Data Quality Summary: Wyckoff 3rd 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	2208-S194	EPA600/R-95/136	chronic bioassay

Notes:
Enthalpy = Enthalpy AnalyticalPolycyclic aromatic hydrocarbons
SDG = Sample Delivery Group

One water sample was collected for bioassay on August 23, 2022, for chronic toxicity. The bioassay was 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 an equivalent species of mussel to what was specified in the QAPP was used.

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 (No Observed Effect Concentration) of 72.5 (the highest concentration tested) and a TUc of less than 1.4. There was a statistically significant effect detected in the 72.5 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 72.5 (the highest concentration tested).

A CH2M Subject Matter Expert (SME) reviewed the results for performance criteria. The reviewer concluded that the test was considered valid and usable as it met the minimum control performance criteria.

Attachment 1
Bioassay Report

ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: August 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: **September 22, 2022**

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	**082322**
Enthalpy Log-in Number	22-1105
Collection Date; Time	8/23/22; 1011h
Receipt Date; Time	8/24/22; 1020h
Receipt Temperature (°C)	2.3
Dissolved Oxygen (mg/L)	9.1
pH	7.62
Conductivity (µS/cm)	5,100
Salinity (ppt)	3.2
Alkalinity (mg/L $CaCO_3$)	460
Total Chlorine (mg/L)	0.04
Total Ammonia (mg/L as N)	4.1

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	8/24/22, 1700h to 8/26/22, 1645h
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 100.8 ppt
Test Concentrations (% sample)	72.5[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 72.5 (the highest concentration tested) and a TU_c of less than 1.4.

There was a statistically significant effect detected in the 72.5 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	72.5	2.9	> 72.5
	Survival	72.5	> 72.5	< 1.4	> 72.5

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)	84.0	97.1
0 (Lab Control)	85.2	95.6
2	92.8	95.3
4	94.5	96.9
9	94.2	96.6
18	92.2	96.7
35	88.7	96.3
72.5[a]	80.7	**77.2**

[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 within the required 36-hour holding time, in good condition, and within the appropriate temperature range of 0-6°C. 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	23.5	26.9 ± 9.06	16.9
Bivalve Normal Development	5	10.3	9.74 ± 4.85	24.9

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. 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:	07-9280-9596	**Test Type:**	Development-Survival	**Analyst:**			
Start Date:	24 Aug-22 17:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater		
Ending Date:	26 Aug-22 16:45	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater		
Test Length:	48h	**Taxon:**		**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	08-8636-4664	**Code:**	22-1105	**Project:**	
Sample Date:	23 Aug-22 10:11 PDT	**Material:**	Effluent Sample	**Source:**	Jacobs
Receipt Date:	24 Aug-22 10:20 PDT	**CAS (PC):**		**Station:**	Wyckoff
Sample Age:	31h (2.3 °C)	**Client:**	Jacobs		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	TU	S
02-4962-3187	Combined Development Rat	Dunnett Multiple Comparison Test		35	72.5	50.37	13.6%	2.9	1
09-9973-1790	Development Rate	Dunnett Multiple Comparison Test		35	72.5	50.37	3.44%	2.9	1
09-3004-8819	Survival Rate	Dunnett Multiple Comparison Test		72.5	>72.5	---	19.1%	<1.4	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	%	95% LCL	95% UCL	TU	S
19-4289-3573	Combined Development Rat	Linear Interpolation (ICPIN)		EC25	66.3	58.1	---	1.5	1
				EC50	>72.5	---	---	<1.4	
13-5196-6972	Development Rate	Linear Interpolation (ICPIN)		EC25	>72.5	---	---	<1.4	1
				EC50	>72.5	---	---	<1.4	
12-4770-7639	Survival Rate	Linear Interpolation (ICPIN)		EC25	>72.5	---	---	<1.4	1
				EC50	>72.5	---	---	<1.4	

Test Acceptability

				TAC Limits			
Analysis ID	**Endpoint**	**Attribute**	**Test Stat**	**Lower**	**Upper**	**Overlap**	**Decision**
09-9973-1790	Development Rate	Control Resp	0.956	0.9	<<	Yes	Passes Criteria
13-5196-6972	Development Rate	Control Resp	0.956	0.9	<<	Yes	Passes Criteria
09-3004-8819	Survival Rate	Control Resp	0.84	0.5	<<	Yes	Passes Criteria
12-4770-7639	Survival Rate	Control Resp	0.84	0.5	<<	Yes	Passes Criteria
02-4962-3187	Combined Development Rat	PMSD	0.136	<<	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	0.803	0.724	0.882	0.730	0.894	0.029	0.064	7.92%	0.00%
0	LC	5	0.828	0.763	0.892	0.762	0.889	0.023	0.052	6.24%	-3.03%
2		5	0.884	0.811	0.957	0.810	0.953	0.026	0.059	6.66%	-10.02%
4		5	0.915	0.846	0.983	0.836	0.963	0.025	0.056	6.06%	-13.87%
9		5	0.909	0.875	0.943	0.878	0.948	0.012	0.028	3.04%	-13.20%
18		5	0.891	0.814	0.968	0.820	0.964	0.028	0.062	6.94%	-10.95%
35		5	0.854	0.772	0.936	0.772	0.937	0.030	0.066	7.75%	-6.32%
72.5		5	0.622	0.558	0.686	0.566	0.704	0.023	0.052	8.30%	22.53%

Development Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	BC	5	0.956	0.929	0.983	0.931	0.986	0.010	0.022	2.28%	0.00%
0	LC	5	0.971	0.963	0.980	0.966	0.982	0.003	0.007	0.73%	-1.56%
2		5	0.953	0.940	0.965	0.936	0.964	0.005	0.010	1.08%	0.40%
4		5	0.969	0.952	0.985	0.957	0.988	0.006	0.013	1.35%	-1.27%
9		5	0.966	0.952	0.979	0.948	0.977	0.005	0.011	1.12%	-0.97%
18		5	0.967	0.945	0.990	0.942	0.988	0.008	0.018	1.88%	-1.14%
35		5	0.963	0.953	0.972	0.953	0.973	0.003	0.008	0.80%	-0.68%
72.5		5	0.772	0.684	0.861	0.704	0.875	0.032	0.071	9.22%	19.24%

Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	BC	5	0.840	0.754	0.926	0.741	0.921	0.031	0.069	8.25%	0.00%
0	LC	5	0.852	0.788	0.916	0.788	0.921	0.023	0.052	6.07%	-1.39%
2		5	0.928	0.843	1.010	0.847	1.000	0.031	0.068	7.35%	-10.45%
4		5	0.945	0.859	1.030	0.847	1.000	0.031	0.069	7.29%	-12.47%
9		5	0.942	0.895	0.989	0.905	1.000	0.017	0.038	4.01%	-12.09%
18		5	0.922	0.836	1.010	0.836	1.000	0.031	0.069	7.50%	-9.70%
35		5	0.887	0.804	0.969	0.804	0.963	0.030	0.067	7.51%	-5.54%
72.5		5	0.807	0.743	0.871	0.730	0.873	0.023	0.052	6.38%	3.90%

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Combined Development Rate Detail MD5: 337EC4AADCEB27F2727C492090A27E4E

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	0.788	0.730	0.894	0.836	0.767
0	LC	0.788	0.841	0.762	0.889	0.857
2		0.889	0.841	0.810	0.953	0.926
4		0.937	0.963	0.878	0.959	0.836
9		0.894	0.899	0.948	0.926	0.878
18		0.964	0.873	0.852	0.820	0.947
35		0.810	0.937	0.899	0.852	0.772
72.5		0.624	0.624	0.566	0.593	0.704

Development Rate Detail MD5: 2CE74B52B51BB7C1DA7234DE2C6CA190

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	0.931	0.986	0.971	0.946	0.948
0	LC	0.968	0.975	0.966	0.966	0.982
2		0.955	0.964	0.956	0.953	0.936
4		0.957	0.963	0.976	0.959	0.988
9		0.966	0.977	0.948	0.967	0.971
18		0.964	0.988	0.942	0.981	0.962
35		0.968	0.973	0.960	0.953	0.961
72.5		0.715	0.756	0.704	0.812	0.875

Survival Rate Detail MD5: A82E4F8BFB21AAAFCAFCC354BBC6F795

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	0.847	0.741	0.921	0.884	0.810
0	LC	0.815	0.862	0.788	0.921	0.873
2		0.931	0.873	0.847	1.000	0.989
4		0.979	1.000	0.899	1.000	0.847
9		0.926	0.921	1.000	0.958	0.905
18		1.000	0.884	0.905	0.836	0.984
35		0.836	0.963	0.937	0.894	0.804
72.5		0.873	0.825	0.804	0.730	0.804

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

Combined Development Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	149/189	138/189	169/189	158/189	145/189
0	LC	149/189	159/189	144/189	168/189	162/189
2		168/189	159/189	153/189	181/190	175/189
4		177/189	182/189	166/189	188/196	158/189
9		169/189	170/189	182/192	175/189	166/189
18		186/193	165/189	161/189	155/189	179/189
35		153/189	177/189	170/189	161/189	146/189
72.5		118/189	118/189	107/189	112/189	133/189

Development Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	149/160	138/140	169/174	158/167	145/153
0	LC	149/154	159/163	144/149	168/174	162/165
2		168/176	159/165	153/160	181/190	175/187
4		177/185	182/189	166/170	188/196	158/160
9		169/175	170/174	182/192	175/181	166/171
18		186/193	165/167	161/171	155/158	179/186
35		153/158	177/182	170/177	161/169	146/152
72.5		118/165	118/156	107/152	112/138	133/152

Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	BC	160/189	140/189	174/189	167/189	153/189
0	LC	154/189	163/189	149/189	174/189	165/189
2		176/189	165/189	160/189	189/189	187/189
4		185/189	189/189	170/189	189/189	160/189
9		175/189	174/189	189/189	181/189	171/189
18		189/189	167/189	171/189	158/189	186/189
35		158/189	182/189	177/189	169/189	152/189
72.5		165/189	156/189	152/189	138/189	152/189

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

Analysis ID:	02-4962-3187	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	46BD23481B982D8DD1C2EAC34CE9A253	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	35	72.5	50.37	2.9	0.109	13.62%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Brine Control		2	8	-2.13	2.41	0.131	CDF	0.9997	Non-Significant Effect
		4	8	-3.12	2.41	0.131	CDF	1.0000	Non-Significant Effect
		9	8	-2.79	2.41	0.131	CDF	1.0000	Non-Significant Effect
		18	8	-2.4	2.41	0.131	CDF	0.9999	Non-Significant Effect
		35	8	-1.3	2.41	0.131	CDF	0.9952	Non-Significant Effect
		72.5*	8	3.78	2.41	0.131	CDF	0.0020	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.516271	0.0860451	6	11.6	<1.0E-05	Significant Effect
Error	0.20775	0.0074196	28			
Total	0.72402		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	3.46	16.8	0.7496	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.953	0.915	0.1399	Normal Distribution

