TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-75

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: 9-14-16, 1410 hrs.

Test Ending: 9-16-16, 1430 hrs.

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

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

TEST MATERIAL

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

NAS Sample No.	5709G
Collection Date	9-13-16
Receipt Date	9-14-16
Temperature (°C)	8.7
pH	7.9
Dissolved oxygen (mg/L)	10.7
Salinity (‰)	4.0

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon seawater.

Date of Collection: 9-13-16

Water Quality: Salinity, 30.0 ‰; pH, 8.2

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

BRINE USED FOR DILUTION WATER AND SALINITY CONTROL

Source: Filtered Yaquina Bay, Oregon, sea water

Salinity: 100.0 ‰

Date of Preparation: 8-5-16

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 9-7-16 and placed in trays with flowing seawater. Holding conditions for the week prior to testing were: temperature, 18.2 ± 0.2°C; pH, 8.0 ± 0.2; salinity, 32.7 ± 0.8‰; and dissolved oxygen, 6.9 ± 0.4 mg/L. Photoperiod was natural daylight.
Source of Gametes: 2 females and 1 male.

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: 23.2/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, 15.9 ± 0.5°C; pH, 8.1 ± 0.2; salinity, 30.1 ± 0.2‰; 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.: 999-3599
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: 9-14-16
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ppt, pH 8.2
Results: EC50, 11.4 µg/L; NOEC, 8 µg/L; IC25, 9.96 µg/L. The EC50 result was within the laboratory's control chart warning limits (8.74 – 12.2 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 (%)	35 (TU_c=2.86)	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 35% 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 — 9-27-16 — Date

Study Director — 9-27-16 — Date

Assistant Laboratory Director — Linda Nemeth — Date 9-27-16

Quality Assurance Unit — Julie R. Fiore — 9-27-16 — Date

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

Test Material					Combined Proportion Normal*		Proportion Survived*	
Concentration (%)	Repl.	Norm.	Abn.	Total		Mean		Mean
70	1	227	2	229	0.978		0.987	
	2	221	3	224	0.953		0.966	
	3	213	2	215	0.918		0.927	
	4	194	3	197	0.836	0.921†	0.849	0.932
35	1	241	3	244	0.988		1.000	
	2	201	1	202	0.866		0.871	
	3	216	1	217	0.931		0.935	
	4	227	0	227	0.978	0.941	0.978	0.946
18	1	233	2	235	0.992		1.000	
	2	227	1	228	0.978		0.983	
	3	228	1	229	0.983		0.987	
	4	213	1	214	0.918	0.968	0.922	0.973
9	1	221	1	222	0.953		0.957	
	2	219	4	223	0.944		0.961	
	3	224	3	227	0.966		0.978	
	4	214	2	216	0.922	0.946	0.931	0.957
4	1	237	1	238	0.996		1.000	
	2	243	4	247	0.984		1.000	
	3	227	0	227	0.978		0.978	
	4	228	6	234	0.983	0.985	1.000	0.995
2	1	222	3	225	0.957		0.970	
	2	240	3	243	0.988		1.000	
	3	211	1	212	0.910		0.914	
	4	217	2	219	0.935	0.947	0.944	0.957
Normal Control	1	247	2	249	0.992		1.000	
	2	223	2	225	0.961		0.970	
	3	251	1	252	0.996		1.000	
	4	230	1	231	0.991	0.985	0.996	0.991
Brine Control[1]	1	239	3	242	0.988		1.000	
	2	211	2	213	0.910		0.918	
	3	234	2	236	0.992		1.000	
	4	224	3	227	0.966	0.964	0.978	0.974

* Based on an average initial count of 232 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

REVISED PAGES 1-14 -GJI

Test No. 658-75 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. Yuri Nelokhina Y 2. J. BROWN JB

3. 4.

Study Schedule:

Test Beginning: 9-14-16 1410 Test Ending: 9-16-16 1430

TEST MATERIAL

Description:	SP-11			
NAS Sample No.	57096			
Date of Collection:	9-13-16			
Date of Receipt:	9-14-16			
Temperature (deg C):	8.7			
pH:	7.9			
Dissolved oxygen (mg/L):	10.7			
Conductivity (umhos/cm):	–			
Hardness (mg/L):	–			
Alkalinity (mg/L):	–			
Salinity (ppt):	4.0			
Total chlorine (mg/L):				
Total ammonia-N (mg/L):				
:				

DILUTION WATER

Description: Yaquina Bay, OR

Date of Collection: 9-13-16 Salinity (ppt) 30.0 pH 8.2

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

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 9-7-16

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
9-7-16	15.1	8.1	33.0	6.9	Held outside in trays of
9-8-16	15.3	8.0	32.0	7.1	flowing seawater
9-9-16	15.1	8.0	34.0	6.9	
9-12-16	15.4	8.0	32.5	7.3	
9-14-16	17.9	7.7	32.0	6.3	
Mean	15.2	8.0	32.7	6.9	
S.D.	0.2	0.2	0.8	0.4	
(N)	5	5	5	5	

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 dale
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. 658-75 Client CH2M Hill - Wyckoff Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 1030 Final: 1100 Fertilization: 1140

Number of organisms used: females: 2 males: 1

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 54 2. 61 3. 54 Mean: 56.3

Dilution factor = DF (mean x 100/2500) = 2.3

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): 23.2 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	18	Brine Ctl	4	35	ϕ	9	2	70		
B	9	ϕ	2	70	Brine Ctl	18	35	4		
C	2	35	9	4	70	18	Brine Ctl	ϕ		
D	35	70	Brine Ctl	4	9	18	ϕ	2		

