### TOXICITY TEST REPORT

# **TEST IDENTIFICATION**

Test No.: 658-84

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

<u>Protocol No.</u>: 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. Stanley Warner

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. Irissarri, B.S., Aq. Toxicol; J. B. Brown, B.S., D.V.M., Assoc. Aq. Toxicol.; Y. Nakahama, Sr. Tech. Study Schedule:

Test Beginning: 11-1-17, 1210 hrs. Test Ending: 11-5-17, 1140 hrs.

<u>Disposition of Study Records</u>: 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.	6036G
Collection Date	10-31-17
Receipt Date	11-1-17
Temperature (°C)	2.8
pН	7.5
Dissolved oxygen (mg/L)	11.5
Salinity (‰)	5.5

<u>Treatments</u>: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

#### DILUTION WATER

Source: Artificial seawater

Date of Preparation: 10-27-17

Water Quality: Salinity, 30.0 %; pH 8.4

Pretreatment: Prepared with Tropic Marin<sup>®</sup> sea salts and MilliO<sup>®</sup> deionized water, aerated.

#### **TEST ORGANISMS**

Species: Menidia beryllina, inland silversides.

Age: 13 days at test initiation.

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

Acclimation: Fish were received on 10-31-17. The water quality, including receiving water, prior to testing averaged: Temperature, 19.3°C; pH, 7.9; salinity, 30.0 ‰; dissolved oxygen, 9.5 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,  $20.0 \pm 0.4$ °C; pH,  $8.3 \pm 0.1$ ; salinity,  $30.4 \pm 0.7$  %; dissolved oxygen,  $7.0 \pm 0.1$  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-Karber method. The statistical software employed for these calculations was CETIS, v.1.8.7.4, Tidepool Scientific Software.

# PROTOCOL DEVIATIONS

The salinity of the 100% test concentration on day four was 33.0 % which is above the protocol limits of  $30.0 \pm 2.0$  %.

#### 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.: 999-3724

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

5-16-16.

Test Date: 11-1-17

Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ‰, pH 8.0.

Results: LC50, 166 μg/L Cu. This result is within the laboratory's control chart warning limits (84.2 – 197).

#### 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  $\geq$  90%.

NOEC (%)	100
LOEC (%)	>100
96-hr LC50 (%)	>100
(95% C.I.)	

Method By Data Inspection

STUDY APPROVAL

Richard A Coldward 11/16/m Laboratory Director Date

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

			Numbe	r of fish su	rviving		96-hr % Si	ırvival
Effluent	•							
Conc. (%)	Replicate	0-hr	24-hr	48-hr	72-hr	96-hr	Individual	Mean
100	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	100
50	1	10	10	10	10	10	100	
	2	10	10	10	10	9	90.0	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	97.5
25	1	10	10	10	10	10	100	
	2	10	10	10	10	9	90.0	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	97.5
12.5	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	100
6.25	1	10	10	9	9	9	90.0	
	2	10	10	10	10	10	100	
	3	10 -	10	10	10	10	100	
	4	10	10	10	10	10	100	97.5
Control	1	10	10	10	10	10	100	
	2	10	10	10	10	10	100	
	3	10	10	10	10	10	100	
	4	10	10	10	10	10	100	100

# APPENDIX I

**PROTOCOL** 

# TEST PROTOCOL

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

# 1 INTRODUCTION

- 1.1 <u>Purpose of Study</u>: 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 <u>Referenced Method</u>: 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 <u>Proposed Study Schedule</u>: 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

PROTOCOL NO. NAS-XXX-MB1 Revision 3 (7-1-12)

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%  $\pm$  10% is recommended for *M. beryllina* (15-32%  $\pm$  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<sup>®</sup> (or equivalent) artificial seawater may also be used.

# 5 <u>TEST ORGANISMS</u>

- 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 <u>Acclimation and Pretest Observation</u>: 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 <u>Preparation of Test Concentrations</u>: 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 <u>Effect Criterion</u>: 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  $20 \pm 1^{\circ}$ C or  $25 \pm 1^{\circ}$ C. The salinity should be in the range of 1-32‰ ± 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 <u>Preparation of Test Concentrations</u>: 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 <u>Beginning of Test</u>: 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 <u>Test Duration, Type and Frequency of Observations, and Methods</u>: 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
BIOLOGICAL DATA	
Survival (in each test container).	Daily.
PHYSICAL AND CHEMICAL DATA	
Dissolved oxygen, pH, temperature, & salinity	Daily.
(in one replicate of each test level and the	
control).	
Total ammonia-N (in sample where toxicity may	Prior to use in test (optional as required).
be contributed by unionized ammonia; i.e., total	-11
ammonia ≥ 5 mg/L)	

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-Karber, or Trimmed Spearman-Karber (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

PROTOCOL NO. NAS-XXX-MB1 Revision 3 (7-1-12)

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.

