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TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: March 2019

Prepared for: Jacobs
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Date Submitted: May 13, 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.

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Introduction

A toxicity test was performed using a groundwater composite sample collected on March 19, 2019 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 March 20 and 22, 2019.

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	031919
Nautilus Log-in Number	19-0392
Collection Date; Time	3/19/2019; 0933h
Receipt Date; Time	3/20/2019; 0950h
Receipt Temperature (°C)	2.5
Dissolved Oxygen (mg/L)	8.6
pH	7.47
Conductivity (µS/cm)	11,110
Salinity (ppt)	7.2
Alkalinity (mg/L CaCO ₃)	413
Total Chlorine (mg/L)	0.04
Total Ammonia (mg/L)	1.2

Test Methods

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

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

Test Period	3/20/2019, 1525h to 3/22/2019, 1500h
Test Organism	<i>Mytilus galloprovincialis</i>
Test Organism Source	Mission Bay (San Diego, CA)
Test Organism Age	4 hours post fertilization
Test Duration	48 ± 2 hours
Test Type	Static
Test Chamber, Test Solution Volume	30 mL glass vial, 10 mL
Test Temperature	15 ± 1°C
Dilution Water	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake)
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 93.3
Test Concentrations (% sample)	73.5 ^a , 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper sulfate (per project QAPP) ^b
Statistical Software	CETIS™ 1.8.7.20

^a Highest concentration tested due to the addition of hypersaline brine

^b Enthelpy typically uses copper chloride for reference toxicant testing

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 73.50 (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	73.5	> 73.5	< 1.36	> 73.5
	Survival	73.5	> 73.5	< 1.36	> 73.5

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 (IC₂₅) = 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)	97.3	96.4
0 (Lab Control)	99.1	97.9
2	99.0	97.0
4	99.7	98.3
9	99.2	97.5
18	100	97.4
35	97.3	97.8
73.5 ^a	99.0	97.1

^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 tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. The results for the copper chloride reference toxicant test were within the acceptable range of the mean historical test results plus or minus two standard deviations. However, the results for the copper sulfate reference toxicant test were greater than three standard deviations of the mean historical test results. Reference toxicant warning and control chart limits were recalculated based on the 75th percentile interlaboratory coefficient of variation, as defined in EPA-833-R-00-003, for comparison purposes only, and the effect concentrations were within three standard deviations of the historical means. Copper chloride reference toxicant results are reported in addition to the copper sulfate results as a point of comparison and to provide additional support for the appreciable sensitivity of the test organism batch. It should also be noted that the control chart for copper sulfate is still in development as a full 20-point history is not yet available.

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

Table 5. Reference Toxicant Test Results

Species and Material	Endpoint	EC ₅₀ (µg/L Copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve / Copper Sulfate	Development Rate	11.9	6.12 ± 1.27	10.4
	Survival Rate	29.4	23.6 ± 0.458	0.97
Bivalve / Copper Chloride	Development Rate	8.33	7.65 ± 1.85	12.1
	Survival Rate	29.2	28.0 ± 6.68	11.9

Effect Concentration 50 (EC₅₀) = 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

Report Date: 03 Apr-19 10:21 (p 1 of 2)
 Test Code: 1903-S059 | 01-5343-3513

Bivalve Larval Survival and Development Test							Nautilus Environmental (CA)				
Batch ID:	02-8063-4317	Test Type:	Development-Survival			Analyst:					
Start Date:	20 Mar-19 15:25	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Diluted Natural Seawater				
Ending Date:	22 Mar-19 15:00	Species:	Mytilus galloprovincialis			Brine:	Frozen Seawater				
Duration:	48h	Source:	Mission Bay			Age:					
Sample ID:	17-9611-0978	Code:	19-0392			Client:	Jacobs				
Sample Date:	19 Mar-19 09:33	Material:	Effluent Sample			Project:					
Receive Date:	20 Mar-19 09:50	Source:	Jacobs								
Sample Age:	30h (2.5 °C)	Station:	Wyckoff <i>Eagle Harbor GWTP Effluent</i>								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
21-1968-8609	Development Rate	73.5	>73.5	NA	1.9%	< 1.361	Dunnett Multiple Comparison Test				
12-4129-7583	Survival Rate	73.5	>73.5	NA	3.16%	< 1.361	Steel Many-One Rank Sum Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
15-3713-8684	Development Rate	EC25	>73.5	N/A	N/A	<1.361	Linear Interpolation (ICPIN)				
		EC50	>73.5	N/A	N/A	<1.361					
11-8603-6224	Survival Rate	EC25	>73.5	N/A	N/A	<1.361	Linear Interpolation (ICPIN)				
		EC50	>73.5	N/A	N/A	<1.361					
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits	Overlap	Decision				
15-3713-8684	Development Rate	Control Resp		0.9641	0.9 - NL	Yes	Passes Acceptability Criteria				
21-1968-8609	Development Rate	Control Resp		0.9641	0.9 - NL	Yes	Passes Acceptability Criteria				
11-8603-6224	Survival Rate	Control Resp		0.9725	0.5 - NL	Yes	Passes Acceptability Criteria				
12-4129-7583	Survival Rate	Control Resp		0.9725	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	0.9641	0.9461	0.9822	0.9415	0.977	0.0065	0.01453	1.51%	0.0%
0	Lab Control	5	0.9788	0.964	0.9936	0.9646	0.995	0.00532	0.0119	1.22%	-1.52%
2		5	0.9698	0.955	0.9845	0.9545	0.9832	0.005323	0.0119	1.23%	-0.59%
4		5	0.9829	0.9739	0.9919	0.9758	0.9949	0.003227	0.007216	0.73%	-1.95%
9		5	0.9749	0.9637	0.9861	0.9659	0.9848	0.004047	0.009049	0.93%	-1.12%
18		5	0.9735	0.9672	0.9798	0.9683	0.9804	0.00228	0.005098	0.52%	-0.97%
35		5	0.9782	0.9688	0.9877	0.9688	0.9882	0.003406	0.007615	0.78%	-1.46%
73.5		5	0.9713	0.9575	0.985	0.957	0.9827	0.004956	0.01108	1.14%	-0.74%
Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9725	0.8963	1	0.8626	1	0.02747	0.06143	6.32%	0.0%
0	Lab Control	5	0.9912	0.9668	1	0.956	1	0.008791	0.01966	1.98%	-1.92%
2		5	0.9901	0.9718	1	0.967	1	0.006593	0.01474	1.49%	-1.81%
4		5	0.9967	0.9876	1	0.9835	1	0.003297	0.007372	0.74%	-2.49%
9		5	0.9923	0.971	1	0.9615	1	0.007692	0.0172	1.73%	-2.03%
18		5	1	1	1	1	1	0	0	0.0%	-2.83%
35		5	0.9725	0.9258	1	0.9286	1	0.01685	0.03767	3.87%	0.0%
73.5		5	0.9901	0.9627	1	0.9505	1	0.00989	0.02211	2.23%	-1.81%

CETIS Summary Report

Report Date: 03 Apr-19 10:21 (p 2 of 2)
 Test Code: 1903-S059 | 01-5343-3513

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	0.9618	0.9769	0.9635	0.977	0.9415	
0	Lab Control	0.9857	0.995	0.9646	0.977	0.9716	
2		0.9697	0.9794	0.962	0.9545	0.9832	
4		0.9949	0.9797	0.9832	0.9758	0.9809	
9		0.9681	0.9844	0.9659	0.9714	0.9848	
18		0.9683	0.9724	0.9695	0.977	0.9804	
35		0.9688	0.9787	0.9731	0.9882	0.9824	
73.5		0.9795	0.957	0.9747	0.9624	0.9827	
Survival Rate Detail							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.8626	1	1	1	1	
0	Lab Control	1	1	1	0.956	1	
2		1	1	1	0.967	0.9835	
4		1	1	0.9835	1	1	
9		1	1	1	0.9615	1	
18		1	1	1	1	1	
35		1	1	1	0.9286	0.9341	
73.5		1	1	1	1	0.9505	
Development Rate Binomials							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	151/157	211/216	211/219	212/217	177/188	
0	Lab Control	207/210	199/200	191/198	170/174	205/211	
2		192/198	190/194	177/184	168/176	176/179	
4		195/196	193/197	176/179	202/207	205/209	
9		182/188	189/192	198/205	170/175	194/197	
18		183/189	211/217	191/197	212/217	200/204	
35		186/192	184/188	181/186	167/169	167/170	
73.5		191/195	178/186	193/198	205/213	170/173	
Survival Rate Binomials							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	157/182	182/182	182/182	182/182	182/182	
0	Lab Control	182/182	182/182	182/182	174/182	182/182	
2		182/182	182/182	182/182	176/182	179/182	
4		182/182	182/182	179/182	182/182	182/182	
9		182/182	182/182	182/182	175/182	182/182	
18		182/182	182/182	182/182	182/182	182/182	
35		182/182	182/182	182/182	169/182	170/182	
73.5		182/182	182/182	182/182	182/182	173/182	

CETIS Analytical Report

Report Date: 03 Apr-19 10:20 (p 1 of 4)
 Test Code: 1903-S059 | 01-5343-3513

Bivalve Larval Survival and Development Test				Nautilus Environmental (CA)			
Analysis ID: 21-1968-8609	Endpoint: Development Rate			CETIS Version: CETISv1.8.7			
Analyzed: 03 Apr-19 10:18	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	1.9%	73.5	>73.5	NA	1.361

Dunnett Multiple Comparison Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-0.7989	2.407	0.047	8	0.9781	CDF	Non-Significant Effect
		4	-3.048	2.407	0.047	8	1.0000	CDF	Non-Significant Effect
		9	-1.569	2.407	0.047	8	0.9980	CDF	Non-Significant Effect
		18	-1.263	2.407	0.047	8	0.9945	CDF	Non-Significant Effect
		35	-2.112	2.407	0.047	8	0.9997	CDF	Non-Significant Effect
		73.5	-1.01	2.407	0.047	8	0.9880	CDF	Non-Significant Effect

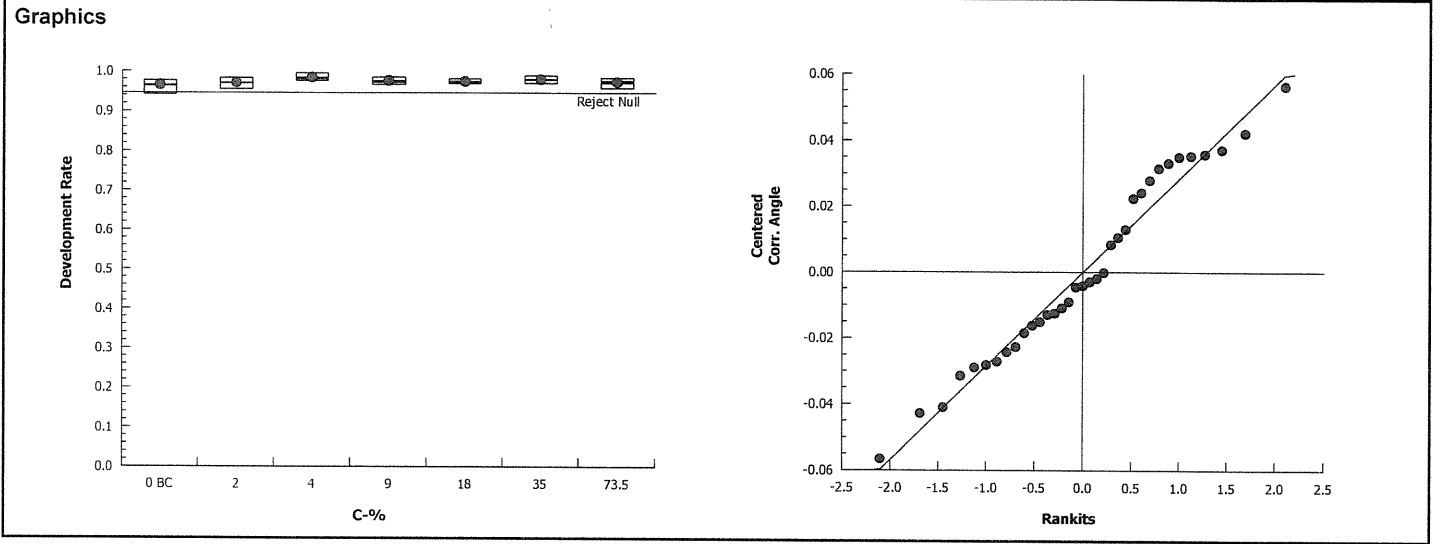
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01108306	0.001847177	6	1.915	0.1133	Non-Significant Effect
Error	0.0270081	0.0009645749	28			
Total	0.03809116		34			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	2.769	16.81	0.8373	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9674	0.9146	0.3754	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	0.9641	0.9461	0.9822	0.9635	0.9415	0.977	0.0065	1.51%	0.0%
2		5	0.9698	0.955	0.9845	0.9697	0.9545	0.9832	0.005323	1.23%	-0.59%
4		5	0.9829	0.9739	0.9919	0.9809	0.9758	0.9949	0.003227	0.73%	-1.95%
9		5	0.9749	0.9637	0.9861	0.9714	0.9659	0.9848	0.004047	0.93%	-1.12%
18		5	0.9735	0.9672	0.9798	0.9724	0.9683	0.9804	0.002279	0.52%	-0.97%
35		5	0.9782	0.9688	0.9877	0.9787	0.9688	0.9882	0.003406	0.78%	-1.46%
73.5		5	0.9713	0.9575	0.985	0.9747	0.957	0.9827	0.004956	1.14%	-0.74%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.383	1.336	1.43	1.378	1.326	1.418	0.017	2.75%	0.0%
2		5	1.399	1.355	1.443	1.396	1.356	1.441	0.0158	2.53%	-1.14%
4		5	1.443	1.402	1.484	1.432	1.415	1.499	0.0147	2.28%	-4.33%
9		5	1.414	1.377	1.451	1.401	1.385	1.447	0.01345	2.13%	-2.23%
18		5	1.408	1.388	1.428	1.404	1.392	1.43	0.007247	1.15%	-1.79%
35		5	1.425	1.391	1.458	1.424	1.393	1.462	0.01201	1.89%	-3.0%
73.5		5	1.403	1.362	1.444	1.411	1.362	1.439	0.01476	2.35%	-1.43%

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 21-1968-8609	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 03 Apr-19 10:18	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

Report Date: 03 Apr-19 10:21 (p 3 of 4)
 Test Code: 1903-S059 | 01-5343-3513

Bivalve Larval Survival and Development Test							Nautilus Environmental (CA)				
Analysis ID: 12-4129-7583		Endpoint: Survival Rate			CETIS Version: CETISv1.8.7						
Analyzed: 03 Apr-19 10:18		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	3.16%	73.5	>73.5	NA	1.361

Steel Many-One Rank Sum Test									
Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	26	16	1	8	0.7547	Asymp	Non-Significant Effect
		4	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		9	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		18	30	16	1	8	0.9557	Asymp	Non-Significant Effect
		35	26	16	1	8	0.7547	Asymp	Non-Significant Effect
		73.5	28	16	1	8	0.8838	Asymp	Non-Significant Effect

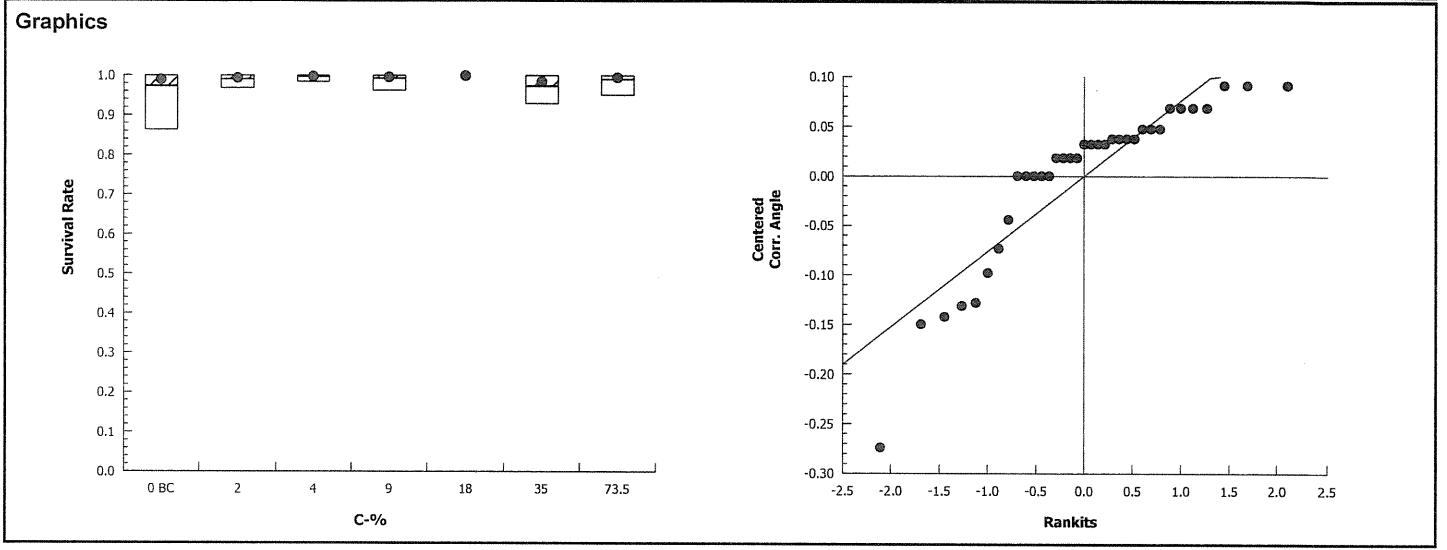
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02799448	0.004665747	6	0.568	0.7522	Non-Significant Effect
Error	0.2300117	0.008214704	28			
Total	0.2580062		34			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	115.6	16.81	<0.0001	Unequal Variances	
Distribution	Shapiro-Wilk W Normality	0.8242	0.9146	<0.0001	Non-normal Distribution	

Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9725	0.8963	1	1	0.8626	1	0.02747	6.32%	0.0%
2		5	0.9901	0.9718	1	1	0.967	1	0.006593	1.49%	-1.81%
4		5	0.9967	0.9876	1	1	0.9835	1	0.003296	0.74%	-2.49%
9		5	0.9923	0.971	1	1	0.9615	1	0.007692	1.73%	-2.03%
18		5	1	1	1	1	1	1	0	0.0%	-2.83%
35		5	0.9725	0.9258	1	1	0.9286	1	0.01685	3.87%	0.0%
73.5		5	0.9901	0.9627	1	1	0.9505	1	0.00989	2.23%	-1.81%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.465	1.275	1.655	1.534	1.191	1.534	0.06852	10.46%	0.0%
2		5	1.486	1.402	1.57	1.534	1.388	1.534	0.03027	4.55%	-1.44%
4		5	1.515	1.464	1.566	1.534	1.442	1.534	0.01833	2.71%	-3.43%
9		5	1.502	1.413	1.591	1.534	1.373	1.534	0.03206	4.78%	-2.49%
18		5	1.534	1.534	1.534	1.534	1.534	1.534	0	0.0%	-4.68%
35		5	1.443	1.287	1.598	1.534	1.3	1.534	0.05589	8.66%	1.55%
73.5		5	1.496	1.392	1.6	1.534	1.347	1.534	0.03744	5.59%	-2.12%

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 12-4129-7583	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7	
Analyzed: 03 Apr-19 10:18	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

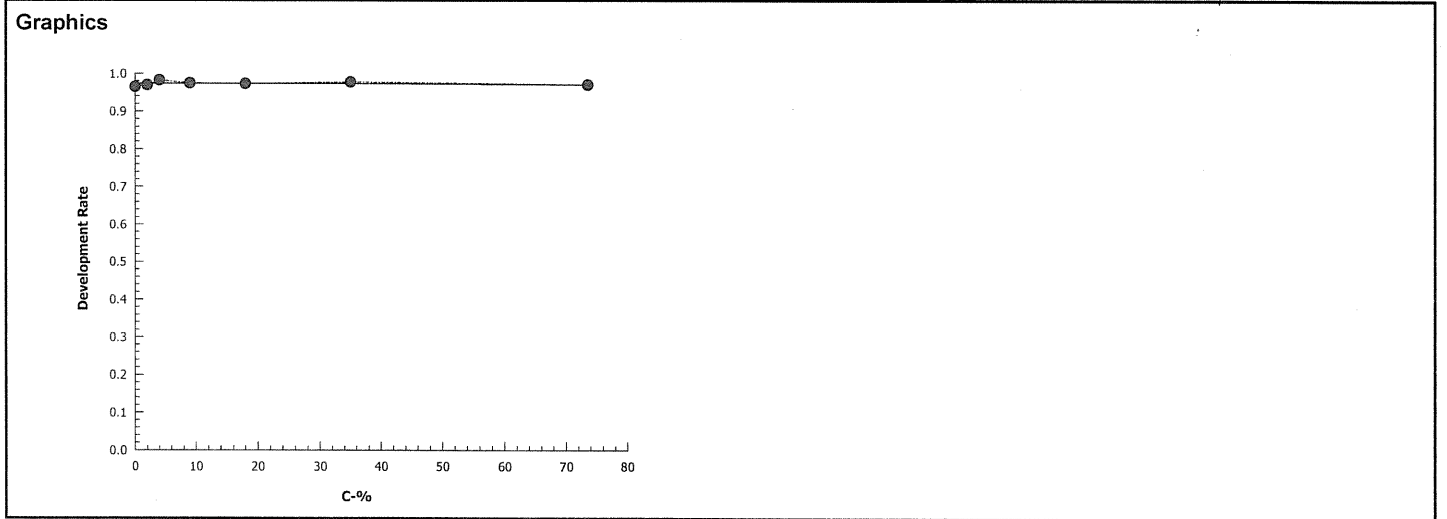
Report Date: 03 Apr-19 10:21 (p 1 of 2)
 Test Code: 1903-S059 | 01-5343-3513

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 15-3713-8684	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 03 Apr-19 10:16	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1967278	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.5	N/A	N/A	<1.361	NA	NA
EC50	>73.5	N/A	N/A	<1.361	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	0.9641	0.9415	0.977	0.0065	0.01453	1.51%	0.0%	962	997
2		5	0.9698	0.9545	0.9832	0.005323	0.0119	1.23%	-0.59%	903	931
4		5	0.9829	0.9758	0.9949	0.003227	0.007217	0.73%	-1.95%	971	988
9		5	0.9749	0.9659	0.9848	0.004047	0.00905	0.93%	-1.12%	933	957
18		5	0.9735	0.9683	0.9804	0.002279	0.005097	0.52%	-0.97%	997	1024
35		5	0.9782	0.9688	0.9882	0.003406	0.007616	0.78%	-1.46%	885	905
73.5		5	0.9713	0.957	0.9827	0.004956	0.01108	1.14%	-0.74%	937	965



CETIS Analytical Report

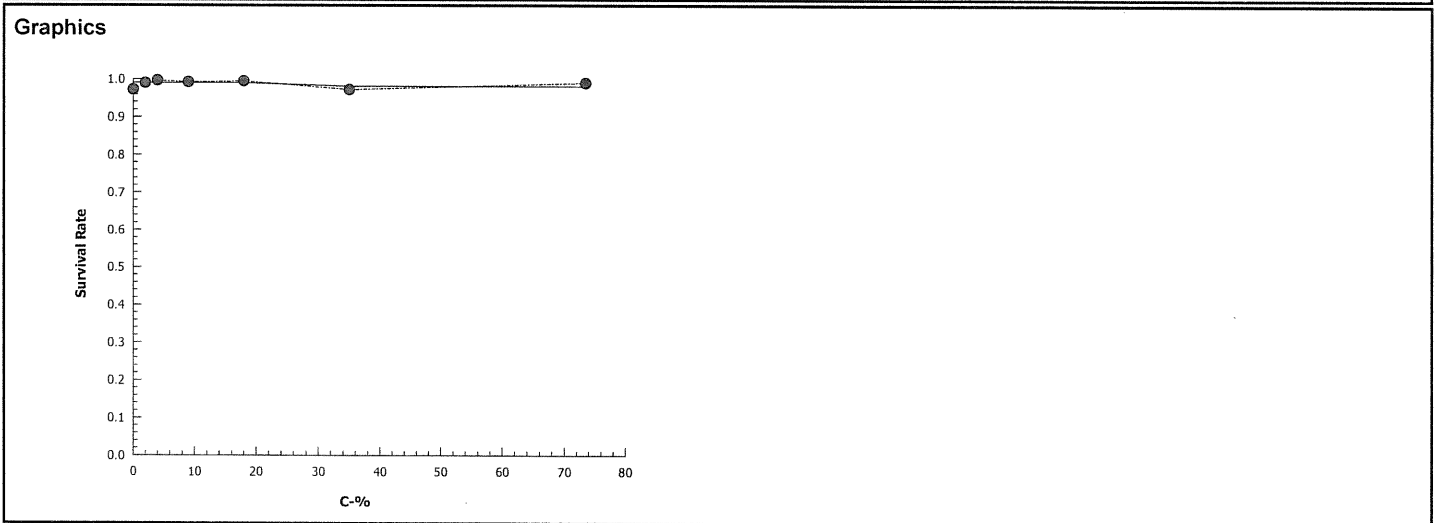
Report Date: 03 Apr-19 10:21 (p 2 of 2)
 Test Code: 1903-S059 | 01-5343-3513

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 11-8603-6224	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 03 Apr-19 10:16	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	675368	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.5	N/A	N/A	<1.361	NA	NA
EC50	>73.5	N/A	N/A	<1.361	NA	NA

Survival Rate Summary			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.9725	0.8626	1	0.02747	0.06143	6.32%	0.0%	885	910
2		5	0.9901	0.967	1	0.006593	0.01474	1.49%	-1.81%	901	910
4		5	0.9967	0.9835	1	0.003296	0.007371	0.74%	-2.49%	907	910
9		5	0.9923	0.9615	1	0.007692	0.0172	1.73%	-2.03%	903	910
18		5	1	1	1	0	0	0.0%	-2.83%	910	910
35		5	0.9725	0.9286	1	0.01685	0.03767	3.87%	0.0%	885	910
73.5		5	0.9901	0.9505	1	0.00989	0.02211	2.23%	-1.81%	901	910



CETIS Test Data Worksheet

Report Date: 15 Mar-19 15:31 (p 1 of 1)

Test Code: 1963-5059 01-5343-3513/92535A9

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 20 Mar-19

Species: *Mytilus galloprovincialis*

Sample Code: 19- 0392

End Date: 22 Mar-19

Protocol: EPA/600/R-95/136 (1995)

Sample Source: Jacobs

Sample Date: 19 Mar-19

Material: Effluent Sample

Sample Station: Wyckoff G.WTP

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			61			175	170	3/29/19
			62			184	177	
			63			189	183	
			64			217	212	
			65			192	186	
			66			188	182	
			67			198	191	
			68			204	200	
			69			197	191	
			70			217	211	
			71			179	176	
			72			200	199	
			73			198	192	
			74			196	195	
			75			211	205	
			76			188	184	
			77			213	205	
			78			219	211	
			79			186	181	
			80			195	191	
			81			210	207	
			82			217	212	
			83			209	205	
			84			157	151	
			85			197	194	
			86			170	167	
			87			174	170	
			88			188	177	
			89			192	189	
			90			207	202	
			91			179	176	
			92			173	170	
			93			194	190	
			94			197	193	
			95			176	168	
			96			216	211	
			97			205	198	
			98			169	167	
			99			198	193	
			100			186	178	

CETIS Test Data Worksheet

Report Date: 15 Mar-19 15:31 (p 1 of 1)
 Test Code: 1403-5059 01-5343-3513/92535A9

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 20 Mar-19
 End Date: 22 Mar-19
 Sample Date: 19 Mar-19

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

Sample Code: 19-0392
 Sample Source: Jacobs
 Sample Station: Wyckoff *GWTP*

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	84					
0	BC	2	96					
0	BC	3	78			191	185	RT 3/22/19
0	BC	4	82					
0	BC	5	88					
0	LC	1	81					
0	LC	2	72					
0	LC	3	67			177	170	RT 3/23/19
0	LC	4	87					
0	LC	5	75					
2		1	73					
2		2	93					
2		3	62			166	160	RT 3/23/19
2		4	95					
2		5	91					
4		1	74					
4		2	94					
4		3	71			160	156	RT 3/23/19
4		4	90					
4		5	83					
9		1	66					
9		2	89					
9		3	97			192	184	RT 3/23/19
9		4	61					
9		5	85					
18		1	63					
18		2	70					
18		3	69					
18		4	64			186	180	RT 3/23/19
18		5	68					
35		1	65					
35		2	76					
35		3	79					
35		4	98			154	151	RT 3/23/19
35		5	86					
73.5	74	1	80					
73.5	74	2	100					
73.5	74	3	99					
73.5	74	4	77			186	178	RT 3/22/19
73.5	74	5	92					

73.5 High Brine @
 73.5
 73.5
 73.5

QC = BO

@Q18 BO 3/15/19

Marine Chronic Bioassay

Water Quality Measurements

Client: Jacobs
 Sample ID: Wyckoff Eagle Harbor WTP Effluent
 Sample Log No.: 19-0392
 Test No.: 1903-5059

Test Species: M. galloprovincialis
 Start Date/Time: 3/20/2019 1525
 End Date/Time: 3/22/2019 1500

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	30.0	29.9	29.8	15.6	15.2	15.1	8.3	7.9	8.0	7.98	8.00	7.94
Brine Control	30.3	30.3	30.3	15.5	15.1	15.0	8.0	7.9	8.1	8.12	8.06	7.97
2	30.0	30.1	30.0	16.0	15.1	14.8	8.2	8.0	8.1	7.96	8.03	7.99
4	30.1	30.2	30.2	16.0	15.5	15.1	8.2	7.9	8.2	7.98	8.05	8.01
9	30.1	30.2	30.3	16.0	15.4	15.1	8.2	7.9	8.2	7.92	8.07	8.07
18	30.1	30.1	30.2	15.9	15.3	15.1	8.2	8.0	8.2	7.85	8.08	8.12
35	30.0	29.9	30.1	15.8	15.5	15.3	8.2	7.9	8.1	7.76	8.11	8.20
73.5% High Brine (A)	29.9	29.7	29.8	15.6	15.6	15.2	8.2	7.8	8.2	7.65	8.11	8.26

Technician Initials: _____
 WQ Readings:

0	24	48
BO	JBS	BO

 Dilutions made by:

BO		
----	--	--

Comments: 0 hrs: (A) EG Q18 4/2/19
 24 hrs: _____
 48 hrs: _____

QC Check: EG 4/2/19 Final Review: AC 4/17/19

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: JACOBS

Analyst: BO

Sample ID: Wyckoff Eagle Harbor GWTP Eff.

Test Date: 3/20/2019

Test No: 1903-S059

Test Type: Mussel Development

Salinity of Effluent 7.2

Salinity of Brine 93.3

Date of Brine used: 1/30/2019

Target Salinity 30

Alkalinity of Brine Control: 106 mg/L as CaCO3

Test Dilution Volume 250

	<u>Effluent</u>	<u>Brine Control</u>
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	<u>0.36</u>	<u>0.47</u>
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	0.36	1.8	250
4	10.0	0.36	3.6	250
9	22.5	0.36	8.1	250
18	45.0	0.36	16.2	250
35	87.5	0.36	31.5	250
73.5	183.8	0.36	66.2	250

DI Volume				
Brine Control	139.7	0.47	66.2	250

Total Brine Volume Required (ml): 193.6

QC Check: Ea 4/2/19

Final Review: AC 4/17/19

Client: Jacobs-Wyckoff G/TP
 Test No.: 1903-S059
 Test Species: M. galloprovincialis
 Animal Source: Mission Bay
 Date Received: 3/5/19
 Test Chambers: 30 ml shell vial
 Sample Volume: 10 mL

Start Date/Time: 3/20/2019 1525
 End Date/Time: 3/22/2019 1500
 Technician Initials: BD/KS/FG

Spawn Information

First Gamete Release Time: 1113

Sex	Number Spawning
Male	4
Female	4

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1,3,4	good motility and density
Female 1	1	pale yellow color, good density, some round
Female 2	2	white color, good density, mostly round
Female 3	3	orange color, good density, mostly round

Egg Fertilization Time: 1253

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	N/A
Female 2	N/A
Female 3	100%

Stock(s) chosen for testing: 3

Embryo Inoculum Preparation

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

Number Counted:

<u>15</u>	<u>15</u>
<u>18</u>	<u>19</u>
<u>19</u>	<u>18</u>
<u>17</u>	<u>19</u>
<u>19</u>	<u>19</u>

Mean: 17.8

Mean 17.8 × 50 = 890 embryos/ml

Initial Density: 890 = 2.96 (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
1	186	187	99	99%
2	168	169	99	
3	189	191	99	
4	192	194	99	
5	166	168	99	
6	192	193	99	

48-h QC: 169/174 = 97.1%

Comments:

$\bar{x} = 182.2$

QC Check: EG 4/2/19

Final Review: AK 4/17/19

Appendix B
Sample Check-In Information

Nautilus Environmental
 4340 Vandever Avenue
 San Diego, CA 92120

Client: JACOBS
 Sample ID: WycKoff - 031919 Eagle Harbor GWTP Effluent
 Test ID No(s): 1903-5059

Sample Check-In Information

Sample Description:
A: COLORLESS, CLEAR, ODORLESS, NO DEBRIS

Sample (A, B, C):	<u>A</u>			
Log-in No. (19-xxxx):	<u>0392</u>			
Sample Collection Date & Time:	<u>3/19/19 0933</u>			
Sample Receipt Date & Time:	<u>3/20/19 0950</u>			
Number of Containers & Container Type:	<u>1-1L CURB</u>			
Approx. Total Volume Received (L):	<u>~1L</u>			
Check-in Temperature (°C)	<u>2.5</u>			
Temperature OK? ¹	<u>(Y) N</u>	Y N	Y N	Y N
DO (mg/L)	<u>8.6</u>			
pH (units)	<u>7.47</u>			
Conductivity (µS/cm)	<u>11,110</u>			
Salinity (ppt)	<u>7.2</u>			
Alkalinity (mg/L) ²	<u>413</u>			
Hardness (mg/L) ^{2,3}	<u>—</u>			
Total Chlorine (mg/L)	<u>0.04</u>			
Technician Initials	<u>TN</u>			

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:			

Cl₂ 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 TN B — C —

QC Check: EG 4/2/19

Final Review: 5/13/19

Test Performed: Mussel Development Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____

Alkalinity: 106 Hardness or Salinity: 30 ppt

Additional Control? (Y) N = Brine Control Alkalinity: 106 Hardness or Salinity: 30 ppt

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: _____

**Total Ammonia Analysis
Freshwater**

Overlying Water

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 3/20/2019

Analyst: NM/EG
Analysis Date: 5/7/19

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 NH₃)		NA	NA	9.0	11.0
Wyckoff		3/20/2019	Check In	1.2	1.5
Spike Check (10 mg/L NH₃)		NA	NA		
Sample Duplicate ^a		NA	NA	1.1	1.3
Sample Duplicate + Spike ^a		NA	NA	10.5	12.8
Spike Check (10 mg/L NH₃)		NA	NA	9.0	11.0

Relative Percent Difference (RPD) = $\frac{[\text{sample}] (\text{mg/L}) - [\text{sample duplicate}] (\text{mg/L})}{[\text{average ammonia}] (\text{mg/L})} \times 100$

Acceptable Range: 0-20%

Percent Recovery = $\frac{[\text{spiked sample}] (\text{mg/L}) - [\text{sample}] (\text{mg/L})}{\text{nominal} [\text{spike}] (\text{mg/L})} \times 100$

Acceptable Range: 80-120%^b

QC Sample ID	[NH ₃]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0	NA	11.0	10	NA	110
Wyckoff	1.5	1.3	12.8	10	14.3	113

Comments: _____

Notes: ^aUnless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

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

^cCalculation not performed due to one or both values below the method detection limit.

Method Detection Limit = 0.5 mg/L

QC Check: EG 5/10/19

Final Review: EG 5/11/19

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)

Date Shipped: 3/19/2019

Carrier Name: FedEx

Airbill No: 774740017282

CHAIN OF CUSTODY RECORD

Wyckoff Eagle Harbor GWTP 2019/WA

Project Code: WEH-025Z

Cooler #: 1 of 1

No: 10-031919-095235-0352

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
031919		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	03/19/2019 09:33	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
	<i>Keith Allers</i> JACKBS	3-19-2019 1000	<i>Taylor</i> ^{NAUTICUS} TAYLOR NASU (EA)	0950 3/20/19	RECEIPT TEMP 2.5°C

NAUTICUS ID: 19-0392

Appendix D
Reference Toxicant Test Results

**Bivalve Larval Development Test
Copper Sulfate**

CETIS Summary Report

Report Date: 09 Apr-19 09:31 (p 1 of 3)
 Test Code: 190320msdvSO | 02-0163-9394

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Batch ID:	15-2490-3772	Test Type:	Development-Survival	Analyst:	
Start Date:	20 Mar-19 15:25	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Diluted Natural Seawater
Ending Date:	22 Mar-19 15:00	Species:	Mytilus galloprovincialis	Brine:	Not Applicable
Duration:	48h	Source:	Taylor Shellfish Mission Bay	Age:	
Sample ID:	17-2428-2167	Code:	190320msdvSO	Client:	Internal
Sample Date:	20 Mar-19	Material:	Copper sulfate	Project:	
Receive Date:	20 Mar-19	Source:	Reference Toxicant		
Sample Age:	15h	Station:	Copper Sulfate		

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-2821-2322	Combined Development Ra	5	10	7.071	4.47%		Dunnett Multiple Comparison Test
11-9614-8486	Development Rate	5	10	7.071	1.92%		Dunnett Multiple Comparison Test
14-4724-2580	Survival Rate	20	40	28.28	5.2%		Steel Many-One Rank Sum Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
10-2896-8877	Combined Development Ra	EC25	8.057	7.412	8.772		Linear Interpolation (ICPIN)
		EC50	11.63	10.5	12.62		
06-2977-8138	Development Rate	EC25	8.199	7.748	8.766		Linear Interpolation (ICPIN)
		EC50	11.9	11.17	12.65		
15-3118-7055	Survival Rate	EC25	24.09	22.74	24.96		Linear Interpolation (ICPIN)
		EC50	29.39	28.5	29.97		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
06-2977-8138	Development Rate	Control Resp	0.9745	0.9 - NL	Yes	Passes Acceptability Criteria	
11-9614-8486	Development Rate	Control Resp	0.9745	0.9 - NL	Yes	Passes Acceptability Criteria	
14-4724-2580	Survival Rate	Control Resp	0.9802	0.5 - NL	Yes	Passes Acceptability Criteria	
15-3118-7055	Survival Rate	Control Resp	0.9802	0.5 - NL	Yes	Passes Acceptability Criteria	
13-2821-2322	Combined Development Ra	PMSD	0.04475	NL - 0.25	No	Passes Acceptability Criteria	

Ⓞ vs 5/2/19 Q18

CETIS Summary Report

Report Date: 09 Apr-19 09:31 (p 2 of 3)
 Test Code: 190320msdvSO | 02-0163-9394

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	0.9553	0.9147	0.9959	0.9176	0.9891	0.01462	0.0327	3.42%	0.0%
2.5		5	0.932	0.878	0.986	0.8846	0.9701	0.01944	0.04348	4.67%	2.44%
5		5	0.9607	0.9393	0.9821	0.94	0.9785	0.007717	0.01726	1.8%	-0.57%
10		5	0.5714	0.4953	0.6476	0.5055	0.6593	0.02742	0.06131	10.73%	40.18%
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	0.9745	0.9611	0.9878	0.9598	0.9891	0.0048	0.01073	1.1%	0.0%
2.5		5	0.9673	0.9587	0.9759	0.9583	0.9758	0.003091	0.006911	0.71%	0.73%
5		5	0.9607	0.9393	0.9821	0.94	0.9785	0.007717	0.01726	1.8%	1.41%
10		5	0.6009	0.5424	0.6595	0.5444	0.6742	0.02109	0.04716	7.85%	38.33%
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	0.9802	0.9462	1	0.9451	1	0.01224	0.02736	2.79%	0.0%
2.5		5	0.9637	0.902	1	0.9066	1	0.02222	0.04969	5.16%	1.68%
5		5	1	1	1	1	1	0	0	0.0%	-2.02%
10		5	0.9495	0.9036	0.9953	0.8956	0.9835	0.01652	0.03694	3.89%	3.14%
20		5	0.9253	0.8549	0.9956	0.8571	1	0.02533	0.05665	6.12%	5.61%
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	0.9891	0.97	0.9766	0.9176	0.9231					
2.5		0.8846	0.9701	0.9624	0.8846	0.9583					
5		0.9447	0.9785	0.9676	0.94	0.9727					
10		0.6593	0.522	0.5934	0.5055	0.5769					
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	0.9891	0.97	0.9766	0.9598	0.9767					
2.5		0.9699	0.9701	0.9624	0.9758	0.9583					
5		0.9447	0.9785	0.9676	0.94	0.9727					
10		0.6742	0.5828	0.6034	0.5444	0.6					
20		0	0	0	0	0					
40		0	0	0	0	0					
Survival Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	1	1	1	0.956	0.9451					
2.5		0.9121	1	1	0.9066	1					
5		1	1	1	1	1					
10		0.978	0.8956	0.9835	0.9286	0.9615					
20		0.9176	0.8901	0.9615	0.8571	1					
40		0	0	0	0	0					

CETIS Summary Report

Report Date: 09 Apr-19 09:31 (p 3 of 3)
 Test Code: 190320msdvSO | 02-0163-9394

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	182/184	194/200	209/214	167/182	168/182
2.5		161/182	195/201	179/186	161/182	184/192
5		188/199	182/186	179/185	188/200	178/183
10		120/182	95/182	108/182	92/182	105/182
20		0/182	0/182	0/182	0/182	0/194
40		0/182	0/182	0/182	0/182	0/182
Development Rate Binomials						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	182/184	194/200	209/214	167/174	168/172
2.5		161/166	195/201	179/186	161/165	184/192
5		188/199	182/186	179/185	188/200	178/183
10		120/178	95/163	108/179	92/169	105/175
20		0/167	0/162	0/175	0/156	0/194
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	182/182	182/182	182/182	174/182	172/182
2.5		166/182	182/182	182/182	165/182	182/182
5		182/182	182/182	182/182	182/182	182/182
10		178/182	163/182	179/182	169/182	175/182
20		167/182	162/182	175/182	156/182	182/182
40		0/182	0/182	0/182	0/182	0/182

CETIS Analytical Report

Report Date: 09 Apr-19 09:31 (p 1 of 4)
 Test Code: 190320msdvSO | 02-0163-9394

Bivalve Larval Survival and Development Test Nautilus Environmental (CA)

Analysis ID: 13-2821-2322 Endpoint: Combined Development Rate CETIS Version: CETISv1.8.7
 Analyzed: 09 Apr-19 9:27 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	4.47%	5	10	7.071	

Dunnnett Multiple Comparison Test

Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	2.5	1.18	2.227	0.099	8	0.2655	CDF	Non-Significant Effect
	5	-0.1124	2.227	0.099	8	0.7885	CDF	Non-Significant Effect
	10*	11.48	2.227	0.099	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.9346469	0.311549	3	62.57	<0.0001	Significant Effect
Error	0.07966667	0.004979167	16			
Total	1.014314		19			

Distributional Tests

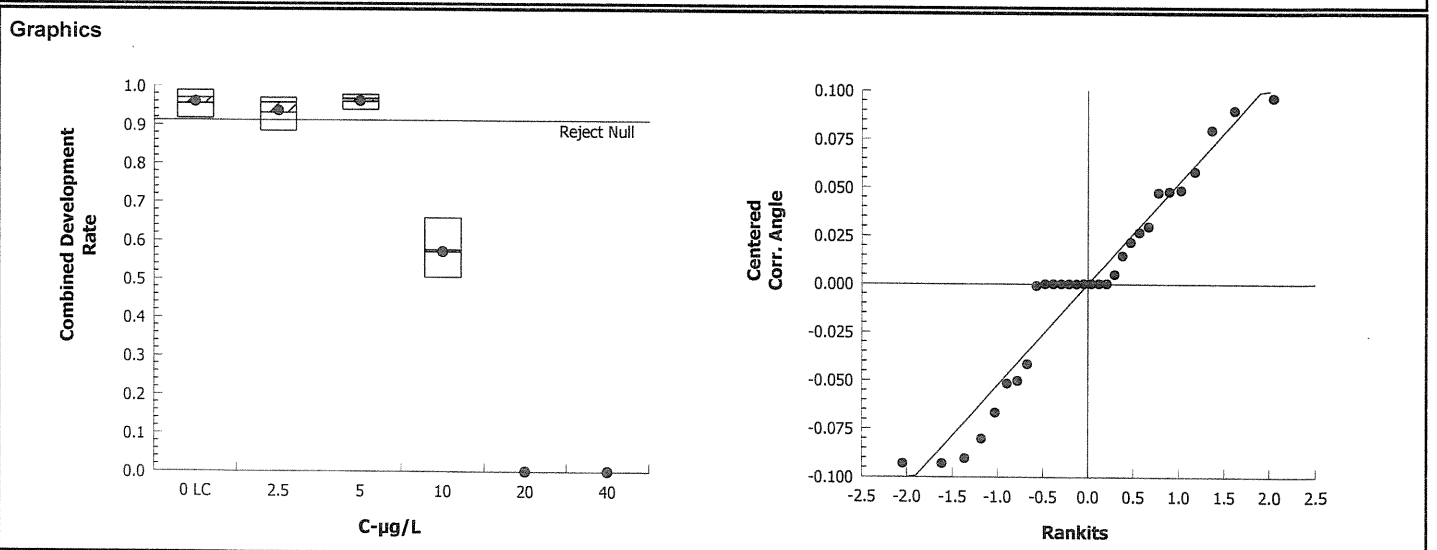
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.77	11.34	0.6216	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.913	0.866	0.0727	Normal Distribution

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	0.9553	0.9147	0.9959	0.97	0.9176	0.9891	0.01462	3.42%	0.0%
2.5		5	0.932	0.878	0.986	0.9583	0.8846	0.9701	0.01944	4.67%	2.44%
5		5	0.9607	0.9393	0.9821	0.9676	0.94	0.9785	0.007717	1.8%	-0.57%
10		5	0.5714	0.4953	0.6476	0.5769	0.5055	0.6593	0.02742	10.73%	40.18%
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	1.37	1.268	1.472	1.397	1.28	1.466	0.03664	5.98%	0.0%
2.5		5	1.317	1.211	1.424	1.365	1.224	1.397	0.03834	6.51%	3.85%
5		5	1.375	1.32	1.43	1.39	1.323	1.424	0.01982	3.22%	-0.37%
10		5	0.8576	0.7801	0.935	0.8626	0.7909	0.9476	0.0279	7.27%	37.4%
20		5	0.03684	0.03619	0.03748	0.03707	0.03591	0.03707	0.000233	1.42%	97.31%
40		5	0.03707	0.03706	0.03708	0.03707	0.03707	0.03707	0	0.0%	97.29%



CETIS Analytical Report

Report Date: 09 Apr-19 09:31 (p 2 of 4)
 Test Code: 190320msdvSO | 02-0163-9394

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

Analysis ID: 11-9614-8486 Endpoint: Development Rate CETIS Version: CETISv1.8.7
 Analyzed: 09 Apr-19 9:28 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	1.92%	5	10	7.071	

Dunnett Multiple Comparison Test

Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	2.5	0.967	2.227	0.054	8	0.3447	CDF	Non-Significant Effect
	5	1.572	2.227	0.054	8	0.1524	CDF	Non-Significant Effect
	10*	21.51	2.227	0.054	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.9610296	0.3203432	3	214.4	<0.0001	Significant Effect
Error	0.02390367	0.001493979	16			
Total	0.9849333		19			

Distributional Tests

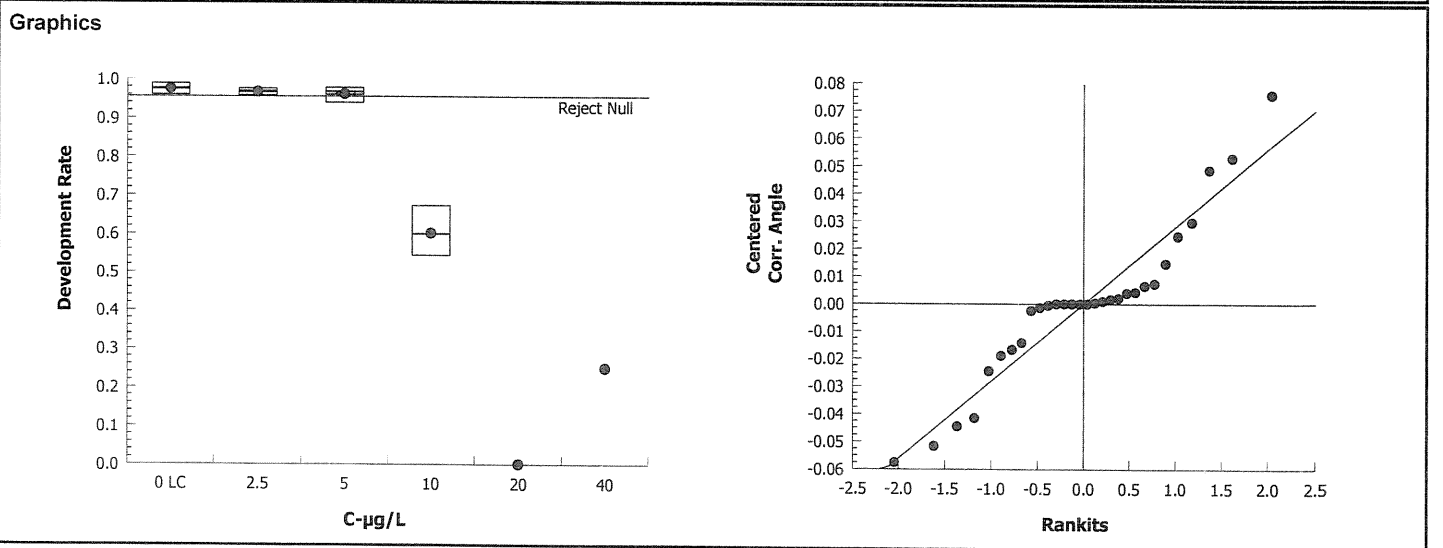
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.907	11.34	0.4061	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9727	0.866	0.8098	Normal Distribution

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	0.9745	0.9611	0.9878	0.9766	0.9598	0.9891	0.0048	1.1%	0.0%
2.5		5	0.9673	0.9587	0.9759	0.9699	0.9583	0.9758	0.003091	0.71%	0.73%
5		5	0.9607	0.9393	0.9821	0.9676	0.94	0.9785	0.007717	1.8%	1.41%
10		5	0.6009	0.5424	0.6595	0.6	0.5444	0.6742	0.02109	7.85%	38.33%
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	1.413	1.369	1.458	1.417	1.369	1.466	0.01597	2.53%	0.0%
2.5		5	1.39	1.366	1.414	1.396	1.365	1.414	0.008694	1.4%	1.67%
5		5	1.375	1.32	1.43	1.39	1.323	1.424	0.01982	3.22%	2.72%
10		5	0.8875	0.8272	0.9478	0.8861	0.8298	0.9633	0.02172	5.47%	37.21%
20		5	0.03835	0.03637	0.04033	0.0387	0.03591	0.04004	0.000712	4.15%	97.29%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	62.95%



CETIS Analytical Report

Report Date: 09 Apr-19 09:31 (p 3 of 4)
 Test Code: 190320msdvSO | 02-0163-9394

Bivalve Larval Survival and Development Test										Nautilus Environmental (CA)	
Analysis ID: 14-4724-2580		Endpoint: Survival Rate			CETIS Version: CETISv1.8.7						
Analyzed: 09 Apr-19 9:28		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	5.2%	20	40	28.28			

Steel Many-One Rank Sum Test									
Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	25.5	17	1	8	0.6377	Asymp	Non-Significant Effect
		5	32.5	17	1	8	0.9812	Asymp	Non-Significant Effect
		10	21	17	0	8	0.2314	Asymp	Non-Significant Effect
		20	20.5	17	1	8	0.1966	Asymp	Non-Significant Effect

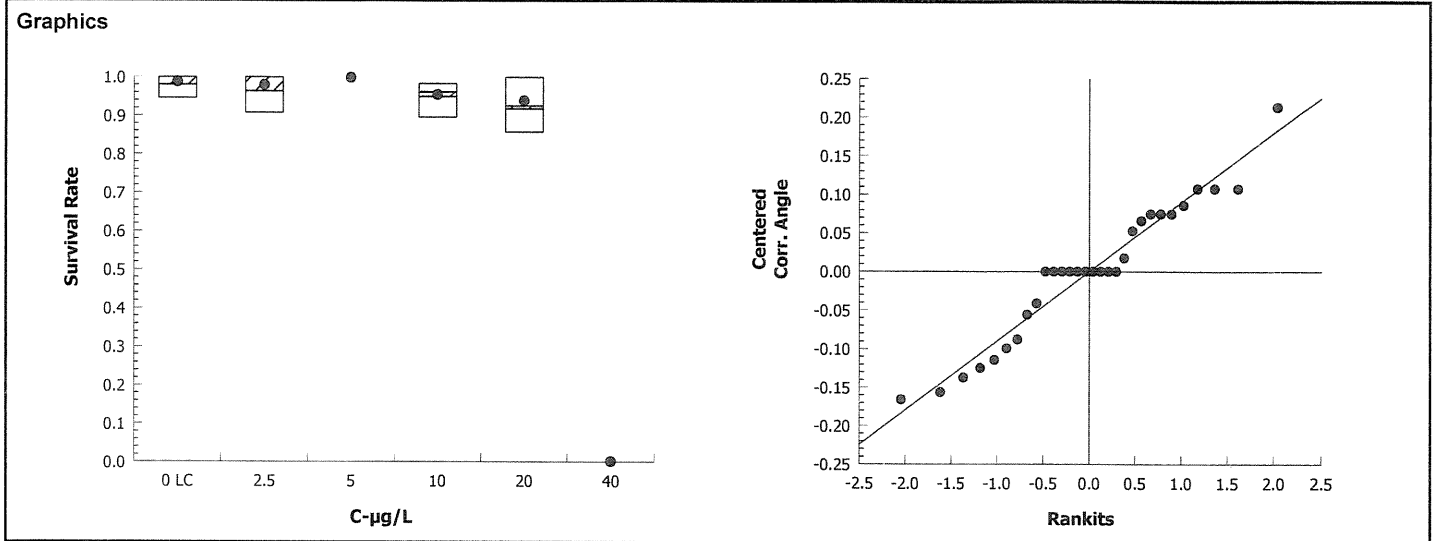
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1424184	0.03560461	4	3.048	0.0410	Significant Effect
Error	0.2336005	0.01168003	20			
Total	0.3760189		24			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	110.5	13.28	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.956	0.8877	0.3398	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	0.9802	0.9462	1	1	0.9451	1	0.01224	2.79%	0.0%
2.5		5	0.9637	0.902	1	1	0.9066	1	0.02222	5.16%	1.68%
5		5	1	1	1	1	1	1	0	0.0%	-2.02%
10		5	0.9495	0.9036	0.9953	0.9615	0.8956	0.9835	0.01652	3.89%	3.14%
20		5	0.9253	0.8549	0.9956	0.9176	0.8571	1	0.02533	6.12%	5.61%
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	1.459	1.331	1.587	1.534	1.334	1.534	0.04594	7.04%	0.0%
2.5		5	1.426	1.243	1.609	1.534	1.26	1.534	0.06584	10.32%	2.25%
5		5	1.534	1.534	1.534	1.534	1.534	1.534	0	0.0%	-5.12%
10		5	1.356	1.252	1.46	1.373	1.242	1.442	0.03756	6.19%	7.07%
20		5	1.321	1.149	1.492	1.28	1.183	1.534	0.06183	10.47%	9.49%
40		5	0.03707	0.03706	0.03708	0.03707	0.03707	0.03707	0	0.0%	97.46%

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 14-4724-2580	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7	
Analyzed: 09 Apr-19 9:28	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

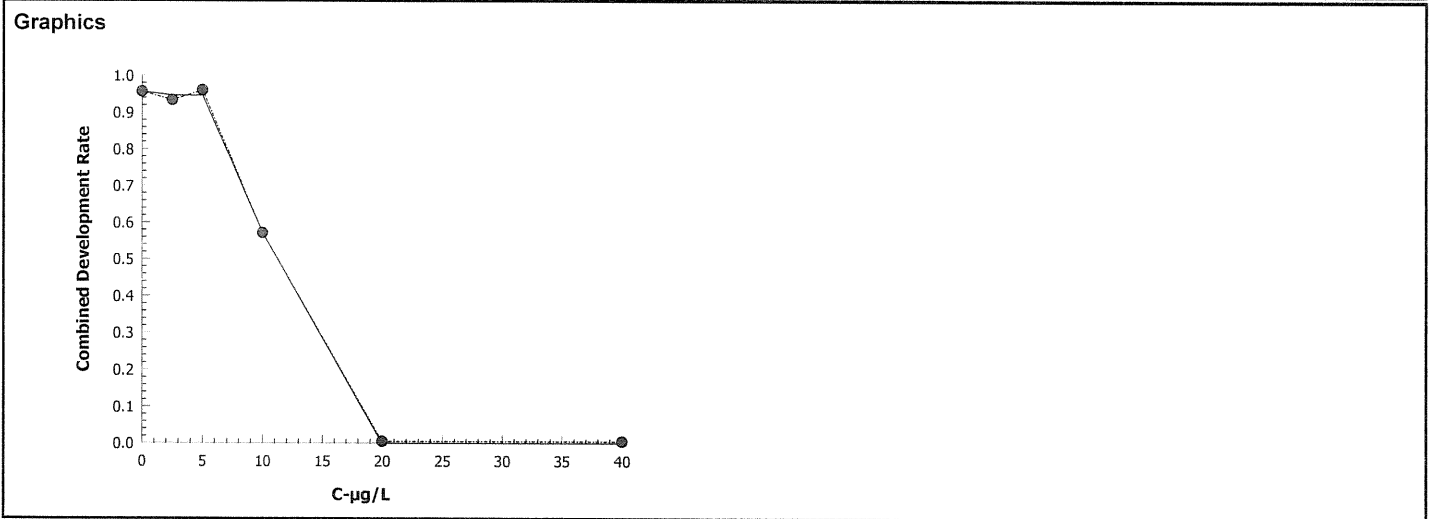
Report Date: 09 Apr-19 09:31 (p 1 of 3)
 Test Code: 190320msdvSO | 02-0163-9394

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 10-2896-8877	Endpoint: Combined Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 09 Apr-19 9:28	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1786700	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	8.057	7.412	8.772
EC50	11.63	10.5	12.62

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	0.9553	0.9176	0.9891	0.01462	0.0327	3.42%	0.0%	920	962
2.5		5	0.932	0.8846	0.9701	0.01944	0.04348	4.67%	2.44%	880	943
5		5	0.9607	0.94	0.9785	0.007717	0.01725	1.8%	-0.57%	915	953
10		5	0.5714	0.5055	0.6593	0.02742	0.06131	10.73%	40.18%	520	910
20		5	0	0	0	0	0		100.0%	0	922
40		5	0	0	0	0	0		100.0%	0	910



CETIS Analytical Report

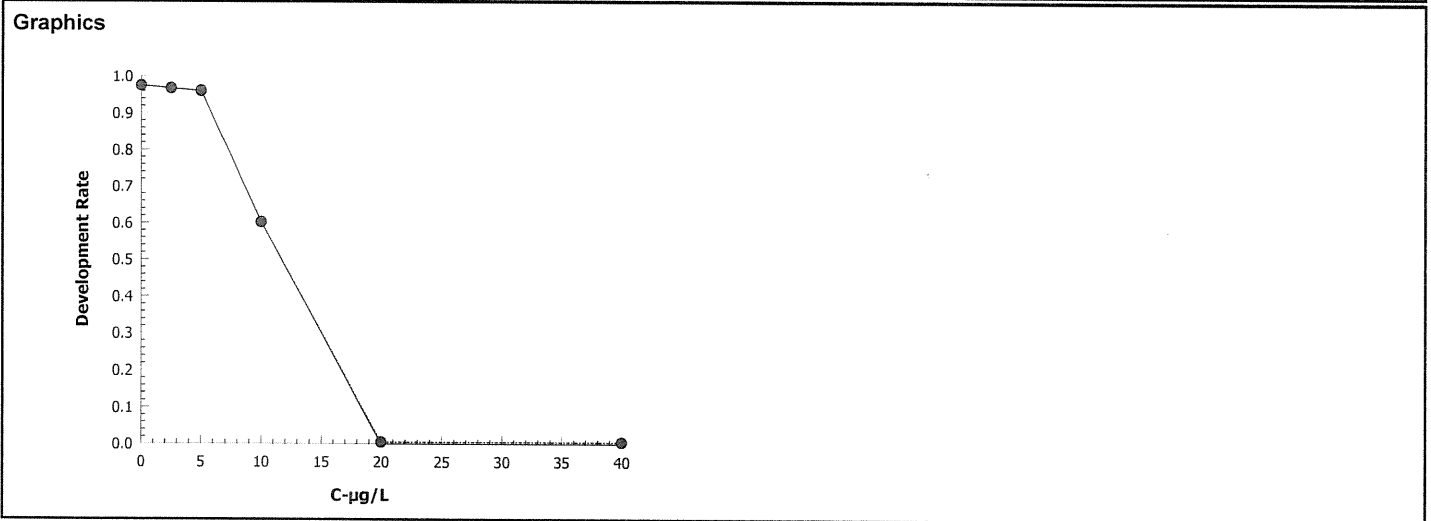
Report Date: 09 Apr-19 09:31 (p 2 of 3)
 Test Code: 190320msdvSO | 02-0163-9394

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 06-2977-8138	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 09 Apr-19 9:28	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2012004	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	8.199	7.748	8.766
EC50	11.9	11.17	12.65

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	0.9745	0.9598	0.9891	0.0048	0.01073	1.1%	0.0%	920	944
2.5		5	0.9673	0.9583	0.9758	0.003091	0.006911	0.71%	0.73%	880	910
5		5	0.9607	0.94	0.9785	0.007717	0.01725	1.8%	1.41%	915	953
10		5	0.6009	0.5444	0.6742	0.02109	0.04716	7.85%	38.33%	520	864
20		5	0	0	0	0	0		100.0%	0	854
40		5	0	0	0	0	0		100.0%	0	5



CETIS Analytical Report

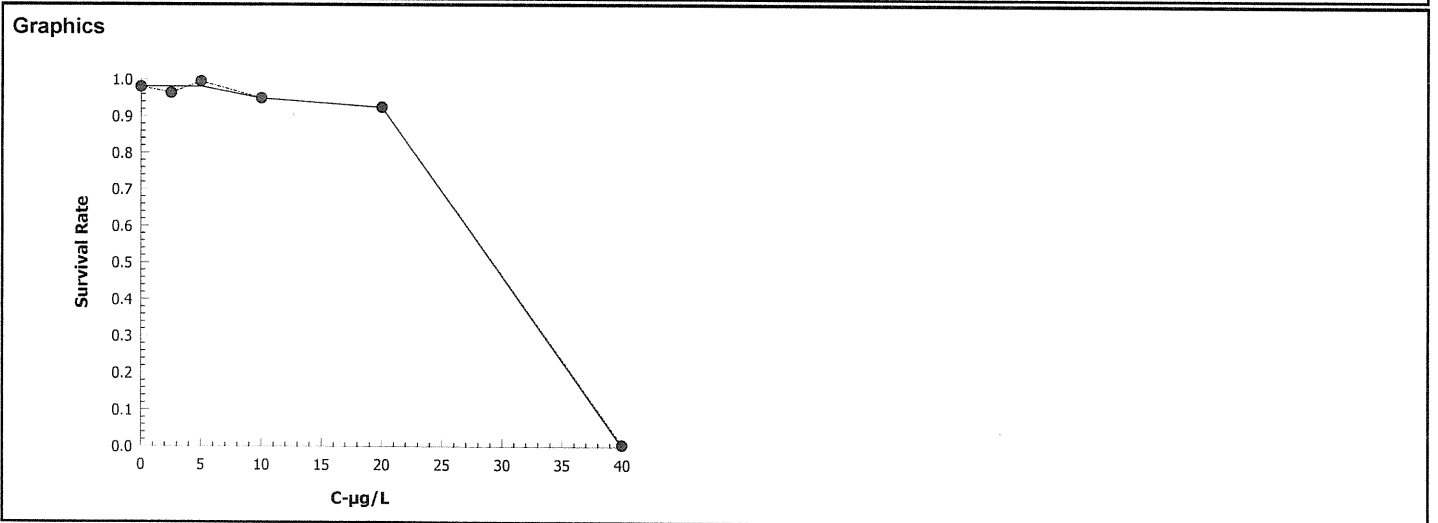
Report Date: 09 Apr-19 09:31 (p 3 of 3)
 Test Code: 190320msdvSO | 02-0163-9394

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 15-3118-7055	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 09 Apr-19 9:28	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1642468	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	24.09	22.74	24.96
EC50	29.39	28.5	29.97

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	0.9802	0.9451	1	0.01224	0.02736	2.79%	0.0%	892	910
2.5		5	0.9637	0.9066	1	0.02222	0.04969	5.16%	1.68%	877	910
5		5	1	1	1	0	0	0.0%	-2.02%	910	910
10		5	0.9495	0.8956	0.9835	0.01652	0.03694	3.89%	3.14%	864	910
20		5	0.9253	0.8571	1	0.02533	0.05665	6.12%	5.61%	842	910
40		5	0	0	0	0	0		100.0%	0	910



Bivalve Larval Survival and Development Test

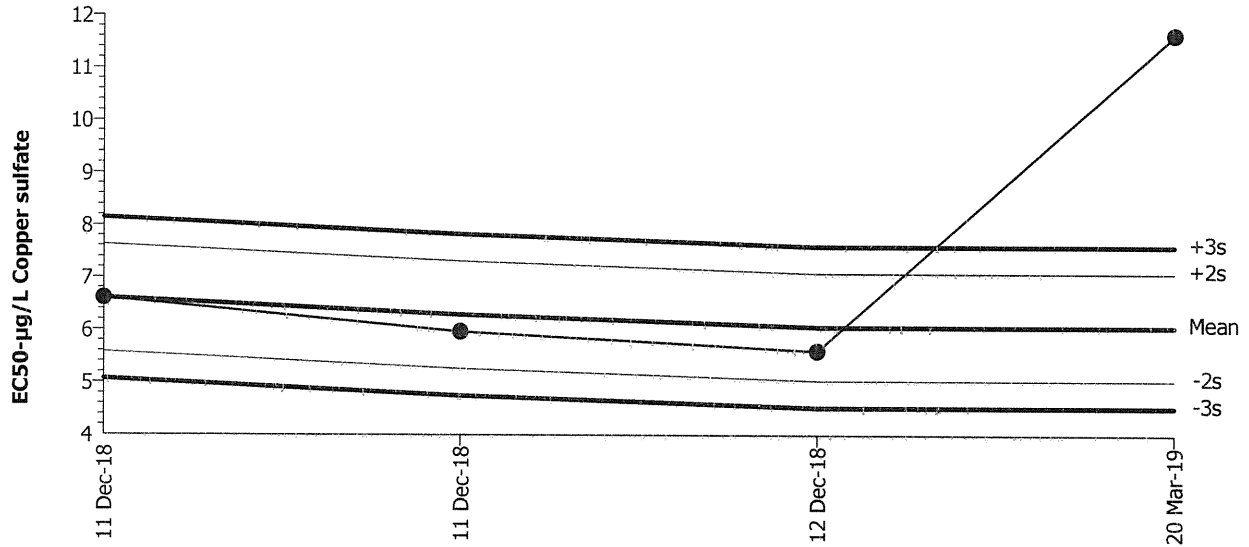
Nautilus Environmental (CA)

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis (Bay Mussel)
 Endpoint: Combined Development Rate

Material: Copper sulfate
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 6.053 Count: 3 -2s Warning Limit: 5.027 -3s Action Limit: 4.514
 Sigma: 0.5128 CV: 8.47% +2s Warning Limit: 7.078 +3s Action Limit: 7.591

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	0.5529	1.078			09-7408-5780	08-1757-8045
2			11	12:30	5.959	-0.09393	-0.1832			01-7940-4185	15-9822-3312
3			12	13:55	5.593	-0.4598	-0.8966			11-6161-8836	02-1891-3936
4	2019	Mar	20	15:25	11.63	5.579	10.88	(+)	(+)	02-0163-9394	10-2896-8877

Bivalve Larval Survival and Development Test

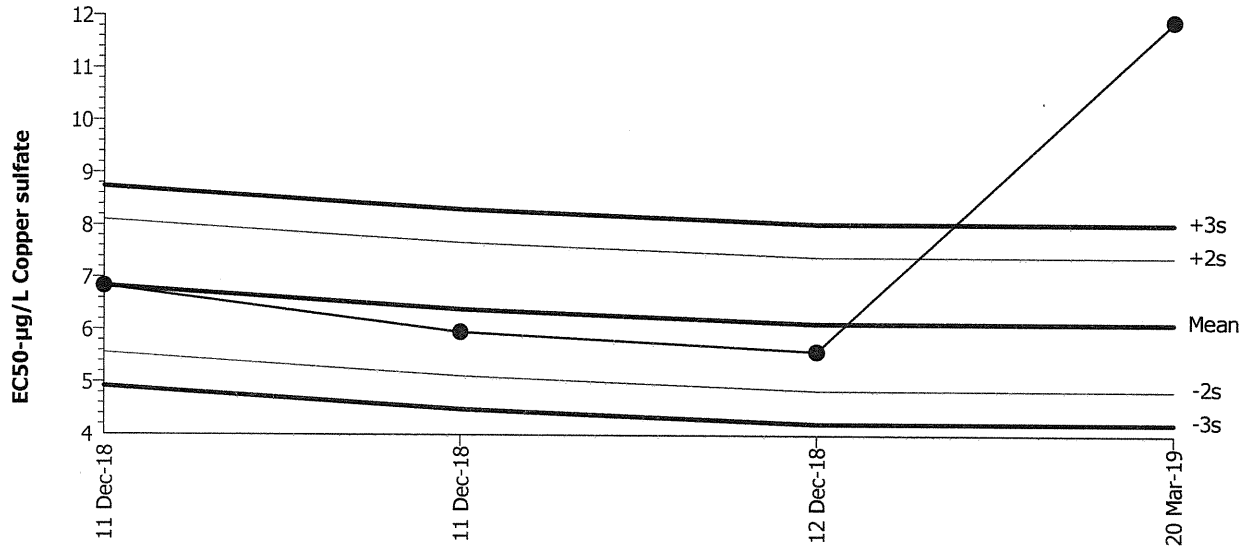
Nautilus Environmental (CA)

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis (Bay Mussel)
 Endpoint: Development Rate

Material: Copper sulfate
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 6.123 Count: 3 -2s Warning Limit: 4.85 -3s Action Limit: 4.213
 Sigma: 0.6367 CV: 10.40% +2s Warning Limit: 7.397 +3s Action Limit: 8.033

Quality Control Data

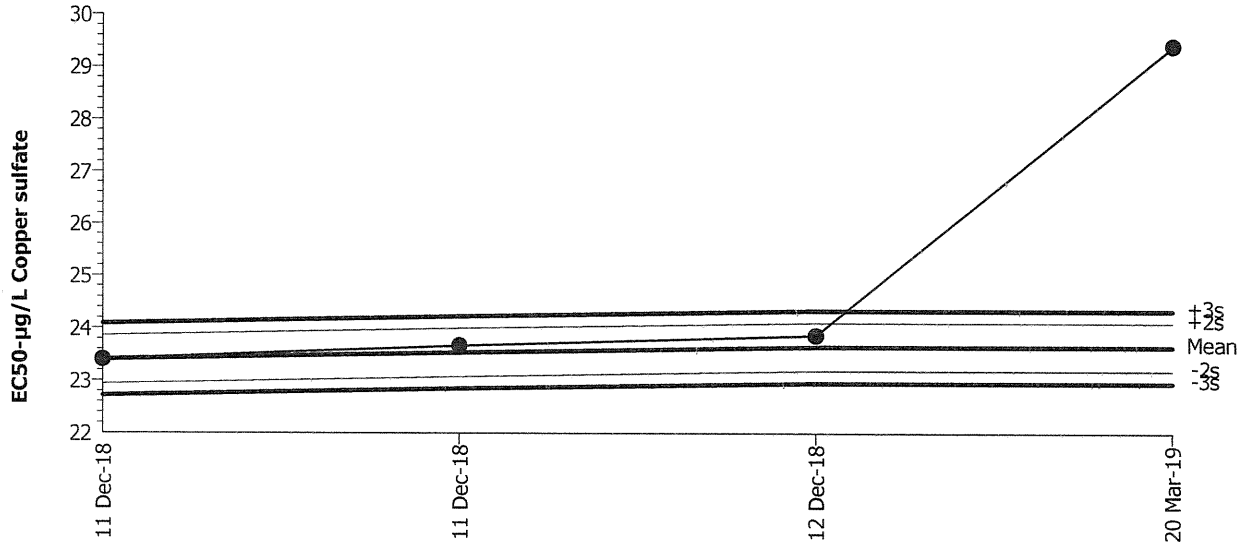
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Dec	11	11:55	6.828	0.7049	1.107			09-7408-5780	03-3077-4520
2			11	12:30	5.952	-0.1707	-0.2681			01-7940-4185	03-0677-9138
3			12	13:55	5.589	-0.5336	-0.8381			11-6161-8836	13-7938-6780
4	2019	Mar	20	15:25	11.9	5.781	9.079	(+)	(+)	02-0163-9394	06-2977-8138

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

Bivalve Larval Survival and Development Test



Mean: 23.64 Count: 3 -2s Warning Limit: 23.18 -3s Action Limit: 22.95
 Sigma: 0.2292 CV: 0.97% +2s Warning Limit: 24.1 +3s Action Limit: 24.33

Quality Control Data

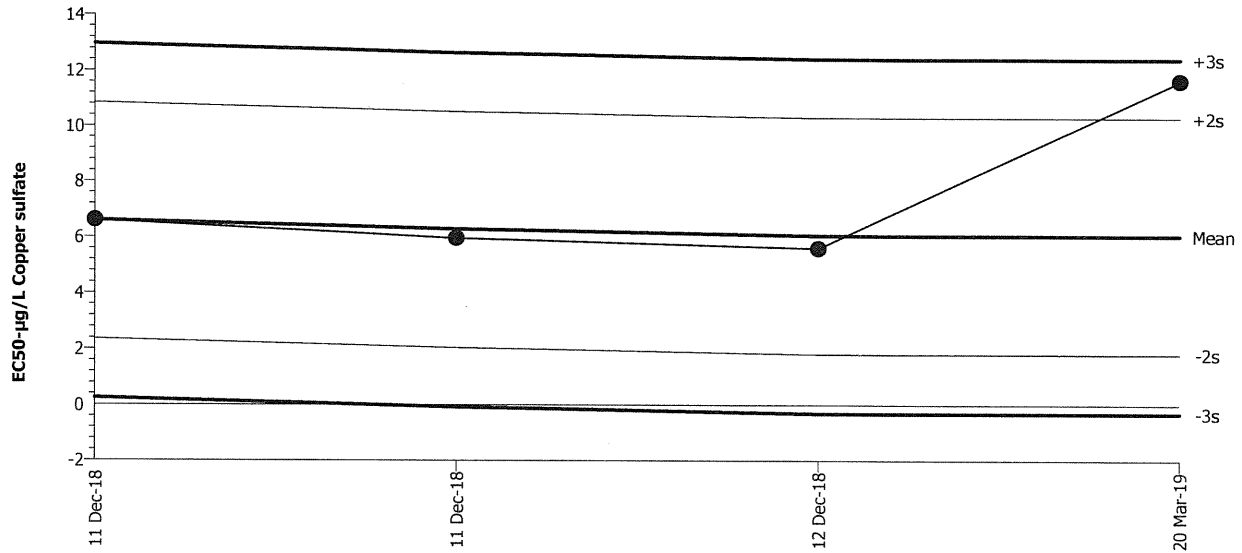
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Dec	11	11:55	23.4	-0.2395	-1.045			09-7408-5780	12-1829-1326
2			11	12:30	23.66	0.02379	0.1038			01-7940-4185	12-4826-8052
3			12	13:55	23.86	0.2171	0.9474			11-6161-8836	20-5520-1077
4	2019	Mar	20	15:25	29.39	5.754	25.11	(+)	(+)	02-0163-9394	15-3118-7055

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

Bivalve Larval Survival and Development Test



Mean: 6.053 Count: 3 -2s Warning Limit: 1.816 -3s Action Limit: -0.3029
 Sigma: 2.119 CV: 35.00% +2s Warning Limit: 10.29 +3s Action Limit: 12.41

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	0.5529	0.261			09-7408-5780	08-1757-8045
2			11	12:30	5.959	-0.09393	-0.04434			01-7940-4185	15-9822-3312
3			12	13:55	5.593	-0.4598	-0.217			11-6161-8836	02-1891-3936
4	2019	Mar	20	15:25	11.63	5.579	2.633	(+)		02-0163-9394	10-2896-8877

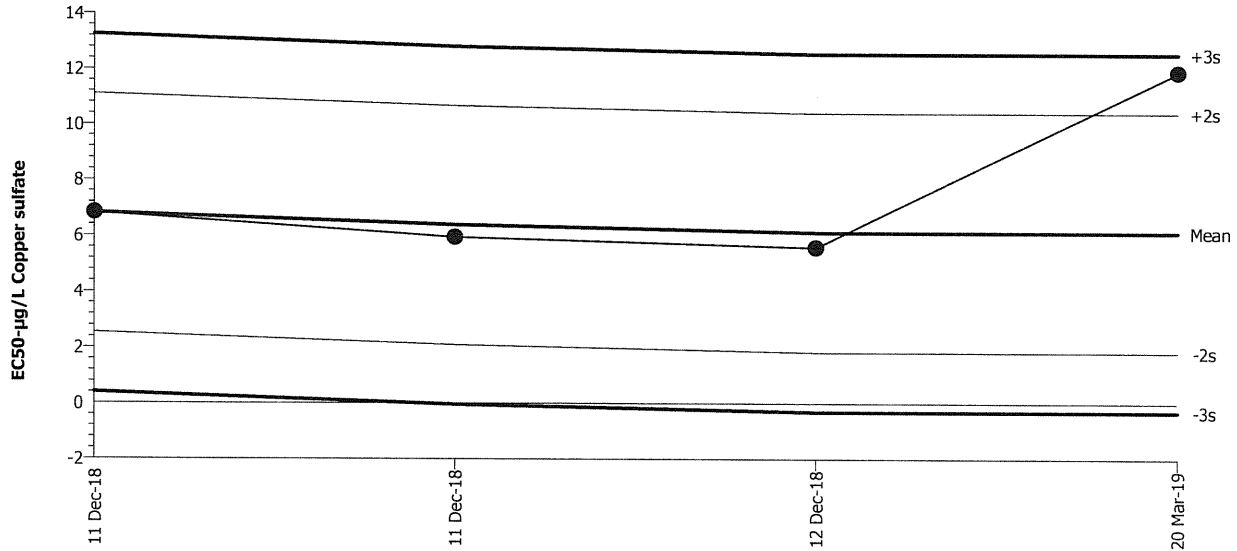
* Reference toxicant warning and control chart limits recalculated based on 75th percentile inter laboratory ^{coefficient} ~~coefficient~~ of variation, as defined in EPA-833-R-00-003, for comparison purposes only.

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

Bivalve Larval Survival and Development Test



Mean: 6.123 Count: 3 -2s Warning Limit: 1.837 -3s Action Limit: -0.306
 Sigma: 2.143 CV: 35.00% +2s Warning Limit: 10.41 +3s Action Limit: 12.55

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	0.7049	0.3289			09-7408-5780	03-3077-4520
2			11	12:30	5.952	-0.1707	-0.07966			01-7940-4185	03-0677-9138
3			12	13:55	5.589	-0.5336	-0.249			11-6161-8836	13-7938-6780
4	2019	Mar	20	15:25	11.9	5.781	2.697	(+)		02-0163-9394	06-2977-8138

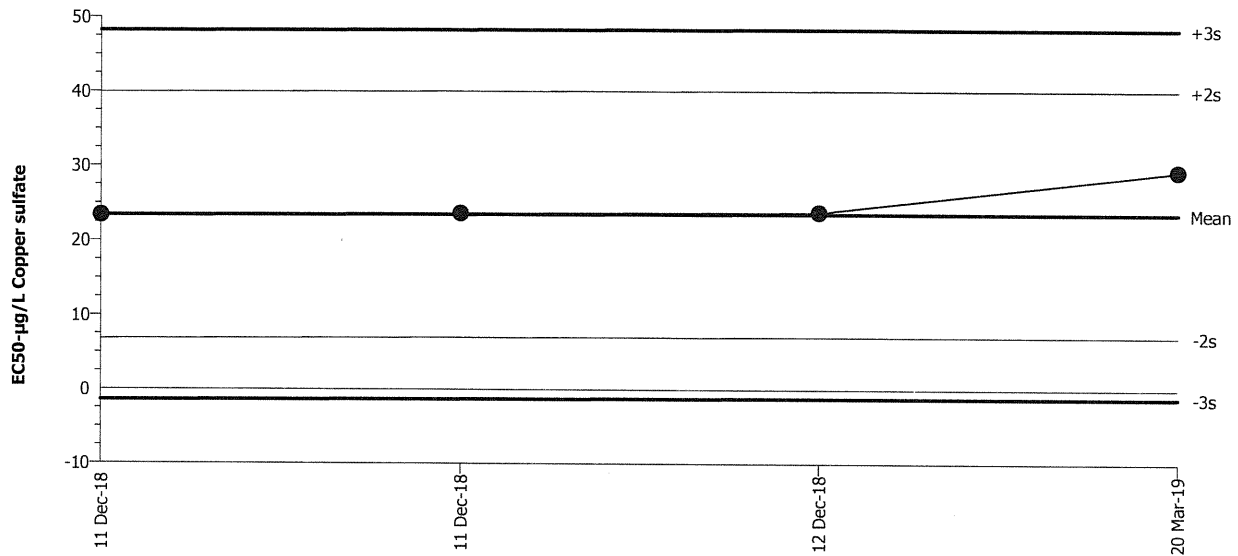
* Reference toxicant warning and control chart limits recalculated based on 75th percentile interlaboratory coefficient of variation, as defined in EPA-833-R-00-003, for comparison purposes only.

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

Bivalve Larval Survival and Development Test



Mean: 23.64 Count: 3 -2s Warning Limit: 7.092 -3s Action Limit: -1.182
 Sigma: 8.274 CV: 35.00% +2s Warning Limit: 40.19 +3s Action Limit: 48.46

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	-0.2395	-0.02894			09-7408-5780	12-1829-1326
2			11	12:30	23.66	0.02379	0.002875			01-7940-4185	12-4826-8052
3			12	13:55	23.86	0.2171	0.02624			11-6161-8836	20-5520-1077
4	2019	Mar	20	15:25	29.39	5.754	0.6955			02-0163-9394	15-3118-7055

* Reference toxicant warning and control chart limits recalculated based on 75th percentile inter laboratory coefficient of variation, as defined in EPA-833-R-00-003, for comparison purposes only.

CETIS Test Data Worksheet

Report Date: 26 Mar-19 14:35 (p 1 of 1)
 Test Code: 02-0163-9394/190320msdvSO

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 20 Mar-19 Species: Mytilus galloprovincialis Sample Code: 190320msdvSO
 End Date: 22 Mar-19 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 20 Mar-19 Material: Copper sulfate Sample Station: Copper Sulfate

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31	Ⓐ 178	Ⓐ 131	178	Ⓒ 120	NM 4/4/19
			32			184	182	
			33			200	194	
			34			175	0	
			35			201	195	
			36			166	161	
			37			0	0	Cell lysed
			38			163	95	
			39			199	188	
			40			186	179	
			41			Ⓐ 156	Ⓐ 156	
			42			0	0	Cells lysed
			43			174	167	
			44			185	179	
			45			186	182	
			46			179	108	
			47			214	209	
			48			162	0	
			49			165	161	
			50			175	105	
			51			0	0	Cells lysed
			52			192	184	
			53			172	168	
			54			167	0	
			55			183	178	
			56			184 169	177 92	Ⓑ 4/5/19
			57			200	188	
			58			0	0	Cells lysed
			59			0	0	Cells lysed
			60			194	0	

Ⓐ Q18 NM 3/28/19
 Ⓑ Q18 NM 4/5/19
 Ⓒ Q18 NM 4/8/19

CETIS Test Data Worksheet

Report Date: 20 Mar-19 10:55 (p 1 of 1)
 Test Code: 02-0163-9394/190320msdvSO

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 20 Mar-19 Species: *Mytilus galloprovincialis* Sample Code: 190320msdvSO
 End Date: 22 Mar-19 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 20 Mar-19 Material: Copper sulfate Sample Station: Copper Sulfate

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	32					
0	LC	2	33					
0	LC	3	47			208	200	RT 3/23/19
0	LC	4	43					
0	LC	5	53					
2.5		1	36					
2.5		2	35					
2.5		3	40			185	176	RT 3/23/19
2.5		4	49					
2.5		5	52					
5		1	39					
5		2	45					
5		3	44			182	175	RT 3/23/19
5		4	57					
5		5	55					
10		1	31					
10		2	38					
10		3	46					
10		4	56			165	69	RT 3/23/19
10		5	50					
20		1	54					
20		2	48					
20		3	34					
20		4	41			156	0	RT 3/23/19
20		5	60					
40		1	51					
40		2	58					
40		3	42			0	0	RT 3/23/19
40		4	37					
40		5	59					

acc:BO

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: CuSO₄
 Test No.: 190320msdvSO

Test Species: M. galloprovincialis
 Start Date/Time: 3/20/2019 1525
 End Date/Time: 3/22/2019 1500

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	31.6	31.4	31.3	15.5	15.4	15.1	8.2	7.9	8.3	7.96	8.01	7.96
2.5	31.9	31.9	31.9	15.4	15.1	14.8	8.1	8.0	8.3	7.98	8.01	7.95
5	31.9	32.0	32.1	15.1	15.1	14.8	8.1	7.9	8.3	7.98	8.02	7.98
10	31.9	31.9	31.9	15.4	15.2	14.8	7.9	7.9	8.3	7.99	8.02	7.98
20	31.9	31.8	31.9	15.7	15.2	14.8	8.0	7.9	8.3	8.00	8.04	7.99
40	31.8	31.7	31.8	15.7	15.3	14.9	8.0	7.9	8.2	8.01	8.04	7.99

Technician Initials: _____
 WQ Readings:

0	24	48
BO	SBS	BO
BO		

 Dilutions made by: _____

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	2.5
Final Volume (mL):	500
Cu stock concentration (µg/L):	8,000

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

QC Check: EG 4/11/19 Final Review: BS 5/3/19

Marine Chronic Bioassay

Larval Development Worksheet

Client: Internal - Copper Sulfate
 Test No.: 190320 msdv SO
 Test Species: M. galloprovincialis
 Animal Source: Mission Bay
 Date Received: 3/5/19
 Test Chambers: 30 ml shell vial
 Sample Volume: 10 ml

Start Date/Time: 3/20/2019 1525
 End Date/Time: 3/22/2019 1500
 Technician Initials: BD/KS/FG

Spawn Information

First Gamete Release Time: 1113

Sex	Number Spawning
Male	4
Female	4

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1,3,4	good motility and density
Female 1	1	pale yellow color, good density, some round
Female 2	2	white color, good density, mostly round
Female 3	3	orange color, good density, mostly round

Egg Fertilization Time: 1253

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	N/A
Female 2	N/A
Female 3	100%

Stock(s) chosen for testing: 3

Embryo Inoculum Preparation

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

Number Counted:

<u>15</u>	<u>15</u>
<u>18</u>	<u>19</u>
<u>19</u>	<u>18</u>
<u>17</u>	<u>19</u>
<u>19</u>	<u>19</u>

Mean: 17.8

Mean 17.8 X 50 = 890 embryos/ml

Initial Density: 890 = 2.96 (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
1	186	187	99	99%
2	168	169	99	
3	189	191	99	
4	192	194	99	
5	166	168	99	
6	192	193	99	

48-h QC: 169/174 = 97.1%

Comments:

x = 182.2

QC Check: EG 4/11/19

Final Review: KS/3/19

**Bivalve Larval Development Test
Copper Chloride**

CETIS Summary Report

Report Date: 28 Mar-19 09:13 (p 1 of 3)
 Test Code: 190320msdv | 00-9922-7600

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

Batch ID: 15-2791-8671	Test Type: Development-Survival	Analyst:
Start Date: 20 Mar-19 15:25	Protocol: EPA/600/R-95/136 (1995)	Diluent: Diluted Natural Seawater
Ending Date: 22 Mar-19 15:00	Species: Mytilus galloprovincialis	Brine: Not Applicable
Duration: 48h	Source: Taylor Shellfish <i>MISSION Bay</i>	Age:

Sample ID: 21-4405-9954	Code: 190320msdv	Client: Internal
Sample Date: 20 Mar-19	Material: Copper chloride	Project:
Receive Date: 20 Mar-19	Source: Reference Toxicant	
Sample Age: 15h	Station: Copper Chloride	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-4071-9030	Combined Development Ra	5	10	7.071	7.61%		Dunnett Multiple Comparison Test
08-3081-0323	Development Rate	2.5	5	3.536	4.82%		Steel Many-One Rank Sum Test
00-7449-8257	Survival Rate	20	40	28.28	7.02%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
12-2384-4421	Combined Development Ra	EC25	6.512	6.049	6.913		Linear Interpolation (ICPIN)
		EC50	8.284	7.795	8.754		
07-3949-1564	Development Rate	EC25	6.616	6.429	6.8		Linear Interpolation (ICPIN)
		EC50	8.334	7.978	8.7		
05-5691-6970	Survival Rate	EC25	23.75	21.71	25.4		Linear Interpolation (ICPIN)
		EC50	29.16	27.81	30.26		

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-3949-1564	Development Rate	Control Resp	0.9728	0.9 - NL	Yes	Passes Acceptability Criteria
08-3081-0323	Development Rate	Control Resp	0.9728	0.9 - NL	Yes	Passes Acceptability Criteria
00-7449-8257	Survival Rate	Control Resp	0.9681	0.5 - NL	Yes	Passes Acceptability Criteria
05-5691-6970	Survival Rate	Control Resp	0.9681	0.5 - NL	Yes	Passes Acceptability Criteria
17-4071-9030	Combined Development Ra	PMSD	0.07614	NL - 0.25	No	Passes Acceptability Criteria

AKP 4/2/19

CETIS Summary Report

Report Date: 28 Mar-19 09:13 (p 2 of 3)
 Test Code: 190320msdv | 00-9922-7600

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	0.9418	0.8561	1	0.8187	0.9788	0.03085	0.06899	7.33%	0.0%
2.5		5	0.8995	0.8488	0.9502	0.8571	0.9424	0.01827	0.04085	4.54%	4.49%
5		5	0.9163	0.8603	0.9722	0.8571	0.9728	0.02016	0.04508	4.92%	2.71%
10		5	0.2408	0.1476	0.3341	0.1319	0.3194	0.03359	0.0751	31.18%	74.43%
20		5	0.02521	0	0.08426	0	0.1099	0.02127	0.04755	188.6%	97.32%
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	0.9728	0.9665	0.9791	0.9648	0.9788	0.002256	0.005044	0.52%	0.0%
2.5		5	0.9621	0.9432	0.9811	0.9424	0.9827	0.00681	0.01523	1.58%	1.1%
5		5	0.9583	0.9423	0.9743	0.9436	0.9728	0.00577	0.0129	1.35%	1.49%
10		5	0.2462	0.1607	0.3317	0.1463	0.3194	0.03079	0.06886	27.96%	74.69%
20		5	0.02913	0	0.09818	0	0.1282	0.02487	0.05561	190.9%	97.01%
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	0.9681	0.8797	1	0.8407	1	0.03187	0.07126	7.36%	0.0%
2.5		5	0.9352	0.8754	0.9949	0.8846	1	0.02152	0.04812	5.15%	3.41%
5		5	0.956	0.9023	1	0.9011	1	0.01935	0.04326	4.53%	1.25%
10		5	0.9692	0.9135	1	0.9011	1	0.02008	0.04491	4.63%	-0.11%
20		5	0.8934	0.8113	0.9755	0.8242	1	0.02957	0.06612	7.4%	7.72%
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	0.9735	0.9648	0.8187	0.9788	0.973					
2.5		0.8571	0.9066	0.8571	0.9341	0.9424					
5		0.9176	0.9436	0.8901	0.9728	0.8571					
10		0.1319	0.3194	0.2143	0.2363	0.3024					
20		0	0.005181	0.1099	0	0.01099					
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	0.9735	0.9648	0.9739	0.9788	0.973					
2.5		0.963	0.9538	0.9689	0.9827	0.9424					
5		0.9709	0.9436	0.9529	0.9728	0.9512					
10		0.1463	0.3194	0.2267	0.2363	0.3024					
20		0	0.005181	0.1282	0	0.01227					
40		0	0	0	0	0					
Survival Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	1	1	0.8407	1	1					
2.5		0.8901	0.9505	0.8846	0.9505	1					
5		0.9451	1	0.9341	1	0.9011					
10		0.9011	1	0.9451	1	1					
20		0.8901	1	0.8571	0.8242	0.8956					
40		0	0	0	0	0					

CETIS Summary Report

Report Date: 28 Mar-19 09:13 (p 3 of 3)
 Test Code: 190320msdv | 00-9922-7600

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	184/189	192/199	149/182	185/189	180/185	
2.5		156/182	165/182	156/182	170/182	180/191	
5		167/182	184/195	162/182	179/184	156/182	
10		24/182	61/191	39/182	43/182	62/205	
20		0/182	1/193	20/182	0/182	2/182	
40		0/182	0/182	0/182	0/182	0/182	
Development Rate Binomials							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	184/189	192/199	149/153	185/189	180/185	
2.5		156/162	165/173	156/161	170/173	180/191	
5		167/172	184/195	162/170	179/184	156/164	
10		24/164	61/191	39/172	43/182	62/205	
20		0/162	1/193	20/156	0/150	2/163	
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	182/182	182/182	153/182	182/182	182/182	
2.5		162/182	173/182	161/182	173/182	182/182	
5		172/182	182/182	170/182	182/182	164/182	
10		164/182	182/182	172/182	182/182	182/182	
20		162/182	182/182	156/182	150/182	163/182	
40		0/182	0/182	0/182	0/182	0/182	

CETIS Analytical Report

Report Date: 28 Mar-19 09:13 (p 1 of 6)
 Test Code: 190320msdv | 00-9922-7600

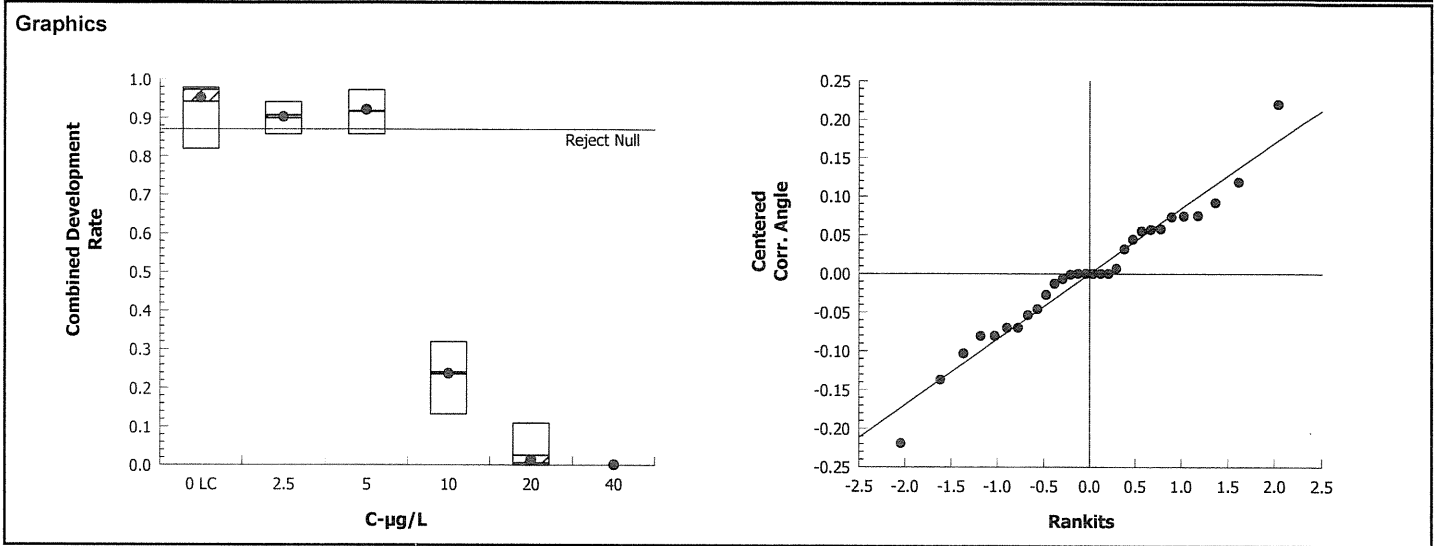
Bivalve Larval Survival and Development Test										Nautilus Environmental (CA)	
Analysis ID: 17-4071-9030		Endpoint: Combined Development Rate				CETIS Version: CETISv1.8.7					
Analyzed: 28 Mar-19 9:10		Analysis: Parametric-Control vs Treatments				Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	7.61%	5	10	7.071			
Dunnnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	1.508	2.305	0.148	8	0.1968	CDF	Non-Significant Effect		
		5	0.9924	2.305	0.148	8	0.3840	CDF	Non-Significant Effect		
		10*	13.09	2.305	0.148	8	<0.0001	CDF	Significant Effect		
		20*	19.17	2.305	0.148	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	6.208095		1.552024		4	150.2	<0.0001	Significant Effect			
Error	0.2066672		0.01033336		20						
Total	6.414762				24						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		1.843	13.28	0.7645	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9807	0.8877	0.8990	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	0.9418	0.8561	1	0.973	0.8187	0.9788	0.03085	7.33%	0.0%
2.5		5	0.8995	0.8488	0.9502	0.9066	0.8571	0.9424	0.01827	4.54%	4.49%
5		5	0.9163	0.8603	0.9722	0.9176	0.8571	0.9728	0.02016	4.92%	2.71%
10		5	0.2408	0.1476	0.3341	0.2363	0.1319	0.3194	0.03359	31.18%	74.43%
20		5	0.02521	0	0.08426	0.005181	0	0.1099	0.02127	188.6%	97.32%
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	1.35	1.197	1.504	1.406	1.131	1.425	0.05523	9.15%	0.0%
2.5		5	1.253	1.168	1.339	1.26	1.183	1.328	0.03071	5.48%	7.18%
5		5	1.286	1.179	1.393	1.28	1.183	1.405	0.03851	6.69%	4.73%
10		5	0.5087	0.3952	0.6221	0.5076	0.3716	0.6006	0.04087	17.96%	62.33%
20		5	0.1178	-0.03891	0.2745	0.07204	0.03707	0.3379	0.05645	107.1%	91.27%
40		5	0.03707	0.03706	0.03708	0.03707	0.03707	0.03707	0	0.0%	97.25%

CETIS Analytical Report

Report Date: 28 Mar-19 09:13 (p 2 of 6)

Test Code: 190320msdv | 00-9922-7600

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 17-4071-9030	Endpoint: Combined Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 28 Mar-19 9:10	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

Report Date: 28 Mar-19 09:13 (p 3 of 6)
 Test Code: 190320msdv | 00-9922-7600

Bivalve Larval Survival and Development Test				Nautilus Environmental (CA)			
Analysis ID: 08-3081-0323	Endpoint: Development Rate			CETIS Version: CETISv1.8.7			
Analyzed: 28 Mar-19 9:10	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	4.82%	2.5	5	3.536	

Steel Many-One Rank Sum Test									
Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	21	17	0	8	0.2314	Asymp	Non-Significant Effect
		5*	17	17	0	8	0.0463	Asymp	Significant Effect
		10*	15	17	0	8	0.0158	Asymp	Significant Effect
		20*	15	17	0	8	0.0158	Asymp	Significant Effect

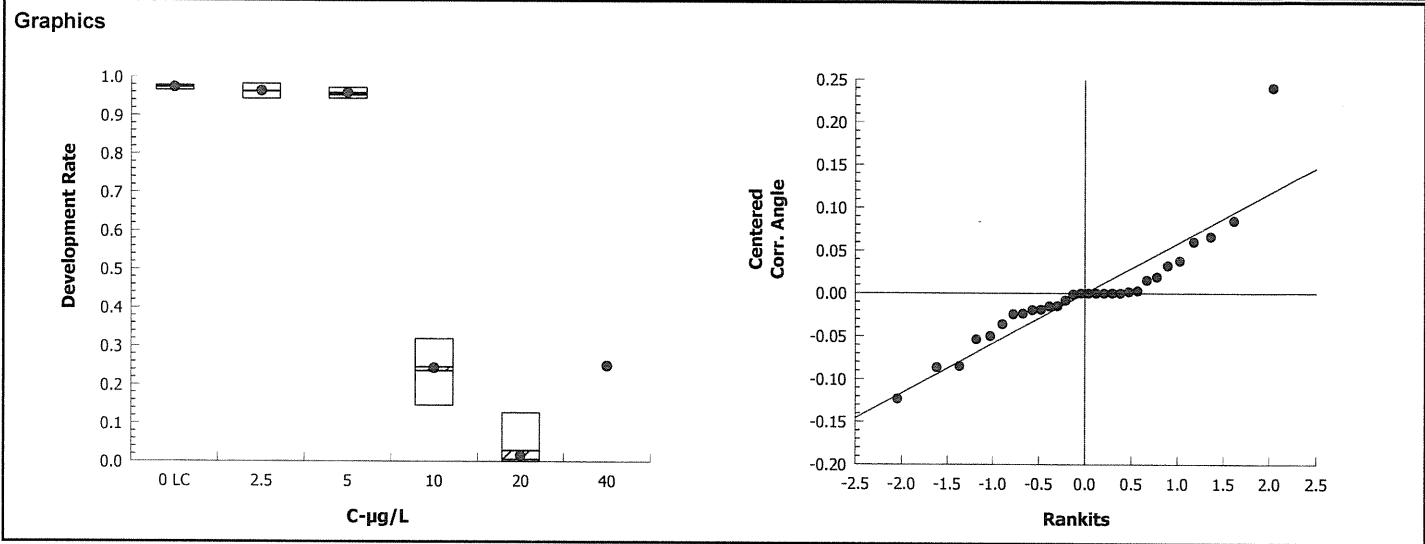
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.16249	1.790622	4	311.1	<0.0001	Significant Effect
Error	0.115125	0.005756249	20			
Total	7.277615		24			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	17.11	13.28	0.0018	Unequal Variances	
Distribution	Shapiro-Wilk W Normality	0.8743	0.8877	0.0053	Non-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	0.9728	0.9665	0.9791	0.9735	0.9648	0.9788	0.002256	0.52%	0.0%
2.5		5	0.9621	0.9432	0.9811	0.963	0.9424	0.9827	0.00681	1.58%	1.1%
5		5	0.9583	0.9423	0.9743	0.9529	0.9436	0.9728	0.00577	1.35%	1.49%
10		5	0.2462	0.1607	0.3317	0.2363	0.1463	0.3194	0.03079	27.96%	74.69%
20		5	0.02913	0	0.09818	0.005181	0	0.1282	0.02487	190.9%	97.01%
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	1.406	1.387	1.425	1.407	1.382	1.425	0.006821	1.09%	0.0%
2.5		5	1.378	1.327	1.43	1.377	1.328	1.439	0.01866	3.03%	1.94%
5		5	1.367	1.326	1.408	1.352	1.331	1.405	0.01481	2.42%	2.74%
10		5	0.5159	0.4134	0.6183	0.5076	0.3926	0.6006	0.03691	16.0%	63.3%
20		5	0.1259	-0.04483	0.2966	0.07204	0.03929	0.3662	0.06148	109.2%	91.05%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	62.75%

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 08-3081-0323	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 28 Mar-19 9:10	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes	



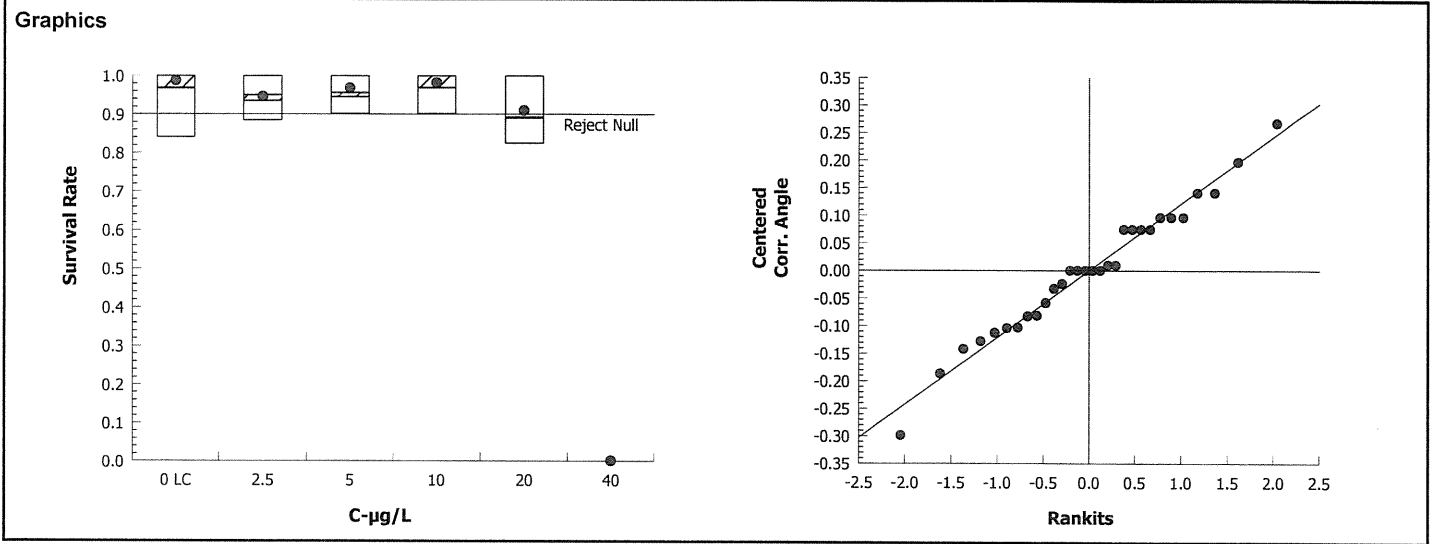
CETIS Analytical Report

Report Date: 28 Mar-19 09:13 (p 5 of 6)

Test Code: 190320msdv | 00-9922-7600

Bivalve Larval Survival and Development Test										Nautilus Environmental (CA)	
Analysis ID: 00-7449-8257		Endpoint: Survival Rate			CETIS Version: CETISv1.8.7						
Analyzed: 28 Mar-19 9:10		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	7.02%	20	40	28.28			
Dunnett Multiple Comparison Test											
Control	vs	C-µg/L	-Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	1.343	2.305	0.21	8	0.2487	CDF	Non-Significant Effect		
		5	0.7286	2.305	0.21	8	0.5006	CDF	Non-Significant Effect		
		10	0.2392	2.305	0.21	8	0.7136	CDF	Non-Significant Effect		
		20	2.122	2.305	0.21	8	0.0705	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.1227251		0.03068128		4	1.483	0.2449	Non-Significant Effect			
Error	0.4138839		0.02069419		20						
Total	0.536609				24						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		0.432	13.28	0.9798	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.979	0.8877	0.8646	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	0.9681	0.8797	1	1	0.8407	1	0.03187	7.36%	0.0%
2.5		5	0.9352	0.8754	0.9949	0.9505	0.8846	1	0.02152	5.15%	3.41%
5		5	0.956	0.9023	1	0.9451	0.9011	1	0.01935	4.53%	1.25%
10		5	0.9692	0.9135	1	1	0.9011	1	0.02008	4.63%	-0.11%
20		5	0.8934	0.8113	0.9755	0.8901	0.8242	1	0.02957	7.4%	7.72%
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	1.459	1.252	1.666	1.534	1.16	1.534	0.07471	11.45%	0.0%
2.5		5	1.337	1.182	1.492	1.347	1.224	1.534	0.05587	9.35%	8.38%
5		5	1.393	1.229	1.557	1.334	1.251	1.534	0.05915	9.5%	4.54%
10		5	1.437	1.269	1.605	1.534	1.251	1.534	0.06053	9.42%	1.49%
20		5	1.266	1.073	1.459	1.233	1.138	1.534	0.06949	12.27%	13.23%
40		5	0.03707	0.03706	0.03708	0.03707	0.03707	0.03707	0	0.0%	97.46%

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 00-7449-8257	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7	
Analyzed: 28 Mar-19 9:10	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

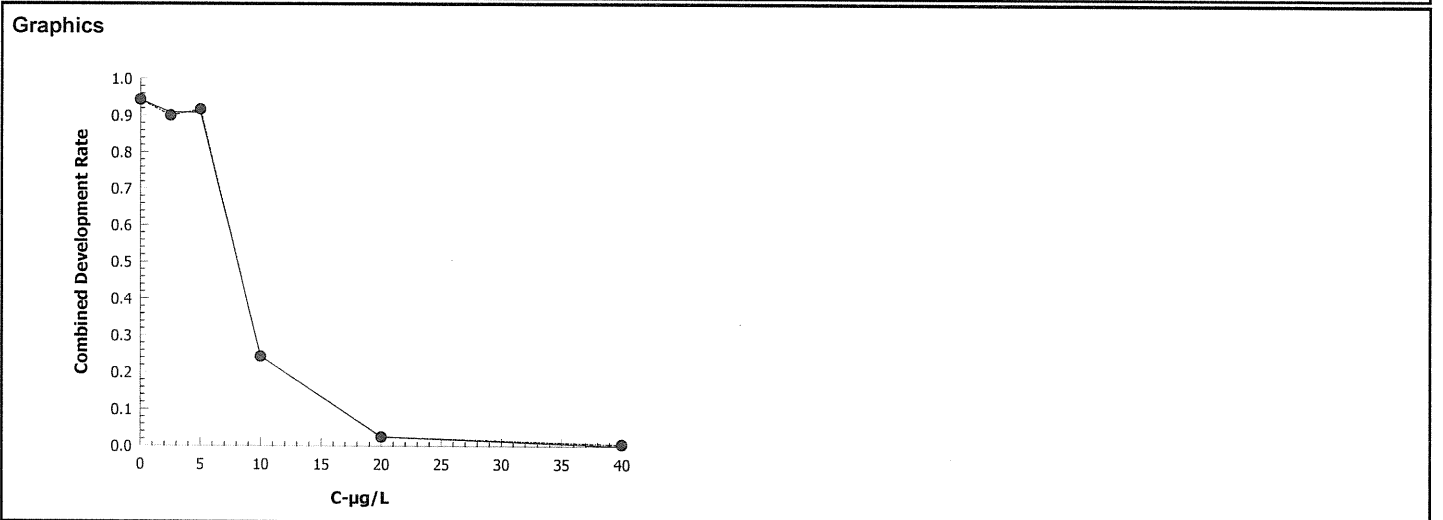
Report Date: 28 Mar-19 09:13 (p 1 of 3)
 Test Code: 190320msdv | 00-9922-7600

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 12-2384-4421	Endpoint: Combined Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 28 Mar-19 9:11	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	234830	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	6.512	6.049	6.913
EC50	8.284	7.795	8.754

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	0.9418	0.8187	0.9788	0.03085	0.06899	7.33%	0.0%	890	944	
2.5		5	0.8995	0.8571	0.9424	0.01827	0.04085	4.54%	4.49%	827	919	
5		5	0.9163	0.8571	0.9728	0.02016	0.04508	4.92%	2.71%	848	925	
10		5	0.2408	0.1319	0.3194	0.03359	0.0751	31.18%	74.43%	229	942	
20		5	0.02521	0	0.1099	0.02127	0.04755	188.6%	97.32%	23	921	
40		5	0	0	0	0	0		100.0%	0	910	



CETIS Analytical Report

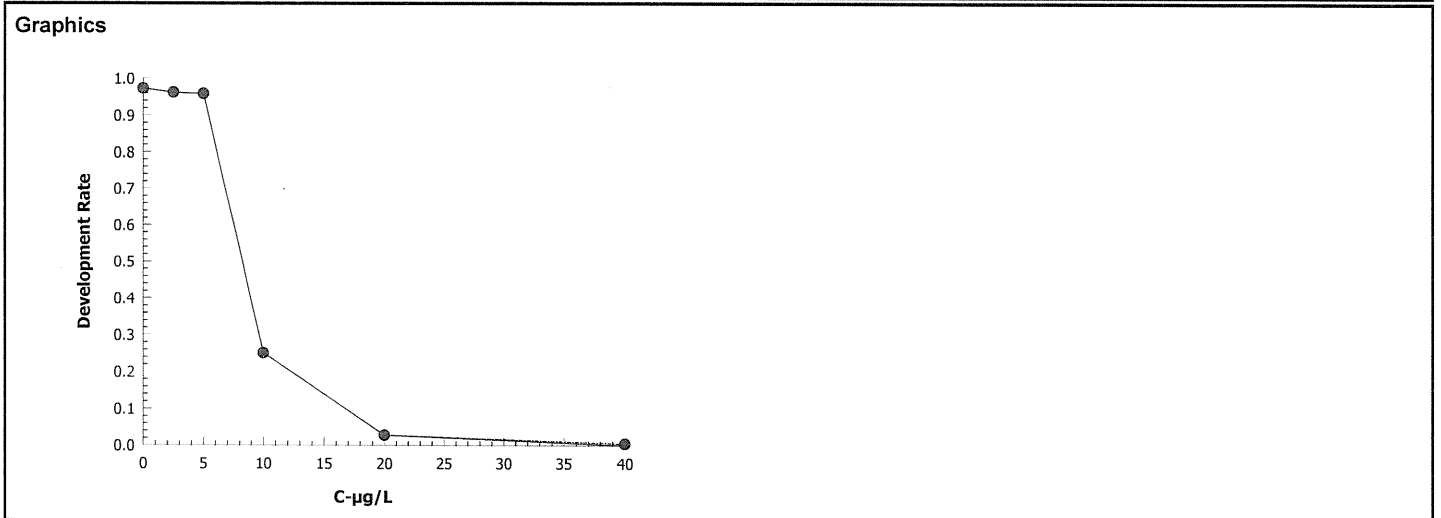
Report Date: 28 Mar-19 09:13 (p 2 of 3)
 Test Code: 190320msdv | 00-9922-7600

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 07-3949-1564	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 28 Mar-19 9:11	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	895165	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	6.616	6.429	6.8
EC50	8.334	7.978	8.7

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	0.9728	0.9648	0.9788	0.002256	0.005043	0.52%	0.0%	890	915
2.5		5	0.9621	0.9424	0.9827	0.00681	0.01523	1.58%	1.1%	827	860
5		5	0.9583	0.9436	0.9728	0.00577	0.0129	1.35%	1.49%	848	885
10		5	0.2462	0.1463	0.3194	0.03079	0.06886	27.96%	74.69%	229	914
20		5	0.02913	0	0.1282	0.02487	0.05561	190.9%	97.01%	23	824
40		5	0	0	0	0	0		100.0%	0	5



CETIS Analytical Report

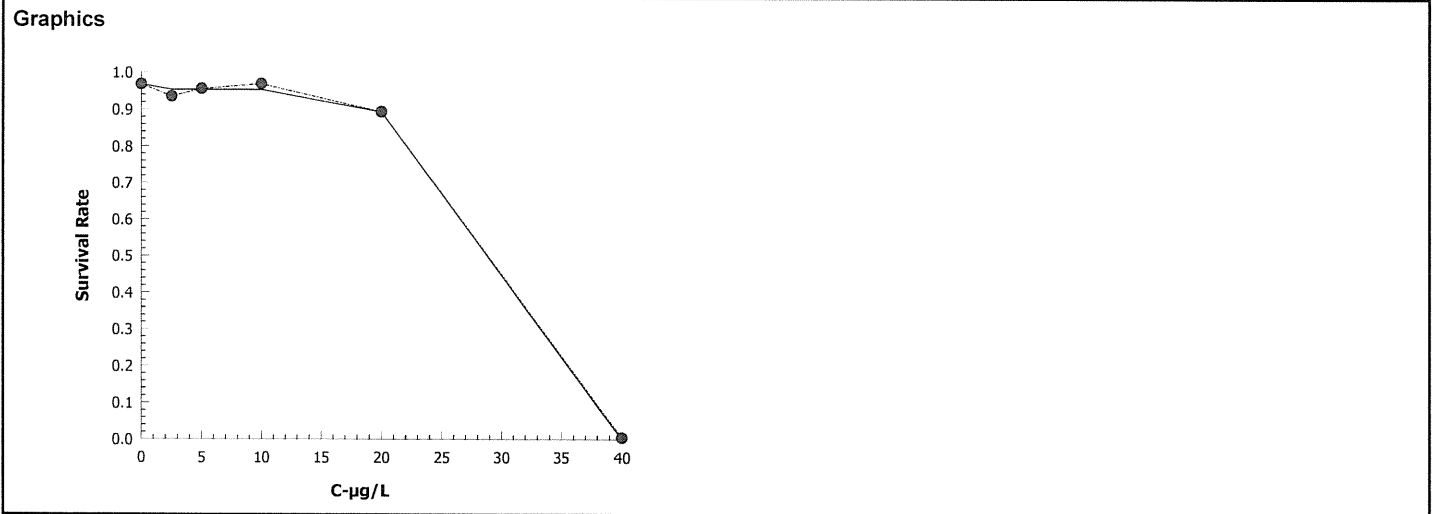
Report Date: 28 Mar-19 09:13 (p 3 of 3)
 Test Code: 190320msdv | 00-9922-7600

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 05-5691-6970	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 28 Mar-19 9:10	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	866266	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	23.75	21.71	25.4
EC50	29.16	27.81	30.26

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	0.9681	0.8407	1	0.03187	0.07126	7.36%	0.0%	881	910
2.5		5	0.9352	0.8846	1	0.02152	0.04812	5.15%	3.41%	851	910
5		5	0.956	0.9011	1	0.01935	0.04326	4.53%	1.25%	870	910
10		5	0.9692	0.9011	1	0.02008	0.04491	4.63%	-0.11%	882	910
20		5	0.8934	0.8242	1	0.02957	0.06612	7.4%	7.72%	813	910
40		5	0	0	0	0	0		100.0%	0	910



Bivalve Larval Survival and Development Test

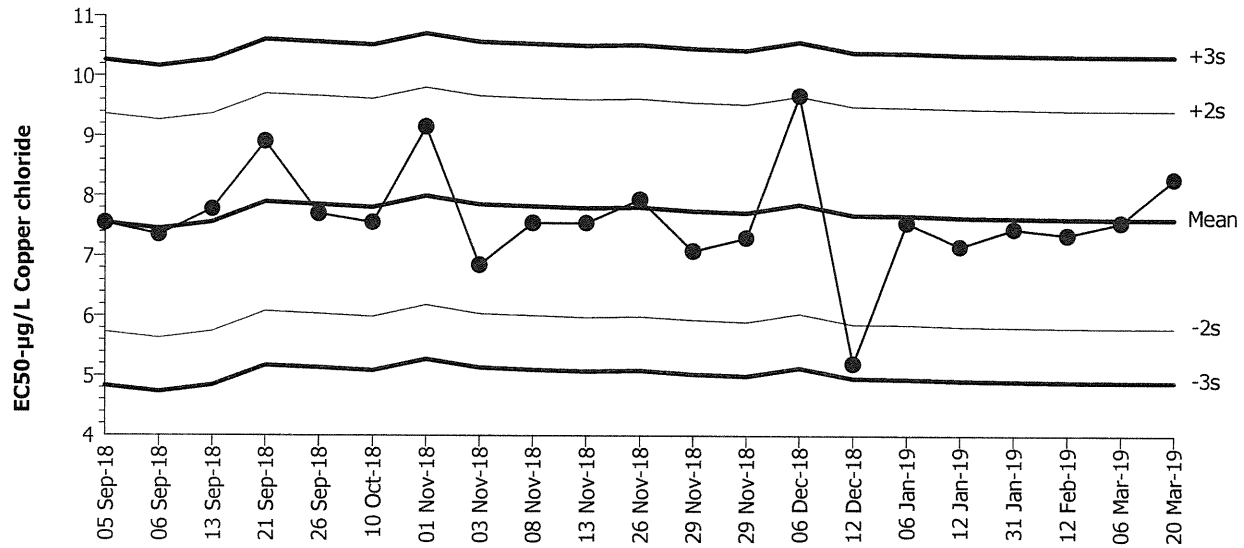
Nautilus Environmental (CA)

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis (Bay Mussel)
 Endpoint: Combined Development Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 7.609 Count: 20 -2s Warning Limit: 5.796 -3s Action Limit: 4.89
 Sigma: 0.9064 CV: 11.90% +2s Warning Limit: 9.422 +3s Action Limit: 10.33

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Sep	5	13:20	7.546	-0.06347	-0.07003			19-1012-9409	17-6300-0161
2			6	14:20	7.352	-0.2569	-0.2834			07-3859-0678	17-1869-4075
3			13	13:45	7.772	0.163	0.1798			05-2434-4016	18-1252-3060
4			21	14:30	8.908	1.299	1.433			02-0289-2516	20-3309-6290
5			26	14:30	7.698	0.08942	0.09866			14-5297-9976	05-2024-1647
6		Oct	10	15:15	7.554	-0.05459	-0.06023			01-3786-4049	16-8922-8681
7		Nov	1	14:30	9.15	1.541	1.701			18-8008-0024	06-2080-3928
8			3	13:30	6.851	-0.7585	-0.8368			13-3014-0314	15-4510-7321
9			8	15:30	7.548	-0.06146	-0.0678			18-8284-7572	06-5807-1390
10			13	13:45	7.548	-0.06146	-0.0678			19-8628-7209	13-8978-9468
11			26	14:50	7.933	0.3245	0.358			05-6256-7096	05-0142-7867
12			29	16:30	7.082	-0.5275	-0.5819			00-5944-3746	07-6331-4646
13			29	20:25	7.3	-0.3093	-0.3412			02-0971-0800	10-0317-7113
14		Dec	6	19:10	9.677	2.068	2.282	(+)		16-3949-4890	04-8210-0425
15			12	13:55	5.202	-2.407	-2.656	(-)		05-3477-8648	14-5219-2764
16	2019	Jan	6	18:10	7.549	-0.05959	-0.06574			00-4814-4263	10-6652-1953
17			12	16:40	7.158	-0.4506	-0.4972			02-7901-0365	04-0206-8014
18			31	20:05	7.448	-0.161	-0.1777			11-0188-3209	19-4158-0070
19		Feb	12	14:30	7.347	-0.2617	-0.2888			05-4773-4064	14-0529-6566
20		Mar	6	14:40	7.555	-0.05373	-0.05928			11-4050-7104	03-7884-0450
21			20	15:25	8.284	0.675	0.7448			00-9922-7600	12-2384-4421

Bivalve Larval Survival and Development Test

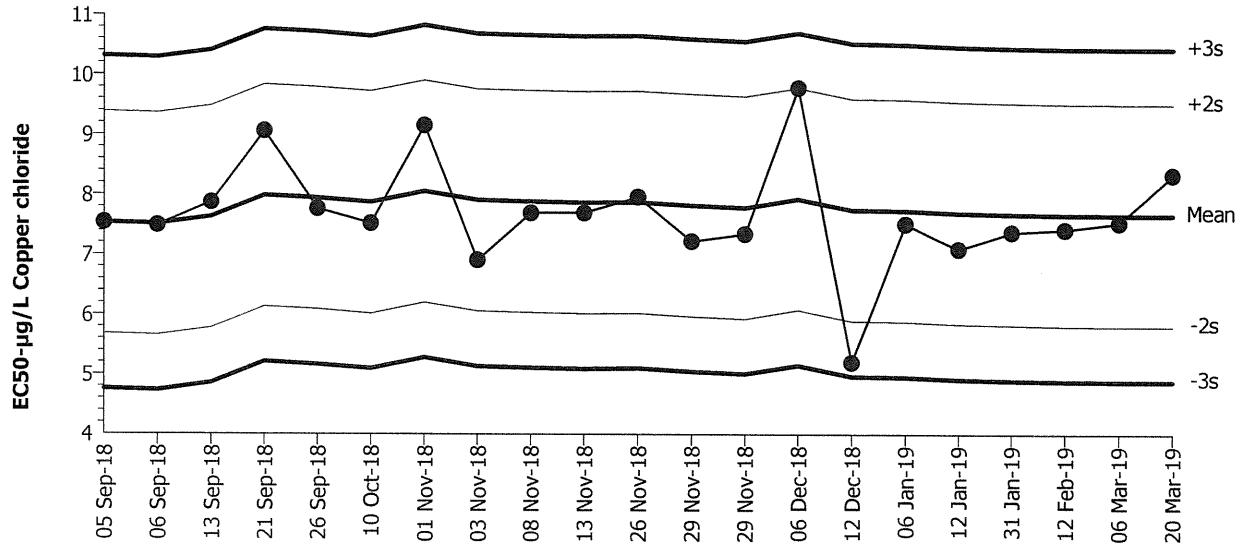
Nautilus Environmental (CA)

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis (Bay Mussel)
 Endpoint: Development Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 7.654 Count: 20 -2s Warning Limit: 5.802 -3s Action Limit: 4.876
 Sigma: 0.926 CV: 12.10% +2s Warning Limit: 9.506 +3s Action Limit: 10.43

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Sep	5	13:20	7.533	-0.1212	-0.1308			19-1012-9409	19-7681-8197
2			6	14:20	7.486	-0.1677	-0.1811			07-3859-0678	06-9273-0813
3			13	13:45	7.865	0.2113	0.2282			05-2434-4016	10-4201-7804
4			21	14:30	9.055	1.401	1.513			02-0289-2516	06-7733-9627
5			26	14:30	7.763	0.1088	0.1175			14-5297-9976	09-2075-5640
6		Oct	10	15:15	7.517	-0.1374	-0.1483			01-3786-4049	18-2433-5674
7		Nov	1	14:30	9.149	1.495	1.615			18-8008-0024	14-9885-9137
8			3	13:30	6.905	-0.7491	-0.809			13-3014-0314	14-1522-9033
9			8	15:30	7.691	0.03688	0.03983			18-8284-7572	13-3039-9929
10			13	13:45	7.691	0.03688	0.03983			19-8628-7209	00-1366-0167
11			26	14:50	7.958	0.3038	0.3281			05-6256-7096	05-6433-7919
12			29	16:30	7.221	-0.4331	-0.4677			00-5944-3746	12-1835-1530
13			29	20:25	7.342	-0.312	-0.337			02-0971-0800	14-6758-9802
14		Dec	6	19:10	9.781	2.127	2.297	(+)		16-3949-4890	03-2778-3085
15			12	13:55	5.198	-2.456	-2.652	(-)		05-3477-8648	07-1234-5247
16	2019	Jan	6	18:10	7.509	-0.1447	-0.1563			00-4814-4263	13-5934-9717
17			12	16:40	7.094	-0.5605	-0.6053			02-7901-0365	06-1305-7196
18			31	20:05	7.377	-0.2775	-0.2997			11-0188-3209	01-6713-0404
19		Feb	12	14:30	7.421	-0.2333	-0.252			05-4773-4064	11-5918-1928
20		Mar	6	14:40	7.531	-0.1225	-0.1323			11-4050-7104	19-8242-5220
21			20	15:25	8.334	0.68	0.7343			00-9922-7600	07-3949-1564

Bivalve Larval Survival and Development Test

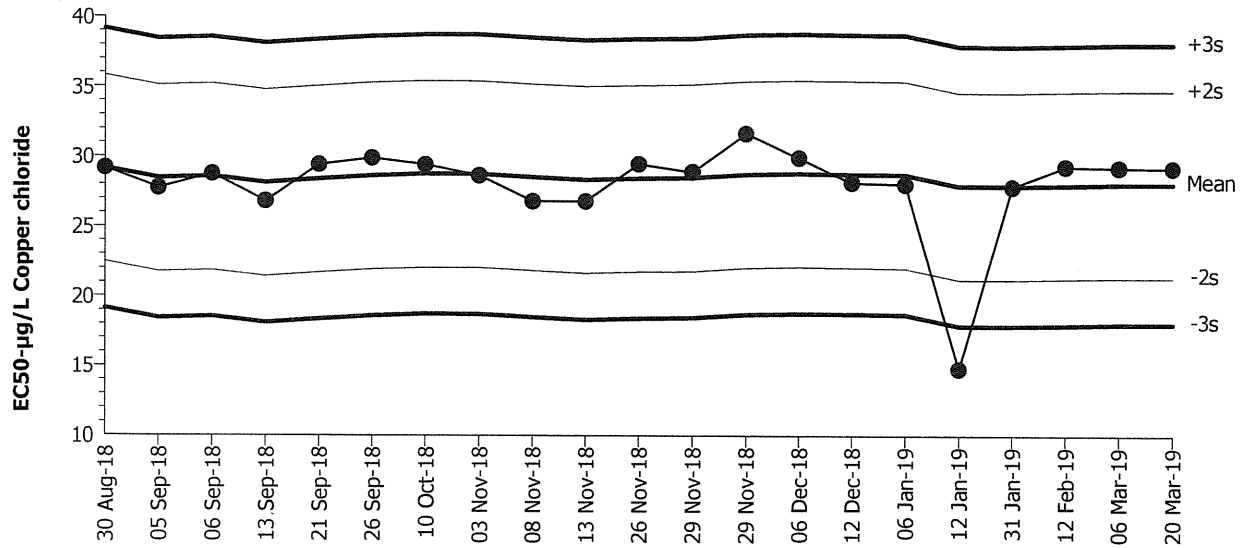
Nautilus Environmental (CA)

Test Type: Development-Survival
 Protocol: EPA/600/R-95/136 (1995)

Organism: Mytilus galloprovincialis (Bay Mussel)
 Endpoint: Survival Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 28.01 Count: 20 -2s Warning Limit: 21.34 -3s Action Limit: 18
 Sigma: 3.338 CV: 11.90% +2s Warning Limit: 34.69 +3s Action Limit: 38.03

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Aug	30	12:40	29.15	1.144	0.3427			04-5323-0718	17-0214-3213
2		Sep	5	13:20	27.72	-0.2859	-0.08564			19-1012-9409	12-4890-7502
3			6	14:20	28.73	0.7187	0.2153			07-3859-0678	08-3450-9811
4			13	13:45	26.78	-1.226	-0.3672			05-2434-4016	13-8372-0203
5			21	14:30	29.39	1.38	0.4135			02-0289-2516	02-1350-9917
6			26	14:30	29.86	1.85	0.5542			14-5297-9976	08-9946-4918
7		Oct	10	15:15	29.38	1.368	0.4099			01-3786-4049	16-7437-4645
8		Nov	3	13:30	28.6	0.5853	0.1753			13-3014-0314	19-1258-0474
9			8	15:30	26.77	-1.24	-0.3715			18-8284-7572	02-4676-6589
10			13	13:45	26.77	-1.24	-0.3715			19-8628-7209	03-9393-7697
11			26	14:50	29.44	1.425	0.4269			05-6256-7096	11-5015-6962
12			29	16:30	28.87	0.8593	0.2574			00-5944-3746	20-1962-0376
13			29	20:25	31.65	3.635	1.089			02-0971-0800	21-2341-9901
14		Dec	6	19:10	29.9	1.887	0.5654			16-3949-4890	19-4410-1396
15			12	13:55	28.13	0.1222	0.03662			05-3477-8648	20-2904-3770
16	2019	Jan	6	18:10	28.01	-0.00389	-0.00117			00-4814-4263	00-6198-2529
17			12	16:40	14.78	-13.23	-3.964	(-)	(-)	02-7901-0365	08-0441-2945
18			31	20:05	27.83	-0.1842	-0.05519			11-0188-3209	12-1004-4079
19		Feb	12	14:30	29.28	1.271	0.3808			05-4773-4064	07-6241-6865
20		Mar	6	14:40	29.2	1.189	0.3562			11-4050-7104	15-7196-8133
21			20	15:25	29.16	1.154	0.3456			00-9922-7600	05-5691-6970

CETIS Test Data Worksheet

Report Date: 15 Mar-19 15:26 (p 1 of 1)
 Test Code: 00-9922-7600/190320msdv

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 20 Mar-19 Species: *Mytilus galloprovincialis* Sample Code: 190320msdv
 End Date: 22 Mar-19 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 20 Mar-19 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			156	2	
			2			191	180	
			3			0	0	Cells lysed
			4			163	2	
			5			173	170	
			6			173	165	
			7			191	61	
			8			170	162	
			9			153	149	
			10			185	180	
			11			162	0	
			12			0	0	Cells lysed
			13			172	167	
			14			0	0	Cells lysed
			15			172	39	
			16			195	184	
			17			164	24	
			18			0	0	Cells lysed
			19			182	43	
			20			162	156	
			21			193	1	
			22			189	185	
			23			150	0	
			24			184	179	
			25			189	184	
			26			199	192	
			27			164	156	
			28			0	0	Cells lysed
			29			161	156	
			30			205	62	

CETIS Test Data Worksheet

Report Date: 15 Mar-19 15:26 (p 1 of 1)
 Test Code: 00-9922-7600/190320msdv

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 20 Mar-19 Species: Mytilus galloprovincialis Sample Code: 190320msdv
 End Date: 22 Mar-19 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 20 Mar-19 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	25					
0	LC	2	26					
0	LC	3	9			163	159	RT 3/23/19
0	LC	4	22					
0	LC	5	10					
2.5		1	20					
2.5		2	6					
2.5		3	29			152	148	RT 3/23/19
2.5		4	5					
2.5		5	2					
5		1	13					
5		2	16					
5		3	8			172	164	RT 3/23/19
5		4	24					
5		5	27					
10		1	17					
10		2	7					
10		3	15			157	39	RT 3/23/19
10		4	19					
10		5	30					
20		1	11					
20		2	21					
20		3	1			172	0	RT 3/23/19
20		4	23					
20		5	4					
40		1	28					
40		2	14					
40		3	3			0	0	RT 3/23/19
40		4	12					
40		5	18					

QC=BO

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: CuCl₂
 Test No.: 190320msdv

Test Species: M. galloprovincialis
 Start Date/Time: 3/20/2019 1525
 End Date/Time: 3/22/2019 1500

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	31.8	31.6 ^{31.6}	31.3	15.5	15.3	15.1	8.1	7.9	8.3	8.00	8.03	7.89
2.5	32.0	31.9	32.0	15.5	15.2	14.6	8.1	7.9	8.3	8.01	8.03	7.94
5	32.0	31.9	32.1	15.5	15.1	14.6	8.1	7.9	8.3	8.01	8.03	7.98
10	32.0	31.9	32.1	15.5	15.3	14.8	8.0	7.9	8.4	8.02	8.04	7.99
20	31.9	31.8	32.0	15.5	15.1	14.8	8.0	8.0	8.4	8.02	8.05	7.99
40	31.7	31.7	31.8	15.5	15.2	14.7	8.0	8.0	8.4	8.03	8.06	8.00

Technician Initials: _____
 WQ Readings:

0	24	48
BO	BO	BO

 Dilutions made by:

BO		
----	--	--

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	2.2
Final Volume (mL):	500
Cu stock concentration (µg/L):	9,000

Comments: 0 hrs: Ⓐ Temperature measured from surrogate vial on tray.
 24 hrs: _____
 48 hrs: @ 0.8 BO 3/22/19

QC Check: VTP 4/2/19 Final Review: EG 4/3/19

Marine Chronic Bioassay

Larval Development Worksheet

Client: Internal CuCl₂
 Test No.: 190320 msdv
 Test Species: M. galloprovincialis
 Animal Source: Mission Bay
 Date Received: 3/15/19
 Test Chambers: 30 ml shell vial
 Sample Volume: 10 mL

Start Date/Time: 3/20/2019 1525
 End Date/Time: 3/22/2019 1500
 Technician Initials: BD/KS/FG

Spawn Information

First Gamete Release Time: 1113

Sex	Number Spawning
Male	4
Female	4

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1,3,4	good motility and density
Female 1	1	pale yellow color, good density, some round
Female 2	2	white color, good density, mostly round
Female 3	3	orange color, good density, mostly round

Egg Fertilization Time: 1253

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	N/A
Female 2	N/A
Female 3	100%

Stock(s) chosen for testing: 3

Embryo Inoculum Preparation

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

Number Counted: 15 15
18 19
19 18
17 19
19 19

Mean: 17.8

Mean 17.8 x 50 = 890 embryos/ml

Initial Density: 890 = 2.96 (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
1	186	187	99	99%
2	168	169	99	
3	189	191	99	
4	192	194	99	
5	166	168	99	
6	192	193	99	

48-h QC: 169/174 = 97.1%

Comments:

$\bar{x} = 182$

QC Check: KTP 4/2/19

Final Review: EG 4/3/19

Appendix E
List of Qualifier Codes

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 $\leq 110\%$
- 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.