ENTHALPY ANALYTICAL
TOXICITY LABORATORY & CONSULTING

Chronic Toxicity Testing Results for Wyckoff Eagle Harbor Groundwater Treatment Plant

Monitoring Period: July 2019

Prepared for: **Jacobs**
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Date Submitted: **August 9, 2019**

Data Quality Assurance:

- Nautilus Environmental 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.

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Results verified by: _____
Eric Green, Project Manager

Introduction

A toxicity test was performed using a groundwater composite sample collected on July 9, 2019 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 (formerly Nautilus Environmental) located in San Diego, California between July 10 and 12, 2019.

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	**070919**
Enthalpy Log-in Number	19-0742
Collection Date; Time	7/9/2019; 1000h
Receipt Date; Time	7/10/2019; 0945h
Receipt Temperature (°C)	3.8
Dissolved Oxygen (mg/L)	7.6
pH	7.66
Conductivity (µS/cm)	NM
Salinity (ppt)	7.0
Alkalinity (mg/L $CaCO_3$)	395
Total Chlorine (mg/L)	0.02
Total Ammonia (mg/L as N)	1.3

NM = not measured

Test Methods

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

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

Test Period	7/10/2019, 1515h to 7/12/2019, 1535h
Test Organism	*Mytilus galloprovincialis*
Test Organism Source	Mission Bay (San Diego, 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 90.3 ppt
Test Concentrations (% sample)	73.5[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 73.5 (the highest concentration tested) and a chronic toxic unit (TU_c) of less than 1.36 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	73.5	> 73.5	< 1.36	> 73.5
	Survival	73.5	> 73.5	< 1.36	> 73.5

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)	98.6	96.3
0 (Lab Control)	99.1	96.7
2	97.0	95.0
4	96.6	95.6
9	100	96.3
18	98.2	96.9
35	97.8	96.0
73.5 [a]	97.5	95.3

[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.

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 D. 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 E.

Table 5. Reference Toxicant Test Results

Species	Endpoint	EC_{50} (µg/L copper)	Historical mean ± 2 SD (µg/L copper)	CV (%)
Bivalve	Normal Development	7.80	7.19 ± 1.70	11.9
	Survival Rate	29.7	28.3 ± 5.20	9.19

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

Bivalve Larval Development Test

CETIS Summary Report

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

Batch ID:	19-3820-1131	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	10 Jul-19 15:15	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	12 Jul-19 15:35	**Species:**	Mytilus galloprovincialis	**Brine:**	Frozen Seawater
Duration:	48h	**Source:**	Mission Bay	**Age:**	

Sample ID:	16-9245-2664	**Code:**	19-0742	**Client:**	Jacobs
Sample Date:	09 Jul-19 10:00	**Material:**	Effluent Sample	**Project:**	
Receive Date:	10 Jul-19 09:45	**Source:**	Jacobs		
Sample Age:	29h (3.8 °C)	**Station:**	Wyckoff		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-9057-3710	Development Rate	73.5	>73.5	NA	1.73%	1.361	Dunnett Multiple Comparison Test
04-3000-7149	Survival Rate	73.5	>73.5	NA	4.69%	1.361	Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
11-3898-7688	Development Rate	EC25	>73.5	N/A	N/A	<1.361	Linear Interpolation (ICPIN)
		EC50	>73.5	N/A	N/A	<1.361	
10-1515-3673	Survival Rate	EC25	>73.5	N/A	N/A	<1.361	Linear Interpolation (ICPIN)
		EC50	>73.5	N/A	N/A	<1.361	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-9057-3710	Development Rate	Control Resp	0.9626	0.9 - NL	Yes	Passes Acceptability Criteria
11-3898-7688	Development Rate	Control Resp	0.9626	0.9 - NL	Yes	Passes Acceptability Criteria
04-3000-7149	Survival Rate	Control Resp	0.9862	0.5 - NL	Yes	Passes Acceptability Criteria
10-1515-3673	Survival Rate	Control Resp	0.9862	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	0.9626	0.9494	0.9757	0.948	0.9779	0.004749	0.01062	1.1%	0.0%
0	Lab Control	5	0.9672	0.9486	0.9857	0.9504	0.991	0.006692	0.01496	1.55%	-0.48%
2		5	0.9497	0.9279	0.9714	0.9245	0.9738	0.007829	0.01751	1.84%	1.34%
4		5	0.9556	0.9355	0.9758	0.9322	0.9744	0.007254	0.01622	1.7%	0.72%
9		5	0.9632	0.9552	0.9712	0.9542	0.9714	0.002885	0.006451	0.67%	-0.07%
18		5	0.9694	0.9608	0.9781	0.9628	0.9807	0.003107	0.006948	0.72%	-0.72%
35		5	0.9602	0.9513	0.9692	0.9486	0.966	0.003233	0.007228	0.75%	0.24%
73.5		5	0.9528	0.9478	0.9577	0.9494	0.9595	0.001777	0.003974	0.42%	1.02%

Survival Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.9862	0.9616	1	0.9569	1	0.008876	0.01985	2.01%	0.0%
0	Lab Control	5	0.9905	0.9642	1	0.9526	1	0.009483	0.0212	2.14%	-0.44%
2		5	0.9698	0.9272	1	0.9138	1	0.01536	0.03435	3.54%	1.66%
4		5	0.9664	0.9179	1	0.9052	1	0.01748	0.03908	4.04%	2.01%
9		5	1	1	1	1	1	0	0	0.0%	-1.4%
18		5	0.9819	0.9425	1	0.9267	1	0.01419	0.03173	3.23%	0.44%
35		5	0.9776	0.935	1	0.9224	1	0.01532	0.03427	3.51%	0.87%
73.5		5	0.975	0.9292	1	0.9181	1	0.01649	0.03688	3.78%	1.14%

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	0.9615	0.9614	0.9779	0.964	0.948
0	Lab Control	0.9679	0.9662	0.9603	0.9504	0.991
2		0.9738	0.9507	0.952	0.9474	0.9245
4		0.9744	0.9322	0.964	0.96	0.9476
9		0.9615	0.9542	0.9668	0.962	0.9714
18		0.9628	0.9685	0.9703	0.9807	0.9649
35		0.9645	0.966	0.9643	0.9578	0.9486
73.5		0.9494	0.9506	0.9513	0.9595	0.9531

Survival Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	1	1	0.9741	0.9569	1
0	Lab Control	1	1	1	1	0.9526
2		0.9871	0.9612	0.9871	1	0.9138
4		1	1	0.9569	0.9698	0.9052
9		1	1	1	1	1
18		0.9267	1	1	1	0.9828
35		1	1	0.9655	1	0.9224
73.5		1	1	1	0.9569	0.9181

Development Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	225/234	224/233	221/226	214/222	237/250
0	Lab Control	241/249	229/237	242/252	249/262	219/221
2		223/229	212/223	218/229	252/266	196/212
4		228/234	220/236	214/222	216/225	199/210
9		225/234	229/240	233/241	228/237	238/245
18		207/215	246/254	229/236	254/259	220/228
35		272/282	227/235	216/224	227/237	203/214
73.5		225/237	231/243	254/267	213/222	203/213

Survival Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	232/232	232/232	226/232	222/232	232/232
0	Lab Control	232/232	232/232	232/232	232/232	221/232
2		229/232	223/232	229/232	232/232	212/232
4		232/232	232/232	222/232	225/232	210/232
9		232/232	232/232	232/232	232/232	232/232
18		215/232	232/232	232/232	232/232	228/232
35		232/232	232/232	224/232	232/232	214/232
73.5		232/232	232/232	232/232	222/232	213/232

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

Analysis ID: 00-9057-3710 **Endpoint:** Development Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Aug-19 8:19 **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	1.73%	73.5	>73.5	NA	1.361

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	1.755	2.407	0.042	8	0.1665	CDF	Non-Significant Effect
		4	0.9479	2.407	0.042	8	0.4763	CDF	Non-Significant Effect
		9	-0.03727	2.407	0.042	8	0.8670	CDF	Non-Significant Effect
		18	-1.066	2.407	0.042	8	0.9899	CDF	Non-Significant Effect
		35	0.4068	2.407	0.042	8	0.7190	CDF	Non-Significant Effect
		73.5	1.494	2.407	0.042	8	0.2478	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00861149	0.001435248	6	1.921	0.1123	Non-Significant Effect
Error	0.02091907	0.0007471096	28			
Total	0.02953056		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.911	16.81	0.1284	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9578	0.9146	0.1960	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	0.9626	0.9494	0.9757	0.9615	0.948	0.9779	0.004749	1.1%	0.0%
2		5	0.9497	0.9279	0.9714	0.9507	0.9245	0.9738	0.007829	1.84%	1.34%
4		5	0.9556	0.9355	0.9758	0.96	0.9322	0.9744	0.007254	1.7%	0.72%
9		5	0.9632	0.9552	0.9712	0.962	0.9542	0.9714	0.002885	0.67%	-0.07%
18		5	0.9694	0.9608	0.9781	0.9685	0.9628	0.9807	0.003107	0.72%	-0.72%
35		5	0.9602	0.9513	0.9692	0.9643	0.9486	0.966	0.003232	0.75%	0.24%
73.5		5	0.9528	0.9478	0.9577	0.9513	0.9494	0.9595	0.001778	0.42%	1.02%

