ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: May 2021

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: **June 11, 2021**

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 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	**051121**
Enthalpy Log-in Number	21-0530
Collection Date; Time	5/11/2021; 0943h
Receipt Date; Time	5/12/2021; 1000h
Receipt Temperature (°C)	3.7
Dissolved Oxygen (mg/L)	9.4
pH	7.78
Conductivity (µS/cm)	11,600
Salinity (ppt)	7.2
Alkalinity (mg/L $CaCO_3$)	421
Total Chlorine (mg/L)	0.04
Total Ammonia (mg/L as N)	1.1

NM = not measured

Test Methods

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

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

Test Period	5/12/2021, 1500h to 5/14/2021, 1340h
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 94.9 ppt
Test Concentrations (% sample)	72.4[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™ 1.8.7.20

[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.

Results

There were no statistically significant effects detected in any effluent concentration tested for the survival or development endpoint of the bivalve test. This results in a no observed effect concentration (NOEC) of 72.4 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.38 for both endpoints.

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_{25} (% effluent)
Bivalve	Normal Development	72.4	> 72.4	< 1.38	> 72.4
	Survival	72.4	> 72.4	< 1.38	> 72.4

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

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

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	98.2	99.4
0 (Lab Control)	96.1	99.6
2	99.0	99.6
4	96.5	99.6
9	97.2	99.8
18	97.1	99.6
35	95.0	99.6
72.4[a]	100	99.9

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

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. The EC_{50} for survival was over three standard deviations above the historical mean; indicating organisms may have been less sensitive than typical for the survival endpoint. 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	20	39.2	29.5 ± 4.47	7.57
Bivalve Normal Development	5	14.3	9.18 ± 5.21	28.4

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.

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. 2000-2013. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20.

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

CETIS Summary Report

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

Batch ID:	11-3036-1120	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 May-21 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	14 May-21 13:40	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	19-0164-1007	**Code:**	21-0530	**Client:**	Jacobs
Sample Date:	11 May-21 09:43	**Material:**	Effluent Sample	**Project:**	
Receive Date:	12 May-21 10:00	**Source:**	Jacobs		
Sample Age:	29h (3.7 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-0254-6995	Combined Development Ra	72.4	>72.4	NA	7.24%	≤1.381	Steel Many-One Rank Sum Test
06-2787-9475	Development Rate	72.4	>72.4	NA	0.83%	≤1.381	Steel Many-One Rank Sum Test
12-7314-4397	Survival Rate	72.4	>72.4	NA	6.44%	≤1.381	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
13-8722-7064	Combined Development Ra	EC25	>72.4	N/A	N/A	<1.381	Linear Interpolation (ICPIN)
		EC50	>72.4	N/A	N/A	<1.381	
18-7960-6538	Development Rate	EC25	>72.4	N/A	N/A	<1.381	Linear Interpolation (ICPIN)
		EC50	>72.4	N/A	N/A	<1.381	
17-3343-9816	Survival Rate	EC25	>72.4	N/A	N/A	<1.381	Linear Interpolation (ICPIN)
		EC50	>72.4	N/A	N/A	<1.381	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-2787-9475	Development Rate	Control Resp	0.9942	0.9 - NL	Yes	Passes Acceptability Criteria
18-7960-6538	Development Rate	Control Resp	0.9942	0.9 - NL	Yes	Passes Acceptability Criteria
12-7314-4397	Survival Rate	Control Resp	0.9824	0.5 - NL	Yes	Passes Acceptability Criteria
17-3343-9816	Survival Rate	Control Resp	0.9824	0.5 - NL	Yes	Passes Acceptability Criteria
16-0254-6995	Combined Development Ra	PMSD	0.07241	NL - 0.25	No	Passes Acceptability Criteria

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

Combined Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9766	0.9311	1	0.9119	1	0.01639	0.03665	3.75%	0.0%
0	Lab Control	5	0.9575	0.8472	1	0.7987	1	0.0397	0.08877	9.27%	1.96%
2		5	0.9862	0.952	1	0.9371	1	0.01233	0.02758	2.8%	-0.99%
4		5	0.9603	0.8655	1	0.8239	1	0.03415	0.07636	7.95%	1.67%
9		5	0.97	0.9143	1	0.8931	1	0.02007	0.04488	4.63%	0.67%
18		5	0.9674	0.922	1	0.9245	1	0.01636	0.03658	3.78%	0.94%
35		5	0.946	0.8547	1	0.8428	1	0.03288	0.07351	7.77%	3.13%
72.4		5	0.9988	0.9955	1	0.9941	1	0.001183	0.002646	0.26%	-2.28%

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9942	0.9855	1	0.9829	1	0.003131	0.007001	0.7%	0.0%
0	Lab Control	5	0.9961	0.9916	1	0.9922	1	0.001619	0.00362	0.36%	-0.2%
2		5	0.9961	0.9889	1	0.9868	1	0.002622	0.005862	0.59%	-0.2%
4		5	0.9955	0.9896	1	0.9886	1	0.002134	0.004773	0.48%	-0.13%
9		5	0.9977	0.9912	1	0.9884	1	0.002326	0.0052	0.52%	-0.35%
18		5	0.9963	0.9894	1	0.9875	1	0.002487	0.005562	0.56%	-0.21%
35		5	0.996	0.9913	1	0.9926	1	0.001668	0.003729	0.37%	-0.18%
72.4		5	0.9988	0.9955	1	0.9941	1	0.001183	0.002646	0.26%	-0.47%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9824	0.9335	1	0.9119	1	0.01761	0.03938	4.01%	0.0%
0	Lab Control	5	0.961	0.8527	1	0.805	1	0.03899	0.08719	9.07%	2.18%
2		5	0.9899	0.962	1	0.9497	1	0.01006	0.0225	2.27%	-0.77%
4		5	0.9648	0.867	1	0.8239	1	0.03522	0.07875	8.16%	1.79%
9		5	0.9723	0.9148	1	0.8931	1	0.02073	0.04634	4.77%	1.02%
18		5	0.9711	0.9218	1	0.9245	1	0.01774	0.03968	4.09%	1.15%
35		5	0.9497	0.8613	1	0.8491	1	0.03182	0.07116	7.49%	3.33%
72.4		5	1	1	1	1	1	0	0	0.0%	-1.79%

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

Combined Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9939	0.9829	0.9941	1	0.9119
0	Lab Control	0.9943	1	1	0.9942	0.7987
2		1	1	0.9371	0.994	1
4		0.9886	0.8239	0.9951	0.9939	1
9		0.9884	0.9686	0.8931	1	1
18		0.994	1	0.9245	0.9875	0.9308
35		0.8428	0.9943	0.8931	1	1
72.4		0.9941	1	1	1	1

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9939	0.9829	0.9941	1	1
0	Lab Control	0.9943	1	1	0.9942	0.9922
2		1	1	0.9868	0.994	1
4		0.9886	1	0.9951	0.9939	1
9		0.9884	1	1	1	1
18		0.994	1	1	0.9875	1
35		0.9926	0.9943	0.993	1	1
72.4		0.9941	1	1	1	1

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	1	1	1	0.9119
0	Lab Control	1	1	1	1	0.805
2		1	1	0.9497	1	1
4		1	0.8239	1	1	1
9		1	0.9686	0.8931	1	1
18		1	1	0.9245	1	0.9308
35		0.8491	1	0.8994	1	1
72.4		1	1	1	1	1

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

Combined Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	163/164	172/175	169/170	172/172	145/159
0	Lab Control	175/176	177/177	179/179	172/173	127/159
2		163/163	162/162	149/159	165/166	177/177
4		173/175	131/159	202/203	164/165	188/188
9		170/172	154/159	142/159	177/177	173/173
18		166/167	159/159	147/159	158/160	148/159
35		134/159	173/174	142/159	175/175	168/168
72.4		168/169	175/175	163/163	175/175	171/171

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	163/164	172/175	169/170	172/172	145/145
0	Lab Control	175/176	177/177	179/179	172/173	127/128
2		163/163	162/162	149/151	165/166	177/177
4		173/175	131/131	202/203	164/165	188/188
9		170/172	154/154	142/142	177/177	173/173
18		166/167	159/159	147/147	158/160	148/148
35		134/135	173/174	142/143	175/175	168/168
72.4		168/169	175/175	163/163	175/175	171/171

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	159/159	159/159	159/159	159/159	145/159
0	Lab Control	159/159	159/159	159/159	159/159	128/159
2		159/159	159/159	151/159	159/159	159/159
4		159/159	131/159	159/159	159/159	159/159
9		159/159	154/159	142/159	159/159	159/159
18		159/159	159/159	147/159	159/159	148/159
35		135/159	159/159	143/159	159/159	159/159
72.4		159/159	159/159	159/159	159/159	159/159

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

Analysis ID:	16-0254-6995	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.24%	72.4	>72.4	NA	1.381

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	32.5	16	1	8	0.9904	Asymp	Non-Significant Effect
		4	28.5	16	1	8	0.9067	Asymp	Non-Significant Effect
		9	27	16	1	8	0.8267	Asymp	Non-Significant Effect
		18	26.5	16	1	8	0.7925	Asymp	Non-Significant Effect
		35	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		72.4	36	16	1	8	0.9994	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.05928196	0.009880327	6	0.6569	0.6845	Non-Significant Effect
Error	0.4211631	0.01504154	28			
Total	0.480445		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	13.46	16.81	0.0363	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8854	0.9146	0.0016	Non-normal Distribution

