ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: September 2021

Prepared for: **Jacobs**
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Date Submitted: **October 22, 2021**

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: _____
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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	**092821**
Enthalpy Log-in Number	21-0991
Collection Date; Time	9/28/2021; 0928h
Receipt Date; Time	9/29/2021; 0940h
Receipt Temperature (°C)	4.0
Dissolved Oxygen (mg/L)	7.9
pH	7.56
Conductivity (µS/cm)	NM
Salinity (ppt)	10.6
Alkalinity (mg/L $CaCO_3$)	409
Total Chlorine (mg/L)	< 0.02
Total Ammonia (mg/L as N)	2.3

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	9/29/2021, 1545h to 10/1/2021, 1440h
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 94.9 ppt
Test Concentrations (% sample)	76.1[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. See QA section.

Results

There were no statistically significant effects detected 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 76.1 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.3 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	76.1	> 76.1	< 1.3	> 76.1
	Survival	76.1	> 76.1	< 1.3	> 76.1

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)	89.2	99.3
0 (Lab Control)	91.8	99.5
2	96.9	99.6
4	94.9	99.6
9	87.5	99.5
18	92.2	99.3
35	90.8	99.4
76.1[a]	95.3	99.6

[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 and survival. 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 Survival Rate	10	28.5	28.3 ± 10.2	18.0
Bivalve Normal Development	5	9.72	8.85 ± 5.21	29.4

NOEC = No Observed Effect Concentration

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

Historical Mean ± 2 SD = The mean EC_{50} from the previous 20 tests performed by the laboratory, plus or minus two standard deviations (SD)

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:	15-5337-3785	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	29 Sep-21 15:45	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	01 Oct-21 14:40	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	17-6733-5211	**Code:**	21-0991	**Client:**	Jacobs
Sample Date:	28 Sep-21 09:28	**Material:**	Effluent Sample	**Project:**	
Receive Date:	29 Sep-21 09:40	**Source:**	Jacobs		
Sample Age:	30h (4 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-6404-9811	Combined Development Ra	76.1	>76.1	NA	20.4%	1.314	Dunnett Multiple Comparison Test
16-5680-0379	Development Rate	76.1	>76.1	NA	1.13%	1.314	Steel Many-One Rank Sum Test
09-6800-5518	Survival Rate	76.1	>76.1	NA	20.1%	1.314	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
01-3694-7731	Combined Development Ra	EC25	>76.1	N/A	N/A	<1.314	Linear Interpolation (ICPIN)
		EC50	>76.1	N/A	N/A	<1.314	
13-0826-8706	Development Rate	EC25	>76.1	N/A	N/A	<1.314	Linear Interpolation (ICPIN)
		EC50	>76.1	N/A	N/A	<1.314	
04-3330-6583	Survival Rate	EC25	>76.1	N/A	N/A	<1.314	Linear Interpolation (ICPIN)
		EC50	>76.1	N/A	N/A	<1.314	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
13-0826-8706	Development Rate	Control Resp	0.9932	0.9 - NL	Yes	Passes Acceptability Criteria
16-5680-0379	Development Rate	Control Resp	0.9932	0.9 - NL	Yes	Passes Acceptability Criteria
04-3330-6583	Survival Rate	Control Resp	0.8922	0.5 - NL	Yes	Passes Acceptability Criteria
09-6800-5518	Survival Rate	Control Resp	0.8922	0.5 - NL	Yes	Passes Acceptability Criteria
15-6404-9811	Combined Development Ra	PMSD	0.2038	NL - 0.25	No	Passes Acceptability Criteria

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

Combined Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.8863	0.7647	1	0.7451	0.9902	0.0438	0.09794	11.05%	0.0%
0	Lab Control	5	0.9137	0.8044	1	0.7941	1	0.03939	0.08807	9.64%	-3.1%
2		5	0.9651	0.9048	1	0.8824	1	0.02172	0.04856	5.03%	-8.89%
4		5	0.9453	0.8588	1	0.8431	1	0.03117	0.06969	7.37%	-6.66%
9		5	0.8706	0.7199	1	0.7059	1	0.05427	0.1213	13.94%	1.77%
18		5	0.9157	0.809	1	0.8235	1	0.03842	0.08592	9.38%	-3.32%
35		5	0.902	0.7824	1	0.7549	1	0.04307	0.09631	10.68%	-1.77%
76.1		5	0.949	0.8783	1	0.8627	1	0.02546	0.05693	6.0%	-7.08%

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9932	0.9811	1	0.9789	1	0.00436	0.009749	0.98%	0.0%
0	Lab Control	5	0.9954	0.9876	1	0.9878	1	0.00282	0.006305	0.63%	-0.22%
2		5	0.9964	0.9903	1	0.9907	1	0.002192	0.004902	0.49%	-0.33%
4		5	0.9961	0.9894	1	0.9892	1	0.00242	0.00541	0.54%	-0.29%
9		5	0.995	0.9863	1	0.9863	1	0.003105	0.006943	0.7%	-0.18%
18		5	0.993	0.9801	1	0.9767	1	0.004651	0.0104	1.05%	0.02%
35		5	0.9936	0.9864	1	0.9888	1	0.002599	0.005812	0.58%	-0.05%
76.1		5	0.9961	0.9894	1	0.99	1	0.002414	0.005398	0.54%	-0.29%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.8922	0.7729	1	0.7549	0.9902	0.04296	0.09606	10.77%	0.0%
0	Lab Control	5	0.9176	0.8127	1	0.8039	1	0.03779	0.08451	9.21%	-2.86%
2		5	0.9686	0.9051	1	0.8824	1	0.02287	0.05113	5.28%	-8.57%
4		5	0.949	0.8623	1	0.8431	1	0.03122	0.06981	7.36%	-6.37%
9		5	0.8745	0.7286	1	0.7157	1	0.05256	0.1175	13.44%	1.98%
18		5	0.9216	0.8242	1	0.8431	1	0.03508	0.07843	8.51%	-3.3%
35		5	0.9078	0.7865	1	0.7549	1	0.04369	0.0977	10.76%	-1.76%
76.1		5	0.9529	0.8787	1	0.8627	1	0.02674	0.0598	6.28%	-6.81%

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

Combined Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.7451	0.8333	0.9902	0.951	0.9118
0	Lab Control	0.7941	0.902	1	1	0.8725
2		0.9907	0.8824	1	0.9608	0.9914
4		0.9912	1	0.902	0.8431	0.9902
9		0.7059	0.9804	1	0.8235	0.8431
18		1	0.8235	1	0.9216	0.8333
35		0.8627	0.9608	1	0.7549	0.9314
76.1		0.8627	1	0.9706	0.9216	0.9903

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.987	1	1	1	0.9789
0	Lab Control	0.9878	0.9892	1	1	1
2		0.9907	1	1	1	0.9914
4		0.9912	1	0.9892	1	1
9		0.9863	1	1	1	0.9885
18		1	0.9767	1	1	0.9884
35		0.9888	0.9899	1	1	0.9896
76.1		1	1	0.99	1	0.9903

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.7549	0.8333	0.9902	0.951	0.9314
0	Lab Control	0.8039	0.9118	1	1	0.8725
2		1	0.8824	1	0.9608	1
4		1	1	0.9118	0.8431	0.9902
9		0.7157	0.9804	1	0.8235	0.8529
18		1	0.8431	1	0.9216	0.8431
35		0.8725	0.9706	1	0.7549	0.9412
76.1		0.8627	1	0.9804	0.9216	1

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

Combined Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	76/102	85/102	101/102	97/102	93/102
0	Lab Control	81/102	92/102	117/117	111/111	89/102
2		107/108	90/102	102/102	98/102	115/116
4		112/113	102/102	92/102	86/102	101/102
9		72/102	100/102	114/114	84/102	86/102
18		104/104	84/102	102/102	94/102	85/102
35		88/102	98/102	113/113	77/102	95/102
76.1		88/102	109/109	99/102	94/102	102/103

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	76/77	85/85	101/101	97/97	93/95
0	Lab Control	81/82	92/93	117/117	111/111	89/89
2		107/108	90/90	102/102	98/98	115/116
4		112/113	102/102	92/93	86/86	101/101
9		72/73	100/100	114/114	84/84	86/87
18		104/104	84/86	102/102	94/94	85/86
35		88/89	98/99	113/113	77/77	95/96
76.1		88/88	109/109	99/100	94/94	102/103

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	77/102	85/102	101/102	97/102	95/102
0	Lab Control	82/102	93/102	102/102	102/102	89/102
2		102/102	90/102	102/102	98/102	102/102
4		102/102	102/102	93/102	86/102	101/102
9		73/102	100/102	102/102	84/102	87/102
18		102/102	86/102	102/102	94/102	86/102
35		89/102	99/102	102/102	77/102	96/102
76.1		88/102	102/102	100/102	94/102	102/102

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

Analysis ID: 15-6404-9811 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-21 11:50 **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	20.4%	76.1	>76.1	NA	1.314

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-1.462	2.407	0.259	8	0.9972	CDF	Non-Significant Effect
		4	-1.127	2.407	0.259	8	0.9916	CDF	Non-Significant Effect
		9	0.05118	2.407	0.259	8	0.8428	CDF	Non-Significant Effect
		18	-0.6278	2.407	0.259	8	0.9653	CDF	Non-Significant Effect
		35	-0.3069	2.407	0.259	8	0.9244	CDF	Non-Significant Effect
		76.1	-1.101	2.407	0.259	8	0.9909	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1224971	0.02041619	6	0.7074	0.6463	Non-Significant Effect
Error	0.8081338	0.02886192	28			
Total	0.9306309		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.717	16.81	0.9438	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9498	0.9146	0.1115	Normal Distribution

