

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-80Title: 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 WarnerTesting 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
pH	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: 3-14-17Water Quality: Salinity, 30.0 ‰; pH, 8.0Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, 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 waterSalinity: 100.0 ‰Date of Preparation: 2-20-17Method of Preparation: Freezing method

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).Age: 3.5 hrs post-fertilization.

Source: Carlsbad Aquafarm, Carlsbad, CA.

Conditioning: 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, $14.5 \pm 2.9^\circ\text{C}$; pH, 7.9 ± 0.1 ; salinity, $18.1 \pm 6.3\text{‰}$; and dissolved oxygen, $8.8 \pm 0.8 \text{ mg/L}$. 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.

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

Brine Control: A brine control was run in which salinity-adjusted Milli-Q[®] 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

Initial Concentration of Test Organisms: 21.1/ml.

Volume of Subsamples Taken for Counting: 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, $15.5 \pm 0.4^\circ\text{C}$; pH, 8.1 ± 0.2 ; salinity, $30.0 \pm 0.2\text{‰}$; and dissolved oxygen, $8.0 \pm 0.1 \text{ mg/L}$. 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-Kärber 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_c) were computed as $100/\text{NOEC}$, $100/\text{EC50}$, or $100/\text{IC25}$.

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 $\text{CuSO}_4 \cdot 5\text{H}_2\text{O}$, 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 $\mu\text{g/L}$; NOEC, 8 $\mu\text{g/L}$; IC25, 9.59 $\mu\text{g/L}$. The EC50 result was within the laboratory's control chart warning limits (8.96 – 12.2 $\mu\text{g/L}$).

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 _c =1.43)	70 (TU _c =1.43)
LOEC (%)	>70 (TU _c <1.43)	>70 (TU _c <1.43)
EC50/LC50 (%)	>70 (TU _c <1.43)	>70 (TU _c <1.43)
(95% C.I.)	---	---
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%)	>70 (TU _c <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

Guy Bubb 3-23-17
Project Manager Date

Gualdo Lusvardi 3-23-17
Study Director Date

Richard A. Caldwell 3/23/17
Assistant Laboratory Director Date
RSC

Julie R. Fine 3-23-17
Quality Assurance Unit Date

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

Test Material Concentration (%)	Repl.	Norm.	Abn.	Total	Combined Proportion Normal*		Proportion Survived*	
					Mean	Mean	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

* 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).

† Result significantly different ($P \leq 0.05$) from the control.

[†] Salinity-adjusted Milli Q[®] 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. INTRODUCTION

1.1 Purpose of Study: 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 Referenced Method: 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. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

2.2 Sponsor's Study Monitor:

2.3 Name of Testing Laboratory:

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

2.4 Test Location:

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

Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Study Schedule: 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 ($\leq 0.45 \mu\text{m}$) 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 ($\leq 0.45 \mu\text{m}$) 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 Source: 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 Conditioning of Adult Oysters: 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 μm) to remove feces and pseudofeces 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 μm 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 ($16 \pm 1^\circ\text{C}$ 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

6. DESCRIPTION OF TEST SYSTEM

6.1 Preparation of Test Concentrations: 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 Test Chambers and Environmental Control: 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 Cleaning: All laboratory glassware, including test chambers, is cleaned as described in EPA/600/4-90/027F. New glassware and test systems are soaked 15 minutes in tap water and scrubbed with detergent (or cleaned in automatic dishwasher); rinsed twice with tap water; carefully rinsed once with fresh, dilute (10%, V:V) hydrochloric or nitric acid to remove scale, metals, and bases; rinsed twice with deionized water; rinsed once with acetone to remove organic compounds (using a fume hood or canopy); and rinsed three times with deionized water. Test systems and chambers are rinsed again with dilution water just before use. 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 Test Procedure: 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 $20 \pm 1^\circ\text{C}$ ($16 \pm 1^\circ\text{C}$ 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 Effect Criteria: 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 $20 \pm 1^\circ\text{C}$ for oysters, $16 \pm 1^\circ\text{C}$ for blue mussels. The test temperatures specified by EPA (EPA/600/R-95/136) are $15 \pm 1^\circ\text{C}$ or $18 \pm 1^\circ\text{C}$, but these specifications were based on erroneous assumptions of the agency authors. Consequently, this protocol specifies $16 \pm 1^\circ\text{C}$. The salinity is $30 \pm 2\text{‰}$. 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 Beginning of Test: 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 Test Duration, Type and Frequency of Observations, and Methods: 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:

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

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

1. $\geq 70\%$ of embryos introduced into a required control treatment result in live larvae.
2. normal shell development in surviving controls is $\geq 90\%$.
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 $\leq 15\%$.

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. $\geq 70\%$ 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 $\leq 1^\circ\text{C}$.

10. 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).