# 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
14 Fooding root-	minimums).
14. Feeding regime	Artemia nauplii are made available while holding prior to the test;
15 Test showher sleeping	add 0.1 mL Artemia 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
17. Dilution water	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:
	$1-32\% \pm 10\%$ for M. beryllina;
10 T	15-32‰ ± 10% for other Menidia sp.
18. Test concentrations	Effluents: minimum of five concentrations and a control. Receiving
10 Dil di	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	Samples are first used within 36 hr of completion of sampling
requirements	period (required for effluents and recommended for receiving
00.0.1.1.	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

NORTHWESTERN AQUATIC SCIENCES	2

PROTOCOL NO. NAS-XXX-MB1

ACLITE	TOXICITY	TECT	ZALL	<b>CDECIES</b>
ACUIE		IESI	IMLL	SPECIES

Test No. 69 Test Type ( Species	(rangefii	Clie nding/de <b>f</b> i <i>lenidia b</i> e	nitive)	CH2M-\	Wyckoff			Inves Test Lengt		96
Test Loc	Ctudy M Laborat cation: N	H2M-Wy Ionitor: tory: North	Mr. Stanwestern A aboratory	nley Warn quatic Scie	er ences	eosote Plac	_	bridge Islar	nd, WA 981	10
	Mgr./Stu fficer // chedule	dy Dir. (28) Ma - BVV	L.K. Ne al Cellan vn S	meth	-6	4.	IRISSARI		1140	
NAS Date Date Ten Diss pH: Con Hare Alka	scription S Sample e of Colle e of Reconperatur solved o	le No. lection: ceipt: re (deg C) exygen (m y (umhos/ mg/L): ng/L):	Alos/ ): g/L):	/o-3 //-	CH7,M 0366 11-17 1-17 2-8 15 1-5	HU-W	YCKOFF:	Trestper	nt Alent	M Grandun
Date Wat H	cription e of Pre ter Qual	: paration/0 ity: Cond. s (mg/L as	Collection: (umhos/cr s CaCO₃):	·	/ <i>2-<del>35 1</del></i> N/A N/A		alinity (ppt) k <b>alinity</b> (mg	30.0 /L as CaCo	D <sub>3</sub> ):	<b>8 . 4</b> N/A d.
	est cond	ducted in	(circle one)	: room 1	Poom 2	2 trailer	water ba	ath other	r:	
D	25	50	6.25	100	12.5	25				
B	625	6.25	50	25	125	100				
	<del></del> -						_			

Error codes: 1) Correction of handwriting error

<sup>2)</sup> Written in wrong location; entry deleted

<sup>3)</sup> Wrong date deleted; replaced with correct date

					ACUTE	OXICITY	TEST (ALL SPECIE	S)	
Test N	o	658-84	Client		CH2M-W	yckoff		Invest	igator
Spe		Aqu	Menic afic	dia beryll BNS บ	ina 15/cms	Inco	Age Ft-Colling, CO	e: 13 duys Date received:	Size:
Acc	climat	ion Data:							
	ate	Temp. (deg.C)		salinity (ppt)	DO (mg/L)	amount	Feeding description	Water changes	Comments
/1	<i>3เ¬Դ</i> ~ <i>[๚Դ</i> lean	129	7.5	29.0 31.0 30.0	12-1	2 1/2 mL	Artun	405	Ker' data
S	S.D. (N)	(2)	(2)	/ (2)	(2)				
Pho	otoper	riod durin	g accl	imation:	16:8 L:D				
rest f	PROC	EDURE:	S AND	CONDI	TIONS				
Tes	st con	centratio	ns (50°	% series	recomme	ended):	100, 50, 25, 12.5, 6	.25 & 0%	
Tes	st cha	mber:	600 m	ıL glass	beakers		Test volume	: 250 mL	
		es/treatm		4		0	Organisms/tre <b>atment</b>		
	-	er chang			Yes@48			tion during test:	None

# **MISCELLANEOUS NOTES**

# **Test Concentration Preparation:**

Duration: 24-hr, 48-hr, 96-hr Beaker placement: Stratified randomization

Feeding: Yes@48 hours ~2 hours prior to test change

	Test Concentration (%)	Volume of effluent* (mL)	Volume of Dilution water (mL)
17 17 17	100 50	1,000 500	0 500
	25	250	750
12	12.5	125	875
Y_	6.25	62.5	937.5
	0	0	1,000
	*Effluent salinity	adjusted to 30 ppt with	Tropic Marin <sup>®</sup> Sea salts.

Test temperature (deg.C): 20 +/- 1

Photoperiod: 16:8 L:D

# ACUTE TOXICITY TEST (ALL SPECIES)

Test No.	658-84	Client	CH2M-Wyo	koff	Inve	stigator		
		D/	AILY RECOR	D SHEET				
Day 0 (1¢ /	1/17/5	_						
Conc.	Temp.		Sal.	DO	Υ	Sun	vivors	
( % )	(deg.C)	рН	(ppt)	(ppm)	Α	В	С	D
1. 100	20.7	8.0	30.0	6.9	10	10	10	10
2. 50	20.7	81.	30.0	7-1	10	10	10	10
3. 25	20.6	8-5	29.5	7.1	10	10	10	10
4. 12.5	20.7	8.3	29.1	71	10	10	10	10
5. 6.25	20.8	8.3	291	7-1	10	10	10	10
6. 0	70.9	8.3	29.5	7-1	10	10	iv	10
5 44 -4	_	600						
Day 1 ( 1[/		140	Cal	- DA				
Conc. (%)	Temp. (deg.C)	рΗ	Sal. (ppt)	DO (ppm)	A	B	vivors C	_D
1. 100	20,3	8-4	30,0	7-0	2	10		10
					60	45	10	
2. 50	20.2	8-3	30.5	7-1	7.0	10		10
3. 25	10.0	<del>- 3 - &gt;</del>	30,0	7.1	10	10	10	10
4. 12.5	203	8-5	30.0	12-0	10	10	10	10
5. 6.25	20.3	8.3	305	7-1	10	10	10	10
6. 0	20.2	8.3	30-5	72	0	(0)	10	10
Day 2 ( 11/	3 117103	110						
Conc.	Temp.	(	Sal.	DO		Sur	vivors	
( % )	(deg.C)	pН	(ppt)	(ppm)	Α	В	С	D
1. 100	199	8.6	30.5	6.8	10	10	10	10
2. 50	19.7	8.5	305	69	10	10	10	10
3. 25	19-8	8-4	300	69	10	10	10	10
4. 12.5	198	8.3	305	6.8	10	10	10	10
5. 6.25	19-9	8.3	31.0	6-7			10	10
6. 0	192	83	31.0	1,9	70	10	70	10
0. 0		1)-/	3/.0	(J=1	1.70	10	10	70
Day <b>3</b> ( j / /	4170							
Conc.	Temp.		Sal.	DO			vivors	
(%)	(deg.C)	pΗ	(ppt)	(ppm)	Α	В	C	D
1. 100	19.4	8.5	30,5	7-0	10	10	10	(0
2. 50	195	8.4	30,0	7.1	10	w	10	10
3. 25	19-5	8.4	30.0	20	10	10	ن	10
4. 12.5	19-5	8-4	300	7-0	10	10	10	10
5. 6.25	19.6	8-4	30.0	7-1	9	10	10	Į O
6. <b>0</b>	19-10	8-3	30-0	7-1	10	10	10	10
D=4 ( ** 4:	,			·	······•			<u>i</u>
Day 4 ( 11 /			Sol.			Cur	vivoro	
Conc. ( % )	Temp. (deg.C)	pН	Sal.	DO (ppm)	A	B	vivors C	D
1. 100		8.6	(ppt)	(P) (P)	10	lò	10	10
2. 50	19.7	85	33.0		<del>1</del>			
	19.7		32.0	7.0	10	9 (ib		10
3. 25	19.7	355		69	10	9(10		10
4. 12.5	19,8	814	30.5	6.9	10	10	10	10
5. 6.25	19.8	8.3	30.5	17.0	9	10	10	ιO
6. 0	19.7	8,2	30.0	17.1	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**

Rec'0-31-12

DATE	10/3	0/2017	
SPECIES	Men	idia beryllina	
AGE	I I da	ay	
LIFE STAGE	Juve	nile	
HATCH DATE.	10/19	9/2017	
BEGAN FEEDING	lmme	ediately	
FOOD:	Rotif	ers, Artemia sp.	
Water Chemistry Record	:	Current	Range
TEM	PERATURE: _	25 ℃	23-26 °C
SALINITY/CONI	DUCTIVITY: _	25 ppt**	23-26 ppt
TOTAL HARDNESS	S (as CaCO <sub>3</sub> ):		
TOTAL ALKALINITY	' (as CaCO3):	200 mg/l	165-210 mg/l
	pH:	8.26	7.87-8.24
Comments:	** Acclimated	to 30 ppt on 10/30/17.	
		Facility Supervisor	

