TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-82

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; Y. Nakahama, Sr. Tech.

Study Schedule:

Test Beginning: 9-20-17, 1350 hrs.

Test Ending: 9-22-17, 1435 hrs.

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

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

TEST MATERIAL

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

NAS Sample No.	6000G
Collection Date	9-19-17
Receipt Date	9-20-17
Temperature (°C)	1.7
pH	7.7
Dissolved oxygen (mg/L)	11.0
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: 9-19-17

Water Quality: Salinity, 30.0 ‰; pH, 8.1

Pretreatment: Filtered to ≤0.45 µm, aerated, salinity adjusted Milli-Q deionized water.

BRINE USED FOR DILUTION WATER AND SALINITY CONTROL

Source: Filtered Yaquina Bay, Oregon, sea water

Salinity: 100.0 ‰

Date of Preparation: 8-8-17

Method of Preparation: Freezing method

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).

Age: 3.0 hrs post-fertilization.

Source: Kamilche Sea Farms, Shelton, WA.

Conditioning: Adult mussels were received on 9-6-17 and placed in trays with flowing seawater. Holding conditions for the two weeks prior to testing were: temperature, 16.2 ± 2.0°C; pH, 7.8 ± 0.2; salinity, 33.6 ± 0.8‰; and dissolved oxygen, 7.4 ± 0.5 mg/L. Photoperiod was natural daylight.
Source of Gametes: 2 females and 4 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 (4.0 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: 21.4/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, 15.4 ± 0.3°C; pH, 8.1 ± 0.2; salinity, 30.0 ± 0.2‰; and dissolved oxygen, 7.9 ± 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_c) 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.: 999-3711
Reference Toxicant and Source: Copper as $CuSO_4$-$5H_2O$, Argent Lot No. 0195, 1.0 mg/ml stock prepared 5-16-16.
Test Date: 9-20-17
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ppt, pH 8.1
Results: EC50, 8.39 µg/L; NOEC, 4 µg/L; IC25, 6.37 µg/L. The EC50 result (8.63 µg Cu/L) was outside the laboratory's control chart warning limits (EC50, 9.23 – 12.1 µg Cu/L) and also slightly below the control chart

lower action limit (8.63 µg Cu/L). There was no evidence that these organisms were unusual in any way and a previous reference toxicant test with the same batch of organisms was within limits. No laboratory error in the test procedure could be identified. The reference test prior to this one and the next reference toxicant test were both within control chart limits. Control chart warning limits of ± 2 SD will be exceeded 5% of the time by chance alone, and ± 3 SD approximately 1% of the time. The EC50 control chart limits were also fairly narrow with a C.V. of 7.0%. Reference toxicant test results should not be used as a *de facto* criterion for test rejection; effluent test results should be reviewed and interpreted in the light of reference toxicant test results (EPA-821-R-02-014). This reference toxicant test result suggests the animals were more sensitive than usual.

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

Project Manager — Date 10-17-17

Study Director — Date 10-17-17

Laboratory Director — Date 10/17/17

Quality Assurance Unit — Date 10/18/17

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*		Proportion Survived*	
(%)	Repl.	Norm.	Abn.	Total		Mean		Mean
70	1	198	11	209	0.925		0.977	
	2	189	13	202	0.883		0.944	
	3	186	7	193	0.869		0.902	
	4	229	6	235	0.975	0.913	1.000	0.956
35	1	191	6	197	0.893		0.921	
	2	199	8	207	0.930		0.967	
	3	202	4	206	0.944		0.963	
	4	216	9	225	0.960	0.932	1.000	0.963
18	1	206	6	212	0.963		0.991	
	2	202	10	212	0.944		0.991	
	3	193	5	198	0.902		0.925	
	4	197	5	202	0.921	0.932	0.944	0.963
9	1	187	8	195	0.874		0.911	
	2	189	7	196	0.883		0.916	
	3	217	8	225	0.964		1.000	
	4	212	5	217	0.991	0.928	1.000	0.957
4	1	211	11	222	0.986		1.000	
	2	192	8	200	0.897		0.935	
	3	183	9	192	0.855		0.897	
	4	217	11	228	0.952	0.923	1.000	0.958
2	1	205	9	214	0.958		1.000	
	2	217	10	227	0.956		1.000	
	3	198	8	206	0.925		0.963	
	4	210	10	220	0.981	0.955	1.000	0.991
Normal Control	1	206	10	216	0.963		1.000	
	2	198	5	203	0.925		0.949	
	3	192	8	200	0.897		0.935	
	4	195	6	201	0.911	0.924	0.939	0.956
Brine Control[1]	1	205	11	216	0.958		1.000	
	2	191	3	194	0.893		0.907	
	3	209	10	219	0.977		1.000	
	4	191	13	204	0.893	0.930	0.953	0.965

* Based on an average initial count of 214 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≤0.05) from the control.

[1] Salinity-adjusted Milli Q® deionized water (4.0 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 (≤0.45 µm) Yaquina Bay seawater or other suitable seawater, adjusted to 30 ± 2 ‰ salinity with deionized water and/or hypersaline brine. Hypersaline brine is prepared from filtered (≤0.45 µm) 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 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 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 ± 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

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 ± 1°C (16 ± 1°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 ± 1°C for oysters, 16 ± 1°C for blue mussels. The test temperatures specified by EPA (EPA/600/R-95/136) are 15 ± 1°C or 18 ± 1°C, but these specifications were based on erroneous assumptions of the agency authors. Consequently, this protocol specifies 16 ± 1°C. The salinity is 30 ± 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 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. ≥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:
1. ≥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.

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-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_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

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CG/MG2

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

REVIEWED PAGES 1-14 GJI

Test No. 658-82 Client: CH2M Hill - Wyckoff Investigator GJI

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 GB /G.J. Irissarri GJI

QA Officer L.K. Nemeth

1. Yves McCalvin 2.

3. 4.

