ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: April 2020

Prepared for: **Jacobs**
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Prepared by: **Enthalpy Analytical (formerly Nautilus Environmental)**
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Date Submitted: **June 1, 2020**

Data Quality Assurance:

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

Results verified by: _____
Eric Green, Project Manager

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Introduction

A toxicity test was performed using a groundwater composite sample collected on April 14, 2020 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	**041420**
Enthalpy Log-in Number	20-0416
Collection Date; Time	4/14/2020; 0857h
Receipt Date; Time	4/15/2020; 0930h
Receipt Temperature (°C)	2.4
Dissolved Oxygen (mg/L)	8.9
pH	7.54
Conductivity (µS/cm)	11,880
Salinity (ppt)	7.1
Alkalinity (mg/L $CaCO_3$)	452
Total Chlorine (mg/L)	< 0.02
Total Ammonia (mg/L as N)	1.1

NM = not measured

Test Methods

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

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

Test Period	4/15/2020, 1325h to 4/17/2020, 1525h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	Taylor Shellfish (Shelton, WA)
Test Organism Age	4 hours post fertilization
Test Duration	48 ± 2 hours
Test Type	Static
Test Chamber, Test Solution Volume	30 mL glass vial, 10 mL
Test Temperature	15 ± 1°C
Dilution Water	Laboratory Seawater (Source: Scripps Institution of Oceanography [SIO] intake) diluted with de-ionized water
Additional Control	Brine Control (de-ionized water and hypersaline brine)
Test Salinity	30 ± 2 ppt
Source of Salinity	Hypersaline brine made by freezing seawater to a salinity of 94.1 ppt
Test Concentrations (% sample)	75.1[a], 35, 18, 9, 4, and 2%, lab and brine controls
Number of Replicates	5
Photoperiod	16 hours light/8 hours dark
Test Protocol	EPA/600/R-95/136
Test Acceptability Criteria for Controls	≥ 50% mean survival, ≥ 90% mean development rate
Reference Toxicant	Copper chloride [b]
Statistical Software	CETIS™ 1.8.7.20

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

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

Results

There were no statistically significant effects observed in any effluent concentration tested for the survival or development endpoint of the bivalve test. This results in a no observed effect concentration (NOEC) of 75.1 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.33 for both endpoints.

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

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

Species	Endpoint	NOEC (% effluent)	LOEC (% effluent)	Toxic Unit (TU_c)	EC_{25} (% effluent)
Bivalve	Normal Development	75.1	> 75.1	< 1.33	> 75.1
	Survival	75.1	> 75.1	< 1.33	> 75.1

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

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

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

Concentration (% Effluent)	Mean Survival (%)	Mean Normal Development (%)
0 (Brine Control)	100	98.2
0 (Lab Control)	100	97.4
2	99.9	97.2
4	98.9	97.8
9	100	98.0
18	100	98.2
35	99.1	98.8
75.1[a]	99.1	97.1

[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. The reference toxicant statistical summaries and laboratory bench sheets are provided in Appendix E.

Table 5. Reference Toxicant Test Results

Species and Endpoint	NOEC (%)	EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve Normal Development	5	11.7	8.94 ± 6.42	35.9
Bivalve Survival Rate	20	30.0	29.6 ± 5.09	8.60

NOEC = No Observed Effect Concentration

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

CV = Coefficient of Variation

References

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

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

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

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

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

Appendix A
Statistical Summaries and Raw Bench Sheets

CETIS Summary Report

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

Batch ID:	07-0492-5892	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	15 Apr-20 13:25	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	17 Apr-20 15:25	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	50h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	03-1250-2921	**Code:**	20-0416	**Client:**	Jacobs
Sample Date:	14 Apr-20 08:57	**Material:**	Effluent Sample	**Project:**	
Receive Date:	15 Apr-20 09:30	**Source:**	Jacobs		
Sample Age:	28h (2.4 °C)	**Station:**	Wyckoff (041420) GWTP Effluent		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-2587-1171	Development Rate	75.1	>75.1	NA	1.68%	<1.332	Dunnett Multiple Comparison Test
18-6051-8845	Survival Rate	75.1	>75.1	NA	1.32%	<1.332	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
03-0901-5666	Development Rate	EC25	>75.1	N/A	N/A	<1.332	Linear Interpolation (ICPIN)
		EC50	>75.1	N/A	N/A	<1.332	
08-9188-7387	Survival Rate	EC25	>75.1	N/A	N/A	<1.332	Linear Interpolation (ICPIN)
		EC50	>75.1	N/A	N/A	<1.332	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-0901-5666	Development Rate	Control Resp	0.9818	0.9 - NL	Yes	Passes Acceptability Criteria
14-2587-1171	Development Rate	Control Resp	0.9818	0.9 - NL	Yes	Passes Acceptability Criteria
08-9188-7387	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
18-6051-8845	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria

Development Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9818	0.9702	0.9934	0.9722	0.9912	0.004181	0.00935	0.95%	0.0%
0	Lab Control	5	0.9738	0.96	0.9877	0.9583	0.9865	0.004988	0.01115	1.15%	0.81%
2		5	0.9723	0.9534	0.9912	0.9575	0.9954	0.006815	0.01524	1.57%	0.97%
4		5	0.9783	0.9687	0.988	0.9707	0.9903	0.003486	0.007795	0.8%	0.35%
9		5	0.9797	0.9718	0.9876	0.9717	0.9871	0.002861	0.006398	0.65%	0.21%
18		5	0.9824	0.9726	0.9922	0.9703	0.9906	0.003526	0.007884	0.8%	-0.06%
35		5	0.9882	0.9771	0.9993	0.9801	1	0.003996	0.008934	0.9%	-0.65%
75.1		5	0.9706	0.9624	0.9787	0.9652	0.9815	0.002943	0.00658	0.68%	1.14%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	1	1	1	1	1	0	0	0.0%	0.0%
0	Lab Control	5	1	1	1	1	1	0	0	0.0%	0.0%
2		5	0.9991	0.9964	1	0.9953	1	0.000948	0.002119	0.21%	0.09%
4		5	0.9886	0.9676	1	0.9621	1	0.007583	0.01696	1.72%	1.14%
9		5	1	1	1	1	1	0	0	0.0%	0.0%
18		5	1	1	1	1	1	0	0	0.0%	0.0%
35		5	0.9905	0.9642	1	0.9526	1	0.009479	0.02119	2.14%	0.95%
75.1		5	0.9905	0.9642	1	0.9526	1	0.009479	0.02119	2.14%	0.95%

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

Development Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.9722	0.9911	0.9727	0.9912	0.9817
0	Lab Control	0.9802	0.9583	0.9769	0.9671	0.9865
2		0.9762	0.9954	0.9575	0.9729	0.9595
4		0.9737	0.9903	0.9816	0.9754	0.9707
9		0.9828	0.9717	0.9825	0.9745	0.9871
18		0.9703	0.9906	0.9836	0.9877	0.9797
35		0.9831	0.9822	0.9801	0.9955	1
75.1		0.9658	0.9712	0.9815	0.9652	0.9692

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	1	1	1	1
0	Lab Control	1	1	1	1	1
2		0.9953	1	1	1	1
4		1	0.981	1	0.9621	1
9		1	1	1	1	1
18		1	1	1	1	1
35		1	1	0.9526	1	1
75.1		1	1	1	0.9526	1

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	210/216	223/225	214/220	226/228	214/218
0	Lab Control	248/253	207/216	211/216	235/243	220/223
2		205/210	215/216	203/212	215/221	237/247
4		222/228	205/207	213/217	198/203	232/239
9		229/233	240/247	224/228	229/235	229/232
18		229/236	210/212	240/244	240/243	241/246
35		233/237	221/225	197/201	220/221	217/217
75.1		226/234	236/243	212/216	194/201	220/227

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	211/211	211/211	211/211	211/211	211/211
0	Lab Control	211/211	211/211	211/211	211/211	211/211
2		210/211	211/211	211/211	211/211	211/211
4		211/211	207/211	211/211	203/211	211/211
9		211/211	211/211	211/211	211/211	211/211
18		211/211	211/211	211/211	211/211	211/211
35		211/211	211/211	201/211	211/211	211/211
75.1		211/211	211/211	211/211	201/211	211/211

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

Analysis ID:	14-2587-1171	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-20 8:53	**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	1.68%	75.1	>75.1	NA	1.332

