Wyckoff Groundwater Treatment Plant: Fourth Quarter 2023 Bioassay Monitoring

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1. Introduction

This technical memorandum summarizes information obtained from the fourth quarter 2023 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 Site's National Pollutant Discharge Elimination System (NPDES).

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 NPDES permit.

The current NPDES permit does not include effluent limits for acute or chronic toxicity. Both acute and chronic toxicity testing were conducted on the effluent samples collected on October 3, 2023 and November 8, 2023 per the requirements outlined in the NPDES permit.

The current NPDES permit does not include specific dilution series for chronic toxicity tests. With the exception of the first and second quarter of 2023, all previous mussel larvae chronic toxicity testing were conducted with hypersaline brine (HSB) addition 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). The highest test concentration for these HSB adjusted samples therefore were less than 100% (ranged from approximately 69 to 76%). Mussel larvae chronic toxicity testing conducted for the first and second quarter of 2023 used samples adjusted with artificial salts only.

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 fourth quarter 2023 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). 73.5 percent effluent is the highest concentration tested using the HSB for the fourth quarter 2023 testing.

For the acute toxicity bioassay (estuarine fish), no statistically significant effects on survival were detected for any concentration tested. This result indicates the median lethal concentration to cause 50% mortality (LC50) in the test population is greater than 100 percent of the effluent concentration and

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

an acute toxic unit (TU) of less than 1.0. No statistically significant effects on the survival or development endpoints were observed for all test concentrations for the mussel larvae chronic toxicity testing, indicating no evidence of the presence of chronic toxicity.

As stated above, the current NPDES permit does not include effluent limit for acute or chronic toxicity. The acute toxicity test requirement section of the permit (Section I.5) specifies the following:

"*If the test demonstrates the presence of acute toxicity, EPA will undertake the following actions as needed to determine the source of toxicity:*

(a) Chemical analysis.

(b) Evaluation of treatment processes and chemicals used.

(c) Physical inspection of facility for proper operation of treatment units, spills, etc.

(d) Examination of records.

(e) Interviews with plant personnel to determine if toxicant releases occurred through spills, unusual operating conditions, etc.

If any toxicity remains after conducting the above steps, additional monitoring or treatment may be required."

The chronic toxicity test requirement section of the permit (Section II.8) specifies the following:

"*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 acute and chronic endpoints would not trigger these requirements.

2. Sampling and Analysis Results

Bioassay samples were collected per the monitoring frequency included in the NPDES permit. Samples were collected from a 24-hr. autosampler composite collection point at the effluent discharge point of the treatment system. One composite water sample was collected on October 3, 2023, for acute toxicity testing, and another sample collected on November 8, 2023, for chronic toxicity testing. Chemical testing was conducted in conjunction with each sample collected for bioassay testing for performance and NPDES compliance requirements. The bioassay analyses were performed by EcoAnalysts, Inc. (EcoAnalysts), of Port Gamble, Washington, a Washington State Department of Ecology accredited lab. Table 1 lists the sample Laboratory ID and sampling analysis methods. EcoAnalysts sampling analyses report for acute toxicity and chronic toxicity testing are provided in Attachments 1 and 2, respectively.

Table 1. Biological Testing Summary

Laboratory	Laboratory ID	Method	Test Type/Descriptor/Species
EcoAnalysts	P231003.08	EPA-821-R-02-012; Test Method 2006.0; EPA/600/4-90/027F; SOP TOX013.08	Acute/96-hr Survival/*Menidia beryllina* (Inland Silverside)
EcoAnalysts	P231108.01	EPA/600/R-95-136 Method 1005.0; ASTM E724-89 TOX042.12	Chronic/48-hr Survival and Development/*Mytilus galloprovincialis* (Mussel)

For the acute toxicity bioassay (estuarine fish), no statistically significant effects on survival were detected for any concentration tested (i.e. LC50 is greater than 100 percent of the effluent concentration and TU is less than 1.0). For the mussel larvae chronic toxicity testing, no statistically significant effects were detected in any effluent concentration tested for the survival or development endpoint. This result indicates a No Observed Effect Concentration (NOEC) of 100 percent (TU is less than 1.0) and 73.5 percent (the highest concentration tested; TU is 1.4 for both endpoints) of the effluent concentration for the salts and HSB adjusted samples, respectively. The Effect Concentration expected to affect 50 percent of the organisms (EC50) is greater than 100 percent and 73.5 percent of the effluent concentration, respectively for the salts and HSB adjusted samples, respectively.

3. Laboratory Quality Data Review

A CH2M chemist validated the bioassay results Stage 2A in accordance with the CH2M 2022 QAPP. This QAPP was cited by EcoAnalysts and the appropriate species of estuarine fish and mussel specified in the QAPP were 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) Artificial Salts Proportion Normal for the mussel larvae chronic toxicity test was significantly lower (2.0 percent) relative to the laboratory (dilution water) control (98.2 percent). Since no toxicity was observed in any test concentrations, indicating artificial salts did not contribute to any negative biological effects, therefore there is no impact to the test concentrations.

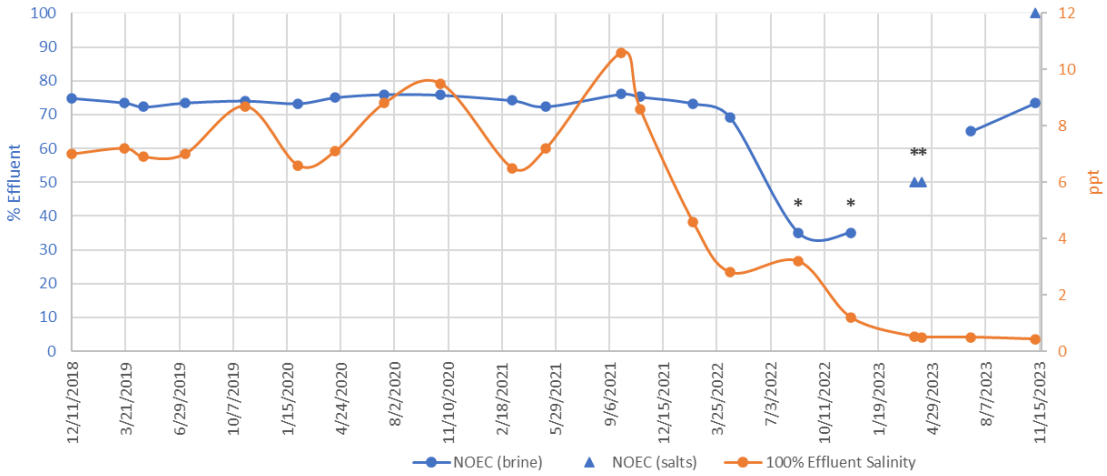
4. Trends

A review of bioassay data collected from 2007 through the fourth quarter of 2023 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 November 2023. 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

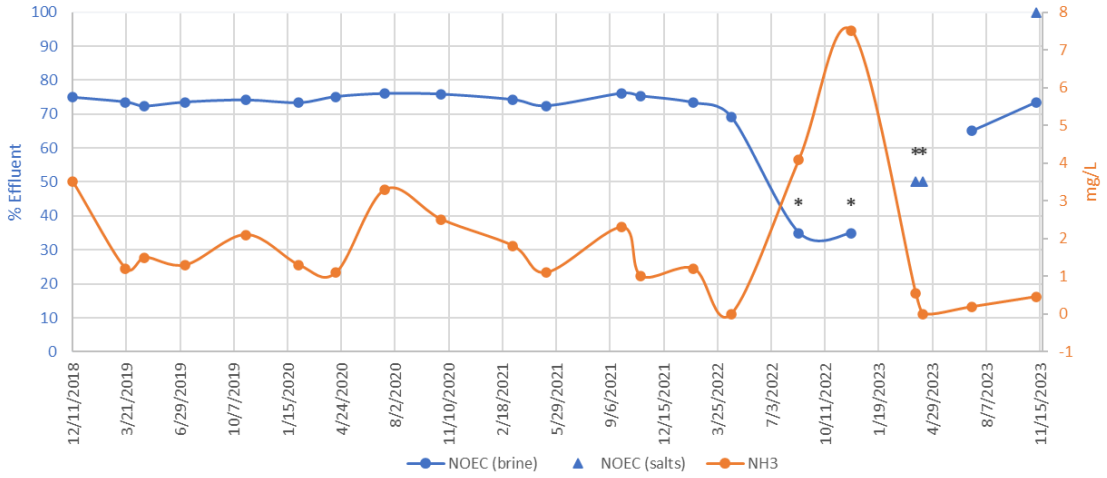
sampling events. For the fourth quarter of 2023, concurrent tests were conducted (one test using artificial salts and the other test using HSB) for the split samples (see Figure 1 for NOEC for samples with salinity adjustment using brine and salts). A review of the water quality parameters measured for the bioassay samples indicated the lowest detected salinity levels were detected in the samples collected from the most recent six 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- Nov 2023)



* 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- Nov 2023)



* Statistically significant effect detected in the maximum test concentration

5. Overall Assessment

While the current NPDES permit 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 acute toxicity test met the WET performance standard because survival rates were within acceptable limits. 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. Due to the recently observed toxicity from third quarter 2022 through second quarter of 2023, CH2M recommends triggering of an accelerated testing if the next testing meets EPA test acceptability criteria and a statistically significant effect is detected when compared to the lab control. As there are no established chronic toxicity criteria included in the permit, CH2M recommends an accelerated schedule of WET testing to establish whether a pattern of chronic toxicity exists. Consistent with WAC 173-205-090(1)(b), it is recommended that the accelerated testing to be conducted monthly for three months using the same toxicity test as in the routine effluent WET testing where a statistically significant effect is detected.

Due to the deviation of the QAPP quality control requirement for the Artificial Salts Proportion Normal for the mussel larvae chronic toxicity test, CH2M recommends continued concurrent mussel chronic bioassay testing using both artificial salt and brine for salinity adjustments in 2024 Q1. The following lists the recommended test dilution series:

<u>Brine</u>
Maximum test concentration, 50%, 25%, 12.5%, 6.25%, and control

<u>Artificial Salt</u>
100%, Maximum test concentration for the chronic toxicity testing using brine for salinity adjustment, 50%, 25%, 12.5%, 6.25%, and control.

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.

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.

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 for Wyckoff/Eagle Harbor Superfund Groundwater Treatment Plant

TOXICITY TESTING RESULTS

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

NPDES TOXICITY TESTING: 4TH QUARTER 2023

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Submittal Date: December 15, 2023

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.

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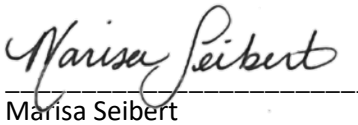

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TABLES

APPENDICES

ACRONYMS AND ABBREVIATIONS

ABS:	Aquatic BioSystems
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.

No statistically significant biological responses of the test organisms were detected in the 96-hour acute test as well as at the highest test concentrations (100% and 73.5% effluent) for any of the chronic endpoints tested (Table 1-1).

Table 1-1. Toxicity Test Results Summary.

Test		NOEL (%)	LOEL (%)	LC_{50}/EC_{50} (%)
Acute	*Menidia beryllina* 96-Hour Survival	100	>100	>100
Chronic - Brine	*Mytilus galloprovincialis* 48-Hour Proportion Survived	73.5	>73.5	>73.5
	Mytilus galloprovincialis 48-Hour Proportion Normal	73.5	>73.5	>73.5
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 the United States Environmental Protection Agency (USEPA) document *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms* (EPA-821-R-02-012), which is the most recently promulgated version of *Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms*, (EPA/600/4-90/027F), and 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 a sample on October 3, 2023, which was used for the *Menidia beryllina* acute test. A second round of sampling was collected on November 8, 2023, which was 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 temperature upon receipt for the October sample was 5.1°C and was 3.6°C for the sample collected in November. Both samples were within the recommended temperature range.

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	100323	110823
Laboratory ID	P231003.08	P231108.01
Date/Time sampled	10/03/23; 0114	11/08/23; 0115
Date/Time received	10/03/23; 1305	11/08/23; 1220
Dissolved Oxygen (mg/L) Recommended: >4.0 mg/L	8.5	9.6
Temperature (°C) Recommended: 0 – 6°C	5.1	3.6
pH (units) Recommended: 6 – 9	7.5	7.3
Conductivity (µS/cm)	1008	909
Salinity (ppt)	0.5	0.432
Total Chlorine (mg/L)	0.01	0.03
Total Ammonia (mg/L)	0.592	0.458

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
Acute	96-Hour Survival	*Menidia beryllina* Inland Silverside	EPA-821-R-02-012; Test Method 2006.0; EPA/600/4-90/027F; SOP TOX013.08
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 November 6, 2023. They were delivered via Taylor Shellfish personnel and maintained under ambient seawater flow-through conditions at 12 ± 3˚C until utilized for testing. *Menidia beryllina* (inland silversides) were purchased from Aquatic BioSystems Inc. (ABS) in Fort Collins, Colorado. ABS is a commercial supplier of test organisms that are used routinely for toxicity testing. Water quality measurements were collected from transport containers and the overall health of the organisms was visually confirmed by a laboratory technician.

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 100323 was received at a salinity of 0.5 ppt and sample 110823 was received at a salinity of 0.432 ppt. The salinity of the effluent sample was increased by the addition of Crystal Sea® MarineMix bioassay grade artificial salt for the *M. beryllina* and 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
100323: Collected 10/03/23	*Menidia beryllina* 96-Hour Survival	0.5 ppt	30 ± 2	Artificial Salt
110823: Collected 11/08/23	*Mytilus galloprovincialis* 48-Hour Survival and Development	0.4 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 Inland Silverside (*Menidia beryllina*) Acute Test Results

The acute toxicity test with *M. beryllina* was initiated on October 3, 2023. The test met the control acceptability criteria of ≥90% mean survival with a mean control survival of 95%. Mean survival for all treatments is summarized in Table 3-1. The test conditions are summarized in Table 3-2**.**

Concentrations of 6.25, 12.5, 25, 50, and 100% effluent were prepared utilizing laboratory water. Sample 100323 (received 10/03/23) was used for test initiation and test solution renewals at 48-hours.

Water quality parameters were within the acceptable limits throughout the duration of the 96-hour static-renewal test. The test chamber for 50% replicate 3 was partially spilled during water quality measurements on Day 2, and 4 fish were lost. These fish were removed from the statistical analysis.

There was no significant difference observed between the laboratory control and the salt control indicating that artificial salts should not have contributed to any negative biological effects, if observed.

The LC_{50} for the copper chloride reference-toxicant test was 165.7 µg Cu/L. These results were within two standard deviations of the laboratory mean at the time of testing (Table 3-5). This indicates that the organisms obtained from this supplier were of similar sensitivity to those previously tested at the EcoAnalysts laboratory.

Table 3-1. Endpoint Summary for the *Menidia beryllina* Acute Test

Conc. (%)	100323				
	Mean Survival (%)	Standard Deviation	NOEL (%)	LOEL (%)	LC_{50} Value (%)
Control (0)	95.0	5.8	100	>100	>100
Salt Control	95.0	5.8			
6.25	90.0	14.1			
12.5	87.5	12.6			
25	95.0	10.0			
50	91.7	16.7			
100	97.5	5.0			

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

Table 3-2. Test Condition Summary for *Menidia beryllina* Acute Test

Test Duration / Type		96-hour / Static-Renewal
Species		*Menidia beryllina*
Supplier		Aquatic Biosystems
Date acquired		10/03/23
Test Dates		10/03/23 – 10/07/23
Age at test initiation (Recommended: 9 - 14 days)		9 Days
Samples used:		100323; P231003.08
Sample Holding Time at Initiation: Recommended: <36 hours; Not to exceed 72 hours		16 hours
Test Procedures		EPA-821-R-02-012, Test Method 2006.0; EPA/600/4-90/027F; SOP TOX013.08
Test location		EcoAnalysts Port Gamble Laboratory
Control water / Diluent		0.45 µm-filtered, North Hood Canal seawater
Test Lighting		16 hour light / 8 hour dark
Test Chamber		12 oz. Plastic Chamber
Exposure volume		250 mL
Replicates/treatment		4
Concentration/treatment		6.25, 12.5, 25, 50, 100%
Organisms/replicate		10
Feeding		0.1 mL concentrated *Artemia* nauplii daily
Test solution renewal		Day 2
Test Dissolved Oxygen (Recommended: ≥4.0 mg/L)		6.1 – 8.4 mg/L
Test Temperature (Recommended: 20 ± 1°C)		18.7 – 20.4°C
Test Salinity (Recommended: 30 ± 2 ppt)		28 – 32 ppt
Test pH (Range not specified) Targeted Range: 6 – 9 units		7.6 – 8.3 units
Quality Assurance		
Control performance standards Survival (Recommended): ≥ 90%		95.0%; meets acceptability criterion
Reference Toxicant Date		10/03/23
Survival	Reference Toxicant LC_{50}	165.7 µg/L copper
	Laboratory Mean $LC_{50;}$ Range LC_{50} (±2 SD)	227.5 (129 – 401 µg/L copper)
Deviations from Test Protocol		4 fish lost from 50% replicate 3

3.2 *Mytilus galloprovincialis* Test Results

The chronic toxicity test with *M. galloprovincialis* was conducted on November 8, 2023 with sample 110823. 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 98.7% proportion survived, 97.9% proportion normal, and 2.1% PMSD for proportion normal in the laboratory control. The test conducted with artificial salts resulted in 97.2% proportion survived, 98.2% proportion normal, and 1.5% PMSD for proportion normal in the laboratory control. Mean survival and proportion normal are summarized in Table 3-3 (brine) and Table 3-4 (salt). The test conditions are summarized in Table 3-5.

Concentrations of 6.25, 12.5, 25, 50, and 73.5% effluent were prepared utilizing laboratory water. A 100% test concentration was also included for the test with artificial salts. Sample P231108.01 (received 11/08/23) was used for test initiation. Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static test.

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

An interrupted dose response occurred in the brine test for survival, where the 12.5% concentration was significantly different than the control, but no other concentration was. As the % effect was low (8%), the result was deemed anomalous and the higher NOEL/LOEL pair was selected for reporting.

The EC_{50} for the ammonia reference toxicant test was 9.3 mg/L total ammonia and was within two standard deviations of the laboratory mean (Table 3-2) at the time of testing. This indicates that the organisms are of a similar sensitivity to those previously tested at the EcoAnalysts laboratory.

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

Conc. (%)	Mean Proportion Survived (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC_{50} Value (%)
Control	98.7	2.6	73.5	>73.5	>73.5
Brine Control	98.4	2.1			
6.25	98.5	2.0			
12.5	90.9	1.6			
25	99.4	1.2			
50	97.6	4.4			
73.5	97.7	2.2			
Conc. (%)	**Mean Proportion Normal (%)**	**Standard Deviation**	**NOEL (%)**	**LOEL (%)**	**EC_{50} Value (%)**
Control	97.9	1.8	73.5	>73.5	>73.5
Brine Control	96.2	2.1			
6.25	98.2	1.2			
12.5	97.3	0.7			
25	98.3	0.7			
50	97.8	0.4			
73.5	97.7	1.3			

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 3-4. Results Summary for *Mytilus galloprovincialis* Embryo Development Test (Salt)

Conc. (%)	Mean Proportion Survived (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC_{50} Value (%)
Control	97.2	3.6	100	>100	>100
Salt Control	92.3	6.5			
6.25	97.3	4.0			
12.5	98.4	3.2			
25	94.8	8.5			
50	98.4	3.2			
73.5	98.2	2.1			
100	99.1	1.0			
Conc. (%)	**Mean Proportion Normal (%)**	**Standard Deviation**	**NOEL (%)**	**LOEL (%)**	**EC_{50} Value (%)**
Control	98.2	0.5	100	>100	>100
Salt Control	2.0	2.1			
6.25	98.1	0.7			
12.5	97.3	0.8			
25	97.6	0.4			
50	97.0	1.7			
73.5	98.0	0.5			
100	98.2	0.4			

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 3-5. Test Condition Summary for *Mytilus galloprovincialis* Embryo Development Test.

Test Duration / Type	**48-Hour; Static**	
Species	*Mytilus galloprovincialis*	
Supplier	Taylor Shellfish	
Date acquired	11/06/23	
Test Dates	11/08/23 – 11/10/23	
Age at test initiation Recommended: <4-hour embryos	<4 hours	
Sample(s) used:	110823; P231108.01	
Holding Time at Initiation: Recommended: < 36 hours	15 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: 254
Replicates/treatment	4	
Concentration/treatment	6.25, 12.5, 25, 50, and 73.5% (brine) 6.25, 12.5, 25, 50, 73.5 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), 7.8 – 8.7 mg/L (salt)
Test Temperature	Recommended: 16 ± 1°C	Actual: 15.9 – 16.8 °C (brine), 15.6 – 16.8 °C (salt)
Test pH	Recommended: 7 – 9	Actual: 7.6 – 8.3 (brine), 7.7 – 8.2 (salt)
Test Salinity	Recommended: 30 ± 2 ppt	Actual: 29 – 31 ppt (brine), 30 – 32 ppt (salt)
Control performance standard (Survival, Normal shell development, PMSD)	Recommended: ≥50% survival, ≥90% normal development, <25% PMSD	Actual: Brine: 98.7% survival, 97.9% normal development, 2.1% PMSD; Salt: 97.2% survival, 98.2% normal development, 1.5% PMSD
Reference Toxicant Date	11/08/23	
Reference Toxicant EC_{50}	9.3 mg/L total ammonia	
Laboratory Mean EC_{50}	7.0 mg/L total ammonia	
Acceptable Range EC_{50} (± 2 SD)	3.8 – 12.9 mg/L total ammonia (within range)	
Deviations from Test Protocol	Salt Control Proportion Normal	

4. REFERENCES

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

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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.

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APPENDIX A

STATISTICAL COMPARISONS AND LABORATORY DOCUMENTS

APPENDIX A.1

MENIDIA BERYLLINA 96-HOUR SURVIVAL TEST

STATISTICAL COMPARISON AND LABORATORY DATA SHEETS

CETIS Summary Report

Inland Silverside 96-h Acute Survival Test — EcoAnalysts

Batch ID:	13-4916-0729	**Test Type:**	Survival (96h)	**Analyst:**	Marisa Seibert
Start Date:	03 Oct-23 17:43	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Laboratory Seawater
Ending Date:	07 Oct-23 16:21	**Species:**	Menidia beryllina	**Brine:**	Crystal Sea Marine Mix
Test Length:	95h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO **Age:** 9d
Sample ID:	10-8719-6579	**Code:**	P231003.08M.b.	**Project:**	Wyckoff Eagle Harbor GWTP 2023/W
Sample Date:	03 Oct-23 01:14	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	03 Oct-23 13:05	**CAS (PC):**		**Station:**	100323
Sample Age:	16h	**Client:**	Jacobs Wyckoff		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
19-6960-0762	96h Proportion Survived	Steel Many-One Rank Sum Test		100	>100	---	20.6%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
19-3255-6303	96h Proportion Survived	Linear Interpolation (ICPIN)		EC15	>100	---	---	<1	1
				EC20	>100	---	---	<1	
				EC25	>100	---	---	<1	
				EC40	>100	---	---	<1	
				EC50	>100	---	---	<1	

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
19-3255-6303	96h Proportion Survived	Control Resp	0.95	0.9	<<	Yes	Passes Criteria
19-6960-0762	96h Proportion Survived	Control Resp	0.95	0.9	<<	Yes	Passes Criteria

96h Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	0.00%
0	SC	4	0.9500	0.8581	1.0420	0.9000	1.0000	0.0289	0.0577	6.08%	0.00%
6.25		4	0.9000	0.6750	1.1250	0.7000	1.0000	0.0707	0.1414	15.71%	5.26%
12.5		4	0.8750	0.6748	1.0750	0.7000	1.0000	0.0629	0.1258	14.38%	7.89%
25		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	0.00%
50		4	0.9167	0.6515	1.1820	0.6667	1.0000	0.0833	0.1667	18.18%	3.51%
100		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	-2.63%

96h Proportion Survived Detail — MD5: 5DB1FEEBBD461E0435C8FDAD6004E375

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	0.9000	0.9000
0	SC	1.0000	1.0000	0.9000	0.9000
6.25		1.0000	0.9000	0.7000	1.0000
12.5		0.9000	0.9000	1.0000	0.7000
25		1.0000	0.8000	1.0000	1.0000
50		1.0000	1.0000	0.6667	1.0000
100		1.0000	1.0000	0.9000	1.0000

96h Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	9/10	9/10
0	SC	10/10	10/10	9/10	9/10
6.25		10/10	9/10	7/10	10/10
12.5		9/10	9/10	10/10	7/10
25		10/10	8/10	10/10	10/10
50		10/10	10/10	4/6	10/10
100		10/10	10/10	9/10	10/10

Inland Silverside 96-h Acute Survival Test

EcoAnalysts

Analysis ID:	06-8614-6850	**Endpoint:**	96h Proportion Survived	**CETIS Version:**	CETISv2.1.4
Analyzed:	25 Oct-23 12:29	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Edit Date:	25 Oct-23 12:21	**MD5 Hash:**	8F3C26FE85F66BFDE2E0DE5FD24D2868	**Editor ID:**	003-841-189-5
Batch ID:	13-4916-0729	**Test Type:**	Survival (96h)	**Analyst:**	Marisa Seibert
Start Date:	03 Oct-23 17:43	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Laboratory Seawater
Ending Date:	07 Oct-23 16:21	**Species:**	Menidia beryllina	**Brine:**	Crystal Sea Marine Mix
Test Length:	95h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO **Age:** 9d
Sample ID:	10-8719-6579	**Code:**	P231003.08M.b.	**Project:**	Wyckoff Eagle Harbor GWTP 2023/W
Sample Date:	03 Oct-23 01:14	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	03 Oct-23 13:05	**CAS (PC):**		**Station:**	100323
Sample Age:	16h	**Client:**	Jacobs Wyckoff		

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed 96h proportion survived endpoint	8.47%

Wilcoxon Rank Sum Two-Sample Test

Control I	vs	Control II	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	18	---	3	Exact	0.7571	Non-Significant Effect

Test Acceptability Criteria

		TAC Limits			
Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.95	0.9	<<	Yes	Passes Criteria
Control Resp	0.95	0.9	<<	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	0	1.0000	Non-Significant Effect
Error	0.0531187	0.0088531	6			
Total	0.0531187		7			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Variance Ratio F Test	1	47.47	1.0000	Equal Variances
Distribution	Anderson-Darling A2 Test	1.422	3.878	0.0005	Non-Normal Distribution
	Kolmogorov-Smirnov D Test	0.3252	0.3313	0.0127	Normal Distribution
	Shapiro-Wilk W Normality Test	0.6647	0.6451	0.0009	Non-Normal Distribution

96h Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9500	0.8581	1.0000	0.9500	0.9000	1.0000	0.0289	6.08%	0.00%
0	SC	4	0.9500	0.8581	1.0000	0.9500	0.9000	1.0000	0.0289	6.08%	0.00%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	1.3310	1.1810	1.4800	1.3310	1.2490	1.4120	0.0471	7.07%	0.00%
0	SC	4	1.3310	1.1810	1.4800	1.3310	1.2490	1.4120	0.0471	7.07%	0.00%

96h Proportion Survived Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	0.9000	0.9000
0	SC	1.0000	1.0000	0.9000	0.9000

Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.4120	1.4120	1.2490	1.2490
0	SC	1.4120	1.4120	1.2490	1.2490

Inland Silverside 96-h Acute Survival Test

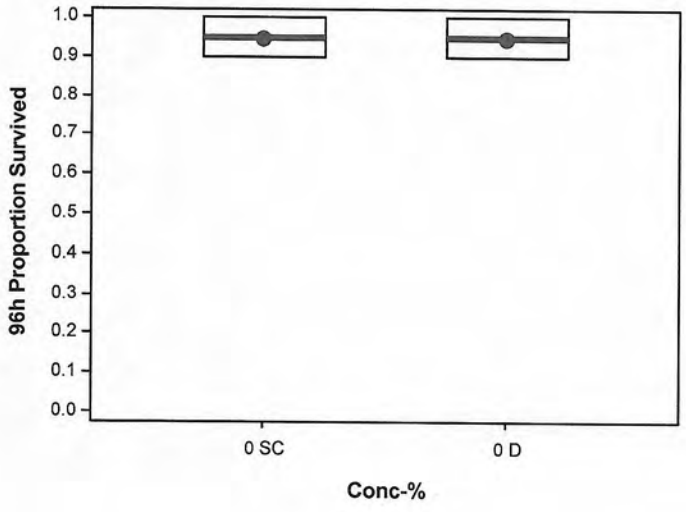
EcoAnalysts

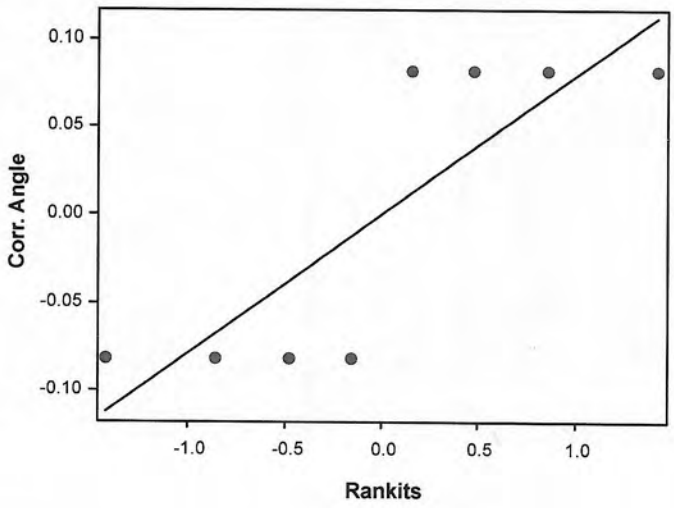
Analysis ID:	06-8614-6850	**Endpoint:**	96h Proportion Survived	**CETIS Version:**	CETISv2.1.4
Analyzed:	25 Oct-23 12:29	**Analysis:**	Nonparametric-Two Sample	**Status Level:**	1
Edit Date:	25 Oct-23 12:21	**MD5 Hash:**	8F3C26FE85F66BFDE2E0DE5FD24D2868	**Editor ID:**	003-841-189-5

96h Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	9/10	9/10
0	SC	10/10	10/10	9/10	9/10

Graphics





CETIS Test Data Worksheet

Report Date: 25 Oct-23 12:29 (p 1 of 1)
Test Code/ID: P231003.08M.b. / 05-3541-5024

Inland Silverside 96-h Acute Survival Test — **EcoAnalysts**

Start Date:	03 Oct-23 17:43	**Species:**	Menidia beryllina	**Sample Code:**	P231003.08M.b.
End Date:	07 Oct-23 16:21	**Protocol:**	EPA/821/R-02-012 (2002)	**Sample Source:**	Jacobs Wyckoff
Sample Date:	03 Oct-23 01:14	**Material:**	Treated Groundwater	**Sample Station:**	100323

Conc-%	Code	Rep	Pos	# Exposed	24h Survival	48h Survival	72h Survival	96h Survival	Notes
0	D	1	4	10	10	10	10	10	
0	D	2	13	10	10	10	10	10	
0	D	3	11	10	10	10	10	9	
0	D	4	19	10	9	9	9	9	
0	SC	1	22	10	10	10	10	10	
0	SC	2	1	10	10	10	10	10	
0	SC	3	6	10	10	10	10	9	
0	SC	4	10	10	10	10	9	9	
6.25		1	21	10	10	10	10	10	
6.25		2	8	10	10	9	9	9	
6.25		3	25	10	8	7	7	7	
6.25		4	16	10	10	10	10	10	
12.5		1	15	10	9	9	9	9	
12.5		2	23	10	9	9	9	9	
12.5		3	27	10	10	10	10	10	
12.5		4	18	10	10	8	7	7	
25		1	9	10	10	10	10	10	
25		2	5	10	9	8	8	8	
25		3	26	10	10	10	10	10	
25		4	14	10	10	10	10	10	
50		1	12	10	10	10	10	10	
50		2	28	10	10	10	10	10	
50		3	3	6	4	4	4	4	
50		4	24	10	10	10	10	10	
100		1	20	10	10	10	10	10	
100		2	7	10	10	10	10	10	
100		3	17	10	9	9	9	9	
100		4	2	10	10	10	10	10	

EcoAnalysts, Inc.

7 Day Chronic WET Test

Version V.3

GENERAL

Client	Jacobs Wyckoff
Project	Wyckoff Eagle Harbor GWTP 2023/WA
Project Number	PG1799
Project Manager	M. Seibert
Date Sample Received	10/3/2023
Test type	96-Hour Acute Toxicity with Menidia
Matrix	Liquid
Test Acceptability	≥ 90% average survival of control
Test Start Date	10/03/23
Test Species	*Menidia beryllina*
Organism Batch	ABS100323
Organism Acquired	10/3/2023
Organism Acclimation	0
Organism Age	9 days
Test Protocol	TOX 013
Test Location	Bath 4
Light Intensity	50-100 foot candles
Light Cycle	16L:8D
Water Description	0.45 um filtered seawater
Organisms per Replicate	10
Test Chamber Size	12 oz. cup
Exposure Volume	250 mL
Feeding Information	0.1 mL Artemia daily
Test Dissolved Oxygen	> 4.0
Test Temperature	20 ± 1
Test Salinity	30 ± 2
Test pH	7.5 ± 1.5

Food Batch ID
281729.00

CSMM Batch #
62123.00

Note: input lowest and highest decimal for temp

Test Parameters		
	Min	Max
DO	4.0	
Temp	18.5	21.4
Salinity	28	32
pH	6	9

TEST START TIME/INIT: 1743 CG/JC

TEST END TIME/INIT: 1621 NL

CLIENT SAMPLE ID	LAB ID
100323	P231003.08

Concentrations

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

Copy and Past VALUES

Treatment	Rep	Chamber
Control	1	18
Control	2	25
Control	3	6
Control	4	12
Salt Control	1	8
Salt Control	2	22
Salt Control	3	21
Salt Control	4	17
6.25%	1	24
6.25%	2	26
6.25%	3	10
6.25%	4	28
12.5%	1	4
12.5%	2	23
12.5%	3	15
12.5%	4	11
25%	1	5
25%	2	20
25%	3	9
25%	4	16
50%	1	1
50%	2	27
50%	3	2
50%	4	14
100%	1	7
100%	2	19
100%	3	13
100%	4	3

ECO ANALYSTS, INC.

96-Hour Acute WET Test

V3

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	10/3/23	**PROTOCOL**	TOX 013
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	10/3/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	100323	**TEST END DATE**	10/7/23	**SPECIES**	*Menidia beryllina*
LAB SAMPLE ID	P231003.08	**MATRIX**	Liquid	**NO. OF ORGANISMS**	10

96-Hour Acute Toxicity with Menidia

		DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
	Concentration (%)	> 4.0	19 - 21	28 - 32	6 - 9
Day 0	Control	① ~~8.0~~ 7.6	18.9	32	7.8
Stock	Salt Control	8.2	19.2	30	8.2
Date 10/3/23	6.25%	7.6	19.4	32	7.7
Time 1520	12.5%	7.8	19.1	32	7.7
Tech MS	25%	① ~~7.9~~ 8.0	18.9	① ~~31~~ 32	7.7
Meter # 8	50%	8.0	18.7	31	7.7
	100%	② 8.4	18.9	31	7.6
Day 1	Control	② ~~7.3~~ 7.1	② ~~19.8~~ 19.6	~~31~~	7.8
Rep 1	Salt Control	7.0	19.9	29	8.1
Date 10/4/23	6.25%	7.0	19.5	31	7.9
Time 0953	12.5%	6.9	19.7	31	7.9
Tech JI	25%	7.0	19.7	31	8.0
Meter # 9	50%	7.0	19.7	31	8.0
	100%	7.0	19.8	30	8.0
Day 2	Control	6.3	19.9	31	7.8
Rep 2	Salt Control	6.8	19.6	29	8.0
Date 10/05/23	6.25%	6.8	19.7	31	7.9
Time 1034	12.5%	6.7	19.5	31	8.0
Tech SR	25%	6.6	19.6	31	8.1
Meter # 8	50%	6.5	19.6	30	8.2
	100%	6.6	19.6	30	8.3
Day 2	Control	7.5	20.4	28	7.9
Renewal Stock	Salt Control	7.5	20.3	30	8.2
Date 10/05/23	6.25%	7.6	20.3	29	7.9
Time 1522	12.5%	7.6	20.3	29	7.9
Tech SR	25%	7.7	20.3	29	7.9
Meter # 9	50%	7.7	20.3	29	7.8
	100%	7.8	20.2	30	7.8

① MR-MS 10/3
② IE-MS 10/3, JI 10/4/23

ECOANALYSTS, INC.

96-Hour Acute WET Test

V3

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	10/3/23	**PROTOCOL**	TOX 013
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	10/3/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	100323	**TEST END DATE**	10/7/23	**SPECIES**	*Menidia beryllina*
LAB SAMPLE ID	P231003.08	**MATRIX**	Liquid	**NO. OF ORGANISMS**	10

96-Hour Acute Toxicity with Menidia

Day 3	Control	6.8	19.7	29	7.8
Rep 3	Salt Control	7.0	19.7	29	8.0
Date 10/06/23	6.25%	7.0	19.7	29	7.9
Time 1039	12.5%	6.9	19.7	29	8.0
Tech SR	25%	6.7	19.7	29	8.0
Meter # 8	50%	7.0	19.7	29	8.1
	100%	6.8	19.7	30	8.2
Day 4	Control	6.3	20.1	29	7.8
Rep 4	Salt Control	6.2	20.2	29	7.9
Date 10/7/23	6.25%	6.1	20.1	29	7.9
Time 1105	12.5%	6.2	20.1	29	8.0
Tech NL	25%	6.2	20.2	29	8.1
Meter # 8	50%	6.2	20.1	29	8.2
	100%	6.1	20.1	30	8.3

ECO ANALYSTS, INC.

96-Hour Acute WET Test

V3

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	10/3/23	**PROTOCOL**	TOX 013
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	10/3/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	100323	**TEST END DATE**	10/7/23	**SPECIES**	*Menidia beryllina*
LAB SAMPLE ID	P231003.08	**MATRIX**	Liquid	**NO. OF ORGANISMS**	10

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

96-Hour Acute Toxicity with Menidia

		Day 1		Day 2		Day 3		Day 4	
		Date	10/4/23	Date	20105/23	Date	20106/23	Date	10/7
		Time	1015	Time	2533	Time	2068	Time	1621
		Tech	JI	Tech	SR	Tech	SR	Tech	NL
Concentration (%)	**REP**	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	9	1
	4	9	1	9	0	9	0	9	0
Salt Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	9	1
	4	10	0	10	0	9	1 NB	9	0
6.25%	1	10	0	10	0	10	0	10	0
	2	10	0	9	1	9	0	9	0
	3	8	2	7	1	7	0	7	0
	4	10	0	10	0	10	0	10	0
12.5%	1	9	1	9	0	9	0	9	0
	2	9	1	9	0	9	0	9	0
	3	10	0	10	0	10	0	10	0
	4	10	0	8	2	7	0	7	0
25%	1	10	0	10	0	10	0	10	0
	2	9	1	8	1	8	0	8	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
50%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	8	2	4	4 NB	4	0	4	0
	4	10	0	10	0	10	0	10	0

@ cup spilled during WQ - SR 20105/23

ECO ANALYSTS, INC.

96-Hour Acute WET Test

V3

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	10/3/23	**PROTOCOL**	TOX 013
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	10/3/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	100323	**TEST END DATE**	10/7/23	**SPECIES**	*Menidia beryllina*
LAB SAMPLE ID	P231003.08	**MATRIX**	Liquid	**NO. OF ORGANISMS**	10

Abbreviation Key:
NB = No Body
FB = Found Body
ST = Stranded

96-Hour Acute Toxicity with Menidia

		Day 1		Day 2		Day 3		Day 4	
		Date	10/4/23	Date	20105123	Date	20106123	Date	1017
		Time	1015	Time	1533	Time	1058	Time	1621
		Tech	JI	Tech	SR	Tech	SR	Tech	ML
Concentration (%)	**REP**	Alive	Dead	Alive	Dead	Alive	Dead	Alive	Dead
100%	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	9	0 NB	9	0	9	0	9	0
	4	10	0	10	0	10	0	10	0
Feed (Init.)	AM	DM		JI 0844		JI 0838		SR	
0.1 mL Artemia daily	PM								

EcoAnalysts, Inc.

96-Hour Acute WET Test

V3

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	10/3/23	**PROTOCOL**	TOX 013
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	10/3/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	100323	**TEST END DATE**	10/7/23	**SPECIES**	*Menidia beryllina*
LAB SAMPLE ID	P231003.08	**MATRIX**	Liquid	**NO. OF ORGANISMS**	10

96-Hour Acute Toxicity with Menidia

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)	Diluent Type	FSW
0	0%	0	1000	1000		
	Salt Control	#VALUE!	#VALUE!	1000		
	6.25%	62.5	937.5	1000		
	12.5%	125	875	1000		
	25%	250	750	1000		
	50%	500	500	1000		
	100%	1000	0	1000		

Day of Test	Concentration	Vol. Effluent Sample Added (mL)	Vol. Diluent Added (mL)	Total Volume (mL)
2	0%	0	800	800
	Salt Control	#VALUE!	#VALUE!	800
	6.25%	50	750	800
	12.5%	100	700	800
	25%	200	600	800
	50%	400	400	800
	100%	800	0	800

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
10/3/23	#7	P231003.08	FSW100323.01	KB
10/05/23	7	P231003.08	FSW100323.01	SR

ORGANISM RECEIPT LOG

Date:	Time:	Batch No.
10/3/23	1200	ABS100323.01

Organism: Mysidia beryllina

Source / Supplier: Aquatic BioSystems

No. Ordered:	No. Received:	Source Batch: Collection date, hatch date, etc.):
630	690	9/24/23

Condition of Organisms:	Approximate Size or Age: (Days from hatch, life stage, size class, etc.):
Good	9 days
Shipper: UPS	**B of L (Tracking No.)** 1Z F46 73R 01 9017 9536
Condition of Container: Good	**Received By:** DM

Container	D.O. (mg/L)	Temp. (°C)	Cond. or Sal. (Include Units) PPT	pH (Units)	# Dead	% Dead*	Tech. (Initials)
1	15.6	21.1	27	7.0	8	—	DM
2	19.2	21.1	27	7.1	16	3.5%	DM

*if >10% contact lab manager

Notes:

1300 Blue Spruce Drive, Suite C
Fort Collins, Colorado 80524

Toll Free: 800/331-5916
Tel: 970/484-5091 Fax:970/484-2514

ORGANISM HISTORY

DATE: 10/2/2023

SPECIES: *Menidia beryllina*

AGE: 8 day

LIFE STAGE: Juvenile

HATCH DATE: 9/24/2023

BEGAN FEEDING: Immediately

FOOD: Rotifers, *Artemia* sp.

Water Chemistry Record:	**Current**	**Range**
TEMPERATURE:	24°C	23-26 °C
SALINITY/CONDUCTIVITY:	25 ppt	23-27 ppt
TOTAL HARDNESS (as $CaCO_3$):	--	--
TOTAL ALKALINITY (as $CaCO_3$):	155 mg/l	150-200 mg/l
pH:	7.93	7.60-8.00

Comments:

Facility Supervisor

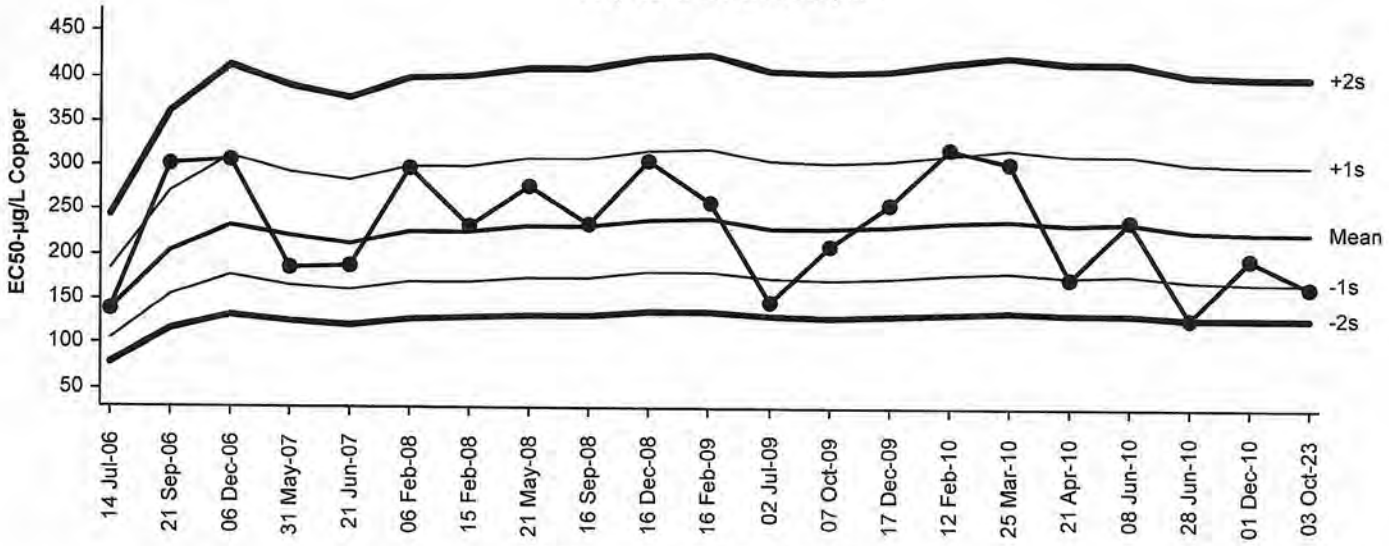
CETIS QC Plot

Report Date: 25 Oct-23 13:05 (1 of 1)

Reference Toxicant 96-h Acute Survival Test — **All Matching Labs**

Test Type:	Survival	**Organism:**	Menidia beryllina	**Material:**	Copper
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	Proportion Survived	**Source:**	Reference Toxicant-REF

Reference Toxicant 96-h Acute Survival Test
Proportion Survived Endpoint



Lognormal Cumulative Mean Plot

Mean:	227.5	**Count:**	20	**-1s Warning Limit:**	171	**-2s Action Limit:**	129
Sigma:	NA	**CV:**	29.00%	**+1s Warning Limit:**	302	**+2s Action Limit:**	401

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2006	Jul	14	15:30	138.8	-88.68	-1.741	(-)		12-3489-2800	05-4639-7387	NewFields
2		Sep	21	17:00	301.5	74.03	0.9932			13-2437-4560	13-4184-0272	NewFields
3		Dec	6	15:30	306.3	78.84	1.049	(+)		13-1351-8433	14-1844-9693	NewFields
4	2007	May	31	18:00	185.8	-41.65	-0.7129			15-1085-6486	07-8998-8487	NewFields
5		Jun	21	17:00	187.9	-39.6	-0.6742			11-4444-5191	09-2989-1578	NewFields
6	2008	Feb	6	16:00	298.9	71.41	0.9623			09-0873-1841	02-2843-3056	NewFields
7			15	16:30	232.3	4.847	0.07432			02-3273-3535	02-4532-0088	NewFields
8		May	21	13:00	277.7	50.26	0.7037			09-4275-9770	06-5552-2016	NewFields
9		Sep	16	14:00	234.5	6.985	0.1066			15-9104-3417	05-6930-9029	NewFields
10		Dec	16	0:00	306.7	79.24	1.054	(+)		14-9978-8744	16-1416-6951	NewFields
11	2009	Feb	16	17:50	259.8	32.29	0.4679			04-7138-1635	03-2527-8796	NewFields
12		Jul	2	15:30	148.7	-78.77	-1.498	(-)		17-6566-4048	02-5341-7743	NewFields
13		Oct	7	16:00	209.6	-17.88	-0.2885			09-2813-8584	10-4729-1377	NewFields
14		Dec	17	17:00	258.8	31.33	0.4548			08-9947-0669	06-8788-0639	NewFields
15	2010	Feb	12	15:50	319.7	92.2	1.199	(+)		16-3220-3330	11-4810-9360	NewFields
16		Mar	25	15:40	305.4	77.97	1.039	(+)		01-9529-2111	16-5352-1628	NewFields
17		Apr	21	14:30	175.7	-51.72	-0.9094			11-3599-7700	11-8587-2436	NewFields
18		Jun	8	15:00	239.1	11.67	0.1763			18-8197-0368	01-7499-5876	NewFields
19			28	18:35	128.9	-98.57	-2.002	(-)	(-)	12-9890-0591	14-9318-8371	NewFields
20		Dec	1	17:00	198.2	-29.31	-0.4862			19-6200-1517	14-1520-5779	NewFields
21	2023	Oct	3	17:05	165.7	-61.76	-1.117	(-)		08-1424-5683	14-5140-9073	EcoAnalysts

CETIS Summary Report

Report Date: 14 Dec-23 14:06 (p 1 of 1)
Test Code/ID: P220110.117 / 08-1424-5683

Reference Toxicant 96-h Acute Survival Test

EcoAnalysts

Batch ID:	17-0645-1239	**Test Type:**	Survival	**Analyst:**	Marisa Seibert		
Start Date:	03 Oct-23 17:05	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Laboratory Seawater		
Ending Date:	07 Oct-23 15:33	**Species:**	Menidia beryllina	**Brine:**	Not Applicable		
Test Length:	94h	**Taxon:**	Actinopterygii	**Source:**	Aquatic Biosystems, CO	**Age:**	9d
Sample ID:	16-0405-5557	**Code:**	P220110.117	**Project:**	Reference Toxicant		
Sample Date:	10 Jan-22	**Material:**	Copper	**Source:**	Reference Toxicant		
Receipt Date:	10 Jan-22	**CAS (PC):**		**Station:**	P220110.117		
Sample Age:	631d 17h	**Client:**	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
21-0621-4474	Proportion Survived	Steel Many-One Rank Sum Test		125	250	176.8	16.9%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
14-5140-9073	Proportion Survived	Linear Interpolation (ICPIN)		EC15	108.9	34.68	154.4	1
				EC20	127.7	55.39	151.4	
				EC25	133.4	70.39	156.7	
				EC40	152	114.5	171.9	
				EC50	165.7	130.4	183.5	

Proportion Survived Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
31.25		4	0.9750	0.8954	1.0550	0.9000	1.0000	0.0250	0.0500	5.13%	2.50%
62.5		4	0.9500	0.7909	1.1090	0.8000	1.0000	0.0500	0.1000	10.53%	5.00%
125		4	0.8250	0.4490	1.2010	0.5000	1.0000	0.1181	0.2363	28.64%	17.50%
250		4	0.0250	-0.0546	0.1046	0.0000	0.1000	0.0250	0.0500	200.00%	97.50%
500		4	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Proportion Survived Detail

MD5: AFD3B05C60E1133FCF9BA7CB3FC4C075

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	1.0000
31.25		1.0000	1.0000	0.9000	1.0000
62.5		1.0000	1.0000	0.8000	1.0000
125		1.0000	0.5000	1.0000	0.8000
250		0.1000	0.0000	0.0000	0.0000
500		0.0000	0.0000	0.0000	0.0000

Proportion Survived Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	10/10	10/10	10/10	10/10
31.25		10/10	10/10	9/10	10/10
62.5		10/10	10/10	8/10	10/10
125		10/10	5/10	10/10	8/10
250		1/10	0/10	0/10	0/10
500		0/10	0/10	0/10	0/10

CETIS Test Data Worksheet

Reference Toxicant 96-h Acute Survival Test — EcoAnalysts

Start Date:	03 Oct-23 17:05	**Species:**	Menidia beryllina
End Date:	07 Oct-23 15:33	**Protocol:**	EPA/821/R-02-012 (2002)
Sample Date:	10 Jan-22	**Material:**	Copper
Sample Code:	P220110.117		
Sample Source:	Reference Toxicant		
Sample Station:	P220110.117		

Conc-µg/L	Code	Rep	Pos	# Exposed	# Survived	Notes
0	D	1	2	10	10	
0	D	2	5	10	10	
0	D	3	23	10	10	
0	D	4	15	10	10	
31.25		1	10	10	10	
31.25		2	22	10	10	
31.25		3	11	10	9	
31.25		4	6	10	10	
62.5		1	16	10	10	
62.5		2	21	10	10	
62.5		3	9	10	8	
62.5		4	24	10	10	
125		1	17	10	10	
125		2	18	10	5	
125		3	1	10	10	
125		4	20	10	8	
250		1	8	10	1	
250		2	19	10	0	
250		3	13	10	0	
250		4	14	10	0	
500		1	12	10	0	
500		2	4	10	0	
500		3	7	10	0	
500		4	3	10	0	

ECO ANALYSTS

96-Hour Menidia Copper Reference Toxicant Test

Toxicant:	Copper Chloride
Ref Tox ID:	P220110.117
Protocol:	TOX099
Replicates:	4

Date Test Started:	10/3/2023
Date Test Ended:	10/7/2023
Matrix:	Liquid
Species:	*Menidia beryllina*
No. of Org. per Chamber:	10

Toxicant: Copper Chloride (400,000 ug Cu/L CuCl2)	Lot #: MKLK7155	Date Prepared: 10/3/23 Renewal: 10/05/23	Initials: LG Initials: JI
Target Concentration: 500 ug/L	Quantity of Stock Target: 2.5 mL	Quantity of Diluent Target: 2000 mL	
Serial Dilute by 50%			

	Conc.	Meter #:	DO (mg/L) (>4.6)	Meter #:	Temp (°C) (20±1°C)	Meter #:	Salinity (ppt) (30±2ppt)	Meter #:	pH (7 - 9)
Day 0 (Stock)	Control	8	7.3	8	19.0	8	32	8	7.7
Date: 10/3/23	31.25		7.5		19.0		32		7.7
Time: 1505	62.5		7.6		20.1		32		7.7
Technician: MS	125		7.6		18.8		32		7.7
	250		7.6		19.0		32		7.7
	500		7.6		19.4		32		7.7
			Day 1		Day 2		Day 3		Day 4
Temperature (OLD)			0745 18.8		19.4		19.2		—
Temperature (NEW)					18.6				
Feed: 0.1mL *Artemia* (Time/Init.)	AM		DM 0915		JI 0843		JI 0838		SR 0820
	PM								
Day 4	Control	8	6.6	8	20.3	8	28	8	7.7
Date: 10/7/23	31.25		6.5		20.2		28		7.8
Time: 1058	62.5		6.5		20.1		28		7.8
Replicate No.: 1	125		6.6		20.0		28		7.8
Technician: NL	250		6.7		20.2		28		7.9
	500		—		—		—		—

Start Time:	1705 LG/JI
End Time:	1533 NL
Test Acceptability:	≥90% survival in control

Test Location:	Batch 3
Dilution Water Batch:	FSW100323.01
Supplier:	Aquatic BioSystems
Organism Batch:	ABS100823.01 Age: 9 days
Chamber Size/Type:	12 oz. Cup
Exposure Volume:	250 mL

DIE JI 10/4/23

96-Hour Menidia Copper Reference Toxicant Test

EcoAnalysts, Inc.

Toxicant:	Copper Chloride
Ref Tox ID:	P220110.117
Species:	① ~~Americamysis bahia~~ Menidia beryllina

Date Test Started:	10/3/2023
Date Test Ended:	10/7/2023

Concentration	Rep	Day 1 Date: 10/4/23 Time: 1034		Day 2 Date: 10/5/2023 Time: 1205		Day 3 Date: 20106/23 Time: 2021		Day 4 Date: 10/7/23 Time: 1533	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
31.25	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	9	1	9	0
	4	10	0	10	0	10	0	10	0
62.5	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	8	2	8	0	8	0	8	0
	4	10	0	10	0	10	0	10	0
125	1	10	0	10	0	10	0	10	0
	2	5	5	5	0	5	0	5	0
	3	10	0	10	0	10	0	10	0
	4	8	2	8	0	8	0	8	0
250	1	1	9	1	0	1	0	1	0
	2	2	8	0	1	—	—	—	—
	3	0	10	—	—	—	—	—	—
	4	1	9	0	1	—	—	—	—
500	1	0	10	—	—	—	—	—	—
	2	0	10	—	—	—	—	—	—
	3	0	10	—	—	—	—	—	—
	4	0	10	—	—	—	—	—	—
INITIALS:		JI		TW		IE SR		NL	

① IE - LG 10/3/23

① IE - SR 20/06/23

1	24
2	11
3	9
4	23
5	5
6	16
7	22
8	2
9	8
10	6
11	13
12	18
13	12
14	17
15	1
16	4
17	21
18	14
19	19
20	10
21	3
22	20
23	15
24	7

P#: P220110.117

APPENDIX A.2

MYTILUS GALLOPROVINCIALIS 48-HOUR SURVIVAL AND DEVELOPMENT TEST

STATISTICAL COMPARISON AND LABORATORY DATA SHEETS

CETIS Summary Report

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	15-1536-2742	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert
Start Date:	08 Nov-23 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	10 Nov-23 14:01	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Test Length:	46h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish **Age:**
Sample ID:	02-4638-8827	**Code:**	P231108.01BC	**Project:**	Wyckoff Eagle Harbor GWTP 2023/W
Sample Date:	08 Nov-23 01:15	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	08 Nov-23 12:20	**CAS (PC):**		**Station:**	110823
Sample Age:	15h	**Client:**	Jacobs Wyckoff		

Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
13-9060-0578	Proportion Normal	Equal Variance t Two-Sample Test	0.1151	Brine Control passed proportion normal	1
08-7899-9229	Proportion Survived	Equal Variance t Two-Sample Test	0.3711	Brine Control passed proportion survived	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
11-3332-1115	Proportion Normal	Dunnett Multiple Comparison Test		73.5	>73.5	---	2.06%	1.4	1
16-7561-2672	Proportion Survived	Dunnett Multiple Comparison Test		73.5	>73.5	---	3.88%	1.4	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
06-7464-2626	Proportion Normal	Linear Interpolation (ICPIN)	✓	EC15	>73.5	---	---	<1.4	1
			✓	EC20	>73.5	---	---	<1.4	
			✓	EC25	>73.5	---	---	<1.4	
			✓	EC40	>73.5	---	---	<1.4	
			✓	EC50	>73.5	---	---	<1.4	
11-5239-8639	Proportion Survived	Linear Interpolation (ICPIN)	✓	EC15	>73.5	---	---	<1.4	1
			✓	EC20	>73.5	---	---	<1.4	
			✓	EC25	>73.5	---	---	<1.4	
			✓	EC40	>73.5	---	---	<1.4	
			✓	EC50	>73.5	---	---	<1.4	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
06-7464-2626	Proportion Normal	Control Resp	0.9789	0.9	<<	Yes	Passes Criteria
11-3332-1115	Proportion Normal	Control Resp	0.9789	0.9	<<	Yes	Passes Criteria
13-9060-0578	Proportion Normal	Control Resp	0.9789	0.9	<<	Yes	Passes Criteria
	Proportion Normal	Control Resp	0.9618	0.9	<<	Yes	Passes Criteria
08-7899-9229	Proportion Survived	Control Resp	0.9872	0.5	<<	Yes	Passes Criteria
	Proportion Survived	Control Resp	0.9843	0.5	<<	Yes	Passes Criteria
11-5239-8639	Proportion Survived	Control Resp	0.9872	0.5	<<	Yes	Passes Criteria
16-7561-2672	Proportion Survived	Control Resp	0.9872	0.5	<<	Yes	Passes Criteria

Bivalve Larval Survival and Development Test
EcoAnalysts

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9789	0.9498	1.0080	0.9544	0.9961	0.0091	0.0183	1.87%	0.00%
0	BC	4	0.9618	0.9287	0.9949	0.9356	0.9799	0.0104	0.0208	2.16%	1.74%
6.25		4	0.9822	0.9625	1.0020	0.9641	0.9918	0.0062	0.0124	1.26%	-0.34%
12.5		4	0.9730	0.9616	0.9843	0.9654	0.9823	0.0036	0.0071	0.73%	0.60%
25		4	0.9833	0.9717	0.9949	0.9759	0.9928	0.0037	0.0073	0.74%	-0.45%
50		4	0.9783	0.9715	0.9852	0.9723	0.9821	0.0021	0.0043	0.44%	0.06%
73.5		4	0.9771	0.9559	0.9982	0.9627	0.9920	0.0066	0.0133	1.36%	0.18%

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9872	0.9465	1.0280	0.9488	1.0000	0.0128	0.0256	2.59%	0.00%
0	BC	4	0.9843	0.9515	1.0170	0.9567	1.0000	0.0103	0.0206	2.09%	0.30%
6.25		4	0.9852	0.9540	1.0170	0.9567	1.0000	0.0098	0.0197	1.99%	0.20%
12.5		4	0.9085	0.8827	0.9342	0.8898	0.9291	0.0081	0.0162	1.78%	7.98%
25		4	0.9941	0.9753	1.0130	0.9764	1.0000	0.0059	0.0118	1.19%	-0.70%
50		4	0.9764	0.9053	1.0470	0.9094	1.0000	0.0223	0.0447	4.57%	1.10%
73.5		4	0.9774	0.9422	1.0130	0.9488	1.0000	0.0111	0.0221	2.26%	1.00%

Proportion Normal Detail

MD5: 35B7970CE6C31F7EEFA4C338E798FB46

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9763	0.9888	0.9961	0.9544
0	BC	0.9770	0.9547	0.9799	0.9356
6.25		0.9918	0.9849	0.9641	0.9881
12.5		0.9739	0.9703	0.9823	0.9654
25		0.9759	0.9847	0.9798	0.9928
50		0.9821	0.9723	0.9806	0.9784
73.5		0.9838	0.9627	0.9698	0.9920

Proportion Survived Detail

MD5: 72C8EA5E8B2F88229FC3E1CEC4F8C916

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	1.0000	1.0000	0.9488
0	BC	1.0000	0.9567	0.9803	1.0000
6.25		0.9567	1.0000	0.9882	0.9961
12.5		0.9055	0.9291	0.8898	0.9094
25		1.0000	1.0000	0.9764	1.0000
50		1.0000	0.9961	1.0000	0.9094
73.5		0.9724	0.9488	1.0000	0.9882

Bivalve Larval Survival and Development Test
EcoAnalysts

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	288/295	265/268	256/257	230/241
0	BC	255/261	232/243	244/249	247/264
6.25		241/243	261/265	242/251	250/253
12.5		224/230	229/236	222/226	223/231
25		283/290	257/261	243/248	277/279
50		274/279	246/253	253/258	226/231
73.5		243/247	232/241	257/265	249/251

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	254/254	254/254	254/254	241/254
0	BC	254/254	243/254	249/254	254/254
6.25		243/254	254/254	251/254	253/254
12.5		230/254	236/254	226/254	231/254
25		254/254	254/254	248/254	254/254
50		254/254	253/254	254/254	231/254
73.5		247/254	241/254	254/254	251/254

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — EcoAnalysts

Start Date:	08 Nov-23 15:50	**Species:**	Mytilus galloprovincialis	**Sample Code:**	P231108.01BC
End Date:	10 Nov-23 14:01	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs Wyckoff
Sample Date:	08 Nov-23 01:15	**Material:**	Treated Groundwater	**Sample Station:**	110823

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	19	254	261	261	255	
0	BC	2	15	254	243	243	232	
0	BC	3	11	254	249	249	244	
0	BC	4	12	254	264	264	247	
0	D	1	20	254	295	295	288	
0	D	2	16	254	268	268	265	
0	D	3	13	254	257	257	256	
0	D	4	3	254	241	241	230	
6.25		1	5	254	243	243	241	
6.25		2	24	254	265	265	261	
6.25		3	2	254	251	251	242	
6.25		4	27	254	253	253	250	
12.5		1	26	254	230	230	224	
12.5		2	25	254	236	236	229	
12.5		3	10	254	226	226	222	
12.5		4	23	254	231	231	223	
25		1	6	254	290	290	283	
25		2	21	254	261	261	257	
25		3	7	254	248	248	243	
25		4	28	254	279	279	277	
50		1	4	254	279	279	274	
50		2	1	254	253	253	246	
50		3	17	254	258	258	253	
50		4	9	254	231	231	226	
73.5		1	18	254	247	247	243	
73.5		2	8	254	241	241	232	
73.5		3	22	254	265	265	257	
73.5		4	14	254	251	251	249	

48-Hour Chronic WET Test

Version V.2

GENERAL

Client	Jacobs Wyckoff
Project	Wyckoff Eagle Harbor GWTP 2023/WA
Project Number	PG1799
Project Manager	M. Seibert
Date Sample Received	11/8/2023
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	11/08/23
Test Species	Mytilus spp.
Organism Batch	TS110623
Organism Acquired	11/6/2023
Organism Acclimation	2
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: 1550 MS

TEST END TIME/INIT: 1401 NL

CLIENT SAMPLE ID	LAB ID
110823	P231108.01

Salinity Adjustment CSMM Batch #
NA

Formalin Lot #
220304-50

Rose Bangel Batch #
5135

	Concentrations
1	Control
2	BRINE Control
3	6.25%
4	12.5%
5	25%
6	50%
7	73.5%
8	.
9	.

P231108.01

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	110823	11/8/23

Volume per Concentration (mls) - 200

Test Parameters	ppt
Salinity of Brine	112.00
Salinity of Sample	0.50
Test Salinity	30.00

Salinity Adjustment Multiplier = **0.36**

		grams added
mls. Sample*	350.00	349.5
mls. Brine	125.91	136.5
*Adjust volume so C16>F19		**486.02**

Post Adjustment Concentration (%) = **73.54**

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

Concentration (%)	Amount of Adjusted Sample (gms.)	Amount of Seawater (gms.)
73.50	204.1	0.1
50.00	138.9	65.4
25.00	69.4	134.8
12.50	34.7	169.5
6.25	17.4	186.9
	0.0	204.2
	464.49	

Brine Control Preparation

Salinity Adjustment			highest	Amount Brine	Amount DI	Amount Seawater
Sample Number/Name	Multiplier	Volume BC	concentration	(grams)	(grams)	(grams)
	0.36	200	73.5	56.2	141.5	6.6

Worksheet Preparation Date / Initials

11/8/2023	MS

Dilution Preparation Date / Initials

11/8/2023	MS

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	DATE RECEIVED	11/8/23	PROTOCOL	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	TEST START DATE	11/8/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	110823	TEST END DATE	11/10/23	SPECIES	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	MATRIX	Liquid	NO. OF ORGANISMS	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

SPAWNING METHOD Heat Shock		INITIAL SPAWNING TIME 1250	FINAL SPAWNING TIME 1348
MALES 7	FEMALES 4	SPERM VIABILITY Good	EGG CONDITION Good
BEGIN FERTILIZATION 1348		END FERTILIZATION 1550	CONDITION OF EMBRYOS Good

TIME OF INITIATION	INITIALS
15:50	MS

EMBRYO DENSITY CALCULATIONS

of embryos in 1 mL of 100X diluted embryo stock

Count 1	Count 2	Mean
275	238	256.5

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

25650

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)

4.210526316 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	11/8/23	PROTOCOL	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	TEST START DATE	11/8/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	110823	TEST END DATE	11/10/23	SPECIES	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	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	15.9	30	7.6
Stock	BRINE Control	8.2	16.1	31	7.9
Date 11/8/23	6.25%	8.3	16.1	30	7.8
Time 1450	12.5%	8.4	16.2	30	7.7
Tech MS	25%	8.4	16.2	30	7.7
Meter # 7	50%	8.5	16.3	29	7.6
	73.5%	8.5	16.3	29	7.6
Day 1	Control		16.8		
Surrogate	BRINE Control		16.8		
Date 11/09/23	6.25%		16.8		
Time 1639	12.5%		16.8		
Tech SR	25%		16.8		
Meter # T16	50%		16.8		
	73.5%		16.8		
Day 2	Control	7.9	16.3 ①	31	8.3
Surrogate	BRINE Control	7.9	16.3 ①	30	8.1
Date 11/10/23	6.25%	7.8	16.3 ①	30	8.0
Time 1359	12.5%	7.8	16.3 ①	30	8.0
Tech NL	25%	7.9	16.3 ①	30	8.0
Meter # 9/T14	50%	7.9	16.3 ①	29	8.1
	73.5%	7.9	16.3 ①	29	②~~8.1~~ 8.2

①Temp blank used - NL 11/10/23

②M.R. - NL 11/10/23

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	DATE RECEIVED	11/8/23	PROTOCOL	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	TEST START DATE	11/8/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	110823	TEST END DATE	11/10/23	SPECIES	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	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	264		11.16.23	MAHL	$\bar{X}$ = 253.5
	2	258		11.16.23	MAHL	
	3	254		11.16.23	MAHL	
	4	250		11.16.23	MAHL	
	5	242		11.16.23	MAHL	
	6	253		11.16.23	MAHL	
Control	1	288	7	11.16.23	MAHL	
	2	265	3	11.16.23	MAHL	
	3	256	1	11.16.23	MAHL	QA: 257 / 1 12/8 MS Δ = 0%
	4	230	11	11.16.23	MAHL	
BRINE Control	1	255	6	11.16.23	MAHL	
	2	232	11	11.16.23	MAHL	
	3	244	5	11.16.23	MAHL	
	4	247	17	11.16.23	MAHL	
6.25%	1	241	2	11/26/23	LG	
	2	261	4	11/26/23	LG	
	3	242	9	12/7	NL	
	4	250	3	12/7	NL	
12.5%	1	224	6	12/7	NL	QA: 228 / 6 12/8 MS Δ = 0%
	2	229	7	12/7	NL	
	3	222	4	12/7	NL	
	4	223	8	12/7	NL	
25%	1	283	7	12/7	NL	
	2	257	4	12/7	NL	
	3	243	5	12/7	NL	
	4	277	2	12/7	NL	
50%	1	274	5	12/7	NL	
	2	246	7	12/9	DM	
	3	253	5	12/9	DM	
	4	276	5	12/9	DM	
73.5%	1	243	4	11.16.23	MAHL	
	2	232	9	11.16.23	MAHL	QA: 238 / 10 12/8 MS Δ = 0%
	3	257	8	11.16.23	MAHL	
	4	249	2	11.16.23	MAHL	

CETIS Summary Report

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	19-3718-7221	**Test Type:**	Development-Survival	**Analyst:**	Marisa Seibert
Start Date:	08 Nov-23 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	10 Nov-23 14:01	**Species:**	Mytilus galloprovincialis	**Brine:**	Crystal Sea Marine Mix
Test Length:	46h	**Taxon:**	Bivalvia	**Source:**	Taylor Shellfish **Age:**
Sample ID:	06-0086-2324	**Code:**	P231108.01SC	**Project:**	Wyckoff Eagle Harbor GWTP 2023/W
Sample Date:	08 Nov-23 01:15	**Material:**	Treated Groundwater	**Source:**	Jacobs Wyckoff
Receipt Date:	08 Nov-23 12:20	**CAS (PC):**		**Station:**	110823
Sample Age:	15h	**Client:**	Jacobs Wyckoff		

Single Comparison Summary

Analysis ID	Endpoint	Comparison Method	P-Value	Comparison Result	S
10-1809-4788	Proportion Normal	Equal Variance t Two-Sample Test	<1.0E-05	Salt Control failed proportion normal	1
12-3806-8469	Proportion Survived	Equal Variance t Two-Sample Test	0.1516	Salt Control passed proportion survived	1

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
07-9423-0414	Proportion Normal	Dunnett Multiple Comparison Test		100	>100	---	1.48%	1	1
21-1756-9394	Proportion Survived	Steel Many-One Rank Sum Test		100	>100	---	8.73%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
01-8548-5736	Proportion Normal	Linear Interpolation (ICPIN)	✓	EC15	>100	---	---	<1	1
			✓	EC20	>100	---	---	<1	
			✓	EC25	>100	---	---	<1	
			✓	EC40	>100	---	---	<1	
			✓	EC50	>100	---	---	<1	
21-2959-4880	Proportion Survived	Linear Interpolation (ICPIN)	✓	EC15	>100	---	---	<1	1
			✓	EC20	>100	---	---	<1	
			✓	EC25	>100	---	---	<1	
			✓	EC40	>100	---	---	<1	
			✓	EC50	>100	---	---	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
01-8548-5736	Proportion Normal	Control Resp	0.9819	0.9	<<	Yes	Passes Criteria
07-9423-0414	Proportion Normal	Control Resp	0.9819	0.9	<<	Yes	Passes Criteria
10-1809-4788	Proportion Normal	Control Resp	0.01994	0.9	<<	Yes	Below Criteria
	Proportion Normal	Control Resp	0.9819	0.9	<<	Yes	Passes Criteria
12-3806-8469	Proportion Survived	Control Resp	0.9232	0.5	<<	Yes	Passes Criteria
	Proportion Survived	Control Resp	0.9715	0.5	<<	Yes	Passes Criteria
21-1756-9394	Proportion Survived	Control Resp	0.9715	0.5	<<	Yes	Passes Criteria
21-2959-4880	Proportion Survived	Control Resp	0.9715	0.5	<<	Yes	Passes Criteria

Bivalve Larval Survival and Development Test
EcoAnalysts

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9819	0.9734	0.9904	0.9767	0.9887	0.0027	0.0053	0.54%	0.00%
0	SC	4	0.0199	-0.0135	0.0534	0.0084	0.0514	0.0105	0.0210	105.43%	97.97%
6.25		4	0.9811	0.9708	0.9914	0.9721	0.9871	0.0032	0.0065	0.66%	0.09%
12.5		4	0.9727	0.9608	0.9847	0.9662	0.9832	0.0038	0.0075	0.77%	0.94%
25		4	0.9756	0.9696	0.9816	0.9715	0.9805	0.0019	0.0037	0.38%	0.65%
50		4	0.9698	0.9431	0.9964	0.9509	0.9916	0.0084	0.0168	1.73%	1.24%
73.5		4	0.9804	0.9722	0.9885	0.9770	0.9879	0.0026	0.0051	0.52%	0.16%
100		4	0.9815	0.9751	0.9880	0.9766	0.9857	0.0020	0.0041	0.42%	0.04%

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9715	0.9142	1.0290	0.9252	1.0000	0.0180	0.0360	3.71%	0.00%
0	SC	4	0.9232	0.8197	1.0270	0.8425	1.0000	0.0325	0.0651	7.05%	4.96%
6.25		4	0.9734	0.9093	1.0380	0.9134	1.0000	0.0202	0.0403	4.14%	-0.20%
12.5		4	0.9843	0.9341	1.0340	0.9370	1.0000	0.0158	0.0315	3.20%	-1.32%
25		4	0.9478	0.8131	1.0830	0.8228	1.0000	0.0423	0.0847	8.93%	2.43%
50		4	0.9843	0.9341	1.0340	0.9370	1.0000	0.0158	0.0315	3.20%	-1.32%
73.5		4	0.9823	0.9482	1.0160	0.9567	1.0000	0.0107	0.0214	2.18%	-1.11%
100		4	0.9911	0.9747	1.0080	0.9803	1.0000	0.0052	0.0104	1.04%	-2.03%

Proportion Normal Detail

MD5: 28D64F9F516365AB99085434AFFD742E

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9787	0.9887	0.9767	0.9836
0	SC	0.0514	0.0113	0.0086	0.0084
6.25		0.9810	0.9841	0.9721	0.9871
12.5		0.9685	0.9832	0.9731	0.9662
25		0.9715	0.9761	0.9805	0.9743
50		0.9916	0.9700	0.9509	0.9665
73.5		0.9772	0.9794	0.9770	0.9879
100		0.9839	0.9800	0.9857	0.9766

Proportion Survived Detail

MD5: F51E3D9FBFEE11B5D88909B3E4965A44

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9252	1.0000	1.0000	0.9606
0	SC	0.8425	1.0000	0.9134	0.9370
6.25		1.0000	0.9921	0.9882	0.9134
12.5		1.0000	0.9370	1.0000	1.0000
25		0.9685	0.8228	1.0000	1.0000
50		0.9370	1.0000	1.0000	1.0000
73.5		1.0000	0.9567	1.0000	0.9724
100		0.9803	0.9843	1.0000	1.0000

Bivalve Larval Survival and Development Test
EcoAnalysts

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	230/235	262/265	252/258	240/244
0	SC	11/214	3/265	2/232	2/238
6.25		258/263	248/252	244/251	229/232
12.5		277/286	234/238	253/260	257/266
25		239/246	204/209	251/256	265/272
50		236/238	259/267	252/265	260/269
73.5		257/263	238/243	255/261	244/247
100		245/249	245/250	275/279	250/256

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	235/254	254/254	254/254	244/254
0	SC	214/254	254/254	232/254	238/254
6.25		254/254	252/254	251/254	232/254
12.5		254/254	238/254	254/254	254/254
25		246/254	209/254	254/254	254/254
50		238/254	254/254	254/254	254/254
73.5		254/254	243/254	254/254	247/254
100		249/254	250/254	254/254	254/254

CETIS Test Data Worksheet

Report Date: 14 Dec-23 13:50 (p 1 of 1)
Test Code/ID: P231108.01SC / 00-7776-3026

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

Start Date: 08 Nov-23 15:50
End Date: 10 Nov-23 14:01
Sample Date: 08 Nov-23 01:15
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Treated Groundwater
Sample Code: P231108.01SC
Sample Source: Jacobs Wyckoff
Sample Station: 110823

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	1	254	235	235	230	
0	D	2	26	254	265	265	262	
0	D	3	21	254	258	258	252	
0	D	4	5	254	244	244	240	
0	SC	1	18	254	214	214	11	
0	SC	2	32	254	265	265	3	
0	SC	3	17	254	232	232	2	
0	SC	4	28	254	238	238	2	
6.25		1	8	254	263	263	258	
6.25		2	27	254	252	252	248	
6.25		3	14	254	251	251	244	
6.25		4	29	254	232	232	229	
12.5		1	19	254	286	286	277	
12.5		2	30	254	238	238	234	
12.5		3	23	254	260	260	253	
12.5		4	9	254	266	266	257	
25		1	20	254	246	246	239	
25		2	6	254	209	209	204	
25		3	7	254	256	256	251	
25		4	11	254	272	272	265	
50		1	24	254	238	238	236	
50		2	12	254	267	267	259	
50		3	13	254	265	265	252	
50		4	2	254	269	269	260	
73.5		1	10	254	263	263	257	
73.5		2	4	254	243	243	238	
73.5		3	25	254	261	261	255	
73.5		4	16	254	247	247	244	
100		1	15	254	249	249	245	
100		2	22	254	250	250	245	
100		3	3	254	279	279	275	
100		4	31	254	256	256	250	

EcoAnalysts, Inc.

48-Hour Chronic WET Test

Version V.2

GENERAL

Client	Jacobs Wyckoff
Project	Wyckoff Eagle Harbor GWTP 2023/WA
Project Number	PG1799
Project Manager	M. Seibert
Date Sample Received	11/8/2023
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	11/08/23
Test Species	Mytilus spp.
Organism Batch	TS110623
Organism Acquired	11/6/2023
Organism Acclimation	2
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:	1550 MG
TEST END TIME/INIT:	1401 NL

CLIENT SAMPLE ID	LAB ID
110823	P231108.01

Salinity Adjustment CSMM Batch #
62123

Formalin Lot #
220304-50

Rose Bangel Batch #
5135

	Concentrations
1	Control
2	SALT Control
3	6.25%
4	12.5%
5	25%
6	50%
7	73.5%
8	100%
9	.

ECOANALYSTS, INC.

48-Hour Chronic WET Test

v.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	11/8/23	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	11/8/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	110823	**TEST END DATE**	11/10/23	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	**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		
	73.5%	147	53.0	200		
	100%	200	0.0	200		

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
11/8/23	#7	P231108.01	FSW110823.01	NS

48-Hour Chronic WET Test

v.2

CLIENT	Jacobs Wyckoff	DATE RECEIVED	11/8/23	PROTOCOL	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	TEST START DATE	11/8/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	110823	TEST END DATE	11/10/23	SPECIES	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	MATRIX	Liquid	NO. OF ORGANISMS	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

SPAWNING METHOD Heat Shock		INITIAL SPAWNING TIME 1250	FINAL SPAWNING TIME 1348
MALES 7	FEMALES 4	SPERM VIABILITY Good	EGG CONDITION Good
BEGIN FERTILIZATION 1348		END FERTILIZATION 1550	CONDITION OF EMBRYOS Good

TIME OF INITIATION	INITIALS
15:50	MS

EMBRYO DENSITY CALCULATIONS

of embryos in 1 mL of 100X diluted embryo stock

Count 1	Count 2	Mean
275	238	256.5

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

25650

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)

4.210526316 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	11/8/23	PROTOCOL	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	TEST START DATE	11/8/23	PROJECT MANAGER	M. Seibert
CLIENT SAMPLE ID	110823	TEST END DATE	11/10/23	SPECIES	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	MATRIX	Liquid	NO. OF ORGANISMS	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

	Concentration (%)	DO (mg/L)	TEMP (°C)	SALINITY (ppt)	pH
		> 4.0	15 - 17	28 - 32	7 - 9
Day 0	Control	8.3	16.7	30	7.8
Stock	SALT Control	8.2	16.8	30	8.2
Date 11/8/23	6.25%	8.2	16.8	31	7.9
Time 1458	12.5%	8.3	16.7	31	7.8
Tech MS	25%	8.4	16.5	31	7.8
Meter # 7	50%	8.5	16.3	31	7.7
	73.5%	8.6	16.3	31	7.7
	100%	8.7	15.6	31	7.7
Day 1	Control		16.8		
Surrogate	SALT Control		16.8		
Date 11/09/23	6.25%		16.8		
Time 1639	12.5%		16.8		
Tech SR	25%		16.8		
Meter # ~~70~~ T16	50%		16.8		
	73.5%		16.8		
	100%		16.8		
Day 2	Control	8.0	16.3 ②	31	7.9
Surrogate	SALT Control	7.9	16.3 ②	30	7.9
Date 11/10/23	6.25%	7.9	16.3 ②	31	7.9
Time 1354	12.5%	7.9	16.3 ②	30	7.9
Tech NL	25%	7.9	16.3 ②	30	8.0
Meter # 9/T14	50%	7.9	16.3 ②	30	8.1
	73.5%	7.8	16.3 ②	31	8.2
	100%	7.8	16.3 ②	32	8.2

② IE-SR 11/09/23

② Temp blank used - NL 11/10/23

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	11/8/23	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	11/8/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	110823	**TEST END DATE**	11/10/23	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	**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	264		11.16.23	MARH	$\bar{X}$ = 253.5
	2	258		11.16.23	MARH	
	3	254		11.16.23	MARH	
	4	250		11.16.23	MARH	
	5	242		11.16.23	MARH	
	6	253		11.16.23	MARH	
Control	1	230	5	11.16.23	MARH	
	2	262	3	11.16.23	MARH	QA: 262/3 Δ = 0%. 12/8 MP
	3	252	6	11.16.23	MARH	
	4	240	4	11.16.23	MARH	
SALT Control	1	~~230~~ 11 ①	~~5~~ 203 ①	11.16.23	MARH	
	2	3	262	11.16.23	MARH	
	3	2	230	11.16.23	MARH	
	4	2	236	11.16.23	MARH	
6.25%	1	~~250~~ 244 ①	5 ~~7~~ ①	12/8/23	DM	
	2	240	4	12/8/23	DM	QA: 242/5 Δ = 0%. 12/8
	3	244	7	12/8/23	DM	
	4	229	3	12/8/23	DM	
12.5%	1	247	9	12/8/23	DM	
	2	234	4	12/8/23	DM	
	3	253	7	12/8/23	DM	
	4	257	9	12/8/23	DM	
25%	1	239	7	12/8/23	DM	
	2	204	5	12/8/23	DM	
	3	251	5	12/8/23	DM	
	4	265	7	12/8/23	DM	
50%	1	②~~210~~236	2	12/9/23	NL	
	2	259	8	12/9/23	NL	
	3	252	13	12/9/23	NL	
	4	260	9	12/9/23	NL	

① WL 11.16.23 MARH, DM - 12/8/23

②IE - NL 12/9/23

ECO ANALYSTS, INC.

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs Wyckoff	**DATE RECEIVED**	11/8/23	**PROTOCOL**	TOX 042
PROJECT	Wyckoff Eagle Harbor GWTP 2023/WA	**TEST START DATE**	11/8/23	**PROJECT MANAGER**	M. Seibert
CLIENT SAMPLE ID	110823	**TEST END DATE**	11/10/23	**SPECIES**	*Mytilus spp.*
LAB SAMPLE ID	P231108.01	**MATRIX**	Liquid	**NO. OF ORGANISMS**	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

Concentration (%)	REP	Normal	Abnormal	Date	Tech	Comments/QA Counts
73.5%	1	① 245 257	6	12/9	NL	
	2	238	5	12/9	NL	
	3	255	6	12/9	NL	
	4	244	3	12/9	NL	
100%	1	245	4	11.16.23	MARH	QA: 249/4 Δ=0% 12/8 MS
	2	245	5	11.16.23	MARH	
	3	275	4	11.16.23	MARH	
	4	250	6	11.16.23	MARH	

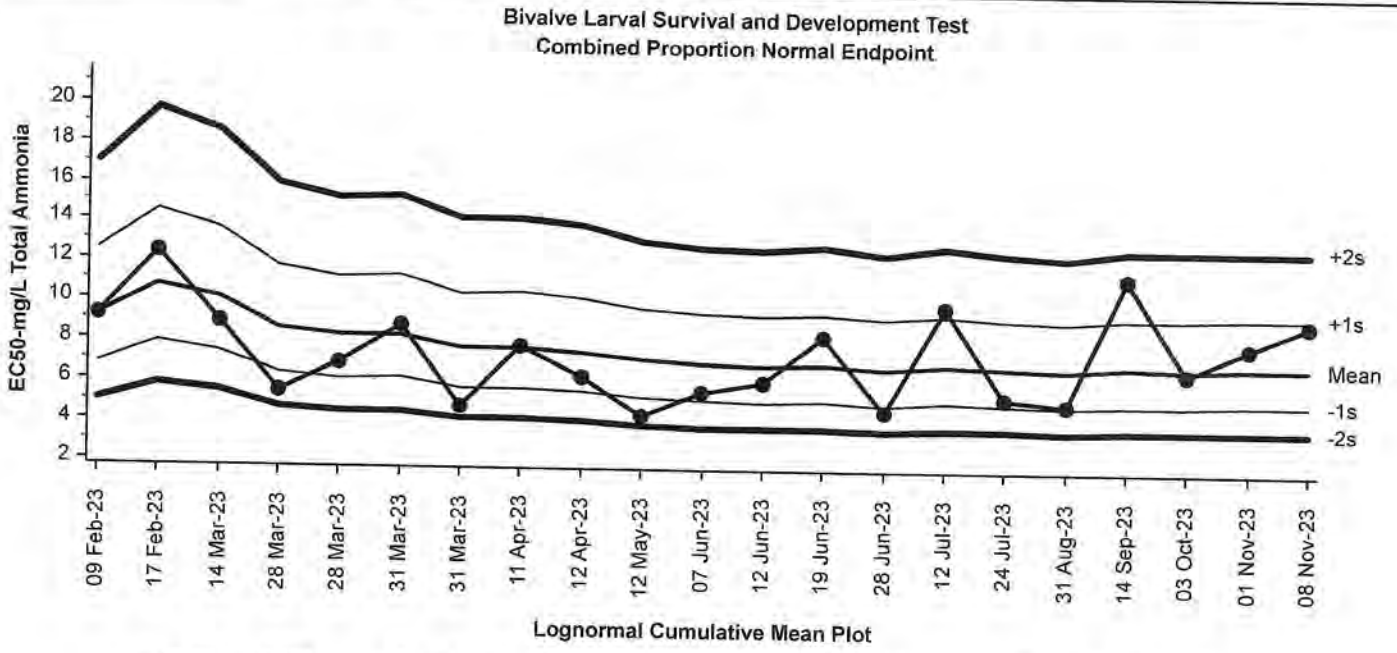
① QC 11.16.23 MARH

CETIS QC Plot

Report Date: 15 Dec-23 11:26 (1 of 1)

Bivalve Larval Survival and Development Test — All Matching Labs

Test Type: Development-Survival | **Organism:** Mytilus galloprovincialis | **Material:** Total Ammonia
Protocol: EPA/600/R-95/136 (1995) | **Endpoint:** Combined Proportion Normal | **Source:** Reference Toxicant-REF



Mean:	7.008	**Count:**	20	**-1s Warning Limit:**	5.17	**-2s Action Limit:**	3.81
Sigma:	NA	**CV:**	31.20%	**+1s Warning Limit:**	9.5	**+2s Action Limit:**	12.9

Quality Control Data

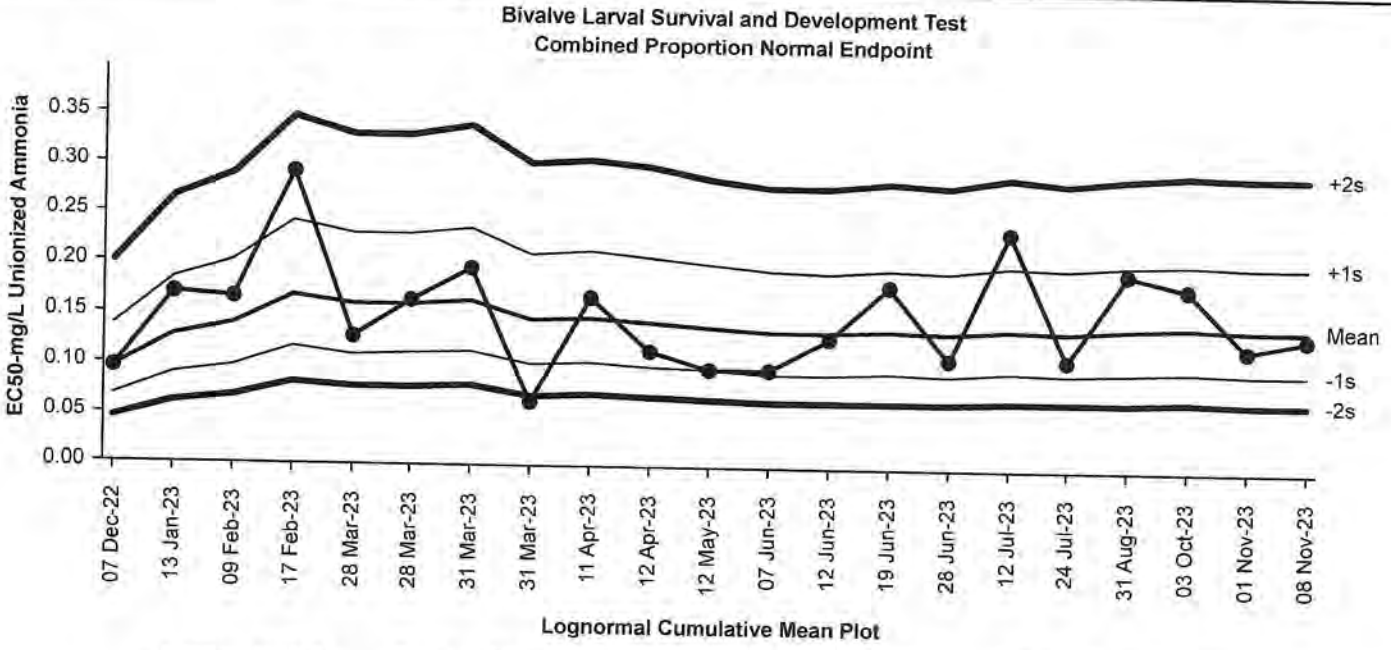
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2023	Feb	9	15:28	9.246	2.239	0.9101			00-8572-7368	10-5325-0783	EcoAnalysts
2			17	14:30	12.4	5.39	1.873	(+)		20-3891-7103	06-7296-3936	EcoAnalysts
3		Mar	14	15:15	8.955	1.948	0.8051			00-9622-9067	21-3408-3763	EcoAnalysts
4			28	15:46	5.455	-1.553	-0.8223			02-2233-3890	16-3797-4494	EcoAnalysts
5			28	15:47	6.941	-0.06703	-0.03155			01-6969-0938	06-4639-7696	EcoAnalysts
6			31	16:52	8.774	1.766	0.738			21-2826-5425	10-8042-3972	EcoAnalysts
7			31	16:54	4.818	-2.19	-1.23	(-)		13-8989-7877	05-5295-3514	EcoAnalysts
8		Apr	11	16:37	7.809	0.8016	0.3556			14-1713-1401	15-2064-5147	EcoAnalysts
9			12	15:13	6.298	-0.7092	-0.3503			21-2394-6995	12-4981-2785	EcoAnalysts
10		May	12	15:35	4.42	-2.588	-1.513	(-)		02-3839-1595	05-0285-3181	EcoAnalysts
11		Jun	7	16:24	5.621	-1.386	-0.7237			16-8311-5218	04-7873-2197	EcoAnalysts
12			12	18:29	6.154	-0.8536	-0.4265			19-7480-8941	04-9719-6422	EcoAnalysts
13			19	16:20	8.423	1.415	0.6039			16-3224-4662	15-6769-3694	EcoAnalysts
14			28	15:18	4.725	-2.283	-1.294	(-)		10-1014-4768	17-1187-2841	EcoAnalysts
15		Jul	12	12:57	9.89	2.883	1.131	(+)		02-0009-8192	04-6529-8407	EcoAnalysts
16			24	17:06	5.374	-1.634	-0.8714			05-3985-4386	13-9086-0827	EcoAnalysts
17		Aug	31	16:54	5.053	-1.955	-1.074	(-)		16-1472-3265	15-9433-1311	EcoAnalysts
18		Sep	14	13:50	11.43	4.421	1.606	(+)		10-9810-7803	01-3503-3195	EcoAnalysts
19		Oct	3	17:04	6.706	-0.3011	-0.1442			15-2407-3570	09-1135-7427	EcoAnalysts
20		Nov	1	17:40	8.055	1.047	0.4572			08-2875-4322	08-8063-5388	EcoAnalysts
21			8	15:55	9.251	2.244	0.9119			13-4824-7359	00-4887-4658	EcoAnalysts

CETIS QC Plot

Report Date: 15 Dec-23 11:31 (1 of 1)

Bivalve Larval Survival and Development Test — All Matching Labs

Test Type:	Development-Survival	**Organism:**	Mytilus galloprovincialis	**Material:**	Unionized Ammonia
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Combined Proportion Normal	**Source:**	Reference Toxicant-REF



Lognormal Cumulative Mean Plot

Mean:	0.1418	**Count:**	20	**-1s Warning Limit:**	0.0987	**-2s Action Limit:**	0.0687
Sigma:	NA	**CV:**	37.50%	**+1s Warning Limit:**	0.204	**+2s Action Limit:**	0.293

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Dec	7	17:43	0.09634	-0.04551	-1.066	(-)		15-6747-3203	15-5237-0673	EcoAnalysts
2	2023	Jan	13	15:30	0.1703	0.02847	0.5039			14-6111-3358	19-5184-9524	EcoAnalysts
3		Feb	9	15:28	0.1664	0.02451	0.4391			11-1705-9064	00-9866-2896	EcoAnalysts
4			17	14:30	0.2912	0.1494	1.982	(+)		05-8051-1741	00-4535-0428	EcoAnalysts
5		Mar	28	15:46	0.1275	-0.01438	-0.2944			08-8126-4059	10-2993-2407	EcoAnalysts
6			28	15:47	0.1637	0.02182	0.3942			03-3638-8838	12-4289-2851	EcoAnalysts
7			31	16:52	0.1949	0.05305	0.8752			14-3337-1963	13-8011-4764	EcoAnalysts
8			31	16:54	0.06349	-0.07836	-2.214	(-)	(-)	01-2022-2925	11-3364-1842	EcoAnalysts
9		Apr	11	16:37	0.1673	0.0255	0.4553			13-1124-3474	18-0348-0749	EcoAnalysts
10			12	15:13	0.1148	-0.02705	-0.5829			18-5662-1396	07-7214-9910	EcoAnalysts
11		May	12	15:35	0.09858	-0.04326	-1.002	(-)		08-2245-0872	03-4589-6060	EcoAnalysts
12		Jun	7	16:24	0.0976	-0.04424	-1.03	(-)		18-8939-1974	09-3314-9652	EcoAnalysts
13			12	18:29	0.1293	-0.01252	-0.2546			09-8773-2984	16-9381-4730	EcoAnalysts
14			19	16:20	0.182	0.04011	0.686			21-4361-0458	04-8703-0787	EcoAnalysts
15			28	15:18	0.1088	-0.033	-0.7294			16-9844-0501	06-2488-5585	EcoAnalysts
16		Jul	12	12:57	0.2364	0.09451	1.407	(+)		13-3479-3905	05-2583-6446	EcoAnalysts
17			24	17:06	0.1104	-0.03146	-0.6907			08-8951-5421	04-1308-9826	EcoAnalysts
18		Aug	31	16:54	0.1956	0.05375	0.8851			07-4158-0358	11-0996-2376	EcoAnalysts
19		Oct	3	17:04	0.182	0.04014	0.6865			19-5530-4547	06-1309-3566	EcoAnalysts
20		Nov	1	17:40	0.1213	-0.0205	-0.43			06-2464-1457	05-4038-7195	EcoAnalysts
21			8	15:55	0.1338	-0.00809	-0.1617			13-8700-3666	02-0586-1811	EcoAnalysts

CETIS Summary Report

Report Date: 15 Dec-23 11:26 (p 1 of 1)
Test Code/ID: P220819.87 / 13-4824-7359

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	15-1536-2742	Test Type:	Development-Survival	Analyst:	Marisa Seibert		
Start Date:	08 Nov-23 15:55	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater		
Ending Date:	10 Nov-23 14:00	Species:	Mytilus galloprovincialis	Brine:	Frozen Seawater		
Test Length:	46h	Taxon:	Bivalvia	Source:	Taylor Shellfish	Age:	
Sample ID:	03-7674-1881	Code:	P220819.87	Project:	Reference Toxicant		
Sample Date:	19 Aug-22	Material:	Total Ammonia	Source:	Reference Toxicant		
Receipt Date:	19 Aug-22	CAS (PC):		Station:	P220819.87		
Sample Age:	446d 16h	Client:	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
03-5328-5509	Combined Proportion Norma	Dunnett Multiple Comparison Test		<1.88	1.88	---	5.07%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg/L	95% LCL	95% UCL	S
00-4887-4658	Combined Proportion Norma	Linear Interpolation (ICPIN)		EC15	3.133	0.5603	4.826	1
				EC20	4.711	3.135	9.173	
				EC25	6.891	4.003	8.494	
				EC40	8.526	7.943	9.002	
				EC50	9.251	8.777	9.699	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
03-5328-5509	Combined Proportion Norma	PMSD	0.05066	<<	0.25	No	Passes Criteria

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	0.9370	0.8800	0.9940	0.8898	0.9764	0.0179	0.0358	3.82%	0.00%
1.88		4	0.7864	0.7373	0.8356	0.7559	0.8150	0.0155	0.0309	3.93%	16.07%
2.88		4	0.8248	0.7735	0.8761	0.7992	0.8661	0.0161	0.0322	3.91%	11.97%
7.62		4	0.6900	0.5719	0.8080	0.5866	0.7598	0.0371	0.0742	10.75%	26.37%
12.3		4	0.1358	0.0851	0.1866	0.0945	0.1693	0.0160	0.0319	23.49%	85.50%
24.9		4	0.0020	-0.0016	0.0056	0.0000	0.0039	0.0011	0.0023	115.47%	99.79%

Combined Proportion Normal Detail

MD5: 251DFF7A2814ED619D88CABF0DD2DEC8

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9449	0.8898	0.9764	0.9370
1.88		0.7559	0.8110	0.8150	0.7638
2.88		0.8346	0.8661	0.7992	0.7992
7.62		0.5866	0.7205	0.6929	0.7598
12.3		0.1496	0.1693	0.0945	0.1299
24.9		0.0039	0.0039	0.0000	0.0000

Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	240/254	226/254	248/254	238/254
1.88		192/254	206/254	207/254	194/254
2.88		212/254	220/254	203/254	203/254
7.62		149/254	183/254	176/254	193/254
12.3		38/254	43/254	24/254	33/254
24.9		1/254	1/254	0/254	0/254

CETIS Summary Report

Report Date: 15 Dec-23 11:31 (p 1 of 1)
Test Code/ID: P220819.87UIA / 13-8700-3666

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID:	15-1536-2742	Test Type:	Development-Survival	Analyst:	Marisa Seibert		
Start Date:	08 Nov-23 15:55	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater		
Ending Date:	10 Nov-23 14:00	Species:	Mytilus galloprovincialis	Brine:	Frozen Seawater		
Test Length:	46h	Taxon:	Bivalvia	Source:	Taylor Shellfish	Age:	
Sample ID:	20-9028-6493	Code:	P220819.87UIA	Project:	Reference Toxicant		
Sample Date:	19 Aug-22	Material:	Unionized Ammonia	Source:	Reference Toxicant		
Receipt Date:	19 Aug-22	CAS (PC):		Station:	P220819.87UIA		
Sample Age:	446d 16h	Client:	Internal Lab				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
10-1992-8396	Combined Proportion Norma	Dunnett Multiple Comparison Test		<0.027	0.027	---	5.07%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	mg/L	95% LCL	95% UCL	S
02-0586-1811	Combined Proportion Norma	Linear Interpolation (ICPIN)		EC15	0.04623	0.008459	0.07358	1
				EC20	0.07339	0.04237	0.1299	
				EC25	0.1013	0.06057	0.121	
				EC40	0.1232	0.1132	0.1303	
				EC50	0.1338	0.1256	0.1398	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits Lower	Upper	Overlap	Decision
10-1992-8396	Combined Proportion Norma	PMSD	0.05066	<<	0.25	No	Passes Criteria

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	0.9370	0.8800	0.9940	0.8898	0.9764	0.0179	0.0358	3.82%	0.00%
0.027		4	0.7864	0.7373	0.8356	0.7559	0.8150	0.0155	0.0309	3.93%	16.07%
0.041		4	0.8248	0.7735	0.8761	0.7992	0.8661	0.0161	0.0322	3.91%	11.97%
0.109		4	0.6900	0.5719	0.8080	0.5866	0.7598	0.0371	0.0742	10.75%	26.37%
0.172		4	0.1358	0.0851	0.1866	0.0945	0.1693	0.0160	0.0319	23.49%	85.50%
0.335		4	0.0020	-0.0016	0.0056	0.0000	0.0039	0.0011	0.0023	115.47%	99.79%

Combined Proportion Normal Detail

MD5: A72E8E08A2FF7E4EA79060426B974A4E

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9449	0.8898	0.9764	0.9370
0.027		0.7559	0.8110	0.8150	0.7638
0.041		0.8346	0.8661	0.7992	0.7992
0.109		0.5866	0.7205	0.6929	0.7598
0.172		0.1496	0.1693	0.0945	0.1299
0.335		0.0039	0.0039	0.0000	0.0000

Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	240/254	226/254	248/254	238/254
0.027		192/254	206/254	207/254	194/254
0.041		212/254	220/254	203/254	203/254
0.109		149/254	183/254	176/254	193/254
0.172		38/254	43/254	24/254	33/254
0.335		1/254	1/254	0/254	0/254

CETIS Test Data Worksheet

Report Date: 15 Dec-23 11:25 (p 1 of 1)
Test Code/ID: P220819.87 / 13-4824-7359

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

Start Date: 08 Nov-23 15:55
End Date: 10 Nov-23 14:00
Sample Date: 19 Aug-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Total Ammonia
Sample Code: P220819.87
Sample Source: Reference Toxicant
Sample Station: P220819.87

Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	18	254	242	242	240	
0	D	2	6	254	229	229	226	
0	D	3	16	254	256	256	248	
0	D	4	22	254	245	245	238	
1.88		1	9	254	231	231	192	
1.88		2	3	254	247	247	206	
1.88		3	5	254	252	252	207	
1.88		4	7	254	242	242	194	
2.88		1	19	254	257	257	212	
2.88		2	8	254	257	257	220	
2.88		3	4	254	247	247	203	
2.88		4	23	254	247	247	203	
7.62		1	17	254	204	204	149	
7.62		2	10	254	258	258	183	
7.62		3	20	254	248	248	176	
7.62		4	1	254	271	271	193	
12.3		1	11	254	251	251	38	
12.3		2	21	254	239	239	43	
12.3		3	13	254	202	202	24	
12.3		4	2	254	256	256	33	
24.9		1	14	254	267	267	1	
24.9		2	12	254	227	227	1	
24.9		3	24	254	250	250	0	
24.9		4	15	254	255	255	0	

CETIS Test Data Worksheet

Report Date: 15 Dec-23 11:31 (p 1 of 1)
Test Code/ID: P220819.87UIA / 13-8700-3666

Bivalve Larval Survival and Development Test — EcoAnalysts

Start Date: 08 Nov-23 15:55
End Date: 10 Nov-23 14:00
Sample Date: 19 Aug-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Unionized Ammonia
Sample Code: P220819.87UIA
Sample Source: Reference Toxicant
Sample Station: P220819.87UIA

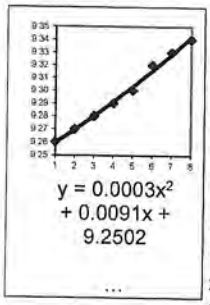
Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	24	254	242	242	240	
0	D	2	18	254	229	229	226	
0	D	3	4	254	256	256	248	
0	D	4	11	254	245	245	238	
0.027		1	21	254	231	231	192	
0.027		2	15	254	247	247	206	
0.027		3	7	254	252	252	207	
0.027		4	20	254	242	242	194	
0.041		1	22	254	257	257	212	
0.041		2	19	254	257	257	220	
0.041		3	23	254	247	247	203	
0.041		4	14	254	247	247	203	
0.109		1	3	254	204	204	149	
0.109		2	16	254	258	258	183	
0.109		3	1	254	248	248	176	
0.109		4	8	254	271	271	193	
0.172		1	6	254	251	251	38	
0.172		2	5	254	239	239	43	
0.172		3	17	254	202	202	24	
0.172		4	10	254	256	256	33	
0.335		1	9	254	267	267	1	
0.335		2	2	254	227	227	1	
0.335		3	13	254	250	250	0	
0.335		4	12	254	255	255	0	

Un-ionized Ammonia Calculator

CLIENT:	Jacobs Wyckoff	Date of Test:	November 8, 2023
PROJECT:	Wyckoff Eagle Harbor GWTP 2023/WA	Test Type:	*Mytilus galloprovinvialis*
COMMENTS:	P220819.87		

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		1.88	31	7.7	16.1	289.25	9.2561		0.027
3	3		2.88	31	7.7	16.0	289.15	9.2561		0.041
4	6		7.62	31	7.7	16.1	289.25	9.2561		0.109
5	12		12.3	31	7.7	15.8	288.95	9.2561		0.172
6	18		24.9	31	7.7	15.3	288.45	9.2561		0.335
7										
8										
9										
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Ammonia Reference Toxicant
Spiking Worksheet

Reference Toxicant ID: P220819.B7
Date Prepared: 11/8/23
Technician Initials: MS

Biv / Echino NH_3 RT

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

Date: 10/11/2023
Measurement: 8456.666667

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
1.88	**1.5**	250		**0.067**
2.88	**3**	250		**0.133**
7.62	**6**	250		**0.266**
12.3	**12**	250		**0.532**
24.9	**18**	250		**0.798**

48 Hour Bivalve Development Reference Toxicant Test

Test ID: P220819.B7	Replicates: 4	Study Director: M. Seibert	Location: INC. 1
Dilution Water Batch: FSW110823.01	Organism Batch: TS110623	Associated Test(s): Jacobs Wyckoff	Organism: M. sp.
Chamber Size/Type: 30 ml shell vial	Exposure Volume: 10 ml		
Toxicant: Ammonium Chloride		Date Prepared: 11/8/23	Initials: MS
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: 250 mL

SPAWNING DATA

Initial Spawning Time: 1250	Final Spawning Time: 1348	Fertilization Time: 1348	No. of Females: ~~5~~ 4	No. of Males: 7
Embryo Density (count/mL):	1. 275	2. 283	3. —	Mean: 279

Stocking Volume Calculation: 2700/27900 = 0.097 x 40 = 3.88 mL egg stock : 36.12mL FSW del. 100µL

0 Hours	Date: 11/8/23	WQ Time: 1149	Start Time: 1555	Initials: MS

STOCK

	Control	1.5	3	6	12	18
D.O. (%) (>4.0 mg/L)	8.4	8.2	7.9	7.8	7.8	7.7
Temperature (16 ± 1°C)	16.5	16.1	16.0	16.1	15.8	15.3
Salinity (30 ± 2 ppt)	30	31	31	31	31	31
pH (6-9)	7.7	7.7	7.7	7.7	7.7	7.7
Meter #	8	8	8	8	8	8

Day 1	Temperature (16 ± 1°C) 16.8	Meter # T16		Initials: SR
Day 2	Date: ~~11~~ 11/10/23	WQ Time: 1346 NL	End Time: 1400	Initials: NL
		Formalin Lot #: 220304-50	Rose Bengal Lot #: 5135	

STOCK

	Control	1.5	3	6	12	18
D.O. (%) (>4.0 mg/L)	7.9	7.9	7.9	7.9	7.9	8.0
Temperature (16 ± 1°C) (2)	16.~~0~~3	16.3	16.3	16.3	16.3	16.3
Salinity (30 ± 2 ppt)	30	31	31	31	31	31
pH (6-9)	7.9	7.9	7.9	7.9	7.9	7.9
Meter #	9	9	9	9	9	9

(1) E-MS 11/8, NL 11/10

(2) Temp blank used - NL 11/10/23

48 Hour Bivalve Development Reference Toxicant Test

Test ID: P220819.87

Conc.	Rep	Number Normal	Number Abnormal	Date	Initials
Control	1	240	2	12/10/23	NL
	2	226	3	12/10/23	NL
	3	248	8	12/10/23	NL
	4	238	7	12/10/23	NL
1.5	1	192	39	12/10/23	NL
	2	206	41	12/11/23	DM
	3	207	45	12/11/23	DM
	4	194	48	12/12/23	NS
3	1	212	45	12/10/23	NL
	2	220	37	12/12/23	NS
	3	203	44	12/13/23	NS
	4	203	44	12/13/23	NS
6	1	149	55	12/10/23	NL
	2	183	75	12/13/23	NS
	3	176	72	12/13/23	NS
	4	193	78	12/13/23	NS
12	1	38	213	12/14/23	NS
	2	43	196	12/14/23	NS
	3	24	178	12/15/23	DM
	4	33	223	12/15/23	DM
18	1	1	266	12/15/23	DM
	2	1	226	12/15/23	DM
	3	0	250	12/15/23	DM
	4	0	255	12/15/23	DM

QA: 226 N 1 A
226/227 = 99.6%
%A: 0.9% DM

QA: 190 N 53 A
190/243 = 78.2%
%A: 2% DM

Stocking Density

Rep	Count	Init.
1	264	MARH
2	258	MARH
3	254	MARH
4	250	MARH
5	242	MARH
6	253	MARH
Mean:	253.5	

ORGANISM RECEIPT LOG

Date:	Time:	Batch No.
11/6/23	1625	TS110623.9

Organism: Mytilus spp.

Source / Supplier: Taylor Shellfish

No. Ordered:	No. Received:	Source Batch: Collection date, hatch date, etc.):
14 lbs	14 lbs	11/6/23

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

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

*if >10% contact lab manager

Notes: ① Received dry - LG 11/6/23

TAYLOR SHELLFISH FARMS

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

HARVEST DATE: [illegible]

HARVEST AREA: [illegible]

HARVEST ITEM:

Dept ID: M[illegible] FARM CODE: [illegible]

QUANTITY: 14 ☐ Dozens ☑ Pounds ☐ Tubs ☐ Sacks

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

Harvest Hour	10
Harvest Minute	30
Refer Date	11/6/13
Refer Hour	11
Refer Minute	30

APPENDIX B

CHAIN-OF-CUSTODY AND SAMPLE RECEIPT FORMS

EcoAnalysts Inc. (REGION COPY)
DateShipped: 10/3/2023
CarrierName: EcoAnalysts (hand delivery)
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2023/WA
Project Code: WEH-032C
Cooler #: 1 of 1

No: 10-100323-102728-0733
IFD10W2LA0010PXTSDDD2
Contact Name: Daniel Baca
Contact Phone: 661-313-3807

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
100323		Ground Water/ D. Baca	Composite	ACTOX-CHRTOX(8 Weeks)	N (1)	SP-11	10/03/2023 01:14	Field Sample

Special Instructions: 2023 Week40-Q4

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

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

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	DJACOBS	10-3-23 @1100	EcoAnalysts	10/3/23 1305	5.1°C P231003.08

EcoAnalysts, Inc. (REGION COPY)
DateShipped: 11/8/2023
CarrierName: EcoAnalysts (hand delivery)
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2023/WA
Project Code: WEH-032D
Cooler #: 1 of 1

No: 10-110823-103825-0744
IFD10W2LA0010PXTSDDD2
Contact Name: Daniel Baca
Contact Phone: 661-313-3807

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
110823		Ground Water/ D. Baca	Composite	ACTOX-CHRTOX(8 Weeks)	N (1)	SP-11	11/08/2023 01:15	Field Sample

Special Instructions: 2023 Week 45-NovQ4

Shipment for Case Complete? N

Samples Transferred From Chain of Custody #

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

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	@ JACOBS	11-8-23 @ 1100	EcoAnalysts	11/8/23 1220	P231108.01 3.6°C

SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:	
Jacobs, Wyckoff	100323	P231003.08		
Project:				
Wyckoff Eagle Harbor GWTP 2023/WA				
Date/Time Received:		10/3/23 1305		
Airbill #:		NA		
Shipper Tracking Information Kept for Records: (Y/N/NA)		NA		
Collection Date/Time:		10/3/23 0114		
Sample Holding Time (must be ≤36 hours at test initiation)		Y		
Condition of Shipping Container:		Good		
Type and Capacity of Sample Container:		10 L wbi		
Total Sample Volume (L):		10L		
Condition of Sampling Container:		Good		
Sample Container Appropriate: (Y/N)		Y		
Custody Seals Intact: (Intact/Broken/Not Present)		Intact		
Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N)		Y		
Sampler's Name Present on COC Form: (Print Name/Not Present)		D. Baca		
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
P231003.08	T21	5.1	8	8.5	8	7.5	8	1008	8	0.5	—	—	0.01	0.592	MS/DW

*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:

V.1

SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:		
Jacobs, Wyckoff	110823	P231108.01			
Project:					
Wyckoff Eagle Harbor GWTP2023/WA					
Date/Time Received:		11/8/23 1220			
Airbill #:		courier			
Shipper Tracking Information Kept for Records: (Y/N/NA)		NA			
Collection Date/Time:		11/8/23 0115			
Sample Holding Time (must be ≤36 hours at test initiation)		Y			
Condition of Shipping Container:		Good			
Type and Capacity of Sample Container:		4L cubi			
Total Sample Volume (L):		4L			
Condition of Sampling Container:		Good			
Sample Container Appropriate: (Y/N)		Y			
Custody Seals Intact: (Intact/Broken/Not Present)		Intact			
Frozen Wet or Blue Ice Present During Shipment/Transport: (Y/N)		Y			
Sampler's Name Present on COC Form: (Print Name/Not Present)		D. Baca			
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
P231108.01	T2	3.6°C	9	9.6	9	7.3	9	909	9	0.432	—	—	0.03	① 0.458	LG/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 IE-MS 11/8

V.1