TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-78

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: 11-30-16, 1300 hrs.

Test Ending: 12-2-16, 1320 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.	5748G
Collection Date	11-29-16
Receipt Date	11-30-16
Temperature (°C)	2.0
pH	7.9
Dissolved oxygen (mg/L)	12.3
Salinity (‰)	5.0

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon seawater.

Date of Collection: 11-29-16

Water Quality: Salinity, 30.0 ‰; pH, 8.0

Pretreatment: Filtered to ≤0.45 µm, aerated, salinity adjusted with 100.0 ppt brine prepared 10-12-16.

BRINE USED FOR DILUTION WATER AND SALINITY CONTROL

Source: Filtered Yaquina Bay, Oregon, sea water

Salinity: 100.0 ‰

Date of Preparation: 10-12-16

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, 11.3 ± 1.2°C; pH, 7.9 ± 0.1; salinity, 24.2 ± 3.3‰; and dissolved oxygen, 8.8 ± 0.5 mg/L. Photoperiod was natural daylight.
Source of Gametes: 1 female and 3 males.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.
Test Concentrations: 70, 35, 18, 9, 4, 2, and 0% (Control).
Brine Control: A brine control was run in which salinity-adjusted Milli-Q® deionized water (5.0 ppt) was substituted for effluent in the preparation of the highest test solution concentration. As a result, the amount of brine in the brine control was the same as used in the 70.0% effluent test concentration.
Replicates/Treatment: 4
Initial Concentration of Test Organisms: 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, 15.1 ± 0.1°C; pH, 8.1 ± 0.2; salinity, 30.6 ± 0.4‰; and dissolved oxygen, 8.1 ± 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.: 999-3623
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: 11-30-16
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ppt, pH 8.0
Results: EC50, 9.07 µg/L; NOEC, 4 µg/L; IC25, 7.19 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 (%)	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: 12-13-16

Study Director — Date: 12-12-16

Assistant Laboratory Director — Date: 12-12-16
for Linda Nemith

Quality Assurance Unit — Date: 12-7-16

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	220	7	227	0.948		0.978	
	2	214	7	221	0.922		0.953	
	3	219	10	229	0.944		0.987	
	4	218	9	227	0.940	0.939	0.978	0.974
35	1	197	5	202	0.849		0.871	
	2	216	14	230	0.931		0.991	
	3	235	6	241	0.975		1.000	
	4	220	12	232	0.948	0.926	1.000	0.966
18	1	204	8	212	0.879		0.914	
	2	212	14	226	0.914		0.974	
	3	193	7	200	0.832		0.862	
	4	198	7	205	0.853	0.870	0.884	0.908
9	1	206	12	218	0.888		0.940	
	2	189	9	198	0.815		0.853	
	3	212	7	219	0.914		0.944	
	4	228	12	240	0.983	0.900	1.000	0.934
4	1	216	8	224	0.931		0.966	
	2	204	9	213	0.879		0.918	
	3	227	13	240	0.978		1.000	
	4	217	15	232	0.935	0.931	1.000	0.971
2	1	200	4	204	0.862		0.879	
	2	208	13	221	0.897		0.953	
	3	224	15	239	0.966		1.000	
	4	221	9	230	0.953	0.919	0.991	0.956
Normal Control	1	223	6	229	0.961		0.987	
	2	202	8	210	0.871		0.905	
	3	216	4	220	0.931		0.948	
	4	222	10	232	0.957	0.930	1.000	0.960
Brine Control[1]	1	219	13	232	0.944		1.000	
	2	215	8	223	0.927		0.961	
	3	196	10	206	0.845		0.888	
	4	212	5	217	0.914	0.907	0.935	0.946

* 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 (5.0 ppt) was substituted for effluent so that the brine concentration is equivalent to that for the 70.0% effluent concentration.

APPENDIX I

PROTOCOL

TEST PROTOCOL

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

1. INTRODUCTION

1.1 Purpose of Study: The purpose of this test is to estimate chronic toxicity of effluents, receiving waters, or other test materials using bivalve larval development in a 48-hr static test.

1.2 Referenced Method: This protocol complies with the U.S. EPA West Coast chronic toxicity manual (EPA/600/R-95/136), ASTM bivalve toxicity method (E 724-89), and the WDOE toxicity guidance manual (WQ-R-95-80). Amendments may be incorporated to meet other methods or regulatory requirements as needed.

1.3 Summary of Method: Pacific oyster or blue mussel larvae (<4-hr-old) are exposed for 48-hr to different concentrations of test material in a static test. Salinity adjustment and brine controls are used when testing low salinity effluents. The test chambers are 30 ml borosilicate glass vials each containing 10 ml of test solution. Four replicate chambers each with 15-30 larvae per milliliter of test solution are employed at each test concentration. Test results are based on abnormal shell development and mortality. Data analysis normally consists of the calculation of an EC50 and IC25 for "percent normal", the calculation of an LC50 for percent survival, and the determination of NOECs and LOECs for both criteria. Special requirements may apply for the State of Washington or other regulatory entities. A test summary table is appended to the end of this protocol.

2. STUDY MANAGEMENT

2.1 Sponsor's Name and Address:

_____
_____
_____

2.2 Sponsor's Study Monitor:

_____

2.3 Name of Testing Laboratory:
Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

2.4 Test Location:

_____

2.5 Laboratory's Personnel to be Assigned to the Study:
Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

2.6 Proposed Study Schedule: Effluent/receiving water tests must begin within 36 hours of the end of the sample collection period. In no case should the test be started more than 72 hours after sample collection.