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: 8-5-16 ; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

$$VB = VE \frac{(TS - SE)}{(SB - TS)} = VE \frac{(30 - 4)}{(100 - 30)} = VE\,(0.371)$$

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

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

Test Conc. (%)	Effluent (ml/100ml)	Brine (ml/100ml)	Dilution Water (ml/100ml)
70	70	26.0	Brought up to a final
35	35	13.0	volume of 100 ml
18	18	6.7	with dilution water
9	9	3.3	
4	4	1.5	
2	2	0.7	
0	0	0	
Brine Control	0	26.0	

9-14-16
652

THE BRINE CONTROL WAS MADE UP OF SALINITY ADJUSTED MILLI-Q DEIONIZED WATER (4.0 PPT) SIMULAR TO THE EFFLUENT, AS A RESULT, THE AMOUNT OF BRINE IN THE BRINE CONTROL WAS THE SAME USED IN THE 70% EFFLUENT CONCENTRATION.

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

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

Test No. 658-75 Client CH2M Hill - Wyckoff Investigator

WATER QUALITY DATA

	Date: 9-14-16 initials: GSJ				Date: 9-16-16 initials: YC			
Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	16.0	7.8	30.0	8.0	15.4	8.6	30.0	7.9
35	16.2	7.8	30.5	8.0	15.4	8.5	30.5	7.9
18	16.2	7.9	30.5	8.0	15.3	8.4	30.5	7.9
9	16.3	8.0	30.0	8.1	15.4	8.3	30.0	7.9
4	16.4	8.0	30.0	8.1	15.3	8.3	30.0	8.0
2	16.4	8.0	30.0	8.1	15.4	8.2	30.0	8.1
Control	16.4	8.0	30.0	8.0	15.4	8.2	30.0	8.1
Brine control	16.7	8.1	30.0	7.9	15.4	8.2	30.0	8.1

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.9	0.5	16
pH	8.1	0.2	16
Salinity (ppt):	30.1	0.2	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 16.4 Day 0: 16.4
Day 1: 15.6 Day 1: 15.6
Day 2: 15.4 Day 2: 15.4

LARVAL COUNT DATA

← 9-20-16 JS | → 9-22-16 JS | 9-25-16 GSJ

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	227	2	221	3	213	2	194	3
35	241	3	201	1	216	1	227	0
18	233	2	227	1	228	1	213	1
9	221	1	219	4	224	3	214	2
4	237	1	243	4	227	0	228	6
2	222	3	240	3	211	1	217	2
Control	247	2	223	2	251	1	230	1
Brine control	239	3	211	2	234	2	224	3
Zero time	214	236	229	248	239	224	—	—

Zero time: Mean 232 SD 12 N 6 CV=(sd/mean)x100 5.2%

Remarks:

To fit 771 DU-O-VUE® Envelope, fold along bottom of shaded strip

PRODUCT 108

KAMILCHE
SEA FARMS
Kamilche Sea Farms, Inc.
2741 SE Bloomfield Road • Shelton, WA 98584
360 427 5774 • Fax 360 427 0810
WA Cert. #217-SS
Harvested: Totten Inlet, Puget Sound

36289

TO Northwest Aquatic Sciences

DATE 9-6-16
CUSTOMER ORDER NO
SALESPERSON
VIA

TERMS

QUANTITY	DESCRIPTION	PRICE	AMOUNT
10 lbs	**Mussels - Beard On** 1 @ 10 lbs	[redacted]	[redacted]
		Total	[redacted]
	Rec'd 9-7-16 YG		

Thank You!

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	18-1994-5734	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	26 Sep-16 9:06	Analysis:	Parametric-Two Sample	Official Results:	Yes
Batch ID:	16-3831-3512	Test Type:	Development-Survival	Analyst:	
Start Date:	14 Sep-16 14:10	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	16 Sep-16 14:30	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	
Sample ID:	03-0633-8162	Code:	12425972	Client:	Wyckoff Treatment Plant
Sample Date:	13 Sep-16 09:15	Material:	Industrial Effluent	Project:	
Receive Date:	14 Sep-16 10:10	Source:	Wyckoff		
Sample Age:	29h (8.7 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	5.17%	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	0.7074	2.447	0.171	6	0.5059	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.801	2.127	0.3257	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.004865057	0.004865057	1	0.5004	0.5059	Non-Significant Effect
Error	0.05833959	0.009723265	6			
Total	0.06320465		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	3.305	47.47	0.3524	Equal Variances
Variances	Mod Levene Equality of Variance	1.206	13.75	0.3143	Equal Variances
Variances	Levene Equality of Variance	1.638	13.75	0.2479	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9024	0.6451	0.3039	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1837	0.3313	0.7316	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.4027	3.878	0.3618	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	0.9914	0.9683	1	0.9978	0.9698	1	0.007256	1.46%	0.0%
0	Brine Reagent	4	0.9741	0.9125	1	0.9978	0.9181	1	0.01936	3.97%	1.74%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.494	1.387	1.601	1.522	1.396	1.538	0.0336	4.5%	0.0%
0	Brine Reagent	4	1.445	1.251	1.639	1.522	1.281	1.538	0.06109	8.46%	3.3%

Proportion Survived Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	0.9698	1	0.9957
0	Brine Reagent	1	0.9181	1	0.9784

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.538	1.396	1.538	1.505
0	Brine Reagent	1.538	1.281	1.538	1.423