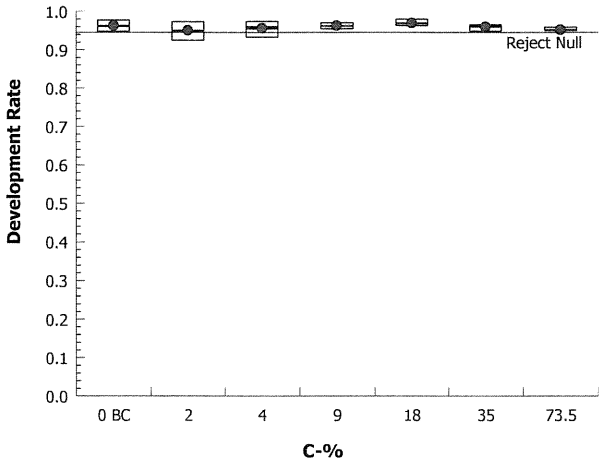
Angular (Corrected) Transformed Summary

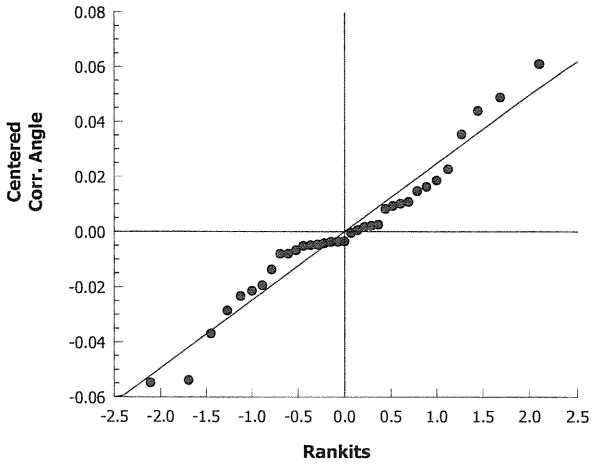
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.378	1.342	1.414	1.373	1.341	1.422	0.0129	2.09%	0.0%
2		5	1.347	1.296	1.398	1.347	1.292	1.408	0.01842	3.06%	2.2%
4		5	1.361	1.313	1.41	1.369	1.307	1.41	0.01753	2.88%	1.19%
9		5	1.378	1.357	1.4	1.375	1.355	1.401	0.007673	1.25%	-0.05%
18		5	1.396	1.37	1.423	1.392	1.377	1.431	0.009561	1.53%	-1.34%
35		5	1.371	1.348	1.393	1.381	1.342	1.385	0.008027	1.31%	0.51%
73.5		5	1.352	1.34	1.364	1.348	1.344	1.368	0.00428	0.71%	1.87%

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

Analysis ID:	00-9057-3710	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Aug-19 8:19	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 04-3000-7149 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Aug-19 8:19 **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	4.69%	73.5	>73.5	NA	1.361

Steel Many-One Rank Sum Test

Control	vs	C-%	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Brine Control		2	23.5	16	1	8	0.5252	Asymp	Non-Significant Effect
		4	23.5	16	2	8	0.5252	Asymp	Non-Significant Effect
		9	32.5	16	1	8	0.9904	Asymp	Non-Significant Effect
		18	27.5	16	1	8	0.8571	Asymp	Non-Significant Effect
		35	26.5	16	1	8	0.7925	Asymp	Non-Significant Effect
		73.5	26	16	2	8	0.7547	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.04959219	0.008265365	6	0.8102	0.5708	Non-Significant Effect
Error	0.2856392	0.0102014	28			
Total	0.3352314		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	0.6739	3.812	0.6720	Equal Variances
Variances	Levene Equality of Variance	3.555	3.528	0.0096	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9065	0.9146	0.0059	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	0.9862	0.9616	1	1	0.9569	1	0.008876	2.01%	0.0%
2		5	0.9698	0.9272	1	0.9871	0.9138	1	0.01536	3.54%	1.66%
4		5	0.9664	0.9179	1	0.9698	0.9052	1	0.01748	4.04%	2.01%
9		5	1	1	1	1	1	1	0	0.0%	-1.4%
18		5	0.9819	0.9425	1	1	0.9267	1	0.01419	3.23%	0.44%
35		5	0.9776	0.935	1	1	0.9224	1	0.01532	3.51%	0.87%
73.5		5	0.975	0.9292	1	1	0.9181	1	0.01649	3.78%	1.14%

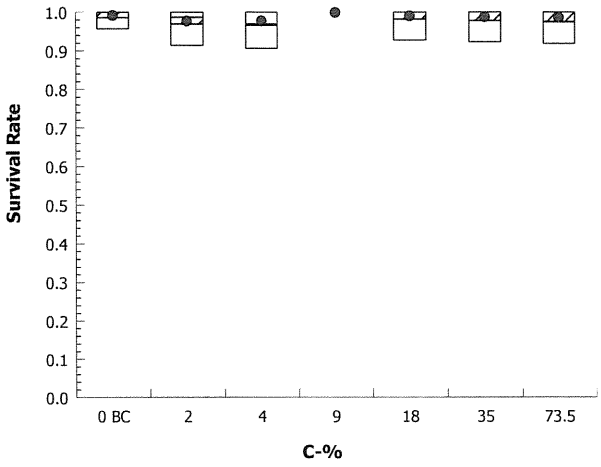
Angular (Corrected) Transformed Summary

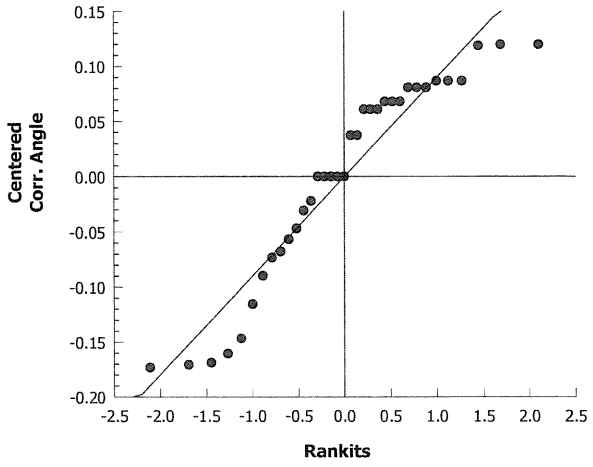
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Brine Control	5	1.477	1.371	1.583	1.538	1.362	1.538	0.0381	5.77%	0.0%
2		5	1.419	1.294	1.544	1.457	1.273	1.538	0.04503	7.09%	3.9%
4		5	1.418	1.269	1.568	1.396	1.258	1.538	0.0539	8.5%	3.97%
9		5	1.538	1.538	1.538	1.538	1.538	1.538	0	0.0%	-4.13%
18		5	1.47	1.338	1.601	1.538	1.297	1.538	0.04736	7.2%	0.48%
35		5	1.457	1.314	1.601	1.538	1.289	1.538	0.05166	7.93%	1.33%
73.5		5	1.451	1.3	1.603	1.538	1.281	1.538	0.05464	8.42%	1.74%

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

Analysis ID:	04-3000-7149	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Aug-19 8:19	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	11-3898-7688	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Aug-19 8:19	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1716485	1000	Yes	Two-Point Interpolation

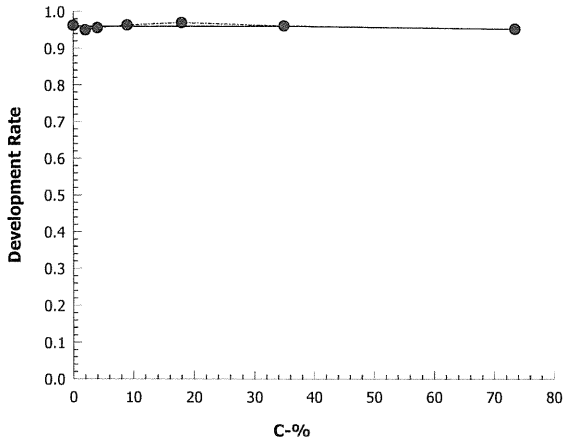
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.5	N/A	N/A	<1.361	NA	NA
EC50	>73.5	N/A	N/A	<1.361	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	0.9626	0.948	0.9779	0.004749	0.01062	1.1%	0.0%	1121	1165
2		5	0.9497	0.9245	0.9738	0.007829	0.01751	1.84%	1.34%	1101	1159
4		5	0.9556	0.9322	0.9744	0.007254	0.01622	1.7%	0.72%	1077	1127
9		5	0.9632	0.9542	0.9714	0.002885	0.006451	0.67%	-0.07%	1153	1197
18		5	0.9694	0.9628	0.9807	0.003107	0.006948	0.72%	-0.72%	1156	1192
35		5	0.9602	0.9486	0.966	0.003232	0.007228	0.75%	0.24%	1145	1192
73.5		5	0.9528	0.9494	0.9595	0.001778	0.003975	0.42%	1.02%	1126	1182

