#### TOXICITY TEST REPORT

#### TEST IDENTIFICATION

Test No.: 658-80

Title: Mussel (Mytilus galloprovincialis) larval test using static 48-hr exposure to CH2M Hill-Wyckoff

Treatment Plant SP11 Field Sample. EPA permit number WAD009248295.

Protocol No.: NAS-XXX-CG/MG2, August 28, 1990, Revision 3 (9-8-01). This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136) and the ASTM bivalve toxicity method (E 724-89).

## STUDY MANAGEMENT

Study Sponsor: CH2M 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. Man.; G.J. Irissarri, B.S., Study Dir.; L.K. Nemeth, B.A., M.B.A., QA Officer; R.S. Caldwell, PhD, Sr. Aq. Toxicol.; J. B. Brown, B.S., D.V.M., Assoc. Aq.

Toxicol.; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 3-15-17, 1400 hrs. Test Ending: 3-17-17, 1405 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 Field Sample. Details are as follows:

NAS Sample No.	5845G
Collection Date	3-14-17
Receipt Date	3-15-17
Temperature (°C)	2.4
pН	7.4
Dissolved oxygen (mg/L)	10.6
Salinity (%)	5.0

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

#### DILUTION WATER

Source: Yaquina Bay, Oregon seawater.

Date of Collection: 3-14-17

Water Quality: Salinity, 30.0 %; pH, 8.0

Pretreatment: Filtered to ≤0.45 μm, aerated, salinity adjusted with 100.0 ppt brine prepared 2-20-17.

#### BRINE USED FOR DILUTION WATER AND SALINITY CONTROL

Source: Filtered Yaquina Bay, Oregon, sea water

Salinity: 100.0 %

Date of Preparation: 2-20-17

Method of Preparation: Freezing method

#### TEST ORGANISMS

Species: Mussel (Mytilus galloprovincialis).

Age: 3.5 hrs post-fertilization.

Source: Carlsbad Aquafarm, Carlsbad, CA.

<u>Conditioning</u>: Adult mussels were received on 3-8-17 and placed in trays with flowing seawater. Holding conditions for the week prior to testing were: temperature,  $14.5 \pm 2.9$ °C; pH,  $7.9 \pm 0.1$ ; salinity,  $18.1 \pm 6.3$ %; and dissolved oxygen,  $8.8 \pm 0.8$  mg/L. Photoperiod was natural daylight.

Source of Gametes: 3 females and 3 males.

#### TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.

<u>Test Concentrations</u>: 70, 35, 18, 9, 4, 2, and 0% (Control).

Brine Control: A brine control was run in which salinity-adjusted Milli-Q<sup>®</sup> deionized water (5.0 ppt) was substituted for effluent in the preparation of the highest test solution concentration. As a result, the amount of brine in the brine control was the same as used in the 70.0% effluent test concentration.

Replicates/Treatment: 4

<u>Initial Concentration of Test Organisms</u>: 21.1/ml. <u>Volume of Subsamples Taken for Counting</u>: NA

Water Volume Changes per 24 hr: None (non-renewal static test).

Aeration: None Feeding: None

Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.

Water Quality and Other Test Conditions: Temperature,  $15.5 \pm 0.4$ °C; pH,  $8.1 \pm 0.2$ ; salinity,  $30.0 \pm 0.2$ %; and dissolved oxygen,  $8.0 \pm 0.1$  mg/L. Photoperiod 16:8 hr, L:D.

#### DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.8.7.4, Tidepool Scientific Software. Toxic units (TU<sub>c</sub>) were computed as 100/NOEC, 100/EC50, or 100/IC25.

#### 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.: 999-3654

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: 3-15-17

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

Results: EC50, 10.7  $\mu$ g/L; NOEC, 8  $\mu$ g/L; IC25, 9.59  $\mu$ g/L. The EC50 result was within the laboratory's control chart warning limits (8.96 – 12.2  $\mu$ g/L).

#### TEST RESULTS

Detailed tabulations of the test results are given in Table 1. The biological effects, given as the NOEC, LOEC, EC50/LC50 for normality and survival, and IC25 for normality are summarized below.

	Combined Proportion Normal	Survival
NOEC (%)	70 (TU <sub>c</sub> =1.43)	70 (TU <sub>c</sub> =1.43)
LOEC (%)	>70 (TU <sub>c</sub> <1.43)	>70 (TU <sub>c</sub> <1.43)
EC50/LC50 (%)	>70 (TU <sub>c</sub> <1.43)	>70 (TU <sub>c</sub> <1.43)
(95% C.I.)	on one	
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>70 (TU <sub>c</sub> <1.43)	
(95% C.I.)		
Method of Calculation	Linear Interpolation	

#### DISCUSSION/CONCLUSIONS

The NOEC for combined proportion normal was 70% effluent. The EC50 and IC25 for abnormal development were both >70%. The brine control test indicated that the brine did not contribute to effluent toxicity.

#### STUDY APPROVAL

Table 1. Test response of mussel (*Mytilus galloprovincialis*) larvae exposed to CH2M Hill-Wyckoff Treatment Plant SP11 Field Sample.

Test Material Concentration					Combined Proportion Normal*			ortion ived*
(%)	Repl.	Norm.	Abn.	Total		Mean		Mean
70	1	198	2	200	0.938		0.948	
	2	208	4	212	0.986		1.000	
	3	196	5	201	0.929		0.953	
	4	216	0	216	1.000	0.963	1.000	0.975
35	1	211	3	214	1.000		1.000	
	2	194	6	200	0.919		0.948	
	3	214	5	219	0.977		1.000	
	4	199	8	207	0.943	0.960	0.981	0.982
18	1	192	3	195	0.910		0.924	
	2	217	5	222	0.978		1.000	
	3	185	1	186	0.877		0.882	
	4	187	3	190	0.886	0.913	0.901	0.927
9	1	189	0	189	0.896		0.896	
	2	204	1	205	0.967		0.972	
	3	193	3	196	0.915		0.929	
	4	209	4	213	0.991	0.942	1.000	0.949
4	1	211	1	212	1.000		1.000	
	2	210	2	212	0.995		1.000	
	3	201	0	201	0.953		0.953	
	4	197	6	203	0.934	0.970	0.962	0.979
2	1	220	1	221	0.996		1.000	
	2	200	0	200	0.948		0.948	
	3	179	1	180	0.848		0.853	
	4	184	3	187	0.872	0.916	0.886	0.922
Normal Control	1	193	2	195	0.915		0.924	
	2	174	2	176	0.825		0.834	
	3	198	1	199	0.938		0.943	
	4	191	1	192	0.905	0.896	0.910	0.903
Brine Control	1	175	3	178	0.829		0.844	
	2	210	3	213	0.995		1.000	
	3	205	4	209	0.972		0.991	
	4	209	3	212	0.991	0.947	1.000	0.959

<sup>\*</sup> Based on an average initial count of 211 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal>average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

<sup>†</sup> Result significantly different (P≤0.05) from the control.

Salinity-adjusted Milli Q<sup>®</sup> deionized water (5.0 ppt) was substituted for effluent so that the brine concentration is equivalent to that for the 70.0% effluent concentration.

# APPENDIX I

**PROTOCOL** 

#### **TEST PROTOCOL**

#### BIVALVE, PACIFIC OYSTER OR BLUE MUSSEL, 48-HR LARVAL DEVELOPMENT TEST

#### 1. <u>INTRODUCTION</u>

2. STUDY MANAGEMENT

- 1.1 <u>Purpose of Study</u>: The purpose of this test is to estimate chronic toxicity of effluents, receiving waters, or other test materials using bivalve larval development in a 48-hr static test.
- 1.2 <u>Referenced Method</u>: This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136), ASTM bivalve toxicity method (E 724-89), and the WDOE toxicity guidance manual (WQ-R-95-80). Amendments may be incorporated to meet other methods or regulatory requirements as needed.
- 1.3 Summary of Method: Pacific oyster or blue mussel larvae (<4-hr-old) are exposed for 48-hr to different concentrations of test material in a static test. Salinity adjustment and brine controls are used when testing low salinity effluents. The test chambers are 30 ml borosilicate glass vials each containing 10 ml of test solution. Four replicate chambers each with 15-30 larvae per milliliter of test solution are employed at each test concentration. Test results are based on abnormal shell development and mortality. Data analysis normally consists of the calculation of an EC50 and IC25 for "percent normal", the calculation of an LC50 for percent survival, and the determination of NOECs and LOECs for both criteria. Special requirements may apply for the State of Washington or other regulatory entities. A test summary table is appended to the end of this protocol.

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 <u>Laboratory's Personnel to be Assigned to the Study:</u>
Study Diseases
Quality 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 the test be started more than 72 hours after sample collection.
- 2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

#### 3. TEST MATERIAL

An effluent, receiving water sample, pore water or elutriate sample, or other test material as requested. A reference toxicant test is run concurrently.

#### 4. DILUTION WATER

Dilution water is filtered ( $\leq 0.45 \, \mu m$ ) Yaquina Bay seawater or other suitable seawater, adjusted to  $30 \pm 2 \, \%$  salinity with deionized water and/or hypersaline brine. Hypersaline brine is prepared from filtered ( $\leq 0.45 \, \mu m$ ) Yaquina Bay water adjusted to 100 % by the freezing method. When testing low salinity effluents, hypersaline brine is administered with dilution water for salinity adjustment.

#### 5. TEST ORGANISMS

- 5.1 Species: Commonly used West Coast species are Pacific oyster, Crassostrea gigas, or blue mussel, Mytilus edulis, M. galloprovincialis, or M. trossulus. These three Mytilus species were formerly all believed to be a single cosmopolitan species, M. edulis (Geller et al., 1993; McDonald & Koehn, 1988; McDonald et al., 1991). The test conditions specified in this protocol apply to the aforementioned species. Other species (e.g. M. californianus, C. virginica and Mercenaria mercenaria) are allowed by one or more of the referenced methods applicable to this protocol, but their use may require modified test conditions or procedures.
- 5.2 <u>Source</u>: Adult oysters are purchased from commercial sources. Mussels are purchased from commercial sources or field collected as required.
- 5.3 Age at Study Initiation: <4-hr-old embryos.
- 5.4 <u>Conditioning of Adult Oysters</u>: Adult oysters may be conditioned if needed by holding for one to eight weeks in seasoned plastic tubs supplied with about 1-2 L/min of unfiltered Yaquina Bay, OR water (25-32 ‰) at a temperature of approximately 20°C. For mussels, conditioning is not ordinarily required.
- 5.5 Spawning and Fertilization: Adult bivalves are cleaned by brushing and placed into spawning trays supplied with seawater. Oysters are spawned by gradually increasing the water temperature to 25-28°C (23-25°C for mussels) over approximately a one-hour period. Sperm from a sacrificed male may be added to the spawning tray to aid stimulation of natural spawning in oysters. If spawning does not occur, the water is cooled to about 20°C (16°C for mussels) and the cycle is repeated. Bivalves that begin spawning are isolated in clean seawater for collection of gametes. After spawning is complete, the temperature is returned to approximately 20°C (16°C for mussels).

Eggs from two or more females are combined and filtered (200-300  $\mu$ m) to remove feces and psuedofeces and adjusted in concentration to about 2500-6000/ml. Eggs are then fertilized by addition of sperm from two or more males at a concentration of  $10^5$  to  $10^7$ /ml. For mussels, ten minutes after adding sperm, the egg and sperm mixture is poured through a 25  $\mu$ m screen to remove excess sperm; then the eggs are rinsed and resuspended in dilution water. Next, the embryo density is adjusted to between 1500 and 3000/ml. Embryos are kept suspended by frequent gentle agitation with a perforated plunger and the temperature is maintained at approximately 20°C ( $16 \pm 1$ °C for mussels). The quality of the embryos is verified before testing by microscopic examination. Embryos are used to initiate the test within 4 hours of fertilization

#### 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 or with a combination of hypersaline brine and dilution water. Hypersaline brine may be required when testing dilute effluents to adjust the salinity of the test solutions to the appropriate salinity. Stock test solutions are prepared then distributed to appropriate replicate test chambers. The method for determining the appropriate volume of test material, brine and dilution water to be used in preparing the stock test solution is described in the laboratory SOP for salinity adjustment using hypersaline brine. Prior to mixing, the test material and dilution water are brought to test temperature. Effluents may not be aerated, or are aerated only if necessary to maintain a minimal dissolved oxygen concentration. When necessary, a brine control is prepared at the highest test concentration by substituting for the effluent deionized water to which has been added sufficient dilution water to achieve a salinity equal to that of the effluent.
- 6.2 <u>Test Chambers and Environmental Control</u>: Larvae are tested in 30 ml glass vials containing 10 ml of the test solutions. Temperature control of test chambers is provided by placement in a constant temperature room. No aeration is required. The required photoperiod is achieved by timer control of the room lights.
- 6.3 <u>Cleaning</u>: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use. For this test, there is an exception in that the test chambers are used new and unwashed

#### 7. EXPERIMENTAL DESIGN AND TEST PROCEDURES

- 7.1 Experimental Design: The test involves exposure of test embryos, within 4 hr of fertilization, to five or more test material concentrations and a dilution water control. Low salinity effluents require brine adjustment of salinity. Brine controls (substituting Milli-Q or low salinity water for the effluent) are run when brine is used to test effluent concentrations up to 70% effluent. A typical effluent concentration series might be 70%, 35%, 18%, 9%, 4%, 2%, 1%, and control. Exposures are for approximately 48 hours, but in no case shall the duration of exposure exceed 54 hours. Each treatment and control consists of four replicate 30 ml test vessels containing 10 ml of test solution. The final density of the embryos is between 15 and 30 embryos/ml in the test solutions. A stratified random design is employed to position vials in the temperature control chamber.
- 7.2 <u>Test Procedure</u>: Each test container is filled with 10 ml of test solution to which is added embryos at a final density of 15-30 embryos/ml. The embryos are incubated at  $20 \pm 1^{\circ}$ C ( $16 \pm 1^{\circ}$ C for mussels) for approximately 48 hr to permit development into prodissoconch I larvae. Larvae are subsequently counted to determine the total number of abnormal and normal surviving larvae. These data are used for calculating the EC50s and LC50s.
- 7.3 <u>Effect Criteria</u>: The effect criteria are: 1) failure of embryos to survive and produce completely developed shells (abnormal/dead); and 2) mortality of the embryos.
- 7.4 Test Conditions: The test temperature is  $20 \pm 1^{\circ}$ C for oysters,  $16 \pm 1^{\circ}$ C for blue mussels. The test temperatures specified by EPA (EPA/600/R-95/136) are  $15 \pm 1^{\circ}$ C or  $18 \pm 1^{\circ}$ C, but these specifications were based on erroneous assumptions of the agency authors. Consequently, this protocol specifies  $16 \pm 1^{\circ}$ C. The salinity is 30  $\pm$  2 ‰. The dissolved oxygen concentration should be at least 60% of saturation at the test temperature and salinity. The photoperiod is a 16:8 hr, L/D cycle of fluorescent light. Test chambers are 30 ml glass vials held in a constant temperature room to obtain precise temperature control.
- 7.5 <u>Beginning of Test</u>: 10 ml of each test concentration is dispensed to each of the corresponding four replicate test vials. The test is then started by the addition of 0.1 ml of a suspension (1,500-3,000 embryos/ml) of <4-hr-old

embryos to the test chambers. Six extra vials of seawater controls are preserved with 5% buffered formalin for establishing the initial count of embryos in the test vessels.

