ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: February 2020

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

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

Date Submitted: **March 18, 2020**

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: _____
Eric Green, 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 on February 4, 2020 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	**020420**
Enthalpy Log-in Number	20-0143
Collection Date; Time	2/4/2020; 0926h
Receipt Date; Time	2/5/2020; 0950h
Receipt Temperature (°C)	2.8
Dissolved Oxygen (mg/L)	9.4
pH	7.42
Conductivity (µS/cm)	10,710
Salinity (ppt)	6.6
Alkalinity (mg/L $CaCO_3$)	409
Total Chlorine (mg/L)	0.02
Total Ammonia (mg/L as N)	1.3

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	2/5/2020, 1310h to 2/7/2020, 1230h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	Taylor Shellfish (Shelton, WA)
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 94.1 ppt
Test Concentrations (% sample)	73.3[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 observed 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 73.3 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.36 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	73.3	> 73.5	< 1.36	> 73.3
	Survival	73.3	> 73.5	< 1.36	> 73.3

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 (IC_{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)	92.4	99.0
0 (Lab Control)	93.5	98.4
2	94.3	98.6
4	99.7	98.8
9	96.9	98.7
18	96.1	98.0
35	93.7	99.0
73.3[a]	97.7	98.6

[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. The reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 5. Reference Toxicant Test Results

Species	Endpoint	EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve	Normal Development	7.13	8.96 ± 6.03	33.7
	Survival Rate	29.8	29.8 ± 4.88	8.19

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

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:	11-1242-0616	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	05 Feb-20 13:10	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	07 Feb-20 12:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	15-3935-9903	**Code:**	20-0143	**Client:**	Jacobs
Sample Date:	04 Feb-20 09:26	**Material:**	Effluent Sample	**Project:**	
Receive Date:	05 Feb-20 09:50	**Source:**	Jacobs		
Sample Age:	28h (2.8 °C)	**Station:**	Wyckoff GWTP effluent		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-1815-1513	Development Rate	73.3	>73.3	NA	1.72%	<1.364	Dunnett Multiple Comparison Test
04-4290-4136	Survival Rate	73.3	>73.3	NA	12.8%	<1.364	Dunnett Multiple Comparison Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-1815-1513	Development Rate	Control Resp	0.9895	0.9 - NL	Yes	Passes Acceptability Criteria
04-4290-4136	Survival Rate	Control Resp	0.9236	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	0.9895	0.9807	0.9983	0.982	1	0.003161	0.007069	0.71%	0.0%
0	Lab Control	5	0.9838	0.9667	1	0.9608	0.9926	0.006158	0.01377	1.4%	0.58%
2		5	0.9862	0.979	0.9934	0.9792	0.9926	0.002595	0.005804	0.59%	0.34%
4		5	0.9882	0.9742	1	0.9752	1	0.00505	0.01129	1.14%	0.13%
9		5	0.9872	0.9818	0.9926	0.9828	0.9924	0.001934	0.004323	0.44%	0.23%
18		5	0.9797	0.9577	1	0.9576	1	0.007949	0.01777	1.81%	0.99%
35		5	0.9899	0.979	1	0.9758	1	0.003927	0.008781	0.89%	-0.04%
73.3		5	0.9858	0.9732	0.9984	0.9741	1	0.004546	0.01016	1.03%	0.37%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9236	0.8306	1	0.8049	1	0.03348	0.07487	8.11%	0.0%
0	Lab Control	5	0.935	0.8284	1	0.8293	1	0.03839	0.08585	9.18%	-1.23%
2		5	0.9431	0.8275	1	0.7805	1	0.04161	0.09305	9.87%	-2.11%
4		5	0.9967	0.9877	1	0.9837	1	0.003252	0.007272	0.73%	-7.92%
9		5	0.9691	0.9136	1	0.9024	1	0.01998	0.04468	4.61%	-4.93%
18		5	0.961	0.9118	1	0.9106	1	0.0177	0.03958	4.12%	-4.05%
35		5	0.9366	0.844	1	0.8211	1	0.03336	0.0746	7.97%	-1.41%
73.3		5	0.9772	0.9385	1	0.9431	1	0.01394	0.03117	3.19%	-5.81%

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	0.982	0.9841	0.9899	0.9915	1
0	Lab Control	0.9926	0.9608	0.9918	0.981	0.9926
2		0.9792	0.9926	0.9917	0.9829	0.9845
4		0.9752	0.9846	1	1	0.9811
9		0.9828	0.9924	0.9837	0.986	0.991
18		0.9576	0.9911	0.9847	1	0.9652
35		0.9911	1	0.9924	0.9901	0.9758
73.3		0.9793	1	0.9914	0.9843	0.9741

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9024	1	0.8049	0.9593	0.9512
0	Lab Control	1	0.8293	0.9919	0.8537	1
2		0.7805	1	0.9837	0.9512	1
4		0.9837	1	1	1	1
9		0.9431	1	1	1	0.9024
18		0.9593	0.9106	1	1	0.935
35		0.9106	0.9512	1	0.8211	1
73.3		1	1	0.9431	1	0.9431

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	109/111	124/126	98/99	117/118	117/117
0	Lab Control	135/136	98/102	121/122	103/105	135/136
2		94/96	134/135	120/121	115/117	127/129
4		118/121	128/130	140/140	140/140	156/159
9		114/116	131/132	121/123	141/143	110/111
18		113/118	111/112	129/131	125/125	111/115
35		111/112	117/117	131/132	100/101	121/124
73.3		142/145	123/123	115/116	125/127	113/116

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	111/123	123/123	99/123	118/123	117/123
0	Lab Control	123/123	102/123	122/123	105/123	123/123
2		96/123	123/123	121/123	117/123	123/123
4		121/123	123/123	123/123	123/123	123/123
9		116/123	123/123	123/123	123/123	111/123
18		118/123	112/123	123/123	123/123	115/123
35		112/123	117/123	123/123	101/123	123/123
73.3		123/123	123/123	116/123	123/123	116/123

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

Analysis ID: 07-1815-1513 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 25 Feb-20 11:04 **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	1.72%	73.3	>73.3	NA	1.364

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	0.5629	2.407	0.067	8	0.6531	CDF	Non-Significant Effect
		4	0.03426	2.407	0.067	8	0.8477	CDF	Non-Significant Effect
		9	0.435	2.407	0.067	8	0.7075	CDF	Non-Significant Effect
		18	1.142	2.407	0.067	8	0.3883	CDF	Non-Significant Effect
		35	-0.117	2.407	0.067	8	0.8864	CDF	Non-Significant Effect
		73.3	0.5034	2.407	0.067	8	0.6789	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.004377651	0.0007296085	6	0.3806	0.8851	Non-Significant Effect
Error	0.05367923	0.001917115	28			
Total	0.05805688		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.978	16.81	0.3229	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.984	0.9146	0.8798	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	0.9895	0.9807	0.9983	0.9899	0.982	1	0.003162	0.71%	0.0%
2		5	0.9862	0.979	0.9934	0.9845	0.9792	0.9926	0.002596	0.59%	0.34%
4		5	0.9882	0.9742	1	0.9846	0.9752	1	0.00505	1.14%	0.13%
9		5	0.9872	0.9818	0.9926	0.986	0.9828	0.9924	0.001934	0.44%	0.23%
18		5	0.9797	0.9577	1	0.9847	0.9576	1	0.007949	1.81%	0.99%
35		5	0.9899	0.979	1	0.9911	0.9758	1	0.003927	0.89%	-0.04%
73.3		5	0.9858	0.9732	0.9984	0.9843	0.9741	1	0.004546	1.03%	0.37%