Combined Development Rate Summary

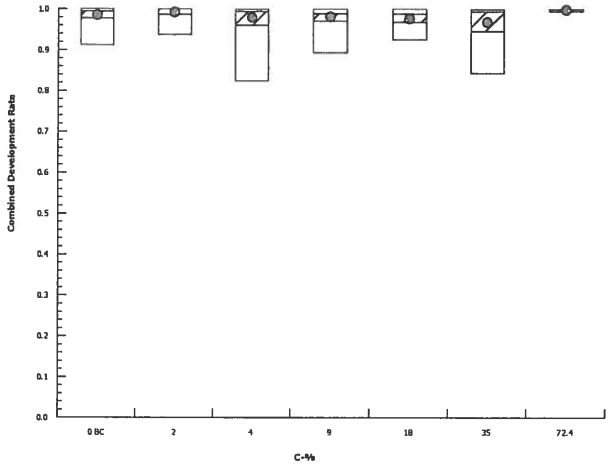
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9766	0.9311	1	0.9939	0.9119	1	0.01639	3.75%	0.0%
2		5	0.9862	0.952	1	1	0.9371	1	0.01233	2.8%	-0.99%
4		5	0.9603	0.8655	1	0.9939	0.8239	1	0.03415	7.95%	1.67%
9		5	0.97	0.9143	1	0.9884	0.8931	1	0.02007	4.63%	0.67%
18		5	0.9674	0.922	1	0.9875	0.9245	1	0.01636	3.78%	0.94%
35		5	0.946	0.8547	1	0.9943	0.8428	1	0.03288	7.77%	3.13%
72.4		5	0.9988	0.9955	1	1	0.9941	1	0.001183	0.26%	-2.28%

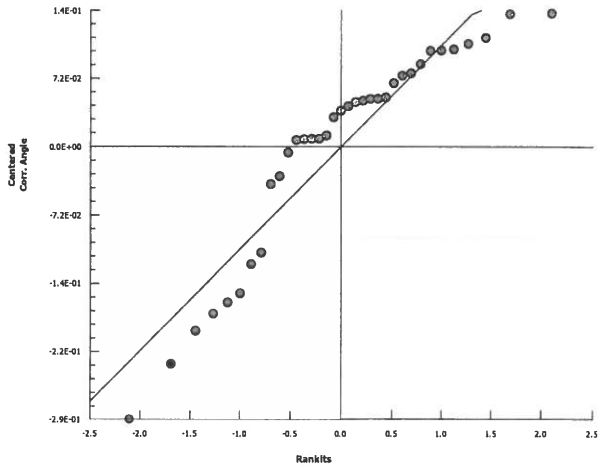
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.446	1.317	1.575	1.493	1.27	1.533	0.04646	7.19%	0.0%
2		5	1.481	1.366	1.597	1.532	1.317	1.533	0.0417	6.3%	-2.47%
4		5	1.426	1.223	1.628	1.493	1.138	1.534	0.0729	11.43%	1.37%
9		5	1.432	1.279	1.585	1.463	1.238	1.533	0.05507	8.6%	0.96%
18		5	1.416	1.279	1.553	1.459	1.292	1.531	0.04936	7.79%	2.05%
35		5	1.392	1.171	1.613	1.495	1.163	1.533	0.07948	12.77%	3.7%
72.4		5	1.525	1.503	1.546	1.533	1.494	1.533	0.007752	1.14%	-5.47%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	16-0254-6995	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	06-2787-9475	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	0.83%	72.4	>72.4	NA	1.381

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	30	16	2	8	0.9557	Asymp	Non-Significant Effect
		4	29	16	2	8	0.9262	Asymp	Non-Significant Effect
		9	32	16	2	8	0.9866	Asymp	Non-Significant Effect
		18	30	16	2	8	0.9557	Asymp	Non-Significant Effect
		35	28	16	2	8	0.8838	Asymp	Non-Significant Effect
		72.4	33	16	2	8	0.9932	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.002427117	0.0004045195	6	0.4527	0.8369	Non-Significant Effect
Error	0.02502079	0.0008935998	28			
Total	0.02744791		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.501	16.81	0.8683	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9002	0.9146	0.0040	Non-normal Distribution

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9942	0.9855	1	0.9941	0.9829	1	0.003131	0.7%	0.0%
2		5	0.9961	0.9889	1	1	0.9868	1	0.002622	0.59%	-0.2%
4		5	0.9955	0.9896	1	0.9951	0.9886	1	0.002134	0.48%	-0.13%
9		5	0.9977	0.9912	1	1	0.9884	1	0.002325	0.52%	-0.35%
18		5	0.9963	0.9894	1	1	0.9875	1	0.002487	0.56%	-0.21%
35		5	0.996	0.9913	1	0.9943	0.9926	1	0.001667	0.37%	-0.18%
72.4		5	0.9988	0.9955	1	1	0.9941	1	0.001183	0.26%	-0.47%

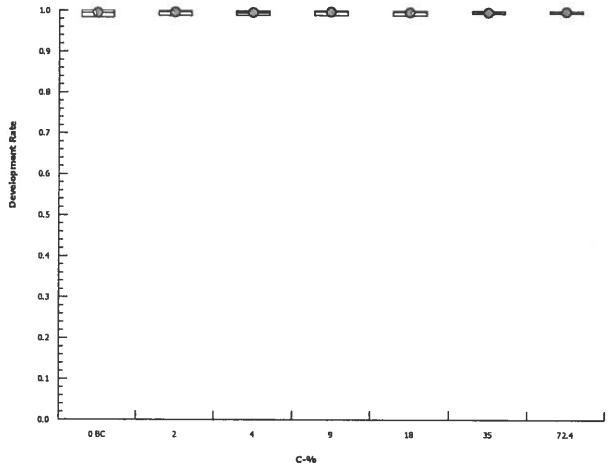
Angular (Corrected) Transformed Summary

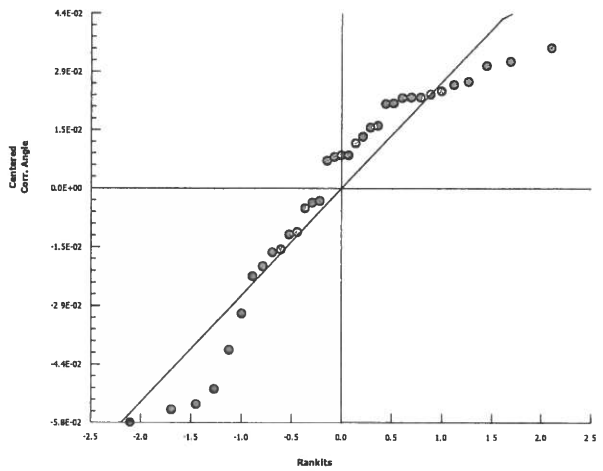
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.498	1.451	1.544	1.494	1.439	1.533	0.0168	2.51%	0.0%
2		5	1.509	1.466	1.552	1.532	1.455	1.533	0.01537	2.28%	-0.76%
4		5	1.504	1.469	1.539	1.501	1.464	1.534	0.01268	1.89%	-0.41%
9		5	1.518	1.479	1.556	1.53	1.463	1.533	0.01374	2.02%	-1.34%
18		5	1.508	1.469	1.548	1.53	1.459	1.531	0.01433	2.12%	-0.73%
35		5	1.506	1.476	1.536	1.495	1.485	1.533	0.01085	1.61%	-0.58%
72.4		5	1.525	1.503	1.546	1.533	1.494	1.533	0.007752	1.14%	-1.82%

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

Analysis ID:	06-2787-9475	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 12-7314-4397 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 11 Jun-21 10:53 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	6.44%	72.4	>72.4	NA	1.381

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		4	27	16	1	8	0.8267	Asymp	Non-Significant Effect
		9	25	16	1	8	0.6693	Asymp	Non-Significant Effect
		18	26	16	1	8	0.7547	Asymp	Non-Significant Effect
		35	24	16	1	8	0.5746	Asymp	Non-Significant Effect
		72.4	30	16	1	8	0.9557	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.05275378	0.008792298	6	0.5282	0.7821	Non-Significant Effect
Error	0.4661114	0.01664684	28			
Total	0.5188652		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	112.8	16.81	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.841	0.9146	0.0001	Non-normal Distribution

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9824	0.9335	1	1	0.9119	1	0.01761	4.01%	0.0%
2		5	0.9899	0.962	1	1	0.9497	1	0.01006	2.27%	-0.77%
4		5	0.9648	0.867	1	1	0.8239	1	0.03522	8.16%	1.79%
9		5	0.9723	0.9148	1	1	0.8931	1	0.02073	4.77%	1.02%
18		5	0.9711	0.9218	1	1	0.9245	1	0.01774	4.09%	1.15%
35		5	0.9497	0.8613	1	1	0.8491	1	0.03182	7.49%	3.33%
72.4		5	1	1	1	1	1	1	0	0.0%	-1.79%

