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TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: April 2019

Prepared for: Jacobs
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Date Submitted: May 21, 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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Results verified by: _____
Eric Green, Project Manager

Introduction

A toxicity test was performed using a groundwater composite sample collected on April 23, 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 April 24 and 26, 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	042319
Enthalpy Log-in Number	19-0517
Collection Date; Time	4/23/2019; 0944h
Receipt Date; Time	4/24/2019; 0830h
Receipt Temperature (°C)	1.0
Dissolved Oxygen (mg/L)	9.3
pH	7.46
Conductivity (µS/cm)	11,870
Salinity (ppt)	6.9
Alkalinity (mg/L CaCO ₃)	415
Total Chlorine (mg/L)	0.05
Total Ammonia (mg/L as N)	1.5

Test Methods

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

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

Test Period	4/24/2019, 1425h to 4/26/2019, 1400h
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 90.3 ppt
Test Concentrations (% sample)	72.3 ^a , 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper sulfate (per project QAPP) ^b
Statistical Software	CETIS™ 1.8.7.20

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

^b Enthlpy 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 72.3 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.38 for both endpoints.

Results for the chronic toxicity test are summarized in Tables 3 and 4. Individual statistical summaries for the test and copies of the laboratory bench sheets are provided in Appendix A. The sample check-in sheet and COC form are provided in Appendices B and C, respectively.

Table 3. Summary of Statistical Results for the Chronic Toxicity Tests

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU _c)	EC ₂₅ (% effluent)
Bivalve	Normal Development	72.3	> 72.3	< 1.38	> 72.3
	Survival	72.3	> 72.3	< 1.38	> 72.3

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

Effect Concentration 25 (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)	92.5	98.2
0 (Lab Control)	96.0	98.6
2	96.5	97.7
4	97.2	98.4
9	99.5	98.9
18	96.1	98.4
35	94.1	98.4
72.3 ^a	98.2	98.1

^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 concurrent reference toxicant test were within the acceptable range of the mean historical test results plus or minus two standard deviations. The reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix 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	Endpoint	EC ₅₀ (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve	Normal Development	7.59	7.57 ± 5.87	38.8
	Survival Rate	30.1	25.1 ± 5.77	11.5

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: 02 May-19 14:05 (p 1 of 2)
 Test Code: 1904-S108 | 19-7850-1717

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

Batch ID: 01-0697-7244	Test Type: Development-Survival	Analyst:
Start Date: 24 Apr-19 14:25	Protocol: EPA/600/R-95/136 (1995)	Diluent: Diluted Natural Seawater
Ending Date: 26 Apr-19 14:00	Species: Mytilus galloprovincialis	Brine: Frozen Seawater
Duration: 48h	Source: Mission Bay	Age:

Sample ID: 17-8136-2383	Code: 19-0517	Client: Jacobs
Sample Date: 23 Apr-19 09:44	Material: (K) Effluent Sample Groundwater	Project:
Receive Date: 24 Apr-19 08:30	Source: Jacobs	
Sample Age: 29h (1 °C)	Station: Wyckoff	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-1928-6631	Development Rate	72.3	>72.3	NA	1.82%	< 1.383	Dunnett Multiple Comparison Test
12-6674-4267	Survival Rate	72.3	>72.3	NA	11.3%	< 1.383	Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
12-2669-1218	Development Rate	EC25	>72.3	N/A	N/A	<1.383	Linear Interpolation (ICPIN)
		EC50	>72.3	N/A	N/A	<1.383	
04-6863-8242	Survival Rate	EC25	>72.3	N/A	N/A	<1.383	Linear Interpolation (ICPIN)
		EC50	>72.3	N/A	N/A	<1.383	

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
11-1928-6631	Development Rate	Control Resp	0.9819	0.9 - NL	Yes	Passes Acceptability Criteria
12-2669-1218	Development Rate	Control Resp	0.9819	0.9 - NL	Yes	Passes Acceptability Criteria
04-6863-8242	Survival Rate	Control Resp	0.9252	0.5 - NL	Yes	Passes Acceptability Criteria
12-6674-4267	Survival Rate	Control Resp	0.9252	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	0.9819	0.9588	1	0.9493	0.9945	0.008317	0.0186	1.89%	0.0%
0	Lab Control	5	0.986	0.9761	0.9958	0.9774	0.9938	0.003547	0.00793	0.8%	-0.42%
2		5	0.9773	0.9603	0.9943	0.9559	0.9893	0.006109	0.01366	1.4%	0.47%
4		5	0.9837	0.9767	0.9906	0.9787	0.9932	0.00251	0.005613	0.57%	-0.18%
9		5	0.9888	0.9774	1	0.9756	1	0.004105	0.009178	0.93%	-0.7%
18		5	0.9843	0.9727	0.9959	0.9762	1	0.004184	0.009355	0.95%	-0.25%
35		5	0.9839	0.9699	0.9978	0.9699	1	0.005023	0.01123	1.14%	-0.21%
72.3		5	0.9812	0.9742	0.9881	0.9735	0.989	0.002502	0.005595	0.57%	0.07%

Survival Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9252	0.8395	1	0.8645	1	0.03085	0.06897	7.46%	0.0%
0	Lab Control	5	0.96	0.8827	1	0.8581	1	0.02785	0.06228	6.49%	-3.77%
2		5	0.9652	0.8982	1	0.8774	1	0.02411	0.0539	5.59%	-4.32%
4		5	0.9716	0.9204	1	0.9097	1	0.01843	0.04121	4.24%	-5.02%
9		5	0.9948	0.9805	1	0.9742	1	0.005161	0.01154	1.16%	-7.53%
18		5	0.9613	0.8952	1	0.871	1	0.02379	0.0532	5.53%	-3.91%
35		5	0.9406	0.8585	1	0.8581	1	0.02959	0.06617	7.04%	-1.67%
72.3		5	0.9819	0.9468	1	0.9355	1	0.01264	0.02827	2.88%	-6.14%

(K) Eh Q18 5/17/19

CETIS Summary Report

Report Date: 02 May-19 14:05 (p 2 of 2)
 Test Code: 1904-S108 | 19-7850-1717

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	0.9493	0.9926	0.9945	0.9879	0.9851	
0	Lab Control	0.9863	0.9938	0.9785	0.9774	0.9938	
2		0.9806	0.9728	0.9559	0.9879	0.9893	
4		0.9932	0.9832	0.9787	0.9825	0.9806	
9		0.9871	0.9756	1	0.9868	0.9943	
18		1	0.9852	0.9799	0.9762	0.9801	
35		0.9866	0.9854	1	0.9699	0.9775	
72.3		0.9793	0.989	0.9814	0.9735	0.9828	
Survival Rate Detail							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.8903	0.871	1	1	0.8645	
0	Lab Control	0.9419	1	1	0.8581	1	
2		1	0.9484	0.8774	1	1	
4		0.9484	1	0.9097	1	1	
9		1	1	1	0.9742	1	
18		1	0.871	0.9613	1	0.9742	
35		0.9613	0.8839	1	0.8581	1	
72.3		0.9355	1	1	0.9742	1	
Development Rate Binomials							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	131/138	134/135	181/182	163/165	132/134	
0	Lab Control	144/146	161/162	182/186	130/133	160/161	
2		152/155	143/147	130/136	163/165	185/187	
4		146/147	176/179	138/141	168/171	152/155	
9		153/155	160/164	174/174	149/151	175/176	
18		166/166	133/135	146/149	164/168	148/151	
35		147/149	135/137	160/160	129/133	174/178	
72.3		142/145	179/181	158/161	147/151	171/174	
Survival Rate Binomials							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	138/155	135/155	155/155	155/155	134/155	
0	Lab Control	146/155	155/155	155/155	133/155	155/155	
2		155/155	147/155	136/155	155/155	155/155	
4		147/155	155/155	141/155	155/155	155/155	
9		155/155	155/155	155/155	151/155	155/155	
18		155/155	135/155	149/155	155/155	151/155	
35		149/155	137/155	155/155	133/155	155/155	
72.3		145/155	155/155	155/155	151/155	155/155	

CETIS Analytical Report

Report Date: 02 May-19 14:05 (p 1 of 4)
 Test Code: 1904-S108 | 19-7850-1717

Bivalve Larval Survival and Development Test							Nautilus Environmental (CA)				
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Analysis ID: 11-1928-6631	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 02 May-19 14:04	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	1.82%	72.3	>72.3	NA	1.383

Dunnett Multiple Comparison Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	0.7947	2.407	0.067	8	0.5477	CDF	Non-Significant Effect
		4	0.07817	2.407	0.067	8	0.8349	CDF	Non-Significant Effect
		9	-0.87	2.407	0.067	8	0.9820	CDF	Non-Significant Effect
		18	-0.1494	2.407	0.067	8	0.8937	CDF	Non-Significant Effect
		35	-0.135	2.407	0.067	8	0.8906	CDF	Non-Significant Effect
		72.3	0.4417	2.407	0.067	8	0.7048	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.006289693	0.001048282	6	0.5422	0.7716	Non-Significant Effect
Error	0.05413731	0.001933475	28			
Total	0.060427		34			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	5.314	16.81	0.5042	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9761	0.9146	0.6308	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	0.9819	0.9588	1	0.9879	0.9493	0.9945	0.008317	1.89%	0.0%
2		5	0.9773	0.9603	0.9943	0.9806	0.9559	0.9893	0.006109	1.4%	0.47%
4		5	0.9837	0.9767	0.9906	0.9825	0.9787	0.9932	0.002511	0.57%	-0.18%
9		5	0.9888	0.9774	1	0.9871	0.9756	1	0.004104	0.93%	-0.7%
18		5	0.9843	0.9727	0.9959	0.9801	0.9762	1	0.004183	0.95%	-0.25%
35		5	0.9839	0.9699	0.9978	0.9854	0.9699	1	0.005023	1.14%	-0.21%
72.3		5	0.9812	0.9742	0.9881	0.9814	0.9735	0.989	0.002502	0.57%	0.07%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.447	1.371	1.522	1.46	1.344	1.497	0.02715	4.2%	0.0%
2		5	1.425	1.37	1.479	1.431	1.359	1.467	0.01976	3.1%	1.53%
4		5	1.445	1.413	1.476	1.438	1.424	1.488	0.01129	1.75%	0.15%
9		5	1.471	1.415	1.527	1.457	1.414	1.533	0.02014	3.06%	-1.67%
18		5	1.451	1.393	1.509	1.429	1.416	1.532	0.02094	3.23%	-0.29%
35		5	1.45	1.387	1.514	1.45	1.396	1.531	0.02277	3.51%	-0.26%
72.3		5	1.434	1.408	1.461	1.434	1.407	1.465	0.009449	1.47%	0.85%

