% (Table 2). Mean mortality of test sediments JT-SS-06, JT-SS-08 and JT-SS-10 was 22.5%, 20.0% and 13.8%, respectively. None of these sediments exceeded the criteria for mortality for SCO or CSL. The biomass of test sediments JT-SS-06 and JT-SS-08 were both above that of the control sediment (Table 3). JT-SS-10 did result in an individual biomass of 0.28 mg that was statistically significantly lower than that of the control. However, JT-SS-10 did not exceed either the SCO or CSL criteria for growth.

## RESULTS OF MIDGE, *CHIRONOMUS DILUTUS*, 10-DAY SURVIVAL AND GROWTH TEST (862-3)

All water quality observations were within the protocol specified ranges (Table 1, Section B). The test met all other applicable acceptability criteria including positive control performance (Section B).

The mean mortality in the control sediment in the *Chironomid* test was 6.3% (Table 4). Mean mortality of test sediments JT-SS-06, JT-SS-08 and JT-SS-10 was 10.0%, 15.0% and 8.8%, respectively. None of these were more than 20% over the control mortality. Therefore none of the sediments exceeded the SCO or CSL criteria for mortality. The control average individual ash-free dry weight was 1.22 mg (Table 5). All three test sediments resulted in growth that was statistically significantly lower than control growth. Two of the three test sediments, JT-SS-08 and JT-SS-10, resulted in exceedances under the SCO guidelines for growth with ash-free dry weights of 0.93 and 0.91 mg/individual, respectively. No test sediment failed the CSL criteria for growth.

## SUMMARY OF SEDIMENT BIOASSAY RESULTS

The Sediment Management Standards includes Sediment Cleanup Objectives (SCO) and Cleanup Screening Levels (CSL) biological criteria to identify sediments that have adverse effects on biological resources. The SCO establishes a no adverse effects level, including no acute or chronic adverse effect, to the benthic community. The CSL establishes a minor adverse effects level, including acute or chronic effects, to the benthic community.

The SCO biological criteria are exceeded when one of the biological tests results is above the SCO for that bioassay endpoint. Two test sediments, JT-SS-08, and JT-SS-10, resulted in an exceedance under the SCO guidelines for growth in the *Chironomus* bioassay.

The CSL biological criteria is exceeded when 1) any two bioassay test results are above the SCO criteria; or 2) when one of the bioassay test results is above the CSL criteria. None of the test sediments exceeded the CSL criteria by resulting in either one test result that was a CSL exceedance or two test results that were SCO exceedances (Table 6).

**STUDY APPROVAL**

for Linda Neuth
Assistant Laboratory Director Date 3-19-15

Project Manager 3-19-15
Date

Table 2. Mortality results of *Hyalella* 28-day toxicity test and data interpretation using guidelines from the Washington State SMS (2013).

| Sample description | Percent mortality (Mean ± SD) | Significantly higher than control sediment at $\alpha$=0.05? | Percent higher (absolute) than control sediment | Exceedance under SCO? [1] | Exceedance under one-test criteria for CSL? [2] |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 5.0 ± 5.3 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 22.5 ± 21.9 | No | 17.5 | No | No |
| JT-SS-08 (NAS# 5193G) | 20.0 ± 20.7 | No | 15.0 | No | No |
| JT-SS-10 (NAS# 5194G) | 13.8 ± 16.0 | No | 8.8 | No | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >10%.
[2] Cleanup Screening Levels (CSL) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >25%.

Table 3. Growth results of *Hyalella* 28-day toxicity test and data interpretation using guidelines from the Washington State SMS (2013).

| Sample description | Average dry wt/amphipod (Mean ± SD) | Significantly lower than control sediment at $\alpha$=0.05? | Percent lower (absolute) than control sediment | Exceedance under SCO? [1] ($MIG_C$-$MIG_T$)/$MIG_C$>0.25 and SD | Exceedance under one-test criteria for CSL? [2] ($MIG_C$-$MIG_T$)/$MIG_C$>0.40 and SD |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 0.32 ± 0.03 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 0.41 ± 0.08 | No | -28.0 | No | No |
| JT-SS-08 (NAS# 5193G) | 0.37 ± 0.08 | No | -15.6 | No | No |
| JT-SS-10 (NAS# 5194G) | 0.28 ± 0.04 | Yes | 12.5 | No | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean growth is significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth and the difference is >25%.
[2] Cleanup Screening Levels (CSL) exceedance (one-test criteria) if the test sediment mean growth is significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth and the difference is >40%.

Table 4. Mortality results of *Chironomus* toxicity test and data interpretation using guidelines from the Washington State SMS (2013).

| Sample description | Percent mortality (Mean ± SD) | Significantly higher than control sediment at $\alpha$=0.05? | Percent higher (absolute) than control sediment | Exceedance under SCO? [1] | Exceedance under one-test criteria for CSL? [2] |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 6.3 ± 5.2 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 10.0 ± 14.1 | No | 3.7 | No | No |
| JT-SS-08 (NAS# 5193G) | 15.0 ± 16.9 | No | 8.7 | No | No |
| JT-SS-10 (NAS# 5194G) | 8.8 ± 11.3 | No | 2.5 | No | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >20%.
[2] Cleanup Screening Levels (CSL) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >30%.

Table 5. Growth results of *Chironomus* toxicity test and data interpretation using guidelines from the Washington State SMS (2013).

| Sample description | Average ash-free dry wt/midge (mg)* (Mean ± SD) | Statistically significantly lower than control sediment at $\alpha$=0.05? | Percent lower than control sediment | Exceedance under SCO?[1] ($MIG_C$-$MIG_T$/$MIG_C$>0.20) | Exceedance under one-test criteria for CSL?[2] ($MIG_C$-$MIG_T$/$MIG_C$>0.30) |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 1.22 ± 0.09 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 0.98 ± 0.11 | Yes | 19.7 | No | No |
| JT-SS-08 (NAS# 5193G) | 0.93 ± 0.15 | Yes | 23.8 | **Yes** | No |
| JT-SS-10 (NAS# 5194G) | 0.91 ± 0.12 | Yes | 25.4 | **Yes** | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean growth is significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth and the difference is >20%.
[2] Cleanup Screening Level (CSL) exceedance (one-test criteria) if the test sediment mean growth is significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth and the difference is >30%.

Table 6. Interpretation of bioassay test results for Jacobson Terminals Property based on Washington State Sediment Management Standards - Sediment Cleanup Objectives (SCO) and Cleanup Screening Level (CSL) criteria.

| Sample Description | SCO Exceedance / CSL Exceedance[1] | | | | CSL Exceedance by two SCO Exceedances[2] |
|---|---|---|---|---|---|
| | Test No. 862-1 *Hyalella* 28-day Survival | Test No. 862-1 *Hyalella* 28-day Growth | Test No. 862-3 *Chironomus* 10-day Survival | Test No. 862-3 *Chironomus* 10-day Growth | |
| | SCO / CSL | SCO / CSL | SCO / CSL | SCO / CSL | CSL |
| Control (NAS# 5195G) | --- | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | No / No | No / No | No / No | No / No | No |
| JT-SS-08 (NAS# 5193G) | No / No | No / No | No / No | **Yes** / No | No |
| JT-SS-10 (NAS# 5194G) | No / No | No / No | No / No | **Yes** / No | No |

[1]One test result must exceed an SCO or a CSO criterion. Two test sediments exceeded the SCO criterion for *Chironomus* growth. No other single criterion exceedances occurred under either SCO or CSO criteria.

[2]Two test results must exceed the SCO criteria to exceed the CSL under this interpretation. No test sediments exceeded the CSL by having two SCO exceedances.

# SECTION A

## Amphipod (*Hyalella azteca*) 28-day sediment bioassay 862-1 data report

# TOXICITY TEST REPORT

## TEST IDENTIFICATION

Test No.: 862-1

Title: Toxicity of freshwater sediments using a 28-day amphipod, *Hyalella azteca*, sediment bioassay as part of the Remedial Investigation at the Jacobson Terminals Property in Seattle, WA.

Protocol No.: NAS-XXX-HA4c, February 11, 2000. Revision 3 (4-26-05). 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 100.1 (Methods for measuring the toxicity and bioaccumulation of sediment-associated contaminants with freshwater invertebrates, EPA/600/R-99/064). Washington State Sediment Management Standards (SMS) (Chapter 173-204 WAC, Last Update: 2/25/13).

## STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 1700 Westlake Ave. North, Suite 200, Seattle, WA 98109.

Sponsor's Study Monitor: Mr. Philip Cordell

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. Mngr./ Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, Ph.D., Sr. Aq. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; J.B. Brown, B.S., D.V.M, Assoc. Aq. Toxicol.; Y. Nakahama, Sr. Tech.; L. Brady, Tech.

Study Schedule:

Test Beginning: 1-23-15, 0905 hrs.

Test Ending: 2-20-15, 1000 hrs.

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

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 of the Remedial Investigation at the Jacobson Terminals Property in Seattle, WA. Details are as follows:

| NAS Sample No. | 5192G | 5193G | 5194G |
|---|---|---|---|
| Description | JT-SS-06 | JT-SS-08 | JT-SS-10 |
| Collection Date | 1/12/15 | 1/12/15 | 1/12/15 |
| Receipt Date | 1/14/15 | 1/14/15 | 1/14/15 |

Control Sediment: The negative control sediment (NAS#5195G) was collected on 1-19-15 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.

Dates of Preparation: 1-19-15 and 2-6-15

Water Quality:

pH: 7.7, 7.5

conductivity: 108, 119 µmhos/cm

hardness: 26, 26 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: *Hyalella azteca,* amphipod.
Age/Size: 7-8 days old
Source: Chesapeake Cultures, Hayes, VA; received 1-21-15
Acclimation: Holding conditions for the three days prior to testing averaged: Temperature, 19.5 ± 1.7 °C; dissolved oxygen, 10.8 ± 3.7 mg/L; pH, 7.2 ± 0.4; conductivity, 358 ± 207 µmhos/cm; hardness, 111 ± 67 mg/L as $CaCO_3$; and alkalinity, 103 ± 67 mg/L as $CaCO_3$. Photoperiod, 16:8, L:D. Half of the water was replaced daily with dechlorinated municipal tap water during holding. Animals were fed YTC daily during holding.

## 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
Organisms/Treatment: 80
Water Volume Changes: 2 water volumes per day
Aeration: None.
Feeding: Animals are fed 1.0 ml of YTC suspension per beaker daily.
Acceptance Criteria: Results are valid if mean control mortality does not exceed 20%, and the mean individual dry weight at test termination is ≥ 0.15 mg.
Effects Criteria: 1) survival after 28 days, and 2) average individual dry weight after 28 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 28 of the test. Temperature was measured daily, pH and dissolved oxygen three times per week, and conductivity weekly, in the overlying water of one replicate test container per treatment. Hardness and alkalinity were measured with titrimetric methods. 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

Percent survival, percent mortality and average individual dry weight were calculated for each replicate as follows:

percent survival = 100 x (number surviving/initial number tested)
percent mortality = 100 x (number dead/initial number tested)

average individual dry weight = (final wt. - tare wt.)/number weighed,
where:
final wt. = tare wt. + dry weight of organisms recovered on day 28, in mg

Means and standard deviations for the biological endpoints described above, and for water quality data, were computed using Microsoft Excel 2010. Mean individual dry weight and mean percent mortality in each test sediment were statistically compared to the control sediment. Where appropriate, an arcsine square root transformation was performed on percent mortality data before analysis. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Mann-Whitney or Approximate T test was conducted at the 0.05 level of significance. The statistical software used was BioStat (version Feb 9, 2006 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District.

## 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.: 999-3381
Reference Toxicant and Source: Potassium Chloride (KCl), Fisher Lot #114689.
Test Date: 1-23-15.
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 0.50 g/L. This result is slightly outside the laboratory's control chart action limits (0.25 – 0.48 g/L). A review of test organisms and test conditions and procedures indicated that there were no unusual circumstances that may have affected the test results.

## 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. The means and standard deviations of percent mortality of *Hyalella* exposed for 28 days to sediments are summarized in Table 2. The means and standard deviations of average individual dry weight of *Hyalella* exposed for 28 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 0.9 mg/L for all day 0 and day 28 measurements.

The test met the acceptability criteria specified in the SMS with 5.0 % mean control mortality (≤20% required) and a final control sediment growth of 0.32 mg/individual (≥ 0.15 mg required). The reference toxicant (positive control) LC50 result was slightly outside the laboratory's control chart action limits (0.50 g/L; control chart mean ± 2 S.D. = 0.36 ± 0.12). A review of test conditions and procedures did not detect any unusual circumstances.

Interpretation was based on guidelines from the Washington State Sediment Management Standards (Chapter 173-204 WAC, Last Update: 2/25/13). The SMS includes Sediment Cleanup Objectives (SCO) and Cleanup Screening Levels (CSL) biological criteria. The Sediment Cleanup Objectives establish a no adverse effects level, including no acute or chronic adverse effect, to the benthic community. The Cleanup Screening Levels establish a minor adverse effects level, including acute or chronic effects, to the benthic community. To qualify as an adverse effect under the SCO for mortality the mean mortality in the test sediment is greater than 10 percent over the mean control and statistically different from the reference ($p = 0.05$). For the growth endpoint, a mean reduction in the biomass that is greater than 25 percent of the control sediment response and statistically different from the control ($p=0.05$). For the CSL adverse effects criteria mean mortality in the test sediment is greater than 25 percent over the mean control and statistically different from the control ($p = 0.05$). For the growth endpoint, a mean reduction in the biomass that is greater than 40 percent from the control and statistically different from the control ($p = 0.05$).

Although sediment JT-SS-10 resulted in mean dry weight that was statistically significantly lower than that of the control, the mean dry weight did not exceed either the SCO or CSL criteria. None of the test sediments resulted in an exceedance under either the SCO or the CSL of the SMS guidelines.

**STUDY APPROVAL**

Project Manager/Study Director — Date: 3-19-15

Quality Assurance Unit — Date: 3-19-15

Assistant Laboratory Director — Date: 3-19-15

Table 1. Summary of water quality conditions during tests of the amphipod, *Hyalella azteca*, exposed to freshwater sediments.

| Water Quality Parameter | Mean ± S.D. | Minimum | Maximum | N |
|---|---|---|---|---|
| Temperature (°C) | 22.4 ± 0.4 | 22.0 | 23.9 | 116 |
| Dissolved oxygen (mg/L) | 7.0 ± 0.5 | 6.1 | 8.5 | 52 |
| Conductivity (µmhos/cm) | 123 ± 22 | 110 | 219 | 24 |
| pH | 7.2 ± 0.2 | 6.6 | 8.0 | 52 |
| Hardness (mg/L as $CaCO_3$) | 32 ± 4 | 26 | 34 | 8 |
| Alkalinity (mg/L as $CaCO_3$) | 35 ± 5 | 30 | 40 | 8 |
| Total ammonia (mg/L) | --- | <0.1 | 0.9 | 8 |

Table 2. Mortality results of *Hyalella* 28-day toxicity test and data interpretation using guidelines from the Washington State SMS (2013).

| Sample description | Percent mortality (Mean ± SD) | Significantly higher than the control sediment at α=0.05? | Percent higher (absolute) than control sediment | Exceedance under SCO?[1] | Exceedance under one-test criteria for CSL[2] |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 5.0 ± 5.3 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 22.5 ± 21.9 | No | 17.5 | No | No |
| JT-SS-08 (NAS# 5193G) | 20.0 ± 20.7 | No | 15.0 | No | No |
| JT-SS-10 (NAS# 5194G) | 13.8 ± 16.0 | No | 8.8 | No | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >10%.
[2] Cleanup Screening Levels (CSL) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >25%.

Table 3. Growth results of *Hyalella* 28-day toxicity test and data interpretation using guidelines from the Washington State SMS (2013).

| Sample description | Average Individual Dry Weight (mg) (Mean ± SD) [1] | Statistically significantly lower than control sediment at α=0.05? | Percent lower than control sediment | Exceedance under SCO?[1] ($MIG_C$-$MIG_T$/$MIG_C$>0.25) | Exceedance under one-test criteria for CSL?[2] ($MIG_C$-$MIG_T$/$MIG_C$>0.40) |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 0.32 ± 0.03 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 0.41 ± 0.08 | No | -28.0 | No | No |
| JT-SS-08 (NAS# 5193G) | 0.37 ± 0.08 | No | -15.6 | No | No |
| JT-SS-10 (NAS# 5194G) | 0.28 ± 0.04 | Yes | 12.5 | No | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean growth is significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth, and the difference is >25%.
[2] Cleanup Screening Levels (CSL) exceedance (one-test criteria) if the test sediment mean individual growth in significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth, and the difference is >40%.

# APPENDIX I

# PROTOCOL

# TEST PROTOCOL

## FRESHWATER AMPHIPOD, *HYALELLA AZTECA*, 28-DAY SEDIMENT SURVIVAL AND GROWTH TEST

## 1. INTRODUCTION

1.1 Purpose of Study: The purpose of this study is to characterize the chronic toxicity of freshwater sediments using a 28-day exposure and survival and growth endpoints with the amphipod, *Hyalella azteca*.

1.2 Referenced Method: This protocol is based on ASTM Method E 1706-00 (ASTM 2001) and EPA Method 100.1 (EPA/600/R-99/064)

1.3 Summary of Method: A summary of test conditions for the amphipod 28-day sediment survival and growth test is tabulated below. The test with *Hyalella azteca* is conducted at 23 ± 1°C with a 16L:8D photoperiod at an illuminance of about 100-1000 lux. Test chambers are 300-mL high-form lipless beakers containing 100 mL of sediment and 175 mL of overlying water. Ten 7-8day old amphipods are used in each replicate. The number of replicates/treatment depends on the objective of the test. Eight replicates are recommended for routine testing. Amphipods in each test chamber are fed 1.0 mL of YCT food daily. Each chamber receives two volume additions per day of overlying water. Test endpoints include survival and 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 normally begun within 14 days of sample collection. 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 8 weeks prior to testing, depending on the testing requirements. 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 dechlorinated tap water or moderately hard synthetic water. Synthetic dilution water is prepared from Milli-Q reagent grade water and reagent grade chemicals. Test water may also be well water, surface water, site water, or other water depending on the study design. The hardness or other water quality parameters of the dilution water may need to be adjusted to meet the study design.

## 5. TEST ORGANISMS

5.1 Species: amphipod, *Hyalella azteca*.

5.2 Source: Cultured at NAS. Alternatively, animals may be purchased from a reputable commercial supplier.

5.3 Age: 7-8 days old at start of test

5.4 Acclimation and Pretest Observation: Cultures are maintained at 23 ± 1°C under a 16:8 L:D photoperiod. Cultured amphipods are fed dried maple leaves with YTC. Rabbit chow, Tetramin® or TetraFin-® flakes may also be used. Acclimation of test organisms to the test water may be desirable, depending on culture water, but it is not required. If test organisms are to be acclimated, fifty percent of the holding water is changed daily with the addition of test water.

## 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. 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 100-1000 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 amphipods 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 28 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 amphipods are introduced to the test chambers. Initial water quality measurements are taken prior to the addition of test organisms.

7.3 Effect Criterion: The effect criteria used in the 28-day amphipod bioassay are mortality and growth. Death is defined as the lack of movement of body or appendages on response to tactile stimulation. Growth is measured as change in dry weight.

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

7.5 Beginning the Test: On the day the test begins, amphipods are impartially counted into small containers of test water (10/container). The test is begun by rinsing test organisms into the equilibrated test containers. For the growth endpoint, time-zero weight data should be collected.

7.6 Feeding: Amphipods are fed 1.0 mL of YCT daily per test chamber. 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 toxicity test is 28 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 28 |
| *PHYSICAL AND CHEMICAL DATA* | |
| Hardness, alkalinity, conductivity, and ammonia-N | Beginning and end of test in overlying water of one replicate beaker from each treatment. |
| Temperature | Daily in overlying water of one replicate beaker from each treatment. |
| Conductivity | Weekly |
| Dissolved oxygen and pH | 3X/week |
| Optional pore water ammonia and/or sulfide | In test sediments prior to initiating the tests. Optionally in sediments from sacrificial test chambers at test beginning and/or end. |

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. Total soluble sulfide and total ammonia-N were

measured using Hach test kits based on the methylene blue (EPA Method 376.2) and salicylate (Clin. Chim. Acta 14:403, 1996) colorimetric methods, respectively; samples were not distilled prior to analysis.

Overlying water should be sampled just before water renewal from about 1 to 2 cm above the sediment surface using a pipet. It may be necessay to pool water samples from individual replicates. The pipet should be checked to make sure no organisms are removed during sampling of overlying water.

7.8 Test Termination: At test termination, the contents of each test container are sieved through a #35 (500 µm mesh) sieve to recover the amphipods. Amphipods from each replicate are put into a 30 mL plastic cup, rinsed with DI water, gently blotted and place into the appropriate tared aluminum weighing pan. The number of survivors for each container is recorded on the datasheet.

7.9 Growth Measurement: Growth is measured as average dry weight of animals in a test replicate at the end of the test on day 28. Pooled animals from each test replicate are gently blotted and placed into tared aluminum weigh pans. The pans are dried at 60-90°C to constant weight. The dried amphipods 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 amphipods in each pan is divided by the number of amphipods weighed to obtain an average dry weight per surviving amphipod per replicate.

## 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 80%.

## 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 amphipod 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 28-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 11, Test Method 100.1, *Hyalella azteca* 10-d Survival and Growth Test for Sediments, pp. 47-54 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 | 28 days |
| 3. Temperature | 23 ± 1°C |
| 4. Light quality | daylight fluorescent 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 | 7-8 days old at test initiation |
| 12. Organisms per test chamber | 10 |
| 13. Replicates per treatment | 8 recommended for routine testing (depends on design) |
| 14. Organisms per treatment | 80 |
| 15. Feeding regime | YCT food, fed 1.0 mL daily/chamber |
| 16. Cleaning | if screens are used, clean as needed |
| 17. Aeration | None, unless DO falls below 2.5 mg/L |
| 18. Overlying (test) water | Dechlorinated tap water, culture water, well water, surface water, site water or reconstituted water, depending on study design. |
| 19. Water quality | Hardness, alkalinity, conductivity, ammonia-N beginning and end; temperature daily; conductivity weekly; DO & pH 3X/wk |
| 20. Endpoints | Survival & growth (based on weight) |
| 21. Test acceptability criteria | Minimum control survival of 80% |
| 22. Sample holding | 14 days at 4°C in the dark (recommended) |
| 23. Sample volume required | 1L (800 mL per sediment) |
| 24. Reference toxicant | Concurrent testing required |

# APPENDIX II

# RAW DATA

# TEST DESCRIPTION, MONITORING, AND RESULTS BENCHSHEETS

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator

**STUDY MANAGEMENT**

Client: Hart Crowser, Inc., 1700 Westlake Ave. North, Suite 200, Seattle, WA 98109

Client's Study Monitor: Mr. Philip Cordell

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. Yves Katalans 2. GA Butler GAB
3. J. Brown JB 4. Logan Brady LB
5. 6. U.S. Caldwell USC
7. 8.