Study Schedule:

Test Beginning: 9-20-17 1350 Test Ending: 9-22-17 1435

TEST MATERIAL

Description: GROUND WATER COMPOSITE SP-11

NAS Sample No.	60006			
Date of Collection:	9-19-17			
Date of Receipt:	9-20-17			
Temperature (deg C):	1.7			
pH:	7.7			
Dissolved oxygen (mg/L):	11.0			
Conductivity (umhos/cm):	–			
Hardness (mg/L):	–			
Alkalinity (mg/L):	–			
Salinity (ppt):	5.0			
Total chlorine (mg/L):				
Total ammonia-N (mg/L):				
:				

DILUTION WATER

Description: Yaquina Bay, OR

Date of Collection: 9-19-17 Salinity (ppt) 30.0 pH 8.1

Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q deionized water

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 9-6-17

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
9-8-17	17.1	7.9	33.5	7.1	Held outside in trays of
9-11-17	15.5	7.8	34.0	6.7	flowing seawater
9-12-17	14.4	7.7	34.0	7.7	
9-15-17	14.2	7.7	34.0	7.7	
9-18-17	15.2	7.8	34.0	7.3	
9-20-17	14.9	8.1	32.0	8.1	
Mean	16.2	7.8	33.6	7.4	
S.D.	2.0	0.2	0.8	0.5	
(N)	6	6	6	6	

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

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CG/MG2

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

Test No. 658-82 Client CH2M Hill - Wyckoff Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0940 Final: 1005 Fertilization: 1050

Number of organisms used: females: 2 males: 4

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 43 2. 40 3. 34 Mean: 39

Dilution factor = DF (mean x 100/2500) = 1.6

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): 21.4 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	0	70	4	BRINE CONTROL	9	2	18	35		
B	2	18	35	4	BRINE CONTROL	0	9	70		
C	35	BRINE CONTROL	2	9	18	70	0	4		
D	70	9	0	35	2	18	4	BRINE CONTROL		

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: 8-8-17 ; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

$$VB = VE \frac{(TS - SE)}{(SB - TS)} = VE \frac{(30 - 5)}{(100 - 30)} = VE\ (0.357)$$

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.

9-20-17 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	0	
Brine Control	0	25.0	

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CG/MG2

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

Test No. 658-82 Client CH2M Hill - Wyckoff Investigator

WATER QUALITY DATA

	Date: 9-20-17 initials:				Date: 9-21-17 initials:			
Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.0	7.7	300	7.9	15.4	8.6	30.0	7.7
35	15.2	7.8	30.5	8.0	15.3	8.4	30.0	7.8
18	15.5	7.9	30.0	8.1	15.3	8.4	30.5	7.8
9	15.6	8.0	300	8.1	15.3	8.3	300	7.8
4	15.7	8.0	30.0	8.1	15.2	8.2	300	7.8
2	15.7	8.1	30.0	8.1	15.4	8.2	30.0	7.8
Control	15.7	8.1	30.0	8.1	15.3	8.1	30.0	7.9
Brine control	16.3	8.0	29.5	8.1	15.2	8.1	300	7.8

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.4	0.3	16
pH	8.1	0.2	16
Salinity (ppt):	30.0	0.2	16
DO (mg/L):	7.9	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 15.7 Day 0: 15.7
Day 1: 15.3 Day 1: 15.4
Day 2: 15.3 Day 2: 15.4

LARVAL COUNT DATA

9-25-17 GJZ — 9-27-17 GJZ | 9-27-17 JS

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	198	11	189	13	186	7	229	6
35	191	6	199	8	202	4	216	9
18	206	6	202	10	193	5	197	5
9	187	8	189	7	217	8	212	5
4	211	11	192	8	183	9	217	11
2	205	9	217	10	198	8	210	10
Control	206	10	198	5	192	8	195	6
Brine control	205	11	191	3	209	10	191	13
Zero time	213	226	195	206	236	205	—	—

Zero time: Mean 214 SD 15 N 6 CV=(sd/mean)x100 7.0%

Remarks:

PRODUCT 108

KAMILCHE
SEA FARMS

Kamilche Sea Farms, Inc.
2741 SE Bloomfield Road • Shelton, WA 98584
360 427 5774 • Fax 360 427 0610
WA Cert. #217-SS
Harvested: Totten Inlet, Puget **Sound**

38303

TO NorthWest Aquatic Sciences
(Gerald)

DATE	9-6-17
CUSTOMER ORDER NO	
SALESPERSON	
VIA	

TERMS

QUANTITY	DESCRIPTION		PRICE	AMOUNT
10 lbs.	Mussels - Beard On	1 @ 10 lbs.	[redacted]	[redacted]
			Total	[redacted]

Rec'd
9-6-17

Thank You!

Bivalve Larval Survival and Development Test					Northwestern Aquatic Sciences
Analysis ID:	11-8704-6299	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Oct-17 8:53	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Batch ID:	07-5095-1409	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	20 Sep-17 13:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	22 Sep-17 14:35	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	49h	**Source:**	Carlsbad Aquafarms	**Age:**	

Sample ID:	02-7867-3813	**Code:**	109C3995	**Client:**	Wyckoff Treatment Plant
Sample Date:	19 Sep-17 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	20 Sep-17 11:30	**Source:**	Wyckoff		
Sample Age:	28h (1.7 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	11.7%	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	0.4979	2.447	0.213	6	0.6362	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.414	2.127	1.0000	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003768686	0.003768686	1	0.2479	0.6362	Non-Significant Effect
Error	0.09119768	0.01519961	6			
Total	0.09496637		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.687	47.47	0.6782	Equal Variances
Variances	Mod Levene Equality of Variance	1.024	13.75	0.3505	Equal Variances
Variances	Levene Equality of Variance	1.172	13.75	0.3207	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8877	0.6451	0.2225	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.249	0.3313	0.1645	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.5728	3.878	0.1408	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	0.9556	0.9076	1	0.9509	0.9346	1	0.01508	3.16%	0.0%
0	Brine Reagent	4	0.965	0.8938	1	0.9509	0.9065	1	0.02237	4.64%	-0.98%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.378	1.209	1.547	1.347	1.312	1.537	0.05319	7.72%	0.0%
0	Brine Reagent	4	1.422	1.202	1.641	1.347	1.26	1.537	0.06907	9.72%	-3.15%

