ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Acute and Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: October 2020

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

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

Date Submitted: **December 16, 2020**

Data Quality Assurance:

- Enthalpy Analytical is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (ORELAP ID 4053). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective US EPA protocols, unless otherwise noted in this report.
- All tests have met internal Quality Assurance Program requirements.

Results verified by: _____
Eric Green, Lab Manager

California
4340 Vandever Avenue
San Diego, California 92120
858.587.7333
fax: 619.279.5919

Introduction

Acute and chronic toxicity tests were performed using a groundwater composite sample collected on October 27, 2020 from the Wyckoff Eagle Harbor Groundwater Treatment Plant on Bainbridge Island in Washington. The tests were performed to satisfy quarterly and annual monitoring requirements according to the project Quality Assurance Project Plan (QAPP 2013). The chronic bioassay was conducted using the bivalve *Mytilus galloprovincialis* (Mediterranean mussel) and the acute bioassay was conducted using the fish *Menidia beryllina* (inland silverside). Testing was performed at Enthalpy Analytical located in San Diego, California between October 28 and November 1, 2020.

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	**102720; Location SP-11**
Enthalpy Log-in Number	20-1180
Collection Date; Time	10/27/2020; 0918h
Receipt Date; Time	10/28/2020; 0935h
Receipt Temperature (°C)	3.4
Dissolved Oxygen (mg/L)	7.8
pH	7.54
Salinity (ppt)	9.5
Alkalinity (mg/L $CaCO_3$)	396
Total Chlorine (mg/L)	0.02
Total Ammonia (mg/L as N)	2.5

Test Methods

Chronic toxicity testing was conducted according to the method set forth in USEPA 1995 and acute toxicity testing was conducted according to the method set forth in USEPA 2002. The methods are summarized in Tables 2 and 3.

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

Test Period	10/28/2020, 1550h to 10/30/2020, 1500h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	Taylor Shellfish (Shelton, WA)
Test Organism Age	4 hours post fertilization
Test Duration	48 ± 2 hours
Test Type	Static
Test Chamber, Test Solution Volume	30 mL glass vial, 10 mL
Test Temperature	15 ± 1°C
Dilution Water	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) diluted with de-ionized water
Additional Control	Brine Control (de-ionized water and hypersaline brine)
Test Salinity	30 ± 2 ppt
Source of Salinity	Hypersaline brine made by freezing seawater to a salinity of 94.3 ppt
Test Concentrations (% sample)	75.8[a], 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper chloride [b]
Statistical Software	CETIS™ 1.8.7.20

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

[b] A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride instead of copper sulfate.

Table 3. Summary of Methods for the Inland Silverside Acute Survival Test

Test Period	10/28/2020, 1545h to 11/1/2020, 1445h
Test Organism	*Menidia beryllina*
Test Organism Source	Aquatic Indicators (St. Augustine, FL)
Test Organism Age	14 days
Test Duration	96 ± 2 hours
Test Type	Static - renewal
Test Chamber, Test Solution Volume	500mL Plastic Cup, 250mL
Test Temperature	25 ± 1°C
Dilution Water	Salt Control (Instant Ocean™ brand sea salts added to de-ionized water)
Additional Control	Laboratory Seawater (Source: SIO intake) diluted with de-ionized water
Test Salinity	30 ± 2 ppt
Source of Salinity	Instant Ocean™ brand sea salts
Test Concentrations (% sample)	100, 50, 25, 12.5, and 6.25%, lab and salt controls
Number of Replicates	4
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/821/R-02/012, 2002 Acute Manual
Test Acceptability Criteria for Controls	≥ 90% mean survival
Reference Toxicant	Copper chloride
Statistical Software	CETIS™ 1.8.7.20

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 75.8 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.32 for both endpoints.

There were no statistically significant effects observed in any effluent concentration tested for the survival endpoint of the inland silverside test. This results in a no observed effect concentration (NOEC) of 100 and an acute toxic unit (TU_a) of 1.0.

Statistical results for the acute and chronic toxicity tests are summarized in Table 4. Detailed summaries of the acute and chronic toxicity tests are provided in Tables 5 and 6, respectively. Individual statistical summaries for the tests 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 4. Summary of Statistical Results for the Chronic Toxicity Tests

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_a/TU_c)	EC_{25} (% effluent)
Inland Silverside	Survival	100	> 100	1.0	> 100
Bivalve	Normal Development	75.8	> 75.8	< 1.32	> 75.8
	Survival	75.8	> 75.8	< 1.32	> 75.8

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

Acute Toxic Unit (TU_a) = 100/LC_{50}. A TU_a of 1.0 indicates no toxicity in the sample.

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

Effect Concentration 25 (IC_{25}) = Concentration expected to cause an effect to 25% of the organisms

Table 5. Detailed Results for the Inland Silverside Acute Survival Test

Concentration (% Effluent)	Mean Survival (%)
0 (Salt Control)	100
0 (Lab Control)	100
6.25	95.0
12.5	100
25	100
50	100
100	100

Table 6. Detailed Results for the Bivalve Development Chronic Toxicity Test

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	99.8	96.8
0 (Lab Control)	99.0	95.6
2	99.2	95.9
4	99.3	96.7
9	98.4	97.2
18	96.1	96.5
35	99.9	97.8
75.8[a]	97.9	96.5

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

Quality Assurance

The sample was received in good condition and within the appropriate temperature range of 0-6°C. Testing occurred within the required 36 hour holding time. 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 responses observed, the calculated effects concentrations were deemed reliable. Minor QA/QC issues that were unlikely to have any bearing on the final test results, such as slight temperature deviations, are noted on the data sheets and a list of qualifier codes used on bench data sheets is presented in Appendix D.

Reference Toxicant

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 7. The reference toxicant tests met all test acceptability criteria. Additionally, the results for both reference toxicant tests were within the acceptable range of the mean historical test results plus or minus two standard deviations. This indicates that the sensitivity of these batches of organisms was typical for our laboratory. The reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 7. Reference Toxicant Test Results

Species	Endpoint	EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve	Normal Development	7.26	10.2 ± 6.48	31.8
	Survival	29.8	29.5 ± 4.41	7.48
Inland Silverside	Survival	137	186 ± 93.2	25.1

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

References

CH2MHill. 2013. Quality Assurance Project Plan – Groundwater Treatment Plant Operations, Maintenance, Bainbridge, Washington. Prepared for USEPA Region 10 June 5, 2013.

Standard Guide for Conducting Static Acute Toxicity Tests with Embryos of Four Species of Saltwater Bivalve Molluscs. 1989. ASTM Standard E 724-89.

Tidepool Scientific Software. 2000 -2013. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20.

USEPA. 1995. Short-Term Method for Estimating the Chronic Toxicity of Effluents and Receiving Waters to the West Coast Marine and Estuarine Organisms. EPA/600/R-95/136. pp. 209-258 and 389-465.

USEPA. 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms, Fifth Edition. United States Environmental Protection Agency Office of Water, Washington DC (EPA-821-R-02-012).

Washington State Department of Ecology. 2016. Laboratory Guidance and Whole Effluent Toxicity Test Review Criteria. Publication No. WQ-R-95-80. Revised June 2016

Appendix A
Statistical Summaries and Raw Bench Sheets

Bivalve Larval Development Test

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

Batch ID:	09-0976-5985	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	28 Oct-20 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	30 Oct-20 15:00	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	20-2216-8976	**Code:**	20-1180	**Client:**	Jacobs
Sample Date:	27 Oct-20 09:18	**Material:**	Effluent Sample	**Project:**	
Receive Date:	28 Oct-20 09:35	**Source:**	Jacobs		
Sample Age:	31h (3.4 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-9069-1216	Development Rate	75.8	>75.8	NA	2.41%	≤1.319	Dunnett Multiple Comparison Test
20-6639-0887	Survival Rate	75.8	>75.8	NA	3.16%	≤1.319	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
15-3007-2344	Development Rate	EC25	>75.8	N/A	N/A	<1.319	Linear Interpolation (ICPIN)
		EC50	>75.8	N/A	N/A	<1.319	
19-0694-7295	Survival Rate	EC25	>75.8	N/A	N/A	<1.319	Linear Interpolation (ICPIN)
		EC50	>75.8	N/A	N/A	<1.319	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
15-3007-2344	Development Rate	Control Resp	0.9676	0.9 - NL	Yes	Passes Acceptability Criteria
18-9069-1216	Development Rate	Control Resp	0.9676	0.9 - NL	Yes	Passes Acceptability Criteria
19-0694-7295	Survival Rate	Control Resp	0.9977	0.5 - NL	Yes	Passes Acceptability Criteria
20-6639-0887	Survival Rate	Control Resp	0.9977	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	0.9676	0.9568	0.9784	0.9535	0.9763	0.003898	0.008716	0.9%	0.0%
0	Lab Control	5	0.9556	0.9423	0.969	0.9399	0.9643	0.004814	0.01076	1.13%	1.24%
2		5	0.9592	0.9321	0.9864	0.924	0.9845	0.009783	0.02188	2.28%	0.87%
4		5	0.9666	0.9494	0.9838	0.9468	0.9827	0.006201	0.01386	1.43%	0.1%
9		5	0.9716	0.959	0.9843	0.962	0.9828	0.00455	0.01017	1.05%	-0.41%
18		5	0.965	0.9433	0.9866	0.9351	0.9806	0.00779	0.01742	1.81%	0.28%
35		5	0.9776	0.9634	0.9919	0.9647	0.9896	0.005133	0.01148	1.17%	-1.03%
75.8		5	0.9651	0.9529	0.9774	0.9524	0.977	0.004414	0.00987	1.02%	0.26%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9977	0.9912	1	0.9883	1	0.002339	0.005231	0.52%	0.0%
0	Lab Control	5	0.9895	0.97	1	0.9649	1	0.007018	0.01569	1.59%	0.82%
2		5	0.9918	0.9691	1	0.9591	1	0.008187	0.01831	1.85%	0.59%
4		5	0.993	0.9735	1	0.9649	1	0.007018	0.01569	1.58%	0.47%
9		5	0.9836	0.9421	1	0.924	1	0.01496	0.03344	3.4%	1.41%
18		5	0.9614	0.8957	1	0.9006	1	0.02365	0.05289	5.5%	3.63%
35		5	0.9988	0.9956	1	0.9942	1	0.00117	0.002615	0.26%	-0.12%
75.8		5	0.9789	0.9487	1	0.9415	1	0.01088	0.02432	2.49%	1.88%

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	0.9535	0.9763	0.9659	0.9713	0.9711
0	Lab Control	0.9636	0.9399	0.9643	0.9613	0.949
2		0.924	0.9626	0.9617	0.9845	0.9634
4		0.9758	0.9607	0.9827	0.9468	0.9672
9		0.9828	0.962	0.9826	0.9647	0.9661
18		0.9718	0.9683	0.9691	0.9806	0.9351
35		0.9771	0.9647	0.9896	0.9886	0.9679
75.8		0.977	0.9689	0.9524	0.9694	0.9578

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	0.9883	1	1	1
0	Lab Control	0.9649	1	0.9825	1	1
2		1	1	1	1	0.9591
4		0.9649	1	1	1	1
9		1	0.924	1	0.9942	1
18		1	1	1	0.9064	0.9006
35		1	0.9942	1	1	1
75.8		1	0.9415	0.9825	1	0.9708