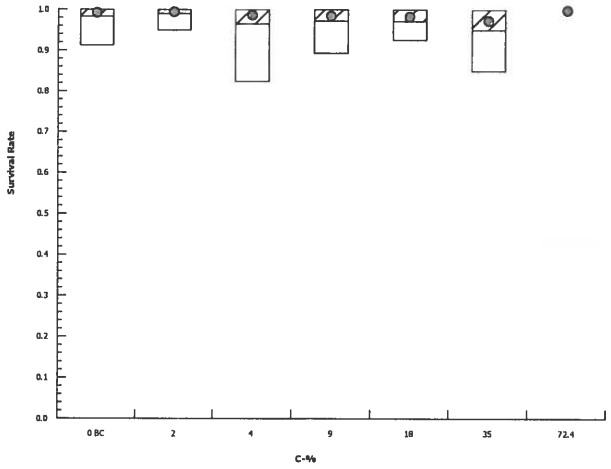
Angular (Corrected) Transformed Summary

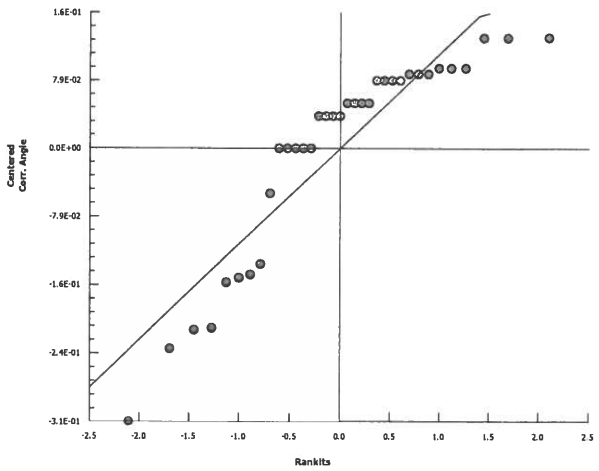
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.479	1.334	1.624	1.531	1.27	1.531	0.05232	7.91%	0.0%
2		5	1.494	1.39	1.597	1.531	1.345	1.531	0.03731	5.59%	-1.02%
4		5	1.452	1.234	1.671	1.531	1.138	1.531	0.07868	12.11%	1.78%
9		5	1.445	1.283	1.607	1.531	1.238	1.531	0.0583	9.02%	2.31%
18		5	1.438	1.28	1.596	1.531	1.292	1.531	0.057	8.86%	2.75%
35		5	1.403	1.182	1.624	1.531	1.172	1.531	0.07961	12.69%	5.15%
72.4		5	1.531	1.531	1.531	1.531	1.531	1.531	0	0.0%	-3.54%

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

Analysis ID:	12-7314-4397	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 2105-S081 | 07-3309-7869

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

Analysis ID:	13-8722-7064	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1415993	1000	Yes	Two-Point Interpolation

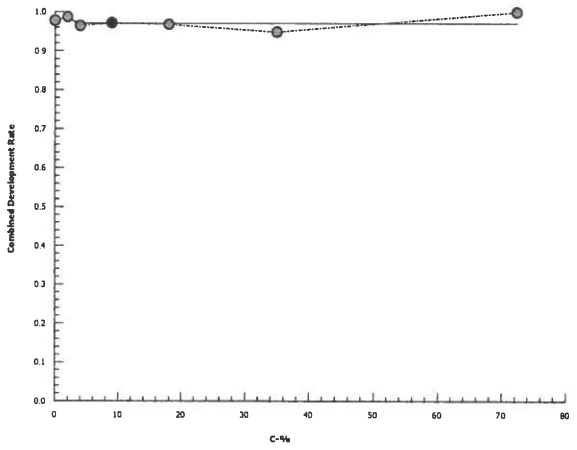
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>72.4	N/A	N/A	<1.381	NA	NA
EC50	>72.4	N/A	N/A	<1.381	NA	NA

Combined Development Rate Summary — Calculated Variate(A/B)

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.9766	0.9119	1	0.01639	0.03665	3.75%	0.0%	821	840
2		5	0.9862	0.9371	1	0.01233	0.02758	2.8%	-0.99%	816	827
4		5	0.9603	0.8239	1	0.03415	0.07636	7.95%	1.67%	858	890
9		5	0.97	0.8931	1	0.02007	0.04488	4.63%	0.67%	816	840
18		5	0.9674	0.9245	1	0.01636	0.03658	3.78%	0.94%	778	804
35		5	0.946	0.8428	1	0.03288	0.07351	7.77%	3.13%	792	835
72.4		5	0.9988	0.9941	1	0.001183	0.002645	0.26%	-2.28%	852	853

Graphics



 Analyst: JL QA: BOG/11/21

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

Analysis ID:	18-7960-6538	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	642997	1000	Yes	Two-Point Interpolation

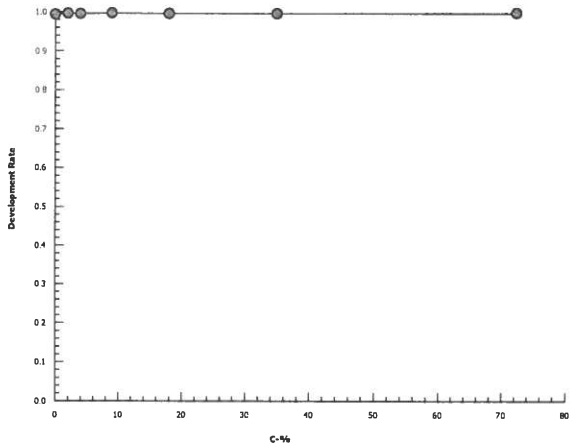
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>72.4	N/A	N/A	<1.381	NA	NA
EC50	>72.4	N/A	N/A	<1.381	NA	NA

Development Rate Summary

			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.9942	0.9829	1	0.003131	0.007001	0.7%	0.0%	821	826
2		5	0.9961	0.9868	1	0.002622	0.005862	0.59%	-0.2%	816	819
4		5	0.9955	0.9886	1	0.002134	0.004772	0.48%	-0.13%	858	862
9		5	0.9977	0.9884	1	0.002325	0.0052	0.52%	-0.35%	816	818
18		5	0.9963	0.9875	1	0.002487	0.005562	0.56%	-0.21%	778	781
35		5	0.996	0.9926	1	0.001667	0.003729	0.37%	-0.18%	792	795
72.4		5	0.9988	0.9941	1	0.001183	0.002645	0.26%	-0.47%	852	853

Graphics



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

Analysis ID:	17-3343-9816	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Jun-21 10:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	60032	1000	Yes	Two-Point Interpolation

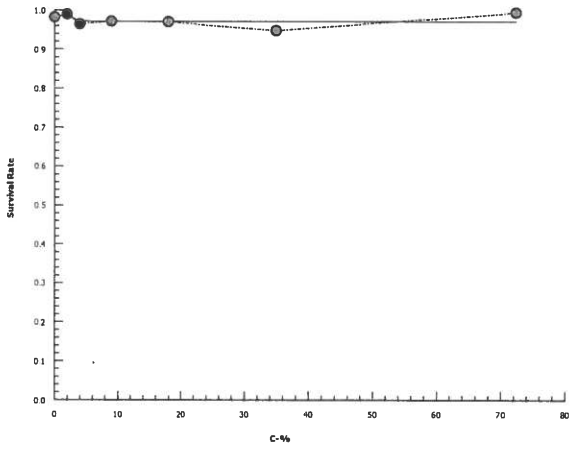
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>72.4	N/A	N/A	<1.381	NA	NA
EC50	>72.4	N/A	N/A	<1.381	NA	NA

Survival Rate Summary

			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.9824	0.9119	1	0.01761	0.03938	4.01%	0.0%	781	795
2		5	0.9899	0.9497	1	0.01006	0.0225	2.27%	-0.77%	787	795
4		5	0.9648	0.8239	1	0.03522	0.07875	8.16%	1.79%	767	795
9		5	0.9723	0.8931	1	0.02073	0.04635	4.77%	1.02%	773	795
18		5	0.9711	0.9245	1	0.01774	0.03968	4.09%	1.15%	772	795
35		5	0.9497	0.8491	1	0.03182	0.07116	7.49%	3.33%	755	795
72.4		5	1	1	1	0	0	0.0%	-1.79%	795	795

Graphics



CETIS Test Data Worksheet

Report Date: 07 May-21 15:35 (p 1 of 1)
Test Code: 2105-508 | ~~07-3309-7859/2BB22F8D~~ QA 6/9/21

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 12 May-21
End Date: 14 May-21
Sample Date: 11 May-21

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

Sample Code: 21-0570
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			165	164	GM 6/2/21
			32			173	172	
			33			147	147	
			34			163	163	
			35			177	177	
			36			174	173	
			37			175	172	
			38			143	142	
			39			177	177	
			40			169	168	
			41			131	131	
			42			176	~~170~~ 175 (A)	
			43			167	166	
			44			176	175	
			45			164	163	
			46			175	173	
			47			173	173	
			48			188	188	GM 6/3/21
			49			179	179	
			50			163	163	
			51			172	172	GM 6/4/21
			52			166	165	
			53			175	175	
			54			170	169	
			55			128	127	
			56			142	142	
			57			154	154	
			58			162	162	
			59			168	168	
			60			171	171	
			61			175	175	GM 6/5/21
			62			151	149	
			63			159	159	
			64			177	177	
			65			203	202	
			66			148	148	GM 6/7/21
			67			172	170	
			68			135	134	
			69			160	158	
			70			145	145	

(A) Q18 GM 6/21/21

CETIS Test Data Worksheet

Report Date: 08 May-21 09:35 (p 1 of 1)
Test Code: 2105.5081 ~~07-3309-7869/2BB22F8D~~ QW AHS 5/27/21

