NAUTILUS ENVIRONMENTAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: December 2018

Prepared for: **Jacobs**
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Date Submitted: **January 11, 2019**

Data Quality Assurance:

- Nautilus Environmental 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 sample collected on December 11, 2018 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 (formerly Nautilus Environmental) located in San Diego, California between December 12 and 14, 2018.

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	**121118**
Nautilus Log-in Number	18-1357
Collection Date; Time	12/11/2018; 0925h
Receipt Date; Time	12/12/2018; 0910h
Receipt Temperature (°C)	4.2
Dissolved Oxygen (mg/L)	9.6
pH	7.53
Conductivity (µS/cm)	11,500
Salinity (ppt)	7.0
Alkalinity (mg/L $CaCO_3$)	606
Total Chlorine (mg/L)	< 0.02
Total Ammonia (mg/L)	3.5

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	12/12/2018, 1355h to 12/14/2018, 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)
Additional Control	Brine Control (deionized water and hypersaline brine)
Test Salinity	30 ± 2 ppt
Source of Salinity	Hypersaline brine made by freezing seawater to a salinity of 98.7
Test Concentrations (% sample)	74.9[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 sulfate (per project QAPP)
Statistical Software	CETIS™ 1.8.7.20

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

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 74.9 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.34 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	74.9	> 74.9	< 1.34	> 74.9
	Survival	74.9	> 74.9	< 1.34	> 74.9

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

Chronic Toxic Unit (TU_c) = 100% sample/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)	Bivalve	
	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	100	97.9
0 (Lab Control)	99.2	98.8
2	100	98.9
4	99.2	98.2
9	100	98.7
18	100	97.9
35	99.2	99.0
74.9 [a]	100	98.5

[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.

Results for the reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. The results for the reference toxicant tests 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 D, and a list of qualifier codes is 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	Development Rate	5.59	6.39 ± 1.24	9.69
	Survival Rate	23.9	23.5 ± 0.372	0.79

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

Bivalve Larval Development Test

CETIS Summary Report

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

Batch ID:	10-5797-1018	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 Dec-18 13:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	14 Dec-18 12:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	03-0912-0974	**Code:**	18-1357	**Client:**	Jacobs
Sample Date:	11 Dec-18 09:25	**Material:**	Effluent Sample	**Project:**	
Receive Date:	12 Dec-18 09:10	**Source:**	Jacobs		
Sample Age:	28h (4.2 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-3682-5542	Development Rate	74.9	>74.9	NA	2.14%	<1.335	Dunnett Multiple Comparison Test
07-6427-6312	Survival Rate	74.9	>74.9	NA	0.96%	<1.335	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
10-1716-4191	Development Rate	EC25	>74.9	N/A	N/A	<1.335	Linear Interpolation (ICPIN)
		EC50	>74.9	N/A	N/A	<1.335	
02-7171-0200	Survival Rate	EC25	>74.9	N/A	N/A	<1.335	Linear Interpolation (ICPIN)
		EC50	>74.9	N/A	N/A	<1.335	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-3682-5542	Development Rate	Control Resp	0.9788	0.9 - NL	Yes	Passes Acceptability Criteria
10-1716-4191	Development Rate	Control Resp	0.9788	0.9 - NL	Yes	Passes Acceptability Criteria
02-7171-0200	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
07-6427-6312	Survival Rate	Control Resp	1	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	0.9788	0.9619	0.9957	0.9577	0.9926	0.006092	0.01362	1.39%	0.0%
0	Lab Control	5	0.9883	0.9766	1	0.9746	1	0.004205	0.009403	0.95%	-0.97%
2		5	0.9888	0.9773	1	0.9792	1	0.004157	0.009295	0.94%	-1.03%
4		5	0.9815	0.9622	1	0.959	1	0.006958	0.01556	1.59%	-0.28%
9		5	0.9869	0.9752	0.9985	0.9781	1	0.004201	0.009393	0.95%	-0.83%
18		5	0.9792	0.9685	0.99	0.9701	0.9936	0.003868	0.008648	0.88%	-0.05%
35		5	0.9898	0.9846	0.9951	0.9852	0.9939	0.001901	0.004251	0.43%	-1.13%
74.9		5	0.9846	0.9711	0.998	0.9667	0.9931	0.004832	0.0108	1.1%	-0.59%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	1	1	1	1	1	0	0	0.0%	0.0%
0	Lab Control	5	0.9919	0.9693	1	0.9593	1	0.00813	0.01818	1.83%	0.81%
2		5	1	1	1	1	1	0	0	0.0%	0.0%
4		5	0.9919	0.9744	1	0.9675	1	0.006298	0.01408	1.42%	0.81%
9		5	1	1	1	1	1	0	0	0.0%	0.0%
18		5	1	1	1	1	1	0	0	0.0%	0.0%
35		5	0.9919	0.9693	1	0.9593	1	0.00813	0.01818	1.83%	0.81%
74.9		5	1	1	1	1	1	0	0	0.0%	0.0%

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	0.9926	0.9577	0.9837	0.9735	0.9863
0	Lab Control	0.9859	0.9931	0.988	0.9746	1
2		1	0.9933	0.9792	0.9792	0.9925
4		0.959	0.9781	1	0.9789	0.9916
9		1	0.9862	0.9781	0.9781	0.992
18		0.9769	0.9771	0.9784	0.9701	0.9936
35		0.9854	0.9932	0.9852	0.9939	0.9915
74.9		0.986	0.984	0.9931	0.993	0.9667

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	1	1	1	1
0	Lab Control	1	1	1	0.9593	1
2		1	1	1	1	1
4		0.9919	1	1	1	0.9675
9		1	1	1	1	1
18		1	1	1	1	1
35		1	1	1	1	0.9593
74.9		1	1	1	1	1

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	134/135	136/142	121/123	147/151	144/146
0	Lab Control	140/142	143/144	164/166	115/118	137/137
2		170/170	149/150	141/144	141/144	133/134
4		117/122	134/137	140/140	139/142	118/119
9		124/124	143/145	134/137	134/137	124/125
18		127/130	128/131	136/139	130/134	155/156
35		135/137	146/147	133/135	164/165	117/118
74.9		141/143	123/125	144/145	142/143	145/150

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	123/123	123/123	123/123	123/123	123/123
0	Lab Control	123/123	123/123	123/123	118/123	123/123
2		123/123	123/123	123/123	123/123	123/123
4		122/123	123/123	123/123	123/123	119/123
9		123/123	123/123	123/123	123/123	123/123
18		123/123	123/123	123/123	123/123	123/123
35		123/123	123/123	123/123	123/123	118/123
74.9		123/123	123/123	123/123	123/123	123/123

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

Analysis ID: 00-3682-5542 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 21 Dec-18 10:20 **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	2.14%	74.9	>74.9	NA	1.335

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-1.489	2.407	0.066	8	0.9974	CDF	Non-Significant Effect
		4	-0.5038	2.407	0.066	8	0.9524	CDF	Non-Significant Effect
		9	-1.105	2.407	0.066	8	0.9910	CDF	Non-Significant Effect
		18	0.02745	2.407	0.066	8	0.8496	CDF	Non-Significant Effect
		35	-1.492	2.407	0.066	8	0.9974	CDF	Non-Significant Effect
		74.9	-0.768	2.407	0.066	8	0.9761	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.009342185	0.001557031	6	0.8169	0.5661	Non-Significant Effect
Error	0.05336814	0.001906005	28			
Total	0.06271032		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.946	16.81	0.6839	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9847	0.9146	0.8977	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	0.9788	0.9619	0.9957	0.9837	0.9577	0.9926	0.006092	1.39%	0.0%
2		5	0.9888	0.9773	1	0.9925	0.9792	1	0.004157	0.94%	-1.03%
4		5	0.9815	0.9622	1	0.9789	0.959	1	0.006958	1.59%	-0.28%
9		5	0.9869	0.9752	0.9985	0.9862	0.9781	1	0.004201	0.95%	-0.83%
18		5	0.9792	0.9685	0.99	0.9771	0.9701	0.9936	0.003868	0.88%	-0.05%
35		5	0.9898	0.9846	0.9951	0.9915	0.9852	0.9939	0.001901	0.43%	-1.13%
74.9		5	0.9846	0.9711	0.998	0.986	0.9667	0.9931	0.004832	1.1%	-0.59%