9. 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-Kärber 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_a), which is 100/LC50, or toxic unit chronic (TU_c), 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;
4. 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. REFERENCES AND GUIDELINES

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

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 ± 1°C mussels (ASTM), 15 or 18 ± 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

REVIEWED
 PAGES 1-14
 -638

Test No. 658-80 Client: CH2M Hill - Wyckoff Investigator _____

STUDY MANAGEMENT

Client: CH2M Hill Wyckoff Treatment Plant, 5350 Creosote Place NE, Bainbridge Island, WA 98110
 Client's Study Monitor: Mr. Stanley Warner
 Testing Laboratory: Northwestern Aquatic Sciences
 Test Location: Newport Laboratory
 Laboratory's Study Personnel:
 Proj. Mgr./Study Dir. G.A. Buhler ⁶³⁸ / G.J. Irissarri ⁶³¹
 QA Officer L.K. Nemeth

1. V. N. Nakama 2. J. Brown
 3. _____ 4. G.S. Hill

Study Schedule:
 Test Beginning: 3-15-17 1400 Test Ending: 3-17-17 1405

TEST MATERIAL

Description:	<u>GROUND WATER SP-11</u>			
NAS Sample No.	<u>58456</u>			
Date of Collection:	<u>3-14-17</u>			
Date of Receipt:	<u>3-15-17</u>			
Temperature (deg C):	<u>2.4</u>			
pH:	<u>7.4</u>			
Dissolved oxygen (mg/L):	<u>10.6</u>			
Conductivity (umhos/cm):	<u>-</u>			
Hardness (mg/L):	<u>-</u>			
Alkalinity (mg/L):	<u>-</u>			
Salinity (ppt):	<u>5.0</u>			
Total chlorine (mg/L):				
Total ammonia-N (mg/L):				

DILUTION WATER

Description: Yaquina Bay, OR
 Date of Collection: 3-14-17 Salinity (ppt) 30.0 pH 8.0
 Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q deionized water BRINE
(DATE OF PREPARATION 2-20-17, SALINITY 100.0)

TEST ORGANISMS

Species: Mytilus galloprovincialis Date Received: 3-8-17
 Source: Carlsbad Aquafarm, Carlsbad, CA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
<u>3-8-17</u>	<u>10.8</u>	<u>7.9</u>	<u>15.0</u>	<u>9.9</u>	<u>Held outside in trays of flowing seawater</u>
<u>3-10-17</u>	<u>18.0</u>	<u>7.9</u>	<u>12.0</u>	<u>9.7</u>	
<u>3-13-17</u>	<u>14.7</u>	<u>7.8</u>	<u>19.0</u>	<u>8.7</u>	
<u>3-15-17</u>	<u>14.5</u>	<u>7.8</u>	<u>26.5</u>	<u>8.0</u>	
	<u>14.5</u>				
Mean	<u>14.8</u>	<u>7.9</u>	<u>18.1</u>	<u>8.8</u>	
S.D.	<u>2.9</u>	<u>0.1</u>	<u>6.3</u>	<u>0.8</u>	
(N)	<u>4</u>	<u>4</u>	<u>4</u>	<u>4</u>	

Photoperiod during acclimation: Outdoor ambient conditions

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

Test No. 658-80 Client CH2M Hill - Wyckoff Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0910 Final: 0950 Fertilization: 1030
 Number of organisms used: females: 3 males: 3
 Egg Dilution (1 ml diluted to 100 ml):
 Count/ml of dilution: 1. 37 2. 49 3. 39 Mean: 41.7
 Dilution factor = DF (mean x 100/2500) = 1.7

TEST PROCEDURES AND CONDITIONS

Test concentrations (50% series recommended): 70, 35, 18, 9, 4, 2, 0% + Brine Control

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4
 Organisms/ml (15-30): 21.1 Test water changes: None Aeration during test: None
 Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt
 Temperature: 20 +/- 1 °C, oysters; 16 +/- 1 °C, mussels Beaker placement: Stratified randomization

RANDOMIZATION CHART

A	70	9	∅	BC	4	35	2	18		
B	2	4	18	9	70	∅	35	BC		
C	35	2	BC	4	18	70	∅	9		
D	∅	18	4	35	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: 2-26-17 ; brine salinity (ppt) 100.0
 Source of seawater: Yaquina Bay, Oregon

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

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

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

3-15-17
 GJL

Test Conc. (%)	Effluent (ml/100ml)	Brine (ml/100ml)	Dilution Water (ml/100ml)
70	70	25.0	Brought up to a final
35	35	12.5	volume of 100 ml
18	18	6.4	with dilution water
9	9	3.2	
4	4	1.4	
2	2	0.7	
0	0	∅	
Brine Control	0	25.0	

THE BRINE CONTROL WAS MADE UP OF SALINITY ADJUSTED MILLI-Q DEIONIZED WATER (5.0ppt) SIMILAR TO THE EFFLUENT. AS A RESULT, THE AMOUNT OF BRINE IN THE BRINE CONTROL WAS THE SAME USED IN THE 70% EFFLUENT CONCENTRATION.

-63L

Test No. 658-80 Client CH2M Hill - Wyckoff Investigator _____

WATER QUALITY DATA

Date: 3-15-17 initials: 6SL Date: 3-17-17 initials: 6SL

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.4	7.7	30.0	7.9	15.2	8.5	30.0	8.0
35	15.6	7.7	30.0	7.9	15.2	8.4	30.0	8.0
18	15.6	7.9	30.0	7.9	15.2	8.3	30.5	8.0
9	15.8	8.0	30.0	8.0	15.2	8.2	30.0	8.1
4	15.8	8.0	30.0	8.0	15.2	8.1	30.0	8.1
2	16.0	8.0	30.0	8.0	15.2	8.1	30.0	8.0
Control	16.1	8.0	30.0	8.1	15.2	8.1	30.0	8.1
Brine control	16.6	8.1	29.5	8.1	15.2	8.1	30.0	8.0

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.5	0.4	16
pH	8.1	0.2	16
Salinity (ppt):	30.0	0.2	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 16.1 Day 0: 16.1
 Day 1: 15.9 Day 1: 16.0
 Day 2: 15.2 Day 2: 15.5

LARVAL COUNT DATA

3-21-17 JS → Rec 3/19/17

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	198	2	208	4	196	5	216	0
35	211	3	194	6	214	5	199	4
18	192	3	217	5	185	1	187	3
9	189	0	204	1	193	3	209	4
4	211	1	210	2	201	0	197	6
2	220	1	200	0	179	1	184	3
Control	193	2	174	2	198	1	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

CARLSBAD

AQUAFARM INC.

