ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: October 2019

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

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

Date Submitted: **November 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.

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

Results verified by: _____
Eric Green, Project Manager

Introduction

Acute and chronic toxicity tests were performed using a groundwater composite sample collected on October 29, 2019 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 (formerly Nautilus Environmental) located in San Diego, California between October 30 and November 3, 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	**102919; Location SP-11**
Enthalpy Log-in Number	19-1164
Collection Date; Time	10/29/2019; 0950h
Receipt Date; Time	10/30/2019; 1000h
Receipt Temperature (°C)	2.0
Dissolved Oxygen (mg/L)	8.5
pH	7.51
Salinity (ppt)	8.7
Alkalinity (mg/L $CaCO_3$)	387
Total Chlorine (mg/L)	0.07
Total Ammonia (mg/L as N)	2.1

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	10/30/2019, 1230h to 11/1/2019, 1130h
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 91.0 ppt
Test Concentrations (% sample)	74.1[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	10/30/2019, 1445h to 11/3/2019, 1410h
Test Organism	*Menidia beryllina*
Test Organism Source	Aquatic Indicators (St. Augustine, FL)
Test Organism Age	12 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	Laboratory Seawater (Source: SIO intake) diluted with de-ionized water
Additional Control	Salt Control (Instant Ocean™ brand sea salts added to 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 74.1 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.35 for both endpoints.

There were no statistically significant effects observed in any effluent concentration tested for the survival endpoint of the inland silverside test, and all concentrations resulted in 100 percent survival. 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	74.1	> 74.1	< 1.35	> 74.1
	Survival	74.1	> 74.1	< 1.35	> 74.1

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	100
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)	99.2	99.0
0 (Lab Control)	97.6	98.2
2	94.8	98.5
4	96.2	97.9
9	92.7	98.8
18	94.2	97.7
35	91.0	98.0
74.1[a]	99.0	98.7

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

Quality Assurance

The sample was received and tested 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 responses 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 7.The results for the bivalve reference toxicant test were within the acceptable range of the mean historical test results plus or minus two standard deviations. The result for the inland silverside reference toxicant test was below two, but within three, standard deviations of the historical mean. This indicates that this batch of organisms was slightly more sensitive than typical for our laboratory.

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 7. Reference Toxicant Test Results

Species	Endpoint	EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve	Normal Development	7.48	8.45 ± 4.45	26.3
	Survival	29.3	29.7 ± 4.97	8.36
Inland Silverside	Survival	115	196 ± 76.8	19.6

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

CETIS Summary Report

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

Batch ID:	19-0640-4065	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	30 Oct-19 12:30	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	01 Nov-19 11:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	16-3418-8863	**Code:**	19-1164	**Client:**	Jacobs
Sample Date:	29 Oct-19 09:50	**Material:**	Effluent Sample	**Project:**	
Receive Date:	30 Oct-19 10:00	**Source:**	Jacobs		
Sample Age:	27h (2 °C)	**Station:**	Wyckoff (SP-11)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-1360-5611	Development Rate	74.1	>74.1	NA	1.63%	< 1.35	Dunnett Multiple Comparison Test
00-6811-3566	Survival Rate	74.1	>74.1	NA	7.03%	< 1.35	Dunnett Multiple Comparison Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
14-1360-5611	Development Rate	Control Resp	0.9903	0.9 - NL	Yes	Passes Acceptability Criteria
00-6811-3566	Survival Rate	Control Resp	0.9915	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	0.9903	0.985	0.9956	0.9854	0.9935	0.001911	0.004274	0.43%	0.0%
0	Lab Control	5	0.9816	0.9712	0.9919	0.971	0.9935	0.003722	0.008322	0.85%	0.88%
2		5	0.9851	0.9737	0.9964	0.9762	1	0.004103	0.009175	0.93%	0.53%
4		5	0.979	0.9692	0.9888	0.9735	0.9928	0.003522	0.007875	0.8%	1.14%
9		5	0.9877	0.9787	0.9967	0.9767	0.993	0.003241	0.007248	0.73%	0.27%
18		5	0.9772	0.9498	1	0.9474	0.9934	0.009843	0.02201	2.25%	1.32%
35		5	0.9796	0.9625	0.9966	0.96	0.9933	0.00614	0.01373	1.4%	1.08%
74.1		5	0.9868	0.9815	0.992	0.9806	0.9926	0.001893	0.004233	0.43%	0.36%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9915	0.9726	1	0.9648	1	0.006828	0.01527	1.54%	0.0%
0	Lab Control	5	0.9761	0.9402	1	0.9296	1	0.01291	0.02886	2.96%	1.56%
2		5	0.9479	0.8778	1	0.8662	1	0.02525	0.05647	5.96%	4.4%
4		5	0.962	0.9009	1	0.8803	1	0.022	0.0492	5.11%	2.98%
9		5	0.9268	0.8629	0.9906	0.8592	1	0.02299	0.05141	5.55%	6.53%
18		5	0.9423	0.8337	1	0.8028	1	0.03911	0.08745	9.28%	4.97%
35		5	0.9099	0.8318	0.9879	0.838	1	0.02812	0.06287	6.91%	8.24%
74.1		5	0.9901	0.9628	1	0.9507	1	0.009859	0.02205	2.23%	0.14%

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	0.9933	0.9854	0.9935	0.9934	0.9858
0	Lab Control	0.971	0.98	0.9935	0.9848	0.9784
2		1	0.9762	0.9847	0.9852	0.9792
4		0.9928	0.9735	0.976	0.9779	0.9747
9		0.9925	0.9767	0.9924	0.993	0.9836
18		0.9474	0.9931	0.9934	0.9597	0.9922
35		0.9921	0.9776	0.96	0.9748	0.9933
74.1		0.9865	0.9806	0.9871	0.9926	0.987

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	0.9648	1	1	0.993
0	Lab Control	0.9718	1	1	0.9296	0.9789
2		0.8662	1	0.9225	0.9507	1
4		0.9718	1	0.8803	0.9577	1
9		0.9437	0.9085	0.9225	1	0.8592
18		0.8028	1	1	1	0.9085
35		0.8873	0.9437	0.8803	0.838	1
74.1		1	1	1	0.9507	1

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	149/150	135/137	152/153	151/152	139/141
0	Lab Control	134/138	147/150	153/154	130/132	136/139
2		123/123	164/168	129/131	133/135	141/144
4		137/138	147/151	122/125	133/136	154/158
9		133/134	126/129	130/131	142/143	120/122
18		108/114	144/145	151/152	143/149	128/129
35		125/126	131/134	120/125	116/119	148/149
74.1		146/148	152/155	153/155	134/135	152/154

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	142/142	137/142	142/142	142/142	141/142
0	Lab Control	138/142	142/142	142/142	132/142	139/142
2		123/142	142/142	131/142	135/142	142/142
4		138/142	142/142	125/142	136/142	142/142
9		134/142	129/142	131/142	142/142	122/142
18		114/142	142/142	142/142	142/142	129/142
35		126/142	134/142	125/142	119/142	142/142
74.1		142/142	142/142	142/142	135/142	142/142

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

Analysis ID:	14-1360-5611	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Nov-19 8:00	**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	1.63%	74.1	>74.1	NA	1.35

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	0.7918	2.407	0.065	8	0.5490	CDF	Non-Significant Effect
		4	1.703	2.407	0.065	8	0.1812	CDF	Non-Significant Effect
		9	0.4079	2.407	0.065	8	0.7186	CDF	Non-Significant Effect
		18	1.501	2.407	0.065	8	0.2454	CDF	Non-Significant Effect
		35	1.473	2.407	0.065	8	0.2554	CDF	Non-Significant Effect
		74.1	0.6396	2.407	0.065	8	0.6190	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.008873828	0.001478971	6	0.8193	0.5644	Non-Significant Effect
Error	0.05054396	0.001805142	28			
Total	0.05941779		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.764	16.81	0.1349	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.972	0.9146	0.4998	Normal Distribution

Development Rate Summary

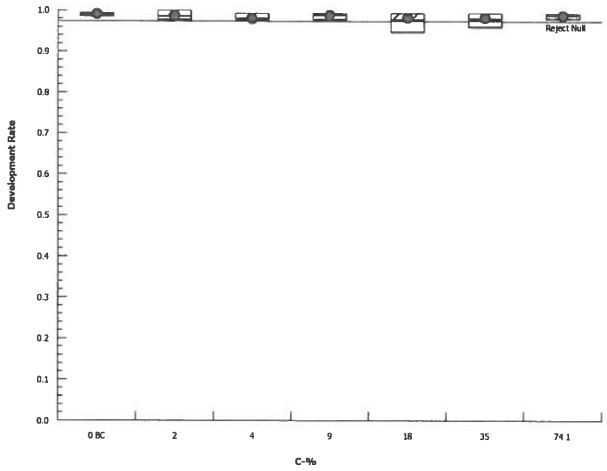
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9903	0.985	0.9956	0.9933	0.9854	0.9935	0.001912	0.43%	0.0%
2		5	0.9851	0.9737	0.9964	0.9847	0.9762	1	0.004103	0.93%	0.53%
4		5	0.979	0.9692	0.9888	0.976	0.9735	0.9928	0.003522	0.8%	1.14%
9		5	0.9877	0.9787	0.9967	0.9924	0.9767	0.993	0.003241	0.73%	0.27%
18		5	0.9772	0.9498	1	0.9922	0.9474	0.9934	0.009843	2.25%	1.32%
35		5	0.9796	0.9625	0.9966	0.9776	0.96	0.9933	0.00614	1.4%	1.08%
74.1		5	0.9868	0.9815	0.992	0.987	0.9806	0.9926	0.001894	0.43%	0.36%

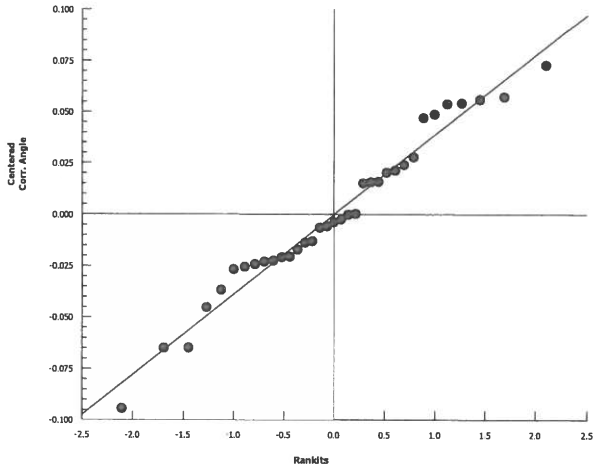
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.474	1.447	1.5	1.489	1.45	1.49	0.009548	1.45%	0.0%
2		5	1.453	1.399	1.506	1.447	1.416	1.526	0.0193	2.97%	1.44%
4		5	1.428	1.388	1.469	1.415	1.407	1.486	0.01455	2.28%	3.1%
9		5	1.463	1.424	1.502	1.483	1.418	1.487	0.014	2.14%	0.74%
18		5	1.434	1.342	1.525	1.483	1.339	1.49	0.03283	5.12%	2.74%
35		5	1.434	1.372	1.497	1.421	1.369	1.489	0.0225	3.51%	2.69%
74.1		5	1.457	1.433	1.48	1.457	1.431	1.485	0.00847	1.3%	1.17%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	14-1360-5611	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Nov-19 8:00	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	00-6811-3566	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Nov-19 8:00	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.03%	74.1	>74.1	NA	1.35