Proportion Survived Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	0.9486	0.9346	0.9393
0	Brine Reagent	1	0.9065	1	0.9533

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.537	1.342	1.312	1.322
0	Brine Reagent	1.537	1.26	1.537	1.353

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	01-1252-8614	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Oct-17 8:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	07-5095-1409	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	20 Sep-17 13:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	22 Sep-17 14:35	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	49h	**Source:**	Carlsbad Aquafarms	**Age:**	
Sample ID:	02-7867-3813	**Code:**	109C3995	**Client:**	Wyckoff Treatment Plant
Sample Date:	19 Sep-17 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	20 Sep-17 11:30	**Source:**	Wyckoff		
Sample Age:	28h (1.7 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.1%	70	>70	NA	1.429

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	-1.41	2.448	0.205	6	0.9963	CDF	Non-Significant Effect
		4	-0.349	2.448	0.205	6	0.9310	CDF	Non-Significant Effect
		9	-0.3139	2.448	0.205	6	0.9253	CDF	Non-Significant Effect
		18	-0.1814	2.448	0.205	6	0.9005	CDF	Non-Significant Effect
		35	-0.2211	2.448	0.205	6	0.9085	CDF	Non-Significant Effect
		70	-0.07519	2.448	0.205	6	0.8765	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.558	2.876	1.0000	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03851885	0.006419808	6	0.4557	0.8328	Non-Significant Effect
Error	0.295845	0.01408786	21			
Total	0.3343638		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.81	16.81	0.9363	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9198	0.8975	0.0343	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	0.9556	0.9076	1	0.9439	0.9346	1	0.01508	3.16%	0.0%
2		4	0.9907	0.9609	1	1	0.9626	1	0.009346	1.89%	-3.67%
4		4	0.9579	0.8769	1	0.9673	0.8972	1	0.02545	5.31%	-0.24%
9		4	0.9568	0.8773	1	0.9579	0.9112	1	0.02497	5.22%	-0.12%
18		4	0.9626	0.9097	1	0.9673	0.9252	0.9907	0.01663	3.46%	-0.73%
35		4	0.9626	0.9107	1	0.965	0.9206	1	0.0163	3.39%	-0.73%
70		4	0.9556	0.8679	1	0.9603	0.9019	1	0.02129	4.46%	0.0%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.378	1.209	1.547	1.332	1.312	1.537	0.05319	7.72%	0.0%
2		4	1.497	1.369	1.624	1.537	1.376	1.537	0.0401	5.36%	-8.59%
4		4	1.407	1.166	1.649	1.424	1.244	1.537	0.07585	10.78%	-2.13%
9		4	1.405	1.162	1.647	1.407	1.268	1.537	0.07629	10.86%	-1.91%
18		4	1.393	1.243	1.543	1.403	1.294	1.474	0.04717	6.77%	-1.11%
35		4	1.397	1.231	1.562	1.383	1.285	1.537	0.05205	7.45%	-1.35%
70		4	1.384	1.191	1.578	1.375	1.252	1.537	0.0609	8.8%	-0.46%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	01-1252-8614	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Oct-17 8:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

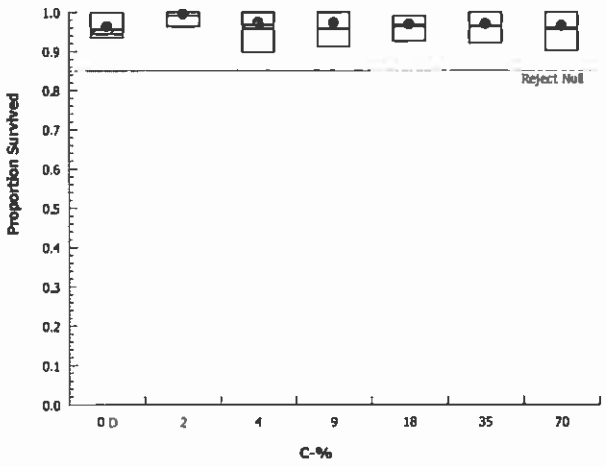
Proportion Survived Detail

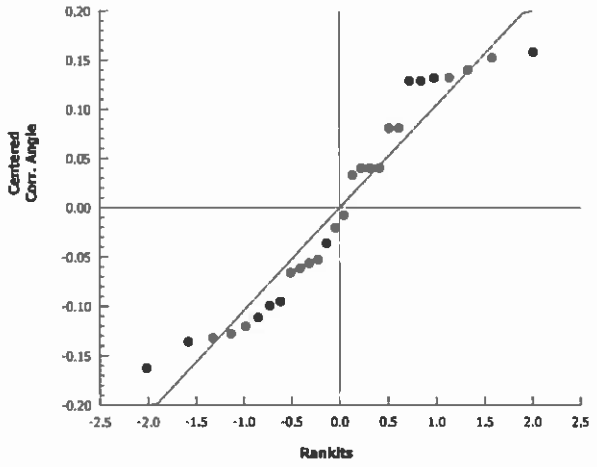
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	0.9486	0.9346	0.9393
2		1	1	0.9626	1
4		1	0.9346	0.8972	1
9		0.9112	0.9159	1	1
18		0.9907	0.9907	0.9252	0.9439
35		0.9206	0.9673	0.9626	1
70		0.9766	0.9439	0.9019	1

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.537	1.342	1.312	1.322
2		1.537	1.537	1.376	1.537
4		1.537	1.312	1.244	1.537
9		1.268	1.277	1.537	1.537
18		1.474	1.474	1.294	1.332
35		1.285	1.389	1.376	1.537
70		1.417	1.332	1.252	1.537

