POSEIDON WATER

Appendix G
Acute Toxicity Study

Renewal of NPDES CA0109223
Carlsbad Desalination Project

Nautilus Environmental

Poseidon Acute Salinity Tolerance Toxicity Test Results

Prepared for: Poseidon Water
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Prepared by: Nautilus Environmental
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Report Submitted: July 29, 2015, revised September 3, 2015

Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053-001). 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 EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

Results verified by: Adrienne Cibar

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INTRODUCTION

Acute survival bioassay screening tests were conducted to determine the sensitivity of two marine organisms to salinity increases above ambient seawater levels. Testing was performed to satisfy acute salinity tolerance requirements described in the Poseidon Resources [Channelside] LP (Poseidon), Carlsbad Desalination Project permit that was adopted in 2006 (Order No. R9-2006-0065).

Poseidon has contracted with Nautilus Environmental (Nautilus) to conduct the salinity studies. Acute testing was conducted using the mysid shrimp (*Americamysis bahia),* and Pacific topsmelt *(Atherinops affinis)* at Nautilus in San Diego, California in February and March, 2015. This round of testing was performed as confirmation of results from previous salinity-related toxicity threshold studies conducted by Weston Solutions, Inc. in 2007.

MATERIALS AND METHODS

Test Material

Test material consisted of hypersaline brine (HSB) prepared at Nautilus using methods described in United States Environmental Protection Agency (USEPA) protocols. Briefly, natural seawater collected from the intake at Scripps Institution of Oceanography (SIO) was filtered through a 20-μm in-line filter. Filtered seawater was then partially frozen in a -20 degrees Celsius (°C) freezer overnight. The remaining liquid (now concentrated in salinity) was decanted from the ice, which is composed mainly of fresh water. The HSB used for this study was approximately twice the salinity of ambient seawater. HSB was added to natural seawater to achieve the desired salinity test concentrations.

Acute Screening Bioassay

The study was performed in accordance with the United States Environmental Protection Agency (USEPA) protocol “Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms” (EPA/821/R-02/012, October 2002). Test methods are summarized in Table 1. All test parameters were equal for both species unless otherwise specified in the table.

Table 1. Acute Test Specifications

Test Organisms, Age:	*Americamysis bahia* (mysid shrimp), 4-5 days old at initiation *Atherinops affinis* (Pacific topsmelt), 12-15 days old at initiation
Test Period:	Mysid Tests: 02/05/2015, 15:45 to 02/09/2015, 13:50 03/03/2015, 15:45 to 03/07/2015, 14:30 Topsmelt Tests: 02/10/2015, 13:40 to 02/14/2015, 11:50 03/05/2015, 15:50 to 03/09/2015, 14:40
Test Organism Source:	Aquatic Biosystems, Inc. (Fort Collins, Colorado)
Organism Acclimation:	Acclimated to laboratory seawater (source: Scripps Institution of Oceanography intake, held at Nautilus in a laboratory flow-through system with a 20-µm in-line fiber filter and a chiller unit). Salinity approximately 33 parts per thousand (ppt).
Protocol:	USEPA Acute Manual (EPA/821/R-02/012, 2002) 96-hr static-renewal test; 80% test solution renewal at 48 hours
Control Water:	Lab Control: laboratory seawater Brine Control: hypersaline brine and de-ionized (DI) water were added to natural seawater at the highest brine volume tested
Aeration:	None
Test Concentrations:	February: 38, 40, 42, and 44 ppt, plus lab and brine controls March: 40, 42, 43, and 44 ppt, plus lab and brine controls
Number of Replicates, Organisms per Replicate:	6 replicates, 5 animals per replicate
Test Temperature:	Mysid Tests: 25 ±1 °C Topsmelt Tests: 20 ±1 °C
Feeding:	Mysid Tests: *Artemia* nauplii, twice daily Topsmelt Tests*: Artemia* nauplii, once daily
Test Acceptability:	Mean survival must be 90% or greater in the control
Statistical Analysis Software:	GraphPad Prism, v. 4.02 and CETIS version 1.8.7.20

Data Analysis and Reporting

Toxicity test responses were statistically evaluated using the Comprehensive Environmental Toxicity Information System™ (CETIS) program by Tidepool Scientific Software according to flowchart specifications provided in method guidance (USEPA 2002). Results were used to calculate the No Observed Effect Concentration (NOEC) and Lowest Observed Effect Concentration (LOEC) values. Since we are interested in effects relative to ambient seawater salinity, organism performance in the test concentrations was compared to that in the lab control rather than the brine control. In cases where the brine control result was less than lab control, a statistical comparison was made between the two controls to ensure no adverse effects were produced due to the addition of HSB.

Additionally, for a more robust analysis, data for each salinity concentration were analyzed using the Test of Significant Toxicity (TST) t-test approach specified in the National Pollutant

Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA 2010). The TST applies a modified t-test that takes into account both the statistical power of the test and magnitude of biological effects in determining the presence of a response. Results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST.

RESULTS

There were no statistically significant effects observed for Pacific topsmelt survival at any of the salinity concentrations tested during both rounds of testing. A NOEC of 44 ppt and a LOEC of >44 ppt is reported for this species for both rounds of testing.

For the test initiated February 5, 2015 survival of mysid shrimp in the highest test concentration (44 ppt) was 86.7 percent; this result was significantly decreased relative to the lab control according to the TST but was not statistically significant using statistical methods outlined in the statistical flowchart of the EPA 2002 test protocol. The TST resulted in a NOEC of 42 and a LOEC of 44, while the EPA 2002 flowchart method resulted in a NOEC of 44 and a LOEC of >44. During the March 2015 testing, no statistically significant effects were observed for mysid shrimp survival at any of the salinity concentrations tested, resulting in a NOEC of 44 and a LOEC of >44.

Mean survival results for the February and March 2015 tests for both species are presented in Figure 1. Tabular summaries of mean test results for the mysid shrimp and Pacific topsmelt toxicity tests are provided in Tables 2 and 3. A summary of the statistical analysis results for both species is provided in Table 4. Detailed toxicity test results and statistical summaries are provided in Appendix A.

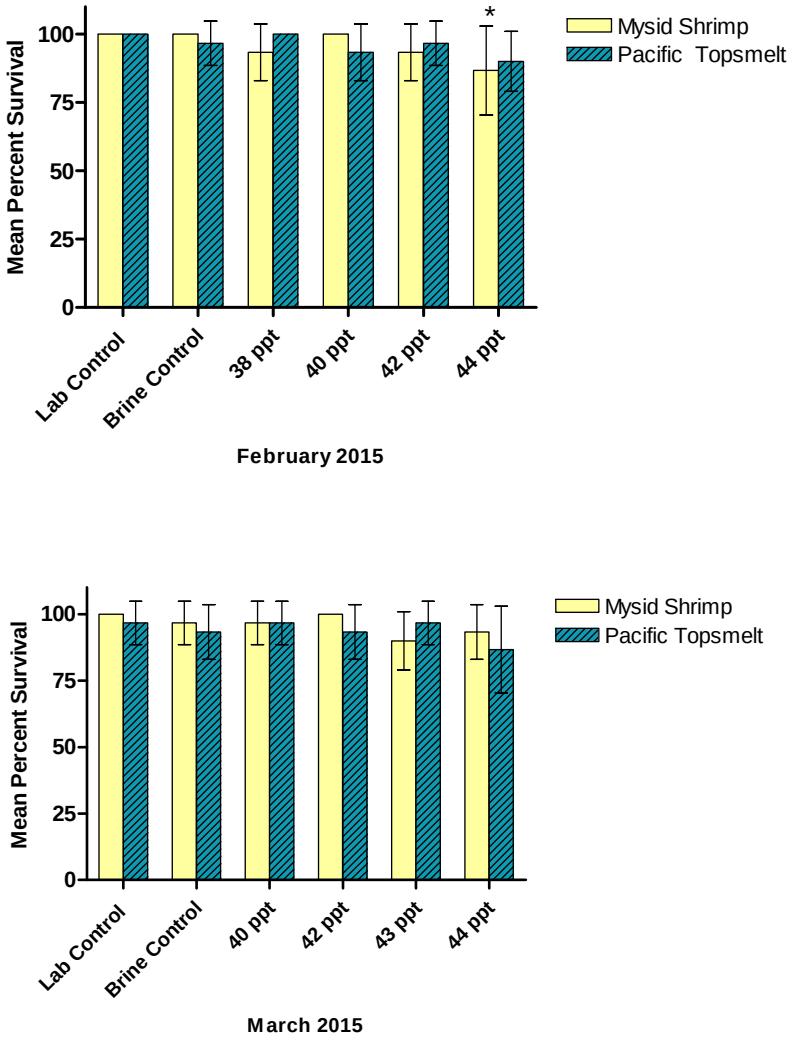


Figure 1. Summary of acute survival results for mysid and topsmelt tests conducted in February and March 2015 (mean ± SD). *The 44 ppt concentration in the February 2015 mysid test was statistically significant according to the TST, but was not significant using the USEPA 2002 flowchart statistical method (Steel Many-One Rank Sum Test).

Table 2. Summary of 96-hr Acute Survival Toxicity Test Results – February 2015

Test Concentration (ppt)	Mysid Shrimp			Pacific Topsmelt		
	Mean Survival (%)	Standard Deviation	Percent Effect[a]	Mean Survival (%)	Standard Deviation	Percent Effect[a]
Brine Control	100	0.0	0.0	97	8.2	0.0
Lab Control	100	0.0	0.0	100	0.0	-3.5
38	93	10	6.7	100	0.0	-3.5
40	100	0.0	0.0	93	10	3.5
42	93	10	6.7	97	8.2	0.0
44	**87***	16	13	90	11	6.9

n=6

[a] Percent Effect = ((Mean Lab Control Response – Mean Test Concentration Response)/Mean Lab Control Response)*100. A negative value indicates better organism performance in the test concentrations than in the lab control.

*** Bold** values indicate a statistically significant effect compared to the lab control using the Test of Significant Toxicity calculation. This value was not found to be statistically significant using the traditional EPA flowchart statistical approach.

Table 3. Summary of 96-hr Acute Survival Toxicity Test Results – March 2015

Test Concentration (ppt)	Mysid Shrimp			Pacific Topsmelt		
	Mean Survival (%)	Standard Deviation	Percent Effect[a]	Mean Survival (%)	Standard Deviation	Percent Effect[a]
Brine Control	97	8.2	0.0	93	10	0.0
Lab Control	100	0.0	-3.5	97	8.2	-3.6
40	97	8.2	0.0	97	8.2	-3.6
42	100	0.0	-3.5	93	10	0.0
43	90	11	6.9	97	8.2	-3.6
44	93	10	3.5	87	16	7.1

n=6

[a] Percent Effect = ((Mean Lab Control Response – Mean Test Concentration Response)/Mean Lab Control Response)*100. A negative value indicates better organism performance in the test concentrations than in the lab control.

Table 4. Summary of Statistical Results for 96-hr Acute Mysid Shrimp and Pacific Topsmelt Toxicity Tests

Test Start Date	Statistical Test Used	NOEC (ppt)	LOEC (ppt)
Mysid Shrimp			
02/05/2015	Steel Many-One Rank Sum Test	44	>44
	Test of Significant Toxicity	42	44
03/03/2015	Steel Many-One Rank Sum Test	44	>44
	Test of Significant Toxicity	44	>44
Pacific Topsmelt			
02/10/2015	Steel Many-One Rank Sum Test	44	>44
	Test of Significant Toxicity	44	>44
03/05/2015	Steel Many-One Rank Sum Test	44	>44
	Test of Significant Toxicity	44	>44

NOEC = No Observed Effect Level; the highest test concentration resulting in no observed effect
LOEC = Lowest Observed Effect Level; the lowest test concentration resulting in an observed effect

QUALITY ASSURANCE

Laboratory and brine controls met the minimum test acceptability criterion of 90 percent mean survival. Variability among replicates was minimal, and the ability to detect a statistical difference was deemed appropriate. Additionally, appropriate alpha levels were used to calculate TST results for this test per the 2010 EPA TST Implementation Guidance.

The formula for the brine control on the dilution preparation worksheet was incorrect for both February acute tests. In error, the technician adjusted the salinity of the brine control with seawater instead of DI water. Therefore, the volume of brine in the brine control was below that in the highest test concentration and the addition of brine was not properly controlled for. However, dilutions for all test concentrations were prepared correctly and there were no effects in any of the topsmelt concentrations, and only in the highest test concentration for mysid shrimp. The statistically significant decrease in the 44 ppt concentration of the mysid test is similar to results from previous studies using this species (Philips, et al. 2012). Additionally, all test concentrations were compared to the seawater lab control for statistical analysis; therefore, this deviation should not affect the interpretation of results.

Although there were no deviations in temperature from internal standard operating procedures for these tests, some evaporation occurred in the test chambers, particularly in the mysid test which is conducted at 25°C. This resulted in the final salinity levels approximately 0.5 to 1.0 ppt

higher at the end of the test than at initiation in some concentrations. Regardless, no effects were observed except for the highest test concentration in the mysid test.

Reference Toxicant Test

The reference toxicant tests met the acceptability criterion for survival. Additionally, the median lethal concentrations (LC_{50}) calculated for all reference toxicant tests were within two standard deviations of the internal control chart means, indicating typical test organism sensitivity to copper. Reference toxicant results are summarized in Table 5, and provided in full in Appendix B. A glossary of qualifier codes used on raw datasheets is available in Appendix C.

Table 5. Summary of Reference Toxicant Statistical Results

Test Endpoint	Test Start Date	NOEC (µg/L Cu)	LOEC (µg/L Cu)	LC_{50} value (µg/L Cu)	Mean LC_{50} ± 2 SD (µg/L Cu)
Mysid Shrimp 96-hour Survival	02/05/2015	100	200	258	212 ± 79.7
	03/03/2015	50	100	150	207 ± 80.6
Pacific Topsmelt 96-hour Survival	02/10/2015	100	200	91.0	121 ± 60.2
	03/05/2015	50	100	128	120 ± 61.7

NOEC = No Observed Effect Level; the highest test concentration resulting in no observed effect

LOEC = Lowest Observed Effect Level; the lowest test concentration resulting in an observed effect

LC_{50} = Lethal concentration 50, concentration expected to cause mortality to 50 percent of test organisms

Mean LC_{50} ± 2 SD = Historical mean of LC_{50} data for previous tests conducted at Nautilus, plus or minus two standard deviations

REFERENCES

Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Grand Canyon.

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

USEPA 2002. Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms. EPA/821/R-02/012, October 2002.

USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Weston Solutions. 2007. Toxicity Testing Results – Test Substance RO Concentrate Comp. January 17, 2007.

APPENDIX A

Raw Data and Statistical Analyses

Mysid Acute Salinity Tolerance Test
February 2015

CETIS Summary Report

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

Batch ID:	14-3042-9926	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	05 Feb-15 15:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Natural Seawater
Ending Date:	09 Feb-15 13:50	**Species:**	Americamysis bahia	**Brine:**	Frozen Seawater
Duration:	94h	**Source:**	Aquatic Biosystems, CO	**Age:**	5 d

Sample ID:	12-6866-9927	**Code:**	Salinity Study	**Client:**	Poseidon
Sample Date:	05 Feb-15	**Material:**	Natural Seawater	**Project:**	
Receive Date:	05 Feb-15	**Source:**	Poseidon		
Sample Age:	16h	**Station:**	Nautilus Brine		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-4638-4426	96h Survival Rate	44	>44	NA	13.5%		Steel Many-One Rank Sum Test
17-1981-1937	96h Survival Rate	42	44	42.99	11.1%		TST-Welch's t Test

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	6	1	1	1	1	1	0	0	0.0%	0.0%
0	Lab Control	6	1	1	1	1	1	0	0	0.0%	0.0%
38		6	0.9333	0.8249	1	0.8	1	0.04216	0.1033	11.07%	6.67%
40		6	1	1	1	1	1	0	0	0.0%	0.0%
42		6	0.9333	0.8249	1	0.8	1	0.04216	0.1033	11.07%	6.67%
44		6	0.8667	0.6953	1	0.6	1	0.06667	0.1633	18.84%	13.33%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1	1	1	1	1	1
0	Lab Control	1	1	1	1	1	1
38		0.8	1	0.8	1	1	1
40		1	1	1	1	1	1
42		0.8	0.8	1	1	1	1
44		1	0.6	0.8	1	0.8	1

Mysid 96-h Acute Survival Test — Nautilus Environmental (CA)

Analysis ID: 17-1981-1937 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 17:09 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.8	11.1%	42	44	42.99	

TST-Welch's t Test

Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:10%)
Lab Control		38*	3.778	1.476	0.074	5	0.0065	CDF	Non-Significant Effect
		40*	0.2691	1.476		5	<0.1	CDF	Non-Significant Effect
		42*	3.778	1.476	0.074	5	0.0065	CDF	Non-Significant Effect
		44	1.467	1.476	0.114	5	0.1011	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.102643	0.02566075	4	1.946	0.1340	Non-Significant Effect
Error	0.3296548	0.01318619	25			
Total	0.4322978		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.342	4.177	0.0253	Equal Variances
Variances	Levene Equality of Variance	14.95	4.177	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8889	0.9031	0.0046	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
38		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%
40		6	1	1	1	1	1	1	0	0.0%	0.0%
42		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%
44		6	0.8667	0.6953	1	0.9	0.6	1	0.06667	18.84%	13.33%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
38		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%
40		6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
42		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%
44		6	1.189	0.9911	1.388	1.226	0.8861	1.345	0.07712	15.88%	11.59%

