

## TOXICITY TEST REPORT

## TEST IDENTIFICATION

Test No.: 658-89

Title: Inland silverside, *Menidia beryllina*, 96-hr acute toxicity test using SP-11 final effluent sample. EPA NPDES permit number WAD009248295.

Protocol No.: NAS-XXX-MB1, September 10, 1991, Revision 3 (7-1-12). Based on Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. EPA 821-R-02-012.

## STUDY MANAGEMENT

Study Sponsor: CH2M-Hill Wyckoff Treatment Plant, 5350 Creosote Place NE, Bainbridge Island, WA 98110.

Sponsor's Study Monitor: Mr. Ken Scheffler

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

Test Location: Newport laboratory.

Laboratory's Study Personnel: G.A. Buhler, B.S., Proj. Mgr./Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; G.J. Irissari, B.S., Aq. Toxicol; J. B. Brown, B.S., D.V.M., Assoc. Aq. Toxicol.

Study Schedule:

Test Beginning: 9-12-18, 1450 hrs.

Test Ending: 9-16-18, 1430 hrs.

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

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

## TEST MATERIAL

Description: CH2M Hill-Wyckoff Treatment Plant SP11 Ground Water Sample. Details are as follows:

NAS Sample No.	6239G
Collection Date	9-11-18
Receipt Date	9-12-18
Temperature (°C)	4.9
pH	7.6
Dissolved oxygen (mg/L)	9.2
Salinity (‰)	6.0

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

## DILUTION WATER

Source: Artificial seawater

Date of Preparation: 9-7-18

Water Quality: Salinity, 30.0 ‰; pH 8.3

Pretreatment: Prepared with Tropic Marin® sea salts and MilliQ® deionized water, aerated.

## TEST ORGANISMS

Species: *Menidia beryllina*, inland silversides.

Age: 12 days at test initiation.

Source: Aquatic BioSystems, Inc. Fort Collins, CO.

Acclimation: Fish were received on 9-11-18. The water quality, including receiving water, prior to testing averaged: Temperature, 19.4°C; pH, 7.6; salinity, 29.3 ‰; dissolved oxygen, 10.4 mg/L. During acclimation, silverside larvae were fed *Artemia* nauplii daily and 50% of the holding water was changed daily.

**TEST PROCEDURES AND CONDITIONS**

Test Chambers: 600 mL glass beakers containing 250 mL of test solutions.  
Test Concentrations: 100, 50, 25, 12.5, 6.25, and 0% (control).  
Salt Control: None.  
Replicates/Treatment: 4  
Organisms/Treatment: 40  
Aeration: None  
Feeding: *Artemia* nauplii 2 hrs. prior to test solution renewal at 48 hrs.  
Water Volume Changes: One at 48 hours.  
Effect Criterion: Mortality, defined as the lack of respiratory movement in response to tactile stimulation.  
Water Quality and Other Test Conditions: Temperature, 20.3 ± 0.2°C; pH, 8.1 ± 0.1; salinity, 30.5 ± 0.4 ‰; dissolved oxygen, 7.1 ± 0.3 mg/L; and photoperiod 16:8 hr, L:D.

**DATA ANALYSIS METHODS**

Percent survival was calculated for each treatment replicate from the raw data and the means were obtained for each treatment level. The LC50 was calculated, where data permitted, either by the Probit or the Trimmed Spearman-Kärber method. The statistical software employed for these calculations was CETIS, v.1.8.7.4, Tidepool Scientific Software.

**PROTOCOL DEVIATIONS**

None.

**REFERENCE TOXICANT TEST**

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

Test No.: 999-3810  
Reference Toxicant and Source: Copper as CuSO<sub>4</sub>•5H<sub>2</sub>O, Argent Lot No. 0195, 1.0 mg/mL stock prepared 3-27-18.  
Test Date: 9-12-18  
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.1.  
Results: LC50, 164 µg/L Cu. This result is within the laboratory's control chart warning limits (83.5 – 204).

**TEST RESULTS**

A detailed tabulation of the test results is given in Table 1. In this test, 100% of the organisms exposed to a 100% concentration of the effluent survived the 96-hour period. Survival in the dilution water controls was 100%, which met the test acceptability criteria of ≥ 90%.

NOEC (%)	100
LOEC (%)	>100
96-hr LC50 (%)	>100
(95% C.I.)	--
Method	By Data Inspection

**STUDY APPROVAL**

Greg Bubler 9-24-18  
 Project Manager/Study Director Date

Julie R. Fouse 9-24-18  
 Quality Assurance Unit Date

Richard A. Caldwell 9/25/18  
 Laboratory Director Date

Table 1. Survival of *Menidia beryllina* exposed to SP-11 final effluent sample from Wyckoff Treatment Plant.

Effluent Conc. (%)	Replicate	Number of fish surviving					96-hr % Survival	
		0-hr	24-hr	48-hr	72-hr	96-hr	Individual	Mean
100	1	10	10	10	10	10	100	100
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
50	1	10	10	10	10	10	100	100
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
25	1	10	10	10	10	10	100	100
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
12.5	1	10	10	10	10	10	100	100
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
6.25	1	10	10	10	10	10	100	100
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	
Control	1	10	10	10	10	10	100	100
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	

**APPENDIX I**

**PROTOCOL**

TEST PROTOCOL

**SILVERSIDE (*MENIDIA BERYLLINA*, *M. MENIDIA*, AND *M. PENINSULAE*)  
ACUTE TOXICITY TEST**

1 INTRODUCTION

- 1.1 Purpose of Study: The purpose of this test is to measure the acute toxicity of effluents and/or receiving waters using the silverside, *Menidia beryllina* (or *M. menidia*, *M. peninsulae*). With certain modifications this method is also applicable to other uses such as TIE testing, product testing and registration, control charting, etc.
- 1.2 Referenced Method: This protocol is based primarily on the U.S. EPA acute toxicity manual (EPA-821-R-02-012). Amendments may be incorporated to meet other methods or regulatory requirements as needed.
- 1.3 Summary of Method: Larval silverside (9-14 days old) are exposed for 24, 48, or 96-hr to different concentrations of effluent, receiving water, or a reference toxicant. The test may be static non-renewal, static renewal, or flow-through. The test chambers are 250 mL or larger beakers, each containing 200 mL or more of test solution. Two replicate beakers (four for the receiving water test), each with 10 organisms, are employed at each test concentration. A standard experimental design is employed consisting of exposure of the test animals to a minimum of five concentrations and a dilution water control in the definitive test, with a minimum 0.5 dilution series (100% and control in the single-concentration test). Mortality is the effect criterion. The data analysis normally consists of calculation of the LC50 and 95% confidence intervals and/or NOEC in the definitive test (pass/fail in a single concentration test). A test summary table is appended to the end of this protocol.

