

TOXICITY TEST REPORT

TEST IDENTIFICATION

Test No.: 658-85

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: 3-1-18, 1350 hrs.

Test Ending: 3-3-18, 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 Ground Water Sample. Details are as follows:

NAS Sample No.	6135G
Collection Date	2-28-18
Receipt Date	3-1-18
Temperature (°C)	2.0
pH	7.5
Dissolved oxygen (mg/L)	10.9
Salinity (‰)	4.5

Treatments: Samples briefly temperature-equilibrated prior to use.

Storage: Used date of receipt.

DILUTION WATER

Source: Yaquina Bay, Oregon seawater.

Date of Collection: 2-28-18

Water Quality: Salinity, 30.0 ‰; pH, 8.0

Pretreatment: Filtered to $\leq 0.45 \mu\text{m}$, 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: 2-9-18

Method of Preparation: Freezing method

TEST ORGANISMS

Species: Mussel (*Mytilus galloprovincialis*).

Age: 2.0 hrs post-fertilization.

Source: Taylor Shellfish Farms, Shelton, WA.

Conditioning: Adult mussels were received on 2-27-18 and placed in trays with flowing seawater. Holding conditions the day of testing were: temperature, 8.1°C; pH, 8.1; salinity, 27.0‰; and dissolved oxygen, 9.1 mg/L. Photoperiod was natural daylight.

Source of Gametes: 2 females and 3 males.

TEST PROCEDURES AND CONDITIONS

Test Chambers: 30 ml borosilicate glass vials containing 10 ml of test solutions.

Test Concentrations: 70, 35, 18, 9, 4, 2, and 0% (Control).

Brine Control: A brine control was run in which salinity-adjusted Milli-Q® deionized water (4.5 ppt) was substituted for effluent in the preparation of the highest test solution concentration. As a result, the amount of brine in the brine control was the same as used in the 70.0% effluent test concentration.

Replicates/Treatment: 4

Initial Concentration of Test Organisms: 24.7/ml.

Volume of Subsamples Taken for Counting: NA

Water Volume Changes per 24 hr: None (non-renewal static test).

Aeration: None

Feeding: None

Effects Criteria: The effect criteria used were: 1) ability of embryos to survive and produce completely developed shells; and 2) survival. Data collected were: 1) the initial embryo density; 2) the number of abnormal larvae observed; and 3) the number of normal (live with completely developed shells) larvae observed.

Water Quality and Other Test Conditions: Temperature, 15.3 ± 0.2°C; pH, 8.1 ± 0.2; salinity, 29.7 ± 0.6‰; 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-Kärber 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.: 999-3760

Reference Toxicant and Source: Copper as CuSO₄·5H₂O, Argent Lot No. 0195, 1.0 mg/ml stock prepared 5-16-16.

Test Date: 3-1-18

Dilution Water Used: Yaquina Bay, OR seawater. Salinity 30.0 ppt, pH 8.0

Results: EC50, 9.04 µg/L; NOEC, 4 µg/L; IC25, 7.09 µg/L. The EC50 result was within the laboratory's control chart warning limits (8.65 – 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 (%) (95% C.I.)	>70 (TU _c <1.43) ---	>70 (TU _c <1.43) ---
Method of Calculation	By Data Inspection	By Data Inspection
IC25 (%) (95% C.I.)	>70 (TU _c <1.43) ---	
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

Gay Butcher 3-7-18
Project Manager Date

Muhammad Alwisari 3-7-18
Study Director Date

Richard A. Caldwell 3/7/18
Laboratory Director Date

Julie R. Xione 3-7-18
Quality Assurance Unit Date

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

Test Material Concentration (%)	Repl.	Norm.	Abn.	Total	Combined Proportion Normal*		Proportion Survived*	
					Mean		Mean	
70	1	212	14	226	0.858		0.915	
	2	218	16	234	0.883		0.947	
	3	217	4	221	0.879		0.895	
	4	228	8	236	0.923	0.886	0.956	0.928
35	1	197	9	206	0.798		0.834	
	2	215	7	222	0.870		0.899	
	3	210	7	217	0.850		0.879	
	4	198	9	207	0.802	0.830	0.838	0.862
18	1	198	6	204	0.802		0.826	
	2	216	10	226	0.875		0.915	
	3	225	9	234	0.911		0.947	
	4	213	12	225	0.862	0.862	0.911	0.900
9	1	197	12	209	0.798		0.846	
	2	225	9	234	0.911		0.947	
	3	192	6	198	0.777		0.802	
	4	217	9	226	0.879	0.841	0.915	0.878
4	1	191	11	202	0.773		0.818	
	2	187	12	199	0.757		0.806	
	3	189	11	200	0.765		0.810	
	4	201	9	210	0.814	0.777	0.850	0.821
2	1	199	14	213	0.806		0.862	
	2	206	11	217	0.834		0.879	
	3	226	6	232	0.915		0.939	
	4	201	11	212	0.814	0.842	0.858	0.885
Normal Control	1	220	13	233	0.891		0.943	
	2	190	10	200	0.769		0.810	
	3	231	14	245	0.935		0.992	
	4	221	7	228	0.895	0.873	0.923	0.917
Brine Control ¹	1	207	9	216	0.838		0.875	
	2	210	8	218	0.850		0.883	
	3	221	11	232	0.895		0.939	
	4	200	13	213	0.810	0.848	0.862	0.890

* Based on an average initial count of 247 embryos per 10 ml sample, except that for the case in the combined proportion normal endpoint where number normal > average initial count, number normal is divided by the total count (as per EPA/600/R-95/136).

† Result significantly different ($P \leq 0.05$) from the control.

¹ Salinity-adjusted Milli Q[®] deionized water (5.5 ppt) was substituted for effluent so that the brine concentration is equivalent to that for the 70.0% effluent concentration.

APPENDIX I
PROTOCOL

TEST PROTOCOL

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

1. **INTRODUCTION**

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

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

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

2. **STUDY MANAGEMENT**

2.1 **Sponsor's Name and Address:**

2.2 **Sponsor's Study Monitor:**

2.3 **Name of Testing Laboratory:**

Northwestern Aquatic Sciences
3814 Yaquina Bay Road
P.O. Box 1437
Newport, OR 97365

2.4 **Test Location:**

2.5 **Laboratory's Personnel to be Assigned to the Study:**

Study Director: _____
Quality Assurance Unit: _____
Aquatic Toxicologist: _____
Aquatic Toxicologist: _____

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

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

3. TEST MATERIAL

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

4. DILUTION WATER

Dilution water is filtered ($\leq 0.45 \mu\text{m}$) Yaquina Bay seawater or other suitable seawater, adjusted to $30 \pm 2 \%$ salinity with deionized water and/or hypersaline brine. Hypersaline brine is prepared from filtered ($\leq 0.45 \mu\text{m}$) 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 pseudofeces 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 ($16 \pm 1^\circ\text{C}$ 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 $20 \pm 1^\circ\text{C}$ ($16 \pm 1^\circ\text{C}$ 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 $20 \pm 1^\circ\text{C}$ for oysters, $16 \pm 1^\circ\text{C}$ for blue mussels. The test temperatures specified by EPA (EPA/600/R-95/136) are $15 \pm 1^\circ\text{C}$ or $18 \pm 1^\circ\text{C}$, but these specifications were based on erroneous assumptions of the agency authors. Consequently, this protocol specifies $16 \pm 1^\circ\text{C}$. 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. $\geq 70\%$ of embryos introduced into a required control treatment result in live larvae ($\geq 50\%$ for mussels).
2. normal shell development in surviving controls is $\geq 90\%$.