Study Schedule:

Test Beginning: 1-23-15 0905 Test Ending: 2-20-15 1000

**TEST MATERIAL**

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

| | | | | | |
|---|---|---|---|---|---|
| NAS Sample No.: | 5192G | 5193G | 5194G | | |
| Description: | JT-SS-06 | JT-SS-08 | JT-SS-10 | | |
| Collection Date: | 1/12/15 | 1/12/15 | 1/12/15 | | |
| Receipt Date: | 1/14/15 | 1/14/15 | 1/14/15 | | |
| : | | | | | |
| NAS Sample No.: | | | | | |
| Description: | | | | | |
| Collection Date: | | | | | |
| Receipt Date: | | | | | |
| : | | | | | |
| NAS Sample No.: | | | | | |
| Description: | | | | | |
| Collection Date: | | | | | |
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| NAS Sample No.: | | | | | |
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| Collection Date: | | | | | |
| Receipt Date: | | | | | |
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| 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

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator

SEDIMENT DESCRIPTIONS -- SUPPLEMENTAL NOTES

| Sample No. | Description |
|---|---|
| 5195G | FINE BLACK MUD |
| 5192G | Dark Brown sandy mud w/ woody fragments, some plastic film |
| 5193G | Watery sandy black mud w/ wood fragments |
| 5194G | BLACK MUDDY SAND w/ WOOD FRAGMENTS, BLACK PLASTIC FILM, ALUMINUM FOIL |

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator

**TEST WATER**

Source: Dechlorinated Newport, OR tap water

Date of Collection/Preparation: 1-19-15, 2-6-15

pH 7.7, 7.5

Cond (umhos/cm2) 108, 119

Hardness (mg/La0 26, 26

Alkalinity (mg/L) 30, 30

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

Treatments: Aerated ≥ 24 hrs

**TEST ORGANISMS**

Species: *Hyalella azteca* Age: 7-8 DAYS Date received: 1-21-15

Source: Chesapeake Cultures, Hayes, VA

Acclimation Data:

| Date | Temp. (deg.C) | pH | DO (mg/L) | Cond. umhos/cm | Hardness (mg/L) | Alkalinity (mg/L) | Feeding Amount | Feeding description | Water changes |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 1-21-15 | 17.5 | 6.8 | >15.0 | 596 | 188 | 180 | 10 mls | YTC | Rec'd day in - yes 2 50% |
| 1-22-15 | 20.2 | 7.3 | 8.9 | 255 | 77 | 70 | " | " | " |
| 1-23-15 | 20.7 | 7.5 | 8.4 | 223 | 68 | 60 | " | " | |
| Mean | 19.5 | 7.2 | 10.8 | 358 | 111 | 103 | | | |
| S.D. | 1.7 | 0.4 | 3.7 | 207 | 67 | 67 | | | |
| (N) | 3 | 3 | 3 | 3 | 3 | 3 | | | |

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 Organisms/treatment: (80) 80 (10/REP)

Test water changes: Twice daily

Aeration: only if DO falls below 2.5 mg/L

Feeding: everyday beginning with day zero

Test temperature (deg.C): 23

Beaker placement: Total randomization

Photoperiod: 16:8, L:D

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: 1/19/15

Sieved through 0.5 -mm screen

Storage: 4°C in the dark in closed containers. NAS# 5195G

**MISCELLANEOUS NOTES**

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator _____

Test conducted in (circle one): (room 1) room 2 trailer water bath other:_____

Randomization chart: TOP SHELF

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 10 | 15 | 20 | 25 | 30 | | | | |
| 4 | 9 | 14 | 19 | 24 | 29 | | | | |
| 3 | 8 | 13 | 18 | 23 | 28 | | | | |
| 2 | 7 | 12 | 17 | 22 | 27 | 32 | | | |
| 1 | 6 | 11 | 16 | 21 | 26 | 31 | | | |

Randomization chart: FRONT

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
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Randomization chart:

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Randomization chart:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
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NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 0 ( / 12/3/15) [illegible]

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness* (mg/L) | Alkalinity* (mg/L) | NH3* (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.2 | 7.6 | 219 | 6.8 | 34 | 30 | | Each beaker fed 1.0 ml |
| 7 | 22.2 | 7.7 | 113 | 6.6 | 34 | 30 | | YTC suspension |
| 17 | 22.3 | 7.6 | 133 | 6.6 | 34 | 40 | | Initials: [illegible] |
| 18 | 22.3 | 7.6 | 115 | 6.6 | 34 | 40 | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0515 |
| | | | | | | | | Initials: [illegible] |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1600 |
| | | | | | | | | Initials: [illegible] |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 1 ( / 12/4/15) [illegible]

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.2 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.3 | | | | | | | YTC suspension |
| 17 | 22.2 | | | | | | | Initials: [illegible] |
| 18 | 22.2 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0515 |
| | | | | | | | | Initials: [illegible] |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1630 |
| | | | | | | | | Initials: [illegible] |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 2 ( 1 /25/15) GJL

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.2 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.2 | | | | | | | YTC suspension |
| 17 | 22.1 | | | | | | | Initials: GJL |
| 18 | 22.1 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0450 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1620 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 3 ( 1 /26/15) GJL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.3 | 7.0 | | 7.1 | | | | Each beaker fed 1.0 ml |
| 7 | 22.2 | 7.0 | | 7.1 | | | | YTC suspension |
| 17 | 22.1 | 7.0 | | 7.1 | | | | Initials: GJL |
| 18 | 22.1 | 7.2 | | 7.2 | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1625 |
| | | | | | | | | Initials: |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 4 (1 12/7/15) ✓

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.6 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.4 | | | | | | | YTC suspension |
| 17 | 22.2 | | | | | | | Initials: ✓ |
| 18 | 22.2 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0520 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1645 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 5 (1 12/8/15) ✓

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 23.0 | 7.3 | 139 | 7.2 | | | | Each beaker fed 1.0 ml |
| 7 | 22.8 | 7.1 | 124 | 7.2 | | | | YTC suspension |
| 17 | 22.7 | 7.1 | 124 | 7.2 | | | | Initials: ✓ |
| 18 | 22.7 | 7.1 | 120 | 7.2 | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0540 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1650 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 6 ( 1 12/9/15) YV

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 23.1 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 23.0 | | | | | | | YTC suspension |
| 17 | 22.8 | | | | | | | Initials: YV |
| 18 | 22.8 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0525 |
| | | | | | | | | Initials: YV |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1615 |
| | | | | | | | | Initials: JS |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 7 ( 1 / 30/15) YV

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.5 | 7.3 | | 7.1 | | | | Each beaker fed 1.0 ml |
| 7 | 22.4 | 6.9 | | 7.0 | | | | YTC suspension |
| 17 | 22.2 | 6.7 | | 7.0 | | | | Initials: GB |
| 18 | 22.2 | 6.8 | | 7.1 | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: UB |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1645 |
| | | | | | | | | Initials: US |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 8 ( 1 /31 /15) GAS

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.5 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.2 | | | | | | | YTC suspension |
| 17 | 22.2 | | | | | | | Initials: GAS |
| 18 | 22.3 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0515 |
| | | | | | | | | Initials: GAS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1700 |
| | | | | | | | | Initials: JS |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 9 ( 2 / 1 /15) GJ

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.3 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.1 | | | | | | | YTC suspension |
| 17 | 22.0 | | | | | | | Initials: GJ |
| 18 | 22.1 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0500 |
| | | | | | | | | Initials: GJ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: GJ |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 10 (2/2/15) EJL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.4 | 7.4 | | 7.2 | | | | Each beaker fed 1.0 ml |
| 7 | 22.2 | 7.4 | | 7.1 | | | | YTC suspension |
| 17 | 22.1 | 7.0 | | 7.1 | | | | Initials: EJL |
| 18 | 22.1 | 7.1 | | 7.1 | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: EJL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1105 |
| | | | | | | | | Initials: US |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 11 (2/3/15) US

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.6 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.7 | | | | | | | YTC suspension |
| 17 | 22.5 | | | | | | | Initials: |
| 18 | 22.5 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0515 |
| | | | | | | | | Initials: Y |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: US |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 12 (2/4/15) LB

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.6 | 6.3 | 126 | 7.1 | | | | Each beaker fed 1.0 ml |
| 7 | 22.6 | 6.3 | 119 | 7.0 | | | | YTC suspension |
| 17 | 22.4 | 6.1 | 122 | 7.0 | | | | Initials: LB |
| 18 | 22.4 | 6.2 | 119 | 7.0 | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0520 |
| | | | | | | | | Initials: LB |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1605 |
| | | | | | | | | Initials: LB |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 13 (2/5/15) LB

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 23.0 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 23.1 | | | | | | | YTC suspension |
| 17 | 23.1 | | | | | | | Initials: LB |
| 18 | 23.1 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0500 |
| | | | | | | | | Initials: LB |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: LB |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 14 (2/6/15) YC/GS

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 23.9 | 7.0 | | 7.6 | | | | Each beaker fed 1.0 ml |
| 7 | 23.6 | 6.7 | | 7.3 | | | | YTC suspension |
| 17 | 23.4 | 6.6 | | 7.3 | | | | Initials: GS |
| 18 | 23.4 | 6.7 | | 7.3 | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: GS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: YC |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 15 (2/7/15) GS

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.4 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.5 | | | | | | | YTC suspension |
| 17 | 22.3 | | | | | | | Initials: GS |
| 18 | 22.3 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1615 |
| | | | | | | | | Initials: VS |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 16 (2/8/15) GJL

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.2 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.1 | | | | | | | YTC suspension |
| 17 | 22.1 | | | | | | | Initials: GJL |
| 18 | 22.0 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1605 |
| | | | | | | | | Initials: |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 17 (2/9/15) GJL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.6 | 6.8 | | 7.3 | | | | Each beaker fed 1.0 ml |
| 7 | 22.4 | 6.6 | | 7.1 | | | | YTC suspension |
| 17 | 22.3 | 6.6 | | 7.1 | | | | Initials: GJL |
| 18 | 22.3 | 6.9 | | 7.1 | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1640 |
| | | | | | | | | Initials: |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 18 (2/10/15) YC

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.2 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.0 | | | | | | | YTC suspension |
| 17 | 22.0 | | | | | | | Initials: YC |
| 18 | 22.0 | | | | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: YC |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1620 |
| | | | | | | | | Initials: CM |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 19 (2/11/15) CMS

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.1 | 6.8 | 117 | 7.2 | | | | Each beaker fed 1.0 ml |
| 7 | 22.1 | 6.6 | 111 | 7.3 | | | | YTC suspension |
| 17 | 22.2 | 6.7 | 113 | 7.3 | | | | Initials: CMS |
| 18 | 22.2 | 6.6 | 110 | 7.2 | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0520 |
| | | | | | | | | Initials: CMS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1605 |
| | | | | | | | | Initials: CM |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 20 (2/12/15)

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.0 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.0 | | | | | | | YTC suspension |
| 17 | 22.0 | | | | | | | Initials: ✓ |
| 18 | 22.0 | | | | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1630 |
| | | | | | | | | Initials: VB |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 21 (2/13/15) ✓

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.7 | 7.3 | | 7.5 | | | | Each beaker fed 1.0 ml |
| 7 | 22.6 | 7.1 | | 7.2 | | | | YTC suspension |
| 17 | 22.4 | 6.7 | | 7.2 | | | | Initials: VB |
| 18 | 22.3 | 7.1 | | 7.2 | | | | |
| | | | | | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: VB |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1615 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 22 ( 2/14/15 GS

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.1 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.1 | | | | | | | YTC suspension |
| 17 | 22.0 | | | | | | | Initials: GS |
| 18 | 22.0 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0520 |
| | | | | | | | | Initials: GS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1630 |
| | | | | | | | | Initials: GS |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 23 ( 2/15/15) GSL

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.3 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.3 | 22.4 | | | | | | YTC suspension |
| 17 | 22.3 | | | | | | | Initials: GSL |
| 18 | 22.2 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1620 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |

2-15-15 GSL

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 24 (2/16/15) GJL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.4 | 6.9 | | 7.4 | | | | Each beaker fed 1.0 ml |
| 7 | 22.2 | 6.6 | | 7.2 | | | | YTC suspension |
| 17 | 22.2 | 6.6 | | 7.2 | | | | Initials: GJL |
| 18 | 22.1 | 6.8 | | 7.2 | | | | |
| | | | | | | | | |
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| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1600 |
| | | | | | | | | Initials: LB |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 25 (2/17/15) GJL/LB

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.7 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.4 | | | | | | | YTC suspension |
| 17 | 22.4 | | | | | | | Initials: L |
| 18 | 22.2 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0540 |
| | | | | | | | | Initials: L |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: GJL |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 26 (2/18/15) LMS

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.9 | 7.2 | 118 | 7.2 | | | | Each beaker fed 1.0 ml |
| 7 | 22.9 | 6.4 | 114 | 7.1 | | | | YTC suspension |
| 17 | 22.8 | 6.3 | 115 | 7.1 | | | | Initials: LMS |
| 18 | 22.9 | 6.9 | 112 | 7.2 | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: JB |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1640 |
| | | | | | | | | Initials: LMS |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 27 (2/19/15) ✓

| Beaker No. | Temp.* (deg.C) | DO (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.0 | | | | | | | Each beaker fed 1.0 ml |
| 7 | 22.4 | | | | | | | YTC suspension |
| 17 | 22.4 | | | | | | | Initials: ✓ |
| 18 | 22.3 | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: JB |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No 862-1 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 28 (2/20/15) MB/YL/LS

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness* (mg/L) | Alkalinity* (mg/L) | NH3* (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 3 | 22.7 | 8.5 | 122 | 8.0 | 34 | 30 | | |
| 7 | 22.6 | 7.5 | 113 | 7.3 | 26 | 30 | | |
| 17 | 22.6 | 7.5 | 118 | 7.3 | 34 | 40 | | |
| 18 | 22.5 | 8.1 | 113 | 7.5 | 26 | 40 | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all beakers. Time: 0500 Initials: MB |

*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 |
|---|---|---|---|---|---|---|---|---|
| | | | | | | | | |
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*Water quality measurements to be taken.

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator _____

DAY 28 TEST TERMINATION SHEET

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

| Beaker No. | Number of survivors | Initials |
|---|---|---|
| | | |

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator _____

## ZERO-TIME WEIGHING DATA SHEET

Tare: Date 1-22-15 Oven temp ( C.) 61 Drying time (hr.) 24 Initials JRF
Standard Weights: 10 mg: 10.007 100mg: 100.014

Final: Date 1-26-15 Oven temp ( C.) 62 Drying time (hr.) 24 Initials JRF
Standard Weights: 10 mg: 10.008 100mg: 100.015

Equip. used: Oven: Blue M #1 Balance: Sartorius M3P

(Dry overnight at 60-90 degrees C)

| Pan # | Tare wt. (mg) | Total wt. (mg) | #weighed | Comments |
|---|---|---|---|---|
| 1 | 32.244 | 32.891 | 10 | |
| 2 | 30.550 | 31.202 | 10 | |
| 3 | 41.342 | 42.029 | 10 | |
| 4 | 35.318 | 35.956 | 10 | |
| 5 | 29.978 | 30.727 | 10 | |

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA4c

HYALELLA AZTECA 28-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-1 Client Hart Crowser Investigator

**WEIGHING DATA SHEET**

Tare: Date 1-22-15 Oven temp ( C.) 61 Drying time (hr.) 24 Initials JRF
Standard Weights: 10 mg: 10.007 100mg: 100.014

Final #1: Date 2-3-15 Oven temp ( C.) 62 Drying time (hr.) 24 Initials JRF
Standard Weights: 10 mg: 10.008 100mg: 100.019

Final #2: Date 2-24-15 Oven temp ( C.) 64 Drying time (hr.) 24 Initials JRF
Standard Weights: 10 mg: 10.007 100mg: 100.018

Equip. used: Oven BLUE M #1 Balance Sartorius M3P
(Dry overnight at 60-90 degrees C)

| Bkr. # | Pan # | Tare wt. (mg) | Total wt. (mg) 1 | Total wt. (mg) 2 | no. weighed | put into pans-initials | Comments |
|---|---|---|---|---|---|---|---|
| 1 | 1 | 34.035 | ~~106.04~~ 37.127 | 37.049 | 10 | LB | |
| 2 | 2 | 33.915 | ~~97~~, 36.436 | 36.374 | 9 | LB | |
| 3 | 3 | 32.674 | ~~36.43~~ 35.888 | 35.812 | 10 | LB | |
| 4 | 4 | 33.239 | 37.387 | 37.301 | 10 | LB | ~~35.863~~ JRF |
| 5 | 5 | 32.927 | 36.~~36~~141 | 36.075 | 9 | LB | |
| 6 | 6 | 31.841 | 34.039 | 33.992 | 4 | LB | |
| 7 | 7 | 32.235 | 35.172 | 35.104 | 10 | LB | |
| 8 | 8 | 31.388 | 34.274 | 34.219 | 9 | LB | |
| 9 | 9 | 32.283 | 34.618 | 34.580 | 9 | LB | |
| 10 | 10 | 31.860 | 35.142 | 35.088 | 10 | LB | |
| 11 | 11 | 32.240 | 35.003 | 34.951 | 9 | LB | |
| 12 | 12 | 32.070 | 34.617 | 34.577 | 8 | LB | |
| 13 | 13 | 33.570 | 36.182 | 36.144 | 10 | LB | |
| 14 | 14 | 33.263 | 36.391 | 36.367 | 10 | LB | |
| 15 | 15 | 36.412 | 40.377 | 40.319 | 10 | LB | |
| 16 | 16 | 31.668 | 34.408 | 34.388 | 8 | LB | |
| 17 | 17 | 34.924 | 36.554 | 36.534 | 5 | LB | |
| 18 | 18 | 35.345 | 37.587 | 37.558 | 7 | LB | |
| 19 | 19 | 28.557 | 30.975 | 30.941 | 8 | LB | |
| 20 | 20 | 33.640 | 36.293 | 36.258 | 9 | LB | |
| 21 | 21 | 34.089 | 38.001 | 37.952 | 10 | LB | |
| 22 | 22 | 32.079 | 34.675 | 34.646 | 8 | LB | |
| 23 | 23 | 32.435 | 36.005 | 35.941 | 7 | LB | |
| 24 | 24 | 34.733 | 37.900 | 37.854 | 10 | LB | |
| 25 | 25 | 36.924 | 39.485 | 39.454 | 9 | LB | |
| 26 | 26 | 33.049 | 34.931 | 34.913 | 4 | LB | |
| 27 | 27 | 38.156 | 41.077 | 41.043 | 6 | LB | |
| 28 | 28 | 34.510 | 37.985 | 37.923 | 10 | LB | |
| 29 | 29 | 32.536 | 35.698 | 35.627 | 7 | LB | |
| 30 | 30 | 34.453 | 37.205 | 37.139 | 10 | LB | |
| 31 | 31 | 30.870 | 33.051 | 32.997 | 9 | LB | |
| 32 | 32 | 34.577 | 37.923 | 37.857 | 7 | LB | |
| | | | | | | | |

# *Chesapeake Cultures*

P.O. Box 507 Hayes, VA 23072 (804)693-4046 (804)694-4704 fax
www.c-cultures.com
growfish@c-cultures.com

NAS
Shipment Information

Rcvd 1-21-15

Species Hyalella azteca Date 1/20/15

Age ~ 4-5 d.; ~ 1.5 mm P.O. No. verbal

Quantity 530+ Invoice No. 8585

Temperature 24°C Salinity ~ pH 7.88

Notes Thank you!