2.7 Good Laboratory Practices: The test is conducted following the principles of Good Laboratory Practices (GLP) as defined in the EPA/TSCA Good Laboratory Practice regulations revised August 17, 1989 (40 CFR Part 792).

3. TEST MATERIAL

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

4. DILUTION WATER

Dilution water is filtered (≤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 GJL

Test No. 658-78 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 (GAB) /G.J. Irissarri GJI

QA Officer L.K. Nemeth

1. [illegible] 2. J. Brown JB

3. 4.

Study Schedule:

Test Beginning: 11-30-16 1300 Test Ending: 12-2-16 1320

TEST MATERIAL

Description:	COMPOSITE SP-11			
NAS Sample No.	5748G			
Date of Collection:	11-29-16			
Date of Receipt:	11-30-16			
Temperature (deg C):	2.0			
pH:	7.9			
Dissolved oxygen (mg/L):	12.3			
Conductivity (umhos/cm):	–			
Hardness (mg/L):	–			
Alkalinity (mg/L):	–			
Salinity (ppt):	5.0			
Total chlorine (mg/L):				
Total ammonia-N (mg/L):				
:				

DILUTION WATER

Description: Yaquina Bay, OR

Date of Collection: 11-29-16 Salinity (ppt) 30.0 pH 8.0

Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with ~~Milli-Q deionized water~~ 100.0 ppt BRINE PREPARED 10-12-16.

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
11-18-16	12.1	8.0	29.0	8.1	Held outside in trays of
11-21-16	12.9	7.8	25.5	8.3	flowing seawater
11-23-16	12.1	8.0	25.5	8.5	
11-25-16	10.4	7.8	24.0	9.1	
11-28-16	10.1	7.8	21.0	9.3	
11-30-16	10.3	7.8	20.0	9.3	
Mean	11.3	7.9	24.2	8.8	
S.D.	1.2	0.1	3.3	0.5	
(N)	6	6	6	6	

Photoperiod during acclimation: Outdoor ambient conditions

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

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

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

Test No. 658-78 Client CH2M Hill - Wyckoff Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0850 Final: 0940 Fertilization: 1030

Number of organisms used: females: 1 males: 3

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 42 2. 40 3. 36 Mean: 39.3

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): 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	ϕ	2	9	4	70	BRINE CONTROL	18	35		
B	2	70	BRINE CONTROL	ϕ	9	4	35	18		
C	70	35	2	9	ϕ	18	BRINE CONTROL	4		
D	18	9	4	35	BRINE CONTROL	70	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: 10-12-16 ; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

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

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

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

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

11-30-16 651

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

651

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

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

Test No. 658-78 Client CH2M Hill - Wyckoff Investigator _____

WATER QUALITY DATA

	Date: 11-30-16 initials: IC				Date: 12-2-16 initials: GT			
Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.3	7.8	31.0	8.2	15.0	8.5	31.5	8.1
35	15.0	7.9	31.0	8.1	15.0	8.4	30.5	8.0
18	15.2	7.9	30.0	8.1	15.0	8.3	30.5	8.0
9	15.2	8.0	30.5	8.1	15.0	8.2	30.5	8.1
4	15.0	8.0	30.5	8.1	15.0	8.2	30.5	8.0
2	15.1	8.0	30.5	8.2	15.0	8.2	30.5	8.1
Control	15.1	8.1	30.5	8.2	15.0	8.1	31.0	8.1
Brine control	15.2	8.2	30.0	8.2	15.0	8.1	30.0	8.0

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.1	0.1	16
pH	8.1	0.2	16
Salinity (ppt):	30.56	0.4	16
DO (mg/L):	~~8.2~~ 8.1	0.1	16

12-7-16 8.1

Room/ Water bath temperature: (°C)

Day 0: 15.1 Day 0: 15.3

Day 1: 15.3 Day 1: 15.3

Day 2: 15.0 Day 2: 15.2

LARVAL COUNT DATA

12-4-16 631 ← 12-6-16 1155 →

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	220	7	214	7	219	10	218	9
35	197	5	216	14	235	6	220	12
18	204	8	212	14	193	7	195	7
9	206	12	189	9	212	7	228	12
4	216	8	204	9	227	13	217	15
2	200	4	208	13	224	15	221	9
Control	223	6	202	8	216	4	222	10
Brine control	219	13	215	8	194	10	212	5
Zero time	219	235	244	228	228	236	—	—

Zero time: Mean 232 SD 9 N 6 CV=(sd/mean)x100 3.7%

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

Analysis ID:	11-5427-9024	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	07 Dec-16 12:25	Analysis:	Parametric-Two Sample	Official Results:	Yes
Batch ID:	07-9756-2087	Test Type:	Development-Survival	Analyst:	
Start Date:	30 Nov-16 13:00	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	02 Dec-16 13:20	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	
Sample ID:	13-4782-2628	Code:	50562424	Client:	Wyckoff Treatment Plant
Sample Date:	29 Nov-16 09:20	Material:	Industrial Effluent	Project:	
Receive Date:	30 Nov-16 10:50	Source:	Wyckoff		
Sample Age:	28h (2 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	11.1%	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	0.3903	2.447	0.22	6	0.7098	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.484	2.127	0.9299	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.002459961	0.002459961	1	0.1523	0.7098	Non-Significant Effect
Error	0.09689554	0.01614926	6			
Total	0.0993555		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.11	47.47	0.9337	Equal Variances
Variances	Mod Levene Equality of Variance	0.02341	13.75	0.8834	Equal Variances
Variances	Levene Equality of Variance	0.02502	13.75	0.8795	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9338	0.6451	0.5509	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1637	0.3313	1.0000	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.2684	3.878	0.7106	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	0.9601	0.8921	1	0.9547	0.9052	1	0.02136	4.45%	0.0%
0	Brine Reagent	4	0.9461	0.8713	1	0.9547	0.8879	1	0.02351	4.97%	1.46%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.398	1.202	1.595	1.357	1.258	1.538	0.06186	8.85%	0.0%
0	Brine Reagent	4	1.363	1.156	1.571	1.357	1.229	1.538	0.06517	9.56%	2.51%

