

# TOXICITY TESTING RESULTS

**LOTT CLEAN WATER ALLIANCE  
OLYMPIA, WASHINGTON**

**JUNE 2022 WET**

**Prepared for**

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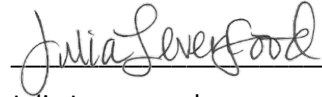
**Submittal Date:** July 6, 2022



Accredited in accordance with  
NELAP, ORELAP ID 4165

All testing reported herein was performed consistent with our laboratory's quality assurance program. All results are intended to be considered in their entirety, and EcoAnalysts is not responsible for use of less than the complete report. The test results summarized in this report apply only to the sample(s) evaluated. This document is uncontrolled when printed or accessed from electronic distribution.

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## ACRONYMS AND ABBREVIATIONS

|                                    |   |
|------------------------------------|---|
| ABS                                | Aquatic BioSystems, Inc.                              |
| ACEC                               | Acute Critical Effluent Concentration                 |
| CCEC                               | Chronic Critical Effluent Concentration               |
| EPA                                | Environmental Protection Agency                       |
| LC <sub>50</sub> /EC <sub>50</sub> | Lethal/Effect Concentration to 50% of Test Population |
| LOEL                               | Lowest Observed Effect Level                          |
| mg/L                               | Milligrams per Liter                                  |
| NOEL                               | No Observed Effect Level                              |
| NPDES                              | National Pollutant Discharge Elimination System       |
| QM                                 | Quality Manual  |
| SOP                                | Standard Operating Practices                          |
| WDOE                               | Washington Department of Ecology                      |
| WET                                | Whole Effluent Toxicity                               |
| WWTP                               | Wastewater Treatment Plant                            |



## 1. EXECUTIVE SUMMARY

EcoAnalysts conducted Whole Effluent Toxicity (WET) testing on three effluent samples collected by LOTT Clean Water Alliance personnel as part of the effluent characterization. The objective of this program was to assess the potential toxicity of primary discharge water to selected aquatic organisms following procedures defined under the facility’s National Pollutant Discharge Elimination System (NPDES) permit. The results of the biological testing are contained in this report.

Statistically significant biological response of the test organisms was not detected at or below the acute critical effluent concentration (ACEC) of 2.8% or the chronic critical effluent concentration (CCEC) of 2.0% for the chronic tests performed (Table 1-1). This effluent sample does not exceed the defined permit requirements (Table 1-2).

**Table 1-1. Toxicity Test Results Summary.**

| Test    |   | NOEL (%) | LOEL (%) | LC <sub>50</sub> /EC <sub>50</sub> (%) |
|---------|---|----------|----------|--|
| Chronic | <i>Atherinops affinis</i><br>7-Day Survival | 100      | >100     | >100                                   |
|         | <i>Atherinops affinis</i><br>7-Day Biomass  | 100      | >100     | >100                                   |
|         | <i>Americamysis bahia</i><br>7-Day Survival | 100      | >100     | >100                                   |
|         | <i>Americamysis bahia</i><br>7-Day Biomass  | 100      | >100     | >100                                   |

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>50</sub>/EC<sub>50</sub> = Lethal/Effect Concentration to 50% of test population

**Table 1-2. Permit Compliance Results.**

|                           |   |
|---------------------------|---|
| <b>Permit Requirement</b> | <i>The Permittee must:<br/>           Conduct chronic toxicity testing on the final effluent annually. Conduct chronic toxicity testing during effluent characterization on a series of at least five concentrations of effluent and a control. This series of dilutions must include the acute critical effluent concentration (ACEC). The ACEC equals 2.8 percent effluent. The series of dilutions should also contain the CCEC of 2.0 percent effluent. Compare the ACEC to the control using hypothesis testing at the 0.05 level of significance.</i> |
| <b>Result</b>             | No statistically significant biological effects were detected at or below the acute critical effluent concentration (ACEC) of 2.8% effluent or the chronic critical effluent concentration (CCEC) of 2.0% effluent.   |

## 2. METHODS

The samples were analyzed for toxicity using criteria outlined in the Washington Department of Ecology’s (WDOE) Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria (WDOE WQ-R-95-80). These criteria are further defined through the Environmental Protection Agency’s (EPA) most recently promulgated effluent guidance documents outlined in Section 4.

To evaluate the relative sensitivity of the organisms, a reference toxicity test was performed using standard reference toxicants (Lee 1980).

### 2.1 Sample Collection and Storage

LOTT Clean Water Alliance personnel collected three 24-hour composite samples ending on June 13, 15, and 17, 2022. The samples were transported by FedEx and were received at the laboratory the day following collection. Sample temperatures upon receipt ranged from 0.5 – 0.8°C. Additional sample conditions are summarized in Table 2-1. The samples were held in a walk-in cold room at 4 ± 2 °C in the dark until utilized for testing.

Table 2-1. Sample Conditions upon Receipt

| Sample  | Final Effluent |               |               |
|---|----------------|---------------|---------------|
|   | P220614.01     | P220616.01    | P220618.01    |
| Laboratory ID                                     | P220614.01     | P220616.01    | P220618.01    |
| Date/Time sampled                                 | 6/13/22; 0600  | 6/15/22; 0600 | 6/17/22; 0600 |
| Date/Time received                                | 6/14/22; 1210  | 6/16/22; 1205 | 6/18/22; 1106 |
| Dissolved Oxygen (mg/L)<br>Recommended: >4.0 mg/L | 10.7           | 10.6          | 8.8           |
| Temperature (°C)<br>Recommended: 0 – 6°C          | 0.5            | 0.5           | 0.8           |
| pH (units)<br>Recommended: 6 – 9                  | 7.2            | 7.0           | 7.3           |
| Conductivity (µS/cm)                              | 463            | 438           | 389           |
| Salinity (ppt)                                    | 0.3            | 0.2           | 0.2           |
| Total Chlorine (mg/L)                             | 0.02           | 0.01          | 0.04          |
| Total Ammonia (mg/L)                              | 0.075          | 0.223         | 0.088         |

### 2.2 Bioassay Testing

Bioassay testing for this project consisted of two chronic bioassays. The tests conducted in support of this project are summarized in Table 2-2.

Table 2-2. Biological Testing Performed

| Test Type | Test Descriptor           | Species                                    | Method   |
|-----------|---------------------------|--|--|
| Chronic   | 7-Day Survival and Growth | <i>Atherinops affinis</i> (Topsmelt)       | WDOE WQ-R-95-80; EPA 600/R-95/136; Test Method 1006.0; SOP TOX002.10 |
|           |                           | <i>Americamysis bahia</i> (Opossum Shrimp) | WDOE WQ-R-95-80; EPA-821-R-02-014; Test Method 1007.0; SOP TOX014.12 |

### 2.3 Organisms for Testing

*Americamysis bahia* (mysids) and *Atherinops affinis* (topsmelt) were purchased from Aquatic BioSystems Inc. (ABS) in Fort Collins, Colorado. ABS is a commercial supplier of test organisms that are used routinely for toxicity testing. Water quality measurements were collected from transport containers and the overall health of the organisms was visually confirmed by a laboratory technician.

### 2.4 Water for Bioassay Testing

Seawater diluent used in this study came from the northern Hood Canal at Port Gamble, Washington. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation of contaminants from this water supply. Chemical analysis of this water source is conducted and reviewed on an annual basis.

### 2.5 Sample Adjustment

Salinity adjustments were necessary to bring the samples within the recommended test salinity for each marine test species. The effluent discharge samples arrived at a salinity of 0.2 – 0.3 ppt. The salinity of the LOTT effluent samples was adjusted to the desired test salinity with Crystal Sea® MarineMix bioassay grade artificial salt. Table 2-3 summarizes the salinity adjustments performed on the project samples to create a salinity range with the tolerance limits of test species.

An artificial salt control sample was created to evaluate any potential negative impacts to the test organisms from the salinity adjustment alone. This sample was designated “Salt Control” and the results are discussed in Section 3.

**Table 2-3. Salinity Adjustment of Project Samples**

| Sample ID: Final Effluent   | Sample Salinity Upon Receipt (ppt) | Sample Salinity Adjustment (ppt) |
|-----------------------------|------------------------------------|----------------------------------|
| Sample 1: Collected 6/13/22 | 0.3                                | 30 ± 2                           |
| Sample 2: Collected 6/15/22 | 0.2                                | 30 ± 2                           |
| Sample 3: Collected 6/17/22 | 0.2                                | 30 ± 2                           |

### 2.6 Data Management and Analysis

Endpoint data was calculated for each replicate, and the mean value and standard deviation were determined for each sample concentration. All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting was reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the EPA guidance (EPA 2002). Statistical comparisons were performed using CETIS™ software.

### 2.7 Quality Assurance/Quality Control

The quality assurance objectives for toxicity testing conducted by the testing laboratory are detailed in the method specific guidance documents and the laboratory’s quality manual (QM). These objectives for accuracy and precision involve all aspects of the testing process, including the following:

- Source and Condition of Test Organisms
- Condition of Equipment
- Test Conditions
- Instrument Calibration

- Use of Reference Toxicants
- Record Keeping
- Data Evaluation

The batch of test organisms obtained was evaluated in a reference toxicant test that was run concurrently with the test period to establish the sensitivity of the test organisms. The reference toxicant LC<sub>50</sub> or EC<sub>50</sub> should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

The methods employed in every phase of the toxicity testing program are detailed in the EcoAnalysts Standard Operating Practices (SOP). All EcoAnalysts staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced as a result of these analyses were recorded on approved data sheets. If an aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

### 3. RESULTS

The results of the effluent testing are presented in this section. Statistical comparisons and laboratory documents are provided in Appendix A. Chain-of-custody and sample receipt logs are provided in Appendix B.

#### 3.1 Topsmelt (*Atherinops affinis*) Chronic Test Results

The chronic toxicity test with *A. affinis* was initiated on June 14, 2022. The test was validated by 100% mean control survival, meeting the control acceptability criterion of ≥80% mean survival. The control treatment had a mean dry weight of 1.285 mg and a mean dry biomass of 1.285 mg, meeting the recommended growth criterion of ≥ 0.850 mg mean dry weight.

Mean survival and growth endpoints are summarized in Table 3-1. The statistical results are summarized in Table 3-2 and the test conditions are summarized in Table 3-3.

Concentrations of 2.0, 2.8, 10, 30, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the CCEC of 2.0% and ACEC of 2.8% effluent. The initial sample (received 6/14/22) was used for test initiation and the second and third samples (received 6/16/22 and 6/18/22) were used for test solution renewals.

Water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test.

No significant differences were observed between the laboratory control and the salt control for any of the biological endpoints tested. This indicates that artificial salts should not have contributed significantly to any negative biological effects, if observed.

The LC<sub>50</sub> for the copper chloride reference-toxicant test was 96.5 µg Cu/L for survival and 106.3 µg Cu/L for mean dry biomass. These results were within two standard deviations of the laboratory mean for survival and mean dry biomass (Table 3-3). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

**Table 3-1. Endpoint Summary for the *Atherinops affinis* Chronic Test**

| Conc. (%)        | Final Effluent    |                               |                                |
|------------------|-------------------|-------------------------------|--------------------------------|
|                  | Mean Survival (%) | Mean Growth (mg) <sup>1</sup> | Mean Biomass (mg) <sup>2</sup> |
| Control (0)      | 100               | 1.285                         | 1.285                          |
| Salt Control     | 100               | 1.204                         | 1.204                          |
| 2.0 <sup>3</sup> | 96                | 1.297                         | 1.245                          |
| 2.8 <sup>4</sup> | 100               | 1.232                         | 1.232                          |
| 10               | 100               | 1.252                         | 1.252                          |
| 30               | 96                | 1.177                         | 1.130                          |
| 100              | 96                | 1.300                         | 1.242                          |

<sup>1</sup> Average weight (mg) per survivor.

<sup>2</sup> Average weight (mg) per original number of animals stocked (Biomass).

<sup>3</sup> Chronic Critical Effluent Concentration (CCEC).

<sup>4</sup> Acute Critical Effluent Concentration (ACEC).

**Table 3-2. Statistical Results Summary for *Atherinops affinis* Chronic Test**

| Endpoint                                | Final Effluent |        |         |
|---|----------------|--------|---------|
|   | Survival       | Growth | Biomass |
| NOEL (%)                                | 100            | 100    | 100     |
| LOEL (%)                                | >100           | >100   | >100    |
| LC <sub>50</sub> / EC <sub>50</sub> (%) | >100           | >100   | >100    |

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>50</sub>/EC<sub>50</sub> = Lethal/Effect Concentration to 50% of test population

**Table 3-3. Test Condition Summary for *Atherinops affinis* Chronic Test**

| Test Duration / Type   |   | 7-Day / Static-Renewal   |   |
|--|---|--|---|
| Species  |   | <i>Atherinops affinis</i>  |   |
| Supplier   |   | Aquatic Bio Systems, Inc.  |   |
| Date acquired  |   | 6/14/22  |   |
| Test Dates   |   | 6/14/22 – 6/21/22  |   |
| Age at test initiation (Recommended: 9-15 days)              |   | 10 Days  |   |
| Samples used:  |   | Final Effluent #1 ; P220614.01<br>Final Effluent #2 ; P220616.01<br>Final Effluent #3 ; P220618.01 |   |
| Sample Holding Time at Initiation:<br>Recommended: <36 hours |   | 34 hours   |   |
| <b>Test Procedures</b>                                       |   | WDOE WQ-R-95-80; EPA 600/R-95/136; Test Method 1006.0; SOP TOX002.10                               |   |
| Test location  |   | EcoAnalysts; Port Gamble, WA   |   |
| Control water / Diluent                                      |   | 0.45 µm-filtered, North Hood Canal seawater  |   |
| Test Lighting  |   | 16-hour light / 8-hour dark  |   |
| Test Chamber   |   | 20 oz. cup   |   |
| Exposure volume  |   | 250 mL   |   |
| Replicates/treatment   |   | 5  |   |
| Concentration/treatment                                      |   | 2.0, 2.8, 10, 30 and 100%  |   |
| Organisms/replicate  |   | 5  |   |
| Feeding  |   | 250 Artemia nauplii am / 500 pm, except Day 7  |   |
| Test solution renewal  |   | Daily (Days 1-6)   |   |
| <b>Test Water Quality</b>                                    |   |  |   |
| Test Dissolved Oxygen  |   | Recommended: > 4.0 mg/L  | 5.9 – 8.7 mg/L                                  |
| Test Temperature   |   | Recommended: 20 ± 1°C  | 19.4 – 21.4 °C                                  |
| Test Salinity  |   | Recommended: 30 ± 2 ppt  | 29 – 32 ppt                                     |
| Test pH  |   | Recommended: 6 - 9 units   | 7.6 – 8.3                                       |
| <b>Quality Assurance</b>                                     |   |  |   |
| Control performance standards                                |   | Survival Recommended: ≥ 80%  | Actual: 100%; meets acceptability criterion     |
|  |   | Growth Recommended: ≥ 0.850 mg   | Actual: 1.285 mg, meets acceptability criterion |
|  |   | Power Standard: ≤39% (Growth)  | -1%; meets criterion                            |
| <b>Reference Toxicant</b>                                    |   | <b>Copper Chloride</b>   |   |
| Reference Toxicant Date                                      |   | 6/14/22  |   |
| Survival   | Reference Toxicant LC <sub>50</sub> (must be < 205 µg/L)          | 96.5 µg Cu/L   |   |
|  | Laboratory Mean LC <sub>50</sub> ; Range LC <sub>50</sub> (±2 SD) | 117.7 µg Cu/L (77.7 – 178.1 µg Cu/L)   |   |
|  | PMSD (must be <25%)   | 14.6%  |   |
| Biomass  | Reference Toxicant LC <sub>50</sub>                               | 106.3 µg Cu/L  |   |
|  | Laboratory Mean LC <sub>50</sub> ; Range LC <sub>50</sub> (±2 SD) | 113.6 µg Cu/L (75.7 – 170.7 µg Cu/L)   |   |
|  | PMSD (must be <50%)   | 17.0%  |   |
| <b>Deviations from Test Protocol</b>                         |   | None   |   |

### 3.2 Mysid (*Americamysis bahia*) Chronic Test Results

The chronic toxicity test with *A. bahia* was initiated on June 14, 2022. Mean survival in the control was 97.5%, meeting the control acceptability criterion of  $\geq 80\%$  mean survival. The control treatment had a mean dry weight of 0.329 mg and a mean dry biomass of 0.321 mg, meeting the recommended growth criterion of  $\geq 0.200$  mg mean dry weight.

Mean survival and growth endpoints are summarized in Table 3-4. The statistical results are summarized in Table 3-5 and the test conditions are summarized in Table 3-6.

Concentrations of 2.0, 2.8, 10, 30, and 100% effluent were prepared utilizing laboratory water. This concentration series includes the ACEC of 2.8% and CCEC of 2.0% effluent. The initial sample (received 6/14/22) was used for test initiation and the second and third samples (received 6/16/22 and 6/18/22) were used for test solution renewals.

Water quality parameters were within the acceptable limits throughout the duration of the 7-day static-renewal test. One test organism was found stranded above the water line on Day 5 in Replicate 5 of the 10% concentration; the start count was adjusted accordingly for statistical analysis.

No significant differences were observed between the laboratory control and the salt control for any of the biological endpoints tested. This indicates that artificial salts should not have contributed significantly to any negative biological effects, if observed.

The LC<sub>50</sub> for the copper chloride reference-toxicant test was 262.7  $\mu\text{g Cu/L}$  for survival and 172.8  $\mu\text{g Cu/L}$  for mean dry biomass. These results were within two standard deviations of the laboratory mean for survival and mean dry biomass (Table 3-6). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

**Table 3-4. Endpoint Summary for the *Americamysis bahia* Chronic Test**

| Conc. (%)        | Final Effluent    |                               |                                |
|------------------|-------------------|-------------------------------|--------------------------------|
|                  | Mean Survival (%) | Mean Growth (mg) <sup>1</sup> | Mean Biomass (mg) <sup>2</sup> |
| Control (0)      | 97.5              | 0.329                         | 0.321                          |
| Salt Control     | 95.0              | 0.336                         | 0.320                          |
| 2.0 <sup>3</sup> | 100               | 0.359                         | 0.359                          |
| 2.8 <sup>4</sup> | 97.5              | 0.349                         | 0.340                          |
| 10               | 97.5              | 0.342                         | 0.333                          |
| 30               | 95.0              | 0.376                         | 0.362                          |
| 100              | 95.0              | 0.338                         | 0.319                          |

<sup>1</sup> Average weight (mg) per survivor.

<sup>2</sup> Average weight (mg) per original number of animals stocked (Biomass).

<sup>3</sup> Chronic Critical Effluent Concentration (CCEC).

<sup>4</sup> Acute Critical Effluent Concentration (ACEC).

**Table 3-5. Statistical Results Summary for *Americamysis bahia* Chronic Test**

| Endpoint                                | Final Effluent |        |         |
|---|----------------|--------|---------|
|   | Survival       | Growth | Biomass |
| NOEL (%)                                | 100            | 100    | 100     |
| LOEL (%)                                | >100           | >100   | >100    |
| LC <sub>50</sub> / EC <sub>50</sub> (%) | >100           | >100   | >100    |

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC<sub>50</sub>/EC<sub>50</sub> = Lethal/Effect Concentration to 50% of test population

**Table 3-6. Test Condition Summary for *Americamysis bahia* Chronic Test**

| Test Duration / Type   |   | 7-Day / Static-Renewal   |                |
|--|---|--|----------------|
| Species  |   | <i>Americamysis bahia</i>  |                |
| Supplier   |   | Aquatic Bio Systems, Inc.  |                |
| Date acquired  |   | 6/14/22  |                |
| Test Dates   |   | 6/14/22 – 6/21/22  |                |
| Age at test initiation (Recommended: 7 days)                 |   | 7 Days   |                |
| Samples used:  |   | Final Effluent #1 ; P220614.01<br>Final Effluent #2 ; P220616.01<br>Final Effluent #3 ; P220618.01 |                |
| Sample Holding Time at Initiation:<br>Recommended: <36 hours |   | 35 hours   |                |
| <b>Test Procedures</b>                                       |   | WDOE WQ-R-95-80; EPA-821-R-02-014; Test Method 1007.0; SOP TOX014.12                               |                |
| Test location  |   | EcoAnalysts; Port Gamble, WA   |                |
| Control water / Diluent                                      |   | 0.45 µm-filtered, North Hood Canal seawater  |                |
| Test Lighting  |   | 16-hour light / 8-hour dark  |                |
| Test Chamber   |   | 12 oz. cup   |                |
| Exposure volume  |   | 250 mL   |                |
| Replicates/treatment   |   | 8  |                |
| Concentration/treatment                                      |   | 2.0, 2.8, 10, 30, and 100%   |                |
| Organisms/replicate  |   | 5  |                |
| Feeding  |   | 375 <i>Artemia</i> nauplii twice daily, except Day 7   |                |
| Test solution renewal  |   | Daily (Days 1-6)   |                |
| <b>Test Water Quality</b>                                    |   |  |                |
| Test Dissolved Oxygen  |   | Recommended: > 4.0 mg/L  | 5.4 – 8.7 mg/L |
| Test Temperature   |   | Recommended: 26 ± 1°C  | 24.5 – 26.2 °C |
| Test Salinity  |   | Recommended: 30 ± 2 ppt  | 28 – 32 ppt    |
| Test pH  |   | Recommended: 6 - 9 units   | 7.6 – 8.3      |
| <b>Quality Assurance</b>                                     |   |  |                |
| <b>Control performance standards</b>                         |   |  |                |
| Survival (Recommended): ≥ 80%                                |   | 97.5%; meets acceptability criterion   |                |
| Growth (Recommended): ≥ 0.200 mg                             |   | 0.329 mg, meets acceptability criterion  |                |
| Power Standard: ≤39% (Growth)                                |   | -9%; meets criterion   |                |
| <b>Reference Toxicant</b>                                    |   | <b>Copper Chloride</b>   |                |
| Reference Toxicant Date                                      |   | 6/14/22  |                |
| Survival   | Reference Toxicant LC <sub>50</sub>                               | 262.7 µg Cu/L  |                |
|  | Laboratory Mean LC <sub>50</sub> ; Range LC <sub>50</sub> (±2 SD) | 241.5 µg Cu/L (177.6 – 328.3 µg Cu/L)  |                |
| Mean Dry Biomass   | Reference Toxicant LC <sub>50</sub>                               | 172.8 µg Cu/L  |                |
|  | Laboratory Mean LC <sub>50</sub> ; Range LC <sub>50</sub> (±2 SD) | 181.6 µg Cu/L (136.9 – 241.0 µg Cu/L)  |                |
| <b>Deviations from Test Protocol</b>                         |   | Test organism stranded above water line  |                |



## 4. REFERENCES

- CETIS. 2016. CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.
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## **APPENDIX A**

Statistical Comparisons

Laboratory Documents

## **APPENDIX A1.1**

LOTT Final Effluent  
*Atherinops affinis* (Topsmelt)  
7-day Chronic Test

# CETIS Summary Report

Report Date: 06 Jul-22 08:35 (p 1 of 3)  
 Test Code/ID: P220614.01 / 06-5866-0941

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

|                                 |                                   |   |
|---------------------------------|-----------------------------------|---|
| Batch ID: 01-5957-2535 ✓        | Test Type: Growth-Survival (7d)   | Analyst: Marisa Seibert                       |
| Start Date: 14 Jun-22 16:01 ✓   | Protocol: EPA/600/R-95/136 (1995) | Diluent: Laboratory Seawater                  |
| Ending Date: 21 Jun-22 14:48 ✓  | Species: Atherinops affinis       | Brine: Crystal Sea Marine Mix                 |
| Test Length: 6d 23h             | Taxon: Actinopterygii             | Source: Aquatic Biosystems, CO Age: 10d ✓     |
| Sample ID: 06-3141-7399         | Code: P220614.01 ✓                | Project: NPDES                                |
| Sample Date: 13 Jun-22 06:00 ✓  | Material: POTW Effluent           | Source: LOTT Clean Water Alliance (WA00370) ✓ |
| Receipt Date: 14 Jun-22 12:10 ✓ | CAS (PC):                         | Station: Final Effluent #1 ✓                  |
| Sample Age: 34h (0.5 °C) ✓      | Client: LOTT                      |   |

## Multiple Comparison Summary

| Analysis ID  | Endpoint               | Comparison Method                | ✓ NOEL | LOEL | TOEL | TU | PMSD  | S |
|--------------|------------------------|----------------------------------|--------|------|------|----|-------|---|
| 13-2883-4578 | 7d Proportion Survived | Steel Many-One Rank Sum Test     | 100    | >100 | n/a  | 1  | 11.0% | 1 |
| 18-6513-2620 | Mean Dry Biomass-mg    | Dunnett Multiple Comparison Test | 100    | >100 | n/a  | 1  | 14.6% | 1 |
| 01-7698-8861 | Mean Dry Weight-mg     | Steel Many-One Rank Sum Test     | 100    | >100 | n/a  | 1  | 12.9% | 1 |

## Point Estimate Summary

| Analysis ID  | Endpoint               | Point Estimate Method        | ✓ Level | %     | 95% LCL | 95% UCL | TU    | S |
|--------------|------------------------|------------------------------|---------|-------|---------|---------|-------|---|
| 01-9090-4251 | 7d Proportion Survived | Linear Interpolation (ICPIN) | ✓ EC5   | >100  | n/a     | n/a     | <1    | 1 |
|              |                        |                              | ✓ EC10  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ EC15  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ EC20  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ EC25  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ EC40  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ EC50  | >100  | n/a     | n/a     | <1    |   |
| 11-3911-7539 | Mean Dry Biomass-mg    | Linear Interpolation (ICPIN) | ✓ IC5   | 15.26 | n/a     | n/a     | 6.554 | 1 |
|              |                        |                              | ✓ IC10  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC15  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC20  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC25  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC40  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC50  | >100  | n/a     | n/a     | <1    |   |
| 02-4208-0216 | Mean Dry Weight-mg     | Linear Interpolation (ICPIN) | IC5     | >100  | n/a     | n/a     | <1    | ↑ |
|              |                        |                              | ✓ IC10  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC15  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC20  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC25  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC40  | >100  | n/a     | n/a     | <1    |   |
|              |                        |                              | ✓ IC50  | >100  | n/a     | n/a     | <1    |   |

## Test Acceptability

| Analysis ID  | Endpoint               | Attribute    | Test Stat | TAC Limits |       | Overlap | Decision        |
|--------------|------------------------|--------------|-----------|------------|-------|---------|-----------------|
|              |                        |              |           | Lower      | Upper |         |                 |
| 01-9090-4251 | 7d Proportion Survived | Control Resp | 1         | 0.8        | >>    | Yes     | Passes Criteria |
| 13-2883-4578 | 7d Proportion Survived | Control Resp | 1         | 0.8        | >>    | Yes     | Passes Criteria |
| 11-3911-7539 | Mean Dry Biomass-mg    | Control Resp | 1.285     | 0.85       | >>    | Yes     | Passes Criteria |
| 18-6513-2620 | Mean Dry Biomass-mg    | Control Resp | 1.285     | 0.85       | >>    | Yes     | Passes Criteria |

**CETIS Summary Report**

Report Date: 06 Jul-22 08:35 (p 2 of 3)  
 Test Code/ID: P220614.01 / 06-5866-0941

**Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

**7d Proportion Survived Summary**

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Min    | Max    | Std Err | Std Dev | CV%   | %Effect |
|--------|------|-------|--------|---------|---------|--------|--------|---------|---------|-------|---------|
| 0      | D    | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00% | 0.00%   |
| 0      | SC   | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00% | 0.00%   |
| 2      |      | 5     | 0.9600 | 0.8489  | 1.0000  | 0.8000 | 1.0000 | 0.0400  | 0.0894  | 9.32% | 4.00%   |
| 2.8    |      | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00% | 0.00%   |
| 10     |      | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00% | 0.00%   |
| 30     |      | 5     | 0.9600 | 0.8489  | 1.0000  | 0.8000 | 1.0000 | 0.0400  | 0.0894  | 9.32% | 4.00%   |
| 100    |      | 5     | 0.9600 | 0.8489  | 1.0000  | 0.8000 | 1.0000 | 0.0400  | 0.0894  | 9.32% | 4.00%   |

**Mean Dry Biomass-mg Summary**

| Conc-% | Code | Count | Mean  | 95% LCL | 95% UCL | Min   | Max   | Std Err | Std Dev | CV%    | %Effect |
|--------|------|-------|-------|---------|---------|-------|-------|---------|---------|--------|---------|
| 0      | D    | 5     | 1.285 | 1.088   | 1.482   | 1.036 | 1.476 | 0.07097 | 0.1587  | 12.35% | 0.00%   |
| 0      | SC   | 5     | 1.204 | 1.144   | 1.264   | 1.146 | 1.272 | 0.02157 | 0.04823 | 4.01%  | 6.29%   |
| 2      |      | 5     | 1.245 | 1.081   | 1.409   | 1.028 | 1.382 | 0.059   | 0.1319  | 10.59% | 3.08%   |
| 2.8    |      | 5     | 1.232 | 1.038   | 1.425   | 0.97  | 1.35  | 0.06973 | 0.1559  | 12.66% | 4.14%   |
| 10     |      | 5     | 1.252 | 1.176   | 1.328   | 1.17  | 1.328 | 0.02736 | 0.06118 | 4.89%  | 2.58%   |
| 30     |      | 5     | 1.13  | 1.004   | 1.256   | 0.952 | 1.21  | 0.04539 | 0.1015  | 8.99%  | 12.08%  |
| 100    |      | 5     | 1.242 | 1.098   | 1.386   | 1.104 | 1.386 | 0.05193 | 0.1161  | 9.35%  | 3.33%   |

**Mean Dry Weight-mg Summary**

| Conc-% | Code | Count | Mean  | 95% LCL | 95% UCL | Min   | Max   | Std Err  | Std Dev | CV%    | %Effect |
|--------|------|-------|-------|---------|---------|-------|-------|----------|---------|--------|---------|
| 0      | D    | 5     | 1.285 | 1.088   | 1.482   | 1.036 | 1.476 | 0.07097  | 0.1587  | 12.35% | 0.00%   |
| 0      | SC   | 5     | 1.204 | 1.144   | 1.264   | 1.146 | 1.272 | 0.02157  | 0.04823 | 4.01%  | 6.29%   |
| 2      |      | 5     | 1.297 | 1.232   | 1.361   | 1.252 | 1.382 | 0.02325  | 0.05199 | 4.01%  | -0.92%  |
| 2.8    |      | 5     | 1.232 | 1.038   | 1.425   | 0.97  | 1.35  | 0.06973  | 0.1559  | 12.66% | 4.14%   |
| 10     |      | 5     | 1.252 | 1.176   | 1.328   | 1.17  | 1.328 | 0.02736  | 0.06118 | 4.89%  | 2.58%   |
| 30     |      | 5     | 1.177 | 1.15    | 1.205   | 1.158 | 1.21  | 0.009972 | 0.0223  | 1.89%  | 8.37%   |
| 100    |      | 5     | 1.3   | 1.136   | 1.464   | 1.104 | 1.443 | 0.05905  | 0.132   | 10.16% | -1.16%  |

# CETIS Summary Report

Report Date: 06 Jul-22 08:35 (p 3 of 3)  
 Test Code/ID: P220614.01 / 06-5866-0941

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

### 7d Proportion Survived Detail

| Conc-% | Code | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |
|--------|------|--------|--------|--------|--------|--------|
| 0      | D    | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0      | SC   | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2      |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |
| 2.8    |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 10     |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 30     |      | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 |
| 100    |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |

### Mean Dry Biomass-mg Detail

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 1.32  | 1.036 | 1.316 | 1.276 | 1.476 |
| 0      | SC   | 1.272 | 1.178 | 1.196 | 1.146 | 1.228 |
| 2      |      | 1.26  | 1.252 | 1.304 | 1.382 | 1.028 |
| 2.8    |      | 1.348 | 1.266 | 1.35  | 1.224 | 0.97  |
| 10     |      | 1.17  | 1.22  | 1.328 | 1.25  | 1.29  |
| 30     |      | 1.168 | 1.16  | 1.21  | 0.952 | 1.158 |
| 100    |      | 1.104 | 1.322 | 1.386 | 1.244 | 1.154 |

### Mean Dry Weight-mg Detail

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 1.32  | 1.036 | 1.316 | 1.276 | 1.476 |
| 0      | SC   | 1.272 | 1.178 | 1.196 | 1.146 | 1.228 |
| 2      |      | 1.26  | 1.252 | 1.304 | 1.382 | 1.285 |
| 2.8    |      | 1.348 | 1.266 | 1.35  | 1.224 | 0.97  |
| 10     |      | 1.17  | 1.22  | 1.328 | 1.25  | 1.29  |
| 30     |      | 1.168 | 1.16  | 1.21  | 1.19  | 1.158 |
| 100    |      | 1.104 | 1.322 | 1.386 | 1.244 | 1.443 |

