TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-74

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: 4-27-16, 1340 hrs.

Test Ending: 4-29-16, 1400 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.	5582G
Collection Date	4-26-16
Receipt Date	4-27-16
Temperature (°C)	5.1
pH	7.7
Dissolved oxygen (mg/L)	9.3
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: 4-26-16

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: 4-20-16

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 4-19-16 and placed in trays with flowing seawater. Holding conditions for the week prior to testing were: temperature, 14.0 ± 0.7°C; pH, 8.1 ± 0.1; salinity, 29.1 ± 1.5‰; and dissolved oxygen, 7.2 ± 0.2 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: 24.1/ml.
Volume of Subsamples Taken for Counting: NA
Water Volume Changes per 24 hr: None (non-renewal static test).
Aeration: None
Feeding: None
Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.
Water Quality and Other Test Conditions: Temperature, 15.4 ± 0.4°C; pH, 8.2 ± 0.2; salinity, 30.0 ± 0.2‰; and dissolved oxygen, 7.9 ± 0.0 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.: 999-3551
Reference Toxicant and Source: Copper as $CuSO_4$-$5H_2O$, Argent Lot No. 0195, 1.0 mg/ml stock prepared 6-19-14.
Test Date: 4-27-16
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ppt, pH 8.2
Results: EC50, 10.4 µg/L; NOEC, 4 µg/L; IC25, 9.13 µg/L. The EC50 result was within the laboratory's control chart warning limits (8.18 – 12.0 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: 5-17-16

Study Director — Date: 5-17-16

Assistant Laboratory Director — for Linda Nemoth — Date: 5-17-16

Quality Assurance Unit — Julie R. Fiore — Date: 5-17-16

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	202	6	208	0.838		0.863	
	2	260	3	263	0.989		1.000	
	3	204	2	206	0.847		0.855	
	4	212	4	216	0.880	0.888	0.896	0.904
35	1	212	2	214	0.880		0.888	
	2	211	4	215	0.876		0.892	
	3	221	3	224	0.917		0.930	
	4	219	3	222	0.909	0.895	0.921	0.908
18	1	230	4	234	0.954		0.971	
	2	205	2	207	0.851		0.859	
	3	228	4	232	0.946		0.963	
	4	205	3	208	0.851	0.900	0.863	0.914
9	1	231	3	234	0.959		0.971	
	2	250	0	250	1.000		1.000	
	3	227	3	230	0.942		0.954	
	4	204	4	208	0.847	0.937	0.863	0.947
4	1	232	5	237	0.963		0.983	
	2	235	0	235	0.975		0.975	
	3	219	2	221	0.909		0.917	
	4	191	3	194	0.793	0.910	0.805	0.920
2	1	230	1	231	0.954		0.959	
	2	223	4	227	0.925		0.942	
	3	212	4	216	0.880		0.896	
	4	224	3	227	0.930	0.922	0.942	0.935
Normal Control	1	226	4	230	0.938		0.954	
	2	238	2	240	0.988		0.996	
	3	224	5	229	0.930		0.950	
	4	225	3	228	0.934	0.947	0.946	0.962
Brine Control[1]	1	226	2	228	0.938		0.946	
	2	214	7	221	0.888		0.917	
	3	235	5	240	0.975		0.996	
	4	240	10	250	0.996	0.949	1.000	0.965

* Based on an average initial count of 241 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 GJI

Test No. 658-74 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. Y.O.S Kalakheve 2.

3. 4.

Study Schedule:

Test Beginning: 4-27-16 1340 Test Ending: 4-29-16 1400

TEST MATERIAL

Description: SP-11 FIELD SAMPLE

NAS Sample No.	55826			
Date of Collection:	4-26-16			
Date of Receipt:	4-27-16			
Temperature (deg C):	5.1			
pH:	7.7			
Dissolved oxygen (mg/L):	9.3			
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: 4-26-16 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: 4-19-16

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
4-20-16	14.7	8.1	30.0	6.9	Held outside in trays of
4-22-16	14.4	8.1	GJI 5-12-16 30.0	7.3	flowing seawater
4-25-16	13.2	8.1	29.0	7.3	
4-27-16	13.7	8.0	27.0	7.3	
Mean	14.0	8.1	GJI 5-12-16 ~~29.0~~ 29.1	7.2	
S.D.	0.7	0.1	~~1.4~~ 1.5	0.2	
(N)	4	4	4	4	

Photoperiod during acclimation: Outdoor ambient conditions

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

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

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

Test No. 658-74 Client CH2M Hill - Wyckoff Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0940 Final: 1020 Fertilization: 1110

Number of organisms used: females: 1 males: 3

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 34 2. 40 3. 35 Mean: 36.3

Dilution factor = DF (mean x 100/2500) = 1.5

TEST PROCEDURES AND CONDITIONS

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

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4

Organisms/ml (15-30): 24.1 Test water changes: None Aeration during test: None

Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt

Temperature: 20 +/- 1 °C, oysters; (16 +/- 1 °C, mussels) Beaker placement:Stratified randomization

RANDOMIZATION CHART

A	18	ϕ	9	2	4	35	70	BRINE CONTROL		
B	BRINE CONTROL	2	ϕ	35	18	70	9	4		
C	ϕ	4	35	BRINE CONTROL	9	18	2	70		
D	35	70	4	9	BRINE CONTROL	2	ϕ	18		