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	1.216	2.407	0.056	8	0.3561	CDF	Non-Significant Effect
		4	0.6004	2.407	0.056	8	0.6365	CDF	Non-Significant Effect
		9	0.434	2.407	0.056	8	0.7080	CDF	Non-Significant Effect
		18	-0.03696	2.407	0.056	8	0.8669	CDF	Non-Significant Effect
		35	-1.297	2.407	0.056	8	0.9951	CDF	Non-Significant Effect
		75.1	1.721	2.407	0.056	8	0.1760	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01531583	0.002552638	6	1.893	0.1172	Non-Significant Effect
Error	0.03775032	0.001348226	28			
Total	0.05306615		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.973	16.81	0.4263	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9482	0.9146	0.0998	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	0.9818	0.9702	0.9934	0.9817	0.9722	0.9912	0.004181	0.95%	0.0%
2		5	0.9723	0.9534	0.9912	0.9729	0.9575	0.9954	0.006815	1.57%	0.97%
4		5	0.9783	0.9687	0.988	0.9754	0.9707	0.9903	0.003486	0.8%	0.35%
9		5	0.9797	0.9718	0.9876	0.9825	0.9717	0.9871	0.002862	0.65%	0.21%
18		5	0.9824	0.9726	0.9922	0.9836	0.9703	0.9906	0.003526	0.8%	-0.06%
35		5	0.9882	0.9771	0.9993	0.9831	0.9801	1	0.003996	0.9%	-0.65%
75.1		5	0.9706	0.9624	0.9787	0.9692	0.9652	0.9815	0.002943	0.68%	1.14%

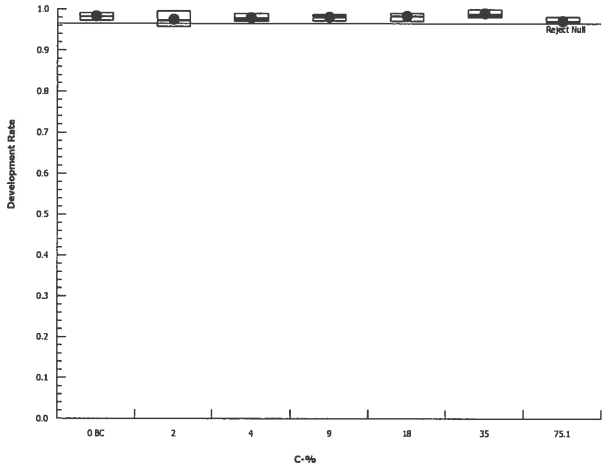
Angular (Corrected) Transformed Summary

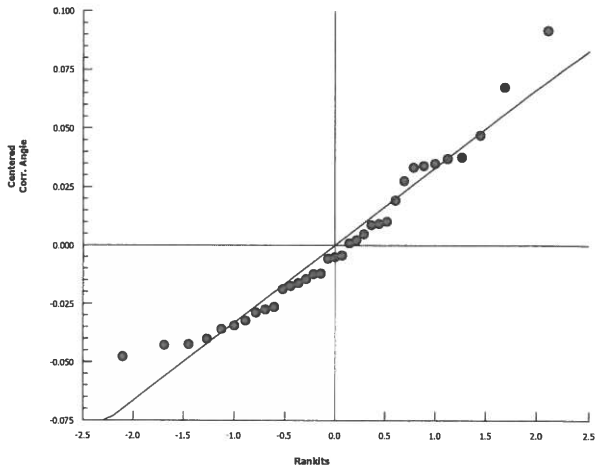
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.439	1.394	1.484	1.435	1.403	1.477	0.01627	2.53%	0.0%
2		5	1.411	1.341	1.481	1.405	1.363	1.503	0.02508	3.97%	1.96%
4		5	1.425	1.389	1.462	1.413	1.399	1.472	0.01314	2.06%	0.97%
9		5	1.429	1.401	1.457	1.438	1.402	1.457	0.01015	1.59%	0.7%
18		5	1.44	1.404	1.477	1.442	1.398	1.474	0.01313	2.04%	-0.06%
35		5	1.469	1.41	1.529	1.441	1.429	1.537	0.02145	3.26%	-2.09%
75.1		5	1.399	1.374	1.425	1.394	1.383	1.434	0.009282	1.48%	2.78%

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

Analysis ID:	14-2587-1171	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-20 8:53	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: JL QA: BO 5/27/20 / EG 6/1/20

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

Analysis ID:	18-6051-8845	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-20 8:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	1.32%	75.1	>75.1	NA	1.332

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	25	16	1	8	0.6693	Asymp	Non-Significant Effect
		4	22.5	16	1	8	0.4265	Asymp	Non-Significant Effect
		9	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		18	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		35	25	16	1	8	0.6693	Asymp	Non-Significant Effect
		75.1	25	16	1	8	0.6693	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01518538	0.002530896	6	0.9027	0.5070	Non-Significant Effect
Error	0.07850601	0.002803786	28			
Total	0.09369139		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.9291	3.812	0.4946	Equal Variances
Variances	Levene Equality of Variance	6.054	3.528	0.0004	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.7259	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	1	1	1	1	1	1	0	0.0%	0.0%
2		5	0.9991	0.9964	1	1	0.9953	1	0.000948	0.21%	0.09%
4		5	0.9886	0.9676	1	1	0.9621	1	0.007583	1.72%	1.14%
9		5	1	1	1	1	1	1	0	0.0%	0.0%
18		5	1	1	1	1	1	1	0	0.0%	0.0%
35		5	0.9905	0.9642	1	1	0.9526	1	0.009479	2.14%	0.95%
75.1		5	0.9905	0.9642	1	1	0.9526	1	0.009479	2.14%	0.95%

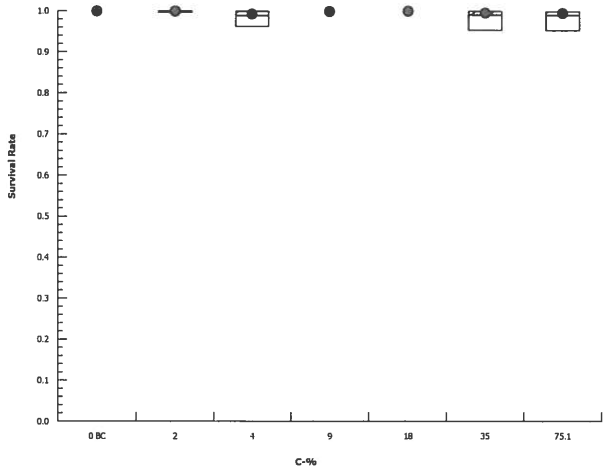
Angular (Corrected) Transformed Summary

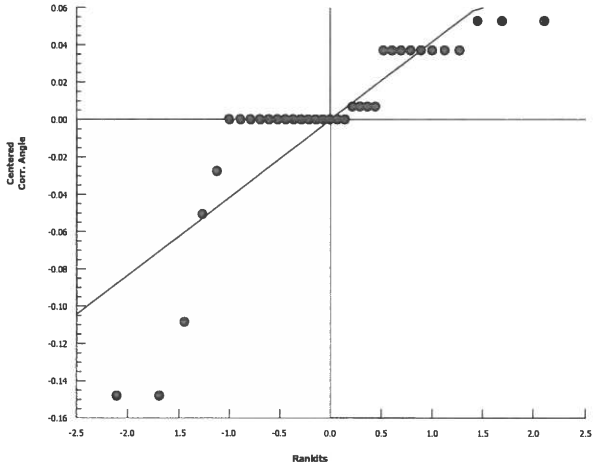
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
2		5	1.529	1.51	1.549	1.536	1.502	1.536	0.006895	1.01%	0.45%
4		5	1.483	1.39	1.577	1.536	1.375	1.536	0.03375	5.09%	3.45%
9		5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
18		5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
35		5	1.499	1.397	1.602	1.536	1.351	1.536	0.03701	5.52%	2.41%
75.1		5	1.499	1.397	1.602	1.536	1.351	1.536	0.03701	5.52%	2.41%

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

Analysis ID:	18-6051-8845	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-20 8:53	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: JU QA: BO 5/27/20 EG 6/1/20

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	03-0901-5666	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-20 8:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1052937	1000	Yes	Two-Point Interpolation

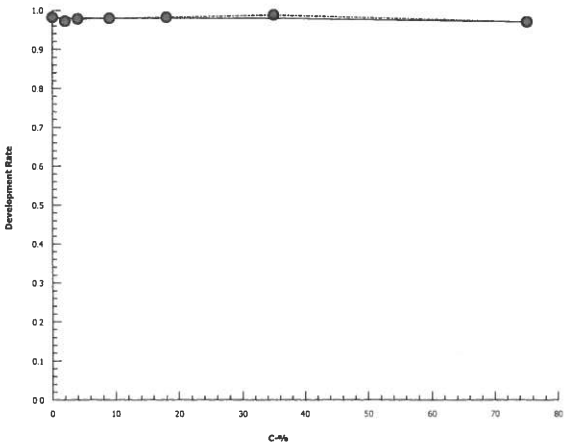
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.1	N/A	N/A	<1.332	NA	NA
EC50	>75.1	N/A	N/A	<1.332	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	0.9818	0.9722	0.9912	0.004181	0.009349	0.95%	0.0%	1087	1107
2		5	0.9723	0.9575	0.9954	0.006815	0.01524	1.57%	0.97%	1075	1106
4		5	0.9783	0.9707	0.9903	0.003486	0.007796	0.8%	0.35%	1070	1094
9		5	0.9797	0.9717	0.9871	0.002862	0.006399	0.65%	0.21%	1151	1175
18		5	0.9824	0.9703	0.9906	0.003526	0.007884	0.8%	-0.06%	1160	1181
35		5	0.9882	0.9801	1	0.003996	0.008934	0.9%	-0.65%	1088	1101
75.1		5	0.9706	0.9652	0.9815	0.002943	0.00658	0.68%	1.14%	1088	1121