### 7d Proportion Survived Binomials

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 0      | SC   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 2      |      | 5/5   | 5/5   | 5/5   | 5/5   | 4/5   |
| 2.8    |      | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 10     |      | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 30     |      | 5/5   | 5/5   | 5/5   | 4/5   | 5/5   |
| 100    |      | 5/5   | 5/5   | 5/5   | 5/5   | 4/5   |

# CETIS Analytical Report

Report Date: 06 Jul-22 08:34 (p 1 of 6)  
 Test Code/ID: P220614.01 / 06-5866-0941

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

|                               |                                    |   |
|-------------------------------|------------------------------------|---|
| Analysis ID: 08-2754-5857     | Endpoint: 7d Proportion Survived   | CETIS Version: CETISv1.9.4                  |
| Analyzed: 06 Jul-22 8:33      | Analysis: Nonparametric-Two Sample | Status Level: 1                             |
| Batch ID: 01-5957-2535        | Test Type: Growth-Survival (7d)    | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:01   | Protocol: EPA/600/R-95/136 (1995)  | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 14:48  | Species: Atherinops affinis        | Brine: Crystal Sea Marine Mix               |
| Test Length: 6d 23h           | Taxon: Actinopterygii              | Source: Aquatic Biosystems, CO Age: 10d     |
| Sample ID: 06-3141-7399       | Code: P220614.01                   | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent            | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                          | Station: Final Effluent #1                  |
| Sample Age: 34h (0.5 °C)      | Client: LOTT                       |   |

|                     |         |  |
|---------------------|---------|--|
| Data Transform      | Alt Hyp | Comparison Result                          |
| Angular (Corrected) | C > T   | Salt Control passed 7d proportion survived |

### Wilcoxon Rank Sum Two-Sample Test

| Control        | vs | Control II   | Test Stat | Critical | Ties | DF | P-Type | P-Value | Decision(α:5%)         |
|----------------|----|--------------|-----------|----------|------|----|--------|---------|------------------------|
| Dilution Water |    | Salt Control | 27.5      | n/a      | 1    | 8  | Exact  | 1.0000  | Non-Significant Effect |

### Test Acceptability Criteria

| Attribute    | Test Stat | TAC Limits |       | Overlap | Decision        |
|--------------|-----------|------------|-------|---------|-----------------|
|              |           | Lower      | Upper |         |                 |
| Control Resp | 1         | 0.8        | >>    | Yes     | Passes Criteria |
| Control Resp | 1         | 0.8        | >>    | Yes     | Passes Criteria |

### ANOVA Table

| Source  | Sum Squares | Mean Square | DF | F Stat | P-Value  | Decision(α:5%)     |
|---------|-------------|-------------|----|--------|----------|--------------------|
| Between | 0           | 0           | 1  | 65540  | <1.0E-37 | Significant Effect |
| Error   | 0           | 0           | 8  |        |          |                    |
| Total   | 0           |             | 9  |        |          |                    |

### 7d Proportion Survived Summary

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Median | Min    | Max    | Std Err | CV%   | %Effect |
|--------|------|-------|--------|---------|---------|--------|--------|--------|---------|-------|---------|
| 0      | D    | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 1.0000 | 0.0000  | 0.00% | 0.00%   |
| 0      | SC   | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 1.0000 | 0.0000  | 0.00% | 0.00%   |

### Angular (Corrected) Transformed Summary

| Conc-% | Code | Count | Mean  | 95% LCL | 95% UCL | Median | Min   | Max   | Std Err | CV%   | %Effect |
|--------|------|-------|-------|---------|---------|--------|-------|-------|---------|-------|---------|
| 0      | D    | 5     | 1.345 | 1.345   | 1.346   | 1.345  | 1.345 | 1.345 | 0       | 0.00% | 0.00%   |
| 0      | SC   | 5     | 1.345 | 1.345   | 1.346   | 1.345  | 1.345 | 1.345 | 0       | 0.00% | 0.00%   |

### 7d Proportion Survived Detail

| Conc-% | Code | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |
|--------|------|--------|--------|--------|--------|--------|
| 0      | D    | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0      | SC   | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |

### Angular (Corrected) Transformed Detail

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 1.345 | 1.345 | 1.345 | 1.345 | 1.345 |
| 0      | SC   | 1.345 | 1.345 | 1.345 | 1.345 | 1.345 |

### 7d Proportion Survived Binomials

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 0      | SC   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |

# CETIS Analytical Report

Report Date: 06 Jul-22 08:34 (p 2 of 6)  
Test Code/ID: P220614.01 / 06-5866-0941

## Topsmelt 7-d Survival and Growth Test

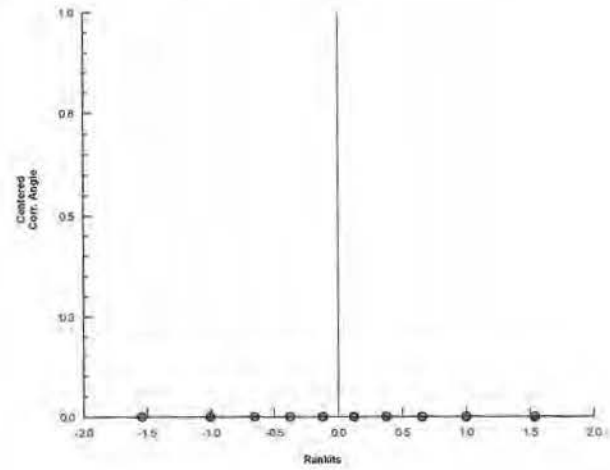
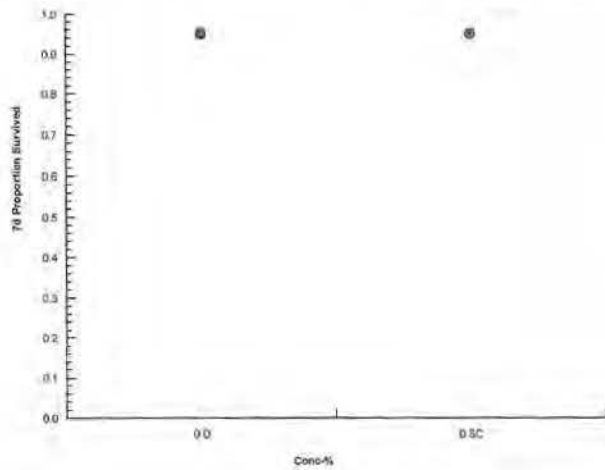
EcoAnalysts

Analysis ID: 08-2754-5857  
Analyzed: 06 Jul-22 8:33

Endpoint: 7d Proportion Survived  
Analysis: Nonparametric-Two Sample

CETIS Version: CETISv1.9.4  
Status Level: 1

### Graphics





**CETIS Analytical Report**

Report Date: 06 Jul-22 08:34 (p 3 of 6)  
 Test Code/ID: P220614.01 / 06-5866-0941

**Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

|                               |                                   |   |
|-------------------------------|-----------------------------------|---|
| Analysis ID: 09-2752-8425     | Endpoint: Mean Dry Biomass-mg     | CETIS Version: CETISv1.9.4                  |
| Analyzed: 06 Jul-22 8:33      | Analysis: Parametric-Two Sample   | Status Level: 1                             |
| Batch ID: 01-5957-2535        | Test Type: Growth-Survival (7d)   | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:01   | Protocol: EPA/600/R-95/136 (1995) | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 14:48  | Species: Atherinops affinis       | Brine: Crystal Sea Marine Mix               |
| Test Length: 6d 23h           | Taxon: Actinopterygii             | Source: Aquatic Biosystems, CO Age: 10d     |
| Sample ID: 06-3141-7399       | Code: P220614.01                  | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent           | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                         | Station: Final Effluent #1                  |
| Sample Age: 34h (0.5 °C)      | Client: LOTT                      |   |

| Data Transform | Alt Hyp | Comparison Result                       | PMSD   |
|----------------|---------|---|--------|
| Untransformed  | C > T   | Salt Control passed mean dry biomass-mg | 10.74% |

**Equal Variance t Two-Sample Test**

| Control        | vs | Control II   | Test Stat | Critical | MSD   | DF | P-Type | P-Value | Decision(α:5%)         |
|----------------|----|--------------|-----------|----------|-------|----|--------|---------|------------------------|
| Dilution Water |    | Salt Control | 1.089     | 1.86     | 0.138 | 8  | CDF    | 0.1539  | Non-Significant Effect |

**Test Acceptability Criteria**

| Attribute    | Test Stat | TAC Limits |       |         | Decision        |
|--------------|-----------|------------|-------|---------|-----------------|
|              |           | Lower      | Upper | Overlap |                 |
| Control Resp | 1.204     | 0.85       | >>    | Yes     | Passes Criteria |
| Control Resp | 1.285     | 0.85       | >>    | Yes     | Passes Criteria |

**ANOVA Table**

| Source  | Sum Squares | Mean Square | DF | F Stat | P-Value | Decision(α:5%)         |
|---------|-------------|-------------|----|--------|---------|------------------------|
| Between | 0.0163214   | 0.0163214   | 1  | 1.186  | 0.3078  | Non-Significant Effect |
| Error   | 0.110053    | 0.0137566   | 8  |        |         |                        |
| Total   | 0.126374    |             | 9  |        |         |                        |

**Distributional Tests**

| Attribute    | Test                                 | Test Stat | Critical | P-Value | Decision(α:1%)      |
|--------------|--------------------------------------|-----------|----------|---------|---------------------|
| Variances    | Levene Equality of Variance Test     | 1.748     | 11.26    | 0.2226  | Equal Variances     |
| Variances    | Mod Levene Equality of Variance Test | 1.454     | 13.75    | 0.2733  | Equal Variances     |
| Variances    | Variance Ratio F Test                | 10.83     | 23.15    | 0.0405  | Equal Variances     |
| Distribution | Anderson-Darling A2 Normality Test   | 0.6888    | 3.878    | 0.0721  | Normal Distribution |
| Distribution | D'Agostino Skewness Test             | 1.251     | 2.576    | 0.2109  | Normal Distribution |
| Distribution | Kolmogorov-Smirnov D Test            | 0.2071    | 0.3025   | 0.2806  | Normal Distribution |
| Distribution | Shapiro-Wilk W Normality Test        | 0.8913    | 0.7411   | 0.1755  | Normal Distribution |

**Mean Dry Biomass-mg Summary**

| Conc-% | Code | Count | Mean  | 95% LCL | 95% UCL | Median | Min   | Max   | Std Err | CV%    | %Effect |
|--------|------|-------|-------|---------|---------|--------|-------|-------|---------|--------|---------|
| 0      | D    | 5     | 1.285 | 1.088   | 1.482   | 1.316  | 1.036 | 1.476 | 0.07097 | 12.35% | 0.00%   |
| 0      | SC   | 5     | 1.204 | 1.144   | 1.264   | 1.196  | 1.146 | 1.272 | 0.02157 | 4.01%  | 6.29%   |

**Mean Dry Biomass-mg Detail**

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 1.32  | 1.036 | 1.316 | 1.276 | 1.476 |
| 0      | SC   | 1.272 | 1.178 | 1.196 | 1.146 | 1.228 |

Topsmelt 7-d Survival and Growth Test

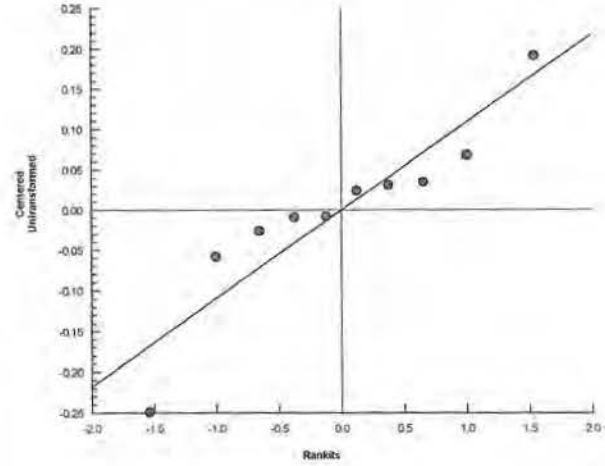
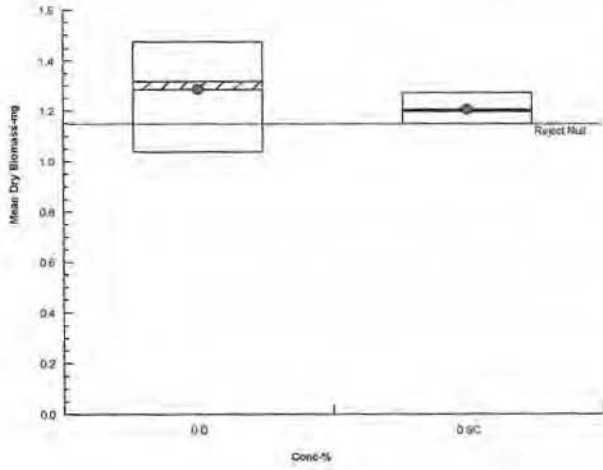
EcoAnalysts

Analysis ID: 09-2752-8425  
Analyzed: 06 Jul-22 8:33

Endpoint: Mean Dry Biomass-mg  
Analysis: Parametric-Two Sample

CETIS Version: CETISv1.9.4  
Status Level: 1

Graphics



**CETIS Analytical Report**

Report Date: 06 Jul-22 08:34 (p 5 of 6)  
 Test Code/ID: P220614.01 / 06-5866-0941

**Topsmelt 7-d Survival and Growth Test**

EcoAnalysts

|                               |                                   |   |
|-------------------------------|-----------------------------------|---|
| Analysis ID: 21-2469-8350     | Endpoint: Mean Dry Weight-mg      | CETIS Version: CETISv1.9.4                  |
| Analyzed: 06 Jul-22 8:33      | Analysis: Parametric-Two Sample   | Status Level: 1                             |
| Batch ID: 01-5957-2535        | Test Type: Growth-Survival (7d)   | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:01   | Protocol: EPA/600/R-95/136 (1995) | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 14:48  | Species: Atherinops affinis       | Brine: Crystal Sea Marine Mix               |
| Test Length: 6d 23h           | Taxon: Actinopterygii             | Source: Aquatic Biosystems, CO Age: 10d     |
| Sample ID: 06-3141-7399       | Code: P220614.01                  | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent           | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                         | Station: Final Effluent #1                  |
| Sample Age: 34h (0.5 °C)      | Client: LOTT                      |   |

| Data Transform | Alt Hyp | Comparison Result                      | PMSD   |
|----------------|---------|--|--------|
| Untransformed  | C > T   | Salt Control passed mean dry weight-mg | 10.74% |

**Equal Variance t Two-Sample Test**

| Control        | vs | Control II   | Test Stat | Critical | MSD   | DF | P-Type | P-Value | Decision(α:5%)         |
|----------------|----|--------------|-----------|----------|-------|----|--------|---------|------------------------|
| Dilution Water |    | Salt Control | 1.089     | 1.86     | 0.138 | 8  | CDF    | 0.1539  | Non-Significant Effect |

**ANOVA Table**

| Source  | Sum Squares | Mean Square | DF | F Stat | P-Value | Decision(α:5%)         |
|---------|-------------|-------------|----|--------|---------|------------------------|
| Between | 0.0163214   | 0.0163214   | 1  | 1.186  | 0.3078  | Non-Significant Effect |
| Error   | 0.110053    | 0.0137566   | 8  |        |         |                        |
| Total   | 0.126374    |             | 9  |        |         |                        |

**Distributional Tests**

| Attribute    | Test                                 | Test Stat | Critical | P-Value | Decision(α:1%)      |
|--------------|--------------------------------------|-----------|----------|---------|---------------------|
| Variances    | Levene Equality of Variance Test     | 1.748     | 11.26    | 0.2226  | Equal Variances     |
| Variances    | Mod Levene Equality of Variance Test | 1.454     | 13.75    | 0.2733  | Equal Variances     |
| Variances    | Variance Ratio F Test                | 10.83     | 23.15    | 0.0405  | Equal Variances     |
| Distribution | Anderson-Darling A2 Normality Test   | 0.6888    | 3.878    | 0.0721  | Normal Distribution |
| Distribution | D'Agostino Skewness Test             | 1.251     | 2.576    | 0.2109  | Normal Distribution |
| Distribution | Kolmogorov-Smirnov D Test            | 0.2071    | 0.3025   | 0.2806  | Normal Distribution |
| Distribution | Shapiro-Wilk W Normality Test        | 0.8913    | 0.7411   | 0.1755  | Normal Distribution |

**Mean Dry Weight-mg Summary**

| Conc-% | Code | Count | Mean  | 95% LCL | 95% UCL | Median | Min   | Max   | Std Err | CV%    | %Effect |
|--------|------|-------|-------|---------|---------|--------|-------|-------|---------|--------|---------|
| 0      | D    | 5     | 1.285 | 1.088   | 1.482   | 1.316  | 1.036 | 1.476 | 0.07097 | 12.35% | 0.00%   |
| 0      | SC   | 5     | 1.204 | 1.144   | 1.264   | 1.196  | 1.146 | 1.272 | 0.02157 | 4.01%  | 6.29%   |

**Mean Dry Weight-mg Detail**

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|--------|------|-------|-------|-------|-------|-------|
| 0      | D    | 1.32  | 1.036 | 1.316 | 1.276 | 1.476 |
| 0      | SC   | 1.272 | 1.178 | 1.196 | 1.146 | 1.228 |

Topsmelt 7-d Survival and Growth Test

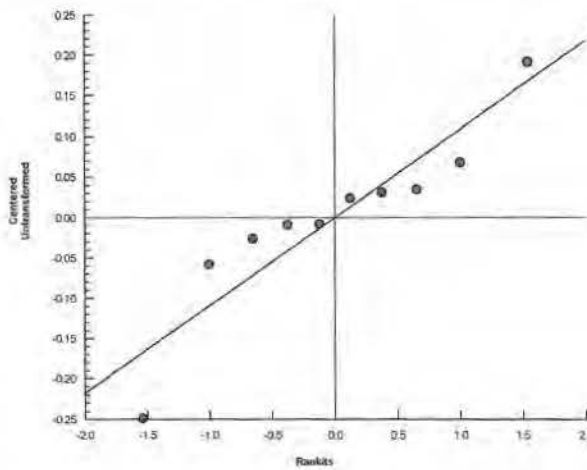
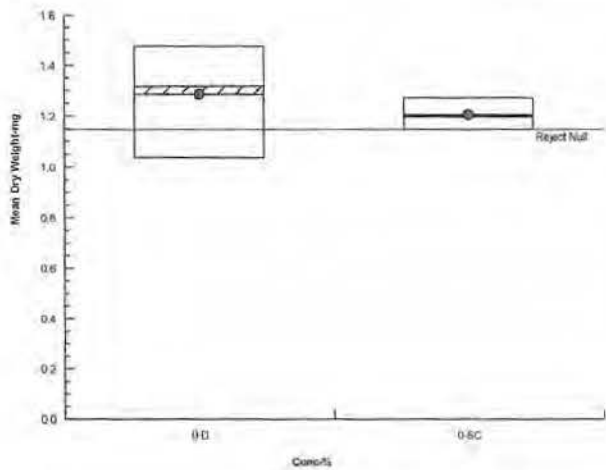
EcoAnalysts

Analysis ID: 21-2469-8350  
Analyzed: 06 Jul-22 8:33

Endpoint: Mean Dry Weight-mg  
Analysis: Parametric-Two Sample

CETIS Version: CETISv1.9.4  
Status Level: 1

Graphics



CETIS Test Data Worksheet

Report Date: 06 Jul-22 08:32 (p 1 of 2)  
 Test Code/ID: P220614.01 / 06-5866-0941

Topsmelt 7-d Survival and Growth Test

EcoAnalysts

Start Date: 14 Jun-22 16:01 Species: Atherinops affinis Sample Code: P220614.01  
 End Date: 21 Jun-22 14:48 Protocol: EPA/600/R-95/136 (1995) Sample Source: LOTT Clean Water Alliance  
 Sample Date: 13 Jun-22 06:00 Material: POTW Effluent Sample Station: Final Effluent #1

| Conc-% | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg Total | Weight-mg Tare | Pan Count | Notes |
|--------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|-----------|-------|
| 0      | D    | 1   | 15  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 75.31           | 68.71          | 5         |       |
| 0      | D    | 2   | 26  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 62.3            | 57.12          | 5         |       |
| 0      | D    | 3   | 4   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 63.13           | 56.55          | 5         |       |
| 0      | D    | 4   | 18  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 56.67           | 50.29          | 5         |       |
| 0      | D    | 5   | 29  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 58.47           | 51.09          | 5         |       |
| 0      | SC   | 1   | 25  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 68.8            | 62.44          | 5         |       |
| 0      | SC   | 2   | 11  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 65.01           | 59.12          | 5         |       |
| 0      | SC   | 3   | 14  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 60.34           | 54.36          | 5         |       |
| 0      | SC   | 4   | 7   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 61.45           | 55.72          | 5         |       |
| 0      | SC   | 5   | 9   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 66.44           | 60.3           | 5         |       |
| 2      |      | 1   | 5   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 54.84           | 48.54          | 5         |       |
| 2      |      | 2   | 33  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 63.01           | 56.75          | 5         |       |
| 2      |      | 3   | 22  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 59.63           | 53.11          | 5         |       |
| 2      |      | 4   | 8   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 60.96           | 54.05          | 5         |       |
| 2      |      | 5   | 27  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 4           | 58.14           | 53             | 4         |       |
| 2.8    |      | 1   | 16  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 56.82           | 50.08          | 5         |       |
| 2.8    |      | 2   | 31  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 54.64           | 48.31          | 5         |       |
| 2.8    |      | 3   | 13  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 58.04           | 51.29          | 5         |       |
| 2.8    |      | 4   | 2   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 55.56           | 49.44          | 5         |       |
| 2.8    |      | 5   | 34  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 56.44           | 51.59          | 5         |       |
| 10     |      | 1   | 32  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 63.22           | 57.37          | 5         |       |
| 10     |      | 2   | 21  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 65.5            | 59.4           | 5         |       |
| 10     |      | 3   | 30  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 59.83           | 53.19          | 5         |       |
| 10     |      | 4   | 23  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 55.6            | 49.35          | 5         |       |
| 10     |      | 5   | 24  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 55.7            | 49.25          | 5         |       |
| 30     |      | 1   | 3   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 54.65           | 48.81          | 5         |       |
| 30     |      | 2   | 17  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 62.19           | 56.39          | 5         |       |
| 30     |      | 3   | 19  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 52.52           | 46.47          | 5         |       |
| 30     |      | 4   | 10  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 4           | 33.15           | 28.39          | 4         |       |
| 30     |      | 5   | 20  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 39.49           | 33.7           | 5         |       |
| 100    |      | 1   | 35  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 41.69           | 36.17          | 5         |       |
| 100    |      | 2   | 28  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 41.78           | 35.17          | 5         |       |

**CETIS Test Data Worksheet**

Report Date: 06 Jul-22 08:32 (p 2 of 2)

Test Code/ID: P220614.01 / 06-5866-0941

| Conc-% | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg<br>Total | Weight-mg<br>Tare | Pan Count | Notes |
|--------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------------|-------------------|-----------|-------|
| 100    |      | 3   | 6   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 55.39              | 48.46             | 5         |       |
| 100    |      | 4   | 1   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 54.06              | 47.84             | 5         |       |
| 100    |      | 5   | 12  | 5         | 5           | 5           | 5           | 4           | 4           | 4           | 4           | 57.8               | 52.03             | 4         |       |

Version V.3

GENERAL

|                         |   |
|-------------------------|---|
| Client                  | LOTT Clean Water Alliance   |
| Project                 | NPDES   |
| Project Number          | PG1602  |
| Project Manager         | J. Levensgood   |
| Date Sample Received    | 6/14/2022   |
| Test type               | 7 Day Chronic Toxicity with Topsmelt  |
| Matrix                  | Liquid  |
| Test Acceptability      | ≥ 80% average survival in control<br>Average dry weight is > 0.85 mg per surviving fish |
| Test Start Date         | 06/14/22  |
| Test Species            | Atherinops affinis  |
| Organism Batch          | ABS061422.01  |
| Organism Acquired       | 6/14/2022   |
| Organism Acclimation    | 0   |
| Organism Age            | Larvae (9-15 days post-hatch)   |
| Test Protocol           | TOX 002   |
| Regional Protocol       | WDOE WQ-R-95-80   |
| Test Location           | Bath 3  |
| Light Intensity         | 50-100 foot candles   |
| Light Cycle             | 16L:8D  |
| Water Description       | 0.45 um filtered seawater   |
| Organisms per Replicate | 5   |
| Test Chamber Size       | 20 oz.  |
| Exposure Volume         | 250 mL  |
| Feeding Information     | 250 nauplii/chamber am<br>500 nauplii/chamber pm  |
| Test Dissolved Oxygen   | > 4.0   |
| Test Temperature        | 20 ± 1  |
| Test Salinity           | 30 ± 2  |
| Test pH                 | 7.5 ± 1.5   |

Note: input lowest and highest decimal for temp

| Test Parameters |     |     |
|-----------------|-----|-----|
|                 | Min | Max |
| DO              | 4.0 |     |
| Temp            | 19  | 21  |
| Salinity        | 28  | 32  |
| pH              | 6   | 9   |

TEST START TIME/INIT: **10:01 AM, RP**  
 TEST END TIME/INIT: **1:48 PM**

| CLIENT SAMPLE ID  | LAB ID     |
|-------------------|------------|
| Final Effluent #1 | P220614.01 |
| Final Effluent #2 | P220616.01 |
| Final Effluent #3 | P220618.01 |

| Concentrations |              |
|----------------|--------------|
| 1              | Control      |
| 2              | Salt Control |
| 3              | 2%           |
| 4              | 2.8%         |
| 5              | 10%          |
| 6              | 30%          |
| 7              | 100%         |
| 8              | -            |
| 9              | -            |

| Food Batch ID |
|---------------|
| 251523        |

| CSMM Batch # |
|--------------|
| C4444255     |

Copy and Past VALUES from

| Treatment    | Rep | Chamber |
|--------------|-----|---------|
| Control      | 1   | 21      |
| Control      | 2   | 32      |
| Control      | 3   | 35      |
| Control      | 4   | 9       |
| Control      | 5   | 28      |
| Salt Control | 1   | 3       |
| Salt Control | 2   | 30      |
| Salt Control | 3   | 29      |
| Salt Control | 4   | 22      |
| Salt Control | 5   | 34      |
| 2%           | 1   | 15      |
| 2%           | 2   | 18      |
| 2%           | 3   | 20      |
| 2%           | 4   | 19      |
| 2%           | 5   | 25      |
| 2.8%         | 1   | 1       |
| 2.8%         | 2   | 26      |
| 2.8%         | 3   | 8       |
| 2.8%         | 4   | 31      |
| 2.8%         | 5   | 4       |
| 10%          | 1   | 13      |
| 10%          | 2   | 16      |
| 10%          | 3   | 14      |
| 10%          | 4   | 12      |
| 10%          | 5   | 7       |
| 30%          | 1   | 11      |
| 30%          | 2   | 10      |
| 30%          | 3   | 2       |
| 30%          | 4   | 23      |
| 30%          | 5   | 5       |
| 100%         | 1   | 33      |
| 100%         | 2   | 17      |
| 100%         | 3   | 24      |
| 100%         | 4   | 27      |
| 100%         | 5   | 6       |



|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| V.3 CLIENT       | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood             |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Atherinops affinis</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

**7 Day Chronic Toxicity with Topsmelt**

| Day of Test | Concentration | Vol. Effluent Sample Added (mL) | Vol. Diluent Added (mL) | Total Volume (mL) | Diluent Type | FSW |
|-------------|---------------|---------------------------------|-------------------------|-------------------|--------------|-----|
| 0           | 0%            | 0                               | 1250.0                  | 1250              |              |     |
|             | Salt Control  | #VALUE!                         | #VALUE!                 | 1250              |              |     |
|             | 2%            | 25                              | 1225.0                  | 1250              |              |     |
|             | 2.8%          | 35                              | 1215.0                  | 1250              |              |     |
|             | 10%           | 125                             | 1125.0                  | 1250              |              |     |
|             | 30%           | 375                             | 875.0                   | 1250              |              |     |
|             | 100%          | 1250                            | 0.0                     | 1250              |              |     |

| Day of Test | Concentration | Vol. Effluent Sample Added (mL) | Vol. Diluent Added (mL) | Total Volume (mL) |
|-------------|---------------|---------------------------------|-------------------------|-------------------|
| 1 - 6       | 0%            | 0                               | 1250.0                  | 1250              |
|             | Salt Control  | #VALUE!                         | #VALUE!                 | 1250              |
|             | 2%            | 25                              | 1225.0                  | 1250              |
|             | 2.8%          | 35                              | 1215.0                  | 1250              |
|             | 10%           | 125                             | 1125.0                  | 1250              |
|             | 30%           | 375                             | 875.0                   | 1250              |
|             | 100%          | 1250                            | 0.0                     | 1250              |

**Test Dilution Prep**

| Date    | Balance ID | Sample ID (P#) | Water Batch ID | Initials |
|---------|------------|----------------|----------------|----------|
| 6/14    | #1         | P220614.01     | FSW061222.0    | RP       |
| 6/15    | #1         | P220614.01     | FSW061222.01   | RP       |
| 6/16/22 | #1         | P220614.01     | FSW061222.01   | SZ/MS    |
| 6/17/22 | #1         | P220610.01     | FSW061622.01   | MS       |
| 6/18/22 | #1         | P220616.01     | FSW061622.01   | RP       |
| 6/19/22 | #1         | P220618.01     | FSW061622.01   | SZ       |
| 6/20/22 | #1         | P220618.01     | FSW061922.01   | RP       |



|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood      |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Atherinops affinis |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

7 Day Chronic Toxicity with Topsmelt

| Concentration (%)                                | REP  | Day 1 |      | Day 2 |      | Day 3 |      | Day 4 |      | Day 5 |      | Day 6 |      | Day 7 |      |
|--|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|
|  |      | Date  | 6/15 | Date  | 6/16 | Date  | 6/17 | Date  | 6/18 | Date  | 6/19 | Date  | 6/20 | Date  | 6/21 |
|  |      | Time  | 1113 | Time  | 1510 | Time  | 1304 | Time  | 1043 | Time  | 1445 | Time  | 1103 | Time  | 1211 |
|  |      | Tech  | RP   | Tech  | MS   | Tech  | NL   | Tech  | RP   | Tech  | 62   | Tech  | RP   | Tech  | DM   |
| Alive  | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead |
| Control  | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| Salt Control                                     | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| 2%   | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| 2.8%   | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| 10%  | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| 30%  | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| 100%   | 1    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 2    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 3    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 4    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
|  | 5    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    | S     | 0    |
| Feed (Init.)                                     | AM   | RP    |      | MS    |      | MS    |      | NL    |      | NL    |      | RP    |      | NONE  |      |
| 250 nauplii/chamber am<br>500 nauplii/chamber pm | PM   | RP    |      | NL    |      | NL    |      | NL    |      | NL    |      | MK    |      | NONE  |      |

① IE-DM-6/21/22

v.3

|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood             |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Atherinops affinis</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

**7 Day Chronic Toxicity with Topsmelt**

| Concentration (%) | REP | Boat Number | Weight Empty Boat (mg)   | Weight Boat & Animals (mg) | Pan Count |
|-------------------|-----|-------------|--------------------------|----------------------------|-----------|
| Control           | 1   | 1           | 68.71                    | 75.31                      | 5         |
|                   | 2   | 2           | 57.12                    | 62.30                      | 5         |
|                   | 3   | 3           | 56.55                    | 63.13                      | 5         |
|                   | 4   | 4           | 50.29                    | <del>56.70</del> 67        | 5         |
|                   | 5   | 5           | 51.09                    | 58.47                      | 5         |
| Salt Control      | 1   | 6           | 62.44                    | 68.80                      | 5         |
|                   | 2   | 7           | 59.12                    | 65.01                      | 5         |
|                   | 3   | 8           | 54.36                    | 60.34                      | 5         |
|                   | 4   | 9           | <del>55.72</del> 57.72 ① | 61.45                      | 5         |
|                   | 5   | 10          | 60.30                    | 66.44                      | 5         |
| 2%                | 1   | 11          | <del>48.54</del> 48.55 ② | 54.84                      | 5         |
|                   | 2   | 12          | 56.75 56.75 ②            | 63.01                      | 5         |
|                   | 3   | 13          | 53.11 53.10 ②            | 59.63                      | 5         |
|                   | 4   | 14          | 54.05 54.06 ②            | 60.96                      | 5         |
|                   | 5   | 15          | 53.00                    | 58.14                      | 4         |
| 2.8%              | 1   | 16          | 50.08                    | 56.82                      | 5         |
|                   | 2   | 17          | 48.31                    | 54.64                      | 5         |
|                   | 3   | 18          | 51.29                    | 58.04                      | 5         |
|                   | 4   | 19          | 49.44                    | 55.56                      | 5         |
|                   | 5   | 20          | 51.59                    | 56.44                      | 5         |
| 10%               | 1   | 21          | 57.37                    | 63.22                      | 5         |
|                   | 2   | 22          | 59.40                    | 65.50                      | 5         |
|                   | 3   | 23          | 53.19                    | 59.83                      | 5         |
|                   | 4   | 24          | 49.35                    | 55.60                      | 5         |
|                   | 5   | 25          | 49.25                    | 55.70                      | 5         |
| 30%               | 1   | 26          | 48.81                    | 54.65                      | 5         |
|                   | 2   | 27          | 56.39                    | 62.19                      | 5         |
|                   | 3   | 28          | 46.47                    | 52.52                      | 5         |
|                   | 4   | 29          | 28.39                    | 37.15                      | 4         |
|                   | 5   | 30          | 33.70                    | 39.49                      | 5         |