2 STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

2.2 Sponsor's Study Monitor:

\_\_\_\_\_

2.3 Name of Testing Laboratory:

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

2.4 Test Location: \_\_\_\_\_

2.5 Laboratory's Personnel to be Assigned to the Study:

Study Director: \_\_\_\_\_  
Qual. Assurance Unit: \_\_\_\_\_  
Aquatic Toxicologist: \_\_\_\_\_  
Aquatic Toxicologist: \_\_\_\_\_

2.6 Proposed Study Schedule: Effluent/receiving water tests must begin within 36 hours of the end of the sample collection period. In no case should an effluent test be started more than 72 hours after the sample collection. Holding times for other materials depend upon the material and the project design.

2.7 Quality Assurance: The test data are reviewed by the Quality Assurance Unit to assure that the studies are performed in accordance with the protocol and standard operating procedures and that reports accurately

reflect the raw data. Studies are conducted in a manner consistent with the general principles of GLP methods.

### 3 TEST MATERIAL

Test materials can include effluents, reference toxicants, receiving waters, sediment porewaters, formulated chemicals, etc. Samples are stored with minimum headspace at 0-6°C in the dark until used. For use in NPDES program testing, the lapsed time from sample collection to first use must not exceed 36 h. In static-renewal tests samples may be used up to 72 h after first use if stored as above.

### 4 DILUTION WATER

The choice of dilution water depends on test requirements. Clean natural filtered seawater is preferred. A salinity range of 1-32‰ ± 10% is recommended for *M. beryllina* (15-32‰ ± 10% for *M. menidia* and *M. peninsulae*). Artificial sea salts, hypersaline brine, and/or deionized Milli-Q water may be added to natural seawater or effluent/receiving water as needed for salinity adjustment. Modified GP2 or Tropic Marin® (or equivalent) artificial seawater may also be used.

### 5 TEST ORGANISMS

5.1 Species: Inland silverside, *Menidia beryllina*; or other silversides: *M. menidia* and *M. peninsulae*.

5.2 Source: The animals are purchased from commercial suppliers.

5.3 Age at Study Initiation: 9-14 days; ≤24-hr range in age.

5.4 Acclimation and Pretest Observation: Test organisms should be held in the laboratory in well aerated dilution water in order to acclimate to test conditions, if necessary. Pretest mortality should not exceed 10% per day prior to testing. Water quality should be monitored and recorded daily during acclimation.

### 6 DESCRIPTION OF TEST SYSTEM

6.1 Preparation of Test Concentrations: Test concentrations are prepared by manual dilution of test material with dilution water. The solution is made in excess and appropriate test volume aliquots are transferred into the test chambers. Prior to mixing, test material and dilution water are brought to test temperature and only aerated if necessary.

6.2 Test Chambers and Environmental Control: Test chambers are 250 mL or larger beakers holding 200 mL or more of test solution. Test chambers are maintained at constant temperature by partial immersion in a temperature-controlled water bath or by holding in a constant temperature room. Aeration is not employed unless dissolved oxygen falls below 4.0 mg/L. If aerated, rate should not exceed 100 bubbles/minute. Effluents/receiving waters are aerated prior to testing if necessary. Photoperiod control of test chambers is provided.

6.3 Cleaning: All laboratory glassware, including test chambers, is cleaned based on the method described in EPA-821-R-02-012. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed three times with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed three times with tap water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with tap water, then once with deionized water. Test systems and chambers are rinsed again with dilution water just before use.

### 7 EXPERIMENTAL DESIGN AND TEST PROCEDURES

7.1 Experimental Design: The test involves exposure of fish to five or more test concentrations (≤0.5 dilution series) and a dilution water control (or 100% and control for the single concentration receiving water test).

Exposures are for 24, 48, or 96 hours. Each treatment consists of two replicate test containers (four for the single-concentration test), each containing 10 fish. A stratified random design is used for the placement of beakers in the test area. Test organisms are impartially distributed to the test chambers by adding one or two animals to each chamber and repeating the process until each contains 10 organisms.

- 7.2 Effect Criterion: The effect criterion used in the silverside acute test is mortality, defined as the lack of body movement in response to tactile stimulation.
- 7.3 Test Conditions: The dissolved oxygen concentration in each test container must be greater than 4.0 mg/L throughout the test. The test temperature employed is  $20 \pm 1^\circ\text{C}$  or  $25 \pm 1^\circ\text{C}$ . The salinity should be in the range of  $1-32\text{‰} \pm 10\%$  (*M. beryllina*). The photoperiod is 16 hours of light and 8 hours of darkness. Illumination is supplied by daylight fluorescent lamps at an intensity of 50-100 ft candles. If the test is a 96-hr test, the test solutions must be renewed at 48 hours.
- 7.4 Preparation of Test Concentrations: The procedure will depend on factors including the salinity of the test material, the test salinity desired and the procedures selected for salinity adjustment.
- 7.5 Beginning of Test: The test is begun by adding the organisms to the equilibrated test containers as previously described.
- 7.6 Feeding: *Artemia* nauplii are made available while holding prior to the test. During a 96-hr test, 0.1 mL *Artemia* nauplii concentrate per beaker is provided 2 hours prior to test solution renewal at 48 hours.
- 7.7 Test Duration, Type and Frequency of Observations, and Methods: The test duration of the acute toxicity test is 24, 48 or 96-hours. The type and frequency of observations to be made during the test are summarized as follows:

TYPE OF OBSERVATION	TIMES OF OBSERVATION
<b>BIOLOGICAL DATA</b>	
Survival (in each test container).	Daily.
<b>PHYSICAL AND CHEMICAL DATA</b>	
Dissolved oxygen, pH, temperature, & salinity (in one replicate of each test level and the control).	Daily.
Total ammonia-N (in sample where toxicity may be contributed by unionized ammonia; i.e., total ammonia $\geq 5$ mg/L)	Prior to use in test (optional as required).

During the test, dead organisms are removed at least every 24 hours. Dissolved oxygen is directly measured in test beakers using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. The pH and temperature are measured directly in the test beakers by careful use of a combined pH/temperature probe and a properly calibrated meter with scale divisions of 0.1 pH units. Salinity is measured with a refractometer.

- 7.8 Criterion of Test Acceptance: The test results are acceptable if survival in the controls is at least 90%.

