Wyckoff Groundwater Treatment Plant: Second Quarter 2024 Bioassay Monitoring

PREPARED FOR: Kristen Reed/Washington State Department of Ecology

COPY: Jacob Moersen/U.S. Environmental Protection Agency
Nicole Caveny/U.S. Environmental Protection Agency

PREPARED BY: Joy Chen/CH2M HILL Engineers, Inc
Mark Fesler/CH2M HILL Engineers, Inc.

DATE: June 12, 2024

1. Introduction

This technical memorandum summarizes information obtained from the second quarter 2024 sampling event performed at the U.S. Environmental Protection Agency (EPA) Wyckoff/Eagle Harbor Superfund Site (the Site) groundwater treatment plant (GWTP) located at 5350 Creosote Place NE, Bainbridge Island, Washington. CH2M HILL Engineers, Inc. (CH2M)[1] conducted this sampling event to support the current biomonitoring requirements of the substantive condition as presented in the site's Record of Decision (EPA 2000), hereinafter referred to as "substantive condition".

Sampling was generally conducted in accordance with the final *Quality Assurance Project Plan, Groundwater Treatment Plant Operations and Maintenance* (QAPP; CH2M, 2022). While there were deviations from the QAPP as noted in the Laboratory Quality Data Review section, the data is deemed usable, and the sampling is considered to have met the monitoring requirements of the substantive condition.

The current substantive condition does not include effluent limits for chronic toxicity. Chronic toxicity testing was conducted on the effluent samples per the requirements outlined in the substantive condition. The current substantive condition does not include specific dilution series for chronic toxicity tests. For the mussel larvae chronic toxicity testing conducted during the second quarter 2024 sampling event, 57.55 percent effluent was the highest concentration tested due to the addition of hypersaline brine (HSB) to achieve a salinity of 30 parts per trillion (ppt) per the *Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms* (EPA/600/R-95/136, 1995).

Due to the recent toxicity observed in the highest test concentrations for the mussel larvae chronic toxicity testing, concurrent tests were conducted (one test using artificial salts and the other test using HSB) during the second quarter 2024 sampling event to evaluate potential toxicity for undiluted sample (i.e. salts) while maintaining comparability of results from this quarter to previous test results (i.e. HSB). 57.55 percent effluent is the highest concentration tested using the HSB for the second quarter 2024 testing.

No statistically significant effects on the survival or development endpoints were observed for all test concentrations, indicating no evidence of the presence of chronic toxicity.

As stated above, the current substantive condition does not include effluent limit for chronic toxicity. The chronic toxicity test requirement section of the substantive condition (Section II.8) specifies the following:

[1] CH2M HILL Engineers, Inc. is now a wholly owned subsidiary of Jacobs Engineering Group Inc.

"*EPA and Ecology will evaluate the results to determine whether they indicate the occurrence of chronic toxicity outside the mixing zone. If it appears that this may be occurring, a toxicity evaluation and reduction plan will be prepared within 90 days. The evaluation portion of the plan may include additional toxicity testing if needed to follow up on initial results or gather information for a possible toxicity limit in the future.*"

The observed results for the chronic developmental endpoint would not trigger this requirement.

2. Sampling and Analysis Results

Biomonitoring samples were collected per the monitoring frequency included in the substantive condition. Samples were collected from a 24-hr. autosampler collection point at the effluent tank of the treatment system. Water samples were collected on April 16, 2024. Chemical testing was conducted on a split of each sample collected for bioassay testing per the substantive condition requirement. The bioassays were performed by EcoAnalysts, Inc. (EcoAnalysts), Port Gamble, Washington, a Washington State Department of Ecology accredited lab. Table 1 lists the sample Laboratory ID and sampling analysis methods. EcoAnalysts sampling analysis report for chronic toxicity testing is provided in Attachment 1.

Table 1. Biological Testing Summary

Laboratory	Laboratory ID	Method	Test Type/Descriptor/Species
EcoAnalysts	P240416.03	EPA/600/R-95-136 Method 1005.0; ASTM E724-89 TOX042.12	Chronic/48-hr Survival and Development/*Mytilus galloprovincialis* (Mussel)

No statistically significant effects were detected in any effluent concentration tested for the survival or development endpoint of the bivalve test. This result indicates a No Observed Effect Concentration of 57.55 percent (the highest concentration tested) of the effluent concentration and a chronic toxic unit of 1.7 for both endpoints. The Effect Concentration expected to affect 50 percent of the organisms (EC50) is greater than 100 percent and 57.55 percent of the effluent concentration, respectively for the salt and HSB adjusted samples, respectively.

3. Laboratory Quality Data Review

A CH2M chemist validated the bioassay results Stage 2A in accordance with the QAPP. The QAPP (CH2M 2022) was cited by EcoAnalysts and the appropriate species of mussel specified in the QAPP was used for the analytical testing.

The data were 100 percent complete, and method and QAPP quality control requirements were met, with the following exceptions noted:

(1) The QAPP reference toxicant copper sulfate was not used. The reference toxicant utilized was ammonia. A review of the total and unionized ammonia quality control data indicates the ammonia reference toxicant test results were within two standard deviations of the laboratory mean at the time of testing. There is no impact to the data and an addendum to the QAPP to utilize ammonia as reference toxicant was requested in May 2023 after these samples were collected and analyzed.

(2) Replicate 2 of the 12.5% effluent concentration in the brine test was removed from statistical analysis because the vial was compromised from potential contamination. Test data is considered usable because no statistically significant biological response of the test organisms was not detected at any of the test concentrations.

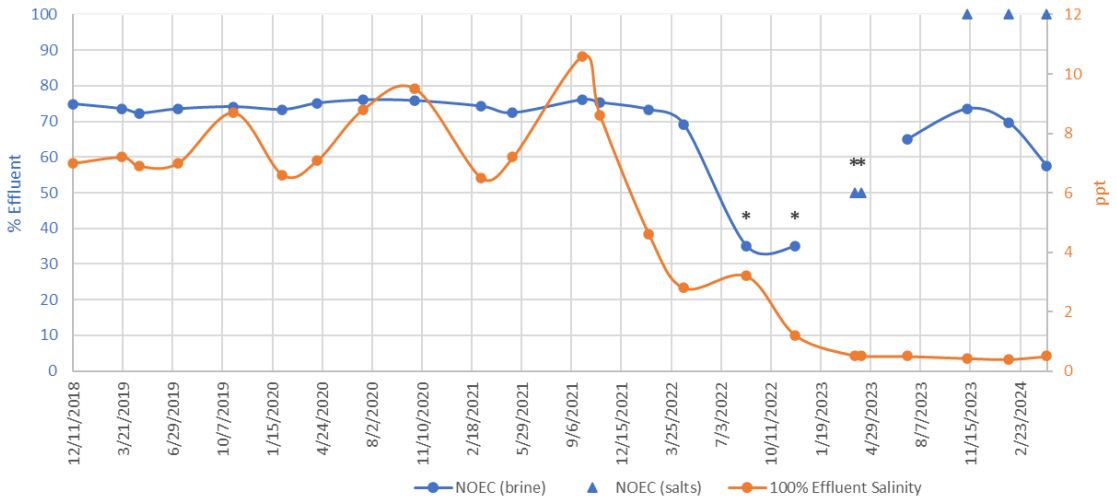
(3) There was a significant difference between the laboratory (dilution water) control and brine control. Test data is considered usable because there was no effect in any of the test concentrations and the difference between the lab and brine controls was relatively low (2.58%); hypersaline brine did not contribute to any negative biological effects.

4. Trends

A review of bioassay data collected from 2007 through the second quarter of 2024 indicated there were no statistically significant effect detected for the survival endpoint for any test concentrations and species. No statistically significant effect was detected for the sublethal endpoints with the exception for the sampling events from the third quarter of 2022 through second quarter of 2023. For these four sampling events, statistically significant effects were detected in the maximum test concentrations for the developmental endpoint of the chronic bioassay test.

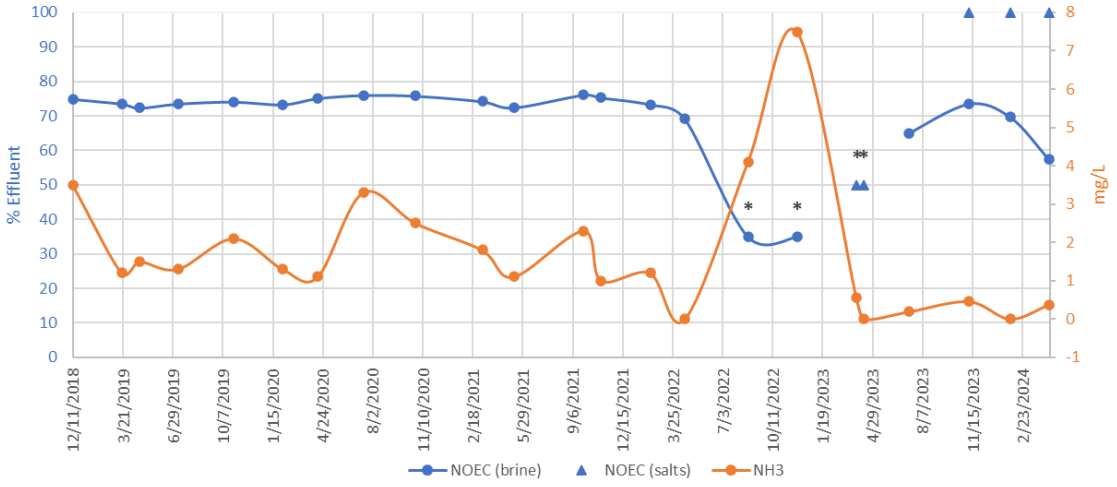
Figure 1 shows the bivalve chronic bioassay NOEC and salinity for the 100 percent effluent samples from December 2018 through April 2024. NOEC for bivalve chronic bioassay tests conducted prior to December 2018 were 70 percent. Hypersaline brine (HSB) with a fixed concentration was used for the salinity adjustment for chronic toxicity testing conducted prior to December 2018, therefore the maximum test concentrations remained the same for that test period. The laboratories that conducted the testing from December 2018 to November 2022 used HSB created at their laboratory (i.e. concentration varies slightly from batch to batch), therefore the resulting maximum test concentrations varies slightly for the different monitoring events. The maximum test concentration for the first and second quarter of 2023 is higher than previous monitoring events as well as third quarter of 2023 (i.e. 100 percent versus ~70 percent) due to the use of artificial sea salts as opposed to HSB. This resulted in a higher NOEC than those reported for the third and fourth quarter of 2022 despite a statistically significant effect was only observed in the maximum test concentration in the samples in all four sampling events. For the second quarter of 2024, concurrent tests were conducted (one test using artificial salts and the other test using HSB) for the split samples (see Figure 1 for NOEC for samples with salinity adjustment using brine and salts). A review of the water quality parameters measured for the bioassay samples indicated the lowest detected salinity levels were detected in the samples collected from the most recent seven sampling events (see Figure 1). While the elevated ammonia concentrations detected in the third and fourth quarter 2022 may have contributed to the observed toxicity during those sampling events, ammonia does not appear to be contributing to the toxicity observed in the monitoring events for the first and second quarter of 2023 (see Figure 2).

Figure 1. Trends in Bivalve (*Mytilus galloprovincialis*) Chronic Bioassay NOEC and 100% Effluent Salinity (Dec 2018- Apr 2024)



* Statistically significant effect detected in the maximum test concentration

Figure 2. Trends in Bivalve (*Mytilus galloprovincialis*) Chronic Bioassay NOEC and 100% Effluent Ammonia Concentration (Dec 2018- Apr 2024)



* Statistically significant effect detected in the maximum test concentration

5. Overall Assessment

While the current substantive condition does not include specific whole effluent toxicity (WET) limits, the Washington Administrative Code (WAC) 173-205-020 specifies the following:

> *"Whole effluent toxicity performance standard" means a level of effluent toxicity that is consistently so much lower than is necessary to meet state water quality standards (chapter 173-201A WAC) that no reasonable potential exists to violate the water quality standards. For acute toxicity, the performance standard is the median survival in one hundred percent effluent being equal to or greater than eighty percent and no individual*

> *test result showing less than sixty-five percent survival in one hundred percent effluent. For chronic toxicity, the performance standard is no chronic toxicity test demonstrating a statistically significant difference in response between the control and a test concentration equal to the acute critical effluent concentration. For permittees that are ineligible for an approved mixing zone, the performance standard will equal or be close to equal (in the case of acute toxicity) the water quality-based effluent toxicity limit.*

Based on sampling results, the survival and development endpoints of the chronic toxicity test met the WET performance standard because survival rates and proportion normal development were within acceptable limits. Result from the second quarter of 2024 is consistent with those from the past three quarters, where there was no evidence of the presence of chronic toxicity. Despite site groundwater does not appear to have returned to conditions prior to third quarter 2022, as indicated by the continued trend of relatively low salinity following second quarter 2023 (see Figure 1), there is no evidence of chronic toxicity in recent monitoring samples. Therefore, CH2M recommends continuation of the quarterly monitoring frequency included in the substantive condition.

Concurrent mussel chronic bioassay testing using both artificial salt and HSB for salinity adjustments were conducted for the fourth quarter 2023 and first and second quarter 2024. Results from these three quarters were the same using artificial salt and HSB for salinity adjustments, indicating testing results using artificial salt is comparable to previous test results (i.e. HSB). Both EPA's Effluent Toxicity Testing Methodologies (EPA 1995) and Ecology's guidance (Ecology 2016) allow for either brine or artificial sea salt to be used. CH2M recommends using only artificial salt for salinity adjustments in third quarter 2024 as it allows for testing to higher effluent concentrations. The recommended test dilution series are as follows: 100%, 50%, 25%, 12.5%, 6.25%, and control.

More than one replicate was removed from statistical analysis in recent quarters because the vial was compromised from potential contamination. Due to short supply of vials in recent years, the lab does not currently have the ability to solicit other suppliers. Baking vials in a muffle furnace prior to use has in some instances provided moderate success in removing organic, volatile films that can interfere with testing, if present for some labs. CH2M recommends the lab to bake the test vials in a muffle furnace prior to use for future testing.

6. References

ASTM. 1989. Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of

Saltwater Bivalve Molluscs, E724-89. ASTM International, West Conshohocken, PA.

CH2M HILL Engineers, Inc. (CH2M, now a wholly owned subsidiary of Jacobs Engineering Group Inc.). 2022. *Quality Assurance Project Plan, Groundwater Treatment Plant Operations and Maintenance.* Final. Prepared for Wyckoff/Eagle Harbor Superfund Site, Bainbridge Island, Washington, U.S. Environmental Protection Agency, Region 10, Seattle, Washington. January.

Ecology. 2016. Whole Effluent Toxicity Testing Guidance and Test Review Criteria. WQ-R-95-80. Washington State Department of Ecology, Water Quality Program. Olympia, Washington. June. Available at: https://fortress.wa.gov/ecy/publications/SummaryPages/9580.html

EPA. 1995. *Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms, 1st ed.* EPA/600/R-95/136. U.S. Environmental Protection Agency, National Exposure Research Laboratory, Cincinnati, OH.

EPA. 2000. *Record of Decision: Wyckoff/Eagle Harbor Superfund Site Soil and Groundwater Operable Units, Bainbridge Island, Washington*. EPA/ROD/R10-00/047. U.S. Environmental Protection Agency Region 10, Seattle, WA.

Washington Administrative Code (WAC) 173-205-020, "*Definitions*." Available at: https://app.leg.wa.gov/WAC/default.aspx?cite=173-205-020

WAC 173-205-090, "*Response to noncompliance with whole effluent toxicity limits.*" Available at: https://app.leg.wa.gov/WAC/default.aspx?cite=173-205-090

Attachment 1
EcoAnalysts Toxicity Testing Results
Wyckoff/Eagle Harbor Superfund Groundwater Treatment Plant

EcoAnalysts, Inc.