Combined Development Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	0.803	0.724	0.882	0.788	0.730	0.894	0.029	7.92%	0.00%
2		5	0.884	0.811	0.957	0.889	0.810	0.953	0.026	6.66%	-10.02%
4		5	0.915	0.846	0.983	0.937	0.836	0.963	0.025	6.06%	-13.87%
9		5	0.909	0.875	0.943	0.899	0.878	0.948	0.012	3.04%	-13.20%
18		5	0.891	0.814	0.968	0.873	0.820	0.964	0.028	6.94%	-10.95%
35		5	0.854	0.772	0.936	0.852	0.772	0.937	0.030	7.75%	-6.32%
72.5		5	0.622	0.558	0.686	0.624	0.566	0.704	0.023	8.30%	22.53%

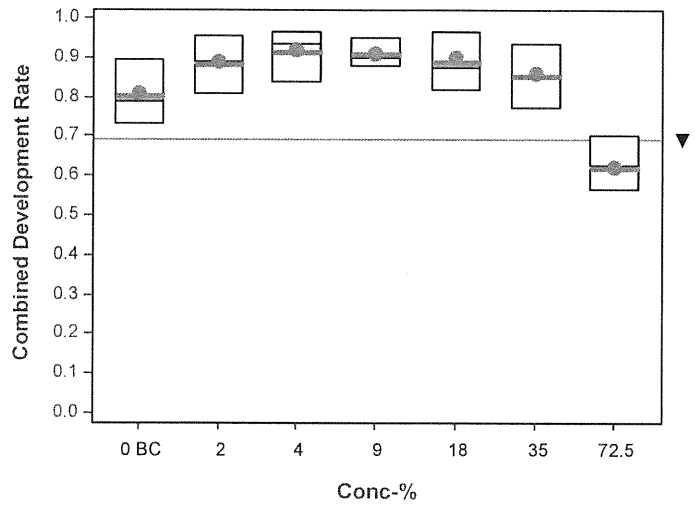
Angular (Corrected) Transformed Summary

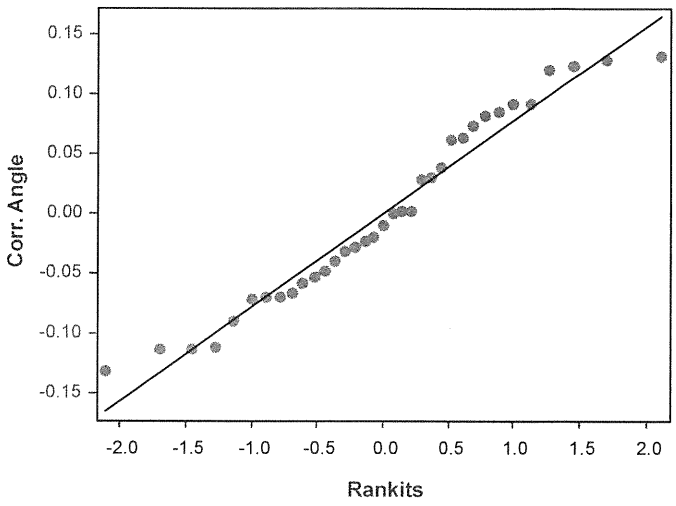
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	1.120	1.010	1.220	1.090	1.020	1.240	0.037	7.49%	0.00%
2		5	1.230	1.110	1.350	1.230	1.120	1.350	0.042	7.70%	-10.39%
4		5	1.290	1.160	1.410	1.320	1.150	1.380	0.044	7.62%	-15.26%
9		5	1.270	1.210	1.330	1.250	1.210	1.340	0.022	3.96%	-13.62%
18		5	1.250	1.110	1.380	1.210	1.130	1.380	0.048	8.57%	-11.74%
35		5	1.190	1.070	1.310	1.180	1.070	1.320	0.044	8.21%	-6.36%
72.5		5	0.910	0.843	0.977	0.911	0.852	0.995	0.024	5.93%	18.47%

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

Analysis ID:	02-4962-3187	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	46BD23481B982D8DD1C2EAC34CE9A253	**Editor ID:**	007-926-968-0

Graphics





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

Analysis ID:	09-9973-1790	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	CD02D2B9A1FC1B8356BAD1446CC3BD5	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	35	72.5	50.37	2.9	0.0329	3.44%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Brine Control		2	8	0.458	2.41	0.0762	CDF	0.6980	Non-Significant Effect
		4	8	-0.934	2.41	0.0762	CDF	0.9851	Non-Significant Effect
		9	8	-0.612	2.41	0.0762	CDF	0.9638	Non-Significant Effect
		18	8	-0.894	2.41	0.0762	CDF	0.9832	Non-Significant Effect
		35	8	-0.351	2.41	0.0762	CDF	0.9316	Non-Significant Effect
		72.5*	8	9.13	2.41	0.0762	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.396136	0.0660227	6	26.4	<1.0E-05	Significant Effect
Error	0.0701286	0.0025046	28			
Total	0.466265		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	11.8	16.8	0.0654	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.972	0.915	0.5084	Normal Distribution

Development Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	0.956	0.929	0.983	0.948	0.931	0.986	0.010	2.28%	0.00%
2		5	0.953	0.940	0.965	0.955	0.936	0.964	0.005	1.08%	0.40%
4		5	0.969	0.952	0.985	0.963	0.957	0.988	0.006	1.35%	-1.27%
9		5	0.966	0.952	0.979	0.967	0.948	0.977	0.005	1.12%	-0.97%
18		5	0.967	0.945	0.990	0.964	0.942	0.988	0.008	1.88%	-1.14%
35		5	0.963	0.953	0.972	0.961	0.953	0.973	0.003	0.80%	-0.68%
72.5		5	0.772	0.684	0.861	0.756	0.704	0.875	0.032	9.22%	19.24%

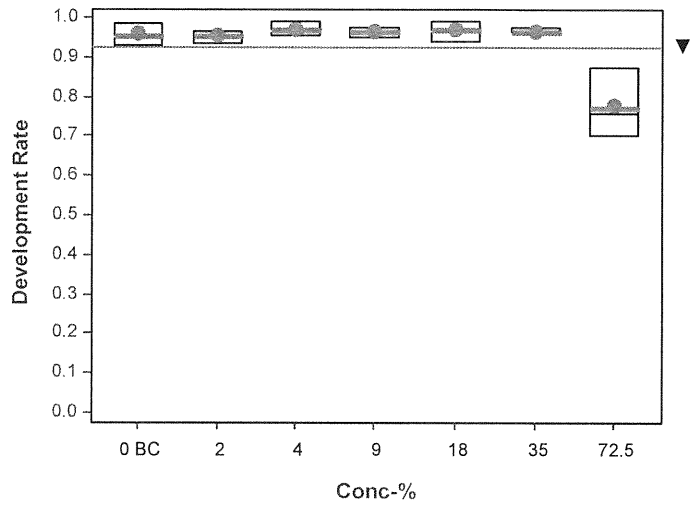
Angular (Corrected) Transformed Summary

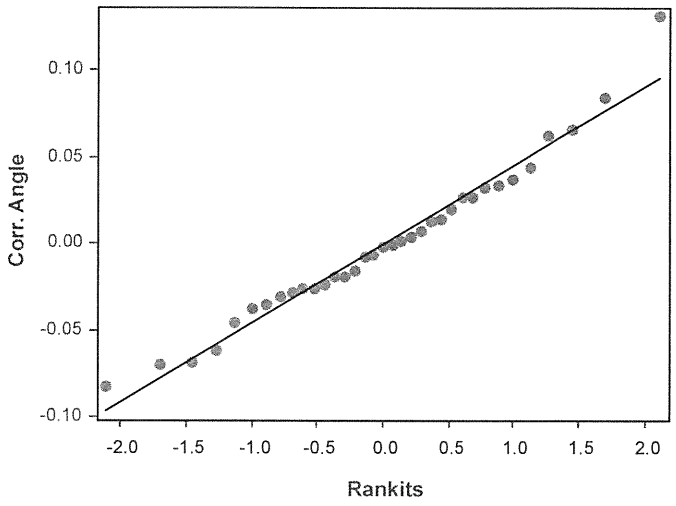
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	1.370	1.290	1.440	1.340	1.310	1.450	0.026	4.27%	0.00%
2		5	1.350	1.320	1.380	1.360	1.310	1.380	0.011	1.73%	1.06%
4		5	1.400	1.350	1.450	1.380	1.360	1.460	0.018	2.94%	-2.16%
9		5	1.390	1.350	1.420	1.390	1.340	1.420	0.013	2.07%	-1.42%
18		5	1.390	1.330	1.460	1.380	1.330	1.460	0.024	3.78%	-2.07%
35		5	1.380	1.350	1.400	1.370	1.350	1.400	0.009	1.49%	-0.81%
72.5		5	1.080	0.968	1.190	1.050	0.995	1.210	0.040	8.23%	21.14%

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

Analysis ID:	09-9973-1790	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	CD02D2B9A1FC1B8356BAD1446CC3BD5	**Editor ID:**	007-926-968-0

Graphics





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

Analysis ID:	09-3004-8819	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	699AE8001E9E2564972A450232BB658B	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	72.5	>72.5	---	1.4	0.16	19.08%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Brine Control		2	8	-2.08	2.41	0.197	CDF	0.9997	Non-Significant Effect
		4	8	-2.64	2.41	0.197	CDF	1.0000	Non-Significant Effect
		9	8	-2.21	2.41	0.197	CDF	0.9999	Non-Significant Effect
		18	8	-1.91	2.41	0.197	CDF	0.9994	Non-Significant Effect
		35	8	-0.897	2.41	0.197	CDF	0.9834	Non-Significant Effect
		72.5	8	0.577	2.41	0.197	CDF	0.6468	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.299988	0.0499981	6	2.99	0.0220	Significant Effect
Error	0.468662	0.0167379	28			
Total	0.76865		34			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	4.49	16.8	0.6106	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.964	0.915	0.2927	Normal Distribution

Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	0.840	0.754	0.926	0.847	0.741	0.921	0.031	8.25%	0.00%
2		5	0.928	0.843	1.000	0.931	0.847	1.000	0.031	7.35%	-10.45%
4		5	0.945	0.859	1.000	0.979	0.847	1.000	0.031	7.29%	-12.47%
9		5	0.942	0.895	0.989	0.926	0.905	1.000	0.017	4.01%	-12.09%
18		5	0.922	0.836	1.000	0.905	0.836	1.000	0.031	7.50%	-9.70%
35		5	0.887	0.804	0.969	0.894	0.804	0.963	0.030	7.51%	-5.54%
72.5		5	0.807	0.743	0.871	0.804	0.730	0.873	0.023	6.38%	3.90%

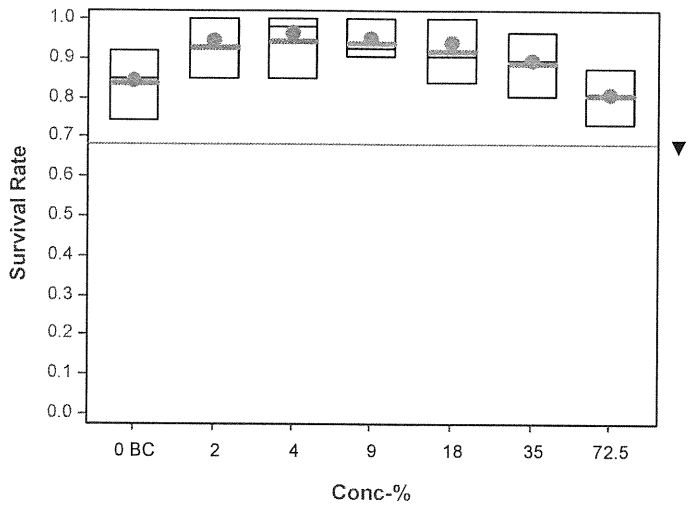
Angular (Corrected) Transformed Summary

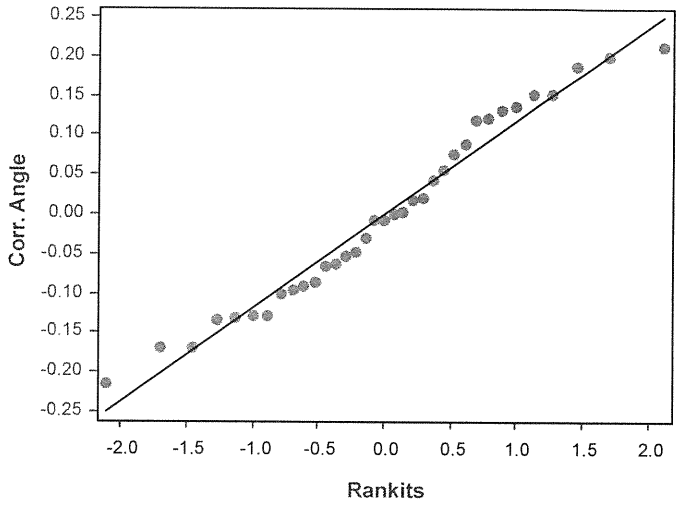
Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	BC	5	1.170	1.050	1.280	1.170	1.040	1.290	0.043	8.18%	0.00%
2		5	1.340	1.140	1.540	1.310	1.170	1.530	0.072	11.98%	-14.58%
4		5	1.380	1.170	1.590	1.420	1.170	1.530	0.075	12.10%	-18.49%
9		5	1.350	1.210	1.490	1.300	1.260	1.530	0.050	8.30%	-15.49%
18		5	1.320	1.120	1.520	1.260	1.150	1.530	0.072	12.10%	-13.38%
35		5	1.240	1.100	1.380	1.240	1.110	1.380	0.049	8.86%	-6.29%
72.5		5	1.120	1.040	1.200	1.110	1.020	1.210	0.029	5.84%	4.05%

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

Analysis ID:	09-3004-8819	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	699AE8001E9E2564972A450232BB658B	**Editor ID:**	007-926-968-0

Graphics





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

Analysis ID:	19-4289-3573	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	46BD23481B982D8DD1C2EAC34CE9A253	**Editor ID:**	007-926-968-0

Linear Interpolation Options

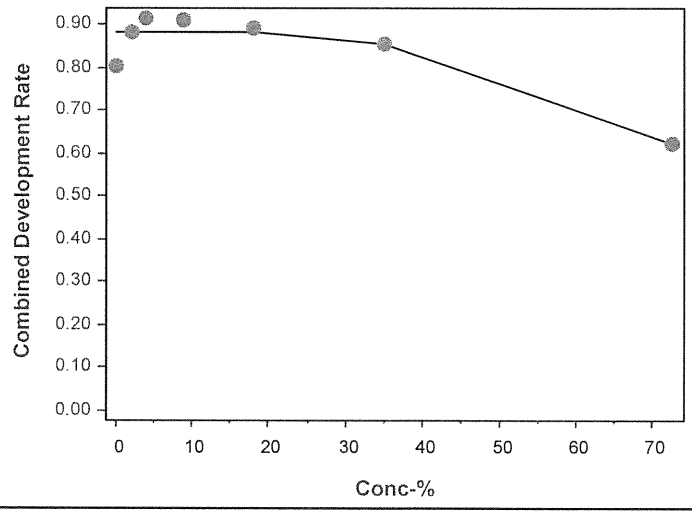
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1460066	1000	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	66.3	58.1	---	1.5	---	1.7
EC50	>72.5	---	---	<1.4	---	---

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	0.803	0.788	0.730	0.894	7.92%	0.00%	759/945	0.881	0.00%
2		5	0.884	0.889	0.810	0.953	6.66%	-10.02%	836/946	0.881	0.00%
4		5	0.915	0.937	0.836	0.963	6.06%	-13.87%	871/952	0.881	0.00%
9		5	0.909	0.899	0.878	0.948	3.04%	-13.20%	862/948	0.881	0.00%
18		5	0.891	0.873	0.820	0.964	6.94%	-10.95%	846/949	0.881	0.00%
35		5	0.854	0.852	0.772	0.937	7.75%	-6.32%	807/945	0.854	3.06%
72.5		5	0.622	0.624	0.566	0.704	8.30%	22.53%	588/945	0.622	29.40%

Graphics



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

Analysis ID:	13-5196-6972	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	CD02D2B9A1FC1B8356BAD1446CC3BD5	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1895024	1000	Yes	Two-Point Interpolation

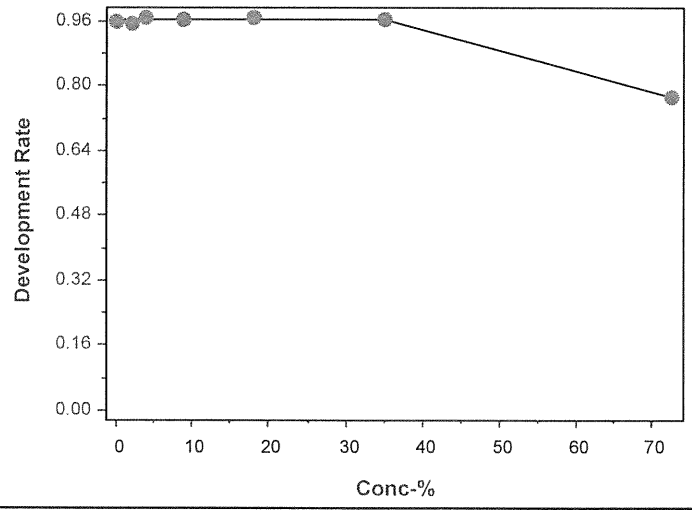
Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	>72.5	---	---	<1.4	---	---
EC50	>72.5	---	---	<1.4	---	---

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	0.956	0.948	0.931	0.986	2.28%	0.00%	759/794	0.962	0.00%
2		5	0.953	0.955	0.936	0.964	1.08%	0.40%	836/878	0.962	0.00%
4		5	0.969	0.963	0.957	0.988	1.35%	-1.27%	871/900	0.962	0.00%
9		5	0.966	0.967	0.948	0.977	1.12%	-0.97%	862/893	0.962	0.00%
18		5	0.967	0.964	0.942	0.988	1.88%	-1.14%	846/875	0.962	0.00%
35		5	0.963	0.961	0.953	0.973	0.80%	-0.68%	807/838	0.962	0.00%
72.5		5	0.772	0.756	0.704	0.875	9.22%	19.24%	588/763	0.771	19.85%

Graphics



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

Analysis ID:	12-4770-7639	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	19 Sep-22 11:18	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	19 Sep-22 11:11	**MD5 Hash:**	699AE8001E9E2564972A450232BB658B	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1416080	1000	Yes	Two-Point Interpolation

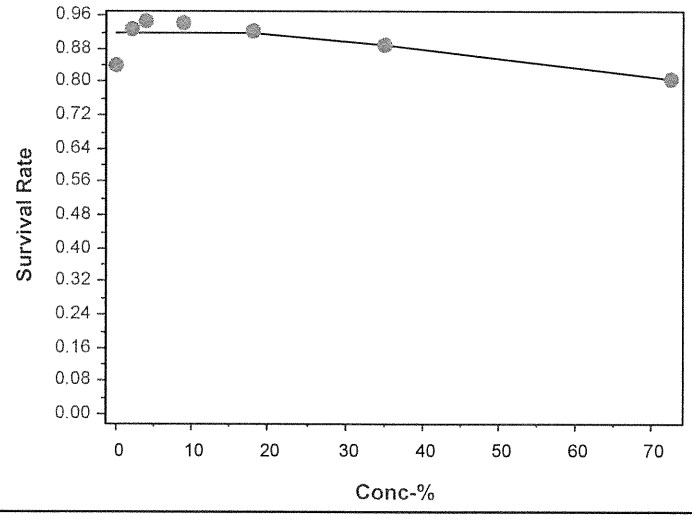
Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
EC25	>72.5	---	---	<1.4	---	---
EC50	>72.5	---	---	<1.4	---	---

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	0.840	0.847	0.741	0.921	8.25%	0.00%	794/945	0.915	0.00%
2		5	0.928	0.931	0.847	1.000	7.35%	-10.45%	877/945	0.915	0.00%
4		5	0.945	0.979	0.847	1.000	7.29%	-12.47%	893/945	0.915	0.00%
9		5	0.942	0.926	0.905	1.000	4.01%	-12.09%	890/945	0.915	0.00%
18		5	0.922	0.905	0.836	1.000	7.50%	-9.70%	871/945	0.915	0.00%
35		5	0.887	0.894	0.804	0.963	7.51%	-5.54%	838/945	0.887	3.06%
72.5		5	0.807	0.804	0.730	0.873	6.38%	3.90%	763/945	0.807	11.80%

Graphics



CETIS Test Data Worksheet

Report Date: 20 Aug-22 12:57 (p 1 of 1)
Test Code: 1105-5194 05-3891-7529/201F3A99

