Data Quality Summary: Wyckoff 2nd Quarter 2022 Groundwater Treatment Plant Bioassay Sampling

Samples were collected and analyzed in support of the Wyckoff Groundwater Treatment Plant. All analytical data were evaluated in accordance with the following guidance:

- *Wyckoff Groundwater Treatment Plant Operations and Maintenance Quality Assurance Project Plan* (QAPP), *Bainbridge Island, Washington* (CH2M, 2022).

This data quality summary presents the findings of the data validation activities.

Analytical Data

The methods, sample delivery group (SDG) number and laboratory name for all analyses are presented in Table 1. These reports can be found in Attachment 1.

Table 1. Analytical Data Summary
Data Quality Summary: Wyckoff Groundwater Treatment Plant Operations and Maintenance Q4 Bioassay

Laboratory	SDG	Method	Analyte
Enthalpy	2204-S143	EPA600/R-95/136	chronic bioassay

Notes:
Enthalpy = Enthalpy AnalyticalPolycyclic aromatic hydrocarbons
SDG = Sample Delivery Group

A CH2M chemist validated the bioassay results Stage 2A in accordance with the QAPP. The data were 100% complete, method and QAPP quality control requirements were met.

Table 2 provides a summary of the final test results.

Table 2 Summary of Chronic Test Results

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	TUc	EC_{50} (% effluent)	Was there statistically significan effects in effluent concentration tested for the survival or development endpoint species test?
Mytilus galloprovincialis (Mediterranean mussel)	**Normal Developent**	**69.1**	**>69.1**	**<1.4**	**>69.1**	**No**
	Survival	**69.1**	**>69.1**	**<1.4**	**>69.1**	**No**

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
Chronic Toxic Unit (TU_c) = 100/NOEC. NOTE: Since 100% sample was not tested, the TU_c value can only be calculated up to the highest concentration tested. If no toxicity is observed at this concentration, the TU_c is reported as less than the calculated value.
Effect Concentration 50 (EC_{50}) = Concentration expected to cause an effect to 50% of the organisms

There were no statistically significant effects detected in the effluent sample 041922 tested for the survival or development endpoint of the bivalve test. This results in a no observed effect concentration (NOEC) of 69.1 (the highest concentration tested) and a chronic toxic unit (TUc) of less than 1.4 for both endpoints. The mean survival rate was greater than 50% (98.5%) as specified in the QAPP.

Attachment 1
Bioassay Report

ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: April 2022

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

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

Date Submitted: **May 11, 2022**

Data Quality Assurance:

- Enthalpy Analytical is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (ORELAP ID 4053). It is also certified by the State of California Water Resources Control Board Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552). Specific fields of testing applicable to each accreditation are available upon request.
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective US EPA protocols, unless otherwise noted in this report.
- All tests have met internal Quality Assurance Program requirements.

Verified by:

Kasey Skrivseth

Kasey Skrivseth, Project Manager

California
4340 Vandever Avenue
San Diego, California 92120
858.587.7333

Introduction

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

Materials and Methods

The groundwater sample was collected into a low-density polyethylene cubitainer by Jacobs personnel, packed in a cooler containing ice, and shipped overnight to Enthalpy. Appropriate chain-of-custody (COC) procedures were employed during collection and transport. Upon arrival at the laboratory, the cooler was opened, the sample inspected, and the contents verified against information on the COC form. Standard water quality parameters were measured and recorded on a sample check-in form and are summarized in Table 1. The sample was stored at 4°C in the dark until used for testing.

Table 1. Sample Information

Sample ID	**041922**
Enthalpy Log-in Number	22-0545
Collection Date; Time	4/19/22; 0934h
Receipt Date; Time	4/20/22; 0853h
Receipt Temperature (°C)	5.2
Dissolved Oxygen (mg/L)	10.8
pH	7.58
Conductivity (µS/cm)	4,910
Salinity (ppt)	2.8
Alkalinity (mg/L $CaCO_3$)	440
Total Chlorine (mg/L)	0.02
Total Ammonia (mg/L as N)	<0.5

Test Methods

Chronic toxicity testing was conducted according to the method set forth in USEPA (1995) and WDOE (2016). This method is summarized in Table 2.

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

Test Period	4/20/22, 1430h to 4/22/22, 1400h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	M-Rep (Carlsbad, CA)
Test Organism Age	4 hours post fertilization
Test Duration	48 ± 2 hours
Test Type	Static
Test Chamber, Test Solution Volume	30 mL glass vial, 10 mL
Test Temperature	15 ± 1°C
Dilution Water	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) diluted with de-ionized water
Additional Control	Brine Control (de-ionized water and hypersaline brine)
Test Salinity	30 ± 2 ppt
Source of Salinity	Hypersaline brine made by freezing seawater to a salinity of 99.6 ppt
Test Concentrations (% sample)	69.1[a], 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper chloride [b]
Statistical Software	CETIS™ 1.8.7.20

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

[b] A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride. See QA section.

Statistical Methods

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

Results

There were no statistically significant effects detected in any effluent concentration tested for the survival or development endpoint of the bivalve test. This results in a NOEC of 69.1 (the highest concentration tested) and a TU_c of less than 1.4 for both endpoints.

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

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

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_c)	EC_{50} (% effluent)
Bivalve	Normal Development	69.1	> 69.1	< 1.4	> 69.1
	Survival	69.1	> 69.1	< 1.4	> 69.1

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

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

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

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	98.5	94.6
0 (Lab Control)	98.0	96.1
2	97.2	93.3
4	99.6	94.8
9	99.5	94.2
18	99.2	93.7
35	100	95.0
69.1[a]	98.5	93.9

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

Quality Assurance

The sample was received within the required 36-hour holding time, in good condition, and within the appropriate temperature range of 0-6°C. All control acceptability criteria were met, and water quality parameters remained within the appropriate ranges throughout the test. Statistical analyses followed standard USEPA flowchart selections. Dose-response relationships were reviewed to ensure the reliability of the results. Based on the dose response observed, the calculated effects concentrations were deemed reliable. Minor QA/QC issues that were unlikely to have any bearing on the final test results, such as slight temperature deviations, are noted on the data sheets and a list of qualifier codes used on bench data sheets is presented in Appendix D.

Reference Toxicant

Results for the reference toxicant tests used to monitor laboratory performance and test organism sensitivity are summarized in Table 5. A deviation to the QAPP was approved by USEPA and Washington Department of Ecology to conduct reference toxicant testing with copper chloride rather than copper sulfate. The results for the concurrent reference toxicant test were within the acceptable range of the mean historical test results plus or minus two standard deviations for development and survival. Reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 5. Reference Toxicant Test Results

Species and Endpoint	**NOEC** (%)	**EC_{50}** (µg/L copper)	**Historical Mean ± 2 SD** (µg/L copper)	**CV** (%)
Bivalve Survival Rate	10	27.4	27.5 ± 10.9	19.9
Bivalve Normal Development	5	7.53	8.49 ± 3.48	20.5

NOEC = No Observed Effect Concentration

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

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

CV = Coefficient of Variation

References

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

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

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

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

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

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

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

Batch ID:	18-5412-5543	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	20 Apr-22 14:30	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	22 Apr-22 14:00	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	48h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	13-8767-1317	**Code:**	22-0545	**Client:**	Jacobs
Sample Date:	19 Apr-22 09:34	**Material:**	Effluent Sample	**Project:**	
Receive Date:	20 Apr-22 08:53	**Source:**	Jacobs		
Sample Age:	29h (5.2 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-6551-1353	Combined Development Ra	69.1	>69.1	NA	4.14%	1.447	Dunnett Multiple Comparison Test
09-6584-5807	Development Rate	69.1	>69.1	NA	3.12%	1.447	Dunnett Multiple Comparison Test
13-9563-0486	Survival Rate	69.1	>69.1	NA	2.76%	1.447	Steel Many-One Rank Sum Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-6584-5807	Development Rate	Control Resp	0.9461	0.9 - NL	Yes	Passes Acceptability Criteria
13-9563-0486	Survival Rate	Control Resp	0.9853	0.5 - NL	Yes	Passes Acceptability Criteria
06-6551-1353	Combined Development Ra	PMSD	0.04141	NL - 0.25	No	Passes Acceptability Criteria

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	0.9323	0.8912	0.9734	0.8733	0.9516	0.0148	0.0331	3.55%	0.0%
0	Lab Control	5	0.9418	0.891	0.9925	0.8733	0.9752	0.01828	0.04088	4.34%	-1.02%
2		5	0.906	0.8791	0.9329	0.8733	0.9333	0.009686	0.02166	2.39%	2.82%
4		5	0.9442	0.9188	0.9696	0.9231	0.9737	0.009145	0.02045	2.17%	-1.28%
9		5	0.937	0.9079	0.966	0.902	0.9627	0.01046	0.02339	2.5%	-0.5%
18		5	0.9297	0.8939	0.9655	0.8867	0.9671	0.01288	0.0288	3.1%	0.28%
35		5	0.9498	0.9315	0.9681	0.9351	0.9679	0.006591	0.01474	1.55%	-1.88%
69.1		5	0.925	0.8909	0.959	0.88	0.9492	0.01226	0.02741	2.96%	0.78%

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9461	0.9416	0.9506	0.9424	0.9516	0.001626	0.003637	0.38%	0.0%
0	Lab Control	5	0.9612	0.9422	0.9802	0.9357	0.9752	0.006834	0.01528	1.59%	-1.59%
2		5	0.9327	0.8998	0.9656	0.9032	0.9589	0.01185	0.0265	2.84%	1.42%
4		5	0.948	0.9257	0.9703	0.9231	0.9737	0.008045	0.01799	1.9%	-0.2%
9		5	0.942	0.9124	0.9717	0.902	0.9627	0.01067	0.02387	2.53%	0.43%
18		5	0.9371	0.9153	0.9589	0.9236	0.9671	0.007843	0.01754	1.87%	0.95%
35		5	0.9498	0.9315	0.9681	0.9351	0.9679	0.006591	0.01474	1.55%	-0.39%
69.1		5	0.9389	0.9234	0.9544	0.9195	0.9496	0.00557	0.01245	1.33%	0.76%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9853	0.9446	1	0.9267	1	0.01467	0.0328	3.33%	0.0%
0	Lab Control	5	0.98	0.9245	1	0.9	1	0.02	0.04472	4.56%	0.54%
2		5	0.972	0.928	1	0.9133	1	0.01583	0.0354	3.64%	1.35%
4		5	0.996	0.9849	1	0.98	1	0.004	0.008944	0.9%	-1.08%
9		5	0.9947	0.9839	1	0.98	1	0.003887	0.008692	0.87%	-0.95%
18		5	0.992	0.9698	1	0.96	1	0.008	0.01789	1.8%	-0.68%
35		5	1	1	1	1	1	0	0	0.0%	-1.49%
69.1		5	0.9853	0.9446	1	0.9267	1	0.01467	0.0328	3.33%	0.0%

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	0.9516	0.8733	0.9467	0.9467	0.9432
0	Lab Control	0.9618	0.963	0.9752	0.8733	0.9357
2		0.9133	0.8733	0.9333	0.9032	0.9068
4		0.9506	0.9267	0.9471	0.9231	0.9737
9		0.9627	0.9535	0.9333	0.902	0.9333
18		0.9342	0.9255	0.8867	0.9351	0.9671
35		0.9351	0.9632	0.9405	0.9423	0.9679
69.1		0.9195	0.88	0.9346	0.9492	0.9416