Graphics



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

Analysis ID:	08-9188-7387	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 May-20 8:53	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	470460	1000	Yes	Two-Point Interpolation

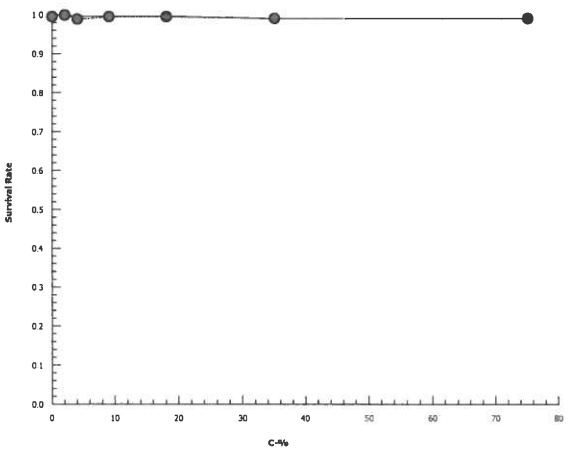
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>75.1	N/A	N/A	<1.332	NA	NA
EC50	>75.1	N/A	N/A	<1.332	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	1	1	1	0	0	0.0%	0.0%	1055	1055
2		5	0.9991	0.9953	1	0.000948	0.00212	0.21%	0.09%	1054	1055
4		5	0.9886	0.9621	1	0.007583	0.01696	1.72%	1.14%	1043	1055
9		5	1	1	1	0	0	0.0%	0.0%	1055	1055
18		5	1	1	1	0	0	0.0%	0.0%	1055	1055
35		5	0.9905	0.9526	1	0.009479	0.0212	2.14%	0.95%	1045	1055
75.1		5	0.9905	0.9526	1	0.009479	0.0212	2.14%	0.95%	1045	1055

Graphics



CETIS Test Data Worksheet

Report Date: 11 Apr-20 15:53 (p 1 of 1)
Test Code: 2004-5085 14-6973-7134/579A68AE

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

Start Date: 15 Apr-20 | **Species:** Mytilus galloprovincialis | **Sample Code:** 20- 0416
End Date: 17 Apr-20 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Jacobs
Sample Date: 14 Apr-20 | **Material:** Effluent Sample | **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			31			221	215	RT 5/6/20
			32			243	235	
			33			216	210	
			34			216	215	
			35			216	212	
			36			212	203	
			37			216	211	
			38			243	236	
			39			228	224	
			40			220	214	
			41			246	241	
			42			228	222	
			43			210	205	
			44			223	220	
			45			225	221	
			46			237	233	
			47			217	217	
			48			227	220	
			49			247	240	
			50			201	197	
			51			225	223	
			52			217	213	
			53			234	226	
			54			228	226	
			55			218	214	
			56			244	240	
			57			239	232	
			58			201	194	
			59			203	198	
			60			247	237	
			61			216	207	RT 5/26/20
			62			232	229	
			63			233	229	
			64			236	229	
			65			221	220	
			66			253	248	
			67			212	210	
			68			235	229	
			69			243	240	
			70			207	205	

Analyst: BO RT QA: BO 5/27/20 / EG 6/1/20

CETIS Test Data Worksheet

Report Date: 11 Apr-20 15:53 (p 1 of 1)
Test Code: 2004-5085 14-6973-7134/579A68AE

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

Start Date: 15 Apr-20 | **Species:** Mytilus galloprovincialis | **Sample Code:** 20-0416
End Date: 17 Apr-20 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Jacobs
Sample Date: 14 Apr-20 | **Material:** Effluent Sample | **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	33					
0	BC	2	51					
0	BC	3	40					
0	BC	4	54					
0	BC	5	55			222	218	ACS 4/19/2020
0	LC	1	66					
0	LC	2	61					
0	LC	3	37					
0	LC	4	32			249	244	ACS 4/19/2020
0	LC	5	44					
2		1	43					
2		2	34					
2		3	36			217	210	ACS 4/19/2020
2		4	31					
2		5	60					
4		1	42					
4		2	70			209	206	ACS 4/19/2020
4		3	52					
4		4	59					
4		5	57					
9		1	63			241	236	ACS 4/19/2020
9		2	49					
9		3	39					
9		4	68					
9		5	62					
18		1	64					
18		2	67					
18		3	56					
18		4	69					
18		5	41			245	241	ACS 4/19/2020
35		1	46					
35		2	45					
35		3	50			198	195	ACS 4/19/2020
35		4	65					
35		5	47					
73.3		1	53					
73.3		2	38					
73.3		3	35					
73.3		4	58			202	198	ACS 4/19/2020
73.3		5	48					

75.1

@19 ACS 4/19/20

QC = BO

Analyst: RT QA: BO 5/20/20 EG 6/1/20

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Jacobs

Sample ID: Wyckoff GWTP Effluent

Sample Log No.: 20-0416

Test No.: 2004-5085

Test Species: *M. galloprovincialis*

Start Date/Time: 4/15/2020 1325

End Date/Time: 4/17/2020 1525

Concentration (% sample)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	30.3	30.3	30.1	15.2	15.1	15.2	8.9	8.6	8.6	8.06	8.02	8.05
Brine Control	30.6	30.5	30.5	15.6	14.9	14.9	8.4	8.6	8.6	8.33	8.10	8.06
2	30.4	30.4	30.5	15.4	14.8	14.8	8.8	8.6	8.6	8.10	8.04	8.07
4	30.5	30.4	30.4	15.4	15.1	15.0	8.8	8.6	8.6	8.09	8.05	8.10
9	30.5	30.4	30.5	15.1	14.9	14.9	8.9	8.6	8.6	8.02	8.05	8.14
18	30.5	30.4	30.4	15.3	14.9	14.9	8.8	8.6	8.5	7.94	8.07	8.19
35	30.5	30.5	30.7	15.6	14.9	15.0	8.7	8.6	8.6	7.81	8.12	8.20
75.1	30.2	30.0	30.0	15.8	15.0	15.0	8.2	8.5	8.5	7.68	8.17	8.32

	0	24	48
WQ Readings:	BO	RT	KL
Dilutions made by:	BO		

Technician Initials:

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs:

QC Check: BO 5/27/20

Final Review: EG 6/1/20

Marine Chronic Bioassay
DC-010

Brine Dilution Worksheet

Project: JACOBS **Analyst:** BO

Sample ID: Wyckoff GWTP Effluent **Test Date:** 4/15/2020

Test No: 2004-S085 **Test Type:** Mussel Development

Salinity of Effluent 7.1

Salinity of Brine 99.2 **Date of Brine used:** 1/14/2020

Target Salinity 30 **Alkalinity of Brine Control:** 98 mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**0.33**	**0.43**

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

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Control	NA	NA	NA	250
2	5.0	0.33	1.7	250
4	10.0	0.33	3.3	250
9	22.5	0.33	7.4	250
18	45.0	0.33	14.9	250
35	87.5	0.33	29.0	250
75.1	187.8	0.33	62.2	250
	DI Volume			
Brine Control	143.4	0.43	62.2	250

Total Brine Volume Required (ml): **180.6**

QC Check: BO 5/27/20 **Final Review:** EL 6/1/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Jacobs / Wyckoff GWTP Effluent
Test No.: 2004-S085
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: MREP 12B
Date Received: 3/3/20
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 4/15/2020 1325
End Date/Time: 4/17/2020 1525
Technician Initials: BO

Spawn Information

First Gamete Release Time: 0955

Sex	Number Spawning
Male	3
Female	2

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1, 2, 3	good motility, good density
Female 1	1	yellow color, round shape, okay density
Female 2	2	white color, round shape, low density
Female 3	—	—

Egg Fertilization Time: 1100

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	-
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:		Mean: 8.8
10	9	
8	9	
7	8	
10	10	
10	7	

Mean 8.8 X 50 = 440 embryos/ml

Initial Density: 440 = 1.47 (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 208	Total 208	% Dividing	Mean % Dividing
TØ A	~~174~~ @	~~175~~ @	100	99.5
TØ B	198	199	99.5	
TØ C	206	208	99.0	
TØ D	219	220	99.5	
TØ E	220	220	100	
TØ F	212	214	99.1	
X̄ =	211			