Biologist

*Please inspect shipment and report any problem immediately*

# TEST DATA ANALYSIS RECORDS

Test Number: P862-1

Freshwater Sediment Test
28-day Hyalella azteca

data entry verified against laboratory benchsheets 3-9-15 JRF

# 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

| INITIAL WEIGHT | | | | |
|---|---|---|---|---|
| pan # | tare wt (mg) | final wt (mg) | wt count | avg. wt/ organism |
| 1 | 32.244 | 32.891 | 10 | 0.0647 |
| 2 | 30.550 | 31.202 | 10 | 0.0652 |
| 3 | 41.342 | 42.029 | 10 | 0.0687 |
| 4 | 35.318 | 35.956 | 10 | 0.0638 |
| 5 | 29.978 | 30.727 | 10 | 0.0749 |
| | | | Mean | 0.067 |

| INDEX | BKR | NAS SMPL | CLIENT DESCRIP | REPL | | INIT | SURV | MORT | PSURV | PMORT | TARE WT (mg) | WT COUNT | DRY WT (mg) | TWT (mg) | WT (mg) | | SURV | MORT | PSURV | PMORT | WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| 1 | 24 | 5195G | Contol | 1 | | 10 | 10 | 0 | 100.0 | 0.0 | 34.733 | 10 | 37.854 | 3.12 | 0.31 | | | | | | |
| 2 | 11 | 5195G | Contol | 2 | | 10 | 9 | 1 | 90.0 | 10.0 | 32.240 | 9 | 34.951 | 2.71 | 0.30 | | | | | | |
| 3 | 2 | 5195G | Contol | 3 | | 10 | 9 | 1 | 90.0 | 10.0 | 33.915 | 9 | 36.374 | 2.46 | 0.27 | | | | | | |
| 4 | 5 | 5195G | Contol | 4 | | 10 | 9 | 1 | 90.0 | 10.0 | 32.927 | 9 | 36.075 | 3.15 | 0.35 | | | | | | |
| 5 | 8 | 5195G | Contol | 5 | | 10 | 9 | 1 | 90.0 | 10.0 | 31.388 | 9 | 34.219 | 2.63 | 0.31 | Mean | 9.5 | 0.5 | 95.0 | 5.0 | 0.32 |
| 6 | 21 | 5195G | Contol | 6 | | 10 | 10 | 0 | 100.0 | 0.0 | 34.089 | 10 | 37.952 | 3.86 | 0.39 | SD | 0.5 | 0.5 | 5.3 | 5.3 | 0.03 |
| 7 | 1 | 5195G | Contol | 7 | | 10 | 10 | 0 | 100.0 | 0.0 | 34.035 | 10 | 37.049 | 3.01 | 0.30 | n | 8 | 8 | 8 | 8 | 8 |
| 8 | 3 | 5195G | Contol | 8 | wq replicate | 10 | 10 | 0 | 100.0 | 0.0 | 32.674 | 10 | 35.812 | 3.14 | 0.31 | | | | | | |
| 9 | 28 | 5192G | JT-SS-06 | 1 | | 10 | 10 | 0 | 100.0 | 0.0 | 34.510 | 10 | 37.923 | 3.41 | 0.34 | | | | | | |
| 10 | 27 | 5192G | JT-SS-06 | 2 | | 10 | 6 | 4 | 60.0 | 40.0 | 38.156 | 6 | 41.043 | 2.89 | 0.48 | | | | | | |
| 11 | 26 | 5192G | JT-SS-06 | 3 | | 10 | 4 | 6 | 40.0 | 60.0 | 33.049 | 4 | 34.913 | 1.86 | 0.47 | | | | | | |
| 12 | 32 | 5192G | JT-SS-06 | 4 | | 10 | 7 | 3 | 70.0 | 30.0 | 34.577 | 7 | 37.857 | 3.28 | 0.47 | | | | | | |
| 13 | 12 | 5192G | JT-SS-06 | 5 | | 10 | 8 | 2 | 80.0 | 20.0 | 32.070 | 8 | 34.577 | 2.51 | 0.31 | Mean | 7.8 | 2.3 | 77.5 | 22.5 | 0.41 |
| 14 | 4 | 5192G | JT-SS-06 | 6 | | 10 | 10 | 0 | 100.0 | 0.0 | 33.239 | 10 | 37.301 | 4.06 | 0.41 | SD | 2.2 | 2.2 | 21.9 | 21.9 | 0.08 |
| 15 | 23 | 5192G | JT-SS-06 | 7 | | 10 | 7 | 3 | 70.0 | 30.0 | 32.435 | 7 | 35.941 | 3.51 | 0.50 | n | 8 | 8 | 8 | 8 | 8 |
| 16 | 7 | 5192G | JT-SS-06 | 8 | wq replicate | 10 | 10 | 0 | 100.0 | 0.0 | 32.235 | 10 | 35.104 | 2.87 | 0.29 | | | | | | |
| 17 | 22 | 5193G | JT-SS-08 | 1 | | 10 | 8 | 2 | 80.0 | 20.0 | 32.079 | 8 | 34.646 | 2.57 | 0.32 | | | | | | |
| 18 | 19 | 5193G | JT-SS-08 | 2 | | 10 | 8 | 2 | 80.0 | 20.0 | 28.557 | 8 | 30.941 | 2.38 | 0.30 | | | | | | |
| 19 | 15 | 5193G | JT-SS-08 | 3 | | 10 | 10 | 0 | 100.0 | 0.0 | 36.412 | 10 | 40.319 | 3.91 | 0.39 | | | | | | |
| 20 | 29 | 5193G | JT-SS-08 | 4 | | 10 | 7 | 3 | 70.0 | 30.0 | 32.536 | 7 | 35.627 | 3.09 | 0.44 | | | | | | |
| 21 | 10 | 5193G | JT-SS-08 | 5 | | 10 | 10 | 0 | 100.0 | 0.0 | 31.860 | 10 | 35.088 | 3.23 | 0.32 | Mean | 8.0 | 2.0 | 80.0 | 20.0 | 0.37 |
| 22 | 14 | 5193G | JT-SS-08 | 6 | | 10 | 10 | 0 | 100.0 | 0.0 | 33.263 | 10 | 36.367 | 3.10 | 0.31 | SD | 2.1 | 2.1 | 20.7 | 20.7 | 0.08 |
| 23 | 6 | 5193G | JT-SS-08 | 7 | | 10 | 4 | 6 | 40.0 | 60.0 | 31.841 | 4 | 33.992 | 2.15 | 0.54 | n | 8 | 8 | 8 | 8 | 8 |
| 24 | 18 | 5193G | JT-SS-08 | 8 | wq replicate | 10 | 7 | 3 | 70.0 | 30.0 | 35.345 | 7 | 37.558 | 2.21 | 0.32 | | | | | | |
| 25 | 20 | 5194G | JT-SS-10 | 1 | | 10 | 9 | 1 | 90.0 | 10.0 | 33.640 | 9 | 36.258 | 2.62 | 0.29 | | | | | | |
| 26 | 30 | 5194G | JT-SS-10 | 2 | | 10 | 10 | 0 | 100.0 | 0.0 | 34.453 | 10 | 37.139 | 2.69 | 0.27 | | | | | | |
| 27 | 25 | 5194G | JT-SS-10 | 3 | | 10 | 9 | 1 | 90.0 | 10.0 | 36.924 | 9 | 39.454 | 2.53 | 0.28 | | | | | | |
| 28 | 13 | 5194G | JT-SS-10 | 4 | | 10 | 10 | 0 | 100.0 | 0.0 | 33.570 | 10 | 36.144 | 2.57 | 0.26 | | | | | | |
| 29 | 9 | 5194G | JT-SS-10 | 5 | | 10 | 9 | 1 | 90.0 | 10.0 | 32.283 | 9 | 34.580 | 2.30 | 0.26 | Mean | 8.6 | 1.4 | 86.3 | 13.8 | 0.28 |
| 30 | 16 | 5194G | JT-SS-10 | 6 | | 10 | 8 | 2 | 80.0 | 20.0 | 31.668 | 8 | 34.388 | 2.72 | 0.34 | SD | 1.6 | 1.6 | 16.0 | 16.0 | 0.04 |
| 31 | 31 | 5194G | JT-SS-10 | 7 | | 10 | 9 | 1 | 90.0 | 10.0 | 30.870 | 9 | 32.997 | 2.13 | 0.24 | n | 8 | 8 | 8 | 8 | 8 |
| 32 | 17 | 5194G | JT-SS-10 | 8 | wq replicate | 10 | 5 | 5 | 50.0 | 50.0 | 34.924 | 5 | 36.534 | 1.61 | 0.32 | | | | | | |

Project Name: P862-1 Hyalella % Mortality

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-06 |
| Alias: | NAS# 5192G |
| Replicates: | 8 |
| Mean: | 22.5 |
| SD: | 21.876 |
| Tr Mean: | 22.873 |
| Trans SD: | 20.152 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 5 |
| SD: | 5.345 |
| Tr Mean: | 9.217 |
| Trans SD: | 9.854 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 13.616 |
| SS: | 3522.352 |
| K: | 8 |
| b: | 56.462 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.9051 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 17.155 |
| Test Residual SD: | 8.352 |
| Ref. Residual Mean: | 9.217 |
| Ref. Residual SD: | 0 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 2.688 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | No |

**Test Results:**

| | |
|---|---|
| Statistic: | Approximate t |
| Balanced Design: | Yes |
| Transformation: | ArcSin |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 <= x2 |
| Alternate: | x1 > x2 |
| Degrees of Freedom: | 10 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 1.7219 |
| Critical Value: | >= 1.812 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 0 | 0 | 0 | 22.873 | 9.217 | | | -22.873 |
| 2 | 40 | 39.232 | 10 | 18.435 | 16.358 | 9.217 | | | -22.873 |
| 3 | 60 | 50.768 | 10 | 18.435 | 27.895 | 9.217 | | | -22.873 |
| 4 | 30 | 33.211 | 10 | 18.435 | 10.338 | 9.217 | | | -9.217 |
| 5 | 20 | 26.565 | 10 | 18.435 | 3.692 | 9.217 | | | -9.217 |
| 6 | 0 | 0 | 0 | 0 | 22.873 | 9.217 | | | -9.217 |
| 7 | 30 | 33.211 | 0 | 0 | 10.338 | 9.217 | | | -9.217 |
| 8 | 0 | 0 | 0 | 0 | 22.873 | 9.217 | | | 3.692 |
| 9 | | | | | | | | | 9.217 |
| 10 | | | | | | | | | 9.217 |
| 11 | | | | | | | | | 9.217 |
| 12 | | | | | | | | | 9.217 |
| 13 | | | | | | | | | 10.338 |
| 14 | | | | | | | | | 10.338 |
| 15 | | | | | | | | | 16.358 |
| 16 | | | | | | | | | 27.895 |

The percent mortality in test sediment JT-SS-06 was not significantly higher that that of the control sediment at $\alpha$=0.05.

Project Name: P862-1 Hyalella % Mortality

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-08 |
| Alias: | NAS# 5193G |
| Replicates: | 8 |
| Mean: | 20 |
| SD: | 20.702 |
| Tr Mean: | 21.29 |
| Trans SD: | 19.156 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 5 |
| SD: | 5.345 |
| Tr Mean: | 9.217 |
| Trans SD: | 9.854 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 13.075 |
| SS: | 3248.336 |
| K: | 8 |
| b: | 54.155 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.9029 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 15.968 |
| Test Residual SD: | 8.693 |
| Ref. Residual Mean: | 9.217 |
| Ref. Residual SD: | 0 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 2.1963 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | No |

**Test Results:**

| | |
|---|---|
| Statistic: | Approximate t |
| Balanced Design: | Yes |
| Transformation: | ArcSin |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 <= x2 |
| Alternate: | x1 > x2 |
| Degrees of Freedom: | 10 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 1.5851 |
| Critical Value: | >= 1.812 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 26.565 | 0 | 0 | 5.275 | 9.217 | | | -21.29 |
| 2 | 20 | 26.565 | 10 | 18.435 | 5.275 | 9.217 | | | -21.29 |
| 3 | 0 | 0 | 10 | 18.435 | 21.29 | 9.217 | | | -21.29 |
| 4 | 30 | 33.211 | 10 | 18.435 | 11.921 | 9.217 | | | -9.217 |
| 5 | 0 | 0 | 10 | 18.435 | 21.29 | 9.217 | | | -9.217 |
| 6 | 0 | 0 | 0 | 0 | 21.29 | 9.217 | | | -9.217 |
| 7 | 60 | 50.768 | 0 | 0 | 29.478 | 9.217 | | | -9.217 |
| 8 | 30 | 33.211 | 0 | 0 | 11.921 | 9.217 | | | 5.275 |
| 9 | | | | | | | | | 5.275 |
| 10 | | | | | | | | | 9.217 |
| 11 | | | | | | | | | 9.217 |
| 12 | | | | | | | | | 9.217 |
| 13 | | | | | | | | | 9.217 |
| 14 | | | | | | | | | 11.921 |
| 15 | | | | | | | | | 11.921 |
| 16 | | | | | | | | | 29.478 |

The percent mortality in test sediment JT-SS-08 was not significantly higher that that of the control sediment at α=0.05. -631

Project Name: P862-1 Hyalella % Mortality

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-10 |
| Alias: | NAS# 5194G |
| Replicates: | 8 |
| Mean: | 13.75 |
| SD: | 15.98 |
| Tr Mean: | 18.163 |
| Trans SD: | 14.397 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 5 |
| SD: | 5.345 |
| Tr Mean: | 9.217 |
| Trans SD: | 9.854 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 10.589 |
| SS: | 2130.599 |
| K: | 8 |
| b: | 44.42 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.9261 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 9.082 |
| Test Residual SD: | 10.631 |
| Ref. Residual Mean: | 9.217 |
| Ref. Residual SD: | 0 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 0.0362 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Student's t |
| Balanced Design: | Yes |
| Transformation: | ArcSin |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 <= x2 |
| Alternate: | x1 > x2 |
| Degrees of Freedom: | 14 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 1.4503 |
| Critical Value: | >= 1.761 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 10 | 18.435 | 0 | 0 | 0.272 | 9.217 | | | -18.163 |
| 2 | 0 | 0 | 10 | 18.435 | 18.163 | 9.217 | | | -18.163 |
| 3 | 10 | 18.435 | 10 | 18.435 | 0.272 | 9.217 | | | -9.217 |
| 4 | 0 | 0 | 10 | 18.435 | 18.163 | 9.217 | | | -9.217 |
| 5 | 10 | 18.435 | 10 | 18.435 | 0.272 | 9.217 | | | -9.217 |
| 6 | 20 | 26.565 | 0 | 0 | 8.402 | 9.217 | | | -9.217 |
| 7 | 10 | 18.435 | 0 | 0 | 0.272 | 9.217 | | | 0.272 |
| 8 | 50 | 45 | 0 | 0 | 26.837 | 9.217 | | | 0.272 |
| 9 | | | | | | | | | 0.272 |
| 10 | | | | | | | | | 0.272 |
| 11 | | | | | | | | | 8.402 |
| 12 | | | | | | | | | 9.217 |
| 13 | | | | | | | | | 9.217 |
| 14 | | | | | | | | | 9.217 |
| 15 | | | | | | | | | 9.217 |
| 16 | | | | | | | | | 26.837 |

The percent mortality in test sediment JT-SS-10 was not significantly higher that that of the control sediment at α=0.05. — 651

Project Name: P862-I Hyalella Growth (dry wt)

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-06 |
| Alias: | NAS# 5192G |
| Replicates: | 8 |
| Mean: | 0.409 |
| SD: | 0.084 |
| Tr Mean: | 0.409 |
| Trans SD: | 0.084 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 0.318 |
| SD: | 0.037 |
| Tr Mean: | 0.318 |
| Trans SD: | 0.037 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 0.056 |
| SS: | 0.059 |
| K: | 8 |
| b: | 0.236 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.9453 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 0.072 |
| Test Residual SD: | 0.035 |
| Ref. Residual Mean: | 0.026 |
| Ref. Residual SD: | 0.023 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 3.0506 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | No |

**Test Results:**

| | |
|---|---|
| Statistic: | Approximate t |
| Balanced Design: | Yes |
| Transformation: | No Transformation |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 >= x2 |
| Alternate: | x1 < x2 |
| Degrees of Freedom: | 10 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | -2.8151 |
| Critical Value: | >= 1.812 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.34 | 0.34 | 0.31 | 0.31 | 0.069 | 0.008 | | | -0.119 |
| 2 | 0.48 | 0.48 | 0.3 | 0.3 | 0.071 | 0.018 | | | -0.099 |
| 3 | 0.47 | 0.47 | 0.27 | 0.27 | 0.061 | 0.048 | | | -0.069 |
| 4 | 0.47 | 0.47 | 0.35 | 0.35 | 0.061 | 0.033 | | | -0.048 |
| 5 | 0.31 | 0.31 | 0.31 | 0.31 | 0.099 | 0.008 | | | -0.018 |
| 6 | 0.41 | 0.41 | 0.39 | 0.39 | 0.001 | 0.073 | | | -0.018 |
| 7 | 0.5 | 0.5 | 0.3 | 0.3 | 0.091 | 0.018 | | | -0.008 |
| 8 | 0.29 | 0.29 | 0.31 | 0.31 | 0.119 | 0.008 | | | -0.008 |
| 9 | | | | | | | | | -0.008 |
| 10 | | | | | | | | | 0.001 |
| 11 | | | | | | | | | 0.033 |
| 12 | | | | | | | | | 0.061 |
| 13 | | | | | | | | | 0.061 |
| 14 | | | | | | | | | 0.071 |
| 15 | | | | | | | | | 0.073 |
| 16 | | | | | | | | | 0.091 |

Average individual growth (dry wt) in test sediment JT-SS-06 is not significantly less than that in the control sediment at α=0.05. -6.51

Project Name: P862-1 Hyalella Growth (dry wt)

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-08 |
| Alias: | NAS# 5193G |
| Replicates: | 8 |
| Mean: | 0.368 |
| SD: | 0.085 |
| Tr Mean: | 0.413 |
| Trans SD: | 0.884 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 0.318 |
| SD: | 0.037 |
| Tr Mean: | -0.413 |
| Trans SD: | 0.862 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | |
| Residual SD: | |
| SS: | |
| K: | |
| b: | |
| Alpha Level: | N/A |
| Calculated Value: | N/A |
| Critical Value: | N/A |
| Normally Distributed: | N/A |
| Override Option: | Not Invoked |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 0.672 |
| Test Residual SD: | 0.515 |
| Ref. Residual Mean: | 0.638 |
| Ref. Residual SD: | 0.527 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 0.1307 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Student's t |
| Balanced Design: | Yes |
| Transformation: | Rankits |
| Experimental Hypothesis | |
| Null: | x1 >= x2 |
| Alternate: | x1 < x2 |
| Degrees of Freedom: | 14 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | -1.8911 |
| Critical Value: | >= 1.761 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.32 | 0.236 | 0.31 | -0.319 | 0.177 | 0.094 | | -1.766 | |
| 2 | 0.3 | -1.013 | 0.3 | -1.013 | 1.425 | 0.6 | | -1.013 | |
| 3 | 0.39 | 0.877 | 0.27 | -1.766 | 0.464 | 1.353 | | -1.013 | |
| 4 | 0.44 | 1.285 | 0.35 | 0.57 | 0.872 | 0.983 | | -1.013 | |
| 5 | 0.32 | 0.236 | 0.31 | -0.319 | 0.177 | 0.094 | | -0.319 | |
| 6 | 0.31 | -0.319 | 0.39 | 0.877 | 0.732 | 1.289 | | -0.319 | |
| 7 | 0.54 | 1.766 | 0.3 | -1.013 | 1.353 | 0.6 | | -0.319 | |
| 8 | 0.32 | 0.236 | 0.31 | -0.319 | 0.177 | 0.094 | | -0.319 | |
| 9 | | | | | | | | 0.236 | |
| 10 | | | | | | | | 0.236 | |
| 11 | | | | | | | | 0.236 | |
| 12 | | | | | | | | 0.57 | |
| 13 | | | | | | | | 0.877 | |
| 14 | | | | | | | | 0.877 | |
| 15 | | | | | | | | 1.285 | |
| 16 | | | | | | | | 1.766 | |

Average individual growth (dry wt) in test sediment JT-SS-08 is not significantly less than that in the control sediment at α=0.05. -631

Project Name: P862-1 Hyalella Growth (dry wt)

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-10 |
| Alias: | NAS# 5194G |
| Replicates: | 8 |
| Mean: | 0.283 |
| SD: | 0.033 |
| Tr Mean: | 0.283 |
| Trans SD: | 0.033 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 0.318 |
| SD: | 0.037 |
| Tr Mean: | 0.318 |
| Trans SD: | 0.037 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 0.03 |
| SS: | 0.017 |
| K: | 8 |
| b: | 0.125 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.9106 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 0.026 |
| Test Residual SD: | 0.019 |
| Ref. Residual Mean: | 0.026 |
| Ref. Residual SD: | 0.023 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 0.0588 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Student's t |
| Balanced Design: | Yes |
| Transformation: | No Transformation |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 >= x2 |
| Alternate: | x1 < x2 |
| Degrees of Freedom: | 14 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 2.0029 |
| Critical Value: | >= 1.761 |
| Accept Null Hypothesis: | No |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.29 | 0.29 | 0.31 | 0.31 | 0.007 | 0.008 | | | -0.048 |
| 2 | 0.27 | 0.27 | 0.3 | 0.3 | 0.013 | 0.018 | | | -0.043 |
| 3 | 0.28 | 0.28 | 0.27 | 0.27 | 0.003 | 0.048 | | | -0.023 |
| 4 | 0.26 | 0.26 | 0.35 | 0.35 | 0.023 | 0.033 | | | -0.023 |
| 5 | 0.26 | 0.26 | 0.31 | 0.31 | 0.023 | 0.008 | | | -0.018 |
| 6 | 0.34 | 0.34 | 0.39 | 0.39 | 0.058 | 0.073 | | | -0.018 |
| 7 | 0.24 | 0.24 | 0.3 | 0.3 | 0.043 | 0.018 | | | -0.013 |
| 8 | 0.32 | 0.32 | 0.31 | 0.31 | 0.038 | 0.008 | | | -0.008 |
| 9 | | | | | | | | | -0.008 |
| 10 | | | | | | | | | -0.008 |
| 11 | | | | | | | | | -0.003 |
| 12 | | | | | | | | | 0.007 |
| 13 | | | | | | | | | 0.033 |
| 14 | | | | | | | | | 0.038 |
| 15 | | | | | | | | | 0.058 |
| 16 | | | | | | | | | 0.073 |