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: 4-20-16 ; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

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

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	

4-27-16 GJ

THE BRINE CONTROL WAS MADE UP OF SALINITY ADJUSTED MILLI-Q DEIONIZED WATER (5.0 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. 658-74 Client CH2M Hill - Wyckoff Investigator

WATER QUALITY DATA

	Date: 4/27/16 initials: YC				Date: 4-29-16 initials: YC			
Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.1	7.8	30.5	7.8	15.4	8.6	29.5	7.9
35	15.0	7.9	30.0	7.9	15.4	8.5	30.0	7.9
18	15.2	8.0	30.0	7.9	15.3	8.4	30.0	7.9
9	15.3	8.0	30.0	7.9	15.4	8.2	30.0	7.9
4	15.4	8.1	30.0	7.9	15.3	8.2	30.0	7.9
2	15.6	8.1	30.0	7.9	15.4	8.2	30.0	7.9
Control	15.7	8.2	30.0	7.9	15.3	8.2	30.0	7.9
Brine control	16.9	8.2	30.0	7.9	15.3	8.2	30.0	7.9

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.4	0.4	16
pH	8.2	0.2	16
Salinity (ppt):	30.0	0.2	16
DO (mg/L):	7.9	0.0	16

Room/ Water bath temperature: (°C)

Day 0: 15.7 Day 0: 15.1

Day 1: 15.5 Day 1: 15.7

Day 2: 15.3 Day 2: 15.4

LARVAL COUNT DATA

	5-5-16 YC		5-6-16 YC		5-5-16 GJL			
Conc.	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
(%)	N	A	N	A	N	A	N	A
70	202	6	260	3	204	2	212	4
35	212	2	211	4	221	3	219	3
18	230	4	205	2	228	4	205	3
9	231	3	250	0	227	3	204	4
4	252	5	235	0	219	2	191	3
2	230	1	223	4	212	4	224	3
Control	226	4	238	2	224	5	225	3
Brine control	226	2	214	7	235	5	240	10
Zero time	252	214	253	239	247	238	—	—

Zero time: Mean 241 SD 14 N 6 CV=(sd/mean)x100 6.0%

Remarks:

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

INVOICE

35637

TO NWAS Gerald

DATE 4-19-16
CUSTOMER ORDER NO.
SALESPERSON
VIA

TERMS:

QUANTITY	DESCRIPTION	PRICE	AMOUNT
10 lbs.	Mussels - Beard On 1 @ 10 lbs	[redacted]	[redacted]
		Total	[redacted]
	Rec' 4-19-16 JC		

Thank You!

Bivalve Larval Survival and Development Test					Northwestern Aquatic Sciences
Analysis ID:	12-3583-2647	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	11 May-16 9:49	Analysis:	Parametric-Two Sample	Official Results:	Yes
Batch ID:	04-5029-0558	Test Type:	Development-Survival	Analyst:	
Start Date:	27 Apr-16 13:40	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	29 Apr-16 14:00	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	
Sample ID:	00-6564-0337	Code:	3E99791	Client:	Wyckoff Treatment Plant
Sample Date:	26 Apr-16 09:03	Material:	Industrial Effluent	Project:	
Receive Date:	27 Apr-16 10:55	Source:	Wyckoff		
Sample Age:	29h (5.1 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	9.53%	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	0.3852	2.447	0.184	6	0.7133	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.001679364	0.001679364	1	0.1484	0.7133	Non-Significant Effect
Error	0.0678938	0.01131563	6			
Total	0.06957316		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.483	47.47	0.4747	Equal Variances
Variances	Mod Levene Equality of Variance	2.501	13.75	0.1648	Equal Variances
Variances	Levene Equality of Variance	3.731	13.75	0.1016	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8875	0.6451	0.2217	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.2466	0.3313	0.1756	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.5104	3.878	0.2005	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	0.9616	0.9249	0.9983	0.9523	0.9461	0.9959	0.01154	2.4%	0.0%
0	Brine Reagent	4	0.9647	0.9008	1	0.9523	0.917	1	0.02008	4.16%	-0.32%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.386	1.258	1.514	1.351	1.336	1.506	0.0403	5.82%	0.0%
0	Brine Reagent	4	1.415	1.213	1.617	1.351	1.279	1.539	0.06351	8.98%	-2.09%

Proportion Survived Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9544	0.9959	0.9502	0.9461
0	Brine Reagent	0.9461	0.917	0.9959	1

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.355	1.506	1.346	1.336
0	Brine Reagent	1.336	1.279	1.506	1.539

