



VIA: Electronic Delivery

Ms. Paula Skryja
BP Olympic Pipeline Company LLC
Environmental Coordinator

March 18, 2025

RE: Bioassay Report Transmittal

Dear Ms. Skryja,

Attached, please find the Olympic Pipeline Gasoline Spill and Remediation Project Sediment Evaluation – Toxicity Testing Report, dated March 11, 2025. The report documents the testing of sediment collected in November 2024 as per the Bioassay Plan (March 2024) approved by the Washington Department of Ecology (WDOE). WDOE participated in the sample location designation and the sample collection of sediment for this toxicity test. No exceedences of any cleanup or risk criteria were present in the testing results. Based on these findings there does not appear to be any residual hydrocarbons from the gasoline spill present in the sediment of Hill Ditch, in the vicinity of the spill site, that presents a risk to invertebrates or other organisms that may consume them. Based on these findings we recommend that no further action is required for sediment evaluated under this plan.

Please let me know if you have any questions,

Sincerely,

Tony Palagyi

Tony Palagyi
Senior Environmental Response Consultant
CTEH, L.L.C.

Olympic Pipeline Gasoline Spill and Remediation Project Sediment Evaluation – Toxicity Testing Report

Sample IDs: MVWH1122P001 and MVWH1122P002

Sample Collection: November 2024

Prepared for: CTEH
5120 Northshore Drive
North Little Rock, AR 72118

Prepared by: Nautilus Environmental
(previously Enthalpy Analytical)
San Diego Bioassay Lab
4340 Vandever Avenue
San Diego, CA 92120

Date Submitted: March 11, 2025

Data Quality Assurance:

- Nautilus Environmental is a member of the IEH Laboratories & Consulting Group. IEH acquired the San Diego aquatic toxicology group from Enthalpy Analytical on December 7, 2024. The testing that is being reported under this cover was performed under the name Enthalpy Analytical and is being reported by Nautilus Environmental.
- Nautilus Environmental (previously Enthalpy Analytical) is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053). It is also certified by the State of California Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Verified by:



Kasey Skrivseth, Project Manager

INTRODUCTION

Sediment toxicity bioassay procedures using the midge *Chironomus dilutus* (*Chironomus*) and the amphipod *Hyalella azteca* (*Hyalella*) were performed on two samples in support of the Olympic Pipeline Gasoline Spill and Remediation Project located near Conway, Washington. Bioassay testing was conducted between December 19, 2024, and January 16, 2025, at Nautilus Environmental, Inc. (Nautilus; formerly Enthalpy Analytical), located in San Diego, California.

METHODS AND MATERIALS

Sample Collection, Receipt and Preparation

Two samples were collected on November 22, 2024. Following collection, the sample material was placed in double-layered, labeled polyethylene plastic bag and sealed to minimize headspace. Bags were placed in ice chests with wet ice and shipped to Nautilus via FedEx. The samples were received at Nautilus on November 26, 2024. The contents of each cooler were inspected to ensure samples were intact and matched the chain-of-custody (COC) form. Sample temperatures ranged from 6.2 to 6.5 degrees Celsius (°C) upon arrival. Laboratory sample log-in numbers were added to all bags, and the labeling process was checked by a second analyst as part of the sample check in QC process. The sample was promptly transferred to storage and held at 4°C in the dark until used for testing.

Prior to testing, the samples were thoroughly homogenized and sieved through a 0.5-millimeter (mm) Nitex® mesh screen to remove native organisms and mitigate potential interferences with testing (e.g., competition with or predation of test organisms). An aliquot of each homogenized sediment was centrifuged to separate sediment particles from interstitial pore water for sulfide and total ammonia analysis. For total ammonia analysis, pore water pH was measured, and then the subsample was preserved with sulfuric acid.

A summary of sample information can be found in Table 1. Copies of the COC and sample check-in information are provided in Appendices A and B, respectively.

Table 1. Sample ID, Receipt, and Testing Information

Sample ID	Date/Time Collected	Date/Time Received	Receipt Temperature (°C)	Hyalella Test Initiation Date	Chironomid Test Initiation Date
MVWA1122P001 (sample)	11/22/24, 10:05	11/26/24, 09:45	6.2	12/19/24	12/20/24
MVWA1122P002 (reference)	11/22/24, 11:30		6.5		

Bioassays were conducted in accordance with American Society for Testing and Materials “Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates” Method E1706-20 (ASTM 2020) Test methodology is summarized in Table 2 and 3.

Table 2. Toxicity Test Methodology and QA/QC Requirements for the 10-day Solid Phase Midge Larvae Toxicity Test

Test organism	Midge larvae <i>Chironomus dilutus</i> (formerly <i>C. tentans</i>)
Test organism source	Aquatic BioSystems, Inc.; Fort Collins, CO
Test organism age at initiation	10 days
Test duration; endpoints	10 days; survival
Test temperature	23 ± 1°C daily mean, 23 ± 3°C instantaneous limit
Photoperiod	16 hours light:8 hours dark
Test chamber	500-mL glass jar
Sediment depth	2.5 cm
Overlying water volume	250 mL
Overlying water	Activated carbon filtered water (CFW)
Overlying water renewal	Twice daily full volume exchanges using Zumwalt apparatus
Overlying water quality monitoring	pH, temperature, conductivity, and dissolved oxygen (DO) daily; total ammonia, total sulfide, hardness and alkalinity at test initiation and termination
Number of organisms/chamber	10
Number of replicates	5, plus one surrogate test chamber for water quality readings and two surrogate chambers for porewater subsampling
Feeding	Chambers were fed 1 mL Tetrafin® slurry: 0.60g/100mL days -1 and 7-9, 0.20g/100mL days 0-3, and 0.40g/100mL days 4-6
Negative control	Beach sand collected from San Diego, CA; sieved through a 0.5-mm screen and rinsed with deionized water and CFW; 0.5-1.0 tbsp DI soaked peat moss added to each chamber
Aeration	Continuous (2-3 bubbles per second)
Test Protocol	ASTM E1706-20 (ASTM 2020)
Test acceptability criteria	≥ 80 percent mean control survival
Reference toxicant	Ammonium chloride; water-only exposure

cm – centimeter
°C – degrees Celsius
g – gram
L – Liter
mg – milligram
mL – milliliter
mm – millimeter
tbsp – tablespoon

USEPA – United States Environmental Protection Agency

Table 3. Toxicity Test Methodology and QA/QC Requirements for the 28-day Solid Phase Amphipod Toxicity Test

Test organism	Freshwater amphipod - <i>Hyalella azteca</i>
Test organism source	Aquatic Research Organisms; Hampton, NH
Test organism age at initiation	9 days
Test duration; endpoints	28 days; survival and growth (as dry weight)
Test temperature	23 ± 1°C mean, 23 ± 3°C instantaneous limit
Photoperiod	16 hours light/8 hours dark
Test chamber	1-L glass jar
Sediment depth	2.0 cm
Overlying water volume	450 mL
Overlying water	CFW
Overlying water renewal	Twice daily full volume exchanges using Zumwalt apparatus
Overlying water quality monitoring	pH, temperature, conductivity, and dissolved oxygen (DO) daily; hardness and alkalinity at test initiation and termination; total ammonia and sulfides on Day 0 and Day 28 ^a .
Number of organisms/chamber	10
Number of replicates per sample	5, plus one surrogate test chamber for water quality readings and two surrogate chambers for porewater subsampling
Feeding	Chambers were fed 1 mL Yeast-Trout-chow-Cereal leaves (YTC) and 1 mL wheat grass slurry (0.025 g/100 mL week 1, 0.05g/100mL week 2, 0.10g/100mL week 3, and 0.15g/100mL week 4) daily
Negative control	Beach sand collected from San Diego, CA; sieved through a 0.5-mm screen and rinsed with deionized water and CFW
Aeration	Continuous (2-3 bubbles per second)
Test Protocol	ASTM E1706-20 (ASTM 2020)
Test acceptability criteria	≥ 80 percent mean survival; mean weight in controls should be ≥0.35 mg/organism
Reference toxicant	Copper chloride; water-only exposure

cm – centimeter

°C – degrees Celsius

g – gram

L – Liter

mg – milligram

mL – milliliter

USEPA – United States Environmental Protection Agency

^a Sulfides were not measured in the overlying on day 28

Statistical Analyses

One control was included in the test design for each species and was associated with the samples both spatially within the environmental chamber and for statistical analyses. Mean survival and growth in each sample were compared to the mean control survival and growth. Furthermore, the site sample was compared to the reference sample. Survival data, expressed as a proportion, were arcsine square-root transformed prior to analysis to normalize the distribution of the data and satisfy statistical assumptions for analysis. Statistical assumptions were evaluated prior to analysis using Brown-Forsythe test for differences in variance and D'Agostino Pearson Omnibus test for normality. Unpaired t-test comparisons were performed to identify significant differences between the lab control or reference sample and the site sample when the site sample's mean survival or growth was less than the reference sample or lab control. If parametric assumptions were not met, the data were initially tested with Kruskal-Wallis test, followed by the Mann-Whitney U-test.

Statistical analyses of test data were performed using GraphPad Prism, Version 6.05. Statistical analyses of reference toxicant data were performed using Comprehensive Environmental Toxicity Information System Software (CETIS™), Version 2.1.4.11. (Tidepool Scientific Software 2000-2022). Analysis for this test followed standard USEPA flow chart methods specified for this test type and the dose-responses were reviewed.

RESULTS AND DISCUSSION

Summaries of toxicity test results are provided in Tables 4 and 5. Detailed results are provided in Appendix C. Raw datasheets including water quality measurements, ammonia analyses, and sulfide analysis are provided in Appendix D. Summaries of statistical analyses are provided in Appendix E.

***Chironomus* Day 10 Survival Results**

Mean survival in the sediment laboratory control was 96 percent, exceeding the ASTM guideline survival criterion of 80 percent. Mean survival in the MVWA1122P001 sample was 94 percent (Figure 1 and Table 4). A Mann-Whitney test failed to detect a statistically significant difference between the MVWA1122P001 sample and the laboratory control or the reference site ($p=0.5000$, Appendix E). The reference site MVWA1122P002 resulted in 96 percent survival, which was equal to the laboratory control and, therefore, not significantly reduced (Appendix E). Survival in the sample was less than a 20 percent difference from both the control and the reference, which is below both the Sediment Cleanup Objective (SCO) of a greater than 20 percent difference and the Cleanup Screening Level (CSL) of a greater than 30 percent difference. The *Chironomus* test met all control and reference performance standards and was below any action levels for cleanup outlined in the Sediment Clean Up User Manual (SCUM).

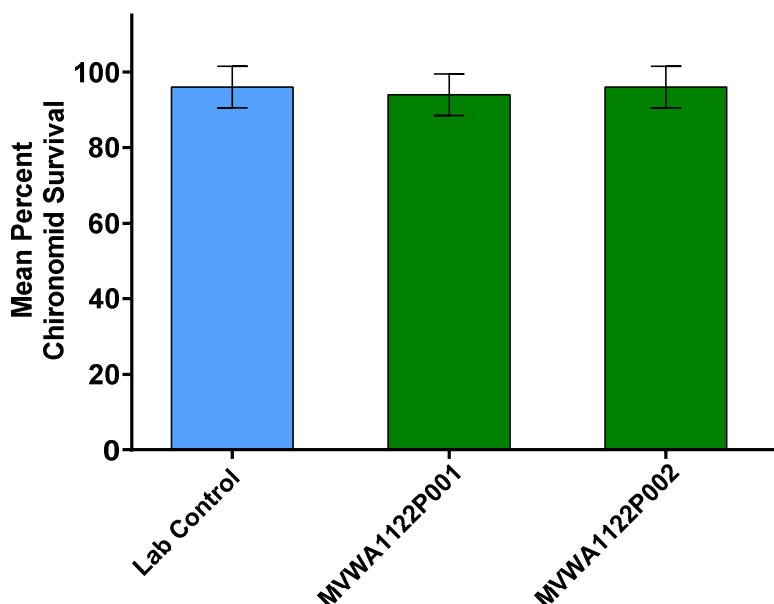


Figure 1. Summary of *Chironomus* 10-Day Survival (mean percent survival $\pm$ 95% S.D.; n=5).

Table 4. Summary of Test Results for *Chironomus* Survival

Sample ID	Mean Percent Survival	Mean Percent Mortality
Lab Control	96.0	4.0
MVWA1122P001	94.0	6.0
MVWA1122P002	96.0	4.0

***Hyalella* Day 28 Survival Results**

Mean survival in the sediment laboratory control was 100 percent, exceeding the ASTM guideline survival criterion of 80 percent. Mean survival in the MVWA1122P002 reference sample was 90 percent (Figure 2 and Table 5). An unpaired t-test with Welch's correction detected a statistically significant difference between the MVWA1122P002 reference sample and the laboratory control ($p=0.0431$, Appendix E). Mean survival in the MVWA1122P001 sample was 98.0 (Figure 2 and Table 5). An unpaired t-test failed to detect a statistically significant difference between the MVWA1122P001 sample and the laboratory control ($p=0.1869$, Appendix E). Survival in the MVWA1122P001 sample was greater than in the MVWA1122P002 reference sample, and therefore not significantly reduced (Appendix E). Survival in the sample was exactly 10 percent different from the control and less than 10 percent different from the reference, which is below both the Sediment Cleanup Objective (SCO) of a greater than 10 percent difference and the

Cleanup Screening Level (CSL) of a greater than 25 percent difference for amphipod survival. Survival in the amphipod test met all control and reference performance standards and was below any action levels for cleanup outlined in the Sediment Clean Up User Manual (SCUM).

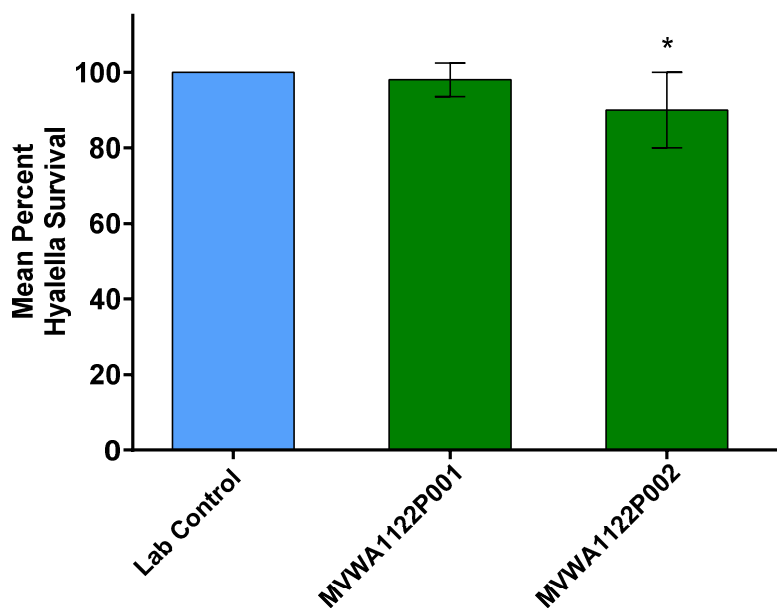


Figure 2. Summary of *Hyalella* 28-Day Survival (mean percent survival $\pm$ 95% S.D.; n=5).

*An asterisk indicates a statistically significant reduction in survival from the lab control.