Graphics





$LC_{50} > 70\%$ BY DATA INSPECTION
-GJ1

CETIS Analytical Report

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	19-1141-7290	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Oct-17 8:53	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	07-5095-1409	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	20 Sep-17 13:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	22 Sep-17 14:35	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	49h	**Source:**	Carlsbad Aquafarms	**Age:**	
Sample ID:	02-7867-3813	**Code:**	109C3995	**Client:**	Wyckoff Treatment Plant
Sample Date:	19 Sep-17 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	20 Sep-17 11:30	**Source:**	Wyckoff		
Sample Age:	28h (1.7 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	6.68%	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	-0.3355	1.943	0.105	6	0.6257	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.464	2.127	0.9786	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0006564991	0.0006564991	1	0.1125	0.7487	Non-Significant Effect
Error	0.03500277	0.005833795	6			
Total	0.03565927		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.545	47.47	0.4632	Equal Variances
Variances	Mod Levene Equality of Variance	2.559	13.75	0.1608	Equal Variances
Variances	Levene Equality of Variance	3.377	13.75	0.1157	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9087	0.6451	0.3449	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.151	0.3313	1.0000	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.3487	3.878	0.4800	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	0.9241	0.8793	0.9688	0.9162	0.8972	0.9626	0.01407	3.05%	0.0%
0	Brine Reagent	4	0.9299	0.8602	0.9997	0.9162	0.8925	0.9766	0.02192	4.71%	-0.63%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.296	1.204	1.387	1.281	1.244	1.376	0.02868	4.43%	0.0%
0	Brine Reagent	4	1.314	1.168	1.459	1.281	1.237	1.417	0.04576	6.97%	-1.4%

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9626	0.9252	0.8972	0.9112
0	Brine Reagent	0.9579	0.8925	0.9766	0.8925

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.376	1.294	1.244	1.268
0	Brine Reagent	1.364	1.237	1.417	1.237

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	06-6491-7236	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Oct-17 8:53	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	07-5095-1409	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	20 Sep-17 13:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	22 Sep-17 14:35	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	49h	**Source:**	Carlsbad Aquafarms	**Age:**	
Sample ID:	02-7867-3813	**Code:**	109C3995	**Client:**	Wyckoff Treatment Plant
Sample Date:	19 Sep-17 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	20 Sep-17 11:30	**Source:**	Wyckoff		
Sample Age:	28h (1.7 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.2%	70	>70	NA	1.429

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	-1.097	2.448	0.15	6	0.9904	CDF	Non-Significant Effect
		4	-0.1778	2.448	0.15	6	0.8997	CDF	Non-Significant Effect
		9	-0.4169	2.448	0.15	6	0.9410	CDF	Non-Significant Effect
		18	-0.2554	2.448	0.15	6	0.9150	CDF	Non-Significant Effect
		35	-0.2374	2.448	0.15	6	0.9116	CDF	Non-Significant Effect
		70	0.2284	2.448	0.15	6	0.7865	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.003	2.876	1.0000	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01550184	0.00258364	6	0.3452	0.9048	Non-Significant Effect
Error	0.1571912	0.007485294	21			
Total	0.172693		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.896	16.81	0.5572	Equal Variances
Variances	Mod Levene Equality of Variance	1.942	3.812	0.1207	Equal Variances
Variances	Levene Equality of Variance	2.272	3.812	0.0758	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9667	0.8975	0.4960	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1063	0.1914	0.5762	Normal Distribution
Distribution	D'Agostino Skewness	0.836	2.576	0.4031	Normal Distribution
Distribution	D'Agostino Kurtosis	0.6546	2.576	0.5127	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	1.128	9.21	0.5691	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.3083	3.878	0.5878	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	0.9241	0.8793	0.9688	0.9182	0.8972	0.9626	0.01407	3.05%	0.0%
2		4	0.9551	0.9185	0.9917	0.9569	0.9252	0.9813	0.0115	2.41%	-3.36%
4		4	0.9225	0.8304	1	0.9245	0.8551	0.986	0.02896	6.28%	0.17%
9		4	0.928	0.8353	1	0.9238	0.8738	0.9907	0.02915	6.28%	-0.43%
18		4	0.9322	0.89	0.9745	0.9322	0.9019	0.9626	0.01329	2.85%	-0.89%
35		4	0.9316	0.8858	0.9774	0.9369	0.8925	0.96	0.0144	3.09%	-0.81%
70		4	0.913	0.8376	0.9884	0.9042	0.8692	0.9745	0.0237	5.19%	1.2%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	06-6491-7236	Endpoint:	Combined Proportion Normal
Analyzed:	17 Oct-17 8:53	Analysis:	Parametric-Control vs Treatments
CETIS Version:	CETISv1.8.7		
Official Results:	Yes		

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.296	1.204	1.387	1.281	1.244	1.376	0.02868	4.43%	0.0%
2		4	1.363	1.272	1.454	1.362	1.294	1.434	0.02856	4.19%	-5.18%
4		4	1.307	1.116	1.497	1.297	1.18	1.452	0.05973	9.14%	-0.84%
9		4	1.321	1.116	1.526	1.302	1.208	1.474	0.06433	9.74%	-1.97%
18		4	1.311	1.225	1.398	1.308	1.252	1.376	0.0271	4.13%	-1.21%
35		4	1.31	1.221	1.399	1.317	1.237	1.369	0.02801	4.28%	-1.12%
70		4	1.282	1.131	1.432	1.258	1.201	1.41	0.04728	7.38%	1.08%