- 7.6 Feeding: Embryos are not fed during the test.
- 7.7 <u>Test Duration, Type and Frequency of Observations, and Methods</u>: The test duration is approximately 48 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	
Initial number of embryos/10 ml	At start of test in six 0-time vials
Number of live abnormal larvae/10 ml	At end of test (48 hr)
Number of live normal larvae/10 ml	At end of test (48 hr)
Physical and Chemical Data	
Temperature	Daily - in water bath or two locations in the temperature
	control room. Beginning & end of test - in the beaker
	reservoirs of each test concentration and controls.
Dissolved oxygen, salinity & pH	Beginning & end of test - in the beaker reservoirs of each test
	concentration and controls.

The initial number of embryos is determined according to method 2 (Sect. 11.4.6.2) of ASTM 1989. This consists of the average count of all embryos exhibiting cell division in six extra test containers at time zero. Live abnormal larvae are those observed at 48 hr in which shell development is incomplete. Live normal larvae are those observed at 48 hr that have completely developed shells containing meat. Larvae possessing misshapen or otherwise malformed shells are considered normal, provided shell development has been completed.

Temperature is measured using a thermister thermometer. Dissolved oxygen is measured using a polarographic oxygen probe calibrated according to the manufacturer's recommendations. Salinity is measured using a refractometer. The pH is measured with a pH probe and a calibrated meter with scale divisions of 0.1 pH units.

#### 8. CRITERIA OF TEST ACCEPTANCE:

For the EPA West Coast bivalve toxicity method (EPA/600/R-95/136) the test is considered acceptable if:

- ≥70% of embryos introduced into a required control treatment result in live larvae (≥50% for mussels).
- 2. normal shell development in surviving controls is ≥90%.

For the WDOE bivalve toxicity method (Publication No. WQ-R-95-80) the test is considered acceptable if:

- ≥70% of embryos introduced into a required control treatment result in live larvae.
- 2. normal shell development in surviving controls is ≥90%.
- 3. the test must achieve a minimum significant difference (%MSD) of <25% relative to the control.
- 4. the coefficient of variation of the six zero time counts must be ≤15%.

For the ASTM bivalve toxicity method (E 724-89) the test is considered acceptable if:

- 1. All test chambers were identical.
- 2. Treatments were randomly assigned to individual test chamber locations.
- 3. Either a dilution water or solvent control was included.
- 4. All brood stock animals came from the same location.
- 5. Embryos were used at <4 hr after fertilization.
- 6. ≥70% of embryos introduced into a required control treatment resulted in live larvae with completely developed shells at the end of the test.
- 7. The DO and temperature were measured as specified in Sect. 7.7 of the method.
- 8. Every measured DO concentration was between 60% and 100% saturation.
- 9. The difference between the time-weighted average measured temperatures for any two test chambers from the beginning to the end of the test was ≤1°C.

- Any single measured temperature was not more than 3°C different from the mean of the time-weighted average measured temperatures for individual test chambers.
- 11. At any one time, the difference between the measured temperatures in any two chambers was not more than 2°C.
- 12. Each data set must have at least one mean treatment response, corrected for controls, that is <37% and one that is >63% (not applicable for many applications).

#### DATA ANALYSIS

The proportion of normal larvae and the proportion of surviving larvae are calculated for each treatment replicate.

The means are obtained for each treatment level and the latter are then corrected for control response using Abbott's formula.

For ASTM (ASTM Standard E 724-89) and EPA (EPA/600/R-95/136) the LC50 (survival) and the EC50 (normal) are calculated, where data permits, using either the Maximum Likelihood Probit or the Trimmed Spearman-Karber methods (EPA 600/4-90-027F). An IC25 is calculated by linear interpolation with bootstrapping (EPA 600/4-89/001a). NOEC and LOEC values for survival and normality are computed using either Dunnett's test, T-test with Bonferroni's Adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni's Adjustment. The appropriate test is selected after evaluating the data for normality and homogeneity of variance. An arcsine square root transformation is performed on the data prior to statistical analysis. The statistical software employed for these calculations is ToxCalc, (most recent version), Tidepool Scientific Software.

For special endpoints requirements applicable in the State of Washington, refer to the WDOE guidance manual (Publication No. WQ-R-95-80, Revised December 1998) or latest version.

Some agencies require that toxic units (TU) be reported. This is reported as either toxic unit acute (TU<sub>a</sub>), which is 100/LC50, or toxic unit chronic (TU<sub>c</sub>), which is 100/NOEC.

#### 10. REPORTING

A report of the test results must include all of the following standard information at a minimum:

- 1. Name and identification of the test; the investigator and laboratory;
- 2. Information on the test material;
- 3. Information on the dilution water;
- Detailed information about the test organisms including acclimation conditions;
- 5. A description of the experimental design and test chambers and other test conditions including water quality;
- 6. Information about any aeration that may have been required;
- 7. Definition of the effect criteria and other observations;
- 8. Responses, if any, in the control treatment;
- 9. Tabulation and statistical analysis of measured responses;
- 10. A description of the statistical methods used;
- 11. Any unusual information about the test or deviations from procedures;
- 12. Reference toxicant testing information.

#### 11. STUDY DESIGN ALTERATION

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

#### 12. REFERENCE TOXICANT

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

#### 13. <u>REFERENCES AND GUIDELINES</u>

Geller, J.B. *et al.* 1993. Interspecific and intrapopulation variation in mitochondrial ribosomal DNA sequences of *Mytilus* spp. (Bivalvia: Mollusca). Molecular Marine Biology and Biotechnology, 2:44-50.

McDonald, J.H. and R.K. Koehn. 1988. The mussels *Mytilus galloprovincialis* and *M. trossulus* on the Pacific coast of North America. Marine Biology. 99:111-118.

McDonald, J.H. et al. 1991. Allozymes and morphometric characters of three species of *Mytilus* in the northern and southern hemispheres. Marine Biology.

Standard Guide for Conducting Static Acute Toxicity Tests with Embryos of Four Species of Saltwater Bivalve Molluscs. 1989. ASTM Standard E 724-89.

U.S. Environmental Protection Agency. 1989. Supplement to "Short-term methods for estimating the chronic toxicity of effluents and surface waters to freshwater organisms". Revision 1. EPA/600/4-89/001a.

Washington State Department of Ecology. 1998. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Publication No. WQ-R-95-80. Revised December 1998.

Weber, C.I. (Ed.) 1993. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms (Fourth Edition). EPA/600/4-90/027F.

U.S. Environmental Protection Agency. 1995. Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms (First Edition). EPA/600/R-95/136.

#### 14. APPROVALS

		for
Name	Date	
		for Northwestern Aquatic Sciences
Name	Date	<del></del>

# Appendix A Test Conditions Summary

1. Test type:	Static non-renewal
2. Test duration:	48 hours, or until complete development up to 54 hours
3. Temperature:	20 ± 1°C oysters
	$16 \pm 1$ °C mussels (ASTM), 15 or $18 \pm 1$ °C (EPA 1995)
4. Dissolved oxygen:	≥ 60% saturation
5. Salinity:	30 ± 2‰
6. Light quality & intensity:	Ambient laboratory light (50-100 ft-c)
7. Photoperiod:	16:8 hr L/D
8. Test chambers:	30 ml glass vials
9. Test solution volume:	10 ml per replicate
10. Renewal of test solutions:	None
11. Age of test organisms:	<4 hr old embryos
12. No. of larvae/container:	150-300
13. No. of replicates/treatment:	4
14. No. of zero time replicates:	6
15. Feeding regime:	Organisms are not fed during the test.
16. Aeration:	None. Initially aerated if necessary to achieve >60% saturation.
17. Dilution water:	Filtered Yaquina Bay seawater, salinity adjusted to 30 ± 2‰ and filtered to
	≤0.45 μm.
18. Effects measured:	Survival and normal shell development.
19. Test acceptability:	≥70% of embryos introduced into a required control treatment resulted in
	live larvae (≥50% for mussels, EPA 1995); ≥90% normal shell development
	in surviving controls; must achieve minimum significant difference
	(%MSD) of <25% relative to the control. The cv of six zero time counts
	must be ≤15%.
20. Sample volume required:	1 L normally requested.

# APPENDIX II RAW DATA

# BIVALVE LARVAL TEST BASED ON EPA/600/R-95/136

Test No	658-	-80Clier	t:	CH2M Hill - Wy	ckoff		Investigato	L
STUDY M	ANAGE	MENT		-		_		
Client:	(	CH2M Hill W	yckoff <b>Trea</b> f	tment Plant, 53	50 Creos	sote Place NE, E	Bainbridge Island,	WA 98110
Client's	_	Monitor:		ey Warner		,		
				atic Sciences		<u> </u>		
		Newport Lat						
		tudy Personi	-	Sa				
	-	udy Dir.	G.A. Buhl	er /G.J. Iriss	arri 677			
	officer	•	Nemeth	70.0.11133	<u> </u>			
1.	illicei ;		Calcam		2.	+ 0 -	12	
3.		10-67 100	Chilland		$-\frac{2}{4}$ $-$	75. CALVA		
	2 a la a al col			<del>-</del>	— <sup>4.</sup> —	475- CALAU	211	
-	Schedul					T	> 15 15	111-
l est Be	eginning	g:	3-15-17	1400		Test Ending:	3-17-17	1405
TEST MA			C=>					
	scription		GROUND A	PATER SP-1	<u> </u>			
	S Samı			5845G				
		ollection:		3-14-17				
	te of Re			3-15-17				
	•	ure (deg C):		2,4				
pН				7,4				
		oxygen (mg/		10,6				
Co	nductivi	ity (umhos/cı	n): _	-				
Ha	rdness	(mg/L):	_	_				
Alk	calinity (	mg/L):						
Sa	linity (p	pt):		5,0				<del></del>
To	tal chloi	rine (mg/L):						
To	tal amm	nonia-N (mg/	L):					
		, -						
DILUTION	WATE	R						
	scriptio		Yaquina I	Ray OR				
	•	ollection:	raquina	3-14-17		Salinity (ppt)	30 0 pl	H 9,0
	eatment		tod filtorod		colinity o		i <del>-Q deienized wa</del> t	
116	eauniein					•		C PENE
_		( j	TALE OF	トにモドルモ井フル	7-2	0-17, SALIN	114 100.0	
TEST OR	GANISI	VIS						
Sp	ecies:	Myti	lus galloprov	vincialis		D	ate Received:	3-8-17
•	urce:			farm, Carlsbad	. CA		_	2 - 1
	_	n Data:	· · · · · · · · · · · · · · · · · · ·	,				
<u>-</u>		Temp (d <b>eg.(</b>	c) pH	Sal (ppt)		D.O. (mg/L)	Comn	nents
-2	1-8-17	ionp (dog.c	7.9	15.0		9.9	Held outside in t	
		19.0	7.9	12.0		8.7	flowing seaw	
_	1-13-17		7.7			<u></u>	HOWING SEAW	uci -
	$\overline{}$							
3_	-15-17	14.5	7.8	26.	-	8.0		
_		138 at .						
_		95 T4.5				43.0		
_	Mean	14.8	7.9	18.		8.8		
_	S.D.	29130	0,1	6:3	5	0,8	ļ	
_	(N)	4	4	4		<u>4</u>	<u></u>	
Ph	otoperio	nd during acc	rlimation:	Outdoor at	mbient c	onditions		

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

# BIVALVE LARVAL TEST BASED ON EPA/600/R-95/136

Test No.	658-80	Client	CH2M Hill - Wyckoff			Investigator		
SPAWNIN	G AND GA	METE HAND	LING					
Spawn	ing: Initial:	0910	Final:	0950		Fertilization:	1.0	030
			females: 3		males:	3		
		diluted to 100		-	•			
	Count/m	l of dilution:	1, 37 2	. 49	3.	39	Mean:	41.7
	Dilution f	actor = DF (m	ean x 100/2500) =	1.7	- -		_	
TEST PRO	CEDURES	S AND COND	ITIONS					
Test co	oncentration	ns (50% serie	s recommended):	70, 35, 18,	9, 4, 2, 0%	+ Brine Con	trol	
	<u>,—_</u>							
Test cl	hamber: 30	ml glass vials	Test volu	me: 10 ml		Replicates/tre	eatment	(4): <u>-</u> 4
Organi	isms/ml (15	-30): <u>21.</u>	Test water	er changes: No	one	Aeration duri	ng test: N	Vone
Feedin	ıg: None		Photoper	iod: 16L:8D		Salinity: 30 +	/- 2 ppt	
Tempe	erature: 20 -	+/- 1 <sup>º</sup> C, oyste	rs; 16 +/- 1 °C, mus	sels	Beaker pl	acement:Stra	atified rar	ndomization

#### RANDOMIZATION CHART

Α	10	9	Þ	Be	Y	35	2	18	
В	2	7	18	9	70	Ø	35	BC	
С	35	2	3 C	4	18	70	Ý	G	
D	Ø	18	4	38	2	BC	9	70	

#### PREPARATION OF TEST SOLUTIONS

This test uses a brine control \_\_\_\_\_; a salinity control \_\_\_\_\_;

If a brine control is used, follow SOP #6208 to prepare test solutions

Date of brine preparation: 2-20-17; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

VB = VE  $\frac{(TS - SE)}{(SB - TS)} = VE \frac{(30 - 5)}{(100 - 50)} = VE (0.36)$ 

Where:

VB=volume brine VE=volume effluent SB=salinity of brine SE=salinity of effluent TS=target salinity

In making up either a brine control or a salinity control, use salinity-adjusted deionized water in place of the effluent.