48-h QC: 204/208 = 98.1%

Comments: @@18BO 4/16/20

QC Check: AC 4/28/20

Final Review: BO 5/27/20 / EG 6/1/20

Appendix B
Sample Check-In Information

Enthalpy Analytical
4340 Vandever Avenue
San Diego, CA 92120

Client: JACOBS
Sample ID: Wyckoff (041420)
Test ID No(s).: 2604-5085

Sample Check-In Information
DC-005

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

Sample (A, B, C):	A			
Log-in No. (20-xxxx):	0416			
Sample Collection Date & Time:	4/14/20 0857			
Sample Receipt Date & Time:	4/15/20 0930			
Number of Containers & Container Type:	1, 1Lwbi			
Approx. Total Volume Received (L):	~1			
Check-in Temperature (°C)	2.4			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.9			
pH (units)	7.54			
Conductivity (µS/cm)	11880			
Salinity (ppt)	(A) ~~7.0~~ 7.1			
Alkalinity (mg/L) [2]	(B) 226 x2 = 452			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	<0.02			
Technician Initials	KL			

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

Filtration? Y (N)
Initials: A) B) C)
Pore Size: ___
Organisms or Debris

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

pH Adjustment? Y (N)

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

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

Test Performed: Chronic Mussel Development — Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: ___
Alkalinity: 105 Hardness or Salinity: 30ppt
Additional Control? (Y) N = Brine Alkalinity: 98 Hardness or Salinity: 30ppt

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

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

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

Additional Comments: (A) @18KL 4/15/20 (B) performed a 1:2 dilution due to not enough sample volume

QC Check: BO 4/18/20
Final Review: EG 6/1/20

Total Ammonia Analysis
Freshwater

Overlying Water

DC-001

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 4/15/2020

Analyst: DM
Analysis Date: 4/16/20

Sample ID	Nautilus 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	7.6	9.3
Wyckoff	20-0416	4/15/2020	Check In	1.1	1.3
Spike Check (10 mg/L NH_3)		NA	NA	7.6	9.3
Batch QA Sample	20-0421	4/3/20	check-in	0.5	0.6
Sample Duplicate[a]	20-0421	NA	NA	0.4	<0.5
Sample Duplicate + Spike[a]		NA	NA	8.1	9.9
Spike Check (10 mg/L NH_3)		NA	NA	7.6	9.3

Relative Percent Difference (RPD) = $\frac{\text{[sample] (mg/L) - [sample duplicate] (mg/L)}}{\text{[average ammonia] (mg/L)}} \times 100$ **Acceptable Range: 0-20%**

Percent Recovery = $\frac{\text{[spiked sample] (mg/L) - [sample] (mg/L)}}{\text{nominal [spike] (mg/L)}} \times 100$ **Acceptable Range: 80-120%[b]**

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	9.3	10	NA	93
Batch QA Sample	0.6	<0.5	9.9	10	C	93

Comments:

Notes: [a]Unless otherwise noted, the last sample listed on the datasheet is used for duplicate and duplicate + spike QC check.

[b] Acceptable range for % recovery applies only to the blank spike. Spike recoveries in samples may vary based on sample matrix and are for information only.

[c] Calculation not performed due to one or both values below the method detection limit.

Method Detection Limit = 0.5 mg/L

QC Check: BO 4/18/20

Final Review: EH 6/1/20

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 4/14/2020
CarrierName: FedEx
AirbillNo: 7702 3407 2763

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

No: 10-041420-091921-0456
2020T10P000DD210W2LA00
Contact Name: Keith Allers
Contact Phone: 206-780-1711

Sample Identifier	CLP Sample No.	Matrix/Sampler	Coll. Method	Analysis/Turnaround (Days)	Tag/Preservative/Bottles	Location	Collection Date/Time	Sample Type
041420		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	04/14/2020 08:57	Field Sample

Special Instructions:

Shipment for Case Complete? N

Samples Transferred From Chain of Custody #

Analysis Key: CHRTOX=Chronic Toxicity

Items/Reason	Relinquished by (Signature and Organization)	Date/Time	Received by (Signature and Organization)	Date/Time	Sample Condition Upon Receipt
	JACOBS	4-14-2020 0928	Nautilus	4/15/20 0930	

receipt temp: 2.4°C
NautilusID: 20-0416

Appendix D
List of Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

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

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

Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L

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

Q5 - Test initiated with aeration due to an anticipated drop in D.O.

Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred

Q7 - Salinity out of recommended range

Q8 - Spilled test chamber/ Unable to recover test organism(s)

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

Q11 - Sample out of holding time; refer to QA section of report

Q12 - Replicate(s) not initiated; excluded from data analysis

Q13 - Survival counts not recorded due to poor visibility or heavy debris

Q14 - D.O. percent saturation was checked and was ≤ 110%

Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.

Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.

Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

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

Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Appendix E
Reference Toxicant Test Results

CETIS Summary Report

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

Batch ID:	19-3832-8294	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	15 Apr-20 13:25	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	17 Apr-20 15:25	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	50h	**Source:**	M-Rep, Carlsbad, CA	**Age:**	

Sample ID:	15-8891-9863	**Code:**	200415msdv	**Client:**	Internal
Sample Date:	15 Apr-20	**Material:**	Copper chloride	**Project:**	
Receive Date:	15 Apr-20	**Source:**	Reference Toxicant		
Sample Age:	13h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
01-8577-8962	Combined Development Ra	5	10	7.071	3.45%		Dunnett Multiple Comparison Test
08-5737-8335	Development Rate	5	10	7.071	3.45%		Dunnett Multiple Comparison Test
00-1254-4681	Survival Rate	20	40	28.28	NA		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
11-3098-9850	Combined Development Ra	EC25	8.134	7.523	8.961		Linear Interpolation (ICPIN)
		EC50	11.68	10.39	12.83		
19-2371-7781	Development Rate	EC25	8.134	7.514	8.851		Linear Interpolation (ICPIN)
		EC50	11.68	10.35	12.7		
00-5465-8677	Survival Rate	EC25	25	25	25		Linear Interpolation (ICPIN)
		EC50	30	30	30		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-5737-8335	Development Rate	Control Resp	0.967	0.9 - NL	Yes	Passes Acceptability Criteria
19-2371-7781	Development Rate	Control Resp	0.967	0.9 - NL	Yes	Passes Acceptability Criteria
00-1254-4681	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
00-5465-8677	Survival Rate	Control Resp	1	0.5 - NL	Yes	Passes Acceptability Criteria
01-8577-8962	Combined Development Ra	PMSD	0.03455	NL - 0.25	No	Passes Acceptability Criteria

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

Combined Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.967	0.9551	0.9788	0.952	0.9779	0.004257	0.009519	0.98%	0.0%
2.5		5	0.9556	0.9166	0.9945	0.9031	0.9817	0.01403	0.03136	3.28%	1.18%
5		5	0.9796	0.9671	0.9921	0.9679	0.9917	0.004493	0.01005	1.03%	-1.31%
10		5	0.5804	0.4889	0.6719	0.4851	0.6736	0.03296	0.0737	12.7%	39.98%
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	0.967	0.9551	0.9788	0.952	0.9779	0.004257	0.009519	0.98%	0.0%
2.5		5	0.9556	0.9166	0.9945	0.9031	0.9817	0.01403	0.03136	3.28%	1.18%
5		5	0.9796	0.9671	0.9921	0.9679	0.9917	0.004493	0.01005	1.03%	-1.31%
10		5	0.5804	0.4889	0.6719	0.4851	0.6736	0.03296	0.0737	12.7%	39.98%
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	1	1	1	1	1	0	0	0.0%	0.0%
2.5		5	1	1	1	1	1	0	0	0.0%	0.0%
5		5	1	1	1	1	1	0	0	0.0%	0.0%
10		5	1	1	1	1	1	0	0	0.0%	0.0%
20		5	1	1	1	1	1	0	0	0.0%	0.0%
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	0.9683	0.9779	0.971	0.9657	0.952
2.5		0.9031	0.9524	0.9655	0.9817	0.9752
5		0.9917	0.9679	0.9863	0.9809	0.9711
10		0.4851	0.5625	0.6318	0.5489	0.6736
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	0.9683	0.9779	0.971	0.9657	0.952
2.5		0.9031	0.9524	0.9655	0.9817	0.9752
5		0.9917	0.9679	0.9863	0.9809	0.9711
10		0.4851	0.5625	0.6318	0.5489	0.6736
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	1	1	1	1	1
2.5		1	1	1	1	1
5		1	1	1	1	1
10		1	1	1	1	1
20		1	1	1	1	1
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	214/221	221/226	234/241	225/233	238/250
2.5		205/227	220/231	252/261	215/219	236/242
5		240/242	211/218	216/219	257/262	235/242
10		114/235	135/240	163/258	129/235	161/239
20		0/217	0/227	0/221	0/238	0/228
40		0/211	0/211	0/211	0/211	0/211

