ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: July 2020

Prepared for: **Jacobs**
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Date Submitted: **August 18, 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, Project Manager

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Introduction

A toxicity test was performed using a groundwater composite sample collected from the Wyckoff Eagle Harbor Groundwater Treatment Plant on Bainbridge Island in Washington. This test was performed to satisfy quarterly monitoring requirements according to the project Quality Assurance Project Plan (QAPP 2013). The chronic bioassay was conducted using the bivalve *Mytilus galloprovincialis* (Mediterranean mussel). Testing was performed at Enthalpy Analytical located in San Diego, California.

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	**071420**
Enthalpy Log-in Number	20-0770
Collection Date; Time	7/14/2020; 0936h
Receipt Date; Time	7/15/2020; 0915h
Receipt Temperature (°C)	5.0
Dissolved Oxygen (mg/L)	7.4
pH	7.38
Conductivity (µS/cm)	14,120
Salinity (ppt)	8.8
Alkalinity (mg/L $CaCO_3$)	396
Total Chlorine (mg/L)	0.02
Total Ammonia (mg/L as N)	3.3

NM = not measured

Test Methods

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

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

Test Period	7/15/2020, 1355h to 7/17/2020, 1310h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	M-Rep (Carlsbad, CA)
Test Organism Age	4 hours post fertilization
Test Duration	48 ± 2 hours
Test Type	Static
Test Chamber, Test Solution Volume	30 mL glass vial, 10 mL
Test Temperature	15 ± 1°C
Dilution Water	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) 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 97.2 ppt
Test Concentrations (% sample)	76.0[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. See QA section.

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

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

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

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_c)	EC_{25} (% effluent)
Bivalve	Normal Development	76.0	> 76.0	< 1.32	> 76.0
	Survival	76.0	> 76.0	< 1.32	> 76.0

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

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

Table 4. Detailed Results for the Bivalve Development Chronic Toxicity Test

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	91.1	97.9
0 (Lab Control)	94.1	98.1
2	95.9	98.3
4	92.5	98.8
9	96.6	98.2
18	96.6	98.2
35	94.6	98.7
76.0[a]	91.6	97.4

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

Quality Assurance

The sample was received within the required 36-hour holding time, in good condition, and within the appropriate temperature range of 0-6°C. All control acceptability criteria were met and water quality parameters remained within the appropriate ranges throughout the test. Statistical analyses followed standard USEPA flowchart selections. Dose-response relationships were reviewed to ensure the reliability of the results. Based on the dose response observed, the calculated effects concentrations were deemed reliable. 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 5. A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride rather than copper sulfate. The results for the concurrent reference toxicant test were within the acceptable range of the mean historical test results plus or minus two standard deviations for development. The EC_{50} for survival was greater than the highest concentration tested; indicating organisms may have been less sensitive than typical for the survival endpoint. Reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 5. Reference Toxicant Test Results

Species and Endpoint	**NOEC** (%)	**EC_{50}** (µg/L copper)	**Historical mean ± 2 SD** (µg/L copper)	**CV** (%)
Bivalve Normal Development	5	13.9	9.03 ± 6.47	35.8
Bivalve Survival Rate	20	> 40.0	29.7 ± 5.08	8.56

NOEC = No Observed Effect Concentration

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

CV = Coefficient of Variation

References

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

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

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

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

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

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

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

Batch ID:	16-0484-7554	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	15 Jul-20 13:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	17 Jul-20 13:10	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	02-9423-5209	**Code:**	20-0770	**Client:**	Jacobs
Sample Date:	14 Jul-20 09:36	**Material:**	Effluent Sample	**Project:**	
Receive Date:	15 Jul-20 09:15	**Source:**	Jacobs		
Sample Age:	28h (5 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-4229-2286	Development Rate	76	>76	NA	2.0%	1.316	Dunnett Multiple Comparison Test
11-2108-7541	Survival Rate	76	>76	NA	13.7%	1.316	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
02-6789-9030	Development Rate	EC25	>76	N/A	N/A	<1.316	Linear Interpolation (ICPIN)
		EC50	>76	N/A	N/A	<1.316	
17-4635-1121	Survival Rate	EC25	>76	N/A	N/A	<1.316	Linear Interpolation (ICPIN)
		EC50	>76	N/A	N/A	<1.316	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-6789-9030	Development Rate	Control Resp	0.9788	0.9 - NL	Yes	Passes Acceptability Criteria
03-4229-2286	Development Rate	Control Resp	0.9788	0.9 - NL	Yes	Passes Acceptability Criteria
11-2108-7541	Survival Rate	Control Resp	0.9107	0.5 - NL	Yes	Passes Acceptability Criteria
17-4635-1121	Survival Rate	Control Resp	0.9107	0.5 - NL	Yes	Passes Acceptability Criteria

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9788	0.9637	0.9939	0.971	1	0.005439	0.01216	1.24%	0.0%
0	Lab Control	5	0.981	0.9677	0.9943	0.9636	0.9923	0.004781	0.01069	1.09%	-0.23%
2		5	0.9832	0.9764	0.99	0.9797	0.9929	0.002448	0.005475	0.56%	-0.45%
4		5	0.9878	0.9739	1	0.9732	1	0.005017	0.01122	1.14%	-0.92%
9		5	0.9819	0.9726	0.9911	0.9745	0.9942	0.003331	0.007449	0.76%	-0.32%
18		5	0.9821	0.9718	0.9924	0.9739	0.994	0.003707	0.00829	0.84%	-0.34%
35		5	0.9871	0.977	0.9971	0.9789	1	0.00362	0.008095	0.82%	-0.85%
76		5	0.9742	0.9539	0.9944	0.9551	0.9865	0.007305	0.01633	1.68%	0.47%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9107	0.8308	0.9906	0.8491	1	0.02878	0.06435	7.07%	0.0%
0	Lab Control	5	0.9409	0.8426	1	0.8176	1	0.03539	0.07913	8.41%	-3.32%
2		5	0.9585	0.8986	1	0.8868	1	0.02157	0.04823	5.03%	-5.25%
4		5	0.9245	0.8664	0.9827	0.8805	1	0.02095	0.04685	5.07%	-1.52%
9		5	0.966	0.8929	1	0.8616	1	0.02636	0.05893	6.1%	-6.08%
18		5	0.966	0.9077	1	0.8868	1	0.02101	0.04698	4.86%	-6.08%
35		5	0.9459	0.8587	1	0.8491	1	0.03142	0.07026	7.43%	-3.87%
76		5	0.9157	0.8566	0.9748	0.8553	0.9811	0.02129	0.04761	5.2%	-0.55%

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	0.9778	0.971	0.9714	0.9737	1
0	Lab Control	0.9636	0.9923	0.9861	0.9808	0.9822
2		0.9809	0.9809	0.9815	0.9797	0.9929
4		0.9938	1	0.979	0.9732	0.9931
9		0.9817	0.9808	0.9781	0.9745	0.9942
18		0.9872	0.994	0.9767	0.9787	0.9739
35		0.9818	0.9874	0.9789	1	0.9873
76		0.9574	0.9551	0.9865	0.9853	0.9864

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.8491	0.8679	0.8805	0.956	1
0	Lab Control	1	0.8176	0.9057	0.9811	1
2		0.9874	0.9874	1	0.9308	0.8868
4		1	0.8805	0.8994	0.9371	0.9057
9		1	0.9811	0.8616	0.9874	1
18		0.9811	1	1	0.8868	0.9623
35		1	1	0.8931	0.8491	0.9874
76		0.8868	0.9811	0.9308	0.8553	0.9245

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	132/135	134/138	136/140	148/152	172/172
0	Lab Control	159/165	129/130	142/144	153/156	166/169
2		154/157	154/157	159/162	145/148	140/141
4		159/160	140/140	140/143	145/149	143/144
9		161/164	153/156	134/137	153/157	172/173
18		154/156	165/166	168/172	138/141	149/153
35		162/165	157/159	139/142	135/135	155/157
76		135/141	149/156	146/148	134/136	145/147

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	135/159	138/159	140/159	152/159	159/159
0	Lab Control	159/159	130/159	144/159	156/159	159/159
2		157/159	157/159	159/159	148/159	141/159
4		159/159	140/159	143/159	149/159	144/159
9		159/159	156/159	137/159	157/159	159/159
18		156/159	159/159	159/159	141/159	153/159
35		159/159	159/159	142/159	135/159	157/159
76		141/159	156/159	148/159	136/159	147/159

CETIS Analytical Report

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

Analysis ID:	03-4229-2286	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Aug-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	2.0%	76	>76	NA	1.316

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-0.3739	2.407	0.065	8	0.9351	CDF	Non-Significant Effect
		4	-1.307	2.407	0.065	8	0.9952	CDF	Non-Significant Effect
		9	-0.2358	2.407	0.065	8	0.9115	CDF	Non-Significant Effect
		18	-0.2851	2.407	0.065	8	0.9206	CDF	Non-Significant Effect
		35	-1.049	2.407	0.065	8	0.9894	CDF	Non-Significant Effect
		76	0.6249	2.407	0.065	8	0.6256	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.009122896	0.001520483	6	0.83	0.5568	Non-Significant Effect
Error	0.05129094	0.001831819	28			
Total	0.06041384		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.499	16.81	0.7441	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9487	0.9146	0.1032	Normal Distribution

