TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-88

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. Ken Scheffler

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.

Study Schedule:

Test Beginning: 9-12-18, 1550 hrs.

Test Ending: 9-14-18, 1550 hrs.

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

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

TEST MATERIAL

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

NAS Sample No.	6239G
Collection Date	9-11-18
Receipt Date	9-12-18
Temperature (°C)	4.9
pH	7.6
Dissolved oxygen (mg/L)	9.2
Salinity (‰)	6.0

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon seawater.

Date of Collection: 9-11-18

Water Quality: Salinity, 31.0 ‰; pH, 8.0

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

BRINE USED FOR DILUTION WATER AND SALINITY CONTROL

Source: Filtered Yaquina Bay, Oregon, sea water

Salinity: 100.0 ‰

Date of Preparation: 5-1-18

Method of Preparation: Freezing method

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).

Age: 1.5 hrs post-fertilization.

Source: Taylor Shellfish Farms, Shelton, WA.

Conditioning: Adult mussels were received on 9-11-18 and placed in trays with flowing seawater. Holding conditions prior to testing were: temperature, 15.9 °C; pH, 7.9; salinity, 34.5‰; and dissolved oxygen, 6.6 mg/L. Photoperiod was natural daylight.
Source of Gametes: 2 females and 2 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 (6.0 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: 22.9/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, 15.8 ± 0.5°C; pH, 8.1 ± 0.2; salinity, 30.8 ± 0.7‰; and dissolved oxygen, 8.0 ± 0.1 mg/L. Photoperiod 16:8 hr, L:D.

DATA ANALYSIS METHODS

The proportion of surviving larvae, and the proportion of normal surviving larvae were calculated for each treatment replicate. The calculation used for the proportion of normal surviving larvae, Combined Proportion Normal, was the combined endpoint specified by EPA/600/R-95/136. The means were obtained for each treatment level and the latter were then corrected for control response using Abbott's formula. The LC50 (survival) and the EC50 (normality) were calculated, where data permitted, using either the Maximum-Likelihood Probit or the Trimmed Spearman-Karber methods. An IC25 was determined by linear interpolation with bootstrapping. NOEC and LOEC values for survival and normality were computed using either Dunnett's test, T-test with Bonferroni's adjustment, Steel's Many-One Rank Test, or Wilcoxon Rank Sum Test with Bonferroni Adjustment. The appropriate test was selected after evaluating the data for normality and homogeneity of variance. An arcsine-square root (angular) transformation was performed on the data prior to statistical analysis. The statistical software employed for these calculations was CETIS, v1.8.7.4, Tidepool Scientific Software. Toxic units (TU_c) were computed as 100/NOEC, 100/EC50, or 100/IC25.

PROTOCOL DEVIATIONS

None.

REFERENCE TOXICANT TEST

The routine reference toxicant test is a standard multi-concentration toxicity test using copper sulfate to evaluate the performance of the test organisms used in the effluent toxicity test. The performance is evaluated by comparing the results of this test with historical results obtained at the laboratory. A summary of the reference toxicant test result is given below. The reference toxicant test raw data are found in Appendix III.

Test No.: 999-3809
Reference Toxicant and Source: Copper as $CuSO_4$-$5H_2O$, Argent Lot No. 0195, 1.0 mg/ml stock prepared 3-27-18.
Test Date: 9-12-18
Dilution Water Used: Yaquina Bay, OR seawater. Salinity 31.0 ppt, pH 8.0
Results: EC50, 10.7 µg/L; NOEC, 4 µg/L; IC25, 9.53 µg/L. The EC50 result was within the laboratory's control chart warning limits (8.60 – 11.7 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: 9-25-18

Study Director — Date: 9-20-18

Laboratory Director — Date: 9/25/18

Quality Assurance Unit — Date: 9-20-18

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	221	7	228	0.965		0.996	
	2	206	2	208	0.900		0.908	
	3	231	5	236	0.979		1.000	
	4	215	5	220	0.939	0.946	0.961	0.966
35	1	242	3	245	0.988		1.000	
	2	229	3	232	1.000		1.000	
	3	252	4	256	0.984		1.000	
	4	241	2	243	0.992	0.991	1.000	1.000
18	1	223	6	229	0.974		1.000	
	2	249	4	253	0.984		1.000	
	3	241	5	246	0.980		1.000	
	4	237	2	239	0.992	0.982	1.000	1.000
9	1	241	4	245	0.984		1.000	
	2	210	5	215	0.917		0.939	
	3	238	3	241	0.988		1.000	
	4	225	3	228	0.983	0.968	0.996	0.984
4	1	242	2	244	0.992		1.000	
	2	221	7	228	0.965		0.996	
	3	222	3	225	0.969		0.983	
	4	235	2	237	0.992	0.980	1.000	0.995
2	1	231	5	236	0.979		1.000	
	2	230	2	232	0.991		1.000	
	3	233	4	237	0.983		1.000	
	4	235	4	239	0.983	0.984	1.000	1.000
Normal Control	1	248	6	254	0.976		1.000	
	2	230	5	235	0.979		1.000	
	3	239	1	240	0.996		1.000	
	4	216	6	222	0.943	0.974	0.969	0.992
Brine Control[1]	1	234	3	237	0.987		1.000	
	2	235	2	237	0.992		1.000	
	3	243	3	246	0.988		1.000	
	4	224	6	230	0.978	0.986	1.000	1.000

* Based on an average initial count of 229 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 (6.0 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. 658-88 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. J. Brown JB 2.
3. 4.