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	214/221	221/226	234/241	225/233	238/250
2.5		205/227	220/231	252/261	215/219	236/242
5		240/242	211/218	216/219	257/262	235/242
10		114/235	135/240	163/258	129/235	161/239
20		0/217	0/227	0/221	0/238	0/228
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	211/211	211/211	211/211	211/211	211/211
2.5		211/211	211/211	211/211	211/211	211/211
5		211/211	211/211	211/211	211/211	211/211
10		211/211	211/211	211/211	211/211	211/211
20		211/211	211/211	211/211	211/211	211/211
40		0/211	0/211	0/211	0/211	0/211

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

Analysis ID: 01-8577-8962 **Endpoint:** Combined Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 01 May-20 10:05 **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	3.45%	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	0.619	2.227	0.079	8	0.4921	CDF	Non-Significant Effect
		5	-1.172	2.227	0.079	8	0.9746	CDF	Non-Significant Effect
		10*	14.66	2.227	0.079	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.060375	0.3534582	3	111.3	<0.0001	Significant Effect
Error	0.05079353	0.003174596	16			
Total	1.111168		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.888	11.34	0.1801	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.975	0.866	0.8548	Normal Distribution

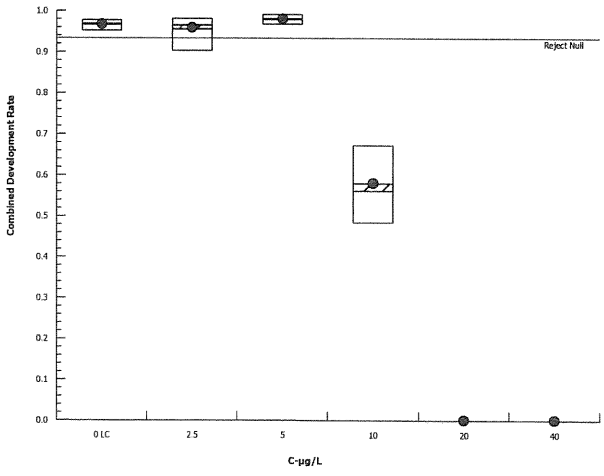
Combined Development Rate Summary

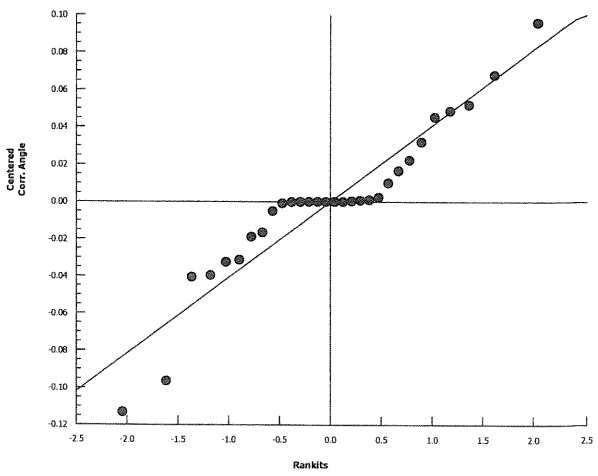
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.967	0.9551	0.9788	0.9683	0.952	0.9779	0.004257	0.98%	0.0%
2.5		5	0.9556	0.9166	0.9945	0.9655	0.9031	0.9817	0.01403	3.28%	1.18%
5		5	0.9796	0.9671	0.9921	0.9809	0.9679	0.9917	0.004493	1.03%	-1.31%
10		5	0.5804	0.4889	0.6719	0.5625	0.4851	0.6736	0.03296	12.7%	39.98%
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	1.389	1.357	1.422	1.392	1.35	1.422	0.01167	1.88%	0.0%
2.5		5	1.367	1.28	1.455	1.384	1.254	1.435	0.03163	5.17%	1.59%
5		5	1.431	1.385	1.477	1.432	1.391	1.48	0.01656	2.59%	-3.01%
10		5	0.8669	0.7736	0.9602	0.8481	0.7705	0.9627	0.0336	8.67%	37.61%
20		5	0.03326	0.03254	0.03399	0.03319	0.03242	0.03395	0.000260	1.75%	97.61%
40		5	0.03443	0.03442	0.03444	0.03443	0.03443	0.03443	0	0.0%	97.52%

Graphics





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

Analysis ID: 08-5737-8335 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 01 May-20 10:05 **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	3.45%	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	0.619	2.227	0.079	8	0.4921	CDF	Non-Significant Effect
		5	-1.172	2.227	0.079	8	0.9746	CDF	Non-Significant Effect
		10*	14.66	2.227	0.079	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.060375	0.3534582	3	111.3	<0.0001	Significant Effect
Error	0.05079353	0.003174596	16			
Total	1.111168		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.888	11.34	0.1801	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.975	0.866	0.8548	Normal Distribution

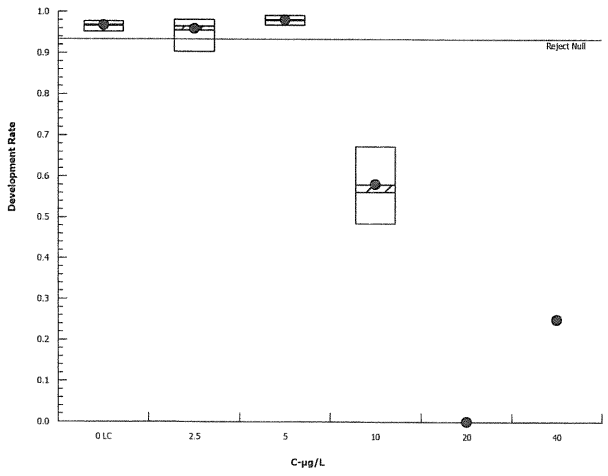
Development Rate Summary

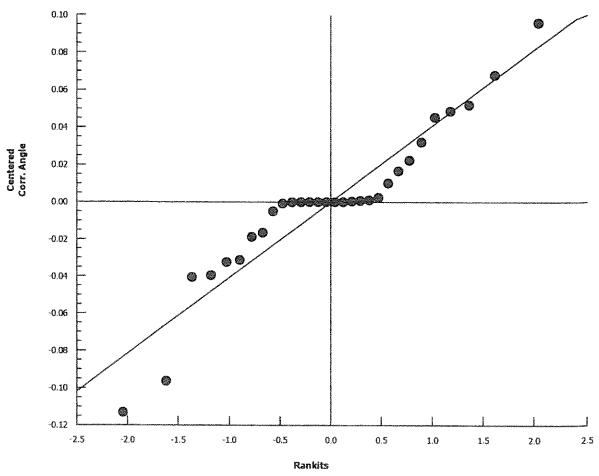
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.967	0.9551	0.9788	0.9683	0.952	0.9779	0.004257	0.98%	0.0%
2.5		5	0.9556	0.9166	0.9945	0.9655	0.9031	0.9817	0.01403	3.28%	1.18%
5		5	0.9796	0.9671	0.9921	0.9809	0.9679	0.9917	0.004493	1.03%	-1.31%
10		5	0.5804	0.4889	0.6719	0.5625	0.4851	0.6736	0.03296	12.7%	39.98%
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	1.389	1.357	1.422	1.392	1.35	1.422	0.01167	1.88%	0.0%
2.5		5	1.367	1.28	1.455	1.384	1.254	1.435	0.03163	5.17%	1.59%
5		5	1.431	1.385	1.477	1.432	1.391	1.48	0.01656	2.59%	-3.01%
10		5	0.8669	0.7736	0.9602	0.8481	0.7705	0.9627	0.0336	8.67%	37.61%
20		5	0.03326	0.03254	0.03399	0.03319	0.03242	0.03395	0.000260	1.75%	97.61%
40		5	0.5236	0.5234	0.5238	0.5236	0.5236	0.5236	0	0.0%	62.32%

Graphics





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

Analysis ID: 00-1254-4681 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 01 May-20 10:06 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

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

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	27.5	17	1	8	0.8000	Asymp	Non-Significant Effect
		5	27.5	17	1	8	0.8000	Asymp	Non-Significant Effect
		10	27.5	17	1	8	0.8000	Asymp	Non-Significant Effect
		20	27.5	17	1	8	0.8000	Asymp	Non-Significant Effect