Bivalve Larval Survival and Development Test

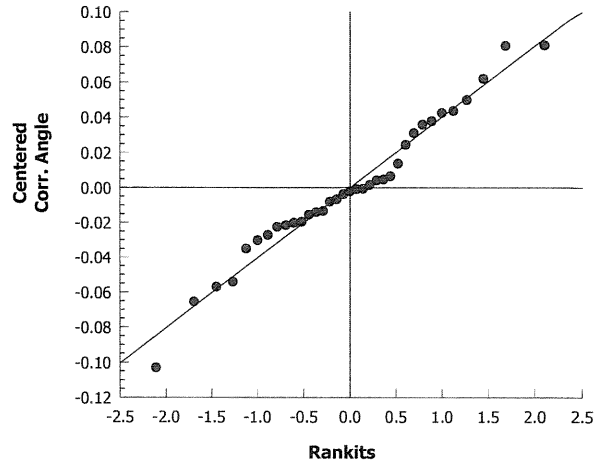
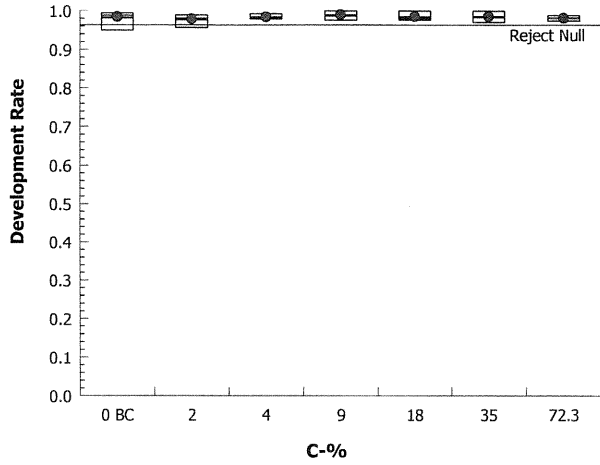
Nautilus Environmental (CA)

Analysis ID: 11-1928-6631
Analyzed: 02 May-19 14:04

Endpoint: Development Rate
Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 02 May-19 14:05 (p 3 of 4)
 Test Code: 1904-S108 | 19-7850-1717

Bivalve Larval Survival and Development Test				Nautilus Environmental (CA)			
Analysis ID: 12-6674-4267	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7					
Analyzed: 02 May-19 14:04	Analysis: Parametric-Control vs Treatments	Official Results: Yes					

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.3%	72.3	>72.3	NA	1.383

Dunnett Multiple Comparison Test									
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-1.071	2.407	0.205	8	0.9900	CDF	Non-Significant Effect
		4	-1.194	2.407	0.205	8	0.9932	CDF	Non-Significant Effect
		9	-1.978	2.407	0.205	8	0.9995	CDF	Non-Significant Effect
		18	-0.8359	2.407	0.205	8	0.9802	CDF	Non-Significant Effect
		35	-0.3527	2.407	0.205	8	0.9318	CDF	Non-Significant Effect
		72.3	-1.468	2.407	0.205	8	0.9972	CDF	Non-Significant Effect

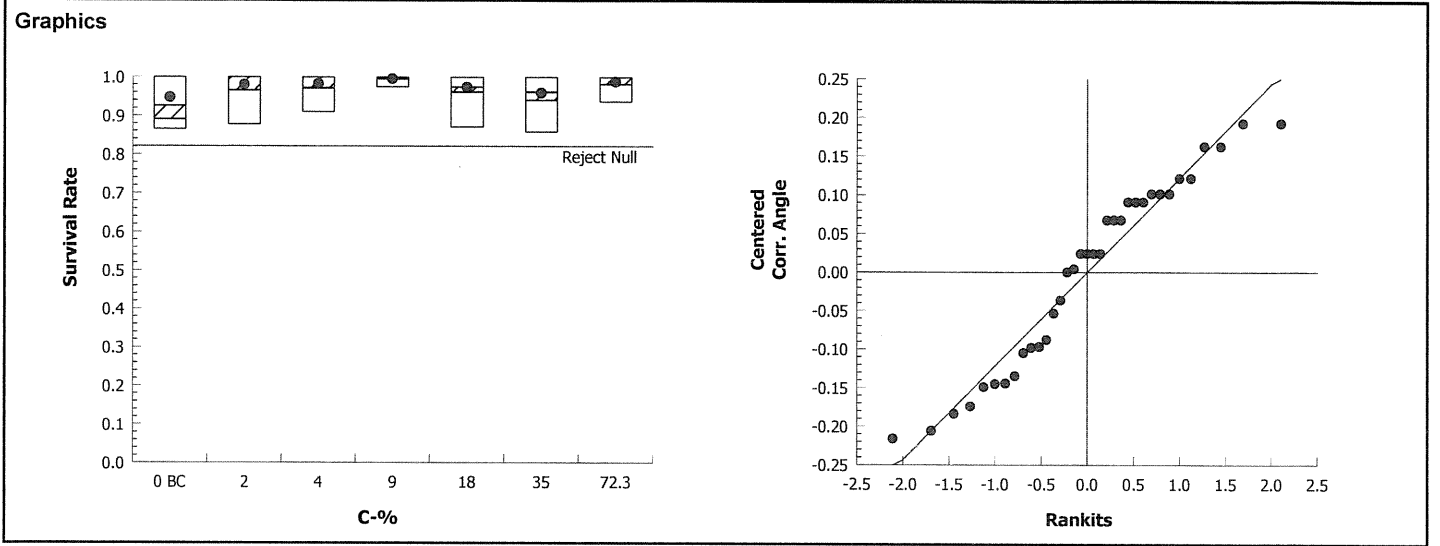
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.09609102	0.01601517	6	0.8873	0.5173	Non-Significant Effect
Error	0.5053791	0.01804925	28			
Total	0.6014701		34			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	5.207	16.81	0.5175	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9414	0.9146	0.0618	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	0.9252	0.8395	1	0.8903	0.8645	1	0.03085	7.46%	0.0%
2		5	0.9652	0.8982	1	1	0.8774	1	0.02411	5.59%	-4.32%
4		5	0.9716	0.9204	1	1	0.9097	1	0.01843	4.24%	-5.02%
9		5	0.9948	0.9805	1	1	0.9742	1	0.005161	1.16%	-7.53%
18		5	0.9613	0.8952	1	0.9742	0.871	1	0.02379	5.53%	-3.91%
35		5	0.9406	0.8585	1	0.9613	0.8581	1	0.02959	7.04%	-1.67%
72.3		5	0.9819	0.9468	1	1	0.9355	1	0.01264	2.88%	-6.14%

Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.338	1.12	1.557	1.233	1.194	1.531	0.07877	13.16%	0.0%
2		5	1.429	1.248	1.611	1.531	1.213	1.531	0.06528	10.21%	-6.8%
4		5	1.44	1.282	1.598	1.531	1.266	1.531	0.0569	8.84%	-7.58%
9		5	1.506	1.439	1.574	1.531	1.409	1.531	0.02423	3.6%	-12.56%
18		5	1.409	1.241	1.577	1.409	1.203	1.531	0.06049	9.6%	-5.31%
35		5	1.368	1.165	1.572	1.373	1.185	1.531	0.07335	11.99%	-2.24%
72.3		5	1.463	1.341	1.585	1.531	1.314	1.531	0.04404	6.73%	-9.32%

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)	
Analysis ID: 12-6674-4267	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7	
Analyzed: 02 May-19 14:04	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

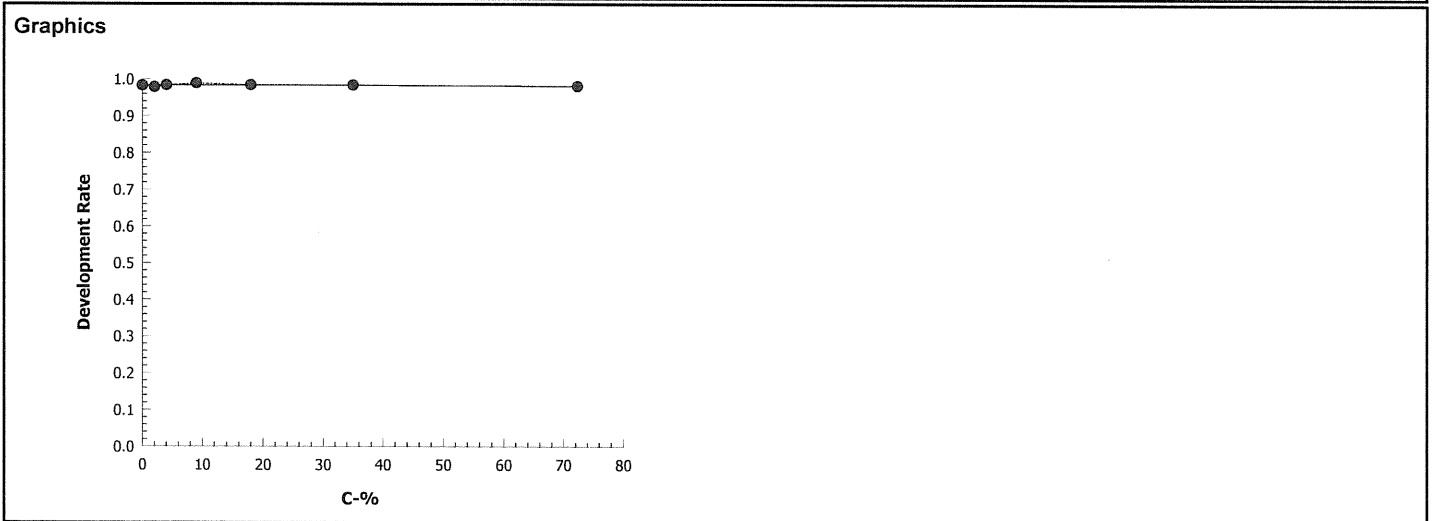
Report Date: 02 May-19 14:05 (p 1 of 2)
 Test Code: 1904-S108 | 19-7850-1717