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	164/172	165/169	170/176	169/174	168/173
0	Lab Control	159/165	172/183	162/168	174/181	186/196
2		158/171	180/187	176/183	190/193	158/164
4		161/165	171/178	170/173	178/188	177/183
9		171/174	152/158	169/172	164/170	171/177
18		172/177	183/189	188/194	152/155	144/154
35		171/175	164/170	191/193	174/176	181/187
75.8		170/174	156/161	160/168	190/196	159/166

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	171/171	169/171	171/171	171/171	171/171
0	Lab Control	165/171	171/171	168/171	171/171	171/171
2		171/171	171/171	171/171	171/171	164/171
4		165/171	171/171	171/171	171/171	171/171
9		171/171	158/171	171/171	170/171	171/171
18		171/171	171/171	171/171	155/171	154/171
35		171/171	170/171	171/171	171/171	171/171
75.8		171/171	161/171	168/171	171/171	166/171

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

Analysis ID:	18-9069-1216	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-20 13:57	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.41%	75.8	>75.8	NA	1.319

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	0.7363	2.407	0.059	8	0.5748	CDF	Non-Significant Effect
		4	0.03392	2.407	0.059	8	0.8478	CDF	Non-Significant Effect
		9	-0.5279	2.407	0.059	8	0.9552	CDF	Non-Significant Effect
		18	0.1915	2.407	0.059	8	0.7989	CDF	Non-Significant Effect
		35	-1.389	2.407	0.059	8	0.9964	CDF	Non-Significant Effect
		75.8	0.2737	2.407	0.059	8	0.7701	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.008288882	0.00138148	6	0.9319	0.4877	Non-Significant Effect
Error	0.0415079	0.001482425	28			
Total	0.04979678		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.538	16.81	0.7389	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9818	0.9146	0.8175	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	0.9676	0.9568	0.9784	0.9711	0.9535	0.9763	0.003898	0.9%	0.0%
2		5	0.9592	0.9321	0.9864	0.9626	0.924	0.9845	0.009783	2.28%	0.87%
4		5	0.9666	0.9494	0.9838	0.9672	0.9468	0.9827	0.0062	1.43%	0.1%
9		5	0.9716	0.959	0.9843	0.9661	0.962	0.9828	0.00455	1.05%	-0.41%
18		5	0.965	0.9433	0.9866	0.9691	0.9351	0.9806	0.00779	1.81%	0.28%
35		5	0.9776	0.9634	0.9919	0.9771	0.9647	0.9896	0.005133	1.17%	-1.03%
75.8		5	0.9651	0.9529	0.9774	0.9689	0.9524	0.977	0.004414	1.02%	0.26%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.391	1.362	1.421	1.4	1.353	1.416	0.01063	1.71%	0.0%
2		5	1.373	1.305	1.441	1.376	1.291	1.446	0.02448	3.99%	1.29%
4		5	1.39	1.342	1.438	1.389	1.338	1.439	0.01736	2.79%	0.06%
9		5	1.404	1.364	1.444	1.386	1.375	1.439	0.01432	2.28%	-0.92%
18		5	1.386	1.332	1.441	1.394	1.313	1.431	0.01963	3.17%	0.34%
35		5	1.425	1.375	1.475	1.419	1.382	1.469	0.01805	2.83%	-2.43%
75.8		5	1.384	1.351	1.418	1.394	1.351	1.419	0.01206	1.95%	0.48%

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

Analysis ID:	20-6639-0887	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-20 13:57	**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%	75.8	>75.8	NA	1.319

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	27	16	1	8	0.8267	Asymp	Non-Significant Effect
		4	27	16	1	8	0.8267	Asymp	Non-Significant Effect
		9	25	16	1	8	0.6693	Asymp	Non-Significant Effect
		18	24	16	1	8	0.5746	Asymp	Non-Significant Effect
		35	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		75.8	21	16	1	8	0.2891	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04434509	0.007390848	6	0.9712	0.4626	Non-Significant Effect
Error	0.2130777	0.007609919	28			
Total	0.2574228		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	16.94	16.81	0.0095	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.879	0.9146	0.0011	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	0.9977	0.9912	1	1	0.9883	1	0.002339	0.52%	0.0%
2		5	0.9918	0.9691	1	1	0.9591	1	0.008187	1.85%	0.59%
4		5	0.993	0.9735	1	1	0.9649	1	0.007017	1.58%	0.47%
9		5	0.9836	0.9421	1	1	0.924	1	0.01496	3.4%	1.41%
18		5	0.9614	0.8957	1	1	0.9006	1	0.02365	5.5%	3.63%
35		5	0.9988	0.9956	1	1	0.9942	1	0.00117	0.26%	-0.12%
75.8		5	0.9789	0.9487	1	0.9825	0.9415	1	0.01088	2.49%	1.88%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.519	1.48	1.557	1.533	1.462	1.533	0.01402	2.07%	0.0%
2		5	1.499	1.408	1.591	1.533	1.367	1.533	0.0331	4.94%	1.26%
4		5	1.503	1.419	1.586	1.533	1.382	1.533	0.03004	4.47%	1.06%
9		5	1.477	1.346	1.607	1.533	1.291	1.533	0.04689	7.1%	2.76%
18		5	1.422	1.233	1.61	1.533	1.25	1.533	0.06801	10.7%	6.39%
35		5	1.525	1.504	1.546	1.533	1.494	1.533	0.00766	1.12%	-0.42%
75.8		5	1.446	1.335	1.556	1.438	1.327	1.533	0.0397	6.14%	4.8%

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

Analysis ID:	15-3007-2344	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-20 13:57	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	188155	1000	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.8	N/A	N/A	<1.319	NA	NA
EC50	>75.8	N/A	N/A	<1.319	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	0.9676	0.9535	0.9763	0.003898	0.008717	0.9%	0.0%	836	864
2		5	0.9592	0.924	0.9845	0.009783	0.02188	2.28%	0.87%	862	898
4		5	0.9666	0.9468	0.9827	0.0062	0.01386	1.43%	0.1%	857	887
9		5	0.9716	0.962	0.9828	0.00455	0.01017	1.05%	-0.41%	827	851
18		5	0.965	0.9351	0.9806	0.00779	0.01742	1.81%	0.28%	839	869
35		5	0.9776	0.9647	0.9896	0.005133	0.01148	1.17%	-1.03%	881	901
75.8		5	0.9651	0.9524	0.977	0.004414	0.00987	1.02%	0.26%	835	865

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

Analysis ID:	19-0694-7295	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-20 13:57	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1337627	1000	Yes	Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.8	N/A	N/A	<1.319	NA	NA
EC50	>75.8	N/A	N/A	<1.319	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	0.9977	0.9883	1	0.002339	0.005231	0.52%	0.0%	853	855
2		5	0.9918	0.9591	1	0.008187	0.01831	1.85%	0.59%	848	855
4		5	0.993	0.9649	1	0.007017	0.01569	1.58%	0.47%	849	855
9		5	0.9836	0.924	1	0.01496	0.03344	3.4%	1.41%	841	855
18		5	0.9614	0.9006	1	0.02365	0.05289	5.5%	3.63%	822	855
35		5	0.9988	0.9942	1	0.00117	0.002616	0.26%	-0.12%	854	855
75.8		5	0.9789	0.9415	1	0.01088	0.02432	2.49%	1.88%	837	855

CETIS Test Data Worksheet

Report Date: 27 Oct-20 11:38 (p 1 of 1)
Test Code: 2010-5165 13-3633-9954/4FA6EDF2

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

Start Date: 28 Oct-20
End Date: 30 Oct-20
Sample Date: 27 Oct-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 20-1180
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			155	152	DM 11/17/20
			32			168	160	
			33			173	168	
			34			161	156	
			35			187	181	
			36			169	165	
			37			173	170	
			38			166	159	
			39			177	171	
			40			170	164	
			41			164	158	
			42			196	190	
			43			181	174	
			44			168	162	
			45			177	172	
			46			175	171	
			47			183	177	
			48			172	169	
			49			158	152	
			50			178	171	
			51			188	178	
			52			196	186	
			53			165	161	
			54			170	164	
			55			194	188	
			56			184	180	
			57			193	191	
			58			174	169	
			59			189	183	
			60			154	144	
			61			165	159	
			62			176	170	
			63			174	170	
			64			172	164	
			65			176	174	
			66			171	158	
			67			183	176	
			68			183	172	
			69			174	171	
			70			193	190	

CETIS Test Data Worksheet

Report Date: 27 Oct-20 11:38 (p 1 of 1)
Test Code: 2010-S165 ~~13-3633-9954/4FA6EDF2~~ (6)

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

Start Date: 28 Oct-20
End Date: 30 Oct-20
Sample Date: 27 Oct-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 20-1180
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	64					
0	BC	2	36					
0	BC	3	62			180	173	RT 10/31/20
0	BC	4	58					
0	BC	5	33					
0	LC	1	61					
0	LC	2	68					
0	LC	3	44			164	159	RT
0	LC	4	43					
0	LC	5	52					
2		1	66					
2		2	56					
2		3	67			186	178	RT
2		4	70					
2		5	41					
4		1	53					
4		2	50					
4		3	37			179	176	RT
4		4	51					
4		5	47					
9		1	69					
9		2	49					
9		3	48			178	175	RT
9		4	40					
9		5	39					
18		1	45					
18		2	59					
18		3	55			183	175	RT
18		4	31					
18		5	60					
35		1	46					
35		2	54					
35		3	57			193	189	RT
35		4	65					
35		5	35					
75.8 76		1	63					
76		2	34					
76 (A)		3	32			175	169	RT
76		4	42					
76		5	38					

QC: RT

(A) QS RT 10/28/20 (B) QUALACS 11/3/2020

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Jacobs

Sample ID: Wyckoff

Sample Log No.: 20- 1180

Test No.: 2010-S165

Test Species: *M. galloprovincialis*

Start Date/Time: 10/28/2020 1550

End Date/Time: 10/30/2020 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	30.1	29.8	29.8	14.0	14.2	15.3	8.9	8.9	8.3	8.15	8.03	8.04
Brine Control	30.4	30.1	30.1	14.3	14.2	15.0	8.6	8.9	8.4	8.23	8.10	8.06
2	29.7	29.9	29.9	14.4	14.2	15.1	8.8	8.8	8.4	8.14	8.06	8.06
4	29.8	29.9	29.9	14.3	14.2	15.2	8.9	8.8	8.3	8.10	8.06	8.09
9	29.9	29.9	29.9	14.1	14.2	15.1	8.9	8.8	8.3	8.06	8.05	8.12
18	29.9	30.0	30.1	14.3	14.2	15.2	8.9	8.8	8.3	7.97	8.07	8.18
35	29.8	29.9	30.0	14.0	14.3	15.1	8.8	8.8	8.3	7.86	8.07	8.23
75.8	30.1	30.2	30.2	14.1	14.4	15.2	8.9	8.7	8.3	7.74	8.06	8.31

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

Technician Initials:

Environmental Chamber: D

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: ACS 11/3/2020

Final Review: BO 12/3/20

Marine Chronic Bioassay **Brine Dilution Worksheet**

DC-010

Project: JACOBS **Analyst:** RT

Sample ID: Wyckoff **Test Date:** 10/28/2020

Test No: 2010-S165 **Test Type:** Mussel Development

Salinity of Effluent 9.5

Salinity of Brine 94.3 **Date of Brine used:** 9/1/2020

Target Salinity 30 **Alkalinity of Brine Control:** 112 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.32**	**0.47**