TOXICITY TESTING RESULTS

WYCKOFF/EAGLE HARBOR SUPERFUND SITE GROUNDWATER TREATMENT PLANT
BAINBRIDGE ISLAND, WA

NPDES TOXICITY TESTING: 2ND QUARTER 2024

Prepared for
Jacobs
1100 112th Avenue NE, Suite 400
Bellevue, WA 98004

Prepared by
EcoAnalysts, Inc.
PO Box 216
4770 NE View Drive
Port Gamble, WA 98364

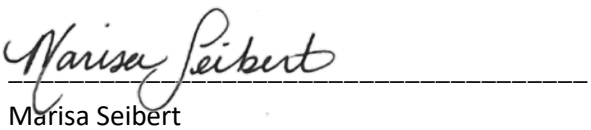
EcoAnalysts Report ID: PG1958Q2.01

Submittal Date: May 16, 2024

NELAP ACCREDITED TNI LABORATORY
Accredited in accordance with NELAP, ORELAP ID 4165

All testing reported herein was performed consistent with our laboratory's quality assurance program. All results are intended to be considered in their entirety, and EcoAnalysts is not responsible for use of less than the complete report. The test results summarized in this report apply only to the sample(s) evaluated. This document is uncontrolled when printed or accessed from electronic distribution.

APPROVED BY



Marisa Seibert

Laboratory Manager/ Project Manager

Author(s):

Marisa Seibert

QA Review:

Dani Mulligan

CONTENTS

TABLES

APPENDICES

ACRONYMS AND ABBREVIATIONS

EC_{50}:	Effect Concentration to 50% of test population
EPA:	Environmental Protection Agency
LC_{50}:	Lethal Concentration to 50% of test population
LOEL:	Lowest Observed Effect Level
NOEL:	No Observed Effect Level
NPDES:	National Pollutant Discharge Elimination System
PMSD:	Percent Minimum Significant Difference
QAPP:	Quality Assurance Project Plan
QM:	Quality Manual
SOP:	Standard Operating Procedures
WET:	Whole Effluent Toxicity

1. EXECUTIVE SUMMARY

EcoAnalysts conducted Whole Effluent Toxicity (WET) testing as part of the biological compliance monitoring for Wyckoff/Eagle Harbor Superfund Site, in Bainbridge Island, Washington. The objective of this program was to assess the potential toxicity of discharge water to selected aquatic organisms following procedures defined under the facility's Quality Assurance Project Plan (QAPP) (CH2M HILL 2022). The results of the toxicity testing are contained in this report.

The bivalve development was conducted as a side-by-side test, with one aliquot of effluent sample adjusted to test salinity with hypersaline brine, and another aliquot adjusted with artificial salts.

A statistically significant biological response of the test organisms was not detected at the 57.55% (brine, highest concentration achievable) and 100% (salt) effluent sample concentrations, for the proportion survived or proportion normal endpoints (Table 1-1).

Table 1-1. Toxicity Test Results Summary.

Test		NOEL (%)	LOEL (%)	LC_{50}/EC_{50} (%)
Chronic - Brine	*Mytilus galloprovincialis* 48-Hour Proportion Survived	57.55	>57.55	>57.55
	Mytilus galloprovincialis 48-Hour Proportion Normal	57.55	>57.55	>57.55
Chronic - Salt	*Mytilus galloprovincialis* 48-Hour Proportion Survived	100	>100	>100
	Mytilus galloprovincialis 48-Hour Proportion Normal	100	>100	>100

NOEL = No Observed Effect Level
LOEL = Lowest Observed Effect Level
LC_{50}/EC_{50} = Lethal/Effect Concentration to 50% of test population

2. METHODS

The sample was analyzed for toxicity using criteria outlined in ASTM E724-89 and the Environmental Protection Agency's (EPA) most recently promulgated effluent guidance documents outlined in Section 4.

To evaluate the relative sensitivity of the organisms, reference toxicity tests were performed using standard reference toxicants (Lee 1980).

2.1 Sample Collection and Storage

Jacobs personnel collected two samples on April 16, 2024, which were used to conduct the Bivalve Survival and Development side-by-side test. The samples were transported by EcoAnalysts personnel and received at the laboratory on the same day as collection. The sample temperatures upon receipt were 4.7°C and was 5.5°C. Both samples were within the recommended temperature range since they were received within 4 hours of collection. Samples were composited and used for testing.

Additional sample conditions are summarized in Table 2-1. The samples were held in a walk-in cold room at 4 ± 2 °C in the dark until utilized for testing.

Table 2-1. Sample Conditions upon Receipt

Sample	24162146_1
Laboratory ID	P240416.03
Date/Time sampled	4/16/24; 0928
Date/Time received	4/16/24; 1154
Dissolved Oxygen (mg/L) Recommended: >4.0 mg/L	8.3
Temperature (°C) Recommended: 0 – 6°C	4.7 – 5.5
pH (units) Recommended: 6 – 9	7.5
Conductivity (µS/cm)	NM
Salinity (ppt)	0.5
Total Chlorine (mg/L)	0.03
Total Ammonia (mg/L)	0.37

NM = Not Measured

2.2 Bioassay Testing

Bioassay testing for this project consisted of one chronic bioassay. The test conducted in support of this project is summarized in Table 2-2.

Table 2-2. Biological Testing Performed

Test Type	Test Descriptor	Species	Method
Chronic	48-Hour Survival and Development	*Mytilus galloprovincialis* Mussel	EPA/600/R-95-136 Method 1005.0; ASTM E724-89; TOX042.12

2.3 Organisms for Testing

Adult mussels (*Mytilus galloprovincialis*) were obtained from Taylor Shellfish in Shelton, Washington on April 3, 2024. They were delivered via Taylor Shellfish personnel and the overall health of the organisms was visually confirmed by a laboratory technician. The organisms were maintained under ambient seawater flow-through conditions at 12 ± 3˚C until utilized for testing. Water quality and observations were conducted every other day to ensure organisms remained in good health.

2.4 Water for Bioassay Testing

Seawater diluent used in this study came from the northern Hood Canal at Port Gamble, Washington. This water source has been used successfully on similar bioassay testing programs. Extensive testing on a variety of test species has shown that there is no significant potential for toxicity or bioaccumulation from this water supply. Chemical analysis of each water source is conducted and reviewed on an annual basis.

2.5 Sample Adjustment

The effluent sample 24162146_1 was received at a salinity of 0.5 ppt. The salinity of the effluent sample was increased by the addition of Crystal Sea® MarineMix bioassay grade artificial salt for the salt portion of the side-by-side bivalve test. A separate aliquot was adjusted to the desired test salinity using hypersaline brine for the other half of the side-by-side bivalve test. Table 2-3 summarizes the salinity adjustments performed on the project sample in relation to marine test species.

An artificial salt control sample was created to evaluate any potential negative impacts to the test organisms from the salinity adjustment alone. This sample was designated “Salt Control”. A “Brine Control” was also prepared for the bivalve test that included an equal proportion of hypersaline brine added to a mixture of natural seawater (Lab Control) and deionized water. The results of this additional control are discussed in the sections below.

Table 2-3. Salinity Adjustment of Project Samples

Sample ID	Test	Sample Salinity Upon Receipt	Sample Salinity Adjustment (ppt)	Salinity Adjustment Media
24162146_1: Collected 4/16/24	*Mytilus galloprovincialis* 48-Hour Survival and Development	0.5 ppt	30 ± 2	Hypersaline Brine
				Artificial Salt

2.6 Data Management and Analysis

Endpoint data was calculated for each replicate, and the mean value and standard deviation were determined for each sample concentration. All hand-entered data was reviewed for data entry errors, which were corrected prior to summary calculations. A minimum of 10% of all calculations and data sorting was reviewed for errors. Review counts were conducted on any apparent outliers.

Statistical comparisons were made according to the EPA guidance. Statistical comparisons were performed using CETIS™ software.

2.7 Quality Assurance/Quality Control

The quality assurance objectives for toxicity testing conducted by the testing laboratory are detailed in the method specific guidance documents and the laboratory’s quality manual (QM). These objectives for accuracy and precision involve all aspects of the testing process, including the following:

- Source and Condition of Test Organisms
- Condition of Equipment

- Test Conditions
- Instrument Calibration
- Use of Reference Toxicants
- Record Keeping
- Data Evaluation

The batch of test organisms obtained was evaluated in a reference toxicant test that was run concurrently with the test period to establish the sensitivity of the test organisms. The reference toxicant LC_{50} or EC_{50} should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

The methods employed in every phase of the toxicity testing program are detailed in the EcoAnalysts Standard Operating Procedures (SOP). All EcoAnalysts staff members receive regular, documented training in all SOPs and test methods. Finally, all data collected and produced because of these analyses were recorded on approved data sheets. If an aspect of a test deviated from protocol, the test was evaluated to determine whether it was valid according to the regulatory agencies responsible for approval of the proposed permitting action.

3. RESULTS

The results of the effluent testing are presented in this section. Statistical comparisons and laboratory documents are provided in Appendix A. Chain-of-custody and sample receipt logs are provided in Appendix B.

3.1 *Mytilus galloprovincialis* Test Results

The chronic toxicity test with *M. galloprovincialis* was conducted on April 16, 2024, with sample 24162146_1. The test was conducted as a side-by-side exposure with one aliquot of sample adjusted to test salinity with hypersaline brine and a second aliquot adjusted with artificial salts. Both tests met EPA test acceptability criteria of ≥90% proportion normal, ≥50% proportion survived, and <25% Percent Minimum Significant Difference (PMSD). The test conducted with hypersaline brine resulted in 93.2% proportion survived, 92.1% proportion normal, and 4.7% PMSD for proportion normal in the laboratory control. The test conducted with artificial salts resulted in 93.8% proportion survived, 93.9% proportion normal, and 6.1% PMSD for proportion normal in the laboratory control. Mean survival and proportion normal are summarized in Table 3-1 (brine) and Table 3-2 (salt). The test conditions are summarized in Table 3-3.

Concentrations of 6.25, 12.5, 25, 50, and 57.55% effluent were prepared utilizing laboratory water. A 100% test concentration was also included for the test with artificial salts. Sample P240416.03 (received 4/16/24) was used for test initiation. Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static test. Replicate 2 of the 12.5% effluent concentration in the salt test was removed from statistical analysis because the vial was compromised.

No significant differences were observed between the laboratory (dilution water) control and salt control indicating that the addition of artificial salt did not contribute to any negative biological effects. There was a significant difference between the laboratory (dilution water) control and brine control. However, since there wasn't an effect in any of the test concentrations, hypersaline brine did not contribute to any negative biological effects.

The EC_{50} for the ammonia reference toxicant test was 6.4 mg/L total ammonia and was within two standard deviations of the laboratory mean (Table 3-3) at the time of testing. This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-1. Results Summary for *Mytilus galloprovincialis* Embryo Development Test (Brine)

Conc. (%)	Mean Proportion Survived (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC_{50} Value (%)
Control	93.2	6.9	57.55	>57.55	>57.55
Brine Control	94.5	6.9			
6.25	98.5	3.0			
12.5	89.7	4.4			
25	95.7	5.5			
50	92.7	6.8			
57.55	96.7	3.9			
Conc. (%)	**Mean Proportion Normal (%)**	**Standard Deviation**	**NOEL (%)**	**LOEL (%)**	**EC_{50} Value (%)**
Control	92.1	1.2	57.55	>57.55	>57.55
Brine Control	89.0	2.4			
6.25	95.3	2.8			
12.5	94.0	2.0			
25	93.9	1.4			
50	93.9	2.3			
57.55	92.7	1.9			

NOEL = No Observed Effect Level;
LOEL = Lowest Observed Effect Level;
LC_{50}/EC_{50} = Lethal/Effect Concentration to 50% of test population;
Proportion survived = total counted / stocking density;
Proportion normal = number normal/total counted

Table 3-2. Results Summary for *Mytilus galloprovincialis* Embryo Development Test (Salt)

Conc. (%)	Mean Proportion Survived (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC_{50} Value (%)
Control	93.8	5.5	100	>100	>100
Salt Control	88.2	10.3			
6.25	99.3	1.0			
12.5	90.9	2.0			
25	97.2	4.8			
50	92.5	6.4			
57.55	89.8	7.0			
100	90.8	7.2			
Conc. (%)	**Mean Proportion Normal (%)**	**Standard Deviation**	**NOEL (%)**	**LOEL (%)**	**EC_{50} Value (%)**
Control	93.9	1.3	100	>100	>100
Salt Control	95.4	2.7			
6.25	94.8	1.2			
12.5	92.9	3.1			
25	95.8	1.9			
50	91.4	0.9			
57.55	95.4	3.8			
100	92.5	1.6			

NOEL = No Observed Effect Level;
LOEL = Lowest Observed Effect Level;
LC_{50}/EC_{50} = Lethal/Effect Concentration to 50% of test population;
Proportion survived = total counted / stocking density;
Proportion normal = number normal/total counted

Table 3-3. Test Condition Summary for *Mytilus galloprovincialis* Embryo Development Test.

Test Duration / Type	**48-Hour; Static**	
Species	*Mytilus galloprovincialis*	
Supplier	Taylor Shellfish	
Date acquired	4/03/24	
Test Dates	4/16/24 – 4/18/24	
Age at test initiation Recommended: <4-hour embryos	<4 hours	
Sample(s) used:	24162146_1; P240416.03	
Holding Time at Initiation: Recommended: < 36 hours	7 hours	
Test Procedures	**EPA/600/R-95-136, Method 1005.0; SOP: TOX042.12**	
Test location	EcoAnalysts, Port Gamble, WA	
Control water / Diluent	0.45 µm-filtered, North Hood Canal seawater	
Test Lighting	16 hour light / 8 hour dark	
Test Chamber	30-mL Chamber	
Exposure volume	10 mL	
Organisms/replicate	Recommended: 150 –300	Actual: 150
Replicates/treatment	4	
Concentration/treatment	6.25, 12.5, 25, 50, and 57.55% (brine) 6.25, 12.5, 25, 50, 57.55 and 100% (salt)	
Feeding	None	
Test solution renewal	None	
Test Water Quality		
Test Dissolved Oxygen	Recommended: > 4.0 mg/L	Actual: 7.8 – 8.5 mg/L (brine), 7.9 – 8.8 mg/L (salt)
Test Temperature	Recommended: 16 ± 1°C	Actual: 16.1 – 17.4 °C (brine), 16.1 – 17.3 °C (salt)
Test pH	Recommended: 7 – 9	Actual: 7.5 – 8.1 (brine), 7.7 – 8.3 (salt)
Test Salinity	Recommended: 30 ± 2 ppt	Actual: 28 – 32 ppt (brine), 28 – 32 ppt (salt)
Control performance standard (Survival, Normal shell development, PMSD)	Recommended: ≥50% survival, ≥90% normal development, <25% PMSD	Actual: Brine: 93.2% survival, 92.1% normal development, 4.7% PMSD; Salt: 93.8% survival, 93.9% normal development, 6.1% PMSD
Reference Toxicant Date	4/16/24	
Reference Toxicant EC_{50}	6.4 mg/L total ammonia	
Laboratory Mean EC_{50}	7.6 mg/L total ammonia	
Acceptable Range EC_{50} (± 2 SD)	4.3 – 13.3 mg/L total ammonia (within range)	
Deviations from Test Protocol	Salt 12.5% Replicate 2 removed from analysis	

4. REFERENCES

ASTM. 1989. Standard Guide for Conducting Static Acute Toxicity Tests Starting with Embryos of Saltwater Bivalve Molluscs, E724-89. ASTM International, West Conshohocken, PA.

CETIS. 2022. CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.

CH2M HILL. 2022. Quality Assurance Project Plan, Groundwater Treatment Plant Operations and Maintenance, Final. Wyckoff/Eagle Harbor Superfund Site. Bainbridge Island, Washington.

USEPA. 1995. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine Organisms and Estuarine Organisms, First Edition. EPA-600-R-95-136.