- ① IE - 6/21/22 IM
- ② MR - 6/21/22 IM
- ③ MR - 6/23/22 IM

|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood             |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Atherinops affinis</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

7 Day Chronic Toxicity with Topsmelt

| Concentration (%) | REP | Boat Number | Weight Empty Boat (mg) | Weight Boat & Animals (mg) | Pan Count |
|-------------------|-----|-------------|------------------------|----------------------------|-----------|
| 100%              | 1   | 31          | 36.17                  | 41.69                      | 5         |
|                   | 2   | 32          | 35.17                  | 41.78                      | 5         |
|                   | 3   | 33          | 48.46                  | 55.39                      | 5         |
|                   | 4   | 34          | 47.84                  | 54.06                      | 5         |
|                   | 5   | 35          | 52.03                  | 57.80                      | 4         |

|                                   | Oven Event 1     | Oven Event 2  |
|-----------------------------------|------------------|---------------|
| Oven ID:                          | BEELZEBUB        | Beelzebub     |
| Date/Time/Initials In Oven:       | 6/21/22 0945 RP  | 6/21/22 1645  |
| Oven Temp °C:                     | 107°C            | 102°C         |
| Date/Time/Initials Out Oven into: | 6/21/22 1203 RP  | 6/22 1150 JL  |
| Date/Time/Initials Weighed:       | 6/21/22 IM 13:20 | 6/23 13:36 IM |
| Balance ID:                       | 3                | 3             |

Boat Number weight Empty Boat (mg)  
 1x 61.14  
 2x 75.70

|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| v.3 CLIENT       | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood             |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Atherinops affinis</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

**7 Day Chronic Toxicity with Topsmelt**

|                   | DO (mg/L)    | TEMP (°C) | SALINITY (ppt) | pH    |     |
|-------------------|--------------|-----------|----------------|-------|-----|
| Concentration (%) | > 4.0        | 19 - 21   | 28 - 32        | 6 - 9 |     |
| <b>Day 0</b>      |              |           |                |       |     |
| Stock             | Control      | 7.5       | 20.3           | 29    | 8.0 |
| Date 6/14/22      | Salt Control | 7.6       | 19.9           | 29    | 8.3 |
| Time 1458         | 2%           | 7.7       | 20.4           | 29    | 8.1 |
| Tech RP           | 2.8%         | 7.7       | 20.4           | 29    | 8.1 |
| Meter # 8         | 10%          | 7.7       | 20.2           | 29    | 8.1 |
| Feed DM           | 30%          | 7.7       | 19.8           | 29    | 7.9 |
|                   | 100%         | 6.5       | 20.8           | 30    | 7.8 |
| <b>Day 1</b>      | Control      | 7.0       | 20.2           | 30    | 7.9 |
| Rep 1             | Salt Control | 6.8       | 20.5           | 30    | 8.2 |
| Date 6/15/22      | 2%           | 6.7       | 20.3           | 30    | 8.0 |
| Time 0914         | 2.8%         | 6.8       | 20.5           | 30    | 8.0 |
| Tech RP           | 10%          | 6.9       | 20.4           | 30    | 8.0 |
| Meter # 9         | 30%          | 6.9       | 20.4           | 30    | 8.0 |
|                   | 100%         | 6.8       | 20.3           | 30    | 8.0 |
| <b>Day 1</b>      | Control      | 7.6       | 19.7           | 30    | 8.0 |
| Renewal Stock     | Salt Control | 7.6       | 19.9           | 30    | 8.2 |
| Date 6/15/22      | 2%           | 7.6       | 19.4           | 30    | 8.1 |
| Time 0902         | 2.8%         | 7.6       | 19.4           | 30    | 8.1 |
| Tech MR RP        | 10%          | 7.6       | 19.4           | 30    | 8.1 |
| Meter # 9         | 30%          | 7.9       | 19.5           | 20    | 8.0 |
|                   | 100%         | 8.7       | 19.9           | 30    | 7.9 |
| <b>Day 2</b>      | Control      | 6.8       | 20.7           | 30    | 7.9 |
| Rep 2             | Salt Control | 6.8       | 20.7           | 31    | 8.0 |
| Date 6/16/22      | 2%           | 6.9       | 20.6           | 30    | 7.9 |
| Time 1215         | 2.8%         | 6.9       | 20.7           | 30    | 7.9 |
| Tech MS           | 10%          | 6.8       | 20.7           | 30    | 7.9 |
| Meter # 8         | 30%          | 6.9       | 20.7           | 30    | 8.0 |
|                   | 100%         | ② 6.8 6.6 | 20.6           | 31    | 8.0 |
| <b>Day 2</b>      | Control      | 7.5       | 19.9           | 31    | 8.1 |
| Renewal Stock     | Salt Control | 7.5       | 20.3           | 30    | 8.2 |
| Date 6/16/22      | 2%           | 7.6       | 21.0           | 31    | 8.1 |
| Time 1210         | 2.8%         | 7.6       | 21.1           | 31    | 8.1 |
| Tech MS           | 10%          | 7.6       | 20.9           | 31    | 8.1 |
| Meter # 8         | 30%          | 7.8       | 20.3           | 31    | 8.0 |
|                   | 100%         | 7.9       | 21.2           | 31    | 7.9 |

① Δ techs - RP 6/15  
 ② MR - MS 6/16



|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| V.3 CLIENT       | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood             |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Atherinops affinis</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

**7 Day Chronic Toxicity with Topsmelt**

|               | Concentration (%) | DO (mg/L)            | TEMP (°C)              | SALINITY (ppt)     | pH  |
|---------------|-------------------|----------------------|------------------------|--------------------|-----|
| <b>Day 3</b>  | Control           | 6.3                  | 19.8                   | 31                 | 7.9 |
| Rep 3         | Salt Control      | 6.0                  | 20.2                   | 31                 | 7.9 |
| Date 6/17/22  | 2%                | 6.4                  | 20.0                   | 31                 | 7.8 |
| Time 10:55 am | 2.8%              | 6.1                  | 20.1                   | 31                 | 7.9 |
| Tech I.M./JL  | 10%               | 6.1                  | 20.1                   | 31                 | 7.9 |
| Meter # 9     | 30%               | 6.5                  | 20.2                   | 31                 | 8.0 |
|               | 100%              | 6.2                  | 20.2                   | 31                 | 8.0 |
| <b>Day 3</b>  | Control           | 7.6                  | 19.6                   | 32                 | 7.9 |
| Renewal Stock | Salt Control      | 7.3 <del>7.4</del> ① | 20.2 <del>19.8</del> ② | 31 <del>30</del> ③ | 8.2 |
| Date 6/17/22  | 2%                | 7.6                  | 19.7                   | 32                 | 7.9 |
| Time 11:27 am | 2.8%              | 7.7                  | 19.7                   | 32                 | 7.9 |
| Tech I.M./JL  | 10%               | 7.7                  | 19.8                   | 32                 | 7.9 |
| Meter # 9     | 30%               | 7.8                  | 20.6                   | 31                 | 7.9 |
|               | 100%              | 8.4                  | 20.3                   | 31                 | 7.8 |
| <b>Day 4</b>  | Control           | 6.1                  | 20.3                   | 31                 | 7.8 |
| Rep 4         | Salt Control      | 6.3                  | 20.3                   | 31                 | 7.9 |
| Date 6/18/22  | 2%                | 6.3                  | 20.3                   | 31                 | 7.8 |
| Time 1030     | 2.8%              | 6.3                  | 20.4                   | 31                 | 7.9 |
| Tech RP       | 10%               | 6.4                  | 20.3                   | 31                 | 7.9 |
| Meter # 6     | 30%               | 6.4                  | 20.3                   | 31                 | 7.9 |
|               | 100%              | 6.4                  | 20.4                   | 30                 | 8.0 |
| <b>Day 4</b>  | Control           | 7.5                  | 19.6                   | 31                 | 7.9 |
| Renewal Stock | Salt Control      | 7.4                  | 20.4                   | 30                 | 8.2 |
| Date 6/18/22  | 2%                | 7.6                  | 19.5                   | 31                 | 8.0 |
| Time 1014     | 2.8%              | 7.6                  | 19.4                   | 31                 | 8.0 |
| Tech RP       | 10%               | 7.5                  | 20.5                   | 31                 | 8.0 |
| Meter # 8     | 30%               | 7.6                  | 20.5                   | 31                 | 7.9 |
|               | 100%              | 8.2                  | 20.7                   | 29                 | 7.9 |
| <b>Day 5</b>  | Control           | 6.3                  | 20.4                   | 32                 | 7.7 |
| Rep 5         | Salt Control      | 6.6                  | 20.4                   | 31                 | 7.9 |
| Date 6/19/22  | 2%                | 6.4                  | 20.3                   | 32                 | 7.8 |
| Time 1415     | 2.8%              | 6.5                  | 20.5                   | 32                 | 7.8 |
| Tech SZ       | 10%               | 6.1                  | 20.4                   | 32                 | 7.8 |
| Meter # 9     | 30%               | 6.2                  | 20.5                   | 31                 | 7.9 |
|               | 100%              | 5.9                  | 20.5                   | 30                 | 8.0 |

① MR-IM 6/17/22  
 ② IE-IM 6/17/22, RP 6/18  
 ③

V.3

|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 002                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood             |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Atherinops affinis</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

**7 Day Chronic Toxicity with Topsmelt**

|               | Concentration (%) | DO (mg/L) | TEMP (°C) | SALINITY (ppt) | pH      |
|---------------|-------------------|-----------|-----------|----------------|---------|
|               |                   | > 4.0     | 19 - 21   | 28 - 32        | 6 - 9   |
| <b>Day 5</b>  | Control           | 7.5       | 19.8      | 32             | 8.0     |
| Renewal Stock | Salt Control      | 7.5       | 20.0      | 31             | 8.1     |
| Date 6/19     | 2%                | 7.5       | 19.8      | 32             | 8.1     |
| Time 1430     | 2.8%              | 7.5       | 19.8      | 32             | 8.1     |
| Tech 52       | 10%               | 7.5       | 19.9      | 32             | 8.0     |
| Meter # 9     | 30%               | 7.6       | 19.9      | 31             | 8.0     |
|               | 100%              | 8.1       | 20.0      | 31             | 7.9     |
| <b>Day 6</b>  | Control           | 6.3       | 20.3      | 31             | 7.8     |
| Rep 1         | Salt Control      | 6.3       | 20.4      | 30             | 7.9     |
| Date 6/20     | 2%                | 6.3       | 20.3      | 31             | 7.9     |
| Time 1043     | 2.8%              | 6.3       | 20.4      | 31             | 7.9     |
| Tech RP       | 10%               | 6.3       | 20.3      | 31             | 7.9     |
| Meter # 8     | 30%               | 6.3       | 20.4      | 31             | 7.9     |
|               | 100%              | 6.3       | 20.4      | 30             | 8.0     |
| <b>Day 6</b>  | Control           | 7.0       | 20.3      | 30             | 7.9     |
| Renewal Stock | Salt Control      | 7.0       | 20.2      | 29             | 7.7 8.3 |
| Date 6/20     | 2%                | 7.7       | 20.2      | 30             | 8.0     |
| Time 1052     | 2.8%              | 7.8       | 20.2      | 30             | 7.9     |
| Tech RP       | 10%               | 7.8       | 20.0      | 30             | 7.9     |
| Meter # 8     | 30%               | 7.9       | 19.9      | 30             | 7.9     |
|               | 100%              | 8.5       | 19.8      | 30             | 7.8     |
| <b>Day 7</b>  | Control           | 6.2       | 21.4      | 30             | 7.6     |
| Rep 2         | Salt Control      | 6.2       | 21.3      | 29             | 7.8     |
| Date 6/21/22  | 2%                | 6.3       | 21.1      | 30             | 7.8     |
| Time 1428     | 2.8%              | 5.9       | 21.1      | 30             | 7.7     |
| Tech 52       | 10%               | 6.2       | 21.1      | 30             | 7.7     |
| Meter # 8     | 30%               | 6.4       | 21.2      | 30             | 7.8     |
|               | 100%              | 6.5       | 21.1      | 29             | 7.9     |

① MR - RP 6/20/22

## Daily Quality Assurance Checks

Project name: LOTT

Test: Topsmelt Chronic

Lab ID: P 220014.01

| Day of Test |   | Initials | Date | Comments |
|-------------|---|----------|------|----------|
| 0           | Test datasheets checked for completeness and legibility                                       | DM       | 6/14 |          |
|             | Headers/ footers filled in, visual check of test chambers, cover test, ensure proper lighting | DM       | 6/14 |          |
|             | Test data within acceptable ranges  | DM       | 6/14 |          |
| 1           | Test datasheets checked for completeness and legibility                                       | RP       | 6/15 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 2           | Test datasheets checked for completeness and legibility                                       | ML       | 6/14 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 3           | Test datasheets checked for completeness and legibility                                       | DM       | 6/17 |          |
|             | Test data within acceptable ranges  | DM       | 6/17 |          |
|             |   |          |      |          |
| 4           | Test datasheets checked for completeness and legibility                                       | NL       | 6/18 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 5           | Test datasheets checked for completeness and legibility                                       | NL       | 6/19 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 6           | Test datasheets checked for completeness and legibility                                       | DM       | 6/20 |          |
|             | Test data within acceptable ranges  | DM       | 6/20 |          |
|             |   |          |      |          |
| 7           | Test datasheets checked for completeness and legibility                                       | MLC      | 6/21 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |

**POWER STANDARD CALCULATIONS**

**Topsmelt Mean Growth per Survivor**

Chronic Power Standard Calculation

|            | average growth/survivor |       |       |       |       |        |
|------------|-------------------------|-------|-------|-------|-------|--------|
| Replicate  | 1                       | 2     | 3     | 4     | 5     | Mean   |
| CCEC (2.0) | 1.26                    | 1.252 | 1.304 | 1.382 | 1.285 | 1.2966 |
| Control    | 1.32                    | 1.036 | 1.316 | 1.276 | 1.476 | 1.2848 |

Control Mean - CCEC Mean

-0.0118

Difference Divided by Control Mean

-0.00918431

Express as %

-1%

≤39% meets the power standard

Pass



## ORGANISM RECEIPT LOG

| <b>Date:</b><br>6/14/22                              | <b>Time:</b><br>1326              | <b>Batch No.</b><br>ABS061422.01   |  |               |        |         |                     |
|--|-----------------------------------|--|--|---------------|--------|---------|---------------------|
| <b>Organism:</b><br><br>Atherinops affinis           |                                   |  |  |               |        |         |                     |
| <b>Source / Supplier:</b><br><br>Aquatic Bio Systems |                                   |  |  |               |        |         |                     |
| <b>No. Ordered:</b><br><br>390                       | <b>No. Received:</b><br><br>390 + | <b>Source Batch:</b><br>Collection date, <u>hatch date</u> , etc.):<br><br>6/4/22                  |  |               |        |         |                     |
| <b>Condition of Organisms:</b><br><br>Good           |                                   | <b>Approximate Size or Age:</b><br>(Days from hatch, life stage, size class, etc.):<br><br>10 days |  |               |        |         |                     |
| <b>Shipper:</b><br><br>UPS                           |                                   | <b>B of L (Tracking No.)</b><br><br>1Z F46 73R 01 9568 4678  |  |               |        |         |                     |
| <b>Condition of Container:</b><br><br>Good           |                                   | <b>Received By:</b><br><br>RP  |  |               |        |         |                     |
| Container  | D.O.<br>(mg/L)                    | Temp.<br>(°C)  | Cond. or<br>Sal.<br>(Include<br>Units) | pH<br>(Units) | # Dead | % Dead* | Tech.<br>(Initials) |
| 1  | 15.4                              | 20.1   | 27 ppt                                 | 6.9           | 7      | —       | RP                  |
|  |                                   |  |  |               |        |         |                     |
|  |                                   |  |  |               |        |         |                     |
|  |                                   |  |  |               |        |         |                     |
|  |                                   |  |  |               |        |         |                     |
| <small>*if &gt;10% contact lab manager</small>       |                                   |  |  |               |        |         |                     |
| <b>Notes:</b>  |                                   |  |  |               |        |         |                     |

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: 6/13/2022

SPECIES: *Atherinops affinis*

AGE: 9 day

LIFE STAGE: Larvae

HATCH DATE: 6/4/2021

BEGAN FEEDING: Immediately


FOOD: *Artemia* sp.

### Water Chemistry Record:

|   | Current          | Range               |
|---|------------------|---------------------|
| TEMPERATURE:                              | <u>20°C</u>      | <u>17-20°C</u>      |
| SALINITY/CONDUCTIVITY:                    | <u>30 ppt **</u> | <u>28-33 ppt</u>    |
| TOTAL HARDNESS (as CaCO <sub>3</sub> ):   | <u>--</u>        | <u>--</u>           |
| TOTAL ALKALINITY (as CaCO <sub>3</sub> ): | <u>150 mg/l</u>  | <u>140-160 mg/l</u> |
| pH:                                       | <u>7.60</u>      | <u>7.60-8.20</u>    |

### Comments:

\*\* Acclimated to 28 ppt 6/13/2022

  
\_\_\_\_\_  
Facility Supervisor

## **APPENDIX A1.2**

Reference Toxicant  
*Atherinops affinis* (Topsmelt)  
7-day Chronic Test

**Topsmelt 7-d Survival and Growth Test**

All Matching Labs

Test Type: Growth-Survival (7d)

Organism: *Atherinops affinis* (Topsmelt)

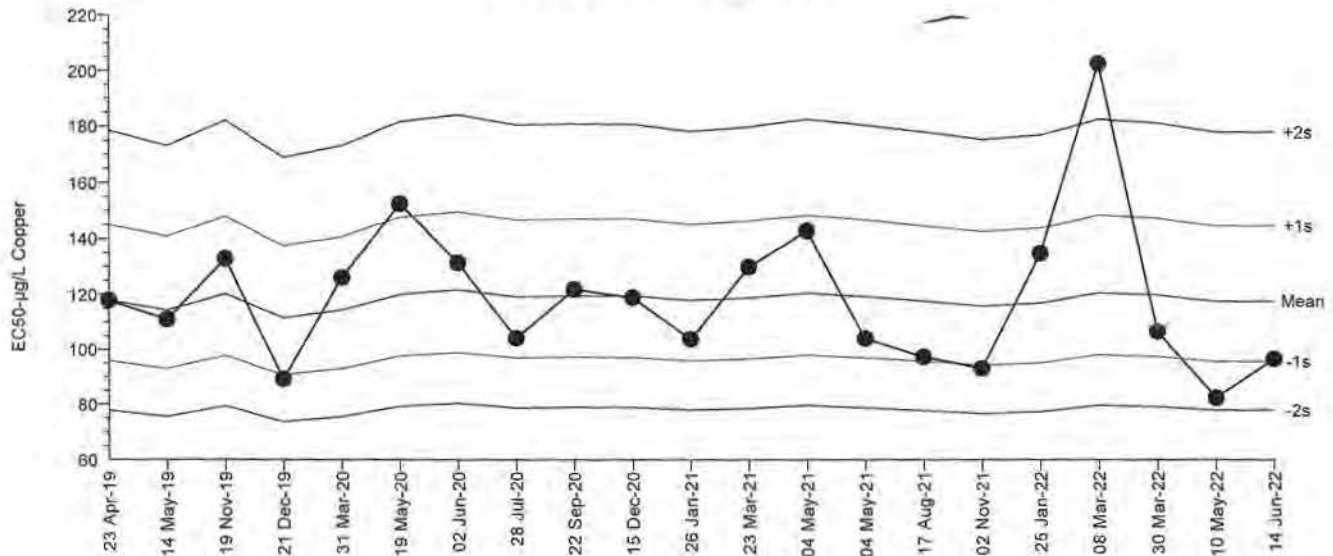
Material: Copper

Protocol: EPA/600/R-95/136 (1995)

Endpoint: 7d Proportion Survived

Source: Reference Toxicant-REF

Topsmelt 7-d Survival and Growth Test



Mean: 117.7      Count: 20      -1s Warning Limit: 95.63      -2s Action Limit: 77.72  
 Sigma: n/a      CV: 21.00%      +1s Warning Limit: 144.8      +2s Action Limit: 178.1

**Quality Control Data**

| Point | Year | Month | Day | Time  | QC Data | Delta  | Sigma    | Warning | Action | Test ID      | Analysis ID  | Laboratory  |
|-------|------|-------|-----|-------|---------|--------|----------|---------|--------|--------------|--------------|-------------|
| 1     | 2019 | Apr   | 23  | 14:15 | 117.9   | 0.2227 | 0.009119 |         |        | 19-2734-4710 | 14-7071-7534 | EcoAnalysts |
| 2     |      | May   | 14  | 14:50 | 111     | -6.645 | -0.2804  |         |        | 20-8835-0675 | 18-7974-5235 | EcoAnalysts |
| 3     |      | Nov   | 19  | 14:55 | 133     | 15.27  | 0.5886   |         |        | 12-1607-8407 | 07-6431-0514 | EcoAnalysts |
| 4     |      | Dec   | 21  | 17:38 | 89.3    | -28.38 | -1.331   | (-)     |        | 06-4648-5486 | 05-5048-1740 | EcoAnalysts |
| 5     | 2020 | Mar   | 31  | 15:42 | 126.2   | 8.486  | 0.3359   |         |        | 04-5562-6739 | 10-7693-9560 | EcoAnalysts |
| 6     |      | May   | 19  | 15:43 | 152.4   | 34.74  | 1.248    | (+)     |        | 06-7292-1758 | 14-9561-8656 | EcoAnalysts |
| 7     |      | Jun   | 2   | 16:40 | 131.4   | 13.75  | 0.5329   |         |        | 08-5783-4440 | 14-4652-5572 | EcoAnalysts |
| 8     |      | Jul   | 28  | 16:00 | 104.1   | -13.58 | -0.5913  |         |        | 14-0144-0053 | 09-7965-4210 | EcoAnalysts |
| 9     |      | Sep   | 22  | 16:11 | 121.9   | 4.222  | 0.17     |         |        | 00-4076-0952 | 20-7772-2356 | EcoAnalysts |
| 10    |      | Dec   | 15  | 16:31 | 119     | 1.281  | 0.05223  |         |        | 02-1504-6456 | 06-3581-5524 | EcoAnalysts |
| 11    | 2021 | Jan   | 26  | 15:44 | 103.7   | -13.94 | -0.608   |         |        | 09-7279-9629 | 04-4959-8826 | EcoAnalysts |
| 12    |      | Mar   | 23  | 15:37 | 130     | 12.33  | 0.4807   |         |        | 20-9800-6970 | 10-7439-4071 | EcoAnalysts |
| 13    |      | May   | 4   | 12:50 | 142.9   | 25.21  | 0.9362   |         |        | 19-6922-3129 | 19-0566-9610 | EcoAnalysts |
| 14    |      |       | 4   | 13:00 | 103.9   | -13.75 | -0.5995  |         |        | 00-2283-9750 | 18-5475-8618 | EcoAnalysts |
| 15    |      | Aug   | 17  | 19:07 | 97.27   | -20.41 | -0.919   |         |        | 09-0043-0470 | 14-6071-0348 | EcoAnalysts |
| 16    |      | Nov   | 2   | 16:06 | 93.18   | -24.5  | -1.126   | (-)     |        | 14-6437-8110 | 20-9597-1265 | EcoAnalysts |
| 17    | 2022 | Jan   | 25  | 16:44 | 135     | 17.31  | 0.6621   |         |        | 06-0817-6358 | 16-0044-1200 | EcoAnalysts |
| 18    |      | Mar   | 8   | 14:43 | 202.7   | 85.02  | 2.623    | (+)     | (+)    | 07-6546-0140 | 03-2149-8422 | EcoAnalysts |
| 19    |      |       | 30  | 13:20 | 106.5   | -11.2  | -0.4826  |         |        | 17-8194-4154 | 17-1422-7340 | EcoAnalysts |
| 20    |      | May   | 10  | 16:20 | 82.35   | -35.33 | -1.722   | (-)     |        | 13-9380-0579 | 14-9529-7356 | EcoAnalysts |
| 21    |      | Jun   | 14  | 16:08 | 96.48   | -21.2  | -0.9582  |         |        | 04-7024-7990 | 02-4116-4567 | EcoAnalysts |

Topsmelt 7-d Survival and Growth Test

All Matching Labs

Test Type: Growth-Survival (7d)

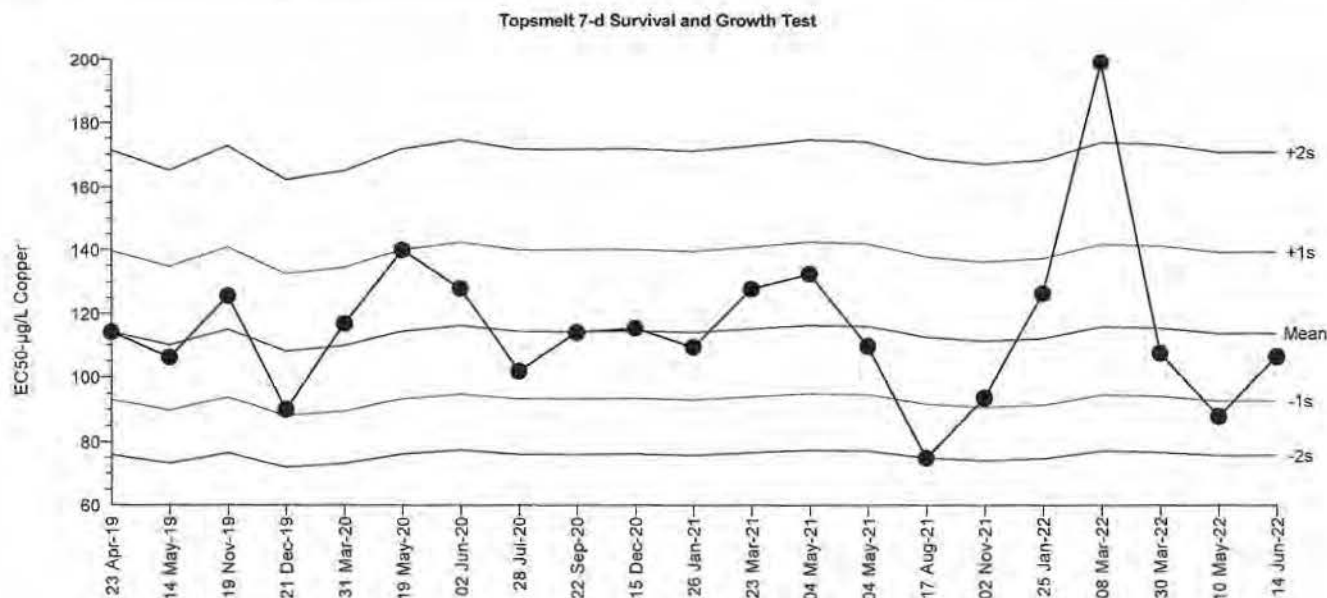
Organism: *Atherinops affinis* (Topsmelt)

Material: Copper

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Mean Dry Biomass-mg

Source: Reference Toxicant-REF



Mean: 113.6      Count: 20      -1s Warning Limit: 92.73      -2s Action Limit: 75.65  
 Sigma: n/a      CV: 20.60%      +1s Warning Limit: 139.3      +2s Action Limit: 170.7

Quality Control Data

| Point | Year | Month | Day | Time  | QC Data | Delta  | Sigma   | Warning | Action | Test ID      | Analysis ID  | Laboratory  |
|-------|------|-------|-----|-------|---------|--------|---------|---------|--------|--------------|--------------|-------------|
| 1     | 2019 | Apr   | 23  | 14:15 | 114     | 0.3613 | 0.0156  |         |        | 19-2734-4710 | 21-1263-7712 | EcoAnalysts |
| 2     |      | May   | 14  | 14:50 | 106.1   | -7.523 | -0.3366 |         |        | 20-8835-0675 | 00-2478-6615 | EcoAnalysts |
| 3     |      | Nov   | 19  | 14:55 | 125.6   | 11.98  | 0.4925  |         |        | 12-1607-8407 | 05-4939-6380 | EcoAnalysts |
| 4     |      | Dec   | 21  | 17:38 | 89.97   | -23.67 | -1.148  | (-)     |        | 06-4648-5486 | 08-1893-2493 | EcoAnalysts |
| 5     | 2020 | Mar   | 31  | 15:42 | 116.8   | 3.163  | 0.1349  |         |        | 04-5562-6739 | 02-3921-4208 | EcoAnalysts |
| 6     |      | May   | 19  | 15:43 | 139.9   | 26.27  | 1.022   | (+)     |        | 06-7292-1758 | 11-4404-5934 | EcoAnalysts |
| 7     |      | Jun   | 2   | 16:40 | 128.1   | 14.42  | 0.5872  |         |        | 08-5783-4440 | 19-9648-3850 | EcoAnalysts |
| 8     |      | Jul   | 28  | 16:00 | 102     | -11.68 | -0.533  |         |        | 14-0144-0053 | 19-9128-9339 | EcoAnalysts |
| 9     |      | Sep   | 22  | 16:11 | 114     | 0.3949 | 0.01705 |         |        | 00-4076-0952 | 19-6273-6753 | EcoAnalysts |
| 10    |      | Dec   | 15  | 16:31 | 115.3   | 1.645  | 0.07065 |         |        | 02-1504-6456 | 04-8535-5615 | EcoAnalysts |
| 11    | 2021 | Jan   | 26  | 15:44 | 109.3   | -4.356 | -0.1921 |         |        | 09-7279-9629 | 01-3400-5429 | EcoAnalysts |
| 12    |      | Mar   | 23  | 15:37 | 127.8   | 14.2   | 0.5785  |         |        | 20-9800-6970 | 16-5581-3702 | EcoAnalysts |
| 13    |      | May   | 4   | 12:50 | 132.6   | 18.94  | 0.7576  |         |        | 19-6922-3129 | 15-2736-9544 | EcoAnalysts |
| 14    |      |       | 4   | 13:00 | 109.6   | -4.034 | -0.1776 |         |        | 00-2283-9750 | 04-8302-3972 | EcoAnalysts |
| 15    |      | Aug   | 17  | 19:07 | 74.85   | -38.78 | -2.051  | (-)     | (-)    | 09-0043-0470 | 02-4979-2645 | EcoAnalysts |
| 16    |      | Nov   | 2   | 16:06 | 93.57   | -20.06 | -0.9547 |         |        | 14-6437-8110 | 21-0684-8201 | EcoAnalysts |
| 17    | 2022 | Jan   | 25  | 16:44 | 126.3   | 12.69  | 0.5201  |         |        | 06-0817-6358 | 07-1146-4932 | EcoAnalysts |
| 18    |      | Mar   | 8   | 14:43 | 199     | 85.4   | 2.754   | (+)     | (+)    | 07-6546-0140 | 14-1523-7409 | EcoAnalysts |
| 19    |      |       | 30  | 13:20 | 107.4   | -6.206 | -0.276  |         |        | 17-8194-4154 | 16-8645-1277 | EcoAnalysts |
| 20    |      | May   | 10  | 16:20 | 87.83   | -25.81 | -1.266  | (-)     |        | 13-9380-0579 | 13-1069-1982 | EcoAnalysts |
| 21    |      | Jun   | 14  | 16:08 | 106.3   | -7.295 | -0.326  |         |        | 04-7024-7990 | 12-0762-5164 | EcoAnalysts |

# CETIS Summary Report

Report Date: 06 Jul-22 09:07 (p 1 of 3)  
 Test Code/ID: P190603.175 / 04-7024-7990

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

|                              |                                   |   |
|------------------------------|-----------------------------------|---|
| Batch ID: 01-5957-2535       | Test Type: Growth-Survival (7d)   | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:08  | Protocol: EPA/600/R-95/136 (1995) | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 15:08 | Species: Atherinops affinis       | Brine: Crystal Sea Marine Mix               |
| Test Length: 6d 23h          | Taxon: Actinopterygii             | Source: Aquatic Biosystems, CO ✓ Age: 10d ✓ |
| Sample ID: 15-2597-6268      | Code: P190603.175                 | Project: Reference Toxicant                 |
| Sample Date: 03 Jun-19       | Material: Copper                  | Source: Reference Toxicant                  |
| Receipt Date: 03 Jun-19      | CAS (PC):                         | Station: P190603.175                        |
| Sample Age: 1107d 16h        | Client: Internal Lab              |   |