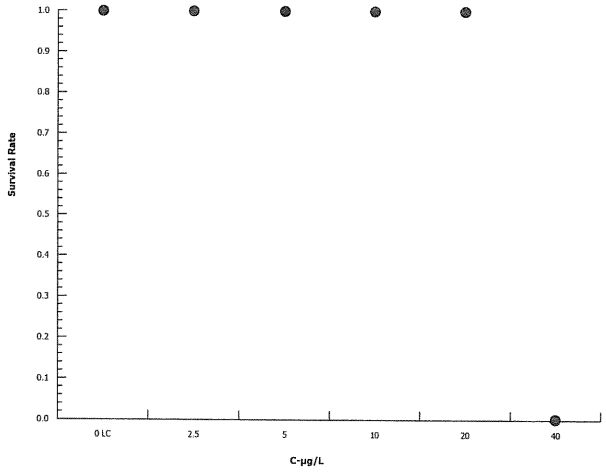
Survival Rate Summary

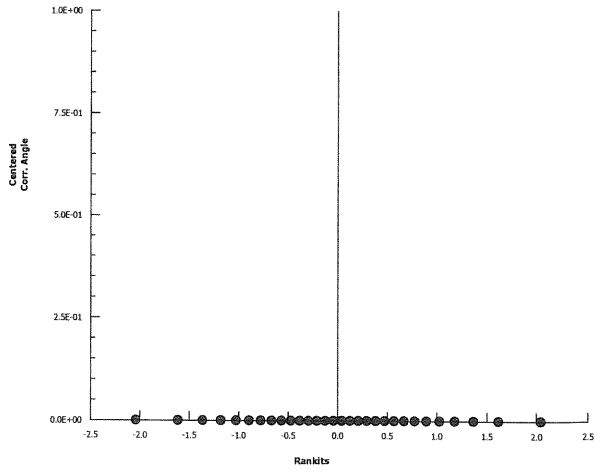
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1	1	1	1	1	1	0	0.0%	0.0%
2.5		5	1	1	1	1	1	1	0	0.0%	0.0%
5		5	1	1	1	1	1	1	0	0.0%	0.0%
10		5	1	1	1	1	1	1	0	0.0%	0.0%
20		5	1	1	1	1	1	1	0	0.0%	0.0%
40		5	0	0	0	0	0	0	0		100.0%

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	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
2.5		5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
5		5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
10		5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
20		5	1.536	1.536	1.537	1.536	1.536	1.536	0	0.0%	0.0%
40		5	0.03443	0.03442	0.03444	0.03443	0.03443	0.03443	0	0.0%	97.76%

Graphics





CETIS Analytical Report

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

Analysis ID:	11-3098-9850	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 May-20 10:06	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	423860	1000	Yes	Two-Point Interpolation

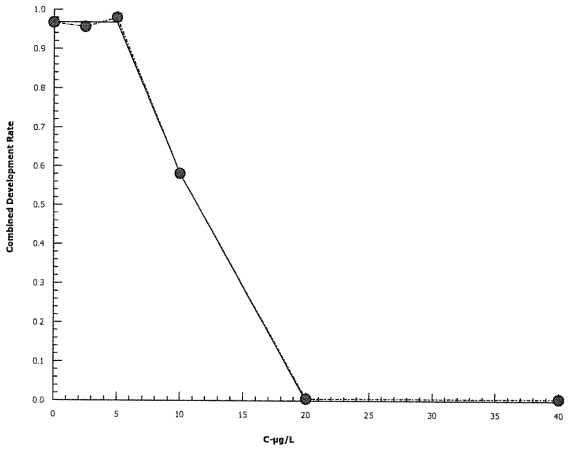
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	8.134	7.523	8.961
EC50	11.68	10.39	12.83

Combined Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.967	0.952	0.9779	0.004257	0.009519	0.98%	0.0%	1132	1171
2.5		5	0.9556	0.9031	0.9817	0.01403	0.03136	3.28%	1.18%	1128	1180
5		5	0.9796	0.9679	0.9917	0.004493	0.01005	1.03%	-1.31%	1159	1183
10		5	0.5804	0.4851	0.6736	0.03296	0.0737	12.7%	39.98%	702	1207
20		5	0	0	0	0	0		100.0%	0	1131
40		5	0	0	0	0	0		100.0%	0	1055

Graphics



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

Analysis ID:	19-2371-7781	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 May-20 10:06	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1236071	1000	Yes	Two-Point Interpolation

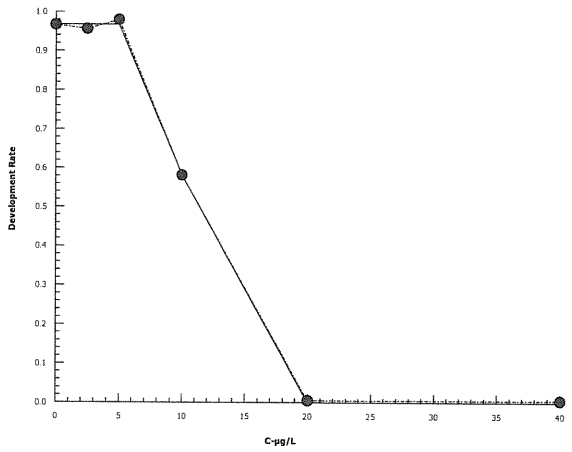
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	8.134	7.514	8.851
EC50	11.68	10.35	12.7

Development Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.967	0.952	0.9779	0.004257	0.009519	0.98%	0.0%	1132	1171
2.5		5	0.9556	0.9031	0.9817	0.01403	0.03136	3.28%	1.18%	1128	1180
5		5	0.9796	0.9679	0.9917	0.004493	0.01005	1.03%	-1.31%	1159	1183
10		5	0.5804	0.4851	0.6736	0.03296	0.0737	12.7%	39.98%	702	1207
20		5	0	0	0	0	0		100.0%	0	1131
40		5	0	0	0	0	0		100.0%	0	5

Graphics



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

Analysis ID:	00-5465-8677	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 May-20 10:06	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	394465	1000	Yes	Two-Point Interpolation

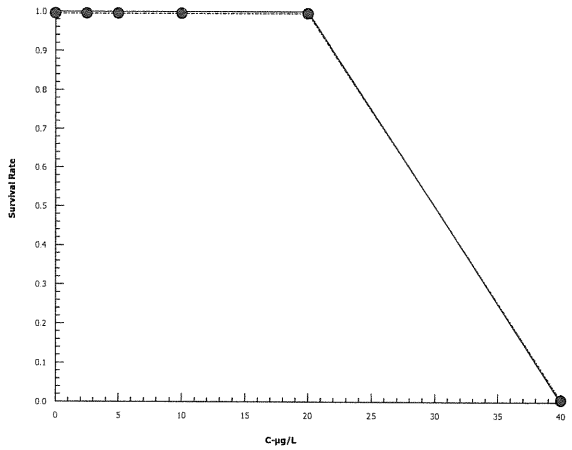
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	25	25	25
EC50	30	30	30

Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	1	1	1	0	0	0.0%	0.0%	1055	1055
2.5		5	1	1	1	0	0	0.0%	0.0%	1055	1055
5		5	1	1	1	0	0	0.0%	0.0%	1055	1055
10		5	1	1	1	0	0	0.0%	0.0%	1055	1055
20		5	1	1	1	0	0	0.0%	0.0%	1055	1055
40		5	0	0	0	0	0		100.0%	0	1055

Graphics

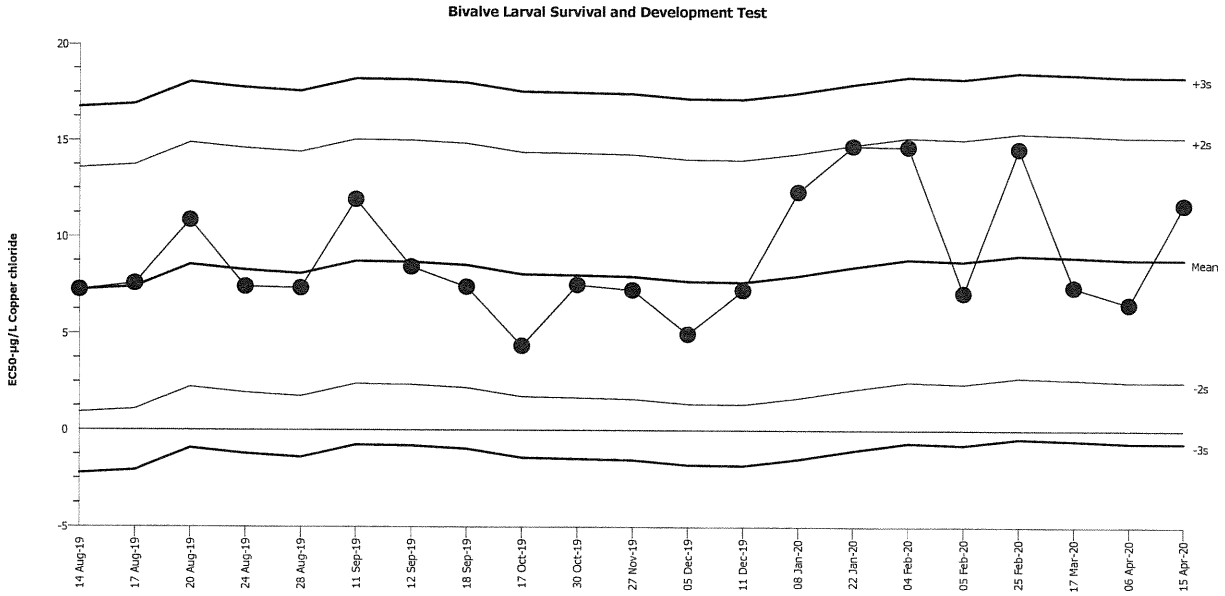


CETIS QC Plot

Report Date: 01 May-20 10:07 (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:	8.848	**Count:**	20	**-2s Warning Limit:**	2.52	**-3s Action Limit:**	-0.6437
Sigma:	3.164	**CV:**	35.80%	**+2s Warning Limit:**	15.18	**+3s Action Limit:**	18.34