Graphics



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

Analysis ID:	10-1515-3673	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Aug-19 8:20	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1423709	1000	Yes	Two-Point Interpolation

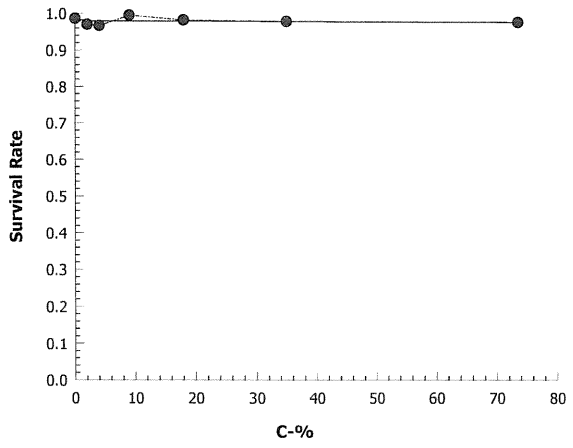
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>73.5	N/A	N/A	<1.361	NA	NA
EC50	>73.5	N/A	N/A	<1.361	NA	NA

Survival Rate Summary — Calculated Variate(A/B)

C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.9862	0.9569	1	0.008876	0.01985	2.01%	0.0%	1144	1160
2		5	0.9698	0.9138	1	0.01536	0.03435	3.54%	1.66%	1125	1160
4		5	0.9664	0.9052	1	0.01748	0.03908	4.04%	2.01%	1121	1160
9		5	1	1	1	0	0	0.0%	-1.4%	1160	1160
18		5	0.9819	0.9267	1	0.01419	0.03173	3.23%	0.44%	1139	1160
35		5	0.9776	0.9224	1	0.01532	0.03427	3.51%	0.87%	1134	1160
73.5		5	0.975	0.9181	1	0.01649	0.03688	3.78%	1.14%	1131	1160

Graphics



CETIS Test Data Worksheet

Report Date: 06 Jul-19 14:59 (p 1 of 1)
Test Code: 1907-5060 03-8657-3583/170AA50F

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

Start Date: 10 Jul-19
End Date: 12 Jul-19
Sample Date: 09 Jul-19
Species: Mytilus galloprovincialis
Protocol: EPA/600/R-95/136 (1995)
Material: Effluent Sample
Sample Code: 19- 0742
Sample Source: Jacobs
Sample Station: Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			181			254	246	JL 7/19/19
			182			213	203	
			183			249	241	
			184			267	254	
			185			266	252	
			186			225	216	
			187			252	242	
			188			212	196	
			189			240	229	
			190			221	219	
			191			215	207	
			192			282	272	
			193			224	216	
			194			228	220	
			195			262	249	
			196			236	229	
			197			237	229	
			198			222	213	
			199			214	203	
			200			223	212	
			201			210	199	
			202			222	214	
			203			229	223	
			204			250	237	
			205			222	214	
			206			229	218	JL 7/26/19
			207			233	224	
			208			245	238	
			209			234	225	
			210			234	225	
			211			234	228	
			212			236	220	
			213			237	228	
			214			235	227	
			215			237	227	
			216			226	221	
			217			243	231	
			218			241	233	
			219			259	254	
			220			237	225	

CETIS Test Data Worksheet

Report Date: 06 Jul-19 14:59 (p 1 of 1)
Test Code: 1907-5060 03-8657-3583/170AA50F

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

Start Date: 10 Jul-19 **Species:** Mytilus galloprovincialis **Sample Code:** 19- 0742
End Date: 12 Jul-19 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Jacobs
Sample Date: 09 Jul-19 **Material:** Effluent Sample **Sample Station:** Wyckoff

C-%	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	BC	1	209			242	233	AC 7/12/19
0	BC	2	207					
0	BC	3	216					
0	BC	4	202					
0	BC	5	204					
0	LC	1	183					
0	LC	2	197					
0	LC	3	187			251	242	JW 7/25/19
0	LC	4	195					
0	LC	5	190					
2		1	203					
2		2	200					
2		3	206					
2		4	185					
2		5	188					
4		1	211					
4		2	212					
4		3	205					
4		4	186					
4		5	201					
9		1	210			240	232	JW 7/25/19
9		2	189					
9		3	218					
9		4	213					
9		5	208					
18		1	191					
18		2	181					
18		3	196					
18		4	219					
18		5	194					
35		1	192					
35		2	214					
35		3	193					
35		4	215					
35		5	199					
~~72.3~~ 73.5		1	220					
~~72.3~~		2	217					
~~72.3~~		3	184					
~~72.3~~		4	198			220	211	JW 7/25/19
~~72.3~~		5	182					

@KFP 8/8 8/1/19 QC= EG

Analyst: RT QA: KFP 8/1/19

Marine Chronic Bioassay

Water Quality Measurements

Client: Jacobs
Sample ID: Wyckoff
Sample Log No.: 19- 0742
Test No.: 1907-5060

Test Species: *M. galloprovincialis*
Start Date/Time: 7/10/2019 1515
End Date/Time: 7/12/2019 1535

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	30.1	30.1	30.0	15.8	14.9	15.0	8.2	8.1	8.2	8.00	8.00	7.98
Brine Control	30.6	30.4	30.6	16.0	14.8	14.8	7.8	8.1	8.3	8.18	8.09	8.04
2	30.3	30.3	30.3	15.7	14.8	14.9	8.1	8.0	8.4	8.00	8.00	8.01
4	~~29.9~~ 30.3 Ⓐ	30.1	30.3	15.7	14.8	14.9	8.2	8.1	8.4	7.97	7.99	8.01
9	30.3	30.2	30.3	15.7	14.7	14.9	8.2	8.1	8.3	7.91	7.98	8.05
18	30.3	30.3	30.3	15.6	14.9	15.0	8.3	8.0	8.4	7.83	8.01	8.10
35	30.3	30.3	30.3	15.7	14.8	15.1	8.0	8.1	8.3	7.74	8.02	8.13
73.5	30.7	30.7	30.7	15.8	14.8	15.0	7.9	8.0	8.3	7.65	8.03	8.21

Technician Initials:		0	24	48
	WQ Readings:	EG	EG OBO RT	DM
	Dilutions made by:	EG	—	—

Comments:
0 hrs: Ⓐ EG 9:18 7/10/19
24 hrs:
48 hrs:

QC Check: KFP 8/1/19

Final Review: EG 8/9/19

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: JACOBS **Analyst:** EG

Sample ID: Wyckoff **Test Date:** 7/10/2019

Test No: 1907-S 060 **Test Type:** Mussel Development

Salinity of Effluent 7.0

Salinity of Brine 93.8 **Date of Brine used:** 6/11/2019

Target Salinity 30 **Alkalinity of Brine Control:** (A) mg/L as CaCO3

Test Dilution Volume 250

	Effluent	Brine Control
Salinity Adjustment Factor:		
(TS - SE)/(SB - TS) =	**0.36**	**0.47**

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

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Control	NA	NA	NA	250
2	5.0	0.36	1.8	250
4	10.0	0.36	3.6	250
9	22.5	0.36	8.1	250
18	45.0	0.36	16.2	250
35	87.5	0.36	31.5	250
73.5	183.8	0.36	66.2	250

	DI Volume			
Brine Control	140.9	0.47	66.2	250

(A) Alkalinity not measured; technician error.

Total Brine Volume Required (ml): **193.8**

QC Check: KTP 8/1/19 **Final Review:** EG 8/9/19

Marine Chronic Bioassay

Larval Development Worksheet

Client: Jacobs-Wyckoff
Test No.: 1907-5060
Test Species: *Mytilus galloprovincialis*
Animal Source: Mission Bay
Date Received: 5/29/19 13AB combined tank
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 7/10/2019 1515
End Date/Time: 7/12/2019 1535
Technician Initials: AC/EG

Spawn Information

First Gamete Release Time: 1140

Sex	Number Spawning
Male	6
Female	2 MB, 1 Cat

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1	Good density & motility
Female 1	MB1	good density + color
Female 2	MB2	light color, good density, then very orange, excellent density
Female 3	Cat 1	

Egg Fertilization Time: 1230

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	90
Female 3	80

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

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

Number Counted:		
	27	33
	27	25
	32	32
	32	40
	25	44

Mean: 31.7

Mean 31.7 X 50 = 1,585 embryos/ml

Initial Density: 1585 / Desired Final Density: 300 = 5.28 (dilution factor)
(to inoculate with 0.5 ml)

When mean percent dividing is ≥ 90, 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

Rep	No. Dividing	Total	% Dividing	Mean % Dividing
TØ A	219	219	100	100
TØ B	261	261	100	
TØ C	232	232	100	
TØ D	211	211	100	
TØ E	233	233	100	
TØ F	238	238	100	

48-h QC: 229/236 = 97%

Comments: $\bar{x}$ = 232.