For the WDOE bivalve toxicity method (Publication No. WQ-R-95-80) the test is considered acceptable if:

1. $\geq 70\%$ of embryos introduced into a required control treatment result in live larvae.
2. normal shell development in surviving controls is $\geq 90\%$.
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 $\leq 15\%$.

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. $\geq 70\%$ 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 $\leq 1^\circ\text{C}$.

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-Kärber 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

REVIEWED
 PAGES 1-14
 -651

Test No. 658-85 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. Irissari ⁶⁵¹

QA Officer L.K. Nemeth

1. Alex Katsikaris

2. J. Brown

3.

4.

Study Schedule:

Test Beginning: 3-1-18 1350

Test Ending: 3-3-18 1400

TEST MATERIAL

Description: GROUND WATER COMPOSITE SP-11

NAS Sample No. 61356

Date of Collection: 2-28-18

Date of Receipt: 3-1-18

Temperature (deg C): 2.0

pH: 7.5

Dissolved oxygen (mg/L): 10.9

Conductivity (umhos/cm): -

Hardness (mg/L): -

Alkalinity (mg/L): -

Salinity (ppt): 4.5

Total chlorine (mg/L): -

Total ammonia-N (mg/L): -

DILUTION WATER

Description: Yaquina Bay, OR

Date of Collection: 2-28-18 Salinity (ppt) 30.0 pH 8.0

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

TEST ORGANISMS

Species: Mytilus galloprovincialis Date Received: 2-27-18

Source: Taylor Shellfish Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
3-1-18	8.1	8.1	27.0	9.1	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

Test No. 658-85 Client CH2M Hill - Wyckoff Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 1110 Final: 1125 Fertilization: 1150
 Number of organisms used: females: 2 males: 3
 Egg Dilution (1 ml diluted to 100 ml):
 Count/ml of dilution: 1. 38 2. 39 3. 44 Mean: 40.3
 Dilution factor = DF (mean x 100/2500) = 1.6

TEST PROCEDURES AND CONDITIONS

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

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4
 Organisms/ml (15-30): 24.7 Test water changes: None Aeration during test: None
 Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt
 Temperature: 20 +/- 1 °C, oysters; 16 +/- 1 °C, mussels Beaker placement: Stratified randomization

RANDOMIZATION CHART

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

PREPARATION OF TEST SOLUTIONS

This test uses a brine control ; a salinity control _____
 If a brine control is used, follow SOP #6208 to prepare test solutions
 Date of brine preparation: 2-9-18; brine salinity (ppt) 100.0
 Source of seawater: Yaquina Bay, Oregon

$$VB = VE \frac{(TS - SE)}{(SB - TS)} = VE \frac{(30 - 4.5)}{(100 - 30)} = VE (0.364)$$

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.

3-1-18
 GJ

Test Conc. (%)	Effluent (ml/100ml)	Brine (ml/100ml)	Dilution Water (ml/100ml)
70	70	25.5	Brought up to a final
35	35	12.7	volume of 100 ml
18	18	6.6	with dilution water
9	9	3.3	
4	4	1.5	
2	2	0.7	
0	0	∅	
Brine Control	0	25.5	

Test No. 658-85 Client CH2M Hill - Wyckoff Investigator _____

WATER QUALITY DATA

Date: 3-17-18 initials: Y

Date: 3-3-18 initials: B

Conc. (%)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
70	15.0	7.6	29.0	7.9	15.2	8.6	29.5	8.1
35	15.1	7.7	29.5	7.9	15-3	8.4	29.5	8.1
18	15.1	7.8	30.0	7.9	15-2	8.2	30.0	8.1
9	15.0	8.0	30.0	7.9	15-5	8.2	30.0	8.1
4	15.0	8.0	30.0	7.9	15-4	8.2	30.0	8.1
2	15.4	8.0	30.0	7.9	15-3	8.2	30.0	8.1
Control	15.4	8.1	28.0	7.9	15-5	8.2	30.0	8.2
Brine control	15.1	8.1	28.0	7.9	15-5	8.2	29.0	8.0

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.3	0.2	16
pH	8.1	0.2	16
Salinity (ppt):	29.7	0.6	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0:	16.0	Day 0:	15.4
Day 1:	16.0	Day 1:	15.8
Day 2:	15.8	Day 2:	15.5

LARVAL COUNT DATA

3-5-18 632 ← 3-6-18 95 →

Conc. (%)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
70	212	14	218	16	217	4	228	8
35	197	9	215	7	210	7	198	9
18	198	6	216	10	225	7	213	12
9	197	12	225	9	192	6	217	9
4	191	11	187	12	189	11	201	9
2	199	14	206	11	226	6	201	11
Control	220	13	190	10	231	14	221	7
Brine control	207	9	210	8	221	11	200	13
Zero time	229	254	249	270	240	241	-	-

Zero time: Mean 247 SD 14 N 6 CV=(sd/mean)x100 5.7%

Remarks:



130 SE Lynch Rd.
Shelton WA 98584

Order	ORD00390924
Date	2/28/2018
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	2/26/2018	379,288
Ordered	Shipped	Item Number	Description	Unit Price	Ext. Price	
10	10	10264	MS Mediterranean Regular			
			RECEIVED 2-27-18 -651			

Subtotal	
Misc	\$0.00
Tax	\$0.00
Freight	\$0.00
Trade Discount	\$0.00
Total	

CETIS Analytical Report

Report Date: 06 Mar-18 14:18 (p 2 of 2)

Test Code: 658-85 | 16-8466-7613

Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID:	04-7952-2676		Endpoint:	Proportion Survived		CETIS Version:	CETISv1.8.7				
Analyzed:	06 Mar-18 14:18		Analysis:	Parametric-Two Sample		Official Results:	Yes				
Batch ID:	15-1631-3211		Test Type:	Development-Survival		Analyst:					
Start Date:	01 Mar-18 13:50		Protocol:	EPA/600/R-95/136 (1995)		Diluent:	Yaquina Bay Seawater				
Ending Date:	03 Mar-18 14:00		Species:	Mytilis galloprovincialis		Brine:					
Duration:	48h		Source:	Kamilche Sea Farms, WA		Age:					
Sample ID:	17-3365-4727		Code:	675578C7		Client:	Wyckoff Treatment Plant				
Sample Date:	28 Feb-18 09:15		Material:	Industrial Effluent		Project:					
Receive Date:	01 Mar-18 12:55		Source:	Wyckoff							
Sample Age:	29h (2 °C)		Station:								
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result					
Angular (Corrected)	NA	C <> T	NA	NA	12.5%	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	0.8702	2.447	0.196	6	0.4176	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.009664159	0.009664159	1	0.7573	0.4176	Non-Significant Effect					
Error	0.07657292	0.01276215	6								
Total	0.08623709		7								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Variance Ratio F	6.374	47.47	0.1625	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.9468	0.6451	0.6791	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	0.917	0.7943	1	0.9028	0.8097	0.9919	0.03857	8.41%	0.0%
0	Brine Reagent	4	0.8897	0.8354	0.9439	0.9028	0.8623	0.9393	0.01705	3.83%	2.98%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.305	1.069	1.541	1.255	1.119	1.481	0.07427	11.38%	0.0%
0	Brine Reagent	4	1.236	1.142	1.329	1.255	1.191	1.322	0.02942	4.76%	5.33%
Proportion Survived Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	0.9433	0.8097	0.9919	0.9231						
0	Brine Reagent	0.8745	0.8826	0.9393	0.8623						
Angular (Corrected) Transformed Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	1.33	1.119	1.481	1.29						
0	Brine Reagent	1.209	1.221	1.322	1.191						