Quality Control Data

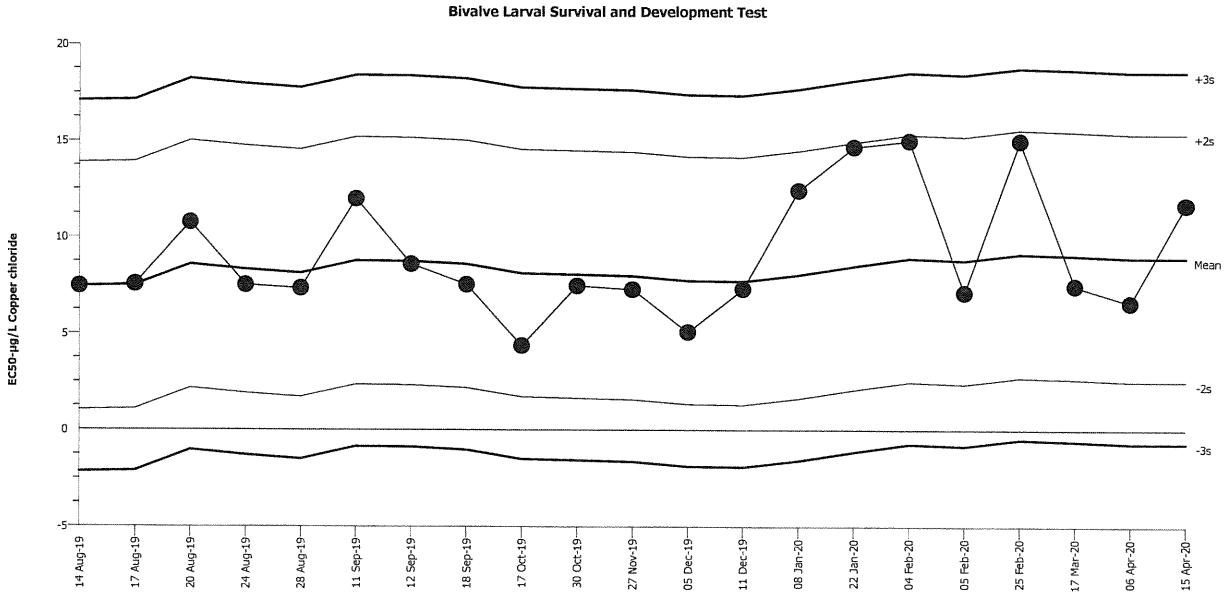
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	14	14:15	7.255	-1.593	-0.5034			18-5609-6564	17-5885-5207
2			17	14:00	7.582	-1.266	-0.4001			15-9584-4385	11-8998-1524
3			20	14:15	10.86	2.014	0.6364			14-8361-1578	03-1832-9380
4			24	16:00	7.414	-1.434	-0.4531			19-4374-5817	01-6546-9581
5			28	14:30	7.348	-1.5	-0.4741			01-0546-0046	21-3090-7111
6		Sep	11	14:30	11.93	3.087	0.9755			09-2717-2159	04-2480-9094
7			12	14:25	8.444	-0.4036	-0.1275			19-6218-6352	07-5188-6358
8			18	13:20	7.4	-1.448	-0.4576			10-9359-1611	21-3838-7021
9		Oct	17	12:30	4.368	-4.48	-1.416			01-8239-7270	07-0806-0577
10			30	12:30	7.518	-1.33	-0.4204			07-8198-2858	11-8079-0492
11		Nov	27	20:00	7.249	-1.599	-0.5055			12-9914-0499	16-0529-7707
12		Dec	5	13:15	4.982	-3.866	-1.222			04-7411-4445	13-6587-0425
13			11	13:35	7.245	-1.603	-0.5066			10-8800-1613	10-7929-5811
14	2020	Jan	8	13:40	12.34	3.492	1.104			07-8444-5322	01-1422-4896
15			22	13:25	14.72	5.872	1.856			02-1152-2212	07-1224-7163
16		Feb	4	16:30	14.68	5.828	1.842			19-9078-6483	21-0369-4045
17			5	13:10	7.103	-1.745	-0.5515			06-6849-2235	04-8167-3886
18			25	14:15	14.58	5.733	1.812			09-2101-6353	02-3593-4650
19		Mar	17	14:20	7.408	-1.44	-0.4552			14-6169-3689	18-9939-7640
20		Apr	6	17:15	6.537	-2.311	-0.7304			02-0082-4673	13-2096-3831
21			15	13:25	11.68	2.835	0.896			16-4614-0901	11-3098-9850

CETIS QC Plot

Report Date: 01 May-20 10:08 (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:	8.935	**Count:**	20	**-2s Warning Limit:**	2.513	**-3s Action Limit:**	-0.6975
Sigma:	3.211	**CV:**	35.90%	**+2s Warning Limit:**	15.36	**+3s Action Limit:**	18.57

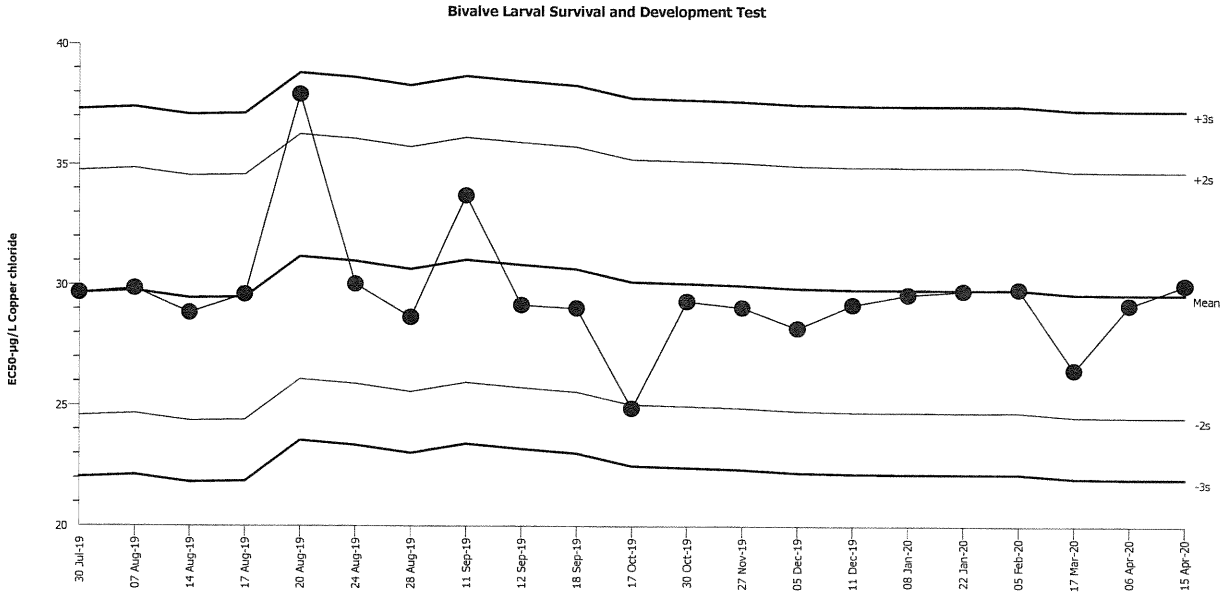
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Aug	14	14:15	7.466	-1.469	-0.4575			18-5609-6564	14-6389-5644
2			17	14:00	7.563	-1.372	-0.4272			15-9584-4385	19-0402-2449
3			20	14:15	10.76	1.829	0.5695			14-8361-1578	12-0750-5104
4			24	16:00	7.521	-1.414	-0.4404			19-4374-5817	04-6745-5945
5			28	14:30	7.351	-1.584	-0.4934			01-0546-0046	10-3410-8075
6		Sep	11	14:30	11.98	3.048	0.9491			09-2717-2159	17-4622-9429
7			12	14:25	8.608	-0.3274	-0.102			19-6218-6352	06-5225-4823
8			18	13:20	7.546	-1.389	-0.4327			10-9359-1611	16-7089-5314
9		Oct	17	12:30	4.375	-4.56	-1.42			01-8239-7270	19-1864-9270
10			30	12:30	7.481	-1.454	-0.4528			07-8198-2858	15-7183-3565
11		Nov	27	20:00	7.297	-1.638	-0.5101			12-9914-0499	01-7534-7240
12		Dec	5	13:15	5.087	-3.848	-1.198			04-7411-4445	10-0471-4567
13			11	13:35	7.32	-1.615	-0.5029			10-8800-1613	20-9346-8800
14	2020	Jan	8	13:40	12.43	3.494	1.088			07-8444-5322	06-2499-4329
15			22	13:25	14.68	5.746	1.79			02-1152-2212	04-4145-0874
16		Feb	4	16:30	15.01	6.073	1.891			19-9078-6483	06-3219-7963
17			5	13:10	7.132	-1.803	-0.5615			06-6849-2235	20-3119-3253
18			25	14:15	15	6.065	1.889			09-2101-6353	13-1093-9538
19		Mar	17	14:20	7.489	-1.446	-0.4504			14-6169-3689	12-6636-5212
20		Apr	6	17:15	6.609	-2.326	-0.7243			02-0082-4673	11-5300-1558
21			15	13:25	11.68	2.748	0.8558			16-4614-0901	19-2371-7781