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9516	0.9424	0.9467	0.9467	0.9432
0	Lab Control	0.9618	0.963	0.9752	0.9704	0.9357
2		0.9384	0.9562	0.9589	0.9032	0.9068
4		0.9506	0.9456	0.9471	0.9231	0.9737
9		0.9627	0.9535	0.9524	0.902	0.9396
18		0.9342	0.9255	0.9236	0.9351	0.9671
35		0.9351	0.9632	0.9405	0.9423	0.9679
69.1		0.9195	0.9496	0.9346	0.9492	0.9416

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	0.9267	1	1	1
0	Lab Control	1	1	1	0.9	1
2		0.9733	0.9133	0.9733	1	1
4		1	0.98	1	1	1
9		1	1	0.98	1	0.9933
18		1	1	0.96	1	1
35		1	1	1	1	1
69.1		1	0.9267	1	1	1

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

Combined Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	177/186	131/150	142/150	142/150	166/176
0	Lab Control	151/157	156/162	157/161	131/150	160/171
2		137/150	131/150	140/150	140/155	146/161
4		154/162	139/150	179/189	156/169	148/152
9		155/161	164/172	140/150	138/153	140/150
18		142/152	149/161	133/150	144/154	147/152
35		144/154	157/163	158/168	147/156	151/156
69.1		160/174	132/150	143/153	168/177	145/154

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	177/186	131/139	142/150	142/150	166/176
0	Lab Control	151/157	156/162	157/161	131/135	160/171
2		137/146	131/137	140/146	140/155	146/161
4		154/162	139/147	179/189	156/169	148/152
9		155/161	164/172	140/147	138/153	140/149
18		142/152	149/161	133/144	144/154	147/152
35		144/154	157/163	158/168	147/156	151/156
69.1		160/174	132/139	143/153	168/177	145/154

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	150/150	139/150	150/150	150/150	150/150
0	Lab Control	150/150	150/150	150/150	135/150	150/150
2		146/150	137/150	146/150	150/150	150/150
4		150/150	147/150	150/150	150/150	150/150
9		150/150	150/150	147/150	150/150	149/150
18		150/150	150/150	144/150	150/150	150/150
35		150/150	150/150	150/150	150/150	150/150
69.1		150/150	139/150	150/150	150/150	150/150

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

Analysis ID: 06-6551-1353 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 10 May-22 13:05 **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	4.14%	69.1	>69.1	NA	1.447

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	1.686	2.407	0.074	8	0.1858	CDF	Non-Significant Effect
		4	-0.7718	2.407	0.074	8	0.9763	CDF	Non-Significant Effect
		9	-0.2616	2.407	0.074	8	0.9163	CDF	Non-Significant Effect
		18	0.1758	2.407	0.074	8	0.8042	CDF	Non-Significant Effect
		35	-1.129	2.407	0.074	8	0.9917	CDF	Non-Significant Effect
		69.1	0.5169	2.407	0.074	8	0.6731	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02374072	0.003956786	6	1.692	0.1600	Non-Significant Effect
Error	0.06549774	0.002339205	28			
Total	0.08923846		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.679	16.81	0.9468	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9773	0.9146	0.6689	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	0.9323	0.8912	0.9734	0.9467	0.8733	0.9516	0.0148	3.55%	0.0%
2		5	0.906	0.8791	0.9329	0.9068	0.8733	0.9333	0.009686	2.39%	2.82%
4		5	0.9442	0.9188	0.9696	0.9471	0.9231	0.9737	0.009144	2.17%	-1.28%
9		5	0.937	0.9079	0.966	0.9333	0.902	0.9627	0.01046	2.5%	-0.5%
18		5	0.9297	0.8939	0.9655	0.9342	0.8867	0.9671	0.01288	3.1%	0.28%
35		5	0.9498	0.9315	0.9681	0.9423	0.9351	0.9679	0.006591	1.55%	-1.88%
69.1		5	0.925	0.8909	0.959	0.9346	0.88	0.9492	0.01226	2.96%	0.78%

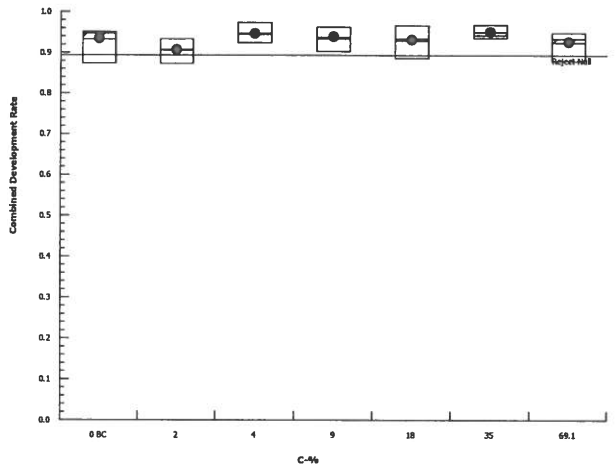
Angular (Corrected) Transformed Summary

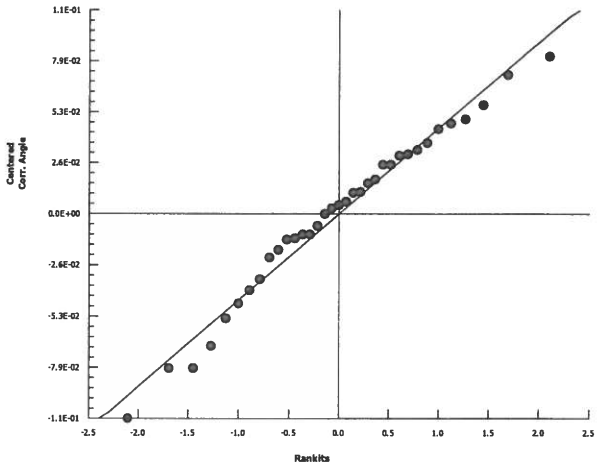
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.312	1.239	1.386	1.338	1.207	1.349	0.02652	4.52%	0.0%
2		5	1.261	1.215	1.307	1.261	1.207	1.31	0.01651	2.93%	3.93%
4		5	1.336	1.277	1.395	1.339	1.29	1.408	0.02119	3.55%	-1.8%
9		5	1.32	1.261	1.38	1.31	1.252	1.377	0.02134	3.61%	-0.61%
18		5	1.307	1.236	1.378	1.311	1.227	1.388	0.02567	4.39%	0.41%
35		5	1.347	1.303	1.39	1.328	1.313	1.391	0.01561	2.59%	-2.63%
69.1		5	1.296	1.235	1.358	1.312	1.217	1.343	0.02218	3.83%	1.21%

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

Analysis ID:	06-6551-1353	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 13:05	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 09-6584-5807 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 10 May-22 13:05 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.12%	69.1	>69.1	NA	1.447

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	0.9962	2.407	0.059	8	0.4540	CDF	Non-Significant Effect
		4	-0.2884	2.407	0.059	8	0.9212	CDF	Non-Significant Effect
		9	0.2261	2.407	0.059	8	0.7871	CDF	Non-Significant Effect
		18	0.6979	2.407	0.059	8	0.5924	CDF	Non-Significant Effect
		35	-0.4185	2.407	0.059	8	0.9415	CDF	Non-Significant Effect
		69.1	0.6003	2.407	0.059	8	0.6366	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.005002312	0.0008337187	6	0.5598	0.7583	Non-Significant Effect
Error	0.04170026	0.001489295	28			
Total	0.04670257		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.36	16.81	0.1103	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9825	0.9146	0.8372	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	0.9461	0.9416	0.9506	0.9467	0.9424	0.9516	0.001627	0.38%	0.0%
2		5	0.9327	0.8998	0.9656	0.9384	0.9032	0.9589	0.01185	2.84%	1.42%
4		5	0.948	0.9257	0.9703	0.9471	0.9231	0.9737	0.008045	1.9%	-0.2%
9		5	0.942	0.9124	0.9717	0.9524	0.902	0.9627	0.01067	2.53%	0.43%
18		5	0.9371	0.9153	0.9589	0.9342	0.9236	0.9671	0.007842	1.87%	0.95%
35		5	0.9498	0.9315	0.9681	0.9423	0.9351	0.9679	0.006591	1.55%	-0.39%
69.1		5	0.9389	0.9234	0.9544	0.9416	0.9195	0.9496	0.00557	1.33%	0.76%

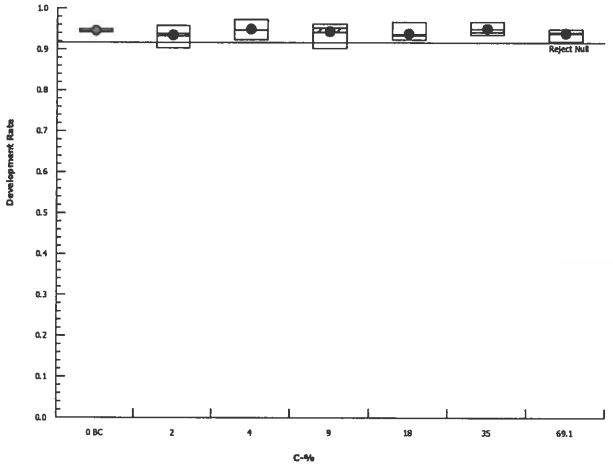
Angular (Corrected) Transformed Summary

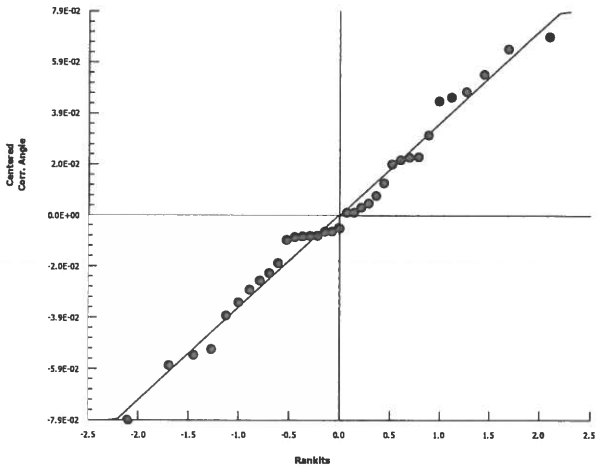
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.337	1.327	1.347	1.338	1.329	1.349	0.003634	0.61%	0.0%
2		5	1.312	1.246	1.378	1.32	1.254	1.367	0.02377	4.05%	1.82%
4		5	1.344	1.291	1.396	1.339	1.29	1.408	0.01888	3.14%	-0.53%
9		5	1.331	1.271	1.391	1.351	1.252	1.377	0.02148	3.61%	0.41%
18		5	1.32	1.27	1.369	1.311	1.291	1.388	0.01777	3.01%	1.27%
35		5	1.347	1.303	1.39	1.328	1.313	1.391	0.01561	2.59%	-0.76%
69.1		5	1.322	1.29	1.354	1.327	1.283	1.344	0.01136	1.92%	1.1%

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

Analysis ID:	09-6584-5807	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 13:05	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 13-9563-0486 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 10 May-22 13:05 **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	2.76%	69.1	>69.1	NA	1.447