TS = target salinity
SE = salinity of effluent
SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Control	NA	NA	NA	250
2	5.0	0.32	1.6	250
4	10.0	0.32	3.2	250
9	22.5	0.32	7.2	250
18	45.0	0.32	14.3	250
35	87.5	0.32	27.9	250
75.8	189.6	0.32	60.4	250
	DI Volume			
Brine Control	129.5	0.47	60.4	250

Total Brine Volume Required (ml): **175.1**

QC Check: AES 11/3/2020 **Final Review:** BO 12/3/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs / Wyckoff
Test No.: 2010-S165
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: Taylor / 53.5C
Date Received: 7/16/2020
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 10/28/2020 1550
End Date/Time: 10/30/2020 1500
Technician Initials: RT/EG

Spawn Information

First Gamete Release Time: 1055

Sex	Number Spawning
Male	9+
Female	5

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	3,4,5,8	average density, good motility
Female 1	2	good density, yellow, mostly round
Female 2	4	average density, pale yellow, mostly round
Female 3	—	—

Egg Fertilization Time: 1215

Embryo Stock Selection

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

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted:		
	5	5
	8	6
	6	6
	6	6
	7	5

Mean: 6.0

Mean 6 X 50 = 300 embryos/ml
CIB RT 10/28/20

Initial Density: 300 = 1.0 (dilution factor)
Desired Final Density: 300
(to inoculate with 0.5 ml)

Prepare the embryo inoculum according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Time Zero Control Counts

TØ Vial No.	No. Dividing	Total	% Dividing	Mean % Dividing
TØ A	151	152	99.3%	99.4
TØ B	188	189	99.5%	
TØ C	197	197	100.0%	
TØ D	169	170	99.4%	
TØ E	160	162	98.8%	
TØ F	160	161	99.4%	
$\bar{X}$ =	171			

48-h QC: 166/170 = 97.6%

Comments:

QC Check: AES 11/3/2020

Final Review: BD 12/3/20

Inland Silverside Acute Survival Test

CETIS Summary Report

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	02-3600-7228	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	28 Oct-20 15:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Artificial Diluted ~~Natural~~ Seawater
Ending Date:	01 Nov-20 14:45	**Species:**	Menidia beryllina	**Brine:**	~~Not Applicable~~ Instant ocean
Duration:	95h	**Source:**	Aquatic Indicators, FL	**Age:**	14d

Sample ID:	18-8031-0064	**Code:**	20-1180	**Client:**	Jacobs
Sample Date:	27 Oct-20 09:18	**Material:**	Effluent Sample	**Project:**	
Receive Date:	28 Oct-20 09:35	**Source:**	Jacobs		
Sample Age:	30h (3.4 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-2473-0169	96h Survival Rate	100	>100	NA	9.2%	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-2473-0169	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
0	Salt Control	4	1	1	1	1	1	0	0	0.0%	0.0%
6.25		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	5.0%
12.5		4	1	1	1	1	1	0	0	0.0%	0.0%
25		4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	1	1	1	1	1	0	0	0.0%	0.0%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
0	Salt Control	1	1	1	1
6.25		1	0.8	1	1
12.5		1	1	1	1
25		1	1	1	1
50		1	1	1	1
100		1	1	1	1

@QA BC 12/3/20

CETIS Analytical Report

Test Code: 2010-S166 | 09-9963-8423

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	00-2473-0169	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	04 Nov-20 14:24	**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	9.2%	100	>100	NA	1

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Salt Control		6.25	16	10	1	6	0.6105	Asymp	Non-Significant Effect
		12.5	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		25	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		50	18	10	1	6	0.8333	Asymp	Non-Significant Effect
		100	18	10	1	6	0.8333	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01181415	0.002362829	5	1	0.4457	Non-Significant Effect
Error	0.04253092	0.002362829	18			
Total	0.05434507		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	4.248	0.4457	Equal Variances
Variances	Levene Equality of Variance	9	4.248	0.0002	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.4634	0.884	<0.0001	Non-normal Distribution

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1	1	1	1	1	1	0	0.0%	0.0%
6.25		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	5.0%
12.5		4	1	1	1	1	1	1	0	0.0%	0.0%
25		4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	0.0%

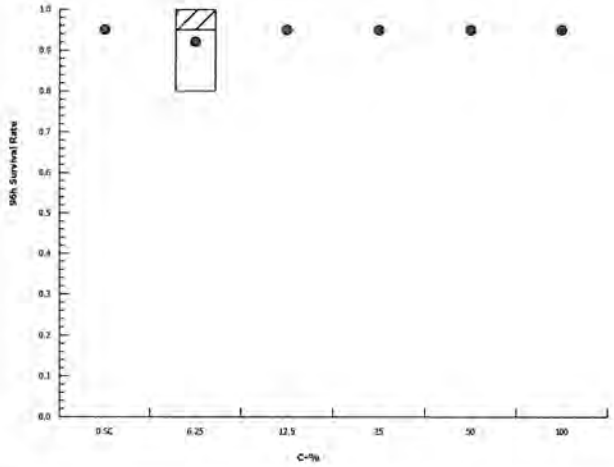
Angular (Corrected) Transformed Summary

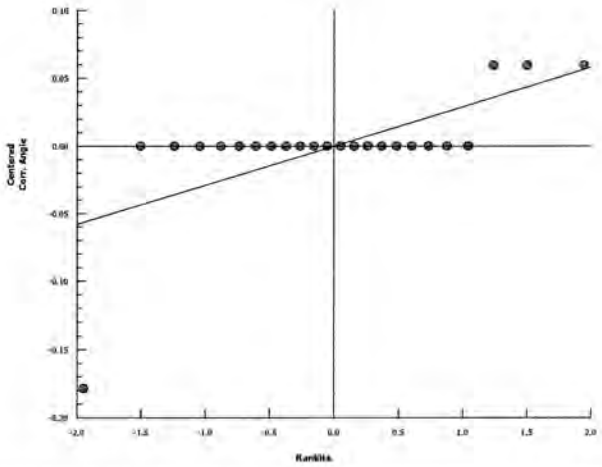
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
6.25		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	4.43%
12.5		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
25		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
50		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
100		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	00-2473-0169	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	04 Nov-20 14:24	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: Jacobs
Sample ID: Wyckoff
Sample Log-in No.: 20- 1180
Test No.: 2010-S166

Test Species: (A) ~~A. affinis~~ M. beryllina
Start Date/Time: 10/28/2020 1545
End Date/Time: 11/1/2020 1445

	Tech Initials				
	0	24	48	72	96
Counts:	SR	RT	SR	DM	AS
Readings:	AS	RT	RT	DM	KL
Dilutions made by:	AS		RT		

Concentration (%)	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	30.0	29.9	i 29.1	30.2	30.5	29.1	29.3	i 24.1	24.2	24.3	7.3	5.8	i 6.5	6.0	4.7	8.05	7.86	i 8.06	7.78	7.75
	B	5	5	5	5	5			f 30.5					f 24.6					f 5.6					f 7.80		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
Salt Control	A	5	5	5	5	5	30.1	30.3	i 30.5	30.7	31.1	24.4	24.6	i 24.2	24.5	24.6	7.1	5.7	i 6.5	5.2	4.4	8.18	7.99	i 8.24	7.95	7.96
	B	5	5	5	5	5			f 30.6					f 24.9					f 4.9					f 7.92		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
6.25%	A	5	5	5	5	5	30.0	30.0	i 30.4	30.4	31.0	25.0	24.8	i 24.2	24.7	24.7	7.0	5.3	i 6.5	4.9	4.4	8.12	8.03	i 8.20	8.01	8.01
	B	5	5	4	~~5~~ (C) 4	4			f 30.3					f 25.1					f 4.7					f 8.02		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
12.5%	A	5	5	5	5	5	29.9	29.9	i 30.3	30.5	30.9	25.1	24.9	i 24.2	24.8	24.7	7.0	5.2	i 6.5	4.8	4.5	8.07	8.05	i 8.16	8.07	8.07
	B	5	5	5	5	5			f 30.1					f 25.1					f 4.7					f 8.07		
	C	5	5	5	5	5																				
	D	5	5	5	5	5			30.3 (B)																	
25	A	5	5	5	5	5	29.6	29.8	~~29.9~~ 30.6		30.9	24.2	24.9	i 24.0	24.8	24.9	7.1	5.1	i 6.7	5.0	4.7	8.00	8.06	i 8.06	8.12	8.16
	B	5	5	5	5	5			f 30.0					f 25.1					f 4.7					f 8.14		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
50	A	5	5	5	5	5	29.4	29.5	i 30.0	30.3	31.0	24.1	24.9	i 24.1	24.5	24.8	7.2	5.1	i 7.0	5.0	4.5	7.88	8.10	i 7.97	8.19	8.27
	B	5	5	5	5	5			f 29.8					f 24.9					f 4.8					f 8.23		
	C	5	5	5	5	5																				
	D	5	5	5	5	5	30.1																			
100	A	5	5	5	5	5	~~28.7~~	29.9	i 29.4	29.9	30.5	24.0	24.9	i 24.1	24.5	24.9	7.6	4.9	i 7.6	4.9	4.3	7.75	8.11	i 7.82	8.20	8.37
	B	5	5	5	5	5	(A)		f 30.2					f 25.1					f 4.6					f 8.31		
	C	5	5	5	5	5																				
	D	5	5	5	~~5~~ 4	5																				

Initial Counts QC'd by: DM/GH/RT SR 10/31/20
Initiated by: SR

Environmental Chamber: A

Animal Source/Date Received: Aquatic Indicaters 10/27/20
Age at Initiation: 14d

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / (none)

Feeding Times					
	0	24	48	72	96
AM:		0900	0910	0850	0840
PM:	1720				

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal

Organisms fed prior to initiation, circle one (y) / n) (A) Q18 AS 10/28/2020 (B) Q18 RT 10/30/20

@21 organisms experienced >3ppt | 3°c shift within 24hrs of application. SR 10/29/20

(C) Q18 DM 11/3/20

BO 12/3/20

QC Check: AS 11/3/2020

Final Review:

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: Jacobs, ⑥ ~~Wyckoff~~
Sample ID: ⑥ ~~102720~~ Wyckoff (102720)
Test ID No(s).: 2010-5165 to -5166

Sample Check-In Information
DC-005

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

Sample (A, B, C):	A			
Log-in No. (20-xxxx):	1180			
Sample Collection Date & Time:	10/27/20 0918			
Sample Receipt Date & Time:	10/28/20 0935			
Number of Containers & Container Type:	1-4L cubi			
Approx. Total Volume Received (L):	4			
Check-in Temperature (°C)	3.4			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.8			
pH (units)	7.54			
Conductivity (µS/cm)	15,500			
Salinity (ppt)	(A) ~~9.8~~ 9.5			
Alkalinity (mg/L) [2]	396			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	GR			

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

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

Salinity Adjustment? (Y) N
Test: Mussel Source: Brine Target ppt: 30
Test: Menidia Source: Instant Ocean Target ppt: 30
Test: Source: Target ppt:

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl_2 Adjustment? Y (N)

	A	B	C
Initial Free Cl_2:			
STS added:			
Final Free Cl_2:			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