QC Check: KTP 8/1/19

Final Review: WS 8/15/19

Appendix B
Sample Check-In Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: Jacobs
Sample ID: Wyckoff (070919)
Test ID No(s).: 1907 - 5060

Sample (A, B, C):	A			
Log-in No. (19-xxxx):	0742			
Sample Collection Date & Time:	7/9/19 1000			
Sample Receipt Date & Time:	7/10/19 0945			
Number of Containers & Container Type:	1 1L cubi			
Approx. Total Volume Received (L):	1			
Check-in Temperature (°C)	3.8			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.6			
pH (units)	7.66			
Conductivity (µS/cm)	—			
Salinity (ppt)	7.0			
Alkalinity (mg/L) [2]	395			
Hardness (mg/L) [2, 3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	KL			

Test Performed: Mussel Development **Control/Dilution Water:** 8:2 / Lab SW / Lab ART **Other:** _____
Alkalinity: 117 Hardness or Salinity: 30 ppt
Additional Control? (Y) N = Brine Control Alkalinity: (A) Hardness or Salinity: 30 ppt

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) Alkalinity of brine control not measured; technician error.

Sample Check-In Information

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

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

Filtration? Y (N)
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___

QC Check: KTP 8/11/19
Final Review: WS 8/15/19

Total Ammonia Analysis
Freshwater

Overlying Water

Client: JACOBS
Project: Wyckoff
Test Type: Mussel Development

DI Blank: 0.0
Test Start Date: 7/10/2019

Analyst: KL/KS
Analysis Date: 7/22/19

Sample ID	Nautilus ID	Sub-Sample Date	Test Day	NH3-N (mg/L)	Ammonia (mg/L) N x 1.22
Blank Spike (10 mg/L NH_3)		NA	NA	7.4	9.0
Wyckoff	19-0742	7/10/2019	Check In	1.3	1.6
Spike Check (10 mg/L NH_3)		NA	NA		
Batch QA Sample	19-0773	7/16/19	N/A	36.0	43.9
Sample Duplicate[a]	Batch QA	NA	NA	36.3	44.3
Sample Duplicate + Spike[a]		NA	NA	43.5	53.1
Spike Check (10 mg/L NH_3)		NA	NA	—	—

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

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

QC Sample ID	[NH_3]	[Sample Dup]	Measured [Spike]	Nominal [Spike]	RPD	% Recovery
Blank	0.0	NA	9.0	10	NA	90
Batch QA ~~(Meastam)~~	43.9	44.3	53.1	10	0.9	92

QIB AC 8/1/19

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: AC 8/1/19

Final Review: KFP 8/1/19

Appendix C
Chain-of-Custody Form

Enthalpy Analytical (REGION COPY)
DateShipped: 7/9/2019
CarrierName: FedEx
AirbillNo: 7756 6640 3707

CHAIN OF CUSTODY RECORD
Wyckoff Eagle Harbor GWTP 2019/WA
Project Code: WEH-029D
Cooler #: 1 of 1

No: 10-070919-102015-0381
2019T10P000DD210W2LA00
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
070919		Ground Water/ K.Allers	Composite	CHRTOX(8 Weeks)	(< 6 C) (1)	SP-11	07/09/2019 10:00	Field Sample

3.8°C

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
	Keith Allers JACOBS	7-9-2019 1025	KS Surface Nautilus	7/10/19 0945	good

LabID: 19-0742

Appendix D
Reference Toxicant Test Results

Bivalve Larval Development Test

CETIS Summary Report

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

Batch ID:	14-8712-8092	**Test Type:**	Development-Survival	**Analyst:**	
Start Date:	10 Jul-19 15:15	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Diluted Natural Seawater
Ending Date:	12 Jul-19 15:35	**Species:**	Mytilus galloprovincialis	**Brine:**	Not Applicable
Duration:	48h	**Source:**	Mission Bay	**Age:**	

Sample ID:	07-5193-5536	**Code:**	190710msdv	**Client:**	Internal
Sample Date:	10 Jul-19	**Material:**	Copper chloride	**Project:**	
Receive Date:	10 Jul-19	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-6066-2134	Combined Development Ra	5	10	7.071	5.24%		Dunnett Multiple Comparison Test
10-2312-9373	Development Rate	5	10	7.071	1.95%		Dunnett Multiple Comparison Test
02-2288-3584	Survival Rate	10	20	14.14	6.49%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
08-2094-6054	Combined Development Ra	EC25	6.361	6.053	6.458		Linear Interpolation (ICPIN)
		EC50	7.786	7.579	7.898		
02-8661-6124	Development Rate	EC25	6.368	6.239	6.452		Linear Interpolation (ICPIN)
		EC50	7.8	7.692	7.885		
18-9463-9841	Survival Rate	EC25	23.15	19.48	25.51		Linear Interpolation (ICPIN)
		EC50	29.69	27.42	31.18		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-8661-6124	Development Rate	Control Resp	0.9707	0.9 - NL	Yes	Passes Acceptability Criteria
10-2312-9373	Development Rate	Control Resp	0.9707	0.9 - NL	Yes	Passes Acceptability Criteria
02-2288-3584	Survival Rate	Control Resp	0.9716	0.5 - NL	Yes	Passes Acceptability Criteria
18-9463-9841	Survival Rate	Control Resp	0.9716	0.5 - NL	Yes	Passes Acceptability Criteria
14-6066-2134	Combined Development Ra	PMSD	0.05238	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	0.9432	0.8937	0.9927	0.8836	0.9872	0.01783	0.03986	4.23%	0.0%
2.5		5	0.9532	0.9255	0.981	0.9224	0.9828	0.01	0.02236	2.35%	-1.07%
5		5	0.9369	0.8814	0.9924	0.8793	0.9835	0.02	0.04472	4.77%	0.67%
10		5	0.1057	0.07905	0.1324	0.0819	0.1293	0.009599	0.02146	20.31%	88.79%
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	0.9707	0.955	0.9865	0.9515	0.9872	0.005686	0.01271	1.31%	0.0%
2.5		5	0.9691	0.9587	0.9795	0.9607	0.9828	0.003752	0.008391	0.87%	0.17%
5		5	0.9598	0.9368	0.9828	0.9409	0.9835	0.00829	0.01854	1.93%	1.13%
10		5	0.1118	0.08853	0.135	0.09314	0.1357	0.008373	0.01872	16.75%	88.49%
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	0.9716	0.9254	1	0.9095	1	0.01662	0.03715	3.82%	0.0%
2.5		5	0.9836	0.9593	1	0.9526	1	0.00877	0.01961	1.99%	-1.24%
5		5	0.9759	0.9333	1	0.9267	1	0.01534	0.03429	3.51%	-0.44%
10		5	0.9414	0.887	0.9958	0.8793	1	0.01959	0.04381	4.65%	3.11%
20		5	0.8509	0.7151	0.9867	0.7414	0.9741	0.04891	0.1094	12.85%	12.42%
40		5	0.1034	0.08784	0.1191	0.08621	0.1207	0.00562	0.01257	12.15%	89.35%

Combined Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9701	0.944	0.931	0.8836	0.9872
2.5		0.9483	0.9224	0.9644	0.9828	0.9483
5		0.8793	0.9835	0.9409	0.9052	0.9754
10		0.09483	0.09483	0.0819	0.1293	0.1277
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	0.9701	0.9733	0.9515	0.9716	0.9872
2.5		0.9607	0.9683	0.9644	0.9828	0.9692
5		0.9488	0.9835	0.9409	0.9502	0.9754
10		0.1023	0.1	0.09314	0.1357	0.1277
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	0.9698	0.9784	0.9095	1
2.5		0.9871	0.9526	1	1	0.9784
5		0.9267	1	1	0.9526	1
10		0.9267	0.9483	0.8793	0.9526	1
20		0.8534	0.9741	0.7414	0.7414	0.944
40		0.08621	0.1078	0.1207	0.1034	0.09914

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	227/234	219/232	216/232	205/232	231/234
2.5		220/232	214/232	244/253	229/233	220/232
5		204/232	239/243	239/254	210/232	238/244
10		22/232	22/232	19/232	30/232	30/235
20		0/232	0/232	0/232	0/232	0/232
40		0/232	0/232	0/232	0/232	0/232

Development Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	227/234	219/225	216/227	205/211	231/234
2.5		220/229	214/221	244/253	229/233	220/227
5		204/215	239/243	239/254	210/221	238/244
10		22/215	22/220	19/204	30/221	30/235
20		0/198	0/226	0/172	0/172	0/219
40		0/20	0/25	0/28	0/24	0/23

Survival Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	232/232	225/232	227/232	211/232	232/232
2.5		229/232	221/232	232/232	232/232	227/232
5		215/232	232/232	232/232	221/232	232/232
10		215/232	220/232	204/232	221/232	232/232
20		198/232	226/232	172/232	172/232	219/232
40		20/232	25/232	28/232	24/232	23/232