CETIS Analytical Report

Report Date: 06 Mar-18 14:04 (p 3 of 3)

Test Code: 658-85 16-8466-7613

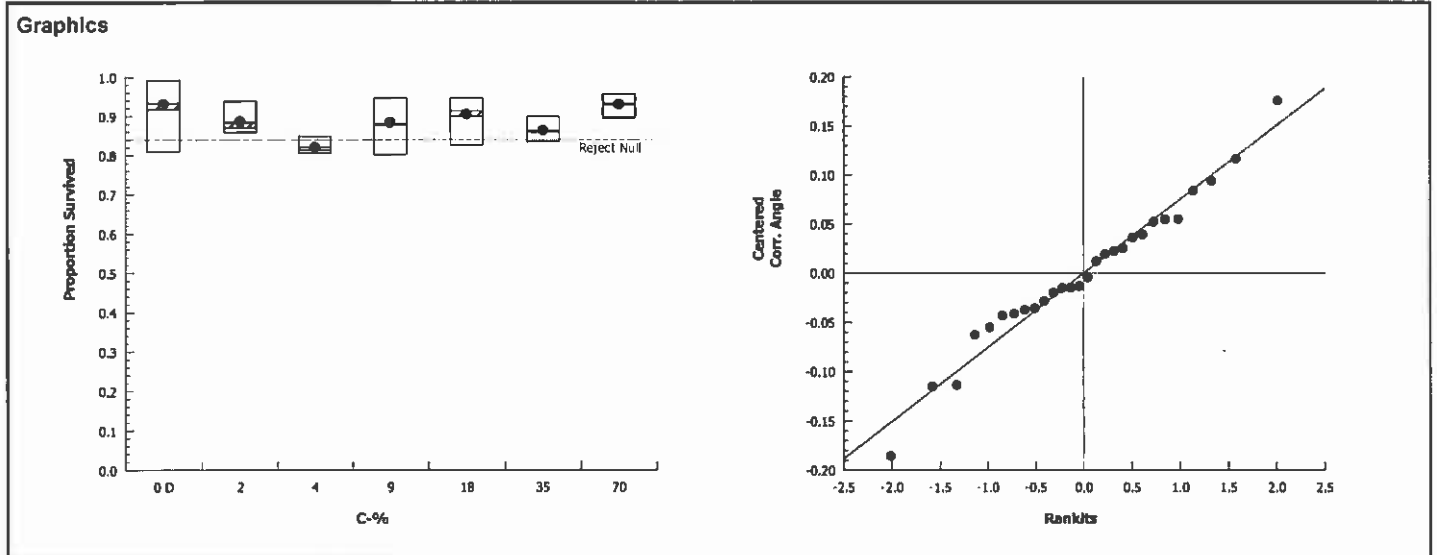
Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID:	07-4732-0291	Endpoint:	Proportion Survived			CETIS Version:	CETISv1.8.7				
Analyzed:	06 Mar-18 14:02	Analysis:	Parametric-Control vs Treatments			Official Results:	Yes				
Batch ID:	15-1631-3211	Test Type:	Development-Survival			Analyst:					
Start Date:	01 Mar-18 13:50	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Yaquina Bay Seawater				
Ending Date:	03 Mar-18 14:00	Species:	Mytilis galloprovincialis			Brine:					
Duration:	48h	Source:	Kamilche Sea Farms, WA			Age:					
Sample ID:	17-3365-4727	Code:	675578C7			Client:	Wyckoff Treatment Plant				
Sample Date:	28 Feb-18 09:15	Material:	Industrial Effluent			Project:					
Receive Date:	01 Mar-18 12:55	Source:	Wyckoff								
Sample Age:	29h (2 °C)	Station:									
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	8.4%	70	>70	NA	1.429		
Dunnnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Dilution Water		2	1.293	2.448	0.146	6	0.3756	CDF	Non-Significant Effect		
		4*	2.867	2.448	0.146	6	0.0213	CDF	Significant Effect		
		9	1.381	2.448	0.146	6	0.2925	CDF	Non-Significant Effect		
		18	0.8303	2.448	0.146	6	0.5322	CDF	Non-Significant Effect		
		35	1.89	2.448	0.146	6	0.1374	CDF	Non-Significant Effect		
		70	0.03145	2.448	0.146	6	0.8485	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.08860122	0.01476687	6	2.079	0.0995	Non-Significant Effect					
Error	0.1491608	0.007102897	21								
Total	0.237762		27								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Bartlett Equality of Variance	8.952	16.81	0.1763	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.9815	0.8975	0.8848	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	0.917	0.7943	1	0.9332	0.8097	0.9919	0.03857	8.41%	0.0%
2		4	0.8846	0.825	0.9442	0.8704	0.8583	0.9393	0.01874	4.24%	3.53%
4		4	0.8209	0.7887	0.853	0.8138	0.8057	0.8502	0.0101	2.46%	10.49%
9		4	0.8775	0.7727	0.9824	0.8806	0.8016	0.9474	0.03295	7.51%	4.31%
18		4	0.8998	0.8172	0.9824	0.913	0.8259	0.9474	0.02594	5.77%	1.88%
35		4	0.8623	0.8122	0.9125	0.8583	0.834	0.8988	0.01577	3.66%	5.96%
70		4	0.9281	0.8831	0.9732	0.9312	0.8947	0.9555	0.01416	3.05%	-1.21%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.305	1.069	1.541	1.31	1.119	1.481	0.07427	11.38%	0.0%
2		4	1.228	1.126	1.33	1.203	1.185	1.322	0.03192	5.2%	5.9%
4		4	1.134	1.091	1.177	1.125	1.114	1.173	0.01345	2.37%	13.09%
9		4	1.223	1.058	1.388	1.221	1.109	1.339	0.05183	8.48%	6.31%
18		4	1.256	1.123	1.388	1.271	1.14	1.339	0.04164	6.63%	3.79%
35		4	1.192	1.119	1.266	1.186	1.151	1.247	0.02321	3.89%	8.63%
70		4	1.303	1.216	1.391	1.307	1.24	1.358	0.0275	4.22%	0.14%

Bivalve Larval Survival and Development Test Northwestern Aquatic Sciences

Analysis ID: 07-4732-0291 **Endpoint:** Proportion Survived CETIS Version: CETISv1.8.7
 Analyzed: 06 Mar-18 14:02 Official Results: Yes
 Analysis: Parametric-Control vs Treatments

Proportion Survived Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.9433	0.8097	0.9919	0.9231
2		0.8623	0.8785	0.9393	0.8583
4		0.8178	0.8057	0.8097	0.8502
9		0.8462	0.9474	0.8016	0.915
18		0.8259	0.915	0.9474	0.9109
35		0.834	0.8988	0.8785	0.8381
70		0.915	0.9474	0.8947	0.9555

Angular (Corrected) Transformed Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.33	1.119	1.481	1.29
2		1.191	1.215	1.322	1.185
4		1.13	1.114	1.119	1.173
9		1.168	1.339	1.109	1.275
18		1.14	1.275	1.339	1.268
35		1.151	1.247	1.215	1.157
70		1.275	1.339	1.24	1.358



LC50 > 70% BY DATA INSPECTION
 -621

CETIS Analytical Report

Report Date: 06 Mar-18 14:18 (p 1 of 2)

Test Code: 658-85 | 16-8466-7613

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

Analysis ID: 06-1314-0330	Endpoint: Combined Proportion Normal	CETIS Version: CETISv1.8.7
Analyzed: 06 Mar-18 14:18	Analysis: Parametric-Two Sample	Official Results: Yes

Batch ID: 15-1631-3211	Test Type: Development-Survival	Analyst:
Start Date: 01 Mar-18 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Yaquina Bay Seawater
Ending Date: 03 Mar-18 14:00	Species: Mytilis galloprovincialis	Brine:
Duration: 48h	Source: Kamilche Sea Farms, WA	Age:

Sample ID: 17-3365-4727	Code: 675578C7	Client: Wyckoff Treatment Plant
Sample Date: 28 Feb-18 09:15	Material: Industrial Effluent	Project:
Receive Date: 01 Mar-18 12:55	Source: Wyckoff	
Sample Age: 29h (2 °C)	Station:	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C <> T	NA	NA	11.4%	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	0.7303	2.447	0.140	6	0.4927	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003509655	0.003509655	1	0.5334	0.4927	Non-Significant Effect
Error	0.03947853	0.006579755	6			
Total	0.04298818		7			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	4.142	47.47	0.2736	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9545	0.6451	0.7565	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	0.8725	0.7584	0.9866	0.8704	0.7692	0.9352	0.03585	8.22%	0.0%
0	Brine Reagent	4	0.8482	0.7919	0.9045	0.8704	0.8097	0.8947	0.01769	4.17%	2.78%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Dilution Water	4	1.214	1.051	1.378	1.204	1.07	1.313	0.05148	8.48%	0.0%
0	Brine Reagent	4	1.172	1.092	1.253	1.204	1.119	1.24	0.0253	4.32%	3.45%

Combined Proportion Normal Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.8907	0.7692	0.9352	0.8947
0	Brine Reagent	0.8381	0.8502	0.8947	0.8097

Angular (Corrected) Transformed Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.234	1.07	1.313	1.24
0	Brine Reagent	1.157	1.173	1.24	1.119

CETIS Analytical Report

Report Date: 06 Mar-18 14:04 (p 1 of 3)

Test Code: 658-85 16-8466-7613

Bivalve Larval Survival and Development Test						Northwestern Aquatic Sciences					
Analysis ID:	04-1228-3858	Endpoint:	Combined Proportion Normal			CETIS Version:	CETISv1.8.7				
Analyzed:	06 Mar-18 14:03	Analysis:	Parametric-Control vs Treatments			Official Results:	Yes				
Batch ID:	15-1631-3211	Test Type:	Development-Survival			Analyst:					
Start Date:	01 Mar-18 13:50	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Yaquina Bay Seawater				
Ending Date:	03 Mar-18 14:00	Species:	Mytilis galloprovincialis			Brine:					
Duration:	48h	Source:	Kamilche Sea Farms, WA			Age:					
Sample ID:	17-3365-4727	Code:	675578C7			Client:	Wyckoff Treatment Plant				
Sample Date:	28 Feb-18 09:15	Material:	Industrial Effluent			Project:					
Receive Date:	01 Mar-18 12:55	Source:	Wyckoff								
Sample Age:	29h (2 °C)	Station:									
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	9.5%	70	70	NA	1.429		
Dunnnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Dilution Water		2	0.981	2.448	0.12	6	0.4625	CDF	Non-Significant Effect		
		4*	2.742	2.448	0.12	6	0.0276	CDF	Significant Effect		
		9	0.9753	2.448	0.12	6	0.4651	CDF	Non-Significant Effect		
		18	0.4134	2.448	0.12	6	0.7167	CDF	Non-Significant Effect		
		35	1.367	2.448	0.12	6	0.2978	CDF	Non-Significant Effect		
		70	-0.2709	2.448	0.12	6	0.9178	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.0581069	0.009684484	6	2.014	0.1091	Non-Significant Effect					
Error	0.1010001	0.004809526	21								
Total	0.159107		27								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Bartlett Equality of Variance	5.092	16.81	0.5320	Equal Variances						
Variances	Mod Levene Equality of Variance	0.7228	3.812	0.6360	Equal Variances						
Variances	Levene Equality of Variance	1.288	3.812	0.3053	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.981	0.8975	0.8735	Normal Distribution						
Distribution	Kolmogorov-Smirnov D	0.09342	0.1914	0.8115	Normal Distribution						
Distribution	D'Agostino Skewness	0.2694	2.576	0.7876	Normal Distribution						
Distribution	D'Agostino Kurtosis	0.1026	2.576	0.9183	Normal Distribution						
Distribution	D'Agostino-Pearson K2 Omnibus	0.08309	9.21	0.9593	Normal Distribution						
Distribution	Anderson-Darling A2 Normality	0.2019	3.878	0.9227	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	0.8725	0.7584	0.9866	0.8927	0.7692	0.9352	0.03585	8.22%	0.0%
2		4	0.8421	0.7625	0.9217	0.8239	0.8057	0.915	0.02501	5.94%	3.48%
4		4	0.7773	0.7373	0.8174	0.7692	0.7571	0.8138	0.01259	3.24%	10.9%
9		4	0.8411	0.7395	0.9427	0.8381	0.7773	0.9109	0.03194	7.59%	3.6%
18		4	0.8623	0.79	0.9347	0.8684	0.8016	0.9109	0.02272	5.27%	1.16%
35		4	0.83	0.7726	0.8873	0.8259	0.7976	0.8704	0.01803	4.35%	4.87%
70		4	0.8856	0.8425	0.9288	0.8806	0.8583	0.9231	0.01357	3.06%	-1.51%

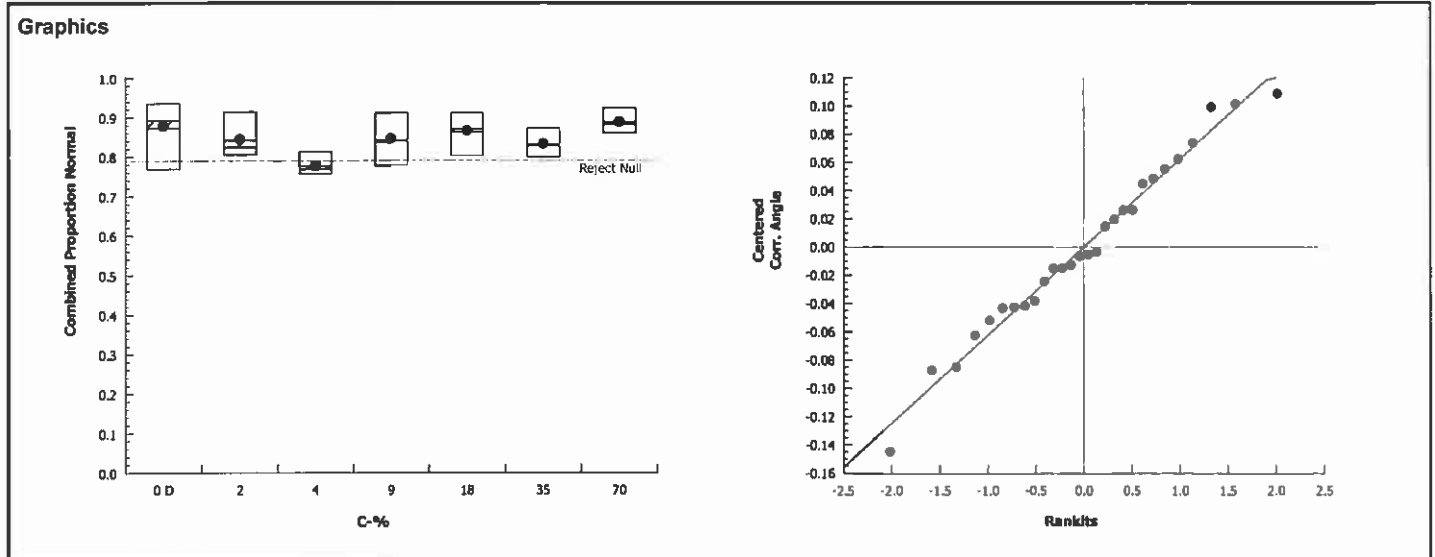
Bivalve Larval Survival and Development Test Northwestern Aquatic Sciences

Analysis ID: 04-1228-3858 Endpoint: Combined Proportion Normal CETIS Version: CETISv1.8.7
 Analyzed: 06 Mar-18 14:03 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	1.214	1.051	1.378	1.237	1.07	1.313	0.05148	8.48%	0.0%
2		4	1.166	1.048	1.284	1.138	1.114	1.275	0.03705	6.35%	3.96%
4		4	1.08	1.031	1.129	1.07	1.055	1.125	0.01541	2.85%	11.07%
9		4	1.167	1.024	1.309	1.159	1.079	1.268	0.04478	7.68%	3.94%
18		4	1.194	1.09	1.298	1.2	1.109	1.268	0.03273	5.48%	1.67%
35		4	1.147	1.07	1.224	1.141	1.104	1.203	0.02425	4.23%	5.52%
70		4	1.228	1.157	1.298	1.218	1.185	1.29	0.02217	3.61%	-1.09%

Combined Proportion Normal Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.8907	0.7692	0.9352	0.8947
2		0.8057	0.834	0.915	0.8138
4		0.7733	0.7571	0.7652	0.8138
9		0.7976	0.9109	0.7773	0.8785
18		0.8016	0.8745	0.9109	0.8623
35		0.7976	0.8704	0.8502	0.8016
70		0.8583	0.8826	0.8785	0.9231

Angular (Corrected) Transformed Detail					
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	1.234	1.07	1.313	1.24
2		1.114	1.151	1.275	1.125
4		1.075	1.055	1.065	1.125
9		1.104	1.268	1.079	1.215
18		1.109	1.209	1.268	1.191
35		1.104	1.203	1.173	1.109
70		1.185	1.221	1.215	1.29



EC₅₀ > 70% BY DATA INSPECTION.
-621

CETIS Analytical Report

Report Date: 06 Mar-18 14:04 (p 1 of 1)

Test Code: 658-85 16-8466-7613

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

Analysis ID: 02-9176-0113 Endpoint: Combined Proportion Normal CETIS Version: CETISv1.8.7
 Analyzed: 06 Mar-18 14:03 Analysis: Linear Interpolation (ICPIN) Official Results: Yes

Batch ID: 15-1631-3211 Test Type: Development-Survival Analyst:
 Start Date: 01 Mar-18 13:50 Protocol: EPA/600/R-95/136 (1995) Diluent: Yaquina Bay Seawater
 Ending Date: 03 Mar-18 14:00 Species: Mytilis galloprovincialis Brine:
 Duration: 48h Source: Kamllche Sea Farms, WA Age:

Sample ID: 17-3365-4727 Code: 675578C7 Client: Wyckoff Treatment Plant
 Sample Date: 28 Feb-18 09:15 Material: Industrial Effluent Project:
 Receive Date: 01 Mar-18 12:55 Source: Wyckoff
 Sample Age: 29h (2 °C) Station:

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1099397	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	0.8725	0.7692	0.9352	0.03585	0.0717	8.22%	0.0%	862	988
2		4	0.8421	0.8057	0.915	0.02501	0.05002	5.94%	3.48%	832	988
4		4	0.7773	0.7571	0.8138	0.01259	0.02518	3.24%	10.9%	768	988
9		4	0.8411	0.7773	0.9109	0.03194	0.06387	7.59%	3.6%	831	988
18		4	0.8623	0.8016	0.9109	0.02272	0.04545	5.27%	1.16%	852	988
35		4	0.83	0.7976	0.8704	0.01803	0.03606	4.35%	4.67%	820	988
70		4	0.8856	0.8583	0.9231	0.01357	0.02713	3.06%	-1.51%	875	988

Combined Proportion Normal Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.8907	0.7692	0.9352	0.8947
2		0.8057	0.834	0.915	0.8138
4		0.7733	0.7571	0.7652	0.8138
9		0.7976	0.9109	0.7773	0.8785
18		0.8016	0.8745	0.9109	0.8623
35		0.7976	0.8704	0.8502	0.8016
70		0.8583	0.8826	0.8785	0.9231

CETIS Test Data Worksheet

Report Date: 06 Mar-18 14:04 (p 1 of 1)
 Test Code: 16-8466-7615/658-85

Bivalve Larval Survival and Development Test Northwestern Aquatic Sciences

Start Date: 01 Mar-18 13:50 Species: Mytilus galloprovincialis Sample Code: 675578C7
 End Date: 03 Mar-18 14:00 Protocol: EPA/600/R-95/136 (1995) Sample Source: Wyckoff
 Sample Date: 28 Feb-18 09:15 Material: Industrial Effluent Sample Station:

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	B	1	32	247	216	216	207	
0	B	2	11	247	218	218	210	
0	B	3	12	247	232	232	221	
0	B	4	3	247	213	213	200	
0	D	1	10	247	233	233	220	
0	D	2	2	247	200	200	190	
0	D	3	23	247	245	245	231	
0	D	4	9	247	228	228	221	
2		1	18	247	213	213	199	
2		2	27	247	217	217	206	
2		3	22	247	232	232	226	
2		4	30	247	212	212	201	
4		1	28	247	202	202	191	
4		2	15	247	199	199	187	
4		3	14	247	200	200	189	
4		4	24	247	210	210	201	
9		1	6	247	209	209	197	
9		2	29	247	234	234	225	
9		3	5	247	198	198	192	
9		4	17	247	226	226	217	
18		1	18	247	204	204	198	
18		2	13	247	226	226	216	
18		3	1	247	234	234	225	
18		4	31	247	225	225	213	
35		1	7	247	208	206	197	
35		2	28	247	222	222	215	
35		3	21	247	217	217	210	
35		4	8	247	207	207	198	
70		1	4	247	228	228	212	
70		2	20	247	234	234	218	
70		3	19	247	221	221	217	
70		4	25	247	238	236	228	

data entry verified against laboratory bench sheets 3-7-18 JRF

Northwestern Aquatic Sciences (REGION COPY)

Date Shipped: 2/28/2018
 Carrier Name: FedEx
 Airbill No: 7716 3761 8883

CHAIN OF CUSTODY RECORD

Wyckoff Eagle Harbor GWTP 2017AWA
 Project Code: WEH-025M
 Cooler #: 1 of 1

No: 10-022818-094122-0238

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 1st Quarter		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	02/28/2018 09:15	Field Sample

Special Instructions: *NARS# 61356*

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
	<i>Keith Allers CH2M</i>	<i>2-28-2018 0950</i>	<i>Loy Bubala MMS</i>	<i>3-1-18 1255</i>	<i>In Test</i>

ORIGIN ID: LKEA (206) 780-1711
KEITH ALLERS
CP2M HILL INC
5350 CREOSOTE PLACE N.E.
BAINBRIDGE ISLAND, WA 98110
UNITED STATES US

SHIP DATE: 28FEB18
ACTWGT: 8.00 LB
CAD: 111531780 WXSJ3100
DIMS: 11x9x13 IN
BILL SENDER

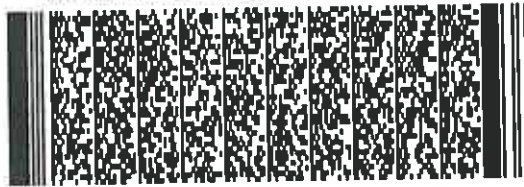
TO **GEARLD IRISSARRI**
NORTHWESTERN AQUATIC SCIENCES
3814 YAQUINA BAY ROAD

NEWPORT OR 97365

(541) 265-7225
INV:
PO:

REF: PN: 438558.FP.Y7.01

DEPT:



FedEx
Express



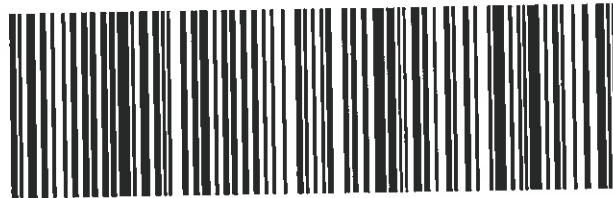
THU - 01 MAR 12:00P
PRIORITY OVERNIGHT

TRK# 7716 3761 8883
0201

86 ONPA

97365

OR-US PDX



CUSTODY SEAL

Date:	2-28-2018
Signature:	<i>Keith Allers</i>



APPENDIX III

RAW DATA – REFERENCE TOXICANT TEST

Test No. 999-3760 Client: QC Test

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. Irissari⁶³¹
 QA Officer L.K. Nemeth
 1. YES water taken 2. GARH LA AB
 3. J. Brown JS 4. _____
 Study Schedule:
 Test Beginning: 3-1-18 1350 Test Ending: 3-3-18 1400

TEST MATERIAL

Description: Copper as CuSO₄·5H₂O, Argent Lot# 0195,
 NAS Sample No. _____ 1.0 mg/ml stock prepared: 5-16-16
 Date of Collection: _____
 Date of Receipt: _____
 Temperature (deg C): _____
 pH: _____
 Dissolved oxygen (mg/L): _____
 Conductivity (umhos/cm): _____
 Hardness (mg/L): _____
 Alkalinity (mg/L): _____
 Salinity (ppt): _____
 Total chlorine (mg/L): _____
 Total ammonia-N (mg/L): _____

DILUTION WATER

Description: Yaquina Bay, OR Seawater
 Date of Collection: 2-29-18 Salinity (ppt) 30.0 pH 8.0
 Treatments: Aerated, filtered to ≤ 0.45 um, salinity adjusted with Milli-Q® deionized water

TEST ORGANISMS

Species: Mytilus galloprovincialis Date Received: 2-27-18
 Source: Taylor Shellfish Farms, Shelton, WA

Acclimation Data:

Date	Temp (deg.C)	pH	Sal (ppt)	D.O. (mg/L)	Comments
3-1-18	8.1	8.1	27.0	9.1	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

Test No. 999-3760 Client _____ QC Test _____ Investigator _____

SPAWNING AND GAMETE HANDLING

Spawning: Initial: 1110 Final: 1125 Fertilization: 1150
 Number of organisms used: females: 2 males: 3
 Egg Dilution (1 ml diluted to 100 ml):
 Count/ml of dilution: 1. 38 2. 39 3. 44 Mean: 40.3
 Dilution factor = DF (mean x 100/2500) = 1.6

TEST PROCEDURES AND CONDITIONS

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

Test chamber: 30 ml glass vials Test volume: 10 ml Replicates/treatment (4): 4
 Organisms/ml (15-30): 24.7 Test water changes: None Aeration during test: None
 Feeding: None Photoperiod: 16L:8D Salinity: 30 +/- 2 ppt
 Temperature: 20 +/- 1 °C, oysters, 16 +/- 1 °C, mussels Beaker placement: Stratified randomization

RANDOMIZATION CHART

A	∅	64	1	32	4	2	16	8		
B	2	16	8	4	64	∅	32	1		
C	32	1	∅	16	2	8	64	4		
D	64	1	32	8	2	4	∅	16		

PREPARATION OF TEST SOLUTIONS

3-1-18
651

Test Conc. (Cu, ug/L)	ml of working stock #2 (2 ug/mL)	Dilution water (ml/100mL)
64	3.2	Brought up to a
32	1.6	final volume of
16	0.8	100 ml with
8	0.4	dilution water.
4	0.2	
2	0.1	
1	0.05	
0	0	

1st working stock made by 1:99 (1.0 mL ↑ 100mL) dilution of concentrated 1 mg/mL stock solution. Final concentration 10 ug/mL.
 2nd working stock made (working stock #2) made by 20:80 (20 mL ↑ 100mL) dilution of 1st working stock. Final concentration 2 ug/mL.