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9788	0.9637	0.9939	0.9737	0.971	1	0.005439	1.24%	0.0%
2		5	0.9832	0.9764	0.99	0.9809	0.9797	0.9929	0.002448	0.56%	-0.45%
4		5	0.9878	0.9739	1	0.9931	0.9732	1	0.005017	1.14%	-0.92%
9		5	0.9819	0.9726	0.9911	0.9808	0.9745	0.9942	0.003331	0.76%	-0.32%
18		5	0.9821	0.9718	0.9924	0.9787	0.9739	0.994	0.003708	0.84%	-0.34%
35		5	0.9871	0.977	0.9971	0.9873	0.9789	1	0.00362	0.82%	-0.85%
76		5	0.9742	0.9539	0.9944	0.9853	0.9551	0.9865	0.007305	1.68%	0.47%

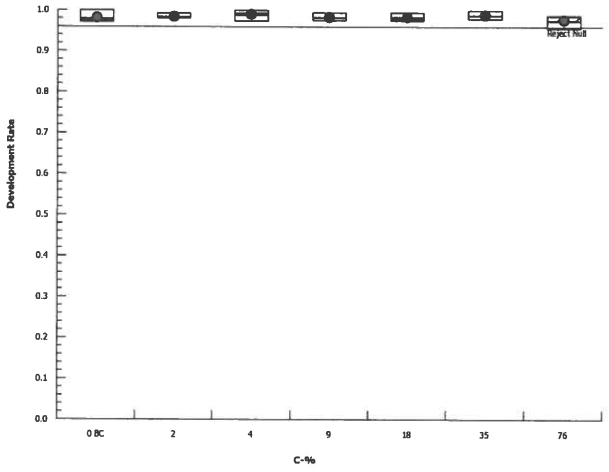
Angular (Corrected) Transformed Summary

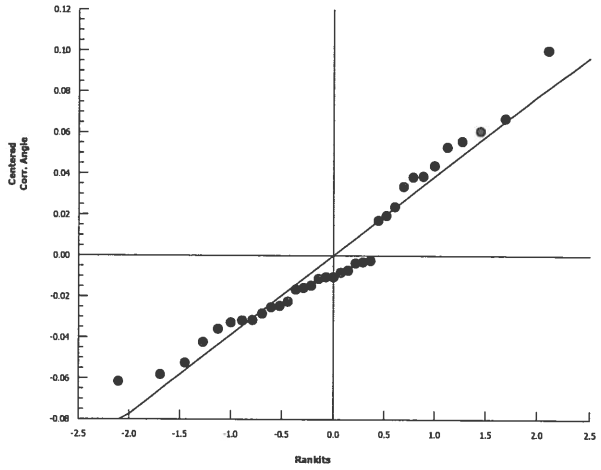
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.432	1.362	1.503	1.408	1.4	1.533	0.02534	3.96%	0.0%
2		5	1.443	1.412	1.473	1.432	1.428	1.486	0.01102	1.71%	-0.71%
4		5	1.468	1.405	1.531	1.487	1.406	1.529	0.02261	3.45%	-2.47%
9		5	1.439	1.398	1.479	1.432	1.41	1.495	0.0146	2.27%	-0.45%
18		5	1.44	1.397	1.483	1.424	1.408	1.493	0.01559	2.42%	-0.54%
35		5	1.461	1.411	1.511	1.458	1.425	1.528	0.01792	2.74%	-1.98%
76		5	1.416	1.353	1.478	1.449	1.357	1.454	0.02264	3.58%	1.18%

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

Analysis ID:	03-4229-2286	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Aug-20 12:10	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 11-2108-7541 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Aug-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	13.7%	76	>76	NA	1.316

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-1.173	2.407	0.206	8	0.9927	CDF	Non-Significant Effect
		4	-0.2196	2.407	0.206	8	0.9084	CDF	Non-Significant Effect
		9	-1.554	2.407	0.206	8	0.9979	CDF	Non-Significant Effect
		18	-1.449	2.407	0.206	8	0.9970	CDF	Non-Significant Effect
		35	-1.056	2.407	0.206	8	0.9896	CDF	Non-Significant Effect
		76	0.09497	2.407	0.206	8	0.8299	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1082672	0.01804453	6	0.9878	0.4523	Non-Significant Effect
Error	0.5115049	0.01826803	28			
Total	0.6197721		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.355	16.81	0.9685	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9777	0.9146	0.6838	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	0.9107	0.8308	0.9906	0.8805	0.8491	1	0.02878	7.07%	0.0%
2		5	0.9585	0.8986	1	0.9874	0.8868	1	0.02157	5.03%	-5.25%
4		5	0.9245	0.8664	0.9827	0.9057	0.8805	1	0.02095	5.07%	-1.52%
9		5	0.966	0.8929	1	0.9874	0.8616	1	0.02636	6.1%	-6.08%
18		5	0.966	0.9077	1	0.9811	0.8868	1	0.02101	4.86%	-6.08%
35		5	0.9459	0.8587	1	0.9874	0.8491	1	0.03142	7.43%	-3.87%
76		5	0.9157	0.8566	0.9748	0.9245	0.8553	0.9811	0.02129	5.2%	-0.55%

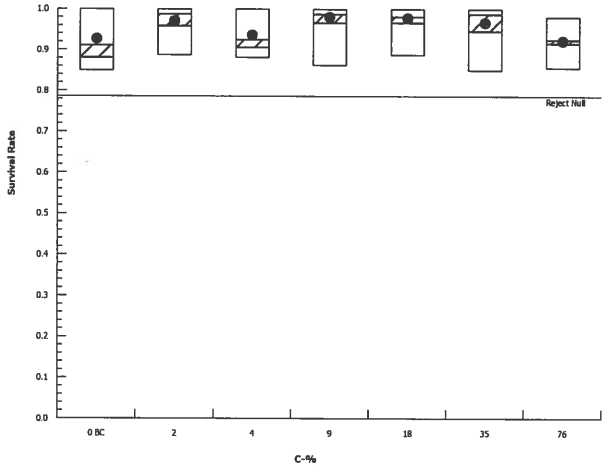
Angular (Corrected) Transformed Summary

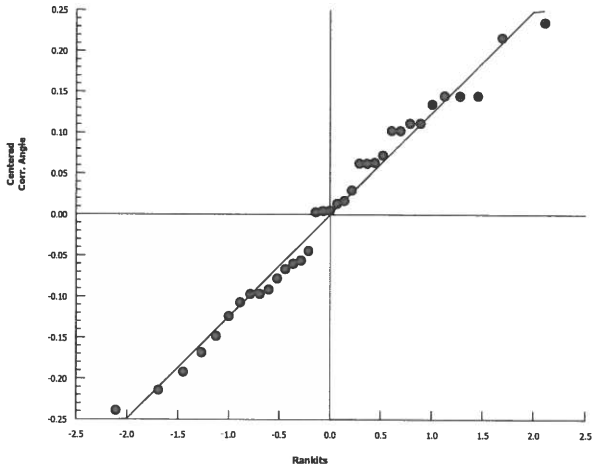
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.296	1.109	1.482	1.218	1.172	1.531	0.06719	11.59%	0.0%
2		5	1.396	1.241	1.552	1.458	1.228	1.531	0.05601	8.97%	-7.74%
4		5	1.315	1.158	1.471	1.259	1.218	1.531	0.05649	9.61%	-1.45%
9		5	1.429	1.254	1.603	1.458	1.19	1.531	0.06286	9.84%	-10.25%
18		5	1.42	1.263	1.577	1.433	1.228	1.531	0.05651	8.9%	-9.56%
35		5	1.386	1.175	1.597	1.458	1.172	1.531	0.07591	12.25%	-6.96%
76		5	1.288	1.169	1.406	1.292	1.181	1.433	0.04272	7.42%	0.63%

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

Analysis ID:	11-2108-7541	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Aug-20 12:10	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	02-6789-9030	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Aug-20 8:35	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1766866	1000	Yes	Two-Point Interpolation

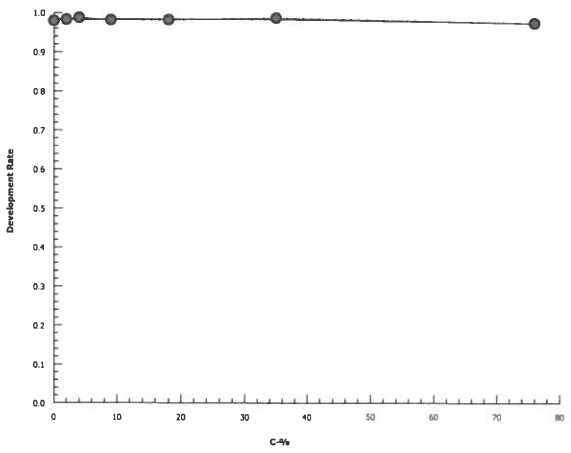
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>76	N/A	N/A	<1.316	NA	NA
EC50	>76	N/A	N/A	<1.316	NA	NA

Development Rate Summary — **Calculated Variate(A/B)**

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.9788	0.971	1	0.005439	0.01216	1.24%	0.0%	722	737
2		5	0.9832	0.9797	0.9929	0.002448	0.005474	0.56%	-0.45%	752	765
4		5	0.9878	0.9732	1	0.005017	0.01122	1.14%	-0.92%	727	736
9		5	0.9819	0.9745	0.9942	0.003331	0.007449	0.76%	-0.32%	773	787
18		5	0.9821	0.9739	0.994	0.003708	0.00829	0.84%	-0.34%	774	788
35		5	0.9871	0.9789	1	0.00362	0.008095	0.82%	-0.85%	748	758
76		5	0.9742	0.9551	0.9865	0.007305	0.01633	1.68%	0.47%	709	728