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

Test Performed: Mussel Development **Control/Dilution Water:** 8:2 / **Lab SW** / **Lab ART** **Other:** ___
Alkalinity: 105 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 112 Hardness or Salinity: 30ppt

Test Performed: Acute Menidia **Control/Dilution Water:** 8:2 / **Lab SW** / ~~Lab ART~~ **Other:** ___
Alkalinity: 156 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Lab SW Alkalinity: 105 Hardness or Salinity: 30ppt

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

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: (A) GR GR 10/28/20 (B) GR 8AcS 10/28/2020

QC Check: BOORO RCS 11/3/20
Final Review: BO 12/3/20

Total Ammonia Analysis **Overlying Water**

Marine

DC-001

Client: Jacobs

Project: Wyckoff

Test Type: Menidia acute and mussel development

DI Blank: —— **Test Start Date:** 10/28/2020 **Analyst:** ER

SW Blank: 0.0 **Analysis Date:** 11/4/20

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	9.2	11.2
Jacobs Wyckoff	20-1180	10/28/2020	0	2.5	3.1
Spike Check (10 mg/L NH_3)		NA	NA		
BATCH QA SAMPLE	20-1185	10/30/20	4	3.0	3.7
Sample Duplicate[a]	20-1185	NA	NA	3.2	3.9
Sample Duplicate + Spike[a]		NA	NA	12.3	15.0
Spike Check (10 mg/L NH_3)		NA	NA	9.2	11.2

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

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

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	11.2	10	NA	112
BATCH QA SAMPLE	3.7	3.9	15.0	10	5.3	113

Comments:

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

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

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

Method Detection Limit (MDL) = 0.5 mg/L

QC Check: ACS 11/4/2020 **Final Review:** BO 12/3/20

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 10/27/2020
CarrierName: FedEx
AirbillNo: 7719 1306 6217

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

No: 10-102720-095252-0490
2020T10P000DD210W2LA00
Contact Name: Keith Allers
Contact Phone: 206-780-1711

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
102720		Ground Water/ K.Allers	Composite	ACTOX-CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	10/27/2020 09:18	Field Sample

Special Instructions:	**Shipment for Case Complete? N**
	Samples Transferred From Chain of Custody #
Analysis Key: ACTOX-CHRTOX=Acute Toxicity, Chronic Toxicity	

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	JACOBS	10-27-2020 1005	Enthalpy SD	10/28/20 0935	Receipt Temp: 3.4°C Log # 20-1180

Appendix D
List of Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

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

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

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

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

Q5 - Test initiated with aeration due to an anticipated drop in D.O.

Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred

Q7 - Salinity out of recommended range

Q8 - Spilled test chamber/ Unable to recover test organism(s)

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

Q11 - Sample out of holding time; refer to QA section of report

Q12 - Replicate(s) not initiated; excluded from data analysis

Q13 - Survival counts not recorded due to poor visibility or heavy debris

Q14 - D.O. percent saturation was checked and was ≤ 110%

Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.

Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.

Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

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

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

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

Appendix E
Reference Toxicant Test Results

Bivalve Larval Development Test

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

Batch ID:	15-7930-3986	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	28 Oct-20 15:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	30 Oct-20 15:00	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	00-1074-6764	**Code:**	201028msdv	**Client:**	Internal
Sample Date:	28 Oct-20	**Material:**	Copper chloride	**Project:**	
Receive Date:	28 Oct-20	**Source:**	Reference Toxicant		
Sample Age:	16h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
19-7661-4518	Combined Development Ra	2.5	5	3.536	3.96%		Dunnett Multiple Comparison Test
17-7885-6687	Development Rate	2.5	5	3.536	2.24%		Dunnett Multiple Comparison Test
17-2684-3616	Survival Rate	20	40	28.28	3.11%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
02-6542-7057	Combined Development Ra	EC25	5.816	5.634	5.988		Linear Interpolation (ICPIN)
		EC50	7.269	7.148	7.383		
12-0840-2779	Development Rate	EC25	5.798	5.642	5.954		Linear Interpolation (ICPIN)
		EC50	7.257	7.156	7.362		
15-7574-6891	Survival Rate	EC25	24.73	24.32	25.06		Linear Interpolation (ICPIN)
		EC50	29.82	29.54	30.04		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
12-0840-2779	Development Rate	Control Resp	0.9694	0.9 - NL	Yes	Passes Acceptability Criteria
17-7885-6687	Development Rate	Control Resp	0.9694	0.9 - NL	Yes	Passes Acceptability Criteria
15-7574-6891	Survival Rate	Control Resp	0.986	0.5 - NL	Yes	Passes Acceptability Criteria
17-2684-3616	Survival Rate	Control Resp	0.986	0.5 - NL	Yes	Passes Acceptability Criteria
19-7661-4518	Combined Development Ra	PMSD	0.03965	NL - 0.25	No	Passes Acceptability Criteria

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

Combined Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.9557	0.9376	0.9738	0.9415	0.9734	0.006515	0.01457	1.52%	0.0%
2.5		5	0.971	0.9246	1	0.9064	0.9946	0.01672	0.03738	3.85%	-1.61%
5		5	0.8582	0.8268	0.8896	0.8298	0.8962	0.01131	0.02528	2.95%	10.2%
10		5	0.02901	0.01884	0.03918	0.01744	0.03846	0.003663	0.00819	28.23%	96.96%
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	0.9694	0.9572	0.9816	0.9548	0.9817	0.004391	0.009818	1.01%	0.0%
2.5		5	0.9811	0.9604	1	0.9568	0.9946	0.007456	0.01667	1.7%	-1.21%
5		5	0.8654	0.8348	0.896	0.8298	0.8962	0.01103	0.02466	2.85%	10.73%
10		5	0.02936	0.01876	0.03996	0.01744	0.03846	0.003818	0.008538	29.08%	96.97%
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	0.986	0.9616	1	0.9591	1	0.008791	0.01966	1.99%	0.0%
2.5		5	0.9895	0.9602	1	0.9474	1	0.01053	0.02354	2.38%	-0.36%
5		5	0.9918	0.9691	1	0.9591	1	0.008187	0.01831	1.85%	-0.59%
10		5	0.9906	0.9647	1	0.9532	1	0.009357	0.02092	2.11%	-0.47%
20		5	0.9719	0.9505	0.9933	0.9532	1	0.007714	0.01725	1.78%	1.42%
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	0.9734	0.9672	0.9415	0.9548	0.9415
2.5		0.9064	0.9943	0.989	0.9709	0.9946
5		0.8652	0.8579	0.8298	0.8421	0.8962
10		0.0266	0.03846	0.02747	0.03509	0.01744
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	0.9734	0.9672	0.9699	0.9548	0.9817
2.5		0.9568	0.9943	0.989	0.9709	0.9946
5		0.8652	0.8579	0.8298	0.878	0.8962
10		0.0266	0.03846	0.02747	0.03681	0.01744
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	0.9708	1	0.9591
2.5		0.9474	1	1	1	1
5		1	1	1	0.9591	1
10		1	1	1	0.9532	1
20		0.9532	0.9708	1	0.9708	0.9649
40		0	0	0	0	0

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

Combined Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	183/188	177/183	161/171	169/177	161/171
2.5		155/171	173/174	179/181	167/172	185/186
5		154/178	163/190	156/188	144/171	164/183
10		5/188	7/182	5/182	6/171	3/172
20		0/171	0/171	0/182	0/171	0/171
40		0/171	0/171	0/171	0/171	0/171

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	183/188	177/183	161/166	169/177	161/164
2.5		155/162	173/174	179/181	167/172	185/186
5		154/178	163/190	156/188	144/164	164/183
10		5/188	7/182	5/182	6/163	3/172
20		0/163	0/166	0/182	0/166	0/165
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	171/171	171/171	166/171	171/171	164/171
2.5		162/171	171/171	171/171	171/171	171/171
5		171/171	171/171	171/171	164/171	171/171
10		171/171	171/171	171/171	163/171	171/171
20		163/171	166/171	171/171	166/171	165/171
40		0/171	0/171	0/171	0/171	0/171

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

Analysis ID:	19-7661-4518	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	**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	3.96%	2.5	5	3.536	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.713	2.227	0.081	8	0.9935	CDF	Non-Significant Effect
		5*	4.813	2.227	0.081	8	0.0003	CDF	Significant Effect
		10*	32.73	2.227	0.081	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.143419	1.714473	3	517.7	<0.0001	Significant Effect
Error	0.05298996	0.003311873	16			
Total	5.196409		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.513	11.34	0.0365	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8852	0.866	0.0220	Normal Distribution

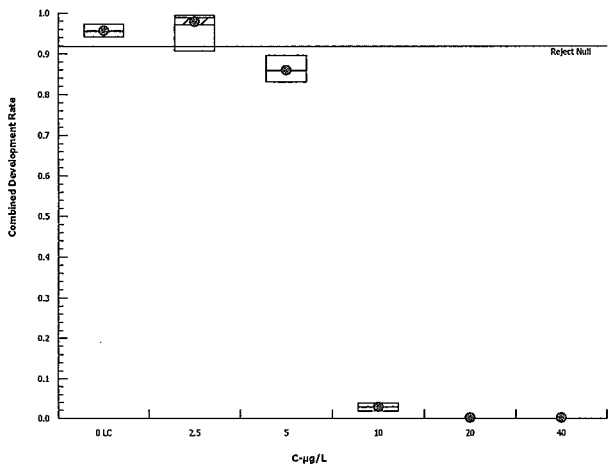
Combined Development Rate Summary

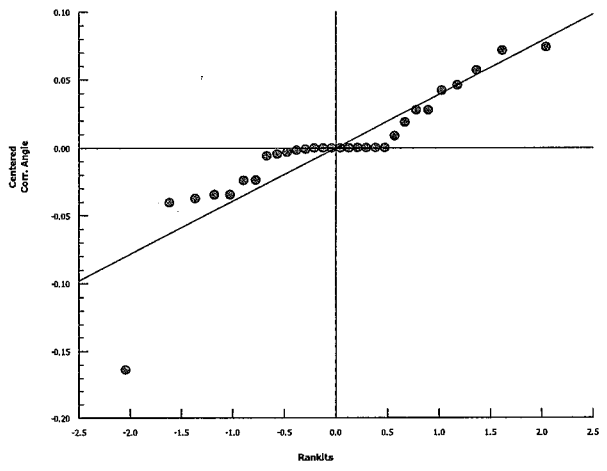
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9557	0.9376	0.9738	0.9548	0.9415	0.9734	0.006515	1.52%	0.0%
2.5		5	0.971	0.9246	1	0.989	0.9064	0.9946	0.01672	3.85%	-1.61%
5		5	0.8582	0.8268	0.8896	0.8579	0.8298	0.8962	0.01131	2.95%	10.2%
10		5	0.02901	0.01884	0.03918	0.02747	0.01744	0.03846	0.003663	28.23%	96.96%
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	1.361	1.316	1.406	1.357	1.327	1.407	0.01624	2.67%	0.0%
2.5		5	1.423	1.3	1.547	1.465	1.26	1.497	0.04453	7.0%	-4.58%
5		5	1.186	1.14	1.232	1.184	1.146	1.243	0.01659	3.13%	12.87%
10		5	0.1697	0.1384	0.2011	0.1665	0.1325	0.1974	0.01129	14.88%	87.53%
20		5	0.03801	0.03736	0.03866	0.03825	0.03707	0.03825	0.000235	1.38%	97.21%
40		5	0.03825	0.03824	0.03825	0.03825	0.03825	0.03825	0	0.0%	97.19%