Study Schedule:

Test Beginning: 9-12-18 1550 Test Ending: 9-19-18 1530

TEST MATERIAL

Description: GROUND WATER COMPOSITE SP-11

NAS Sample No.	62396				
Date of Collection:	9-11-18				
Date of Receipt:	9-12-18				
Temperature (deg C):	4.9				
pH:	7.6				
Dissolved oxygen (mg/L):	9.2				
Conductivity (umhos/cm):	—				
Hardness (mg/L):	—				
Alkalinity (mg/L):	—				
Salinity (ppt):	6.0				
Total chlorine (mg/L):					
Total ammonia-N (mg/L):					
:					

DILUTION WATER

Description: Yaquina Bay, OR

Date of Collection: 9-11-18 Salinity (ppt) 31.0 pH 8.0

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

TEST ORGANISMS

Species: MYTILUS GALLOPROVINCIALIS Date Received: 9-11-18

Source: TAYLOR SHELLFISH FARMS, SHELTON, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
9-12-18	15.9	7.9	34.5	6.6	Held outside in trays of flowing seawater
Mean					
S.D.					
(N)					

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. 658-88 Client CH2M Hill - Wyckoff Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 1130 Final: 1245 Fertilization: 1420

Number of organisms used: females: 2 males: 2

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 34 2. 31 3. 30 Mean: 31.7

Dilution factor = DF (mean x 100/2500) = 1.3

TEST PROCEDURES AND CONDITIONS

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

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

Organisms/ml (15-30): 22.9 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	9	35	BRINE CONTROL	70	ϕ	2	18	4		
B	18	70	20	BRINE CONTROL	9	4	ϕ	35		
C	70	2	18	ϕ	4	BRINE CONTROL	35	9		
D	BRINE CONTROL	9	0	35	2	70	4	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: 5-1-18 ; brine salinity (ppt) 100.0

Source of seawater: Yaquina Bay, Oregon

$$VB = VE \frac{(TS - SE)}{(SB - TS)} = VE \frac{(30 - 6)}{(102 - 30)} = VE\ (0.333)$$

Where:
VB=volume brine
VE=volume effluent
SB=sallnity 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	24.0	Brought up to a final
35	35	12.0	volume of 100 ml
18	18	6.2	with dilution water
9	9	3.1	
4	4	1.4	
2	2	0.7	
0	0	ϕ	
Brine Control *	ϕ	24.0	

9-12-18 652

* 70 ML OF SALINITY ADJUSTED MILLI-Q DEIONIZED WATER AT 6.0 PPT WAS MIXED W/ 24.0 ML OF BRINE AND BROUGHT UP TO 100 ML WITH DILUTION WATER

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

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

Test No. 658-88 Client CH2M Hill - Wyckoff Investigator

WATER QUALITY DATA

	Date: 9-12-18 initials: JB				Date: 9-14-18 initials: JB			
Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.2	7.7	31.0	7.8	15.4	8.4	31.0	8.0
35	15.7	7.8	31.0	7.9	15.4	8.3	31.0	8.0
18	15.9	7.9	29.5	7.9	15.5	8.3	29.0	8.0
9	16.2	7.9	30.0	7.9	15.4	8.2	30.5	8.0
4	16.3	8.0	30.5	8.0	15.5	8.1	31.0	8.0
2	16.3	8.0	31.0	8.0	15.5	8.1	31.0	8.0
Control	16.1	8.0	31.5	8.0	15.5	8.1	31.5	8.0
Brine control	17.0	8.0	31.5	8.0	15.5	8.0	31.0	8.0

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.8	0.5	16
pH	8.1	0.2	16
Salinity (ppt):	30.8	0.7	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 16.1 Day 0: 16.4

Day 1: 15.4 Day 1: 15.5

Day 2: 15.2 Day 2: 15.8

LARVAL COUNT DATA

9-17-18 BJ → ← 9-19-18 JB →

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	221	7	206	2	231	5	215	5
35	242	3	229	3	252	4	241	2
18	223	6	249	4	241	5	237	2
9	241	4	210	5	238	3	225	3
4	242	2	221	7	222	3	235	2
2	231	5	230	2	233	4	235	4
Control	248	6	230	5	239	1	216	6
Brine control	234	3	235	2	243	3	224	6
Zero time	226	237	222	219	231	237	—	—

Zero time: Mean 229 SD 8 N 6 CV=(sd/mean)x100 3.3%

Remarks:

TAYLOR SHELLFISH Farms

130 SE Lynch Rd.
Shelton WA 98584

Order	ORD00405054
Date	9/10/2018
Page	1

Bill To:

NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY RD
NEWPORT OR 97365

Ship To:

NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY RD
NEWPORT OR 97365

Customer ID	Purchase Order No.	Salesperson ID	Shipping Method	Payment Terms	Req Ship Date	Master No.
11117		A931	FED EX	CREDIT CARD	9/10/2018	396,649

Ordered	Shipped	Item Number	Description	Unit Price	Ext. Price
10	10	10264	MS Totten Inlet Mussel Regular	[redacted]	[redacted]

RECEIVED 9-11-18

Subtotal	[redacted]
Misc	$0.00
Tax	$0.00
Freight	$0.00
Trade Discount	$0.00
Total	[redacted]

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	11-8164-0850	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Sep-18 9:37	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes
Batch ID:	09-9972-9530	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 Sep-18 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	14 Sep-18 15:50	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	13-5101-8713	**Code:**	5086E8D9	**Client:**	Wyckoff Treatment Plant
Sample Date:	11 Sep-18 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	12 Sep-18 14:30	**Source:**	Wyckoff		
Sample Age:	30h (4.9 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	1.66%	Passes proportion survived

Wilcoxon Rank Sum Two-Sample Test

Control	vs	Control	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		Brine Reagent	16	NA	1	6	1.0000	Exact	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.291	2.127	0.0077	Outlier Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.002545141	0.002545141	1	1	0.3559	Non-Significant Effect
Error	0.01527085	0.002545141	6			
Total	0.01781599		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	13.75	0.3559	Equal Variances
Variances	Levene Equality of Variance	9	13.75	0.0240	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7065	0.6451	0.0027	Non-normal Distribution
Distribution	Kolmogorov-Smirnov D	0.375	0.3313	0.0015	Non-normal Distribution
Distribution	Anderson-Darling A2 Normality	1.162	3.878	0.0049	Non-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	0.9924	0.968	1	1	0.9694	1	0.007642	1.54%	0.0%
0	Brine Reagent	4	1	1	1	1	1	1	0	0.0%	-0.77%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.502	1.389	1.616	1.538	1.395	1.538	0.03567	4.75%	0.0%
0	Brine Reagent	4	1.538	1.537	1.538	1.538	1.538	1.538	0	0.0%	-2.38%