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	1.338	2.407	0.204	8	0.3063	CDF	Non-Significant Effect
		4	0.9794	2.407	0.204	8	0.4617	CDF	Non-Significant Effect
		9	2.027	2.407	0.204	8	0.1045	CDF	Non-Significant Effect
		18	1.17	2.407	0.204	8	0.3761	CDF	Non-Significant Effect
		35	2.347	2.407	0.204	8	0.0565	CDF	Non-Significant Effect
		74.1	-0.01636	2.407	0.204	8	0.8615	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1768408	0.02947347	6	1.648	0.1711	Non-Significant Effect
Error	0.5008067	0.01788595	28			
Total	0.6776475		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.442	16.81	0.4885	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9789	0.9146	0.7238	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	0.9915	0.9726	1	1	0.9648	1	0.006828	1.54%	0.0%
2		5	0.9479	0.8778	1	0.9507	0.8662	1	0.02525	5.96%	4.4%
4		5	0.962	0.9009	1	0.9718	0.8803	1	0.022	5.11%	2.98%
9		5	0.9268	0.8629	0.9906	0.9225	0.8592	1	0.02299	5.55%	6.53%
18		5	0.9423	0.8337	1	1	0.8028	1	0.03911	9.28%	4.97%
35		5	0.9099	0.8318	0.9879	0.8873	0.838	1	0.02812	6.91%	8.24%
74.1		5	0.9901	0.9628	1	1	0.9507	1	0.009859	2.23%	0.14%

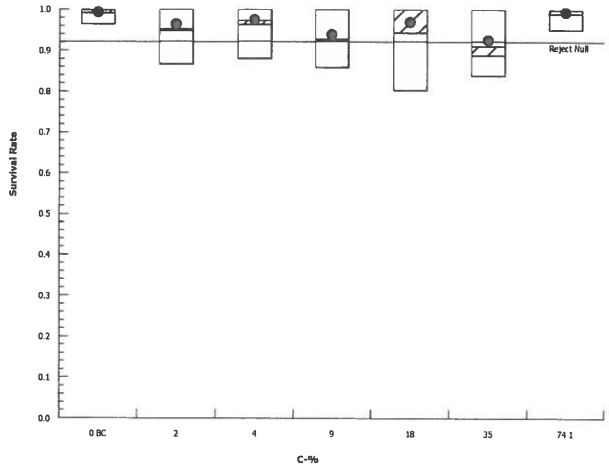
Angular (Corrected) Transformed Summary

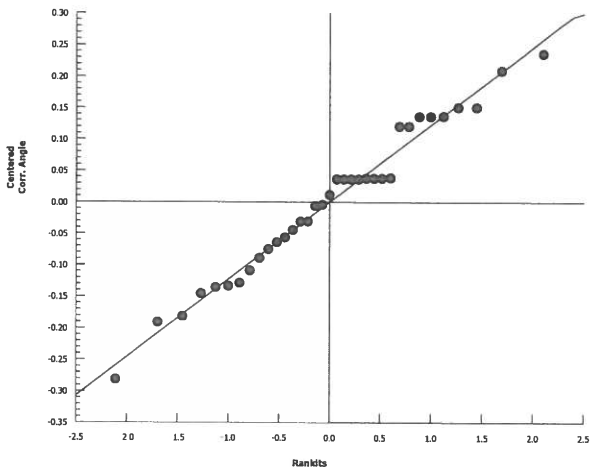
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.491	1.412	1.57	1.529	1.382	1.529	0.02845	4.27%	0.0%
2		5	1.378	1.194	1.562	1.347	1.196	1.529	0.06612	10.73%	7.59%
4		5	1.408	1.247	1.569	1.402	1.217	1.529	0.05809	9.22%	5.56%
9		5	1.32	1.16	1.479	1.289	1.186	1.529	0.05738	9.72%	11.5%
18		5	1.392	1.15	1.634	1.529	1.111	1.529	0.08713	14.0%	6.64%
35		5	1.293	1.111	1.474	1.228	1.157	1.529	0.0654	11.31%	13.32%
74.1		5	1.492	1.391	1.593	1.529	1.347	1.529	0.03638	5.45%	-0.09%

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

Analysis ID:	00-6811-3566	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Nov-19 8:00	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Test Data Worksheet

Report Date: 26 Oct-19 16:34 (p 1 of 1)
Test Code: 1910-5148 14-0607-3357/53CEFA0D

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

Start Date: 30 Oct-19
End Date: 01 Nov-19
Sample Date: 29 Oct-19
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 19- 1164
Sample Source: Jacobs
Sample Station: Wyckoff SP-11

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			154	153	11/8/19 JCL
			32			158	154	
			33			139	136	
			34			152	151	
			35			144	141	
			36			131	129	
			37			149	143	
			38			150	149	
			39			150	147	
			40			145	144	
			41			138	137	
			42			153	152	
			43			143	142	
			44			155	153	
			45			114	108	
			46			123	123	
			47			122	120	
			48			137	135	
			49			152	151	
			50			129	128	
			51			151	147	
			52			134	131	
			53			119	116	
			54			154	152	
			55			131	130	
			56			155	152	
			57			148	146	
			58			125	120	
			59			141	139	
			60			126	125	
			61			132	130	
			62			135	134	
			63			135	133	
			64			129	126	
			65			136	133	
			66			134	133	
			67			149	148	
			68			125	122	
			69			168	164	
			70			138	134	

CETIS Test Data Worksheet

Report Date: 26 Oct-19 16:34 (p 1 of 1)
Test Code: 1910-5148 14-0607-3357/53CEFA0D

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

Start Date: 30 Oct-19
End Date: 01 Nov-19
Sample Date: 29 Oct-19
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 19-1164
Sample Source: Jacobs
Sample Station: Wyckoff SP-11

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	38					
0	BC	2	48					
0	BC	3	42					
0	BC	4	49			148	148	RT 11/1/19
0	BC	5	59					
0	LC	1	70					
0	LC	2	39					
0	LC	3	31			150	149	RT
0	LC	4	61					
0	LC	5	33					
2		1	46					
2		2	69					
2		3	36			123	123	RT
2		4	63					
2		5	35					
4		1	41					
4		2	51					
4		3	68			126	123	RT
4		4	65					
4		5	32					
9		1	66					
9		2	64					
9		3	55			131	130	RT
9		4	43					
9		5	47					
18		1	45					
18		2	40					
18		3	34			154	154	RT
18		4	37					
18		5	50					
35		1	60					
35		2	52					
35		3	58			119	116	RT
35		4	53					
35		5	67					
73.5		1	57					
73.5		2	56					
73.5		3	44			168	165	RT
73.5		4	62					
73.5 (A)		5	54					

74.1

QC-BO

(A) QLB ACS 10/27/19

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Jacobs

Sample ID: Wyckoff (SP-11)

Sample Log No.: 19- 1164

Test No.: 1910-5148

Test Species: *M. galloprovincialis*

Start Date/Time: 10/30/2019 1230

End Date/Time: 11/1/2019 1130

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	29.8	29.8	15.8	14.5	14.5	8.4	8.4	9.2	8.01	8.00	7.96
Brine Control	30.6	30.7	30.9	15.6	14.6	14.7	8.6	8.3	9.2	8.17	8.05	8.01
2	30.0	30.2	30.2	15.8	14.5	14.6	8.6	8.3	9.3	8.06	8.03	7.99
4	30.0	30.2	30.2	15.6	14.5	14.8	8.5	8.3	9.2	8.03	8.03	8.02
9	30.1	30.2	30.3	15.5	14.5	14.6	8.6	8.3	9.3	7.98	8.03	8.06
18	30.2	30.3	30.4	15.6	14.6	14.9	8.6	8.2	9.3	7.89	8.04	8.10
35	30.3	30.5	30.7	15.2	14.5	14.8	8.8	8.3	9.3	7.79	8.04	8.19
74.1	30.6	30.9	30.9	14.6	14.6	14.9	8.8	8.2	9.2	7.68	8.07	8.26

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

Technician Initials:

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: EM 11/15/19

Final Review: AC 11/20/19

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: JACOBS **Analyst:** BO

Sample ID: Wyckoff (SP-11) **Test Date:** 10/30/2019

Test No: 1910-S148 **Test Type:** Mussel Development

Salinity of Effluent 8.7

Salinity of Brine 91 **Date of Brine used:** 9/27/2019

Target Salinity 30 **Alkalinity of Brine Control:** 93 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.35**	**0.49**

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	0.35	1.7	250
4	10.0	0.35	3.5	250
9	22.5	0.35	7.9	250
18	45.0	0.35	15.7	250
35	87.5	0.35	30.6	250
74.1	185.3	0.35	64.7	250
	DI Volume			
Brine Control	131.6	0.49	64.7	250

Total Brine Volume Required (ml): **188.8**

QC Check: EA 11/15/19 **Final Review:** AC 11/20/19

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

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs / Wyckoff (SP-11)
Test No.: 1910-5148
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: Taylor Shellfish / 17B
Date Received: 8/14/19
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 10/30/2019 1230
End Date/Time: 11/1/2019 1130
Technician Initials: BO

Spawn Information

First Gamete Release Time: 0945

Sex	Number Spawning
Male	4+
Female	4

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	3,4	good density + motility
Female 1	4	yellow color, good density, mostly round
Female 2	3	pink color, good density, mostly round
Female 3	–	–

Egg Fertilization Time: 1040

Embryo Stock Selection

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

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

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

Mean: 6.8

Mean 6.8 X 50 = 340 embryos/ml

Initial Density: 340 = 1.13 (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	141	141	100	99.8
TØ B	154	154	100	
TØ C	130	130	100	
TØ D	150	150	100	
TØ E	133	134	99.2	
TØ F	142	143	99.3	
$\bar{X}$ =	142			

48-h QC: 140/143 = 97.9%

Comments:

QC Check: Eq 11/15/19

Final Review: AC 11/20/19

Inland Silverside Acute Survival Test

CETIS Summary Report

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

Batch ID:	04-5621-4720	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	30 Oct-19 14:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Artificial seawater ~~Diluted Natural Seawater~~
Ending Date:	03 Nov-19 14:10	**Species:**	Menidia beryllina	**Brine:**	(A) ~~Not Applicable~~ Instant Ocean
Duration:	95h	**Source:**	Aquatic Indicators	**Age:**	12d

