TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-83

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

Study Schedule:

Test Beginning: 11-1-17, 1335 hrs.

Test Ending: 11-3-17, 1335 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.	6036G
Collection Date	10-31-17
Receipt Date	11-1-17
Temperature (°C)	2.8
pH	7.5
Dissolved oxygen (mg/L)	11.5
Salinity (‰)	5.5

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon seawater.

Date of Collection: 10-31-17

Water Quality: Salinity, 30.0 ‰; pH, 7.9

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: 10-3-17

Method of Preparation: Freezing method

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).

Age: 2.5 hrs post-fertilization.

Source: Kamilche Sea Farms, Shelton, WA.

Conditioning: Adult mussels were received on 10-25-17 and placed in trays with flowing seawater. Holding conditions prior to testing were: temperature, 13.6 ± 0.7°C; pH, 7.9 ± 0.1; salinity, 28.4 ± 2.3‰; and dissolved oxygen, 7.9 ± 0.2 mg/L. Photoperiod was natural daylight.
Source of Gametes: 2 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 (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: 24.7/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, 15.7 ± 0.2°C; pH, 8.2 ± 0.2; salinity, 30.5 ± 0.5‰; and dissolved oxygen, 8.0 ± 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.: 999-3723
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: 11-1-17
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ppt, pH 7.9
Results: EC50, 11.2 µg/L; NOEC, 8 µg/L; IC25, 9.84 µg/L. The EC50 result was within the laboratory's control chart warning limits (8.88 – 12.5 µg/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

Project Manager — Date 11-16-17

Study Director — Date 11-16-17

Laboratory Director — Date 11/16/17

Quality Assurance Unit — Date 11-16-17
for Linda Nemeth

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	215	2	217	0.870		0.879	
	2	234	4	238	0.947		0.964	
	3	247	3	250	1.000		1.000	
	4	233	6	239	0.943	0.940	0.968	0.952
35	1	221	3	224	0.895		0.907	
	2	251	5	256	0.981		1.000	
	3	259	4	263	0.985		1.000	
	4	230	1	231	0.931	0.948	0.935	0.961
18	1	237	1	238	0.960		0.964	
	2	227	2	229	0.919		0.927	
	3	245	1	246	0.992		0.996	
	4	241	6	247	0.976	0.962	1.000	0.972
9	1	202	3	205	0.818		0.830	
	2	228	5	233	0.923		0.943	
	3	213	4	217	0.862		0.879	
	4	255	2	257	0.992	0.899	1.000	0.913
4	1	245	2	247	0.992		1.000	
	2	237	3	240	0.960		0.972	
	3	279	1	280	0.996		1.000	
	4	228	4	232	0.923	0.968	0.939	0.978
2	1	221	2	223	0.895		0.903	
	2	265	2	267	0.993		1.000	
	3	262	0	262	1.000		1.000	
	4	224	6	230	0.907	0.949	0.931	0.959
Normal Control	1	244	5	249	0.988		1.000	
	2	246	1	247	0.996		1.000	
	3	255	1	256	0.996		1.000	
	4	229	3	232	0.927	0.977	0.939	0.985
Brine Control[1]	1	205	30	235	0.830		0.951	
	2	265	1	266	0.996		1.000	
	3	225	2	227	0.911		0.919	
	4	219	3	222	0.887	0.906	0.899	0.942

* Based on an average initial count of 247 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 (5.5 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. 658-83 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 /G.J. Irissarri GJI

QA Officer L.K. Nemeth

1. [signature] 2. J. Brown JB

3. 4.

Study Schedule:

Test Beginning: 11-1-17 1335 Test Ending: 11-7-17 1335

TEST MATERIAL

Description: GROUND WATER COMPOSITE SP-11

NAS Sample No.	60366				
Date of Collection:	10-31-17				
Date of Receipt:	11-1-17				
Temperature (deg C):	2.8				
pH:	7.5				
Dissolved oxygen (mg/L):	11.5				
Conductivity (umhos/cm):	–				
Hardness (mg/L):	–				
Alkalinity (mg/L):	–				
Salinity (ppt):	5.5				
Total chlorine (mg/L):					
Total ammonia-N (mg/L):					
:					

DILUTION WATER

Description: Yaquina Bay, OR

Date of Collection: 10-31-17 Salinity (ppt) 30.0 pH 7.9

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

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 10-25-17

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
10-25-17	12.9	7.8	26.0	8.0	Held outside in trays of
10-27-17	14.5	7.8	27.0	8.1	flowing seawater
10-30-17	13.7	8.0	29.5	7.7	
11-1-17	13.4	7.9	31.0	7.9	
Mean	13.6	7.9	28.4	7.9	
S.D.	0.7	0.1	2.3	0.2	
(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

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

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

Test No. 658-83 Client CH2M Hill - Wyckoff Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0945 Final: 1020 Fertilization: 1105

Number of organisms used: females: 2 males: 3

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 40 2. 41 3. 40 Mean: 40.3

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): 24.7 | 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	35	9	Brine Ctl	70	Ø	18	4	2		
B	18	4	2	35	Brine Ctl	9	70	Ø		
C	70	2	4	18	Ø	35	Brine Ctl	9		
D	Brine Ctl	18	Ø	70	9	2	35	4		

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: 10-3-17 ; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

$$VB = VE \frac{(TS - SE)}{(SB - TS)} = VE \frac{(30 - 5.5)}{(100 - 30)} = VE\,(0.35)$$

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.