Proportion Survived Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9694
0	Brine Reagent	1	1	1	1

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.538	1.538	1.538	1.395
0	Brine Reagent	1.538	1.538	1.538	1.538

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences	
Analysis ID:	17-3896-0561	Endpoint:	Proportion Survived	CETIS Version:	CETISv1.8.7
Analyzed:	20 Sep-18 9:37	Analysis:	Nonparametric-Control vs Treatments	Official Results:	Yes
Batch ID:	09-9972-9530	Test Type:	Development-Survival	Analyst:	
Start Date:	12 Sep-18 15:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	14 Sep-18 15:50	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	
Sample ID:	13-5101-8713	Code:	5086E8D9	Client:	Wyckoff Treatment Plant
Sample Date:	11 Sep-18 09:20	Material:	Industrial Effluent	Project:	
Receive Date:	12 Sep-18 14:30	Source:	Wyckoff		
Sample Age:	30h (4.9 °C)	Station:			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.81%	70	>70	NA	1.429

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Dilution Water		2	20	10	1	6	0.9616	Asymp	Non-Significant Effect
		4	17	10	1	6	0.7639	Asymp	Non-Significant Effect
		9	16	10	1	6	0.6451	Asymp	Non-Significant Effect
		18	20	10	1	6	0.9616	Asymp	Non-Significant Effect
		35	20	10	1	6	0.9616	Asymp	Non-Significant Effect
		70	13.5	10	1	6	0.3133	Asymp	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.534	2.876	0.2009	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0456452	0.007607534	6	1.561	0.2078	Non-Significant Effect
Error	0.1023722	0.004874864	21			
Total	0.1480174		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.412	3.812	0.0624	Equal Variances
Variances	Levene Equality of Variance	6.795	3.812	0.0004	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8578	0.8975	0.0014	Non-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	0.9924	0.968	1	1	0.9694	1	0.007642	1.54%	0.0%
2		4	1	1	1	1	1	1	0	0.0%	-0.77%
4		4	0.9945	0.9814	1	0.9978	0.9825	1	0.004133	0.83%	-0.22%
9		4	0.9836	0.936	1	0.9978	0.9389	1	0.01496	3.04%	0.88%
18		4	1	1	1	1	1	1	0	0.0%	-0.77%
35		4	1	1	1	1	1	1	0	0.0%	-0.77%
70		4	0.9662	0.8987	1	0.9782	0.9083	1	0.0212	4.39%	2.64%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	17-3896-0561	Endpoint: Proportion Survived	CETIS Version: CETISv1.8.7
Analyzed:	20 Sep-18 9:37	Analysis: Nonparametric-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	1.502	1.389	1.616	1.538	1.395	1.538	0.03567	4.75%	0.0%
2		4	1.538	1.537	1.538	1.538	1.538	1.538	0	0.0%	-2.38%
4		4	1.505	1.43	1.579	1.521	1.438	1.538	0.02345	3.12%	-0.17%
9		4	1.475	1.31	1.641	1.521	1.321	1.538	0.05203	7.05%	1.78%
18		4	1.538	1.537	1.538	1.538	1.538	1.538	0	0.0%	-2.38%
35		4	1.538	1.537	1.538	1.538	1.538	1.538	0	0.0%	-2.38%
70		4	1.419	1.218	1.621	1.438	1.263	1.538	0.06325	8.91%	5.52%

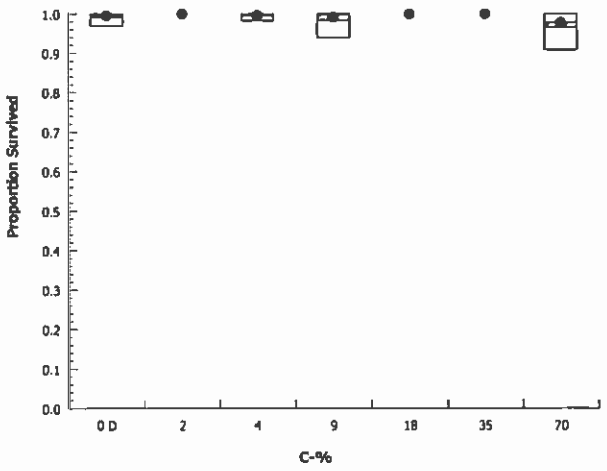
Proportion Survived Detail

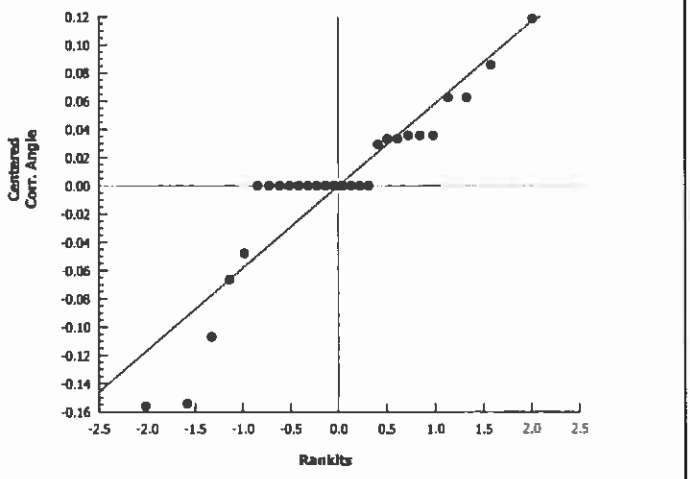
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1	1	1	0.9694
2		1	1	1	1
4		1	0.9956	0.9825	1
9		1	0.9389	1	0.9956
18		1	1	1	1
35		1	1	1	1
70		0.9956	0.9083	1	0.9607