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

Analysis ID:	14-6066-2134	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Jul-19 11:39	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	5.24%	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	-0.3336	2.227	0.104	8	0.8529	CDF	Non-Significant Effect
		5	0.2539	2.227	0.104	8	0.6511	CDF	Non-Significant Effect
		10*	21.7	2.227	0.104	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	3.858434	1.286145	3	236.1	<0.0001	Significant Effect
Error	0.08715706	0.005447316	16			
Total	3.945591		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.066	11.34	0.2544	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9628	0.866	0.6022	Normal Distribution

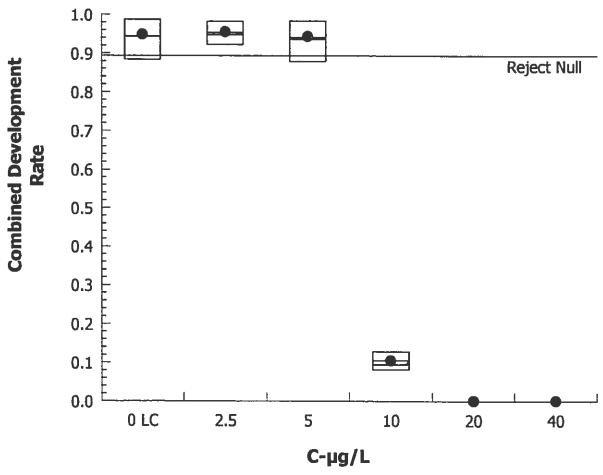
Combined Development Rate Summary

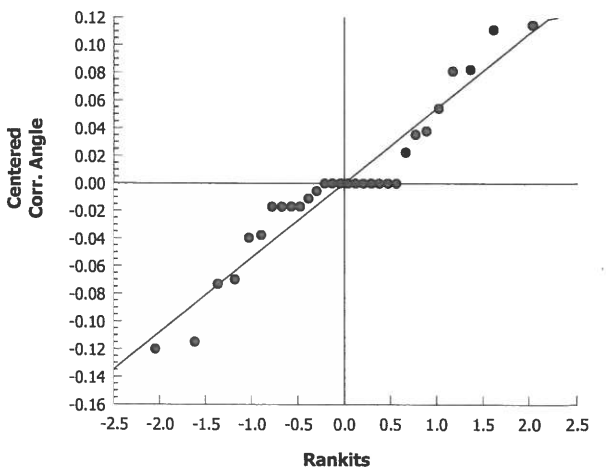
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9432	0.8937	0.9927	0.944	0.8836	0.9872	0.01783	4.23%	0.0%
2.5		5	0.9532	0.9255	0.981	0.9483	0.9224	0.9828	0.01	2.35%	-1.07%
5		5	0.9369	0.8814	0.9924	0.9409	0.8793	0.9835	0.02	4.77%	0.67%
10		5	0.1057	0.07905	0.1324	0.09483	0.0819	0.1293	0.009599	20.31%	88.79%
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	1.343	1.232	1.454	1.332	1.223	1.457	0.04002	6.66%	0.0%
2.5		5	1.358	1.289	1.428	1.341	1.289	1.439	0.02504	4.12%	-1.16%
5		5	1.331	1.21	1.452	1.325	1.216	1.442	0.04345	7.3%	0.88%
10		5	0.3299	0.2867	0.3731	0.313	0.2902	0.3678	0.01556	10.54%	75.43%
20		5	0.03283	0.03283	0.03283	0.03283	0.03283	0.03283	0	0.0%	97.55%
40		5	0.03283	0.03283	0.03283	0.03283	0.03283	0.03283	0	0.0%	97.55%

Graphics





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

Analysis ID:	10-2312-9373	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Jul-19 11:39	**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	1.95%	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	0.2821	2.227	0.053	8	0.6393	CDF	Non-Significant Effect
		5	1.198	2.227	0.053	8	0.2595	CDF	Non-Significant Effect
		10*	44.81	2.227	0.053	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.140649	1.380216	3	982.3	<0.0001	Significant Effect
Error	0.02248156	0.001405098	16			
Total	4.163131		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.947	11.34	0.5836	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9494	0.866	0.3582	Normal Distribution

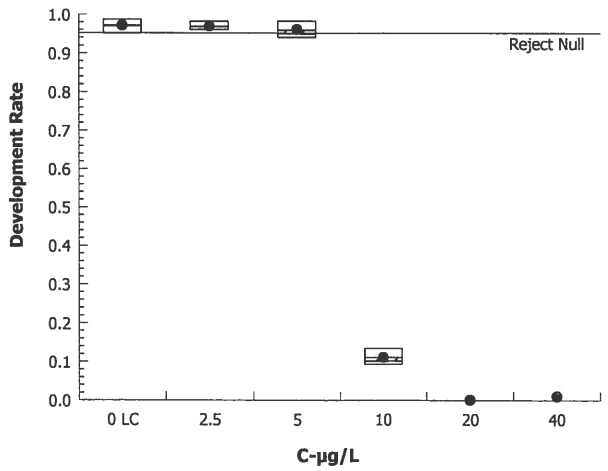
Development Rate Summary

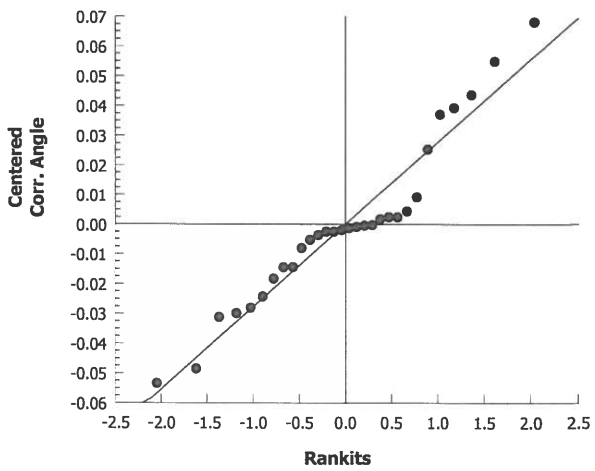
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9707	0.955	0.9865	0.9716	0.9515	0.9872	0.005686	1.31%	0.0%
2.5		5	0.9691	0.9587	0.9795	0.9683	0.9607	0.9828	0.003753	0.87%	0.17%
5		5	0.9598	0.9368	0.9828	0.9502	0.9409	0.9835	0.00829	1.93%	1.13%
10		5	0.1118	0.08853	0.135	0.1023	0.09314	0.1357	0.008373	16.75%	88.49%
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	1.402	1.354	1.45	1.401	1.349	1.457	0.01723	2.75%	0.0%
2.5		5	1.396	1.363	1.428	1.392	1.371	1.439	0.0117	1.88%	0.48%
5		5	1.374	1.311	1.437	1.346	1.325	1.442	0.02274	3.7%	2.03%
10		5	0.34	0.3035	0.3766	0.3256	0.3101	0.3773	0.01317	8.66%	75.75%
20		5	0.03577	0.0329	0.03865	0.03554	0.03327	0.03813	0.001035	6.47%	97.45%
40		5	0.1027	0.0948	0.1106	0.1022	0.09463	0.112	0.002846	6.2%	92.68%

Graphics





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

Analysis ID: 02-2288-3584 **Endpoint:** Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 30 Jul-19 11:40 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	6.49%	10	20	14.14	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.411	2.362	0.169	8	0.9267	CDF	Non-Significant Effect
		5	-0.2831	2.362	0.169	8	0.9035	CDF	Non-Significant Effect
		10	1.169	2.362	0.169	8	0.3469	CDF	Non-Significant Effect
		20*	3.269	2.362	0.169	8	0.0069	CDF	Significant Effect
		40*	15.48	2.362	0.169	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.850606	0.9701213	5	76.12	<0.0001	Significant Effect
Error	0.3058812	0.01274505	24			
Total	5.156487		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	11.19	15.09	0.0476	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9592	0.9031	0.2958	Normal Distribution

Survival Rate Summary

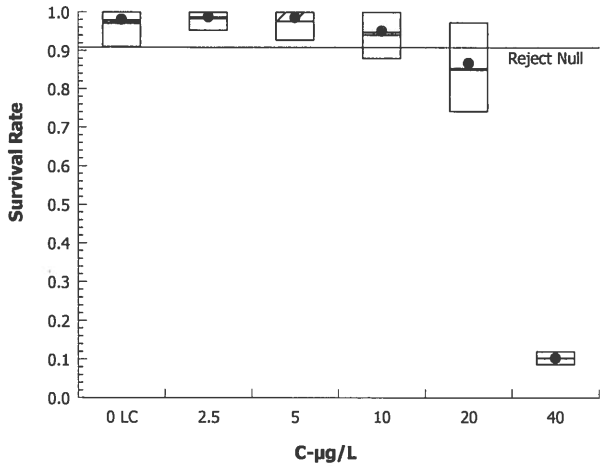
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.9716	0.9254	1	0.9784	0.9095	1	0.01662	3.82%	0.0%
2.5		5	0.9836	0.9593	1	0.9871	0.9526	1	0.00877	1.99%	-1.24%
5		5	0.9759	0.9333	1	1	0.9267	1	0.01534	3.51%	-0.44%
10		5	0.9414	0.887	0.9958	0.9483	0.8793	1	0.01959	4.65%	3.11%
20		5	0.8509	0.7151	0.9867	0.8534	0.7414	0.9741	0.04891	12.85%	12.42%
40		5	0.1034	0.08784	0.1191	0.1034	0.08621	0.1207	0.00562	12.15%	89.35%