Graphics





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

Analysis ID:	17-7885-6687	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	**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	2.24%	2.5	5	3.536	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.846	2.227	0.057	8	0.9955	CDF	Non-Significant Effect
		5*	7.878	2.227	0.057	8	<0.0001	CDF	Significant Effect
		10*	48.18	2.227	0.057	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.349535	1.783178	3	1101	<0.0001	Significant Effect
Error	0.02590531	0.001619082	16			
Total	5.375441		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.431	11.34	0.3298	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9598	0.866	0.5404	Normal Distribution

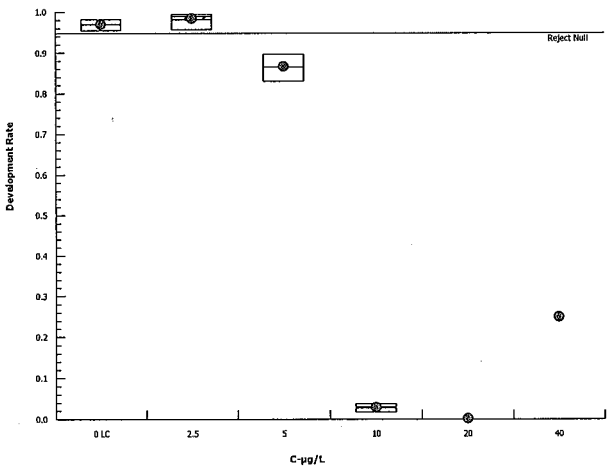
Development Rate Summary

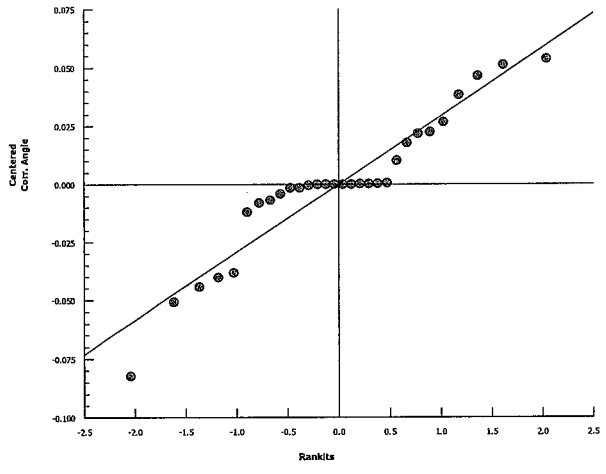
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9694	0.9572	0.9816	0.9699	0.9548	0.9817	0.004391	1.01%	0.0%
2.5		5	0.9811	0.9604	1	0.989	0.9568	0.9946	0.007456	1.7%	-1.21%
5		5	0.8654	0.8348	0.896	0.8652	0.8298	0.8962	0.01103	2.85%	10.73%
10		5	0.02936	0.01876	0.03996	0.02747	0.01744	0.03846	0.003818	29.08%	96.97%
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	1.397	1.361	1.432	1.396	1.357	1.435	0.01276	2.04%	0.0%
2.5		5	1.444	1.368	1.519	1.465	1.361	1.497	0.02712	4.2%	-3.36%
5		5	1.196	1.152	1.241	1.195	1.146	1.243	0.01612	3.01%	14.35%
10		5	0.1706	0.1381	0.2031	0.1665	0.1325	0.1974	0.0117	15.34%	87.78%
20		5	0.03856	0.03751	0.03961	0.03882	0.03707	0.03917	0.000379	2.2%	97.24%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	62.51%

Graphics





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

Analysis ID:	17-2684-3616	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	**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	3.11%	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	29	17	1	8	0.8870	Asymp	Non-Significant Effect
		5	29.5	17	2	8	0.9089	Asymp	Non-Significant Effect
		10	29	17	1	8	0.8870	Asymp	Non-Significant Effect
		20	22.5	17	2	8	0.3541	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02627879	0.006569698	4	1.058	0.4030	Non-Significant Effect
Error	0.1242299	0.006211495	20			
Total	0.1505087		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.2223	13.28	0.9943	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8429	0.8877	0.0013	Non-normal Distribution

Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.986	0.9616	1	1	0.9591	1	0.008791	1.99%	0.0%
2.5		5	0.9895	0.9602	1	1	0.9474	1	0.01053	2.38%	-0.36%
5		5	0.9918	0.9691	1	1	0.9591	1	0.008187	1.85%	-0.59%
10		5	0.9906	0.9647	1	1	0.9532	1	0.009357	2.11%	-0.47%
20		5	0.9719	0.9505	0.9933	0.9708	0.9532	1	0.007714	1.78%	1.42%
40		5	0	0	0	0	0	0	0		100.0%

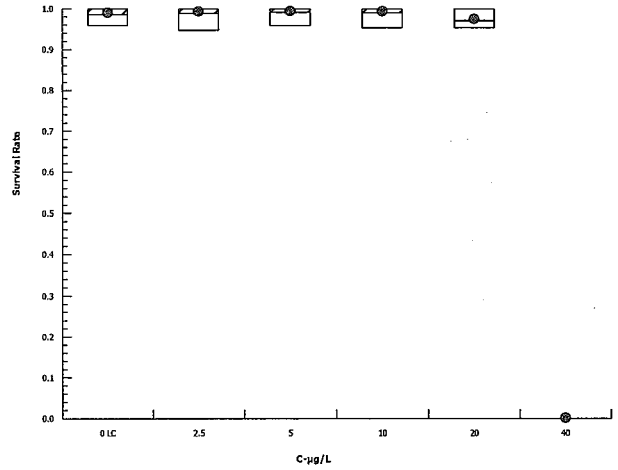
Angular (Corrected) Transformed Summary

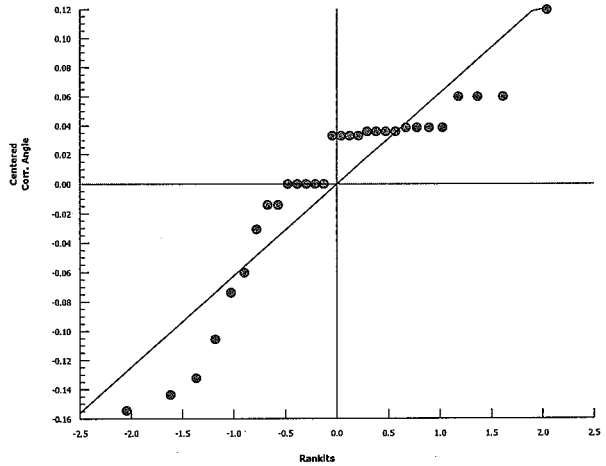
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.473	1.37	1.575	1.533	1.367	1.533	0.03698	5.61%	0.0%
2.5		5	1.494	1.387	1.601	1.533	1.339	1.533	0.03865	5.79%	-1.44%
5		5	1.499	1.408	1.591	1.533	1.367	1.533	0.0331	4.94%	-1.81%
10		5	1.497	1.397	1.596	1.533	1.353	1.533	0.03595	5.37%	-1.62%
20		5	1.413	1.327	1.499	1.399	1.353	1.533	0.03103	4.91%	4.05%
40		5	0.03825	0.03824	0.03825	0.03825	0.03825	0.03825	0	0.0%	97.4%

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

Analysis ID:	17-2684-3616	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	02-6542-7057	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	163891	1000	Yes	Two-Point Interpolation

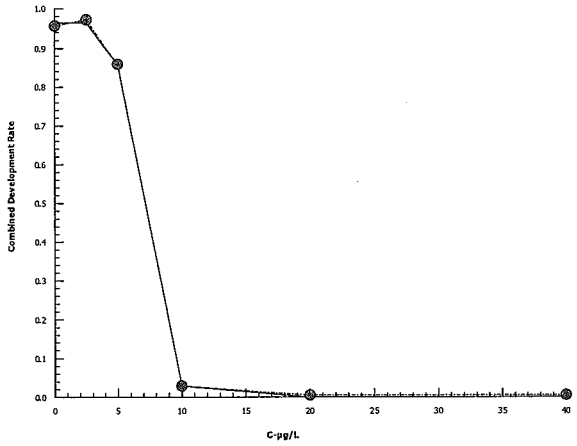
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	5.816	5.634	5.988
EC50	7.269	7.148	7.383

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	0.9557	0.9415	0.9734	0.006515	0.01457	1.52%	0.0%	851	890
2.5		5	0.971	0.9064	0.9946	0.01672	0.03738	3.85%	-1.61%	859	884
5		5	0.8582	0.8298	0.8962	0.01131	0.02528	2.95%	10.2%	781	910
10		5	0.02901	0.01744	0.03846	0.003663	0.00819	28.23%	96.96%	26	895
20		5	0	0	0	0	0		100.0%	0	866
40		5	0	0	0	0	0		100.0%	0	855

Graphics



 Analyst: JL QA AC 11/16/20

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	12-0840-2779	Endpoint:	Development Rate	CETIS Version:	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1961268	1000	Yes	Two-Point Interpolation

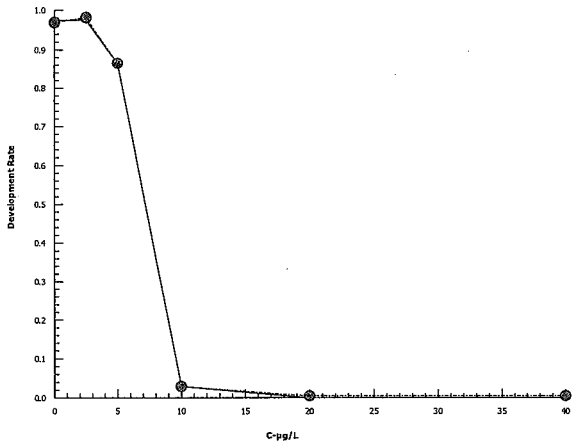
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	5.798	5.642	5.954
EC50	7.257	7.156	7.362

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	0.9694	0.9548	0.9817	0.004391	0.009818	1.01%	0.0%	851	878
2.5		5	0.9811	0.9568	0.9946	0.007456	0.01667	1.7%	-1.21%	859	875
5		5	0.8654	0.8298	0.8962	0.01103	0.02466	2.85%	10.73%	781	903
10		5	0.02936	0.01744	0.03846	0.003818	0.008538	29.08%	96.97%	26	887
20		5	0	0	0	0	0		100.0%	0	842
40		5	0	0	0	0	0		100.0%	0	5

Graphics



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

Analysis ID:	15-7574-6891	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Nov-20 12:10	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1370855	1000	Yes	Two-Point Interpolation

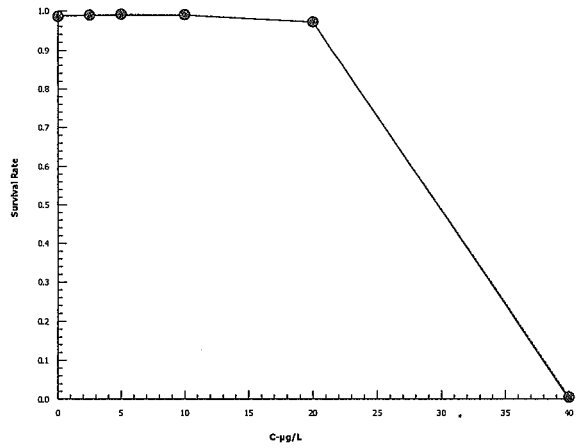
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	24.73	24.32	25.06
EC50	29.82	29.54	30.04