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.538	1.538	1.538	1.395
2		1.538	1.538	1.538	1.538
4		1.538	1.505	1.438	1.538
9		1.538	1.321	1.538	1.505
18		1.538	1.538	1.538	1.538
35		1.538	1.538	1.538	1.538
70		1.505	1.263	1.538	1.371

Graphics





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

-GSI

CETIS Analytical Report

Test Code: 658-88 03-9579-0496

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	12-4759-4159	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Sep-18 9:37	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Batch ID:	09-9972-9530	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 Sep-18 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	14 Sep-18 15:50	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	

Sample ID:	13-5101-8713	**Code:**	5086E8D9	**Client:**	Wyckoff Treatment Plant
Sample Date:	11 Sep-18 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	12 Sep-18 14:30	**Source:**	Wyckoff		
Sample Age:	30h (4.9 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	3.28%	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	0.9393	2.447	0.093	6	0.3838	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	1.799	2.127	0.3289	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.002526167	0.002526167	1	0.8822	0.3838	Non-Significant Effect
Error	0.01718015	0.002863359	6			
Total	0.01970632		7			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	9.355	47.47	0.0989	Equal Variances
Variances	Mod Levene Equality of Variance	1.486	13.75	0.2686	Equal Variances
Variances	Levene Equality of Variance	1.387	13.75	0.2836	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9214	0.6451	0.4416	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.2271	0.3313	0.2913	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.5114	3.878	0.1995	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	0.9735	0.9386	1	0.983	0.9432	0.9958	0.01099	2.26%	0.0%
0	Brine Reagent	4	0.9862	0.9772	0.9953	0.983	0.9782	0.9916	0.002845	0.58%	-1.3%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.419	1.305	1.534	1.441	1.33	1.506	0.03596	5.07%	0.0%
0	Brine Reagent	4	1.455	1.417	1.492	1.441	1.422	1.479	0.01176	1.62%	-2.5%

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9764	0.9787	0.9958	0.9432
0	Brine Reagent	0.9873	0.9916	0.9878	0.9782

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.416	1.424	1.506	1.33
0	Brine Reagent	1.458	1.479	1.46	1.422

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID:	14-9228-5364	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Sep-18 9:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	09-9972-9530	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 Sep-18 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	14 Sep-18 15:50	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	
Sample ID:	13-5101-8713	**Code:**	5086E8D9	**Client:**	Wyckoff Treatment Plant
Sample Date:	11 Sep-18 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	12 Sep-18 14:30	**Source:**	Wyckoff		
Sample Age:	30h (4.9 °C)	**Station:**			

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.71%	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	-0.6445	2.448	0.101	6	0.9663	CDF	Non-Significant Effect
		4	-0.3588	2.448	0.101	6	0.9325	CDF	Non-Significant Effect
		9	0.3548	2.448	0.101	6	0.7399	CDF	Non-Significant Effect
		18	-0.4982	2.448	0.101	6	0.9514	CDF	Non-Significant Effect
		35	-1.483	2.448	0.101	6	0.9971	CDF	Non-Significant Effect
		70	1.814	2.448	0.101	6	0.1557	CDF	Non-Significant Effect

Auxiliary Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.439	2.876	0.2826	No Outliers Detected

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04333178	0.007221963	6	2.104	0.0960	Non-Significant Effect
Error	0.07208219	0.003432485	21			
Total	0.115414		27			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.698	16.81	0.3496	Equal Variances
Variances	Mod Levene Equality of Variance	0.6817	3.812	0.6662	Equal Variances
Variances	Levene Equality of Variance	1.471	3.812	0.2361	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9621	0.8975	0.3907	Normal Distribution
Distribution	Kolmogorov-Smirnov D	0.1236	0.1914	0.3254	Normal Distribution
Distribution	D'Agostino Skewness	1.31	2.576	0.1903	Normal Distribution
Distribution	D'Agostino Kurtosis	0.4648	2.576	0.6420	Normal Distribution
Distribution	D'Agostino-Pearson K2 Omnibus	1.932	9.21	0.3807	Normal Distribution
Distribution	Anderson-Darling A2 Normality	0.4241	3.878	0.3227	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	0.9735	0.9386	1	0.9776	0.9432	0.9958	0.01099	2.26%	0.0%
2		4	0.9841	0.9758	0.9925	0.9832	0.9788	0.9914	0.002623	0.53%	-1.09%
4		4	0.9795	0.9568	1	0.9805	0.9651	0.9918	0.007109	1.45%	-0.61%
9		4	0.9677	0.9138	1	0.9831	0.917	0.9876	0.01692	3.5%	0.6%
18		4	0.9823	0.9704	0.9943	0.9819	0.9738	0.9916	0.003762	0.77%	-0.9%
35		4	0.991	0.9803	1	0.9898	0.9844	1	0.003367	0.68%	-1.79%
70		4	0.9456	0.8901	1	0.952	0.8996	0.9788	0.01743	3.69%	2.87%

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Analysis ID: 14-9228-5364 Endpoint: Combined Proportion Normal CETIS Version: CETISv1.8.7
Analyzed: 20 Sep-18 9:38 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	1.419	1.305	1.534	1.42	1.33	1.506	0.03596	5.07%	0.0%
2		4	1.446	1.41	1.482	1.441	1.425	1.478	0.01125	1.56%	-1.88%
4		4	1.434	1.351	1.518	1.437	1.383	1.48	0.02626	3.66%	-1.05%
9		4	1.405	1.27	1.539	1.44	1.279	1.459	0.04224	6.02%	1.04%
18		4	1.44	1.392	1.488	1.436	1.408	1.479	0.01505	2.09%	-1.45%
35		4	1.481	1.416	1.545	1.47	1.445	1.538	0.02027	2.74%	-4.33%
70		4	1.344	1.222	1.466	1.352	1.248	1.425	0.03841	5.72%	5.29%