APPENDIX A

STATISTICAL COMPARISONS AND LABORATORY DOCUMENTS

APPENDIX A.1

MYTILUS GALLOPROVINCIALIS 48-HOUR SURVIVAL AND DEVELOPMENT TEST

STATISTICAL COMPARISON AND LABORATORY DATA SHEETS

Bivalve Larval Survival and Development Test **EcoAnalysts**

Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**			
Start Date:	16 Apr-24 16:39	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater		
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater		
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish	**Age:**	
Sample ID:	09-7058-0144	**Code:**	P240416.03	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W		
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff		
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1		
Sample Age:	7h	**Client:**	Jacobs Wyckoff				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
10-0241-7906	Proportion Normal	Dunnett Multiple Comparison Test		57.55	>57.55	---	4.68%	1.7	1
21-1390-8293	Proportion Survived	Dunnett Multiple Comparison Test		57.55	>57.55	---	13.3%	1.7	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
03-9675-8499	Proportion Normal	Linear Interpolation (ICPIN)	✓	EC25	>57.55	---	---	<1.7	1
			✓	EC50	>57.55	---	---	<1.7	
17-7236-7661	Proportion Survived	Linear Interpolation (ICPIN)	✓	EC25	>57.55	---	---	<1.7	1
			✓	EC50	>57.55	---	---	<1.7	

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
03-9675-8499	Proportion Normal	Control Resp	0.9214	0.9	<<	Yes	Passes Criteria
10-0241-7906	Proportion Normal	Control Resp	0.9214	0.9	<<	Yes	Passes Criteria
17-7236-7661	Proportion Survived	Control Resp	0.9317	0.5	<<	Yes	Passes Criteria
21-1390-8293	Proportion Survived	Control Resp	0.9317	0.5	<<	Yes	Passes Criteria

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9214	0.9029	0.9398	0.9134	0.9384	0.0058	0.0116	1.26%	0.00%
0	BC	4	0.8896	0.8520	0.9271	0.8583	0.9128	0.0118	0.0236	2.65%	3.45%
6.25		4	0.9529	0.9087	0.9970	0.9149	0.9812	0.0139	0.0278	2.91%	-3.42%
12.5		4	0.9400	0.9080	0.9720	0.9200	0.9643	0.0101	0.0201	2.14%	-2.02%
25		4	0.9393	0.9172	0.9614	0.9248	0.9574	0.0070	0.0139	1.48%	-1.94%
50		4	0.9392	0.9022	0.9762	0.9051	0.9562	0.0116	0.0233	2.48%	-1.93%
57.55		4	0.9272	0.8972	0.9573	0.9030	0.9429	0.0094	0.0189	2.04%	-0.63%

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9317	0.8220	1.0410	0.8467	1.0000	0.0345	0.0689	7.40%	0.00%
0	BC	4	0.9450	0.8350	1.0550	0.8467	0.9933	0.0346	0.0692	7.32%	-1.43%
6.25		4	0.9850	0.9373	1.0330	0.9400	1.0000	0.0150	0.0300	3.05%	-5.72%
12.5		4	0.8967	0.8260	0.9673	0.8333	0.9333	0.0222	0.0444	4.95%	3.76%
25		4	0.9567	0.8698	1.0430	0.8867	1.0000	0.0273	0.0546	5.70%	-2.68%
50		4	0.9267	0.8188	1.0350	0.8400	1.0000	0.0339	0.0678	7.31%	0.54%
57.55		4	0.9667	0.9054	1.0280	0.9333	1.0000	0.0193	0.0385	3.98%	-3.76%

Bivalve Larval Survival and Development Test

EcoAnalysts

Proportion Normal Detail

MD5: 2996717A20F40F21FEE003D468193276

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9134	0.9191	0.9384	0.9146
0	BC	0.9128	0.8583	0.9014	0.8859
6.25		0.9149	0.9605	0.9812	0.9548
12.5		0.9643	0.9275	0.9200	0.9481
25		0.9248	0.9333	0.9416	0.9574
50		0.9444	0.9051	0.9562	0.9510
57.55		0.9429	0.9214	0.9030	0.9416

Proportion Survived Detail

MD5: D9AAD70D0AC03F87EFCD01B7BF355E8C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8467	0.9067	0.9733	1.0000
0	BC	0.9933	0.8467	0.9467	0.9933
6.25		0.9400	1.0000	1.0000	1.0000
12.5		0.9333	0.9200	0.8333	0.9000
25		0.8867	1.0000	1.0000	0.9400
50		0.8400	1.0000	0.9133	0.9533
57.55		0.9333	0.9333	1.0000	1.0000

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	116/127	125/136	137/146	150/164
0	BC	136/149	109/127	128/142	132/149
6.25		129/141	146/152	157/160	148/155
12.5		135/140	128/138	115/125	128/135
25		123/133	140/150	145/154	135/141
50		119/126	143/158	131/137	136/143
57.55		132/140	129/140	149/165	145/154

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	127/150	136/150	146/150	150/150
0	BC	149/150	127/150	142/150	149/150
6.25		141/150	150/150	150/150	150/150
12.5		140/150	138/150	125/150	135/150
25		133/150	150/150	150/150	141/150
50		126/150	150/150	137/150	143/150
57.55		140/150	140/150	150/150	150/150

CETIS Analytical Report

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	06-4775-3194	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	09 May-24 9:45	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:26	**MD5 Hash:**	4DE3D7CA55B5706E5252276D6FB47DCE	**Editor ID:**	003-841-189-5
Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	16 Apr-24 16:39	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish **Age:**
Sample ID:	09-7058-0144	**Code:**	P240416.03	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1
Sample Age:	7h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Brine Control passed combined proportion normal endpoi	15.69%

Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Brine Control	6	0.7793	1.943	0.2188	CDF	0.2327	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
PMSD	0.1569	<<	0.25	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0153974	0.0153974	1	0.6073	0.4654	Non-Significant Effect
Error	0.152134	0.0253556	6			
Total	0.167531		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	1.258	13.75	0.3048	Equal Variances
	Mod Levene Equality of Variance Test	1.093	13.75	0.3360	Equal Variances
	Variance Ratio F Test	3.647	47.47	0.3161	Equal Variances
Distribution	Anderson-Darling A2 Test	0.2891	3.878	0.6451	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1489	0.3313	1.0000	Normal Distribution
	Shapiro-Wilk W Normality Test	0.943	0.6451	0.6408	Normal Distribution

Combined Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	4	0.8417	0.7148	0.9685	0.8667	0.7267	0.9067	0.0399	9.47%	4.36%
0	D	4	0.8800	0.7234	1.0000	0.8733	0.7733	1.0000	0.0492	11.19%	0.00%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	4	1.1690	1.0030	1.3350	1.1970	1.0210	1.2600	0.0522	8.94%	6.98%
0	D	4	1.2570	0.9393	1.5740	1.2110	1.0750	1.5300	0.0998	15.87%	0.00%

Combined Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	0.9067	0.7267	0.8533	0.8800
0	D	0.7733	0.8333	0.9133	1.0000

Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	1.2600	1.0210	1.1780	1.2170
0	D	1.0750	1.1500	1.2720	1.5300

Bivalve Larval Survival and Development Test

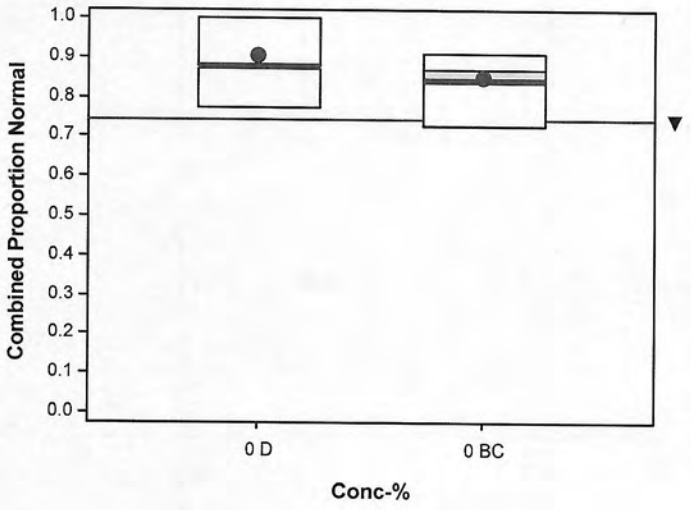
EcoAnalysts

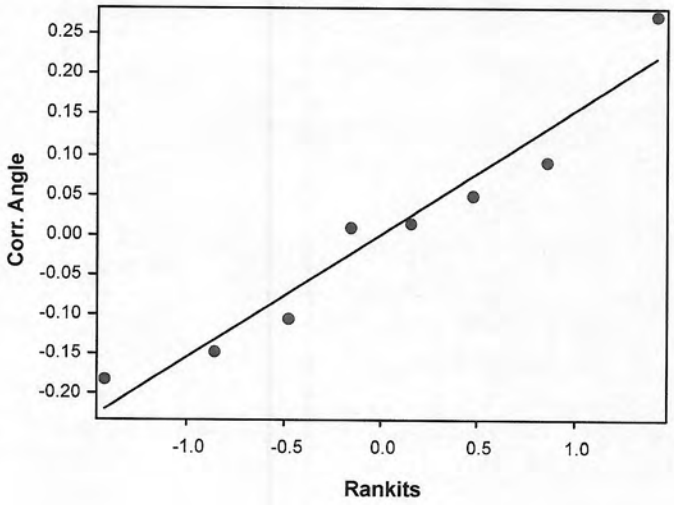
Analysis ID:	06-4775-3194	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	09 May-24 9:45	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:26	**MD5 Hash:**	4DE3D7CA55B5706E5252276D6FB47DCE	**Editor ID:**	003-841-189-5

Combined Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	136/150	109/150	128/150	132/150
0	D	116/150	125/150	137/150	150/150

Graphics





Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	13-3750-3764	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	09 May-24 9:30	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:26	**MD5 Hash:**	5D4A3909DCDFE6823EFE0D43C7D273A5	**Editor ID:**	003-841-189-5
Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	16 Apr-24 16:39	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish **Age:**
Sample ID:	09-7058-0144	**Code:**	P240416.03	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1
Sample Age:	7h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Brine Control failed proportion normal endpoint	2.58%

Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Brine Control*	6	2.484	1.943	0.0421	CDF	0.0238	Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.9214	0.9	<<	Yes	Passes Criteria
Control Resp	0.8896	0.9	<<	Yes	Below Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0057931	0.0057931	1	6.171	0.0475	Significant Effect
Error	0.0056328	0.0009388	6			
Total	0.0114259		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	1.076	13.75	0.3396	Equal Variances
	Mod Levene Equality of Variance Test	1.052	13.75	0.3446	Equal Variances
	Variance Ratio F Test	2.788	47.47	0.4221	Equal Variances
Distribution	Anderson-Darling A2 Test	0.3238	3.878	0.5444	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1915	0.3313	0.6336	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9406	0.6451	0.6175	Normal Distribution

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	4	0.8896	0.8520	0.9271	0.8937	0.8583	0.9128	0.0118	2.65%	3.45%
0	D	4	0.9214	0.9029	0.9398	0.9169	0.9134	0.9384	0.0058	1.26%	0.00%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	4	1.2330	1.1740	1.2930	1.2390	1.1850	1.2710	0.0186	3.01%	4.18%
0	D	4	1.2870	1.2520	1.3230	1.2780	1.2720	1.3200	0.0111	1.73%	0.00%

Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	0.9128	0.8583	0.9014	0.8859
0	D	0.9134	0.9191	0.9384	0.9146

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	13-3750-3764	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	09 May-24 9:30	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:26	**MD5 Hash:**	5D4A3909DCDFE6823EFE0D43C7D273A5	**Editor ID:**	003-841-189-5

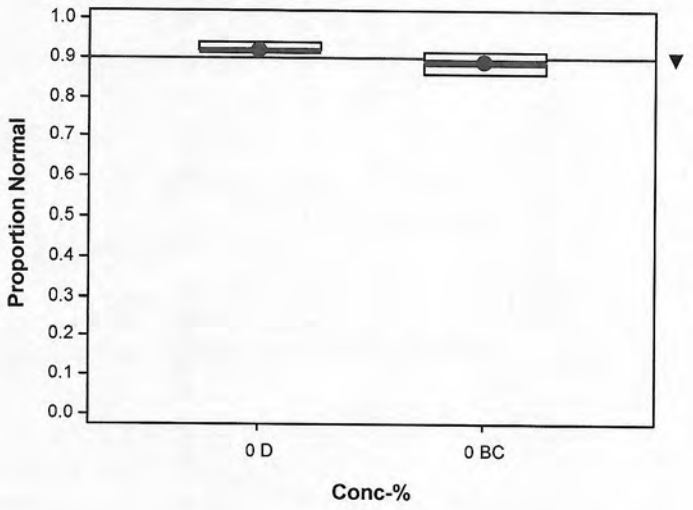
Angular (Corrected) Transformed Detail

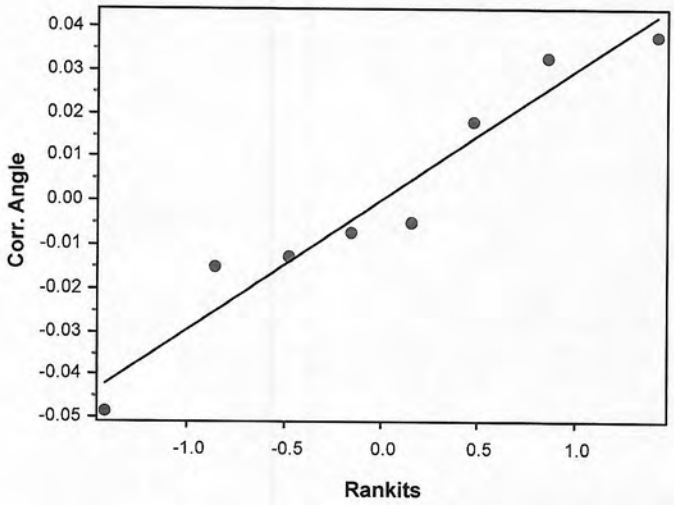
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	1.2710	1.1850	1.2510	1.2260
0	D	1.2720	1.2820	1.3200	1.2740

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	136/149	109/127	128/142	132/149
0	D	116/127	125/136	137/146	150/164

Graphics





Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	17-5447-6838	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv2.1.4
Analyzed:	09 May-24 9:30	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:26	**MD5 Hash:**	7670037C034DFC9D30470810E642B673	**Editor ID:**	003-841-189-5
Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	16 Apr-24 16:39	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish **Age:**
Sample ID:	09-7058-0144	**Code:**	P240416.03	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1
Sample Age:	7h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Brine Control passed proportion survived endpoint	12.47%

Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Brine Control	6	-0.269	1.943	0.2146	CDF	0.6015	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.9317	0.5	<<	Yes	Passes Criteria
Control Resp	0.945	0.5	<<	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0017646	0.0017646	1	0.07236	0.7969	Non-Significant Effect
Error	0.146318	0.0243862	6			
Total	0.148082		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	0.03726	13.75	0.8533	Equal Variances
	Mod Levene Equality of Variance Test	0.02903	13.75	0.8703	Equal Variances
	Variance Ratio F Test	1.09	47.47	0.9451	Equal Variances
Distribution	Anderson-Darling A2 Test	0.3081	3.878	0.5884	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1745	0.3313	0.8586	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9313	0.6451	0.5283	Normal Distribution

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	4	0.9450	0.8350	1.0000	0.9778	0.8467	0.9933	0.0346	7.32%	-1.43%
0	D	4	0.9317	0.8220	1.0000	0.9400	0.8467	1.0000	0.0345	7.40%	0.00%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	4	1.3710	1.1280	1.6140	1.4390	1.1680	1.4890	0.0764	11.14%	-2.21%
0	D	4	1.3410	1.0880	1.5950	1.3340	1.1680	1.5300	0.0798	11.89%	0.00%

Proportion Survived Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	0.9933	0.8467	0.9467	0.9933
0	D	0.8467	0.9067	0.9733	1.0000

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	17-5447-6838	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv2.1.4
Analyzed:	09 May-24 9:30	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:26	**MD5 Hash:**	7670037C034DFC9D30470810E642B673	**Editor ID:**	003-841-189-5