Average individual growth (dry wt) in test sediment JT-SS-10 is significantly less than that in the control sediment at α=0.05. -6.31

data entry verified against laboratory bench sheets 3-9-15 JRF 2/24/2015

## Water Quality Data

| BKR | NAS SMPL | CLIENT DESCRIP | REPL | DAY | Overlying water TEMP | DO | COND | pH | NH3 | HARD | ALK |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 5195G | Control | 8 | 0 | 22.2 | 7.6 | 219 | 6.8 | 0.9 | 34 | 30 |
| 7 | 5192G | JT-SS-06 | 8 | 0 | 22.2 | 7.7 | 113 | 6.6 | 0.2 | 34 | 30 |
| 17 | 5194G | JT-SS-10 | 8 | 0 | 22.3 | 7.6 | 133 | 6.6 | 0.3 | 34 | 40 |
| 18 | 5193G | JT-SS-08 | 8 | 0 | 22.3 | 7.6 | 115 | 6.6 | 0.1 | 34 | 40 |
| 3 | 5195G | Control | 8 | 1 | 22.2 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 1 | 22.3 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 1 | 22.2 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 1 | 22.2 | | | | | | |
| 3 | 5195G | Control | 8 | 2 | 22.2 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 2 | 22.2 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 2 | 22.1 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 2 | 22.1 | | | | | | |
| 3 | 5195G | Control | 8 | 3 | 22.3 | 7.0 | | 7.1 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 3 | 22.2 | 7.0 | | 7.1 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 3 | 22.1 | 7.0 | | 7.1 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 3 | 22.1 | 7.2 | | 7.2 | | | |
| 3 | 5195G | Control | 8 | 4 | 22.6 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 4 | 22.4 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 4 | 22.2 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 4 | 22.2 | | | | | | |
| 3 | 5195G | Control | 8 | 5 | 23.0 | 7.3 | 139 | 7.2 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 5 | 22.8 | 7.1 | 124 | 7.2 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 5 | 22.7 | 7.1 | 124 | 7.2 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 5 | 22.7 | 7.1 | 120 | 7.2 | | | |
| 3 | 5195G | Control | 8 | 6 | 23.1 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 6 | 23.0 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 6 | 22.8 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 6 | 22.8 | | | | | | |
| 3 | 5195G | Control | 8 | 7 | 22.5 | 7.3 | | 7.1 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 7 | 22.4 | 6.9 | | 7.0 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 7 | 22.2 | 6.7 | | 7.0 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 7 | 22.2 | 6.8 | | 7.1 | | | |
| 3 | 5195G | Control | 8 | 8 | 22.5 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 8 | 22.2 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 8 | 22.2 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 8 | 22.3 | | | | | | |
| 3 | 5195G | Control | 8 | 9 | 22.3 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 9 | 22.1 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 9 | 22.0 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 9 | 22.1 | | | | | | |
| 3 | 5195G | Control | 8 | 10 | 22.4 | 7.4 | | 7.2 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 10 | 22.2 | 7.4 | | 7.1 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 10 | 22.1 | 7.0 | | 7.1 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 10 | 22.1 | 7.1 | | 7.1 | | | |
| 3 | 5195G | Control | 8 | 11 | 22.6 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 11 | 22.7 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 11 | 22.5 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 11 | 22.5 | | | | | | |

| | | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| 3 | 5195G | Control | 8 | 12 | 22.6 | 6.3 | 126 | 7.1 |
| 7 | 5192G | JT-SS-06 | 8 | 12 | 22.6 | 6.3 | 119 | 7.0 |
| 17 | 5194G | JT-SS-10 | 8 | 12 | 22.4 | 6.1 | 122 | 7.0 |
| 18 | 5193G | JT-SS-08 | 8 | 12 | 22.4 | 6.2 | 119 | 7.0 |
| 3 | 5195G | Control | 8 | 13 | 23.0 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 13 | 23.1 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 13 | 23.1 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 13 | 23.1 | | | |
| 3 | 5195G | Control | 8 | 14 | 23.9 | 7.0 | | 7.6 |
| 7 | 5192G | JT-SS-06 | 8 | 14 | 23.6 | 6.7 | | 7.3 |
| 17 | 5194G | JT-SS-10 | 8 | 14 | 23.4 | 6.6 | | 7.3 |
| 18 | 5193G | JT-SS-08 | 8 | 14 | 23.4 | 6.7 | | 7.3 |
| 3 | 5195G | Control | 8 | 15 | 22.4 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 15 | 22.5 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 15 | 22.3 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 15 | 22.3 | | | |
| 3 | 5195G | Control | 8 | 16 | 22.2 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 16 | 22.1 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 16 | 22.1 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 16 | 22.0 | | | |
| 3 | 5195G | Control | 8 | 17 | 22.6 | 6.8 | | 7.3 |
| 7 | 5192G | JT-SS-06 | 8 | 17 | 22.4 | 6.6 | | 7.1 |
| 17 | 5194G | JT-SS-10 | 8 | 17 | 22.3 | 6.6 | | 7.1 |
| 18 | 5193G | JT-SS-08 | 8 | 17 | 22.3 | 6.9 | | 7.1 |
| 3 | 5195G | Control | 8 | 18 | 22.2 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 18 | 22.0 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 18 | 22.0 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 18 | 22.0 | | | |
| 3 | 5195G | Control | 8 | 19 | 22.1 | 6.8 | 117 | 7.2 |
| 7 | 5192G | JT-SS-06 | 8 | 19 | 22.1 | 6.6 | 111 | 7.3 |
| 17 | 5194G | JT-SS-10 | 8 | 19 | 22.2 | 6.7 | 113 | 7.3 |
| 18 | 5193G | JT-SS-08 | 8 | 19 | 22.2 | 6.6 | 110 | 7.2 |
| 3 | 5195G | Control | 8 | 20 | 22.0 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 20 | 22.0 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 20 | 22.0 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 20 | 22.0 | | | |
| 3 | 5195G | Control | 8 | 21 | 22.7 | 7.3 | | 7.5 |
| 7 | 5192G | JT-SS-06 | 8 | 21 | 22.6 | 7.1 | | 7.2 |
| 17 | 5194G | JT-SS-10 | 8 | 21 | 22.4 | 6.7 | | 7.2 |
| 18 | 5193G | JT-SS-08 | 8 | 21 | 22.3 | 7.1 | | 7.2 |
| 3 | 5195G | Control | 8 | 22 | 22.1 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 22 | 22.1 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 22 | 22.0 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 22 | 22.0 | | | |
| 3 | 5195G | Control | 8 | 23 | 22.3 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 23 | 22.4 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 23 | 22.3 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 23 | 22.2 | | | |
| 3 | 5195G | Control | 8 | 24 | 22.4 | 6.9 | | 7.4 |
| 7 | 5192G | JT-SS-06 | 8 | 24 | 22.2 | 6.6 | | 7.2 |
| 17 | 5194G | JT-SS-10 | 8 | 24 | 22.2 | 6.6 | | 7.2 |
| 18 | 5193G | JT-SS-08 | 8 | 24 | 22.1 | 6.8 | | 7.2 |

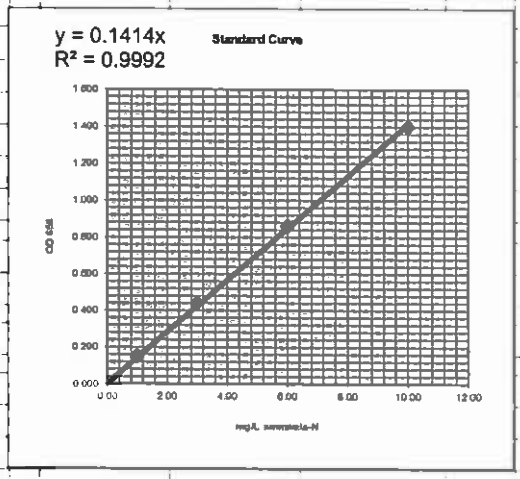
| | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 3 | 5195G | Control | 8 | 25 | 22.7 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 25 | 22.4 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 25 | 22.4 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 25 | 22.2 | | | | | | |
| 3 | 5195G | Control | 8 | 26 | 22.9 | 7.2 | 118 | 7.2 | | | |
| 7 | 5192G | JT-SS-06 | 8 | 26 | 22.9 | 6.4 | 114 | 7.1 | | | |
| 17 | 5194G | JT-SS-10 | 8 | 26 | 22.8 | 6.3 | 115 | 7.1 | | | |
| 18 | 5193G | JT-SS-08 | 8 | 26 | 22.9 | 6.9 | 112 | 7.2 | | | |
| 3 | 5195G | Control | 8 | 27 | 22.0 | | | | | | |
| 7 | 5192G | JT-SS-06 | 8 | 27 | 22.4 | | | | | | |
| 17 | 5194G | JT-SS-10 | 8 | 27 | 22.4 | | | | | | |
| 18 | 5193G | JT-SS-08 | 8 | 27 | 22.3 | | | | | | |
| 3 | 5195G | Control | 8 | 28 | 22.7 | 8.5 | 122 | 8.0 | <0.1 | 34 | 30 |
| 7 | 5192G | JT-SS-06 | 8 | 28 | 22.6 | 7.5 | 113 | 7.3 | <0.1 | 26 | 30 |
| 17 | 5194G | JT-SS-10 | 8 | 28 | 22.6 | 7.5 | 118 | 7.3 | <0.1 | 34 | 40 |
| 18 | 5193G | JT-SS-08 | 8 | 28 | 22.5 | 8.1 | 113 | 7.5 | <0.1 | 26 | 40 |
| | | | | | | | | | | | |
| | | | | **Mean** | 22.4 | 7.0 | 123 | 7.2 | --- | 32 | 35 |
| | | | | **SD** | 0.4 | 0.5 | 22 | 0.2 | --- | 4 | 5 |
| | | | | **n** | 116 | 52 | 24 | 52 | 8 | 8 | 8 |
| | | | | **Min** | 22.0 | 6.1 | 110 | 6.6 | <0.1 | 26 | 30 |
| | | | | **Max** | 23.9 | 8.5 | 219 | 8.0 | 0.9 | 34 | 40 |

# AMMONIA EXPOSURE BENCHSHEETS AND ANALYSIS

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

**Result**

| | Sample description | Dilution factor | $OD_{655}$ | $NH_3$-N (mg/L) | pH | Salinity (ppt) |
|---|---|---|---|---|---|---|
| | Blank | ----- | ----- | ----- | | |
| | 1.0 mg/L $NH_3$-N Std. | ----- | 0.151 | 1.00 | | |
| | 3.0 mg/L $NH_3$-N Std. | ----- | 0.440 | 3.00 | | |
| | 6.0 mg/L $NH_3$-N Std. | ----- | 0.862 | 6.00 | | |
| | 10.0 mg/L $NH_3$-N Std. | ----- | 1.400 | 10.00 | | |
| | 3.0 mg/L spike | ----- | 0.449 | 3.17 | | |
| | 3.0 mg/L spike dupl. | ----- | 0.440 | 3.11 | | |
| | 5.0 mg/L 2nd source | | 0.710 | 5.02 | | |
| 1 | Day 0 (1-23-15) | | | | | |
| 2 | 3 | 1 | 0.120 | 0.85 | | |
| 3 | 7 | 1 | 0.023 | 0.16 | | |
| 4 | 17 | 1 | 0.040 | 0.28 | | |
| 5 | 18 | 1 | 0.020 | 0.14 | | |
| 6 | | | | | | |
| 7 | Day 28 (2-20-15) | | | | | |
| 8 | 3 | 1 | 0.009 | ND | | |
| 9 | 7 | 1 | 0.002 | ND | | |
| 10 | 17 | 1 | 0.000 | ND | | |
| 11 | 18 | 1 | 0.000 | ND | | |




| | |
|---|---|
| Reporting limit (mg/L) = | 0.10 |
| Recovery (%) = | 104.8 |
| Precision (RPD) = | 2.02 |
| 2nd source (%) = | 100.4 |
| Sample volume (ml): | 0.50 |
| Dilution factor | 1 |

**Sample Set Description:**

Test No.: 862-1
Test Day: 0,& 28
Species: *Hyalella*

**Sample Type (check)**

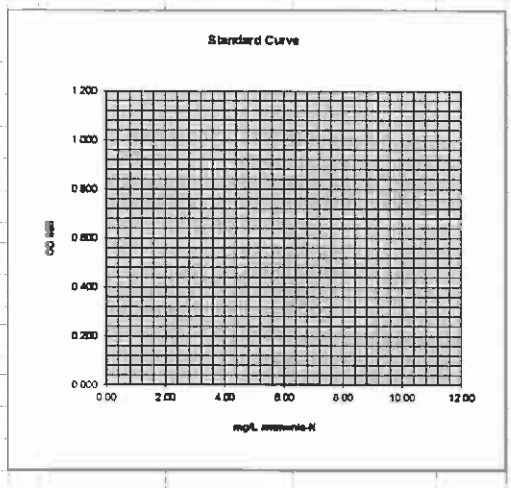
| | |
|---|---|
| | Bulk Sediment Porewaters |
| | Test Beaker Porewaters |
| X | Overlying Water |

Analyst: JB
Date analysed: 2/20/2015

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

**Result**

| | Sample description | Dilution factor | $OD_{655}$ | $NH_3$-N (mg/L) | pH | Salinity (ppt) |
|---|---|---|---|---|---|---|
| | Blank | ----- | ---- | ---- | | |
| | 1.0 mg/L $NH_3$-N Std. | ---- | .151 | 1.00 | | |
| | 3.0 mg/L $NH_3$-N Std. | ----- | .440 | 3.00 | | |
| | 6.0 mg/L $NH_3$-N Std. | ---- | .862 | 6.00 | | |
| | 10.0 mg/L $NH_3$-N Std. | ---- | 1.4 | 10.00 | | |
| | 3.0 mg/L spike | ---- | .449 | | | |
| | 3.0 mg/L spike dupl. | ----- | .446 | | | |
| | 5.0 mg/L 2nd source | | .710 | | | |
| 1 | Day 0 (1-23-15) | | | | | |
| 2 | 3 | 1 | .012 | | | |
| 3 | 7 | 1 | .023 | | | |
| 4 | 17 | 1 | .040 | | | |
| 5 | 18 | 1 | .020 | | | |
| 6 | | | | | | |
| 7 | Day 28 (2-20-15) | | | | | |
| 8 | 3 | 1 | .009 | | | |
| 9 | 7 | 1 | .002 | | | |
| 10 | 17 | 1 | .000 | | | |
| 11 | 18 | 1 | .000 | | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |
| 18 | | | | | | |
| 19 | | | | | | |
| 20 | | | | | | |
| 21 | | | | | | |
| 22 | | | | | | |
| 23 | | | | | | |
| 24 | | | | | | |
| 25 | | | | | | |
| 26 | | | | | | |
| 27 | | | | | | |
| 28 | | | | | | |
| 29 | | | | | | |
| 30 | | | | | | |
| 31 | | | | | | |
| 32 | | | | | | |
| 33 | | | | | | |
| 34 | | | | | | |
| 35 | | | | | | |
| 36 | | | | | | |




Reporting limit (mg/L) = 0.10

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

Sample volume (ml): 0.50
Dilution factor 1

**Sample Set Description:**
Test No.: 862-1
Test Day: 0,& 28
Species: *Hyalella*

**Sample Type (check)**
Bulk Sediment Porewaters
Test Beaker Porewaters
X Overlying Water

Analyst: JB
Date analysed: 2/20/2015

# CHAIN-OF-CUSTODY RECORDS

# Sample Custody Record

Samples Shipped to: NW Aquatic Sciences

HARTCROWSER

Hart Crowser, Inc.
1700 Westlake Avenue North, Suite 200
Seattle, Washington 98109-6212
Office: 206.324.9530 • Fax 206.328.5581

JOB 17800-56 LAB NUMBER

PROJECT NAME Jacobsen Terminals

HART CROWSER CONTACT Phil Cordell

SAMPLED BY: PRL

| LAB NO. | SAMPLE ID | DESCRIPTION | DATE | TIME | MATRIX | REQUESTED ANALYSIS: 10-day Chironomus dilution survival and growth* | 28-day Hyalella dilution survival and growth* | NO. OF CONTAINERS | OBSERVATIONS/COMMENTS/ COMPOSITING INSTRUCTIONS |
|---|---|---|---|---|---|---|---|---|---|
| | JT-SS-06 | 5192G | 1/12/15 | 1159 | Sediment | X | X | 4 | 4 liters sediment |
| | JT-SS-08 | 5193G | ↓ | 1058 | ↓ | X | X | 4 | " " |
| | JT-SS-10 | 5194G | ↓ | 1400 | ↓ | X | X | 4 | " " |

30 of 34

| RELINQUISHED BY | DATE | RECEIVED BY | DATE |
|---|---|---|---|
| SIGNATURE Phil Cordell | 1/13/15 | SIGNATURE GERALD IRISSARRI | 1-14-15 |
| PRINT NAME HC | TIME 1000 | PRINT NAME NAS | TIME 1140 |
| COMPANY | | COMPANY NAS | |
| RELINQUISHED BY | DATE | RECEIVED BY | DATE |
| SIGNATURE | TIME | SIGNATURE | TIME |
| PRINT NAME | | PRINT NAME | |
| COMPANY | | COMPANY | |

SPECIAL SHIPMENT HANDLING OR STORAGE REQUIREMENTS:

COOLER NO.: STORAGE LOCATION:

See Lab Work Order No. _____ for Other Contract Requirements

12 TOTAL NUMBER OF CONTAINERS

SAMPLE RECEIPT INFORMATION
CUSTODY SEALS: ☒YES ☐NO ☐N/A
GOOD CONDITION ☒YES ☐NO
TEMPERATURE 2.5 °C
SHIPMENT METHOD: ☐HAND ☐COURIER ☒OVERNIGHT

TURNAROUND TIME:
☐ 24 HOURS ☐ 1 WEEK
☐48 HOURS ☒STANDARD
☐72 HOURS OTHER _____

White and Yellow Copies to Lab Pink to Project Manager Lab to Return White Copy to Hart Crowser Gold to Sample Custodian

From: (206) 826-4527 Origin ID: LKEA
Phil Cordell
Hart Crowser, Inc
1700 Westlake Avenue North
Suite 200
Seattle, WA 98109

FedEx Express

Ship Date: 13JAN15
ActWgt: 75.0 LB
CAD: 4596184/INET3550

Delivery Address Bar Code

SHIP TO: (206) 324-9530 BILL SENDER
Gerald Irissarri
Northwest Aquatics Scientists
3814 Yakima Rd

NEWPORT, OR 97365

Ref # 17800-56-02
Invoice #
PO #
Dept #

WED - 14 JAN AM
STANDARD OVERNIGHT

TRK# 0201 7725 6121 8674

86 ONPA

97365
OR-US
PDX

522G18F15BA09

Rcvd 1130 1-14-15

**After printing this label**
1. Use the 'Print' button on this page to print your label to your laser or inkjet printer
2. Fold the printed page along the horizontal line.
3. Place label in shipping pouch and affix it to your shipment so that the barcode portion of the label can be read and scanned

**Warning** Use only the printed original label for shipping. Using a photocopy of this label for shipping purposes is fraudulent and could result in additional billing charges, along with the cancellation of your FedEx account number.
Use of this system constitutes your agreement to the service conditions in the current FedEx Service Guide, available on fedex.com. FedEx will not be responsible for any claim in excess of $100 per package, whether the result of loss, damage, delay, non-delivery, misdelivery, or misinformation, unless you declare a higher value, pay an additional charge, document your actual loss and file a timely claim. Limitations found in the current FedEx Service Guide apply. Your right to recover from FedEx for any loss, including intrinsic value of the package, loss of sales, income interest, profit, attorney's fees, costs, and other forms of damage whether direct, incidental, consequential, or special is limited to the greater of $100 or the authorized declared value. Recovery cannot exceed actual documented loss. Maximum for items of extraordinary value is $1,000, e.g. jewelry, precious metals, negotiable instruments and other items listed in our ServiceGuide. Written claims must be filed within strict time limits, see current FedEx Service Guide.

CUSTODY SEAL CUSTODY SEAL CUSTODY SEAL CUSTODY SEAL

Date 1/13/15

Initials

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# APPENDIX III

# RAW DATA – REFERENCE TOXICANT TEST

NORTHWESTERN AQUATIC SCIENCES — PROTOCOL NO. NAS-XXX-HA1

ACUTE TOXICITY TEST (ALL SPECIES)

REVIEWED PAGES 1-9 -GJI

Test No. 999-3381 Client: QC Test Investigator

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

Species *Hyalella azteca*

## 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. Yves Nakahara YN 2. GA Bulaber GAB

3. 4.

Test Beginning: 1-23-15 0915 Test Ending: 1-27-15 1030

## TEST MATERIAL

Description: Potassium Chloride Crystals - Fisher Lot No.: 114689

| | | | | |
|---|---|---|---|---|
| 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: 1-15-15

Water Quality: Cond. (umhos/cm): 283 Salinity (ppt) pH 7.5

Hardness (mg/L as $CaCO_3$): 77 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:

**Randomization chart:**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 0.5 | 0.125 | 0.063 | 1.0 | 0.25 | Ø | | | | |
| 0.125 | 0.25 | Ø | 0.063 | 0.5 | 1.0 | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |

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

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-HA1

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3381 Client QC Test Investigator

**TEST ORGANISMS**

Species: *Hyalella azteca* Age: 7-8 DAYS Size:

Source: Chesapeake Cultures, Hayes, VA Date received: 1-21-15

Acclimation Data:

| Date | Temp. (deg.C) | pH | DO (mg/L) | Cond. umhos/cm | Hardness (mg/L) | Alkalinity (mg/L) | Feeding Amount | Feeding description | Water changes |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
| | | | | | | | | | |
| | | | | | | | | | |
| 1-21-15 | 17.5 | 6.8 | >15.0 | 596 | 188 | 180 | 10mLs | 4 hr | Rec'd daph - 4 of 30 °C |
| 1-22-15 | 20.2 | 7.3 | 8.9 | 255 | 77 | 70 | " | " | 4 |
| 1-23-15 | 20.7 | 7.5 | 8.4 | 223 | 68 | 60 | " | " | |
| Mean | 19.5 | 7.2 | 10.8 | 358 | 111 | 103 | | | |
| S.D. | 1.7 | 0.4 | 3.7 | 207 | 67 | 67 | | | |
| (N) | 3 | 3 | 3 | 3 | 3 | 3 | | | |

Photoperiod during acclimation: 16:8, L:D

**TEST PROCEDURES AND CONDITIONS**

Test concentrations (50% series recommended): 1, 0.5, 0.25, 0.125, 0.063 0 g/L

Test chamber: 250 ml glass beakers Test volume: 100 ml

Replicates/treatment: 2 Organisms/treatment: 20 (10/rep)

Test water changes: None Aeration during test: None

Feeding: 0.5 ml YTC suspension per beaker on days 0 and 2

Duration: 24-hr, 48-hr, (96-hr) Test temperature (deg.C): (23 ± 1 or 20 ± 1)

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

**MISCELLANEOUS NOTES**

Test solution preparation:

Working stock: Dissolve 0.5g KCl crystals in dilution water and dilute to 500 mL.
Final conc.: 1.0 g/L.

| Test concentration (g/L) | KCl working stock (ml/200ml) | Dilution water |
|---|---|---|
| 1 | 200 | Brought up to final volume of 200 ml with dilution water and distributed evenly between two replicates |
| 0.5 | 100 | |
| 0.25 | 50 | |
| 0.125 | 25 | |
| 0.063 | 12.5 | |
| 0 | 0 | |

1-23-15 (89)

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3381 Client QC Test

DAILY RECORD SHEET

Day 0 ( 1 /23/15) GB/GJ

| Conc. (g/L) | Temp. (deg.C) | pH | Cond. (umhos/cm) | DO (ppm) | Hardness (mg/L) | Alkalinity (mg/L) | Survivors A | Survivors B |
|---|---|---|---|---|---|---|---|---|
| 1. 1 | 22.7 | 7.6 | 1990 | 8.5 | 86 | 70 | 10 | 10 |
| 2. 0.5 | 22.7 | 7.4 | 1160 | 8.6 | | | 10 | 10 |
| 3. 0.25 | 22.7 | 7.3 | 728 | 8.7 | | | 10 | 10 |
| 4. 0.125 | 22.8 | 7.3 | 510 | 8.6 | | | 10 | 10 |
| 5. 0.063 | 22.7 | 7.1 | 395 | 8.6 | | | 10 | 10 |
| 6. 0 | 22.4 | 7.1 | 282 | 8.5 | 86 | 60 | 10 | 10 |
| Each beaker fed 0.5 ml YTC suspension. Initials: GB | | | | | | | | |