Graphics



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

Analysis ID:	17-4635-1121	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	17 Aug-20 8:36	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	772937	1000	Yes	Two-Point Interpolation

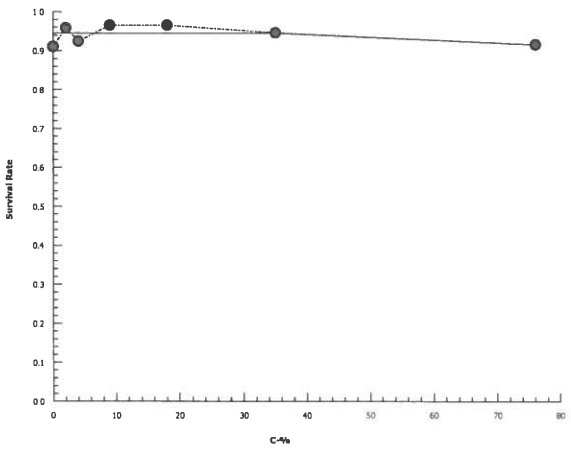
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>76	N/A	N/A	<1.316	NA	NA
EC50	>76	N/A	N/A	<1.316	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	0.9107	0.8491	1	0.02878	0.06435	7.07%	0.0%	724	795
2		5	0.9585	0.8868	1	0.02157	0.04823	5.03%	-5.25%	762	795
4		5	0.9245	0.8805	1	0.02095	0.04685	5.07%	-1.52%	735	795
9		5	0.966	0.8616	1	0.02636	0.05893	6.1%	-6.08%	768	795
18		5	0.966	0.8868	1	0.02101	0.04698	4.86%	-6.08%	768	795
35		5	0.9459	0.8491	1	0.03142	0.07026	7.43%	-3.87%	752	795
76		5	0.9157	0.8553	0.9811	0.02129	0.04761	5.2%	-0.55%	728	795

Graphics



CETIS Test Data Worksheet

Report Date: 11 Jul-20 17:57 (p 1 of 1)
Test Code: 2007-5059 11-6319-6188/4554F71C

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

Start Date:	15 Jul-20	**Species:**	Mytilus galloprovincialis	**Sample Code:**	20-0770
End Date:	17 Jul-20	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs
Sample Date:	14 Jul-20	**Material:**	Effluent Sample	**Sample Station:**	Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			157	154	JL 8/6/20
			32			135	135	
			33			143	140	
			34			136	134	
			35			157	155	
			36			149	145	
			37			135	132	
			38			137	134	
			39			172	172	
			40			172	168	
			41			148	146	
			42			173	172	
			43			144	143	
			44			152	148	
			45			169	166	
			46			157	153	
			47			140	136	
			48			144	142	
			49			148	145	
			50			156	154	
			51			165	162	
			52			160	159	
			53			141	135	
			54			159	157	
			55			140	140	
			56			153	149	
			57			162	159	
			58			165	159	
			59			147	145	
			60			142	139	
			61			164	161	
			62			156	153	
			63			156	153	
			64			156	149	
			65			166	165	
			66			141	138	
			67			138	134	
			68			157	154	
			69			141	140	
			70			130	129	

CETIS Test Data Worksheet

Report Date: 11 Jul-20 17:57 (p 1 of 1)
Test Code: 2007-5059 11-6319-6188/4554F71C

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

Start Date: 15 Jul-20
End Date: 17 Jul-20
Sample Date: 14 Jul-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 20-0770
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	37			144	141	DM 7/18/20
0	BC	2	67					
0	BC	3	47					
0	BC	4	44					
0	BC	5	39					
0	LC	1	58			172	168	
0	LC	2	70					
0	LC	3	48					
0	LC	4	63					
0	LC	5	45					
2		1	31			164	162	
2		2	68					
2		3	57					
2		4	49					
2		5	69					
4		1	52			172	171	
4		2	55					
4		3	33					
4		4	36					
4		5	43					
9		1	61			153	150	
9		2	62					
9		3	38					
9		4	46					
9		5	42					
18		1	50			158	155	
18		2	65					
18		3	40					
18		4	66					
18		5	56					
35		1	51			176	173	
35		2	54					
35		3	60					
35		4	32					
35		5	35					
76 75.1		1	53			145	141	DM 7/18/20
76 75.1		2	64					
76 75.1		3	41					
76 75.1		4	34					
76 75.1 Ⓐ		5	59					

QC: EG

Ⓐ Q18 JA 8/7/20

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS

Sample ID: Wyckoff

Sample Log No.: 20- 0770

Test No.: 2007-S 059

Test Species: *M. galloprovincialis*

Start Date/Time: 7/15/20 1355

End Date/Time: 7/17/20 1310

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	29.7	29.6	16.0	16.1	15.6	8.5	8.2	8.4	8.11	~~7.95~~ (B) 8.05	7.80
Brine Control	30.4	30.0	30.3	15.8 (A)	15.9	15.4	7.9	8.1	8.5	8.17	~~7.81~~ (B) 8.11	7.82
2	30.1	29.9	30.2	16.0	(B) ~~16.0~~ 15.9	15.4	8.1	8.2	8.4	8.09	~~7.77~~ (B) 8.11	7.81
4	30.2	29.9	30.2	16.0	15.8	15.4	8.3	8.1	8.4	8.07	~~7.77~~ (B) 8.08	7.84
9	30.2	29.9	30.2	16.0	16.1	15.6	8.3	8.1	8.4	8.00	~~7.76~~ (B) 8.02	7.85
18	30.2	29.8	30.2	16.0	16.1	15.5	8.3	8.1	8.4	7.90	~~7.76~~ (B) 8.06	7.91
35	30.1	29.8	30.2	16.0	16.1	15.5	8.3	8.1	8.4	7.83	~~7.77~~ (B) 8.06	7.97
76	30.1	29.8	30.2	16.0	16.0	15.6	8.2	8.0	8.3	7.77	~~7.77~~ (B) 8.04	8.03

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

Technician Initials:

Environmental Chamber: D

Comments:

0 hrs: (A) Temperature measured using surrogate vial RT 7/15/20

24 hrs: (B) QIBKL 7/16/20

48 hrs:

QC Check: EG 7/17/20

Final Review: AC 8/6/20

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** EG/RT

Sample ID: Wyckoff **Test Date:** 7/15/2020

Test No: 2007-S 059 **Test Type:** Mussel Development

Salinity of Effluent 8.8

Salinity of Brine 97.2 **Date of Brine used:** 6/9/2020

Target Salinity 30 **Alkalinity of Brine Control:** 95 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.32**	**0.45**

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	7.1	250
18	45.0	0.32	14.2	250
35	87.5	0.32	27.6	250
76.0	190.0	0.32	60.0	250

	DI Volume			
Brine Control	134.3	0.45	60.0	250

Total Brine Volume Required (ml): **173.5**

QC Check: RT 7/18/20 **Final Review:** AC 8/6/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: JACOBS / Wyckoff
Test No.: 2007-5059
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-rep / 3A
Date Received: 4/21/20
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 7/15/2020 1355
End Date/Time: 7/17/2020 1310
Technician Initials: EG/RT

Spawn Information

First Gamete Release Time: 1007

Sex	Number Spawning
Male	3
Female	3+

Gamete Selection

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

Egg Fertilization Time: 1105

Embryo Stock Selection

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

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

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

Number Counted:		
	19	11
	19	12
	24	16
	19	15
	22	16

Mean: 17.3

Mean 17.3 X 50 = 865 embryos/ml

Initial Density: 865 = 2.88 (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	179	181	98.9	99.8
TØ B	139	139	100	
TØ C	166	166	100	
TØ D	167	167	100	
TØ E	147	147	100	
TØ F	155	155	100	
$\bar{X}$ =	159			

48-h QC: 133/136 = 97.8%

Comments:

QC Check: RT 7/18/20

Final Review: AC 8/6/20

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wychoff Eagle Harbor GWTP Eff.
Test ID No(s).: 2007-5059

Sample Check-In Information
DC-005

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

Sample (A, B, C):	A			
Log-in No. (20-xxxx):	0770			
Sample Collection Date & Time:	7/14/20 0936			
Sample Receipt Date & Time:	7/15/20 0915			
Number of Containers & Container Type:	1, 1L wbi			
Approx. Total Volume Received (L):	~1			
Check-in Temperature (°C)	5.0			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.4			
pH (units)	7.38			
Conductivity (µS/cm)	(A) 7/14/20			
Salinity (ppt)	8.8			
Alkalinity (mg/L) [2]	396			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	KL			

Test Performed: Mussel Development **Control/Dilution Water:** 8:2 / ~~Lab SW~~ / Lab ART Other: _____
Alkalinity: 101 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 95 Hardness or Salinity: 30ppt

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

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

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: (A) Q18 KL 7/16/20

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: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

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

QC Check: RT 7/18/20
Final Review: AC 8/6/20

Total Ammonia Analysis
Freshwater

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 7/15/2020

Analyst: GP
Analysis Date: 7/21/20

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	N x 1.22 Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	9.5	11.6
Wyckoff	20- 0770	7/15/2020	Check In	3.3	4.0
Spike Check (10 mg/L NH_3)		NA	NA		
Batch QA Sample	20-0971			36.7	44.8
Sample Duplicate[a]	20-0971	NA	NA	36.3	44.3
Sample Duplicate + Spike[a]		NA	NA	43.3	52.8
Spike Check (10 mg/L NH_3)		NA	NA	9.5	11.6

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	11.6	10	NA	116
Batch QA Sample	44.8	44.3	52.8	10	1.1	80

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: RT 8/15/20

Final Review: BO 8/15/20

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 7/14/2020
CarrierName: FedEx
AirbillNo: 7709 4990 2064

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

No: 10-071420-101314-0479
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
071420		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	07/14/2020 09:36	Field Sample

Special Instructions:	**Shipment for Case Complete? N**
	Samples Transferred From Chain of Custody #

Analysis Key: CHRTOX=Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	Keith Allers JACOBS	7-14-2020 1020	Nautilus	7/15/2020 0915	

Recipt temp: 5.0°C
Nautilus ID: 20-0770

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

CETIS Summary Report

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

Batch ID:	08-3335-0228	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	15 Jul-20 13:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	17 Jul-20 13:10	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	04-7267-3004	**Code:**	200715msdv	**Client:**	Internal
Sample Date:	15 Jul-20	**Material:**	Copper chloride	**Project:**	
Receive Date:	15 Jul-20	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-5603-4025	Combined Development Ra	10	20	14.14	10.2%		Dunnett Multiple Comparison Test
19-8463-7267	Development Rate	5	10	7.071	2.41%		Dunnett Multiple Comparison Test
01-3736-1147	Survival Rate	20	40	28.28	11.4%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
11-0488-5403	Combined Development Ra	EC25	11.24	10.31	12.09		Linear Interpolation (ICPIN)
		EC50	14.16	13.54	14.73		
14-0926-7215	Development Rate	EC25	10.9	10.08	11.53		Linear Interpolation (ICPIN)
		EC50	13.94	13.39	14.36		
14-7805-7540	Survival Rate	EC25	>40	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>40	N/A	N/A		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
14-0926-7215	Development Rate	Control Resp	0.9869	0.9 - NL	Yes	Passes Acceptability Criteria
19-8463-7267	Development Rate	Control Resp	0.9869	0.9 - NL	Yes	Passes Acceptability Criteria
01-3736-1147	Survival Rate	Control Resp	0.8994	0.5 - NL	Yes	Passes Acceptability Criteria
14-7805-7540	Survival Rate	Control Resp	0.8994	0.5 - NL	Yes	Passes Acceptability Criteria
14-5603-4025	Combined Development Ra	PMSD	0.1015	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	0.887	0.8062	0.9678	0.8239	0.9695	0.02909	0.06506	7.33%	0.0%
2.5		5	0.948	0.9109	0.9852	0.9119	0.9944	0.01337	0.0299	3.15%	-6.88%
5		5	0.9525	0.8925	1	0.8742	0.9885	0.02159	0.04827	5.07%	-7.39%
10		5	0.7944	0.7146	0.8741	0.7233	0.8802	0.02874	0.06426	8.09%	10.44%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0.001258	0	0.00475	0	0.006289	0.001258	0.002813	223.6%	99.86%

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	0.9869	0.9729	1	0.9695	1	0.005032	0.01125	1.14%	0.0%
2.5		5	0.9817	0.9559	1	0.9471	1	0.009274	0.02074	2.11%	0.52%
5		5	0.9823	0.9669	0.9977	0.9613	0.9929	0.005556	0.01242	1.27%	0.46%
10		5	0.8115	0.7426	0.8805	0.7325	0.8802	0.02483	0.05553	6.84%	17.77%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0.001639	0	0.006191	0	0.008197	0.001639	0.003666	223.6%	99.83%

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	0.8994	0.8066	0.9921	0.8302	1	0.0334	0.07468	8.3%	0.0%
2.5		5	0.966	0.9227	1	0.9245	1	0.01561	0.0349	3.61%	-7.41%
5		5	0.9698	0.9065	1	0.8805	1	0.0228	0.05098	5.26%	-7.83%
10		5	0.9786	0.9397	1	0.9245	1	0.01401	0.03132	3.2%	-8.81%
20		5	0.9006	0.8647	0.9366	0.8553	0.9308	0.01295	0.02896	3.22%	-0.14%
40		5	0.761	0.6428	0.8792	0.6415	0.9057	0.04256	0.09517	12.51%	15.38%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8428	0.9695	0.8239	0.8553	0.9434
2.5		0.9371	0.9944	0.9497	0.9119	0.9471
5		0.9811	0.9371	0.8742	0.9885	0.9815
10		0.7233	0.7547	0.8802	0.7736	0.84
20		0	0	0	0	0
40		0	0.006289	0	0	0

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1	0.9695	0.9924	0.9855	0.9868
2.5		1	0.9944	0.9805	0.9864	0.9471
5		0.9873	0.9613	0.9929	0.9885	0.9815
10		0.7325	0.8163	0.8802	0.7885	0.84
20		0	0	0	0	0
40		0	0.008197	0	0	0

Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8428	1	0.8302	0.8679	0.956
2.5		0.9371	1	0.9686	0.9245	1
5		0.9937	0.9748	0.8805	1	1
10		0.9874	0.9245	1	0.9811	1
20		0.8931	0.9308	0.9182	0.9057	0.8553
40		0.6415	0.7673	0.7296	0.761	0.9057

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	134/159	159/164	131/159	136/159	150/159
2.5		149/159	178/179	151/159	145/159	161/170
5		156/159	149/159	139/159	172/174	159/162
10		115/159	120/159	147/167	123/159	147/175
20		0/159	0/159	0/159	0/159	0/159
40		0/159	1/159	0/159	0/159	0/159

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	134/134	159/164	131/132	136/138	150/152
2.5		149/149	178/179	151/154	145/147	161/170
5		156/158	149/155	139/140	172/174	159/162
10		115/157	120/147	147/167	123/156	147/175
20		0/142	0/148	0/146	0/144	0/136
40		0/102	1/122	0/116	0/121	0/144

Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	134/159	159/159	132/159	138/159	152/159
2.5		149/159	159/159	154/159	147/159	159/159
5		158/159	155/159	140/159	159/159	159/159
10		157/159	147/159	159/159	156/159	159/159
20		142/159	148/159	146/159	144/159	136/159
40		102/159	122/159	116/159	121/159	144/159

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

Analysis ID: 14-5603-4025 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 12 Aug-20 11:54 **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	10.2%	10	20	14.14	

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	-1.801	2.227	0.138	8	0.9949	CDF	Non-Significant Effect
		5	-2.092	2.227	0.138	8	0.9977	CDF	Non-Significant Effect
		10	2.212	2.227	0.138	8	0.0514	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2272284	0.07574282	3	7.873	0.0019	Significant Effect
Error	0.1539264	0.0096204	16			
Total	0.3811548		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.5808	11.34	0.9008	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9514	0.866	0.3890	Normal Distribution

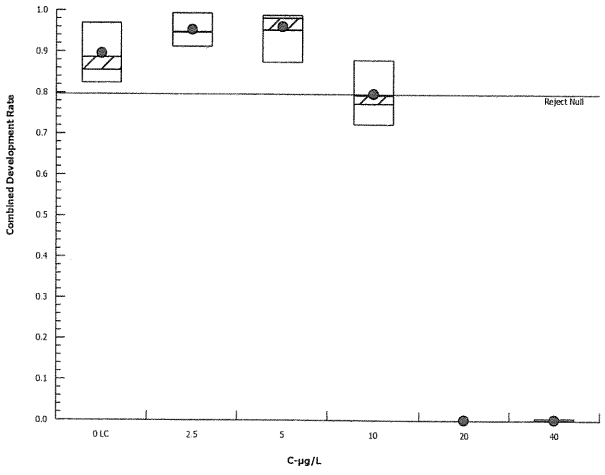
Combined Development Rate Summary

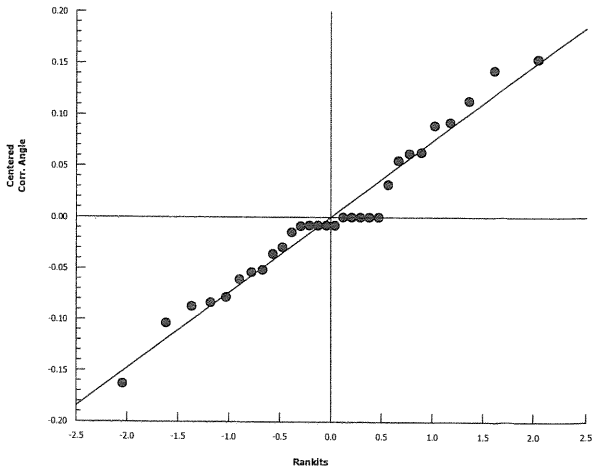
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.887	0.8062	0.9678	0.8553	0.8239	0.9695	0.02909	7.33%	0.0%
2.5		5	0.948	0.9109	0.9852	0.9471	0.9119	0.9944	0.01337	3.15%	-6.88%
5		5	0.9525	0.8925	1	0.9811	0.8742	0.9885	0.02159	5.07%	-7.39%
10		5	0.7944	0.7146	0.8741	0.7736	0.7233	0.8802	0.02874	8.09%	10.44%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0.001258	0	0.00475	0	0	0.006289	0.001258	223.6%	99.86%