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.	778547239894
QUANTITY	ITEM	DESCRIPTION		PRICE LBS.	AMOUNT
1	Bio Assay Mus	Mytilus galloprovincialis		140.00	140.00
1	BOX CHARGE	Box Charge		0.00	0.00
		RECEIVED 3-8-17 -652			
				Total	\$140.00

THIS IS YOUR INVOICE

CETIS Analytical Report

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

Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID: 08-8549-8548		Endpoint: <u>Proportion Survived</u>		CETIS Version: CETISv1.8.7							
Analyzed: 22 Mar-17 8:47		Analysis: Parametric-Two Sample		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: 48h		Source: Carlsbad Aquafarms		Age:							
Sample ID: 12-3464-8860		Code: 49973F1C		Client: 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	Alt Hyp	Trials	Seed	PMSD	Test Result					
Angular (Corrected)	NA	C <> T	NA	NA	19.3%	Passes proportion survived					
Equal Variance t Two-Sample Test											
Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Dilution Water		Brine Reagent	1.73	2.447	0.237	6	0.1344	CDF	Non-Significant Effect		
Auxiliary Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:5%)					
Extreme Value	Grubbs Extreme Value		2.074	2.127	0.0751	No Outliers Detected					
ANOVA Table											
Source	Sum Squares		Mean Square	DF	F Stat	P-Value	Decision(α:5%)				
Between	0.05629235		0.05629235	1	2.992	0.1344	Non-Significant Effect				
Error	0.1128788		0.01881314	6							
Total	0.1691712			7							
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Variance Ratio F		5.356	47.47	0.2016	Equal Variances					
Variances	Mod Levene Equality of Variance		0.4967	13.75	0.5074	Equal Variances					
Variances	Levene Equality of Variance		2.265	13.75	0.1830	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.8289	0.6451	0.0578	Normal Distribution					
Distribution	Kolmogorov-Smirnov D		0.2696	0.3313	0.0899	Normal Distribution					
Distribution	Anderson-Darling A2 Normality		0.6769	3.878	0.0772	Normal Distribution					
Proportion Survived Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.9028	0.8268	0.9789	0.9336	0.8341	0.9431	0.02389	5.29%	0.0%
0	Brine Reagent	4	0.9585	0.8364	1	0.9336	0.8436	1	0.03837	8.01%	-6.17%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.26	1.137	1.382	1.311	1.151	1.33	0.03847	6.11%	0.0%
0	Brine Reagent	4	1.428	1.144	1.711	1.311	1.164	1.536	0.08903	12.47%	-13.32%
Proportion Survived Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	0.9242	0.8341	0.9431	0.91						
0	Brine Reagent	0.8436	1	0.9905	1						
Angular (Corrected) Transformed Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	1.292	1.151	1.33	1.266						
0	Brine Reagent	1.164	1.536	1.473	1.536						

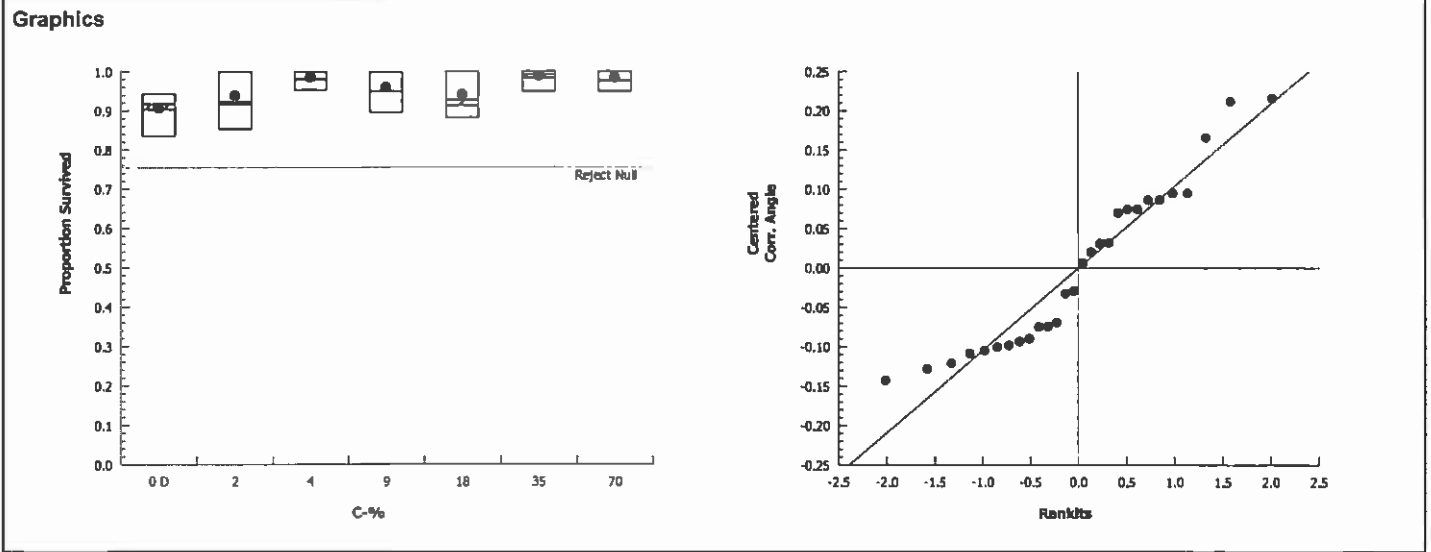
Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID:	12-1613-9556	Endpoint:	Proportion Survived			CETIS Version:	CETISv1.8.7				
Analyzed:	22 Mar-17 8:44	Analysis:	Parametric-Control vs Treatments			Official Results:	Yes				
Batch ID:	05-1514-6467	Test Type:	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:	48h	Source:	Carlsbad Aquafarms			Age:					
Sample ID:	12-3464-8860	Code:	49973F1C			Client:	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	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	16.4%	70	>70	NA	1.429		
Dunnnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Dilution Water		2	-0.7158	2.448	0.207	6	0.9720	CDF	Non-Significant Effect		
		4	-2.248	2.448	0.207	6	0.9998	CDF	Non-Significant Effect		
		9	-1.306	2.448	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	-2.147	2.448	0.207	6	0.9997	CDF	Non-Significant Effect		
Auxiliary Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:5%)					
Extreme Value	Grubbs Extreme Value		2.051	2.876	0.9511	No Outliers Detected					
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.1451618	0.02419363	6	1.695	0.1716	Non-Significant Effect					
Error	0.2997454	0.01427359	21								
Total	0.4449072		27								
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		2.009	16.81	0.9189	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9214	0.8975	0.0376	Normal Distribution					
Proportion Survived Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	0.9028	0.8268	0.9789	0.9171	0.8341	0.9431	0.02389	5.29%	0.0%
2		4	0.9218	0.8179	1	0.9171	0.8531	1	0.03263	7.08%	-2.1%
4		4	0.9787	0.939	1	0.981	0.9526	1	0.01246	2.55%	-8.4%
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		4	0.9751	0.9293	1	0.9763	0.9479	1	0.0144	2.95%	-8.01%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.26	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	1.242	1.536	0.06443	9.41%	-8.76%
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%