## Multiple Comparison Summary

| Analysis ID  | Endpoint               | Comparison Method                | ✓ NOEL | LOEL | TOEL  | TU | PMSD  | S |
|--------------|------------------------|----------------------------------|--------|------|-------|----|-------|---|
| 02-5827-0401 | 7d Proportion Survived | Dunnett Multiple Comparison Test | ✓ 40   | 80   | 56.57 |    | 14.6% | 1 |
| 05-4344-8272 | Mean Dry Biomass-mg    | Dunnett Multiple Comparison Test | ✓ 40   | 80   | 56.57 |    | 17.0% | 1 |
| 01-7169-6981 | Mean Dry Weight-mg     | Bonferroni Adj t Test            | 160    | >160 | n/a   |    | 29.9% | 1 |

## Point Estimate Summary

| Analysis ID  | Endpoint               | Point Estimate Method        | ✓ Level | µg/L  | 95% LCL | 95% UCL | TU | S |
|--------------|------------------------|------------------------------|---------|-------|---------|---------|----|---|
| 02-4116-4567 | 7d Proportion Survived | Spearman-Kärber              | ✓ EC50  | 96.48 | 83.13   | 112     |    | 1 |
| 12-0762-5164 | Mean Dry Biomass-mg    | Linear Interpolation (ICPIN) | ✓ IC5   | 46.76 | 28.68   | 53.27   |    | 1 |
|              |                        |                              | ✓ IC10  | 54.64 | 42.26   | 72.27   |    |   |
|              |                        |                              | ✓ IC15  | 63.81 | 47.99   | 89.78   |    |   |
|              |                        |                              | ✓ IC20  | 74.5  | 52.75   | 90.24   |    |   |
|              |                        |                              | ✓ IC25  | 82.25 | 58.48   | 92.63   |    |   |
|              |                        |                              | ✓ IC40  | 95.97 | 81.1    | 107.7   |    |   |
| 12-3149-3797 | Mean Dry Weight-mg     | Linear Interpolation (ICPIN) | IC5     | >160  | n/a     | n/a     |    | 1 |
|              |                        |                              | IC10    | >160  | n/a     | n/a     |    |   |
|              |                        |                              | IC15    | >160  | n/a     | n/a     |    |   |
|              |                        |                              | IC20    | >160  | n/a     | n/a     |    |   |
|              |                        |                              | IC25    | >160  | n/a     | n/a     |    |   |
|              |                        |                              | IC50    | >160  | n/a     | n/a     |    |   |

## Test Acceptability

| Analysis ID  | Endpoint               | Attribute    | Test Stat | TAC Limits |       |         | Decision        |
|--------------|------------------------|--------------|-----------|------------|-------|---------|-----------------|
|              |                        |              |           | Lower      | Upper | Overlap |                 |
| 02-4116-4567 | 7d Proportion Survived | Control Resp | 0.96      | 0.8        | >>    | Yes     | Passes Criteria |
| 02-5827-0401 | 7d Proportion Survived | Control Resp | 0.96      | 0.8        | >>    | Yes     | Passes Criteria |
| 05-4344-8272 | Mean Dry Biomass-mg    | Control Resp | 1.16      | 0.85       | >>    | Yes     | Passes Criteria |
| 12-0762-5164 | Mean Dry Biomass-mg    | Control Resp | 1.16      | 0.85       | >>    | Yes     | Passes Criteria |
| 02-5827-0401 | 7d Proportion Survived | PMSD         | 0.1462    | <<         | 0.25  | No      | Passes Criteria |
| 05-4344-8272 | Mean Dry Biomass-mg    | PMSD         | 0.1702    | <<         | 0.5   | No      | Passes Criteria |



# CETIS Summary Report

Report Date: 06 Jul-22 09:07 (p 2 of 3)  
 Test Code/ID: P190603.175 / 04-7024-7990

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

### 7d Proportion Survived Summary

| Conc-µg/L | Code | Count | Mean   | 95% LCL | 95% UCL | Min    | Max    | Std Err | Std Dev | CV%     | %Effect |
|-----------|------|-------|--------|---------|---------|--------|--------|---------|---------|---------|---------|
| 0         | D    | 5     | 0.9600 | 0.8489  | 1.0000  | 0.8000 | 1.0000 | 0.0400  | 0.0894  | 9.32%   | 0.00%   |
| 20        |      | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00%   | -4.17%  |
| 40        |      | 5     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00%   | -4.17%  |
| 80        |      | 5     | 0.6800 | 0.4579  | 0.9021  | 0.4000 | 0.8000 | 0.0800  | 0.1789  | 26.31%  | 29.17%  |
| 160       |      | 5     | 0.0800 | 0.0000  | 0.2160  | 0.0000 | 0.2000 | 0.0490  | 0.1095  | 136.93% | 91.67%  |
| 320       |      | 5     | 0.0000 | 0.0000  | 0.0000  | 0.0000 | 0.0000 | 0.0000  | 0.0000  |         | 100.00% |

### Mean Dry Biomass-mg Summary

| Conc-µg/L | Code | Count | Mean   | 95% LCL  | 95% UCL | Min   | Max   | Std Err | Std Dev | CV%     | %Effect |
|-----------|------|-------|--------|----------|---------|-------|-------|---------|---------|---------|---------|
| 0         | D    | 5     | 1.16   | 1.074    | 1.246   | 1.08  | 1.232 | 0.03093 | 0.06915 | 5.96%   | 0.00%   |
| 20        |      | 5     | 1.204  | 1.089    | 1.318   | 1.088 | 1.318 | 0.04137 | 0.09251 | 7.69%   | -3.76%  |
| 40        |      | 5     | 1.229  | 1.03     | 1.428   | 1.052 | 1.452 | 0.07176 | 0.1605  | 13.06%  | -5.93%  |
| 80        |      | 5     | 0.9304 | 0.7341   | 1.127   | 0.672 | 1.062 | 0.07071 | 0.1581  | 16.99%  | 19.79%  |
| 160       |      | 5     | 0.1212 | -0.08518 | 0.3276  | 0     | 0.316 | 0.07433 | 0.1662  | 137.14% | 89.55%  |
| 320       |      | 5     | 0      | 0        | 0       | 0     | 0     | 0       | 0       |         | 100.00% |

### Mean Dry Weight-mg Summary

| Conc-µg/L | Code | Count | Mean  | 95% LCL | 95% UCL | Min   | Max   | Std Err | Std Dev | CV%    | %Effect |
|-----------|------|-------|-------|---------|---------|-------|-------|---------|---------|--------|---------|
| 0         | D    | 5     | 1.215 | 1.073   | 1.358   | 1.08  | 1.387 | 0.05147 | 0.1151  | 9.47%  | 0.00%   |
| 20        |      | 5     | 1.204 | 1.089   | 1.318   | 1.088 | 1.318 | 0.04137 | 0.09251 | 7.69%  | 0.98%   |
| 40        |      | 5     | 1.229 | 1.03    | 1.428   | 1.052 | 1.452 | 0.07176 | 0.1605  | 13.06% | -1.09%  |
| 80        |      | 5     | 1.419 | 1.062   | 1.777   | 1.132 | 1.77  | 0.1288  | 0.2879  | 20.28% | -16.78% |
| 160       |      | 2     | 1.515 | 0.6891  | 2.341   | 1.45  | 1.58  | 0.065   | 0.09192 | 6.07%  | -24.64% |

### 7d Proportion Survived Detail

| Conc-µg/L | Code | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  |
|-----------|------|--------|--------|--------|--------|--------|
| 0         | D    | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 |
| 20        |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 40        |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 80        |      | 0.4000 | 0.6000 | 0.8000 | 0.8000 | 0.8000 |
| 160       |      | 0.0000 | 0.2000 | 0.0000 | 0.0000 | 0.2000 |
| 320       |      | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

### Mean Dry Biomass-mg Detail

| Conc-µg/L | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|-----------|------|-------|-------|-------|-------|-------|
| 0         | D    | 1.232 | 1.08  | 1.148 | 1.11  | 1.23  |
| 20        |      | 1.212 | 1.318 | 1.262 | 1.088 | 1.138 |
| 40        |      | 1.052 | 1.246 | 1.452 | 1.098 | 1.296 |
| 80        |      | 0.672 | 1.062 | 0.962 | 0.906 | 1.05  |
| 160       |      | 0     | 0.316 | 0     | 0     | 0.29  |
| 320       |      | 0     | 0     | 0     | 0     | 0     |

### Mean Dry Weight-mg Detail

| Conc-µg/L | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|-----------|------|-------|-------|-------|-------|-------|
| 0         | D    | 1.232 | 1.08  | 1.148 | 1.387 | 1.23  |
| 20        |      | 1.212 | 1.318 | 1.262 | 1.088 | 1.138 |
| 40        |      | 1.052 | 1.246 | 1.452 | 1.098 | 1.296 |
| 80        |      | 1.68  | 1.77  | 1.203 | 1.132 | 1.312 |
| 160       |      |       | 1.58  |       |       | 1.45  |
| 320       |      |       |       |       |       |       |

# CETIS Summary Report

Report Date: 06 Jul-22 09:07 (p 3 of 3)  
Test Code/ID: P190603.175 / 04-7024-7990

## Topsmelt 7-d Survival and Growth Test

EcoAnalysts

### 7d Proportion Survived Binomials

| Conc- $\mu$ g/L | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 |
|-----------------|------|-------|-------|-------|-------|-------|
| 0               | D    | 5/5   | 5/5   | 5/5   | 4/5   | 5/5   |
| 20              |      | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 40              |      | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 80              |      | 2/5   | 3/5   | 4/5   | 4/5   | 4/5   |
| 160             |      | 0/5   | 1/5   | 0/5   | 0/5   | 1/5   |
| 320             |      | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   |



CETIS Test Data Worksheet

Report Date: 06 Jul-22 09:06 (p 1 of 1)  
 Test Code/ID: P190603.175 / 04-7024-7990

**Topsmelt 7-d Survival and Growth Test** EcoAnalysts

Start Date: 14 Jun-22 16:08 / Species: *Atherinops affinis* / Sample Code: P190603.175 /  
 End Date: 21 Jun-22 15:08 / Protocol: EPA/600/R-95/136 (1995) / Sample Source: Reference Toxicant /  
 Sample Date: 03 Jun-19 / Material: Copper / Sample Station: P190603.175 /

| Conc-µg/L | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg Total | Weight-mg Tare | Pan Count | Notes |
|-----------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|-----------|-------|
| 0         | D    | 1   | 5   | 5         |             |             |             |             |             |             | 5           | 60.07           | 53.91          | 5         |       |
| 0         | D    | 2   | 16  | 5         |             |             |             |             |             |             | 5           | 57.59           | 52.19          | 5         |       |
| 0         | D    | 3   | 11  | 5         |             |             |             |             |             |             | 5           | 59.6            | 53.86          | 5         |       |
| 0         | D    | 4   | 15  | 5         |             |             |             |             |             |             | 4           | 68.34           | 62.79          | 4         |       |
| 0         | D    | 5   | 18  | 5         |             |             |             |             |             |             | 5           | 55.92           | 49.77          | 5         |       |
| 20        |      | 1   | 4   | 5         |             |             |             |             |             |             | 5           | 63.89           | 57.83          | 5         |       |
| 20        |      | 2   | 27  | 5         |             |             |             |             |             |             | 5           | 64.18           | 57.59          | 5         |       |
| 20        |      | 3   | 26  | 5         |             |             |             |             |             |             | 5           | 61.92           | 55.61          | 5         |       |
| 20        |      | 4   | 1   | 5         |             |             |             |             |             |             | 5           | 59.96           | 54.52          | 5         |       |
| 20        |      | 5   | 30  | 5         |             |             |             |             |             |             | 5           | 55.89           | 50.2           | 5         |       |
| 40        |      | 1   | 14  | 5         |             |             |             |             |             |             | 5           | 49.12           | 43.86          | 5         |       |
| 40        |      | 2   | 21  | 5         |             |             |             |             |             |             | 5           | 55.93           | 49.7           | 5         |       |
| 40        |      | 3   | 24  | 5         |             |             |             |             |             |             | 5           | 60.91           | 53.65          | 5         |       |
| 40        |      | 4   | 2   | 5         |             |             |             |             |             |             | 5           | 56.3            | 50.81          | 5         |       |
| 40        |      | 5   | 22  | 5         |             |             |             |             |             |             | 5           | 60.64           | 54.16          | 5         |       |
| 80        |      | 1   | 17  | 5         |             |             |             |             |             |             | 2           | 63.46           | 60.1           | 2         |       |
| 80        |      | 2   | 9   | 5         |             |             |             |             |             |             | 3           | 64.84           | 59.53          | 3         |       |
| 80        |      | 3   | 23  | 5         |             |             |             |             |             |             | 4           | 60.06           | 55.25          | 4         |       |
| 80        |      | 4   | 3   | 5         |             |             |             |             |             |             | 4           | 59              | 54.47          | 4         |       |
| 80        |      | 5   | 29  | 5         |             |             |             |             |             |             | 4           | 59.44           | 54.19          | 4         |       |
| 160       |      | 1   | 28  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 160       |      | 2   | 6   | 5         |             |             |             |             |             |             | 1           | 40.59           | 39.01          | 1         |       |
| 160       |      | 3   | 13  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 160       |      | 4   | 8   | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 160       |      | 5   | 7   | 5         |             |             |             |             |             |             | 1           | 42.97           | 41.52          | 1         |       |
| 320       |      | 1   | 19  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 320       |      | 2   | 20  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 320       |      | 3   | 10  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 320       |      | 4   | 12  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |
| 320       |      | 5   | 25  | 5         |             |             |             |             |             |             | 0           | 0               | 0              |           |       |

7 Day Chronic Survival and Growth Test

|             |                 |
|-------------|-----------------|
| Toxicant:   | Copper Chloride |
| Ref Tox ID: | P190603.175     |
| Lot #:      | S0014419        |
| Protocol:   | TOX002          |
| Replicates: | 5               |

|                          |                           |
|--------------------------|---------------------------|
| Date Test Started:       | 6/14/2022                 |
| Date Test Ended:         | 6/21/2022                 |
| Matrix:                  | Liquid                    |
| Species:                 | <i>Atherinops affinis</i> |
| No. of Org. per Chamber: | 5                         |

|                       | Conc. (µg/L)     | Meter #: | DO (mg/L) (>4.0) | Meter #: | Temp (°C) (20±1°C) | Meter #: | Salinity (ppt) (30±2ppt) | Meter #: | pH (6 - 9) |       |
|-----------------------|------------------|----------|------------------|----------|--------------------|----------|--------------------------|----------|------------|-------|
| Day 0 (Stock)         | Control          | 8        | 7.5              | 8        | 20.1               | 8        | 29                       | 8        | 8.0        |       |
| Date: 6/14/22         | 20               |          | 7.6              |          | 20.0               |          | 29                       |          | 8.1        |       |
| Time: 1146            | 40               |          | 7.6              |          | 20.1               |          | 29                       |          | 8.1        |       |
| Technician: RP        | 80               |          | 7.6              |          | 20.1               |          | 29                       |          | 8.1        |       |
| Feed: DM              | 160              |          | 7.6              |          | 20.1               |          | 29                       |          | 8.1        |       |
| (500 nauplii/chamber) | 320              |          | 7.6              |          | 20.1               |          | 29                       |          | 8.1        |       |
|                       | Day 1            |          | Day 2            |          | Day 3              |          | Day 4                    |          | Day 5      | Day 6 |
| Temperature (OLD)     | 20.8             |          | 20.8             |          | 20.5               |          | 20.5                     |          | 20.4       | 20.5  |
| Temperature (NEW)     | 19.7             |          | 19.1             |          | 19.7               |          | 19.4                     |          | 19.7       | 19.8  |
| Day 7                 | Control          | 9        | 6.1              | 9        | 21.3               | 9        | 30                       | 9        | 7.6        |       |
| Date: 6/21/22         | 20               |          | 6.3              |          | 21.5               |          | 30                       |          | 7.7        |       |
| Time: 1454            | 40               |          | 6.5              |          | 21.4               |          | 30                       |          | 7.8        |       |
| Replicate No.: 1      | 80               |          | 6.7              |          | 21.4               |          | 30                       |          | 7.8        |       |
| Technician: SZ        | 160 <sup>②</sup> |          | 7.0              |          | 21.4               |          | 30                       |          | 7.9        |       |
|                       | 320              |          | —                |          | —                  |          | —                        |          | —          |       |

Dilution Preparation (Serial dilute by 50%)

| CuCl <sub>2</sub> *2H <sub>2</sub> O Stock Solution: | Target Stock Solution Conc. | Volume of Stock Solution | Amt. of Toxicant |
|--|-----------------------------|--------------------------|------------------|
| 400,000 µg/L   | 320 µg/L                    | 2500 g                   | 2 g              |
| 400,000 µg/L   | 160 µg/L                    | 2500 g                   | 1 g              |

| Day | Date | Init. | Highest Conc. | Day | Date | Init. | Highest Conc. |
|-----|------|-------|---------------|-----|------|-------|---------------|
| 0   | 6/14 | RP    | 320 µg/L      | 4   | 6/18 | RP    | 160 µg/L      |
| 1   | 6/15 | RP    | 320 µg/L      | 5   | 6/19 | SZ    | 160 µg/L      |
| 2   | 6/16 | SZ/MS | 320 µg/L      | 6   | 6/20 | RP    | 160 µg/L      |
| 3   | 6/17 | MS    | 160 µg/L      |     |      |       |               |

|                       |                 |
|-----------------------|-----------------|
| Start Time:           | 1608 DM, SZ, RP |
| End Time:             | 1508 DM, SZ     |
| Test Location:        | Bath 4          |
| Dilution Water Batch: | FSND61222.01    |

|                    |                                    |
|--------------------|------------------------------------|
| Supplier:          | Aquatic Biosystems                 |
| Organism Batch:    | ABS061422.01 Age 3-15 d post hatch |
| Chamber Size/Type: | 20 oz. cup                         |
| Exposure Volume:   | 250 mL                             |

① IE - RP 6/15

② NG taken from rep 2 - SZ 6/21/22

③ age @ init start: 10d

-MS 7/5  
1 of 1

**7 Day Chronic Survival and Growth Test**

|             |                 |
|-------------|-----------------|
| Toxicant:   | Copper Chloride |
| Ref Tox ID: | PA0603.175      |

|                    |           |
|--------------------|-----------|
| Date Test Started: | 6/14/2022 |
| Date Test Ended:   | 6/21/2022 |

|          |                           |
|----------|---------------------------|
| Species: | <i>Atherinops affinis</i> |
|----------|---------------------------|

| Concentration (µg/L)                     | Rep | Day 1      |        | Day 2        |        | Day 3         |        | Day 4      |        | Day 5      |        | Day 6      |        | Day 7      |        |
|--|-----|------------|--------|--------------|--------|---------------|--------|------------|--------|------------|--------|------------|--------|------------|--------|
|  |     | Date: 6/15 |        | Date: 6/16   |        | Date: 6/17/22 |        | Date: 6/18 |        | Date: 6/19 |        | Date: 6/20 |        | Date: 6/21 |        |
|  |     | Time: 1038 |        | Time: 1510   |        | Time: 115     |        | Time: 1119 |        | Time: 1340 |        | Time: 1129 |        | Time: 1505 |        |
|  |     | Tech.: RP  |        | Tech.: SZ/MS |        | Tech.: NL     |        | Tech.: RP  |        | Tech.: SZ  |        | Tech.: RP  |        | Tech.: SZ  |        |
|  |     | # Alive    | # Dead | # Alive      | # Dead | # Alive       | # Dead | # Alive    | # Dead | # Alive    | # Dead | # Alive    | # Dead | # Alive    | # Dead |
| Control                                  | 1   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 2   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 3   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 4   | 5          | 0      | 5            | 0      | 4             | 1      | 4          | 0      | 4          | 0      | 4          | 0      | 4          | 0      |
|  | 5   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
| 20                                       | 1   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 2   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 3   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 4   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 5   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
| 40                                       | 1   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 2   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 3   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 4   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
|  | 5   | 5          | 0      | 5            | 0      | 5             | 0      | 5          | 0      | 5          | 0      | 5          | 0      | 5          | 0      |
| 80                                       | 1   | 5          | 0      | 4            | 1      | 2             | 2      | 2          | 0      | 2          | 0      | 2          | 0      | 2          | 0      |
|  | 2   | 5          | 0      | 3            | 2      | 3             | 0      | 3          | 0      | 3          | 0      | 3          | 0      | 3          | 0      |
|  | 3   | 5          | 0      | 4            | 1      | 4             | 0      | 4          | 0      | 4          | 0      | 4          | 0      | 4          | 0      |
|  | 4   | 5          | 0      | 5            | 0      | 4             | 1      | 4          | 0      | 4          | 0      | 4          | 0      | 4          | 0      |
|  | 5   | 5          | 0      | 4            | 1      | 4             | 0      | 4          | 0      | 4          | 0      | 4          | 0      | 4          | 0      |
| 160                                      | 1   | 4          | 1      | 1            | 3      | 1             | 0      | 0          | 1      | -          | -      | -          | -      | -          | -      |
|  | 2   | 3          | 2      | 1            | 2      | 1             | 0      | 1          | 0      | 1          | 0      | 1          | 0      | 1          | 0      |
|  | 3   | 4          | 1      | 1            | 3      | 0             | 1      | -          | -      | -          | -      | -          | -      | -          | -      |
|  | 4   | 5          | 0      | 1            | 4      | 0             | 1      | -          | -      | -          | -      | -          | -      | -          | -      |
|  | 5   | 5          | 0      | 3            | 2      | 3             | 0      | 1          | 2      | 1          | 0      | 1          | 0      | 1          | 0      |
| 320                                      | 1   | 0          | 5      | -            | -      | -             | -      | -          | -      | -          | -      | -          | -      | -          | -      |
|  | 2   | 1          | 4      | 0            | 1      | -             | -      | -          | -      | -          | -      | -          | -      | -          | -      |
|  | 3   | 1          | 4      | 0            | 1      | -             | -      | -          | -      | -          | -      | -          | -      | -          | -      |
|  | 4   | 2          | 3      | 0            | 2      | -             | -      | -          | -      | -          | -      | -          | -      | -          | -      |
|  | 5   | 1          | 4      | 0            | 1      | -             | -      | -          | -      | -          | -      | -          | -      | -          | -      |
| Feeding: 250 am/500 pm (artemia/chamber) | AM  | RP         |        | MS (1)       |        | MS (1)        |        | NL (1)     |        | NL (1)     |        | RP (1)     |        | None       |        |
|  | PM  | RP         |        | NL (1)       |        | NL (1)        |        | NL (1)     |        | NL (1)     |        | MK (1)     |        | None       |        |

0 chambers w/ 2 fish are fed 1/2 ration of food. MS 6/16 in Velle, MS 6/17, NL 6/17, NL 6/18, NL 6/19, RP 6/20, MK 6/20

**7 Day Chronic Survival and Growth Test**

Toxicant: Copper Chloride  
 Ref Tox ID: P190603.175

Date Test Started: 6/14/2022  
 Date Test Ended: 6/21/2022  
 Species: *D. rerio*

| Concentration (µg/L) | Replicate | Boat Number | Weight Empty Boat (mg) | Weight Boat & Animals (mg) | Pan Count |
|----------------------|-----------|-------------|------------------------|----------------------------|-----------|
| Control              | 1         | 1           | 53.91                  | 60.07                      | 5         |
|                      | 2         | 2           | 52.19                  | 57.59                      | 5         |
|                      | 3         | 3           | 53.86                  | 59.60                      | 5         |
|                      | 4         | X X 1 (2)   | 62.79 41.13 (2)        | 63.39 68.34 (2)            | 4         |
|                      | 5         | 5           | 49.77                  | 55.92                      | 5         |
| 20                   | 1         | 6           | 57.83                  | 63.81                      | 5         |
|                      | 2         | 7           | 57.59                  | 64.18                      | 5         |
|                      | 3         | 8           | 55.61                  | 61.92                      | 5         |
|                      | 4         | 9           | 54.52                  | 59.96                      | 5         |
|                      | 5         | 10          | 50.20                  | 55.89                      | 5         |
| 40                   | 1         | 11          | 43.86                  | 49.12                      | 5         |
|                      | 2         | 12          | 49.70                  | 55.93                      | 5         |
|                      | 3         | 13          | 53.65                  | 60.91                      | 5         |
|                      | 4         | 14          | 50.81                  | 56.30                      | 5         |
|                      | 5         | 15          | 54.16                  | 60.64                      | 5         |
| 80                   | 1         | 16          | 60.10                  | 63.46                      | 2         |
|                      | 2         | 17          | 59.53                  | 64.84                      | 3         |
|                      | 3         | 18          | 55.25                  | 60.06                      | 4         |
|                      | 4         | 19          | 54.47                  | 59.00                      | 4         |
|                      | 5         | 20          | 54.19                  | 59.44                      | 4         |
| 160                  | 1         |             |                        |                            |           |
|                      | 2         | 21          | 39.01                  | 40.59                      | 1         |
|                      | 3         |             |                        |                            |           |
|                      | 4         |             |                        |                            |           |
|                      | 5         | 22          | 41.52                  | 42.97                      | 1         |
| 320                  | 1         |             |                        |                            |           |
|                      | 2         |             |                        |                            |           |
|                      | 3         |             |                        |                            |           |
|                      | 4         |             |                        |                            |           |
|                      | 5         |             |                        |                            |           |

|   |              |    |              |    |
|---|--------------|----|--------------|----|
| Date/Time in oven: [Init.]                                      | 6/21/22 0945 | RP | 6/21/22 1645 | MR |
| Oven Temp: [Init.]  | 107°C        | RP | 102°C        | MR |
| Date/Time removed from oven (and placed in desiccator): [Init.] | 6/21/22 1233 | RP | 6/22 1150    | UL |
| Weight date and time (removed from desiccator): [Init.]         | 6/21/22      | IM | 6/22 1554    | SR |

(2) remeasured due to questionable difference 05/14/15 in weight. MS 7/16

1x 62.79  
 2x 59.58

1330

01E 7.5.22 MMR



## Daily Quality Assurance Checks

Project name: **VARIOUS**Test: **Topsoil Cu Cl<sub>2</sub> RT**Lab ID: **P190603.175**

| Day of Test |   | Initials | Date | Comments |
|-------------|---|----------|------|----------|
| 0           | Test datasheets checked for completeness and legibility                                       | DM       | 6/14 |          |
|             | Headers/ footers filled in, visual check of test chambers, cover test, ensure proper lighting | DM       | 6/14 |          |
|             | Test data within acceptable ranges  | DM       | 6/14 |          |
| 1           | Test datasheets checked for completeness and legibility                                       | RP       | 6/15 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 2           | Test datasheets checked for completeness and legibility                                       | NL       | 6/16 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 3           | Test datasheets checked for completeness and legibility                                       | DM       | 6/17 |          |
|             | Test data within acceptable ranges  | DM       | 6/17 |          |
|             |   |          |      |          |
| 4           | Test datasheets checked for completeness and legibility                                       | NL       | 6/18 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 5           | Test datasheets checked for completeness and legibility                                       | NL       | 6/19 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 6           | Test datasheets checked for completeness and legibility                                       | DM       | 6/20 |          |
|             | Test data within acceptable ranges  | DM       | 6/20 |          |
|             |   |          |      |          |
| 7           | Test datasheets checked for completeness and legibility                                       | MK       | 6/21 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |

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

Topsmest cncz RT

6/14-6/21/22

P190603.175

## **APPENDIX A2.1**

LOTT Final Effluent  
*Americamysis bahia* (Opossum Shrimp)  
7-day Chronic Test

# CETIS Summary Report

Report Date: 05 Jul-22 11:18 (p 1 of 3)  
 Test Code/ID: P220614.01A.b. / 15-6194-2791

## Mysidopsis 7-d Survival, Growth and Fecundity Test

EcoAnalysts

|                               |                                     |   |
|-------------------------------|-------------------------------------|---|
| Batch ID: 06-3547-3663        | Test Type: Growth-Survival-Fec (7d) | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:31   | Protocol: EPA/821/R-02-014 (2002)   | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 16:10  | Species: Americamysis bahia         | Brine: Crystal Sea Marine Mix               |
| Test Length: 7d               | Taxon: Malacostraca                 | Source: Aquatic Biosystems, CO Age: 7d      |
| Sample ID: 12-5027-6102       | Code: P220614.01A.b.                | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent             | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                           | Station: Final Effluent #1                  |
| Sample Age: 35h (0.5 °C)      | Client: LOTT                        |   |

### Multiple Comparison Summary

| Analysis ID  | Endpoint               | Comparison Method                | ✓ NOEL | LOEL | TOEL | TU | PMSD  | S |
|--------------|------------------------|----------------------------------|--------|------|------|----|-------|---|
| 06-1740-2601 | 7d Proportion Survived | Steel Many-One Rank Sum Test     | 100    | >100 | n/a  | 1  | 10.9% | 1 |
| 07-8970-6227 | Mean Dry Biomass-mg    | Steel Many-One Rank Sum Test     | 100    | >100 | n/a  | 1  | 16.9% | 1 |
| 10-6424-7622 | Mean Dry Weight-mg     | Dunnett Multiple Comparison Test | 100    | >100 | n/a  | 1  | 13.1% | 1 |

### Point Estimate Summary

| Analysis ID  | Endpoint               | Point Estimate Method        | ✓ Level | %     | 95% LCL | 95% UCL | TU   | S |
|--------------|------------------------|------------------------------|---------|-------|---------|---------|------|---|
| 05-5434-3201 | 7d Proportion Survived | Linear Interpolation (ICPIN) | ✓ EC5   | >100  | n/a     | n/a     | <1   | 1 |
|              |                        |                              | ✓ EC10  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ EC15  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ EC20  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ EC25  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ EC40  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ EC50  | >100  | n/a     | n/a     | <1   |   |
| 06-0425-6440 | Mean Dry Biomass-mg    | Linear Interpolation (ICPIN) | ✓ IC5   | 71.95 | 7.715   | n/a     | 1.39 | 1 |
|              |                        |                              | ✓ IC10  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC15  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC20  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC25  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC40  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC50  | >100  | n/a     | n/a     | <1   |   |
| 13-7832-9689 | Mean Dry Weight-mg     | Linear Interpolation (ICPIN) | IC5     | >100  | n/a     | n/a     | <1   | 1 |
|              |                        |                              | ✓ IC10  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC15  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC20  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC25  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC40  | >100  | n/a     | n/a     | <1   |   |
|              |                        |                              | ✓ IC50  | >100  | n/a     | n/a     | <1   |   |

### Test Acceptability

| Analysis ID  | Endpoint               | Attribute    | Test Stat | TAC Limits |       | Overlap | Decision        |
|--------------|------------------------|--------------|-----------|------------|-------|---------|-----------------|
|              |                        |              |           | Lower      | Upper |         |                 |
| 05-5434-3201 | 7d Proportion Survived | Control Resp | 0.975     | 0.8        | >>    | Yes     | Passes Criteria |
| 06-1740-2601 | 7d Proportion Survived | Control Resp | 0.975     | 0.8        | >>    | Yes     | Passes Criteria |
| 06-0425-6440 | Mean Dry Biomass-mg    | Control Resp | 0.3205    | 0.2        | >>    | Yes     | Passes Criteria |
| 07-8970-6227 | Mean Dry Biomass-mg    | Control Resp | 0.3205    | 0.2        | >>    | Yes     | Passes Criteria |



# CETIS Summary Report

Report Date: 05 Jul-22 11:18 (p 2 of 3)  
 Test Code/ID: P220614.01A.b / 15-6194-2791

## Mysidopsis 7-d Survival, Growth and Fecundity Test

EcoAnalysts

### 7d Proportion Survived Summary

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Min    | Max    | Std Err | Std Dev | CV%    | %Effect |
|--------|------|-------|--------|---------|---------|--------|--------|---------|---------|--------|---------|
| 0      | D    | 8     | 0.9750 | 0.9159  | 1.0000  | 0.8000 | 1.0000 | 0.0250  | 0.0707  | 7.25%  | 0.00%   |
| 0      | SC   | 8     | 0.9500 | 0.8318  | 1.0000  | 0.6000 | 1.0000 | 0.0500  | 0.1414  | 14.89% | 2.56%   |
| 2      |      | 8     | 1.0000 | 1.0000  | 1.0000  | 1.0000 | 1.0000 | 0.0000  | 0.0000  | 0.00%  | -2.56%  |
| 2.8    |      | 8     | 0.9750 | 0.9159  | 1.0000  | 0.8000 | 1.0000 | 0.0250  | 0.0707  | 7.25%  | 0.00%   |
| 10     |      | 8     | 0.9750 | 0.9159  | 1.0000  | 0.8000 | 1.0000 | 0.0250  | 0.0707  | 7.25%  | 0.00%   |
| 30     |      | 8     | 0.9500 | 0.8318  | 1.0000  | 0.6000 | 1.0000 | 0.0500  | 0.1414  | 14.89% | 2.56%   |
| 100    |      | 8     | 0.9500 | 0.8726  | 1.0000  | 0.8000 | 1.0000 | 0.0327  | 0.0926  | 9.75%  | 2.56%   |

