ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: March 2021

Prepared for: **Jacobs**
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Date Submitted: **April 7, 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: _____
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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	**030921**
Enthalpy Log-in Number	21-0281
Collection Date; Time	3/9/2021; 0915h
Receipt Date; Time	3/10/2021; 0915h
Receipt Temperature (°C)	1.9
Dissolved Oxygen (mg/L)	9.6
pH	7.70
Conductivity (µS/cm)	11,390
Salinity (ppt)	6.5
Alkalinity (mg/L $CaCO_3$)	327
Total Chlorine (mg/L)	<0.02
Total Ammonia (mg/L as N)	1.8

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	3/10/2021, 1415h to 3/12/2021, 1315h
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)	74.2[a], 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper chloride [b]
Statistical Software	CETIS™ 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 74.2 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.35 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	74.2	> 74.2	< 1.35	> 74.2
	Survival	74.2	> 74.2	< 1.35	> 74.2

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

Effect Concentration 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)	76.4	98.6
0 (Lab Control)	91.5	97.0
2	87.9	97.4
4	83.0	98.1
9	77.6	98.8
18	85.7	97.8
35	84.9	97.7
74.2[a]	88.5	96.8

[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 greater than the highest concentration tested; 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	> 40.0	29.6 ± 4.41	7.46
Bivalve Normal Development	5	9.69	9.42 ± 5.85	31.0

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

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

Batch ID:	16-5403-8639	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	10 Mar-21 14:15	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	12 Mar-21 13:15	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	16-5966-5760	**Code:**	21-0281	**Client:**	Jacobs
Sample Date:	09 Mar-21 09:15	**Material:**	Effluent Sample	**Project:**	
Receive Date:	10 Mar-21 09:15	**Source:**	Jacobs		
Sample Age:	29h (1.9 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-2387-1345	Development Rate	74.2	>74.2	NA	1.74%	<1.348	Dunnett Multiple Comparison Test
02-0804-8648	Survival Rate	74.2	>74.2	NA	27.2%	<1.348	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-9634-7858	Development Rate	EC25	>74.2	N/A	N/A	<1.348	Linear Interpolation (ICPIN)
		EC50	>74.2	N/A	N/A	<1.348	
16-3953-4561	Survival Rate	EC25	>74.2	N/A	N/A	<1.348	Linear Interpolation (ICPIN)
		EC50	>74.2	N/A	N/A	<1.348	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-2387-1345	Development Rate	Control Resp	0.9862	0.9 - NL	Yes	Passes Acceptability Criteria
07-9634-7858	Development Rate	Control Resp	0.9862	0.9 - NL	Yes	Passes Acceptability Criteria
02-0804-8648	Survival Rate	Control Resp	0.7642	0.5 - NL	Yes	Passes Acceptability Criteria
16-3953-4561	Survival Rate	Control Resp	0.7642	0.5 - NL	Yes	Passes Acceptability Criteria

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9862	0.9719	1	0.9697	1	0.005159	0.01154	1.17%	0.0%
0	Lab Control	5	0.97	0.9463	0.9937	0.9402	0.9926	0.008535	0.01909	1.97%	1.65%
2		5	0.9743	0.9671	0.9816	0.9697	0.9846	0.002624	0.005867	0.6%	1.2%
4		5	0.9807	0.9639	0.9975	0.9636	0.9918	0.006043	0.01351	1.38%	0.55%
9		5	0.9883	0.972	1	0.9688	1	0.005872	0.01313	1.33%	-0.21%
18		5	0.9776	0.9625	0.9928	0.9593	0.9908	0.005448	0.01218	1.25%	0.87%
35		5	0.9765	0.9609	0.9922	0.9608	0.9926	0.005637	0.0126	1.29%	0.98%
74.2		5	0.9684	0.9554	0.9815	0.9533	0.9818	0.004688	0.01048	1.08%	1.8%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.7642	0.7409	0.7874	0.7388	0.791	0.008377	0.01873	2.45%	0.0%
0	Lab Control	5	0.9149	0.8198	1	0.806	1	0.03425	0.07658	8.37%	-19.73%
2		5	0.8791	0.7671	0.9911	0.806	0.9851	0.04035	0.09023	10.26%	-15.04%
4		5	0.8299	0.707	0.9527	0.7164	0.9478	0.04424	0.09892	11.92%	-8.59%
9		5	0.7761	0.702	0.8502	0.7164	0.8657	0.0267	0.0597	7.69%	-1.56%
18		5	0.8567	0.7901	0.9233	0.806	0.9179	0.024	0.05366	6.26%	-12.11%
35		5	0.8493	0.7211	0.9774	0.7612	1	0.04615	0.1032	12.15%	-11.13%
74.2		5	0.8851	0.7544	1	0.7985	1	0.04706	0.1052	11.89%	-15.82%

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

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9697	1	0.9906	0.9903	0.9804
0	Lab Control	0.9402	0.9722	0.968	0.9767	0.9926
2		0.9697	0.9725	0.9722	0.9727	0.9846
4		0.9636	0.9685	0.9896	0.9918	0.9901
9		1	0.9828	0.9688	1	0.99
18		0.9821	0.9836	0.9722	0.9908	0.9593
35		0.9608	0.9926	0.9815	0.9806	0.9672
74.2		0.9533	0.9701	0.9722	0.9648	0.9818

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.7388	0.7612	0.791	0.7687	0.7612
0	Lab Control	0.8731	0.806	0.9328	0.9627	1
2		0.9851	0.8134	0.806	0.8209	0.9701
4		0.8209	0.9478	0.7164	0.9104	0.7537
9		0.7463	0.8657	0.7164	0.806	0.7463
18		0.8358	0.9104	0.806	0.8134	0.9179
35		0.7612	1	0.806	0.7687	0.9104
74.2		0.7985	1	0.806	1	0.8209

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	96/99	102/102	105/106	102/103	100/102
0	Lab Control	110/117	105/108	121/125	126/129	135/136
2		128/132	106/109	105/108	107/110	128/130
4		106/110	123/127	95/96	121/122	100/101
9		100/100	114/116	93/96	108/108	99/100
18		110/112	120/122	105/108	108/109	118/123
35		98/102	135/136	106/108	101/103	118/122
74.2		102/107	130/134	105/108	137/142	108/110

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	99/134	102/134	106/134	103/134	102/134
0	Lab Control	117/134	108/134	125/134	129/134	134/134
2		132/134	109/134	108/134	110/134	130/134
4		110/134	127/134	96/134	122/134	101/134
9		100/134	116/134	96/134	108/134	100/134
18		112/134	122/134	108/134	109/134	123/134
35		102/134	134/134	108/134	103/134	122/134
74.2		107/134	134/134	108/134	134/134	110/134

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

Analysis ID:	02-0804-8648	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 9:52	**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	27.2%	74.2	>74.2	NA	1.348

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	40	16	0	8	1.0000	Asymp	Non-Significant Effect
		4	31	16	0	8	0.9749	Asymp	Non-Significant Effect
		9	27	16	0	8	0.8267	Asymp	Non-Significant Effect
		18	40	16	0	8	1.0000	Asymp	Non-Significant Effect
		35	35.5	16	2	8	0.9991	Asymp	Non-Significant Effect
		74.2	40	16	0	8	1.0000	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1935783	0.03226306	6	1.52	0.2082	Non-Significant Effect
Error	0.5944435	0.02123013	28			
Total	0.7880218		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	17.06	16.81	0.0091	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9351	0.9146	0.0398	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	0.7642	0.7409	0.7874	0.7612	0.7388	0.791	0.008377	2.45%	0.0%
2		5	0.8791	0.7671	0.9911	0.8209	0.806	0.9851	0.04035	10.26%	-15.04%
4		5	0.8299	0.707	0.9527	0.8209	0.7164	0.9478	0.04424	11.92%	-8.59%
9		5	0.7761	0.702	0.8502	0.7463	0.7164	0.8657	0.0267	7.69%	-1.56%
18		5	0.8567	0.7901	0.9233	0.8358	0.806	0.9179	0.024	6.26%	-12.11%
35		5	0.8493	0.7211	0.9774	0.806	0.7612	1	0.04615	12.15%	-11.13%
74.2		5	0.8851	0.7544	1	0.8209	0.7985	1	0.04706	11.89%	-15.82%