Sample ID:	14-1353-6260	**Code:**	19-1164	**Client:**	Jacobs
Sample Date:	29 Oct-19 09:50	**Material:**	Effluent Sample	**Project:**	
Receive Date:	30 Oct-19 10:00	**Source:**	Jacobs		
Sample Age:	29h (2 °C)	**Station:**	Wyckoff (SP-11)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-5978-7537	96h Survival Rate	100	>100	NA	NA	1	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-5978-7537	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	1	1	1	1	1	0	0	0.0%	0.0%
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	1	1	1
12.5		1	1	1	1
25		1	1	1	1
50		1	1	1	1
100		1	1	1	1

(A) EG Q18 11/15/19

CETIS Analytical Report

Test Code: 1910-S149 | 08-3055-1545

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

Analysis ID:	02-5978-7537	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Nov-19 8:54	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

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

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Salt Control		6.25	18	10	1	6	0.8333	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	0	5	65540	<0.0001	Significant Effect
Error	0	0	18			
Total	0		23			

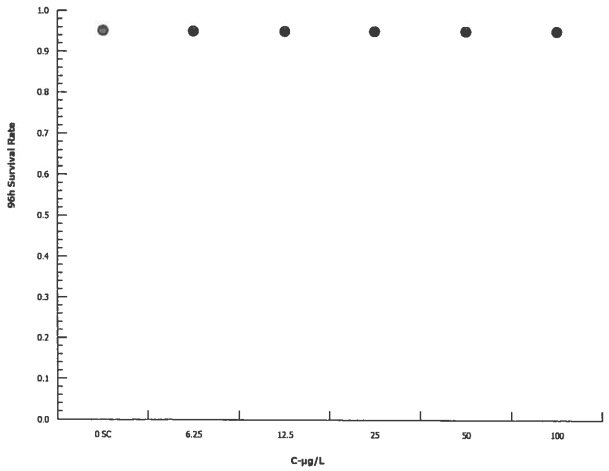
96h Survival Rate Summary

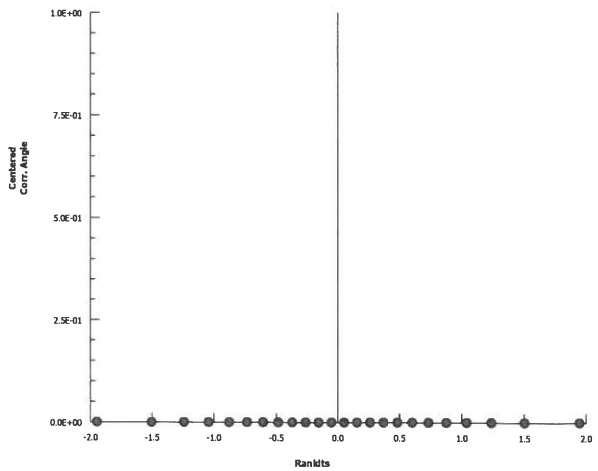
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1	1	1	1	1	1	0	0.0%	0.0%
6.25		4	1	1	1	1	1	1	0	0.0%	0.0%
12.5		4	1	1	1	1	1	1	0	0.0%	0.0%
25		4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	1	1	1	1	1	1	0	0.0%	0.0%

Angular (Corrected) Transformed Summary

C-µg/L	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	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
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%

Graphics





Analyst: JCL QA: EG 11/15/19

Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: Jacobs
Sample ID: Wyckoff SP-11
Sample Log-in No.: 19-~~1194~~ 1164
Test No.: 1910-5149

Test Species: *M. beryllina*
Start Date/Time: 10/30/2019 1445
End Date/Time: 11/3/2019 1410

Tech Initials	0	24	48	72	96
Counts:	DM	RT	RT	RT	MS
Readings:	HH	HH	KL	RT	DM
Dilutions made by:	HH		TN		

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	30.8	30.9	i 29.1	30.4	30.9	24.1	24.3	i 24.0	24.3	24.2	6.5	5.1	i 7.7	6.1	5.8	7.71	7.7	i 8.05	7.77	7.91
	B	5	5	5	5	5			f 31.5					f 24.1					f 6.4					f 7.78		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
Salt Control	A	5	5	5	5	5	30.5	30.7	i 30.8	31.5	31.5	24.0	24.5	i 24.5	24.6	24.4	10.7	5.0	i 7.1	5.7	5.5	8.16	7.99	i 8.19	7.93	7.99
	B	5	5	5	5	5			f 31.3					f 24.0					6.2					f 7.85		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
6.25	A	5	5	5	5	5	30.5	30.6	i 30.7	31.3	31.3	24.1	24.3	i 24.6	24.8	24.6	10.8	5.0	i 7.2	5.5	5.3	8.10	7.9	i 8.15	7.97	8.09
	B	5	5	5	5	5			f 31.2					f 24.1					f 6.0					f 8.01		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
12.5	A	5	5	5	5	5	30.4	30.4	30.6	31.4	31.6	24.0	24.4	i 24.5	24.8	24.7	10.9	5.2	i 7.2	5.4	5.4	8.05	8.03	i 8.11	8.01	8.15
	B	5	5	5	5	5			f 31.2					f 24.2					f 6.1					f 8.07		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
25	A	5	5	5	5	5	30.3	30.3	i 30.5	31.0	31.1	24.0	24.4	i 24.3	24.8	24.7	7.1	5.1	i 7.3	5.5	5.4	7.96	8.04	i 8.06	8.07	8.18
	B	5	5	5	5	5			f 30.8					f 24.3					f 6.0					f 8.14		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
50	A	5	5	5	5	5	30.0	30.1	i 30.2	30.9	31.1	24.1	24.0	i 24.0	24.8	24.5	7.5	4.9	i 7.6	5.3	5.4	7.84	8.04	i 7.97	8.12	8.28
	B	5	5	5	5	5			f 29.7					f 24.4					f 5.9					f 8.22		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
100	A	5	5	5	5	5	29.5	29.9	i 29.5	30.9	31.6	24.2	24.3	i 24.2	24.5	24.3	8.5	5.3	i 8.3	5.5	5.5	7.72	8.13	i 7.85	8.21	8.40
	B	5	5	5	5	5			f 29.8					f 24.3					f 5.9					f 8.32		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				

Initial Counts QC'd by: HH
Initiated by: DM

Environmental Chambe

Animal Source/Date Received: Aquatic Ind. Labs., 10/24/19 Age at Initiatio

Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23

Feeding Times	0	24	48	72	96
		0900	0930	1000	0945
	1715				

Comments: i = initial reading in fresh test solution, f = final reading in test chamb

Organisms fed prior to initiation, circle one (y / n) AE 0218 / 5/19

QC Check: BO 11/11/19

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: Jacobs
Sample ID: Wyckoff (SP-11)
Test ID No(s).: 1910 - S148 and S149

Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (19-xxxx):	1164			
Sample Collection Date & Time:	10/29/19 09:50			
Sample Receipt Date & Time:	10/30/19 10:00			
Number of Containers & Container Type:	1 4L cubi			
Approx. Total Volume Received (L):	4			
Check-in Temperature (°C)	2.0			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.5			
pH (units)	7.51			
Conductivity (µS/cm)	—			
Salinity (ppt)	8.7			
Alkalinity (mg/L) [2]	387			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.07			
Technician Initials	AC/ER			

Test Performed: Mussel Development **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART **Other:** _____
Alkalinity: 102 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Control Alkalinity: 93 Hardness or Salinity: 30ppt

Test Performed: Acute Menidia **Control/Dilution Water:** 8:2 / Lab SW / (Lab ART) **Other:** _____
Alkalinity: 147 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Lab SW Alkalinity: 102 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: _____

Sample Description: Colorless, clear, no odor, no debris

COC Complete (Y/N)?
A Y B — C —

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

Salinity Adjustment? (Y) N

Test: Mussel	Source: Brine	Target ppt: 36	
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 AC B___ C___

QC Check: BO 11/11/19
Final Review: EG 11/15/19

Total Ammonia Analysis
Freshwater

Overlying Water

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 10/30/2019

Analyst: QR
Analysis Date: 11/14/19

Sample ID	Nautilus ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L) N x 1.22
Blank Spike (10 mg/L NH_3)		NA	NA	8.0	9.8
Wyckoff	19-1164	10/30/2019	Check In	2.1	2.6
Spike Check (10 mg/L NH_3)		NA	NA		
Sample Duplicate[a]	19-1164	NA	NA	2.0	2.4
Sample Duplicate + Spike[a]		NA	NA	9.8	12.0
Spike Check (10 mg/L NH_3)		NA	NA	8.0	9.8

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

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

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	9.8	10	NA	98
Wyckoff	2.6	2.4	12.0	10	8.0	94

Comments:

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

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

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

Method Detection Limit = 0.5 mg/L

QC Check: EA 11/15/19

Final Review: AC 11/20/19

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 10/29/2019
CarrierName: FedEx
AirbillNo: 7768 4265 7340

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

No: 10-102919-101727-0409
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
102919		Ground Water/ K.Allers	Composite	ACTOX-CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	10/29/2019 09:50	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
	[signature] JACOBS	10-29-2019 1030	[signature]	10/30/19 10:00	good Temp °C 2.0

Log-in # 19-1164

Appendix D
Reference Toxicant Test Results

Bivalve Larval Development Test

CETIS Summary Report

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

Batch ID:	09-4506-2647	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	30 Oct-19 12:30	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	01 Nov-19 11:30	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	Taylor Shellfish	**Age:**	