Day 1 ( 1 /24/15) GB

| Conc. (g/L) | Temp. (deg.C) | pH | Cond. (umhos/cm) | DO (ppm) | Hardness (mg/L) | Alkalinity (mg/L) | Survivors A | Survivors B |
|---|---|---|---|---|---|---|---|---|
| 1. 1 | 23.2 | 7.7 | 1980 | 8.2 | | | 4(6D) | 5(5D) |
| 2. 0.5 | 23.2 | 7.6 | 1208 | 8.2 | | | 10 | 8(2D) |
| 3. 0.25 | 23.2 | 7.6 | 747 | 8.5 | | | 10 | 10 |
| 4. 0.125 | 23.2 | 7.6 | 521 | 8.3 | | | 10 | 10 |
| 5. 0.063 | 23.1 | 7.6 | 412 | 8.4 | | | 10 | 10 |
| 6. 0 | 23.0 | 7.5 | 300 | 8.2 | | | 10 | 10 |

Day 2 ( 1 /25/15) GJ

| Conc. (g/L) | Temp. (deg.C) | pH | Cond. (umhos/cm) | DO (ppm) | Hardness (mg/L) | Alkalinity (mg/L) | Survivors A | Survivors B |
|---|---|---|---|---|---|---|---|---|
| 1. 1 | 23.3 | 7.8 | 2080 | 8.0 | | | ϕ (4D) | ϕ (5D) |
| 2. 0.5 | 23.4 | 7.8 | 1264 | 8.0 | | | 7(3D) | 4(4D) |
| 3. 0.25 | 23.3 | 7.7 | 793 | 8.1 | | | 10 | 10 |
| 4. 0.125 | 23.4 | 7.7 | 555 | 8.1 | | | 10 | 10 |
| 5. 0.063 | 23.4 | 7.7 | 442 | 8.2 | | | 10 | 10 |
| 6. 0 | 23.3 | 7.5 | 309 | 8.2 | | | 10 | 10 |
| Each beaker fed 0.5 ml YTC suspension. Initials: GJ | | | | | | | | |

Day 3 ( 1 /26/15) GJ

| Conc. (g/L) | Temp. (deg.C) | pH | Cond. (umhos/cm) | DO (ppm) | Hardness (mg/L) | Alkalinity (mg/L) | Survivors A | Survivors B |
|---|---|---|---|---|---|---|---|---|
| 1. 1 | — | — | — | — | | | | |
| 2. 0.5 | 23.6 | 7.7 | 1282 | 7.6 | | | INADVERTENTLY | |
| 3. 0.25 | 23.6 | 7.7 | 804 | 7.6 | | | NOT ASSESSED | |
| 4. 0.125 | 23.6 | 7.6 | 560 | 7.6 | | | -GJ | |
| 5. 0.063 | 23.5 | 7.6 | 454 | 7.7 | | | | |
| 6. 0 | 23.4 | 7.5 | 316 | 7.8 | | | | |

Day 4 ( 1 /29/15) JK

| Conc. (g/L) | Temp. (deg.C) | pH | Cond. (umhos/cm) | DO (ppm) | Hardness (mg/L) | Alkalinity (mg/L) | Survivors A | Survivors B |
|---|---|---|---|---|---|---|---|---|
| 1. 1 | — | — | — | — | — | — | 0 | 0 |
| 2. 0.5 | 23.3 | 7.9 | 1269 | 8.1 | | | 7 | 3(1D) |
| 3. 0.25 | 23.2 | 7.9 | 785 | 8.1 | | | 10 | 10 |
| 4. 0.125 | 23.2 | 7.7 | 536 | 8.1 | | | 10 | 10 |
| 5. 0.063 | 23.3 | 7.8 | 446 | 8.1 | | | 10 | 10 |
| 6. 0 | 23.1 | 7.6 | 338 | 8.1 | 86 | 70 | 10 | 10 |
| Mean | | | | | | | | |
| SD | | | | | | | | |
| n | | | | | | | | |

(SEE PAGE 5)

# *Chesapeake Cultures*

P.O. Box 507 Hayes, VA 23072 (804)693-4046 (804)694-4704 fax
www.c-cultures.com
growfish@c-cultures.com

NAS
Shipment Information

Rcvd 1-21-15
K

Species Hyalella azteca Date 1/20/15

Age ~4-5 d.; ~1.5 mm P.O. No. verbal

Quantity 530+ Invoice No. 8585

Temperature 24°C Salinity ~ pH 7.88

Notes Thank you!

Biologist

*Please inspect shipment and report any problem immediately*

| Water Quality Data - test #999-338 Hyalella KCl QC test | | | | | | | |
|---|---|---|---|---|---|---|---|
| Day | Concentration (g/L) | Temperature | pH | Conductivity | DO | Hardness | Alkalinity |
| 0 | 1 | 22.7 | 7.6 | 1990 | 8.5 | 86 | 70 |
| 0 | 0.5 | 22.7 | 7.4 | 1160 | 8.6 | | |
| 0 | 0.25 | 22.7 | 7.3 | 728 | 8.7 | | |
| 0 | 0.125 | 22.8 | 7.3 | 510 | 8.6 | | |
| 0 | 0.063 | 22.7 | 7.1 | 395 | 8.6 | | |
| 0 | 0 | 22.4 | 7.1 | 282 | 8.5 | 86 | 60 |
| 1 | 1 | 23.2 | 7.7 | 1980 | 8.2 | | |
| 1 | 0.5 | 23.2 | 7.6 | 1208 | 8.2 | | |
| 1 | 0.25 | 23.2 | 7.6 | 747 | 8.5 | | |
| 1 | 0.125 | 23.2 | 7.6 | 521 | 8.3 | | |
| 1 | 0.063 | 23.1 | 7.6 | 412 | 8.4 | | |
| 1 | 0 | 23.0 | 7.5 | 300 | 8.2 | | |
| 2 | 1 | 23.3 | 7.8 | 2080 | 8.0 | | |
| 2 | 0.5 | 23.4 | 7.8 | 1264 | 8.0 | | |
| 2 | 0.25 | 23.3 | 7.8 | 793 | 8.1 | | |
| 2 | 0.125 | 23.4 | 7.7 | 555 | 8.1 | | |
| 2 | 0.063 | 23.4 | 7.7 | 442 | 8.2 | | |
| 2 | 0 | 23.3 | 7.5 | 309 | 8.2 | | |
| 3 | 1 | | | | | | |
| 3 | 0.5 | 23.6 | 7.7 | 1282 | 7.6 | | |
| 3 | 0.25 | 23.6 | 7.7 | 804 | 7.6 | | |
| 3 | 0.125 | 23.6 | 7.6 | 560 | 7.6 | | |
| 3 | 0.063 | 23.5 | 7.6 | 454 | 7.7 | | |
| 3 | 0 | 23.4 | 7.5 | 316 | 7.8 | | |
| 4 | 1 | | | | | | |
| 4 | 0.5 | 23.3 | 7.9 | 1269 | 8.1 | | |
| 4 | 0.25 | 23.2 | 7.9 | 785 | 8.1 | | |
| 4 | 0.125 | 23.2 | 7.7 | 536 | 8.1 | | |
| 4 | 0.063 | 23.3 | 7.8 | 446 | 8.1 | | |
| 4 | 0 | 23.1 | 7.6 | 338 | 8.1 | 86 | 70 |
| | MEAN | 23.2 | 7.6 | | 8.2 | 86 | 67 |
| | SD | 0.3 | 0.2 | | 0.3 | 0 | 6 |
| | N | 28 | 28 | | 28 | 3 | 3 |
| | MIN | 22.4 | 7.1 | | 7.6 | 86 | 60 |
| | MAX | 23.6 | 7.9 | | 8.7 | 86 | 70 |
| | | MEAN 1.0 g/L | | 2017 | | | |
| | | SD | | | | | |
| | | N | | 3 | | | |
| | | MEAN 0 g/L | | 309 | | | |
| | | SD | | 21 | | | |
| | | N | | 5 | | | |

## Acute 96-hr Toxicity Test-96 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 1/23/2015 09:15 | Test ID: | 999-3381 | Sample ID: | REF-Ref Toxicant |
| End Date: | 1/27/2015 10:30 | Lab ID: | ORNAS-Northwestern Aquati | Sample Type: | KCL-Potassium chloride |
| Sample Date: | | Protocol: | NASXXXHA1-Hyalella azteca | Test Species: | HA-Hyalella azteca |
| Comments: | | | | | |

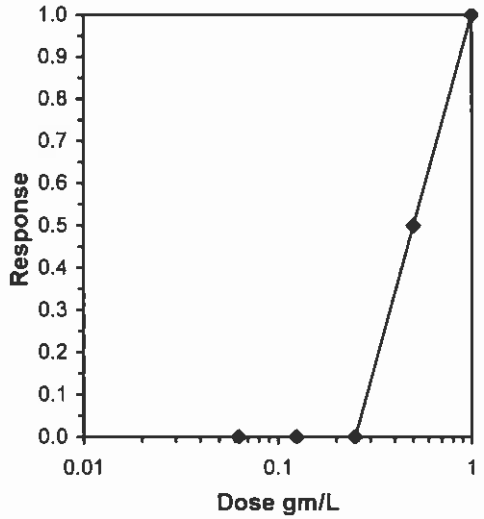
| Conc-gm/L | 1 | 2 |
|---|---|---|
| D-Control | 1.0000 | 1.0000 |
| 0.063 | 1.0000 | 1.0000 |
| 0.125 | 1.0000 | 1.0000 |
| 0.25 | 1.0000 | 1.0000 |
| 0.5 | 0.7000 | 0.3000 |
| 1 | 0.0000 | 0.0000 |

| | | | Transform: Arcsin Square Root | | | | | Number | Total |
|---|---|---|---|---|---|---|---|---|---|
| Conc-gm/L | Mean | N-Mean | Mean | Min | Max | CV% | N | Resp | Number |
| D-Control | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0 | 20 |
| 0.063 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0 | 20 |
| 0.125 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0 | 20 |
| 0.25 | 1.0000 | 1.0000 | 1.4120 | 1.4120 | 1.4120 | 0.000 | 2 | 0 | 20 |
| 0.5 | 0.5000 | 0.5000 | 0.7854 | 0.5796 | 0.9912 | 37.050 | 2 | 10 | 20 |
| 1 | 0.0000 | 0.0000 | 0.1588 | 0.1588 | 0.1588 | 0.000 | 2 | 20 | 20 |

| Auxiliary Tests | Statistic | Critical | Skew | Kurt |
|---|---|---|---|---|
| Normality of the data set cannot be confirmed | | | | |
| Equality of variance cannot be confirmed | | | | |

Trimmed Spearman-Karber

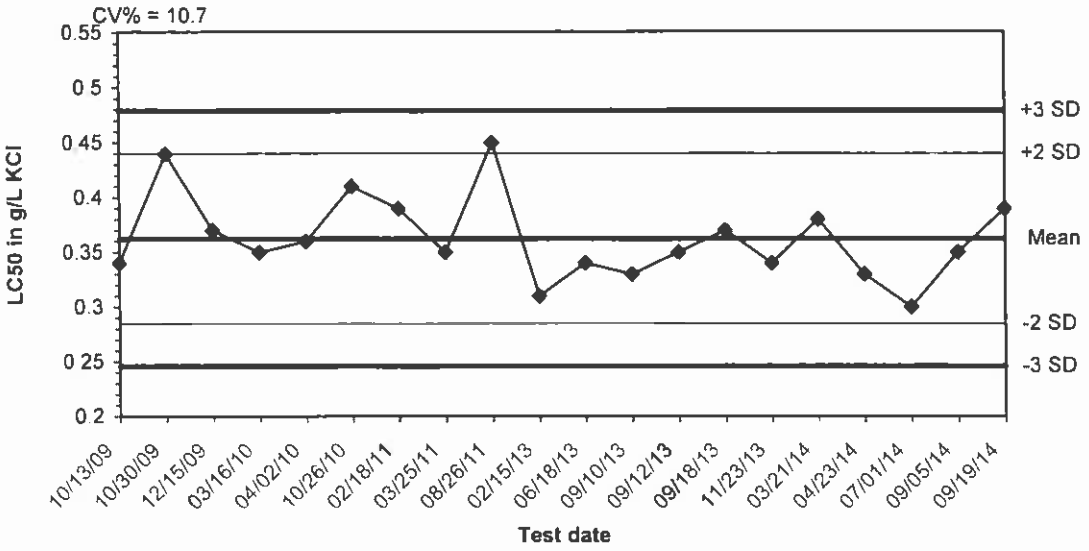
| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 0.5000 | 0.4282 | 0.5838 |
| 5.0% | 0.5000 | 0.4209 | 0.5940 |
| 10.0% | 0.5000 | 0.4119 | 0.6069 |
| 20.0% | 0.5000 | 0.3862 | 0.6474 |
| Auto-0.0% | 0.5000 | 0.4282 | 0.5838 |



| Test: AT-Acute 96-hr Toxicity Test | | | | | | | Test ID: 999-3381 | | |
|---|---|---|---|---|---|---|---|---|---|
| Species: HA-Hyalella azteca | | | | | | | Protocol: NASXXXHA1-Hyalella azteca acute | | |
| Sample ID: REF-Ref Toxicant | | | | | | | Sample Type: KCL-Potassium chloride | | |
| Start Date: 1/23/2015 09:15 | | | End Date: 1/27/2015 10:3 | | | | 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 | 2 | D-Control | 10 | | | | 10 | |
| | 3 | 1 | 0.063 | 10 | | | | 10 | |
| | 4 | 2 | 0.063 | 10 | | | | 10 | |
| | 5 | 1 | 0.125 | 10 | | | | 10 | |
| | 6 | 2 | 0.125 | 10 | | | | 10 | |
| | 7 | 1 | 0.250 | 10 | | | | 10 | |
| | 8 | 2 | 0.250 | 10 | | | | 10 | |
| | 9 | 1 | 0.500 | 10 | | | | 7 | |
| | 10 | 2 | 0.500 | 10 | | | | 3 | |
| | 11 | 1 | 1.000 | 10 | | | | 0 | |
| | 12 | 2 | 1.000 | 10 | | | | 0 | |

Comments: data entry verified against laboratory bench sheets 3-5-15 JRF

## Amphipod, Hyalella azteca, acute reference toxicant test




| Dates | Values | Mean | -2 SD | -3 SD | +2 SD | +3 SD |
|---|---|---|---|---|---|---|
| 10/13/09 | 0.3400 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 10/30/09 | 0.4400 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 12/15/09 | 0.3700 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 03/16/10 | 0.3500 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 04/02/10 | 0.3600 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 10/26/10 | 0.4100 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 02/18/11 | 0.3900 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 03/25/11 | 0.3500 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 08/26/11 | 0.4500 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 02/15/13 | 0.3100 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 06/18/13 | 0.3400 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 09/10/13 | 0.3300 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 09/12/13 | 0.3500 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 09/18/13 | 0.3700 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 11/23/13 | 0.3400 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 03/21/14 | 0.3800 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 04/23/14 | 0.3300 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 07/01/14 | 0.3000 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 09/05/14 | 0.3500 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |
| 09/19/14 | 0.3900 | 0.3625 | 0.2847 | 0.2458 | 0.4403 | 0.4792 |

# SECTION B

## Midge (*Chironomus dilutus*) 10-day sediment bioassay 862-3 data report

# TOXICITY TEST REPORT

## TEST IDENTIFICATION

Test No.: 862-3

Title: Toxicity of freshwater sediments using a 10-day midge, *Chironomus dilutus*, sediment bioassay as part of the Remedial Investigation at the Jacobson Terminals Property in Seattle, WA

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). Washington State Sediment Management Standards (SMS) (Chapter 173-204 WAC, Last Update: 2/25/13).

## STUDY MANAGEMENT

Study Sponsor: Hart Crowser, Inc., 1700 Westlake Ave. North, Suite 200, Seattle, WA 98109.

Sponsor's Study Monitor: Mr. Philip Cordell

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. Mngr./ Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, Ph.D., Sr. Aq. Toxicol.; G.A. Buhler, B.S., Aq. Toxicol.; J.B. Brown, B.S., D.V.M, Assoc. Aq. Toxicol.; Y. Nakahama, Sr. Tech.; L. Brady, Tech.

Study Schedule:

Test Beginning: 2-6-15, 1005 hrs.

Test Ending: 2-16-15, 1045 hrs.

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

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 of the Remedial Investigation at the Jacobson Terminals Property in Seattle, WA. Details are as follows:

| NAS Sample No. | 5192G | 5193G | 5194G |
|---|---|---|---|
| Description | JT-SS-06 | JT-SS-08 | JT-SS-10 |
| Collection Date | 1/12/15 | 1/12/15 | 1/12/15 |
| Receipt Date | 1/14/15 | 1/14/15 | 1/14/15 |

Control Sediment: The negative control sediment (NAS#5195G) was collected on 1-19-15 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.

Dates of Preparation: 1-19-15 and 2-6-15

Water Quality:

pH: 7.7, 7.5

conductivity: 108, 119 µmhos/cm

hardness: 26, 26 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: 2nd to 3rd instar, mean initial wt: 0.21 ± 0.01 mg
Source: Aquatic BioSystems, Fort Collins, CO
Acclimation: Holding conditions prior to testing averaged: Temperature, 22.7 °C; dissolved oxygen, 6.5 mg/L; pH, 7.3; conductivity, 376 µmhos/cm; hardness, 129 mg/L as $CaCO_3$; and alkalinity, 90 mg/L as $CaCO_3$. Photoperiod was 16:8, L:D. Half of the water in culture tanks was replaced 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
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.
Acceptance Criteria: Results are valid if mean control mortality does not exceed 30%, and the mean individual ash-free dry weight at test termination is ≥ 0.48 mg.
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. 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

Percent survival and average individual ash-free dry weight 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 2010. The values for mortality and individual ash-free dry wt for the test sediment were statistically compared against the reference sediment. Where appropriate, an arcsine square root transformation was performed on proportional mortality data before analysis. Following determination of normality and homogeneity of variances, a one-tailed Student T-test, Mann-Whitney or Approximate T test was conducted at the 0.05 level of significance. The statistical software used was BioStat (version Feb 9, 2006 (EXCEL)) bioassay software developed by the U.S. Army Corps of Engineers, Seattle District.

## 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.: 999-3391
Reference Toxicant and Source: Potassium Chloride (KCl), Fisher Lot #114689.
Test Date: 2-6-15.
Dilution Water Used: Moderately hard synthetic water prepared from Milli-Q® deionized water.
Result: 96-hr LC50, 5.74 g/L. This result is within the laboratory's control chart warning limits (2.71 –7.48 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. 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 Tables 2 and 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.

The test met the acceptability criteria specified in the SMS with 6.3% mean control mortality (≤30% required) and a control mean ash-free dry weight of 1.22 mg per larvae (≥0.48 mg required). The reference toxicant (positive control) EC50 result was within the laboratory's control chart limits (5.74 g/L; control chart mean ± 2 S.D. = 5.10 ± 2.39).

Interpretation was based on guidelines from the Washington State Sediment Management Standards (SMS) (Chapter 173-204 WAC, Last Update: 2/25/13). The SMS includes Sediment Cleanup Objectives (SCO) and Cleanup Screening Levels (CSL) biological criteria. The Sediment Cleanup Objectives establish a no adverse effects level, including no acute or chronic adverse effect, to the benthic community. The Cleanup Screening Levels establish a minor adverse effects level, including acute or chronic effects, to the benthic community. To qualify as an adverse effect under the SCO for mortality the mean mortality in the test sediment is greater than 20 percent over the mean control and statistically different from the control ($p = 0.05$). For the growth endpoint, a mean reduction in the biomass that is greater than 20 percent of the control sediment response and statistically different from the control ($p=0.05$). For the CSL adverse effects criteria mean mortality in the test sediment is greater than 30 percent over the mean control and statistically different from the control ($p = 0.05$). For the growth endpoint, a mean reduction in the biomass that is greater than 30 percent and statistically different from the control ($p = 0.05$).

Mean mortality of test sediments JT-SS-06, JT-SS-08 and JT-SS-10 was 10.0%, 15.0% and 8.8%, respectively. None of these were more than 20% above the control mortality; therefore none of the sediments exceeded the SCO or CSL criteria for mortality. For the growth endpoint, all three test sediments were significantly different from the control, but only two test sediments, JT-SS-08, and JT-SS-10, resulted in and exceeded under the SCO guidelines with ash-free dry weights of 0.93 and 0.91 mg/individual, respectively. No sediment failed the CSL criteria for growth since none were >30% different from the control.

**STUDY APPROVAL**

Project Manager/Study Director — Date: 3-19-15

Quality Assurance Unit — Date: 3-19-15

Assistant Laboratory Director — Date: 3-19-15

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) | 22.7 ± 0.5 | 22.0 | 24.0 | 44 |
| Dissolved oxygen (mg/L) | 6.1 ± 0.7 | 4.5 | 7.4 | 44 |
| Conductivity (µmhos/cm) | 129 ± 29 | 108 | 217 | 12 |
| pH | 7.2 ± 0.1 | 7.1 | 7.4 | 12 |
| Hardness (mg/L as $CaCO_3$) | 29 ± 4 | 26 | 34 | 8 |
| Alkalinity (mg/L as $CaCO_3$) | 36 ± 7 | 30 | 50 | 8 |
| Total ammonia (mg/L) | --- | <0.1 | 0.3 | 8 |

Table 2. Mortality results of *Chironomus* toxicity test and data interpretation using guidelines from the Washington State SMS.

| Sample description | Percent mortality (Mean ± SD) | Significantly higher than the control sediment at α=0.05? | Percent higher (absolute) than control sediment | Exceedance under SCO?[1] | Exceedance under one-test criteria for CSL[2] |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 6.3 ± 5.2 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 10.0 ± 14.1 | No | 3.7 | No | No |
| JT-SS-08 (NAS# 5193G) | 15.0 ± 16.9 | No | 8.7 | No | No |
| JT-SS-10 (NAS# 5194G) | 8.8 ± 11.3 | No | 2.5 | No | No |

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >20%.

[2] Cleanup Screening Levels (CSL) exceedance if the test sediment mean mortality is significantly higher (1-tailed t-test at P≤0.05) than the control sediment mean mortality and the absolute difference is >30%.