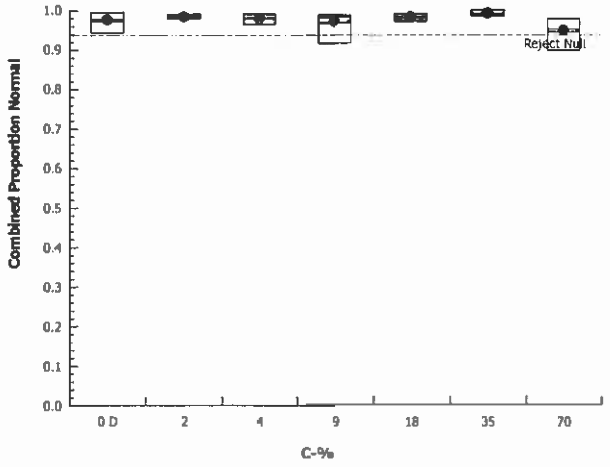
Combined Proportion Normal Detail

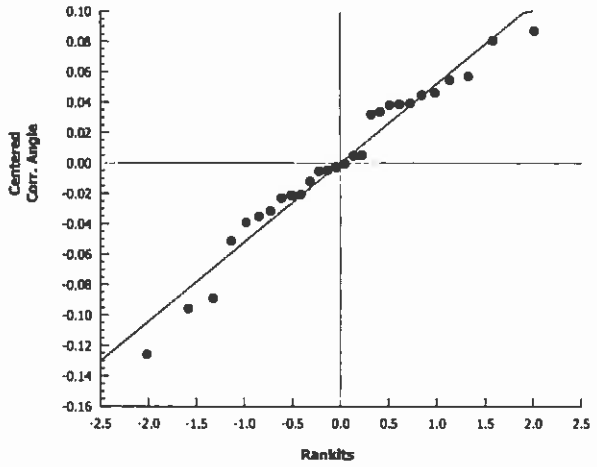
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9764	0.9787	0.9958	0.9432
2		0.9788	0.9914	0.9831	0.9833
4		0.9918	0.9651	0.9694	0.9916
9		0.9837	0.917	0.9876	0.9825
18		0.9738	0.9842	0.9797	0.9916
35		0.9878	1	0.9844	0.9918
70		0.9651	0.8996	0.9788	0.9389

Angular (Corrected) Transformed Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.416	1.424	1.506	1.33
2		1.425	1.478	1.441	1.441
4		1.48	1.383	1.395	1.479
9		1.443	1.279	1.459	1.438
18		1.408	1.445	1.428	1.479
35		1.46	1.538	1.445	1.48
70		1.383	1.248	1.425	1.321

Graphics





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

CETIS Analytical Report

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

Analysis ID:	02-0236-2241	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Sep-18 9:38	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Batch ID:	09-9972-9530	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 Sep-18 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Yaquina Bay Seawater
Ending Date:	14 Sep-18 15:50	**Species:**	Mytilis galloprovincialis	**Brine:**	
Duration:	48h	**Source:**	Kamilche Sea Farms, WA	**Age:**	

Sample ID:	13-5101-8713	**Code:**	5086E8D9	**Client:**	Wyckoff Treatment Plant
Sample Date:	11 Sep-18 09:20	**Material:**	Industrial Effluent	**Project:**	
Receive Date:	12 Sep-18 14:30	**Source:**	Wyckoff		
Sample Age:	30h (4.9 °C)	**Station:**			

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	315039	280	Yes	Two-Point Interpolation

Residual Analysis

Attribute	Method	Test Stat	Critical	P-Value	Decision(α:5%)
Extreme Value	Grubbs Extreme Value	2.439	2.876	0.2826	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	0.9735	0.9432	0.9958	0.01099	0.02199	2.26%	0.0%	933	958
2		4	0.9841	0.9788	0.9914	0.002623	0.005247	0.53%	-1.09%	929	944
4		4	0.9795	0.9651	0.9918	0.007109	0.01422	1.45%	-0.61%	920	939
9		4	0.9677	0.917	0.9876	0.01692	0.03385	3.5%	0.6%	914	944
18		4	0.9823	0.9738	0.9916	0.003762	0.007524	0.77%	-0.9%	950	967
35		4	0.991	0.9844	1	0.003367	0.006733	0.68%	-1.79%	964	973
70		4	0.9456	0.8996	0.9788	0.01743	0.03487	3.69%	2.87%	873	923

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9764	0.9787	0.9958	0.9432
2		0.9788	0.9914	0.9831	0.9833
4		0.9918	0.9651	0.9694	0.9916
9		0.9837	0.917	0.9876	0.9825
18		0.9738	0.9842	0.9797	0.9916
35		0.9878	1	0.9844	0.9918
70		0.9651	0.8996	0.9788	0.9389

CETIS Test Data Worksheet

Report Date: 20 Sep-18 09:36 (p 1 of 1)
Test Code: 03-9579-0496/658-88

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences
Start Date:	12 Sep-18 15:50	Species:	Mytilis galloprovincialis	Sample Code: 5086E8D9
End Date:	14 Sep-18 15:50	Protocol:	EPA/600/R-95/136 (1995)	Sample Source: Wyckoff
Sample Date:	11 Sep-18 09:20	Material:	Industrial Effluent	Sample Station:

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	229	237	237	234	
0	B	2	11	229	237	237	235	
0	B	3	12	229	246	246	243	
0	B	4	3	229	230	230	224	
0	D	1	10	229	254	254	248	
0	D	2	2	229	235	235	230	
0	D	3	23	229	240	240	239	
0	D	4	9	229	222	222	216	
2		1	18	229	236	236	231	
2		2	27	229	232	232	230	
2		3	22	229	237	237	233	
2		4	30	229	239	239	235	
4		1	28	229	244	244	242	
4		2	15	229	228	228	221	
4		3	14	229	225	225	222	
4		4	24	229	237	237	235	
9		1	6	229	245	245	241	
9		2	29	229	215	215	210	
9		3	5	229	241	241	238	
9		4	17	229	228	228	225	
18		1	16	229	229	229	223	
18		2	13	229	253	253	249	
18		3	1	229	246	246	241	
18		4	31	229	239	239	237	
35		1	7	229	245	245	242	
35		2	26	229	232	232	229	
35		3	21	229	256	256	252	
35		4	8	229	243	243	241	
70		1	4	229	228	228	221	
70		2	20	229	208	208	206	
70		3	19	229	236	236	231	
70		4	25	229	220	220	215	

data entry verified against laboratory bench sheets 9-20-18 JRF

Northwestern Aquatic Sciences (REGION COPY)
DateShipped: 9/11/2018
CarrierName: FedEx
AirbillNo: 7827 2950 7782

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2018/WA
Project Code: WEH-025T
Cooler # 1 of 1

No: 10-091118-095627-0301
2018T10P303DD210W2LA00
Contact Name: Keith Allers
Contact Phone: 206-780-1711

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
658 3rd Quarter 2018		Ground Water/ K Allers	Composite	ACTOX-CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	09/11/2018 09:20	Field Sample

PAGE 13 OF 14

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

Special Instructions

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

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	Keith Allers CH2M	9-11-2018 1000	Craig Bullock NWS NWS # 62396	9-12-18 1430	Intact

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

BAINBRIDGE ISLAND WA 98110
UNITED STATES US

SHIP DATE: 11SEP18
ACTWGT: 46.00 LB
CAD: 111531780/WSXI3100
DIMS: 22x15x15 IN

BILL SENDER

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

NEWPORT OR 97365
(541) 265-7225

REF: PN 707869 FP Y1 OP 01

INV:
PO:
DEPT:

552117-78C/DCA5

FedEx Express

WED - 12 SEP 12:00P
PRIORITY OVERNIGHT

TRK# 0201 7827 2950 7782

86 ONPA

97365
OR-US PDX

APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

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

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

REVIEWED PALES 1-8 -631

Test No. 999-3809 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 631

QA Officer L.K. Nemeth

1. J. Brown JB 2.

3. 4.

Study Schedule:

Test Beginning: 9-12-18 1550 Test Ending: 9-17-18 1550

TEST MATERIAL

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

NAS Sample No. 1.0 mg/ml stock prepared: 3-27-18

Date of Collection:

Date of Receipt:

Temperature (deg C):

pH:

Dissolved oxygen (mg/L):

Conductivity (umhos/cm):

Hardness (mg/L):

Alkalinity (mg/L):

Salinity (ppt):

Total chlorine (mg/L):

Total ammonia-N (mg/L):

:

DILUTION WATER

Description: Yaquina Bay, OR Seawater

Date of Collection: 9-11-18 Salinity (ppt) 31.0 pH 8.0

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

TEST ORGANISMS

Species: *Mytilus galloprovincialis* Date Received: 9-11-18

Source: ~~Kamilche Sea Farms, Shelton, WA~~ TAYLOR SHELLFISH FARMS, SHELTON, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
9-12-18	15.9	7.9	34.5	6.6	Held outside in trays of flowing seawater
Mean					
S.D.					
(N)					

Photoperiod during acclimation: Outdoor ambient conditions

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

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

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

Test No. 999-3809 Client QC Test Investigator

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 1130 Final: 1245 Fertilization: 1420

Number of organisms used: females: 2 males: 2

Egg Dilution (1 ml diluted to 100 ml):

Count/ml of dilution: 1. 34 2. 31 3. 30 Mean: 31.7

Dilution factor = DF (mean x 100/2500) = 1.3

TEST PROCEDURES AND CONDITIONS

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

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

Organisms/ml (15-30): 22.9 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	32	8	Ø	16	64	1	2		
B	32	Ø	64	4	1	2	8	16		
C	8	16	2	32	64	1	Ø	4		
D	1	64	4	16	32	8	2	Ø		

PREPARATION OF TEST SOLUTIONS

9-12-18
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:

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

Test No. 999-3809 Client QC Test Investigator

WATER QUALITY DATA

	Date: 9-12-18 initials:				Date: 9-14-18 initials:			
Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	16.0	8.1	31.0	8.0	15.6	8.1	31.0	8.1
32	16.1	8.1	31.0	8.1	15.6	8.1	30.5	8.0
16	16.2	8.1	31.0	8.1	15.6	8.1	31.0	8.1
8	16.1	8.1	31.0	8.1	15.6	8.1	31.0	8.1
4	16.3	8.1	31.0	8.1	15.6	8.1	31.0	8.0
2	16.3	8.1	31.0	8.1	15.7	8.0	31.0	8.0
1	16.2	8.1	31.0	8.0	15.7	8.0	31.0	8.0
Control	16.4	8.0	31.0	8.0	15.8	8.0	31.0	8.0
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.9	0.3	16
pH:	8.1	0.0	16
Salinity (ppt):	31.1	0.3	16
DO (mg/L):	8.1	0.1	16

Room/ Water bath temperature: (°C)

Day 0: 16.1 Day 0: 16.4
Day 1: 15.4 Day 1: 15.5
Day 2: 15.2 Day 2: 15.8

LARVAL COUNT DATA

9-16-18 KS → ← 9-17-18 KS →

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	ϕ	1	ϕ	ϕ	ϕ	1	ϕ	1
32	ϕ	86	ϕ	74	ϕ	118	ϕ	83
16	ϕ	224	ϕ	242	ϕ	247	ϕ	201
8	203	12	200	11	195	11	215	14
4	234	2	243	5	204	3	216	3
2	236	7	219	5	214	4	226	7
1	218	3	233	8	211	4	224	3
Control	206	4	221	6	241	6	230	4
Brine control	—	---	---	---	---	---	---	---
Zero time	226	237	222	219	231	237	—	—

Zero time: Mean 229 SD 8 N 6 CV=(sd/mean)x100 3.3%

Remarks:

TAYLOR SHELLFISH Farms

130 SE Lynch Rd.
Shelton WA 98584

Order	ORD00405054
Date	9/10/2018
Page	1

Bill To:

NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY RD
NEWPORT OR 97365

Ship To:

NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY RD
NEWPORT OR 97365

Customer ID	Purchase Order No.	Salesperson ID	Shipping Method	Payment Terms	Req Ship Date	Master No.
11117		A931	FED EX	CREDIT CARD	9/10/2018	396,649

Ordered	Shipped	Item Number	Description	Unit Price	Ext. Price
10	10	10264	MS Totten Inlet Mussel Regular	[redacted]	[redacted]

RECEIVED 9-11-18

Subtotal	[redacted]
Misc	$0.00
Tax	$0.00
Freight	$0.00
Trade Discount	$0.00
Total	[redacted]

CETIS Summary Report

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

Batch ID:	09-9972-9530	Test Type:	Development-Survival	Analyst:	
Start Date:	12 Sep-18 15:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Yaquina Bay Seawater
Ending Date:	14 Sep-18 15:50	Species:	Mytilis galloprovincialis	Brine:	
Duration:	48h	Source:	Kamilche Sea Farms, WA	Age:	

Sample ID:	06-3658-0230	Code:	25F17186	Client:	Internal Lab
Sample Date:	12 Sep-18 15:50	Material:	Copper sulfate	Project:	
Receive Date:	12 Sep-18 15:50	Source:	Reference Toxicant		
Sample Age:	NA	Station:			

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-1843-8007	Combined Proportion Norm	4	8	5.657	6.28%		Dunnett Multiple Comparison Test
11-1742-4239	Proportion Normal	4	8	5.657	1.17%		Bonferroni Adj t Test
18-9820-6985	Proportion Survived	16	32	22.63	7.13%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
06-7348-0523	Combined Proportion Norm	EC25	9.525	9.07	9.892		Linear Interpolation (ICPIN)
11-7187-6407	Combined Proportion Norm	EC50	10.71	10.58	10.84		Spearman-Kärber
19-4709-1684	Proportion Normal	EC25	9.774	9.715	9.819		Linear Interpolation (ICPIN)
20-8554-4545	Proportion Normal	EC50	11.03	10.94	11.13		Spearman-Kärber
07-6968-3996	Proportion Survived	EC50	28.79	28.07	29.53		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	0.9558	0.895	1	0.8996	0.9829	0.0191	0.03821	4.0%	0.0%
1		4	0.9546	0.9155	0.9937	0.9214	0.9782	0.01229	0.02459	2.58%	0.13%
2		4	0.9622	0.9267	0.9977	0.9345	0.9869	0.01115	0.02231	2.32%	-0.67%
4		4	0.9514	0.8793	1	0.8908	0.9915	0.02265	0.04529	4.76%	0.47%
8		4	0.8876	0.8285	0.9466	0.8515	0.9389	0.01856	0.03712	4.18%	7.14%
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	0.9771	0.913	1	0.917	1	0.02012	0.04024	4.12%	0.0%
1		4	0.9738	0.9299	1	0.9389	1	0.01381	0.02762	2.84%	0.34%
2		4	0.9825	0.9462	1	0.952	1	0.01142	0.02283	2.32%	-0.56%
4		4	0.9651	0.8924	1	0.9039	1	0.02283	0.04566	4.73%	1.23%
8		4	0.94	0.8713	1	0.8996	1	0.02157	0.04314	4.59%	3.8%
16		4	0.964	0.871	1	0.8777	1	0.02921	0.05841	6.06%	1.34%
32		4	0.3941	0.2608	0.5274	0.3231	0.5153	0.0419	0.0838	21.26%	59.66%
64		4	0.003275	0	0.006749	0	0.004367	0.001092	0.002183	66.67%	99.66%

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	0.8996	0.9651	0.9757	0.9829
1		0.952	0.9668	0.9214	0.9782
2		0.9712	0.9563	0.9345	0.9869
4		0.9915	0.9798	0.8908	0.9432
8		0.8865	0.8734	0.8515	0.9389
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	0.917	0.9913	1	1
1		0.9651	1	0.9389	0.9913
2		1	0.9782	0.952	1
4		1	1	0.9039	0.9563
8		0.9389	0.9214	0.8996	1
16		0.9782	1	1	0.8777
32		0.3755	0.3231	0.5153	0.3624
64		0.004367	0	0.004367	0.004367

Combined Proportion Normal Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	206/229	221/229	241/247	230/234
1		218/229	233/241	211/229	224/229
2		236/243	219/229	214/229	226/229
4		234/236	243/248	204/229	216/229
8		203/229	200/229	195/229	215/229
16		0/229	0/229	0/229	0/229
32		0/229	0/229	0/229	0/229
64		0/229	0/229	0/229	0/229

Proportion Survived Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	210/229	227/229	229/229	229/229
1		221/229	229/229	215/229	227/229
2		229/229	224/229	218/229	229/229
4		229/229	229/229	207/229	219/229
8		215/229	211/229	206/229	229/229
16		224/229	229/229	229/229	201/229
32		86/229	74/229	118/229	83/229
64		1/229	0/229	1/229	1/229