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	1.241	1.1	1.383	1.181	1.138	1.395	0.05109	9.2%	0.0%
2.5		5	1.353	1.248	1.459	1.339	1.27	1.496	0.03805	6.29%	-9.0%
5		5	1.371	1.238	1.504	1.433	1.208	1.463	0.04783	7.8%	-10.45%
10		5	1.104	1.002	1.206	1.075	1.017	1.217	0.03674	7.44%	11.05%
20		5	0.03966	0.03965	0.03967	0.03966	0.03966	0.03966	0	0.0%	96.81%
40		5	0.04761	0.02555	0.06967	0.03966	0.03966	0.07939	0.007945	37.32%	96.17%

Graphics





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

Analysis ID:	19-8463-7267	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Aug-20 11:54	**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%	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	0.3315	2.227	0.085	8	0.6183	CDF	Non-Significant Effect
		5	0.5115	2.227	0.085	8	0.5397	CDF	Non-Significant Effect
		10*	8.826	2.227	0.085	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.4015166	0.1338388	3	36.6	<0.0001	Significant Effect
Error	0.05851293	0.003657058	16			
Total	0.4600295		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.467	11.34	0.6900	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9684	0.866	0.7218	Normal Distribution

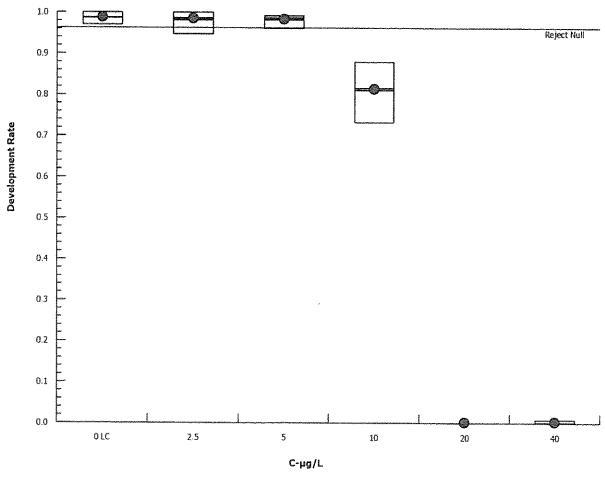
Development Rate Summary

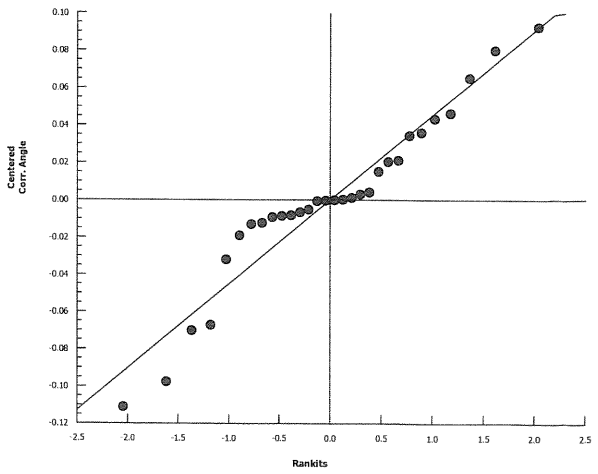
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9869	0.9729	1	0.9868	0.9695	1	0.005032	1.14%	0.0%
2.5		5	0.9817	0.9559	1	0.9864	0.9471	1	0.009274	2.11%	0.52%
5		5	0.9823	0.9669	0.9977	0.9873	0.9613	0.9929	0.005556	1.27%	0.46%
10		5	0.8115	0.7426	0.8805	0.8163	0.7325	0.8802	0.02483	6.84%	17.77%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0.001639	0	0.006191	0	0	0.008197	0.001639	223.6%	99.83%

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	1.462	1.402	1.523	1.456	1.395	1.528	0.02168	3.31%	0.0%
2.5		5	1.45	1.359	1.54	1.454	1.339	1.53	0.03262	5.03%	0.87%
5		5	1.443	1.389	1.497	1.458	1.373	1.486	0.01939	3.0%	1.34%
10		5	1.125	1.036	1.213	1.128	1.027	1.217	0.03188	6.34%	23.08%
20		5	0.04181	0.04096	0.04266	0.04168	0.04111	0.04289	0.000306	1.63%	97.14%
40		5	0.05476	0.02959	0.07992	0.04644	0.04168	0.09066	0.009063	37.01%	96.26%

Graphics





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

Analysis ID: 01-3736-1147 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 12 Aug-20 11:54 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.4%	20	40	28.28	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.786	2.362	0.176	8	0.9985	CDF	Non-Significant Effect
		5	-2.103	2.362	0.176	8	0.9995	CDF	Non-Significant Effect
		10	-2.273	2.362	0.176	8	0.9997	CDF	Non-Significant Effect
		20	0.365	2.362	0.176	8	0.7040	CDF	Non-Significant Effect
		40*	2.841	2.362	0.176	8	0.0182	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5433294	0.1086659	5	7.81	0.0002	Significant Effect
Error	0.3339113	0.01391297	24			
Total	0.8772408		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.935	15.09	0.4239	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9863	0.9031	0.9571	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	0.8994	0.8066	0.9921	0.8679	0.8302	1	0.0334	8.3%	0.0%
2.5		5	0.966	0.9227	1	0.9686	0.9245	1	0.01561	3.61%	-7.41%
5		5	0.9698	0.9065	1	0.9937	0.8805	1	0.0228	5.26%	-7.83%
10		5	0.9786	0.9397	1	0.9874	0.9245	1	0.01401	3.2%	-8.81%
20		5	0.9006	0.8647	0.9366	0.9057	0.8553	0.9308	0.01295	3.22%	-0.14%
40		5	0.761	0.6428	0.8792	0.761	0.6415	0.9057	0.04256	12.51%	15.38%

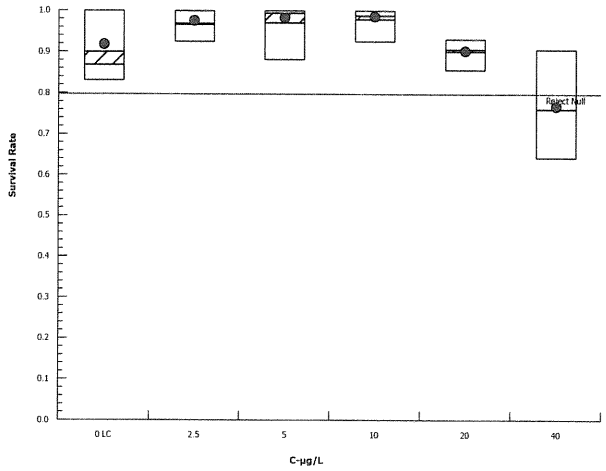
Angular (Corrected) Transformed Summary

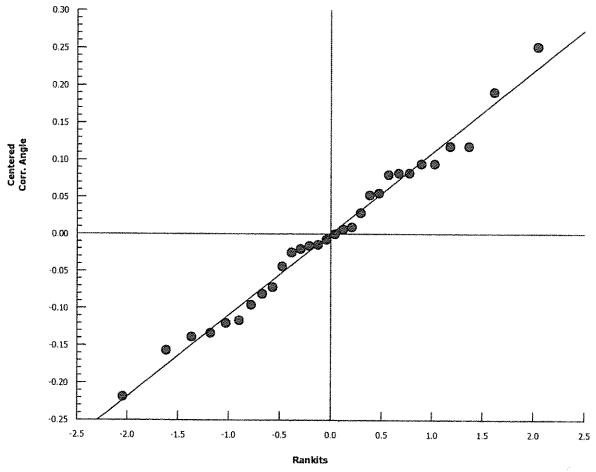
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.28	1.076	1.483	1.199	1.146	1.531	0.07334	12.81%	0.0%
2.5		5	1.413	1.271	1.555	1.393	1.292	1.531	0.05099	8.07%	-10.41%
5		5	1.437	1.273	1.6	1.491	1.218	1.531	0.05889	9.17%	-12.26%
10		5	1.449	1.328	1.571	1.458	1.292	1.531	0.04378	6.76%	-13.25%
20		5	1.252	1.194	1.311	1.259	1.181	1.305	0.02114	3.78%	2.13%
40		5	1.068	0.9186	1.217	1.06	0.9289	1.259	0.05371	11.25%	16.56%

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

Analysis ID:	01-3736-1147	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Aug-20 11:54	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: JL QA: BO 8/18/20

CETIS Analytical Report

Test Code: 200715msdv | 17-4780-3294

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

Analysis ID:	11-0488-5403	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Aug-20 11:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	666566	1000	Yes	Two-Point Interpolation

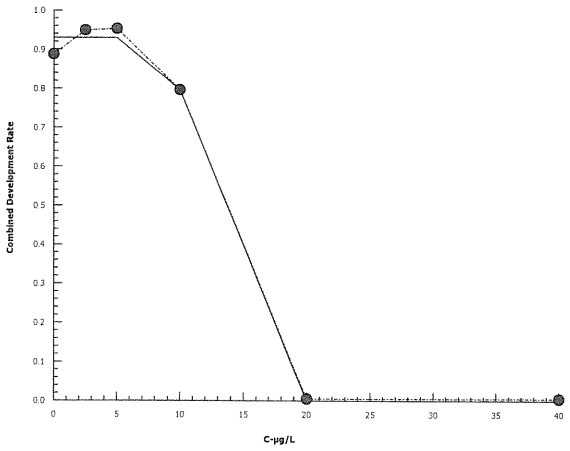
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	11.24	10.31	12.09
EC50	14.16	13.54	14.73