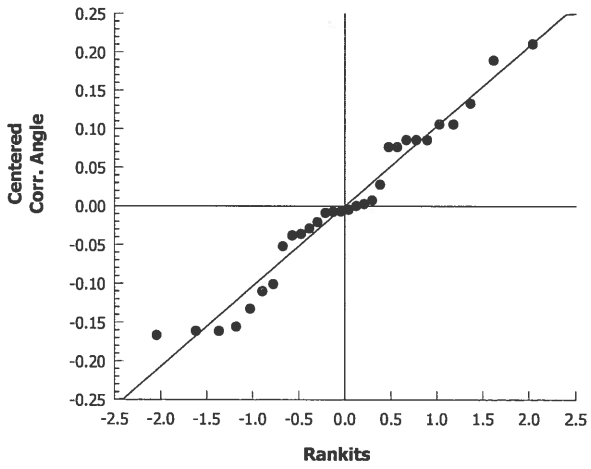
Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.432	1.291	1.573	1.423	1.265	1.538	0.05081	7.93%	0.0%
2.5		5	1.462	1.363	1.56	1.457	1.351	1.538	0.03557	5.44%	-2.05%
5		5	1.452	1.305	1.6	1.538	1.297	1.538	0.05312	8.18%	-1.41%
10		5	1.349	1.201	1.496	1.341	1.216	1.538	0.05301	8.79%	5.83%
20		5	1.199	0.9886	1.409	1.178	1.037	1.409	0.07569	14.12%	16.3%
40		5	0.327	0.3013	0.3527	0.3275	0.298	0.3548	0.009259	6.33%	77.17%

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	02-2288-3584	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Jul-19 11:40	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	08-2094-6054	**Endpoint:**	Combined Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Jul-19 11:40	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	492075	1000	Yes	Two-Point Interpolation

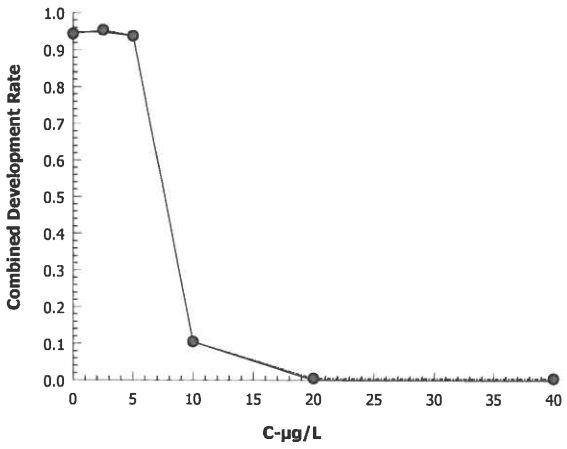
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.361	6.053	6.458
EC50	7.786	7.579	7.898

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	0.9432	0.8836	0.9872	0.01783	0.03986	4.23%	0.0%	1098	1164
2.5		5	0.9532	0.9224	0.9828	0.01	0.02236	2.35%	-1.07%	1127	1182
5		5	0.9369	0.8793	0.9835	0.02	0.04472	4.77%	0.67%	1130	1205
10		5	0.1057	0.0819	0.1293	0.009599	0.02146	20.31%	88.79%	122	1163
20		5	0	0	0	0	0		100.0%	0	1160
40		5	0	0	0	0	0		100.0%	0	1160

Graphics



Analyst: JA QA: KFP 8/1/19

Bivalve Larval Survival and Development Test					Nautilus Environmental (CA)
Analysis ID:	02-8661-6124	**Endpoint:**	Development Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Jul-19 11:40	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1713316	1000	Yes	Two-Point Interpolation

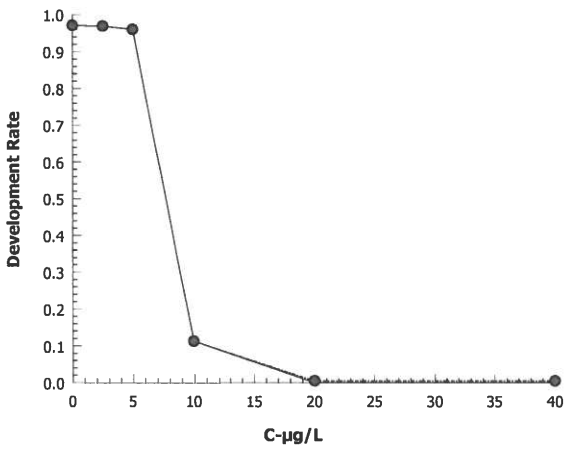
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	6.368	6.239	6.452
EC50	7.8	7.692	7.885

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	0.9707	0.9515	0.9872	0.005686	0.01271	1.31%	0.0%	1098	1131
2.5		5	0.9691	0.9607	0.9828	0.003753	0.008392	0.87%	0.17%	1127	1163
5		5	0.9598	0.9409	0.9835	0.00829	0.01854	1.93%	1.13%	1130	1177
10		5	0.1118	0.09314	0.1357	0.008373	0.01872	16.75%	88.49%	123	1095
20		5	0	0	0	0	0		100.0%	0	987
40		5	0	0	0	0	0		100.0%	0	120

Graphics



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

Analysis ID:	18-9463-9841	**Endpoint:**	Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	30 Jul-19 11:40	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1584768	1000	Yes	Two-Point Interpolation

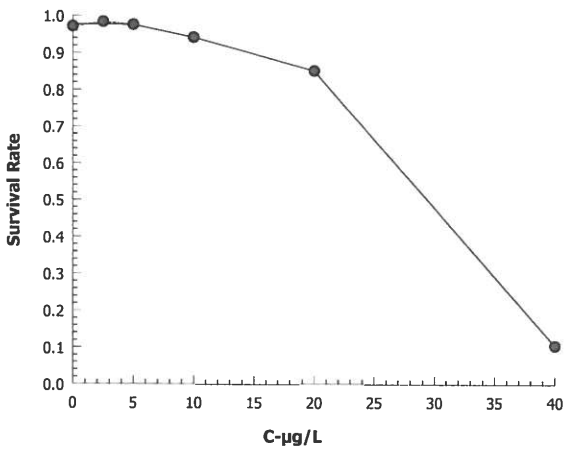
Point Estimates

Level	µg/L	95% LCL	95% UCL
EC25	23.15	19.48	25.51
EC50	29.69	27.42	31.18

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	0.9716	0.9095	1	0.01662	0.03715	3.82%	0.0%	1127	1160
2.5		5	0.9836	0.9526	1	0.00877	0.01961	1.99%	-1.24%	1141	1160
5		5	0.9759	0.9267	1	0.01534	0.03429	3.51%	-0.44%	1132	1160
10		5	0.9414	0.8793	1	0.01959	0.04381	4.65%	3.11%	1092	1160
20		5	0.8509	0.7414	0.9741	0.04891	0.1094	12.85%	12.42%	987	1160
40		5	0.1034	0.08621	0.1207	0.00562	0.01257	12.15%	89.35%	120	1160

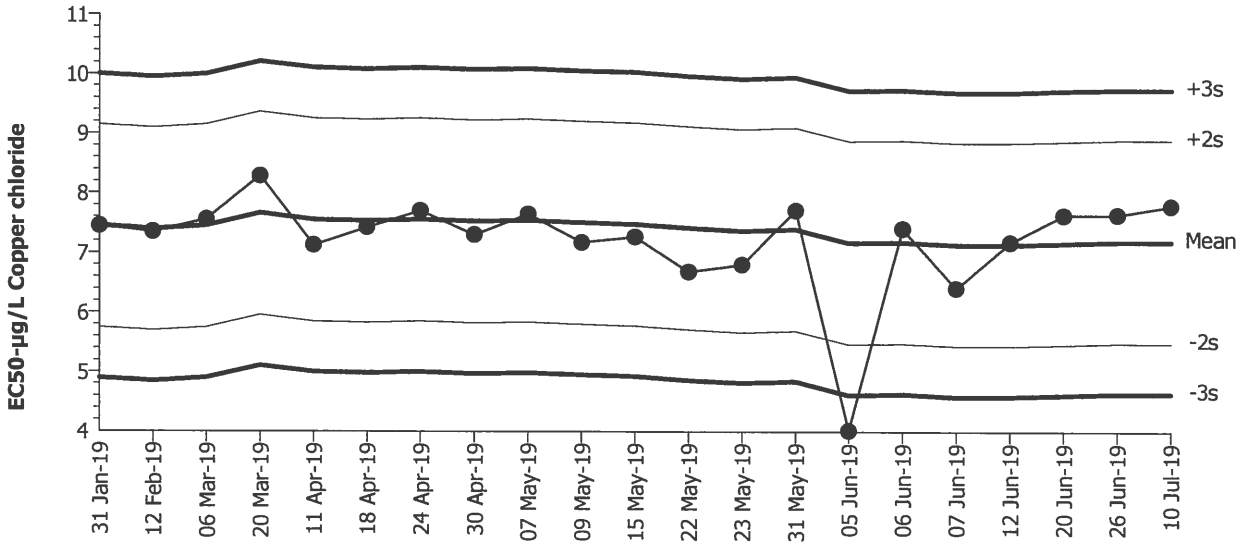
Graphics



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

Bivalve Larval Survival and Development Test



Mean:	7.186	**Count:**	20	**-2s Warning Limit:**	5.485	**-3s Action Limit:**	4.634
Sigma:	0.8505	**CV:**	11.80%	**+2s Warning Limit:**	8.887	**+3s Action Limit:**	9.737