Aquatic BioSystems, Inc • Quality Research Organisms

Report Date: Test Code: 09 Nov-17 15:16 (p 1 of 2) 658-84 | 03-9583-7319

	-								Test	Code:		658-84   0	3-95 <b>83-7319</b>
Inland Silver	side 96-h Acute	Surviv	al Test							-	Northwest	ern Aquat	lc Sciences
Analysis ID:	20-5206-6673		Endpoint	96h	Proportion	Survive	d		CETI	S Versio	on: CETISv1	.8.7	
Analyzed:	09 Nov-17 15:	16	Analysis:	-	parametric-			reatments		ial Resu			
Batch ID:	11-5542-7334		Test Type	Sur	vival (96h)				Anal	vst:			
Start Date:	01 Nov-17 12:1	0	Protocol:		A/821/R-02-	012 (20	02)		Dilue	-	Reconstituted V	Vater	
Ending Date:			Species:		nidia beryllin	•	,		Brine		ropic Marin		
Duration:	95h		Source:		uatic Indicato				Age:		<b>-</b>		
Sample ID:	16-3976-7545		Code:	61E	BCDDF9				Clier	nt: V	Wyckoff Treatm	ent Plant	
	31 Oct-17 09:3	0	Material:		ustrial Efflue	nt			Proje		VET Quarterly		e Test (40)
-	: 01 Nov-17 11:0		Source:		ckoff								
Sample Age:	27h		Station:	·						-			
Data Transfo	m	Zeta	Alt	Тур	Trials	Seed			PMSD /	NOEL	LOEL	TOEL	TU
Angular (Corre	ected)	NA	C > .	Г	NA	NA			6.45%	100	>100	/NA	1
Steel Many-O	ne Rank Sum T	est										<u></u>	
Control	vs C-%		Test	Stat	Critical	Ties	DF	P-Value	P-Type	Decisi	on(a:5%)		
Dilution Water	6.25		16		10	1	6	0.6105	Asymp		gnificant Effect	t	
	12.5		18		10	1	6	0.8333	Asymp		gnificant Effect		
	25		16		10	1	6	0.6105	Asymp	Non-Si	gnificant Effect	i	
	50		16		10	1	6	0.6105	Asymp	Non-Si	gnificant Effect	t	
	100		18		10	1	6	0.8333	Asymp	Non-Si	gnificant Effect	İ	
ANOVA Table													
Source	Sum Squ	ares	Mea	n Squ	ıare	DF		F Stat	P-Value	Decisi	on(α:5%)		
Between	0.0099597	749	0.00	19919	95	5		0.6	0.7006	Non-Si	gnificant Effect	t .	
Error	0.059758	5	0.00	33199	917	18		т.					
Total	0.0697182	24				23		_					
Distributiona	l Tests				_								-
Attribute	Test				Test Stat	Critic	al	P-Value	Decision(	(a:1%)			
Variances	Mod Leve	ene Eq	uality of Var	iance	0.6	4.248		0.7006	Equal Var	iances			
Variances			of Variance	•	5.4	4.248		0.0033	Unequal V				
Distribution	Shapiro-\	Wilk W	Normality		0.6694	0.884		<0.0001	Non-norm	al Distrib	ution		
96h Proportio	on Survived Sun	nmary											
C-%	Control Type	Cou	nt Mea	n	95% LCL	95% l	JCL	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	0.97	5	0.8954	1		1	0.9	1	0.025	5.13%	2.5%
12.5		4	1	-	1	1		1	1	1	0	0.0%	0.0%
25		4	0.97		0.8954	1		1	0.9	1	0.025	5.13%	2.5%
50 100		4	0.97 1		0.8954 1	1 1		1	0.9 1	1	0.025	5.13%	2.5%
		7				'	==	1	1		0	0.0%	0.0%
,	rected) Transfor		•	_	059/ 1.01	050/ 1	IC!	Madia	581-	Men	04-1-5-	O) m/	0/5/
C-%	Control Type Dilution Water	Cour			95% LCL			Median 1.412	Min 1.412	Max	Std Err	CV%	%Effect
6.25	Distriction vyaler	4	1.41: 1.37		1.412 1.242	1.412 1.501		1.412	1.412 1.249	1.412 1.412	0 0.04074	0.0% 5.94%	0.0%
12.5		4	1.41		1.2 <del>4</del> 2 1.412	1.412		1.412	1.412	1.412	0.04074	5.94% 0.0%	2.89% 0.0%
25		4	1.37		1.242	1.501		1.412	1.249	1.412	0.04074	5.94%	2.89%
50		4	1.37		1.242	1.501		1.412	1.249	1.412	0.04074	5.94%	2.89%
100			4 44		4 446	4 440		4 440	4.440				