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	0.887	0.8239	0.9695	0.02909	0.06506	7.33%	0.0%	710	800
2.5		5	0.948	0.9119	0.9944	0.01337	0.0299	3.15%	-6.88%	784	826
5		5	0.9525	0.8742	0.9885	0.02159	0.04827	5.07%	-7.39%	775	813
10		5	0.7944	0.7233	0.8802	0.02874	0.06426	8.09%	10.44%	652	819
20		5	0	0	0	0	0		100.0%	0	795
40		5	0.001258	0	0.006289	0.001258	0.002813	223.6%	99.86%	0	795

Graphics



Analyst: JL QA: BO 8/18/20

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

Analysis ID:	14-0926-7215	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Aug-20 11:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	818614	1000	Yes	Two-Point Interpolation

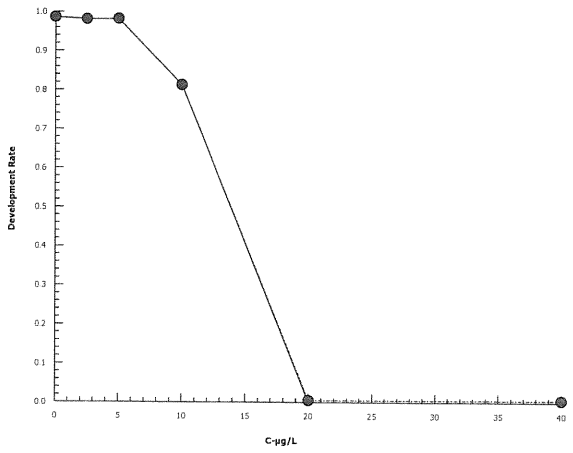
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	10.9	10.08	11.53
EC50	13.94	13.39	14.36

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	0.9869	0.9695	1	0.005032	0.01125	1.14%	0.0%	710	720
2.5		5	0.9817	0.9471	1	0.009274	0.02074	2.11%	0.52%	784	799
5		5	0.9823	0.9613	0.9929	0.005556	0.01242	1.27%	0.46%	775	789
10		5	0.8115	0.7325	0.8802	0.02483	0.05553	6.84%	17.77%	652	802
20		5	0	0	0	0	0		100.0%	0	716
40		5	0.001639	0	0.008197	0.001639	0.003666	223.6%	99.83%	0	605

Graphics



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

Analysis ID:	14-7805-7540	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	12 Aug-20 11:54	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1852204	1000	Yes	Two-Point Interpolation

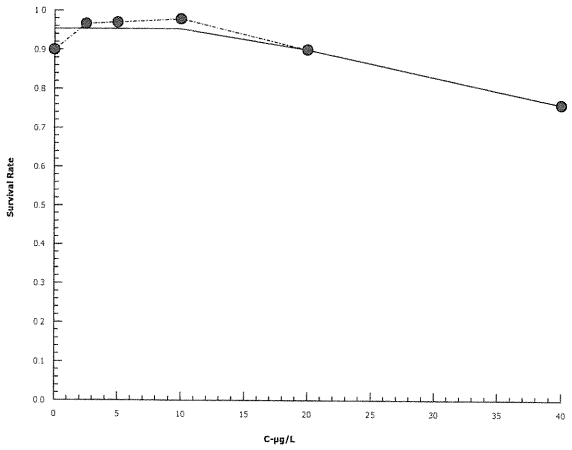
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	>40	N/A	N/A
EC50	>40	N/A	N/A

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	0.8994	0.8302	1	0.0334	0.07468	8.3%	0.0%	715	795
2.5		5	0.966	0.9245	1	0.01561	0.0349	3.61%	-7.41%	768	795
5		5	0.9698	0.8805	1	0.0228	0.05098	5.26%	-7.83%	771	795
10		5	0.9786	0.9245	1	0.01401	0.03132	3.2%	-8.81%	778	795
20		5	0.9006	0.8553	0.9308	0.01295	0.02896	3.22%	-0.14%	716	795
40		5	0.761	0.6415	0.9057	0.04256	0.09517	12.51%	15.38%	604	795

Graphics

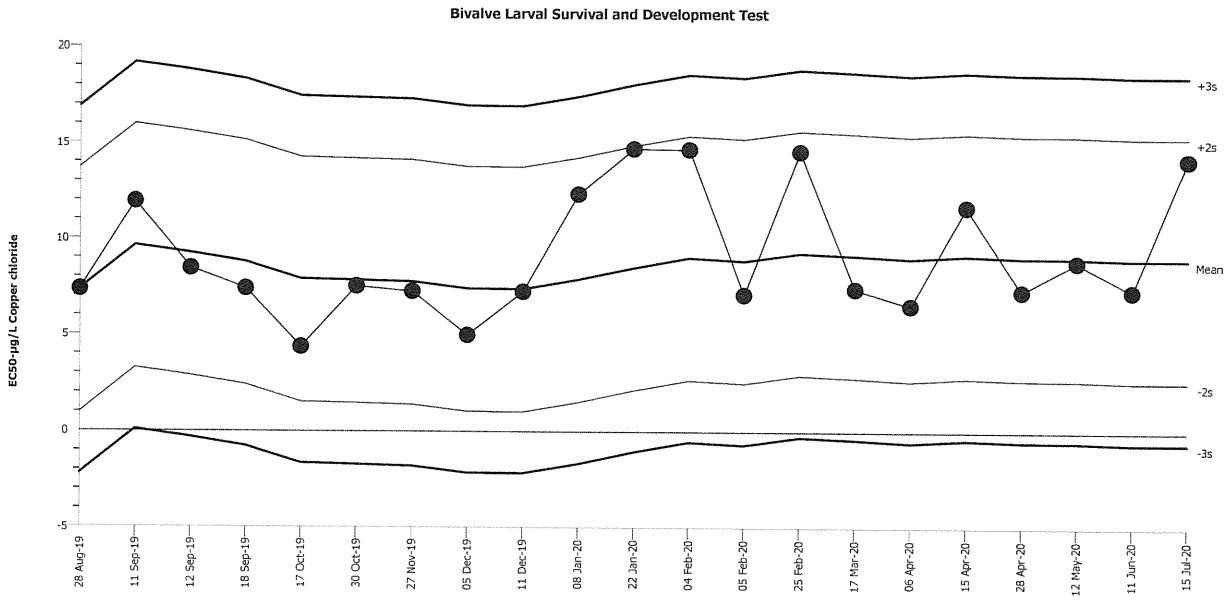


CETIS QC Plot

Report Date: 12 Aug-20 11:57 (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:	8.948	**Count:**	20	**-2s Warning Limit:**	2.582	**-3s Action Limit:**	-0.6014
Sigma:	3.183	**CV:**	35.60%	**+2s Warning Limit:**	15.31	**+3s Action Limit:**	18.5

Quality Control Data

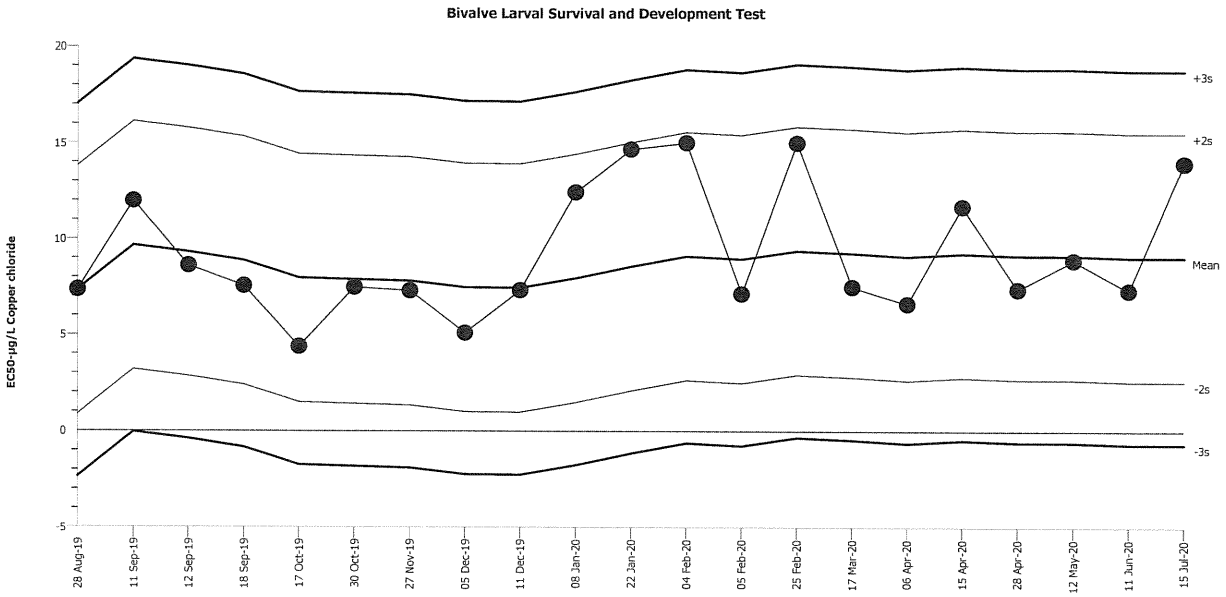
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	28	14:30	7.348	-1.6	-0.5027			01-0546-0046	21-3090-7111
2		Sep	11	14:30	11.93	2.987	0.9383			09-2717-2159	04-2480-9094
3			12	14:25	8.444	-0.5036	-0.1582			19-6218-6352	07-5188-6358
4			18	13:20	7.4	-1.548	-0.4863			10-9359-1611	21-3838-7021
5		Oct	17	12:30	4.368	-4.58	-1.439			01-8239-7270	07-0806-0577
6			30	12:30	7.518	-1.43	-0.4493			07-8198-2858	11-8079-0492
7		Nov	27	20:00	7.249	-1.699	-0.5339			12-9914-0499	16-0529-7707
8		Dec	5	13:15	4.982	-3.966	-1.246			04-7411-4445	13-6587-0425
9			11	13:35	7.245	-1.703	-0.535			10-8800-1613	10-7929-5811
10	2020	Jan	8	13:40	12.34	3.392	1.066			07-8444-5322	01-1422-4896
11			22	13:25	14.72	5.772	1.813			02-1152-2212	07-1224-7163
12		Feb	4	16:30	14.68	5.728	1.799			19-9078-6483	21-0369-4045
13			5	13:10	7.103	-1.845	-0.5797			06-6849-2235	04-8167-3886
14			25	14:15	14.58	5.633	1.77			09-2101-6353	02-3593-4650
15		Mar	17	14:20	7.408	-1.54	-0.4839			14-6169-3689	18-9939-7640
16		Apr	6	17:15	6.537	-2.411	-0.7574			02-0082-4673	13-2096-3831
17			15	13:25	11.68	2.735	0.8592			16-4614-0901	11-3098-9850
18			28	13:25	7.292	-1.656	-0.5204			06-8086-6028	13-2749-2065
19		May	12	16:15	8.819	-0.1291	-0.04056			12-3773-8150	00-4087-7530
20		Jun	11	15:45	7.306	-1.642	-0.516			20-6521-9403	10-1893-3875
21		Jul	15	13:55	14.16	5.214	1.638			17-4780-3294	11-0488-5403

CETIS QC Plot

Report Date: 12 Aug-20 11:57 (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:	9.031	**Count:**	20	**-2s Warning Limit:**	2.559	**-3s Action Limit:**	-0.6767
Sigma:	3.236	**CV:**	35.80%	**+2s Warning Limit:**	15.5	**+3s Action Limit:**	18.74

Quality Control Data

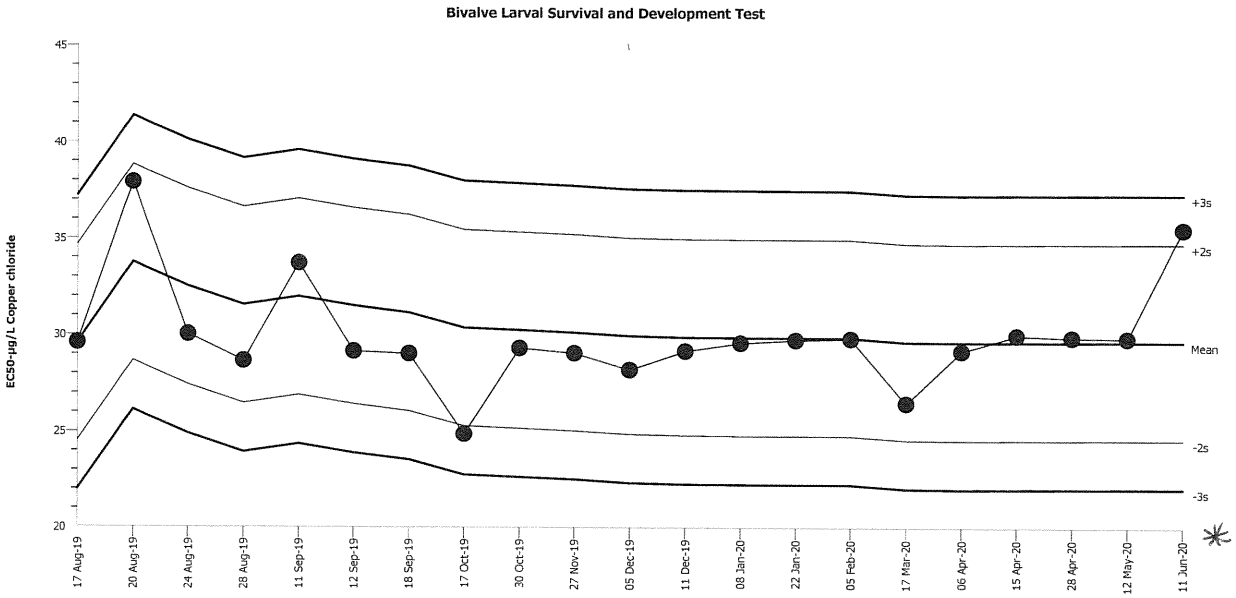
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	28	14:30	7.351	-1.68	-0.5192			01-0546-0046	10-3410-8075
2		Sep	11	14:30	11.98	2.952	0.9121			09-2717-2159	17-4622-9429
3			12	14:25	8.608	-0.4234	-0.1308			19-6218-6352	06-5225-4823
4			18	13:20	7.546	-1.485	-0.459			10-9359-1611	16-7089-5314
5		Oct	17	12:30	4.375	-4.656	-1.439			01-8239-7270	19-1864-9270
6			30	12:30	7.481	-1.55	-0.4789			07-8198-2858	15-7183-3565
7		Nov	27	20:00	7.297	-1.734	-0.5358			12-9914-0499	01-7534-7240
8		Dec	5	13:15	5.087	-3.944	-1.219			04-7411-4445	10-0471-4567
9			11	13:35	7.32	-1.711	-0.5287			10-8800-1613	20-9346-8800
10	2020	Jan	8	13:40	12.43	3.398	1.05			07-8444-5322	06-2499-4329
11			22	13:25	14.68	5.65	1.746			02-1152-2212	04-4145-0874
12		Feb	4	16:30	15.01	5.977	1.847			19-9078-6483	06-3219-7963
13			5	13:10	7.132	-1.899	-0.5868			06-6849-2235	20-3119-3253
14			25	14:15	15	5.969	1.845			09-2101-6353	13-1093-9538
15		Mar	17	14:20	7.489	-1.542	-0.4766			14-6169-3689	12-6636-5212
16		Apr	6	17:15	6.609	-2.422	-0.7483			02-0082-4673	11-5300-1558
17			15	13:25	11.68	2.652	0.8195			16-4614-0901	19-2371-7781
18			28	13:25	7.365	-1.666	-0.5148			06-8086-6028	17-1633-3832
19		May	12	16:15	8.876	-0.1547	-0.04782			12-3773-8150	04-4023-9067
20		Jun	11	15:45	7.306	-1.725	-0.5332			20-6521-9403	18-5947-9043
21		Jul	15	13:55	13.94	4.908	1.517			17-4780-3294	14-0926-7215

CETIS QC Plot

Report Date: 12 Aug-20 11:57 (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:	29.67	**Count:**	20	**-2s Warning Limit:**	24.59	**-3s Action Limit:**	22.05
Sigma:	2.54	**CV:**	8.56%	**+2s Warning Limit:**	34.75	**+3s Action Limit:**	37.29

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	17	14:00	29.6	-0.06634	-0.02612			15-9584-4385	20-0172-5237
2			20	14:15	37.92	8.249	3.248	(+)	(+)	14-8361-1578	02-5800-6574
3			24	16:00	30.04	0.3674	0.1446			19-4374-5817	17-7461-0750
4			28	14:30	28.66	-1.005	-0.3958			01-0546-0046	13-4512-6481
5		Sep	11	14:30	33.71	4.045	1.592			09-2717-2159	01-1883-2964
6			12	14:25	29.16	-0.5059	-0.1992			19-6218-6352	02-6393-7831
7			18	13:20	29.04	-0.631	-0.2484			10-9359-1611	04-3365-2341
8		Oct	17	12:30	24.88	-4.788	-1.885			01-8239-7270	13-2801-3685
9			30	12:30	29.32	-0.3471	-0.1366			07-8198-2858	20-5233-5110
10		Nov	27	20:00	29.07	-0.6033	-0.2375			12-9914-0499	00-1104-7300
11		Dec	5	13:15	28.21	-1.456	-0.5731			04-7411-4445	20-5035-4724
12			11	13:35	29.18	-0.4907	-0.1932			10-8800-1613	02-9848-3585
13	2020	Jan	8	13:40	29.6	-0.06894	-0.02714			07-8444-5322	01-5655-1706
14			22	13:25	29.76	0.08561	0.0337			02-1152-2212	19-4150-8988
15		Feb	5	13:10	29.83	0.1563	0.06154			06-6849-2235	07-0404-6516
16		Mar	17	14:20	26.48	-3.188	-1.255			14-6169-3689	14-2151-4803
17		Apr	6	17:15	29.18	-0.4932	-0.1942			02-0082-4673	12-2147-8498
18			15	13:25	30	0.33	0.1299			16-4614-0901	00-5465-8677
19			28	13:25	29.9	0.226	0.08896			06-8086-6028	08-1083-2165
20		May	12	16:15	29.85	0.181	0.07127			12-3773-8150	18-0143-0286
21		Jun	11	15:45	35.5	5.829	2.295	(+)		20-6521-9403	17-6494-5506