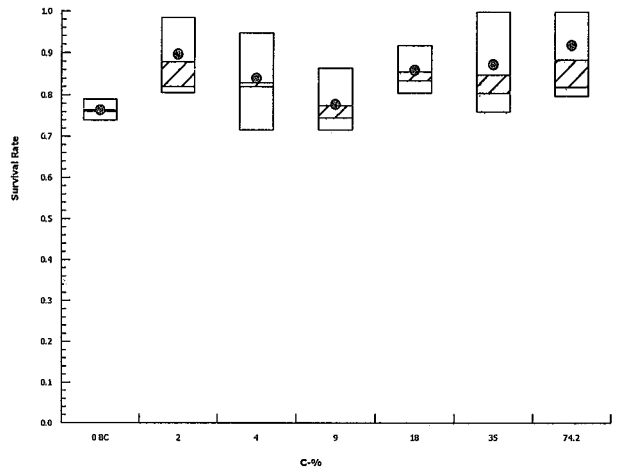
Angular (Corrected) Transformed Summary

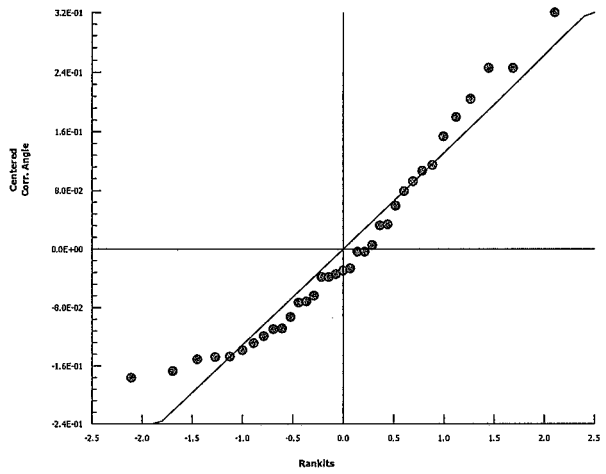
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.064	1.036	1.091	1.06	1.034	1.096	0.009897	2.08%	0.0%
2		5	1.244	1.039	1.448	1.134	1.115	1.448	0.07363	13.24%	-16.88%
4		5	1.16	0.9857	1.335	1.134	1.009	1.34	0.06288	12.12%	-9.06%
9		5	1.081	0.9883	1.174	1.043	1.009	1.196	0.0334	6.91%	-1.6%
18		5	1.188	1.089	1.287	1.154	1.115	1.28	0.03561	6.7%	-11.65%
35		5	1.208	0.9629	1.452	1.115	1.06	1.528	0.08817	16.32%	-13.51%
74.2		5	1.282	1.003	1.561	1.134	1.105	1.528	0.1005	17.52%	-20.47%

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

Analysis ID:	02-0804-8648	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 9:52	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	03-2387-1345	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 9:52	**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	1.74%	74.2	>74.2	NA	1.348

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	1.775	2.407	0.065	8	0.1612	CDF	Non-Significant Effect
		4	0.7588	2.407	0.065	8	0.5643	CDF	Non-Significant Effect
		9	-0.3965	2.407	0.065	8	0.9384	CDF	Non-Significant Effect
		18	1.25	2.407	0.065	8	0.3419	CDF	Non-Significant Effect
		35	1.36	2.407	0.065	8	0.2974	CDF	Non-Significant Effect
		74.2	2.398	2.407	0.065	8	0.0509	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02074923	0.003458204	6	1.918	0.1128	Non-Significant Effect
Error	0.05048063	0.00180288	28			
Total	0.07122986		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.163	16.81	0.6546	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9693	0.9146	0.4240	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	0.9862	0.9719	1	0.9903	0.9697	1	0.005159	1.17%	0.0%
2		5	0.9743	0.9671	0.9816	0.9725	0.9697	0.9846	0.002624	0.6%	1.2%
4		5	0.9807	0.9639	0.9975	0.9896	0.9636	0.9918	0.006043	1.38%	0.55%
9		5	0.9883	0.972	1	0.99	0.9688	1	0.005872	1.33%	-0.21%
18		5	0.9776	0.9625	0.9928	0.9821	0.9593	0.9908	0.005448	1.25%	0.87%
35		5	0.9765	0.9609	0.9922	0.9806	0.9608	0.9926	0.005636	1.29%	0.98%
74.2		5	0.9684	0.9554	0.9815	0.9701	0.9533	0.9818	0.004689	1.08%	1.8%

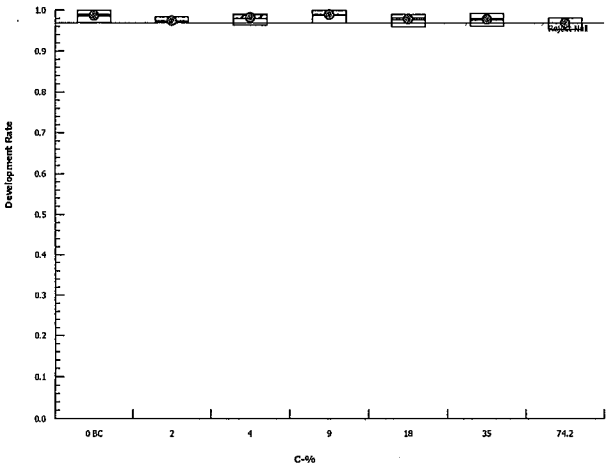
Angular (Corrected) Transformed Summary

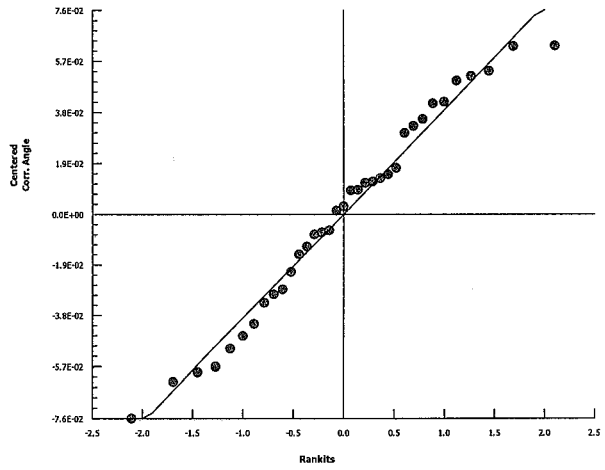
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.459	1.399	1.518	1.472	1.396	1.521	0.0213	3.27%	0.0%
2		5	1.411	1.386	1.436	1.404	1.396	1.446	0.009026	1.43%	3.27%
4		5	1.438	1.378	1.498	1.469	1.379	1.48	0.02165	3.37%	1.4%
9		5	1.469	1.401	1.538	1.471	1.393	1.523	0.02472	3.76%	-0.73%
18		5	1.425	1.374	1.476	1.437	1.368	1.475	0.01826	2.87%	2.3%
35		5	1.422	1.367	1.477	1.431	1.371	1.485	0.01982	3.12%	2.5%
74.2		5	1.394	1.357	1.432	1.397	1.353	1.436	0.01352	2.17%	4.42%

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

Analysis ID:	03-2387-1345	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 9:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	16-3953-4561	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 9:52	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1655526	1000	Yes	Two-Point Interpolation

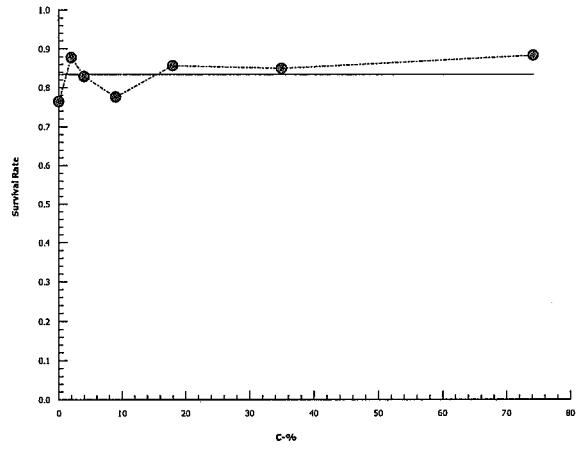
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>74.2	N/A	N/A	<1.348	NA	NA
EC50	>74.2	N/A	N/A	<1.348	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	0.7642	0.7388	0.791	0.008377	0.01873	2.45%	0.0%	512	670
2		5	0.8791	0.806	0.9851	0.04035	0.09023	10.26%	-15.04%	588	670
4		5	0.8299	0.7164	0.9478	0.04424	0.09892	11.92%	-8.59%	555	670
9		5	0.7761	0.7164	0.8657	0.0267	0.0597	7.69%	-1.56%	520	670
18		5	0.8567	0.806	0.9179	0.024	0.05366	6.26%	-12.11%	574	670
35		5	0.8493	0.7612	1	0.04615	0.1032	12.15%	-11.13%	569	670
74.2		5	0.8851	0.7985	1	0.04706	0.1052	11.89%	-15.82%	592	670