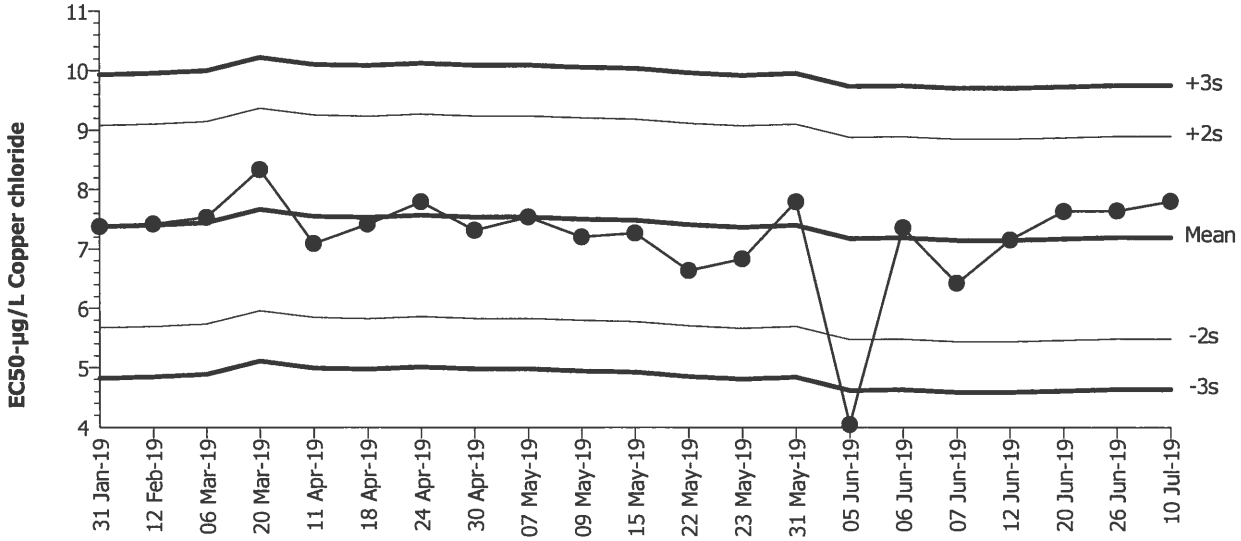
Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jan	31	20:05	7.448	0.262	0.308			11-0188-3209	19-4158-0070
2		Feb	12	14:30	7.347	0.1613	0.1896			05-4773-4064	14-0529-6566
3		Mar	6	14:40	7.555	0.3693	0.4342			11-4050-7104	03-7884-0450
4			20	15:25	8.284	1.098	1.291			00-9922-7600	12-2384-4421
5		Apr	11	17:38	7.124	-0.06153	-0.07235			03-4197-9533	19-1101-0677
6			18	15:00	7.422	0.2363	0.2778			07-6552-9366	19-9120-3565
7			24	14:25	7.7	0.5144	0.6048			00-2566-7560	04-1354-8994
8			30	14:45	7.298	0.1119	0.1315			20-4872-1616	19-4433-9185
9		May	7	23:15	7.642	0.4555	0.5356			01-8514-7589	19-5887-7680
10			9	16:40	7.169	-0.01678	-0.01973			16-2457-2225	00-5727-2653
11			15	15:10	7.264	0.07801	0.09172			12-3612-1420	09-9400-1021
12			22	14:35	6.682	-0.5037	-0.5923			07-8876-2604	11-8156-7920
13			23	14:30	6.802	-0.3835	-0.451			04-4294-0564	00-1747-4708
14			31	14:45	7.705	0.5187	0.6099			03-7170-8467	07-0824-5140
15		Jun	5	14:30	4.018	-3.168	-3.725	(-)	(-)	14-9865-1579	13-4485-4153
16			6	15:45	7.404	0.2176	0.2558			20-7222-8009	13-0272-9420
17			7	13:35	6.408	-0.7782	-0.915			16-0229-2669	15-6829-1413
18			12	15:00	7.174	-0.01208	-0.0142			20-8735-2782	03-4367-9827
19			20	15:00	7.627	0.441	0.5186			00-4624-1892	07-7845-5970
20			26	15:55	7.636	0.4503	0.5295			00-8415-2643	12-3790-3484
21		Jul	10	15:15	7.786	0.5999	0.7053			02-0190-4206	08-2094-6054

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

Bivalve Larval Survival and Development Test



Mean:	7.188	**Count:**	20	**-2s Warning Limit:**	5.484	**-3s Action Limit:**	4.633
Sigma:	0.8519	**CV:**	11.90%	**+2s Warning Limit:**	8.892	**+3s Action Limit:**	9.744

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jan	31	20:05	7.377	0.1885	0.2213			11-0188-3209	01-6713-0404
2		Feb	12	14:30	7.421	0.2327	0.2731			05-4773-4064	11-5918-1928
3		Mar	6	14:40	7.531	0.3435	0.4032			11-4050-7104	19-8242-5220
4			20	15:25	8.334	1.146	1.345			00-9922-7600	07-3949-1564
5		Apr	11	17:38	7.092	-0.09631	-0.1131			03-4197-9533	11-6047-7524
6			18	15:00	7.416	0.2277	0.2673			07-6552-9366	15-9786-1055
7			24	14:25	7.793	0.605	0.7101			00-2566-7560	21-1882-8302
8			30	14:45	7.316	0.1277	0.1498			20-4872-1616	19-2600-3345
9		May	7	23:15	7.538	0.3503	0.4112			01-8514-7589	17-7584-8304
10			9	16:40	7.2	0.01215	0.01426			16-2457-2225	15-1559-8997
11			15	15:10	7.264	0.07575	0.08892			12-3612-1420	11-8863-7865
12			22	14:35	6.635	-0.5528	-0.6489			07-8876-2604	17-1974-2634
13			23	14:30	6.832	-0.3556	-0.4175			04-4294-0564	17-8215-3713
14			31	14:45	7.79	0.6019	0.7065			03-7170-8467	14-6790-8476
15		Jun	5	14:30	4.043	-3.145	-3.691	(-)	(-)	14-9865-1579	11-4839-0192
16			6	15:45	7.353	0.1653	0.194			20-7222-8009	04-5983-4829
17			7	13:35	6.42	-0.7681	-0.9016			16-0229-2669	12-7601-7003
18			12	15:00	7.147	-0.04149	-0.0487			20-8735-2782	10-3598-4911
19			20	15:00	7.627	0.4393	0.5156			00-4624-1892	15-3942-3527
20			26	15:55	7.636	0.4482	0.5261			00-8415-2643	06-7718-5278
21		Jul	10	15:15	7.8	0.6115	0.7179			02-0190-4206	02-8661-6124

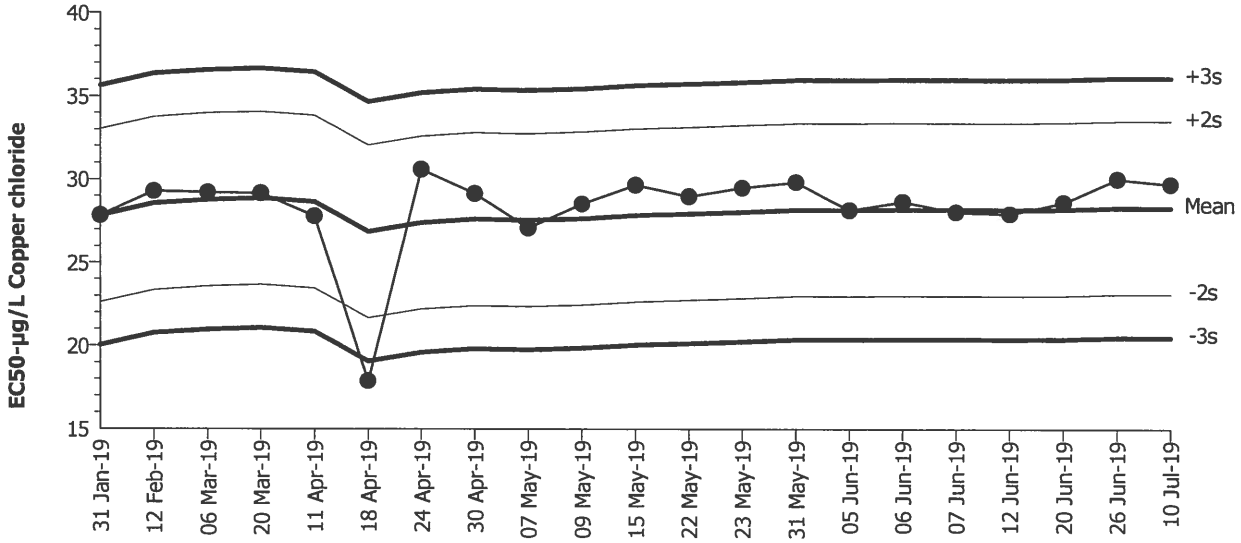
CETIS QC Plot

Report Date: 30 Jul-19 11:42 (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

Bivalve Larval Survival and Development Test



Mean:	28.28	**Count:**	20	**-2s Warning Limit:**	23.08	**-3s Action Limit:**	20.48
Sigma:	2.6	**CV:**	9.19%	**+2s Warning Limit:**	33.48	**+3s Action Limit:**	36.08

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2019	Jan	31	20:05	27.83	-0.4542	-0.1747			11-0188-3209	12-1004-4079
2		Feb	12	14:30	29.28	1.001	0.385			05-4773-4064	07-6241-6865
3		Mar	6	14:40	29.2	0.9189	0.3534			11-4050-7104	15-7196-8133
4			20	15:25	29.16	0.8836	0.3398			00-9922-7600	05-5691-6970
5		Apr	11	17:38	27.79	-0.495	-0.1904			03-4197-9533	08-6961-6395
6			18	15:00	17.87	-10.41	-4.002	(-)	(-)	07-6552-9366	08-9162-0556
7			24	14:25	30.58	2.3	0.8845			00-2566-7560	12-6072-8549
8			30	14:45	29.13	0.8524	0.3279			20-4872-1616	08-0824-0389
9		May	7	23:15	27.08	-1.205	-0.4633			01-8514-7589	14-6629-9437
10			9	16:40	28.51	0.2344	0.09017			16-2457-2225	07-4498-9014
11			15	15:10	29.64	1.359	0.5227			12-3612-1420	00-6090-1816
12			22	14:35	28.95	0.6734	0.259			07-8876-2604	08-9025-9816
13			23	14:30	29.48	1.2	0.4614			04-4294-0564	02-9635-2644
14			31	14:45	29.82	1.545	0.5941			03-7170-8467	20-0663-5874
15		Jun	5	14:30	28.14	-0.1374	-0.05284			14-9865-1579	15-6459-4753
16			6	15:45	28.65	0.3668	0.1411			20-7222-8009	14-8023-9336
17			7	13:35	28.03	-0.2521	-0.09697			16-0229-2669	01-4873-8064
18			12	15:00	27.92	-0.3649	-0.1404			20-8735-2782	03-5721-7619
19			20	15:00	28.59	0.3111	0.1197			00-4624-1892	18-2643-8450
20			26	15:55	30	1.72	0.6615			00-8415-2643	15-8975-2294
21		Jul	10	15:15	29.69	1.409	0.5418			02-0190-4206	18-9463-9841

Analyst: JC QA: KKP 8/1/19

CETIS Test Data Worksheet

Report Date: 06 Jul-19 14:55 (p 1 of 1)
Test Code: 02-0190-4206/190710msdv

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

Start Date: 10 Jul-19 ~~14:48~~ Q18 JA 7/6/19
End Date: 12 Jul-19
Sample Date: 10 Jul-19

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

Sample Code: 190710msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
			1			225	219	JA 7/17/19
			2			~~320~~ 221 Ⓐ	~~40~~ 30 Ⓐ	
			3			244	238	
			4			221	214	
			5			226	0	
			6			233	229	
			7			219	0	
			8			221	210	
			9			215	204	
			10			229	220	
			11			198	0	
			12			235	30	
			13			254	239	
			14			234	231	
			15			243	239	
			16			220	22	
			17			211	205	
			18			227	216	
			19			172	0	
			20			172	0	
			21			24	0	JA 7/18/19 some cells lysed
			22			234	227	
			23			28	0	some cells lysed
			24			25	0	some cells lysed
			25			20	0	some cells lysed
			26			204	19	
			27			215	22	
			28			253	244	
			29			227	220	
			30			23	0	some cells lysed

Ⓐ Q18 JA 7/17/19

CETIS Test Data Worksheet

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

Start Date: 10 Jul-19 ~~14:48~~ 0918 Ac 7/6/19 **Species:** Mytilus galloprovincialis
End Date: 12 Jul-19 **Protocol:** EPA/600/R-95/136 (1995)
Sample Date: 10 Jul-19 **Material:** Copper chloride

Sample Code: 190710msdv
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	Initial Density	Final Density	# Counted	# Normal	Notes
0	LC	1	22			235	229	Ac 7/12/19
0	LC	2	1					
0	LC	3	18					
0	LC	4	17					
0	LC	5	14					
2.5		1	10			226	217	
2.5		2	4					
2.5		3	28					
2.5		4	6					
2.5		5	29					
5		1	9			212	196	
5		2	15					
5		3	13					
5		4	8					
5		5	3					
10		1	27			222	42	
10		2	16					
10		3	26					
10		4	2					
10		5	12					
20		1	11			203	0	
20		2	5					
20		3	19					
20		4	20					
20		5	7					
40		1	25			0	0	cells lysed
40		2	24					
40		3	23					
40		4	21					
40		5	30					

QC = Ac

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
Sample ID: $CuCl_2$
Test No.: 190710msdv

Test Species: *M. galloprovincialis*
Start Date/Time: 7/10/2019 1515
End Date/Time: 7/12/2019 1535

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	31.7	31.7	31.6	16.0	14.8	15.2	8.5	8.0	8.2	8.00	7.98	7.96
2.5	31.8	32.0	31.9	15.7	14.8	15.0	8.4	8.0	8.3	8.01	7.98	7.98
5	31.8	32.0	31.9	15.7	14.7	15.0	8.5	8.0	8.3	8.01	7.98	7.98
10	31.9	32.4	31.9	15.8	14.7	14.9	8.4	8.0	8.6	8.00	7.97	7.99
20	31.8	31.9	31.9	15.7	14.7	14.9	8.4	8.0	8.3	7.99	7.97	7.99
40	31.8	31.8	31.8	15.8	14.7	15.0	8.4	7.9	8.3	8.01	7.92	8.00

	0	24	48
WQ Readings:	~~AC~~/EL	RT	DM
Dilutions made by:	EL		

Technician Initials:

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

Comments:
0 hrs:
24 hrs:
48 hrs:

QC Check: RH 7/31/19

Final Review: ~~VP~~ 8/1/19

Marine Chronic Bioassay | **Larval Development Worksheet**

Client: Internal / CuCl2
Test No.: 190710 msdv
Test Species: *Mytilus galloprovincialis*
Animal Source: Mission Bay
Date Received: 5/29/19 13A+B combined tank
Test Chambers: 30 mL glass shell vials
Sample Volume: 10 mL

Start Date/Time: 7/10/2019 1515
End Date/Time: 7/12/2019 1535
Technician Initials: AC / EG

Spawn Information

First Gamete Release Time: 1140

Sex	Number Spawning
Male	6
Female	2 MB, 1 Cat

Gamete Selection

Sex	Beaker Number(s)	Condition (sperm motility, egg density, color, shape, etc.)
Male	1	good density + motility
Female 1	MB1	good density + color
Female 2	MB2	light color, good density, chain
Female 3	Cat 1	very orange, excellent density

Egg Fertilization Time: 1230

Embryo Stock Selection

Stock Number	% of embryos at 2-cell division stage
Female 1	100
Female 2	90
Female 3	80

Stock(s) chosen for testing: 1

Embryo Inoculum Preparation

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

Number Counted:		
	27	33
	27	25
	32	32
	32	40
	25	44

Mean: 31.7

Mean 31.7 X 50 = 1,585 embryos/ml

Initial Density: 1585 / Desired Final Density: 300 (to inoculate with 0.5 ml) = 5.28 (dilution factor)

When mean percent dividing is ≥ 90, 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

Rep	No. Dividing	Total	% Dividing	Mean % Dividing
TØ A	219	219	100	100
TØ B	261	261	100	
TØ C	232	232	100	
TØ D	211	211	100	
TØ E	233	233	100	
TØ F	238	238	100	

48-h QC: 229/236 = 97%

Comments: $\bar{x}$ = 232.

QC Check: RH 7/31/19

Final Review: KFP 8/1/19

Appendix E
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 <u>below</u> 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 <u>above</u> 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% <u>mortality</u> 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 <u>temperature</u> 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 <u>salinity</u> 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.