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	0.986	0.9591	1	0.008791	0.01966	1.99%	0.0%	843	855
2.5		5	0.9895	0.9474	1	0.01053	0.02354	2.38%	-0.36%	846	855
5		5	0.9918	0.9591	1	0.008187	0.01831	1.85%	-0.59%	848	855
10		5	0.9906	0.9532	1	0.009357	0.02092	2.11%	-0.47%	847	855
20		5	0.9719	0.9532	1	0.007714	0.01725	1.78%	1.42%	831	855
40		5	0	0	0	0	0		100.0%	0	855

Graphics

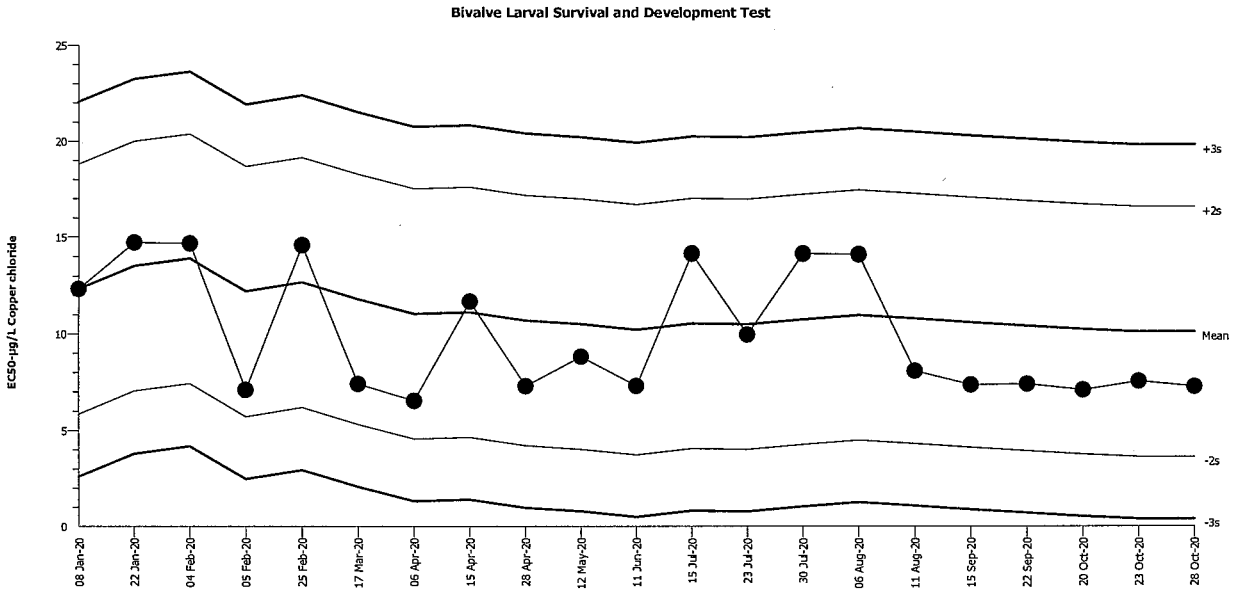


CETIS QC Plot

Report Date: 16 Nov-20 12:40 (1 of 1)

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

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



Mean:	10.12	**Count:**	20	**-2s Warning Limit:**	3.636	**-3s Action Limit:**	0.3937
Sigma:	3.242	**CV:**	32.00%	**+2s Warning Limit:**	16.6	**+3s Action Limit:**	19.85

Quality Control Data

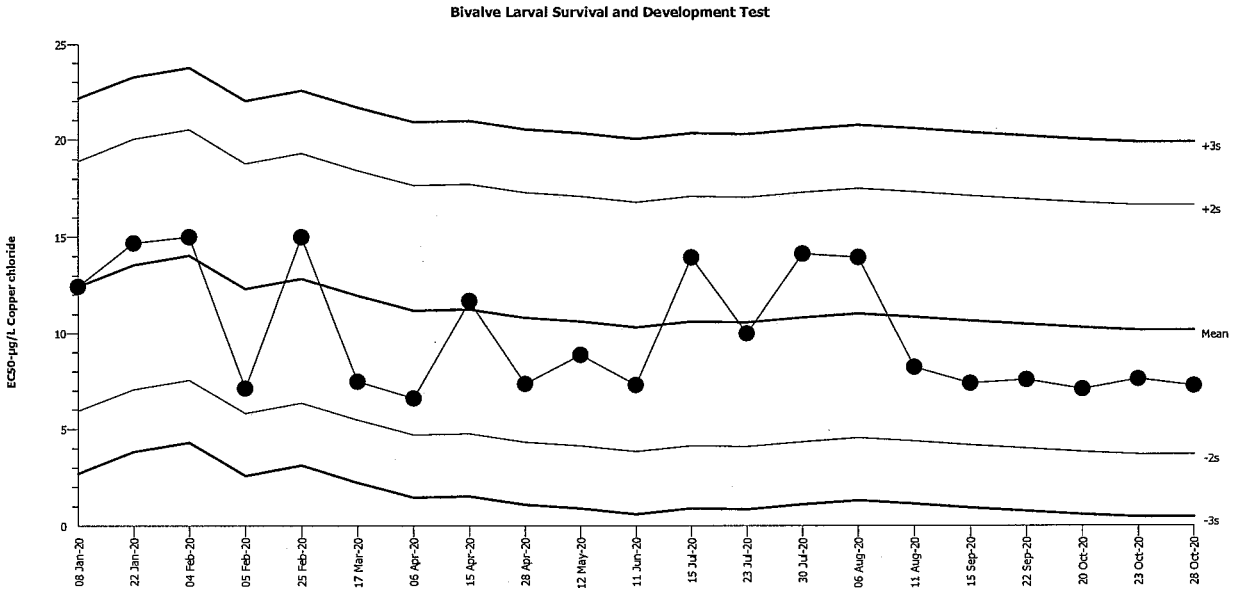
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jan	8	13:40	12.34	2.22	0.6847			07-8444-5322	01-1422-4896
2			22	13:25	14.72	4.6	1.419			02-1152-2212	07-1224-7163
3		Feb	4	16:30	14.68	4.556	1.405			19-9078-6483	21-0369-4045
4			5	13:10	7.103	-3.017	-0.9306			06-6849-2235	04-8167-3886
5			25	14:15	14.58	4.461	1.376			09-2101-6353	02-3593-4650
6		Mar	17	14:20	7.408	-2.712	-0.8366			14-6169-3689	18-9939-7640
7		Apr	6	17:15	6.537	-3.583	-1.105			02-0082-4673	13-2096-3831
8			15	13:25	11.68	1.563	0.4821			16-4614-0901	11-3098-9850
9			28	13:25	7.292	-2.828	-0.8724			06-8086-6028	13-2749-2065
10		May	12	16:15	8.819	-1.301	-0.4013			12-3773-8150	00-4087-7530
11		Jun	11	15:45	7.306	-2.814	-0.8681			20-6521-9403	10-1893-3875
12		Jul	15	13:55	14.16	4.042	1.247			17-4780-3294	11-0488-5403
13			23	15:00	9.974	-0.1456	-0.04492			06-0741-6264	07-6012-8216
14			30	15:35	14.17	4.045	1.248			00-9901-5729	19-4020-2576
15		Aug	6	15:40	14.13	4.005	1.235			01-4440-0014	02-9592-9535
16			11	14:30	8.085	-2.035	-0.6276			21-4043-5119	05-6052-3343
17		Sep	15	0:00	7.365	-2.755	-0.8498			19-9833-0655	18-5101-1090
18			22	14:40	7.405	-2.715	-0.8375			04-0347-9113	09-6026-9613
19		Oct	20	14:25	7.1	-3.02	-0.9314			08-8652-5764	17-2783-6415
20			23	13:45	7.548	-2.572	-0.7932			09-8413-3498	19-3049-9702
21			28	15:50	7.269	-2.851	-0.8794			09-4043-4676	02-6542-7057

CETIS QC Plot

Report Date: 16 Nov-20 12:40 (1 of 1)

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

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



Mean:	10.18	**Count:**	20	**-2s Warning Limit:**	3.692	**-3s Action Limit:**	0.45
Sigma:	3.242	**CV:**	31.80%	**+2s Warning Limit:**	16.66	**+3s Action Limit:**	19.9

Quality Control Data

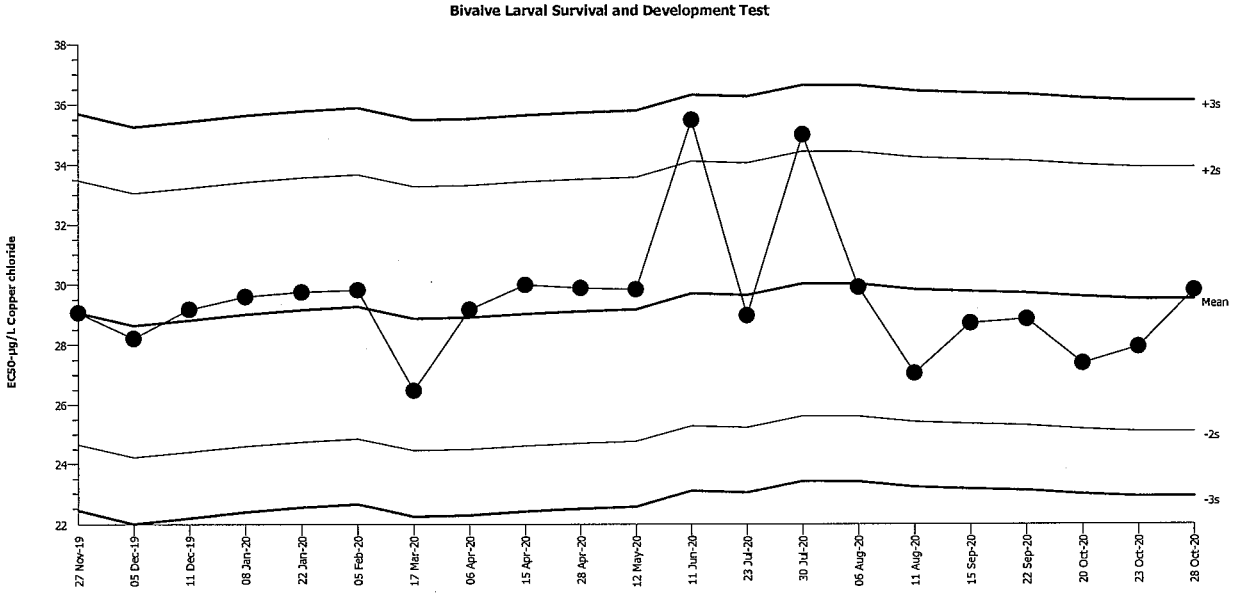
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jan	8	13:40	12.43	2.249	0.6937			07-8444-5322	06-2499-4329
2			22	13:25	14.68	4.501	1.388			02-1152-2212	04-4145-0874
3		Feb	4	16:30	15.01	4.828	1.489			19-9078-6483	06-3219-7963
4			5	13:10	7.132	-3.048	-0.9401			06-6849-2235	20-3119-3253
5			25	14:15	15	4.82	1.487			09-2101-6353	13-1093-9538
6		Mar	17	14:20	7.489	-2.691	-0.8301			14-6169-3689	12-6636-5212
7		Apr	6	17:15	6.609	-3.571	-1.101			02-0082-4673	11-5300-1558
8			15	13:25	11.68	1.503	0.4636			16-4614-0901	19-2371-7781
9			28	13:25	7.365	-2.815	-0.8683			06-8086-6028	17-1633-3832
10		May	12	16:15	8.876	-1.304	-0.4021			12-3773-8150	04-4023-9067
11		Jun	11	15:45	7.306	-2.874	-0.8866			20-6521-9403	18-5947-9043
12		Jul	15	13:55	13.94	3.759	1.16			17-4780-3294	14-0926-7215
13			23	15:00	9.999	-0.1809	-0.05579			06-0741-6264	12-5816-3058
14			30	15:35	14.14	3.957	1.22			00-9901-5729	02-7058-2757
15		Aug	6	15:40	13.95	3.77	1.163			01-4440-0014	13-7910-6508
16			11	14:30	8.237	-1.943	-0.5993			21-4043-5119	01-1240-7098
17		Sep	15	0:00	7.397	-2.783	-0.8584			19-9833-0655	03-7616-5506
18			22	14:40	7.576	-2.604	-0.8031			04-0347-9113	01-0437-7711
19		Oct	20	14:25	7.089	-3.091	-0.9533			08-8652-5764	06-9681-8469
20			23	13:45	7.616	-2.564	-0.7909			09-8413-3498	17-5257-3346
21			28	15:50	7.257	-2.923	-0.9017			09-4043-4676	12-0840-2779

CETIS QC Plot

Report Date: 16 Nov-20 12:40 (1 of 1)

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

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



Mean:	29.52	**Count:**	20	**-2s Warning Limit:**	25.11	**-3s Action Limit:**	22.9
Sigma:	2.207	**CV:**	7.48%	**+2s Warning Limit:**	33.94	**+3s Action Limit:**	36.14

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Nov	27	20:00	29.07	-0.4533	-0.2054			12-9914-0499	00-1104-7300
2		Dec	5	13:15	28.21	-1.306	-0.5916			04-7411-4445	20-5035-4724
3			11	13:35	29.18	-0.3407	-0.1544			10-8800-1613	02-9848-3585
4	2020	Jan	8	13:40	29.6	0.08106	0.03673			07-8444-5322	01-5655-1706
5			22	13:25	29.76	0.2356	0.1068			02-1152-2212	19-4150-8988
6		Feb	5	13:10	29.83	0.3063	0.1388			06-6849-2235	07-0404-6516
7		Mar	17	14:20	26.48	-3.038	-1.377			14-6169-3689	14-2151-4803
8		Apr	6	17:15	29.18	-0.3432	-0.1555			02-0082-4673	12-2147-8498
9			15	13:25	30	0.48	0.2175			16-4614-0901	00-5465-8677
10			28	13:25	29.9	0.376	0.1703			06-8086-6028	08-1083-2165
11		May	12	16:15	29.85	0.331	0.15			12-3773-8150	18-0143-0286
12		Jun	11	15:45	35.5	5.979	2.709	(+)		20-6521-9403	17-6494-5506
13		Jul	23	15:00	28.98	-0.5402	-0.2448			06-0741-6264	11-2012-0880
14			30	15:35	35.02	5.498	2.491	(+)		00-9901-5729	18-8992-7280
15		Aug	6	15:40	29.92	0.4032	0.1827			01-4440-0014	05-9348-7696
16			11	14:30	27.06	-2.461	-1.115			21-4043-5119	16-7506-8565
17		Sep	15	0:00	28.73	-0.7943	-0.3599			19-9833-0655	01-9900-7404
18			22	14:40	28.86	-0.6564	-0.2974			04-0347-9113	03-4439-9784
19		Oct	20	14:25	27.4	-2.124	-0.9624			08-8652-5764	01-6350-7777
20			23	13:45	27.94	-1.578	-0.7152			09-8413-3498	02-1232-2390
21			28	15:50	29.82	0.2995	0.1357			09-4043-4676	15-7574-6891

Analyst: JM QA: 11/16/20

CETIS Test Data Worksheet

Report Date: 27 Oct-20 11:37 (p 1 of 1)
Test Code: 09-4043-4676/201028msdv

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

Start Date: 28 Oct-20 | **Species:** Mytilus galloprovincialis | **Sample Code:** 201028msdv
End Date: 30 Oct-20 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 28 Oct-20 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			164	144	NM 11/13/20
			2			172	167	
			3			181	179	
			4			166	0	
			5			178	154	
			6			0	0	Cells lysed
			7			0	0	Cells lysed
			8			190	163	
			9			174	173	
			10			166	161	
			11			188	156	
			12			0	0	Cells lysed
			13			172	3	
			14			164	161	
			15			182	0	
			16			186	185	
			17			183	177	
			18			177	169	
			19			188	183	
			20			163	0	
			21			166	0	
			22			163	6	
			23			0	0	Cells lysed
			24			188	5	
			25			183	164	
			26			182	5	
			27			0	0	Cells lysed
			28			182	7	
			29			162	155	
			30			165	0	

CETIS Test Data Worksheet

Report Date: 27 Oct-20 11:37 (p 1 of 1)
Test Code: 09-4043-4676/201028msdv

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

Start Date: 28 Oct-20 | **Species:** Mytilus galloprovincialis | **Sample Code:** 201028msdv
End Date: 30 Oct-20 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 28 Oct-20 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	19					
0	LC	2	17					
0	LC	3	10			152	150	RT 10/30/20
0	LC	4	18					
0	LC	5	14					
2.5		1	29					
2.5		2	9					
2.5		3	3			166	166	RT
2.5		4	2					
2.5		5	16					
5		1	5					
5		2	8					
5		3	11			170	153	RT
5		4	1					
5		5	25					
10		1	24					
10		2	28					
10		3	26			179	13	RT
10		4	22					
10		5	13					
20		1	20					
20		2	21					
20		3	15			176	0	RT
20		4	4					
20		5	30					
40		1	6					
40		2	12					
40		3	27			0	0	RT
40		4	23					
40		5	7					

QC: RT

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 201028msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 10/28/2020 1550

End Date/Time: 10/30/2020 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	32.2	31.9	31.7	14.6	14.5	15.4	8.7	8.6	8.3	8.04	8.01	8.04
2.5	32.4	32.0	32.1	14.4	14.3	15.1	8.9	8.8	8.4	8.06	8.03	8.05
5	32.5	32.0	32.1	14.3	14.3	15.1	8.9	8.8	8.4	8.09	8.03	8.06
10	32.4	32.1	32.1	14.5	14.6	15.3	8.8	8.6	8.3	8.10	8.05	8.07
20	32.4	32.1	32.1	14.4	14.5	15.2	8.8	8.7	8.4	8.11	8.06	8.07
40	32.5	32.1	32.0	14.4	14.4	15.2	8.8	8.7	8.4	8.12	8.06	8.06

Technician Initials:		0	24	48
	WQ Readings:	RT	RT	RT
	Dilutions made by:	RT		

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

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: ACS 11/3/2020

Final Review: AC 11/16/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / $CaCl_2$
Test No.: 201028msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: Taylor / SB-SC
Date Received: 7/16/2020
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 10/28/2020 1550
End Date/Time: 10/30/2020 1500
Technician Initials: RT/EC

Spawn Information

First Gamete Release Time: 1055

Sex	Number Spawning
Male	9+
Female	5

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	3,4,5,8	average density, good motility
Female 1	2	good density, yellow, mostly round
Female 2	4	averge density, pale yellow, mostly round
Female 3	—	

Egg Fertilization Time: 1215

Embryo Stock Selection

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

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted:		
	5	5
	8	6
	6	6
	6	6
	7	5

Mean: 6.0

Mean 36 X 50 = 300 embryos/ml
CIS RT 10/28/20

Initial Density: 300 = 1.0 (dilution factor)
Desired Final Density: 300
(to inoculate with 0.5 ml)

Prepare the embryo inoculum according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Time Zero Control Counts

TØ Vial No.	No. Dividing	Total	% Dividing	Mean % Dividing
TØ A	151	152	99.3%	99.4
TØ B	188	189	99.5%	
TØ C	197	197	100.0%	
TØ D	169	170	99.4%	
TØ E	160	162	98.8%	
TØ F	160	161	99.4%	
$\bar{X}$ =	171			

48-h QC: 166/170 = 97.6%

Comments:

QC Check: AYS 11/3/2020

Final Review: AC 11/16/20

Inland Silverside Acute Survival Test

CETIS Summary Report

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	20-5415-1393	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	28 Oct-20 16:35	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	01 Nov-20 15:40	**Species:**	Menidia beryllina	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Indicators, FL	**Age:**	14d

Sample ID:	00-8038-1860	**Code:**	201028mbra	**Client:**	Internal
Sample Date:	28 Oct-20	**Material:**	Copper chloride	**Project:**	
Receive Date:	28 Oct-20	**Source:**	Reference Toxicant		
Sample Age:	17h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
01-8091-4658	96h Survival Rate	100	200	141.4	13.3%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
10-4215-8111	96h Survival Rate	EC50	136.6	121.8	153.2		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
01-8091-4658	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria
10-4215-8111	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria

96h 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	4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.9	0.7163	1	0.8	1	0.05774	0.1155	12.83%	10.0%
200		4	0.05	0	0.2091	0	0.2	0.05	0.1	200.0%	95.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	1
100		1	0.8	0.8	1
200		0	0	0	0.2
400		0	0	0	0
800		0	0	0	0

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 01-8091-4658 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Nov-20 10:23 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	13.3%	100	200	141.4	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		50	0	2.287	0.147	6	0.7500	CDF	Non-Significant Effect
		100	1.852	2.287	0.147	6	0.1028	CDF	Non-Significant Effect
		200*	16.49	2.287	0.147	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.162353	1.054118	3	127.5	<0.0001	Significant Effect
Error	0.09923882	0.008269902	12			
Total	3.261592		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.667	5.953	0.0439	Equal Variances
Variances	Levene Equality of Variance	17	5.953	0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8711	0.8408	0.0283	Normal Distribution

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	0.9	0.7163	1	0.9	0.8	1	0.05774	12.83%	10.0%
200		4	0.05	0	0.2091	0	0	0.2	0.05	200.0%	95.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

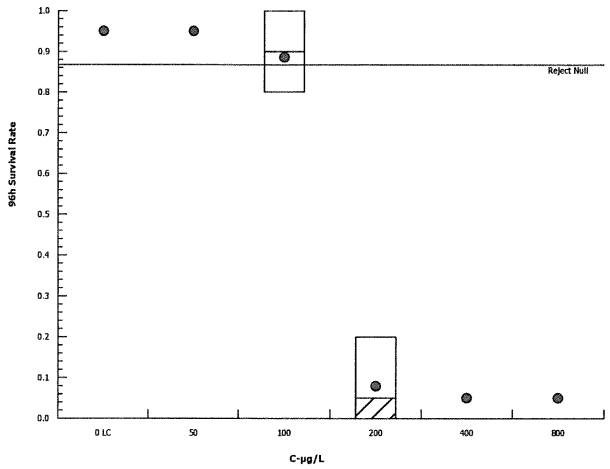
Angular (Corrected) Transformed Summary

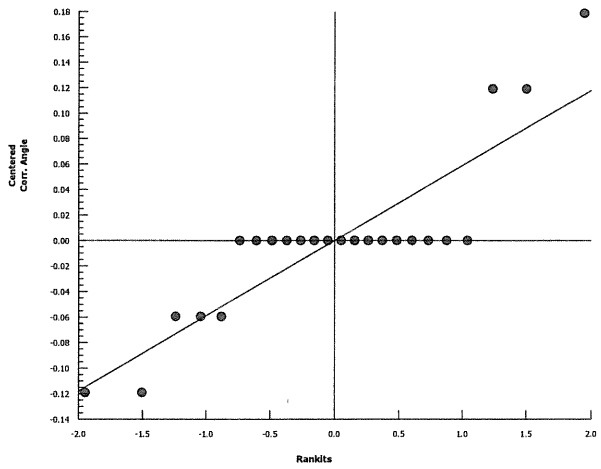
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
50		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
100		4	1.226	1.007	1.445	1.226	1.107	1.345	0.06874	11.21%	8.85%
200		4	0.285	0.09558	0.4745	0.2255	0.2255	0.4636	0.05953	41.77%	78.81%
400		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
800		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	01-8091-4658	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Nov-20 10:23	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	10-4215-8111	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Nov-20 10:23	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

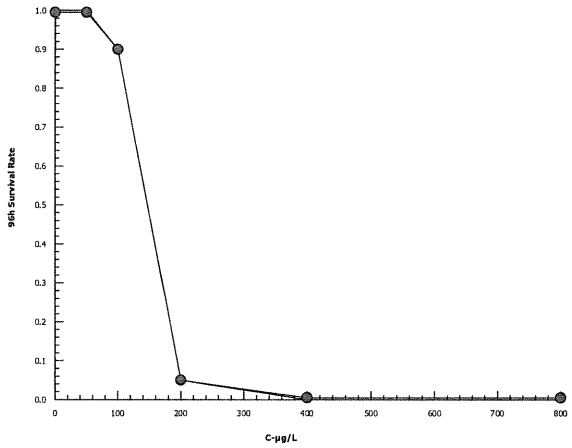
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.135	0.02496	136.6	121.8	153.2

96h 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	4	1	1	1	0	0	0.0%	0.0%	20	20
50		4	1	1	1	0	0	0.0%	0.0%	20	20
100		4	0.9	0.8	1	0.05774	0.1155	12.83%	10.0%	18	20
200		4	0.05	0	0.2	0.05	0.1	200.0%	95.0%	1	20
400		4	0	0	0	0	0		100.0%	0	20
800		4	0	0	0	0	0		100.0%	0	20

Graphics

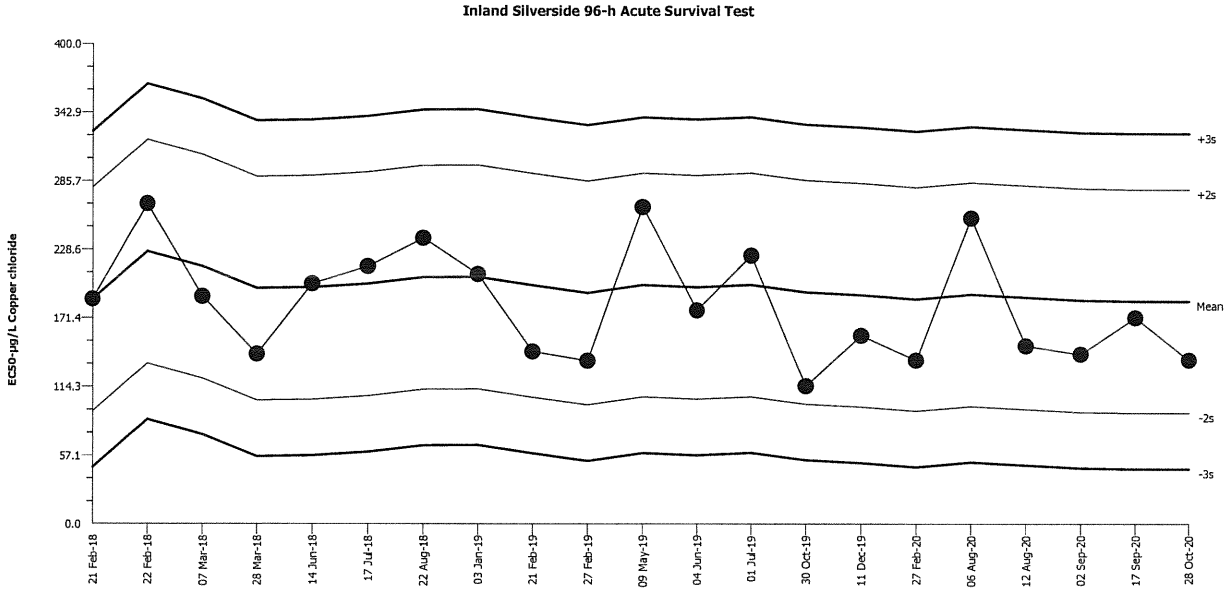


CETIS QC Plot

Report Date: 04 Nov-20 11:41 (1 of 1)

Inland Silverside 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Test Type:	Survival (96h)	**Organism:**	Menidia beryllina (Inland Silverside)	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	185.7	**Count:**	20	**-2s Warning Limit:**	92.48	**-3s Action Limit:**	45.87
Sigma:	46.61	**CV:**	25.10%	**+2s Warning Limit:**	278.9	**+3s Action Limit:**	325.5

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Feb	21	12:25	187.2	1.478	0.03171			20-0148-6736	18-8740-2809
2			22	17:20	266.7	81.01	1.738			21-2244-9573	15-2512-9013
3		Mar	7	16:25	189.3	3.65	0.0783			06-3891-7579	03-5981-6406
4			28	17:15	141.4	-44.28	-0.95			18-3798-9831	05-5342-2351
5		Jun	14	14:35	200	14.3	0.3068			01-9952-0614	00-3575-1747
6		Jul	17	14:30	214.4	28.65	0.6148			11-1445-3115	12-3693-5336
7		Aug	22	16:25	237.8	52.14	1.119			08-6172-7555	12-4329-0617
8	2019	Jan	3	16:50	207.9	22.15	0.4753			16-0506-4055	11-1190-1934
9		Feb	21	16:05	143.5	-42.22	-0.9058			10-4228-2556	08-7111-9529
10			27	16:25	135.8	-49.93	-1.071			14-0947-0420	00-4247-8099
11		May	9	19:10	263.9	78.2	1.678			03-9779-6453	09-3747-7536
12		Jun	4	14:50	177.8	-7.945	-0.1705			00-2136-1210	01-4264-5145
13		Jul	1	15:55	223.6	37.92	0.8135			04-4319-5710	17-4098-1084
14		Oct	30	14:45	114.9	-70.83	-1.52			05-0159-0485	07-6888-5964
15		Dec	11	16:30	156.9	-28.78	-0.6175			11-0566-6524	14-4935-0865
16	2020	Feb	27	17:15	136.4	-49.34	-1.059			00-2639-4829	10-5059-8408
17		Aug	6	16:00	254.9	69.21	1.485			13-3377-6823	09-5433-0150
18			12	15:20	148.4	-37.34	-0.8012			02-5307-3356	11-5066-6205
19		Sep	2	15:25	141.4	-44.28	-0.95			09-8373-9144	18-7650-2455
20			17	14:45	172	-13.74	-0.2948			07-8442-4358	02-9347-5784
21		Oct	28	16:35	136.6	-49.1	-1.053			10-9446-3954	10-4215-8111

Analyst: JU QA: JS 11/4/20

Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: Internal
Test Species: *M. beryllina*
Sample ID: $CuCl_2$
Start Date/Time: 10/28/2020 1635
Test No.: 201028mbra
End Date/Time: 11/1/2020 1540

	Tech Initials				
	0	24	48	72	96
Counts:	AR	RT	AR	DM	AS
Readings:	AC /	RT	RT	DM	KL
Dilutions made by:	AS		RT		
High conc. made (µg/L):	800	--	200	--	--
Vol. Cu stock added (mL):	15.0	--	3.8	--	--
Final Volume (mL):	2000	--	2000	--	--

Cu stock concentration (µg/L): 107,000

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	4	5	5	5	5	5	30.1	30.3	i 29.4	30.4	31.0	24.0	24.3	i 24.1	24.2	24.4	7.3	5.8	i 6.8	5.2	4.8 (C)	8.04	7.83	i 8.02	7.77	7.72
	10	5	5	5	5	5			f 30.7					f 24.5					f 5.0					f 7.79		
	5	5	5	5	5	5																				
	9	5	5	5	5	5																				
50	23	5	5	5	5	5	29.9	30.2	i 29.2	30.5	30.9	24.9	24.2	i 24.1	24.5	24.4	7.1	5.9	i 6.8	5.2	4.3	8.04	7.83	i 8.04	7.77	7.77
	18	5	5	5	5	5			f 30.8					f 24.7					f 5.0					f 7.79		
	3	5	5	5	5	5																				
	6	5	5	5	5	5																				
100	19	5	5	5	5	5	29.8	30.0	i 29.3	30.0	30.1	25.3	24.6	i 24.2	24.7	24.7	7.2	5.5	i 6.9	5.0	4.4	8.03	7.80	i 8.05	7.75	7.71
	14	5	4	4	4	4			f 30.0					f 25.1					f 4.8					f 7.70		
	2	5	4	4	4	4																				
	17	5	5	5	5	5																				
200	20	5	2	0	–	–	29.9	30.1	i 29.3	30.1	30.7	25.5	24.4	i 24.1	24.6	24.4	7.2	5.5	i 7.0	5.3	5.4	8.03	7.87	i 8.04	7.88	7.90
	11	5	0	–	–	–			f 30.7					f 24.7					f 4.9					f 7.82		
	7	5	0	–	–	–																				
	22	5	1	1	1	1	29.8																			
400	8	5	0				~~8.03~~ (B)	30.2	i –	–	–	24.2	24.3	i –	–	–	7.4	5.9	i –	–	–	8.03	7.92	i –	–	–
	15	5	0						f –					f –					f –					f –		
	1	5	0																							
	21	5	0																							
800	13	5	0				29.7	29.8	i –	–	–	24.6	24.8	i –	–	–	7.2	5.9	i –	–	–	8.00	7.89	i –	–	–
	16	5	0						f –					f –					f –					f –		
	24	5	0																							
	12	5	0																							

Rand # QC: DM
Initial Counts QC'd by: DM/HH
Initiated by: AR

Environmental Chamber: A

Animal Source/Date Received: Aquatic Indicaters 10/27/20
Age at Initiation: 14 d

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / (none)

Feeding Times					
	0	24	48	72	96
AM:		0900	0910	0850	0840
PM:	1720				

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal (A) Q19 AS 10/28/2020

Organisms fed prior to initiation, circle one (y) / n) Q21 organisms experienced >3ppt shift and >3°c shift during the firts 24 hrs. of acclimation. AR 10/28/20

(B) Q18 KL 11/1/20 (C) Q18 AS 11/1/2020

QC Check: AS 11/1/2020

Final Review: VS 11/4/20

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