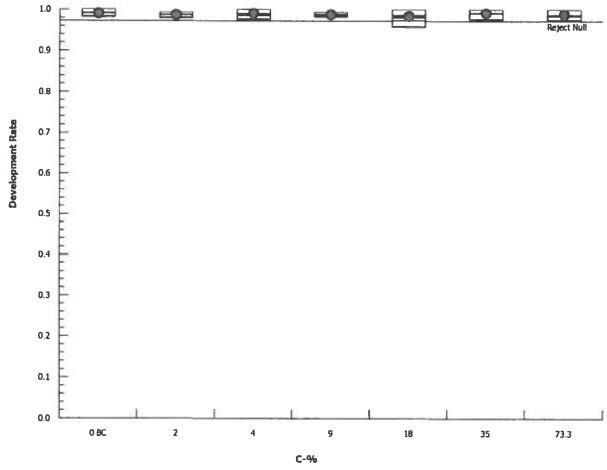
Angular (Corrected) Transformed Summary

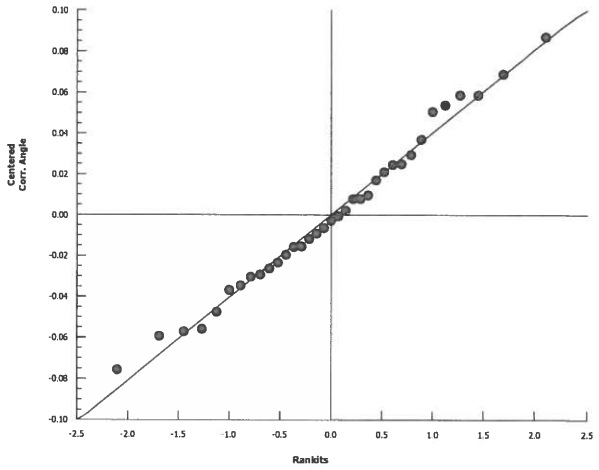
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.471	1.428	1.514	1.47	1.436	1.525	0.01556	2.37%	0.0%
2		5	1.455	1.423	1.487	1.446	1.426	1.485	0.01151	1.77%	1.06%
4		5	1.47	1.402	1.538	1.446	1.413	1.529	0.02456	3.74%	0.06%
9		5	1.459	1.434	1.483	1.452	1.439	1.484	0.008907	1.37%	0.82%
18		5	1.439	1.356	1.522	1.447	1.363	1.526	0.02987	4.64%	2.15%
35		5	1.474	1.425	1.523	1.476	1.415	1.525	0.01759	2.67%	-0.22%
73.3		5	1.457	1.4	1.514	1.445	1.409	1.526	0.02062	3.17%	0.95%

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

Analysis ID:	07-1815-1513	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Feb-20 11:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 04-4290-4136 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 25 Feb-20 11:04 **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	12.8%	73.3	>73.3	NA	1.364

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-0.7365	2.407	0.208	8	0.9740	CDF	Non-Significant Effect
		4	-2.174	2.407	0.208	8	0.9998	CDF	Non-Significant Effect
		9	-1.28	2.407	0.208	8	0.9948	CDF	Non-Significant Effect
		18	-0.9071	2.407	0.208	8	0.9838	CDF	Non-Significant Effect
		35	-0.4469	2.407	0.208	8	0.9454	CDF	Non-Significant Effect
		73.3	-1.458	2.407	0.208	8	0.9971	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.113521	0.01892017	6	1.017	0.4344	Non-Significant Effect
Error	0.5207969	0.01859989	28			
Total	0.6343179		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.921	16.81	0.2439	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9664	0.9146	0.3519	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	0.9236	0.8306	1	0.9512	0.8049	1	0.03348	8.11%	0.0%
2		5	0.9431	0.8275	1	0.9837	0.7805	1	0.04161	9.87%	-2.11%
4		5	0.9967	0.9877	1	1	0.9837	1	0.003252	0.73%	-7.92%
9		5	0.9691	0.9136	1	1	0.9024	1	0.01998	4.61%	-4.93%
18		5	0.961	0.9118	1	0.9593	0.9106	1	0.0177	4.12%	-4.05%
35		5	0.9366	0.844	1	0.9512	0.8211	1	0.03336	7.97%	-1.41%
73.3		5	0.9772	0.9385	1	1	0.9431	1	0.01394	3.19%	-5.81%

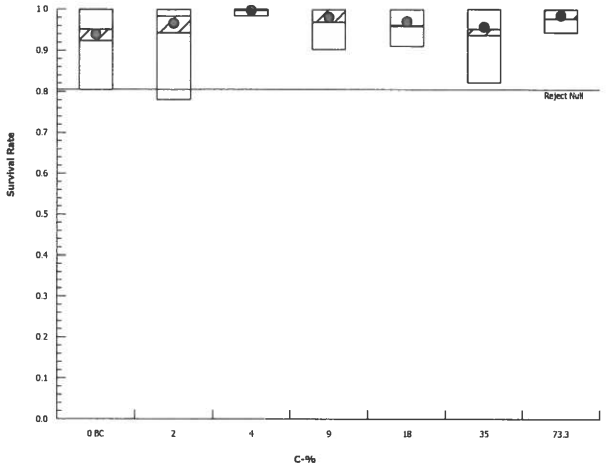
Angular (Corrected) Transformed Summary

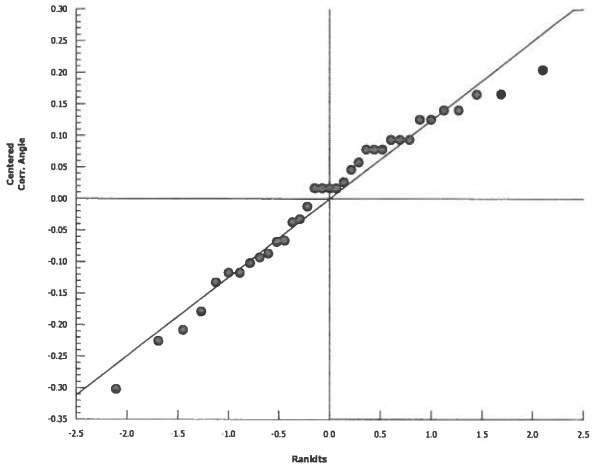
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.322	1.133	1.51	1.348	1.113	1.526	0.06804	11.51%	0.0%
2		5	1.385	1.157	1.614	1.443	1.083	1.526	0.08228	13.28%	-4.81%
4		5	1.509	1.463	1.555	1.526	1.443	1.526	0.01655	2.45%	-14.19%
9		5	1.432	1.269	1.595	1.526	1.253	1.526	0.05863	9.16%	-8.36%
18		5	1.4	1.25	1.549	1.368	1.267	1.526	0.0538	8.59%	-5.92%
35		5	1.36	1.15	1.57	1.348	1.134	1.526	0.07573	12.45%	-2.92%
73.3		5	1.447	1.314	1.581	1.526	1.33	1.526	0.04796	7.41%	-9.52%

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

Analysis ID:	04-4290-4136	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Feb-20 11:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Test Data Worksheet

Report Date: 02 Feb-20 12:41 (p 1 of 1)
Test Code: 2002 - 5016 11-4010-6265/43F4A419

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

Start Date: 05 Feb-20 | Species: Mytilus galloprovincialis | Sample Code: 20- 0143
End Date: 07 Feb-20 | Protocol: EPA/600/R-95/136 (1995) | Sample Source: Jacobs
Sample Date: 04 Feb-20 | Material: Effluent Sample | Sample Station: Wyckoff GWTP Effluent

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			256			123	121	RT 2/19/20
			257			121	118	
			258			127	125	
			259			112	111	
			260			143	141	
			261			145	142	
			262			111	109	
			263			132	131	
			264			123	123	
			265			116	113	
			266			101	100	
			267			129	127	
			268			135	134	
			269			99	98	
			270			116	115	
			271			117	117	
			272			102	98	
			273			112	111	
			274			105	103	
			275			121	120	
			276			117	117	
			277			125	125	
			278			124	121	
			279			130	128	
			280			136	135	
			281			116	114	
			282			115	111	
			283			96	94	
			284			118	113	
			285			126	124	
			286			131	129	RT 2/21/20
			287			111	110	
			288			140	140	ACS 2/24/2020
			289			159	156	
			290			132	131	
			291			136	135	
			292			117	115	
			293			118	117	
			294			140	140	
			295			122	121	