## 8 DATA ANALYSIS

Ordinarily, the following data analysis is performed. Due to special requirements, alternative methods may be used. Percent survival is calculated for each treatment replicate from the raw data and the means are obtained for each treatment level. For multi-concentration tests, the LC50 and/or NOEC are calculated. The LC50 is calculated using Maximum-Likelihood Probit, Spearman-Kärber, or Trimmed Spearman-Kärber (EPA-821-R-02-012, p 73). The NOEC is calculated according to the EPA flowchart (EPA-821-R-02-012, p 87). In the single concentration test, a pass/fail analysis is performed according to the EPA flowchart (EPA-821-R-02-012, p 86). An arcsine transformation is performed on survival data prior to

analysis. The statistical software employed for these calculations is either CETIS or ToxCalc, both by Tidepool Scientific Software. Some agencies require that toxic units (TU) be reported. The toxic unit acute (TU<sub>a</sub>) is 100/LC50.

9 REPORTING

The final report of the test results includes the following standard information at a minimum: name and identification of the test including a reference to the test protocol or method; the client and client's study monitor; the investigator and laboratory; information on the test material; information on the dilution water; detailed information about the test organisms including acclimation conditions; a description of the experimental design and test chambers and other test conditions including water quality; information about any aeration that may have been required; definition of the effect criteria and other observations; responses in the control treatments; tabulation and statistical analysis of measured responses; a description of the statistical methods used; any unusual information about the test or deviations from procedures; reference toxicant testing information. In order to provide for independent outside QA evaluation, the final report should also include appended raw data records including A) a copy of the test protocol or other appropriate method description; B) copies of all of the test raw data including test bench sheets, data analysis printouts, and chain-of-custody records, and C) copies of all similar raw data pertaining to the reference toxicant test including the current control chart. The final report should also contain the approval signatures of the Study Director, Project Manager, QA Unit, and Laboratory Director.

10 STUDY DESIGN ALTERATION

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

11 REFERENCE TOXICANT

Reference toxicant (positive control) testing should be included with each study or at regular intervals as defined in the Quality Assurance Program of the laboratory.

12 REFERENCES AND GUIDELINES

Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. October 2002, Fifth Edition. EPA 821-R-02-012.

13 APPROVALS

\_\_\_\_\_ for \_\_\_\_\_  
Name Date Client

\_\_\_\_\_ for NORTHWESTERN AQUATIC SCIENCES  
Name Date



Appendix A  
Test Conditions Summary

1. Test type	Static non-renewal, static renewal, or flow-through.
2. Test duration	24, 48, or 96 hrs.
3. Temperature	20 ± 1°C; or 25 ± 1°C (recommended). Temperature maximum deviation of 3°C during test (required).
4. Light quality	Ambient laboratory illumination (recommended).
5. Light intensity	50-100 footcandles (recommended).
6. Photoperiod	16 hr light, 8 hr dark (recommended).
7. Test chamber size	250 mL (recommended minimum).
8. Test solution volume	200 mL (recommended minimum).
9. Renewal of test solutions	After 48 hrs (required minimum). Alternatives may be required.
10. Age of test organisms	9-14 days; ≤24-h age range (required).
11. No. organisms per test chamber	10 for effluent and receiving water tests (required minimum).
12. No. replicate chambers per concentration	2 for effluent tests; 4 for receiving water tests (required minimums).
13. No. organisms per concentration	20 for effluent tests; 40 for receiving water tests (required minimums).
14. Feeding regime	<i>Artemia nauplii</i> are made available while holding prior to the test; add 0.1 mL <i>Artemia</i> concentrate 2 hr prior to renewal at 48 hr.
15. Test chamber cleaning	Cleaning not required.
16. Test chamber aeration	None, unless DO concentration falls below 4.0 mg/L; rate should not exceed 100 bubbles/min (recommended).
17. Dilution water	Uncontaminated natural seawater, filtered to ≤0.45µm, adjusted with hypersaline brine and/or Milli-Q® deionized water; or receiving water: 1-32‰ ± 10% for <i>M. beryllina</i> ; 15-32‰ ± 10% for other <i>Menidia</i> sp.
18. Test concentrations	Effluents: minimum of five concentrations and a control. Receiving waters: 100% receiving water and a control.
19. Dilution series	Effluents: ≥0.5 dilution series (recommended). Receiving waters: none or ≥0.5 dilution series (recommended).
20. Endpoint	Mortality (required).
21. Sampling and sample holding requirements	Samples are first used within 36 hr of completion of sampling period (required for effluents and recommended for receiving waters).
22. Sample volumes required	1 L (recommended); 2 L for receiving waters (recommended).
23. Test acceptability criterion	≥ 90% survival in controls (required).
24. Reference toxicant	Run concurrently.

## **APPENDIX II**

### **RAW DATA**

Test No. 658-89 Client: CH2M-Wyckoff Investigator Revised PAT-10 65  
 Test Type (ranging/definitive) Test Length (hr) 96  
 Species Meridia beryllina

**STUDY MANAGEMENT**

Client: CH2M-Wyckoff Treatment Plant, 5350 Creosote Place NE, Bainbridge Island, WA 98110  
 Client's Study Monitor: Mr. Stanley Warner Mr. Ken Schaffner  
 Testing Laboratory: Northwestern Aquatic Sciences  
 Test Location: Newport Laboratory  
 Laboratory's Study Personnel:  
 Proj. Mgr./Study Dir. GA Buhler 03  
 QA Officer L.K. Nemeth  
 1. G.J. KRISARRI 632 2. J. Brown 83  
 3. 4.  
 Study Schedule:  
 Test Beginning: 9-12-78 1450 Test Ending: 9-16-78 1430

**TEST MATERIAL**

Description: Ground water composite SP-11  
 NAS Sample No. 62396  
 Date of Collection: 9-11-78  
 Date of Receipt: 9-12-78  
 Temperature (deg C): 4.9  
 Dissolved oxygen (mg/L): 9.2  
 pH: 7.6  
 Conductivity (umhos/cm): -  
 Hardness (mg/L): -  
 Alkalinity (mg/L): -  
 Salinity (ppt): 6.0

**DILUTION WATER**

Description: Artificial seawater  
 Date of Preparation/Collection: 9-7-78  
 Water Quality: Cond. (umhos/cm): N/A Salinity (ppt) 30.0 pH 8.3  
 Hardness (mg/L as CaCO<sub>3</sub>): N/A Alkalinity (mg/L as CaCO<sub>3</sub>): N/A  
 Treatments: Prepared with Tropic Marin sea salts and MilliQ deionized water, aerated.