Graphics



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

Analysis ID:	07-9634-7858	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 9:52	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	254027	1000	Yes	Two-Point Interpolation

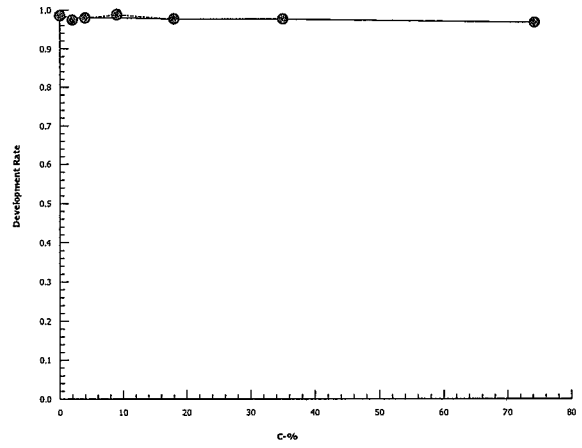
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>74.2	N/A	N/A	<1.348	NA	NA
EC50	>74.2	N/A	N/A	<1.348	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	0.9862	0.9697	1	0.005159	0.01154	1.17%	0.0%	505	512
2		5	0.9743	0.9697	0.9846	0.002624	0.005867	0.6%	1.2%	574	589
4		5	0.9807	0.9636	0.9918	0.006043	0.01351	1.38%	0.55%	545	556
9		5	0.9883	0.9688	1	0.005872	0.01313	1.33%	-0.21%	514	520
18		5	0.9776	0.9593	0.9908	0.005448	0.01218	1.25%	0.87%	561	574
35		5	0.9765	0.9608	0.9926	0.005636	0.0126	1.29%	0.98%	558	571
74.2		5	0.9684	0.9533	0.9818	0.004689	0.01048	1.08%	1.8%	582	601

Graphics



CETIS Test Data Worksheet

Report Date: 06 Mar-21 13:12 (p 1 of 1)
Test Code: 203-5063 ~~06-3498-6822/25D92146~~ @18 #5 3/16/21

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

Start Date: 10 Mar-21 | **Species:** Mytilus galloprovincialis | **Sample Code:** 21-0281
End Date: 12 Mar-21 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Jacobs
Sample Date: 09 Mar-21 | **Material:** Effluent Sample | **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			125	121	RT 4/2/21
			32			116	114	
			33			108	106	
			34			134	130	
			35			106	105	
			36			110	106	
			37			117	110	
			38			101	100	
			39			127	123	
			40			130	128	
			41			102	98	
			42			108	105	
			43			129	126	
			44			108	105	
			45			96	93	
			46			122	120	
			47			100	100	
			48			99	96	
			49			96	95	
			50			102	102	
			51			136	135	
			52			110	108	
			53			100	99	
			54			109	108	
			55			103	101	
			56			103	102	
			57			108	108	
			58			122	121	
			59			122	118	
			60			123	118	
			61			132	128	
			62			142	137	
			63			112	110	
			64			110	107	
			65			102	100	
			66			136	135	
			67			108	105	
			68			109	106	
			69			107	102	
			70			108	105	

CETIS Test Data Worksheet

Report Date: 06 Mar-21 13:12 (p 1 of 1)
Test Code: 2103-5063 ~~06-3498-6822/25D92146~~ Q18 HFS 3/16/21

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

Start Date: 10 Mar-21 | **Species:** Mytilus galloprovincialis | **Sample Code:** 21-0281
End Date: 12 Mar-21 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Jacobs
Sample Date: 09 Mar-21 | **Material:** Effluent Sample | **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	48					
0	BC	2	50					
0	BC	3	35			111	110	BO 3/12/21
0	BC	4	56					
0	BC	5	65					
0	LC	1	37			110	102	
0	LC	2	70			~~102~~	~~110~~ ⓑ	DM 3/13/21
0	LC	3	31					
0	LC	4	43					
0	LC	5	66					
2		1	61			128	125	
2		2	68					
2		3	67					
2		4	64					
2		5	40					
4		1	36			109	104	
4		2	39					
4		3	49					
4		4	58					
4		5	38					
9		1	47			102	102	
9		2	32					
9		3	45					
9		4	57					
9		5	53					
18		1	63			127	123	
18		2	46					
18		3	42					
18		4	54					
18		5	60					
35		1	41			106	103	
35		2	51					
35		3	33					
35		4	55					
35		5	59					
75.8		1	69			109	102	DM 3/13/21
75.8		2	34					
75.8		3	44					
75.8		4	62					
75.8		5	52					

74.2 ~~72.4~~ @ @

QC = BO

@ Q18 BO 3/10/21 ⓑ Q19 AHS OBO DM 4/6/21

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Jacobs

Sample ID: ~~Wycoff~~ Wyckoff

Sample Log No.: 21- 0281

Test No.: 2103-5063

Test Species: *M. galloprovincialis*

Start Date/Time: 3/10/2021 1415

End Date/Time: 3/12/2021 1315

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	30.1	30.2	30.2	14.6	15.0	14.9	8.6	8.0	8.4	8.05	7.99	7.90
Brine Control	29.9	29.9	29.9	15.8	15.0	14.8	8.0	8.0	8.4	8.10	8.09	7.94
2	30.2	30.4	30.4	14.8	14.8	14.8	8.6	8.0	8.3	8.02	8.03	8.01
4	30.3	30.4	30.4	14.9	14.9	14.8	8.7	8.0	8.3	8.02	8.02	8.03
9	30.3	30.4	30.4	14.9	14.8	14.8	8.7	8.0	8.3	7.98	8.02	8.07
18	30.2	30.3	30.3	15.0	15.0	15.0	8.5	8.0	8.3	7.92	8.04	8.13
35	30.1	30.2	30.2	15.6	14.9	14.9	8.3	8.0	8.3	7.89	8.09	8.19
74.2 ~~73.4~~ @	29.9	29.9	29.9	15.8	14.9	14.8	7.9	8.0	8.3	7.86	~~8.16~~	8.29

	0	24	48
WQ Readings:	BO	RT	RT
Dilutions made by:	BO		

Technician Initials:

Environmental Chamber: D.

Comments:

0 hrs: @ Q18 BO 3/10/21 ® Q18 MS 3/16/21

24 hrs:

48 hrs:

QC Check: MS 3/16/21

Final Review: BO 4/6/21

Marine Chronic Bioassay

DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** BO

Sample ID: Wyckoff **Test Date:** 3/10/2021

Test No: 2103- $063 **Test Type:** Mussel Development

Salinity of Effluent 7.4

Salinity of Brine 94.9 **Date of Brine used:** 1/5/2021

Target Salinity 30 **Alkalinity of Brine Control:** 102 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**0.35**	**0.46**

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	0.35	1.7	250
4	10.0	0.35	3.5	250
9	22.5	0.35	7.8	250
18	45.0	0.35	15.7	250
35	87.5	0.35	30.5	250
74.2	185.4	0.35	64.6	250

	DI Volume			
Brine Control	139.7	0.46	64.6	250

Total Brine Volume Required (ml): **188.3**

QC Check: AYS 3/16/21 **Final Review:** BO 4/6/21

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

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs/Wyckoff
Test No.: 2103-5063
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REF / 1B
Date Received: 1/19/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 3/10/2021 1415
End Date/Time: 3/12/2021 1315
Technician Initials: BO

Spawn Information

First Gamete Release Time: 1100

Sex	Number Spawning
Male	4
Female	3

Gamete Selection

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

Egg Fertilization Time: 1145

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	100
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:		
	5	6
	4	4
	3	3
	5	3
	5	5

Mean: 4.3

Mean 4.3 X 50 = 215 embryos/ml

Initial Density: 215 = 0.72 (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	136	140	97.1	99.2
TØ B	143	143	100	
TØ C	133	134	99.3	
TØ D	140 @	141 @	99.3	
TØ E	~~129~~ 122	~~130~~ 122	100	
TØ F	129	130	99.2	
$\bar{x}$ =	133.8			

48-h QC: 139/143 = 97.2%

Comments: @ @18 BO 3/10/21

QC Check: MS 3/16/21

Final Review: BO 4/6/21

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: Jacobs
Sample ID: Wyckoff
Test ID No(s).: 2103-S063

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (21-xxxx):	0281			
Sample Collection Date & Time:	3/9/21 0915			
Sample Receipt Date & Time:	3/10/21 0916			
Number of Containers & Container Type:	1, 1L cubi			
Approx. Total Volume Received (L):	~1			
Check-in Temperature (°C)	1.9			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.6			
pH (units)	7.70			
Conductivity (µS/cm)	11,590			
Salinity (ppt)	6.5			
Alkalinity (mg/L) [2]	327			
Hardness (mg/L) [2, 3]	—			
Total Chlorine (mg/L)	<0.02			
Technician Initials	DM			

