ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: February 2022

Prepared for: **Jacobs**
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Date Submitted: **March 3, 2022**

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	**020822**
Enthalpy Log-in Number	22-0142
Collection Date; Time	2/8/22; 0935h
Receipt Date; Time	2/9/22; 1057h
Receipt Temperature (°C)	2.7
Dissolved Oxygen (mg/L)	7.7
pH	7.41
Conductivity (µS/cm)	7,740
Salinity (ppt)	4.6
Alkalinity (mg/L $CaCO_3$)	472
Total Chlorine (mg/L)	0.02
Total Ammonia (mg/L as N)	1.2

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	2/9/22, 1625h to 2/11/22, 1625h
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 99.6 ppt
Test Concentrations (% sample)	73.3[a], 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper 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.

Statistical Methods

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in the sample was compared to that observed in the brine control. Results were used to calculate the No Observed Effect Concentration (NOEC) and the concentrations expected to cause an adverse effect to 50 percent of test organisms (EC_{50}). The chronic toxic unit (TUc) value was calculated as 100/NOEC, as specified in the permit. The statistical analyses were performed using the Comprehensive Environmental Toxicity Information System™ (CETIS), version 1.8.7.20 by Tidepool Scientific Software.

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 73.3 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.4 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_{50} (% effluent)
Bivalve	Normal Development	73.3	> 73.3	< 1.4	> 73.3
	Survival	73.3	> 73.3	< 1.4	> 73.3

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

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

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

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	97.7	95.0
0 (Lab Control)	96.7	96.5
2	98.5	96.2
4	97.7	96.9
9	99.2	97.2
18	98.8	97.2
35	99.8	97.3
73.3[a]	99.5	96.8

[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	28.9	27.3 ± 10.8	19.8
Bivalve Normal Development	5	8.10	8.55 ± 3.98	23.3

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:	06-1417-9146	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	09 Feb-22 16:25	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	11 Feb-22 16:25	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	48h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	11-5102-2408	**Code:**	22-0142	**Client:**	Jacobs
Sample Date:	08 Feb-22 09:35	**Material:**	Effluent Sample	**Project:**	
Receive Date:	09 Feb-22 10:57	**Source:**	Jacobs		
Sample Age:	31h (2.7 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-8886-6790	Combined Development Ra	73.3	>73.3	NA	5.49%	1.364	Dunnett Multiple Comparison Test
02-2892-3513	Development Rate	73.3	>73.3	NA	2.91%	1.364	Dunnett Multiple Comparison Test
18-5342-4762	Survival Rate	73.3	>73.3	NA	3.64%	1.364	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
06-3515-8270	Combined Development Ra	EC25	>73.3	N/A	N/A	<1.364	Linear Interpolation (ICPIN)
		EC50	>73.3	N/A	N/A	<1.364	
00-9187-5556	Development Rate	EC25	>73.3	N/A	N/A	<1.364	Linear Interpolation (ICPIN)
		EC50	>73.3	N/A	N/A	<1.364	
12-0974-5156	Survival Rate	EC25	>73.3	N/A	N/A	<1.364	Linear Interpolation (ICPIN)
		EC50	>73.3	N/A	N/A	<1.364	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-9187-5556	Development Rate	Control Resp	0.9496	0.9 - NL	Yes	Passes Acceptability Criteria
02-2892-3513	Development Rate	Control Resp	0.9496	0.9 - NL	Yes	Passes Acceptability Criteria
12-0974-5156	Survival Rate	Control Resp	0.9772	0.5 - NL	Yes	Passes Acceptability Criteria
18-5342-4762	Survival Rate	Control Resp	0.9772	0.5 - NL	Yes	Passes Acceptability Criteria
12-8886-6790	Combined Development Ra	PMSD	0.05494	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	0.9282	0.8763	0.9801	0.8762	0.9778	0.01871	0.04183	4.51%	0.0%
0	Lab Control	5	0.933	0.873	0.993	0.8515	0.9667	0.02161	0.04833	5.18%	-0.51%
2		5	0.9476	0.9194	0.9758	0.9208	0.9741	0.01016	0.02271	2.4%	-2.09%
4		5	0.9468	0.8941	0.9994	0.8713	0.9703	0.01898	0.04244	4.48%	-2.0%
9		5	0.9645	0.9355	0.9935	0.9307	0.9865	0.01045	0.02336	2.42%	-3.92%
18		5	0.9603	0.9375	0.9831	0.9356	0.9755	0.008226	0.01839	1.92%	-3.46%
35		5	0.9712	0.9522	0.9903	0.9517	0.9853	0.006868	0.01536	1.58%	-4.64%
73.3		5	0.9636	0.935	0.9922	0.9257	0.9863	0.0103	0.02303	2.39%	-3.81%

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9496	0.9277	0.9715	0.9352	0.9778	0.007887	0.01764	1.86%	0.0%
0	Lab Control	5	0.9646	0.9598	0.9694	0.96	0.9689	0.001732	0.003874	0.4%	-1.58%
2		5	0.9619	0.9476	0.9762	0.9459	0.9741	0.005141	0.01149	1.2%	-1.3%
4		5	0.9688	0.9533	0.9842	0.9581	0.9899	0.005554	0.01242	1.28%	-2.02%
9		5	0.9721	0.9569	0.9874	0.9592	0.9865	0.005482	0.01226	1.26%	-2.37%
18		5	0.9718	0.9662	0.9774	0.9643	0.9755	0.002009	0.004492	0.46%	-2.34%
35		5	0.9732	0.9517	0.9947	0.9517	0.99	0.007732	0.01729	1.78%	-2.49%
73.3		5	0.9683	0.9509	0.9857	0.9492	0.9863	0.006268	0.01402	1.45%	-1.97%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9772	0.937	1	0.9307	1	0.01448	0.03239	3.31%	0.0%
0	Lab Control	5	0.9673	0.9029	1	0.8812	1	0.0232	0.05187	5.36%	1.01%
2		5	0.9851	0.9577	1	0.9505	1	0.009901	0.02214	2.25%	-0.81%
4		5	0.9772	0.9266	1	0.9059	1	0.01823	0.04076	4.17%	0.0%
9		5	0.9921	0.9761	1	0.9703	1	0.005773	0.01291	1.3%	-1.52%
18		5	0.9881	0.9679	1	0.9703	1	0.007276	0.01627	1.65%	-1.11%
35		5	0.998	0.9925	1	0.9901	1	0.00198	0.004428	0.44%	-2.13%
73.3		5	0.995	0.9813	1	0.9752	1	0.00495	0.01107	1.11%	-1.82%

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	0.9778	0.896	0.9558	0.8762	0.9352
0	Lab Control	0.96	0.9667	0.961	0.9257	0.8515
2		0.9459	0.9741	0.9208	0.9307	0.9663
4		0.968	0.9703	0.966	0.9581	0.8713
9		0.9809	0.9865	0.9741	0.9307	0.9505
18		0.9755	0.9736	0.9713	0.9455	0.9356
35		0.9802	0.9813	0.9517	0.9577	0.9853
73.3		0.9735	0.9606	0.9718	0.9863	0.9257

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9778	0.9378	0.9558	0.9415	0.9352
0	Lab Control	0.96	0.9667	0.961	0.9689	0.9663
2		0.9459	0.9741	0.9688	0.9543	0.9663
4		0.968	0.9899	0.966	0.9581	0.9617
9		0.9809	0.9865	0.9741	0.9592	0.96
18		0.9755	0.9736	0.9713	0.9745	0.9643
35		0.99	0.9813	0.9517	0.9577	0.9853
73.3		0.9735	0.9606	0.9718	0.9863	0.9492

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	0.9554	1	0.9307	1
0	Lab Control	1	1	1	0.9554	0.8812
2		1	1	0.9505	0.9752	1
4		1	0.9802	1	1	0.9059
9		1	1	1	0.9703	0.9901
18		1	1	1	0.9703	0.9703
35		0.9901	1	1	1	1
73.3		1	1	1	1	0.9752

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	220/225	181/202	216/226	177/202	202/216
0	Lab Control	216/225	203/210	197/205	187/202	172/202
2		210/222	226/232	186/202	188/202	201/208
4		212/219	196/202	199/206	206/215	176/202
9		205/209	219/222	226/232	188/202	192/202
18		199/204	221/227	203/209	191/202	189/202
35		198/202	210/214	197/207	204/213	201/204
73.3		220/226	195/203	207/213	216/219	187/202

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	220/225	181/193	216/226	177/188	202/216
0	Lab Control	216/225	203/210	197/205	187/193	172/178
2		210/222	226/232	186/192	188/197	201/208
4		212/219	196/198	199/206	206/215	176/183
9		205/209	219/222	226/232	188/196	192/200
18		199/204	221/227	203/209	191/196	189/196
35		198/200	210/214	197/207	204/213	201/204
73.3		220/226	195/203	207/213	216/219	187/197

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	202/202	193/202	202/202	188/202	202/202
0	Lab Control	202/202	202/202	202/202	193/202	178/202
2		202/202	202/202	192/202	197/202	202/202
4		202/202	198/202	202/202	202/202	183/202
9		202/202	202/202	202/202	196/202	200/202
18		202/202	202/202	202/202	196/202	196/202
35		200/202	202/202	202/202	202/202	202/202
73.3		202/202	202/202	202/202	202/202	197/202

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

Analysis ID:	12-8886-6790	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	5.49%	73.3	>73.3	NA	1.364