CETIS Test Data Worksheet

Report Date: 02 Feb-20 12:40 (p 1 of 1)
Test Code: 2002-S016 11-4010-6265/43F4A419

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

Start Date:	05 Feb-20	**Species:**	Mytilus galloprovincialis	**Sample Code:**	20-0143
End Date:	07 Feb-20	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs
Sample Date:	04 Feb-20	**Material:**	Effluent Sample	**Sample Station:**	Wyckoff GWTP Effluent

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	262					
0	BC	2	285					
0	BC	3	269			106	106	NM 2/7/20
0	BC	4	293					
0	BC	5	271					
0	LC	1	291					
0	LC	2	272					
0	LC	3	295			123	122	NM
0	LC	4	274					
0	LC	5	280					
2		1	283					
2		2	268					
2		3	275			117	117	NM
2		4	292					
2		5	267					
4		1	257					
4		2	279					
4		3	294			140	139	NM
4		4	288					
4		5	289					
9		1	281					
9		2	263					
9		3	256			118	116	NM
9		4	260					
9		5	287					
18		1	284					
18		2	273					
18		3	286			130	128	NM
18		4	277					
18		5	282					
35		1	259					
35		2	276					
35		3	290			137	135	NM
35		4	266					
35		5	278					
~~74.1~~ 73.3		1	261					
~~74.1~~		2	264					
~~74.1~~		3	270			117	117	NM
~~74.1~~		4	258					
~~74.1~~ (A)		5	265					

(A) ~~ACS~~ QC= EG

(A) GLY ACS 2/2/2020

000-089-187-4 CETIS™ v1.8.7.20 Analyst: ACS QA: EG 2/27/20

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Jacobs

Sample ID: Wyckoff GWTP Effluent

Sample Log No.: 20-0143

Test No.: 2002-S010

Test Species: *M. galloprovincialis*

Start Date/Time: 2/5/2020 1310

End Date/Time: 2/7/2020 1230

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	30.3	30.0	29.9	14.3	14.5	14.7	8.7	8.5	8.4	8.08	8.04	7.97
Brine Control	30.5	30.2	30.4	14.9	14.2	14.5	8.3	8.6	8.4	8.24	8.09	8.01
2	30.3	30.0	30.2	14.8	14.7	14.8	8.4	8.5	8.3	8.07	8.06	8.00
4	30.3	30.0	30.1	14.5	14.5	14.6	8.5	8.6	8.4	8.04	8.06	8.03
9	30.3	30.1	30.1	14.3	14.4	14.6	8.4	8.6	8.4	7.94	8.06	8.07
18	30.3	30.1	30.2	14.4	14.7	14.9	8.4	8.5	8.3	7.84	8.06	8.12
35	30.3	30.0	30.2	14.1	14.6	14.8	8.3	8.5	8.3	7.69	8.07	8.19
73.3	30.5	30.3	30.4	14.0	14.6	14.7	8.4	8.5	8.4	7.60	8.09	8.27

	0	24	48
WQ Readings:	EG	DM	RH 0800 KL
Dilutions made by:	EG	—	—

Technician Initials:

Environmental Chamber: D

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: EG 2/27/20

Final Review: RH 3/3/20 // AC 3/6/20

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** EG

Sample ID: Wyckoff GWTP Effluent **Test Date:** 2/5/2020

Test No: 2002-S016 **Test Type:** Mussel Development

Salinity of Effluent 6.6

Salinity of Brine 94.1 **Date of Brine used:** 12/20/2019

Target Salinity 30 **Alkalinity of Brine Control:** 83 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**0.37**	**0.47**

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	0.37	1.8	250
4	10.0	0.37	3.7	250
9	22.5	0.37	8.2	250
18	45.0	0.37	16.4	250
35	87.5	0.37	31.9	250
73.3	183.1	0.37	66.9	250

	DI Volume			
Brine Control	142.9	0.47	66.9	250

Total Brine Volume Required (ml): **195.8**

QC Check: EG 2/27/20 **Final Review:** RH 3/3/20 / AK 3/6/20

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

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs/Wyckoff GWTP Effluent
Test No.: 2002-SO16
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: Taylor / 19B
Date Received: 11/20/19
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 2/5/2020 1310
End Date/Time: 2/7/2020 1230
Technician Initials: EG

Spawn Information

First Gamete Release Time: 0935

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	good density + motility
Female 1	1	OK shape, good density, pink color
Female 2	2	great shape, OK density, pale color
Female 3		

Egg Fertilization Time: 1025

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	—
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: 13, 9, 10, 8, 12 / 4, 12, 11, 10, 8

Mean: 9.7

Mean 9.7 X 50 = 485 embryos/ml

Initial Density: 485 / Desired Final Density: 300 (to inoculate with 0.5 ml) = 1.62 (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	119	119	100	100
TØ B	144	144	100	
TØ C	119	119	100	
TØ D	127	127	100	
TØ E	121	121	100	
TØ F	107	107	100	
$\bar{X}$ =	123			

48-h QC: 116/118 = 98.3%

Comments:

QC Check: EG 2/27/20

Final Review: RH 3/3/20 // AC 3/6/20

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: Jacobs
Sample ID: Wyckoff GWTP effluent (020420)
Test ID No(s).: 2002-5016

Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (20-xxxx):	0143			
Sample Collection Date & Time:	2/4/20 0926			
Sample Receipt Date & Time:	2/5/20 0950			
Number of Containers & Container Type:	1 1L cubi			
Approx. Total Volume Received (L):	~1L			
Check-in Temperature (°C)	2.8			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.4			
pH (units)	7.42			
Conductivity (µS/cm)	10,710			
Salinity (ppt)	6.6			
Alkalinity (mg/L) [2]	409			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	RT/ER/HS			

Test Performed: Mussel Development **Control/Dilution Water:** 8:2 / Lab SW / Lab ART **Other:** _____
Alkalinity: 97 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Control Alkalinity: 83 Hardness or Salinity: 30ppt

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

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

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

Additional Comments: _____

Sample Description:
A: no color, clear, no oder, no debris

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

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

Salinity Adjustment? (Y) N
Test: 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.			

Subsamples for Additional Chemistry Required? (Y) N
(NH3) Other _____
Tech Initials A HS B___ C___

QC Check: EG 2/26/20
Final Review: RH 3/3/20 // AK 3/6/20

Total Ammonia Analysis
Freshwater

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 2/5/2020

Analyst: DM
Analysis Date: 2/13/20

Sample ID	Nautilus ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	N x 1.22 Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	7.4	9.0
Wyckoff	20-0193	2/5/2020	Check In	1.3	1.6
Spike Check (10 mg/L NH_3)		NA	NA	7.4	9.0
Sample Duplicate[a]		NA	NA	1.2	1.5
Sample Duplicate + Spike[a]		NA	NA	8.7	10.6
Spike Check (10 mg/L NH_3)		NA	NA	7.4	9.0

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

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

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	9.0	10	NA	90
Wyckoff	1.6	1.5	10.6	10	6.5	90

Comments:

Notes: [a]Unless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

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

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

Method Detection Limit = 0.5 mg/L

QC Check: BO 2/28/20

Final Review: EG 2/28/20

Appendix C
Chain-of-Custody Form

Enthalpy Analyyical (REGION COPY)
DateShipped: 2/4/2020
CarrierName: FedEx
AirbillNo: 7776 8157 0320

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2019/WA
Project Code: WEH-029K
Cooler #: 1 of 1

No: 10-020420-095301-0439
2020T10P000DD210W2LA00
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
020420		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	02/04/2020 09:26	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	2-4-2020 1000	Elmyla Russ Enthalpy	2/5/20 0950	GOOD / 2.8°C

ID#: 20-014

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 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.