11-1-17 GJL

Test Conc. (%)	Effluent (ml/100ml)	Brine (ml/100ml)	Dilution Water (ml/100ml)
70	70	24.5	Brought up to a final
35	35	12.3	volume of 100 ml
18	18	6.3	with dilution water
9	9	3.2	
4	4	1.4	
2	2	0.7	
0	0	0	
Brine Control	0	24.5	

THE BRINE CONTROL WAS MADE UP OF SALINITY ADJUSTED MILLI-Q DEIONIZED WATER (5.5 ppt) SIMULAR TO THE EFFLUENT. AS A RESULT, THE AMOUNT OF BRINE IN THE BRINE CONTROL WAS THE SAME USED IN THE 70% EFFLENT CONCENTRATION.

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

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

Test No. 658-83 Client CH2M Hill - Wyckoff Investigator

WATER QUALITY DATA

Conc. (%)	Date: 11-1-17 initials:				Date: 11-3-17 initials:			
	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.7	7.8	31.0	7.9	15.5	8.6	31.5	7.9
35	15.7	7.9	30.5	7.9	15.3	8.5	31.0	7.5
18	15.8	8.0	30.5	7.9	15.6	8.4	30.5	7.9
9	15.8	8.1	30.5	7.9	15.7	8.4	30.5	8.0
4	15.7	8.1	30.0	7.9	15.6	8.3	30.5	8.0
2	15.9	8.1	30.0	7.9	15.8	8.3	30.0	8.1
Control	15.8	8.2	30.0	7.9	15.7	8.2	30.0	8.1
Brine control	16.0	8.2	31.0	8.0	15.8	8.2	31.0	8.1

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.7	0.2	16
pH	8.2	0.2	16
Salinity (ppt):	30.5	0.5	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 16.0 Day 0: 15.7
Day 1: 15.8 Day 1: 15.8
Day 2: 15.7 Day 2: 15.5

LARVAL COUNT DATA

← 11-7-17 JS | 11-7-17 GJ →

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	215	2	234	4	247	3	233	6
35	221	3	251	5	259	4	230	1
18	237	1	227	2	245	1	241	6
9	202	3	228	5	213	4	255	2
4	245	2	237	3	279	1	228	4
2	221	2	265	2	262	0	224	6
Control	244	5	246	1	255	1	229	3
Brine control	205	30	265	1	225	2	219	3
Zero time	270	247	233	239	253	237	—	—

Zero time: Mean 247 SD 14 N 6 CV=(sd/mean)x100 5.5%

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

38630

TO Northwest Aquatic Sciences
attn: Gerald

DATE 10/24/17
CUSTOMER ORDER NO.
SALESPERSON
VIA

TERMS

QUANTITY	DESCRIPTION	PRICE	AMOUNT
10	Mussels - Beard On	[redacted]	[redacted]

RECEIVED 10-25-17
-GJ

Thank You!

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	09-1848-1329	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	07 Nov-17 14:44	Analysis:	Parametric-Two Sample	Official Results:	Yes
Batch ID:	17-2910-2311	Test Type:	Development-Survival	Analyst:	
Start Date:	01 Nov-17 13:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	03 Nov-17 13:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Carlsbad Aquafarms	Age:	
Sample ID:	02-1796-4113	Code:	CFDDE51	Client:	Wyckoff Treatment Plant
Sample Date:	31 Oct-17 09:30	Material:	Industrial Effluent	Project:	
Receive Date:	01 Nov-17 11:00	Source:	Wyckoff		
Sample Age:	28h (2.8 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	6.96%	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	1.539	2.447	0.207	6	0.1746	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.665	2.127	0.5401	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03404954	0.03404954	1	2.37	0.1746	Non-Significant Effect
Error	0.08621112	0.01436852	6			
Total	0.1202607		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.437	47.47	0.7729	Equal Variances
Variances	Mod Levene Equality of Variance	0.2482	13.75	0.6361	Equal Variances
Variances	Levene Equality of Variance	0.05629	13.75	0.8204	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9549	0.6451	0.7603	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1877	0.3313	0.6812	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.2993	3.878	0.6142	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	0.9848	0.9365	1	0.9757	0.9393	1	0.01518	3.08%	0.0%
0	Brine Reagent	4	0.9423	0.8721	1	0.9757	0.8988	1	0.02207	4.69%	4.32%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.485	1.312	1.657	1.444	1.322	1.539	0.05429	7.31%	0.0%
0	Brine Reagent	4	1.354	1.147	1.561	1.444	1.247	1.539	0.06509	9.61%	8.79%

Proportion Survived Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9393
0	Brine Reagent	0.9514	1	0.919	0.8988

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.539	1.539	1.539	1.322
0	Brine Reagent	1.349	1.539	1.282	1.247