Comments:

Test No. 999-3760 Client QC Test Investigator

WATER QUALITY DATA

Date: 3-1-18 initials: Z

Date: 3-3-18 initials: UJS

Conc. (ug/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)	Temp. (deg.C)	pH	Sal. (ppt)	DO (mg/L)
64	15.9	8.1	29.5	7.9	15.6	8.2	29.5	8.1
32	15.9	8.1	29.5	7.9	15.5	8.2	30.0	8.1
16	16.0	8.1	30.0	7.9	15.5	8.2	30.0	8.1
8	15.9	8.1	30.0	7.9	15.6	8.2	30.0	8.2
4	15.8	8.1	30.0	7.9	15.5	8.2	30.5	8.2
2	15.9	8.1	30.0	7.9	15.5	8.2	30.0	8.2
1	15.9	8.1	30.0	7.9	15.5	8.2	30.0	8.1
Control	16.0	8.0	30.0	7.9	15.8	8.2	30.0	8.2
Brine control	---	---	---	---	---	---	---	---

WATER QUALITY:

	Mean	SD	N
Temperature (°C):	15.7	0.2	16
pH:	8.1	0.1	16
Salinity (ppt):	29.9	0.3	16
DO (mg/L):	8.0	0.1	16

Room/ Water bath temperature: (°C)

Day 0:	16.0	Day 0:	15.4
Day 1:	16.0	Day 1:	15.8
Day 2:	15.8	Day 2:	15.5

LARVAL COUNT DATA

3-5-18 62I

3-5-18 83

Conc. (ug/L)	Replicate 1		Replicate 2		Replicate 3		Replicate 4	
	N	A	N	A	N	A	N	A
64	∅	1	∅	∅	∅	∅	∅	∅
32	∅	50	∅	73	∅	62	∅	74
16	∅	206	∅	225	∅	185	∅	222
8	165	52	149	71	126	66	152	57
4	210	11	231	11	220	10	231	7
2	205	14	219	13	234	8	211	10
1	236	12	220	15	210	7	207	13
Control	202	9	217	11	231	7	220	10
Brine control	---	---	---	---	---	---	---	---
Zero time	229	254	249	270	240	241	-	-

Zero time: Mean 247 SD 14 N 6

CV=(sd/mean)x100 5.7%

Remarks:



130 SE Lynch Rd.
Shelton WA 98584

Order	ORD00390924
Date	2/26/2018
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	2/26/2018	379,288
Ordered	Shipped	Item Number	Description	Unit Price	Ext. Price	
10	10	10264	MS Mediterranean Regular			
			RECEIVED 2-27-18 -651			

Subtotal	
Misc	\$0.00
Tax	\$0.00
Freight	\$0.00
Trade Discount	\$0.00
Total	

CETIS Summary Report

Report Date: 05 Mar-18 14:21 (p 1 of 2)
 Test Code: 999-3760 17-0778-1414

Bivalve Larval Survival and Development Test Northwestern Aquatic Sciences

Batch ID: 15-1631-3211	Test Type: Development-Survival	Analyst:
Start Date: 01 Mar-18 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Yaquina Bay Seawater
Ending Date: 03 Mar-17 14:00	Species: Mytilis galloprovincialis	Brine:
Duration: NA	Source: Kamilche Sea Farms, WA	Age:

Sample ID: 03-1968-5423	Code: 130E032F	Client: Internal Lab
Sample Date: 01 Mar-18 13:50	Material: Copper sulfate	Project:
Receive Date: 01 Mar-18 13:50	Source: Reference Toxicant	
Sample Age: NA	Station:	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
19-2338-8869	Combined Proportion Normal	4	8	5.657	10.4%		Dunnett Multiple Comparison Test
18-9848-1589	Proportion Normal	4	8	5.657	2.78%		Bonferroni Adj t Test
20-9328-0766	Proportion Survived	16	32	22.63	10.6%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
07-2704-1665	Combined Proportion Normal	EC25	7.085	6.021	8.009		Linear Interpolation (ICPIN)
17-6952-8158	Combined Proportion Normal	EC50	9.037	8.852	9.225		Spearman-Kärber
06-0467-9395	Proportion Normal	EC25	7.789	6.95	8.581		Linear Interpolation (ICPIN)
03-0888-9917	Proportion Normal	EC50	9.424	9.224	9.629		Trimmed Spearman-Kärber
19-0796-7634	Proportion Survived	EC50	24.42	23.78	25.08		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	0.8806	0.8035	0.9576	0.8178	0.9352	0.02421	0.04841	5.5%	0.0%
1		4	0.8836	0.7994	0.9678	0.8381	0.9555	0.02646	0.05293	5.99%	-0.34%
2		4	0.8796	0.7987	0.9604	0.83	0.9474	0.02541	0.05082	5.78%	0.11%
4		4	0.9028	0.8378	0.9679	0.8502	0.9352	0.02044	0.04089	4.53%	-2.53%
8		4	0.5992	0.4946	0.7037	0.5101	0.668	0.03285	0.0657	10.96%	31.95%
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	0.918	0.8449	0.9912	0.8543	0.9636	0.02298	0.04597	5.01%	0.0%
1		4	0.9302	0.8404	1	0.8785	1	0.02821	0.05643	6.07%	-1.32%
2		4	0.9251	0.8564	0.9938	0.8866	0.9798	0.02158	0.04316	4.67%	-0.77%
4		4	0.9423	0.8825	1	0.8947	0.9798	0.0188	0.0376	3.99%	-2.65%
8		4	0.8482	0.7673	0.9291	0.7773	0.8907	0.02542	0.05084	5.99%	7.61%
16		4	0.8482	0.73	0.9663	0.749	0.9109	0.03712	0.07425	8.75%	7.61%
32		4	0.2621	0.1898	0.3345	0.2024	0.2996	0.02275	0.04549	17.35%	71.44%
64		4	0.001012	0	0.004233	0	0.004049	0.001012	0.002024	200.0%	99.89%

CETIS Summary Report

Report Date: 05 Mar-18 14:21 (p 2 of 2)
 Test Code: 999-3760 | 17-0778-1414

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	0.8178	0.8785	0.9352	0.8907	
1		0.9555	0.8907	0.8502	0.8381	
2		0.83	0.8866	0.9474	0.8543	
4		0.8502	0.9352	0.8907	0.9352	
8		0.668	0.6032	0.5101	0.6154	
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	0.8543	0.9231	0.9636	0.9312	
1		1	0.9514	0.8785	0.8907	
2		0.8866	0.9393	0.9798	0.8947	
4		0.8947	0.9798	0.9312	0.9636	
8		0.8785	0.8907	0.7773	0.8462	
16		0.834	0.9109	0.749	0.8988	
32		0.2024	0.2955	0.251	0.2996	
64		0.004049	0	0	0	
Combined Proportion Normal Binomials						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	
0	Dilution Water	202/247	217/247	231/247	220/247	
1		236/247	220/247	210/247	207/247	
2		205/247	219/247	234/247	211/247	
4		210/247	231/247	220/247	231/247	
8		165/247	149/247	126/247	152/247	
16		0/247	0/247	0/247	0/247	
32		0/247	0/247	0/247	0/247	
64		0/247	0/247	0/247	0/247	
Proportion Survived Binomials						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	
0	Dilution Water	211/247	228/247	238/247	230/247	
1		247/247	235/247	217/247	220/247	
2		219/247	232/247	242/247	221/247	
4		221/247	242/247	230/247	238/247	
8		217/247	220/247	192/247	209/247	
16		206/247	225/247	185/247	222/247	
32		50/247	73/247	62/247	74/247	
64		1/247	0/247	0/247	0/247	