Combined Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.8863	0.7647	1	0.9118	0.7451	0.9902	0.0438	11.05%	0.0%
2		5	0.9651	0.9048	1	0.9907	0.8824	1	0.02172	5.03%	-8.89%
4		5	0.9453	0.8588	1	0.9902	0.8431	1	0.03117	7.37%	-6.66%
9		5	0.8706	0.7199	1	0.8431	0.7059	1	0.05427	13.94%	1.77%
18		5	0.9157	0.809	1	0.9216	0.8235	1	0.03842	9.38%	-3.32%
35		5	0.902	0.7824	1	0.9314	0.7549	1	0.04307	10.68%	-1.77%
76.1		5	0.949	0.8784	1	0.9706	0.8627	1	0.02546	6.0%	-7.08%

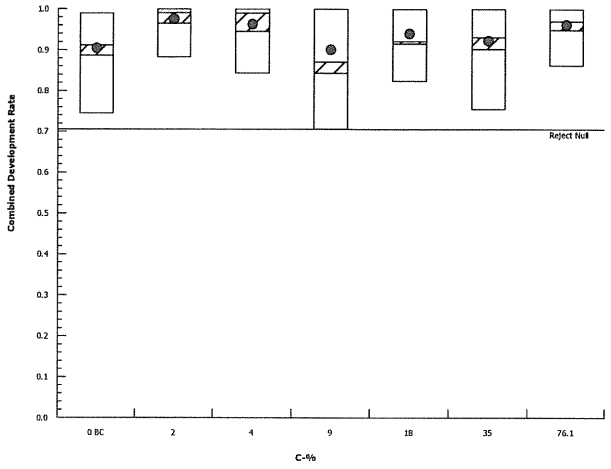
Angular (Corrected) Transformed Summary

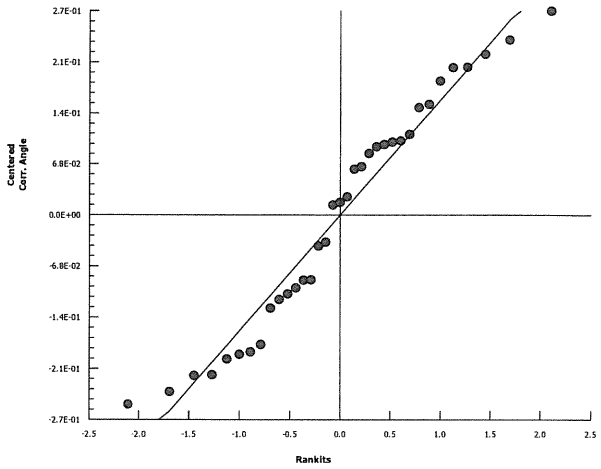
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.256	1.048	1.464	1.269	1.042	1.472	0.0749	13.33%	0.0%
2		5	1.413	1.263	1.563	1.474	1.221	1.521	0.05404	8.55%	-12.51%
4		5	1.377	1.18	1.574	1.472	1.164	1.521	0.07098	11.53%	-9.64%
9		5	1.251	0.9789	1.522	1.164	0.9976	1.524	0.09784	17.49%	0.44%
18		5	1.323	1.088	1.559	1.287	1.137	1.522	0.08498	14.36%	-5.37%
35		5	1.289	1.067	1.511	1.306	1.053	1.524	0.07984	13.85%	-2.63%
76.1		5	1.374	1.206	1.542	1.398	1.191	1.523	0.06056	9.85%	-9.42%

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

Analysis ID:	15-6404-9811	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 16-5680-0379 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-21 11:50 **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	1.13%	76.1	>76.1	NA	1.314

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	29.5	16	1	8	0.9424	Asymp	Non-Significant Effect
		4	29.5	16	1	8	0.9424	Asymp	Non-Significant Effect
		9	28.5	16	1	8	0.9067	Asymp	Non-Significant Effect
		18	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		35	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		76.1	29.5	16	1	8	0.9424	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.001270365	0.0002117274	6	0.1825	0.9793	Non-Significant Effect
Error	0.03248619	0.001160221	28			
Total	0.03375655		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.252	16.81	0.7766	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8603	0.9146	0.0004	Non-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	0.9932	0.9811	1	1	0.9789	1	0.00436	0.98%	0.0%
2		5	0.9964	0.9903	1	1	0.9907	1	0.002192	0.49%	-0.33%
4		5	0.9961	0.9894	1	1	0.9892	1	0.00242	0.54%	-0.29%
9		5	0.995	0.9863	1	1	0.9863	1	0.003105	0.7%	-0.18%
18		5	0.993	0.9801	1	1	0.9767	1	0.004651	1.05%	0.02%
35		5	0.9936	0.9864	1	0.9899	0.9888	1	0.002599	0.58%	-0.05%
76.1		5	0.9961	0.9894	1	1	0.99	1	0.002414	0.54%	-0.29%

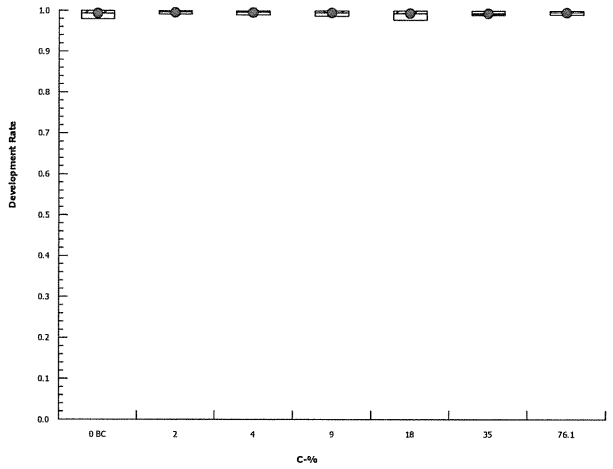
Angular (Corrected) Transformed Summary

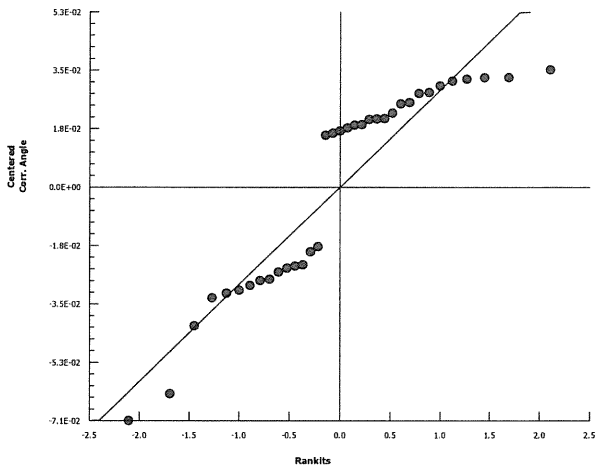
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.488	1.433	1.543	1.517	1.425	1.521	0.01983	2.98%	0.0%
2		5	1.502	1.473	1.532	1.518	1.474	1.521	0.01074	1.6%	-0.97%
4		5	1.501	1.468	1.533	1.517	1.467	1.521	0.01187	1.77%	-0.85%
9		5	1.496	1.453	1.538	1.516	1.453	1.524	0.01529	2.29%	-0.52%
18		5	1.489	1.43	1.547	1.519	1.418	1.522	0.02097	3.15%	-0.04%
35		5	1.488	1.453	1.523	1.47	1.465	1.524	0.01262	1.9%	-0.02%
76.1		5	1.5	1.467	1.534	1.517	1.471	1.523	0.01191	1.78%	-0.85%

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

Analysis ID:	16-5680-0379	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	09-6800-5518	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**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	20.1%	76.1	>76.1	NA	1.314

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-1.533	2.407	0.26	8	0.9977	CDF	Non-Significant Effect
		4	-1.146	2.407	0.26	8	0.9921	CDF	Non-Significant Effect
		9	0.09918	2.407	0.26	8	0.8286	CDF	Non-Significant Effect
		18	-0.6084	2.407	0.26	8	0.9635	CDF	Non-Significant Effect
		35	-0.3261	2.407	0.26	8	0.9276	CDF	Non-Significant Effect
		76.1	-1.154	2.407	0.26	8	0.9923	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1381171	0.02301952	6	0.7892	0.5860	Non-Significant Effect
Error	0.816757	0.02916989	28			
Total	0.9548741		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.001	16.81	0.9856	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9351	0.9146	0.0399	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	0.8922	0.7729	1	0.9314	0.7549	0.9902	0.04296	10.77%	0.0%
2		5	0.9686	0.9051	1	1	0.8824	1	0.02287	5.28%	-8.57%
4		5	0.949	0.8623	1	0.9902	0.8431	1	0.03122	7.36%	-6.37%
9		5	0.8745	0.7286	1	0.8529	0.7157	1	0.05256	13.44%	1.98%
18		5	0.9216	0.8242	1	0.9216	0.8431	1	0.03508	8.51%	-3.3%
35		5	0.9078	0.7865	1	0.9412	0.7549	1	0.04369	10.76%	-1.76%
76.1		5	0.9529	0.8787	1	0.9804	0.8627	1	0.02674	6.28%	-6.81%

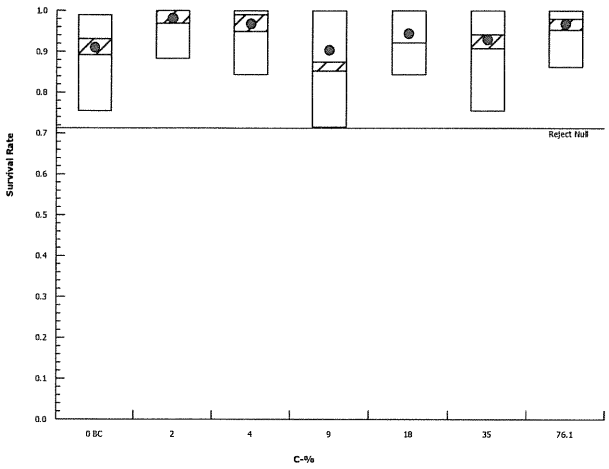
Angular (Corrected) Transformed Summary

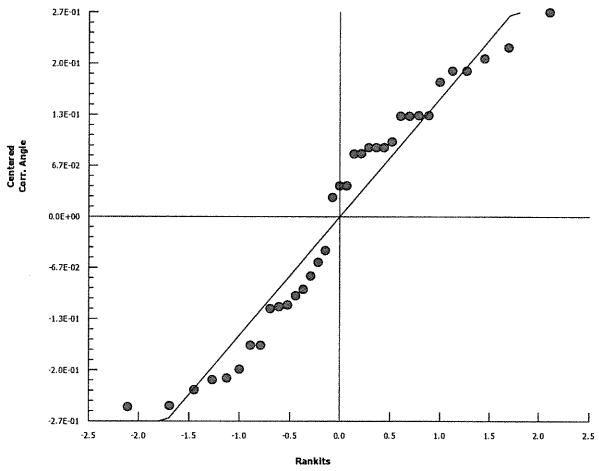
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.266	1.06	1.471	1.306	1.053	1.472	0.07393	13.06%	0.0%
2		5	1.431	1.264	1.598	1.521	1.221	1.521	0.06009	9.39%	-13.08%
4		5	1.389	1.186	1.592	1.472	1.164	1.521	0.07314	11.77%	-9.78%
9		5	1.255	0.9898	1.52	1.177	1.008	1.521	0.09549	17.01%	0.85%
18		5	1.331	1.107	1.556	1.287	1.164	1.521	0.08075	13.56%	-5.19%
35		5	1.301	1.078	1.524	1.326	1.053	1.521	0.0804	13.82%	-2.78%
76.1		5	1.39	1.208	1.572	1.43	1.191	1.521	0.06565	10.56%	-9.85%

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

Analysis ID:	09-6800-5518	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	01-3694-7731	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1409208	1000	Yes	Two-Point Interpolation

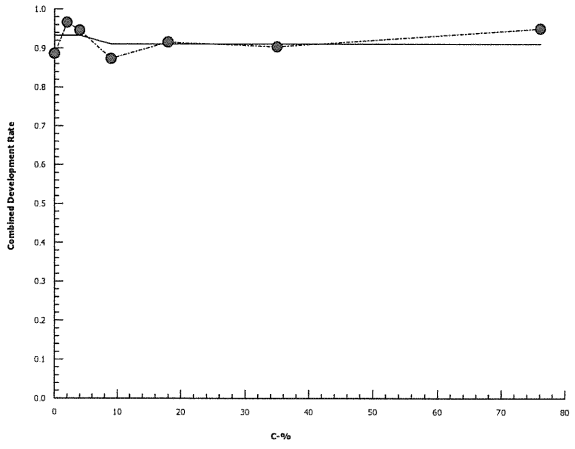
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>76.1	N/A	N/A	<1.314	NA	NA
EC50	>76.1	N/A	N/A	<1.314	NA	NA

Combined 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	0.8863	0.7451	0.9902	0.0438	0.09794	11.05%	0.0%	452	510
2		5	0.9651	0.8824	1	0.02172	0.04856	5.03%	-8.89%	512	530
4		5	0.9453	0.8431	1	0.03117	0.06969	7.37%	-6.66%	493	521
9		5	0.8706	0.7059	1	0.05427	0.1213	13.94%	1.77%	456	522
18		5	0.9157	0.8235	1	0.03842	0.08592	9.38%	-3.32%	469	512
35		5	0.902	0.7549	1	0.04307	0.09631	10.68%	-1.77%	471	521
76.1		5	0.949	0.8627	1	0.02546	0.05693	6.0%	-7.08%	492	518

Graphics



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

Analysis ID:	13-0826-8706	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	309100	1000	Yes	Two-Point Interpolation

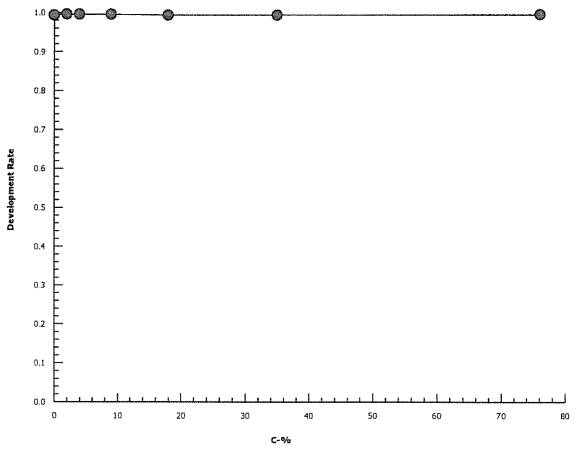
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>76.1	N/A	N/A	<1.314	NA	NA
EC50	>76.1	N/A	N/A	<1.314	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	0.9932	0.9789	1	0.00436	0.009749	0.98%	0.0%	452	455
2		5	0.9964	0.9907	1	0.002192	0.004902	0.49%	-0.33%	512	514
4		5	0.9961	0.9892	1	0.00242	0.005411	0.54%	-0.29%	493	495
9		5	0.995	0.9863	1	0.003105	0.006943	0.7%	-0.18%	456	458
18		5	0.993	0.9767	1	0.004651	0.0104	1.05%	0.02%	469	472
35		5	0.9936	0.9888	1	0.002599	0.005812	0.58%	-0.05%	471	474
76.1		5	0.9961	0.99	1	0.002414	0.005398	0.54%	-0.29%	492	494

Graphics



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

Analysis ID:	04-3330-6583	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 11:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1369266	1000	Yes	Two-Point Interpolation

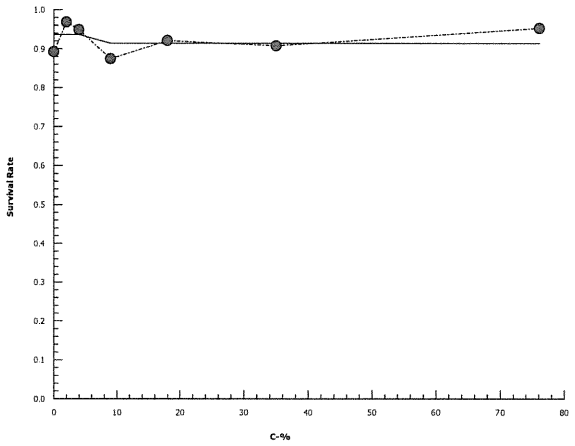
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>76.1	N/A	N/A	<1.314	NA	NA
EC50	>76.1	N/A	N/A	<1.314	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	0.8922	0.7549	0.9902	0.04296	0.09606	10.77%	0.0%	455	510
2		5	0.9686	0.8824	1	0.02287	0.05113	5.28%	-8.57%	494	510
4		5	0.949	0.8431	1	0.03122	0.06981	7.36%	-6.37%	484	510
9		5	0.8745	0.7157	1	0.05256	0.1175	13.44%	1.98%	446	510
18		5	0.9216	0.8431	1	0.03508	0.07843	8.51%	-3.3%	470	510
35		5	0.9078	0.7549	1	0.04369	0.0977	10.76%	-1.76%	463	510
76.1		5	0.9529	0.8627	1	0.02674	0.0598	6.28%	-6.81%	486	510

Graphics



CETIS Test Data Worksheet

Report Date: 21 Sep-21 14:11 (p 1 of 1)
Test Code: 2109-S088 20-8006-6372/2109-S088

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

Start Date: 29 22 Sep-21 **Species:** Mytilus galloprovincialis **Sample Code:** 21- 0991
End Date: @01 24 ~~Sep~~ Oct-21 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Jacobs
Sample Date: 25 21 Sep-21 **Material:** Effluent Sample **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			94	94	AFS 10/19/21
			2			95	93	
			3			102	102	
			4			96	95	
			5			117	117	
			6			77	76	
			7			98	98	
			8			116	115	
			9			89	88	
			10			103	102	
			11			113	112	
			12			99	98	
			13			93	92	
			14			109	109	
			15			89	89	
			16			85	85	
			17			86	84	
			18			100	100	
			19			111	111	
			20			100	99	
			21			86	85	
			22			90	90	
			23			104	104	
			24			101	101	
			25			88	88	
			26			101	101	
			27			77	77	
			28			82	81	
			29			114	114	
			30			108	107	
			31			102	102	
			32			113	113	
			33			102	102	
			34			93	92	
			35			94	94	
			36			97	97	
			37			86	86	
			38			73	72	
			39			84	84	
			40			87	86	

@QA BS 10/21/21

Analyst: AFS 10 QA: BS 10/21/21
QA AFS 10/19/21

CETIS Test Data Worksheet

Test Code: 2109-S088 20-8006-6372/2109-S088

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

Start Date: 29~~22~~ Sep-21 Species: Mytilus galloprovincialis Sample Code: 21- 0991
End Date: @ ~~24 Sep-21~~ 1-Oct-21 Protocol: EPA/600/R-95/136 (1995) Sample Source: Jacobs
Sample Date: ~~21~~ 28 Sep-21 Material: Effluent Sample Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	6					
0	BC	2	16					
0	BC	3	24			98	98	10/2/21
0	BC	4	36					
0	BC	5	2					
0	LC	1	28					
0	LC	2	13			113	113	
0	LC	3	5					
0	LC	4	19					
0	LC	5	15					
2		1	30					
2		2	22					
2		3	31			92	92	
2		4	7					
2		5	8					
4		1	11					
4		2	33					
4		3	34			81	80	
4		4	37					
4		5	26					
9		1	38					
9		2	18					
9		3	29			104	104	
9		4	39					
9		5	40					
18		1	23					
18		2	17					
18		3	3			91	91	
18		4	1					
18		5	21					
35		1	9					
35		2	12					
35		3	32			105	104	
35		4	27					
35		5	4					
72.4		1	25					
72.4		2	14					
72.4		3	20			84	83	
72.4		4	35					
72.4		5	10					

76.1

Ⓐ QC = OK

@QC BB 9/25/21

Ⓐ QC AS 9/21/21

Marine Chronic Bioassay
DM-014

Water Quality Measurements

Client: JACOBS
Sample ID: Wyckoff
Sample Log No.: 21- 0991
Test No.: 2109-S088

Test Species: *M. galloprovincialis*
Start Date/Time: ~~9/22/2021~~ 9/29/21 1545
End Date/Time: ~~9/24/2021~~ @ 10/1/21 1440

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	30.0	30.1	30.1	15.1	15.4	15.2	8.6	8.7	8.5	8.04	7.87	7.98
Brine Control	29.8	30.1	30.1	15.0	15.2	15.0	8.6	8.7	8.5	8.09	7.91	7.99
2	30.0	30.4	30.4	15.1	15.3	15.2	8.6	8.7	8.4	8.06	7.92	7.99
4	30.0	30.3	30.4	15.0	15.3	15.1	8.6	8.7	8.5	8.01	7.93	8.04
9	30.1	30.5	30.6	14.8	15.1	14.9	8.7	8.7	8.5	7.97	7.95	8.07
18	29.9	30.3	30.3	14.9	15.3	15.2	8.7	8.7	8.4	7.89	7.95	8.13
35	29.5	29.9	29.9	14.6	15.4	15.3	8.8	8.7	8.4	7.79	7.98	8.19
76.1 ⑥	~~29.5~~	29.9	30.0	14.2	15.2	15.0	8.9	8.7	8.5	7.71	8.04	8.28

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

Technician Initials:

Environmental Chamber: D.

Comments:
0 hrs: @ @18BO 9/23/21 ⑥ @ 8 RT 9/24/21
24 hrs:
48 hrs:

QC Check: JL 10/11/21

Final Review: AFS 10/19/21

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** KS

Sample ID: Wyckoff **Test Date:** 9/29/2021

Test No: 2109- SO 88 **Test Type:** Mussel Development

Salinity of Effluent 10.6

Salinity of Brine 91.8 **Date of Brine used:** 9/28/2021

Target Salinity 30 **Alkalinity of Brine Control:** 116 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.31**	**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	0.31	1.6	250
4	10.0	0.31	3.1	250
9	22.5	0.31	7.1	250
18	45.0	0.31	14.1	250
35	87.5	0.31	27.5	250
76.1	190.3	0.31	59.7	250

	DI Volume			
Brine Control	123.0	0.49	59.7	250

Total Brine Volume Required (ml): **172.8**

QC Check: JL 10/11/21 **Final Review:** AS 10/19/21

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

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: JACOBS / WYCKOFF
Test No.: 2109-5088
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP SB
Date Received: 9/14/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 9/29/2021 1545
End Date/Time: 10/1/2021 1440
Technician Initials: BS

Spawn Information

First Gamete Release Time: 11:05

Sex	Number Spawning
Male	2
Female	2

Gamete Selection

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

Egg Fertilization Time: 12:00

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: 1

Embryo Inoculum Preparation

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

Number Counted:		
	4	4
	6	3
	8	9
	9	5
	8	5

Mean: 6.1

Mean 6.1 X 50 = 305 embryos/ml

Initial Density: 305 = 1.02 (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	113	113	100.0	100.0
TØ B	99	99	100.0	
TØ C	108	108	100.0	
TØ D	94	94	100.0	
TØ E	98	98	100.0	
TØ F	101	101	100.0	
$\bar{x}$ =	102			

48-h QC: 103/103 100%

Comments:

QC Check: JL 10/11/21

Final Review: ACS 10/19/21

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff Eagle Harbor GWTP
Test ID No(s).: 2109-5088 (092821)

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample Description: A: no color, clear, no odor, no debris

Sample (A, B, C):	A			
Log-in No. (21-xxxx):	0991			
Sample Collection Date & Time:	9/28/21 0925			
Sample Receipt Date & Time:	9/29/21 0940			
Number of Containers & Container Type:	HDPE			
Approx. Total Volume Received (L):	~1			
Check-in Temperature (°C)	4.0			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.9			
pH (units)	7.56			
Conductivity (µS/cm)	—			
Salinity (ppt)	10.6			
Alkalinity (mg/L) [2]	109			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	<0.02			
Technician Initials	HM			

Subsamples for Additional Chemistry Required:
(NH3) (always required)
Other _____
Tech Initials A HM B___ C___

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

Filtration? Y (N) Initials: _____
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.			

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 Alkalinity: 116 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 at receipt.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: _____

QC Check: JL 10/4/21
Final Review: AS 10/19/21

Total Ammonia Analysis
Marine

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
SW Blank: 0.0

Test Start Date: 9/29/21

Analyst: KB
Analysis Date: 20/21/21

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	8.8	10.7
Wyckoff	21-0991	~~9/22/2021~~ 9/29/21	0	2.3	2.8
Spike Check (10 mg/L NH_3)		NA	NA	8.8	10.7
Wyckoff	21-0991	9/29/21	0	2.3	2.8
Sample Duplicate[a]		NA	NA	2.5	~~3.0~~ @2 3.1
Sample Duplicate + Spike[a]		NA	NA	11.3	13.8
Spike Check (10 mg/L NH_3)		NA	NA	8.8	10.7

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	10.7	10	NA	107
21-0991	2.8	~~@2 3.0~~ 3.1	13.8	10	~~@2 6.9~~ 10.2	110

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A1053	A1183	A1189

Comments: @Q8 BO 9/23/21 @2 Q8 BO 10/21/21

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.

HACH Ammonia Nitrogen Test Kit, Test 'N Tube™ Vials. Method 10031. Method Detection Limit = 0.5 mg/L

QC Check: ~~BO 10/21/21~~ Q8 RT 10/21/21 RT 10/21/21

Final Review: BO 10/22/21

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 9/28/2021
CarrierName: FedEx
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2020/WA
Project Code: WEH-031D
Cooler #: 1 of 1

No: 10-092821-103857-0557
2021T10P000DD210W2LA00
Contact Name: Daniel Baca
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
092821		Ground Water/ D. Baca	Composite	CHRTOX(8 Weeks)	N (< 6 C) (1)	SP-11	09/28/2021 09:28	Field Sample

Special Instructions: 2021-Week40 Tox2

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
	JACOBS	9/28/21 @ 1102	Haylie Reinemann GA SD	9/29/21 0940	

Receipt temp. 4.0°C
ID: 21-0991

Appendix D
List of Qualifier Codes

Glossary of Qualifier Codes

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

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

Q3 - Sample pH adjusted to within range of 6-9 with reagent grade NaOH or HCl, as needed

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

Q5 - Test initiated with continuous 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, partial 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. Test results were reviewed and reported in accordance with guidance found in EPA-833-R-00-003, 2000 unless otherwise specified.

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. Test results were reviewed and reported in accordance with EPA-833-R-00-003, 2000 guidance unless otherwise specified.

Q18 - Incorrect or illegible Entry

Q19 - Miscalculation

Q20 - PMSD criteria do not apply to the test of significant toxicity (TST) analysis

Q21 - Other (provide reason in comments section)

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

Q23 - Test organisms experienced a temperature shift greater than 3°C within 1 day or were received at a temperature greater than 3°C outside the recommended test temperature range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.

Q24 - Test organisms experienced a salinity shift greater than 3 ppt within 1 day or were received at a salinity greater than 3 ppt outside the recommended test salinity range and had minimal time to acclimate prior to test initiation. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate test(s). Organisms were ultimately deemed fit to use for testing.

Appendix E
Reference Toxicant Test Results

CETIS Summary Report

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

Batch ID:	04-0234-6365	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	29 Sep-21 15:45	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	01 Oct-21 14:40	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	47h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	03-2935-6953	**Code:**	210929msdv	**Client:**	Internal
Sample Date:	29 Sep-21	**Material:**	Copper chloride	**Project:**	
Receive Date:	29 Sep-21	**Source:**	Reference Toxicant		
Sample Age:	16h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-4465-0530	Combined Development Ra	5	10	7.071	7.19%		Dunnett Multiple Comparison Test
03-7320-3141	Development Rate	5	10	7.071	2.13%		Dunnett Multiple Comparison Test
15-1856-8764	Survival Rate	10	20	14.14	7.23%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
18-2247-7613	Combined Development Ra	EC25	7.261	6.428	7.863		Linear Interpolation (ICPIN)
		EC50	9.809	8.858	11.27		
04-7958-3381	Development Rate	EC25	7.359	6.946	7.806		Linear Interpolation (ICPIN)
		EC50	9.718	8.899	10.99		
17-8563-2416	Survival Rate	EC25	22.73	18.51	25.31		Linear Interpolation (ICPIN)
		EC50	28.5	25.72	30.22		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-7320-3141	Development Rate	Control Resp	0.9857	0.9 - NL	Yes	Passes Acceptability Criteria
04-7958-3381	Development Rate	Control Resp	0.9857	0.9 - NL	Yes	Passes Acceptability Criteria
15-1856-8764	Survival Rate	Control Resp	0.9804	0.5 - NL	Yes	Passes Acceptability Criteria
17-8563-2416	Survival Rate	Control Resp	0.9804	0.5 - NL	Yes	Passes Acceptability Criteria
13-4465-0530	Combined Development Ra	PMSD	0.07189	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	0.967	0.8945	1	0.8627	1	0.02612	0.0584	6.04%	0.0%
2.5		5	0.9392	0.8476	1	0.8333	1	0.03301	0.07382	7.86%	2.87%
5		5	0.9392	0.8574	1	0.8431	1	0.02948	0.06591	7.02%	2.87%
10		5	0.4663	0.3686	0.5641	0.3545	0.5463	0.03521	0.07874	16.88%	51.77%
20		5	0.001961	0	0.007405	0	0.009804	0.001961	0.004384	223.6%	99.8%
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	0.9857	0.9648	1	0.9565	1	0.007521	0.01682	1.71%	0.0%
2.5		5	0.9916	0.9744	1	0.9681	1	0.006193	0.01385	1.4%	-0.6%
5		5	0.9956	0.988	1	0.9885	1	0.002714	0.006069	0.61%	-1.0%
10		5	0.4663	0.3686	0.5641	0.3545	0.5463	0.03521	0.07874	16.88%	52.69%
20		5	0.002564	0	0.009683	0	0.01282	0.002564	0.005734	223.6%	99.74%
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	0.9804	0.926	1	0.902	1	0.01961	0.04384	4.47%	0.0%
2.5		5	0.9471	0.8586	1	0.8333	1	0.03186	0.07124	7.52%	3.4%
5		5	0.9431	0.8669	1	0.8529	1	0.02745	0.06138	6.51%	3.8%
10		5	1	1	1	1	1	0	0	0.0%	-2.0%
20		5	0.851	0.6905	1	0.6765	1	0.05778	0.1292	15.18%	13.2%
40		5	0.001961	0	0.007405	0	0.009804	0.001961	0.004384	223.6%	99.8%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8627	0.9906	0.9904	1	0.9912
2.5		0.8333	0.9706	1	1	0.8922
5		1	0.9118	0.9412	0.8431	1
10		0.5463	0.5357	0.3545	0.4336	0.4615
20		0	0	0	0.009804	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	0.9565	0.9906	0.9904	1	0.9912
2.5		1	0.99	1	1	0.9681
5		1	0.9894	1	0.9885	1
10		0.5463	0.5357	0.3545	0.4336	0.4615
20		0	0	0	0.01282	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	0.902	1	1	1	1
2.5		0.8333	0.9804	1	1	0.9216
5		1	0.9216	0.9412	0.8529	1
10		1	1	1	1	1
20		0.8922	0.6765	1	0.7647	0.9216
40		0	0.009804	0	0	0

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

Combined Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	88/102	105/106	103/104	117/117	112/113
2.5		85/102	99/102	118/118	111/111	91/102
5		106/106	93/102	96/102	86/102	117/117
10		59/108	60/112	39/110	49/113	48/104
20		0/102	0/102	0/103	1/102	0/102
40		0/102	0/102	0/102	0/102	0/102

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	88/92	105/106	103/104	117/117	112/113
2.5		85/85	99/100	118/118	111/111	91/94
5		106/106	93/94	96/96	86/87	117/117
10		59/108	60/112	39/110	49/113	48/104
20		0/91	0/69	0/103	1/78	0/94
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	92/102	102/102	102/102	102/102	102/102
2.5		85/102	100/102	102/102	102/102	94/102
5		102/102	94/102	96/102	87/102	102/102
10		102/102	102/102	102/102	102/102	102/102
20		91/102	69/102	102/102	78/102	94/102
40		0/102	1/102	0/102	0/102	0/102

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

Analysis ID: 13-4465-0530 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-21 14:25 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.19%	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	0.7701	2.305	0.183	8	0.4819	CDF	Non-Significant Effect
		5	0.8397	2.305	0.183	8	0.4506	CDF	Non-Significant Effect
		10*	8.524	2.305	0.183	8	<0.0001	CDF	Significant Effect
		20*	17.24	2.305	0.183	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	6.970155	1.742539	4	110.7	<0.0001	Significant Effect
Error	0.3148416	0.01574208	20			
Total	7.284997		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	11.52	13.28	0.0213	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9402	0.8877	0.1500	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	0.967	0.8945	1	0.9906	0.8627	1	0.02612	6.04%	0.0%
2.5		5	0.9392	0.8476	1	0.9706	0.8333	1	0.03301	7.86%	2.87%
5		5	0.9392	0.8574	1	0.9412	0.8431	1	0.02948	7.02%	2.87%
10		5	0.4663	0.3686	0.5641	0.4615	0.3545	0.5463	0.03521	16.88%	51.77%
20		5	0.001961	0	0.007405	0	0	0.009804	0.001961	223.6%	99.8%
40		5	0	0	0	0	0	0	0		100.0%

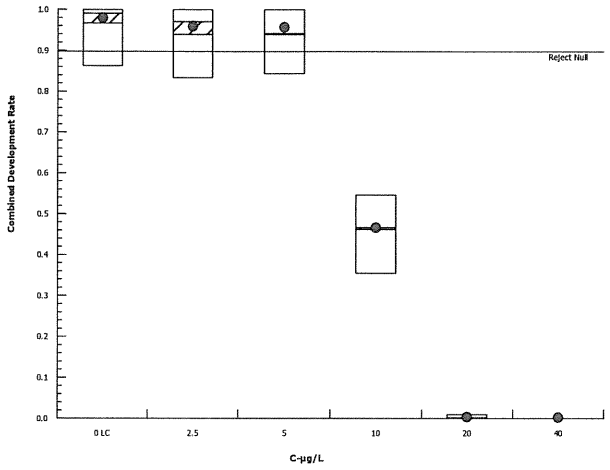
Angular (Corrected) Transformed Summary

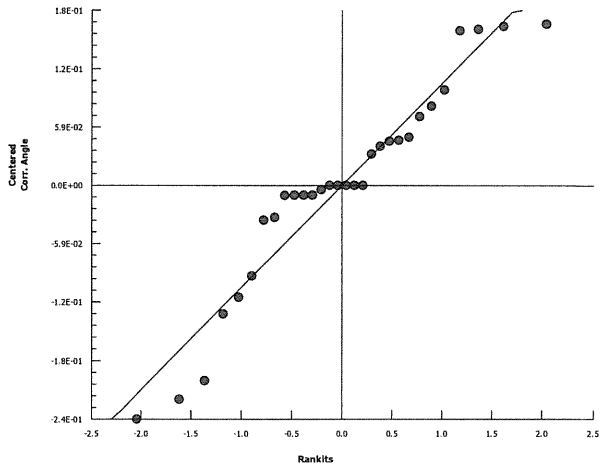
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.428	1.261	1.594	1.474	1.191	1.525	0.05991	9.38%	0.0%
2.5		5	1.367	1.157	1.577	1.398	1.15	1.525	0.07563	12.37%	4.28%
5		5	1.361	1.163	1.559	1.326	1.164	1.525	0.0712	11.7%	4.67%
10		5	0.7513	0.6525	0.85	0.7469	0.6378	0.8318	0.03557	10.59%	47.38%
20		5	0.05941	0.03181	0.08701	0.04953	0.04929	0.09918	0.009942	37.42%	95.84%
40		5	0.04953	0.04952	0.04954	0.04953	0.04953	0.04953	0	0.0%	96.53%

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

Analysis ID:	13-4465-0530	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 14:25	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 03-7320-3141 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-21 14:25 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.13%	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	-0.6849	2.305	0.08	8	0.9482	CDF	Non-Significant Effect
		5	-1.095	2.305	0.08	8	0.9812	CDF	Non-Significant Effect
		10*	20.54	2.305	0.08	8	<0.0001	CDF	Significant Effect
		20*	40.37	2.305	0.08	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.097662	2.024415	4	676.8	<0.0001	Significant Effect
Error	0.05981922	0.002990961	20			
Total	8.157481		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.405	13.28	0.2482	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9405	0.8877	0.1519	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	0.9857	0.9648	1	0.9906	0.9565	1	0.007521	1.71%	0.0%
2.5		5	0.9916	0.9744	1	1	0.9681	1	0.006194	1.4%	-0.6%
5		5	0.9956	0.988	1	1	0.9885	1	0.002714	0.61%	-1.0%
10		5	0.4663	0.3686	0.5641	0.4615	0.3545	0.5463	0.03521	16.88%	52.69%
20		5	0.002564	0	0.009683	0	0	0.01282	0.002564	223.6%	99.74%
40		5	0	0	0	0	0	0	0		100.0%

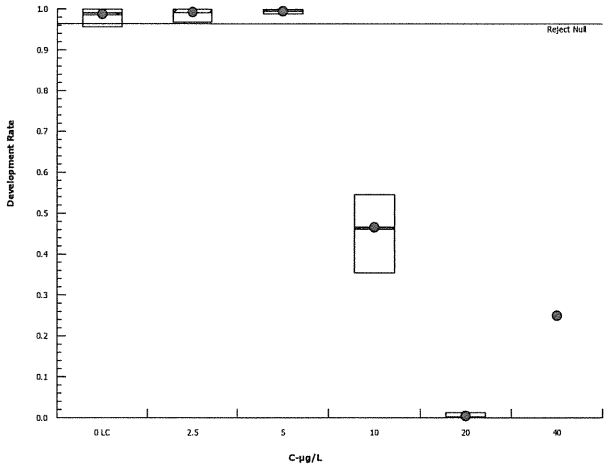
Angular (Corrected) Transformed Summary

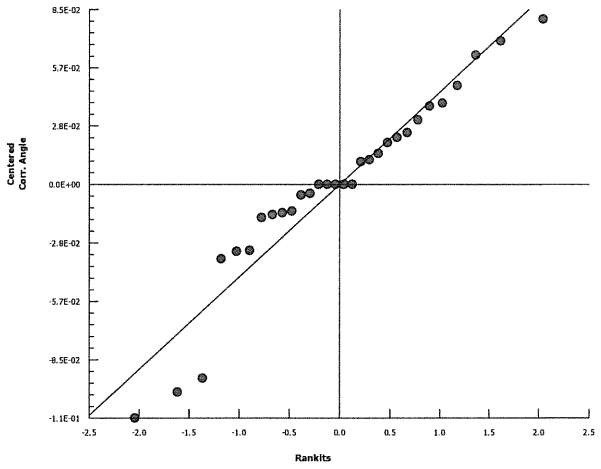
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.462	1.387	1.537	1.474	1.361	1.525	0.02704	4.14%	0.0%
2.5		5	1.485	1.414	1.556	1.517	1.391	1.525	0.02554	3.85%	-1.62%
5		5	1.499	1.461	1.538	1.52	1.463	1.525	0.01394	2.08%	-2.59%
10		5	0.7513	0.6525	0.85	0.7469	0.6378	0.8318	0.03557	10.59%	48.6%
20		5	0.0654	0.03165	0.09916	0.05244	0.04929	0.1135	0.01216	41.56%	95.53%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	64.18%