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

Start Date:	12 May-21	**Species:**	Mytilus galloprovincialis	**Sample Code:**	21-0530
End Date:	14 May-21	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs
Sample Date:	11 May-21	**Material:**	Effluent Sample	**Sample Station:**	Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	45					
0	BC	2	37					
0	BC	3	54			170	169	RK 5/15/21
0	BC	4	51					
0	BC	5	70					
0	LC	1	44					
0	LC	2	39					
0	LC	3	49			174	174	
0	LC	4	32					
0	LC	5	55					
2		1	34					
2		2	58					
2		3	62			146	144	
2		4	52					
2		5	64					
4		1	46					
4		2	41					
4		3	65			188	187	
4		4	31					
4		5	48					
9		1	67					
9		2	57					
9		3	56			139	139	
9		4	35					
9		5	47					
18		1	43					
18		2	63					
18		3	33			148	148	
18		4	69					
18		5	66					
35		1	68					
35		2	36					
35		3	38			136	135	
35		4	53					
35		5	59					
72.4	74.2	1	40					
72.4	74.2	2	42					
72.4	74.2	3	50			158	158	
72.4	74.2	4	61					
72.4	74.2	5	60					

QA 5/12/21 QCW

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS

Sample ID: Wyckoff

Sample Log No.: 21-0530

Test No.: 2105-S081

Test Species: *M. galloprovincialis*

Start Date/Time: 5/12/2021 1500

End Date/Time: 5/14/2021 ~~1340~~ ~~1510~~ 1340
Q18 RT 5/14/21 Q16 RT 5/14/21

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	29.8	(A) ~~29.8~~ 29.7	29.4	16.0	15.4	15.9	8.8	8.7	8.5	8.02	7.92	7.87
Brine Control	30.9	31.1	31.0	15.5	15.4	15.8	8.9	8.8	8.5	8.17	8.02	7.93
2	29.9	29.9	29.8	16.0	15.6	15.8	8.6	8.8	8.6	8.05	7.93	7.94
4	30.0	30.0	30.0	15.9	15.6	15.9	8.5	8.8	8.6	8.00	7.94	7.95
9	29.9	30.0	29.9	15.9	15.7	15.7	8.7	8.7	8.6	7.95	7.95	8.00
18	30.1	30.1	30.0	15.8	15.7	15.8	8.8	8.7	8.6	7.88	7.98	8.05
35	30.3	30.3	30.3	15.6	15.8	15.7	8.8	8.7	8.6	7.80	7.98	8.11
12.4	30.6	30.4	30.4	15.5	16.0	15.8	8.8	8.8	8.5	7.68	7.98	8.19

	0	24	48
WQ Readings:	VS	KL	ACS 0800 RT
Dilutions made by:	VS	—	—

Technician Initials:

Environmental Chamber: D

Comments:

0 hrs:

24 hrs: (A) Q18 KL 5/13/21

48 hrs:

QC Check: ACS 5/27/21

Final Review: BO 6/11/21

Marine Chronic Bioassay — **Brine Dilution Worksheet**

DC-010

Project: JACOBS — **Analyst:** KS

Sample ID: Wyckoff — **Test Date:** 5/12/2021

Test No: 2105-S081 — **Test Type:** Mussel Development

Salinity of Effluent 7.2

Salinity of Brine 89.7 — **Date of Brine used:** 4/13/2021

Target Salinity 30 — **Alkalinity of Brine Control:** ~~103~~ 106 mg/L as CaCO3

Test Dilution Volume 250

@ Q18 BS 5/14/21

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.38**	**0.50**

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	8.6	250
18	45.0	0.38	17.2	250
35	87.5	0.38	33.4	250
72.4	180.9	0.38	69.1	250
	DI Volume			
Brine Control	137.5	0.50	69.1	250

Total Brine Volume Required (ml): **203.1**

QC Check: AIS 5/27/21 — **Final Review:** BO 6/11/21

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs/Wyckoff
Test No.: 2105-5081
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP / 3A
Date Received: 4/19/21
Test Chambers: shell vials
Sample Volume: 10 mL

Start Date/Time: 5/12/21 1500
End Date/Time: 5/14/21 ~~1510~~ 1340 QS RT 5/14/21
Technician Initials: KS

Spawn Information

First Gamete Release Time: 11:30

Sex	Number Spawning
Male	3
Female	2

Gamete Selection

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

Egg Fertilization Time: 12:30

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	—
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:		
	10	9
	7	11
	5	11
	7	12
	6	13

Mean: 9.1

Mean 9.1 ~~9.5~~ QS RT 5/12/21 X 50 = 455 embryos/ml

Initial Density: 455
Desired Final Density: 300
(to inoculate with 0.5 ml)
= 1.52 (dilution factor)

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	180	180	100	99.8
TØ B	189	191	99.0	
TØ C	131	131	100	
TØ D	165	165	100	
TØ E	141	141	100	
TØ F	147	147	100	
$\bar{X}$ =	159			

48-h QC: 153/153

Comments:

QC Check: ACS 5/27/21

Final Review: BO 6/11/21

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff (051121)
Test ID No(s).: 2105-5081

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (21-xxxx):	0530 0543	(A) (B)		
Sample Collection Date & Time:	5/11/21 1015	5/12/21 (A) 1000		
Sample Receipt Date & Time:	5/12/21 1000			
Number of Containers & Container Type:	1 x 1L cubi			
Approx. Total Volume Received (L):	~1			
Check-in Temperature (°C)	3.7			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.4			
pH (units)	7.78			
Conductivity (µS/cm)	11,600			
Salinity (ppt)	7.2			
Alkalinity (mg/L) [2]	421			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.04			
Technician Initials	GM			

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

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

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

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

Salinity Adjustment? (Y) N (B)
Test: mussel Source: brine Target ppt: 30 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.			

Test Performed: Mussel Development **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: (B) 98 99 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 106 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 CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: (A) QI8 GM 5/12/21 (B) QI8 AJS 5/27/21

QC Check: AJS 5/27/21
Final Review: BO 6/11/21

Total Ammonia Analysis
Marine

Overlying Water

DC-001

Client: Jacobs
Project: Wyckoff
Test Type: Mussel development

DI Blank: 0.0
SW Blank: 0.0

Test Start Date: 5/12/2021

Analyst: KB
Analysis Date: 5/27/21

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L) N x 1.22
Blank Spike (10 mg/L NH_3)		NA	NA	9.5	11.6
Wyckoff	21-0530	5/12/2021	check-in	1.1	1.3
Spike Check (10 mg/L NH_3)		NA	NA	9.5	11.6
Batch QC	19			2.2	2.7
Sample Duplicate[a]	19	NA	NA	2.2	2.7
Sample Duplicate + Spike[a]		NA	NA	11.2	13.7
Spike Check (10 mg/L NH_3)		NA	NA	9.5	11.6

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 (A)
Blank	0.0	NA	11.6	10	NA	(A) ~~120~~ ~~116~~ 116
Batch QC	2.7	2.7	13.7	10	0	110

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A8236	A8249	A1056

Comments: (A) Q18 KB 5/28/21

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 6/1/21

Final Review: BO 6/11/21

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 5/11/2021
CarrierName: FedEx
AirbillNo: 7736 9092 7629

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2020/WA
Project Code: WEH-029Z
Cooler #: 1 of 1

No: 10-051121-100930-0543
2021T10P000DD210W2LA00
Contact Name: Keith Allers
Contact Phone: 206-780-1711

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
051121		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	05/11/2021 09:43	Field Sample

Special Instructions:

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
	Keith JACOBS	5-11-21 1015	EASD	5/12/21 1000	received in good condition

Receipt temp. 3.7°C
Log-in number: 21-0530

Appendix D
List of Qualifier Codes

ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

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 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 in holding 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 in holding 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:	02-7409-9943	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 May-21 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	14 May-21 13:40	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	00-8857-4692	**Code:**	210512msdv	**Client:**	Internal
Sample Date:	12 May-21	**Material:**	Copper chloride	**Project:**	
Receive Date:	12 May-21	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-3594-9466	Combined Development Ra	5	10	7.071	1.06%		Dunnett Multiple Comparison Test
17-5934-7608	Development Rate	5	10	7.071	1.06%		Dunnett Multiple Comparison Test
01-3180-9279	Survival Rate	20	40	28.28	2.12%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
00-9727-8504	Combined Development Ra	EC25	11.4	10.88	11.83		Linear Interpolation (ICPIN)
		EC50	14.27	13.92	14.55		
12-3891-6641	Development Rate	EC25	11.4	10.86	11.83		Linear Interpolation (ICPIN)
		EC50	14.27	13.91	14.55		
18-1677-8776	Survival Rate	EC25	29.62	26.56	33.67		Linear Interpolation (ICPIN)
		EC50	39.23	33.11	N/A		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
12-3891-6641	Development Rate	Control Resp	0.9952	0.9 - NL	Yes	Passes Acceptability Criteria
17-5934-7608	Development Rate	Control Resp	0.9952	0.9 - NL	Yes	Passes Acceptability Criteria
01-3180-9279	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
18-1677-8776	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
08-3594-9466	Combined Development Ra	PMSD	0.01055	NL - 0.25	No	Passes Acceptability Criteria