**TEST LOCATION**

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

**Randomization chart:**

D	100	25	12.5	∅	625	50			
C	50	6.25	100	12.5	25	∅			
B	∅	50	25	625	12.5	100			
A	∅	12.5	625	100	50	25			

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

Test No. 658-89 Client CH2M-Wyckoff Investigator \_\_\_\_\_

**TEST ORGANISMS**

Species: Menidia beryllina Age: 12 days Size: \_\_\_\_\_  
Source: Aquatic Biosystems, PT Collins, CO Date received: 9-11-18

**Acclimation Data:**

Date	Temp. (deg.C)	pH	salinity (ppt)	DO (mg/L)	Feeding		Water changes	Comments
					amount	description		
9-11-18	18.7	7.4	28.0	13.9	2/amt	Artemia	yes	Rec'd date
9-12-18	20.1	7.8	30.5	16.8	"	"	"	
Mean	19.4	7.6	29.3	15.4				
S.D.								
(N)	(2)	(2)	(2)	(2)				

Photoperiod during acclimation: 16:8 L:D

**TEST PROCEDURES AND CONDITIONS**

Test concentrations (50% series recommended): 100, 50, 25, 12.5, 6.25 & 0%

Test chamber: 600 mL glass beakers Test volume: 250 mL  
Replicates/treatment: 4 Organisms/treatment: 40 (10/repl)  
Test water changes: Yes@48 hours Aeration during test: None  
Feeding: Yes@48 hours ~2 hours prior to test change

Duration: 24-hr, 48-hr, 96-hr Test temperature (deg.C): 20 +/- 1  
Beaker placement: Stratified randomization Photoperiod: 16:8 L:D

**MISCELLANEOUS NOTES**

Test Concentration Preparation:

Test Concentration (%)	Volume of effluent* (mL)	Volume of Dilution water (mL)
100	1,000	0
50	500	500
25	250	750
12.5	125	875
6.25	62.5	937.5
0	0	1,000

\*Effluent salinity adjusted to 30 ppt with Tropic Marin® Sea salts.

NORTHWESTERN AQUATIC SCIENCES  
ACUTE TOXICITY TEST (ALL SPECIES)

PROTOCOL NO. NAS-XXX-MB1

Test No. 658-89 Client CH2M-Wyckoff Investigator \_\_\_\_\_

DAILY RECORD SHEET

Day 0 (9/12/18) AB

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 100	20.8	8.0	30.0	7.6	10	10	10	10
2. 50	20.8	8.0	30.0	7.7	10	10	10	10
3. 25	20.6	8.0	30.0	7.7	10	10	10	10
4. 12.5	20.6	8.1	30.0	7.7	10	10	10	10
5. 6.25	20.5	8.1	30.0	7.7	10	10	10	10
6. 0	20.5	8.2	30.0	7.6	10	10	10	10

Day 1 (9/13/18) AB

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 100	20.3	8.2	30.5	7.2	10	10	10	10
2. 50	20.3	8.1	30.0	7.0	10	10	10	10
3. 25	20.3	8.1	30.5	7.2	10	10	10	10
4. 12.5	20.3	8.0	30.5	7.1	10	10	10	10
5. 6.25	20.3	8.0	30.0	7.2	10	10	10	10
6. 0	20.4	8.0	30.5	7.3	10	10	10	10

Day 2 (9/14/18) AB

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 100	20.1	8.4	31.0	6.9	10	10	10	10
2. 50	20.1	8.3	30.5	6.9	10	10	10	10
3. 25	20.2	8.2	31.0	7.0	10	10	10	10
4. 12.5	20.0	8.1	31.0	6.7	10	10	10	10
5. 6.25	20.1	8.1	31.0	6.8	10	10	10	10
6. 0	20.0	8.0	30.5	6.9	10	10	10	10

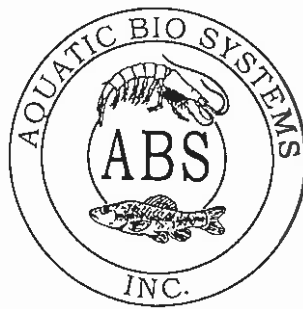
Day 3 (9/15/18) AB

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 100	20.2	8.3	30.5	6.9	10	10	10	10
2. 50	20.1	8.2	30.5	7.1	10	10	10	10
3. 25	20.1	8.1	30.5	7.0	10	10	10	10
4. 12.5	20.1	8.1	31.0	6.9	10	10	10	10
5. 6.25	20.2	8.1	30.5	6.8	10	10	10	10
6. 0	20.2	8.0	31.0	7.0	10	10	10	10

Day 4 (9/16/18) AB

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (ppm)	Survivors			
					A	B	C	D
1. 100	20.2	8.4	31.0	6.8	10	10	10	10
2. 50	20.3	8.3	30.5	6.8	10	10	10	10
3. 25	20.3	8.2	30.5	6.8	10	10	10	10
4. 12.5	20.2	8.1	30.5	6.7	10	10	10	10
5. 6.25	20.3	8.1	30.5	6.9	10	10	10	10
6. 0	20.2	8.0	31.0	7.0	10	10	10	10

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



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

### ORGANISM HISTORY

DATE: 9/10/2018  
SPECIES: Menidia beryllina  
AGE: 10 day  
LIFE STAGE: Juvenile  
HATCH DATE: 8/31/2018  
BEGAN FEEDING: Immediately  
FOOD: Rotifers, Artemia sp.

*Feed  
9-11-18*

### Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>26 °C</u>	<u>23-26 °C</u>
SALINITY/CONDUCTIVITY:	<u>25 ppt**</u>	<u>23-26 ppt</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>--</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>165 mg/l</u>	<u>160-210 mg/l</u>
pH:	<u>8.10</u>	<u>7.87-8.25</u>

### Comments:

\*\* Acclimated to 28 ppt on 9/10/18.

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

*4510*

**CETIS Analytical Report**

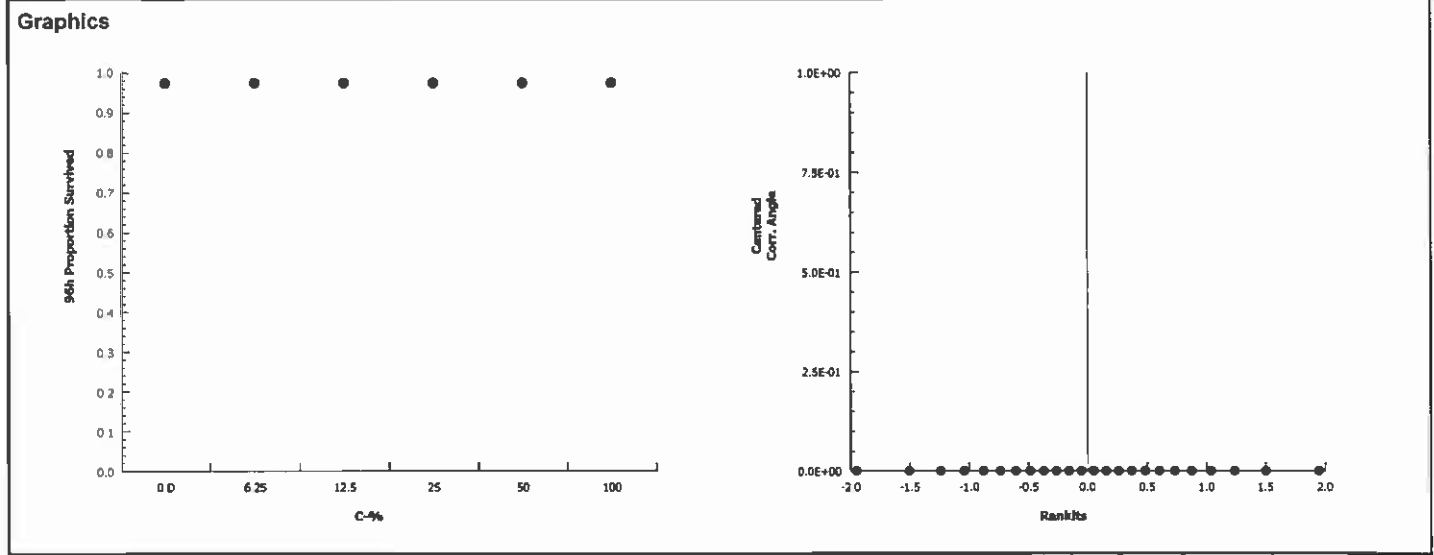
Report Date: 21 Sep-18 09:11 (p 1 of 2)  
 Test Code: 658-89 | 08-1507-3595

Inland Silverside 96-h Acute Survival Test						Northwestern Aquatic Sciences					
Analysis ID:	15-6735-7282	Endpoint:	96h Proportion Survived	CETIS Version:	CETISv1.8.7	Analyst:					
Analyzed:	21 Sep-18 9:11	Analysis:	Nonparametric-Control vs Treatments	Official Results:	Yes						
Batch ID:	07-3825-6946	Test Type:	Survival (96h)	Diluent:	Reconstituted Water						
Start Date:	12 Sep-18 14:50	Protocol:	EPA/821/R-02-012 (2002)	Brine:	Tropic Marin						
Ending Date:	16 Sep-18 14:30	Species:	Menidia beryllina	Age:							
Duration:	96h	Source:	Aquatic Indicators, FL								
Sample ID:	14-4240-5520	Code:	55F95C90	Client:	Wyckoff Treatment Plant						
Sample Date:	11 Sep-18 09:20	Material:	Industrial Effluent	Project:	WET Quarterly Compliance Test (3Q)						
Receive Date:	12 Sep-18 14:30	Source:	Wyckoff								
Sample Age:	29h	Station:									
Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU			
Angular (Corrected)	NA	C > T	NA	NA	100	>100	NA	1			
<b>Steel Many-One Rank Sum Test</b>											
Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)		
Dilution Water		6.25	18	10	1	6	0.8333	Asymp	Non-Significant Effect		
		12.5	18	10	1	6	0.8333	Asymp	Non-Significant Effect		
		25	18	10	1	6	0.8333	Asymp	Non-Significant Effect		
		50	18	10	1	6	0.8333	Asymp	Non-Significant Effect		
		100	18	10	1	6	0.8333	Asymp	Non-Significant Effect		
<b>ANOVA Table</b>											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0	0	5	65540	<0.0001	Significant Effect					
Error	0	0	18								
Total	0		23								
<b>96h Proportion Survived Summary</b>											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1	1	1	1	1	1	0	0.0%	0.0%
6.25		4	1	1	1	1	1	1	0	0.0%	0.0%
12.5		4	1	1	1	1	1	1	0	0.0%	0.0%
25		4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	0.0%
<b>Angular (Corrected) Transformed Summary</b>											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
6.25		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
12.5		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
25		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
50		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
100		4	1.412	1.412	1.412	1.412	1.412	1.412	0	0.0%	0.0%
<b>96h Proportion Survived Detail</b>											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	1	1	1	1						
6.25		1	1	1	1						
12.5		1	1	1	1						
25		1	1	1	1						
50		1	1	1	1						
100		1	1	1	1						

Inland Silverside 96-h Acute Survival Test		Northwestern Aquatic Sciences	
Analysis ID: 15-6735-7282	Endpoint: 96h Proportion Survived	CETIS Version: CETISv1.8.7	
Analyzed: 21 Sep-18 9:11	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes	

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
6.25		1.412	1.412	1.412	1.412
12.5		1.412	1.412	1.412	1.412
25		1.412	1.412	1.412	1.412
50		1.412	1.412	1.412	1.412
100		1.412	1.412	1.412	1.412



LLSD > 100% log data inspection.

9-21-18 JS



**CETIS Test Data Worksheet**

Report Date: 21 Sep-18 09:11 (p 1 of 1)  
 Test Code: 08-1507-3595/658-89

<b>Inland Silverside 96-h Acute Survival Test</b>				<b>Northwestern Aquatic Sciences</b>			
<b>Start Date:</b>	12 Sep-18 14:50	<b>Species:</b>	Menidia beryllina	<b>Sample Code:</b>	55F95C90		
<b>End Date:</b>	16 Sep-18 14:30	<b>Protocol:</b>	EPA/821/R-02-012 (2002)	<b>Sample Source:</b>	Wyckoff		
<b>Sample Date:</b>	11 Sep-18 09:20	<b>Material:</b>	Industrial Effluent	<b>Sample Station:</b>			

C-%	Code	Rep	Pos	# Exposed	24h Survival	48h Survival	72h Survival	96h Survival	Notes
0	D	1	4	10				10	
0	D	2	23	10				10	
0	D	3	2	10				10	
0	D	4	18	10				10	
6.25		1	21	10				10	
6.25		2	6	10				10	
6.25		3	11	10				10	
6.25		4	20	10				10	
12.5		1	14	10				10	
12.5		2	16	10				10	
12.5		3	15	10				10	
12.5		4	1	10				10	
25		1	3	10				10	
25		2	9	10				10	
25		3	5	10				10	
25		4	13	10				10	
50		1	24	10				10	
50		2	19	10				10	
50		3	17	10				10	
50		4	22	10				10	
100		1	12	10				10	
100		2	10	10				10	
100		3	8	10				10	
100		4	7	10				10	

*data entry verified against laboratory bench sheets 9-24-18 JLF*

Water Quality Data - test #658-89, Menidia acute test					
Day	Concentration	Temperature	pH	Salinity	DO
0	100	20.8	8.0	30.0	7.6
0	50	20.8	8.0	30.0	7.7
0	25	20.6	8.0	30.0	7.7
0	12.2	20.6	8.1	30.0	7.7
0	6.25	20.5	8.1	30.0	7.7
0	0	20.5	8.2	30.0	7.6
1	100	20.3	8.2	30.5	7.2
1	50	20.3	8.1	30.0	7.0
1	25	20.3	8.1	30.5	7.2
1	12.5	20.3	8.0	30.5	7.1
1	6.25	20.3	8.0	30.0	7.2
1	0	20.4	8.0	30.5	7.3
2	100	20.1	8.4	31.0	6.9
2	50	20.1	8.3	30.5	6.9
2	25	20.2	8.2	31.0	7.0
2	12.5	20.0	8.1	31.0	6.7
2	6.25	20.1	8.1	31.0	6.8
2	0	20.0	8.0	30.5	6.9
3	100	20.2	8.3	30.5	6.9
3	50	20.1	8.2	30.5	7.1
3	25	20.1	8.1	30.5	7.0
3	12.5	20.1	8.1	31.0	6.9
3	6.25	20.2	8.1	30.5	6.8
3	0	20.2	8.0	31.0	7.0
4	100	20.2	8.4	31.0	6.8
4	50	20.3	8.3	30.5	6.8
4	25	20.3	8.2	30.5	6.8
4	12.5	20.2	8.1	30.5	6.7
4	6.25	20.3	8.1	30.5	6.9
4	0	20.2	8.0	31.0	7.0
	MEAN	20.3	8.1	30.5	7.1
	SD	0.2	0.1	0.4	0.3
	N	30	30	30	30
	MIN	20.0	8.0	30.0	6.7
	MAX	20.8	8.4	31.0	7.7

Data entry verified against laboratory bench sheets

11-25-18 JRC

Northwestern Aquatic Sciences (REGION COPY)

CHAIN OF CUSTODY RECORD

No: 10-091118-095627-0301

Date Shipped: 9/11/2018

Wyckoff Eagle Harbor GWTP 2018/WA

2018T10P303DD210W2LA00

Carrier Name: FedEx

Project Code: WEH-025T

Contact Name: Keith Allers

Airbill No: 7827 2950 7782

Cooler # 1 of 1

Contact Phone: 206-780-1711

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
658 3rd Quarter 2018		Ground Water/ K Allers	Composite	ACTOX-CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	09/11/2018 09:20	Field Sample

9510

Shipment for Case Complete? N  
Samples Transferred From Chain of Custody #

Special Instructions

Analysis Key: ACTOX-CHRTOX=Acute Toxicity, Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	<i>Keith Allers</i> CHZM	9-11-2018 1600	<i>Eugene Sullivan</i> MMS	9-12-18 1430	<i>Intact</i>
			MMS # 62396		



**APPENDIX III**

**RAW DATA – REFERENCE TOXICANT TEST**

Test No. 999-3810 Client: QC Test Investigator Review PPTB  
 Test Type (range-finding/definitive) \_\_\_\_\_ Test Length (hr) 48  
 Species Menidia beryllina (Inland silverside)

**STUDY MANAGEMENT**

Client: QC Test  
 Client's Study Monitor: N/A  
 Testing Laboratory: Northwestern Aquatic Sciences  
 Test Location: Newport Laboratory  
 Laboratory's Study Personnel:  
 Proj. Mgr./Study Dir. GA Bullock (AS)  
 QA Officer L.K. Nemeth  
 1. J. Brown 2. \_\_\_\_\_  
 3. \_\_\_\_\_ 4. \_\_\_\_\_  
 Study Schedule:  
 Test Beginning: 9-12-18 1205 Test Ending: 9-14-18 1242

**TEST MATERIAL**

Description: Copper as: CuSO<sub>4</sub>-5H<sub>2</sub>O Argent Reagent lot #0195  
 1.0 mg/mL stock prep: 3-27-18  
 NAS Sample No. \_\_\_\_\_  
 Date of Collection: \_\_\_\_\_  
 Date of Receipt: \_\_\_\_\_  
 Temperature (deg C): \_\_\_\_\_  
 Dissolved oxygen (mg/L): \_\_\_\_\_  
 pH: \_\_\_\_\_  
 Conductivity (umhos/cm): \_\_\_\_\_  
 Hardness (mg/L): \_\_\_\_\_  
 Alkalinity (mg/L): \_\_\_\_\_  
 Salinity (ppt): \_\_\_\_\_  
 Total chlorine (mg/L): \_\_\_\_\_  
 Total ammonia-N (mg/L): \_\_\_\_\_

**DILUTION WATER**

Description: Yaquina Bay Seawater  
 Date of Preparation/Collection: 9-7-18  
 Water Quality: Cond. (umhos/cm) N/A Salinity (ppt) 30.0 pH 8.1  
 Hardness (mg/L as CaCO<sub>3</sub>): N/A Alkalinity (mg/L as CaCO<sub>3</sub>): N/A  
 Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q® deionized water.

**TEST LOCATION**

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

**Randomization chart:**

B	100	10	300	1000	30	Ø			
A	Ø	100	30	300	1000	10			

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

Test No. 999-3810 Client \_\_\_\_\_ QC Test \_\_\_\_\_ Investigator \_\_\_\_\_

**TEST ORGANISMS**

Species: Menidia beryllina Age: 12 days Size: \_\_\_\_\_  
Source: Aquatic BioSystems, Ft Collins, CO Date received: 9-11-18

**Acclimation Data:**

Date	Temp. (deg.C)	pH	salinity (ppt)	DO (mg/L)	Feeding		Water changes	Comments
					amount	description		
9-11-18	18.7	7.4	28.0	13.9	2 1/2 ml	Artemia	403	Rec'd date
9-12-18	20.1	7.8	30.5	6.8	"	"	"	
Mean	19.4	7.6	29.3	10.4				
S.D.	-	-	-	-				
(N)	(2)	(2)	(2)	(2)				

Photoperiod during acclimation: 16:8 L:D

**TEST PROCEDURES AND CONDITIONS**

Test concentrations (50% series recommended): 1,000, 300, 100, 30, 10 & 0 ug/L

Test chamber: 600 mL beakers Test volume: 250 mL

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

Test water changes: None Aeration during test: None

Feeding: ~2 hrs prior to test initiation

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

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

**MISCELLANEOUS NOTES**

Test solution perpetration:

Test Concentration (ug/L)	Volume of W.S.* (mL)	Volume of Dilution water (mL)
1,000	5.0	Appropriate amount of W.S. added to a graduated cylinder then brought up to volume (500mL) with dilution water.
300	1.5	
100	0.5	
30	0.15	
10	0.05	
0	0	

*GRB 9-12-18*

\*Working stock (W.S.) made by 5:45 (5mL ↑ 50 mL) dilution of concentrated (1.0 mg/mL) Cu stock with Milli-Q DI water. Final concentration: 100 ug/mL Cu.