96h Survival Rate Detail

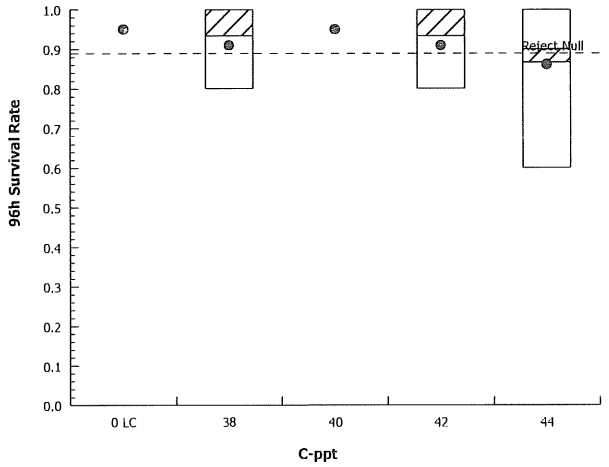
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1	1	1	1	1	1
38		0.8	1	0.8	1	1	1
40		1	1	1	1	1	1
42		0.8	0.8	1	1	1	1
44		1	0.6	0.8	1	0.8	1

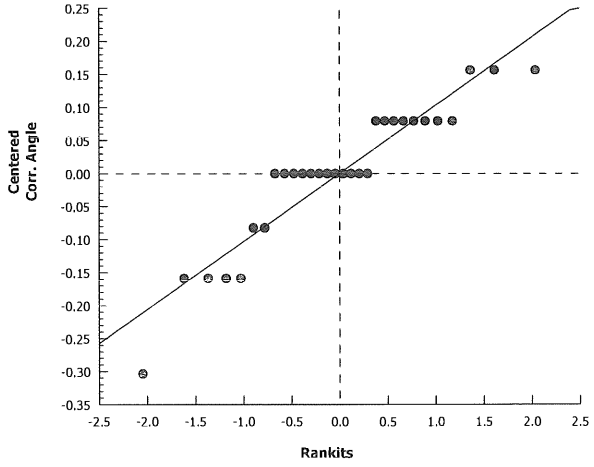
Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345
38		1.107	1.345	1.107	1.345	1.345	1.345
40		1.345	1.345	1.345	1.345	1.345	1.345
42		1.107	1.107	1.345	1.345	1.345	1.345
44		1.345	0.8861	1.107	1.345	1.107	1.345

Mysid 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	17-1981-1937	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:09	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Graphics





Mysid 96-h Acute Survival Test — Nautilus Environmental (CA)

Analysis ID: 05-4638-4426 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 17:09 **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	13.5%	44	>44	NA	

Steel Many-One Rank Sum Test

Control	vs	C-ppt	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		38	33	25	1	10	0.3906	Asymp	Non-Significant Effect
		40	39	25	1	10	0.8000	Asymp	Non-Significant Effect
		42	33	25	1	10	0.3906	Asymp	Non-Significant Effect
		44	30	25	1	10	0.2033	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.102643	0.02566075	4	1.946	0.1340	Non-Significant Effect
Error	0.3296548	0.01318619	25			
Total	0.4322978		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.342	4.177	0.0253	Equal Variances
Variances	Levene Equality of Variance	14.95	4.177	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8889	0.9031	0.0046	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
38		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%
40		6	1	1	1	1	1	1	0	0.0%	0.0%
42		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%
44		6	0.8667	0.6953	1	0.9	0.6	1	0.06667	18.84%	13.33%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
38		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%
40		6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
42		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%
44		6	1.189	0.9911	1.388	1.226	0.8861	1.345	0.07712	15.88%	11.59%

96h Survival Rate Detail

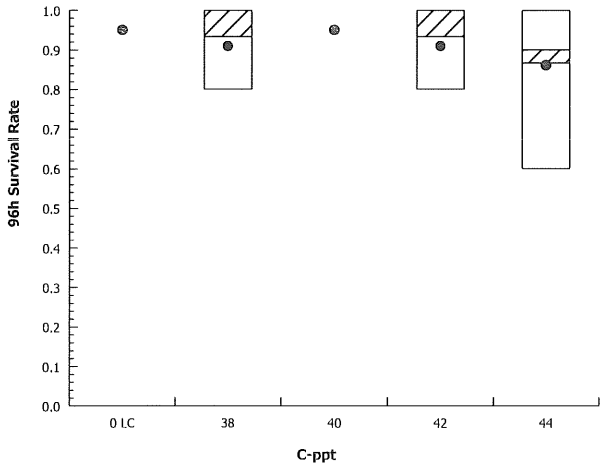
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1	1	1	1	1	1
38		0.8	1	0.8	1	1	1
40		1	1	1	1	1	1
42		0.8	0.8	1	1	1	1
44		1	0.6	0.8	1	0.8	1

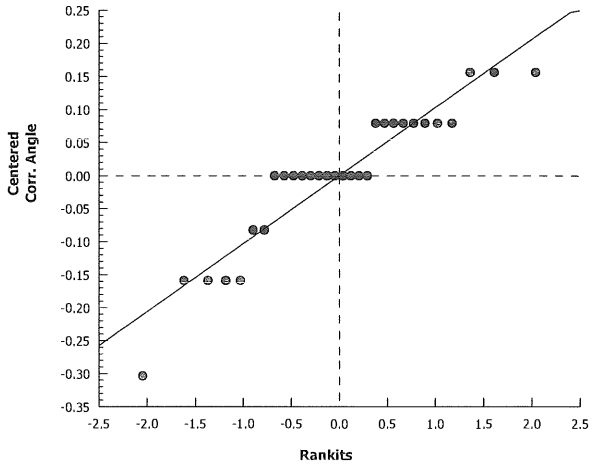
Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345
38		1.107	1.345	1.107	1.345	1.345	1.345
40		1.345	1.345	1.345	1.345	1.345	1.345
42		1.107	1.107	1.345	1.345	1.345	1.345
44		1.345	0.8861	1.107	1.345	1.107	1.345

Mysid 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	05-4638-4426	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:09	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1502-S025 | 08-9077-1636

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

Analysis ID:	08-2120-2616	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:10	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	Test Result
Angular (Corrected)	NA	C > T	NA	NA	Passes 96h survival rate

Wilcoxon Rank Sum Two-Sample Test

Control	vs	Control	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		Brine Control	39	NA	1	10	1.0000	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0	0	1	65540	<0.0001	Significant Effect
Error	0	0	10			
Total	0		11			

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
0	Brine Control	6	1	1	1	1	1	1	0	0.0%	0.0%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
0	Brine Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%

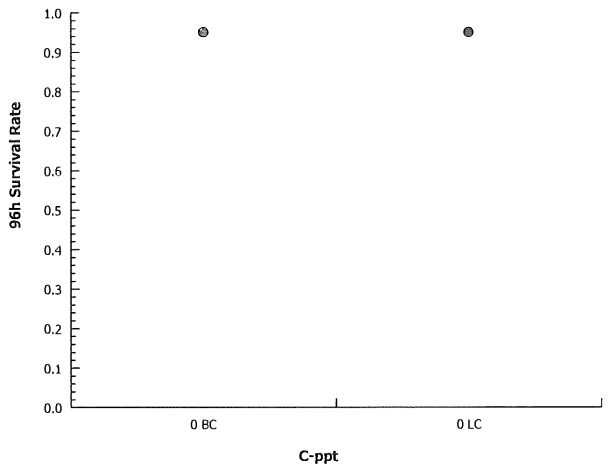
96h Survival Rate Detail

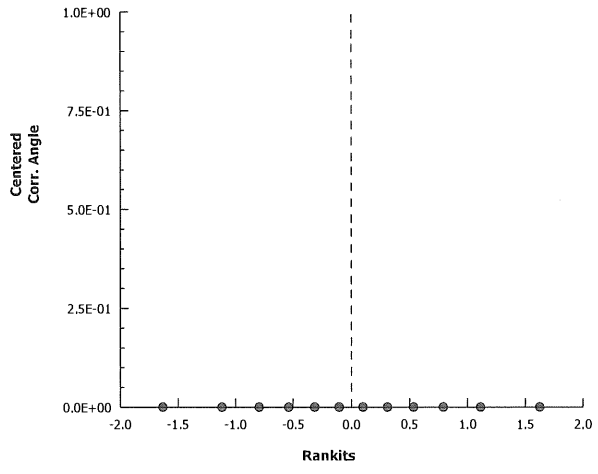
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1	1	1	1	1	1
0	Lab Control	1	1	1	1	1	1

Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1.345	1.345	1.345	1.345	1.345	1.345
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345

Graphics





 Analyst: AC QA: 3/16/15

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: Nautilus Frozen Brine
Test No.: 1502-5025

Test Species: *A. bahia*
Start Date/Time: 2/5/2015 1545
End Date/Time: 2/9/2015 1350

	Tech Initials				
	0	24	48	72	96
Counts:	NH	EG	NH	CH	AG
Readings:	NH	NH	NH	BK	BK
Dilutions made by:	NH/AW		NH		

Concentration ____ppt____	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	33.1	33.4	i33.0	33.6	34.1	25.7	25.6	i24.3	25.2	25.1	7.2	6.2	i6.2	6.1	5.8	7.97	8.00	i7.96	7.97	7.94
	B	5	5	5	5	5			f33.9					f24.8					f6.1					f7.95		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
Brine Control	A	5	5	5	5	5	34.1	34.3	i34.1	34.3	34.8	24.6	25.4	i24.2	25.2	25.3	7.4	6.0	i6.8	6.1	5.6	7.94	7.99	i7.98	8.00	7.97
	B	5	5	5	5	5			f34.8					f25.1					f6.0					f7.99		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
38.0 ppt	A	5	5	5	5	4	37.0	37.1	i38.0	38.4	38.8	25.3	25.4	i24.1	25.3	25.4	7.2	5.8	i6.7	5.9	5.6	7.94	8.00	i7.95	8.02	7.98
	B	5	5	5	5	5			f38.7					f25.2					f6.0					f8.01		
	C	5	5	5	4	4																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
40.0 ppt	A	5	5	5	5	5	40.0	40.1	i40.0	40.4	40.7	25.5	25.8	i24.2	25.2	25.5	7.2	5.6	i6.7	6.1	5.5	7.92	8.00	i7.95	8.03	8.00
	B	5	5	5	5	5			f40.8					f25.3					f6.6					f8.02		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
42.0 ppt	A	5	4	4	4	4	42.0	42.2	i42.0	42.3	42.9	25.4	25.8	i24.1	25.2	25.5	7.2	5.8	i6.8	6.0	5.9	7.90	8.00	i7.94	8.03	8.02
	B	5	4	4	4	4			f42.9					f25.2					f6.6					f8.03		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				

Initial Counts
QC'd by: AW

Animal Source/Date Received: 2/4/15 / ABS
Age at Initiation: 5 days

	Feeding Times				
	0	24	48	72	96
AM:		0845	0830	0920	0815
PM:	1600	1900	1530	1530	–

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y) / n) Hach Sension 5 Salinity Meter

QC Check: KB 2/12/15
Final Review: AL 4/29/15

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements & Test Organism Survival

Client: Poseidon
Sample ID: Nautilus Frozen Brine
Test No.: 1502-S025

Test Species: *A. bahia*
Start Date/Time: 2/5/2015 1545
End Date/Time: 2/9/2015 1350

Tech Initials	0	24	48	72	96
Counts:	NH	EG	NH	CH	AG
Readings:	NH	NH	NH	BK	BK
Dilutions made by:	NH/AL		NH		

Concentration ___ppt___	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
44.0 ppt	A	5	5	5	5	5	44.0	44.2	i 44.0	44.2	44.4	25.5	25.6	i 24.1	25.1	25.5	7.2	5.8	i 6.7	5.9	5.8	7.85	8.00	i 7.94	8.06	8.02
(a)	B	5	5	5	5	3			f 44.0					f 25.1					f 5.8					f 8.07		
	C	5	5	5	5	4																				
	D	5	5	5	5	5																				
	E	5	5	4	4	4																				
	F	5	5	5	5	5																				
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
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	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5	KB	KB																						

2/12/15

Initial Counts QC'd by: AW

Animal Source/Date Received: 2/4/15 / ABS

Age at Initiation: 5 days

Feeding Times	0	24	48	72	96
AM:		0845	0830	0920	0815
PM:	1600	1520	1530	1530	–

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y) / n) Hach Sension 5 Salinity Meter
(a) Subsample diluted w/ DI at 1:1 ratio to obtain salinity measurement for all days.

QC Check: KB 2/12/15

Final Review: AC 4/29/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon **Analyst:** NH

Sample ID: frozen seawater **Test Date:** 2/5/2015 (Initiation)

Test No: 1502-5025 **Test Type:** My-a (Acute Mysid)

Salinity of Seawater 33.1

Salinity of Brine 87.3 **Date of Brine used:** 1/7/15

Test Dilution Volume 2500 **Alkalinity of Brine Control:** 114 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Lab Control	100.0	250	NA	NA	2500
38.0	91.0	2274.0	0.10	226.0	2500
40.0	87.3	2181.7	0.15	318.3	2500
42.0	83.6	2089.5	0.20	410.5	2500
44.0	79.9	1997.2	0.25	502.8	2500

	DI Volume			
Brine Control	~~494.8~~	~~1.02~~	~~502.8~~	~~2500~~

AC Q18, Q21 4/28/15

1960.4

Total Brine Volume Required (ml): ~~1005.5~~

Q20 KB 2/12/15

QC Check: KB 2/12/15 **Final Review:** AC 2/25/15

Q21: The brine control formula was incorrect and the resulting salinity was high (44 ppt). The technician reduced salinity with seawater instead of DI. Therefore, the brine control did not accurately control for the maximum addition of brine. See QA section of report.

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon **Analyst:** NH

Sample ID: frozen seawater **Test Date:** 2/5/2015 (Renewal)

Test No: 1502-5025 **Test Type:** My-a (Acute mysid)

Salinity of Seawater 33.0

Salinity of Brine 90.9 **Date of Brine used:** 12/8/14

Test Dilution Volume 1500 **Alkalinity of Brine Control:** 108 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
Lab Control	100.0	250	NA	NA	1500
38.0	91.4	1370.5	0.09	129.5	1500
40.0	87.9	1318.7	0.14	181.3	1500
42.0	84.5	1266.8	0.18	233.2	1500
44.0	81.0	1215.0	0.23	285.0	1500

	DI Volume			
Brine Control	~~303.8~~	~~0.94~~	285.0	~~1500~~

AC Q18Q21 4/29/15

Total Brine Volume Required (ml): **1114.0**

QC Check: KB 2/12/15 **Final Review:** AQ 2/25/15

Q21: The brine control formula was incorrect and the resulting salinity was high. The technician ~~reduced~~ adjusted salinity with seawater instead of DI. Therefore, the brine control did not accurately control for the maximum addition of brine. There was limited volume of the brine used for the initiation.

Q21:

Topsmelt Acute Salinity Tolerance Test
February 2015

CETIS Summary Report

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	09-5191-8844	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	10 Feb-15 13:40	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Natural Seawater
Ending Date:	14 Feb-15 11:50	**Species:**	Atherinops affinis	**Brine:**	Frozen Seawater
Duration:	94h	**Source:**	Aquatic Biosystems, CO	**Age:**	15 d

Sample ID:	18-6431-5094	**Code:**	Salinity Study	**Client:**	Poseidon
Sample Date:	10 Feb-15	**Material:**	Natural Seawater	**Project:**	
Receive Date:	10 Feb-15	**Source:**	Poseidon		
Sample Age:	14h	**Station:**	Nautilus Brine		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-0664-6286	96h Survival Rate	44	>44	NA	11.5%		Steel Many-One Rank Sum Test
08-8280-6545	96h Survival Rate	44	>44	NA	8.97%		TST-Welch's t Test

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	0.0%
0	Lab Control	6	1	1	1	1	1	0	0	0.0%	-3.45%
38		6	1	1	1	1	1	0	0	0.0%	-3.45%
40		6	0.9333	0.8249	1	0.8	1	0.04216	0.1033	11.07%	3.45%
42		6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	0.0%
44		6	0.9	0.785	1	0.8	1	0.04472	0.1095	12.17%	6.9%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1	1	1	0.8	1	1
0	Lab Control	1	1	1	1	1	1
38		1	1	1	1	1	1
40		1	1	1	0.8	0.8	1
42		1	0.8	1	1	1	1
44		1	0.8	0.8	1	0.8	1

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 20-0664-6286 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 17:05 **Analysis:** Nonparametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	11.5%	44	>44	NA	

Steel Many-One Rank Sum Test

Control	vs	C-ppt	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		38	39	25	1	10	0.8000	Asymp	Non-Significant Effect
		40	33	25	1	10	0.3906	Asymp	Non-Significant Effect
		42	36	25	1	10	0.6101	Asymp	Non-Significant Effect
		44	30	25	1	10	0.2033	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06426895	0.01606724	4	1.932	0.1363	Non-Significant Effect
Error	0.207929	0.008317159	25			
Total	0.2721979		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.269	4.177	0.0275	Equal Variances
Variances	Levene Equality of Variance	16.34	4.177	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8773	0.9031	0.0025	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
38		6	1	1	1	1	1	1	0	0.0%	0.0%
40		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%
42		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	3.33%
44		6	0.9	0.785	1	0.9	0.8	1	0.04472	12.17%	10.0%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
38		6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
40		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%
42		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	2.95%
44		6	1.226	1.089	1.363	1.226	1.107	1.345	0.05325	10.64%	8.85%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1	1	1	1	1	1
38		1	1	1	1	1	1
40		1	1	1	0.8	0.8	1
42		1	0.8	1	1	1	1
44		1	0.8	0.8	1	0.8	1