### Mean Dry Biomass-mg Summary

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Min   | Max   | Std Err  | Std Dev | CV%    | %Effect |
|--------|------|-------|--------|---------|---------|-------|-------|----------|---------|--------|---------|
| 0      | D    | 8     | 0.3205 | 0.2949  | 0.3461  | 0.264 | 0.356 | 0.01081  | 0.03059 | 9.54%  | 0.00%   |
| 0      | SC   | 8     | 0.3195 | 0.2751  | 0.3639  | 0.194 | 0.368 | 0.01877  | 0.05308 | 16.61% | 0.31%   |
| 2      |      | 8     | 0.3587 | 0.3371  | 0.3804  | 0.33  | 0.406 | 0.009149 | 0.02588 | 7.21%  | -11.93% |
| 2.8    |      | 8     | 0.3398 | 0.3136  | 0.3659  | 0.3   | 0.396 | 0.01107  | 0.0313  | 9.21%  | -6.01%  |
| 10     |      | 8     | 0.3333 | 0.3054  | 0.3612  | 0.276 | 0.382 | 0.0118   | 0.03339 | 10.02% | -4.00%  |
| 30     |      | 8     | 0.3623 | 0.2904  | 0.4341  | 0.168 | 0.444 | 0.0304   | 0.08597 | 23.73% | -13.03% |
| 100    |      | 8     | 0.3192 | 0.2802  | 0.3583  | 0.252 | 0.38  | 0.0165   | 0.04667 | 14.62% | 0.39%   |

### Mean Dry Weight-mg Summary

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Min    | Max   | Std Err  | Std Dev | CV%    | %Effect |
|--------|------|-------|--------|---------|---------|--------|-------|----------|---------|--------|---------|
| 0      | D    | 8     | 0.3287 | 0.3117  | 0.3458  | 0.296  | 0.356 | 0.007201 | 0.02037 | 6.20%  | 0.00%   |
| 0      | SC   | 8     | 0.3357 | 0.3219  | 0.3494  | 0.32   | 0.368 | 0.005824 | 0.01647 | 4.91%  | -2.10%  |
| 2      |      | 8     | 0.3587 | 0.3371  | 0.3804  | 0.33   | 0.406 | 0.009149 | 0.02588 | 7.21%  | -9.13%  |
| 2.8    |      | 8     | 0.3491 | 0.325   | 0.3732  | 0.31   | 0.396 | 0.01019  | 0.02883 | 8.26%  | -6.20%  |
| 10     |      | 8     | 0.3419 | 0.3218  | 0.3621  | 0.3125 | 0.382 | 0.008514 | 0.02408 | 7.04%  | -4.01%  |
| 30     |      | 8     | 0.3763 | 0.3325  | 0.42    | 0.28   | 0.444 | 0.01852  | 0.05238 | 13.92% | -14.45% |
| 100    |      | 8     | 0.3382 | 0.291   | 0.3855  | 0.272  | 0.445 | 0.01996  | 0.05646 | 16.69% | -2.89%  |

**CETIS Summary Report**

Report Date: 05 Jul-22 11:18 (p 3 of 3)  
 Test Code/ID: P220614.01A.b. / 15-6194-2791

**Mysidopsis 7-d Survival, Growth and Fecundity Test**

EcoAnalysts

**7d Proportion Survived Detail**

| Conc-% | Code | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  | Rep 6  | Rep 7  | Rep 8  |
|--------|------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0      | D    | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0      | SC   | 1.0000 | 1.0000 | 0.6000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2      |      | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 2.8    |      | 1.0000 | 1.0000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 10     |      | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 30     |      | 1.0000 | 1.0000 | 0.6000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 100    |      | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 | 0.8000 | 1.0000 | 1.0000 |

**Mean Dry Biomass-mg Detail**

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5  | Rep 6 | Rep 7 | Rep 8 |
|--------|------|-------|-------|-------|-------|--------|-------|-------|-------|
| 0      | D    | 0.322 | 0.304 | 0.356 | 0.264 | 0.296  | 0.34  | 0.342 | 0.34  |
| 0      | SC   | 0.352 | 0.328 | 0.194 | 0.338 | 0.368  | 0.33  | 0.326 | 0.32  |
| 2      |      | 0.364 | 0.368 | 0.406 | 0.33  | 0.336  | 0.338 | 0.348 | 0.38  |
| 2.8    |      | 0.396 | 0.368 | 0.3   | 0.31  | 0.346  | 0.332 | 0.322 | 0.344 |
| 10     |      | 0.328 | 0.382 | 0.334 | 0.276 | 0.3125 | 0.332 | 0.328 | 0.374 |
| 30     |      | 0.324 | 0.378 | 0.168 | 0.416 | 0.392  | 0.372 | 0.404 | 0.444 |
| 100    |      | 0.288 | 0.302 | 0.356 | 0.356 | 0.272  | 0.252 | 0.348 | 0.38  |

**Mean Dry Weight-mg Detail**

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3  | Rep 4 | Rep 5  | Rep 6 | Rep 7 | Rep 8 |
|--------|------|-------|-------|--------|-------|--------|-------|-------|-------|
| 0      | D    | 0.322 | 0.304 | 0.356  | 0.33  | 0.296  | 0.34  | 0.342 | 0.34  |
| 0      | SC   | 0.352 | 0.328 | 0.3233 | 0.338 | 0.368  | 0.33  | 0.326 | 0.32  |
| 2      |      | 0.364 | 0.368 | 0.406  | 0.33  | 0.336  | 0.338 | 0.348 | 0.38  |
| 2.8    |      | 0.396 | 0.368 | 0.375  | 0.31  | 0.346  | 0.332 | 0.322 | 0.344 |
| 10     |      | 0.328 | 0.382 | 0.334  | 0.345 | 0.3125 | 0.332 | 0.328 | 0.374 |
| 30     |      | 0.324 | 0.378 | 0.28   | 0.416 | 0.392  | 0.372 | 0.404 | 0.444 |
| 100    |      | 0.288 | 0.302 | 0.356  | 0.445 | 0.272  | 0.315 | 0.348 | 0.38  |

**7d Proportion Survived Binomials**

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|--------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0      | D    | 5/5   | 5/5   | 5/5   | 4/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 0      | SC   | 5/5   | 5/5   | 3/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 2      |      | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 2.8    |      | 5/5   | 5/5   | 4/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 10     |      | 5/5   | 5/5   | 5/5   | 4/5   | 4/4   | 5/5   | 5/5   | 5/5   |
| 30     |      | 5/5   | 5/5   | 3/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 100    |      | 5/5   | 5/5   | 5/5   | 4/5   | 5/5   | 4/5   | 5/5   | 5/5   |

**CETIS Analytical Report**

Report Date: 05 Jul-22 11:17 (p 1 of 6)  
 Test Code/ID: P220614.01A.b. / 15-6194-2791

**Mysidopsis 7-d Survival, Growth and Fecundity Test** EcoAnalysts

|                               |                                     |   |
|-------------------------------|-------------------------------------|---|
| Analysis ID: 19-3988-3495     | Endpoint: 7d Proportion Survived    | CETIS Version: CETISv1.9.4                  |
| Analyzed: 05 Jul-22 11:16     | Analysis: Nonparametric-Two Sample  | Status Level: 1                             |
| Batch ID: 06-3547-3663        | Test Type: Growth-Survival-Fec (7d) | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:31   | Protocol: EPA/821/R-02-014 (2002)   | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 16:10  | Species: Americamysis bahia         | Brine: Crystal Sea Marine Mix               |
| Test Length: 7d               | Taxon: Malacostraca                 | Source: Aquatic Biosystems, CO Age: 7d      |
| Sample ID: 12-5027-6102       | Code: P220614.01A.b.                | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent             | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                           | Station: Final Effluent #1                  |
| Sample Age: 35h (0.5 °C)      | Client: LOTT                        |   |

| Data Transform      | Alt Hyp | Comparison Result                          | PMSD   |
|---------------------|---------|--|--------|
| Angular (Corrected) | C > T   | Salt Control passed 7d proportion survived | 10.79% |

**Wilcoxon Rank Sum Two-Sample Test**

| Control        | vs | Control II   | Test Stat | Critical | Ties | DF | P-Type | P-Value | Decision(α:5%)         |
|----------------|----|--------------|-----------|----------|------|----|--------|---------|------------------------|
| Dilution Water |    | Salt Control | 67.5      | n/a      | 1    | 14 | Exact  | 0.5000  | Non-Significant Effect |

**Test Acceptability Criteria**

| Attribute    | Test Stat | TAC Limits |       | Overlap | Decision        |
|--------------|-----------|------------|-------|---------|-----------------|
|              |           | Lower      | Upper |         |                 |
| Control Resp | 0.95      | 0.8        | >>    | Yes     | Passes Criteria |
| Control Resp | 0.975     | 0.8        | >>    | Yes     | Passes Criteria |

**ANOVA Table**

| Source  | Sum Squares | Mean Square | DF | F Stat | P-Value | Decision(α:5%)         |
|---------|-------------|-------------|----|--------|---------|------------------------|
| Between | 0.0030545   | 0.0030545   | 1  | 0.1826 | 0.6756  | Non-Significant Effect |
| Error   | 0.234131    | 0.0167236   | 14 |        |         |                        |
| Total   | 0.237185    |             | 15 |        |         |                        |

**Distributional Tests**

| Attribute    | Test                                 | Test Stat | Critical | P-Value  | Decision(α:1%)          |
|--------------|--------------------------------------|-----------|----------|----------|-------------------------|
| Variances    | Levene Equality of Variance Test     | 0.9944    | 8.862    | 0.3356   | Equal Variances         |
| Variances    | Mod Levene Equality of Variance Test | 0.1826    | 8.862    | 0.6756   | Equal Variances         |
| Variances    | Variance Ratio F Test                | 3.719     | 8.885    | 0.1044   | Equal Variances         |
| Distribution | Anderson-Darling A2 Normality Test   | 4.191     | 3.878    | <1.0E-37 | Non-Normal Distribution |
| Distribution | D'Agostino Skewness Test             | 3.993     | 2.576    | 6.5E-05  | Non-Normal Distribution |
| Distribution | Kolmogorov-Smirnov D Test            | 0.4692    | 0.2471   | 1.4E-10  | Non-Normal Distribution |
| Distribution | Shapiro-Wilk W Normality Test        | 0.4951    | 0.8408   | 1.9E-06  | Non-Normal Distribution |

**7d Proportion Survived Summary**

| Conc.-% | Code | Count | Mean   | 95% LCL | 95% UCL | Median | Min    | Max    | Std Err | CV%    | %Effect |
|---------|------|-------|--------|---------|---------|--------|--------|--------|---------|--------|---------|
| 0       | D    | 8     | 0.9750 | 0.9159  | 1.0000  | 1.0000 | 0.8000 | 1.0000 | 0.0250  | 7.25%  | 0.00%   |
| 0       | SC   | 8     | 0.9500 | 0.8318  | 1.0000  | 1.0000 | 0.6000 | 1.0000 | 0.0500  | 14.89% | 2.56%   |

**Angular (Corrected) Transformed Summary**

| Conc.-% | Code | Count | Mean  | 95% LCL | 95% UCL | Median | Min    | Max   | Std Err | CV%    | %Effect |
|---------|------|-------|-------|---------|---------|--------|--------|-------|---------|--------|---------|
| 0       | D    | 8     | 1.316 | 1.245   | 1.386   | 1.345  | 1.107  | 1.345 | 0.02977 | 6.40%  | 0.00%   |
| 0       | SC   | 8     | 1.288 | 1.152   | 1.424   | 1.345  | 0.8861 | 1.345 | 0.0574  | 12.61% | 2.10%   |

**7d Proportion Survived Detail**

| Conc.-% | Code | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  | Rep 6  | Rep 7  | Rep 8  |
|---------|------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0       | D    | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |
| 0       | SC   | 1.0000 | 1.0000 | 0.6000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 |

**Angular (Corrected) Transformed Detail**

| Conc.-% | Code | Rep 1 | Rep 2 | Rep 3  | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|---------|------|-------|-------|--------|-------|-------|-------|-------|-------|
| 0       | D    | 1.345 | 1.345 | 1.345  | 1.107 | 1.345 | 1.345 | 1.345 | 1.345 |
| 0       | SC   | 1.345 | 1.345 | 0.8861 | 1.345 | 1.345 | 1.345 | 1.345 | 1.345 |

# CETIS Analytical Report

Report Date: 05 Jul-22 11:17 (p 2 of 6)  
 Test Code/ID: P220614.01A.b / 15-6194-2791

## Mysidopsis 7-d Survival, Growth and Fecundity Test

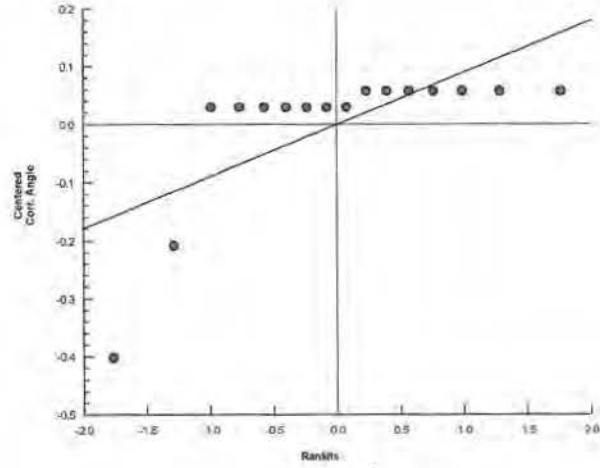
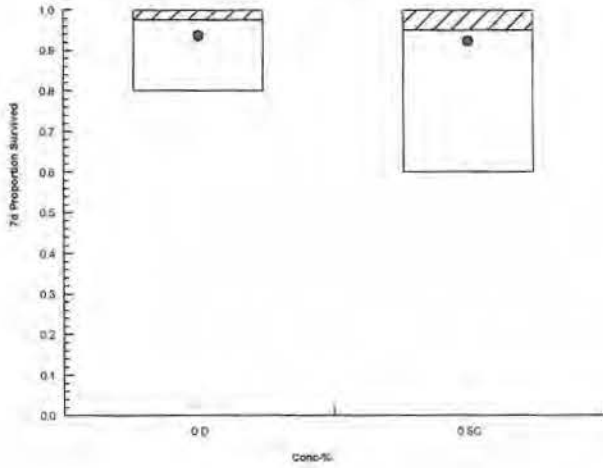
EcoAnalysts

Analysis ID: 19-3988-3495      Endpoint: 7d Proportion Survived      CETIS Version: CETISv1.9.4  
 Analyzed: 05 Jul-22 11:16      Analysis: Nonparametric-Two Sample      Status Level: 1

### 7d Proportion Survived Binomials

| Conc.-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|---------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0       | D    | 5/5   | 5/5   | 5/5   | 4/5   | 5/5   | 5/5   | 5/5   | 5/5   |
| 0       | SC   | 5/5   | 5/5   | 3/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   |

### Graphics



**CETIS Analytical Report**

Report Date: 05 Jul-22 11:18 (p 3 of 6)  
 Test Code/ID: P220614.01A.b. / 15-6194-2791

**Mysidopsis 7-d Survival, Growth and Fecundity Test**

EcoAnalysts

|                               |                                     |   |
|-------------------------------|-------------------------------------|---|
| Analysis ID: 06-5471-3177     | Endpoint: Mean Dry Biomass-mg       | CETIS Version: CETISv1.9.4                  |
| Analyzed: 05 Jul-22 11:16     | Analysis: Nonparametric-Two Sample  | Status Level: 1                             |
| Batch ID: 06-3547-3663        | Test Type: Growth-Survival-Fec (7d) | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:31   | Protocol: EPA/821/R-02-014 (2002)   | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 16:10  | Species: Americamysis bahia         | Brine: Crystal Sea Marine Mix               |
| Test Length: 7d               | Taxon: Malacostraca                 | Source: Aquatic Biosystems, CO Age: 7d      |
| Sample ID: 12-5027-6102       | Code: P220614.01A.b.                | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent             | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                           | Station: Final Effluent #1                  |
| Sample Age: 35h (0.5 °C)      | Client: LOTT                        |   |

| Data Transform | Alt Hyp | Comparison Result                       | PMSD   |
|----------------|---------|---|--------|
| Untransformed  | C > T   | Salt Control passed mean dry biomass-mg | 11.90% |

**Wilcoxon Rank Sum Two-Sample Test**

| Control        | vs | Control II   | Test Stat | Critical | Ties | DF | P-Type | P-Value | Decision(α:5%)         |
|----------------|----|--------------|-----------|----------|------|----|--------|---------|------------------------|
| Dilution Water |    | Salt Control | 70        | n/a      | 0    | 14 | Exact  | 0.6008  | Non-Significant Effect |

**Test Acceptability Criteria**

**TAC Limits**

| Attribute    | Test Stat | Lower | Upper | Overlap | Decision        |
|--------------|-----------|-------|-------|---------|-----------------|
| Control Resp | 0.3195    | 0.2   | >>    | Yes     | Passes Criteria |
| Control Resp | 0.3205    | 0.2   | >>    | Yes     | Passes Criteria |

**ANOVA Table**

| Source  | Sum Squares | Mean Square | DF | F Stat   | P-Value | Decision(α:5%)         |
|---------|-------------|-------------|----|----------|---------|------------------------|
| Between | 3.999E-06   | 3.999E-06   | 1  | 0.002131 | 0.9638  | Non-Significant Effect |
| Error   | 0.026276    | 0.0018769   | 14 |          |         |                        |
| Total   | 0.02628     |             | 15 |          |         |                        |

**Distributional Tests**

| Attribute    | Test                                 | Test Stat | Critical | P-Value | Decision(α:1%)          |
|--------------|--------------------------------------|-----------|----------|---------|-------------------------|
| Variances    | Levene Equality of Variance Test     | 0.2011    | 8.862    | 0.6607  | Equal Variances         |
| Variances    | Mod Levene Equality of Variance Test | 0.03983   | 8.862    | 0.8447  | Equal Variances         |
| Variances    | Variance Ratio F Test                | 3.012     | 8.885    | 0.1690  | Equal Variances         |
| Distribution | Anderson-Darling A2 Normality Test   | 1.301     | 3.878    | 0.0018  | Non-Normal Distribution |
| Distribution | D'Agostino Skewness Test             | 3.168     | 2.576    | 0.0015  | Non-Normal Distribution |
| Distribution | Kolmogorov-Smirnov D Test            | 0.2548    | 0.2471   | 0.0066  | Non-Normal Distribution |
| Distribution | Shapiro-Wilk W Normality Test        | 0.8034    | 0.8408   | 0.0030  | Non-Normal Distribution |

**Mean Dry Biomass-mg Summary**

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Median | Min   | Max   | Std Err | CV%    | %Effect |
|--------|------|-------|--------|---------|---------|--------|-------|-------|---------|--------|---------|
| 0      | D    | 8     | 0.3205 | 0.2949  | 0.3461  | 0.331  | 0.264 | 0.356 | 0.01081 | 9.54%  | 0.00%   |
| 0      | SC   | 8     | 0.3195 | 0.2751  | 0.3639  | 0.329  | 0.194 | 0.368 | 0.01877 | 16.61% | 0.31%   |

**Mean Dry Biomass-mg Detail**

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|--------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0      | D    | 0.322 | 0.304 | 0.356 | 0.264 | 0.296 | 0.34  | 0.342 | 0.34  |
| 0      | SC   | 0.352 | 0.328 | 0.194 | 0.338 | 0.368 | 0.33  | 0.326 | 0.32  |

Mysidopsis 7-d Survival, Growth and Fecundity Test

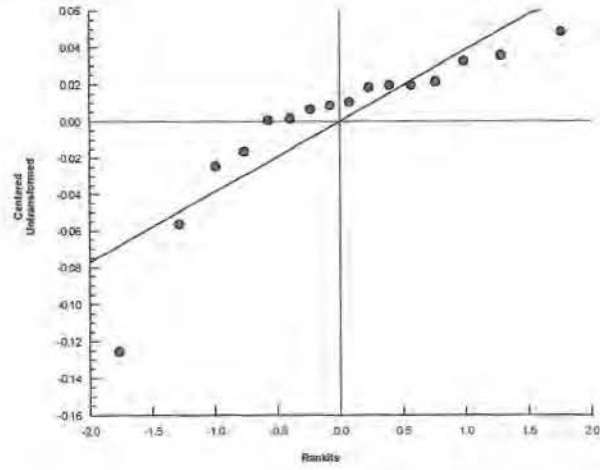
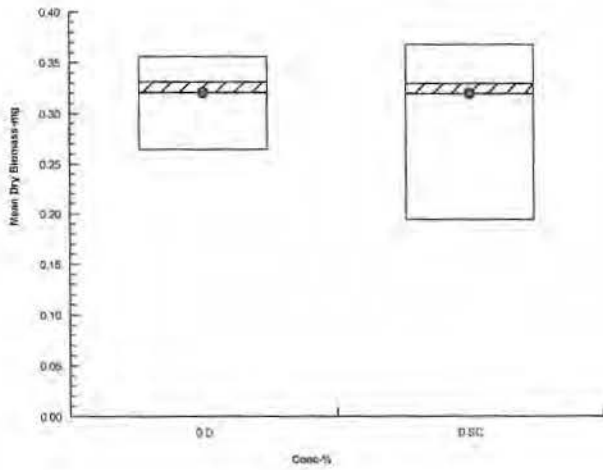
EcoAnalysts

Analysis ID: 06-5471-3177  
Analyzed: 05 Jul-22 11:16

Endpoint: Mean Dry Biomass-mg  
Analysis: Nonparametric-Two Sample

CETIS Version: CETISv1.9.4  
Status Level: 1

Graphics





# CETIS Analytical Report

Report Date: 05 Jul-22 11:18 (p 5 of 6)  
 Test Code/ID: P220614.01A.b. / 15-6194-2791

## Mysidopsis 7-d Survival, Growth and Fecundity Test

EcoAnalysts

|                               |                                     |   |
|-------------------------------|-------------------------------------|---|
| Analysis ID: 02-8109-1809     | Endpoint: Mean Dry Weight-mg        | CETIS Version: CETISv1.9.4                  |
| Analyzed: 05 Jul-22 11:16     | Analysis: Parametric-Two Sample     | Status Level: 1                             |
| Batch ID: 06-3547-3663        | Test Type: Growth-Survival-Fec (7d) | Analyst: Marisa Seibert                     |
| Start Date: 14 Jun-22 16:31   | Protocol: EPA/821/R-02-014 (2002)   | Diluent: Laboratory Seawater                |
| Ending Date: 21 Jun-22 16:10  | Species: Americamysis bahia         | Brine: Crystal Sea Marine Mix               |
| Test Length: 7d               | Taxon: Malacostraca                 | Source: Aquatic Biosystems, CO Age: 7d      |
| Sample ID: 12-5027-6102       | Code: P220614.01A.b.                | Project: NPDES                              |
| Sample Date: 13 Jun-22 06:00  | Material: POTW Effluent             | Source: LOTT Clean Water Alliance (WA00370) |
| Receipt Date: 14 Jun-22 12:10 | CAS (PC):                           | Station: Final Effluent #1                  |
| Sample Age: 35h (0.5 °C)      | Client: LOTT                        |   |

| Data Transform | Alt Hyp | Comparison Result                      | PMSD  |
|----------------|---------|--|-------|
| Untransformed  | C > T   | Salt Control passed mean dry weight-mg | 4.96% |

### Equal Variance t Two-Sample Test

| Control        | vs | Control II   | Test Stat | Critical | MSD   | DF | P-Type | P-Value | Decision(α:5%)         |
|----------------|----|--------------|-----------|----------|-------|----|--------|---------|------------------------|
| Dilution Water |    | Salt Control | -0.7469   | 1.761    | 0.016 | 14 | CDF    | 0.7662  | Non-Significant Effect |

### ANOVA Table

| Source  | Sum Squares | Mean Square | DF | F Stat | P-Value | Decision(α:5%)         |
|---------|-------------|-------------|----|--------|---------|------------------------|
| Between | 0.0001914   | 0.0001914   | 1  | 0.5578 | 0.4675  | Non-Significant Effect |
| Error   | 0.0048030   | 0.0003431   | 14 |        |         |                        |
| Total   | 0.0049944   |             | 15 |        |         |                        |

### Distributional Tests

| Attribute    | Test                                 | Test Stat | Critical | P-Value | Decision(α:1%)      |
|--------------|--------------------------------------|-----------|----------|---------|---------------------|
| Variances    | Levene Equality of Variance Test     | 0.4271    | 8.862    | 0.5240  | Equal Variances     |
| Variances    | Mod Levene Equality of Variance Test | 0.4442    | 8.862    | 0.5159  | Equal Variances     |
| Variances    | Variance Ratio F Test                | 1.529     | 8.885    | 0.5894  | Equal Variances     |
| Distribution | Anderson-Darling A2 Normality Test   | 0.1994    | 3.878    | 0.9294  | Normal Distribution |
| Distribution | D'Agostino Skewness Test             | 0.143     | 2.576    | 0.8863  | Normal Distribution |
| Distribution | Kolmogorov-Smirnov D Test            | 0.1243    | 0.2471   | 0.8240  | Normal Distribution |
| Distribution | Shapiro-Wilk W Normality Test        | 0.9813    | 0.8408   | 0.9730  | Normal Distribution |

### Mean Dry Weight-mg Summary

| Conc-% | Code | Count | Mean   | 95% LCL | 95% UCL | Median | Min   | Max   | Std Err  | CV%   | %Effect |
|--------|------|-------|--------|---------|---------|--------|-------|-------|----------|-------|---------|
| 0      | D    | 8     | 0.3287 | 0.3117  | 0.3458  | 0.335  | 0.296 | 0.356 | 0.007201 | 6.20% | 0.00%   |
| 0      | SC   | 8     | 0.3357 | 0.3219  | 0.3494  | 0.329  | 0.32  | 0.368 | 0.005824 | 4.91% | -2.10%  |

### Mean Dry Weight-mg Detail

| Conc-% | Code | Rep 1 | Rep 2 | Rep 3  | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|--------|------|-------|-------|--------|-------|-------|-------|-------|-------|
| 0      | D    | 0.322 | 0.304 | 0.356  | 0.33  | 0.296 | 0.34  | 0.342 | 0.34  |
| 0      | SC   | 0.352 | 0.328 | 0.3233 | 0.338 | 0.368 | 0.33  | 0.326 | 0.32  |

Mysidopsis 7-d Survival, Growth and Fecundity Test

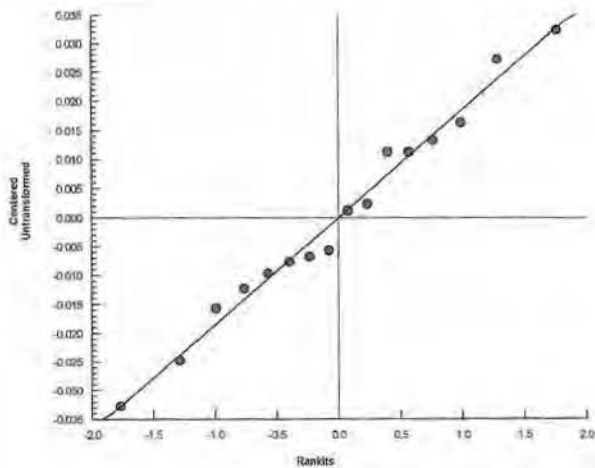
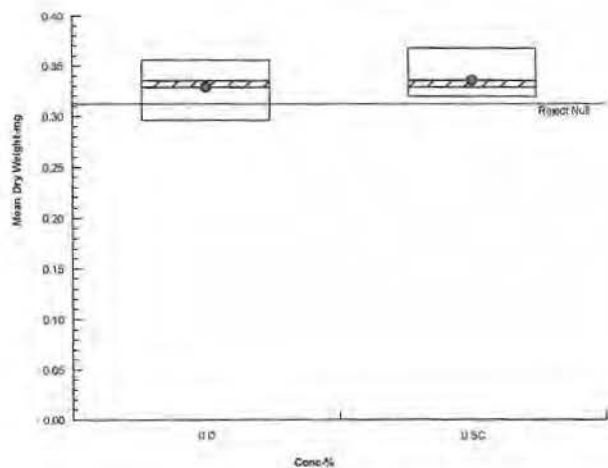
EcoAnalysts

Analysis ID: 02-8109-1809  
Analyzed: 05 Jul-22 11:16

Endpoint: Mean Dry Weight-mg  
Analysis: Parametric-Two Sample

CETIS Version: CETISv1.9.4  
Status Level: 1

Graphics





**CETIS Test Data Worksheet**

Report Date: 05 Jul-22 11:16 (p 1 of 2)  
 Test Code/ID: P220614.01A.b. / 15-6194-2791

**Mysidopsis 7-d Survival, Growth and Fecundity Test**

EcoAnalysts

Start Date: 14 Jun-22 16:31 Species: Americamysis bahia Sample Code: P220614.01A.b.  
 End Date: 21 Jun-22 16:10 Protocol: EPA/821/R-02-014 (2002) Sample Source: LOTT Clean Water Alliance  
 Sample Date: 13 Jun-22 06:00 Material: POTW Effluent Sample Station: Final Effluent #1

| Conc-% | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg Total | Weight-mg Tare | Pan Count | Females Total | Gravid Females | Notes |
|--------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|-----------|---------------|----------------|-------|
| 0      | D    | 1   | 21  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 24.34           | 22.73          | 5         |               |                |       |
| 0      | D    | 2   | 50  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 23.27           | 21.75          | 5         |               |                |       |
| 0      | D    | 3   | 53  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 25.73           | 23.95          | 5         |               |                |       |
| 0      | D    | 4   | 56  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 4           | 32.18           | 30.86          | 4         |               |                |       |
| 0      | D    | 5   | 16  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 30.47           | 28.99          | 5         |               |                |       |
| 0      | D    | 6   | 38  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 30.61           | 28.91          | 5         |               |                |       |
| 0      | D    | 7   | 3   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 24.07           | 22.36          | 5         |               |                |       |
| 0      | D    | 8   | 48  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 26.63           | 24.93          | 5         |               |                |       |
| 0      | SC   | 1   | 18  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 33.13           | 31.37          | 5         |               |                |       |
| 0      | SC   | 2   | 39  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 36.59           | 34.95          | 5         |               |                |       |
| 0      | SC   | 3   | 55  | 5         | 5           | 5           | 4           | 4           | 4           | 3           | 3           | 31.99           | 31.02          | 3         |               |                |       |
| 0      | SC   | 4   | 40  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 32.06           | 30.37          | 5         |               |                |       |
| 0      | SC   | 5   | 33  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 28.07           | 26.23          | 5         |               |                |       |
| 0      | SC   | 6   | 12  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 28.77           | 27.12          | 5         |               |                |       |
| 0      | SC   | 7   | 35  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 33.58           | 31.95          | 5         |               |                |       |
| 0      | SC   | 8   | 15  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 31.53           | 29.93          | 5         |               |                |       |
| 2      |      | 1   | 13  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 28.63           | 26.81          | 5         |               |                |       |
| 2      |      | 2   | 20  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 29.3            | 27.46          | 5         |               |                |       |
| 2      |      | 3   | 44  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 36.52           | 34.49          | 5         |               |                |       |
| 2      |      | 4   | 36  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 28.17           | 26.52          | 5         |               |                |       |
| 2      |      | 5   | 1   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 32.3            | 30.62          | 5         |               |                |       |
| 2      |      | 6   | 32  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 29.48           | 27.79          | 5         |               |                |       |
| 2      |      | 7   | 45  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 31.49           | 29.75          | 5         |               |                |       |
| 2      |      | 8   | 47  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 33.77           | 31.87          | 5         |               |                |       |
| 2.8    |      | 1   | 22  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 36.84           | 34.86          | 5         |               |                |       |
| 2.8    |      | 2   | 11  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 39.22           | 37.38          | 5         |               |                |       |
| 2.8    |      | 3   | 4   | 5         | 4           | 4           | 4           | 4           | 4           | 4           | 4           | 32.71           | 31.21          | 4         |               |                |       |
| 2.8    |      | 4   | 2   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 34.33           | 32.78          | 5         |               |                |       |
| 2.8    |      | 5   | 9   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 36.2            | 34.47          | 5         |               |                |       |
| 2.8    |      | 6   | 26  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 35.28           | 33.62          | 5         |               |                |       |
| 2.8    |      | 7   | 52  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 36.14           | 34.53          | 5         |               |                |       |
| 2.8    |      | 8   | 7   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 28.16           | 26.44          | 5         |               |                |       |

CETIS Test Data Worksheet

Report Date: 05 Jul-22 11:16 (p 2 of 2)