	Test Conc.	Effluent	Brine	Dilution Water
	(%)	(ml/100ml)	(ml/100ml)	(ml/100ml)
c-17	70	70	25.0	Brought up to a final
3-15-17	35	35	12.5	volume of 100 ml
63	18	18	6,4	with dilution water
	9	9	3,2	
	4	4	1,4	
	2	2	0.7	
	0	0	φ	
	Brine Control	0	25.0	

THE BRINE CONTROL WAS MADE UP OF SAUNTY ADJUSTED MILLI-O DEIGNIZED WATER (5.0 PPT) STAVLAR TO THE EFFLUENT. AS A RESULT, THE AMOUNT OF BRINE IN THE BRINE CONTROL WAS THE SAME USED IN THE FOR EFFLUENT CONCENTRATION.

-631

# BIVALVE LARVAL TEST BASED ON EPA/600/R-95/136

Test No. 658-80 Client CH2M Hill - Wyckoff

Investigator

#### **WATER QUALITY DATA**

	Date: 3-15-17 initials: 631			631	Date: 37717 initials:			5
Conc. ( % )	Temp. (deg.C)	рН	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	рН	Sal. (ppt)	DO (mg/L)
70	15.4	7.7	30,0	7.9	15-2	8-5	300	8.0
35	15.6	7.7	30,0	7.9	15-2	8.4	300	8-0
18	15,6	7.9	30,0	7.9	15-2	8-3	305	8-0
9	15.8	7,0	30,0	8,0	15.2	8-2-	300	8-1
4	15.8	8.0	30,0	8,0	15-2	8-1	300	8-1
2	16.0	7.0	30,0	8,0	15-2	8-1	300	8-0
Control	16,1	₹, ₽	30.0	8.1	15-2	87	300	8-1
Brine control	16.6	8.1	29.5	8.1	15-2	8-1	300	8-0

**WATER QUALITY:** Mean <u>SD</u> <u>N</u> Temperature ( ℃): 15.5 0.4 рΗ 8,1 0,2 16 Salinity (ppt): 30,0 16 0.2 DO (mg/L): 16 8,0 0.1

Room/ Water bath temperature: (°C)

Day 0: 16.1 Day 0: 16.1 Day 1: 15.2 Day 1: 16.1 Day 2: 15.5

	<del>/</del>		LARVA ろっとノーノ	L COUNTY	PATA —		1 Ace:	3/19/17
Conc.	Replic	cate 1	Replic	cate 2	Replic	ate 3	Repli	cate 4
(%)	N	Α	N	Α	N	Α	N	Α
70	198	2_	208	4	196	5	216	Ø
35	211	3	194	6	2/4	5	199	E/
18	19.2	3	2/7	5-	185	1	187	3
9	189	Ø	204	7	193	3	201	Ÿ
4	211	7	210	2	201	Ø	197	6
2	220	1	200	CE	179	/	184	3
Control	193	2	174	2	198		191	1
Brine control	175	3	210	3	205	4	209	3
Zero time	218	214	186	215	204	227	-	

Zero time: Mean 211 SD 14 N 6

CV=(sd/mean)x100 6,7%

Remarks:

# Invoice



**DATE** INVOICE # 3/1/2017 195377

Sustainable Mariculture ~ Preserving Our Oceans' Resources

MAIL TO:

Carlsbad Aquafarm
P.O Box 2600
Carlsbad, CA 92018
760-438-2444
info@carlsbadaquafarm.com

**BILL TO:** 

Northwestern Aquatic Sciences 3814 Yaquina Bay Road Newport, Oregon 97365

P.O. #	TERMS	SHIP DATE	SHIP VIA	F.	O.B.	A	IRBILL#
	Net 7	3/1/2017		s	.D.	778	547239894
QUANTITY	ITEM	DES	CRIPTION		PRICE	LBS.	AMOUNT
1	Bio Assay Mus BOX CHARGE	Вох	alloprovinci c Charge  3-8-17  -631			_	
THIS IS YOU	JR INVOICE		· ·		Tota	al	\$140.00

# **CETIS Analytical Report**

Report Date: Test Code: 22 Mar-17 08:48 (p 2 of 2) 658-80 02-8040-0265

Bivalve Larva										
	al Survival and Develo	pment Test						Northwest	ern Aquati	Sciences
Analysis ID: Analyzed:	08-8549-6548 22 Mar-17 8:47	Endpoint: Pro	portion Surv				S Version:		.8.7	
Batch ID:	05-1514-6467	Test Type: Dev		<del></del>						
Start Date:	15 Mar-17 14:00	• • •	V600/R-95/			Anal		juina Bay Se	anuator	
Ending Date:			ilis galloprov			Brine		luma bay Se	awalei	
Duration:	48h	- •	ins galloprot Isbad Aquaf			Age:				
Sample ID:	12-3464-8860		73F1C			Clier	=	ckoff Treatm	ent Plant	
•	: 14 Mar-17 09:20 :: 15 Mar-17 12:25		ustrial Efflue ckoff	nt		Proje	BCT:			
_	29h (2.4 °C)	Station:	CKUII							
								_		<u>.</u>
Data Transfo Angular (Corre		Alt Hyp	Trials NA	Seed NA		PMSD 19.3%	Test Res		nivod	
			19/5	- N/A		15.376	Passes p	roportion su	rviveu	
	ce t Two-Sample Test									
Control	vs Control	. Test Stat			P-Value	P-Type	Decision	· · ·		_
Dilution Water	r Brine Reagent	1.73	2.447	0.237 6	0.1344	CDF	Non-Sign	ificant Effect		
<b>Auxiliary Tes</b>	ts									
Attribute	Test		Test Stat	Critical	P-Value	Decision(	(a:5%)			
Extreme Value	e Grubbs Extrem	e Value	2.074	2.127	0.0751	No Outlier	s Detected			
ANOVA Table	3		<del></del> -							
Source	Sum Squares	Mean Squ		DF	F Stat	P-Value	Decision	·		
Between	0.05629235	0.0562923		1	2.992	0.1344	Non-Sign	ificant Effect	ł	
Error	0.1128788	0.0188131	4	6						
Total	0.1691712			7						
Distributiona	l Tests									
Attribute	Test		Test Stat	Critical	P-Value	Declaion(	(a:1%)			
Attribute Variances	Test Variance Ratio	F	Test Stat 5.356	Critical 47.47	P-Value 0.2016	Decision( Equal Var	· ·			
Variances Variances	Variance Ratio Mod Levene Ed	quality of Variance	5.356 0.4967	47.47 13.75	0.2016 0.5074	Equal Var Equal Var	iances			
Variances Variances Variances	Variance Ratio Mod Levene Ed Levene Equalit	quality of Variance y of Variance	5.356 0.4967 2.265	47.47 13.75 13.75	0.2016 0.5074 0.1830	Equal Var Equal Var Equal Var	iances iances iances			
Variances Variances Variances Distribution	Variance Ratio Mod Levene Ed Levene Equality Shapiro-Wilk W	quality of Variance y of Variance V Normality	5.356 0.4967 2.265 0.8289	47.47 13.75 13.75 0.6451	0.2016 0.5074 0.1830 0.0578	Equal Var Equal Var Equal Var Normal Di	iances iances iances istribution			
Variances Variances Variances Distribution Distribution	Variance Ratio Mod Levene Ed Levene Equalit Shaplro-Wilk W Kolmogorov-Sr	quality of Variance y of Variance V Normality mirnov D	5.356 0.4967 2.265 0.8289 0.2696	47.47 13.75 13.75 0.6451 0.3313	0.2016 0.5074 0.1830 0.0578 0.0899	Equal Var Equal Var Equal Var Normal Di Normal Di	iances iances iances istribution istribution		_	
Variances Variances Variances Distribution Distribution Distribution	Variance Ratio Mod Levene Ec Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli	quality of Variance y of Variance V Normality	5.356 0.4967 2.265 0.8289	47.47 13.75 13.75 0.6451	0.2016 0.5074 0.1830 0.0578	Equal Var Equal Var Equal Var Normal Di	iances iances iances istribution istribution			
Variances Variances Variances Distribution Distribution Distribution Proportion S	Variance Ratio Mod Levene Ec Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli	quality of Variance y of Variance V Normality nimov D ing A2 Normality	5.356 0.4967 2.265 0.8289 0.2696 0.6769	47.47 13.75 13.75 0.6451 0.3313 3.878	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772	Equal Var Equal Var Equal Var Normal Di Normal Di	iances iances iances istribution istribution			
Variances Variances Variances Distribution Distribution Distribution Proportion S C-%	Variance Ratio Mod Levene Ec Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou	quality of Variance y of Variance V Normality mimov D ing A2 Normality	5.356 0.4967 2.265 0.8289 0.2696 0.6769	47.47 13.75 13.75 0.6451 0.3313 3.878	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772	Equal Var Equal Var Equal Var Normal Di Normal Di	iances iances iances iances istribution istribution	Std Err	CV%	%Effect
Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0	Variance Ratio Mod Levene Ec Levene Equality Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou	quality of Variance y of Variance V Normality mirrov D ing A2 Normality  Int Mean 0.9028	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Min 0.8341	iances iances iances iances istribution istribution Max 0,9431	0.02389	5.29%	0.0%
Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0	Variance Ratio Mod Levene Equalit Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4	quality of Variance y of Variance V Normality mirnov D ing A2 Normality  Int Mean 0.9028 0.9585	5.356 0.4967 2.265 0.8289 0.2696 0.6769	47.47 13.75 13.75 0.6451 0.3313 3.878	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772	Equal Var Equal Var Equal Var Normal Di Normal Di	iances iances iances iances istribution istribution			
Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Con	Variance Ratio Mod Levene Equality Shaplro-Wilk Wilder School Sch	quality of Variance y of Variance V Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution Max 0.9431	0.02389 0.03837	5.29% 8.01%	0.0% -6.17%
Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Corn	Variance Ratio Mod Levene Ec Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4 Tected) Transformed S Control Type Cou	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution Max 0.9431 1	0.02389 0.03837 Std Err	5.29% 8.01% CV%	0.0% -6.17% %Effect
Variances Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Con	Variance Ratio Mod Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4  rected) Transformed S Control Type Cou	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Cort C-% 0 0	Variance Ratio Mod Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4 Tected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution Max 0.9431 1	0.02389 0.03837 Std Err	5.29% 8.01% CV%	0.0% -6.17% %Effect
Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Cort C-% 0 0	Variance Ratio Mod Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4  rected) Transformed S Control Type Cou	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Variances Distribution Distribution Proportion S C-% 0 0 Angular (Corr C-% 0 0 Proportion S C-%	Variance Ratio Mod Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4  rected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4  urvived Detail Rep	quality of Variance y of Variance y Normality mirrov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26 1.428	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1 95% UCL 1.382 1.711	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Cort C-% 0 0 Proportion S C-%	Variance Ratio Mod Levene Equality Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli  urvived Summary  Control Type Cou Dilution Water 4 Brine Reagent 4  Tected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4  urvived Detail Control Type Rep Dilution Water 0.92	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26 1.428	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1 1 95% UCL 1.382 1.711	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Variances Distribution Distribution Proportion S C-% 0 0 Angular (Corr C-% 0 0 Proportion S C-%	Variance Ratio Mod Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4  rected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4  urvived Detail Rep	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26 1.428	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1 95% UCL 1.382 1.711	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Variances Dlstribution Distribution Proportion S C-% 0 0 Angular (Corr C-% 0 0 Proportion S C-% 0 0	Variance Ratio Mod Levene Equality Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli  urvived Summary  Control Type Cou Dilution Water 4 Brine Reagent 4  Tected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4  urvived Detail Control Type Rep Dilution Water 0.92	quality of Variance y of Variance y Normality mirrov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26 1.428  1 Rep 2 122 123 136 1	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1 1 95% UCL 1.382 1.711	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436 Min 1.151	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Variances Distribution Distribution Distribution Proportion S C-% 0 0 Angular (Corr C-% 0 0 Proportion S C-% 0 0 Angular (Corr C-% 0 0 Angular (Corr	Variance Ratio Mod Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4  rected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4  urvived Detail Control Type Rep Dilution Water 0.92 Brine Reagent 0.84	quality of Variance y of Variance y Normality mimov D ing A2 Normality  Int Mean 0,9028 0,9585  Summary Int Mean 1,26 1,428  1 Rep 2 142 0,8341 136 1  Detail	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144	47.47 13.75 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1 1 95% UCL 1.382 1.711	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436 Min 1.151	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%
Variances Variances Variances Variances Distribution Distribution Proportion S C-% 0 0 Angular (Cort C-% 0 0 Proportion S C-% 0 0	Variance Ratio Mod Levene Ec Levene Equalit Shaplro-Wilk W Kolmogorov-Sr Anderson-Darli urvived Summary Control Type Cou Dilution Water 4 Brine Reagent 4  Trected) Transformed S Control Type Cou Dilution Water 4 Brine Reagent 4  urvived Detail Control Type Rep Dilution Water 0.92 Brine Reagent 0.84  Trected) Transformed I	quality of Variance y of Variance y of Variance y Normality mirrov D ing A2 Normality  Int Mean 0.9028 0.9585  Summary Int Mean 1.26 1.428  1 Rep 2 142 0.8341 136 1  Detail 1 Rep 2	5.356 0.4967 2.265 0.8289 0.2696 0.6769 95% LCL 0.8268 0.8364 95% LCL 1.137 1.144 Rep 3 0.9431 0.9905	47.47 13.75 0.6451 0.3313 3.878 95% UCL 0.9789 1 95% UCL 1.382 1.711 Rep 4 0.91	0.2016 0.5074 0.1830 0.0578 0.0899 0.0772 Median 0.9336 0.9336	Equal Var Equal Var Equal Var Normal Di Normal Di Normal Di Min 0.8341 0.8436 Min 1.151	iances iances iances iances istribution istribution istribution  Max 0.9431 1  Max 1.33	0.02389 0.03837 Std Err 0.03847	5.29% 8.01% CV% 6.11%	0.0% -6.17% %Effect 0.0%