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

Subsamples for Additional Chemistry Required:
(NH3) (always required)
Other _____
Tech Initials A DM 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: 119 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 102 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: _____

QC Check: AS 3/16/21
Final Review: BO 4/6/21

Total Ammonia Analysis **Overlying Water**
Freshwater

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0 **Analyst:** KN
Test Start Date: 3/10/2021 **Analysis Date:** 3/19/21

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	8.9	10.9
Wyckoff	21- 0281	3/10/2021	Check In	1.8	~~2.2~~ <0.5 (A) 2.2 (B)
Spike Check (10 mg/L NH_3)		NA	NA		
Batch sample QC	21-0169			0.1	~~0.1~~ <0.5 (A)
Sample Duplicate[a]		NA	NA	0.1	~~0.1~~ <0.5 (A)
Sample Duplicate + Spike[a]		NA	NA	9.2	11.2
Spike Check (10 mg/L NH_3)		NA	NA	8.9	10.7

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	10.9	10	NA	109
Batch sample QC	~~0.1~~ <0.5 (A)	~~0.1~~ <0.5 (A)	11.2	10	~~0~~ C (A)	~~111~~ C (A)

Comments: lot #5: R1: A0268 R2: A0127 Test Tubes: A0195

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.

Method Detection Limit = 0.5 mg/L

QC Check: AS 3/22/21 **Final Review:** BO 4/6/21

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

(A) Q18 KN 3/19/21
(B) Q10 KN 3/22/21

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 3/9/2021
CarrierName: FedEx
AirbillNo: 7731 0833 2105

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

No: 10-030921-093518-0522
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
030921		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	03/09/2021 09:15	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 Allers JACOBS	3-9-21 0940	Kayla EA-SD	3/10/21 0915	

log-in #: 21-0281
Receipt temp: 1.9°C

Appendix D
List of Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log

Q2 - Temperatures out of recommended range; no action taken, test terminated same day

Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L

Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L

Q5 - Test initiated with 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, 50% 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 <u>below</u> 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.

Q17 - Percent minimum significant difference (PMSD) was <u>above</u> 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.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

Q22 - Greater than 10% <u>mortality</u> observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.

Q23 - Test organisms received at a <u>temperature</u> greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Q24 - Test organisms received at <u>salinity</u> greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Appendix E
Reference Toxicant Test Results

CETIS Summary Report

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

Batch ID:	08-4396-8015	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	10 Mar-21 14:15	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	12 Mar-21 13:15	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	01-2417-0500	**Code:**	210310msdv	**Client:**	Internal
Sample Date:	10 Mar-21	**Material:**	Copper chloride	**Project:**	
Receive Date:	10 Mar-21	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-6519-0936	Combined Development Ra	5	10	7.071	12.5%		Dunnett Multiple Comparison Test
13-4134-2422	Development Rate	5	10	7.071	2.73%		Dunnett Multiple Comparison Test
18-8943-0292	Survival Rate	20	40	28.28	13.6%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
10-0885-9755	Combined Development Ra	EC25	6.682	5.741	7.601		Linear Interpolation (ICPIN)
		EC50	9.481	8.639	10.58		
08-4869-7631	Development Rate	EC25	7.308	7.009	7.641		Linear Interpolation (ICPIN)
		EC50	9.694	9.109	10.56		
03-1123-6073	Survival Rate	EC25	>40	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>40	N/A	N/A		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-4869-7631	Development Rate	Control Resp	0.9625	0.9 - NL	Yes	Passes Acceptability Criteria
13-4134-2422	Development Rate	Control Resp	0.9625	0.9 - NL	Yes	Passes Acceptability Criteria
03-1123-6073	Survival Rate	Control Resp	0.8612	0.5 - NL	Yes	Passes Acceptability Criteria
18-8943-0292	Survival Rate	Control Resp	0.8612	0.5 - NL	Yes	Passes Acceptability Criteria
12-6519-0936	Combined Development Ra	PMSD	0.1254	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	0.8299	0.6983	0.9614	0.6567	0.903	0.0474	0.106	12.77%	0.0%
2.5		5	0.9002	0.8273	0.9732	0.8507	0.9784	0.02629	0.05878	6.53%	-8.48%
5		5	0.7791	0.7127	0.8455	0.709	0.8433	0.0239	0.05345	6.86%	6.12%
10		5	0.3925	0.32	0.4651	0.3209	0.4701	0.02613	0.05843	14.88%	52.7%
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	0.9625	0.944	0.9809	0.9462	0.9758	0.006649	0.01487	1.55%	0.0%
2.5		5	0.9717	0.9518	0.9917	0.9481	0.9915	0.007175	0.01604	1.65%	-0.96%
5		5	0.9596	0.9482	0.9711	0.9496	0.9735	0.00412	0.009212	0.96%	0.29%
10		5	0.4518	0.3795	0.5241	0.3805	0.525	0.02604	0.05824	12.89%	53.06%
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	0.8612	0.7375	0.9849	0.694	0.9254	0.04456	0.09965	11.57%	0.0%
2.5		5	0.9269	0.8439	1	0.8731	1	0.02989	0.06683	7.21%	-7.63%
5		5	0.8119	0.7419	0.882	0.7388	0.8881	0.02524	0.05644	6.95%	5.72%
10		5	0.8672	0.8415	0.8929	0.8433	0.8955	0.009261	0.02071	2.39%	-0.69%
20		5	0.8866	0.7988	0.9743	0.7836	0.9776	0.03161	0.07068	7.97%	-2.95%
40		5	0.6955	0.6364	0.7546	0.6418	0.7687	0.02129	0.04761	6.85%	19.24%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.903	0.8955	0.6567	0.8955	0.7985
2.5		0.9784	0.8582	0.8657	0.8507	0.9481
5		0.7612	0.8433	0.709	0.8209	0.7612
10		0.403	0.4701	0.3209	0.3507	0.4179
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	0.9758	0.9756	0.9462	0.9677	0.9469
2.5		0.9784	0.9746	0.9915	0.9661	0.9481
5		0.9623	0.9496	0.9596	0.9735	0.9533
10		0.4576	0.525	0.3805	0.4087	0.487
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	0.9254	0.9179	0.694	0.9254	0.8433
2.5		1	0.8806	0.8731	0.8806	1
5		0.791	0.8881	0.7388	0.8433	0.7985
10		0.8806	0.8955	0.8433	0.8582	0.8582
20		0.8657	0.9104	0.7836	0.8955	0.9776
40		0.709	0.6866	0.6716	0.7687	0.6418

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	121/134	120/134	88/134	120/134	107/134
2.5		136/139	115/134	116/134	114/134	128/135
5		102/134	113/134	95/134	110/134	102/134
10		54/134	63/134	43/134	47/134	56/134
20		0/134	0/134	0/134	0/134	0/134
40		0/134	0/134	0/134	0/134	0/134

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	121/124	120/123	88/93	120/124	107/113
2.5		136/139	115/118	116/117	114/118	128/135
5		102/106	113/119	95/99	110/113	102/107
10		54/118	63/120	43/113	47/115	56/115
20		0/116	0/122	0/105	0/120	0/131
40		0/95	0/92	0/90	0/103	0/86

Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	124/134	123/134	93/134	124/134	113/134
2.5		134/134	118/134	117/134	118/134	134/134
5		106/134	119/134	99/134	113/134	107/134
10		118/134	120/134	113/134	115/134	115/134
20		116/134	122/134	105/134	120/134	131/134
40		95/134	92/134	90/134	103/134	86/134

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

Analysis ID:	12-6519-0936	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 10:43	**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	12.5%	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	-1.718	2.227	0.138	8	0.9936	CDF	Non-Significant Effect
		5	1.191	2.227	0.138	8	0.2619	CDF	Non-Significant Effect
		10*	7.775	2.227	0.138	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.9892086	0.3297362	3	34.46	<0.0001	Significant Effect
Error	0.1531111	0.009569443	16			
Total	1.14232		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.251	11.34	0.3545	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9474	0.866	0.3287	Normal Distribution