Blvalve Larval Survival and Development Test Northwestern Aquatic Sciences

Analysis ID: 12-1613-9556 Endpoint: Proportion Survived CETIS Version: CETISv1.8.7
 Analyzed: 22 Mar-17 8:44 Analysis: Parametric-Control vs Treatments Official Results: Yes

Proportion Survived Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9242	0.8341	0.9431	0.91
2		1	0.9479	0.8531	0.8863
4		1	1	0.9526	0.9621
9		0.8957	0.9716	0.9289	1
18		0.9242	1	0.8815	0.9005
35		1	0.9479	1	0.981
70		0.9479	1	0.9526	1

Angular (Corrected) Transformed Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.292	1.151	1.33	1.266
2		1.536	1.34	1.177	1.227
4		1.536	1.536	1.351	1.375
9		1.242	1.401	1.301	1.536
18		1.292	1.536	1.219	1.25
35		1.536	1.34	1.536	1.433
70		1.34	1.536	1.351	1.536



LC₅₀ > 70% BY DATA INSPECTION
 -631

CETIS Analytical Report

Report Date: 22 Mar-17 08:48 (p 1 of 2)
 Test Code: 658-80 02-8040-0265

Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID:	15-0070-7739	Endpoint:	Combined Proportion Normal			CETIS Version:	CETISv1.8.7				
Analyzed:	22 Mar-17 8:47	Analysis:	Parametric-Two Sample			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:	48h	Source:	Carlsbad Aquafarms			Age:					
Sample ID:	12-3464-8860	Code:	49973F1C			Client:	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	Alt Hyp	Trials	Seed	PMSD	Test Result					
Angular (Corrected)	NA	C <> T	NA	NA	18.2%	Passes combined proportion normal					
Equal Variance t Two-Sample Test											
Control	vs Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)			
Dilution Water	Brine Reagent	1.474	2.447	0.220	6	0.1909	CDF	Non-Significant Effect			
Auxiliary Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)						
Extreme Value	Grubbs Extreme Value	1.998	2.127	0.1232	No Outliers Detected						
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.03519318	0.03519318	1	2.173	0.1909	Non-Significant Effect					
Error	0.09716019	0.01619337	6								
Total	0.1323534		7								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Variance Ratio F	4.424	47.47	0.2534	Equal Variances						
Variances	Mod Levene Equality of Variance	0.7184	13.75	0.4292	Equal Variances						
Variances	Levene Equality of Variance	1.604	13.75	0.2522	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.8717	0.6451	0.1565	Normal Distribution						
Distribution	Kolmogorov-Smimov D	0.2842	0.3313	0.0563	Normal Distribution						
Distribution	Anderson-Darling A2 Normality	0.559	3.878	0.1523	Normal Distribution						
Combined Proportion Normal Summary											
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 (Corrected) Transformed Summary											
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%
0	Brine Reagent	4	1.38	1.122	1.639	1.297	1.145	1.502	0.08126	11.77%	-10.63%
Combined Proportion Normal Detail											
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						
Angular (Corrected) Transformed Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	1.274	1.139	1.32	1.258						
0	Brine Reagent	1.145	1.502	1.401	1.473						