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-0.8687	2.407	0.097	8	0.9820	CDF	Non-Significant Effect
		4	-0.9733	2.407	0.097	8	0.9867	CDF	Non-Significant Effect
		9	-1.988	2.407	0.097	8	0.9995	CDF	Non-Significant Effect
		18	-1.614	2.407	0.097	8	0.9983	CDF	Non-Significant Effect
		35	-2.382	2.407	0.097	8	0.9999	CDF	Non-Significant Effect
		73.3	-1.899	2.407	0.097	8	0.9994	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03247905	0.005413175	6	1.343	0.2718	Non-Significant Effect
Error	0.1128756	0.004031271	28			
Total	0.1453546		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.903	16.81	0.8209	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9468	0.9146	0.0901	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	0.9282	0.8763	0.9801	0.9352	0.8762	0.9778	0.01871	4.51%	0.0%
2		5	0.9476	0.9194	0.9758	0.9459	0.9208	0.9741	0.01016	2.4%	-2.09%
4		5	0.9468	0.8941	0.9994	0.966	0.8713	0.9703	0.01898	4.48%	-2.0%
9		5	0.9645	0.9355	0.9935	0.9741	0.9307	0.9865	0.01045	2.42%	-3.92%
18		5	0.9603	0.9375	0.9831	0.9713	0.9356	0.9755	0.008226	1.92%	-3.46%
35		5	0.9712	0.9522	0.9903	0.9802	0.9517	0.9853	0.006868	1.58%	-4.64%
73.3		5	0.9636	0.935	0.9922	0.9718	0.9257	0.9863	0.0103	2.39%	-3.81%

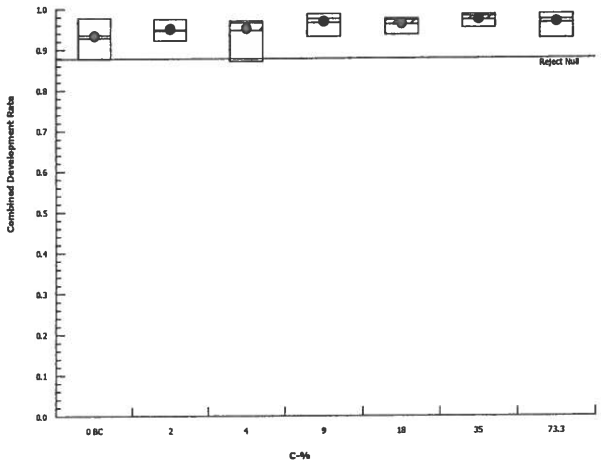
Angular (Corrected) Transformed Summary

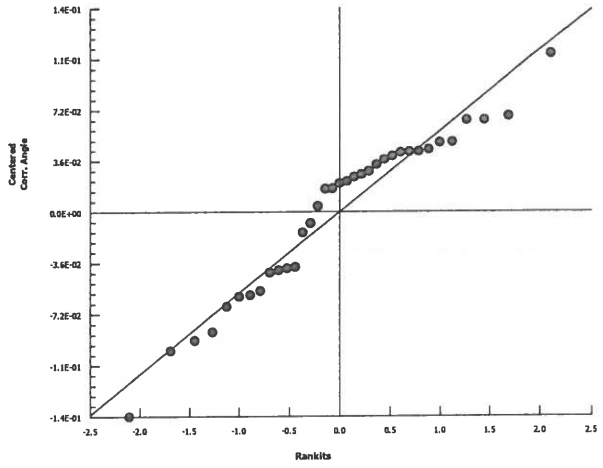
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.309	1.204	1.415	1.313	1.211	1.421	0.03811	6.51%	0.0%
2		5	1.344	1.279	1.41	1.336	1.286	1.409	0.02356	3.92%	-2.66%
4		5	1.349	1.247	1.45	1.385	1.204	1.398	0.03658	6.07%	-2.99%
9		5	1.389	1.312	1.467	1.409	1.304	1.454	0.02783	4.48%	-6.1%
18		5	1.374	1.317	1.431	1.401	1.314	1.414	0.02056	3.35%	-4.95%
35		5	1.405	1.349	1.461	1.43	1.349	1.449	0.02025	3.22%	-7.3%
73.3		5	1.386	1.313	1.459	1.402	1.295	1.453	0.02627	4.24%	-5.82%

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

Analysis ID:	12-8886-6790	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 02-2892-3513 Endpoint: Development Rate CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-22 8:52 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	2.91%	73.3	>73.3	NA	1.364

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-1.135	2.407	0.060	8	0.9918	CDF	Non-Significant Effect
		4	-1.978	2.407	0.060	8	0.9995	CDF	Non-Significant Effect
		9	-2.356	2.407	0.060	8	0.9999	CDF	Non-Significant Effect
		18	-2.198	2.407	0.060	8	0.9998	CDF	Non-Significant Effect
		35	-2.629	2.407	0.060	8	1.0000	CDF	Non-Significant Effect
		73.3	-1.914	2.407	0.060	8	0.9994	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01510645	0.002517741	6	1.62	0.1788	Non-Significant Effect
Error	0.04352988	0.001554638	28			
Total	0.05863633		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.327	16.81	0.3876	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9681	0.9146	0.3941	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	0.9496	0.9277	0.9715	0.9415	0.9352	0.9778	0.007887	1.86%	0.0%
2		5	0.9619	0.9476	0.9762	0.9663	0.9459	0.9741	0.00514	1.2%	-1.3%
4		5	0.9688	0.9533	0.9842	0.966	0.9581	0.9899	0.005554	1.28%	-2.02%
9		5	0.9721	0.9569	0.9874	0.9741	0.9592	0.9865	0.005482	1.26%	-2.37%
18		5	0.9718	0.9662	0.9774	0.9736	0.9643	0.9755	0.002008	0.46%	-2.34%
35		5	0.9732	0.9517	0.9947	0.9813	0.9517	0.99	0.007732	1.78%	-2.49%
73.3		5	0.9683	0.9509	0.9857	0.9718	0.9492	0.9863	0.006268	1.45%	-1.97%

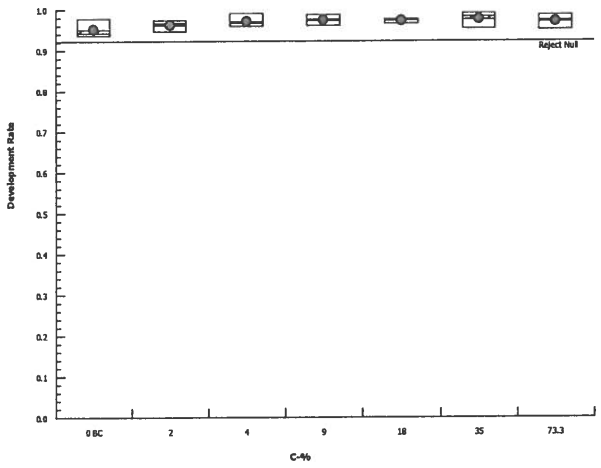
Angular (Corrected) Transformed Summary

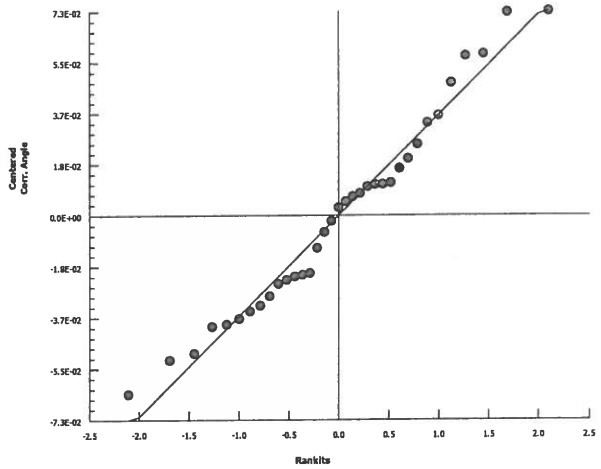
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.348	1.292	1.403	1.326	1.313	1.421	0.01998	3.32%	0.0%
2		5	1.376	1.339	1.413	1.386	1.336	1.409	0.01326	2.16%	-2.1%
4		5	1.397	1.345	1.449	1.385	1.365	1.47	0.01883	3.01%	-3.66%
9		5	1.406	1.359	1.454	1.409	1.367	1.454	0.0171	2.72%	-4.36%
18		5	1.403	1.386	1.419	1.407	1.381	1.414	0.005876	0.94%	-4.07%
35		5	1.413	1.347	1.48	1.434	1.349	1.471	0.02404	3.8%	-4.86%
73.3		5	1.395	1.344	1.447	1.402	1.344	1.453	0.0185	2.96%	-3.54%

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

Analysis ID:	02-2892-3513	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	18-5342-4762	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.64%	73.3	>73.3	NA	1.364

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	28.5	16	1	8	0.9067	Asymp	Non-Significant Effect
		4	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		9	29.5	16	1	8	0.9424	Asymp	Non-Significant Effect
		18	29.5	16	1	8	0.9424	Asymp	Non-Significant Effect
		35	31	16	1	8	0.9749	Asymp	Non-Significant Effect
		73.3	31	16	1	8	0.9749	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02001472	0.003335787	6	0.4798	0.8176	Non-Significant Effect
Error	0.1946549	0.006951959	28			
Total	0.2146696		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.362	16.81	0.2127	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.883	0.9146	0.0014	Non-normal Distribution