Table 3. Growth results of *Chironomus* toxicity test and data interpretation using guidelines from the Washington State SMS.

| Sample description | Average ash-free dry wt/midge (mg)* (Mean ± SD) | Statistically significantly lower than control sediment at α=0.05? | Percent lower than control sediment | Exceedance under SCO?[1] ($MIG_C$-$MIG_T$/$MIG_C$>0.20) | Exceedance under one-test criteria for CSL?[2] ($MIG_C$-$MIG_T$/$MIG_C$>0.30) |
|---|---|---|---|---|---|
| Control (NAS# 5195G) | 1.22 ± 0.09 | --- | --- | --- | --- |
| JT-SS-06 (NAS# 5192G) | 0.98 ± 0.11 | Yes | 19.7 | No | No |
| JT-SS-08 (NAS# 5193G) | 0.93 ± 0.15 | Yes | 23.8 | Yes | No |
| JT-SS-10 (NAS# 5194G) | 0.91 ± 0.12 | Yes | 25.4 | Yes | No |

* 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)

[1] Sediment Cleanup Objectives (SCO) exceedance if the test sediment mean growth is significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth, and the difference is >20%.

[2] Cleanup Screening Levels (CSL) exceedance (one-test criteria) if the test sediment mean individual growth in significantly lower (1-tailed t-test at P≤0.05) than the control sediment mean growth, and the difference is >30%.

# 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

Test No. 862-3 Client Hart Crowser Investigator

**STUDY MANAGEMENT**

Client: Hart Crowser, Inc., 1700 Westlake Ave. North, Suite 200, Seattle, WA 98109

Client's Study Monitor: Mr. Philip Cordell

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. [illegible]
3. [illegible] 4. Lauren Brady LB
5. [illegible] 6.
7. 8.

Study Schedule:

Test Beginning: 2-6-15 1005 Test Ending: 2-16-15 1045

**TEST MATERIAL**

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

| | | | | | |
|---|---|---|---|---|---|
| NAS Sample No.: | 5192G | 5193G | 5194G | | |
| Description: | JT-SS-06 | JT-SS-08 | JT-SS-10 | | |
| Collection Date: | 1/12/15 | 1/12/15 | 1/12/15 | | |
| Receipt Date: | 1/14/15 | 1/14/15 | 1/14/15 | | |
| : | | | | | |
| NAS Sample No.: | | | | | |
| Description: | | | | | |
| Collection Date: | | | | | |
| Receipt Date: | | | | | |
| : | | | | | |
| NAS Sample No.: | | | | | |
| Description: | | | | | |
| Collection Date: | | | | | |
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| 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

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator _____

SEDIMENT DESCRIPTIONS -- SUPPLEMENTAL NOTES

| Sample No. | Description |
|---|---|
| 5195G | FINE BLACK MUD |
| 5192G | DARK BROWN SANDY MUD W/ WOODY FRAGMENTS, SOME PLASTIC FILM |
| 5193G | WATERY SANDY BLACK MUD W/ WOODY FRAGMENTS |
| 5194G | BLACK MUDDY SAND W/ WOOD FRAGMENTS, BLACK PLASTIC FILM, ALUMINUM FOIL |

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

**TEST WATER**

Source: Dechlorinated municipal tap water

Date of Collection: 1-19-15, 2-6-15

pH 7.7, 7.5

Cond (umhos/cm2) 108, 119

Hardness (mg/L) 26, 26

Alkalinity (mg/L) 30, 30

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

Treatments: Dechlorinated, aerated

**TEST ORGANISMS**

Species: *Chironomus dilutus* Age: 2ND TO 3rd instar

Source: ~~NAS cultures~~ AQUATIC BIOSYSTEMS Date received: 2-5-15

Acclimation Data:

| Date | Temp. (deg.C) | pH | DO (mg/L) | Cond. umhos/cm | Hardness (mg/L) | Alkalinity (mg/L) | Feeding | Water changes |
|---|---|---|---|---|---|---|---|---|
| 2-5-15 | 20.6 | 6.9 | 6.2 | 436 | 171 | 110 | Animals fed Tetra Fin | YES |
| 2-6-15 | 24.8 | 7.6 | 6.9 | 316 | 86 | 70 | and *Selenastrum* | |
| | | | | | | | Details recorded on | |
| | | | | | | | Chironomid culture | |
| | | | | | | | data sheets | |
| | | | | | | | | |
| Mean | 22.7 | 7.3 | 6.5 | 376 | 129 | 90 | | |
| S.D. | – | – | – | – | – | – | | |
| (N) | 2 | 2 | 2 | 2 | 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 Organisms/treatment: (80) 80 (10/REP)

Test water changes: Twice daily

Aeration: only if DO falls below 2.5 mg/L

Feeding: everyday beginning with day zero

Test temperature (°C): 23 ± 1

Beaker placement: Total randomization

Photoperiod: 16:8, L:D

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: 1/19/15

Sieved through 0.5 -mm screen

Storage: darkness at 4°C, in sealed containers NAS# 5195G

**MISCELLANEOUS NOTES**

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

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

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 | 32 | | | | |
| 1 | 7 | 13 | 19 | 25 | 31 | | | | |

Randomization chart: FRONT

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | |
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Randomization chart:

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
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NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 0 (2/6/15) 1/2/15

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness* (mg/L) | Alkalinity* (mg/L) | NH3* (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | ~3.6 | 7.0 | 118 | 7.1 | 26 | 30 | | Each beaker fed 1.5 ml |
| 12 | 24.0 | 7.4 | 217 | 7.4 | 26 | 30 | | Tetra Fin suspension |
| 16 | 23.8 | 7.2 | 120 | 7.3 | 34 | 30 | | Initials: GSL |
| 28 | 24.0 | 6.7 | 136 | 7.2 | 34 | 30 | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: GAB |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1610 |
| | | | | | | | | Initials: KA |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 1 (2/7/15) 0815

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.2 | 6.6 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.6 | 6.8 | | | | | | Tetra Fin suspension |
| 16 | 22.3 | 6.8 | | | | | | Initials: GSL |
| 28 | 23.0 | 6.4 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1615 |
| | | | | | | | | Initials: KA |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 2 (2/8/15) GSL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.2 | 6.7 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.6 | 6.4 | | | | | | Tetra Fin suspension |
| 16 | 22.4 | 6.8 | | | | | | Initials: GSL |
| 28 | 22.9 | 6.8 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1605 |
| | | | | | | | | Initials: |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 3 (2/9/15) GSL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.4 | 6.5 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.9 | 6.1 | | | | | | Tetra Fin suspension |
| 16 | 22.7 | 6.2 | | | | | | Initials: GSL |
| 28 | 23.2 | 6.1 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1606 |
| | | | | | | | | Initials: |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 4 (2/10/15) ✓

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.1 | 6.5 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.4 | 6.1 | | | | | | Tetra Fin suspension |
| 16 | 22.2 | 6.3 | | | | | | Initials: ✓ |
| 28 | 22.7 | 6.7 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1620 |
| | | | | | | | | Initials: CMS |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 5 (2/11/15) BB

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.0 | 6.6 | 108 | 7.2 | | | | Each beaker fed 1.5 ml |
| 12 | 22.4 | 6.3 | 139 | 7.1 | | | | Tetra Fin suspension |
| 16 | 22.2 | 6.2 | 112 | 7.2 | | | | Initials: BB |
| 28 | 22.5 | 6.6 | 117 | 7.1 | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: CMS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1605 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 6 (2/4/15) ✓ LS

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.1 | 4.8 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.8 | 4.6 | | | | | | Tetra Fin suspension |
| 16 | 22.4 | 4.6 | | | | | | Initials: ✓ |
| 28 | 23.4 | 4.5 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1030 |
| | | | | | | | | Initials: LS |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 7 (2/13/15) ✓

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.6 | 5.5 | | | | | | Each beaker fed 1.5 ml |
| 12 | 23.1 | 5.3 | | | | | | Tetra Fin suspension |
| 16 | 22.8 | 5.3 | | | | | | Initials: GAS |
| 28 | 23.6 | 5.9 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0505 |
| | | | | | | | | Initials: GAS |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1615 |
| | | | | | | | | Initials: ✓ |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

DAILY RECORD SHEET

Day 8 (2/14/15) GB

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.1 | 5.4 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.6 | 5.6 | | | | | | Tetra Fin suspension |
| 16 | 22.3 | 5.7 | | | | | | Initials: 0500 GB |
| 28 | 22.9 | 5.9 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 615 |
| | | | | | | | | Initials: GSW |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1630 |
| | | | | | | | | Initials: GB |
| | | | | | | | | |

*Water quality measurements to be taken.

Day 9 (2/15/15) GSL

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond. (umhos/cm) | pH | Hardness (mg/L) | Alkalinity (mg/L) | NH3 (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.4 | 5.6 | | | | | | Each beaker fed 1.5 ml |
| 12 | 22.8 | 5.4 | | | | | | Tetra Fin suspension |
| 16 | 22.6 | 5.4 | | | | | | Initials: GSL |
| 28 | 23.2 | 5.3 | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |
| | | | | | | | | |
| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 1620 |
| | | | | | | | | Initials: GSL |
| | | | | | | | | |

*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator _____

DAILY RECORD SHEET

Day 10 (2/16/15) GJ

| Beaker No. | Temp.* (deg.C) | DO* (ppm) | Cond.* (umhos/cm) | pH* | Hardness* (mg/L) | Alkalinity* (mg/L) | NH3* (ppm) | Comments |
|---|---|---|---|---|---|---|---|---|
| 9 | 22.3 | 6.5 | 116 | 7.1 | 26 | 40 | | |
| 12 | 22.9 | 6.3 | 129 | 7.1 | 26 | 40 | | |
| 16 | 22.6 | 6.4 | 117 | 7.1 | 26 | 50 | | |
| 28 | 23.4 | 6.2 | 123 | 7.1 | 34 | 40 | | |
| | | | | | | | | |
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| | | | | | | | | Water changed in all |
| | | | | | | | | beakers. |
| | | | | | | | | Time: 0510 |
| | | | | | | | | Initials: GJ |
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*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 |
|---|---|---|---|---|---|---|---|---|
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*Water quality measurements to be taken.

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

DAY 10 TEST TERMINATION SHEET

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

| Beaker No. | Number of survivors | Initials |
|---|---|---|
| | | |

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator _____

Tare: Date 2-6-15 Oven temp ( C.) 550 Drying time (hr.) 2 Initials GJL
Standard Weights: 10 mg: 10.006 100mg: 100.016

Final: #1 Date 2-9-15 Oven temp ( C.) 62 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.007 100mg: 100.015

Final: #2 Date 2-11-15 Oven temp ( C.) 63 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.006 100mg: 10.016

Ashed Date 2-12-15 Oven temp ( C.) 555 Drying time (hr.) 2 Initials GJL
Standard Weights: 10 mg: 10.007 100mg: 100.015

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

**(Dry overnight at 60-90 °C, ash for 2 hrs at 550°C)**

| Pan # | Tare wt. (mg) | Total wt. (mg) 1 | Total wt. (mg) 2 | Ashed total wt. (mg) | #Weighed | Comments |
|---|---|---|---|---|---|---|
| 1 | 50.59 | 52.65 | 52.61 | 50.75 | 10 | |
| 2 | 45.38 | 47.68 | 47.68 | 45.52 | 10 | |
| 3 | 47.92 | 50.08 | 50.03 | 48.06 | 10 | |
| 4 | 48.84 | 50.81 | 50.78 | 49.00 | 10 | |
| 5 | 41.81 | 43.80 | 43.79 | 41.96 | 10 | |

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CT4b

CHIRONOMUS DILUTUS 10-DAY SOLID PHASE SEDIMENT TEST

Test No. 862-3 Client Hart Crowser Investigator

**WEIGHING DATA SHEET**

Tare: Date 2-9-15 Oven temp ( C.) 550 Drying time (hr.) 2 Initials JRF
Standard Weights: 10 mg: 10.007 100mg: 100.009

Final #1: Date 2-17-15 Oven temp ( C.) 62 Drying time (hr.) 24 Initials GJL
Standard Weights: 10 mg: 10.007 100mg: 100.017

Final #2: Date 2-18-15 Oven temp ( C.) 61 Drying time (hr.) 22 Initials GJL
Standard Weights: 10 mg: 10.005 100mg: 100.015

Final #3: Date 2-19-15 Oven temp ( C.) 550 Drying time (hr.) 2 Initials JRF
Standard Weights: 10 mg: 10.006 100mg: 100.008

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 | 91.57 | 99.16 | 99.12 | 9 | LB | 93.18 | |
| 2 | 2 | 94.72 | 110.65 | 110.59 | 9 | LB | 99.34 | |
| 3 | 3 | 91.38 | 104.78 | 104.72 | 10 | LB | 94.54 | |
| 4 | 4 | 95.13 | 110.63 | 110.57 | 9 | LB | 99.60 | |
| 5 | 5 | 97.48 | 112.61 | 112.47 | 10 | LB | 101.97 | |
| 6 | 6 | 86.54 | 97.96 | 97.91 | 10 | LB | 89.22 | |
| 7 | 7 | 102.37 | 119.25 | 119.18 | 9 | LB | 107.17 | |
| 8 | 8 | 97.82 | 108.74 | 108.69 | 10 | LB | 100.38 | |
| 9 | 9 | 95.79 | 107.71 | 107.66 | 10 | LB | 98.43 | |
| 10 | 10 | 86.88 | 96.42 | 96.39 | 10 | LB | 89.04 | |
| 11 | 11 | 60.69 | 69.14 | 69.11 | 6 | LB | 62.54 | |
| 12 | 12 | 91.27 | 107.62 | 107.57 | 10 | LB | 96.15 | |
| 13 | 13 | 103.87 | 111.46 | 111.44 | 7 | LB | 105.52 | |
| 14 | 14 | 89.36 | 106.79 | 106.74 | 10 | LB | 94.24 | |
| 15 | 15 | 92.85 | 106.20 | 106.15 | 9 | LB | 95.78 | |
| 16 | 16 | 92.47 | 101.20 | 101.14 | 6 | LB | 94.32 | |
| 17 | 17 | 89.93 | 101.72 | 101.64 | 10 | LB | 92.78 | |
| 18 | 18 | 93.05 | 108.27 | 108.20 | 9 | LB | 97.35 | |
| 19 | 19 | 90.24 | 102.97 | 102.91 | 10 | LB | 93.19 | |
| 20 | 20 | 96.56 | 109.33 | 109.27 | 10 | LB | 99.53 | |
| 21 | 21 | 95.31 | 107.66 | 107.61 | 9 | LB | 98.38 | |
| 22 | 22 | 91.56 | 104.09 | 104.05 | 10 | LB | 94.44 | |
| 23 | 23 | 85.64 | 99.39 | 99.35 | 10 | LB | 88.84 | |
| 24 | 24 | 95.40 | 106.30 | 106.27 | 8 | LB | 97.95 | |
| 25 | 25 | 88.72 | 105.18 | 105.13 | 9 | LB | 93.38 | |
| 26 | 26 | 95.60 | 104.35 | 104.33 | 9 | LB | 97.60 | |
| 27 | 27 | 94.09 | 106.45 | 106.40 | 10 | LB | 97.20 | |
| 28 | 28 | 93.17 | 102.93 | 102.89 | 8 | LB | 95.36 | |
| 29 | 29 | 94.74 | 105.63 | 105.60 | 9 | LB | 97.34 | |
| 30 | 30 | 101.96 | 112.46 | 112.43 | 8 | LB | 104.36 | |
| 31 | 31 | 94.64 | 102.47 | 102.45 | 6 | LB | 96.44 | |
| 32 | 32 | 93.81 | 103.85 | 103.84 | 9 | LB | 96.14 | |
| | | | | | | | | |

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Toll Free: 800/331-5916
Tel:970/484-5091 Fax:970/484-2514

## ORGANISM HISTORY

DATE: 2/4/2015

SPECIES: *Chironomus dilutus (formerly C. tentans)*

AGE: Deposited 1/24/2015

LIFE STAGE: Second Instar 2/4/2015

HATCH DATE: Emergent date 2/17/2015

BEGAN FEEDING: Immediately

FOOD: *Selenastrum* sp., Flake slurry

RECEIVED 2-5-15
-GL

| Water Chemistry Record: | Current | Range |
|---|---|---|
| TEMPERATURE: | 22°C | 22-26°C |
| SALINITY/CONDUCTIVITY: | -- | -- |
| TOTAL HARDNESS (as $CaCO_3$): | 190 mg/l | 100-190 mg/l |
| TOTAL ALKALINITY (as $CaCO_3$): | 60 mg/l | 50-90 mg/l |
| pH: | 7.50 | 7.50-8.20 |

**Comments:**

*Facility Supervisor*

# TEST DATA ANALYSIS RECORDS

data entry verified against laboratory bench sheets
3-17-15
JRF

Test Number: 862-3

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 | ashed wt (mg) | avg. AFDW/ organism |
|---|---|---|---|---|---|---|
| 1 | 50.59 | 52.61 | 10 | 0.20 | 50.75 | 0.19 |
| 2 | 45.38 | 47.68 | 10 | 0.23 | 45.52 | 0.22 |
| 3 | 47.92 | 50.03 | 10 | 0.21 | 48.06 | 0.20 |
| 4 | 48.84 | 50.78 | 10 | 0.19 | 49.00 | 0.18 |
| 5 | 41.81 | 43.79 | 10 | 0.20 | 41.96 | 0.18 |
| | | | Mean | 0.21 | | 0.19 |
| | | | SD | 0.01 | | 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 | 5 | 5195G | Control | 1 | | 10 | 10 | 0 | 100.0 | 0.0 | 97.48 | 10 | 112.47 | 101.97 | 14.99 | 1.50 | 10.50 | 1.05 | | | | | | | |
| 2 | 4 | 5195G | Control | 2 | | 10 | 9 | 1 | 90.0 | 10.0 | 95.13 | 9 | 110.57 | 99.60 | 15.44 | 1.72 | 10.97 | 1.22 | | | | | | | |
| 3 | 25 | 5195G | Control | 3 | | 10 | 9 | 1 | 90.0 | 10.0 | 88.72 | 9 | 105.13 | 93.38 | 16.41 | 1.82 | 11.75 | 1.31 | | | | | | | |
| 4 | 14 | 5195G | Control | 4 | | 10 | 10 | 0 | 100.0 | 0.0 | 89.36 | 10 | 106.74 | 94.24 | 17.38 | 1.74 | 12.50 | 1.25 | | | | | | | |
| 5 | 2 | 5195G | Control | 5 | | 10 | 9 | 1 | 90.0 | 10.0 | 94.72 | 9 | 110.59 | 99.34 | 15.87 | 1.76 | 11.25 | 1.25 | Mean | 9.4 | 0.6 | 93.8 | 6.3 | 1.72 | 1.22 |
| 6 | 18 | 5195G | Control | 6 | | 10 | 9 | 1 | 90.0 | 10.0 | 93.05 | 9 | 108.20 | 97.35 | 15.15 | 1.68 | 10.85 | 1.21 | SD | 0.5 | 0.5 | 5.2 | 5.2 | 0.12 | 0.09 |
| 7 | 7 | 5195G | Control | 7 | | 10 | 9 | 1 | 90.0 | 10.0 | 102.37 | 9 | 119.18 | 107.17 | 16.81 | 1.87 | 12.01 | 1.33 | n | 8 | 8 | 8 | 8 | 8 | 8 |
| 8 | 12 | 5195G | Control | 8 | wq replicate | 10 | 10 | 0 | 100.0 | 0.0 | 91.27 | 10 | 107.57 | 96.15 | 16.30 | 1.63 | 11.42 | 1.14 | | | | | | | |
| 9 | 8 | 5192G | JT-SS-06 | 1 | | 10 | 10 | 0 | 100.0 | 0.0 | 97.82 | 10 | 108.69 | 100.38 | 10.87 | 1.09 | 8.31 | 0.83 | | | | | | | |
| 10 | 31 | 5192G | JT-SS-06 | 2 | | 10 | 6 | 4 | 60.0 | 40.0 | 94.64 | 6 | 102.45 | 96.44 | 7.81 | 1.30 | 6.01 | 1.00 | | | | | | | |
| 11 | 24 | 5192G | JT-SS-06 | 3 | | 10 | 8 | 2 | 80.0 | 20.0 | 95.40 | 8 | 106.27 | 97.95 | 10.87 | 1.36 | 8.32 | 1.04 | | | | | | | |
| 12 | 23 | 5192G | JT-SS-06 | 4 | | 10 | 10 | 0 | 100.0 | 0.0 | 85.64 | 10 | 99.35 | 88.84 | 13.71 | 1.37 | 10.51 | 1.05 | | | | | | | |
| 13 | 19 | 5192G | JT-SS-06 | 5 | | 10 | 10 | 0 | 100.0 | 0.0 | 90.24 | 10 | 102.91 | 93.19 | 12.67 | 1.27 | 9.72 | 0.97 | Mean | 9.0 | 1.0 | 90.0 | 10.0 | 1.27 | 0.98 |
| 14 | 32 | 5192G | JT-SS-06 | 6 | | 10 | 9 | 1 | 90.0 | 10.0 | 93.81 | 9 | 103.84 | 96.14 | 10.03 | 1.11 | 7.70 | 0.86 | SD | 1.4 | 1.4 | 14.1 | 14.1 | 0.13 | 0.11 |
| 15 | 15 | 5192G | JT-SS-06 | 7 | | 10 | 9 | 1 | 90.0 | 10.0 | 92.85 | 9 | 106.15 | 95.78 | 13.30 | 1.48 | 10.37 | 1.15 | n | 8 | 8 | 8 | 8 | 8 | 8 |
| 16 | 9 | 5192G | JT-SS-06 | 8 | wq replicate | 10 | 10 | 0 | 100.0 | 0.0 | 95.79 | 10 | 107.66 | 98.43 | 11.87 | 1.19 | 9.23 | 0.92 | | | | | | | |
| 17 | 30 | 5193G | JT-SS-08 | 1 | | 10 | 8 | 2 | 80.0 | 20.0 | 101.96 | 8 | 112.43 | 104.36 | 10.47 | 1.31 | 8.07 | 1.01 | | | | | | | |
| 18 | 29 | 5193G | JT-SS-08 | 2 | | 10 | 9 | 1 | 90.0 | 10.0 | 94.74 | 9 | 105.60 | 97.34 | 10.86 | 1.21 | 8.26 | 0.92 | | | | | | | |
| 19 | 10 | 5193G | JT-SS-08 | 3 | | 10 | 10 | 0 | 100.0 | 0.0 | 86.88 | 10 | 96.39 | 89.04 | 9.51 | 0.95 | 7.35 | 0.73 | | | | | | | |
| 20 | 26 | 5193G | JT-SS-08 | 4 | | 10 | 9 | 1 | 90.0 | 10.0 | 95.60 | 9 | 104.33 | 97.60 | 8.73 | 0.97 | 6.73 | 0.75 | | | | | | | |
| 21 | 11 | 5193G | JT-SS-08 | 5 | | 10 | 6 | 4 | 60.0 | 40.0 | 60.69 | 6 | 69.11 | 62.54 | 8.42 | 1.40 | 6.57 | 1.10 | Mean | 8.5 | 1.5 | 85.0 | 15.0 | 1.21 | 0.93 |
| 22 | 6 | 5193G | JT-SS-08 | 6 | | 10 | 10 | 0 | 100.0 | 0.0 | 86.54 | 10 | 97.91 | 89.22 | 11.37 | 1.14 | 8.69 | 0.87 | SD | 1.7 | 1.7 | 16.9 | 16.9 | 0.18 | 0.15 |
| 23 | 27 | 5193G | JT-SS-08 | 7 | | 10 | 10 | 0 | 100.0 | 0.0 | 94.09 | 10 | 106.40 | 97.20 | 12.31 | 1.23 | 9.20 | 0.92 | n | 8 | 8 | 8 | 8 | 8 | 8 |
| 24 | 16 | 5193G | JT-SS-08 | 8 | wq replicate | 10 | 6 | 4 | 60.0 | 40.0 | 92.47 | 6 | 101.14 | 94.32 | 8.67 | 1.45 | 6.82 | 1.14 | | | | | | | |
| 25 | 13 | 5194G | JT-SS-10 | 1 | | 10 | 7 | 3 | 70.0 | 30.0 | 103.87 | 7 | 111.44 | 105.52 | 7.57 | 1.08 | 5.92 | 0.85 | | | | | | | |
| 26 | 21 | 5194G | JT-SS-10 | 2 | | 10 | 9 | 1 | 90.0 | 10.0 | 95.31 | 9 | 107.61 | 98.38 | 12.30 | 1.37 | 9.23 | 1.03 | | | | | | | |
| 27 | 17 | 5194G | JT-SS-10 | 3 | | 10 | 10 | 0 | 100.0 | 0.0 | 89.93 | 10 | 101.64 | 92.78 | 11.71 | 1.17 | 8.86 | 0.89 | | | | | | | |
| 28 | 1 | 5194G | JT-SS-10 | 4 | | 10 | 9 | 1 | 90.0 | 10.0 | 91.57 | 9 | 99.12 | 93.18 | 7.55 | 0.84 | 5.94 | 0.66 | | | | | | | |
| 29 | 20 | 5194G | JT-SS-10 | 5 | | 10 | 10 | 0 | 100.0 | 0.0 | 96.56 | 10 | 109.27 | 99.53 | 12.71 | 1.27 | 9.74 | 0.97 | Mean | 9.1 | 0.9 | 91.3 | 8.8 | 1.19 | 0.91 |
| 30 | 3 | 5194G | JT-SS-10 | 6 | | 10 | 10 | 0 | 100.0 | 0.0 | 91.38 | 10 | 104.72 | 94.54 | 13.34 | 1.33 | 10.18 | 1.02 | SD | 1.1 | 1.1 | 11.3 | 11.3 | 0.17 | 0.12 |
| 31 | 22 | 5194G | JT-SS-10 | 7 | | 10 | 10 | 0 | 100.0 | 0.0 | 91.56 | 10 | 104.05 | 94.44 | 12.49 | 1.25 | 9.61 | 0.96 | n | 8 | 8 | 8 | 8 | 8 | 8 |
| 32 | 28 | 5194G | JT-SS-10 | 8 | wq replicate | 10 | 8 | 2 | 80.0 | 20.0 | 93.17 | 8 | 102.89 | 95.36 | 9.72 | 1.22 | 7.53 | 0.94 | | | | | | | |