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.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

Q22 - Greater than 10% mortality 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 temperature 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 salinity 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:	10-5409-8412	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	05 Feb-20 13:10	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	07 Feb-20 12:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	04-8842-7133	**Code:**	200205msdv	**Client:**	Internal
Sample Date:	05 Feb-20	**Material:**	Copper chloride	**Project:**	
Receive Date:	05 Feb-20	**Source:**	Reference Toxicant		
Sample Age:	13h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-0959-6796	Combined Development Ra	2.5	5	3.536	11.2%		Dunnett Multiple Comparison Test
06-6130-0163	Development Rate	2.5	5	3.536	2.67%		Dunnett Multiple Comparison Test
12-4039-9276	Survival Rate	20	40	28.28	18.1%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
04-8167-3886	Combined Development Ra	EC25	5.654	4.978	6.09		Linear Interpolation (ICPIN)
		EC50	7.103	6.652	7.393		
20-3119-3253	Development Rate	EC25	5.698	5.291	6.115		Linear Interpolation (ICPIN)
		EC50	7.132	6.86	7.41		
07-0404-6516	Survival Rate	EC25	24.74	22.76	25.13		Linear Interpolation (ICPIN)
		EC50	29.83	28.51	30.09		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-6130-0163	Development Rate	Control Resp	0.9893	0.9 - NL	Yes	Passes Acceptability Criteria
20-3119-3253	Development Rate	Control Resp	0.9893	0.9 - NL	Yes	Passes Acceptability Criteria
07-0404-6516	Survival Rate	Control Resp	0.9024	0.5 - NL	Yes	Passes Acceptability Criteria
12-4039-9276	Survival Rate	Control Resp	0.9024	0.5 - NL	Yes	Passes Acceptability Criteria
18-0959-6796	Combined Development Ra	PMSD	0.112	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	0.8927	0.8197	0.9656	0.7886	0.9268	0.02627	0.05874	6.58%	0.0%
2.5		5	0.8944	0.8014	0.9873	0.8049	0.9922	0.03348	0.07486	8.37%	-0.19%
5		5	0.7715	0.6788	0.8641	0.6423	0.8293	0.03338	0.07464	9.68%	13.58%
10		5	0	0	0	0	0	0	0		100.0%
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	0.9893	0.9804	0.9982	0.9828	1	0.003192	0.007137	0.72%	0.0%
2.5		5	0.9858	0.9683	1	0.963	1	0.006318	0.01413	1.43%	0.35%
5		5	0.8669	0.7847	0.9491	0.776	0.9519	0.02962	0.06623	7.64%	12.37%
10		5	0	0	0	0	0	0	0		100.0%
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	0.9024	0.8269	0.978	0.7967	0.9431	0.02721	0.06084	6.74%	0.0%
2.5		5	0.9073	0.8135	1	0.8049	1	0.03378	0.07553	8.32%	-0.54%
5		5	0.8943	0.7559	1	0.7236	1	0.04985	0.1115	12.46%	0.9%
10		5	0.8829	0.7738	0.992	0.7642	0.9919	0.03929	0.08787	9.95%	2.16%
20		5	0.8959	0.8087	0.9832	0.8293	1	0.03142	0.07027	7.84%	0.72%
40		5	0	0	0	0	0	0	0		100.0%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9106	0.9268	0.9106	0.9268	0.7886
2.5		0.9431	0.8455	0.8862	0.8049	0.9922
5		0.8049	0.8049	0.8293	0.776	0.6423
10		0	0	0	0	0
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	0.9912	0.9828	1	0.9828	0.9898
2.5		0.9831	0.963	0.9909	1	0.9922
5		0.8319	0.9519	0.887	0.776	0.8876
10		0	0	0	0	0
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	0.9187	0.9431	0.9106	0.9431	0.7967
2.5		0.9593	0.878	0.8943	0.8049	1
5		0.9675	0.8455	0.935	1	0.7236
10		0.9919	0.7642	0.9187	0.9106	0.8293
20		0.935	1	0.8618	0.8537	0.8293
40		0	0	0	0	0

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	112/123	114/123	112/123	114/123	97/123
2.5		116/123	104/123	109/123	99/123	128/129
5		99/123	99/123	102/123	97/125	79/123
10		0/123	0/123	0/123	0/123	0/123
20		0/123	0/129	0/123	0/123	0/123
40		0/123	0/123	0/123	0/123	0/123

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	112/113	114/116	112/112	114/116	97/98
2.5		116/118	104/108	109/110	99/99	128/129
5		99/119	99/104	102/115	97/125	79/89
10		0/122	0/94	0/113	0/112	0/102
20		0/115	0/129	0/106	0/105	0/102
40		0/1	0/1	0/1	0/1	0/1

Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	113/123	116/123	112/123	116/123	98/123
2.5		118/123	108/123	110/123	99/123	123/123
5		119/123	104/123	115/123	123/123	89/123
10		122/123	94/123	113/123	112/123	102/123
20		115/123	123/123	106/123	105/123	102/123
40		0/123	0/123	0/123	0/123	0/123

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

Analysis ID: 18-0959-6796 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-20 15:04 **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	11.2%	2.5	5	3.536	

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	-0.2838	2.108	0.146	8	0.7688	CDF	Non-Significant Effect
		5*	2.431	2.108	0.146	8	0.0284	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.106906	0.05345298	2	4.452	0.0358	Significant Effect
Error	0.1440656	0.01200546	12			
Total	0.2509716		14			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.501	9.21	0.4722	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8999	0.8328	0.0948	Normal Distribution

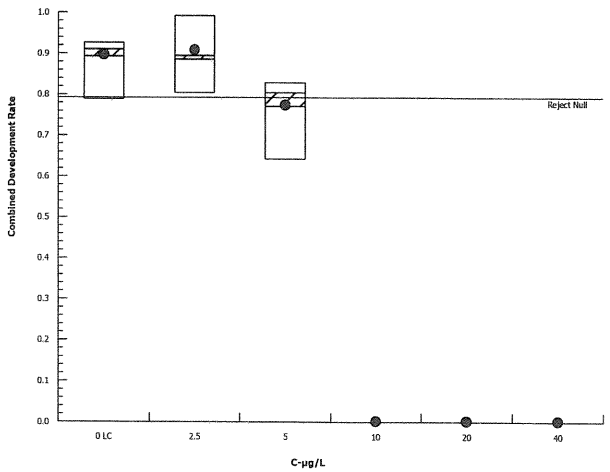
Combined Development Rate Summary

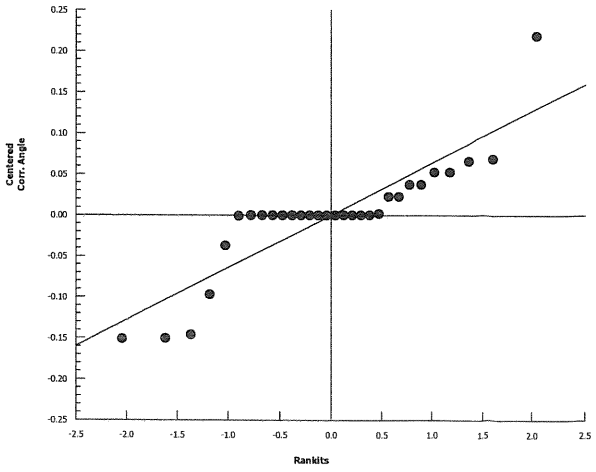
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.8927	0.8197	0.9656	0.9106	0.7886	0.9268	0.02627	6.58%	0.0%
2.5		5	0.8944	0.8014	0.9873	0.8862	0.8049	0.9922	0.03348	8.37%	-0.19%
5		5	0.7715	0.6788	0.8641	0.8049	0.6423	0.8293	0.03338	9.68%	13.58%
10		5	0	0	0	0	0	0	0		100.0%
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	1.244	1.138	1.351	1.267	1.093	1.297	0.03837	6.9%	0.0%
2.5		5	1.264	1.082	1.446	1.227	1.113	1.483	0.06546	11.58%	-1.58%
5		5	1.076	0.9702	1.181	1.113	0.9297	1.145	0.03803	7.91%	13.54%
10		5	0.0451	0.04509	0.04511	0.0451	0.0451	0.0451	0	0.0%	96.38%
20		5	0.04489	0.0443	0.04548	0.0451	0.04404	0.0451	0.000212	1.06%	96.39%
40		5	0.0451	0.04509	0.04511	0.0451	0.0451	0.0451	0	0.0%	96.38%