Test Code/ID: P220614.01A.b. / 15-6194-2791

| Conc-% | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg Total | Weight-mg Tare | Pan Count | Females Total | Gravid Females | Notes |
|--------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|-----------|---------------|----------------|-------|
| 10     |      | 1   | 54  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 25.91           | 24.27          | 5         |               |                |       |
| 10     |      | 2   | 14  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 30.44           | 28.53          | 5         |               |                |       |
| 10     |      | 3   | 43  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 29.85           | 28.18          | 5         |               |                |       |
| 10     |      | 4   | 51  | 5         | 5           | 5           | 5           | 5           | 5           | 4           | 4           | 36.06           | 34.68          | 4         |               |                |       |
| 10     |      | 5   | 28  | 4         | 4           | 4           | 4           | 4           | 4           | 4           | 4           | 35.02           | 33.77          | 4         |               |                |       |
| 10     |      | 6   | 23  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 32.1            | 30.44          | 5         |               |                |       |
| 10     |      | 7   | 29  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 28.74           | 27.1           | 5         |               |                |       |
| 10     |      | 8   | 27  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 32.52           | 30.65          | 5         |               |                |       |
| 30     |      | 1   | 17  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 30.07           | 28.45          | 5         |               |                |       |
| 30     |      | 2   | 41  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 37.12           | 35.23          | 5         |               |                |       |
| 30     |      | 3   | 49  | 5         | 5           | 5           | 5           | 4           | 4           | 3           | 3           | 38.58           | 37.74          | 3         |               |                |       |
| 30     |      | 4   | 5   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 38.4            | 36.32          | 5         |               |                |       |
| 30     |      | 5   | 37  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 34.31           | 32.35          | 5         |               |                |       |
| 30     |      | 6   | 31  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 31.47           | 29.61          | 5         |               |                |       |
| 30     |      | 7   | 6   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 31.79           | 29.77          | 5         |               |                |       |
| 30     |      | 8   | 10  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 27.46           | 25.24          | 5         |               |                |       |
| 100    |      | 1   | 34  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 27.57           | 26.13          | 5         |               |                |       |
| 100    |      | 2   | 30  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 31.49           | 29.98          | 5         |               |                |       |
| 100    |      | 3   | 24  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 27.82           | 26.04          | 5         |               |                |       |
| 100    |      | 4   | 42  | 5         | 5           | 5           | 5           | 5           | 4           | 4           | 4           | 26.34           | 24.56          | 4         |               |                |       |
| 100    |      | 5   | 25  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 26.6            | 25.24          | 5         |               |                |       |
| 100    |      | 6   | 19  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 4           | 35.28           | 34.02          | 4         |               |                |       |
| 100    |      | 7   | 8   | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 22.18           | 20.44          | 5         |               |                |       |
| 100    |      | 8   | 46  | 5         | 5           | 5           | 5           | 5           | 5           | 5           | 5           | 22.15           | 20.25          | 5         |               |                |       |

Version V.2

GENERAL

|                         |  |
|-------------------------|--|
| Client                  | LOTT Clean Water Alliance  |
| Project                 | NPDES  |
| Project Number          | PG1602   |
| Project Manager         | J. Levensgood  |
| Date Sample Received    | 6/14/2022  |
| Test type               | 7 Day Chronic Toxicity with Mysid  |
| Matrix                  | Liquid   |
| Test Acceptability      | ≥ 80% average survival of control mysid<br>Average dry weight is 0.20 mg or greater per surviving control mysids |
| Test Start Date         | 06/14/22   |
| Test Species            | Americanysis bahia   |
| Organism Batch          | ABS061422.02   |
| Organism Acquired       | 6/14/2022  |
| Organism Acclimation    | 0  |
| Organism Age            | 7 day old  |
| Test Protocol           | TOX 014  |
| Test Location           | Temp Control Room  |
| Light Intensity         | 50-100 foot candles  |
| Light Cycle             | 16L:8D   |
| Water Description       | 0.45 um filtered seawater  |
| Organisms per Replicate | 5  |
| Test Chamber Size       | 12 oz.   |
| Exposure Volume         | 250 mL   |
| Feeding Information     | 375 nauplii/chamber twice per day except Day 7   |
| Test Dissolved Oxygen   | > 4.0  |
| Test Temperature        | 26 ± 1   |
| Test Salinity           | 30 ± 2   |
| Test pH                 | 7.5 ± 1.5  |

Note: input lowest and highest decimal for temp

| Test Parameters |     |     |
|-----------------|-----|-----|
|                 | Min | Max |
| DO              | 4.0 |     |
| Temp            | 25  | 27  |
| Salinity        | 28  | 32  |
| pH              | 6   | 9   |

TEST START TIME/INIT: 11:31 AM, RP  
TEST END TIME/INIT: 11:10 PM, SZ

| CLIENT SAMPLE ID  | LAB ID     |
|-------------------|------------|
| Final Effluent #1 | P220614.01 |
| Final Effluent #2 | P220616.01 |
| Final Effluent #3 | P220616.01 |

| Concentrations |              |
|----------------|--------------|
| 1              | Control      |
| 2              | Salt Control |
| 3              | 2%           |
| 4              | 2.8%         |
| 5              | 10%          |
| 6              | 30%          |
| 7              | 100%         |
| 8              |              |
| 9              |              |

| Food Batch ID |
|---------------|
| 251523        |

| CSMM Batch # |
|--------------|
| C4444255     |

Copy and Past VALUES

| Treatment    | Rep | Chamber |
|--------------|-----|---------|
| Control      | 1   | 37      |
| Control      | 2   | 50      |
| Control      | 3   | 21      |
| Control      | 4   | 11      |
| Control      | 5   | 14      |
| Control      | 6   | 6       |
| Control      | 7   | 16      |
| Control      | 8   | 22      |
| Salt Control | 1   | 13      |
| Salt Control | 2   | 41      |
| Salt Control | 3   | 28      |
| Salt Control | 4   | 34      |
| Salt Control | 5   | 1       |
| Salt Control | 6   | 33      |
| Salt Control | 7   | 39      |
| Salt Control | 8   | 25      |
| 2.0%         | 1   | 51      |
| 2.0%         | 2   | 9       |
| 2.0%         | 3   | 31      |
| 2.0%         | 4   | 38      |
| 2.0%         | 5   | 45      |
| 2.0%         | 6   | 52      |
| 2.0%         | 7   | 36      |
| 2.0%         | 8   | 40      |
| 2.8%         | 1   | 4       |
| 2.8%         | 2   | 44      |
| 2.8%         | 3   | 26      |
| 2.8%         | 4   | 2       |
| 2.8%         | 5   | 20      |
| 2.8%         | 6   | 48      |
| 2.8%         | 7   | 3       |
| 2.8%         | 8   | 47      |
| 10%          | 1   | 24      |
| 10%          | 2   | 17      |
| 10%          | 3   | 42      |
| 10%          | 4   | 54      |
| 10%          | 5   | 23      |
| 10%          | 6   | 56      |
| 10%          | 7   | 5       |
| 10%          | 8   | 32      |
| 30%          | 1   | 55      |
| 30%          | 2   | 12      |
| 30%          | 3   | 7       |
| 30%          | 4   | 29      |
| 30%          | 5   | 27      |
| 30%          | 6   | 35      |
| 30%          | 7   | 46      |
| 30%          | 8   | 30      |
| 100%         | 1   | 43      |
| 100%         | 2   | 18      |
| 100%         | 3   | 8       |
| 100%         | 4   | 53      |
| 100%         | 5   | 19      |
| 100%         | 6   | 10      |
| 100%         | 7   | 15      |
| 100%         | 8   | 49      |

|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| v.2 CLIENT       | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levengood       |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

**7 Day Chronic Toxicity with Mysid**

| Day of Test | Concentration | Vol. Effluent Sample Added (mL) | Vol. Diluent Added (mL) | Total Volume (mL) | Diluent Type | FSW |
|-------------|---------------|---------------------------------|-------------------------|-------------------|--------------|-----|
| 0           | 0%            | 0                               | 2000.0                  | 2000              |              |     |
|             | Salt Control  | #VALUE!                         | #VALUE!                 | 2000              |              |     |
|             | 2.0%          | 40                              | 1960.0                  | 2000              |              |     |
|             | 2.8%          | 56                              | 1944.0                  | 2000              |              |     |
|             | 10%           | 200                             | 1800.0                  | 2000              |              |     |
|             | 30%           | 600                             | 1400.0                  | 2000              |              |     |
|             | 100%          | 2000                            | 0.0                     | 2000              |              |     |

| Day of Test | Concentration | Vol. Effluent Sample Added (mL) | Vol. Diluent Added (mL) | Total Volume (mL) |
|-------------|---------------|---------------------------------|-------------------------|-------------------|
| 1 - 6       | 0%            | 0                               | 2000.0                  | 2000              |
|             | Salt Control  | #VALUE!                         | #VALUE!                 | 2000              |
|             | 2.0%          | 40                              | 1960.0                  | 2000              |
|             | 2.8%          | 56                              | 1944.0                  | 2000              |
|             | 10%           | 200                             | 1800.0                  | 2000              |
|             | 30%           | 600                             | 1400.0                  | 2000              |
|             | 100%          | 2000                            | 0.0                     | 2000              |

**Test Dilution Prep**

| Date    | Balance ID | Sample ID (P#) | Water Batch ID | Initials |
|---------|------------|----------------|----------------|----------|
| 6/14    | 1          | P220614.01     | FSW061222.01   | RP       |
| 6/15    | 1          | P220614.01     | FSW061227.01   | RP       |
| 6/16/22 | 1          | P220614.01     | FSW061222.01   | SZ       |
| 6/17/22 | 1          | P220616.01     | FSW061622.01   | MS       |
| 6/18/22 | 1          | P220618.01     | FSW0611622.01  | RP       |
| 6/19/22 | 1          | P220618.01     | FSW061622.01   | SZ       |
| 6/20/22 | 1          | P220618.01     | FSW061922.01   | RP       |

① IE-SZ 061622

v.2

|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood      |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

7 Day Chronic Toxicity with Mysid

| Concentration (%) | REP | Day 1 |      | Day 2 |      | Day 3 |      | Day 4 |      | Day 5 |      | Day 6 |      | Day 7 |      |
|-------------------|-----|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|
|                   |     | Date  | Time | Date  | Time | Date  | Time | Date  | Time | Date  | Time | Date  | Time | Date  | Time |
|                   |     | Tech  |      | Tech  |      | Tech  |      | Tech  |      | Tech  |      | Tech  |      | Tech  |      |
| Control           | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 3   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 4     | 1NB  |
|                   | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
| Salt Control      | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 3   | 5     | 0    | 5     | 0    | 4     | 1NB  | 4     | 0    | 4     | 0    | 3     | 1NB  | 3     | 0    |
|                   | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
| 2.0%              | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 3   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
| 2.8%              | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 3   | 5     | 0    | 5     | 0    | 4     | 0    | 4     | 0    | 4     | 0    | 4     | 0    | 4     | 0    |
|                   | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
| 10%               | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 3   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 4     | 0    |
|                   | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 4     | 1    | 4     | 0    | 4     | 0    |
|                   | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |
|                   | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    |

① IE-NL 6/15, RP 6/18, RP 6/20      ③ IW-RP 6/20  
 ② One found dored out on side of coop -SZ 6/14



|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levengood       |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

Abbreviation Key:

NB = No Body  
 FB = Found Body  
 ST = Stranded

7 Day Chronic Toxicity with Mysid

| Concentration (%)                              | REP | Day 1 |      | Day 2 |      | Day 3 |      | Day 4 |      | Day 5 |      | Day 6 |      | Day 7 |       |
|--|-----|-------|------|-------|------|-------|------|-------|------|-------|------|-------|------|-------|-------|
|  |     | Date  | 6/15 | Date  | 6/16 | Date  | 6/17 | Date  | 6/18 | Date  | 6/19 | Date  | 6/20 | Date  | 6/21  |
|  |     | Time  | 1047 | Time  | 1330 | Time  | 125  | Time  | 1329 | Time  | 1145 | Time  | 121  | Time  | 1610  |
|  |     | Tech  | NL   | Tech  | MS   | Tech  | MS   | Tech  | RP   | Tech  | SZ   | Tech  | RP   | Tech  | DM/SZ |
|  |     | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead | Alive | Dead  |
| 30%  | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 3     | 0    | 5     | 0     |
|  | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 6     | 0    | 5     | 0    | 5     | 0    | 045   | 0     |
|  | 3   | 5     | 0    | 5     | 0    | 5     | 0    | 4     | 1    | 4     | 0    | 3     | INB  | 3     | 0     |
|  | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 6     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 6     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
| 100%   | 1   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 2   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 3   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 4   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 4     | INB  | 4     | 0    | 4     | 0     |
|  | 5   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 6   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 4     | 1     |
|  | 7   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
|  | 8   | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0    | 5     | 0     |
| Feed (Init.)                                   | AM  | RP    |      | MS    |      | MS    |      | NL    |      | NL    |      | RP    |      | NONE  |       |
| 375 nauplii/chamber twice per day except Day 7 | PM  | RP    |      | NL    |      | NL    |      | NL    |      | NL    |      | MK    |      | NONE  |       |

① IE-NL 6/15, DM - 6/21/22

|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood      |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

7 Day Chronic Toxicity with Mysid

| Concentration (%) | REP | Boat Number | Weight Empty Boat (mg) | Weight Boat & Animals (mg) | Pan Count |
|-------------------|-----|-------------|------------------------|----------------------------|-----------|
| Control           | 1   | 1           | 22.73                  | 24.34                      | 5         |
|                   | 2   | 2           | 21.75                  | 23.27                      | 5         |
|                   | 3   | 3           | 23.95                  | 25.73                      | 5         |
|                   | 4   | 4           | 30.86                  | 32.18                      | ① 5 4     |
|                   | 5   | 5           | 28.99                  | 30.47                      | 5         |
|                   | 6   | 6           | 28.91                  | 30.61                      | 5         |
|                   | 7   | 7           | 22.36                  | 24.07                      | 5         |
|                   | 8   | 8           | 24.93                  | 26.63                      | 5         |
| Salt Control      | 1   | 9           | 31.37                  | 33.13                      | 5         |
|                   | 2   | 10          | 34.95                  | 36.59                      | ① 5 5     |
|                   | 3   | 11          | 31.02                  | 31.99                      | 3         |
|                   | 4   | 12          | 30.37                  | 32.06                      | 5         |
|                   | 5   | 13          | 26.23                  | 28.07                      | 5         |
|                   | 6   | 14          | 27.12                  | 28.77                      | 5         |
|                   | 7   | 15          | 31.95                  | 33.58                      | 5         |
|                   | 8   | 16          | 29.93                  | 31.53                      | 5         |
| 2.0%              | 1   | 17          | 26.81                  | 28.63                      | 5         |
|                   | 2   | 18          | 27.46                  | 29.30                      | 5         |
|                   | 3   | 19          | 34.49                  | 36.52                      | 5         |
|                   | 4   | 20          | 26.52                  | 28.17                      | 5         |
|                   | 5   | 21          | 30.62                  | 32.30                      | 5         |
|                   | 6   | 22          | 27.79                  | 29.48                      | 5         |
|                   | 7   | 23          | 29.75                  | 31.49                      | 5         |
|                   | 8   | 24          | 31.87                  | 33.77                      | 5         |
| 2.8%              | 1   | 25          | 34.86                  | 36.84                      | 5         |
|                   | 2   | 26          | 37.38                  | 39.22                      | 5         |
|                   | 3   | 27          | 31.21                  | 32.71                      | 4         |
|                   | 4   | 28          | 32.78                  | 34.33                      | 5         |
|                   | 5   | 29          | 34.47                  | 36.20                      | 5         |
|                   | 6   | 30          | 33.62                  | 35.28                      | 5         |
|                   | 7   | 31          | 34.53                  | 36.14                      | 5         |
|                   | 8   | 32          | 26.44                  | 28.16                      | 5         |
| 10%               | 1   | 33          | 24.27                  | 25.91                      | 5         |
|                   | 2   | 34          | 28.53                  | 30.44                      | 5         |
|                   | 3   | 35          | 28.18                  | 29.85                      | 5         |
|                   | 4   | 36          | 34.68                  | 36.06                      | 4         |
|                   | 5   | 37          | 33.77                  | 35.02                      | 4         |
|                   | 6   | 38          | 30.44                  | 32.10                      | 5         |
|                   | 7   | 39          | 27.10                  | 28.74                      | 5         |
|                   | 8   | 40          | 30.65                  | 32.52                      | 5         |

① checked pan count & adjusted corrected. Ms 7/5

|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levengood       |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

7 Day Chronic Toxicity with Mysid

| Concentration (%) | REP | Boat Number | Weight Empty Boat (mg) | Weight Boat & Animals (mg) | Pan Count |
|-------------------|-----|-------------|------------------------|----------------------------|-----------|
| 30%               | 1   | 41          | 28.45                  | 30.07                      | 5         |
|                   | 2   | 42          | 35.23                  | 37.12                      | 5         |
|                   | 3   | 43          | 37.74                  | 38.98                      | 3         |
|                   | 4   | 44          | 36.32                  | 38.40                      | 5         |
|                   | 5   | 45          | 32.35                  | 34.31                      | 5         |
|                   | 6   | 46          | 29.61                  | 31.48                      | 5         |
|                   | 7   | 47          | 29.77                  | 31.79                      | 5         |
|                   | 8   | 48          | 25.24                  | 27.46                      | 5         |
| 100%              | 1   | 49          | 26.13                  | 27.57                      | 5         |
|                   | 2   | 50          | 29.98                  | 31.49                      | 5         |
|                   | 3   | 51          | 26.04                  | 27.82 (4) (4)              | 5         |
|                   | 4   | 52          | 24.56                  | 26.34 (3) (3)              | 4         |
|                   | 5   | 53          | 25.24                  | 26.60                      | 5         |
|                   | 6   | 54          | 34.02                  | 35.28                      | 4         |
|                   | 7   | 55          | 20.44                  | 22.18                      | 5         |
|                   | 8   | 56          | 20.25                  | 22.15                      | 3         |

|  | Oven Event 1    | Oven Event 2        |
|--|-----------------|---------------------|
| Oven ID:                                     | BEEZERUB        | BEEZERUB            |
| Date/Time/Initials In Oven:                  | 6/21/22 DMH RP  | 6/21/22 1723 DMH/SZ |
| Oven Temp °C:                                | 107C            | 102.104             |
| Date/Time/Initials Out Oven into Dessicator: | 6/21/22 1203 RP | 6/22 1150 JL        |
| Date/Time/Initials Weighed:                  | 6/21/22 IM      | 6/23 14:41 IM       |
| Balance ID:                                  | 3               | 3                   |

② ~~1x 34.0~~  
2x 62.12

① MR - 6/21/22 IM

② Boat discarded

③ DM - 6/21/22 - IE

④ IE - 6/23/22 IM



|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| V.2 CLIENT       | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood      |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

7 Day Chronic Toxicity with Mysid

|               | Concentration (%) | DO (mg/L) | TEMP (°C) | SALINITY (ppt) | pH    |
|---------------|-------------------|-----------|-----------|----------------|-------|
|               |                   | > 4.0     | 25 - 27   | 28 - 32        | 6 - 9 |
| <b>Day 0</b>  | Control           | 7.5       | 25.5      | 30             | 8.0   |
| Stock         | Salt Control      | 7.6       | 25.6      | 29             | 8.3   |
| Date 6/14/22  | 2.0%              | 7.7       | 25.9      | 29             | 8.0   |
| Time 1610     | 2.8%              | 7.6       | 25.9      | 29             | 8.0   |
| Tech RP       | 10%               | 7.6       | 26.3      | 30             | 8.0   |
| Meter # 8     | 30%               | 7.6       | 25.7      | 29             | 7.9   |
| Feed DM       | 100%              | 8.5       | 26.1      | 30             | 7.7   |
| <b>Day 1</b>  | Control           | 6.5       | 24.5      | 31             | 7.9   |
| Rep 1         | Salt Control      | 6.4       | 25.0      | 31             | 8.1   |
| Date 6/15/22  | 2.0%              | 6.7       | 24.5      | 31             | 7.9   |
| Time 0858     | 2.8%              | 6.5       | 25.1      | 31             | 7.9   |
| Tech NL       | 10%               | 6.4       | 25.1      | 31             | 7.9   |
| Meter # 8     | 30%               | 6.3       | 25.3      | 31             | 7.9   |
|               | 100%              | 6.1       | 25.1      | 31             | 7.9   |
| <b>Day 1</b>  | Control           | 7.3       | 25.3      | 30             | 8.0   |
| Renewal Stock | Salt Control      | 7.3       | 25.5      | 30             | 8.1   |
| Date 6/15/22  | 2.0%              | 7.4       | 25.9      | 30             | 8.0   |
| Time 0924     | 2.8%              | 7.4       | 25.7      | 30             | 8.0   |
| Tech NL       | 10%               | 7.5       | 25.5      | 30             | 8.0   |
| Meter # 8     | 30%               | 7.7       | 25.5      | 30             | 7.9   |
|               | 100%              | 8.2       | 25.5      | 31             | 7.8   |
| <b>Day 2</b>  | Control           | 6.0       | 25.1      | 31             | 7.7   |
| Rep 2         | Salt Control      | 5.7       | 25.4      | 31             | 7.8   |
| Date 6/16/22  | 2.0%              | 5.7       | 25.5      | 31             | 7.8   |
| Time 1140     | 2.8%              | 5.8       | 25.5      | 31             | 7.8   |
| Tech SZ/MS    | 10%               | 5.9       | 25.4      | 31             | 7.8   |
| Meter # 9     | 30%               | 5.8       | 25.5      | 31             | 7.8   |
|               | 100%              | 5.6       | 25.5      | 31             | 7.9   |
| <b>Day 2</b>  | Control           | 7.40      | 25.0      | 31             | 7.9   |
| Renewal Stock | Salt Control      | 7.3       | 25.1      | 31             | 8.1   |
| Date 6/16/22  | 2.0%              | 7.4       | 25.0      | 31             | 8.0   |
| Time 1140     | 2.8%              | 7.4       | 25.0      | 31             | 8.0   |
| Tech MS/SZ    | 10%               | 7.5       | 25.4      | 31             | 8.0   |
| Meter # 9     | 30%               | 7.8       | 25.1      | 31             | 7.9   |
|               | 100%              | 8.7       | 25.4      | 31             | 7.8   |

|                  |                           |                 |         |                  |                           |
|------------------|---------------------------|-----------------|---------|------------------|---------------------------|
| CLIENT           | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014                   |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levengood              |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | <i>Americamysis bahia</i> |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                         |

**7 Day Chronic Toxicity with Mysid**

|               | Concentration (%) | DO (mg/L) | TEMP (°C) | SALINITY (ppt) | pH    |
|---------------|-------------------|-----------|-----------|----------------|-------|
|               |                   | > 4.0     | 25 - 27   | 28 - 32        | 6 - 9 |
| <b>Day 3</b>  | Control           | 6.3       | 25.5      | 31             | 7.8   |
| Rep 3         | Salt Control      | 6.0       | 25.8      | 31             | 7.9   |
| Date 6/17/22  | 2.0%              | 6.2       | 25.5      | 32             | 7.8   |
| Time 1110     | 2.8%              | 5.9       | 25.8      | 31             | 7.8   |
| Tech MS       | 10%               | 5.9       | 25.0      | 31             | 7.8   |
| Meter # 8     | 30%               | 6.0       | 25.6      | 31             | 7.9   |
|               | 100%              | 5.9       | 25.7      | 31             | 8.0   |
| <b>Day 3</b>  | Control           | 7.5       | 25.2      | 31             | 7.8   |
| Renewal Stock | Salt Control      | 7.3       | 25.7      | 31             | 8.1   |
| Date 6/17/22  | 2.0%              | 7.6       | 25.4      | 31             | 7.9   |
| Time 1105     | 2.8%              | 7.5       | 25.3      | 31             | 7.9   |
| Tech MS       | 10%               | 7.6       | 25.6      | 31             | 7.9   |
| Meter # 8     | 30%               | 7.6       | 25.6      | 31             | 7.8   |
|               | 100%              | 8.5       | 25.1      | 29             | 7.8   |
| <b>Day 4</b>  | Control           | 8.6       | 25.0      | 32             | 7.8   |
| Rep 4         | Salt Control      | 5.8       | 25.2      | 31             | 7.9   |
| Date 6/18/22  | 2%                | 6.0       | 25.2      | 32             | 7.9   |
| Time 0949     | 2.8%              | 5.7       | 25.2      | 32             | 7.8   |
| Tech RP       | 10%               | 5.7       | 25.2      | 32             | 7.8   |
| Meter # 9     | 30%               | 5.5       | 25.3      | 31             | 7.8   |
|               | 100%              | 5.6       | 25.2      | 30             | 8.0   |
| <b>Day 4</b>  | Control           | 7.4       | 25.4      | 32             | 8.0   |
| Renewal Stock | Salt Control      | 7.2       | 25.2      | 31             | 8.1   |
| Date 6/18/22  | 2%                | 7.4       | 25.2      | 32             | 8.0   |
| Time 1001     | 2.8%              | 7.4       | 25.4      | 32             | 8.0   |
| Tech RP       | 10%               | 7.5       | 25.6      | 32             | 7.9   |
| Meter # 9     | 30%               | 7.7       | 25.6      | 31             | 7.9   |
|               | 100%              | 8.0       | 25.3      | 30             | 7.8   |

① IE-MS 6/17, RP 6/18

|                  |                           |                 |         |                  |                    |
|------------------|---------------------------|-----------------|---------|------------------|--------------------|
| V.2 CLIENT       | LOTT Clean Water Alliance | DATE RECEIVED   | 6/14/22 | PROTOCOL         | TOX 014            |
| PROJECT          | NPDES                     | TEST START DATE | 6/14/22 | PROJECT MANAGER  | J. Levensgood      |
| CLIENT SAMPLE ID | Final Effluent #1         | TEST END DATE   | 6/21/22 | SPECIES          | Americamysis bahia |
| LAB SAMPLE ID    | P220614.01                | MATRIX          | Liquid  | NO. OF ORGANISMS | 5                  |

**7 Day Chronic Toxicity with Mysid**

|               | Concentration (%) | DO (mg/L) | TEMP (°C) | SALINITY (ppt)       | pH    |
|---------------|-------------------|-----------|-----------|----------------------|-------|
|               |                   | > 4.0     | 25 - 27   | 28 - 32              | 6 - 9 |
| <b>Day 5</b>  | Control           | 5.9       | 25.3      | <sup>D</sup> 31.5 32 | 7.8   |
| Rep 5         | Salt Control      | 5.5       | 25.5      | <sup>D</sup> 30.8 31 | 7.9   |
| Date 6/19/22  | 2%                | 6.1       | 25.4      | 32                   | 7.9   |
| Time 11:05    | 2.8%              | 5.6       | 25.3      | 32                   | 7.8   |
| Tech SZ       | 10%               | 5.8       | 25.4      | 32                   | 7.9   |
| Meter # 8     | 30%               | 5.6       | 25.3      | 31                   | 7.8   |
|               | 100%              | 5.4       | 25.4      | 30                   | 8.0   |
| <b>Day 5</b>  | Control           | 7.2       | 25.2      | 31                   | 8.0   |
| Renewal Stock | Salt Control      | 7.2       | 25.5      | 31                   | 8.1   |
| Date 6/19/22  | 2%                | 7.2       | 25.6      | 31                   | 8.0   |
| Time 11:20    | 2.8%              | 7.2       | 25.6      | 31                   | 8.0   |
| Tech SZ       | 10%               | 7.4       | 25.6      | 31                   | 8.0   |
| Meter # 8     | 30%               | 7.6       | 25.8      | 31                   | 7.9   |
|               | 100%              | 8.3       | 25.6      | 31                   | 7.8   |
| <b>Day 6</b>  | Control           | 5.9       | 25.0      | 31                   | 7.8   |
| Rep 6         | Salt Control      | 5.8       | 25.4      | 30                   | 7.9   |
| Date 6/20/22  | 2%                | 5.8       | 25.0      | 30                   | 7.8   |
| Time 10:24    | 2.8%              | 5.7       | 25.3      | 31                   | 7.8   |
| Tech RP       | 10%               | 5.4       | 25.4      | 30                   | 7.8   |
| Meter # 9     | 30%               | 5.6       | 25.3      | 30                   | 7.9   |
|               | 100%              | 5.7       | 25.4      | 29                   | 8.0   |
| <b>Day 6</b>  | Control           | 7.5       | 26.2      | 28                   | 7.9   |
| Renewal Stock | Salt Control      | 7.3       | 25.7      | 29                   | 8.2   |
| Date 6/20/22  | 2%                | 7.4       | 25.2      | 30                   | 7.9   |
| Time 10:13    | 2.8%              | 7.8       | 25.1      | 29                   | 7.8   |
| Tech RP       | 10%               | 7.8       | 25.3      | 30                   | 7.8   |
| Meter # 9     | 30%               | 8.1       | 25.0      | 29                   | 7.8   |
|               | 100%              | 8.5       | 25.0      | 29                   | 7.6   |
| <b>Day 7</b>  | Control           | 6.2       | 25.5      | 29                   | 7.6   |
| Rep 7         | Salt Control      | 6.3       | 25.6      | 30                   | 7.9   |
| Date 6/21/22  | 2%                | 6.3       | 25.9      | 31                   | 7.8   |
| Time 15:30    | 2.8%              | 6.1       | 25.5      | 31                   | 7.8   |
| Tech SR/JL    | 10%               | 6.3       | 25.4      | 31                   | 7.9   |
| Meter # 8     | 30%               | 6.2       | 25.4      | 31                   | 7.9   |
|               | 100%              | 5.6       | 25.8      | 30                   | 8.0   |

① IE - SZ 6/19

## Daily Quality Assurance Checks

Project name: LOTT

Test: Mysid Chronic

Lab ID: P220614.01

| Day of Test |   | Initials | Date | Comments |
|-------------|---|----------|------|----------|
| 0           | Test datasheets checked for completeness and legibility                                       | DM       | 6/14 |          |
|             | Headers/ footers filled in, visual check of test chambers, cover test, ensure proper lighting | DM       | 6/14 |          |
|             | Test data within acceptable ranges  | DM       | 6/14 |          |
| 1           | Test datasheets checked for completeness and legibility                                       | RP       | 6/15 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 2           | Test datasheets checked for completeness and legibility                                       | NL       | 6/16 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 3           | Test datasheets checked for completeness and legibility                                       | DM       | 6/17 |          |
|             | Test data within acceptable ranges  | DM       | 6/17 |          |
|             |   |          |      |          |
| 4           | Test datasheets checked for completeness and legibility                                       | NL       | 6/19 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 5           | Test datasheets checked for completeness and legibility                                       | NL       | 6/19 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 6           | Test datasheets checked for completeness and legibility                                       | DM       | 6/20 |          |
|             | Test data within acceptable ranges  | DM       | 6/20 |          |
|             |   |          |      |          |
| 7           | Test datasheets checked for completeness and legibility                                       | RP       | 6/21 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |

**POWER STANDARD CALCULATIONS**

**Mysid Mean Growth per Survivor**

Chronic Power Standard Calculation

|            | average growth/survivor |       |       |      |       |       |       |      |         |
|------------|-------------------------|-------|-------|------|-------|-------|-------|------|---------|
| Replicate  | 1                       | 2     | 3     | 4    | 5     | 6     | 7     | 8    | Mean    |
| CCEC (2.0) | 0.364                   | 0.368 | 0.406 | 0.33 | 0.336 | 0.338 | 0.348 | 0.38 | 0.35875 |
| Control    | 0.322                   | 0.304 | 0.356 | 0.33 | 0.296 | 0.34  | 0.342 | 0.34 | 0.32875 |

Control Mean - CCEC Mean

-0.03

Difference Divided by Control Mean

-0.09125475

Express as %

-9%

≤39% meets the power standard

Pass

## ORGANISM RECEIPT LOG

| Date:<br>6/14/22                          |                | Time:<br>1326          |   | Batch No.<br>ABS061422.02   |        |         |                     |
|---|----------------|------------------------|---|---|--------|---------|---------------------|
| Organism:<br>Americamysis bahia           |                |                        |   |   |        |         |                     |
| Source / Supplier:<br>Aquatic Bio Systems |                |                        |   |   |        |         |                     |
| No. Ordered:<br>575                       |                | No. Received:<br>575 + |   | Source Batch:<br>Collection date, <del>hatch date</del> , etc.):<br>6/7/22                          |        |         |                     |
| Condition of Organisms:<br>Good           |                |                        |   | Approximate Size or Age:<br>(Days from hatch, life stage, size class, etc.):<br>7 <sup>0</sup> days |        |         |                     |
| Shipper:<br>UPS                           |                |                        |   | B of L (Tracking No.)<br>1Z F46 73R 01 9568 4678  |        |         |                     |
| Condition of Container:<br>Good           |                |                        |   | Received By:<br>RP  |        |         |                     |
| Container                                 | D.O.<br>(mg/L) | Temp.<br>(°C)          | Cond. or<br><del>Sal.</del><br>(Include<br>Units) | pH<br>(Units)   | # Dead | % Dead* | Tech.<br>(Initials) |
| 1   | 16.1           | 20.2                   | 28 ppt  | 7.1   | 0      | -       | RP                  |
|   |                |                        |   |   |        |         |                     |
|   |                |                        |   |   |        |         |                     |
|   |                |                        |   |   |        |         |                     |
|   |                |                        |   |   |        |         |                     |
| *if >10% contact lab manager              |                |                        |   |   |        |         |                     |
| Notes:                                    |                |                        |   |   |        |         |                     |

① IE-RP 6/14



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: 6/13/2022

SPECIES: Americamysis bahia (formerly Mysisidopsis)

AGE: 6 day

LIFE STAGE: Juvenile

HATCH DATE: 6/7/2022

BEGAN FEEDING: Immediately


FOOD: Artemia sp.