NORTHWESTERN AQUATIC SCIENCES  
ACUTE TOXICITY TEST (ALL SPECIES)

PROTOCOL NO. NAS-XXX-MB1

Test No. 999-3810 Client QC Test Investigator \_\_\_\_\_

DAILY RECORD SHEET

Day 0 (9/12/18)

Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Survivors	
					A	B
1. 1,000	20.1	8.0	30.5	7.6	10	10
2. 300	20.1	8.0	30.5	7.6	10	10
3. 100	20.3	8.0	30.5	7.7	10	10
4. 30	20.2	8.0	30.5	7.7	10	10
5. 10	20.2	8.0	30.5	7.6	10	10
6. 0	20.3	8.0	30.5	7.7	10	10

Day 1 (9/13/18)

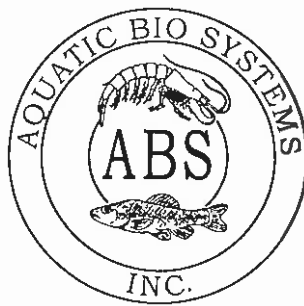
Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Survivors	
					A	B
1. 1,000	20.2	8.0	30.5	7.2	0(100)	0(100)
2. 300	20.3	8.0	31.0	7.4	1(90)	0(100)
3. 100	20.3	8.0	30.5	7.6	10	10
4. 30	20.3	8.0	30.5	7.6	10	10
5. 10	20.2	8.0	30.5	7.6	10	10
6. 0	20.3	8.0	30.5	7.5	10	10

Day 2 (9/14/18) (A3)

Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Survivors	
					A	B
1. 1,000	—	—	—	—	0	0
2. 300	20.0	8.0	31.5	6.6	0(10)	0
3. 100	20.1	8.0	31.0	6.9	9(10)	10
4. 30	20.0	8.0	31.5	6.8	10	10
5. 10	20.1	8.0	31.0	6.8	10	10
6. 0	20.2	8.0	31.0	6.9	10	10



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Tel: 970/484-5091 Fax: 970/484-2514

### ORGANISM HISTORY

DATE: 9/10/2018

SPECIES: Menidia beryllina

AGE: 10 day

LIFE STAGE: Juvenile

HATCH DATE: 8/31/2018

BEGAN FEEDING: Immediately

FOOD: Rotifers, Artemia sp.

*Recd  
9-11-18*

### Water Chemistry Record:

	Current	Range
TEMPERATURE:	<u>26 °C</u>	<u>23-26 °C</u>
SALINITY/CONDUCTIVITY:	<u>25 ppt**</u>	<u>23-26 ppt</u>
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	<u>--</u>	<u>--</u>
TOTAL ALKALINITY (as CaCO <sub>3</sub> ):	<u>165 mg/l</u>	<u>160-210 mg/l</u>
pH:	<u>8.10</u>	<u>7.87-8.25</u>

### Comments:

\*\* Acclimated to 28 ppt on 9/10/18.

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

**CETIS Summary Report**

Report Date: 21 Sep-18 08:59 (p 1 of 1)  
 Test Code: 999-3810 | 10-4067-5467

Reference Toxicant 48-h Acute Survival Test						Northwestern Aquatic Sciences																		
Batch ID:	08-8793-3656	Test Type:	Survival (48h)	Analyst:		Start Date:	12 Sep-18 12:05	Protocol:	EPA7821/R-02-012 (2002)	Diluent:	Yaquina Bay Seawater	Ending Date:	14 Sep-18 12:42	Species:	Menidia beryllina	Brine:		Duration:	49h	Source:	Aquatic Indicators, FL	Age:		
Sample ID:	06-5399-6487	Code:	26FB31C7	Client:	Internal Lab	Sample Date:	12 Sep-18 12:05	Material:	Copper sulfate	Project:		Receive Date:	12 Sep-18 12:05	Source:	Reference Toxicant	Sample Age:	NA	Station:						
Comparison Summary																								
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method																	
19-3004-9531	48h Proportion Survived	100	300	173.2	NA		Dunn/Bonferroni Test																	
Point Estimate Summary																								
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method																	
06-2711-2603	48h Proportion Survived	EC50	163.5	146.2	182.9		Spearman-Kärber																	
48h Proportion Survived Summary																								
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect													
0	Dilution Water	2	1	1	1	1	1	0	0	0.0%	0.0%													
10		2	1	1	1	1	1	0	0	0.0%	0.0%													
30		2	1	1	1	1	1	0	0	0.0%	0.0%													
100		2	0.95	0.3147	1	0.9	1	0.05	0.07071	7.44%	5.0%													
300		2	0	0	0	0	0	0	0		100.0%													
1000		2	0	0	0	0	0	0	0		100.0%													
48h Proportion Survived Detail																								
C-µg/L	Control Type	Rep 1	Rep 2																					
0	Dilution Water	1	1																					
10		1	1																					
30		1	1																					
100		0.9	1																					
300		0	0																					
1000		0	0																					

**CETIS Test Data Worksheet**

Report Date: 21 Sep-18 08:58 (p. 1 of 1)  
 Test Code: 10-4067-5467/999-3810

<b>Reference Toxicant 48-h Acute Survival Test</b>				<b>Northwestern Aquatic Sciences</b>			
Start Date:	12 Sep-18 12:05	Species:	Menidia beryllina	Sample Code:	26FB31C7		
End Date:	14 Sep-18 12:42	Protocol:	EPA/821/R-02-012 (2002)	Sample Source:	Reference Toxicant		
Sample Date:	12 Sep-18 12:05	Material:	Copper sulfate	Sample Station:			

C-µg/L	Code	Rep	Pos	# Exposed	24h Survival	48h Survival	Notes
0	D	1	11	10		10	
0	D	2	8	10		10	
10		1	9	10		10	
10		2	12	10		10	
30		1	6	10		10	
30		2	1	10		10	
100		1	5	10		9	
100		2	4	10		10	
300		1	2	10		0	
300		2	3	10		0	
1000		1	10	10		0	
1000		2	7	10		0	