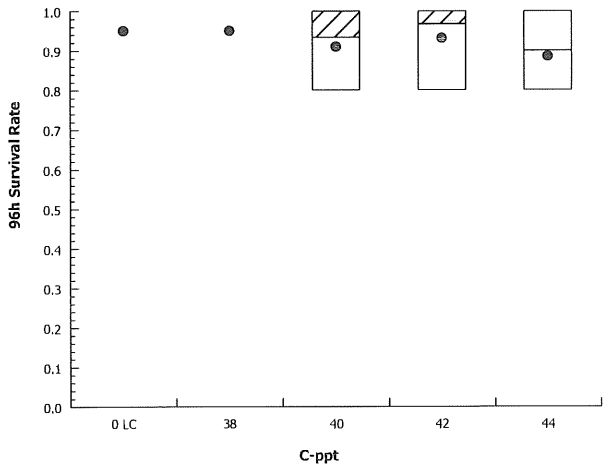
Angular (Corrected) Transformed Detail

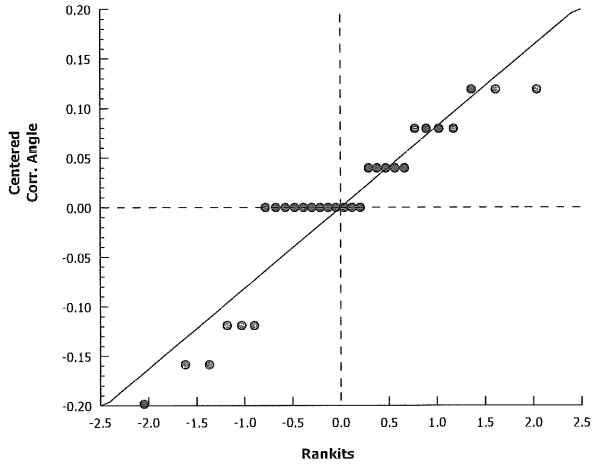
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345
38		1.345	1.345	1.345	1.345	1.345	1.345
40		1.345	1.345	1.345	1.107	1.107	1.345
42		1.345	1.107	1.345	1.345	1.345	1.345
44		1.345	1.107	1.107	1.345	1.107	1.345

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	20-0664-6286	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:05	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 08-8280-6545 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 17:05 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.8	8.97%	44	>44	NA	

TST-Welch's t Test

Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:10%)
Lab Control		38*	0.2691	NA			<0.1		Non-Significant Effect
		40*	3.778	1.476	0.074	5	0.0065	CDF	Non-Significant Effect
		42*	5.779	1.476	0.059	5	0.0011	CDF	Non-Significant Effect
		44*	2.817	1.476	0.079	5	0.0186	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06426895	0.01606724	4	1.932	0.1363	Non-Significant Effect
Error	0.207929	0.008317159	25			
Total	0.2721979		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.269	4.177	0.0275	Equal Variances
Variances	Levene Equality of Variance	16.34	4.177	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8773	0.9031	0.0025	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
38		6	1	1	1	1	1	1	0	0.0%	0.0%
40		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%
42		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	3.33%
44		6	0.9	0.785	1	0.9	0.8	1	0.04472	12.17%	10.0%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
38		6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
40		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%
42		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	2.95%
44		6	1.226	1.089	1.363	1.226	1.107	1.345	0.05325	10.64%	8.85%

96h Survival Rate Detail

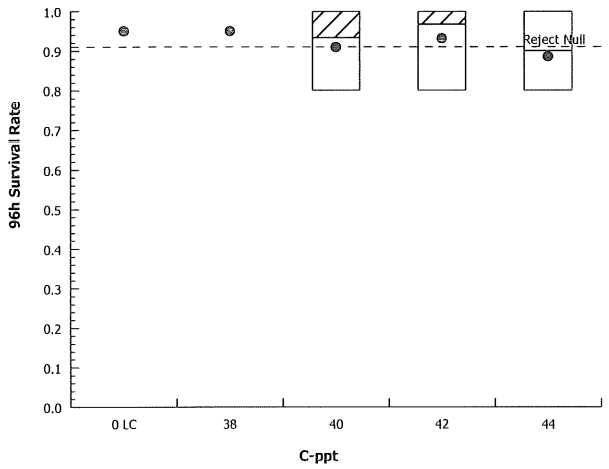
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1	1	1	1	1	1
38		1	1	1	1	1	1
40		1	1	1	0.8	0.8	1
42		1	0.8	1	1	1	1
44		1	0.8	0.8	1	0.8	1

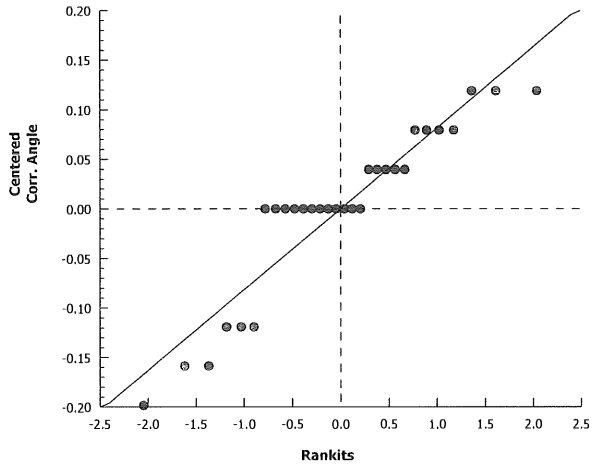
Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345
38		1.345	1.345	1.345	1.345	1.345	1.345
40		1.345	1.345	1.345	1.107	1.107	1.345
42		1.345	1.107	1.345	1.345	1.345	1.345
44		1.345	1.107	1.107	1.345	1.107	1.345

Pacific Topsmelt 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	08-8280-6545	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:05	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Graphics





Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	09-8475-5533	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:11	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	8.59%	Passes 96h survival rate

Wilcoxon Rank Sum Two-Sample Test

Control	vs	Control	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		Brine Control	36	NA	1	10	0.5000	Exact	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.004725658	0.004725658	1	1	0.3409	Non-Significant Effect
Error	0.04725658	0.004725658	10			
Total	0.05198224		11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1	10.04	0.3409	Equal Variances
Variances	Levene Equality of Variance	6.25	10.04	0.0314	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.5612	0.8025	<0.0001	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
0	Brine Control	6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	3.33%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
0	Brine Control	6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	2.95%

96h Survival Rate Detail

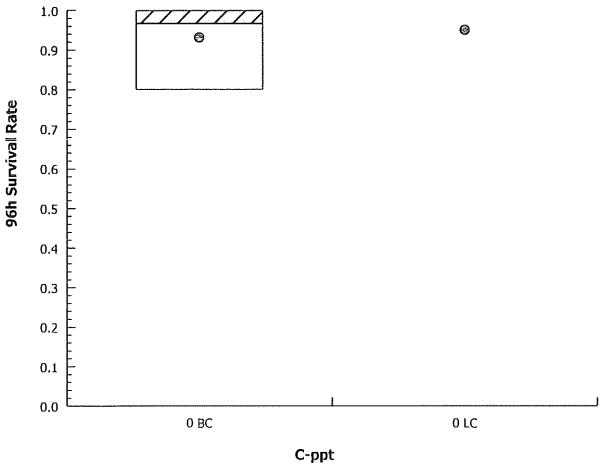
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1	1	1	0.8	1	1
0	Lab Control	1	1	1	1	1	1

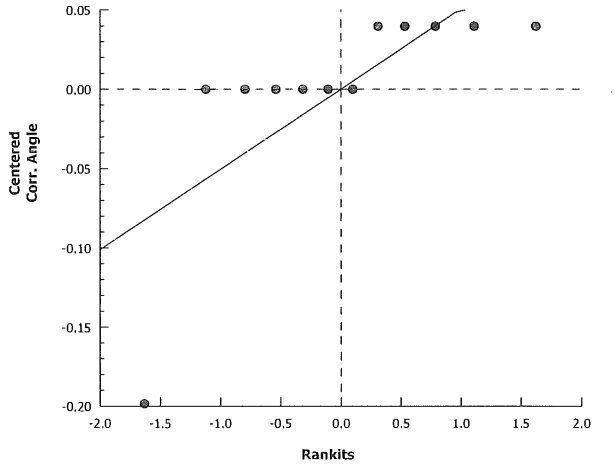
Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1.345	1.345	1.345	1.107	1.345	1.345
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345

Pacific Topsmelt 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	09-8475-5533	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 17:11	**Analysis:**	Nonparametric-Two Sample	**Official Results:**	Yes

Graphics





Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: Frozen SW Brine
Test No.: 1502-S026

Test Species: *A. affinis*
Start Date/Time: 2/10/2015 1340
End Date/Time: 2/14/2015 1150

Tech Initials	0	24	48	72	96
Counts:	AP	NH	NH	NH	AB
Readings:	NH	NH	AB	NH	AB
Dilutions made by:	NH		NH		

Concentration (AC - % ppt 418 2/10)	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	5	5	5	5	33.0	33.1	i 33.0	33.4	33.9	20.0	20.8	i 19.0	20.6	20.6	7.4	6.6	i 8.1	6.3	6.4	7.96	7.80	i 8.00	7.86	7.84
	B	5	5	5	5	5			f 33.4					f 20.9					f 6.3					f 7.89		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
Brine Control	A	5	5	5	5	5	34.0	34.2	i 34.1	34.3	34.8	20.0	20.8	i 19.1	20.8	20.7	7.4	6.3	i 8.1	6.3	6.4	7.96	7.81	i 8.01	7.86	7.88
	B	5	5	5	5	5			f 33.1					f 20.5					f 6.3					f 7.88		
	C	5	5	5	5	5																				
	D	5	5	5	5	4																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
38.0 ppt	A	5	5	5	5	5	38.0	38.1	i 38.0	38.2	38.6	20.1	20.7	i 19.1	20.8	20.8	7.4	6.1	i 8.0	6.3	6.3	7.94	7.82	i 7.98	7.90	7.92
	B	5	5	5	5	5			f 38.4					f 20.7					f 6.2					f 7.91		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5			40.0																	
40.0 ppt	A	5	5	5	5	5	40.0	40.2	i ~~34.4~~	40.1	40.6	19.9	20.7	i 19.1	20.8	20.9	7.3	6.1	i 8.0	6.3	6.3	7.93	7.83	i 7.97	7.91	7.90
	B	5	5	5	5	5			f 40.4	NH [illegible]				f 20.6					f 6.1					f 7.91		
	C	5	5	5	5	5				2/12/15																
	D	5	5	5	5	4																				
	E	5	4	4	4	4																				
	F	5	5	5	5	5																				
42.0 ppt	A	5	5	5	5	5	42.0	42.2	i 42.0	42.3	43.6	19.9	20.5	i 19.2	20.6	20.4	7.3	6.0	i 8.0	6.2	5.8	7.91	7.82	i 7.94	7.91	7.90
	B	5	4	4	4	4			f 42.6					f 20.9					f 5.9					f 7.90		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				

Initial Counts QC'd by: NH

Animal Source/Date Received: 2/6/15 / ABS

Age at Initiation: 15d

Feeding Times	0	24	48	72	96
AM:		0820	0805	0915	0900
PM:	1505	—	—	—	—

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y / n) — y circled. Hach Sension 5 Salinity Meter

QC Check: KB 2/19/15

Final Review: [illegible] 3/16/15

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: Frozen seawater brine
Test No.: 1502-S026

Test Species: *A. affinis*
Start Date/Time: 2/10/2015 1340
End Date/Time: 2/14/2015 1150

Tech Initials	0	24	48	72	96
Counts:	AD	NH	NH	NA	AB
Readings:	NH	NH	AW	NH	AB
Dilutions made by:	NH		NH		

Concentration ~~%~~ ppt AF 2/10	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
44.0 ppt	A	5	5	5	5	5	44.0	44.2	i 44.0	44.2	45.4	19.9	20.5	i 19.2	20.4	20.2	7.3	6.1	i 8.0	6.2	6.1	7.91	7.84	i 7.96	7.92	7.93
(a)	B	5	5	5	5	4			f 44.2					f 20.5					f 5.9					f 7.90		
	C	5	4	4	4	4																				
	D	5	5	5	5	5																				
	E	5	5	5	5	4																				
	F	5	5	5	5	3																				
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								

Initial Counts QC'd by: NA

Animal Source/Date Received: 2/6/15 / ABS

Age at Initiation: 15d

Feeding Times	0	24	48	72	96
AM:		1820	0805	0915	0900
PM:	1505	—	—	—	—

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one ((y) / n) Hach Sension5 Salinity Meter
(a) Subsample diluted ~~LT~~ w/DI at 1:1 ratio to obtain salinity measurement for all days (initialed, 3/16/15)

QC Check: KB 2/19/15

Final Review: [initials] 3/16/15

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon **Analyst:** NH

Sample ID: frozen seawater - initiation **Test Date:** 2/10/2015

Test No: 1502-5026 **Test Type:** Aa-a (topsmelt)

Salinity of Seawater 33.0

Salinity of Brine 86.1 **Date of Brine used:** 12/2/14

Test Dilution Volume 1500 **Alkalinity of Brine Control:** 107 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
34.0	100.0	**250**	NA	**NA**	1500
38.0	90.6	**1358.8**	0.10	**141.2**	1500
40.0	86.8	**1302.3**	0.15	**197.7**	1500
42.0	83.1	**1245.8**	0.20	**254.2**	1500
44.0	79.3	**1189.3**	0.26	**310.7**	1500

	DI Volume			
Brine Control	~~297.3~~	1.05	310.7	~~1500~~

AC Q18, Q20 4/29/15

Q20

Total Brine Volume Required (ml): **1214.7**

QC Check: KB 2/19/15 **Final Review:** AC 3/19/15

Q20- Brine control calculation incorrect. See QA section of report.

Marine Chronic Bioassay **Brine Dilution Worksheet**

Project: Poseidon **Analyst:** NH

Sample ID: frozen seawater - renewal **Test Date:** 2/10/2015

Test No: 1502-SQ26 **Test Type:** Aa-a (Topsmelt)

Salinity of Seawater 33.0

Salinity of Brine 85.2 **Date of Brine used:** 12/2/14

Test Dilution Volume 1500 **Alkalinity of Brine Control:** 111 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
34.0	100.0	**250**	NA	**NA**	1500
38.0	90.4	**1356.3**	0.11	**143.7**	1500
40.0	86.6	**1298.9**	0.15	**201.1**	1500
42.0	82.8	**1241.4**	0.21	**258.6**	1500
44.0	78.9	**1183.9**	0.27	**316.1**	1500

	DI Volume			
Brine Control	~~296.0~~	~~1.07~~	~~316.1~~	~~1500~~

AC Q18,Q21 4/29/15

Total Brine Volume Required (ml): **1235.6**

QC Check: KB 2/19/15 **Final Review:** AC 3/19/15

Q21: Brine control formula was incorrect and salinity was high (44ppt). ~~Da~~The technician reduced salinity with seawater instead of DI. Therefore, the brine control did not accurately control for the maximum addition of brine. See QA section of report.

Mysid Acute Salinity Tolerance Test
March 2015

CETIS Summary Report

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

Batch ID:	18-6763-0526	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	03 Mar-15 15:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Natural Seawater
Ending Date:	07 Mar-15 14:30	**Species:**	Americamysis bahia	**Brine:**	Frozen Seawater
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	4d

Sample ID:	15-5878-5286	**Code:**	1503-S014	**Client:**	Poseidon
Sample Date:	03 Mar-15	**Material:**	Natural Seawater	**Project:**	
Receive Date:	03 Mar-15	**Source:**	Poseidon		
Sample Age:	16h	**Station:**	Nautilus Brine		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-5966-3321	96h Survival Rate	44	>44	NA	11.5%		Steel Many-One Rank Sum Test
03-1884-4572	96h Survival Rate	44	>44	NA	8.71%		TST-Welch's t Test

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	0.0%
0	Lab Control	6	1	1	1	1	1	0	0	0.0%	-3.45%
40		6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	0.0%
42		6	1	1	1	1	1	0	0	0.0%	-3.45%
43		6	0.9	0.785	1	0.8	1	0.04472	0.1095	12.17%	6.9%
44		6	0.9333	0.8249	1	0.8	1	0.04216	0.1033	11.07%	3.45%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1	1	1	0.8	1	1
0	Lab Control	1	1	1	1	1	1
40		1	1	0.8	1	1	1
42		1	1	1	1	1	1
43		0.8	0.8	1	1	1	0.8
44		1	1	1	0.8	1	0.8

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

Analysis ID:	08-5966-3321	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:36	**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	11.5%	44	>44	NA	

Steel Many-One Rank Sum Test

Control	vs	C-ppt	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		40	36	25	1	10	0.6101	Asymp	Non-Significant Effect
		42	39	25	1	10	0.8000	Asymp	Non-Significant Effect
		43	30	25	1	10	0.2033	Asymp	Non-Significant Effect
		44	33	25	1	10	0.3906	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06426895	0.01606724	4	1.932	0.1363	Non-Significant Effect
Error	0.207929	0.008317159	25			
Total	0.2721979		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.269	4.177	0.0275	Equal Variances
Variances	Levene Equality of Variance	16.34	4.177	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8773	0.9031	0.0025	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
40		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	3.33%
42		6	1	1	1	1	1	1	0	0.0%	0.0%
43		6	0.9	0.785	1	0.9	0.8	1	0.04472	12.17%	10.0%
44		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
40		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	2.95%
42		6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
43		6	1.226	1.089	1.363	1.226	1.107	1.345	0.05325	10.64%	8.85%
44		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%