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 12-2669-1218	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 02 May-19 14:04	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1960171	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>72.3	N/A	N/A	<1.383	NA	NA
EC50	>72.3	N/A	N/A	<1.383	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	0.9819	0.9493	0.9945	0.008317	0.0186	1.89%	0.0%	741	754	
2		5	0.9773	0.9559	0.9893	0.006109	0.01366	1.4%	0.47%	773	790	
4		5	0.9837	0.9787	0.9932	0.002511	0.005614	0.57%	-0.18%	780	793	
9		5	0.9888	0.9756	1	0.004104	0.009178	0.93%	-0.7%	811	820	
18		5	0.9843	0.9762	1	0.004183	0.009354	0.95%	-0.25%	757	769	
35		5	0.9839	0.9699	1	0.005023	0.01123	1.14%	-0.21%	745	757	
72.3		5	0.9812	0.9735	0.989	0.002502	0.005595	0.57%	0.07%	797	812	



CETIS Analytical Report

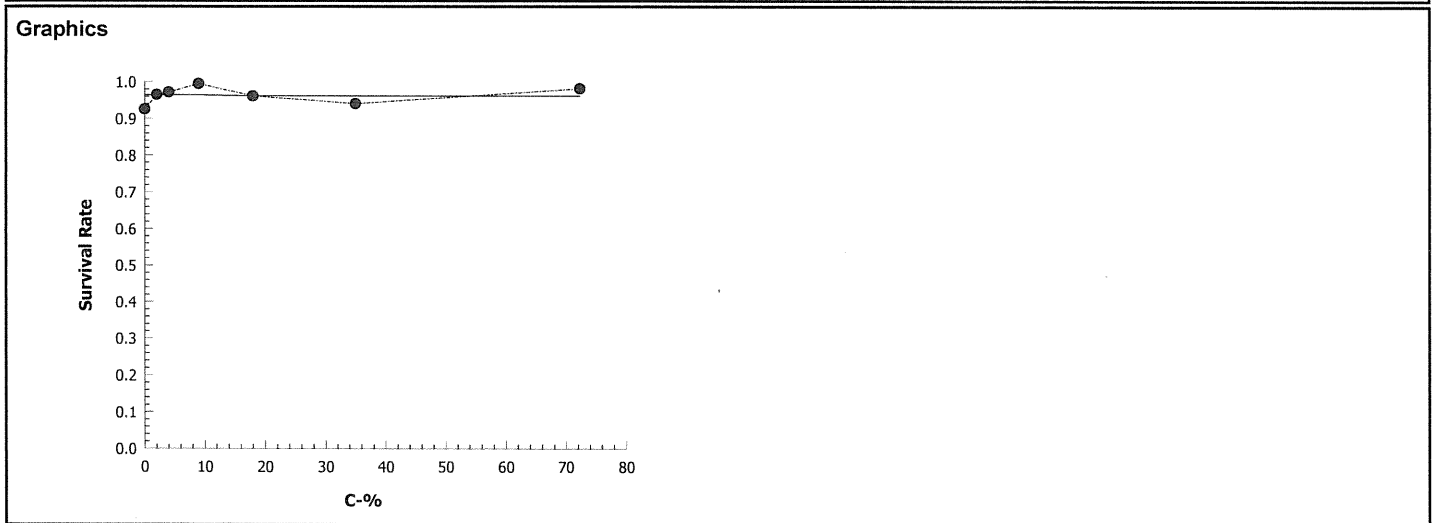
Report Date: 02 May-19 14:05 (p 2 of 2)
 Test Code: 1904-S108 | 19-7850-1717

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 04-6863-8242	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 02 May-19 14:04	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1896575	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>72.3	N/A	N/A	<1.383	NA	NA
EC50	>72.3	N/A	N/A	<1.383	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	0.9252	0.8645	1	0.03085	0.06897	7.46%	0.0%	717	775	
2		5	0.9652	0.8774	1	0.02411	0.0539	5.59%	-4.32%	748	775	
4		5	0.9716	0.9097	1	0.01843	0.04121	4.24%	-5.02%	753	775	
9		5	0.9948	0.9742	1	0.005161	0.01154	1.16%	-7.53%	771	775	
18		5	0.9613	0.871	1	0.02379	0.0532	5.53%	-3.91%	745	775	
35		5	0.9406	0.8581	1	0.02959	0.06617	7.04%	-1.67%	729	775	
72.3		5	0.9819	0.9355	1	0.01264	0.02827	2.88%	-6.14%	761	775	



CETIS Test Data Worksheet

Report Date: 23 Apr-19 09:50 (p 1 of 1)

Test Code: 1904-S108 19-7850-1717/75ED8A55

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 24 Apr-19
 End Date: 26 Apr-19
 Sample Date: 23 Apr-19

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

Sample Code: 19-0517
 Sample Source: Jacobs
 Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			61			137	135	JUL 5/2/19
			62			182	181	
			63			138	131	
			64			④ 146 147	146	
			65			168	164	
			66			165	163	
			67			171	168	
			68			174	171	
			69			151	147	
			70			155	153	
			71			133	130	
			72			161	158	
			73			155	152	
			74			187	185	
			75			141	138	
			76			160	160	
			77			166	166	
			78			149	147	
			79			134	132	
			80			164	160	
			81			135	134	
			82			151	148	
			83			181	179	
			84			174	174	
			85			147	143	
			86			145	142	
			87			165	163	
			88			149	146	
			89			186	182	
			90			135	133	
			91			151	149	
			92			179	176	
			93			162	161	
			94			136	130	
			95			155	152	
			96			146	144	
			97			133	129	
			98			161	160	
			99			178	174	
			100			176	175	

④ Q18 JUL 5/2/19

⑤ EG Q18 5/17/19

CETIS Test Data Worksheet

Report Date: 23 Apr-19 09:50 (p 1 of 1)
 Test Code: 1904-S108 19-7850-1717/75ED8A55

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 24 Apr-19
 End Date: 26 Apr-19
 Sample Date: 23 Apr-19

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

Sample Code: 19-0517
 Sample Source: Jacobs
 Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	63					
0	BC	2	81					
0	BC	3	62			164	163	RT 4/26/19
0	BC	4	87					
0	BC	5	79					
0	LC	1	96					
0	LC	2	93					
0	LC	3	89			185	182	RT
0	LC	4	71					
0	LC	5	98					
2		1	95					
2		2	85					
2		3	94			119	115	RT
2		4	66					
2		5	74					
4		1	64					
4		2	92			160	157	RT
4		3	75					
4		4	67					
4		5	73					
9		1	70					
9		2	80					
9		3	84			146	146	RT
9		4	91					
9		5	100					
18		1	77					
18		2	90					
18		3	88			143	141	RT
18		4	65					
18		5	82					
35		1	78					
35		2	61					
35		3	76			145	145	RT
35		4	97					
35		5	99					
72.3 73.5		1	86					
72.3 73.5		2	83					
72.3 73.5		3	72			147	145	RT
72.3 73.5		4	69					
72.3 73.5		5	68					

QC = BC

EG Q18 5/17/19

Q18 ACS 4/23/19

Marine Chronic Bioassay

Water Quality Measurements

Client: Jacobs
 Sample ID: Wyckoff
 Sample Log No.: 19-0517
 Test No.: 1904-5108

Test Species: M. galloprovincialis
 Start Date/Time: 4/24/2019 1425
 End Date/Time: 4/26/2019 1400

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	29.7	29.7	29.8	15.2	14.9	14.7	8.6	8.8	8.3	8.01	7.99	8.00
Brine Control	30.1	30.1	30.2	15.7	14.8	14.8	8.3	8.8	8.3	8.12	8.10	8.04
2	30.0	30.1	30.2	14.9	14.6	14.6	8.6	8.8	8.4	8.03	8.03	8.03
4	30.0	30.1	30.2	14.8	14.8	14.7	8.6	8.8	8.4	8.00	8.03	8.05
9	30.1	30.2	30.2	14.6	14.7	14.6	8.6	8.8	8.4	7.94	8.03	8.08
ⓐ 18	30.2	30.3	30.4	14.2	14.7	14.6	8.7	8.8	8.4	7.86	8.01	8.10
35	30.5	30.6	30.7	14.0	14.8	14.7	8.7	8.8	8.3	7.75	8.05	8.18
72.3	30.9	31.0	31.1	14.0	14.7	14.7	8.7	8.7	8.3	7.64	8.02	8.22

Technician Initials: _____
 WQ Readings:

	0	24	48
EH		RT	RT

 Dilutions made by:

	0	24	48
BO		—	—

Comments: 0 hrs: ⓐ EH 9:18 4/24/19
 24 hrs: _____
 48 hrs: _____

QC Check: EH 5/17/19

Final Review: KFF 5/21/19

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: JACOBS

Analyst: BO

Sample ID: Wyckoff

Test Date: 4/24/2019

Test No: 1904-5108

Test Type: Mussel Development

Salinity of Effluent 6.9

Salinity of Brine 90.3

Date of Brine used: 3/29/2019

Target Salinity 30

Alkalinity of Brine Control: 96 mg/L as CaCO3

Test Dilution Volume 250

	<u>Effluent</u>	<u>Brine Control</u>
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	<u>0.38</u>	<u>0.50</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	0.38	1.9	250
4	10.0	0.38	3.8	250
9	22.5	0.38	8.6	250
18	45.0	0.38	17.2	250
35	87.5	0.38	33.5	250
72.3	180.8	0.38	69.2	250