Proportion Survived Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9871	0.9052	0.9483	1
0	Brine Reagent	1	0.9612	0.8879	0.9353

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.457	1.258	1.341	1.538
0	Brine Reagent	1.538	1.373	1.229	1.314

Bivalve Larval Survival and Development Test					Northwestern Aquatic Sciences
Analysis ID:	11-4844-8502	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	07 Dec-16 12:24	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Batch ID:	07-9756-2087	Test Type:	Development-Survival	Analyst:	
Start Date:	30 Nov-16 13:00	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	02 Dec-16 13:20	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	

Sample ID:	13-4782-2628	Code:	50562424	Client:	Wyckoff Treatment Plant
Sample Date:	29 Nov-16 09:20	Material:	Industrial Effluent	Project:	
Receive Date:	30 Nov-16 10:50	Source:	Wyckoff		
Sample Age:	28h (2 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.9%	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	0.03057	2.448	0.217	6	0.8487	CDF	Non-Significant Effect
		4	-0.4126	2.448	0.217	6	0.9404	CDF	Non-Significant Effect
		9	0.6294	2.448	0.217	6	0.6242	CDF	Non-Significant Effect
		18	1.404	2.448	0.217	6	0.2836	CDF	Non-Significant Effect
		35	-0.4582	2.448	0.217	6	0.9465	CDF	Non-Significant Effect
		70	-0.172	2.448	0.217	6	0.8985	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.132	2.876	0.7532	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0829802	0.01383003	6	0.8766	0.5286	Non-Significant Effect
Error	0.3313173	0.01577701	21			
Total	0.4142975		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.135	16.81	0.6583	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9713	0.8975	0.6166	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	0.9601	0.8921	1	0.9677	0.9052	1	0.02136	4.45%	0.0%
2		4	0.9558	0.8683	1	0.972	0.8793	1	0.02751	5.76%	0.45%
4		4	0.9709	0.9092	1	0.9828	0.9181	1	0.01939	3.99%	-1.12%
9		4	0.9343	0.838	1	0.9418	0.8534	1	0.03024	6.47%	2.69%
18		4	0.9084	0.8309	0.9859	0.8987	0.8621	0.9741	0.02434	5.36%	5.39%
35		4	0.9655	0.8647	1	0.9957	0.8707	1	0.03167	6.56%	-0.56%
70		4	0.9741	0.9504	0.9979	0.9784	0.9526	0.9871	0.007466	1.53%	-1.46%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.398	1.202	1.595	1.399	1.258	1.538	0.06186	8.85%	0.0%
2		4	1.396	1.168	1.623	1.415	1.216	1.538	0.07144	10.24%	0.19%
4		4	1.435	1.235	1.636	1.461	1.281	1.538	0.06302	8.78%	-2.62%
9		4	1.343	1.107	1.578	1.327	1.178	1.538	0.07405	11.03%	4.0%
18		4	1.274	1.12	1.427	1.248	1.19	1.409	0.04826	7.58%	8.92%
35		4	1.439	1.185	1.694	1.508	1.203	1.538	0.08	11.12%	-2.91%
70		4	1.414	1.343	1.485	1.423	1.351	1.457	0.02226	3.15%	-1.09%

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences
Analysis ID:	11-4844-8502	Endpoint:	Proportion Survived	CETIS Version: CETISv1.8.7
Analyzed:	07 Dec-16 12:24	Analysis:	Parametric-Control vs Treatments	Official Results: Yes

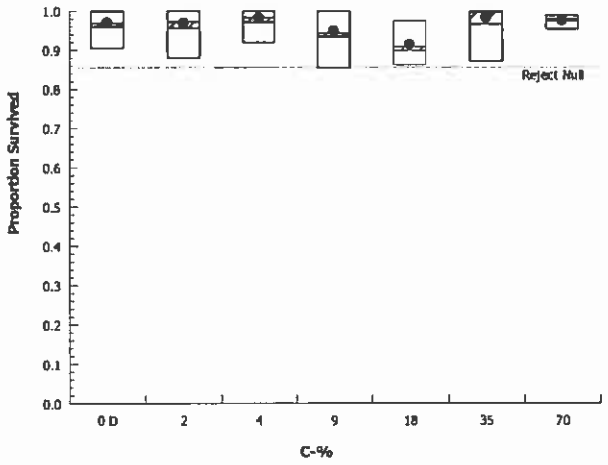
Proportion Survived Detail

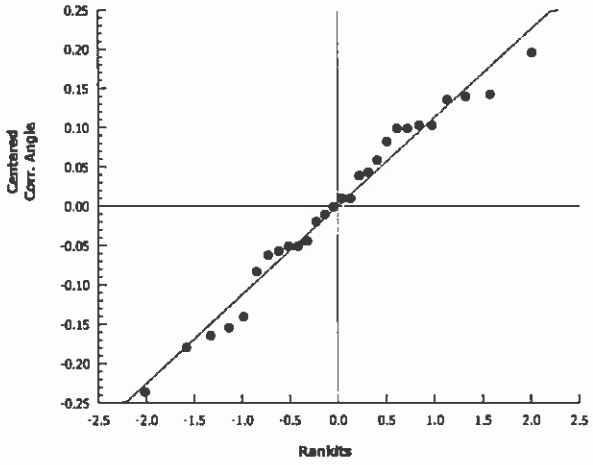
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9871	0.9052	0.9483	1
2		0.8793	0.9526	1	0.9914
4		0.9655	0.9181	1	1
9		0.9397	0.8534	0.944	1
18		0.9138	0.9741	0.8621	0.8836
35		0.8707	0.9914	1	1
70		0.9784	0.9526	0.9871	0.9784