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	00-3513-4227	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	11 May-16 9:48	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes
Batch ID:	04-5029-0558	Test Type:	Development-Survival	Analyst:	
Start Date:	27 Apr-16 13:40	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	29 Apr-16 14:00	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	
Sample ID:	00-6564-0337	Code:	3E99791	Client:	Wyckoff Treatment Plant
Sample Date:	26 Apr-16 09:03	Material:	Industrial Effluent	Project:	
Receive Date:	27 Apr-16 10:55	Source:	Wyckoff		
Sample Age:	29h (5.1 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.8%	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	0.8505	2.448	0.202	6	0.5228	CDF	Non-Significant Effect
		4	0.9031	2.448	0.202	6	0.4984	CDF	Non-Significant Effect
		9	0.1777	2.448	0.202	6	0.8037	CDF	Non-Significant Effect
		18	1.184	2.448	0.202	6	0.3724	CDF	Non-Significant Effect
		35	1.485	2.448	0.202	6	0.2543	CDF	Non-Significant Effect
		70	1.185	2.448	0.202	6	0.3720	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04902507	0.008170846	6	0.6001	0.7271	Non-Significant Effect
Error	0.2859159	0.01361504	21			
Total	0.334941		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.206	16.81	0.2234	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9806	0.8975	0.8656	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	0.9616	0.9249	0.9983	0.9523	0.9461	0.9959	0.01154	2.4%	0.0%
2		4	0.9346	0.8921	0.9772	0.9419	0.8963	0.9585	0.01338	2.86%	2.81%
4		4	0.9201	0.7892	1	0.9461	0.805	0.9834	0.04112	8.94%	4.32%
9		4	0.9471	0.853	1	0.9627	0.8631	1	0.02955	6.24%	1.51%
18		4	0.9139	0.8165	1	0.9129	0.8589	0.971	0.0306	6.7%	4.96%
35		4	0.9077	0.8747	0.9406	0.9066	0.888	0.9295	0.01036	2.28%	5.61%
70		4	0.9035	0.7973	1	0.8797	0.8548	1	0.03338	7.39%	6.04%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.386	1.258	1.514	1.351	1.336	1.506	0.0403	5.82%	0.0%
2		4	1.316	1.233	1.398	1.327	1.243	1.366	0.02594	3.94%	5.06%
4		4	1.311	1.073	1.55	1.345	1.113	1.442	0.07496	11.43%	5.38%
9		4	1.371	1.144	1.599	1.378	1.192	1.539	0.07146	10.42%	1.06%
18		4	1.288	1.105	1.472	1.284	1.186	1.4	0.05771	8.96%	7.05%
35		4	1.263	1.206	1.321	1.261	1.229	1.302	0.01803	2.85%	8.84%
70		4	1.288	1.019	1.557	1.217	1.18	1.539	0.08456	13.13%	7.05%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	00-3513-4227	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 May-16 9:48	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

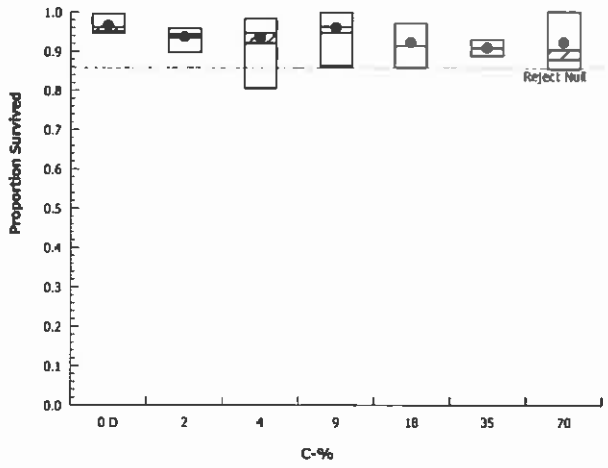
Proportion Survived Detail

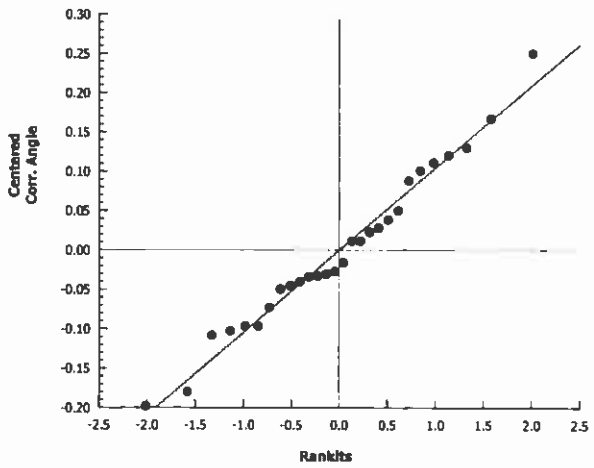
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9544	0.9959	0.9502	0.9461
2		0.9585	0.9419	0.8963	0.9419
4		0.9834	0.9751	0.917	0.805
9		0.971	1	0.9544	0.8631
18		0.971	0.8589	0.9627	0.8631
35		0.888	0.8921	0.9295	0.9212
70		0.8631	1	0.8548	0.8963

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.355	1.506	1.346	1.336
2		1.366	1.327	1.243	1.327
4		1.442	1.412	1.279	1.113
9		1.4	1.539	1.355	1.192
18		1.4	1.186	1.376	1.192
35		1.229	1.236	1.302	1.286
70		1.192	1.539	1.18	1.243