CETIS Analytical Report

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

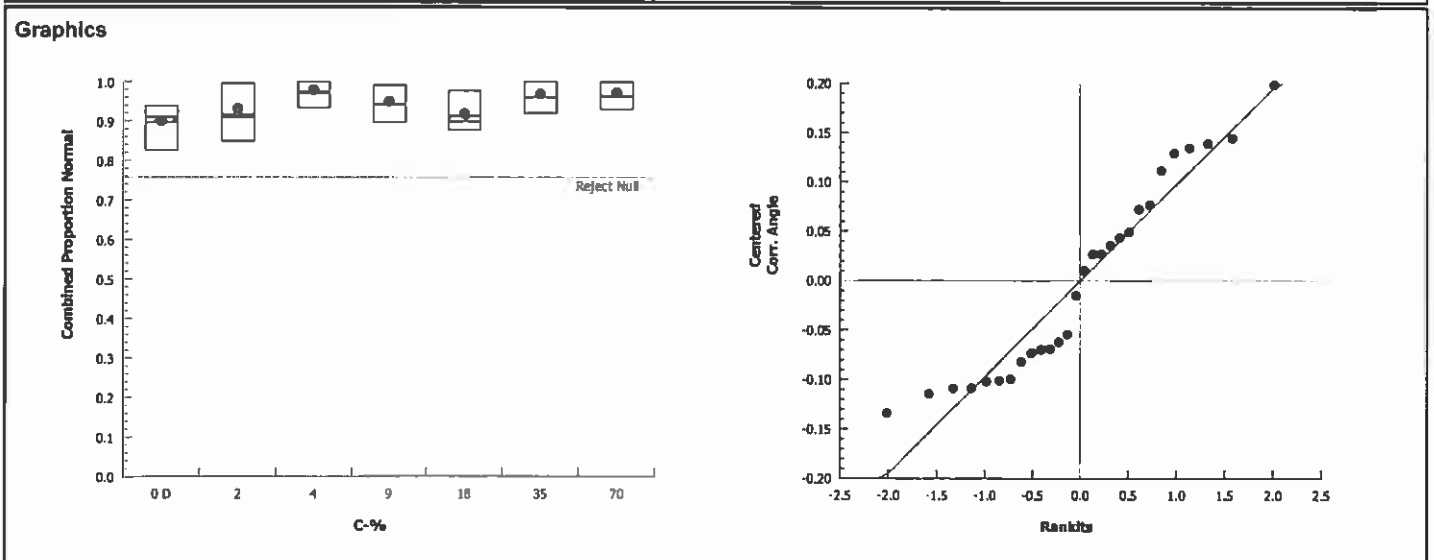
Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID: 09-0535-7845		Endpoint: Combined Proportion Normal				CETIS Version: CETISv1.8.7					
Analyzed: 22 Mar-17 8:47		Analysis: Parametric-Control vs Treatments				Official Results: Yes					
Batch ID: 05-1514-6467		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: 48h		Source: Carlsbad Aquafarms				Age:					
Sample ID: 12-3464-8860		Code: 49973F1C				Client: 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	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	15.5%	70	>70	NA	1.429		
Dunnnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Dilution Water		2	-0.7276	2.448	0.192	6	0.9729	CDF	Non-Significant Effect		
		4	-2.255	2.448	0.192	6	0.9998	CDF	Non-Significant Effect		
		9	-1.229	2.448	0.192	6	0.9936	CDF	Non-Significant Effect		
		18	-0.4268	2.448	0.192	6	0.9424	CDF	Non-Significant Effect		
		35	-1.837	2.448	0.192	6	0.9991	CDF	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(α: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(α: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(α:1%)					
Variances	Bartlett Equality of Variance		1.313	16.81	0.9709	Equal Variances					
Variances	Mod Levene Equality of Variance		0.6364	3.812	0.6999	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					
Distribution	Kolmogorov-Smirnov D		0.1751	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					
Distribution	Anderson-Darling A2 Normality		0.8898	3.878	0.0229	Normal Distribution					
Combined Proportion Normal Summary											
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	0.91	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	1	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	1	0.9621	0.9289	1	0.01745	3.62%	-7.54%

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences			
Analysis ID: 09-0535-7845	Endpoint: Combined Proportion Normal	CETIS Version: CETISv1.8.7					
Analyzed: 22 Mar-17 8:47	Analysis: Parametric-Control vs Treatments	Official Results: Yes					

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	1.266	1.139	1.32	0.03864	6.19%	0.0%
2		4	1.305	1.064	1.546	1.273	1.171	1.503	0.07565	11.59%	-4.58%
4		4	1.425	1.248	1.601	1.427	1.31	1.536	0.05546	7.78%	-14.21%
9		4	1.344	1.175	1.513	1.331	1.242	1.473	0.05312	7.9%	-7.74%
18		4	1.281	1.13	1.433	1.246	1.212	1.42	0.04767	7.44%	-2.69%
35		4	1.392	1.215	1.57	1.375	1.283	1.536	0.05576	8.01%	-11.57%
70		4	1.402	1.224	1.58	1.386	1.301	1.537	0.05593	7.98%	-12.38%

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9147	0.8246	0.9384	0.9052
2		0.9955	0.9479	0.8483	0.872
4		1	0.9953	0.9526	0.9336
9		0.8957	0.9668	0.9147	0.9905
18		0.91	0.9775	0.8768	0.8863
35		1	0.9194	0.9772	0.9431
70		0.9384	0.9858	0.9289	1

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	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
9		1.242	1.388	1.274	1.473
18		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