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.457	1.258	1.341	1.538
2		1.216	1.351	1.538	1.478
4		1.384	1.281	1.538	1.538
9		1.323	1.178	1.332	1.538
18		1.273	1.409	1.19	1.223
35		1.203	1.478	1.538	1.538
70		1.423	1.351	1.457	1.423

Graphics





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

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	19-7178-8764	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Dec-16 12:25	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	07-9756-2087	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	30 Nov-16 13:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	02 Dec-16 13:20	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	13-4782-2628	**Code:**	50562424	**Client:**	Wyckoff Treatment Plant
Sample Date:	29 Nov-16 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	30 Nov-16 10:50	**Source:**	Wyckoff		
Sample Age:	28h (2 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	7.96%	Passes combined proportion normal

Equal Variance t Two-Sample Test

Control	vs	Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		Brine Reagent	0.8295	2.447	0.129	6	0.4386	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.558	2.127	0.7558	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003829708	0.003829708	1	0.6881	0.4386	Non-Significant Effect
Error	0.03339617	0.005566029	6			
Total	0.03722588		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	1.178	47.47	0.8962	Equal Variances
Variances	Mod Levene Equality of Variance	0.06631	13.75	0.8054	Equal Variances
Variances	Levene Equality of Variance	0.04278	13.75	0.8430	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8373	0.6451	0.0706	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.2183	0.3313	0.3594	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.6259	3.878	0.1037	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	0.93	0.8636	0.9963	0.9289	0.8707	0.9612	0.02085	4.48%	0.0%
0	Brine Reagent	4	0.9073	0.8382	0.9765	0.9289	0.8448	0.944	0.02173	4.79%	2.43%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.311	1.187	1.434	1.301	1.203	1.373	0.0388	5.92%	0.0%
0	Brine Reagent	4	1.267	1.153	1.381	1.301	1.166	1.332	0.03575	5.64%	3.34%

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9612	0.8707	0.931	0.9569
0	Brine Reagent	0.944	0.9267	0.8448	0.9138

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.373	1.203	1.305	1.362
0	Brine Reagent	1.332	1.297	1.166	1.273

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	12-2603-7322	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Dec-16 12:24	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	07-9756-2087	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	30 Nov-16 13:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	02 Dec-16 13:20	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	13-4782-2628	**Code:**	50562424	**Client:**	Wyckoff Treatment Plant
Sample Date:	29 Nov-16 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	30 Nov-16 10:50	**Source:**	Wyckoff		
Sample Age:	28h (2 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	9.52%	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	0.3004	2.448	0.149	6	0.7605	CDF	Non-Significant Effect
		4	-0.06555	2.448	0.149	6	0.8741	CDF	Non-Significant Effect
		9	0.718	2.448	0.149	6	0.5840	CDF	Non-Significant Effect
		18	1.751	2.448	0.149	6	0.1719	CDF	Non-Significant Effect
		35	0.0474	2.448	0.149	6	0.8440	CDF	Non-Significant Effect
		70	-0.1724	2.448	0.149	6	0.8986	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.266	2.876	0.5020	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04103262	0.00683877	6	0.9193	0.5008	Non-Significant Effect
Error	0.1562211	0.0074391	21			
Total	0.1972537		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.893	16.81	0.3309	Equal Variances
Variances	Mod Levene Equality of Variance	0.8956	3.812	0.5161	Equal Variances
Variances	Levene Equality of Variance	0.9638	3.812	0.4730	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9798	0.8975	0.8452	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.09322	0.1914	0.8152	Normal Distribution
Distribution	D'Agostino Skewness	0.07078	2.576	0.9436	Normal Distribution
Distribution	D'Agostino Kurtosis	0.1031	2.576	0.9179	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	0.01563	9.21	0.9922	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.2584	3.878	0.7433	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	0.93	0.8636	0.9963	0.944	0.8707	0.9612	0.02085	4.48%	0.0%
2		4	0.9192	0.8421	0.9962	0.9246	0.8621	0.9655	0.02422	5.27%	1.16%
4		4	0.931	0.8665	0.9956	0.9332	0.8793	0.9784	0.02029	4.36%	-0.12%
9		4	0.8998	0.7893	1	0.9009	0.8147	0.9828	0.03472	7.72%	3.24%
18		4	0.8696	0.8135	0.9257	0.8664	0.8319	0.9138	0.01763	4.06%	6.49%
35		4	0.9259	0.8395	1	0.9397	0.8491	0.9751	0.02714	5.86%	0.44%
70		4	0.9386	0.9205	0.9566	0.9418	0.9224	0.9483	0.005668	1.21%	-0.93%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID: 12-2603-7322 Endpoint: Combined Proportion Normal CETIS Version: CETISv1.8.7
Analyzed: 07 Dec-16 12:24 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	1.311	1.187	1.434	1.333	1.203	1.373	0.0388	5.92%	0.0%
2		4	1.292	1.148	1.437	1.297	1.19	1.384	0.04536	7.02%	1.4%
4		4	1.315	1.179	1.45	1.309	1.216	1.423	0.04249	6.47%	-0.31%
9		4	1.267	1.059	1.474	1.251	1.126	1.439	0.06521	10.29%	3.34%
18		4	1.204	1.118	1.289	1.197	1.148	1.273	0.02685	4.46%	8.15%
35		4	1.308	1.147	1.468	1.323	1.172	1.412	0.05044	7.72%	0.22%
70		4	1.321	1.284	1.358	1.327	1.289	1.341	0.01151	1.74%	-0.8%