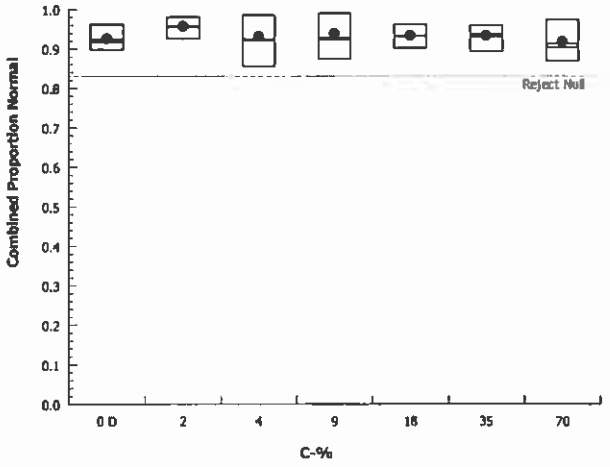
Combined Proportion Normal Detail

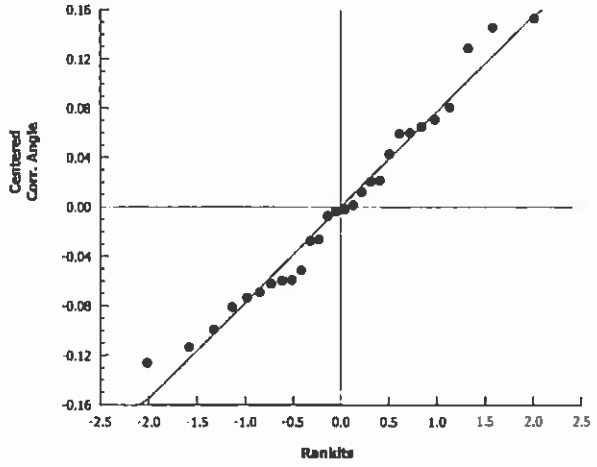
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9626	0.9252	0.8972	0.9112
2		0.9579	0.9559	0.9252	0.9813
4		0.986	0.8972	0.8551	0.9518
9		0.8738	0.8832	0.9644	0.9907
18		0.9626	0.9439	0.9019	0.9206
35		0.8925	0.9299	0.9439	0.96
70		0.9252	0.8832	0.8692	0.9745

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.376	1.294	1.244	1.268
2		1.364	1.359	1.294	1.434
4		1.452	1.244	1.18	1.349
9		1.208	1.222	1.381	1.474
18		1.376	1.332	1.252	1.285
35		1.237	1.303	1.332	1.369
70		1.294	1.222	1.201	1.41

Graphics





$EC_{50} > 70\%$ BY DATA INSPECTION -GJ

CETIS Analytical Report

Report Date: 17 Oct-17 08:54 (p 1 of 1)
Test Code: 658-82 | 05-5208-6613

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	08-6236-2201	Endpoint:	Combined Proportion Normal	CETIS Version:	CETISv1.8.7
Analyzed:	17 Oct-17 8:53	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Batch ID:	07-5095-1409	Test Type:	Development-Survival	Analyst:	
Start Date:	20 Sep-17 13:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	22 Sep-17 14:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	49h	Source:	Carlsbad Aquafarms	Age:	

Sample ID:	02-7867-3813	Code:	109C3995	Client:	Wyckoff Treatment Plant
Sample Date:	19 Sep-17 09:20	Material:	Industrial Effluent	Project:	
Receive Date:	20 Sep-17 11:30	Source:	Wyckoff		
Sample Age:	28h (1.7 °C)	Station:			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	863800	280	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.003	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	0.9241	0.8972	0.9626	0.01407	0.02813	3.05%	0.0%	791	856
2		4	0.9551	0.9252	0.9813	0.0115	0.02301	2.41%	-3.36%	830	869
4		4	0.9225	0.8551	0.986	0.02896	0.05792	6.28%	0.17%	803	870
9		4	0.928	0.8738	0.9907	0.02915	0.0583	6.28%	-0.43%	805	867
18		4	0.9322	0.9019	0.9626	0.01329	0.02657	2.85%	-0.89%	798	856
35		4	0.9316	0.8925	0.96	0.0144	0.0288	3.09%	-0.81%	808	867
70		4	0.913	0.8692	0.9745	0.0237	0.0474	5.19%	1.2%	802	877

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9626	0.9252	0.8972	0.9112
2		0.9579	0.9559	0.9252	0.9813
4		0.986	0.8972	0.8551	0.9518
9		0.8738	0.8832	0.9644	0.9907
18		0.9626	0.9439	0.9019	0.9206
35		0.8925	0.9299	0.9439	0.96
70		0.9252	0.8832	0.8692	0.9745

CETIS Test Data Worksheet

Report Date: 17 Oct-17 08:51 (p 1 of 1)
Test Code: 05-5208-6612/658-82

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences
Start Date:	20 Sep-17 13:50	Species:	Mytilis galloprovincialis	Sample Code: 109C3995
End Date:	22 Sep-17 14:35	Protocol:	EPA/600/R-95/136 (1995)	Sample Source: Wyckoff
Sample Date:	19 Sep-17 09:20	Material:	Industrial Effluent	Sample Station:

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	214	216	216	205	
0	B	2	11	214	194	194	191	
0	B	3	12	214	219	219	209	
0	B	4	3	214	204	204	191	
0	D	1	10	214	216	216	206	
0	D	2	2	214	203	203	198	
0	D	3	23	214	200	200	192	
0	D	4	9	214	201	201	195	
2		1	18	214	214	214	205	
2		2	27	214	227	227	217	
2		3	22	214	206	206	198	
2		4	30	214	220	220	210	
4		1	28	214	222	222	211	
4		2	15	214	200	200	192	
4		3	14	214	192	192	183	
4		4	24	214	228	228	217	
9		1	6	214	195	195	187	
9		2	29	214	196	196	189	
9		3	5	214	225	225	217	
9		4	17	214	217	217	212	
18		1	16	214	212	212	206	
18		2	13	214	212	212	202	
18		3	1	214	198	198	193	
18		4	31	214	202	202	197	
35		1	7	214	197	197	191	
35		2	26	214	207	207	199	
35		3	21	214	206	206	202	
35		4	8	214	225	225	216	
70		1	4	214	209	209	198	
70		2	20	214	202	202	189	
70		3	19	214	193	193	186	
70		4	25	214	235	235	229	

Northwestern Aquatic Sciences (REGION COPY)
DateShipped: 9/19/2017
CarrierName: FedEx
AirbillNo: 7877 7852 1087

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2017/WA
Project Code: WEH-025H
Cooler #: 1 of 1

No: 10-091917-095026-0196
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 Third Quarter		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	09/19/2017 09:20	Field Sample
					NAS# 60000			

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
	Keith Allers CHZM	9-19-2017 0955	Cory Bubba	9-2017 1130	AR

PAGE 13 OF 14

ORIGIN ID:BFIA (206) 780-1711
KEITH ALLERS
CH2MHILL INC
5350 CREOSOTE PLACE N.E.