DI Volume				
Brine Control	139.0	0.50	69.2	250

Total Brine Volume Required (ml): 203.3

QC Check: EG 5/17/19

Final Review: KFP 5/21/19

Marine Chronic Bioassay

Larval Development Worksheet

Client: Jacobs / Wyckoff
 Test No.: 1904 - 5108
 Test Species: M. galloprovincialis
 Animal Source: Mission Bay
 Date Received: 4/23/19
 Test Chambers: 30 mL shell vials
 Sample Volume: 10 mL

Start Date/Time: 4/24/2019 1425
 End Date/Time: 4/26/2019 1400
 Technician Initials: BO / EG

Spawn Information

First Gamete Release Time: 1030

Sex	Number Spawning
Male	<u>3</u>
Female	<u>8</u>

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	<u>1, 2</u>	<u>good motility, great density</u>
Female 1	<u>3</u>	<u>orange color, mostly round, good den</u>
Female 2	<u>6</u>	<u>orange color, mostly round, fair density</u>
Female 3	<u>—</u>	<u>—</u>

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	<u>99%</u>
Female 2	<u>100%</u>
Female 3	<u>—</u>

Egg Fertilization Time: 1120

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted: 17 17
15 14
12 15
12 15
16 16

Mean: 14.9

Mean 14.9 x 50 = 745 embryos/ml

Initial Density: 745 = 2.48 (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
<u>1</u>	<u>148</u>	<u>148</u>	<u>100</u>	<u>100</u>
<u>2</u>	<u>152</u>	<u>152</u>	<u>100</u>	
<u>3</u>	<u>159</u>	<u>160</u>	<u>99</u>	
<u>4</u>	<u>156</u>	<u>156</u>	<u>100</u>	
<u>5</u>	<u>143</u>	<u>143</u>	<u>100</u>	
<u>6</u>	<u>169</u>	<u>169</u>	<u>100</u>	

48-h QC: 152/154 = 99%

Comments:

$\bar{x} = 155$

QC Check:

EA 5/17/19

Final Review:

KPS/2/1/19

Appendix B
Sample Check-In Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff - 042319
Test ID No(s): 1904-5108

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>0517</u>			
Sample Collection Date & Time:	<u>4/23/19 0944</u>			
Sample Receipt Date & Time:	<u>4/24/19 0830</u>			
Number of Containers & Container Type:	<u>1 1L cubi</u>			
Approx. Total Volume Received (L):	<u>~1L</u>			
Check-in Temperature (°C)	<u>1.0</u>			
Temperature OK? ¹	<u>(Y) N</u>	<u>Y N</u>	<u>Y N</u>	<u>Y N</u>
DO (mg/L)	<u>9.3</u>			
pH (units)	<u>7.46</u>			
Conductivity (µS/cm)	<u>11,870</u>			
Salinity (ppt)	<u>6.94^e</u>			
Alkalinity (mg/L) ²	<u>415</u>			
Hardness (mg/L) ^{2,3}	<u>—</u>			
Total Chlorine (mg/L)	<u>0.05</u>			
Technician Initials	<u>HH</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 AHH B — C —

QC Check: EA 5/17/19

Final Review: KTP 5/21/19

Test Performed: Mussel Development Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: 94 Hardness or Salinity: 30 ppt

Additional Control? (Y) N = None Control Alkalinity: 96 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: KTP 5/21/19

**Total Ammonia Analysis
Freshwater**

Overlying Water

Client: JACOBS
 Project: Wyckoff
 Test Type: Mussel Development

DI Blank: 0.0
 Test Start Date: 4/17/2019 4/24/19
 Analyst: KL
 Analysis Date: 4/29/19

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 NH ₃)		NA	NA	6.7	8.2
Wyckoff		4/17/2019 4/24/19	Check In	1.5	1.8
Spike Check (10 mg/L NH ₃)		NA	NA	6.7	8.2
Sample Duplicate ^a		NA	NA	1.6	2.0
Sample Duplicate + Spike ^a		NA	NA	7.7	9.4
Spike Check (10 mg/L NH ₃)		NA	NA	6.7	8.2

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	0.0	NA	8.2	10	NA	80-82
Wyckoff	1.8	2.0	9.4	10	10.5	76

Comments: A EG Q18 4/22/19 B Q16 4/21/19 C Q18 4/21/19

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

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

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

Method Detection Limit = 0.5 mg/L

QC Check: VS 4/29/19

Final Review: EG 5/17/19

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)

DateShipped: 4/23/2019

CarrierName: FedEx

AirbillNo: 775037485152

CHAIN OF CUSTODY RECORD

Wyckoff Eagle Harbor GWTP 2019/WA

Project Code: WEH-029A

Cooler #: 1 of 1

No: 10-042319-102126-0363

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
042319		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	04/23/2019 09:44	Field Sample

Shipment for Case Complete? N
 Samples Transferred From Chain of Custody #

Special Instructions:

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> JACOBS	4-23-2019 1025	<i>Hayden A Nauticus</i>	4/24/19 0830	temp: 10°C

19-0517

Appendix D
Reference Toxicant Test Results

CETIS Summary Report

Report Date: 09 May-19 13:26 (p 1 of 3)
 Test Code: 190424msdvSO | 14-4098-8496

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

Batch ID: 04-0967-6710	Test Type: Development-Survival	Analyst:
Start Date: 24 Apr-19 14:25	Protocol: EPA/600/R-95/136 (1995)	Diluent: Diluted Natural Seawater
Ending Date: 26 Apr-19 14:00	Species: Mytilus galloprovincialis	Brine: Not Applicable
Duration: 48h	Source: Mission Bay	Age:

Sample ID: 04-6938-7195	Code: 190424msdvSO	Client: Internal
Sample Date: 24 Apr-19	Material: Copper sulfate	Project:
Receive Date: 24 Apr-19	Source: Reference Toxicant	
Sample Age: 14h	Station: Copper Sulfate	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-3870-8526	Combined Development Ra	5	10	7.071	5.85%		Dunnett Multiple Comparison Test
00-7240-4995	Development Rate	5	10	7.071	1.96%		Dunnett Multiple Comparison Test
12-9807-3384	Survival Rate	20	40	28.28	6.92%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
15-6766-2617	Combined Development Ra	EC25	6.247	5.964	6.342		Linear Interpolation (ICPIN)
		EC50	7.565	7.378	7.649		
17-2116-2275	Development Rate	EC25	6.279	6.205	6.325		Linear Interpolation (ICPIN)
		EC50	7.586	7.511	7.64		
03-3240-7186	Survival Rate	EC25	24.99	24.05	25.14		Linear Interpolation (ICPIN)
		EC50	30.08	29.44	30.23		

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-7240-4995	Development Rate	Control Resp	0.9864	0.9 - NL	Yes	Passes Acceptability Criteria
17-2116-2275	Development Rate	Control Resp	0.9864	0.9 - NL	Yes	Passes Acceptability Criteria
03-3240-7186	Survival Rate	Control Resp	0.9652	0.5 - NL	Yes	Passes Acceptability Criteria
12-9807-3384	Survival Rate	Control Resp	0.9652	0.5 - NL	Yes	Passes Acceptability Criteria
14-3870-8526	Combined Development Ra	PMSD	0.05855	NL - 0.25	No	Passes Acceptability Criteria

CETIS Summary Report

Report Date: 09 May-19 13:26 (p 2 of 3)
 Test Code: 190424msdvSO | 14-4098-8496

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	0.9523	0.8927	1	0.8839	1	0.02149	0.04805	5.05%	0.0%
2.5		5	0.9182	0.845	0.9915	0.8516	0.9937	0.02637	0.05896	6.42%	3.58%
5		5	0.9602	0.9337	0.9866	0.9355	0.9814	0.009531	0.02131	2.22%	-0.82%
10		5	0.03637	0.02039	0.05236	0.01923	0.05161	0.005757	0.01287	35.39%	96.18%
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	0.9864	0.9742	0.9987	0.9786	1	0.004402	0.009844	1.0%	0.0%
2.5		5	0.9792	0.955	1	0.9517	0.9937	0.008714	0.01948	1.99%	0.73%
5		5	0.9844	0.9721	0.9968	0.9733	1	0.004437	0.009922	1.01%	0.2%
10		5	0.03799	0.01966	0.05632	0.01923	0.05797	0.006602	0.01476	38.86%	96.15%
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	0.9652	0.9146	1	0.9032	1	0.0182	0.0407	4.22%	0.0%
2.5		5	0.9381	0.8601	1	0.8581	1	0.02809	0.06282	6.7%	2.81%
5		5	0.9755	0.9423	1	0.9355	1	0.01197	0.02676	2.74%	-1.07%
10		5	0.9677	0.9105	1	0.8903	1	0.0206	0.04607	4.76%	-0.27%
20		5	0.96	0.9288	0.9912	0.9355	1	0.01125	0.02515	2.62%	0.53%
40		5	0.0129	0.000237	0.02557	0	0.02581	0.004562	0.0102	79.06%	98.66%
Combined Development Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.929	0.9548	0.8839	0.9939	1					
2.5		0.8903	0.9937	0.8516	0.9653	0.8903					
5		0.9808	0.9613	0.9355	0.9814	0.9419					
10		0.03226	0.04651	0.05161	0.01923	0.03226					
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	0.9796	0.9801	0.9786	0.9939	1					
2.5		0.9928	0.9937	0.9925	0.9653	0.9517					
5		0.9808	0.9868	1	0.9814	0.9733					
10		0.03268	0.04651	0.05797	0.01923	0.03356					
20		0	0	0	0	0					
40		0	0	0	0	0					
Survival Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.9484	0.9742	0.9032	1	1					
2.5		0.8968	1	0.8581	1	0.9355					
5		1	0.9742	0.9355	1	0.9677					
10		0.9871	1	0.8903	1	0.9613					
20		0.9419	0.9355	1	0.9613	0.9613					
40		0.0129	0.02581	0	0.01935	0.006452					