96h Survival Rate Detail

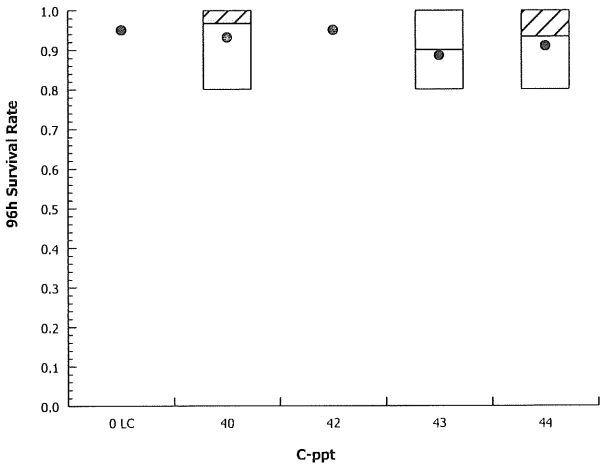
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1	1	1	1	1	1
40		1	1	0.8	1	1	1
42		1	1	1	1	1	1
43		0.8	0.8	1	1	1	0.8
44		1	1	1	0.8	1	0.8

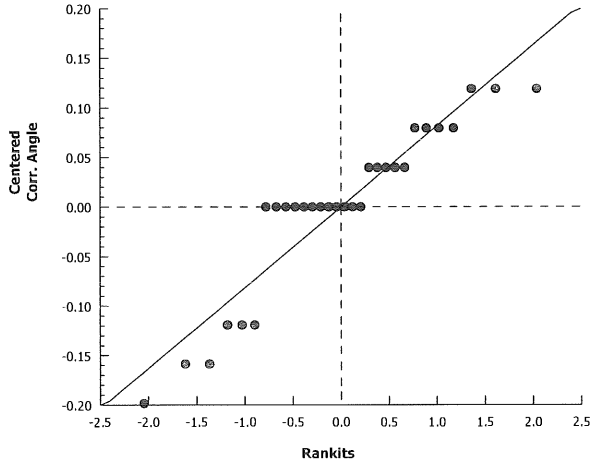
Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345
40		1.345	1.345	1.107	1.345	1.345	1.345
42		1.345	1.345	1.345	1.345	1.345	1.345
43		1.107	1.107	1.345	1.345	1.345	1.107
44		1.345	1.345	1.345	1.107	1.345	1.107

Mysid 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	08-5966-3321	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:36	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 03-1884-4572 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 11:37 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.8	8.71%	44	>44	NA	

TST-Welch's t Test

Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:10%)
Lab Control		40*	5.779	1.476	0.059	5	0.0011	CDF	Non-Significant Effect
		42*	0.2691	1.476		5	<0.1	CDF	Non-Significant Effect
		43*	2.817	1.476	0.079	5	0.0186	CDF	Non-Significant Effect
		44*	3.778	1.476	0.074	5	0.0065	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06426895	0.01606724	4	1.932	0.1363	Non-Significant Effect
Error	0.207929	0.008317159	25			
Total	0.2721979		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.269	4.177	0.0275	Equal Variances
Variances	Levene Equality of Variance	16.34	4.177	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8773	0.9031	0.0025	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1	1	1	1	1	1	0	0.0%	0.0%
40		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	3.33%
42		6	1	1	1	1	1	1	0	0.0%	0.0%
43		6	0.9	0.785	1	0.9	0.8	1	0.04472	12.17%	10.0%
44		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	6.67%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
40		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	2.95%
42		6	1.345	1.345	1.345	1.345	1.345	1.345	0	0.0%	0.0%
43		6	1.226	1.089	1.363	1.226	1.107	1.345	0.05325	10.64%	8.85%
44		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	5.9%

96h Survival Rate Detail

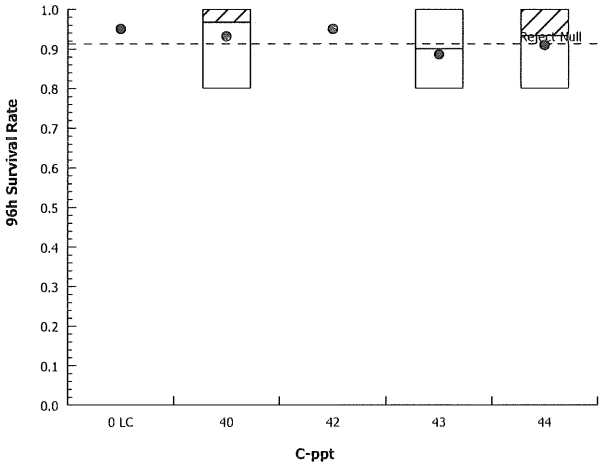
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1	1	1	1	1	1
40		1	1	0.8	1	1	1
42		1	1	1	1	1	1
43		0.8	0.8	1	1	1	0.8
44		1	1	1	0.8	1	0.8

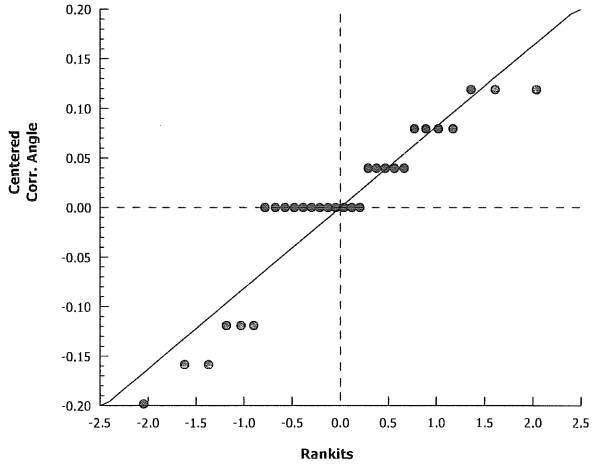
Angular (Corrected) Transformed Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.345	1.345	1.345	1.345	1.345	1.345
40		1.345	1.345	1.107	1.345	1.345	1.345
42		1.345	1.345	1.345	1.345	1.345	1.345
43		1.107	1.107	1.345	1.345	1.345	1.107
44		1.345	1.345	1.345	1.107	1.345	1.107

Mysid 96-h Acute Survival Test					Nautilus Environmental (CA)
Analysis ID:	03-1884-4572	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:37	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Graphics





TST Summary Sheet

Lab Name	Naut	**Client Name**	Pos
Test ID	1503-S015	**Test Species**	*A. bahia (mysid shrimp)*
Test Date	3/3/2015	**Test Type**	Acute
Test Duration	96h	**Endpoint**	Survival
Critical Conc.	43		

Statistic	Control	Critical Concentration
Percent Mean of Raw Data	1.00	0.90
Mean used in Calcuation (transformed)	1.35	1.23
Variance used in Calcuation (transformed)	0.000	0.017
Standard Deviation of Transformed Data	0.000	0.130
CV of Transformed Data	0.000	0.106
n	6	6

Mean % Effect at Critical Conc.

10.00

Calculated t-value	Degrees of Freedom	Table t-value	Percent Difference
2.8168	5	1.4759	

Results

Pass Sample is Non-toxic

Raw Data

Control Data		Critical Concentration Data	
No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)	No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)
5	5	5	4
5	5	5	4
5	5	5	5
5	5	5	5
5	5	5	5
5	5	5	4

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: brine - Nautilus frozen seawater
Test No.: 1503-SD14

Test Species: *A. bahia*
Start Date/Time: 3/3/2015 1545
End Date/Time: 3/7/2015 1430

Tech Initials	0	24	48	72	96
Counts:	EG	CH	EG	KS	AD
Readings:	AC	VS	AG	VS	AB
Dilutions made by:	AC		EG		

CH CH 3/5/15 Q18

Concentration ____ppt____	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab	A	5	5	5	5	5	33.3	33.6	i 33.2	34.1	35.3	26.0	25.0	i 25.8	24.9	24.4	6.9	6.3	i 6.6	5.7	6.6	7.96	7.98	i 8.02	7.89	7.96
Control	B	5	5	5	5	5			f 34.2					f 23.7	Q1				f 5.8					f 7.93		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
Brine	A	5	5	5	5	5	33.3	33.7	i 34.1	34.6	35.4	26.0	25.5	i 24.7	24.8	24.7	7.1	6.4	i 6.7	5.7	6.1	7.91	7.99	i 8.03	7.92	8.03
Control	B	5	5	5	5	5			f 34.0					f 24.0					f 5.6					f 7.94		
	C	5	5	5	5	5																				
	D	5	5	5	4	4																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
40 ppt	A	5	5	5	5	5	40.0	40.0	i 40.8	40.6	41.5	24.8	25.7	i 25.1	24.9	24.7	6.7	6.2	i 6.5	6.1	6.3	7.86	7.98	i 7.96	7.94	8.02
	B	5	5	5	5	5			f 40.3					f 24.2					f 5.5					f 7.97		
	C	5	5	4	4	4																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
42 ppt	A	5	5	5	5	5	42.0	42.1	i 42.0	42.6	42.3	25.8	25.8	i 25.3	24.8	25.0	6.7	6.4	i 6.5	5.3	5.8	7.89	7.98	i 7.94	7.94	7.99
	B	5	5	5	5	5			f 42.4					f 24.2					f 5.7					f 7.95		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	6	5																				
43 ppt	A	5	5	5	5	4	43.0	43.0	i 43.0	@ 44.2	@ 44.2	25.9	25.9	i 25.5	25.0	25.4	7.0	6.4	i 6.6	5.2	5.6	7.90	7.97	i 7.93	7.94	7.99
	B	5	5	4	4	4		@	f 43.6					f 24.5					f 5.5					f 7.98		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	4																				

Initial Counts QC'd by: VCR

Animal Source/Date Received: ABS | 3/3/15

Age at Initiation: 4d

Feeding Times	0	24	48	72	96
AM:		0820	0830	0830	0900
PM:	1445	1615	1520	1550	—

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

Organisms fed prior to initiation, circle one ((y) / n) Hach Sensions

@ Subsample diluted w/ DI water 1:1 to obtain salinity measurement Salinity meter VS 3/6/15

QC Check: KB 3/10/15

Final Review: AS 3/16/15

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: brine - Nautilus frozen seawater
Test No.: 1503-S014

Test Species: *A. bahia*
Start Date/Time: 3/3/2015 1545
End Date/Time: 3/7/2015 1430

Tech Initials	0	24	48	72	96
Counts:	EG	CH	EG	KS	AB
Readings:	AC	KS CH	CH	KS	AB
Dilutions made by:	AC		CH		

Concentration ____ppt____	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
44ppt	A	5	5	5	5	5	@ 44.0	@KS 44.0	i @ 44.0	@KS 44.0	@ 44.0	25.8	25.9	i 25.0	25.0	25.5	7.1	6.5	i 6.6	5.3	5.6	7.90	7.99	i 7.94	7.95	7.99
@	B	5	5	5	5	5			f @ 44.2					f 24.5					f 5.8					f 7.99		
	C	5	5	5	5	5																				
	D	5	5	5	5	4																				
	E	5	5	5	5	5																				
	F	5	4	4	4	4																				
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								
	A	5							i					i					i					i		
	B	5							f					f					f					f		
	C	5																								
	D	5																								
	E	5																								
	F	5																								

Initial Counts QC'd by: VCR Q18 KS 3/10/15

Animal Source/Date Received: ABS 3/3/15

Age at Initiation: 4d

Feeding Times	0	24	48	72	96
AM:		0820	0830	0830	0900
PM:	1145	1615	1500	1550	—

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one ((y) / n)
@ subsample diluted w/ DI water 1:1 to obtain salinity measurement KS 3/4/15

QC Check: KB 3/10/15

Final Review: B 3/16/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: CH

Sample ID: frozen seawater

Test Date: 3/3/15

Test No: 1503-5014

Test Type: mysid acute/ 48hr renewal test initiation and

Salinity of Seawater 33.1

Salinity of Brine 93.0

Test Dilution Volume 1500

Date of Brine used: ~~2/26/15~~ 12/24/14, 12/31/14, 1/2/15, 1/19/15, 2/11/15, 2/13/15 (a) CH 3/12/15 QB

Alkalinity of Brine Control: 111 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
33.1	100.0	**250**	NA	**NA**	1500
40.0	88.5	**1327.2**	0.13	**172.8**	1500
42.0	85.1	**1277.1**	0.17	**222.9**	1500
43.0	83.5	**1252.1**	0.20	**247.9**	1500
44.0	81.8	**1227.0**	0.22	**273.0**	1500

	DI Volume			
Brine Control	~~444.6~~ AC Q1e 4/29 494.0	~~0.61~~ 0.55 AC Q18 4/29	273.0	1500

Total Brine Volume Required (ml): ~~545.9~~ 1169.6 5/16/15

QC Check: KB 3/10/15

Final Review: AC 4/29/15

(a) Brine was mixed the day prior to test initiation from brine made on multiple dates in order to have enough volume for test initiation & 48hr renewal

Topsmelt Acute Salinity Tolerance Test
March 2015

CETIS Summary Report

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	01-9359-5620	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	05 Mar-15 15:50	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Natural Seawater
Ending Date:	09 Mar-15 14:40	**Species:**	Atherinops affinis	**Brine:**	Frozen Seawater
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	12d

Sample ID:	14-5497-7546	**Code:**	1503-S015	**Client:**	Poseidon
Sample Date:	05 Mar-15	**Material:**	Natural Seawater	**Project:**	
Receive Date:	05 Mar-15	**Source:**	Poseidon		
Sample Age:	16h	**Station:**	Nautilus Brine		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-6335-4659	96h Survival Rate	44	>44	NA	14.6%		Steel Many-One Rank Sum Test
21-3466-5940	96h Survival Rate	44	>44	NA	11.2%		TST-Welch's t Test

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	6	0.9333	0.8249	1	0.8	1	0.04216	0.1033	11.07%	0.0%
0	Lab Control	6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	-3.57%
40		6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	-3.57%
42		6	0.9333	0.8249	1	0.8	1	0.04216	0.1033	11.07%	0.0%
43		6	0.9667	0.881	1	0.8	1	0.03333	0.08165	8.45%	-3.57%
44		6	0.8667	0.6953	1	0.6	1	0.06667	0.1633	18.84%	7.14%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Brine Control	1	1	0.8	1	1	0.8
0	Lab Control	0.8	1	1	1	1	1
40		1	1	1	0.8	1	1
42		0.8	1	0.8	1	1	1
43		1	1	1	0.8	1	1
44		1	0.8	1	1	0.6	0.8

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 11-6335-4659 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 11:29 **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	14.6%	44	>44	NA	

Steel Many-One Rank Sum Test

Control	vs	C-ppt	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		40	39	25	2	10	0.8000	Asymp	Non-Significant Effect
		42	36	25	2	10	0.6101	Asymp	Non-Significant Effect
		43	39	25	2	10	0.8000	Asymp	Non-Significant Effect
		44	32.5	25	2	10	0.3556	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06132809	0.01533202	4	0.9684	0.4422	Non-Significant Effect
Error	0.395814	0.01583256	25			
Total	0.4571421		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.615	13.28	0.4605	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8174	0.9031	0.0001	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	0.0%
40		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	0.0%
42		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	3.45%
43		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	0.0%
44		6	0.8667	0.6953	1	0.9	0.6	1	0.06667	18.84%	10.34%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	0.0%
40		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	0.0%
42		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	3.04%
43		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	0.0%
44		6	1.189	0.9911	1.388	1.226	0.8861	1.345	0.07712	15.88%	8.9%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	0.8	1	1	1	1	1
40		1	1	1	0.8	1	1
42		0.8	1	0.8	1	1	1
43		1	1	1	0.8	1	1
44		1	0.8	1	1	0.6	0.8

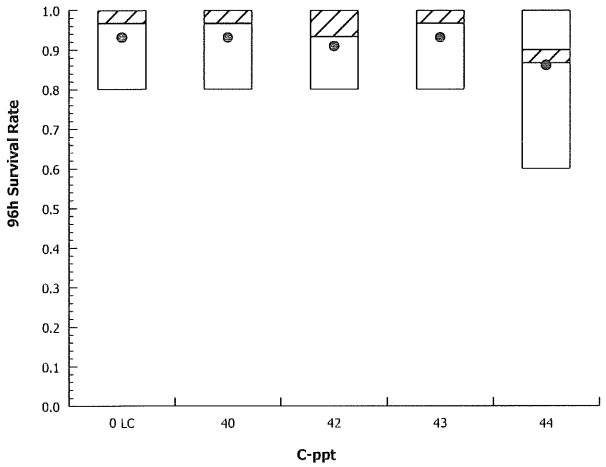
Angular (Corrected) Transformed Detail

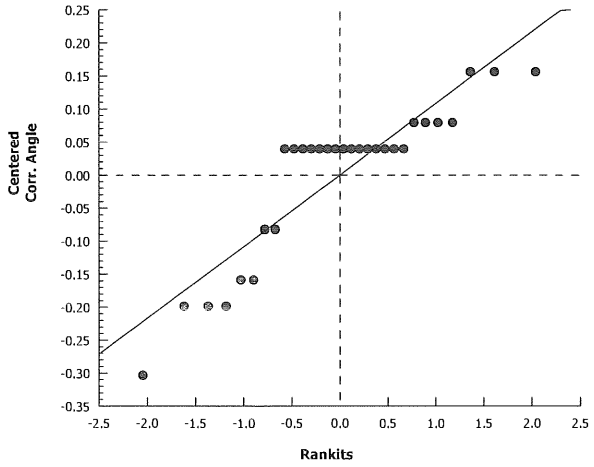
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.107	1.345	1.345	1.345	1.345	1.345
40		1.345	1.345	1.345	1.107	1.345	1.345
42		1.107	1.345	1.107	1.345	1.345	1.345
43		1.345	1.345	1.345	1.107	1.345	1.345
44		1.345	1.107	1.345	1.345	0.8861	1.107