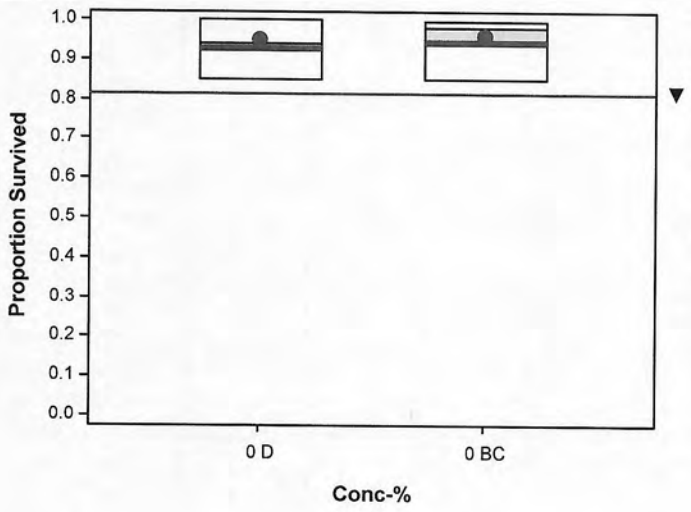
Angular (Corrected) Transformed Detail

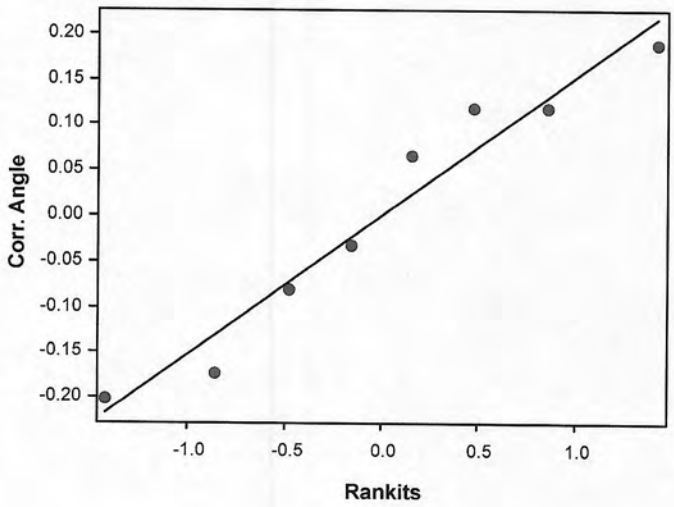
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	1.4890	1.1680	1.3380	1.4890
0	D	1.1680	1.2600	1.4070	1.5300

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	BC	149/150	127/150	142/150	149/150
0	D	127/150	136/150	146/150	150/150

Graphics





CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — EcoAnalysts

Start Date:	16 Apr-24 16:39	**Species:**	Mytilus galloprovincialis	**Sample Code:**	P240416.03
End Date:	18 Apr-24 16:32	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs Wyckoff
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Sample Station:**	24162146_1

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	12	150	149	149	136	
0	BC	2	7	150	127	127	109	
0	BC	3	11	150	142	142	128	
0	BC	4	3	150	149	149	132	
0	D	1	18	150	127	127	116	
0	D	2	26	150	136	136	125	
0	D	3	16	150	146	146	137	
0	D	4	17	150	164	164	150	
6.25		1	6	150	141	141	129	
6.25		2	14	150	152	152	146	
6.25		3	8	150	160	160	157	
6.25		4	9	150	155	155	148	
12.5		1	5	150	140	140	135	
12.5		2	27	150	138	138	128	
12.5		3	2	150	125	125	115	
12.5		4	23	150	135	135	128	
25		1	24	150	133	133	123	
25		2	25	150	150	150	140	
25		3	28	150	154	154	145	
25		4	20	150	141	141	135	
50		1	15	150	126	126	119	
50		2	21	150	158	158	143	
50		3	13	150	137	137	131	
50		4	1	150	143	143	136	
57.55		1	4	150	140	140	132	
57.55		2	10	150	140	140	129	
57.55		3	19	150	165	165	149	
57.55		4	22	150	154	154	145	

Analyst: _____ QA: _____

EcoAnalysts, Inc.

48-Hour Chronic WET Test

Version V.2

GENERAL

Client	Jacobs Wyckoff
Project	Wyckoff Eagle Harbor GWTP 2024/WA
Project Number	PG1958
Project Manager	**M. Seibert**
Date Sample Received	4/16/2024
Test type	**48-Hour Chronic Toxicity Using Bivalve Larvae**
Matrix	**Liquid**
Test Acceptability	≥90% normal shell development, ≥50% survival (mussels) or ≥70% survival (oysters), MSD <25%
Test Start Date	04/16/24
Test Species	**Mytilus spp.**
Organism Batch	**TS040324.01**
Organism Acquired	4/3/2024
Organism Acclimation	13
Organism Age	<4 hr old embryos
Test Protocol	**TOX 042**
Test Location	**Incubator 1**
Light Intensity	**50-100 foot candles**
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	150 - 300
Test Chamber Size	30 mL
Exposure Volume	10 mL
Test Dissolved Oxygen	> 4.0
Test Temperature	16 ± 1
Test Salinity	30 ± 2
Test pH	8 ± 1

Note: input lowest and highest decimal for temp

Test Parameters	Min	Max
DO	4.0	
Temp	15	17
Salinity	28	32
pH	7	9

TEST START TIME/INIT:	1639 MS
TEST END TIME/INIT:	1632 RG/TVL

CLIENT SAMPLE ID	LAB ID
24162146_1	P240416.03

Salinity Adjustment CSMM Batch #
62123

Formalin Lot #
230724-07

Rose Bangel Batch #
5135

Concentrations

1	Control
2	Brine Control
3	6.25%
4	12.5%
5	25%
6	50%
7	57.55%
8	.
9	.

SALINITY ADJUSTMENT AND TEST DILUTION PREPARATION WORKSHEET

Only red characters and green cells are changeable.

ORGANISM	CLIENT	CLIENT SAMPLE ID	DATE
M. sp.	Jacobs Wyckoff	24162146_1	4/16/24

Volume per Concentration (mls) - 200

Test Parameters	ppt
Salinity of Brine	70.00
Salinity of Sample	0.50
Test Salinity	30.00

Salinity Adjustment Multiplier = **0.74**

		grams added
mls. Sample*	500.00	499.3
mls. Brine	368.75	387.9
*Adjust volume so C16>F19		**887.19**
Post Adjustment Concentration (%) =		**57.55**

Test Dilution Preparation **(List highest to lowest!)**

Concentration (%)	Amount of Adjusted Sample (gms.)	Amount of Seawater (gms.)
57.55	204.2	0.0
50.00	177.4	26.8
25.00	88.7	115.5
12.50	44.4	159.9
6.25	22.2	182.1
	0.0	204.2
.	**536.93**	

Brine Control Preparation

Salinity Adjustment			highest	Amount Brine	Amount DI	Amount Seawater
Sample Number/Name	Multiplier	Volume BC	concentration	(grams)	(grams)	(grams)
24162146_1	0.74	200	57.6	87.4	110.8	6.0

Worksheet Preparation Date / Initials

4/16/2024	MS

Dilution Preparation Date / Initials

4/16/2024	MS CS

① D'd techs - MS 4/16

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	DATE RECEIVED	4/16/24	PROTOCOL	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2024/WA	TEST START DATE	4/16/24	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	24162146_1	TEST END DATE	4/18/24	SPECIES	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	MATRIX	Liquid	NO. OF ORGANISMS	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

SPAWNING METHOD Heat shock		INITIAL SPAWNING TIME 1327	FINAL SPAWNING TIME 1440
MALES 4	FEMALES 3	SPERM VIABILITY Good	EGG CONDITION Good
BEGIN FERTILIZATION 1440		END FERTILIZATION 1643	CONDITION OF EMBRYOS Good

TIME OF INITIATION	INITIALS
16:39	MS

EMBRYO DENSITY CALCULATIONS

of embryos in 1 mL of 100X diluted embryo stock

Count 1	Count 2	Mean
243	246	244.5

embryos in original stock = # of embryos in diluted stock x 100

24450

Percentage of embryo stock needed = 2700 embryos per 1 mL/# embryos in original stock

0.11

mL of egg stock to add to FSW to achieve total volume = percentage of embro stock needed * 40 mL (or desired volume of embryo stock)

4.417177914 Add this volume to beaker and dilute to 40 mL (or desired volume of embryo stock) with FSW = final embryo stock

Add 0.1 mL of final embryo stock to test chambers

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	koff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

		DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
	Concentration (%)	> 4.0	15 - 17	28 - 32	7 - 9
Day 0	Control	8.3	17.4	29	7.8
Stock	Brine Control	8.3	17.2	29	7.8
Date 4/16/24	6.25%	8.4	17.3	28	7.8
Time 1621	12.5%	8.4	17.3	28	7.8
Tech MS	25%	8.5	17.0	28	7.7
Meter # 7	50%	8.5	16.9	29	7.5
	57.55%	8.5	16.8	30	7.5
Day 1	Control		16.1 ①		
Surrogate	Brine Control		16.1 ①		
Date 4/17/24	6.25%		16.1 ①		
Time 1410	12.5%		16.1 ①		
Tech WD	25%		16.1 ①		
Meter # T33	50%		16.1 ①		
	57.55%		16.1 ①		
Day 2	Control	8.0	16.1 ①	30	7.8
Surrogate	Brine Control	7.8	16.1 ①	31	7.8
Date 4/18/24	6.25%	7.9	16.1 ①	30	7.9
Time 0950	12.5%	8.1	16.1 ①	30	8.0
Tech NL	25%	8.0	16.1 ①	30	8.1
Meter # 8/T33	50%	7.9	16.1 ①	31	8.1
	57.55%	7.9	16.1 ①	32	8.1

① - TEMP BLANK USED - WD 4/17/24, NL 4/18

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

Concentration (%)	REP	Normal	Abnormal	Date	Tech	Comments/QA Counts
Stocking Density	1	134		5/4/24	SR	
	2	136		5/4/24	SR	
	3	158		5/4/24	SR	
	4	153		5/4/24	SR	
	5	166		5/4/24	SR	
	6	150		5/4/24	SR	
Control	1	116	11	4/27/24	NL	
	2	125	11	4/27/24	NL	
	3	137	9	4/27/24	NL	QA-136N 9AB-DM 5/8/24
	4	150	14	4/27/24	NL	
Brine Control	1	136	13	5/6/24	NS	
	2	109	18	5/6/24	NS	
	3	120	14	5/6/24	NS	
	4	132	17	5/6/24	NS	
6.25%	1	129	12	5/7/24	MS	① QA: 137N 11AB-DM 5/8/24
	2	146	6	5/7/24	MS	
	3	157	3	5/7/24	MS	
	4	148	7	5/7/24	MS	
12.5%	1	135	5	5/7/24	MS	QA-130N 6AB-DM-5/8/24
	2	120	10	5/8/24	DM	
	3	115	10	5/8/24	DM	
	4	120	7	5/8/24	DM	
25%	1	123	10	5/7/24	MS	QA: 119N 12AB-NL 5/8/24
	2	140	10	5/8/24	DM	
	3	145	9	5/8/24	DM	
	4	135	6	5/8/24	DM	
50%	1	119	7	5/7/24	MS	
	2	143	15	5/8/24	DM	
	3	131	6	5/8/24	DM	
	4	136	9	5/8/24	DM	
57.55%	1	132	8	4/27/24	NL	
	2	129	11	4/27/24	NL	
	3	149	16	4/27/24	NL	
	4	145	9	4/27/24	NL	

① wrong sheet - DM - 5/8/24

CETIS Summary Report

SALT TEST

Test Code/ID: P240416.03SC / 16-9188-6602

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert		
Start Date:	16 Apr-24 16:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater		
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Crystal Sea Marine Mix		
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish	**Age:**	
Sample ID:	12-7656-3299	**Code:**	P240416.03SC	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W		
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff		
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1		
Sample Age:	7h	**Client:**	Jacobs Wyckoff				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
04-2173-0603	Proportion Normal	Bonferroni Adj t Test		100	>100	---	6.06%	1	1
01-7098-6746	Proportion Survived	Bonferroni Adj t Test		100	>100	---	15.4%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
15-1869-2605	Proportion Normal	Linear Interpolation (ICPIN)	✓	EC25	>100	---	---	<1	1
			✓	EC50	>100	---	---	<1	
01-7704-1777	Proportion Survived	Linear Interpolation (ICPIN)	✓	EC25	>100	---	---	<1	1
			✓	EC50	>100	---	---	<1	

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
04-2173-0603	Proportion Normal	Control Resp	0.9394	0.9	<<	Yes	Passes Criteria
15-1869-2605	Proportion Normal	Control Resp	0.9394	0.9	<<	Yes	Passes Criteria
01-7098-6746	Proportion Survived	Control Resp	0.9383	0.5	<<	Yes	Passes Criteria
01-7704-1777	Proportion Survived	Control Resp	0.9383	0.5	<<	Yes	Passes Criteria

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9394	0.9192	0.9596	0.9231	0.9510	0.0064	0.0127	1.35%	0.00%
0	SC	4	0.9541	0.9116	0.9966	0.9211	0.9843	0.0134	0.0267	2.80%	-1.57%
6.25		4	0.9481	0.9284	0.9678	0.9396	0.9660	0.0062	0.0124	1.31%	-0.93%
12.5		3	0.9288	0.8515	1.0060	0.9051	0.9640	0.0180	0.0311	3.35%	1.13%
25		4	0.9582	0.9275	0.9889	0.9355	0.9816	0.0096	0.0193	2.01%	-2.00%
50		4	0.9141	0.9001	0.9282	0.9023	0.9235	0.0044	0.0088	0.97%	2.69%
57.55		4	0.9540	0.8935	1.0140	0.9070	1.0000	0.0190	0.0380	3.98%	-1.55%
100		4	0.9254	0.8999	0.9509	0.9067	0.9416	0.0080	0.0160	1.73%	1.49%

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9383	0.8503	1.0260	0.8667	1.0000	0.0277	0.0553	5.90%	0.00%
0	SC	4	0.8817	0.7186	1.0450	0.7600	1.0000	0.0512	0.1025	11.62%	6.04%
6.25		4	0.9933	0.9783	1.0080	0.9800	1.0000	0.0047	0.0094	0.95%	-5.86%
12.5		3	0.9089	0.8583	0.9595	0.8867	0.9267	0.0118	0.0204	2.24%	3.14%
25		4	0.9717	0.8950	1.0480	0.9000	1.0000	0.0241	0.0482	4.96%	-3.55%
50		4	0.9250	0.8239	1.0260	0.8600	1.0000	0.0318	0.0636	6.87%	1.42%
57.55		4	0.8983	0.7873	1.0090	0.8467	1.0000	0.0349	0.0698	7.77%	4.26%
100		4	0.9083	0.7946	1.0220	0.8267	1.0000	0.0357	0.0715	7.87%	3.20%

Bivalve Larval Survival and Development Test EcoAnalysts

Proportion Normal Detail MD5: E5CCEDC710A450EF44321FEB215EAC36

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9357	0.9231	0.9510	0.9477
0	SC	0.9638	0.9474	0.9843	0.9211
6.25		0.9396	0.9660	0.9400	0.9467
12.5		0.9173	0.9640	0.9051	
25		0.9355	0.9630	0.9527	0.9816
50		0.9161	0.9147	0.9023	0.9235
57.55		0.9070	0.9539	1.0000	0.9549
100		0.9067	0.9416	0.9355	0.9179

Proportion Survived Detail MD5: D4981AC91DC6D4BEA964D6847FBD9F22

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9333	0.8667	0.9533	1.0000
0	SC	0.9200	1.0000	0.8467	0.7600
6.25		0.9933	0.9800	1.0000	1.0000
12.5		0.8867	0.9267	0.9133	
25		1.0000	0.9000	0.9867	1.0000
50		0.9533	0.8600	0.8867	1.0000
57.55		0.8600	1.0000	0.8467	0.8867
100		1.0000	0.9133	0.8267	0.8933

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	131/140	120/130	136/143	145/153
0	SC	133/138	144/152	125/127	105/114
6.25		140/149	142/147	141/150	160/169
12.5		122/133	134/139	124/137	
25		145/155	130/135	141/148	160/163
50		131/143	118/129	120/133	157/170
57.55		117/129	145/152	127/127	127/133
100		136/150	129/137	116/124	123/134

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	140/150	130/150	143/150	150/150
0	SC	138/150	150/150	127/150	114/150
6.25		149/150	147/150	150/150	150/150
12.5		133/150	139/150	137/150	
25		150/150	135/150	148/150	150/150
50		143/150	129/150	133/150	150/150
57.55		129/150	150/150	127/150	133/150
100		150/150	137/150	124/150	134/150

CETIS Analytical Report

Bivalve Larval Survival and Development Test — EcoAnalysts

Analysis ID:	10-6976-1990	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	15 May-24 15:42	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:36	**MD5 Hash:**	842DFD0AE9C6443F48788645DA856E2B	**Editor ID:**	003-841-189-5
Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert
Start Date:	16 Apr-24 16:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Crystal Sea Marine Mix
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish **Age:**
Sample ID:	12-7656-3299	**Code:**	P240416.03SC	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1
Sample Age:	7h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed combined proportion normal endpoint	15.16%

Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	0.5692	1.943	0.1906	CDF	0.2949	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
PMSD	0.1516	<<	0.25	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0062323	0.0062323	1	0.324	0.5899	Non-Significant Effect
Error	0.115422	0.019237	6			
Total	0.121654		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	0.3122	13.75	0.5966	Equal Variances
	Mod Levene Equality of Variance Test	0.3099	13.75	0.5979	Equal Variances
	Variance Ratio F Test	1.827	47.47	0.6328	Equal Variances
Distribution	Anderson-Darling A2 Test	0.2043	3.878	0.9161	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1447	0.3313	1.0000	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9665	0.6451	0.8689	Normal Distribution

Combined Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.8867	0.7761	0.9972	0.8900	0.8000	0.9667	0.0348	7.84%	0.00%
0	SC	4	0.8450	0.6704	1.0000	0.8600	0.7000	0.9600	0.0549	12.99%	4.70%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.2400	1.0550	1.4260	1.2340	1.1070	1.3870	0.0583	9.40%	0.00%
0	SC	4	1.1850	0.9337	1.4350	1.1890	0.9912	1.3690	0.0789	13.31%	4.50%

Combined Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8733	0.8000	0.9067	0.9667
0	SC	0.8867	0.9600	0.8333	0.7000

Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.2070	1.1070	1.2600	1.3870
0	SC	1.2270	1.3690	1.1500	0.9912

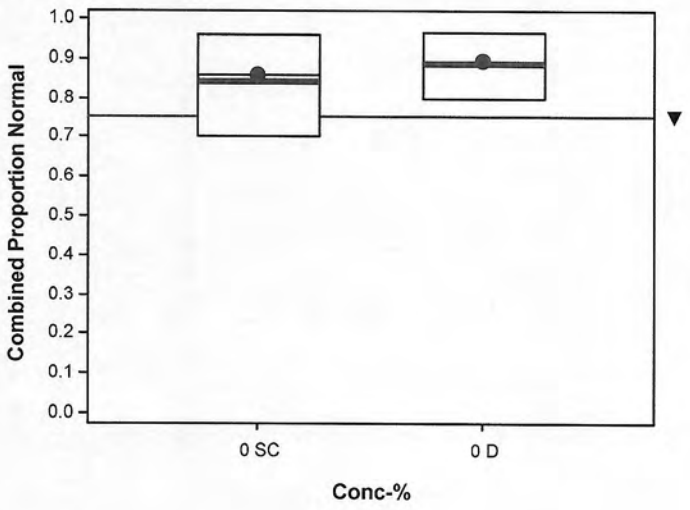
Bivalve Larval Survival and Development Test — **EcoAnalysts**

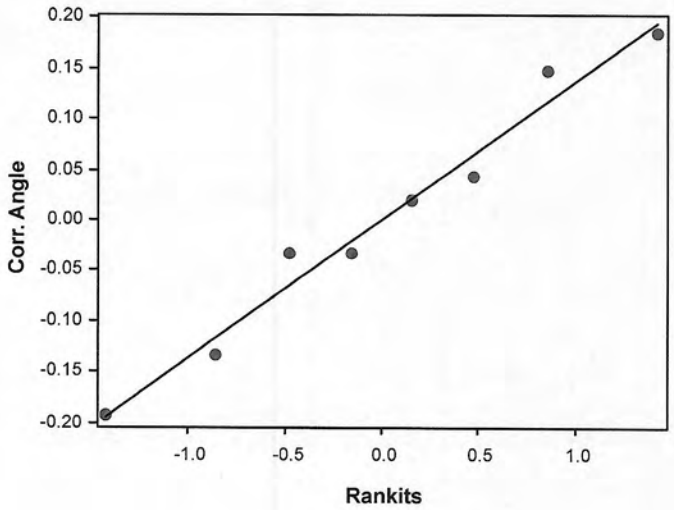
Analysis ID:	10-6976-1990	**Endpoint:**	Combined Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	15 May-24 15:42	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:36	**MD5 Hash:**	842DFD0AE9C6443F48788645DA856E2B	**Editor ID:**	003-841-189-5

Combined Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	131/150	120/150	136/150	145/150
0	SC	133/150	144/150	125/150	105/150

Graphics





Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	14-6564-4593	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	15 May-24 15:42	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:36	**MD5 Hash:**	369965426D5A1CD7304F01BCA1C649AF	**Editor ID:**	003-841-189-5
Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert
Start Date:	16 Apr-24 16:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Crystal Sea Marine Mix
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish
					Age:
Sample ID:	12-7656-3299	**Code:**	P240416.03SC	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1
Sample Age:	7h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed proportion normal endpoint	3.94%

Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	-1.094	1.943	0.06996	CDF	0.8420	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.9541	0.9	<<	Yes	Passes Criteria
Control Resp	0.9394	0.9	<<	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0031029	0.0031029	1	1.197	0.3159	Non-Significant Effect
Error	0.0155553	0.0025926	6			
Total	0.0186583		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	2.551	13.75	0.1614	Equal Variances
	Mod Levene Equality of Variance Test	2.48	13.75	0.1663	Equal Variances
	Variance Ratio F Test	6.478	47.47	0.1592	Equal Variances
Distribution	Anderson-Darling A2 Test	0.246	3.878	0.7842	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1751	0.3313	0.8489	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9725	0.6451	0.9168	Normal Distribution

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9394	0.9192	0.9596	0.9417	0.9231	0.9510	0.0064	1.35%	0.00%
0	SC	4	0.9541	0.9116	0.9966	0.9556	0.9211	0.9843	0.0134	2.80%	-1.57%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.3230	1.2810	1.3650	1.3270	1.2900	1.3480	0.0132	1.99%	0.00%
0	SC	4	1.3620	1.2560	1.4690	1.3590	1.2860	1.4450	0.0335	4.92%	-2.98%

Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9357	0.9231	0.9510	0.9477
0	SC	0.9638	0.9474	0.9843	0.9211

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	14-6564-4593	**Endpoint:**	Proportion Normal	**CETIS Version:**	CETISv2.1.4
Analyzed:	15 May-24 15:42	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:36	**MD5 Hash:**	369965426D5A1CD7304F01BCA1C649AF	**Editor ID:**	003-841-189-5

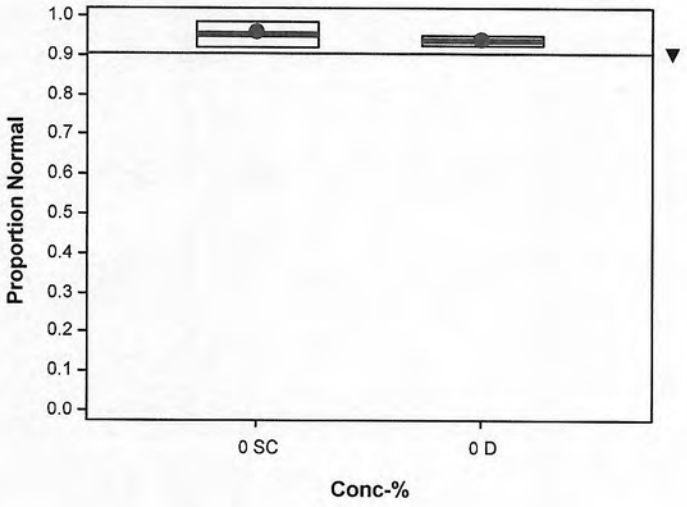
Angular (Corrected) Transformed Detail

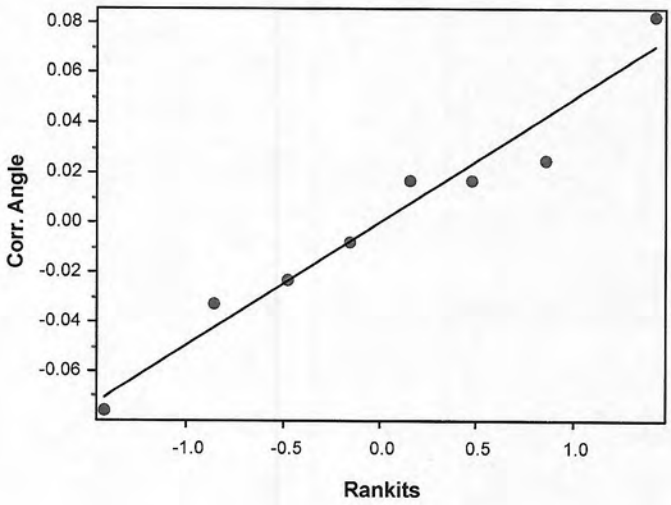
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.3140	1.2900	1.3480	1.3400
0	SC	1.3790	1.3390	1.4450	1.2860

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	131/140	120/130	136/143	145/153
0	SC	133/138	144/152	125/127	105/114

Graphics





Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID:	14-3047-3403	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv2.1.4
Analyzed:	15 May-24 15:42	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:36	**MD5 Hash:**	094DAFFC9A0373A5DB42979C5907175B	**Editor ID:**	003-841-189-5
Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert
Start Date:	16 Apr-24 16:40	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	18 Apr-24 16:32	**Species:**	Mytilus galloprovincialis	**Brine:**	Crystal Sea Marine Mix
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish
Sample ID:	12-7656-3299	**Code:**	P240416.03SC	**Project:**	Wyckoff Eagle Harbor GWTP 2024/W
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	16 Apr-24 11:54	**CAS (PC):**		**Station:**	24162146_1
Sample Age:	7h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed proportion survived endpoint	14.53%

Equal Variance t Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	0.7117	1.943	0.2378	CDF	0.2517	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
Control Resp	0.8817	0.5	<<	Yes	Passes Criteria
Control Resp	0.9383	0.5	<<	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0151715	0.0151715	1	0.5065	0.5034	Non-Significant Effect
Error	0.179714	0.0299524	6			
Total	0.194886		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Levene Equality of Variance Test	0.5698	13.75	0.4789	Equal Variances
	Mod Levene Equality of Variance Test	0.5192	13.75	0.4983	Equal Variances
	Variance Ratio F Test	2.131	47.47	0.5504	Equal Variances
Distribution	Anderson-Darling A2 Test	0.2609	3.878	0.7350	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1912	0.3313	0.6379	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9495	0.6451	0.7057	Normal Distribution

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9383	0.8503	1.0000	0.9433	0.8667	1.0000	0.0277	5.90%	0.00%
0	SC	4	0.8817	0.7186	1.0000	0.8833	0.7600	1.0000	0.0512	11.62%	6.04%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.3470	1.1270	1.5680	1.3310	1.1970	1.5300	0.0692	10.27%	0.00%
0	SC	4	1.2600	0.9390	1.5820	1.2260	1.0590	1.5300	0.1010	16.02%	6.46%

Proportion Survived Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9333	0.8667	0.9533	1.0000
0	SC	0.9200	1.0000	0.8467	0.7600

Bivalve Larval Survival and Development Test EcoAnalysts

Analysis ID:	14-3047-3403	**Endpoint:**	Proportion Survived	**CETIS Version:**	CETISv2.1.4
Analyzed:	15 May-24 15:42	**Analysis:**	Parametric-Two Sample	**Status Level:**	1
Edit Date:	09 May-24 9:36	**MD5 Hash:**	094DAFFC9A0373A5DB42979C5907175B	**Editor ID:**	003-841-189-5

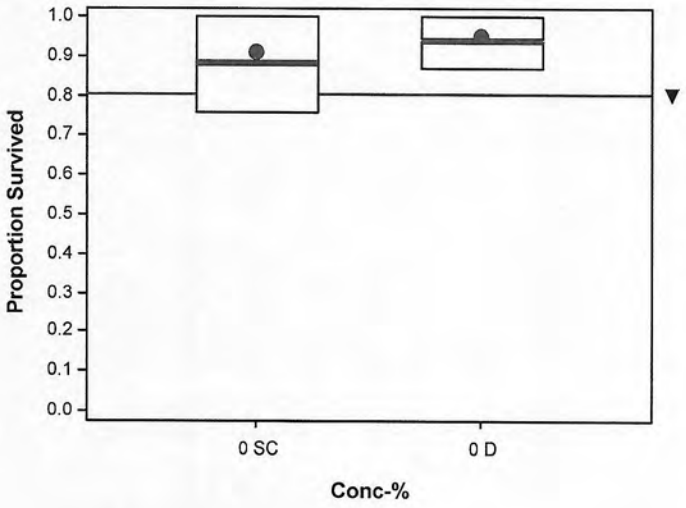
Angular (Corrected) Transformed Detail

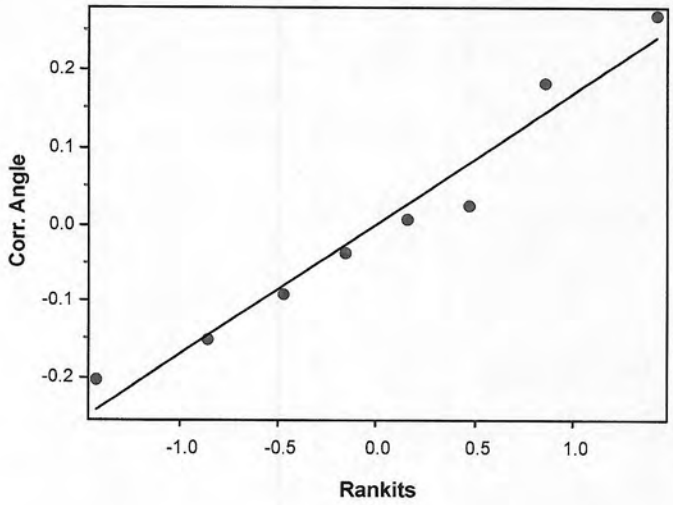
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.3100	1.1970	1.3530	1.5300
0	SC	1.2840	1.5300	1.1680	1.0590

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	140/150	130/150	143/150	150/150
0	SC	138/150	150/150	127/150	114/150

Graphics





CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — EcoAnalysts

Start Date:	16 Apr-24 16:40	**Species:**	Mytilus galloprovincialis	**Sample Code:**	P240416.03SC
End Date:	18 Apr-24 16:32	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs Wyckoff
Sample Date:	16 Apr-24 09:28	**Material:**	Treated Groundwater	**Sample Station:**	24162146_1

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	18	150	140	140	131	
0	D	2	12	150	130	130	120	
0	D	3	20	150	143	143	136	
0	D	4	7	150	153	153	145	
0	SC	1	17	150	138	138	133	
0	SC	2	11	150	152	152	144	
0	SC	3	8	150	127	127	125	
0	SC	4	21	150	114	114	105	
6.25		1	16	150	149	149	140	
6.25		2	19	150	147	147	142	
6.25		3	30	150	150	150	141	
6.25		4	28	150	169	169	160	
12.5		1	22	150	133	133	122	
12.5		2	14	150	139	139	134	
12.5		3	25	150	137	137	124	
25		1	15	150	155	155	145	
25		2	26	150	135	135	130	
25		3	29	150	148	148	141	
25		4	1	150	163	163	160	
50		1	4	150	143	143	131	
50		2	6	150	129	129	118	
50		3	9	150	133	133	120	
50		4	5	150	170	170	157	
57.55		1	3	150	129	129	117	
57.55		2	31	150	152	152	145	
57.55		3	27	150	127	127	127	
57.55		4	13	150	133	133	127	
100		1	24	150	150	150	136	
100		2	2	150	137	137	129	
100		3	23	150	124	124	116	
100		4	10	150	134	134	123	

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

Version V.2

GENERAL

Client	Jacobs Wyckoff
Project	Wyckoff Eagle Harbor GWTP 2024/WA
Project Number	PG1958
Project Manager	M. Seibert
Date Sample Received	4/16/2024
Test type	48-Hour Chronic Toxicity Using Bivalve Larvae
Matrix	Liquid
Test Acceptability	≥90% normal shell development, ≥50% survival (mussels) or ≥70% survival (oysters), MSD <25%
Test Start Date	04/16/24
Test Species	Mytilus spp.
Organism Batch	TS040324.01
Organism Acquired	4/3/2024
Organism Acclimation	13
Organism Age	<4 hr old embryos
Test Protocol	TOX 042
Test Location	Incubator 1
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	150 - 300
Test Chamber Size	30 mL
Exposure Volume	10 mL
Test Dissolved Oxygen	> 4.0
Test Temperature	16 ± 1
Test Salinity	30 ± 2
Test pH	8 ± 1

Note: input lowest and highest decimal for temp

Test Parameters	Min	Max
DO	4.0	
Temp	15	17
Salinity	28	32
pH	7	9

TEST START TIME/INIT:	1640 MS
TEST END TIME/INIT:	1632 PG/TVL

CLIENT SAMPLE ID	LAB ID
24162146_1	P240416.03

Salinity Adjustment CSMM Batch #
62123

Formalin Lot #
230724-07

Rose Bangel Batch #
5135

	Concentrations
1	Control
2	Salt Control
3	6.25%
4	12.5%
5	25%
6	50%
7	57.55%
8	100%
9	.