BAINBRIDGE ISLAND, WA 98110
UNITED STATES US

SHIP DATE: 19SEP17
ACTWGT: 11.00 LB
CAD: 111531780/WSXI3100
DIMS: 15x12x13 IN

BILL SENDER

TO GEARLD IRSSARRI
NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD

NEWPORT OR 97365
(541) 265-7225
INV:
PO:
REF: PN: 436558 FP Y6.01
DEPT:

549J1/FF19/104C

FedEx Express

WED - 20 SEP 12:00P
PRIORITY OVERNIGHT

TRK# 0201 7877 7852 1087

86 ONPA

97365
OR-US PDX

CUSTODY SEAL

Date: 9-19-2017

Signature: Keith Allers

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

NORTHWESTERN AQUATIC SCIENCES PROTOCOL NO. NAS-XXX-CG/MG2

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

REVIEWED PAGES 1-9 GJI

Test No. 999-3711 Client: QC Test Investigator

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. Irissarri GJI

QA Officer L.K. Nemeth

1. Yves Letourneau YL 2. BA Buhrow

3. 4.

Study Schedule:

Test Beginning: 9-20-17 1350 Test Ending: 9-22-17 1435

TEST MATERIAL

Description: Copper as $CuSO_4 \cdot 5H_2O$, 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: 9-19-17 Salinity (ppt) 30.0 pH 8.1

Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q® deionized water

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 9-6-17

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
9-8-17	17.1	7.9	33.5	7.1	Held outside in trays of
9-11-17	15.5	7.8	34.0	6.7	flowing seawater
9-12-17	14.4	7.7	34.0	7.7	
9-15-17	14.2	7.7	34.0	7.7	
9-19-17	18.2	7.8	34.0	7.3	
9-20-17	14.9	8.1	32.0	8.1	
Mean	16.2	7.8	33.6	7.4	
S.D.	2.0	0.2	0.8	0.5	
(N)	6	6	6	6	

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. 999-3711 Client QC Test Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0940 Final: 1005 Fertilization: 1050
Number of organisms used: females: 2 males: 4
Egg Dilution (1 ml diluted to 100 ml):
Count/ml of dilution: 1. 43 2. 40 3. 34 Mean: 39
Dilution factor = DF (mean x 100/2500) = 1.6

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): 21.4 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	4	16	ϕ	8	32	1	64	2		
B	32	1	4	64	2	16	ϕ	8		
C	64	ϕ	8	2	16	4	32	1		
D	2	1	32	0	8	64	4	16		

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	

9-20-17
631

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. 999-3711 Client QC Test Investigator _____

WATER QUALITY DATA

	Date: 9-20-17 initials: [illegible]				Date: 9-22-17 initials: CG/GJ			
Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	15.4	8.1	29.5	8.1	15.4	8.1	300	7.8
32	15.4	8.1	29.5	8.1	15.4	8.1	300	7.9
16	15.5	8.1	30.0	8.1	15.4	8.1	300	7.9
8	15.4	8.1	30.0	8.1	15.4	8.1	300	8.0
4	15.4	8.1	30.0	8.1	15.4	8.1	300	7.9
2	15.5	8.1	30.0	8.1	15.4	8.1	300	8.0
1	15.5	8.1	30.0	8.1	15.4	8.1	300	8.0
Control	15.7	8.1	30.0	8.1	15.5	8.1	300	8.0
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.4	0.1	16
pH:	8.1	0.0	16
Salinity (ppt):	29.9	0.2	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 15.7 Day 0: 15.4
Day 1: 15.3 Day 1: 15.4
Day 2: 15.3 Day 2: 15.5

LARVAL COUNT DATA

	← 9-24-17 GJ				9-26-17 GJ		9-27-17 RC →	
Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	Ø	1	Ø	1	Ø	1	Ø	2
32	Ø	38	Ø	40	Ø	34	Ø	65
16	Ø	169	Ø	174	Ø	198	Ø	201
8	107	79	106	82	128	83	122	94
4	196	7	201	12	205	10	217	14
2	194	12	189	3	193	10	200	9
1	210	8	197	9	192	10	196	9
Control	200	6	194	3	208	5	193	7
Brine control	---	---	---	---	---	---	---	---
Zero time	213	226	195	206	236	205	—	—

Zero time: Mean 214 SD 15 N 6 CV=(sd/mean)x100 7.0%

Remarks:

PRODUCT 108

KAMILCHE
SEA FARMS

Kamilche Sea Farms, Inc.
2741 SE Bloomfield Road • Shelton, WA 98584
360 427 5774 • Fax 360 427 0310
WA Cert. #217-SS
Harvested: Totten Inlet, Puget Sound

38303

TO NorthWest Aquatic Sciences
(Gerald)

DATE 9-6-17
CUSTOMER ORDER NO
SALESPERSON
VIA

TERMS

QUANTITY	DESCRIPTION		PRICE	AMOUNT
10 lbs.	Mussels - Beard On	1 @ 10 lbs.	[redacted]	[redacted]
			Total	[redacted]

Rec'd
9-6-17

Thank You!