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	23	16	1	8	0.4756	Asymp	Non-Significant Effect
		4	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		9	26	16	1	8	0.7547	Asymp	Non-Significant Effect
		18	28	16	1	8	0.8838	Asymp	Non-Significant Effect
		35	30	16	1	8	0.9557	Asymp	Non-Significant Effect
		69.1	27.5	16	2	8	0.8571	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02990003	0.004983338	6	0.8216	0.5627	Non-Significant Effect
Error	0.169824	0.006065141	28			
Total	0.199724		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.5934	3.812	0.7321	Equal Variances
Variances	Levene Equality of Variance	2.131	3.528	0.0812	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8088	0.9146	<0.0001	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	0.9853	0.9446	1	1	0.9267	1	0.01467	3.33%	0.0%
2		5	0.972	0.928	1	0.9733	0.9133	1	0.01583	3.64%	1.35%
4		5	0.996	0.9849	1	1	0.98	1	0.004	0.9%	-1.08%
9		5	0.9947	0.9839	1	1	0.98	1	0.003887	0.87%	-0.95%
18		5	0.992	0.9698	1	1	0.96	1	0.008	1.8%	-0.68%
35		5	1	1	1	1	1	1	0	0.0%	-1.49%
69.1		5	0.9853	0.9446	1	1	0.9267	1	0.01467	3.33%	0.0%

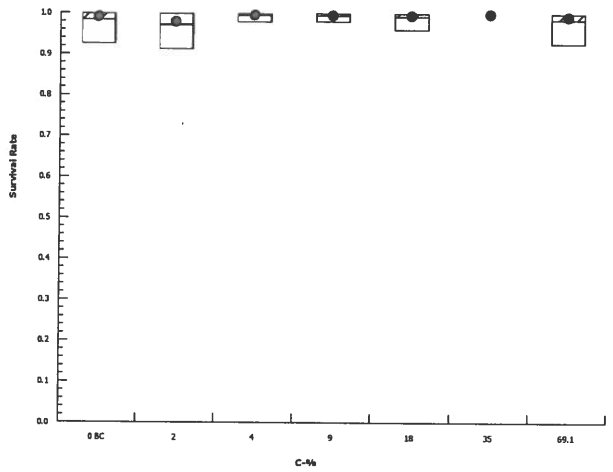
Angular (Corrected) Transformed Summary

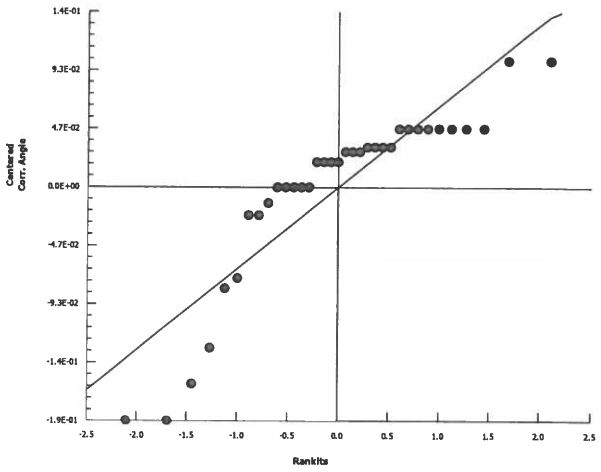
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.483	1.354	1.613	1.53	1.297	1.53	0.04668	7.04%	0.0%
2		5	1.429	1.296	1.562	1.407	1.272	1.53	0.04797	7.51%	3.65%
4		5	1.51	1.454	1.566	1.53	1.429	1.53	0.02021	2.99%	-1.78%
9		5	1.502	1.447	1.557	1.53	1.429	1.53	0.01982	2.95%	-1.23%
18		5	1.498	1.409	1.587	1.53	1.369	1.53	0.0321	4.79%	-0.98%
35		5	1.53	1.53	1.53	1.53	1.53	1.53	0	0.0%	-3.15%
69.1		5	1.483	1.354	1.613	1.53	1.297	1.53	0.04668	7.04%	0.0%

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

Analysis ID:	13-9563-0486	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 13:05	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Test Data Worksheet

Report Date: 17 Apr-22 11:55 (p 1 of 1)
Test Code: 2204-S143 13-0564-5860/4DD29324

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

Start Date: 20 Apr-22 | **Species:** Mytilus galloprovincialis | **Sample Code:** 22- 0545
End Date: 22 Apr-22 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Jacobs
Sample Date: 19 Apr-22 | **Material:** Effluent Sample | **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			161	149	AS 5/9/22
			32			172	164	
			33			152	148	
			34			147	140	
			35			186	177	
			36			152	142	
			37			154	144	
			38			171	160	
			39			139	132	
			40			163	157	
			41			152	147	
			42			153	138	
			43			156	147	
			44			150	142	
			45			162	156	AJS 5/10/22
			46			176	166	
			47			146	140	
			48			161	157	
			49			146	137	
			50			157	151	
			51			154	145	
			52			189	179	
			53			147	139	
			54			161	155	
			55			169	156	
			56			155	140	
			57			161	146	
			58			137	131	
			59			154	144	
			60			149	140	
			61			135	131	
			62			174	160	
			63			153	143	
			64			139	131	
			65			162	154	
			66			168	158	
			67			156	151	
			68			144	133	
			69			177	168	
			70			150	142	

CETIS Test Data Worksheet

Report Date: 17 Apr-22 11:55 (p 1 of 1)
Test Code: 2204-5143 13-0564-5860/4DD29324

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

Start Date: 20 Apr-22
End Date: 22 Apr-22
Sample Date: 19 Apr-22

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

Sample Code: 22-0545
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	35					
0	BC	2	64					
0	BC	3	70			148	140	WF
0	BC	4	44					
0	BC	5	46					
0	LC	1	50					
0	LC	2	45					
0	LC	3	48			161	157	WF
0	LC	4	61					
0	LC	5	38					
2		1	49					
2		2	58					
2		3	47			142	140	WF
2		4	56					
2		5	57					
4		1	65					
4		2	53					
4		3	52			179	169	WF
4		4	55					
4		5	33					
9		1	54					
9		2	32					
9		3	34			145	142	WF
9		4	42					
9		5	60					
18		1	36					
18		2	31					
18		3	68			143	132	WF
18		4	37					
18		5	41					
35		1	59					
35		2	40					
35		3	66			162	160	WF
35		4	43					
35		5	67					
73.3		1	62					
73.3		2	39					
73.3		3	63			153	144	WF
73.3		4	69					
73.3		5	51					

QC 18 BO 4/20/22 69.1%

QC=BO

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** BO

Sample ID: Wyckoff **Test Date:** 4/20/2022

Test No: 2204-S143 **Test Type:** Mussel Development

Salinity of Effluent 2.8

Salinity of Brine 90.9 **Date of Brine used:** 12/27/2021

Target Salinity 30 **Alkalinity of Brine Control:** 108 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**0.45**	**0.49**

TS = target salinity
SE = salinity of effluent
SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Control	NA	NA	NA	250
2	5.0	0.45	2.2	250
4	10.0	0.45	4.5	250
9	22.5	0.45	10.0	250
18	45.0	0.45	20.1	250
35	87.5	0.45	39.1	250
69.1	172.8	0.45	77.2	250

	DI Volume			
Brine Control	156.7	0.49	77.2	250

Total Brine Volume Required (ml): **230.3**

QC Check: JU 5/5/22 **Final Review:** AFS 5/11/22

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: JACOBS

Sample ID: Wyckoff

Sample Log No.: 22-0915

Test No.: 2204-S143

Test Species: *M. galloprovincialis*

Start Date/Time: 4/20/22 1430

End Date/Time: 4/22/22 1400

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	29.7	30.0	30.0	15.4	14.6	14.8	8.1	8.7	8.6	7.96	7.93	7.98
Brine Control	30.6	30.8	30.7	14.5	14.4	14.6	8.4	8.8	8.6	8.18	8.08	8.10
2	29.9	30.1	30.0	15.0	14.4	14.6	8.2	8.8	8.7	7.95	7.96	8.00
4	30.0	30.1	30.0	15.1	14.5	14.6	8.2	8.8	8.8	7.92	7.95	8.01
9	30.0	30.2	30.1	15.0	14.5	14.6	8.3	8.8	8.8	7.86	7.95	8.01
18	30.2	30.3	30.2	15.0	14.7	14.7	8.3	8.7	8.7	7.79	7.95	8.01
35	29.9	30.5	30.3	15.0	14.6	14.8	8.3	8.7	8.7	7.71	7.94	8.03
69.1	30.2	30.8	30.4	14.3	14.6	14.8	8.4	8.7	8.7	7.69	7.94	8.04

	0	24	48
WQ Readings:	BO	HH600 pM	BO
Dilutions made by:	BO	—	—

Technician Initials:

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: ac 5/5/22

Final Review: AFS 5/11/22

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: JACOBS / Wyckoff
Test No.: 2204-S143
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: MREP / 6A
Date Received: 11/17/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 4/20/22 1430
End Date/Time: 4/22/22 1400
Technician Initials: BO

Spawn Information

First Gamete Release Time: 1100

Sex	Number Spawning
Male	5
Female	3

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1, 2, 3	good motility, very dense
Female 1	2	some round, some oval, white, average density
Female 2	3	round, white, very dense
Female 3	—	—

Egg Fertilization Time: 1200

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	80
Female 2	100
Female 3	—

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted:

9	9
8	8
7	10
7	6
10	8

Mean: 8.2

Mean 8.2 X 50 = 410 embryos/ml

Initial Density: 410 = 1.4 (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	169	169	100	99.5
TØ B	141	141	100	
TØ C	167	169	99	
TØ D	133	133	100	
TØ E	143	144	99	
TØ F	147	149	99	
$\bar{X}$ =	150			

48-h QC: 168/175 = 96%

Comments:

QC Check: OL 5/5/22

Final Review: AJS 5/11/22

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff Eagle Harbor GWTP
Test ID No(s).: 2204-S143

NORTHWEST CLIENTS
Sample Check-In Information
DC-005

Sample (A, B, C):	A			
Log-in No. (22-xxxx):	0545			
Sample Collection Date & Time:	4/19/22 0934			
Sample Receipt Date & Time:	4/20/22 0853			
Number of Containers & Container Type:	1 x 1L cubi			
Approx. Total Volume Received (L):	~1L			
Check-in Temperature (°C)	5.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	10.0			
pH (units)	7.58			
Conductivity (µS/cm)	4910			
Salinity (ppt)	2.8			
Alkalinity (mg/L) [2]	440			
Hardness (mg/L) [2,3]	(A) ~~487~~ 587			
Total Chlorine (mg/L)	0.02			
Technician Initials	KR			

Test Performed: Mussel development **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART **Other:** _____
Alkalinity: 103 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 108 Hardness or Salinity: 30ppt

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

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

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

Additional Comments: (A) DO, pH KR 4/20/22

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

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

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

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

Salinity Adjustment? (Y) N
Test: Mussel Source: Brine Target ppt: 30
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

QC Check: QL 5/5/22
Final Review: AYS 5/11/22

Total Ammonia Analysis **Overlying Water**
Marine
DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: N/A **Test Start Date:** 4/20/22 **Analyst:** BS
SW Blank: 0.0 **Analysis Date:** 5/6/22