Survival Rate Summary

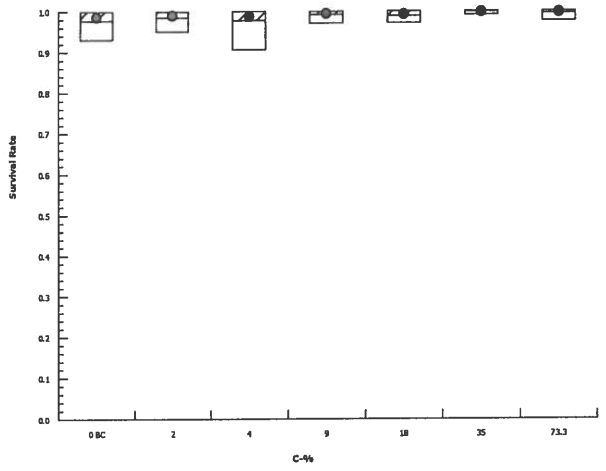
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	0.9772	0.937	1	1	0.9307	1	0.01448	3.31%	0.0%
2		5	0.9851	0.9577	1	1	0.9505	1	0.009901	2.25%	-0.81%
4		5	0.9772	0.9266	1	1	0.9059	1	0.01823	4.17%	0.0%
9		5	0.9921	0.976	1	1	0.9703	1	0.005773	1.3%	-1.52%
18		5	0.9881	0.9679	1	1	0.9703	1	0.007276	1.65%	-1.11%
35		5	0.998	0.9925	1	1	0.9901	1	0.00198	0.44%	-2.13%
73.3		5	0.995	0.9813	1	1	0.9752	1	0.00495	1.11%	-1.82%

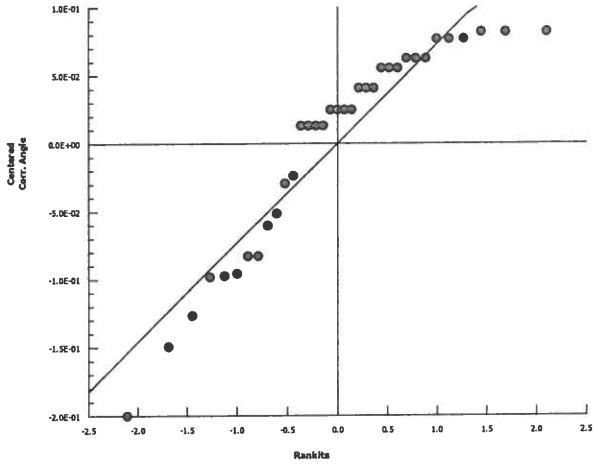
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.454	1.313	1.595	1.536	1.304	1.536	0.05077	7.81%	0.0%
2		5	1.473	1.363	1.583	1.536	1.346	1.536	0.03963	6.01%	-1.33%
4		5	1.459	1.309	1.609	1.536	1.259	1.536	0.05405	8.28%	-0.36%
9		5	1.495	1.419	1.571	1.536	1.398	1.536	0.02739	4.1%	-2.84%
18		5	1.48	1.387	1.574	1.536	1.398	1.536	0.03381	5.11%	-1.83%
35		5	1.523	1.487	1.559	1.536	1.471	1.536	0.0129	1.89%	-4.74%
73.3		5	1.511	1.443	1.579	1.536	1.413	1.536	0.02456	3.63%	-3.93%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	18-5342-4762	Endpoint:	Survival Rate	CETIS Version:	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	Analysis:	Nonparametric-Control vs Treatments	Official Results:	Yes

Graphics





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

Analysis ID:	06-3515-8270	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1449849	1000	Yes	Two-Point Interpolation

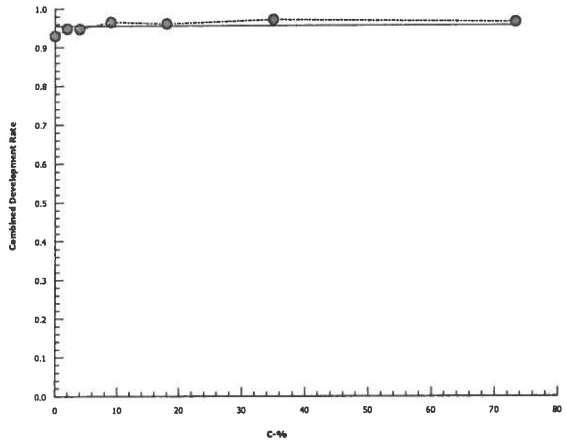
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.3	N/A	N/A	<1.364	NA	NA
EC50	>73.3	N/A	N/A	<1.364	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	0.9282	0.8762	0.9778	0.01871	0.04183	4.51%	0.0%	996	1071
2		5	0.9476	0.9208	0.9741	0.01016	0.02271	2.4%	-2.09%	1011	1066
4		5	0.9468	0.8713	0.9703	0.01898	0.04244	4.48%	-2.0%	989	1044
9		5	0.9645	0.9307	0.9865	0.01045	0.02336	2.42%	-3.92%	1030	1067
18		5	0.9603	0.9356	0.9755	0.008226	0.01839	1.92%	-3.46%	1003	1044
35		5	0.9712	0.9517	0.9853	0.006868	0.01536	1.58%	-4.64%	1010	1040
73.3		5	0.9636	0.9257	0.9863	0.0103	0.02303	2.39%	-3.81%	1025	1063

Graphics



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

Analysis ID:	00-9187-5556	Endpoint:	Development Rate	CETIS Version:	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	990245	1000	Yes	Two-Point Interpolation

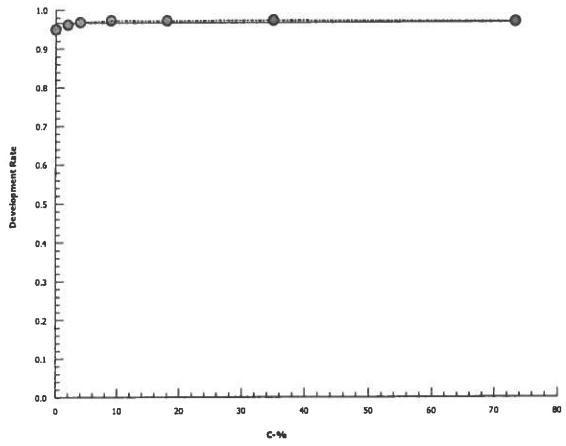
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.3	N/A	N/A	<1.364	NA	NA
EC50	>73.3	N/A	N/A	<1.364	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	0.9496	0.9352	0.9778	0.007887	0.01764	1.86%	0.0%	996	1048
2		5	0.9619	0.9459	0.9741	0.00514	0.01149	1.2%	-1.3%	1011	1051
4		5	0.9688	0.9581	0.9899	0.005554	0.01242	1.28%	-2.02%	989	1021
9		5	0.9721	0.9592	0.9865	0.005482	0.01226	1.26%	-2.37%	1030	1059
18		5	0.9718	0.9643	0.9755	0.002008	0.004491	0.46%	-2.34%	1003	1032
35		5	0.9732	0.9517	0.99	0.007732	0.01729	1.78%	-2.49%	1010	1038
73.3		5	0.9683	0.9492	0.9863	0.006268	0.01402	1.45%	-1.97%	1025	1058

Graphics



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

Analysis ID:	12-0974-5156	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 8:52	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1404582	1000	Yes	Two-Point Interpolation

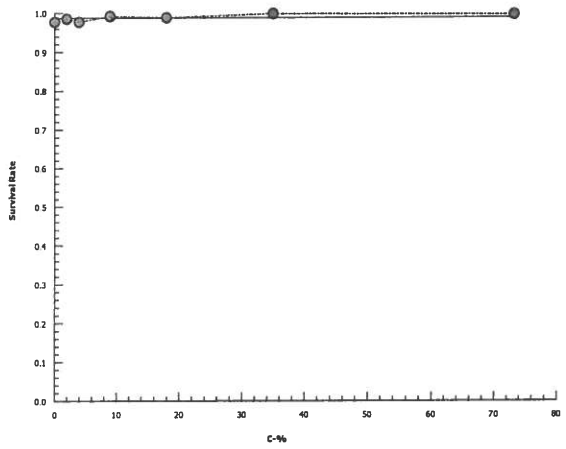
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.3	N/A	N/A	<1.364	NA	NA
EC50	>73.3	N/A	N/A	<1.364	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	0.9772	0.9307	1	0.01448	0.03239	3.31%	0.0%	987	1010
2		5	0.9851	0.9505	1	0.009901	0.02214	2.25%	-0.81%	995	1010
4		5	0.9772	0.9059	1	0.01823	0.04076	4.17%	0.0%	987	1010
9		5	0.9921	0.9703	1	0.005773	0.01291	1.3%	-1.52%	1002	1010
18		5	0.9881	0.9703	1	0.007276	0.01627	1.65%	-1.11%	998	1010
35		5	0.998	0.9901	1	0.00198	0.004428	0.44%	-2.13%	1008	1010
73.3		5	0.995	0.9752	1	0.00495	0.01107	1.11%	-1.82%	1005	1010

Graphics



CETIS Test Data Worksheet

Report Date: 05 Feb-22 14:37 (p 1 of 1)
Test Code: 2202-S067 07-8877-2263/2F03B5A7

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

Start Date: 09 Feb-22
End Date: 11 Feb-22
Sample Date: 08 Feb-22

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

Sample Code: 22-0142
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			232	226	RT 2/27/22
			32			207	197	
			33			222	219	
			34			215	206	
			35			197	188	
			36			209	203	
			37			210	203	
			38			232	226	
			39			196	188	
			40			219	216	
			41			213	204	
			42			206	199	
			43			188	177	
			44			214	210	
			45			216	202	
			46			227	221	
			47			209	205	
			48			208	201	
			49			198	196	
			50			203	195	
			51			200	192	GM 2/28/22
			52			213	207	
			53			225	220	
			54			204	199	
			55			219	212	
			56			200	198	
			57			192	186	
			58			196	189	
			59			193	181	
			60			196	191	
			61			222	210	
			62			226	220	
			63			205	197	
			64			178	172	
			65			193	187	
			66			197	187	
			67			204	201	
			68			225	216	
			69			226	216	QC: 191 normal/202 total AES 3/1/22
			70			183	176	

Analyst: AES 0830 RT/GM QA: AES 3/1/22

CETIS Test Data Worksheet

Report Date: 05 Feb-22 14:37 (p 1 of 1)
Test Code: 2202-5067 07-8877-2263/2F03B5A7

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

Start Date: 09 Feb-22
End Date: 11 Feb-22
Sample Date: 08 Feb-22

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

Sample Code: 22- 0142
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	53			1		
0	BC	2	59					
0	BC	3	69			191	179	WF 2/15/22
0	BC	4	43					
0	BC	5	45					
0	LC	1	68					
0	LC	2	37			~~206~~	~~198~~	Q18WF 2/15/22
0	LC	3	63			206	198	
0	LC	4	65					
0	LC	5	64					
2		1	61					
2		2	31					
2		3	57			214	205	
2		4	35					
2		5	48					
4		1	55					
4		2	49					
4		3	42			197	190	
4		4	34					
4		5	70					
9		1	47					
9		2	33					
9		3	38			217	209	
9		4	39					
9		5	51					
18		1	54					
18		2	46					
18		3	36			209	202	
18		4	60					
18		5	58					
35		1	56					
35		2	44					
35		3	32			~~209~~ 217	~~202~~ 207	
35		4	41					
35		5	67					
75.3		1	62					
75.3		2	50					
75.3		3	52			216	209	
75.3		4	40					
75.3		5	66					

73.3

Q18 KS 2/9/22 QL

@Q18 WF 3/1/22 Total: 217
Normal: 207

Analyst: WF BD QA: AHS 3/1/22
QB PT

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS

Sample ID: Wyckoff

Sample Log No.: 22-0142

Test No.: 2202-5067

Test Species: *M. galloprovincialis*

Start Date/Time: 2/9/2022 1625

End Date/Time: 2/11/2022 1625

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	29.8	29.5	30.1	14.8	14.6	14.4	8.7	8.8	8.5	7.98	7.97	7.90
Brine Control	30.2	30.1	30.3	14.4	14.6	14.4	8.8	8.7	8.4	8.10	8.06	7.90
2	30.0	29.9	30.3	14.8	14.4	14.4	8.7	8.7	8.5	7.99	7.96	7.91
4	30.0	30.4	30.8	14.8	14.0	14.4	8.7	8.8	8.5	7.97	7.98	7.93
9	30.1	30.2	30.6	14.7	14.4	14.3	8.6	8.6	8.5	7.93	8.00	7.98
18	30.2	30.3	30.7	14.6	14.3	14.4	8.6	8.6	8.5	7.87	8.02	8.05
35	30.2	30.3	30.8	14.7	14.2	14.4	8.6	8.7	8.5	7.77	8.05	8.20
733	30.5	30.9	30.8	14.6	14.0	14.5	8.5	8.7	8.5	7.65	8.11	8.22

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

Technician Initials:

Environmental Chamber: D

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: JL 3/1/22

Final Review: ACS 3/1/22

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** KS

Sample ID: Wyckoff **Test Date:** 2/9/2022 1625

Test No: 2202-S067 **Test Type:** Mussel Development

Salinity of Effluent 4.7

Salinity of Brine 99.6 **Date of Brine used:** 9/28/2021

Target Salinity 30 **Alkalinity of Brine Control:** 110 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.36**	**0.43**

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	0.36	1.8	250
4	10.0	0.36	3.6	250
9	22.5	0.36	8.2	250
18	45.0	0.36	16.4	250
35	87.5	0.36	31.9	250
73.3	183.3	0.36	66.7	250
	DI Volume			
Brine Control	154.8	0.43	66.7	250

Total Brine Volume Required (ml): **195.4**

QC Check: JL 3/1/22 **Final Review:** AS 3/1/22

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

BJE JH 5/1/22

Client/Sample: Jacobs / Wyckoff
Test No.: 2202-SO67
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP / 6A/B
Date Received: 11/17/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 2/9/2022 1625 ~~1750~~
End Date/Time: 2/11/2022 1625
Technician Initials: KS / BO

Spawn Information

First Gamete Release Time: 1300

Sex	Number Spawning
Male	3+
Female	3+

Gamete Selection

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

Egg Fertilization Time: 1400

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:		
	8	8
	7	9
	10	8
	10	8
	8	14

Mean: 9.0

Mean 9.0 X 50 = 450 embryos/ml

Initial Density: 450 = 1.5 (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	172	172	100	100
TØ B	238	238	100	
TØ C	188	188	100	
TØ D	201	201	100	
TØ E	201	201	100	
TØ F	212	212	100	
$\bar{X}$ =	202			

48-h QC: 235/241 = 97.5%

Comments:

QC Check: CM 3/1/22

Final Review: AS 3/1/22

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JAWOS
Sample ID: Wyckoff
Test ID No(s).: 2202-5067

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):	0142			
Sample Collection Date & Time:	2/8/22 0938			
Sample Receipt Date & Time:	2/9/22 1057			
Number of Containers & Container Type:	1 x 4L cubi			
Approx. Total Volume Received (L):	~4L			
Check-in Temperature (°C)	2.7			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.7			
pH (units)	7.41			
Conductivity (µS/cm)	7740			
Salinity (ppt)	4.6			
Alkalinity (mg/L) [2]	472			
Hardness (mg/L) [2, 3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	DR			

Subsamples for Additional Chemistry Required:
(NH3) (always required)
Other _____
Tech Initials A RT 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: 96 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 110 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 3/1/22
Final Review: AFS 3/1/22

Total Ammonia Analysis
Marine

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: —
SW Blank: 0.0

Test Start Date: 2/9/2022

Analyst: RT/WF
Analysis Date: 2/12/22

N x 1.22

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	9.6	11.7
020822 Wyckoff	22-0142	2/9/22	0	1.2	1.5
Spike Check (10 mg/L NH_3)		NA	NA		
Sample Duplicate[a]		NA	NA	1.1	1.3
Sample Duplicate + Spike[a]		NA	NA	10.2	12.4
Spike Check (10 mg/L NH_3)		NA	NA	9.6	11.7

Relative Percent Difference (RPD) = [sample] (mg/L) - [sample duplicate] (mg/L) x 100
[average ammonia] (mg/L)

Acceptable Range: 0-20%

Percent Recovery = [spiked sample] (mg/L) - [sample] (mg/L) x 100
nominal [spike] (mg/L)

Acceptable Range: 80-120%[b]

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	11.7	10	NA	117
020822 Wyckoff	1.5	1.3	12.4	10	14.3	109

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A1210	A1228	A1301

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.

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

QC Check: 2/12/22

Final Review: AS 3/1/22

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (LAB COPY)
DateShipped: 2/8/2022
CarrierName: JACOBS (hand delivery)
AirbillNo:

Jacobs, Wyckoff-

Project Code: WEH-031I
Cooler #: Enthalpy

No: 10-020822-111624-0595
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	For Lab Use Only
020822		Ground Water/ D. Baca	Composite	CHRTOX(8 Weeks)	A (< 6 C) (1)	SP-11	02/08/2022 09:35	

Special Instructions: 2022 Week 07-Q1

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	2-8-22 @1130	EA-SD	2/9/22 1057	Temp: 2.7°C Reciept

Log # 22-0142

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:	13-5890-5177	Test Type:	Development-Survival	Analyst:	
Start Date:	09 Feb-22 16:25	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Diluted Natural Seawater
Ending Date:	11 Feb-22 16:25	Species:	Mytilus galloprovincialis	Brine:	Not Applicable
Duration:	48h	Source:	M-Rep, Carlsbad, CA	Age:	

Sample ID:	18-7108-1056	Code:	220209msdv	Client:	Internal
Sample Date:	09 Feb-22	Material:	Copper chloride	Project:	
Receive Date:	09 Feb-22	Source:	Reference Toxicant		
Sample Age:	16h	Station:	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
19-0549-9771	Combined Development Ra	5	10	7.071	2.98%		Dunnett Multiple Comparison Test
18-2586-1415	Development Rate	5	10	7.071	3.04%		Dunnett Multiple Comparison Test
09-5309-5366	Survival Rate	10	20	14.14	1.3%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
13-7282-5479	Combined Development Ra	EC25	6.494	6.291	6.697		Linear Interpolation (ICPIN)
		EC50	8.083	7.83	8.443		
03-6791-7638	Development Rate	EC25	6.507	6.314	6.709		Linear Interpolation (ICPIN)
		EC50	8.097	7.848	8.461		
05-7427-9529	Survival Rate	EC25	23.23	22.25	24.32		Linear Interpolation (ICPIN)
		EC50	28.86	28.19	29.56		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-6791-7638	Development Rate	Control Resp	0.9698	0.9 - NL	Yes	Passes Acceptability Criteria
18-2586-1415	Development Rate	Control Resp	0.9698	0.9 - NL	Yes	Passes Acceptability Criteria
05-7427-9529	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
09-5309-5366	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
19-0549-9771	Combined Development Ra	PMSD	0.02985	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	0.9698	0.9561	0.9834	0.9595	0.9835	0.004921	0.011	1.14%	0.0%
2.5		5	0.9622	0.9406	0.9838	0.9409	0.9833	0.007779	0.01739	1.81%	0.78%
5		5	0.9561	0.9297	0.9825	0.9206	0.9716	0.009506	0.02126	2.22%	1.41%
10		5	0.1901	0.1144	0.2658	0.1535	0.2949	0.02725	0.06094	32.05%	80.4%
20		5	0.00297	0	0.01122	0	0.01485	0.00297	0.006642	223.6%	99.69%
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	0.9698	0.9561	0.9834	0.9595	0.9835	0.004921	0.011	1.14%	0.0%
2.5		5	0.9641	0.9439	0.9843	0.9409	0.9833	0.007281	0.01628	1.69%	0.59%
5		5	0.958	0.9298	0.9862	0.9206	0.975	0.01015	0.0227	2.37%	1.21%
10		5	0.1924	0.1177	0.267	0.1535	0.2949	0.02689	0.06012	31.26%	80.16%
20		5	0.00355	0	0.01341	0	0.01775	0.00355	0.007939	223.6%	99.63%
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	1	1	1	1	1	0	0	0.0%	0.0%
2.5		5	0.998	0.9925	1	0.9901	1	0.00198	0.004428	0.44%	0.2%
5		5	0.998	0.9925	1	0.9901	1	0.00198	0.004428	0.44%	0.2%
10		5	0.9871	0.9643	1	0.9604	1	0.008224	0.01839	1.86%	1.29%
20		5	0.8931	0.8348	0.9513	0.8366	0.9604	0.02098	0.04691	5.25%	10.69%
40		5	0.005941	0	0.01604	0	0.0198	0.003638	0.008134	136.9%	99.41%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9798	0.9633	0.9835	0.9628	0.9595
2.5		0.9505	0.9833	0.9754	0.9409	0.9609
5		0.9524	0.9716	0.9206	0.9704	0.9653
10		0.2949	0.1557	0.1931	0.1535	0.1535
20		0.01485	0	0	0	0
40		0	0	0	0	0

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9798	0.9633	0.9835	0.9628	0.9595
2.5		0.96	0.9833	0.9754	0.9409	0.9609
5		0.9524	0.9716	0.9206	0.9704	0.975
10		0.2949	0.1557	0.198	0.1535	0.1598
20		0.01775	0	0	0	0
40		0	0	0	0	0

Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1	1	1	1	1
2.5		0.9901	1	1	1	1
5		1	1	1	1	0.9901
10		1	1	0.9752	1	0.9604
20		0.8366	0.9158	0.8762	0.9604	0.8762
40		0	0.0198	0.00495	0.00495	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	242/247	210/218	238/242	207/215	213/222
2.5		192/202	235/239	198/203	191/203	221/230
5		200/210	205/211	197/214	197/203	195/202
10		69/234	33/212	39/202	33/215	31/202
20		3/202	0/202	0/202	0/202	0/202
40		0/202	0/202	0/202	0/202	0/202

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	242/247	210/218	238/242	207/215	213/222
2.5		192/200	235/239	198/203	191/203	221/230
5		200/210	205/211	197/214	197/203	195/200
10		69/234	33/212	39/197	33/215	31/194
20		3/169	0/185	0/177	0/194	0/177
40		0/1	0/4	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	202/202	202/202	202/202	202/202	202/202
2.5		200/202	202/202	202/202	202/202	202/202
5		202/202	202/202	202/202	202/202	200/202
10		202/202	202/202	197/202	202/202	194/202
20		169/202	185/202	177/202	194/202	177/202
40		0/202	4/202	1/202	1/202	0/202

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

Analysis ID:	19-0549-9771	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.98%	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	0.5966	2.305	0.074	8	0.5604	CDF	Non-Significant Effect
		5	1.09	2.305	0.074	8	0.3433	CDF	Non-Significant Effect
		10*	29.81	2.305	0.074	8	<0.0001	CDF	Significant Effect
		20*	42.2	2.305	0.074	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.059924	2.014981	4	792.4	<0.0001	Significant Effect
Error	0.05085971	0.002542986	20			
Total	8.110784		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.755	13.28	0.5996	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9446	0.8877	0.1885	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	0.9698	0.9561	0.9834	0.9633	0.9595	0.9835	0.004921	1.14%	0.0%
2.5		5	0.9622	0.9406	0.9838	0.9609	0.9409	0.9833	0.007779	1.81%	0.78%
5		5	0.9561	0.9297	0.9825	0.9653	0.9206	0.9716	0.009506	2.22%	1.41%
10		5	0.1901	0.1144	0.2658	0.1557	0.1535	0.2949	0.02725	32.05%	80.4%
20		5	0.00297	0	0.01122	0	0	0.01485	0.00297	223.6%	99.69%
40		5	0	0	0	0	0	0	0		100.0%

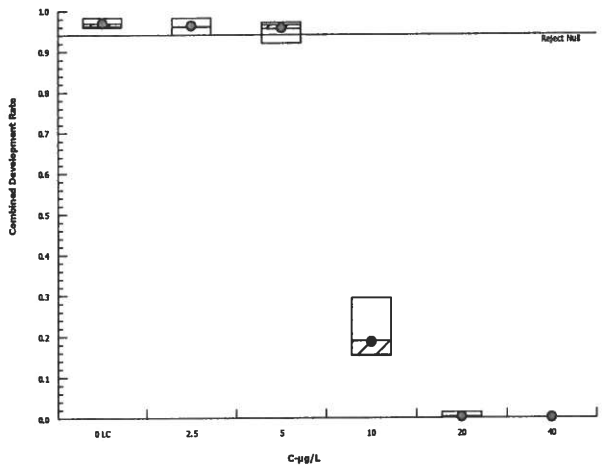
Angular (Corrected) Transformed Summary

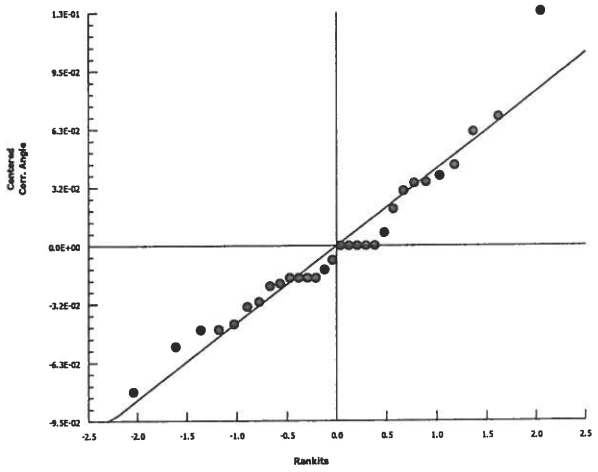
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.399	1.357	1.441	1.378	1.368	1.442	0.01512	2.42%	0.0%
2.5		5	1.38	1.321	1.439	1.372	1.325	1.441	0.02125	3.44%	1.36%
5		5	1.364	1.304	1.424	1.384	1.285	1.401	0.02161	3.54%	2.49%
10		5	0.4479	0.3561	0.5397	0.4056	0.4025	0.574	0.03306	16.51%	67.97%
20		5	0.05258	0.004283	0.1009	0.03519	0.03519	0.1222	0.0174	73.98%	96.24%
40		5	0.03519	0.03518	0.03519	0.03519	0.03519	0.03519	0	0.0%	97.48%

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

Analysis ID:	19-0549-9771	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID:	18-2586-1415	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.04%	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	0.4459	2.305	0.075	8	0.6275	CDF	Non-Significant Effect
		5	0.8991	2.305	0.075	8	0.4244	CDF	Non-Significant Effect
		10*	29.29	2.305	0.075	8	<0.0001	CDF	Significant Effect
		20*	41.49	2.305	0.075	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.058728	2.014682	4	770.1	<0.0001	Significant Effect
Error	0.05232569	0.002616285	20			
Total	8.111053		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.449	13.28	0.6537	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9462	0.8877	0.2053	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	0.9698	0.9561	0.9834	0.9633	0.9595	0.9835	0.004921	1.14%	0.0%
2.5		5	0.9641	0.9439	0.9843	0.9609	0.9409	0.9833	0.007281	1.69%	0.59%
5		5	0.958	0.9298	0.9862	0.9704	0.9206	0.975	0.01015	2.37%	1.21%
10		5	0.1924	0.1177	0.267	0.1598	0.1535	0.2949	0.02689	31.26%	80.16%
20		5	0.00355	0	0.01341	0	0	0.01775	0.00355	223.6%	99.63%
40		5	0	0	0	0	0	0	0		100.0%

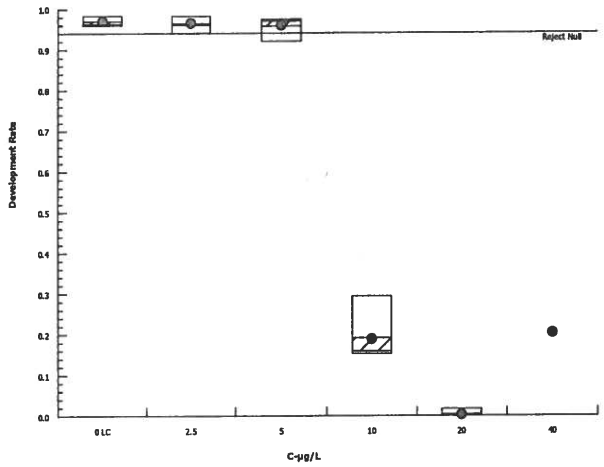
Angular (Corrected) Transformed Summary

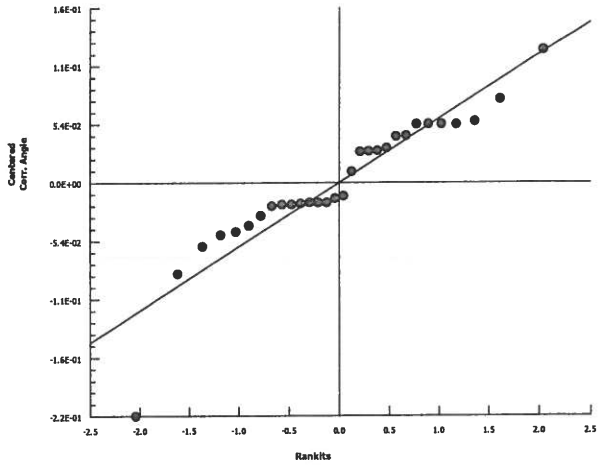
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.399	1.357	1.441	1.378	1.368	1.442	0.01512	2.42%	0.0%
2.5		5	1.384	1.329	1.439	1.372	1.325	1.441	0.01991	3.22%	1.03%
5		5	1.369	1.304	1.435	1.398	1.285	1.412	0.02357	3.85%	2.08%
10		5	0.4509	0.3604	0.5414	0.4112	0.4026	0.574	0.03258	16.16%	67.76%
20		5	0.0563	0.002612	0.11	0.03759	0.03591	0.1336	0.01934	76.8%	95.97%
40		5	0.4694	0.319	0.6199	0.5236	0.2527	0.5236	0.05418	25.81%	66.44%

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

Analysis ID:	18-2586-1415	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 09-5309-5366 Endpoint: Survival Rate CETIS Version: CETISv1.8.7
Analyzed: 02 Mar-22 9:04 Analysis: Parametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	1.3%	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	0.3857	2.362	0.079	8	0.6955	CDF	Non-Significant Effect
		5	0.3857	2.362	0.079	8	0.6955	CDF	Non-Significant Effect
		10	1.723	2.362	0.079	8	0.1593	CDF	Non-Significant Effect
		20*	8.697	2.362	0.079	8	<0.0001	CDF	Significant Effect
		40*	43.82	2.362	0.079	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.354871	1.670974	5	598	<0.0001	Significant Effect
Error	0.06706765	0.002794486	24			
Total	8.421939		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.508	4.248	0.2366	Equal Variances
Variances	Levene Equality of Variance	5.721	3.895	0.0013	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9501	0.9031	0.1699	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	1	1	1	1	1	1	0	0.0%	0.0%
2.5		5	0.998	0.9925	1	1	0.9901	1	0.00198	0.44%	0.2%
5		5	0.998	0.9925	1	1	0.9901	1	0.00198	0.44%	0.2%
10		5	0.9871	0.9643	1	1	0.9604	1	0.008224	1.86%	1.29%
20		5	0.8931	0.8348	0.9513	0.8762	0.8366	0.9604	0.02098	5.25%	10.69%
40		5	0.005941	0	0.01604	0.00495	0	0.0198	0.003638	136.9%	99.41%

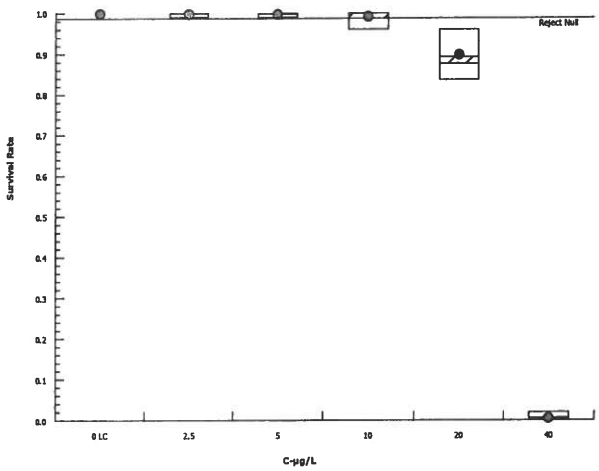
Angular (Corrected) Transformed Summary

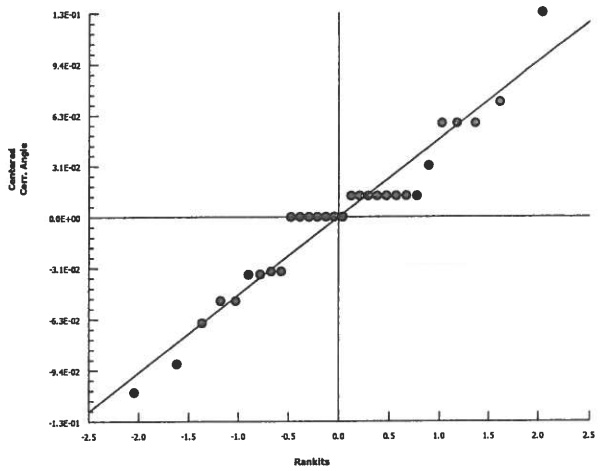
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.536	1.535	1.536	1.536	1.536	1.536	0	0.0%	0.0%
2.5		5	1.523	1.487	1.559	1.536	1.471	1.536	0.0129	1.89%	0.84%
5		5	1.523	1.487	1.559	1.536	1.471	1.536	0.0129	1.89%	0.84%
10		5	1.478	1.378	1.578	1.536	1.37	1.536	0.0359	5.43%	3.75%
20		5	1.245	1.143	1.347	1.211	1.155	1.37	0.03685	6.62%	18.93%
40		5	0.07048	0.01675	0.1242	0.07042	0.03519	0.1412	0.01935	61.4%	95.41%

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

Analysis ID:	09-5309-5366	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 220209msdv | 20-6883-0287

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

Analysis ID:	13-7282-5479	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1940413	1000	Yes	Two-Point Interpolation

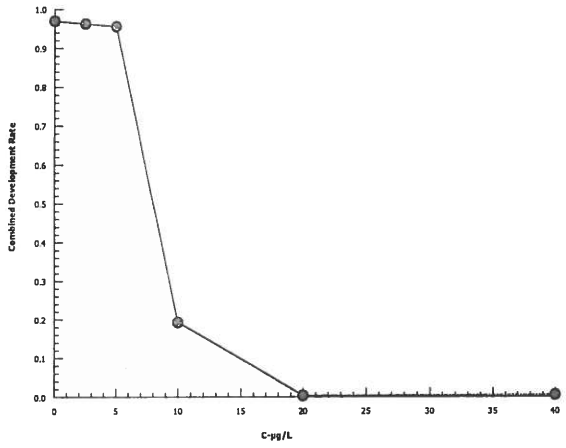
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.494	6.291	6.697
EC50	8.083	7.83	8.443

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	0.9698	0.9595	0.9835	0.004921	0.011	1.14%	0.0%	1110	1144
2.5		5	0.9622	0.9409	0.9833	0.007779	0.01739	1.81%	0.78%	1037	1077
5		5	0.9561	0.9206	0.9716	0.009506	0.02126	2.22%	1.41%	994	1040
10		5	0.1901	0.1535	0.2949	0.02725	0.06094	32.05%	80.4%	205	1065
20		5	0.00297	0	0.01485	0.00297	0.006642	223.6%	99.69%	3	1010
40		5	0	0	0	0	0		100.0%	0	1010

Graphics



Analyst: JW QA: AJS 3/2/22

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

Analysis ID:	03-6791-7638	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2038522	1000	Yes	Two-Point Interpolation

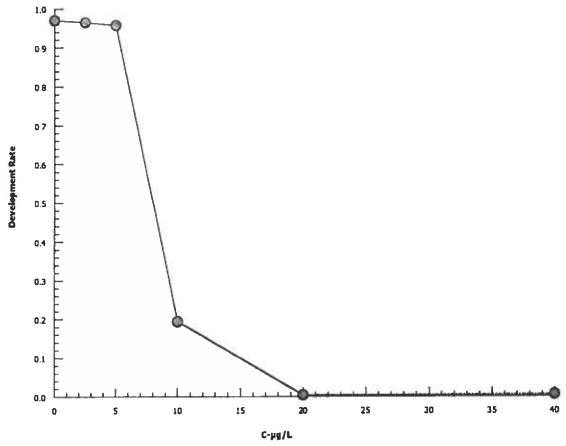
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.507	6.314	6.709
EC50	8.097	7.848	8.461

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	0.9698	0.9595	0.9835	0.004921	0.011	1.14%	0.0%	1110	1144
2.5		5	0.9641	0.9409	0.9833	0.007281	0.01628	1.69%	0.59%	1037	1075
5		5	0.958	0.9206	0.975	0.01015	0.0227	2.37%	1.21%	994	1038
10		5	0.1924	0.1535	0.2949	0.02689	0.06012	31.26%	80.16%	204	1052
20		5	0.00355	0	0.01775	0.00355	0.007939	223.6%	99.63%	3	902
40		5	0	0	0	0	0		100.0%	0	8

Graphics



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

Analysis ID:	05-7427-9529	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Mar-22 9:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1915300	1000	Yes	Two-Point Interpolation

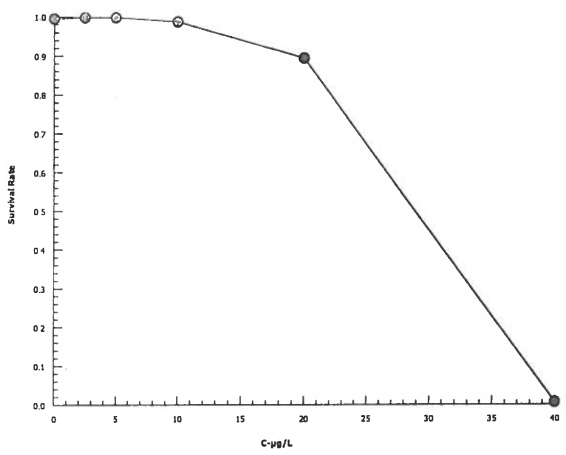
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	23.23	22.25	24.32
EC50	28.86	28.19	29.56

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	1	1	1	0	0	0.0%	0.0%	1010	1010
2.5		5	0.998	0.9901	1	0.00198	0.004428	0.44%	0.2%	1008	1010
5		5	0.998	0.9901	1	0.00198	0.004428	0.44%	0.2%	1008	1010
10		5	0.9871	0.9604	1	0.008224	0.01839	1.86%	1.29%	997	1010
20		5	0.8931	0.8366	0.9604	0.02098	0.04691	5.25%	10.69%	902	1010
40		5	0.005941	0	0.0198	0.003638	0.008134	136.9%	99.41%	6	1010

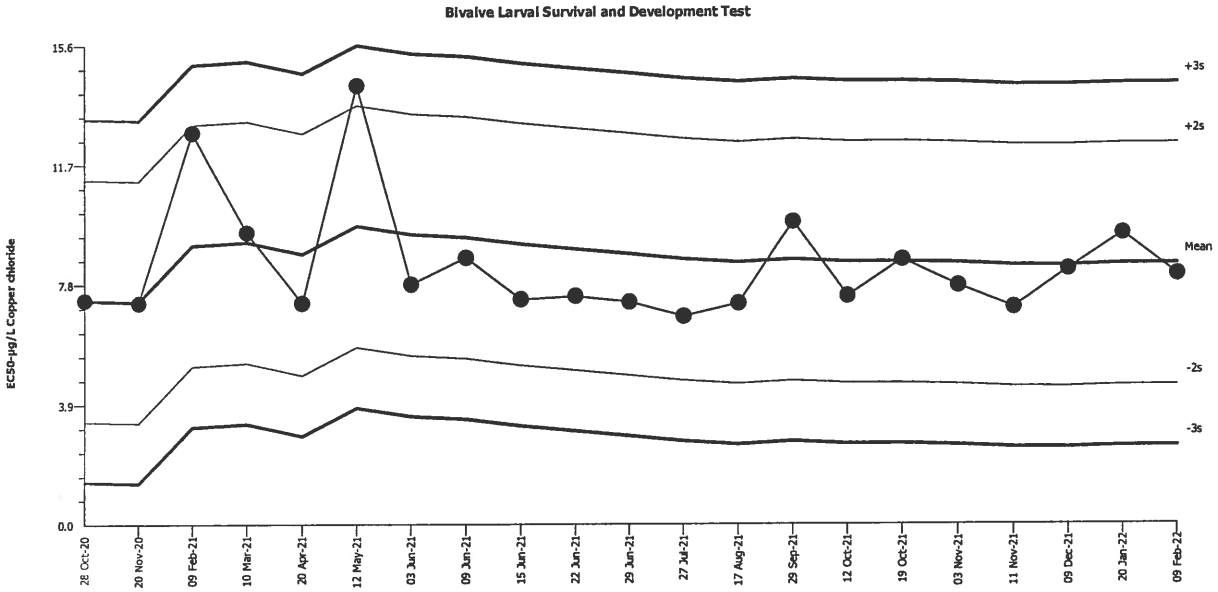
Graphics



CETIS QC Plot

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



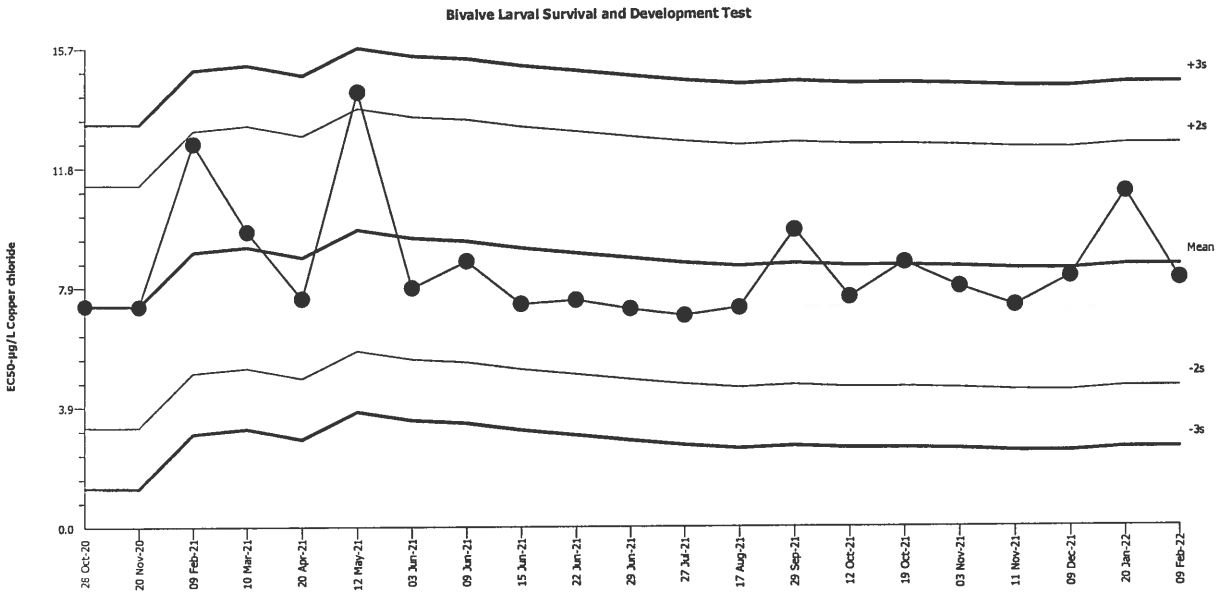
Mean:	8.432	**Count:**	20	**-2s Warning Limit:**	4.502	**-3s Action Limit:**	2.537
Sigma:	1.965	**CV:**	23.30%	**+2s Warning Limit:**	12.36	**+3s Action Limit:**	14.33

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Oct	28	15:50	7.269	-1.163	-0.5919			09-4043-4676	02-6542-7057
2		Nov	20	16:00	7.187	-1.245	-0.6335			13-7696-8009	10-4367-1427
3	2021	Feb	9	15:15	12.74	4.307	2.192	(+)		12-5648-6062	18-1503-3303
4		Mar	10	14:15	9.481	1.049	0.5337			13-7922-5399	10-0885-9755
5		Apr	20	16:15	7.185	-1.247	-0.6347			06-7450-9711	18-3353-6875
6		May	12	15:00	14.27	5.836	2.97	(+)		15-4594-3065	00-9727-8504
7		Jun	3	15:50	7.791	-0.6408	-0.3261			07-9391-2508	21-2212-7050
8			9	14:00	8.654	0.2215	0.1127			18-5736-8495	04-4549-3405
9			15	15:40	7.302	-1.13	-0.5752			00-2993-6780	17-7654-7354
10			22	13:45	7.404	-1.028	-0.5232			16-6840-3553	15-2803-6917
11			29	14:55	7.211	-1.221	-0.6213			07-2040-2693	08-8247-6801
12		Jul	27	16:30	6.748	-1.684	-0.8568			16-6019-6958	06-5859-7928
13		Aug	17	14:25	7.168	-1.264	-0.6435			07-7298-7649	09-6648-5411
14		Sep	29	15:45	9.809	1.377	0.7008			12-3450-8829	18-2247-7613
15		Oct	12	15:00	7.395	-1.037	-0.5277			14-7239-9185	01-1367-5722
16			19	17:00	8.581	0.1489	0.07576			17-5798-2248	09-1208-0351
17		Nov	3	15:00	7.733	-0.6995	-0.356			14-6395-1490	06-4040-2968
18			11	14:35	7.03	-1.402	-0.7137			00-1546-1531	12-7713-2161
19		Dec	9	15:50	8.264	-0.1677	-0.08537			06-2693-6580	11-5581-5612
20	2022	Jan	20	15:15	9.426	0.9945	0.5061			06-1599-8254	16-9050-7435
21		Feb	9	16:25	8.083	-0.3491	-0.1776			20-6883-0287	13-7282-5479

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:	8.554	Count:	20	-2s Warning Limit:	4.57	-3s Action Limit:	2.578
Sigma:	1.992	CV:	23.30%	+2s Warning Limit:	12.54	+3s Action Limit:	14.53

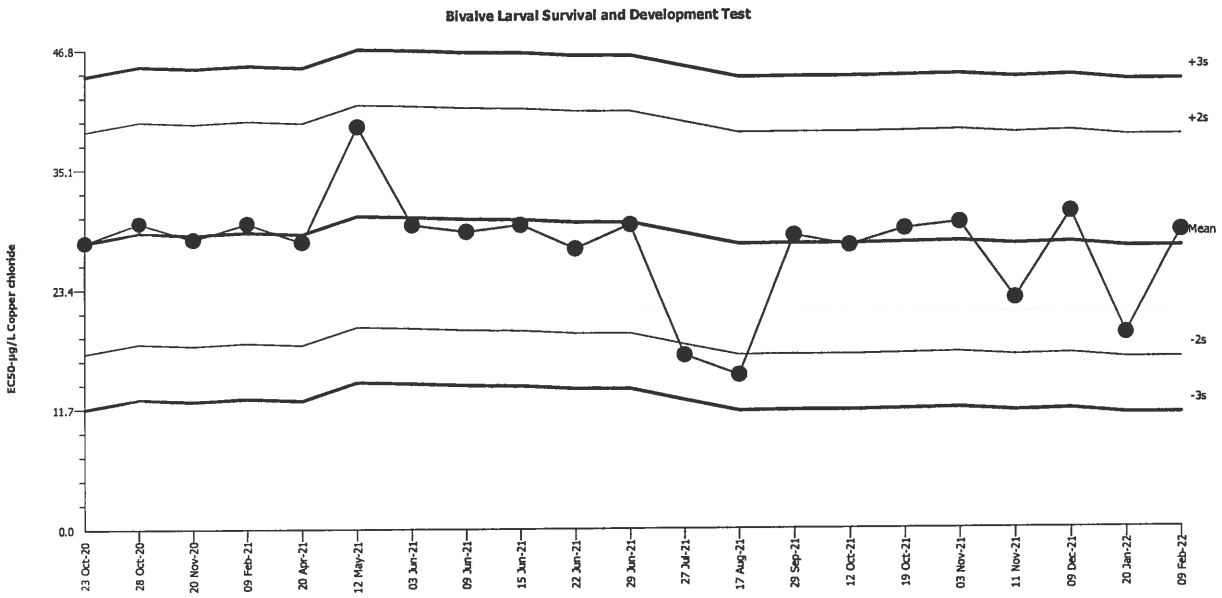
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Oct	28	15:50	7.257	-1.297	-0.6512			09-4043-4676	12-0840-2779
2		Nov	20	16:00	7.23	-1.324	-0.6648			13-7696-8009	11-4264-3018
3	2021	Feb	9	15:15	12.58	4.029	2.023	(+)		12-5648-6062	01-5747-2564
4		Mar	10	14:15	9.694	1.14	0.5721			13-7922-5399	08-4869-7631
5		Apr	20	16:15	7.482	-1.072	-0.5382			06-7450-9711	17-9210-1733
6		May	12	15:00	14.27	5.714	2.868	(+)		15-4594-3065	12-3891-6641
7		Jun	3	15:50	7.832	-0.7219	-0.3624			07-9391-2508	11-7075-1183
8			9	14:00	8.715	0.1614	0.08101			18-5736-8495	18-6125-5477
9			15	15:40	7.302	-1.252	-0.6287			00-2993-6780	13-6998-5313
10			22	13:45	7.427	-1.127	-0.5659			16-6840-3553	07-3347-2243
11			29	14:55	7.132	-1.422	-0.7139			07-2040-2693	17-0989-5973
12		Jul	27	16:30	6.912	-1.642	-0.8245			16-6019-6958	03-0913-6262
13		Aug	17	14:25	7.168	-1.386	-0.696			07-7298-7649	11-4901-9823
14		Sep	29	15:45	9.718	1.164	0.5843			12-3450-8829	04-7958-3381
15		Oct	12	15:00	7.509	-1.045	-0.5244			14-7239-9185	04-3282-5514
16			19	17:00	8.648	0.09356	0.04697			17-5798-2248	05-0981-9303
17		Nov	3	15:00	7.85	-0.7037	-0.3533			14-6395-1490	11-9492-7222
18			11	14:35	7.225	-1.329	-0.6672			00-1546-1531	03-5898-7126
19		Dec	9	15:50	8.177	-0.3769	-0.1892			06-2693-6580	19-9748-5087
20	2022	Jan	20	15:15	10.94	2.39	1.2			06-1599-8254	16-8693-8465
21		Feb	9	16:25	8.097	-0.4568	-0.2293			20-6883-0287	03-6791-7638

CETIS QC Plot

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:	27.33	Count:	20	-2s Warning Limit:	16.48	-3s Action Limit:	11.06
Sigma:	5.421	CV:	19.80%	+2s Warning Limit:	38.17	+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	Oct	23	13:45	27.94	0.6117	0.1128			09-8413-3498	02-1232-2390
2			28	15:50	29.82	2.489	0.4592			09-4043-4676	15-7574-6891
3		Nov	20	16:00	28.24	0.9137	0.1686			13-7696-8009	21-0824-4197
4	2021	Feb	9	15:15	29.8	2.475	0.4565			12-5648-6062	08-9593-0094
5		Apr	20	16:15	27.97	0.6408	0.1182			06-7450-9711	02-2099-4435
6		May	12	15:00	39.23	11.9	2.195	(+)		15-4594-3065	18-1677-8776
7		Jun	3	15:50	29.62	2.288	0.422			07-9391-2508	05-7225-1680
8			9	14:00	28.97	1.636	0.3019			18-5736-8495	17-4075-5383
9			15	15:40	29.61	2.281	0.4209			00-2993-6780	11-7676-4213
10			22	13:45	27.27	-0.06302	-0.01163			16-6840-3553	00-7652-1305
11			29	14:55	29.58	2.255	0.416			07-2040-2693	20-9452-4039
12		Jul	27	16:30	16.82	-10.51	-1.939			16-6019-6958	09-3317-6652
13		Aug	17	14:25	14.86	-12.47	-2.3	(-)		07-7298-7649	12-6822-1646
14		Sep	29	15:45	28.5	1.169	0.2156			12-3450-8829	17-8563-2416
15		Oct	12	15:00	27.53	0.1971	0.03636			14-7239-9185	11-8743-4626
16			19	17:00	29.13	1.8	0.332			17-5798-2248	01-7668-6950
17		Nov	3	15:00	29.71	2.376	0.4383			14-6395-1490	03-1145-8832
18			11	14:35	22.33	-5.005	-0.9233			00-1546-1531	07-6640-8098
19		Dec	9	15:50	30.73	3.398	0.6268			06-2693-6580	02-3744-1694
20	2022	Jan	20	15:15	18.86	-8.474	-1.563			06-1599-8254	12-6429-5476
21		Feb	9	16:25	28.86	1.532	0.2825			20-6883-0287	05-7427-9529

Analyst: SCL QA: AHS 3/2/22

CETIS Test Data Worksheet

Report Date: 05 Feb-22 14:36 (p 1 of 1)
Test Code: 20-6883-0287/220209msdv

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

Start Date: 09 Feb-22
End Date: 11 Feb-22
Sample Date: 09 Feb-22

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

Sample Code: ~~22209msdv~~ 220209msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			214	197	WF 3/1/22
			2			203	191	
			3			1	0	
			4			230	221	
			5			215	33	
			6			218	210	
			7			185	0	
			8			222	213	
			9			210	200	
			10			212	33	
			11			177	0	
			12			1	0	
			13			0	0	
			14			~~180~~ 200	192	
			15			194	31	
			16			203	197	
			17			0	0	
			18			177	0	
			19			239	235	
			20			215	207	
			21			169	3	
			22			242	238	
			23			200	195	
			24			247	242	
			25			194	0	
			26			197	39	
			27			203	198	
			28			211	205	
			29			4	0	
			30			~~134~~ 234	69	QC = 70/232

(C) ~~[illegible]~~

(A) Q18 WF 3/1/22 TOTAL: 200

(B) Q18 AS 3/1/22

(C) Q18 WF 3/1/22 TOTAL: 234

CETIS Test Data Worksheet

Report Date: 05 Feb-22 14:36 (p 1 of 1)
Test Code: 20-6883-0287/220209msdv

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

Start Date: 09 Feb-22
End Date: 11 Feb-22
Sample Date: 09 Feb-22

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

Sample Code: (A) ~~22209msdv~~ 220209msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	24					
0	LC	2	6					
0	LC	3	22			209	205	RT OBS WF 2/15/22
0	LC	4	20					
0	LC	5	8					
2.5		1	14					
2.5		2	19					
2.5		3	27			200	196	
2.5		4	2					
2.5		5	4					
5		1	9					
5		2	28					
5		3	1			197	190	
5		4	16					
5		5	23					
10		1	30					
10		2	10					
10		3	26			192	26	
10		4	5					
10		5	15					
20		1	21					
20		2	7					
20		3	11			169	0	
20		4	25					
20		5	18					
40		1	17					
40		2	29					
40		3	12			2	0	
40		4	3					
40		5	13					

QC'd

(A) QIP AFS 3/1/22

CETIS™ v1.8.7.20

Analyst: WFBO QIP RT 2 HCl 1 QA: AFS 3/1/22

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 220209msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 2/9/2022 1625

End Date/Time: 2/11/2022 1625

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	31.6	31.8	31.9	14.6	14.6	14.6	8.5	8.6	8.5	8.01	7.96	7.89
2.5	31.9	32.1	32.4	14.5	14.5	14.6	8.6	8.6	8.5	7.98	7.96	7.90
5	32.0	32.2	32.5	14.4	14.5	14.6	8.6	8.6	8.5	8.00	7.96	7.91
10	32.0	32.1	32.5	14.8	14.5	14.6	8.5	8.7	8.5	8.00	7.96	7.91
20	31.9	32.1	32.5	14.7	14.4	14.6	8.6	8.7	8.5	8.01	8.00	7.91
40	31.9	32.1	32.4	14.6	14.5	14.7	8.6	8.7	8.5	8.00	8.00	7.91

	0	24	48
WQ Readings:	BO	KD	RT
Dilutions made by:	KB		

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:

24 hrs:

48 hrs:

QC Check: JH 3/2/22

Final Review: AMS 3/1/22

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

QA JL 3/1/22

Client/Sample: Internal / CuCl2
Test No.: 220209msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP / 6A/B
Date Received: 11/17/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 2/9/2022 1625 ~~1750~~
End Date/Time: 2/11/2022 1625
Technician Initials: YS / BD

Spawn Information

First Gamete Release Time: 1300

Sex	Number Spawning
Male	3+
Female	3+

Gamete Selection

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

Egg Fertilization Time: 1400

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: 8, 7, 10, 10, 8 | 8, 9, 8, 8, 14

Mean: 9.0

Mean 9.0 X 50 = 450 embryos/ml

Initial Density: 450 = 1.5 (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	172	172	100	100
TØ B	238	238	100	
TØ C	188	188	100	
TØ D	201	201	100	
TØ E	201	201	100	
TØ F	212	212	100	
$\bar{X}$ =	202			

48-h QC: 235/241 = 97.5%

Comments:

QC Check: JL 3/1/22

Final Review: AS 3/1/22