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	11-6335-4659	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:29	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Pacific Topsmelt 96-h Acute Survival Test TST **Nautilus Environmental (CA)**

Analysis ID: 21-3466-5940 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Mar-15 11:30 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.8	11.2%	44	>44	NA	

TST-Welch's t Test

Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:10%)
Lab Control		40*	5.137	1.383	0.070	9	0.0003	CDF	Non-Significant Effect
		42*	3.728	1.397	0.083	8	0.0029	CDF	Non-Significant Effect
		43*	5.137	1.383	0.070	9	0.0003	CDF	Non-Significant Effect
		44*	1.737	1.44	0.120	6	0.0665	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06132809	0.01533202	4	0.9684	0.4422	Non-Significant Effect
Error	0.395814	0.01583256	25			
Total	0.4571421		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.615	13.28	0.4605	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8174	0.9031	0.0001	Non-normal Distribution

96h Survival Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	0.0%
40		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	0.0%
42		6	0.9333	0.8249	1	1	0.8	1	0.04216	11.07%	3.45%
43		6	0.9667	0.881	1	1	0.8	1	0.03333	8.45%	0.0%
44		6	0.8667	0.6953	1	0.9	0.6	1	0.06667	18.84%	10.34%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	0.0%
40		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	0.0%
42		6	1.266	1.137	1.395	1.345	1.107	1.345	0.0502	9.71%	3.04%
43		6	1.306	1.204	1.408	1.345	1.107	1.345	0.03969	7.45%	0.0%
44		6	1.189	0.9911	1.388	1.226	0.8861	1.345	0.07712	15.88%	8.9%

96h Survival Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	0.8	1	1	1	1	1
40		1	1	1	0.8	1	1
42		0.8	1	0.8	1	1	1
43		1	1	1	0.8	1	1
44		1	0.8	1	1	0.6	0.8

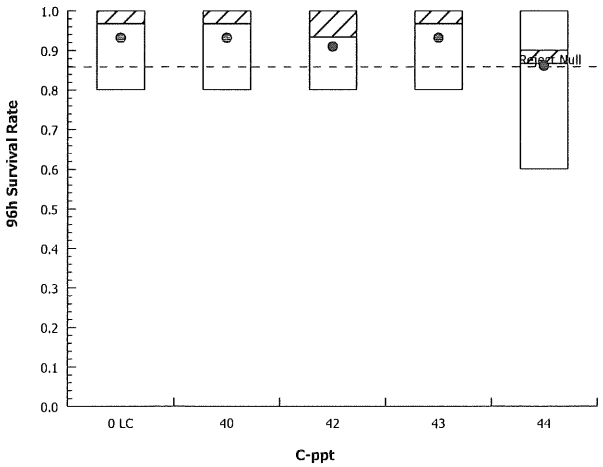
Angular (Corrected) Transformed Detail

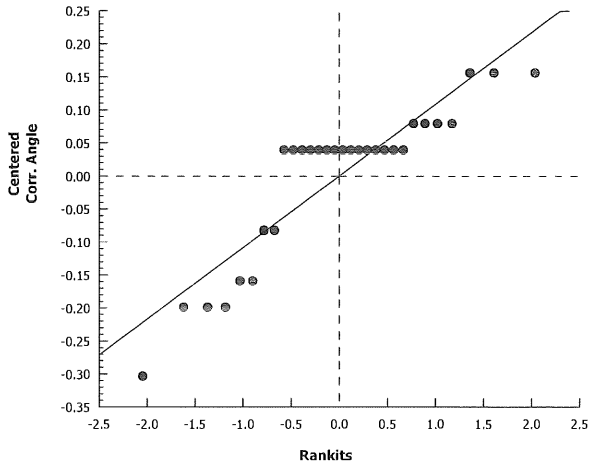
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6
0	Lab Control	1.107	1.345	1.345	1.345	1.345	1.345
40		1.345	1.345	1.345	1.107	1.345	1.345
42		1.107	1.345	1.107	1.345	1.345	1.345
43		1.345	1.345	1.345	1.107	1.345	1.345
44		1.345	1.107	1.345	1.345	0.8861	1.107

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	21-3466-5940	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:30	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Graphics





Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: brine - Nautilus frozen seawater
Test No.: 1503-S015

Test Species: *A. affinis*
Start Date/Time: 3/5/2015 1550
End Date/Time: 3/9/2015 1440

Tech Initials	0	24	48	72	96
Counts:	CH	KB	AO	AB	BK
Readings:	CH	KS	AO/AB	AB	AG
Dilutions made by:	CH		NH		

Concentration ___ppt___	Rep	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	A	5	4	4	4	4	32.9	33.0	i 33.0	33.1	33.2	19.9	20.1	i 19.2	19.5	19.8	7.6	6.6	i 7.8	6.7	6.8	8.05	7.93	i 8.04	7.99	7.91
	B	5	5	5	5	5			f 33.0					f 20.7					f 6.4					f 7.89		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
Brine Control	A	5	5	5	5	5	32.9	33.0	i 33.1	33.4	33.4	19.9	19.5	i 19.3	19.6	19.6	7.6	6.8	i 7.8	6.7	6.8	8.09	7.94	i 8.06	8.00	7.99
	B	5	5	5	5	5			f 33.1					f 20.7					f 6.3					f 7.89		
	C	5	4	4	4	4																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	4	4	4	4																				
40 ppt	A	5	5	5	5	5	40.0	40.0	i 40.0	40.2	40.2	19.8	19.9	i 19.8	19.6	19.5	7.2	6.5	i 7.2	6.7	6.9	8.01	7.94	i 8.03	8.01	8.01
	B	5	5	5	5	5			f 40.0					f 20.6					f 6.2					f 7.92		
	C	5	5	5	5	5																				
	D	5	4	4	4	4																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				
42 ppt	A	5	4	4	4	4	42.0	42.0	i 42.0	42.3	42.3	19.7	19.9	i 19.7	19.6	19.5	7.2	6.5	i 7.2	6.7	7.0	8.01	7.94	i 8.02	8.02	8.02
	B	5	5	5	5	5			f 42.0					f 20.7					f 6.2					f 7.94		
	C	5	4	4	4	4																				
	D	5	5	5	5	5																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5				Q.8 AB 3/8/15																
43 ppt	A	5	5	5	5	5	43.0	43.0	i 43.0	~~44.0~~	43.2	19.5	19.5	i 19.8	19.5	19.4	7.1	6.5	i 7.2	6.7	7.0	8.01	7.93	i 8.02	8.01	8.01
	B	5	5	5	5	5			f 43.4	43.2				f 20.6					f 6.3					f 7.96		
	C	5	5	5	5	5																				
	D	5	4	4	4	4																				
	E	5	5	5	5	5																				
	F	5	5	5	5	5																				

Initial Counts QC'd by: CH

Animal Source/Date Received: ABS/ 3/3/15

Age at Initiation: 12d

Feeding Times	0	24	48	72	96
AM:		~~1600~~ 0830 (A)	~~0835~~ 0900 (A)	0835	0815
PM:	1550	–	–	–	–

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

Organisms fed prior to initiation, circle one (y / n) — (y) circled. Hach Sension 5 Salinity Meter

(A) AO Q.8 3/7/15

QC Check: KB 3/10/15

Final Review: [illegible] 3/16/15

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Poseidon
Sample ID: brine - Nautilus frozen seawater
Test No.: 1503-S015

Test Species: *A. affinis*
Start Date/Time: 3/5/2015 1550
End Date/Time: 3/9/2015 1440

Tech Initials	0	24	48	72	96
Counts:	CH	KB	AD	AB	BK
Readings:	CH	KS	AD/AB	AB	AG
Dilutions made by:	CH		NH		

Concentration ___ppt___	Rep	Number of Live Organisms					Salinity (ppt)ⓐ					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
44 ppt	A	5	5	5	5	5	44.0	44.0	i 44.8	44.0	44.2	19.5	19.5	i 19.7	19.4	19.3	7.1	6.7	i 7.2	6.7	7.2	8.01	7.95	i 8.03	8.03	8.02
	B	5	4	4	4	4			f 44.0					f 20.4					f 6.3					f 7.96		
	C	5	5	5	5	5																				
	D	5	5	5	5	5																				
	E	5	3	3 ⊛	3	3																				
	F	5	5	4	4	4																				
	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									
	E																									
	F																									
	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									
	E																									
	F																									
	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									
	E																									
	F																									
	A								i					i					i					i		
	B								f					f					f					f		
	C																									
	D																									
	E																									
	F																									

Initial Counts QC'd by: CH

Animal Source/Date Received: ABS / 3/3/15

Age at Initiation: 12d

Feeding Times	0	24	48	72	96
AM:		0830	0900	0855	0815
PM:	1550	—	—	—	—

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

Organisms fed prior to initiation, circle one (ⓨ / n)

ⓐ subsample diluted w/ DI water 1:1 to obtain salinity measurement KS

⊛ QA AB 3/8/15

QC Check: KB 3/10/15

Final Review: G 3/16/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: CH

Sample ID: frozen seawater

Test Date: 3/5/15

Test No: 1303-S015

Test Type: topsmelt acute / test initiation

Salinity of Seawater 33.1

Salinity of Brine 84.0

Test Dilution Volume 1500

Date of Brine used: @ 3/5/15 (QA# 3/12/15 Q18) 11/5/14, 11/19/14, 11/25/14, 12/1/14, 3/5/15 3/4/15 AC Q18 3/13/15

Alkalinity of Brine Control: 116 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
33.1	100.0	250	NA	NA	1500
40.0	86.4	1296.7	0.16	203.3	1500
42.0	82.5	1237.7	0.21	262.3	1500
43.0	80.6	1208.3	0.24	291.7	1500
44.0	78.6	1178.8	0.27	321.2	1500

	DI Volume			
Brine Control	~~#DIV/0!~~ AC 523	@183/5/15 ~~1.10~~ ~~477.2~~ AC 3/5 Q18 0.67	321.2	1500

Total Brine Volume Required (ml): @ 3/6/15 ~~642.4~~ 1399.7

QC Check: AC 3/13/15

Final Review: 3/16/15

@ Brine was mixed the day prior to test initiation from brine made on multiple dates to have enough volume for test initiation & renewal.

Marine Chronic Bioassay **Brine Dilution Worksheet**

Project: Poseidon **Analyst:** NH

Sample ID: frozen seawater **Test Date:** 3/5/2015

Test No: 1503-5015 **Test Type:** Topsmelt Acute / Renewal 48 hr

Salinity of Seawater 33.0

Salinity of Brine 84.0

Test Dilution Volume 1500

@ **Date of Brine used:** ~~3/5/15~~ CH 3/12/15 Q18 11/5/14, 11/18/14, 11/25/14, 12/11/14, ~~3/5/15~~ 3/4/15 AC Q18 3/13

Alkalinity of Brine Control: 111 mg/L as CaCO3

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

Target Salinity ppt	Concentration % seawater	Seawater Volume (ml)	Salinity Adjustment Factor	Brine Volume (ml)	Dilute to: (ml)
33.~~1~~0 Q18 3/16/15	100.0	**250**	NA	**NA**	1500
40.0	86.3	**1294.1**	0.16	**205.9**	1500
42.0	82.4	**1235.3**	0.21	**264.7**	1500
43.0	80.4	**1205.9**	0.24	**294.1**	1500
44.0	78.4	**1176.5**	0.28	**323.5**	1500

	DI Volume			
Brine Control	Q18 3/16/15 ~~294.1~~ 500.1	Q18 3/16/15 ~~1.10~~ 0.65	323.5	1500

Total Brine Volume Required (ml): **1411.8**

QC Check: AC 3/13/15 **Final Review:** [initials] 4/29/15

@ Brine was mixed the day prior to test initiation from brine made on multiple dates to have enough volume for test initiation & renewal.

APPENDIX B

Reference Toxicant Test Data

Mysid Acute Reference Toxicant Test
February 2015

CETIS Summary Report

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

Batch ID:	04-3347-7787	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	05 Feb-15 15:55	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	09 Feb-15 14:00	**Species:**	Americamysis bahia	**Brine:**	Not Applicable
Duration:	94h	**Source:**	Aquatic Biosystems, CO	**Age:**	5 d

Sample ID:	17-2429-4896	**Code:**	150205myra	**Client:**	Internal
Sample Date:	05 Feb-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	05 Feb-15	**Source:**	Reference Toxicant		
Sample Age:	16h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-3206-3714	96h Survival Rate	100	200	141.4	18.7%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
20-9020-4206	96h Survival Rate	EC50	257.5	219	302.7		Spearman-Kärber

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	0.0%
50		4	1	1	1	1	1	0	0	0.0%	-5.26%
100		4	1	1	1	1	1	0	0	0.0%	-5.26%
200		4	0.75	0.4453	1	0.6	1	0.09574	0.1915	25.53%	21.05%
400		4	0.1	0	0.2837	0	0.2	0.05774	0.1155	115.5%	89.47%
800		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	0.8	1	1
50		1	1	1	1
100		1	1	1	1
200		0.6	1	0.8	0.6
400		0.2	0	0.2	0
800		0	0	0	0

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

Analysis ID: 07-3206-3714 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Feb-15 13:42 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	18.7%	100	200	141.4	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		50	-0.6611	2.356	0.212	6	0.9445	CDF	Non-Significant Effect
		100	-0.6611	2.356	0.212	6	0.9445	CDF	Non-Significant Effect
		200*	2.55	2.356	0.212	6	0.0350	CDF	Significant Effect
		400*	10.45	2.356	0.212	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.897553	0.7243882	4	44.66	<0.0001	Significant Effect
Error	0.2432871	0.01621914	15			
Total	3.14084		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.938	4.893	0.0222	Equal Variances
Variances	Levene Equality of Variance	8.631	4.893	0.0008	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9034	0.866	0.0478	Normal Distribution

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	-5.26%
100		4	1	1	1	1	1	1	0	0.0%	-5.26%
200		4	0.75	0.4453	1	0.7	0.6	1	0.09574	25.53%	21.05%
400		4	0.1	0	0.2837	0.1	0	0.2	0.05774	115.5%	89.47%
800		4	0	0	0	0	0	0	0		100.0%

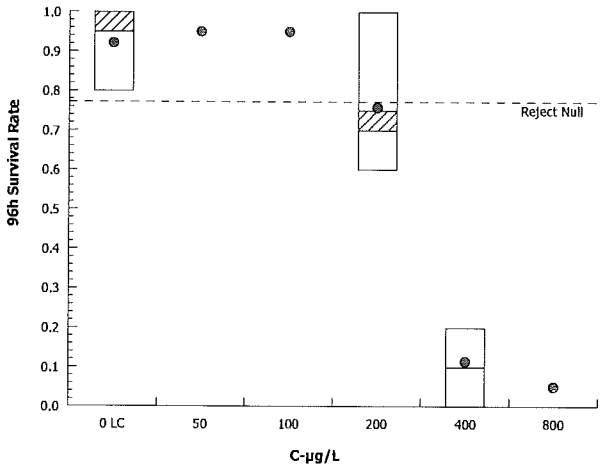
Angular (Corrected) Transformed Summary

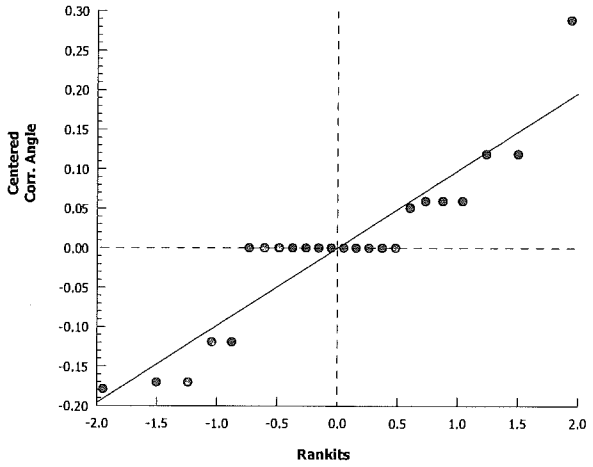
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	0.0%
50		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-4.63%
100		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-4.63%
200		4	1.056	0.7075	1.405	0.9966	0.8861	1.345	0.1096	20.75%	17.86%
400		4	0.3446	0.1258	0.5634	0.3446	0.2255	0.4636	0.06874	39.9%	73.2%
800		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	82.46%

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

Analysis ID:	07-3206-3714	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Feb-15 13:42	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 150205myra | 14-1381-7090

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

Analysis ID: 20-9020-4206 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 13 Feb-15 13:42 **Analysis:** Untrimmed Spearman-Kärber **Official Results:** Yes

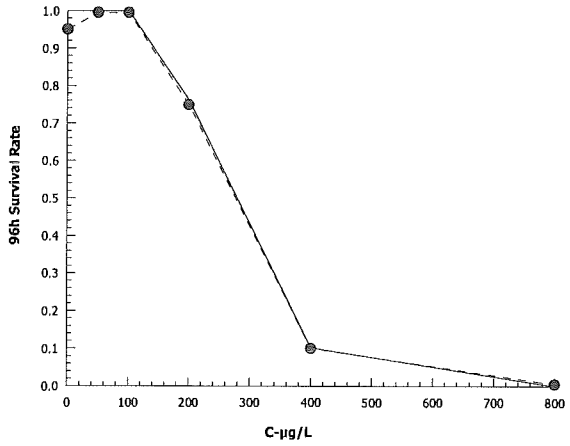
Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.05	0.00%	2.411	0.03513	257.5	219	302.7