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

Start Date: 24 Aug-22
End Date: 26 Aug-22
Sample Date: 23 Aug-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 22- 1105
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			61			152	~~141~~ (A) 107	TT 9/4/22
			62			189	182	
			63			171	161	
			64			174	169	
			65			140	138	
			66			196	188	
			67			138	112	
			68			158	153	
			69			165	162	
			70			176	168	
			71			152	133	
			72			167	158	
			73			174	170	
			74			158	155	
			75			170	166	
			76			177	170	
			77			165	118	
			78			185	177	
			79			175	169	
			80			182	177	
			81			190	181	
			82			181	175	
			83			165	159	
			84			166	153	
			85			153	145	
			86			193	186	
			87			160	158	
			88			149	144	
			89			154	149	
			90			152	146	
			91			186	179	
			92			163	159	
			93			174	168	
			94			169	161	
			95			167	165	
			96			192	182	
			97			156	118	
			98			171	166	
			99			160	149	
			100			187	175	

(A) Q18 TT 9/4/22

CETIS Test Data Worksheet

Report Date: 20 Aug-22 12:57 (p 1 of 1)
Test Code: 05-3891-7529/201F3A99

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 24 Aug-22
End Date: 26 Aug-22
Sample Date: 23 Aug-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 22-1105
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	99					
0	BC	2	65					
0	BC	3	64			155	153	RT 9/6/22
0	BC	4	72					
0	BC	5	85					
0	LC	1	89					
0	LC	2	92					
0	LC	3	88			140	136	
0	LC	4	93					
0	LC	5	69					
2		1	70					
2		2	83					
2		3	84			163	154	
2		4	81					
2		5	100					
4		1	78					
4		2	62					
4		3	75			166	158	
4		4	66					
4		5	87					
9		1	79					
9		2	73					
9		3	96			180	170	
9		4	82					
9		5	98					
18		1	86					
18		2	95					
18		3	63			167	158	
18		4	74					
18		5	91					
35		1	68					
35		2	80					
35		3	76			178	170	
35		4	94					
35		5	90					
69.1		1	77					
69.1		2	97					
69.1 (A)		3	61			154	118	
69.1		4	67					
69.1		5	71					

125

QC: RT

(A) CV 18 HM 8/22/22

CETIS™ v1.8.7.20

Analyst: HM QA: BO 9/22/22

Marine Chronic Bioassay

DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** RT

Sample ID: Wyckoff **Test Date:** 8/24/2022

Test No: 2208-S194 **Test Type:** Mussel Development

Salinity of Effluent 3.2

Salinity of Brine 100.8 **Date of Brine used:** 8/3/2022

Target Salinity 30 **Alkalinity of Brine Control:** 114 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.38**	**0.42**

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	0.38	1.9	250
4	10.0	0.38	3.8	250
9	22.5	0.38	8.5	250
18	45.0	0.38	17.0	250
35	87.5	0.38	33.1	250
72.5	181.4	0.38	68.6	250
	DI Volume			
Brine Control	162.0	0.42	68.6	250

Total Brine Volume Required (ml): **201.6**

QC Check: VS 9/9/22 **Final Review:** BO 9/22/22

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS

Sample ID: Wyckoff

Sample Log No.: 22-1105

Test No.: 2208-5194

Test Species: *M. galloprovincialis*

Start Date/Time: 8/24/22 1700

End Date/Time: 8/26/22 1645

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	29.4	29.2	29.1	16.0	14.7	15.2	7.9	8.4	8.7	8.04	8.03	7.97
Brine Control	30.5	30.5	30.4	15.7	14.6	14.9	8.1	8.4	8.7	8.18	8.12	8.04
2	29.7	29.7	29.6	15.9	14.6	14.9	8.0	8.3	8.7	8.03	8.03	8.01
4	29.6	29.6	29.6	15.8	14.6	15.0	8.0	8.3	8.7	8.00	8.03	8.02
9	29.8	29.8	29.7	15.8	14.6	15.1	7.9	8.3	8.7	7.92	8.02	8.06
18	29.7	29.8	29.7	15.8	14.6	15.1	7.9	8.3	8.7	7.84	8.01	8.09
35	30.0	30.0	30.0	15.8	14.6	15.1	7.8	8.1	8.6	7.76	7.97	8.10
72.5	30.4	30.4	30.3	15.8	14.6	15.1	7.8	7.6	8.5	7.64	7.89	8.13

Technician Initials:

	0	24	48
WQ Readings:	RT	KD	GM
Dilutions made by:	RT	–	–

Environmental Chamber: D

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: 9/9/22

Final Review: 9/22/22

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs / Wychoff
Test No.: 2208-5104
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: 4A 6T M-REP
Date Received: 6/7/22 QA/AS 6/24/22
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 8/24/22 1700
End Date/Time: 8/26/22 1645
Technician Initials: RT

Spawn Information

First Gamete Release Time: 1225

Sex	Number Spawning
Male	1
Female	2

Gamete Selection

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

Egg Fertilization Time: ~~1305~~ 1525
QA/AS 8/24/22

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	80
Female 2	99
Female 3	

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted:		
	6	6
	6	7
	11	1
	6	5
	7	15

Mean: 7

Mean 7 X 50 = 350 embryos/ml

Initial Density: 350 = 1.17 (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	201	201	100	100
TØ B	~~148~~ 177	~~148~~ 177	100	
TØ C	178	178	100	
TØ D	185	185	100	
TØ E	179	179	100	
TØ F	213	213	100	
$\bar{X}$ =	189			

48-h QC: 169/173 97.7%

Comments: QA/AS 8/26/22

QC Check: VS 9/9/22

Final Review: BO 9/22/22

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff
Test ID No(s).: 2208-5194

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

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

Sample (A, B, C):	A			
Log-in No. (22-xxxx):	1105			
Sample Collection Date & Time:	8/23/22 1011			
Sample Receipt Date & Time:	8/24/22 1020			
Number of Containers & Container Type:	1x 2L cubi			
Approx. Total Volume Received (L):	1			
Check-in Temperature (°C)	2.3			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.1			
pH (units)	7.62			
Conductivity (µS/cm)	5100			
Salinity (ppt)	3.2			
Alkalinity (mg/L) [2]	460			
Hardness (mg/L) [2, 3]	—			
Total Chlorine (mg/L)	0.04			
Technician Initials	WF			

Subsamples for Additional Chemistry Required:
(NH3) (always required)
Other _____
Tech Initials A WF B___ C___

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

Filtration? Y (N) **Initials:** _____
Pore Size: _____
Organisms or **Debris**

Salinity Adjustment? (Y) N
Test: Mussel Source: Brine 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.			

Test Performed: Mussel development **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART **Other:** _____
Alkalinity: 114 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 114 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 at receipt.
[2] mg/L as $CaCO_3$, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: _____

QC Check: BS 9/9/22
Final Review: BO 9/22/22

Total Ammonia Analysis
Marine

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: N/A
SW Blank: 0.0

Test Start Date: 8/24/22

Analyst: BS
Analysis Date: 9/19/22

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	8.2	10.0
Wyckoff Effluent	22-1105	22-1105	check in	4.1	5.0
Spike Check (10 mg/L NH_3)		NA	NA		
Sample Duplicate[a]	22-1105	NA	NA	3.8	4.6
Sample Duplicate + Spike[a]		NA	NA	13.2	16.1
Spike Check (10 mg/L NH_3)		NA	NA	8.1	9.9

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	10.0	10	NA	100
22-1105	5.0	4.6	16.1	10	8.3	111

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A2055	A2055	A2071

Comments:

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: AKS 9/9/22

Final Review: BO 9/22/22

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 8/23/2022
CarrierName: FedEx
AirbillNo:

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

No: 10-082322-113216-0650
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
082322		Ground Water/ D. Baca	Composite	CHRTOX(8 Weeks)	N (1)	SP-11	08/23/2022 10:11	Field Sample

Special Instructions: 2022 Week 35-Q3 Sample(3)

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	8-23-22 @11:56	EASD	8/24/22 1020	Good 2.3°C 22-1105

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:	11-9077-7653	**Test Type:**	Development-Survival	**Analyst:**			
Start Date:	24 Aug-22 17:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater		
Ending Date:	26 Aug-22 16:45	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable		
Test Length:	48h	**Taxon:**		**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	17-4831-2678	**Code:**	220824msdv	**Project:**	
Sample Date:	24 Aug-22	**Material:**	Copper chloride	**Source:**	Reference Toxicant
Receipt Date:	24 Aug-22	**CAS (PC):**		**Station:**	Copper Chloride
Sample Age:	17h	**Client:**	Internal		

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	✓	NOEL	LOEL	TOEL	PMSD	S
08-2749-7117	Combined Development Rat	Dunnett Multiple Comparison Test		5	10	7.071	16.5%	1
03-0796-5613	Development Rate	Steel Many-One Rank Sum Test		5	10	7.071	9.37%	1
16-2156-7634	Survival Rate	Dunnett Multiple Comparison Test		10	20	14.14	11.5%	1

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	✓	Level	µg/L	95% LCL	95% UCL	S
09-5721-9456	Combined Development Rat	Linear Interpolation (ICPIN)		EC25	7.09	5.9	9.37	1
				EC50	9.83	7.79	13.8	
18-8831-9322	Development Rate	Linear Interpolation (ICPIN)		EC25	7.56	6.47	10.2	1
				EC50	10.3	7.92	14.3	
07-4875-9471	Survival Rate	Linear Interpolation (ICPIN)		EC25	15.6	12.3	18.5	1
				EC50	23.5	19.2	26.6	