0.0%

0.0%

1.412

1.412

1.412

1.412

0

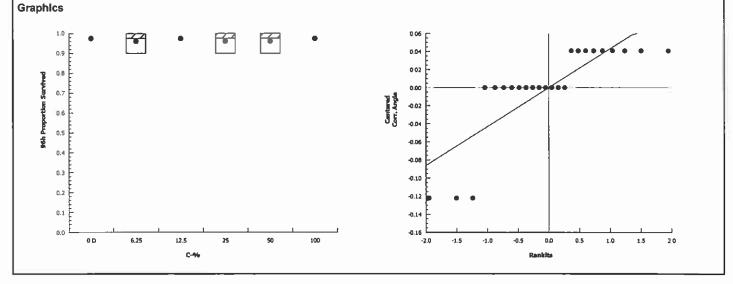
1.412

1.412

Report Date: Test Code: 09 Nov-17-15:16 (p 2 of 2) 658-84 | 03-9583-7319

Inland Silverside 96-h Acute Survival Test Northwestern Aquatic Sciences 20-5206-6673 Endpoint 96h Proportion Survived CETIS Version: **CETISv1.8.7** Analysis ID: 09 Nov-17 15:16 Analysis: Nonparametric-Control vs Treatments Official Results: Yes Analyzed: 96h Proportion Survived Detail **Control Type** Rep 1 Rep 2 Rep 3 Rep 4 0 **Dilution Water** 1 1 1 6.25 0.9 1 1 1 12.5 1 1 1 1 0.9 25 1 1 1 50 0.9 1 100 1 1 1

Angular (	Corrected) Transfor	med Detai	II		
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.412	1.412	1.412	1.412
6.25		1.249	1.412	1.412	1.412
12.5		1.412	1.412	1.412	1.412
25		1.412	1.249	1.412	1.412
50		1.412	1,249	1.412	1.412
100		1.412	1.412	1.412	1.412



LC50 7 10020 bo data respection.

119-17 85

Report Date: Test Code: 09 Nov-17 15:15 (p 1 of 1)

03-9583-73 (9/658-84

								rest code.		03-9303-73 (91030-04
Inland Silve	rside 9	6-h A	cute \$	Survival Tes	t				Northweste	rn Aquatic Sciences
Start Date: End Date: Sample Date	05 1	Nov-1	7 12:1 7 11:4 7 09:3	0 Protoc	ol: EPA/821/F	R-02-012 (2002)		Sample Code: Sample Source Sample Station	•	
C-%	Code	Rep	Pos	# Exposed	24h Survival	48h Survival	72h Survival	96h Survival	N	otes
0	D	1	1	10				10		
0	D	2	4	10		1		10		
0	D	3	2	10				10		
0	D	4	24	10		Į.		10		
6.25		1	15	10				9		
6.25		2	20	10				10		
6.25		3	16	10		Į.		10		
6.25		4	3	10		l		10	-	
12 5		1	8	10				10		
12.5		2	17	10				10		
12.5	-	3	13	10				10		
12.5		4	18	10				10		
25		1	23	10				10		
25		2	6	10				9		
25		3	9	10				10		
25		4	11	10				10		
50		1	21	10				10		
50		2	5	10				9		
50		3	19	10				10		
50		4	10	10				10		
100		1	12	10	1			10		
100		2	14	10				10		
100		3	7	10				10		



Day	Concentration	Temperature	рН	Salinity	DO
Ö	100	20.7	8.0	30.0	
0	50	20.7	8.1	30.0	7.1
0	25	20.6	8.2	29.5	7.1
0	12.2	20.7	8.3	29.5	7.1
0	6.25	20.8	8.3	29.5	7.1
0	0	20.9	8.3	29.5	7.1
_ 1	100	20.3	8.4		7.0
1	50	20.2	8.3	30.5	7.1
1	25	20.2	8.3	30.0	7.1
1	12.5	20.3	8.3	30.0	7.0
1	6.25	20.3	8.3	30.5	7.1
1	0	20.2	8.3	30.5	7.2
2	100	19.9		30.5	6.8
2	50	19.8	8.5	30.5	6.9
_ 2	25	19.8	8.4	30.0	6.9
2	12.5	19.8	8.3	30.5	6.8
2	6.25	19.9	8.3	31.0	6.7
2	0	19.8	8.3	31.0	6.9
3	100	19.4	8.5	30.5	7.0
_ 3	50	19.5	8.4	30.0	7.1
_ 3	25	19.5	8.4	30.0	7.0
3	12.5	19.5	8.4	30.0	7.0
3	6.25	19.6	8.3	30.0	7.1
3	0	19.6	8.3		7.1
4	100	19.7	8.6	33.0	6.9
4	50	19.7	8.5	32.0	7.0
4	25	19.7	8.4	31.0	6.9
_ 4	12.5	19.8	8.4		6.9
4	6.25	19.8	8.3		7.0
4	0	19.7	8.2	30.0	7.1
	MEAN	20.0	8.3		7.0
	SD	0.4	0.1	0.7	0.1
	N	30	30	30	30
	MIN	19.4	8.0	29.5	6.7
	MAX	20.9	8.6	33.0	7.2

Page 1 of 1

Northwestern Aquatic Sciences (REGION COPY)