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Batch ID:	07-5095-1409	Test Type:	Development-Survival	Analyst:	
Start Date:	20 Sep-17 13:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	22 Sep-17 14:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	49h	Source:	Carlsbad Aquafarms	Age:	

Sample ID:	11-3820-2694	Code:	43D79846	Client:	Internal Lab
Sample Date:	20 Sep-17 13:50	Material:	Copper sulfate	Project:	
Receive Date:	20 Sep-17 13:50	Source:	Reference Toxicant		
Sample Age:	NA	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-7374-1818	Combined Proportion Norm	4	8	5.657	5.82%		Dunnett Multiple Comparison Test
18-7098-6234	Proportion Normal	2	4	2.828	1.86%		Dunnett Multiple Comparison Test
12-3204-2141	Proportion Survived	16	32	22.63	9.35%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
06-6343-7481	Combined Proportion Norm	EC25	6.368	5.829	6.84		Linear Interpolation (ICPIN)
06-3387-4553	Combined Proportion Norm	EC50	8.39	8.191	8.594		Spearman-Kärber
05-0535-8724	Proportion Normal	EC25	6.359	6.183	6.554		Linear Interpolation (ICPIN)
15-2190-9348	Proportion Normal	EC50	8.457	8.238	8.683		Trimmed Spearman-Kärber
13-4117-6377	Proportion Survived	EC50	24.15	23.54	24.78		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	0.9287	0.8774	0.98	0.9019	0.972	0.01612	0.03223	3.47%	0.0%
1		4	0.9287	0.8707	0.9868	0.8972	0.9813	0.01824	0.03647	3.93%	0.0%
2		4	0.9065	0.8727	0.9403	0.8832	0.9346	0.01062	0.02124	2.34%	2.39%
4		4	0.9381	0.9107	0.9655	0.9159	0.9579	0.008613	0.01723	1.84%	-1.01%
8		4	0.5409	0.4593	0.6224	0.4953	0.5981	0.02562	0.05124	9.47%	41.76%
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 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	0.9743	0.9609	0.9877	0.965	0.9848	0.004211	0.008423	0.86%	0.0%
1		4	0.9566	0.9482	0.9649	0.9505	0.9633	0.002622	0.005245	0.55%	1.82%
2		4	0.9584	0.9292	0.9877	0.9417	0.9844	0.009187	0.01837	1.92%	1.63%
4		4	0.9505	0.932	0.969	0.9394	0.9655	0.005806	0.01161	1.22%	2.44%
8		4	0.5776	0.5458	0.6095	0.5638	0.6066	0.01001	0.02001	3.47%	40.71%
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	0.9533	0.9007	1	0.9206	0.9953	0.01652	0.03304	3.47%	0.0%
1		4	0.9661	0.928	1	0.9439	1	0.01197	0.02394	2.48%	-1.35%
2		4	0.9463	0.8911	1	0.8972	0.9766	0.01733	0.03466	3.66%	0.74%
4		4	0.986	0.9462	1	0.9486	1	0.01251	0.02502	2.54%	-3.43%
8		4	0.9334	0.8234	1	0.8692	1	0.03457	0.06914	7.41%	2.08%
16		4	0.8668	0.7453	0.9883	0.7897	0.9393	0.03818	0.07636	8.81%	9.07%
32		4	0.2068	0.1023	0.3113	0.1589	0.3037	0.03284	0.06568	31.77%	78.31%
64		4	0.005841	0.002123	0.009559	0.004673	0.009346	0.001168	0.002336	40.0%	99.39%

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	0.9346	0.9065	0.972	0.9019
1		0.9813	0.9206	0.8972	0.9159
2		0.9065	0.8832	0.9019	0.9346
4		0.9159	0.9393	0.9579	0.9394
8		0.5	0.4953	0.5981	0.5701
16		0	0	0	0
32		0	0	0	0
64		0	0	0	0

Proportion Normal Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9709	0.9848	0.9765	0.965
1		0.9633	0.9563	0.9505	0.9561
2		0.9417	0.9844	0.9507	0.9569
4		0.9655	0.9437	0.9535	0.9394
8		0.5753	0.5638	0.6066	0.5648
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	0.9626	0.9206	0.9953	0.9346
1		1	0.9626	0.9439	0.9579
2		0.9626	0.8972	0.9486	0.9766
4		0.9486	0.9953	1	1
8		0.8692	0.8785	0.986	1
16		0.7897	0.8131	0.9252	0.9393
32		0.1776	0.1869	0.1589	0.3037
64		0.004673	0.004673	0.004673	0.009346

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	200/214	194/214	208/214	193/214
1		210/214	197/214	192/214	196/214
2		194/214	189/214	193/214	200/214
4		196/214	201/214	205/214	217/231
8		107/214	106/214	128/214	122/214
16		0/214	0/214	0/214	0/214
32		0/214	0/214	0/214	0/214
64		0/214	0/214	0/214	0/214

Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	200/206	194/197	208/213	193/200
1		210/218	197/206	192/202	196/205
2		194/206	189/192	193/203	200/209
4		196/203	201/213	205/215	217/231
8		107/186	106/188	128/211	122/216
16		0/169	0/174	0/198	0/201
32		0/38	0/40	0/34	0/65
64		0/1	0/1	0/1	0/2

Proportion Survived Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	206/214	197/214	213/214	200/214
1		214/214	206/214	202/214	205/214
2		206/214	192/214	203/214	209/214
4		203/214	213/214	214/214	214/214
8		186/214	188/214	211/214	214/214
16		169/214	174/214	198/214	201/214
32		38/214	40/214	34/214	65/214
64		1/214	1/214	1/214	2/214