EC₅₀ > 70% BY DATA INSPECTION
 -653

CETIS Analytical Report

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

Bivalve Larval Survival and Development Test **Northwestern Aquatic Sciences**

Analysis ID: 10-9527-5584 Endpoint: Combined Proportion Normal
 Analyzed: 22 Mar-17 8:47 Analysis: Linear Interpolation (ICPIN) CETIS Version: CETISv1.8.7
 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: 48h Source: Carlsbad Aquafarms Age:

Sample ID: 12-3464-8860 Code: 49973F1C Client: 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:

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	671997	280	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.026	2.876	1.0000	No Outliers Detected

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>70	N/A	N/A	<1.429	NA	NA

Combined Proportion Normal Summary **Calculated Variate(A/B)**

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Dilution Water	4	0.8957	0.8246	0.9384	0.0247	0.0494	5.52%	0.0%	756	844
2		4	0.9159	0.8483	0.9955	0.03396	0.06793	7.42%	-2.26%	783	854
4		4	0.9704	0.9336	1	0.01623	0.03246	3.35%	-8.33%	819	844
9		4	0.9419	0.8957	0.9905	0.02209	0.04418	4.69%	-5.16%	795	844
18		4	0.9126	0.8768	0.9775	0.02272	0.04544	4.98%	-1.89%	781	855
35		4	0.9599	0.9194	1	0.01785	0.03571	3.72%	-7.17%	818	852
70		4	0.9633	0.9289	1	0.01745	0.03491	3.62%	-7.54%	818	849

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9147	0.8246	0.9384	0.9052
2		0.9955	0.9479	0.8483	0.872
4		1	0.9953	0.9526	0.9336
9		0.8957	0.9668	0.9147	0.9905
18		0.91	0.9775	0.8768	0.8863
35		1	0.9194	0.9772	0.9431
70		0.9384	0.9858	0.9289	1

CETIS Test Data Worksheet

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

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences			
Start Date:	15 Mar-17 14:00	Species:	Mytilus galloprovincialis	Sample Code:	49973F1C		
End Date:	17 Mar-17 14:05	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Wyckoff		
Sample Date:	14 Mar-17 09:20	Material:	Industrial Effluent	Sample Station:			

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	211	178	178	175	
0	B	2	11	211	213	213	210	
0	B	3	12	211	209	209	205	
0	B	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	199	
70		1	4	211	200	200	198	
70		2	20	211	212	212	208	
70		3	19	211	201	201	198	
70		4	25	211	216	216	216	

data entry verified against laboratory bench sheets 3-23-17 JRF

Northwestern Aquatic Sciences (REGION COPY)

Date Shipped: 3/14/2017

Carrier Name: FedEx

Airbill No: 7859 0360 1601

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 Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
658 1st Quarter-2		Ground Water/ K,Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	03/14/2017 09:20	Field Sample

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

Analysis Key: CHRTOX=Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	<i>Keith Allers - CH2M</i>	3-14-2017 0955	<i>Gary Behler LMS</i>	3-15-17 1225	<i>In Ref</i>

ORIGIN ID: BFIA (206) 780-1711
KEITH ALLERS
CH2M HILL INC
5350 CREOSOTE PLACE N.E.
BAINBRIDGE ISLAND, WA 98110
UNITED STATES US

SHIP DATE: 14MAR17
ACTWGT: 12.00 LB
CAD: 103963466 WSI3100
DIMS: 12x15x13 IN
BILL SENDER

TO GEARLD IRISSARRI
NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD

5846134/ADR/ISSC1

NEWPORT OR 97365

(541) 265-7225
INV:
PO:

REF: PN: 436558.FP.Y5.01

DEPT:



FedEx
Express



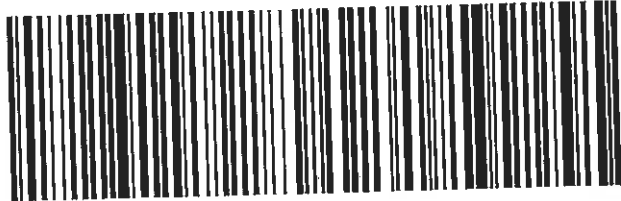
WED - 15 MAR 12:00P
PRIORITY OVERNIGHT

TRK# 7859 0360 1601
0201

86 ONPA

97365

OR-US PDX



NORTHWESTERN AQUATIC SCIENCES A Division of NAS Associates, Inc.		SAMPLE NO <u>658</u>	DATE <u>3-14-17</u>	SEAL BROKEN BY <u>GD</u> DATE <u>3-15-17</u>
		SIGNATURE <u>Keith Allers</u>		
		PRINT NAME AND TITLE <u>Keith Allers Industrial Tech</u>		

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

Test No. 999-3654 Client: QC Test

Investigator REVIEWED PAGES 1-8 -651

STUDY MANAGEMENT

Client: QC Test
 Client's Study Monitor: QC Test
 Testing Laboratory: Northwestern Aquatic Sciences
 Test Location: Newport Laboratory
 Laboratory's Study Personnel:
 Proj. Mgr./Study Dir. G.J. Irissari ⁶⁵¹
 QA Officer L.K. Nemeth
 1. G.A. Butler ^{UB} 2. J. Brown ^{JS}
 3. _____ 4. R.S. Piquini ^{RS}
 Study Schedule:
 Test Beginning: 3-15-17 1400 Test Ending: 3-17-17 1405