Sample ID:	02-6673-8220	**Code:**	191030msdv	**Client:**	Internal
Sample Date:	30 Oct-19	**Material:**	Copper chloride	**Project:**	
Receive Date:	30 Oct-19	**Source:**	Reference Toxicant		
Sample Age:	13h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-3686-0042	Combined Development Ra	5	10	7.071	6.68%		Dunnett Multiple Comparison Test
18-3591-5974	Development Rate	2.5	5	3.536	1.11%		Dunnett Multiple Comparison Test
10-2770-1957	Survival Rate	20	40	28.28	8.59%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
11-8079-0492	Combined Development Ra	EC25	6.259	6.084	6.273		Linear Interpolation (ICPIN)
		EC50	7.518	7.405	7.545		
15-7183-3565	Development Rate	EC25	6.204	6.15	6.254		Linear Interpolation (ICPIN)
		EC50	7.481	7.439	7.522		
20-5233-5110	Survival Rate	EC25	23.94	22.13	25.33		Linear Interpolation (ICPIN)
		EC50	29.32	28.14	30.24		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
15-7183-3565	Development Rate	Control Resp	0.9844	0.9 - NL	Yes	Passes Acceptability Criteria
18-3591-5974	Development Rate	Control Resp	0.9844	0.9 - NL	Yes	Passes Acceptability Criteria
10-2770-1957	Survival Rate	Control Resp	0.9479	0.5 - NL	Yes	Passes Acceptability Criteria
20-5233-5110	Survival Rate	Control Resp	0.9479	0.5 - NL	Yes	Passes Acceptability Criteria
11-3686-0042	Combined Development Ra	PMSD	0.06684	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	0.933	0.8529	1	0.8592	0.9871	0.02885	0.06451	6.91%	0.0%
2.5		5	0.9523	0.8827	1	0.8521	0.98	0.02509	0.05609	5.89%	-2.07%
5		5	0.9616	0.9289	0.9943	0.9155	0.981	0.01178	0.02635	2.74%	-3.06%
10		5	0.006761	0	0.01704	0	0.02027	0.003702	0.008278	122.4%	99.28%
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	0.9844	0.9769	0.9918	0.9762	0.9919	0.002688	0.00601	0.61%	0.0%
2.5		5	0.9787	0.9739	0.9835	0.9737	0.9837	0.001726	0.00386	0.39%	0.58%
5		5	0.9697	0.9579	0.9814	0.9559	0.981	0.004232	0.009464	0.98%	1.49%
10		5	0.006891	0	0.01718	0	0.02027	0.003707	0.008288	120.3%	99.3%
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	0.9479	0.8664	1	0.8662	1	0.02934	0.06561	6.92%	0.0%
2.5		5	0.9732	0.8989	1	0.8662	1	0.02676	0.05984	6.15%	-2.68%
5		5	0.9915	0.9681	1	0.9577	1	0.008451	0.0189	1.91%	-4.61%
10		5	0.9676	0.9124	1	0.9155	1	0.01987	0.04443	4.59%	-2.08%
20		5	0.9056	0.8168	0.9945	0.8169	1	0.03201	0.07158	7.9%	4.46%
40		5	0.004225	0	0.009015	0	0.007042	0.001725	0.003857	91.29%	99.55%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8662	0.8592	0.9871	0.9718	0.9809
2.5		0.98	0.9797	0.9762	0.9737	0.8521
5		0.981	0.9735	0.9724	0.9655	0.9155
10		0	0.006494	0	0.02027	0.007042
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	0.9762	0.9919	0.9871	0.9857	0.9809
2.5		0.98	0.9797	0.9762	0.9737	0.9837
5		0.981	0.9735	0.9724	0.9655	0.9559
10		0	0.006494	0	0.02027	0.007692
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	0.8873	0.8662	1	0.9859	1
2.5		1	1	1	1	0.8662
5		1	1	1	1	0.9577
10		1	1	0.9225	1	0.9155
20		0.8944	0.9507	1	0.8662	0.8169
40		0.007042	0.007042	0.007042	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	123/142	122/142	153/155	138/142	154/157
2.5		147/150	145/148	164/168	148/152	121/142
5		155/158	147/151	141/145	140/145	130/142
10		0/151	1/154	0/142	3/148	1/142
20		0/142	0/142	0/152	0/142	0/142
40		0/142	0/142	0/142	0/142	0/142

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	123/126	122/123	153/155	138/140	154/157
2.5		147/150	145/148	164/168	148/152	121/123
5		155/158	147/151	141/145	140/145	130/136
10		0/151	1/154	0/131	3/148	1/130
20		0/127	0/135	0/152	0/123	0/116
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	126/142	123/142	142/142	140/142	142/142
2.5		142/142	142/142	142/142	142/142	123/142
5		142/142	142/142	142/142	142/142	136/142
10		142/142	142/142	131/142	142/142	130/142
20		127/142	135/142	142/142	123/142	116/142
40		1/142	1/142	1/142	0/142	0/142

CETIS Analytical Report

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

Analysis ID: 11-3686-0042 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 08 Nov-19 11:47 **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	6.68%	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	-0.6185	2.227	0.132	8	0.9138	CDF	Non-Significant Effect
		5	-0.7774	2.227	0.132	8	0.9379	CDF	Non-Significant Effect
		10*	21.24	2.227	0.132	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.190468	2.063489	3	235.7	<0.0001	Significant Effect
Error	0.1400762	0.008754763	16			
Total	6.330544		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.288	11.34	0.1519	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9002	0.866	0.0415	Normal Distribution

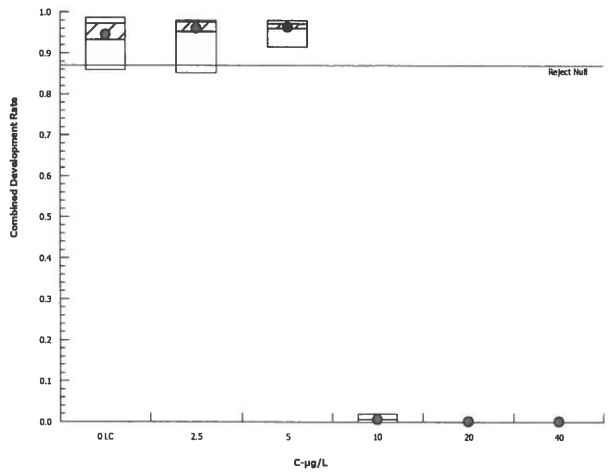
Combined Development Rate Summary

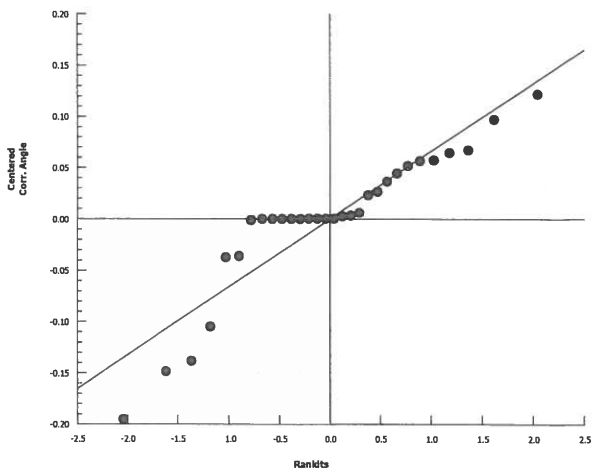
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.933	0.8529	1	0.9718	0.8592	0.9871	0.02885	6.91%	0.0%
2.5		5	0.9523	0.8827	1	0.9762	0.8521	0.98	0.02509	5.89%	-2.07%
5		5	0.9616	0.9289	0.9943	0.9724	0.9155	0.981	0.01178	2.74%	-3.06%
10		5	0.006761	0	0.01704	0.006494	0	0.02027	0.003702	122.4%	99.28%
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	1.335	1.17	1.499	1.402	1.186	1.457	0.05926	9.93%	0.0%
2.5		5	1.371	1.235	1.507	1.416	1.176	1.429	0.04897	7.99%	-2.74%
5		5	1.381	1.305	1.457	1.404	1.276	1.433	0.02734	4.43%	-3.45%
10		5	0.07804	0.02633	0.1298	0.08067	0.0407	0.1429	0.01863	53.37%	94.15%
20		5	0.04169	0.04091	0.04247	0.04197	0.04057	0.04197	0.000281	1.51%	96.88%
40		5	0.04197	0.04196	0.04198	0.04197	0.04197	0.04197	0	0.0%	96.86%

Graphics





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

Analysis ID: 18-3591-5974 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 08 Nov-19 11:47 **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	1.11%	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	1.251	2.227	0.040	8	0.2419	CDF	Non-Significant Effect
		5*	2.763	2.227	0.040	8	0.0179	CDF	Significant Effect
		10*	75.78	2.227	0.040	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.779801	2.259934	3	2773	<0.0001	Significant Effect
Error	0.0130385	0.0008149062	16			
Total	6.79284		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.105	11.34	0.2503	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9475	0.866	0.3310	Normal Distribution

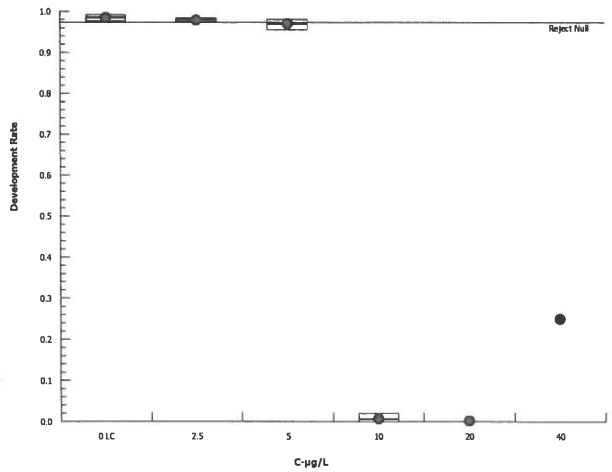
Development Rate Summary

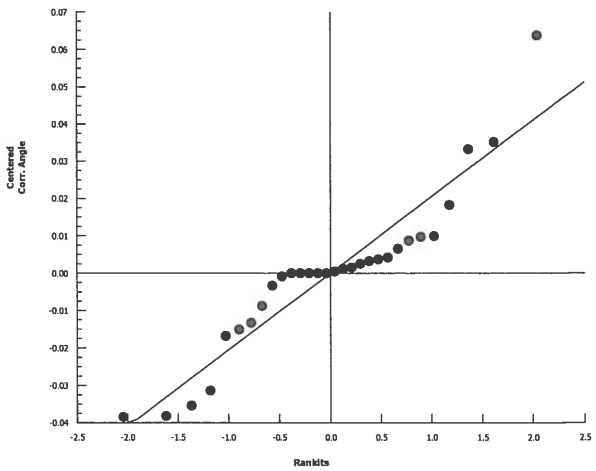
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9844	0.9769	0.9918	0.9857	0.9762	0.9919	0.002688	0.61%	0.0%
2.5		5	0.9787	0.9739	0.9835	0.9797	0.9737	0.9837	0.001727	0.39%	0.58%
5		5	0.9697	0.9579	0.9814	0.9724	0.9559	0.981	0.004233	0.98%	1.49%
10		5	0.006891	0	0.01718	0.006494	0	0.02027	0.003707	120.3%	99.3%
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	1.447	1.417	1.478	1.451	1.416	1.481	0.01102	1.7%	0.0%
2.5		5	1.425	1.408	1.441	1.428	1.408	1.443	0.006009	0.94%	1.56%
5		5	1.397	1.363	1.432	1.404	1.359	1.433	0.01228	1.97%	3.45%
10		5	0.07915	0.02769	0.1306	0.08067	0.0407	0.1429	0.01854	52.37%	94.53%
20		5	0.04391	0.04113	0.04668	0.04438	0.04057	0.04644	0.000999	5.09%	96.97%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	63.82%

Graphics





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

Analysis ID: 10-2770-1957 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 08 Nov-19 11:47 **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	8.59%	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	-0.9374	2.362	0.190	8	0.9802	CDF	Non-Significant Effect
		5	-1.353	2.362	0.190	8	0.9941	CDF	Non-Significant Effect
		10	-0.5385	2.362	0.190	8	0.9452	CDF	Non-Significant Effect
		20	1.227	2.362	0.190	8	0.3235	CDF	Non-Significant Effect
		40*	16.39	2.362	0.190	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.67212	1.534424	5	94.69	<0.0001	Significant Effect
Error	0.3889071	0.01620446	24			
Total	8.061027		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	11.59	15.09	0.0409	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9467	0.9031	0.1374	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	0.9479	0.8664	1	0.9859	0.8662	1	0.02934	6.92%	0.0%
2.5		5	0.9732	0.8989	1	1	0.8662	1	0.02676	6.15%	-2.68%
5		5	0.9915	0.9681	1	1	0.9577	1	0.008451	1.91%	-4.61%
10		5	0.9676	0.9124	1	1	0.9155	1	0.01987	4.59%	-2.08%
20		5	0.9056	0.8168	0.9945	0.8944	0.8169	1	0.03201	7.9%	4.46%
40		5	0.004225	0	0.009015	0.007042	0	0.007042	0.001725	91.29%	99.55%

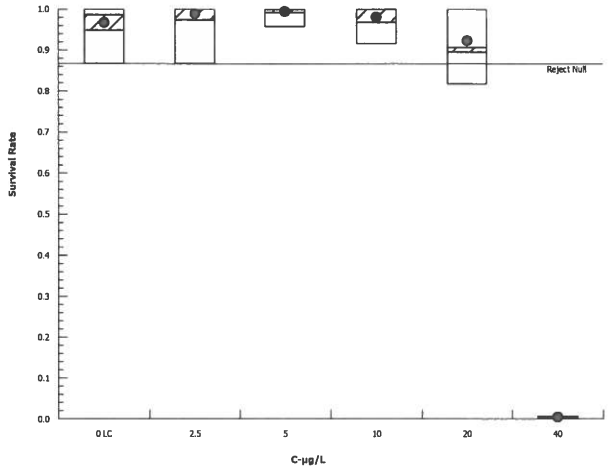
Angular (Corrected) Transformed Summary

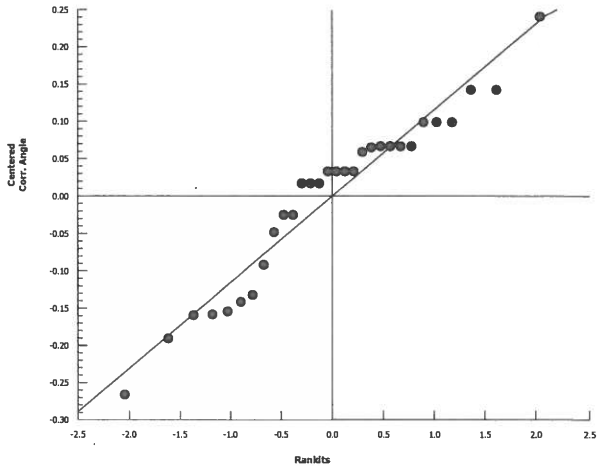
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.387	1.185	1.589	1.452	1.196	1.529	0.07277	11.73%	0.0%
2.5		5	1.462	1.278	1.647	1.529	1.196	1.529	0.0665	10.17%	-5.44%
5		5	1.496	1.404	1.587	1.529	1.364	1.529	0.03301	4.94%	-7.86%
10		5	1.43	1.262	1.598	1.529	1.276	1.529	0.06042	9.45%	-3.13%
20		5	1.288	1.094	1.482	1.24	1.129	1.529	0.06986	12.13%	7.12%
40		5	0.0672	0.0386	0.09579	0.08402	0.04197	0.08402	0.0103	34.27%	95.15%

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

Analysis ID:	10-2770-1957	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	08 Nov-19 11:47	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	11-8079-0492	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	08 Nov-19 11:47	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	450191	1000	Yes	Two-Point Interpolation

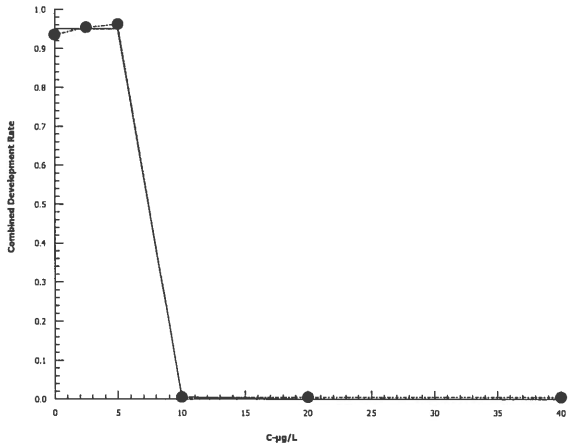
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.259	6.084	6.273
EC50	7.518	7.405	7.545

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	0.933	0.8592	0.9871	0.02885	0.06451	6.91%	0.0%	690	738
2.5		5	0.9523	0.8521	0.98	0.02509	0.05609	5.89%	-2.07%	725	760
5		5	0.9616	0.9155	0.981	0.01178	0.02635	2.74%	-3.06%	713	741
10		5	0.006761	0	0.02027	0.003702	0.008278	122.4%	99.28%	4	737
20		5	0	0	0	0	0		100.0%	0	720
40		5	0	0	0	0	0		100.0%	0	710

Graphics



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

Analysis ID:	15-7183-3565	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	08 Nov-19 11:48	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	567986	1000	Yes	Two-Point Interpolation

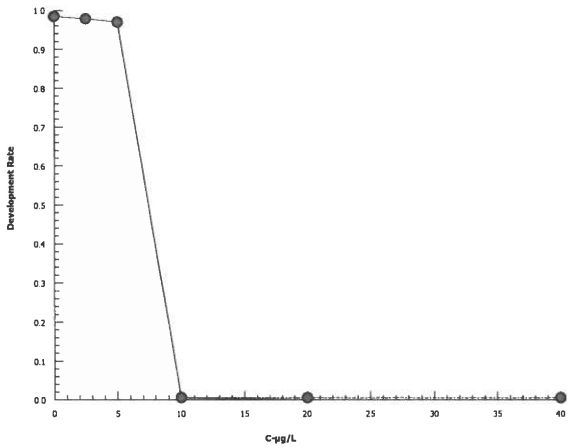
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.204	6.15	6.254
EC50	7.481	7.439	7.522

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	0.9844	0.9762	0.9919	0.002688	0.00601	0.61%	0.0%	690	701
2.5		5	0.9787	0.9737	0.9837	0.001727	0.003861	0.39%	0.58%	725	741
5		5	0.9697	0.9559	0.981	0.004233	0.009464	0.98%	1.49%	713	735
10		5	0.006891	0	0.02027	0.003707	0.008288	120.3%	99.3%	4	714
20		5	0	0	0	0	0		100.0%	0	653
40		5	0	0	0	0	0		100.0%	0	5

Graphics



Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	20-5233-5110	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	08 Nov-19 11:47	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1995347	1000	Yes	Two-Point Interpolation

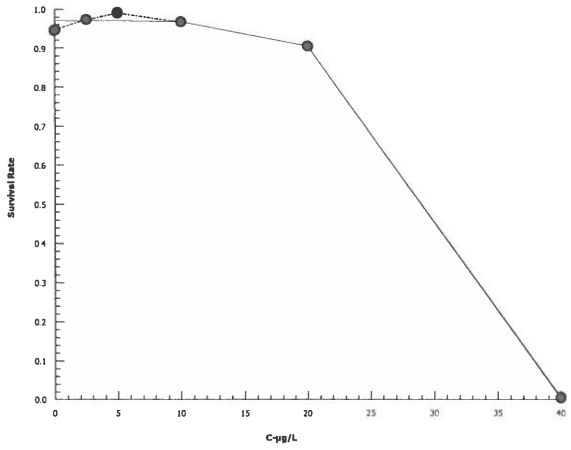
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	23.94	22.13	25.33
EC50	29.32	28.14	30.24

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	0.9479	0.8662	1	0.02934	0.06561	6.92%	0.0%	672	710
2.5		5	0.9732	0.8662	1	0.02676	0.05984	6.15%	-2.68%	691	710
5		5	0.9915	0.9577	1	0.008451	0.0189	1.91%	-4.61%	704	710
10		5	0.9676	0.9155	1	0.01987	0.04443	4.59%	-2.08%	687	710
20		5	0.9056	0.8169	1	0.03201	0.07158	7.9%	4.46%	643	710
40		5	0.004225	0	0.007042	0.001725	0.003857	91.29%	99.55%	3	710

Graphics

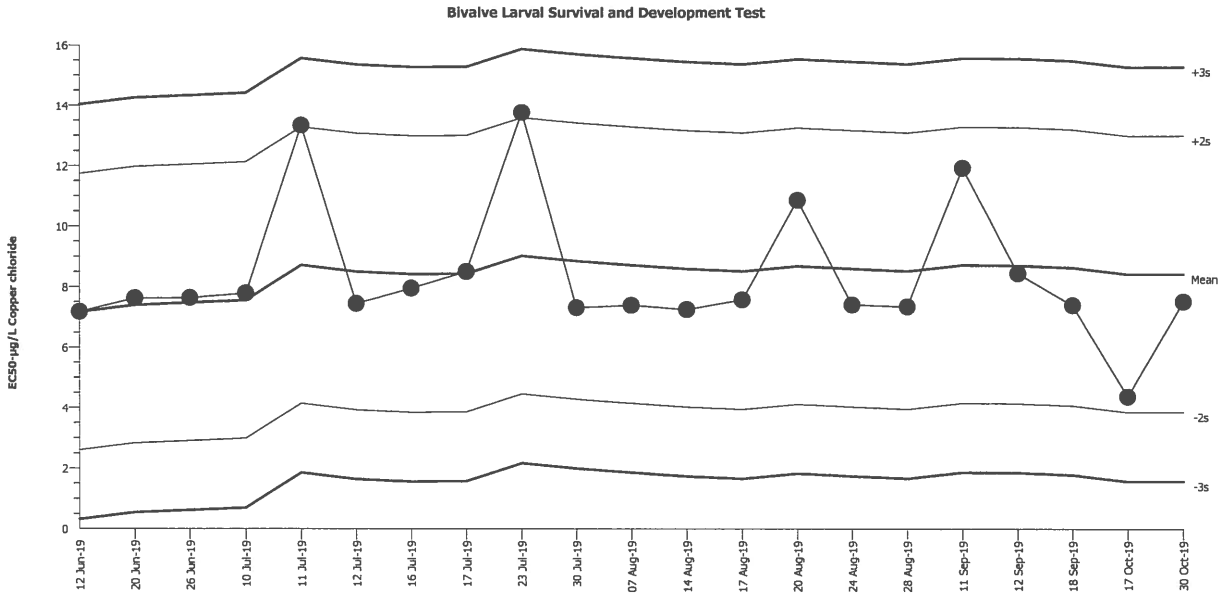


CETIS QC Plot

Report Date: 08 Nov-19 11:49 (1 of 1)

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

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



Mean:	8.426	**Count:**	20	**-2s Warning Limit:**	3.854	**-3s Action Limit:**	1.568
Sigma:	2.286	**CV:**	27.10%	**+2s Warning Limit:**	13	**+3s Action Limit:**	15.28

