

Wyckoff Groundwater Treatment Plant: First Quarter 2023 Bioassay Monitoring

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DATE: June 20, 2023

1. Introduction

This technical memorandum summarizes information obtained from the first 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 chronic toxicity. Chronic toxicity testing was conducted on the effluent samples per the requirements outlined in the NPDES permit. The current NPDES permit does not include specific dilution series for chronic toxicity tests. For the mussel larvae chronic toxicity testing conducted during the first quarter 2023 sampling event, 100 percent effluent is the highest concentration tested due to the addition of artificial sea salts 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).

No statistically significant effects on the survival endpoint were observed for all test concentrations and species, indicating no evidence of the presence of acute toxicity or chronic toxicity for the survival endpoint. A statistically significant effect was detected at the highest test concentration (100 percent effluent concentration) for the development endpoint of the chronic toxicity test.

As stated above, the current NPDES permit does not include effluent limit for chronic toxicity. 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 chronic developmental endpoint would therefore trigger this requirement.

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

2. Sampling and Analysis Results

Biomonitoring samples were collected per the monitoring frequency included in the NPDES permit. Samples were collected from a 24-hr. autosampler collection point at the effluent tank of the treatment system. Water samples were collected on March 28, 2023. Chemical testing was conducted on a split of each sample collected for bioassay testing per the NPDES permit requirement. The bioassays were performed by EcoAnalysts, Inc. (EcoAnalysts), Port Gamble, Washington, a Washington State Department of Ecology accredited lab. Table 1 lists the sample Laboratory ID and sampling analysis methods. EcoAnalysts sampling analysis report for chronic toxicity testing is provided in Attachment 1.

Table 1. Biological Testing Summary

Laboratory	Laboratory ID	Method	Test Type/Descriptor/Species
EcoAnalysts	P230328.01	EPA/600/R-95-136 Method 1005.0; ASTM E724-89 TOX042.12	Chronic/48-hr Survival and Development/ <i>Mytilus galloprovincialis</i> (Mussel)

No statistically significant effects were detected in any effluent concentration tested for the survival endpoint of the bivalve test. This result indicates a No Observed Effect Concentration of 100 percent of the effluent concentration and a chronic toxic unit of 1. A statistically significant effect was detected in the 100 percent effluent concentration for the development endpoint, which results in a No Observed Effect Concentration (NOEC) of 50 and a chronic toxic unit of 2. Overall, the Effect Concentration 50 is expected to affect 50 percent of the organisms and determined to be greater than 100 percent of the effluent concentration.

3. Laboratory Quality Data Review

A CH2M chemist validated the bioassay results Stage 2A in accordance with the QAPP. Additional examination of the data beyond the data validation scope was performed because a statistically significant biological response by the test organism was observed. The QAPP (CH2M 2022) was cited by EcoAnalysts and the appropriate species of mussel specified in the QAPP was used for the analytical testing.

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

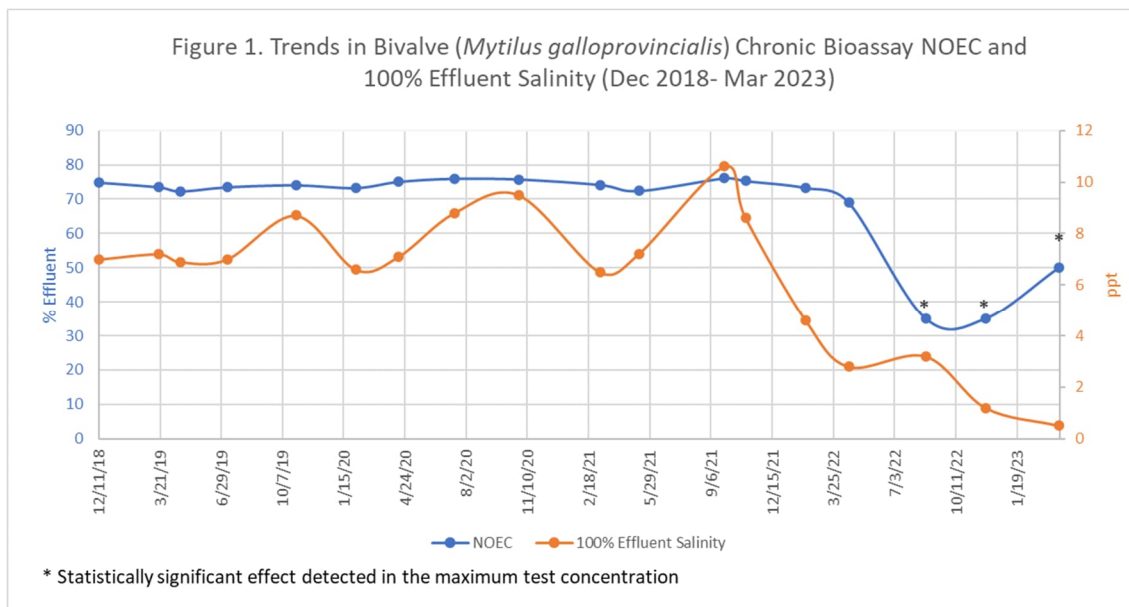
1. The QAPP reference toxicant copper sulfate was not used. The reference toxicant utilized was ammonia. A review of the total and unionized ammonia quality control data indicates the ammonia reference toxicant test results were within two standard deviations of the laboratory mean at the time of testing. There is no impact to the data and an addendum to the QAPP to utilize ammonia as reference toxicant has been requested.
2. The EPA method indicates that hypersaline brine (HSB) is to be used to adjust salinity. EcoAnalysts utilized artificial sea salts to adjust salinity. The method indicates that the use of artificial sea salts is only necessary when high effluent concentrations preclude the use of HSB alone. A request was made to the EcoAnalysts to compare test samples to both lab and salt control to evaluate potential effects of artificial salt on toxicity. As noted in the report the artificial salt used to increase the salinity may have contributed to toxicity of the sample when compared to the lab control. The use of HSB was discussed with EcoAnalysts and they indicated they will follow method specification going forward.
3. The test failed to meet EPA test acceptability criteria of greater than or equal to 90 percent normal shell development. Percent normal for shell development endpoint for the lab control

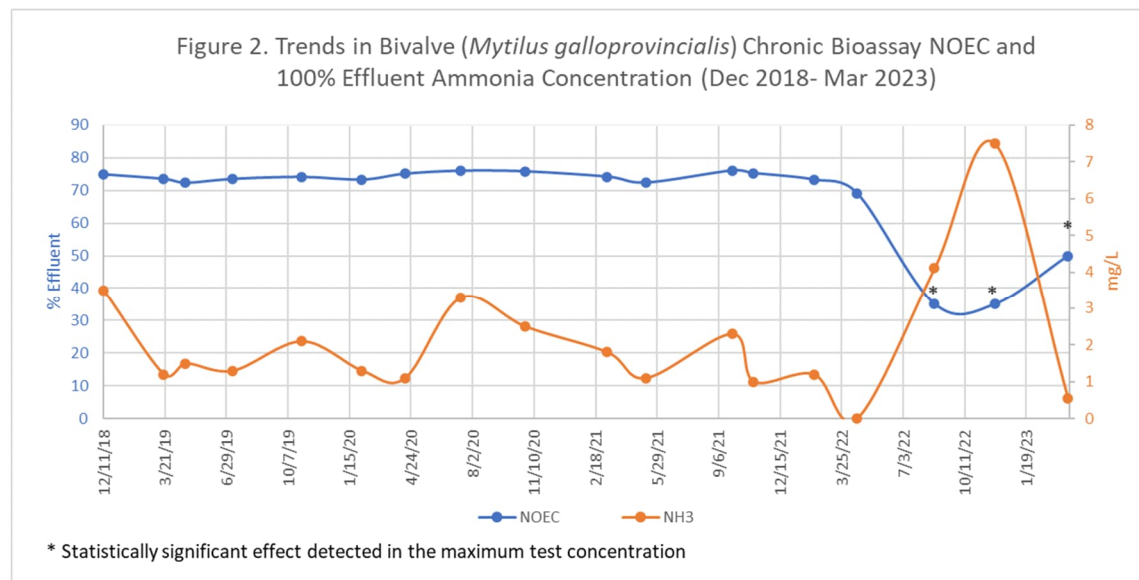
sample was 70 percent, which met the Washington Department of Ecology’s (WDOE) test acceptability criteria of greater than or equal to 70 percent combined normal shell development (WDOE WQ-R-95-80). Test data is considered generally usable, however toxicity may be overestimated or underestimated due to uncertainty from reduced species performance.

4. Trends

A review of bioassay data collected from 2007 through the first 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 since the third quarter of 2022. For these three 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 March 2023. NOEC for bivalve chronic bioassay tests conducted prior to December 2018 were 70 percent. 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 current quarter (i.e. first quarter of 2023) is higher than previous monitoring events (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 three sampling events. 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 three 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 current monitoring event (see Figure 2).





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 survival endpoint of the chronic toxicity test met the WET performance standard because survival rates were within acceptable limits. While the development endpoint of the chronic toxicity test showed a statistically significant response relative to the lab control, uncertainty exists as the observed toxicity may be related to poor species performance and/or salinity adjustment using artificial sea salts. 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 uncertainties with the toxicity results relating to the deviations identified in Section 3, CH2M recommends triggering of the accelerated testing: 1) after EcoAnalysts switch to the use of HSB for salinity adjustment, 2) if the testing meets EPA test acceptability criteria, and 3) a statistically significant effect is detected when compared to the lab control.

Statistical analysis for monitoring events from December 2018 to November 2022 compared test samples to HSB data as opposed to the dilution water control (i.e. lab control) per the recommendation

included in EPA/600/R96-136 Section 13.10.8.4.2 "*Statistical analysis should use the appropriate dilution water control data.*" Review of the third and fourth quarter 2022 results indicates the differences in the mean percent normal development results for HSB and lab controls are not expected to change the conclusion of the associated toxicity reports.

CH2M will provide recommendations for next steps after review of bioassay data collected after EcoAnalysts makes the necessary adjustments to the testing procedures listed in Section 3.

6. References

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
Wyckoff/Eagle Harbor Superfund Groundwater
Treatment Plant

TOXICITY TESTING RESULTS

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

TOXICITY TESTING: 1ST QUARTER 2023

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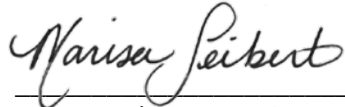
2nd Revision Date: May 30th, 2023



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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APPENDICES

- Appendix A: Statistical Comparison and Laboratory Documents
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ACRONYMS AND ABBREVIATIONS

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

1. EXECUTIVE SUMMARY

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

A statistically significant biological response of the test organisms was detected at the 100% test concentration when compared to the dilution water (laboratory) control. However, no statistically significant biological response of the test organisms was detected at any of the concentrations tested when compared to the salt control. (Table 1-1). See Section 3.1 for further information.

Table 1-1. Toxicity Test Results Summary.

Test		NOEL (%)	LOEL (%)	LC ₅₀ /EC ₅₀ (%)
Dilution Water Control Comparison	<i>Mytilus galloprovincialis</i> 48-Hour Proportion Survived	100	>100	>100
	<i>Mytilus galloprovincialis</i> 48-Hour Proportion Normal	50	100	>100
Salt Control Comparison	<i>Mytilus galloprovincialis</i> 48-Hour Proportion Survived	100	>100	>100
	<i>Mytilus galloprovincialis</i> 48-Hour Proportion Normal	100	>100	>100

NOEL = No Observed Effect Level

LOEL = Lowest Observed Effect Level

LC₅₀/EC₅₀ = Lethal/Effect Concentration to 50% of test population

2. METHODS

The sample was analyzed for toxicity using criteria outlined in the Washington Department of Ecology’s (WDOE) Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria (WDOE WQ-R-95-80). These criteria are further defined through 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 March 28, 2023. The sample was transported by courier and received at the laboratory on the same day as collection. The sample temperature upon receipt was 2.3°C, below the 6°C threshold.