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	07-6188-5797	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	07 Nov-17 14:43	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	17-2910-2311	Test Type:	Development-Survival	Analyst:	
Start Date:	01 Nov-17 13:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	03 Nov-17 13:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Carlsbad Aquafarms	Age:	
Sample ID:	02-1796-4113	Code:	CFDDE51	Client:	Wyckoff Treatment Plant
Sample Date:	31 Oct-17 09:30	Material:	Industrial Effluent	Project:	
Receive Date:	01 Nov-17 11:00	Source:	Wyckoff		
Sample Age:	28h (2.8 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	6.5%	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	0.7897	2.448	0.234	6	0.5510	CDF	Non-Significant Effect
		4	0.3595	2.448	0.234	6	0.7381	CDF	Non-Significant Effect
		9	1.856	2.448	0.234	6	0.1455	CDF	Non-Significant Effect
		18	0.5667	2.448	0.234	6	0.6521	CDF	Non-Significant Effect
		35	0.7504	2.448	0.234	6	0.5691	CDF	Non-Significant Effect
		70	1.09	2.448	0.234	6	0.4132	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.944	2.876	1.0000	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.07602561	0.01267093	6	0.6948	0.6565	Non-Significant Effect
Error	0.3829809	0.01823719	21			
Total	0.4590065		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.105	16.81	0.9813	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9247	0.8975	0.0454	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	0.9848	0.9365	1	1	0.9393	1	0.01518	3.08%	0.0%
2		4	0.9585	0.8801	1	0.9656	0.9028	1	0.02465	5.14%	2.67%
4		4	0.9777	0.9317	1	0.9858	0.9393	1	0.01446	2.96%	0.72%
9		4	0.913	0.7947	1	0.9109	0.83	1	0.03716	8.14%	7.3%
18		4	0.9717	0.9178	1	0.9798	0.9271	1	0.01694	3.49%	1.34%
35		4	0.9605	0.8857	1	0.9676	0.9069	1	0.02351	4.9%	2.47%
70		4	0.9524	0.8699	1	0.9656	0.8785	1	0.02594	5.45%	3.29%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.485	1.312	1.657	1.539	1.322	1.539	0.05429	7.31%	0.0%
2		4	1.409	1.169	1.65	1.422	1.254	1.539	0.07562	10.73%	5.08%
4		4	1.45	1.279	1.621	1.47	1.322	1.539	0.0537	7.41%	2.31%
9		4	1.307	1.034	1.581	1.273	1.146	1.539	0.08605	13.16%	11.93%
18		4	1.431	1.251	1.61	1.443	1.297	1.539	0.05629	7.87%	3.65%
35		4	1.413	1.179	1.647	1.426	1.261	1.539	0.07351	10.4%	4.83%
70		4	1.381	1.17	1.591	1.384	1.215	1.539	0.06624	9.6%	7.01%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	07-6188-5797	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	07 Nov-17 14:43	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

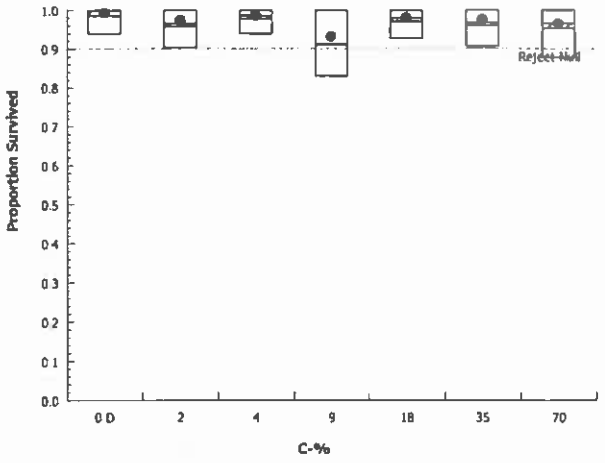
Proportion Survived Detail

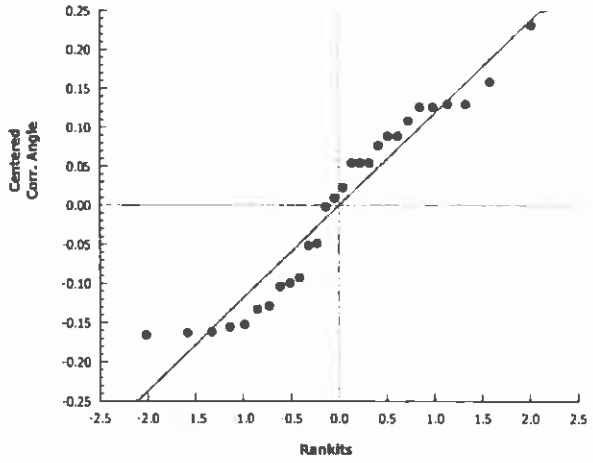
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9393
2		0.9028	1	1	0.9312
4		1	0.9717	1	0.9393
9		0.83	0.9433	0.8785	1
18		0.9636	0.9271	0.996	1
35		0.9069	1	1	0.9352
70		0.8785	0.9636	1	0.9676

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.539	1.539	1.539	1.322
2		1.254	1.539	1.539	1.305
4		1.539	1.402	1.539	1.322
9		1.146	1.33	1.215	1.539
18		1.379	1.297	1.507	1.539
35		1.261	1.539	1.539	1.313
70		1.215	1.379	1.539	1.39

Graphics





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

Bivalve Larval Survival and Development Test					Northwestern Aquatic Sciences
Analysis ID:	07-9152-3592	Endpoint:	Combined Proportion Normal	CETIS Version:	CETISv1.8.7
Analyzed:	07 Nov-17 14:44	Analysis:	Parametric-Two Sample	Official Results:	Yes