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	0.95	0.8	1	0.05	0.1	10.53%	0.0%	19	20
50		4	1	1	1	0	0	0.0%	-5.26%	20	20
100		4	1	1	1	0	0	0.0%	-5.26%	20	20
200		4	0.75	0.6	1	0.09574	0.1915	25.53%	21.05%	15	20
400		4	0.1	0	0.2	0.05774	0.1155	115.5%	89.47%	2	20
800		4	0	0	0	0	0		100.0%	0	20

Graphics



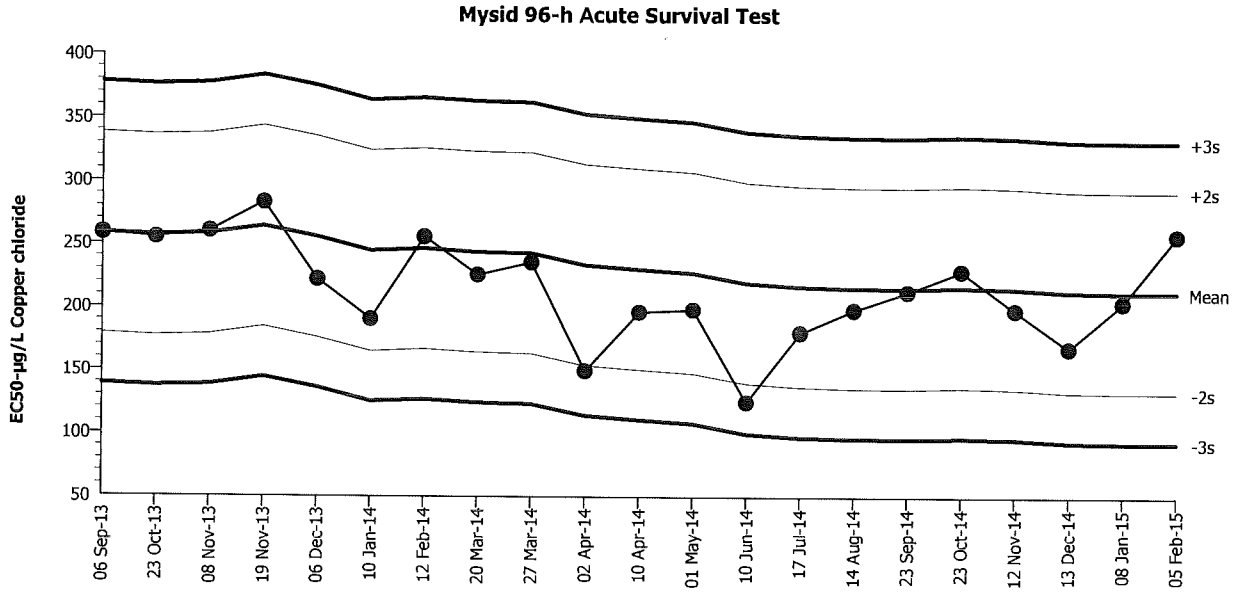
 Analyst: CB QA: KAP 2/19/15

CETIS QC Plot

Report Date: 13 Feb-15 13:43 (1 of 1)

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

Test Type:	Survival (96h)	**Organism:**	Americamysis bahia (Opossum Shri	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF



Mean:	212.3	**Count:**	20	**-2s Warning Limit:**	132.6	**-3s Action Limit:**	92.8
Sigma:	39.84	**CV:**	18.80%	**+2s Warning Limit:**	292	**+3s Action Limit:**	331.8

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Sep	6	15:05	258.5	46.24	1.161			13-4471-4704	00-6973-4292
2		Oct	23	13:30	254.9	42.61	1.07			16-6624-9217	07-4356-3093
3		Nov	8	14:45	260.1	47.84	1.201			01-3268-3774	14-6511-6184
4			19	18:00	282.8	70.54	1.771			18-1597-6213	02-6078-0521
5		Dec	6	16:15	221.9	9.614	0.2413			15-9430-7362	18-2355-9881
6	2014	Jan	10	14:40	190.3	-21.96	-0.5512			09-8692-0569	06-8811-0955
7		Feb	12	17:15	255.8	43.46	1.091			12-4495-5726	14-4047-7239
8		Mar	20	17:05	225.7	13.39	0.3361			04-4343-4707	19-2134-2594
9			27	17:15	235.7	23.38	0.5869			18-1249-5190	11-5455-8999
10		Apr	2	15:45	149.6	-62.7	-1.574			20-2330-3139	08-0863-3116
11			10	17:10	196.5	-15.77	-0.3958			16-5864-1833	17-8475-7680
12		May	1	15:15	198.3	-14.03	-0.352			13-9584-4772	21-4495-8860
13		Jun	10	16:15	124.9	-87.42	-2.194	(-)		00-0683-8894	06-8677-9461
14		Jul	17	16:45	180.3	-32.05	-0.8045			21-4298-8152	14-5683-3222
15		Aug	14	17:30	198.2	-14.07	-0.3531			18-8849-8580	16-2876-9748
16		Sep	23	16:35	212.8	0.5362	0.01346			12-4500-2750	04-3355-5833
17		Oct	23	15:15	229.4	17.1	0.4291			14-7022-2326	19-3230-6931
18		Nov	12	15:05	198.2	-14.07	-0.3531			15-3145-0944	01-6220-6073
19		Dec	13	16:30	168.2	-44.12	-1.107			11-0699-8383	10-8975-0476
20	2015	Jan	8	13:15	204.2	-8.055	-0.2022			16-0054-0041	01-6807-2003
21		Feb	5	15:55	257.5	45.17	1.134			14-1381-7090	20-9020-4206

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 150205myra

Test Species: *A. bahia*
Start Date/Time: 2/5/2015 1535
End Date/Time: 2/9/2015 1400

Cu stock concentration (µg/L): 860,900

	Tech Initials				
	0	24	48	72	96
Counts:	BG	EG	AP	CH	AG
Readings:	ALB	VH	AB	BK	BK
Dilutions made by:	AG		NH		
High conc. made (µg/L):	800	--	800	--	--
Vol. Cu stock added (mL):	18.4	--	18.4	--	--
Final Volume (mL):	2000	--	2000	--	--

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	8	5	5	5	5	5	29.8	30.1	i 29.3	29.8	30.0	25.8	25.9	i 24.4	25.0	25.3	6.4	5.9	i 6.8	6.3	5.9	7.98	7.94	i 7.86	7.94	7.91
	7	5	5	5	4	4			f 30.7					f 24.8					f 5.7					f 7.86		
	15	5	5	5	5	5																				
	4	5	5	5	5	5																				
50	11	5	5	5	5	5	29.9	30.1	i 29.2	29.7	29.8	25.6	25.9	i 24.2	25.3	25.6	6.4	5.9	i 6.7	6.1	5.5	8.00	7.96	i 7.99	7.97	7.91
	12	5	5	5	5	5			f 30.4					f 25.5					f 5.3					f 7.86		
	2	5	5	5	5	5																				
	21	5	5	5	5	5																				
100	10	5	5	5	5	5	29.9	30.2	i 29.2	29.7	30.2	26.0	25.9	i 24.1	25.3	25.5	6.4	5.6	i 6.8	5.9	5.5	7.99	7.96	i 7.99	7.97	7.93
	13	5	5	5	5	5			f 30.4					f 25.3					5.3					f 7.87		
	1	5	5	5	5	5																				
	23	5	5	5	5	5							Q1													
200	20	5	5	4	4	3	29.9	30.0	i 29.2	29.8	30.3	26.0	26.1	i 24.2	25.3	25.4	6.4	5.9	i 6.7	6.1	5.5	7.98	7.96	i 7.99	7.97	7.97
	14	5	5	5	5	5			f 30.5					f 25.4					f 5.5					f 7.93		
	6	5	5	5	5	4																				
	22	5	5	4	4	3																				
400	3	5	4	2	1	1	29.7	30.0	i 29.1	29.7	30.3	25.2	25.9	i 24.1	25.2	25.4	6.5	6.0	i 6.7	6.2	5.9	7.98	7.96	i 7.98	7.94	7.98
	19	5	4	2	1	0			f 30.7				AB 2/13	f ~~25.0~~					f 5.6					f 7.91		
	16	5	4	3	2	1							2/7/15	25.2												
	9	5	5	2	2	0																				
800	5	5	1	0		DEAD	29.6	30.1	i 28.9	-	-	24.9	25.7	i 24.2	-	-	6.5	6.1	i 6.7	-	-	7.96	7.96	i 7.96	-	-
	24	5	0	-	ALL				f 30.2					f 25.5					f 3.6					f 7.91		
	18	5	1	0																						
	17	5	1	0																						

Rand # QC: BG
Initial Count QC: BG

Animal Source/Date Received: ABS / 2/4/15
Age at Initiation: 5d

	Feeding Times				
	0	24	48	72	96
AM:	-	0845	0830	0920	0815
PM:	1600	1930	1530	1530	-

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

QC Check: KB 2/12/15
Final Review: KB 2/19/15

Topsmelt Acute Reference Toxicant Test
February 2015

CETIS Summary Report

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	11-5441-5565	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	10 Feb-15 14:45	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	14 Feb-15 12:45	**Species:**	Atherinops affinis	**Brine:**	Not Applicable
Duration:	94h	**Source:**	Aquatic Biosystems, CO	**Age:**	15 d

Sample ID:	19-5120-7043	**Code:**	150210aara	**Client:**	Internal
Sample Date:	10 Feb-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	10 Feb-15	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-7551-7551	96h Survival Rate	100	200	141.4	43.9%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
21-3305-5409	96h Survival Rate	EC50	91.04	76.69	108.1		Trimmed Spearman-Kärber

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	5.0%
100		4	0.4	0	1	0	1	0.2449	0.4899	122.5%	60.0%
200		4	0	0	0	0	0	0	0		100.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

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

CETIS Analytical Report

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID: 04-7551-7551 **Endpoint:** 96h Survival Rate **CETIS Version:** CETISv1.8.7
Analyzed: 20 Feb-15 12:06 **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	43.9%	100	200	141.4	

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		50	16	11	1	6	0.4206	Asymp	Non-Significant Effect
		100	12	11	1	6	0.0738	Asymp	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.116211	0.5581056	2	5.342	0.0296	Significant Effect
Error	0.9403625	0.1044847	9			
Total	2.056574		11			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	10.47	8.022	0.0045	Unequal Variances
Variances	Levene Equality of Variance	17.2	8.022	0.0008	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8681	0.8025	0.0618	Normal Distribution

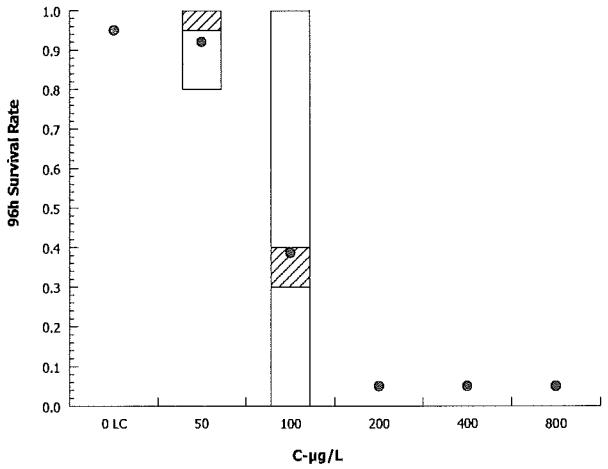
96h Survival Rate Summary

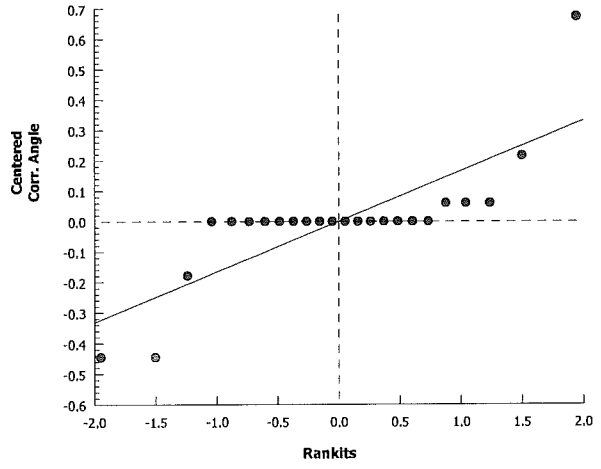
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	5.0%
100		4	0.4	0	1	0.3	0	1	0.2449	122.5%	60.0%
200		4	0	0	0	0	0	0	0		100.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
50		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	4.43%
100		4	0.6706	-0.1999	1.541	0.5558	0.2255	1.345	0.2735	81.58%	50.15%
200		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
400		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
800		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

Graphics





CETIS Analytical Report

Test Code: 150210aara | 05-4867-3928

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Analysis ID:	21-3305-5409	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Feb-15 12:06	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

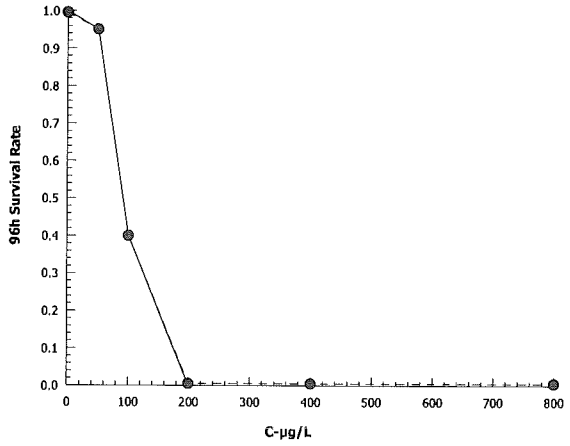
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	1.959	0.03726	91.04	76.69	108.1

96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	1	1	1	0	0	0.0%	0.0%	20	20
50		4	0.95	0.8	1	0.05	0.1	10.53%	5.0%	19	20
100		4	0.4	0	1	0.2449	0.4899	122.5%	60.0%	8	20
200		4	0	0	0	0	0		100.0%	0	20
400		4	0	0	0	0	0		100.0%	0	20
800		4	0	0	0	0	0		100.0%	0	20

Graphics



 Analyst: VB QA: KFP 2/26/15

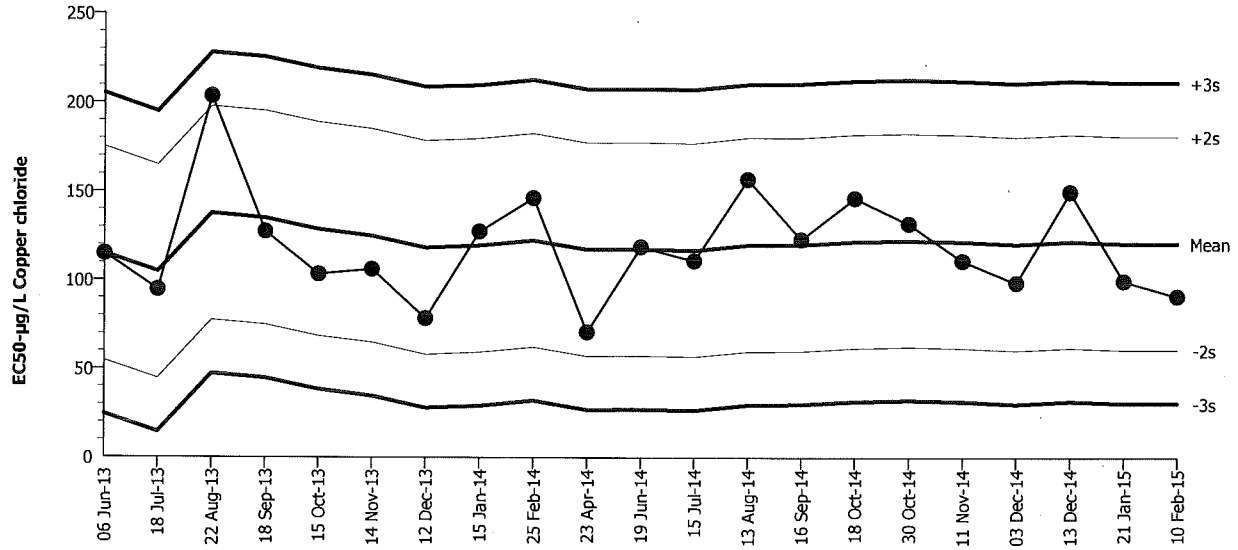
CETIS QC Plot

Report Date: 20 Feb-15 12:07 (1 of 1)

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

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

Pacific Topsmelt 96-h Acute Survival Test



Mean:	121	**Count:**	20	**-2s Warning Limit:**	60.8	**-3s Action Limit:**	30.68
Sigma:	30.12	**CV:**	24.90%	**+2s Warning Limit:**	181.3	**+3s Action Limit:**	211.4