# **CETIS Analytical Report**

000-091-187-4

Report Date: Test Code: 22 Mar-17 08:48 (p 3 of 3) 658-80 02-8040-0265

**Bivalve Larval Survival and Development Test** Northwestern Aquatic Sciences Endpoint Proportion Survived Analysis ID: 12-1613-9556 **CETIS Version: CETISv1.8.7** Parametric-Control vs Treatments Analyzed: 22 Mar-17 8:44 Analysis: Official Results: Yes Batch ID: Test Type: Development-Survival 05-1514-6467 Analyst: Start Date: 15 Mar-17 14:00 Protocol: EPA/600/R-95/136 (1995) **Diluent:** Yaquina Bay Seawater Ending Date: 17 Mar-17 14:05 Species: Mytilis galloprovincialis Brine: **Duration:** Source: Carlsbad Aquafarms Age: 12-3464-8860 Sample ID: Code: 49973F1C Cllent: Wyckoff Treatment Plant Sample Date: 14 Mar-17 09:20 Material: Industrial Effluent Project: Receive Date: 15 Mar-17 12:25 Source: Wyckoff Sample Age: 29h (2.4 °C) Station: **Data Transform** Zeta Trials Seed **PMSD** NOEL) LOEL **TOEL** TU Alt Hyp Angular (Corrected) NA C > T NA NA 16.4% 70 >70 NA 1.429 **Dunnett Multiple Comparison Test** Control C-% Critical ٧S **Test Stat** MSD **DF P-Value** P-Type Decision(a:5%) **Dllution Water** 2 -0.7158 2.448 0.207 6 0.9720 CDF Non-Significant Effect -2.2482.448 0.207 6 CDF 4 0.9998 Non-Significant Effect 9 -1.3062.448 0.207 6 0.9949 CDF Non-Significant Effect 18 -0.7643 2.448 0.207 6 0.9754 **CDF** Non-Significant Effect 35 -2.387 2.448 0.207 6 0.9999 CDF Non-Significant Effect 70 2.448 0.207 6 CDF -2.1470.9997 Non-Significant Effect **Auxiliary Tests** Attribute Test Test Stat Critical P-Value Decision(a:5%) Grubbs Extreme Value 2.051 2.876 Extreme Value 0.9511 No Outliers Detected **ANOVA Table** Source Sum Squares DF F Stat Mean Square P-Value Decision(a:5%) 6 Between 0.1451618 0.02419363 1.695 0.1716 Non-Significant Effect 21 Ептог 0.2997454 0.01427359 Total 0.4449072 27 Distributional Tests **Test Stat** Critical Attribute Test P-Value Decision(a:1%) **Bartlett Equality of Variance** 2.009 Variances 16.81 0.9189 Equal Variances Shapiro-Wilk W Normality 0.9214 0.8975 0.0376 Distribution **Normal Distribution Proportion Survived Summary** C-% **Control Type** 95% UCL Min Count Mean 95% LCL Median Max Std Err CV% %Effect **Dilution Water** 0.9028 0.9789 0.8341 0.9431 5.29% 0.0% 0 4 0.8268 0.9171 0.02389 2 0.9218 0.8179 0.9171 0.8531 7.08% -2.1% 4 1 1 0.03263 4 4 0.9787 0.939 1 0.981 0.9526 0.01246 2.55% -8.4% 1 9 4 0.9491 0.8758 1 0.9502 0.8957 1 0.02301 4.85% -5.12% 18 4 0.9265 0.8438 1 0.9123 0.8815 1 0.02599 5.61% -2.63% 35 4 0.9822 0.9431 1 0.9905 0.9479 1 0.01229 2.5% -8.79% 70 0.9751 0.9293 0.9763 0.9479 -8.01% 1 1 0.0144 2.95% Angular (Corrected) Transformed Summary C-% **Control Type** Count 95% UCL Mean 95% LCL Median Min Max Std Err CV% %Effect Dilution Water 1.26 0 4 1.137 1.382 1.279 1.151 1.33 0.03847 6.11% 0.0% 2 4 1.32 1.067 1.574 1.284 1.177 1.536 0.07971 12.08% 4.8% 4 4 1.45 1,29 1.61 1.456 1.351 1.536 0.05025 6.93% -15.08% 9 4 1.37 1.165 1.575 1.351 1.536 0.06443 9.41% -8.76% 1.242 18 4 1.324 1.095 1.554 1.271 1.219 1.536 0.07221 10.91% -5.13% 35 4 1.461 1.311 1.612 1.485 1.34 1.536 0.04717 6.46% -16.01% 70 4 1.441 1.266 1.616 1.444 1.34 1.536 0.05503 7.64% -14.39%

Report Date:

22 Mar-17 08:48 (p 4 of 3) 658-80 102-8040-0265

12-1613-9556 22 Mar-17 8:44 urvived Detail	End	point: Pr	oportion Sun	vived )	CETIS Version:	Northwestern Aquatic Sciences
22 Mar-17 8:44 urvived Detail				/ived	CETIC Vomica:	OFTIO 4 0.7
urvived Detail	Ana	lysis: Pa			CETIO Version:	CETISv1.8.7
			rametric-Coi	itrol vs Treatments	Official Results:	Yes
						<del></del>
Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
Dilution Water	0.9242	0.8341	0.9431	0.91		
	1	0.9479	0.8531	0.8863		
	1	1	0.9526	0.9621		
	0.8957	0.9716	0.9289	1		
	0.9242	1	0.8815	0.9005		
	1	0.9479	1	0.981		
	0.9479	1	0.9526	1		
Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
Dilution Water	1.292	1.151	1.33	1.266		
	1.34	1.536	1.351	1.536		
<b>287</b>	5	•	Reject Null	0.25		• •
	rected) Transford Control Type Dilution Water	Dilution Water 0.9242 1 0.8957 0.9242 1 0.9479  rected) Transformed Detail  Control Type Rep 1 Dilution Water 1.292 1.536 1.536 1.242 1.292 1.536 1.34	Dilution Water 0.9242 0.8341 1 0.9479 1 1 0.8957 0.9716 0.9242 1 1 0.9479 0.9479 1  rected) Transformed Detail  Control Type Rep 1 Rep 2  Dilution Water 1.292 1.151 1.536 1.34 1.536 1.536 1.242 1.401 1.292 1.536 1.536 1.34 1.536 1.34 1.536 1.34 1.536 1.34	Dilution Water 0.9242 0.8341 0.9431 1 0.9479 0.8531 1 1 0.9526 0.8957 0.9716 0.9289 0.9242 1 0.8815 1 0.9479 1 0.9526  rected) Transformed Detail  Control Type Rep 1 Rep 2 Rep 3 Dilution Water 1.292 1.151 1.33 1.536 1.34 1.177 1.536 1.536 1.351 1.242 1.401 1.301 1.292 1.536 1.219 1.536 1.34 1.536 1.34 1.536 1.34 1.536	Dilution Water 0.9242 0.8341 0.9431 0.91  1 0.9479 0.8531 0.8863 1 1 0.9526 0.9621 0.8957 0.9716 0.9289 1 0.9242 1 0.8815 0.9005 1 0.9479 1 0.981 0.9479 1 0.9526 1   rected) Transformed Detail  Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 1.292 1.151 1.33 1.266 1.536 1.34 1.177 1.227 1.536 1.536 1.351 1.375 1.242 1.401 1.301 1.536 1.292 1.536 1.219 1.25 1.536 1.34 1.536 1.433 1.34 1.536 1.351 1.536	Dilution Water 0.9242 0.8341 0.9431 0.91  1 0.9479 0.8531 0.8863 1 1 0.9526 0.9621 0.8957 0.9716 0.9289 1 0.9242 1 0.8815 0.9005 1 0.9479 1 0.981 0.9479 1 0.9526 1   rected) Transformed Detail  Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 1.292 1.151 1.33 1.266 1.536 1.34 1.177 1.227 1.536 1.536 1.351 1.375 1.242 1.401 1.301 1.536 1.292 1.536 1.219 1.25 1.536 1.34 1.536 1.433 1.34 1.536 1.351 1.536

0.05 0.00 -0.05 -0.10 -0.15

-0.20 -0.25

-2.5 -2.0

-1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0

LC 50 > 70% BY DATA INSPECTION -631

C-%

0.1

Report Date:

22 Mar-17 08:48 (p 1 of 2)

Test Code:

658-80 02-8040-0265

Bivalve Larva	al Survival and D	evelopm	ent Test						Northwest	ern Aquati	c Sciences
Analysis ID:	15-0070-7739	Er	ndpoint: Co	mbined Prop	ortion Norm	nal )	CET	S Version	: CETISv1	.8.7	
Analyzed:	22 Mar-17 8:47			ametric-Two			Offic	ial Results	s: Yes		_
Batch ID:	05-1514-6467	Te	st Type: De	velopment-S	urvival		Anal	 vst:			
Start Date:	15 Mar-17 14:0			A/600/R-95/			Dilu		quina Bay Se	eawater	
Ending Date:				tilis gallopro	, ,		Brin			, a , , a , a , a , a , a , a , a , a ,	
Duration:	48h	- •		risbad Aquat			Age:				
Sample ID:	12-3464-8860	C	<del></del>	73F1C			Clier		ckoff Treatm	ont Bloot	·
	: 14 Mar-17 09:2			ustrial Efflue	ant		Proje		ckon rreau	ent Plant	
	e: 15 Mar-17 12:2			ckoff	711L		Proje	sci.			
Sample Age:	••	-	ation:	CKOII							
Data Transfo		Zeta NA	Alt Hyp C <> T	Trials NA	Seed		PMSD	Test Res			
Angular (Corr		INA		NA	NA .		18.2%	Passes o	ombined pro	poπion nor	mai
	ice t Two-Sample	Test									
Control	vs Control	1	Test Stat			P-Value	P-Type	Decision	• •	+	
Dilution Wate	r Brine Rea	agent)	1.474	2.447	0.220 6	0.1909	CDF (	Non-Sigr	ificant Effect	1	
Auxiliary Tes	is .										
Attribute	Test			Test Stat	Critical	P-Value	Decision	a:5%)			
Extreme Valu		xtreme Va	alue	1.998	2,127	0.1232		s Detected			
ANOVA Table											
	_		Maan Car		DE	F 64-4	D.V-I	B	( . E0()		
Source	Sum Squ		Mean Squ		DF	F Stat	P-Value	Decision	<del></del>	1	
Between	0.0351931		0.035193		1	2.173	0.1909	Non-Sign	ificant Effect	I	
Total	0.0971601 0.1323534		0.0161933	<u> </u>	6 7						
							_				
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	a:1%)			
Variances	Variance			4.424	47.47	0.2534	Equal Var	iances			
Variances		-	ity of Variance		13.75	0.4292	Equal Var				
Variances		quality of		1.604	13.75	0.2522	Equal Var	iances			
Distribution		Vilk W No		0.8717	0.6451	0.1565	Normal D				
Distribution	_	rov-Smirne		0.2842	0.3313	0.0563	Normal D				
Distribution	Andersor	n-Darling A	V2 Normality	0.559	3.87B 	0.1523	Normal Di	stribution			
Combined P	roportion Norma	l Summai	ry								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.8957	0.8171	0.9743	0.9265	0.8246	0.9384	0.0247	5.52%	0.0%
0	Brine Reagent	4	0.9467	0.8212	1	0.9265	0.8294	0.9953	0.03943	8.33%	-5.69%
Angular (Cor	rected) Transfor	med Sum	mary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.248	1.125	1.371	1.297	1.139	1.32	0.03864	6.19%	0.0%
ō	Brine Reagent	4	1.38	1.122	1.639	1.297	1.145	1.502	0.08126	11.77%	-10.63%
Combined Pr	roportion Norma	l Detail				<del></del>		•			
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	0.9147	0.8246	0.9384	0.9052						
0	Brine Reagent	0.8294	0.9953	0.9716	0.9905						
	-										
	rected) Transfor	med Deta	ii .								
Angular (Cor	•			Rep 3	Ren 4						
Angular (Cor	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
Angular (Cor	•			Rep 3 1.32 1.401	Rep 4 1.258 1.473						