Graphics





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

Analysis ID: 06-6130-0163 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-20 15:04 **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	2.67%	2.5	5	3.536	

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	0.2372	2.108	0.093	8	0.5698	CDF	Non-Significant Effect
		5*	5.987	2.108	0.093	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2218482	0.1109241	2	22.99	<0.0001	Significant Effect
Error	0.05790585	0.004825487	12			
Total	0.279754		14			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.061	9.21	0.1312	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9697	0.8328	0.8539	Normal Distribution

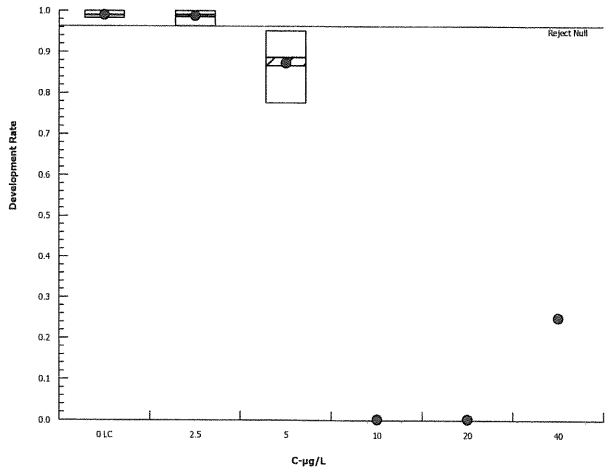
Development Rate Summary

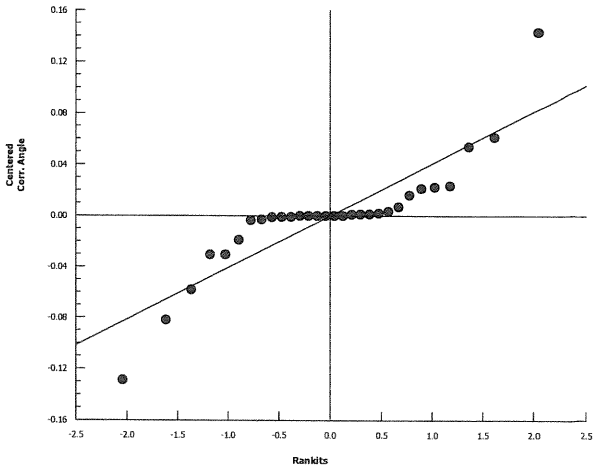
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9893	0.9804	0.9982	0.9898	0.9828	1	0.003192	0.72%	0.0%
2.5		5	0.9858	0.9683	1	0.9909	0.963	1	0.006318	1.43%	0.35%
5		5	0.8669	0.7847	0.9491	0.887	0.776	0.9519	0.02962	7.64%	12.37%
10		5	0	0	0	0	0	0	0		100.0%
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	1.47	1.427	1.513	1.47	1.439	1.524	0.01552	2.36%	0.0%
2.5		5	1.459	1.392	1.526	1.475	1.377	1.521	0.02415	3.7%	0.71%
5		5	1.207	1.08	1.333	1.228	1.078	1.35	0.04551	8.44%	17.9%
10		5	0.04814	0.04511	0.05118	0.04726	0.04528	0.05159	0.001095	5.08%	96.72%
20		5	0.04752	0.04476	0.05028	0.04858	0.04404	0.04953	0.000994	4.68%	96.77%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	64.37%

Graphics





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

Analysis ID: 12-4039-9276 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 11 Mar-20 15:05 **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	18.1%	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	-0.3009	2.305	0.228	8	0.8829	CDF	Non-Significant Effect
		5	-0.2032	2.305	0.228	8	0.8593	CDF	Non-Significant Effect
		10	0.1492	2.305	0.228	8	0.7481	CDF	Non-Significant Effect
		20	-0.08069	2.305	0.228	8	0.8252	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00600022	0.001500055	4	0.06134	0.9925	Non-Significant Effect
Error	0.489131	0.02445655	20			
Total	0.4951312		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.868	13.28	0.7600	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9544	0.8877	0.3148	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	0.9024	0.8269	0.978	0.9187	0.7967	0.9431	0.02721	6.74%	0.0%
2.5		5	0.9073	0.8135	1	0.8943	0.8049	1	0.03378	8.32%	-0.54%
5		5	0.8943	0.7559	1	0.935	0.7236	1	0.04985	12.46%	0.9%
10		5	0.8829	0.7738	0.992	0.9106	0.7642	0.9919	0.03929	9.95%	2.16%
20		5	0.8959	0.8087	0.9832	0.8618	0.8293	1	0.03142	7.84%	0.72%
40		5	0	0	0	0	0	0	0		100.0%

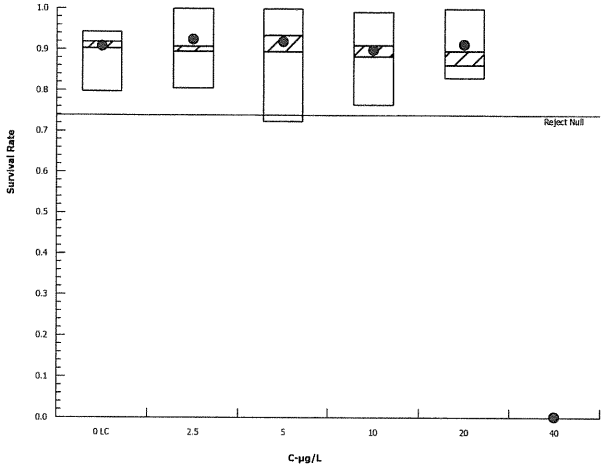
Angular (Corrected) Transformed Summary

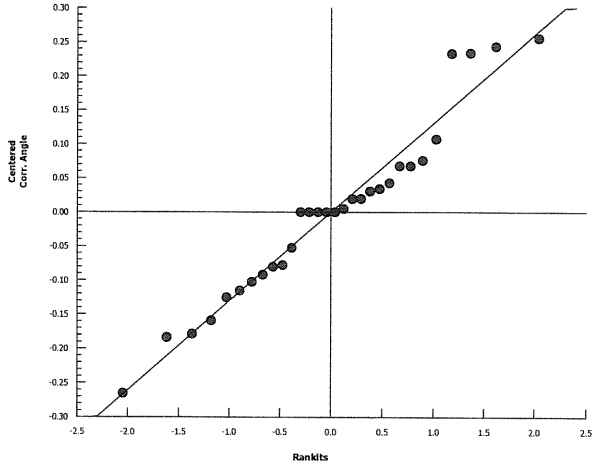
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.262	1.146	1.378	1.282	1.103	1.33	0.04177	7.4%	0.0%
2.5		5	1.292	1.095	1.49	1.24	1.113	1.526	0.0711	12.3%	-2.36%
5		5	1.282	1.038	1.527	1.313	1.017	1.526	0.08811	15.36%	-1.59%
10		5	1.248	1.051	1.444	1.267	1.064	1.481	0.07076	12.68%	1.17%
20		5	1.27	1.076	1.464	1.19	1.145	1.526	0.0699	12.3%	-0.63%
40		5	0.0451	0.04509	0.04511	0.0451	0.0451	0.0451	0	0.0%	96.43%