CETIS Summary Report

Report Date: 09 May-19 13:26 (p 3 of 3)

Test Code: 190424msdvSO | 14-4098-8496

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	144/155	148/155	137/155	162/163	158/158	
2.5		138/155	157/158	132/155	167/173	138/155	
5		153/156	149/155	145/155	158/161	146/155	
10		5/155	8/172	8/155	3/156	5/155	
20		0/155	0/155	0/216	0/155	0/155	
40		0/155	0/155	0/155	0/155	0/155	
Development Rate Binomials							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	144/147	148/151	137/140	162/163	158/158	
2.5		138/139	157/158	132/133	167/173	138/145	
5		153/156	149/151	145/145	158/161	146/150	
10		5/153	8/172	8/138	3/156	5/149	
20		0/146	0/145	0/216	0/149	0/149	
40		0/2	0/4	0/1	0/3	0/1	
Survival Rate Binomials							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	147/155	151/155	140/155	155/155	155/155	
2.5		139/155	155/155	133/155	155/155	145/155	
5		155/155	151/155	145/155	155/155	150/155	
10		153/155	155/155	138/155	155/155	149/155	
20		146/155	145/155	155/155	149/155	149/155	
40		2/155	4/155	0/155	3/155	1/155	

CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 1 of 4)
 Test Code: 190424msdVSO | 14-4098-8496

Bivalve Larval Survival and Development Test				Nautilus Environmental (CA)					
Analysis ID: 14-3870-8526	Endpoint: Combined Development Rate			CETIS Version: CETISv1.8.7					
Analyzed: 09 May-19 13:24	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	5.85%	5	10	7.071	

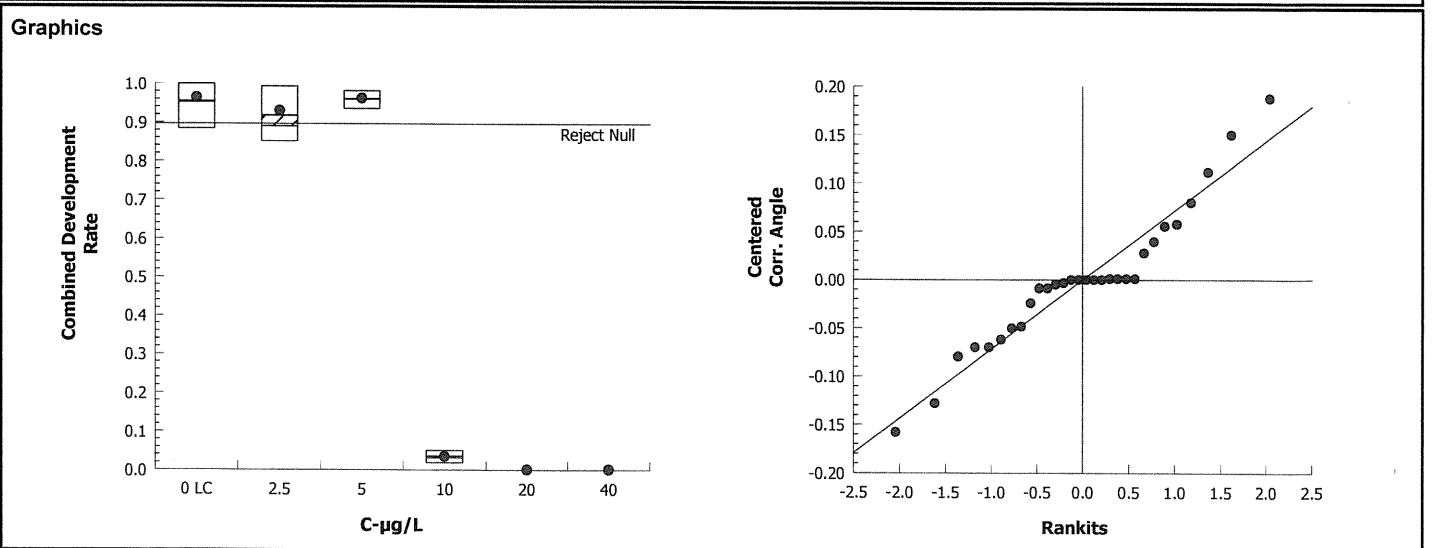
Dunnnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	1.256	2.227	0.138	8	0.2402	CDF	Non-Significant Effect
		5	0.07948	2.227	0.138	8	0.7207	CDF	Non-Significant Effect
		10*	19.3	2.227	0.138	8	<0.0001	CDF	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.09965	1.699883	3	178.4	<0.0001	Significant Effect
Error	0.1524397	0.009527481	16			
Total	5.25209		19			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	7.187	11.34	0.0662	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9778	0.866	0.9020	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	0.9523	0.8927	1	0.9548	0.8839	1	0.02149	5.05%	0.0%
2.5		5	0.9182	0.845	0.9915	0.8903	0.8516	0.9937	0.02637	6.42%	3.58%
5		5	0.9602	0.9337	0.9866	0.9613	0.9355	0.9814	0.009531	2.22%	-0.82%
10		5	0.03637	0.02039	0.05236	0.03226	0.01923	0.05161	0.005757	35.39%	96.18%
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	1.381	1.22	1.541	1.357	1.223	1.531	0.0578	9.36%	0.0%
2.5		5	1.303	1.142	1.465	1.233	1.175	1.491	0.05827	10.0%	5.62%
5		5	1.376	1.306	1.446	1.373	1.314	1.434	0.02516	4.09%	0.36%
10		5	0.1894	0.1453	0.2335	0.1806	0.1391	0.2292	0.01589	18.76%	86.29%
20		5	0.03894	0.03553	0.04235	0.04017	0.03403	0.04017	0.001229	7.06%	97.18%
40		5	0.04017	0.04016	0.04018	0.04017	0.04017	0.04017	0	0.0%	97.09%



CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 2 of 4)
 Test Code: 190424msdVSO | 14-4098-8496

Bivalve Larval Survival and Development Test							Nautilus Environmental (CA)			
Analysis ID: 00-7240-4995		Endpoint: Development Rate			CETIS Version: CETISv1.8.7					
Analyzed: 09 May-19 13:24		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	1.96%	5	10	7.071		

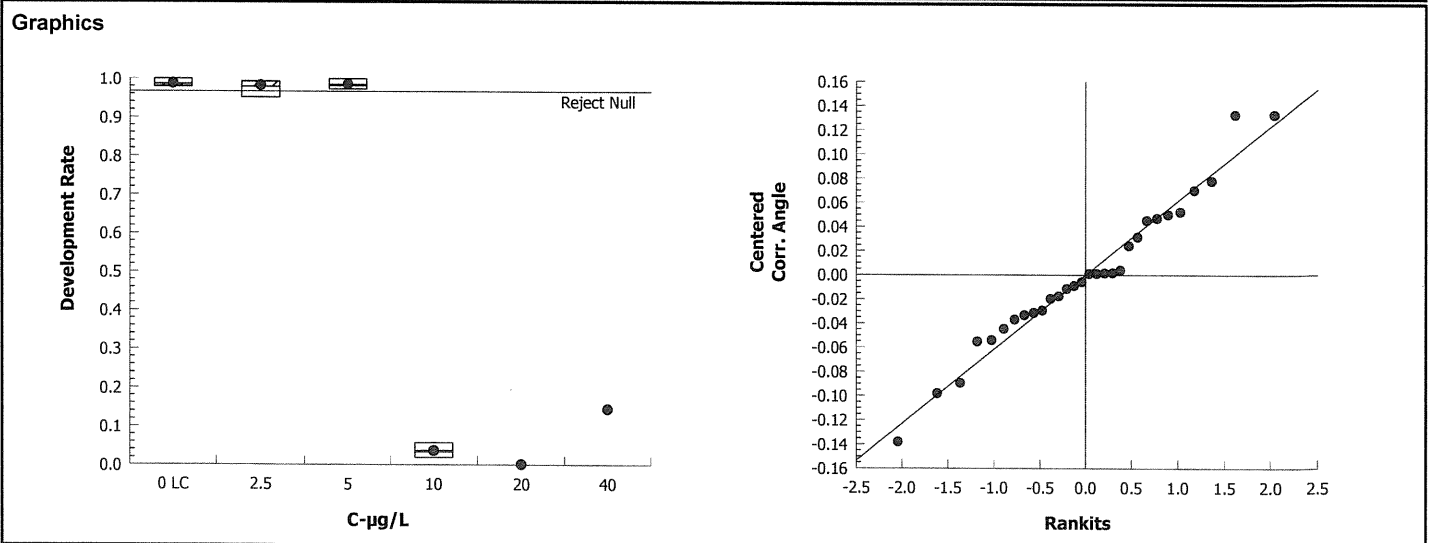
Dunnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.6775	2.227	0.073	8	0.4664	CDF	Non-Significant Effect
		5	0.2891	2.227	0.073	8	0.6363	CDF	Non-Significant Effect
		10*	38.91	2.227	0.073	8	<0.0001	CDF	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.928229	1.976076	3	744.8	<0.0001	Significant Effect
Error	0.04244851	0.002653032	16			
Total	5.970677		19			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	1.143	11.34	0.7666	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.958	0.866	0.5051	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	0.9864	0.9742	0.9987	0.9801	0.9786	1	0.004402	1.0%	0.0%
2.5		5	0.9792	0.955	1	0.9925	0.9517	0.9937	0.008713	1.99%	0.73%
5		5	0.9844	0.9721	0.9968	0.9814	0.9733	1	0.004437	1.01%	0.2%
10		5	0.03799	0.01966	0.05632	0.03356	0.01923	0.05797	0.006602	38.86%	96.15%
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	1.461	1.401	1.521	1.429	1.424	1.531	0.02167	3.32%	0.0%
2.5		5	1.439	1.355	1.522	1.484	1.349	1.491	0.03006	4.67%	1.51%
5		5	1.451	1.393	1.51	1.434	1.407	1.529	0.02094	3.23%	0.64%
10		5	0.1931	0.1442	0.2421	0.1842	0.1391	0.2432	0.01763	20.41%	86.78%
20		5	0.03978	0.03577	0.04379	0.04097	0.03403	0.04153	0.001443	8.11%	97.28%
40		5	0.3908	0.2328	0.5489	0.3614	0.2527	0.5236	0.05693	32.57%	73.25%



CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 3 of 4)

Test Code: 190424msdvSO | 14-4098-8496

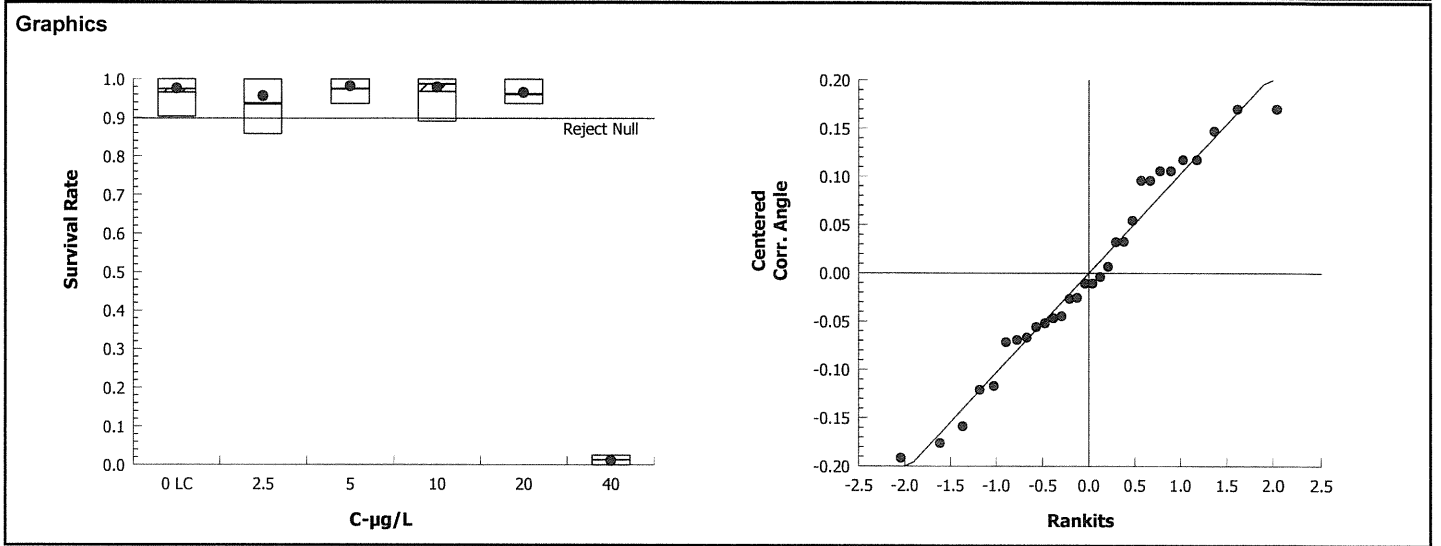
Bivalve Larval Survival and Development Test										Nautilus Environmental (CA)	
Analysis ID: 12-9807-3384		Endpoint: Survival Rate				CETIS Version: CETISv1.8.7					
Analyzed: 09 May-19 13:25		Analysis: Parametric-Control vs Treatments				Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	6.92%	20	40	28.28			
Dunnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	0.7447	2.362	0.167	8	0.5365	CDF	Non-Significant Effect		
		5	-0.3058	2.362	0.167	8	0.9079	CDF	Non-Significant Effect		
		10	-0.1624	2.362	0.167	8	0.8769	CDF	Non-Significant Effect		
		20	0.422	2.362	0.167	8	0.6804	CDF	Non-Significant Effect		
		40*	18.47	2.362	0.167	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	7.021692		1.404338		5	112.3	<0.0001	Significant Effect			
Error	0.300084		0.0125035		24						
Total	7.321776				29						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		5.222	15.09	0.3894	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.964	0.9031	0.3904	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	0.9652	0.9146	1	0.9742	0.9032	1	0.0182	4.22%	0.0%
2.5		5	0.9381	0.8601	1	0.9355	0.8581	1	0.02809	6.7%	2.81%
5		5	0.9755	0.9423	1	0.9742	0.9355	1	0.01197	2.74%	-1.07%
10		5	0.9677	0.9105	1	0.9871	0.8903	1	0.0206	4.76%	-0.27%
20		5	0.96	0.9288	0.9912	0.9613	0.9355	1	0.01125	2.62%	0.53%
40		5	0.0129	0.000237	0.02557	0.0129	0	0.02581	0.004562	79.06%	98.66%
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	1.264	1.563	1.409	1.254	1.531	0.05381	8.51%	0.0%
2.5		5	1.361	1.16	1.562	1.314	1.185	1.531	0.07234	11.89%	3.73%
5		5	1.435	1.318	1.552	1.409	1.314	1.531	0.04219	6.57%	-1.53%
10		5	1.425	1.269	1.58	1.457	1.233	1.531	0.05605	8.8%	-0.81%
20		5	1.384	1.276	1.491	1.373	1.314	1.531	0.03863	6.24%	2.11%
40		5	0.1071	0.04741	0.1667	0.1138	0.04017	0.1613	0.02149	44.88%	92.42%

CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 4 of 4)

Test Code: 190424msdvSO | 14-4098-8496

Bivalve Larval Survival and Development Test		Nautilus Environmental (CA)
Analysis ID: 12-9807-3384	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7
Analyzed: 09 May-19 13:25	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 1 of 3)

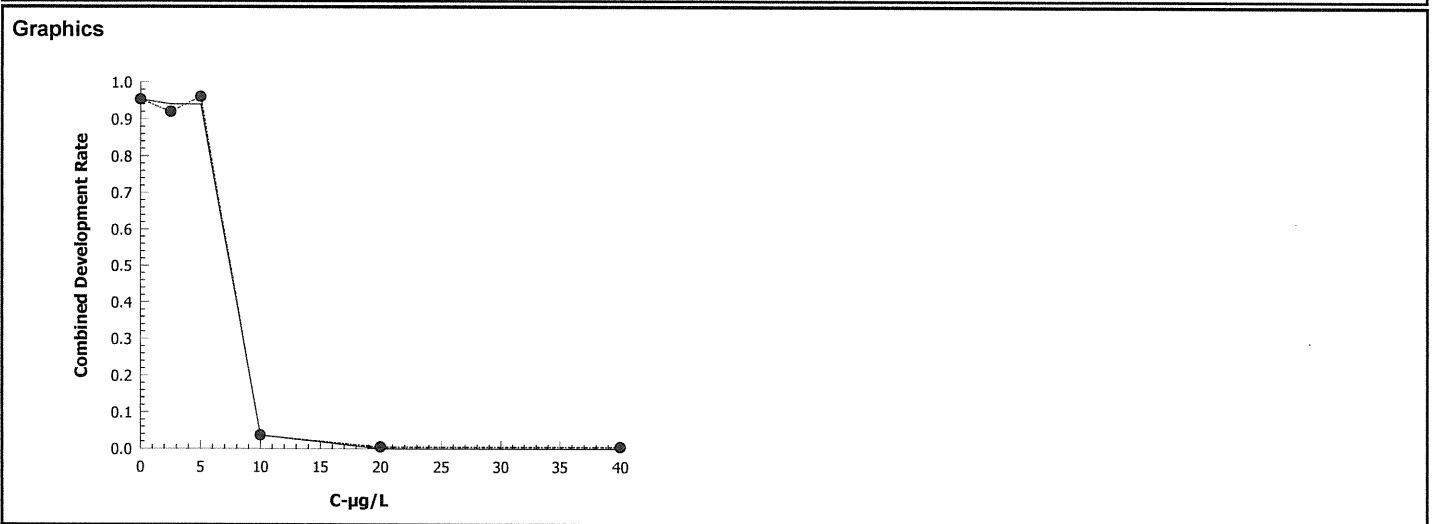
Test Code: 190424msdvSO | 14-4098-8496

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 15-6766-2617	Endpoint: Combined Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 09 May-19 13:25	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1685698	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	6.247	5.964	6.342
EC50	7.565	7.378	7.649

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	0.9523	0.8839	1	0.02149	0.04805	5.05%	0.0%	749	786	
2.5		5	0.9182	0.8516	0.9937	0.02637	0.05896	6.42%	3.58%	732	796	
5		5	0.9602	0.9355	0.9814	0.009531	0.02131	2.22%	-0.82%	751	782	
10		5	0.03637	0.01923	0.05161	0.005757	0.01287	35.39%	96.18%	29	793	
20		5	0	0	0	0	0		100.0%	0	836	
40		5	0	0	0	0	0		100.0%	0	775	



CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 2 of 3)

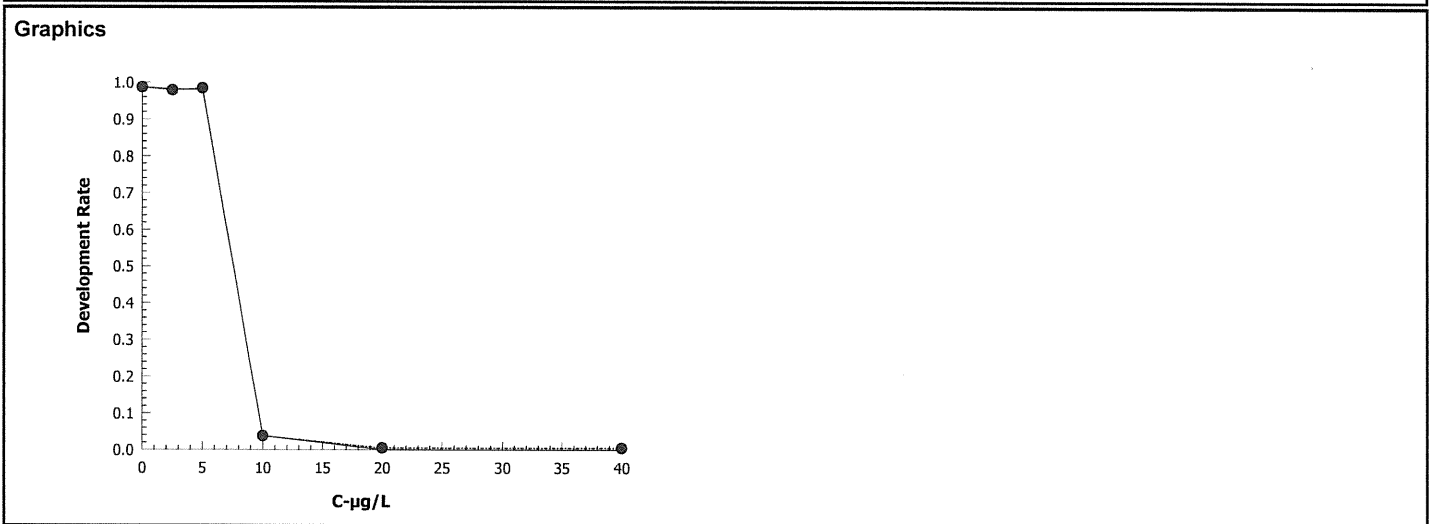
Test Code: 190424msdvSO | 14-4098-8496

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 17-2116-2275	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 09 May-19 13:25	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	444410	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	6.279	6.205	6.325
EC50	7.586	7.511	7.64

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	0.9864	0.9786	1	0.004402	0.009844	1.0%	0.0%	749	759	
2.5		5	0.9792	0.9517	0.9937	0.008713	0.01948	1.99%	0.73%	732	748	
5		5	0.9844	0.9733	1	0.004437	0.009921	1.01%	0.2%	751	763	
10		5	0.03799	0.01923	0.05797	0.006602	0.01476	38.86%	96.15%	29	768	
20		5	0	0	0	0	0		100.0%	0	805	
40		5	0	0	0	0	0		100.0%	0	11	



CETIS Analytical Report

Report Date: 09 May-19 13:25 (p 3 of 3)

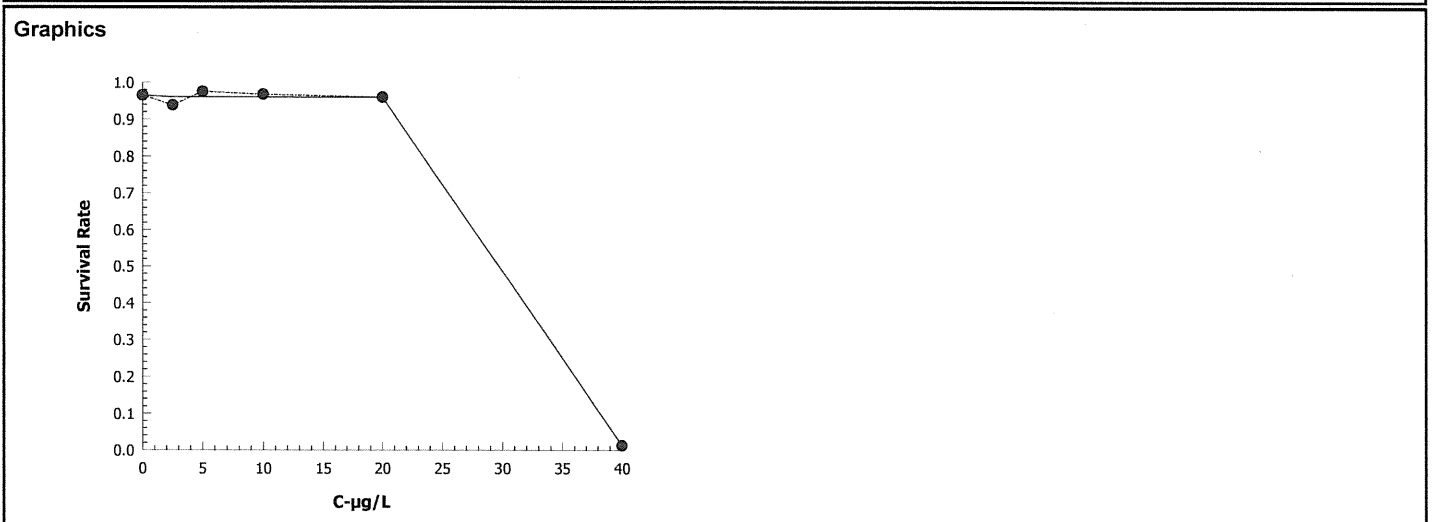
Test Code: 190424msdvSO | 14-4098-8496

Bivalve Larval Survival and Development Test			Nautilus Environmental (CA)		
Analysis ID: 03-3240-7186	Endpoint: Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 09 May-19 13:25	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1064279	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
EC25	24.99	24.05	25.14
EC50	30.08	29.44	30.23

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	0.9652	0.9032	1	0.0182	0.0407	4.22%	0.0%	748	775	
2.5		5	0.9381	0.8581	1	0.02809	0.06282	6.7%	2.81%	727	775	
5		5	0.9755	0.9355	1	0.01197	0.02676	2.74%	-1.07%	756	775	
10		5	0.9677	0.8903	1	0.0206	0.04607	4.76%	-0.27%	750	775	
20		5	0.96	0.9355	1	0.01125	0.02515	2.62%	0.53%	744	775	
40		5	0.0129	0	0.02581	0.004562	0.0102	79.06%	98.66%	10	775	



Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Test Type: Development-Survival

Organism: Mytilus galloprovincialis (Bay Mussel)

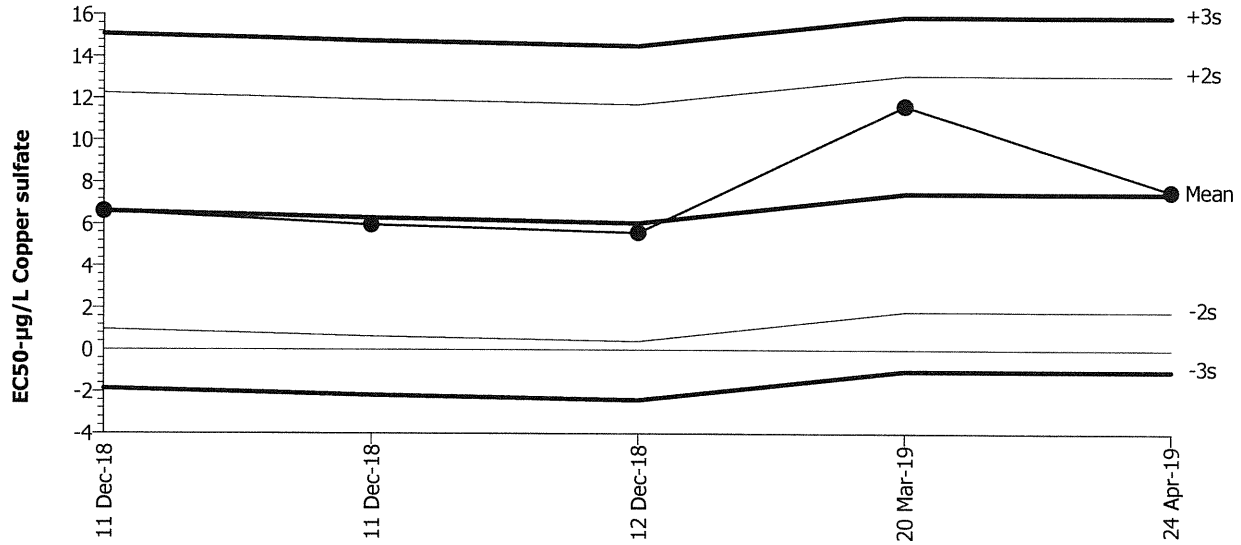
Material: Copper sulfate

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

Endpoint: Combined Development Rate

Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 7.448 Count: 4 -2s Warning Limit: 1.806 -3s Action Limit: -1.015
 Sigma: 2.821 CV: 37.90% +2s Warning Limit: 13.09 +3s Action Limit: 15.91

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	-0.8421	-0.2985			09-7408-5780	08-1757-8045
2			11	12:30	5.959	-1.489	-0.5278			01-7940-4185	15-9822-3312
3			12	13:55	5.593	-1.855	-0.6575			11-6161-8836	02-1891-3936
4	2019	Mar	20	15:25	11.63	4.184	1.483			02-0163-9394	10-2896-8877
5		Apr	24	14:25	7.565	0.1174	0.04161			14-4098-8496	15-6766-2617