TEST MATERIAL

Description: Copper as CuSO₄·5H₂O, Argent Lot# 0195,
 NAS Sample No. _____ 1.0 mg/ml stock prepared: 5-16-16
 Date of Collection: _____
 Date of Receipt: _____
 Temperature (deg C): _____
 pH: _____
 Dissolved oxygen (mg/L): _____
 Conductivity (umhos/cm): _____
 Hardness (mg/L): _____
 Alkalinity (mg/L): _____
 Salinity (ppt): _____
 Total chlorine (mg/L): _____
 Total ammonia-N (mg/L): _____

DILUTION WATER

Description: Yaquina Bay, OR Seawater
 Date of Collection: 3-14-17 Salinity (ppt) 30.0 pH 8.0
 Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q® deionized water
100.0 ppt BRINE, BRINE PREPARED 2-20-17

TEST ORGANISMS

Species: Mytilus galloprovincialis Date Received: 3-8-17
 Source: Carlsbad Aquafarms, Carlsbad, CA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
3-8-17	10.9	7.9	15.0	9.9	Held outside in trays of
3-10-17	13.0	7.9	12.0	8.7	flowing seawater
3-13-17	14.7	7.8	19.0	8.7	
3-15-17	14.5	7.8	26.5	8.0	
	6.3 ^{2.15} 14.5				
Mean	14.8	7.9	18.1	8.8	
S.D.	2.9 3.0	0.1	6.3	0.8	
(N)	4	4	4	4	

Photoperiod during acclimation: Outdoor ambient conditions

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

Test No. 999-3654 Client _____ QC Test _____ Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0950 Final: 0950 Fertilization: 1030
 Number of organisms used: females: 3 males: 3
 Egg Dilution (1 ml diluted to 100 ml):
 Count/ml of dilution: 1. 37 2. 49 3. 39 Mean: 41.7
 Dilution factor = DF (mean x 100/2500) = 1.7

TEST PROCEDURES AND CONDITIONS

Test concentrations (50% series recommended): 64, 32, 16, 8, 4, 2, 1 and 0 ug/L

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4
 Organisms/ml (15-30): 21.1 Test water changes: None Aeration during test: None
 Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt
 Temperature: 20 +/- 1 °C, oysters; 16 +/- 1 °C, mussels Beaker placement: Stratified randomization

RANDOMIZATION CHART

A	8	32	1	64	∅	16	2	4		
B	1	8	16	4	32	2	∅	64		
C	2	4	64	16	1	8	32	∅		
D	4	64	∅	32	2	16	1	8		

PREPARATION OF TEST SOLUTIONS

Test Conc. (Cu, ug/L)	ml of working stock #2 (2 ug/mL)	Dilution water (ml/100mL)
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	

3-15-17
651

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: WSL

Date: 3-17-17 initials: GD

Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	16.0	8.0	29.5	8.1	15.2	8.1	30.0	8.0
32	15.9	8.0	29.5	8.1	15.3	8.1	29.5	8.1
16	16.0	8.0	30.0	8.1	15.3	8.1	30.0	8.0
8	15.9	8.0	29.5	8.1	15.2	8.1	30.0	8.0
4	15.9	8.0	30.0	8.1	15.2	8.1	30.0	8.1
2	16.0	8.0	30.0	8.1	15.3	8.1	30.0	8.1
1	16.0	8.0	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	30.0	8.0
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.6	0.4	16
pH:	8.1	0.1	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: 15.9 Day 1: 16.0
 Day 2: 15.2 Day 2: 15.5

LARVAL COUNT DATA

← 3-21-17 WS → 3/19/17

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	0	0	0	0	0	0	0	0
32	0	47	0	46	0	59	0	62
16	0	196	0	190	0	188	0	181
8	201	1	212	1	204	3	173	6
4	182	3	189	4	198	2	193	2
2	181	3	210	5	222	3	227	2
1	214	4	203	1	199	3	176	4
Control	208	4	202	2	221	0	207	2
Brine control	---	---	---	---	---	---	---	---
Zero time	218	214	186	215	204	227	---	---

Zero time: Mean 211 SD 14 N 6

CV=(sd/mean)x100 6.7%

Remarks:

Invoice

CARLSBAD AQUAFARM INC.

Sustainable Mariculture ~ Preserving Our Oceans' Resources

DATE	INVOICE #
3/1/2017	195377

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.	778547239894
QUANTITY	ITEM	DESCRIPTION	PRICE LBS.	AMOUNT	
1 1	Bio Assay Mus BOX CHARGE	Mytilus galloprovincialis Box Charge RECEIVED 3-8-17 -651	140.00 140.00	140.00 140.00	
THIS IS YOUR INVOICE				Total	\$140.00

CETIS Summary Report

Report Date: 22 Mar-17 08:29 (p 1 of 2)
 Test Code: 999-3654 | 02-0711-3850

Bivalve Larval Survival and Development Test			Northwestern Aquatic Sciences		
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: 48h	Source: Carlsbad Aquafarms	Age:			
Sample ID: 18-1959-5246	Code: 6C74D1EE	Client: Internal Lab			
Sample Date: 15 Mar-17 14:00	Material: Copper sulfate	Project:			
Receive Date: 15 Mar-17 14:00	Source: Reference Toxicant				
Sample Age: NA	Station:				

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-0379-4549	Combined Proportion Norm	8	16	11.31	8.18%		Dunnett Multiple Comparison Test
20-1354-4304	Proportion Survived	8	16	11.31	6.54%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
05-0354-9501	Combined Proportion Norm	EC25	9.589	8.847	9.978		Linear Interpolation (ICPIN)
03-7078-9692	Combined Proportion Norm	EC50	10.72	10.53	10.92		Trimmed Spearman-Kärber
00-4990-3080	Proportion Survived	EC50	24.29	23.53	25.08		Trimmed Spearman-Kärber