DateShipped: 10/31/2017 CarrierName: FedEx

AirbillNo: 788277595497

CHAIN OF CUSTODY RECORD

Wyckoff Eagle Harbor GWTP 2017/WA Project Code: WEH-025! Cooler #: 1 of 1

No: 10-103117-100104-0209 2018T10P303DD210W2LA00 Contact Phone: 206-780-1711

Contact Name: Keith Allers

	Sample No.		Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	
		Ground Water/ K.Allers	Composite	ACTOX-CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	10/31/2017 09:30	Field Sample
_								
-								
+								
-								
_								
		,						
-								
_								

	1,004 60211	Shipment for Case Complete? N
Special Instructions:	Sac ca #SAC	Samples Transferred From Chain of Custody #
Analysis Key: ACTOX-CHRTOX=Acute Toxicity, Chronic Toxicit	Toxicity, Chronic Toxicity	

Date/Time Sample Condition Upon Receipt In Just Received by (Signature and Organization) | Ilems/Reason | Relinquished by (Signature and Organization) | Date/Filme 1005 CHZMO

(206) 780-1711

SHIP DATE: 310CT17 ACTWGT: 49 00 LB CAD: 111531780WSXI3100 DIMS: 21x15x16 IN

BILL SENDER

BAINBRIDGE ISLAND, WA 98110 UNITED STATES US

GEARLD IRSSARRI NORTHWESTERN AQUATIC SCIENCES 3814 YAQUINA BAY ROAD

NEWPORT OR 97365 (541) 265-7225 INV: PO:

REF: PN: 436558.FP.Y5.01





TRK# 7882 7759 5497

WED - 01 NOV 12:00P PRIORITY OVERNIGHT

86 ONPA

97365

OR-US PDX



# APPENDIX III RAW DATA – REFERENCE TOXICANT TEST

# ACUTE TOXICITY TEST (ALL SPECIES)

Client: QC Test Client's Study Monitor: N/A Testing Laboratory: Northwestern Aquatic Sciences Test Location: Newport Laboratory Laboratory's Study Personnel:
STUDY MANAGEMENT  Client: QC Test  Client's Study Monitor: N/A  Testing Laboratory: Northwestern Aquatic Sciences  Test Location: Newport Laboratory  Laboratory's Study Personnel:
Client's Study Monitor: N/A Testing Laboratory: Northwestern Aquatic Sciences Test Location: Newport Laboratory Laboratory's Study Personnel:
Test Location: Newport Laboratory Laboratory's Study Personnel:
Laboratory's Study Personnel:
Proj. Mgr./Study Dir. GABMEN 6FS
QA Officer L.K. Nemeth
1. Vastalana 2. T. Brown 3
34
Study Schedule:
Test Beginning:
•
TEST MATERIAL Argent Reagent lot #0195
Description: Copper as: CuSO₄-5H₂O 1.0 mg/mL stock prep: \$\(\sigma^2\)/67
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: / ~ 30-17-
Water Quality: Cond. (umhos/cm) N/A Salinity (ppt) 30.0 pH 8-
Hardness (mg/L as CaCO₃): N/A Alkalinity (mg/L as CaCO₃): N/A
Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q® deionized water.
TEST LOCATION  Test conducted in (circle one): oom 1 room 2 trailer water bath other:  Randomization chart:
R 00 (00 30 300 (000
B 0 (0 (00 30 300 (000
A 30 @ (000 100 10 300 )
· '
<del>                                     </del>
<del>                                     </del>

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

# ACUTE TOXICITY TEST (ALL SPECIES)

Test No. 999_	3724 Client	QC Test		Inve	stigator
TEST ORGAI		n dlin a		1	
·	Menidia be			Age: 13 days	Size:
Source:	Aguatu Bio!	ystems, Inc., F	+ Collins, co	Date received	1: 10-31-17

Acclimation Data:

	Temp.		salinity	DO	F	eeding	Water	
Date	(deg.C)	_pH	(ppt)	(mg/L)	amount	description	changes	Comments
7.	104		- 4 -	10.	1		<u></u>	
1001-17	179	7.5	24.0	12-1	2/1200	Av teine	40	Nec' darty
11-1-17	2-200	8-1	32-0	6.9	u	ч	1 (	
Mean	192	7-8	30-5	9-5				
S.D.	_							
(N)	(1)	(W	CU	(L)				

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 m	L 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**

# <u>Test solution perpetration:</u>

	Test Concentration(ug/L)	Volume of W.S.* (mL)	Volume of Dilution water (mL)
11-1-17	1,000	5.0	Appropriate amount of
8	300	1.5	W.S. added to a
	100	0.5	graduated cylinder
	30	0.15	then brought up to
	10	0.05	volume (500mL) with
	0	0	dilution water.

\*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.