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

Combined Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.9952	0.9897	1	0.9906	1	0.001995	0.004461	0.45%	0.0%
2.5		5	0.9936	0.9857	1	0.9866	1	0.002825	0.006316	0.64%	0.16%
5		5	0.9903	0.9865	0.994	0.9866	0.9931	0.001351	0.003021	0.31%	0.49%
10		5	0.8709	0.8198	0.922	0.8072	0.9134	0.0184	0.04114	4.72%	12.49%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.9952	0.9897	1	0.9906	1	0.001995	0.004461	0.45%	0.0%
2.5		5	0.9936	0.9857	1	0.9866	1	0.002825	0.006316	0.64%	0.16%
5		5	0.9903	0.9865	0.994	0.9866	0.9931	0.001351	0.003021	0.31%	0.49%
10		5	0.8709	0.8198	0.922	0.8072	0.9134	0.0184	0.04114	4.72%	12.49%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	1	1	1	1	1	0	0	0.0%	0.0%
2.5		5	1	1	1	1	1	0	0	0.0%	0.0%
5		5	1	1	1	1	1	0	0	0.0%	0.0%
10		5	1	1	1	1	1	0	0	0.0%	0.0%
20		5	1	1	1	1	1	0	0	0.0%	0.0%
40		5	0.48	0.2893	0.6707	0.27	0.63	0.0687	0.1536	32.0%	52.0%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9929	1	1	0.9906	0.9926
2.5		0.9931	1	1	0.9866	0.9883
5		0.9925	0.9875	0.9931	0.9866	0.9919
10		0.9134	0.8993	0.8611	0.8072	0.8735
20		0	0	0	0	0
40		0	0	0	0	0

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9929	1	1	0.9906	0.9926
2.5		0.9931	1	1	0.9866	0.9883
5		0.9925	0.9875	0.9931	0.9866	0.9919
10		0.9134	0.8993	0.8611	0.8072	0.8735
20		0	0	0	0	0
40		0	0	0	0	0

Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1	1	1	1	1
2.5		1	1	1	1	1
5		1	1	1	1	1
10		1	1	1	1	1
20		1	1	1	1	1
40		0.51	0.38	0.27	0.63	0.61

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

Combined Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	140/141	130/130	156/156	105/106	134/135
2.5		143/144	151/151	135/135	147/149	169/171
5		132/133	158/160	143/144	147/149	122/123
10		116/127	134/149	124/144	134/166	145/166
20		0/143	0/118	0/170	0/159	0/156
40		0/100	0/100	0/100	0/100	0/100

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	140/141	130/130	156/156	105/106	134/135
2.5		143/144	151/151	135/135	147/149	169/171
5		132/133	158/160	143/144	147/149	122/123
10		116/127	134/149	124/144	134/166	145/166
20		0/143	0/118	0/170	0/159	0/156
40		0/51	0/38	0/27	0/63	0/61

Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	100/100	100/100	100/100	100/100	100/100
2.5		100/100	100/100	100/100	100/100	100/100
5		100/100	100/100	100/100	100/100	100/100
10		100/100	100/100	100/100	100/100	100/100
20		100/100	100/100	100/100	100/100	100/100
40		51/100	38/100	27/100	63/100	61/100

CETIS Analytical Report

Test Code: 210512msdv | 15-4594-3065

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

Analysis ID:	08-3594-9466	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-21 11:21	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	1.06%	5	10	7.071	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.332	2.227	0.054	8	0.6181	CDF	Non-Significant Effect
		5	1.138	2.227	0.054	8	0.2804	CDF	Non-Significant Effect
		10*	12.21	2.227	0.054	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3006229	0.1002076	3	69.11	<0.0001	Significant Effect
Error	0.02320029	0.001450018	16			
Total	0.3238232		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.527	11.34	0.0886	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9628	0.866	0.6011	Normal Distribution

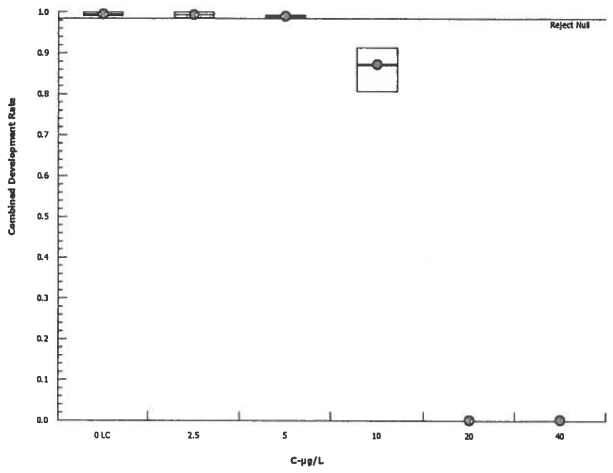
Combined Development Rate Summary

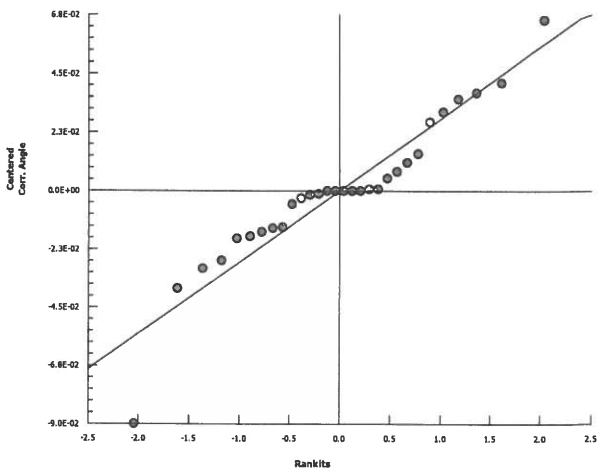
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9952	0.9897	1	0.9929	0.9906	1	0.001995	0.45%	0.0%
2.5		5	0.9936	0.9857	1	0.9931	0.9866	1	0.002825	0.64%	0.16%
5		5	0.9903	0.9865	0.994	0.9919	0.9866	0.9931	0.00135	0.3%	0.49%
10		5	0.8709	0.8198	0.922	0.8735	0.8072	0.9134	0.0184	4.72%	12.49%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.5	1.468	1.533	1.486	1.474	1.531	0.01181	1.76%	0.0%
2.5		5	1.492	1.448	1.536	1.487	1.455	1.53	0.01584	2.37%	0.53%
5		5	1.473	1.454	1.492	1.481	1.455	1.487	0.006787	1.03%	1.83%
10		5	1.206	1.132	1.281	1.207	1.116	1.272	0.0269	4.99%	19.59%
20		5	0.04119	0.03748	0.04489	0.04004	0.03836	0.04605	0.001335	7.25%	97.26%
40		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.67%

Graphics





Analyst: JM QA: AK 6/7/21

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

Analysis ID:	17-5934-7608	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-21 11:21	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	1.06%	5	10	7.071	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.332	2.227	0.054	8	0.6181	CDF	Non-Significant Effect
		5	1.138	2.227	0.054	8	0.2804	CDF	Non-Significant Effect
		10*	12.21	2.227	0.054	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3006229	0.1002076	3	69.11	<0.0001	Significant Effect
Error	0.02320029	0.001450018	16			
Total	0.3238232		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.527	11.34	0.0886	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9628	0.866	0.6011	Normal Distribution

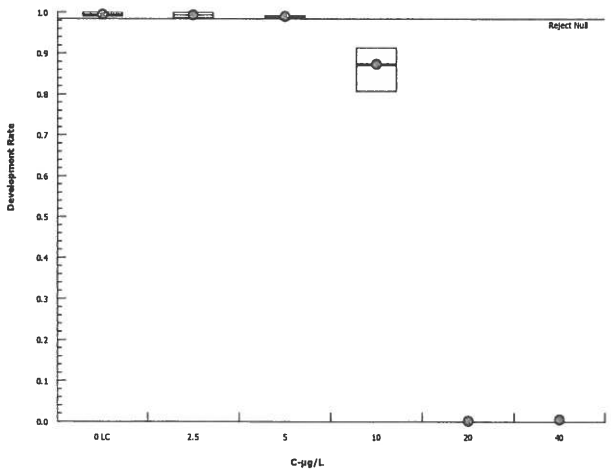
Development Rate Summary

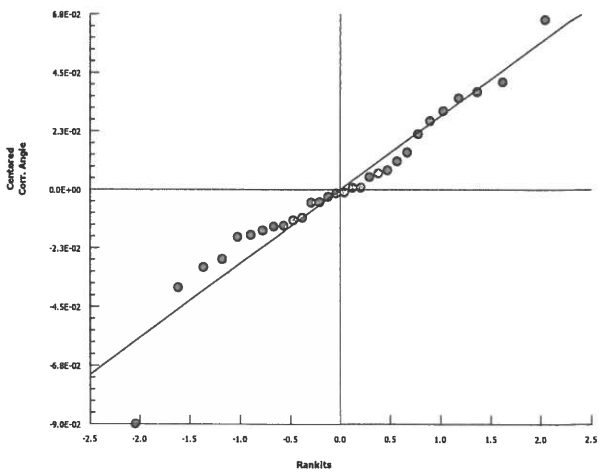
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9952	0.9897	1	0.9929	0.9906	1	0.001995	0.45%	0.0%
2.5		5	0.9936	0.9857	1	0.9931	0.9866	1	0.002825	0.64%	0.16%
5		5	0.9903	0.9865	0.994	0.9919	0.9866	0.9931	0.00135	0.3%	0.49%
10		5	0.8709	0.8198	0.922	0.8735	0.8072	0.9134	0.0184	4.72%	12.49%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.5	1.468	1.533	1.486	1.474	1.531	0.01181	1.76%	0.0%
2.5		5	1.492	1.448	1.536	1.487	1.455	1.53	0.01584	2.37%	0.53%
5		5	1.473	1.454	1.492	1.481	1.455	1.487	0.006787	1.03%	1.83%
10		5	1.206	1.132	1.281	1.207	1.116	1.272	0.0269	4.99%	19.59%
20		5	0.04119	0.03748	0.04489	0.04004	0.03836	0.04605	0.001335	7.25%	97.26%
40		5	0.07495	0.05758	0.09231	0.07007	0.06304	0.09637	0.006254	18.66%	95.0%