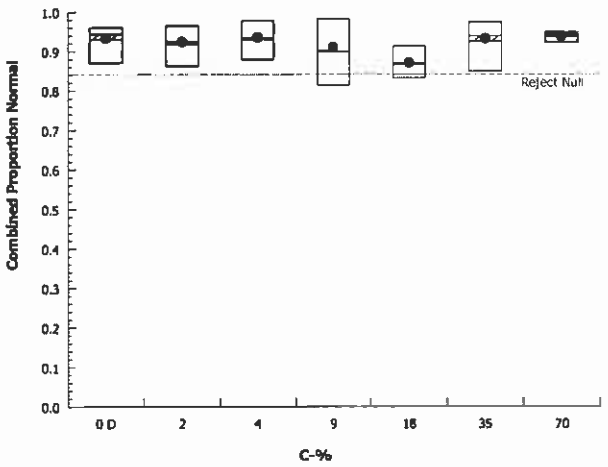
Combined Proportion Normal Detail

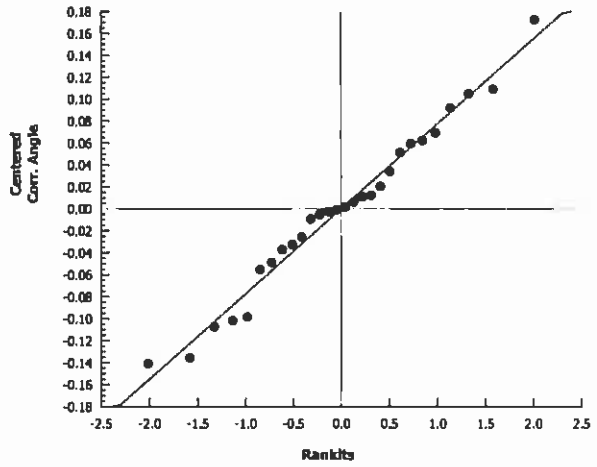
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9612	0.8707	0.931	0.9569
2		0.8621	0.8966	0.9655	0.9526
4		0.931	0.8793	0.9784	0.9353
9		0.8879	0.8147	0.9138	0.9828
18		0.8793	0.9138	0.8319	0.8534
35		0.8491	0.931	0.9751	0.9483
70		0.9483	0.9224	0.944	0.9397

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.373	1.203	1.305	1.362
2		1.19	1.243	1.384	1.351
4		1.305	1.216	1.423	1.314
9		1.229	1.126	1.273	1.439
18		1.216	1.273	1.148	1.178
35		1.172	1.305	1.412	1.341
70		1.341	1.289	1.332	1.323

Graphics





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

CETIS Analytical Report

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

Analysis ID:	13-5908-8399	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Dec-16 12:24	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	07-9756-2087	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	30 Nov-16 13:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	02 Dec-16 13:20	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	

Sample ID:	13-4782-2628	**Code:**	50562424	**Client:**	Wyckoff Treatment Plant
Sample Date:	29 Nov-16 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	30 Nov-16 10:50	**Source:**	Wyckoff		
Sample Age:	28h (2 °C)	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1435497	280	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.266	2.876	0.5020	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	0.93	0.8707	0.9612	0.02085	0.0417	4.48%	0.0%	863	928
2		4	0.9192	0.8621	0.9655	0.02422	0.04843	5.27%	1.16%	853	928
4		4	0.931	0.8793	0.9784	0.02029	0.04059	4.36%	-0.12%	864	928
9		4	0.8998	0.8147	0.9828	0.03472	0.06945	7.72%	3.24%	835	928
18		4	0.8696	0.8319	0.9138	0.01763	0.03526	4.06%	6.49%	807	928
35		4	0.9259	0.8491	0.9751	0.02714	0.05428	5.86%	0.44%	868	937
70		4	0.9386	0.9224	0.9483	0.005668	0.01134	1.21%	-0.93%	871	928

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9612	0.8707	0.931	0.9569
2		0.8621	0.8966	0.9655	0.9526
4		0.931	0.8793	0.9784	0.9353
9		0.8879	0.8147	0.9138	0.9828
18		0.8793	0.9138	0.8319	0.8534
35		0.8491	0.931	0.9751	0.9483
70		0.9483	0.9224	0.944	0.9397

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Start Date:	30 Nov-16 13:00	**Species:**	Mytilis galloprovincialis	**Sample Code:**	50562424
End Date:	02 Dec-16 13:20	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Wyckoff
Sample Date:	29 Nov-16 09:20	**Material:**	Industrial Effluent	**Sample Station:**	