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

Analysis ID:	12-4039-9276	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-20 15:05	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	04-8167-3886	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-20 15:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	36123	1000	Yes	Two-Point Interpolation

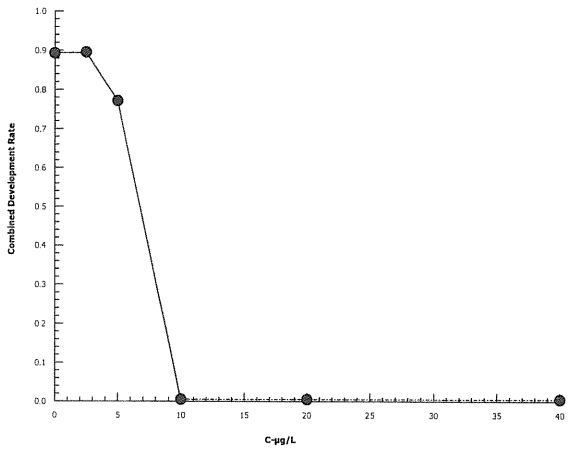
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	5.654	4.978	6.09
EC50	7.103	6.652	7.393

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	0.8927	0.7886	0.9268	0.02627	0.05874	6.58%	0.0%	549	615
2.5		5	0.8944	0.8049	0.9922	0.03348	0.07486	8.37%	-0.19%	556	621
5		5	0.7715	0.6423	0.8293	0.03338	0.07464	9.68%	13.58%	476	617
10		5	0	0	0	0	0		100.0%	0	615
20		5	0	0	0	0	0		100.0%	0	621
40		5	0	0	0	0	0		100.0%	0	615

Graphics



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

Analysis ID:	20-3119-3253	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-20 15:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	372019	1000	Yes	Two-Point Interpolation

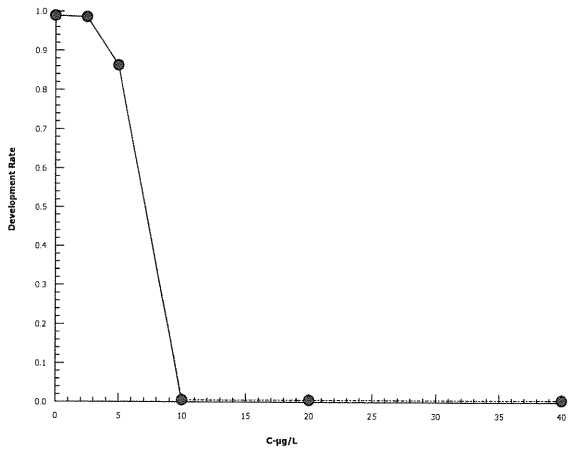
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	5.698	5.291	6.115
EC50	7.132	6.86	7.41

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	0.9893	0.9828	1	0.003192	0.007136	0.72%	0.0%	549	555
2.5		5	0.9858	0.963	1	0.006318	0.01413	1.43%	0.35%	556	564
5		5	0.8669	0.776	0.9519	0.02962	0.06623	7.64%	12.37%	476	552
10		5	0	0	0	0	0		100.0%	0	543
20		5	0	0	0	0	0		100.0%	0	557
40		5	0	0	0	0	0		100.0%	0	5

Graphics



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

Analysis ID:	07-0404-6516	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	11 Mar-20 15:05	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	664263	1000	Yes	Two-Point Interpolation

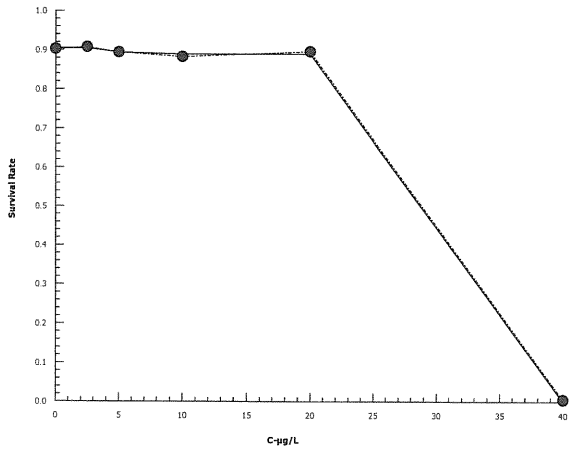
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	24.74	22.76	25.13
EC50	29.83	28.51	30.09

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	0.9024	0.7967	0.9431	0.02721	0.06084	6.74%	0.0%	555	615
2.5		5	0.9073	0.8049	1	0.03378	0.07553	8.32%	-0.54%	558	615
5		5	0.8943	0.7236	1	0.04985	0.1115	12.46%	0.9%	550	615
10		5	0.8829	0.7642	0.9919	0.03929	0.08787	9.95%	2.16%	543	615
20		5	0.8959	0.8293	1	0.03142	0.07027	7.84%	0.72%	551	615
40		5	0	0	0	0	0		100.0%	0	615

Graphics



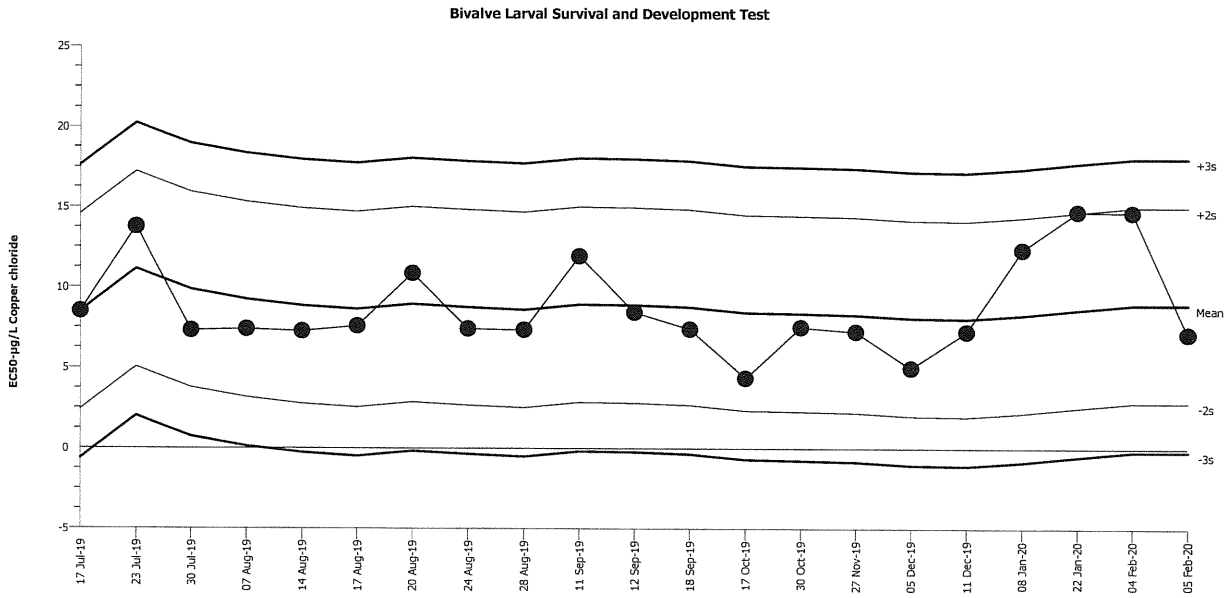
Analyst: JC QA: YS 3/12/20

CETIS QC Plot

Report Date: 11 Mar-20 15:07 (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:	8.915	**Count:**	20	**-2s Warning Limit:**	2.837	**-3s Action Limit:**	-0.2019
Sigma:	3.039	**CV:**	34.10%	**+2s Warning Limit:**	14.99	**+3s Action Limit:**	18.03