Graphics





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

Analysis ID: 01-3180-9279 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 27 May-21 11:21 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.12%	20	40	28.28	

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		5	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		10	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		20	27.5	16	1	8	0.8333	Asymp	Non-Significant Effect
		40*	15	16	0	8	0.0191	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.38726	0.477452	5	115.5	<0.0001	Significant Effect
Error	0.09917824	0.004132427	24			
Total	2.486439		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	20.36	4.248	<0.0001	Unequal Variances
Variances	Levene Equality of Variance	17.16	3.895	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.5426	0.9031	<0.0001	Non-normal Distribution

Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1	1	1	1	1	1	0	0.0%	0.0%
2.5		5	1	1	1	1	1	1	0	0.0%	0.0%
5		5	1	1	1	1	1	1	0	0.0%	0.0%
10		5	1	1	1	1	1	1	0	0.0%	0.0%
20		5	1	1	1	1	1	1	0	0.0%	0.0%
40		5	0.48	0.2893	0.6707	0.51	0.27	0.63	0.0687	32.0%	52.0%

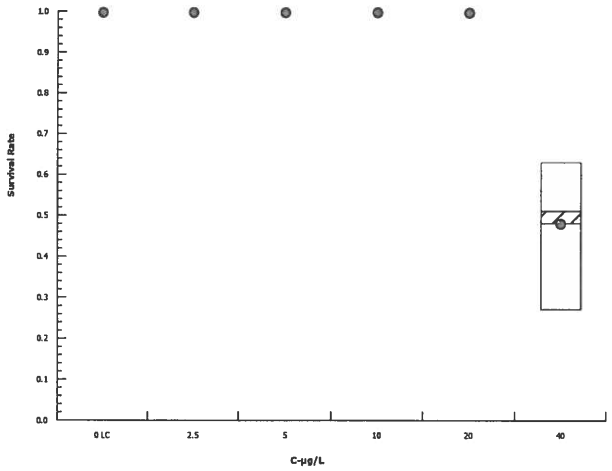
Angular (Corrected) Transformed Summary

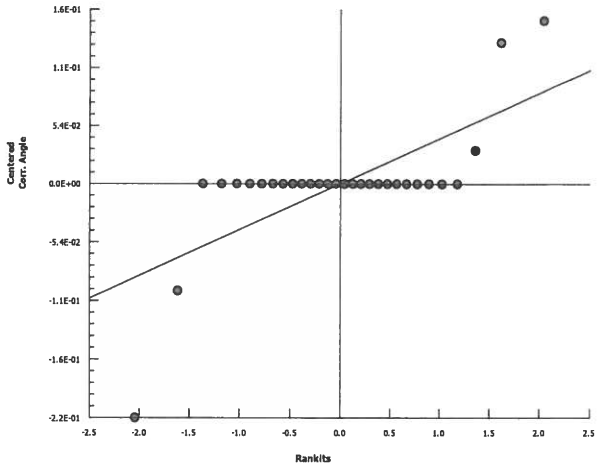
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.521	1.521	1.521	1.521	1.521	1.521	0	0.0%	0.0%
2.5		5	1.521	1.521	1.521	1.521	1.521	1.521	0	0.0%	0.0%
5		5	1.521	1.521	1.521	1.521	1.521	1.521	0	0.0%	0.0%
10		5	1.521	1.521	1.521	1.521	1.521	1.521	0	0.0%	0.0%
20		5	1.521	1.521	1.521	1.521	1.521	1.521	0	0.0%	0.0%
40		5	0.7638	0.5683	0.9594	0.7954	0.5464	0.9169	0.07042	20.61%	49.77%

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

Analysis ID:	01-3180-9279	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-21 11:21	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	00-9727-8504	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-21 11:22	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	724345	1000	Yes	Two-Point Interpolation

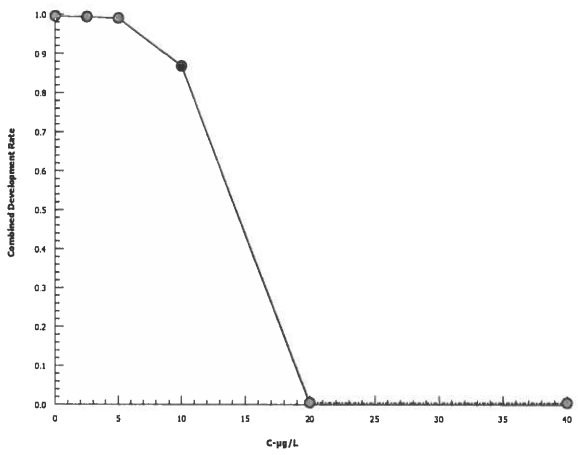
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	11.4	10.88	11.83
EC50	14.27	13.92	14.55

Combined Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.9952	0.9906	1	0.001995	0.004462	0.45%	0.0%	665	668
2.5		5	0.9936	0.9866	1	0.002825	0.006316	0.64%	0.16%	745	750
5		5	0.9903	0.9866	0.9931	0.00135	0.00302	0.3%	0.49%	702	709
10		5	0.8709	0.8072	0.9134	0.0184	0.04114	4.72%	12.49%	653	752
20		5	0	0	0	0	0		100.0%	0	746
40		5	0	0	0	0	0		100.0%	0	500

Graphics



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

Analysis ID:	12-3891-6641	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-21 11:22	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1769803	1000	Yes	Two-Point Interpolation

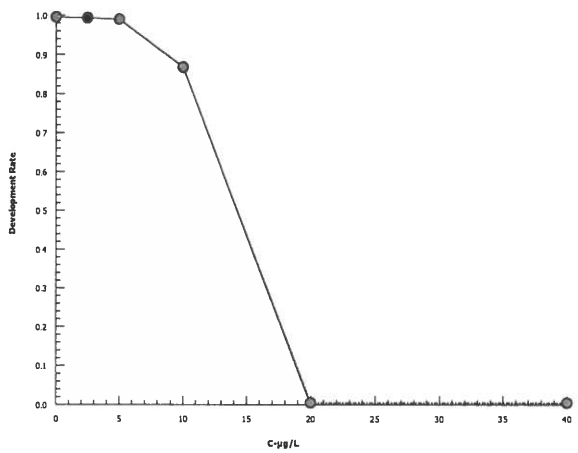
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	11.4	10.86	11.83
EC50	14.27	13.91	14.55

Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.9952	0.9906	1	0.001995	0.004462	0.45%	0.0%	665	668
2.5		5	0.9936	0.9866	1	0.002825	0.006316	0.64%	0.16%	745	750
5		5	0.9903	0.9866	0.9931	0.00135	0.00302	0.3%	0.49%	702	709
10		5	0.8709	0.8072	0.9134	0.0184	0.04114	4.72%	12.49%	653	752
20		5	0	0	0	0	0		100.0%	0	746
40		5	0	0	0	0	0		100.0%	0	240

Graphics



Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	18-1677-8776	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-21 11:21	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1793240	1000	Yes	Two-Point Interpolation

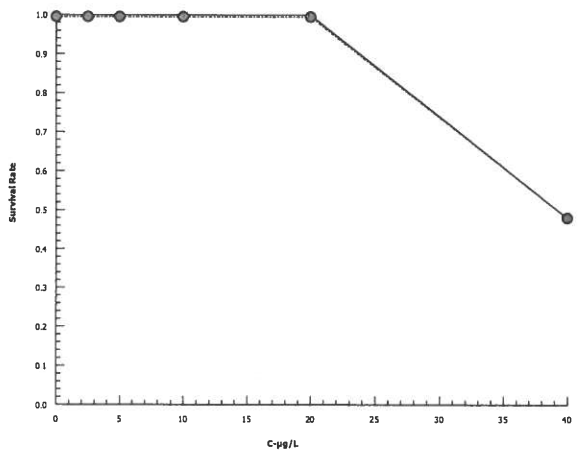
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	29.62	26.56	33.67
EC50	39.23	33.11	N/A

Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	1	1	1	0	0	0.0%	0.0%	500	500
2.5		5	1	1	1	0	0	0.0%	0.0%	500	500
5		5	1	1	1	0	0	0.0%	0.0%	500	500
10		5	1	1	1	0	0	0.0%	0.0%	500	500
20		5	1	1	1	0	0	0.0%	0.0%	500	500
40		5	0.48	0.27	0.63	0.0687	0.1536	32.0%	52.0%	240	500