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	09-8205-9580	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Sep-16 9:05	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	16-3831-3512	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	14 Sep-16 14:10	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	16 Sep-16 14:30	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	03-0633-8162	**Code:**	12425972	**Client:**	Wyckoff Treatment Plant
Sample Date:	13 Sep-16 09:15	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	14 Sep-16 10:10	**Source:**	Wyckoff		
Sample Age:	29h (8.7 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	5.27%	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	1.553	2.448	0.173	6	0.2311	CDF	Non-Significant Effect
		4	-0.213	2.448	0.173	6	0.9069	CDF	Non-Significant Effect
		9	1.823	2.448	0.173	6	0.1534	CDF	Non-Significant Effect
		18	0.9028	2.448	0.173	6	0.4985	CDF	Non-Significant Effect
		35	1.769	2.448	0.173	6	0.1673	CDF	Non-Significant Effect
		70	2.366	2.448	0.173	6	0.0586	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.914	2.876	1.0000	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1138183	0.01896971	6	1.905	0.1273	Non-Significant Effect
Error	0.2091037	0.009957318	21			
Total	0.322922		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.922	16.81	0.5539	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9666	0.8975	0.4931	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	0.9914	0.9683	1	0.9978	0.9698	1	0.007256	1.46%	0.0%
2		4	0.9569	0.8984	1	0.9569	0.9138	1	0.01837	3.84%	3.48%
4		4	0.9946	0.9775	1	1	0.9784	1	0.005388	1.08%	-0.33%
9		4	0.9569	0.9257	0.9881	0.9591	0.931	0.9784	0.009798	2.05%	3.48%
18		4	0.9731	0.9181	1	0.9849	0.9224	1	0.01728	3.55%	1.85%
35		4	0.9461	0.8554	1	0.9569	0.8707	1	0.02851	6.03%	4.57%
70		4	0.9321	0.8355	1	0.9461	0.8491	0.9871	0.03035	6.51%	5.98%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.494	1.387	1.601	1.522	1.396	1.538	0.0336	4.5%	0.0%
2		4	1.385	1.203	1.566	1.364	1.273	1.538	0.05697	8.23%	7.34%
4		4	1.509	1.418	1.6	1.538	1.423	1.538	0.02863	3.79%	-1.01%
9		4	1.366	1.288	1.443	1.367	1.305	1.423	0.02428	3.56%	8.61%
18		4	1.431	1.265	1.596	1.448	1.289	1.538	0.05202	7.27%	4.26%
35		4	1.37	1.141	1.599	1.369	1.203	1.538	0.07196	10.51%	8.35%
70		4	1.327	1.132	1.522	1.34	1.172	1.457	0.0613	9.24%	11.17%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	09-8205-9580	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Sep-16 9:05	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

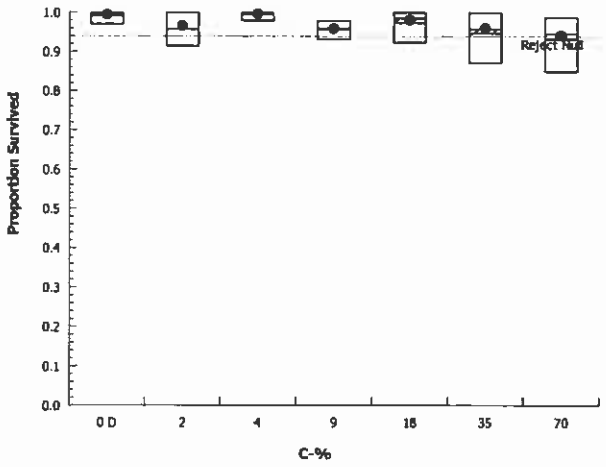
Proportion Survived Detail

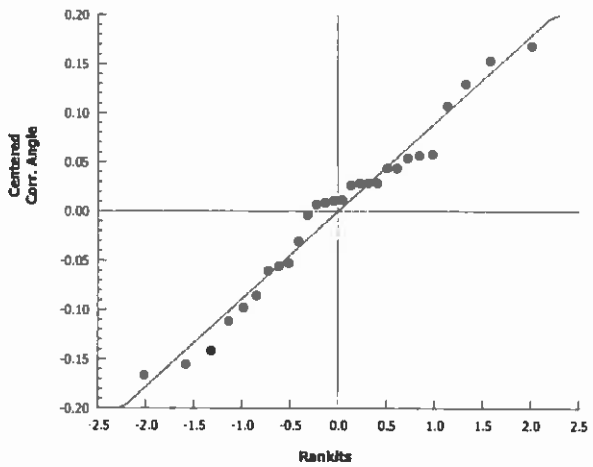
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	0.9698	1	0.9957
2		0.9698	1	0.9138	0.944
4		1	1	0.9784	1
9		0.9569	0.9612	0.9784	0.931
18		1	0.9828	0.9871	0.9224
35		1	0.8707	0.9353	0.9784
70		0.9871	0.9655	0.9267	0.8491

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.538	1.396	1.538	1.505
2		1.396	1.538	1.273	1.332
4		1.538	1.538	1.423	1.538
9		1.362	1.373	1.423	1.305
18		1.538	1.439	1.457	1.289
35		1.538	1.203	1.314	1.423
70		1.457	1.384	1.297	1.172