Graphics





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

-GSL

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences	
Analysis ID:	13-1249-3666	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 May-16 9:49	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes
Batch ID:	04-5029-0558	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	27 Apr-16 13:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	29 Apr-16 14:00	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	00-6564-0337	**Code:**	3E99791	**Client:**	Wyckoff Treatment Plant
Sample Date:	26 Apr-16 09:03	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	27 Apr-16 10:55	**Source:**	Wyckoff		
Sample Age:	29h (5.1 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	10.1%	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	0.2736	2.447	0.172	6	0.7936	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0007414265	0.0007414265	1	0.07484	0.7936	Non-Significant Effect
Error	0.05944255	0.009907091	6			
Total	0.06018397		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	2.554	47.47	0.4616	Equal Variances
Variances	Mod Levene Equality of Variance	1.393	13.75	0.2826	Equal Variances
Variances	Levene Equality of Variance	1.286	13.75	0.3000	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9245	0.6451	0.4671	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.2477	0.3313	0.1705	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.4267	3.878	0.3182	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	0.9471	0.9038	0.9903	0.9378	0.9295	0.9876	0.01359	2.87%	0.0%
0	Brine Reagent	4	0.9492	0.8738	1	0.9378	0.888	0.9959	0.02368	4.99%	-0.22%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.347	1.229	1.466	1.319	1.302	1.459	0.03733	5.54%	0.0%
0	Brine Reagent	4	1.367	1.177	1.557	1.319	1.229	1.506	0.05966	8.73%	-1.43%

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9378	0.9876	0.9295	0.9336
0	Brine Reagent	0.9378	0.888	0.9751	0.9959

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.319	1.459	1.302	1.31
0	Brine Reagent	1.319	1.229	1.412	1.506

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	20-0792-5515	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 May-16 9:49	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	04-5029-0558	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	27 Apr-16 13:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	29 Apr-16 14:00	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	00-6564-0337	**Code:**	3E99791	**Client:**	Wyckoff Treatment Plant
Sample Date:	26 Apr-16 09:03	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	27 Apr-16 10:55	**Source:**	Wyckoff		
Sample Age:	29h (5.1 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.4%	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	0.7165	2.448	0.19	6	0.5847	CDF	Non-Significant Effect
		4	0.7733	2.448	0.19	6	0.5586	CDF	Non-Significant Effect
		9	-0.03415	2.448	0.19	6	0.8662	CDF	Non-Significant Effect
		18	1.131	2.448	0.19	6	0.3954	CDF	Non-Significant Effect
		35	1.359	2.448	0.19	6	0.3010	CDF	Non-Significant Effect
		70	1.242	2.448	0.19	6	0.3478	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04638238	0.007730396	6	0.6453	0.6933	Non-Significant Effect
Error	0.2515802	0.01198001	21			
Total	0.2979626		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.626	16.81	0.2668	Equal Variances
Variances	Mod Levene Equality of Variance	0.8408	3.812	0.5526	Equal Variances
Variances	Levene Equality of Variance	1.387	3.812	0.2657	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9719	0.8975	0.6310	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1287	0.1914	0.2682	Normal Distribution
Distribution	D'Agostino Skewness	0.6109	2.576	0.5413	Normal Distribution
Distribution	D'Agostino Kurtosis	0.4066	2.576	0.6843	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	0.5385	9.21	0.7639	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.382	3.878	0.4038	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	0.9471	0.9038	0.9903	0.9357	0.9295	0.9876	0.01359	2.87%	0.0%
2		4	0.9222	0.8727	0.9717	0.9274	0.8797	0.9544	0.01556	3.38%	2.63%
4		4	0.9098	0.7772	1	0.9357	0.7925	0.9751	0.04164	9.16%	3.94%
9		4	0.9367	0.8334	1	0.9502	0.8465	1	0.03247	6.93%	1.1%
18		4	0.9004	0.8088	0.9921	0.8983	0.8506	0.9544	0.0288	6.4%	4.93%
35		4	0.8952	0.8623	0.9282	0.8942	0.8755	0.917	0.01036	2.31%	5.48%
70		4	0.8882	0.778	0.9985	0.8631	0.8382	0.9886	0.03464	7.8%	6.22%

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

Analysis ID:	20-0792-5515	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 May-16 9:49	**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	1.347	1.229	1.466	1.314	1.302	1.459	0.03733	5.54%	0.0%
2		4	1.292	1.201	1.383	1.298	1.217	1.355	0.02862	4.43%	4.12%
4		4	1.288	1.063	1.513	1.32	1.098	1.412	0.07071	10.98%	4.44%
9		4	1.35	1.108	1.592	1.347	1.168	1.539	0.07616	11.28%	-0.2%
18		4	1.26	1.101	1.418	1.255	1.174	1.355	0.0498	7.91%	6.49%
35		4	1.242	1.188	1.296	1.24	1.21	1.279	0.01702	2.74%	7.8%
70		4	1.251	1.022	1.48	1.192	1.157	1.464	0.072	11.51%	7.13%