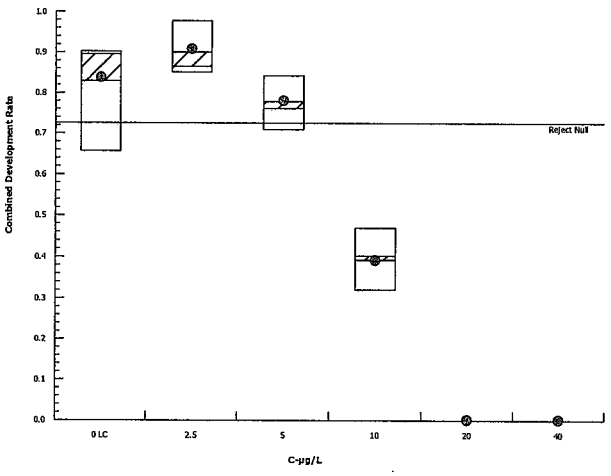
Combined Development Rate Summary

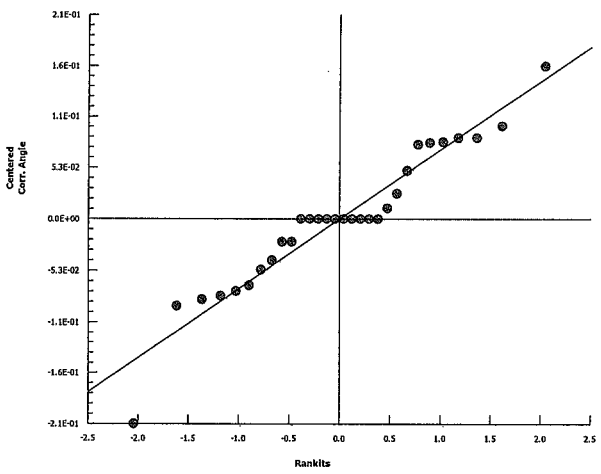
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.8299	0.6983	0.9614	0.8955	0.6567	0.903	0.0474	12.77%	0.0%
2.5		5	0.9002	0.8273	0.9732	0.8657	0.8507	0.9784	0.02629	6.53%	-8.48%
5		5	0.7791	0.7127	0.8455	0.7612	0.709	0.8433	0.0239	6.86%	6.12%
10		5	0.3925	0.32	0.4651	0.403	0.3209	0.4701	0.02613	14.88%	52.7%
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	1.157	0.9915	1.323	1.242	0.9448	1.254	0.05977	11.55%	0.0%
2.5		5	1.264	1.124	1.403	1.196	1.174	1.423	0.05019	8.88%	-9.18%
5		5	1.084	1.003	1.164	1.06	1.001	1.164	0.02904	5.99%	6.37%
10		5	0.6765	0.6019	0.751	0.6878	0.6022	0.7555	0.02684	8.87%	41.56%
20		5	0.04321	0.0432	0.04322	0.04321	0.04321	0.04321	0	0.0%	96.27%
40		5	0.04321	0.0432	0.04322	0.04321	0.04321	0.04321	0	0.0%	96.27%

Graphics





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

Analysis ID:	13-4134-2422	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 10:43	**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	2.73%	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	-1.02	2.227	0.063	8	0.9637	CDF	Non-Significant Effect
		5	0.3223	2.227	0.063	8	0.6223	CDF	Non-Significant Effect
		10*	22.57	2.227	0.063	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.581414	0.5271379	3	260.7	<0.0001	Significant Effect
Error	0.03234775	0.002021734	16			
Total	1.613761		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.754	11.34	0.4311	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9687	0.866	0.7264	Normal Distribution

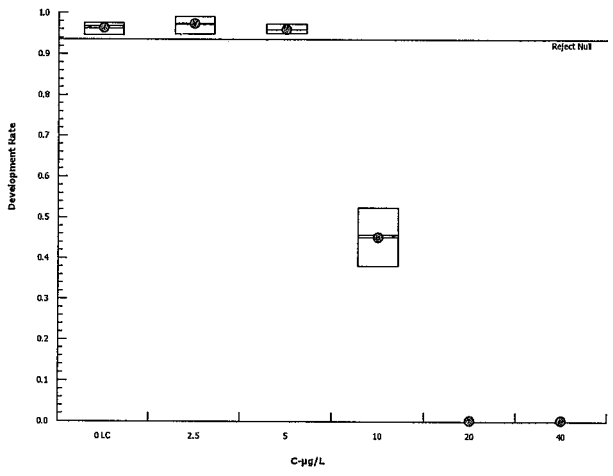
Development Rate Summary

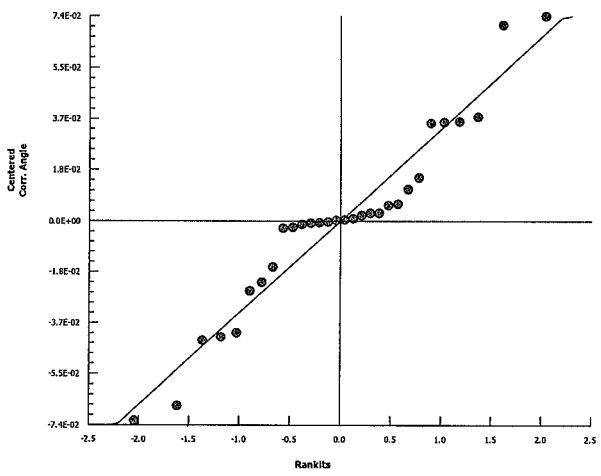
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9625	0.944	0.9809	0.9677	0.9462	0.9758	0.006649	1.55%	0.0%
2.5		5	0.9717	0.9518	0.9917	0.9746	0.9481	0.9915	0.007175	1.65%	-0.96%
5		5	0.9596	0.9482	0.9711	0.9596	0.9496	0.9735	0.00412	0.96%	0.29%
10		5	0.4518	0.3795	0.5241	0.4576	0.3805	0.525	0.02604	12.89%	53.06%
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	1.379	1.33	1.427	1.39	1.337	1.415	0.0174	2.82%	0.0%
2.5		5	1.408	1.345	1.47	1.411	1.341	1.478	0.02253	3.58%	-2.1%
5		5	1.37	1.339	1.4	1.368	1.344	1.407	0.01086	1.77%	0.66%
10		5	0.7368	0.6639	0.8097	0.743	0.6648	0.8104	0.02625	7.97%	46.56%
20		5	0.04598	0.04365	0.04831	0.04566	0.0437	0.04881	0.0008378	4.07%	96.67%
40		5	0.05189	0.04973	0.05404	0.05215	0.04929	0.05394	0.0007774	3.35%	96.24%

Graphics





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

Analysis ID: 18-8943-0292 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 06 Apr-21 10:43 **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	13.6%	20	40	28.28	

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	-1.975	2.362	0.163	8	0.9992	CDF	Non-Significant Effect
		5	1.125	2.362	0.163	8	0.3656	CDF	Non-Significant Effect
		10	0.06959	2.362	0.163	8	0.8119	CDF	Non-Significant Effect
		20	-0.565	2.362	0.163	8	0.9486	CDF	Non-Significant Effect
		40*	3.134	2.362	0.163	8	0.0094	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3513181	0.07026362	5	5.912	0.0011	Significant Effect
Error	0.2852361	0.01188484	24			
Total	0.6365541		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	11.74	15.09	0.0385	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9755	0.9031	0.6970	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	0.8612	0.7375	0.9849	0.9179	0.694	0.9254	0.04456	11.57%	0.0%
2.5		5	0.9269	0.8439	1	0.8806	0.8731	1	0.02989	7.21%	-7.63%
5		5	0.8119	0.7419	0.882	0.7985	0.7388	0.8881	0.02524	6.95%	5.72%
10		5	0.8672	0.8415	0.8929	0.8582	0.8433	0.8955	0.009261	2.39%	-0.69%
20		5	0.8866	0.7988	0.9743	0.8955	0.7836	0.9776	0.03161	7.97%	-2.95%
40		5	0.6955	0.6364	0.7546	0.6866	0.6418	0.7687	0.02129	6.85%	19.24%