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

Analysis ID:	03-7320-3141	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 14:25	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 15-1856-8764 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-21 14:25 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.23%	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	0.9985	2.362	0.202	8	0.4206	CDF	Non-Significant Effect
		5	1.18	2.362	0.202	8	0.3427	CDF	Non-Significant Effect
		10	-0.6284	2.362	0.202	8	0.9558	CDF	Non-Significant Effect
		20*	2.951	2.362	0.202	8	0.0143	CDF	Significant Effect
		40*	16.45	2.362	0.202	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.653782	1.530756	5	83.56	<0.0001	Significant Effect
Error	0.4396555	0.01831898	24			
Total	8.093437		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.52	4.248	0.0215	Equal Variances
Variances	Levene Equality of Variance	4.565	3.895	0.0046	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9432	0.9031	0.1106	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	0.9804	0.926	1	1	0.902	1	0.01961	4.47%	0.0%
2.5		5	0.9471	0.8586	1	0.9804	0.8333	1	0.03186	7.52%	3.4%
5		5	0.9431	0.8669	1	0.9412	0.8529	1	0.02745	6.51%	3.8%
10		5	1	1	1	1	1	1	0	0.0%	-2.0%
20		5	0.851	0.6905	1	0.8922	0.6765	1	0.05778	15.18%	13.2%
40		5	0.001961	0	0.007405	0	0	0.009804	0.001961	223.6%	99.8%

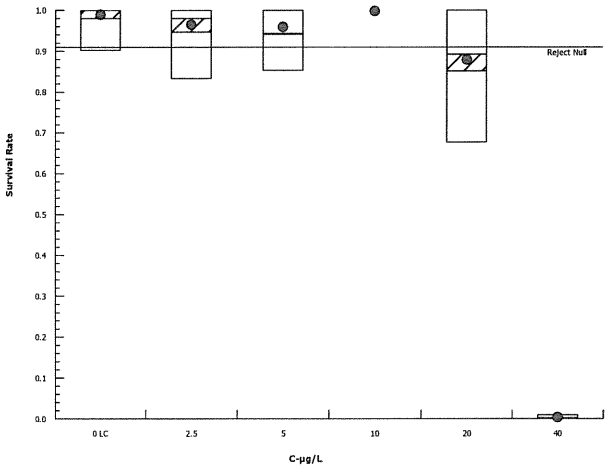
Angular (Corrected) Transformed Summary

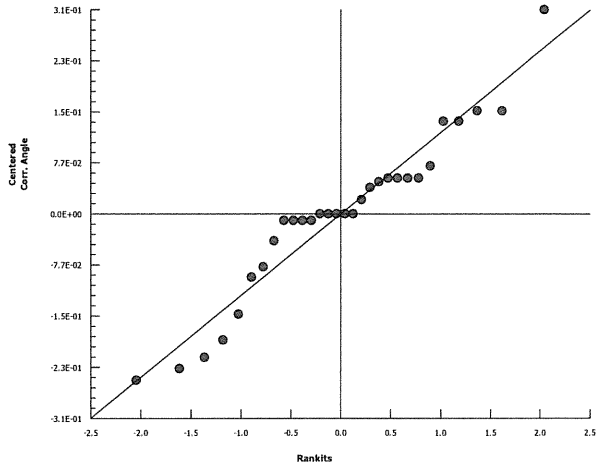
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.467	1.318	1.617	1.521	1.252	1.521	0.05379	8.2%	0.0%
2.5		5	1.382	1.182	1.582	1.43	1.15	1.521	0.07206	11.66%	5.82%
5		5	1.367	1.178	1.555	1.326	1.177	1.521	0.06772	11.08%	6.88%
10		5	1.521	1.521	1.522	1.521	1.521	1.521	0	0.0%	-3.67%
20		5	1.215	0.9484	1.481	1.236	0.9658	1.521	0.09598	17.67%	17.21%
40		5	0.05946	0.03189	0.08703	0.04953	0.04953	0.09918	0.00993	37.34%	95.95%

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

Analysis ID:	15-1856-8764	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 14:25	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	18-2247-7613	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 14:27	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	598715	1000	Yes	Two-Point Interpolation

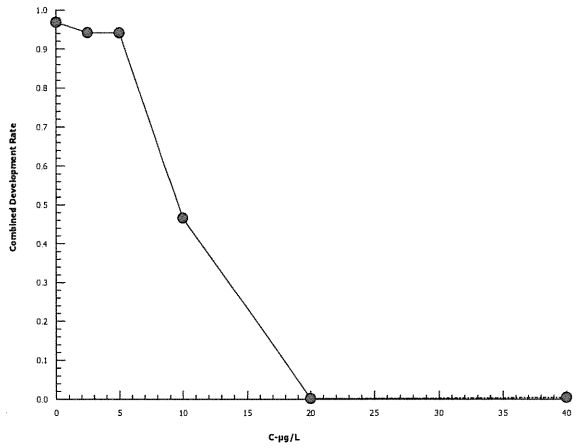
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	7.261	6.428	7.863
EC50	9.809	8.858	11.27

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	0.967	0.8627	1	0.02612	0.0584	6.04%	0.0%	525	542
2.5		5	0.9392	0.8333	1	0.03301	0.07382	7.86%	2.87%	504	535
5		5	0.9392	0.8431	1	0.02948	0.06591	7.02%	2.87%	498	529
10		5	0.4663	0.3545	0.5463	0.03521	0.07874	16.88%	51.77%	255	547
20		5	0.001961	0	0.009804	0.001961	0.004384	223.6%	99.8%	1	511
40		5	0	0	0	0	0		100.0%	0	510

Graphics



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

Analysis ID:	04-7958-3381	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 14:28	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	295463	1000	Yes	Two-Point Interpolation

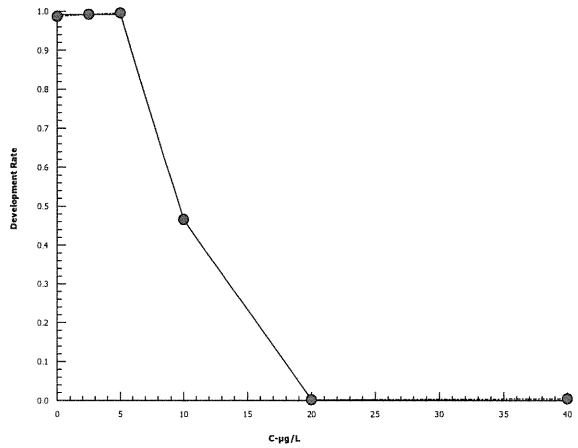
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	7.359	6.946	7.806
EC50	9.718	8.899	10.99

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	0.9857	0.9565	1	0.007521	0.01682	1.71%	0.0%	525	532
2.5		5	0.9916	0.9681	1	0.006194	0.01385	1.4%	-0.6%	504	508
5		5	0.9956	0.9885	1	0.002714	0.00607	0.61%	-1.0%	498	500
10		5	0.4663	0.3545	0.5463	0.03521	0.07874	16.88%	52.69%	255	547
20		5	0.002564	0	0.01282	0.002564	0.005734	223.6%	99.74%	1	435
40		5	0	0	0	0	0		100.0%	0	5

Graphics



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

Analysis ID:	17-8563-2416	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-21 14:27	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	420427	1000	Yes	Two-Point Interpolation

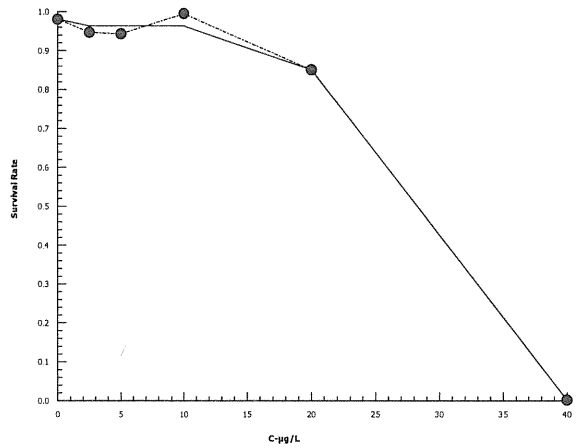
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	22.73	18.51	25.31
EC50	28.5	25.72	30.22

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	0.9804	0.902	1	0.01961	0.04384	4.47%	0.0%	500	510
2.5		5	0.9471	0.8333	1	0.03186	0.07124	7.52%	3.4%	483	510
5		5	0.9431	0.8529	1	0.02745	0.06138	6.51%	3.8%	481	510
10		5	1	1	1	0	0	0.0%	-2.0%	510	510
20		5	0.851	0.6765	1	0.05778	0.1292	15.18%	13.2%	434	510
40		5	0.001961	0	0.009804	0.001961	0.004384	223.6%	99.8%	1	510

Graphics

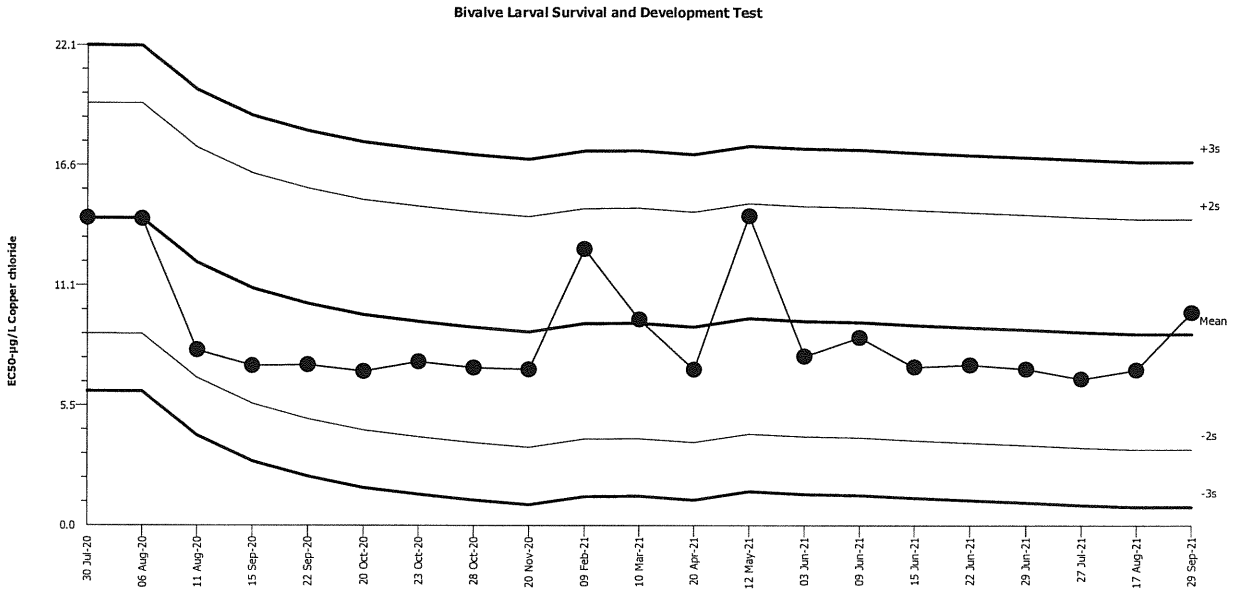


CETIS QC Plot

Report Date: 19 Oct-21 14:29 (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:	8.81	**Count:**	20	**-2s Warning Limit:**	3.496	**-3s Action Limit:**	0.839
Sigma:	2.657	**CV:**	30.20%	**+2s Warning Limit:**	14.12	**+3s Action Limit:**	16.78

Quality Control Data

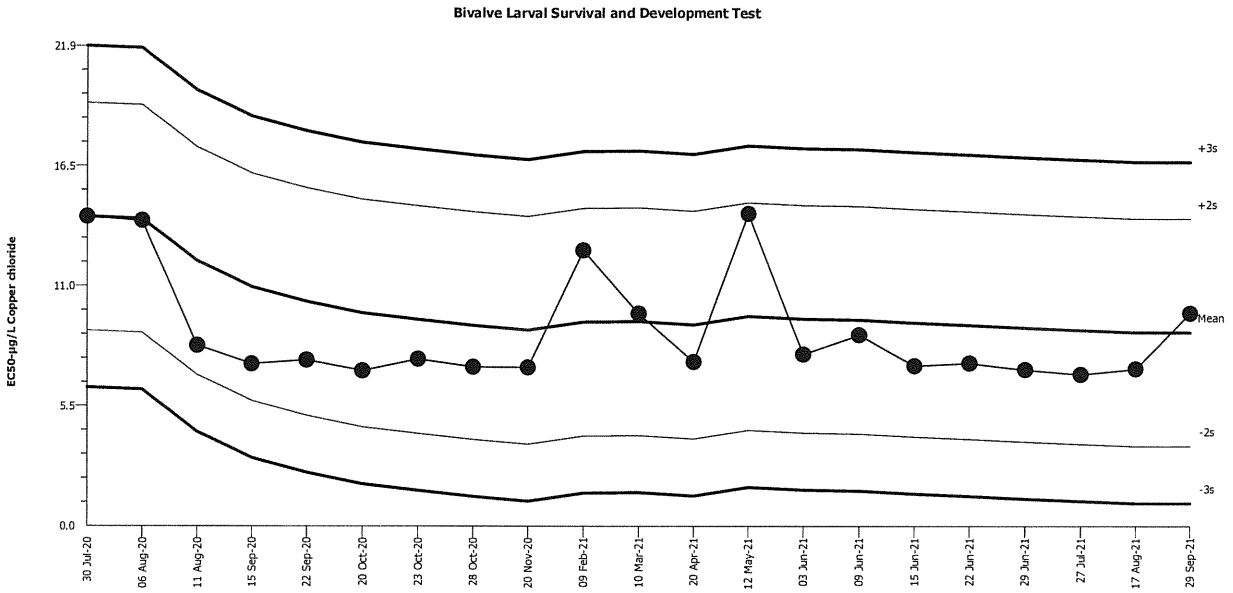
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jul	30	15:35	14.17	5.355	2.015	(+)		00-9901-5729	19-4020-2576
2		Aug	6	15:40	14.13	5.315	2.001	(+)		01-4440-0014	02-9592-9535
3			11	14:30	8.085	-0.7246	-0.2727			21-4043-5119	05-6052-3343
4		Sep	15	0:00	7.365	-1.445	-0.5438			19-9833-0655	18-5101-1090
5			22	14:40	7.405	-1.405	-0.5288			04-0347-9113	09-6026-9613
6		Oct	20	14:25	7.1	-1.71	-0.6434			08-8652-5764	17-2783-6415
7			23	13:45	7.548	-1.262	-0.4748			09-8413-3498	19-3049-9702
8			28	15:50	7.269	-1.541	-0.58			09-4043-4676	02-6542-7057
9		Nov	20	16:00	7.187	-1.623	-0.6108			13-7696-8009	10-4367-1427
10	2021	Feb	9	15:15	12.74	3.929	1.479			12-5648-6062	18-1503-3303
11		Mar	10	14:15	9.481	0.6707	0.2524			13-7922-5399	10-0885-9755
12		Apr	20	16:15	7.185	-1.625	-0.6116			06-7450-9711	18-3353-6875
13		May	12	15:00	14.27	5.458	2.054	(+)		15-4594-3065	00-9727-8504
14		Jun	3	15:50	7.791	-1.019	-0.3834			07-9391-2508	21-2212-7050
15			9	14:00	8.654	-0.1565	-0.05889			18-5736-8495	04-4549-3405
16			15	15:40	7.302	-1.508	-0.5677			00-2993-6780	17-7654-7354
17			22	13:45	7.404	-1.406	-0.5292			16-6840-3553	15-2803-6917
18			29	14:55	7.211	-1.599	-0.6017			07-2040-2693	08-8247-6801
19		Jul	27	16:30	6.748	-2.062	-0.7759			16-6019-6958	06-5859-7928
20		Aug	17	14:25	7.168	-1.642	-0.6182			07-7298-7649	09-6648-5411
21		Sep	29	15:45	9.809	0.9992	0.376			12-3450-8829	18-2247-7613

CETIS QC Plot

Report Date: 19 Oct-21 14:30 (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:	8.85	**Count:**	20	**-2s Warning Limit:**	3.644	**-3s Action Limit:**	1.041
Sigma:	2.603	**CV:**	29.40%	**+2s Warning Limit:**	14.06	**+3s Action Limit:**	16.66