### Water Chemistry Record:

|   | Current         | Range               |
|---|-----------------|---------------------|
| TEMPERATURE:                              | <u>25°C</u>     | <u>24-26 °C</u>     |
| SALINITY/CONDUCTIVITY:                    | <u>25 ppt**</u> | <u>21-30 ppt</u>    |
| TOTAL HARDNESS (as CaCO <sub>3</sub> ):   | <u>--</u>       | <u>--</u>           |
| TOTAL ALKALINITY (as CaCO <sub>3</sub> ): | <u>125 mg/l</u> | <u>120-155 mg/l</u> |
| pH:                                       | <u>8.20</u>     | <u>7.60-8.20</u>    |

### Comments:

\*\* Acclimated to 28 ppt on 6/13/2022.

  
\_\_\_\_\_  
Facility Supervisor

## **APPENDIX A2.2**

Reference Toxicant  
*Americamysis bahia* (Opossum Shrimp)  
7-day Chronic Test

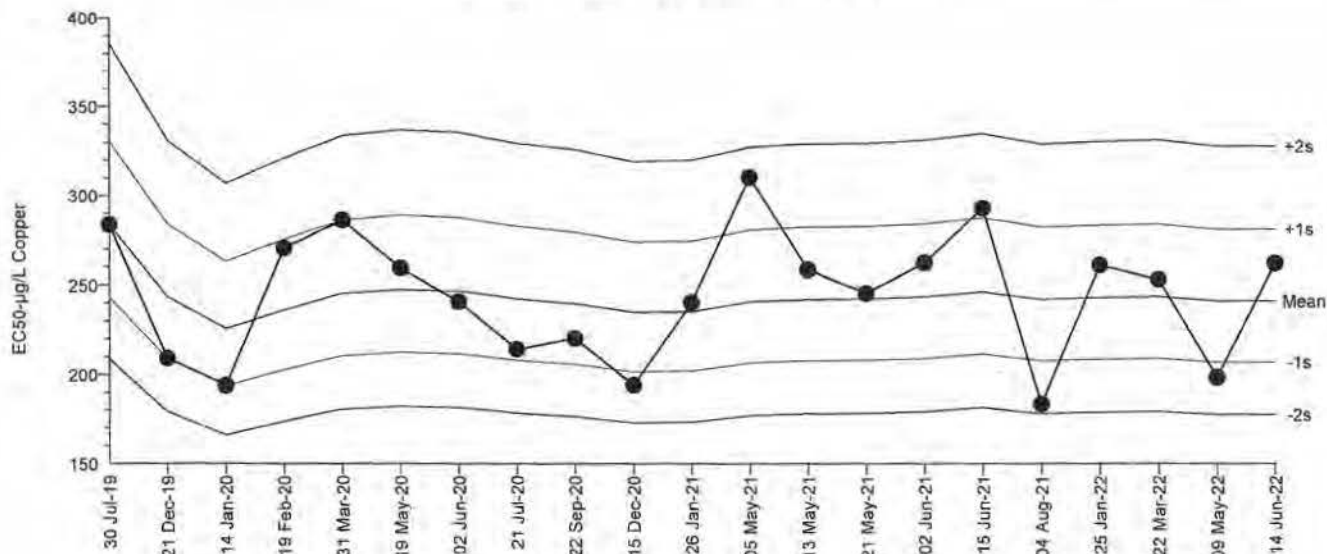


Mysidopsis 7-d Survival, Growth and Fecundity Test

All Matching Labs

Test Type: Growth-Survival-Fec (7d) Organism: Americamysis bahia (Atlantic Mysid) Material: Copper  
 Protocol: EPA/821/R-02-014 (2002) Endpoint: 7d Proportion Survived Source: Reference Toxicant-REF

Mysidopsis 7-d Survival, Growth and Fecundity Test



Mean: 241.5      Count: 20      -1s Warning Limit: 207.1      -2s Action Limit: 177.6  
 Sigma: n/a      CV: 15.50%      +1s Warning Limit: 281.5      +2s Action Limit: 328.3

Quality Control Data

| Point | Year | Month | Day | Time  | QC Data | Delta   | Sigma    | Warning | Action | Test ID      | Analysis ID  | Laboratory  |
|-------|------|-------|-----|-------|---------|---------|----------|---------|--------|--------------|--------------|-------------|
| 1     | 2019 | Jul   | 30  | 16:20 | 283.6   | 42.08   | 1.046    | (+)     |        | 21-4716-1578 | 15-8960-0029 | EcoAnalysts |
| 2     |      | Dec   | 21  | 16:39 | 209.2   | -32.29  | -0.9343  |         |        | 15-0976-9668 | 01-1173-6021 | EcoAnalysts |
| 3     | 2020 | Jan   | 14  | 15:00 | 194     | -47.5   | -1.426   | (-)     |        | 14-9113-8081 | 08-6296-9558 | EcoAnalysts |
| 4     |      | Feb   | 19  | 14:05 | 270.8   | 29.28   | 0.745    |         |        | 17-7253-3227 | 08-6985-9007 | EcoAnalysts |
| 5     |      | Mar   | 31  | 16:50 | 286.4   | 44.89   | 1.11     | (+)     |        | 07-7649-3400 | 14-2171-3388 | EcoAnalysts |
| 6     |      | May   | 19  | 16:35 | 259.7   | 18.12   | 0.4711   |         |        | 07-3270-5732 | 08-5506-4436 | EcoAnalysts |
| 7     |      | Jun   | 2   | 16:05 | 241     | -0.4875 | -0.01315 |         |        | 14-5486-5100 | 09-7785-9196 | EcoAnalysts |
| 8     |      | Jul   | 21  | 15:49 | 214.2   | -27.32  | -0.7816  |         |        | 12-8664-0663 | 09-9237-2726 | EcoAnalysts |
| 9     |      | Sep   | 22  | 15:45 | 220.4   | -21.15  | -0.5965  |         |        | 05-7885-1555 | 00-5105-2202 | EcoAnalysts |
| 10    |      | Dec   | 15  | 16:21 | 194.1   | -47.4   | -1.422   | (-)     |        | 08-3458-2726 | 14-5395-8847 | EcoAnalysts |
| 11    | 2021 | Jan   | 26  | 16:40 | 240.4   | -1.137  | -0.03071 |         |        | 07-1117-8190 | 18-1766-3913 | EcoAnalysts |
| 12    |      | May   | 5   | 13:55 | 310.6   | 69.08   | 1.638    | (+)     |        | 17-8663-7553 | 11-7697-5970 | EcoAnalysts |
| 13    |      |       | 13  | 11:05 | 258.8   | 17.29   | 0.4502   |         |        | 19-7700-2362 | 11-9997-1744 | EcoAnalysts |
| 14    |      |       | 21  | 12:14 | 245.7   | 4.139   | 0.1106   |         |        | 17-0876-1948 | 07-6006-4617 | EcoAnalysts |
| 15    |      | Jun   | 2   | 14:52 | 262.7   | 21.17   | 0.547    |         |        | 17-9119-3773 | 16-5196-6847 | EcoAnalysts |
| 16    |      |       | 15  | 15:45 | 293.2   | 51.72   | 1.263    | (+)     |        | 03-5233-7318 | 00-1595-5137 | EcoAnalysts |
| 17    |      | Aug   | 4   | 14:22 | 183.5   | -58.01  | -1.788   | (-)     |        | 11-9220-8292 | 11-5521-2623 | EcoAnalysts |
| 18    | 2022 | Jan   | 25  | 14:54 | 261.6   | 20.07   | 0.5196   |         |        | 07-3642-0806 | 18-1546-2888 | EcoAnalysts |
| 19    |      | Mar   | 22  | 14:41 | 253.6   | 12.04   | 0.3167   |         |        | 04-5639-7943 | 10-6451-7186 | EcoAnalysts |
| 20    |      | May   | 9   | 16:19 | 198.5   | -43.05  | -1.278   | (-)     |        | 19-7074-6899 | 02-8778-9883 | EcoAnalysts |
| 21    |      | Jun   | 14  | 16:43 | 262.7   | 21.17   | 0.547    |         |        | 18-6733-3888 | 19-0995-8777 | EcoAnalysts |

Mysidopsis 7-d Survival, Growth and Fecundity Test

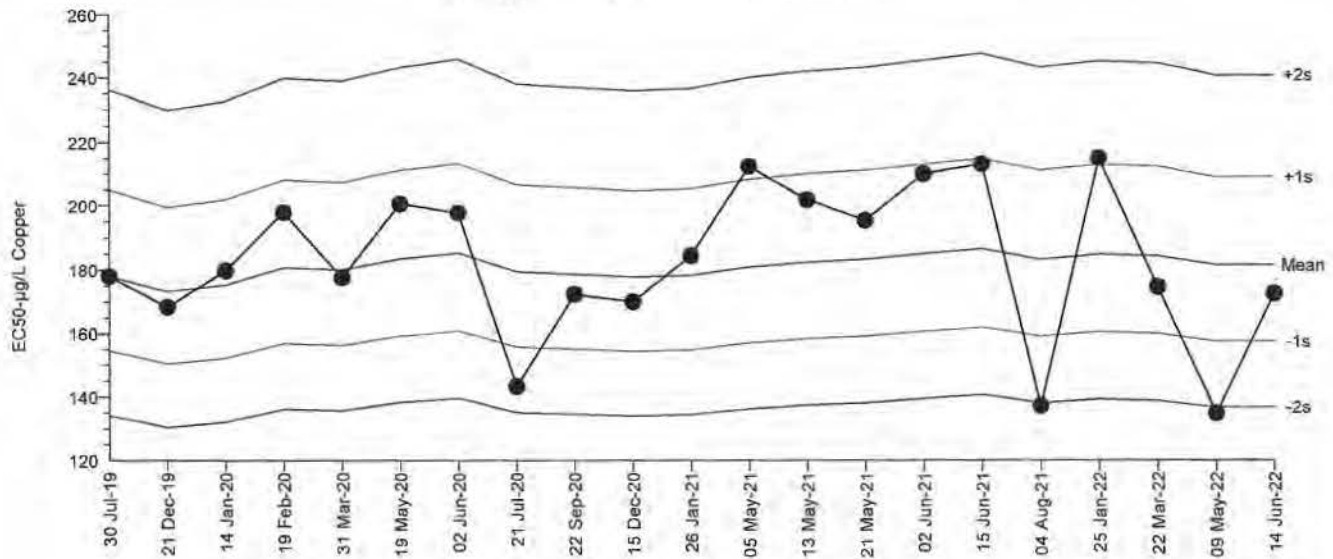
All Matching Labs

Test Type: Growth-Survival-Fec (7d)  
 Protocol: EPA/821/R-02-014 (2002)

Organism: Americamysis bahia (Atlantic Mysid)  
 Endpoint: Mean Dry Biomass-mg

Material: Copper  
 Source: Reference Toxicant-REF

Mysidopsis 7-d Survival, Growth and Fecundity Test



Mean: 181.6      Count: 20      -1s Warning Limit: 157.7      -2s Action Limit: 136.9  
 Sigma: n/a      CV: 14.20%      +1s Warning Limit: 209.2      +2s Action Limit: 241

Quality Control Data

| Point | Year | Month | Day | Time  | QC Data | Delta  | Sigma    | Warning | Action | Test ID      | Analysis ID  | Laboratory  |
|-------|------|-------|-----|-------|---------|--------|----------|---------|--------|--------------|--------------|-------------|
| 1     | 2019 | Jul   | 30  | 16:20 | 178     | -3.61  | -0.1419  |         |        | 21-4716-1578 | 08-2317-2246 | EcoAnalysts |
| 2     |      | Dec   | 21  | 16:39 | 168.4   | -13.27 | -0.536   |         |        | 15-0976-9668 | 15-2130-9544 | EcoAnalysts |
| 3     | 2020 | Jan   | 14  | 15:00 | 179.7   | -1.95  | -0.07626 |         |        | 14-9113-8081 | 07-6380-9313 | EcoAnalysts |
| 4     |      | Feb   | 19  | 14:05 | 197.9   | 16.22  | 0.6045   |         |        | 17-7253-3227 | 11-8162-9937 | EcoAnalysts |
| 5     |      | Mar   | 31  | 16:50 | 177.6   | -4.047 | -0.1593  |         |        | 07-7649-3400 | 15-3133-9567 | EcoAnalysts |
| 6     |      | May   | 19  | 16:35 | 200.6   | 18.97  | 0.7021   |         |        | 07-3270-5732 | 02-5392-0671 | EcoAnalysts |
| 7     |      | Jun   | 2   | 16:05 | 197.7   | 16.1   | 0.6002   |         |        | 14-5486-5100 | 02-7706-4638 | EcoAnalysts |
| 8     |      | Jul   | 21  | 15:49 | 143.3   | -38.35 | -1.676   | (-)     |        | 12-8664-0663 | 08-6549-5897 | EcoAnalysts |
| 9     |      | Sep   | 22  | 15:45 | 172.4   | -9.276 | -0.3705  |         |        | 05-7885-1555 | 16-3328-8093 | EcoAnalysts |
| 10    |      | Dec   | 15  | 16:21 | 170.1   | -11.55 | -0.4644  |         |        | 08-3458-2726 | 14-7371-9500 | EcoAnalysts |
| 11    | 2021 | Jan   | 26  | 16:40 | 184.5   | 2.824  | 0.109    |         |        | 07-1117-8190 | 17-9170-4283 | EcoAnalysts |
| 12    |      | May   | 5   | 13:55 | 212.5   | 30.91  | 1.111    | (+)     |        | 17-8663-7553 | 10-0342-0386 | EcoAnalysts |
| 13    |      |       | 13  | 11:05 | 202     | 20.37  | 0.7513   |         |        | 19-7700-2362 | 03-8519-3421 | EcoAnalysts |
| 14    |      |       | 21  | 12:14 | 195.5   | 13.9   | 0.5212   |         |        | 17-0876-1948 | 05-2904-3898 | EcoAnalysts |
| 15    |      | Jun   | 2   | 14:52 | 210.2   | 28.53  | 1.031    | (+)     |        | 17-9119-3773 | 04-0234-8908 | EcoAnalysts |
| 16    |      |       | 15  | 15:45 | 213.3   | 31.69  | 1.136    | (+)     |        | 03-5233-7318 | 16-1998-7202 | EcoAnalysts |
| 17    |      | Aug   | 4   | 14:22 | 137.3   | -44.38 | -1.98    | (-)     |        | 11-9220-8292 | 11-1291-6081 | EcoAnalysts |
| 18    | 2022 | Jan   | 25  | 14:54 | 215.1   | 33.49  | 1.196    | (+)     |        | 07-3642-0806 | 07-5288-1253 | EcoAnalysts |
| 19    |      | Mar   | 22  | 14:41 | 174.8   | -6.839 | -0.2712  |         |        | 04-5639-7943 | 19-2364-7596 | EcoAnalysts |
| 20    |      | May   | 9   | 16:19 | 134.9   | -46.69 | -2.1     | (-)     | (-)    | 19-7074-6899 | 18-1195-6589 | EcoAnalysts |
| 21    |      | Jun   | 14  | 16:43 | 172.8   | -8.846 | -0.3528  |         |        | 18-6733-3888 | 06-0726-9833 | EcoAnalysts |

# CETIS Summary Report

Report Date: 05 Jul-22 11:31 (p 1 of 3)  
 Test Code/ID: P190603.176 / 18-6733-3888

## Mysidopsis 7-d Survival, Growth and Fecundity Test

EcoAnalysts

|                              |                                     |  |
|------------------------------|-------------------------------------|--|
| Batch ID: 06-3547-3663       | Test Type: Growth-Survival-Fec (7d) | Analyst: Marisa Seibert                |
| Start Date: 14 Jun-22 16:43  | Protocol: EPA/821/R-02-014 (2002)   | Diluent: Laboratory Seawater           |
| Ending Date: 21 Jun-22 16:09 | Species: Americamysis bahia         | Brine: Crystal Sea Marine Mix          |
| Test Length: 6d 23h          | Taxon: Malacostraca                 | Source: Aquatic Biosystems, CO Age: 7d |
| Sample ID: 10-0572-0296      | Code: P190603.176                   | Project: Reference Toxicant            |
| Sample Date: 03 Jun-19       | Material: Copper                    | Source: Reference Toxicant             |
| Receipt Date: 03 Jun-19      | CAS (PC):                           | Station: P190603.176                   |
| Sample Age: 1107d 17h        | Client: Internal Lab                |  |

## Multiple Comparison Summary

| Analysis ID  | Endpoint               | Comparison Method                | ✓ NOEL  | LOEL | TOEL  | TU | PMSD  | S |
|--------------|------------------------|----------------------------------|---------|------|-------|----|-------|---|
| 04-8020-2437 | 7d Proportion Survived | Dunnett Multiple Comparison Test | 125     | 250  | 176.8 |    | 14.9% | 1 |
| 09-4972-5538 | Mean Dry Biomass-mg    | Dunnett Multiple Comparison Test | ✓ <62.5 | 62.5 | n/a   |    | 11.6% | 1 |
| 19-4976-2719 | Mean Dry Weight-mg     | Dunnett Multiple Comparison Test | ✓ <62.5 | 62.5 | n/a   |    | 9.28% | 1 |

## Point Estimate Summary

| Analysis ID  | Endpoint               | Point Estimate Method        | ✓ Level | µg/L  | 95% LCL | 95% UCL | TU | S |
|--------------|------------------------|------------------------------|---------|-------|---------|---------|----|---|
| 19-0995-8777 | 7d Proportion Survived | Linear Interpolation (ICPIN) | EC5     | 86.93 | 6.969   | 135.9   |    | 1 |
|              |                        |                              | EC10    | 130.3 | 78.79   | 149.2   |    |   |
|              |                        |                              | EC15    | 142.6 | 125     | 168.2   |    |   |
|              |                        |                              | EC20    | 156.1 | 138.7   | 194.9   |    |   |
|              |                        |                              | EC25    | 170.8 | 150.1   | 221.5   |    |   |
|              |                        |                              | EC40    | 223.8 | 188.1   | 278.5   |    |   |
|              |                        |                              | EC50    | 262.7 | 213.9   | 307     |    |   |
| 06-0726-9833 | Mean Dry Biomass-mg    | Linear Interpolation (ICPIN) | ✓ IC5   | 1.797 | 1.122   | 4.465   |    | 1 |
|              |                        |                              | ✓ IC10  | 6.821 | 3.505   | 28.87   |    |   |
|              |                        |                              | ✓ IC15  | 20.87 | 8.562   | 68.49   |    |   |
|              |                        |                              | ✓ IC20  | 60.17 | 19.29   | 82.89   |    |   |
|              |                        |                              | ✓ IC25  | 80.16 | 42.07   | 114.3   |    |   |
|              |                        |                              | ✓ IC40  | 141.8 | 125.9   | 158.9   |    |   |
|              |                        |                              | ✓ IC50  | 172.8 | 153.6   | 195.7   |    |   |
| 06-9022-2558 | Mean Dry Weight-mg     | Linear Interpolation (ICPIN) | IC5     | 2.132 | 1.545   | 3.529   |    | 1 |
|              |                        |                              | IC10    | 8.807 | 5.479   | 19.51   |    |   |
|              |                        |                              | IC15    | 29.71 | 15.49   | 66.4    |    |   |
|              |                        |                              | IC20    | 71.2  | 40.97   | 95.07   |    |   |
|              |                        |                              | IC25    | 101.8 | 80.66   | 145.7   |    |   |
|              |                        |                              | IC40    | 227.2 | 181.6   | n/a     |    |   |
|              |                        |                              | IC50    | >250  | n/a     | n/a     |    |   |

## Test Acceptability

| Analysis ID  | Endpoint               | Attribute    | Test Stat | TAC Limits |       |     | Overlap         | Decision |
|--------------|------------------------|--------------|-----------|------------|-------|-----|-----------------|----------|
|              |                        |              |           | Lower      | Upper |     |                 |          |
| 04-8020-2437 | 7d Proportion Survived | Control Resp | 0.975     | 0.8        | >>    | Yes | Passes Criteria |          |
| 19-0995-8777 | 7d Proportion Survived | Control Resp | 0.975     | 0.8        | >>    | Yes | Passes Criteria |          |
| 06-0726-9833 | Mean Dry Biomass-mg    | Control Resp | 0.3308    | 0.2        | >>    | Yes | Passes Criteria |          |
| 09-4972-5538 | Mean Dry Biomass-mg    | Control Resp | 0.3308    | 0.2        | >>    | Yes | Passes Criteria |          |

**CETIS Summary Report**

Report Date: 05 Jul-22 11:31 (p 2 of 3)  
 Test Code/ID: P190603.176 / 18-6733-3888

**Mysidopsis 7-d Survival, Growth and Fecundity Test**

EcoAnalysts

**7d Proportion Survived Summary**

| Conc-µg/L | Code | Count | Mean   | 95% LCL | 95% UCL | Min    | Max    | Std Err | Std Dev | CV%    | %Effect |
|-----------|------|-------|--------|---------|---------|--------|--------|---------|---------|--------|---------|
| 0         | D    | 8     | 0.9750 | 0.9159  | 1.0000  | 0.8000 | 1.0000 | 0.0250  | 0.0707  | 7.25%  | 0.00%   |
| 62.5      |      | 8     | 0.9500 | 0.8726  | 1.0000  | 0.8000 | 1.0000 | 0.0327  | 0.0926  | 9.75%  | 2.56%   |
| 125       |      | 8     | 0.9000 | 0.8106  | 0.9894  | 0.8000 | 1.0000 | 0.0378  | 0.1069  | 11.88% | 7.69%   |
| 250       |      | 8     | 0.5250 | 0.3264  | 0.7236  | 0.2000 | 0.8000 | 0.0840  | 0.2375  | 45.25% | 46.15%  |
| 500       |      | 8     | 0.0000 | 0.0000  | 0.0000  | 0.0000 | 0.0000 | 0.0000  | 0.0000  |        | 100.00% |
| 1000      |      | 8     | 0.0000 | 0.0000  | 0.0000  | 0.0000 | 0.0000 | 0.0000  | 0.0000  |        | 100.00% |

**Mean Dry Biomass-mg Summary**

| Conc-µg/L | Code | Count | Mean   | 95% LCL | 95% UCL | Min   | Max   | Std Err  | Std Dev | CV%    | %Effect |
|-----------|------|-------|--------|---------|---------|-------|-------|----------|---------|--------|---------|
| 0         | D    | 8     | 0.3307 | 0.3122  | 0.3493  | 0.284 | 0.352 | 0.007837 | 0.02217 | 6.70%  | 0.00%   |
| 62.5      |      | 8     | 0.264  | 0.2338  | 0.2942  | 0.216 | 0.314 | 0.01279  | 0.03617 | 13.70% | 20.18%  |
| 125       |      | 8     | 0.2195 | 0.1893  | 0.2497  | 0.174 | 0.3   | 0.01277  | 0.03612 | 16.46% | 33.64%  |
| 250       |      | 8     | 0.1035 | 0.06602 | 0.141   | 0.028 | 0.162 | 0.01585  | 0.04483 | 43.32% | 68.71%  |
| 500       |      | 8     | 0      | 0       | 0       | 0     | 0     | 0        | 0       |        | 100.00% |
| 1000      |      | 8     | 0      | 0       | 0       | 0     | 0     | 0        | 0       |        | 100.00% |

**Mean Dry Weight-mg Summary**

| Conc-µg/L | Code | Count | Mean   | 95% LCL | 95% UCL | Min   | Max   | Std Err  | Std Dev | CV%    | %Effect |
|-----------|------|-------|--------|---------|---------|-------|-------|----------|---------|--------|---------|
| 0         | D    | 8     | 0.3396 | 0.3286  | 0.3506  | 0.316 | 0.355 | 0.004652 | 0.01316 | 3.87%  | 0.00%   |
| 62.5      |      | 8     | 0.2779 | 0.2567  | 0.2991  | 0.244 | 0.314 | 0.008961 | 0.02535 | 9.12%  | 18.18%  |
| 125       |      | 8     | 0.2449 | 0.2155  | 0.2743  | 0.21  | 0.3   | 0.01243  | 0.03515 | 14.35% | 27.88%  |
| 250       |      | 8     | 0.1972 | 0.1662  | 0.2282  | 0.14  | 0.25  | 0.01311  | 0.03707 | 18.80% | 41.94%  |

**7d Proportion Survived Detail**

| Conc-µg/L | Code | Rep 1  | Rep 2  | Rep 3  | Rep 4  | Rep 5  | Rep 6  | Rep 7  | Rep 8  |
|-----------|------|--------|--------|--------|--------|--------|--------|--------|--------|
| 0         | D    | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 1.0000 |
| 62.5      |      | 1.0000 | 0.8000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 1.0000 | 0.8000 |
| 125       |      | 1.0000 | 1.0000 | 1.0000 | 0.8000 | 0.8000 | 0.8000 | 1.0000 | 0.8000 |
| 250       |      | 0.8000 | 0.8000 | 0.4000 | 0.4000 | 0.2000 | 0.8000 | 0.4000 | 0.4000 |
| 500       |      | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |
| 1000      |      | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 | 0.0000 |

**Mean Dry Biomass-mg Detail**

| Conc-µg/L | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|-----------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0         | D    | 0.328 | 0.342 | 0.35  | 0.352 | 0.316 | 0.336 | 0.284 | 0.338 |
| 62.5      |      | 0.264 | 0.228 | 0.254 | 0.244 | 0.312 | 0.28  | 0.314 | 0.216 |
| 125       |      | 0.3   | 0.21  | 0.216 | 0.198 | 0.222 | 0.22  | 0.216 | 0.174 |
| 250       |      | 0.162 | 0.14  | 0.1   | 0.096 | 0.028 | 0.148 | 0.086 | 0.068 |
| 500       |      | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |
| 1000      |      | 0     | 0     | 0     | 0     | 0     | 0     | 0     | 0     |

**Mean Dry Weight-mg Detail**

| Conc-µg/L | Code | Rep 1  | Rep 2 | Rep 3 | Rep 4  | Rep 5  | Rep 6 | Rep 7 | Rep 8  |
|-----------|------|--------|-------|-------|--------|--------|-------|-------|--------|
| 0         | D    | 0.328  | 0.342 | 0.35  | 0.352  | 0.316  | 0.336 | 0.355 | 0.338  |
| 62.5      |      | 0.264  | 0.285 | 0.254 | 0.244  | 0.312  | 0.28  | 0.314 | 0.27   |
| 125       |      | 0.3    | 0.21  | 0.216 | 0.2475 | 0.2775 | 0.275 | 0.216 | 0.2175 |
| 250       |      | 0.2025 | 0.175 | 0.25  | 0.24   | 0.14   | 0.185 | 0.215 | 0.17   |
| 500       |      |        |       |       |        |        |       |       |        |
| 1000      |      |        |       |       |        |        |       |       |        |

# CETIS Summary Report

Report Date: 05 Jul-22 11:31 (p 3 of 3)  
Test Code/ID: P190603.176 / 18-6733-3888

## Mysidopsis 7-d Survival, Growth and Fecundity Test

EcoAnalysts

### 7d Proportion Survived Binomials

| Conc- $\mu$ g/L | Code | Rep 1 | Rep 2 | Rep 3 | Rep 4 | Rep 5 | Rep 6 | Rep 7 | Rep 8 |
|-----------------|------|-------|-------|-------|-------|-------|-------|-------|-------|
| 0               | D    | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   | 4/5   | 5/5   |
| 62.5            |      | 5/5   | 4/5   | 5/5   | 5/5   | 5/5   | 5/5   | 5/5   | 4/5   |
| 125             |      | 5/5   | 5/5   | 5/5   | 4/5   | 4/5   | 4/5   | 5/5   | 4/5   |
| 250             |      | 4/5   | 4/5   | 2/5   | 2/5   | 1/5   | 4/5   | 2/5   | 2/5   |
| 500             |      | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   |
| 1000            |      | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   | 0/5   |



CETIS Test Data Worksheet

Report Date: 05 Jul-22 11:30 (p 1 of 2)  
 Test Code/ID: P190603.176 / 18-6733-3888

Mysidopsis 7-d Survival, Growth and Fecundity Test

EcoAnalysts

Start Date: 14 Jun-22 16:43 Species: Americamysis bahia Sample Code: P190603.176  
 End Date: 21 Jun-22 16:09 Protocol: EPA/821/R-02-014 (2002) Sample Source: Reference Toxicant  
 Sample Date: 03 Jun-19 Material: Copper Sample Station: P190603.176

| Conc-µg/L | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg Total | Weight-mg Tare | Pan Count | Females Total | Females Gravid | Notes |
|-----------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|-----------------|----------------|-----------|---------------|----------------|-------|
| 0         | D    | 1   | 22  | 5         | 5           |             |             |             |             |             | 5           | 26.97           | 25.33          | 5         |               |                |       |
| 0         | D    | 2   | 35  | 5         | 5           |             |             |             |             |             | 5           | 29.25           | 27.54          | 5         |               |                |       |
| 0         | D    | 3   | 39  | 5         | 5           |             |             |             |             |             | 5           | 27.71           | 25.96          | 5         |               |                |       |
| 0         | D    | 4   | 38  | 5         | 5           |             |             |             |             |             | 5           | 27.12           | 25.36          | 5         |               |                |       |
| 0         | D    | 5   | 37  | 5         | 5           |             |             |             |             |             | 5           | 28.49           | 26.91          | 5         |               |                |       |
| 0         | D    | 6   | 18  | 5         | 5           |             |             |             |             |             | 5           | 30.07           | 28.39          | 5         |               |                |       |
| 0         | D    | 7   | 29  | 5         | 4           |             |             |             |             |             | 4           | 30.35           | 28.93          | 4         |               |                |       |
| 0         | D    | 8   | 14  | 5         | 5           |             |             |             |             |             | 5           | 28.19           | 26.5           | 5         |               |                |       |
| 62.5      |      | 1   | 48  | 5         | 5           |             |             |             |             |             | 5           | 30.92           | 29.6           | 5         |               |                |       |
| 62.5      |      | 2   | 7   | 5         | 5           |             |             |             |             |             | 4           | 26.48           | 25.34          | 4         |               |                |       |
| 62.5      |      | 3   | 30  | 5         | 5           |             |             |             |             |             | 5           | 33.2            | 31.93          | 5         |               |                |       |
| 62.5      |      | 4   | 26  | 5         | 5           |             |             |             |             |             | 5           | 29.54           | 28.32          | 5         |               |                |       |
| 62.5      |      | 5   | 19  | 5         | 5           |             |             |             |             |             | 5           | 25.95           | 24.39          | 5         |               |                |       |
| 62.5      |      | 6   | 20  | 5         | 5           |             |             |             |             |             | 5           | 34.06           | 32.66          | 5         |               |                |       |
| 62.5      |      | 7   | 41  | 5         | 5           |             |             |             |             |             | 5           | 36.3            | 34.73          | 5         |               |                |       |
| 62.5      |      | 8   | 43  | 5         | 4           |             |             |             |             |             | 4           | 23.76           | 22.68          | 4         |               |                |       |
| 125       |      | 1   | 13  | 5         | 5           |             |             |             |             |             | 5           | 26.98           | 25.48          | 5         |               |                |       |
| 125       |      | 2   | 17  | 5         | 5           |             |             |             |             |             | 5           | 28.15           | 27.1           | 5         |               |                |       |
| 125       |      | 3   | 6   | 5         | 5           |             |             |             |             |             | 5           | 28.45           | 27.37          | 5         |               |                |       |
| 125       |      | 4   | 25  | 5         | 5           |             |             |             |             |             | 4           | 27.05           | 26.06          | 4         |               |                |       |
| 125       |      | 5   | 4   | 5         | 5           |             |             |             |             |             | 4           | 32.87           | 31.76          | 4         |               |                |       |
| 125       |      | 6   | 23  | 5         | 5           |             |             |             |             |             | 4           | 26.31           | 25.21          | 4         |               |                |       |
| 125       |      | 7   | 28  | 5         | 5           |             |             |             |             |             | 5           | 28.32           | 27.24          | 5         |               |                |       |
| 125       |      | 8   | 3   | 5         | 5           |             |             |             |             |             | 4           | 26.52           | 25.65          | 4         |               |                |       |
| 250       |      | 1   | 16  | 5         | 5           |             |             |             |             |             | 4           | 28.65           | 27.84          | 4         |               |                |       |
| 250       |      | 2   | 21  | 5         | 5           |             |             |             |             |             | 4           | 27.4            | 26.7           | 4         |               |                |       |
| 250       |      | 3   | 15  | 5         | 5           |             |             |             |             |             | 2           | 27.73           | 27.23          | 2         |               |                |       |
| 250       |      | 4   | 42  | 5         | 5           |             |             |             |             |             | 2           | 34.11           | 33.63          | 2         |               |                |       |
| 250       |      | 5   | 12  | 5         | 5           |             |             |             |             |             | 1           | 28.91           | 28.77          | 1         |               |                |       |
| 250       |      | 6   | 24  | 5         | 5           |             |             |             |             |             | 4           | 31.12           | 30.38          | 4         |               |                |       |
| 250       |      | 7   | 32  | 5         | 4           |             |             |             |             |             | 2           | 31.49           | 31.06          | 2         |               |                |       |
| 250       |      | 8   | 10  | 5         | 5           |             |             |             |             |             | 2           | 36.02           | 35.68          | 2         |               |                |       |