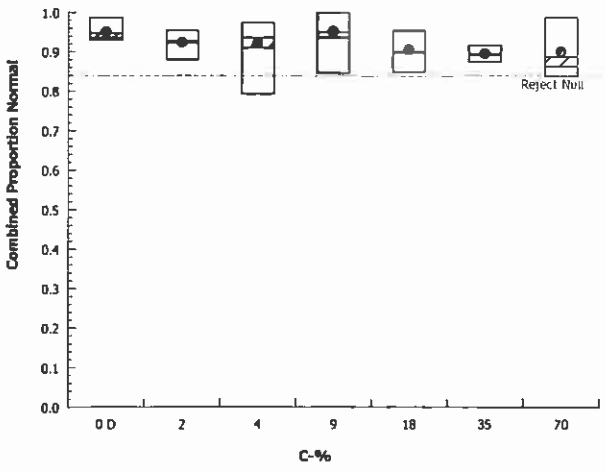
Combined Proportion Normal Detail

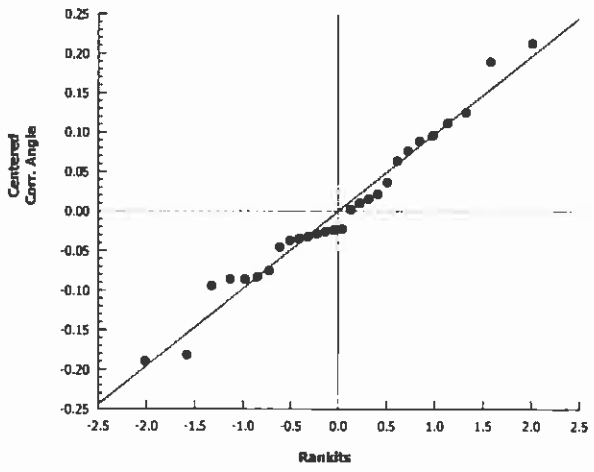
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9378	0.9876	0.9295	0.9336
2		0.9544	0.9253	0.8797	0.9295
4		0.9627	0.9751	0.9087	0.7925
9		0.9585	1	0.9419	0.8465
18		0.9544	0.8506	0.9461	0.8506
35		0.8797	0.8755	0.917	0.9087
70		0.8382	0.9886	0.8465	0.8797

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.319	1.459	1.302	1.31
2		1.355	1.294	1.217	1.302
4		1.376	1.412	1.264	1.098
9		1.366	1.539	1.327	1.168
18		1.355	1.174	1.336	1.174
35		1.217	1.21	1.279	1.264
70		1.157	1.464	1.168	1.217

Graphics





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

Bivalve Larval Survival and Development Test					Northwestern Aquatic Sciences
Analysis ID:	13-3657-8118	Endpoint:	Combined Proportion Normal	CETIS Version:	CETISv1.8.7
Analyzed:	11 May-16 9:49	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Batch ID:	04-5029-0558	Test Type:	Development-Survival	Analyst:	
Start Date:	27 Apr-16 13:40	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	29 Apr-16 14:00	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	

Sample ID:	00-6564-0337	Code:	3E99791	Client:	Wyckoff Treatment Plant
Sample Date:	26 Apr-16 09:03	Material:	Industrial Effluent	Project:	
Receive Date:	27 Apr-16 10:55	Source:	Wyckoff		
Sample Age:	29h (5.1 °C)	Station:			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1647373	280	Yes	Two-Point Interpolation

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	0.9471	0.9295	0.9876	0.01359	0.02718	2.87%	0.0%	913	964
2		4	0.9222	0.8797	0.9544	0.01556	0.03112	3.38%	2.63%	889	964
4		4	0.9098	0.7925	0.9751	0.04164	0.08329	9.16%	3.94%	877	964
9		4	0.9367	0.8465	1	0.03247	0.06494	6.93%	1.1%	912	973
18		4	0.9004	0.8506	0.9544	0.0288	0.0576	6.4%	4.93%	868	964
35		4	0.8952	0.8755	0.917	0.01036	0.02071	2.31%	5.48%	863	964
70		4	0.8882	0.8382	0.9886	0.03464	0.06927	7.8%	6.22%	878	986

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9378	0.9876	0.9295	0.9336
2		0.9544	0.9253	0.8797	0.9295
4		0.9627	0.9751	0.9087	0.7925
9		0.9585	1	0.9419	0.8465
18		0.9544	0.8506	0.9461	0.8506
35		0.8797	0.8755	0.917	0.9087
70		0.8382	0.9886	0.8465	0.8797

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Start Date:	27 Apr-16 13:40	**Species:**	Mytilis galloprovincialis	**Sample Code:**	3E99791
End Date:	29 Apr-16 14:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Wyckoff
Sample Date:	26 Apr-16 09:03	**Material:**	Industrial Effluent	**Sample Station:**	

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	241	228	228	226	
0	B	2	11	241	221	221	214	
0	B	3	12	241	240	240	235	
0	B	4	3	241	250	250	240	
0	D	1	10	241	230	230	226	
0	D	2	2	241	240	240	238	
0	D	3	23	241	229	229	224	
0	D	4	9	241	228	228	225	
2		1	18	241	231	231	230	
2		2	27	241	227	227	223	
2		3	22	241	216	216	212	
2		4	30	241	227	227	224	
4		1	28	241	237	237	232	
4		2	15	241	235	235	235	
4		3	14	241	221	221	219	
4		4	24	241	194	194	191	
9		1	6	241	234	234	231	
9		2	29	241	250	250	250	
9		3	5	241	230	230	227	
9		4	17	241	208	208	204	
18		1	16	241	234	234	230	
18		2	13	241	207	207	205	
18		3	1	241	232	232	228	
18		4	31	241	208	208	205	
35		1	7	241	214	214	212	
35		2	26	241	215	215	211	
35		3	21	241	224	224	221	
35		4	8	241	222	222	219	
70		1	4	241	208	208	202	
70		2	20	241	263	263	260	
70		3	19	241	206	206	204	
70		4	25	241	216	216	212	

data entry verified against laboratory bench sheets 5-12-16 JRF

Northwestern Aquatic Sciences (REGION COPY)
DateShipped: 4/26/2016
CarrierName: FedEx
AirbillNo: 7829 2118 6504

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
658-2nd Quarter		Ground Water/ K Allers	Composite	CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	04/26/2016 09:03	Field Sample