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Jun	6	14:25	114.9	-6.13	-0.2035			12-0033-0929	20-0252-9346
2		Jul	18	12:50	94.59	-26.41	-0.8768			19-2632-6339	04-8526-8990
3		Aug	22	14:40	203.7	82.68	2.745	(+)		16-8357-2725	11-7110-5550
4		Sep	18	13:50	127.5	6.456	0.2143			09-9085-4812	11-5673-1751
5		Oct	15	15:20	103.5	-17.47	-0.5801			00-5901-5898	17-6384-6991
6		Nov	14	9:40	106.1	-14.88	-0.4941			06-5418-8921	10-2371-6330
7		Dec	12	13:20	78.46	-42.54	-1.412			12-4998-2305	03-2148-1441
8	2014	Jan	15	15:25	127.5	6.456	0.2143			13-3854-5258	05-1070-1044
9		Feb	25	13:55	146.4	25.41	0.8436			20-0325-5939	07-6658-0335
10		Apr	23	16:00	70.71	-50.29	-1.67			11-8272-9093	14-5541-7971
11		Jun	19	15:35	118.9	-2.079	-0.06903			11-2944-5183	19-5384-3170
12		Jul	15	14:15	111	-10.04	-0.3334			00-8730-8108	10-9428-5566
13		Aug	13	14:10	156.9	35.92	1.192			12-9208-7415	02-1974-5349
14		Sep	16	13:10	123.1	2.114	0.0702			05-7478-8365	11-8140-9628
15		Oct	18	10:45	146.4	25.41	0.8436			18-6908-7115	01-0100-8379
16			30	11:50	132	10.95	0.3636			17-2734-9303	12-4790-6162
17		Nov	11	10:50	111	-10.04	-0.3334			19-6246-9477	14-2586-1124
18		Dec	3	16:45	98.53	-22.47	-0.746			07-3639-8754	16-6449-6521
19			13	15:30	150.1	29.08	0.9654			03-6652-6590	12-5127-9321
20	2015	Jan	21	15:35	99.71	-21.29	-0.7068			16-8270-8063	13-6137-3732
21		Feb	10	14:45	91.04	-29.96	-0.9947			05-4867-3928	21-3305-5409

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 150209aara (16) (R)

Test Species: *A. affinis*
Start Date/Time: ~~2/9/2015~~ (R) 2/10/15 1446
End Date/Time: ~~2/13/2015~~ (R) 2/14/15 1245

Cu stock concentration (µg/L): 16,900

	Tech Initials				
	0	24	48	72	96
Counts:	ALB	MH	MH	MH	MH
Readings:	BK	MH	ALB	MH	AB
Dilutions made by:	ALB	–	BK	–	–
High conc. made (µg/L):	800	--	200	--	--
Vol. Cu stock added (mL):	18.4	--	4.6	--	--
Final Volume (mL):	2000	--	2000	--	--

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C) Q2					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	22	5	5	5	5	5	29.9	30.1	i 29.9	30.8	32.0	20.3	20.9	i 20.0	20.4	20.5	7.4	6.4	i 6.9	6.4	6.7	7.98	7.80	i 7.99	7.88	7.70
	2	5	5	5	5	5			f 30.2					f 20.9					f 6.1					f 7.71		
	10	5	5	5	5	5																				
	15	5	5	5	5	5																				
50	24	5	5	5	5	5	29.8	30.2	i 29.9	30.2	30.4	20.3	20.8	i 19.9	20.8	21.1	7.3	6.3	i 6.8	6.2	6.2	7.96	7.79	i 7.99	7.85	7.72
	8	5	5	5	5	5			f 30.7					f 20.7					f 6.2					f 7.84		
	17	5	4	4	4	4																				
	11	5	5	5	5	5																				
100	12	5	5	4	4	3	29.8	29.9	i 29.8	30.3	31.8	20.3	20.6	i 20.1	20.4	20.4	7.3	6.4	i 6.9	6.5	6.4	7.98	7.77	i 8.00	7.91	7.78
	4	5	3	1	0	–			f 30.5					f 20.3					f 6.4					f 7.86		
	1	5	4	1	1	0																				
	19	5	5	5	5	5																				
200	23	5	0	–	–	–	29.8	29.7	i 29.8	–	–	20.3	20.6	i 20.0	–	–	7.3	6.3	i 6.9	–	–	7.96	7.78	i 7.99	–	–
	13	5	1	0	–	–			f 30.1					f 20.7					f 6.3					f 7.87		
	14	5	0	–	–	–																				
	18	5	0	–	–	–																				
400	9	5	0	ALL DEAD			29.7	29.8	i –	–	–	20.4	20.6	i –	–	–	7.5	6.3	i –	–	–	7.97	7.77	i –	–	–
	16	5	0						f –					f –					f –					f –		
	7	5	0																							
	5	5	0																							
800	3	5	0	ALL DEAD			29.6	29.6	i –	–	–	20.3	20.7	i –	–	–	7.3	6.3	i –	–	–	7.98	7.71	i –	–	–
	21	5	0						f –					f –					f –					f –		
	6	5	0																							
	20	5	0																							

Rand # QC: ALB
Initial Count QC: MH

Animal Source/Date Received: ABG 2/6/15
Age at Initiation: 15 days

	Feeding Times				
	0	24	48	72	96
AM:	--	0820	0805	0915	0900
PM:	1505	--	--	--	--

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (y) n (R) BG 2/6/15
Lab control and 100% salinities slightly higher at 96 hours. Values confirmed with a second salinity meter.

QC Check: KB 2/20/15
Final Review: KPP 2/26/15

Mysid Acute Reference Toxicant Test
March 2015

CETIS Summary Report

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

Batch ID:	17-5434-7167	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	03 Mar-15 15:10	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	07 Mar-15 13:55	**Species:**	Americamysis bahia	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	4d

Sample ID:	14-7479-8613	**Code:**	150303myra	**Client:**	Internal
Sample Date:	03 Mar-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	03 Mar-15	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-4701-4628	96h Survival Rate	50	100	70.71	12.9%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
10-4165-9670	96h Survival Rate	EC50	150.3	121.7	185.7		Trimmed Spearman-Kärber

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	0.95	0.7909	1	0.8	1	0.05	0.1	10.53%	5.0%
100		4	0.8	0.8	0.8	0.8	0.8	0	0	0.0%	20.0%
200		4	0.3	0.1163	0.4837	0.2	0.4	0.05774	0.1155	38.49%	70.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	0.8
100		0.8	0.8	0.8	0.8
200		0.4	0.2	0.2	0.4
400		0	0	0	0
800		0	0	0	0

Analyst: AC QA: KPP 3/24/15

CETIS Analytical Report

Mysid 96-h Acute Survival Test — Nautilus Environmental (CA)

Analysis ID:	00-4701-4628	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:14	**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	12.9%	50	100	70.71	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		50	0.9647	2.287	0.141	6	0.3485	CDF	Non-Significant Effect
		100*	3.859	2.287	0.141	6	0.0030	CDF	Significant Effect
		200*	12.49	2.287	0.141	6	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.477123	0.4923743	3	64.64	<0.0001	Significant Effect
Error	0.09140357	0.007616965	12			
Total	1.568527		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	3.21	5.953	0.0618	Equal Variances
Variances	Levene Equality of Variance	15.36	5.953	0.0002	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8697	0.8408	0.0269	Normal Distribution

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	0.95	0.7909	1	1	0.8	1	0.05	10.53%	5.0%
100		4	0.8	0.7997	0.8003	0.8	0.8	0.8	0	0.0%	20.0%
200		4	0.3	0.1163	0.4837	0.3	0.2	0.4	0.05774	38.49%	70.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
50		4	1.286	1.096	1.475	1.345	1.107	1.345	0.05953	9.26%	4.43%
100		4	1.107	1.107	1.108	1.107	1.107	1.107	0	0.0%	17.7%
200		4	0.5742	0.3711	0.7773	0.5742	0.4636	0.6847	0.06382	22.23%	57.32%
400		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
800		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	0.8
100		0.8	0.8	0.8	0.8
200		0.4	0.2	0.2	0.4
400		0	0	0	0
800		0	0	0	0

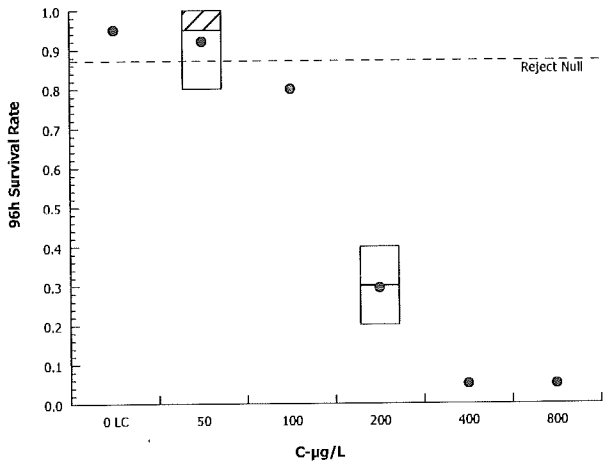
Mysid 96-h Acute Survival Test — Nautilus Environmental (CA)

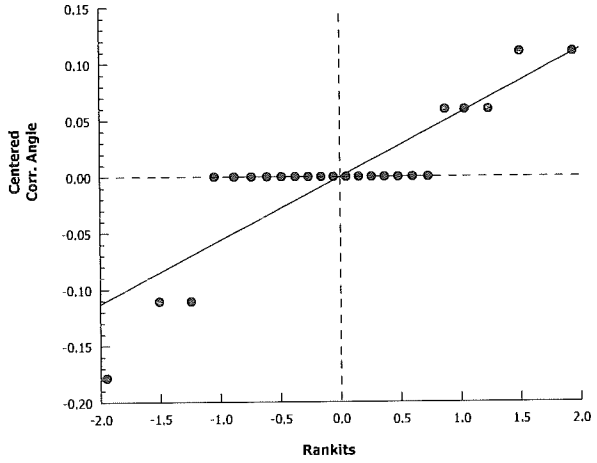
Analysis ID:	00-4701-4628	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:14	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Angular (Corrected) Transformed Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.345	1.345	1.345	1.345
50		1.345	1.345	1.345	1.107
100		1.107	1.107	1.107	1.107
200		0.6847	0.4636	0.4636	0.6847
400		0.2255	0.2255	0.2255	0.2255
800		0.2255	0.2255	0.2255	0.2255

Graphics





CETIS Analytical Report

Test Code: 150303myra | 16-0363-1107

Mysid 96-h Acute Survival Test — Nautilus Environmental (CA)

Analysis ID:	10-4165-9670	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:14	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	5.00%	2.177	0.04587	150.3	121.7	185.7

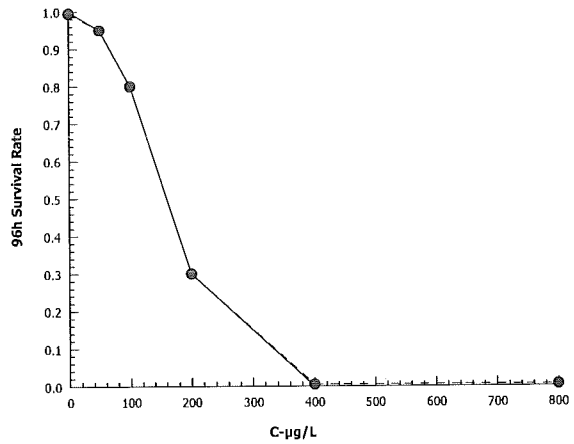
96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	1	1	1	0	0	0.0%	0.0%	20	20
50		4	0.95	0.8	1	0.05	0.1	10.53%	5.0%	19	20
100		4	0.8	0.8	0.8	0	0	0.0%	20.0%	16	20
200		4	0.3	0.2	0.4	0.05774	0.1155	38.49%	70.0%	6	20
400		4	0	0	0	0	0		100.0%	0	20
800		4	0	0	0	0	0		100.0%	0	20

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	0.8
100		0.8	0.8	0.8	0.8
200		0.4	0.2	0.2	0.4
400		0	0	0	0
800		0	0	0	0

Graphics



Analyst: AC QA: 3/26/15

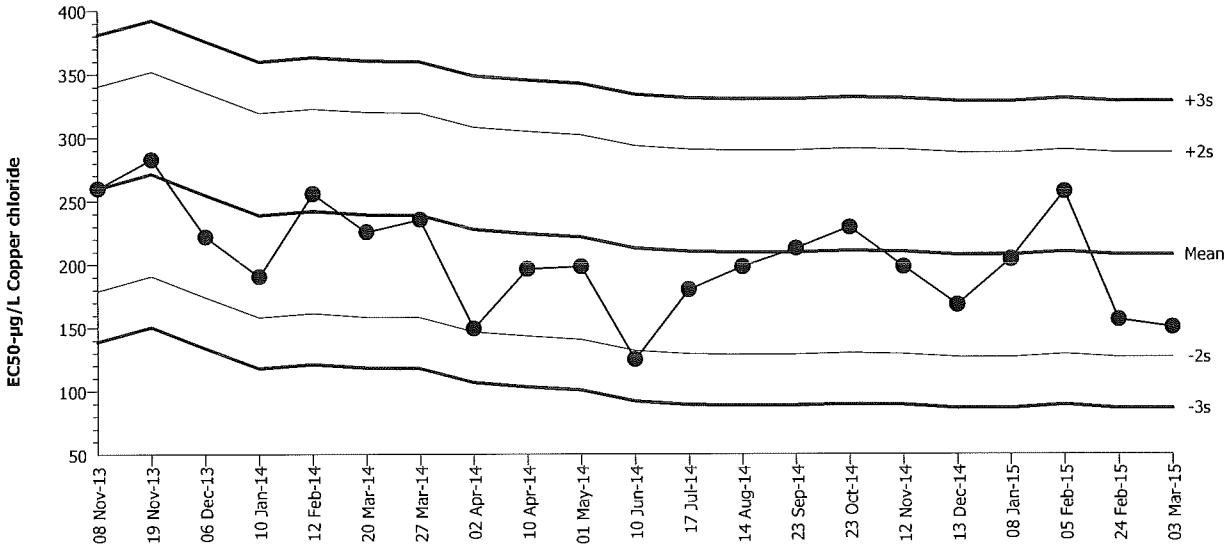
CETIS QC Plot

Report Date: 13 Mar-15 11:17 (1 of 1)

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

Test Type:	Survival (96h)	**Organism:**	Americamysis bahia (Opossum Shri	**Material:**	Copper chloride
Protocol:	EPA/821/R-02-012 (2002)	**Endpoint:**	96h Survival Rate	**Source:**	Reference Toxicant-REF

Mysid 96-h Acute Survival Test



Mean:	207.3	**Count:**	20	**-2s Warning Limit:**	126.7	**-3s Action Limit:**	86.38
Sigma:	40.32	**CV:**	19.50%	**+2s Warning Limit:**	288	**+3s Action Limit:**	328.3

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Nov	8	14:45	260.1	52.84	1.311			01-3268-3774	14-6511-6184
2			19	18:00	282.8	75.54	1.874			18-1597-6213	02-6078-0521
3		Dec	6	16:15	221.9	14.61	0.3624			15-9430-7362	18-2355-9881
4	2014	Jan	10	14:40	190.3	-16.96	-0.4207			09-8692-0569	06-8811-0955
5		Feb	12	17:15	255.8	48.46	1.202			12-4495-5726	14-4047-7239
6		Mar	20	17:05	225.7	18.39	0.4561			04-4343-4707	19-2134-2594
7			27	17:15	235.7	28.38	0.7039			18-1249-5190	11-5455-8999
8		Apr	2	15:45	149.6	-57.7	-1.431			20-2330-3139	08-0863-3116
9			10	17:10	196.5	-10.77	-0.2671			16-5864-1833	17-8475-7680
10		May	1	15:15	198.3	-9.025	-0.2238			13-9584-4772	21-4495-8860
11		Jun	10	16:15	124.9	-82.42	-2.044	(-)		00-0683-8894	06-8677-9461
12		Jul	17	16:45	180.3	-27.05	-0.6709			21-4298-8152	14-5683-3222
13		Aug	14	17:30	198.2	-9.069	-0.2249			18-8849-8580	16-2876-9748
14		Sep	23	16:35	212.8	5.536	0.1373			12-4500-2750	04-3355-5833
15		Oct	23	15:15	229.4	22.1	0.548			14-7022-2326	19-3230-6931
16		Nov	12	15:05	198.2	-9.069	-0.2249			15-3145-0944	01-6220-6073
17		Dec	13	16:30	168.2	-39.12	-0.9703			11-0699-8383	10-8975-0476
18	2015	Jan	8	13:15	204.2	-3.055	-0.07576			16-0054-0041	01-6807-2003
19		Feb	5	15:55	257.5	50.17	1.244			14-1381-7090	20-9020-4206
20			24	14:45	156.3	-50.99	-1.265			00-7545-3960	18-6061-5275
21		Mar	3	15:10	150.3	-56.99	-1.413			16-0363-1107	10-4165-9670

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements & Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 150303myra

Test Species: *A. bahia*
Start Date/Time: 3/3/2015 1510
End Date/Time: 3/7/2015 1355

Tech Initials	0	24	48	72	96
Counts:	EG	CH	EG	KB	AD
Readings:	AC	KB	AG	KB	AB
Dilutions made by:	AC		EG		
High conc. made (µg/L):	800	--	800	--	--
Vol. Cu stock added (mL):	18.4	--	18.4	--	--
Final Volume (mL):	2000	--	2000	--	--