Analyst:\_\_\_\_\_ QA:\_\_\_\_

# **CETIS Analytical Report**

Report Date: Test Code: 22 Mar-17 08:48 (p 1 of 3) 658-80 02-8040-0265

Bivaive Larval Survival and Development Test Northwestern Aquatic Sciences 09-0535-7845 Endpoint: Combined Proportion Normal **CETIS Version:** Analysis ID: CETISv1.8.7 Analyzed: 22 Mar-17 8:47 Parametric-Control vs Treatments Analysis: Official Results: Yes Batch ID: 05-1514-6467 Test Type: Development-Survival Analyst: Start Date: 15 Mar-17 14:00 Protocol: EPA/600/R-95/136 (1995) Diluent: Yaquina Bay Seawater Ending Date: 17 Mar-17 14:05 Species: Mytilis galloprovincialis Brine: **Duration:** Source: Carlsbad Aquafarms Age: Sample ID: 12-3464-8860 Code: 49973F1C Wyckoff Treatment Plant Cllent: Sample Date: 14 Mar-17 09:20 Material: Industrial Effluent Project: Receive Date: 15 Mar-17 12:25 Source: Wyckoff Sample Age: 29h (2.4 °C) Station: Data Transform Zeta NOEL Trials Seed **PMSD** LOEL TOEL Alt Hyp TU Angular (Corrected) NA C > T NA NA 15.5% 70 >70 NA 1.429 **Dunnett Multiple Comparison Test** Control C-% **Test Stat** Critical MSD DF P-Value P-Type Decision(a:5%) **Dilution Water** 2 -0.7276 2.448 0.192 0.9729 CDF 6 Non-Significant Effect 4 -2.2552.448 0.192 6 0.9998 CDF Non-Significant Effect 9 -1.2292.448 0.192 6 0.9936 CDF Non-Significant Effect -0.4268 18 2.448 0.192 6 0.9424 CDF Non-Significant Effect 35 -1.8372.448 CDF 0.192 6 0.9991 Non-Significant Effect 70 -1.966 2.448 0.192 6 0.9994 CDF Non-Significant Effect **Auxiliary Tests** Attribute Test Test Stat Critical P-Value Decision(a:5%) Extreme Value Grubbs Extreme Value 2.026 2.876 1.0000 No Outliers Detected **ANOVA Table** Source **Sum Squares** Mean Square DF F Stat P-Value Decision(a:5%) Between 0.107906 0.01798433 6 1.456 0.2412 Non-Significant Effect Error 0.259468 0.01235562 21 Total 0.367374 27 **Distributional Tests Attribute** Test Test Stat Critical P-Value Decision(a:1%) Variances **Bartlett Equality of Variance** 1.313 16.81 0.9709 **Equal Variances** Variances 0.6999 Mod Levene Equality of Variance 0.6364 3.812 **Equal Variances** Variances Levene Equality of Variance 0.7191 3.812 0.6387 **Equal Variances** Distribution Shapiro-Wilk W Normality 0.9215 0.8975 0.0377 **Normal Distribution** 0.1751 Distribution Kolmogorov-Smirnov D 0.1914 0.0277 **Normal Distribution** Distribution D'Agostino Skewness 0.8968 2.576 0.3698 Normal Distribution Distribution D'Agostino Kurtosis 2.025 2.576 0.0429 **Normal Distribution** Distribution D'Agostino-Pearson K2 Omnibus 4.903 9.21 0.0862 Normal Distribution 0.0229 Distribution Anderson-Darling A2 Normality 0.8898 3.878 **Normal Distribution Combined Proportion Normal Summary** Control Type C-% Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effect 0 Dilution Water 4 0.8957 0.8171 0.9743 0.91 0.8246 0.9384 0.0247 5.52% 0.0% 2 4 0.9159 0.8078 1 0.91 0.8483 0.9955 0.03396 7.42% -2.26% 4 4 0.9704 0.9187 1 0.9739 0.9336 0.01623 3.35% -8.33% 9 4 0.9419 0.8716 1 0.9408 0.8957 0.9905 0.02209 4.69% -5.16% 18 4 0.9126 0.8403 0.9849 0.8981 0.8768 0.9775 0.02272 4.98% -1.89% 35 4 0.9599 0.9031 1 0.9601 0.9194 1 0.01785 3.72% -7.17% 70 4 0.9633 0.9077 0.9621 0.9289 1 1 0.01745 3.62% -7.54%

000-091-187-4

CETIS™ V1.8.7.4
PAGE 9 OF 14

Analyst:\_\_\_\_\_ QA:\_\_\_\_

Report Date: Test Code:

22 Mar-17 08:48 (p 2 of 3) 658-80 02-8040-0265

Biraham   Development   Test   Survival and Development   Test   Survival   Survival   Analysis   Parametric Cornor vs   Trashmoth   Survival   Northwestern Aquatic Science   Analysis   Parametric Cornor vs   Trashmoth   Survival   Northwestern   Survival   Northwestern   N								Tes	t Code:		658-80 02	-8040-026
Analyzer Corrected) Transformed Variable (Corrected) Variabl	Bivalve Larva	al Survival and D	)evelopmer	nt Test					-	Northwest	ern Aquatio	Sciences
Analyses: Parametric Countries Treatments	Analysis ID:	09-0535-7845	End	point: (Co	mbined Prop	ortion Norm	nal \	CEI	TIS Version:	CETISv1	.8.7	
Angular (Corrected) Transformed Summary  C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% 95% COMPARIAN	_				•			Offi	cial Results:			
Control Type   Count   Mean   95% LCL   95% UCL   Median   Mile   Max   Std Err   CV%   %.Effect   CV%   Median   Mile   Max   Std Err   CV%   Median   Mile   Std Err   CV%   Median   Mile   Std Err   CV%   Median   Mile   Std Err   CV%   Median   Std Err   CV%   Median   Std				_				<del></del>				
Dilution Water		· –		-	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
4 1.305 1.064 1.546 1.273 1.171 1.503 0.07585 11.59% 4.88% 4.89% 4												
1												
4 1.344 1.175 1.513 1.331 1.242 1.473 0.05312 7.9% 7.74% 5.656 8.0 4 1.281 1.13 1.433 1.246 1.212 1.42 0.478 7.44% 2.69% 5.6 4 1.392 1.215 1.57 1.375 1.283 1.536 0.06576 8.0 1% -1.157% 5.0 4 1.402 1.224 1.58 1.385 1.301 1.537 0.05593 7.98% 5.158% 5.0 1.5 1.395 1.3			4									
1			4									
4 1.392 1.215 1.57 1.375 1.283 1.536 0.05576 8.01% -11.57% 0.000 4 1.402 1.224 1.58 1.386 1.301 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.000 1.537 0.05593 7.98% -12.38% 0.000 1.537 0.000 1.53			4									
Combined Proportion Normal Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  0 Dilution Water 0.9147 0.8246 0.9384 0.9052  0 0.80557 0.9868 0.9147 0.9905  1 0.9975 0.8768 0.8863  1 0.910 0.9775 0.8768 0.8863  1 0.910 0.9772 0.9431  1 0.9384 0.9052  1 0.9384 0.9052  1 0.9384 0.9668 0.9147 0.9905  18 0.911 0.9775 0.8768 0.8863  15 0 0.91 0.9775 0.8768 0.8863  15 0 0.91 0.9775 0.8768 0.8863  15 0 0.91 0.9780 0.9789 1  Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  1 1.536 1.502 1.351 1.311  1 1.242 1.388 1.274 1.473  1 1.536 1.502 1.351 1.311  1 1.266 1.42 1.212 1.227  1 1.536 1.536 1.532 1.419 1.33  1 1.32 1.565  1 1.536 1.532 1.419 1.33  1 1.32 1.565 1.351 1.311  1 1.546 1.42 1.212 1.227  1 1.536 1.536 1.532 1.419 1.33  1 1.536 1.536 1.532 1.537    Sraphics	35		4									
Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 0.9147 0.8246 0.9384 0.9052 1 0.9955 0.9479 0.8483 0.872 1 0.9955 0.9479 0.8483 0.872 2 0.8957 0.9668 0.9147 0.9905 18 0.91 0.9775 0.8768 0.8863 18 0.91 0.9772 0.8768 0.8863 18 0.9384 0.9858 0.9289 1  Control Type Rep 1 Rep 2 Rep 3 Rep 4  Control Type Rep 1 1.274 1.139 1.258 1.503 1.34 1.171 1.205 1.535 1.502 1.351 1.31 1.242 1.388 1.274 1.473 1.265 1.42 1.212 1.227 1.536 1.283 1.419 1.33 1.31 1.32 1.451 1.301 1.537  Control Type Rep 1 Rep 2 Rep 3 Rep 4  Control Type R	70		4									
Dilution Water 0.9147 0.8246 0.9384 0.9052 0.9959 0.9479 0.8483 0.872 0.9955 0.9479 0.8483 0.872 0.9336 0.872 0.9668 0.9147 0.9905 88 0.91 0.9775 0.8768 0.8863 0.9289 1 0.9384 0.9858 0.9289 1 0.9384 0.9858 0.9289 1 0.9384 0.9858 0.9289 1 0.9431 0.9938 0.9289 1 0.9431 0.9384 0.9858 0.9289 1 0.9431 0.9384 0.9858 0.9289 1 0.9431 0.9384 0.9858 0.9289 1 0.9431 0.9384 0.9858 0.9289 1 0.9431 0.9384 0.9858 0.9289 1 0.9431 0.94	ombined P	roportion Norma	l Detail									
0.9955 0.9479 0.8483 0.872 1 0.9955 0.9336 0.9857 0.9668 0.91347 0.9905 8 0.91 0.9775 0.8768 0.8863 5 1 0.9194 0.9772 0.9431 0 0.9384 0.9858 0.9289 1  Inquiar (Corrected) Transformed Detail	-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
1 0.9953 0.9526 0.9336 0.8957 0.9668 0.9147 0.9905 18 0.91 0.9775 0.9668 0.8863 15 1 0.9194 0.9772 0.9431 10 0.9384 0.9858 0.9289 1  Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  1.503 1.34 1.171 1.205 1.536 1.502 1.351 1.31 1.242 1.388 1.274 1.473 1.8 1.266 1.42 1.212 1.227 1.55 1.536 1.283 1.419 1.33 1.0 1.32 1.451 1.301 1.537  Sraphics	)	Dilution Water	0.9147	0.8246	0.9384	0.9052						
0.8957 0.9668 0.9147 0.9905 0.91 0.9775 0.8768 0.8863 0.914 0.9772 0.9431 0.9384 0.9858 0.9289 1  Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 1.274 1.139 1.32 1.258 1.503 1.34 1.171 1.205 1.536 1.502 1.351 1.31 1.242 1.388 1.274 1.473 1.88 1.266 1.42 1.212 1.227 1.55 1.536 1.283 1.419 1.33 1.70 1.32 1.451 1.301 1.537  Sraphics  Sraphics	2		0.9955	0.9479	0.8483	0.872						
18	1		1	0.9953	0.9526	0.9336						
1 0.9194 0.9772 0.9431 0.9384 0.9858 0.9289 1  Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  0 Dilution Water 1.274 1.139 1.32 1.258 1.536 1.502 1.351 1.31 1.242 1.388 1.274 1.473 18 1.266 1.42 1.212 1.227 1.55 1.556 1.283 1.419 1.33 1.70 1.32 1.451 1.301 1.537   Graphics  Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  1.536 1.502 1.351 1.31 1.536 1.502 1.351 1.31 1.537 1.536 1.502 1.351 1.31 1.538 1.274 1.473 1.538 1.274 1.473 1.536 1.283 1.419 1.33 1.537 1.537 1.537 1.537	)		0.8957	0.9668	0.9147	0.9905						
Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 1.274 1.139 1.32 1.258 2 1.503 1.34 1.171 1.205 4 1.536 1.502 1.351 1.31 6 1.266 1.42 1.212 1.227 1.536 1.283 1.419 1.33 1.507 1.32 1.451 1.301 1.537   Graphics  Graphics  Angular (Corrected) Transformed Detail  3-	18		0.91	0.9775	0.8768	0.8863						
Angular (Corrected) Transformed Detail  2-% Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 1.274 1.139 1.32 1.258 2 1.503 1.34 1.171 1.205 4 1.536 1.502 1.351 1.31 6 1.266 1.42 1.212 1.227 1.536 1.283 1.419 1.33 1.507 1.32 1.451 1.301 1.537   Graphics  Graphics  Angular (Corrected) Transformed Detail  3-	35		1	0.9194	0.9772	0.9431						
Control Type Rep 1 Rep 2 Rep 3 Rep 4  Dilution Water 1.274 1.139 1.32 1.258  1.503 1.34 1.171 1.205  1.536 1.502 1.351 1.31  1.244 1.473  1.88 1.266 1.42 1.212 1.227  35 1.536 1.283 1.419 1.33  70 1.32 1.451 1.301 1.537   Graphics  Rep 4 1 Rep 2 Rep 3 Rep 4 1.258  Rep 3 Rep 4 1.258  Rep 5 1.502 1.351 1.31  Rep 6 1.473 1.314  Rep 7 1.205  Rep 7 1.205  Rep 8 1.205  Rep 8 1.205  Rep 8 1.205  Rep 9 1 Rep 2 Rep 3 Rep 4 1.258  Rep 4 1.258  Rep 6 1.42 1.215  Rep 7 1.205  Rep 6 1.42 1.215  Rep 7 1.205  Rep 8 1.205	<b>7</b> 0		0.9384	0.9858		1						
Dilution Water 1.274 1.139 1.32 1.258  1.503 1.34 1.171 1.205  1.536 1.502 1.351 1.31  1.242 1.388 1.274 1.473  188 1.265 1.536 1.283 1.419 1.33  70 1.32 1.451 1.301 1.537   Graphics  Reject Null  0.3  0.0  0.0  0.0  0.0  0.0  0.0  0	Angular (Cor	rected) Transfor	med Detail							· · · · · · · · · · · · · · · · · · ·		
1.503 1.34 1.171 1.205 1.536 1.502 1.351 1.31 1.242 1.388 1.274 1.473 1.88 1.266 1.42 1.212 1.227 35 1.536 1.283 1.419 1.33 70 1.32 1.451 1.301 1.537  Graphics  Reject Null  0.30 0.00 0.00 0.00 0.00 0.00 0.00 0	J-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
1.536 1.502 1.351 1.31 1.242 1.388 1.274 1.473 1.88 1.266 1.42 1.212 1.227 1.536 1.536 1.283 1.419 1.33 1.32 1.451 1.301 1.537   Graphics  Reject No.8  Reject No	)	Dilution Water	1.274	1.139	1.32	1.258		_				
1.242 1.388 1.274 1.473 1.266 1.42 1.212 1.227 1.556 1.536 1.283 1.419 1.33 1.301 1.537   Graphics  Refert No.8  0.20 0.00 0.00 0.00 0.00 0.00 0.00 0	2		1.503	1.34	1.171	1.205						
1.266 1.42 1.212 1.227 3.5 1.536 1.283 1.419 1.33 7.0 1.32 1.451 1.301 1.537   Graphics  Reject Null  Reject Null  O	ţ		1.536	1.502	1.351	1.31						
1.536 1.283 1.419 1.33 70 1.32 1.451 1.301 1.537  Graphics  Reject Null  O	3		1.242	1.388	1.274	1.473						
To 1.32 1.451 1.301 1.537  Graphics  Reject Null	18		1.266	1.42	1.212	1.227						
Straphics  1.0  1.0  1.0  1.0  1.0  1.0  1.0  1.	35		1.536	1.283	1.419	1.33						
1.0 0.20 0.20 0.15 0.15 0.00 0.5 1.0 1.5 2.0 2.5	70		1,32	1.451	1.301	1.537						
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0.8 Reject Mull 0.10 0.00 0.00 0.00 0.00 0.00 0.00 0.	E						[		ĺ			
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0.2	8 0.4						-0.0s (-		/.			
0.2 0.1 0.0 0.0 0.0 0.0 0.0 0.0 0.0	0.3						[		10000			
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			C-%	20	- 10		-13	.E.O113	Rankity	V.5 1.0	1.0 £.U	43

EC 50 > 70% BY DATA INSPECTION

-631

# **CETIS Analytical Report**

Report Date: Test Code: 22 Mar-17 08:48 (p 1 of 1)

(658-80 )02-804**0-0265** 

Bivalve	e Larval	Survival and D	evelopmer	nt Test						Northwest	ern Aquat	ic Sciences
Analysi	is ID:	10-9527-5584	End	point: (	Combined Prop	orlion Norm	nal	CETI	S Version	n: CETISv1	8.7	
Analyze		22 Mar-17 8:47			Linear Interpola				ial Result		.0.7	
Andryze		22 11101 17 0, 11	7110	tyois, .	Elizat interpole	10011 (101 114)	,		iai itosaii	<u>ta. 100</u>		
Batch II	ID:	05-1514-6467	Tes	t Type:	Development-S	urvival		Anal	yst:			
Start Da	ate:	15 Mar-17 14:00	) Pro	tocol:	EPA/600/R-95/	136 (1995)		Dllue		aquina Bay Se	awater	
Ending	Date:	17 Mar-17 14:0	Spe		Mytilis galloprov			Brine				
Duratio		48h	•		Carlsbad Aqual			Age:				
Sample	e ID:	12-3464-8860	Cod	le: 4	49973F1C			Clier	it: W	yckoff Treatm	ent Plant	
Sample	e Date:	14 Mar-17 09:20	) Mat	erial:	Industrial Efflue	ent		Proje	ect:			
Receive	e Date:	15 Mar-17 12:2	5 Sou	rce:	Wyckoff							
Sample	e Age:	29h (2.4 °C)	Stat	don:								
Linear	Interpo	lation Options										
X Trans	sform	Y Transform	See	d I	Resamples	Exp <b>95</b> %	CL Meth	od				
Linear		Linear	671		280	Yes		Point Interp	olation			
Residu	al Analy	ysis					<u>-</u>					
Attribut	te	Method			Test Stat	Critical	P-Value	Decision(	a:5%)			
Extreme		Grubbs Ex	Ireme Valu	e	2.026	2.876	1.0000	No Outlier	<u> </u>	d		
								,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,		-		
Point E		?S			95%   Cl	95% UCI				<u>.                                    </u>		
Point E	%	95% LCL	95% UCL	TU	95% LCL					·		
Point E Level EC25	% >70	95% LCL N/A	95% UCL N/A			NA						
Point E Level EC25 Combin	% >70	95% LCL	95% UCL N/A	TU		NA Calcu	lated Varia	te(A/B)	_			
Point E Level EC25	>70	95% LCL N/A	95% UCL N/A	TU		NA	lated Varia Std Err	Std Dev	CV%	%Effect	A	В
Point E Level EC25 Combin	% >70 ned Pro	95% LCL N/A	95% UCL N/A Summary	TU <1.429	NA Min	NA Calcu			CV% 5.52%	%Effect	A 756	B 844
Point E Level EC25 Combin C-% 0	% >70 ned Pro	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count	TU <1.429 Mean 0.8957 0.9159	Min 0.8246 0.8483	NA Calcu Max	Std Err	Std Dev				
Point E Level EC25 Combin C-%	% >70 ned Pro	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count 4	TU <1.429 Mean 0.8957	Min 0.8246 0.8483	Calcu Max 0.9384	Std Err 0.0247	Std Dev 0.0494	5.52%	0.0%	756	844
Point E Level EC25 Combin C-% 0 2 4	% >70 ned Pro	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count 4	TU <1.429 Mean 0.8957 0.9159	Min 0.8246 0.8483 0.9336	Max 0.9384 0.9955	Std Err 0.0247 0.03396	Std Dev 0.0494 0.06793	5.52% 7.42%	0.0% -2.26%	756 783	844 854
Point E Level EC25 Combin C-% 0	% >70 ned Pro	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count 4 4	TU <1.429 Mean 0.8957 0.9159 0.9704	Min 0.8246 0.8483 0.9336 0.8957	Calcu Max 0.9384 0.9955	Std Err 0.0247 0.03396 0.01623	0.0494 0.06793 0.03246	5.52% 7.42% 3.35%	0.0% -2.26% -8.33%	756 783 819	844 854 844
Point E Level EC25 Combin C-% 0 2 4 9	% >70 ned Pro	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4	TU <1.429 Mean 0.8957 0.9159 0.9704 0.9419	Min 0.8246 0.8483 0.9336 0.8957 0.8768	Calcu Max 0.9384 0.9955 1 0.9905	Std Err 0.0247 0.03396 0.01623 0.02209	Std Dev 0.0494 0.06793 0.03246 0.04418	5.52% 7.42% 3.35% 4.69%	0.0% -2.26% -8.33% -5.16%	756 783 819 795	844 854 844 844
Point E Level EC25 Combin C-% 0 2 4 9 18	% >70 ned Pro	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4	TU <1.429 Mean 0.8957 0.9159 0.9704 0.9419 0.9126	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194	Max 0.9384 0.9955 1 0.9905 0.9775	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272	0.0494 0.06793 0.03246 0.04418 0.04544	5.52% 7.42% 3.35% 4.69% 4.98%	0.0% -2.26% -8.33% -5.16% -1.89%	756 783 819 795 781	844 854 844 844 855
Point E Level EC25 Combin 0 2 4 9 18 35 70	>70 ned Pro C D	95% LCL N/A portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4	TU <1.429 Mean 0.8957 0.9159 0.9704 0.9419 0.9126 0.9599	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194	Max 0.9384 0.9955 1 0.9905 0.9775	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E Level EC25 Combin 0 2 4 9 18 35 70	>70 ned Pro C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4	TU <1.429 Mean 0.8957 0.9159 0.9704 0.9419 0.9126 0.9599	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194	Max 0.9384 0.9955 1 0.9905 0.9775	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E  EC25  Combir  C-%  0  2  4  9  18  35  70  Combir  C-%	>70 ned Pro  C D	95% LCL N/A portion Normal ontrol Type ilution Water	95% UCL N/A Summary Count 4 4 4 4 4 4 4 4 4 4	TU <1.429 Mean 0.8957 0.9159 0.9704 0.9419 0.9126 0.9599 0.9633	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289	NA Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E Level EC25 Combin 0 2 4 9 18 35 70 Combin	>70 ned Pro  C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4 4 4 Detall	TU <1.429  Mean 0.8957 0.9159 0.9704 0.9419 0.9126 0.9599 0.9633	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289 Rep 3 0.9384	NA  Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1 Rep 4	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E  EC25  Combir  C-%  0  2  4  9  18  35  70  Combir  C-%  0  2	>70 ned Pro  C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4 4 4 Detall Rep 1 0.9147	TU <1.429  Mean 0.8957 0.9159 0.9704 0.9419 0.9633  Rep 2 0.8246 0.9479	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289 Rep 3 0.9384 0.8483	NA  Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1  Rep 4 0.9052 0.872	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E Level EC25 Combin 0 2 4 9 18 35 70 Combin C-% 0 2 4	>70 ned Pro  C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4 4 7 Detall Rep 1 0.9147 0.9955	TU <1.429  Mean 0.8957 0.9159 0.9704 0.9419 0.9126 0.9599 0.9633  Rep 2 0.8246 0.9479 0.9953	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289  Rep 3 0.9384 0.8483 0.9526	NA  Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1  Rep 4 0.9052 0.872 0.9336	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E Level EC25 Combin 0 2 4 9 18 35 70 Combin C-% 0 2 4 9	>70 ned Pro  C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4 4 7 Detall Rep 1 0.9147 0.9955 1 0.8957	TU <1.429 Mean 0.8957 0.9159 0.9419 0.9126 0.9599 0.9633 Rep 2 0.8246 0.9479 0.9953 0.9668	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289  Rep 3 0.9384 0.8483 0.9526 0.9147	NA  Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1  Rep 4 0.9052 0.872 0.9336 0.9905	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E  EC25  Combin  C-%  0  2  4  9  18  35  70  Combin  C-%  0  2  4  9  18	>70 ned Pro  C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4 4 7 Detall Rep 1 0.9147 0.9955 1 0.8957 0.91	TU <1.429  Mean 0.8957 0.9159 0.9704 0.9419 0.9599 0.9633  Rep 2 0.8246 0.9479 0.9953 0.9668 0.9775	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289  Rep 3 0.9384 0.8483 0.9526 0.9147 0.8768	NA  Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1  Rep 4 0.9052 0.872 0.9336 0.9905 0.8863	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852
Point E Level EC25 Combin 0 2 4 9 18 35 70 Combin C-% 0 2 4 9	>70 ned Pro  C D	95% LCL N/A  portion Normal ontrol Type ilution Water  portion Normal ontrol Type	95% UCL N/A Summary Count 4 4 4 4 4 4 4 7 Detall Rep 1 0.9147 0.9955 1 0.8957	TU <1.429 Mean 0.8957 0.9159 0.9419 0.9126 0.9599 0.9633 Rep 2 0.8246 0.9479 0.9953 0.9668	Min 0.8246 0.8483 0.9336 0.8957 0.8768 0.9194 0.9289  Rep 3 0.9384 0.8483 0.9526 0.9147 0.8768 0.9772	NA  Calcu Max 0.9384 0.9955 1 0.9905 0.9775 1 1  Rep 4 0.9052 0.872 0.9336 0.9905	Std Err 0.0247 0.03396 0.01623 0.02209 0.02272 0.01785	Std Dev 0.0494 0.06793 0.03246 0.04418 0.04544 0.03571	5.52% 7.42% 3.35% 4.69% 4.98% 3.72%	0.0% -2.26% -8.33% -5.16% -1.89% -7.17%	756 783 819 795 781 818	844 854 844 844 855 852

Analyst:\_\_\_\_\_ QA:\_\_\_\_

**Bivalve Larval Survival and Development Test** 

Report Date: **Test Code:** 

22 Mar-17 08:42 (p 1 of 1) 02-8040-0266/658-80

Northwestern Aquatic Sciences

Start Date: 15 Mar-17 14:00 Species: Mytilis galloprovincialis Sample Code: 17 Mar-17 14:05 Protocol: EPA/600/R-95/136 (1995) End Date: Sample Source: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	В	1	32	211	178	178	175	
0	В	2	11	211	213	213	210	
0	В	3	12	211	209	209	205	
0	В	4	3	211	212	212	209	
0	D	1	10	211	195	195	193	
0	D	2	2	211	176	176	174	
0	D	3	23	211	199	199	198	
0	D	4	9	211	192	192	191	
2		1	18	211	221	221	220	
2		2	27	211	200	200	200	
2		3	22	211	180	180	179	
2		4	30	211	187	187	184	
4		1	28	211	212	212	211	
4		2	15	211	212	212	210	
4		3	14	211	201	201	201	
4		4	24	211	203	203	197	
9		1	6	211	189	189	189	-
9		2	29	211	205	205	204	
9		3	5	211	196	196	193	
9		4	17	211	213	213	209	
18		1	16	211	195	195	192	
16		2	13	211	222	222	217	
18		3	1	211	186	186	185	
18		4	31	211	190	190	187	
35		1	7	211	214	214	211	
35		2	26	211	200	200	194	
35		3	21	211	219	219	214	
35		4	8	211	207	207	109	
70		1	4	211	200	200	198	
70		2	20	211	212	212	208	
70		3	19	211	201	201	196	
70		4	25	211	216	216	216	

data entry verified against laboratory bench shock 3-23-17 JAP

QA:\_\_\_ Analyst:\_\_\_\_

Page 1 of 1

Northwestern Aquatic Sciences (REGION COPY)

DateShipped: 3/14/2017 CarrierName: FedEx

CHAIN OF CUSTODY RECORD

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

No: 10-030717-094613-0162 2017T10P303DD210W2LA00 Contact Name: Keith Allers Contact Phone: 206-780-1711

Sample No.	Method	(Days)		Locanoli	Collection Date/Time	защые туре
Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	03/14/2017 09:20	Field Sample

				Shipment for Case Complete? N	Somplete? N
Special Instructions:	ns:			Samples Transferre	Samples Transferred From Chain of Custody #
Analysis Key: CH	Analysis Key: CHRTOX=Chronic Toxicity				
Items/Reason	Relinquished by (Signature and Organization)	Date/Time 3-14-2017 0455	Received by (Signature and Organization)	S-15-73	Sample Condition Upon Receipt

ORIGIN ID:BFIA (206) KEITH ALLERS CH2MHILLINC 5350 CREOSOTE PLACE N.E.