PAGE 13 OF 14

Special Instructions: NAS # 55826

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

Analysis Key: CHRTOX=Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	Keith Allers CH2M	4-26-16 0955	NAS	4-27-16 1055	

NORTHWESTERN AQUATIC SCIENCES A Division of NAS Associates, Inc.	SAMPLE NO. 658	DATE 4-26-2016	SEAL BROKEN BY	DATE 4-27-16
	SIGNATURE Keith Allers			
	PRINT NAME AND TITLE Keith Allers Industrial Tech			

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 1-6 GJI

Test No. 999-3551 Client: QC Test Investigator

STUDY MANAGEMENT

Client: QC Test

Client's Study Monitor: QC Test

Testing Laboratory: Northwestern Aquatic Sciences

Test Location: Newport Laboratory

Laboratory's Study Personnel:

Proj. Mgr./Study Dir. G.J. Irissarri GJI

QA Officer L.K. Nemeth

1. [signature] 2. [signature]

3. 4.

Study Schedule:

Test Beginning: 4-27-16 1340 Test Ending: 5-2-16 1400

TEST MATERIAL

Description: Copper as $CuSO_4 \cdot 5H_2O$, Argent Lot# 0195,

NAS Sample No.		1.0 mg/ml stock prepared: 6-19-14		
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: 4-26-16 Salinity (ppt) 30.0 pH 8.1

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

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 4-19-16

Source: Kamilche Sea Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
4-20-16	14.7	8.1	30.0	6.9	Held outside in trays of
4-22-16	14.4	8.1	30.5	7.3	flowing seawater
4-25-16	13.2	8.1	29.0	7.3	
4-27-16	13.7	8.0	27.0	7.3	
Mean	14.0	8.1	GJI 5-12-16 ~~29.0~~ 29.1	7.2	
S.D.	0.7	0.1	~~1.4~~ 1.5	0.2	
(N)	4	4	4	4	

Photoperiod during acclimation: Outdoor ambient conditions

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

Test No. 999-3551 Client QC Test Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 0940 Final: 1020 Fertilization: 1110
Number of organisms used: females: 1 males: 3
Egg Dilution (1 ml diluted to 100 ml):
Count/ml of dilution: 1. 34 2. 40 3. 35 Mean: 36.3
Dilution factor = DF (mean x 100/2500) = 1.5

TEST PROCEDURES AND CONDITIONS

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

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4
Organisms/ml (15-30): 24.1 Test water changes: None Aeration during test: None
Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt
Temperature: 20 +/- 1 °C, oysters; (16 +/- 1 °C, mussels) Beaker placement:Stratified randomization

RANDOMIZATION CHART

A	4	1	2	64	8	16	32	ϕ		
B	16	64	1	4	32	ϕ	2	8		
C	64	8	ϕ	32	1	2	16	4		
D	2	16	32	8	4	1	0	64		

PREPARATION OF TEST SOLUTIONS

4-27-16
632

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:

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

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

Test No. 999-3551 Client QC Test Investigator

WATER QUALITY DATA

	Date: 4-27-16 Initials:				Date: 4-29-16 Initials:			
Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	15.1	8.2	29.5	7.9	15.3	8.2	29.5	7.9
32	15.1	8.2	30.0	7.9	15.3	8.2	30.0	7.9
16	15.1	8.2	30.0	7.9	15.3	8.2	30.0	7.9
8	15.0	8.2	30.0	7.9	15.3	8.2	30.0	7.9
4	15.0	8.2	30.0	7.9	15.3	8.2	30.0	7.9
2	15.0	8.2	30.0	7.9	15.3	8.2	30.0	7.9
1	15.0	8.2	30.0	7.9	15.4	8.2	30.0	7.9
Control	15.1	8.1	30.0	7.9	15.4	8.2	30.0	7.9
Brine control	---	---	---	—	---	---	---	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.2	0.2	16
pH:	8.2	0.0	16
Salinity (ppt):	29.9	0.2	16
DO (mg/L):	7.9	0.0	16

Room/ Water bath temperature: (°C)

Day 0: 15.7 Day 0: 15.1
Day 1: 15.5 Day 1: 15.7
Day 2: 15.3 Day 2: 15.4

LARVAL COUNT DATA

← 5-5-16 GC → | 5-6-16 GC | 5-8-16 GJL

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	Ø	Ø	Ø	2	Ø	2	Ø	2
32	Ø	136	Ø	158	Ø	121	Ø	113
16	Ø	217	Ø	231	Ø	220	Ø	195
8	272	29	187	34	~~208~~ 208	31	187	35
4	227	6	253	8	251	3	223	8
2	220	4	239	5	250	6	248	4
1	259	10	234	3	234	8	222	1
Control	221	1	270	7	228	5	221	2
Brine control	---	---	---	---	---	---	---	---
Zero time	252	214	253	239	247	238	—	—

Zero time: Mean 241 SD 14 N 6 CV=(sd/mean)x100 6.0 %

Remarks:

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

INVOICE

35637

TO NWAS Gerald

DATE 4-19-16
CUSTOMER ORDER NO
SALESPERSON
VIA

TERMS:

QUANTITY	DESCRIPTION	PRICE	AMOUNT
10 lbs.	Mussels - Beard On 1 @ 10 lbs	[redacted]	[redacted]
		Total	[redacted]
	Rec' 4-19-16 [illegible]		

Thank You!