Quality Control Data

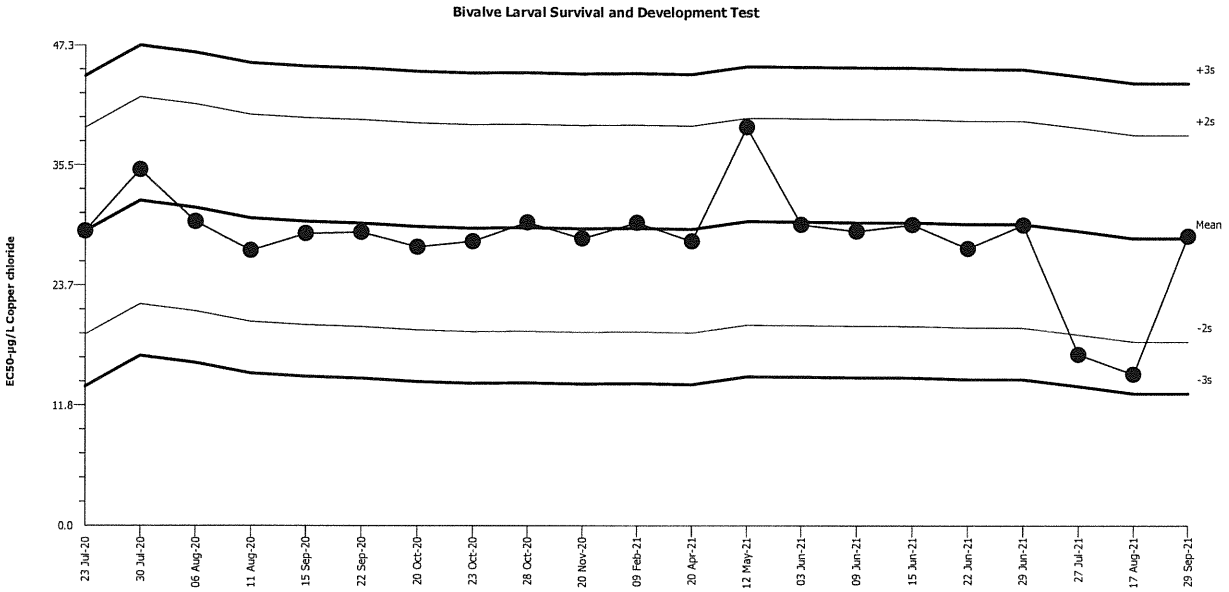
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jul	30	15:35	14.14	5.287	2.031	(+)		00-9901-5729	02-7058-2757
2		Aug	6	15:40	13.95	5.1	1.959			01-4440-0014	13-7910-6508
3			11	14:30	8.237	-0.613	-0.2355			21-4043-5119	01-1240-7098
4		Sep	15	0:00	7.397	-1.453	-0.5581			19-9833-0655	03-7616-5506
5			22	14:40	7.576	-1.274	-0.4893			04-0347-9113	01-0437-7711
6		Oct	20	14:25	7.089	-1.761	-0.6763			08-8652-5764	06-9681-8469
7			23	13:45	7.616	-1.234	-0.4741			09-8413-3498	17-5257-3346
8			28	15:50	7.257	-1.593	-0.612			09-4043-4676	12-0840-2779
9		Nov	20	16:00	7.23	-1.62	-0.6225			13-7696-8009	11-4264-3018
10	2021	Feb	9	15:15	12.58	3.733	1.434			12-5648-6062	01-5747-2564
11		Mar	10	14:15	9.694	0.8437	0.3241			13-7922-5399	08-4869-7631
12		Apr	20	16:15	7.482	-1.368	-0.5256			06-7450-9711	17-9210-1733
13		May	12	15:00	14.27	5.418	2.081	(+)		15-4594-3065	12-3891-6641
14		Jun	3	15:50	7.832	-1.018	-0.391			07-9391-2508	11-7075-1183
15			9	14:00	8.715	-0.1346	-0.05172			18-5736-8495	18-6125-5477
16			15	15:40	7.302	-1.548	-0.5948			00-2993-6780	13-6998-5313
17			22	13:45	7.427	-1.423	-0.5468			16-6840-3553	07-3347-2243
18			29	14:55	7.132	-1.718	-0.6601			07-2040-2693	17-0989-5973
19		Jul	27	16:30	6.912	-1.938	-0.7447			16-6019-6958	03-0913-6262
20		Aug	17	14:25	7.168	-1.682	-0.6464			07-7298-7649	11-4901-9823
21		Sep	29	15:45	9.718	0.8679	0.3334			12-3450-8829	04-7958-3381

CETIS QC Plot

Report Date: 19 Oct-21 14:30 (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:	28.29	**Count:**	20	**-2s Warning Limit:**	18.08	**-3s Action Limit:**	12.98
Sigma:	5.101	**CV:**	18.00%	**+2s Warning Limit:**	38.49	**+3s Action Limit:**	43.59

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Jul	23	15:00	28.98	0.6898	0.1352			06-0741-6264	11-2012-0880
2			30	15:35	35.02	6.728	1.319			00-9901-5729	18-8992-7280
3		Aug	6	15:40	29.92	1.633	0.3202			01-4440-0014	05-9348-7696
4			11	14:30	27.06	-1.231	-0.2414			21-4043-5119	16-7506-8565
5		Sep	15	0:00	28.73	0.4357	0.08541			19-9833-0655	01-9900-7404
6			22	14:40	28.86	0.5736	0.1125			04-0347-9113	03-4439-9784
7		Oct	20	14:25	27.4	-0.8939	-0.1752			08-8652-5764	01-6350-7777
8			23	13:45	27.94	-0.3483	-0.06829			09-8413-3498	02-1232-2390
9			28	15:50	29.82	1.529	0.2998			09-4043-4676	15-7574-6891
10		Nov	20	16:00	28.24	-0.04627	-0.009071			13-7696-8009	21-0824-4197
11	2021	Feb	9	15:15	29.8	1.515	0.2969			12-5648-6062	08-9593-0094
12		Apr	20	16:15	27.97	-0.3192	-0.06259			06-7450-9711	02-2099-4435
13		May	12	15:00	39.23	10.94	2.145	(+)		15-4594-3065	18-1677-8776
14		Jun	3	15:50	29.62	1.328	0.2603			07-9391-2508	05-7225-1680
15			9	14:00	28.97	0.6764	0.1326			18-5736-8495	17-4075-5383
16			15	15:40	29.61	1.321	0.2591			00-2993-6780	11-7676-4213
17			22	13:45	27.27	-1.023	-0.2006			16-6840-3553	00-7652-1305
18			29	14:55	29.58	1.295	0.2538			07-2040-2693	20-9452-4039
19		Jul	27	16:30	16.82	-11.47	-2.249	(-)		16-6019-6958	09-3317-6652
20		Aug	17	14:25	14.86	-13.43	-2.632	(-)		07-7298-7649	12-6822-1646
21		Sep	29	15:45	28.5	0.2089	0.04094			12-3450-8829	17-8563-2416

CETIS Test Data Worksheet

Report Date: 21 Sep-21 14:16 (p 1 of 1)
Test Code: 12-3450-8829/~~210922msdv~~ @ 210929msdv

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

Start Date: 29 (A) 22 Sep-21
End Date: 01 (A) 24 Sep-21
Sample Date: 29 (A) 22 Sep-21

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

Sample Code: (A) ~~210922msdv~~ 210929 msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			41			78	1	AS 10/19/21
			42			106	105	
			43			103	0	
			44			1	0	Cells in lysis
			45			0	0	Cells in lysis
			46			94	91	
			47			106	106	
			48			118	118	
			49			108	59	
			50			112	60	
			51			104	48	
			52			0	0	Cells in lysis
			53			94	93	
			54			69	0	
			55			117	117	
			56			91	0	
			57			104	103	
			58			113	112	
			59			87	86	
			60			0	0	Cells in lysis
			61			117	117	
			62			0	0	Cells in lysis
			63			110	39	
			64			92	88	
			65			94	0	
			66			111	111	
			67			85	85	
			68			100	99	
			69			96	96	
			70			113	49	

@QIR RT 10/19/21

@QIR BD 10/21/21

CETIS Test Data Worksheet

Report Date: 21 Sep-21 14:14 (p 1 of 1)
Test Code: 12-3450-8829/~~210922msdv~~ @ 210929msdv

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

Start Date: 29 ~~22~~ Sep-21 **Species:** Mytilus galloprovincialis **Sample Code:** @ ~~210922msdv~~ 210929msdv
End Date: @ ~~24 Sep-21~~ 1-Oct-21 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 29 ~~22~~ Sep-21 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	64					
0	LC	2	42					
0	LC	3	57			95	94	RT 10/2/21
0	LC	4	55					
0	LC	5	58					
2.5		1	67					
2.5		2	68					
2.5		3	48			110	109	
2.5		4	66					
2.5		5	46					
5		1	47					
5		2	53					
5		3	69			86	86	
5		4	59					
5		5	61					
10		1	49					
10		2	50					
10		3	63			109	18	
10		4	70					
10		5	51					
20		1	56					
20		2	54					
20		3	43			85	0	
20		4	41					
20		5	65					
40		1	62					
40		2	44					
40		3	52			0	0	
40		4	45					
40		5	60					

QC = YS

@ Q18 BO 9/25/21
(B) Q18 ACS 10/19/21

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: ~~210922msdv~~ ~~210929MSDV~~ 210929msdv @

Test Species: *M. galloprovincialis*

Start Date/Time: ~~9/22/2021~~ 9/29/21 1545 @

End Date/Time: ~~9/24/2021~~ 10/1/21 1440

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	32.1	32.0	15.8	15.4	14.7	8.6	8.6	8.6	7.99	7.89	7.99
2.5	32.1	32.4	32.5	15.7	15.2	14.5	8.7	8.7	8.6	8.00	7.89	8.01
5	32.0	32.5	32.6	15.4	15.0	14.5	8.7	8.8	8.6	8.01	7.90	8.01
10	32.0	32.5	32.5	15.5	15.6	14.7	8.6	8.7	8.6	8.01	7.91	8.01
20	32.0	32.5	32.5	15.4	15.7	14.6	8.7	8.7	8.6	8.02	7.92	8.01
40	31.9	32.3	32.4	15.2	15.4	14.6	8.7	8.7	8.6	8.02	7.92	8.01

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

Technician Initials:

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	2.0
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,000

Environmental Chamber: D

Comments:

0 hrs: @1428 9/25/21

24 hrs:

48 hrs:

QC Check: JL 10/11/21

Final Review: AES 10/19/21

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Interval / CuCl2
Test No.: ~~210001msdv~~ (A) 210929msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP SB
Date Received: 9/14/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 9/29/2021 1545
End Date/Time: 10/1/2021 1440
Technician Initials: KS

Spawn Information

First Gamete Release Time: 11:05

Sex	Number Spawning
Male	2
Female	2

Gamete Selection

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

Egg Fertilization Time: 12:00

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: 1

Embryo Inoculum Preparation

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

Number Counted:		
	4	4
	6	3
	8	9
	9	5
	8	5

Mean: 6.1

Mean 6.1 X 50 = 305 embryos/ml

Initial Density: 305 = 1.02 (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	113	113	100.0	100.0
TØ B	99	99	100.0	
TØ C	108	108	100.0	
TØ D	94	94	100.0	
TØ E	98	98	100.0	
TØ F	101	101	100.0	
$\bar{X}$ =	102			

48-h QC: 103/103 100%

Comments: (A) 018 JU 10/11/21

QC Check: JU 10/11/21

Final Review: AES 10/19/21