CETIS Test Data Worksheet

Report Date: 05 Jul-22 11:30 (p 2 of 2)  
 Test Code/ID: P190603.176 / 18-6733-3888

| Conc-µg/L | Code | Rep | Pos | # Exposed | 1d Survival | 2d Survival | 3d Survival | 4d Survival | 5d Survival | 6d Survival | 7d Survival | Weight-mg<br>Total | Weight-mg<br>Tare | Pan Count | Total<br>Females | Gravid<br>Females | Notes |
|-----------|------|-----|-----|-----------|-------------|-------------|-------------|-------------|-------------|-------------|-------------|--------------------|-------------------|-----------|------------------|-------------------|-------|
| 500       |      | 1   | 2   | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 2   | 47  | 5         | 3           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 3   | 45  | 5         | 2           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 4   | 44  | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 5   | 40  | 5         | 3           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 6   | 46  | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 7   | 9   | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 500       |      | 8   | 5   | 5         | 3           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 1   | 34  | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 2   | 33  | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 3   | 36  | 5         | 0           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 4   | 8   | 5         | 2           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 5   | 1   | 5         | 0           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 6   | 11  | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 7   | 27  | 5         | 0           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |
| 1000      |      | 8   | 31  | 5         | 1           |             |             |             |             |             | 0           | 0                  | 0                 |           |                  |                   |       |



# 7 Day Chronic Survival and Growth Test

Toxicant: Copper Chloride  
 Ref Tox ID: P190603.176

Date Test Started: 6/14/2022  
 Date Test Ended: 6/21/2022

Species: *Americamysis bahia*

| Concentration     | Rep | Day 1<br>Date: 6/15/22<br>Time: 1018<br>Tech: SZ/MS |        | Day 2<br>Date: 6/16/22<br>Time: 1310<br>Tech: SZ/MS |        | Day 3<br>Date: 6/17/22<br>Time: 1200<br>Tech: MS |        | Day 4<br>Date: 6/18<br>Time: 1257<br>Tech: RP |        | Day 5<br>Date: 6/19<br>Time: 140<br>Tech: NL |        | Day 6<br>Date: 6/20<br>Time: 1145<br>Tech: RP |        | Day 7<br>Date: 6/21<br>Time: 1608<br>Tech: SZ/DM |        | Pan Count<br>Date:<br>Time:<br>Tech: |
|-------------------|-----|---|--------|---|--------|--|--------|---|--------|--|--------|---|--------|--|--------|--------------------------------------|
|                   |     | # Alive   | # Dead | # Alive   | # Dead | # Alive  | # Dead | # Alive                                       | # Dead | # Alive                                      | # Dead | # Alive                                       | # Dead | # Alive  | # Dead |                                      |
| Control           | 1   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 2   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 3   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 4   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 5   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 6   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 7   | 4   | 1      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      |                                      |
|                   | 8   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
| 62.5              | 1   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 2   | 5   | 0      | 5   | 0      | 4  | INB    | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 3   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 4   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 5   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 6   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 7   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 8   | 4   | 1      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      |                                      |
| 125               | 1   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 2   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 3   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 4   | 5   | 0      | 4   | INB    | 4  | 0      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      |                                      |
|                   | 5   | 5   | 0      | 5   | 0      | 4  | 1      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      |                                      |
|                   | 6   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 7   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 8   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
| 250               | 1   | 5   | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      | 5   | 0      | 5  | 0      |                                      |
|                   | 2   | 5   | 0      | 5   | 0      | 4  | 1      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      |                                      |
|                   | 3   | 5   | 0      | 4   | 1      | 3  | 1      | 2   | 1      | 2  | 0      | 2   | 0      | 2  | 0      |                                      |
|                   | 4   | 5   | 0      | 4   | 1      | 4  | 0      | 3   | 1      | 2  | 0      | 2   | 0      | 2  | 0      |                                      |
|                   | 5   | 5   | 0      | 3   | 2      | 2  | 1      | 1   | 1      | 1  | 0      | 1   | 0      | 1  | 0      |                                      |
|                   | 6   | 5   | 0      | 4   | INB    | 4  | 0      | 4   | 0      | 4  | 0      | 4   | 0      | 4  | 0      |                                      |
|                   | 7   | 4   | 1      | 2   | 2NB    | 2  | 0      | 2   | 0      | 2  | 0      | 2   | 0      | 2  | 0      |                                      |
|                   | 8   | 5   | 0      | 5   | 0      | 4  | 1      | 4   | 0      | 2  | 2      | 2   | 0      | 2  | 0      |                                      |
| 500<br>sz 6/15/22 | 1   | 2   | 3      | 0   | 1      | 0  | 1      |   |        |  |        |   |        |  |        |                                      |
|                   | 2   | 3   | 2      | 1   | 2      | 0  | 1      |   |        |  |        |   |        |  |        |                                      |
|                   | 3   | 2   | 3      | 0   | 2      |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 4   | 1   | 4      | 0   | 1      |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 5   | 3   | 2      | 2   | INB    | 0  | 2      |   |        |  |        |   |        |  |        |                                      |
|                   | 6   | 1   | 4      | 1   | 0      | 0  | 1      |   |        |  |        |   |        |  |        |                                      |
|                   | 7   | 1   | 4      | 0   | 1      |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 8   | 3   | 2      | 1   | 2NB    | 0  | 1      |   |        |  |        |   |        |  |        |                                      |
| 1000              | 1   | 1   | 4      | 1   | 0      | 0  | 1      |   |        |  |        |   |        |  |        |                                      |
|                   | 2   | 1   | 4      | 0   | 1      |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 3   | 0   | 5      |   |        |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 4   | 2   | 3      | 0   | 2      |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 5   | 0   | 5      |   |        |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 6   | 1   | 4      | 0   | 1      |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 7   | 0   | 5      |   |        |  |        |   |        |  |        |   |        |  |        |                                      |
|                   | 8   | 1   | 4      | 0   | 1      |  |        |   |        |  |        |   |        |  |        |                                      |

|                     |    |        |        |        |        |        |        |      |      |
|---------------------|----|--------|--------|--------|--------|--------|--------|------|------|
| Feed                | AM | RP     | MS (1) | MS (1) | NL (1) | NL (1) | RP (1) | None | None |
| 375 nauplii/chamber | PM | RP (1) | NL (1) | NL (1) | NL (1) | NL (1) | NL (1) | None | None |

① chambers with 2 fish are fed half the amount - RP 6/15, MS 6/16, NL 6/17  
 ② Recant - sz 6/15  
 - MS 6/17  
 - NL 6/17  
 - NL 6/19  
 - NL 6/19  
 - MK 6/20

### 7 Day Chronic Survival and Growth Test

|             |                 |
|-------------|-----------------|
| Toxicant:   | Copper Chloride |
| Ref Tox ID: | P190603.176     |

|                    |                    |
|--------------------|--------------------|
| Date Test Started: | 6/14/2022          |
| Date Test Ended:   | 6/21/2022          |
| Species:           | Americamysis bahia |

| Concentration (µg/L) | Replicate | Boat Number | Weight Empty Boat (mg) | Weight Boat & Animals (mg) | Pan Count |
|----------------------|-----------|-------------|------------------------|----------------------------|-----------|
| Control              | 1         | 1 ✓         | 25.33                  | 26.97                      | 5         |
|                      | 2         | 2           | 27.54                  | 29.25                      | 5         |
|                      | 3         | 3           | 25.96                  | 27.71                      | 5         |
|                      | 4         | 4           | 25.36                  | 27.12                      | 5         |
|                      | 5         | 5           | 26.91                  | 28.49                      | 5         |
|                      | 6         | 6           | 28.39                  | 30.07                      | 5         |
|                      | 7         | 7           | 28.963                 | 30.35                      | 4         |
|                      | 8         | 8           | 26.50                  | 28.19                      | 5         |
| 62.5                 | 1         | 9           | 29.60                  | 30.92                      | 5         |
|                      | 2         | 10          | 25.34                  | 26.48                      | 4         |
|                      | 3         | 11          | 31.93                  | 33.20                      | 5         |
|                      | 4         | 12          | 28.32                  | 29.54                      | 5         |
|                      | 5         | 13          | 24.39                  | 25.95                      | 5         |
|                      | 6         | 14          | 32.66                  | 34.06                      | 5         |
|                      | 7         | 15          | 34.73                  | 36.30                      | 5         |
|                      | 8         | 16          | 22.68                  | 23.76                      | 4         |
| 125                  | 1         | 17          | 25.48                  | 26.98                      | 5         |
|                      | 2         | 18          | 27.10                  | 28.15                      | 5         |
|                      | 3         | 19          | 27.37                  | 28.45                      | 5         |
|                      | 4         | 20          | 26.08                  | 27.05                      | 4         |
|                      | 5         | 21          | 31.76                  | 32.87                      | 4         |
|                      | 6         | 22          | 25.21                  | 26.31                      | 4         |
|                      | 7         | 23          | 27.24                  | 28.32                      | 5         |
|                      | 8         | 24          | 25.65                  | 26.52                      | 4         |
| 250                  | 1         | 25          | 27.84                  | 28.65                      | 4         |
|                      | 2         | 26          | 26.70                  | 27.40                      | 4         |
|                      | 3         | 27          | 27.23                  | 27.73                      | 2         |
|                      | 4         | 28          | 39.63                  | 34.11                      | 2         |
|                      | 5         | 29          | 28.77                  | 28.91                      | 1         |
|                      | 6         | 30          | 30.38                  | 31.12                      | 4         |
|                      | 7         | 31          | 31.06                  | 31.49                      | 2         |
|                      | 8         | 32          | 35.68                  | 36.02                      | 2         |

① IE-6/21/22 IM

### 7 Day Chronic Survival and Growth Test

|             |                 |
|-------------|-----------------|
| Toxicant:   | Copper Chloride |
| Ref Tox ID: | P190603.170     |

|                    |                    |
|--------------------|--------------------|
| Date Test Started: | 6/14/2022          |
| Date Test Ended:   | 6/21/2022          |
| Species:           | Americamysis bahia |

| Concentration (µg/L) | Replicate | Boat Number | Weight Empty Boat (mg) | Weight Boat & Animals (mg) | Pan Count |
|----------------------|-----------|-------------|------------------------|----------------------------|-----------|
| 500                  | 1         |             |                        |                            |           |
|                      | 2         |             |                        |                            |           |
|                      | 3         |             |                        |                            |           |
|                      | 4         |             |                        |                            |           |
|                      | 5         |             |                        |                            |           |
|                      | 6         |             |                        |                            |           |
|                      | 7         |             |                        |                            |           |
|                      | 8         |             |                        |                            |           |
| 1000                 | 1         |             |                        |                            |           |
|                      | 2         |             |                        |                            |           |
|                      | 3         |             |                        |                            |           |
|                      | 4         |             |                        |                            |           |
|                      | 5         |             |                        |                            |           |
|                      | 6         |             |                        |                            |           |
|                      | 7         |             |                        |                            |           |
|                      | 8         |             |                        |                            |           |

|   |                  |    |               |    |
|---|------------------|----|---------------|----|
| Date/Time in oven: [Init.]                                      | 6/21/22<br>09:45 | RP | 6/21/22 17:23 | SZ |
| OvenTemp: [Init.]   | 107°C            | RP | 104°C         | SZ |
| Date/Time removed from oven (and placed in dessicator): [Init.] | 6/21/22<br>12:03 | RP | 6/22 11:50    | JC |
| Weight date and time (removed from dessicator): [Init.]         | 6/21/22<br>14:50 | IM | 6/23 14:12    | IM |

① IW-RP6/21  
 1x 28.75  
 2x 35.84

### 7 Day Chronic Survival and Growth Test

|             |                 |
|-------------|-----------------|
| Toxicant:   | Copper Chloride |
| Ref Tox ID: | P190603.176     |
| Lot #:      | S0D1448         |
| Protocol:   | TOX099          |
| Replicates: | 8               |

|                          |                    |
|--------------------------|--------------------|
| Date Test Started:       | 6/14/2022          |
| Date Test Ended:         | 6/21/2022          |
| Matrix:                  | Liquid             |
| Species:                 | Americamysis bahia |
| No. of Org. per Chamber: | 5                  |

|                      | Conc.   | Meter #: | DO (mg/L)<br>(>4.0) | Meter #: | Temp (°C)<br>(26±1°C) | Meter #: | Salinity (ppt)<br>(30±2ppt) | Meter #: | pH (6 - 9) |       |
|----------------------|---------|----------|---------------------|----------|-----------------------|----------|-----------------------------|----------|------------|-------|
| <b>Day 0 (Stock)</b> | Control | 8        | 7.3                 | 8        | 25.4                  | 8        | 30                          | 8        | 8.0        |       |
| Date: 6/14/22        | 62.5    |          | 7.4                 |          | 25.4                  |          | 29                          |          | 8.0        |       |
| Time: 1104           | 125     |          | 7.5                 |          | 25.3                  |          | 29                          |          | 8.0        |       |
| Technician: RP       | 250     |          | 7.6                 |          | 25.9                  |          | 29                          |          | 8.0        |       |
| PM Feed: DM          | 500     |          | 7.6                 |          | 25.6                  |          | 29                          |          | 8.0        |       |
|                      | 1000    |          | 7.5                 |          | 26.5                  |          | 29                          |          | 8.0        |       |
|                      | Day 1   |          | Day 2               |          | Day 3                 |          | Day 4                       |          | Day 5      | Day 6 |
| Temperature (OLD)    | 25.4    |          | 25.1                |          | 25.4                  |          | 25.6                        |          | 25.4       | 25.6  |
| Temperature (NEW)    | 25.7    |          | 25.2                |          | 25.4                  |          | 25.1                        |          | 25.4       | 25.7  |
| <b>Day 7</b>         | Control | 8        | 6.22                | 8        | 25.2                  | 8        | 31                          | 8        | 7.8        |       |
| Date: 6/21/22        | 62.5    |          | 6.2                 |          | 25.3                  |          | 31                          |          | 7.8        |       |
| Time: 15:55          | 125     |          | 6.3                 |          | 25.5                  |          | 31                          |          | 7.8        |       |
| Replicate No.: 1     | 250     |          | 6.3                 |          | 25.6                  |          | 31                          |          | 7.8        |       |
| Technician: SR/JL    | 500     |          |                     |          |                       |          |                             |          |            |       |
|                      | 1000    |          |                     |          |                       |          |                             |          |            |       |

#### Dilution Preparation (Serial dilute by 50%)

| CuCl <sub>2</sub> *2H <sub>2</sub> O Stock Solution: | Target Stock Solution Conc. | Volume of Stock Solution | Amt. of Toxicant |
|--|-----------------------------|--------------------------|------------------|
| 400,000 µg/L   | 1000 µg/L                   | 4000 g                   | 10 g             |
| 400,000 µg/L   | 500 µg/L                    | 4000 g                   | 5 g              |
| 400,000 µg/L   | 250 µg/L                    | 4000 g                   | 2.5 g            |

| Day | Date | Init. | Highest Conc. | Day | Date | Init. | Highest Conc. |
|-----|------|-------|---------------|-----|------|-------|---------------|
| 0   | 6/14 | RP    | 1000 mg/L     | 4   | 6/18 | RP    | 250 mg/L      |
| 1   | 6/15 | SZ/MS | 1000 mg/L     | 5   | 6/19 | SZ    | 250 mg/L      |
| 2   | 6/16 | SZ/MS | 1000 mg/L     | 6   | 6/20 | RP    | 250 mg/L      |
| 3   | 6/17 | MS    | 250 mg/L      |     |      |       |               |

|                     |  |
|---------------------|--|
| Start Time:         | 11:43 AM, SZ, RP                               |
| End Time:           | 1:09 PM, SZ                                    |
| Test Acceptability: | ≥80% Survival in control                       |
|                     | ≥Mean 0.20 mg dry weight / organism in control |
|                     | PMSD <50% (recommended)                        |

|                       |                       |
|-----------------------|-----------------------|
| Test Location:        | Test Room             |
| Dilution Water Batch: | FSN061222.01          |
| Supplier:             | Aquatic BioSystems    |
| Organism Batch:       | AB-061222.02 Age: 7 d |
| Chamber Size/Type:    | 12 oz cup             |
| Exposure Volume:      | 250 mL                |



## Daily Quality Assurance Checks

Project name: **Various**Test: **Mysid cnc12 RT**Lab ID: **P190603.176**

| Day of Test |   | Initials | Date | Comments |
|-------------|---|----------|------|----------|
| 0           | Test datasheets checked for completeness and legibility                                       | DM       | 6/14 |          |
|             | Headers/ footers filled in, visual check of test chambers, cover test, ensure proper lighting | DM       | 6/14 |          |
|             | Test data within acceptable ranges  | DM       | 6/14 |          |
| 1           | Test datasheets checked for completeness and legibility                                       | RP       | 6/15 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 2           | Test datasheets checked for completeness and legibility                                       | NL       | 6/16 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 3           | Test datasheets checked for completeness and legibility                                       | DM       | 6/17 |          |
|             | Test data within acceptable ranges  | DM       | 6/17 |          |
|             |   |          |      |          |
| 4           | Test datasheets checked for completeness and legibility                                       | NL       | 6/18 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 5           | Test datasheets checked for completeness and legibility                                       | NL       | 6/19 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |
| 6           | Test datasheets checked for completeness and legibility                                       | DM       | 6/20 |          |
|             | Test data within acceptable ranges  | DM       | 6/20 |          |
|             |   |          |      |          |
| 7           | Test datasheets checked for completeness and legibility                                       | RP       | 6/21 |          |
|             | Test data within acceptable ranges  | ↓        | ↓    |          |
|             |   |          |      |          |

|       |    |
|-------|----|
| 1     | 21 |
| 2     | 11 |
| 3     | 22 |
| 4     | 47 |
| 5     | 24 |
| 6     | 39 |
| 7     | 34 |
| 8     | 32 |
| <hr/> |    |
| 9     | 16 |
| 10    | 23 |
| 11    | 15 |
| 12    | 19 |
| 13    | 37 |
| 14    | 38 |
| 15    | 48 |
| 16    | 27 |
| <hr/> |    |
| 17    | 12 |
| 18    | 33 |
| 19    | 36 |
| 20    | 45 |
| 21    | 6  |
| 22    | 31 |
| 23    | 17 |
| 24    | 3  |
| <hr/> |    |
| 25    | 30 |
| 26    | 20 |
| 27    | 42 |
| 28    | 18 |
| 29    | 8  |
| 30    | 14 |
| 31    | 25 |
| 32    | 13 |
| <hr/> |    |
| 33    | 10 |
| 34    | 28 |
| 35    | 46 |
| 36    | 1  |
| 37    | 5  |
| 38    | 44 |
| 39    | 7  |
| 40    | 26 |
| <hr/> |    |
| 41    | 4  |
| 42    | 41 |
| 43    | 43 |
| 44    | 35 |
| 45    | 9  |
| 46    | 2  |
| 47    | 29 |
| 48    | 40 |
| <hr/> |    |

Mysid CuCl<sub>2</sub> RT  
6/14-6/21/22

PI 90603.176

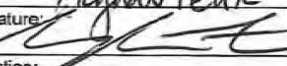
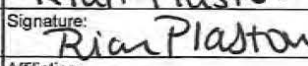
## **APPENDIX B**

Chain of Custody  
Sample Receipt Forms



|                      |  |  |   |
|----------------------|--|--|---|
| Destination:         | Sample Originator (Organization): <b>LOTT Clean Water Alliance</b> | Report Results To: <b>LOTT Clean Water Alliance</b>                            | Phone: <b>360 528 5760</b>                  |
| Destination Contact: | PERSON WHO COLLECTED SAMPLE: <b>MEGHAN FEUK</b>                    | Contact Name: <b>MEGHAN FEUK</b>   | Fax:  |
| Date:                | Address: <b>500 ADAMS ST NE<br/>OLYMPIA, WA 98501</b>              | Address: <b>500 ADAMS ST NE<br/>OLYMPIA, WA 98501</b>                          | Email: <b>meghanfeuk@lottcleanwater.org</b> |
| Turn-Around-Time:    | Phone: <b>360 528 5760</b>   | Analyses:  | Invoicing To:                               |
| Project Name:        | Fax:   | Chironomid<br>Toxicity<br>Survival + Growth<br>Chironomid<br>Survival + Growth | Comments or Special Instructions:           |
| Contract/PO:         | E-mail: <b>meghanfeuk@lottcleanwater.org</b>                       |  |   |

| No. | Sample ID         | Matrix | Volume & Type of Container | Date & Time  |   |   | Preservation | Sample Temp Upon Receipt | LAB ID     |
|-----|-------------------|--------|----------------------------|--------------|---|---|--------------|--------------------------|------------|
|     |                   |        |                            |              |   |   |              |                          |            |
| 1   | FINAL EFFLUENT #1 | liquid | 20L cube                   | 6/13/22 0600 | X | X | wet ice      | 0.5°C                    | P220614.01 |
| 2   |                   |        |                            |              |   |   |              |                          |            |
| 3   |                   |        |                            |              |   |   |              |                          |            |
| 4   |                   |        |                            |              |   |   |              |                          |            |
| 5   |                   |        |                            |              |   |   |              |                          |            |
| 6   |                   |        |                            |              |   |   |              |                          |            |
| 7   |                   |        |                            |              |   |   |              |                          |            |
| 8   |                   |        |                            |              |   |   |              |                          |            |
| 9   |                   |        |                            |              |   |   |              |                          |            |
| 10  |                   |        |                            |              |   |   |              |                          |            |
| 11  |                   |        |                            |              |   |   |              |                          |            |
| 12  |                   |        |                            |              |   |   |              |                          |            |
| 13  |                   |        |                            |              |   |   |              |                          |            |
| 14  |                   |        |                            |              |   |   |              |                          |            |
| 15  |                   |        |                            |              |   |   |              |                          |            |
| 16  |                   |        |                            |              |   |   |              |                          |            |
| 17  |                   |        |                            |              |   |   |              |                          |            |
| 18  |                   |        |                            |              |   |   |              |                          |            |
| 19  |                   |        |                            |              |   |   |              |                          |            |
| 20  |                   |        |                            |              |   |   |              |                          |            |

|   |  |                        |              |   |
|---|--|------------------------|--------------|---|
| Relinquished by:  | Received by:   | Relinquished by:       | Received by: | <b>Matrix Codes</b><br><br>FW = Fresh Water<br><br>SB = Salt & Brackish Water<br><br>SS = Soil & Sediment |
| Print Name: <b>Meghan Feuk</b>  | Print Name: <b>Rian Plastow</b>  | Print Name:            | Print Name:  |   |
| Signature:  | Signature:  | Signature:             | Signature:   |   |
| Affiliation: <b>LOTT CLEAN WATER ALLIANCE</b>   | Affiliation: <b>EcoAnalysts</b>  | Affiliation:           | Affiliation: |   |
| Date/Time: <b>6/13/22 0730</b>  | Date/Time: <b>6/14/22 1210</b>   | Date/Time: <b>6/14</b> | Date/Time:   |   |

# CHAIN OF CUSTODY



EcoAnalysts, Inc.  
4770 NE View Dr., Portland, WA. 98364  
Tel: (360) 297-6040

|                      |  |  |   |
|----------------------|--|--|---|
| Destination:         | Sample Originator (Organization): <i>LOTT Clean Water Alliance</i> | Report Results To: <i>LOTT Clean Water Alliance</i>                                  | Phone: <i>360 528 5760</i>                  |
| Destination Contact: | PERSON WHO COLLECTED SAMPLE: <i>Meghan Feuk</i>                    | Contact Name: <i>Meghan Feuk</i>   | Fax:  |
| Date:                | Address: <i>500 Adams St NE<br/>Olympia, WA 98501</i>              | Address: <i>500 Adams St NE<br/>Olympia, WA 98501</i>                                | Email: <i>meghanfeuk@lottcleanwater.org</i> |
| Turn-Around-Time:    | Phone: <i>360 528 5760</i>   | Analyses:  | Involving To:                               |
| Project Name:        | Fax:   | <i>Chronic Toxicity Survival + Growth</i><br><i>Chronic Myriad Survival + Growth</i> | Comments or Special Instructions:           |
| Contract/PO:         | E-mail: <i>meghanfeuk@lottcleanwater.org</i>                       |  |   |

| No. | Sample ID                | Matrix        | Volume & Type of Container | Date & Time         | <i>Chronic Toxicity Survival + Growth</i> | <i>Chronic Myriad Survival + Growth</i> | Analyses: | Involving To: | Preservation  | Sample Temp Upon Receipt | LAB ID            |
|-----|--------------------------|---------------|----------------------------|---------------------|---|---|-----------|---------------|---------------|--------------------------|-------------------|
| 1   | <i>FINAL EFFLUENT #2</i> | <i>liquid</i> | <i>20L cubi</i>            | <i>6/15/22 0600</i> | <i>X</i>                                  | <i>X</i>                                |           |               | <i>on ice</i> | <i>0.5°C</i>             | <i>P220616.01</i> |
| 2   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 3   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 4   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 5   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 6   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 7   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 8   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 9   |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 10  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 11  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 12  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 13  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 14  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 15  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 16  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 17  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 18  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 19  |                          |               |                            |                     |   |   |           |               |               |                          |                   |
| 20  |                          |               |                            |                     |   |   |           |               |               |                          |                   |

|                                      |                                 |              |              |                  |              |              |              |                            |
|--------------------------------------|---------------------------------|--------------|--------------|------------------|--------------|--------------|--------------|----------------------------|
| Relinquished by:                     |                                 | Received by: |              | Relinquished by: |              | Received by: |              | Matrix Codes               |
| Print Name: <i>Meghan Feuk</i>       | Print Name: <i>Rian Plaston</i> | Print Name:  | Print Name:  | Print Name:      | Print Name:  | Print Name:  | Print Name:  | PW = Fresh Water           |
| Signature: <i>[Signature]</i>        | Signature: <i>[Signature]</i>   | Signature:   | Signature:   | Signature:       | Signature:   | Signature:   | Signature:   | SB = Salt & Brackish Water |
| Affiliation: <i>LOTT Clean Water</i> | Affiliation: <i>EcoAnalysts</i> | Affiliation: | Affiliation: | Affiliation:     | Affiliation: | Affiliation: | Affiliation: | SS = Soil & Sediment       |
| Date/Time: <i>6/15/22 0730</i>       | Date/Time: <i>6/16/22 1205</i>  | Date/Time:   | Date/Time:   | Date/Time:       | Date/Time:   | Date/Time:   | Date/Time:   |                            |

|                      |  |   |   |
|----------------------|--|---|---|
| Destination:         | Sample Originator (Organization): <b>LOTT Clean Water Alliance</b> | Report Results To: <b>LOTT Clean Water Alliance</b>   | Phone: <b>360 528 5760</b>                  |
| Destination Contact: | PERSON WHO COLLECTED SAMPLE: <b>Faith Rasmussen</b>                | Contact Name: <b>Meghan Feek</b>                      | Fax:  |
| Date:                | Address: <b>500 Adams St NE<br/>Olympia WA 98501</b>               | Address: <b>500 Adams St NE<br/>Olympia, WA 98501</b> | Email: <b>meghanfeek@lottcleanwater.org</b> |
| Turn-Around-Time:    | Phone: <b>360 528 5770</b>   | Analyses:   | Invoicing To:                               |
| Project Name:        | Fax:   |   |   |
| Contract/PO:         | E-mail: <b>faithrasmussen@lottcleanwater.org</b>                   |   |   |

| No. | Sample ID         | Matrix | Volume & Type of Container | Date & Time  | Chronic Toxicity | Survival + Growth |  |  |  |  | Preservation | Sample Temp Upon Receipt | LAB ID    |
|-----|-------------------|--------|----------------------------|--------------|------------------|-------------------|--|--|--|--|--------------|--------------------------|-----------|
| 1   | Final Effluent #3 | liquid | 20 Lube                    | 6/17/22 0600 | X                | X                 |  |  |  |  | wet ice      | 0.8                      | P22061801 |
| 2   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 3   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 4   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 5   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 6   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 7   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 8   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 9   |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 10  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 11  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 12  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 13  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 14  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 15  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 16  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 17  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 18  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 19  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |
| 20  |                   |        |                            |              |                  |                   |  |  |  |  |              |                          |           |

| Relinquished by:                              |                                    | Received by: |              | Relinquished by: |              | Received by: |  | Matrix Codes<br>FW = Fresh Water<br>SB = Salt & Brackish Water<br>SS = Soil & Sediment |
|---|------------------------------------|--------------|--------------|------------------|--------------|--------------|--|--|
| Print Name: <b>Faith Rasmussen</b>            | Print Name: <b>Nicole Lundgren</b> | Print Name:  | Print Name:  | Print Name:      | Print Name:  |              |  |  |
| Signature: <i>[Signature]</i>                 | Signature: <i>[Signature]</i>      | Signature:   | Signature:   | Signature:       | Signature:   |              |  |  |
| Affiliation: <b>LOTT Clean Water Alliance</b> | Affiliation: <b>ECO.A.</b>         | Affiliation: | Affiliation: | Affiliation:     | Affiliation: |              |  |  |
| Date/Time: <b>3/17/2022 0745</b>              | Date/Time: <b>6/18/22 1100</b>     | Date/Time:   | Date/Time:   | Date/Time:       | Date/Time:   |              |  |  |

### SAMPLE RECEIPT

|   |                   |                      |                      |
|---|-------------------|----------------------|----------------------|
| Client:   | Client ID:        | Lab ID:              | Renewals:            |
| LOTT Clean Water Alliance                                       | Final Effluent #1 | P220614.01           |                      |
| Project:  | Final Effluent #2 |                      | P220616.01           |
| NPDES   | Final Effluent #3 |                      | P220618.01           |
| Date/Time Received:   |                   | 6/14/22 1210         | 6/16/22 1205         |
| Airbill #:  |                   | 274267814206         | 274378830914         |
| Shipper Tracking Information Kept for Records: (Y/N/NA)         |                   | N                    | N                    |
| Collection Date/Time:   |                   | 6/13/22 0600         | 6/15/22 0600         |
| Sample Holding Time (must be ≤36 hours at test initiation)      |                   | 30h 30h <sup>②</sup> | 30h 30h <sup>②</sup> |
| Condition of Shipping Container:                                |                   | Good                 | Good                 |
| Type and Capacity of Sample Container:                          |                   | 20 L cubi            | 20 L cubi            |
| Total Sample Volume (L):  |                   | ~18 L                | ~18 L                |
| Condition of Sampling Container:                                |                   | Good                 | Good                 |
| Sample Container Appropriate: (Y/N)                             |                   | Y                    | Y                    |
| Custody Seals Intact: (Intact/Broken/Not Present)               |                   | Not present          | Not present          |
| Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N) |                   | Y                    | Y                    |
| Sampler's Name Present on COC Form: (Print Name/Not Present)    |                   | Meghan Feuk          | Meghan Feuk          |
| Color:  |                   | pale yellow          | yellow               |

#### TAKE THE FOLLOWING MEASUREMENTS UPON ARRIVAL

| LAB ID     | Meter # | Temp. (°C) *<br>(0-6°C) | Meter # | Dissolved Oxygen (mg/L) | Meter # | pH  | Meter # | Cond. (µS/cm) | Meter # | Sal. (ppt) | Hardness (mg CaCO <sub>3</sub> /L) | Alkalinity (mg CaCO <sub>3</sub> /L) | Total Chlorine (mg/L) | Total NH <sub>3</sub> (mg/L) | Tech |
|------------|---------|-------------------------|---------|-------------------------|---------|-----|---------|---------------|---------|------------|------------------------------------|--------------------------------------|-----------------------|------------------------------|------|
| P220614.01 | 15      | 0.5                     | 8       | 10.7                    | 8       | 7.2 | 8       | 4103          | 8       | 0.268      | 710                                | 98                                   | 0.02                  | 0.076                        | RP   |
| P220616.01 | 5       | 0.5                     | 8       | 10.6                    | 8       | 7.0 | 8       | 438           | 8       | 0.192      | —                                  | —                                    | 0.01                  | 0.223                        | MS   |
| P220618.01 | 15      | 0.8                     | 9       | 8.8                     | 9       | 7.3 | 9       | 389           | 9       | 0.163      | —                                  | —                                    | 0.04                  | 0.076                        | NL   |

\*Notify project manager or study director of temperatures above 6°C or ≥36 hours holding time. Client must be notified ASAP.

|   |                    |
|---|--------------------|
| If there are sample receipt problems, complete the following: |                    |
| Reason for unacceptability:                                   |                    |
| Name of Client Contact:                                       | Contacted by:      |
| Client Response and/or Action to be Taken:                    | Date Action Taken: |

① preserved - RP 6/14 MS 6/16  
 ② IE - RP 6/16 6/18 RP 6/23  
 ③ ran on 6/23/22 - Page 1 of 2  
 ④ NH<sub>3</sub> = 0.088 mg/l RP 6/23