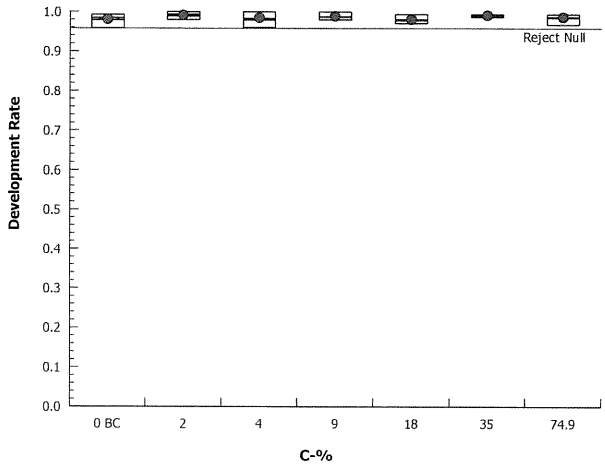
Angular (Corrected) Transformed Summary

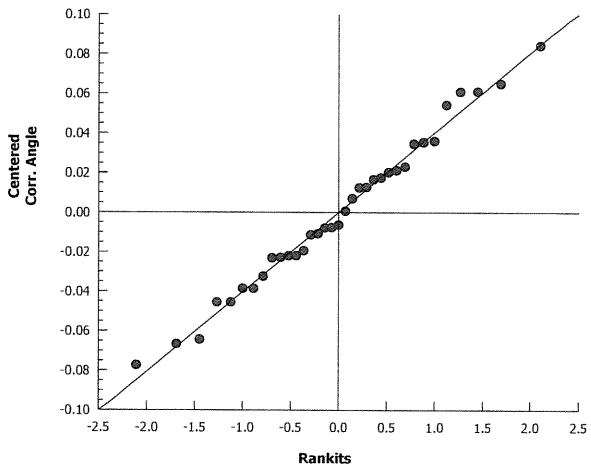
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.43	1.373	1.488	1.443	1.364	1.485	0.02074	3.24%	0.0%
2		5	1.472	1.415	1.528	1.484	1.426	1.532	0.02041	3.1%	-2.87%
4		5	1.444	1.368	1.521	1.425	1.367	1.529	0.02752	4.26%	-0.97%
9		5	1.461	1.407	1.515	1.453	1.422	1.526	0.0196	3.0%	-2.13%
18		5	1.43	1.385	1.474	1.419	1.397	1.491	0.01591	2.49%	0.05%
35		5	1.472	1.445	1.498	1.479	1.449	1.493	0.009432	1.43%	-2.88%
74.9		5	1.452	1.401	1.503	1.452	1.387	1.488	0.01839	2.83%	-1.48%

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

Analysis ID:	00-3682-5542	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Dec-18 10:20	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 07-6427-6312 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 21 Dec-18 10:20 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	0.96%	74.9	>74.9	NA	1.335

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		4	22.5	16	1	8	0.4265	Asymp	Non-Significant Effect
		9	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		18	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		35	25	16	1	8	0.6693	Asymp	Non-Significant Effect
		74.9	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.008279897	0.001379983	6	1.138	0.3668	Non-Significant Effect
Error	0.03396659	0.001213092	28			
Total	0.04224648		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.166	3.812	0.3613	Equal Variances
Variances	Levene Equality of Variance	6.341	3.528	0.0003	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.5609	0.9146	<0.0001	Non-normal Distribution

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1	1	1	1	1	1	0	0.0%	0.0%
2		5	1	1	1	1	1	1	0	0.0%	0.0%
4		5	0.9919	0.9744	1	1	0.9675	1	0.006298	1.42%	0.81%
9		5	1	1	1	1	1	1	0	0.0%	0.0%
18		5	1	1	1	1	1	1	0	0.0%	0.0%
35		5	0.9919	0.9693	1	1	0.9593	1	0.00813	1.83%	0.81%
74.9		5	1	1	1	1	1	1	0	0.0%	0.0%

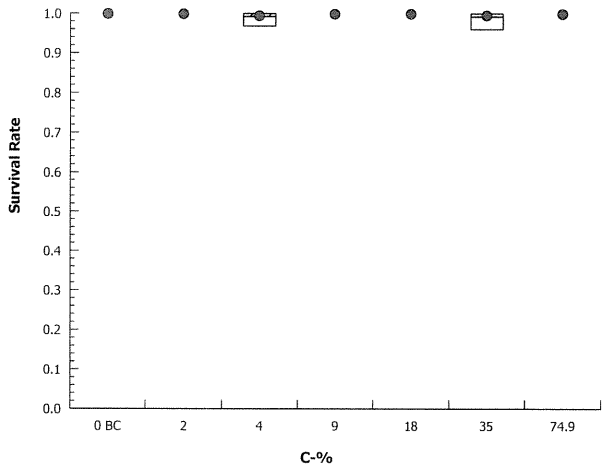
Angular (Corrected) Transformed Summary

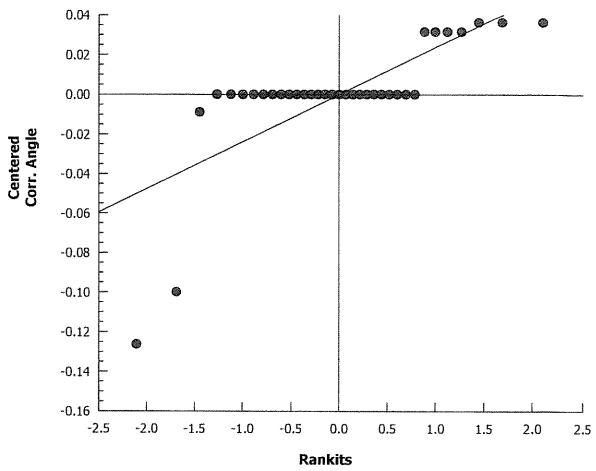
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.526	1.525	1.526	1.526	1.526	1.526	0	0.0%	0.0%
2		5	1.526	1.525	1.526	1.526	1.526	1.526	0	0.0%	0.0%
4		5	1.489	1.416	1.563	1.526	1.389	1.526	0.02647	3.98%	2.38%
9		5	1.526	1.525	1.526	1.526	1.526	1.526	0	0.0%	0.0%
18		5	1.526	1.525	1.526	1.526	1.526	1.526	0	0.0%	0.0%
35		5	1.494	1.406	1.582	1.526	1.368	1.526	0.03158	4.73%	2.07%
74.9		5	1.526	1.525	1.526	1.526	1.526	1.526	0	0.0%	0.0%

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

Analysis ID:	07-6427-6312	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Dec-18 10:20	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1812-S072 | 05-4652-3733

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

Analysis ID:	10-1716-4191	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Dec-18 10:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1638278	1000	Yes	Two-Point Interpolation