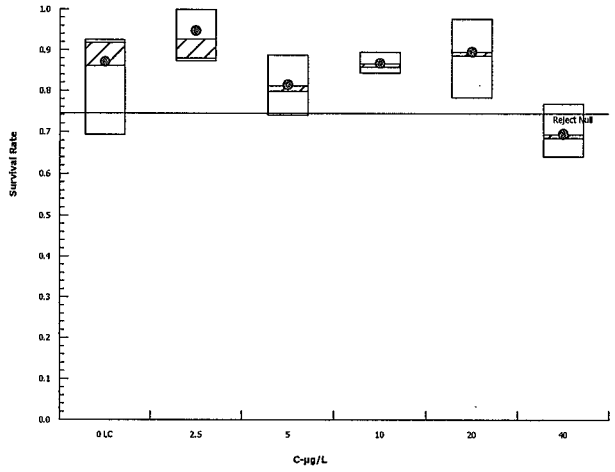
Angular (Corrected) Transformed Summary

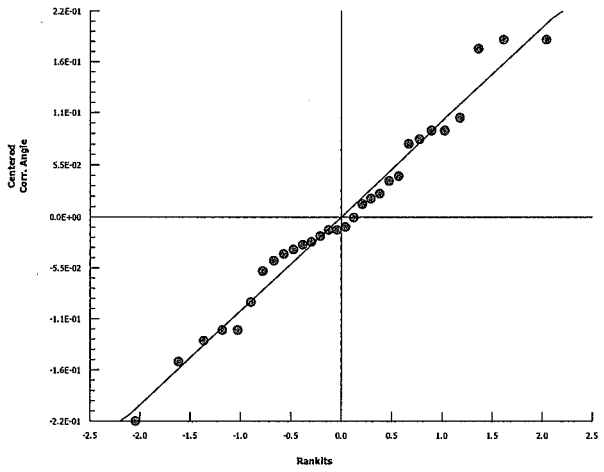
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.203	1.037	1.37	1.28	0.9847	1.294	0.0599	11.13%	0.0%
2.5		5	1.34	1.126	1.553	1.218	1.207	1.528	0.0768	12.82%	-11.32%
5		5	1.126	1.034	1.218	1.105	1.034	1.23	0.03308	6.57%	6.44%
10		5	1.199	1.16	1.237	1.185	1.164	1.242	0.01383	2.58%	0.4%
20		5	1.242	1.092	1.393	1.242	1.087	1.421	0.05419	9.75%	-3.24%
40		5	0.9873	0.9219	1.053	0.9766	0.9292	1.069	0.02353	5.33%	17.96%

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

Analysis ID:	18-8943-0292	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 10:43	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	10-0885-9755	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 10:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1455771	1000	Yes	Two-Point Interpolation

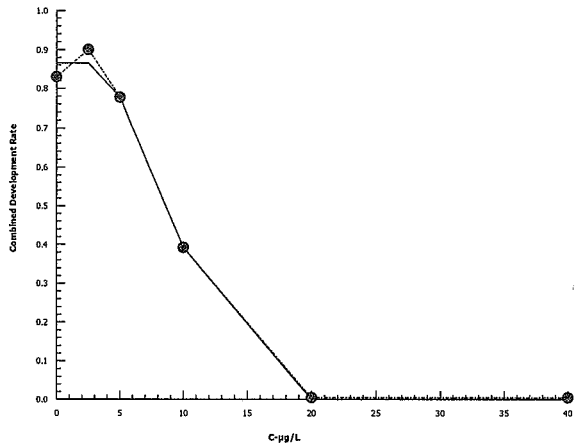
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.682	5.741	7.601
EC50	9.481	8.639	10.58

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	0.8299	0.6567	0.903	0.0474	0.106	12.77%	0.0%	556	670
2.5		5	0.9002	0.8507	0.9784	0.02629	0.05878	6.53%	-8.48%	609	676
5		5	0.7791	0.709	0.8433	0.0239	0.05345	6.86%	6.12%	521	670
10		5	0.3925	0.3209	0.4701	0.02613	0.05843	14.88%	52.7%	263	670
20		5	0	0	0	0	0		100.0%	0	670
40		5	0	0	0	0	0		100.0%	0	670

Graphics



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

Analysis ID:	08-4869-7631	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 10:44	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	864583	1000	Yes	Two-Point Interpolation

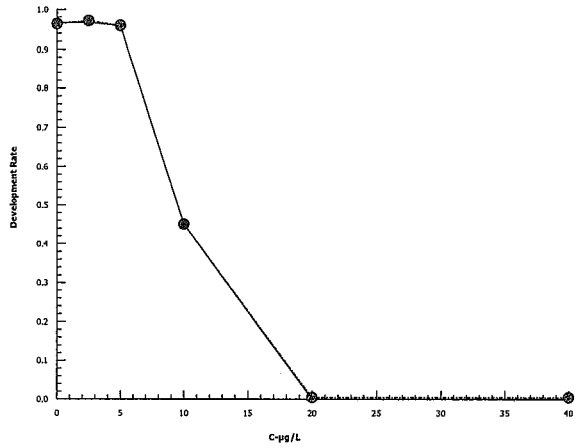
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	7.308	7.009	7.641
EC50	9.694	9.109	10.56

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	0.9625	0.9462	0.9758	0.006649	0.01487	1.55%	0.0%	556	577
2.5		5	0.9717	0.9481	0.9915	0.007175	0.01604	1.65%	-0.96%	609	627
5		5	0.9596	0.9496	0.9735	0.00412	0.009212	0.96%	0.29%	522	544
10		5	0.4518	0.3805	0.525	0.02604	0.05824	12.89%	53.06%	262	581
20		5	0	0	0	0	0		100.0%	0	594
40		5	0	0	0	0	0		100.0%	0	466

Graphics



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

Analysis ID:	03-1123-6073	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Apr-21 10:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	276224	1000	Yes	Two-Point Interpolation

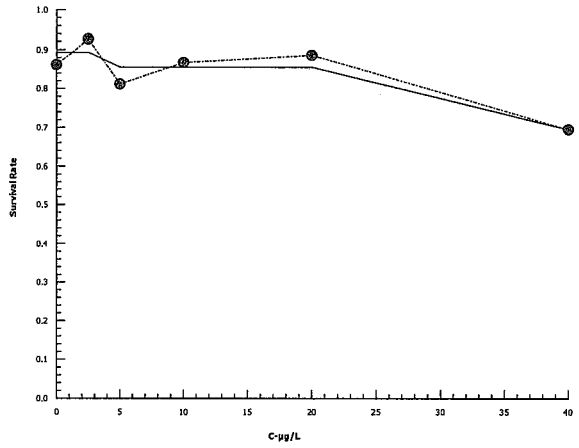
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	>40	N/A	N/A
EC50	>40	N/A	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	0.8612	0.694	0.9254	0.04456	0.09965	11.57%	0.0%	577	670
2.5		5	0.9269	0.8731	1	0.02989	0.06683	7.21%	-7.63%	621	670
5		5	0.8119	0.7388	0.8881	0.02524	0.05644	6.95%	5.72%	544	670
10		5	0.8672	0.8433	0.8955	0.009261	0.02071	2.39%	-0.69%	581	670
20		5	0.8866	0.7836	0.9776	0.03161	0.07068	7.97%	-2.95%	594	670
40		5	0.6955	0.6418	0.7687	0.02129	0.04761	6.85%	19.24%	466	670

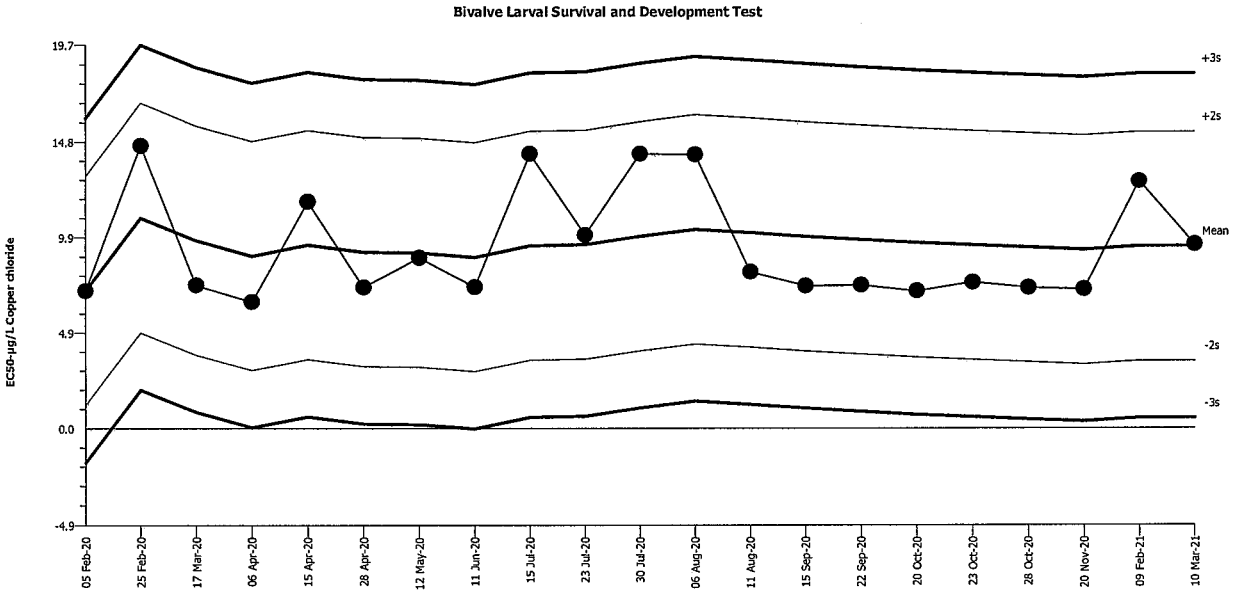
Graphics



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:**	Combined Development Rate	**Source:**	Reference Toxicant-REF