Graphics





LC50 > 70% BY DATA INSPECTION
-GJZ

CETIS Analytical Report

Test Code: 658-75 | 15-1168-7781

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	19-7432-6057	Endpoint:	Combined Proportion Normal	CETIS Version:	CETISv1.8.7
Analyzed:	26 Sep-16 9:06	Analysis:	Parametric-Two Sample	Official Results:	Yes
Batch ID:	16-3831-3512	Test Type:	Development-Survival	Analyst:	
Start Date:	14 Sep-16 14:10	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	16 Sep-16 14:30	Species:	Mytills galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	
Sample ID:	03-0633-8162	Code:	12425972	Client:	Wyckoff Treatment Plant
Sample Date:	13 Sep-16 09:15	Material:	Industrial Effluent	Project:	
Receive Date:	14 Sep-16 10:10	Source:	Wyckoff		
Sample Age:	29h (8.7 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	4.71%	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	1.109	2.447	0.139	6	0.3099	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.768	2.127	0.3719	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.007945473	0.007945473	1	1.23	0.3099	Non-Significant Effect
Error	0.03876178	0.006460296	6			
Total	0.04670725		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.626	47.47	0.4488	Equal Variances
Variances	Mod Levene Equality of Variance	0.9855	13.75	0.3592	Equal Variances
Variances	Levene Equality of Variance	0.9459	13.75	0.3683	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.905	0.6451	0.3201	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.2207	0.3313	0.3401	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.4258	3.878	0.3198	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	0.9851	0.9595	1	0.9895	0.9612	0.996	0.008046	1.63%	0.0%
0	Brine Reagent	4	0.9635	0.9034	1	0.9895	0.9095	0.9915	0.0189	3.92%	2.19%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.46	1.365	1.555	1.469	1.373	1.508	0.02985	4.09%	0.0%
0	Brine Reagent	4	1.397	1.243	1.551	1.469	1.265	1.479	0.04837	6.93%	4.32%

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.992	0.9612	0.996	0.9914
0	Brine Reagent	0.9876	0.9095	0.9915	0.9655

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.481	1.373	1.508	1.478
0	Brine Reagent	1.459	1.265	1.479	1.384

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences	
Analysis ID:	01-8887-6439	Endpoint:	Combined Proportion Normal	CETIS Version:	CETISv1.8.7
Analyzed:	26 Sep-16 9:05	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	16-3831-3512	Test Type:	Development-Survival	Analyst:	
Start Date:	14 Sep-16 14:10	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	16 Sep-16 14:30	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	

Sample ID:	03-0633-8162	Code:	12425972	Client:	Wyckoff Treatment Plant
Sample Date:	13 Sep-16 09:15	Material:	Industrial Effluent	Project:	
Receive Date:	14 Sep-16 10:10	Source:	Wyckoff		
Sample Age:	29h (8.7 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.94%	35	70	49.5	2.857

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	1.869	2.448	0.144	6	0.1422	CDF	Non-Significant Effect
		4	0.117	2.448	0.144	6	0.8232	CDF	Non-Significant Effect
		9	2.058	2.448	0.144	6	0.1032	CDF	Non-Significant Effect
		18	0.9264	2.448	0.144	6	0.4876	CDF	Non-Significant Effect
		35	1.934	2.448	0.144	6	0.1277	CDF	Non-Significant Effect
		70*	2.681	2.448	0.144	6	0.0314	CDF	Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.043	2.876	0.9740	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08833917	0.0147232	6	2.134	0.0920	Non-Significant Effect
Error	0.1448655	0.006898355	21			
Total	0.2332046		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.198	16.81	0.4014	Equal Variances
Variances	Mod Levene Equality of Variance	1.312	3.812	0.2953	Equal Variances
Variances	Levene Equality of Variance	1.654	3.812	0.1820	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9639	0.8975	0.4297	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.09894	0.1914	0.7073	Normal Distribution
Distribution	D'Agostino Skewness	1.003	2.576	0.3160	Normal Distribution
Distribution	D'Agostino Kurtosis	0.05312	2.576	0.9576	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	1.008	9.21	0.6040	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.3232	3.878	0.5459	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	0.9851	0.9595	1	0.9917	0.9612	0.996	0.008046	1.63%	0.0%
2		4	0.9473	0.8946	1	0.9461	0.9095	0.9877	0.01657	3.5%	3.84%
4		4	0.9852	0.9734	0.997	0.9833	0.9784	0.9958	0.003717	0.75%	-0.01%
9		4	0.9461	0.9173	0.9749	0.9483	0.9224	0.9655	0.009059	1.92%	3.96%
18		4	0.9677	0.9144	1	0.9806	0.9181	0.9915	0.01675	3.46%	1.77%
35		4	0.9409	0.8525	1	0.9547	0.8664	0.9877	0.02777	5.9%	4.49%
70		4	0.9213	0.8228	1	0.9353	0.8362	0.9784	0.03095	6.72%	6.48%

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

Analysis ID: 01-8887-6439 **Endpoint:** Combined Proportion Normal **CETIS Version:** CETISv1.8.7
Analyzed: 26 Sep-16 9:05 **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	1.46	1.365	1.555	1.479	1.373	1.508	0.02985	4.09%	0.0%
2		4	1.35	1.218	1.482	1.338	1.265	1.459	0.04146	6.14%	7.52%
4		4	1.453	1.395	1.511	1.441	1.423	1.506	0.01817	2.5%	0.47%
9		4	1.339	1.275	1.402	1.342	1.289	1.384	0.01995	2.98%	8.28%
18		4	1.405	1.268	1.543	1.431	1.281	1.478	0.04318	6.15%	3.73%
35		4	1.346	1.156	1.537	1.364	1.197	1.46	0.05981	8.89%	7.78%
70		4	1.302	1.12	1.485	1.316	1.154	1.423	0.05736	8.81%	10.78%

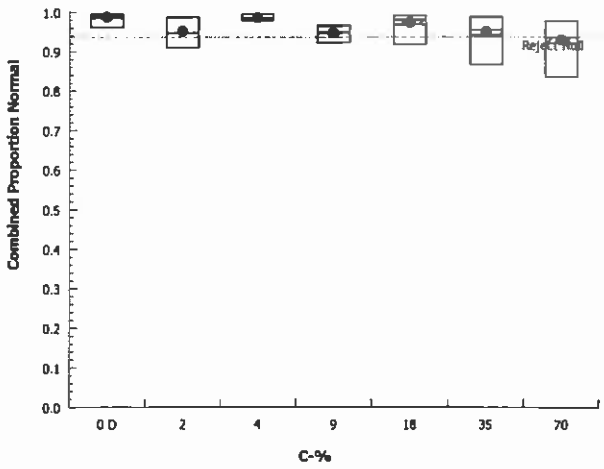
Combined Proportion Normal Detail

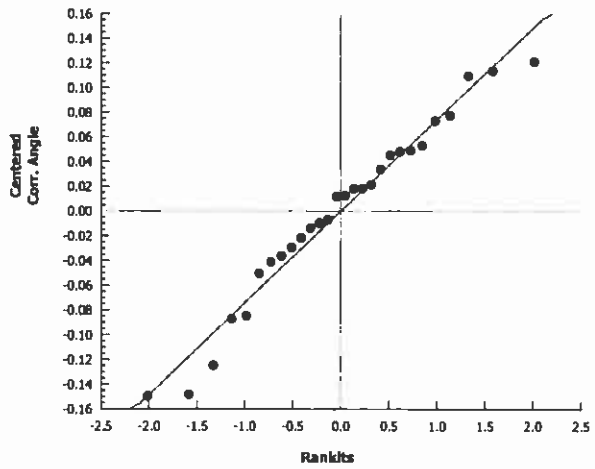
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.992	0.9612	0.996	0.9914
2		0.9569	0.9877	0.9095	0.9353
4		0.9958	0.9838	0.9784	0.9828
9		0.9526	0.944	0.9655	0.9224
18		0.9915	0.9784	0.9828	0.9181
35		0.9877	0.8664	0.931	0.9784
70		0.9784	0.9526	0.9181	0.8362

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.481	1.373	1.508	1.478
2		1.362	1.459	1.265	1.314
4		1.506	1.443	1.423	1.439
9		1.351	1.332	1.384	1.289
18		1.478	1.423	1.439	1.281
35		1.46	1.197	1.305	1.423
70		1.423	1.351	1.281	1.154

Graphics





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

CETIS Analytical Report

Test Code: 658-75 15-1168-7781

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	00-3944-7763	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Sep-16 9:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	16-3831-3512	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	14 Sep-16 14:10	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	16 Sep-16 14:30	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	

Sample ID:	03-0633-8162	**Code:**	12425972	**Client:**	Wyckoff Treatment Plant
Sample Date:	13 Sep-16 09:15	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	14 Sep-16 10:10	**Source:**	Wyckoff		
Sample Age:	29h (8.7 °C)	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1582626	280	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.043	2.876	0.9740	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	0.9851	0.9612	0.996	0.008046	0.01609	1.63%	0.0%	951	965
2		4	0.9473	0.9095	0.9877	0.01657	0.03313	3.5%	3.84%	890	939
4		4	0.9852	0.9784	0.9958	0.003717	0.007434	0.75%	-0.01%	935	949
9		4	0.9461	0.9224	0.9655	0.009059	0.01812	1.92%	3.96%	878	928
18		4	0.9677	0.9181	0.9915	0.01675	0.03351	3.46%	1.77%	901	931
35		4	0.9409	0.8664	0.9877	0.02777	0.05553	5.9%	4.49%	885	940
70		4	0.9213	0.8362	0.9784	0.03095	0.0619	6.72%	6.48%	855	928

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.992	0.9612	0.996	0.9914
2		0.9569	0.9877	0.9095	0.9353
4		0.9958	0.9838	0.9784	0.9828
9		0.9526	0.944	0.9655	0.9224
18		0.9915	0.9784	0.9828	0.9181
35		0.9877	0.8664	0.931	0.9784
70		0.9784	0.9526	0.9181	0.8362

CETIS Test Data Worksheet

Report Date: 26 Sep-16 09:05 (p 1 of 1)
Test Code: 15-1168-7781/658-75

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences
Start Date:	14 Sep-16 14:10	Species:	Mytilis galloprovincialis	Sample Code: 12425972
End Date:	16 Sep-16 14:30	Protocol:	EPA/600/R-95/136 (1995)	Sample Source: Wyckoff
Sample Date:	13 Sep-16 09:15	Material:	Industrial Effluent	Sample Station:

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	232	242	242	239	
0	B	2	11	232	213	213	211	
0	B	3	12	232	236	236	234	
0	B	4	3	232	227	227	224	
0	D	1	10	232	249	249	247	
0	D	2	2	232	225	225	223	
0	D	3	23	232	252	252	251	
0	D	4	9	232	231	231	230	
2		1	18	232	225	225	222	
2		2	27	232	243	243	240	
2		3	22	232	212	212	211	
2		4	30	232	219	219	217	
4		1	28	232	238	238	237	
4		2	15	232	247	247	243	
4		3	14	232	227	227	227	
4		4	24	232	234	234	228	
9		1	6	232	222	222	221	
9		2	29	232	223	223	219	
9		3	5	232	227	227	224	
9		4	17	232	216	216	214	
18		1	16	232	235	235	233	
18		2	13	232	228	228	227	
18		3	1	232	229	229	228	
18		4	31	232	214	214	213	
35		1	7	232	244	244	241	
35		2	26	232	202	202	201	
35		3	21	232	217	217	216	
35		4	8	232	227	227	227	
70		1	4	232	229	229	227	
70		2	20	232	224	224	221	
70		3	19	232	215	215	213	
70		4	25	232	197	197	194	

data entry verified against laboratory bench sheets 9-26-16 JRF

Northwestern Aquatic Sciences (REGION COPY)
DateShipped. 9/13/2016
CarrierName FedEx
AirbillNo 7840 7318 3739

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2015/WA
Project Code WEH-024U
Cooler # 1 of 1

No: 10-042616-092946-0104
2016T10P303DD210W2LA00
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
658-3rd Quarter	NAS# 5709G	Ground Water/ K Allers	Composite	CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	09/13/2016 09 15	Field Sample

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

Special Instructions

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 CH2M	9-13-16 10:00	[signature]	9-14-16 1010	INTACT

ORIGIN ID BFIA (206) 780-1711
KEITH ALLERS
CH2M
5350 CREOSOTE PLACE N.E.

BAINBRIDGE ISLAND, WA 98110
UNITED STATES US

SHIP DATE 13SEP16
ACTWGT 7.00 LB
CAD 103963466/WSXI2500
DIMS 10x8x7 IN

BILL SENDER

TO GEARLD IRISSARRI
NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD

NEWPORT OR 97365
(541) 265-7225 REF 436558 FP Y5 01
INV
PO DEPT

544J1/A053/14E8

FedEx Express

WED - 14 SEP 12:00P
PRIORITY OVERNIGHT

TRK# 0201 7840 7318 3739

86 ONPA

97365
OR-US PDX

PAGE 14 OF 14

NORTHWESTERN AQUATIC SCIENCES
A Division of NAS Associates, Inc

SAMPLE NO 658
SIGNATURE
PRINT NAME AND TITLE Keith Allers Industrial Tech
DATE 9-13-16
SEAL BROKEN BY
DATE 9-14-16

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. 999-3599 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 Matalanis 2. J. Brown JB

3. 4.

Study Schedule:

Test Beginning: 9-14-16 1410 Test Ending: 9-16-16 1430

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: 9-13-16 Salinity (ppt) 30.0 pH 8.2

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

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 9-7-16

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
9-7-16	15.1	8.1	33.0	6.9	Held outside in trays of
9-8-16	15.3	8.0	32.0	7.1	flowing seawater
9-9-16	15.1	8.0	34.0	6.9	
9-12-16	15.4	8.0	32.5	7.3	
9-14-16	17.9	7.7	32.0	6.3	
Mean	15.2	8.0	32.7	6.9	
S.D.	0.2	0.2	0.8	0.4	
(N)	5	5	5	5	

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. 999-3599 Client QC Test Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 1030 Final: 1100 Fertilization: 1140

Number of organisms used: females: 2 males: 1

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 54 2. 61 3. 54 Mean: 56.3

Dilution factor = DF (mean x 100/2500) = 2.3

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): 23.2 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	32	2	8	64	4	16	1	Ø		
B	2	16	Ø	1	32	64	8	4		
C	8	64	1	4	2	Ø	32	16		
D	16	4	32	8	Ø	1	2	64		

PREPARATION OF TEST SOLUTIONS

9-14-16
GSL

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	

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. 999-3599 Client QC Test Investigator

WATER QUALITY DATA

	Date: 9-14-16 initials: GJ				Date: 9-16-16 initials: [illegible]			
Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	16.2	8.0	29.0	7.8	15.4	8.2	29.5	8.1
32	16.2	8.0	29.5	7.8	15.4	8.2	29.5	8.1
16	16.2	8.0	30.0	7.8	15.4	8.2	30.0	8.1
8	16.1	8.0	30.0	7.8	15.3	8.2	30.0	8.1
4	16.2	8.0	30.0	7.8	15.3	8.2	30.0	8.1
2	16.2	8.0	30.0	7.8	15.4	8.2	30.0	8.1
1	16.2	8.0	30.0	7.8	15.3	8.2	30.0	8.1
Control	16.4	8.0	30.0	7.9	15.4	8.2	30.0	8.1
Brine control	---	—	—	—	---	—	—	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.8	0.4	16
pH:	8.1	0.1	16
Salinity (ppt): 29.8	~~29.8~~	0.3	16
DO (mg/L): 9-26-16 GJ	8.0	0.2	16

Room/ Water bath temperature: (°C)

Day 0: 16.4 Day 0: 16.4
Day 1: 15.6 Day 1: 15.6
Day 2: 15.4 Day 2: 15.4

LARVAL COUNT DATA

← 9-20-16 JS → 9-22-16 JS | 9-25-16 GJ

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	Ø	2	Ø	Ø	Ø	Ø	Ø	1
32	Ø	153	Ø	129	Ø	151	Ø	126
16	9	213	16	204	15	210	4	201
8	226	2	241	1	221	1	210	4
4	250	3	227	2	218	1	205	1
2	230	Ø	219	1	201	1	225	2
1	234	3	236	3	239	3	199	Ø
Control	240	3	239	4	228	Ø	216	4
Brine control	--	--	—	—	---	—	—	—
Zero time	214	236	229	248	239	224	~	—

Zero time: Mean 232 SD 12 N 6 CV=(sd/mean)x100 5.2%

Remarks:

To fit 771 DU-O-VUE® Envelope, fold along bottom of shaded strip

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

36289

TO Northwest Aquatic Sciences

DATE 9-6-16

CUSTOMER ORDER NO

SALESPERSON

VIA

TERMS

QUANTITY	DESCRIPTION	PRICE	AMOUNT
10 lbs	Mussels - Beard On 1 @ 10 lbs	[redacted]	[redacted]
		Total	[redacted]
	Rec'd 9-7-16 YS		

Thank You!

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Batch ID:	16-3831-3512	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	14 Sep-16 14:10	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	16 Sep-16 14:30	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	

Sample ID:	11-7437-4557	**Code:**	45FF889D	**Client:**	Internal Lab
Sample Date:	14 Sep-16 14:10	**Material:**	Copper sulfate	**Project:**	
Receive Date:	14 Sep-16 14:10	**Source:**	Reference Toxicant		
Sample Age:	NA	**Station:**			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-5831-3530	Combined Proportion Norm	8	16	11.31	8.21%		Dunnett Multiple Comparison Test
00-0990-2625	Proportion Survived	16	32	22.63	7.01%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
02-5069-8037	Combined Proportion Norm	EC25	9.959	9.539	10.19		Linear Interpolation (ICPIN)
15-4984-2250	Combined Proportion Norm	EC50	11.38	11.2	11.56		Trimmed Spearman-Kärber
03-8246-0195	Proportion Survived	EC50	33.27	32.33	34.23		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	0.9712	0.9285	1	0.931	0.9877	0.01345	0.02689	2.77%	0.0%
1		4	0.955	0.8518	1	0.8578	0.9876	0.03243	0.06485	6.79%	1.67%
2		4	0.9429	0.8561	1	0.8664	0.9914	0.02728	0.05456	5.79%	2.92%
4		4	0.9475	0.872	1	0.8836	0.9881	0.02372	0.04744	5.01%	2.45%
8		4	0.9569	0.8952	1	0.9052	0.9959	0.01939	0.03877	4.05%	1.47%
16		4	0.04741	0.009021	0.08581	0.01724	0.06897	0.01206	0.02413	50.89%	95.12%
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	0.9828	0.944	1	0.9483	1	0.01219	0.02438	2.48%	0.0%
1		4	0.9644	0.8513	1	0.8578	1	0.03556	0.07112	7.37%	1.86%
2		4	0.9472	0.8611	1	0.8707	0.9914	0.02705	0.05411	5.71%	3.62%
4		4	0.9547	0.8743	1	0.8879	1	0.02529	0.05057	5.3%	2.85%
8		4	0.9655	0.9118	1	0.9224	1	0.01688	0.03376	3.5%	1.75%
16		4	0.9397	0.8786	1	0.8836	0.9698	0.0192	0.03839	4.09%	4.39%
32		4	0.6024	0.5048	0.6999	0.5431	0.6595	0.03065	0.0613	10.18%	38.71%
64		4	0.003233	0	0.009799	0	0.008621	0.002063	0.004127	127.7%	99.67%

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	0.9877	0.9835	0.9828	0.931
1		0.9873	0.9874	0.9876	0.8578
2		0.9914	0.944	0.8664	0.9698
4		0.9881	0.9784	0.9397	0.8836
8		0.9741	0.9959	0.9526	0.9052
16		0.03879	0.06897	0.06466	0.01724
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	1	0.9828	0.9483
1		1	1	1	0.8578
2		0.9914	0.9483	0.8707	0.9784
4		1	0.9871	0.944	0.8879
8		0.9828	1	0.9569	0.9224
16		0.9569	0.9483	0.9698	0.8836
32		0.6595	0.556	0.6509	0.5431
64		0.008621	0	0	0.00431

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	240/243	239/243	228/232	216/232
1		234/237	236/239	239/242	199/232
2		230/232	219/232	201/232	225/232
4		250/253	227/232	218/232	205/232
8		226/232	241/242	221/232	210/232
16		9/232	16/232	15/232	4/232
32		0/232	0/232	0/232	0/232
64		0/232	0/232	0/232	0/232

Proportion Survived Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	232/232	232/232	228/232	220/232
1		232/232	232/232	232/232	199/232
2		230/232	220/232	202/232	227/232
4		232/232	229/232	219/232	206/232
8		228/232	232/232	222/232	214/232
16		222/232	220/232	225/232	205/232
32		153/232	129/232	151/232	126/232
64		2/232	0/232	0/232	1/232

CETIS Test Data Worksheet

Test Code: 07-6290-8502999-3599

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

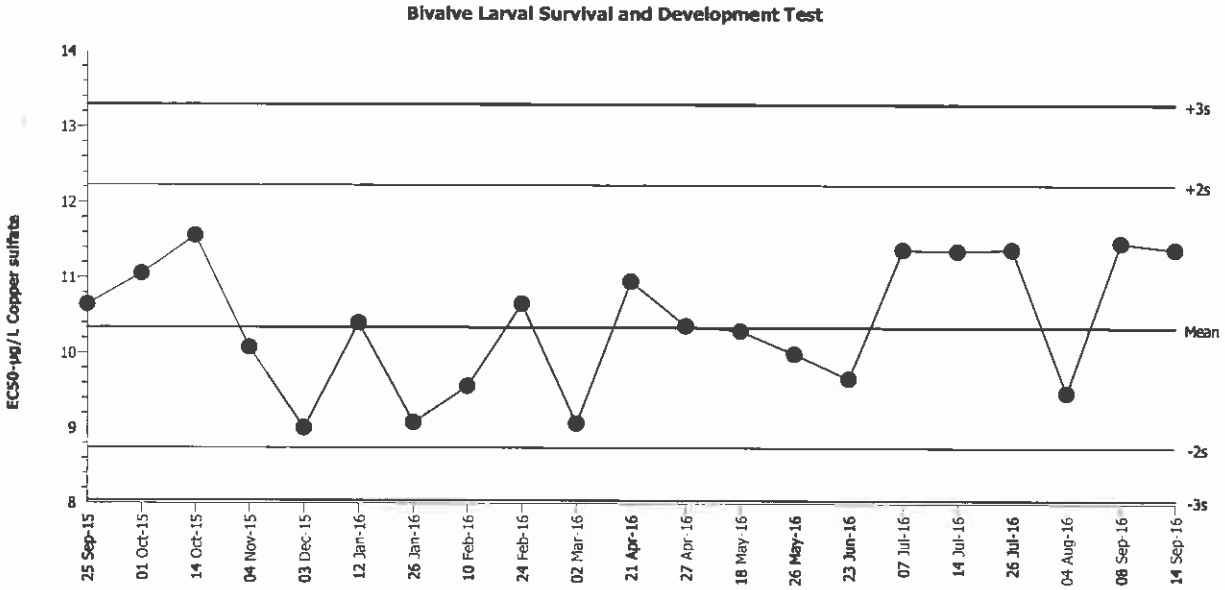
Start Date:	14 Sep-16 14:10	**Species:**	Mytilis galloprovincialis
End Date:	16 Sep-16 14:30	**Protocol:**	EPA/600/R-95/136 (1995)
Sample Date:	14 Sep-16 14:10	**Material:**	Copper sulfate
Sample Code:	45FF889D		
Sample Source:	Reference Toxicant		
Sample Station:			

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	8	232	243	243	240	
0	D	2	14	232	243	243	239	
0	D	3	18	232	228	228	228	
0	D	4	25	232	220	220	216	
1		1	16	232	237	237	234	
1		2	32	232	239	239	236	
1		3	27	232	242	242	239	
1		4	7	232	199	199	199	
2		1	23	232	230	230	230	
2		2	4	232	220	220	219	
2		3	2	232	202	202	201	
2		4	24	232	227	227	225	
4		1	1	232	253	253	250	
4		2	29	232	229	229	227	
4		3	9	232	219	219	218	
4		4	31	232	206	206	205	
8		1	10	232	228	228	226	
8		2	6	232	242	242	241	
8		3	5	232	222	222	221	
8		4	11	232	214	214	210	
16		1	30	232	222	222	9	
16		2	15	232	220	220	16	
16		3	28	232	225	225	15	
16		4	12	232	205	205	4	
32		1	19	232	153	153	0	
32		2	26	232	129	129	0	
32		3	17	232	151	151	0	
32		4	20	232	126	126	0	
64		1	13	232	2	2	0	
64		2	22	232	0	0	0	
64		3	3	232	0	0	0	
64		4	21	232	1	1	0	

data entry verified against laboratory bench sheets 9-26-16 JMF

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:	10.34	**Count:**	20	**-2s Warning Limit:**	8.743	**-3s Action Limit:**	8.04
Sigma:	NA	**CV:**	8.75%	**+2s Warning Limit:**	12.23	**+3s Action Limit:**	13.3

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2015	Sep	25	14:45	10.64	0.3045	0.3461			08-8031-4805	17-8304-1379
2		Oct	1	15:00	11.05	0.7141	0.7964			02-0435-7572	17-5283-9042
3			14	14:20	11.56	1.218	1.328			04-2701-8188	06-5641-7689
4		Nov	4	14:35	10.07	-0.2655	-0.3103			01-4945-0913	12-4167-7901
5		Dec	3	12:10	9.003	-1.337	-1.651			03-1706-3107	08-0944-2928
6	2016	Jan	12	14:40	10.4	0.0586	0.06738			10-6038-5674	20-9220-6975
7			26	13:20	9.075	-1.265	-1.556			18-1116-9330	06-8399-0866
8		Feb	10	14:45	9.559	-0.7811	-0.9367			18-2305-5641	14-7293-2864
9			24	14:50	10.66	0.3167	0.3598			01-7836-7496	17-6653-1457
10		Mar	2	14:40	9.062	-1.277	-1.572			03-1233-9663	15-4736-9649
11		Apr	21	12:50	10.95	0.6119	0.6856			06-2981-4615	06-1924-7869
12			27	13:40	10.36	0.02227	0.02565			10-3412-7541	03-6222-0465
13		May	18	13:50	10.3	-0.04422	-0.05112			00-5077-6110	04-6829-6018
14			26	15:05	9.988	-0.3513	-0.4122			00-2461-1517	20-1645-6539
15		Jun	23	13:50	9.66	-0.6793	-0.8104			20-2900-3441	12-0442-8997
16		Jul	7	14:20	11.37	1.034	1.137			20-3374-2875	12-6544-3567
17			14	14:40	11.36	1.016	1.118			14-8632-5752	09-0596-6683
18			26	17:45	11.38	1.038	1.141			03-9839-1525	11-6670-0698
19		Aug	4	14:30	9.471	-0.869	-1.047			08-0477-0132	01-3411-5201
20		Sep	8	14:35	11.46	1.124	1.231			17-2952-4030	02-3236-2087
21			14	14:10	11.38	1.038	1.14			07-6290-8502	15-4984-2250