# ACUTE TOXICITY TEST (ALL SPECIES)

Test No.	999-3724	Client	QC Te	st	Investigator	. <u> </u>							
DAILY RECORD SHEET													
Day 0 ( / ( )	Day 0 ( / ( / ( / ( / ( / ( / ( / ( / ( / (												
Conc.	Temp.		Sal.	DO	Survi	vors							
( ug/L )	(deg.C)	pН	(ppt)	(mg/L)	A	В							
1. 1,000	20.7	8.5	30.0	オ・(	10	10							
2. 300	20,6	8.2	30,0	7.1	10	10							
3. 100	20.7	8.2	30.0	7-1	10	10							
4. 30	20.8	8.2	300	7-1	10	10							
5. 10	20,7	8.2	300	7-1	10	10							
6. 0	20.8	8.2	30.0	7-1	10	iB							
Day 1 ( 11/2/17) 043 PM													
Conc.	Temp.		Sal.	DO	Survi	_							
( ug/L )	(deg.C)	pH	(ppt)	(mg/L)	A	В							
1. 1,000	20-2	1.8.	31.0	7-1	O(((O(b)))	0(100)							
2. 300	202	8.2	30-5	7-1	OX (00)	O(00)							
3. 100	200	8.1	30-5	7.1	9(U)	10							
4. 30	20.2	8.1	30.5	7.2	10	10							
5. 10	20.2	85	31-0	71	9(1m)	10							
6. 0	20.2	2.1	30.5	72	10	10							
Day 2 ( / l /	13 /14-) 04	1/1/2		·									
Conc.	Temp.	v	Sal.	DO	Survi								
( ug/L )	(deg.C)	рН	(ppt)	(mg/L)	Α	В							
1. 1,000		{	)	)	0	Ø							
	1 —				$\mathcal{O}$	0							
3. 100	8201	8.2	310	6-7	9	10							
4. 30	201	82	31-5	70	10	10							
5. 10	20.1	8.2	31-0	69	q	10							
6. 0	20.1	82	310	70	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**

Rec'0-3+12

DATE:	10/30/2017	
SPECIES:	Menidia beryllina	
AGE:	11 day	
LIFE STAGE:	Juvenile	
HATCH DATE:	10/19/2017	
BEGAN FEEDING:	Immediately	
FOOD:	Rotifers, Artemia sp.	
Water Chemistry Record:	Current	Range
TEMPERATURE	25 °C	23-26 °C
SALINITY/CONDUCTIVITY:	25 ppt**	23-26 ppt
TOTAL HARDNESS (as CaCO <sub>3</sub> ):	:	
TOTAL ALKALINITY (as CaCO <sub>1</sub> ):	200 mg/l	165-210 mg/l
pI ł:	8.26	7.87-8.24
Comments: ** Acclin	mated to 30 ppt on 10/30/17.	7 
	Facility Supervisor	

# **CETIS Summary Report**

Report Date: Test Code: <del>-09 Nov-17</del> 14:35 (p 1 of 1) 999-3724 | 11-8493-3239

Ending Date: 03 Nov-17 12:05 Species: Menidia beryllina Brine:	Bay Seawater
Duration: 49h Source: Aquatic Indicators, FL Age:	
Sample ID: 12-7576-7347 Code: 4C0AAA33 Client: Internal L Sample Date: 01 Nov-17 11:30 Material: Copper sulfate Project: Receive Date: 01 Nov-17 11:30 Source: Reference Toxicant Sample Age: NA Station:	_ab
Comparison Summary	
Analysis ID Endpoint NOEL LOEL TOEL PMSD TU Method	
18-7023-1343 48h Proportion Survived 100 300 173.2 16.5% Bonferroni Adj	t Test
Point Estimate Summary  Analysis ID Endpoint Level µg/L 95% LCL 95% UCL TU Method	
07-3474-6376 48h Proportion Survived EC50 165.7 146.1 188 Trimmed Spea	ıman-Kärber
48h Proportion Survived Summary	
	d Dev CV% %Effect
0 Dilution Water 2 1 1 1 1 1 0 0	0.0% 0.0%
	7.44% 5.0%
30 2 1 1 1 1 1 0 0	0.0% 0.0%
	7071 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	
0 Dilution Water 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	
10 0.9 1	



1000

0

# **CETIS Test Data Worksheet**

Report Date:

09 Nov-17 14:34 (p-1-of-17

Northwestern Aquatic Sciences

**Test Code:** 

11-8493-3239/999-3724

Reference	Toxicant	48-h Acute	Survival	Test
-----------	----------	------------	----------	------

03 Nov-17 12:05

Start Date: 01 Nov-17 11:30 End Date:

Menidia beryllina Species:

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

Sample Code:

4C0AAA33

Sample Source: Reference Toxicant

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



: Date: 09 Nov-17 14:36 ( 1 of 1)