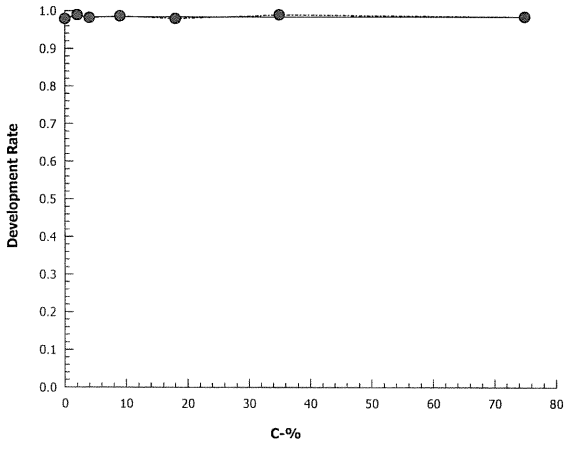
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>74.9	N/A	N/A	<1.335	NA	NA
EC50	>74.9	N/A	N/A	<1.335	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	0.9788	0.9577	0.9926	0.006092	0.01362	1.39%	0.0%	682	697
2		5	0.9888	0.9792	1	0.004157	0.009295	0.94%	-1.03%	734	742
4		5	0.9815	0.959	1	0.006958	0.01556	1.59%	-0.28%	648	660
9		5	0.9869	0.9781	1	0.004201	0.009393	0.95%	-0.83%	659	668
18		5	0.9792	0.9701	0.9936	0.003868	0.008649	0.88%	-0.05%	676	690
35		5	0.9898	0.9852	0.9939	0.001901	0.00425	0.43%	-1.13%	695	702
74.9		5	0.9846	0.9667	0.9931	0.004832	0.0108	1.1%	-0.59%	695	706

Graphics



Analyst: JCL QA: KB 1/7/19

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	02-7171-0200	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Dec-18 10:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2044787	1000	Yes	Two-Point Interpolation

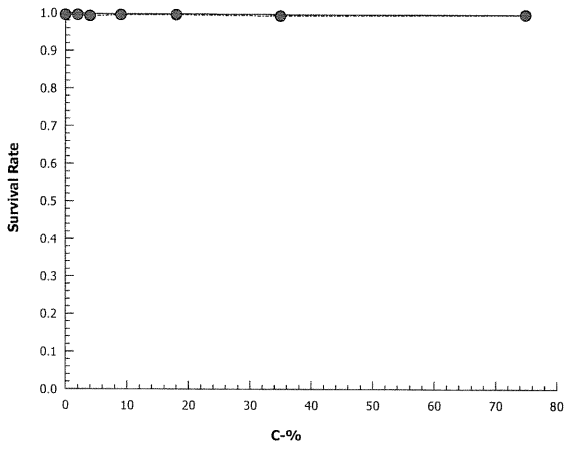
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>74.9	N/A	N/A	<1.335	NA	NA
EC50	>74.9	N/A	N/A	<1.335	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	1	1	1	0	0	0.0%	0.0%	615	615
2		5	1	1	1	0	0	0.0%	0.0%	615	615
4		5	0.9919	0.9675	1	0.006298	0.01408	1.42%	0.81%	610	615
9		5	1	1	1	0	0	0.0%	0.0%	615	615
18		5	1	1	1	0	0	0.0%	0.0%	615	615
35		5	0.9919	0.9593	1	0.00813	0.01818	1.83%	0.81%	610	615
74.9		5	1	1	1	0	0	0.0%	0.0%	615	615

Graphics



CETIS Test Data Worksheet

Report Date: 08 Dec-18 15:06 (p 1 of 1)
Test Code: 1812-5072 05-4652-3733/20934A55

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

Start Date: 12 Dec-18 | **Species:** Mytilus galloprovincialis | **Sample Code:** 18- 1357
End Date: 14 Dec-18 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Jacobs
Sample Date: 11 Dec-18 | **Material:** Effluent Sample | **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			134	130	AES 12/20/18
			32			145	143	
			33			142	136	
			34			135	133	
			35			142	139	
			36			143	141	
			37			150	149	
			38			135	134	
			39			125	123	
			40			137	134	
			41			137	135	
			42			137	137	
			43			124	124	
			44			144	143	
			45			146	144	
			46			170	170	
			47			123	121	
			48			122	117	
			49			134	133	
			50			145	144	
			51			150	145	
			52			119	118	
			53			144	141	
			54			151	147	
			55			137	134	
			56			147	116	
			57			118	117	
			58			156	155	
			59			130	127	
			60			165	164	
			61			137	134	
			62			139 ~~140~~ (A)	136	
			63			144	141	
			64			125	124	
			65			131	128	
			66			118	115	
			67			166	164	
			68			143	142	
			69			140	140	
			70			142	140	

(A) QA'd AES 12/20/18

CETIS Test Data Worksheet

Report Date: 08 Dec-18 15:06 (p 1 of 1)
Test Code: 1812-5072 05-4652-3733/20934A55

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

Start Date: 12 Dec-18
End Date: 14 Dec-18
Sample Date: 11 Dec-18
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 18- 1357
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	38			133	133	EG 12/14/18
0	BC	2	33			(A) ~~X~~		
0	BC	3	47					
0	BC	4	54					
0	BC	5	45					
0	LC	1	70					
0	LC	2	44			140	140	SG 12/15/18
0	LC	3	67					
0	LC	4	66					
0	LC	5	42					
2		1	46					
2		2	37					
2		3	63			146	143	SG 12/15/18
2		4	53					
2		5	49					
4		1	48			128	123	SG 12/15/18
4		2	61			(A) 146	(A) ~~X~~	
4		3	69					
4		4	35					
4		5	52					
9		1	43					
9		2	32			146	144	SG 12/15/18
9		3	40					
9		4	55					
9		5	64					
18		1	59					
18		2	65					
18		3	62			141	138	SG 12/15/18
18		4	31					
18		5	58					
35		1	41					
35		2	56			153	152	SG 12/15/18
35		3	34					
35		4	60					
35		5	57					
74.9 70		1	36			143	142	EG
74.9 70		2	39					
74.9 70		3	50					
74.9 70		4	68					
74.9 70		5	51					

EG Q18 12/21/18

QC = EG

(A) Q18 SG 12/15/18

Marine Chronic Bioassay

Water Quality Measurements

Client: Jacobs
Sample ID: Wyckoff
Sample Log No.: 18- 1357
Test No.: 1812-8072

Test Species: *M. galloprovincialis*
Start Date/Time: 12/12/2018 1355
End Date/Time: 12/14/2018 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	30.4	30.3	14.7	15.0	14.8	8.7	8.4	8.0	8.12	7.94	7.92
Brine Control	30.6	30.8	30.7	14.1	14.9	14.8	8.6	8.3	7.9	8.44	8.13	8.00
2	30.3	30.6	30.6	15.0	14.9	14.7	8.5	8.3	7.9	8.08	7.97	7.94
4	30.4	30.6	30.6	14.6	15.0	14.7	8.6	8.4	7.9	8.08	7.98	7.96
9	30.5	30.7	30.6	14.7	14.9	14.8	8.6	8.3	7.9	8.05	7.98	8.00
18	30.5	30.6	30.6	15.0	15.2	14.8	8.5	8.3	7.9	7.98	8.00	8.04
35	30.5	30.7	30.7	15.0	15.1	14.8	8.4	8.3	7.9	7.91	8.01	8.12
(A) ~~70~~ 74.9	30.9	31.1	31.1	15.2	15.1	14.8	8.1	8.2	7.9	7.83	8.04	8.18

Technician Initials:		0	24	48
	WQ Readings:	BO	KL	RT
	Dilutions made by:	EG	-	-

Comments:
0 hrs: (A) EG Q18 12/21/18
24 hrs:
48 hrs:

QC Check: EG 12/21/18

Final Review: KS 1/4/19

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: JACOBS **Analyst:** EG

Sample ID: Wyckoff **Test Date:** 12/12/2018

Test No: 1812-S072 **Test Type:** Mussel Development

Salinity of Effluent 7.0

Salinity of Brine 98.7 **Date of Brine used:** 7/24/2018

Target Salinity 30 **Alkalinity of Brine Control:** 82 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.33**	**0.44**

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	0.33	1.7	250
4	10.0	0.33	3.3	250
9	22.5	0.33	7.5	250
18	45.0	0.33	15.1	250
35	87.5	0.33	29.3	250
74.9	187.3	0.33	62.7	250

	DI Volume			
Brine Control	143.6	0.44	62.7	250

Total Brine Volume Required (ml): **182.3**

QC Check: EG 12/21/18 **Final Review:** KS 1/4/19

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

Marine Chronic Bioassay

Larval Development Worksheet

Client: Jacobs - Wyckoff
Test No.: 1812-5072
Test Species: M. galloprovincialis
Animal Source: Taylor Shellfish
Date Received: 9/25/18 and 10/30/18
Test Chambers: 30 mL sheet vial
Sample Volume: 10 mL

Start Date/Time: 12/12/2018 1355
End Date/Time: 12/14/2018 1230
Technician Initials: EG

Spawn Information

First Gamete Release Time: 1130

Sex	Number Spawning
Male	6
Female	2

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	2, 3, 6	good density and mot. lity
Female 1	1	good density, round, pale color
Female 2	2	good trick density, round, pale color
Female 3	—	0 —

Egg Fertilization Time: 1210

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: 1

Embryo Inoculum Preparation

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

Number Counted:		
	6	12
	4	10
	5	6
	7	12
	5	12

Mean: 7.9

Mean 7.9 X 50 = 395 embryos/ml

Initial Density: 395 = 1.32 (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

Rand. No.	No. Dividing	Total	% Dividing	Mean % Dividing
T#1	132	132	100	100
T#2	114	114	100	
T#3	106	106	100	
T#4	134	134	100	
T#5	124	124	100	
T#6	127	127	100	
	$\bar{x}$ 122.8			

48-h QC: 141/142 99.3%

Comments:

QC Check: EG 12/21/18

Final Review: YB 1/4/19

Appendix B
Sample Check-In Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff 121118
Test ID No(s).: 1812 - 5072

Sample (A, B, C):	A			
Log-in No. (18-xxxx):	1357			
Sample Collection Date & Time:	12/11/18 0925			
Sample Receipt Date & Time:	12/12/18 0910			
Number of Containers & Container Type:	1 1L cube			
Approx. Total Volume Received (L):	~1L			
Check-in Temperature (°C)	4.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.6			
pH (units)	7.53			
Conductivity (µS/cm)	11,500			
Salinity (ppt)	7.0			
Alkalinity (mg/L) [2]	606			
Hardness (mg/L) [2, 3]	—			
Total Chlorine (mg/L)	≤0.02			
Technician Initials	AES			

Test Performed: Chronic Mussel Development **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: 125 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Control Alkalinity: 82 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 Check-In Information

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

COC Complete (Y/N)?
A Y B____ C____

Filtration? Y (N)
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 AES B____ C____

QC Check: EG 12/21/18
Final Review: KS 1/4/19

Total Ammonia Analysis
Freshwater

Overlying Water

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 12/12/18

Analyst: SG
Analysis Date: 12/12/18

N x 1.22

Sample ID	Nautilus ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	8.8	10.7
18-1357	1	12/12/2018	0	2.9	3.5
Spike Check (10 mg/L NH_3)		NA	NA	8.8	10.7
Sample Duplicate[a]	1	NA	NA	2.6	3.2
Sample Duplicate + Spike[a]		NA	NA	10.4	12.7
Spike Check (10 mg/L NH_3)		NA	NA	8.8	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	10.7	10	NA	107
1	3.5	3.2	12.7	10	9.0	92

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: EG 12/21/18

Final Review: YS 1/4/19

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 12/11/2018
CarrierName: FedEx
AirbillNo: 773939295943

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2018/WA
Project Code: WEH-025W
Cooler #: 1 of 1

No: 10-121118-100928-0327
2019T10P000DD210W2LA00
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
121118		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	12/11/2018 09:25	Field Sample

Rec Temp 4.2

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	12-11-18 1045	Nautilus	12/12/18 0910	Good
	0				

Nautilus ID= 18-1357

Appendix D
Reference Toxicant Test Results

CETIS Summary Report

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

Batch ID:	03-2541-7463	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	12 Dec-18 13:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	14 Dec-18 12:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	04-7899-2830	**Code:**	181212msdvSO	**Client:**	Internal
Sample Date:	12 Dec-18	**Material:**	Copper sulfate	**Project:**	
Receive Date:	12 Dec-18	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Sulfate		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-5760-9453	Combined Development Ra	2	4	2.828	1.82%		Dunnett Multiple Comparison Test
11-3000-4340	Development Rate	2	4	2.828	1.21%		Dunnett Multiple Comparison Test
00-5033-9234	Survival Rate	16	32	22.63	1.64%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
02-1891-3936	Combined Development Ra	EC25	4.383	4.234	4.498		Linear Interpolation (ICPIN)
		EC50	5.593	5.494	5.672		
13-7938-6780	Development Rate	EC25	4.377	4.236	4.49		Linear Interpolation (ICPIN)
		EC50	5.589	5.494	5.664		
20-5520-1077	Survival Rate	EC25	19.79	19.41	20.1		Linear Interpolation (ICPIN)
		EC50	23.86	23.61	24.07		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
11-3000-4340	Development Rate	Control Resp	0.9889	0.9 - NL	Yes	Passes Acceptability Criteria
13-7938-6780	Development Rate	Control Resp	0.9889	0.9 - NL	Yes	Passes Acceptability Criteria
00-5033-9234	Survival Rate	Control Resp	0.9951	0.5 - NL	Yes	Passes Acceptability Criteria
20-5520-1077	Survival Rate	Control Resp	0.9951	0.5 - NL	Yes	Passes Acceptability Criteria
20-5760-9453	Combined Development Ra	PMSD	0.01822	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	0.984	0.9696	0.9985	0.9724	1	0.005204	0.01164	1.18%	0.0%
2		5	0.9815	0.9582	1	0.9512	1	0.008385	0.01875	1.91%	0.26%
4		5	0.8138	0.7839	0.8438	0.7846	0.8457	0.01077	0.02409	2.96%	17.29%
8		5	0.002694	0	0.007311	0	0.007407	0.001663	0.003719	138.1%	99.73%
16		5	0	0	0	0	0	0	0		100.0%
32		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	0.9889	0.9736	1	0.9724	1	0.005511	0.01232	1.25%	0.0%
2		5	0.9879	0.9774	0.9984	0.9787	1	0.003791	0.008476	0.86%	0.1%
4		5	0.8165	0.7886	0.8443	0.7846	0.8457	0.01002	0.02241	2.75%	17.44%
8		5	0.002694	0	0.007311	0	0.007407	0.001663	0.003719	138.1%	99.73%
16		5	0	0	0	0	0	0	0		100.0%
32		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	0.9951	0.9816	1	0.9756	1	0.004878	0.01091	1.1%	0.0%
2		5	0.9935	0.9754	1	0.9675	1	0.006504	0.01454	1.46%	0.16%
4		5	0.9967	0.9877	1	0.9837	1	0.003252	0.007272	0.73%	-0.16%
8		5	1	1	1	1	1	0	0	0.0%	-0.49%
16		5	0.9789	0.9498	1	0.9431	1	0.01047	0.02342	2.39%	1.63%
32		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	0.9921	0.9756	1	0.98	0.9724
2		0.9787	1	0.9512	0.9847	0.9929
4		0.8457	0.7846	0.8264	0.8158	0.7967
8		0	0.007407	0	0.006061	0
16		0	0	0	0	0
32		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	0.9921	1	1	0.98	0.9724
2		0.9787	1	0.9832	0.9847	0.9929
4		0.8457	0.7846	0.8264	0.8158	0.8099
8		0	0.007407	0	0.006061	0
16		0	0	0	0	0
32		0	0	0	0	0

Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1	0.9756	1	1	1
2		1	1	0.9675	1	1
4		1	1	1	1	0.9837
8		1	1	1	1	1
16		0.9756	1	0.9431	1	0.9756
32		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	125/126	120/123	140/140	147/150	141/145
2		138/141	127/127	117/123	129/131	139/140
4		137/162	102/130	119/144	124/152	98/123
8		0/156	1/135	0/129	1/165	0/126
16		0/123	0/153	0/123	0/155	0/123
32		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	125/126	120/120	140/140	147/150	141/145
2		138/141	127/127	117/119	129/131	139/140
4		137/162	102/130	119/144	124/152	98/121
8		0/156	1/135	0/129	1/165	0/126
16		0/120	0/153	0/116	0/155	0/120
32		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	123/123	120/123	123/123	123/123	123/123
2		123/123	123/123	119/123	123/123	123/123
4		123/123	123/123	123/123	123/123	121/123
8		123/123	123/123	123/123	123/123	123/123
16		120/123	123/123	116/123	123/123	120/123
32		0/123	0/123	0/123	0/123	0/123

CETIS Analytical Report

Test Code: 181212msdvSO | 11-6161-8836

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

Analysis ID:	20-5760-9453	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**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	1.82%	2	4	2.828	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2	0.1686	2.227	0.066	8	0.6859	CDF	Non-Significant Effect
		4*	11.04	2.227	0.066	8	<0.0001	CDF	Significant Effect
		8*	47.15	2.227	0.066	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.516973	2.172324	3	995.7	<0.0001	Significant Effect
Error	0.03490622	0.002181639	16			
Total	6.551879		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.001	11.34	0.1718	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9682	0.866	0.7164	Normal Distribution

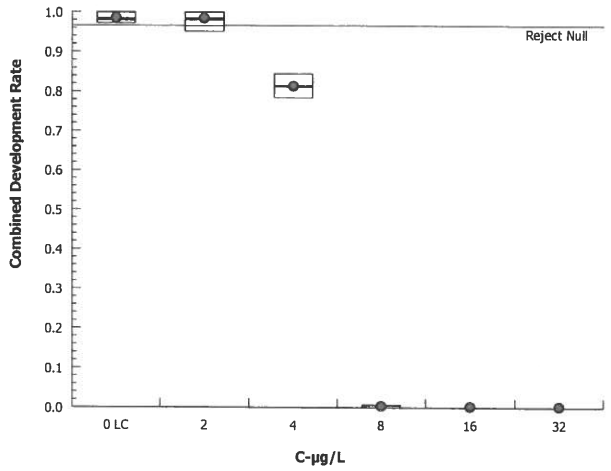
Combined Development Rate Summary

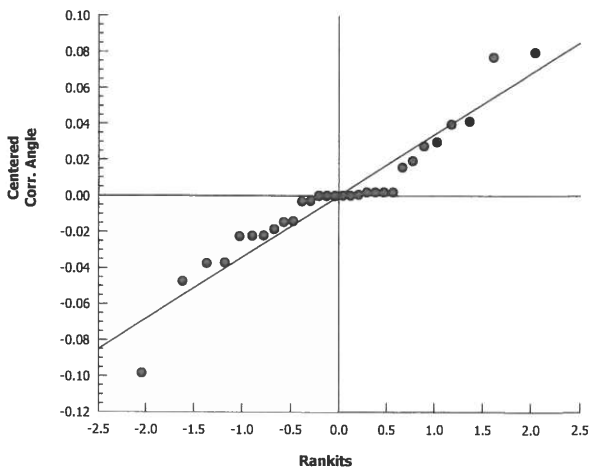
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.984	0.9696	0.9985	0.98	0.9724	1	0.005204	1.18%	0.0%
2		5	0.9815	0.9582	1	0.9847	0.9512	1	0.008386	1.91%	0.26%
4		5	0.8138	0.7839	0.8438	0.8158	0.7846	0.8457	0.01077	2.96%	17.29%
8		5	0.002694	0	0.007311	0	0	0.007407	0.001663	138.1%	99.73%
16		5	0	0	0	0	0	0	0		100.0%
32		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	1.451	1.386	1.517	1.429	1.404	1.529	0.02347	3.62%	0.0%
2		5	1.446	1.363	1.53	1.447	1.348	1.526	0.03011	4.66%	0.34%
4		5	1.125	1.087	1.164	1.127	1.088	1.167	0.01391	2.76%	22.47%
8		5	0.05855	0.03158	0.08552	0.04456	0.04004	0.08617	0.009715	37.1%	95.97%
16		5	0.04318	0.03992	0.04644	0.0451	0.04017	0.0451	0.001176	6.09%	97.02%
32		5	0.0451	0.04509	0.04511	0.0451	0.0451	0.0451	0	0.0%	96.89%

Graphics





Analyst: EG QA: AC 1/11/19

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

Analysis ID:	11-3000-4340	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**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	1.21%	2	4	2.828	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2	0.3497	2.227	0.055	8	0.6105	CDF	Non-Significant Effect
		4*	13.91	2.227	0.055	8	<0.0001	CDF	Significant Effect
		8*	57.05	2.227	0.055	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.697048	2.232349	3	1451	<0.0001	Significant Effect
Error	0.02460741	0.001537963	16			
Total	6.721655		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.535	11.34	0.3163	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.975	0.866	0.8557	Normal Distribution

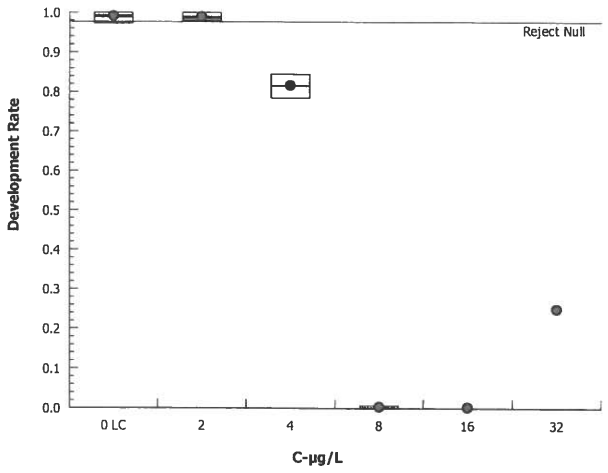
Development Rate Summary

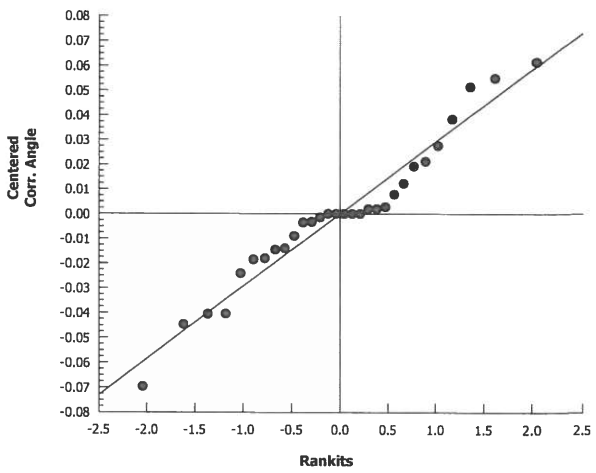
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9889	0.9736	1	0.9921	0.9724	1	0.005511	1.25%	0.0%
2		5	0.9879	0.9774	0.9984	0.9847	0.9787	1	0.003791	0.86%	0.1%
4		5	0.8165	0.7886	0.8443	0.8158	0.7846	0.8457	0.01002	2.75%	17.44%
8		5	0.002694	0	0.007311	0	0	0.007407	0.001663	138.1%	99.73%
16		5	0	0	0	0	0	0	0		100.0%
32		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	1.474	1.404	1.543	1.482	1.404	1.529	0.02509	3.81%	0.0%
2		5	1.465	1.414	1.516	1.447	1.424	1.526	0.01841	2.81%	0.59%
4		5	1.129	1.093	1.165	1.127	1.088	1.167	0.01294	2.56%	23.41%
8		5	0.05855	0.03158	0.08552	0.04456	0.04004	0.08617	0.009715	37.1%	96.03%
16		5	0.04367	0.03983	0.04752	0.04566	0.04017	0.04644	0.001384	7.09%	97.04%
32		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	64.47%

Graphics





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

Analysis ID:	00-5033-9234	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**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	1.64%	16	32	22.63	

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2	27	17	1	8	0.7639	Asymp	Non-Significant Effect
		4	28	17	1	8	0.8326	Asymp	Non-Significant Effect
		8	30	17	1	8	0.9275	Asymp	Non-Significant Effect
		16	22	17	2	8	0.3104	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02023075	0.005057688	4	1.73	0.1828	Non-Significant Effect
Error	0.05845465	0.002922733	20			
Total	0.0786854		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.299	4.893	0.3149	Equal Variances
Variances	Levene Equality of Variance	3.623	4.431	0.0224	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8573	0.8877	0.0024	Non-normal Distribution

Survival Rate Summary

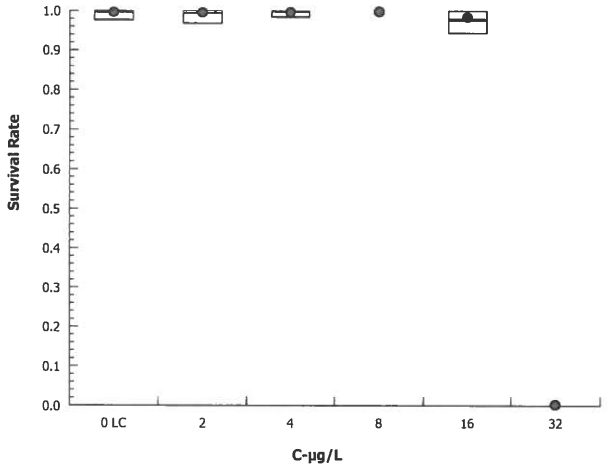
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9951	0.9816	1	1	0.9756	1	0.004878	1.1%	0.0%
2		5	0.9935	0.9754	1	1	0.9675	1	0.006504	1.46%	0.16%
4		5	0.9967	0.9877	1	1	0.9837	1	0.003252	0.73%	-0.16%
8		5	1	1	1	1	1	1	0	0.0%	-0.49%
16		5	0.9789	0.9498	1	0.9756	0.9431	1	0.01047	2.39%	1.63%
32		5	0	0	0	0	0	0	0		100.0%

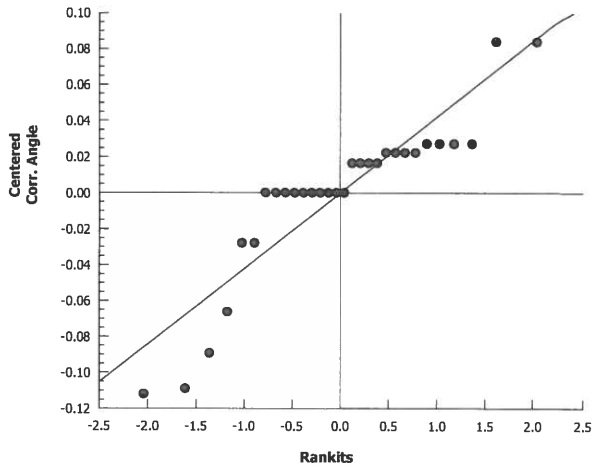
Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.503	1.441	1.565	1.526	1.414	1.526	0.02234	3.32%	0.0%
2		5	1.498	1.423	1.574	1.526	1.389	1.526	0.02725	4.07%	0.33%
4		5	1.509	1.463	1.555	1.526	1.443	1.526	0.01655	2.45%	-0.39%
8		5	1.526	1.525	1.526	1.526	1.526	1.526	0	0.0%	-1.49%
16		5	1.442	1.338	1.546	1.414	1.33	1.526	0.03751	5.82%	4.09%
32		5	0.0451	0.04509	0.04511	0.0451	0.0451	0.0451	0	0.0%	97.0%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	00-5033-9234	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	02-1891-3936	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	20280	1000	Yes	Two-Point Interpolation

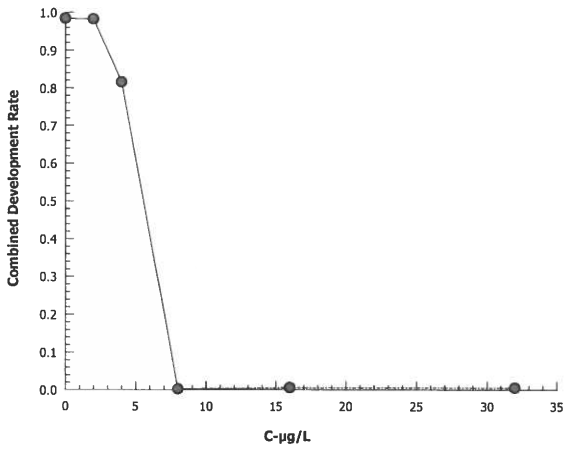
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	4.383	4.234	4.498
EC50	5.593	5.494	5.672

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	0.984	0.9724	1	0.005204	0.01164	1.18%	0.0%	673	684
2		5	0.9815	0.9512	1	0.008386	0.01875	1.91%	0.26%	650	662
4		5	0.8138	0.7846	0.8457	0.01077	0.02409	2.96%	17.29%	580	711
8		5	0.002694	0	0.007407	0.001663	0.003719	138.1%	99.73%	1	711
16		5	0	0	0	0	0		100.0%	0	677
32		5	0	0	0	0	0		100.0%	0	615

Graphics



Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	13-7938-6780	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1200573	1000	Yes	Two-Point Interpolation

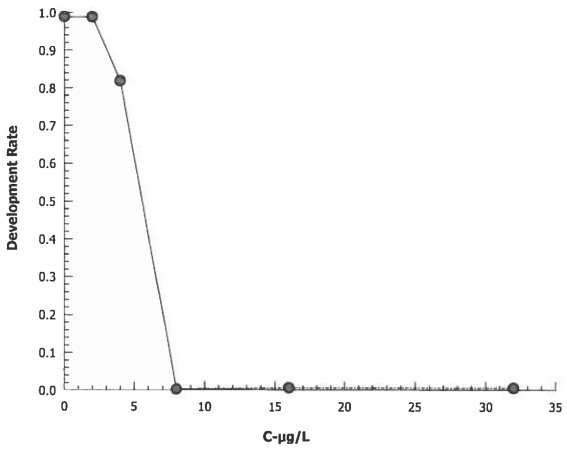
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	4.377	4.236	4.49
EC50	5.589	5.494	5.664

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	0.9889	0.9724	1	0.005511	0.01232	1.25%	0.0%	673	681
2		5	0.9879	0.9787	1	0.003791	0.008477	0.86%	0.1%	650	658
4		5	0.8165	0.7846	0.8457	0.01002	0.02241	2.75%	17.44%	580	709
8		5	0.002694	0	0.007407	0.001663	0.003719	138.1%	99.73%	1	711
16		5	0	0	0	0	0		100.0%	0	664
32		5	0	0	0	0	0		100.0%	0	5

Graphics



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

Analysis ID:	20-5520-1077	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	31 Dec-18 14:58	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1706025	1000	Yes	Two-Point Interpolation

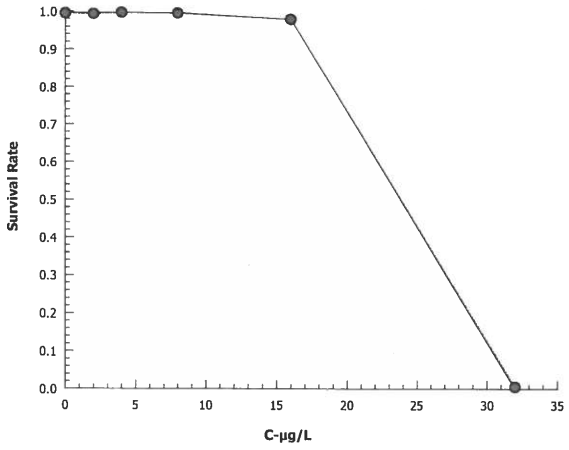
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	19.79	19.41	20.1
EC50	23.86	23.61	24.07

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	0.9951	0.9756	1	0.004878	0.01091	1.1%	0.0%	612	615
2		5	0.9935	0.9675	1	0.006504	0.01454	1.46%	0.16%	611	615
4		5	0.9967	0.9837	1	0.003252	0.007272	0.73%	-0.16%	613	615
8		5	1	1	1	0	0	0.0%	-0.49%	615	615
16		5	0.9789	0.9431	1	0.01047	0.02342	2.39%	1.63%	602	615
32		5	0	0	0	0	0		100.0%	0	615

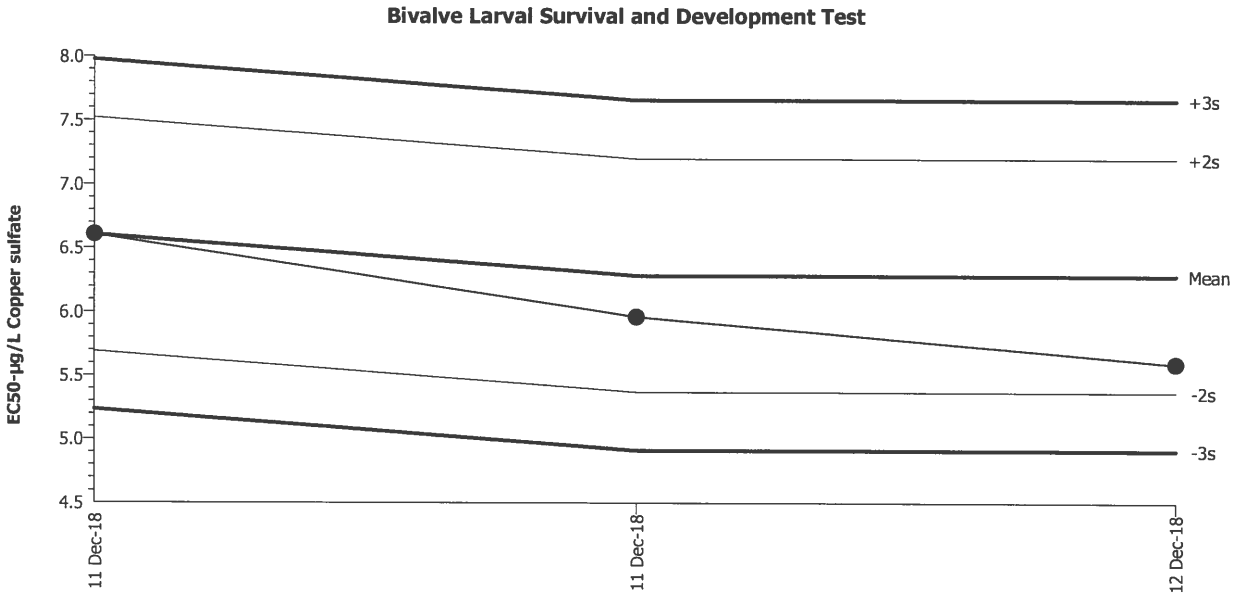
Graphics



CETIS QC Plot

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

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



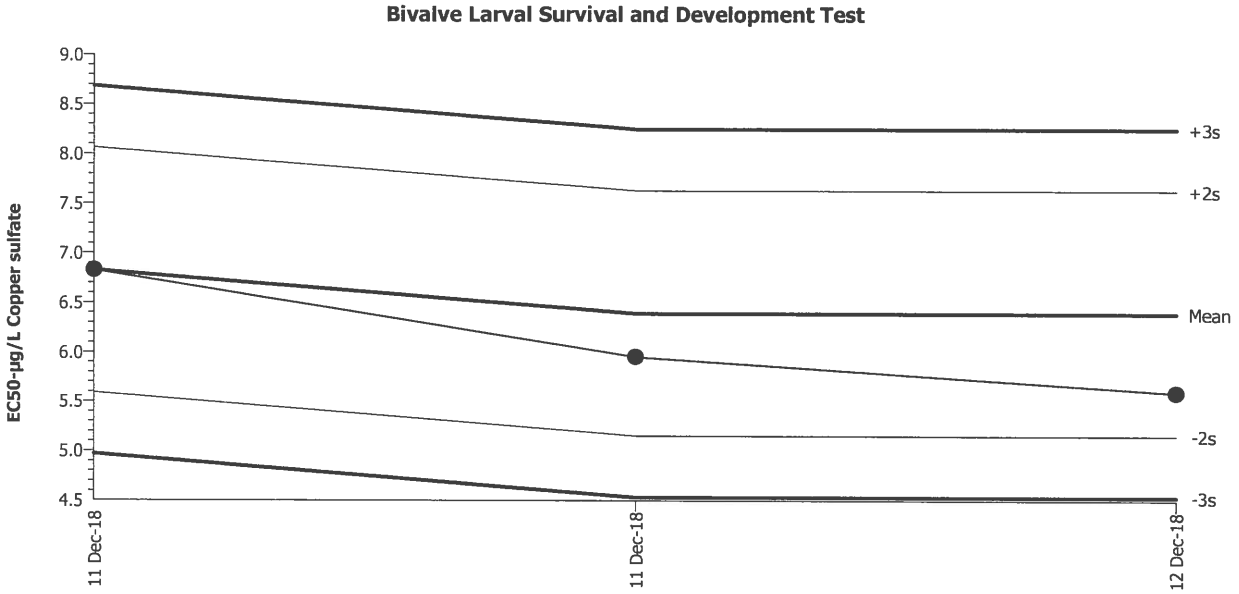
Mean:	6.282	**Count:**	2	**-2s Warning Limit:**	5.368	**-3s Action Limit:**	4.91
Sigma:	0.4574	**CV:**	7.28%	**+2s Warning Limit:**	7.197	**+3s Action Limit:**	7.655

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Dec	11	11:55	6.606	0.3239	0.7082			09-7408-5780	08-1757-8045
2			11	12:30	5.959	-0.3229	-0.706			01-7940-4185	15-9822-3312
3			12	13:55	5.593	-0.6888	-1.506			11-6161-8836	02-1891-3936

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

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



Mean:	6.39	**Count:**	2	**-2s Warning Limit:**	5.152	**-3s Action Limit:**	4.533
Sigma:	0.6192	**CV:**	9.69%	**+2s Warning Limit:**	7.629	**+3s Action Limit:**	8.248

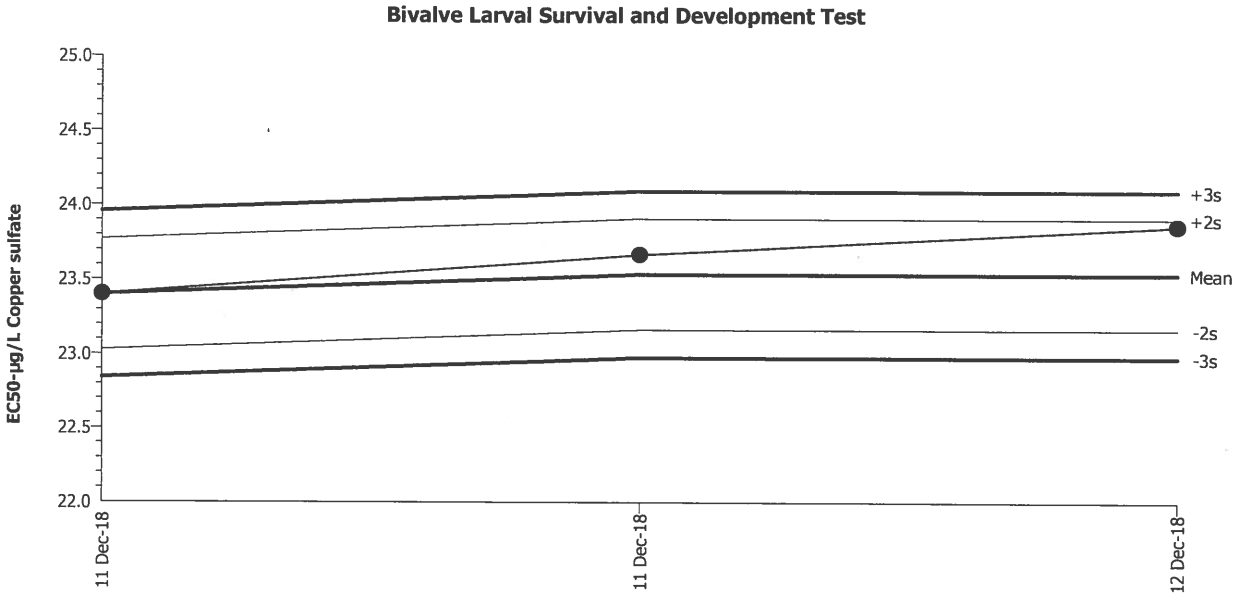
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Dec	11	11:55	6.828	0.4379	0.7072			09-7408-5780	03-3077-4520
2			11	12:30	5.952	-0.4377	-0.7069			01-7940-4185	03-0677-9138
3			12	13:55	5.589	-0.8006	-1.293			11-6161-8836	13-7938-6780

CETIS QC Plot

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

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



Mean:	23.53	**Count:**	2	**-2s Warning Limit:**	23.16	**-3s Action Limit:**	22.97
Sigma:	0.1862	**CV:**	0.79%	**+2s Warning Limit:**	23.9	**+3s Action Limit:**	24.09

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Dec	11	11:55	23.4	-0.1295	-0.6953			09-7408-5780	12-1829-1326
2			11	12:30	23.66	0.1338	0.7185			01-7940-4185	12-4826-8052
3			12	13:55	23.86	0.3271	1.757			11-6161-8836	20-5520-1077

CETIS Test Data Worksheet

Report Date: 12 Dec-18 07:55 (p 1 of 1)
Test Code: 11-6161-8836/181212msdvSO

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

Start Date: 12 Dec-18
End Date: 14 Dec-18
Sample Date: 12 Dec-18
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper sulfate
Sample Code: 181212msdvSO
Sample Source: Reference Toxicant
Sample Station: Copper Sulfate

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			71			155	~~0~~	12/22/18
			72			120	~~0~~	
			73			153	~~153~~ Ⓐ	Re-read JCL 12/27/18
			74			145	141	
			75			141	138	
			76			127	127	
			77			126	125	
			78			120	~~0~~	
			79			152	124	
			80			135	1	
			81			116	~~0~~	
			82			~~0~~	~~0~~	
			83			150	147	
			84			~~0~~	~~0~~	
			85			~~0~~	~~0~~	
			86			120	120	
			87			129	~~0~~	
			88			165	1	
			89			126	~~0~~	
			90			~~0~~	~~0~~	
			91			156	~~0~~	
			92			~~0~~	~~0~~	
			93			162	137	
			94			140	139	
			95			121	98	
			96			140	140	
			97			119	117	
			98			131	129	
			99			144	119	
			100			130	102	

Ⓐ Q18 JCL 12/27/18

CETIS Test Data Worksheet

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

Start Date: 12 Dec-18 | **Species:** Mytilus galloprovincialis | **Sample Code:** 181212msdvSO
End Date: 14 Dec-18 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 12 Dec-18 | **Material:** Copper sulfate | **Sample Station:** Copper Sulfate

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	77					
0	LC	2	86					
0	LC	3	96			143	143	SG 12/15/18
0	LC	4	83					
0	LC	5	74					
2.5		1	75					
2.5		2	76					
2.5		3	97					
2.5		4	98			126	124	SG 12/15/18
2.5		5	94					
5		1	93					
5		2	100					
5		3	99			148	118	SG 12/15/18
5		4	79					
5		5	95					
10		1	91					
10		2	80					
10		3	87					
10		4	88			166	2	SG 12/15/18
10		5	89					
20		1	72					
20		2	73					
20		3	81					
20		4	71			142	0	SG 12/15/18
20		5	78					
40		1	92					
40		2	85					
40		3	84			0	0	SG 12/15/18
40		4	82					
40		5	90					

QC: EG

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
Sample ID: $CuSO_4$
Test No.: 181212msdvSO

Test Species: *M. galloprovincialis*
Start Date/Time: 12/12/2018 1355
End Date/Time: 12/14/2018 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	31.2	31.4	31.4	15.2	15.3	15.2	8.5	8.4	7.9	8.05	7.98	7.93
2.5 2	31.5	31.6	31.6	15.2	15.2	15.0	8.4	8.4	7.9	8.06	7.97	7.92
5 4	31.4	31.7	31.6	15.1	15.3	15.0	8.4	8.3	7.9	8.06	7.98	7.92
10 8	31.4	31.6	31.6	15.1	15.5	15.1	8.5	8.3	7.9	8.07	7.98	7.92
20 16	31.4	31.6	31.6	15.2	15.5	15.2	8.4	8.2	7.9	8.07	7.98	7.92
40 32	31.4	31.6	31.5	15.2	15.5	15.2	8.4	8.2	7.8	8.08	7.99	7.92
(A)												

Technician Initials:

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

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	2.0
Final Volume (mL):	500
Cu stock concentration (µg/L):	(A) ~~10,000~~ 8,000

Comments:
0 hrs: (A) EG 1218 12/31/18; adjusted for measured stock concentration
24 hrs:
48 hrs:

QC Check: MH/EG 12/31/18

Final Review: EC 1/11/19

Marine Chronic Bioassay | **Larval Development Worksheet**

Client: Internal
Test No.: 181212 msdv50 ~~A~~ EC 12/31/18
Test Species: M. galloprovincialis
Animal Source: Taylor Shellfish
Date Received: 9/25/18 and 10/30/18
Test Chambers: 30 mL shell vial
Sample Volume: 10 mL

Start Date/Time: 12/12/2018 1355
End Date/Time: 12/14/2018 1230
Technician Initials: EC

Spawn Information

First Gamete Release Time: 1130

Sex	Number Spawning
Male	6
Female	2

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	2, 3, 6	good density and motility
Female 1	1	good density, round, pale color
Female 2	2	good to ok density, round, pale color
Female 3	–	–

Egg Fertilization Time: 1210

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: 1

Embryo Inoculum Preparation

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

Number Counted:		
	6	12
	4	10
	5	6
	7	12
	5	12

Mean: 7.9

Mean 7.9 X 50 = 395 embryos/ml

Initial Density: 395
Desired Final Density: 300
(to inoculate with 0.5 ml)
= 1.32 (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

Rand. No.	No. Dividing	Total	% Dividing	Mean % Dividing
T#1	132	132	100	100
T#2	114	114	100	
T#3	106	106	100	
T#4	134	134	100	
T#5	124	124	100	
T#6	127	127	100	

$\bar{x}$ 122.8

48-h QC: 141/142
—

Comments:

QC Check: PH/EC 12/31/18

Final Review: AC 1/1/19

Appendix E
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.