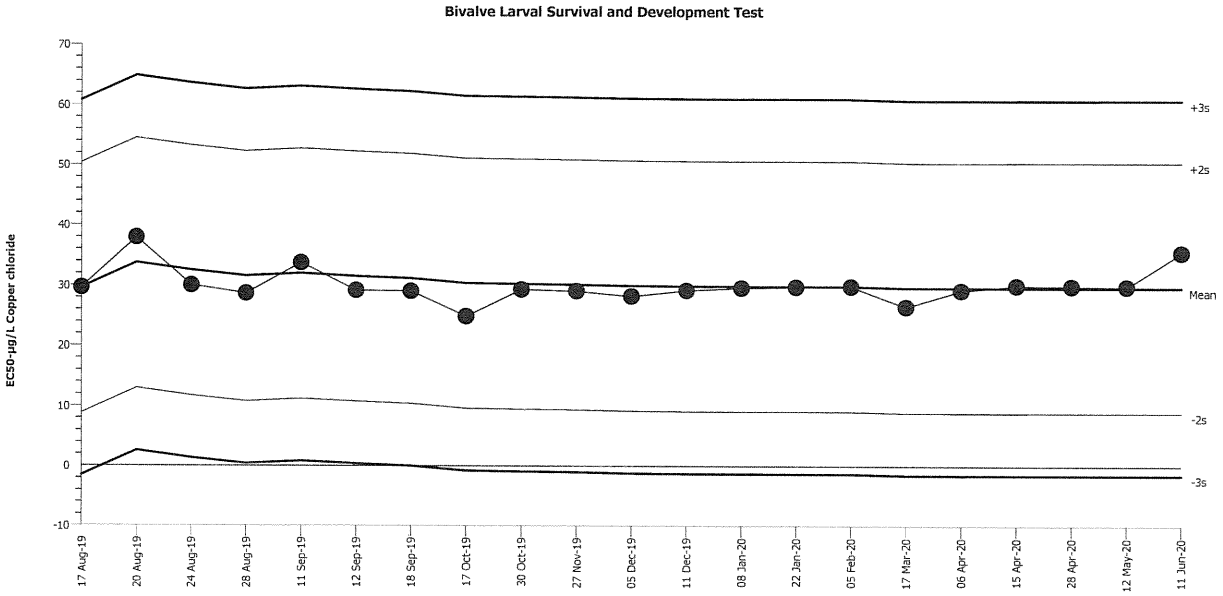
*EC50 for survival is greater than highest concentration tested on 7/15/20

CETIS QC Plot

Report Date: 12 Aug-20 11:58 (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:	29.67	**Count:**	20	**-2s Warning Limit:**	8.901	**-3s Action Limit:**	-1.484
Sigma:	10.38	**CV:**	35.00%	**+2s Warning Limit:**	50.44	**+3s Action Limit:**	60.82

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	17	14:00	29.6	-0.06634	-0.00639			15-9584-4385	20-0172-5237
2			20	14:15	37.92	8.249	0.7943			14-8361-1578	02-5800-6574
3			24	16:00	30.04	0.3674	0.03538			19-4374-5817	17-7461-0750
4			28	14:30	28.66	-1.005	-0.09682			01-0546-0046	13-4512-6481
5		Sep	11	14:30	33.71	4.045	0.3895			09-2717-2159	01-1883-2964
6			12	14:25	29.16	-0.5059	-0.04872			19-6218-6352	02-6393-7831
7			18	13:20	29.04	-0.631	-0.06077			10-9359-1611	04-3365-2341
8		Oct	17	12:30	24.88	-4.788	-0.461			01-8239-7270	13-2801-3685
9			30	12:30	29.32	-0.3471	-0.03342			07-8198-2858	20-5233-5110
10		Nov	27	20:00	29.07	-0.6033	-0.0581			12-9914-0499	00-1104-7300
11		Dec	5	13:15	28.21	-1.456	-0.1402			04-7411-4445	20-5035-4724
12			11	13:35	29.18	-0.4907	-0.04726			10-8800-1613	02-9848-3585
13	2020	Jan	8	13:40	29.6	-0.06894	-0.00664			07-8444-5322	01-5655-1706
14			22	13:25	29.76	0.08561	0.008244			02-1152-2212	19-4150-8988
15		Feb	5	13:10	29.83	0.1563	0.01505			06-6849-2235	07-0404-6516
16		Mar	17	14:20	26.48	-3.188	-0.307			14-6169-3689	14-2151-4803
17		Apr	6	17:15	29.18	-0.4932	-0.04749			02-0082-4673	12-2147-8498
18			15	13:25	30	0.33	0.03178			16-4614-0901	00-5465-8677
19			28	13:25	29.9	0.226	0.02176			06-8086-6028	08-1083-2165
20		May	12	16:15	29.85	0.181	0.01743			12-3773-8150	18-0143-0286
21		Jun	11	15:45	35.5	5.829	0.5613			20-6521-9403	17-6494-5506

* 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

CETIS Test Data Worksheet

Report Date: 11 Jul-20 17:57 (p 1 of 1)
Test Code: 17-4780-3294/200715msdv

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

Start Date: 15 Jul-20 | **Species:** Mytilus galloprovincialis | **Sample Code:** 200715msdv
End Date: 17 Jul-20 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 15 Jul-20 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			140	139	JL OBO DM 8/12/20
			2			132	131	↓
			3			167	147	
			4			116	0	JL 8/12/20
			5			122	1	
			6			148	0	
			7			170	161	
			8			175	147	
			9			146	0	
			10			136	0	
			11			152	150	
			12			154	151	
			13			147	120	
			14			134	134	
			15			138	136	
			16			144	0	
			17			179	178	
			18			174	172	
			19			102	0	
			20			147	145	
			21			149	149	
			22			158	156	
			23			155	149	
			24			121	0	
			25			142	0	
			26			156	123	
			27			164	159	
			28			162	159	
			29			157	115	
			30			144	0	

Analyst: BO OBO JL QA: BO 8/18/20

CETIS Test Data Worksheet

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

Start Date: 15 Jul-20 | **Species:** Mytilus galloprovincialis | **Sample Code:** 200715msdv
End Date: 17 Jul-20 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 15 Jul-20 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	14			130	130	DM 7/18/20
0	LC	2	27					
0	LC	3	2					
0	LC	4	15					
0	LC	5	11					
2.5		1	21			153	152	
2.5		2	17					
2.5		3	12					
2.5		4	20					
2.5		5	7					
5		1	22			153	150	
5		2	23					
5		3	1					
5		4	18					
5		5	28					
10		1	29					
10		2	13			143	123	
10		3	3					
10		4	26					
10		5	8					
20		1	25			145	0	
20		2	6					
20		3	9					
20		4	16					
20		5	10					
40		1	19			98	98 0	cells lysed
40		2	5					
40		3	4					
40		4	24					
40		5	30					

QC: EG

Ⓐ QB DM 8/18/20

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 200715msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 7/15/2020 1355

End Date/Time: 7/17/2020 1310

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	31.9	31.4	31.4	15.9	16.3	15.5	8.8	8.3	8.4	8.09	~~8.03~~ 7.91 (A)	7.86
2.5	32.1	31.7	32.2	15.7	15.8	15.2	8.7	8.4	8.4	8.10	~~8.05~~ 7.75 (A)	7.90
5	32.1	31.7	32.2	15.6	15.8	15.2	8.8	8.2	8.3	8.11	~~8.06~~ 7.76 (A)	7.94
10	32.1	31.7	32.2	15.9	16.0	15.4	8.6	8.2	8.3	8.11	~~8.06~~ 7.77 (A)	7.93
20	32.1	31.7	32.2	15.7	15.9	15.4	8.7	8.3	8.3	8.12	~~8.06~~ 7.78 (A)	7.93
40	32.0	31.6	32.1	15.7	15.9	15.4	8.7	8.2	8.3	8.12	~~8.06~~ 7.80 (A)	7.93

	0	24	48
WQ Readings:	EL	KL	ER
Dilutions made by:	RT		

Technician Initials:

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: (A) @18 KL 7/16/20

48 hrs:

QC Check: EL 7/17/20

Final Review: BO 8/18/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / CuCl2
Test No.: 200715msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-rep / 3A
Date Received: 4/21/20
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 7/15/2020 1355
End Date/Time: 7/17/2020 1310
Technician Initials: EG/RT

Spawn Information

First Gamete Release Time: 1007

Sex	Number Spawning
Male	3
Female	3+

Gamete Selection

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

Egg Fertilization Time: 1105

Embryo Stock Selection

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

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

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

Number Counted:		
	19	11
	19	12
	24	16
	19	15
	22	16

Mean: 17.3

Mean 17.3 X 50 = 865 embryos/ml

Initial Density: 865 = 2.88 (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	179	181	98.9	99.8
TØ B	139	139	100	
TØ C	166	166	100	
TØ D	167	167	100	
TØ E	147	147	100	
TØ F	155	155	100	
$\bar{X}$ =	159			

48-h QC: 133/136 = 97.8%

Comments:

QC Check: RT 7/18/20

Final Review: BO 8/18/20