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:	29.6	**Count:**	20	**-2s Warning Limit:**	24.51	**-3s Action Limit:**	21.97
Sigma:	2.545	**CV:**	8.60%	**+2s Warning Limit:**	34.69	**+3s Action Limit:**	37.24

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jul	30	15:30	29.67	0.07303	0.0287			15-3542-8276	20-8453-4017
2		Aug	7	15:30	29.85	0.2549	0.1002			01-2834-9487	07-4855-2818
3			14	14:15	28.85	-0.7545	-0.2965			18-5609-6564	13-1367-1354
4			17	14:00	29.6	0.00366	0.001438			15-9584-4385	20-0172-5237
5			20	14:15	37.92	8.319	3.269	(+)	(+)	14-8361-1578	02-5800-6574
6			24	16:00	30.04	0.4374	0.1719			19-4374-5817	17-7461-0750
7			28	14:30	28.66	-0.9354	-0.3676			01-0546-0046	13-4512-6481
8		Sep	11	14:30	33.71	4.115	1.617			09-2717-2159	01-1883-2964
9			12	14:25	29.16	-0.4359	-0.1713			19-6218-6352	02-6393-7831
10			18	13:20	29.04	-0.561	-0.2204			10-9359-1611	04-3365-2341
11		Oct	17	12:30	24.88	-4.718	-1.854			01-8239-7270	13-2801-3685
12			30	12:30	29.32	-0.2771	-0.1089			07-8198-2858	20-5233-5110
13		Nov	27	20:00	29.07	-0.5333	-0.2096			12-9914-0499	00-1104-7300
14		Dec	5	13:15	28.21	-1.386	-0.5445			04-7411-4445	20-5035-4724
15			11	13:35	29.18	-0.4207	-0.1653			10-8800-1613	02-9848-3585
16	2020	Jan	8	13:40	29.6	0.00106	0.000417			07-8444-5322	01-5655-1706
17			22	13:25	29.76	0.1556	0.06114			02-1152-2212	19-4150-8988
18		Feb	5	13:10	29.83	0.2263	0.08893			06-6849-2235	07-0404-6516
19		Mar	17	14:20	26.48	-3.118	-1.225			14-6169-3689	14-2151-4803
20		Apr	6	17:15	29.18	-0.4232	-0.1663			02-0082-4673	12-2147-8498
21			15	13:25	30	0.4	0.1572			16-4614-0901	00-5465-8677

CETIS Test Data Worksheet

Report Date: 11 Apr-20 15:53 (p 1 of 1)
Test Code: 16-4614-0901/200415msdv

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

Start Date: 15 Apr-20
End Date: 17 Apr-20
Sample Date: 15 Apr-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 200415msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			231	220	DM 4/20/20
			2			221	214	
			3			241	234	
			4			242	240	
			5			218	211	
			6			217	0	
			7			262	257	
			8			238	0	
			9			242	236 (A)	
			10			235	114	
			11			0	0	cells lysed
			12			227	0	
			13			258	163	
			14			242	235	
			15			240	135	DM 4/27/20
			16			239	161	
			17			219	215	
			18			261	252	
			19			0	0	cells lysed
			20			0	0	cells lysed
			21		221	~~0~~ (B) (B) ~~221~~	0	
			22			219	216	
			23			235	129	
			24			0	0	cells lysed
			25			226	221	
			26			233	225	
			27			227	205	
			28			0	0	cells lysed
			29			250	238	
			30			228	0	

(A) Q18 DM 4/22/20
(B) Q18 AC OBO DM 4/28/20

CETIS Test Data Worksheet

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

Start Date: 15 Apr-20
End Date: 17 Apr-20
Sample Date: 15 Apr-20
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 200415msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	2			221	217	AES 4/19/2020
0	LC	2	25					
0	LC	3	3					
0	LC	4	26					
0	LC	5	29					
2.5		1	27					
2.5		2	1			231	225	AES 4/19/2020
2.5		3	18					
2.5		4	17					
2.5		5	9					
5		1	4					
5		2	5					
5		3	22			227	223	AES 4/19/2020
5		4	7					
5		5	14					
10		1	10					
10		2	15					
10		3	13					
10		4	23			~~233~~ 238	~~175~~ 132 (A)	AES 4/19/2020
10		5	16					
20		1	6					
20		2	12					
20		3	21					
20		4	8			254	0	AES 4/19/2020
20		5	30					
40		1	19					
40		2	28					
40		3	24					
40		4	20					
40		5	11			57	0	AES 4/19/2020 cells dissolved into lysis

QC = BO

(A) Gia AES 4/19/2020

Marine Chronic Bioassay

DM-014

Water Quality Measurements

Client: Internal

Sample ID: $CuCl_2$

Test No.: 200415msdv

Test Species: *M. galloprovincialis*

Start Date/Time: 4/15/2020 1325

End Date/Time: 4/17/2020 1525

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	31.3	31.2	31.4	15.4	15.3	~~15.0~~ 15.0 (A)	8.6	8.6	8.6	8.01	8.00	8.03
2.5	31.5	31.5	31.5	15.3	15.0	15.0	8.8	8.7	8.6	8.03	8.01	8.05
5	31.5	31.5	31.5	15.4	15.1	15.0	8.8	8.6	8.6	8.03	8.02	8.05
10	31.6	31.5	31.5	15.6	15.4	15.2	8.6	8.5	8.5	8.06	8.02	8.05
20	31.5	31.4	31.4	15.8	15.3	15.1	8.6	8.5	8.6	8.07	8.02	8.06
40	31.4	31.3	31.3	15.8	15.2	15.1	8.6	8.5	8.6	8.08	8.03	8.06

	0	24	48
WQ Readings:	BO	RT	KL
Dilutions made by:	BO		

High conc. made (µg/L):	40
Vol. Cu stock added (mL):	1.9
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,400

Technician Initials:

Environmental Chamber: D.

Comments:

0 hrs:

24 hrs:

48 hrs: (A) QIBKL 4/17/20

QC Check: AC 4/28/20

Final Review: YS 5/19/20

Marine Chronic Bioassay
DM-013

Larval Development Worksheet

Client/Sample: Internal / CuCl2
Test No.: 200415msdv
Test Species: *Mytilus galloprovincialis*
Animal Source/Batch Tank: MREP / ZB
Date Received: 3/3/20
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 4/15/2020 1325
End Date/Time: 4/17/2020 1525
Technician Initials: BO

Spawn Information

First Gamete Release Time: 0955

Sex	Number Spawning
Male	3
Female	2

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1, 2, 3	good motility, good density
Female 1	1	yellow color, round shape, okay density
Female 2	2	white color, round shape, low density
Female 3		

Egg Fertilization Time: 1100

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	-
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:		
	10	9
	8	9
	7	8
	10	10
	10	7

Mean: 8.8

Mean 8.8 X 50 = 440 embryos/ml

Initial Density: 440 = 1.47 (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 208	Total 208	% Dividing	Mean % Dividing
TØ A	~~174~~ @	~~175~~ @	100	99.5
TØ B	198	199	99.5	
TØ C	206	208	99.0	
TØ D	219	220	99.5	
TØ E	220	220	100	
TØ F	212	214	99.1	
X̄ =	211			

48-h QC: 204/208 = 98.1%

Comments: @@18BO 4/16/20

QC Check: AC 4/28/20

Final Review: YS 5/19/20