Test Acceptability

				TAC Limits			
Analysis ID	Endpoint	Attribute	Test Stat	Lower	Upper	Overlap	Decision
03-0796-5613	Development Rate	Control Resp	0.969	0.9	<<	Yes	Passes Criteria
18-8831-9322	Development Rate	Control Resp	0.969	0.9	<<	Yes	Passes Criteria
07-4875-9471	Survival Rate	Control Resp	0.895	0.5	<<	Yes	Passes Criteria
16-2156-7634	Survival Rate	Control Resp	0.895	0.5	<<	Yes	Passes Criteria
08-2749-7117	Combined Development Rat	PMSD	0.165	<<	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	0.868	0.781	0.954	0.783	0.937	0.031	0.070	8.03%	0.00%
2.5		5	0.844	0.769	0.920	0.772	0.915	0.027	0.061	7.21%	2.68%
5		5	0.817	0.745	0.888	0.762	0.894	0.026	0.058	7.04%	5.85%
10		5	0.420	0.159	0.681	0.212	0.720	0.094	0.210	50.04%	51.59%
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	0.969	0.950	0.989	0.950	0.983	0.007	0.016	1.62%	0.00%
2.5		5	0.971	0.953	0.988	0.949	0.986	0.006	0.014	1.46%	-0.18%
5		5	0.965	0.955	0.975	0.954	0.976	0.004	0.008	0.82%	0.44%
10		5	0.497	0.205	0.788	0.245	0.850	0.105	0.235	47.26%	48.74%
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	0.895	0.811	0.979	0.799	0.952	0.030	0.068	7.56%	0.00%
2.5		5	0.870	0.792	0.948	0.783	0.937	0.028	0.063	7.20%	2.84%
5		5	0.847	0.777	0.916	0.788	0.926	0.025	0.056	6.64%	5.44%
10		5	0.839	0.736	0.943	0.762	0.963	0.037	0.084	9.95%	6.26%
20		5	0.538	0.425	0.650	0.444	0.640	0.041	0.091	16.87%	39.95%
40		5	0.018	-0.028	0.064	0.000	0.085	0.017	0.037	207.56%	97.99%

Combined Development Rate Detail MD5: 868BE36FADC3D29F16373F2D867B7C3E

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	0.783	0.878	0.810	0.931	0.937
2.5		0.772	0.852	0.915	0.794	0.889
5		0.762	0.852	0.894	0.762	0.815
10		0.386	0.212	0.249	0.720	0.534
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: AA1654EA81BA29F6C1E13916525D109A

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	0.980	0.954	0.950	0.978	0.983
2.5		0.986	0.976	0.977	0.949	0.966
5		0.954	0.976	0.966	0.966	0.962
10		0.507	0.245	0.326	0.850	0.555
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: F1CEE03BC3EDFEC963FB751F0881A86A

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	0.799	0.921	0.852	0.952	0.952
2.5		0.783	0.873	0.937	0.836	0.921
5		0.799	0.873	0.926	0.788	0.847
10		0.762	0.862	0.762	0.847	0.963
20		0.492	0.481	0.444	0.630	0.640
40		0.085	0.000	0.000	0.000	0.005

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	148/189	166/189	153/189	176/189	177/189
2.5		146/189	161/189	173/189	150/189	168/189
5		144/189	161/189	169/189	144/189	154/189
10		73/189	40/189	47/189	136/189	101/189
20		0/189	0/189	0/189	0/189	0/189
40		0/189	0/189	0/189	0/189	0/189

Development Rate Binomials

Conc-µg/L	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	LC	148/151	166/174	153/161	176/180	177/180
2.5		146/148	161/165	173/177	150/158	168/174
5		144/151	161/165	169/175	144/149	154/160
10		73/144	40/163	47/144	136/160	101/182
20		0/93	0/91	0/84	0/119	0/121
40		0/16	0/1	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	151/189	174/189	161/189	180/189	180/189
2.5		148/189	165/189	177/189	158/189	174/189
5		151/189	165/189	175/189	149/189	160/189
10		144/189	163/189	144/189	160/189	182/189
20		93/189	91/189	84/189	119/189	121/189
40		16/189	0/189	0/189	0/189	1/189

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

Analysis ID:	08-2749-7117	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	868BE36FADC3D29F16373F2D867B7C3E	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	5	10	7.071	---	0.143	16.48%

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	0.437	2.23	0.19	CDF	0.5724	Non-Significant Effect
		5	8	0.887	2.23	0.19	CDF	0.3768	Non-Significant Effect
		10*	8	5.96	2.23	0.19	CDF	2.9E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.842638	0.280879	3	15.5	5.5E-05	Significant Effect
Error	0.290602	0.0181626	16			
Total	1.13324		19			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	5.65	11.3	0.1300	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.962	0.866	0.5765	Normal Distribution

Combined Development Rate Summary

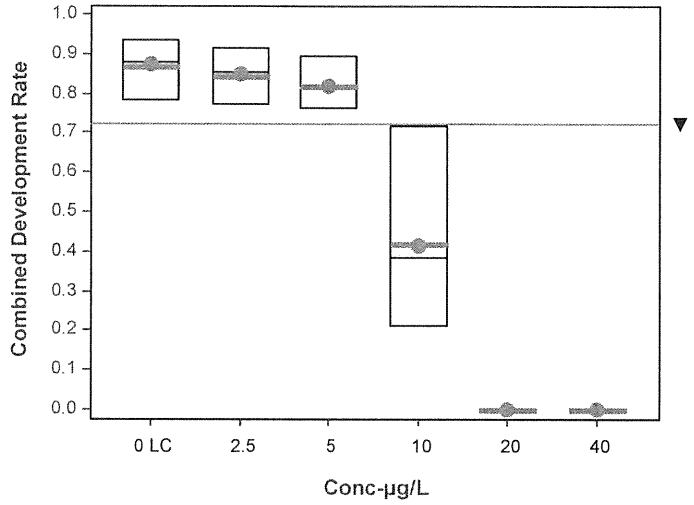
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	0.868	0.781	0.954	0.878	0.783	0.937	0.031	8.03%	0.00%
2.5		5	0.844	0.769	0.920	0.852	0.772	0.915	0.027	7.21%	2.68%
5		5	0.817	0.745	0.888	0.815	0.762	0.894	0.026	7.04%	5.85%
10		5	0.420	0.159	0.681	0.386	0.212	0.720	0.094	50.04%	51.59%
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%

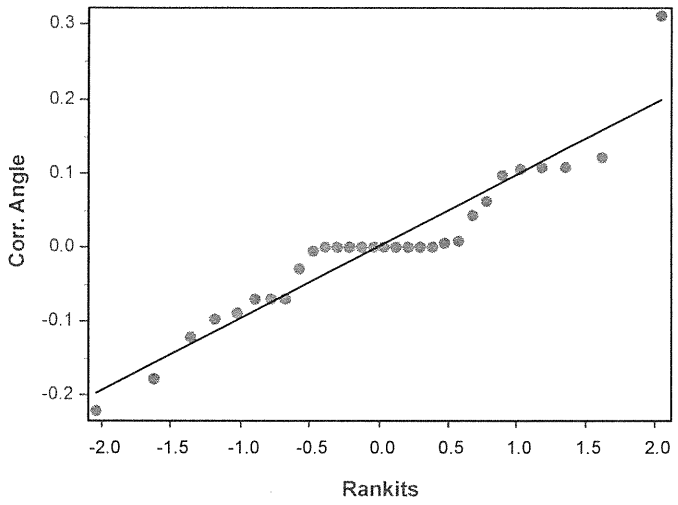
Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	1.210	1.080	1.340	1.210	1.090	1.320	0.047	8.67%	0.00%
2.5		5	1.170	1.060	1.280	1.180	1.070	1.280	0.038	7.29%	3.08%
5		5	1.130	1.040	1.230	1.130	1.060	1.240	0.034	6.78%	6.26%
10		5	0.701	0.427	0.974	0.671	0.478	1.010	0.099	31.43%	42.01%
20		5	0.036	0.036	0.036	0.036	0.036	0.036	0.000	0.00%	96.99%
40		5	0.036	0.036	0.036	0.036	0.036	0.036	0.000	0.00%	96.99%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	08-2749-7117	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	868BE36FADC3D29F16373F2D867B7C3E	**Editor ID:**	007-926-968-0

Graphics





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

Analysis ID:	03-0796-5613	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	AA1654EA81BA29F6C1E13916525D109A	**Editor ID:**	007-926-968-0

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	5	10	7.071	---	0.0908	9.37%

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	26	17	0	CDF	0.6242	Non-Significant Effect
		5	8	24	17	0	CDF	0.4372	Non-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	1.38966	0.463222	3	27.2	<1.0E-05	Significant Effect
Error	0.272972	0.0170607	16			
Total	1.66264		19			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	24.2	11.3	2.2E-05	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.775	0.866	0.0004	Non-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	0.969	0.950	0.989	0.978	0.950	0.983	0.007	1.62%	0.00%
2.5		5	0.971	0.953	0.988	0.976	0.949	0.986	0.006	1.46%	-0.18%
5		5	0.965	0.955	0.975	0.966	0.954	0.976	0.004	0.82%	0.44%
10		5	0.497	0.205	0.788	0.507	0.245	0.850	0.105	47.26%	48.74%
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%

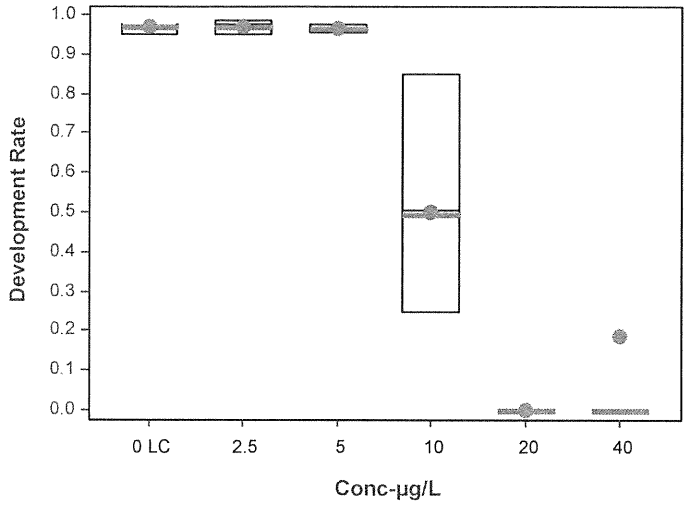
Angular (Corrected) Transformed Summary

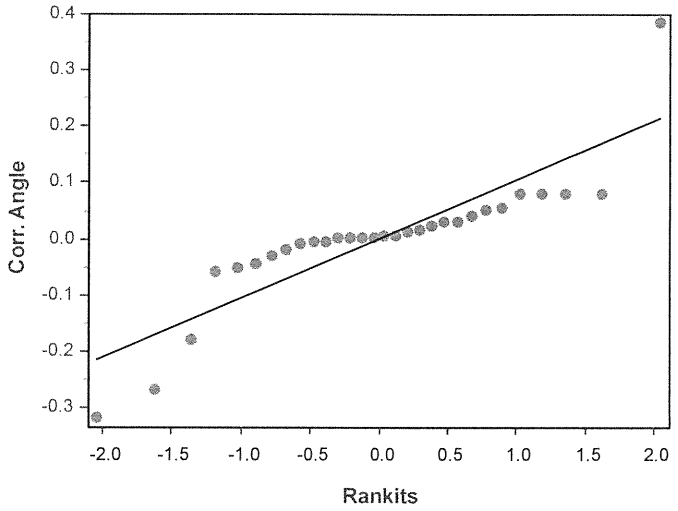
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	1.400	1.340	1.450	1.420	1.350	1.440	0.020	3.19%	0.00%
2.5		5	1.400	1.350	1.450	1.410	1.340	1.450	0.019	2.96%	-0.34%
5		5	1.380	1.360	1.410	1.380	1.350	1.410	0.010	1.58%	1.11%
10		5	0.786	0.472	1.100	0.792	0.518	1.170	0.113	32.18%	43.77%
20		5	0.050	0.045	0.055	0.052	0.046	0.055	0.002	8.25%	96.42%
40		5	0.444	0.223	0.665	0.524	0.125	0.524	0.080	40.12%	68.26%

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

Analysis ID:	03-0796-5613	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Nonparametric-Control vs Treatments	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	AA1654EA81BA29F6C1E13916525D109A	**Editor ID:**	007-926-968-0

Graphics





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

Analysis ID:	16-2156-7634	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	F1CEE03BC3EDFEC963FB751F0881A86A	**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	---	0.103	11.49%

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	0.668	2.36	0.156	CDF	0.5718	Non-Significant Effect
		5	8	1.21	2.36	0.156	CDF	0.3299	Non-Significant Effect
		10	8	1.24	2.36	0.156	CDF	0.3176	Non-Significant Effect
		20*	8	6.51	2.36	0.156	CDF	<1.0E-05	Significant Effect
		40*	8	17.5	2.36	0.156	CDF	<1.0E-05	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.02451	1.0049	5	92.1	<1.0E-05	Significant Effect
Error	0.261742	0.0109059	24			
Total	5.28625		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	1	15.1	0.9625	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.942	0.903	0.1000	Normal Distribution

Survival Rate Summary

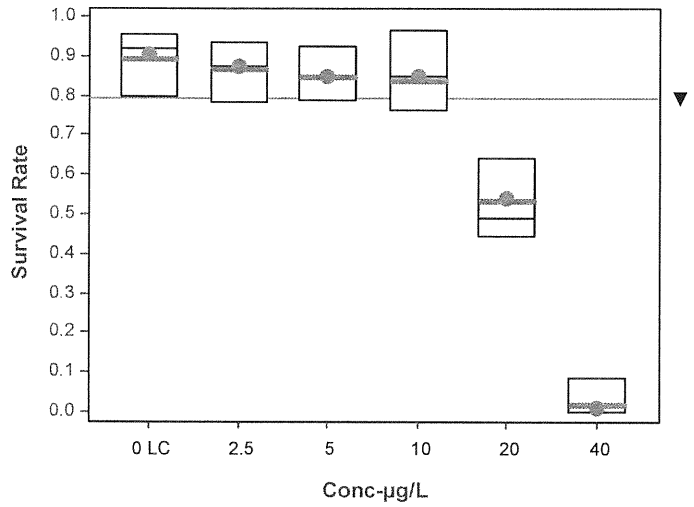
Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	0.895	0.811	0.979	0.921	0.799	0.952	0.030	7.56%	0.00%
2.5		5	0.870	0.792	0.948	0.873	0.783	0.937	0.028	7.20%	2.84%
5		5	0.847	0.777	0.916	0.847	0.788	0.926	0.025	6.64%	5.44%
10		5	0.839	0.736	0.943	0.847	0.762	0.963	0.037	9.95%	6.26%
20		5	0.538	0.425	0.650	0.492	0.444	0.640	0.041	16.87%	39.95%
40		5	0.018	0.000	0.064	0.000	0.000	0.085	0.017	207.56%	97.99%

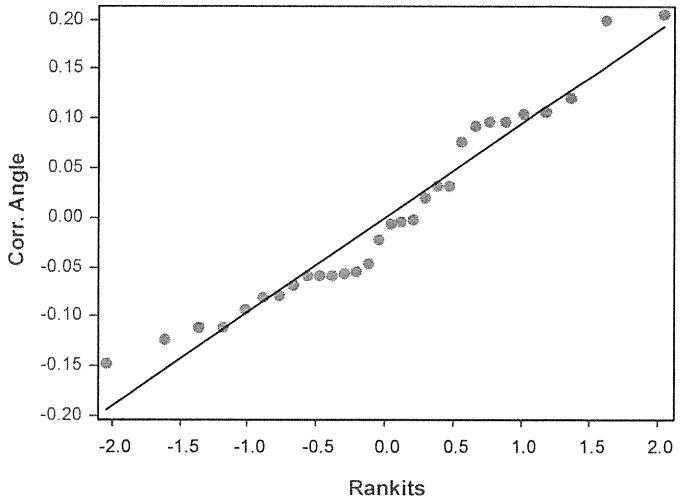
Angular (Corrected) Transformed Summary

Conc-µg/L	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	LC	5	1.250	1.120	1.390	1.290	1.110	1.350	0.049	8.72%	0.00%
2.5		5	1.210	1.090	1.330	1.210	1.090	1.320	0.042	7.77%	3.52%
5		5	1.170	1.070	1.280	1.170	1.090	1.300	0.037	7.00%	6.38%
10		5	1.170	1.010	1.330	1.170	1.060	1.380	0.058	11.05%	6.54%
20		5	0.824	0.710	0.937	0.777	0.730	0.928	0.041	11.13%	34.30%
40		5	0.095	-0.045	0.235	0.036	0.036	0.295	0.050	118.19%	92.39%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	16-2156-7634	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Parametric-Control vs Treatments	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	F1CEE03BC3EDFEC963FB751F0881A86A	**Editor ID:**	007-926-968-0

Graphics





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

Analysis ID:	09-5721-9456	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	868BE36FADC3D29F16373F2D867B7C3E	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1331939	1000	Yes	Two-Point Interpolation

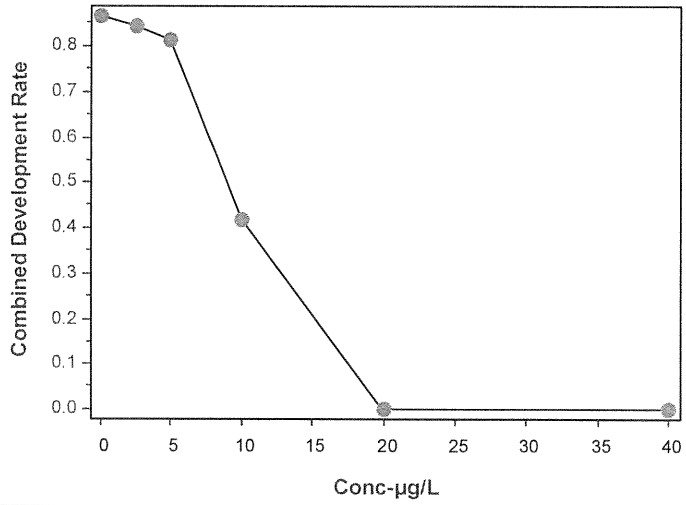
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	7.09	5.9	9.37
EC50	9.83	7.79	13.8

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	0.868	0.878	0.783	0.937	8.03%	0.00%	820/945	0.868	0.00%
2.5		5	0.844	0.852	0.772	0.915	7.21%	2.68%	798/945	0.844	2.76%
5		5	0.817	0.815	0.762	0.894	7.04%	5.85%	772/945	0.817	5.88%
10		5	0.420	0.386	0.212	0.720	50.04%	51.59%	397/945	0.420	51.61%
20		5	0.000	0.000	0.000	0.000	---	100.00%	0/945	0.000	100.00%
40		5	0.000	0.000	0.000	0.000	---	100.00%	0/945	0.000	100.00%

Graphics



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

Analysis ID:	18-8831-9322	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	AA1654EA81BA29F6C1E13916525D109A	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1086328	1000	Yes	Two-Point Interpolation

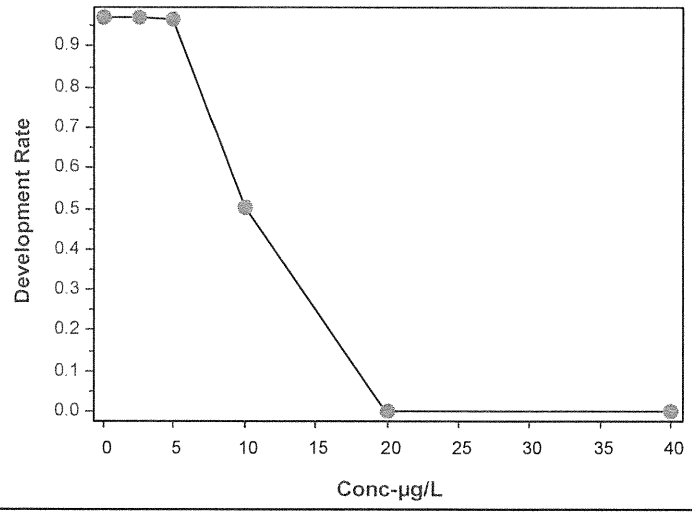
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	7.56	6.47	10.2
EC50	10.3	7.92	14.3

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	0.969	0.978	0.950	0.983	1.62%	0.00%	820/846	0.970	0.00%
2.5		5	0.971	0.976	0.949	0.986	1.46%	-0.18%	798/822	0.970	0.00%
5		5	0.965	0.966	0.954	0.976	0.82%	0.44%	772/800	0.965	0.52%
10		5	0.497	0.507	0.245	0.850	47.26%	48.74%	397/793	0.501	48.35%
20		5	0.000	0.000	0.000	0.000	---	100.00%	0/508	0.000	100.00%
40		5	0.000	0.000	0.000	0.000	---	100.00%	0/20	0.000	100.00%

Graphics



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

Analysis ID:	07-4875-9471	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv2.1.2
Analyzed:	15 Sep-22 14:36	**Analysis:**	Linear Interpolation (ICPIN)	**Status Level:**	1
Edit Date:	15 Sep-22 14:32	**MD5 Hash:**	F1CEE03BC3EDFEC963FB751F0881A86A	**Editor ID:**	007-926-968-0

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	476860	1000	Yes	Two-Point Interpolation

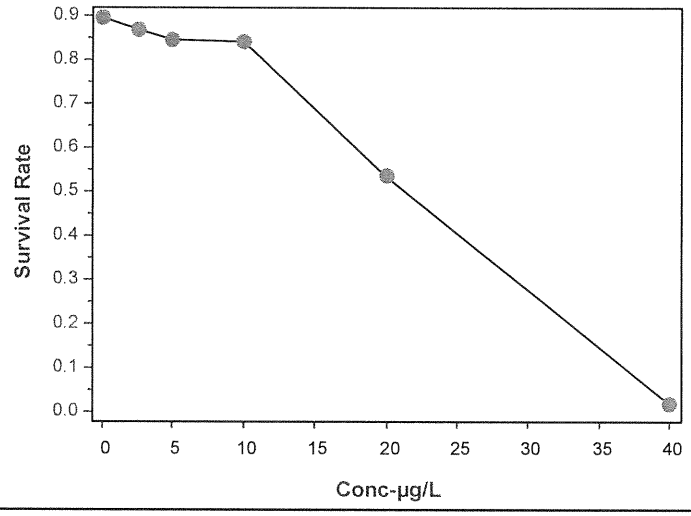
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	15.6	12.3	18.5
EC50	23.5	19.2	26.6

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	0.895	0.921	0.799	0.952	7.56%	0.00%	846/945	0.895	0.00%
2.5		5	0.870	0.873	0.783	0.937	7.20%	2.84%	822/945	0.870	2.79%
5		5	0.847	0.847	0.788	0.926	6.64%	5.44%	800/945	0.847	5.36%
10		5	0.839	0.847	0.762	0.963	9.95%	6.26%	793/945	0.839	6.26%
20		5	0.538	0.492	0.444	0.640	16.87%	39.95%	508/945	0.538	39.89%
40		5	0.018	0.000	0.000	0.085	207.56%	97.99%	17/945	0.018	97.99%

Graphics

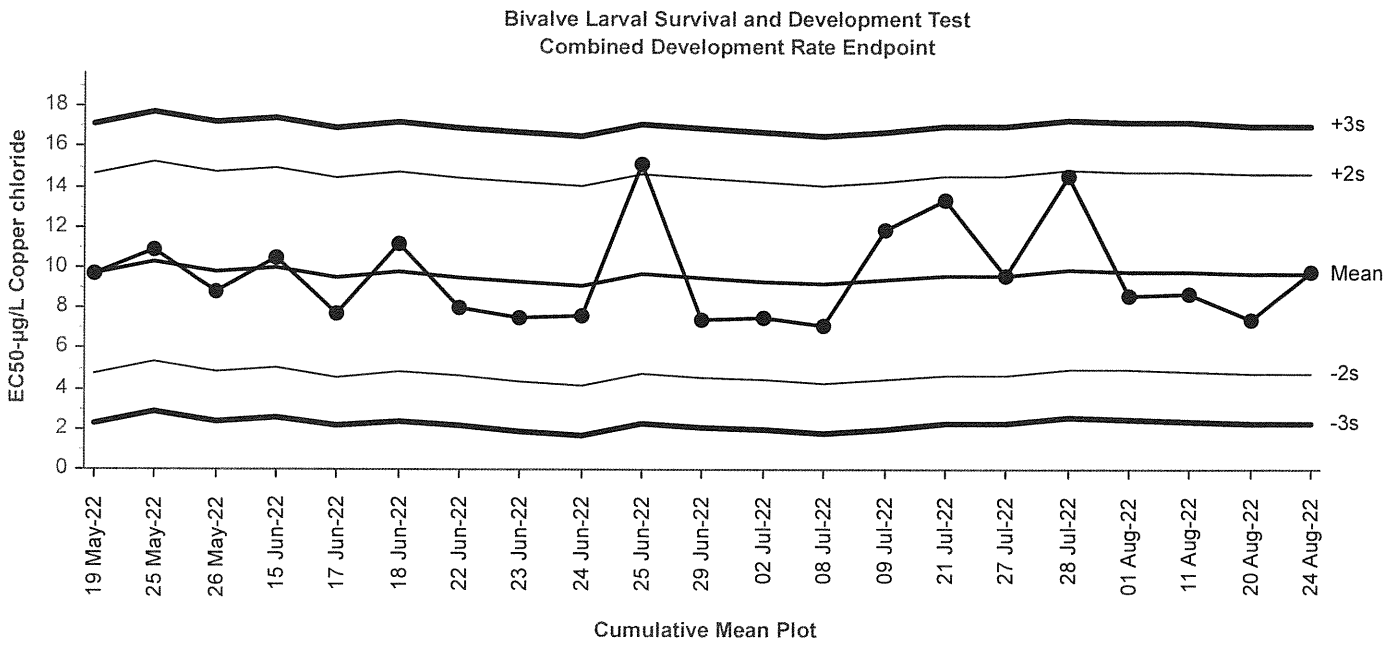


CETIS QC Plot

Report Date: 15 Sep-22 14:38 (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:	9.669	**Count:**	20	**-2s Warning Limit:**	4.74	**-3s Action Limit:**	2.27
Sigma:	2.465	**CV:**	25.50%	**+2s Warning Limit:**	14.6	**+3s Action Limit:**	17.1

Quality Control Data

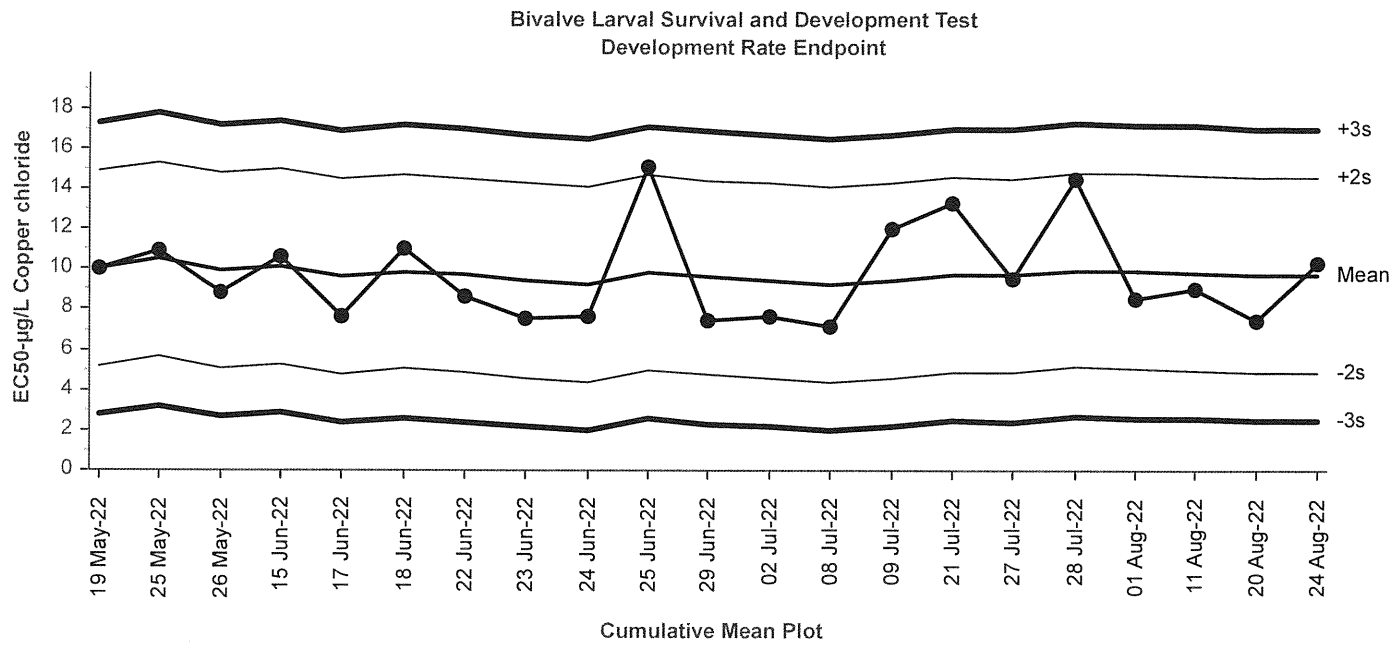
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	May	19	15:00	9.697	0.0284	0.01152			09-6277-1477	09-2283-7570
2			25	16:15	10.9	1.235	0.5012			08-1713-3780	00-8795-8727
3			26	16:10	8.857	-0.8122	-0.3295			06-7934-6987	05-3456-5364
4		Jun	15	16:05	10.51	0.839	0.3404			00-0973-0859	10-3057-0474
5			17	17:00	7.692	-1.977	-0.8019			11-9754-7815	20-7892-8869
6			18	13:45	11.14	1.475	0.5985			12-5222-9594	05-5453-0539
7			22	15:20	8.015	-1.654	-0.6709			13-5029-6239	16-6455-0791
8			23	16:30	7.551	-2.118	-0.859			13-4807-6858	00-5631-5384
9			24	17:35	7.661	-2.008	-0.8147			18-6610-9703	15-9489-9679
10			25	13:30	15.14	5.467	2.218	(+)		00-9942-7497	07-3022-3622
11			29	15:30	7.394	-2.275	-0.9227			10-7570-4279	01-8774-7760
12		Jul	2	15:50	7.549	-2.12	-0.8601			11-2833-5057	03-6227-8823
13			8	16:10	7.125	-2.544	-1.032			02-6577-2570	18-5175-4691
14			9	15:20	11.88	2.207	0.8954			00-5850-8514	13-7194-8786
15			21	15:20	13.35	3.678	1.492			19-9779-3969	07-3733-9935
16			27	17:10	9.634	-0.03496	-0.01418			10-2942-6187	02-0896-6713
17			28	16:10	14.54	4.873	1.977			12-6417-7061	20-3705-6551
18		Aug	1	14:15	8.601	-1.068	-0.4331			13-9611-9448	10-0347-5354
19			11	17:15	8.711	-0.9581	-0.3887			00-9864-7136	07-4996-9021
20			20	13:15	7.437	-2.232	-0.9054			17-3409-3229	13-5013-5598
21			24	17:00	9.827	0.1577	0.06396			00-6269-9880	09-5721-9456

CETIS QC Plot

Report Date: 15 Sep-22 14:38 (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:	9.739	**Count:**	20	**-2s Warning Limit:**	4.89	**-3s Action Limit:**	2.46
Sigma:	2.426	**CV:**	24.90%	**+2s Warning Limit:**	14.6	**+3s Action Limit:**	17