(206) 780-1711

SHIP DATE: 14MAR17 ACTWGT: 12.00 LB CAD: 103963466WSXI3100

BILL SENDER

BAINBRIDGE ISLAND, WA 98110 UNITED STATES US

TO GEARLD IRISSARRI NORTHWESTERN AQUATIC SCIENCES 3814 YAQUINA BAY ROAD

**NEWPORT OR 97365** 

(541) **265-7225** INV: PO:

REF: PN: 436558.FP.Y5,01





WED - 15 MAR 12:00P PRIORITY OVERNIGHT

7859 0360 1601

97365

PDX OR-US

86 ONPA



**NORTHWESTERN** AQUATIC SCIENCES

A Division of NAS Associates, Inc.

PRINT NAME

# APPENDIX III RAW DATA – REFERENCE TOXICANT TEST

### NORTHWESTERN AQUATIC SCIENCES

PROTOCOL NO. NAS-XXX-CG/MG2

- · · · · · · · · · · · · · · · ·		-		
	BIVALVE L	ARVAL TEST	BASED ON E	EPA/600/R-95/13

Test No.	999-36	S54 Client	·	QC Tes	t			Investigator	PABC
STUDY N	//ANAGI	EMENT			-	•			
Client		QC To	est						
Client'	s Study	Monitor:		QC Tes	t				
Testin	g Labor	atory: Northwe	stern Aqu	ratic Scie	ences				
		Newport Labo							
Labora	atory's S	Study Personne	el:		. 1 1				
		tudy Dir.		G.J. Iris	الائة sarri				
	Officer	•	L.K. Nen	neth					
1.	GA	BAler	UB			2.	N. BROW	n 3	
3.						4.	12.5. P.14 ULM		
Study	Schedu	le:					2 11 12 2		
	Beginnin		-15-17	1400	5		Test Ending:	3-17-17	1405
TEST MA	ATERIA	L							
D	escriptio	in: Coppi	er as CuS	O <sub>4</sub> ·5H <sub>2</sub> C	), Argent Le	ot#	0195,		
N.	AS Sam	ple No.				1.0	mg/ml stock prep	ared: 5-16-16	
		ollection:		-					
D	ate of R	eceipt:							
		ure (deg C):							
	H:	, ,							
•		oxygen (mg/L	.):				_		
		rity (umhos/cm							
	ardness	• •	,					-	
	lkalinity								
	alinity (p								
		rine (mg/L):							
		nonia-N (mg/L	λ:						
			:						
			<b>-</b> '						
DILUTIO									
	escriptic		Yaquina		R Seawater				
		ollection:			1-17		Salinity (ppt)		
Ti	reatmen	ts:	Aerated,	filtered	to ≤ 0.45 u		salinity adjusted w		ized water
			100,0	PPT P	FRINE.	В	RINE PREPAR	2-20-17	
TEST OF									
	pecies:		is gallopro				D:	ate Received:	3-8-17
	ource:		sbad Aqua	afarms, (	Carlsbad, C	Α	- <u> </u>		
Α	cclimation								
	Date	Temp (deg.C)	) PH		Sal (ppt)		D.O. (mg/L)	Comm	
	3-8-17	10.5	7.9	7	15.0		99	Held outside in to	rays of
	3-10-17	18.0	7.	9	12.0		8.7	flowing seawa	ater
,	3-16-17	14.7	7.	.8	19,0		8.7		
	3-15-17		7,		26,5		8,0		
		-							
		654 14,5							
	Mean	1.15" 14.85	7.9	i l	18.1		8.8		
	S.D.	2.9 3,0	0.1		6.3		0,8	1	
	(N)	4	4		4		4		
Р		od during accl		Oi		ien	t conditions	-	

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

### PROTOCOL NO. NAS-XXX-CG/MG2

# NORTHWESTERN AQUATIC SCIENCES

#### BIVALVE LARVAL TEST BASED ON EPA/600/R-95/136

Test No.	999-3654	Client		Q	C Test			_ Inves	tigator_	
Number of Egg Dilut	g: Initial: of organisi tion (1 ml o Count/ml o	ns used: filuted to 1 of dilution: ctor = DF	femal 00 ml): 1	Final: es: <u>3</u>	 24		Ferti nales: 3 <u>2</u>	ilization: 3	lo _ Mean:	30 41,7
Test cha Organism Feeding:	mber: 30 rns/ml (15-3	AND CON s (50% ser nl glass via	ies recom	Test v Test w	olume: 10 vater chan period: 16	ml ges: None L:8D	Aera	licates/tre ation durin hity: 30 +/	eatment (4 ng test: No '- 2 ppt	one
RANDOMIZA	ATION CH	ART								
A	8	32	1	64	Ø	16	2	4		
В	1	8	16	4	32	2	Ø	4		
С	2	4	64	16	/	8	32	Ø		
D	4	64	Þ	32	2	16	)	8		

#### PREPARATION OF TEST SOLUTIONS

	(Cu, u
_	64
7-15-17	32
3-1632	16
13-	8
	4

Test Conc.	ml of working stock #2	Dilution water			
(Cu, ug/L)	(2 ug/mL)	(ml/100 <b>mL</b> )			
64	3.2	Brought up to a			
32	1.6	final volume of			
16	0.8	100 ml with			
8	0.4	dilution water.			
4	0.2				
2	0.1				
1	0.05				
0	0				

1st working stock made by 1:99 (1.0 mL 100mL) dilution of concentrated 1 mg/mL stock solution. Final concentration 10 ug/mL.

2nd working stock made (working stock #2) made by 20:80 (20 mL 100mL) dilution of 1st working stock. Final concentration 2 ug/mL.

Comments:

Test No. 999-3654 Client QC Test Investigator

## **WATER QUALITY DATA**

	Date:	3-15-17	initials:	اده_	Date:	initials:	<u>ch</u>	
Conc. ( ug/L )	Temp. (deg.C)	рН	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	рН	Sal. (ppt)	DO (mg/L)
64	16,0	7.0	29.5	8.1	15-2	8-1	300	8-0
32	15.9	8.0	29.5	8.1	15-3	8-1	295	8.1
16	16,0	8.0	30,0	8.1	15-3	8-1	30.0	20
8	15.9	9,0	29,5	8.1	15.2	8-1	300	80
4	15.9	8,0	30,0	જ. ા	15-2	8.1	300	8.1
2	16.0	9,0	30,0	1,8	15-3	8-1	300	8-1
1	16.0	7,0	30,0	8,1	15-5	8.1	30-0	8-1
Control	16.1	8.0	30,0	8,1	15-5	8-1	300	8.0
Brine control						_	***	

**WATER QUALITY:** <u>SD</u> <u>Mean</u> N Temperature ( ℃): 15.6 4,0 pH: 웅. 1 6.1 16 Salinity (ppt): 29.9 0.2 16 DO (mg/L): 8.1 0,0 16 Room/ Water bath temperature: (°C)

Day 0: 16.1 Day 0: 16.1 Day 1: 16.1 Day 2: 15-5

1	3-21-17 33 - 3/19/17												
Conc.	Replic	cate 1	Replic	caté 2	Replic	cate 3	Replic	cate/4					
( ug/L )	N	Α	N	Α	N	Α	N	Α					
64	Ø	0	Ø	05	Ø	Ø		Ø					
32	\&	47	Ø	76	Ø	59	Ø	62					
16	Ø	196	Ø	190	Ø	188	Ż	181					
8	201	1	212	/	204	3	173	6					
4	182	3	189	4	198	2	193	$\sim$					
2	181	3	210	5	222	3	227	2					
1	214	4	203	1	199	3	176	4					
Control	208	4	202	2	221	Ø	207	v					
Brine control			_					_					
Zero time	218	214	186	215	204	227	-	_					
7 4!	M 2		.1	Al .	,		\ 100	- 04					

Zero time: Mean 211 SD 14 N 6 CV=(sd/mean)x100 6.7 %

Remarks:

# Invoice



DATE	INVOICE #
3/1/2017	195377

Sustainable Mariculture ~ Preserving Our Oceans' Resources

MAIL TO:

Carlsbad Aquafarm
P.O Box 2600
Carlsbad, CA 92018
760-438-2444
info@carlsbadaquafarm.com

BILL TO:

Northwestern Aquatic Sciences 3814 Yaquina Bay Road Newport, Oregon 97365

P.O. #	TERMS	SHIP DATE	SHIP VIA	F.	O.B.	AIRBILL #	
	Net 7	3/1/2017		S	.D.	77854723989	
QUANTITY	ITEM	DESC	CRIPTION		PRICE	LBS.	AMOUNT
1 1	Bio Assay Mus BOX CHARGE	Вох	Charge  3-8-17 -631	alis			
THIS IS YOU	JR INVOICE				Tota	al	\$140.00

# **CETIS Summary Report**

Report Date: Test Code: 22 Mar-17 08:29 (p 1 of 2)