CETIS Test Data Worksheet

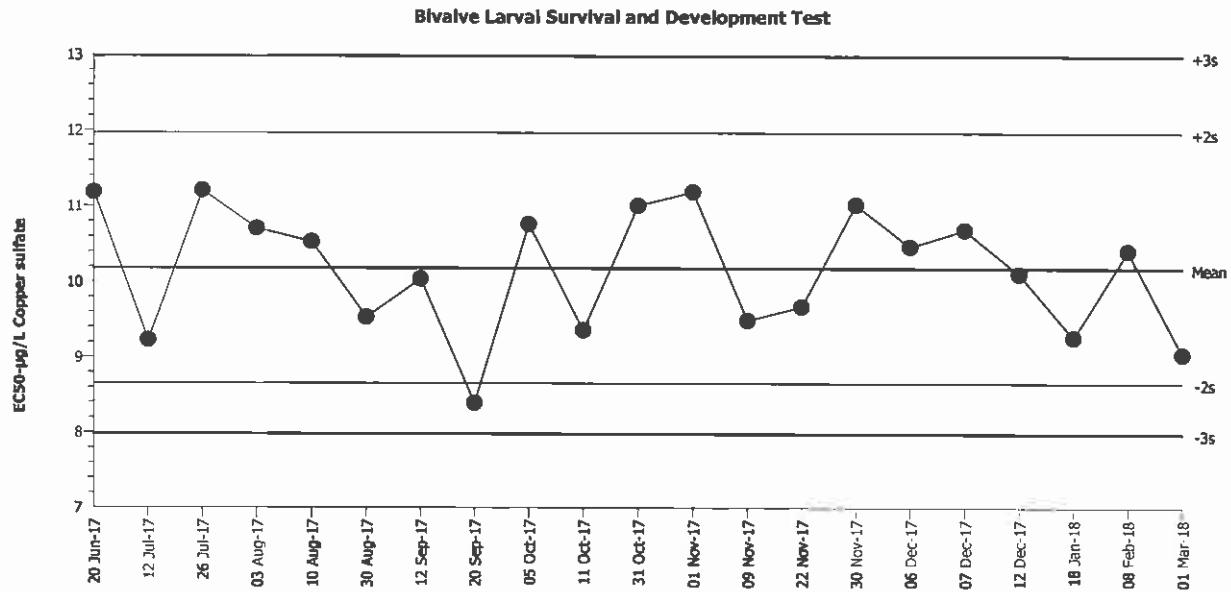
Report Date: 05 Mar-18 14:22 (p 1 of 1)
 Test Code: 17-0778-141/999-3760

Bivalve Larval Survival and Development Test				Northwestern Aquatic Sciences			
Start Date:	01 Mar-18 13:50	Species:	Mytilis galloprovincialis	Sample Code:	130E032F		
End Date:	03 Mar-17 14:00	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant		
Sample Date:	01 Mar-18 13:50	Material:	Copper sulfate	Sample Station:			

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	5	247	211	211	202	
0	D	2	31	247	228	228	217	
0	D	3	25	247	238	238	231	
0	D	4	28	247	230	230	220	
1		1	8	247	248	248	236	
1		2	21	247	235	235	220	
1		3	15	247	217	217	210	
1		4	10	247	220	220	207	
2		1	24	247	219	219	205	
2		2	27	247	232	232	219	
2		3	17	247	242	242	234	
2		4	26	247	221	221	211	
4		1	22	247	221	221	210	
4		2	4	247	242	242	231	
4		3	6	247	230	230	220	
4		4	9	247	238	238	231	
8		1	29	247	217	217	165	
8		2	7	247	220	220	149	
8		3	13	247	192	192	128	
8		4	2	247	209	209	152	
16		1	32	247	206	206	0	
16		2	20	247	225	225	0	
16		3	23	247	185	185	0	
16		4	11	247	222	222	0	
32		1	3	247	50	50	0	
32		2	18	247	73	73	0	
32		3	16	247	62	62	0	
32		4	12	247	74	74	0	
64		1	30	247	1	1	0	
64		2	19	247	0	0	0	
64		3	1	247	0	0	0	
64		4	14	247	0	0	0	

data entry verified against laboratory bench sheets 3-7-18 SKF

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: 10.18 **Count:** 20 **-2s Warning Limit:** 8.652 **-3s Action Limit:** 7.978
Sigma: NA **CV:** 8.45% **+2s Warning Limit:** 11.97 **+3s Action Limit:** 12.98

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jun	20	16:50	11.18	1.006	1.162			02-1409-7276	08-4079-1566
2		Jul	12	13:25	9.224	-0.9519	-1.211			02-4818-7084	16-2581-4533
3			26	14:10	11.2	1.028	1.186			17-8688-1039	02-1882-3377
4		Aug	3	13:30	10.7	0.5269	0.6223			12-5107-4745	07-3767-8523
5			10	15:10	10.53	0.3502	0.4171			14-0880-4376	18-5220-7572
6			30	14:35	9.527	-0.6487	-0.812			21-1251-6624	08-5861-5172
7		Sep	12	14:00	10.03	-0.1432	-0.1747			10-5774-5128	09-7081-1600
8			20	13:50	8.39	-1.786	-2.379	(-)		12-1488-7812	06-3387-4553
9		Oct	5	15:20	10.76	0.5849	0.689			01-0974-4829	16-8936-6824
10			11	17:10	9.355	-0.8206	-1.036			06-4503-4555	15-6316-6751
11			31	13:55	11	0.8281	0.9645			19-0668-1049	19-2975-1853
12		Nov	1	13:35	11.19	1.011	1.168			17-3365-4072	06-0748-0919
13			9	14:05	9.488	-0.6874	-0.8623			02-6639-7760	19-3779-2145
14			22	14:00	9.666	-0.5099	-0.6338			14-5021-8992	06-6921-5494
15			30	13:40	11.02	0.8407	0.9786			00-8984-2368	13-5466-3879
16		Dec	6	13:45	10.46	0.2862	0.3419			20-9204-7376	08-8218-5639
17			7	13:00	10.69	0.5099	0.6027			17-2684-2601	21-0626-4507
18			12	14:20	10.1	-0.07377	-0.08969			13-4785-2533	00-4154-6333
19	2018	Jan	18	17:30	9.259	-0.9164	-1.163			18-1463-9193	16-7842-9995
20		Feb	8	12:30	10.41	0.2316	0.2774			12-4129-6067	04-6683-9588
21		Mar	1	13:50	9.037	-1.139	-1.463			17-0778-1414	17-6952-8158