Quality Control Data

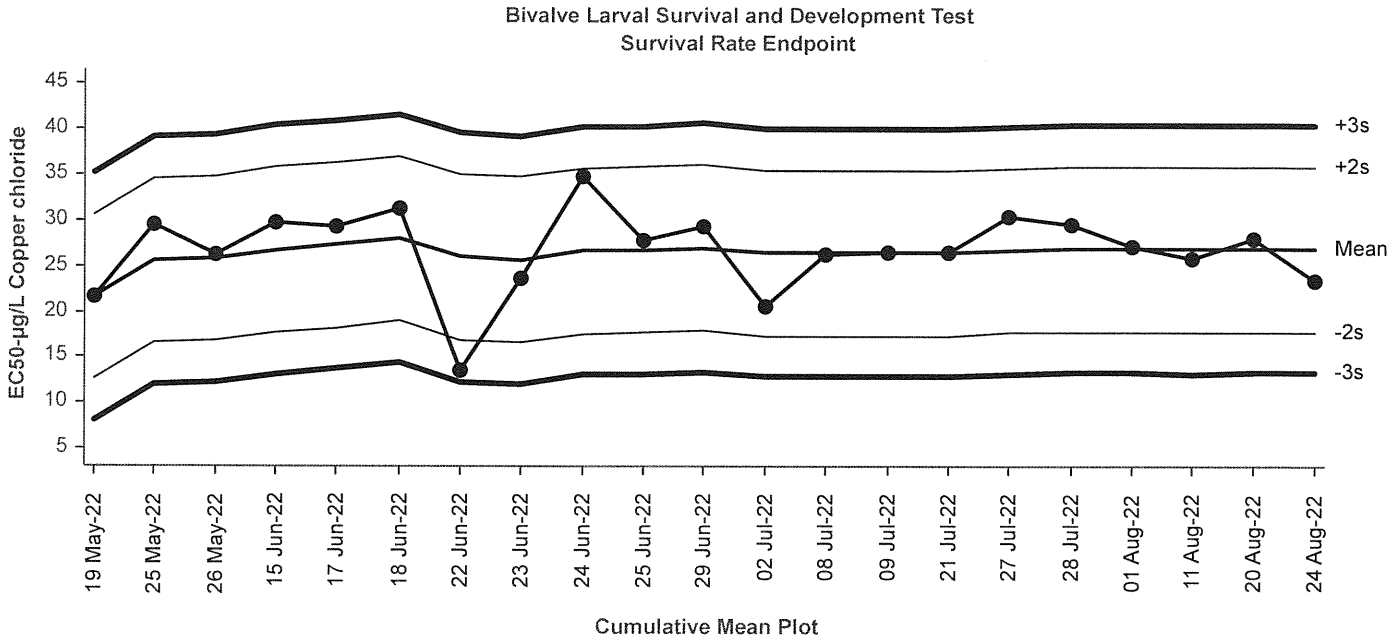
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	May	19	15:00	10.03	0.2864	0.118			09-6277-1477	17-0036-0143
2			25	16:15	10.96	1.22	0.5028			08-1713-3780	16-8618-6676
3			26	16:10	8.888	-0.8505	-0.3506			06-7934-6987	21-4293-9672
4		Jun	15	16:05	10.66	0.9165	0.3778			00-0973-0859	04-9614-9810
5			17	17:00	7.682	-2.057	-0.8477			11-9754-7815	18-3364-3843
6			18	13:45	11.03	1.293	0.533			12-5222-9594	18-0234-7285
7			22	15:20	8.609	-1.13	-0.4659			13-5029-6239	07-9450-2650
8			23	16:30	7.567	-2.172	-0.8954			13-4807-6858	11-5051-8358
9			24	17:35	7.649	-2.09	-0.8613			18-6610-9703	09-0986-8018
10			25	13:30	15.07	5.332	2.198	(+)		00-9942-7497	00-5372-4029
11			29	15:30	7.456	-2.283	-0.9412			10-7570-4279	02-5324-4545
12		Jul	2	15:50	7.62	-2.119	-0.8735			11-2833-5057	19-6651-0291
13			8	16:10	7.156	-2.583	-1.065			02-6577-2570	18-8979-5628
14			9	15:20	12.06	2.318	0.9553			00-5850-8514	02-4671-5320
15			21	15:20	13.28	3.536	1.458			19-9779-3969	12-5751-9718
16			27	17:10	9.524	-0.2148	-0.08855			10-2942-6187	20-1467-5696
17			28	16:10	14.48	4.741	1.954			12-6417-7061	16-6165-4739
18		Aug	1	14:15	8.58	-1.159	-0.4778			13-9611-9448	13-0914-2180
19			11	17:15	9.087	-0.6516	-0.2686			00-9864-7136	10-5564-7447
20			20	13:15	7.411	-2.328	-0.9598			17-3409-3229	01-3382-1740
21			24	17:00	10.31	0.5729	0.2361			00-6269-9880	18-8831-9322

CETIS QC Plot

Report Date: 15 Sep-22 14:38 (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:	26.87	**Count:**	20	**-2s Warning Limit:**	17.8	**-3s Action Limit:**	13.3
Sigma:	4.528	**CV:**	16.90%	**+2s Warning Limit:**	35.9	**+3s Action Limit:**	40.5

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2022	May	19	15:00	21.66	-5.213	-1.151			09-6277-1477	18-6597-6314
2			25	16:15	29.51	2.64	0.5831			08-1713-3780	03-6065-1842
3			26	16:10	26.24	-0.6256	-0.1382			06-7934-6987	18-6979-1322
4		Jun	15	16:05	29.69	2.824	0.6237			00-0973-0859	11-2376-5459
5			17	17:00	29.39	2.523	0.5572			11-9754-7815	05-1683-8844
6			18	13:45	31.37	4.499	0.9936			12-5222-9594	03-1487-9330
7			22	15:20	13.51	-13.36	-2.95	(-)		13-5029-6239	15-3025-9504
8			23	16:30	23.57	-3.295	-0.7277			13-4807-6858	20-7545-6113
9			24	17:35	34.8	7.933	1.752			18-6610-9703	10-3591-3624
10			25	13:30	27.71	0.8414	0.1858			00-9942-7497	14-2833-3108
11			29	15:30	29.2	2.326	0.5136			10-7570-4279	12-8684-5948
12		Jul	2	15:50	20.58	-6.291	-1.389			11-2833-5057	04-8946-4726
13			8	16:10	26.3	-0.5684	-0.1255			02-6577-2570	10-6839-1725
14			9	15:20	26.41	-0.4638	-0.1024			00-5850-8514	00-6028-0150
15			21	15:20	26.53	-0.3422	-0.07558			19-9779-3969	06-3587-0808
16			27	17:10	30.34	3.471	0.7666			10-2942-6187	08-5991-2538
17			28	16:10	29.61	2.738	0.6048			12-6417-7061	16-9619-1003
18		Aug	1	14:15	27.1	0.2312	0.05105			13-9611-9448	19-4797-3651
19			11	17:15	25.91	-0.9609	-0.2122			00-9864-7136	03-2596-2467
20			20	13:15	28.03	1.16	0.2561			17-3409-3229	00-2953-9784
21			24	17:00	23.46	-3.408	-0.7526			00-6269-9880	07-4875-9471

CETIS Test Data Worksheet

Report Date: 20 Aug-22 12:57 (p 1 of 1)
Test Code: 00-6269-9880/220824msdv

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

Start Date: 24 Aug-22
End Date: 26 Aug-22
Sample Date: 24 Aug-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 220824msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			10 (A) 0	0	TT 9/1/22
			2			175	169	
			3			16	0	
			4			~~163~~ (A) 182	~~40~~ (A) 101	
			5			163	40	
			6			144	47	
			7			180	177	
			8			~~12~~ (A) 0	0	
			9			1	0	
			10			177	173	
			11			148	146	
			12			91	0	
			13			84	0	
			14			165	161	
			15			180	176	
			16			174	166	
			17			158	150	
			18			151	144	
			19			144	73	
			20			160	154	
			21			160	136	
			22			121	0	
			23			119	0	
			24			149	144	
			25			174	168	
			26			151	148	
			27			161	153	
			28			0	0	
			29			93	0	
			30			165	161	

(A) Q18 TT 9/1/22

CETIS Test Data Worksheet

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

Start Date: 24 Aug-22
End Date: 26 Aug-22
Sample Date: 24 Aug-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 220824msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	26					
0	LC	2	16					
0	LC	3	27			155	148	RT ~~9/2/22~~ 9/6/22
0	LC	4	15					Q13 RT 9/6/22
0	LC	5	7					
2.5		1	11					
2.5		2	30					
2.5		3	10			161	156	
2.5		4	17					
2.5		5	25					
5		1	18					
5		2	14					
5		3	2			169	165	
5		4	24					
5		5	20					
10		1	19					
10		2	5					
10		3	6			135	120	137 / 133 YS 9/9/22
10		4	21					
10		5	4					
20		1	29					
20		2	12					
20		3	13			84	0	
20		4	23					
20		5	22					
40		1	3					
40		2	1					
40		3	28			0	0	
40		4	8					
40		5	9					

QC: RT

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 220824msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 8/24/22 1700

End Date/Time: 8/26/22 1645

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	31.8	31.8	31.8	15.2	14.9	14.9	8.6	8.5	8.6	7.95	7.99	8.00
2.5	32.1	32.2	32.2	15.0	14.6	14.7	8.4	8.4	8.6	7.97	8.00	8.00
5	32.1	32.2	32.2	15.0	14.6	14.7	8.3	8.4	8.6	7.98	8.00	8.00
10	32.2	32.2	32.2	15.1	14.7	14.8	8.2	8.4	8.6	7.98	8.01	8.00
20	32.1	32.1	32.1	15.1	14.6	14.8	8.2	8.4	8.6	7.99	8.02	8.00
40	32.1	32.1	32.1	15.1	14.6	14.8	8.2	8.4	8.6	8.00	8.02	8.00

Technician Initials:		0	24	48
	WQ Readings:	RT	KD	GM
	Dilutions made by:	RT		

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	2.0
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,000

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: ACS 9/14/22

Final Review: BO 9/22/22

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Entrena / Cu12
Test No.: 220824msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: 4A ~~ST~~ M-REP
Date Received: 6/7/22 QA TM 6/24/22
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 8/24/22 1700
End Date/Time: 8/26/22 1645
Technician Initials: RT

Spawn Information

First Gamete Release Time: 1225

Sex	Number Spawning
Male	1
Female	2

Gamete Selection

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

Egg Fertilization Time: ~~1305~~ 1525
QA TM 8/24/22

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	80
Female 2	99
Female 3	

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted:		
	6	6
	6	7
	11	1
	6	5
	7	15

Mean: 7

Mean 7 X 50 = 350 embryos/ml

Initial Density: 350 = 1.17 (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	201	201	100	100
TØ B	~~148~~ 177	~~148~~ 177	100	
TØ C	178	178	100	
TØ D	185	185	100	
TØ E	179	179	100	
TØ F	213	213	100	
$\bar{X}$ =	189			

48-h QC: 169/173 97.7%

Comments: ⑥ QA TS 8/26/22

QC Check: ARS 9/14/22

Final Review: BO 9/22/22