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

Batch ID:	04-5029-0558	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	27 Apr-16 13:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	29 Apr-16 14:00	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	

Sample ID:	20-4684-5129	**Code:**	7A0060C9	**Client:**	Internal Lab
Sample Date:	27 Apr-16 13:40	**Material:**	Copper sulfate	**Project:**	
Receive Date:	27 Apr-16 13:40	**Source:**	Reference Toxicant		
Sample Age:	NA	**Station:**			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-6549-7029	Combined Proportion Norm	4	8	5.657	7.44%		Dunnett Multiple Comparison Test
07-3754-5189	Proportion Survived	16	32	22.63	9.25%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
03-1552-6985	Combined Proportion Norm	EC25	9.129	8.453	9.76		Linear Interpolation (ICPIN)
03-6222-0465	Combined Proportion Norm	EC50	10.36	10.21	10.51		Spearman-Kärber
19-0074-0746	Proportion Survived	EC50	31.56	30.75	32.4		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	0.9387	0.8947	0.9827	0.917	0.9747	0.01382	0.02765	2.95%	0.0%
1		4	0.9565	0.9185	0.9944	0.9212	0.971	0.01193	0.02385	2.49%	-1.89%
2		4	0.9663	0.9088	1	0.9129	0.9917	0.01808	0.03617	3.74%	-2.94%
4		4	0.9562	0.9116	1	0.9253	0.9882	0.01401	0.02802	2.93%	-1.86%
8		4	0.8296	0.7275	0.9318	0.7759	0.9037	0.0321	0.0642	7.74%	11.62%
16		4	0	0	0	0	0	0	0		100.0%
32		4	0	0	0	0	0	0	0		100.0%
64		4	0	0	0	0	0	0	0		100.0%

Proportion Survived Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.9533	0.8939	1	0.9212	1	0.01866	0.03733	3.92%	0.0%
1		4	0.9772	0.9208	1	0.9253	1	0.01773	0.03545	3.63%	-2.5%
2		4	0.9824	0.9262	1	0.9295	1	0.01763	0.03527	3.59%	-3.05%
4		4	0.9813	0.9466	1	0.9585	1	0.01091	0.02183	2.22%	-2.94%
8		4	0.9575	0.8867	1	0.917	1	0.02224	0.04448	4.65%	-0.44%
16		4	0.8952	0.7956	0.9948	0.8091	0.9585	0.0313	0.0626	6.99%	6.09%
32		4	0.5477	0.4171	0.6783	0.4689	0.6556	0.04104	0.08208	14.99%	42.55%
64		4	0.006224	0	0.01283	0	0.008299	0.002075	0.004149	66.67%	99.35%

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	0.917	0.9747	0.9461	0.917
1		0.9628	0.971	0.971	0.9212
2		0.9129	0.9917	0.9766	0.9841
4		0.9419	0.9693	0.9882	0.9253
8		0.9037	0.7759	0.8631	0.7759
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	0.9212	1	0.9668	0.9253
1		1	0.9834	1	0.9253
2		0.9295	1	1	1
4		0.9668	1	1	0.9585
8		1	0.917	0.9917	0.9212
16		0.9004	0.9585	0.9129	0.8091
32		0.5643	0.6556	0.5021	0.4689
64		0	0.008299	0.008299	0.008299

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	221/241	270/277	228/241	221/241
1		259/269	234/241	234/241	222/241
2		220/241	239/241	250/256	248/252
4		227/241	253/261	251/254	223/241
8		272/301	187/241	208/241	187/241
16		0/241	0/241	0/241	0/241
32		0/241	0/241	0/241	0/241
64		0/241	0/241	0/241	0/241

Proportion Survived Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	222/241	241/241	233/241	223/241
1		241/241	237/241	241/241	223/241
2		224/241	241/241	241/241	241/241
4		233/241	241/241	241/241	231/241
8		241/241	221/241	239/241	222/241
16		217/241	231/241	220/241	195/241
32		136/241	158/241	121/241	113/241
64		0/241	2/241	2/241	2/241

CETIS Test Data Worksheet

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

Start Date:	27 Apr-16 13:40	**Species:**	Mytilis galloprovincialis	**Sample Code:**	7A0060C9
End Date:	29 Apr-16 14:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Reference Toxicant
Sample Date:	27 Apr-16 13:40	**Material:**	Copper sulfate	**Sample Station:**	

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	8	241	222	222	221	
0	D	2	14	241	277	277	270	
0	D	3	18	241	233	233	228	
0	D	4	25	241	223	223	221	
1		1	16	241	269	269	259	
1		2	32	241	237	237	234	
1		3	27	241	242	242	234	
1		4	7	241	223	223	222	
2		1	23	241	224	224	220	
2		2	4	241	244	244	239	
2		3	2	241	256	256	250	
2		4	24	241	252	252	248	
4		1	1	241	233	233	227	
4		2	29	241	261	261	253	
4		3	9	241	254	254	251	
4		4	31	241	231	231	223	
8		1	10	241	301	301	272	
8		2	6	241	221	221	187	
8		3	5	241	239	239	208	
8		4	11	241	222	222	187	
16		1	30	241	217	217	0	
16		2	15	241	231	231	0	
16		3	28	241	220	220	0	
16		4	12	241	195	195	0	
32		1	19	241	136	136	0	
32		2	26	241	158	158	0	
32		3	17	241	121	121	0	
32		4	20	241	113	113	0	
64		1	13	241	0	0	0	
64		2	22	241	2	2	0	
64		3	3	241	2	2	0	
64		4	21	241	2	2	0	