Mean:	9.393	**Count:**	20	**-2s Warning Limit:**	3.483	**-3s Action Limit:**	0.5277
Sigma:	2.955	**CV:**	31.50%	**+2s Warning Limit:**	15.3	**+3s Action Limit:**	18.26

Quality Control Data

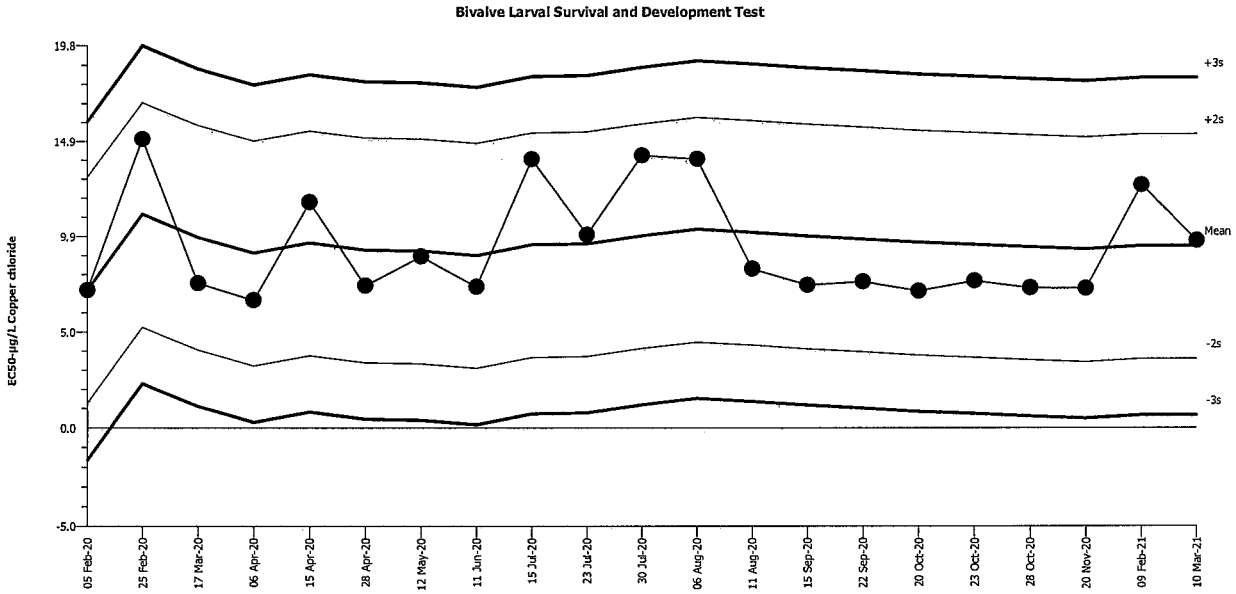
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Feb	5	13:10	7.103	-2.29	-0.775			06-6849-2235	04-8167-3886
2			25	14:15	14.58	5.188	1.756			09-2101-6353	02-3593-4650
3		Mar	17	14:20	7.408	-1.985	-0.6719			14-6169-3689	18-9939-7640
4		Apr	6	17:15	6.537	-2.856	-0.9665			02-0082-4673	13-2096-3831
5			15	13:25	11.68	2.29	0.775			16-4614-0901	11-3098-9850
6			28	13:25	7.292	-2.101	-0.7111			06-8086-6028	13-2749-2065
7		May	12	16:15	8.819	-0.5741	-0.1943			12-3773-8150	00-4087-7530
8		Jun	11	15:45	7.306	-2.087	-0.7064			20-6521-9403	10-1893-3875
9		Jul	15	13:55	14.16	4.769	1.614			17-4780-3294	11-0488-5403
10			23	15:00	9.974	0.5814	0.1967			06-0741-6264	07-6012-8216
11			30	15:35	14.17	4.772	1.615			00-9901-5729	19-4020-2576
12		Aug	6	15:40	14.13	4.732	1.601			01-4440-0014	02-9592-9535
13			11	14:30	8.085	-1.308	-0.4425			21-4043-5119	05-6052-3343
14		Sep	15	0:00	7.365	-2.028	-0.6863			19-9833-0655	18-5101-1090
15			22	14:40	7.405	-1.988	-0.6728			04-0347-9113	09-6026-9613
16		Oct	20	14:25	7.1	-2.293	-0.7758			08-8652-5764	17-2783-6415
17			23	13:45	7.548	-1.845	-0.6242			09-8413-3498	19-3049-9702
18			28	15:50	7.269	-2.124	-0.7188			09-4043-4676	02-6542-7057
19		Nov	20	16:00	7.187	-2.206	-0.7465			13-7696-8009	10-4367-1427
20	2021	Feb	9	15:15	12.74	3.346	1.132			12-5648-6062	18-1503-3303
21		Mar	10	14:15	9.481	0.08772	0.02969			13-7922-5399	10-0885-9755

CETIS QC Plot

Report Date: 06 Apr-21 10:50 (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:	9.424	**Count:**	20	**-2s Warning Limit:**	3.572	**-3s Action Limit:**	0.6456
Sigma:	2.926	**CV:**	31.00%	**+2s Warning Limit:**	15.28	**+3s Action Limit:**	18.2

Quality Control Data

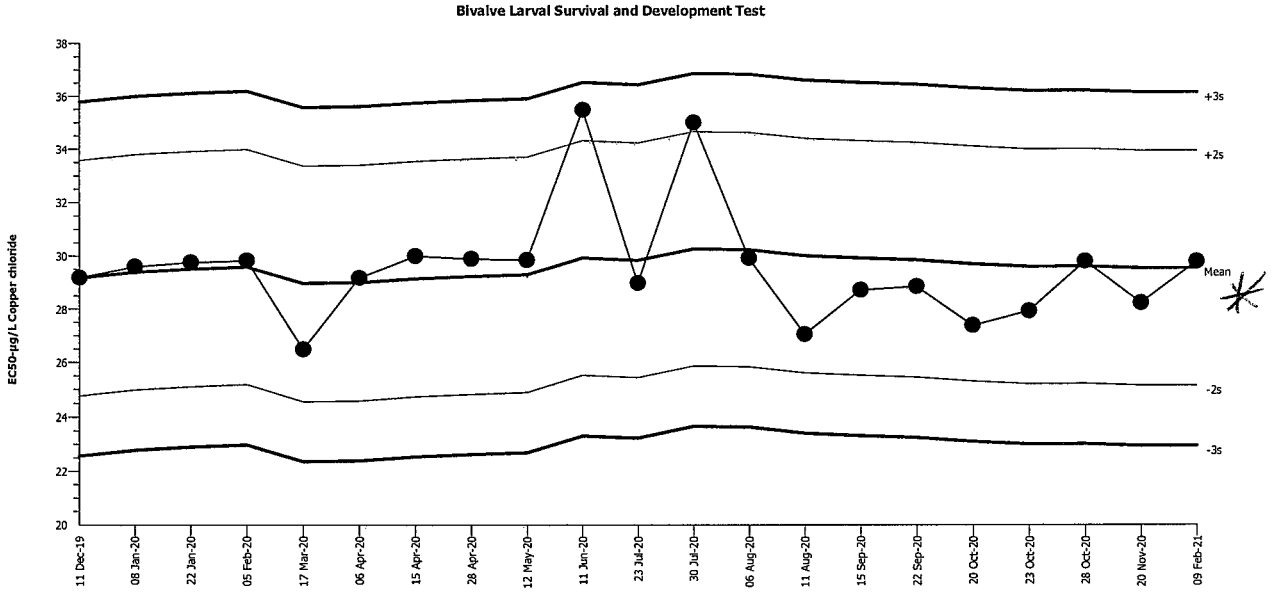
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Feb	5	13:10	7.132	-2.292	-0.7833			06-6849-2235	20-3119-3253
2			25	14:15	15	5.576	1.906			09-2101-6353	13-1093-9538
3		Mar	17	14:20	7.489	-1.935	-0.6614			14-6169-3689	12-6636-5212
4		Apr	6	17:15	6.609	-2.815	-0.9619			02-0082-4673	11-5300-1558
5			15	13:25	11.68	2.259	0.772			16-4614-0901	19-2371-7781
6			28	13:25	7.365	-2.059	-0.7037			06-8086-6028	17-1633-3832
7		May	12	16:15	8.876	-0.5477	-0.1872			12-3773-8150	04-4023-9067
8		Jun	11	15:45	7.306	-2.118	-0.724			20-6521-9403	18-5947-9043
9		Jul	15	13:55	13.94	4.515	1.543			17-4780-3294	14-0926-7215
10			23	15:00	9.999	0.5751	0.1966			06-0741-6264	12-5816-3058
11			30	15:35	14.14	4.713	1.611			00-9901-5729	02-7058-2757
12		Aug	6	15:40	13.95	4.526	1.547			01-4440-0014	13-7910-6508
13			11	14:30	8.237	-1.187	-0.4057			21-4043-5119	01-1240-7098
14		Sep	15	0:00	7.397	-2.027	-0.6927			19-9833-0655	03-7616-5506
15			22	14:40	7.576	-1.848	-0.6315			04-0347-9113	01-0437-7711
16		Oct	20	14:25	7.089	-2.335	-0.7978			08-8652-5764	06-9681-8469
17			23	13:45	7.616	-1.808	-0.6179			09-8413-3498	17-5257-3346
18			28	15:50	7.257	-2.167	-0.7407			09-4043-4676	12-0840-2779
19		Nov	20	16:00	7.23	-2.194	-0.7499			13-7696-8009	11-4264-3018
20	2021	Feb	9	15:15	12.58	3.159	1.08			12-5648-6062	01-5747-2564
21		Mar	10	14:15	9.694	0.2697	0.09216			13-7922-5399	08-4869-7631