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	232	232	232	219	
0	B	2	11	232	223	223	215	
0	B	3	12	232	206	206	196	
0	B	4	3	232	217	217	212	
0	D	1	10	232	229	229	223	
0	D	2	2	232	210	210	202	
0	D	3	23	232	220	220	216	
0	D	4	9	232	232	232	222	
2		1	18	232	204	204	200	
2		2	27	232	221	221	208	
2		3	22	232	239	239	224	
2		4	30	232	230	230	221	
4		1	28	232	224	224	216	
4		2	15	232	213	213	204	
4		3	14	232	240	240	227	
4		4	24	232	232	232	217	
9		1	6	232	218	218	206	
9		2	29	232	198	198	189	
9		3	5	232	219	219	212	
9		4	17	232	240	240	228	
18		1	16	232	212	212	204	
18		2	13	232	226	226	212	
18		3	1	232	200	200	193	
18		4	31	232	205	205	198	
35		1	7	232	202	202	197	
35		2	26	232	230	230	216	
35		3	21	232	241	241	235	
35		4	8	232	232	232	220	
70		1	4	232	227	227	220	
70		2	20	232	221	221	214	
70		3	19	232	229	229	219	
70		4	25	232	227	227	218	

Data entry verified against laboratory bench sheets 12-8-16 JRF

Northwestern Aquatic Sciences (REGION COPY)
DateShipped: 11/29/2016
CarrierName: FedEx
AirbillNo: 7847 9613 6387

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2017/WA
Project Code: WEH-024Z
Cooler #: 1 of 1

No: 10-112916-100213-0140
2017T10P303DD210W2LA00
Contact Name Keith Allers
Contact Phone: 206-780-1711

Sample Identifier	~~CLP~~ NAS Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
658 4th Quarter -2	57486	Ground Water/ K Allers	Composite	CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	11/29/2016	Field Sample

PAGE 13 OF 14

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 CHZM	11-29-2016 1010	Gerald Lissaarri NORTHWESTERN AQUATIC SCIENCES	11-30-16 1050	

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

BAINBRIDGE ISLAND WA 98110
UNITED STATES US

SHIP DATE 29NOV16
ACTWGT 8.00 LB
CAD 103963466/WSXI3100
DIMS. 11x8x12 IN

BILL SENDER

TO **GEARLD IRISSARRI**
NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD

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

544J1/D42F/14E8

FedEx Express

TRK# 0201 **7847 9613 6387**

WED - 30 NOV 12:00P
PRIORITY OVERNIGHT

86 ONPA

97365
OR-US PDX

NORTHWESTERN AQUATIC SCIENCES
A Division of NAS Associates, Inc.

SAMPLE NO 3
SIGNATURE
PRINT NAME Keith Allers Industrial Tech 3
DATE 11-29-2016
SEAL BROKEN BY KA
DATE 11-30-16

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 -632

Test No. 999-3623 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. Kelli Nakahara KN 2. GA Buhler GAB

3. J. Brown JB 4.

Study Schedule:

Test Beginning: 11-30-16 1300 Test Ending: 12-2-16 1320

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: 11-29-16 Salinity (ppt) 30.0 pH 8.0

Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with ~~Milli-Q® deionized water~~ 100.0 ppt BRINE PREPARED 10-12-16.

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
11-15-16	12.1	8.0	29.0	8.1	Held outside in trays of
11-21-16	12.1	7.8	25.5	8.3	flowing seawater
11-23-16	12.1	8.0	25.5	8.5	
11-25-16	10.4	7.8	24.0	9.1	
11-28-16	10.1	7.8	21.0	9.3	
11-30-16	10.3	7.8	20.0	9.3	
Mean	11.3	7.9	24.2	8.8	
S.D.	1.2	0.1	3.3	0.5	
(N)	6	6	6	6	

Photoperiod during acclimation: Outdoor ambient conditions

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

Test No. 999-3623 Client QC Test Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0850 Final: 0940 Fertilization: 1030
Number of organisms used: females: 1 males: 3
Egg Dilution (1 ml diluted to 100 ml):
Count/ml of dilution: 1. 42 2. 40 3. 36 Mean: 39.3
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): 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	16	ϕ	64	2	4	1	8	32		
B	64	2	8	4	32	ϕ	16	1		
C	16	4	32	ϕ	1	2	64	8		
D	2	8	32	1	64	16	4	ϕ		

PREPARATION OF TEST SOLUTIONS

11-30-16
631

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. 999-3623 Client QC Test Investigator

WATER QUALITY DATA

Conc. (ug/L)	Date: 12-3-16 initials:				Date: 12-2-16 initials: GD			
	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	15.1	8.0	29.5	8.1	15-2	8.1	30.0	8.1
32	15.1	8.0	30.0	8.1	15-2	8.1	30.0	8.1
16	15.2	8.0	30.0	8.1	15-2	8.1	30.5	8.2
8	15.1	8.0	30.0	8.1	15-2	8.1	30.5	8.2
4	15.1	8.0	30.0	8.1	15-2	8.1	30.0	8.1
2	15.1	8.0	30.0	8.1	15-1	8.1	30.0	8.1
1	15.1	8.0	30.0	8.1	15-1	8.1	30.0	8.2
Control	15.3	8.0	30.0	8.1	15-2	8.1	30.0	8.1
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.2	0.1	16
pH:	8.1	0.1	16
Salinity (ppt):	30.0	0.2	16
DO (mg/L):	8.1	0.0	16

Room/ Water bath temperature: (°C)

Day 0: 15.1 Day 0: 15.3
Day 1: 15-3 Day 1: 15.3
Day 2: 15-0 Day 2: 15-2

LARVAL COUNT DATA

12-4-16 GD ← 12-5-16 JB →

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	∅	∅	∅	∅	∅	∅	∅	∅
32	∅	125	∅	161	∅	152	∅	148
16	∅	186	∅	219	∅	226	∅	244
8	150	82	112	115	169	80	160	78
4	214	12	221	15	203	7	235	19
2	223	9	209	5	213	8	223	9
1	212	6	211	7	206	5	229	10
Control	215	3	206	7	221	10	208	11
Brine control	---	---	---	---	---	---	---	---
Zero time	219	235	244	228	228	236	—	—

Zero time: Mean 232 SD 9 N 6 CV=(sd/mean)x100 3.7%

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 JG		

Thank You!