# Reference Toxicant 48-h Acute Survival Test

Sigma:

NA

CV:

23.70%

Northwestern Aquatic Sciences

Test Type: Survival (48h)

Organism: Menidia beryllina (Inland Silverside)

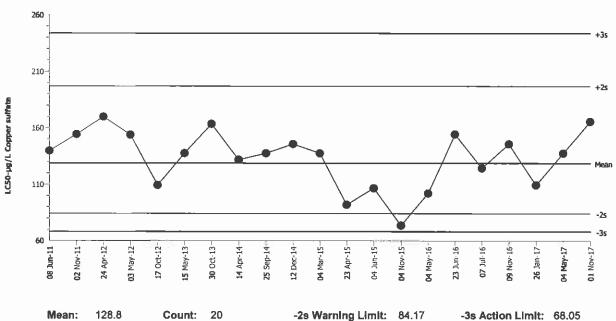
Protocol: EPA/821/R-02-012 (2002) Endpoint: 48h Proportion Survived

Material: Copper sulfate

Source: Reference Toxicant-REF

+3s Action Limit: 243.7

#### Reference Toxicant 48-h Acute Survival Test



+2s Warning Limit:

Qualit	y Con	troi Data	a								
oint	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Jun	8	9:15	139.7	10.98	0.3849			19-8506-0915	02-0234-8313
2		Nov	2	10:25	154.4	25.6	0.853			17-7747-0844	14-4821-2321
3	2012	Apr	24	8:40	169.8	41.03	1.301			09-0894-6453	11-2925-5863
ļ		May	3	8:30	153.9	25.14	0.8387			00-0711-2305	20-7362-3197
5		Oct	17	11:15	109.3	-19.48	-0.7716			10-0444-6311	00-7573-7836
ì	2013	May	15	7:00	137.6	8.815	0.3115			16-6995-0954	16-4582 <del>-44</del> 81
7		Oct	30	13:25	163.5	34.75	1.124			19-3608-8754	00-4003-9076
3	2014	Арг	14	11:30	131.8	3.034	0.1095			20-7450-8590	01-1253-6144
)		Sep	25	10:00	137.6	8.815	0.3115			15-5297-1657	07-1849-0124
0		Dec	12	8:30	145.7	16.97	0.5822			09-0648-4504	05-6807-3777
1	2015	Mar	4	10:40	137.6	8.815	0.3115			21-1719-8305	13-3397-0822
2		Apr	23	8:45	91.95	-36.81	-1.584			10-0925-2273	19-0548-1025
3		Jun	4	13:15	106.7	-22.1	-0.8856			13-8689-1181	03-9820-8141
4		Nov	4	11:50	73.52	-55.25	-2.636	(-)		13-1712-8446	07-9583-1346
5	2016	May	4	8:10	102.1	-26.7	-1.093			04-2416-9651	04-0389-3629
6		Jun	23	12:50	154.4	25.6	0.853			02-4383-5206	09-5052-4145
7		Jul	7	12:00	124.3	-4.46	-0.1658			15-9630-6419	09-4197-3954
8		Nov	9	9:15	145.7	16.97	0.5822			07-2855-3734	07-9706-7591
9	2017	Jan	26	9:50	109.3	-19.48	-0.7716			19-7433-3662	14-4648-6112
20		May	4	11:10	137.6	8.815	0.3115			15-3521-6796	21-0276-8734
21		Nov	1	11:30	165.7	36.92	1.186			11-8493-3239	07-3474-6376

Wate	er Quality Data - test #	999-3724, Me	nidia acı	ute test	
Day	Concentration (g/L)	Temperature	pН	Salinity	DO
0	1000	20.7	8.2	30.0	7.1
0	300	20.6	8.2	30.0	7.1
0	100	20.7	8.2	30.0	7.1
0	30	20.8	8.2	30.0	7.1
0	10	20.7	8.2	30.0	7.1
0	0	20.8	8.2	30.0	7.1
_ 1	1000	20.2	8.1	31.0	7.1
1	300	20.2	8.2	30.5	7.1
1	100	20.2	8.1	30.5	7.1
1	30	20.2	8.1	30.5	7.2
1	10	20.2	8.1	31.0	7.1
1	0	20.2	8.1	30.5	7.2
2	1000	·			
_ 2	300				
2	100	20.1	8.2	31.0	6.7
2	30	20.1	8.2	31.5	7.0
2	10	20.1	8.2	31.0	6.9
2	0	20.1	8.2	31.0	7.0
	MEAN	20.4	8.2	30.5	7.1
	SD	0.3	0.0	0.5	0.1
	N	16	16	16	16
	MIN	20.1	8.1	30.0	6.7
	MAX	20.8	8.2	31.5	7.2