*data entry verified against laboratory bench sheets 9-24-18 JRF*

Reference Toxicant 48-h Acute Survival Test

Northwestern Aquatic Sciences

Test Type: Survival (48h)

Organism: Menidia beryllina (Inland Silverside)

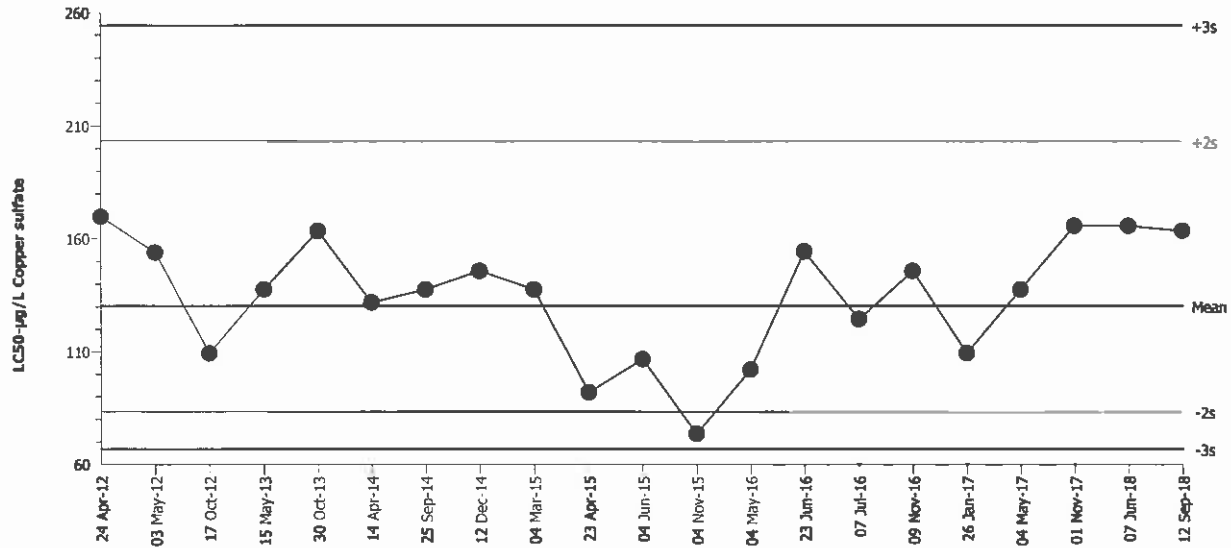
Material: Copper sulfate

Protocol: EPA/821/R-02-012 (2002)

Endpoint: 48h Proportion Survived

Source: Reference Toxicant-REF

Reference Toxicant 48-h Acute Survival Test



Mean: 130.3      Count: 20      -2s Warning Limit: 83.46      -3s Action Limit: 66.79  
 Sigma: NA      CV: 25.00%      +2s Warning Limit: 203.5      +3s Action Limit: 254.3

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2012	Apr	24	8:40	169.8	39.48	1.188			09-0894-6453	11-2925-5863
2		May	3	8:30	153.9	23.58	0.7465			00-0711-2305	20-7362-3197
3		Oct	17	11:15	109.3	-21.04	-0.7901			10-0444-6311	00-7573-7836
4	2013	May	15	7:00	137.6	7.261	0.2433			16-6995-0954	16-4582-4481
5		Oct	30	13:25	163.5	33.2	1.018			19-3608-8754	00-4003-9076
6	2014	Apr	14	11:30	131.8	1.479	0.05067			20-7450-8590	01-1253-6144
7		Sep	25	10:00	137.6	7.261	0.2433			15-5297-1657	07-1849-0124
8		Dec	12	8:30	145.7	15.41	0.5017			09-0648-4504	05-6807-3777
9	2015	Mar	4	10:40	137.6	7.261	0.2433			21-1719-8305	13-3397-0822
10		Apr	23	8:45	91.95	-38.37	-1.565			10-0925-2273	19-0548-1025
11		Jun	4	13:15	106.7	-23.65	-0.8989			13-8689-1181	03-9820-8141
12		Nov	4	11:50	73.52	-56.8	-2.569	(-)		13-1712-8446	07-9583-1346
13	2016	May	4	8:10	102.1	-28.25	-1.097			04-2416-9651	04-0389-3629
14		Jun	23	12:50	154.4	24.05	0.7601			02-4383-5206	09-5052-4145
15		Jul	7	12:00	124.3	-6.015	-0.2121			15-9630-6419	09-4197-3954
16		Nov	9	9:15	145.7	15.41	0.5017			07-2855-3734	07-9706-7591
17	2017	Jan	26	9:50	109.3	-21.04	-0.7901			19-7433-3662	14-4648-6112
18		May	4	11:10	137.6	7.261	0.2433			15-3521-6796	21-0276-8734
19		Nov	1	11:30	165.7	35.37	1.078			11-8493-3239	07-3474-6376
20	2018	Jun	7	11:10	165.7	35.37	1.078			14-7451-0146	00-8489-2453
21		Sep	12	12:05	163.5	33.2	1.018			10-4067-5467	06-2711-2603

*725 8*

Water Quality Data - test #999-3810, Menidia acute test						
Day	Concentration (g/L)	Temperature	pH	Salinity	DO	
0	1000	20.1	8.0	30.5	7.6	
0	300	20.1	8.0	30.5	7.6	
0	100	20.3	8.0	30.5	7.7	
0	30	20.2	8.0	30.5	7.7	
0	10	20.2	8.0	30.5	7.6	
0	0	20.3	8.0	30.5	7.7	
1	1000	20.2	8.0	30.5	7.2	
1	300	20.3	8.0	31.0	7.4	
1	100	20.3	8.0	30.5	7.6	
1	30	20.3	8.0	30.5	7.6	
1	10	20.2	8.0	30.5	7.6	
1	0	20.3	8.0	30.5	7.5	
2	1000					
2	300	20.0	8.0	31.5	6.6	
2	100	20.1	8.0	31.0	6.9	
2	30	20.0	8.0	31.5	6.8	
2	10	20.1	8.0	31.0	6.8	
2	0	20.2	8.0	31.0	6.9	
	MEAN	20.2	8.0	30.7	7.3	
	SD	0.1	0.0	0.4	0.4	
	N	17	17	17	17	
	MIN	20.0	8.0	30.5	6.6	
	MAX	20.3	8.0	31.5	7.7	

data entry verified against laboratory bench sheets

9-24-16 JMF