Sample ID	Enthalpy ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	N x 1.22 Ammonia (mg/L)
Blank Spike (10 mg/L NH_3)		NA	NA	9.0	11.0
~~Wyckoff~~	22-0545	4/20/22	check in	0.3	<0.5
Spike Check (10 mg/L NH_3)		NA	NA		
Sample Duplicate[a]	22-0545	NA	NA	0.4	~~0.5~~ <0.5
Sample Duplicate + Spike[a]		NA	NA	9.3	11.3
Spike Check (10 mg/L NH_3)		NA	NA	9.0	11.0

QCB JU 5/10/22

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

Acceptable Range: 0-20%

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

Acceptable Range: 80-120%[b]

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	11.0	10	NA	110
22-0545	<0.5	ⓓ ~~<0.5~~	11.3	10	C	C

	Reagent 1	Reagent 2	Test Tubes
Standard Lot Number	A1208	A1211	A1319

Comments: ⓓ QB BS 5/6/22

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: JU 5/10/22 **Final Review:** AJS 5/11/22

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 4/19/2022
CarrierName: FedEx
AirbillNo:

Jacobs, Wyckoff-
Wyckoff Eagle Harbor GWTP 2022/WA
Project Code: WEH-031K
Cooler #: 1 of 1

No: 10-041922-111646-0616
2021T10P000DD210W2LA00
Contact Name: Daniel Baca
Contact Phone: 661-313-3807

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type	Receipt Temp (°C)
041922		Ground Water/ D. Baca	Composite	CHRTOX(8 Weeks)	N (1)	SP-11	04/19/2022 09:34	Field Sample	5.2

Special Instructions: 2022 Q2

Shipment for Case Complete? N

Samples Transferred From Chain of Custody #

Analysis Key: CHRTOX=Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	@ JACOBS	04-19-22 @1126	EA-SD	4/20/22 0053	Good

Shipped via: FedEx
Log in #: 22-0545

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:	12-3743-0224	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	20 Apr-22 14:30	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	22 Apr-22 14:00	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	48h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	20-8214-1437	**Code:**	220420msdv	**Client:**	Internal
Sample Date:	20 Apr-22	**Material:**	Copper chloride	**Project:**	
Receive Date:	20 Apr-22	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-5521-5229	Combined Development Ra	5	10	7.071	4.01%		Dunnett Multiple Comparison Test
09-5797-9362	Development Rate	5	10	7.071	3.0%		Dunnett Multiple Comparison Test
14-9901-0252	Survival Rate	10	20	14.14	2.13%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
06-3800-2192	Combined Development Ra	EC25	6.222	6.092	6.315		Linear Interpolation (ICPIN)
		EC50	7.519	7.412	7.613		
16-7080-8967	Development Rate	EC25	6.234	6.122	6.32		Linear Interpolation (ICPIN)
		EC50	7.527	7.434	7.619		
11-4955-0328	Survival Rate	EC25	20.84	18.86	22.36		Linear Interpolation (ICPIN)
		EC50	27.36	26.19	28.41		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-5797-9362	Development Rate	Control Resp	0.9515	0.9 - NL	Yes	Passes Acceptability Criteria
16-7080-8967	Development Rate	Control Resp	0.9515	0.9 - NL	Yes	Passes Acceptability Criteria
11-4955-0328	Survival Rate	Control Resp	0.9973	0.5 - NL	Yes	Passes Acceptability Criteria
14-9901-0252	Survival Rate	Control Resp	0.9973	0.5 - NL	Yes	Passes Acceptability Criteria
10-5521-5229	Combined Development Ra	PMSD	0.04015	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	0.949	0.9326	0.9655	0.9267	0.9578	0.005926	0.01325	1.4%	0.0%
2.5		5	0.9299	0.892	0.9678	0.8933	0.9655	0.01366	0.03054	3.28%	2.01%
5		5	0.9386	0.9065	0.9707	0.9	0.9653	0.01155	0.02583	2.75%	1.1%
10		5	0.02049	0	0.0468	0.006623	0.05732	0.009474	0.02119	103.4%	97.84%
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	0.9515	0.9414	0.9617	0.9392	0.9578	0.003661	0.008187	0.86%	0.0%
2.5		5	0.9566	0.9422	0.971	0.9371	0.9655	0.005172	0.01156	1.21%	-0.53%
5		5	0.9423	0.9181	0.9665	0.9184	0.9653	0.008719	0.0195	2.07%	0.97%
10		5	0.0206	0	0.04685	0.006623	0.05732	0.009454	0.02114	102.6%	97.83%
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	0.9973	0.9899	1	0.9867	1	0.002667	0.005963	0.6%	0.0%
2.5		5	0.972	0.9403	1	0.9533	1	0.01143	0.02556	2.63%	2.54%
5		5	0.996	0.9849	1	0.98	1	0.004	0.008944	0.9%	0.13%
10		5	0.992	0.9698	1	0.96	1	0.008	0.01789	1.8%	0.53%
20		5	0.78	0.7125	0.8475	0.7	0.84	0.02431	0.05437	6.97%	21.79%
40		5	0.016	0	0.03912	0	0.04667	0.008327	0.01862	116.4%	98.4%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9267	0.9578	0.9563	0.9573	0.947
2.5		0.9133	0.92	0.9573	0.9655	0.8933
5		0.9558	0.9448	0.9	0.9272	0.9653
10		0.01852	0.01333	0.006623	0.05732	0.006667
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	0.9392	0.9578	0.9563	0.9573	0.947
2.5		0.958	0.965	0.9573	0.9655	0.9371
5		0.9558	0.9448	0.9184	0.9272	0.9653
10		0.01852	0.01389	0.006623	0.05732	0.006667
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	0.9867	1	1	1	1
2.5		0.9533	0.9533	1	1	0.9533
5		1	1	0.98	1	1
10		1	0.96	1	1	1
20		0.8	0.7	0.7533	0.84	0.8067
40		0.04667	0.006667	0	0.006667	0.02

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	139/150	159/166	153/160	157/164	143/151
2.5		137/150	138/150	157/164	168/174	134/150
5		173/181	154/163	135/150	140/151	167/173
10		3/162	2/150	1/151	9/157	1/150
20		0/150	0/150	0/150	0/150	0/150
40		0/150	0/150	0/150	0/150	0/150

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	139/148	159/166	153/160	157/164	143/151
2.5		137/143	138/143	157/164	168/174	134/143
5		173/181	154/163	135/147	140/151	167/173
10		3/162	2/144	1/151	9/157	1/150
20		0/120	0/105	0/113	0/126	0/121
40		0/7	0/1	0/1	0/1	0/3

Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	148/150	150/150	150/150	150/150	150/150
2.5		143/150	143/150	150/150	150/150	143/150
5		150/150	150/150	147/150	150/150	150/150
10		150/150	144/150	150/150	150/150	150/150
20		120/150	105/150	113/150	126/150	121/150
40		7/150	1/150	0/150	1/150	3/150

CETIS Analytical Report

Test Code: 220420msdv | 07-3521-2032

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

Analysis ID:	10-5521-5229	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 11:17	**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	4.01%	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	1.052	2.227	0.077	8	0.3119	CDF	Non-Significant Effect
		5	0.5767	2.227	0.077	8	0.5108	CDF	Non-Significant Effect
		10*	35.24	2.227	0.077	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.351591	1.783864	3	602.2	<0.0001	Significant Effect
Error	0.04739965	0.002962478	16			
Total	5.398991		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.487	11.34	0.4777	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9701	0.866	0.7566	Normal Distribution

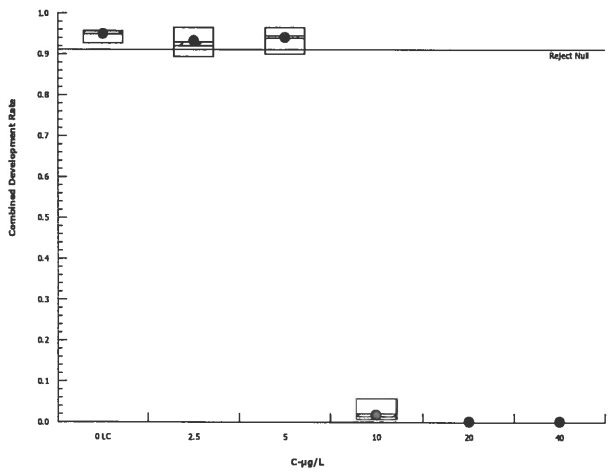
Combined Development Rate Summary

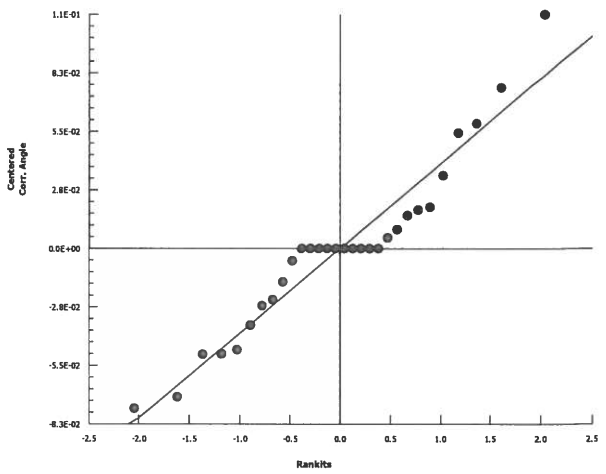
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.949	0.9326	0.9655	0.9563	0.9267	0.9578	0.005926	1.4%	0.0%
2.5		5	0.9299	0.892	0.9678	0.92	0.8933	0.9655	0.01366	3.28%	2.01%
5		5	0.9386	0.9065	0.9707	0.9448	0.9	0.9653	0.01155	2.75%	1.1%
10		5	0.02049	0	0.0468	0.01333	0.006623	0.05732	0.009474	103.4%	97.84%
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	1.344	1.309	1.38	1.36	1.297	1.364	0.01282	2.13%	0.0%
2.5		5	1.308	1.231	1.386	1.284	1.238	1.384	0.02786	4.76%	2.69%
5		5	1.325	1.259	1.39	1.334	1.249	1.383	0.02363	3.99%	1.48%
10		5	0.1314	0.04952	0.2134	0.1157	0.08147	0.2418	0.02951	50.2%	90.22%
20		5	0.04084	0.04083	0.04085	0.04084	0.04084	0.04084	0	0.0%	96.96%
40		5	0.04084	0.04083	0.04085	0.04084	0.04084	0.04084	0	0.0%	96.96%

Graphics





Analyst: _____ QA: AES 5/11/22

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

Analysis ID:	09-5797-9362	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 11:17	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.0%	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	-0.4784	2.227	0.06	8	0.8869	CDF	Non-Significant Effect
		5	0.6878	2.227	0.06	8	0.4618	CDF	Non-Significant Effect
		10*	45.36	2.227	0.06	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.543667	1.847889	3	1026	<0.0001	Significant Effect
Error	0.028821	0.001801313	16			
Total	5.572488		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.061	11.34	0.1087	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9105	0.866	0.0650	Normal Distribution

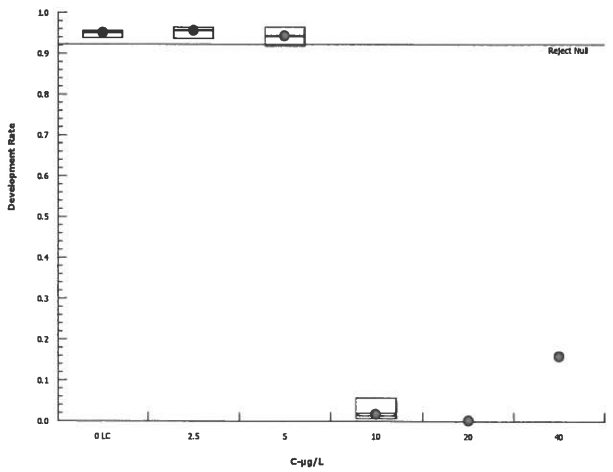
Development Rate Summary

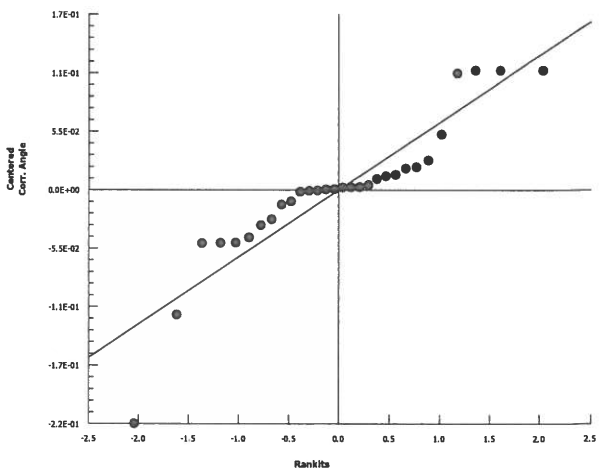
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9515	0.9414	0.9617	0.9563	0.9392	0.9578	0.003661	0.86%	0.0%
2.5		5	0.9566	0.9422	0.971	0.958	0.9371	0.9655	0.005172	1.21%	-0.53%
5		5	0.9423	0.9181	0.9665	0.9448	0.9184	0.9653	0.008719	2.07%	0.97%
10		5	0.0206	0	0.04685	0.01389	0.006623	0.05732	0.009454	102.6%	97.83%
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	1.349	1.326	1.373	1.36	1.322	1.364	0.008344	1.38%	0.0%
2.5		5	1.362	1.329	1.396	1.365	1.317	1.384	0.01209	1.99%	-0.95%
5		5	1.331	1.278	1.383	1.334	1.281	1.383	0.01892	3.18%	1.37%
10		5	0.1319	0.05016	0.2137	0.1181	0.08147	0.2418	0.02945	49.91%	90.22%
20		5	0.04631	0.04425	0.04837	0.04566	0.04456	0.04881	0.0007423	3.58%	96.57%
40		5	0.4108	0.2137	0.6078	0.5236	0.1901	0.5236	0.07099	38.64%	69.56%

Graphics





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

Analysis ID: 14-9901-0252 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 10 May-22 11:17 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.13%	10	20	14.14	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	2.162	2.362	0.1	8	0.0737	CDF	Non-Significant Effect
		5	0.1241	2.362	0.1	8	0.7940	CDF	Non-Significant Effect
		10	0.4061	2.362	0.1	8	0.6870	CDF	Non-Significant Effect
		20*	10.2	2.362	0.1	8	<0.0001	CDF	Significant Effect
		40*	33.25	2.362	0.1	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.643667	1.528733	5	343.9	<0.0001	Significant Effect
Error	0.1066925	0.00444552	24			
Total	7.75036		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.541	15.09	0.4744	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9281	0.9031	0.0438	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	0.9973	0.9899	1	1	0.9867	1	0.002667	0.6%	0.0%
2.5		5	0.972	0.9403	1	0.9533	0.9533	1	0.01143	2.63%	2.54%
5		5	0.996	0.9849	1	1	0.98	1	0.004	0.9%	0.13%
10		5	0.992	0.9698	1	1	0.96	1	0.008	1.8%	0.53%
20		5	0.78	0.7125	0.8475	0.8	0.7	0.84	0.02431	6.97%	21.79%
40		5	0.016	0	0.03912	0.006667	0	0.04667	0.008327	116.4%	98.4%

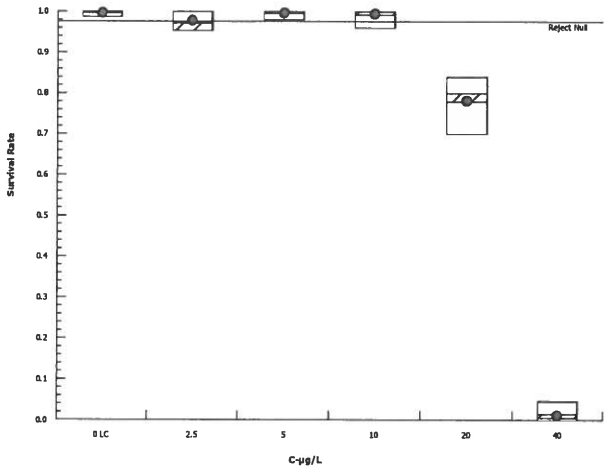
Angular (Corrected) Transformed Summary

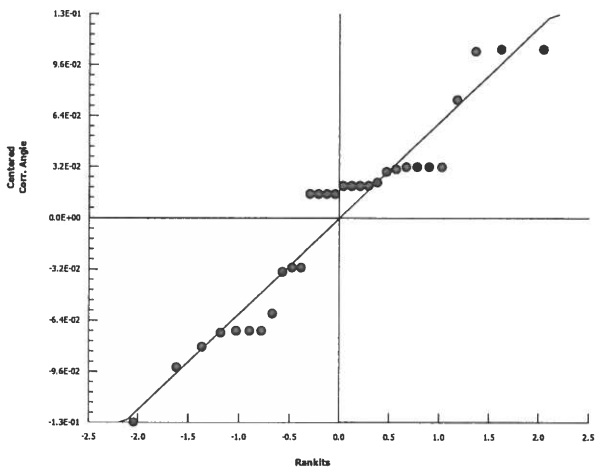
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.515	1.473	1.557	1.53	1.455	1.53	0.01498	2.21%	0.0%
2.5		5	1.424	1.304	1.544	1.353	1.353	1.53	0.04333	6.81%	6.02%
5		5	1.51	1.454	1.566	1.53	1.429	1.53	0.02021	2.99%	0.35%
10		5	1.498	1.409	1.587	1.53	1.369	1.53	0.0321	4.79%	1.13%
20		5	1.085	1.004	1.166	1.107	0.9912	1.159	0.02907	5.99%	28.39%
40		5	0.1128	0.02729	0.1983	0.08174	0.04084	0.2177	0.0308	61.05%	92.55%

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

Analysis ID:	14-9901-0252	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 11:17	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 220420msdv | 07-3521-2032

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	06-3800-2192	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 11:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1911543	1000	Yes	Two-Point Interpolation

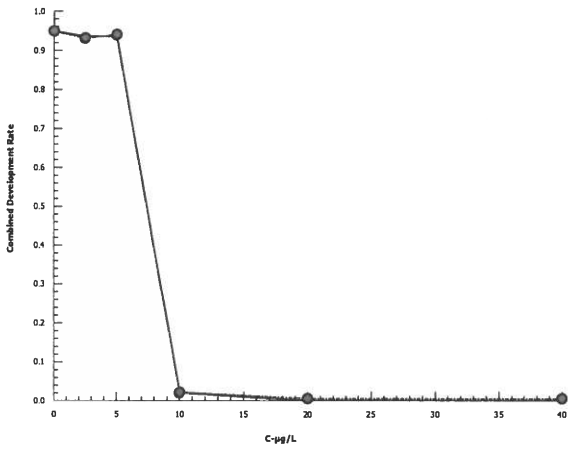
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.222	6.092	6.315
EC50	7.519	7.412	7.613

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	0.949	0.9267	0.9578	0.005926	0.01325	1.4%	0.0%	751	791
2.5		5	0.9299	0.8933	0.9655	0.01366	0.03054	3.28%	2.01%	734	788
5		5	0.9386	0.9	0.9653	0.01155	0.02583	2.75%	1.1%	769	818
10		5	0.02049	0.006623	0.05732	0.009474	0.02119	103.4%	97.84%	16	770
20		5	0	0	0	0	0		100.0%	0	750
40		5	0	0	0	0	0		100.0%	0	750

Graphics



Analyst: _____ QA: AJS 5/11/22

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

Analysis ID:	16-7080-8967	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 11:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1469357	1000	Yes	Two-Point Interpolation

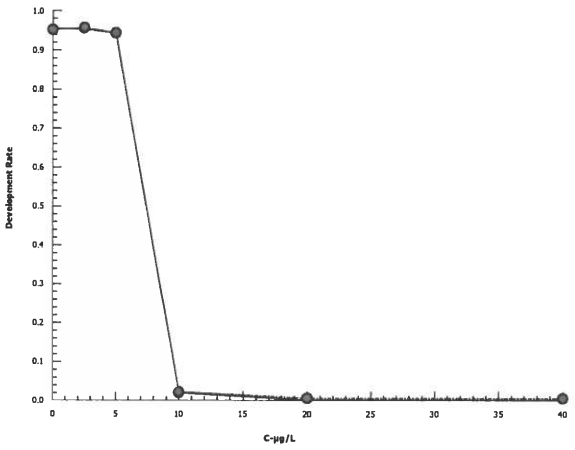
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.234	6.122	6.32
EC50	7.527	7.434	7.619

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	0.9515	0.9392	0.9578	0.003661	0.008187	0.86%	0.0%	751	789
2.5		5	0.9566	0.9371	0.9655	0.005172	0.01156	1.21%	-0.53%	734	767
5		5	0.9423	0.9184	0.9653	0.008719	0.0195	2.07%	0.97%	769	815
10		5	0.0206	0.006623	0.05732	0.009454	0.02114	102.6%	97.83%	16	764
20		5	0	0	0	0	0		100.0%	0	585
40		5	0	0	0	0	0		100.0%	0	13

Graphics



Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	11-4955-0328	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	10 May-22 11:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	390374	1000	Yes	Two-Point Interpolation

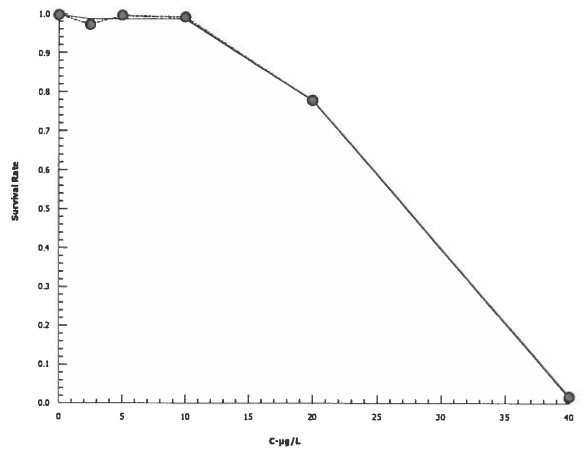
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	20.84	18.86	22.36
EC50	27.36	26.19	28.41

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	0.9973	0.9867	1	0.002667	0.005963	0.6%	0.0%	748	750
2.5		5	0.972	0.9533	1	0.01143	0.02556	2.63%	2.54%	729	750
5		5	0.996	0.98	1	0.004	0.008944	0.9%	0.13%	747	750
10		5	0.992	0.96	1	0.008	0.01789	1.8%	0.53%	744	750
20		5	0.78	0.7	0.84	0.02431	0.05437	6.97%	21.79%	584	750
40		5	0.016	0	0.04667	0.008327	0.01862	116.4%	98.4%	12	750

Graphics

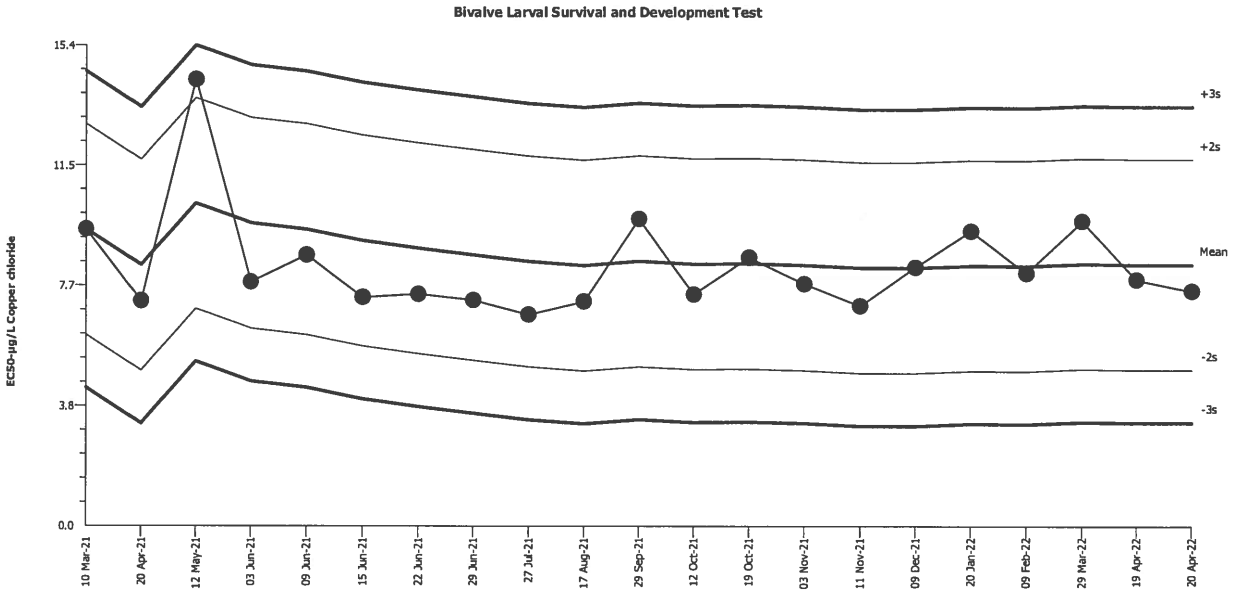


CETIS QC Plot

Report Date: 10 May-22 11:19 (1 of 1)

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

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



Mean:	8.358	**Count:**	20	**-2s Warning Limit:**	4.988	**-3s Action Limit:**	3.303
Sigma:	1.685	**CV:**	20.20%	**+2s Warning Limit:**	11.73	**+3s Action Limit:**	13.41

Quality Control Data

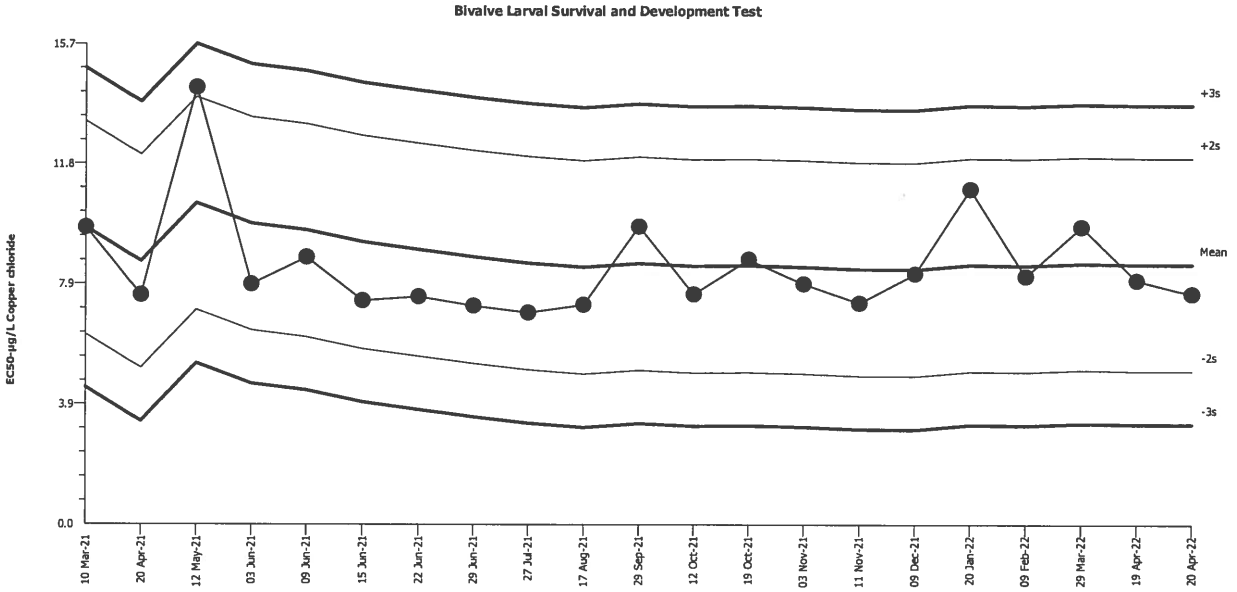
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Mar	10	14:15	9.481	1.123	0.6663			13-7922-5399	10-0885-9755
2		Apr	20	16:15	7.185	-1.173	-0.6962			06-7450-9711	18-3353-6875
3		May	12	15:00	14.27	5.91	3.507	(+)	(+)	15-4594-3065	00-9727-8504
4		Jun	3	15:50	7.791	-0.5668	-0.3364			07-9391-2508	21-2212-7050
5			9	14:00	8.654	0.2955	0.1754			18-5736-8495	04-4549-3405
6			15	15:40	7.302	-1.056	-0.6269			00-2993-6780	17-7654-7354
7			22	13:45	7.404	-0.954	-0.5662			16-6840-3553	15-2803-6917
8			29	14:55	7.211	-1.147	-0.6806			07-2040-2693	08-8247-6801
9		Jul	27	16:30	6.748	-1.61	-0.9552			16-6019-6958	06-5859-7928
10		Aug	17	14:25	7.168	-1.19	-0.7065			07-7298-7649	09-6648-5411
11		Sep	29	15:45	9.809	1.451	0.8612			12-3450-8829	18-2247-7613
12		Oct	12	15:00	7.395	-0.9628	-0.5714			14-7239-9185	01-1367-5722
13			19	17:00	8.581	0.2229	0.1323			17-5798-2248	09-1208-0351
14		Nov	3	15:00	7.733	-0.6255	-0.3712			14-6395-1490	06-4040-2968
15			11	14:35	7.03	-1.328	-0.7884			00-1546-1531	12-7713-2161
16		Dec	9	15:50	8.264	-0.09375	-0.05564			06-2693-6580	11-5581-5612
17	2022	Jan	20	15:15	9.426	1.068	0.6341			06-1599-8254	16-9050-7435
18		Feb	9	16:25	8.083	-0.2751	-0.1632			20-6883-0287	13-7282-5479
19		Mar	29	13:15	9.75	1.392	0.8264			09-4881-8633	10-6557-0477
20		Apr	19	17:40	7.878	-0.4803	-0.2851			20-9064-9386	10-4205-1906
21			20	14:30	7.519	-0.8385	-0.4976			07-3521-2032	06-3800-2192

CETIS QC Plot

Report Date: 10 May-22 11:19 (1 of 1)

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

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



Mean:	8.489	**Count:**	20	**-2s Warning Limit:**	5.009	**-3s Action Limit:**	3.269
Sigma:	1.74	**CV:**	20.50%	**+2s Warning Limit:**	11.97	**+3s Action Limit:**	13.71

Quality Control Data

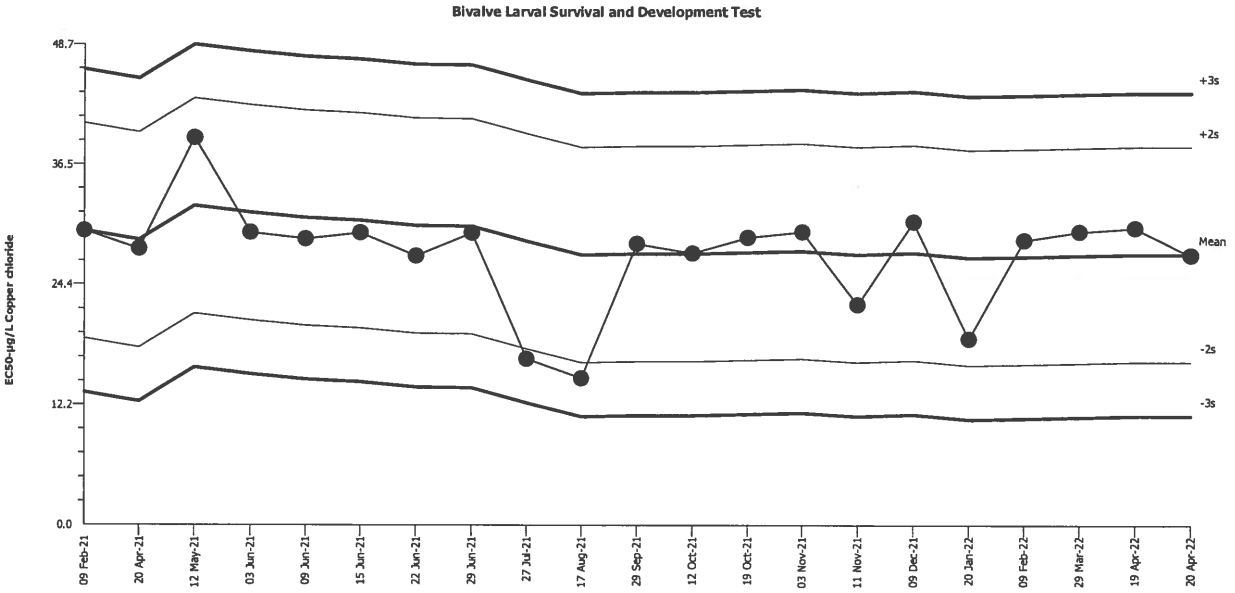
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Mar	10	14:15	9.694	1.205	0.6923			13-7922-5399	08-4869-7631
2		Apr	20	16:15	7.482	-1.007	-0.5788			06-7450-9711	17-9210-1733
3		May	12	15:00	14.27	5.779	3.321	(+)	(+)	15-4594-3065	12-3891-6641
4		Jun	3	15:50	7.832	-0.6569	-0.3775			07-9391-2508	11-7075-1183
5			9	14:00	8.715	0.2264	0.1301			18-5736-8495	18-6125-5477
6			15	15:40	7.302	-1.187	-0.6824			00-2993-6780	13-6998-5313
7			22	13:45	7.427	-1.062	-0.6105			16-6840-3553	07-3347-2243
8			29	14:55	7.132	-1.357	-0.78			07-2040-2693	17-0989-5973
9		Jul	27	16:30	6.912	-1.577	-0.9065			16-6019-6958	03-0913-6262
10		Aug	17	14:25	7.168	-1.321	-0.7595			07-7298-7649	11-4901-9823
11		Sep	29	15:45	9.718	1.229	0.7063			12-3450-8829	04-7958-3381
12		Oct	12	15:00	7.509	-0.9796	-0.563			14-7239-9185	04-3282-5514
13			19	17:00	8.648	0.1586	0.09112			17-5798-2248	05-0981-9303
14		Nov	3	15:00	7.85	-0.6387	-0.3671			14-6395-1490	11-9492-7222
15			11	14:35	7.225	-1.264	-0.7265			00-1546-1531	03-5898-7126
16		Dec	9	15:50	8.177	-0.3119	-0.1793			06-2693-6580	19-9748-5087
17	2022	Jan	20	15:15	10.94	2.455	1.411			06-1599-8254	16-8693-8465
18		Feb	9	16:25	8.097	-0.3918	-0.2252			20-6883-0287	03-6791-7638
19		Mar	29	13:15	9.709	1.22	0.7011			09-4881-8633	12-2799-4519
20		Apr	19	17:40	7.967	-0.5224	-0.3002			20-9064-9386	19-3933-4036
21			20	14:30	7.527	-0.9616	-0.5527			07-3521-2032	16-7080-8967

CETIS QC Plot

Report Date: 10 May-22 11:19 (1 of 1)

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

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



Mean:	27.46	**Count:**	20	**-2s Warning Limit:**	16.54	**-3s Action Limit:**	11.08
Sigma:	5.46	**CV:**	19.90%	**+2s Warning Limit:**	38.38	**+3s Action Limit:**	43.84

Quality Control Data

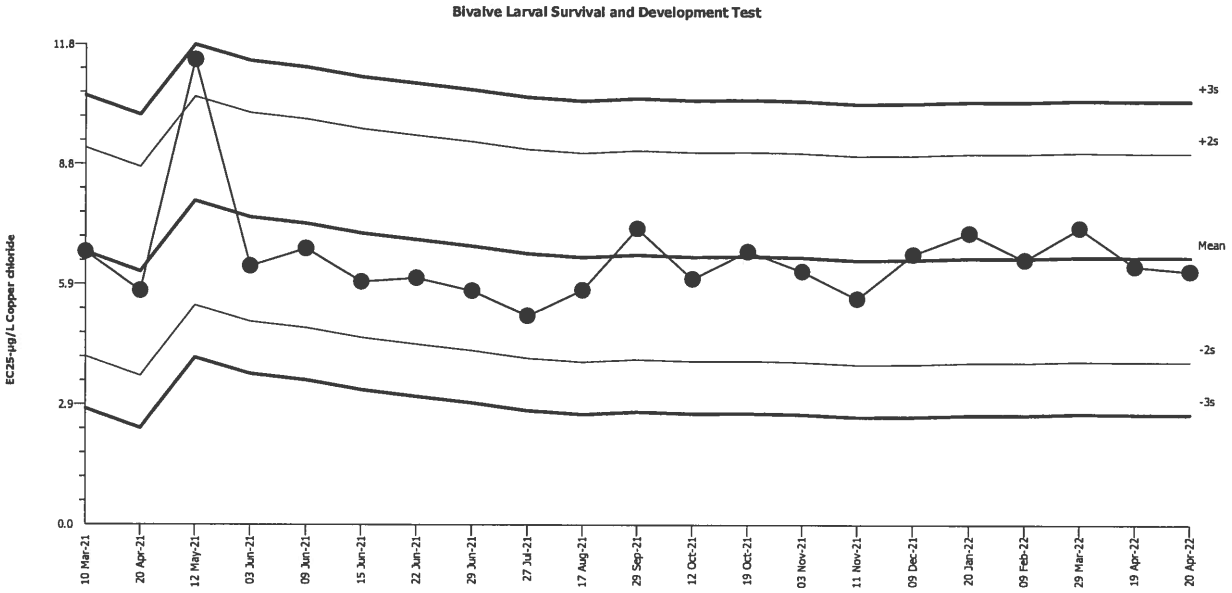
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Feb	9	15:15	29.8	2.345	0.4294			12-5648-6062	08-9593-0094
2		Apr	20	16:15	27.97	0.5108	0.09354			06-7450-9711	02-2099-4435
3		May	12	15:00	39.23	11.77	2.156	(+)		15-4594-3065	18-1677-8776
4		Jun	3	15:50	29.62	2.158	0.3952			07-9391-2508	05-7225-1680
5			9	14:00	28.97	1.506	0.2759			18-5736-8495	17-4075-5383
6			15	15:40	29.61	2.151	0.394			00-2993-6780	11-7676-4213
7			22	13:45	27.27	-0.193	-0.03535			16-6840-3553	00-7652-1305
8			29	14:55	29.58	2.125	0.3892			07-2040-2693	20-9452-4039
9		Jul	27	16:30	16.82	-10.64	-1.949			16-6019-6958	09-3317-6652
10		Aug	17	14:25	14.86	-12.6	-2.307	(-)		07-7298-7649	12-6822-1646
11		Sep	29	15:45	28.5	1.039	0.1903			12-3450-8829	17-8563-2416
12		Oct	12	15:00	27.53	0.06711	0.01229			14-7239-9185	11-8743-4626
13			19	17:00	29.13	1.67	0.3058			17-5798-2248	01-7668-6950
14		Nov	3	15:00	29.71	2.246	0.4113			14-6395-1490	03-1145-8832
15			11	14:35	22.33	-5.135	-0.9405			00-1546-1531	07-6640-8098
16		Dec	9	15:50	30.73	3.268	0.5985			06-2693-6580	02-3744-1694
17	2022	Jan	20	15:15	18.86	-8.604	-1.576			06-1599-8254	12-6429-5476
18		Feb	9	16:25	28.86	1.402	0.2567			20-6883-0287	05-7427-9529
19		Mar	29	13:15	29.74	2.275	0.4167			09-4881-8633	11-4846-6536
20		Apr	19	17:40	30.12	2.656	0.4865			20-9064-9386	11-9782-6263
21			20	14:30	27.36	-0.09525	-0.01745			07-3521-2032	11-4955-0328

CETIS QC Plot

Report Date: 10 May-22 11:19 (1 of 1)

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

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



Mean:	6.559	**Count:**	20	**-2s Warning Limit:**	3.999	**-3s Action Limit:**	2.719
Sigma:	1.28	**CV:**	19.50%	**+2s Warning Limit:**	9.119	**+3s Action Limit:**	10.4

Quality Control Data

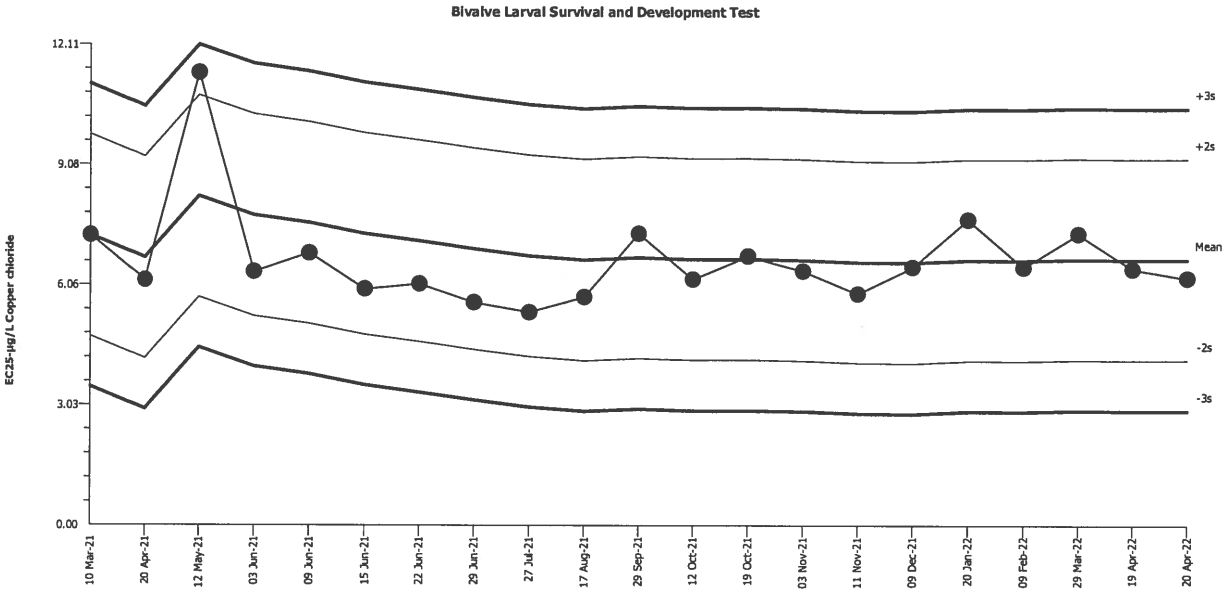
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Mar	10	14:15	6.682	0.1235	0.09646			13-7922-5399	10-0885-9755
2		Apr	20	16:15	5.728	-0.8307	-0.649			06-7450-9711	18-3353-6875
3		May	12	15:00	11.4	4.843	3.783	(+)	(+)	15-4594-3065	00-9727-8504
4		Jun	3	15:50	6.337	-0.2221	-0.1735			07-9391-2508	21-2212-7050
5			9	14:00	6.767	0.2075	0.1621			18-5736-8495	04-4549-3405
6			15	15:40	5.953	-0.6065	-0.4738			00-2993-6780	17-7654-7354
7			22	13:45	6.048	-0.5106	-0.3989			16-6840-3553	15-2803-6917
8			29	14:55	5.736	-0.8235	-0.6433			07-2040-2693	08-8247-6801
9		Jul	27	16:30	5.123	-1.436	-1.122			16-6019-6958	06-5859-7928
10		Aug	17	14:25	5.751	-0.8077	-0.631			07-7298-7649	09-6648-5411
11		Sep	29	15:45	7.261	0.7023	0.5487			12-3450-8829	18-2247-7613
12		Oct	12	15:00	6.03	-0.5294	-0.4136			14-7239-9185	01-1367-5722
13			19	17:00	6.701	0.1421	0.1111			17-5798-2248	09-1208-0351
14		Nov	3	15:00	6.215	-0.3443	-0.269			14-6395-1490	06-4040-2968
15			11	14:35	5.544	-1.015	-0.7927			00-1546-1531	12-7713-2161
16		Dec	9	15:50	6.632	0.07313	0.05713			06-2693-6580	11-5581-5612
17	2022	Jan	20	15:15	7.149	0.5896	0.4607			06-1599-8254	16-9050-7435
18		Feb	9	16:25	6.494	-0.06506	-0.05083			20-6883-0287	13-7282-5479
19		Mar	29	13:15	7.278	0.7193	0.5619			09-4881-8633	10-6557-0477
20		Apr	19	17:40	6.34	-0.2185	-0.1707			20-9064-9386	10-4205-1906
21			20	14:30	6.222	-0.3365	-0.2629			07-3521-2032	06-3800-2192

CETIS QC Plot

Report Date: 10 May-22 11:19 (1 of 1)

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

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



Mean:	6.703	**Count:**	20	**-2s Warning Limit:**	4.159	**-3s Action Limit:**	2.887
Sigma:	1.272	**CV:**	19.00%	**+2s Warning Limit:**	9.247	**+3s Action Limit:**	10.52

Quality Control Data

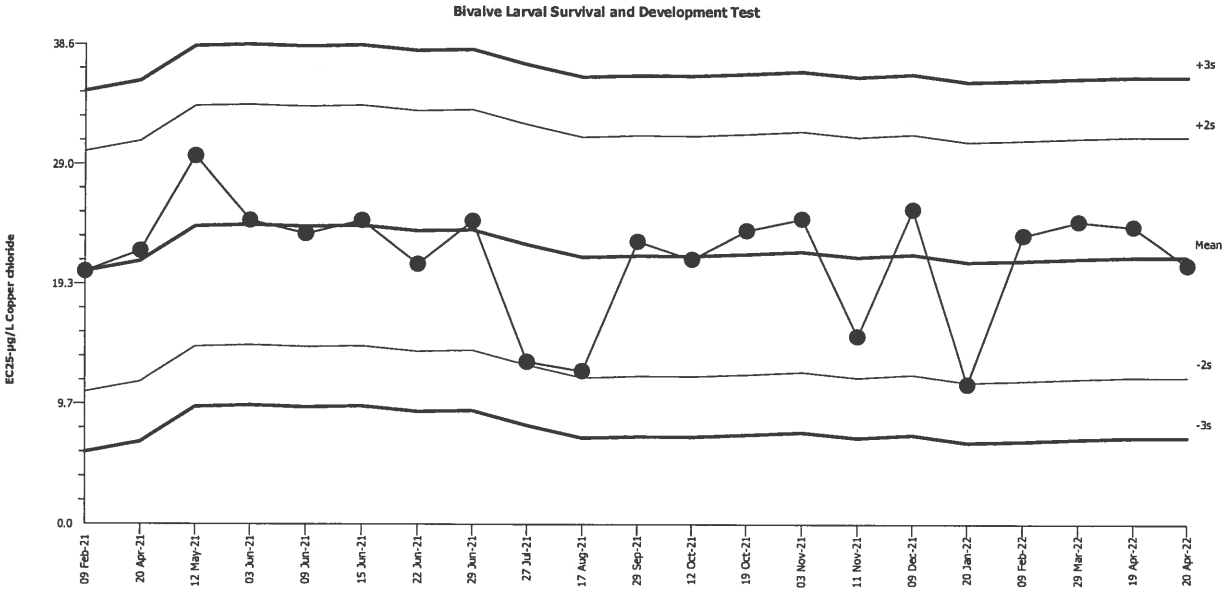
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Mar	10	14:15	7.308	0.6049	0.4756			13-7922-5399	08-4869-7631
2		Apr	20	16:15	6.175	-0.5285	-0.4155			06-7450-9711	17-9210-1733
3		May	12	15:00	11.4	4.699	3.694	(+)	(+)	15-4594-3065	12-3891-6641
4		Jun	3	15:50	6.387	-0.3164	-0.2487			07-9391-2508	11-7075-1183
5			9	14:00	6.858	0.1547	0.1216			18-5736-8495	18-6125-5477
6			15	15:40	5.953	-0.7505	-0.59			00-2993-6780	13-6998-5313
7			22	13:45	6.084	-0.6194	-0.4869			16-6840-3553	07-3347-2243
8			29	14:55	5.615	-1.088	-0.8551			07-2040-2693	17-0989-5973
9		Jul	27	16:30	5.367	-1.336	-1.05			16-6019-6958	03-0913-6262
10		Aug	17	14:25	5.751	-0.9517	-0.7482			07-7298-7649	11-4901-9823
11		Sep	29	15:45	7.359	0.6559	0.5157			12-3450-8829	04-7958-3381
12		Oct	12	15:00	6.202	-0.5012	-0.3941			14-7239-9185	04-3282-5514
13			19	17:00	6.79	0.08685	0.06828			17-5798-2248	05-0981-9303
14		Nov	3	15:00	6.411	-0.2923	-0.2298			14-6395-1490	11-9492-7222
15			11	14:35	5.837	-0.8657	-0.6805			00-1546-1531	03-5898-7126
16		Dec	9	15:50	6.512	-0.1907	-0.1499			06-2693-6580	19-9748-5087
17	2022	Jan	20	15:15	7.713	1.01	0.7941			06-1599-8254	16-8693-8465
18		Feb	9	16:25	6.507	-0.1959	-0.154			20-6883-0287	03-6791-7638
19		Mar	29	13:15	7.354	0.6514	0.5121			09-4881-8633	12-2799-4519
20		Apr	19	17:40	6.469	-0.234	-0.1839			20-9064-9386	19-3933-4036
21			20	14:30	6.234	-0.4687	-0.3685			07-3521-2032	16-7080-8967

CETIS QC Plot

Report Date: 10 May-22 11:19 (1 of 1)

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

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



Mean:	21.5	**Count:**	20	**-2s Warning Limit:**	11.81	**-3s Action Limit:**	6.961
Sigma:	4.847	**CV:**	22.50%	**+2s Warning Limit:**	31.2	**+3s Action Limit:**	36.04

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2021	Feb	9	15:15	20.31	-1.191	-0.2456			12-5648-6062	08-9593-0094
2		Apr	20	16:15	21.96	0.4561	0.09411			06-7450-9711	02-2099-4435
3		May	12	15:00	29.62	8.115	1.674			15-4594-3065	18-1677-8776
4		Jun	3	15:50	24.43	2.926	0.6038			07-9391-2508	05-7225-1680
5			9	14:00	23.34	1.844	0.3804			18-5736-8495	17-4075-5383
6			15	15:40	24.42	2.917	0.6019			00-2993-6780	11-7676-4213
7			22	13:45	20.9	-0.5995	-0.1237			16-6840-3553	00-7652-1305
8			29	14:55	24.38	2.877	0.5936			07-2040-2693	20-9452-4039
9		Jul	27	16:30	13.05	-8.454	-1.744			16-6019-6958	09-3317-6652
10		Aug	17	14:25	12.3	-9.205	-1.899			07-7298-7649	12-6822-1646
11		Sep	29	15:45	22.73	1.225	0.2528			12-3450-8829	17-8563-2416
12		Oct	12	15:00	21.29	-0.2093	-0.04319			14-7239-9185	11-8743-4626
13			19	17:00	23.62	2.119	0.4372			17-5798-2248	01-7668-6950
14		Nov	3	15:00	24.56	3.059	0.6311			14-6395-1490	03-1145-8832
15			11	14:35	15.09	-6.406	-1.322			00-1546-1531	07-6640-8098
16		Dec	9	15:50	25.32	3.825	0.7891			06-2693-6580	02-3744-1694
17	2022	Jan	20	15:15	11.23	-10.27	-2.12	(-)		06-1599-8254	12-6429-5476
18		Feb	9	16:25	23.23	1.725	0.356			20-6883-0287	05-7427-9529
19		Mar	29	13:15	24.35	2.851	0.5883			09-4881-8633	11-4846-6536
20		Apr	19	17:40	23.94	2.44	0.5035			20-9064-9386	11-9782-6263
21			20	14:30	20.84	-0.6623	-0.1366			07-3521-2032	11-4955-0328

CETIS Test Data Worksheet

Report Date: 07 May-22 17:35 (p 1 of 1)
Test Code: 07-3521-2032/220420msdv

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

Start Date: 20 Apr-22
End Date: 22 Apr-22
Sample Date: 20 Apr-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 220420msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			105	0	AFS 5/9/22
			2			181	173	
			3			120	0	
			4			150	1	
			5			166	159	
			6			151	143	
			7			147	135	
			8			3	0	
			9			7	0	
			10			174	168	
			11			151	140	
			12			143	138	
			13			173	167	
			14			126	0	
			15			121	0	
			16			151	1	
			17			144	2	
			18			157	9	
			19			162	3	
			20			143	137	
			21			164	157	
			22			143	134	
			23			160	153	
			24			1	0	
			25			113	0	
			26			1	0	
			27			163	154	
			28			164	157	
			29			0	0	
			30			148	139	

CETIS Test Data Worksheet

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

Start Date: 20 Apr-22
End Date: 22 Apr-22
Sample Date: 20 Apr-22
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 220420msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	30					
0	LC	2	5					
0	LC	3	23			160	153	WF
0	LC	4	28					
0	LC	5	6					
2.5		1	20					
2.5		2	12					
2.5		3	21			159	154	WF
2.5		4	10					
2.5		5	22					
5		1	2					
5		2	27					
5		3	7			140	131	WF
5		4	11					
5		5	13					
10		1	19					
10		2	17					
10		3	16			145	0	WF
10		4	18					
10		5	4					
20		1	3					
20		2	1					
20		3	25			114	0	WF
20		4	14					
20		5	15					
40		1	9					
40		2	24					
40		3	29			0	0	WF
40		4	26					
40		5	8					

QC = B0

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 220420 m3dv

Test Species: *M. galloprovincialis*

Start Date/Time: 1430 4/20/22

End Date/Time: 1400 4/22/22

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	31.2	31.6	31.5	15.5	14.6	14.8	8.2	8.6	8.5	8.00	7.93	7.96
2.5	31.6	31.8	31.7	15.1	14.4	14.6	8.3	8.7	8.6	7.99	7.93	7.98
5	31.6	31.9	31.8	15.0	14.7	14.6	8.4	8.7	8.6	7.99	7.94	8.00
10	31.6	31.9	31.8	15.1	14.7	14.7	8.3	8.6	8.6	7.98	7.94	7.99
20	31.6	31.8	31.8	15.1	14.6	14.8	8.3	8.7	8.6	7.96	7.95	7.99
40	31.5	31.7	31.7	15.0	14.6	14.7	8.3	8.7	8.6	7.96	7.95	7.96

	0	24	48
WQ Readings:	BO	GM	BO
Dilutions made by:	BO		

Technician Initials:

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

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: JL 5/5/22

Final Review: AES 5/11/22

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / CuCl2
Test No.: 220420msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: MREP / 6A
Date Received: 11/17/21
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 4/20/22 1430
End Date/Time: 4/22/22 1400
Technician Initials: BSO

Spawn Information

First Gamete Release Time: 1100

Sex	Number Spawning
Male	5
Female	3

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1, 2, 3	good motility, very dense
Female 1	2	Some round, some oval, white, average density
Female 2	3	round, white, very dense
Female 3	—	—

Egg Fertilization Time: 1200

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	80
Female 2	100
Female 3	—

Stock(s) chosen for testing: 2

Embryo Inoculum Preparation

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

Number Counted:		
	9	9
	8	8
	7	10
	7	6
	10	8

Mean: 8.2

Mean 8.2 X 50 = 410 embryos/ml

Initial Density: 410 = 1.4 (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	169	169	100	99.5
TØ B	141	141	100	
TØ C	167	169	99	
TØ D	133	133	100	
TØ E	143	144	99	
TØ F	147	149	99	
X̄ =	150			

48-h QC: 168/175 = 96%

Comments:

QC Check: 5/5/22

Final Review: AYS 5/11/22