Graphics

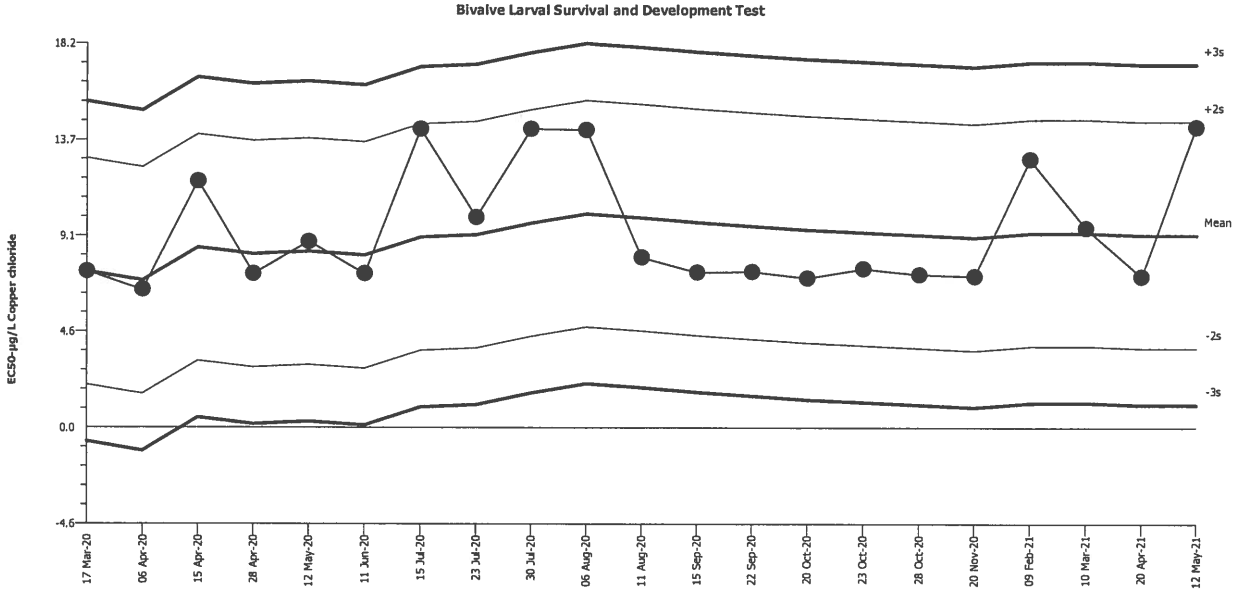


CETIS QC Plot

Report Date: 27 May-21 11:23 (1 of 1)

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

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



Mean:	9.142	**Count:**	20	**-2s Warning Limit:**	3.766	**-3s Action Limit:**	1.078
Sigma:	2.688	**CV:**	29.40%	**+2s Warning Limit:**	14.52	**+3s Action Limit:**	17.21

Quality Control Data

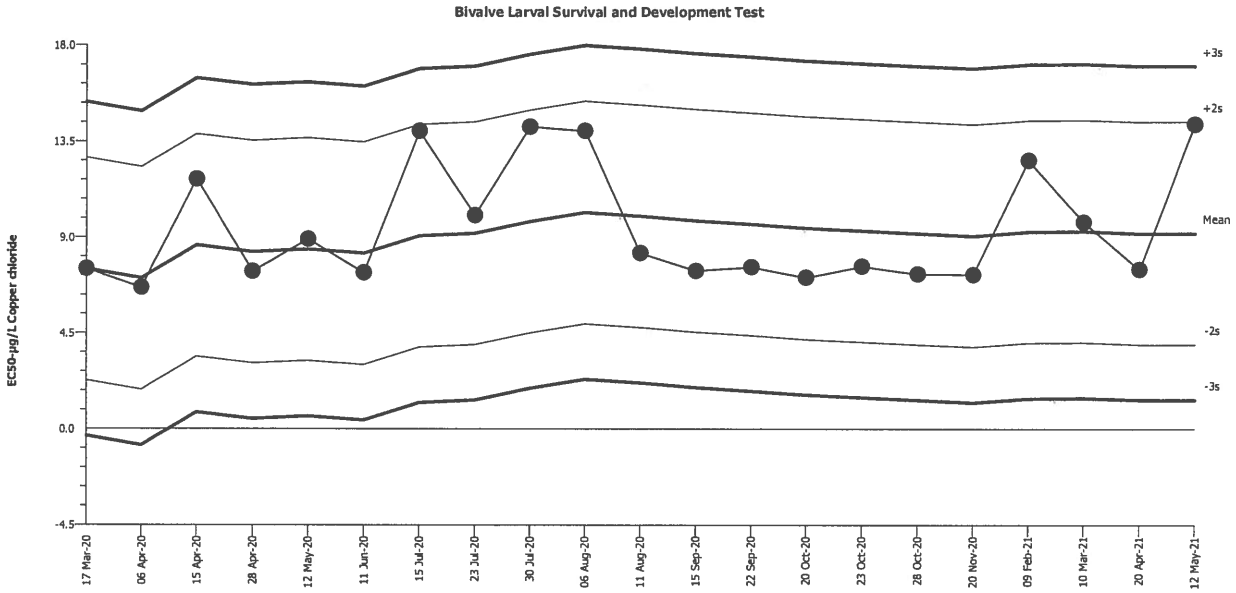
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Mar	17	14:20	7.408	-1.734	-0.6452			14-6169-3689	18-9939-7640
2		Apr	6	17:15	6.537	-2.605	-0.9691			02-0082-4673	13-2096-3831
3			15	13:25	11.68	2.541	0.9453			16-4614-0901	11-3098-9850
4			28	13:25	7.292	-1.85	-0.6884			06-8086-6028	13-2749-2065
5		May	12	16:15	8.819	-0.3231	-0.1202			12-3773-8150	00-4087-7530
6		Jun	11	15:45	7.306	-1.836	-0.6832			20-6521-9403	10-1893-3875
7		Jul	15	13:55	14.16	5.02	1.868			17-4780-3294	11-0488-5403
8			23	15:00	9.974	0.8324	0.3097			06-0741-6264	07-6012-8216
9			30	15:35	14.17	5.023	1.869			00-9901-5729	19-4020-2576
10		Aug	6	15:40	14.13	4.983	1.854			01-4440-0014	02-9592-9535
11			11	14:30	8.085	-1.057	-0.3931			21-4043-5119	05-6052-3343
12		Sep	15	0:00	7.365	-1.777	-0.6611			19-9833-0655	18-5101-1090
13			22	14:40	7.405	-1.737	-0.6462			04-0347-9113	09-6026-9613
14		Oct	20	14:25	7.1	-2.042	-0.7595			08-8652-5764	17-2783-6415
15			23	13:45	7.548	-1.594	-0.5928			09-8413-3498	19-3049-9702
16			28	15:50	7.269	-1.873	-0.6968			09-4043-4676	02-6542-7057
17		Nov	20	16:00	7.187	-1.955	-0.7273			13-7696-8009	10-4367-1427
18	2021	Feb	9	15:15	12.74	3.597	1.338			12-5648-6062	18-1503-3303
19		Mar	10	14:15	9.481	0.3387	0.126			13-7922-5399	10-0885-9755
20		Apr	20	16:15	7.185	-1.957	-0.7281			06-7450-9711	18-3353-6875
21		May	12	15:00	14.27	5.126	1.907			15-4594-3065	00-9727-8504

CETIS QC Plot

Report Date: 27 May-21 11:23 (1 of 1)

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

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



Mean:	9.176	**Count:**	20	**-2s Warning Limit:**	3.966	**-3s Action Limit:**	1.361
Sigma:	2.605	**CV:**	28.40%	**+2s Warning Limit:**	14.39	**+3s Action Limit:**	16.99