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	032823
Laboratory ID	P230328.01
Date/Time sampled	3/28/23; 0138
Date/Time received	3/28/23; 1310
Dissolved Oxygen (mg/L) Recommended: >4.0 mg/L	9.2
Temperature (°C) Recommended: 0 – 6°C	2.3
pH (units) Recommended: 6 – 9	7.6
Conductivity (µS/cm)	1061
Salinity (ppt)	0.513
Total Chlorine (mg/L)	0.05
Total Ammonia (mg/L)	0.553

2.2 Bioassay Testing

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

Table 2-2. Biological Testing Performed

Test Type	Test Descriptor	Species	Method
Chronic	48-Hour Survival and Development	<i>Mytilus galloprovincialis</i> (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 March 22, 2023. They were shipped dry and maintained under ambient seawater flow-through conditions at $12 \pm 3^\circ\text{C}$ until utilized for testing. 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

Salinity adjustment was necessary to bring the sample within the recommended test salinity for the marine test species. The salinity of the effluent sample was adjusted with Crystal Sea® Marinemix bioassay-grade artificial salt to the desired test salinity for the marine acute and chronic tests.

Table 2-3 summarizes the salinity adjustment 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. Filtered seawater was diluted with laboratory deionized water to meet the salinity of the received effluent sample. Next, Crystal Sea® Marinemix was added to adjust the salinity to the test requirement. This sample was designated “Salt Control” and the results are discussed in the sections below.

Table 2-3. Salinity Adjustment of Project Samples

Sample ID: 032823	Sample Salinity Adjustment (ppt)
Sample 1: Collected 3/28/23	30 ± 2

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 LC₅₀ or EC₅₀ should fall within two standard deviations of the historical laboratory mean. Water quality measurements were monitored to ensure that they fell within prescribed limits.

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

3. RESULTS

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

3.1 *Mytilus galloprovincialis* Test Results

The chronic toxicity test with *M. galloprovincialis* was conducted on March 28, 2023. The test failed to meet the EPA test acceptability criteria of $\geq 90\%$ normal shell development but met $\geq 50\%$ survival and $< 25\%$ Percent Minimum Significant Difference (PMSD). The WDOE test acceptability criteria of $\geq 70\%$ combined normal shell development and $< 25\%$ PMSD were met with 98.1% proportion survived, 70.0% proportion normal, 70.0% combined proportion normal and 8.8% PMSD for proportion normal in the laboratory control. Mean survival and proportion normal are 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 P230328.01 (received 3/28/23) was used for test initiation. Water quality parameters were within the acceptable limits throughout the duration of the 48-hour static test.

A significant difference was observed between the laboratory (dilution water) control and the salt control for the proportion normal endpoint, but not the survival or combined proportion normal endpoints. This indicates that the salt used to increase the salinity of the sample may have contributed to the toxicity of the sample, if observed, in the proportion normal endpoint. To counteract this potential effect, the proportion normal for the sample was compared to the salt control for statistical analysis. Though there was a toxic effect observed in the 100% concentration for the proportion normal endpoint when compared to the laboratory control, there was no effect when compared to the salt control. The NOEL/LOEL from the comparison to the salt control are reported below. There was no significant difference in survival or combined endpoint in any concentration of the sample compared to either control, nor was there a difference in the point estimate values (EC_{50}) calculated based on either control for any endpoint.

The EC_{50} for the ammonia reference toxicant test was 5.5 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 3-1. Results Summary for *Mytilus galloprovincialis* Embryo Development Test

Conc. (%)	Mean Proportion Survived (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC_{50} Value (%)
0	98.1	2.9	100 ^{c,d}	>100 ^{c,d}	>100 ^{c,d}
Salt Control	94.6	7.6			
6.25	95.1	3.6			
12.5	98.2	3.6			
25	97.0	3.2			
50	98.2	2.5			
100	99.9	0.2			

Toxicity Testing Results
Wyckoff/Eagle Harbor Superfund Site Groundwater
Treatment Plant

Conc. (%)	Mean Proportion Normal (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC ₅₀ Value (%)
0	70.0	5.8	50 ^c / 100 ^d	100 ^c / >100 ^d	>100 ^{c,d}
Salt Control	61.2^a	5.6			
6.25	68.4	1.6			
12.5	68.0	3.4			
25	68.6	2.9			
50	67.6	2.7			
100	63.0^a	4.3			
Conc. (%)	Mean Combined Proportion Normal (%)	Standard Deviation	NOEL (%)	LOEL (%)	EC ₅₀ Value (%)
0	70.0	2.8	100 ^{c,d}	>100 ^{c,d}	>100 ^{c,d}
Salt Control	60.0	11.0			
6.25	66.0	5.4			
12.5	69.1	4.2			
25	66.9	4.2			
50	67.9	5.4			
100	65.1	2.3			

BOLD = Significantly different than control;

a = significant compared to dilution water control;

b = significant compared to salt control;

c = compared to dilution water control; d = compared to salt control

NOEL = No Observed Effect Level; LOEL = Lowest Observed Effect Level; LC₅₀/EC₅₀ = Lethal/Effect Concentration to 50% of test population;

proportion survived = total counted / stocking density; proportion normal = number normal/total counted;

combined proportion normal = number normal / stocking density

Table 3-2. Test Condition Summary for *Mytilus galloprovincialis* Embryo Development Test.

Test Duration / Type	48-Hour; Static	
Species	<i>Mytilus galloprovincialis</i>	
Supplier	Taylor Shellfish	
Date acquired	3/22/23	
Test Dates	3/28 – 3/30/23	
Age at test initiation Recommended: <4-hour embryos	<4 hours	
Sample(s) used:	032823; P230328.01	
Holding Time at Initiation: Recommended: < 36 hours	14 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: 211
Replicates/treatment	4	
Concentration/treatment	6.25, 12.5, 25, 50 and 100%	
Feeding	None	
Test solution renewal	None	
Test Water Quality		
Test Dissolved Oxygen	Recommended: > 4.0 mg/L	Actual: 7.8 – 8.4 mg/L
Test Temperature	Recommended: 16 ± 1°C	Actual: 15.4 – 16.3 °C
Test pH	Recommended: 7 – 9	Actual: 7.7 – 8.4
Test Salinity	Recommended: 30 ± 2 ppt	Actual: 29 – 31 ppt
Control performance standard (Survival, Normal shell development, PMSD)	Recommended: EPA: ≥50% survival, ≥90% normal development, <25% PMSD; WDOE: ≥70% combined normal development, <25% PMSD	Actual: 98.1% survival, 70.0% normal development, 8.8% PMSD; 70.0% combined normal development EPA: Fail; WDOE: Pass
Reference Toxicant Date	3/28/23	
Reference Toxicant EC ₅₀	5.5 mg/L total ammonia	
Laboratory Mean EC ₅₀	7.0 mg/L total ammonia	
Acceptable Range EC ₅₀ (± 2 SD)	3.6 – 13.7 mg/L total ammonia (within range)	
Deviations from Test Protocol	Control acceptability under EPA requirements	

4. REFERENCES

- CETIS. 2022. CETIS™ Comprehensive Environmental Toxicity Information System User's Guide. Tidepool Scientific Software. McKinleyville, CA.
- CH2M HILL. 2022. Quality Assurance Project Plan, Groundwater Treatment Plant Operations and Maintenance, Final. Wyckoff/Eagle Harbor Superfund Site. Bainbridge Island, Washington.
- USEPA. 1995. Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine Organisms and Estuarine Organisms, First Edition. EPA-600-R-95-136.
- WDOE. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Washington State Department of Ecology. Water Quality Program. Publication number: WQ-R-95-80, Revised June 2016.

APPENDIX A

Statistical Comparisons and Laboratory Documents

APPENDIX A.1

Mytilus galloprovincialis 48-Hour Survival and Development Test

Statistical Comparison and Laboratory Data Sheets

CETIS Summary Report

Report Date: 17 Apr-23 15:08 (p 1 of 4)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 30 Mar-23 14:40	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish
Sample ID: 12-0776-7817	Code: P230328.01	Project: WEH-031V
Sample Date: 28 Mar-23 01:38	Material: Treated Groundwater	Source: Jacobs Wyckoff
Receipt Date: 28 Mar-23 13:10	CAS (PC):	Station: 32823
Sample Age: 14h (2.3 °C)	Client: Jacobs Wyckoff	Age:

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
15-3418-4659	Combined Proportion Norma	Dunnett Multiple Comparison Test	100	>100	---	10.3%	1	1
10-1140-9931	Proportion Normal	Dunnett Multiple Comparison Test	50	100	70.71	8.83%	2	1
08-0886-0540	Proportion Survived	Dunnett Multiple Comparison Test	100	>100	---	5.78%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
19-9657-7128	Combined Proportion Norma	Linear Interpolation (ICPIN)	✓ EC15	>100	---	---	<1	1
			✓ EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	
03-6956-7611	Proportion Normal	Linear Interpolation (ICPIN)	✓ EC15	>100	---	---	<1	1
			✓ EC20	>100	---	---	<1	
			✓ EC25	>100	---	---	<1	
			✓ EC40	>100	---	---	<1	
			✓ EC50	>100	---	---	<1	
12-5279-0442	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		Overlap	Decision
				Lower	Upper		
03-6956-7611	Proportion Normal	Control Resp	0.6999	0.9	<<	Yes	Below Criteria
10-1140-9931	Proportion Normal	Control Resp	0.6999	0.9	<<	Yes	Below Criteria
08-0886-0540	Proportion Survived	Control Resp	0.981	0.5	<<	Yes	Passes Criteria
12-5279-0442	Proportion Survived	Control Resp	0.981	0.5	<<	Yes	Passes Criteria
15-3418-4659	Combined Proportion Norma	PMSD	0.1031	<<	0.25	No	Passes Criteria

CETIS Summary Report

Report Date: 17 Apr-23 15:08 (p 2 of 4)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Combined Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.7002	0.6561	0.7444	0.6682	0.7346	0.0139	0.0277	3.96%	0.00%
0	SC	4	0.5995	0.4247	0.7744	0.5024	0.7062	0.0550	0.1099	18.33%	14.38%
6.25		4	0.6600	0.5743	0.7456	0.6161	0.7346	0.0269	0.0538	8.15%	5.75%
12.5		4	0.6908	0.6246	0.7569	0.6445	0.7441	0.0208	0.0416	6.02%	1.35%
25		4	0.6694	0.6030	0.7359	0.6114	0.7109	0.0209	0.0417	6.24%	4.40%
50		4	0.6789	0.5926	0.7652	0.6114	0.7441	0.0271	0.0542	7.99%	3.05%
100		4	0.6505	0.6139	0.6871	0.6209	0.6730	0.0115	0.0230	3.54%	7.11%

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.6999	0.6075	0.7923	0.6351	0.7525	0.0290	0.0581	8.30%	0.00%
0	SC	4	0.6119	0.5224	0.7013	0.5377	0.6564	0.0281	0.0562	9.18%	12.57%
6.25		4	0.6837	0.6590	0.7085	0.6633	0.6982	0.0078	0.0156	2.28%	2.31%
12.5		4	0.6804	0.6267	0.7340	0.6300	0.7009	0.0169	0.0337	4.96%	2.79%
25		4	0.6856	0.6389	0.7323	0.6482	0.7186	0.0147	0.0294	4.28%	2.05%
50		4	0.6759	0.6336	0.7182	0.6450	0.7040	0.0133	0.0266	3.93%	3.43%
100		4	0.6299	0.5623	0.6975	0.5796	0.6762	0.0213	0.0425	6.75%	10.00%

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9810	0.9346	1.0280	0.9384	1.0000	0.0146	0.0292	2.98%	0.00%
0	SC	4	0.9455	0.8246	1.0660	0.8389	1.0000	0.0380	0.0760	8.04%	3.62%
6.25		4	0.9514	0.8939	1.0090	0.9194	1.0000	0.0181	0.0362	3.80%	3.02%
12.5		4	0.9822	0.9257	1.0390	0.9289	1.0000	0.0178	0.0356	3.62%	-0.12%
25		4	0.9704	0.9202	1.0210	0.9431	1.0000	0.0158	0.0315	3.25%	1.09%
50		4	0.9822	0.9431	1.0210	0.9479	1.0000	0.0123	0.0246	2.50%	-0.12%
100		4	0.9988	0.9950	1.0030	0.9953	1.0000	0.0012	0.0024	0.24%	-1.81%

CETIS Summary Report

Report Date: 17 Apr-23 15:08 (p 3 of 4)

Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Combined Proportion Normal Detail

MD5: 75558110FA6653E4FA062C9B56CB3B61

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.6919	0.7346	0.7062	0.6682
0	SC	0.6825	0.7062	0.5071	0.5024
6.25		0.6161	0.7346	0.6635	0.6256
12.5		0.7441	0.6967	0.6777	0.6445
25		0.7109	0.6114	0.6777	0.6777
50		0.6777	0.6114	0.7441	0.6825
100		0.6209	0.6730	0.6635	0.6445

Proportion Normal Detail

MD5: B0585D7FEBF8132D862044D4E10140BD

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.6667	0.7452	0.7525	0.6351
0	SC	0.6545	0.6564	0.5377	0.5989
6.25		0.6633	0.6982	0.6931	0.6804
12.5		0.7009	0.6967	0.6300	0.6939
25		0.6944	0.6482	0.7186	0.6810
50		0.6908	0.6450	0.7040	0.6636
100		0.5796	0.6762	0.6512	0.6126

Proportion Survived Detail

MD5: 4AEE09CAA5E6EFB9703AECE9C0CF6D5

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	0.9858	0.9384	1.0000
0	SC	1.0000	1.0000	0.9431	0.8389
6.25		0.9289	1.0000	0.9573	0.9194
12.5		1.0000	1.0000	1.0000	0.9289
25		1.0000	0.9431	0.9431	0.9953
50		0.9810	0.9479	1.0000	1.0000
100		1.0000	0.9953	1.0000	1.0000

CETIS Summary Report

Report Date: 17 Apr-23 15:08 (p 4 of 4)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Combined Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	146/211	155/211	149/211	141/211
0	SC	144/211	149/211	107/211	106/211
6.25		130/211	155/211	140/211	132/211
12.5		157/211	147/211	143/211	136/211
25		150/211	129/211	143/211	143/211
50		143/211	129/211	157/211	144/211
100		131/211	142/211	140/211	136/211

Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	146/219	155/208	149/198	141/222
0	SC	144/220	149/227	107/199	106/177
6.25		130/196	155/222	140/202	132/194
12.5		157/224	147/211	143/227	136/196
25		150/216	129/199	143/199	143/210
50		143/207	129/200	157/223	144/217
100		131/226	142/210	140/215	136/222

Proportion Survived Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	211/211	208/211	198/211	211/211
0	SC	211/211	211/211	199/211	177/211
6.25		196/211	211/211	202/211	194/211
12.5		211/211	211/211	211/211	196/211
25		211/211	199/211	199/211	210/211
50		207/211	200/211	211/211	211/211
100		211/211	210/211	211/211	211/211

CETIS Analytical Report

Report Date: 17 Apr-23 15:04 (p 1 of 2)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test			EcoAnalysts	
Analysis ID: 00-2533-0787	Endpoint: Combined Proportion Normal	CETIS Version: CETISv2.1.4		
Analyzed: 17 Apr-23 15:01	Analysis: Parametric-Two Sample	Status Level: 1		
Edit Date: 17 Apr-23 14:42	MD5 Hash: B495C1D26B92C98B4CD6D47AFD606371	Editor ID: 004-221-689-0		
Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke		
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater		
Ending Date: 30 Mar-23 14:40	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix		
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish	Age:	
Sample ID: 12-0776-7817	Code: P230328.01	Project: WEH-031V		
Sample Date: 28 Mar-23 01:38	Material: Treated Groundwater	Source: Jacobs Wyckoff		
Receipt Date: 28 Mar-23 13:10	CAS (PC):	Station: 32823		
Sample Age: 14h (2.3 °C)	Client: Jacobs Wyckoff			

Data Transform	Alt Hyp	Comparison Result	PMSD
Angular (Corrected)	C > T	Salt Control passed combined proportion normal endpoint	15.45%

Equal Variance t Two-Sample Test									
Control I	vs	Control II	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		Salt Control	6	1.779	1.943	0.1137	CDF	0.0627	Non-Significant Effect

Test Acceptability Criteria					
Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
PMSD	0.1545	<<	0.25	No	Passes Criteria

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0216869	0.0216869	1	3.167	0.1255	Non-Significant Effect
Error	0.041093	0.0068488	6			
Total	0.0627799		7			

ANOVA Assumptions Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variance	Levene Equality of Variance Test	58.51	13.75	0.0003	Unequal Variances	
	Mod Levene Equality of Variance Test	53.05	13.75	0.0003	Unequal Variances	
	Variance Ratio F Test	13.86	47.47	0.0580	Equal Variances	
Distribution	Anderson-Darling A2 Test	0.2124	3.878	0.8925	Normal Distribution	
	Kolmogorov-Smirnov D Test	0.1427	0.3313	1.0000	Normal Distribution	
	Shapiro-Wilk W Normality Test	0.9498	0.6451	0.7091	Normal Distribution	

Combined Proportion Normal Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.7002	0.6561	0.7444	0.6991	0.6682	0.7346	0.0139	3.96%	0.00%
0	SC	4	0.5995	0.4247	0.7744	0.5948	0.5024	0.7062	0.0550	18.33%	14.38%

Angular (Corrected) Transformed Summary											
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	4	0.9917	0.9434	1.0400	0.9901	0.9570	1.0300	0.0152	3.06%	0.00%
0	SC	4	0.8876	0.7077	1.0670	0.8823	0.7878	0.9979	0.0565	12.73%	10.50%

Combined Proportion Normal Detail					
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.6919	0.7346	0.7062	0.6682
0	SC	0.6825	0.7062	0.5071	0.5024

Angular (Corrected) Transformed Detail					
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.9824	1.0300	0.9979	0.9570
0	SC	0.9722	0.9979	0.7925	0.7878

CETIS Analytical Report

Report Date: 17 Apr-23 15:04 (p 2 of 2)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

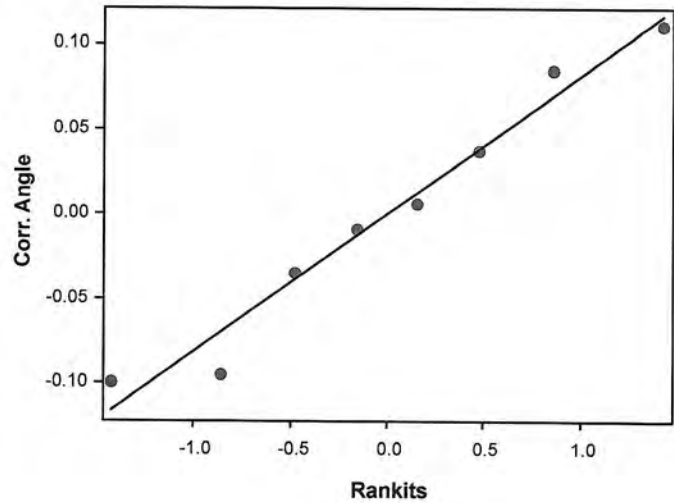
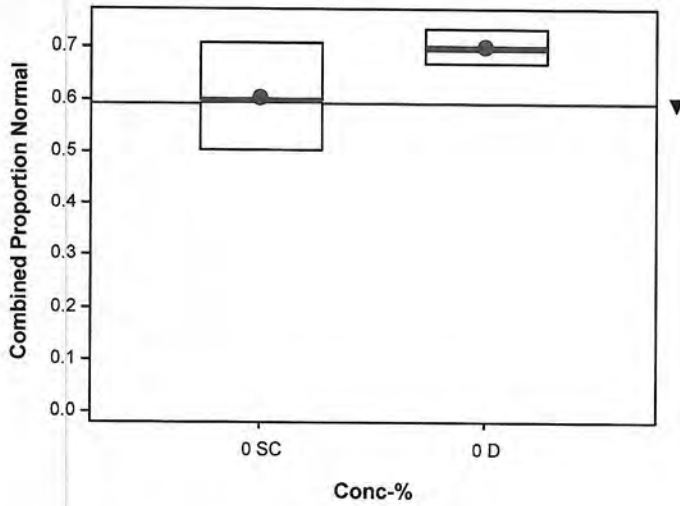
EcoAnalysts

Analysis ID: 00-2533-0787 Endpoint: Combined Proportion Normal CETIS Version: CETISv2.1.4
 Analyzed: 17 Apr-23 15:01 Analysis: Parametric-Two Sample Status Level: 1
 Edit Date: 17 Apr-23 14:42 MD5 Hash: B495C1D26B92C98B4CD6D47AFD606371 Editor ID: 004-221-689-0

Combined Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	146/211	155/211	149/211	141/211
0	SC	144/211	149/211	107/211	106/211

Graphics



CETIS Summary Report

Report Date: 27 Apr-23 14:33 (p 1 of 4)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke	Age:
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater	
Ending Date: 30 Mar-23 14:40	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix	
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish	
Sample ID: 12-0776-7817	Code: P230328.01	Project: WEH-031V	
Sample Date: 28 Mar-23 01:38	Material: Treated Groundwater	Source: Jacobs Wyckoff	
Receipt Date: 28 Mar-23 13:10	CAS (PC):	Station: 32823	
Sample Age: 14h (2.3 °C)	Client: Jacobs Wyckoff		

Compared to Salt Control

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	TU	S
17-7276-5074	Combined Proportion Normal	Dunnett Multiple Comparison Test	100	>100	—	17.5%	1	1
11-7721-4712	Proportion Normal	Dunnett Multiple Comparison Test	100	>100	—	10.3%	1	1
12-1437-7905	Proportion Survived	Dunnett Multiple Comparison Test	100	>100	—	8.76%	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	%	95% LCL	95% UCL	TU	S
10-1272-2961	Combined Proportion Normal	Linear Interpolation (ICPIN)	✓ EC15	>100	—	—	<1	1
			✓ EC20	>100	—	—	<1	
			✓ EC25	>100	—	—	<1	
			✓ EC40	>100	—	—	<1	
			✓ EC50	>100	—	—	<1	
03-5384-3058	Proportion Normal	Linear Interpolation (ICPIN)	✓ EC15	>100	—	—	<1	1
			✓ EC20	>100	—	—	<1	
			✓ EC25	>100	—	—	<1	
			✓ EC40	>100	—	—	<1	
			✓ EC50	>100	—	—	<1	
08-2517-6479	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			Decision
				Lower	Upper	Overlap	
03-5384-3058	Proportion Normal	Control Resp	0.6119	0.9	<<	Yes	Below Criteria
11-7721-4712	Proportion Normal	Control Resp	0.6119	0.9	<<	Yes	Below Criteria
08-2517-6479	Proportion Survived	Control Resp	0.9455	0.5	<<	Yes	Passes Criteria
12-1437-7905	Proportion Survived	Control Resp	0.9455	0.5	<<	Yes	Passes Criteria
17-7276-5074	Combined Proportion Normal	PMSD	0.1754	<<	0.25	No	Passes Criteria

CETIS Summary Report

Report Date: 27 Apr-23 14:33 (p 2 of 4)

Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Combined Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.7002	0.6561	0.7444	0.6682	0.7346	0.0139	0.0277	3.96%	0.00%
0	SC	4	0.5995	0.4247	0.7744	0.5024	0.7062	0.0550	0.1099	18.33%	14.38%
6.25		4	0.6600	0.5743	0.7456	0.6161	0.7346	0.0269	0.0538	8.15%	5.75%
12.5		4	0.6908	0.6246	0.7569	0.6445	0.7441	0.0208	0.0416	6.02%	1.35%
25		4	0.6694	0.6030	0.7359	0.6114	0.7109	0.0209	0.0417	6.24%	4.40%
50		4	0.6789	0.5926	0.7652	0.6114	0.7441	0.0271	0.0542	7.99%	3.05%
100		4	0.6505	0.6139	0.6871	0.6209	0.6730	0.0115	0.0230	3.54%	7.11%

Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.6999	0.6075	0.7923	0.6351	0.7525	0.0290	0.0581	8.30%	0.00%
0	SC	4	0.6119	0.5224	0.7013	0.5377	0.6564	0.0281	0.0562	9.18%	12.57%
6.25		4	0.6837	0.6590	0.7085	0.6633	0.6982	0.0078	0.0156	2.28%	2.31%
12.5		4	0.6804	0.6267	0.7340	0.6300	0.7009	0.0169	0.0337	4.96%	2.79%
25		4	0.6856	0.6389	0.7323	0.6482	0.7186	0.0147	0.0294	4.28%	2.05%
50		4	0.6759	0.6336	0.7182	0.6450	0.7040	0.0133	0.0266	3.93%	3.43%
100		4	0.6299	0.5623	0.6975	0.5796	0.6762	0.0213	0.0425	6.75%	10.00%

Proportion Survived Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	4	0.9810	0.9346	1.0280	0.9384	1.0000	0.0146	0.0292	2.98%	0.00%
0	SC	4	0.9455	0.8246	1.0660	0.8389	1.0000	0.0380	0.0760	8.04%	3.62%
6.25		4	0.9514	0.8939	1.0090	0.9194	1.0000	0.0181	0.0362	3.80%	3.02%
12.5		4	0.9822	0.9257	1.0390	0.9289	1.0000	0.0178	0.0356	3.62%	-0.12%
25		4	0.9704	0.9202	1.0210	0.9431	1.0000	0.0158	0.0315	3.25%	1.09%
50		4	0.9822	0.9431	1.0210	0.9479	1.0000	0.0123	0.0246	2.50%	-0.12%
100		4	0.9988	0.9950	1.0030	0.9953	1.0000	0.0012	0.0024	0.24%	-1.81%

CETIS Summary Report

Report Date: 27 Apr-23 14:33 (p 3 of 4)

Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Combined Proportion Normal Detail

MD5: 75558110FA6653E4FA062C9B56CB3B61

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.6919	0.7346	0.7062	0.6682
0	SC	0.6825	0.7062	0.5071	0.5024
6.25		0.6161	0.7346	0.6635	0.6256
12.5		0.7441	0.6967	0.6777	0.6445
25		0.7109	0.6114	0.6777	0.6777
50		0.6777	0.6114	0.7441	0.6825
100		0.6209	0.6730	0.6635	0.6445

Proportion Normal Detail

MD5: B0585D7FEBF8132D862044D4E10140BD

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	0.6667	0.7452	0.7525	0.6351
0	SC	0.6545	0.6564	0.5377	0.5989
6.25		0.6633	0.6982	0.6931	0.6804
12.5		0.7009	0.6967	0.6300	0.6939
25		0.6944	0.6482	0.7186	0.6810
50		0.6908	0.6450	0.7040	0.6636
100		0.5796	0.6762	0.6512	0.6126

Proportion Survived Detail

MD5: 4AEE09CAA5E6EFB9703AECE9C0CF6D5

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	D	1.0000	0.9858	0.9384	1.0000
0	SC	1.0000	1.0000	0.9431	0.8389
6.25		0.9289	1.0000	0.9573	0.9194
12.5		1.0000	1.0000	1.0000	0.9289
25		1.0000	0.9431	0.9431	0.9953
50		0.9810	0.9479	1.0000	1.0000
100		1.0000	0.9953	1.0000	1.0000

CETIS Analytical Report

Report Date: 27 Apr-23 13:20 (p 1 of 3)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID: 17-7276-5074	Endpoint: Combined Proportion Normal	CETIS Version: CETISv2.1.4
Analyzed: 27 Apr-23 13:19	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 17 Apr-23 14:42	MD5 Hash: 4025593621A1937B2F3EC7A1D523F751	Editor ID: 004-221-689-0
Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 30 Mar-23 14:40	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish Age:
Sample ID: 12-0776-7817	Code: P230328.01	Project: WEH-031V
Sample Date: 28 Mar-23 01:38	Material: Treated Groundwater	Source: Jacobs Wyckoff
Receipt Date: 28 Mar-23 13:10	CAS (PC):	Station: 32823
Sample Age: 14h (2.3 °C)	Client: Jacobs Wyckoff	

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	100	>100	---	1	0.1052	17.54%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Salt Control		6.25	6	-1.374	2.407	0.1078	CDF	0.9940	Non-Significant Effect
		12.5	6	-2.102	2.407	0.1078	CDF	0.9994	Non-Significant Effect
		25	6	-1.589	2.407	0.1078	CDF	0.9968	Non-Significant Effect
		50	6	-1.825	2.407	0.1078	CDF	0.9985	Non-Significant Effect
		100	6	-1.134	2.407	0.1078	CDF	0.9881	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits			Decision
		Lower	Upper	Overlap	
PMSD	0.1754	<<	0.25	No	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0218131	0.0043626	5	1.087	0.4009	Non-Significant Effect
Error	0.0722498	0.0040139	18			
Total	0.0940629		23			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	6.705	15.09	0.2435	Equal Variances
	Levene Equality of Variance Test	4.421	4.248	0.0084	Unequal Variances
	Mod Levene Equality of Variance Test	3.771	4.248	0.0164	Equal Variances
Distribution	Anderson-Darling A2 Test	0.2684	3.878	0.7106	Normal Distribution
	D'Agostino Kurtosis Test	0.4139	2.576	0.6789	Normal Distribution
	D'Agostino Skewness Test	0.2287	2.576	0.8191	Normal Distribution
	D'Agostino-Pearson K2 Omnibus Test	0.2236	9.21	0.8942	Normal Distribution
	Kolmogorov-Smirnov D Test	0.1125	0.2056	0.6148	Normal Distribution
	Shapiro-Wilk W Normality Test	0.9752	0.884	0.7944	Normal Distribution

Combined Proportion Normal Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	SC	4	0.5995	0.4247	0.7744	0.5948	0.5024	0.7062	0.0550	18.33%	0.00%
6.25		4	0.6600	0.5743	0.7456	0.6445	0.6161	0.7346	0.0269	8.15%	-10.08%
12.5		4	0.6908	0.6246	0.7569	0.6872	0.6445	0.7441	0.0208	6.02%	-15.22%
25		4	0.6694	0.6030	0.7359	0.6777	0.6114	0.7109	0.0209	6.24%	-11.66%
50		4	0.6789	0.5926	0.7652	0.6801	0.6114	0.7441	0.0271	7.99%	-13.24%
100		4	0.6505	0.6139	0.6871	0.6540	0.6209	0.6730	0.0115	3.54%	-8.50%

CETIS Analytical Report

Report Date: 27 Apr-23 13:20 (p 2 of 3)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID: 17-7276-5074 Endpoint: Combined Proportion Normal CETIS Version: CETISv2 1.4
 Analyzed: 27 Apr-23 13:19 Analysis: Parametric-Control vs Treatments Status Level: 1
 Edit Date: 17 Apr-23 14:42 MD5 Hash: 4025593621A1937B2F3EC7A1D523F751 Editor ID: 004-221-689-0

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	SC	4	0.8876	0.7077	1.0670	0.8823	0.7878	0.9979	0.0565	12.73%	0.00%
6.25		4	0.9491	0.8572	1.0410	0.9322	0.9026	1.0300	0.0289	6.08%	-6.93%
12.5		4	0.9818	0.9097	1.0540	0.9773	0.9320	1.0400	0.0227	4.61%	-10.61%
25		4	0.9588	0.8886	1.0290	0.9671	0.8977	1.0030	0.0221	4.60%	-8.02%
50		4	0.9693	0.8766	1.0620	0.9696	0.8977	1.0400	0.0291	6.01%	-9.21%
100		4	0.9384	0.9000	0.9767	0.9420	0.9075	0.9620	0.0120	2.57%	-5.72%

Combined Proportion Normal Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	SC	0.6825	0.7062	0.5071	0.5024
6.25		0.6161	0.7346	0.6635	0.6256
12.5		0.7441	0.6967	0.6777	0.6445
25		0.7109	0.6114	0.6777	0.6777
50		0.6777	0.6114	0.7441	0.6825
100		0.6209	0.6730	0.6635	0.6445

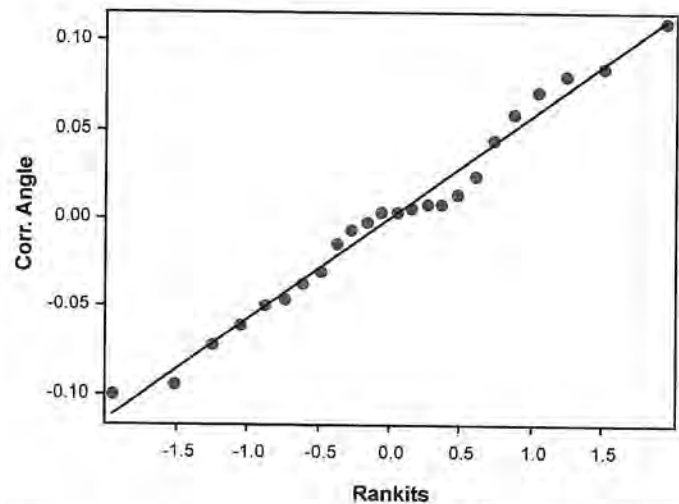
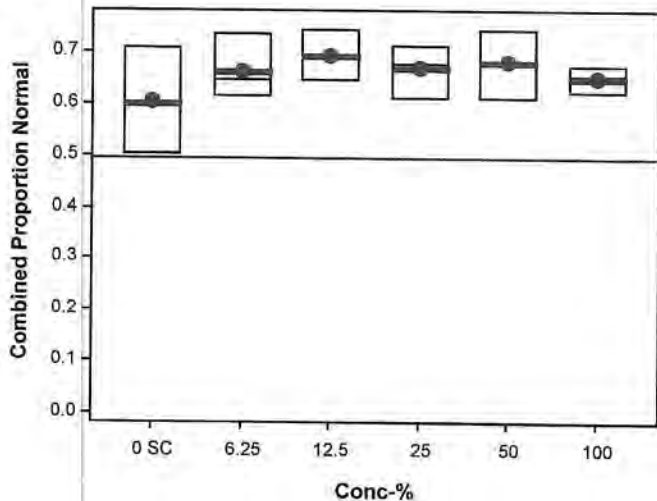
Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	SC	0.9722	0.9979	0.7925	0.7878
6.25		0.9026	1.0300	0.9520	0.9124
12.5		1.0400	0.9875	0.9671	0.9320
25		1.0030	0.8977	0.9671	0.9671
50		0.9671	0.8977	1.0400	0.9722
100		0.9075	0.9620	0.9520	0.9320

Combined Proportion Normal Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	SC	144/211	149/211	107/211	106/211
6.25		130/211	155/211	140/211	132/211
12.5		157/211	147/211	143/211	136/211
25		150/211	129/211	143/211	143/211
50		143/211	129/211	157/211	144/211
100		131/211	142/211	140/211	136/211

Graphics



CETIS Analytical Report

Report Date: 27 Apr-23 13:16 (p 1 of 2)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test				EcoAnalysts
Analysis ID: 10-1272-2961	Endpoint: Combined Proportion Normal	CETIS Version: CETISv2.1.4		
Analyzed: 27 Apr-23 13:15	Analysis: Linear Interpolation (ICPIN)	Status Level: 1		
Edit Date: 17 Apr-23 14:42	MD5 Hash: 4025593621A1937B2F3EC7A1D523F751	Editor ID: 004-221-689-0		
Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke		
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater		
Ending Date: 30 Mar-23 14:40	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix		
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish	Age:	
Sample ID: 12-0776-7817	Code: P230328.01	Project: WEH-031V		
Sample Date: 28 Mar-23 01:38	Material: Treated Groundwater	Source: Jacobs Wyckoff		
Receipt Date: 28 Mar-23 13:10	CAS (PC):	Station: 32823		
Sample Age: 14h (2.3 °C)	Client: Jacobs Wyckoff			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Log(X+1)	Linear	1059744	200	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC15	>100	---	---	<1	---	---
EC20	>100	---	---	<1	---	---
EC25	>100	---	---	<1	---	---
EC40	>100	---	---	<1	---	---
EC50	>100	---	---	<1	---	---

Combined Proportion Normal Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	SC	4	0.5995	0.5948	0.5024	0.7062	18.33%	0.00%	506/844	0.6597	0.00%
6.25		4	0.6600	0.6445	0.6161	0.7346	8.15%	-10.08%	557/844	0.6597	0.00%
12.5		4	0.6908	0.6872	0.6445	0.7441	6.02%	-15.22%	583/844	0.6597	0.00%
25		4	0.6694	0.6777	0.6114	0.7109	6.24%	-11.66%	565/844	0.6597	0.00%
50		4	0.6789	0.6801	0.6114	0.7441	7.99%	-13.24%	573/844	0.6597	0.00%
100		4	0.6505	0.6540	0.6209	0.6730	3.54%	-8.50%	549/844	0.6505	1.39%

Combined Proportion Normal Detail					
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	SC	0.6825	0.7062	0.5071	0.5024
6.25		0.6161	0.7346	0.6635	0.6256
12.5		0.7441	0.6967	0.6777	0.6445
25		0.7109	0.6114	0.6777	0.6777
50		0.6777	0.6114	0.7441	0.6825
100		0.6209	0.6730	0.6635	0.6445

Combined Proportion Normal Binomials					
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	SC	144/211	149/211	107/211	106/211
6.25		130/211	155/211	140/211	132/211
12.5		157/211	147/211	143/211	136/211
25		150/211	129/211	143/211	143/211
50		143/211	129/211	157/211	144/211
100		131/211	142/211	140/211	136/211

CETIS Analytical Report

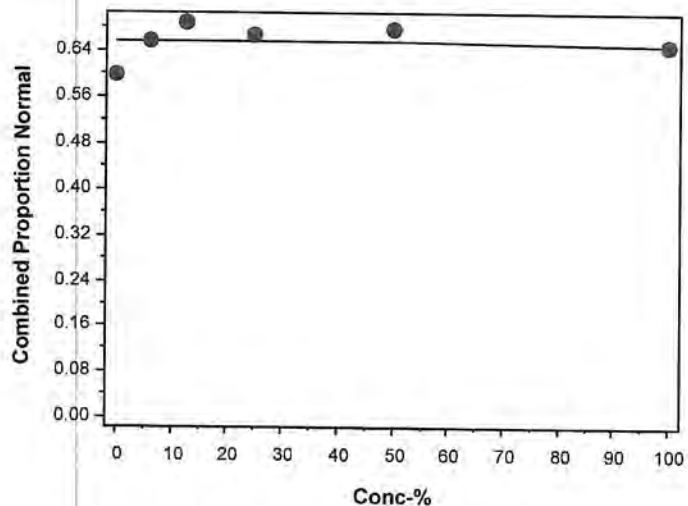
Report Date: 27 Apr-23 13:16 (p 2 of 2)
Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test

EcoAnalysts

Analysis ID: 10-1272-2961	Endpoint: Combined Proportion Normal	CETIS Version: CETISv2.1.4
Analyzed: 27 Apr-23 13:15	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 17 Apr-23 14:42	MD5 Hash: 4025593621A1937B2F3EC7A1D523F751	Editor ID: 004-221-689-0

Graphics



CETIS Test Data Worksheet

Report Date: 17 Apr-23 14:57 (p 1 of 1)
 Test Code/ID: P230328.01 / 04-9403-3749

Bivalve Larval Survival and Development Test					EcoAnalysts				
Start Date:	28 Mar-23 15:46	Species:	Mytilus galloprovincialis		Sample Code:	P230328.01			
End Date:	30 Mar-23 14:40	Protocol:	EPA/600/R-95/136 (1995)		Sample Source:	Jacobs Wyckoff			
Sample Date:	28 Mar-23 01:38	Material:	Treated Groundwater		Sample Station:	32823			

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	5	211	219	219	146	
0	D	2	13	211	208	208	155	
0	D	3	21	211	198	198	149	
0	D	4	19	211	222	222	141	
0	SC	1	20	211	220	220	144	
0	SC	2	27	211	227	227	149	
0	SC	3	11	211	199	199	107	
0	SC	4	10	211	177	177	106	
6.25		1	15	211	196	196	130	
6.25		2	28	211	222	222	155	
6.25		3	17	211	202	202	140	
6.25		4	2	211	194	194	132	
12.5		1	3	211	224	224	157	
12.5		2	16	211	211	211	147	
12.5		3	6	211	227	227	143	
12.5		4	23	211	196	196	136	
25		1	12	211	216	216	150	
25		2	7	211	199	199	129	
25		3	8	211	199	199	143	
25		4	18	211	210	210	143	
50		1	14	211	207	207	143	
50		2	26	211	200	200	129	
50		3	25	211	223	223	157	
50		4	24	211	217	217	144	
100		1	1	211	226	226	131	
100		2	4	211	210	210	142	
100		3	22	211	215	215	140	
100		4	9	211	222	222	136	

Client	Jacobs- Wyckoff
Project	WEH-031V
Project Number	PG1799
Project Manager	J. Levensgood
Date Sample Received	3/28/2023
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	03/28/23
Test Species	Mytilus spp.
Organism Batch	TS032223
Organism Acquired	3/22/2023
Organism Acclimation	6
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: 1546 MS/DM
 TEST END TIME/INIT: 1740 MK

CLIENT SAMPLE ID	LAB ID
32823	P230328.01

Salinity Adjustment CSMM
 Batch #
 01661304

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	100%
8	.
9	.

Treatment	Rep	Chamber
Control	1	
Control	2	
Control	3	
Control	4	
Salt Control	1	
Salt Control	2	
Salt Control	3	
Salt Control	4	
6.25%	1	
6.25%	2	
6.25%	3	
6.25%	4	
12.5%	1	
12.5%	2	
12.5%	3	
12.5%	4	
25%	1	
25%	2	
25%	3	
25%	4	
50%	1	
50%	2	
50%	3	
50%	4	
100%	1	
100%	2	
100%	3	
100%	4	
.	1	
.	2	
.	3	
.	4	
.	1	
.	2	
.	3	
.	4	

V.2 CLIENT	Jacobs- Wyckoff	DATE RECEIVED	3/28/23	PROTOCOL	TOX 042
PROJECT	WEH-031V	TEST START DATE	3/28/23	PROJECT MANAGER	J. Levengood
CLIENT SAMPLE ID	32823	TEST END DATE	3/30/23	SPECIES	<i>Mytilus spp.</i>
LAB SAMPLE ID	P230328.01	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%	25.00	175.00	200		
	25%	50	150.0	200		
	50%	100	100.0	200		
	100%	200	0.0	200		

Test Dilution Prep

Date	Balance ID	Sample ID (P#)	Water Batch ID	Initials
3/28/23	5	P230328.01	FAN 032823.02	DM

48-Hour Chronic WET Test

V.2

CLIENT	Jacobs- Wyckoff	DATE RECEIVED	3/28/23	PROTOCOL	TOX 042
PROJECT	WEH-031V	TEST START DATE	3/28/23	PROJECT MANAGER	J. Levensgood
CLIENT SAMPLE ID	32823	TEST END DATE	3/30/23	SPECIES	<i>Mytilus spp.</i>
LAB SAMPLE ID	P230328.01	MATRIX	Liquid	NO. OF ORGANISMS	150 - 300

48-Hour Chronic Toxicity Using Bivalve Larvae

SPAWNING METHOD Heat Shock		INITIAL SPAWNING TIME See hard copy 1259		FINAL SPAWNING TIME 1353	
MALES 3	FEMALES 4	SPERM VIABILITY Good		EGG CONDITION Good	
BEGIN FERTILIZATION 1353		END FERTILIZATION 1545		CONDITION OF EMBRYOS Good	

TIME OF INITIATION 15:46	INITIALS DM/MS
-----------------------------	-------------------

EMBRYO DENSITY CALCULATIONS

# of embryos in 1 mL of 100X diluted embryo stock			# embryos in original stock = # of embryos in diluted stock x 100
Count 1	Count 2	Mean	
191	245	218	21800
Percentage of embryo stock needed = 2500 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)			
4.587155963 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			

CLIENT	Jacobs- Wyckoff	DATE RECEIVED	3/28/23	PROTOCOL	TOX 042
PROJECT	WEH-031V	TEST START DATE	3/28/23	PROJECT MANAGER	J. Levengood
CLIENT SAMPLE ID	32823	TEST END DATE	3/30/23	SPECIES	<i>Mytilus spp.</i>
LAB SAMPLE ID	P230328.01	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	8.2	① 16.3	31	7.9
Stock				
Salt Control	8.3	① 16.3	30	7.8
Date 3/28/23				
6.25%	8.3	① 16.3	31	7.9
Time 1500				
12.5%	8.3	① 16.3	31	7.9
Tech DM				
25%	8.3	① 16.3	30	7.8
Meter # 9				
50%	8.3	① 16.3	30	7.8
100%	8.4	① 16.3	29	7.7
Day 1				
Control		① 15.4		
Surrogate				
Salt Control		① 15.4		
Date 3/29/23				
6.25%		① 15.4		
Time 0920				
12.5%		① 15.4		
Tech SZ				
25%		① 15.4		
Meter # 716				
50%		① 15.4		
100%		① 15.4		
Day 2				
Control	7.8	① 15.6	31	7.9
Surrogate				
Salt Control	7.9	① 15.6	30	7.8
Date 3/30/23				
6.25%	8.0	① 15.6	31	8.0
Time 1418				
12.5%	8.0	① 15.6	31	8.1
Tech SZ				
25%	8.0	① 15.6	30	8.2
Meter # 8				
50%	8.0	① 15.6	30	8.3
100%	8.1	① 15.6	29	8.4

① used Temp Blank-DM-3/28/23, SZ 3/29/23, SZ 3/30/23

v.2

CLIENT	Jacobs- Wyckoff	DATE RECEIVED	3/28/23	PROTOCOL	TOX 042
PROJECT	WEH-031V	TEST START DATE	3/28/23	PROJECT MANAGER	J. Levengood
CLIENT SAMPLE ID	32823	TEST END DATE	3/30/23	SPECIES	Mytilus spp.
LAB SAMPLE ID	P230328.01	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	108		4/6/23	MK	
	2	204		4/6/23	MK	
	3	228		4/6/23	MK	
	4	215		4/6/23	MK	
	5	230		4/6/23	MK	
	6	218		4/6/23	MK	
Control	1	146	73	4/6/23	MK	QA 135/1212 = 0.64% orig 0.66% Δ=3% ✓ NL 4/13
	2	155	53	4/6/23	MK	
	3	149	49	4/6/23	MK	
	4	141	81	4/6/23	MK	
Salt Control	1	144	76	4/10/23	MS	
	2	149	78	4/13/23	NL	
	3	167	92	4/13	NL	
	4	106	71	4/13	NL	
6.25%	1	130	66	4/10/23	MS	
	2	155	67	4/13	NL	QA 153N/166A 70%:70% Δ=0% ✓
	3	140	62	4/13	NL	
	4	132	62	4/13	NL	
12.5%	1	157	67	4/10/23	MS	
	2	147	64	4/13	NL	
	3	143	84	4/13	NL	142N/87A QA 52% 63%:62% Δ=1% ✓
	4	136	60	4/13	NL	
25%	1	150	66	4/10/23	MS	
	2	129	① 43 70	4/13	NL	
	3	143	56	4/13	NL	
	4	143	67	4/13	NL	
50%	1	143	64	4/10/23	MS	
	2	129	71	4/13	NL	
	3	157	66	4/13	NL	
	4	144	73	4/13	NL	
100%	1	131	95	4/10/23	MS	
	2	① 140 142	① 75 68	4/13	NL	
	3	140	75	4/13	NL	
	4	136	86	4/13	NL	

01E-NL4/13

Bivalve Larval Survival and Development Test

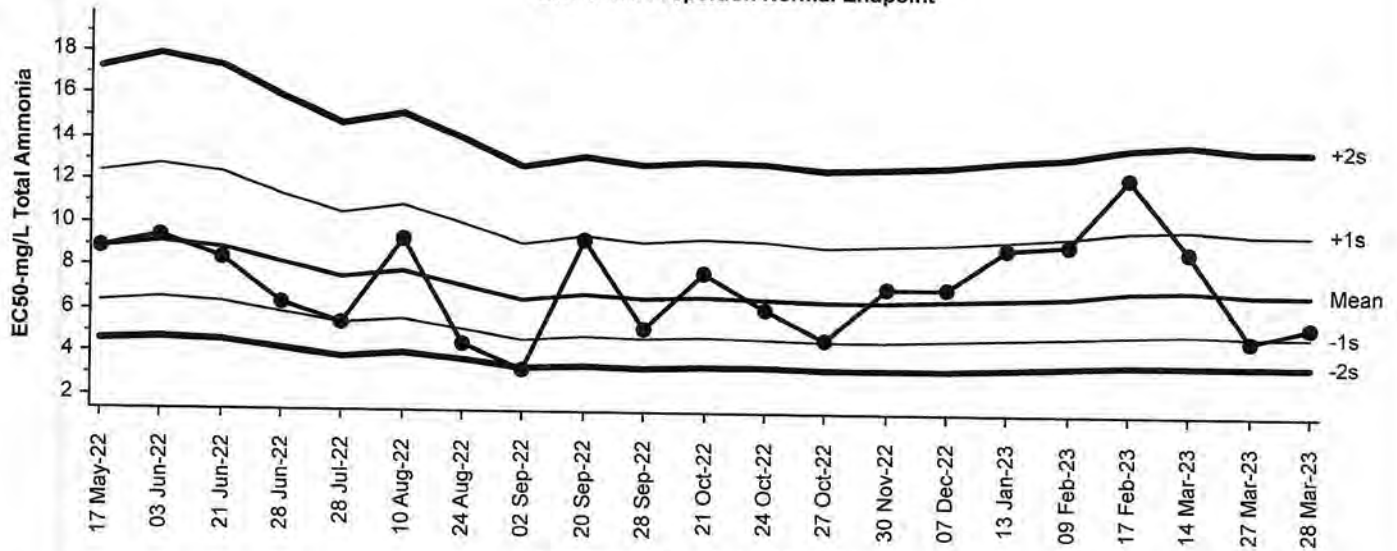
All Matching Labs

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis
 Endpoint: Combined Proportion Normal

Material: Total Ammonia
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test
 Combined Proportion Normal Endpoint



Lognormal Cumulative Mean Plot

Mean: 7.015 Count: 20 -1s Warning Limit: 5.03 -2s Action Limit: 3.6
 Sigma: NA CV: 34.30% +1s Warning Limit: 9.8 +2s Action Limit: 13.7

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	May	17	16:11	8.853	1.839	0.6974			11-5935-3112	11-7802-4839	EcoAnalysts
2		Jun	3	16:12	9.446	2.431	0.8914			08-5591-8618	16-7793-8354	EcoAnalysts
3			21	17:46	8.368	1.353	0.5284			03-7983-1979	17-5961-0612	EcoAnalysts
4			28	16:05	6.362	-0.6526	-0.2926			08-5637-7603	04-5931-5430	EcoAnalysts
5		Jul	28	15:55	5.431	-1.583	-0.7663			19-6544-8440	11-0281-7196	EcoAnalysts
6		Aug	10	16:57	9.323	2.308	0.8521			20-5736-9281	08-2934-0504	EcoAnalysts
7			24	16:43	4.439	-2.576	-1.371	(-)		10-4871-9595	11-0042-4049	EcoAnalysts
8		Sep	2	14:54	3.311	-3.703	-2.249	(-)	(-)	16-0701-8534	00-0124-1152	EcoAnalysts
9			20	16:02	9.267	2.253	0.8343			11-7896-9547	00-7476-6700	EcoAnalysts
10			28	16:31	5.182	-1.833	-0.9073			10-3818-0354	11-9896-8834	EcoAnalysts
11		Oct	21	14:16	7.804	0.7898	0.3196			05-2022-4267	03-4308-3965	EcoAnalysts
12			24	15:17	6.15	-0.8648	-0.3942			01-4864-2336	19-5269-5566	EcoAnalysts
13			27	17:02	4.776	-2.239	-1.152	(-)		12-4527-0974	13-7457-7890	EcoAnalysts
14		Nov	30	14:32	7.166	0.151	0.06382			11-2220-4195	10-4569-3704	EcoAnalysts
15		Dec	7	17:43	7.159	0.1442	0.06097			19-4874-8030	20-9525-0017	EcoAnalysts
16	2023	Jan	13	15:30	9.078	2.063	0.7723			14-2219-3979	18-3945-1944	EcoAnalysts
17		Feb	9	15:28	9.246	2.232	0.8275			00-8572-7368	10-5325-0783	EcoAnalysts
18			17	14:30	12.4	5.383	1.706	(+)		20-3891-7103	06-7296-3936	EcoAnalysts
19		Mar	14	15:15	8.955	1.941	0.7317			00-9622-9067	21-3408-3763	EcoAnalysts
20			27	16:54	4.818	-2.197	-1.125	(-)		13-8989-7877	05-5295-3514	EcoAnalysts
21			28	15:46	5.455	-1.56	-0.7534			02-2233-3890	16-3797-4494	EcoAnalysts

Bivalve Larval Survival and Development Test

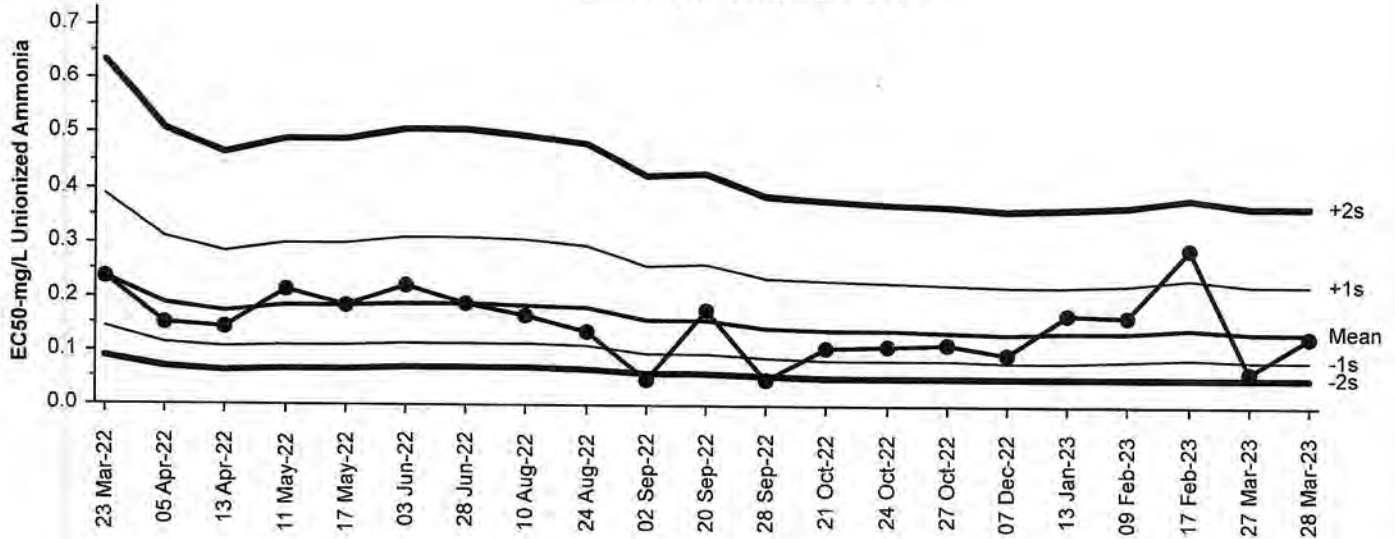
All Matching Labs

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis
 Endpoint: Combined Proportion Normal

Material: Unionized Ammonia
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test
 Combined Proportion Normal Endpoint



Lognormal Cumulative Mean Plot

Mean: 0.1372 Count: 20 -1s Warning Limit: 0.0837 -2s Action Limit: 0.051
 Sigma: NA CV: 52.60% +1s Warning Limit: 0.225 +2s Action Limit: 0.369

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID	Laboratory
1	2022	Mar	23	15:30	0.2359	0.09862	1.095	(+)		18-9877-4146	02-7793-3754	EcoAnalysts
2		Apr	5	15:09	0.1515	0.01422	0.1993			15-5824-5509	00-2065-3937	EcoAnalysts
3			13	15:38	0.1448	0.00755	0.1083			18-9475-6703	13-3543-8688	EcoAnalysts
4		May	11	15:54	0.2152	0.07796	0.9095			19-4844-7090	04-7446-5371	EcoAnalysts
5			17	16:11	0.185	0.04777	0.6039			21-0960-1917	00-4551-7197	EcoAnalysts
6		Jun	3	16:12	0.2219	0.08469	0.9718			21-4199-4121	20-5427-8206	EcoAnalysts
7			28	16:05	0.188	0.05078	0.6365			19-3785-6817	00-8378-9623	EcoAnalysts
8		Aug	10	16:57	0.1651	0.0279	0.3742			09-3839-8015	12-5640-2017	EcoAnalysts
9			24	16:43	0.1359	-0.00138	-0.02037			00-7678-9875	07-1760-4646	EcoAnalysts
10		Sep	2	14:54	0.04851	-0.08873	-2.103	(-)	(-)	13-9573-6141	09-4475-1376	EcoAnalysts
11			20	16:02	0.1767	0.0395	0.5114			13-8303-2046	02-4939-5521	EcoAnalysts
12			28	16:31	0.04973	-0.08751	-2.052	(-)	(-)	14-4835-8902	06-7637-8760	EcoAnalysts
13		Oct	21	14:16	0.1071	-0.03016	-0.5016			20-9426-4253	15-1656-6246	EcoAnalysts
14			24	15:17	0.1096	-0.02765	-0.4549			18-7734-9147	06-4748-9707	EcoAnalysts
15			27	17:02	0.1156	-0.02165	-0.3472			01-3898-0369	19-9850-5740	EcoAnalysts
16		Dec	7	17:43	0.09634	-0.04091	-0.7156			15-6747-3203	15-5237-0673	EcoAnalysts
17	2023	Jan	13	15:30	0.1703	0.03308	0.4366			14-6111-3358	19-5184-9524	EcoAnalysts
18		Feb	9	15:28	0.1664	0.02912	0.389			11-1705-9064	00-9866-2896	EcoAnalysts
19			17	14:30	0.2912	0.154	1.521	(+)		05-8051-1741	00-4535-0428	EcoAnalysts
20		Mar	27	16:54	0.06349	-0.07375	-1.559	(-)		01-2022-2925	11-3364-1842	EcoAnalysts
21			28	15:46	0.1275	-0.00977	-0.1493			08-8126-4059	10-2993-2407	EcoAnalysts

CETIS Summary Report

Report Date: 17 Apr-23 15:32 (p 1 of 1)
 Test Code/ID: P220819.38 / 02-2233-3890

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 30 Mar-23 14:45	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish
Sample ID: 05-0700-7810	Code: P220819.38	Age:
Sample Date: 19 Aug-22	Material: Total Ammonia	Project: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Source: Reference Toxicant
Sample Age: 221d 16h	Client: Internal Lab	Station: P220819.38

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
05-2047-3230	Combined Proportion Norma	Dunnett Multiple Comparison Test	3.27	6.47	4.6	12.5%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
16-3797-4494	Combined Proportion Norma	Linear Interpolation (ICPIN)	EC15	3.83	3.431	3.973	1
			EC20	4.035	3.644	4.242	
			EC25	4.248	3.877	4.515	
			EC40	4.942	4.584	5.443	
			EC50	5.455	5.088	6.151	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
05-2047-3230	Combined Proportion Norma	PMSD	0.1246	<<	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	3	0.6698	0.5747	0.7650	0.6351	0.7109	0.0221	0.0383	5.72%	0.00%
1.49		3	0.6967	0.5888	0.8046	0.6588	0.7441	0.0251	0.0434	6.23%	-4.01%
3.27		3	0.6825	0.6001	0.7649	0.6493	0.7156	0.0192	0.0332	4.86%	-1.89%
6.47		3	0.2212	0.1156	0.3267	0.1943	0.2701	0.0245	0.0425	19.21%	66.98%
12.7		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
19.1		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Combined Proportion Normal Detail

MD5: 947C54FFAB05EC37EF7BC67DB635C3A9

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	0.7109	0.6351	0.6635
1.49		0.6872	0.6588	0.7441
3.27		0.7156	0.6825	0.6493
6.47		0.1991	0.1943	0.2701
12.7		0.0000	0.0000	0.0000
19.1		0.0000	0.0000	0.0000

Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	150/211	134/211	140/211
1.49		145/211	139/211	157/211
3.27		151/211	144/211	137/211
6.47		42/211	41/211	57/211
12.7		0/211	0/211	0/211
19.1		0/211	0/211	0/211

CETIS Summary Report

Report Date: 17 Apr-23 15:35 (p 1 of 1)
 Test Code/ID: P220819.38UIA / 08-8126-4059

Bivalve Larval Survival and Development Test

EcoAnalysts

Batch ID: 02-5396-5048	Test Type: Development-Survival	Analyst: Sarah Zischke
Start Date: 28 Mar-23 15:46	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 30 Mar-23 14:45	Species: Mytilus galloprovincialis	Brine: Crystal Sea Marine Mix
Test Length: 47h	Taxon: Bivalvia	Source: Taylor Shellfish
Sample ID: 15-5779-3495	Code: P220819.38UIA	Age:
Sample Date: 19 Aug-22	Material: Unionized Ammonia	Project: Reference Toxicant
Receipt Date: 19 Aug-22	CAS (PC):	Source: Reference Toxicant
Sample Age: 221d 16h	Client: Internal Lab	Station: P220819.38UIA

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
14-7121-9324	Combined Proportion Norma	Dunnett Multiple Comparison Test	0.074	0.147	0.1043	12.5%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
10-2993-2407	Combined Proportion Norma	Linear Interpolation (ICPIN)	EC15	0.08968	0.07794	0.09299	1
			EC20	0.095	0.08358	0.09982	
			EC25	0.1003	0.08956	0.1063	
			EC40	0.1165	0.1066	0.1261	
			EC50	0.1275	0.1179	0.1397	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
14-7121-9324	Combined Proportion Norma	PMSD	0.1246	<<	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	3	0.6698	0.5747	0.7650	0.6351	0.7109	0.0221	0.0383	5.72%	0.00%
0.034		3	0.6967	0.5888	0.8046	0.6588	0.7441	0.0251	0.0434	6.23%	-4.01%
0.074		3	0.6825	0.6001	0.7649	0.6493	0.7156	0.0192	0.0332	4.86%	-1.89%
0.147		3	0.2212	0.1156	0.3267	0.1943	0.2701	0.0245	0.0425	19.21%	66.98%
0.23		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%
0.347		3	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	0.0000	---	100.00%

Combined Proportion Normal Detail

MD5: 50EFFC8A110FEE10A4558C2ABC203530

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	0.7109	0.6351	0.6635
0.034		0.6872	0.6588	0.7441
0.074		0.7156	0.6825	0.6493
0.147		0.1991	0.1943	0.2701
0.23		0.0000	0.0000	0.0000
0.347		0.0000	0.0000	0.0000

Combined Proportion Normal Binomials

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3
0	D	150/211	134/211	140/211
0.034		145/211	139/211	157/211
0.074		151/211	144/211	137/211
0.147		42/211	41/211	57/211
0.23		0/211	0/211	0/211
0.347		0/211	0/211	0/211

CETIS Test Data Worksheet

Report Date: 17 Apr-23 15:27 (p 1 of 1)

Test Code/ID: P220819.38 / 02-2233-3890

Bivalve Larval Survival and Development Test				EcoAnalysts			
Start Date: 28 Mar-23 15:46	Species: Mytilus galloprovincialis	Sample Code: P220819.38					
End Date: 30 Mar-23 14:45	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Reference Toxicant					
Sample Date: 19 Aug-22	Material: Total Ammonia	Sample Station: P220819.38					

Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	16	211	209	209	150	
0	D	2	3	211	203	203	134	
0	D	3	9	211	211	211	140	
1.49		1	6	211	200	200	145	
1.49		2	13	211	203	203	139	
1.49		3	7	211	219	219	157	
3.27		1	15	211	218	218	151	
3.27		2	11	211	206	206	144	
3.27		3	1	211	220	220	137	
6.47		1	17	211	222	222	42	
6.47		2	2	211	204	204	41	
6.47		3	18	211	220	220	57	
12.7		1	10	211	176	176	0	
12.7		2	5	211	133	133	0	
12.7		3	14	211	138	138	0	
19.1		1	4	211	111	111	0	
19.1		2	12	211	105	105	0	
19.1		3	8	211	88	88	0	

CETIS Test Data Worksheet

Report Date: 17 Apr-23 15:31 (p 1 of 1)

Test Code/ID: P220819.38UIA / 08-8126-4059

Bivalve Larval Survival and Development Test				EcoAnalysts			
Start Date: 28 Mar-23 15:46	Species: Mytilus galloprovincialis	Sample Code: P220819.38UIA					
End Date: 30 Mar-23 14:45	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Reference Toxicant					
Sample Date: 19 Aug-22	Material: Unionized Ammonia	Sample Station: P220819.38UIA					

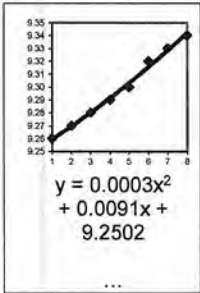
Conc-mg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	D	1	2	211	209	209	150	
0	D	2	7	211	203	203	134	
0	D	3	5	211	211	211	140	
0.034		1	11	211	200	200	145	
0.034		2	15	211	203	203	139	
0.034		3	12	211	219	219	157	
0.074		1	4	211	218	218	151	
0.074		2	13	211	206	206	144	
0.074		3	10	211	220	220	137	
0.147		1	6	211	222	222	42	
0.147		2	1	211	204	204	41	
0.147		3	9	211	220	220	57	
0.23		1	17	211	176	176	0	
0.23		2	16	211	133	133	0	
0.23		3	3	211	138	138	0	
0.347		1	14	211	111	111	0	
0.347		2	8	211	105	105	0	
0.347		3	18	211	88	88	0	

Un-ionized Ammonia Calculator

CLIENT:	Jacobs Wyckoff	Date of Test:	March 28, 2023
PROJECT:	WEH-031V	Test Type:	Bivalve NH3 RT Dev
COMMENTS:	P220819.38		

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 ₃ U (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	1.5	1.49	31	7.9	16.3	289.45	9.2561	0.034
2	3	3.27	31	7.9	16.3	289.45	9.2561	0.074
3	6	6.47	31	7.9	16.3	289.45	9.2561	0.147
4	12	12.7	31	7.8	16.3	289.45	9.2561	0.230
5	18	19.1	31	7.8	16.3	289.45	9.2561	0.347
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QAV MARCH

48 Hour Bivalve Development Reference Toxicant Test

Test ID: P22008 19.38	Replicates: 3	Study Director: J. Levensgood	Location: INC #1
Dilution Water Batch: FSW 032823.02	Organism Batch: T5032223.02	Associated Test(s): Various ①	Organism: M.sp.
Chamber Size/Type: 30 ml shell vial	Exposure Volume: 10 ml		
Toxicant: Ammonium Chloride:	Lot #: 22E3156086	Date Prepared: 3/28/23	Initials: DM
Target Concentrations: See spiking worksheet	Quantity of Stock: Target: See spiking worksheet	Quantity of Diluent: Target: 200 mL	
See spiking worksheet	Actual: See spiking worksheet	Actual:	

SPAWNING DATA

Initial Spawning Time: 1259	Final Spawning Time: 1353	Fertilization Time: 1353	No. of Females: 4	No. of Males: 3
Embryo Density (count/mL):	1. 191	2. 245	3. -	Mean: 218
Stocking Volume Calculation: $\frac{2500}{21800} = 0.11 \times 40 \text{ mL} = 4.6 \text{ mL egg stock in } 35.4 \text{ mL FSW}$				

0 Hours Date: **3/28/23** WQ Time: **1427** Start Time: **1546** Initials: **DM/MS**
STOCK

	Control	1.5	3	6	12	18
D.O. (%) (>4.8 mg/L)	8.1	8.3	8.3	8.4	8.4	8.4
Temperature (16 ± 1°C)	② 16.3	② 16.3	② 16.3	② 16.3	② 16.3	② 16.3
Salinity (30 ± 2 ppt)	30	31	31	31	31	31
pH (7.5-9)	7.8	7.9	7.9	7.9	7.8	7.8

Day 1 Temperature (16 ± 1°C) **15.6^②, 16, 52**

Final Day Date: **3/30/23** WQ Time: **1426** End Time: **1445** Initials: **MM**

Formalin Lot #: **220304-56** Rose Bengal Lot #: **5135**

STOCK

	Control	1.5	3	6	12	18
D.O. (%) (>4.8 mg/L)	7.9	8.0	8.0	8.0	7.9	7.9
Temperature (16 ± 1°C)	16.1^②	16.1^②	16.1^②	16.1^②	16.1^②	16.1^②
Salinity (30 ± 2 ppt)	31	31	31	31	31	31
pH (7.5-9)	8.0	8.0	8.0	8.0	7.9	8.0

① IE-DM-3/28/23

② used temp blank-DM-3/28/23, 52 3/29/23, 52 3/30/23

48 Hour Bivalve Development Reference Toxicant Test

P2208/9.38

Conc.	Rep	Number Normal	Number Abnormal	Date	Initials
Control	1	150	59	4/10/23	MS
	2	134	69	4/13/23	SZ
	3	140	71	4/13/23	SZ
1.5	1	145	55	4/13/23	SZ
	2	139	64	4/13/23	SZ
	3	157	62	4/13/23	SZ
3	1	151	67	4/13/23	SZ
	2	144	62	4/13/23	SZ
	3	137	83	4/13/23	SZ
6	1	42	180	4/13/23	SZ
	2	41	163	4/13/23	SZ
	3	57	163	4/13/23	SZ
12	1	0	176	4/13/23	SZ
	2	0	133	4/13/23	SZ
	3	0	138	4/13/23	SZ
18	1	0	111	4/13/23	SZ
	2	0	105	4/13/23	SZ
	3	0	88	4/13/23	SZ

Stocking Density

Rep	Count	Init.
1	168, Rep 4: 215	MK
2	204, Rep 5: 230	MK
3	228, Rep 6: 218	MK
Mean: ① 200 211		

QA Count Checks:	#2 conc/rep	#3 conc/rep	#4 conc/rep
#1 conc/rep <u>0 repl</u>	<u>1.5 rep 3</u>	<u>6.0 rep 1</u>	_____
# normal <u>146</u>	# normal <u>159</u>	# normal <u>49</u>	# normal _____
# abnormal <u>58</u>	# abnormal <u>61</u>	# abnormal <u>174</u>	# abnormal _____
Tech. Init. <u>NL</u>	Tech. Init. <u>NL</u>	Tech. Init. <u>NL</u>	Tech. Init. _____
Calc. QA: $\frac{146}{204} = 0.72\%$ orig $\frac{150}{209} = 0.72\%$ $\Delta = 0\%$	QA: $\frac{159}{220} = 72\%$ orig = $\frac{157}{219} = 72\%$ $\Delta = 0\%$	QA $\frac{49}{223} = 22\%$ orig $\frac{42}{222} = 19\%$ $\Delta = 3\%$	/

QA Check Acceptability: <5% difference in means of QA & orig. counts

① IE- SZ 4/17/23

**Ammonia Reference Toxicant
Spiking Worksheet**

Reference Toxicant ID: P220819.38
 Date Prepared: 3/20/23
 Technician Initials: DM

Biv / Echino NH₃ RT

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

Date: 3/1/2023
 Measurement: 9853.333333

Test Solutions			Volume of stock to reach desired concentration	
Measured Concentration	Desired Concentration	Volume	mL stock to increase	
mg/L	mg/L	mL		
0.50	0	250		SALT WATER
1.49	1.5	250		0.057
3.27	3	250		0.114
6.47	6	250		0.228
12.7	12	250		0.457
19.1	18	250		0.685

MAINTENANCE LOG FOR FLOW-THROUGH CULTURE TUBS

LOCATION: Bat16

① Organism (A): M.sp	Batch Number: TS021623.01	Date Received: 2/16/23	Initial # of Organisms:	10% Mort =
② Organism (B): M.sp	Batch Number: TS120622.01	Date Received: 12/06/22	Initial # of Organisms:	10% Mort =
Organism (C): M.sp	Batch Number: TS032223.01	Date Received: 3/22/23	Initial # of Organisms:	10% Mort =
③ Organism (D): M.sp	Batch Number: TS032223.01	Date Received: 3/22/23	Initial # of Organisms:	10% Mort =
Organism (E):	Batch Number:	Date Received:	Initial # of Organisms:	10% Mort =

Date	Feed AM/PM	Organism (A, B, C, D, or E)	D.O.	Temp (°C)	Cond/ Sal	pH	H ₂ O Change	Organisms appear healthy (Y/N)	# Mort	Cumulative # Mort*	Init.	Comments
3/9	- ✓	B	8.3	10.4	30	7.9	FT	Y	0	-	NL	
3/9 ¹²	✓ -	A	8.3	11.4	30	7.8	FT	Y	0	-	UG	
3/12	✓ -	B	8.4	11.1	30	7.8	FT	Y	0	-	UG	
3/14	- -	A	8.5	11.3	30	7.8	FT	Y	0	-	SZ	
3/14	- -	B	8.3	11.7	30	7.8	FT	Y	0	-	SZ	
3/16	- ✓	A	7.6	13.5	30	7.7	FT	Y	0	-	UG	
3/16	- ✓	B	8.3	13.0	30	7.8	FT	Y	0	-	UG	
3/19	✓ -	A	7.8	11.7	30	7.7	FT	Y	0	-	UG	
3/21	✓ -	A	8.3	11.8	30	7.8	FT	Y	0	-	UG	
3/23		A	7.0	11.7	30	7.6	FT	Y	0	-	SZ	
3/23		B					FT	Y	0	-	SZ	
3/23	-	C	7.4	10.4	31	7.7	FT	Y	0	-	SZ	culture was dispatched - SZ-3/23/23
3/26	- ✓	A	5.0	13.8	30	7.4	FT	Y	0	-	UG	
3/26	- ✓	B	6.2	13.7	30	7.6	FT	Y	0	-	UG	
3/26	- ✓	C	5.8	13.9	30	7.6	FT	Y	0	-	UG	

FT = Flow-through

*For all days of a given batch; if >10% notify lab manager

9/8/2022

② Culture Maintenance Log V 1.5

① WP-UG 3/12/23

② MS 3/23

③ put in separate tub on 3/23 - MS 3/23

④ culture was dispatched - UG 3/20/23

MAINTENANCE LOG FOR FLOW-THROUGH CULTURE TUBS

LOCATION: Bath 10

Organism (A): ^① <u>TS M.sp</u>	Batch Number: <u>TS032223.01</u>	Date Received: <u>3/22/23</u>	Initial # of Organisms: _____ 10% Mort = _____
Organism (B): <u>M.sp</u>	Batch Number: <u>TS032223.02</u>	Date Received: <u>3/22/23</u>	Initial # of Organisms: _____ 10% Mort = _____
Organism (C): <u>M.sp (Bath 7)</u>	Batch Number: <u>TS040323.01</u>	Date Received: <u>4/3/23</u>	Initial # of Organisms: _____ 10% Mort = _____
Organism (D):	Batch Number:	Date Received:	Initial # of Organisms: _____ 10% Mort = _____
Organism (E):	Batch Number:	Date Received:	Initial # of Organisms: _____ 10% Mort = _____

Date	Feed AM/PM	Organism (A, B, C, D, or E)	D.O.	Temp (°C)	Cond/ (Sal)	pH	H ₂ O Change	Organisms appear healthy (Y/N)	# Mort	Cumulative # Mort*	Init.	Comments	
3/28	-	-	A, B	6.3	14.1	30	7.5	FT	Y	0	-	UG	
3/30	-	✓	A, B	6.3	14.5	30	7.6	FT	Y	0	-	UG	
3/31	-	✓	A, B	7.3	14.3	30	7.6	FT	Y	0	-	DM	
4/2	-	✓	A, B	8.2	13.7	30	7.9	FT	Y	0	-	NL	
4/4	-	✓	A, B	7.8	14.4	30	7.4	FT	Y	0	-	UG	
4/4	-	✓	C	5.3	13.4	30	7.3	FT	Y	0	-	UG	
4/5	-	-	C	4.7	14.3	30	7.3	FT	Y	0	-	UG	
4/7	-	✓	A, B	8.2	13.9	30	7.8	FT	Y	0	-	NL	spawned, did full H ₂ O Δ
4/7	-	✓	C	6.7	13.5	30	7.5	FT	Y	0	-	NL	-UG 4/5/23
4/9	-	✓	B	8.3	13.8	30	7.9	FT	Y	0	-	UG	
4/9	-	✓	C	5.2	14.3	30	7.4	FT	Y	0	-	UG	

FT = Flow-through

*For all days of a given batch; if >10% notify lab manager

APPENDIX B

Chain-of-Custody and Sample Receipt Forms

EcoAnalysts, Inc. (REGION COPY)
 DateShipped: 3/28/2023
 CarrierName: EcoAnalysts (hand delivery)
 AirbillNo:

Jacobs, Wyckoff-
 Wyckoff Eagle Harbor GWTP 2023/WA
 Project Code: WEH-031V
 Cooler #: 1 of 1

No: 10-032823-103023-0678
 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
032823		Ground Water/ D. Baca	Composite	CHRTOX(8 Weeks)	N (1)	SP-11	03/28/2023 01:38	Field Sample

Special Instructions: 2023 Week 13-1st Quarter Bioassay-Chronic Toxicity Bivalve Test.
 SP-11 is our Effluent Sampling Point.

Analysis Key: CHRTOX=Chronic Toxicity

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

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	<i>D. Baca</i> @ JACOBS	3-28-23 @ 11:12	<i>D. Baca</i> EcoAnalysts	3/28/23 13:10	Good

① Lab sample ID: P230328.01

SAMPLE RECEIPT

Client:	Client ID:	Lab ID:	Renewals:
Jacobs Wyckoff	D32823	P230328.01	
Project:			
WEH-031V			
Date/Time Received:		3/28/23 1310	
Airbill #:		Counter	
Shipper Tracking Information Kept for Records: (Y/N/NA)		NA	
Collection Date/Time:		3/28/23 0138	
Sample Holding Time (must be ≤36 hours at test initiation)		Good	
Condition of Shipping Container:		Good	
Type and Capacity of Sample Container:		4L wbi	
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)		Ice	
Sampler's Name Present on COC Form: (Print Name/Not Present)		Daniel 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 NH ₃ (mg/L)	Tech
P230328.01	121	2.3	8	9.2	8	7.6	8	1061	8	0.513	-	-	0.05	0.553	UG/DM

*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: