ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

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

Monitoring Period: November 2021

Prepared for: **Jacobs**
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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

Toxicity tests were 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), and the acute bioassay was conducted using the *Menidia beryllina* (inland silverside). 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	**110221**
Enthalpy Log-in Number	21-1139
Collection Date; Time	11/2/2021; 1020h
Receipt Date; Time	11/3/2021; 1015h
Receipt Temperature (°C)	5.0
Dissolved Oxygen (mg/L)	7.8
pH	7.48
Conductivity (µS/cm)	14,260
Salinity (ppt)	8.6
Alkalinity (mg/L $CaCO_3$)	300
Total Chlorine (mg/L)	< 0.02
Total Ammonia (mg/L as N)	1.0

NM = not measured

Test Methods

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

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

Test Period	11/3/2021, 1500h to 11/5/2021, 1500h
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 95.4 ppt
Test Concentrations (% sample)	75.3[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.

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

Test Period	11/3/2021, 1715 to 11/7/2021, 1635h
Test Organism	*Menidia beryllina*
Test Organism Source	Aquatic Biosystems (Fort Collins, CO)
Test Organism Age	10 days
Test Duration	96 ± 2 hours
Test Type	Static with 48-hr renewal
Test Chamber, Test Solution Volume	500 mL plastic cup, 250 mL
Test Temperature	25 ± 1°C
Dilution Water	Artificial Saltwater (Instant Ocean® salts added to deionized water to 30 ± 2 ppt)[a]
Additional Control	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) diluted with de-ionized water
Test Salinity	30 ± 2 ppt
Source of Salinity	Instant Ocean® salts were added to the sample to raise salinity to 30 ± 2 ppt
Test Concentrations (% sample)	100, 50, 25, 12.5, 6.25%, lab and salt controls
Number of Replicates	4
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/821/R-02/012
Test Acceptability Criteria for Controls	≥ 90% mean survival
Reference Toxicant	Copper chloride[b]
Statistical Software	CETIS™ 1.8.7.20

[a] Test was renewed with diluted laboratory seawater at 48 hours. See QA section of the report

[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 75.3 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.3 for both endpoints. Likewise, there was no statistically significant effect detected to survival for any concentration tested in the inland silverside test. This results in a NOEC of 100 and an acute toxic unit (TU_a) of 1.0.

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

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_c)	EC_{25} (% effluent)
Bivalve	Normal Development	75.3	> 75.3	< 1.3	> 75.3
	Survival	75.3	> 75.3	< 1.3	> 75.3
Inland Silverside	Survival	100	> 100	1.0	> 100

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.

Acute Toxic Unit (TUa) = 100/LC50. A TUa of 1.0 indicates no toxicity in the sample.

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

Table 5. Detailed Results for the Bivalve Development Chronic Toxicity Test

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	96.8	98.3
0 (Lab Control)	98.1	98.6
2	98.6	98.5
4	97.4	97.7
9	100	98.9
18	97.4	97.2
35	100	98.6
75.3[a]	99.0	96.3

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

Table 6. Detailed Results for the Inland Silverside Acute Survival Test

Concentration (% Effluent)	Mean Survival (%)
0 (Salt Control)	95.0
0 (Lab Control)	95.0
6.25	95.0
12.5	100
25	95.0
50	95.0
100	85.0

Quality Assurance

The sample was received in good condition and within the appropriate temperature range of 0-6°C. Both tests were initiated within the required 36-hour holding time. All control acceptability criteria were met, and water quality parameters remained within the appropriate ranges throughout the test. Statistical analyses followed standard USEPA flowchart selections. Dose-response relationships were reviewed to ensure the reliability of the results. Based on the dose response observed, the calculated effects concentrations were deemed reliable.

Due to insufficient volume, a 50 percent renewal was performed at 48 hours for the inland silverside test. Additionally, during the 48-hour renewal, test concentrations were made with diluted natural seawater instead of artificial seawater due to technician error. The test was renewed with these dilutions, due to there being insufficient volume of the sample to remake them. However, since the salt control survival was equal to the lab control, and since there were no significant effects detected in the sample, it is unlikely that this affected test results.

Minor QA/QC issues that were unlikely to have any bearing on the final test results, such as slight temperature deviations, are noted on the data sheets and a list of qualifier codes used on bench data sheets is presented in Appendix D.

Reference Toxicant

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 7. 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 tests were within the acceptable range of the mean historical test results plus or minus two standard deviations for all tests and endpoints. Reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 7. Reference Toxicant Test Results

Species and Endpoint	**NOEC** (%)	**EC_{50}** (µg/L copper)	**Historical Mean ± 2 SD** (µg/L copper)	**CV** (%)
Bivalve Survival Rate	20	29.7	27.9 ± 9.66	17.3
Bivalve Normal Development	5	7.85	8.33 ± 3.88	23.3
Inland Silverside Survival	100	211	187 ± 92.9	24.9

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.

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

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

Appendix A
Statistical Summaries and Raw Bench Sheets

Bivalve Larval Development Test

CETIS Summary Report

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

Batch ID:	09-1175-3863	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	03 Nov-21 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	05 Nov-21 15:00	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	48h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	07-7918-2489	**Code:**	21-1139	**Client:**	Jacobs
Sample Date:	02 Nov-21 10:20	**Material:**	Effluent Sample	**Project:**	
Receive Date:	03 Nov-21 10:15	**Source:**	Jacobs		
Sample Age:	29h (5 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-5824-0438	Combined Development Ra	75.3	>75.3	NA	6.08%	1.328	Dunnett Multiple Comparison Test
20-8202-3703	Development Rate	75.3	>75.3	NA	1.88%	1.328	Dunnett Multiple Comparison Test
18-8630-7874	Survival Rate	75.3	>75.3	NA	5.58%	1.328	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-4338-6363	Combined Development Ra	EC25	>75.3	N/A	N/A	<1.328	Linear Interpolation (ICPIN)
		EC50	>75.3	N/A	N/A	<1.328	
18-2762-8635	Development Rate	EC25	>75.3	N/A	N/A	<1.328	Linear Interpolation (ICPIN)
		EC50	>75.3	N/A	N/A	<1.328	
01-3044-4779	Survival Rate	EC25	>75.3	N/A	N/A	<1.328	Linear Interpolation (ICPIN)
		EC50	>75.3	N/A	N/A	<1.328	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
18-2762-8635	Development Rate	Control Resp	0.9826	0.9 - NL	Yes	Passes Acceptability Criteria
20-8202-3703	Development Rate	Control Resp	0.9826	0.9 - NL	Yes	Passes Acceptability Criteria
01-3044-4779	Survival Rate	Control Resp	0.9684	0.5 - NL	Yes	Passes Acceptability Criteria
18-8630-7874	Survival Rate	Control Resp	0.9684	0.5 - NL	Yes	Passes Acceptability Criteria
03-5824-0438	Combined Development Ra	PMSD	0.06075	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	0.9515	0.9086	0.9945	0.9211	0.9919	0.01546	0.03458	3.63%	0.0%
0	Lab Control	5	0.967	0.9198	1	0.9035	1	0.017	0.03802	3.93%	-1.62%
2		5	0.9715	0.9359	1	0.9211	0.9915	0.01283	0.02869	2.95%	-2.1%
4		5	0.9508	0.8804	1	0.8509	0.9853	0.02537	0.05672	5.97%	0.08%
9		5	0.9889	0.9837	0.9942	0.9843	0.9928	0.001882	0.004209	0.43%	-3.93%
18		5	0.9466	0.8893	1	0.8772	0.9843	0.02064	0.04615	4.88%	0.52%
35		5	0.9861	0.9683	1	0.9627	1	0.00641	0.01433	1.45%	-3.63%
75.3		5	0.9527	0.9112	0.9942	0.9035	0.9915	0.01495	0.03343	3.51%	-0.12%

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9826	0.9686	0.9965	0.964	0.9919	0.005028	0.01124	1.14%	0.0%
0	Lab Control	5	0.9858	0.971	1	0.9717	1	0.005338	0.01194	1.21%	-0.33%
2		5	0.9854	0.978	0.9928	0.9767	0.9915	0.002669	0.005969	0.61%	-0.29%
4		5	0.9766	0.9641	0.989	0.9603	0.9853	0.004485	0.01003	1.03%	0.61%
9		5	0.9889	0.9837	0.9942	0.9843	0.9928	0.001882	0.004209	0.43%	-0.65%
18		5	0.972	0.9584	0.9856	0.9545	0.9843	0.004893	0.01094	1.13%	1.08%
35		5	0.9861	0.9683	1	0.9627	1	0.00641	0.01433	1.45%	-0.36%
75.3		5	0.9627	0.9342	0.9911	0.9279	0.9915	0.01024	0.0229	2.38%	2.03%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9684	0.9272	1	0.9298	1	0.01483	0.03317	3.43%	0.0%
0	Lab Control	5	0.9807	0.9427	1	0.9298	1	0.0137	0.03064	3.12%	-1.27%
2		5	0.986	0.947	1	0.9298	1	0.01404	0.03138	3.18%	-1.81%
4		5	0.9737	0.9006	1	0.8684	1	0.02632	0.05884	6.04%	-0.54%
9		5	1	1	1	1	1	0	0	0.0%	-3.26%
18		5	0.9737	0.9214	1	0.9035	1	0.01881	0.04207	4.32%	-0.54%
35		5	1	1	1	1	1	0	0	0.0%	-3.26%
75.3		5	0.9895	0.9716	1	0.9737	1	0.006446	0.01441	1.46%	-2.17%

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	0.9851	0.9211	0.9919	0.9211	0.9386
0	Lab Control	0.9035	1	0.9913	0.9752	0.9649
2		0.9915	0.9839	0.9844	0.9211	0.9767
4		0.9833	0.8509	0.9853	0.9741	0.9603
9		0.9928	0.992	0.9912	0.9843	0.9845
18		0.9764	0.9211	0.8772	0.9739	0.9843
35		1	0.9627	0.9922	0.9835	0.9921
75.3		0.9386	0.96	0.9699	0.9915	0.9035

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9851	0.9813	0.9919	0.9906	0.964
0	Lab Control	0.9717	1	0.9913	0.9752	0.991
2		0.9915	0.9839	0.9844	0.9906	0.9767
4		0.9833	0.9798	0.9853	0.9741	0.9603
9		0.9928	0.992	0.9912	0.9843	0.9845
18		0.9764	0.9545	0.9709	0.9739	0.9843
35		1	0.9627	0.9922	0.9835	0.9921
75.3		0.964	0.96	0.9699	0.9915	0.9279

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	0.9386	1	0.9298	0.9737
0	Lab Control	0.9298	1	1	1	0.9737
2		1	1	1	0.9298	1
4		1	0.8684	1	1	1
9		1	1	1	1	1
18		1	0.9649	0.9035	1	1
35		1	1	1	1	1
75.3		0.9737	1	1	1	0.9737

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	132/134	105/114	123/124	105/114	107/114
0	Lab Control	103/114	119/119	114/115	118/121	110/114
2		117/118	122/124	126/128	105/114	126/129
4		118/120	97/114	134/136	113/116	121/126
9		137/138	124/125	113/114	125/127	127/129
18		124/127	105/114	100/114	112/115	125/127
35		130/130	129/134	127/128	119/121	126/127
75.3		107/114	120/125	129/133	116/117	103/114

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	132/134	105/107	123/124	105/106	107/111
0	Lab Control	103/106	119/119	114/115	118/121	110/111
2		117/118	122/124	126/128	105/106	126/129
4		118/120	97/99	134/136	113/116	121/126
9		137/138	124/125	113/114	125/127	127/129
18		124/127	105/110	100/103	112/115	125/127
35		130/130	129/134	127/128	119/121	126/127
75.3		107/111	120/125	129/133	116/117	103/111

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	114/114	107/114	114/114	106/114	111/114
0	Lab Control	106/114	114/114	114/114	114/114	111/114
2		114/114	114/114	114/114	106/114	114/114
4		114/114	99/114	114/114	114/114	114/114
9		114/114	114/114	114/114	114/114	114/114
18		114/114	110/114	103/114	114/114	114/114
35		114/114	114/114	114/114	114/114	114/114
75.3		111/114	114/114	114/114	114/114	111/114

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

Analysis ID: 03-5824-0438 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 23 Nov-21 15:04 **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	6.08%	75.3	>75.3	NA	1.328

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-0.9573	2.407	0.126	8	0.9860	CDF	Non-Significant Effect
		4	-0.08767	2.407	0.126	8	0.8795	CDF	Non-Significant Effect
		9	-1.968	2.407	0.126	8	0.9995	CDF	Non-Significant Effect
		18	0.2024	2.407	0.126	8	0.7952	CDF	Non-Significant Effect
		35	-1.871	2.407	0.126	8	0.9993	CDF	Non-Significant Effect
		75.3	0.007972	2.407	0.126	8	0.8550	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.07096888	0.01182815	6	1.739	0.1488	Non-Significant Effect
Error	0.1904587	0.006802098	28			
Total	0.2614276		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.187	16.81	0.1633	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9582	0.9146	0.2014	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	0.9515	0.9086	0.9945	0.9386	0.9211	0.9919	0.01546	3.63%	0.0%
2		5	0.9715	0.9359	1	0.9839	0.9211	0.9915	0.01283	2.95%	-2.1%
4		5	0.9508	0.8804	1	0.9741	0.8509	0.9853	0.02537	5.97%	0.08%
9		5	0.9889	0.9837	0.9942	0.9912	0.9843	0.9928	0.001883	0.43%	-3.93%
18		5	0.9466	0.8893	1	0.9739	0.8772	0.9843	0.02064	4.88%	0.52%
35		5	0.9861	0.9683	1	0.9921	0.9627	1	0.00641	1.45%	-3.63%
75.3		5	0.9527	0.9112	0.9942	0.96	0.9035	0.9915	0.01495	3.51%	-0.12%

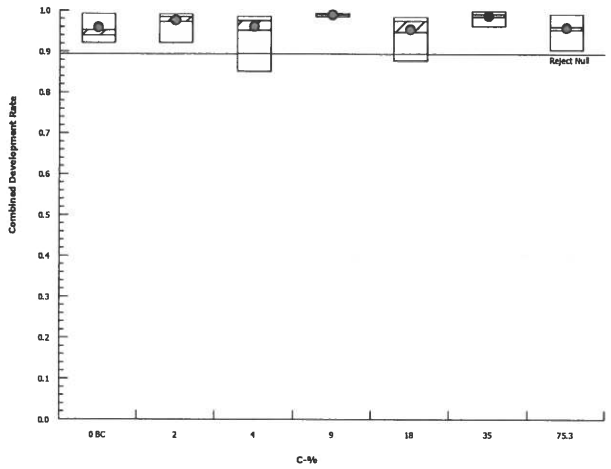
Angular (Corrected) Transformed Summary

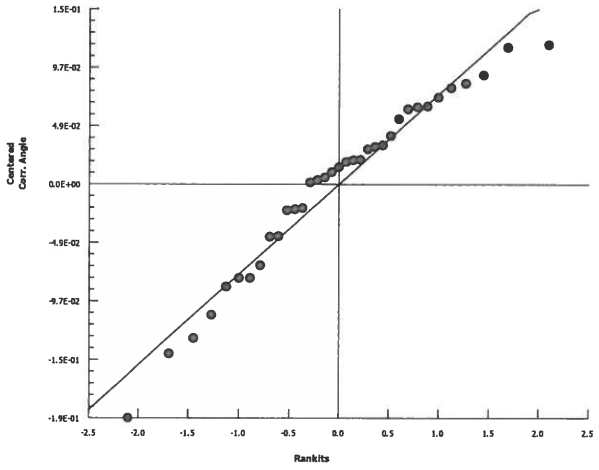
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.364	1.248	1.48	1.32	1.286	1.481	0.04174	6.84%	0.0%
2		5	1.414	1.321	1.507	1.443	1.286	1.479	0.03349	5.3%	-3.66%
4		5	1.369	1.228	1.509	1.409	1.174	1.449	0.05059	8.26%	-0.34%
9		5	1.467	1.442	1.492	1.477	1.445	1.486	0.008875	1.35%	-7.52%
18		5	1.354	1.23	1.478	1.409	1.213	1.445	0.04457	7.36%	0.77%
35		5	1.462	1.392	1.532	1.482	1.376	1.527	0.02526	3.86%	-7.15%
75.3		5	1.364	1.26	1.468	1.369	1.255	1.478	0.03737	6.13%	0.03%

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

Analysis ID:	03-5824-0438	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Nov-21 15:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 20-8202-3703 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 23 Nov-21 15:04 **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	1.88%	75.3	>75.3	NA	1.328

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	-0.3256	2.407	0.063	8	0.9275	CDF	Non-Significant Effect
		4	0.9013	2.407	0.063	8	0.4979	CDF	Non-Significant Effect
		9	-0.9065	2.407	0.063	8	0.9838	CDF	Non-Significant Effect
		18	1.459	2.407	0.063	8	0.2601	CDF	Non-Significant Effect
		35	-0.7132	2.407	0.063	8	0.9723	CDF	Non-Significant Effect
		75.3	2.242	2.407	0.063	8	0.0697	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02872553	0.004787588	6	2.799	0.0292	Significant Effect
Error	0.04790152	0.001710769	28			
Total	0.07662705		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.605	16.81	0.2685	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9612	0.9146	0.2489	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	0.9826	0.9686	0.9965	0.9851	0.964	0.9919	0.005028	1.14%	0.0%
2		5	0.9854	0.978	0.9928	0.9844	0.9767	0.9915	0.00267	0.61%	-0.29%
4		5	0.9766	0.9641	0.989	0.9798	0.9603	0.9853	0.004485	1.03%	0.61%
9		5	0.9889	0.9837	0.9942	0.9912	0.9843	0.9928	0.001883	0.43%	-0.65%
18		5	0.972	0.9584	0.9856	0.9739	0.9545	0.9843	0.004893	1.13%	1.08%
35		5	0.9861	0.9683	1	0.9921	0.9627	1	0.00641	1.45%	-0.36%
75.3		5	0.9627	0.9342	0.9911	0.964	0.9279	0.9915	0.01024	2.38%	2.03%

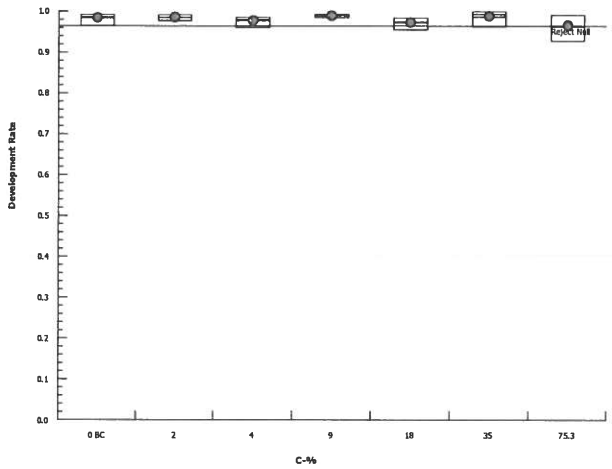
Angular (Corrected) Transformed Summary

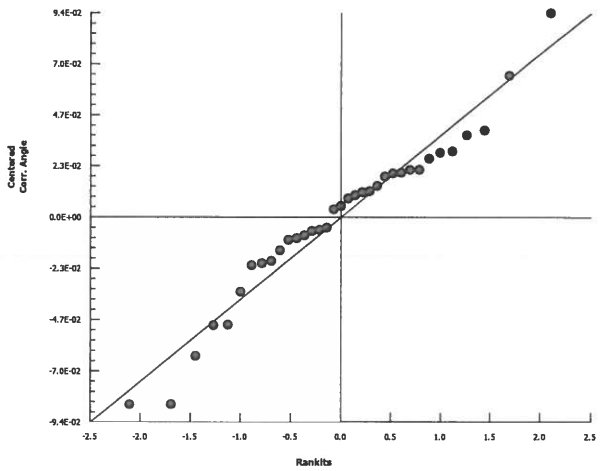
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.443	1.393	1.493	1.448	1.38	1.481	0.01799	2.79%	0.0%
2		5	1.452	1.421	1.483	1.445	1.418	1.479	0.0111	1.71%	-0.59%
4		5	1.42	1.381	1.459	1.428	1.37	1.449	0.01408	2.22%	1.63%
9		5	1.467	1.442	1.492	1.477	1.445	1.486	0.008875	1.35%	-1.64%
18		5	1.405	1.365	1.445	1.409	1.356	1.445	0.01446	2.3%	2.65%
35		5	1.462	1.392	1.532	1.482	1.376	1.527	0.02526	3.86%	-1.29%
75.3		5	1.385	1.305	1.464	1.38	1.299	1.478	0.02871	4.64%	4.06%

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

Analysis ID:	20-8202-3703	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Nov-21 15:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 18-8630-7874 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 23 Nov-21 15:04 **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	5.58%	75.3	>75.3	NA	1.328

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	31.5	16	3	8	0.9815	Asymp	Non-Significant Effect
		4	31	16	2	8	0.9749	Asymp	Non-Significant Effect
		9	35	16	2	8	0.9986	Asymp	Non-Significant Effect
		18	29	16	2	8	0.9262	Asymp	Non-Significant Effect
		35	35	16	2	8	0.9986	Asymp	Non-Significant Effect
		75.3	32	16	3	8	0.9866	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04840661	0.008067769	6	0.9275	0.4906	Non-Significant Effect
Error	0.2435529	0.008698317	28			
Total	0.2919595		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.9014	3.812	0.5123	Equal Variances
Variances	Levene Equality of Variance	4.231	3.528	0.0037	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8657	0.9146	0.0005	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	0.9684	0.9272	1	0.9737	0.9298	1	0.01483	3.43%	0.0%
2		5	0.986	0.947	1	1	0.9298	1	0.01404	3.18%	-1.81%
4		5	0.9737	0.9006	1	1	0.8684	1	0.02632	6.04%	-0.54%
9		5	1	1	1	1	1	1	0	0.0%	-3.26%
18		5	0.9737	0.9214	1	1	0.9035	1	0.01881	4.32%	-0.54%
35		5	1	1	1	1	1	1	0	0.0%	-3.26%
75.3		5	0.9895	0.9716	1	1	0.9737	1	0.006446	1.46%	-2.17%

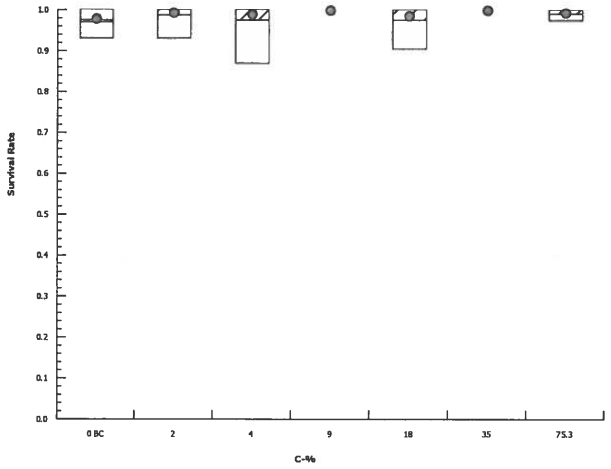
Angular (Corrected) Transformed Summary

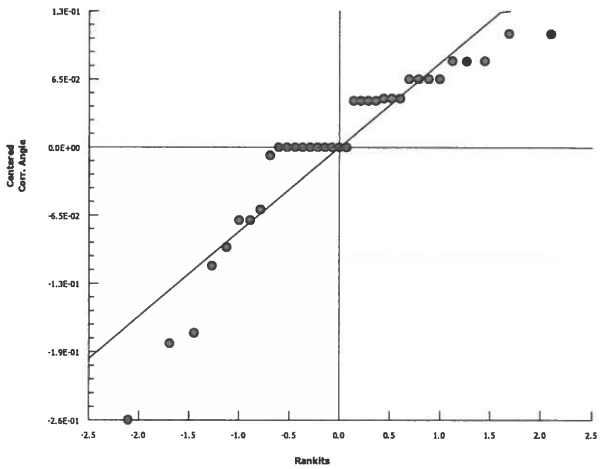
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.416	1.284	1.548	1.408	1.303	1.524	0.04762	7.52%	0.0%
2		5	1.48	1.357	1.603	1.524	1.303	1.524	0.04425	6.69%	-4.52%
4		5	1.459	1.279	1.639	1.524	1.2	1.524	0.06487	9.94%	-3.06%
9		5	1.524	1.524	1.524	1.524	1.524	1.524	0	0.0%	-7.64%
18		5	1.442	1.291	1.592	1.524	1.255	1.524	0.05417	8.4%	-1.84%
35		5	1.524	1.524	1.524	1.524	1.524	1.524	0	0.0%	-7.64%
75.3		5	1.478	1.399	1.556	1.524	1.408	1.524	0.02844	4.3%	-4.36%

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

Analysis ID:	18-8630-7874	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Nov-21 15:04	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 2111-S048 | 08-7751-8703

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

Analysis ID:	07-4338-6363	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Nov-21 15:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1034442	1000	Yes	Two-Point Interpolation

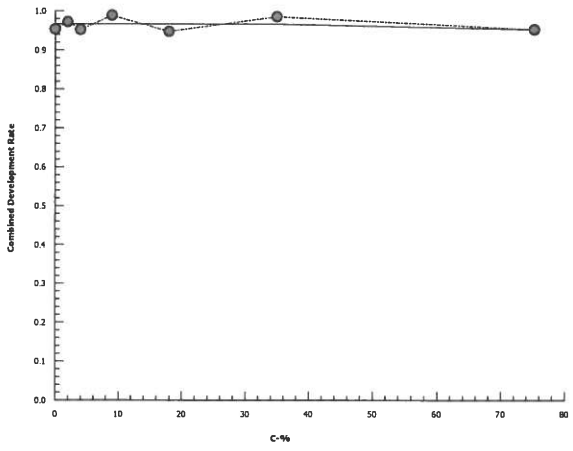
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.3	N/A	N/A	<1.328	NA	NA
EC50	>75.3	N/A	N/A	<1.328	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	0.9515	0.9211	0.9919	0.01546	0.03458	3.63%	0.0%	572	600
2		5	0.9715	0.9211	0.9915	0.01283	0.02869	2.95%	-2.1%	596	613
4		5	0.9508	0.8509	0.9853	0.02537	0.05672	5.97%	0.08%	583	612
9		5	0.9889	0.9843	0.9928	0.001883	0.004211	0.43%	-3.93%	626	633
18		5	0.9466	0.8772	0.9843	0.02064	0.04615	4.88%	0.52%	566	597
35		5	0.9861	0.9627	1	0.00641	0.01433	1.45%	-3.63%	631	640
75.3		5	0.9527	0.9035	0.9915	0.01495	0.03343	3.51%	-0.12%	575	603

Graphics



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

Analysis ID:	18-2762-8635	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Nov-21 15:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	524309	1000	Yes	Two-Point Interpolation

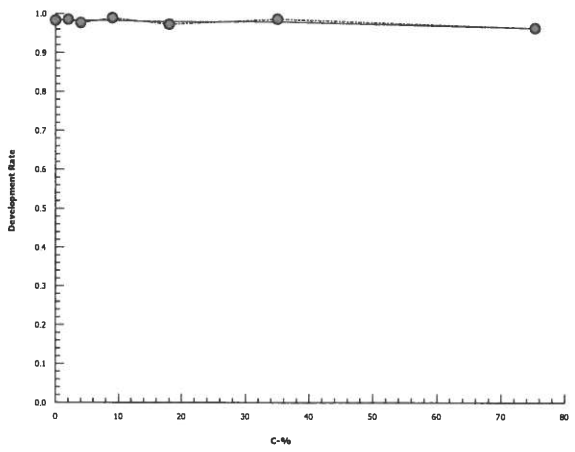
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.3	N/A	N/A	<1.328	NA	NA
EC50	>75.3	N/A	N/A	<1.328	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	0.9826	0.964	0.9919	0.005028	0.01124	1.14%	0.0%	572	582
2		5	0.9854	0.9767	0.9915	0.00267	0.00597	0.61%	-0.29%	596	605
4		5	0.9766	0.9603	0.9853	0.004485	0.01003	1.03%	0.61%	583	597
9		5	0.9889	0.9843	0.9928	0.001883	0.004211	0.43%	-0.65%	626	633
18		5	0.972	0.9545	0.9843	0.004893	0.01094	1.13%	1.08%	566	582
35		5	0.9861	0.9627	1	0.00641	0.01433	1.45%	-0.36%	631	640
75.3		5	0.9627	0.9279	0.9915	0.01024	0.0229	2.38%	2.03%	575	597

Graphics



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

Analysis ID:	01-3044-4779	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Nov-21 15:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2044335	1000	Yes	Two-Point Interpolation

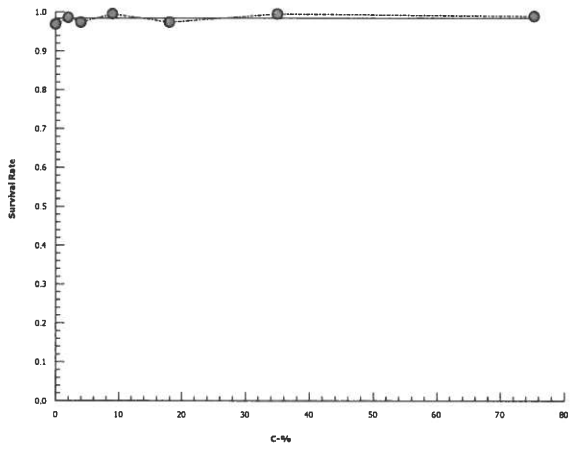
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.3	N/A	N/A	<1.328	NA	NA
EC50	>75.3	N/A	N/A	<1.328	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	0.9684	0.9298	1	0.01483	0.03317	3.43%	0.0%	552	570
2		5	0.986	0.9298	1	0.01404	0.03138	3.18%	-1.81%	562	570
4		5	0.9737	0.8684	1	0.02632	0.05884	6.04%	-0.54%	555	570
9		5	1	1	1	0	0	0.0%	-3.26%	570	570
18		5	0.9737	0.9035	1	0.01881	0.04207	4.32%	-0.54%	555	570
35		5	1	1	1	0	0	0.0%	-3.26%	570	570
75.3		5	0.9895	0.9737	1	0.006446	0.01441	1.46%	-2.17%	564	570

Graphics



CETIS Test Data Worksheet

Test Code: 2111-5048 08-7751-8703/344DDF6F

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

Start Date:	03 Nov-21	**Species:**	Mytilus galloprovincialis	**Sample Code:**	21- 1139
End Date:	05 Nov-21	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Jacobs
Sample Date:	02 Nov-21	**Material:**	Effluent Sample	**Sample Station:**	Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			99	97	RT 11/23/21
			2			110	105	
			3			115	114	
			4			106	105	
			5			125	120	
			6			111	110	
			7			124	123	
			8			117	116	
			9			111	107	
			10			136	134	
			11			128	127	
			12			124	122	
			13			126	121	
			14			115	112	
			15			128	126	
			16			127	126	
			17			120	118	
			18			107	105	
			19			116	113	
			20			121	119	
			21			119	119	
			22			138	137	
			23			111	103	
			24			127	125	
			25			121	118	
			26			129	127	
			27			129	126	
			28			106	105	
			29			134	129	
			30			114	113	
			31			106	103	
			32			103	100	
			33			130	130	
			34			133	129	
			35			127	125	
			36			118	117	
			37			125	124	
			38			111	107	
			39			127	124	
			40			134	132	

CETIS Test Data Worksheet

Report Date: 29 Oct-21 14:21 (p 1 of 1)
Test Code: 2111-5048 08-7751-8703/344DDF6F

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

Start Date: 03 Nov-21
End Date: 05 Nov-21
Sample Date: 02 Nov-21
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 21-1139
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	40					
0	BC	2	18					
0	BC	3	7			103	101	RT 11/5/21
0	BC	4	28					
0	BC	5	38					
0	LC	1	31					
0	LC	2	21					
0	LC	3	3			119	118	WF 11/6/21
0	LC	4	25					
0	LC	5	6					
2		1	36					
2		2	12					
2		3	15			123	122	WF 11/6/21
2		4	4					
2		5	27					
4		1	17					
4		2	1					
4		3	10			135	133	WF 11/6/21
4		4	19					
4		5	13					
9		1	22					
9		2	37					
9		3	30			128	128	WF 11/6/21
9		4	35					
9		5	26					
18		1	39					
18		2	2					
18		3	32			109	105	WF 11/6/21
18		4	14					
18		5	24					
35		1	33					
35		2	29					
35		3	11			135	133	WF 11/6/21
35		4	20					
35		5	16					
75.3 76.1		1	9					
76.1		2	5					
76.1		3	34			130	127	WF 11/6/21
76.1		4	8					
76.1		5	23					

Q18 AT 11/3/21

QC = RT

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** RT

Sample ID: Wyckoff **Test Date:** 11/3/2021

Test No: 2111-5048 **Test Type:** Mussel Development

Salinity of Effluent 8.6

Salinity of Brine 95.4 **Date of Brine used:** 9/28/2021

Target Salinity 30 **Alkalinity of Brine Control:** 114 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.33**	**0.46**

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	0.33	1.6	250
4	10.0	0.33	3.3	250
9	22.5	0.33	7.4	250
18	45.0	0.33	14.7	250
35	87.5	0.33	28.6	250
75.3	188.4	0.33	61.6	250

	DI Volume			
Brine Control	134.4	0.46	61.6	250

Total Brine Volume Required (ml): **178.9**

QC Check: JR 12/1/21 **Final Review:** AES 12/1/21

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS
Sample ID: Wyckoff
Sample Log No.: 21- 1139
Test No.: 2111-S048

Test Species: *M. galloprovincialis*
Start Date/Time: 11/3/2021 1500
End Date/Time: 11/5/2021 1500

Concentration (% sample)	Salinity (ppt)			Temperature (A) (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	29.0	28.9	28.9	15.9	15.1	15.3	9.0	8.4	8.3	7.99	8.00	7.98
Brine Control	30.3	30.2	30.0	15.9	15.5	15.2	9.0	8.3	8.1	8.21	8.07	7.98
2	29.2	29.2	29.1	15.9	14.9	15.1	9.0	8.4	8.0	8.00	7.98	7.97
4	29.2	29.1	29.1	15.9	14.9	15.1	8.9	8.3	7.9	7.96	7.98	7.98
9	29.2	29.3	29.1	15.9	15.0	15.1	8.8	8.2	8.1	7.91	7.98	8.00
18	29.4	29.4	29.3	15.9	15.0	15.1	8.8	8.3	8.1	7.84	7.99	8.02
35	29.6	29.7	29.6	15.9	14.9	15.2	8.7	8.3	7.9	7.77	8.02	8.07
75.3	30.6	30.6	30.4	15.9	15.0	15.3	8.4	8.2	8.0	7.67	8.02	8.12

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

Technician Initials:

Environmental Chamber: D

Comments:
0 hrs: (A) Temperature measured using surrogate vial
24 hrs:
48 hrs:

QC Check: JL 11/9/21

Final Review: ARS 12/1/21

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: JACOBS / Wyckoff
Test No.: 2111-5048
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP SA
Date Received: 9/14/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 11/3/2021 1500
End Date/Time: 11/5/2021 1500
Technician Initials: RT

Spawn Information

First Gamete Release Time: 1140

Sex	Number Spawning
Male	6+
Female	4+

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	3,4,5,6	good motility, good density
Female 1	2	good density, pale orange, round
Female 2	3	good density, pale orange, round
Female 3		

Egg Fertilization Time: 1230

Embryo Stock Selection

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

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

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

Number Counted:

(A) ~~13~~ 15	(A) ~~15~~ 21
(A) ~~14~~ 19	(A) ~~13~~ 20
(A) ~~16~~ 17	(A) ~~16~~ 18
(A) ~~12~~ 20	(A) ~~9~~ 15
(A) ~~13~~ 18	(A) ~~9~~ 15

Mean: 17.8

Mean 17.8 X 50 = 890 embryos/ml

Initial Density: 890 = 2.97 (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	106	106	100	100.0
TØ B	102	102	100	
TØ C	113	113	100	
TØ D	130	130	100	
TØ E	109	109	100	
TØ F	122	122	100	
$\bar{X}$ =	114			

48-h QC: 91/94 = 96.8%

Comments: (A) Q18 RT 11/3/21

QC Check: JL 11/9/21

Final Review: ACS 12/1/21

Inland Silverside Acute Survival Test

CETIS Summary Report

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

Batch ID:	18-5054-1892	**Test Type:**	Survival (96h)	**Analyst:**	Artificial Seawater & Diluted natural
Start Date:	03 Nov-21 17:15	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Ⓐ ~~Diluted Natural Seawater~~ Seawater
Ending Date:	07 Nov-21 16:35	**Species:**	Menidia beryllina	**Brine:**	Ⓐ ~~Not Applicable~~ Instant Ocean
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	10d

Sample ID:	15-2531-1593	**Code:**	21-1139	**Client:**	Jacobs
Sample Date:	02 Nov-21 10:20	**Material:**	Effluent Sample	**Project:**	
Receive Date:	03 Nov-21 10:15	**Source:**	Jacobs		
Sample Age:	31h (5 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-3278-3288	96h Survival Rate	100	>100	NA	19.9%	1	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
17-3636-8621	96h Survival Rate	EC25	>100	N/A	N/A	<1	Linear Interpolation (ICPIN)
		EC50	>100	N/A	N/A	<1	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-3278-3288	96h Survival Rate	Control Resp	0.95	0.9 - NL	Yes	Passes Acceptability Criteria
17-3636-8621	96h Survival Rate	Control Resp	0.95	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
0	Salt Control	4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
6.25		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
12.5		4	1	1	1	1	1	0	0	0.0%	-5.26%
25		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
50		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
100		4	0.85	0.5453	1	0.6	1	0.09574	0.1915	22.53%	10.53%

96h Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.8
0	Salt Control	1	1	0.8	1
6.25		1	0.8	1	1
12.5		1	1	1	1
25		1	1	0.8	1
50		1	1	1	0.8
100		0.8	0.6	1	1

Ⓐ QA AMS 12/1/21

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

Analysis ID: 06-3278-3288 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 15 Nov-21 9:31 **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	19.9%	100	>100	NA	1

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Salt Control		6.25	18	10	2	6	0.8333	Asymp	Non-Significant Effect
		12.5	20	10	1	6	0.9516	Asymp	Non-Significant Effect
		25	18	10	2	6	0.8333	Asymp	Non-Significant Effect
		50	18	10	2	6	0.8333	Asymp	Non-Significant Effect
		100	15.5	10	2	6	0.5438	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06485811	0.01297162	5	0.7386	0.6044	Non-Significant Effect
Error	0.3161308	0.01756282	18			
Total	0.3809889		23			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.129	4.248	0.3806	Equal Variances
Variances	Levene Equality of Variance	3.269	4.248	0.0283	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8244	0.884	0.0008	Non-normal Distribution

96h Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
6.25		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
12.5		4	1	1	1	1	1	1	0	0.0%	-5.26%
25		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
50		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
100		4	0.85	0.5453	1	0.9	0.6	1	0.09574	22.53%	10.53%

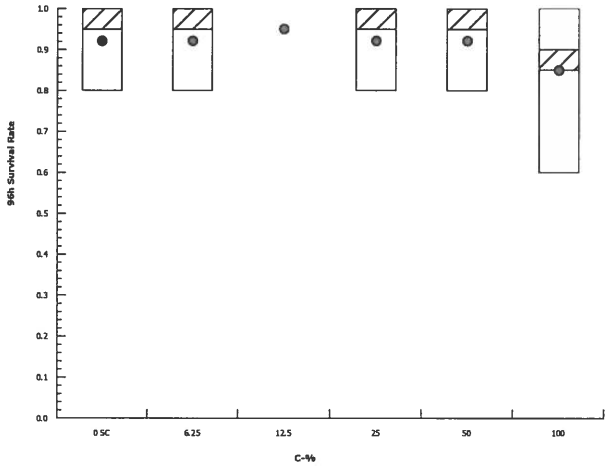
Angular (Corrected) Transformed Summary

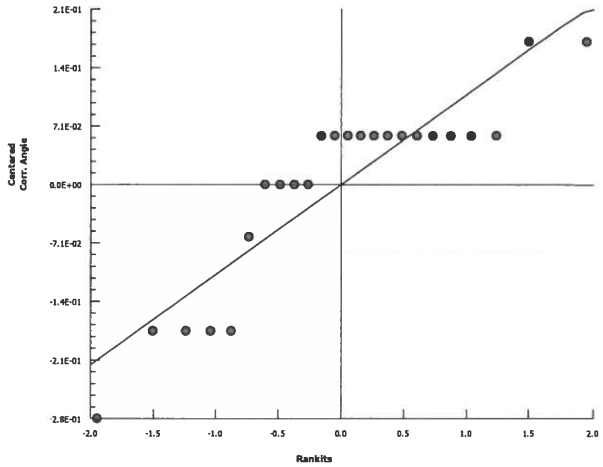
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Salt Control	4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
6.25		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
12.5		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-4.63%
25		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
50		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
100		4	1.171	0.8199	1.522	1.226	0.8861	1.345	0.1103	18.84%	8.93%

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

Analysis ID:	06-3278-3288	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Nov-21 9:31	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	17-3636-8621	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	15 Nov-21 9:31	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1236349	1000	Yes	Two-Point Interpolation

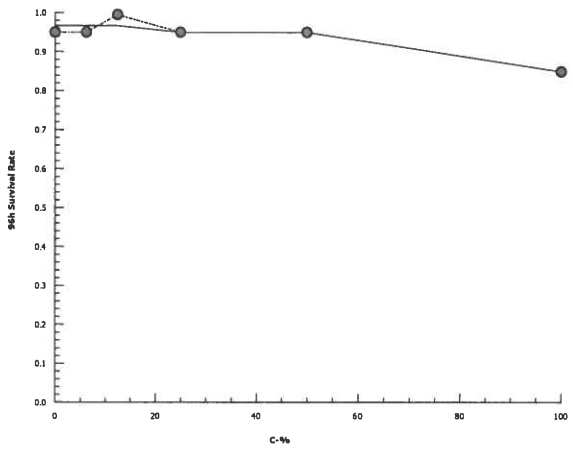
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>100	N/A	N/A	<1	NA	NA
EC50	>100	N/A	N/A	<1	NA	NA

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Salt Control	4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
6.25		4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
12.5		4	1	1	1	0	0	0.0%	-5.26%	20	20
25		4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
50		4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
100		4	0.85	0.6	1	0.09574	0.1915	22.53%	10.53%	17	20

Graphics



Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: JACOBS
Sample ID: Wyckoff
Sample Log-In No.: 21- 1139
Test No.: 2111-5056

Test Species: *M. beryllina*
Start Date/Time: 11/3/2021 1715
End Date/Time: 11/7/2021 1635

Tech Initials	0	24	48	72	96
Counts:	GM	SP	KB	KB	KL
Readings:	GM	SP	KB	KB	KL
Dilutions made by:	GM		SP (A)		

Concentration (%)	Rep	Number of Live Organisms (99)					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	30.1	30.4	i 29.5 f 30.4	30.2	30.4	25.0	24.6	i 24.2 f 24.5	24.3	24.6	6.7	6.2	i 6.6 f 5.3	6.1	5.7	7.91	7.86	i 7.94 f 7.73	7.89	7.74
	B	5	5	5	5	5																				
	C	5	5	5	5	5																				
	D	5	4	4	4	4																				
Salt Control	A	5	5	5	5	5	29.5	29.8	i 29.8 f 30.1	30.2	30.4	24.0	24.6	i 24.1 f 24.5	24.6	24.7	6.4	5.9	i 6.3 f 5.6	5.9	5.5	8.13	8.01	i 8.01 f 7.86	7.92	7.87
	B	5	5	5	5	5																				
	C	5	5	4	4	4																				
	D	5	5	5	5	5																				
6.25%	A	5	5	5	5	5	29.6	29.9	i 29.7 f 30.2	30.1	30.4	24.0	24.6	i 24.1 f 24.5	24.8	24.5	6.4	5.9	i 6.4 f 5.4	6.0	5.6	8.07	8.04	i 8.00 f 7.91	7.88	7.89
	B	5	4	4	4	4																				
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
12.5%	A	5	5	5	5	5	29.6	29.9	i 29.8 f 30.1	30.2	30.0	24.0	24.8	i 24.2 f 24.6	24.7	24.4	6.3	5.9	i 6.5 f 5.2	5.9	5.6	8.01	8.03	i 7.96 f 7.96	7.90	7.89
	B	5	5	5	5	5																				
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
25%	A	5	5	5	5	5	29.6	29.8	i 29.6 f 30.1	30.0	30.3	24.0	25.0	i 24.1 f 25.0	24.8	24.6	6.3	5.7	i 6.5 f 5.3	5.8	5.7	7.97	8.03	i 7.95 f 8.01	7.92	8.04
	B	5	5	5	5	5																				
	C	5	5	4	4	4																				
	D	5	5	5	5	5																				
50%	A	5	5	5	5	5	29.7	29.9	i 29.7 f 30.1	30.0	30.8	24.0	25.0	i 24.1 f 24.9	24.7	24.4	6.3	5.6	i 6.4 f 5.2	5.8	5.7	7.98	8.03	i 7.94 f 8.14	8.01	8.19
	B	5	5	5	5	5																				
	C	5	5	5	5	5																				
	D	5	4	4	4	4																				
100%	A	5	4	4	4	4	29.7	29.9	i 29.7 f 30.2	30.1	30.4	24.0	24.9	i 24.2 f 24.7	24.4	24.3	6.4	5.7	i 6.4 f 5.3	5.9	5.8	7.64	8.04	i 7.92 f 8.23	8.04	8.35
	B	5	3	3	3	3																				
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				

Initial Counts QC'd by: ~~GM~~ Q188 11/3/21 1830
Initiated by: GM

Environmental Chamber: A

Animal Source/Date Received: ABS 11/2/21

Age at Initiation: 10days

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

Feeding Times	0	24	48	72	96
AM:		0830	0845	1300	0850
PM:	1806				

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

Organisms fed prior to initiation, circle one (y / n) (A) Test renewal dilutions made with diluted natural seawater instead of artificial seawater at 48hrs due to tech error ACS 12/1/21

QC Check: JL 11/9/21

ACS 12/1/21
Final Review:

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff (110221)
Test ID No(s).: 2111 - 5048 + 5056

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (21-xxxx):	1139			
Sample Collection Date & Time:	11/2/21 1020			
Sample Receipt Date & Time:	11/3/21 1015			
Number of Containers & Container Type:	1 x 4L cubi			
Approx. Total Volume Received (L):	~4L			
Check-in Temperature (°C)	5.0			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.8			
pH (units)	7.48			
Conductivity (µS/cm)	14,260			
Salinity (ppt)	8.6			
Alkalinity (mg/L) [2]	300			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	< 0.02			
Technician Initials	KB			

Sample Description:
A colorless, clear, No odor, No debris

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

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

Filtration? Y (N) **Initials:** _____
Pore Size: _____
Organisms or Debris

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

pH Adjustment? Y (N)

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

Test Performed: Acute Menidia **Control/Dilution Water:** 8:2 / Lab SW / (Lab ART) **Other:** _____
Alkalinity: 137 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Lab SW Alkalinity: ~~137~~ 99 Hardness or Salinity: 30ppt
QIR JL 11/8/21

Test Performed: mussel development **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART **Other:** _____
Alkalinity: 99 137 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 114 Hardness or Salinity: 30ppt

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

Notes: [1] Temperature of sample should be 0-6°C at receipt.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: A QIR ARS 12/1/21

QC Check: JL 11/8/21
Final Review: ARS 12/1/21

Total Ammonia Analysis
Freshwater

Overlying Water

DC-001

Client: Jacobs
Project: Wyckoff
Test Type: Mussel Development and acute menidia

DI Blank: 0.0
Test Start Date: ~~11/2/2021~~ 11/3/21 ACS 12/1/21

Analyst: BS
Analysis Date: 11/12/21

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L) N x 1.22
Blank Spike (10 mg/L NH_3)		NA	NA	8.1	9.9
Wyckoff Effluent	21-1139	11/3/2021	check in	1.0	1.2
Spike Check (10 mg/L NH_3)		NA	NA		
Sample Duplicate[a]	21-1139	NA	NA	0.7	0.9
Sample Duplicate + Spike[a]		NA	NA	10.4	12.7
Spike Check (10 mg/L NH_3)		NA	NA	8.2	10.0

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

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

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	9.9	10	NA	99
21-1139	1.2	0.9	12.7	10	@ 28.6	115

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A1189	A1197	A1240

Comments: @RPD is out of range due to low ammonia values

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: JL 11/17/21

Final Review: ACS 12/1/21

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 11/2/2021
CarrierName: FedEx
AirbillNo:

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

No: 10-110221-112205-0570
2021T10P000DD210W2LA00
Contact Name: Daniel Baca
Contact Phone: 206-871-8760

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
110221		Ground Water/ D. Baca	Composite	ACTOX-CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	11/02/2021 10:20	Field Sample

Special Instructions: 2021 Week 45 - 4th Quarter

Shipment for Case Complete? N

Samples Transferred From Chain of Custody #

Analysis Key: ACTOX-CHRTOX=Acute Toxicity, Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	JACOBS	11-2-21 @1145	EA-SD	11/3/21 1015	Good 5.0°C

EA login 21-1139

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:	03-9630-7688	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	03 Nov-21 15:00	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	05 Nov-21 15:00	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	48h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	07-7158-7375	**Code:**	211103msdv	**Client:**	Internal
Sample Date:	03 Nov-21	**Material:**	Copper chloride	**Project:**	
Receive Date:	03 Nov-21	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-1574-3505	Combined Development Ra	5	10	7.071	14.9%		Dunnett Multiple Comparison Test
13-9419-9521	Development Rate	5	10	7.071	9.94%		Steel Many-One Rank Sum Test
07-8853-6576	Survival Rate	20	40	28.28	7.57%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
06-4040-2968	Combined Development Ra	EC25	6.215	5.427	6.849		Linear Interpolation (ICPIN)
		EC50	7.733	7.033	8.816		
11-9492-7222	Development Rate	EC25	6.411	6.141	6.918		Linear Interpolation (ICPIN)
		EC50	7.85	7.359	8.933		
03-1145-8832	Survival Rate	EC25	24.56	23.3	25.22		Linear Interpolation (ICPIN)
		EC50	29.71	28.86	30.15		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
11-9492-7222	Development Rate	Control Resp	0.9828	0.9 - NL	Yes	Passes Acceptability Criteria
13-9419-9521	Development Rate	Control Resp	0.9828	0.9 - NL	Yes	Passes Acceptability Criteria
03-1145-8832	Survival Rate	Control Resp	0.9649	0.5 - NL	Yes	Passes Acceptability Criteria
07-8853-6576	Survival Rate	Control Resp	0.9649	0.5 - NL	Yes	Passes Acceptability Criteria
16-1574-3505	Combined Development Ra	PMSD	0.1491	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	0.9485	0.8824	1	0.886	0.9921	0.02384	0.0533	5.62%	0.0%
2.5		5	0.9802	0.9544	1	0.9568	1	0.009281	0.02075	2.12%	-3.33%
5		5	0.9144	0.7844	1	0.7368	0.9925	0.04685	0.1048	11.46%	3.6%
10		5	0.1288	0	0.3556	0	0.4386	0.08168	0.1826	141.8%	86.42%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.9828	0.9734	0.9922	0.9714	0.9921	0.003396	0.007593	0.77%	0.0%
2.5		5	0.9802	0.9544	1	0.9568	1	0.009281	0.02075	2.12%	0.27%
5		5	0.9777	0.9582	0.9971	0.9537	0.9925	0.007017	0.01569	1.61%	0.52%
10		5	0.1373	0	0.3863	0	0.4808	0.08971	0.2006	146.1%	86.03%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.9649	0.9048	1	0.9035	1	0.02167	0.04844	5.02%	0.0%
2.5		5	1	1	1	1	1	0	0	0.0%	-3.64%
5		5	0.9351	0.8068	1	0.7544	1	0.04622	0.1033	11.05%	3.09%
10		5	0.9789	0.9317	1	0.9123	1	0.01701	0.03803	3.89%	-1.46%
20		5	0.9544	0.8916	1	0.8684	0.9912	0.0226	0.05054	5.3%	1.09%
40		5	0	0	0	0	0	0	0		100.0%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8947	0.9852	0.886	0.9847	0.9921
2.5		1	1	0.9606	0.9833	0.9568
5		0.7368	0.9035	0.9744	0.9925	0.9649
10		0.1463	0	0.05036	0.4386	0.008772
20		0	0	0	0	0
40		0	0	0	0	0

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9714	0.9852	0.9806	0.9847	0.9921
2.5		1	1	0.9606	0.9833	0.9568
5		0.9767	0.9537	0.9744	0.9925	0.991
10		0.1463	0	0.05036	0.4808	0.008929
20		0	0	0	0	0
40		0	0	0	0	0

Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9211	1	0.9035	1	1
2.5		1	1	1	1	1
5		0.7544	0.9474	1	1	0.9737
10		1	1	1	0.9123	0.9825
20		0.9649	0.8684	0.9912	0.9912	0.9561
40		0	0	0	0	0

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

Combined Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	102/114	133/135	101/114	129/131	126/127
2.5		120/120	128/128	122/127	118/120	133/139
5		84/114	103/114	114/117	133/134	110/114
10		18/123	0/132	7/139	50/114	1/114
20		0/114	0/114	0/114	0/114	0/114
40		0/114	0/114	0/114	0/114	0/114

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	102/105	133/135	101/103	129/131	126/127
2.5		120/120	128/128	122/127	118/120	133/139
5		84/86	103/108	114/117	133/134	110/111
10		18/123	0/132	7/139	50/104	1/112
20		0/110	0/99	0/113	0/113	0/109
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	105/114	114/114	103/114	114/114	114/114
2.5		114/114	114/114	114/114	114/114	114/114
5		86/114	108/114	114/114	114/114	111/114
10		114/114	114/114	114/114	104/114	112/114
20		110/114	99/114	113/114	113/114	109/114
40		0/114	0/114	0/114	0/114	0/114

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

Analysis ID:	16-1574-3505	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Nov-21 12:20	**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	14.9%	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	-0.6718	2.227	0.253	8	0.9226	CDF	Non-Significant Effect
		5	0.4945	2.227	0.253	8	0.5472	CDF	Non-Significant Effect
		10*	9.453	2.227	0.253	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.414649	1.47155	3	45.69	<0.0001	Significant Effect
Error	0.5152858	0.03220537	16			
Total	4.929935		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.508	11.34	0.1382	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9385	0.866	0.2247	Normal Distribution

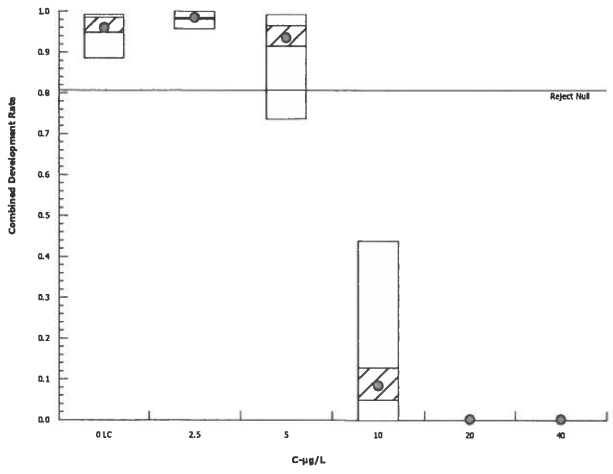
Combined Development Rate Summary

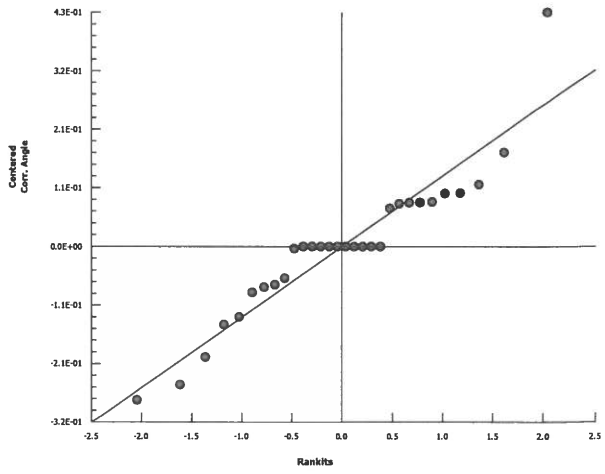
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9485	0.8824	1	0.9847	0.886	0.9921	0.02384	5.62%	0.0%
2.5		5	0.9802	0.9544	1	0.9833	0.9568	1	0.009281	2.12%	-3.33%
5		5	0.9144	0.7844	1	0.9649	0.7368	0.9925	0.04685	11.46%	3.6%
10		5	0.1288	0	0.3556	0.05036	0	0.4386	0.08168	141.8%	86.42%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.369	1.214	1.524	1.447	1.226	1.482	0.05572	9.1%	0.0%
2.5		5	1.445	1.346	1.544	1.441	1.362	1.527	0.03573	5.53%	-5.57%
5		5	1.313	1.093	1.533	1.382	1.032	1.484	0.07931	13.51%	4.1%
10		5	0.296	-0.04508	0.6371	0.2263	0.04353	0.7238	0.1229	92.8%	78.38%
20		5	0.04685	0.04684	0.04685	0.04685	0.04685	0.04685	0	0.0%	96.58%
40		5	0.04685	0.04684	0.04685	0.04685	0.04685	0.04685	0	0.0%	96.58%

Graphics





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

Analysis ID: 13-9419-9521 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 24 Nov-21 12:20 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	9.94%	5	10	7.071	

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	27	17	0	8	0.7105	Asymp	Non-Significant Effect
		5	26	17	0	8	0.6242	Asymp	Non-Significant Effect
		10*	15	17	0	8	0.0123	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.821548	1.607183	3	67.76	<0.0001	Significant Effect
Error	0.3795103	0.02371939	16			
Total	5.201058		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	19.81	11.34	0.0002	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8326	0.866	0.0028	Non-normal Distribution

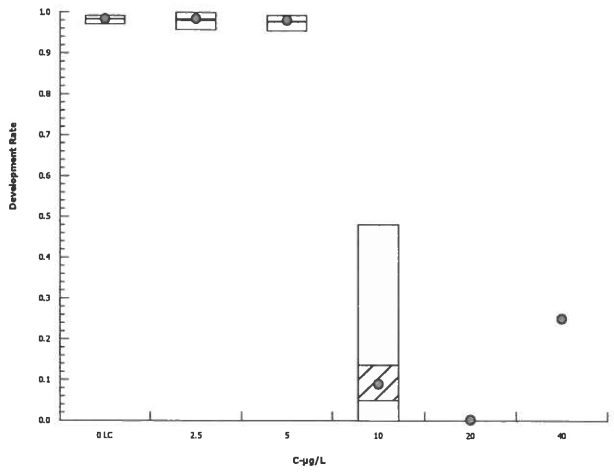
Development Rate Summary

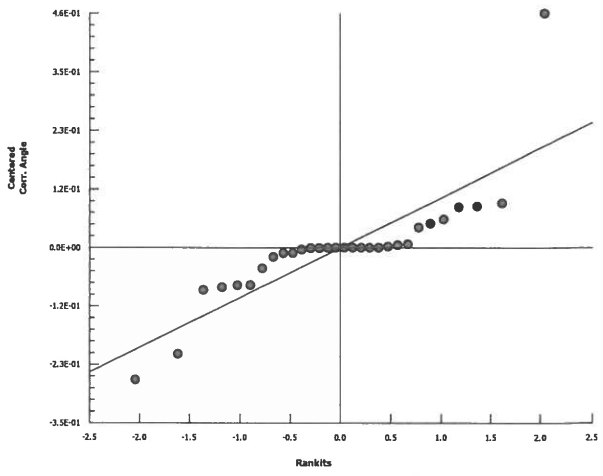
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9828	0.9734	0.9922	0.9847	0.9714	0.9921	0.003396	0.77%	0.0%
2.5		5	0.9802	0.9544	1	0.9833	0.9568	1	0.009281	2.12%	0.27%
5		5	0.9777	0.9582	0.9971	0.9767	0.9537	0.9925	0.007017	1.61%	0.52%
10		5	0.1373	0	0.3863	0.05036	0	0.4808	0.08971	146.1%	86.03%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.442	1.405	1.478	1.447	1.401	1.482	0.01318	2.04%	0.0%
2.5		5	1.445	1.346	1.544	1.441	1.362	1.527	0.03573	5.53%	-0.22%
5		5	1.428	1.362	1.495	1.418	1.354	1.484	0.02384	3.73%	0.94%
10		5	0.3046	-0.0569	0.6662	0.2263	0.04353	0.7662	0.1302	95.58%	78.87%
20		5	0.048	0.04635	0.04965	0.04769	0.04705	0.05027	0.0005943	2.77%	96.67%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	63.69%

Graphics





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

Analysis ID: 07-8853-6576 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 24 Nov-21 12:20 **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	7.57%	20	40	28.28	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.25	2.305	0.187	8	0.9876	CDF	Non-Significant Effect
		5	0.6546	2.305	0.187	8	0.5342	CDF	Non-Significant Effect
		10	-0.4122	2.305	0.187	8	0.9062	CDF	Non-Significant Effect
		20	0.5352	2.305	0.187	8	0.5880	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.07903066	0.01975767	4	1.202	0.3409	Non-Significant Effect
Error	0.3288174	0.01644087	20			
Total	0.4078481		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.309	4.893	0.3114	Equal Variances
Variances	Levene Equality of Variance	3.084	4.431	0.0394	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8939	0.8877	0.0136	Normal Distribution

Survival Rate Summary

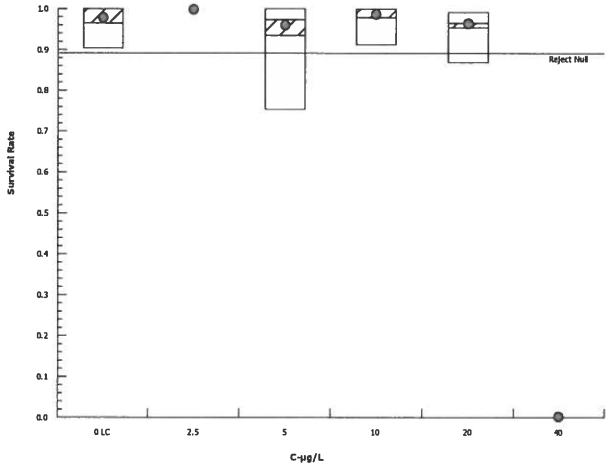
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9649	0.9048	1	1	0.9035	1	0.02167	5.02%	0.0%
2.5		5	1	1	1	1	1	1	0	0.0%	-3.64%
5		5	0.9351	0.8068	1	0.9737	0.7544	1	0.04622	11.05%	3.09%
10		5	0.9789	0.9317	1	1	0.9123	1	0.01701	3.89%	-1.46%
20		5	0.9544	0.8916	1	0.9649	0.8684	0.9912	0.0226	5.3%	1.09%
40		5	0	0	0	0	0	0	0		100.0%

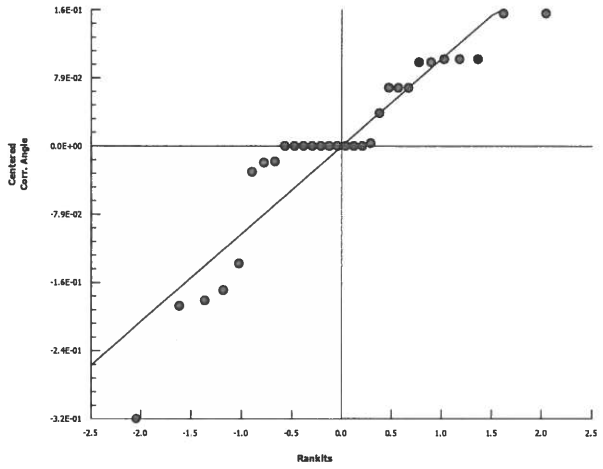
Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.423	1.25	1.595	1.524	1.255	1.524	0.06229	9.79%	0.0%
2.5		5	1.524	1.524	1.524	1.524	1.524	1.524	0	0.0%	-7.13%
5		5	1.369	1.128	1.61	1.408	1.052	1.524	0.08681	14.17%	3.73%
10		5	1.456	1.319	1.593	1.524	1.27	1.524	0.04936	7.58%	-2.35%
20		5	1.379	1.238	1.52	1.382	1.2	1.477	0.05088	8.25%	3.05%
40		5	0.04685	0.04684	0.04685	0.04685	0.04685	0.04685	0	0.0%	96.71%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	07-8853-6576	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Nov-21 12:20	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	06-4040-2968	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Nov-21 12:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1020017	1000	Yes	Two-Point Interpolation

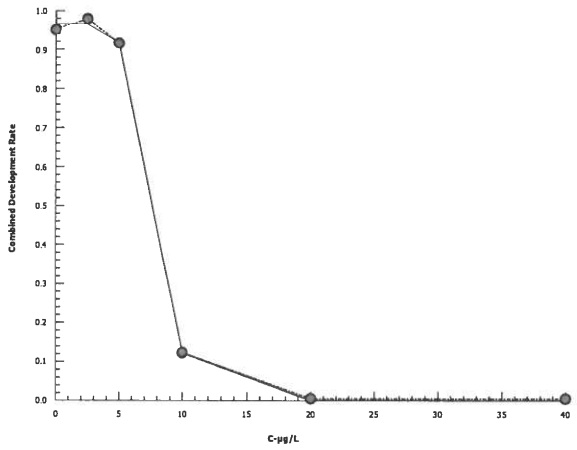
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.215	5.427	6.849
EC50	7.733	7.033	8.816

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	0.9485	0.886	0.9921	0.02384	0.0533	5.62%	0.0%	591	621
2.5		5	0.9802	0.9568	1	0.009281	0.02075	2.12%	-3.33%	621	634
5		5	0.9144	0.7368	0.9925	0.04685	0.1048	11.46%	3.6%	544	593
10		5	0.1288	0	0.4386	0.08168	0.1826	141.8%	86.42%	76	622
20		5	0	0	0	0	0		100.0%	0	570
40		5	0	0	0	0	0		100.0%	0	570

Graphics



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

Analysis ID:	11-9492-7222	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Nov-21 12:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	46101	1000	Yes	Two-Point Interpolation

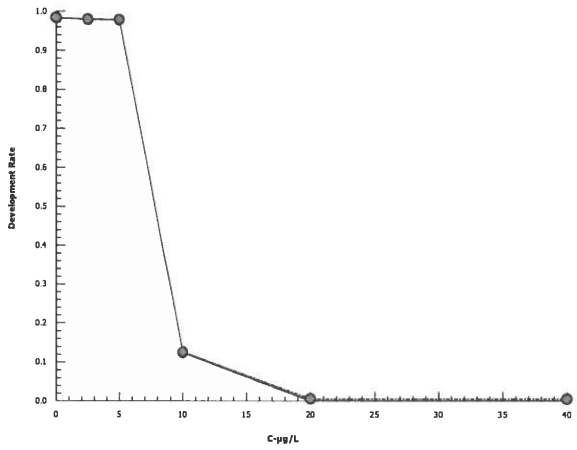
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.411	6.141	6.918
EC50	7.85	7.359	8.933

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	0.9828	0.9714	0.9921	0.003396	0.007594	0.77%	0.0%	591	601
2.5		5	0.9802	0.9568	1	0.009281	0.02075	2.12%	0.27%	621	634
5		5	0.9777	0.9537	0.9925	0.007017	0.01569	1.61%	0.52%	544	556
10		5	0.1373	0	0.4808	0.08971	0.2006	146.1%	86.03%	76	610
20		5	0	0	0	0	0		100.0%	0	544
40		5	0	0	0	0	0		100.0%	0	5

Graphics



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

Analysis ID:	03-1145-8832	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Nov-21 12:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	401743	1000	Yes	Two-Point Interpolation

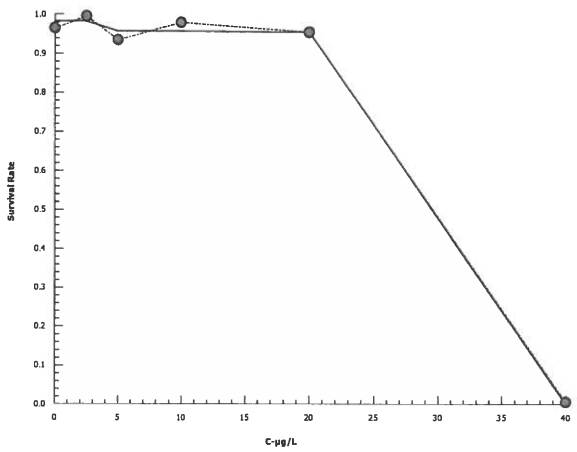
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	24.56	23.3	25.22
EC50	29.71	28.86	30.15

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	0.9649	0.9035	1	0.02167	0.04844	5.02%	0.0%	550	570
2.5		5	1	1	1	0	0	0.0%	-3.64%	570	570
5		5	0.9351	0.7544	1	0.04622	0.1033	11.05%	3.09%	533	570
10		5	0.9789	0.9123	1	0.01701	0.03803	3.89%	-1.46%	558	570
20		5	0.9544	0.8684	0.9912	0.0226	0.05054	5.3%	1.09%	544	570
40		5	0	0	0	0	0		100.0%	0	570

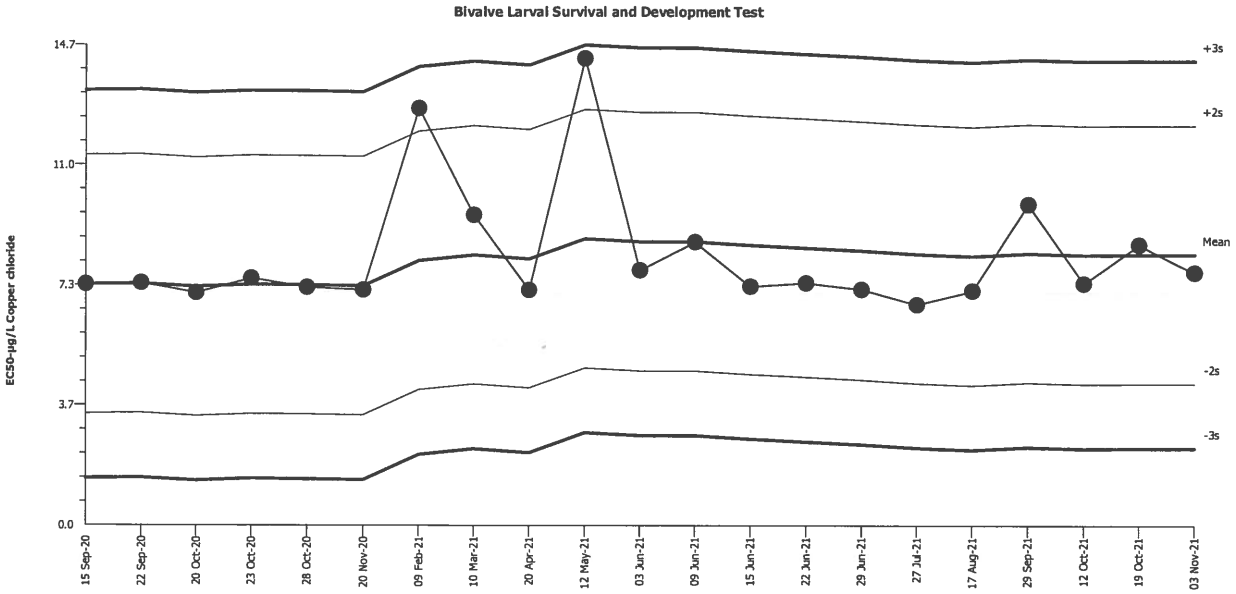
Graphics



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



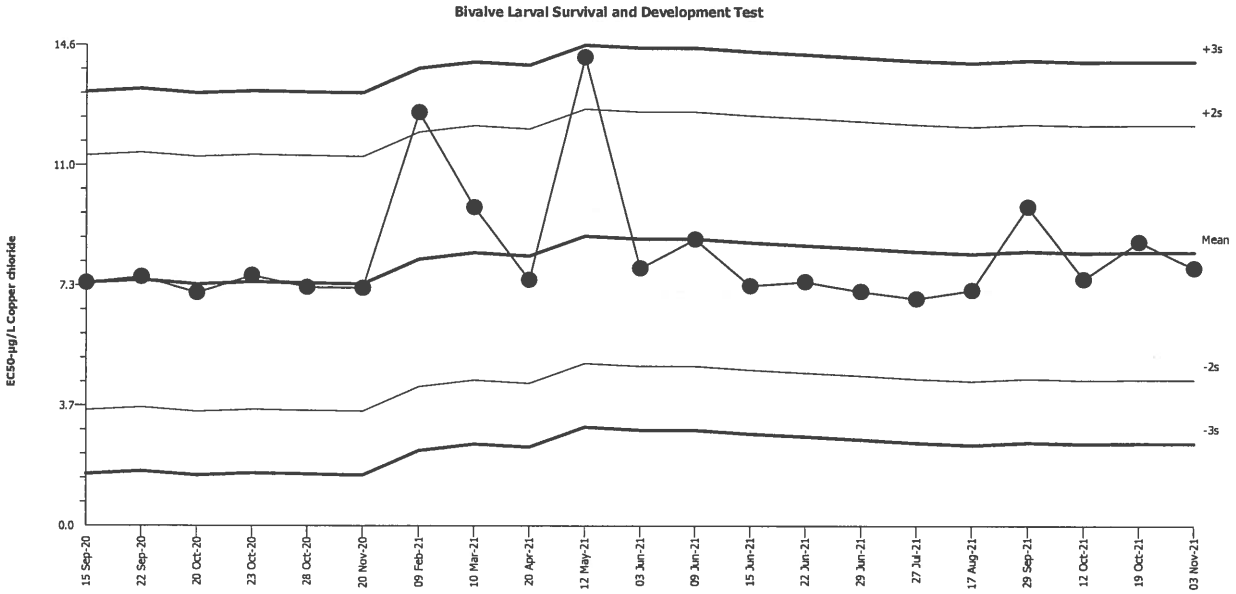
Mean:	8.28	**Count:**	20	**-2s Warning Limit:**	4.328	**-3s Action Limit:**	2.352
Sigma:	1.976	**CV:**	23.90%	**+2s Warning Limit:**	12.23	**+3s Action Limit:**	14.21

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Sep	15	0:00	7.365	-0.9149	-0.463			19-9833-0655	18-5101-1090
2			22	14:40	7.405	-0.8751	-0.4428			04-0347-9113	09-6026-9613
3		Oct	20	14:25	7.1	-1.18	-0.597			08-8652-5764	17-2783-6415
4			23	13:45	7.548	-0.7315	-0.3702			09-8413-3498	19-3049-9702
5			28	15:50	7.269	-1.011	-0.5117			09-4043-4676	02-6542-7057
6		Nov	20	16:00	7.187	-1.093	-0.5531			13-7696-8009	10-4367-1427
7	2021	Feb	9	15:15	12.74	4.459	2.256	(+)		12-5648-6062	18-1503-3303
8		Mar	10	14:15	9.481	1.201	0.6077			13-7922-5399	10-0885-9755
9		Apr	20	16:15	7.185	-1.095	-0.5542			06-7450-9711	18-3353-6875
10		May	12	15:00	14.27	5.988	3.03	(+)	(+)	15-4594-3065	00-9727-8504
11		Jun	3	15:50	7.791	-0.4888	-0.2474			07-9391-2508	21-2212-7050
12			9	14:00	8.654	0.3735	0.189			18-5736-8495	04-4549-3405
13			15	15:40	7.302	-0.9783	-0.4951			00-2993-6780	17-7654-7354
14			22	13:45	7.404	-0.876	-0.4433			16-6840-3553	15-2803-6917
15			29	14:55	7.211	-1.069	-0.5409			07-2040-2693	08-8247-6801
16		Jul	27	16:30	6.748	-1.532	-0.7751			16-6019-6958	06-5859-7928
17		Aug	17	14:25	7.168	-1.112	-0.563			07-7298-7649	09-6648-5411
18		Sep	29	15:45	9.809	1.529	0.7739			12-3450-8829	18-2247-7613
19		Oct	12	15:00	7.395	-0.8848	-0.4478			14-7239-9185	01-1367-5722
20			19	17:00	8.581	0.3009	0.1523			17-5798-2248	09-1208-0351
21		Nov	3	15:00	7.733	-0.5475	-0.2771			14-6395-1490	06-4040-2968

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:	8.328	**Count:**	20	**-2s Warning Limit:**	4.448	**-3s Action Limit:**	2.508
Sigma:	1.94	**CV:**	23.30%	**+2s Warning Limit:**	12.21	**+3s Action Limit:**	14.15

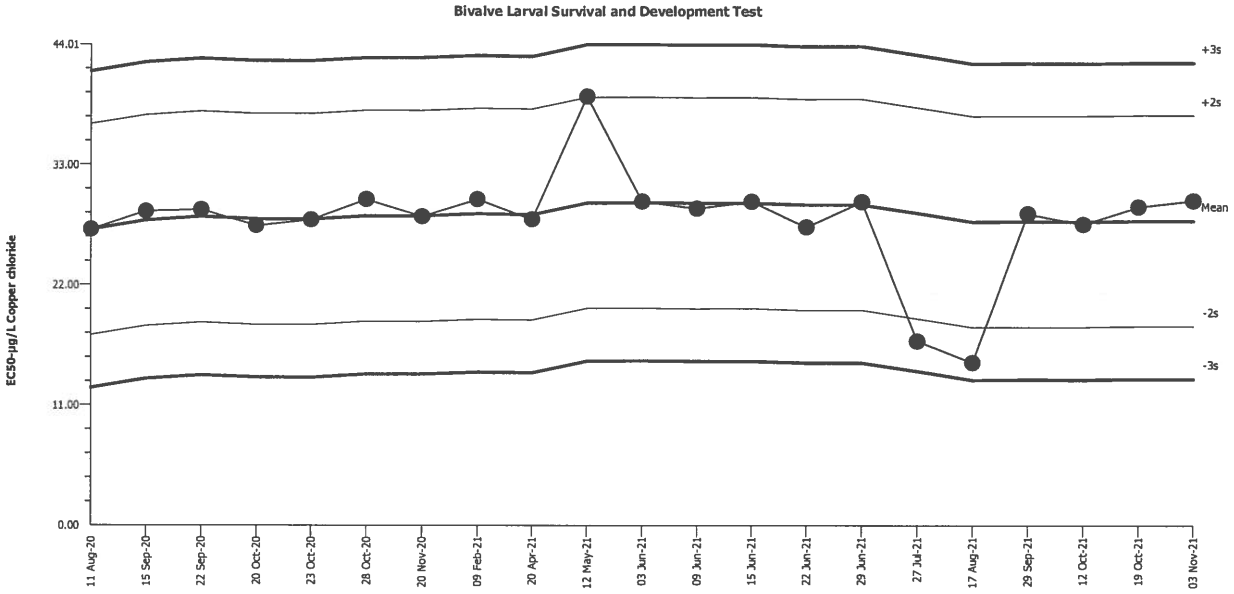
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Sep	15	0:00	7.397	-0.9308	-0.4798			19-9833-0655	03-7616-5506
2			22	14:40	7.576	-0.7517	-0.3875			04-0347-9113	01-0437-7711
3		Oct	20	14:25	7.089	-1.239	-0.6384			08-8652-5764	06-9681-8469
4			23	13:45	7.616	-0.712	-0.367			09-8413-3498	17-5257-3346
5			28	15:50	7.257	-1.071	-0.5521			09-4043-4676	12-0840-2779
6		Nov	20	16:00	7.23	-1.098	-0.5661			13-7696-8009	11-4264-3018
7	2021	Feb	9	15:15	12.58	4.255	2.193	(+)		12-5648-6062	01-5747-2564
8		Mar	10	14:15	9.694	1.366	0.7039			13-7922-5399	08-4869-7631
9		Apr	20	16:15	7.482	-0.8461	-0.4362			06-7450-9711	17-9210-1733
10		May	12	15:00	14.27	5.94	3.062	(+)	(+)	15-4594-3065	12-3891-6641
11		Jun	3	15:50	7.832	-0.4959	-0.2556			07-9391-2508	11-7075-1183
12			9	14:00	8.715	0.3874	0.1997			18-5736-8495	18-6125-5477
13			15	15:40	7.302	-1.026	-0.529			00-2993-6780	13-6998-5313
14			22	13:45	7.427	-0.9013	-0.4646			16-6840-3553	07-3347-2243
15			29	14:55	7.132	-1.196	-0.6166			07-2040-2693	17-0989-5973
16		Jul	27	16:30	6.912	-1.416	-0.7301			16-6019-6958	03-0913-6262
17		Aug	17	14:25	7.168	-1.16	-0.5982			07-7298-7649	11-4901-9823
18		Sep	29	15:45	9.718	1.39	0.7164			12-3450-8829	04-7958-3381
19		Oct	12	15:00	7.509	-0.8186	-0.422			14-7239-9185	04-3282-5514
20			19	17:00	8.648	0.3196	0.1647			17-5798-2248	05-0981-9303
21		Nov	3	15:00	7.85	-0.4777	-0.2462			14-6395-1490	11-9492-7222

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:	27.85	**Count:**	20	**-2s Warning Limit:**	18.19	**-3s Action Limit:**	13.36
Sigma:	4.83	**CV:**	17.30%	**+2s Warning Limit:**	37.51	**+3s Action Limit:**	42.34

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2020	Aug	11	14:30	27.06	-0.7912	-0.1638			21-4043-5119	16-7506-8565
2		Sep	15	0:00	28.73	0.8757	0.1813			19-9833-0655	01-9900-7404
3			22	14:40	28.86	1.014	0.2099			04-0347-9113	03-4439-9784
4		Oct	20	14:25	27.4	-0.4539	-0.09399			08-8652-5764	01-6350-7777
5			23	13:45	27.94	0.09165	0.01898			09-8413-3498	02-1232-2390
6			28	15:50	29.82	1.969	0.4078			09-4043-4676	15-7574-6891
7		Nov	20	16:00	28.24	0.3937	0.08152			13-7696-8009	21-0824-4197
8	2021	Feb	9	15:15	29.8	1.955	0.4047			12-5648-6062	08-9593-0094
9		Apr	20	16:15	27.97	0.1208	0.025			06-7450-9711	02-2099-4435
10		May	12	15:00	39.23	11.38	2.356	(+)		15-4594-3065	18-1677-8776
11		Jun	3	15:50	29.62	1.768	0.366			07-9391-2508	05-7225-1680
12			9	14:00	28.97	1.116	0.2311			18-5736-8495	17-4075-5383
13			15	15:40	29.61	1.761	0.3647			00-2993-6780	11-7676-4213
14			22	13:45	27.27	-0.583	-0.1207			16-6840-3553	00-7652-1305
15			29	14:55	29.58	1.735	0.3592			07-2040-2693	20-9452-4039
16		Jul	27	16:30	16.82	-11.03	-2.284	(-)		16-6019-6958	09-3317-6652
17		Aug	17	14:25	14.86	-12.99	-2.689	(-)		07-7298-7649	12-6822-1646
18		Sep	29	15:45	28.5	0.6489	0.1343			12-3450-8829	17-8563-2416
19		Oct	12	15:00	27.53	-0.3229	-0.06685			14-7239-9185	11-8743-4626
20			19	17:00	29.13	1.28	0.265			17-5798-2248	01-7668-6950
21		Nov	3	15:00	29.71	1.856	0.3842			14-6395-1490	03-1145-8832

CETIS Test Data Worksheet

Report Date: 29 Oct-21 14:23 (p 1 of 1)
Test Code: 14-6395-1490/211103msdv

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

Start Date: 03 Nov-21
End Date: 05 Nov-21
Sample Date: 03 Nov-21
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 211103msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			41			99	0	RT 11/23/21
			42			105	102	
			43			104	50	UC-AC
			44			0	0	
			45			113	0	
			46			108	103	
			47			127	122	
			48			132	0	
			49			127	126	
			50			117	114	
			51			128	128	
			52			120	120	
			53			110	0	
			54			134	133	
			55			0	0	
			56			139	7	
			57			135	133	
			58			103	101	
			59			120	118	
			60			123	18	
			61			109	0	
			62			111	110	
			63			113	0	
			64			0	0	
			65			112	1	
			66			0	0	
			67			139	133	
			68			131	129	
			69			0	0	
			70			86	84	

CETIS Test Data Worksheet

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

Start Date: 03 Nov-21 | **Species:** Mytilus galloprovincialis | **Sample Code:** 211103msdv
End Date: 05 Nov-21 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 03 Nov-21 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	42					
0	LC	2	57					
0	LC	3	58			99	98	11/6/21 WF
0	LC	4	68					
0	LC	5	49					
2.5		1	52					
2.5		2	51					
2.5		3	47			122	116	11/6/21/WF
2.5		4	59					
2.5		5	67					
5		1	70					
5		2	46					
5		3	50			115	110	11/6/21 WF
5		4	54					
5		5	62					
10		1	60					
10		2	48					
10		3	56			135	7	11/6/21 WF
10		4	43			~~135~~ (A)	~~7~~ (A)	11/6/21 WF
10		5	65					
20		1	53					
20		2	41					
20		3	45			108	0	11/6/21 WF
20		4	63					
20		5	61					
40		1	66					
40		2	55					
40		3	69			0	0	11/6/21 WF
40		4	64					
40		5	44					

QC- RT

(A) Q18 WF 11/6

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 211103msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 11/3/2021 1500

End Date/Time: 11/5/2021 1500

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	32.1	31.8	31.5	15.3	15.1	15.1	9.0	8.3	8.1	7.96	7.96	7.94
2.5	32.2	32.2	32.0	15.3	15.0	15.1	8.9	8.3	8.0	7.95	7.95	7.92
5	32.2	32.2	32.0	15.2	15.0	15.1	8.9	8.3	8.0	7.95	7.96	7.93
10	32.2	32.1	32.0	15.3	15.0	15.2	8.9	8.3	8.0	7.96	7.96	7.94
20	32.2	32.2	32.0	15.3	15.0	15.1	8.9	8.3	8.0	7.95	7.96	7.94
40	32.2	32.1	32.0	15.4	15.0	15.1	8.9	8.3	8.0	7.95	7.96	7.94

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

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: JCW 11/9/21

Final Review: AC 11/24/21

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / CuCl2
Test No.: 211103 msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: M-REP SA
Date Received: 9/14/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 11/3/2021 1500
End Date/Time: 11/5/2021 1500
Technician Initials: RT

Spawn Information

First Gamete Release Time: 1140

Sex	Number Spawning
Male	6+
Female	4+

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	3,4,5,6	good motility, good density
Female 1	2	good density, pale orange, round
Female 2	3	good density, pale orange, round
Female 3		

Egg Fertilization Time: 1230

Embryo Stock Selection

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

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

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

Number Counted:

(A) ~~13~~ 15	(A) ~~15~~ 21
(A) ~~19~~ 19	(A) ~~13~~ 20
(A) ~~16~~ 17	(A) ~~16~~ 18
(A) ~~12~~ 20	(A) ~~9~~ 15
(A) ~~13~~ 18	(A) ~~9~~ 15

Mean: 17.8

Mean 17.8 X 50 = 890 embryos/ml

Initial Density: 890 = 2.97 (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	106	106	100	100.0
TØ B	102	102	100	
TØ C	113	113	100	
TØ D	130	130	100	
TØ E	109	109	100	
TØ F	122	122	100	
$\bar{X}$ =	114			

48-h QC: 91/94 = 96.8%

Comments: (A) Q15 RT 11/3/21

QC Check: JL 11/9/21

Final Review: AC 11/24/21

CETIS Summary Report

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

Batch ID:	18-0499-6137	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	03 Nov-21 17:15	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	07 Nov-21 16:40	**Species:**	Menidia beryllina	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	10d

Sample ID:	12-7711-2580	**Code:**	211103mbra	**Client:**	Internal
Sample Date:	03 Nov-21	**Material:**	Copper chloride	**Project:**	
Receive Date:	03 Nov-21	**Source:**	Reference Toxicant		
Sample Age:	17h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-1931-6320	96h Survival Rate	100	200	141.4	20.6%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
13-6085-8539	96h Survival Rate	EC50	211.2	177.7	251.1		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
13-6085-8539	96h Survival Rate	Control Resp	0.95	0.9 - NL	Yes	Passes Acceptability Criteria
16-1931-6320	96h Survival Rate	Control Resp	0.95	0.9 - NL	Yes	Passes Acceptability Criteria

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
50		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
100		4	0.85	0.6909	1	0.8	1	0.05	0.1	11.76%	10.53%
200		4	0.65	0.3453	0.9547	0.4	0.8	0.09574	0.1915	29.46%	31.58%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	0.8
50		1	0.8	1	1
100		0.8	0.8	0.8	1
200		0.4	0.6	0.8	0.8
400		0	0	0	0
800		0	0	0	0

CETIS Analytical Report

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

Analysis ID:	16-1931-6320	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 Nov-21 9:42	**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	20.6%	100	200	141.4	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		50	0	2.287	0.234	6	0.7500	CDF	Non-Significant Effect
		100	1.163	2.287	0.234	6	0.2753	CDF	Non-Significant Effect
		200*	3.317	2.287	0.234	6	0.0080	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.3074218	0.1024739	3	4.892	0.0190	Significant Effect
Error	0.2513887	0.02094906	12			
Total	0.5588105		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.3	11.34	0.7291	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9137	0.8408	0.1334	Normal Distribution

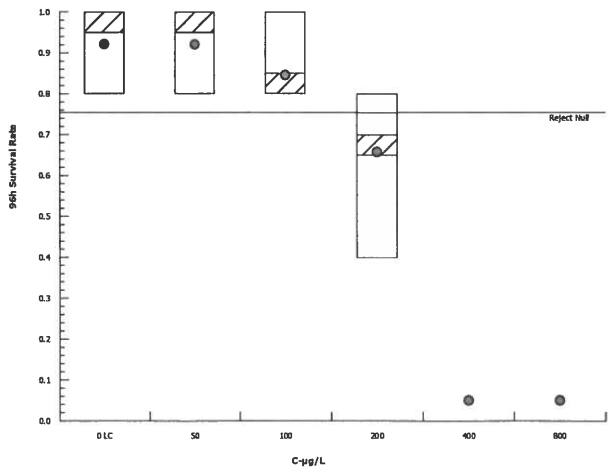
96h Survival Rate Summary

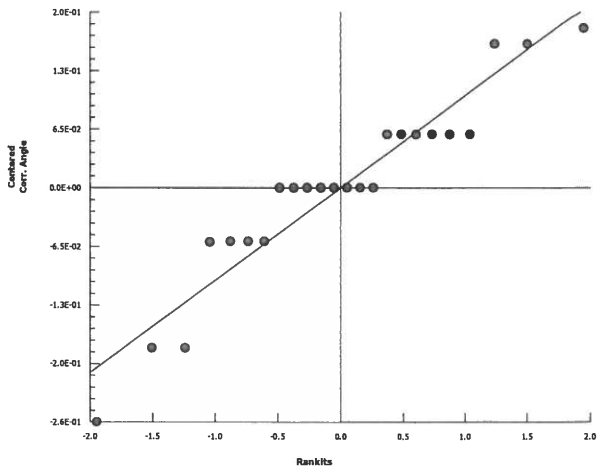
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
50		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
100		4	0.85	0.6909	1	0.8	0.8	1	0.05	11.76%	10.53%
200		4	0.65	0.3453	0.9547	0.7	0.4	0.8	0.09574	29.46%	31.58%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
50		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
100		4	1.167	0.9772	1.356	1.107	1.107	1.345	0.05953	10.21%	9.26%
200		4	0.9463	0.623	1.27	0.9966	0.6847	1.107	0.1016	21.47%	26.4%
400		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	82.46%
800		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	82.46%

Graphics





CETIS Analytical Report

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

Analysis ID:	13-6085-8539	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 Nov-21 9:42	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

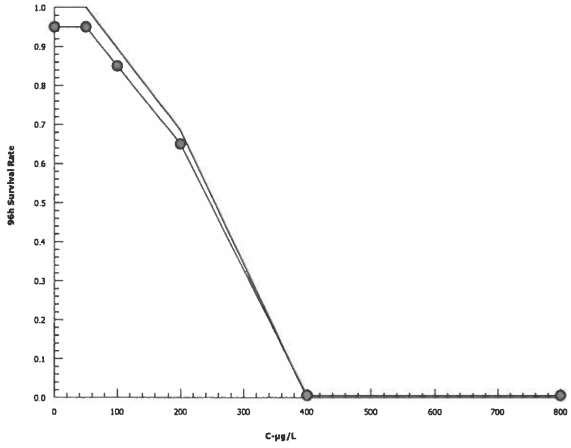
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.05	0.00%	2.325	0.03749	211.2	177.7	251.1

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
50		4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
100		4	0.85	0.8	1	0.05	0.1	11.76%	10.53%	17	20
200		4	0.65	0.4	0.8	0.09574	0.1915	29.46%	31.58%	13	20
400		4	0	0	0	0	0		100.0%	0	20
800		4	0	0	0	0	0		100.0%	0	20

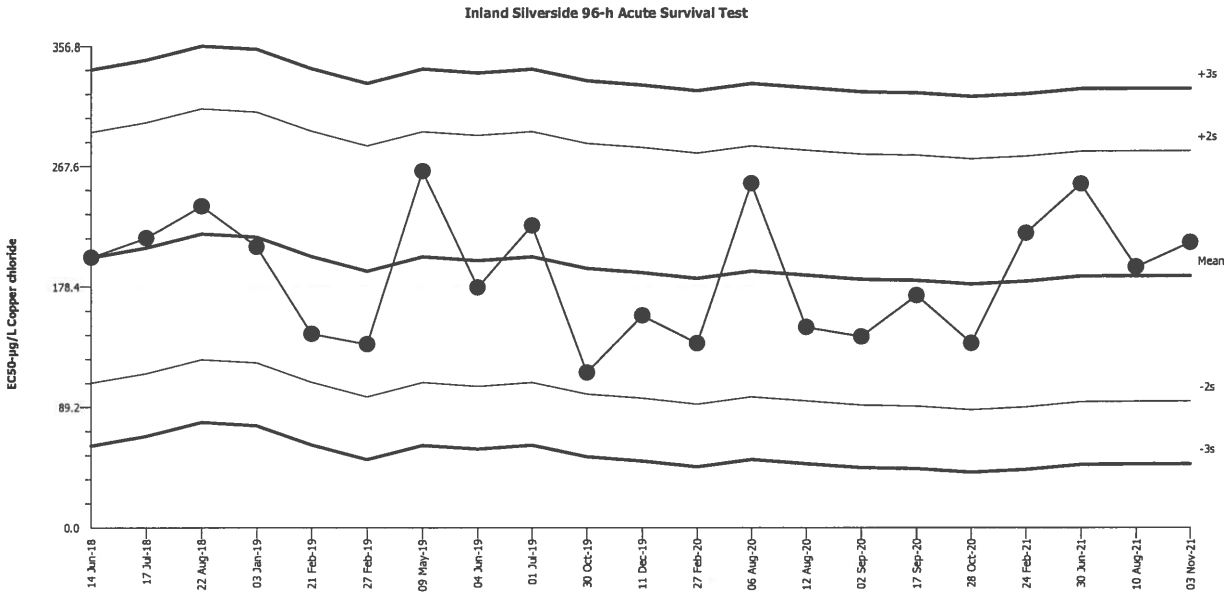
Graphics



CETIS QC Plot

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

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



Mean:	186.6	**Count:**	20	**-2s Warning Limit:**	93.67	**-3s Action Limit:**	47.2
Sigma:	46.47	**CV:**	24.90%	**+2s Warning Limit:**	279.6	**+3s Action Limit:**	326

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2018	Jun	14	14:35	200	13.4	0.2884			01-9952-0614	00-3575-1747
2		Jul	17	14:30	214.4	27.75	0.5973			11-1445-3115	12-3693-5336
3		Aug	22	16:25	237.8	51.24	1.103			08-6172-7555	12-4329-0617
4	2019	Jan	3	16:50	207.9	21.25	0.4573			16-0506-4055	11-1190-1934
5		Feb	21	16:05	143.5	-43.12	-0.9279			10-4228-2556	08-7111-9529
6			27	16:25	135.8	-50.83	-1.094			14-0947-0420	00-4247-8099
7		May	9	19:10	263.9	77.3	1.663			03-9779-6453	09-3747-7536
8		Jun	4	14:50	177.8	-8.845	-0.1903			00-2136-1210	01-4264-5145
9		Jul	1	15:55	223.6	37.02	0.7965			04-4319-5710	17-4098-1084
10		Oct	30	14:45	114.9	-71.73	-1.544			05-0159-0485	07-6888-5964
11		Dec	11	16:30	156.9	-29.68	-0.6388			11-0566-6524	14-4935-0865
12	2020	Feb	27	17:15	136.4	-50.24	-1.081			00-2639-4829	10-5059-8408
13		Aug	6	16:00	254.9	68.31	1.47			13-3377-6823	09-5433-0150
14			12	15:20	148.4	-38.24	-0.8229			02-5307-3356	11-5066-6205
15		Sep	2	15:25	141.4	-45.18	-0.9722			09-8373-9144	18-7650-2455
16			17	14:45	172	-14.64	-0.3151			07-8442-4358	02-9347-5784
17		Oct	28	16:35	136.6	-50	-1.076			10-9446-3954	10-4215-8111
18	2021	Feb	24	17:30	218.2	31.59	0.6798			11-4316-4077	02-1492-4727
19		Jun	30	16:05	254.9	68.31	1.47			01-4075-9626	19-2668-9340
20		Aug	10	14:30	193.2	6.587	0.1418			20-1130-3481	09-5748-8802
21		Nov	3	17:15	211.2	24.65	0.5304			01-2577-3416	13-6085-8539

Marine Acute Bioassay
Static-Renewal Conditions
DM-001

Water Quality Measurements & Test Organism Survival

Client: Internal | Test Species: *M. beryllina*
Sample ID: $CuCl_2$ | Start Date/Time: 11/3/2021 1715
Test No.: 211103mbra | End Date/Time: 11/7/2021 1640
Cu stock concentration (µg/L): 94,000

	Tech Initials				
	0	24	48	72	96
Counts:	BO	SP	KB	KB	KL
Readings:	GM	SP	KB	KB	KL
Dilutions made by:	GM		SP		
High conc. made (µg/L):	800	--	400	--	--
Vol. Cu stock added (mL):	17.0	--	~~9.0~~ (B) 6.5	--	--
Final Volume (mL):	2000	--	2000	--	--

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	4	5	5	5	5	5	30.2	30.4	f 29.6	30.1	30.2	24.0	24.5	~~f 25.1~~ f 24.2 (A)	24.2	24.1	6.6	6.3	f 6.6	6.1	5.7	7.94	7.85	f 7.93	7.88	7.71
	10	5	5	5	5	5			f 30.3					f 24.3					f 6.0					f 7.67		
	5	5	5	5	5	5																				
	9	5	4	4	4	4																				
50	23	5	5	5	5	5	30.1	30.5	f 29.4	30.2	30.2	24.0	24.6	~~f 25.1~~ f 23.3 (C)	24.4	24.6	6.7	6.2	f 6.5	6.0	5.7	7.93	7.85	f 7.94	7.91	7.73
	18	5	4	4	4	4			f 30.4					f 24.4					f 5.8					f 7.68		
	3	5	5	5	5	5																				
	6	5	5	5	5	5																				
100	19	5	4	4	4	4	30.2	30.4	f 29.6	30.0	30.0	24.0	24.8	~~f 25.3~~ f 23.4 (C)	24.5	24.6	6.7	6.1	f 6.7	6.1	5.6	7.94	7.85	f 7.95	7.88	7.74
	14	5	4	4	4	4			f 30.4					f 24.5					f 5.4					f 7.71		
	2	5	4	4	4	4																				
	17	5	(B) 5	5	5	5																				
200	20	5	3	2	2	2	30.1	30.2	f 29.5	29.8	30.2	24.0	24.7	~~f 24.9~~ f 23.3 (C)	24.2	24.3	6.6	6.0	f 6.7	6.7	6.0	7.94	7.84	f 7.95	7.92	~~7.82~~ (C) 7.82
	11	5	5	3	3	3			f 30.4					f 24.6					f 6.0					f 7.73		
	7	5	5	4	4	4																				
	22	5	5	4	4	4																				
400	8	5	1	0	–	–	30.0	30.4	f 29.5	–	–	24.0	24.6	~~f 25.2~~ f 23.3	–	–	6.7	6.1	f 6.7	–	–	7.94	7.83	f 7.94	–	–
	15	5	1	0	–	–			f 30.4					f 24.5					f 6.1					f 7.73		
	1	5	0	–	–	–																				
	21	5	0	–	–	–																				
800	13	5	0				~~29.5~~ 30.0		f			24.0	24.6	f			6.7	6.3	f			7.92	7.84	f		
	16	5	0	All					f					f					f					f		
	24	5	0																							
	12	5	0	Dead																						

Rand # QC: BO
Initial Counts QC'd by: GM
Initiated by: BO

Environmental Chamber: A

Animal Source/Date Received: ABS 11/2/21
Age at Initiation: 10days
Animal Acclimation Qualifiers (circle all that apply): Q22 / Q23 / Q24 / none

Feeding Times					
	0	24	48	72	96
AM:		0830	0845	1700	1450
PM:	1800				

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y / n)

(A) Q18 SP 11/4/21
(B) Q18 SP 11/5/21
(C) Q23 KB 11/5/21 dilutions needed to warm up

QC Check: JL 11/9/21
Final Review: ACS 11/10/21