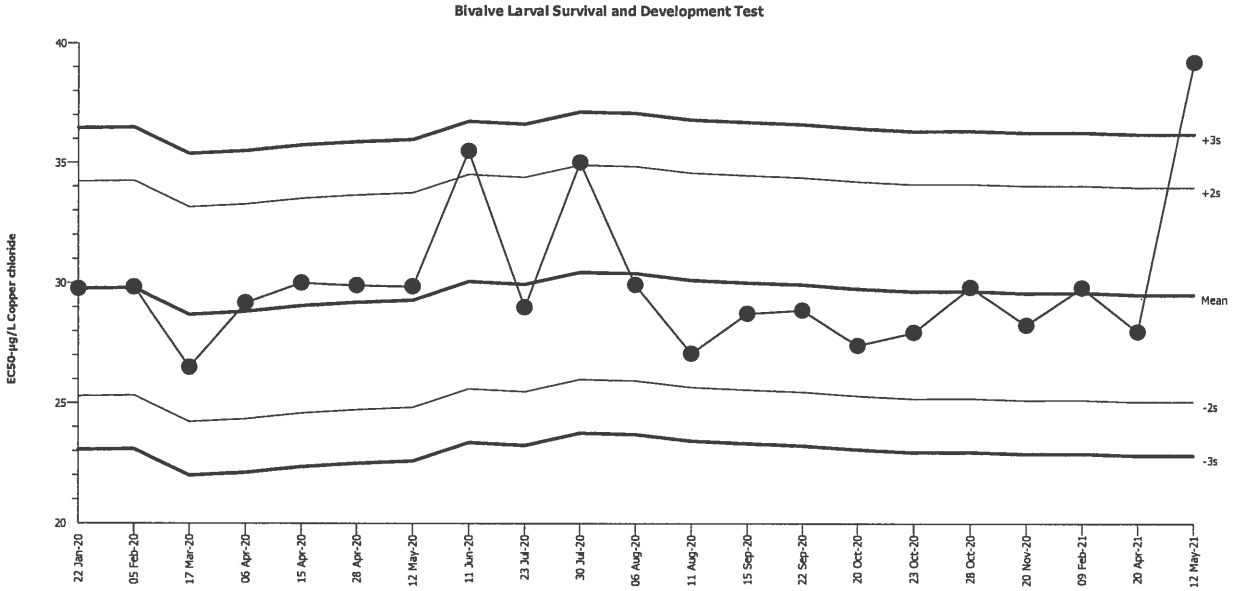
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Mar	17	14:20	7.489	-1.687	-0.6477			14-6169-3689	12-6636-5212
2		Apr	6	17:15	6.609	-2.567	-0.9853			02-0082-4673	11-5300-1558
3			15	13:25	11.68	2.507	0.9624			16-4614-0901	19-2371-7781
4			28	13:25	7.365	-1.811	-0.6952			06-8086-6028	17-1633-3832
5		May	12	16:15	8.876	-0.2997	-0.1151			12-3773-8150	04-4023-9067
6		Jun	11	15:45	7.306	-1.87	-0.718			20-6521-9403	18-5947-9043
7		Jul	15	13:55	13.94	4.763	1.828			17-4780-3294	14-0926-7215
8			23	15:00	9.999	0.8231	0.316			06-0741-6264	12-5816-3058
9			30	15:35	14.14	4.961	1.904			00-9901-5729	02-7058-2757
10		Aug	6	15:40	13.95	4.774	1.833			01-4440-0014	13-7910-6508
11			11	14:30	8.237	-0.939	-0.3604			21-4043-5119	01-1240-7098
12		Sep	15	0:00	7.397	-1.779	-0.6828			19-9833-0655	03-7616-5506
13			22	14:40	7.576	-1.6	-0.6141			04-0347-9113	01-0437-7711
14		Oct	20	14:25	7.089	-2.087	-0.801			08-8652-5764	06-9681-8469
15			23	13:45	7.616	-1.56	-0.5989			09-8413-3498	17-5257-3346
16			28	15:50	7.257	-1.919	-0.7367			09-4043-4676	12-0840-2779
17		Nov	20	16:00	7.23	-1.946	-0.7471			13-7696-8009	11-4264-3018
18	2021	Feb	9	15:15	12.58	3.407	1.308			12-5648-6062	01-5747-2564
19		Mar	10	14:15	9.694	0.5177	0.1987			13-7922-5399	08-4869-7631
20		Apr	20	16:15	7.482	-1.694	-0.6503			06-7450-9711	17-9210-1733
21		May	12	15:00	14.27	5.092	1.955			15-4594-3065	12-3891-6641

CETIS QC Plot

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

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



Mean:	29.51	**Count:**	20	**-2s Warning Limit:**	25.05	**-3s Action Limit:**	22.81
Sigma:	2.233	**CV:**	7.57%	**+2s Warning Limit:**	33.98	**+3s Action Limit:**	36.21

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jan	22	13:25	29.76	0.2456	0.11			02-1152-2212	19-4150-8988
2		Feb	5	13:10	29.83	0.3163	0.1417			06-6849-2235	07-0404-6516
3		Mar	17	14:20	26.48	-3.028	-1.356			14-6169-3689	14-2151-4803
4		Apr	6	17:15	29.18	-0.3332	-0.1492			02-0082-4673	12-2147-8498
5			15	13:25	30	0.49	0.2194			16-4614-0901	00-5465-8677
6			28	13:25	29.9	0.386	0.1728			06-8086-6028	08-1083-2165
7		May	12	16:15	29.85	0.341	0.1527			12-3773-8150	18-0143-0286
8		Jun	11	15:45	35.5	5.989	2.682	(+)		20-6521-9403	17-6494-5506
9		Jul	23	15:00	28.98	-0.5302	-0.2375			06-0741-6264	11-2012-0880
10			30	15:35	35.02	5.508	2.467	(+)		00-9901-5729	18-8992-7280
11		Aug	6	15:40	29.92	0.4132	0.185			01-4440-0014	05-9348-7696
12			11	14:30	27.06	-2.451	-1.098			21-4043-5119	16-7506-8565
13		Sep	15	0:00	28.73	-0.7843	-0.3512			19-9833-0655	01-9900-7404
14			22	14:40	28.86	-0.6464	-0.2895			04-0347-9113	03-4439-9784
15		Oct	20	14:25	27.4	-2.114	-0.9467			08-8652-5764	01-6350-7777
16			23	13:45	27.94	-1.568	-0.7024			09-8413-3498	02-1232-2390
17			28	15:50	29.82	0.3095	0.1386			09-4043-4676	15-7574-6891
18		Nov	20	16:00	28.24	-1.266	-0.5671			13-7696-8009	21-0824-4197
19	2021	Feb	9	15:15	29.8	0.2946	0.1319			12-5648-6062	08-9593-0094
20		Apr	20	16:15	27.97	-1.539	-0.6893			06-7450-9711	02-2099-4435
21		May	12	15:00	39.23	9.721	4.353	(+)	(+)	15-4594-3065	18-1677-8776

CETIS Test Data Worksheet

Report Date: 07 May-21 15:34 (p 1 of 1)
Test Code: 15-4594-3065/210512msdv

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

Start Date: 12 May-21
End Date: 14 May-21
Sample Date: 12 May-21
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 210512msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			106	105	GM 5/25/21
			2			133	132	
			3			135	134	
			4			123	122	
			5			130	130	
			6			61	0	52/0 YS 6/7/21
			7			151	151	
			8			38	0	50/0 YS 6/7/21
			9			141	124	
			10			135	135	
			11			118	0	
			12			127	116	133/131 YS 6/7/21
			13			170	0	
			14			144	143	
			15			27	0	
			16			141	140	
			17			149	147	
			18			166	134	172/162 YS 6/7/21
			19			166	145	
			20			171	169	
			21			149	134	
			22			160	158	
			23			139	0	171/0 YS 6/7/21
			24			156	0	
			25			51	0	
			26			143	0	
			27			156	156	
			28			144	143	
			29			63	0	
			30			149	147	

CETIS Test Data Worksheet

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

Start Date: 12 May-21 **Species:** Mytilus galloprovincialis **Sample Code:** 210512msdv
End Date: 14 May-21 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 12 May-21 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	16					
0	LC	2	5			135	133	133/135 GH 5/15/21
0	LC	3	27					
0	LC	4	1					
0	LC	5	3					
2.5		1	14					
2.5		2	7					
2.5		3	10					
2.5		4	17			153	151	151/153
2.5		5	20					
5		1	2					
5		2	22					
5		3	28					
5		4	30					
5		5	4			136	136	136/138
10		1	12					
10		2	21					
10		3	9					
10		4	18			170	152	152/170
10		5	19					
20		1	26					
20		2	11					
20		3	13					
20		4	23			167	0	0/167
20		5	24					
40		1	25					
40		2	8			58	0	0/58 Most embryos lysed
40		3	15					
40		4	29					
40		5	6					

QC: VS

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 210512msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 5/12/2021 1500

End Date/Time: 5/14/2021 ~~1510~~ 1340

RT 5/14/21

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	31.7	31.5	31.4	15.2	15.5	15.5	8.6	8.9	8.6	7.99	7.90	7.94
2.5	32.0	32.0	31.9	14.9	15.3	15.3	8.6	8.9	8.6	8.02	7.94	7.95
5	32.0	32.0	32.0	14.8	15.4	15.4	8.7	8.8	8.6	8.03	7.95	7.96
10	32.0	32.0	32.0	14.9	15.4	15.4	8.6	8.8	8.5	8.03	7.94	7.96
20	31.9	31.9	31.9	15.0	15.5	15.4	8.7	8.8	8.6	8.03	7.95	7.97
40	31.8	31.8	31.8	14.9	15.6	15.5	8.6	8.8	8.5	8.04	7.96	7.97

	0	24	48
Technician Initials: WQ Readings:	VM	KL	RT
Dilutions made by:	VM		

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	1.2
Final Volume (mL):	500
Cu stock concentration (µg/L):	9,000

Environmental Chamber: D

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: ACS 5/26/21

Final Review: AC 6/7/21

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / $CaCl_2$
Test No.: 210512nsdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP / 3A
Date Received: 4/19/21
Test Chambers: shell vials
Sample Volume: 10 mL

Start Date/Time: 5/12/21 1500
End Date/Time: 5/14/21 ~~1510~~ 1340 QA PT 5/14/21
Technician Initials: KS

Spawn Information

First Gamete Release Time: 11:30

Sex	Number Spawning
Male	3
Female	2

Gamete Selection

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

Egg Fertilization Time: 12:30

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	—
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:		
	10	9
	7	11
	5	11
	7	12
	6	13

Mean: 9.1

Mean 9.1 ~~9.5~~ QA PT 5/12/21 X 50 = 455 embryos/ml

Initial Density: 455
Desired Final Density: 300
(to inoculate with 0.5 ml)
= 1.52 (dilution factor)

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	180	180	100	99.8
TØ B	189	191	99.0	
TØ C	131	131	100	
TØ D	165	165	100	
TØ E	141	141	100	
TØ F	147	147	100	
$\overline{X}$ =	159			

48-h QC: 153/153

Comments:

QC Check: ACS 5/26/21

Final Review: AC 6/7/21