***Hyalella* Day 28 Growth Results**

The mean weight per organism in the sediment laboratory control was 0.86-mg, exceeding the ASTM guideline growth criterion of ≥ 0.35 -mg per individual dry weight. Mean weight per organism in the MVWA1122P002 reference sample was 0.87-mg, which was greater than the laboratory control and therefore, not significantly reduced (Figure 3 and Table 5). Mean weight per organism in the MVWA1122P001 sample was 0.89-mg, which was greater than the laboratory control and the reference sample and, therefore, not significantly reduced (Figure 3 and Table 5). Growth in the sample was greater than both the control and reference, indicating no detrimental effects to amphipod growth. Ratios of sample and control/reference growth were above the SCO and CSL levels; note that action levels for the growth endpoint are expressed as less than <0.75 and <0.60 for the SCO and CSL, respectively, therefore exceeding these ratios indicates the sample was not impacted relative to the control or reference. Growth in the amphipod test met all control and reference performance standards and was below any action levels for cleanup outlined in the Sediment Clean Up User Manual (SCUM).

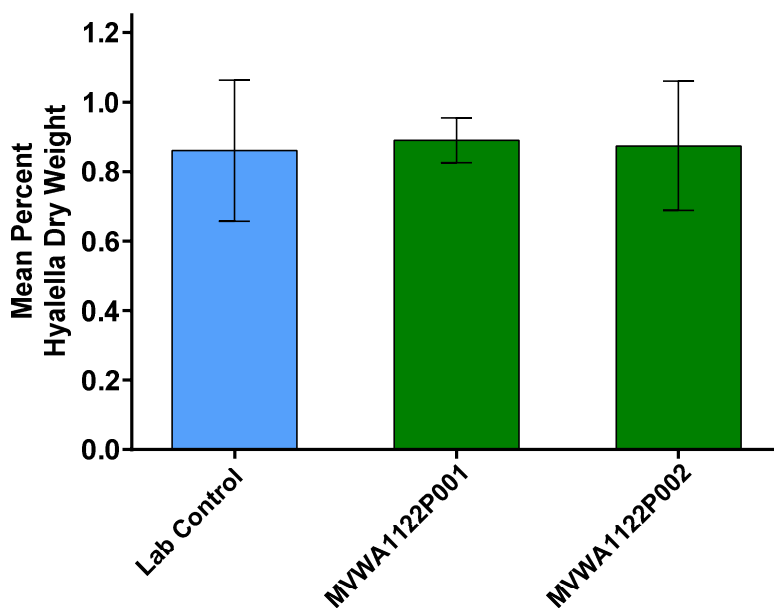


Figure 3. Summary of *Hyalella* 28-Day Growth (mean dry weight per surviving organism $\pm 95\%$ S.D.; n=5).

Table 5. Summary of Test Results for *Hyalella* Survival and Growth

Sample ID	Mean Percent Survival	Mean Percent Mortality	Mean Dry Weight per Organism (mg)
Lab Control	100	0.0	0.86
MVWA1122P001	98.0	2.0	0.89
MVWA1122P002	90.0	10	0.87

Values in **bold** indicate a statistically significant difference when compared to the laboratory control.

QUALITY ASSURANCE

All data presented have been thoroughly reviewed and deemed acceptable for reporting in accordance with relevant USEPA protocols and Nautilus's internal QA/QC program. Discussions and deviations with respect to test conditions are summarized below. Minor QA/QC issues that were unlikely to impact the final test data are noted on test datasheets, and a list of laboratory qualifier codes used on raw datasheets can be found in Appendix F.

Following the 2020 ASTM guidance, *Hyalella* for a 28-day (or 42-day) test the organism age should be between 7 and 8 days old. The test reported herein was initiated with 9-day-old *Hyalella*. The 2020 ASTM guidance indicates that the younger age range for the 42-day test, compared to the 10-day guidance of 7-10 day old, is suggested to avoid reproduction occurring prior to the 28-day endpoint. Reproduction is not an endpoint in this test, therefore it is unlikely that the age affected survival or growth results. Furthermore, the reference toxicant test that was run concurrently had typical results, indicating that slightly older organisms did not affect the sensitivity of the organisms.

It was the laboratories intent to collect ammonia and sulfides from the overlying water of the *Hyalella* test on day 10 in addition to days 0 and 28. Subsamples were not collected on day 10 of the test. Also, overlying sulfides were not collected on day 28 of the *Hyalella* test. Given the low ammonia values in other subsamples, it is not likely that an additional data point from day 10 would provide any value. In addition, there were no effects above the cleanup level standards and amphipod growth was higher in the sample than either the reference or control; this suggests that there were no impacts to the amphipod survival and growth due to ammonia or sulfides.

Minimal information is available on the effects of sulfide on these freshwater test organisms. Sulfide measurements in the porewater of the reference sample MVWA1122P002 were higher compared to sample MVWA1122P001 for both tests and at initiation and termination. It is possible that sulfide influenced the survival effects observed in the reference sample (P002).

All water quality parameters were within specified ranges for the duration of the tests. Overlying water total ammonia concentrations ranged from <0.5 to 1.5 milligrams per liter (mg/L). Interstitial water total ammonia concentrations ranged from <0.5 to 8.2 mg/L. All total ammonia values were well below any reported thresholds for *Chironomus* or *Hyalella* (USEPA 2000).

Reference Toxicant Test

Mean control survival in the concurrent reference toxicant test with *Chironomus* was 100 percent. A median lethal effect concentration (LC₅₀) value of 96.0 mg/L total ammonia was determined using the trimmed Spearman-Kärber method. Mean control survival in the concurrent reference toxicant test with *Hyalella* was 100 percent. A LC₅₀ value of 236 micrograms per liter (µg/L) copper was determined using the trimmed Spearman-Kärber method. Both LC₅₀ values were within plus or minus two standard deviations from the historical mean. This indicates that these batches of organisms were of typical sensitivity to ammonia and copper for this endpoint (96-hour survival) as those historically tested at

Nautilus. Based on the dose response observed, the calculated effect concentration was deemed reliable. A copy of the reference toxicant results is provided in Appendix G.

REFERENCES

- ASTM. 2020. Standard Test Method for Measuring the Toxicity of Sediment-Associated Contaminants with Freshwater Invertebrates. American Society for Testing and Materials, Philadelphia Pennsylvania. ASTM Method E1706-20.
- CTEH, 2024. Bioassay Sampling and Analysis Plan. Olympic Pipeline Gasoline Spill.
- GraphPad Software Inc. 1992-2014. GraphPad Prism, Version 6.05.
- State of Washington Department of Ecology, Sediment Cleanup User's Manual (SCUM), 2021. *Guidance for Implementing the Cleanup Provisions of the Sediment Management Standards, Chapter 173-204 WAC*, Publication No. 12-09-057. Third Revision December 2021
- Tidepool Scientific Software. 2000-2022. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 2.1.4.11.
- Zumwalt, D.C, Dwyer, F.J, Greer, I.E., and Ingersoll, C.G. 1994. A water-renewal system that accurately delivers small volumes of water to exposure chamber. Environ. Toxicol. Chem. 1311-1314.

Appendix A
Chain-of-Custody Forms

Enthalpy Analytical - Environmental Toxicology

4340 Vandever Avenue
San Diego, CA 92120
Phone 858.587.7333

Chain of Custody

Date 11/22/24 Page 1 of 1

Sample Collection By:							ANALYSES REQUIRED																																																																																																														
Report to: Company <u>OPL</u> Address _____ City/State/Zip _____ Contact <u>Paula Skryja</u> Phone <u>425 469-4043</u> Email <u>Paula.Skryja@bp.com</u>				Invoice To: Same as Report to ☐ Company <u>CTEH</u> Address _____ City/State/Zip _____ Contact _____ Phone _____ Email _____			<table border="1" style="width:100%; border-collapse: collapse;"> <tr> <td rowspan="10" style="width: 20px; text-align: center; vertical-align: middle;">Bioassay</td> <td colspan="8"></td> <td colspan="2" style="text-align: center;">Enthalpy Matrix Codes:</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">G = Grab</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">C = Composite</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">FW = Freshwater</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">SW = Seawater</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">Sed = Sediment</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">STRM = Stormwater</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">GW = Groundwater</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">WW = Wastewater</td> </tr> <tr> <td colspan="8"></td> <td colspan="2">O = Other (specify)</td> </tr> </table>										Bioassay									Enthalpy Matrix Codes:										G = Grab										C = Composite										FW = Freshwater										SW = Seawater										Sed = Sediment										STRM = Stormwater										GW = Groundwater										WW = Wastewater										O = Other (specify)	
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PROJECT INFORMATION		SAMPLE RECEIPT		1) RELINQUISHED BY (CLIENT)		2) RECEIVED BY (COURIER)	
Project Name:	MVWA 1122 Bioassay	Total No. of Containers	1	(Signature)	(Time)	(Signature)	(Time)
PO No.:		Received Good Condition?	Y	(Printed Name)	(Date)	(Printed Name)	(Date)
Shipped Via:	FEDEX	Matches Test Schedule?	Y	(Company)		(Company)	
SPECIAL INSTRUCTIONS/COMMENTS:				3) RELINQUISHED BY (COURIER)		4) RECEIVED BY (LABORATORY)	
				(Signature)	(Time)	(Signature)	(Time)
				(Printed Name)	(Date)	(Printed Name)	(Date)
				(Company)		(Company)	
						EA-JD	24-3302

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.
Shaded areas are for lab use only
Report turn-around-time varies depending on length of test; please inquire with your project manager.

<http://enthalpy.com/environmental-toxicology-2/>

Enthalpy Analytical - Environmental Toxicology

4340 Vandever Avenue
San Diego, CA 92120
Phone 858.587.7333

Chain of Custody

Date 11/22/24 Page 1 of 1

Sample Collection By:							ANALYSES REQUIRED										Receipt Temperature (°C)	
Report to:				Invoice To:			Enthalpy Matrix Codes:											
Company <u>OPL</u> Address _____ City/State/Zip _____ Contact <u>Paula Skryja</u> Phone <u>425.469.4043</u> Email <u>Paula.Skryja@hp.com</u>				Company _____ Address _____ City/State/Zip _____ Contact _____ Phone _____ Email _____			G = Grab <u>C</u> = Composite FW = Freshwater SW = Seawater <u>Sed</u> = Sediment STRM = Stormwater GW = Groundwater WW = Wastewater O = Other (specify) _____										X Bioassay	0.2
Same as Report to ☐ <u>CTEH</u>																		
SAMPLE ID	SAMPLE			MATRIX CODE	Container		COMMENTS											
	Date	Time	Type (G or C)	(FW, SW, Sed, STRM, GW, WW, O)	Type	Qty												
1 <u>MVWA 1122 P001</u>	<u>11/22</u>	<u>10:05</u>	<u>C</u>	<u>Sed</u>	<u>20L</u>	<u>1</u>	<u>NSAR ORIGINAL SOURCE AREA</u>											
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PROJECT INFORMATION		SAMPLE RECEIPT		1) RELINQUISHED BY (CLIENT)				2) RECEIVED BY (COURIER)										
Project Name: <u>MVWA 1122 Bioassay</u>		Total No. of Containers <u>1</u>		(Signature) <u>[Signature]</u> (Time) <u>09:50</u>				(Signature) _____ (Time) _____										
PO No.: _____		Received Good Condition? <u>Y</u>		(Printed Name) _____ (Date) _____				(Printed Name) _____ (Date) _____										
Shipped Via: <u>FedEx</u>		Matches Test Schedule? <u>Y</u>		(Company) _____				(Company) _____										
SPECIAL INSTRUCTIONS/COMMENTS:				3) RELINQUISHED BY (COURIER)				4) RECEIVED BY (LABORATORY)										
				(Signature) _____ (Time) _____				(Signature) <u>[Signature]</u> (Time) <u>0945</u>										
				(Printed Name) _____ (Date) _____				(Printed Name) <u>Autumn Dawson</u> (Date) <u>11/26/24</u>										
				(Company) _____				(Company) <u>EA-JD</u> (Log-In #s) <u>24-3301</u>										

Additional costs may be required for sample disposal or storage. Payment net 30 unless otherwise contracted.
Shaded areas are for lab use only
Report turn-around-time varies depending on length of test; please inquire with your project manager.

<http://enthalpy.com/environmental-toxicology-2/>

Appendix B
Sample Receipt Information

Client: CTEH

Test Type(s): 28-day Hyalella Survival + Growth + 10-day Chironomid Survival

Project: MVWA 1122 Bioassay

Test IDs: 2412-S102 to S105

Enthalpy Log-in 24-xxxx	Sample ID	Collection Date & Time	Receipt Date & Time	Receipt Temp. (°C)	No. Containers	Container Type	Approx. Total Volume Received (L)	Sample Description	Tech Initials	QC Initials
3301	MVWA1122 P001	11/22/24 1005	11/26/24 0945	6.2	1	plastic bag	~12	high moisture, dark brown	AD	HY
3302	MVWA1122 P002	11/22/24 1130	11/26/24 0945	6.5	1	plastic bag	~15	high moisture, dark brown	AD	HY

Samples Shipped Via: FedEx

COC Present? ☒ Y ☐ N

Sieving Required? ☒ Y ☐ N Screen Size: 0.5mm

Sub-samples for additional chemistry:

Collect and Preserve Initial Porewater

Other _____

Other _____

Tech Initials AD ALS 060 KL

Tech Initials _____

Tech Initials _____

Comments: Q18WF 12/19/24

QC Check: ALS 2/27/25

Final Review: 2/3/25

Sediment Sample
Sieving Worksheet
DS-047

Client: CTEH

Sieve by Date: 12/13/2024

Project: MVWA 1122 Bioassay

Target Sieve Size: 0.5-mm

Test ID(s): 2412-S102 to S105

Minimum Sieved Volume: 6-L

Test Initiation Date(s): 12/19/24 ; 12/20/24

Test Type(s): 28d Hyalella ; 10d chiron

Sample ID	Enthalpy Log-in	Sample Description	Organisms Present Yes/No ¹	Final Sieve Size Used	Sieved by Date/Initials	QC Initials ²
MVWA1122P001	24-3301	Dark brown, earthy odor, liquidy	Yes; 3 clams, 9 crabs	0.5mm	JR 12/13/24	KL
MVWA1122P002	24-3302	Swampy odor, plant material, liquidy	Yes; 1 fish, 1 worm	0.5mm	K 12/13/24	GM

¹ Include quantity and general description

² 2nd person cross checks that the original sample container label matches the bag of sieved sample

Comments: _____

QC Check: AS 3/2/25

Final Review: VS 3/3/25

Control Sediment Datasheet

DS-054

Client: CTEH

Project: MVWA 1122 Bioassay

Test ID(s): 2412-S102 & S103 & S104 & S105

Test Initiation Date(s): 12/19/24 and 12/20/24

Sieve by Date: 12/18/2024

Target Sieve Size: 0.5-mm

Minimum Sieved Volume: 6-L

Control Sediment Information

Sample Description	Organisms Present (Y/N) ¹	Final Sieve Size Used	Sieved by (Initials/Date)	QC (initials)
Fine brown sand	N	0.5mm	AD 12/18/24	KL

¹ Include quantity and general description

Control Sediment Use & Preparation

Control Sediment Type	Test Type	Enthalpy Log-in #	Rinse Water Type	Rinsed by (Initials/Date)	QC (initials)
SS	28d Hya & 10d Chiro	24-70B	D1/CFW	AD 12/18/24	KL

Control Sediment Type: SS - Scripps Sand Eoh - Eohaustorius Home Sediment MBS - Marine Bioaccum. Sediment MS - Marine Sediment

Comments: _____

QC Check: ACS 3/2/25

Final Review: 3/3/25

DC-001

Client: CTEH

Project: MVWA 1122 Bioassay

Test Type: 28-day *Hyalella* and 10-day *Chironomus*

DI Blank: 0.0

Test Start Date: 12/19/24 & 12/20/24

Analyst: *KL*

Analysis Date: 12/16/24

N x 1.22

16.5

$$\text{Relative Percent Difference (RPD)} = \frac{[\text{sample}] (\text{mg/L}) - [\text{sample duplicate}] (\text{mg/L})}{[\text{average ammonia}] (\text{mg/L})} \times 100$$

Acceptable Range: 0-20%

$$\text{Percent Recovery} = \frac{[\text{spiked sample}] \text{ (mg/L)} - [\text{sample}] \text{ (mg/L)}}{\text{nominal [spike] (mg/L)}} \times 100$$

Acceptable Range: 80-120%^b

16.5

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	7395266	2395466	211945

Comments: (A) 9/12/16/24 (B) 5/11/27/28

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^b Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^c Calculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: AS 2/27/25

Final Review: VS 3/3/25

Pore Water

DI Blank:

Analyst: KL
Analysis Date: 12/16/24

Comments: (A) 12/16/24 (B) Pore water samples collected on 12/16/24.

Method Detection Limit = 5.0 µg/L S²⁻

Final Review: 1/3/25

Nautilus Environmental, Inc. 4340 Vandever Avenue. San Diego, CA 92120.

Appendix C
Summary of Results Tables

CTEH
10-Day *Chironomus* Survival Bioassay
Project: MVWA 1122 Bioassay
Sample Date: 11/22/2024
Test Initiation Date: 12/20/2024

Site ID	Replicate	Random No.	No. Larvae Alive	Total No. Alive ^a	Percent Survival	Mean Percent Survival	Standard Deviation	Percent Mortality	Mean Percent Mortality	Standard Deviation
Lab Control	A	23	10	10	100	96.0	5.5	0.0	4.0	5.5
	B	29	9	9	90.0			10.0		
	C	28	10	10	100			0.0		
	D	21	9	9	90.0			10.0		
	E	17	9	10	100			0.0		
MVWA1122P001	A	30	9	9	90.0	94.0	5.5	10.0	6.0	5.5
	B	20	9	9	90.0			10.0		
	C	24	9	9	90.0			10.0		
	D	26	10	10	100			0.0		
	E	22	10	10	100			0.0		
MVWA1122P002	A	27	10	10	100	96.0	5.5	0.0	4.0	5.5
	B	19	10	10	100			0.0		
	C	25	9	9	90.0			10.0		
	D	18	9	10	100			0.0		
	E	16	9	9	90.0			10.0		

^a Total number alive includes organisms that are pupae or emerged as flies and are used in the percent survival calculation

CTEH
 28-Day *Hyalella* Survival & Growth Bioassay
 Project: MVWA 1122 Bioassay
 Sample Date: 11/22/2024
 Test Initiation Date: 12/19/2024

Site ID	Replicate	Random No.	No. Alive	Percent Survival	Mean Percent Survival	Standard Deviation	Percent Mortality	Mean Percent Mortality	Standard Deviation	Total Dry Weight (mg)	Dry Weight per Org. (mg)	Mean Dry Weight per Org. (mg)	Standard Deviation
Lab Control	A	9	10	100	100	0.0	0.0	0.0	0.0	8.11	0.81	0.86	0.20
	B	6	10	100			0.0			8.20	0.82		
	C	7	10	100			0.0			12.08	1.21		
	D	10	10	100			0.0			7.80	0.78		
	E	5	10	100			0.0			6.78	0.68		
MVWA1122P001	A	4	10	100	98.0	4.5	0.0	2.0	4.5	8.71	0.87	0.89	0.06
	B	15	10	100			0.0			7.86	0.79		
	C	14	9	90.0			10.0			8.66	0.96		
	D	11	10	100			0.0			9.13	0.91		
	E	8	10	100			0.0			9.15	0.92		
MVWA1122P002	A	12	10	100	90.0*	10	0.0	10	10	6.83	0.68	0.87	0.19
	B	1	10	100			0.0			9.23	0.92		
	C	13	8	80.0			20.0			9.38	1.17		
	D	2	8	80.0			20.0			6.37	0.80		
	E	3	9	90.0			10.0			7.19	0.80		

Values in **bold** with an asterisk were found to be statistically significant when compared to the Lab Control

Appendix D
Raw Datasheets

Organism Survival and Growth

Client: CTEH

Test Species: Chironomus dilutus

Project ID: MVWA 1122 Bioassay

Start Date/Time: 12/20/2024

Test No(s).: 2412-8104 and 8105

End Date/Time: 12/30/29

Initial No. Organisms: 10/rep

Random Number	Number Alive	10% QC Check of final counts
16	9	
17	10 (1)	
18	10 (1)	
19	10	10
20	9	
21	9	
22	10	
23	10	
24	9	
25	9	9
26	10	
27	10	
28	10	
29	9	
30	9	9
Tech Initials:	AES / AD	AD AES

Initiation (initials):

T ₀ Weights:	N/A
Counts:	KL / HH OBO KS
Counts QC:	HH OBO KS / KL
Air:	KL

Test Initiated By:	KL
Initiation Check:	HH
Lights (16:8):	KL

T₀ overlying water subsamples: Ammonia: WF Alkalinity & Hardness: WF

Termination (initials):

T₁ overlying water subsamples: Ammonia: ATS Alkalinity & Hardness: ATS

Animal Source/Date Received: ABS 12/18/24 Age at Initiation: 10 days

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / none

Comments: Note: Record # of pupae in parentheses

and exclude from wt. analysis Q18175 2/27/25

Drying Oven Info

Date/Time in:

Date/Time out:

Temp (°C):

QC Check: AS 2/27/25

Final Review:

DS-031C

Daily Observations

Client: CTEH

Test Species: *Chironomus dilutus*

Project ID: MVWA 1122 Bioassay

Start Date/Time: 12/20/2024 1235

Test No.: 2412-5104 and 5105

End Date/Time: 12/30/24 1045

Random Number	Daily Observations (Use Codes Provided)									
	1	2	3	4	5	6	7	8	9	10
16	N S _i	E N S _i	S _i	S _i	S _i	N	N	N	N	N
17 (H)	S _i , N	N	N	N	N	N	N	N	N	N
18	N	E	N	N	N	N	N	N	N	N
19	E	E	N	N	N	N	N	N	N	N
20	E	E	E	N	N	N	N	N	N	N
21	E	E	N	N	N	N	N	N	N	N
22	N	E	N	F	E	N	N	N	N	N
23	E	E	N	N	N	N	N	N	N	N
24	N	N	N	N	N	N	N	N	N	N
25	N	E	N	E	E	N	N	N	N	N
26	F	E	N	N	N	N	N	N	N	N
27	N	N	N	N	N	N	N	N	N	N
28	N	N	N	N	N	N	N	N	N	N
29	N	E N	N	N	N	N	N	N	N	N
30	E	E N E	N	N	N	N	N	N	N	N
Tech	Pd VBO LM	(H)	WF	AD	LM	WE	WF	Gm	AH	AcS

Observations Key:

A = No/low aeration B = Body or molt on sediment surface, specify number C = Cloudy
E = Excess food on sediment surface G = Abnormal growth on or discoloration of sediment surface
N = Normal P = Developed pupae observed S = Organism trapped on surface, specify number

QC Check: AS 2/27/25

Final Review:

(H) Q18LM 12/24/24
(F) Q18AD 12/22/24

CTEH
 10-Day Chironomus Survival Bioassay
 Project: MVWA 1122 Bioassay
 Test Initiation Date: 12/19/2024

Q18 vs 1/27/25
 20

Site	Rep	Rand #
Lab Control	A	23
	B	29
	C	28
	D	21
	E	17
MVWA1122P001	A	30
	B	20
	C	24
	D	26
	E	22
MVWA1122P002	A	27
	B	19
	C	25
	D	18
	E	16

QC: HH

10-Day Freshwater Sediment Bioassay
Static-Renewal Conditions

DS-030S

Water Quality Measurements

Client: CTEH

Project ID: MVWA 1122 Bioassay

Test No(s): 2412-5104 and 5105

Sample ID: Lab Control

Test Species: ~~Hyalomma azteca~~ Chironomus dilutus

Start Date/Time: 12/20/2024 1235

End Date/Time: 12/30/24 1045

Log-in No.: 24-7013

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Alkalinity/Hardness (mg/L)	Technician Initials	Comments
0	8.05	8.4	870	22.3	WF	111 / 216	FM	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)
1	8.02	8.2	942	22.4	LM			
2	8.19	8.0	861	22.5	AD			
3	8.13	8.4	851	22.5	WF			
4	8.14	8.0	883	22.6	AD			
5	8.06	8.0	847	22.4	LM			
6	7.71	7.2 7.0	866	22.9	WF			
7	8.31	8.1	885	23.1	WF			
8	8.36	8.2	860	22.8	GM			
9	8.30	8.2	892	22.8	AD			
10	8.28	8.2	895	22.7	GM	124 / 234	AD	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)

Ⓜ Q1B FM 12/21/24

Environmental Chamber: F

QC Check: ALS 2/27/25 Ⓜ Q1B WF 12/26/24

Final Review: KS 3/3/25

10-Day Freshwater Sediment Bioassay
Static-Renewal Conditions
 DS-030S

Water Quality Measurements

Client: CTEH

Project ID: MVWA 1122 Bioassay

Test No(s): 2412-5104

Sample ID: MVWA1122P001

Test Species: ~~Hyalomma azteca~~ Chironomus dilutus

Start Date/Time: 12/20/2024 1235

End Date/Time: 12/30/24 1045

Log-in No.: 24-3301

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Alkalinity/Hardness (mg/L)	Technician Initials	Comments
0	7.97	8.3	837	22.3	WF	127 / 236	FM	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)
1	8.02	8.2	881	22.8	LM			
2	8.03	8.0	804	22.6	AD			
3	8.05	8.3	812	22.5	WF			
4	8.09	7.9	844	22.6	AD			
5	8.10	8.0	853	22.2	LM			
6	7.26	5.0	818	22.9	WF			
7	7.80	7.2	848	23.1	WF			
8	7.30	4.4	826	22.9	GM			
9	8.06	7.9	855	22.8	AD			
10	8.12	8.0	873	22.8	GM	114 / 228	AD	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)

Ⓢ Q18 FM 12/21/24 Ⓢ Airline failure in surrogate Replent DOs range from 6.8-7.5 mg/L

Environmental Chamber: F

QC Check: ACS 2/27/25

Final Review: KS 3/3/25

Ⓢ Q18 FM 12/21/24

10-Day Freshwater Sediment Bioassay
Static-Renewal Conditions
 DS-030S

Water Quality Measurements

Client: CTEH

Project ID: MVWA 1122 Bioassay

Test No(s): 2412-5105

Sample ID: MVWA1122P002

Test Species: ~~Hyalomma azteca~~ Chironomus dilutus

Start Date/Time: 12/20/2024 1235

End Date/Time: 12/30/24 1045

Log-in No.: 24-3302

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Alkalinity/Hardness (mg/L)	Technician Initials	Comments
0	7.71	7.9	861	22.3	WF	119 / 229	FM	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)
1	7.77	8.0	803	22.6	LM			
2	7.89	7.8	817	22.2	AD			
3	7.81	7.8	812	22.5	WF			
4	7.81	7.6	834	22.6	AD			
5	7.80	7.9	841	22.6	LM			
6	7.22	5.2	815	22.8	WF			
7	7.97	7.7	828	22.9	WF			
8	7.86	7.5	800	22.8	GM			
9	7.97	7.7	850	22.8	AD			
10	7.95	7.7	852	22.8	GM	110 / 223	AD	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)

Ⓢ Q18 FM 12/21/24

Environmental Chamber: F

QC Check: AS 2/27/25

Ⓢ Q18 LM 12/21/24

Final Review: KS 3/3/25

10-Day Freshwater Sediment Bioassay

Static-Renewal Conditions

DS-030

RENEWAL Water Quality Measurements

Client: CTEH

Test Species: *Chironomus dilutus*

Project ID: MVWA 1122 Bioassay

Start Date/Time: 12/20/2024 1235

Test No(s): 2412-5104 and 5105

End Date/Time: 12/30/24 1045

Renewal Water Type: Carbon Filtered Water (CFW)

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Water Change Time		Feeding Time ^a	Comments
						AM	PM		
-1	8.33	8.8	823	22.0	HH		1740	1820	
0	8.35	8.2	968	24.0	LM	0850	1640	1710	
1	8.39	8.4	938	22.8	LM	0825	1510	1558	
2	8.42	8.7	849	22.1	AD	0915	1500	1535	
3	8.34	8.7	840	22.5	WF	0910	1600	1625	
4	8.36	8.3	872	22.8	AD	0850	1425	1445	
5	8.34	8.0	880	24.0	LM	0620	1400	1415	
6	8.37	8.4	842	23.8	WF	1000	1600	1630	
7	8.27	8.1	875	24.0	WF	0820	1540	1635	
8	8.41	8.1	848	24.0	GM	0800	1400	1420	
9	8.45	8.3	885	23.8	AD	0935	1535	1550	
10									

^a Feeding time applies to the test chambers for the entire test.

Environmental Chamber: F

QC Check: ACS 2/27/25

Final Review: JCS 3/3/25

Organism Survival and Growth

Client: CTEH

Test Species: *Hyalella azteca*

Project ID: MVWA 1122 Bioassay

Start Date/Time: 12/19/2024

Test No(s).: 2412-S102 and S103

End Date/Time: 1/16/25 1245

Initial No. Organisms: 10/rep

Random Number	Number Alive	10% QC Check of final counts
1	10	
2	8	8
3	9	
4	10	
5	10	
6	10	
7	10	10
8	10	
9	10	
10	10	
11	10	
12	10	
13	8	
14	9	
15	10	
Tech Initials:	YS / DT A + B DT	

Random Number	Number Alive	10% QC Check of final counts
Tech Initials:		

Initiation (initials):

T ₀ Weights:	WF
Counts:	WF
Counts QC:	KL
Air:	MM

Test Initiated By:	WF
Initiation Check:	HH
Lights (16:8):	HH

T₀ overlying water subsamples: Ammonia: WF Alkalinity & Hardness: WF

Termination (initials):

T₁ overlying water subsamples: Ammonia: WF Alkalinity & Hardness: WF

Animal Source/Date Received: ABO/12/18/24 ^{42c} Size at Initiation: qd

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / none

Comments: (2) Q18 H/L 2/2/25

Drying Oven Info

Date/Time in: 7/16/25 1356

Date/Time out: 1/17/25 1430

Temp (°C): 66.2

QC Check: ATS 3/2/25

Final Review: ✓ 3/3/28

DS-046

Project ID: MVWA 1122 Bioassay

Test No.: 2412-5102 and 5103

Test Species: *Hyalella azteca*

Start Date/Time: 12/19/25 1615

End Date/Time: 1/16/25 1245

[illegible]

Tech Initials:	FM	FM
Date/Time:	1/16/2025 1150	1/17/25 1430

QC Check: ACS 3/2/25
Nautilus Environmental, Inc. 4340 V

Final Review: *yes 3/3/25*

CTEH
28-Day Hyaella Survival & Growth Bioassay
Project: MVWA 1122 Bioassay
Test Initiation Date: 12/18/2024

Site	Rep	Rand #
Lab Control	A	9
	B	6
	C	7
	D	10
	E	5
MVWA1122P001	A	4
	B	15
	C	14
	D	11
	E	8
MVWA1122P002	A	12
	B	1
	C	13
	D	2
	E	3

QC: A)

Ⓐ Q18K 12/18/24

28-Day Freshwater Sediment Bioassay
Static-Renewal Conditions
DS-023

RENEWAL Water Quality Measurements

Client: CTEH

Project ID: MWWA 1122 Bioassay

Test No(s): 1412-5102 and 5103

Renewal Water Type: Carbon Filtered Water

Test Species: *Hyalella azteca*

Start Date/Time: 12/19/2024 1615

End Date/Time: 1/16/25 1245

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Water Change Time		Feeding Time ^a	Comments
						AM	PM		
-1	8.30	8.8	923	20.8 ^b	AD		1030		
0	8.33	8.8	823	22.0	WF	1150	1740	1820	
1	8.35	8.2	964	24.0	LM	0825	1030	1710	
2	8.39	8.4	938	22.8	LM	0820	1510	1555	
3	8.42	8.7	849	22.1	AD	0915	1500	1535	
4	8.34	8.7	840	22.5	WF	0910	1600	1625	
5	8.36	8.3	872	22.8	AD	0850	1425	1445	
6	8.34	8.0	880	24.0	LM	0620	1400	1415	
7	8.37	8.4	842	23.8	WF	1000	1600	1630	
8	8.27	8.1	875	24.0	WF	0820	1540	1635	
9	8.41	8.1	848	24.0	GM	0800	1400	1420	
10	8.45	8.3	885	23.8	AD	0935	1535	1550	
11	8.45	8.4	884	23.4	GM	0815	1515	1620	
12	8.34	8.2	863	23.5	GM	0825	1430	1445	
13	8.42	8.4	868	23.6	AD	0740	1455	1530	
14	8.42	8.3	854	23.6	WF	0815	1720	1735	
15	8.37	8.7	882	22.6	WF	0745	1600	1625	
16	8.44	8.3	862	23.5	LM	0715	1430	1435	
17	8.47	8.1	835	23.7	AD	0855	1510	1520	
18	8.12	8.2	858	24.0	AD	0815	1555	1630	
19	8.30	8.4	864	23.8	AD	0910	1640	1656	
20	8.45	8.7	831	23.7	WF	0815	1455	1540	
21	8.40	8.6	862	23.4	WF	0815	1610	1650	
22	8.45	8.1	862	23.3	WF	0820	1500	1510	
23	8.45	8.3	927	23.2	WF	0845	1455	1505	
24	8.49	8.3	864	23.1	AD	0900	1500	1510	
25	8.41	8.4	878	22.9	SM	0905	1715	1725	
26	8.39	8.4	848	22.9	AD	0910	1555	1605	
27	8.38	8.3	887	22.8	WF	0910	1605	1615	
28									

^a Feeding time applies to the test chambers for the entire test.

^b No organisms exposed to low temperature on day -1 #s 3/2/25

QC Check: AS 3/2/25

Environmental Chamber: F

Final Review: VS 3/3/25

28-Day Freshwater Sediment Bioassay

DS-024

Daily Observations

Client: CTEH

Test Species: *Hyalella azteca*

Project ID: MVWA 1122 Bioassay

Start Date/Time: 12/19/2024 1615

Test No.: 2412-5102 and 5103

End Date/Time: 1/16/25 1245

Random Number	Daily Observations (Use Codes Provided)																												
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	
1	N	N	N	N	N	N	S ₂	N	N	N	N	N B ₂	B ₂	B ₃	B ₃	B ₅	B ₄	B ₆	B ₆	B ₃	B ₂	B ₁	B ₃	B ₆	B ₃	B ₃	B ₂	N	
2	N	N	N	N	N	N	N	N	N	N	N	N	B ₁	B ₂	B ₁	B ₁	B ₂	B ₂	B ₁	B ₂	B ₂	B ₂	B ₁	B ₃	B ₅ S ₁	B ₂	B ₁	N	
3	N	N	N	N	N	N	N	N	N	N	N	N B ₆	B ₃	B ₃	B ₄	B ₄	B ₄	B ₇	B ₉	B ₃	B ₈	B ₅	B ₃	B ₄	B ₄ S ₁	B ₃	B ₂	N	
4	N	N	N	N	N	N	N	N	N	N	N	N	B ₁	B ₁	B ₁	B ₁	B ₁	B ₃	S ₄ B ₁	B ₁	B ₄ S ₂	B ₃	B ₃	B ₁ S ₁	B ₂	B ₃	B ₃	N	
5	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
6	N	N	N	N	N	N	S ₁	N	N	N	N	N	N	N	N	N	N	B ₁	B₁ N	N	N	N	N	S ₁	B ₁	B ₁	N	N	N
7	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
8	N	N	N	N	N	N	S ₁	N	S ₁	N	N	N	N	B ₂	N	N	N	N	N	N	N	N	N	N	N	B ₂	N	B ₁	N
9	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
10	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N	N
11	N	N	N	S ₁	N	N	N	N	N	N	N	N B ₃	B ₄	B ₅	B ₃	B ₁	B ₁	B ₃	N	N	N	N	N	S ₁	B ₁	B ₁	S ₁	N	
12	N	N	N	N	^{PO} _{B₁}	^{PO} _{B₁}	^{PO} _{N₂}	N	N	^{PO} _{B₁}	^{PO} _{B₂}	^{PO} _{B₃}	^{PO} _{B₄}	^{PO} _{B₅}	B ₄	B ₃	B ₂	^{PO} _{B₁}	^{PO} _{B₂}	^{PO} _{B₃}	^{PO} _{B₄}	^{PO} _{B₅}	^{PO} _{B₆}	^{PO} _{B₁}	^{PO} _{B₂}	^{PO} _{B₃}	^{PO} _{B₄}	N	
13	N	N	N	N	N	N	N	N	N	N	N	N	B ₄	B ₄	B ₂	B ₂	B ₈	B ₈	B ₉	B ₅	B ₅	B ₁	B ₅	B ₄	B ₄	B ₅	B ₂	N	
14	N	N	N	N	N	N	N	N	N	N	N	N	N	B ₃	B ₂	B ₂	B ₁	B ₂	B ₂	B ₄	B ₃	B ₃	B ₂	B ₁	B ₁	B ₁	B ₁	N	
15	N	N	N	N	N	N	N	B ₁	B ₁	N	N	N	B ₂	B ₁	B ₃	B ₃	B ₁	B ₁	B ₁	B ₁	B ₂	B ₁	B ₂	N	N	N	N	N	
Tech Initials:	LM	LM	AD	WF	AD	LM	WF	WF	GM	AD	GM	GM	AD	WF	WF	LM	AD	AD	AD	WF	WF	WF	WF	AD	SM	AD	WF	VS	

Observations Key: A = No/low aeration B = Body or molt on sediment surface, specify number C = Cloudy
 G = Abnormal growth on or discoloration of sediment surface N = Normal
 S = Organism trapped on surface, specify number

QC Check: AS 3/2/25

Final Review: VS 3/3/25

Client: CTEH
Project ID: MVWA 1122 Bioassay
Test No(s): ~~1124~~ S102 and S103
Sample ID: Lab Control

Test Species: *Hyalella azteca*
Start Date/Time: 12/19/2024 1615
End Date/Time: 1/16/25 1245
Log-in No.: 24-7013

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Alkalinity/Hardness (mg/L)	Technician Initials	Comments
0	8.22	8.9	980	22.0	WF	128 / 237	FM	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)
1	8.26	8.5	949	20.3	LM			
2	8.30	8.2	938	22.6	LM			
3	8.37	8.4	853	22.6	AD			
4	8.29	8.5	841	22.5	WF			
5	8.29	8.2	874	22.7	AD			
6	8.33	8.1	880	22.5	LM			
7	8.21	8.0	866	22.9	WF			
8	8.26	8.2	874	23.1	WF			
9	8.36	8.3	849	22.8	GM			
10	8.42	8.2	881	22.9	AD			
11	8.40	8.4	885	22.8	GM			
12	8.35	8.3	864	22.9	GM			
13	8.34	8.5	868	22.9	AD			
14	8.33	8.3	855	22.8	WF			
15	8.31	8.6	882	22.6	WF			
16	8.36	8.4	863	22.7	LM			
17	8.41	8.2	836	22.8	AD			
18	8.35	8.7	850	22.8	AD			
19	8.33	8.4	863	22.7	AD			
20	8.37	8.1	832	22.6	WF			
21	8.36	8.5	864	22.6	WF			
22	8.37	8.1	864	22.6	WF			
23	8.32	8.3	928	22.6	WF			
24	8.37	8.3	862	22.6	AD			
25	8.26	8.3	882	22.6	SM			
26	8.22	8.2	850	22.6	AD			
27	8.18	8.1	891	22.5	WF			
28	8.24	8.5	991	22.2	HH	162 / 285	DT	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)

Environmental Chamber: F

QC Check: ACS 3/2/25

81x2
0208 4/21/25

Final Review: VS 3/3/25

28-Day Freshwater Sediment Bioassay
Static-Renewal Conditions
DS-023S

Water Quality Measurements

Client: CTEH
Project ID: MVWA 1122 Bioassay
Test No(s): 342, 5102
Sample ID: MVWA1122P001

Test Species: *Hyalella azteca*
Start Date/Time: 12/19/2024 1615
End Date/Time: 1/16/25 1245
Log-in No.: 24-3301

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Alkalinity/Hardness (mg/L)	Technician Initials	Comments
0	7.85	8.6	960	22.3	WF	118 / 229	WF 560 FM	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)
1	7.88	8.5	919	20.4 R	LM			
2	7.88	8.1	911	22.6	LM			
3	7.91	8.0	829	22.7	AD			
4	7.93	8.3	822	22.6	WF			
5	7.95	8.0	855	22.7	AD			
6	8.05	8.0	841	22.5	LM			
7	7.86	7.06.80	8668/8	22.9	WF			
8	8.20	7.8	876	23.1	WF			
9	8.38	8.0	855	23.0	GM			
10	8.48	8.1	898	22.9	AD			
11	8.50	8.2	908	22.8	GM			
12	8.46	8.2	892	22.8	GM			
13	8.45	8.5	907	22.8	AD			
14	8.40	8.4	883	22.8	WF			
15	8.35	8.5	904	22.7	WF			
16	8.34	8.3	878	22.7	LM			
17	8.32	8.1	848	22.8	AD			
18	8.27	8.6	860	22.7	AD			
19	8.11	8.1	870	22.7	AD			
20	8.17	8.4	835	22.7	WF			
21	8.19	8.2	865	22.6	WF			
22	8.18	7.9	863	22.6	WF			
23	8.12	8.1	926	22.7	WF			
24	8.12	8.1	856	22.7	AD			
25	8.03	8.0	872	22.6	SM			
26	7.98	8.0	841	22.6	AD			
27	7.93	7.8	879	22.6	WF			
28	8.04	8.4	976	22.5	HM	144 / 294	DT	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)

Environmental Chamber: F

QC Check: 8/6 3/2/25

QA18 WF 12/26/24

Final Review:

VS 3/3/25

28-Day Freshwater Sediment Bioassay
Static-Renewal Conditions
DS-023S

Water Quality Measurements

Client: CTEH
Project ID: MVWA 1122 Bioassay
Test No(s): 242-5103
Sample ID: MVWA1122P002

Test Species: *Hyalella azteca*
Start Date/Time: 12/19/2024 1615
End Date/Time: 1/16/25 1245
Log-in No.: 24-3302

Test Day	pH (units)	Dissolved Oxygen (mg/L)	Conductivity (µmhos/cm)	Temperature (°C)	Technician Initials	Alkalinity/Hardness (mg/L)	Technician Initials	Comments
0	8.20	8.9	981	22.4	WF	136 / 239	WF	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)
1	7.63	8.0	954	20.7	LM			
2	7.87	8.0	925	22.5	LM			
3	7.90	7.9	830	22.7	AD			
4	7.85	8.2	819	22.5	WF			
5	7.87	7.8	850	22.7	AD			
6	7.87	7.8	861	22.7	LM			
7	7.22	6.7	825	23.8	WF			
8	8.18	7.9	855	23.1	WF			
9	8.23	8.0	831	22.9	GM			
10	8.39	7.9	872	22.9	AD			
11	8.54	8.1	892	22.8	GM			
12	8.66	8.0	890	22.9	GM			
13	8.73	8.3	902	22.8	AD			
14	8.72	8.1	894	22.8	WF			
15	8.57	8.2	920	22.7	WF			
16	8.53	8.0	903	22.6	LM			
17	8.43	7.9	873	22.8	AD			
18	8.31	8.5	882	22.8	AD			
19	8.24	8.1	886	22.7	AD			
20	8.22	8.4	853	22.7	WF			
21	8.18	8.4	882	22.6	WF			
22	8.17	7.9	875	22.6	WF			
23	8.14	8.0	938	22.7	WF			
24	8.11	8.0	862	22.7	AD			
25	8.03	8.0	875	22.6	SM			
26	7.96	7.9	842	22.5	AD			
27	7.93	7.7	879	22.6	WF			
28	7.98	8.3	973	22.5	HM	144 / 282	DT	Note: Subsample for ammonia, alkalinity, & hardness (initial on count sheet)

Environmental Chamber: F

QC Check: 1/15/25

Final Review: 1/13/25

DC-001

Client: CTEH
Project: MVWA 1122 Bioassay
Test Type: 28-day *Hyalella*

DI Blank: 0.0
Test Start Date: 12/19/2024

Analyst: SM
Analysis Date: 3/3/25

N x 1.22

$$\text{Relative Percent Difference (RPD)} = \frac{[\text{sample}] (\text{mg/L}) - [\text{sample duplicate}] (\text{mg/L})}{[\text{average ammonia}] (\text{mg/L})} \times 100$$
$$\text{Percent Recovery} = \frac{[\text{spiked sample}] (\text{mg/L}) - [\text{sample}] (\text{mg/L})}{\text{nominal spike} (\text{mg/L})} \times 100$$

Comments:

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^b Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^c Calculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: ATS 3/3/25

Final Review: 4/3/25

DC-001

Client: CTEH
Project: MVWA 1122 Bioassay
Test Type: 28-day *Hyaella*

DI Blank: 0.0
Test Start Date: 12/19/2024

Analyst: SM
Analysis Date: 3/3/25

N x 1.22

$$\text{Relative Percent Difference (RPD)} = \frac{[\text{sample}] \text{ (mg/L)} - [\text{sample duplicate}] \text{ (mg/L)}}{[\text{average ammonia}] \text{ (mg/L)}} \times 100$$

Acceptable Range: 0-20%

$$\text{Percent Recovery} = \frac{[\text{spiked sample}] \text{ (mg/L)} - [\text{sample}] \text{ (mg/L)}}{\text{nominal [spike] (mg/L)}} \times 100$$

Acceptable Range: 80-120%^b

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A4198	A213	A4240

Comments: 4 samples not collected due to tech. error.

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^b Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^c Calculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials, Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: 4/5 3/3/26

Final Review: VS 3/3/25

DC-001

Client: CTEH

Project: MVWA 1122 Bioassay

Test Type: 10-day *Chironomous*

DI Blank: 0-0

Test Start Date: 12/20/2024

Analyst: SM

Analysis Date: 3/3/25

N x 1.22

$$\text{Relative Percent Difference (RPD)} = \frac{[\text{sample}] (\text{mg/L}) - [\text{sample duplicate}] (\text{mg/L})}{[\text{average ammonia}] (\text{mg/L})} \times 100$$

Acceptable Range: 0-20%

$$\text{Percent Recovery} = \frac{[\text{spiked sample}] \text{ (mg/L)} - [\text{sample}] \text{ (mg/L)}}{\text{nominal [spike] (mg/L)}} \times 100$$

Acceptable Range: 80-120%^b

Reagent 1

Reagent 2

Test Tubes

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^b Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^c Calculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: Ans 3/3/25

Final Review: 493 | 3 | 25

DC-001

Client: CTEH

Test Type: 10-day *Chironomous*

DI Blank: 0.0

Test Start Date: 12/20/2024

Analyst: SM

Analysis Date: 3/3/25

N x 1.22

$$\text{Relative Percent Difference (RPD)} = \frac{[\text{sample}] \text{ (mg/L)} - [\text{sample duplicate}] \text{ (mg/L)}}{[\text{average ammonia}] \text{ (mg/L)}} \times 100$$

Acceptable Range: 0-20%

$$\text{Percent Recovery} = \frac{[\text{spiked sample}] (\text{mg/L}) - [\text{sample}] (\text{mg/L})}{\text{nominal} [\text{spike}] (\text{mg/L})} \times 100$$

Acceptable Range: 80-120%^b

Reagent 1

Reagent 2

Test Tubes

Comments:

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^b Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^c Calculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: Ans 3/3/25

Final Review: VB 3/3/25

Sulfide Analysis
Freshwater

Overlying Water

Client: CTEH
Project: MVWA 1122 Bioassay
Test Type: 10-day Chironomus

DI Blank: 0 µg/L S²⁻
Analyst: KL
Analysis Date: 12/20/24

Sample ID	Sample Date	Test Day	pH (units)	Sulfide µg/L S ²⁻
Lab Control	12/20/2024	0	8.05	6
MVWA1122P001	12/20/2024	0	7.97	3
MVWA1122P002	12/20/2024	0	7.71	5
Sample Duplicate ^a (P002)	12/20/24	0	7.71	4

Comments:

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 µg/L S²⁻

QC Check: ACS 2/27/25

Final Review: VS 3/3/25

Nautilus Environmental, 4340 Vandever Avenue, San Diego, CA 92120.

Overlying Water

DI Blank:

Analyst: VS
Analysis Date: 12/20/24

Sample ID	Sample Date	Test Day	pH (units)	Sulfide µg/L S²⁻
Lab Control	12/30/2024	10	8.28	A-6 0
MVWA1122P001	12/30/2024	10	8.12	A-264 11
MVWA1122P002	12/30/2024	10	7.95	3
Sample Duplicate ^a	NA	NA		NA

Comments: (A) Q18 VS 12/30/24

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 $\mu\text{g/L S}^{2-}$

QC Check: ACS 2/27/25

Final Review: 45 3/3/25

Nautilus Environmental, 4340 Vandever Avenue, San Diego, CA 92120.

Pore Water

DI Blank: 0 ng/L S²⁻

Analyst: KL
Analysis Date: 12/20/24

Sample ID	Sample Date	Test Day	pH (units)	Sulfide $\mu\text{g/L S}^{2-}$
Lab Control	12/20/2024	0	7.50	16 x2 = 32
MVWA1122P001	12/20/2024	0	6.78	470
MVWA1122P002	12/20/2024	0	6.89	579
Sample Duplicate ^a (P002)	12/20/24	0	6.89	302 x2 = 604

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 µg/L S²⁻

QC Check: ACS 2/27/25

Final Review: 45 3 | 3 | 25

Nautilus Environmental, Inc. 4340 Vandever Avenue. San Diego, CA 92120.

Sulfide Analysis
Freshwater

Pore Water

Client: CTEH
 Project: MVWA 1122 Bioassay
 Test Type: 10-day Chironomus

DI Blank: ☐

Analyst: YS
 Analysis Date: 12/30/24

Sample ID	Sample Date	Test Day	pH (units)	Sulfide $\mu\text{g/L S}^{2-}$
Lab Control	12/30/2024	10	9.08	6
MVWA1122P001	12/30/2024	10	6.52	264
MVWA1122P002	12/30/2024	10	6.73	458
Sample Duplicate ^a P001	12/30/24	10		275

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 $\mu\text{g/L S}^{2-}$

QC Check: ALS 2/27/25

Final Review: YS 3/3/25

Nautilus Environmental, Inc. 4340 Vandever Avenue. San Diego, CA 92120.

Sulfide Analysis
Freshwater

Overlying Water

Client: CTEH
Project: MVWA 1122 Bioassay
Test Type: 28-day Hyalella

DI Blank: 0 µg/L

Analyst: KL/FM
Analysis Date: 12/19/24

Sample ID	Sample Date	Test Day	pH (units)	Sulfide µg/L S ²⁻
Lab Control	12/19/2024	0	8.22	0
MVWA1122P001	12/19/2024	0	7.85	11
MVWA1122P002	12/19/2024	0	8.20	35
Sample Duplicate ^a (P002)	12/19/24	0	8.20	27

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 µg/L S²⁻

QC Check: ACS 2/27/25

Final Review: VS3/3/25

Sulfide Analysis
Freshwater

Pore Water

Client: CTEH

Project: MVWA 1122 Bioassay

Test Type: 28-day Hyalella and 10-day Chironomus

DI Blank: 0 ng/L

Q18 vs 12/17/24

Analyst: KL/FM

Analysis Date: 12/19/24

Sample ID	Sample Date	Test Day	pH (units)	Sulfide $\mu\text{g/L S}^{2-}$
Lab Control	12/19/2024	0	8.04	0 x 2 = 0
MVWA1122P001	12/19/2024	0	6.94	481
MVWA1122P002	12/19/2024	0	6.92	564
Sample Duplicate ^a (P002)	12/19/24	0	6.92	290 x 2 = 580

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 $\mu\text{g/L S}^{2-}$

QC Check: ATS 2/27/25

Final Review: VS 3/3/25

Nautilus Environmental, Inc. 4340 Vandever Avenue. San Diego, CA 92120.

**Sulfide Analysis
Freshwater**

Pore Water

Client: CTEH
Project: MVWA 1122 Bioassay
Test Type: 28-day Hyalella

DI Blank: 0

Analyst: VS
Analysis Date: 1/16/25

Sample ID	Sample Date	Test Day	pH (units)	Sulfide $\mu\text{g/L S}^2$
Lab Control	1/16/2025	28	9.37	28
MVWA1122P001	1/16/2025	28	5.62	179
MVWA1122P002	1/16/2025	28	6.66	212
				211
Sample Duplicate ^a P002	1/16/25	28	NA	211

618
VS 1/16/25

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate

Method Detection Limit = 5.0 $\mu\text{g/L S}^2$

QC Check: ACS 2/27/25

Final Review: VS 3/3/25

Nautilus Environmental, Inc. 4340 Vandever Avenue. San Diego, CA 92120.

Appendix E
Summary of Statistical Analyses

CTEH / MVWA 1122 Bioassay
Toxicity Test Mean Survival and Data Transform Summary

Sample Date: 11/22/24; Test Initiation Date: 12/20/24

Chironomid 10-day Survival

Site ID	Replicate	Number Alive	Percent Survival	Mean Percent Survival
Lab Control	A	10	100	96.0
	B	9	90.0	
	C	10	100	
	D	9	90.0	
	E	10	100.0	
MVWA1122P001	A	9	90.0	94.0
	B	9	90.0	
	C	9	90.0	
	D	10	100	
	E	10	100	
MVWA1122P002	A	10	100	96.0
	B	10	100	
	C	9	90.0	
	D	10	100.0	
	E	9	90.0	

Entry: ACS 3/3/25

Q/C:

vs 3/3/25

Initial Number of Animals: 10

Number Initial	Number Survived	Proportion Alive	Transformed Result
10	10	1	1.412
10	9	0.9	1.249
10	10	1	1.412
10	9	0.9	1.249
10	10	1	1.412
10	9	0.9	1.249
10	9	0.9	1.249
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	9	0.9	1.249
10	10	1	1.412
10	9	0.9	1.249

CTEH
MVWA 1122 Bioassay
Normality Test
Chironomid 10-Day Survival, Initiated 12/20/24

	Residuals
Number of values	15
Minimum	-0.0978
25% Percentile	-0.0978
Median	0.0652
75% Percentile	0.0652
Maximum	0.0978
Mean	0.0
Std. Deviation	0.08266
Std. Error of Mean	0.02134
Lower 95% CI of mean	-0.04577
Upper 95% CI of mean	0.04577
D'Agostino & Pearson omnibus normality test	
K2	14.92
P value	0.0006
Passed normality test (alpha=0.05)?	No
P value summary	***
Sum	0.0

Entry: ACS 3/3/25

QC:

4/3/25

Prism v. 6.05

Nautilus Environmental, Inc.
San Diego, CA

CTEH
MVWA 1122 Bioassay
Variance Test
Chironomid 10-Day Survival, Initiated 12/20/24

Table Analyzed	Survival Transformed
Kruskal-Wallis test	
P value	> 0.9999
Exact or approximate P value?	Exact
P value summary	ns
Do the medians vary signif. ($P < 0.05$)	No
Number of groups	3
Kruskal-Wallis statistic	0.5000
Data summary	
Number of treatments (columns)	3
Number of values (total)	15

Entry: ACS 3/3/25

QC:

VS 3/3/25

Prism v. 6.05

Nautilus Environmental, Inc.
San Diego, CA

CTEH
MVWA 1122 Bioassay
T-Tests
Chironomid 10-Day Survival, Initiated 12/20/24

Table Analyzed	Survival Transformed
Column C	MVWA1122P002
vs.	vs.
Column B	MVWA1122P001
Mann Whitney test	
P value	0.5000
Exact or approximate P value?	Exact
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
Sum of ranks in column B,C	25 , 30
Mann-Whitney U	10
Difference between medians	
Median of column B	1.249, n=5
Median of column C	1.412, n=5
Difference: Actual	0.1630
Difference: Hodges-Lehmann	0.0

Table Analyzed	Survival Transformed
Column B	MVWA1122P001
vs.	vs.
Column A	Lab Control
Mann Whitney test	
P value	0.5000
Exact or approximate P value?	Exact
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
Sum of ranks in column A,B	30 , 25
Mann-Whitney U	10
Difference between medians	
Median of column A	1.412, n=5
Median of column B	1.249, n=5
Difference: Actual	-0.1630
Difference: Hodges-Lehmann	0.0

CTEH
MVWA 1122 Bioassay
Column Statistics
Chironomid 10-Day Survival, Initiated 12/20/24

	Lab Control	MVWA1122P001	MVWA1122P002
Number of values	5	5	5
Minimum	1.249	1.249	1.249
25% Percentile	1.249	1.249	1.249
Median	1.412	1.249	1.412
75% Percentile	1.412	1.412	1.412
Maximum	1.412	1.412	1.412
Mean	1.347	1.314	1.347
Std. Deviation	0.08928	0.08928	0.08928
Std. Error of Mean	0.03993	0.03993	0.03993
Lower 95% CI of mean	1.236	1.203	1.236
Upper 95% CI of mean	1.458	1.425	1.458
Sum	6.734	6.571	6.734

Entry: ACS 3/3/25

QC: *vs 3/3/25*

Prism v. 6.05

Nautilus Environmental, Inc.
San Diego, CA

Hyalella 28-day Survival

Number Initial	Number Survived	Proportion Alive	Transformed Result
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	9	0.9	1.249
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	10	1	1.412
10	8	0.8	1.107
10	8	0.8	1.107
10	9	0.9	1.249

Site ID	Replicate	Number Alive	Percent Survival	Mean Percent Survival
Lab Control	A	10	100	100
	B	10	100	
	C	10	100	
	D	10	100	
	E	10	100	
MVWA1122P001	A	10	100	98.0
	B	10	100	
	C	9	90.0	
	D	10	100	
	E	10	100	
MVWA1122P002	A	10	100	90.0
	B	10	100	
	C	8	80.0	
	D	8	80.0	
	E	9	90.0	

Q/C:

KS 3/3/25

CTEH
MVWA 1122 Bioassay
Normality Test
Hyalella 28-Day Survival, Initiated 12/19/24

	Residuals
Number of values	15
Minimum	-0.1504
25% Percentile	-0.008400
Median	-2.146e-007
75% Percentile	0.0326
Maximum	0.1546
Mean	-1.118e-009
Std. Deviation	0.09038
Std. Error of Mean	0.02334
Lower 95% CI of mean	-0.05005
Upper 95% CI of mean	0.05005
D'Agostino & Pearson omnibus normality test	
K2	0.3243
P value	0.8503
Passed normality test (alpha=0.05)?	Yes
P value summary	ns
Sum	-1.676e-008

Entry: ACS 3/2/25

QC: *✓ 3/3/25*

Prism v. 6.05

Nautilus Environmental, Inc.
San Diego, CA

CTEH
MVWA 1122 Bioassay
Variance Test
Hyalella 28-Day Survival, Initiated 12/19/24

Table Analyzed	Survival Transformed
ANOVA summary	
F	3.484
P value	0.0641
P value summary	ns
Are differences among means statistically significant? ($P < 0.05$)	No
R square	0.3674
Brown-Forsythe test	
F (DFn, DFd)	5.940 (2, 12)
P value	0.0161
P value summary	*
Significantly different standard deviations? ($P < 0.05$)	Yes

ANOVA table	SS	DF	MS	F (DFn, DFd)	P value
Treatment (between columns)	0.06641	2	0.03321	F (2, 12) = 3.484	P = 0.0641
Residual (within columns)	0.1144	12	0.009531		
Total	0.1808	14			
Data summary					
Number of treatments (columns)	3				
Number of values (total)	15				

Entry: ACS 3/2/25

QC:

✓ 3/3/25

Prism v. 6.05

Nautilus Environmental, Inc.
San Diego, CA

CTEH
MVWA 1122 Bioassay
T-Tests
Hyalella 28-Day Survival, Initiated 12/19/24

Table Analyzed	Survival Transformed
Column B	MVWA1122P001
vs.	vs.
Column A	Lab Control
Unpaired t test with Welch's correction	
P value	0.1869
P value summary	ns
Significantly different? (P < 0.05)	No
One- or two-tailed P value?	One-tailed
Welch-corrected t, df	t=1.000 df=4.000
How big is the difference?	
Mean ± SEM of column A	1.412 ± 2.145e-007, n=5
Mean ± SEM of column B	1.379 ± 0.0326, n=5
Difference between means	-0.03260 ± 0.0326
95% confidence interval	-0.1231 to 0.05791
R squared	0.2000
F test to compare variances	
F,DFn, Dfd	2.310e+010, 4, 4
P value	< 0.0001
P value summary	****
Significantly different? (P < 0.05)	Yes

Table Analyzed	Survival Transformed
Column C	MVWA1122P002
vs.	vs.
Column A	Lab Control
Unpaired t test with Welch's correction	
P value	0.0431
P value summary	*
Significantly different? (P < 0.05)	Yes
One- or two-tailed P value?	One-tailed
Welch-corrected t, df	t=2.266 df=4.000
How big is the difference?	
Mean ± SEM of column A	1.412 ± 2.145e-007, n=5
Mean ± SEM of column C	1.257 ± 0.06823, n=5
Difference between means	-0.1546 ± 0.06823
95% confidence interval	-0.3440 to 0.03484
R squared	0.5621
F test to compare variances	
F,DFn, Dfd	1.012e+011, 4, 4
P value	< 0.0001
P value summary	****
Significantly different? (P < 0.05)	Yes

CTEH
MVWA 1122 Bioassay
Column Statistics
Hyalella 28-Day Survival, Initiated 12/19/24

	Lab Control	MVWA1122P001	MVWA1122P002
Number of values	5	5	5
Minimum	1.412	1.249	1.107
25% Percentile	1.412	1.331	1.107
Median	1.412	1.412	1.249
75% Percentile	1.412	1.412	1.412
Maximum	1.412	1.412	1.412
Mean	1.412	1.379	1.257
Std. Deviation	4.805e-007	0.07290	0.1526
Std. Error of Mean	2.149e-007	0.0326	0.06823
Lower 95% CI of mean	1.412	1.289	1.068
Upper 95% CI of mean	1.412	1.470	1.447
Sum	7.060	6.897	6.287

Entry: ACS 3/2/25

QC: *vs 3/3/25*

Appendix F
Laboratory Qualifier Codes

Glossary of Qualifier Codes

- Q1 - Temperature out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 - Temperature out of recommended range; no action taken, test terminated same day
- Q3 - Sample pH adjusted to within range of 6-9 with reagent grade NaOH or HCl, as needed
- Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 - Test initiated with continuous aeration due to an anticipated drop in D.O.
- Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 - Salinity out of recommended range
- Q8 - Spilled test chamber/ Unable to recover test organism(s)
- Q9 - Inadequate sample volume remaining, partial renewal performed
- Q10 - Inadequate sample volume remaining, no renewal performed
- Q11 - Sample out of holding time; refer to QA section of report
- Q12 - Replicate(s) not initiated; excluded from data analysis
- Q13 - Survival counts not recorded due to poor visibility or heavy debris
- Q14 - D.O. percent saturation was checked and was $\leq 110\%$
- Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set. Test results were reviewed and reported in accordance with guidance found in EPA-833-R-00-003, 2000 unless otherwise specified.
- Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set. Test results were reviewed and reported in accordance with EPA-833-R-00-003, 2000 guidance unless otherwise specified.
- Q18 - Incorrect or illegible Entry
- Q19 - Miscalculation
- Q20 - PMSD criteria do not apply to the test of significant toxicity (TST) analysis
- Q21 - Other (provide reason in comments section)
- Q22 - Greater than 10% batch mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Enthalpy and ultimately deemed fit to use for testing.
- Q23 - Test organisms experienced a temperature shift greater than 3°C within 1 day or were received at a temperature greater than 3°C outside the recommended test temperature range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.
- Q24 - Test organisms experienced a salinity shift greater than 3 ppt within 1 day or were received at a salinity greater than 3 ppt outside the recommended test salinity range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.

Appendix G
Reference Toxicant Results

CETIS Summary Report

Report Date: 02 Mar-25 16:33 (p 1 of 1)
 Test Code/ID: 241220ctrNH3SED / 11-5004-4537

Chironomus 96-h Acute Survival Test

Nautilus Environmental (CA)

Batch ID: 04-2898-8790	Test Type: Survival (96h)	Analyst:
Start Date: 20 Dec-24 13:40	Protocol: EPA/600/R-99/064 (2000)	Diluent: Carbon Filtered Water (CFW)
Ending Date: 24 Dec-24 12:15	Species: Chironomus dilutus	Brine: Not Applicable
Test Length: 95h	Taxon:	Source: Aquatic Biosystems, CO Age: 10d

Sample ID: 19-1533-0172	Code: 241220ctrNH3SED	Project:
Sample Date: 20 Dec-24	Material: Total Ammonia	Source: Reference Toxicant
Receipt Date: 20 Dec-24	CAS (PC):	Station: Total Ammonia
Sample Age: 14h	Client: Internal	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
18-7507-2413	96h Survival Rate	Steel Many-One Rank Sum Test	35.1	68.4	49	18.3%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	mg/L	95% LCL	95% UCL	S
07-8997-7725	96h Survival Rate	Trimmed Spearman-Kärber	EC50	96	81.6	113	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
07-8997-7725	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
18-7507-2413	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Survival Rate Summary

Conc-mg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
18.2		4	0.950	0.858	1.040	0.900	1.000	0.029	0.058	6.08%	5.00%
35.1		4	0.950	0.791	1.110	0.800	1.000	0.050	0.100	10.53%	5.00%
68.4		4	0.525	0.373	0.677	0.400	0.600	0.048	0.096	18.24%	47.50%
129.2		4	0.525	0.068	0.982	0.100	0.700	0.144	0.287	54.71%	47.50%
245.6		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
498		4	0.050	-0.042	0.142	0.000	0.100	0.029	0.058	115.47%	95.00%

96h Survival Rate Detail

MD5: D1824E2CF528475E12054C1C87A3D7F4

Conc-mg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
18.2		1.000	1.000	0.900	0.900
35.1		1.000	1.000	0.800	1.000
68.4		0.500	0.600	0.400	0.600
129.2		0.700	0.100	0.700	0.600
245.6		0.000	0.000	0.000	0.000
498		0.100	0.000	0.100	0.000

CETIS Analytical Report

Report Date: 02 Mar-25 16:33 (p 1 of 2)
Test Code/ID: 241220ctrNH3SED / 11-5004-4537

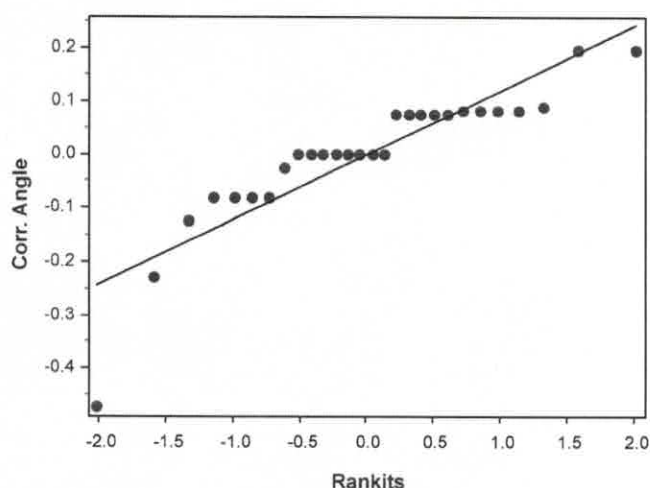
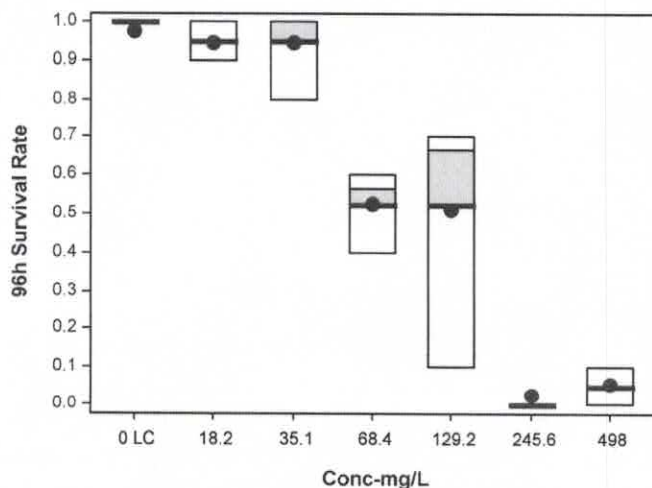
Chironomus 96-h Acute Survival Test							Nautilus Environmental (CA)																
Analysis ID: 18-7507-2413		Endpoint: 96h Survival Rate			CETIS Version: CETISv2.1.4																		
Analyzed: 02 Mar-25 16:33		Analysis: Nonparametric-Control vs Treatments			Status Level: 1																		
Edit Date: 02 Mar-25 16:32		MD5 Hash: D1824E2CF528475E12054C1C87A3D7F4			Editor ID: 007-926-968-0																		
Data Transform		Alt Hyp			NOEL		LOEL		TOEL		Tox Units		MSDu		PMSD								
Angular (Corrected)		C > T			35.1		68.4		49		---		0.183		18.29%								
Steel Many-One Rank Sum Test																							
Control		vs		Conc-mg/L		df		Test Stat		Critical		Ties		P-Type		P-Value		Decision(α:5%)					
Lab Control		18.2		6		14		10		1		CDF		0.3081		Non-Significant Effect							
		35.1		6		16		10		1		CDF		0.5661		Non-Significant Effect							
		68.4*		6		10		10		0		CDF		0.0350		Significant Effect							
		129.2*		6		10		10		0		CDF		0.0350		Significant Effect							
ANOVA Table																							
Source		Sum Squares		Mean Square		DF		F Stat		P-Value		Decision(α:5%)											
Between		1.49761		0.374403		4		13		9.3E-05		Significant Effect											
Error		0.433331		0.0288888		15																	
Total		1.93094				19																	
ANOVA Assumptions Tests																							
Attribute		Test				Test Stat		Critical		P-Value		Decision(α:1%)											
Variance		Bartlett Equality of Variance Test										Indeterminate											
Distribution		Shapiro-Wilk W Normality Test				0.831		0.866		0.0026		Non-Normal Distribution											
96h Survival Rate Summary																							
Conc-mg/L		Code		Count		Mean		95% LCL		95% UCL		Median		Min		Max		Std Err		CV%		%Effect	
0		LC		4		1.000		1.000		1.000		1.000		1.000		1.000		0.000		0.00%		0.00%	
18.2				4		0.950		0.858		1.000		0.950		0.900		1.000		0.029		6.08%		5.00%	
35.1				4		0.950		0.791		1.000		1.000		0.800		1.000		0.050		10.53%		5.00%	
68.4				4		0.525		0.373		0.677		0.567		0.400		0.600		0.048		18.24%		47.50%	
129.2				4		0.525		0.068		0.982		0.667		0.100		0.700		0.144		54.71%		47.50%	
245.6				4		0.000		0.000		0.000		0.000		0.000		0.000		0.000		---		100.00%	
498				4		0.050		0.000		0.142		0.050		0.000		0.100		0.029		115.47%		95.00%	
Angular (Corrected) Transformed Summary																							
Conc-mg/L		Code		Count		Mean		95% LCL		95% UCL		Median		Min		Max		Std Err		CV%		%Effect	
0		LC		4		1.410		1.410		1.410		1.410		1.410		1.410		0.000		0.00%		0.00%	
18.2				4		1.330		1.180		1.480		1.330		1.250		1.410		0.047		7.07%		5.77%	
35.1				4		1.340		1.090		1.580		1.410		1.110		1.410		0.076		11.41%		5.40%	
68.4				4		0.811		0.657		0.964		0.853		0.685		0.886		0.048		11.89%		42.59%	
129.2				4		0.798		0.287		1.310		0.956		0.322		0.991		0.161		40.25%		43.52%	
245.6				4		0.159		0.159		0.159		0.159		0.159		0.159		0.000		0.00%		88.76%	
498				4		0.240		0.091		0.390		0.240		0.159		0.322		0.047		39.16%		82.98%	

CETIS Analytical Report

Report Date: 02 Mar-25 16:33 (p 2 of 2)
 Test Code/ID: 241220ctrNH3SED / 11-5004-4537

Chironomus 96-h Acute Survival Test			Nautilus Environmental (CA)	
Analysis ID: 18-7507-2413	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4		
Analyzed: 02 Mar-25 16:33	Analysis: Nonparametric-Control vs Treatments	Status Level: 1		
Edit Date: 02 Mar-25 16:32	MD5 Hash: D1824E2CF528475E12054C1C87A3D7F4	Editor ID: 007-926-968-0		

Graphics



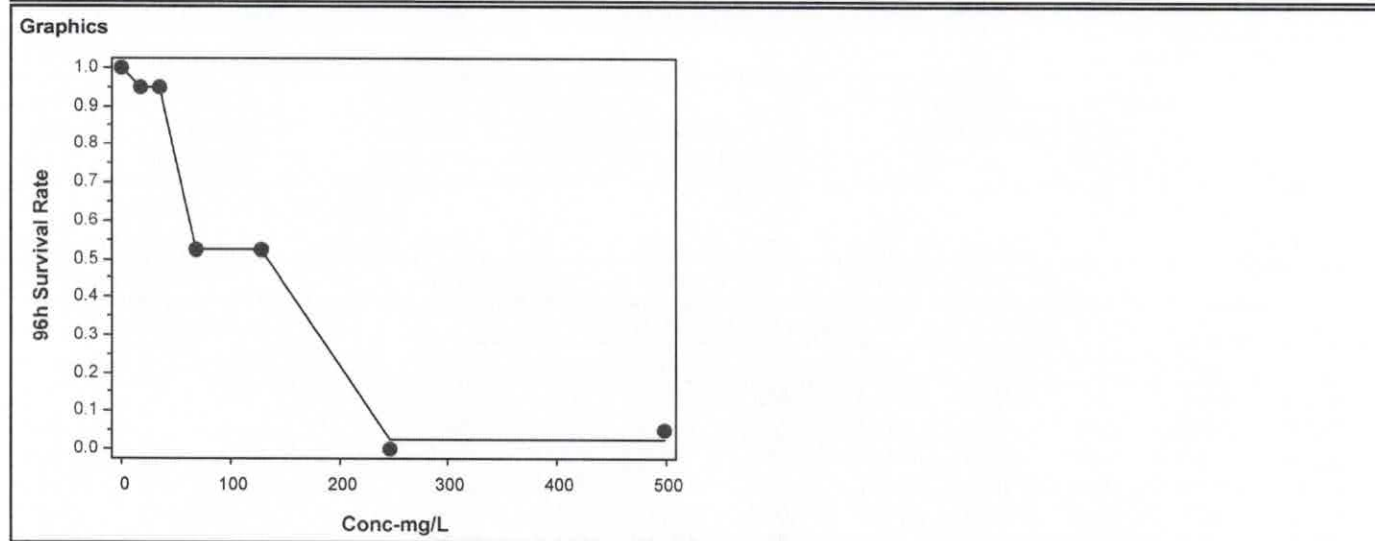
CETIS Analytical Report

Report Date: 02 Mar-25 16:33 (p 1 of 1)
 Test Code/ID: 241220ctrNH3SED / 11-5004-4537

Chironomus 96-h Acute Survival Test				Nautilus Environmental (CA)	
Analysis ID: 07-8997-7725		Endpoint: 96h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 02 Mar-25 16:33		Analysis: Trimmed Spearman-Kärber		Status Level: 1	
Edit Date: 02 Mar-25 16:32		MD5 Hash: D1824E2CF528475E12054C1C87A3D7F4		Editor ID: 007-926-968-0	

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	1.98	0.0354	96	81.6	113

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-mg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	40/40	1.000	0.00%
18.2		4	0.950	0.950	0.900	1.000	6.08%	5.00%	38/40	0.950	5.00%
35.1		4	0.950	1.000	0.800	1.000	10.53%	5.00%	38/40	0.950	5.00%
68.4		4	0.525	0.567	0.400	0.600	18.24%	47.50%	21/40	0.525	47.50%
129.2		4	0.525	0.667	0.100	0.700	54.71%	47.50%	21/40	0.525	47.50%
245.6		4	0.000	0.000	0.000	0.000	---	100.00%	0/40	0.025	97.50%
498		4	0.050	0.050	0.000	0.100	115.47%	95.00%	2/40	0.025	97.50%



CETIS QC Plot

Report Date: 02 Mar-25 16:34 (1 of 1)

Chironomus 96-h Acute Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

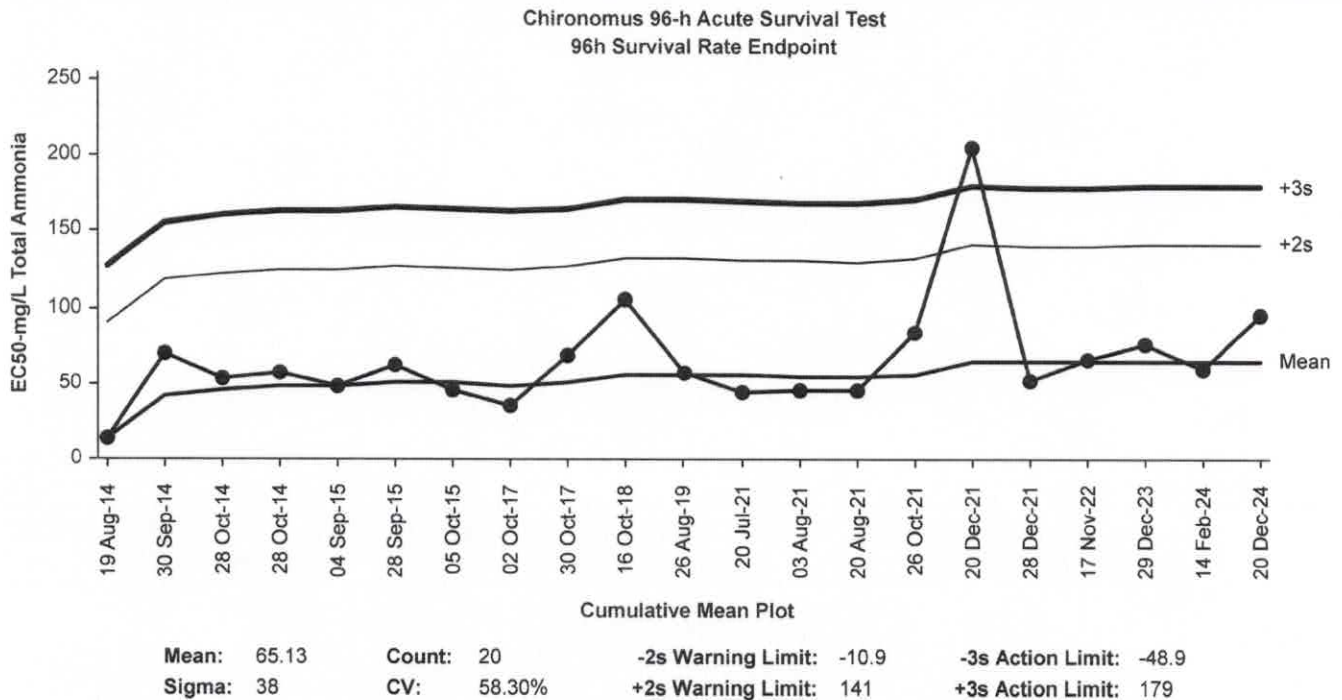
Organism: Chironomus dilutus

Material: Total Ammonia

Protocol: EPA/600/R-99/064 (2000)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF



Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Aug	19	17:35	13.85	-51.28	-1.349			06-6149-7180	09-7696-8711
2		Sep	30	17:00	69.92	4.786	0.126			02-9200-2624	04-0033-0926
3		Oct	28	17:00	54.12	-11.01	-0.2898			09-6234-9603	12-8117-9461
4			28	17:15	57.95	-7.183	-0.189			05-4330-2797	06-7105-1932
5	2015	Sep	4	15:25	48.29	-16.84	-0.4432			14-6837-4354	08-3599-8388
6			28	15:40	62.77	-2.36	-0.06211			09-3006-9944	07-2648-2888
7		Oct	5	17:30	45.89	-19.24	-0.5064			03-9930-1734	08-1584-7208
8	2017		2	15:30	36.13	-29	-0.7631			13-5191-9297	06-9058-0712
9			30	15:40	68.62	3.493	0.09192			00-1769-0978	07-0805-0360
10	2018		16	16:20	106.1	40.97	1.078			15-9035-9078	13-0630-5838
11	2019	Aug	26	15:00	57.25	-7.878	-0.2073			10-0394-8759	15-3104-1914
12	2021	Jul	20	17:00	45.18	-19.95	-0.525			10-2159-3177	00-8996-5427
13		Aug	3	16:10	46.47	-18.66	-0.4911			20-0306-0810	06-2460-6245
14			20	17:45	45.95	-19.18	-0.5048			03-1493-3047	03-2065-0498
15		Oct	26	18:55	84.59	19.46	0.5122			11-0299-7216	02-4594-7205
16		Dec	20	15:00	204.9	139.7	3.678	(+)	(+)	03-6450-9872	04-5264-7968
17			28	16:45	51.86	-13.27	-0.3493			07-8056-3793	09-1111-8136
18	2022	Nov	17	16:40	66.14	1.012	0.02662			15-3915-2035	21-4130-6161
19	2023	Dec	29	11:40	76.81	11.68	0.3075			18-8410-8536	09-6635-0369
20	2024	Feb	14	15:20	59.83	-5.298	-0.1394			12-0941-4704	18-6822-4113
21		Dec	20	13:40	96.02	30.89	0.8128			11-5004-4537	07-8997-7725

96-hour Freshwater Acute Bioassay

Static Conditions

DS-042S (SED)

Water Quality Measurements
& Test Organism SurvivalClient: Internal
Sample ID: NH₃
Test No.: 241220ct, NH3 SEDTest Species: *C. dilutus*
Start Date/Time: 12/20/24 1340
End Date/Time: 12/24/24 1215

Tech Initials					
0	24	48	72	96	
HH	--	--	--	HH	
HH	LM	AD	WF	AD	
HH	--	--	--	--	
500	--	--	--	--	
911	--	--	--	--	
2000	--	--	--	--	

Dilutions made by/Subsample NH₃ day 0:

High conc. made (mg/L):

Vol. NH stock added (mL):

Final Volume (mL):

NH₃ stock concentration (mg/L): 10,980

Concentration mg/L (measured)	Rand #	Number of Live Organisms		pH (units)					Dissolved Oxygen (mg/L)					Conductivity (µmhos/cm)					Temperature (°C)				
		0	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	26	10	10	7.3	7.2	7.2	7.0	7.2	8.2	8.1	8.1	8.0	8.2	8.2	8.2	8.2	8.3	9.31	23.7	22.7	22.9	22.8	22.8
	1	10	10						8.2					8.2									
	8	10	10																				
	12	10	10																				
15.6 (18.2)	23	10	10	8.0	8.2	8.2	8.1	8.2	8.2	8.0	8.2	7.9	8.2	10.8	9.70	9.70	10.2	10.2	23.7	22.7	23.0	23.1	23.1
	25	10	10						8.2					10.3									
	6	10	9																				
	24	10	9																				
31.25 (35.1)	3	10	10	8.4	8.3	8.1	8.0	8.3	8.2	8.1	8.0	8.1	7.9	8.2	12.3	10.78	10.69	11.15	23.7	22.6	23.0	22.7	23.0
	13	10	10						8.2					11.35									
	27	10	8																				
	9	10	10																				
62.5 (68.4)	2	10	5	8.7	8.8	8.1	7.9	8.0	8.2	8.1	7.9	8.0	7.8	8.2	14.4	13.00	12.84	13.3	23.7	22.9	23.1	23.1	23.1
	15	10	6						8.2					13.86									
	14	10	4																				
	22	10	6																				
125 (129.2)	20	10	7	8.0	7.9	8.0	7.8	7.8	8.2	7.9	7.8	7.9	7.8	8.2	19.4	17.4	16.9	17.50	23.5	22.8	23.1	23.0	23.0
	7	10	1						8.2					18.12									
	4	10	7																				
	11	10	6																				
250 (245.6)	19	10	0	7.7	7.7	7.6	7.6	7.6	8.2	7.9	7.7	7.7	7.7	8.2	20.9	20.8	24.50	25.50	23.4	22.7	23.1	23.0	23.1
	16	10	0						8.2					22.00									
	21	10	0																				
	17	10	0																				
500 (498.0)	5	10	1	7.6	7.6	7.4	7.4	7.4	8.2	8.0	7.8	7.8	7.8	8.2	27.7	27.0	41.00	42.70	22.9	22.8	23.1	23.1	23.1
	18	10	0						8.3					24.10									
	10	10	1																				
	28	10	0																				

Rand # QC:

Initial Count QC'd by: KL/KLOORS

Initiated by: KLX

Environmental Chamber: F

Animal Source/Date Received: ABS 12/18/24

Age at Initiation: 10 days

Animal Acclimation Qualifiers (circle all that apply):

Q22 / Q23 / Q24 / none

Comments: 0019 HH 12/24/24

Chambers fed prior to initiation, circle one (y) (n)

y

QC Check:

ACS 3/2/25

Final Review: 018/24/24

Feeding Times					
0	24	48	72	96	

AM:

PM:

Total Ammonia Analysis Freshwater

DC-001

Overlying Water

Client: Internal
Project: 241220 ctr NH3 SED
Test Type: 96-hr Chironomus Ammonia reference toxicant test

DI Blank: 0.0
Test Start Date: 12/20/2024

Analyst: SM
Analysis Date: 2/17/25

N x 1.22					
Sample ID	Nautilus ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L)
Blank Spike (10 mg/L NH ₃)		NA	NA	8.3	10.1
LC	28	12/20/2024	0	0.1	10.5
15.6	29	12/20/2024	0	14.9	18.2
31.25	30	12/20/2024	0	28.8	35.1
62.5	31	12/20/2024	0	28.0	34.2 x 2 = 68.4
125	32	12/20/2024	0	26.5	32.3 x 4 = 129.2
250	33	12/20/2024	0	25.2	30.7 x 8 = 245.6
500	34	12/20/2024	0	40.8	49.8 x 10 = 498.0
Spike Check (10 mg/L NH ₃)		NA	NA		
Sample Duplicate ^a	29	NA	NA	14.8	18.1
Sample Duplicate + Spike ^a		NA	NA	22.4	27.3
Spike Check (10 mg/L NH ₃)		NA	NA	8.3	10.1

Relative Percent Difference (RPD) = $\frac{[\text{sample}] (\text{mg/L}) - [\text{sample duplicate}] (\text{mg/L})}{[\text{average ammonia}] (\text{mg/L})} \times 100$

Acceptable Range: 0-20%

Percent Recovery = $\frac{[\text{spiked sample}] (\text{mg/L}) - [\text{sample}] (\text{mg/L})}{\text{nominal} [\text{spike}] (\text{mg/L})} \times 100$

Acceptable Range: 80-120%^b

QC Sample ID	[NH ₃]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	10.1	10	NA	101
29	18.2	18.1	27.3	10	0.6	91

Reagent 1		Reagent 2		Test Tubes	
Standard Lot Number	A4198		A4213		A4240

Comments: DQ & AHS 3/2/25

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

^bAcceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

^cCalculation not performed due to one or both values below the method detection limit.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: AHS 3/2/25

Final Review: V 3/3/25

CETIS Summary Report

Report Date: 08 Jan-25 11:34 (p 1 of 1)
 Test Code/ID: 241219harSED / 06-3922-3450

Acute Amphipod Survival Test

Nautilus Environmental (CA)

Batch ID: 13-3884-3756	Test Type: Survival (96h)	Analyst:
Start Date: 19 Dec-24 16:30	Protocol: EPA/600/R-99/064 (2000)	Diluent: Carbon Filtered Water (CFW)
Ending Date: 23 Dec-24 16:55	Species: Hyalella azteca	Brine: Not Applicable
Test Length: 4d 0h	Taxon:	Source: Aquatic Research Organism Age: 9d

Sample ID: 19-5539-6046	Code: 241219harSED	Project:
Sample Date: 19 Dec-24	Material: Copper chloride	Source: Reference Toxicant
Receipt Date: 19 Dec-24	CAS (PC):	Station: Copper Chloride
Sample Age: 16h	Client: Internal	

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓ NOEL	LOEL	TOEL	PMSD	S
07-5596-7140	96h Survival Rate	Steel Many-One Rank Sum Test	100	200	141.4	10.6%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓ Level	µg/L	95% LCL	95% UCL	S
08-5703-8091	96h Survival Rate	Trimmed Spearman-Kärber	EC50	236	202	276	1

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
07-5596-7140	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
08-5703-8091	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
100		4	0.975	0.895	1.050	0.900	1.000	0.025	0.050	5.13%	2.50%
200		4	0.475	0.203	0.747	0.300	0.700	0.085	0.171	35.95%	52.50%
400		4	0.275	0.123	0.427	0.200	0.400	0.048	0.096	34.82%	72.50%
800		4	0.025	-0.055	0.105	0.000	0.100	0.025	0.050	200.00%	97.50%
1600		4	0.025	-0.055	0.105	0.000	0.100	0.025	0.050	200.00%	97.50%

96h Survival Rate Detail

MD5: 501C103AC849C61E050645E048467162

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
100		0.900	1.000	1.000	1.000
200		0.300	0.700	0.400	0.500
400		0.200	0.400	0.300	0.200
800		0.100	0.000	0.000	0.000
1600		0.000	0.100	0.000	0.000

CETIS Analytical Report

Report Date: 08 Jan-25 11:34 (p 1 of 2)
 Test Code/ID: 241219harSED / 06-3922-3450

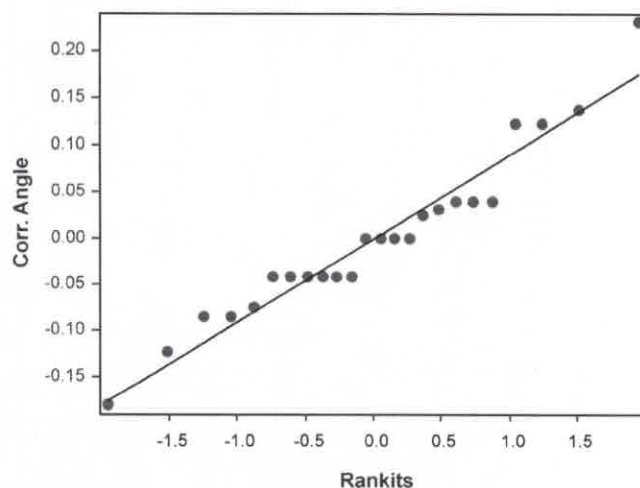
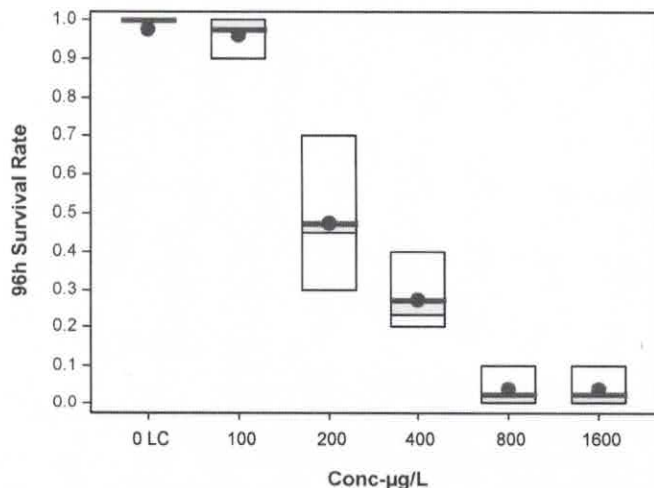
Acute Amphipod Survival Test							Nautilus Environmental (CA)																
Analysis ID: 07-5596-7140		Endpoint: 96h Survival Rate			CETIS Version: CETISv2.1.4																		
Analyzed: 08 Jan-25 11:33		Analysis: Nonparametric-Control vs Treatments			Status Level: 1																		
Edit Date: 08 Jan-25 11:33		MD5 Hash: 501C103AC849C61E050645E048467162			Editor ID: 007-926-968-0																		
Data Transform		Alt Hyp		NOEL		LOEL		TOEL		Tox Units		MSDu		PMSD									
Angular (Corrected)		C > T		100		200		141.4		---		0.106		10.61%									
Steel Many-One Rank Sum Test																							
Control		vs		Conc-µg/L		df		Test Stat		Critical		Ties		P-Type		P-Value		Decision(α:5%)					
Lab Control		100		6		16		10		1		CDF		0.6105		Non-Significant Effect							
		200*		6		10		10		0		CDF		0.0417		Significant Effect							
		400*		6		10		10		0		CDF		0.0417		Significant Effect							
		800*		6		10		10		0		CDF		0.0417		Significant Effect							
		1600*		6		10		10		0		CDF		0.0417		Significant Effect							
ANOVA Table																							
Source		Sum Squares		Mean Square		DF		F Stat		P-Value		Decision(α:5%)											
Between		5.8849		1.17698		5		114		<1.0E-05		Significant Effect											
Error		0.185958		0.010331		18																	
Total		6.07086				23																	
ANOVA Assumptions Tests																							
Attribute		Test		Test Stat		Critical		P-Value		Decision(α:1%)													
Variance		Bartlett Equality of Variance Test								Indeterminate													
Distribution		Shapiro-Wilk W Normality Test		0.955		0.884		0.3436		Normal Distribution													
96h Survival Rate Summary																							
Conc-µg/L		Code		Count		Mean		95% LCL		95% UCL		Median		Min		Max		Std Err		CV%		%Effect	
0		LC		4		1.000		1.000		1.000		1.000		1.000		1.000		0.000		0.00%		0.00%	
100				4		0.975		0.895		1.000		1.000		0.900		1.000		0.025		5.13%		2.50%	
200				4		0.475		0.203		0.747		0.450		0.300		0.700		0.085		35.95%		52.50%	
400				4		0.275		0.123		0.427		0.233		0.200		0.400		0.048		34.82%		72.50%	
800				4		0.025		0.000		0.105		0.000		0.000		0.100		0.025		200.00%		97.50%	
1600				4		0.025		0.000		0.105		0.000		0.000		0.100		0.025		200.00%		97.50%	
Angular (Corrected) Transformed Summary																							
Conc-µg/L		Code		Count		Mean		95% LCL		95% UCL		Median		Min		Max		Std Err		CV%		%Effect	
0		LC		4		1.410		1.410		1.410		1.410		1.410		1.410		0.000		0.00%		0.00%	
100				4		1.370		1.240		1.500		1.410		1.250		1.410		0.041		5.94%		2.89%	
200				4		0.760		0.481		1.040		0.735		0.580		0.991		0.088		23.07%		46.16%	
400				4		0.548		0.379		0.717		0.502		0.464		0.685		0.053		19.41%		61.20%	
800				4		0.200		0.070		0.329		0.159		0.159		0.322		0.041		40.84%		85.87%	
1600				4		0.200		0.070		0.329		0.159		0.159		0.322		0.041		40.84%		85.87%	

CETIS Analytical Report

Report Date: 08 Jan-25 11:34 (p 2 of 2)
 Test Code/ID: 241219harSED / 06-3922-3450

Acute Amphipod Survival Test			Nautilus Environmental (CA)	
Analysis ID: 07-5596-7140	Endpoint: 96h Survival Rate	CETIS Version: CETISv2.1.4		
Analyzed: 08 Jan-25 11:33	Analysis: Nonparametric-Control vs Treatments	Status Level: 1		
Edit Date: 08 Jan-25 11:33	MD5 Hash: 501C103AC849C61E050645E048467162	Editor ID: 007-926-968-0		

Graphics



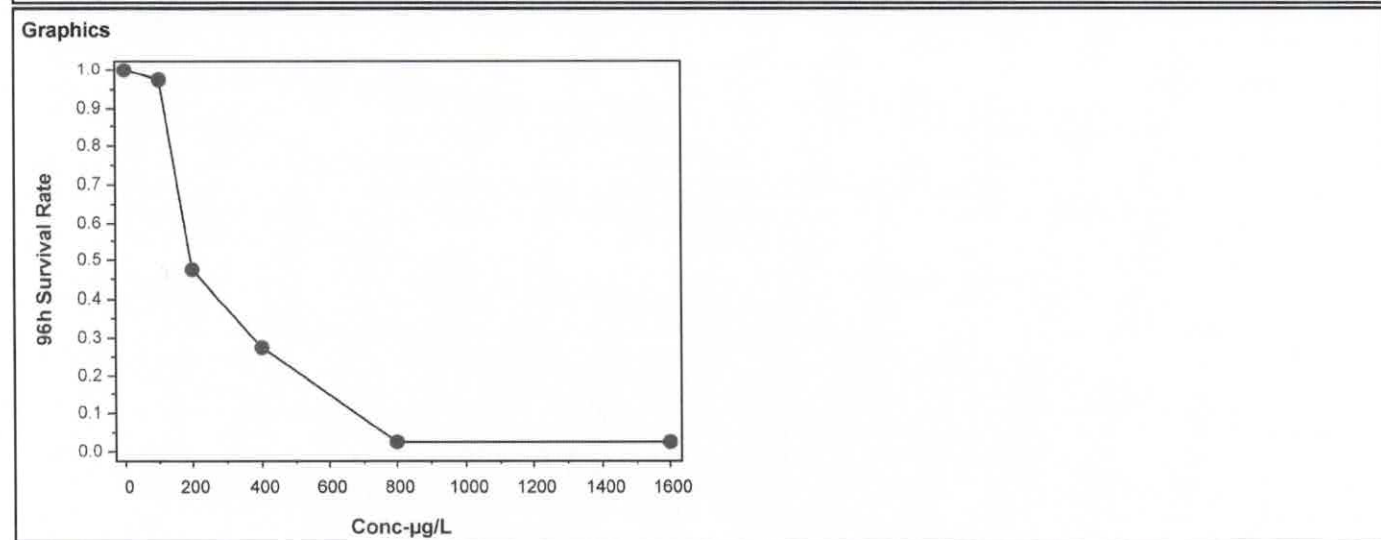
CETIS Analytical Report

Report Date: 08 Jan-25 11:34 (p 1 of 1)
 Test Code/ID: 241219harSED / 06-3922-3450

Acute Amphipod Survival Test				Nautilus Environmental (CA)	
Analysis ID: 08-5703-8091		Endpoint: 96h Survival Rate		CETIS Version: CETISv2.1.4	
Analyzed: 08 Jan-25 11:34		Analysis: Trimmed Spearman-Kärber		Status Level: 1	
Edit Date: 08 Jan-25 11:33		MD5 Hash: 501C103AC849C61E050645E048467162		Editor ID: 007-926-968-0	

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	2.50%	2.37	0.034	236	202	276

96h Survival Rate Summary			Calculated Variate(A/B)							Isotonic Variate	
Conc-µg/L	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	LC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	40/40	1.000	0.00%
100		4	0.975	1.000	0.900	1.000	5.13%	2.50%	39/40	0.975	2.50%
200		4	0.475	0.450	0.300	0.700	35.95%	52.50%	19/40	0.475	52.50%
400		4	0.275	0.233	0.200	0.400	34.82%	72.50%	11/40	0.275	72.50%
800		4	0.025	0.000	0.000	0.100	200.00%	97.50%	1/40	0.025	97.50%
1600		4	0.025	0.000	0.000	0.100	200.00%	97.50%	1/40	0.025	97.50%



CETIS QC Plot

Report Date: 08 Jan-25 11:34 (1 of 1)

Acute Amphipod Survival Test

Nautilus Environmental (CA)

Test Type: Survival (96h)

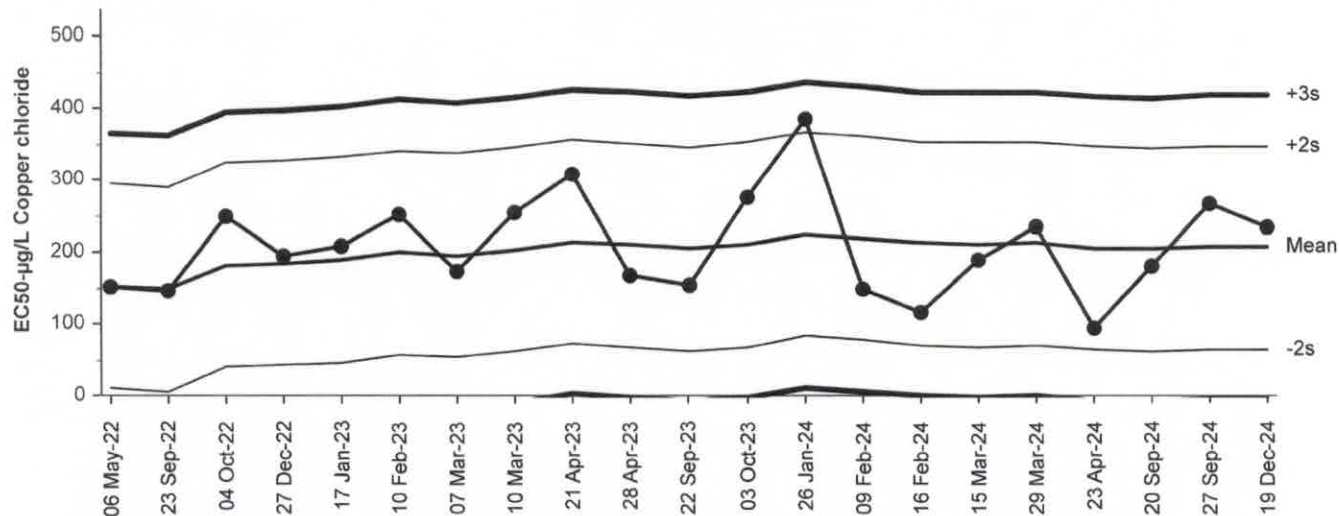
Organism: *Hyalella azteca*

Material: Copper chloride

Protocol: EPA/600/R-99/064 (2000)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF

Acute Amphipod Survival Test
96h Survival Rate Endpoint

Cumulative Mean Plot

Mean: 207.4 Count: 20 -2s Warning Limit: 65.3 -3s Action Limit: -5.72
Sigma: 71.04 CV: 34.30% +2s Warning Limit: 349 +3s Action Limit: 421

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	May	6	15:50	151.8	-55.63	-0.7831			18-9899-3969	02-1827-7020
2		Sep	23	18:10	144.7	-62.74	-0.8831			19-7717-5225	18-7002-2538
3		Oct	4	15:45	247.9	40.46	0.5695			12-1324-5905	16-9032-8146
4		Dec	27	16:30	193.8	-13.6	-0.1915			17-2611-4440	08-0671-0162
5	2023	Jan	17	17:30	207.2	-0.2226	-0.00313			04-7626-4182	05-5044-0652
6		Feb	10	17:00	252	44.58	0.6276			17-2264-1690	06-9899-9279
7		Mar	7	15:15	172.4	-35.01	-0.4928			05-7367-1481	11-4226-5734
8			10	15:40	254.7	47.32	0.666			13-0750-5556	19-2670-8300
9		Apr	21	17:15	308.4	101	1.422			03-0194-2656	10-5075-1011
10			28	15:10	168.2	-39.22	-0.5521			06-5993-5770	04-5746-7376
11		Sep	22	16:10	154.8	-52.64	-0.741			15-0126-4382	10-8932-6756
12		Oct	3	16:30	275.8	68.44	0.9634			14-9349-6936	10-0172-0824
13	2024	Jan	26	14:30	386.9	179.5	2.527	(+)		19-0033-5341	00-6517-8183
14		Feb	9	16:05	148.8	-58.65	-0.8256			00-8657-8400	08-9847-6763
15			16	15:25	114.9	-92.53	-1.303			04-0414-4548	19-6977-4716
16		Mar	15	15:40	188.6	-18.84	-0.2652			20-0438-2868	02-4656-0536
17			29	16:10	235.1	27.71	0.3901			18-6545-5346	19-6564-0167
18		Apr	23	17:55	94.74	-112.7	-1.586			12-0877-9386	17-4796-2357
19		Sep	20	14:15	180.2	-27.23	-0.3832			00-2900-9740	02-6845-2833
20			27	17:00	267.3	59.87	0.8428			01-7978-3149	06-8639-7282
21		Dec	19	16:30	235.7	28.28	0.3981			06-3922-3450	08-5703-8091

Freshwater Acute Bioassay

Static Conditions

DF-011 (SED)

Water Quality Measurements

& Test Organism Survival

Client: InternalTest Species: H. aztecaSample ID: CuCl₂Start Date/Time: 12/19/24 1630Test No.: 241219 har SEDEnd Date/Time: 12/23/24 1655

Tech Initials

0 24 48 72 96

Counts: WF WF WF WF WF

Readings: WF WF WF WF WF

Dilutions made by: WF

High conc. made (µg/L): 1600

Vol. Cu stock added (mL): 6.7

Final Volume (mL): 4000

Cu stock concentration (µg/L): 96,000

Concentration µg/L	Rand #	Number of Live Organisms		pH (units)					Dissolved Oxygen (mg/L)					Conductivity (µmhos/cm)					Temperature (°C)				
		0	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	4	10	10	8.30	8.31	8.34	8.34	8.58	8.6	8.6	8.4	8.2	8.4	822	962	953	844	834	22.0	22.0	22.6	22.7	22.5
	10	10	10																				
	5	10	10																				
	9	10	10																				
100	23	10	9	8.29	8.26	8.26	8.36	8.57	8.6	8.6	8.3	8.1	8.3	822	962	955	847	837	22.0	22.0	22.6	22.6	22.5
	18	10	10																				
	3	10	10																				
	6	10	10																				
200	19	10	3	8.28	8.27	8.25	8.35	8.59	8.7	8.6	8.3	8.1	8.4	822	961	954	846	837	22.1	22.0	22.6	22.5	22.5
	14	10	7																				
	2	10	4																				
	17	10	5																				
400	20	10	2	8.26	8.26	8.25	8.35	8.25	8.6	8.6	8.3	8.2	8.4	821	964	956	847	836	22.1	22.0	22.7	22.7	22.5
	11	10	4																				
	7	10	3																				
	22	10	2																				
800	8	10	1	8.22	8.22	8.20	8.32	8.25	8.6	8.6	8.3	8.1	8.3	819	958	949	841	830	22.1	22.1	22.7	22.7	22.6
	15	10	0																				
	1	10	0																				
	21	10	0																				
1600	13	10	0	8.05	8.16	8.19	8.30	8.23	8.5	8.5	8.3	8.1	8.3	813	951	943	835	825	22.5	22.5	22.9	22.9	22.7
	16	10	1																				
	24	10	0																				
	12	10	0																				

Rand # QC'd: WFCounts QC'd: PLInitiated by: WFEnvironmental Chamber: FAnimal Source/Date Received: ABO/24 12/18/24Age at Initiation: 9d

Animal Acclimation Qualifiers (circle all that apply):

Q22 / Q23 / Q24 / noneComments: Organisms fed prior to initiation, circle one (y/n) (A) 0.8 HH 12/19/24QC Check: ACS 1/8/25Final Review: YS 2/25/25