Bivalve Larval Survival and Development Test

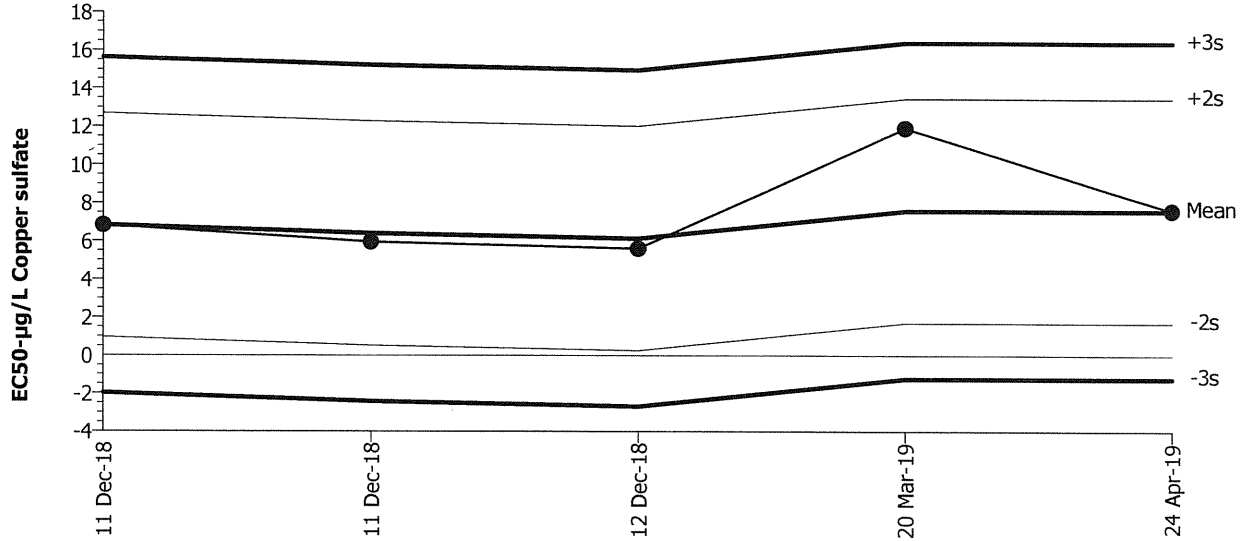
Nautilus Environmental (CA)

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: 7.568 Count: 4 -2s Warning Limit: 1.694 -3s Action Limit: -1.243
 Sigma: 2.937 CV: 38.80% +2s Warning Limit: 13.44 +3s Action Limit: 16.38

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	-0.7401	-0.252			09-7408-5780	03-3077-4520
2			11	12:30	5.952	-1.616	-0.5501			01-7940-4185	03-0677-9138
3			12	13:55	5.589	-1.979	-0.6737			11-6161-8836	13-7938-6780
4	2019	Mar	20	15:25	11.9	4.336	1.476			02-0163-9394	06-2977-8138
5		Apr	24	14:25	7.586	0.01777	0.006051			14-4098-8496	17-2116-2275

Bivalve Larval Survival and Development Test

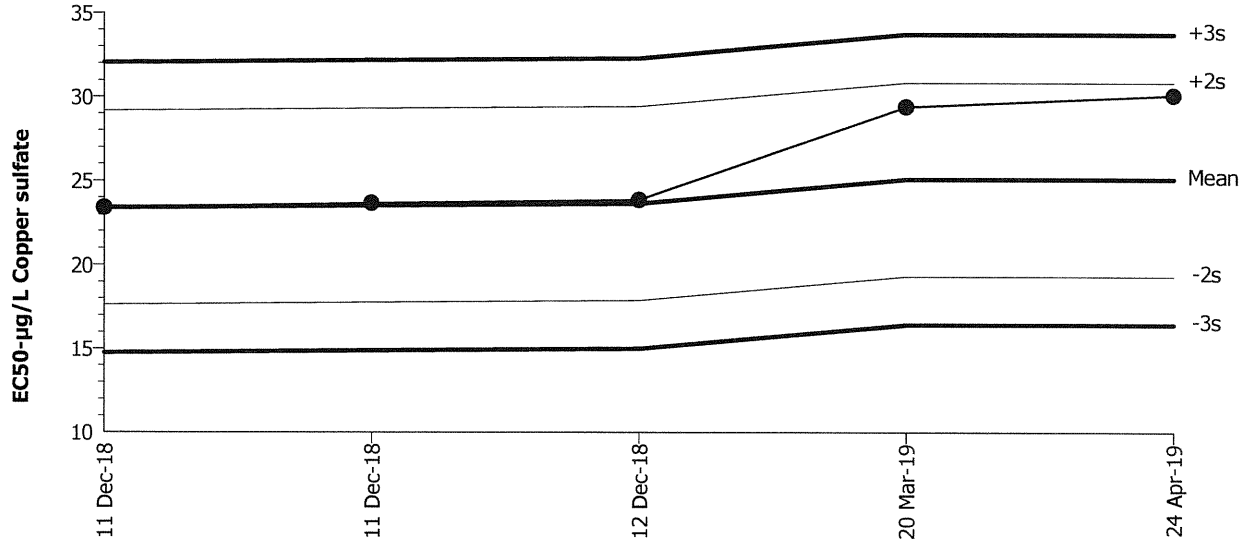
Nautilus Environmental (CA)

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

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

Material: Copper sulfate
 Source: Reference Toxicant-REF

Bivalve Larval Survival and Development Test



Mean: 25.08 Count: 4 -2s Warning Limit: 19.31 -3s Action Limit: 16.43
 Sigma: 2.883 CV: 11.50% +2s Warning Limit: 30.84 +3s Action Limit: 33.73

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	-1.679	-0.5825			09-7408-5780	12-1829-1326
2			11	12:30	23.66	-1.416	-0.4912			01-7940-4185	12-4826-8052
3			12	13:55	23.86	-1.223	-0.4242			11-6161-8836	20-5520-1077
4	2019	Mar	20	15:25	29.39	4.314	1.496			02-0163-9394	15-3118-7055
5		Apr	24	14:25	30.08	5.002	1.735			14-4098-8496	03-3240-7186

CETIS Test Data Worksheet

Report Date: 23 Apr-19 09:49 (p 1 of 1)

Test Code: 14-4098-8496/190424msdvSO

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 24 Apr-19

Species: *Mytilus galloprovincialis*

Sample Code: 190424msdvSO

End Date: 26 Apr-19

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

Sample Source: Reference Toxicant

Sample Date: 24 Apr-19

Material: Copper sulfate

Sample Station: Copper Sulfate

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			163	162	JCL 5/9/19
			32			153	5	
			33			138	8	
			34			173	167	
			35			140	137	
			36			145	0	
			37			1	0	cells lysed
			38			151	149	
			39			2	0	cells lysed
			40			145	145	
			41			4	0	cells lysed
			42			139	138	
			43			172	8	
			44			145	138	
			45			216	0	
			46			151	148	
			47			149	0	
			48			158	158	
			49			150	146	
			50			3	0	cells lysed
			51			158	157	
			52			156	3	
			53			149	5	
			54			149	0	
			55			0	0	cells lysed
			56			156	153	
			57			161	158	
			58			147	144	
			59			146	0	
			60			133	132	

CETIS Test Data Worksheet

Report Date: 23 Apr-19 09:49 (p 1 of 1)
 Test Code: 14-4098-8496/190424msdvSO

Bivalve Larval Survival and Development Test

Nautilus Environmental (CA)

Start Date: 24 Apr-19 Species: *Mytilus galloprovincialis* Sample Code: 190424msdvSO
 End Date: 26 Apr-19 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 24 Apr-19 Material: Copper sulfate Sample Station: Copper Sulfate

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	58					
0	LC	2	46					
0	LC	3	35			124	122	RT 4/26/19
0	LC	4	31					
0	LC	5	48					
2.5		1	42					
2.5		2	51					
2.5		3	60			123	122	RT
2.5		4	34					
2.5		5	44					
5		1	56					
5		2	38					
5		3	40			137	137	RT
5		4	57					
5		5	49					
10		1	32					
10		2	43					
10		3	33			128	8	RT
10		4	52					
10		5	53					
20		1	59					
20		2	36					
20		3	45			114	0	RT
20		4	54					
20		5	47					
40		1	39					
40		2	41					
40		3	55			0	0	RT
40		4	50					
40		5	37					

QC = BD

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: CuCl₂ CuSO₄
 Test No.: 190424msdvSO

Test Species: M. galloprovincialis
 Start Date/Time: 4/24/2019 1425
 End Date/Time: 4/26/2019 1400

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	32.2	32.2	32.2	14.9	14.9	15.0	8.6	8.8	8.3	8.02	8.04	8.00
2.5	32.5	32.5	32.5	14.9	14.7	14.7	8.4	8.8	8.4	8.01	8.04	8.00
5	32.4	32.5	32.5	14.7	14.6	14.6	8.3	8.8	8.4	8.01	8.04	8.01
10	32.5	32.5	32.6	14.8	14.8	14.9	8.3	8.7	8.3	8.02	8.04	8.01
20	32.4	32.5	32.6	14.7	14.7	14.8	8.3	8.8	8.4	8.02	8.04	8.02
40	32.4	32.4	32.5	14.7	14.6	14.9	8.3	8.7	8.4	8.02	8.03	8.02

Technician Initials: _____
 WQ Readings:

0	24	48
EG	RT	RT

 Dilutions made by:

BO		
----	--	--

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: QA18 ACS 4/23/19
 24 hrs: _____
 48 hrs: _____

QC Check: KFP 5/10/19 Final Review: EG 5/17/19

Marine Chronic Bioassay

Larval Development Worksheet

Client: Internal / CuSO₄
 Test No.: 190424 msdvSO
 Test Species: M. galloprovincialis
 Animal Source: Mission Bay
 Date Received: 4/23/19
 Test Chambers: 30ml shell vials
 Sample Volume: 10ml

Start Date/Time: 4/24/2019 1425
 End Date/Time: 4/26/2019 1400
 Technician Initials: BD/EG

Spawn Information

First Gamete Release Time: 1030

Sex	Number Spawning
Male	3
Female	8

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1,2	good motility, great density
Female 1	3	orange color, mostly round, good density
Female 2	6	orange color, mostly round, fair density
Female 3		

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	99%
Female 2	100%
Female 3	

Egg Fertilization Time: 1120

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted: 17 17
15 14
12 15
12 15
16 16

Mean: 14.9

Mean 14.9 X 50 = 745 embryos/ml

Initial Density: 745 = 2.48 (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	148	148	100	100 98.7% 99.8%
2	152	152	100	
3	159	160	99	
4	156	156	100	
5	143	143	100	
6	169	169	100	

48-h QC: 152/154 = 99%

Comments:

$\bar{x} = 155$

@KFPAB 5/10/19

QC Check: KFP 5/10/19

Final Review: EG 5/17/19

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.