CETIS QC Plot

Report Date: 06 Apr-21 10:51 (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:** Survival Rate | **Source:** Reference Toxicant-REF



Mean:	29.56	**Count:**	20	**-2s Warning Limit:**	25.15	**-3s Action Limit:**	22.95
Sigma:	2.204	**CV:**	7.46%	**+2s Warning Limit:**	33.97	**+3s Action Limit:**	36.17

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Dec	11	13:35	29.18	-0.3807	-0.1727			10-8800-1613	02-9848-3585
2	2020	Jan	8	13:40	29.6	0.04106	0.01863			07-8444-5322	01-5655-1706
3			22	13:25	29.76	0.1956	0.08875			02-1152-2212	19-4150-8988
4		Feb	5	13:10	29.83	0.2663	0.1208			06-6849-2235	07-0404-6516
5		Mar	17	14:20	26.48	-3.078	-1.397			14-6169-3689	14-2151-4803
6		Apr	6	17:15	29.18	-0.3832	-0.1739			02-0082-4673	12-2147-8498
7			15	13:25	30	0.44	0.1996			16-4614-0901	00-5465-8677
8			28	13:25	29.9	0.336	0.1524			06-8086-6028	08-1083-2165
9		May	12	16:15	29.85	0.291	0.132			12-3773-8150	18-0143-0286
10		Jun	11	15:45	35.5	5.939	2.695	(+)		20-6521-9403	17-6494-5506
11		Jul	23	15:00	28.98	-0.5802	-0.2633			06-0741-6264	11-2012-0880
12			30	15:35	35.02	5.458	2.477	(+)		00-9901-5729	18-8992-7280
13		Aug	6	15:40	29.92	0.3632	0.1648			01-4440-0014	05-9348-7696
14			11	14:30	27.06	-2.501	-1.135			21-4043-5119	16-7506-8565
15		Sep	15	0:00	28.73	-0.8343	-0.3786			19-9833-0655	01-9900-7404
16			22	14:40	28.86	-0.6964	-0.316			04-0347-9113	03-4439-9784
17		Oct	20	14:25	27.4	-2.164	-0.9818			08-8652-5764	01-6350-7777
18			23	13:45	27.94	-1.618	-0.7343			09-8413-3498	02-1232-2390
19			28	15:50	29.82	0.2595	0.1177			09-4043-4676	15-7574-6891
20		Nov	20	16:00	28.24	-1.316	-0.5972			13-7696-8009	21-0824-4197
21	2021	Feb	9	15:15	29.8	0.2446	0.111			12-5648-6062	08-9593-0094

*EC_{50} for survival endpoint was greater than highest concentration tested. Therefore, it is not included in the control chart.

CETIS Test Data Worksheet

Report Date: 06 Mar-21 13:11 (p 1 of 1)
Test Code: 13-7922-5399/210310msdv

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

Start Date: 10 Mar-21
End Date: 12 Mar-21
Sample Date: 10 Mar-21
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 210310msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			105	0	GH 4/1/21
			2			115	56	
			3			131	(A) 80	
			4			139	136	
			5			86	0	
			6			95	0	
			7			135	128	
			8			122	0	
			9			118	114	
			10			113	107	
			11			113	110	
			12			93	88	
			13			116	0	
			14			123	120	
			15			118	54	
			16			120	63	
			17			92	0	
			18			99	95	
			19			90	0	
			20			106	102	
			21			107	102	
			22			113	43	
			23			124	121	
			24			124	120	
			25			115	47	
			26			118	115	
			27			119	113	
			28			117	116	
			29			103	0	
			30			120	0	

(A) Q18 GH 4/1/21

CETIS Test Data Worksheet

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

Start Date: 10 Mar-21 **Species:** Mytilus galloprovincialis **Sample Code:** 210310msdv
End Date: 12 Mar-21 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 10 Mar-21 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	23			126	121	DM 3/13/21
0	LC	2	14					
0	LC	3	12					
0	LC	4	24					
0	LC	5	10					
2.5		1	4			119	116	
2.5		2	26					
2.5		3	28					
2.5		4	9					
2.5		5	7					
5		1	20			110	105	
5		2	27					
5		3	18					
5		4	11					
5		5	21					
10		1	15			133	49	
10		2	16					
10		3	22					
10		4	25					
10		5	2					
20		1	13			113	6	
20		2	8					
20		3	1					
20		4	30					
20		5	3					
40		1	6			0	0	Cells lysed DM 3/13/21
40		2	17					
40		3	19					
40		4	29					
40		5	5					

QC=BO

Marine Chronic Bioassay
DM-014

Water Quality Measurements

Client: Internal
Sample ID: $CuCl_2$
Test No.: 210310msdv

Test Species: *M. galloprovincialis*
Start Date/Time: 3/10/2021 1415
End Date/Time: 3/12/2021 1315

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	32.2	32.1	14.6	15.0	14.9	8.7	8.0	8.3	8.01	8.02	8.00
2.5	32.2	32.5	32.5	14.3	14.8	14.8	8.7	8.1	8.5	8.02	8.00	7.99
5	32.2	32.5	32.5	14.4	14.8	14.8	8.7	8.1	8.4	8.04	7.99	7.99
10	32.2	32.5	32.5	14.5	14.9	14.8	8.6	8.0	8.5	8.04	7.99	7.99
20	32.2	32.4	32.5	14.4	14.8	14.8	8.6	8.1	8.5	8.05	7.99	7.99
40	32.1	32.4	32.3	14.5	14.8	14.8	8.6	8.0	8.4	8.06	7.99	7.99

	0	24	48
WQ Readings:	BO	RT	RT
Dilutions made by:	BO		

Technician Initials:

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	1.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	11,400

Environmental Chamber: D.

Comments:
0 hrs:
24 hrs:
48 hrs:

QC Check: ACS 3/16/21

Final Review: BO 4/7/21

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / $CuCl_2$
Test No.: 210310msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REF / 1B
Date Received: 1/19/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 3/10/2021 1415
End Date/Time: 3/12/2021 1315
Technician Initials: BO

Spawn Information

First Gamete Release Time: 1100

Sex	Number Spawning
Male	4
Female	3

Gamete Selection

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

Egg Fertilization Time: 1145

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	100
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:		
	5	6
	4	4
	3	3
	5	3
	5	5

Mean: 4.3

Mean 4.3 X 50 = 215 embryos/ml

Initial Density: 215 = 0.72 (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	136	140	97.1	99.2
TØ B	143	143	100	
TØ C	133	134	99.3	
TØ D	140@	141@	99.3	
TØ E	~~129~~ 122	~~130~~ 122	100	
TØ F	129	130	99.2	
$\bar{x}$ =	133.8			

48-h QC: 139/143 = 97.2%

Comments: @ @18 BO 3/10/21

QC Check: AFS 3/16/21

Final Review: BO 4/7/21