Quality Control Data

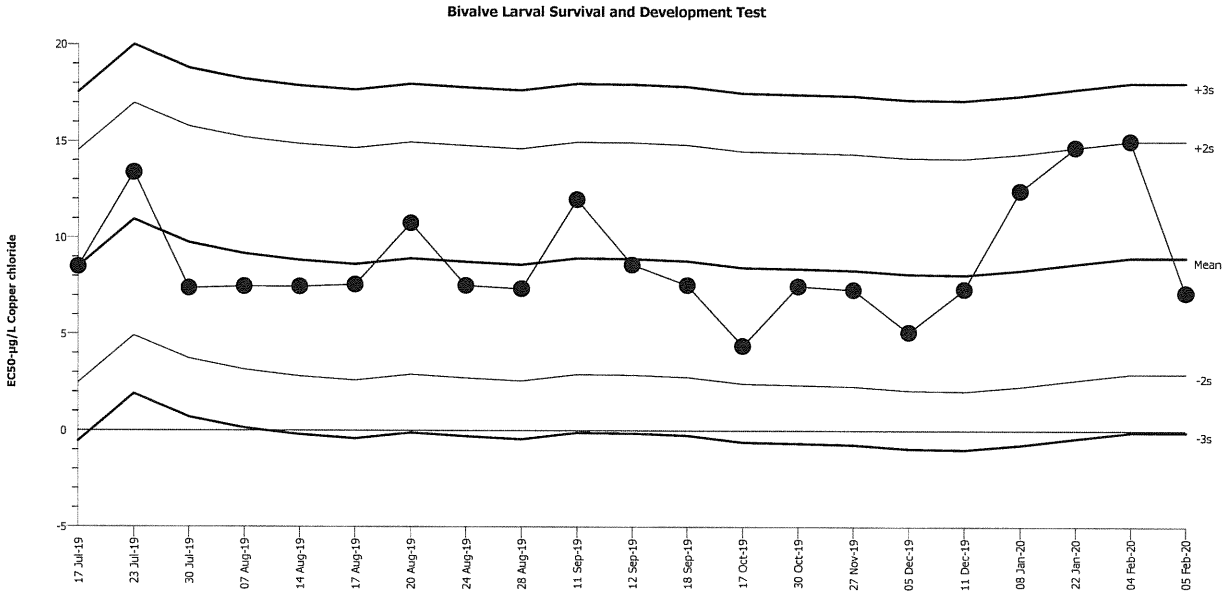
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jul	17	14:50	8.497	-0.4178	-0.1375			04-5072-3133	00-7236-3161
2			23	14:30	13.76	4.846	1.595			07-6771-8781	18-1893-5656
3			30	15:30	7.313	-1.602	-0.5272			15-3542-8276	10-4430-8659
4		Aug	7	15:30	7.395	-1.52	-0.5003			01-2834-9487	15-5629-3220
5			14	14:15	7.255	-1.66	-0.5462			18-5609-6564	17-5885-5207
6			17	14:00	7.582	-1.333	-0.4386			15-9584-4385	11-8998-1524
7			20	14:15	10.86	1.947	0.6405			14-8361-1578	03-1832-9380
8			24	16:00	7.414	-1.501	-0.4938			19-4374-5817	01-6546-9581
9			28	14:30	7.348	-1.567	-0.5156			01-0546-0046	21-3090-7111
10		Sep	11	14:30	11.93	3.02	0.9936			09-2717-2159	04-2480-9094
11			12	14:25	8.444	-0.4706	-0.1548			19-6218-6352	07-5188-6358
12			18	13:20	7.4	-1.515	-0.4985			10-9359-1611	21-3838-7021
13		Oct	17	12:30	4.368	-4.547	-1.496			01-8239-7270	07-0806-0577
14			30	12:30	7.518	-1.397	-0.4597			07-8198-2858	11-8079-0492
15		Nov	27	20:00	7.249	-1.666	-0.5484			12-9914-0499	16-0529-7707
16		Dec	5	13:15	4.982	-3.933	-1.294			04-7411-4445	13-6587-0425
17			11	13:35	7.245	-1.67	-0.5495			10-8800-1613	10-7929-5811
18	2020	Jan	8	13:40	12.34	3.425	1.127			07-8444-5322	01-1422-4896
19			22	13:25	14.72	5.805	1.91			02-1152-2212	07-1224-7163
20		Feb	4	16:30	14.68	5.761	1.896			19-9078-6483	21-0369-4045
21			5	13:10	7.103	-1.812	-0.5963			06-6849-2235	04-8167-3886

CETIS QC Plot

Report Date: 11 Mar-20 15:07 (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:	8.961	**Count:**	20	**-2s Warning Limit:**	2.929	**-3s Action Limit:**	-0.0867
Sigma:	3.016	**CV:**	33.70%	**+2s Warning Limit:**	14.99	**+3s Action Limit:**	18.01

Quality Control Data

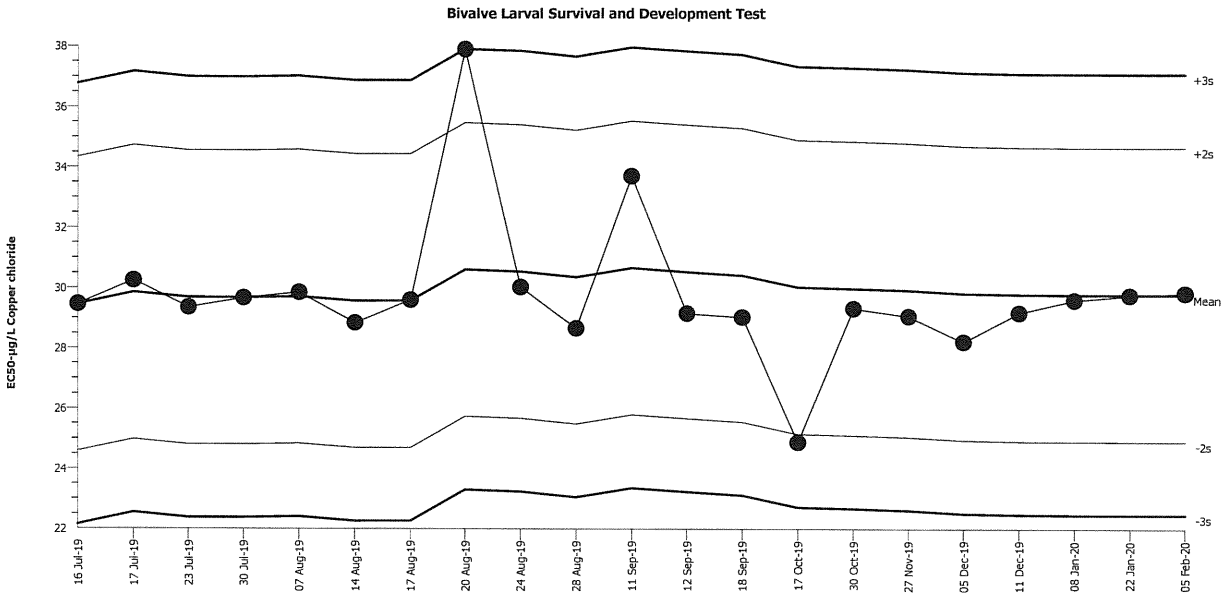
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jul	17	14:50	8.504	-0.4571	-0.1516			04-5072-3133	09-0911-7730
2			23	14:30	13.38	4.42	1.466			07-6771-8781	07-7153-3575
3			30	15:30	7.388	-1.573	-0.5215			15-3542-8276	07-3589-9194
4		Aug	7	15:30	7.473	-1.488	-0.4934			01-2834-9487	19-8086-2685
5			14	14:15	7.466	-1.495	-0.4957			18-5609-6564	14-6389-5644
6			17	14:00	7.563	-1.398	-0.4634			15-9584-4385	19-0402-2449
7			20	14:15	10.76	1.803	0.5976			14-8361-1578	12-0750-5104
8			24	16:00	7.521	-1.44	-0.4775			19-4374-5817	04-6745-5945
9			28	14:30	7.351	-1.61	-0.5339			01-0546-0046	10-3410-8075
10		Sep	11	14:30	11.98	3.022	1.002			09-2717-2159	17-4622-9429
11			12	14:25	8.608	-0.3534	-0.1172			19-6218-6352	06-5225-4823
12			18	13:20	7.546	-1.415	-0.4693			10-9359-1611	16-7089-5314
13		Oct	17	12:30	4.375	-4.586	-1.521			01-8239-7270	19-1864-9270
14			30	12:30	7.481	-1.48	-0.4906			07-8198-2858	15-7183-3565
15		Nov	27	20:00	7.297	-1.664	-0.5517			12-9914-0499	01-7534-7240
16		Dec	5	13:15	5.087	-3.874	-1.284			04-7411-4445	10-0471-4567
17			11	13:35	7.32	-1.641	-0.544			10-8800-1613	20-9346-8800
18	2020	Jan	8	13:40	12.43	3.468	1.15			07-8444-5322	06-2499-4329
19			22	13:25	14.68	5.72	1.897			02-1152-2212	04-4145-0874
20		Feb	4	16:30	15.01	6.047	2.005	(+)		19-9078-6483	06-3219-7963
21			5	13:10	7.132	-1.829	-0.6064			06-6849-2235	20-3119-3253