Quality Control Data

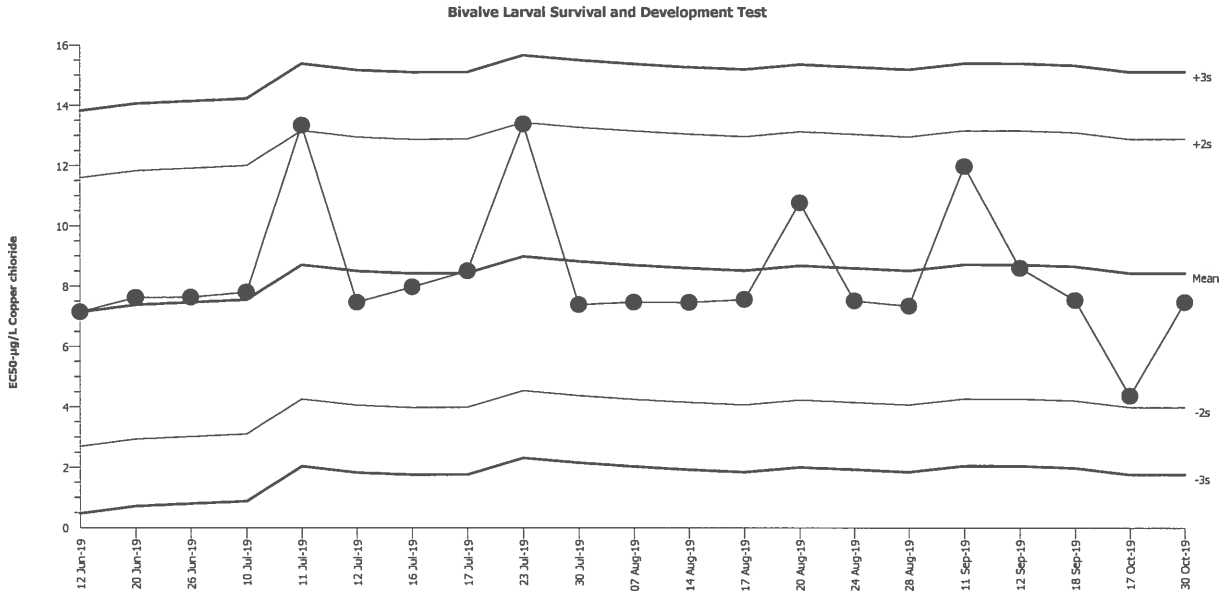
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jun	12	15:00	7.174	-1.252	-0.5477			20-8735-2782	03-4367-9827
2			20	15:00	7.627	-0.799	-0.3495			00-4624-1892	07-7845-5970
3			26	15:55	7.636	-0.7897	-0.3454			00-8415-2643	12-3790-3484
4		Jul	10	15:15	7.786	-0.6401	-0.28			02-0190-4206	08-2094-6054
5			11	14:35	13.33	4.903	2.145	(+)		05-8111-1120	10-3716-8433
6			12	14:45	7.443	-0.983	-0.43			01-0237-5581	01-3582-7031
7			16	14:35	7.951	-0.4749	-0.2077			04-6285-8375	08-7682-6614
8			17	14:50	8.497	0.0712	0.03115			04-5072-3133	00-7236-3161
9			23	14:30	13.76	5.335	2.334	(+)		07-6771-8781	18-1893-5656
10			30	15:30	7.313	-1.113	-0.4869			15-3542-8276	10-4430-8659
11		Aug	7	15:30	7.395	-1.031	-0.4512			01-2834-9487	15-5629-3220
12			14	14:15	7.255	-1.171	-0.5122			18-5609-6564	17-5885-5207
13			17	14:00	7.582	-0.8441	-0.3692			15-9584-4385	11-8998-1524
14			20	14:15	10.86	2.436	1.065			14-8361-1578	03-1832-9380
15			24	16:00	7.414	-1.012	-0.4425			19-4374-5817	01-6546-9581
16			28	14:30	7.348	-1.078	-0.4716			01-0546-0046	21-3090-7111
17		Sep	11	14:30	11.93	3.509	1.535			09-2717-2159	04-2480-9094
18			12	14:25	8.444	0.01845	0.00807			19-6218-6352	07-5188-6358
19			18	13:20	7.4	-1.026	-0.4488			10-9359-1611	21-3838-7021
20		Oct	17	12:30	4.368	-4.058	-1.775			01-8239-7270	07-0806-0577
21			30	12:30	7.518	-0.908	-0.3972			07-8198-2858	11-8079-0492

CETIS QC Plot

Report Date: 08 Nov-19 11:49 (1 of 1)

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

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



Mean:	8.446	**Count:**	20	**-2s Warning Limit:**	3.996	**-3s Action Limit:**	1.771
Sigma:	2.225	**CV:**	26.30%	**+2s Warning Limit:**	12.9	**+3s Action Limit:**	15.12

Quality Control Data

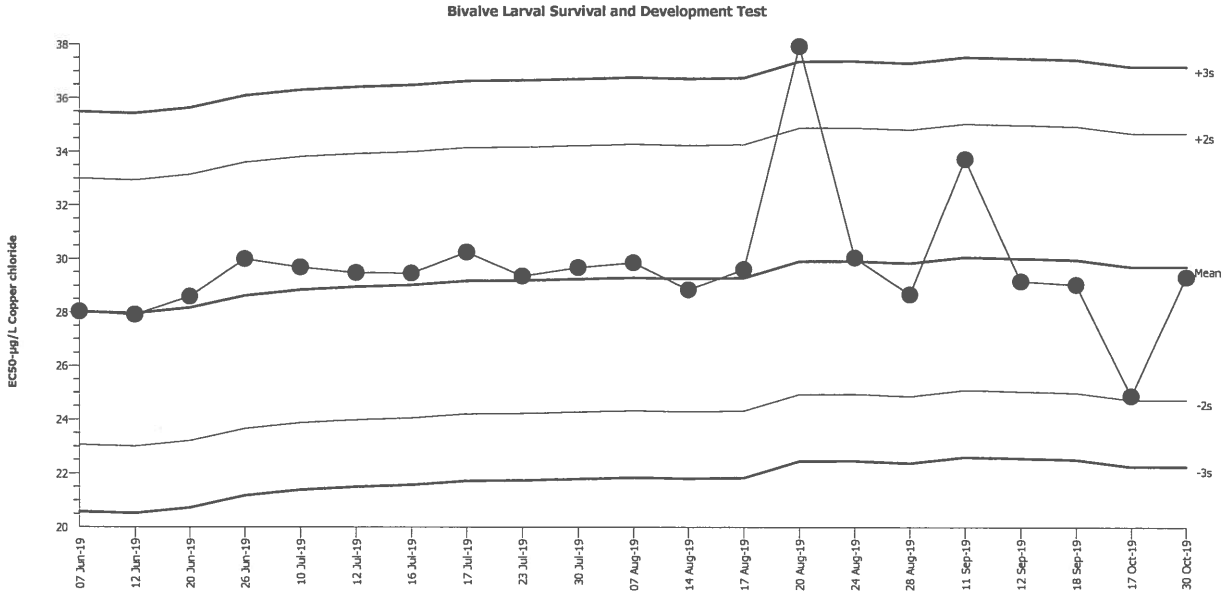
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jun	12	15:00	7.147	-1.299	-0.584			20-8735-2782	10-3598-4911
2			20	15:00	7.627	-0.8187	-0.368			00-4624-1892	15-3942-3527
3			26	15:55	7.636	-0.8098	-0.364			00-8415-2643	06-7718-5278
4		Jul	10	15:15	7.8	-0.6465	-0.2905			02-0190-4206	02-8661-6124
5			11	14:35	13.34	4.894	2.199	(+)		05-8111-1120	03-7465-8007
6			12	14:45	7.467	-0.979	-0.44			01-0237-5581	09-6402-3981
7			16	14:35	7.977	-0.4691	-0.2108			04-6285-8375	01-4279-2596
8			17	14:50	8.504	0.05786	0.026			04-5072-3133	09-0911-7730
9			23	14:30	13.38	4.935	2.218	(+)		07-6771-8781	07-7153-3575
10			30	15:30	7.388	-1.058	-0.4755			15-3542-8276	07-3589-9194
11		Aug	7	15:30	7.473	-0.973	-0.4373			01-2834-9487	19-8086-2685
12			14	14:15	7.466	-0.9801	-0.4405			18-5609-6564	14-6389-5644
13			17	14:00	7.563	-0.8826	-0.3967			15-9584-4385	19-0402-2449
14			20	14:15	10.76	2.318	1.042			14-8361-1578	12-0750-5104
15			24	16:00	7.521	-0.9252	-0.4158			19-4374-5817	04-6745-5945
16			28	14:30	7.351	-1.095	-0.4922			01-0546-0046	10-3410-8075
17		Sep	11	14:30	11.98	3.537	1.589			09-2717-2159	17-4622-9429
18			12	14:25	8.608	0.1616	0.07263			19-6218-6352	06-5225-4823
19			18	13:20	7.546	-0.9003	-0.4046			10-9359-1611	16-7089-5314
20		Oct	17	12:30	4.375	-4.071	-1.83			01-8239-7270	19-1864-9270
21			30	12:30	7.481	-0.9648	-0.4336			07-8198-2858	15-7183-3565

CETIS QC Plot

Report Date: 08 Nov-19 11:49 (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:	29.71	**Count:**	20	**-2s Warning Limit:**	24.74	**-3s Action Limit:**	22.25
Sigma:	2.485	**CV:**	8.36%	**+2s Warning Limit:**	34.68	**+3s Action Limit:**	37.16

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jun	7	13:35	28.03	-1.682	-0.6769			16-0229-2669	01-4873-8064
2			12	15:00	27.92	-1.795	-0.7223			20-8735-2782	03-5721-7619
3			20	15:00	28.59	-1.119	-0.4503			00-4624-1892	18-2643-8450
4			26	15:55	30	0.29	0.1167			00-8415-2643	15-8975-2294
5		Jul	10	15:15	29.69	-0.02142	-0.00862			02-0190-4206	18-9463-9841
6			12	14:45	29.48	-0.2291	-0.0922			01-0237-5581	14-9229-9767
7			16	14:35	29.46	-0.2465	-0.09921			04-6285-8375	17-5419-9497
8			17	14:50	30.25	0.5379	0.2165			04-5072-3133	00-3161-9686
9			23	14:30	29.36	-0.3524	-0.1418			07-6771-8781	15-4437-4125
10			30	15:30	29.67	-0.03697	-0.01488			15-3542-8276	20-8453-4017
11		Aug	7	15:30	29.85	0.1449	0.05832			01-2834-9487	07-4855-2818
12			14	14:15	28.85	-0.8645	-0.3479			18-5609-6564	13-1367-1354
13			17	14:00	29.6	-0.1063	-0.04279			15-9584-4385	20-0172-5237
14			20	14:15	37.92	8.209	3.303	(+)	(+)	14-8361-1578	02-5800-6574
15			24	16:00	30.04	0.3274	0.1317			19-4374-5817	17-7461-0750
16			28	14:30	28.66	-1.045	-0.4207			01-0546-0046	13-4512-6481
17		Sep	11	14:30	33.71	4.005	1.612			09-2717-2159	01-1883-2964
18			12	14:25	29.16	-0.5459	-0.2197			19-6218-6352	02-6393-7831
19			18	13:20	29.04	-0.671	-0.27			10-9359-1611	04-3365-2341
20		Oct	17	12:30	24.88	-4.828	-1.943			01-8239-7270	13-2801-3685
21			30	12:30	29.32	-0.3871	-0.1558			07-8198-2858	20-5233-5110

CETIS Test Data Worksheet

Report Date: 26 Oct-19 16:33 (p 1 of 1)
Test Code: 07-8198-2858/191030msdv

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

Start Date: 30 Oct-19
End Date: 01 Nov-19
Sample Date: 30 Oct-19
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 191030msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			152	0	JCL 11/8/19
			2			123	0	
			3			150	147	
			4			145	141	
			5			127	0	
			6			130	1	
			7			151	0	
			8			148	145	
			9			1	0	most cells lysed
			10			155	153	
			11			0	0	cells lysed
			12			135	0	
			13			154	1	
			14			1	0	most cells lysed
			15			158	155	
			16			126	123	
			17			116	0	
			18			131	0	
			19			151	147	
			20			140	138	
			21			136	130	
			22			168	164	
			23			152	148	
			24			123	122	
			25			148	3	
			26			1	0	most cells lysed
			27			157	154	
			28			145	140	
			29			0	0	
			30			123	121	

CETIS Test Data Worksheet

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

Start Date: 30 Oct-19 | **Species:** Mytilus galloprovincialis | **Sample Code:** 191030msdv
End Date: 01 Nov-19 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 30 Oct-19 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	16					
0	LC	2	24					
0	LC	3	10			151	149	RT 11/1/19
0	LC	4	20					
0	LC	5	27					
2.5		1	3					
2.5		2	8					
2.5		3	22			159	157	RT
2.5		4	23					
2.5		5	30					
5		1	15					
5		2	19					
5		3	4			148	144	RT
5		4	28					
5		5	21					
10		1	7					
10		2	13					
10		3	18			136	0	RT
10		4	25					
10		5	6					
20		1	5					
20		2	12					
20		3	1			158	0	RT
20		4	2					
20		5	17					
40		1	14					
40		2	26					
40		3	9			0	0	RT
40		4	29					
40		5	11					

QC = B0

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 191030msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 10/30/2019 1230

End Date/Time: 11/1/2019 1130

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	31.8	31.7	31.3	15.3	14.8	14.7	8.4	8.6	~~9.3~~ 9.2 (R)	8.08	8.01	7.97
2.5	31.8	31.8	31.9	15.2	14.5	14.5	8.4	8.5	9.2	8.08	8.01	7.99
5	31.9	31.8	31.9	15.3	14.5	14.6	8.6	8.4	9.2	8.08	8.01	7.99
10	31.8	31.8	32.0	15.5	14.6	14.6	8.6	8.3	9.3	8.08	8.02	7.99
20	31.8	31.9	32.1	15.5	14.4	14.5	8.5	8.3	9.4	8.08	8.02	7.99
40	31.7	31.8	31.9	15.5	14.5	14.8	8.5	8.4	9.4	8.08	8.02	7.99

Technician Initials:

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

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

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs: (A) Q18 BO 11/1/19

QC Check: KS 11/8/19

Final Review: ACH 11/19

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / CuCl2
Test No.: 191030 msdV
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: Taylor Shellfish / 17B
Date Received: 8/14/19
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 10/30/2019 1230
End Date/Time: 11/1/2019 1130
Technician Initials: BO

Spawn Information

First Gamete Release Time: 0945

Sex	Number Spawning
Male	4+
Female	4

Gamete Selection

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

Egg Fertilization Time: 1040

Embryo Stock Selection

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

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted: 7, 5, 8, 7, 7 | 8, 6, 6, 6, 8

Mean: 6.8

Mean 6.8 X 50 = 340 embryos/ml

Initial Density: 340 = 1.13 (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	141	141	100	99.8
TØ B	154	154	100	
TØ C	130	130	100	
TØ D	150	150	100	
TØ E	133	134	99.2	
TØ F	142	143	99.3	
$\bar{X}$ =	142			

48-h QC: 140/143 = 97.9%

Comments:

QC Check: JS 11/8/19

Final Review: AC 11/11/19

Inland Silverside Acute Survival Test

CETIS Summary Report

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

Batch ID:	09-3320-6780	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	30 Oct-19 14:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	03 Nov-19 14:15	**Species:**	Menidia beryllina	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Indicators	**Age:**	12d

Sample ID:	07-0984-9504	**Code:**	191030mbra	**Client:**	Internal
Sample Date:	30 Oct-19	**Material:**	Copper chloride	**Project:**	
Receive Date:	30 Oct-19	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-9321-0468	96h Survival Rate	100	200	141.4	28.4%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
07-6888-5964	96h Survival Rate	EC50	114.9	99.66	132.4		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-6888-5964	96h Survival Rate	Control Resp	1	0.9 - NL	Yes	Passes Acceptability Criteria
10-9321-0468	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	0.7	0.1488	1	0.2	1	0.1732	0.3464	49.49%	30.0%
200		4	0	0	0	0	0	0	0		100.0%
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	0.2
200		0	0	0	0
400		0	0	0	0
800		0	0	0	0

CETIS Analytical Report

Test Code: 191030mbra | 05-0159-0485

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

Analysis ID:	10-9321-0468	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Nov-19 9:13	**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	28.4%	100	200	141.4	

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		50	18	11	1	6	0.6667	Asymp	Non-Significant Effect
		100	12	11	1	6	0.0738	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3073171	0.1536585	2	3.218	0.0882	Non-Significant Effect
Error	0.429721	0.04774678	9			
Total	0.7370381		11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	2.108	8.022	0.1774	Equal Variances
Variances	Levene Equality of Variance	6.494	8.022	0.0180	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.6789	0.8025	0.0005	Non-normal Distribution

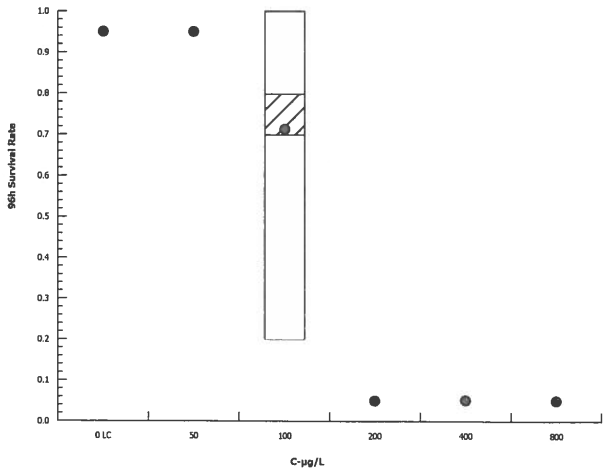
96h Survival Rate Summary

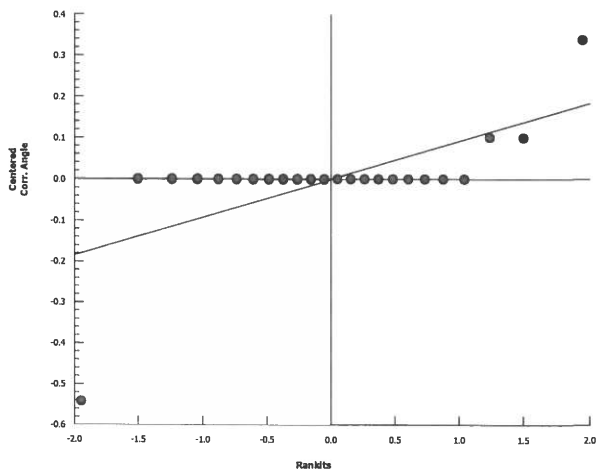
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	0.7	0.1488	1	0.8	0.2	1	0.1732	49.49%	30.0%
200		4	0	0	0	0	0	0	0		100.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

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	1.006	0.4036	1.608	1.107	0.4636	1.345	0.1892	37.63%	25.23%
200		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
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%

Graphics





Analyst: JCL QA: AC 11/1/19

CETIS Analytical Report

Test Code: 191030mbra | 05-0159-0485

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

Analysis ID:	07-6888-5964	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Nov-19 9:14	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

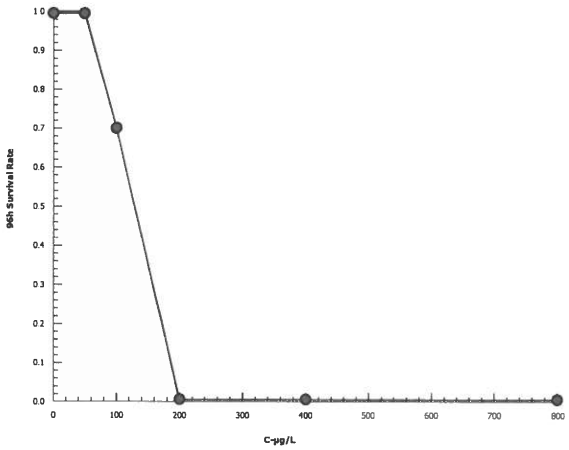
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.06	0.03085	114.9	99.66	132.4

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	0.7	0.2	1	0.1732	0.3464	49.49%	30.0%	14	20
200		4	0	0	0	0	0		100.0%	0	20
400		4	0	0	0	0	0		100.0%	0	20
800		4	0	0	0	0	0		100.0%	0	20

Graphics

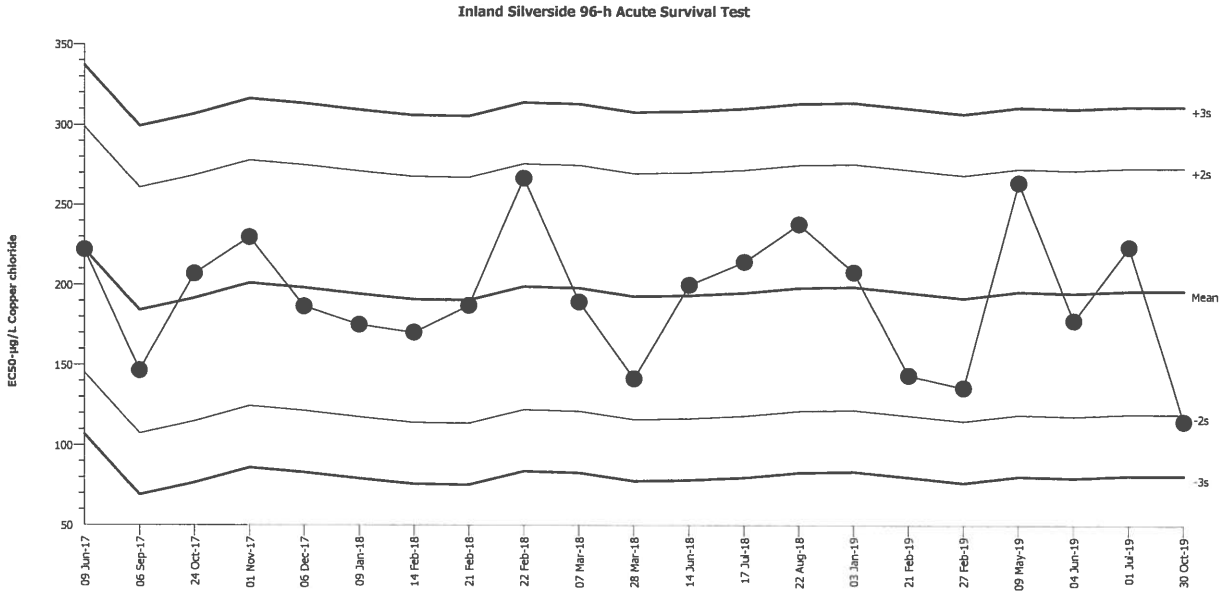


Analyst: JCL QA: AC 11/11/19

CETIS QC Plot

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:	196.3	**Count:**	20	**-2s Warning Limit:**	119.5	**-3s Action Limit:**	81.15
Sigma:	38.39	**CV:**	19.60%	**+2s Warning Limit:**	273.1	**+3s Action Limit:**	311.5

Quality Control Data

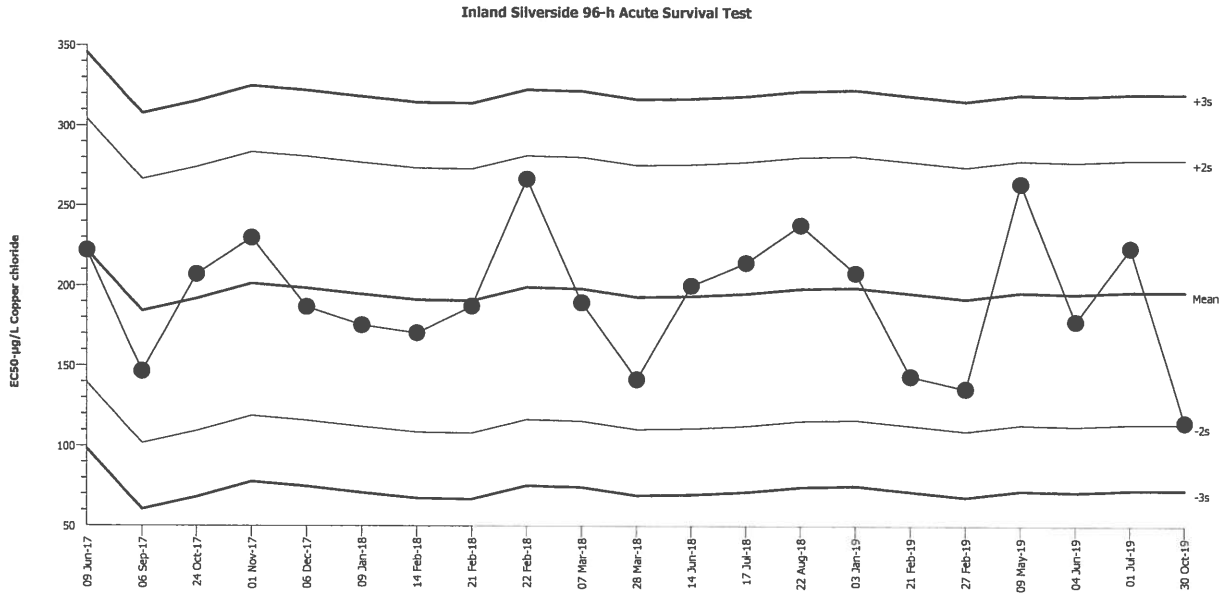
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jun	9	17:15	221.9	25.61	0.6672			04-5405-2533	13-3732-1084
2		Sep	6	15:50	146.4	-49.89	-1.3			01-8301-6131	10-0799-2130
3		Oct	24	16:10	207.1	10.75	0.2801			10-0714-4627	19-6697-7894
4		Nov	1	10:15	229.7	33.44	0.8711			14-0848-4500	09-3507-0741
5		Dec	6	15:25	186.6	-9.693	-0.2525			17-2716-0280	10-6923-1723
6	2018	Jan	9	16:05	175.2	-21.1	-0.5495			15-9782-4320	14-5127-3080
7		Feb	14	14:50	170.3	-26.03	-0.6779			14-7429-6310	14-6416-7425
8			21	12:25	187.2	-9.122	-0.2376			20-0148-6736	18-8740-2809
9			22	17:20	266.7	70.41	1.834			21-2244-9573	15-2512-9013
10		Mar	7	16:25	189.3	-6.95	-0.181			06-3891-7579	03-5981-6406
11			28	17:15	141.4	-54.88	-1.43			18-3798-9831	05-5342-2351
12		Jun	14	14:35	200	3.7	0.09638			01-9952-0614	00-3575-1747
13		Jul	17	14:30	214.4	18.05	0.4703			11-1445-3115	12-3693-5336
14		Aug	22	16:25	237.8	41.54	1.082			08-6172-7555	12-4329-0617
15	2019	Jan	3	16:50	207.9	11.55	0.3009			16-0506-4055	11-1190-1934
16		Feb	21	16:05	143.5	-52.82	-1.376			10-4228-2556	08-7111-9529
17			27	16:25	135.8	-60.53	-1.577			14-0947-0420	00-4247-8099
18		May	9	19:10	263.9	67.6	1.761			03-9779-6453	09-3747-7536
19		Jun	4	14:50	177.8	-18.55	-0.4831			00-2136-1210	01-4264-5145
20		Jul	1	15:55	223.6	27.32	0.7115			04-4319-5710	17-4098-1084
21		Oct	30	14:45	114.9	-81.43	-2.121	(-)		05-0159-0485	07-6888-5964

CETIS QC Plot

Report Date: 06 Nov-19 09:15 (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:	196.3	**Count:**	20	**-2s Warning Limit:**	113.9	**-3s Action Limit:**	72.65
Sigma:	41.22	**CV:**	21.00%	**+2s Warning Limit:**	278.8	**+3s Action Limit:**	320

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jun	9	17:15	221.9	25.61	0.6213			04-5405-2533	13-3732-1084
2		Sep	6	15:50	146.4	-49.89	-1.21			01-8301-6131	10-0799-2130
3		Oct	24	16:10	207.1	10.75	0.2608			10-0714-4627	19-6697-7894
4		Nov	1	10:15	229.7	33.44	0.8112			14-0848-4500	09-3507-0741
5		Dec	6	15:25	186.6	-9.693	-0.2351			17-2716-0280	10-6923-1723
6	2018	Jan	9	16:05	175.2	-21.1	-0.5118			15-9782-4320	14-5127-3080
7		Feb	14	14:50	170.3	-26.03	-0.6313			14-7429-6310	14-6416-7425
8			21	12:25	187.2	-9.122	-0.2213			20-0148-6736	18-8740-2809
9			22	17:20	266.7	70.41	1.708			21-2244-9573	15-2512-9013
10		Mar	7	16:25	189.3	-6.95	-0.1686			06-3891-7579	03-5981-6406
11			28	17:15	141.4	-54.88	-1.331			18-3798-9831	05-5342-2351
12		Jun	14	14:35	200	3.7	0.08976			01-9952-0614	00-3575-1747
13		Jul	17	14:30	214.4	18.05	0.438			11-1445-3115	12-3693-5336
14		Aug	22	16:25	237.8	41.54	1.008			08-6172-7555	12-4329-0617
15	2019	Jan	3	16:50	207.9	11.55	0.2802			16-0506-4055	11-1190-1934
16		Feb	21	16:05	143.5	-52.82	-1.281			10-4228-2556	08-7111-9529
17			27	16:25	135.8	-60.53	-1.468			14-0947-0420	00-4247-8099
18		May	9	19:10	263.9	67.6	1.64			03-9779-6453	09-3747-7536
19		Jun	4	14:50	177.8	-18.55	-0.4499			00-2136-1210	01-4264-5145
20		Jul	1	15:55	223.6	27.32	0.6626			04-4319-5710	17-4098-1084
21		Oct	30	14:45	114.9	-81.43	-1.975			05-0159-0485	07-6888-5964

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

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: 10/30/2019 1445
Test No.: 191030mbra
End Date/Time: 11/3/2019 1415
Cu stock concentration (µg/L): 99,600

	Tech Initials				
	0	24	48	72	96
Counts:	HH	RT	KL	RT	MES
Readings:	RT	HH	KL	RT	DM
Dilutions made by:	HH		TN		
High conc. made (µg/L):	800	–	100	–	–
Vol. Cu stock added (mL):	10	–	2.0	–	–
Final Volume (mL):	2000	–	2000	–	–

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	30.6	31.0	29.1	30.3	29.8	24.1	24.6	27.6	24.6	24.5	6.4	5.2	7.9	5.8	5.7	7.91	7.78	8.02	7.79	7.97
	10	5	5	5	5	5			31.4					28.0					5.7					7.76		
	5	5	5	5	5	5																				
	9	5	5	5	5	5																				
50	23	5	5	5	5	5	30.5	30.6	29.0	30.0	30.1	24.0	25.3	24.6	25.2	24.8	6.3	4.8	7.7	5.4	5.6	7.91	7.76	8.02	7.75	7.89
	18	5	5	5	5	5			30.8					25.3					5.4					7.75		
	3	5	5	5	5	5																				
	6	5	5	5	5	5																				
100	19	5	5	5	5	5	30.5	30.6	29.1	29.9	29.7	24.0	25.2	24.8	25.3	25.0	6.3	4.4	7.8	5.2	5.4	7.91	7.72	8.01	7.72	7.84
	14	5	4	4	4	4			30.7					25.3					5.1					7.71		
	2	5	4	4	4	4																				
	17	5	2	1	1	1																				
200	20	5	0	All dead			30.4	30.8	–	–	–	24.1	24.8	–	–	–	6.4	5.1	–	–	–	7.92	7.79	–	–	–
	11	5	0						–					–					–					–		
	7	5	0																							
	22	5	0																							
400	8	5	0	All dead			30.4	30.6	–	–	–	24.0	24.9	–	–	–	6.4	5.0	–	–	–	7.91	7.79	–	–	–
	15	5	0						–					–					–					–		
	1	5	0																							
	21	5	0																							
800	13	5	0	All dead			30.5	30.4	–	–	–	24.0	24.9	–	–	–	6.3	5.0	–	–	–	7.91	7.78	–	–	–
	16	5	0						–					–					–					–		
	24	5	0																							
	12	5	0																							

Rand # QC: RT
Initial Counts QC'd by: VM
Initiated by: HH

Environmental Chamber: A

Animal Source/Date Received: Aquatic Indicators 10/29/19
Age at Initiation: 12 days
Animal Acclimation Qualifiers (circle all that apply): Q22 Q23 Q24 none

Feeding Times					
	0	24	48	72	96
AM:		0900	0935	1000	0845
PM:	1715				

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) @ Q18/CL 11/1/19

QC Check: BO 11/11/19
Final Review: AC 11/11/19

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

Appendix E
List of Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

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

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

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

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

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

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

Q7 - Salinity out of recommended range

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

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

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

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

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

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

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

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

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

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

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

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

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