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	200.0	200		
	Salt Control	#VALUE!	#VALUE!	200		
	6.25%	12.5	187.5	200		
	12.5%	25	175.0	200		
	25%	50	150.0	200		
	50%	100	100.0	200		
	57.55%	115.1	84.9	200		
	100%	200	0.0	200		

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
4/16/24	~~FSW04~~ 8 ①	P240416.03	FSW041624.01	CS

① IE- CS 4/16/24

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

SPAWNING METHOD Heat Shock		**INITIAL SPAWNING TIME** 1324	**FINAL SPAWNING TIME** 1440
MALES 4	**FEMALES** 3	**SPERM VIABILITY** Good	**EGG CONDITION** Good
BEGIN FERTILIZATION 1440		**END FERTILIZATION** 1643	**CONDITION OF EMBRYOS** Good

TIME OF INITIATION	**INITIALS**
16:40	MS

EMBRYO DENSITY CALCULATIONS

# of embryos in 1 mL of 100X diluted embryo stock

Count 1	Count 2	Mean
243	246	244.5

 # embryos in original stock = # of embryos in diluted stock x 100

24450

Percentage of embryo stock needed = 2700 embryos per 1 mL/# embryos in original stock

0.11

mL of egg stock to add to FSW to achieve total volume = percentage of embro stock needed * 40 mL (or desired volume of embryo stock)

4.417177914 **Add this volume to beaker and dilute to 40 mL (or desired volume of embryo stock) with FSW = final embryo stock**

Add 0.1 mL of final embryo stock to test chambers

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	coff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

		DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
	Concentration (%)	> 4.0	15 - 17	28 - 32	7 - 9
Day 0	Control	8.4	17.0	29	① ~~7.9~~ 8.0
Stock	Salt Control	8.4	17.3	28	8.3
Date 4/16/24	6.25%	8.3	17.2	29	7.9
Time 1630	12.5%	8.4	17.1	29	7.9
Tech MS	25%	8.4	17.0	29	7.8
Meter # 7	50%	8.4	16.9	29	7.7
	57.55%	8.4	17.0	29	7.7
	100%	8.4	16.9	29	7.7
Day 1	Control		16.1 ②		
Surrogate	Salt Control		16.1 ②		
Date 4/17/24	6.25%		16.1 ②		
Time 1410	12.5%		16.1 ②		
Tech WD	25%		16.1 ②		
Meter # T33	50%		16.1 ②		
	57.55%		16.1 ②		
	100%		16.1 ②		
Day 2	Control	7.9	16.1 ②	③ ~~32~~ 30	③ ~~8.1~~ 8.0
Surrogate	Salt Control	8.0	16.1 ②	30	8.1
Date 4/18/24	6.25%	7.9	16.1 ②	31	8.0
Time 1000	12.5%	8.8	16.1 ②	31	8.0
Tech NL	25%	7.9	16.1 ②	30	8.1
Meter # 8/T33	50%	8.0	16.1 ②	30	8.2
	57.55%	③ ~~8.8~~ 7.9	16.1 ②	32	8.2
	100%	8.1	16.1 ②	30	8.3

① MR-MS 4/16

② - TEMP BLANK USED - WD 4/17/24, NL 4/18

③ IE-NL 4/18/24

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

Concentration (%)	REP	Normal	Abnormal	Date	Tech	Comments/QA Counts
Stocking Density	1	134		① ~~134~~ 5/4/24	SR	
	2	130		① ~~130~~ 5/4/24	SR	
	3	158		① ~~158~~ 5/4/24	SR	
	4	153		① ~~153~~ 5/4/24	SR	
	5	160		① ~~160~~ 5/4/24	SR	
	6	150		① ~~150~~ 5/4/24	SR	
Control	1	131	9	4/27/24	NL	
	2	120	10	4/27/24	NL	
	3	136	7	4/27/24	NL	QA: 132N 8AB-DM-5/8/24
	4	145	8	4/27/24	NL	
Salt Control	1	133	5	5/6/24	MS	
	2	144	8	5/6/24	MS	
	3	125	2	5/6/24	MS	
	4	105	9	5/6/24	MS	
6.25%	1	140	9	5/7/24	MS	QA: 137N 11AB-DM-5/8/24
	2	142	5	5/8/24	DM	
	3	141	9	5/8/24	DM	
	4	160	9	5/8/24	DM	
12.5%	1	122	11	5/7/24	MS	
	2	~~134~~ ②	~~05~~ ②	5/8/24 ②	DM ②	
	3	134	5	5/8/24	DM	QA: 136N, 3AB-NL 5/8/24
	4	124	13	5/8/24	DM	
25%	1	145	10	5/6/24	MS	
	2	130	5	5/8/24	DM	
	3	141	7	5/8/24	DM	
	4	160	3	5/8/24	DM	
50%	1	131	12	5/6/24	MS	
	2	110	11	5/8/24	DM	
	3	120	13	5/8/24	DM	
	4	157	13	5/8/24	DM	

① WC-DM-5/8/24

② Vial compromised, not enumerated & removed from statistical analysis - DM-5/8/24

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

v.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	4/16/24	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2024/WA	**TEST START DATE**	4/16/24	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	24162146_1	**TEST END DATE**	4/18/24	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P240416.03	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

Concentration (%)	REP	Normal	Abnormal	Date	Tech	Comments/QA Counts
57.55%	1	117	12	5/6/24	NS	
	2	145	7	5/6/24	NS	
	3	127	0	5/6/24	NS	
	4	127	6	5/6/24	NS	
100%	1	136	14	4/27/24	NL	
	2	129	8	4/27/24	NL	
	3	116	8	4/27/24	NL	
	4	123	11	4/27/24	NL	

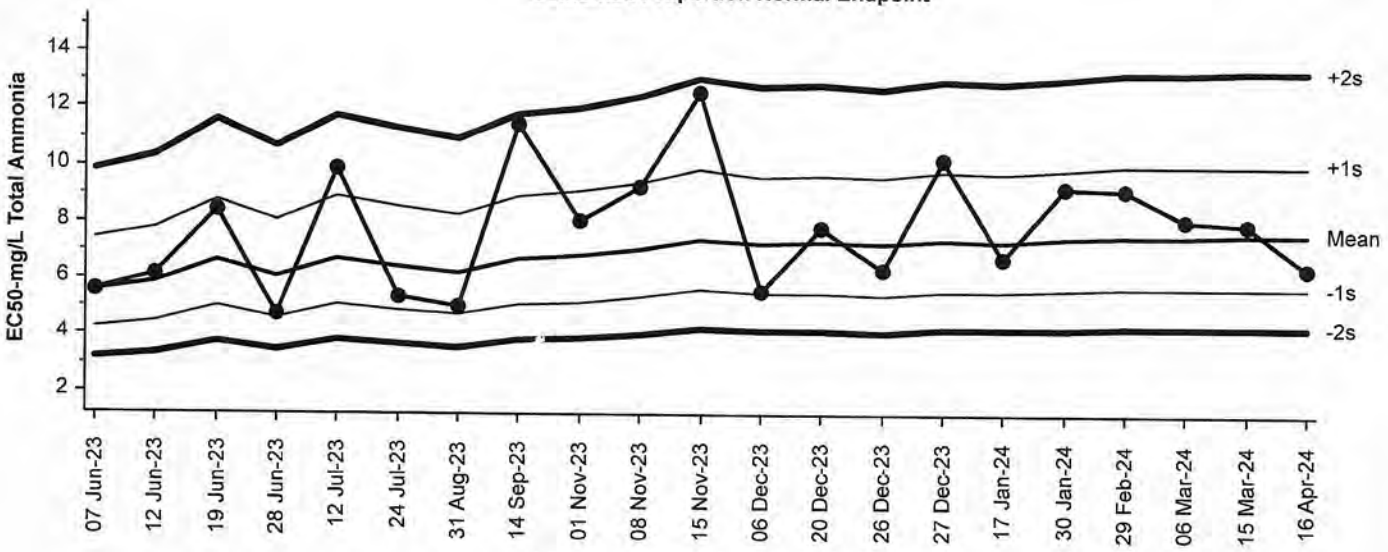
CETIS QC Plot

Report Date: 10 May-24 12:46 (1 of 1)

Bivalve Larval Survival and Development Test — **All Matching Labs**

Test Type:	Development-Survival	**Organism:**	Mytilus galloprovincialis	**Material:**	Total Ammonia
Protocol:	All Protocols	**Endpoint:**	Combined Proportion Normal	**Source:**	Reference Toxicant-REF

Bivalve Larval Survival and Development Test
Combined Proportion Normal Endpoint



Lognormal Cumulative Mean Plot

Mean:	7.606	**Count:**	20	**-1s Warning Limit:**	5.75	**-2s Action Limit:**	4.34
Sigma:	NA	**CV:**	28.60%	**+1s Warning Limit:**	10.1	**+2s Action Limit:**	13.3

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2023	Jun	7	16:24	5.621	-1.985	-1.078	(-)		16-8311-5218	04-7873-2197	EcoAnalysts
2			12	18:29	6.154	-1.452	-0.7552			19-7480-8941	04-9719-6422	EcoAnalysts
3			19	16:20	8.423	0.8163	0.3633			16-3224-4662	15-6769-3694	EcoAnalysts
4			28	15:18	4.725	-2.882	-1.697	(-)		10-1014-4768	17-1187-2841	EcoAnalysts
5		Jul	12	12:57	9.89	2.284	0.9356			02-0009-8192	04-6529-8407	EcoAnalysts
6			24	17:06	5.374	-2.232	-1.238	(-)		05-3985-4386	13-9086-0827	EcoAnalysts
7		Aug	31	16:54	5.053	-2.554	-1.458	(-)		16-1472-3265	15-9433-1311	EcoAnalysts
8		Sep	14	13:50	11.43	3.822	1.451	(+)		10-9810-7803	01-3503-3195	EcoAnalysts
9		Nov	1	17:40	8.055	0.4483	0.2041			08-2875-4322	08-8063-5388	EcoAnalysts
10			8	15:55	9.251	1.645	0.6977			13-4824-7359	00-4887-4658	EcoAnalysts
11			15	14:38	12.55	4.945	1.785	(+)		04-7650-2671	01-5035-4681	EcoAnalysts
12		Dec	6	17:35	5.604	-2.002	-1.089	(-)		07-4908-4729	09-1248-2427	EcoAnalysts
13			20	15:50	7.826	0.2196	0.1014			21-3057-6259	03-0359-1538	EcoAnalysts
14			26	17:01	6.393	-1.214	-0.6196			09-3076-3716	00-6627-3829	EcoAnalysts
15			27	16:43	10.27	2.666	1.071	(+)		05-3736-4406	14-3667-2208	EcoAnalysts
16	2024	Jan	17	15:15	6.76	-0.8462	-0.4203			06-5202-1140	06-9659-2949	EcoAnalysts
17			30	16:45	9.227	1.621	0.6883			00-0328-6111	17-2839-1252	EcoAnalysts
18		Feb	29	16:10	9.166	1.56	0.6647			11-3381-6441	12-8703-1430	EcoAnalysts
19		Mar	6	15:31	8.112	0.5054	0.2293			06-3359-5243	09-6537-8157	EcoAnalysts
20			15	17:03	7.968	0.3614	0.1654			06-5685-3013	05-8374-8533	EcoAnalysts
21		Apr	16	16:43	6.425	-1.181	-0.6014			04-9604-7497	05-8877-4895	EcoAnalysts

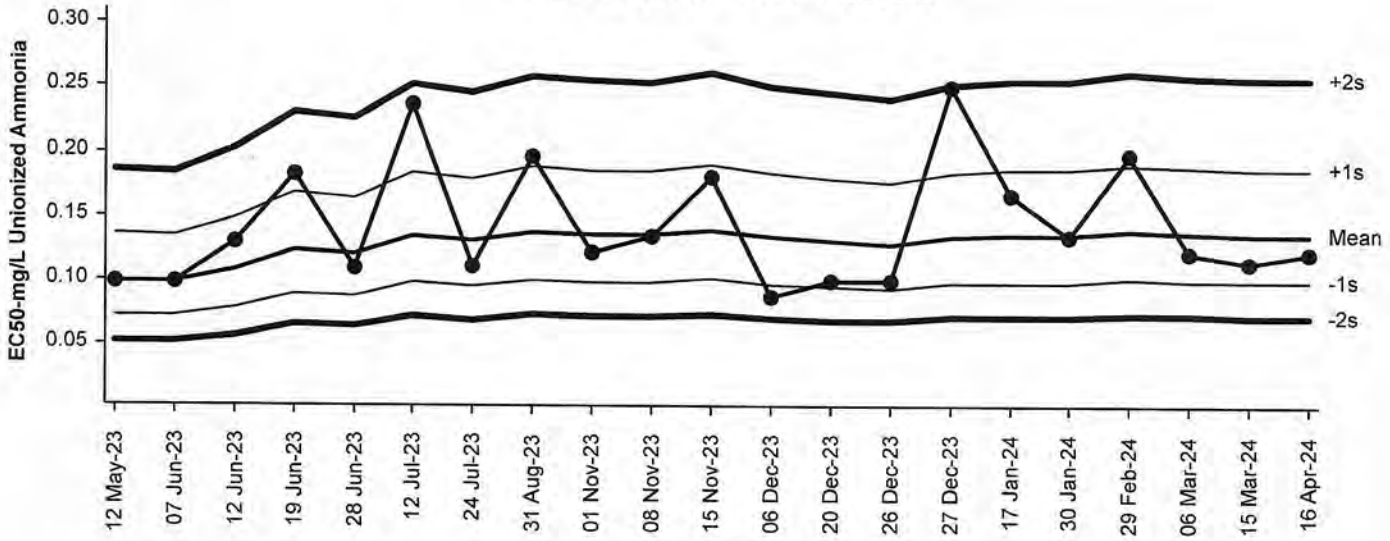
Analyst: MB QA: BM

CETIS QC Plot

Bivalve Larval Survival and Development Test — **All Matching Labs**

Test Type:	Development-Survival	**Organism:**	Mytilus galloprovincialis	**Material:**	Unionized Ammonia
Protocol:	All Protocols	**Endpoint:**	Combined Proportion Normal	**Source:**	Reference Toxicant-REF