CETIS QC Plot

Report Date: 11 Mar-20 15:07 (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:	29.78	**Count:**	20	**-2s Warning Limit:**	24.9	**-3s Action Limit:**	22.47
Sigma:	2.438	**CV:**	8.19%	**+2s Warning Limit:**	34.66	**+3s Action Limit:**	37.09

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jul	16	14:35	29.46	-0.3165	-0.1298			04-6285-8375	17-5419-9497
2			17	14:50	30.25	0.4679	0.1919			04-5072-3133	00-3161-9686
3			23	14:30	29.36	-0.4224	-0.1733			07-6771-8781	15-4437-4125
4			30	15:30	29.67	-0.107	-0.04388			15-3542-8276	20-8453-4017
5		Aug	7	15:30	29.85	0.07493	0.03073			01-2834-9487	07-4855-2818
6			14	14:15	28.85	-0.9345	-0.3833			18-5609-6564	13-1367-1354
7			17	14:00	29.6	-0.1763	-0.07233			15-9584-4385	20-0172-5237
8			20	14:15	37.92	8.139	3.338	(+)	(+)	14-8361-1578	02-5800-6574
9			24	16:00	30.04	0.2574	0.1056			19-4374-5817	17-7461-0750
10			28	14:30	28.66	-1.115	-0.4575			01-0546-0046	13-4512-6481
11		Sep	11	14:30	33.71	3.935	1.614			09-2717-2159	01-1883-2964
12			12	14:25	29.16	-0.6159	-0.2526			19-6218-6352	02-6393-7831
13			18	13:20	29.04	-0.741	-0.304			10-9359-1611	04-3365-2341
14		Oct	17	12:30	24.88	-4.898	-2.009	(-)		01-8239-7270	13-2801-3685
15			30	12:30	29.32	-0.4571	-0.1875			07-8198-2858	20-5233-5110
16		Nov	27	20:00	29.07	-0.7133	-0.2926			12-9914-0499	00-1104-7300
17		Dec	5	13:15	28.21	-1.566	-0.6422			04-7411-4445	20-5035-4724
18			11	13:35	29.18	-0.6007	-0.2464			10-8800-1613	02-9848-3585
19	2020	Jan	8	13:40	29.6	-0.1789	-0.0734			07-8444-5322	01-5655-1706
20			22	13:25	29.76	-0.02439	-0.01			02-1152-2212	19-4150-8988
21		Feb	5	13:10	29.83	0.04632	0.019			06-6849-2235	07-0404-6516

CETIS Test Data Worksheet

Report Date: 02 Feb-20 12:41 (p 1 of 1)
Test Code: 06-6849-2235/200205msdv

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

Start Date: 05 Feb-20
End Date: 07 Feb-20
Sample Date: 05 Feb-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 200205msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			226			115	0	JA 2/21/20
			227			0	0	
			228			113	112	
			229			105	0	
			230			118	116	
			231			125	97	JA 2/28/20
			232			102	0	
			233			106	0	
			234			110	109	
			235			116	114	
			236			112	112	
			237			122	0	
			238			108	104	
			239			0	0	
			240			(A) ~~126~~ 119	(A) ~~0~~ 99	JA 3/6/20 EG QC'd 3/10/20 99/119
			241			(B) ~~102~~ 129	0	VS QC'd 3/11/20 0/129
			242			(A) ~~0~~ 102	0	EG QC'd 3/10/20 0/102.
			243			0	0	
			244			94	0	
			245			104	99	
			246			0	0	
			247			112	0	
			248			113	0	
			249			116	114	
			250			98	97	
			251			115	102	
			252			129	128	
			253			89	79	
			254			0	0	
			255			99	99	

(A) EG QC 3/10/20
(B) VS QC 3/11/20

CETIS Test Data Worksheet

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

Start Date: 05 Feb-20
End Date: 07 Feb-20
Sample Date: 05 Feb-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 200205msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	228					
0	LC	2	249					
0	LC	3	236			110	109	NM 2/7/20
0	LC	4	235					
0	LC	5	250					
2.5		1	230					
2.5		2	238					
2.5		3	234			114	114	NM
2.5		4	255					
2.5		5	252					
5		1	240					
5		2	245					
5		3	251			116	103	NM
5		4	231					
5		5	253					
10		1	237					
10		2	244					
10		3	248			112	0	NM
10		4	247					
10		5	232					
20		1	226					
20		2	241					
20		3	233			105	0	NM
20		4	229					
20		5	242					
40		1	254					
40		2	227					
40		3	243			0	0	Cells lysed NM
40		4	246					
40		5	239					

QC= EG

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 200205msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 2/5/2020 1310

End Date/Time: 2/7/2020 1230

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	31.6	14.6	15.2	14.9	8.9	8.8	8.5	8.04	8.00	7.97
2.5	31.9	31.6	31.7	14.6	14.7	14.7	8.9	8.8	8.5	8.01	8.01	7.98
5	31.9	31.6	31.7	14.6	14.7	14.6	8.6	8.7	8.5	7.99	8.01	7.98
10	31.9	31.8	31.9	14.7	15.1	15.2	8.5	8.6	8.3	7.98	8.01	7.98
20	32.0	31.6	31.7	14.8	15.0	15.0	8.5	8.6	8.4	8.02	8.02	7.99
40	31.9	31.6	31.6	14.7	15.0	14.9	8.5	8.6	8.4	8.04	8.03	@7.9 8.00

	0	24	48
WQ Readings:	EG	DM	RH 0800 KL
Dilutions made by:	EG		

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	1.9
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,400

Technician Initials:

Environmental Chamber: D

Comments:

0 hrs:

24 hrs:

48 hrs: @0818KL 2/7/20

QC Check: RH 03/10/20

Final Review: EG 3/10/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / CuCl2
Test No.: 200205 msdV
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: Taylor / 19B
Date Received: 11/20/19
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 2/5/2020 1310
End Date/Time: 2/7/2020 1230
Technician Initials: EG

Spawn Information

First Gamete Release Time: 0935

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	good density + motility
Female 1	1	Ok shape, good density, pink color
Female 2	2	great shape, Ok density, pale color
Female 3		

Egg Fertilization Time: 1025

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	—
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:		
	13	4
	9	12
	10	11
	8	10
	12	8

Mean: 9.7

Mean 9.7 X 50 = 485 embryos/ml

Initial Density: 485 = 1.62 (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	119	119	100	100
TØ B	144	144	100	
TØ C	119	119	100	
TØ D	127	127	100	
TØ E	121	121	100	
TØ F	107	107	100	
$\bar{X}$ =	123			

48-h QC: 116/118 = 98.3%

Comments:

QC Check: RT 03/10/20

Final Review: EG 3/10/20