Batch ID:	17-2910-2311	Test Type:	Development-Survival	Analyst:	
Start Date:	01 Nov-17 13:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	03 Nov-17 13:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Carlsbad Aquafarms	Age:	

Sample ID:	02-1796-4113	Code:	CFDDE51	Client:	Wyckoff Treatment Plant
Sample Date:	31 Oct-17 09:30	Material:	Industrial Effluent	Project:	
Receive Date:	01 Nov-17 11:00	Source:	Wyckoff		
Sample Age:	28h (2.8 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	9.95%	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	1.679	2.447	0.227	6	0.1441	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.827	2.127	0.2919	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04849564	0.04849564	1	2.819	0.1441	Non-Significant Effect
Error	0.1032098	0.01720162	6			
Total	0.1517054		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.457	47.47	0.4797	Equal Variances
Variances	Mod Levene Equality of Variance	0.2738	13.75	0.6196	Equal Variances
Variances	Levene Equality of Variance	0.5299	13.75	0.4941	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9413	0.6451	0.6240	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1713	0.3313	0.9034	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.2685	3.878	0.7104	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	0.9768	0.9238	1	0.9575	0.9271	0.9961	0.01666	3.41%	0.0%
0	Brine Reagent	4	0.9059	0.796	1	0.9575	0.83	0.9962	0.03455	7.63%	7.25%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.443	1.285	1.602	1.379	1.297	1.508	0.04988	6.91%	0.0%
0	Brine Reagent	4	1.288	1.039	1.536	1.379	1.146	1.509	0.07818	12.14%	10.79%

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9879	0.996	0.9961	0.9271
0	Brine Reagent	0.83	0.9962	0.9109	0.8866

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.46	1.507	1.508	1.297
0	Brine Reagent	1.146	1.509	1.268	1.227

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	11-2606-0516	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Nov-17 14:44	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	17-2910-2311	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	01 Nov-17 13:35	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	03 Nov-17 13:35	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Carlsbad Aquafarms	**Age:**	
Sample ID:	02-1796-4113	**Code:**	CFDDE51	**Client:**	Wyckoff Treatment Plant
Sample Date:	31 Oct-17 09:30	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	01 Nov-17 11:00	**Source:**	Wyckoff		
Sample Age:	28h (2.8 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	8.89%	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	0.7203	2.448	0.211	6	0.5830	CDF	Non-Significant Effect
		4	0.3587	2.448	0.211	6	0.7384	CDF	Non-Significant Effect
		9	1.976	2.448	0.211	6	0.1190	CDF	Non-Significant Effect
		18	0.6613	2.448	0.211	6	0.6099	CDF	Non-Significant Effect
		35	1.015	2.448	0.211	6	0.4468	CDF	Non-Significant Effect
		70	1.051	2.448	0.211	6	0.4308	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.949	2.876	1.0000	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06960741	0.01160123	6	0.7824	0.5931	Non-Significant Effect
Error	0.3113644	0.01482688	21			
Total	0.3809718		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.898	16.81	0.9289	Equal Variances
Variances	Mod Levene Equality of Variance	0.5933	3.812	0.7322	Equal Variances
Variances	Levene Equality of Variance	0.7255	3.812	0.6340	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9438	0.8975	0.1377	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1196	0.1914	0.3768	Normal Distribution
Distribution	D'Agostino Skewness	0.4067	2.576	0.6842	Normal Distribution
Distribution	D'Agostino Kurtosis	1.632	2.576	0.1027	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	2.829	9.21	0.2430	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.5296	3.878	0.1800	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	0.9768	0.9238	1	0.9919	0.9271	0.9961	0.01666	3.41%	0.0%
2		4	0.9485	0.8604	1	0.9497	0.8947	1	0.02771	5.84%	2.89%
4		4	0.9677	0.9136	1	0.9757	0.9231	0.9964	0.017	3.51%	0.92%
9		4	0.8989	0.7784	1	0.8927	0.8178	0.9922	0.03786	8.43%	7.98%
18		4	0.9615	0.9118	1	0.9676	0.919	0.9919	0.01564	3.25%	1.56%
35		4	0.9478	0.8795	1	0.9558	0.8947	0.9848	0.02146	4.53%	2.97%
70		4	0.9403	0.8556	1	0.9453	0.8704	1	0.02662	5.66%	3.73%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	11-2606-0516	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Nov-17 14:44	Analysis:	Parametric-Control vs Treatments	**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	1.443	1.285	1.602	1.484	1.297	1.508	0.04988	6.91%	0.0%
2		4	1.381	1.138	1.625	1.372	1.24	1.54	0.07645	11.07%	4.3%
4		4	1.412	1.25	1.575	1.424	1.29	1.511	0.05114	7.24%	2.14%
9		4	1.273	1.028	1.519	1.24	1.13	1.482	0.07715	12.12%	11.79%
18		4	1.386	1.254	1.519	1.391	1.282	1.481	0.04168	6.01%	3.95%
35		4	1.356	1.197	1.514	1.368	1.24	1.447	0.04984	7.35%	6.06%
70		4	1.353	1.132	1.574	1.335	1.203	1.539	0.06947	10.27%	6.27%