le: 999-3654) 02-0711-3850

											• •
Bivalve Larva	I Survival and D	evelopment	Test						Northweste	ern Aquatic	Sciences
Batch ID: Start Date: Ending Date: Duration:	05-1514-6467 15 Mar-17 14:0 17 Mar-17 14:0 48h	0 Prote	col: EP	evelopment-S PA/600/R-95/1 rtilis galloprov urlsbad Aquaf	136 (1995) rincialis		Anal Dilue Brine Age:	e <b>nt:</b> Yaqı ə:	ıina Bay Se	awater	
•	18-1959-5246 15 Mar-17 14:00 15 Mar-17 14:00 NA		rial: Co ce: Re	74D1EE opper sulfate eference Toxic	cant		Clier Proje		nal Lab		
Comparison S	Summary		-	- 12							. <u>-</u>
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
06-0379-4549	Combined Prop	ortion Norm	В	16	11.31	8.18%		Dunnett M	ultiple Com	parison Tes	
20-1354-4304	Proportion Surv	_	8	16	11.31	6.54%				parison Tes	
Point Estimat	e Summary										
Analysis ID	Endpoint		Level ==	μg/L	95% LCL	95% UCL	TU	Method			
Alialysis IU				0.500	8.847	9.978		Linear Inte	rpolation (10	CPIN)	
	Combined Prop	ortion Norm	EC25	9.589	0.011						
05-0354-9501	Combined Prop			10.72	10.53	10.92		Trimmed S	Spearman-K	,	
05-0354-9501 03-7078-9692( 00-4990-3080	Combined Prop	ortion Norm								(ärber	
05-0354-9501 03-7078-9692( 00-4990-3080	Combined Prop	ortion Norm	EC50	10.72	10.53	10.92			Spearman-K	(ärber	
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pre	Combined Prop Proportion Surv	ortion Norm	EC50	10.72	10.53	10.92	Max		Spearman-K	(ärber	%Effec
05-0354-9501 03-7078-9692 00-4990-3080 Combined Pro C-µg/L	Combined Prop Proportion Surv oportion Normal	rived Summary	EC50 EC50	10.72 )	10.53 23.53	10.92 25.08	Max 1	Trimmed \$	Spearman-K Spearman-K	(ärber	%Effec
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L	Combined Prop Proportion Surv oportion Normal Control Type	rived Summary Count	EC50 EC50 Mean	10.72 ) 24.29 95% LCL 0.9528 0.8253	10.53 23.53 95% UCL	10.92 25.08 Min		Trimmed S	Spearman-K Spearman-K Std Dev	Kärber Kärber CV%	
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L 0	Combined Prop Proportion Surv oportion Normal Control Type	ortion Norm rived  Summary  Count	EC50 EC50 Mean 0.981	10.72 ) 24.29 95% LCL 0.9528	10.53 23.53 95% UCL 1	10.92 25.08 Min 0.9573	1	Std Err 0.008866	Std Dev 0.01773	CV%	0.0%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-μg/L 0 1 2	Combined Prop Proportion Surv oportion Normal Control Type	ortion Norm fived  Summary  Count  4	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028	10.72 ) 24.29 95% LCL 0.9528 0.8253	10.53 23.53 95% UCL 1	10.92 25.08 Min 0.9573 0.8341	1 0.9817	Std Err 0.008866 0.03299	Std Dev 0.01773 0.06598	CV% 1.81% 7.09%	0.0% 5.18%
05-0354-9501 03-7078-9692( 00-4990-3080	Combined Prop Proportion Surv oportion Normal Control Type	ortion Norm fived  Summary  Count  4	EC50 EC50 Mean 0.981 0.9302 0.9578	95% LCL 0.9528 0.8253 0.8516	10.53 23.53 <b>95% UCL</b> 1 1	10.92 25.08 Min 0.9573 0.8341 0.8578	1 0.9817 0.9953	Std Err 0.008866 0.03299 0.03336	Std Dev 0.01773 0.06598 0.06671	CV% 1.81% 7.09% 6.97%	5.18% 2.37%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L 0 1 2 4 8 16	Combined Prop Proportion Surv oportion Normal Control Type	ortion Norm fived  Summary  Count  4	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028	95% LCL 0.9528 0.8253 0.8516 0.8519	10.53 23.53 <b>95% UCL</b> 1 1 1 0.9538	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626	1 0.9817 0.9953 0.9384	Std Err 0.008866 0.03299 0.03336 0.01601	Spearman-K Spearman-K Std Dev 0.01773 0.06598 0.06671 0.03203	CV% 1.81% 7.09% 6.97% 3.55%	0.0% 5.18% 2.37% 7.97% 4.83%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L 0 1 2 4	Combined Prop Proportion Surv oportion Normal Control Type	ortion Norm fived  Summary  Count  4	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028 0.9337	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097	10.53 23.53 95% UCL 1 1 1 0.9538 1	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199	1 0.9817 0.9953 0.9384 0.9953	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894	Spearman-K Spearman-K Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789	CV% 1.81% 7.09% 6.97% 3.55%	0.0% 5.18% 2.37% 7.97%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-μg/L 0 1 2 4 8 16 32	Combined Prop Proportion Surv oportion Normal Control Type	ortion Norm fived  Summary  Count  4	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028 0.9337 0	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097 0	10.53 23.53 95% UCL 1 1 0.9538 1	Min 0.9573 0.8341 0.8578 0.8626 0.8199 0	1 0.9817 0.9953 0.9384 0.9953 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789	CV% 1.81% 7.09% 6.97% 3.55%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L 0 1 2 4 8 16 32 64	Combined Prop Proportion Surv oportion Normal Control Type	Summary Count 4 4 4 4 4 4 4 4 4	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028 0.9337 0	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097 0	10.53 23.53 95% UCL 1 1 0.9538 1 0	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0	1 0.9817 0.9953 0.9384 0.9953 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0	CV% 1.81% 7.09% 6.97% 3.55%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-μg/L 0 1 2 4 8 16 32 64 Proportion Su	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 4 4	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028 0.9337 0	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097 0	10.53 23.53 95% UCL 1 1 0.9538 1 0	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0	1 0.9817 0.9953 0.9384 0.9953 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0	CV% 1.81% 7.09% 6.97% 3.55%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L 0 1 2 4 8 16 32 64	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 4 9	EC50 EC50 Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097 0	10.53 23.53 95% UCL 1 1 1 0.9538 1 0 0	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0	1 0.9817 0.9953 0.9384 0.9953 0 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0	CV% 1.81% 7.09% 6.97% 3.55% 8.34%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0%
05-0354-9501 03-7078-9692( 00-4990-3080  Combined Pro C-µg/L 0 1 2 4 8 16 32 64  Proportion Su C-µg/L 0 1	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	contion Normary  Count  4  4  4  4  4  4  7  Count	EC50 EC50  Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0 0	95% LCL 0.9528 0.8253 0.8516 0.8097 0 0 95% LCL 0.9644 0.8433	10.53 23.53 95% UCL 1 1 1 0.9538 1 0 0 0	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0	1 0.9817 0.9953 0.9384 0.9953 0 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0 0	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0 0 0 Std Dev	CV% 1.81% 7.09% 6.97% 3.55% 8.34%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0%
05-0354-9501 03-7078-9692( 00-4990-3080  Combined Pro C-µg/L 0 1 2 4 8 16 32 64  Proportion Su C-µg/L 0 1	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 7 Count	EC50 EC50  Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0 0  Mean 0.9893	95% LCL 0.9528 0.8253 0.8516 0.8097 0 0 95% LCL 0.9644	10.53 23.53 95% UCL 1 1 1 0.9538 1 0 0 0	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0 0	1 0.9817 0.9953 0.9384 0.9953 0 0 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0 0 0 Std Err 0.007829	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0 0 Std Dev 0.01566	CV% 1.81% 7.09% 6.97% 3.55% 8.34%  CV% 1.58%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0% 100.0%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-µg/L 0 1 2 4 8 16 32 64 Proportion Su C-µg/L 0 1	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	EC50 EC50  Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0  Mean 0.9893 0.9443	95% LCL 0.9528 0.8253 0.8516 0.8097 0 0 95% LCL 0.9644 0.8433	10.53 23.53 95% UCL 1 1 1 0.9538 1 0 0 0	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0 0	1 0.9817 0.9953 0.9384 0.9953 0 0 0 0	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0 0 0 Std Err 0.007829 0.03176	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0 0 Std Dev 0.01566 0.06351	CV% 1.81% 7.09% 6.97% 3.55% 8.34%  CV% 1.58% 6.73%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0% 100.0% 4.55%
05-0354-9501 03-7078-9692( 00-4990-3080  Combined Pro C-µg/L 0 1 2 4 8 16 32 64  Proportion Su C-µg/L 0 1 2 4 4 8 16 32 64	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	EC50 EC50  Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0  Mean 0.9893 0.9443 0.968	95% LCL 0.9528 0.8253 0.8516 0.8097 0 0 95% LCL 0.9644 0.8433 0.8662	10.53 23.53 95% UCL 1 1 1 0.9538 1 0 0 0 95% UCL 1 1	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0 0 Min 0.9668 0.8531 0.872	1 0.9817 0.9953 0.9384 0.9953 0 0 0 Max 1 1	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0 0  Std Err 0.007829 0.03176 0.03199	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0 0 Std Dev 0.01566 0.06351 0.06398	CV% 1.81% 7.09% 6.97% 3.55% 8.34%  CV% 1.58% 6.73% 6.61%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0% 100.0% 4.55% 2.16%
05-0354-9501 03-7078-9692( 00-4990-3080  Combined Pro C-µg/L 0 1 2 4 8 16 32 64  Proportion Su C-µg/L 0 1 2 4 8 8 64	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	EC50 EC50  Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0  Mean 0.9893 0.9443 0.968 0.9159	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097 0 0 0 95% LCL 0.9644 0.8433 0.8662 0.8688	10.53 23.53 95% UCL 1 1 0.9538 1 0 0 0 95% UCL 1 1 1 0.9629	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0 0 0 Min 0.9668 0.8531 0.872 0.8768	1 0.9817 0.9953 0.9384 0.9953 0 0 0 Max 1 1 1 0.9479	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0 0 Std Err 0.007829 0.03176 0.03199 0.01478	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0 0 Std Dev 0.01566 0.06351 0.06398 0.02957	CV% 1.81% 7.09% 6.97% 3.55% 8.34%  CV% 1.58% 6.73% 6.61% 3.23%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0% 100.0% 4.55% 2.16% 7.43%
05-0354-9501 03-7078-9692( 00-4990-3080 Combined Pro C-μg/L 0 1 2 4 8 16 32 64 Proportion Su	Combined Prop Proportion Surv oportion Normal Control Type Dilution Water	Summary Count 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4 4	EC50 EC50  Mean 0.981 0.9302 0.9578 0.9028 0.9337 0 0  Mean 0.9893 0.9443 0.968 0.9159 0.9467	95% LCL 0.9528 0.8253 0.8516 0.8519 0.8097 0 0 0 95% LCL 0.9644 0.8433 0.8662 0.8688 0.8387	10.53 23.53 95% UCL 1 1 0.9538 1 0 0 0 95% UCL 1 1 1 0.9629 1	10.92 25.08 Min 0.9573 0.8341 0.8578 0.8626 0.8199 0 0 0 0 Min 0.9668 0.8531 0.872 0.8768 0.8483	1 0.9817 0.9953 0.9384 0.9953 0 0 0 Max 1 1 1 0.9479	Std Err 0.008866 0.03299 0.03336 0.01601 0.03894 0 0 Std Err 0.007829 0.03176 0.03199 0.01478 0.03392	Std Dev 0.01773 0.06598 0.06671 0.03203 0.07789 0 0 Std Dev 0.01566 0.06351 0.06398 0.02957 0.06784	CV% 1.81% 7.09% 6.97% 3.55% 8.34%  CV% 1.58% 6.73% 6.61% 3.23% 7.17%	0.0% 5.18% 2.37% 7.97% 4.83% 100.0% 100.0% 100.0% 4.55% 2.16% 7.43% 4.31%

Report Date: Test Code:

22 Mar-17 08:29 (p 2 of 2) 999-3654 02-0711-3850

						Test Code:	(999-3654) 02-0711-3850
Bivalve La	rval Survival and D	Developme	nt Test				Northwestern Aquatic Sclences
Combined	Proportion Norma	l Detail					<u> </u>
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
0	Dilution Water	0.9858	0.9573	1	0.981		
1		0.9817	0.9621	0.9431	0.8341		
2		0.8578	0.9953	0.9867	0.9913		
4		0.8626	0.8957	0.9384	0.9147		
В		0.9526	0.9953	0.9668	0.8199		
16		0	0	0	0		
32		0	0	0	0		
64		0	0	0	0		
Proportion	Survived Detail						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
0	Dilution Water	1	0.9668	1	0.9905		-
1		1	0.9668	0.9573	0.8531		
2		0.872	1	1	1		
4		0.8768	0.9147	0.9479	0.9242		
8		0.9573	1	0.981	0.8483		
16		0.9289	0.9005	0.891	0.8578		
32		0.2227	0.218	0.2796	0.2938		
64		0	0	0	0		
Combined	Proportion Norma	l Binomials	5				
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
0	Dilution Water	208/211	202/211	221/221	207/211		
1		214/218	203/211	199/211	176/211		
2		181/211	210/211	222/225	227/229		
4		182/211	189/211	198/211	193/211		
8		201/211	212/213	204/211	173/211		
16		0/211	0/211	0/211	0/211		
32		0/211	0/211	0/211	0/211		
64		0/211	0/211	0/211	0/211		
Proportion	Survived Binomia	als					
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4		
0	Dilution Water	211/211	204/211	211/211	209/211		
1		211/211	204/211	202/211	180/211		
2		184/211	211/211	211/211	211/211		
4		185/211	193/211	200/211	195/211		
		202/244	211/211	207/211	179/211		
8		202/211					
16		196/211	190/211	188/211	181/211		
8 16 32					181/211 62/211		

Report Date: Test Code: 22 Mar-17 08:28 (p 1 of 1) 02-0711-3850(999-3654

Bivalve Larval Survival and Development Test	Northwestern Aquatic Sciences

Start Date: 15 Mar-17 14:00 Species: Mytilis galloprovincialis Sample Code: 6C74D1EE

End Date: 17 Mar-17 14:05 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant

Sample Date: 15 Mar-17 14:00 Material: Copper sulfate Sample Station:

nple Dat	e: 15 I	viar-1	7 14:00	Material:	Copper sulfate			Sample Station:
C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	8	211	212	212	208	
0	D	2	14	211	204	204	202	
0	D	3	18	211	221	221	221	
0	D	4	25	211	209	209	207	
1		1	16	211	218	218	214	
1	Ì	2	32	211	204	204	203	
1		3	27	211	202	202	199	
1		. 4	7	211	180	180	176	
2		1	23	211	184	184	181	
2		2	4	211	215	215	210	
2		3	2	211	225	225	222	
2		4	24	211	229	229	227	
4		1	1	211	185	185	182	
4		2	29	211	193	193	189	
4		3	9	211	200	200	198	
4		4	31	211	195	195	193	
6		1	10	211	202	202	201	
8		2	6	211	213	213	212	
8		3	5	211	207	207	204	
6		4	11	211	179	179	173	
16		1	30	211	196	196	0	
16		2	15	211	190	190	0	
16	-	3	28	211	188	188	0	
16		4	12	211	181	181	0	
32		1	19	211	47	47	0	
32		2	26	211	46	46	0	
32		3	17	211	59	59	0	
32		4	20	211	62	62	0	
64		1	13	211	0	Ō	0	
64		2	22	211	ō	0	0	
64		3	3	211	0	0	0	
64		4	21	211	0	0	. 0	

data entry weifed against laboratory bench sheets 3-23-17 Jak

Report Date: 23 Mar-17 08:39 (1 of 1)

# **Bivalve Larval Survival and Development Test**

#### **Northwestern Aquatic Sciences**

Test Type: Development-Survival Protocol: EPA/600/R-95/136 (1995) Organism: Mytilis galloprovincialis (Bay Mussel)

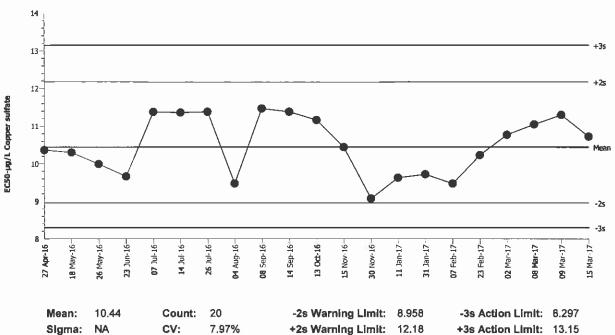
**Endpoint:** Combined Proportion Normal

Material:

Copper sulfate

Source: Reference Toxicant-REF





Quali	ty Con	trol Data	8								-
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Apr	27	13:40	10.36	-0.08165	-0.1023			10-3412-7541	03-6222-0465
2		May	18	13:50	10.3	-0.1481	-0.1862			00-5077-6110	04-6829-6018
3			26	15:05	9.988	-0.4552	-0.5809			00-2461-1517	20-1645-6539
4		Jun	23	13:50	9.66	-0.7832	-1.016			20-2900-3441	12-0442-8997
5		Jul	7	14:20	11.37	0.9304	1.112			20-3374-2875	12-6544-3567
6			14	14:40	11.36	0.9123	1.092			14-8632-5752	09-0596-6683
7			26	17:45	11.38	0.9344	1.117			03-9839-1525	11-6670-0698
8		Aug	4	14:30	9.471	-0.9729	-1.275			08-0477-0132	01-3411-5201
9		Sep	8	14:35	11.46	1.02	1.215			17-2952-4030	02-3236-2087
10			14	14:10	11.38	0.9336	1.116			07-6290-8502	15-4984-2250
11		Oct	13	14:00	11.16	0.7162	0.8646			06-0302-4211	08-1866-7265
12		Nov	15	11:25	10.44	-0.00595	-0.00743			11-4556-2596	05-0436-7942
13			30	13:00	9.074	-1.37	-1.833			08-5747-3287	19-4644-8589
14	2017	Jan	11	12:55	9.624	-0.8193	-1.065			01-6959-8959	07-5347-4966
15			31	12:30	9.717	-0.7263	-0.9395			00-4554-8270	10-7110-0578
16		Feb	7	13:30	9.47	-0.9737	-1.276			11-6796-8451	21-2845-1444
17			23	19:30	10.23	-0.2153	-0.2715			18-8118-8710	02-7913-5800
18		Mar	2	13:00	10.77	0.3243	0.3986			07-2409-7218	16-5427-1566
19			8	15:30	11.04	0.6007	0.7289			11-1093-1447	03-3381-2684
20			9	13:55	11.3	0.8543	1.025			18-3588-3261	02-4714-0912
21			15	14:00	10.72	0.2779	0.3423			02-0711-3850	03-7078-9692