Data Quality Summary: Wyckoff 4th Quarter 2022 Groundwater Treatment Plant Bioassay Sampling

Samples were collected and analyzed in support of the Wyckoff Groundwater Treatment Plant. All analytical data were evaluated in accordance with the following guidance:

- *Wyckoff Groundwater Treatment Plant Operations and Maintenance Quality Assurance Project Plan* (QAPP), *Bainbridge Island, Washington* (CH2M, 2022).

This data quality summary presents the findings of the data validation activities.

Analytical Data

The methods, sample delivery group (SDG) number and laboratory name for all analyses are presented in Table 1. These reports can be found in Attachment 1.

Table 1. Analytical Data Summary
Data Quality Summary: Wyckoff Groundwater Treatment Plant Operations and Maintenance Q4 Bioassay

Laboratory	SDG	Method	Analyte
Enthalpy	2211-S332	EPA600/R-95/136	Chronic bioassay
Enthalpy	2212-S119	EPA821/R-02/012	Acute bioassay

Notes:
Enthalpy = Enthalpy Analytical
SDG = Sample Delivery Group

One water sample was collected on November 29, 2022, for chronic toxicity, and another sample collected on December 13, 2022, for acute toxicity. The bioassays were performed by Enthalpy Analytical, San Diego, California.

A CH2M chemist validated the bioassay results Stage 2A in accordance with the QAPP. The data were 100% complete, method and QAPP quality control requirements were met. The latest version of the QAPP was utilized by the laboratory, and it was confirmed that the appropriate species of mussel and estuarine fish that was specified in the QAPP were used.

For the chronic bioassay, there were no statistically significant effects detected in any effluent concentration tested for the survival endpoint of the bivalve test. This results in a NOEC of 67.2 (the highest concentration tested) and a TUc of less than 1.5. There was a statistically significant effect detected in the 67.2 percent effluent concentration for the development endpoint. This results in a NOEC of 35 and a TUc of 2.9. Overall, the effect concentration (EC_{50}) expected to cause an effect to 50% of the organisms is determined to be greater than 67.2 (the highest concentration tested).

For the acute bioassay, there was no statistically significant effect detected to survival for any concentration tested in the inland silverside test. This results in a LC50 of greater than 100 percent concentration and an acute toxic unit (TUa) of less than 1.0.

Attachment 1
Bioassay Reports

ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: November 2022

Prepared for: **Jacobs**
1100 112th Ave NE Suite 500
Bellevue, WA, 98004

Prepared by: **Enthalpy Analytical**
4340 Vandever Avenue
San Diego, CA 92120
(858) 587-7333

Date Submitted: **January 9, 2023**

Data Quality Assurance:

- Enthalpy Analytical is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (ORELAP ID 4053). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective US EPA protocols, unless otherwise noted in this report.
- All tests have met internal Quality Assurance Program requirements.

Results verified by: _____
Barbara Orelo, Project Manager

California
4340 Vandever Avenue
San Diego, California 92120
858.587.7333
fax: 619.279.5919

Introduction

A toxicity test was performed using a groundwater composite sample collected from the Wyckoff Eagle Harbor Groundwater Treatment Plant on Bainbridge Island in Washington. This test was performed to satisfy quarterly monitoring requirements according to the project Quality Assurance Project Plan (QAPP 2022). The chronic bioassay was conducted using the bivalve *Mytilus galloprovincialis* (Mediterranean mussel). Testing was performed at Enthalpy Analytical located in San Diego, California.

Materials and Methods

The groundwater sample was collected into a low-density polyethylene cubitainer by Jacobs personnel, packed in a cooler containing ice, and shipped overnight to Enthalpy. Appropriate chain-of-custody (COC) procedures were employed during collection and transport. Upon arrival at the laboratory, the cooler was opened, the sample inspected, and the contents verified against information on the COC form. Standard water quality parameters were measured and recorded on a sample check-in form and are summarized in Table 1. The sample was stored at 4°C in the dark until used for testing.

Table 1. Sample Information

Sample ID	**112922**
Enthalpy Log-in Number	22-1486
Collection Date; Time	11/29/22; 1000h
Receipt Date; Time	11/30/22; 1314h
Receipt Temperature (°C)	3.2
Dissolved Oxygen (mg/L)	6.8
pH	7.53
Conductivity (µS/cm)	2,200
Salinity (ppt)	1.2
Alkalinity (mg/L $CaCO_3$)	342
Total Chlorine (mg/L)	0.04
Total Ammonia (mg/L as N)	7.5

Test Methods

Chronic toxicity testing was conducted according to the method set forth in USEPA (1995) and WDOE (2016). This method is summarized in Table 2.

Table 2. Summary of Methods for the Bivalve Larval Development Test

Test Period	11/30/22, 1655h to 12/2/22, 1530h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	M-Rep (Carlsbad, CA)
Test Organism Age	4 hours post fertilization
Test Duration	48 ± 2 hours
Test Type	Static
Test Chamber, Test Solution Volume	30 mL glass vial, 10 mL
Test Temperature	15 ± 1°C
Dilution Water	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) diluted with de-ionized water
Additional Control	Brine Control (de-ionized water and hypersaline brine)
Test Salinity	30 ± 2 ppt
Source of Salinity	Hypersaline brine made by freezing seawater to a salinity of 89.1 ppt
Test Concentrations (% sample)	67.2[a], 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper chloride [b]
Statistical Software	CETIS™ 2.1.2.3

[a] Highest concentration tested due to the addition of hypersaline brine

[b] A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride. See QA section.

Statistical Methods

Statistical analyses were conducted using USEPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in the sample was compared to that observed in the brine control. Results were used to calculate the No Observed Effect Concentration (NOEC) and the concentrations expected to cause an adverse effect to 50 percent of test organisms (EC_{50}). The chronic toxic unit (TU_c) value was calculated as 100/NOEC, as specified in the permit. The statistical analyses were performed using the Comprehensive Environmental Toxicity Information System™ (CETIS), version 2.1.2.3 by Tidepool Scientific Software.

Results

There were no statistically significant effects detected in any effluent concentration tested for the survival endpoint of the bivalve test. This results in a NOEC of 67.2 (the highest concentration tested) and a TU_c of less than 1.5.

There was a statistically significant effect detected in the 67.2 percent effluent concentration for the development endpoint. This results in a NOEC of 35 and a TU_c of 2.9.

Results for the chronic toxicity test are summarized in Tables 3 and 4. Individual statistical summaries for the test and copies of the laboratory bench sheets are provided in Appendix A. The sample check-in sheet and COC form are provided in Appendices B and C, respectively.

Table 3. Summary of Statistical Results for the Chronic Toxicity Tests

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_c)	EC_{50} (% effluent)
Bivalve	Normal Development	35	67.2	2.9	> 67.2
	Survival	67.2	> 67.2	< 1.5	> 67.2

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

Chronic Toxic Unit (TU_c) = 100/NOEC. NOTE: Since 100% sample was not tested, the TU_c value can only be calculated up to the highest concentration tested. If no toxicity is observed at this concentration, the TU_c is reported as less than the calculated value.

Effect Concentration 50 (EC_{50}) = Concentration expected to cause an effect to 50% of the organisms

Table 4. Detailed Results for the Bivalve Development Chronic Toxicity Test

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	91.8	96.9
0 (Lab Control)	95.2	98.3
2	97.7	98.2
4	95.5	98.3
9	98.8	97.8
18	98.6	98.2
35	98.1	98.8
67.2[a]	95.6	**59.4**

[a] Highest concentration tested due to the addition of hypersaline brine

Values in **bold** indicate a statistically significant effect.

Quality Assurance

The sample was received the day after collection, in good condition, and within the appropriate temperature range of 0-6°C. The test was initiated within the required 36-hour holding time. All control acceptability criteria were met, and water quality parameters remained within the appropriate ranges throughout the test. Statistical analyses followed standard USEPA flowchart selections. Dose-response relationships were reviewed to ensure the reliability of the results. Based on the dose response observed, the calculated effects concentrations were deemed reliable. Minor QA/QC issues that were unlikely to have any bearing on the final test results, such as slight temperature deviations, are noted on the data sheets and a list of qualifier codes used on bench data sheets is presented in Appendix D.

Reference Toxicant

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride rather than copper sulfate. The results for the concurrent reference toxicant test were within the acceptable range of the mean historical test results plus or minus two standard deviations for development and survival. Reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 5. Reference Toxicant Test Results

Species and Endpoint	**NOEC** (%)	**EC_{50}** (µg/L copper)	**Historical Mean ± 2 SD** (µg/L copper)	**CV** (%)
Bivalve Survival Rate	10	26.8	26.8 ± 6.54	12.2
Bivalve Normal Development	2.5	7.17	9.24 ± 4.54	24.6

NOEC = No Observed Effect Concentration

Effect Concentration 50 (EC_{50}) = The concentration expected to cause an effect to 50% of the organisms

Historical Mean ± 2 SD = The mean EC_{50} from the previous 20 tests performed by the laboratory, plus or minus two standard deviations (SD)

CV = Coefficient of Variation

References

CH2MHill. 2022. Quality Assurance Project Plan – Groundwater Treatment Plant Operations, Maintenance, Bainbridge, Washington. Prepared for USEPA Region, January 2022.

Standard Guide for Conducting Static Acute Toxicity Tests with Embryos of Four Species of Saltwater Bivalve Molluscs. 1989. ASTM Standard E 724-89.

Tidepool Scientific Software. 2000-2022. CETIS Comprehensive Environmental Toxicity Information System Software, Version 2.1.2.3.

USEPA. 1995. Short-Term Method for Estimating the Chronic Toxicity of Effluents and Receiving Waters to the West Coast Marine and Estuarine Organisms. EPA/600/R-95/136. pp. 209-258 and 389-465.

Washington State Department of Ecology. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Publication No. WQ-R-95-80. Revised June 2016

Appendix A
Statistical Summaries and Raw Bench Sheets

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Batch ID:	07-1916-2625	**Test Type:**	Development-Survival	**Analyst:**			
Start Date:	30 Nov-22 16:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater		
Ending Date:	02 Dec-22 15:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater		
Test Length:	47h	**Taxon:**		**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	16-3753-9921	**Code:**	22-1486	**Project:**	
Sample Date:	29 Nov-22 10:00	**Material:**	Effluent Sample	**Source:**	Jacobs
Receipt Date:	30 Nov-22 13:14	**CAS (PC):**		**Station:**	Wyckoff
Sample Age:	31h (3.2 °C)	**Client:**	Jacobs		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
11-3857-6594	Combined Development Rat	Dunnett Multiple Comparison Test		35	67.2	48.5	9.27%	2.9	1
00-7714-8399	Development Rate	Dunnett Multiple Comparison Test		35	67.2	48.5	1.94%	2.9	1
17-4497-2337	Survival Rate	Dunnett Multiple Comparison Test		67.2	>67.2	---	9.37%	< 1.5	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
03-9255-2551	Combined Development Rat	Linear Interpolation (ICPIN)		EC25	55	52.4	58	1.8	1
				EC50	>67.2	---	---	<1.5	
02-1155-3168	Development Rate	Linear Interpolation (ICPIN)		EC25	55.4	52	59.1	1.8	1
				EC50	>67.2	---	---	<1.5	
01-0736-3836	Survival Rate	Linear Interpolation (ICPIN)		EC25	>67.2	---	---	<1.5	1
				EC50	>67.2	---	---	<1.5	

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
00-7714-8399	Development Rate	Control Resp	0.969	0.9	<<	Yes	Passes Criteria
02-1155-3168	Development Rate	Control Resp	0.969	0.9	<<	Yes	Passes Criteria
01-0736-3836	Survival Rate	Control Resp	0.918	0.5	<<	Yes	Passes Criteria
17-4497-2337	Survival Rate	Control Resp	0.918	0.5	<<	Yes	Passes Criteria
11-3857-6594	Combined Development Rat	PMSD	0.0927	<<	0.25	No	Passes Criteria

Bivalve Larval Survival and Development Test
Nautilus Environmental (CA)

Combined Development Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	BC	5	0.890	0.747	1.030	0.690	0.973	0.051	0.115	12.92%	0.00%
0	LC	5	0.935	0.897	0.974	0.903	0.982	0.014	0.031	3.33%	-5.09%
2		5	0.959	0.906	1.010	0.884	0.987	0.019	0.043	4.43%	-7.74%
4		5	0.938	0.888	0.988	0.875	0.987	0.018	0.040	4.28%	-5.40%
9		5	0.966	0.941	0.992	0.931	0.982	0.009	0.021	2.13%	-8.55%
18		5	0.968	0.936	1.000	0.931	0.996	0.012	0.026	2.69%	-8.78%
35		5	0.970	0.935	1.000	0.940	0.996	0.012	0.028	2.85%	-8.94%
67.2		5	0.567	0.514	0.620	0.502	0.620	0.019	0.043	7.56%	36.28%

Development Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	BC	5	0.969	0.958	0.980	0.955	0.976	0.004	0.009	0.91%	0.00%
0	LC	5	0.983	0.976	0.989	0.976	0.990	0.002	0.005	0.52%	-1.39%
2		5	0.982	0.975	0.989	0.973	0.987	0.002	0.006	0.57%	-1.28%
4		5	0.983	0.977	0.988	0.976	0.987	0.002	0.004	0.45%	-1.39%
9		5	0.978	0.974	0.982	0.974	0.982	0.002	0.003	0.35%	-0.90%
18		5	0.982	0.968	0.995	0.966	0.996	0.005	0.011	1.10%	-1.29%
35		5	0.988	0.981	0.995	0.982	0.996	0.002	0.005	0.55%	-1.92%
67.2		5	0.594	0.523	0.665	0.502	0.654	0.025	0.057	9.58%	38.71%

Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	BC	5	0.918	0.778	1.060	0.722	1.000	0.050	0.112	12.26%	0.00%
0	LC	5	0.952	0.909	0.995	0.917	1.000	0.015	0.034	3.62%	-3.73%
2		5	0.977	0.924	1.030	0.903	1.000	0.019	0.042	4.32%	-6.46%
4		5	0.955	0.904	1.000	0.889	1.000	0.018	0.040	4.23%	-4.04%
9		5	0.988	0.963	1.010	0.954	1.000	0.009	0.020	2.03%	-7.67%
18		5	0.986	0.962	1.010	0.963	1.000	0.009	0.019	1.94%	-7.47%
35		5	0.981	0.950	1.010	0.954	1.000	0.011	0.025	2.58%	-6.96%
67.2		5	0.956	0.919	0.994	0.921	1.000	0.014	0.031	3.19%	-4.24%

Bivalve Larval Survival and Development Test **Nautilus Environmental (CA)**

Combined Development Rate Detail MD5: 8212D4D92BCE9770D2E81A032252E2D7

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	0.954	0.931	0.903	0.973	0.690
0	LC	0.912	0.982	0.935	0.903	0.944
2		0.973	0.983	0.968	0.884	0.987
4		0.940	0.944	0.875	0.944	0.987
9		0.968	0.931	0.982	0.977	0.974
18		0.996	0.978	0.954	0.931	0.983
35		0.991	0.982	0.940	0.940	0.996
67.2		0.502	0.583	0.569	0.560	0.620

Development Rate Detail MD5: 2BDF87BB681BC472C9658C63ACA44F83

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	0.976	0.966	0.975	0.973	0.955
0	LC	0.990	0.982	0.981	0.985	0.976
2		0.973	0.983	0.986	0.979	0.987
4		0.976	0.986	0.984	0.981	0.987
9		0.981	0.976	0.982	0.977	0.974
18		0.996	0.978	0.986	0.966	0.983
35		0.991	0.982	0.985	0.985	0.996
67.2		0.502	0.621	0.586	0.608	0.654

Survival Rate Detail MD5: B5549213E865B7CE0E4BE5D9D9DECF26

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	0.977	0.963	0.926	1.000	0.722
0	LC	0.921	1.000	0.954	0.917	0.968
2		1.000	1.000	0.981	0.903	1.000
4		0.963	0.958	0.889	0.963	1.000
9		0.986	0.954	1.000	1.000	1.000
18		1.000	1.000	0.968	0.963	1.000
35		1.000	1.000	0.954	0.954	1.000
67.2		1.000	0.940	0.972	0.921	0.949

Combined Development Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	206/216	201/216	195/216	220/226	149/216
0	LC	197/216	221/225	202/216	195/216	204/216
2		219/225	225/229	209/216	191/216	232/235
4		203/216	204/216	189/216	204/216	227/230
9		209/216	201/216	213/217	216/221	223/229
18		237/238	226/231	206/216	201/216	229/233
35		220/222	217/221	203/216	203/216	229/230
67.2		109/217	126/216	123/216	121/216	134/216

Development Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	206/211	201/208	195/200	220/226	149/156
0	LC	197/199	221/225	202/206	195/198	204/209
2		219/225	225/229	209/212	191/195	232/235
4		203/208	204/207	189/192	204/208	227/230
9		209/213	201/206	213/217	216/221	223/229
18		237/238	226/231	206/209	201/208	229/233
35		220/222	217/221	203/206	203/206	229/230
67.2		109/217	126/203	123/210	121/199	134/205

Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	211/216	208/216	200/216	216/216	156/216
0	LC	199/216	216/216	206/216	198/216	209/216
2		216/216	216/216	212/216	195/216	216/216
4		208/216	207/216	192/216	208/216	216/216
9		213/216	206/216	216/216	216/216	216/216
18		216/216	216/216	209/216	208/216	216/216
35		216/216	216/216	206/216	206/216	216/216
67.2		216/216	203/216	210/216	199/216	205/216

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	11-3857-6594	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	EFFDC09D775D17F9977D6F6E34552561	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	35	67.2	48.5	2.9	0.0825	9.27%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Brine Control		2	8	-2.07	2.41	0.143	CDF	0.9997	Non-Significant Effect
		4	8	-1.2	2.41	0.143	CDF	0.9932	Non-Significant Effect
		9	8	-2.21	2.41	0.143	CDF	0.9999	Non-Significant Effect
		18	8	-2.45	2.41	0.143	CDF	0.9999	Non-Significant Effect
		35	8	-2.57	2.41	0.143	CDF	0.9999	Non-Significant Effect
		67.2*	8	6.84	2.41	0.143	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.20431	0.200718	6	22.7	<1.0E-05	Significant Effect
Error	0.247635	0.0088441	28			
Total	1.45194		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	8.58	16.8	0.1986	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.928	0.915	0.0238	Normal Distribution

Combined Development Rate Summary

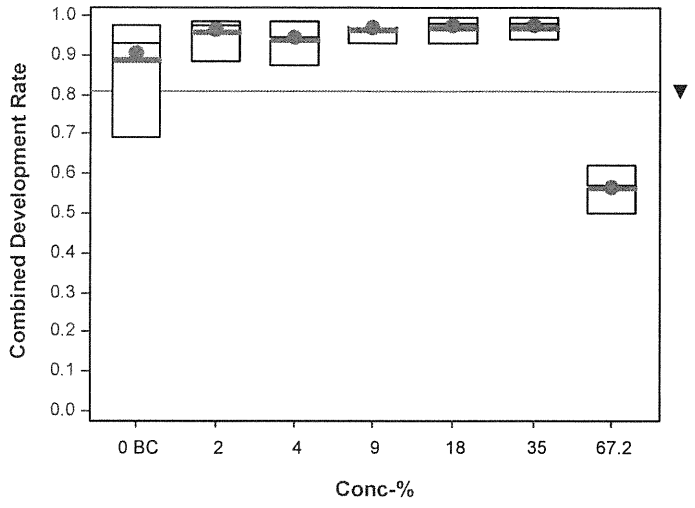
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	0.890	0.747	1.000	0.931	0.690	0.973	0.051	12.92%	0.00%
2		5	0.959	0.906	1.000	0.973	0.884	0.987	0.019	4.43%	-7.74%
4		5	0.938	0.888	0.988	0.944	0.875	0.987	0.018	4.28%	-5.40%
9		5	0.966	0.941	0.992	0.974	0.931	0.982	0.009	2.13%	-8.55%
18		5	0.968	0.936	1.000	0.978	0.931	0.996	0.012	2.69%	-8.78%
35		5	0.970	0.935	1.000	0.982	0.940	0.996	0.012	2.85%	-8.94%
67.2		5	0.567	0.514	0.620	0.569	0.502	0.620	0.019	7.56%	36.28%

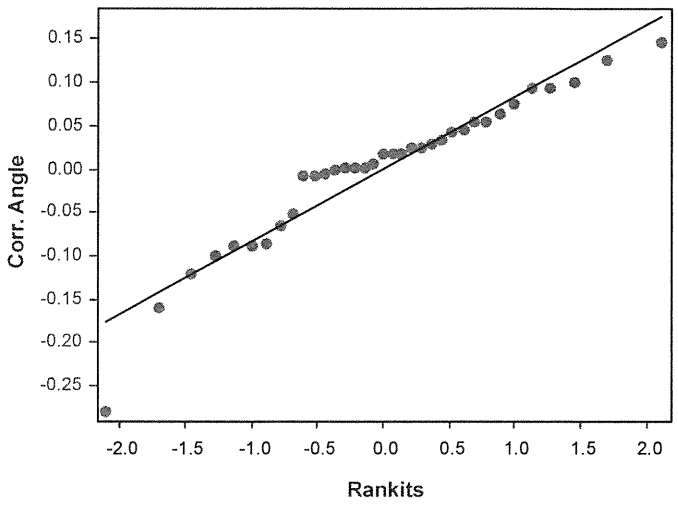
Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	1.260	1.050	1.470	1.300	0.980	1.410	0.074	13.21%	0.00%
2		5	1.380	1.270	1.500	1.410	1.220	1.460	0.042	6.73%	-9.80%
4		5	1.330	1.220	1.440	1.330	1.210	1.460	0.039	6.57%	-5.64%
9		5	1.390	1.330	1.460	1.410	1.300	1.430	0.023	3.70%	-10.44%
18		5	1.410	1.310	1.500	1.420	1.300	1.510	0.035	5.57%	-11.55%
35		5	1.410	1.310	1.520	1.440	1.320	1.500	0.038	6.04%	-12.12%
67.2		5	0.853	0.799	0.907	0.855	0.788	0.907	0.019	5.07%	32.30%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	11-3857-6594	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	EFFDC09D775D17F9977D6F6E34552561	**Editor ID:**	007-926-968-0

Graphics





Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	00-7714-8399	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	6E4EA53C951191645760F53DD80B8235	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	35	67.2	48.5	2.9	0.0188	1.94%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Brine Control		2	8	-1.97	2.41	0.0495	CDF	0.9995	Non-Significant Effect
		4	8	-2.14	2.41	0.0495	CDF	0.9997	Non-Significant Effect
		9	8	-1.28	2.41	0.0495	CDF	0.9948	Non-Significant Effect
		18	8	-2.2	2.41	0.0495	CDF	0.9998	Non-Significant Effect
		35	8	-3.28	2.41	0.0495	CDF	1.0000	Non-Significant Effect
		67.2*	8	25.1	2.41	0.0495	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.32114	0.220189	6	208	<1.0E-05	Significant Effect
Error	0.0296007	0.0010572	28			
Total	1.35074		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	12.9	16.8	0.0440	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.943	0.915	0.0674	Normal Distribution

Development Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	0.969	0.958	0.980	0.973	0.955	0.976	0.004	0.91%	0.00%
2		5	0.982	0.975	0.989	0.983	0.973	0.987	0.002	0.57%	-1.28%
4		5	0.983	0.977	0.988	0.984	0.976	0.987	0.002	0.45%	-1.39%
9		5	0.978	0.974	0.982	0.977	0.974	0.982	0.002	0.35%	-0.90%
18		5	0.982	0.968	0.995	0.983	0.966	0.996	0.005	1.10%	-1.29%
35		5	0.988	0.981	0.995	0.985	0.982	0.996	0.002	0.55%	-1.92%
67.2		5	0.594	0.523	0.665	0.608	0.502	0.654	0.025	9.58%	38.71%

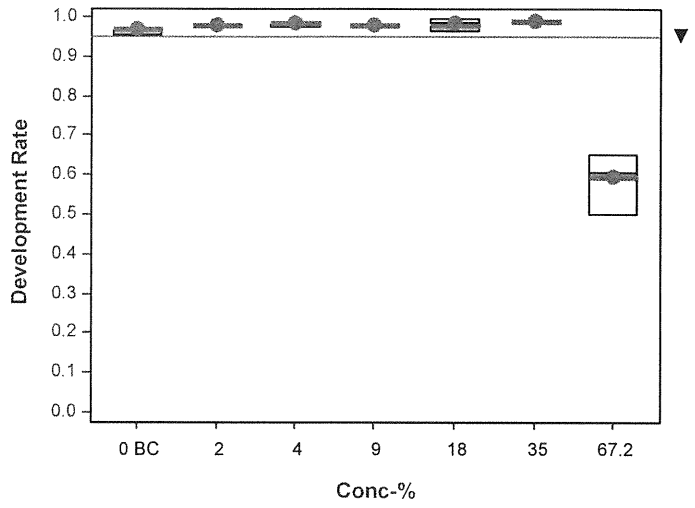
Angular (Corrected) Transformed Summary

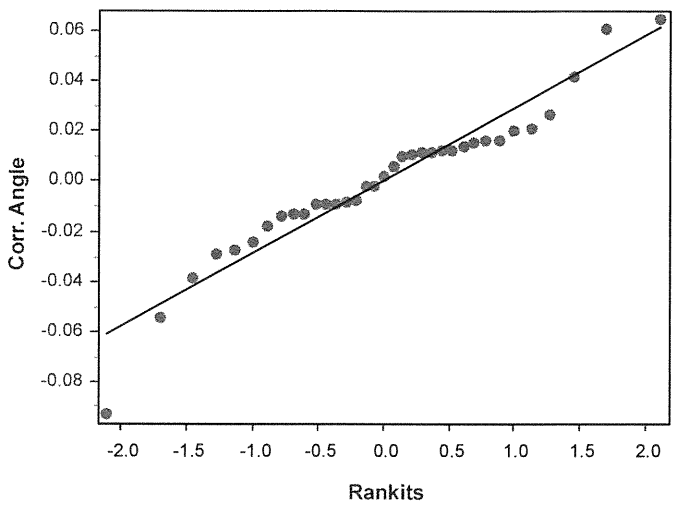
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	1.400	1.370	1.430	1.410	1.360	1.420	0.011	1.75%	0.00%
2		5	1.440	1.410	1.460	1.440	1.410	1.460	0.009	1.41%	-2.90%
4		5	1.440	1.420	1.460	1.450	1.420	1.460	0.007	1.14%	-3.15%
9		5	1.420	1.410	1.440	1.420	1.410	1.430	0.005	0.82%	-1.88%
18		5	1.440	1.390	1.500	1.440	1.390	1.510	0.020	3.03%	-3.24%
35		5	1.460	1.430	1.500	1.450	1.440	1.500	0.012	1.87%	-4.83%
67.2		5	0.880	0.809	0.952	0.894	0.788	0.942	0.026	6.56%	36.92%

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	00-7714-8399	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	6E4EA53C951191645760F53DD80B8235	**Editor ID:**	007-926-968-0

Graphics





Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	17-4497-2337	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	F4F72CB7E06B7C017E4118C577E07F98	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	67.2	>67.2	---	1.5	0.0859	9.37%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Brine Control		2	8	-1.75	2.41	0.181	CDF	0.9989	Non-Significant Effect
		4	8	-0.652	2.41	0.181	CDF	0.9674	Non-Significant Effect
		9	8	-2.06	2.41	0.181	CDF	0.9997	Non-Significant Effect
		18	8	-1.96	2.41	0.181	CDF	0.9995	Non-Significant Effect
		35	8	-1.8	2.41	0.181	CDF	0.9991	Non-Significant Effect
		67.2	8	-0.666	2.41	0.181	CDF	0.9686	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.111795	0.0186325	6	1.32	0.2790	Non-Significant Effect
Error	0.393749	0.0140625	28			
Total	0.505544		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.63	16.8	0.5915	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.951	0.915	0.1193	Normal Distribution

Survival Rate Summary

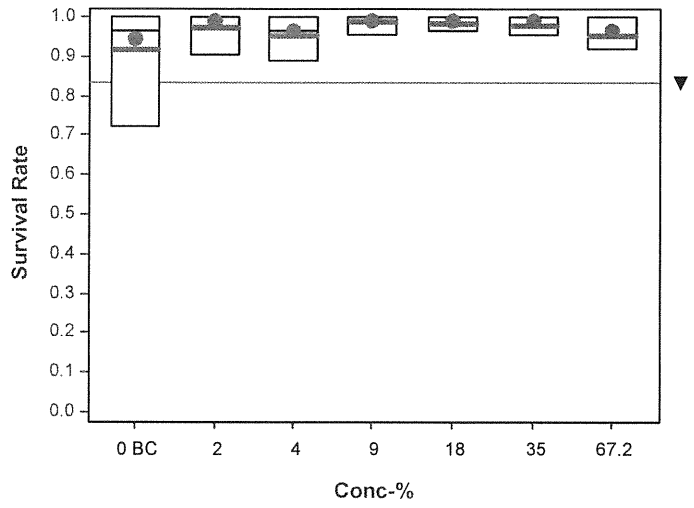
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	0.918	0.778	1.000	0.963	0.722	1.000	0.050	12.26%	0.00%
2		5	0.977	0.924	1.000	1.000	0.903	1.000	0.019	4.32%	-6.46%
4		5	0.955	0.904	1.000	0.963	0.889	1.000	0.018	4.23%	-4.04%
9		5	0.988	0.963	1.000	1.000	0.954	1.000	0.009	2.03%	-7.67%
18		5	0.986	0.962	1.000	1.000	0.963	1.000	0.009	1.94%	-7.47%
35		5	0.981	0.950	1.000	1.000	0.954	1.000	0.011	2.58%	-6.96%
67.2		5	0.956	0.919	0.994	0.949	0.921	1.000	0.014	3.19%	-4.24%

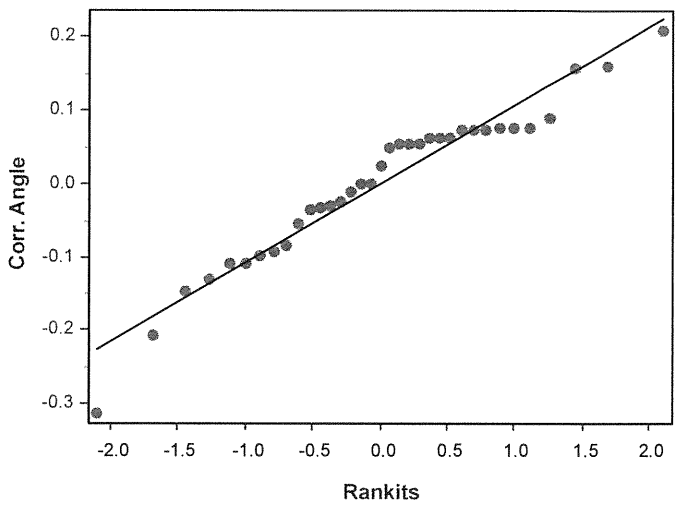
Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	1.330	1.090	1.570	1.380	1.020	1.540	0.087	14.71%	0.00%
2		5	1.460	1.310	1.610	1.540	1.250	1.540	0.055	8.45%	-9.87%
4		5	1.380	1.240	1.510	1.380	1.230	1.540	0.049	7.87%	-3.68%
9		5	1.480	1.380	1.580	1.540	1.350	1.540	0.036	5.46%	-11.65%
18		5	1.480	1.370	1.580	1.540	1.380	1.540	0.038	5.70%	-11.06%
35		5	1.460	1.340	1.590	1.540	1.350	1.540	0.045	6.84%	-10.17%
67.2		5	1.380	1.260	1.500	1.340	1.290	1.540	0.044	7.11%	-3.76%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	17-4497-2337	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	F4F72CB7E06B7C017E4118C577E07F98	**Editor ID:**	007-926-968-0

Graphics





Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	03-9255-2551	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	EFFDC09D775D17F9977D6F6E34552561	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1178517	1000	Yes	Two-Point Interpolation

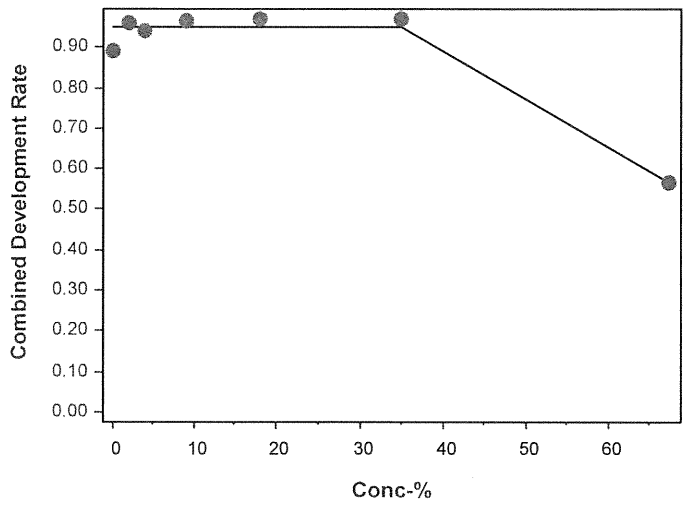
Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	55	52.4	58	1.8	1.7	1.9
EC50	>67.2	---	---	<1.5	---	---

Combined Development Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	BC	5	0.890	0.931	0.690	0.973	12.92%	0.00%	971/1090	0.949	0.00%
2		5	0.959	0.973	0.884	0.987	4.43%	-7.74%	1076/1121	0.949	0.00%
4		5	0.938	0.944	0.875	0.987	4.28%	-5.40%	1027/1094	0.949	0.00%
9		5	0.966	0.974	0.931	0.982	2.13%	-8.55%	1062/1099	0.949	0.00%
18		5	0.968	0.978	0.931	0.996	2.69%	-8.78%	1099/1134	0.949	0.00%
35		5	0.970	0.982	0.940	0.996	2.85%	-8.94%	1072/1105	0.949	0.00%
67.2		5	0.567	0.569	0.502	0.620	7.56%	36.28%	613/1081	0.567	40.25%

Graphics



Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	02-1155-3168	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	6E4EA53C951191645760F53DD80B8235	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1697317	1000	Yes	Two-Point Interpolation

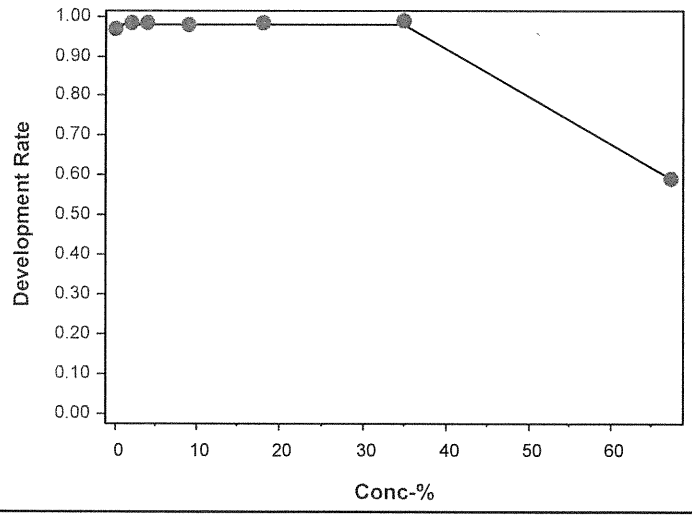
Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	55.4	52	59.1	1.8	1.7	1.9
EC50	>67.2	---	---	<1.5	---	---

Development Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	BC	5	0.969	0.973	0.955	0.976	0.91%	0.00%	971/1001	0.980	0.00%
2		5	0.982	0.983	0.973	0.987	0.57%	-1.28%	1076/1096	0.980	0.00%
4		5	0.983	0.984	0.976	0.987	0.45%	-1.39%	1027/1045	0.980	0.00%
9		5	0.978	0.977	0.974	0.982	0.35%	-0.90%	1062/1086	0.980	0.00%
18		5	0.982	0.983	0.966	0.996	1.10%	-1.29%	1099/1119	0.980	0.00%
35		5	0.988	0.985	0.982	0.996	0.55%	-1.92%	1072/1085	0.980	0.00%
67.2		5	0.594	0.608	0.502	0.654	9.58%	38.71%	613/1034	0.593	39.49%

Graphics



Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	01-0736-3836	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:00	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	06 Jan-23 10:54	**MD5 Hash:**	F4F72CB7E06B7C017E4118C577E07F98	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1425004	1000	Yes	Two-Point Interpolation

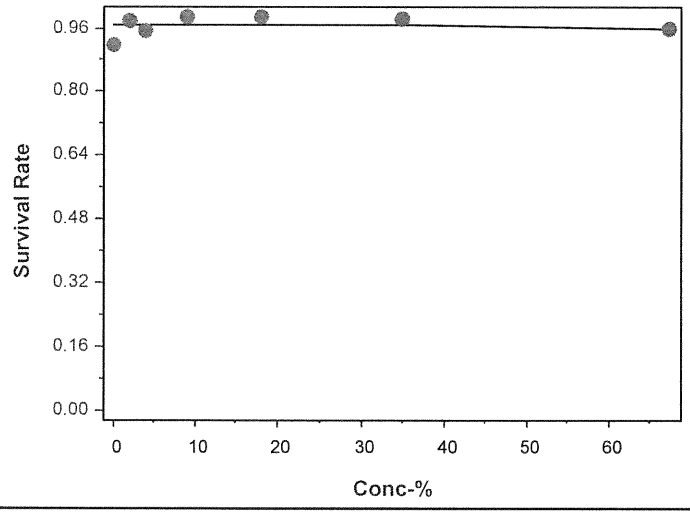
Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	>67.2	---	---	<1.5	---	---
EC50	>67.2	---	---	<1.5	---	---

Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	BC	5	0.918	0.963	0.722	1.000	12.26%	0.00%	991/1080	0.967	0.00%
2		5	0.977	1.000	0.903	1.000	4.32%	-6.46%	1055/1080	0.967	0.00%
4		5	0.955	0.963	0.889	1.000	4.23%	-4.04%	1031/1080	0.967	0.00%
9		5	0.988	1.000	0.954	1.000	2.03%	-7.67%	1067/1080	0.967	0.00%
18		5	0.986	1.000	0.963	1.000	1.94%	-7.47%	1065/1080	0.967	0.00%
35		5	0.981	1.000	0.954	1.000	2.58%	-6.96%	1060/1080	0.967	0.00%
67.2		5	0.956	0.949	0.921	1.000	3.19%	-4.24%	1033/1080	0.956	1.14%

Graphics



CETIS Test Data Worksheet

Report Date: 29 Nov-22 15:54 (p 1 of 1)
Test Code/ID: 2211-832 45260D7 / 00-7250-7607

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Start Date:	30 Nov-22	**Species:**	Mytilus galloprovincialis	**Sample Code:**	22-1486
End Date:	02 Dec-22	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs
Sample Date:	29 Nov-22	**Material:**	Effluent Sample	**Sample Station:**	Wyckoff

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			208	201	MM 12/27/22
			32			206	203	
			33			195	191	
			34			212	209	
			35			230	229	
			36			229	223	
			37			156	149	
			38			225	219	
			39			230	227	
			40			222	220	
			41			211	206	MM 12/28/22
			42			221	216	
			43			238	237	
			44			192	189	
			45			225	221	
			46			233	229	
			47			198	195	
			48			231	226	
			49			208	201	
			50			~~215~~ (B) 210	~~+3~~ (B) 123	
			51			200	195	
			52			226	220	
			53			235	232	
			54			209	209	
			55			208	204	
			56			209	206	
			57			208	203	
			58			213	209	
			59			~~214~~ 214 (B)	~~+5~~ 104 (B)	
			60			~~209~~ (B) 205	~~+6~~ 134 (B)	
			61			217	213	
			62			~~(A) 205~~ 203	~~+6~~ 126	
			63			206 (B)	203	
			64			207	204	
			65			206	201	
			66			221	217	
			67			206	202	
			68			~~19~~ 8 (B) 199	~~+6~~ 121 (B)	
			69			229	225	
			70			199	197	

(A) QC'd MM 12/28/22 (B) QC'd MM 1/5/23

Analyst: ACS OBO MM QA: ACS 1/6/23

CETIS Test Data Worksheet

Report Date: ~~29~~ 3332 Nov-22 15:54 (p 1 of 1)
Test Code/ID: 2211-3332 (A) 45260D7 / 00-7250-7607

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Start Date:	30 Nov-22	**Species:**	Mytilus galloprovincialis	**Sample Code:**	22-1486
End Date:	02 Dec-22	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs
Sample Date:	29 Nov-22	**Material:**	Effluent Sample	**Sample Station:**	Wyckoff

Conc-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	41			221	216	EG 12/6/22
0	BC	2	49					
0	BC	3	51					
0	BC	4	52					
0	BC	5	37					
0	LC	1	70			205	203	
0	LC	2	45					
0	LC	3	67					
0	LC	4	47					
0	LC	5	54					
2		1	38			229	223	
2		2	69					
2		3	34					
2		4	33					
2		5	53					
4		1	57			208	202	
4		2	64					
4		3	44					
4		4	55					
4		5	39					
9		1	58			203	199	
9		2	65					
9		3	61					
9		4	42					
9		5	36					
18		1	43			225	225	
18		2	48					
18		3	56					
18		4	31					
18		5	46					
35		1	40			228	222	
35		2	66					
35		3	32					
35		4	63					
35		5	35					
72.5 (A)		1	59			220	121	
72.5		2	62					
72.5		3	50					
72.5		4	68					
72.5		5	60					

67.2%

QC: RT

(A) QC'd ARS 1/6/23

Analyst: ARS OBO HH QA: ARS 1/6/23

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS

Sample ID: Wyckoff

Sample Log No.: 22- 1486

Test No.: 2211-S332

Test Species: *M. galloprovincialis*

Start Date/Time: 11/30/22 1655

End Date/Time: 12/2/22 1530

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	30.5	30.4	30.3	15.1	14.8	14.8	8.7	8.7	8.7	8.03	8.02	7.96
Brine Control	30.6	30.3	30.3	16.0	14.6	14.6	7.9	8.6	8.7	8.09	8.05	7.97
2	30.4	30.6	30.6	15.6	14.6	14.5	8.7	8.7	8.7	8.01	8.00	7.97
4	30.5	30.6	30.6	15.4	14.7	14.6	8.7	8.6	8.7	7.98	7.99	7.98
9	30.5	30.5	30.5	15.3	14.7	14.6	8.7	8.6	8.7	7.91	7.98	8.00
18	30.5	30.5	30.5	15.1	14.9	14.7	8.6	8.5	8.6	7.80	7.95	8.04
35	30.3	30.5	30.6	14.1	14.7	14.5	8.6	8.5	8.7	7.67	7.99	8.12
~~75~~ 67.2	30.3	30.3	30.3	14.1	14.8	14.7	8.0	8.3	8.6	7.57	7.98	8.17
QB RT 12/2/22												

Technician Initials:

	0	24	48
WQ Readings:	RT	~~RT~~ (A)	GM
Dilutions made by:	RT	—	—

Environmental Chamber: D

Comments:

0 hrs:

24 hrs: (A) QB RT 11/30/22

48 hrs:

QC Check: ACS 1/6/23

Final Review: BO 1/9/23

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** RT

Sample ID: Wyckoff **Test Date:** 11/30/2022

Test No: 2211-5332 **Test Type:** Mussel Development

Salinity of Effluent 1.2

Salinity of Brine 89.1 **Date of Brine used:** 10/11/2022

Target Salinity 30 **Alkalinity of Brine Control:** 117 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.49**	**0.51**

TS = target salinity
SE = salinity of effluent
SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Control	NA	NA	NA	250
2	5.0	0.49	2.4	250
4	10.0	0.49	4.9	250
9	22.5	0.49	11.0	250
18	45.0	0.49	21.9	250
35	87.5	0.49	42.6	250
67.2	168.1	0.49	81.9	250

	DI Volume			
Brine Control	161.4	0.51	81.9	250

Total Brine Volume Required (ml): **246.7**

QC Check: AES 1/6/23 **Final Review:** BO 1/9/23

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs/Wyckoff Eagle Harbor GWTP
Test No.: 2211-6332
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REF 6A + 6B
Date Received: 10/20/22
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 11/30/22 1655
End Date/Time: 12/2/22 1530
Technician Initials: RT

Spawn Information

First Gamete Release Time: 1430

Sex	Number Spawning
Male	2
Female	4+

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1,2	good density, good motility
Female 1	2	good density, orange, mostly round
Female 2	4	good density, pale orange, mostly round
Female 3	—	

Egg Fertilization Time: ~~16~~ 1500
QA AR 11/30/22

Stock(s) chosen for testing: 1

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	99
Female 2	99
Female 3	

Embryo Inoculum Preparation

Target count on Sedgwick-Rafter slide for desired density is 6 embryos

Number Counted: 15, 20, 11, 8, 18, 27, 13, 12, 11, 11

Mean: 14.6

Mean 14.6 X 50 = 730 embryos/ml

Initial Density: 730 = 2.4 (dilution factor)
Desired Final Density: 300
(to inoculate with 0.5 ml)

Prepare the embryo inoculum according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Time Zero Control Counts

TØ Vial No.	No. Dividing	Total	% Dividing	Mean % Dividing
TØ A	218	218	100	100
TØ B	220	220	100	
TØ C	207	207	100	
TØ D	203	203	100	
TØ E	216	216	100	
TØ F	229	229	100	
$\bar{X}$ =	216			

48-h QC: 203/207 = 98.1%

Comments:

QC Check: AS 1/6/23

Final Review: BO 1/9/23

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: Jacobs
Sample ID: Wyckoff Eagle Harbor GWTP
Test ID No(s).: 2211-5332

Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (22-xxxx):	1486			
Sample Collection Date & Time:	11/29/22 1000			
Sample Receipt Date & Time:	11/30/22 1314			
Number of Containers & Container Type:	1x4L cubi			
Approx. Total Volume Received (L):	~4L			
Check-in Temperature (°C)	3.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	6.8			
pH (units)	7.53			
Conductivity (µS/cm)	2200			
Salinity (ppt)	1.2			
Alkalinity (mg/L) [2]	342			
Hardness (mg/L) [2,3]	298			
Total Chlorine (mg/L)	0.04			
Technician Initials	DR			

Sample Description:
A: Colorless, clear, no odor, no debris

COC Complete (Y/N)?
A Y B___ C___

Filtration? Y (N)
Initials: A) B) C)
Pore Size: _____
Organisms or Debris

Salinity Adjustment? Y (N)
Test: Source: Target ppt:
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl_2 Adjustment? Y (N)

	A	B	C
Initial Free Cl_2:			
STS added:			
Final Free Cl_2:			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Measure NH3 via test strip (circle one)? Y (N)

NH3 Strip Result* A:_____ B:_____ C:_____
*(if 6 or more, notify PM)

Subsamples for Additional Chemistry Required? (Y) N
(NH3) Other _____
Tech Initials A DR B_____ C_____

Final Review: BO 1/9/23

Test Performed: Mussel Development — **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 102 Hardness or Salinity: 30 ppt
Additional Control? (Y) N = Brine Alkalinity: 117 Hardness or Salinity: 30 ppt

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: _____

QC Check: ACS 1/6/23

Total Ammonia Analysis
Marine

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: ~~Menidia Acute~~ Mussel Development

DI Blank: —
SW Blank: 0.0

Test Start Date: 11/30/22

Analyst: GG
Analysis Date: 1/6/23

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L) N x 1.22	Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	9.8	12.0
Wyckoff Effluent	22-1486	11/30/22	check in	7.5	9.2
Spike Check (10 mg/L NH_3)		NA	NA	9.8	12.0
Sample Duplicate[a]	22-1486	NA	NA	7.6	9.3
Sample Duplicate + Spike[a]		NA	NA	17.3	21.1
Spike Check (10 mg/L NH_3)		NA	NA	9.8	12.0

Relative Percent Difference (RPD) = [sample] (mg/L) - [sample duplicate] (mg/L) / [average ammonia] (mg/L) x 100 — **Acceptable Range: 0-20%**

Percent Recovery = [spiked sample] (mg/L) - [sample] (mg/L) / nominal [spike] (mg/L) x 100 — **Acceptable Range: 80-120%**[b]

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	12.0	10	NA	120
Wyckoff Effluent	9.2	9.3	21.1	10	1.1	119

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A2223	A2262	A2294

Comments: ⑤ QA/ACS 1/6/23

Notes: [a] Unless 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: ACS 1/6/23

Final Review: BO 1/9/23

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 11/29/2022
CarrierName: FedEx
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2022/WA
Project Code: WEH-031R
Cooler #: 1 of 1

No: 10-112922-114833-0653
2022T10P000DD210W2LA00
Contact Name: Daniel Baca
Contact Phone: 661-313-3807

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
112922		Ground Water/ D. Baca	Composite	CHRTOX(8 Weeks)	N (1)	SP-11	11/29/2022 10:00	Field Sample

Special Instructions: 2022 Q4 Sample

Shipment for Case Complete? N

Samples Transferred From Chain of Custody #

Analysis Key: CHRTOX=Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	@ JACOBS	11-29-22 @1204	Denise Rios FA-SD	11/30/22 1314	Good

Receipt Temp: 3.2

log # 22-1486

Appendix D
List of 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 ≤ 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 E
Reference Toxicant Test Results

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Batch ID:	05-3665-1511	**Test Type:**	Development-Survival	**Analyst:**			
Start Date:	30 Nov-22 16:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater		
Ending Date:	02 Dec-22 15:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable		
Test Length:	47h	**Taxon:**		**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	12-7426-1585	**Code:**	221130msdv	**Project:**	
Sample Date:	30 Nov-22	**Material:**	Copper chloride	**Source:**	Reference Toxicant
Receipt Date:	30 Nov-22	**CAS (PC):**		**Station:**	Copper Chloride
Sample Age:	17h	**Client:**	Internal		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
19-3880-6824	Combined Development Rat	Dunnett Multiple Comparison Test		2.5	5	3.536	9.38%	1
01-5267-8516	Development Rate	Steel Many-One Rank Sum Test		2.5	5	3.536	4.26%	1
02-7738-0819	Survival Rate	Dunnett Multiple Comparison Test		10	20	14.14	7.61%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
20-6599-7993	Combined Development Rat	Linear Interpolation (ICPIN)		EC25	5.4	4.67	5.95	1
				EC50	6.97	6.51	7.34	
04-3978-8699	Development Rate	Linear Interpolation (ICPIN)		EC25	5.71	5.36	6	1
				EC50	7.17	6.92	7.4	
20-7306-6373	Survival Rate	Linear Interpolation (ICPIN)		EC25	20.1	15.1	22.7	1
				EC50	26.8	24.9	28.5	

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
01-5267-8516	Development Rate	Control Resp	0.968	0.9	<<	Yes	Passes Criteria
04-3978-8699	Development Rate	Control Resp	0.968	0.9	<<	Yes	Passes Criteria
02-7738-0819	Survival Rate	Control Resp	0.924	0.5	<<	Yes	Passes Criteria
20-7306-6373	Survival Rate	Control Resp	0.924	0.5	<<	Yes	Passes Criteria
19-3880-6824	Combined Development Rat	PMSD	0.0938	<<	0.25	No	Passes Criteria

Bivalve Larval Survival and Development Test — Nautilus Environmental (CA)

Combined Development Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	5	0.894	0.806	0.983	0.815	0.972	0.032	0.071	7.98%	0.00%
2.5		5	0.919	0.875	0.962	0.875	0.972	0.016	0.035	3.82%	-2.72%
5		5	0.739	0.649	0.829	0.639	0.838	0.033	0.073	9.83%	17.37%
10		5	0.015	-0.020	0.050	0.000	0.065	0.013	0.028	190.60%	98.34%
20		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
40		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

Development Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	5	0.968	0.963	0.972	0.962	0.972	0.002	0.004	0.41%	0.00%
2.5		5	0.971	0.958	0.984	0.959	0.985	0.005	0.011	1.09%	-0.32%
5		5	0.843	0.776	0.910	0.767	0.905	0.024	0.054	6.43%	12.87%
10		5	0.017	-0.024	0.059	0.000	0.077	0.015	0.033	193.67%	98.22%
20		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
40		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	LC	5	0.924	0.835	1.010	0.847	1.000	0.032	0.072	7.75%	0.00%
2.5		5	0.946	0.904	0.989	0.912	1.000	0.015	0.034	3.62%	-2.40%
5		5	0.876	0.807	0.945	0.824	0.944	0.025	0.055	6.32%	5.21%
10		5	0.853	0.776	0.930	0.792	0.954	0.028	0.062	7.28%	7.72%
20		5	0.706	0.616	0.795	0.616	0.815	0.032	0.072	10.19%	23.65%
40		5	0.003	-0.002	0.008	0.000	0.009	0.002	0.004	149.07%	99.70%

Combined Development Rate Detail MD5: 484B892BF9AD462982D390EF0A4C2EBA

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	0.972	0.966	0.870	0.815	0.847
2.5		0.917	0.907	0.921	0.875	0.972
5		0.838	0.722	0.769	0.639	0.727
10		0.065	0.009	0.000	0.000	0.000
20		0.000	0.000	0.000	0.000	0.000
40		0.000	0.000	0.000	0.000	0.000

Development Rate Detail MD5: ED5C13018A8C22821C7452106EF2D6B1

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	0.972	0.966	0.969	0.962	0.968
2.5		0.985	0.975	0.961	0.959	0.972
5		0.905	0.876	0.814	0.767	0.853
10		0.077	0.010	0.000	0.000	0.000
20		0.000	0.000	0.000	0.000	0.000
40		0.000	0.000	0.000	0.000	0.000

Survival Rate Detail MD5: 93FFE20B1EF285543D4FFAB63AB7D089

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	1.000	1.000	0.898	0.847	0.875
2.5		0.931	0.931	0.958	0.912	1.000
5		0.926	0.824	0.944	0.833	0.852
10		0.847	0.954	0.856	0.792	0.815
20		0.616	0.718	0.685	0.815	0.694
40		0.000	0.009	0.005	0.000	0.000

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Combined Development Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	211/217	228/236	188/216	176/216	183/216
2.5		198/216	196/216	199/216	189/216	210/216
5		181/216	156/216	166/216	138/216	157/216
10		14/216	2/216	0/216	0/216	0/216
20		0/216	0/216	0/216	0/216	0/216
40		0/216	0/216	0/216	0/216	0/216

Development Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	211/217	228/236	188/194	176/183	183/189
2.5		198/201	196/201	199/207	189/197	210/216
5		181/200	156/178	166/204	138/180	157/184
10		14/183	2/206	0/185	0/171	0/176
20		0/133	0/155	0/148	0/176	0/150
40		0/1	0/2	0/1	0/1	0/1

Survival Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	216/216	216/216	194/216	183/216	189/216
2.5		201/216	201/216	207/216	197/216	216/216
5		200/216	178/216	204/216	180/216	184/216
10		183/216	206/216	185/216	171/216	176/216
20		133/216	155/216	148/216	176/216	150/216
40		0/216	2/216	1/216	0/216	0/216

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	19-3880-6824	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:19	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	484B892BF9AD462982D390EF0A4C2EBA	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	2.5	5	3.536	---	0.0839	9.38%

Dunnett Multiple Comparison Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Lab Control		2.5	8	-0.492	2.23	0.137	CDF	0.8898	Non-Significant Effect
		5*	8	3.57	2.23	0.137	CDF	0.0035	Significant Effect
		10*	8	18.9	2.23	0.137	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.74928	1.58309	3	167	<1.0E-05	Significant Effect
Error	0.151972	0.0094983	16			
Total	4.90125		19			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1.4	11.3	0.7066	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.911	0.866	0.0673	Normal Distribution

Combined Development Rate Summary

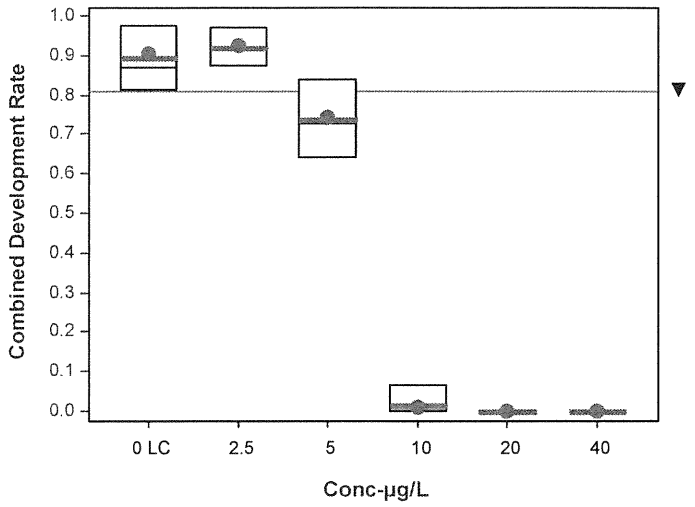
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	0.894	0.806	0.983	0.870	0.815	0.972	0.032	7.98%	0.00%
2.5		5	0.919	0.875	0.962	0.917	0.875	0.972	0.016	3.82%	-2.72%
5		5	0.739	0.649	0.829	0.727	0.639	0.838	0.033	9.83%	17.37%
10		5	0.015	0.000	0.050	0.000	0.000	0.065	0.013	190.60%	98.34%
20		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
40		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

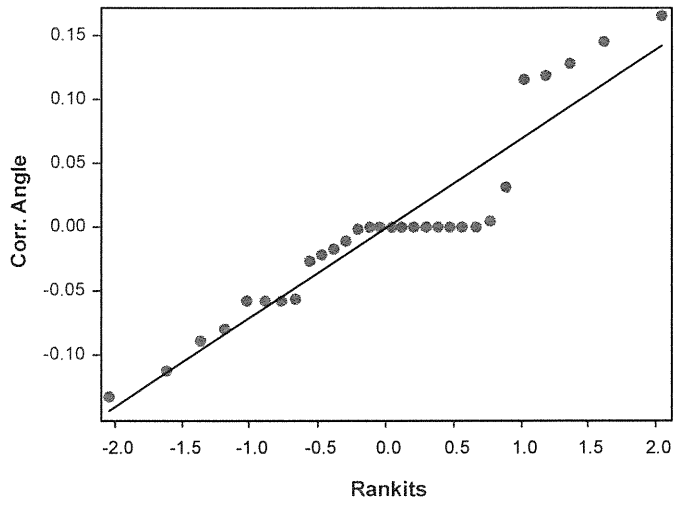
Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	1.260	1.100	1.420	1.200	1.130	1.400	0.057	10.21%	0.00%
2.5		5	1.290	1.200	1.380	1.280	1.210	1.400	0.032	5.53%	-2.41%
5		5	1.040	0.933	1.140	1.020	0.926	1.160	0.038	8.11%	17.48%
10		5	0.091	-0.029	0.211	0.034	0.034	0.257	0.043	106.14%	92.75%
20		5	0.034	0.034	0.034	0.034	0.034	0.034	0.000	0.00%	97.29%
40		5	0.034	0.034	0.034	0.034	0.034	0.034	0.000	0.00%	97.29%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	19-3880-6824	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:19	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	484B892BF9AD462982D390EF0A4C2EBA	**Editor ID:**	007-926-968-0

Graphics





Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	01-5267-8516	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:19	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	ED5C13018A8C22821C7452106EF2D6B1	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	2.5	5	3.536	---	0.0412	4.26%

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		2.5	8	29	17	0	CDF	0.8495	Non-Significant Effect
		5*	8	15	17	0	CDF	0.0123	Significant Effect
		10*	8	15	17	0	CDF	0.0123	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.76508	1.92169	3	433	<1.0E-05	Significant Effect
Error	0.0710671	0.0044417	16			
Total	5.83615		19			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	14.1	11.3	0.0027	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.901	0.866	0.0432	Normal Distribution

Development Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	0.968	0.963	0.972	0.968	0.962	0.972	0.002	0.41%	0.00%
2.5		5	0.971	0.958	0.984	0.972	0.959	0.985	0.005	1.09%	-0.32%
5		5	0.843	0.776	0.910	0.853	0.767	0.905	0.024	6.43%	12.87%
10		5	0.017	0.000	0.059	0.000	0.000	0.077	0.015	193.67%	98.22%
20		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%
40		5	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

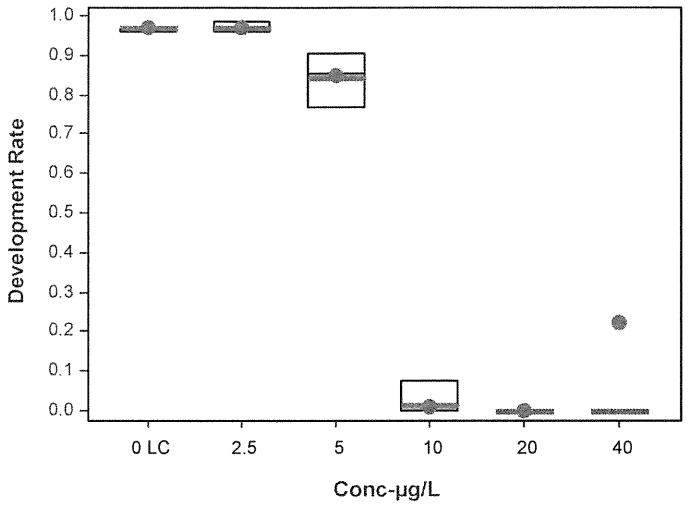
Angular (Corrected) Transformed Summary

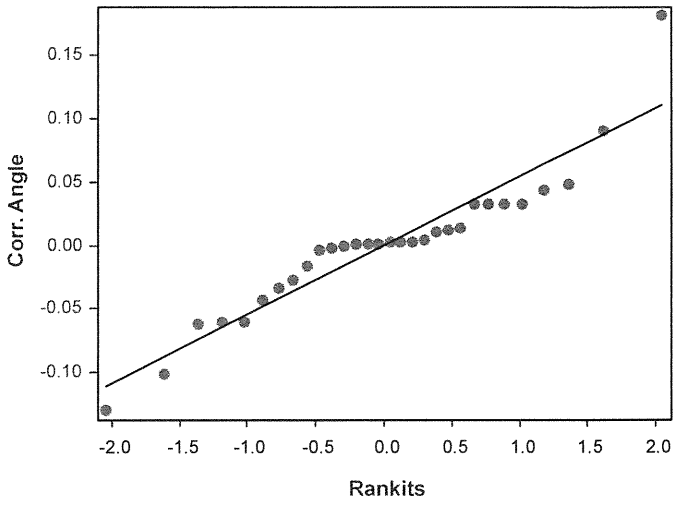
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	1.390	1.380	1.400	1.390	1.370	1.400	0.005	0.79%	0.00%
2.5		5	1.400	1.360	1.440	1.400	1.370	1.450	0.015	2.33%	-0.80%
5		5	1.170	1.080	1.260	1.180	1.070	1.260	0.033	6.37%	15.99%
10		5	0.098	-0.032	0.229	0.038	0.037	0.280	0.047	106.87%	92.92%
20		5	0.041	0.038	0.043	0.041	0.038	0.043	0.001	5.01%	97.08%
40		5	0.491	0.401	0.581	0.524	0.361	0.524	0.032	14.77%	64.66%

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	01-5267-8516	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:19	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	ED5C13018A8C22821C7452106EF2D6B1	**Editor ID:**	007-926-968-0

Graphics





Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	02-7738-0819	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:19	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	93FFE20B1EF285543D4FFAB63AB7D089	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	10	20	14.14	---	0.0703	7.61%

Dunnett Multiple Comparison Test

Control	vs	Conc-µg/L	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Lab Control		2.5	8	-0.24	2.36	0.161	CDF	0.8944	Non-Significant Effect
		5	8	1.78	2.36	0.161	CDF	0.1460	Non-Significant Effect
		10	8	2.26	2.36	0.161	CDF	0.0616	Non-Significant Effect
		20*	8	4.98	2.36	0.161	CDF	0.0001	Significant Effect
		40*	8	18.8	2.36	0.161	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.08281	1.21656	5	104	<1.0E-05	Significant Effect
Error	0.279637	0.0116516	24			
Total	6.36245		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	10.1	15.1	0.0737	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.922	0.903	0.0297	Normal Distribution

Survival Rate Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	0.924	0.835	1.000	0.898	0.847	1.000	0.032	7.75%	0.00%
2.5		5	0.946	0.904	0.989	0.931	0.912	1.000	0.015	3.62%	-2.40%
5		5	0.876	0.807	0.945	0.852	0.824	0.944	0.025	6.32%	5.21%
10		5	0.853	0.776	0.930	0.847	0.792	0.954	0.028	7.28%	7.72%
20		5	0.706	0.616	0.795	0.694	0.616	0.815	0.032	10.19%	23.65%
40		5	0.003	0.000	0.008	0.000	0.000	0.009	0.002	149.07%	99.70%

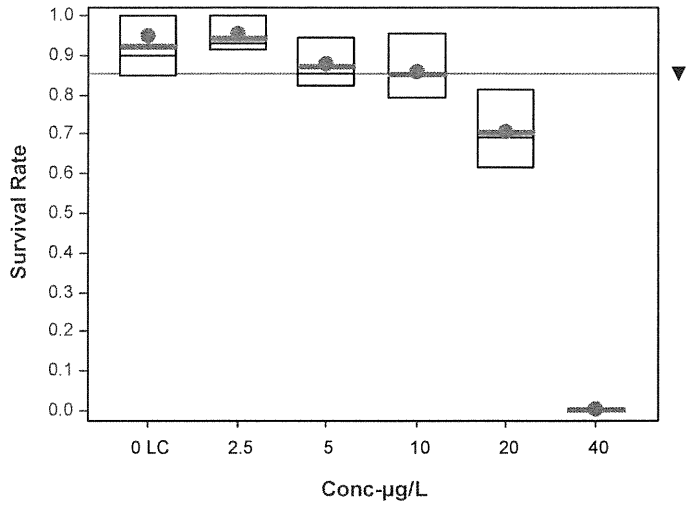
Angular (Corrected) Transformed Summary

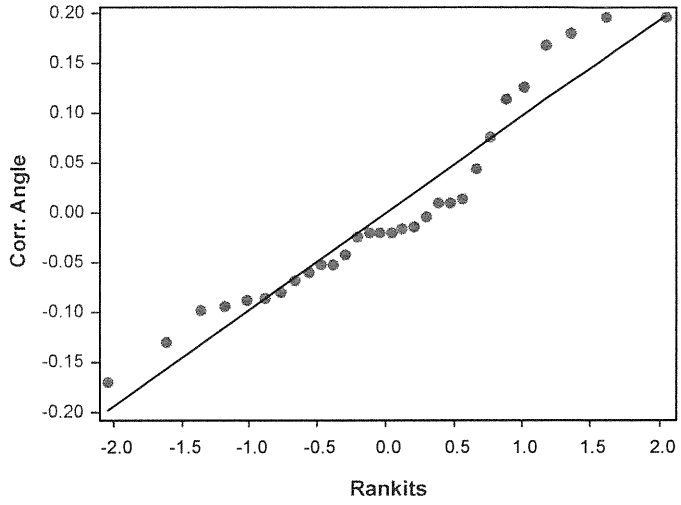
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	1.340	1.110	1.570	1.250	1.170	1.540	0.081	13.59%	0.00%
2.5		5	1.360	1.220	1.490	1.300	1.270	1.540	0.048	7.87%	-1.22%
5		5	1.220	1.110	1.330	1.180	1.140	1.330	0.040	7.33%	9.05%
10		5	1.190	1.060	1.310	1.170	1.100	1.350	0.045	8.44%	11.50%
20		5	1.000	0.899	1.100	0.985	0.902	1.130	0.036	8.12%	25.37%
40		5	0.053	0.018	0.088	0.034	0.034	0.096	0.013	52.96%	96.02%

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	02-7738-0819	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:19	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	93FFE20B1EF285543D4FFAB63AB7D089	**Editor ID:**	007-926-968-0

Graphics





Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	20-6599-7993	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:20	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	484B892BF9AD462982D390EF0A4C2EBA	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1058417	1000	Yes	Two-Point Interpolation

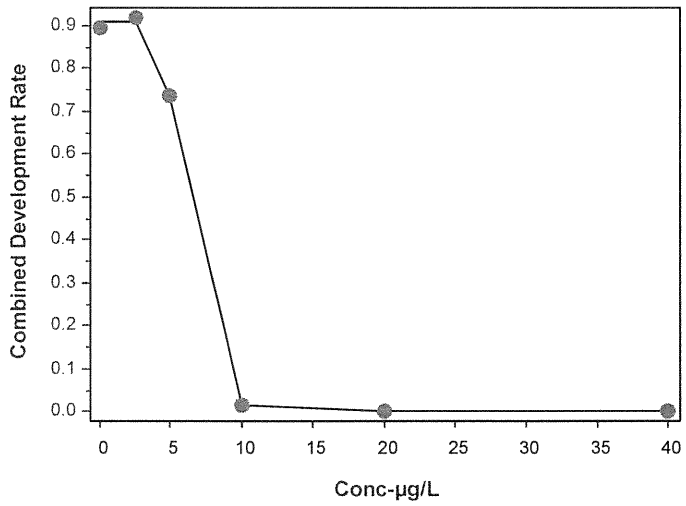
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	5.4	4.67	5.95
EC50	6.97	6.51	7.34

Combined Development 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	5	0.894	0.870	0.815	0.972	7.98%	0.00%	986/1101	0.907	0.00%
2.5		5	0.919	0.917	0.875	0.972	3.82%	-2.72%	992/1080	0.907	0.00%
5		5	0.739	0.727	0.639	0.838	9.83%	17.37%	798/1080	0.739	18.52%
10		5	0.015	0.000	0.000	0.065	190.60%	98.34%	16/1080	0.015	98.37%
20		5	0.000	0.000	0.000	0.000	---	100.00%	0/1080	0.000	100.00%
40		5	0.000	0.000	0.000	0.000	---	100.00%	0/1080	0.000	100.00%

Graphics



Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	04-3978-8699	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:20	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	ED5C13018A8C22821C7452106EF2D6B1	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1333117	1000	Yes	Two-Point Interpolation

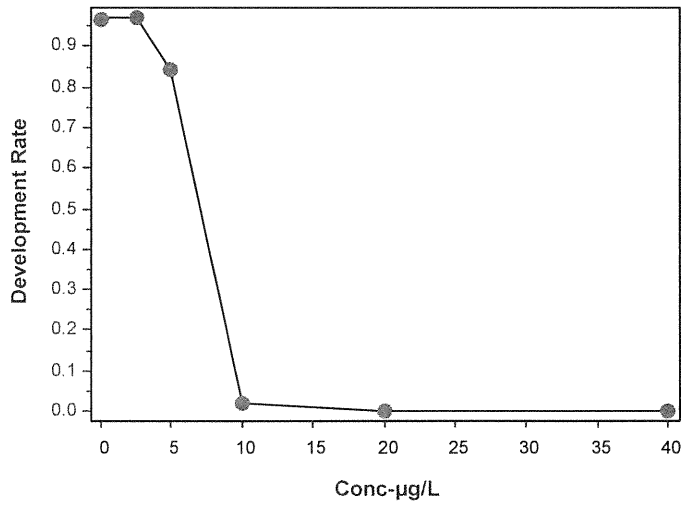
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	5.71	5.36	6
EC50	7.17	6.92	7.4

Development 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	5	0.968	0.968	0.962	0.972	0.41%	0.00%	986/1019	0.969	0.00%
2.5		5	0.971	0.972	0.959	0.985	1.09%	-0.32%	992/1022	0.969	0.00%
5		5	0.843	0.853	0.767	0.905	6.43%	12.87%	798/946	0.844	12.90%
10		5	0.017	0.000	0.000	0.077	193.67%	98.22%	16/921	0.017	98.20%
20		5	0.000	0.000	0.000	0.000	---	100.00%	0/762	0.000	100.00%
40		5	0.000	0.000	0.000	0.000	---	100.00%	0/6	0.000	100.00%

Graphics



Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Analysis ID:	20-7306-6373	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	06 Jan-23 11:20	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	06 Jan-23 11:18	**MD5 Hash:**	93FFE20B1EF285543D4FFAB63AB7D089	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	430683	1000	Yes	Two-Point Interpolation

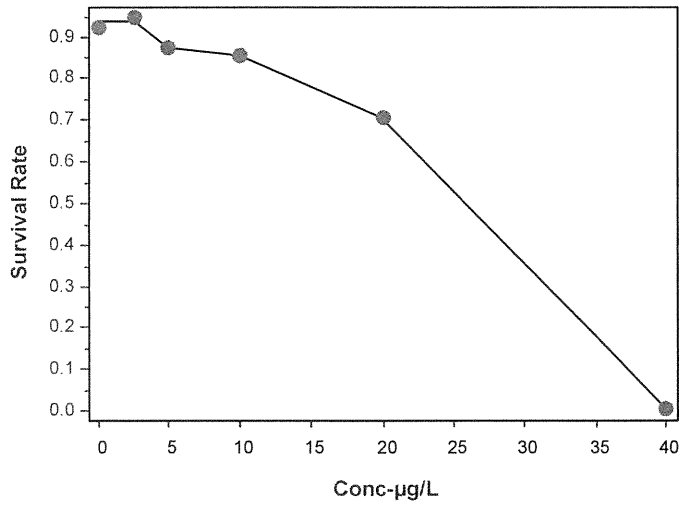
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	20.1	15.1	22.7
EC50	26.8	24.9	28.5

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	5	0.924	0.898	0.847	1.000	7.75%	0.00%	998/1080	0.935	0.00%
2.5		5	0.946	0.931	0.912	1.000	3.62%	-2.40%	1022/1080	0.935	0.00%
5		5	0.876	0.852	0.824	0.944	6.32%	5.21%	946/1080	0.876	6.31%
10		5	0.853	0.847	0.792	0.954	7.28%	7.72%	921/1080	0.853	8.77%
20		5	0.706	0.694	0.616	0.815	10.19%	23.65%	762/1080	0.706	24.49%
40		5	0.003	0.000	0.000	0.009	149.07%	99.70%	3/1080	0.003	99.70%

Graphics

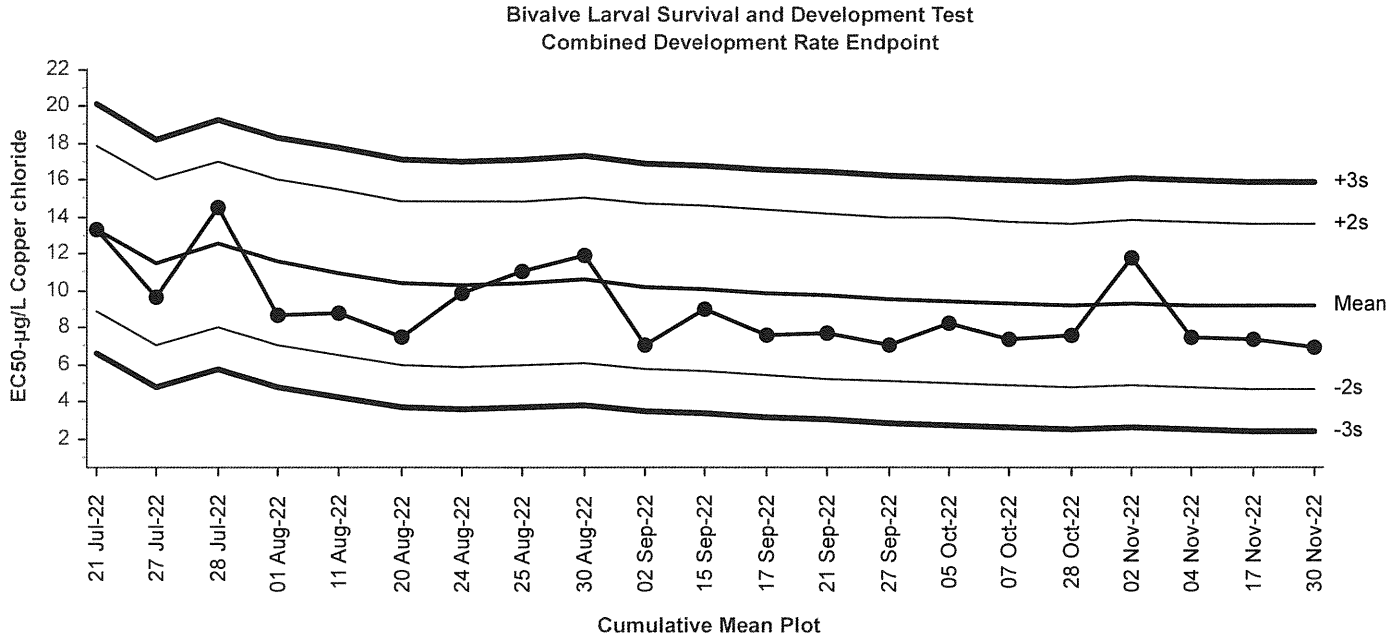


CETIS QC Plot

Report Date: 06 Jan-23 11:23 (1 of 1)

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Test Type:	Development-Survival	**Organism:**	Mytilus galloprovincialis	**Material:**	Copper chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Combined Development Rate	**Source:**	Reference Toxicant-REF



Mean:	9.154	**Count:**	20	**-2s Warning Limit:**	4.67	**-3s Action Limit:**	2.43
Sigma:	2.24	**CV:**	24.50%	**+2s Warning Limit:**	13.6	**+3s Action Limit:**	15.9

Quality Control Data

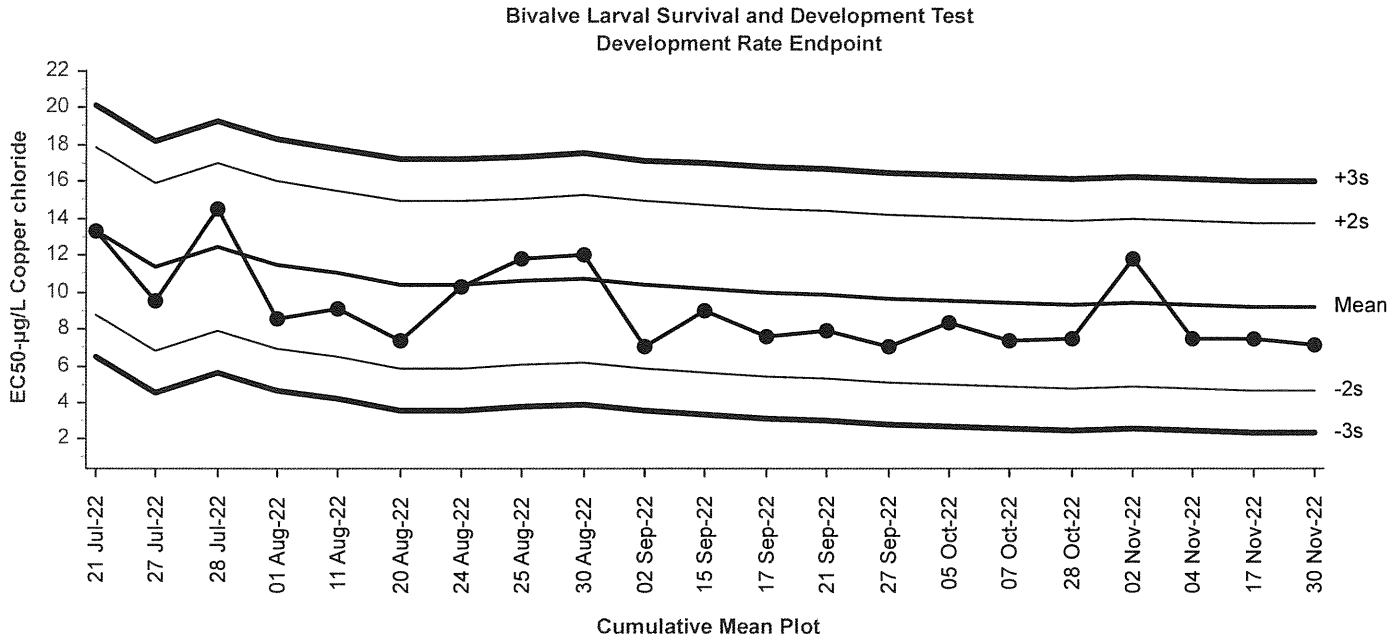
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	Jul	21	15:20	13.35	4.193	1.872			19-9779-3969	07-3733-9935
2			27	17:10	9.634	0.48	0.2143			10-2942-6187	02-0896-6713
3			28	16:10	14.54	5.388	2.405	(+)		12-6417-7061	20-3705-6551
4		Aug	1	14:15	8.601	-0.5526	-0.2467			13-9611-9448	10-0347-5354
5			11	17:15	8.711	-0.4431	-0.1978			00-9864-7136	07-4996-9021
6			20	13:15	7.437	-1.717	-0.7664			17-3409-3229	13-5013-5598
7			24	17:00	9.827	0.6727	0.3003			00-6269-9880	09-5721-9456
8			25	16:10	11.03	1.879	0.8387			09-4049-8245	16-6708-7113
9			30	16:00	11.87	2.72	1.214			19-7189-9086	16-5328-3209
10		Sep	2	15:50	7.006	-2.148	-0.9591			20-7743-0163	03-9271-4476
11			15	17:10	8.93	-0.2239	-0.09996			05-2639-2543	14-5316-2495
12			17	15:50	7.569	-1.585	-0.7075			01-1452-0296	11-5344-5886
13			21	14:00	7.73	-1.424	-0.6355			21-3821-2403	01-0407-9043
14			27	14:45	7.05	-2.104	-0.9392			16-5489-6261	14-2194-1742
15		Oct	5	17:55	8.255	-0.8992	-0.4014			07-3382-0760	11-3283-4631
16			7	16:50	7.327	-1.827	-0.8156			18-9536-1232	04-5872-4598
17			28	14:00	7.533	-1.621	-0.7236			15-6262-8974	16-6274-0594
18		Nov	2	15:45	11.84	2.688	1.2			00-8571-5759	02-8850-9512
19			4	14:20	7.429	-1.725	-0.77			07-4090-5456	06-1005-1310
20			17	17:15	7.395	-1.759	-0.785			10-8063-1957	09-3146-9181
21			30	16:55	6.971	-2.183	-0.9747			18-6969-8385	20-6599-7993

CETIS QC Plot

Report Date: 06 Jan-23 11:23 (1 of 1)

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Test Type:	Development-Survival	**Organism:**	Mytilus galloprovincialis	**Material:**	Copper chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Development Rate	**Source:**	Reference Toxicant-REF



Mean:	9.238	**Count:**	20	**-2s Warning Limit:**	4.69	**-3s Action Limit:**	2.42
Sigma:	2.272	**CV:**	24.60%	**+2s Warning Limit:**	13.8	**+3s Action Limit:**	16.1

Quality Control Data

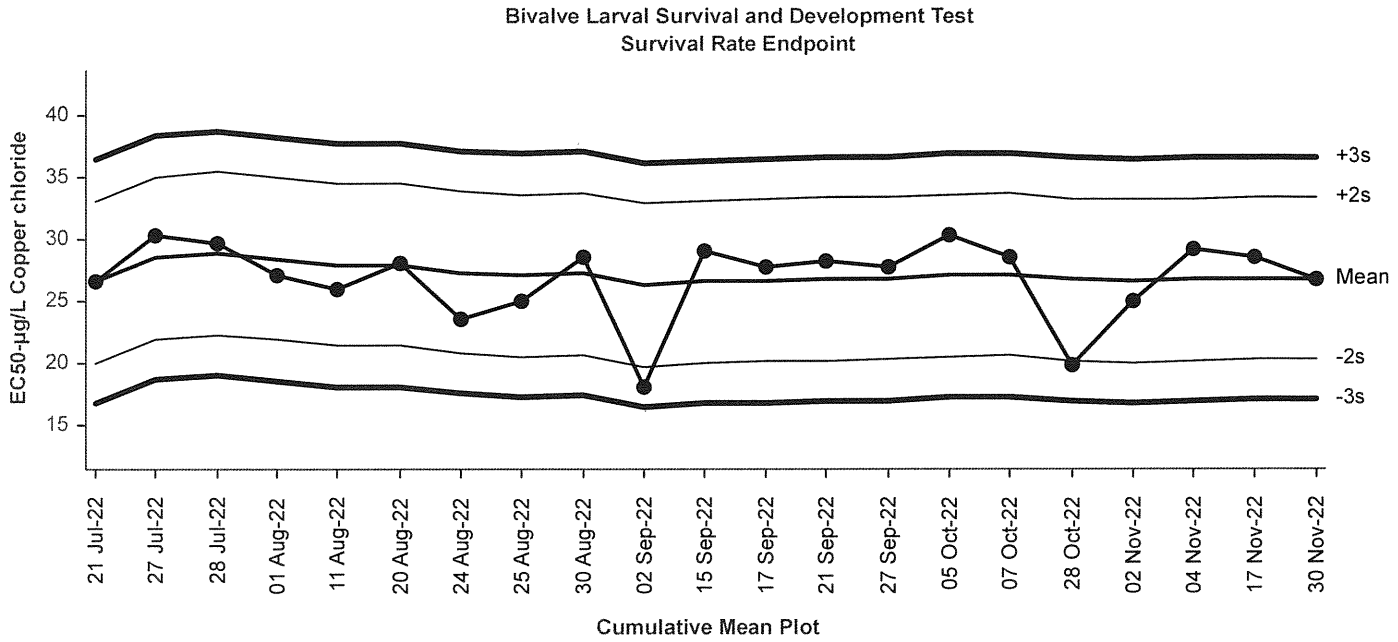
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	Jul	21	15:20	13.28	4.037	1.777			19-9779-3969	12-5751-9718
2			27	17:10	9.524	0.2862	0.126			10-2942-6187	20-1467-5696
3			28	16:10	14.48	5.242	2.307	(+)		12-6417-7061	16-6165-4739
4		Aug	1	14:15	8.58	-0.6582	-0.2897			13-9611-9448	13-0914-2180
5			11	17:15	9.087	-0.1506	-0.06628			00-9864-7136	10-5564-7447
6			20	13:15	7.411	-1.827	-0.8043			17-3409-3229	01-3382-1740
7			24	17:00	10.31	1.074	0.4727			00-6269-9880	18-8831-9322
8			25	16:10	11.78	2.545	1.12			09-4049-8245	09-4327-5326
9			30	16:00	11.99	2.749	1.21			19-7189-9086	10-6237-7089
10		Sep	2	15:50	7.005	-2.233	-0.9828			20-7743-0163	12-4705-1691
11			15	17:10	8.947	-0.2906	-0.1279			05-2639-2543	09-3905-5948
12			17	15:50	7.57	-1.668	-0.7342			01-1452-0296	10-4926-6934
13			21	14:00	7.915	-1.323	-0.5823			21-3821-2403	11-1198-0760
14			27	14:45	7.076	-2.162	-0.9514			16-5489-6261	06-9229-6449
15		Oct	5	17:55	8.312	-0.9261	-0.4076			07-3382-0760	16-5397-8534
16			7	16:50	7.327	-1.911	-0.8411			18-9536-1232	20-1532-2768
17			28	14:00	7.48	-1.758	-0.7738			15-6262-8974	03-0762-2274
18		Nov	2	15:45	11.84	2.607	1.147			00-8571-5759	01-8428-9056
19			4	14:20	7.429	-1.809	-0.7961			07-4090-5456	12-6999-9673
20			17	17:15	7.423	-1.815	-0.7987			10-8063-1957	16-6822-5457
21			30	16:55	7.173	-2.065	-0.9091			18-6969-8385	04-3978-8699

CETIS QC Plot

Report Date: 06 Jan-23 11:23 (1 of 1)

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Test Type:	Development-Survival	**Organism:**	Mytilus galloprovincialis	**Material:**	Copper chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	26.79	**Count:**	20	**-2s Warning Limit:**	20.3	**-3s Action Limit:**	17
Sigma:	3.269	**CV:**	12.20%	**+2s Warning Limit:**	33.3	**+3s Action Limit:**	36.6

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	Jul	21	15:20	26.53	-0.2622	-0.08021			19-9779-3969	06-3587-0808
2			27	17:10	30.34	3.551	1.086			10-2942-6187	08-5991-2538
3			28	16:10	29.61	2.818	0.8622			12-6417-7061	16-9619-1003
4		Aug	1	14:15	27.1	0.3112	0.09519			13-9611-9448	19-4797-3651
5			11	17:15	25.91	-0.8809	-0.2695			00-9864-7136	03-2596-2467
6			20	13:15	28.03	1.24	0.3793			17-3409-3229	00-2953-9784
7			24	17:00	23.46	-3.328	-1.018			00-6269-9880	07-4875-9471
8			25	16:10	24.98	-1.81	-0.5537			09-4049-8245	01-3678-6981
9			30	16:00	28.45	1.657	0.5068			19-7189-9086	04-7142-7490
10		Sep	2	15:50	17.97	-8.816	-2.697	(-)		20-7743-0163	07-3288-6541
11			15	17:10	29	2.207	0.6753			05-2639-2543	11-6800-1324
12			17	15:50	27.77	0.9769	0.2988			01-1452-0296	09-4499-7601
13			21	14:00	28.13	1.344	0.411			21-3821-2403	00-9005-5752
14			27	14:45	27.65	0.856	0.2618			16-5489-6261	09-9794-3011
15		Oct	5	17:55	30.31	3.521	1.077			07-3382-0760	19-5972-4820
16			7	16:50	28.44	1.65	0.5047			18-9536-1232	15-0116-3798
17			28	14:00	19.73	-7.062	-2.16	(-)		15-6262-8974	03-9777-8315
18		Nov	2	15:45	24.89	-1.903	-0.5821			00-8571-5759	08-9990-6478
19			4	14:20	29.08	2.294	0.7017			07-4090-5456	15-9684-3131
20			17	17:15	28.52	1.732	0.5298			10-8063-1957	17-0268-5735
21			30	16:55	26.77	-0.01793	-0.00549			18-6969-8385	20-7306-6373

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal/CuCl2
Test No.: 221130msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REF 6A+6B
Date Received: 10/20/22
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 11/30/22 1655
End Date/Time: 12/2/22 1530
Technician Initials: RT

Spawn Information

First Gamete Release Time: 1430

Sex	Number Spawning
Male	2
Female	4+

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1,2	good density, good motility
Female 1	2	good density, orange, mostly round
Female 2	4	good density, pale orange, mostly round
Female 3	—	—

Egg Fertilization Time: ~~16~~ 1500
QG AR 11/30/22

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	99
Female 2	99
Female 3	

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

Target count on Sedgwick-Rafter slide for desired density is 6 embryos

Number Counted: 15, 20, 11, 8, 18 / 27, 13, 12, 11, 11

Mean: 14.6

Mean 14.6 X 50 = 730 embryos/ml

Initial Density: 730 = 2.4 (dilution factor)
Desired Final Density: 300
(to inoculate with 0.5 ml)

Prepare the embryo inoculum according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Time Zero Control Counts

TØ Vial No.	No. Dividing	Total	% Dividing	Mean % Dividing
TØ A	218	218	100	100
TØ B	220	220	100	
TØ C	207	207	100	
TØ D	203	203	100	
TØ E	216	216	100	
TØ F	229	229	100	
$\bar{X}$ =	216			

48-h QC: 203/207 = 98.1%

Comments:

QC Check: ACS 1/6/23

Final Review: BO 1/9/23

CETIS Test Data Worksheet

Report Date: 23 Nov-22 15:33 (p 1 of 1)
Test Code/ID: 221130msdv / 18-6969-8385

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Start Date: 30 Nov-22
End Date: 2-Dec ~~30 Nov~~-22 (B)
Sample Date: 30 Nov-22

Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride

Sample Code: 221130msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

Conc-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			~~197~~ (A) 217	~~185~~ 211	LM 12-7-22
			2			171	0	
			3			1	0	
			4			236	228	QC: 223/230 RT
			5			133	0	QC: 0/144 RT
			6			148	0	
			7			201	196	
			8			163	14	QC: 22/188 RT
			9			0	0	
			10			~~150~~ 150 (B)	0	
			11			176	0	
			12			0	0	
			13			183	176	
			14			216	218	
			15			0	0	
			16			204	166	QC: 167/200 RT
			17			~~0~~ 181 (B)	~~0~~ 157	
			18			~~154~~ 194	~~157~~ 188	
			19			189	183	
			20			2	0	
			21			206	2	
			22			~~216~~ 176 (A)	0	
			23			185	0	
			24			200	181	
			25			207	179	
			26			201	194	
			27			180	138	
			28			197	189	
			29			178	156	
			30			155	0	

(A): QR CM 12/7/22

(B) QR AES 1/6/23

CETIS Test Data Worksheet

Bivalve Larval Survival and Development Test — **Nautilus Environmental (CA)**

Start Date: 30 Nov-22 | **Species:** Mytilus galloprovincialis | **Sample Code:** 221130msdv
End Date: ~~30 Nov-22~~ 2-Dec (5) | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 30 Nov-22 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

Conc-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	1			209	207	EG 12/6/22
0	LC	2	4					
0	LC	3	18					
0	LC	4	13					
0	LC	5	19					
2.5		1	26			207	200	
2.5		2	7					
2.5		3	25					
2.5		4	28					
2.5		5	14					
5		1	24			209	194	
5		2	29					
5		3	16					
5		4	27					
5		5	17					
10		1	8			211	18	
10		2	21					
10		3	23					
10		4	2					
10		5	11					
20		1	5			161 (4)	160 (4)	
20		2	30					
20		3	6					
20		4	22					
20		5	10					
40		1	9			0	0	
40		2	20					
40		3	3					
40		4	12					
40		5	15					

QC: RT

(4) Q18 AFS 12/8/22
(5) Q18 AFS 1/6/23

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 221130 msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 11/30/22 1655

End Date/Time: 12/2/22 1530

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	30.5	30.3	30.3	(R) ~~8~~ 14.9	15.0	15.1	8.8	8.8	8.8	7.95	7.96	7.92
2.5	30.8	30.6	30.6	14.7	14.7	14.7	8.9	8.8	8.9	7.98	7.99	7.93
5	30.7	30.6	30.6	14.6	14.6	14.6	8.8	8.8	8.9	8.00	8.00	7.94
10	30.7	30.7	30.7	14.7	14.7	14.7	8.7	8.7	8.8	8.02	8.00	7.95
20	30.7	30.7	30.6	14.7	14.7	14.7	8.8	8.7	8.8	8.02	8.01	7.96
40	30.7	30.6	30.6	14.7	14.7	14.6	8.7	8.7	8.8	8.03	8.01	7.96

Technician Initials:

	0	24	48
WQ Readings:	RT	KR	CM
Dilutions made by:	RT		

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	2.2
Final Volume (mL):	500
Cu stock concentration (µg/L):	9200

Environmental Chamber: D

Comments:

0 hrs: (N) QAR KR 11/30/22

24 hrs:

48 hrs:

QC Check: ACS 1/6/23

Final Review: BO 1/9/23

ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Acute Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: December 2022

Prepared for: **Jacobs**
1100 112th Ave NE Suite 500
Bellevue, WA, 98004

Prepared by: **Enthalpy Analytical**
4340 Vandever Avenue
San Diego, CA 92120
(858) 587-7333

Date Submitted: **January 12, 2023**

Data Quality Assurance:

- Enthalpy Analytical is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (ORELAP ID 4053). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective US EPA protocols, unless otherwise noted in this report.
- All tests have met internal Quality Assurance Program requirements.

Results verified by: _____
Barbara Orelo, Project Manager

California
4340 Vandever Avenue
San Diego, California 92120
858.587.7333
fax: 619.279.5919

Introduction

A toxicity test was performed using a groundwater composite sample collected from the Wyckoff Eagle Harbor Groundwater Treatment Plant on Bainbridge Island in Washington. This test was performed to satisfy quarterly monitoring requirements according to the project Quality Assurance Project Plan (QAPP 2013). The acute bioassay was conducted using the *Menidia beryllina* (inland silverside). Testing was performed at Enthalpy Analytical located in San Diego, California.

Materials and Methods

The groundwater sample was collected into a low-density polyethylene cubitainer by Jacobs personnel, packed in a cooler containing ice, and shipped overnight to Enthalpy. Appropriate chain-of-custody (COC) procedures were employed during collection and transport. Upon arrival at the laboratory, the cooler was opened, the sample inspected, and the contents verified against information on the COC form. Standard water quality parameters were measured and recorded on a sample check-in form and are summarized in Table 1. The sample was stored at 4°C in the dark until used for testing.

Table 1. Sample Information

Sample ID	**121322**
Enthalpy Log-in Number	22-1573
Collection Date; Time	12/13/22; 10:40
Receipt Date; Time	12/14/22; 09:10
Receipt Temperature (°C)	3.2
Dissolved Oxygen (mg/L)	8.5
pH	7.68
Conductivity (µS/cm)	1097
Salinity (ppt)	0.6
Alkalinity (mg/L $CaCO_3$)	330
Hardness (mg/L $CaCO_3$)	150
Total Chlorine (mg/L)	0.04
Total Ammonia (mg/L as N)	<0.5

Test Methods

Acute toxicity testing was conducted according to the method set forth in USEPA 2002. This method is summarized in Table 2.

Table 2. Summary of Methods for the Inland Silverside Acute Test

Test Period	12/14/22, 16:45 to 12/18/22, 15:15
Test Organism	*Menidia beryllina*
Test Organism Source	Aquatic Biosystems (Fort Collins, CO)
Test Organism Age	14 days
Test Duration	96 ± 2 hours
Test Type	Static with 48-hr renewal
Test Chamber, Test Solution Volume	500 mL plastic cup, 250 mL
Test Temperature	25 ± 1°C
Dilution Water	Artificial Saltwater (Instant Ocean® salts added to deionized water to 30 ± 2 ppt)
Additional Control	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) diluted with de-ionized water
Test Salinity	30 ± 2 ppt
Source of Salinity	Instant Ocean® salts were added to the sample to raise salinity to 30 ± 2 ppt
Test Concentrations (% sample)	100, 50, 25, 12.5, and 6.25%; plus lab and salt controls
Number of Replicates	4
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/821/R-02/012
Test Acceptability Criteria for Controls	≥ 90% mean survival
Reference Toxicant	Copper chloride[a]
Statistical Software	CETIS™ 2.1.2.3

[a] A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride. See QA section.

Statistical Methods

Statistical analyses were conducted using USEPA flowchart specifications as outlined in the test guidance manual (USEPA 2002). Organism performance in the sample was compared to that observed in the salt control. Results were used to calculate the No Observed Effect Concentration (NOEC) and the concentrations expected to cause a lethal effect to 50 and 25 percent of test organisms (LC_{50} and LC_{25}, respectively). The acute toxic unit (TU_a) value was calculated as $100/LC_{50}$. The statistical analyses were performed using the Comprehensive Environmental Toxicity Information System™ (CETIS), version 2.1.2.3 by Tidepool Scientific Software.

Results

There was no statistically significant effect detected to survival for any concentration tested in the inland silverside test. This results in a LC_{50} of greater than 100 percent concentration and an acute toxic unit (TU_a) of less than 1.0.

Results for the toxicity tests are summarized in Table 3. Detailed summaries of the acute toxicity test are provided in Table 4. Individual statistical summaries for the test and copies of the laboratory bench sheets are provided in Appendix A. The sample check-in sheet and COC form are provided in Appendices B and C, respectively.

Table 3. Summary of Statistical Results for the Toxicity Tests

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_a)	LC_{50} (% effluent)	LC_{25} (% effluent)
Inland Silverside	Survival	100	> 100	<1.0	> 100	> 100

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

Acute Toxic Unit (TUa) = $100/LC_{50}$. A TUa of 1.0 indicates no toxicity in the sample.

Lethal Concentration 50 (LC_{50}) = Concentration expected to cause an effect to 50% of the organisms

Lethal Concentration 25 (LC_{25}) = Concentration expected to cause an effect to 25% of the organisms

Table 4. Detailed Results for the Inland Silverside Acute Survival Test

Concentration (% Effluent)	Mean Survival (%)
0 (Salt Control)	100
0 (Lab Control)	100
6.25	100
12.5	100
25	100
50	100
100	100

Quality Assurance

The sample was received in good condition and within the appropriate temperature range of 0-6°C. The test was initiated within the required 36-hour holding time. Control acceptability criteria was met, and water quality parameters remained within the appropriate ranges throughout the test. Statistical analyses followed standard USEPA flowchart selections. Dose-response relationships were reviewed to ensure the reliability of the results. Based on the dose response observed, the calculated effects concentrations were deemed reliable.

Minor QA/QC issues that were unlikely to have any bearing on the final test results, such as slight temperature deviations, are noted on the data sheets and a list of qualifier codes used on bench data sheets is presented in Appendix D.

Reference Toxicant

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride rather than copper sulfate. The results for the concurrent reference toxicant tests were within the acceptable range of the mean historical test results plus or minus two standard deviations for all tests and endpoints. Reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 5. Reference Toxicant Test Results

Species and Endpoint	**NOEC** (%)	**EC_{50}** (µg/L copper)	**Historical Mean ± 2 SD** (µg/L copper)	**CV** (%)
Inland Silverside Survival	100	192	182 ± 82.9	22.7

NOEC = No Observed Effect Concentration

Effect Concentration 50 (EC_{50}) = Concentration expected to cause an effect to 50% of the organisms

Historical Mean ± 2 SD = The mean EC_{50} from the previous 20 tests performed by the laboratory, plus or minus two standard deviations (SD)

CV = Coefficient of Variation

References

CH2MHill. 2013. Quality Assurance Project Plan – Groundwater Treatment Plant Operations, Maintenance, Bainbridge, Washington. Prepared for USEPA Region 10 June 5, 2013.

Tidepool Scientific Software. 2000-2022. CETIS Comprehensive Environmental Toxicity Information System Software, Version 2.1.2.3.

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. United States Environmental Protection Agency Office of Water, Washington DC. EPA/821/R-02/012.

Washington State Department of Ecology. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Publication No. WQ-R-95-80. Revised June 2016

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

Report Date: 11 Jan-23 16:22 (p 1 of 1)
Test Code/ID: 2212-S119 / 20-8408-8453

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	01-8095-7181	**Test Type:**	Survival (96h)	**Analyst:**	Artificial Saltwater
Start Date:	14 Dec-22 16:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	(A) ~~Diluted Natural Seawater~~
Ending Date:	18 Dec-22 15:15	**Species:**	Menidia beryllina	**Brine:**	Instant Ocean
Test Length:	94h	**Taxon:**		**Source:**	Aquatic Biosystems, CO **Age:** 14d

Sample ID:	00-7055-1751	**Code:**	22-1573	**Project:**	
Sample Date:	13 Dec-22 10:40	**Material:**	Effluent Sample	**Source:**	Jacobs
Receipt Date:	14 Dec-22 09:10	**CAS (PC):**		**Station:**	Wyckoff
Sample Age:	30h (3.2 °C)	**Client:**	Jacobs		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
13-8564-4261	96h Survival Rate	Steel Many-One Rank Sum Test		100	>100	---	---	1	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
19-7584-9697	96h Survival Rate	Linear Interpolation (ICPIN)		EC25	>100	---	---	<1	1
				EC50	>100	---	---	<1	

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
13-8564-4261	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
19-7584-9697	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

96h Survival Rate Summary

Conc-%	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%
0	SC	4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
6.25		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
12.5		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
25		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
50		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%
100		4	1.000	1.000	1.000	1.000	1.000	0.000	0.000	0.00%	0.00%

96h Survival Rate Detail MD5: 2DF63B8F56A38BF2F8B31B242708326E

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
0	SC	1.000	1.000	1.000	1.000
6.25		1.000	1.000	1.000	1.000
12.5		1.000	1.000	1.000	1.000
25		1.000	1.000	1.000	1.000
50		1.000	1.000	1.000	1.000
100		1.000	1.000	1.000	1.000

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	13-8564-4261	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 16:22	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	11 Jan-23 14:29	**MD5 Hash:**	68E117461239090AA7E1427F0F536296	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Angular (Corrected)	C > T	100	>100	---	1

Steel Many-One Rank Sum Test

Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Salt Control		6.25	6	18	10	1	CDF	0.8333	Non-Significant Effect
		12.5	6	18	10	1	CDF	0.8333	Non-Significant Effect
		25	6	18	10	1	CDF	0.8333	Non-Significant Effect
		50	6	18	10	1	CDF	0.8333	Non-Significant Effect
		100	6	18	10	1	CDF	0.8333	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	5			Indeterminate
Error	0	0	18			
Total	0		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				Indeterminate

96h Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	SC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
6.25		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
12.5		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
25		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
100		4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%

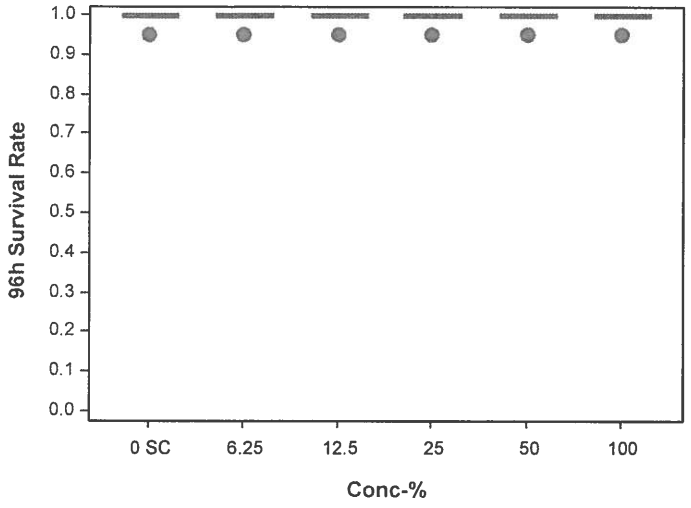
Angular (Corrected) Transformed Summary

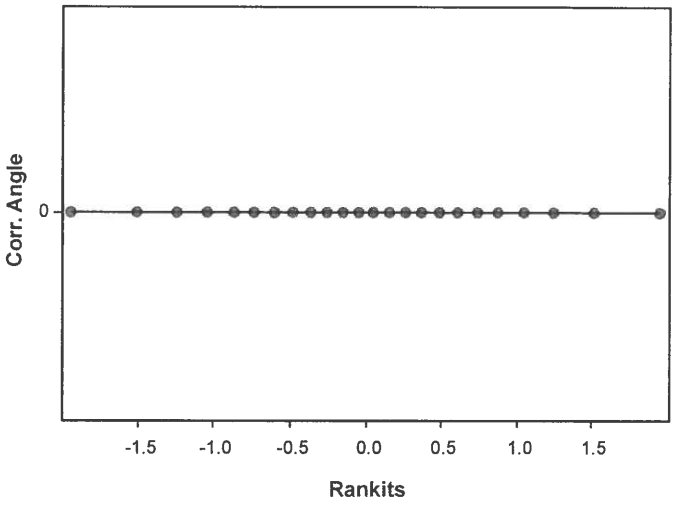
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	SC	4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
6.25		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
12.5		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
25		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
50		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
100		4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	13-8564-4261	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 16:22	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	11 Jan-23 14:29	**MD5 Hash:**	68E117461239090AA7E1427F0F536296	**Editor ID:**	007-803-386-7

Graphics





CETIS Analytical Report

Test Code/ID: 2212-S119 / 20-8408-8453

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	19-7584-9697	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:31	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	11 Jan-23 14:29	**MD5 Hash:**	68E117461239090AA7E1427F0F536296	**Editor ID:**	007-803-386-7

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	290134	1000	Yes	Two-Point Interpolation

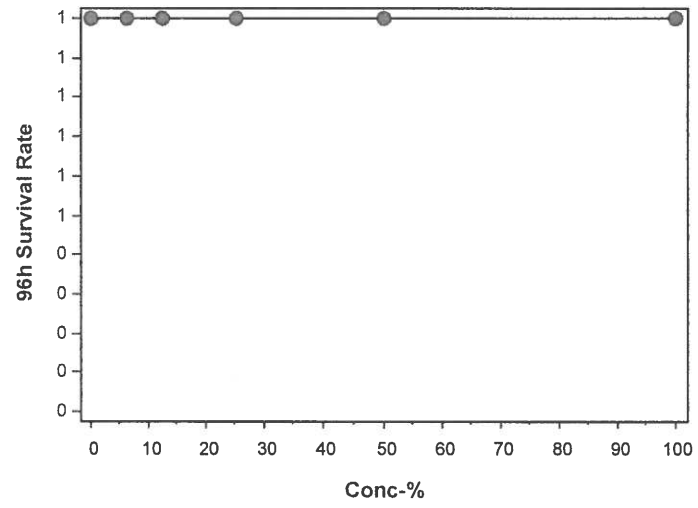
Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	>100	---	---	<1	---	---
EC50	>100	---	---	<1	---	---

96h Survival Rate Summary

			Calculated Variate(A/B)							Isotonic Variate	
Conc-%	Code	Count	Mean	Median	Min	Max	CV%	%Effect	ΣA/ΣB	Mean	%Effect
0	SC	4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
6.25		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
12.5		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
25		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
50		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%
100		4	1.000	1.000	1.000	1.000	0.00%	0.00%	20/20	1.000	0.00%

Graphics



Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: JACOBS
Sample ID: Wyckoff
Sample Log-in No.: 22-1573
Test No.: 2212-S119

Test Species: *M. beryllina*
Start Date/Time: 12/14/22 1645
End Date/Time: 12/18/22 1515

	Tech Initials				
	0	24	48	72	96
Counts:	DR	EG	GM	GM	KR
Readings:	RT	HH	GM	WC	KR
Dilutions made by:	HH		GM		

Concentration (%)	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	29.7	29.8	30.2	30.5	30.7	24.5	25.6	24.7	24.7	24.6	6.5	5.2	6.9	5.2	5.4	7.96	7.80	7.84	7.87	7.79
	B	5	5	5	5	5			f 30.7					f 24.9					f 6.0					f 7.7	7.72	
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
Salt Control	A	5	5	5	5	5	30.2	30.0	29.8	30.3	30.6	24.7	25.6	24.8	24.9	25.0	6.5	5.0	6.5	5.1	5.3	8.23	8.05	8.15	8.03	7.96
	B	5	5	5	5	5			f 31.1					f 25.0					f 5.8					f 7.94		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
6.25%	A	5	5	5	5	5	30.2	30.0	29.8	30.3	30.4	24.0	25.4	24.9	24.7	25.0	6.4	5.1	6.5	5.2	5.2	8.17	8.08	8.12	8.04	8.04
	B	5	5	5	5	5			f 30.9					f 25.1					f 5.7					f 8.00		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
12.5%	A	5	5	5	5	5	30.2	30.0	29.8	30.4	30.5	24.1	25.4	24.9	24.9	25.1	6.5	5.1	6.6	5.1	5.2	8.11	8.09	8.08	8.07	8.09
	B	5	5	5	5	5			f 30.7					f 25.5					f 5.3					f 8.03		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
25%	A	5	5	5	5	5	30.1	30.4	29.8	30.5	30.8	24.2	25.6	24.7	24.8	24.9	6.5	4.9	6.6	5.1	5.1	8.02	8.10	8.03	8.11	8.16
	B	5	5	5	5	5			f 30.5					f 25.6					f 5.0					f 8.08		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
50%	A	5	5	5	5	5	29.9	30.3	29.8	30.5	31.0	24.0	25.6	24.6	24.5	24.7	6.7	5.0	6.8	5.2	5.3	7.87	8.14	7.95	8.18	8.27
	B	5	5	5	5	5			f 30.5					f 25.7					f 5.0					f 8.20		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
100%	A	5	5	5	5	5	29.8	30.0	29.9	30.8	31.5	24.1	25.6	24.4	24.4	24.6	6.9	4.9	7.0	5.3	5.4	7.70	8.10	7.86	8.25	8.38
	B	5	5	5	5	5			f 30.2					f 25.7					f 5.0					f 8.30		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				

Initial Counts QC'd by: KR
Initiated by: DR

Environmental Chamber: A

Animal Source/Date Received: ABS 12/9/22

Age at Initiation: 14 days

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none

Feeding Times					
	0	24	48	72	96
AM:		0900	0910	0855	0825
PM:	1730				

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y) / n) ABS ABOVE 1/11/23

ACS 1/11/23

QC Check: BD 1/11/23

Final Review:

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff (121322)
Test ID No(s).: 2212-S119.

Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (22-xxxx):	1573			
Sample Collection Date & Time:	12/13/22 1040			
Sample Receipt Date & Time:	12/14/22 0910			
Number of Containers & Container Type:	1x10L cubi			
Approx. Total Volume Received (L):	10			
Check-in Temperature (°C)	3.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.5			
pH (units)	7.68			
Conductivity (µS/cm)	1097			
Salinity (ppt)	0.6			
Alkalinity (mg/L) [2]	330			
Hardness (mg/L) [2,3]	150			
Total Chlorine (mg/L)	0.04			
Technician Initials	WF			

Test Performed: Acute ~~Mysid~~ Menidia GIR RC 12/13/22 Control/Dilution Water: 8:2 / Lab SW / (Lab ART) Other: ______
Alkalinity: 152 Hardness or Salinity: 30ppt
Additional Control? (Y) N = LAB SW Alkalinity: 99 Hardness or Salinity: 30ppt

Test Performed: ______ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: ______
Alkalinity: ______ Hardness or Salinity: ______
Additional Control? Y N = ______ Alkalinity: ______ Hardness or Salinity: ______

Test Performed: ______ Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: ______
Alkalinity: ______ Hardness or Salinity: ______
Additional Control? Y N = ______ Alkalinity: ______ Hardness or Salinity: ______

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as $CaCO_3$, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: ______

QC Check: BO 1/11/23

Sample Description: Clear, Colorless, No odor, No debris

COC Complete (Y/N)?
A Y B___ C___

Filtration? Y (N)
Initials: A) ___ B) ___ C) ___
Pore Size: ______
Organisms or Debris

Salinity Adjustment? (Y) N
Test: Menidia Source: Instant ocean Target ppt: 30
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl_2 Adjustment? Y (N)

	A	B	C
Initial Free Cl_2:			
STS added:			
Final Free Cl_2:			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Measure NH3 via test strip (circle one)? Y (N)
NH3 Strip Result* A: ______ B: ______ C: ______
*(If 6 or more, notify PM)

Subsamples for Additional Chemistry Required? (Y) N
(NH3) Other ______
Tech Initials A WF B ___ C ___
Final Review: ACS 1/11/23

Total Ammonia Analysis
Marine
DC-001

Overlying Water

Client: JACOBS
Project: Wyckoff
Test Type: (E) ~~Mussel Development~~ Menidia acute

DI Blank: —
SW Blank: 0.0

Test Start Date: (E) ~~11/30/2022~~ 12/14/22

Analyst: MM
Analysis Date: 12/15/22

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	N x 1.22 Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	9.0	11.0
Wyckoff Effluent	22-1573	(E) ~~11/30/2022~~ 12/14/22	check in	(E) ~~-0.6~~ 0.0	<0.5
Spike Check (10 mg/L NH_3)		NA	NA		
Sample Duplicate[a]	22-1573	NA	NA	(E) ~~-0.2~~ 0.0	<0.5
Sample Duplicate + Spike[a]		NA	NA	8.9	10.9
Spike Check (10 mg/L NH_3)		NA	NA	9.0	11.0

Relative Percent Difference (RPD) = [sample] (mg/L) - [sample duplicate] (mg/L) / [average ammonia] (mg/L) x 100 — **Acceptable Range: 0-20%**

Percent Recovery = [spiked sample] (mg/L) - [sample] (mg/L) / nominal [spike] (mg/L) x 100 — **Acceptable Range: 80-120%[b]**

QC Sample ID	$[NH_3]$	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	11.0	10	NA	110
22-1573	<0.5	~~10.9~~ <0.5 (D)	10.9	10	C	C

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A2117 / A2055	A2117	A2223

Comments: (D) ~~Q18~~ MM 12/15/22 (E) DQ14 ACS 12/30/22

Notes: [a] Unless 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: RL 12/23/22

Final Review: ACS 1/11/23

Enthalpy Analytical. 4340 Vandever Avenue. San Diego, CA 92120.

Appendix C
Chain-of-Custody Form

Enthalphy Analytical (REGION COPY)
DateShipped: 12/13/2022
CarrierName: FedEx
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2022/WA
Project Code: WEH-031S
Cooler #: 1 of 1

No: 10-121322-115003-0658
2022T10P000DD210W2LA00
Contact Name: Daniel Baca
Contact Phone: 661-313-3807

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
121322		Ground Water/ D. Baca	Composite	Acute Toxicity(8 Weeks)	A (< 6 C) (1)	SP-11	12/13/2022 10:40	Field Sample

Special Instructions: 2022 Week 51 Acute Toxicity. Sample 10L Cube filled >50%

Shipment for Case Complete? N

Samples Transferred From Chain of Custody #

Analysis Key

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	@JACOBS	12-13-22 @1200	@ EASD	12/14/22 0910	Good 3.2°C Log # 22-1573

Appendix D
List of 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 ≤ 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 E
Reference Toxicant Test Results

CETIS Summary Report

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	00-5812-1136	**Test Type:**	Survival (96h)	**Analyst:**			
Start Date:	14 Dec-22 17:05	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater		
Ending Date:	18 Dec-22 15:20	**Species:**	Menidia beryllina	**Brine:**	Not Applicable		
Test Length:	94h	**Taxon:**		**Source:**	Aquatic Biosystems, CO	**Age:**	14d

Sample ID:	00-1293-7336	**Code:**	221214mbra	**Project:**			
Sample Date:	14 Dec-22	**Material:**	Copper chloride	**Source:**	Reference Toxicant		
Receipt Date:	14 Dec-22	**CAS (PC):**		**Station:**	Copper Chloride		
Sample Age:	17h	**Client:**	Internal				

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
01-7219-1164	48h Survival Rate	Steel Many-One Rank Sum Test		100	200	141.4	19.2%	1
15-2152-3233	96h Survival Rate	Steel Many-One Rank Sum Test		100	200	141.4	19.2%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
07-1654-3492	48h Survival Rate	Trimmed Spearman-Kärber		EC50	192	154	241	1
15-9199-6379	96h Survival Rate	Trimmed Spearman-Kärber		EC50	192	154	241	1

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
15-2152-3233	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria
15-9199-6379	96h Survival Rate	Control Resp	1	0.9	<<	Yes	Passes Criteria

48h 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%
50		4	0.950	0.791	1.110	0.800	1.000	0.050	0.100	10.53%	5.00%
100		4	0.800	0.540	1.060	0.600	1.000	0.082	0.163	20.41%	20.00%
200		4	0.600	0.340	0.860	0.400	0.800	0.082	0.163	27.22%	40.00%
400		4	0.050	-0.109	0.209	0.000	0.200	0.050	0.100	200.00%	95.00%
800		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

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%
50		4	0.950	0.791	1.110	0.800	1.000	0.050	0.100	10.53%	5.00%
100		4	0.800	0.540	1.060	0.600	1.000	0.082	0.163	20.41%	20.00%
200		4	0.600	0.340	0.860	0.400	0.800	0.082	0.163	27.22%	40.00%
400		4	0.050	-0.109	0.209	0.000	0.200	0.050	0.100	200.00%	95.00%
800		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

48h Survival Rate Detail — MD5: DF7FDE934F83BDDA7A3C88C813BFA558

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
50		1.000	0.800	1.000	1.000
100		1.000	0.800	0.600	0.800
200		0.400	0.600	0.600	0.800
400		0.200	0.000	0.000	0.000
800		0.000	0.000	0.000	0.000

96h Survival Rate Detail — MD5: DF7FDE934F83BDDA7A3C88C813BFA558

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4
0	LC	1.000	1.000	1.000	1.000
50		1.000	0.800	1.000	1.000
100		1.000	0.800	0.600	0.800
200		0.400	0.600	0.600	0.800
400		0.200	0.000	0.000	0.000
800		0.000	0.000	0.000	0.000

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	01-7219-1164	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:36	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	11 Jan-23 14:34	**MD5 Hash:**	DF7FDE934F83BDDA7A3C88C813BFA55	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	100	200	141.4	---	0.192	19.16%

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		50	6	16	10	1	CDF	0.5661	Non-Significant Effect
		100	6	12	10	1	CDF	0.1228	Non-Significant Effect
		200*	6	10	10	0	CDF	0.0350	Significant Effect
		400*	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	2.93998	0.734995	4	39.4	<1.0E-05	Significant Effect
Error	0.27989	0.0186593	15			
Total	3.21987		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	0.917	0.866	0.0850	Normal Distribution

48h Survival Rate Summary

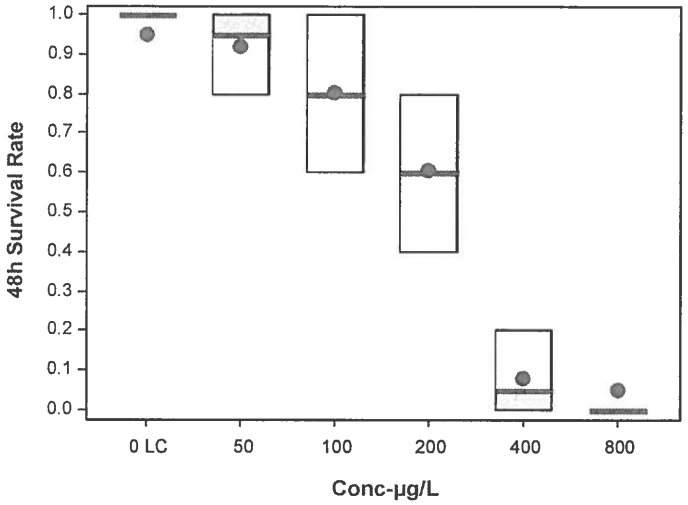
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	5.00%
100		4	0.800	0.540	1.000	0.800	0.600	1.000	0.082	20.41%	20.00%
200		4	0.600	0.340	0.860	0.600	0.400	0.800	0.082	27.22%	40.00%
400		4	0.050	0.000	0.209	0.000	0.000	0.200	0.050	200.00%	95.00%
800		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

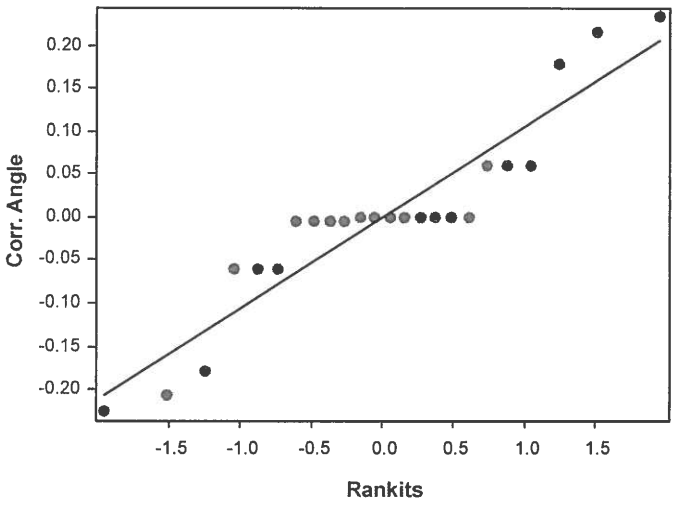
Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
50		4	1.290	1.100	1.480	1.350	1.110	1.350	0.060	9.26%	4.43%
100		4	1.110	0.813	1.410	1.110	0.886	1.350	0.094	16.87%	17.38%
200		4	0.891	0.616	1.170	0.886	0.685	1.110	0.086	19.37%	33.77%
400		4	0.285	0.096	0.475	0.226	0.226	0.464	0.060	41.77%	78.81%
800		4	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%

Inland Silverside 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	01-7219-1164	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:36	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	11 Jan-23 14:34	**MD5 Hash:**	DF7FDE934F83BDDA7A3C88C813BFA55	**Editor ID:**	007-803-386-7

Graphics





Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	15-2152-3233	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:36	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	11 Jan-23 14:34	**MD5 Hash:**	DF7FDE934F83BDDA7A3C88C813BFA55	**Editor ID:**	007-803-386-7

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	100	200	141.4	---	0.192	19.16%

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		50	6	16	10	1	CDF	0.5661	Non-Significant Effect
		100	6	12	10	1	CDF	0.1228	Non-Significant Effect
		200*	6	10	10	0	CDF	0.0350	Significant Effect
		400*	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	2.93998	0.734995	4	39.4	<1.0E-05	Significant Effect
Error	0.27989	0.0186593	15			
Total	3.21987		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	0.917	0.866	0.0850	Normal Distribution

96h Survival Rate Summary

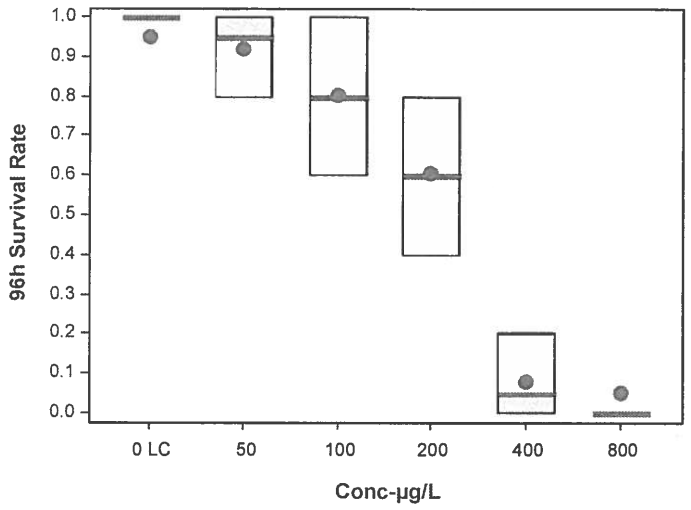
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.000	1.000	1.000	1.000	1.000	1.000	0.000	0.00%	0.00%
50		4	0.950	0.791	1.000	1.000	0.800	1.000	0.050	10.53%	5.00%
100		4	0.800	0.540	1.000	0.800	0.600	1.000	0.082	20.41%	20.00%
200		4	0.600	0.340	0.860	0.600	0.400	0.800	0.082	27.22%	40.00%
400		4	0.050	0.000	0.209	0.000	0.000	0.200	0.050	200.00%	95.00%
800		4	0.000	0.000	0.000	0.000	0.000	0.000	0.000	---	100.00%

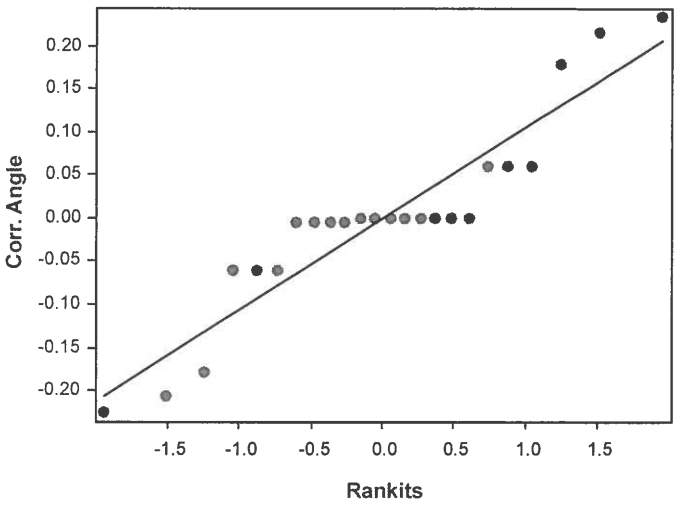
Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	4	1.350	1.340	1.350	1.350	1.350	1.350	0.000	0.00%	0.00%
50		4	1.290	1.100	1.480	1.350	1.110	1.350	0.060	9.26%	4.43%
100		4	1.110	0.813	1.410	1.110	0.886	1.350	0.094	16.87%	17.38%
200		4	0.891	0.616	1.170	0.886	0.685	1.110	0.086	19.37%	33.77%
400		4	0.285	0.096	0.475	0.226	0.226	0.464	0.060	41.77%	78.81%
800		4	0.226	0.225	0.226	0.226	0.226	0.226	0.000	0.00%	83.24%

Inland Silverside 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	15-2152-3233	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:36	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	11 Jan-23 14:34	**MD5 Hash:**	DF7FDE934F83BDDA7A3C88C813BFA55	**Editor ID:**	007-803-386-7

Graphics





CETIS Analytical Report

Test Code/ID: 221214mbra / 05-3190-6319

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	07-1654-3492	**Endpoint:**	48h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:36	**Analysis:**	Trimmed Spearman-Kärber	**Status Level:**	1
Edit Date:	11 Jan-23 14:34	**MD5 Hash:**	DF7FDE934F83BDDA7A3C88C813BFA55	**Editor ID:**	007-803-386-7

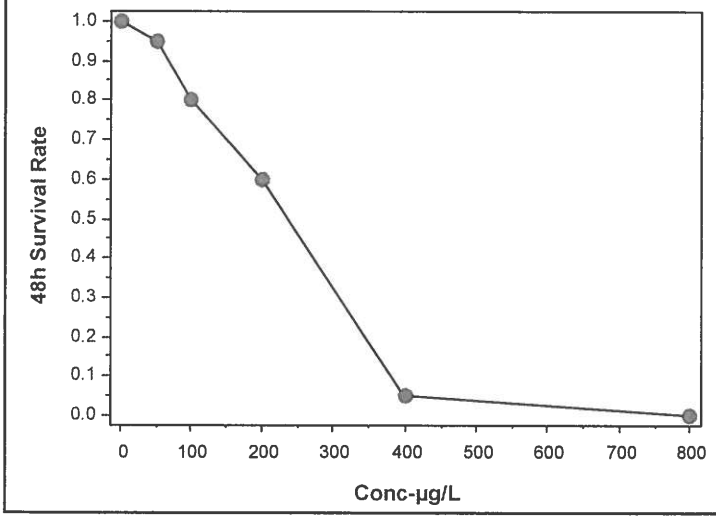
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	2.28	0.0487	192	154	241

48h 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%	20/20	1.000	0.00%
50		4	0.950	1.000	0.800	1.000	10.53%	5.00%	19/20	0.950	5.00%
100		4	0.800	0.800	0.600	1.000	20.41%	20.00%	16/20	0.800	20.00%
200		4	0.600	0.600	0.400	0.800	27.22%	40.00%	12/20	0.600	40.00%
400		4	0.050	0.000	0.000	0.200	200.00%	95.00%	1/20	0.050	95.00%
800		4	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%

Graphics



Analyst: BO QA: ACS 1/11/23

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	15-9199-6379	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	11 Jan-23 14:36	**Analysis:**	Trimmed Spearman-Kärber	**Status Level:**	1
Edit Date:	11 Jan-23 14:34	**MD5 Hash:**	DF7FDE934F83BDDA7A3C88C813BFA55	**Editor ID:**	007-803-386-7

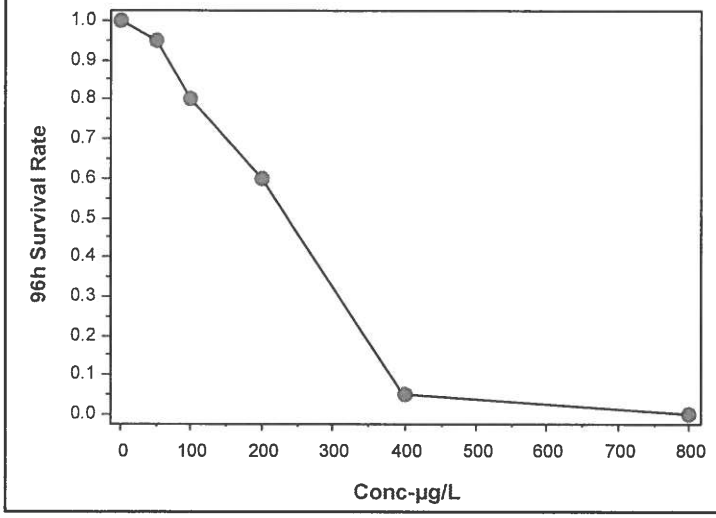
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	2.28	0.0487	192	154	241

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%	20/20	1.000	0.00%
50		4	0.950	1.000	0.800	1.000	10.53%	5.00%	19/20	0.950	5.00%
100		4	0.800	0.800	0.600	1.000	20.41%	20.00%	16/20	0.800	20.00%
200		4	0.600	0.600	0.400	0.800	27.22%	40.00%	12/20	0.600	40.00%
400		4	0.050	0.000	0.000	0.200	200.00%	95.00%	1/20	0.050	95.00%
800		4	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%

Graphics

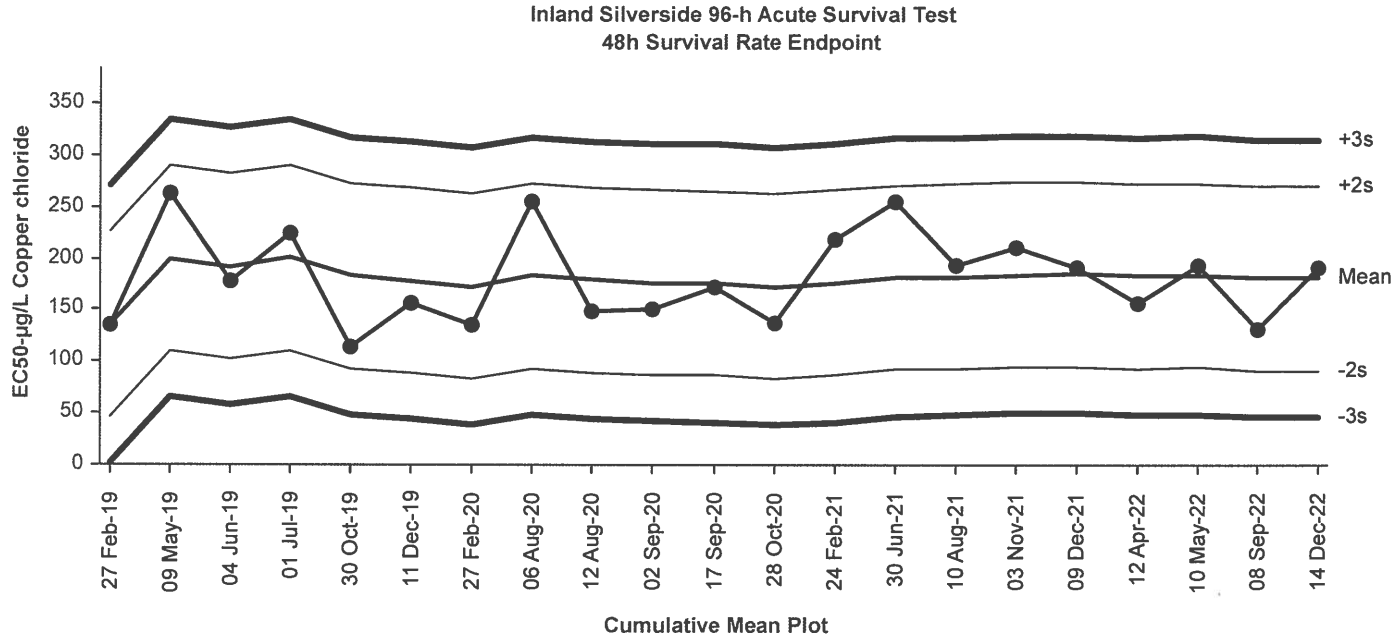


CETIS QC Plot

Report Date: 11 Jan-23 14:37 (1 of 1)

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Test Type:	Survival (96h)	**Organism:**	Menidia beryllina	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	48h Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	181.2	**Count:**	20	**-2s Warning Limit:**	91.4	**-3s Action Limit:**	46.5
Sigma:	44.9	**CV:**	24.80%	**+2s Warning Limit:**	271	**+3s Action Limit:**	316

Quality Control Data

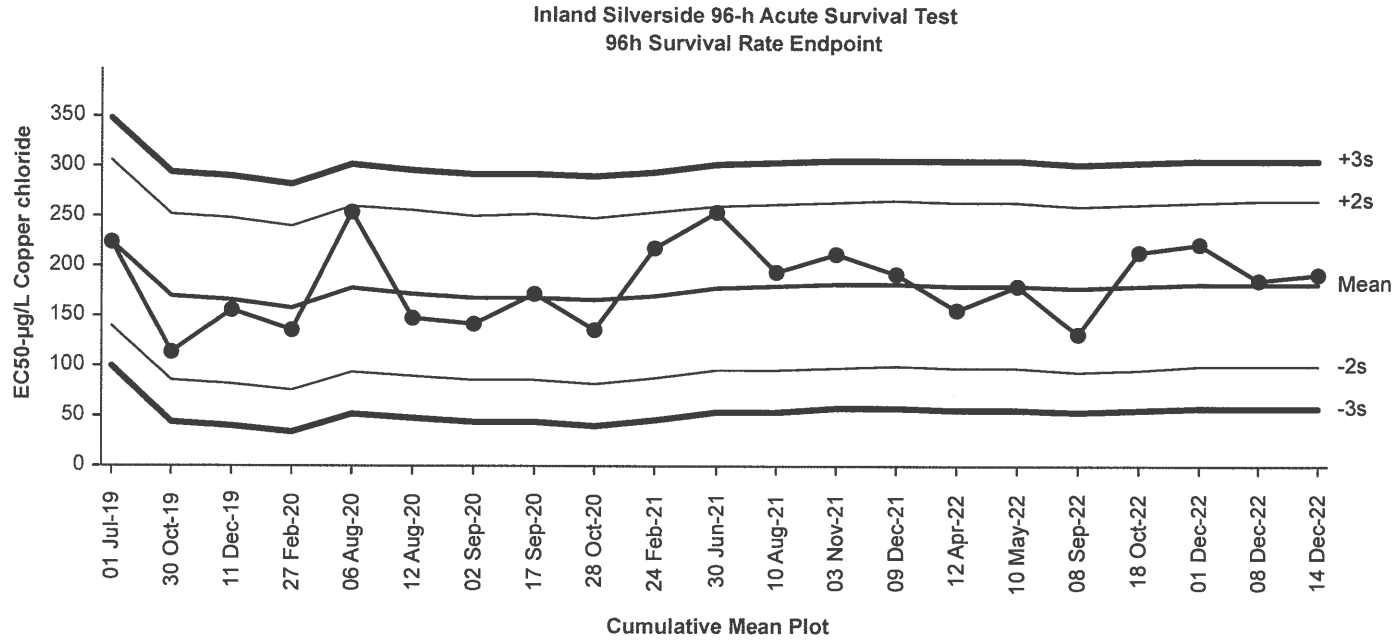
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Feb	27	16:25	135.8	-45.43	-1.012			14-0947-0420	02-5508-4150
2		May	9	19:10	263.9	82.7	1.842			03-9779-6453	03-1351-8680
3		Jun	4	14:50	177.8	-3.445	-0.07673			00-2136-1210	13-3334-6824
4		Jul	1	15:55	223.6	42.42	0.9447			04-4319-5710	18-6869-6221
5		Oct	30	14:45	114.9	-66.33	-1.477			05-0159-0485	12-2414-9813
6		Dec	11	16:30	156.9	-24.28	-0.5408			11-0566-6524	01-2860-3882
7	2020	Feb	27	17:15	136.4	-44.84	-0.9988			00-2639-4829	03-7515-2505
8		Aug	6	16:00	254.9	73.71	1.642			13-3377-6823	17-9944-6476
9			12	15:20	148.4	-32.84	-0.7315			02-5307-3356	02-4132-9895
10		Sep	2	15:25	151.6	-29.63	-0.6599			09-8373-9144	13-7286-8914
11			17	14:45	172	-9.243	-0.2058			07-8442-4358	10-5127-9850
12		Oct	28	16:35	136.6	-44.6	-0.9932			10-9446-3954	17-3578-0181
13	2021	Feb	24	17:30	218.2	36.99	0.8238			11-4316-4077	14-2201-8542
14		Jun	30	16:05	254.9	73.71	1.642			01-4075-9626	08-3028-1140
15		Aug	10	14:30	193.2	11.99	0.267			20-1130-3481	18-0139-1152
16		Nov	3	17:15	211.2	30.05	0.6693			01-2577-3416	15-8121-1522
17		Dec	9	17:20	192.4	11.24	0.2504			15-9690-9061	15-0369-8333
18	2022	Apr	12	17:35	156.9	-24.28	-0.5408			07-5453-0338	01-4100-4645
19		May	10	17:15	193.2	11.99	0.267			13-4082-2694	15-9283-9569
20		Sep	8	18:45	132	-49.25	-1.097			01-2610-4728	12-3529-7018
21		Dec	14	17:05	192.4	11.24	0.2504			05-3190-6319	07-1654-3492

CETIS QC Plot

Report Date: 11 Jan-23 14:37 (1 of 1)

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Test Type:	Survival (96h)	**Organism:**	Menidia beryllina	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	182.3	**Count:**	20	**-2s Warning Limit:**	99.4	**-3s Action Limit:**	57.9
Sigma:	41.45	**CV:**	22.70%	**+2s Warning Limit:**	265	**+3s Action Limit:**	307

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jul	1	15:55	223.6	41.32	0.9967			04-4319-5710	17-4098-1084
2		Oct	30	14:45	114.9	-67.43	-1.627			05-0159-0485	07-6888-5964
3		Dec	11	16:30	156.9	-25.38	-0.6124			11-0566-6524	14-4935-0865
4	2020	Feb	27	17:15	136.4	-45.94	-1.108			00-2639-4829	10-5059-8408
5		Aug	6	16:00	254.9	72.61	1.752			13-3377-6823	09-5433-0150
6			12	15:20	148.4	-33.94	-0.8189			02-5307-3356	11-5066-6205
7		Sep	2	15:25	141.4	-40.88	-0.9862			09-8373-9144	18-7650-2455
8			17	14:45	172	-10.34	-0.2495			07-8442-4358	02-9347-5784
9		Oct	28	16:35	136.6	-45.7	-1.102			10-9446-3954	10-4215-8111
10	2021	Feb	24	17:30	218.2	35.89	0.8658			11-4316-4077	02-1492-4727
11		Jun	30	16:05	254.9	72.61	1.752			01-4075-9626	19-2668-9340
12		Aug	10	14:30	193.2	10.89	0.2627			20-1130-3481	09-5748-8802
13		Nov	3	17:15	211.2	28.95	0.6984			01-2577-3416	13-6085-8539
14		Dec	9	17:20	192.4	10.14	0.2447			15-9690-9061	01-9685-6201
15	2022	Apr	12	17:35	156.9	-25.38	-0.6124			07-5453-0338	19-2336-1516
16		May	10	17:15	180.3	-2.05	-0.04945			13-4082-2694	00-0925-3219
17		Sep	8	18:45	132	-50.35	-1.215			01-2610-4728	13-4659-9428
18		Oct	18	15:45	213.3	30.97	0.7471			04-3098-2404	19-6506-1409
19		Dec	1	18:15	221.9	39.61	0.9557			10-3325-3262	00-5431-5878
20			8	16:42	186.6	4.307	0.1039			14-7600-8927	07-7357-4624
21			14	17:05	192.4	10.14	0.2447			05-3190-6319	15-9199-6379

Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 221214mbra

Test Species: *M. beryllina*
Start Date/Time: 12/14/22 1705
Renewal Date/Time: 12/16/22 1715
End Date/Time: 12/18/22 1520

Cu stock concentration (µg/L): 98,000

	Tech Initials				
	0	24	48	72	96
Counts:	DR	EG	GM	GM	KR
Readings:	RT	HH	GM	WF	KR
Dilutions made by:	HH		GM		
High conc. made (µg/L):	800	–	400	–	–
Vol. Cu stock added (mL):	16.3	–	8.2	–	–
Final Volume (mL):	2000	–	2000	–	–

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	4	5	5	5	5	5	29.7	29.8	i 30.2	30.5	30.7	24.6	25.0	i 24.2	24.5	24.6	6.7	5.2	i 6.9	5.0	5.6	7.93	7.80	i 7.84	7.72	7.79
	10	5	5	5	5	5			f 30.4		(A)	24.5		f 24.9		(A)	6.5		f 6.0		(A)	7.96		f 7.71		
	5	5	5	5	5	5																				
	9	5	5	5	5	5																				
50	23	5	5	5	5	5	29.6	29.8	i 30.7	30.2	30.5	24.6	25.0	i 24.5	25.0	25.1	6.6	5.4	i 6.8	5.5	5.6	7.93	7.80	i 7.84	7.71	7.80
	18	5	4	4	4	4			f 30.0					f 25.4					f 5.5					f 7.68		
	3	5	5	5	5	5																				
	6	5	5	5	5	5																				
100	19	5	5	5	5	5	29.6	29.8	i 30.1	30.1	30.1	24.6	25.0	i 24.5	25.2	25.3	6.6	5.3	i 6.7	5.2	5.3	7.93	7.80	i 7.85	7.72	7.74
	14	5	4	4	4	4			f 29.8					f 25.3					f 5.4					f 7.68		
	2	5	3	3	3	3																				
	17	5	4	4	4	4																				
200	20	5	2	2	2	2	29.5	29.7	i 30.1	30.2	30.3	24.6	25.0	i 24.4	25.2	25.3	6.6	5.2	i 6.7	5.5	5.4	7.93	7.80	i 7.85	7.80	7.84
	11	5	3	3	3	3			f 29.7					f 25.7					f 5.3					f 7.67		
	7	5	3	3	3	3																				
	22	5	4	4	4	4																				
400	8	5	1	1	1	(D) 01	29.4	29.6	i 30.0	30.2	30.2	24.5	25.0	i 24.2	25.1	25.2	6.6	5.5	i 6.8	5.7	5.6	7.92	7.84	i 7.85	7.84	7.87
	15	5	0	–	–	–			f 29.7					f 25.7					f 5.5					f 7.73		
	1	5	0	–	–	–																				
	21	5	1	0	–	–																				
800	13	5	0				29.3	29.6	i –	–	–	24.6	25.0	i –	–	–	6.6	5.5	i –	–	–	7.89	7.83	i –	–	
	16	5	0						f –					f –					f –							
	24	5	0	all dead																						
	12	5	0																							

Rand # QC: KR
Initial Counts QC'd by: HH
Initiated by: DR

Environmental Chamber: A

Animal Source/Date Received: ABS 12/9/22
Age at Initiation: 14 days

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none

Feeding Times					
	0	24	48	72	96
AM:		0900	0910	0855	0835
PM:	1730				

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y) n) (A) Q18 RT 12/14/22
(B) Q18 KR 12/10/22

QC Check: BO 1/11/23
Final Review: AJS 1/11/23

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