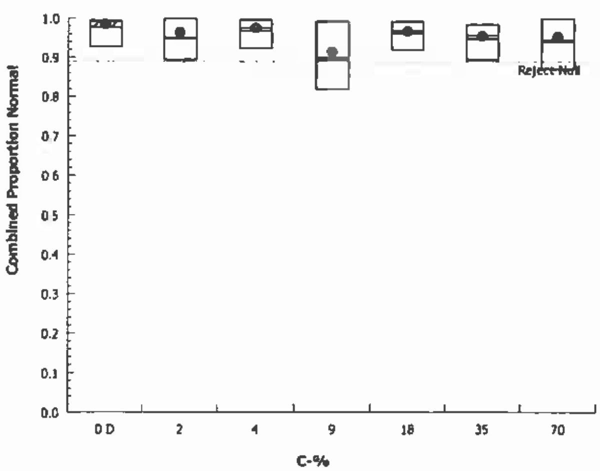
Combined Proportion Normal Detail

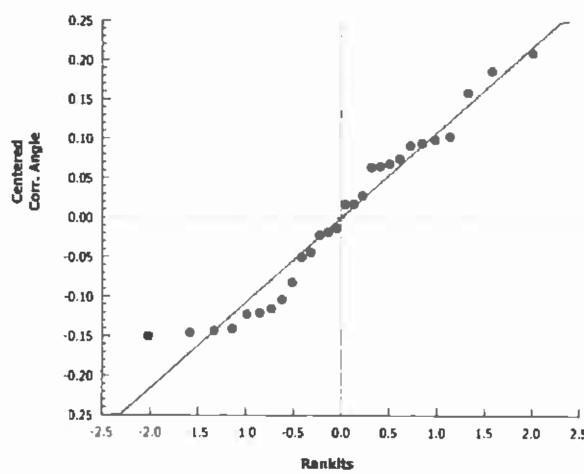
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9879	0.996	0.9961	0.9271
2		0.8947	0.9925	1	0.9069
4		0.9919	0.9595	0.9964	0.9231
9		0.8178	0.9231	0.8623	0.9922
18		0.9595	0.919	0.9919	0.9757
35		0.8947	0.9805	0.9848	0.9312
70		0.8704	0.9474	1	0.9433

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.46	1.507	1.508	1.297
2		1.24	1.484	1.54	1.261
4		1.481	1.368	1.511	1.29
9		1.13	1.29	1.191	1.482
18		1.368	1.282	1.481	1.414
35		1.24	1.431	1.447	1.305
70		1.203	1.339	1.539	1.33

Graphics





$EC_{50} > 70\%$ BY DATA INSPECTION
— [illegible]

CETIS Analytical Report

Report Date: 07 Nov-17 14:45 (p 1 of 1)
Test Code: 658-831 | 12-3949-1835

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	05-4264-6696	Endpoint:	Combined Proportion Normal	CETIS Version:	CETISv1.8.7
Analyzed:	07 Nov-17 14:44	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Batch ID:	17-2910-2311	Test Type:	Development-Survival	Analyst:	
Start Date:	01 Nov-17 13:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	03 Nov-17 13:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Carlsbad Aquafarms	Age:	

Sample ID:	02-1796-4113	Code:	CFDDE51	Client:	Wyckoff Treatment Plant
Sample Date:	31 Oct-17 09:30	Material:	Industrial Effluent	Project:	
Receive Date:	01 Nov-17 11:00	Source:	Wyckoff		
Sample Age:	28h (2.8 °C)	Station:			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1581249	280	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.949	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	0.9768	0.9271	0.9961	0.01666	0.03331	3.41%	0.0%	974	997
2		4	0.9485	0.8947	1	0.02771	0.05541	5.84%	2.89%	972	1023
4		4	0.9677	0.9231	0.9964	0.017	0.03401	3.51%	0.92%	989	1021
9		4	0.8989	0.8178	0.9922	0.03786	0.07573	8.43%	7.98%	898	998
18		4	0.9615	0.919	0.9919	0.01564	0.03127	3.25%	1.56%	950	988
35		4	0.9478	0.8947	0.9848	0.02146	0.04293	4.53%	2.97%	961	1013
70		4	0.9403	0.8704	1	0.02662	0.05324	5.66%	3.73%	929	988

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9879	0.996	0.9961	0.9271
2		0.8947	0.9925	1	0.9069
4		0.9919	0.9595	0.9964	0.9231
9		0.8178	0.9231	0.8623	0.9922
18		0.9595	0.919	0.9919	0.9757
35		0.8947	0.9805	0.9848	0.9312
70		0.8704	0.9474	1	0.9433

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences
Start Date: 01 Nov-17 13:35	Species: Mytilis galloprovincialis		Sample Code: CFDDE51	
End Date: 03 Nov-17 13:35	Protocol: EPA/600/R-95/136 (1995)		Sample Source: Wyckoff	
Sample Date: 31 Oct-17 09:30	Material: Industrial Effluent		Sample Station:	

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	247	235	235	205	
0	B	2	11	247	266	266	265	
0	B	3	12	247	227	227	225	
0	B	4	3	247	222	222	219	
0	D	1	10	247	249	249	244	
0	D	2	2	247	247	247	246	
0	D	3	23	247	256	256	255	
0	D	4	9	247	232	232	229	
2		1	18	247	223	223	221	
2		2	27	247	267	267	265	
2		3	22	247	262	262	262	
2		4	30	247	230	230	224	
4		1	28	247	247	247	245	
4		2	15	247	240	240	237	
4		3	14	247	280	280	279	
4		4	24	247	232	232	228	
9		1	6	247	205	205	202	
9		2	29	247	233	233	228	
9		3	5	247	217	217	213	
9		4	17	247	257	257	255	
18		1	16	247	238	238	237	
18		2	13	247	229	229	227	
18		3	1	247	246	246	245	
18		4	31	247	247	247	241	
35		1	7	247	224	224	221	
35		2	26	247	256	256	251	
35		3	21	247	263	263	259	
35		4	8	247	231	231	230	
70		1	4	247	217	217	215	
70		2	20	247	238	238	234	
70		3	19	247	250	250	247	
70		4	25	247	239	239	233	

Northwestern Aquatic Sciences (REGION COPY)
DateShipped: 10/31/2017
CarrierName: FedEx
AirbillNo: 788277595497

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

No: 10-103117-100104-0209
2018T10P303DD210W2LA00
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 4th Quarter		Ground Water/ K.Allers	Composite	ACTOX-CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	10/31/2017 09:30	Field Sample

PAGE 13 OF 14

Special Instructions: NAS# 6036G

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

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

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	Keith Allers CH2M	10-31-2017 1005	Yur Kolaka NAS	11-1-17/700	Intact

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

BAINBRIDGE ISLAND, WA 98110
UNITED STATES US

SHIP DATE: 31OCT17
ACTWGT: 49.00 LB
CAD: 111531780/WSXI3100
DIMS: 21x15x16 IN

BILL SENDER

TO GEARLD IRSSARRI
NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD

NEWPORT OR 97365
(541) 265-7225

REF: PN 436558 FP Y5 01

INV.
PO.
DEPT:

549J3F877/104C

FedEx Express

WED - 01 NOV 12:00P
PRIORITY OVERNIGHT

TRK# 0201 7882 7759 5497

86 ONPA

97365
OR-US PDX

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 1-8 -GJI

Test No. 999-3723 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. [signature] 2. J. Brown JB

3. 4.

Study Schedule:

Test Beginning: 11-1-17 1335 Test Ending: 11-3-17 1335

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: 10-31-17 Salinity (ppt) 30.0 pH 7.9

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

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 10-25-17

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
10-25-17	12.9	7.8	26.0	8.0	Held outside in trays of
10-27-17	14.5	7.8	27.0	8.1	flowing seawater
10-30-17	13.7	8.0	29.5	7.7	
11-1-17	13.4	7.9	31.0	7.9	
Mean	13.6	7.9	28.4	7.9	
S.D.	0.7	0.1	2.3	0.2	
(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

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

Test No. 999-3723 Client QC Test Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0945 Final: 1020 Fertilization: 1105

Number of organisms used: females: 2 males: 3

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 40 2. 41 3. 40 Mean: 40.3

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): 24.7 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	16	1	2	64	8	32	ϕ	4		
B	32	64	4	ϕ	1	16	2	8		
C	32	ϕ	8	4	2	64	16	1		
D	ϕ	8	1	16	4	2	64	32		

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	

11-1-17 GJ

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. 999-3723 Client QC Test Investigator

WATER QUALITY DATA

	Date: 11-1-17 initials:				Date: 11-3-17 initials:			
Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	15.9	8.2	29.5	7.9	15.7	8.2	29.5	8.0
32	15.9	8.2	30.0	7.9	15.6	8.2	30.0	8.0
16	15.9	8.2	30.0	7.9	15.6	8.2	30.0	8.0
8	15.8	8.2	30.0	7.9	15.6	8.2	30.0	8.1
4	15.9	8.2	30.0	7.9	15.6	8.2	30.0	8.1
2	15.9	8.2	30.0	7.9	15.7	8.2	30.5	8.1
1	15.9	8.2	30.0	7.9	15.6	8.2	30.5	8.1
Control	16.0	8.2	30.0	7.9	15.7	8.2	30.0	8.1
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:	Mean	SD	N
Temperature (°C):	15.8	0.1	16
pH:	8.2	0.0	16
Salinity (ppt):	30.0	0.3	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 16.0 Day 0: 15.7
Day 1: 15.8 Day 1: 15.8
Day 2: 15.7 Day 2: 15.7

LARVAL COUNT DATA

← 11-6-17 JS → 11-7-17 JS / 11-7-17 GS

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	Ø	1	Ø	Ø	Ø	1	Ø	Ø
32	Ø	127	Ø	120	Ø	126	Ø	127
16	Ø	219	Ø	227	7	222	6	207
8	217	7	209	4	242	5	243	7
4	231	5	239	5	257	6	229	5
2	268	3	230	4	228	3	230	6
1	241	1	224	2	222	Ø	216	3
Control	264	3	223	4	286	2	224	Ø
Brine control	---	---	---	---	---	---	---	---
Zero time	270	247	233	239	253	237	—	—

Zero time: Mean 247 SD 14 N 6 CV=(sd/mean)x100 5.5 %

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**

38630

TO Northwest Aquatic Sciences
attn Gerald

DATE 10/24/17
CUSTOMER ORDER NO
SALESPERSON
VIA

TERMS

QUANTITY	DESCRIPTION	PRICE	AMOUNT	
10	**Mussels - Beard On**	[redacted]	[redacted]	[redacted]
	RECEIVED 10-25-17			
	-601			

Thank You!

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Batch ID:	17-2910-2311	Test Type:	Development-Survival	Analyst:	
Start Date:	01 Nov-17 13:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	03 Nov-17 13:35	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Carlsbad Aquafarms	Age:	

Sample ID:	18-3214-5961	Code:	6D345429	Client:	Internal Lab
Sample Date:	01 Nov-17 13:35	Material:	Copper sulfate	Project:	
Receive Date:	01 Nov-17 13:35	Source:	Reference Toxicant		
Sample Age:	NA	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-6690-6427	Combined Proportion Norm	8	16	11.31	8.67%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
18-7430-1728	Combined Proportion Norm	EC25	9.842	8.927	10.16		Linear Interpolation (ICPIN)
06-0748-0919	Combined Proportion Norm	EC50	11.19	11.09	11.28		Trimmed Spearman-Kärber
04-1437-1603	Proportion Normal	EC25	9.925	9.831	9.998		Linear Interpolation (ICPIN)
18-3071-5127	Proportion Normal	EC50	11.12	11	11.24		Spearman-Kärber
18-6061-0060	Proportion Survived	EC50	32.6	31.9	33.28		Linear Regression (MLE)

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	0.9479	0.8687	1	0.9028	0.9931	0.02487	0.04974	5.25%	0.0%
1		4	0.914	0.8449	0.983	0.8745	0.9757	0.0217	0.0434	4.75%	3.58%
2		4	0.9436	0.8951	0.9921	0.9231	0.9889	0.01523	0.03047	3.23%	0.45%
4		4	0.9518	0.913	0.9905	0.9271	0.9772	0.01217	0.02435	2.56%	-0.41%
8		4	0.9221	0.8103	1	0.8462	0.9838	0.03512	0.07023	7.62%	2.72%
16		4	0.01316	0	0.03748	0	0.02834	0.007642	0 01528	116.2%	98.61%
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	0.9565	0.8761	1	0.9069	1	0.02525	0.0505	5.28%	0.0%
1		4	0.92	0.8541	0.986	0.8866	0.9798	0.02073	0.04147	4.51%	3.81%
2		4	0.9595	0.9146	1	0.9352	1	0.01412	0.02824	2.94%	-0.32%
4		4	0.9727	0.9325	1	0.9474	1	0.01263	0.02526	2.6%	-1.69%
8		4	0.9423	0.8324	1	0.8623	1	0.03453	0.06905	7.33%	1.48%
16		4	0.8988	0.8512	0.9464	0.8623	0.9271	0.01497	0.02993	3.33%	6.03%
32		4	0.5061	0.4844	0.5278	0.4858	0.5142	0.006815	0.01363	2.69%	47.09%
64		4	0.002024	0	0.005744	0	0.004049	0.001169	0.002337	115.5%	99.79%

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	0.9888	0.9028	0.9931	0.9069
1		0.9757	0.9069	0.8988	0.8745
2		0.9889	0.9312	0.9231	0.9312
4		0.9352	0.9676	0.9772	0.9271
8		0.8785	0.8462	0.9798	0.9838
16		0	0	0.02834	0.02429
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	0.919	1	0.9069
1		0.9798	0.915	0.8988	0.8866
2		1	0.9474	0.9352	0.9555
4		0.9555	0.9879	1	0.9474
8		0.9069	0.8623	1	1
16		0.8866	0.919	0.9271	0.8623
32		0.5142	0.4858	0.5101	0.5142
64		0.004049	0	0.004049	0

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	264/267	223/247	286/288	224/247
1		241/247	224/247	222/247	216/247
2		268/271	230/247	228/247	230/247
4		231/247	239/247	257/263	229/247
8		217/247	209/247	242/247	243/247
16		0/247	0/247	7/247	6/247
32		0/247	0/247	0/247	0/247
64		0/247	0/247	0/247	0/247

Proportion Survived Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	247/247	227/247	247/247	224/247
1		242/247	226/247	222/247	219/247
2		247/247	234/247	231/247	236/247
4		236/247	244/247	247/247	234/247
8		224/247	213/247	247/247	247/247
16		219/247	227/247	229/247	213/247
32		127/247	120/247	126/247	127/247
64		1/247	0/247	1/247	0/247

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Start Date:	01 Nov-17 13:35	Species:	Mytilis galloprovincialis	Sample Code:	6D345429
End Date:	03 Nov-17 13:35	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant
Sample Date:	01 Nov-17 13:35	Material:	Copper sulfate	Sample Station:	