Cu stock concentration (µg/L): 86,900

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C) Q1					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	8	5	5	5	5	5	30.3	30.8	i 30.2	29.6	29.8	24.3	25.8	i 24.1	25.4	25.5	6.6	6.3	i 7.1	5.3	5.7	7.92	7.90	i 8.03	7.82	7.80
	7	5	5	5	5	5			f 31.0					f 24.9					f 5.8					f 7.81		
	15	5	5	5	5	5																				
	4	5	5	5	5	5																				
50	11	5	5	5	5	5	30.2	30.3	i 30.1	30.9	31.1	24.2	26.3	i 24.7	25.1	25.7	6.7	6.0	i 7.0	5.7	5.5	7.94	7.90	i 8.04	7.84	7.80
	12	5	5	5	5	5			30.5					f 24.6					f 5.8					f 7.80		
	2	5	5	5	5	5																				
	21	5	5	4	4	4																				
100	10	5	5	5	4	4	30.2	30.3	i 30.1	30.2	31.1	24.2	26.2	i 24.4	25.5	25.6	6.7	6.0	i 7.1	5.5	5.7	7.95	7.91	i 8.05	7.84	7.86
	13	5	5	4	4	4			f 30.6					f 24.5					f 5.9					f 7.83		
	1	5	5	5	4	4																				
	23	5	5	4	4	4	30.1																			
200	20	5	4	2	2	2	30.2	30.4	i 30.0	30.1	30.6	24.2	26.3	i 24.7	25.4	25.2	6.7	6.1	i 7.0	5.7	5.7	7.96	7.92	i 8.04	7.87	7.86
	14	5	3	2	2	1	AC @18		f 30.5					f 24.4					f 5.8					f 7.81		
	6	5	1	1	1	1	3/3																			
	22	5	5	4	4	2																				
400	3	5	Ⓐ 3	0			30.0	30.2	i 29.8 30.0 3/5/15	–	–	24.1	26.1	i 25.2	–	–	6.7	6.1	i 6.7	–	–	7.95	7.92	i 8.03	–	–
	19	5	1	0					f 30.5					f 24.8					f 5.8					f 7.83		
	16	5	1	0		all dead																				
	9	5	3	0																						
800	5	5	2	0			29.9	30.1	i 29.5 3/5/15	–	–	24.0	26.3	i 25.4	–	–	6.7	6.2	i 6.7	–	–	7.94	7.92	i 8.00	–	–
	24	5	2	0		all dead	AC		f 30.4 3/5/15					f 24.8					f 5.8					f 7.86		
	18	5	0	–	–		2nd 3/3																			
	17	5	3	0		dead																				

Rand # QC: ALB
Initial Count QC: VTR

Animal Source/Date Received: ABS | 3/3/15
Age at Initiation: 4d

Feeding Times	0	24	48	72	96
AM:	–	0820	0830	0830	0900
PM:	1615	1615	1500	1550	–

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one ((Y) / n)
Ⓐ CH 3/4/15 0918

QC Check: KB 3/10/15
Final Review: APP 3/26/15

Topsmelt Acute Reference Toxicant Test
March 2015

CETIS Summary Report

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

Batch ID:	15-8595-8712	**Test Type:**	Survival (96h)	**Analyst:**	
Start Date:	05 Mar-15 16:00	**Protocol:**	EPA/821/R-02-012 (2002)	**Diluent:**	Diluted Natural Seawater
Ending Date:	09 Mar-15 14:35	**Species:**	Atherinops affinis	**Brine:**	Not Applicable
Duration:	95h	**Source:**	Aquatic Biosystems, CO	**Age:**	12d

Sample ID:	11-0049-8953	**Code:**	150305aara	**Client:**	Internal
Sample Date:	05 Mar-15	**Material:**	Copper chloride	**Project:**	
Receive Date:	05 Mar-15	**Source:**	Reference Toxicant		
Sample Age:	16h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-0019-9527	96h Survival Rate	50	100	70.71	15.3%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
19-8561-4737	96h Survival Rate	EC50	127.5	105.1	154.6		Spearman-Kärber

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	0	0	0.0%	0.0%
50		4	1	1	1	1	1	0	0	0.0%	0.0%
100		4	0.65	0.4909	0.8091	0.6	0.8	0.05	0.1	15.38%	35.0%
200		4	0.2	0	0.4598	0	0.4	0.08165	0.1633	81.65%	80.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	1
100		0.6	0.8	0.6	0.6
200		0	0.2	0.4	0.2
400		0	0	0	0
800		0	0	0	0

Analyst: AC QA: PPP 3/26/15

CETIS Analytical Report

Pacific Topsmelt 96-h Acute Survival Test — Nautilus Environmental (CA)

Analysis ID:	17-0019-9527	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:19	**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	15.3%	50	100	70.71	

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		50	18	10	1	6	0.7500	Asymp	Non-Significant Effect
		100*	10	10	0	6	0.0276	Asymp	Significant Effect
		200*	10	10	0	6	0.0276	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.128261	0.7094204	3	59.88	<0.0001	Significant Effect
Error	0.1421622	0.01184685	12			
Total	2.270423		15			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.609	5.953	0.2391	Equal Variances
Variances	Levene Equality of Variance	2.818	5.953	0.0841	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.7731	0.8408	0.0012	Non-normal Distribution

96h Survival Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1	1	1	1	1	1	0	0.0%	0.0%
50		4	1	1	1	1	1	1	0	0.0%	0.0%
100		4	0.65	0.4909	0.8091	0.6	0.6	0.8	0.05	15.38%	35.0%
200		4	0.2	0	0.4598	0.2	0	0.4	0.08165	81.65%	80.0%
400		4	0	0	0	0	0	0	0		100.0%
800		4	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
50		4	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
100		4	0.9413	0.7655	1.117	0.8861	0.8861	1.107	0.05527	11.74%	30.03%
200		4	0.4594	0.161	0.7578	0.4636	0.2255	0.6847	0.09377	40.82%	65.85%
400		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
800		4	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	1
100		0.6	0.8	0.6	0.6
200		0	0.2	0.4	0.2
400		0	0	0	0
800		0	0	0	0

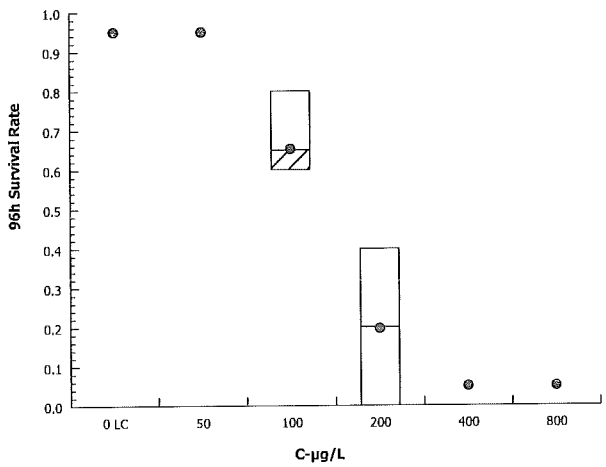
Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

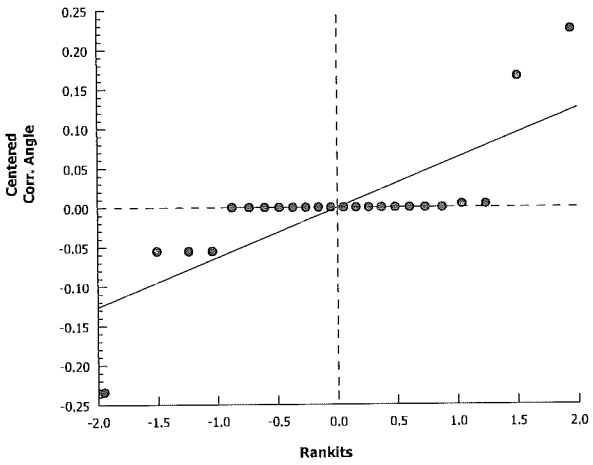
Analysis ID:	17-0019-9527	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:19	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Angular (Corrected) Transformed Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1.345	1.345	1.345	1.345
50		1.345	1.345	1.345	1.345
100		0.8861	1.107	0.8861	0.8861
200		0.2255	0.4636	0.6847	0.4636
400		0.2255	0.2255	0.2255	0.2255
800		0.2255	0.2255	0.2255	0.2255

Graphics





CETIS Analytical Report

Test Code: 150305aara | 19-3155-0811

Pacific Topsmelt 96-h Acute Survival Test — Nautilus Environmental (CA)

Analysis ID:	19-8561-4737	**Endpoint:**	96h Survival Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	13 Mar-15 11:19	**Analysis:**	Untrimmed Spearman-Kärber	**Official Results:**	Yes

Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0	0.00%	2.105	0.0419	127.5	105.1	154.6

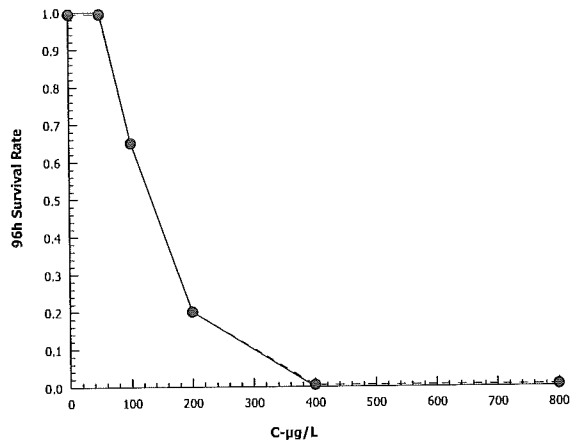
96h Survival Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	4	1	1	1	0	0	0.0%	0.0%	20	20
50		4	1	1	1	0	0	0.0%	0.0%	20	20
100		4	0.65	0.6	0.8	0.05	0.1	15.38%	35.0%	13	20
200		4	0.2	0	0.4	0.08165	0.1633	81.65%	80.0%	4	20
400		4	0	0	0	0	0		100.0%	0	20
800		4	0	0	0	0	0		100.0%	0	20

96h Survival Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Lab Control	1	1	1	1
50		1	1	1	1
100		0.6	0.8	0.6	0.6
200		0	0.2	0.4	0.2
400		0	0	0	0
800		0	0	0	0

Graphics



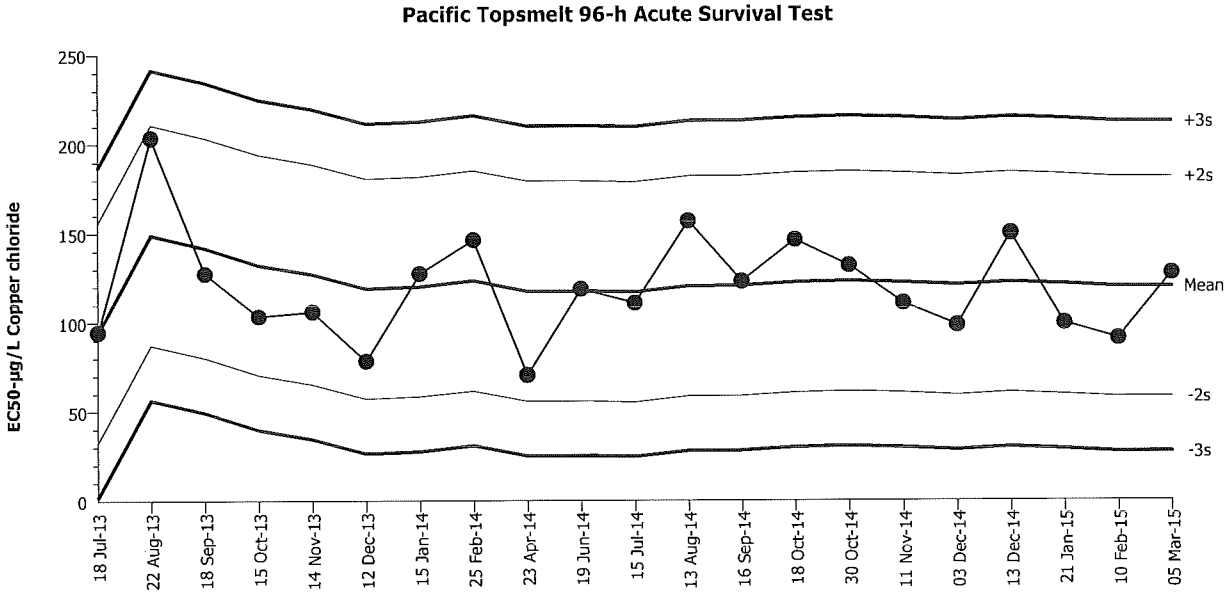
Analyst: AC QA: KFR 3/26/15

CETIS QC Plot

Report Date: 13 Mar-15 11:19 (1 of 1)

Pacific Topsmelt 96-h Acute Survival Test — **Nautilus Environmental (CA)**

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



Mean:	119.8	**Count:**	20	**-2s Warning Limit:**	58.17	**-3s Action Limit:**	27.33
Sigma:	30.84	**CV:**	25.70%	**+2s Warning Limit:**	181.5	**+3s Action Limit:**	212.4

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Jul	18	12:50	94.59	-25.21	-0.8175			19-2632-6339	04-8526-8990
2		Aug	22	14:40	203.7	83.88	2.72	(+)		16-8357-2725	11-7110-5550
3		Sep	18	13:50	127.5	7.656	0.2483			09-9085-4812	11-5673-1751
4		Oct	15	15:20	103.5	-16.27	-0.5277			00-5901-5898	17-6384-6991
5		Nov	14	9:40	106.1	-13.68	-0.4437			06-5418-8921	10-2371-6330
6		Dec	12	13:20	78.46	-41.34	-1.341			12-4998-2305	03-2148-1441
7	2014	Jan	15	15:25	127.5	7.656	0.2483			13-3854-5258	05-1070-1044
8		Feb	25	13:55	146.4	26.61	0.8628			20-0325-5939	07-6658-0335
9		Apr	23	16:00	70.71	-49.09	-1.592			11-8272-9093	14-5541-7971
10		Jun	19	15:35	118.9	-0.8793	-0.02851			11-2944-5183	19-5384-3170
11		Jul	15	14:15	111	-8.843	-0.2867			00-8730-8108	10-9428-5566
12		Aug	13	14:10	156.9	37.12	1.204			12-9208-7415	02-1974-5349
13		Sep	16	13:10	123.1	3.314	0.1075			05-7478-8365	11-8140-9628
14		Oct	18	10:45	146.4	26.61	0.8628			18-6908-7115	01-0100-8379
15			30	11:50	132	12.15	0.394			17-2734-9303	12-4790-6162
16		Nov	11	10:50	111	-8.843	-0.2867			19-6246-9477	14-2586-1124
17		Dec	3	16:45	98.53	-21.27	-0.6897			07-3639-8754	16-6449-6521
18			13	15:30	150.1	30.28	0.9818			03-6652-6590	12-5127-9321
19	2015	Jan	21	15:35	99.71	-20.09	-0.6514			16-8270-8063	13-6137-3732
20		Feb	10	14:45	91.04	-28.76	-0.9325			05-4867-3928	21-3305-5409
21		Mar	5	16:00	127.5	7.656	0.2483			19-3155-0811	19-8561-4737

Analyst: AC QA: PFP 3/26/15

Marine Acute Bioassay
Static-Renewal Conditions

Water Quality Measurements
& Test Organism Survival

Client: Internal
Sample ID: $CuCl_2$
Test No.: 150305aara

Test Species: *A. affinis*
Start Date/Time: 3/5/2015 1600
End Date/Time: 3/9/2015 1435

Tech Initials	0	24	48	72	96
Counts:	CH	KS	AD	AB	EG
Readings:	AG	KS	AD/AB	AB	AG
Dilutions made by:	EG	–	AB	–	–
High conc. made (µg/L):	800	--	200	--	--
Vol. Cu stock added (mL):	18.4	--	4.6	--	--
Final Volume (mL):	2000	--	2000	--	--

Cu stock concentration (µg/L): 86,900

Concentration (µg/L)	Rand #	Number of Live Organisms					Salinity (ppt)					Temperature (°C)					Dissolved Oxygen (mg/L)					pH (units)				
		0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	22	5	5	5	5	5	30.0	30.3	i 29.9	30.7	30.7	19.8	20.8	i 19.2	19.7	19.7	7.2	6.5	i 7.2	7.0	6.9	7.95	7.85	i 8.03	7.89	7.88
	2	5	5	5	5	5			f 30.7					f 20.9					f 6.8					f 7.83		
	10	5	5	5	5	5																				
	15	5	5	5	5	4/5	EG 3/9/15																			
50	24	5	5	5	5	5	29.9	30.3	i 30.0	30.4	30.4	19.9	21.0	i 19.1	20.1	19.8	7.0	6.4	i 7.2	6.9	6.8	7.97	7.85	i 8.04	7.93	7.90
	8	5	5	5	5	5			f 30.6					f 21.2					f 6.7					f 7.86		
	17	5	5	5	5	5																				
	11	5	5	5	5	5																				
100	12	5	3	3	3	3	29.9	30.3	i 30.0	30.5	30.7	19.5	20.9	i 19.0	19.9	19.4	7.0	6.4	i 7.2	7.1	7.0	7.97	7.84	i 8.04	7.95	7.94
	4	5	4	4	4	4			f 30.7					f 20.9					f 6.8					f 7.92		
	1	5	4	4	3	3																				
	19	5	4	3	3	3																				
200	23	5	2	2	2	0	29.9	30.3	i 30.0	30.5	30.8	19.6	20.7	i 19.0	19.7	19.5	7.0	6.4	i 7.2	7.1	7.1	7.97	7.85	i 8.03	7.96	7.95
	13	5	5	2	1	1			f 30.9					f 20.9					f 6.8					f 7.93		
	14	5	4	4	4	2																				
	18	5	1	1	1	1																				
400	9	5	0				29.6	29.9	i			19.6	20.6	i			7.0	6.6	i			7.96	7.86	i		
	16	5	0						f					f					f					f		
	7	5	0																							
	5	5	0																							
800	3	5	0				29.5	30.0	i			19.6	20.4	i			6.9	6.7	i			7.95	7.86	i		
	21	5	0						f					f					f					f		
	6	5	0																							
	20	5	0																							

Rand # QC: AG
Initial Count QC: CH

Animal Source/Date Received: ABS / 3/3/15
Age at Initiation: 12d

Feeding Times	0	24	48	72	96
AM:	--	0830 KS	0900 AD	0835 AB	0815
PM:	1600	--	--	--	--

Comments: i = initial reading in fresh test solution, f = final reading in test chamber prior to renewal
Organisms fed prior to initiation, circle one (Y) / N) AD @18 3/7/15

QC Check: KB 3/10/15
Final Review: KEP 3/26/15

APPENDIX C

Glossary of Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

Laboratory Procedures

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%

Data Analysis/Reporting

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.

Error Correction

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)