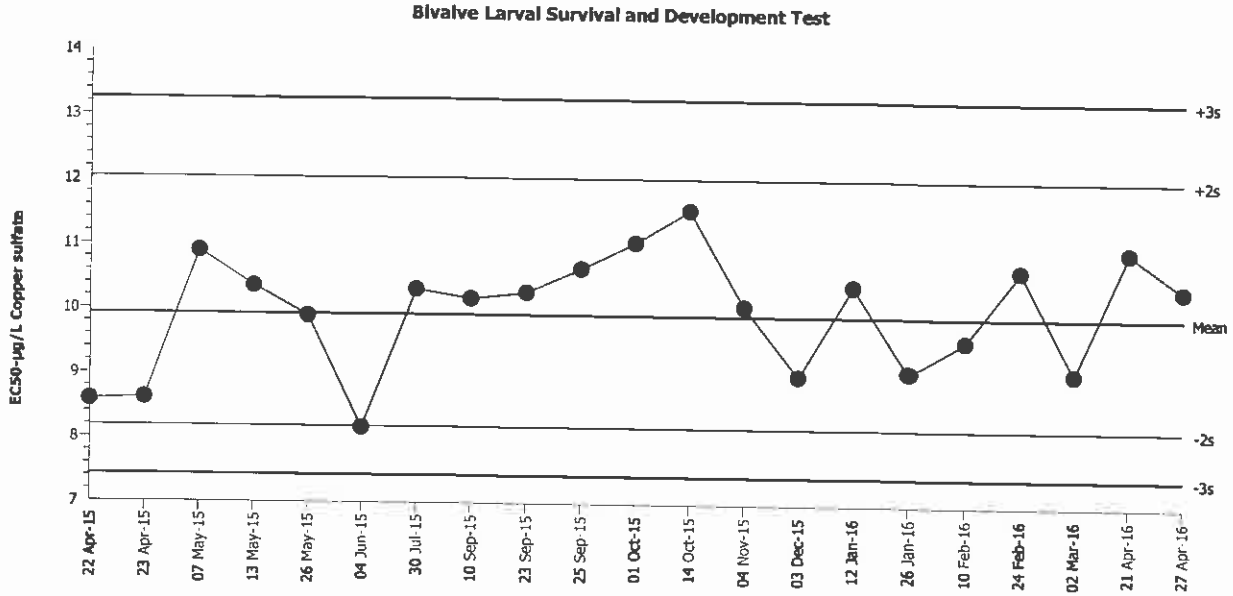
data entry verified against laboratory bench sheets 5-12-16 JMF

CETIS QC Plot

Report Date: 11 May-16 13:37 (1 of 1)

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

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



Mean:	9.924	**Count:**	20	**-2s Warning Limit:**	8.183	**-3s Action Limit:**	7.43
Sigma:	NA	**CV:**	10.10%	**+2s Warning Limit:**	12.04	**+3s Action Limit:**	13.26

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2015	Apr	22	13:40	8.585	-1.339	-1.502			00-3213-1743	14-0335-9169
2			23	14:55	8.612	-1.312	-1.47			04-5651-6084	07-9131-6184
3		May	7	15:35	10.89	0.9697	0.966			00-3523-0479	12-8078-2374
4			13	13:45	10.35	0.4303	0.4398			16-6504-7989	11-4000-6150
5			26	12:40	9.895	-0.02907	-0.0304			15-2938-1576	00-3109-6336
6		Jun	4	16:30	8.168	-1.757	-2.019	(-)		17-2527-9288	05-3466-4089
7		Jul	30	16:35	10.31	0.3883	0.3977			09-9236-7237	16-6604-3815
8		Sep	10	14:20	10.17	0.2481	0.2558			14-2847-3680	18-2495-6117
9			23	15:20	10.27	0.349	0.3581			07-5017-0556	12-5711-0160
10			25	14:45	10.64	0.7198	0.7256			08-8031-4805	17-8304-1379
11		Oct	1	15:00	11.05	1.129	1.117			02-0435-7572	17-5283-9042
12			14	14:20	11.56	1.634	1.579			04-2701-8188	06-5641-7689
13		Nov	4	14:35	10.07	0.1498	0.1553			01-4945-0913	12-4167-7901
14		Dec	3	12:10	9.003	-0.9219	-1.01			03-1706-3107	08-0944-2928
15	2016	Jan	12	14:40	10.4	0.474	0.4834			10-6038-5674	20-9220-6975
16			26	13:20	9.075	-0.8497	-0.9275			18-1116-9330	06-8399-0866
17		Feb	10	14:45	9.559	-0.3658	-0.3892			18-2305-5641	14-7293-2864
18			24	14:50	10.66	0.7321	0.7375			01-7836-7496	17-6653-1457
19		Mar	2	14:40	9.062	-0.8619	-0.9415			03-1233-9663	15-4736-9649
20		Apr	21	12:50	10.95	1.027	1.021			06-2981-4615	06-1924-7869
21			27	13:40	10.36	0.4376	0.4472			10-3412-7541	03-6222-0465

Analyst: _____ QA: _____