Combined Proportion Normal Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.981	0.9528	1	0.9573	1	0.008866	0.01773	1.81%	0.0%
1		4	0.9302	0.8253	1	0.8341	0.9817	0.03299	0.06598	7.09%	5.18%
2		4	0.9578	0.8516	1	0.8578	0.9953	0.03336	0.06671	6.97%	2.37%
4		4	0.9028	0.8519	0.9538	0.8626	0.9384	0.01601	0.03203	3.55%	7.97%
8		4	0.9337	0.8097	1	0.8199	0.9953	0.03894	0.07789	8.34%	4.83%
16		4	0	0	0	0	0	0	0		100.0%
32		4	0	0	0	0	0	0	0		100.0%
64		4	0	0	0	0	0	0	0		100.0%

Proportion Survived Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.9893	0.9644	1	0.9668	1	0.007829	0.01566	1.58%	0.0%
1		4	0.9443	0.8433	1	0.8531	1	0.03176	0.06351	6.73%	4.55%
2		4	0.968	0.8662	1	0.872	1	0.03199	0.06398	6.61%	2.16%
4		4	0.9159	0.8688	0.9629	0.8768	0.9479	0.01478	0.02957	3.23%	7.43%
8		4	0.9467	0.8387	1	0.8483	1	0.03392	0.06784	7.17%	4.31%
16		4	0.8945	0.8479	0.9412	0.8578	0.9289	0.01466	0.02931	3.28%	9.58%
32		4	0.2536	0.1918	0.3153	0.218	0.2938	0.0194	0.03879	15.3%	74.37%
64		4	0	0	0	0	0	0	0		100.0%

CETIS Summary Report

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

Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences
Combined Proportion Normal Detail						
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	
8		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 Normal Binomials						
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 Binomials						
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	
8		202/211	211/211	207/211	179/211	
16		196/211	190/211	188/211	181/211	
32		47/211	46/211	59/211	62/211	
64		0/211	0/211	0/211	0/211	

CETIS Test Data Worksheet

Report Date: 22 Mar-17 08:28 (p 1 of 1)
 Test Code: 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:			

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		2	32	211	204	204	203	
1		3	27	211	202	202	199	
1		4	7	211	180	180	178	
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	185	195	193	
8		1	10	211	202	202	201	
8		2	6	211	213	213	212	
8		3	5	211	207	207	204	
8		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	0	0	
64		2	22	211	0	0	0	
64		3	3	211	0	0	0	
64		4	21	211	0	0	0	

data entry verified against laboratory bench sheets 3-23-17 JZK

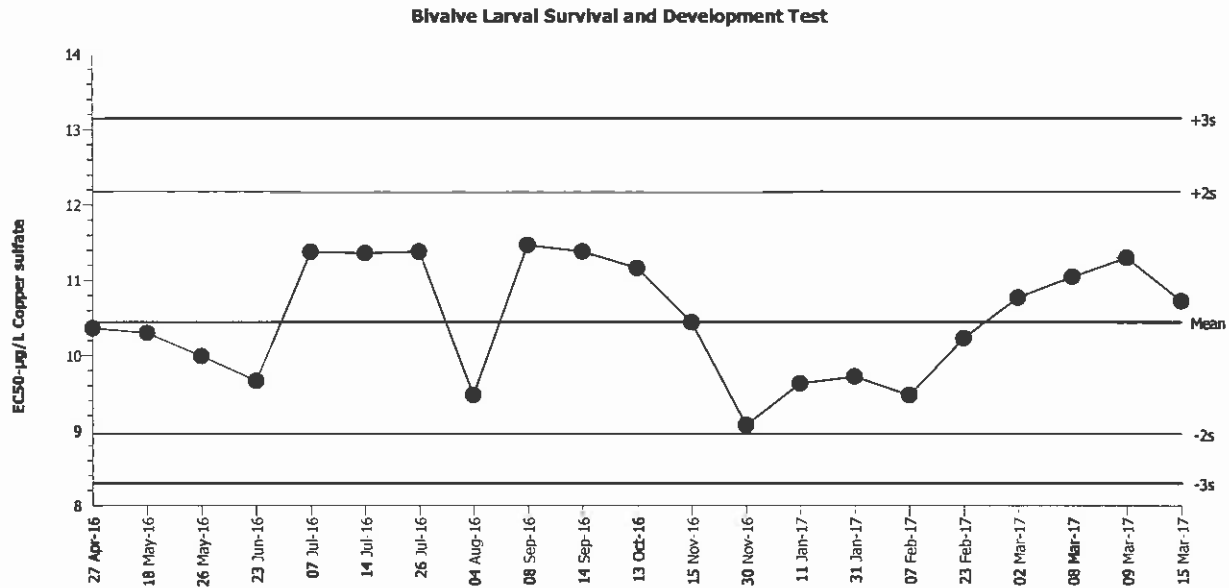
Bivalve Larval Survival and Development Test

Northwestern Aquatic Sciences

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis (Bay Mussel)
 Endpoint: Combined Proportion Normal

Material: Copper sulfate
 Source: Reference Toxicant-REF



Mean: 10.44 Count: 20 -2s Warning Limit: 8.958 -3s Action Limit: 6.297
 Sigma: NA CV: 7.97% +2s Warning Limit: 12.18 +3s Action Limit: 13.15

Quality Control Data

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