Project Name: P862-3 Chironomus % Mortality

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-06 |
| Alias: | NAS# 5192G |
| Replicates: | 8 |
| Mean: | 10 |
| SD: | 14.142 |
| Tr Mean: | N/A |
| Trans SD: | N/A |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 6.25 |
| SD: | 5.175 |
| Tr Mean: | N/A |
| Trans SD: | N/A |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 10.868 |
| SS: | 2244.172 |
| K: | 8 |
| b: | 42.996 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.8237 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | No |
| Override Option: | Not Invoked |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 12.833 |
| Test Residual SD: | 6.43 |
| Ref. Residual Mean: | 8.641 |
| Ref. Residual SD: | 2.385 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 1.7288 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Mann-Whitney |
| Balanced Design: | Yes |
| Transformation: | rank-order |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 <= x2 |
| Alternate: | x1 > x2 |
| Mann-Whitney N1: | 8 |
| Mann-Whitney N2: | 8 |
| Degrees of Freedom: | |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 33 |
| Critical Value: | >= 49.000 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0 | 4 | 0 | 4 | 12.833 | 11.522 | 4 | | -12.833 |
| 2 | 40 | 16 | 10 | 11 | 26.398 | 6.913 | 4 | | -12.833 |
| 3 | 20 | 15 | 10 | 11 | 13.732 | 6.913 | 4 | | -12.833 |
| 4 | 0 | 4 | 0 | 4 | 12.833 | 11.522 | 4 | | -12.833 |
| 5 | 0 | 4 | 10 | 11 | 12.833 | 6.913 | 4 | | -11.522 |
| 6 | 10 | 11 | 10 | 11 | 5.602 | 6.913 | 4 | | -11.522 |
| 7 | 10 | 11 | 10 | 11 | 5.602 | 6.913 | 4 | | -11.522 |
| 8 | 0 | 4 | 0 | 4 | 12.833 | 11.522 | 11 | | 5.602 |
| 9 | | | | | | | 11 | | 5.602 |
| 10 | | | | | | | 11 | | 6.913 |
| 11 | | | | | | | 11 | | 6.913 |
| 12 | | | | | | | 11 | | 6.913 |
| 13 | | | | | | | 11 | | 6.913 |
| 14 | | | | | | | 11 | | 6.913 |
| 15 | | | | | | | 15 | | 13.732 |
| 16 | | | | | | | 16 | | 26.398 |

The percent mortality in test sediment JT-SS-06 was not significantly higher that that of the control sediment at α=0.05. -631

Project Name: P862-3 Chironomus % Mortality

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-08 |
| Alias: | NAS# 5193G |
| Replicates: | 8 |
| Mean: | 15 |
| SD: | 16.903 |
| Tr Mean: | 17.737 |
| Trans SD: | 16.677 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 6.25 |
| SD: | 5.175 |
| Tr Mean: | 11.522 |
| Trans SD: | 9.541 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 11.662 |
| SS: | 2583.955 |
| K: | 8 |
| b: | 48.025 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.8926 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 13.303 |
| Test Residual SD: | 8.71 |
| Ref. Residual Mean: | 8.641 |
| Ref. Residual SD: | 2.385 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 1.4601 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Student's t |
| Balanced Design: | Yes |
| Transformation: | ArcSin |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 <= x2 |
| Alternate: | x1 > x2 |
| Degrees of Freedom: | 14 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 0.915 |
| Critical Value: | >= 1.761 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 20 | 26.565 | 0 | 0 | 8.828 | 11.522 | | | -17.737 |
| 2 | 10 | 18.435 | 10 | 18.435 | 0.698 | 6.913 | | | -17.737 |
| 3 | 0 | 0 | 10 | 18.435 | 17.737 | 6.913 | | | -17.737 |
| 4 | 10 | 18.435 | 0 | 0 | 0.698 | 11.522 | | | -11.522 |
| 5 | 40 | 39.232 | 10 | 18.435 | 21.494 | 6.913 | | | -11.522 |
| 6 | 0 | 0 | 10 | 18.435 | 17.737 | 6.913 | | | -11.522 |
| 7 | 0 | 0 | 10 | 18.435 | 17.737 | 6.913 | | | 0.698 |
| 8 | 40 | 39.232 | 0 | 0 | 21.494 | 11.522 | | | 0.698 |
| 9 | | | | | | | | | 6.913 |
| 10 | | | | | | | | | 6.913 |
| 11 | | | | | | | | | 6.913 |
| 12 | | | | | | | | | 6.913 |
| 13 | | | | | | | | | 6.913 |
| 14 | | | | | | | | | 8.828 |
| 15 | | | | | | | | | 21.494 |
| 16 | | | | | | | | | 21.494 |

The percent mortality in test sediment JT-SS-08 was not significantly higher that that of the control sediment at α=0.05. -631

Project Name: P862-3 Chironomus % Mortality

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-10 |
| Alias: | NAS# 5194G |
| Replicates: | 8 |
| Mean: | 8.75 |
| SD: | 11.26 |
| Tr Mean: | 0.058 |
| Trans SD: | 1.072 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 6.25 |
| SD: | 5.175 |
| Tr Mean: | -0.058 |
| Trans SD: | 0.662 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | |
| Residual SD: | |
| SS: | |
| K: | |
| b: | |
| Alpha Level: | N/A |
| Calculated Value: | N/A |
| Critical Value: | N/A |
| Normally Distributed: | N/A |
| Override Option: | Not Invoked |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 0.916 |
| Test Residual SD: | 0.436 |
| Ref. Residual Mean: | 0.6 |
| Ref. Residual SD: | 0.166 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 1.9139 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | No |

**Test Results:**

| | |
|---|---|
| Statistic: | Approximate t |
| Balanced Design: | Yes |
| Transformation: | Rankits |
| Experimental Hypothesis | |
| Null: | x1 <= x2 |
| Alternate: | x1 > x2 |
| Degrees of Freedom: | 12 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 0.2603 |
| Critical Value: | >= 1.782 |
| Accept Null Hypothesis: | Yes |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 30 | 1.766 | 0 | -0.858 | 1.708 | 0.8 | | -0.858 | |
| 2 | 10 | 0.422 | 10 | 0.422 | 0.364 | 0.48 | | -0.858 | |
| 3 | 0 | -0.858 | 10 | 0.422 | 0.916 | 0.48 | | -0.858 | |
| 4 | 10 | 0.422 | 0 | -0.858 | 0.364 | 0.8 | | -0.858 | |
| 5 | 0 | -0.858 | 10 | 0.422 | 0.916 | 0.48 | | -0.858 | |
| 6 | 0 | -0.858 | 10 | 0.422 | 0.916 | 0.48 | | -0.858 | |
| 7 | 0 | -0.858 | 10 | 0.422 | 0.916 | 0.48 | | -0.858 | |
| 8 | 20 | 1.285 | 0 | -0.858 | 1.227 | 0.8 | | 0.422 | |
| 9 | | | | | | | | 0.422 | |
| 10 | | | | | | | | 0.422 | |
| 11 | | | | | | | | 0.422 | |
| 12 | | | | | | | | 0.422 | |
| 13 | | | | | | | | 0.422 | |
| 14 | | | | | | | | 0.422 | |
| 15 | | | | | | | | 1.285 | |
| 16 | | | | | | | | 1.766 | |

The percent mortality in test sediment JT-SS-10 was not significantly higher that that of the control sediment at α=0.05. -651

Project Name: P862-3 Chironomus Growth (AFDW)

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-06 |
| Alias: | NAS# 5192G |
| Replicates: | 8 |
| Mean: | 0.978 |
| SD: | 0.106 |
| Tr Mean: | 0.978 |
| Trans SD: | 0.106 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 1.22 |
| SD: | 0.091 |
| Tr Mean: | 1.22 |
| Trans SD: | 0.091 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 0.085 |
| SS: | 0.136 |
| K: | 8 |
| b: | 0.364 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.975 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 0.083 |
| Test Residual SD: | 0.058 |
| Ref. Residual Mean: | 0.065 |
| Ref. Residual SD: | 0.058 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 0.6009 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Student's t |
| Balanced Design: | Yes |
| Transformation: | No Transformation |
| Experimental Hypothesis | |
| Null: | x1 >= x2 |
| Alternate: | x1 < x2 |
| Degrees of Freedom: | 14 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 4.9253 |
| Critical Value: | >= 1.761 |
| Accept Null Hypothesis: | No |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 0.83 | 0.83 | 1.05 | 1.05 | 0.148 | 0.17 | | | -0.17 |
| 2 | 1 | 1 | 1.22 | 1.22 | 0.023 | 0 | | | -0.148 |
| 3 | 1.04 | 1.04 | 1.31 | 1.31 | 0.063 | 0.09 | | | -0.118 |
| 4 | 1.05 | 1.05 | 1.25 | 1.25 | 0.073 | 0.03 | | | -0.08 |
| 5 | 0.97 | 0.97 | 1.25 | 1.25 | 0.008 | 0.03 | | | -0.058 |
| 6 | 0.86 | 0.86 | 1.21 | 1.21 | 0.118 | 0.01 | | | -0.01 |
| 7 | 1.15 | 1.15 | 1.33 | 1.33 | 0.173 | 0.11 | | | -0.008 |
| 8 | 0.92 | 0.92 | 1.14 | 1.14 | 0.058 | 0.08 | | | 0 |
| 9 | | | | | | | | | 0.023 |
| 10 | | | | | | | | | 0.03 |
| 11 | | | | | | | | | 0.03 |
| 12 | | | | | | | | | 0.063 |
| 13 | | | | | | | | | 0.073 |
| 14 | | | | | | | | | 0.09 |
| 15 | | | | | | | | | 0.11 |
| 16 | | | | | | | | | 0.173 |

Average individual growth (AFDW) in test sediment JT-SS-06 is significantly less than that in the control sediment at α=0.05. --6) 1

Project Name: P862-3 Chironomus Growth (AFDW)

| | |
|---|---|
| Sample: | x1 |
| Samp ID: | JT-SS-08 |
| Alias: | NAS# 5193G |
| Replicates: | 8 |
| Mean: | 0.93 |
| SD: | 0.149 |
| Tr Mean: | 0.93 |
| Trans SD: | 0.149 |

| | |
|---|---|
| Ref Samp: | x2 |
| Ref ID: | Control |
| Alias: | NAS# 5195G |
| Replicates: | 8 |
| Mean: | 1.22 |
| SD: | 0.091 |
| Tr Mean: | 1.22 |
| Trans SD: | 0.091 |

**Shapiro-Wilk Results:**

| | |
|---|---|
| Residual Mean: | 0 |
| Residual SD: | 0.106 |
| SS: | 0.213 |
| K: | 8 |
| b: | 0.453 |
| Alpha Level: | 0.05 |
| Calculated Value: | 0.9628 |
| Critical Value: | <= 0.887 |
| Normally Distributed: | Yes |
| Override Option: | N/A |

**Levene's Results:**

| | |
|---|---|
| Test Residual Mean: | 0.115 |
| Test Residual SD: | 0.084 |
| Ref. Residual Mean: | 0.065 |
| Ref. Residual SD: | 0.058 |
| Deg. of Freedom: | 14 |
| Alpha Level: | 0.1 |
| Calculated Value: | 1.3811 |
| Critical Value: | >= 1.761 |
| Variances Homogeneous: | Yes |

**Test Results:**

| | |
|---|---|
| Statistic: | Student's t |
| Balanced Design: | Yes |
| Transformation: | No Transformation |

Experimental Hypothesis

| | |
|---|---|
| Null: | x1 >= x2 |
| Alternate: | x1 < x2 |
| Degrees of Freedom: | 14 |
| Experimental Alpha Level: | 0.05 |
| Calculated Value: | 4.7022 |
| Critical Value: | >= 1.761 |
| Accept Null Hypothesis: | No |
| Power: | |
| Min. Difference for Power: | |

| Replicate Number | Test Data | Trans. Test Data | Reference Data | Trans. Reference Data | Levene's Test Residuals | Levene's Reference Residuals | Mann-Whitney Ranks | Rankits | Shipiro-Wilk Residuals |
|---|---|---|---|---|---|---|---|---|---|
| 1 | 1.01 | 1.01 | 1.05 | 1.05 | 0.08 | 0.17 | | | -0.2 |
| 2 | 0.92 | 0.92 | 1.22 | 1.22 | 0.01 | 0 | | | -0.18 |
| 3 | 0.73 | 0.73 | 1.31 | 1.31 | 0.2 | 0.09 | | | -0.17 |
| 4 | 0.75 | 0.75 | 1.25 | 1.25 | 0.18 | 0.03 | | | -0.08 |
| 5 | 1.1 | 1.1 | 1.25 | 1.25 | 0.17 | 0.03 | | | -0.06 |
| 6 | 0.87 | 0.87 | 1.21 | 1.21 | 0.06 | 0.01 | | | -0.01 |
| 7 | 0.92 | 0.92 | 1.33 | 1.33 | 0.01 | 0.11 | | | -0.01 |
| 8 | 1.14 | 1.14 | 1.14 | 1.14 | 0.21 | 0.08 | | | -0.01 |
| 9 | | | | | | | | | 0 |
| 10 | | | | | | | | | 0.03 |
| 11 | | | | | | | | | 0.03 |
| 12 | | | | | | | | | 0.08 |
| 13 | | | | | | | | | 0.09 |
| 14 | | | | | | | | | 0.11 |
| 15 | | | | | | | | | 0.17 |
| 16 | | | | | | | | | 0.21 |

Average individual growth (AFDW) in test sediment JT-SS-08 is significantly less than that in the control sediment at α=0.05. -6J1

data entry verified against laboratory bench sheets 3-4-15 JKF

## Water Quality Data

| BKR | NAS SMPL | CLIENT DESCRIP | REPL | DAY | Overlying water TEMP | DO | COND | pH | NH3 | HARD | ALK |
|---|---|---|---|---|---|---|---|---|---|---|---|
| 9 | 5192G | JT-SS-06 | 8 | 0 | 23.6 | 7.0 | 118 | 7.1 | <0.1 | 26 | 30 |
| 12 | 5195G | Control | 8 | 0 | 24.0 | 7.4 | 217 | 7.4 | 0.3 | 26 | 30 |
| 16 | 5193G | JT-SS-08 | 8 | 0 | 23.8 | 7.2 | 120 | 7.3 | 0.1 | 34 | 30 |
| 28 | 5194G | JT-SS-10 | 8 | 0 | 24.0 | 6.7 | 136 | 7.2 | 0.2 | 34 | 30 |
| 9 | 5192G | JT-SS-06 | 8 | 1 | 22.2 | 6.6 | | | | | |
| 12 | 5195G | Control | 8 | 1 | 22.6 | 6.8 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 1 | 22.3 | 6.8 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 1 | 23.0 | 6.4 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 2 | 22.2 | 6.7 | | | | | |
| 12 | 5195G | Control | 8 | 2 | 22.6 | 6.4 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 2 | 22.4 | 6.8 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 2 | 22.9 | 6.8 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 3 | 22.4 | 6.5 | | | | | |
| 12 | 5195G | Control | 8 | 3 | 22.9 | 6.1 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 3 | 22.7 | 6.2 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 3 | 23.2 | 6.1 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 4 | 22.1 | 6.5 | | | | | |
| 12 | 5195G | Control | 8 | 4 | 22.4 | 6.1 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 4 | 22.2 | 6.3 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 4 | 22.7 | 6.7 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 5 | 22.0 | 6.6 | 108 | 7.2 | | | |
| 12 | 5195G | Control | 8 | 5 | 22.4 | 6.3 | 139 | 7.1 | | | |
| 16 | 5193G | JT-SS-08 | 8 | 5 | 22.2 | 6.2 | 112 | 7.2 | | | |
| 28 | 5194G | JT-SS-10 | 8 | 5 | 22.5 | 6.6 | 117 | 7.1 | | | |
| 9 | 5192G | JT-SS-06 | 8 | 6 | 22.1 | 4.8 | | | | | |
| 12 | 5195G | Control | 8 | 6 | 22.8 | 4.6 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 6 | 22.4 | 4.6 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 6 | 23.4 | 4.5 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 7 | 22.6 | 5.5 | | | | | |
| 12 | 5195G | Control | 8 | 7 | 23.1 | 5.3 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 7 | 22.8 | 5.3 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 7 | 23.6 | 5.9 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 8 | 22.1 | 5.4 | | | | | |
| 12 | 5195G | Control | 8 | 8 | 22.6 | 5.6 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 8 | 22.3 | 5.7 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 8 | 22.9 | 5.9 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 9 | 22.4 | 5.6 | | | | | |
| 12 | 5195G | Control | 8 | 9 | 22.8 | 5.4 | | | | | |
| 16 | 5193G | JT-SS-08 | 8 | 9 | 22.6 | 5.4 | | | | | |
| 28 | 5194G | JT-SS-10 | 8 | 9 | 23.2 | 5.3 | | | | | |
| 9 | 5192G | JT-SS-06 | 8 | 10 | 22.3 | 6.5 | 116 | 7.1 | 0.2 | 26 | 40 |
| 12 | 5195G | Control | 8 | 10 | 22.9 | 6.3 | 129 | 7.1 | 0.1 | 26 | 40 |
| 16 | 5193G | JT-SS-08 | 8 | 10 | 22.6 | 6.4 | 117 | 7.1 | 0.1 | 26 | 50 |
| 28 | 5194G | JT-SS-10 | 8 | 10 | 23.4 | 6.2 | 123 | 7.1 | 0.2 | 34 | 40 |
| | | | | **Mean** | 22.7 | 6.1 | 129 | 7.2 | --- | 29 | 36 |
| | | | | **SD** | 0.5 | 0.7 | 29 | 0.1 | --- | 4 | 7 |
| | | | | **n** | 44 | 44 | 12 | 12 | 8 | 8 | 8 |
| | | | | **Min** | 22.0 | 4.5 | 108 | 7.1 | <0.1 | 26 | 30 |
| | | | | **Max** | 24.0 | 7.4 | 217 | 7.4 | 0.3 | 34 | 50 |