CETIS Test Data Worksheet

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

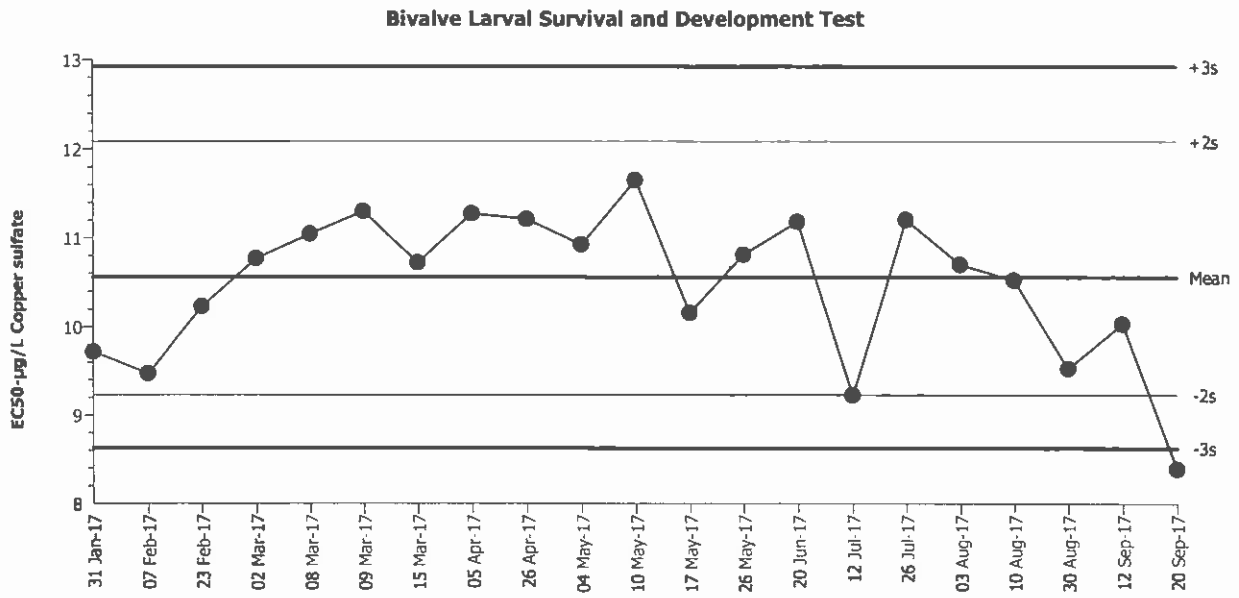
Start Date:	20 Sep-17 13:50	**Species:**	Mytilis galloprovincialis
End Date:	22 Sep-17 14:35	**Protocol:**	EPA/600/R-95/136 (1995)
Sample Date:	20 Sep-17 13:50	**Material:**	Copper sulfate
Sample Code:	43D79846		
Sample Source:	Reference Toxicant		
Sample Station:			

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	5	214	206	206	200	
0	D	2	31	214	197	197	194	
0	D	3	25	214	213	213	208	
0	D	4	28	214	200	200	193	
1		1	8	214	218	218	210	
1		2	21	214	206	206	197	
1		3	15	214	202	202	192	
1		4	10	214	205	205	196	
2		1	24	214	206	206	194	
2		2	27	214	192	192	189	
2		3	17	214	203	203	193	
2		4	26	214	209	209	200	
4		1	22	214	203	203	196	
4		2	4	214	213	213	201	
4		3	6	214	215	215	205	
4		4	9	214	231	231	217	
8		1	29	214	186	186	107	
8		2	7	214	188	188	106	
8		3	13	214	211	211	128	
8		4	2	214	216	216	122	
16		1	32	214	169	169	0	
16		2	20	214	174	174	0	
16		3	23	214	198	198	0	
16		4	11	214	201	201	0	
32		1	3	214	38	38	0	
32		2	18	214	40	40	0	
32		3	16	214	34	34	0	
32		4	12	214	65	65	0	
64		1	30	214	1	1	0	
64		2	19	214	1	1	0	
64		3	1	214	1	1	0	
64		4	14	214	2	2	0	

data entry verified against laboratory bench sheets 10-4-17 JDF

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

Test Type:	Development-Survival	**Organism:**	Mytilis galloprovincialis (Bay Mussel)	**Material:**	Copper sulfate
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Combined Proportion Normal	**Source:**	Reference Toxicant-REF



Mean:	10.56	**Count:**	20	**-2s Warning Limit:**	9.229	**-3s Action Limit:**	8.628
Sigma:	NA	**CV:**	6.97%	**+2s Warning Limit:**	12.08	**+3s Action Limit:**	12.92

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jan	31	12:30	9.717	-0.8418	-1.234			00-4554-8270	10-7110-0578
2		Feb	7	13:30	9.47	-1.089	-1.617			11-6796-8451	21-2845-1444
3			23	19:30	10.23	-0.3308	-0.4727			18-8118-8710	02-7913-5800
4		Mar	2	13:00	10.77	0.2088	0.2908			07-2409-7218	16-5427-1566
5			8	15:30	11.04	0.4852	0.6671			11-1093-1447	03-3381-2684
6			9	13:55	11.3	0.7388	1.004			18-3588-3261	02-4714-0912
7			15	14:00	10.72	0.1624	0.2266			02-0711-3850	03-7078-9692
8		Apr	5	13:15	11.27	0.7131	0.9704			06-1946-6662	08-5835-4084
9			26	13:20	11.21	0.6501	0.8873			07-5946-4689	06-0434-2999
10		May	4	14:30	10.92	0.3646	0.5041			10-0435-4306	18-9328-1898
11			10	14:10	11.65	1.09	1.459			14-1408-5654	20-3711-0314
12			17	13:10	10.15	-0.4044	-0.5798			03-9435-3893	05-7716-5731
13			26	13:20	10.81	0.2511	0.3489			05-6211-0933	16-0073-3043
14		Jun	20	16:50	11.18	0.6225	0.8506			02-1409-7276	08-4079-1566
15		Jul	12	13:25	9.224	-1.335	-2.008	(-)		02-4818-7084	16-2581-4533
16			26	14:10	11.2	0.6444	0.8797			17-8688-1039	02-1882-3377
17		Aug	3	13:30	10.7	0.1433	0.2002			12-5107-4745	07-3767-8523
18			10	15:10	10.53	-0.03333	-0.04694			14-0880-4376	18-5220-7572
19			30	14:35	9.527	-1.032	-1.528			21-1251-6624	08-5861-5172
20		Sep	12	14:00	10.03	-0.5268	-0.7599			10-5774-5128	09-7081-1600
21			20	13:50	8.39	-2.17	-3.415	(-)	(-)	12-1488-7812	06-3387-4553