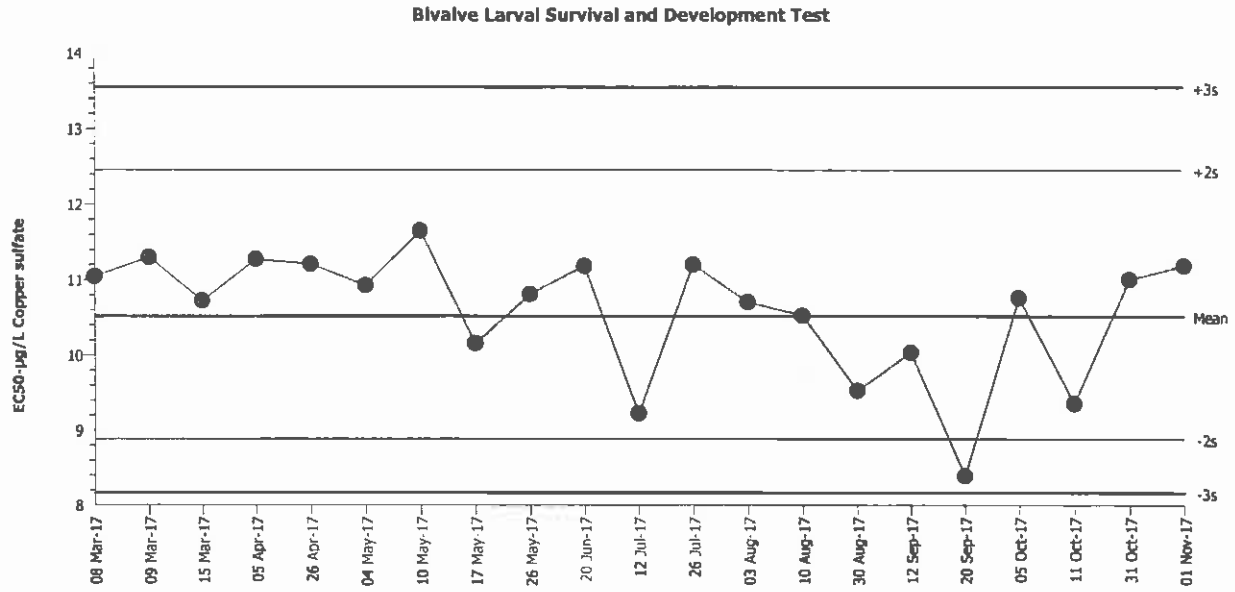
C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	5	247	267	267	264	
0	D	2	31	247	227	227	223	
0	D	3	25	247	288	288	286	
0	D	4	28	247	224	224	224	
1		1	8	247	242	242	241	
1		2	21	247	226	226	224	
1		3	15	247	222	222	222	
1		4	10	247	219	219	216	
2		1	24	247	271	271	268	
2		2	27	247	234	234	230	
2		3	17	247	231	231	228	
2		4	26	247	236	236	230	
4		1	22	247	236	236	231	
4		2	4	247	244	244	239	
4		3	6	247	263	263	257	
4		4	9	247	234	234	229	
8		1	29	247	224	224	217	
8		2	7	247	213	213	209	
8		3	13	247	247	247	242	
8		4	2	247	250	250	243	
16		1	32	247	219	219	0	
16		2	20	247	227	227	0	
16		3	23	247	229	229	7	
16		4	11	247	213	213	6	
32		1	3	247	127	127	0	
32		2	18	247	120	120	0	
32		3	16	247	126	126	0	
32		4	12	247	127	127	0	
64		1	30	247	1	1	0	
64		2	19	247	0	0	0	
64		3	1	247	1	1	0	
64		4	14	247	0	0	0	

CETIS QC Plot

Report Date: 07 Nov-17 14:09 (1 of 1)

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:	10.52	**Count:**	20	**-2s Warning Limit:**	8.884	**-3s Action Limit:**	8.165
Sigma:	NA	**CV:**	8.80%	**+2s Warning Limit:**	12.45	**+3s Action Limit:**	13.55

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Mar	8	15:30	11.04	0.5273	0.5798			11-1093-1447	03-3381-2684
2			9	13:55	11.3	0.7809	0.8488			18-3588-3261	02-4714-0912
3			15	14:00	10.72	0.2045	0.2283			02-0711-3850	03-7078-9692
4		Apr	5	13:15	11.27	0.7552	0.8218			06-1946-6662	08-5835-4084
5			26	13:20	11.21	0.6923	0.7555			07-5946-4689	06-0434-2999
6		May	4	14:30	10.92	0.4068	0.4497			10-0435-4306	18-9328-1898
7			10	14:10	11.65	1.132	1.212			14-1408-5654	20-3711-0314
8			17	13:10	10.15	-0.3622	-0.4153			03-9435-3893	05-7716-5731
9			26	13:20	10.81	0.2932	0.3259			05-6211-0933	16-0073-3043
10		Jun	20	16:50	11.18	0.6646	0.7262			02-1409-7276	08-4079-1566
11		Jul	12	13:25	9.224	-1.293	-1.555			02-4818-7084	16-2581-4533
12			26	14:10	11.2	0.6866	0.7495			17-8688-1039	02-1882-3377
13		Aug	3	13:30	10.7	0.1855	0.2072			12-5107-4745	07-3767-8523
14			10	15:10	10.53	0.008826	0.009942			14-0880-4376	18-5220-7572
15			30	14:35	9.527	-0.9901	-1.172			21-1251-6624	08-5861-5172
16		Sep	12	14:00	10.03	-0.4846	-0.5591			10-5774-5128	09-7081-1600
17			20	13:50	8.39	-2.127	-2.678	(-)		12-1488-7812	06-3387-4553
18		Oct	5	15:20	10.76	0.2435	0.2713			01-0974-4829	16-8936-6824
19			11	17:10	9.355	-1.162	-1.387			06-4503-4555	15-6316-6751
20			31	13:55	11	0.4867	0.5361			19-0668-1049	19-2975-1853
21		Nov	1	13:35	11.19	0.6696	0.7315			17-3365-4072	06-0748-0919