Bivalve Larval Survival and Development Test
Combined Proportion Normal Endpoint



Lognormal Cumulative Mean Plot

Mean:	0.1364	**Count:**	20	**-1s Warning Limit:**	0.0995	**-2s Action Limit:**	0.0725
Sigma:	NA	**CV:**	32.40%	**+1s Warning Limit:**	0.187	**+2s Action Limit:**	0.257

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2023	May	12	15:35	0.09858	-0.03784	-1.029	(-)		08-2245-0872	03-4589-6060	EcoAnalysts
2		Jun	7	16:24	0.0976	-0.03882	-1.06	(-)		18-8939-1974	09-3314-9652	EcoAnalysts
3			12	18:29	0.1293	-0.0071	-0.1692			09-8773-2984	16-9381-4730	EcoAnalysts
4			19	16:20	0.182	0.04553	0.912			21-4361-0458	04-8703-0787	EcoAnalysts
5			28	15:18	0.1088	-0.02757	-0.715			16-9844-0501	06-2488-5585	EcoAnalysts
6		Jul	12	12:57	0.2364	0.09994	1.74	(+)		13-3479-3905	05-2583-6446	EcoAnalysts
7			24	17:06	0.1104	-0.02603	-0.6704			08-8951-5421	04-1308-9826	EcoAnalysts
8		Aug	31	16:54	0.1956	0.05917	1.141	(+)		07-4158-0358	11-0996-2376	EcoAnalysts
9		Nov	1	17:40	0.1213	-0.01508	-0.3708			06-2464-1457	05-4038-7195	EcoAnalysts
10			8	15:55	0.1338	-0.00266	-0.06239			13-8700-3666	02-0586-1811	EcoAnalysts
11			15	14:38	0.1802	0.04373	0.8805			19-3724-7711	00-4487-8422	EcoAnalysts
12		Dec	6	17:35	0.08732	-0.04911	-1.413	(-)		11-7639-2844	02-1522-3004	EcoAnalysts
13			20	15:50	0.1006	-0.03585	-0.9653			09-2413-6838	00-3830-4602	EcoAnalysts
14			26	17:01	0.09993	-0.0365	-0.9858			07-1075-7212	13-4227-6824	EcoAnalysts
15			27	16:43	0.2498	0.1134	1.915	(+)		21-2709-9990	17-0965-3961	EcoAnalysts
16	2024	Jan	17	15:15	0.1665	0.03008	0.6309			15-5848-1090	20-9766-0257	EcoAnalysts
17			30	16:45	0.134	-0.00243	-0.05692			12-6773-1386	01-4900-2989	EcoAnalysts
18		Feb	29	16:10	0.1971	0.06064	1.165	(+)		13-3303-1935	02-6389-9378	EcoAnalysts
19		Mar	6	15:31	0.1223	-0.01414	-0.3465			17-0621-0144	14-8112-7594	EcoAnalysts
20			15	17:03	0.1145	-0.02187	-0.5534			01-3031-4193	06-2698-4373	EcoAnalysts
21		Apr	16	16:43	0.121	-0.01541	-0.3796			03-1250-7032	02-5987-7371	EcoAnalysts

CETIS Summary Report

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert		
Start Date:	16 Apr-24 16:43	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater		
Ending Date:	18 Apr-24 16:26	**Species:**	Mytilus galloprovincialis	**Brine:**			
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish	**Age:**	
Sample ID:	00-3333-2336	**Code:**	P220819.135	**Project:**	Reference Toxicant		
Sample Date:	19 Aug-22	**Material:**	Total Ammonia	**Source:**	Reference Toxicant		
Receipt Date:	19 Aug-22	**CAS (PC):**		**Station:**	P220819.135		
Sample Age:	606d 17h	**Client:**	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
14-5779-2287	Combined Proportion Norma	Dunnett Multiple Comparison Test	✓	3.11	6.7	4.565	11.1%	1
02-8301-9921	Proportion Normal	Dunnett Multiple Comparison Test	✓	3.11	6.7	4.565	2.81%	1
19-9881-5054	Proportion Survived	Dunnett Multiple Comparison Test		6.7	13.5	9.511	15.4%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg/L	95% LCL	95% UCL	S
18-3623-5759	Combined Proportion Norma	Linear Interpolation (ICPIN)		EC10	3.626	3.218	3.672	1
				EC25	4.524	4.112	4.661	
				EC50	6.425	5.965	6.796	
07-1006-0740	Proportion Normal	Linear Interpolation (ICPIN)	✓	EC10	3.518	3.365	3.685	1
			✓	EC25	4.376	4.167	4.603	
			✓	EC50	6.184	5.805	6.654	
17-3136-4443	Proportion Survived	Linear Interpolation (ICPIN)		EC10	9.899	6.596	13.56	1
				EC25	19.12	11.07	---	
				EC50	>21.1	---	---	

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
02-8301-9921	Proportion Normal	Control Resp	0.9512	0.9	<<	Yes	Passes Criteria
07-1006-0740	Proportion Normal	Control Resp	0.9512	0.9	<<	Yes	Passes Criteria
17-3136-4443	Proportion Survived	Control Resp	0.9167	0.5	<<	Yes	Passes Criteria
19-9881-5054	Proportion Survived	Control Resp	0.9167	0.5	<<	Yes	Passes Criteria
14-5779-2287	Combined Proportion Norma	PMSD	0.1115	<<	0.25	No	Passes Criteria

Bivalve Larval Survival and Development Test
EcoAnalysts

Combined Proportion Normal Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.8717	0.7709	0.9724	0.8000	0.9400	0.0317	0.0633	7.27%	0.00%
1.1		4	0.8500	0.7565	0.9435	0.8000	0.9333	0.0294	0.0588	6.91%	2.49%
3.11		4	0.9100	0.8342	0.9858	0.8800	0.9800	0.0238	0.0476	5.23%	-4.40%
6.7		4	0.4117	0.3766	0.4467	0.3867	0.4333	0.0110	0.0220	5.35%	52.77%
13.5		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
21.1		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Proportion Normal Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9512	0.9087	0.9937	0.9130	0.9714	0.0134	0.0267	2.81%	0.00%
1.1		4	0.9358	0.9281	0.9436	0.9302	0.9407	0.0024	0.0049	0.52%	1.62%
3.11		4	0.9333	0.9147	0.9519	0.9167	0.9441	0.0058	0.0117	1.25%	1.88%
6.7		4	0.4222	0.3584	0.4860	0.3810	0.4715	0.0201	0.0401	9.50%	55.62%
13.5		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
21.1		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9167	0.8108	1.0230	0.8267	0.9867	0.0333	0.0666	7.26%	0.00%
1.1		4	0.9083	0.8076	1.0090	0.8600	1.0000	0.0317	0.0633	6.97%	0.91%
3.11		4	0.9633	0.9222	1.0040	0.9400	1.0000	0.0129	0.0258	2.68%	-5.09%
6.7		4	0.9533	0.8118	1.0950	0.8200	1.0000	0.0445	0.0889	9.33%	-4.00%
13.5		4	0.7650	0.6513	0.8787	0.6800	0.8467	0.0357	0.0715	9.34%	16.55%
21.1		4	0.6833	0.5696	0.7971	0.5867	0.7467	0.0358	0.0715	10.46%	25.45%

Combined Proportion Normal Detail
MD5: 1C0575C583258B4F94EB1AF4F950B050

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8000	0.9067	0.9400	0.8400
1.1		0.8467	0.8000	0.9333	0.8200
3.11		0.9000	0.9800	0.8800	0.8800
6.7		0.3867	0.4000	0.4333	0.4267
13.5		0.0000	0.0000	0.0000	0.0000
21.1		0.0000	0.0000	0.0000	0.0000

Proportion Normal Detail
MD5: 4D7360F4F32F9938B7CF3A3249154542

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9677	0.9714	0.9527	0.9130
1.1		0.9407	0.9302	0.9333	0.9389
3.11		0.9441	0.9363	0.9167	0.9362
6.7		0.4715	0.4000	0.4362	0.3810
13.5		0.0000	0.0000	0.0000	0.0000
21.1		0.0000	0.0000	0.0000	0.0000

Proportion Survived Detail
MD5: FD925647C7C8017C80808B096A835829

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8267	0.9333	0.9867	0.9200
1.1		0.9000	0.8600	1.0000	0.8733
3.11		0.9533	1.0000	0.9600	0.9400
6.7		0.8200	1.0000	0.9933	1.0000
13.5		0.6800	0.7933	0.7400	0.8467
21.1		0.5867	0.7467	0.6733	0.7267

Bivalve Larval Survival and Development Test — **EcoAnalysts**

Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	120/150	136/150	141/150	126/150
1.1		127/150	120/150	140/150	123/150
3.11		135/150	147/150	132/150	132/150
6.7		58/150	60/150	65/150	64/150
13.5		0/150	0/150	0/150	0/150
21.1		0/150	0/150	0/150	0/150

Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	120/124	136/140	141/148	126/138
1.1		127/135	120/129	140/150	123/131
3.11		135/143	147/157	132/144	132/141
6.7		58/123	60/150	65/149	64/168
13.5		0/102	0/119	0/111	0/127
21.1		0/88	0/112	0/101	0/109

Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	124/150	140/150	148/150	138/150
1.1		135/150	129/150	150/150	131/150
3.11		143/150	150/150	144/150	141/150
6.7		123/150	150/150	149/150	150/150
13.5		102/150	119/150	111/150	127/150
21.1		88/150	112/150	101/150	109/150

CETIS Summary Report

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	02-2917-9486	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert		
Start Date:	16 Apr-24 16:43	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater		
Ending Date:	18 Apr-24 16:26	**Species:**	Mytilus galloprovincialis	**Brine:**			
Test Length:	48h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish	**Age:**	
Sample ID:	17-6589-5051	**Code:**	P220819.135UIA	**Project:**	Reference Toxicant		
Sample Date:	19 Aug-22	**Material:**	Unionized Ammonia	**Source:**	Reference Toxicant		
Receipt Date:	19 Aug-22	**CAS (PC):**		**Station:**	P220819.135UIA		
Sample Age:	606d 17h	**Client:**	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
12-7234-1536	Combined Proportion Norma	Dunnett Multiple Comparison Test	✓	0.058	0.125	0.08515	11.1%	1
00-7648-2325	Proportion Normal	Dunnett Multiple Comparison Test	✓	0.058	0.125	0.08515	2.81%	1
12-2244-1328	Proportion Survived	Dunnett Multiple Comparison Test		0.125	0.252	0.1775	15.4%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg/L	95% LCL	95% UCL	S
03-5786-4929	Combined Proportion Norma	Linear Interpolation (ICPIN)		EC10	0.07031	0.06021	0.07112	1
				EC25	0.08905	0.08082	0.0911	
				EC50	0.121	0.1145	0.1259	
17-8902-5269	Proportion Normal	Linear Interpolation (ICPIN)	✓	EC10	0.06783	0.06407	0.07126	1
			✓	EC25	0.08616	0.08222	0.09086	
			✓	EC50	0.1174	0.1119	0.1237	
17-7501-2356	Proportion Survived	Linear Interpolation (ICPIN)		EC10	0.193	0.1352	0.257	1
				EC25	0.3617	0.1888	---	
				EC50	>0.395	---	---	

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
00-7648-2325	Proportion Normal	Control Resp	0.9512	0.9	<<	Yes	Passes Criteria
17-8902-5269	Proportion Normal	Control Resp	0.9512	0.9	<<	Yes	Passes Criteria
12-2244-1328	Proportion Survived	Control Resp	0.9167	0.5	<<	Yes	Passes Criteria
17-7501-2356	Proportion Survived	Control Resp	0.9167	0.5	<<	Yes	Passes Criteria
12-7234-1536	Combined Proportion Norma	PMSD	0.1115	<<	0.25	No	Passes Criteria

Bivalve Larval Survival and Development Test — EcoAnalysts

Combined Proportion Normal Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.8717	0.7709	0.9724	0.8000	0.9400	0.0317	0.0633	7.27%	0.00%
0.021		4	0.8500	0.7565	0.9435	0.8000	0.9333	0.0294	0.0588	6.91%	2.49%
0.058		4	0.9100	0.8342	0.9858	0.8800	0.9800	0.0238	0.0476	5.23%	-4.40%
0.125		4	0.4117	0.3766	0.4467	0.3867	0.4333	0.0110	0.0220	5.35%	52.77%
0.252		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
0.395		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Proportion Normal Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9512	0.9087	0.9937	0.9130	0.9714	0.0134	0.0267	2.81%	0.00%
0.021		4	0.9358	0.9281	0.9436	0.9302	0.9407	0.0024	0.0049	0.52%	1.62%
0.058		4	0.9333	0.9147	0.9519	0.9167	0.9441	0.0058	0.0117	1.25%	1.88%
0.125		4	0.4222	0.3584	0.4860	0.3810	0.4715	0.0201	0.0401	9.50%	55.62%
0.252		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
0.395		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Proportion Survived Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9167	0.8108	1.0230	0.8267	0.9867	0.0333	0.0666	7.26%	0.00%
0.021		4	0.9083	0.8076	1.0090	0.8600	1.0000	0.0317	0.0633	6.97%	0.91%
0.058		4	0.9633	0.9222	1.0040	0.9400	1.0000	0.0129	0.0258	2.68%	-5.09%
0.125		4	0.9533	0.8118	1.0950	0.8200	1.0000	0.0445	0.0889	9.33%	-4.00%
0.252		4	0.7650	0.6513	0.8787	0.6800	0.8467	0.0357	0.0715	9.34%	16.55%
0.395		4	0.6833	0.5696	0.7971	0.5867	0.7467	0.0358	0.0715	10.46%	25.45%

Combined Proportion Normal Detail

MD5: 33F6C74CB0322943A28F2E5B84224AE7

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8000	0.9067	0.9400	0.8400
0.021		0.8467	0.8000	0.9333	0.8200
0.058		0.9000	0.9800	0.8800	0.8800
0.125		0.3867	0.4000	0.4333	0.4267
0.252		0.0000	0.0000	0.0000	0.0000
0.395		0.0000	0.0000	0.0000	0.0000

Proportion Normal Detail

MD5: 2AB44B4D7E78319C972CD0F1AE26A1CE

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9677	0.9714	0.9527	0.9130
0.021		0.9407	0.9302	0.9333	0.9389
0.058		0.9441	0.9363	0.9167	0.9362
0.125		0.4715	0.4000	0.4362	0.3810
0.252		0.0000	0.0000	0.0000	0.0000
0.395		0.0000	0.0000	0.0000	0.0000

Proportion Survived Detail

MD5: 8D469CF3A4206B080786C9AAF86BF664

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.8267	0.9333	0.9867	0.9200
0.021		0.9000	0.8600	1.0000	0.8733
0.058		0.9533	1.0000	0.9600	0.9400
0.125		0.8200	1.0000	0.9933	1.0000
0.252		0.6800	0.7933	0.7400	0.8467
0.395		0.5867	0.7467	0.6733	0.7267

Bivalve Larval Survival and Development Test
EcoAnalysts

Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	120/150	136/150	141/150	126/150
0.021		127/150	120/150	140/150	123/150
0.058		135/150	147/150	132/150	132/150
0.125		58/150	60/150	65/150	64/150
0.252		0/150	0/150	0/150	0/150
0.395		0/150	0/150	0/150	0/150

Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	120/124	136/140	141/148	126/138
0.021		127/135	120/129	140/150	123/131
0.058		135/143	147/157	132/144	132/141
0.125		58/123	60/150	65/149	64/168
0.252		0/102	0/119	0/111	0/127
0.395		0/88	0/112	0/101	0/109

Proportion Survived Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	124/150	140/150	148/150	138/150
0.021		135/150	129/150	150/150	131/150
0.058		143/150	150/150	144/150	141/150
0.125		123/150	150/150	149/150	150/150
0.252		102/150	119/150	111/150	127/150
0.395		88/150	112/150	101/150	109/150

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — EcoAnalysts

Start Date:	16 Apr-24 16:43	**Species:**	Mytilus galloprovincialis	**Sample Code:**	P220819.135
End Date:	18 Apr-24 16:26	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Reference Toxicant
Sample Date:	19 Aug-22	**Material:**	Total Ammonia	**Sample Station:**	P220819.135

Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	21	150	124	124	120	
0	D	2	18	150	140	140	136	
0	D	3	2	150	148	148	141	
0	D	4	4	150	138	138	126	
1.1		1	23	150	135	135	127	
1.1		2	22	150	129	129	120	
1.1		3	8	150	150	150	140	
1.1		4	3	150	131	131	123	
3.11		1	15	150	143	143	135	
3.11		2	13	150	157	157	147	
3.11		3	9	150	144	144	132	
3.11		4	17	150	141	141	132	
6.7		1	16	150	123	123	58	
6.7		2	6	150	150	150	60	
6.7		3	11	150	149	149	65	
6.7		4	10	150	168	168	64	
13.5		1	7	150	102	102	0	
13.5		2	1	150	119	119	0	
13.5		3	5	150	111	111	0	
13.5		4	12	150	127	127	0	
21.1		1	24	150	88	88	0	
21.1		2	20	150	112	112	0	
21.1		3	19	150	101	101	0	
21.1		4	14	150	109	109	0	

Analyst: RS QA DM

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — EcoAnalysts

Start Date:	16 Apr-24 16:43	**Species:**	Mytilus galloprovincialis	**Sample Code:**	P220819.135UIA
End Date:	18 Apr-24 16:26	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Reference Toxicant
Sample Date:	19 Aug-22	**Material:**	Unionized Ammonia	**Sample Station:**	P220819.135UIA

Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	9	150	124	124	120	
0	D	2	12	150	140	140	136	
0	D	3	13	150	148	148	141	
0	D	4	22	150	138	138	126	
0.021		1	18	150	135	135	127	
0.021		2	23	150	129	129	120	
0.021		3	2	150	150	150	140	
0.021		4	10	150	131	131	123	
0.058		1	6	150	143	143	135	
0.058		2	4	150	157	157	147	
0.058		3	8	150	144	144	132	
0.058		4	7	150	141	141	132	
0.125		1	17	150	123	123	58	
0.125		2	16	150	150	150	60	
0.125		3	1	150	149	149	65	
0.125		4	19	150	168	168	64	
0.252		1	24	150	102	102	0	
0.252		2	15	150	119	119	0	
0.252		3	11	150	111	111	0	
0.252		4	5	150	127	127	0	
0.395		1	21	150	88	88	0	
0.395		2	14	150	112	112	0	
0.395		3	3	150	101	101	0	
0.395		4	20	150	109	109	0	

48 Hour Bivalve Development Reference Toxicant Test

Test ID: P220819.135	Replicates: 4	Study Director: M. Seibert	Location: Inc. 1
Dilution Water Batch: FSW04162401	Organism Batch: TS040324.01	Associated Test(s): Jacobs Mycroff	Organism: M. sp.
Chamber Size/Type: 30 ml shell vial	Exposure Volume: 10 ml		
Toxicant: Ammonium Chloride		Date Prepared: 4/16/24	Initials: RG
Target Concentrations: **See spiking worksheet**		Quantity of Stock: Target: See spiking worksheet	Quantity of Diluent: Target: 250 mL
See spiking worksheet		Actual: See spiking worksheet	Actual: 200 mL

SPAWNING DATA

Initial Spawning Time: 1327	Final Spawning Time: 1440	Fertilization Time: 1440	No. of Females: 3	No. of Males: 4
Embryo Density (count/mL):	1. 243	2. ②2496	3. —	Mean: 244.5
Stocking Volume Calculation: 2700/24450 = 0.11 × 40mL = 4.4mL egg stock in 35.6mL FSW				

0 Hours	Date: 4/16/24	WQ Time: 1520 MS	Start Time: 1643	Initials: MS

STOCK

	Control	1.5	3	6	12	18
D.O. (%) (>4.0 mg/L)	8.3	8.3	8.4	8.5	8.5	8.4
Temperature (16 ± 1°C) ①	16.7	16.7	16.7	16.7	16.7	16.7
Salinity (30 ± 2 ppt)	29	29	29	29	29	29
pH (6-9)	7.7	7.8	7.8	7.8	7.8	7.8
Meter # 7	7	7	7	7	7	7

Day 1	Temperature (16 ± 1°C)	16.1	Meter # T33	Initials: WD
Day 2	Date: 4/18/24	WQ Time: 1005	End Time: 1626	Initials: NL
		Formalin Lot #: 230724-07	Rose Bengal Lot #:	5135

STOCK

	Control	1.5	3	6	12	18
D.O. (%) (>4.0 mg/L)	7.9	8.0	8.0	8.0	8.1	8.0
Temperature (16 ± 1°C) ①	16.1	16.1	16.1	16.1	16.1	16.1
Salinity (30 ± 2 ppt)	31	30	31	31	30	31
pH (6-9)	8.0	8.0	7.9	7.9	7.9	7.9
Meter #	8|T33	8|T33	8|T33	8|T33	8|T33	8|T33

① temp taken from temp. blank - MS 4/16, NL 4/18

② IE - MS 4/16

Test ID:

48 Hour Bivalve Development Reference Toxicant Test

Test ID: P220819.135

Conc.	Rep	Number Normal	Number Abnormal	Date	Initials
Control	1	120	4	05/04/24	SR
	2	136	4	05/04/24	SR
	3	141	7	05/04/24	SR
	4	126	12	05/04/24	SR
1.5	1	127	8	05/04/24	SR
	2	120	9	05/04/24	SR
	3	140	10	05/04/24	SR
	4	123	8	05/04/24	SR
3	1	135	8	05/04/24	SR
	2	147	10	05/04/24	SR
	3	132	12	05/04/24	SR
	4	132	9	05/04/24	SR
6	1	58	65	05/04/24	SR
	2	60	90	05/04/24	SR
	3	65	84	05/04/24	SR
	4	64	104	05/04/24	SR
12	1	0	102	05/04/24	SR
	2	0	119	05/04/24	SR
	3	0	111	05/04/24	SR
	4	0	127	05/04/24	SR
18	1	0	88	05/04/24	SR
	2	0	112	05/04/24	SR
	3	0	101	05/04/24	SR
	4	0	109	05/04/24	SR

QA: 141 N -DM 7AB 5/8/24

QA: 129 N 7AB -DM 5/8/24

Stocking Density

Rep	Count	Init.
1	134	SR
2	136	SR
3	158	SR
4	153	SR
5	166	SR
6	150	SR
Mean:	150	SR

① IE - SR 05/04/24

Ammonia Reference Toxicant
Spiking Worksheet

Reference Toxicant ID: P220819.135
Date Prepared: 4/16/24
Technician Initials: RG

Biv / Echino NH_3 RT

Assumptions in Model
Stock ammonia concentration is 9,000 mg/L = 9 mg/mL

Date: 3/5/2024
Measurement: 11300

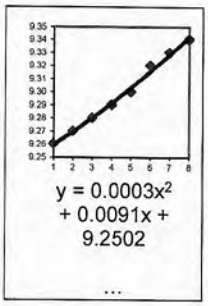
Test Solutions			Volume of stock to reach desired concentration	
Measured Concentration	**Desired Concentration**	**Volume**		
mg/L	mg/L	mL	mL stock to increase	
			[redacted]	SALT WATER
1.1	**1.5**	200		**0.040**
3.11	**3**	200		**0.080**
6.7	**6**	200		**0.159**
13.5	**12**	200		**0.319**
21.1	**18**	200		**0.478**

Un-ionized Ammonia Calculator

CLIENT:	Jacobs Wyckoff	Date of Test:	April 16, 2024
PROJECT:	Wyckoff Eagle Harbor GWTP 2024/WA	Test Type:	*Mytilus galloprovinvialis*
COMMENTS:	P220819.135		

To convert Total Ammonia (mg/L) to Free (un-ionized) Ammonia (mg/L) enter the corresponding total ammonia, salinity, temperature, and pH.

Ionic strength	pKa^s
1	9.26
2	9.27
3	9.28
4	9.29
5	9.30
6	9.32
7	9.33
8	9.34



	Sample	Mod	*NH3T (mg/L)*	*salinity (ppt)*	*pH*	*temp (C)*	*temp (K)*	pKa^s		NH_3U (mg/L)
	Target / Sample Name		Actual	Actual	Actual	Actual	Calculated	Calculated		**Calculated**
	Example 3.5		2.000	10.0	7.5	5.0	278.15	9.2520		**0.008**
1										
2	1.5		1.1	29	7.8	16.7	289.85	9.2557		**0.021**
3	3		3.11	29	7.8	16.7	289.85	9.2557		**0.058**
4	6		6.7	29	7.8	16.7	289.85	9.2557		**0.125**
5	12		13.5	29	7.8	16.7	289.85	9.2557		**0.252**
6	18		21.1	29	7.8	16.7	289.85	9.2557		**0.395**
7										
8										
9										
10										
11										
12										
13										
14										
15										
16										
17										
18										
19										
20										
21										
22										
23										
24										
25										
26										
27										
28										
29										
30										
31										
32										
33										
34										
35										
36										
37										
38										
39										
40										
41										
42										
43										
44										
45										

v.2

Daily Quality Assurance Checks

Project name: Jacobs Wyckoff

Test: Bivalve NH3 RT

Lab ID: P220819.135

Day of Test		Initials	Date	Comments
0	Test datasheets checked for completeness and legibility	NR	4/16	
	Headers/ footers filled in, visual check of test chambers, cover test, ensure proper lighting	↓	↓	
	Test data within acceptable ranges	↓	↓	
1	Test datasheets checked for completeness and legibility	NL	4/17	
	Test data within acceptable ranges	↓	↓	
2	Test datasheets checked for completeness and legibility	LG	4/18	
	Test data within acceptable ranges	↓	↓	

ORGANISM RECEIPT LOG

Date:	Time:	Batch No.
4/3/24	1630	TS040324.01

Organism: M. sp

Source / Supplier: Taylor Shellfish

No. Ordered:	No. Received:	Source Batch: Collection date, hatch date, etc.):
① ~~4lb~~ 10lb	10lb	4/3/24

Condition of Organisms:	Approximate Size or Age: (Days from hatch, life stage, size class, etc.):
Good	Adults
Shipper: Courier	**B of L (Tracking No.)** NA
Condition of Container: Good	**Received By:** NL

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units)	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	②	7.3	②	②	—	—	NL

*if >10% contact lab manager

Notes: ①IE-NL 4/3/24 ②Recieved Dry-NL 4/3

TAYLOR SHELLFISH FARMS

SE 130 LYNCH RD, SHELTON WA 98584
PHONE # (360) 426-6178
WASHINGTON STATE CERT. # : WA46SP

HARVEST DATE: 4/3/24

HARVEST AREA: ☐ N Totten ☐

HARVEST ITEM: ☐ Mussels ☐

Dept ID: M127

FARM CODE:

Harvest Hour	11 a
Harvest Minute	30
Refer Date	4/3/24
Refer Hour	
Refer Minute	

QUANTITY: 10 ☐ Dozens ☑ Pounds ☐ Tubs ☐ Sacks

All Shellstock containers in this lot have the same harvest data and area of harvest.

MAINTENANCE LOG FOR CULTURES

ORGANISM: M. sp

LOCATION: Bath 10

Batch Number: TS040324.01	Date Received: 4/3/24	Initial # of Organisms: 10 lbs	10% mortality = 116

Date	Feed AM	Feed PM	Tub No.	D.O.	Temp (°C)	Cond/ (Sal)	pH	H_2O Change	Organisms appear healthy (Y/N)	# Mort (per tub)	[1]Cumulative # Mort*	Init.	Comments
4/4	✓	–	A	8.3	11.9	28	7.7	FT	Y	0	—	NL	
4/4	✓	–	B	8.5	11.5	28	7.6	FT	Y	0	0	NL	
4/5	—	—	A	08.35	~~11.1~~ 10.9 ①	29	7.7	FT	Y	0	–	RG	
4/5	—	—	B	08.34	~~10.9~~ 10.8 ①	29	7.7	FT	Y	0	0	RG	
4/7	✓	–	A	8.5	12.0	29	7.8	FT	Y	0	–	TW	
4/7	✓	–	B	8.8	11.7	29	7.8	FT	Y	0	0	TW	
4/8	✓	–	A	8.4	11.1	30	7.7	FT	Y	0	–	TW	
4/8	✓	–	B	8.5	10.9	30	7.7	FT	Y	0	0	TW	
4/10	—	—	A	8.4	11.8	30	7.8	FT	Y	0	0	RG	
4/10	—	—	B	8.5	11.4	30	7.9	FT	Y	0	0	RG	
4/12	—	—	A	8.4	~~11.8~~ 11.8 ①	30	7.8	FT	Y	0	0	RG	
4/12	—	—	B	8.5	11.5	~~30~~ ①	7.8	FT	Y	0	0	RG	
4/14	–	✓	A	8.3	12.4	30	7.8	FT	Y	0	–	TW	
4/14	–	✓	B	8.3	11.8	30	7.8	FT	Y	0	0	TW	
						② Disp							

FT = Flow-through

*For all containers and all days for a given batch; if **>10% notify lab manager***

[1] Cumulative # Mort is the running total of the **current day's total mortality + previous cumulative culture mortality** since acquired in lab

① MC RG 4/5, 4/12 RG

② IE-MS 4/17

APPENDIX B

CHAIN-OF-CUSTODY AND SAMPLE RECEIPT FORMS

EPA Manchester Lab (REGION COPY)
DateShipped: 4/16/2024
CarrierName: EcoAaylists (hand delivery)
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2024/WA
Project Code:
Cooler #: Other

No: 10-041624-073555-0795
IFD10W2LA0010PXTSDDD2
Contact Name: Mario Lopez Ramos
Contact Phone:

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
24162146_1		Ground Water/ C.Aguilar	Composite	CHRTOX(8 Weeks)	N (< 6 C) (2)	SP-11	04/16/2024 09:28	Field Sample

Special Instructions: 2024 Week 16 - 2nd Quarter Bioasssay - Chronic Toxicity Bivalve Test

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
	Jacobs	1200 4/16/24			
	Jacobs	1154	EcoA.	4/16 1154	P240416.03a : 4.7°C (D) P240416.03b : 5.5°C

D IE-MS 4/16

SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:	
Jacobs Wykoff	24162146-1	P240416.03		
Project:				
Chronic Toxicity Bivalve Test				
Date/Time Received:		4/16/24 1154		
Airbill #:		NA		
Shipper Tracking Information Kept for Records: (Y/N/NA)		NA		
Collection Date/Time:		4/16/24 0920		
Sample Holding Time (must be ≤36 hours at test initiation)		Y		
Condition of Shipping Container:		Good		
Type and Capacity of Sample Container:		1L Cubi x2		
Total Sample Volume (L):		2L		
Condition of Sampling Container:		Good		
Sample Container Appropriate: (Y/N)		Y		
Custody Seals Intact: (Intact/Broken/Not Present)		Not present		
Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N)		Y		
Sampler's Name Present on COC Form: (Print Name/Not Present)		C. Aguilar		
Color:		Clear		

TAKE THE FOLLOWING MEASUREMENTS UPON ARRIVAL

LAB ID	Meter #	Temp. (°C) (0-6°C) *	Meter #	Dissolved Oxygen (mg/L)	Meter #	pH	Meter #	Cond. (µS/cm)	Meter #	Sal. (ppt)	Hardness (mg $CaCO_3$/L)	Alkalinity (mg $CaCO_3$/L)	Total Chlorine (mg/L)	Total NH3 (mg/L)	Tech
P240416.03a	T21	4.7		—		—		—		—	—	—	—	—	MS
P240416.03b	T21	5.5		—		—		—		—	—	—	—	—	MS
① P240416.03	—	—	7	8.3	7	7.5	—	—	7	0.5	—	—	0.03	0.37	RG/MS

*Notify project manager or study director of temperatures above 6°C or ≥36 hours holding time. Client must be notified ASAP.

If there are sample receipt problems, complete the following:	
Reason for unacceptability:	
Name of Client Contact:	Contacted by:
Client Response and/or Action to be Taken:	Date Action Taken:

① Composited sample after temp. taken - MS 4/16