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Batch ID:	07-9756-2087	Test Type:	Development-Survival	Analyst:	
Start Date:	30 Nov-16 13:00	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	02 Dec-16 13:20	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	

Sample ID:	10-9472-2337	Code:	41402321	Client:	Internal Lab
Sample Date:	30 Nov-16 13:00	Material:	Copper sulfate	Project:	
Receive Date:	30 Nov-16 13:00	Source:	Reference Toxicant		
Sample Age:	NA	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-9908-8537	Combined Proportion Norm	4	8	5.657	9.52%		Dunnett Multiple Comparison Test
11-7608-4392	Proportion Survived	16	32	22.63	11.9%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
08-0843-3013	Combined Proportion Norm	EC25	7.191	5.809	8.69		Linear Interpolation (ICPIN)
19-4644-8589	Combined Proportion Norm	EC50	9.074	8.882	9.27		Spearman-Kärber
05-5220-6708	Proportion Survived	EC50	34.69	33.89	35.51		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	0.9159	0.8689	0.963	0.8879	0.9526	0.01478	0.02955	3.23%	0.0%
1		4	0.9246	0.8559	0.9933	0.8879	0.9871	0.02159	0.04318	4.67%	-0.94%
2		4	0.9353	0.8865	0.9842	0.9009	0.9612	0.01534	0.03068	3.28%	-2.12%
4		4	0.919	0.8677	0.9704	0.875	0.9526	0.01614	0.03228	3.51%	-0.34%
8		4	0.6369	0.4649	0.8088	0.4828	0.7284	0.05402	0.108	16.96%	30.47%
16		4	0	0	0	0	0	0	0		100.0%
32		4	0	0	0	0	0	0	0		100.0%
64		4	0	0	0	0	0	0	0		100.0%

Proportion Survived Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.9494	0.897	1	0.9181	0.9957	0.01645	0.0329	3.47%	0.0%
1		4	0.9472	0.8868	1	0.9095	1	0.01898	0.03797	4.01%	0.23%
2		4	0.9688	0.9081	1	0.9224	1	0.01906	0.03813	3.94%	-2.04%
4		4	0.9698	0.8985	1	0.9052	1	0.0224	0.04479	4.62%	-2.16%
8		4	0.9946	0.9775	1	0.9784	1	0.005388	0.01078	1.08%	-4.77%
16		4	0.93	0.7891	1	0.8017	1	0.04425	0.0885	9.52%	2.04%
32		4	0.6315	0.5263	0.7366	0.5388	0.694	0.03304	0.06608	10.46%	33.48%
64		4	0	0	0	0	0	0	0		100.0%

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	0.9267	0.8879	0.9526	0.8966
1		0.9138	0.9095	0.8879	0.9871
2		0.9612	0.9009	0.9181	0.9612
4		0.9224	0.9526	0.875	0.9261
8		0.6466	0.4828	0.7284	0.6897
16		0	0	0	0
32		0	0	0	0
64		0	0	0	0

Proportion Survived Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9397	0.9181	0.9957	0.944
1		0.9397	0.9397	0.9095	1
2		1	0.9224	0.9526	1
4		0.9741	1	0.9052	1
8		1	0.9784	1	1
16		0.8017	0.944	0.9741	1
32		0.5388	0.694	0.6552	0.6379
64		0	0	0	0

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	215/232	206/232	221/232	208/232
1		212/232	211/232	206/232	229/232
2		223/232	209/232	213/232	223/232
4		214/232	221/232	203/232	238/257
8		150/232	112/232	169/232	160/232
16		0/232	0/232	0/232	0/232
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	218/232	213/232	231/232	219/232
1		218/232	218/232	211/232	232/232
2		232/232	214/232	221/232	232/232
4		226/232	232/232	210/232	232/232
8		232/232	227/232	232/232	232/232
16		186/232	219/232	226/232	232/232
32		125/232	161/232	152/232	148/232
64		0/232	0/232	0/232	0/232

CETIS Test Data Worksheet

Report Date: 05 Dec-16 14:26 (p 1 of 1)
Test Code: 08-5747-3287/999-3623

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

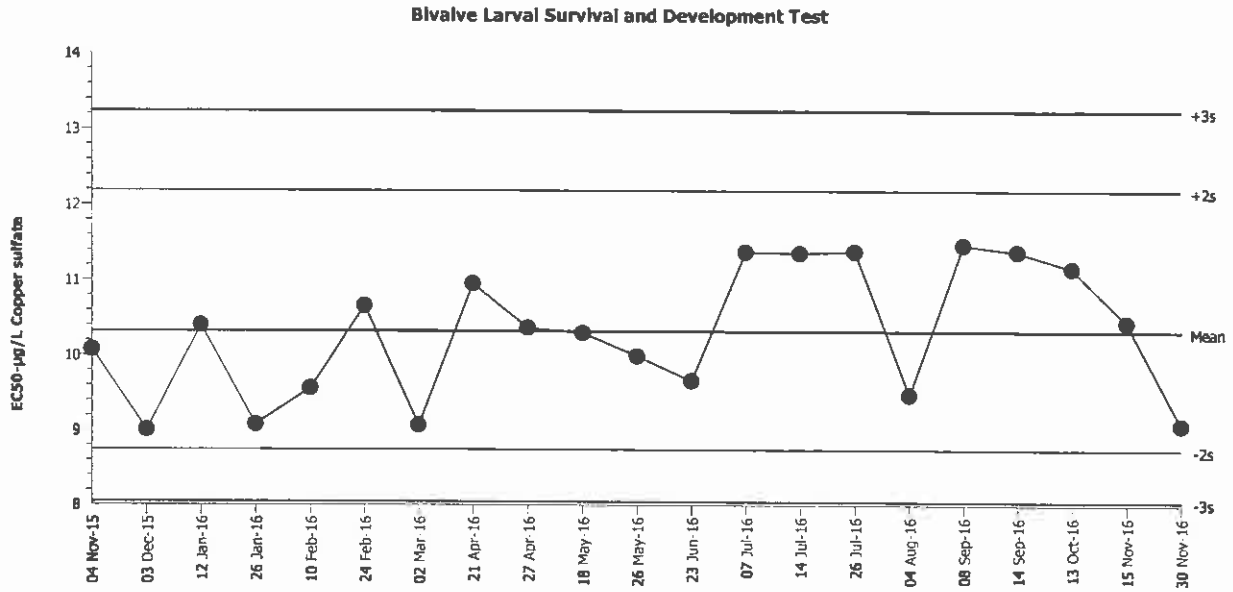
Start Date:	30 Nov-16 13:00	**Species:**	Mytilis galloprovincialis
End Date:	02 Dec-16 13:20	**Protocol:**	EPA/600/R-95/136 (1995)
Sample Date:	30 Nov-16 13:00	**Material:**	Copper sulfate
Sample Code:	41402321		
Sample Source:	Reference Toxicant		
Sample Station:			

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	8	232	218	218	215	
0	D	2	14	232	213	213	206	
0	D	3	18	232	231	231	221	
0	D	4	25	232	219	219	208	
1		1	16	232	218	218	212	
1		2	32	232	218	218	211	
1		3	27	232	211	211	206	
1		4	7	232	239	239	229	
2		1	23	232	232	232	223	
2		2	4	232	214	214	209	
2		3	2	232	221	221	213	
2		4	24	232	232	232	223	
4		1	1	232	226	226	214	
4		2	29	232	236	236	221	
4		3	9	232	210	210	203	
4		4	31	232	257	257	238	
8		1	10	232	232	232	150	
8		2	6	232	227	227	112	
8		3	5	232	249	249	169	
8		4	11	232	238	238	160	
16		1	30	232	186	186	0	
16		2	15	232	219	219	0	
16		3	28	232	226	226	0	
16		4	12	232	244	244	0	
32		1	19	232	125	125	0	
32		2	26	232	161	161	0	
32		3	17	232	152	152	0	
32		4	20	232	148	148	0	
64		1	13	232	0	0	0	
64		2	22	232	0	0	0	
64		3	3	232	0	0	0	
64		4	21	232	0	0	0	

data entry verified against laboratory bench sheets 12-7-16 JRF

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:	10.32	**Count:**	20	**-2s Warning Limit:**	8.741	**-3s Action Limit:**	8.044
Sigma:	NA	**CV:**	8.66%	**+2s Warning Limit:**	12.18	**+3s Action Limit:**	13.24

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2015	Nov	4	14:35	10.07	-0.2449	-0.2893			01-4945-0913	12-4167-7901
2		Dec	3	12:10	9.003	-1.317	-1.644			03-1706-3107	08-0944-2928
3	2016	Jan	12	14:40	10.4	0.07925	0.09217			10-6038-5674	20-9220-6975
4			26	13:20	9.075	-1.244	-1.548			18-1116-9330	06-8399-0866
5		Feb	10	14:45	9.559	-0.7605	-0.9222			18-2305-5641	14-7293-2864
6			24	14:50	10.66	0.3374	0.3875			01-7836-7496	17-6653-1457
7		Mar	2	14:40	9.062	-1.257	-1.564			03-1233-9663	15-4736-9649
8		Apr	21	12:50	10.95	0.6326	0.7168			06-2981-4615	06-1924-7869
9			27	13:40	10.36	0.04292	0.05			10-3412-7541	03-6222-0465
10		May	18	13:50	10.3	-0.02357	-0.02755			00-5077-6110	04-6829-6018
11			26	15:05	9.988	-0.3306	-0.3923			00-2461-1517	20-1645-6539
12		Jun	23	13:50	9.66	-0.6587	-0.7946			20-2900-3441	12-0442-8997
13		Jul	7	14:20	11.37	1.055	1.173			20-3374-2875	12-6544-3567
14			14	14:40	11.36	1.037	1.153			14-8632-5752	09-0596-6683
15			26	17:45	11.38	1.059	1.177			03-9839-1525	11-6670-0698
16		Aug	4	14:30	9.471	-0.8484	-1.033			08-0477-0132	01-3411-5201
17		Sep	8	14:35	11.46	1.145	1.267			17-2952-4030	02-3236-2087
18			14	14:10	11.38	1.058	1.176			07-6290-8502	15-4984-2250
19		Oct	13	14:00	11.16	0.8408	0.9436			06-0302-4211	08-1866-7265
20		Nov	15	11:25	10.44	0.1186	0.1377			11-4556-2596	05-0436-7942
21			30	13:00	9.074	-1.245	-1.549			08-5747-3287	19-4644-8589