# AMMONIA EXPOSURE BENCHSHEETS AND ANALYSIS

# Total Ammonia-N in 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. | ---- | 0.151 | 1.00 | | |
| | 3.0 mg/L NH3-N Std. | ----- | 0.440 | 3.00 | | |
| | 6.0 mg/L NH3-N Std. | ---- | 0.862 | 6.00 | | |
| | 10.0 mg/L NH3-N Std. | ---- | 1.400 | 10.00 | | |
| | 3.0 mg/L spike | ----- | 0.449 | 3.17 | | |
| | 3.0 mg/L spike dupl. | ---- | 0.440 | 3.11 | | |
| | 5.0 mg/L 2nd source | | 0.710 | 5.02 | | |
| 1 | Day 0 (2-6-15) | | | | | |
| 2 | 9 | 1 | 0.014 | ND | | |
| 3 | 12 | 1 | 0.044 | 0.31 | | |
| 4 | 16 | 1 | 0.020 | 0.14 | | |
| 5 | 28 | 1 | 0.029 | 0.21 | | |
| 6 | | | | | | |
| 7 | Day 10 (2-16-15) | | | | | |
| 8 | 9 | 1 | 0.022 | 0.16 | | |
| 9 | 12 | 1 | 0.019 | 0.13 | | |
| 10 | 16 | 1 | 0.019 | 0.13 | | |
| 11 | 28 | 1 | 0.021 | 0.15 | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |
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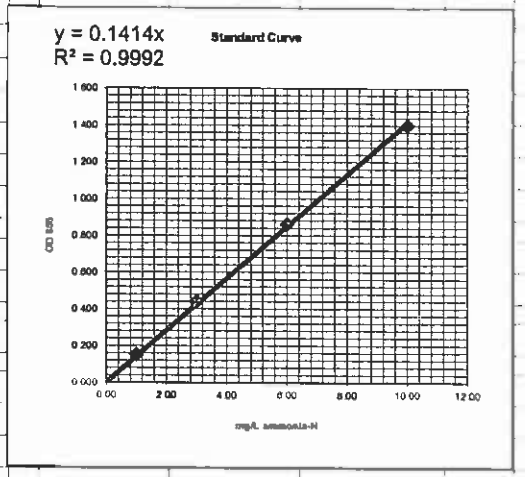
| 30 | | | | | | |
| 31 | | | | | | |
| 32 | | | | | | |
| 33 | | | | | | |
| 34 | | | | | | |
| 35 | | | | | | |
| 36 | | | | | | |




| | |
|---|---|
| Reporting limit (mg/L) = | 0.10 |
| Recovery (%) = | 104.8 |
| Precision (RPD) = | 2.02 |
| 2nd source (%) = | 100.4 |
| Sample volume (ml): | 0.50 |
| Dilution factor | 1 |

**Sample Set Description:**

Test No.: 862-3
Test Day: 0 & 10
Species: *Chironomus*

**Sample Type (check)**

| | |
|---|---|
| | Bulk Sediment Porewaters |
| | Test Beaker Porewaters |
| X | Overlying Water |

Analyst: JB
Date analysed: 2/20/2015

# Total Ammonia-N in 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. | ---- | .151 | 1.00 | | |
| | 3.0 mg/L NH3-N Std. | ---- | .490 | 3.00 | | |
| | 6.0 mg/L NH3-N Std. | ---- | .862 | 6.00 | | |
| | 10.0 mg/L NH3-N Std. | ---- | 1.4 | 10.00 | | |
| | 3.0 mg/L spike | ---- | .449 | | | |
| | 3.0 mg/L spike dupl. | ---- | .440 | | | |
| | 5.0 mg/L 2nd source | | .710 | | | |
| 1 | Day 0 (2-6-15) | | | | | |
| 2 | 9 | 1 | .014 | | | |
| 3 | 12 | 1 | .044 | | | |
| 4 | 16 | 1 | .020 | | | |
| 5 | 28 | 1 | .029 | | | |
| 6 | | | | | | |
| 7 | Day 10 (2-16-15) | | | | | |
| 8 | 9 | 1 | .022 | | | |
| 9 | 12 | 1 | .019 | | | |
| 10 | 16 | 1 | .019 | | | |
| 11 | 28 | | .021 | | | |
| 12 | | | | | | |
| 13 | | | | | | |
| 14 | | | | | | |
| 15 | | | | | | |
| 16 | | | | | | |
| 17 | | | | | | |
| 18 | | | | | | |
| 19 | | | | | | |
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| 21 | | | | | | |
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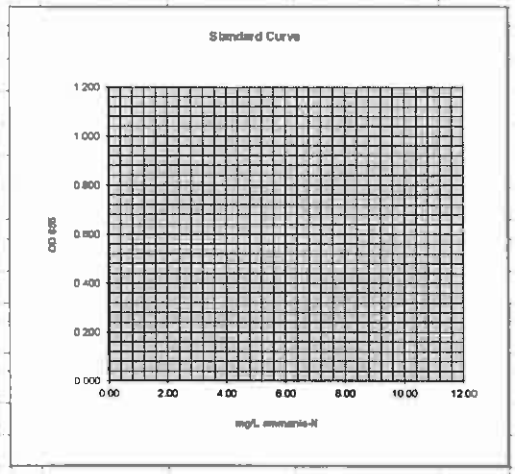
| 34 | | | | | | |
| 35 | | | | | | |
| 36 | | | | | | |




| | |
|---|---|
| Reporting limit (mg/L) = | 0.10 |
| Recovery (%) = | #VALUE! |
| Precision (RPD) = | #VALUE! |
| 2nd source (%) = | #VALUE! |
| Sample volume (ml): | 0.50 |
| Dilution factor | 1 |

**Sample Set Description:**

Test No.: 862-3
Test Day: 0 & 10
Species: *Chironomus*

**Sample Type (check)**

- Bulk Sediment Porewaters
- Test Beaker Porewaters
- X Overlying Water

Analyst: JB
Date analysed: 2/20/2015

# CHAIN-OF-CUSTODY RECORDS

# Sample Custody Record

Samples Shipped to: NW Aquatic Sciences

HARTCROWSER

Hart Crowser, Inc.
1700 Westlake Avenue North, Suite 200
Seattle, Washington 98109-6212
Office: 206.324.9530 • Fax 206.328.5581

JOB 17800-56 LAB NUMBER

PROJECT NAME Jacobsen Terminals

HART CROWSER CONTACT Phil Cordell

SAMPLED BY: PRL

| LAB NO. | SAMPLE ID | DESCRIPTION | DATE | TIME | MATRIX | REQUESTED ANALYSIS * 10-day Chronomus dilution survival and growth | REQUESTED ANALYSIS * 28-day Hyalella Aztera survival and growth | NO. OF CONTAINERS | OBSERVATIONS/COMMENTS/ COMPOSITING INSTRUCTIONS |
|---|---|---|---|---|---|---|---|---|---|
| | JT-SS-06 | 5192G | 1/12/15 | 1159 | Sediment | X | X | 4 | 4 liters sediment |
| | JT-SS-08 | 5193G | ↓ | 1058 | ↓ | X | X | 4 | " " |
| | JT-SS-10 | 5194G | ↓ | 1400 | ↓ | X | X | 4 | " " |

25 of 26

| RELINQUISHED BY | DATE | RECEIVED BY | DATE |
|---|---|---|---|
| SIGNATURE Phil Cordell | 1/13/15 | SIGNATURE GERALD IRISSARRI | 1-14-15 |
| PRINT NAME HC | TIME 1000 | PRINT NAME NAS | TIME 1140 |
| COMPANY | | COMPANY NAS | |
| RELINQUISHED BY | DATE | RECEIVED BY | DATE |
| SIGNATURE | TIME | SIGNATURE | TIME |
| PRINT NAME | | PRINT NAME | |
| COMPANY | | COMPANY | |

SPECIAL SHIPMENT HANDLING OR STORAGE REQUIREMENTS:

12 TOTAL NUMBER OF CONTAINERS

SAMPLE RECEIPT INFORMATION
CUSTODY SEALS: ☒YES ☐NO ☐N/A
GOOD CONDITION ☒YES ☐NO
TEMPERATURE 2.5 °C
SHIPMENT METHOD: ☐HAND ☐COURIER ☒OVERNIGHT

COOLER NO.: STORAGE LOCATION:

See Lab Work Order No. _____ for Other Contract Requirements

TURNAROUND TIME:
☐ 24 HOURS ☐ 1 WEEK
☐48 HOURS ☒STANDARD
☐72 HOURS OTHER _____

White and Yellow Copies to Lab Pink to Project Manager Lab to Return White Copy to Hart Crowser Gold to Sample Custodian

From (206) 826-4527
Phil Cordell
Hart Crowser, Inc
1700 Westlake Avenue North
Suite 200
Seattle WA 98109

Origin ID LKEA

FedEx Express

J1422453633u

Ship Date 13JAN15
ActWgt 75 0 LB
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Ref # 17800-56-02
Invoice #
PO #
Dept #

SHIP TO (206) 324-9530
Gerald Irissarri
Northwest Aquatics Scientists
3814 Yakima Rd

NEWPORT, OR 97365

BILL SENDER

WED - 14 JAN AM
STANDARD OVERNIGHT

TRK# 0201 7725 6121 8674

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OR-US
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522G7/8F15/8A09

RCVD 1130 1-14-15

After printing this label:
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CUSTODY SEAL CUSTODY SEAL CUSTODY SEAL CUSTODY SEAL

Date 1/13/15

Initials

26 of 26

# APPENDIX III

# RAW DATA – REFERENCE TOXICANT TEST

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS- _____

ACUTE TOXICITY TEST (ALL SPECIES)

REVIEWED PAGES 1-5 -GJI

Test No. 999-3391 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. Yves Nakahama 2. [illegible]

3. _____ 4. _____

Study Schedule:

Test Beginning: 2-6-15 0945 Test Ending: 2-10-15 1045

**TEST MATERIAL**

Description: Potassium Chloride Crystals - Fisher Lot No.: 114689

| | | | | |
|---|---|---|---|---|
| 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: 1-30-15

Water Quality: Cond. (umhos/cm): 248 Salinity (ppt) _____ pH 8.1

Hardness (mg/L as $CaCO_3$): 86 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: _____

**Randomization chart:**

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| 5 | 20 | 2.5 | 1.25 | ø | 5 | 5 | 10 | 0 | 0 |
| ø | 5 | 5 | 5 | 20 | 10 | 2.5 | 1.25 | 20 | 10 |
| 2.5 | 10 | 10 | ø | 10 | 20 | ø | ø | 1.25 | 5 |
| 1.25 | ø | 1.25 | 20 | 1.25 | 1.25 | 1.25 | 5 | 10 | 2.5 |
| 10 | 2.5 | 20 | 10 | 5 | ø | 10 | 2.5 | 5 | 1.25 |
| 20 | 1.25 | ø | 2.5 | 2.5 | 2.5 | 20 | 20 | 2.5 | 20 |

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

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3391 Client QC Test Investigator _____

**TEST ORGANISMS**

Species: *Chironomus dilutus* Age: 2ND ~~AND~~ TO 3rd instar (63)

Source: ~~NAS cultures~~ AQUATIC BIOSYSTEMS, FORT COLLINS, CO Date received: 2-5-15

Acclimation Data:

| Date | Temp. (deg.C) | pH | DO (mg/L) | Cond. umhos/cm | Hardness (mg/L) | Alkalinity (mg/L) | Feeding | Water changes |
|---|---|---|---|---|---|---|---|---|
| 2-5-15 | 20.6 | 6.9 | 6.2 | 436 | 171 | 110 | Animals fed Tetra Fin | YES |
| 2-6-15 | 24.8 | 7.6 | 6.7 | 316 | 86 | 70 | and *Selenastrum* | - |
| | | | | | | | ~~Details recorded on~~ | |
| | | | | | | | ~~Chironomid culture~~ | |
| | | | | | | | ~~data sheets~~ | |
| Mean | 22.7 | 7.3 | 6.5 | 376 | 129 | 90 | | |
| S.D. | - | - | - | - | - | - | | |
| (N) | 2 | 2 | 2 | 2 | 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 |
| 10 | 100 | 100 |
| 5 | 50 | 150 |
| 2.5 | 25 | 175 |
| 1.25 | 12.5 | 187.5 |
| 0 | 0 | 0 |

2-6-15 DAM

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3391 Client QC Test

DAILY RECORD SHEET

Day 0 (2/6/15) 1630 Temp Beaker (°C): 23.3

| Conc. ( g/L ) | Temp. (deg.C) | pH | Cond. (umhos/cm) | DO (ppm) | Hardness (mg/L) | Alkalinity (mg/L) | Comments |
|---|---|---|---|---|---|---|---|
| 1. 20 | 23.4 | 7.9 | >20,000 | 8.4 | 86 | 60 | |
| 2. 10 | 23.4 | 8.0 | 14380 | 8.3 | | | |
| 3. 5 | 23.4 | 8.0 | 7830 | 8.3 | | | |
| 4. 2.5 | 23.4 | 8.0 | 4220 | 8.3 | | | |
| 5. 1.25 | 23.5 | 8.0 | 2320 | 8.4 | | | |
| 6. 0 | 23.5 | 7.9 | 273 | 8.4 | 86 | 70 | |
| Each replicate fed 0.25 ml Tetra Fin suspension. Initials: 651 | | | | | | | |

Day 1 (2/7/15) 1633 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 | | | | | | | |

Day 2 (2/8/15) 651 Temp Beaker (°C): 22.6

| 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 | | | | | | | |
| Each replicate fed 0.25 ml Tetra Fin suspension. Initials: 651 | | | | | | | |

Day 3 (2/9/15) 651 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 4 (2/10/15) ~ Temp Beaker (°C): 22.6

| 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 | 7.8 | 8960 | 8.0 | | | |
| 4. 2.5 | 23.2 | 7.8 | 5120 | 8.1 | | | |
| 5. 1.25 | 23.5 | 7.9 | 2800 | 8.2 | | | |
| 6. 0 | 23.3 | 7.9 | 364 | 8.1 | 94 | 80 | |
| Mean | 23.4 | 7.9 | CONTROL 319 | 8.3 | 89 | 70 | |
| SD | 0.1 | 0.1 | — | 0.1 | 5 | 10 | |
| n | 10 | 10 | 2 | 10 | 3 | 3 | |

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-_____

ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999-3391 Client QC Test Investigator _____

DAILY RECORD SHEET - Survivors

Day 0 ( 2/6/15) GJL

| 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 ( 2/7/15) AP

| 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 ( 2/8/15) GJL

| 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 | 0 (10) |
| 2. 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 (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 3 (2/9/15) GJL

| 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 | 0 |
| 2. 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3. 5 | 1 | 1 | 0 | 1 | 1 | 1 | 1 | 1 | 1 | 1 | 9 (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 ( 2/10/15) GJL

| 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 | 0 |
| 2. 10 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
| 3. 5 | 1 | 1 | 0 | 1 | 0 | 0 | 1 | 1 | 1 | 1 | 7 (20) |
| 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 |

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel:970/484-5091 Fax:970/484-2514

## ORGANISM HISTORY

DATE: 2/4/2015

SPECIES: *Chironomus dilutus (formerly C. tentans)*

AGE: Deposited 1/24/2015

LIFE STAGE: Second Instar 2/4/2015

HATCH DATE: Emergent date 2/17/2015

BEGAN FEEDING: Immediately

FOOD: *Selenastrum* sp., Flake slurry

RECEIVED 2-5-15
-GJL

| Water Chemistry Record: | Current | Range |
|---|---|---|
| TEMPERATURE: | 22°C | 22-26°C |
| SALINITY/CONDUCTIVITY: | -- | -- |
| TOTAL HARDNESS (as $CaCO_3$): | 190 mg/l | 100-190 mg/l |
| TOTAL ALKALINITY (as $CaCO_3$): | 60 mg/l | 50-90 mg/l |
| pH: | 7.50 | 7.50-8.20 |

**Comments:**

*Facility Supervisor*

## Acute 96-hr Toxicity Test-96 Hr Survival

| | | | | | |
|---|---|---|---|---|---|
| Start Date: | 2/6/2015 09:45 | Test ID: | 999-3391 | Sample ID: | REF-Ref Toxicant |
| End Date: | 2/10/2015 10:45 | Lab ID: | ORNAS-Northwestern Aquati | Sample Type: | KCL-Potassium chloride |
| Sample Date: | | Protocol: | EPAF 91-EPA Freshwater | Test Species: | CT-Chironomus tentans |
| Comments: | | | | | |

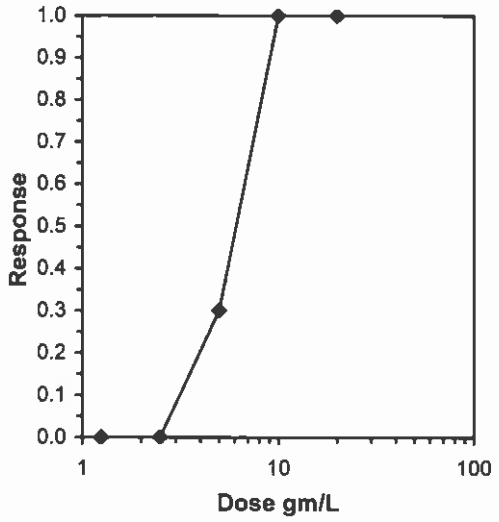
| Conc-gm/L | 1 |
|---|---|
| D-Control | 1.0000 |
| 1.25 | 1.0000 |
| 2.5 | 1.0000 |
| 5 | 0.7000 |
| 10 | 0.0000 |
| 20 | 0.0000 |

| 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 | 1.0000 | 1.0000 | 0 | 10 | 10 | 1 | 1.0000 | 0.0500 | 0 | 10 |
| 2.5 | 1.0000 | 1.0000 | 0 | 10 | 10 | 1 | 1.0000 | 0.0500 | 0 | 10 |
| 5 | 0.7000 | 0.7000 | 3 | 7 | 10 | 1 | 0.1053 | 0.0500 | 3 | 10 |
| 10 | 0.0000 | 0.0000 | 10 | 0 | 10 | 1 | | | 10 | 10 |
| 20 | 0.0000 | 0.0000 | 10 | 0 | 10 | 1 | | | 10 | 10 |

| Hypothesis Test (1-tail, 0.05) | NOEC | LOEC | ChV | TU |
|---|---|---|---|---|
| Fisher's Exact Test | 5 | 10 | 7.07107 | |

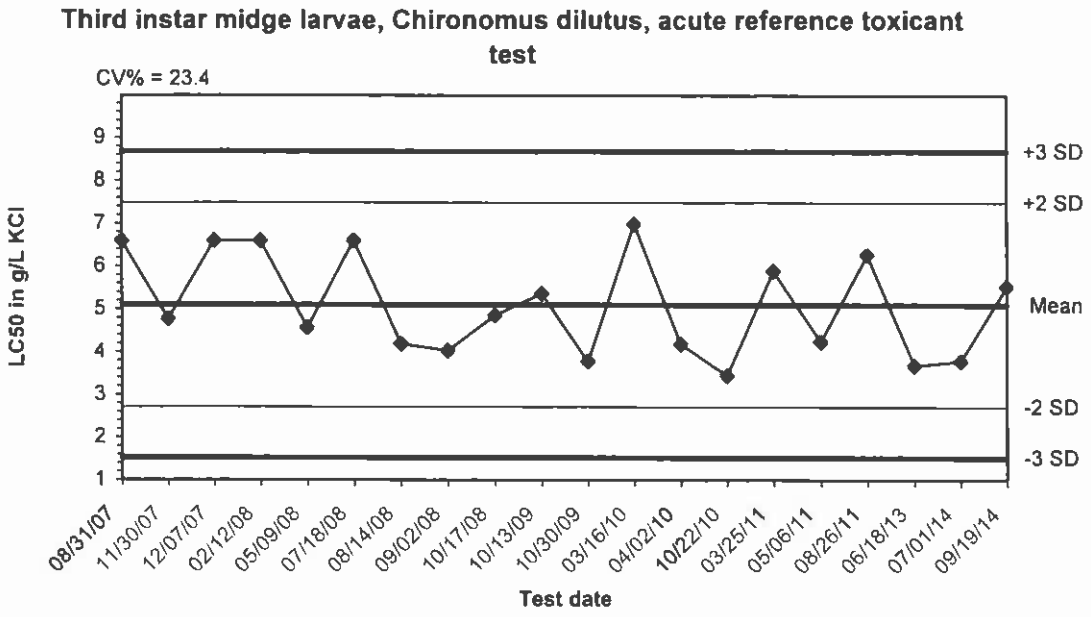
Trimmed Spearman-Karber

| Trim Level | EC50 | 95% CL | |
|---|---|---|---|
| 0.0% | 5.7435 | 4.6982 | 7.0214 |
| 5.0% | 5.8220 | 4.6455 | 7.2964 |
| 10.0% | 5.8972 | 4.5356 | 7.6674 |
| 20.0% | 6.0284 | 4.0589 | 8.9534 |
| Auto-0.0% | 5.7435 | 4.6982 | 7.0214 |



| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Test: AT-Acute 96-hr Toxicity Test | | | | | | Test ID: 999-3391 | | | |
| Species: CT-Chironomus tentans | | | | | | Protocol: EPAF 91-EPA Freshwater | | | |
| Sample ID: REF-Ref Toxicant | | | | | | Sample Type: KCL-Potassium chloride | | | |
| Start Date: 2/6/2015 09:45 | | | End Date: 2/10/2015 10:45 | | | 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 | | | | 10 | |
| | 3 | 1 | 2.500 | 10 | | | | 10 | |
| | 4 | 1 | 5.000 | 10 | | | | 7 | |
| | 5 | 1 | 10.000 | 10 | | | | 0 | |
| | 6 | 1 | 20.000 | 10 | | | | 0 | |

Comments: data entry verified against laboratory bench sheets 3-5-15 JDF




| Dates | Values | Mean | -2 SD | -3 SD | +2 SD | +3 SD |
|---|---|---|---|---|---|---|
| 08/31/07 | 6.6000 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 11/30/07 | 4.7700 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 12/07/07 | 6.6000 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 02/12/08 | 6.6000 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 05/09/08 | 4.5600 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 07/18/08 | 6.6000 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 08/14/08 | 4.1900 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 09/02/08 | 4.0300 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 10/17/08 | 4.8500 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 10/13/09 | 5.3600 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 10/30/09 | 3.7700 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 03/16/10 | 6.9900 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 04/02/10 | 4.1900 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 10/22/10 | 3.4500 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 03/25/11 | 5.8900 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 05/06/11 | 4.2400 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 08/26/11 | 6.2700 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 06/18/13 | 3.6800 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 07/01/14 | 3.7900 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |
| 09/19/14 | 5.5300 | 5.0980 | 2.7119 | 1.5188 | 7.4841 | 8.6772 |