CETIS Test Data Worksheet

Report Date: 18 Sep-18 10:44 (p 1 of 1)
Test Code: 03-0658-4322/999-3809

Bivalve Larval Survival and Development Test — Northwestern Aquatic Sciences

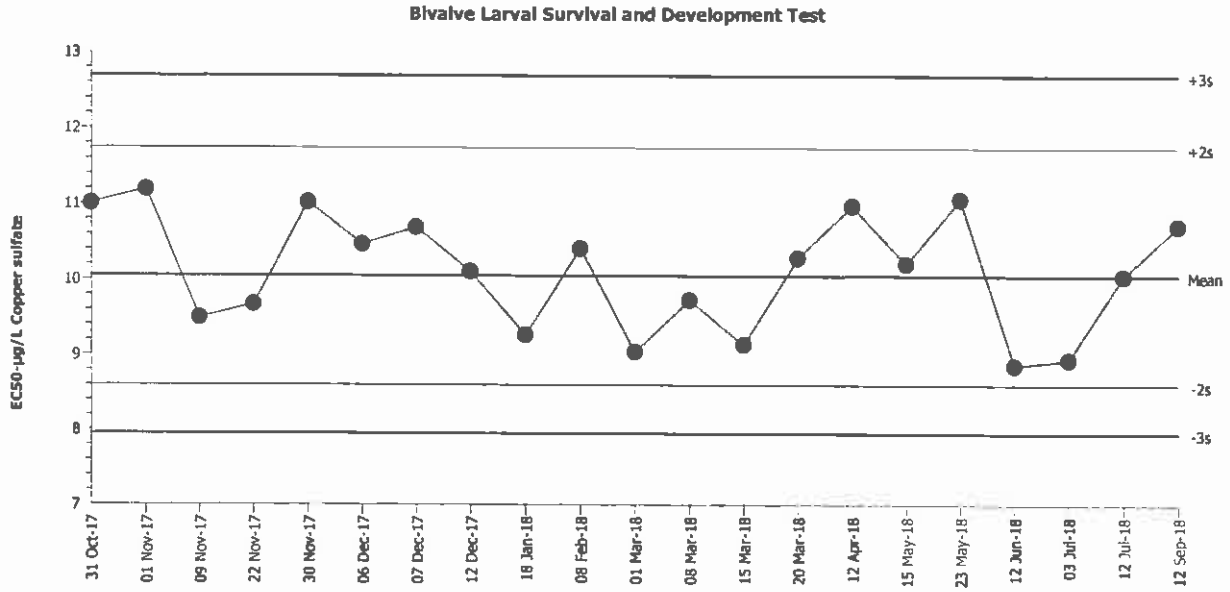
Start Date:	12 Sep-18 15:50	**Species:**	Mytilis galloprovincialis
End Date:	14 Sep-18 15:50	**Protocol:**	EPA/600/R-95/136 (1995)
Sample Date:	12 Sep-18 15:50	**Material:**	Copper sulfate
Sample Code:	25F17186		
Sample Source:	Reference Toxicant		
Sample Station:			

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	5	229	210	210	206	
0	D	2	31	229	227	227	221	
0	D	3	25	229	247	247	241	
0	D	4	28	229	234	234	230	
1		1	8	229	221	221	218	
1		2	21	229	241	241	233	
1		3	15	229	215	215	211	
1		4	10	229	227	227	224	
2		1	24	229	243	243	236	
2		2	27	229	224	224	219	
2		3	17	229	218	218	214	
2		4	26	229	233	233	226	
4		1	22	229	236	236	234	
4		2	4	229	248	248	243	
4		3	6	229	207	207	204	
4		4	9	229	219	219	216	
8		1	29	229	215	215	203	
8		2	7	229	211	211	200	
8		3	13	229	206	206	195	
8		4	2	229	229	229	215	
16		1	32	229	224	224	0	
16		2	20	229	242	242	0	
16		3	23	229	247	247	0	
16		4	11	229	201	201	0	
32		1	3	229	86	86	0	
32		2	18	229	74	74	0	
32		3	16	229	118	118	0	
32		4	12	229	83	83	0	
64		1	30	229	1	1	0	
64		2	19	229	0	0	0	
64		3	1	229	1	1	0	
64		4	14	229	1	1	0	

data entry verified against laboratory bench sheets 9-20-18 JAF

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:	10.04	**Count:**	20	**-2s Warning Limit:**	8.596	**-3s Action Limit:**	7.951
Sigma:	NA	**CV:**	8.10%	**+2s Warning Limit:**	11.74	**+3s Action Limit:**	12.69

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Oct	31	13:55	11	0.9595	1.172			19-0668-1049	19-2975-1853
2		Nov	1	13:35	11.19	1.142	1.383			17-3365-4072	06-0748-0919
3			9	14:05	9.488	-0.556	-0.7312			02-6639-7760	19-3779-2145
4			22	14:00	9.666	-0.3785	-0.4932			14-5021-8992	06-6921-5494
5			30	13:40	11.02	0.9721	1.186			00-8984-2368	13-5466-3879
6		Dec	6	13:45	10.46	0.4176	0.523			20-9204-7376	08-8218-5639
7			7	13:00	10.69	0.6413	0.7947			17-2684-2601	21-0626-4507
8			12	14:20	10.1	0.05765	0.0735			13-4785-2533	00-4154-6333
9	2018	Jan	18	17:30	9.259	-0.785	-1.045			18-1463-9193	16-7842-9995
10		Feb	8	12:30	10.41	0.363	0.4559			12-4129-6067	04-6683-9588
11		Mar	1	13:50	9.037	-1.008	-1.357			17-0778-1414	17-6952-8158
12			8	13:15	9.724	-0.3205	-0.4164			06-2342-5193	14-1701-7153
13			15	13:40	9.132	-0.9126	-1.223			03-6132-1874	05-3263-8438
14			20	14:20	10.28	0.2405	0.3039			04-1206-8235	10-0132-8767
15		Apr	12	13:45	10.97	0.9285	1.135			04-5251-3935	19-5006-5316
16		May	15	13:55	10.2	0.1583	0.2008			00-8499-1435	03-3023-6490
17			23	14:20	11.06	1.015	1.236			01-8034-7341	14-7874-1735
18		Jun	12	13:50	8.858	-1.186	-1.613			20-7731-4517	07-1052-9994
19		Jul	3	13:00	8.935	-1.109	-1.502			15-9311-8936	16-1627-9282
20			12	14:40	10.04	-0.00096	-0.00122			19-6340-6472	19-1532-3200
21		Sep	12	15:50	10.71	0.6652	0.8234			03-0658-4322	11-7187-6407

Analyst: QA: