



Appendix H
Chronic Toxicity Study

Renewal of NPDES CA0109223
Carlsbad Desalination Project



Nautilus Environmental

Poseidon Salinity Tolerance Study Interim Report - Chronic Toxicity Test Results October 2014 through July 2015

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Data Quality Assurance:

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

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INTRODUCTION

Chronic bioassay testing was conducted to determine the sensitivity of several marine organisms to salinity increases above ambient seawater levels. Testing was conducted in response to the amendments made to the Ocean Plan (OP) by the California State Water Quality Control Board (SWQCB); amendments to the Ocean Plan state that at the end of the zone of initial dilution (ZID), receiving water salinity must be within 2,000 mg/L of ambient salinity levels. This limit was based on salinity tolerance studies performed by Phillips et al. (2012) and in a literature review reported by Roberts et al. (2012). Poseidon Water (Poseidon) has contracted with Nautilus Environmental (Nautilus) to conduct salinity studies with the aim of applying for a facility-specific alternative receiving water limit for the Carlsbad Desalination Plant brine discharge.

This study includes a suite of salinity tolerance bioassays using the species and endpoints listed in the OP amendment. These include: purple sea urchin (*Strongylocentrotus purpuratus*) fertilization and development, sand dollar (*Dendraster excentricus*) fertilization and development, red abalone (*Haliotis rufescens*) development, Pacific topsmelt (*Atherinops affinis*) survival and growth, and giant kelp (*Macrocystis pyrifera*) germination and germ-tube growth. The goal of this study is to characterize individual species sensitivity with regards to increased salinity using standard whole effluent toxicity (WET) methods. The approach for this study includes conducting each test three times over the course of approximately one year to address possible seasonal effects and potential differences in batch sensitivities. Results for tests conducted between October 2014 and July 2015 are included in this report. Further testing is scheduled to be completed by February 2016 and will be reported separately.

Tests were spaced several months apart in order to account for potential differences in sensitivity due to seasonal effects. Three tests per year were schedule to generally cover organism spawning, non-spawning, and recruitment periods. Table 1 outlines the testing schedule; including the tests which have already been completed (shaded gray) with results reported herein, and tests scheduled to be completed by February 2016.

Table 1. Salinity Tolerance Test Schedule

Species and Endpoint	Winter	Spring/Summer	Fall
Abalone Development	Dec. 2014	May 2015	Sept. - Oct. 2015
Kelp Germination and Growth	Dec. 2015- Feb. 2016	May 2015	Sept. - Oct. 2015
Topsmelt Survival and Growth	Dec. 2015- Feb. 2016	May 2015	Sept. - Oct. 2015
Urchin Fertilization	Dec. 2015- Feb. 2016	July 2015	Oct. 2014
Sand Dollar Fertilization	Dec. 2015- Feb. 2016	July 2015	Oct. 2014
Urchin Development	Dec. 2015- Feb. 2016	July 2015	Oct. 2014
Sand Dollar Development	Dec. 2015- Feb. 2016	July 2015	Oct. 2014

Dates shaded gray = test already completed and results reported herein

MATERIALS AND METHODS

Test Material

All bioassays were performed in accordance with the United States Environmental Protection Agency (USEPA) protocol “Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms” (EPA/600/R-95/136, August 1995). All organisms were acclimated in the laboratory to, or collected directly from ambient seawater. Species-specific test methods are summarized in Tables 2 through 6.

All testing was conducted using natural seawater collected from the intake at Scripps Institution of Oceanography (SIO) in La Jolla, California. After collection, the seawater is held at Nautilus in a 5,000-gallon storage tank, and is continuously cycled through an in-line 20- μ m fiber filter system and a chiller unit. The ambient salinity of SIO seawater is approximately 33.5 parts per thousand (ppt). Salinity of each test concentration was increased with the addition of hypersaline seawater (brine) prepared at Nautilus using methods described in USEPA protocols. Briefly, filtered seawater is partially frozen in a -20 °C freezer overnight. The liquid that remains after the freezing period (now concentrated in salinity) is decanted from the ice, which is composed mainly of fresh water. The brine used for this study was approximately two to three times the salinity of ambient seawater. A brine control was also tested at ambient salinity by mixing brine with deionized water and seawater. The brine control incorporated the highest percentage brine used in the dilution series and was tested to ensure that any effects observed in the test concentrations were not attributable to the brine itself.

All definitive tests were conducted using the same salinity concentrations. Nominal test concentrations consisted of 35.0, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, and 38.5 ppt, as well as an ambient seawater control (lab control), and a brine control.

After dilutions were mixed, minor salinity adjustments were made by addition of brine or seawater so that the measured salinity levels at test initiation matched the nominal salinity to the nearest 0.1 ppt. A Hach SensION5 meter with 0.1 ppt accuracy, calibrated daily to a certified 35 ppt standard was used for all salinity measurements in this study. Water quality parameters (i.e., pH, dissolved oxygen (DO), salinity, and temperature) were measured in all test concentrations prior to test initiation, and once daily depending on the duration of the test.

Table 2. Red Abalone Chronic Test Specifications

Endpoint:	48-hour larval development
Test Organism, Age:	<i>Haliotis rufescens</i> (red abalone), newly fertilized embryos (within one hour of fertilization)
Test Organism Source:	American Abalone (Davenport, California)
Number of Replicates, Organisms per Replicate:	5 replicates, 150-200 embryos per replicate
Test Chamber Type, Volume per Replicate:	Glass shell vial containing 20 mL of test solution
Feeding:	none
Test Acceptability:	Mean normal development must be 80 percent or greater in the control, must have statistically significant effect at 56 micrograms per liter ($\mu\text{g/L}$) zinc in the reference toxicant; must achieve a percent minimum significant difference (PMSD) of less than 20.

Table 3. Echinoderm Larval Development Chronic Test Specifications

Endpoint:	72-hour larval development
Test Organism, Age:	<i>Strongylocentrotus purpuratus</i> (purple urchin) and <i>Dendraster excentricus</i> (sand dollar), newly fertilized embryos (within one hour of fertilization)
Test Organism Source:	Field Collected (San Diego, California)
Number of Replicates, Organisms per Replicate:	5 replicates, 150-200 embryos per replicate
Test Chamber Type, Volume per Replicate:	Glass shell vial containing 10 mL of test solution
Feeding:	none
Test Acceptability:	Mean normal development must be 80 percent or greater in the control; must achieve a PMSD of less than 25.

Table 4. Echinoderm Fertilization Chronic Test Specifications

Endpoint:	Egg fertilization (20-min sperm exposure, followed by 20-min egg fertilization period)
Test Organism:	<i>Strongylocentrotus purpuratus</i> (purple urchin) and <i>Dendraster excentricus</i> (sand dollar)
Test Organism Source:	Field Collected (San Diego, California)
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Feeding:	none
Test Acceptability:	Mean fertilization must be 70 percent or greater in the control; must achieve a PMSD of less than 25.

Table 5. Giant Kelp Chronic Test Specifications

Endpoints:	48-hour spore germination and growth
Test Organism:	<i>Macrocystis pyrifera</i> (giant kelp)
Test Organism Source:	Field Collected (La Jolla, California)
Number of Replicates, Organisms per Replicate:	5 replicates, ~250,000 spores per replicate
Test Chamber Type, Volume per Replicate:	Glass petri dish containing 30 mL of test solution
Light Intensity; Photoperiod:	200 ± 40 foot-candles (ft-c); 16 h light, 8 h darkness
Feeding:	none
Test Acceptability:	Mean germination in the controls must be equal to or greater than 70 percent, and mean length equal to or greater than 10 micrometers (µm). The no observed effect concentration (NOEC) for mean length in the reference toxicant must be equal to or less than 35 µg/L copper. The PMSD for both endpoints must be less than 20.

Table 6. Pacific Topsmelt Chronic Test Specifications

Endpoints:	7-day survival and growth (as dry biomass)
Test Organism, Age:	<i>Atherinops affinis</i> (Pacific topsmelt), 12-15 days old at initiation
Test Organism Source:	Aquatic Biosystems, Inc. (Fort Collins, Colorado)
Number of Replicates, Organisms per Replicate:	5 replicates, 5 animals per replicate
Test Chamber Type, Volume per Replicate:	500 mL plastic cup containing 250 mL of test solution
Feeding:	<i>Artemia</i> nauplii, twice daily
Test Acceptability:	Mean survival must be 80 percent or greater in the control, 0.85 mg average weight of control larvae; the median lethal effect concentration (LC ₅₀) with copper must be less than 205 µg/L in the reference toxicant; must achieve a PMSD less than 25 for survival and less than 50 for growth.

Data Analysis

Toxicity test responses were statistically evaluated using the Comprehensive Environmental Toxicity Information System™ (CETIS) version 1.8.7.20 program by Tidepool Scientific Software. All comparisons were made according to flowchart specifications provided in method guidance (USEPA 1995). Results were used to calculate the No Observed Effect Concentration (NOEC), Lowest Observed Effect Concentration (LOEC), and point estimates including estimated median lethal concentration (LC₅₀) or effect values (EC₂₅ and EC₅₀; the concentration calculated by the dose-response in the test where 25 or 50 percent of the organisms were adversely effected). All point estimates were calculated using linear interpolation to ensure comparability of statistical method between tests regardless of minor data variations. 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 brine.

Concurrent reference toxicant tests were conducted to evaluate the sensitivity of each batch of organisms. Copper chloride was used as the reference toxicant for all species, except for abalone for which zinc sulfate was used. The NOEC, LOEC, and LC₅₀/EC₅₀ value for each reference toxicant test is reported in the Quality Assurance section. The mean LC₅₀/EC₅₀ value plus or minus two standard deviations is also reported for up to the last twenty tests performed at Nautilus.

RESULTS

In tests conducted to date, there were no statistically significant effects observed in any test concentration of the Pacific topsmelt, giant kelp, purple urchin or sand dollar fertilization, or the sand dollar larval development tests. The larval development endpoint for purple urchins and abalone appear to be the most sensitive of this suite of tests to increased salinity, with the abalone being more sensitive than purple urchins. These findings are similar to those reported by Phillips et al. in 2012.

A summary of the statistical analysis results for all tests conducted to date is provided in Table 7. Detailed summaries of all test results are presented in Appendix A, and raw data and statistical analysis worksheets are provided in Appendix B.

Table 7. Summary of Statistical Results for all Chronic Salinity Tolerance Tests to Date (October 2014 through July 2015)

Species	Endpoint	Test Start Date	Measured Salinity at Test Initiation (ppt)	NOEC (ppt)	LOEC (ppt)	EC ₂₅ (ppt)	95% CI	EC ₅₀ (ppt)	95% CI
Abalone	Development Rate	12/10/14	34.3 (BC), 33.0 (LC), 35.0, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	36.0	36.5	36.4	36.3 - 36.5	36.7	36.6 - 36.8
Abalone	Development Rate	05/20/15	33.3 (BC), 33.2 (LC), 35.0, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	36.0	36.5	37.1	36.8 - 37.2	37.3	37.3 - 37.4
Purple Urchin	Development Rate	10/30/14	33.7 (BC), 33.3 (LC), 34.9, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	36.5	37.0	38.1	37.8 - 38.2	> 38.5	N/A
Purple Urchin	Development Rate	7/22/15	33.4 (BC), 33.5 (LC), 34.9, 35.4, 35.9, 36.4, 36.9, 37.4, 37.9, 38.5	36.9	37.4	37.7	37.5 - 37.9	38.1	38.0 - 38.3
Sand Dollar	Development Rate	10/30/14	33.7 (BC), 33.3 (LC), 34.9, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Sand Dollar	Development Rate	7/22/15	33.4 (BC), 33.5 (LC), 34.9, 35.4, 35.9, 36.4, 36.9, 37.4, 37.9, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Purple Urchin	Fertilization Rate	10/30/14	33.7 (BC), 33.3 (LC), 34.9, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Purple Urchin	Fertilization Rate	7/22/15	33.4 (BC), 33.5 (LC), 34.9, 35.4, 35.9, 36.4, 36.9, 37.4, 37.9, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Sand Dollar	Fertilization Rate	10/30/14	33.7 (BC), 33.3 (LC), 34.9, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Sand Dollar	Fertilization Rate	7/22/15	33.4 (BC), 33.5 (LC), 34.9, 35.4, 35.9, 36.4, 36.9, 37.4, 37.9, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Giant Kelp	Germination	05/12/15	34.0 (BC), 33.0 (LC), 35.0, 35.5, 36.0, 36.6, 37.0, 37.5, 38.1, 38.5	38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
	Growth			38.5	> 38.5	> 38.5	N/A	> 38.5	N/A
Pacific Topsmelt	Survival	05/05/15	33.3 (BC), 33.3 (LC), 35.0, 35.5, 36.0, 36.5, 37.0, 37.5, 38.0, 38.5	38.5	>38.5	>38.5	N/A	> 38.5	N/A
	Growth			38.5	> 38.5	> 38.5	N/A	> 38.5	N/A

ppt = parts per thousand

BC = Brine Control, LC = Lab Control (ambient seawater). The lab control (ambient seawater) was used for all statistical comparisons.

NOEC = No Observed Effect Level; the lowest level or concentration resulting in no observed effect

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

EC₂₅/ EC₅₀ = the concentration at which an adverse effect is observed in 25 or 50 percent of the organisms

95% CI = 95 percent confidence interval; lower and upper confidence limits

N/A = Not applicable; confidence limits cannot be calculated

QUALITY ASSURANCE

All laboratory and brine controls met the minimum test acceptability criteria. Variability among replicates was within the appropriate limits for all tests as described in EPA 1995, and the ability to detect a statistical difference was deemed appropriate. Minor deviations are noted on datasheets and a glossary of qualifier codes is available in Appendix C.

Final salinity readings in most tests were within 0.2 ppt of the initial measurement with two exceptions. The final salinity measured in the kelp test was 0.5 to 0.7 ppt higher than the initial reading, likely due to some evaporation because this test is conducted under a specified light intensity. Final readings in the topsmelt test were a maximum of 0.4 ppt higher than the initial readings. Neither of these two tests resulted in any adverse effects to the test organisms in any of the salinity concentrations tested.

Reference Toxicant Test

All reference toxicant tests included in this study met test acceptability criteria. Additionally, the LC₅₀ or EC₅₀ values calculated for all endpoints were within two standard deviations of the internal control chart means, indicating typical test organism sensitivity to copper (zinc for abalone). Reference toxicant results are summarized in Table 8, and provided in full in Appendix D.

Table 8. Summary of Reference Toxicant Statistical Results

Test Endpoint	Test Start Date	NOEC (µg/L)	LOEC (µg/L)	LC ₅₀ /EC ₅₀ value (µg/L)	Mean LC ₅₀ /EC ₅₀ ± 2 SD (µg/L)
Abalone Development	12/10/14	18	32	40.8	56.7 ± 23.4
	5/20/15	32	56	55.5	56.5 ± 21.8
Purple Urchin Development	10/30/14	<2.5	2.5	6.16	8.97 ± 5.17
	7/22/15	<2.5	2.5	10.4	9.85 ± 4.93
Sand Dollar Development	10/30/14	10	>10	14.0	20.8 ± 10.5
	7/22/15	10	20	14.6	18.5 ± 10.9
Purple Urchin Fertilization	10/30/14	<10	10	19.0	40.8 ± 24.5
	7/22/15	<10	10	35.9	22.9 ± 16.9
Sand Dollar Fertilization	10/30/14	10	20	26.2	23.1 ± 34.6
	7/22/15	<10	10	16.8	24.1 ± 24.7
Kelp Germination Growth	5/13/15	10	32	118	110 ± 63.1
		10	32	280	196 ± 157
Pacific Topsmelt Survival Growth	5/5/15	37.5	75	62.7	83.1 ± 38.5
		37.5	>37.5	63.5	85.3 ± 41.7

The reference toxicant for abalone is zinc sulfate; for all other species copper chloride was used. Values reported are in micrograms per liter zinc or copper.

NOEC = No Observed Effect Level; the lowest level or concentration resulting in no observed effect

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

LC₅₀ = Lethal concentration 50, concentration expected to cause mortality to 50 percent of test organisms.

Mean LC₅₀ ± 2 SD = Historical mean of LC₅₀ data for previous tests conducted at Nautilus, plus or minus two standard deviations.

REFERENCES

- California State Water Resources Control Board (SWRCB) 2014. Draft Staff Report Including the Draft Substitute Environmental Documentation Amendment to the Water Quality Control Plan For Ocean Waters of California Addressing Desalination Facility Intakes , Brine Discharges, and the Incorporation of other Non-substantive Changes . Sacramento, CA, July 2014.
- 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.
- Roberts, P., S. Jenkins, J. Paduan, D. Schlenk and J. Weis. 2012. Management of Brine Discharges to Coastal Waters: Recommendations of a Science Advisory Panel. Environmental Review Panel (ERP). Southern California Coastal Water Research Project (SCCWRP). Costa Mesa, CA. Technical Report 694. http://www.swrcb.ca.gov/water_issues/programs/ocean/desalination/docs/dpr.pdf
- Tidepool Scientific Software. 2000-2013. CETIS Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20.
- USEPA 1995. Short-term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136, August 1995.

APPENDIX A

Chronic Test Data Summaries

**Appendix Table A-1. Summary of Salinity Tolerance Chronic Test Results
Red Abalone (*Haliotis rufescens*) 48-hour Development**

Nominal Test Concentration (ppt)	December 10, 2014 Test Date		May 20, 2015 Test Date	
	Mean Percent Normal Development	Standard Deviation	Mean Percent Normal Development	Standard Deviation
Lab Control (LC)	94.0	2.6	96.6	2.7
Brine Control (BC)	94.6	1.8	97.0	2.6
35.0	95.0	2.6	98.4	1.7
35.5	94.6	2.3	96.8	2.2
36.0	90.6	1.8	96.4	1.7
36.5	63.4	7.7	83.4	7.7
37.0	22.6	7.4	78.6	10
37.5	1.20	2.7	34.2	5.8
38.0	0.00	0.0	7.20	6.4
38.5	0.00	0.0	1.40	1.1
NOEC	36.0		36.0	
LOEC	36.5		36.5	
EC ₂₅ /EC ₅₀	36.4/36.7		37.1/37.3	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

**Appendix Table A-2. Summary of Salinity Tolerance Chronic Test Results
Purple Urchin (*Strongylocentrotus purpuratus*) 72-hour Development**

Nominal Test Concentration (ppt)	October 30, 2014 Test Date		July 22, 2015 Test Date	
	Mean Percent Normal Development	Standard Deviation	Mean Percent Normal Development	Standard Deviation
Lab Control (LC)	95.6	2.1	96.8	1.9
Brine Control (BC)	96.2	1.1	96.4	3.6
35.0	95.6	1.8	95.4	1.8
35.5	95.0	1.9	95.4	2.2
36.0	95.8	2.3	95.0	2.1
36.5	94.0	3.8	94.6	3.9
37.0	87.4	4.5	92.2	5.4
37.5	82.6	7.7	81.6	5.2
38.0	74.4	8.0	63.8	10
38.5	52.4	8.8	26.0	10
NOEC	36.5		36.9	
LOEC	37.0		37.4	
EC ₂₅ /EC ₅₀	38.1/>38.5		37.7/38.1	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

**Appendix Table A-3. Summary of Salinity Tolerance Chronic Test Results
Sand Dollar (*Dendraster excentricus*) 72-hour Development**

Nominal Test Concentration (ppt)	October 30, 2014 Test Date		July 22, 2015 Test Date	
	Mean Percent Normal Development	Standard Deviation	Mean Percent Normal Development	Standard Deviation
Lab Control (LC)	97.2	0.84	96.4	1.5
Brine Control (BC)	96.8	2.7	96.0	3.0
35.0	98.4	1.7	97.6	1.7
35.5	98.4	1.1	96.2	1.8
36.0	98.8	1.6	96.2	1.9
36.5	96.8	1.3	94.4	4.0
37.0	97.8	1.3	96.2	3.1
37.5	98.0	1.2	96.6	0.89
38.0	97.4	3.0	93.0	2.2
38.5	97.0	1.9	94.2	2.5
NOEC	38.5		38.5	
LOEC	>38.5		>38.5	
EC ₂₅ /EC ₅₀	>38.5/>38.5		>38.5/>38.5	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

**Appendix Table A-4. Summary of Salinity Tolerance Chronic Test Results
Purple Urchin (*Strongylocentrotus purpuratus*) Fertilization**

Nominal Test Concentration (ppt)	October 30, 2014 Test Date		July 22, 2015 Test Date	
	Mean Percent Fertilization	Standard Deviation	Mean Percent Fertilization	Standard Deviation
Lab Control (LC)	80.8	6.8	93.6	2.4
Brine Control (BC)	83.6	3.6	93.4	3.1
35.0	86.8	6.3	94.6	3.2
35.5	82.8	4.3	93.4	2.1
36.0	83.2	2.5	95.2	2.8
36.5	84.8	6.3	96.4	1.5
37.0	84.2	4.1	94.0	2.1
37.5	82.2	4.0	95.6	1.5
38.0	82.2	2.5	92.4	2.5
38.5	81.6	3.8	91.8	4.0
NOEC	38.5		38.5	
LOEC	>38.5		>38.5	
EC ₂₅ /EC ₅₀	>38.5/>38.5		>38.5/>38.5	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

**Appendix Table A-5. Summary of Salinity Tolerance Chronic Test Results
Sand Dollar (*Dendraster excentricus*) Fertilization**

Nominal Test Concentration (ppt)	October 30, 2014 Test Date		July 22, 2015 Test Date	
	Mean Percent Fertilization	Standard Deviation	Mean Percent Fertilization	Standard Deviation
Lab Control (LC)	97.0	1.7	88.6	3.1
Brine Control (BC)	96.2	1.6	90.4	3.3
35.0	96.8	1.8	90.6	2.8
35.5	95.2	1.1	91.0	6.1
36.0	94.8	2.4	89.6	3.4
36.5	96.0	1.9	92.6	2.8
37.0	96.4	1.3	92.0	1.4
37.5	93.6	3.4	93.6	1.9
38.0	93.8	4.4	88.6	4.2
38.5	95.6	3.1	91.2	3.3
NOEC	38.5		38.5	
LOEC	>38.5		>38.5	
EC ₂₅ /EC ₅₀	>38.5/>38.5		>38.5/>38.5	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

**Appendix Table A-6. Summary of Salinity Tolerance Chronic Test Results
Giant Kelp (*Macrocystis pyrifera*) 48-hour Germination and Growth**

Nominal Test Concentration (ppt)	May 20, 2015 Test Date			
	Mean Percent Germination	Standard Deviation	Mean Growth (µm)	Standard Deviation
Lab Control (LC)	89.4	4.2	14.6	0.3
Brine Control (BC)	92.2	3.3	14.4	1.1
35.0	92.4	2.8	15.3	1.5
35.5	91.2	3.8	15.3	1.5
36.0	91.0	5.6	15.9	0.68
36.5	94.2	2.8	16.0	0.61
37.0	90.4	2.1	16.0	0.95
37.5	91.8	1.9	16.4	0.52
38.0	93.8	3.3	15.8	0.53
38.5	92.6	5.4	15.3	1.1
NOEC	38.5		38.5	
LOEC	>38.5		>38.5	
EC ₂₅ /EC ₅₀	>38.5/>38.5		>38.5/>38.5	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

**Appendix Table A-7. Summary of Salinity Tolerance Chronic Test Results
Pacific Topsmelt (*Atherinops affinis*) 7-day Survival and Growth**

Nominal Test Concentration (ppt)	May 5, 2015 Test Date			
	Mean Percent Survival	Standard Deviation	Mean Growth (mg/org.)	Standard Deviation
Lab Control (LC)	88.0	18	1.16	0.14
Brine Control (BC)	96.0	8.9	1.08	0.10
35.0	100	0.0	1.04	0.05
35.5	100	0.0	1.11	0.05
36.0	96.0	8.9	1.14	0.21
36.5	100	0.0	1.20	0.23
37.0	92.0	18	0.90	0.14
37.5	92.0	11	1.08	0.12
38.0	96.0	8.9	1.10	0.15
38.5	92.0	11.0	1.07	0.13
NOEC	38.5		38.5	
LOEC	>38.5		>38.5	
EC ₂₅ /EC ₅₀	>38.5/>38.5		>38.5/>38.5	

The measured test concentrations were within 0.1 ppt of each nominal concentration at test initiation. All statistical endpoints (i.e., NOEC, LOEC, EC₂₅/EC₅₀) were calculated based on initial measured test concentrations.

Values in **bold** indicate a statistically significant decrease compared to the lab control using Dunnett's Multiple Comparison Test.

NOEC = No Observed Effect Concentration, or the level at which there is no adverse effect.

LOEC = Lowest Observed Effect Concentration, or the lowest concentration at which there is a statistically significant adverse effect.

EC₂₅/EC₅₀ = The calculated test concentration at which 25 or 50 percent of the test organisms are adversely affected.

APPENDIX B

**Chronic Test Data
Raw Data and Statistical Analyses**

**Red Abalone
48-hour Larval Development**

Test Date: December 10, 2014

CETIS Summary Report

Report Date: 07 Aug-15 11:15 (p 1 of 1)
 Test Code: 1412-S285 | 17-5757-1101

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Batch ID: 14-4277-1885	Test Type: Development	Analyst:
Start Date: 10 Dec-14 15:30	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 12 Dec-14 17:00	Species: Haliotis rufescens	Brine: Not Applicable
Duration: 50h	Source: American Abalone	Age:

Sample ID: 05-4416-6489	Code: <i>to 57715 918</i> 14- <i>Nautilus Brine</i>	Client: Poseidon
Sample Date: 10 Dec-14	Material: Brined seawater	Project:
Receive Date: 10 Dec-14	Source: Poseidon	
Sample Age: 16h	Station:	

Batch Note: Linear interpolation is reported for consistency among statistical point estimate methods over all tests conducted. Linear regression also analyzed for comparison.

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-3414-5689	Development Rate	36	36.5	36.25	5.83%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
15-5698-7246	Development Rate	EC25	36.42	36.36	36.48		Linear Regression (MLE)
		EC50	36.69	36.64	36.73		
02-9593-9815	Development Rate	EC25	36.36	36.26	36.51		Linear Interpolation (ICPIN)
		EC50	36.7	36.62	36.78		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-3414-5689	Development Rate	Control Resp	0.94	0.8 - NL	Yes	Passes Acceptability Criteria
02-9593-9815	Development Rate	Control Resp	0.94	0.8 - NL	Yes	Passes Acceptability Criteria
15-5698-7246	Development Rate	Control Resp	0.94	0.8 - NL	Yes	Passes Acceptability Criteria
00-3414-5689	Development Rate	PMSD	0.05833	NL - 0.2	No	Passes Acceptability Criteria

Development Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.946	0.9234	0.9686	0.92	0.97	0.008124	0.01817	1.92%	0.0%
0	Lab Control	5	0.94	0.9071	0.9729	0.91	0.98	0.01183	0.02646	2.82%	0.63%
35		5	0.95	0.9183	0.9817	0.91	0.98	0.0114	0.0255	2.68%	-0.42%
35.5		5	0.946	0.9174	0.9746	0.91	0.97	0.0103	0.02302	2.43%	0.0%
36		5	0.906	0.8834	0.9286	0.88	0.93	0.008124	0.01817	2.01%	4.23%
36.5		5	0.634	0.5388	0.7292	0.57	0.75	0.03429	0.07668	12.09%	32.98%
37		5	0.226	0.1345	0.3175	0.16	0.32	0.03295	0.07369	32.61%	76.11%
37.5		5	0.012	0	0.04532	0	0.06	0.012	0.02683	223.6%	98.73%
38		5	0	0	0	0	0	0	0		100.0%
38.5		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.97	0.92	0.95	0.95	0.94
0	Lab Control	0.98	0.93	0.95	0.93	0.91
35		0.91	0.95	0.98	0.96	0.95
35.5		0.97	0.96	0.94	0.95	0.91
36		0.88	0.93	0.91	0.91	0.9
36.5		0.67	0.57	0.61	0.57	0.75
37		0.16	0.19	0.32	0.17	0.29
37.5		0.06	0	0	0	0
38		0	0	0	0	0
38.5		0	0	0	0	0

CETIS Analytical Report

Report Date: 30 Jun-15 13:57 (p 1 of 2)
 Test Code: 1412-S285 | 17-5757-1101

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Analysis ID: 00-3414-5689	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 30 Jun-15 13:56	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	5.83%	36	36.5	36.25	

Dunnett Multiple Comparison Test

Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control		35	-0.5021	2.407	0.104	8	0.9522	CDF	Non-Significant Effect
		35.5	-0.2519	2.407	0.104	8	0.9145	CDF	Non-Significant Effect
		36	1.594	2.407	0.104	8	0.2140	CDF	Non-Significant Effect
		36.5*	9.393	2.407	0.104	8	<0.0001	CDF	Significant Effect
		37*	19.36	2.407	0.104	8	<0.0001	CDF	Significant Effect
		37.5*	28.65	2.407	0.104	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	7.50991	1.251652	6	267.3	<0.0001	Significant Effect
Error	0.1311219	0.004682924	28			
Total	7.641032		34			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	5.01	16.81	0.5425	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9331	0.9146	0.0346	Normal Distribution

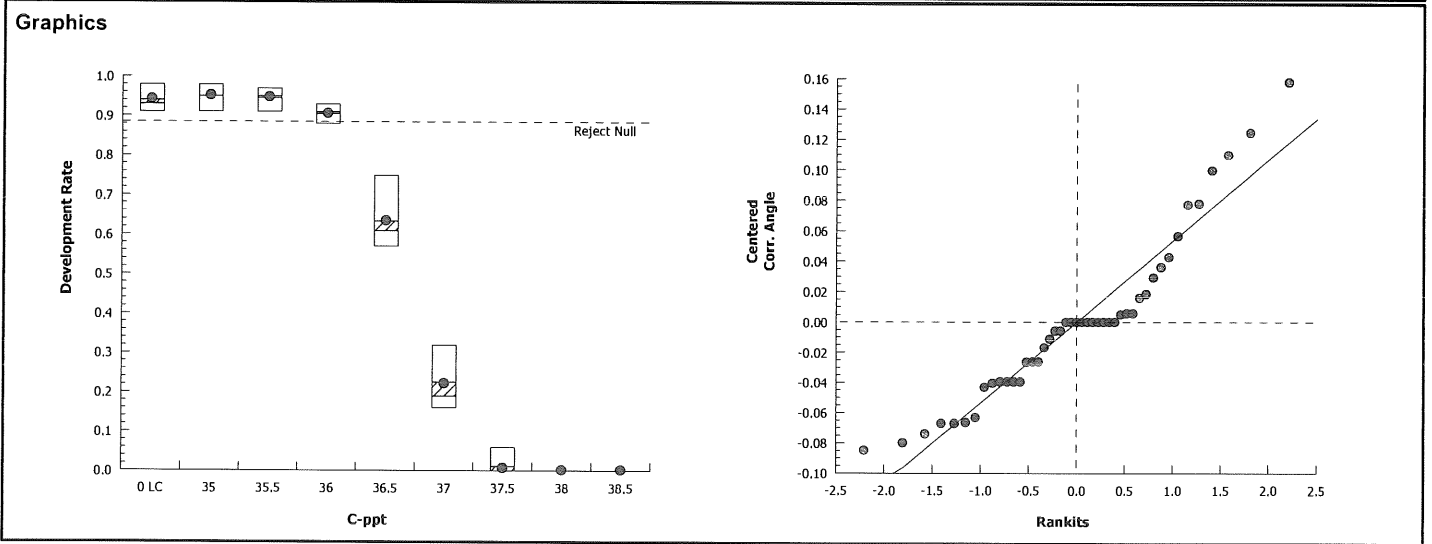
Development Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.94	0.9071	0.9729	0.93	0.91	0.98	0.01183	2.82%	0.0%
35		5	0.95	0.9183	0.9817	0.95	0.91	0.98	0.0114	2.68%	-1.06%
35.5		5	0.946	0.9174	0.9746	0.95	0.91	0.97	0.0103	2.43%	-0.64%
36		5	0.906	0.8834	0.9286	0.91	0.88	0.93	0.008124	2.01%	3.62%
36.5		5	0.634	0.5388	0.7292	0.61	0.57	0.75	0.03429	12.09%	32.55%
37		5	0.226	0.1345	0.3175	0.19	0.16	0.32	0.03295	32.61%	75.96%
37.5		5	0.012	0	0.04532	0	0	0.06	0.012	223.6%	98.72%
38		5	0	0	0	0	0	0	0		100.0%
38.5		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.329	1.252	1.407	1.303	1.266	1.429	0.02788	4.69%	0.0%
35		5	1.351	1.278	1.424	1.345	1.266	1.429	0.02615	4.33%	-1.64%
35.5		5	1.34	1.279	1.402	1.345	1.266	1.397	0.02219	3.7%	-0.82%
36		5	1.26	1.222	1.299	1.266	1.217	1.303	0.01395	2.48%	5.19%
36.5		5	0.9227	0.8217	1.024	0.8963	0.8556	1.047	0.0364	8.82%	30.58%
37		5	0.4915	0.3831	0.5999	0.451	0.4115	0.6013	0.03903	17.76%	63.03%
37.5		5	0.08951	-0.02013	0.1991	0.05002	0.05002	0.2475	0.03949	98.65%	93.27%
38		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.24%
38.5		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.24%

Red Abalone Larval Development Test		Nautilus Environmental (CA)	
Analysis ID: 00-3414-5689	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 30 Jun-15 13:56	Analysis: Parametric-Control vs Treatments	Official Results: Yes	

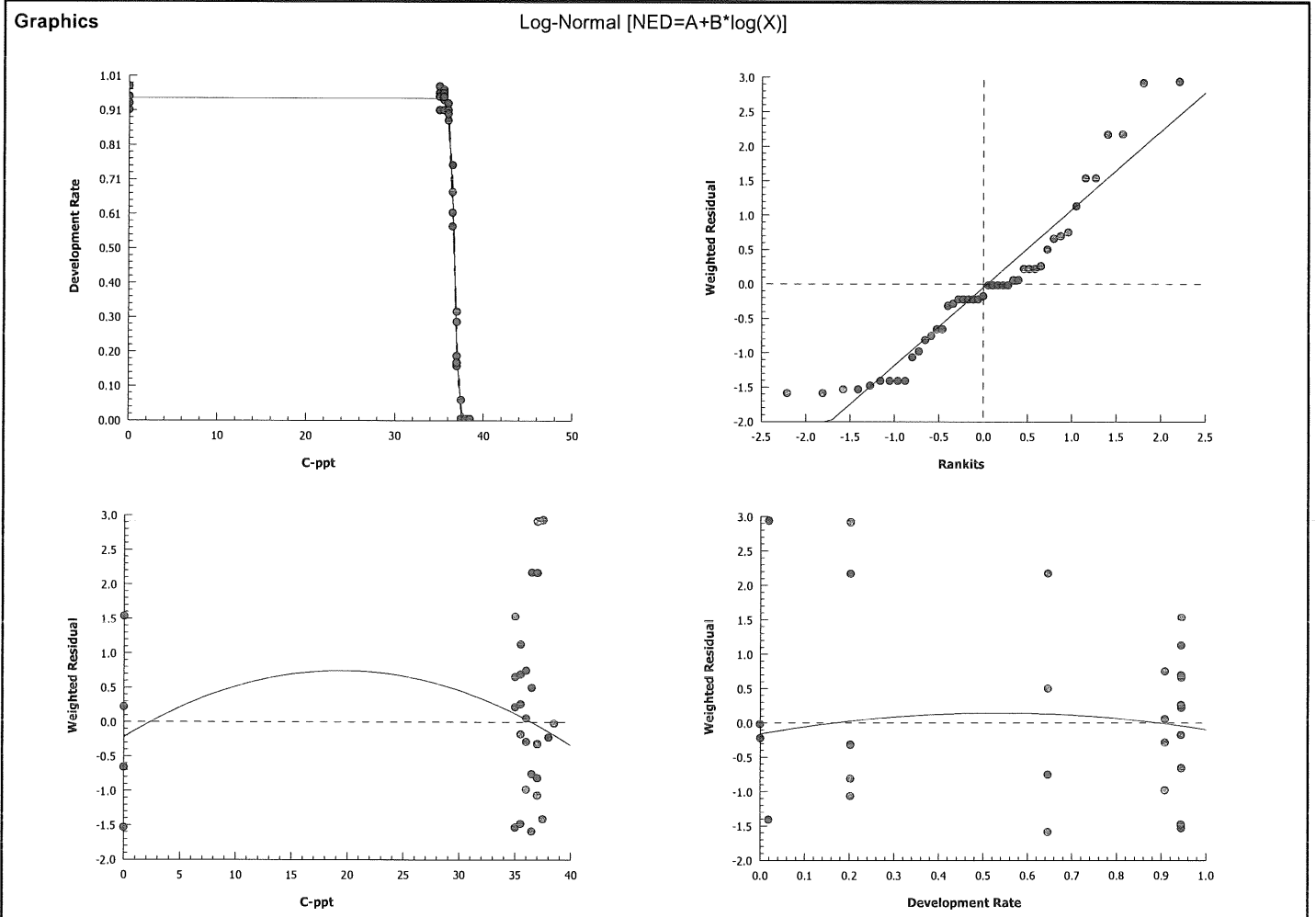


CETIS Analytical Report

Report Date: 30 Jun-15 13:57 (p 1 of 2)
 Test Code: 1412-S285 | 17-5757-1101

Red Abalone Larval Development Test										Nautilus Environmental (CA)	
Analysis ID: 15-5698-7246		Endpoint: Development Rate			CETIS Version: CETISv1.8.7						
Analyzed: 30 Jun-15 13:56		Analysis: Linear Regression (MLE)			Official Results: Yes						
Linear Regression Options											
Model Function		Threshold Option		Threshold		Optimized Pooled		Het Corr		Weighted	
Log-Normal [NED=A+B*log(X)]		Control Threshold		0.06		Yes No		Yes		Yes	
Regression Summary											
Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α:5%)	
7	-1104	2215	2220	1.565	0.004658	0.9807	-0.3747	2.364	1.0000	Non-Significant Lack of Fit	
Point Estimates											
Level	ppt	95% LCL	95% UCL								
EC25	36.42	36.36	36.48								
EC50	36.69	36.64	36.73								
Regression Parameters											
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α:5%)				
Threshold	0.05502	0.006813	0.04127	0.06877	8.076	<0.0001	Significant Parameter				
Slope	214.7	11.12	192.2	237.1	19.3	<0.0001	Significant Parameter				
Intercept	-335.9	17.41	-371	-300.7	-19.29	<0.0001	Significant Parameter				
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Model	3144.959	3144.959	1	2237	<0.0001	Significant					
Lack of Fit	-3.93311	-0.65552	6	-0.3747	1.0000	Non-Significant					
Pure Error	62.98253	1.749515	36								
Residual	59.04942	1.405939	42								
Residual Analysis											
Attribute	Method		Test Stat	Critical	P-Value	Decision(α:5%)					
Goodness-of-Fit	Pearson Chi-Sq GOF		59.05	58.12	0.0422	Significant Heterogeneity					
	Likelihood Ratio GOF		63.56	58.12	0.0174	Significant Heterogeneity					
Variances	Mod Levene Equality of Variance		1.323	2.305	0.2746	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9177	0.9498	0.0035	Non-normal Distribution					
	Anderson-Darling A2 Normality		1.152	2.492	0.0052	Non-normal Distribution					
Development Rate Summary											
Development Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.94	0.91	0.98	0.01183	0.02646	2.82%	0.0%	470	500
35		5	0.95	0.91	0.98	0.0114	0.0255	2.68%	-1.06%	475	500
35.5		5	0.946	0.91	0.97	0.0103	0.02302	2.43%	-0.64%	473	500
36		5	0.906	0.88	0.93	0.008124	0.01817	2.01%	3.62%	453	500
36.5		5	0.634	0.57	0.75	0.03429	0.07668	12.09%	32.55%	317	500
37		5	0.226	0.16	0.32	0.03295	0.07369	32.61%	75.96%	113	500
37.5		5	0.012	0	0.06	0.012	0.02683	223.6%	98.72%	6	500
38		5	0	0	0	0	0		100.0%	0	500
38.5		5	0	0	0	0	0		100.0%	0	500

Red Abalone Larval Development Test		Nautilus Environmental (CA)	
Analysis ID: 15-5698-7246	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 30 Jun-15 13:56	Analysis: Linear Regression (MLE)	Official Results: Yes	



CETIS Analytical Report

Report Date: 07 Aug-15 11:14 (p 1 of 1)
 Test Code: 1412-S285 | 17-5757-1101

Red Abalone Larval Development Test		Nautilus Environmental (CA)	
Analysis ID: 02-9593-9815	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 07 Aug-15 11:13	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	

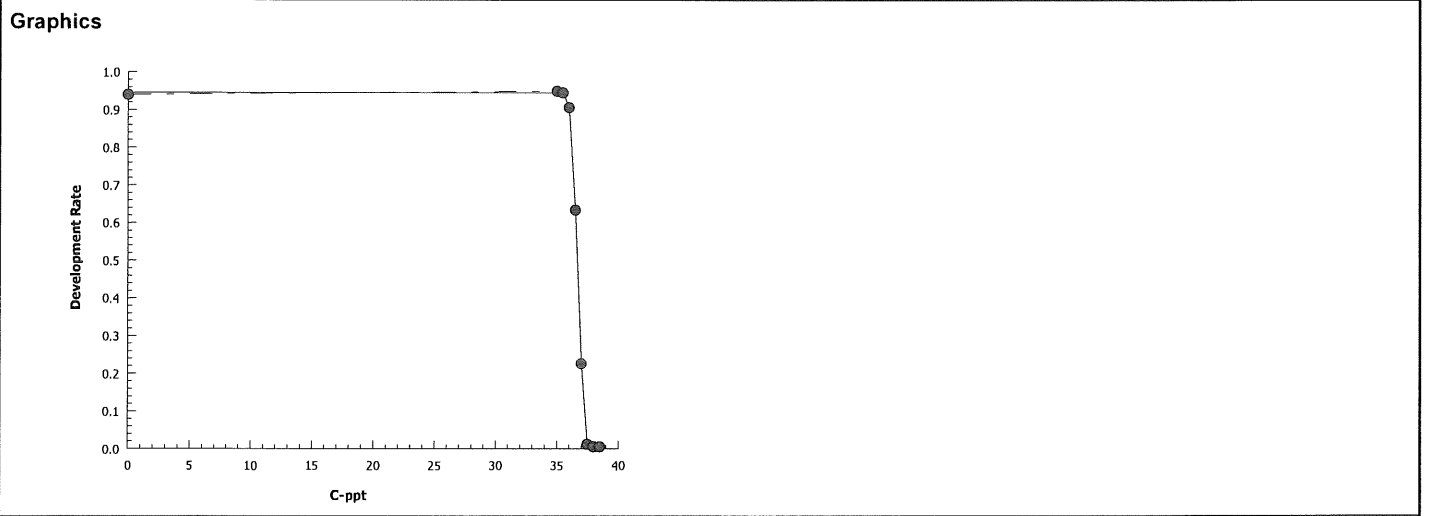
Batch Note: Linear interpolation is reported for consistency among statistical point estimate methods over all tests conducted. Linear regression also analyzed for comparison.

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1835851	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	36.36	36.26	36.51
EC50	36.7	36.62	36.78

Development Rate Summary			Calculated Variate(A/B)									
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
0	Lab Control	5	0.94	0.91	0.98	0.01183	0.02646	2.82%	0.0%	470	500	
35		5	0.95	0.91	0.98	0.0114	0.0255	2.68%	-1.06%	475	500	
35.5		5	0.946	0.91	0.97	0.0103	0.02302	2.43%	-0.64%	473	500	
36		5	0.906	0.88	0.93	0.008124	0.01817	2.01%	3.62%	453	500	
36.5		5	0.634	0.57	0.75	0.03429	0.07668	12.09%	32.55%	317	500	
37		5	0.226	0.16	0.32	0.03295	0.07369	32.61%	75.96%	113	500	
37.5		5	0.012	0	0.06	0.012	0.02683	223.6%	98.72%	6	500	
38		5	0	0	0	0	0		100.0%	0	500	
38.5		5	0	0	0	0	0		100.0%	0	500	

Development Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.98	0.93	0.95	0.93	0.91
35		0.91	0.95	0.98	0.96	0.95
35.5		0.97	0.96	0.94	0.95	0.91
36		0.88	0.93	0.91	0.91	0.9
36.5		0.67	0.57	0.61	0.57	0.75
37		0.16	0.19	0.32	0.17	0.29
37.5		0.06	0	0	0	0
38		0	0	0	0	0
38.5		0	0	0	0	0



CETIS Test Data Worksheet

Report Date: 09 Dec-14 16:04 (p 1 of 2)
 Test Code: 1412-5285 17-5757-1101/1412-S

Red Abalone Larval Development Test Nautilus Environmental (CA)

Start Date: 10 Dec-14 Species: Haliotis rufescens Sample Code: 14-
 End Date: 12 Dec-14 Protocol: EPA/600/R-95/136 (1995) Sample Source: Poseidon
 Sample Date: 10 Dec-14 Material: Brined seawater Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			31	100	0	AD
			32		0	
			33		0	
			34		10	
			35		95	
			36		32	a lot of cellular debris A (-95)
			37		95	
			38		95	
			39		107	cellular debris
			40		95	
			41		59	cellular debris
			42		94	
			43		90	
			44		97	
			45		0	little to no cell division
			46		0	little to no cell division
			47		0	little to no cell division
			48		19	cellular debris
			49		96	
			50		75	cellular debris
			51		91	
			52		57	cellular debris
			53		93	
			54		91	
			55		91	
			56		93	
			57		97	
			58		98	
			59		0	little to no cell division
			60		0	little to no cell division
			61		88	
			62		161	cellular debris
			63		96	
			64		0	little to no cell division
			65		57	cellular debris
			66		92	
			67		93	
			68		16	cellular debris
			69		91	
			70		91	
			71		94	
			72		0	little to no cell division
			73		95	
			74		0	little to no cell division
			75		0	little to no cell division
			76		95/12/16/14	
			77		17	PA -> 80

CETIS Test Data Worksheet

Report Date: 09 Dec-14 16:04 (p 2 of 2)

Test Code: 17-5757-1101/1412-S

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			78	100	0	Very few surviving eggs/amphos, lots of cell debris
			79	100	0	Little to no cell division, some cell debris
			80	100	95	

CETIS Test Data Worksheet

Report Date: 09 Dec-14 16:04 (p 1 of 2)

Test Code: 1412-5285 17-5757-1101/1412-S

Red Abalone Larval Development Test				Nautilus Environmental (CA)			
Start Date: 10 Dec-14	Species: Haliotis rufescens		Sample Code: 14-				
End Date: 12 Dec-14	Protocol: EPA/600/R-95/136 (1995)		Sample Source: Poseidon				
Sample Date: 10 Dec-14	Material: Brined seawater		Sample Station:				

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
0	BC	1	44	100	97	AC 12/12/14
0	BC	2	66			
0	BC	3	35			
0	BC	4	73			
0	BC	5	42			
0	LC	1	58	100	98	AC 12/12/14
0	LC	2	67			
0	LC	3	37			
0	LC	4	56			
0	LC	5	51			
35		1	69	100	95	AC 12/12/14
35		2	38			
35		3	76			
35		4	49			
35		5	40			
35.5		1	57	100	99	AC 12/12/14
35.5		2	63			
35.5		3	71			
35.5		4	80			
35.5		5	55			
36		1	61	99	90	AC 12/12/14
36		2	53			
36		3	70			
36		4	54			
36		5	43			
36.5		1	39			
36.5		2	52	96	68	8/12/12/14 Some cellular debris present
36.5		3	62			
36.5		4	65			
36.5		5	50			
37		1	68			
37		2	48	100	26	8/12/12/14 Lots of cellular debris
37		3	36			
37		4	77			
37		5	41			
37.5		1	34	100	4	8/12/12/14 Very little development
37.5		2	79			
37.5		3	78			
37.5		4	64			
37.5		5	74			
38		1	59	100	0	8/12/12/14 AC ^{no} development Little/No development
38		2	33			
38		3	72			
38		4	75			
38		5	31			
38.5		1	32	100	0	8
38.5		2	45			

CETIS Test Data Worksheet

Report Date: 09 Dec-14 16:04 (p 2 of 2)

Test Code: 17-5757-1101/1412-S

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
38.5		3	60			
38.5		4	47			
38.5		5	46			

QC=VCR

Marine Chronic Bioassay

Water Quality Measurements

Client/Project: Poseidon/Salinity Tolerance Study
 Sample ID: Brine (frozen seawater)
 Test No.: 1412-S285

Test Species: H. rufescens
 Start Date/Time: 12/10/2014 1530
 End Date/Time: 12/12/2014 1700

Concentration (ppt)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	33.0	33.0	33.0	15.0	15.6	15.5	8.2	8.0	7.9	8.08	7.98	7.96
Brine Control	34.3	34.3	34.2	15.6	15.3	15.2	8.0	8.0	7.9	8.06	7.98	7.96
35.0	35.0	35.0	34.9	15.2	15.0	15.1	8.0	8.0	7.9	8.05	7.98	7.96
35.5	35.5	35.5	35.5	15.2	15.0	15.0	8.0	7.9	7.9	8.04	7.98	7.96
36.0	36.0	36.0	36.0	15.3	15.1	15.2	8.0	7.9	7.9	8.03	7.98	7.97
36.5	36.5	36.6	36.6	15.4	14.9	15.0	8.0	8.0	7.9	8.04	7.99	7.98
37.0	37.0	37.0	37.1	15.1	14.9	14.9	8.1	8.0	7.9	8.03	8.00	7.99
37.5	37.5	37.6	37.6	15.0	15.0	15.0	8.0	8.0	7.9	8.02	7.99	7.99
38.0	38.0	38.0	38.1	14.5	14.9	15.0	8.0	8.0	7.9	8.02	7.99	7.99
38.5	38.5	38.5	38.6 38.6	14.6	15.1	15.0	8.1	8.0	7.9	8.02	7.99	7.99
			<small>not used 12/12/14</small>									

Technician Initials: _____
 WQ Readings:

0	24	48
AC/CR	NH	NH

 Dilutions made by:

0	24	48
VCR/AC	-	-

Comments: 0 hrs: Hach Senson5 meter used to measure salinity
 24 hrs: _____
 48 hrs: _____

QC Check: AC 12/16/14

Final Review: E 1-22-15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: PA

Sample ID: frozen seawater

Test Date: 12/10/2014 1530

Test No: 1412-S285

Test Type: Abalone Development

Salinity of Seawater 32.9

Salinity of Brine 91.2

Test Dilution Volume 400

AC Q18 3/20/15 Brine mixture made
Date of Brine used: 1/5 to 1/25; mixed 12/8/14

Alkalinity of Brine Control: 112 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	400
35.0	96.4	385.6	0.04	14.4	400
35.5	95.5	382.2	0.05	17.8	400
36.0	94.7	378.7	0.06	21.3	400
36.5	93.8	375.3	0.07	24.7	400
37.0	93.0	371.9	0.08	28.1	400
37.5	92.1	368.4	0.09	31.6	400
38.0	91.3	365.0	0.10	35.0	400
38.5	90.4	361.6	0.11	38.4	400

DI Volume

Brine Control	52.6	AC Q18 3/20/15 0.73	38.4	400
---------------	------	---------------------	------	-----

68.1 0.56
Total Brine Volume Required (ml): 76.8

QC Check: AC 12/16/14

Final Review: JC 1/22/15

(a) The brine control was originally calculated incorrectly. Additional DI water was added to achieve the appropriate test salinity (33.4 ppt). Therefore, the percent of brine controlled for all test concentrations except the ~~38.0~~ ^{AC Q18 3/20/15} and 38.5 ppt.

Marine Chronic Bioassay

Abalone Embryo-Larval Development

Client: Poseidon Salinity Study

Test Species: Haliotis rufescens

Sample ID: Brine (Frozen seawater)

Start Date/Time: 12/10/2014 15:30

Test No.: 1412-S 285

End Date/Time: 12/12/2014 17:00

Animal Source/Date Received: American Abalone 12/09/14

Number of abalone and condition upon receipt/holding:

Males: 4 / Good condition

Females: 4 / Good condition

	Males:	Females:
Tris & peroxide addition time	1100	1030
Spawn time	1330	1400
Number of spawners	4	1
Condition of spawn (light, moderate, heavy)	Heavy	moderate
Fertilization time	1435	

Embryo counts (per 0.5 ml)	
1	121
2	131
3	145
Mean	132.3

Time of test Initiation: 1530

48 hr. QC 97%

Technician Initials: [Signature]

Comments: _____

QC Check: AC 12/16/14

Final Review: [Signature] 1-22-15

**Red Abalone
48-hour Larval Development**

Test Date: May 20, 2015

CETIS Summary Report

Report Date: 23 Jun-15 10:01 (p 1 of 1)
 Test Code: 1505-S270 | 17-5102-4942

Red Abalone Larval Development Test	Nautilus Environmental (CA)
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Batch ID: 10-1456-6728	Test Type: Development	Analyst:
Start Date: 20 May-15 14:15	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 22 May-15 15:55	Species: Haliotis rufescens	Brine: Frozen Seawater
Duration: 50h	Source: American Abalone	Age:

Sample ID: 02-8083-6653	Code: @10BD3A2D-Nautilus Brine	Client: Poseidon
Sample Date: 20 May-15	Material: Natural Seawater	Project:
Receive Date: 20 May-15	Source: Poseidon	
Sample Age: 14h	Station: Nautilus Brine	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
00-3926-0772	Development Rate	36	36.5	36.25	6.17%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
13-6034-8183	Development Rate	EC25	37.06	36.8	37.15		Linear Interpolation (ICPIN)
		EC50	37.34	37.26	37.39		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
00-3926-0772	Development Rate	Control Resp	0.966	0.8 - NL	Yes	Passes Acceptability Criteria	
13-6034-8183	Development Rate	Control Resp	0.97	0.8 - NL	Yes	Passes Acceptability Criteria	
00-3926-0772	Development Rate	PMSD	0.06168	NL - 0.2	No	Passes Acceptability Criteria	

Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.97	0.9371	1	0.93	1	0.01183	0.02646	2.73%	0.0%
0	Lab Control	5	0.966	0.9325	0.9995	0.94	1	0.01208	0.02702	2.8%	0.41%
35		5	0.984	0.9632	1	0.96	1	0.007483	0.01673	1.7%	-1.44%
35.5		5	0.968	0.9411	0.9949	0.94	0.99	0.009695	0.02168	2.24%	0.21%
36		5	0.964	0.9432	0.9848	0.94	0.98	0.007483	0.01673	1.74%	0.62%
36.5		5	0.834	0.7384	0.9296	0.71	0.9	0.03444	0.07701	9.23%	14.02%
37		5	0.786	0.6577	0.9143	0.66	0.94	0.04622	0.1033	13.15%	18.97%
37.5		5	0.342	0.2699	0.4141	0.25	0.4	0.02596	0.05805	16.97%	64.74%
38		5	0.072	0	0.1512	0.01	0.16	0.02853	0.0638	88.61%	92.58%
38.5		5	0.014	0	0.02816	0	0.03	0.005099	0.0114	81.44%	98.56%

Development Rate Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.96	0.98	1	0.93	0.98	
0	Lab Control	0.95	1	0.94	0.95	0.99	
35		1	0.96	0.98	1	0.98	
35.5		0.94	0.98	0.98	0.99	0.95	
36		0.98	0.96	0.94	0.96	0.98	
36.5		0.71	0.87	0.88	0.81	0.9	
37		0.82	0.66	0.76	0.75	0.94	
37.5		0.38	0.25	0.35	0.4	0.33	
38		0.1	0.01	0.16	0.08	0.01	
38.5		0.03	0	0.01	0.02	0.01	

@Nautilus Brine - @18VOR 6/23/15

CETIS Analytical Report

Report Date: 23 Jun-15 10:00 (p 1 of 2)
 Test Code: 1505-S270 | 17-5102-4942

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Analysis ID: 00-3926-0772 Endpoint: Development Rate CETIS Version: CETISv1.8.7
 Analyzed: 22 Jun-15 16:38 Analysis: Parametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	6.17%	36	36.5	36.25	

Dunnnett Multiple Comparison Test

Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		35	-0.925	2.478	0.141	8	0.9903	CDF	Non-Significant Effect
		35.5	0.029	2.478	0.141	8	0.8820	CDF	Non-Significant Effect
		36	0.2994	2.478	0.141	8	0.8036	CDF	Non-Significant Effect
		36.5*	4.267	2.478	0.141	8	0.0005	CDF	Significant Effect
		37*	5.248	2.478	0.141	8	<0.0001	CDF	Significant Effect
		37.5*	13.65	2.478	0.141	8	<0.0001	CDF	Significant Effect
		38*	20.31	2.478	0.141	8	<0.0001	CDF	Significant Effect
		38.5*	22.6	2.478	0.141	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	11.03475	1.379343	8	170	<0.0001	Significant Effect
Error	0.2921088	0.008114133	36			
Total	11.32686		44			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	10.49	20.09	0.2325	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9729	0.9308	0.3681	Normal Distribution

Development Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.966	0.9325	0.9995	0.95	0.94	1	0.01208	2.8%	0.0%
35		5	0.984	0.9632	1	0.98	0.96	1	0.007483	1.7%	-1.86%
35.5		5	0.968	0.9411	0.9949	0.98	0.94	0.99	0.009695	2.24%	-0.21%
36		5	0.964	0.9432	0.9848	0.96	0.94	0.98	0.007484	1.74%	0.21%
36.5		5	0.834	0.7384	0.9296	0.87	0.71	0.9	0.03444	9.23%	13.66%
37		5	0.786	0.6577	0.9143	0.76	0.66	0.94	0.04622	13.15%	18.63%
37.5		5	0.342	0.2699	0.4141	0.35	0.25	0.4	0.02596	16.97%	64.6%
38		5	0.072	0	0.1512	0.08	0.01	0.16	0.02853	88.61%	92.55%
38.5		5	0.014	0	0.02816	0.01	0	0.03	0.005099	81.44%	98.55%

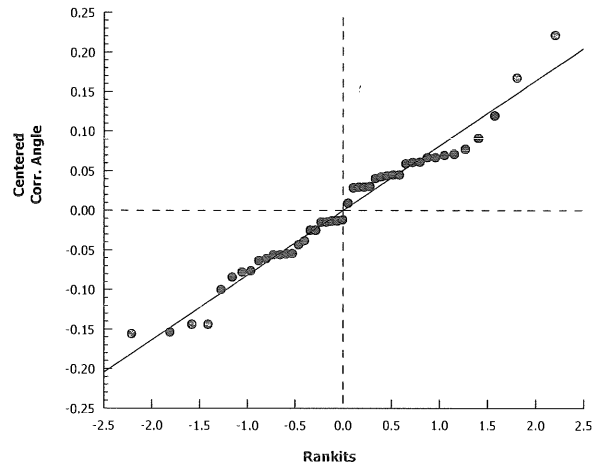
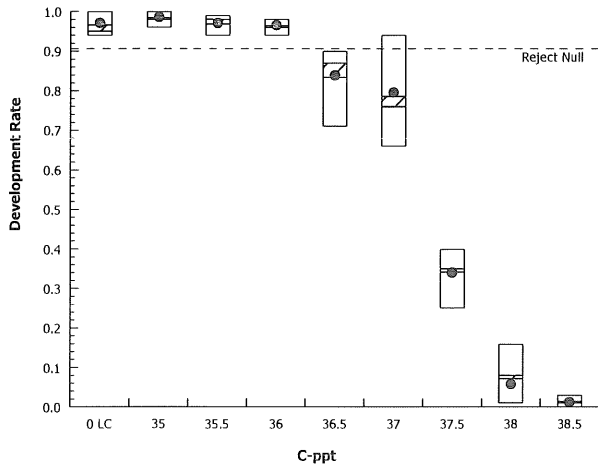
Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.401	1.291	1.511	1.345	1.323	1.521	0.03965	6.33%	0.0%
35		5	1.454	1.372	1.535	1.429	1.369	1.521	0.02944	4.53%	-3.76%
35.5		5	1.399	1.322	1.477	1.429	1.323	1.471	0.02787	4.45%	0.12%
36		5	1.384	1.328	1.44	1.369	1.323	1.429	0.02017	3.26%	1.22%
36.5		5	1.158	1.035	1.281	1.202	1.002	1.249	0.04442	8.58%	17.35%
37		5	1.102	0.9282	1.276	1.059	0.9483	1.323	0.06262	12.71%	21.34%
37.5		5	0.6235	0.5459	0.7011	0.6331	0.5236	0.6847	0.02794	10.02%	55.5%
38		5	0.2441	0.07145	0.4167	0.2868	0.1002	0.4115	0.06217	56.96%	82.58%
38.5		5	0.1133	0.05482	0.1717	0.1002	0.05002	0.1741	0.02105	41.55%	91.92%

Red Abalone Larval Development Test Nautilus Environmental (CA)

Analysis ID: 00-3926-0772 Endpoint: Development Rate CETIS Version: CETISv1.8.7
Analyzed: 22 Jun-15 16:38 Analysis: Parametric-Control vs Treatments Official Results: Yes

Graphics



CETIS Analytical Report

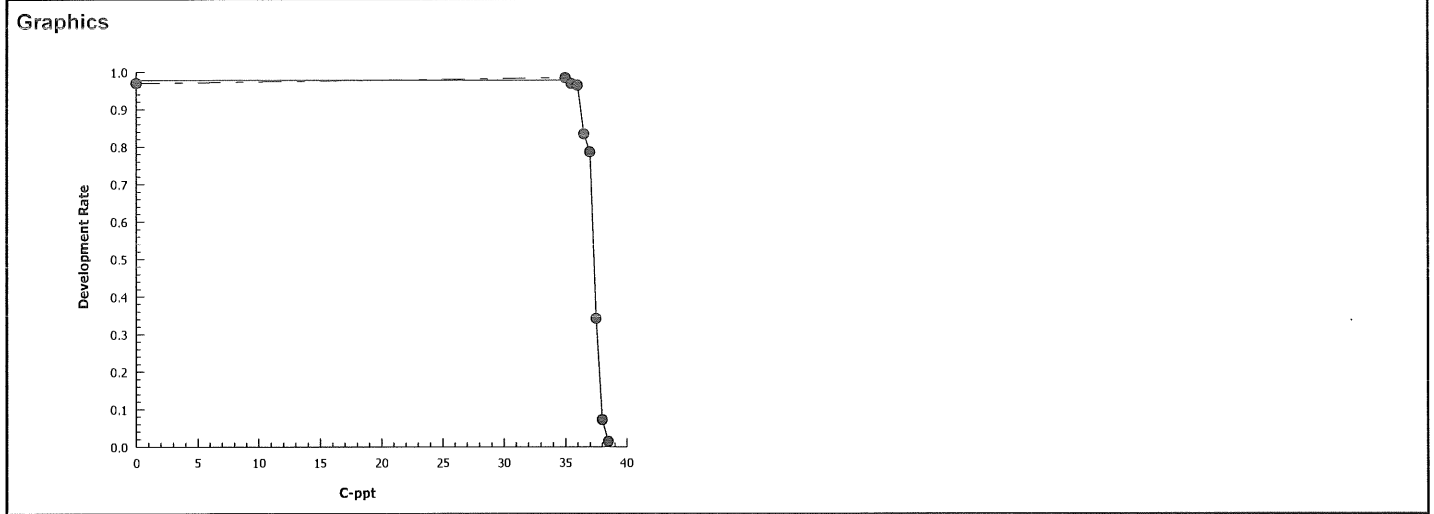
Report Date: 23 Jun-15 10:01 (p 1 of 1)
 Test Code: 1505-S270 | 17-5102-4942

Red Abalone Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 13-6034-8183	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 23 Jun-15 10:00	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	742320	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	37.06	36.8	37.15
EC50	37.34	37.26	37.39

Development Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Brine Control	5	0.97	0.93	1	0.01183	0.02646	2.73%	0.0%	485	500
35		5	0.984	0.96	1	0.007483	0.01673	1.7%	-1.44%	492	500
35.5		5	0.968	0.94	0.99	0.009695	0.02168	2.24%	0.21%	484	500
36		5	0.964	0.94	0.98	0.007484	0.01673	1.74%	0.62%	482	500
36.5		5	0.834	0.71	0.9	0.03444	0.07701	9.23%	14.02%	417	500
37		5	0.786	0.66	0.94	0.04622	0.1033	13.15%	18.97%	393	500
37.5		5	0.342	0.25	0.4	0.02596	0.05805	16.97%	64.74%	171	500
38		5	0.072	0.01	0.16	0.02853	0.0638	88.61%	92.58%	36	500
38.5		5	0.014	0	0.03	0.005099	0.0114	81.44%	98.56%	7	500



CETIS Test Data Worksheet

Report Date: 22 Jun-15 16:29 (p 1 of 2)
 Test Code: 17-5102-4942/1505-S270

Red Abalone Larval Development Test				Nautilus Environmental (CA)	
Start Date:	20 May-15 14:15	Species:	Haliotis rufescens	Sample Code:	10BD3A2D
End Date:	22 May-15 15:55	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Poseidon
Sample Date:	20 May-15	Material:	Natural Seawater	Sample Station:	Nautilus Brine

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			31	100	96	
			32	100	94	
			33	100	10	
			34	100	100	
			35	100	96	
			36	100	3	
			37	100	98	
			38	100	100	
			39	100	33	
			40	100	100	
			41	100	82	
			42	100	94	
			43	100	98	
			44	100	95	
			45	100	1	
			46	100	93	
			47	100	99	
			48	100	71	
			49	100	87	
			50	100	16	
			51	100	90	
			52	100	2	
			53	100	40	
			54	100	35	
			55	100	95	
			56	100	1	
			57	100	76	
			58	100	94	
			59	100	98	
			60	100	98	
			61	100	96	
			62	100	1	
			63	100	38	
			64	100	100	
			65	100	98	
			66	100	0	
			67	100	81	
			68	100	66	
			69	100	88	
			70	100	98	
			71	100	94	
			72	100	1	
			73	100	25	
			74	100	98	
			75	100	99	
			76	100	75	
			77	100	96	

CETIS Test Data Worksheet

Report Date: 22 Jun-15 16:29 (p 2 of 2)
Test Code: 17-5102-4942/1505-S270

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			78	100	8	
			79	100	95	
			80	100	98	

CETIS Test Data Worksheet

Report Date: 19 May-15 15:21 (p 1 of 2)
 Test Code: 185-5270 17-5102-4942/685E852E

Red Abalone Larval Development Test				Nautilus Environmental (CA)	
Start Date: 20 May-15	Species: Haliotis rufescens	Sample Code: 10BD3A2D			
End Date: 22 May-15	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Poseidon			
Sample Date: 20 May-15	Material: Natural Seawater	Sample Station: Nautilus Brine			

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
0	BC	1	31			
0	BC	2	59			
0	BC	3	34			
0	BC	4	46			
0	BC	5	80			
0	LC	1	79			
0	LC	2	64			
0	LC	3	71			
0	LC	4	55			
0	LC	5	75			
35		1	40			
35		2	35			
35		3	65			
35		4	38			
35		5	70			
35.5		1	58			
35.5		2	37			
35.5		3	43			
35.5		4	47			
35.5		5	44			
36		1	60			
36		2	61			
36		3	42			
36		4	77			
36		5	74			
36.5		1	48			
36.5		2	49			
36.5		3	69			
36.5		4	67			
36.5		5	51			
37		1	41			
37		2	68			
37		3	57			
37		4	76			
37		5	32			
37.5		1	63			
37.5		2	73			
37.5		3	54			
37.5		4	53			
37.5		5	39			
38		1	33			
38		2	45			
38		3	50			
38		4	78			
38		5	72			
38.5		1	36			
38.5		2	66			

CETIS Test Data Worksheet

Report Date: 19 May-15 15:21 (p 2 of 2)
Test Code: 17-5102-4942/685E852E

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
38.5		3	62			
38.5		4	52			
38.5		5	56			

QC: AB

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon
 Sample ID: brine (frozen seawater)
 Sample Log No.:
 Test No.: 1505-S270

Test Species: H. rufescens
 Start Date/Time: 5/20/2015 1415
 End Date/Time: 5/22/2015 1555

Concentration Ac 700 ppt 018 6/23/15	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Brine Control	33.3	33.2	33.1	15.2	15.6	15.7	8.3	8.3	8.2	8.08	8.04	8.02
Lab Control	33.2	33.3	33.3	14.8	15.5	15.3	8.5	8.3	8.4	8.10	8.05	8.06
35	35.0	34.9	34.9	14.8	15.3	15.2	8.5	8.3	8.4	8.07	8.06	8.06
35.5	35.5	35.5	35.5	14.7	15.2	15.3	8.5	8.3	8.4	8.07	8.06	8.07
36	36.0	36.0	36.0	14.7	15.3	15.1	8.5	8.4	8.4	8.06	8.06	8.07
36.5	36.5	36.5	36.5	14.8	15.1	15.0	8.5	8.3	8.4	8.06	8.06	8.08
37	37.0	37.0	37.0	14.3	15.1	15.1	8.6	8.4	8.4	8.07	8.06	8.08
37.5	37.5	37.5	37.5	14.4	15.1	15.2	8.5	8.4	8.4	8.06	8.05	8.08
38	38.0	38.1	38.0	15.0	15.2	15.2	8.4	8.3	8.5	8.05	8.06	8.09
38.5	38.5	38.5	38.6	14.9	15.1	15.1	8.5	8.3	8.4	8.05	8.05	8.09

Technician Initials: _____
 WQ Readings:

0	24	48
AC	AG	AD
Dilutions made by:		
AC		

Comments: 0 hrs: Salinity measurements with Hach SensION5 meter
 24 hrs: _____
 48 hrs: _____

QC Check: AC 5/29/15

Final Review: g 6/23/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: AC

Sample ID: frozen seawater

Test Date: 5/20/15

Test No: 1505-5270

Test Type: Acute Abalone Development
AC Q&E 5/22/15

Salinity of Seawater 33.2

Salinity of Brine 84.3

Date of Brine used: 4/28/15

Test Dilution Volume 300

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)
34.0	100.0	250	NA	NA	300
35.0	96.5	289.4	0.04	10.6	300
35.5	95.5	286.5	0.05	13.5	300
36.0	94.5	283.6	0.06	16.4	300
36.5	93.5	280.6	0.07	19.4	300
37.0	92.6	277.7	0.08	22.3	300
37.5	91.6	274.8	0.09	25.2	300
38.0	90.6	271.8	0.10	28.2	300
38.5	89.6	268.9	0.12	31.1	300

0.026418787
 0.033757339
 0.04109589
 0.048434442
 0.055772994
 0.063111546
 0.070450098
 0.07778865

DI Volume

Brine Control	47.9	0.65	31.1	300
----------------------	------	------	------	-----

Total Brine Volume Required (ml): **197.8**

QC Check: AC 5/29/15

Final Review: eg 6/23/15

Marine Chronic Bioassay

Abalone Embryo-Larval Development

Client: Poseidon

Test Species: Haliotis rufescens

Sample ID: Nautilus binne

Start Date/Time: 5/20/2015 1415

Test No.: 1505-8270

End Date/Time: 5/22/2015 1553

Animal Source/Date Received: American Abalone/ 5-14-15

Number of abalone and condition upon receipt/holding:

Males: 4, spawned 5/19/15 in holding

Females: 4

	Males:	Females:
Tris & peroxide addition time	1055	1030
Spawn time	1312	1314
Number of spawners	3	2-4
Condition of spawn (light, moderate, heavy)	moderate	heavy
Fertilization time	1325	

Embryo counts (per 0.5 ml)	
1	124
2	140
3	292
Mean	192

Time of test Initiation: 1415

48 hr. QC 97%

Technician Initials: AC

Comments: _____

QC Check: AC 5/29/15

Final Review: 6/23/15

**Purple Urchin
72-hour Larval Development**

Test Date: October 30, 2014

CETIS Summary Report

Report Date: 30 Jun-15 09:47 (p 1 of 1)
 Test Code: 1410-S149 | 21-4399-6332

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Batch ID: 04-0332-3382	Test Type: Development	Analyst:
Start Date: 30 Oct-14 15:00	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 02 Nov-14 17:00	Species: Strongylocentrotus purpuratus	Brine: Frozen Seawater
Duration: 74h	Source: Pt. Loma	Age:

Sample ID: 20-8391-3010	Code: Brine	Client: Poseidon
Sample Date: 30 Oct-14	Material: Brined seawater	Project:
Receive Date: 30 Oct-14	Source: Poseidon	
Sample Age: 15h	Station: <i>Nautilus Brine</i>	

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-9905-4562	Development Rate	36.5	37	36.75	5.72%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
09-1602-6129	Development Rate	EC25	38.06	37.77	38.22		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
09-1602-6129	Development Rate	Control Resp	0.956	0.8 - NL	Yes	Passes Acceptability Criteria	
18-9905-4562	Development Rate	Control Resp	0.956	0.8 - NL	Yes	Passes Acceptability Criteria	
18-9905-4562	Development Rate	PMSD	0.05724	NL - 0.25	No	Passes Acceptability Criteria	

Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.962	0.9484	0.9756	0.95	0.98	0.004899	0.01095	1.14%	0.0%
0	Lab Control	5	0.956	0.9303	0.9817	0.94	0.99	0.009274	0.02074	2.17%	0.62%
35		5	0.956	0.9334	0.9786	0.93	0.98	0.008124	0.01817	1.9%	0.62%
35.5		5	0.95	0.9268	0.9732	0.93	0.97	0.008367	0.01871	1.97%	1.25%
36		5	0.958	0.9297	0.9863	0.92	0.98	0.0102	0.0228	2.38%	0.42%
36.5		5	0.94	0.8927	0.9873	0.89	0.98	0.01703	0.03808	4.05%	2.29%
37		5	0.874	0.8181	0.9299	0.82	0.93	0.02015	0.04506	5.16%	9.15%
37.5		5	0.826	0.7308	0.9212	0.71	0.89	0.03429	0.07668	9.28%	14.14%
38		5	0.744	0.6448	0.8432	0.65	0.85	0.03572	0.07987	10.74%	22.66%
38.5		5	0.524	0.4141	0.6339	0.39	0.6	0.03957	0.08849	16.89%	45.53%

Development Rate Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.96	0.95	0.96	0.98	0.96	
0	Lab Control	0.94	0.94	0.99	0.96	0.95	
35		0.98	0.95	0.96	0.96	0.93	
35.5		0.93	0.96	0.93	0.96	0.97	
36		0.98	0.97	0.96	0.92	0.96	
36.5		0.96	0.91	0.96	0.89	0.98	
37		0.82	0.85	0.86	0.91	0.93	
37.5		0.85	0.89	0.71	0.89	0.79	
38		0.85	0.71	0.8	0.65	0.71	
38.5		0.6	0.48	0.59	0.39	0.56	

CETIS Analytical Report

Report Date: 30 Jun-15 09:46 (p 1 of 2)
 Test Code: 1410-S149 | 21-4399-6332

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 18-9905-4562	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:46	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	5.72%	36.5	37	36.75	

Dunnnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control		35	0.06846	2.478	0.115	8	0.8722	CDF	Non-Significant Effect
		35.5	0.3886	2.478	0.115	8	0.7720	CDF	Non-Significant Effect
		36	-0.07106	2.478	0.115	8	0.9045	CDF	Non-Significant Effect
		36.5	0.7114	2.478	0.115	8	0.6382	CDF	Non-Significant Effect
		37*	3.311	2.478	0.115	8	0.0069	CDF	Significant Effect
		37.5*	4.717	2.478	0.115	8	0.0001	CDF	Significant Effect
		38*	6.924	2.478	0.115	8	<0.0001	CDF	Significant Effect
		38.5*	11.98	2.478	0.115	8	<0.0001	CDF	Significant Effect

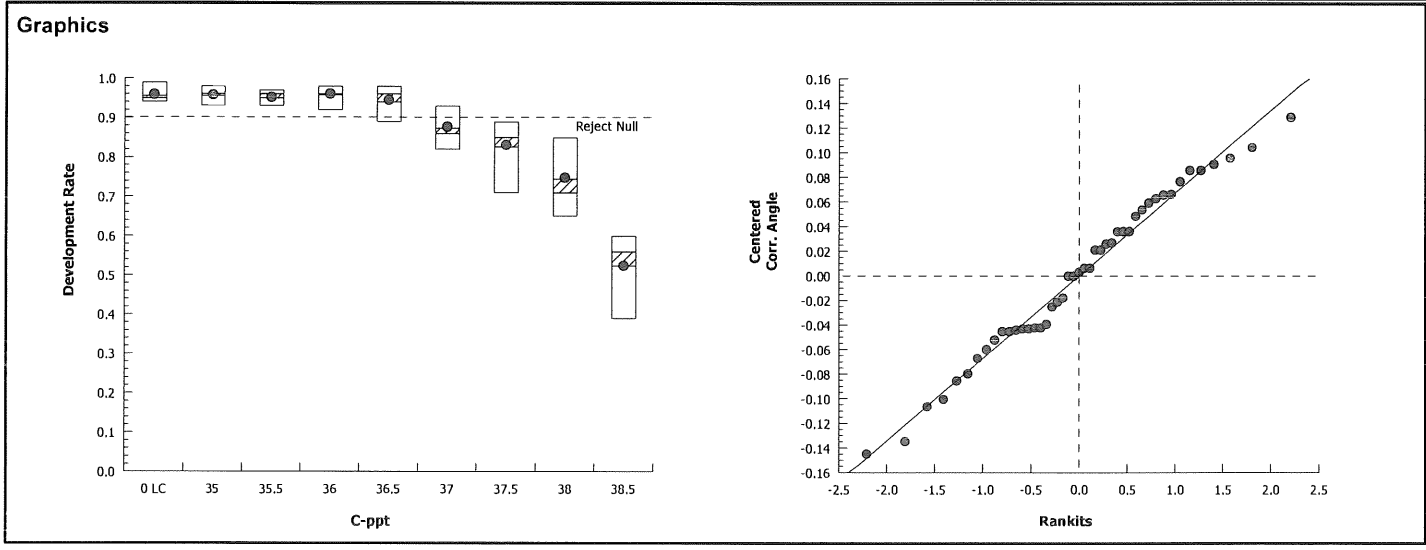
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	1.491822	0.1864777	8	34.5	<0.0001	Significant Effect
Error	0.1945804	0.005405012	36			
Total	1.686402		44			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	5.305	20.09	0.7246	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9797	0.9308	0.6077	Normal Distribution

Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.956	0.9303	0.9817	0.95	0.94	0.99	0.009274	2.17%	0.0%
35		5	0.956	0.9334	0.9786	0.96	0.93	0.98	0.008124	1.9%	0.0%
35.5		5	0.95	0.9268	0.9732	0.96	0.93	0.97	0.008367	1.97%	0.63%
36		5	0.958	0.9297	0.9863	0.96	0.92	0.98	0.0102	2.38%	-0.21%
36.5		5	0.94	0.8927	0.9873	0.96	0.89	0.98	0.01703	4.05%	1.67%
37		5	0.874	0.8181	0.9299	0.86	0.82	0.93	0.02015	5.16%	8.58%
37.5		5	0.826	0.7308	0.9212	0.85	0.71	0.89	0.03429	9.28%	13.6%
38		5	0.744	0.6448	0.8432	0.71	0.65	0.85	0.03572	10.74%	22.18%
38.5		5	0.524	0.4141	0.6339	0.56	0.39	0.6	0.03957	16.89%	45.19%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.366	1.29	1.443	1.345	1.323	1.471	0.02741	4.49%	0.0%
35		5	1.363	1.307	1.42	1.369	1.303	1.429	0.02041	3.35%	0.23%
35.5		5	1.348	1.295	1.402	1.369	1.303	1.397	0.01915	3.18%	1.32%
36		5	1.37	1.303	1.436	1.369	1.284	1.429	0.02405	3.93%	-0.24%
36.5		5	1.333	1.232	1.434	1.369	1.233	1.429	0.03632	6.09%	2.42%
37		5	1.212	1.125	1.299	1.187	1.133	1.303	0.03133	5.78%	11.27%
37.5		5	1.147	1.024	1.27	1.173	1.002	1.233	0.04422	8.62%	16.05%
38		5	1.044	0.9276	1.161	1.002	0.9377	1.173	0.0421	9.01%	23.56%
38.5		5	0.8095	0.6989	0.9201	0.8455	0.6745	0.8861	0.03984	11.01%	40.76%

Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)
Analysis ID: 18-9905-4562	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 30 Jun-15 9:46	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

Report Date: 30 Jun-15 09:47 (p 1 of 1)

Test Code: 1410-S149 | 21-4399-6332

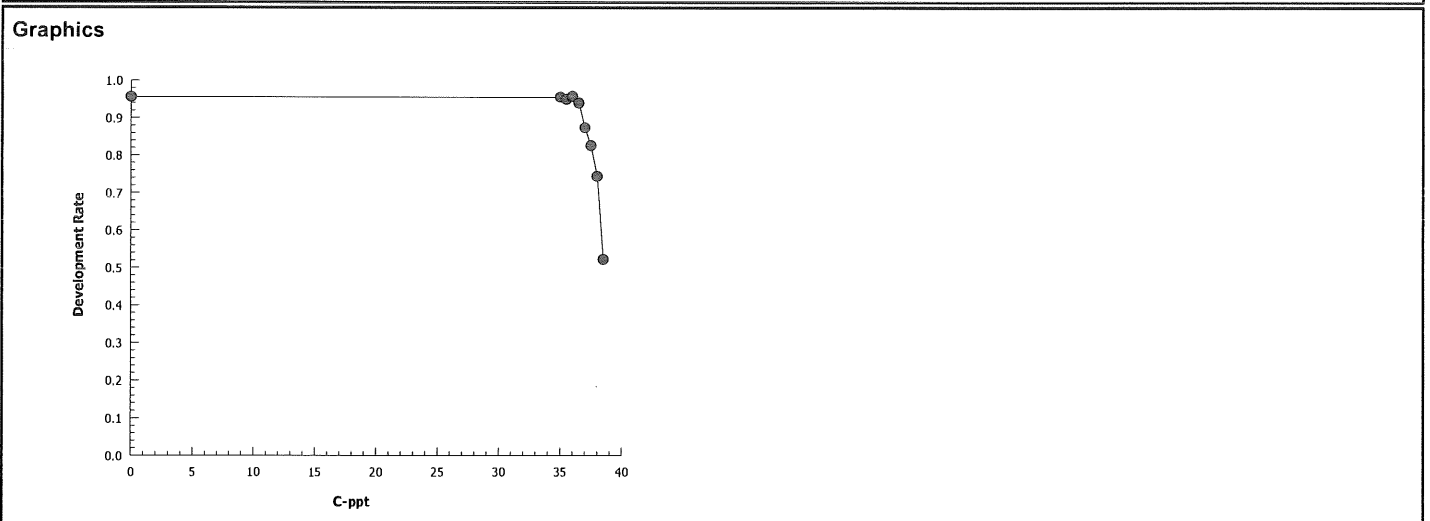
Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 09-1602-6129	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:46	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1731366	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	38.06	37.77	38.22
EC50	>38.5	N/A	N/A

Development Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.956	0.94	0.99	0.009274	0.02074	2.17%	0.0%	478	500
35		5	0.956	0.93	0.98	0.008124	0.01817	1.9%	0.0%	478	500
35.5		5	0.95	0.93	0.97	0.008367	0.01871	1.97%	0.63%	475	500
36		5	0.958	0.92	0.98	0.0102	0.0228	2.38%	-0.21%	479	500
36.5		5	0.94	0.89	0.98	0.01703	0.03808	4.05%	1.67%	470	500
37		5	0.874	0.82	0.93	0.02015	0.04506	5.16%	8.58%	437	500
37.5		5	0.826	0.71	0.89	0.03429	0.07668	9.28%	13.6%	413	500
38		5	0.744	0.65	0.85	0.03572	0.07987	10.74%	22.18%	372	500
38.5		5	0.524	0.39	0.6	0.03957	0.08849	16.89%	45.19%	261	500



CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:36 (p 1 of 2)

Test Code: 1410-S149 21-4399-6332/1410-S149

Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Start Date: 30 Oct-14 Species: Strongylocentrotus purpuratus
 End Date: 03 Nov-14 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 30 Oct-14 Material: Brined seawater

Sample Code: Brine
 Sample Source: Poseidon
 Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	83	SG 11/4/14
			2	100	87	
			3	100	87	
			4	100	77	
			5	100	93	
			6	100	96	
			7	100	98	
			8	100	71	EG 11/5/14
			9	100	78	QC Recounted QC check
			10	100	82	QC ins 86/100
			11	100	92	
			12	100	39	
			13	100	95	
			14	100	48	
			15	100	86	
			16	100	98	
			17	100	79	
			18	100	94	
			19	100	96	
			20	100	96	
			21	100	97	
			22	100	71	
			23	100	91	QC: 88/100
			24	100	56	
			25	100	85	
			26	100	96	
			27	100	60	
			28	100	59	
			29	100	93	
			30	100	96	
			31	100	96	
			32	100	96	
			33	100	95	
			34	100	95	
			35	100	93	
			36	100	89	
			37	100	96	
			38	100	93	
			39	100	85	
			40	100	96	
			41	100	98	
			42	100	97	
			43	100	99	
			44	100	89	QC ins 83/100
			45	100	96	
			46	100	80	
			47	100	89	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:36 (p 2 of 2)
Test Code: 21-4399-6332/1410-S149

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			48	100	85	
			49	100	94	
			50	100	98	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:36 (p 1 of 2)

Test Code: 1410-S149 21-4399-6332/1410-S149

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Start Date: 30 Oct-14 Species: Strongylocentrotus purpuratus Sample Code: Brine
 End Date: 03 Nov-14 Protocol: EPA/600/R-95/136 (1995) Sample Source: Poseidon
 Sample Date: 30 Oct-14 Material: Brined seawater Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
0	BC	1	37	100	98	
0	BC	2	13			
0	BC	3	45			
0	BC	4	7			
0	BC	5	19			
0	LC	1	49			
0	LC	2	18	100	94	
0	LC	3	43			
0	LC	4	31			
0	LC	5	33			
35		1	41			
35		2	34			
35		3	26			
35		4	40			
35		5	5			
35.5		1	29			
35.5		2	3			
35.5		3	38			
35.5		4	6			
35.5		5	42			
36		1	16			
36		2	21			
36		3	20			
36		4	11			
36		5	30			
36.5		1	4			
36.5		2	9			
36.5		3	32			
36.5		4	47			
36.5		5	50			
37		1	10			
37		2	25			
37		3	15	100	93	
37		4	23			
37		5	35			
37.5		1	48			
37.5		2	44			
37.5		3	2			
37.5		4	36			
37.5		5	17			
38		1	39			
38		2	22			
38		3	46			
38		4	1			
38		5	8			
38.5		1	27			
38.5		2	14			

QC=AC

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:36 (p 2 of 2)
Test Code: 21-4399-6332/1410-S149

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
38.5		3	28			
38.5		4	12			
38.5		5	24			

QCEAC

Marine Chronic Bioassay

Water Quality Measurements

Client/Project: Poseidon/Salinity Tolerance Study

Test Species: S.purpuratus

Sample ID: Brine (frozen seawater)

Start Date/Time: 10/30/2014 15:00

Test No.: 1410-S151^{AC 2/5} 1410-S149

End Date/Time: 11/3/2014 17:00
2 11/2/14 Q18

Concentration (ppt)	Salinity (ppt)				Temperature (°C)				Dissolved Oxygen (mg/L)				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	33.3	33.3	33.4	33.6	15.6	15.7	15.3	15.1	8.7	7.9	7.5	7.8	8.04	7.95	8.07	7.93
Brine Control	33.7	33.7	33.8	34.3	15.4	15.7	15.1	14.9	8.8	7.8	7.8	7.8	8.06	8.00	8.11	7.94
35	34.9	34.9	35.0	35.0	14.8	15.8	15.0	15.0	8.8	7.7	7.7	7.9	8.07	7.99	8.11	7.96
35.5	35.5	35.5	35.5	35.5	15.0	15.7	15.0	14.9	8.8	8.0	7.7	7.8	8.07	8.01	8.09	7.98
36	36.0	36.0	36.1	36.1	14.7	15.7	14.8	15.0	8.7	8.1	7.8	7.9	8.13	8.15	8.11	7.98
36.5	36.5	36.5	36.5	36.5	14.7	15.8	14.7	15.0	8.8	7.8	7.8	7.7	8.10	8.05	8.10	7.98
37	37.0	37.0	37.1	37.1	14.8	15.9	14.8	15.0	8.8	7.7	7.9	7.9	8.09	7.99	8.11	7.98
37.5	37.5	37.5	37.4	37.4	14.9	15.9	14.7	15.0	8.8	7.8	7.8	7.9	8.08	8.00	8.19	7.99
38	38.0	37.9	38.0	38.0	15.4	15.9	14.6	15.1	8.7	7.8	7.9	7.9	8.06	8.00	8.17	7.99
38.5	38.5	38.4	38.5	38.5	15.6	16.0	14.7	15.1	8.8	7.8	7.9	7.9	8.05	8.00	8.17	8.00

Technician Initials: WQ Readings:

0	24	48	72
<u>B</u>	<u>AG</u>	<u>KFP</u>	<u>AW</u>

Dilutions made by:

<u>AC</u>			
-----------	--	--	--

Comments: 0 hrs: _____
24 hrs: _____
48 hrs: _____
72 hrs: _____

QC Check: AC 12/5/14

Final Review: KFP 12/5/14

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Pos. eidan / Salinity Tolerance Study
 Sample ID: Brine (from frozen seawater)
 Test No.: 1410-5149

Start Date/Time: 10/30/2014 17:00
 End Date/Time: 11/3/2014 17:14 1200
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 10/6/14

Tech initials: PA
 Injection Time: 14:30

Sperm Absorbance at 400 nm: 0.823 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 18
24
20
25
24
 Mean: 22.2 X 50 = 1110 eggs/ml
 (target counts of ²⁰⁰25 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 1110 eggs/ml = 1.1 dilution factor
 Final density: 1000 eggs/ml = 1.0 part egg stock
0.11 parts seawater
 egg stock 200 ml
 seawater 222 ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Volume of Sperm stock needed to fertilize eggs:

Egg Stock (mL) = 225
 Sperm Stock (μL) = 225
 Egg/Sperm Ratio = 1 ml : 1 μl
 Fertilization Time: 1445

Embryo Stock Fertilization Checks:	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>14:55</u>	<u>100</u>	<u>0</u>	<u>100</u>
20 minutes (2nd fert. If needed)				

Test Initiation Time: 1500 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>97</u>	<u>3</u>	<u>97</u>
End of test QC check			

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test up to 96-hrs (ASTM 1999).

QC Check: KB 11/24/14 Final Review: AC 12/5/14

**Purple Urchin
72-hour Larval Development**

Test Date: July 22, 2015

CETIS Summary Report

Report Date: 04 Aug-15 14:41 (p 1 of 1)
 Test Code: 1507-S081 | 09-7618-8627

Echinoid Embryo-Larval Development Test							Nautilus Environmental (CA)				
Batch ID:	00-7317-1895	Test Type:	Development			Analyst:					
Start Date:	22 Jul-15 16:10	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Natural Seawater				
Ending Date:	25 Jul-15 16:10	Species:	Strongylocentrotus purpuratus			Brine:	Frozen Seawater				
Duration:	72h	Source:	Pt. Loma			Age:					
Sample ID:	13-8856-1048	Code:	1507-S081			Client:	Poseidon				
Sample Date:	22 Jul-15	Material:	Brined seawater			Project:					
Receive Date:	22 Jul-15	Source:	Poseidon								
Sample Age:	16h	Station:									
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
16-2651-6908	Development Rate	36.9	37.4	37.15	5.54%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method				
18-0652-3476	Development Rate	EC25	37.69	37.58	37.77		Linear Regression (MLE)				
		EC50	38.13	38.05	38.21						
01-1762-9710	Development Rate	EC25	37.65	37.49	37.88		Linear Interpolation (ICPIN)				
		EC50	38.14	37.98	38.28						
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
01-1762-9710	Development Rate	Control Resp	0.968	0.8 - NL	Yes	Passes Acceptability Criteria					
16-2651-6908	Development Rate	Control Resp	0.968	0.8 - NL	Yes	Passes Acceptability Criteria					
18-0652-3476	Development Rate	Control Resp	0.968	0.8 - NL	Yes	Passes Acceptability Criteria					
16-2651-6908	Development Rate	PMSD	0.05536	NL - 0.25	No	Passes Acceptability Criteria					
Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
33.4	Brine Control	5	0.964	0.9187	1	0.92	1	0.01631	0.03647	3.78%	0.0%
33.5	Lab Control	5	0.968	0.9441	0.9919	0.95	1	0.008602	0.01924	1.99%	-0.41%
34.9		5	0.954	0.9314	0.9766	0.93	0.98	0.008124	0.01817	1.9%	1.04%
35.4		5	0.954	0.9268	0.9812	0.92	0.98	0.009798	0.02191	2.3%	1.04%
35.9		5	0.95	0.9237	0.9763	0.92	0.97	0.009487	0.02121	2.23%	1.45%
36.4		5	0.946	0.8974	0.9946	0.88	0.98	0.01749	0.03912	4.14%	1.87%
36.9		5	0.922	0.8555	0.9885	0.85	0.98	0.02396	0.05357	5.81%	4.36%
37.4		5	0.816	0.7517	0.8803	0.77	0.89	0.02315	0.05177	6.34%	15.35%
37.9		5	0.638	0.51	0.766	0.47	0.74	0.04609	0.1031	16.15%	33.82%
38.5		5	0.26	0.1331	0.3869	0.19	0.44	0.04572	0.1022	39.32%	73.03%
Development Rate Detail											
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
33.4	Brine Control	0.99	0.93	0.98	1	0.92					
33.5	Lab Control	0.96	0.95	1	0.97	0.96					
34.9		0.95	0.95	0.98	0.93	0.96					
35.4		0.96	0.98	0.92	0.96	0.95					
35.9		0.92	0.97	0.95	0.97	0.94					
36.4		0.98	0.95	0.95	0.88	0.97					
36.9		0.97	0.98	0.85	0.91	0.9					
37.4		0.89	0.77	0.79	0.85	0.78					
37.9		0.47	0.64	0.64	0.74	0.7					
38.5		0.21	0.44	0.24	0.19	0.22					

AC 08/11/15
 Linear regression tests analyzed for comparison of methods. All point estimates reported using linear interpolation for consistency among tests. *Statistical*

CETIS Analytical Report

Report Date: 04 Aug-15 14:41 (p 1 of 2)
Test Code: 1507-S081 | 09-7618-8627

Echinoid Embryo-Larval Development Test										Nautilus Environmental (CA)	
Analysis ID: 16-2651-6908		Endpoint: Development Rate				CETIS Version: CETISv1.8.7					
Analyzed: 04 Aug-15 14:39		Analysis: Parametric-Control vs Treatments				Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	5.54%	36.9	37.4	37.15			
Dunnnett Multiple Comparison Test											
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)		
33.5		34.9	0.822	2.478	0.126	8	0.5873	CDF	Non-Significant Effect		
33.5		35.4	0.8018	2.478	0.126	8	0.5967	CDF	Non-Significant Effect		
33.5		35.9	1.002	2.478	0.126	8	0.5023	CDF	Non-Significant Effect		
33.5		36.4	1.052	2.478	0.126	8	0.4784	CDF	Non-Significant Effect		
33.5		36.9	1.912	2.478	0.126	8	0.1492	CDF	Non-Significant Effect		
33.5		37.4*	5.283	2.478	0.126	8	<0.0001	CDF	Significant Effect		
33.5		37.9*	9.269	2.478	0.126	8	<0.0001	CDF	Significant Effect		
33.5		38.5*	17.05	2.478	0.126	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square	DF	F Stat	P-Value	Decision(α :5%)				
Between	3.356625		0.4195781	8	64.46	<0.0001	Significant Effect				
Error	0.2343234		0.006508983	36							
Total	3.590948		44								
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)					
Variances	Bartlett Equality of Variance		6.686	20.09	0.5709	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9785	0.9308	0.5599	Normal Distribution					
Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	0.968	0.9441	0.9919	0.96	0.95	1	0.008602	1.99%	0.0%
34.9		5	0.954	0.9314	0.9766	0.95	0.93	0.98	0.008124	1.9%	1.45%
35.4		5	0.954	0.9268	0.9812	0.96	0.92	0.98	0.009798	2.3%	1.45%
35.9		5	0.95	0.9237	0.9763	0.95	0.92	0.97	0.009487	2.23%	1.86%
36.4		5	0.946	0.8974	0.9946	0.95	0.88	0.98	0.01749	4.14%	2.27%
36.9		5	0.922	0.8555	0.9885	0.91	0.85	0.98	0.02396	5.81%	4.75%
37.4		5	0.816	0.7517	0.8803	0.79	0.77	0.89	0.02315	6.34%	15.7%
37.9		5	0.638	0.51	0.766	0.64	0.47	0.74	0.04609	16.15%	34.09%
38.5		5	0.26	0.1331	0.3869	0.22	0.19	0.44	0.04572	39.32%	73.14%
Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	1.4	1.314	1.487	1.369	1.345	1.521	0.03119	4.98%	0.0%
34.9		5	1.358	1.301	1.416	1.345	1.303	1.429	0.02062	3.39%	3.0%
35.4		5	1.359	1.295	1.424	1.369	1.284	1.429	0.02336	3.84%	2.92%
35.9		5	1.349	1.289	1.41	1.345	1.284	1.397	0.02173	3.6%	3.65%
36.4		5	1.347	1.246	1.447	1.345	1.217	1.429	0.0361	6.0%	3.83%
36.9		5	1.303	1.17	1.436	1.266	1.173	1.429	0.04784	8.21%	6.97%
37.4		5	1.131	1.044	1.217	1.095	1.071	1.233	0.03116	6.16%	19.25%
37.9		5	0.9274	0.7951	1.06	0.9273	0.7554	1.036	0.04764	11.49%	33.77%
38.5		5	0.5305	0.3926	0.6684	0.4882	0.451	0.7253	0.04967	20.94%	62.12%

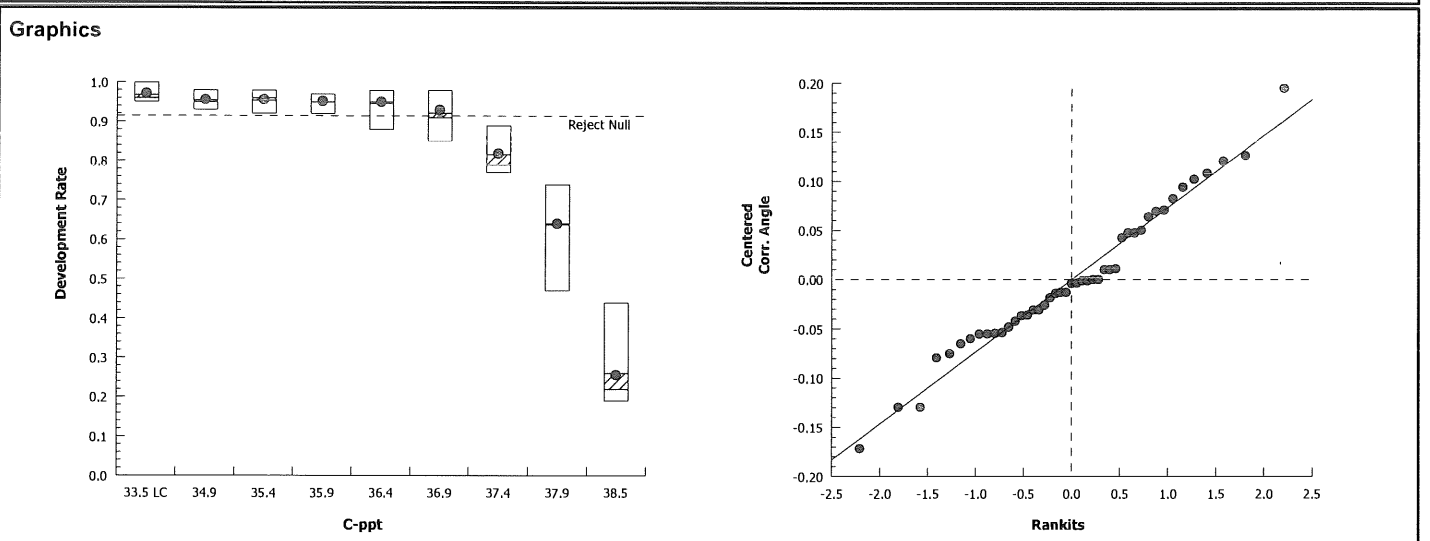
CETIS Analytical Report

Report Date: 04 Aug-15 14:41 (p 2 of 2)
 Test Code: 1507-S081 | 09-7618-8627

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 16-2651-6908	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 04 Aug-15 14:39	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Development Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.96	0.95	1	0.97	0.96
34.9		0.95	0.95	0.98	0.93	0.96
35.4		0.96	0.98	0.92	0.96	0.95
35.9		0.92	0.97	0.95	0.97	0.94
36.4		0.98	0.95	0.95	0.88	0.97
36.9		0.97	0.98	0.85	0.91	0.9
37.4		0.89	0.77	0.79	0.85	0.78
37.9		0.47	0.64	0.64	0.74	0.7
38.5		0.21	0.44	0.24	0.19	0.22

Angular (Corrected) Transformed Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	1.369	1.345	1.521	1.397	1.369
34.9		1.345	1.345	1.429	1.303	1.369
35.4		1.369	1.429	1.284	1.369	1.345
35.9		1.284	1.397	1.345	1.397	1.323
36.4		1.429	1.345	1.345	1.217	1.397
36.9		1.397	1.429	1.173	1.266	1.249
37.4		1.233	1.071	1.095	1.173	1.083
37.9		0.7554	0.9273	0.9273	1.036	0.9912
38.5		0.476	0.7253	0.512	0.451	0.4882



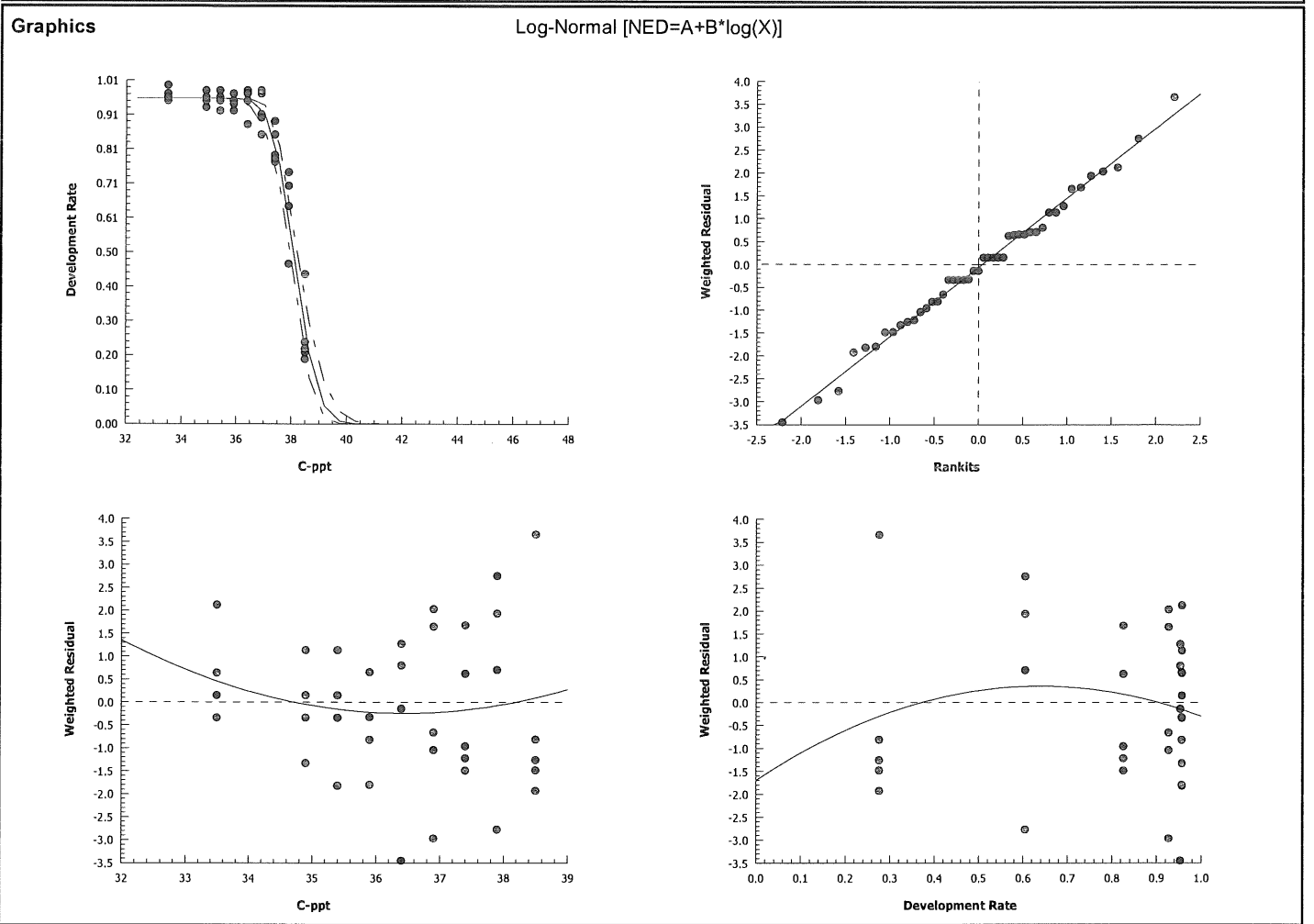
CETIS Analytical Report

Report Date: 04 Aug-15 14:41 (p 1 of 2)
Test Code: 1507-S081 | 09-7618-8627

Echinoid Embryo-Larval Development Test										Nautilus Environmental (CA)	
Analysis ID: 18-0652-3476		Endpoint: Development Rate			CETIS Version: CETISv1.8.7						
Analyzed: 04 Aug-15 14:41		Analysis: Linear Regression (MLE)			Official Results: Yes						
Linear Regression Options											
Model Function		Threshold Option		Threshold		Optimized Pooled		Het Corr		Weighted	
Log-Normal [NED=A+B*log(X)]		Control Threshold		0.032		Yes No		Yes		Yes	
Regression Summary											
Iters	LL	AICc	BIC	Mu	Sigma	Adj R2	F Stat	Critical	P-Value	Decision(α :5%)	
8	-1454	2915	2920	1.581	0.007532	0.9372	0.392	2.364	0.8793	Non-Significant Lack of Fit	
Point Estimates											
Level	ppt	95% LCL	95% UCL								
EC25	37.69	37.58	37.77								
EC50	38.13	38.05	38.21								
Regression Parameters											
Parameter	Estimate	Std Error	95% LCL	95% UCL	t Stat	P-Value	Decision(α :5%)				
Threshold	0.04539	0.006405	0.03247	0.05832	7.087	<0.0001	Significant Parameter				
Slope	132.8	10.96	110.6	154.9	12.11	<0.0001	Significant Parameter				
Intercept	-209.9	17.32	-244.9	-175	-12.12	<0.0001	Significant Parameter				
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)					
Model	1521.97	1521.97	1	658.3	<0.0001	Significant					
Lack of Fit	5.955364	0.992561	6	0.392	0.8793	Non-Significant					
Pure Error	91.1523	2.532008	36								
Residual	97.10767	2.312087	42								
Residual Analysis											
Attribute	Method		Test Stat	Critical	P-Value	Decision(α :5%)					
Goodness-of-Fit	Pearson Chi-Sq GOF		97.11	58.12	<0.0001	Significant Heterogeneity					
	Likelihood Ratio GOF		99.08	58.12	<0.0001	Significant Heterogeneity					
Variances	Bartlett Equality of Variance		8.286	15.51	0.4060	Equal Variances					
	Mod Levene Equality of Variance		0.5334	2.305	0.8209	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9912	0.9498	0.9790	Normal Distribution					
	Anderson-Darling A2 Normality		0.1858	2.492	0.9613	Normal Distribution					
Development Rate Summary											
Development Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
33.5	Lab Control	5	0.968	0.95	1	0.008602	0.01924	1.99%	0.0%	484	500
34.9		5	0.954	0.93	0.98	0.008124	0.01817	1.9%	1.45%	477	500
35.4		5	0.954	0.92	0.98	0.009798	0.02191	2.3%	1.45%	477	500
35.9		5	0.95	0.92	0.97	0.009487	0.02121	2.23%	1.86%	475	500
36.4		5	0.946	0.88	0.98	0.01749	0.03912	4.14%	2.27%	473	500
36.9		5	0.922	0.85	0.98	0.02396	0.05357	5.81%	4.75%	461	500
37.4		5	0.816	0.77	0.89	0.02315	0.05177	6.34%	15.7%	408	500
37.9		5	0.638	0.47	0.74	0.04609	0.1031	16.15%	34.09%	319	500
38.5		5	0.26	0.19	0.44	0.04572	0.1022	39.32%	73.14%	130	500

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 18-0652-3476	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 04 Aug-15 14:41	Analysis: Linear Regression (MLE)	Official Results: Yes			

Development Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.96	0.95	1	0.97	0.96
34.9		0.95	0.95	0.98	0.93	0.96
35.4		0.96	0.98	0.92	0.96	0.95
35.9		0.92	0.97	0.95	0.97	0.94
36.4		0.98	0.95	0.95	0.88	0.97
36.9		0.97	0.98	0.85	0.91	0.9
37.4		0.89	0.77	0.79	0.85	0.78
37.9		0.47	0.64	0.64	0.74	0.7
38.5		0.21	0.44	0.24	0.19	0.22



CETIS Analytical Report

Report Date: 04 Aug-15 14:41 (p 1 of 1)
 Test Code: 1507-S081 | 09-7618-8627

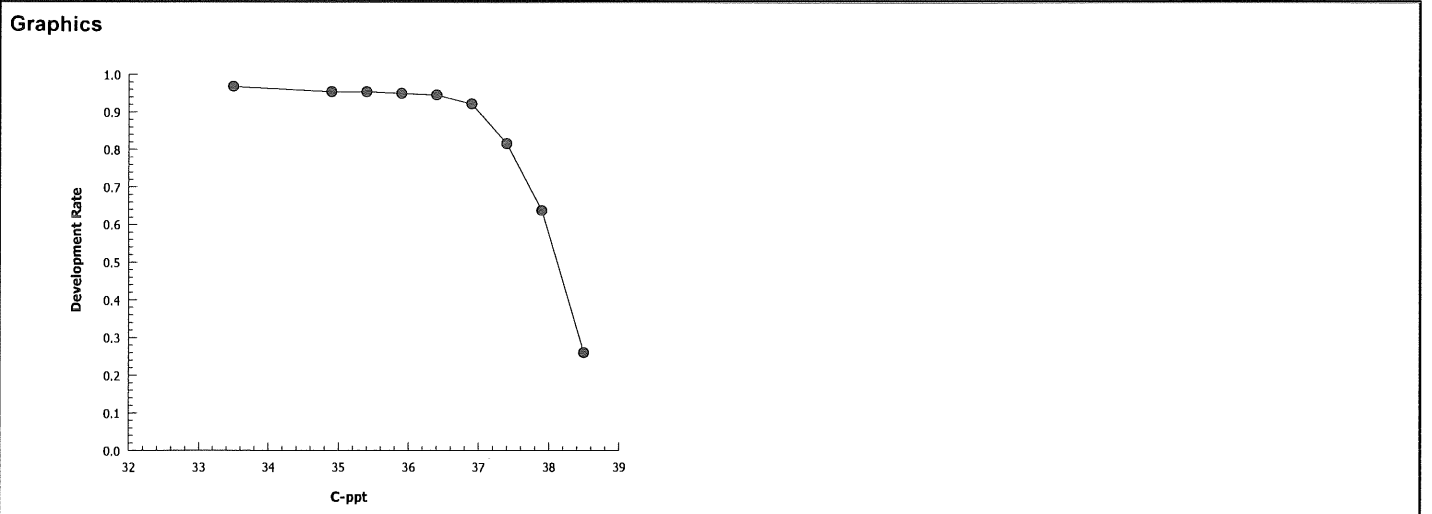
Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 01-1762-9710	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 04 Aug-15 14:41	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1146216	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	37.65	37.49	37.88
EC50	38.14	37.98	38.28

Development Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
33.5	Lab Control	5	0.968	0.95	1	0.008602	0.01924	1.99%	0.0%	484	500
34.9		5	0.954	0.93	0.98	0.008124	0.01817	1.9%	1.45%	477	500
35.4		5	0.954	0.92	0.98	0.009798	0.02191	2.3%	1.45%	477	500
35.9		5	0.95	0.92	0.97	0.009487	0.02121	2.23%	1.86%	475	500
36.4		5	0.946	0.88	0.98	0.01749	0.03912	4.14%	2.27%	473	500
36.9		5	0.922	0.85	0.98	0.02396	0.05357	5.81%	4.75%	461	500
37.4		5	0.816	0.77	0.89	0.02315	0.05177	6.34%	15.7%	408	500
37.9		5	0.638	0.47	0.74	0.04609	0.1031	16.15%	34.09%	319	500
38.5		5	0.26	0.19	0.44	0.04572	0.1022	39.32%	73.14%	130	500

Development Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.96	0.95	1	0.97	0.96
34.9		0.95	0.95	0.98	0.93	0.96
35.4		0.96	0.98	0.92	0.96	0.95
35.9		0.92	0.97	0.95	0.97	0.94
36.4		0.98	0.95	0.95	0.88	0.97
36.9		0.97	0.98	0.85	0.91	0.9
37.4		0.89	0.77	0.79	0.85	0.78
37.9		0.47	0.64	0.64	0.74	0.7
38.5		0.21	0.44	0.24	0.19	0.22



CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:17 (p 1 of 2)

Test Code: ~~1507-5081~~ 09-7618-8627/3A2F74D3

Echinoid Embryo-Larval Development Test				Nautilus Environmental (CA)	
Start Date: 22 Jul-15	Species: Strongylocentrotus purpuratus	Sample Code: 1507-S081		Sample Source: Poseidon	
End Date: 25 Jul-15	Protocol: EPA/600/R-95/136 (1995)	Sample Station:			
Sample Date: 22 Jul-15	Material: Brined seawater				

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	95	organism present in vial (copepod)
			2	100	47	
			3	100	97	
			4	100	93	
			5	100	22	
			6	100	85	
			7	100	74	
			8	100	93	
			9	100	95	
			10	100	85	
			11	100	98	
			12	100	100	
			13	100	97	
			14	100	100	
			15	100	99	
			16	100	94	
			17	100	95	
			18	100	92	
			19	100	79	
			20	100	95	
			21	100	95	
			22	100	96	
			23	100	90	
			24	100	80	
			25	100	21	
			26	100	96	
			27	100	97	
			28	100	64	8/4/15
			29	100	70	
			30	100	97	
			31	100	19	
			32	100	95	
			33	100	91	
			34	100	44	
			35	100	92	
			36	100	96	
			37	100	92	
			38	100	98	
			39	100	98	
			40	100	97	
			41	100	96	
			42	100	95	
			43	100	77	
			44	100	64	
			45	100	98	
			46	100	78	
			47	100	98	

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:17 (p 2 of 2)

Test Code: ~~1507-808~~ 09-7618-8627/3A2F74D3

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			48	100	24	
			49	100	96	8/4/16
			50	100	89	↓

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:17 (p 1 of 2)
 Test Code: 1507-S081 09-7618-8627/3A2F74D3

Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Start Date: 22 Jul-15 Species: Strongylocentrotus purpuratus Sample Code: 1507-S081
 End Date: 25 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Poseidon
 Sample Date: 22 Jul-15 Material: Brined seawater Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
0	BC	1	15	100	99	AC 7/28/15
0	BC	2	4			
0	BC	3	39			
0	BC	4	14			
0	BC	5	18			
0	LC	1	49	100	100	AC 7/28/15
0	LC	2	21			
0	LC	3	12			
0	LC	4	3			
0	LC	5	22			
35		1	42			
35		2	1			
35		3	47			
35		4	8			
35		5	26			
35.5		1	41			
35.5		2	38			
35.5		3	37			
35.5		4	36			
35.5		5	17			
36		1	35			
36		2	30			
36		3	9			
36		4	27			
36		5	16			
36.5		1	45			
36.5		2	20			
36.5		3	32			
36.5		4	24			
36.5		5	13			
37		1	40			
37		2	11			
37		3	10			
37		4	33			
37		5	23			
37.5		1	50			
37.5		2	43			
37.5		3	19			
37.5		4	6			
37.5		5	46			
38		1	2			
38		2	28			
38		3	44			
38		4	7			
38		5	29			
38.5		1	25			
38.5		2	34			

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:17 (p 2 of 2)

Test Code: *1507-3081* 09-7618-8627/3A2F74D3

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
38.5		3	48			
38.5		4	31			
38.5		5	5			

2006

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon

Test Species: *S. purpuratus*

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: 7/21/2015 7/22/15 1610

Sample Log No.: 15NA

End Date/Time: 7/24/2015 7/25/15 1610

Test No.: AC 018 7122
1507-5081

Concentration (ppt)	Salinity (ppt)				Temperature (°C)				Dissolved Oxygen (mg/L)				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	33.5	33.4	33.5	33.3	16.0	15.5	15.2	15.4	8.6	7.9	7.9	8.0	8.09	8.05	8.08	8.03
Brine Control	33.4	33.3	33.4	33.3	16.0	15.2	15.0	15.2	8.4	8.0	7.8	8.2	8.10	8.07	8.08	8.06
35.0	34.9	34.8	34.9	34.8	16.0	15.2	14.9	15.3	8.6	8.0	8.0	8.1	8.09	8.07	8.08	8.06
35.5	35.4	35.4	35.4	35.3	16.0	15.2	14.8	15.2	8.7	8.1	8.0	8.2	8.09	8.08	8.06	8.01
36.0	35.9	35.9	35.9	35.9	15.8	15.2	14.8	15.4	8.8	8.1	8.1	8.2	8.09	8.08	8.07	8.07
36.5	36.4	36.4	36.4	36.3	15.9	15.1	14.8	15.4	8.8	8.1	8.0	8.2	8.09	8.09	8.07	8.07
37.0	36.9	36.9	36.9	36.8	15.9	15.1	14.7	15.4	8.8	8.0	8.0	8.2	8.09	8.09	8.08	8.07
37.5	37.4	37.4	37.4	37.3	15.8	15.1	14.8	15.4	8.8	8.1	8.0	8.2	8.09	8.09	8.08	8.07
38.0	37.9	37.9	37.9	37.8	15.6	15.2	14.7	15.6	8.8	8.0	8.0	8.2	8.09	8.09	8.09	8.07
38.5	38.5	38.5	38.4	38.3	15.6	15.1	14.7	15.6	8.8	8.1	8.0	8.2	8.09	8.09	8.09	8.07

Technician Initials: **WQ Readings:**

0	24	48	72
AD	EG	CH	AD

Dilutions made by:

AC			
----	--	--	--

Comments: 0 hrs: @ AC 018 7/21/15 Hatch Senson 5 Salinity meter
 24 hrs: _____
 48 hrs: _____
 72 hrs: _____

QC Check: AC 8/4/15

Final Review: EG 8/4/15

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Posidon
 Sample ID: Nautilus brine (frozen seawater)
 Test No.: 1507-3081

Start Date/Time: 7/29/15 1610
 End Date/Time: 7/29/15 1610
 Species: S. purpuratus
 Date Collected: 7/19/15

Tech initials: PA/A
 Injection Time: 1525

Sperm Absorbance at 400 nm: 1525 ^{At 918 7122} (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)
0.435

Eggs Counted: 28 Mean: 31 X 50 = 1560 eggs/ml
41
29 (target counts of 20 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)
30
24

Initial density: 1560 eggs/ml = 1.6 dilution factor
 Final density: 1000 eggs/ml = -1.0 stock
0.6 sw
 egg stock ^{At 918 7122} 30100 ml
 seawater 3060 ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Add 100 µL sperm stock per 100mL of egg stock. For example, if you have 60mL of egg stock, add 60µL sperm stock.

Embryo Stock Fertilization Checks (Initiate test only when fertilization is $\geq 90\%$):

	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1605</u>	<u>98</u>	<u>2</u>	<u>98</u>
20 minutes (2nd fert. If needed)	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>

Fertilization Time: 1555

Test Initiation Time: 1610 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>99</u>	<u>1</u>	<u>99</u>
End of test QC check	<u>—</u>	<u>—</u>	<u>—</u>

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test to 96-hrs (ASTM 1999).

QC Check: ACB/4/15 Final Review: [Signature] 8/4/15

**Sand Dollar
72-hour Larval Development**

Test Date: October 30, 2014

CETIS Summary Report

Report Date: 30 Jun-15 09:48 (p 1 of 1)
 Test Code: 1410-S151 | 00-7545-0940

Echinoid Embryo-Larval Development Test	Nautilus Environmental (CA)
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Batch ID: 20-9369-4926	Test Type: Development	Analyst:
Start Date: 30 Oct-14 16:20	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 02 Nov-14 17:20	Species: Dendroaster excentricus	Brine: Frozen Seawater
Duration: 73h	Source: Mission Bay	Age:

Sample ID: 15-8340-2497	Code: Brine	Client: Poseidon
Sample Date: 30 Oct-14	Material: Brined seawater	Project:
Receive Date: 30 Oct-14	Source: Poseidon	
Sample Age: 16h	Station: Nautilus Brine	

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-3618-3366	Development Rate	38.5	>38.5	NA	3.76%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
04-0439-9908	Development Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
04-0439-9908	Development Rate	Control Resp	0.972	0.8 - NL	Yes	Passes Acceptability Criteria	
11-3618-3366	Development Rate	Control Resp	0.972	0.8 - NL	Yes	Passes Acceptability Criteria	
11-3618-3366	Development Rate	PMSD	0.03765	NL - 0.25	No	Passes Acceptability Criteria	

Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.968	0.9347	1	0.92	0.98	0.012	0.02683	2.77%	0.0%
0	Lab Control	5	0.972	0.9616	0.9824	0.96	0.98	0.003742	0.008367	0.86%	-0.41%
35		5	0.984	0.9632	1	0.96	1	0.007483	0.01673	1.7%	-1.65%
35.5		5	0.984	0.9698	0.9982	0.97	1	0.005099	0.0114	1.16%	-1.65%
36		5	0.988	0.9676	1	0.96	1	0.007348	0.01643	1.66%	-2.07%
36.5		5	0.968	0.9518	0.9842	0.95	0.98	0.005831	0.01304	1.35%	0.0%
37		5	0.978	0.9618	0.9942	0.97	1	0.005831	0.01304	1.33%	-1.03%
37.5		5	0.98	0.9648	0.9952	0.97	1	0.005477	0.01225	1.25%	-1.24%
38		5	0.974	0.9372	1	0.93	1	0.01327	0.02966	3.05%	-0.62%
38.5		5	0.97	0.9468	0.9932	0.94	0.99	0.008367	0.01871	1.93%	-0.21%

Development Rate Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.98	0.98	0.98	0.92	0.98	
0	Lab Control	0.97	0.98	0.97	0.98	0.96	
35		0.98	1	1	0.98	0.96	
35.5		0.98	1	0.97	0.98	0.99	
36		0.96	1	0.99	1	0.99	
36.5		0.96	0.97	0.98	0.95	0.98	
37		0.98	0.97	0.97	0.97	1	
37.5		1	0.98	0.98	0.97	0.97	
38		0.93	1	1	0.98	0.96	
38.5		0.99	0.97	0.97	0.98	0.94	

CETIS Analytical Report

Report Date: 30 Jun-15 09:48 (p 1 of 2)
 Test Code: 1410-S151 | 00-7545-0940

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 11-3618-3366	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:47	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.76%	38.5	>38.5	NA	

Dunnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		35	-1.362	2.478	0.090	8	0.9978	CDF	Non-Significant Effect
		35.5	-1.236	2.478	0.090	8	0.9966	CDF	Non-Significant Effect
		36	-1.82	2.478	0.090	8	0.9996	CDF	Non-Significant Effect
		36.5	0.2822	2.478	0.090	8	0.8093	CDF	Non-Significant Effect
		37	-0.6538	2.478	0.090	8	0.9781	CDF	Non-Significant Effect
		37.5	-0.8304	2.478	0.090	8	0.9870	CDF	Non-Significant Effect
		38	-0.6709	2.478	0.090	8	0.9791	CDF	Non-Significant Effect
		38.5	0.02403	2.478	0.090	8	0.8832	CDF	Non-Significant Effect

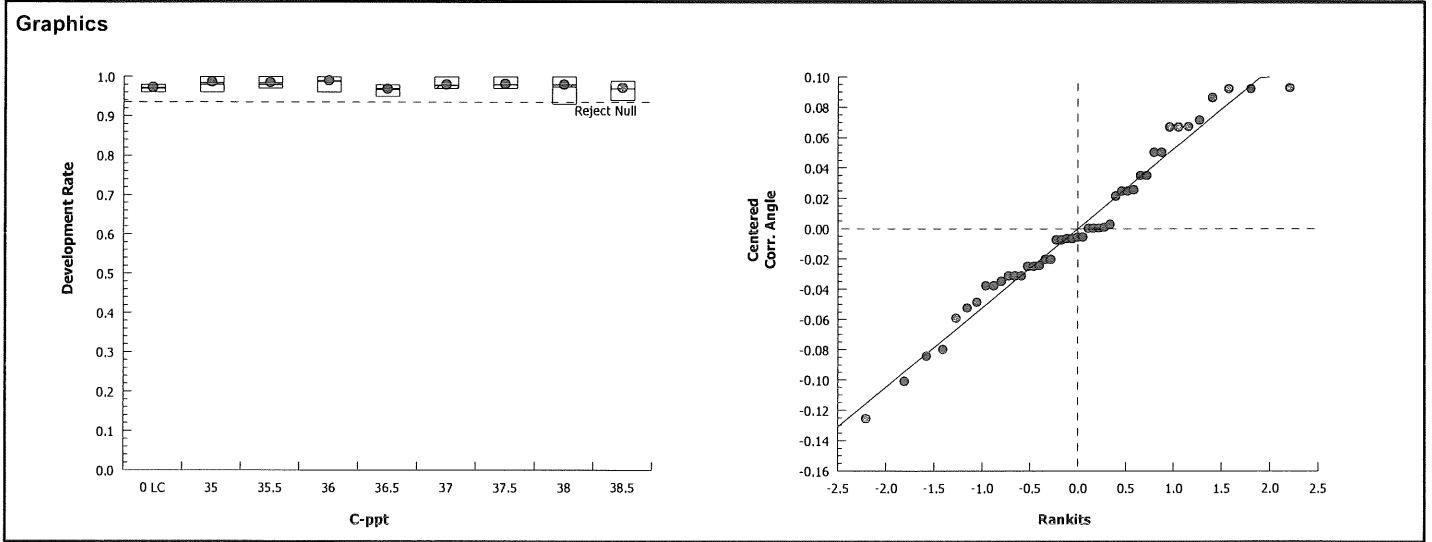
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02641647	0.003302059	8	0.9943	0.4570	Non-Significant Effect
Error	0.1195557	0.003320992	36			
Total	0.1459722		44			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	7.437	20.09	0.4903	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9715	0.9308	0.3283	Normal Distribution	

Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.972	0.9616	0.9824	0.97	0.96	0.98	0.003742	0.86%	0.0%
35		5	0.984	0.9632	1	0.98	0.96	1	0.007483	1.7%	-1.24%
35.5		5	0.984	0.9698	0.9982	0.98	0.97	1	0.005099	1.16%	-1.24%
36		5	0.988	0.9676	1	0.99	0.96	1	0.007349	1.66%	-1.65%
36.5		5	0.968	0.9518	0.9842	0.97	0.95	0.98	0.005831	1.35%	0.41%
37		5	0.978	0.9618	0.9942	0.97	0.97	1	0.005831	1.33%	-0.62%
37.5		5	0.98	0.9648	0.9952	0.98	0.97	1	0.005477	1.25%	-0.82%
38		5	0.974	0.9372	1	0.98	0.93	1	0.01327	3.05%	-0.21%
38.5		5	0.97	0.9468	0.9932	0.97	0.94	0.99	0.008367	1.93%	0.21%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.404	1.373	1.435	1.397	1.369	1.429	0.01127	1.8%	0.0%
35		5	1.454	1.372	1.535	1.429	1.369	1.521	0.02944	4.53%	-3.53%
35.5		5	1.449	1.39	1.509	1.429	1.397	1.521	0.0214	3.3%	-3.21%
36		5	1.47	1.394	1.547	1.471	1.369	1.521	0.02763	4.2%	-4.72%
36.5		5	1.394	1.348	1.44	1.397	1.345	1.429	0.01646	2.64%	0.73%
37		5	1.428	1.361	1.495	1.397	1.397	1.521	0.02403	3.76%	-1.7%
37.5		5	1.434	1.371	1.498	1.429	1.397	1.521	0.02276	3.55%	-2.16%
38		5	1.429	1.31	1.547	1.429	1.303	1.521	0.04258	6.67%	-1.74%
38.5		5	1.403	1.336	1.47	1.397	1.323	1.471	0.02416	3.85%	0.06%

Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)	
Analysis ID: 11-3618-3366	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 30 Jun-15 9:47	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

Report Date: 30 Jun-15 09:48 (p 1 of 1)
 Test Code: 1410-S151 | 00-7545-0940

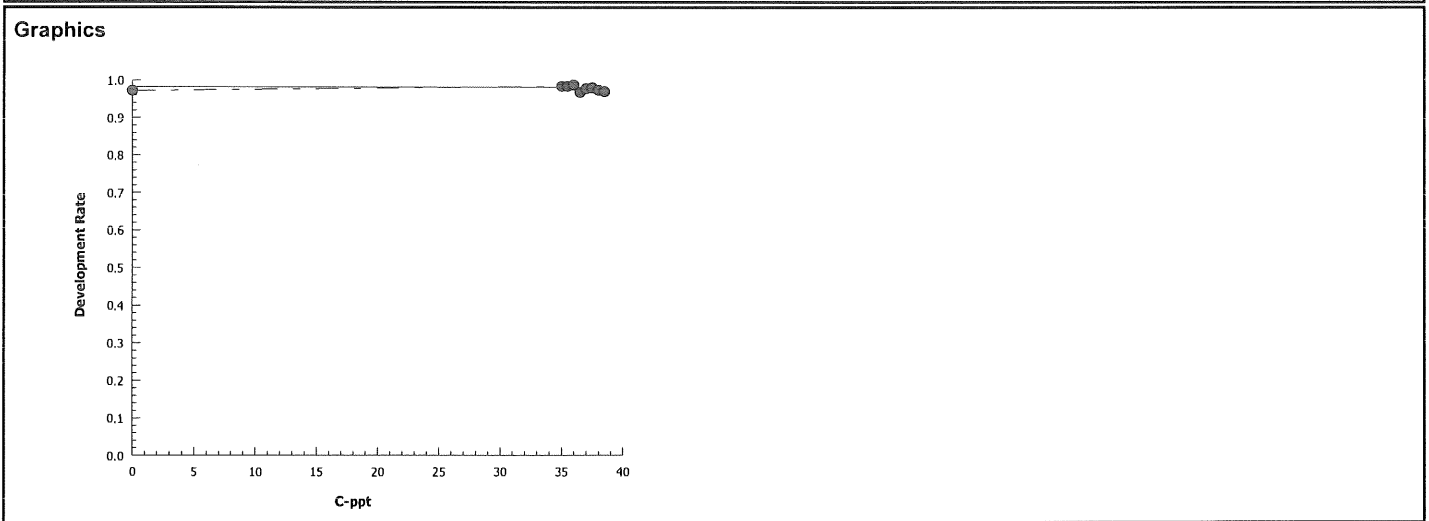
Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 04-0439-9908	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:48	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2735	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Development Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.972	0.96	0.98	0.003742	0.008366	0.86%	0.0%	486	500
35		5	0.984	0.96	1	0.007483	0.01673	1.7%	-1.24%	492	500
35.5		5	0.984	0.97	1	0.005099	0.0114	1.16%	-1.24%	492	500
36		5	0.988	0.96	1	0.007349	0.01643	1.66%	-1.65%	494	500
36.5		5	0.968	0.95	0.98	0.005831	0.01304	1.35%	0.41%	484	500
37		5	0.978	0.97	1	0.005831	0.01304	1.33%	-0.62%	489	500
37.5		5	0.98	0.97	1	0.005477	0.01225	1.25%	-0.82%	490	500
38		5	0.974	0.93	1	0.01327	0.02966	3.05%	-0.21%	487	500
38.5		5	0.97	0.94	0.99	0.008367	0.01871	1.93%	0.21%	485	500



CETIS Test Data Worksheet

Report Date: 24 Nov-14 09:36 (p 1 of 2)
 Test Code: 1410-S151 00-7545-0940/1410-S151

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Start Date: 30 Oct-14	Species: Dendraster excentricus	Sample Code: Brine			
End Date: 03 Nov-14	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Poseidon			
Sample Date: 30 Oct-14	Material: Brined seawater	Sample Station:			

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	100	
			2	100	100	
			3	100	96	
			4	100	98	
			5	100	97	
			6	100	98	
			7	100	99	QC: 99/100
			8	100	97	
			9	100	100	
			10	100	100	
			11	100	99	
			12	100	98	QC: 99/100
			13	100	97	
			14	100	97	
			15	100	98	
			16	100	99	
			17	100	98	
			18	100	96	
			19	100	99	
			20	100	97	
			21	100	97	
			22	100	96	
			23	100	98	
			24	100	100	
			25	100	98	
			26	100	97	
			27	100	96	
			28	100	92	
			29	100	98	
			30	100	100	
			31	100	98	
			32	100	98	
			33	100	98	
			34	100	100	
			35	100	97	
			36	100	98	
			37	100	98	
			38	100	97	
			39	100	98	
			40	100	98	
			41	100	98	QC: 98/100 12/15/14 98/100
			42	100	100	
			43	100	97	
			44	100	100	
			45	100	94	
			46	100	98	
			47	100	97	

CETIS Test Data Worksheet

Report Date: 24 Nov-14 09:36 (p 2 of 2)

Test Code: 00-7545-0940/1410-S151

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			48	100	93	Q.C.: 98/100 ⁰⁻¹⁸ 12/5/14 94/100
			49	100	95	
			50	100	96	Q.C.: 99/100 ⁰⁻¹⁸ 12/5/14 93/100

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:41 (p 1 of 2)
 Test Code: 00-7545-0940/1410-S151

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Start Date: 30 Oct-14	Species: Dendraster excentricus	Sample Code: Brine			
End Date: 03 Nov-14	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Poseidon			
Sample Date: 30 Oct-14	Material: Brined seawater	Sample Station:			

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
0	BC	1	41			
0	BC	2	6			
0	BC	3	32			
0	BC	4	28			
0	BC	5	36			
0	LC	1	35			
0	LC	2	40			
0	LC	3	13			
0	LC	4	46			
0	LC	5	27			
35		1	4			
35		2	24			
35		3	34			
35		4	31			
35		5	3			
35.5		1	15			
35.5		2	42			
35.5		3	8			
35.5		4	29			
35.5		5	16			
36		1	18			
36		2	44			
36		3	11			
36		4	30			
36		5	19			
36.5		1	50			
36.5		2	5			
36.5		3	33			
36.5		4	49			
36.5		5	17			
37		1	12			
37		2	21			
37		3	43			
37		4	47			
37		5	1			
37.5		1	10			
37.5		2	25			
37.5		3	37			
37.5		4	38			
37.5		5	20			
38		1	48			
38		2	9			
38		3	2			
38		4	39			
38		5	22			
38.5		1	7			
38.5		2	14			

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:41 (p 2 of 2)
Test Code: 00-7545-0940/1410-S151

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
38.5		3	26			
38.5		4	23			
38.5		5	45			

QC=AC

Marine Chronic Bioassay

Water Quality Measurements

Client/Project: Poseidon/Salinity Tolerance Study

Test Species: D. excentricus

Sample ID: Brine (frozen seawater)

Start Date/Time: 10/30/2014 16:20

Test No.: 1410-S151

End Date/Time: 11/3/2014 17:20

KFP
Q18
11/2/14

Concentration (ppt)	Salinity (ppt)				Temperature (°C) <small>Q18 11/1/14</small>				Dissolved Oxygen (mg/L) <small>11/1/14</small>				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	33.3	33.3	33.4	33.0	15.6	15.7	KFP 15.3	KFP 15.1	8.7	7.9	KFP 7.9	7.8	8.04	7.98	8.07	7.93
Brine Control	33.7	33.7	33.8	34.3	15.4	15.7	KFP 15.1	KFP 14.9	8.8	7.8	7.8	7.8	8.06	8.00	8.11	7.94
35	34.9	34.9	35.0	35.0	14.8	15.8	KFP 15.0	KFP 15.2	8.8	7.7	7.7	7.9	8.07	7.99	8.11	7.96
35.5	35.5	35.5	35.5	35.5	15.0	15.7	KFP 15.0	14.9	8.8	8.0	7.7	7.8	8.07	8.01	8.09	7.98
36	36.0	36.0	36.1	36.1	14.7	15.7	KFP 14.8	15.0	8.7	8.1	7.8	7.9	8.13	8.15	8.11	7.98
36.5	36.5	36.5	36.5	36.5	14.7	15.8	KFP 14.7	15.0	8.8	7.8	7.8	7.7	8.10	8.05	8.10	7.98
37	37.0	37.0	37.1	37.1	14.8	15.9	KFP 14.8	15.0	8.8	7.7	7.9	7.9	8.09	7.99	8.11	7.98
37.5	37.5	37.5	37.4	37.4	14.9	15.9	KFP 14.7	15.0	8.8	7.8	7.8	7.9	8.08	8.00	8.19	7.99
38	38.0	37.9	38.0	38.0	15.4	15.9	KFP 14.6	15.1	8.7	7.8	7.9	7.9	8.06	8.00	8.17	7.99
38.5	38.5	38.4	38.5	38.5	15.6	16.0	KFP 14.7	15.1	8.8	7.8	7.9	7.9	8.05	8.00	8.17	8.00

Technician Initials: PA WQ Readings: PA AG KFP AW
 Dilutions made by: AC

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____
 72 hrs: _____

QC Check: AC 12/1/14

Final Review: KFP 12/4/14

Marine Chronic Bioassay

Echinoderm Laryal Development Worksheet

Client: Poseldon
 Sample ID: Salinity Tolerance Study (Brine)
 Test No.: 1410-SP51

Start Date/Time: 10/30/2014 11:20
 End Date/Time: 11/3/2014 11:14 1720
 Species: D. excentricus
 Animal Source: Mission Bay
 Date Collected: 10/24/14

Tech initials: AC
 Injection Time: 15:15

Sperm Absorbance at 400 nm: 0.744 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 22
19
21
17
26

Mean: 21 X 50 = 1050 eggs/ml
 (target counts of ²⁰25 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 1050 eggs/ml = 1.05 dilution factor
 Final density: 1000 eggs/ml = 1.0 part egg stock
0.05 parts seawater

egg stock 100 ml
 seawater ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Volume of Sperm stock needed to fertilize eggs:

Egg Stock (mL) = 100
 Sperm Stock (μ L) = 100
 Egg/Sperm Ratio = 1ml:1ul

Fertilization Time: 1555

Embryo Stock Fertilization Checks:	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1605</u>	<u>98</u>	<u>2</u>	<u>98</u>
20 minutes (2nd fert. if needed)	<u> </u>	<u> </u>	<u> </u>	<u> </u>

Test Initiation Time: 16:20 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>97</u>	<u>3</u>	<u>97</u>
End of test QC check	<u> </u>	<u> </u>	<u> </u>

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test up to 96-hrs (ASTM 1999).

QC Check: AC 12/4/14 Final Review: KTP 12/4/14

**Sand Dollar
72-hour Larval Development**

Test Date: July 22, 2015

CETIS Summary Report

Report Date: 04 Aug-15 15:01 (p 1 of 1)
 Test Code: 1507-S083 | 20-0434-3180

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Batch ID: 03-4901-7220	Test Type: Development	Analyst:
Start Date: 22 Jul-15 15:10	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 25 Jul-15 15:40	Species: Dendraster excentricus	Brine: Frozen Seawater
Duration: 73h	Source: Mission Bay	Age:

Sample ID: 15-0490-2480	Code: 1507-S083	Client: Poseidon
Sample Date: 22 Jul-15	Material: Brined seawater	Project:
Receive Date: 22 Jul-15	Source: Poseidon	
Sample Age: 15h	Station:	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-8801-0881	Development Rate	38.5	>38.5	NA	4.29%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
06-2599-1098	Development Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-2599-1098	Development Rate	Control Resp	0.964	0.8 - NL	Yes	Passes Acceptability Criteria
17-8801-0881	Development Rate	Control Resp	0.964	0.8 - NL	Yes	Passes Acceptability Criteria
17-8801-0881	Development Rate	PMSD	0.04292	NL - 0.25	No	Passes Acceptability Criteria

Development Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
33.4	Brine Control	5	0.96	0.9228	0.9972	0.91	0.99	0.01342	0.03	3.13%	0.0%
33.5	Lab Control	5	0.964	0.9452	0.9828	0.94	0.98	0.006782	0.01517	1.57%	-0.42%
34.9		5	0.976	0.9552	0.9968	0.95	0.99	0.007483	0.01673	1.71%	-1.67%
35.4		5	0.962	0.9398	0.9842	0.94	0.99	0.008	0.01789	1.86%	-0.21%
35.9		5	0.962	0.9381	0.9859	0.94	0.99	0.008602	0.01924	2.0%	-0.21%
36.4		5	0.944	0.8939	0.9941	0.9	0.99	0.01806	0.04037	4.28%	1.67%
36.9		5	0.962	0.9233	1	0.91	0.99	0.01393	0.03114	3.24%	-0.21%
37.4		5	0.966	0.9549	0.9771	0.96	0.98	0.004	0.008944	0.93%	-0.63%
37.9		5	0.93	0.9022	0.9578	0.9	0.96	0.01	0.02236	2.4%	3.13%
38.5		5	0.942	0.9111	0.9729	0.92	0.98	0.01114	0.0249	2.64%	1.88%

Development Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.4	Brine Control	0.91	0.97	0.97	0.99	0.96
33.5	Lab Control	0.98	0.97	0.94	0.96	0.97
34.9		0.99	0.97	0.95	0.98	0.99
35.4		0.96	0.99	0.94	0.96	0.96
35.9		0.95	0.94	0.97	0.96	0.99
36.4		0.99	0.98	0.94	0.9	0.91
36.9		0.97	0.98	0.96	0.91	0.99
37.4		0.97	0.96	0.96	0.96	0.98
37.9		0.92	0.93	0.96	0.94	0.9
38.5		0.94	0.92	0.98	0.95	0.92

CETIS Analytical Report

Report Date: 04 Aug-15 15:01 (p 1 of 2)
 Test Code: 1507-S083 | 20-0434-3180

Echinoid Embryo-Larval Development Test					Nautilus Environmental (CA)				
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Analysis ID: 17-8801-0881	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 04 Aug-15 15:00	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.29%	38.5	>38.5	NA	

Dunnnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
33.5		34.9	-1.038	2.478	0.094	8	0.9933	CDF	Non-Significant Effect
33.5		35.4	0.06751	2.478	0.094	8	0.8724	CDF	Non-Significant Effect
33.5		35.9	0.05108	2.478	0.094	8	0.8766	CDF	Non-Significant Effect
33.5		36.4	0.9325	2.478	0.094	8	0.5351	CDF	Non-Significant Effect
33.5		36.9	-0.08789	2.478	0.094	8	0.9079	CDF	Non-Significant Effect
33.5		37.4	-0.09918	2.478	0.094	8	0.9102	CDF	Non-Significant Effect
33.5		37.9	2.034	2.478	0.094	8	0.1203	CDF	Non-Significant Effect
33.5		38.5	1.314	2.478	0.094	8	0.3588	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.04842526	0.006053158	8	1.678	0.1376	Non-Significant Effect
Error	0.1298275	0.00360632	36			
Total	0.1782528		44			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	7.868	20.09	0.4464	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9796	0.9308	0.6045	Normal Distribution

Development Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	0.964	0.9452	0.9828	0.97	0.94	0.98	0.006782	1.57%	0.0%
34.9		5	0.976	0.9552	0.9968	0.98	0.95	0.99	0.007483	1.71%	-1.25%
35.4		5	0.962	0.9398	0.9842	0.96	0.94	0.99	0.008	1.86%	0.21%
35.9		5	0.962	0.9381	0.9859	0.96	0.94	0.99	0.008602	2.0%	0.21%
36.4		5	0.944	0.8939	0.9941	0.94	0.9	0.99	0.01806	4.28%	2.08%
36.9		5	0.962	0.9233	1	0.97	0.91	0.99	0.01393	3.24%	0.21%
37.4		5	0.966	0.9549	0.9771	0.96	0.96	0.98	0.004	0.93%	-0.21%
37.9		5	0.93	0.9022	0.9578	0.93	0.9	0.96	0.01	2.4%	3.53%
38.5		5	0.942	0.9111	0.9729	0.94	0.92	0.98	0.01114	2.64%	2.28%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	1.383	1.334	1.432	1.397	1.323	1.429	0.01765	2.85%	0.0%
34.9		5	1.422	1.356	1.488	1.429	1.345	1.471	0.02377	3.74%	-2.85%
35.4		5	1.38	1.313	1.448	1.369	1.323	1.471	0.02425	3.93%	0.19%
35.9		5	1.381	1.31	1.452	1.369	1.323	1.471	0.02551	4.13%	0.14%
36.4		5	1.348	1.225	1.47	1.323	1.249	1.471	0.04398	7.3%	2.56%
36.9		5	1.386	1.291	1.482	1.397	1.266	1.471	0.03447	5.56%	-0.24%
37.4		5	1.387	1.354	1.419	1.369	1.369	1.429	0.01178	1.9%	-0.27%
37.9		5	1.306	1.25	1.362	1.303	1.249	1.369	0.02007	3.44%	5.59%
38.5		5	1.333	1.259	1.407	1.323	1.284	1.429	0.02668	4.48%	3.61%

CETIS Analytical Report

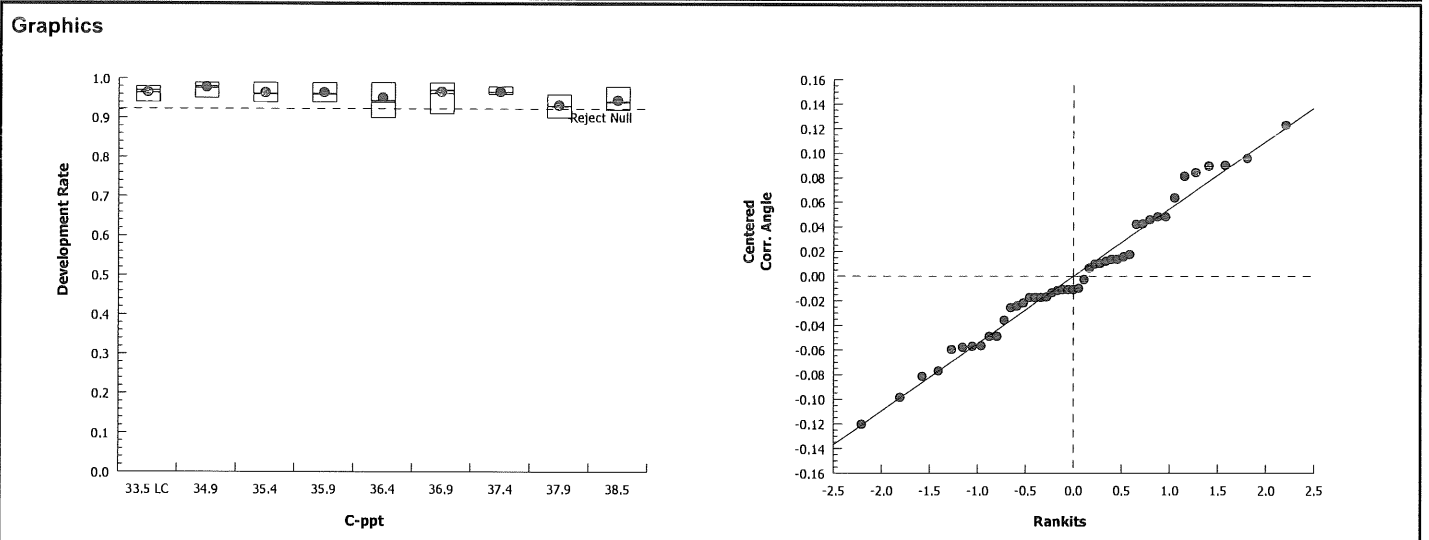
Report Date: 04 Aug-15 15:01 (p 2 of 2)

Test Code: 1507-S083 | 20-0434-3180

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 17-8801-0881	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 04 Aug-15 15:00	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Development Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.98	0.97	0.94	0.96	0.97
34.9		0.99	0.97	0.95	0.98	0.99
35.4		0.96	0.99	0.94	0.96	0.96
35.9		0.95	0.94	0.97	0.96	0.99
36.4		0.99	0.98	0.94	0.9	0.91
36.9		0.97	0.98	0.96	0.91	0.99
37.4		0.97	0.96	0.96	0.96	0.98
37.9		0.92	0.93	0.96	0.94	0.9
38.5		0.94	0.92	0.98	0.95	0.92

Angular (Corrected) Transformed Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	1.429	1.397	1.323	1.369	1.397
34.9		1.471	1.397	1.345	1.429	1.471
35.4		1.369	1.471	1.323	1.369	1.369
35.9		1.345	1.323	1.397	1.369	1.471
36.4		1.471	1.429	1.323	1.249	1.266
36.9		1.397	1.429	1.369	1.266	1.471
37.4		1.397	1.369	1.369	1.369	1.429
37.9		1.284	1.303	1.369	1.323	1.249
38.5		1.323	1.284	1.429	1.345	1.284



CETIS Analytical Report

Report Date: 04 Aug-15 15:01 (p 1 of 1)
 Test Code: 1507-S083 | 20-0434-3180

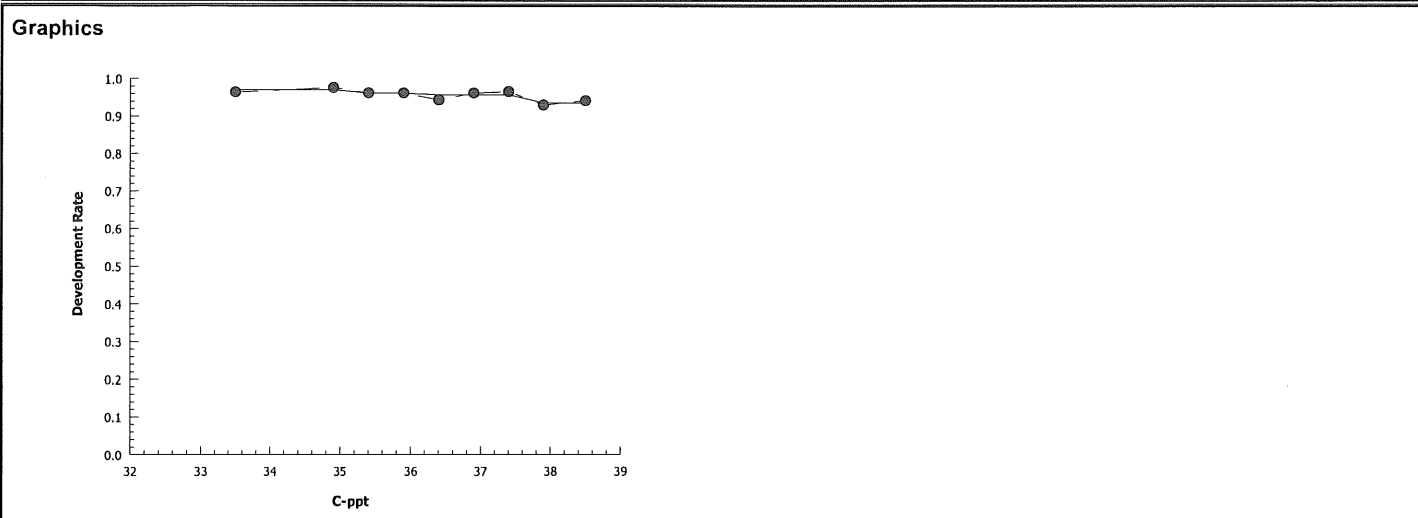
Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 06-2599-1098	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 04 Aug-15 15:01	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1018710	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Development Rate Summary			Calculated Variate(A/B)									
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
33.5	Lab Control	5	0.964	0.94	0.98	0.006782	0.01517	1.57%	0.0%	482	500	
34.9		5	0.976	0.95	0.99	0.007483	0.01673	1.71%	-1.25%	488	500	
35.4		5	0.962	0.94	0.99	0.008	0.01789	1.86%	0.21%	481	500	
35.9		5	0.962	0.94	0.99	0.008602	0.01924	2.0%	0.21%	481	500	
36.4		5	0.944	0.9	0.99	0.01806	0.04037	4.28%	2.08%	472	500	
36.9		5	0.962	0.91	0.99	0.01393	0.03114	3.24%	0.21%	481	500	
37.4		5	0.966	0.96	0.98	0.004	0.008945	0.93%	-0.21%	483	500	
37.9		5	0.93	0.9	0.96	0.01	0.02236	2.4%	3.53%	465	500	
38.5		5	0.942	0.92	0.98	0.01114	0.0249	2.64%	2.28%	471	500	

Development Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.98	0.97	0.94	0.96	0.97
34.9		0.99	0.97	0.95	0.98	0.99
35.4		0.96	0.99	0.94	0.96	0.96
35.9		0.95	0.94	0.97	0.96	0.99
36.4		0.99	0.98	0.94	0.9	0.91
36.9		0.97	0.98	0.96	0.91	0.99
37.4		0.97	0.96	0.96	0.96	0.98
37.9		0.92	0.93	0.96	0.94	0.9
38.5		0.94	0.92	0.98	0.95	0.92



CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:13 (p 1 of 2)
 Test Code: 20-0434-3180/7777D98C

Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Start Date: 22 Jul-15
 End Date: 25 Jul-15
 Sample Date: 22 Jul-15

Species: Dendraster excentricus
 Protocol: EPA/600/R-95/136 (1995)
 Material: Brined seawater

Sample Code: 1507-S 083
 Sample Source: Poseidon
 Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	92	CH 8/3/15
			2	100	90	
			3	100	90	
			4	100	94	
			5	100	94	
			6	100	97	
			7	100	94	
			8	100	98	
			9	100	96	
			10	100	98	
			11	100	96	CH 8/4/15
			12	100	96	
			13	100	99	
			14	100	94	
			15	100	97	
			16	100	96	
			17	100	95	
			18	100	99	
			19	100	99	
			20	100	94	
			21	100	96	
			22	100	92	
			23	100	91	
			24	100	91	
			25	100	97	
			26	100	95	
			27	100	91	
			28	100	96	
			29	100	98	
			30	100	96	
			31	100	96	
			32	100	99	
			33	100	97	
			34	100	96	
			35	100	99	
			36	100	93	
			37	100	96	
			38	100	97	
			39	100	97	
			40	100	95	
			41	100	97	
			42	100	99	
			43	100	98	
			44	100	99	
			45	100	97	
			46	100	92	
			47	100	94	

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:13 (p 2 of 2)

Test Code: | 807-5083 20-0434-3180/7777D98C

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
			48	100	98	CH 8/4/15
			49	100	98	
			50	100	96	↓

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:13 (p 1 of 2)

Test Code: 1507-5083 20-0434-3180/7777D98C

Echinoid Embryo-Larval Development Test				Nautilus Environmental (CA)			
Start Date:	22 Jul-15	Species:	Dendraster excentricus	Sample Code:	1507-S		
End Date:	25 Jul-15	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Poseidon		
Sample Date:	22 Jul-15	Material:	Brined seawater	Sample Station:			

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
0	BC	1	24	100	96	AC 7/28/15
0	BC	2	33			
0	BC	3	25			
0	BC	4	42			
0	BC	5	28			
0	LC	1	10	100	96	AC 7/28/15
0	LC	2	15			
0	LC	3	20			
0	LC	4	30			
0	LC	5	6			
35		1	32			
35		2	39			
35		3	40			
35		4	8			
35		5	19			
35.5		1	12			
35.5		2	44			
35.5		3	5			
35.5		4	21			
35.5		5	11			
36		1	26			
36		2	7			
36		3	45			
36		4	16			
36		5	35			
36.5		1	18			
36.5		2	29			
36.5		3	47			
36.5		4	2			
36.5		5	27			
37		1	38			
37		2	49			
37		3	34			
37		4	23			
37		5	13			
37.5		1	41			
37.5		2	37			
37.5		3	31			
37.5		4	9			
37.5		5	43			
38		1	1			
38		2	36			
38		3	50			
38		4	14			
38		5	3			
38.5		1	4			
38.5		2	46			

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:13 (p 2 of 2)

Test Code: 107-5083 20-0434-3180/7777D98C

C-ppt	Code	Rep	Pos	# Counted	# Normal	Notes
38.5		3	48			
38.5		4	17			
38.5		5	22			

QCing

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon

Test Species: D. excentricus

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: 7/21/2015 1510

Sample Log No.: 15 N/A

End Date/Time: 7/24/2015 1540

Test No.: AC Q18 7122
1507-9083

7/25/15

Concentration (ppt)	Salinity (ppt)				Temperature (°C)				Dissolved Oxygen (mg/L)				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	335	33.4	33.5	33.3	16.0	15.5	15.2	15.4	8.6	7.9	7.9	8.0	8.09	8.05	7.99	8.03
Brine Control	334	33.3	33.4	33.3	16.0	15.2	15.0	15.2	8.4	8.0	7.8	8.2	8.10	8.07	7.99	8.06
35.0	34.9	34.8	34.9	34.8	16.0	15.2	14.9	15.3	8.6	8.0	8.0	8.1	8.09	8.07	7.99	8.06
35.5	35.4	35.4	35.4	35.3	16.0	15.2	14.8	15.2	8.7	8.1	8.0	8.2	8.09	8.08	8.06	8.01
36.0	35.9	35.9	35.9	35.9	15.8	15.2	14.8	15.4	8.8	8.1	8.1	8.2	8.09	8.08	8.07	8.07
36.5	36.4	36.4	36.4	36.3	15.9	15.1	14.8	15.4	8.8	8.1	8.0	8.2	8.09	8.09	8.07	8.07
37.0	36.9	36.9	36.9	36.8	15.9	15.1	14.7	15.4	8.8	8.0	8.0	8.2	8.09	8.09	8.08	8.07
37.5	37.4	37.4	37.4	37.3	15.8	15.1	14.8	15.4	8.8	8.1	8.0	8.2	8.09	8.09	8.08	8.07
38.0	37.9	37.9	37.9	37.8	15.6	15.2	14.7	15.6	8.8	8.0	8.0	8.2	8.09	8.09	8.09	8.07
38.5	38.5	38.5	38.4	38.3	15.6	15.1	14.7	15.6	8.8	8.1	8.0	8.2	8.09	8.09	8.09	8.07

Technician Initials: _____ WQ Readings:

0	24	48	72
AP	EG	CH	AD

 Dilutions made by:

AC			
----	--	--	--

Comments: 0 hrs: Hach Sension 5 salinity meter
 24 hrs: _____
 48 hrs: AC Q18 7/24/15
 72 hrs: _____

QC Check: AC 8/4/15

Final Review: JA 8/4/15

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Poseidon
 Sample ID: Nautilus brine (Frozen seawater)
 Test No.: 1507-SD83

Start Date/Time: 7/22/15 1510
 End Date/Time: 7/25/15 1540
 Species: D. excentricus
 Date Collected: 7/17/15

Tech initials: AC
 Injection Time: 1430

Sperm Absorbance at 400 nm: 1.0 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 16
27
20
18
17
 Mean: 19.6 X 50 = 980 eggs/ml
 (target counts of 20 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 980 eggs/ml = 0.98 dilution factor
 Final density: 1000 eggs/ml
 egg stock 100 ml
 seawater — ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Add 100 µL sperm stock per 100mL of egg stock. For example, if you have 60mL of egg stock, add 60µL sperm stock.

Embryo Stock Fertilization Checks (Initiate test only when fertilization is $\geq 90\%$):

	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1502</u>	<u>47</u>	<u>3</u>	<u>97</u>
20 minutes (2nd fert. If needed)	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>

Fertilization Time: 1452

Test Initiation Time: 1510 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>100</u>	<u>0</u>	<u>100</u>
End of test QC check	<u>—</u>	<u>—</u>	<u>—</u>

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test to 96-hrs (ASTM 1999).

QC Check: AC 8/4/15 Final Review: S 8/4/15

Purple Urchin Fertilization

Test Date: October 30, 2014

CETIS Summary Report

Report Date: 30 Jun-15 09:45 (p 1 of 1)
 Test Code: 1410-S148 | 01-7477-7582

Echinoid Sperm Cell Fertilization Test 15C	Nautilus Environmental (CA)
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Batch ID: 07-7620-3286	Test Type: Fertilization	Analyst:
Start Date: 30 Oct-14 15:05	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 30 Oct-14 15:45	Species: Strongylocentrotus purpuratus	Brine: Frozen Seawater
Duration: 40m	Source: Pt. Loma	Age:

Sample ID: 13-8527-9097	Code: Brine	Client: Poseidon
Sample Date: 30 Oct-14	Material: Brined seawater	Project:
Receive Date: 30 Oct-14	Source: Poseidon	
Sample Age: 15h	Station: <i>Nautilus Brine</i>	

Sample Note: Frozed seawater prepared at Nautilus was used as brine.

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-1180-9983	Fertilization Rate	38.5	>38.5	NA	10.4%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
15-0920-7834	Fertilization Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
15-0920-7834	Fertilization Rate	Control Resp	0.808	0.7 - NL	Yes	Passes Acceptability Criteria	
20-1180-9983	Fertilization Rate	Control Resp	0.808	0.7 - NL	Yes	Passes Acceptability Criteria	
20-1180-9983	Fertilization Rate	PMSD	0.1039	NL - 0.25	No	Passes Acceptability Criteria	

Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.836	0.7907	0.8813	0.78	0.87	0.01631	0.03647	4.36%	0.0%
0	Lab Control	5	0.808	0.7236	0.8924	0.73	0.88	0.0304	0.06797	8.41%	3.35%
35		5	0.868	0.7893	0.9467	0.77	0.94	0.02835	0.0634	7.31%	-3.83%
35.5		5	0.828	0.7743	0.8817	0.76	0.87	0.01934	0.04324	5.22%	0.96%
36		5	0.832	0.8011	0.8629	0.79	0.85	0.01114	0.0249	2.99%	0.48%
36.5		5	0.848	0.7698	0.9262	0.78	0.95	0.02818	0.06301	7.43%	-1.44%
37		5	0.842	0.7905	0.8935	0.79	0.89	0.01855	0.04147	4.93%	-0.72%
37.5		5	0.822	0.772	0.872	0.78	0.86	0.018	0.04025	4.9%	1.68%
38		5	0.822	0.7911	0.8529	0.79	0.86	0.01114	0.0249	3.03%	1.68%
38.5		5	0.816	0.769	0.863	0.77	0.86	0.01691	0.03782	4.63%	2.39%

Fertilization Rate Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.87	0.78	0.82	0.86	0.85	
0	Lab Control	0.75	0.87	0.88	0.73	0.81	
35		0.85	0.89	0.77	0.94	0.89	
35.5		0.86	0.87	0.82	0.76	0.83	
36		0.79	0.85	0.85	0.83	0.84	
36.5		0.78	0.82	0.95	0.84	0.85	
37		0.79	0.89	0.87	0.81	0.85	
37.5		0.78	0.83	0.86	0.78	0.86	
38		0.86	0.82	0.82	0.79	0.82	
38.5		0.8	0.77	0.86	0.85	0.8	

CETIS Analytical Report

Report Date: 30 Jun-15 09:44 (p 1 of 2)
 Test Code: 1410-S148 | 01-7477-7582

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 20-1180-9983	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:44	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Sample Note: Frozed seawater prepared at Nautilus was used as brine.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.4%	38.5	>38.5	NA	

Dunnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control		35	-2.005	2.478	0.104	8	0.9998	CDF	Non-Significant Effect
		35.5	-0.5516	2.478	0.104	8	0.9708	CDF	Non-Significant Effect
		36	-0.6444	2.478	0.104	8	0.9775	CDF	Non-Significant Effect
		36.5	-1.342	2.478	0.104	8	0.9976	CDF	Non-Significant Effect
		37	-1.007	2.478	0.104	8	0.9926	CDF	Non-Significant Effect
		37.5	-0.3573	2.478	0.104	8	0.9512	CDF	Non-Significant Effect
		38	-0.3308	2.478	0.104	8	0.9479	CDF	Non-Significant Effect
		38.5	-0.166	2.478	0.104	8	0.9227	CDF	Non-Significant Effect

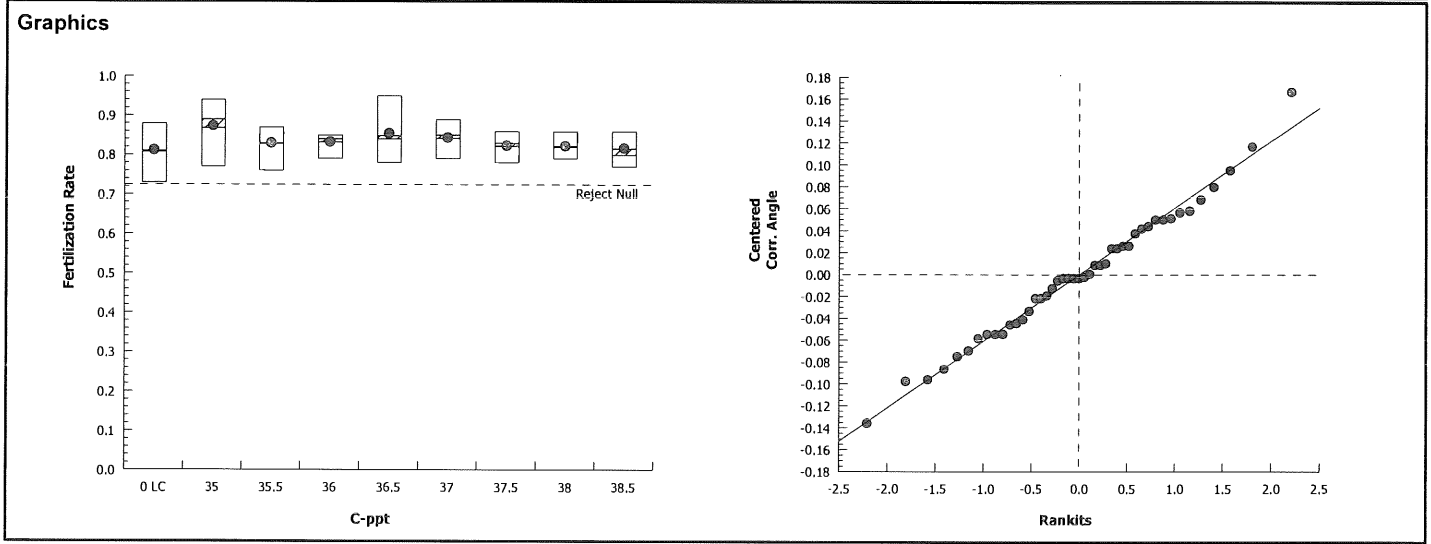
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.02892046	0.003615058	8	0.8153	0.5940	Non-Significant Effect
Error	0.1596273	0.004434091	36			
Total	0.1885477		44			

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)	
Variances	Bartlett Equality of Variance	9.343	20.09	0.3142	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9902	0.9308	0.9652	Normal Distribution	

Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.808	0.7236	0.8924	0.81	0.73	0.88	0.0304	8.41%	0.0%
35		5	0.868	0.7893	0.9467	0.89	0.77	0.94	0.02835	7.31%	-7.43%
35.5		5	0.828	0.7743	0.8817	0.83	0.76	0.87	0.01934	5.22%	-2.48%
36		5	0.832	0.8011	0.8629	0.84	0.79	0.85	0.01114	2.99%	-2.97%
36.5		5	0.848	0.7698	0.9262	0.84	0.78	0.95	0.02818	7.43%	-4.95%
37		5	0.842	0.7905	0.8935	0.85	0.79	0.89	0.01855	4.93%	-4.21%
37.5		5	0.822	0.772	0.872	0.83	0.78	0.86	0.018	4.9%	-1.73%
38		5	0.822	0.7911	0.8529	0.82	0.79	0.86	0.01114	3.03%	-1.73%
38.5		5	0.816	0.769	0.863	0.8	0.77	0.86	0.01691	4.63%	-0.99%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.122	1.014	1.231	1.12	1.024	1.217	0.03908	7.79%	0.0%
35		5	1.207	1.091	1.322	1.233	1.071	1.323	0.0416	7.71%	-7.53%
35.5		5	1.145	1.076	1.215	1.146	1.059	1.202	0.02511	4.9%	-2.07%
36		5	1.149	1.109	1.19	1.159	1.095	1.173	0.01452	2.83%	-2.42%
36.5		5	1.179	1.055	1.302	1.159	1.083	1.345	0.04445	8.43%	-5.04%
37		5	1.164	1.094	1.235	1.173	1.095	1.233	0.02549	4.9%	-3.78%
37.5		5	1.137	1.072	1.202	1.146	1.083	1.187	0.02351	4.62%	-1.34%
38		5	1.136	1.095	1.177	1.133	1.095	1.187	0.01477	2.91%	-1.24%
38.5		5	1.129	1.068	1.19	1.107	1.071	1.187	0.02203	4.36%	-0.62%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 20-1180-9983	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 30 Jun-15 9:44	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

Report Date: 30 Jun-15 09:45 (p 1 of 1)
 Test Code: 1410-S148 | 01-7477-7582

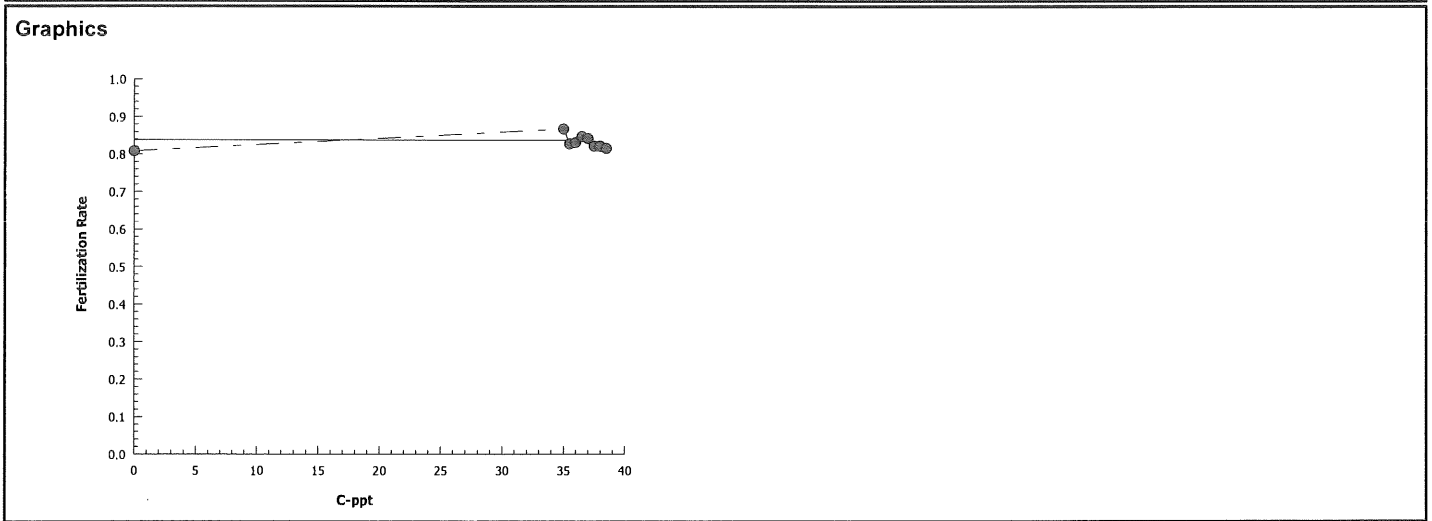
Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 15-0920-7834	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:44	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Sample Note: Frozed seawater prepared at Nautilus was used as brine.

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	842477	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Fertilization Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.808	0.73	0.88	0.0304	0.06797	8.41%	0.0%	404	500
35		5	0.868	0.77	0.94	0.02835	0.0634	7.31%	-7.43%	434	500
35.5		5	0.828	0.76	0.87	0.01934	0.04324	5.22%	-2.48%	414	500
36		5	0.832	0.79	0.85	0.01114	0.0249	2.99%	-2.97%	416	500
36.5		5	0.848	0.78	0.95	0.02818	0.06301	7.43%	-4.95%	424	500
37		5	0.842	0.79	0.89	0.01855	0.04147	4.93%	-4.21%	421	500
37.5		5	0.822	0.78	0.86	0.018	0.04025	4.9%	-1.73%	411	500
38		5	0.822	0.79	0.86	0.01114	0.0249	3.03%	-1.73%	411	500
38.5		5	0.816	0.77	0.86	0.01691	0.03782	4.63%	-0.99%	408	500



CETIS Test Data Worksheet

Report Date:

28 Oct-14 16:43 (p 1 of 2)

Test Code:

01-7477-7582/1410-S148

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Start Date:	30 Oct-14	Species:	Strongylocentrotus purpuratus		Sample Code: Brine
End Date:	30 Oct-14	Protocol:	EPA/600/R-95/136 (1995)		Sample Source: Poseidon
Sample Date:	30 Oct-14	Material:	Brined seawater		Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	83	11/4/14 ↓ 11/5/14 ↓
			2	100	87	
			3	100	87	
			4	100	77	
			5	100	84	
			6	100	85	
			7	100 85	78	
			8	100	81	
			9	100	85	
			10	100	87	
			11	100	82	
			12	100	76	
			13	100	94	
			14	100	86	
			15	100	89	
			16	100	82	
			17	100	87	
			18	100	85	
			19	100	77	
			20	100	85	
			21	100	75	
			22	100	81	
			23	100	79	
			24	100	85	
			25	100	85	
			26	100	82	
			27	100	78	
			28	100	83	
			29	100	80	
			30	100	79	
			31	100	86	
			32	100	86	
			33	100	82	
			34	100	89	
			35	100	82	
			36	100	89	
			37	100	88	
			38	100	82	
			39	100	95	
			40	100	78	
			41	100	86	
			42	100	78	
			43	100	86	
			44	100	86	
			45	100	83	
			46	100	85	
			47	100	84	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:43 (p 2 of 2)
Test Code: 01-7477-7582/1410-S148

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			48	100	79	11/5/14
			49	100	73	
			50	100	80	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:43 (p 1 of 2)
 Test Code: 01-7477-7582/1410-S148

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)		
Start Date:	30 Oct-14	Species:	Strongylocentrotus purpuratus	Sample Code:	Brine	
End Date:	30 Oct-14	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Poseidon	
Sample Date:	30 Oct-14	Material:	Brined seawater	Sample Station:		

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	BC	1	10	100	86	
0	BC	2	40			
0	BC	3	35			
0	BC	4	32			
0	BC	5	9			
0	LC	1	21	100	76	
0	LC	2	3	100	90	
0	LC	3	37			
0	LC	4	49			
0	LC	5	22			
35		1	46	100	84	
35		2	15			
35		3	19			
35		4	13			
35		5	36			
35.5		1	31	100	82	
35.5		2	17			
35.5		3	38			
35.5		4	12			
35.5		5	1			
36		1	48			
36		2	20	100	91	
36		3	25			
36		4	28			
36		5	5			
36.5		1	42	100	80	
36.5		2	16			
36.5		3	39			
36.5		4	47			
36.5		5	24			
37		1	30	100	7186	
37		2	34			
37		3	2			
37		4	8			
37		5	18			
37.5		1	27	100	76	
37.5		2	45			
37.5		3	43			
37.5		4	7			
37.5		5	41			
38		1	44	100	87	
38		2	11			
38		3	33			
38		4	23			
38		5	26			
38.5		1	29	100	60	
38.5		2	4			

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:43 (p 2 of 2)

Test Code: 01-7477-7582/1410-S148

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
38.5		3	14			
38.5		4	6			
38.5		5	50			

RL-AC

Marine Chronic Bioassay

Water Quality Measurements

Client/Project : Poseidon/Salinity Tolerance Study

Test Species: S. purpuratus

Sample ID: Brine (frozen seawater)

Start Date/Time: 10/30/2014 ^{AC} 13:05:05

Sample Log No.: 44 Not Applicable

End Date/Time: 10/30/2014 ^{AC} 13:45:45

Dilutions made by: ^{6/10/14} 11/7/14 IAC

Test No: 1410-S148

Analyst: g

Concentration (ppt)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.7	8.04	33.3	15.6
Brine Control	8.4	8.06	33.7	15.4
35.0	8.4	8.07	34.9	14.8
35.5	8.4	8.07	35.5	15.0
36.0	8.7	8.13	36.0	14.7
36.5	8.4	8.10	36.5	14.7
37.0	8.8	8.09	37.0	14.8
37.5	8.8	8.08	37.5	14.9
38.0	8.7	8.06	38.0	15.4
38.5	8.8	8.05	38.5	15.6

Comments: _____

QC Check: VB 11/10/14

Final Review: KFP 11/18/14

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Poseidon/Salinity Tolerance Study
 Sample ID: Brine (Frozen seawater)
 Test No.: 1410-5148

Start Date/Time: 10/30/2014 / AC 1505 1505
 End Date/Time: 10/30/2014 / AC 1545 1545
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 10/29/14

Tech initials: S/AC
 Injection Time: 1430

Sperm Absorbance at 400 nm: 0.823 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 58 Mean: 63.4 X 50 = 3170 eggs/ml

127
64
57
71

(target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)

Initial density: 3170 eggs/ml
 Final density: 4000 eggs/ml

= 0.79 dilution factor
~~0.67~~ Q20 KB 11/1/14
 - 1.0 part egg stock
0.37 parts seawater
~~0.37~~ Q20 KB 11/1/14

egg stock 100 ml
 seawater — ml
 No dilution required.

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Sperm:Egg Ratio

Range Finder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1440</u>	<u>100:1</u>	<u>73</u>	<u>27</u>
Eggs Added (0.5 ml):	<u>1450</u>	<u>150:1</u>	<u>92</u>	<u>8</u>
Test Ended:	<u>1500</u>	<u>150:1</u>	<u>91</u>	<u>9</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1309 1505</u>	QC1	<u>90</u>	<u>10</u>
Eggs Added (0.5 ml):	<u>1325 1525</u>	QC2	<u>84</u>	<u>16</u>
Test Ended:	<u>1345 1545</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

QC Check: KB 11/10/14

Final Review: KFP 11/18/14

Purple Urchin Fertilization

Test Date: July 22, 2015

CETIS Summary Report

Report Date: 03 Aug-15 16:36 (p 1 of 1)
 Test Code: 1507-S080 | 03-7187-5571

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Batch ID: 04-1054-0985	Test Type: Fertilization	Analyst:
Start Date: 22 Jul-15 16:17	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 25 Jul-15 16:57	Species: Strongylocentrotus purpuratus	Brine: Frozen Seawater
Duration: 73h 40 min	Source: Pt. Loma	Age:

Sample ID: 06-4744-0121	Code: 1507-S080	Client: Poseidon
Sample Date: 22 Jul-15	Material: Brined seawater	Project:
Receive Date: 22 Jul-15	Source: Poseidon	
Sample Age: 16h	Station: Nautilus Brine	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-4148-2778	Fertilization Rate	38.5	>38.5	NA	4.94%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
02-8620-7509	Fertilization Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability						
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-8620-7509	Fertilization Rate	Control Resp	0.936	0.7 - NL	Yes	Passes Acceptability Criteria
17-4148-2778	Fertilization Rate	Control Resp	0.936	0.7 - NL	Yes	Passes Acceptability Criteria
17-4148-2778	Fertilization Rate	PMSD	0.0494	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
33.4	Brine Control	5	0.934	0.8961	0.9719	0.91	0.98	0.01364	0.0305	3.27%	0.0%
33.5	Lab Control	5	0.936	0.9061	0.9659	0.91	0.97	0.01077	0.02408	2.57%	-0.21%
34.9		5	0.946	0.9062	0.9858	0.9	0.98	0.01435	0.03209	3.39%	-1.29%
35.4		5	0.934	0.9083	0.9597	0.91	0.96	0.009274	0.02074	2.22%	0.0%
35.9		5	0.952	0.9175	0.9865	0.91	0.98	0.01241	0.02775	2.92%	-1.93%
36.4		5	0.964	0.9452	0.9828	0.94	0.98	0.006782	0.01517	1.57%	-3.21%
36.9		5	0.94	0.9137	0.9663	0.91	0.96	0.009487	0.02121	2.26%	-0.64%
37.4		5	0.956	0.9372	0.9748	0.94	0.98	0.006782	0.01517	1.59%	-2.36%
37.9		5	0.924	0.8928	0.9552	0.9	0.95	0.01122	0.0251	2.72%	1.07%
38.5		5	0.918	0.8688	0.9672	0.88	0.97	0.01772	0.03962	4.32%	1.71%

Fertilization Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.4	Brine Control	0.92	0.95	0.91	0.91	0.98
33.5	Lab Control	0.93	0.92	0.95	0.91	0.97
34.9		0.9	0.97	0.98	0.93	0.95
35.4		0.95	0.96	0.91	0.93	0.92
35.9		0.94	0.98	0.97	0.96	0.91
36.4		0.98	0.97	0.94	0.97	0.96
36.9		0.93	0.96	0.96	0.94	0.91
37.4		0.98	0.95	0.96	0.94	0.95
37.9		0.95	0.9	0.92	0.95	0.9
38.5		0.9	0.97	0.88	0.95	0.89

Q15 2w 8/7/15

CETIS Analytical Report

Report Date: 03 Aug-15 16:34 (p 1 of 2)
 Test Code: 1507-S080 | 03-7187-5571

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 17-4148-2778		Endpoint: Fertilization Rate			CETIS Version: CETISv1.8.7						
Analyzed: 03 Aug-15 16:29		Analysis: Parametric-Control vs Treatments			Official Results: Yes						
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	4.94%	38.5	>38.5	NA			
Dunnett Multiple Comparison Test											
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)		
33.5		34.9	-0.7306	2.478	0.087	8	0.9825	CDF	Non-Significant Effect		
33.5		35.4	0.1559	2.478	0.087	8	0.8485	CDF	Non-Significant Effect		
33.5		35.9	-1.082	2.478	0.087	8	0.9942	CDF	Non-Significant Effect		
33.5		36.4	-1.829	2.478	0.087	8	0.9996	CDF	Non-Significant Effect		
33.5		36.9	-0.2068	2.478	0.087	8	0.9297	CDF	Non-Significant Effect		
33.5		37.4	-1.241	2.478	0.087	8	0.9966	CDF	Non-Significant Effect		
33.5		37.9	0.7002	2.478	0.087	8	0.6433	CDF	Non-Significant Effect		
33.5		38.5	0.8823	2.478	0.087	8	0.5589	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α :5%)			
Between	0.04086687		0.005108358		8	1.669	0.1400	Non-Significant Effect			
Error	0.1101584		0.003059955		36						
Total	0.1510253				44						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)					
Variances	Bartlett Equality of Variance		4.026	20.09	0.8548	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.974	0.9308	0.4007	Normal Distribution					
Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	0.936	0.9061	0.9659	0.93	0.91	0.97	0.01077	2.57%	0.0%
34.9		5	0.946	0.9062	0.9858	0.95	0.9	0.98	0.01435	3.39%	-1.07%
35.4		5	0.934	0.9083	0.9597	0.93	0.91	0.96	0.009274	2.22%	0.21%
35.9		5	0.952	0.9175	0.9865	0.96	0.91	0.98	0.01241	2.92%	-1.71%
36.4		5	0.964	0.9452	0.9828	0.97	0.94	0.98	0.006782	1.57%	-2.99%
36.9		5	0.94	0.9137	0.9663	0.94	0.91	0.96	0.009487	2.26%	-0.43%
37.4		5	0.956	0.9372	0.9748	0.95	0.94	0.98	0.006782	1.59%	-2.14%
37.9		5	0.924	0.8928	0.9552	0.92	0.9	0.95	0.01122	2.72%	1.28%
38.5		5	0.918	0.8688	0.9672	0.9	0.88	0.97	0.01772	4.32%	1.92%
Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	1.319	1.254	1.384	1.303	1.266	1.397	0.02346	3.98%	0.0%
34.9		5	1.345	1.255	1.434	1.345	1.249	1.429	0.03216	5.35%	-1.94%
35.4		5	1.314	1.26	1.367	1.303	1.266	1.369	0.01919	3.27%	0.41%
35.9		5	1.357	1.278	1.436	1.369	1.266	1.429	0.02855	4.7%	-2.87%
36.4		5	1.383	1.334	1.432	1.397	1.323	1.429	0.01765	2.85%	-4.85%
36.9		5	1.326	1.271	1.381	1.323	1.266	1.369	0.01987	3.35%	-0.55%
37.4		5	1.362	1.312	1.413	1.345	1.323	1.429	0.01814	2.98%	-3.29%
37.9		5	1.295	1.234	1.355	1.284	1.249	1.345	0.02168	3.75%	1.86%
38.5		5	1.288	1.191	1.386	1.249	1.217	1.397	0.03515	6.1%	2.34%

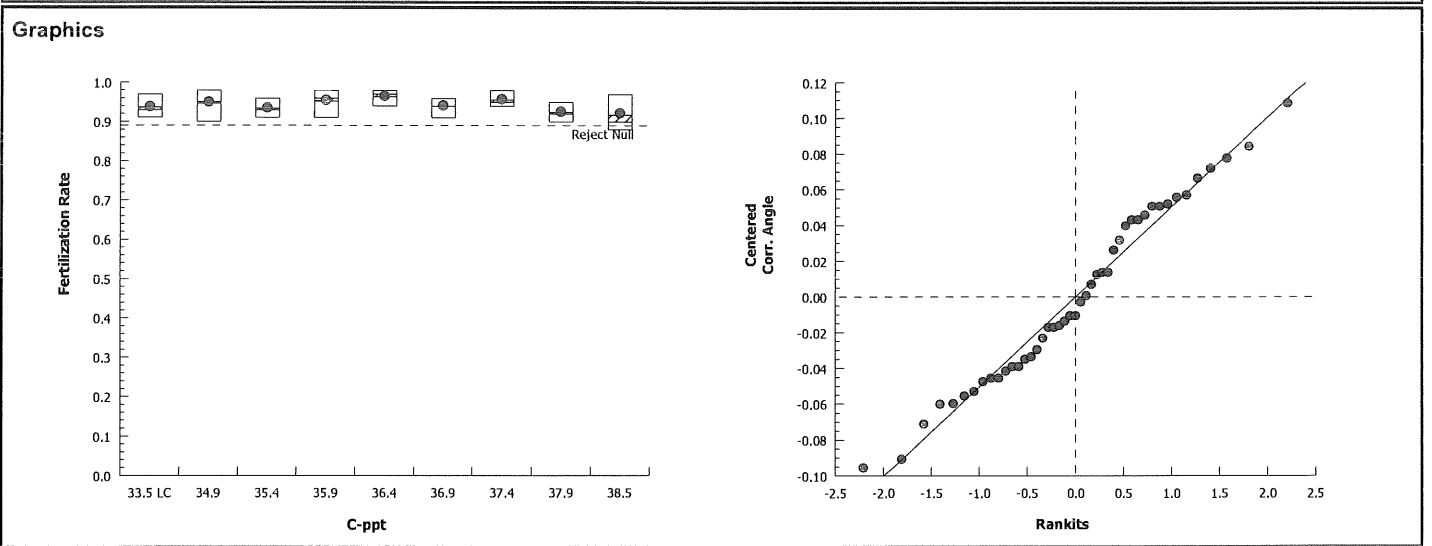
CETIS Analytical Report

Report Date: 03 Aug-15 16:35 (p 2 of 2)
 Test Code: 1507-S080 | 03-7187-5571

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 17-4148-2778	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 03 Aug-15 16:29	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Fertilization Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.93	0.92	0.95	0.91	0.97
34.9		0.9	0.97	0.98	0.93	0.95
35.4		0.95	0.96	0.91	0.93	0.92
35.9		0.94	0.98	0.97	0.96	0.91
36.4		0.98	0.97	0.94	0.97	0.96
36.9		0.93	0.96	0.96	0.94	0.91
37.4		0.98	0.95	0.96	0.94	0.95
37.9		0.95	0.9	0.92	0.95	0.9
38.5		0.9	0.97	0.88	0.95	0.89

Angular (Corrected) Transformed Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	1.303	1.284	1.345	1.266	1.397
34.9		1.249	1.397	1.429	1.303	1.345
35.4		1.345	1.369	1.266	1.303	1.284
35.9		1.323	1.429	1.397	1.369	1.266
36.4		1.429	1.397	1.323	1.397	1.369
36.9		1.303	1.369	1.369	1.323	1.266
37.4		1.429	1.345	1.369	1.323	1.345
37.9		1.345	1.249	1.284	1.345	1.249
38.5		1.249	1.397	1.217	1.345	1.233



CETIS Analytical Report

Report Date: 03 Aug-15 16:36 (p 1 of 1)
 Test Code: 1507-S080 | 03-7187-5571

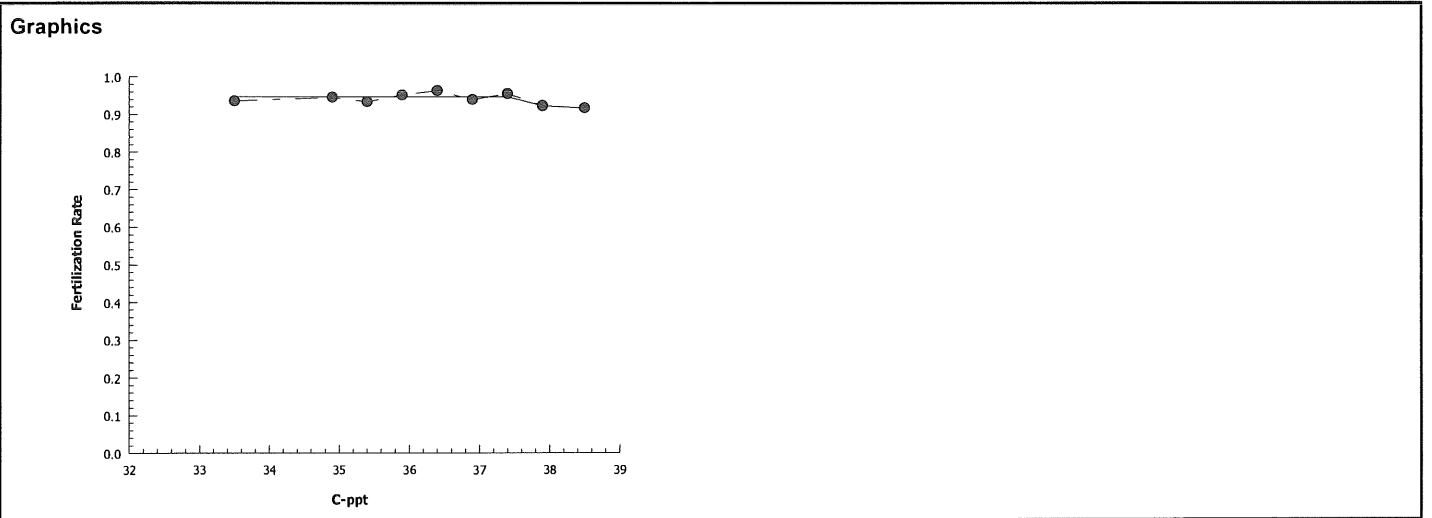
Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 02-8620-7509	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 03 Aug-15 16:35	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	45597	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Fertilization Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
33.5	Lab Control	5	0.936	0.91	0.97	0.01077	0.02408	2.57%	0.0%	468	500
34.9		5	0.946	0.9	0.98	0.01435	0.03209	3.39%	-1.07%	473	500
35.4		5	0.934	0.91	0.96	0.009274	0.02074	2.22%	0.21%	467	500
35.9		5	0.952	0.91	0.98	0.01241	0.02775	2.92%	-1.71%	476	500
36.4		5	0.964	0.94	0.98	0.006782	0.01517	1.57%	-2.99%	482	500
36.9		5	0.94	0.91	0.96	0.009487	0.02121	2.26%	-0.43%	470	500
37.4		5	0.956	0.94	0.98	0.006782	0.01517	1.59%	-2.14%	478	500
37.9		5	0.924	0.9	0.95	0.01122	0.0251	2.72%	1.28%	462	500
38.5		5	0.918	0.88	0.97	0.01772	0.03962	4.32%	1.92%	459	500

Fertilization Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.5	Lab Control	0.93	0.92	0.95	0.91	0.97
34.9		0.9	0.97	0.98	0.93	0.95
35.4		0.95	0.96	0.91	0.93	0.92
35.9		0.94	0.98	0.97	0.96	0.91
36.4		0.98	0.97	0.94	0.97	0.96
36.9		0.93	0.96	0.96	0.94	0.91
37.4		0.98	0.95	0.96	0.94	0.95
37.9		0.95	0.9	0.92	0.95	0.9
38.5		0.9	0.97	0.88	0.95	0.89



CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:18 (p 1 of 2)

Test Code: 1507-S080 03-7187-5571/162A5EF3

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 22 Jul-15

Species: Strongylocentrotus purpuratus

Sample Code: 4507-S N/A

End Date: 22^(a) Jul-15

Protocol: EPA/600/R-95/136 (1995)

Sample Source: Poseidon

Sample Date: 22 Jul-15

Material: Brined seawater

Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	94	7/29/15
			2	100	91	
			3	100	90	
			4	100	95	
			5	100	95	
			6	100	96	
			7	100	92	
			8	100	95	
			9	100	95	
			10	100	94	
			11	100	90	
			12	100	96	
			13	100	98	
			14	100	95	
			15	100	96	
			16	100	98	
			17	100	97	
			18	100	97	
			19	100	97	
			20	100	95	
			21	100	90	
			22	100	91	
			23	100	91	
			24	100	98	
			25	100	95	
			26	100	93	
			27	100	93	
			28	100	93	
			29	100	95	
			30	100	97	
			31	100	96	
			32	100	88	
			33	100	93	
			34	100	95	
			35	100	90	
			36	100	92	
			37	100	97	
			38	100	94	
			39	100	92	
			40	100	96	
			41	100	91	
			42	100	96	
			43	100	98	
			44	100	94	
			45	100	91	
			46	100	89	
			47	100	92	

(a) Q16 JW 8/7/15

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:18 (p 2 of 2)

Test Code: ISOT 5080 03-7187-5571/162A5EF3

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			48	100	91	
			49	100	97	7/29/15
			50	100	98	↓

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:18 (p 1 of 2)

Test Code: 1507-S080 03-7187-5571/162A5EF3

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 22 Jul-15

Species: Strongylocentrotus purpuratus

Sample Code: 1507-S N/A

End Date: 22-25 Jul-15

Protocol: EPA/600/R-95/136 (1995)

Sample Source: Poseidon

Sample Date: 22 Jul-15

Material: Brined seawater

Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	BC	1	39	100	90	AC 7/22/15
0	BC	2	25			AC 7/22/15 AC Q18 7/22/15
0	BC	3	48			
0	BC	4	41			
0	BC	5	16			
0	LC	1	33	100	96	AC 7/22/15
0	LC	2	36			
0	LC	3	4			
0	LC	4	45			
0	LC	5	30			
35		1	35	100	92	AC 7/22/15
35		2	17			
35		3	43			
35		4	26			
35		5	20			
35.5		1	34	100	94	AC 7/22/15
35.5		2	42			
35.5		3	2			
35.5		4	28			
35.5		5	47			
36		1	10	100	94	AC 7/22/15
36		2	50			
36		3	49			
36		4	31			
36		5	22			
36.5		1	24	100	96	AC 7/22/15
36.5		2	37			
36.5		3	44			
36.5		4	18			
36.5		5	12			
37		1	27	100	93	AC 7/22/15
37		2	40			
37		3	6			
37		4	38			
37		5	23			
37.5		1	13	100	97	AC 7/22/15
37.5		2	29			
37.5		3	15			
37.5		4	1			
37.5		5	8			
38		1	9	100	97	AC 7/22/15
38		2	21			
38		3	7			
38		4	14			
38		5	3	100	95	AC 7/22/15
38.5		1	11			
38.5		2	19			

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:18 (p 2 of 2)

Test Code: 1507-5080 03-7187-5571/162A5EF3

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
38.5		3	32			
38.5		4	5			
38.5		5	46			

GLWS

Marine Chronic Bioassay

Water Quality Measurements

Client : Poseidon

Test Species: S. purpuratus

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: 7/21/2015 7/22/15 1617

Sample Log No.: AC R18 7/22
15- N/A

End Date/Time: 7/21/2015 7/22/15 1657

Dilutions made by: PA/AC

Test No: 1507-S080

Analyst: AD

Concentration ppt	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (C)
Lab Control	8.6	8.09	33.5	16.0
Brine Control	8.4	8.10	33.4	16.0
35.0	8.6	8.09	34.9	16.0
35.5	8.7	8.09	35.4	16.0
36.0	8.8	8.09	35.9	15.8
36.5	8.8	8.09	36.4	15.9
37.0	8.8	8.09	36.9	15.9
37.5	8.8	8.09	37.4	15.8
38.0	8.8	8.09	37.9	15.6
38.5	8.8	8.09	38.5	15.6

Comments: Hack session # 15 salinity meter

QC Check: AC 8/3/15

Final Review: JW 8/7/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: AC

Sample ID: Nautilus brine (frozen seawater)

Test Date: 7/22/2015

Test No: 1507-S080

Test Type: urchin fertilization and dev.
sand dollar fertilization and dev.

Salinity of Seawater 33.4

Salinity of Brine 85.2

Date of Brine used: Mix of brine from 5/21 + 5/29/15, blended 7/21/15

Test Dilution Volume 500

Alkalinity of Brine Control: 110 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	500
35.0	96.9	484.6	0.03	15.4	500
35.5	95.9	479.7	0.04	20.3	500
36.0	95.0	474.9	0.05	25.1	500
36.5	94.0	470.1	0.06	29.9	500
37.0	93.1	465.3	0.07	34.7	500
37.5	92.1	460.4	0.09	39.6	500
38.0	91.1	455.6	0.10	44.4	500
38.5	90.2	450.8	0.11	49.2	500

DI Volume

Brine Control	76.3	0.64	49.2	500
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Total Brine Volume Required (ml): 307.9

QC Check: AC 8/3/15

Final Review: rw 8/7/15

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Poseidon
 Sample ID: Nautilus brine (Frozen seawater)
 Test No.: 1507-5080
 Tech initials: Y
 Injection Time: 1525

Start Date/Time: 7/22/15 1617
 End Date/Time: 7/22/15 1657
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 7/9/15

Sperm Absorbance at 400 nm: 0.935 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 87 Mean: 81.8 $\times 50 =$ 4090 eggs/ml
82
80 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter
79 slide for a final density of 4000 eggs/ml)
81

Initial density: 4090 eggs/ml = 1.02 dilution factor egg stock 150 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater ml
0.02 parts seawater

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1538</u>	<u>100:1</u>	<u>63</u>	<u>37</u>
Eggs Added (0.5 ml):	<u>1553</u>	<u>200:1</u>	<u>84</u>	<u>16</u>
Test Ended:	<u>1603</u>	<u>400:1</u>	<u>97</u>	<u>3</u>
		<u>400:1</u>	<u>95</u>	<u>5</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 350:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1617</u>	QC1	<u>91</u>	<u>9</u>
Eggs Added (0.5 ml):	<u>1637</u>	QC2	<u>93</u>	<u>7</u>
Test Ended:	<u>1657</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

QC Check: AC 8/3/15 Final Review: JW 8/7/15

Sand Dollar Fertilization

Test Date: October 30, 2014

CETIS Summary Report

Report Date: 30 Jun-15 09:50 (p 1 of 1)
 Test Code: 1410-S150 | 08-5460-9726

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA)

Batch ID: 03-5643-7558	Test Type: Fertilization	Analyst:
Start Date: 30 Oct-14 16:10	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 30 Oct-14 16:50	Species: Dendraster excentricus	Brine: Frozen Seawater
Duration: 40m	Source: Mission Bay	Age:

Sample ID: 02-2309-2849	Code: Brine	Client: Poseidon
Sample Date: 30 Oct-14	Material: Brined seawater	Project:
Receive Date: 30 Oct-14	Source: Poseidon	
Sample Age: 16h	Station: Nautilus Brine	

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-7819-5516	Fertilization Rate	38.5	>38.5	NA	3.75%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
20-3895-2473	Fertilization Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
17-7819-5516	Fertilization Rate	Control Resp	0.97	0.7 - NL	Yes	Passes Acceptability Criteria	
20-3895-2473	Fertilization Rate	Control Resp	0.97	0.7 - NL	Yes	Passes Acceptability Criteria	
17-7819-5516	Fertilization Rate	PMSD	0.03751	NL - 0.25	No	Passes Acceptability Criteria	

Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.962	0.9416	0.9824	0.94	0.98	0.007348	0.01643	1.71%	0.0%
0	Lab Control	5	0.97	0.9485	0.9915	0.94	0.98	0.007746	0.01732	1.79%	-0.83%
35		5	0.968	0.9458	0.9902	0.95	0.99	0.008	0.01789	1.85%	-0.62%
35.5		5	0.952	0.9384	0.9656	0.94	0.96	0.004899	0.01096	1.15%	1.04%
36		5	0.948	0.9184	0.9776	0.91	0.97	0.01068	0.02387	2.52%	1.46%
36.5		5	0.96	0.9368	0.9832	0.94	0.98	0.008366	0.01871	1.95%	0.21%
37		5	0.964	0.9473	0.9807	0.95	0.98	0.006	0.01342	1.39%	-0.21%
37.5		5	0.936	0.8943	0.9777	0.88	0.97	0.01503	0.03362	3.59%	2.7%
38		5	0.938	0.8836	0.9924	0.87	0.98	0.0196	0.04382	4.67%	2.5%
38.5		5	0.956	0.9181	0.9939	0.92	0.99	0.01364	0.0305	3.19%	0.62%

Fertilization Rate Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	0.97	0.95	0.97	0.94	0.98	
0	Lab Control	0.97	0.98	0.94	0.98	0.98	
35		0.97	0.95	0.98	0.95	0.99	
35.5		0.96	0.94	0.94	0.96	0.96	
36		0.97	0.91	0.96	0.96	0.94	
36.5		0.94	0.97	0.94	0.98	0.97	
37		0.95	0.97	0.97	0.95	0.98	
37.5		0.94	0.97	0.88	0.95	0.94	
38		0.96	0.87	0.92	0.98	0.96	
38.5		0.96	0.92	0.93	0.99	0.98	

CETIS Analytical Report

Report Date: 30 Jun-15 09:50 (p 1 of 2)
 Test Code: 1410-S150 | 08-5460-9726

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 17-7819-5516	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:49	Analysis: Parametric-Control vs Treatments	Official Results: Yes			

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.75%	38.5	>38.5	NA	

Dunnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control		35	0.1084	2.478	0.091	8	0.8617	CDF	Non-Significant Effect
		35.5	1.369	2.478	0.091	8	0.3353	CDF	Non-Significant Effect
		36	1.532	2.478	0.091	8	0.2700	CDF	Non-Significant Effect
		36.5	0.7489	2.478	0.091	8	0.6211	CDF	Non-Significant Effect
		37	0.5102	2.478	0.091	8	0.7249	CDF	Non-Significant Effect
		37.5	2.18	2.478	0.091	8	0.0915	CDF	Non-Significant Effect
		38	1.919	2.478	0.091	8	0.1473	CDF	Non-Significant Effect
		38.5	0.8193	2.478	0.091	8	0.5885	CDF	Non-Significant Effect

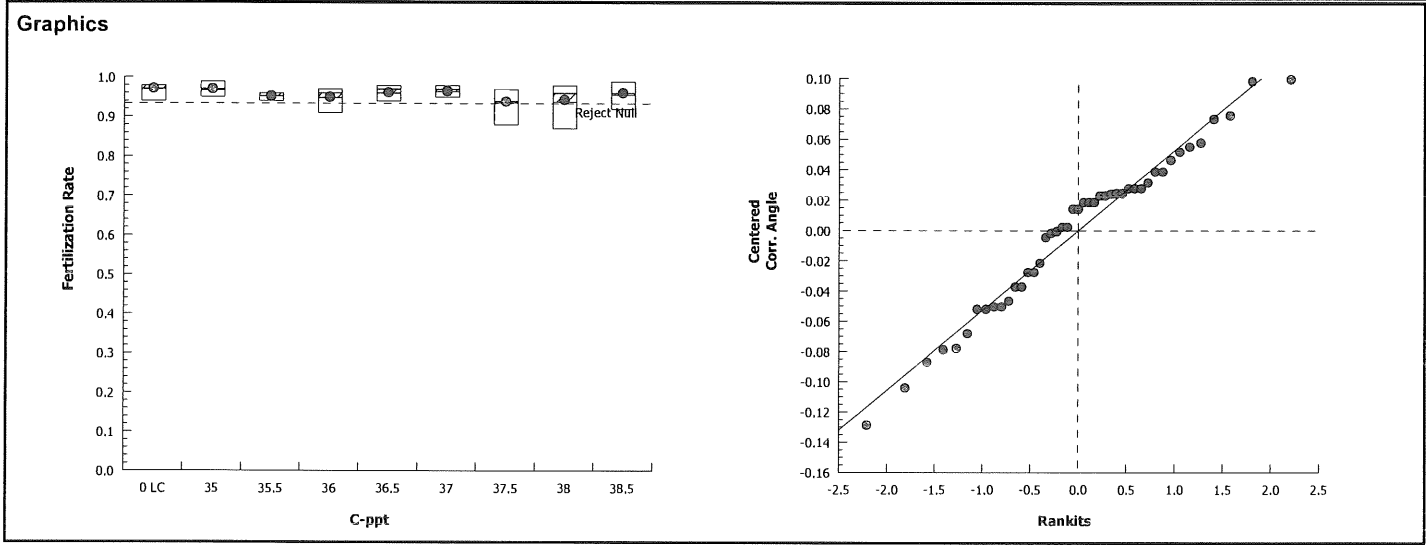
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.03236474	0.004045592	8	1.196	0.3289	Non-Significant Effect
Error	0.1217972	0.003383255	36			
Total	0.1541619		44			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	7.847	20.09	0.4485	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.971	0.9308	0.3158	Normal Distribution

Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.97	0.9485	0.9915	0.98	0.94	0.98	0.007746	1.79%	0.0%
35		5	0.968	0.9458	0.9902	0.97	0.95	0.99	0.008	1.85%	0.21%
35.5		5	0.952	0.9384	0.9656	0.96	0.94	0.96	0.004899	1.15%	1.86%
36		5	0.948	0.9184	0.9776	0.96	0.91	0.97	0.01068	2.52%	2.27%
36.5		5	0.96	0.9368	0.9832	0.97	0.94	0.98	0.008366	1.95%	1.03%
37		5	0.964	0.9473	0.9807	0.97	0.95	0.98	0.006	1.39%	0.62%
37.5		5	0.936	0.8943	0.9777	0.94	0.88	0.97	0.01503	3.59%	3.51%
38		5	0.938	0.8836	0.9924	0.96	0.87	0.98	0.0196	4.67%	3.3%
38.5		5	0.956	0.9181	0.9939	0.96	0.92	0.99	0.01364	3.19%	1.44%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.401	1.344	1.458	1.429	1.323	1.429	0.02048	3.27%	0.0%
35		5	1.397	1.33	1.465	1.397	1.345	1.471	0.02428	3.89%	0.28%
35.5		5	1.351	1.32	1.382	1.369	1.323	1.369	0.01129	1.87%	3.59%
36		5	1.345	1.281	1.409	1.369	1.266	1.397	0.02298	3.82%	4.02%
36.5		5	1.374	1.314	1.433	1.397	1.323	1.429	0.02143	3.49%	1.97%
37		5	1.383	1.337	1.428	1.397	1.345	1.429	0.01632	2.64%	1.34%
37.5		5	1.321	1.24	1.402	1.323	1.217	1.397	0.02927	4.95%	5.72%
38		5	1.331	1.221	1.441	1.369	1.202	1.429	0.03962	6.66%	5.04%
38.5		5	1.371	1.272	1.47	1.369	1.284	1.471	0.03568	5.82%	2.15%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)
Analysis ID: 17-7819-5516	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 30 Jun-15 9:49	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

Report Date: 30 Jun-15 09:50 (p 1 of 1)
 Test Code: 1410-S150 | 08-5460-9726

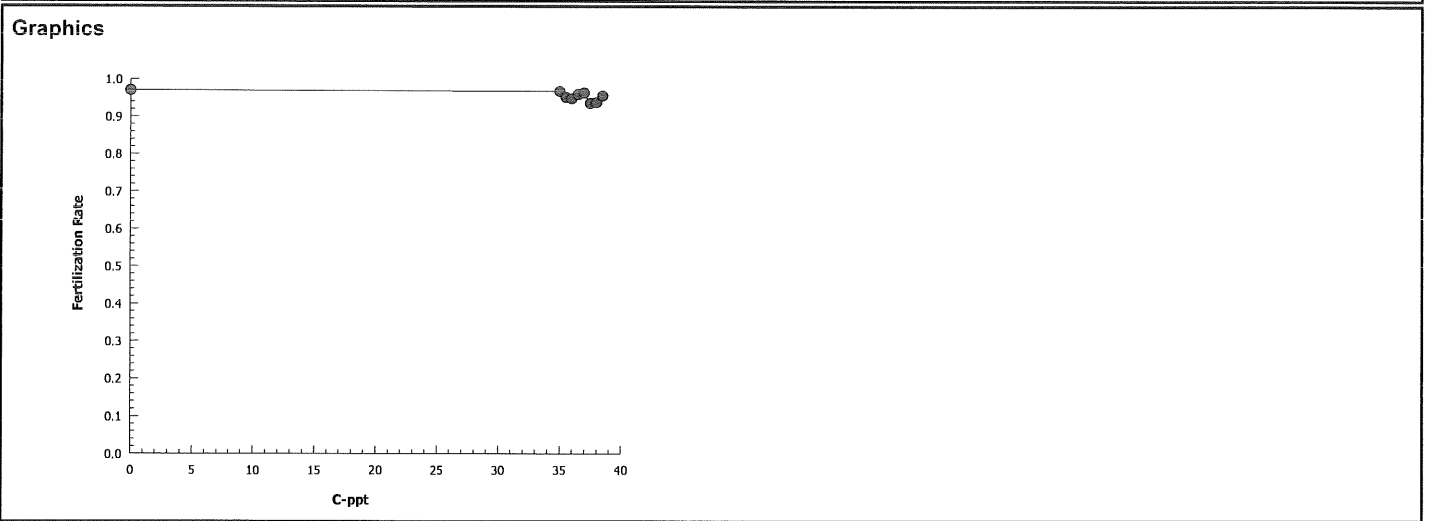
Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 20-3895-2473	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 30 Jun-15 9:49	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Sample Note: Frozen seawater prepared at Nautilus was used as brine.

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1475078	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Fertilization Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.97	0.94	0.98	0.007746	0.01732	1.79%	0.0%	485	500
35		5	0.968	0.95	0.99	0.008	0.01789	1.85%	0.21%	484	500
35.5		5	0.952	0.94	0.96	0.004899	0.01096	1.15%	1.86%	476	500
36		5	0.948	0.91	0.97	0.01068	0.02387	2.52%	2.27%	474	500
36.5		5	0.96	0.94	0.98	0.008366	0.01871	1.95%	1.03%	480	500
37		5	0.964	0.95	0.98	0.006	0.01342	1.39%	0.62%	482	500
37.5		5	0.936	0.88	0.97	0.01503	0.03362	3.59%	3.51%	468	500
38		5	0.938	0.87	0.98	0.0196	0.04382	4.67%	3.3%	469	500
38.5		5	0.956	0.92	0.99	0.01364	0.0305	3.19%	1.44%	478	500



CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:37 (p 1 of 2)
 Test Code: 08-5460-9726/1410-S150

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Start Date: 30 Oct-14	Species: Dendraster excentricus	Sample Code: Brine			
End Date: 30 Oct-14	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Poseidon			
Sample Date: 30 Oct-14	Material: Brined seawater	Sample Station:			

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	95	11/14/14
			2	100	96	
			3	100	97	
			4	100	94	
			5	100	98	
			6	100	98	
			7	100	96	
			8	100	94	
			9	100	97	
			10	100	94	
			11	100	92	
			12	100	99	
			13	100	98	
			14	100	96	
			15	100	99	
			16	100	96	
			17	100	98	
			18	100	94	
			19	100	95	
			20	100	94	
			21	100	95	
			22	100	96	
			23	100	94	
			24	100	98	
			25	100	95	
			26	100	97	
			27	100	97	
			28	100	96	
			29	100	92	
			30	100	94	
			31	100	96	
			32	100	98	
			33	100	97	
			34	100	97	
			35	100	98	
			36	100	97	
			37	100	96	
			38	100	95	
			39	100	94	
			40	100	97	
			41	100	88	
			42	100	94	
			43	100	98	
			44	100	97	
			45	100	98	
			46	100	91	
			47	100	93	

Analyst: ^{QIB} ASG QA: KB 11/14/14
 96 11/14/14

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:37 (p 2 of 2)
Test Code: 08-5460-9726/1410-S150

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			48	100	87	11/14/14 ↓
			49	100	95	
			50	100	97	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:37 (p 1 of 2)
 Test Code: 08-5460-9726/1410-S150

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 30 Oct-14 Species: Dendraster excentricus Sample Code: Brine
 End Date: 30 Oct-14 Protocol: EPA/600/R-95/136 (1995) Sample Source: Poseidon
 Sample Date: 30 Oct-14 Material: Brined seawater Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	BC	1	34			
0	BC	2	19			
0	BC	3	33	100	94	
0	BC	4	39			
0	BC	5	24			
0	LC	1	50			
0	LC	2	17	100	96	
0	LC	3	18			
0	LC	4	32			
0	LC	5	6			
35		1	44			
35		2	25			
35		3	43			
35		4	21			
35		5	12			
35.5		1	16			
35.5		2	10			
35.5		3	8			
35.5		4	7			
35.5		5	31			
36		1	26			
36		2	46			
36		3	2			
36		4	14			
36		5	30			
36.5		1	23			
36.5		2	3			
36.5		3	42			
36.5		4	13			
36.5		5	27			
37		1	49			
37		2	36			
37		3	40			
37		4	38			
37		5	35			
37.5		1	20			
37.5		2	9			
37.5		3	41			
37.5		4	1			
37.5		5	4			
38		1	22			
38		2	48	100	91	
38		3	11			
38		4	45			
38		5	37			
38.5		1	28			
38.5		2	29	100	99	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:37 (p 2 of 2)
Test Code: 08-5460-9726/1410-S150

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
38.5		3	47			
38.5		4	15			
38.5		5	5			

QCEAL

Marine Chronic Bioassay

Water Quality Measurements

Client/Project : Poseidon/Salinity Tolerance Study

Test Species: D. excentricus

Sample ID: Brine (frozen seawater)

Start Date/Time: 10/30/2014 16:10

Sample Log No.: ^{n/10/14} ~~14-~~ N/A

End Date/Time: 10/30/2014 16:50

Dilutions made by: AL

Test No: 1410-S150

Analyst: g

Concentration (ppt)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.7	8.04	33.3	15.6
Brine Control	8.8	8.06	33.7	15.4
35.0	8.8	8.07	34.9	14.8
35.5	8.8	8.07	35.5	15.0
36.0	8.7	8.13	36.0	14.7
36.5	8.8	8.10	36.5	14.7
37.0	8.8	8.09	37.0	14.8
37.5	8.8	8.08	37.5	14.9
38.0	8.7	8.06	38.0	15.4
38.5	8.8	8.05	38.5	15.6

Comments: _____

QC Check: KB 11/14/14

Final Review: KTP 11/18/14

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Poseidon/Salinity Tolerance Study
 Sample ID: Brine (Frozen seawater)
 Test No.: 1410-S150

Start Date/Time: 10/30/2014 / 16:10
 End Date/Time: 10/30/2014 / 16:50
 Species: D. excentricus
 Animal Source: Mission Bay
 Date Collected: 10/24/14

Tech initials: AC
 Injection Time: 15:15

Sperm Absorbance at 400 nm: 0.744 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 37 Mean: 43.8 x 50 = 2190 eggs/ml
41
37 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter
43 slide for a final density of 4000 eggs/ml)
64

Initial density: 2190 eggs/ml = 0.55 dilution factor egg stock - ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater - ml
0.45 parts seawater 45 ml poured of per 100 after settling

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	¹⁵⁰ 100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	3.75	2.5
ml Seawater	0.0	10	20	30	40	45	46.25	47.5
								48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>15:35</u>	<u>50</u>	<u>67</u>	<u>33</u>
Eggs Added (0.5 ml):	<u>15:50</u>	<u>100</u>	<u>90</u>	<u>10</u>
Test Ended:	<u>16:00</u>	<u>150</u>	<u>96</u>	<u>4</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>16:10</u>	QC1	<u>98</u>	<u>2</u>
Eggs Added (0.5 ml):	<u>16:30</u>	QC2	<u>97</u>	<u>3</u>
Test Ended:	<u>16:50</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

QC Check: KB 11/14/14 Final Review: KFP 11/18/14

Sand Dollar Fertilization

Test Date: July 22, 2015

CETIS Summary Report

Report Date: 05 Aug-15 09:24 (p 1 of 1)
 Test Code: 1507-S082 | 00-0483-7190

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Batch ID: 02-7259-2739	Test Type: Fertilization	Analyst:
Start Date: 22 Jul-15 15:20	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 22 Jul-15 16:00	Species: Dendroaster excentricus	Brine: Frozen Seawater
Duration: 40m	Source: Mission Bay	Age:

Sample ID: 15-0490-2480	Code: ① 59B2FD50 1507-S082	Client: Poseidon
Sample Date: 22 Jul-15	Material: Brined seawater	Project:
Receive Date: 22 Jul-15	Source: Poseidon	
Sample Age: 15h	Station: Nautilus Brine	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-3708-0048	Fertilization Rate	38.5	>38.5	NA	7.59%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
18-8484-4421	Fertilization Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
15-3708-0048	Fertilization Rate	Control Resp	0.886	0.7 - NL	Yes	Passes Acceptability Criteria
18-8484-4421	Fertilization Rate	Control Resp	0.886	0.7 - NL	Yes	Passes Acceptability Criteria
15-3708-0048	Fertilization Rate	PMSD	0.07594	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
33.4	Brine Control	5	0.904	0.8632	0.9448	0.88	0.94	0.0147	0.03286	3.64%	0.0%
33.5	Lab Control	5	0.886	0.8481	0.9239	0.85	0.92	0.01364	0.0305	3.44%	1.99%
34.9		5	0.906	0.8713	0.9407	0.86	0.93	0.01249	0.02793	3.08%	-0.22%
35.4		5	0.91	0.834	0.986	0.85	0.98	0.02739	0.06124	6.73%	-0.66%
35.9		5	0.896	0.8533	0.9387	0.84	0.93	0.01536	0.03435	3.83%	0.89%
36.4		5	0.926	0.8913	0.9607	0.9	0.96	0.01249	0.02793	3.02%	-2.43%
36.9		5	0.92	0.9024	0.9376	0.9	0.93	0.006324	0.01414	1.54%	-1.77%
37.4		5	0.936	0.9118	0.9602	0.91	0.96	0.008718	0.01949	2.08%	-3.54%
37.9		5	0.886	0.8336	0.9384	0.82	0.92	0.01887	0.04219	4.76%	1.99%
38.5		5	0.912	0.8704	0.9536	0.87	0.95	0.01497	0.03347	3.67%	-0.89%

Fertilization Rate Detail

C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
33.4	Brine Control	0.88	0.88	0.94	0.88	0.94
33.5	Lab Control	0.92	0.85	0.91	0.86	0.89
34.9		0.92	0.9	0.93	0.86	0.92
35.4		0.85	0.86	0.89	0.97	0.98
35.9		0.84	0.91	0.93	0.91	0.89
36.4		0.9	0.9	0.92	0.96	0.95
36.9		0.93	0.93	0.9	0.93	0.91
37.4		0.91	0.93	0.95	0.96	0.93
37.9		0.87	0.92	0.82	0.92	0.9
38.5		0.95	0.87	0.91	0.94	0.89

① Avg SW 8/7/15

CETIS Analytical Report

Report Date: 05 Aug-15 09:23 (p 1 of 2)

Test Code: 1507-S082 | 00-0483-7190

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
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Analysis ID: 15-3708-0048	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 05 Aug-15 9:23	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.59%	38.5	>38.5	NA	

Dunnnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
33.5		34.9	-0.8331	2.478	0.098	8	0.9871	CDF	Non-Significant Effect
33.5		35.4	-1.398	2.478	0.098	8	0.9981	CDF	Non-Significant Effect
33.5		35.9	-0.4261	2.478	0.098	8	0.9591	CDF	Non-Significant Effect
33.5		36.4	-1.794	2.478	0.098	8	0.9996	CDF	Non-Significant Effect
33.5		36.9	-1.426	2.478	0.098	8	0.9982	CDF	Non-Significant Effect
33.5		37.4	-2.251	2.478	0.098	8	0.9999	CDF	Non-Significant Effect
33.5		37.9	-0.0428	2.478	0.098	8	0.8985	CDF	Non-Significant Effect
33.5		38.5	-1.147	2.478	0.098	8	0.9953	CDF	Non-Significant Effect

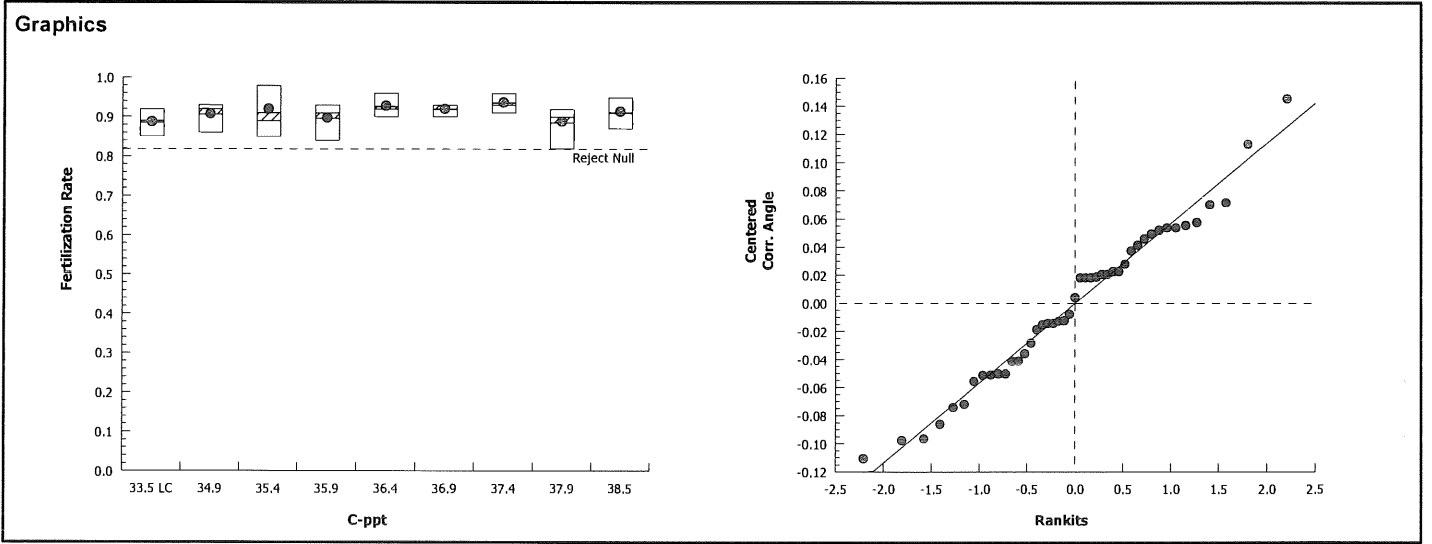
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.03743324	0.004679155	8	1.205	0.3240	Non-Significant Effect
Error	0.1398344	0.00388429	36			
Total	0.1772677		44			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	10.92	20.09	0.2065	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9805	0.9308	0.6393	Normal Distribution

Fertilization Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	0.886	0.8481	0.9239	0.89	0.85	0.92	0.01364	3.44%	0.0%
34.9		5	0.906	0.8713	0.9407	0.92	0.86	0.93	0.01249	3.08%	-2.26%
35.4		5	0.91	0.834	0.986	0.89	0.85	0.98	0.02739	6.73%	-2.71%
35.9		5	0.896	0.8533	0.9387	0.91	0.84	0.93	0.01536	3.83%	-1.13%
36.4		5	0.926	0.8913	0.9607	0.92	0.9	0.96	0.01249	3.02%	-4.52%
36.9		5	0.92	0.9024	0.9376	0.93	0.9	0.93	0.006324	1.54%	-3.84%
37.4		5	0.936	0.9118	0.9602	0.93	0.91	0.96	0.008718	2.08%	-5.64%
37.9		5	0.886	0.8336	0.9384	0.9	0.82	0.92	0.01887	4.76%	0.0%
38.5		5	0.912	0.8704	0.9536	0.91	0.87	0.95	0.01497	3.67%	-2.94%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
33.5	Lab Control	5	1.229	1.169	1.288	1.233	1.173	1.284	0.02154	3.92%	0.0%
34.9		5	1.261	1.205	1.318	1.284	1.187	1.303	0.0205	3.63%	-2.67%
35.4		5	1.284	1.134	1.433	1.233	1.173	1.429	0.05384	9.38%	-4.48%
35.9		5	1.245	1.178	1.313	1.266	1.159	1.303	0.02424	4.35%	-1.37%
36.4		5	1.299	1.23	1.368	1.284	1.249	1.369	0.02482	4.27%	-5.76%
36.9		5	1.285	1.253	1.317	1.303	1.249	1.303	0.01146	1.99%	-4.57%
37.4		5	1.317	1.267	1.368	1.303	1.266	1.369	0.01807	3.07%	-7.22%
37.9		5	1.23	1.151	1.31	1.249	1.133	1.284	0.02869	5.22%	-0.14%
38.5		5	1.274	1.199	1.349	1.266	1.202	1.345	0.0269	4.72%	-3.68%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)
Analysis ID: 15-3708-0048	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 05 Aug-15 9:23	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

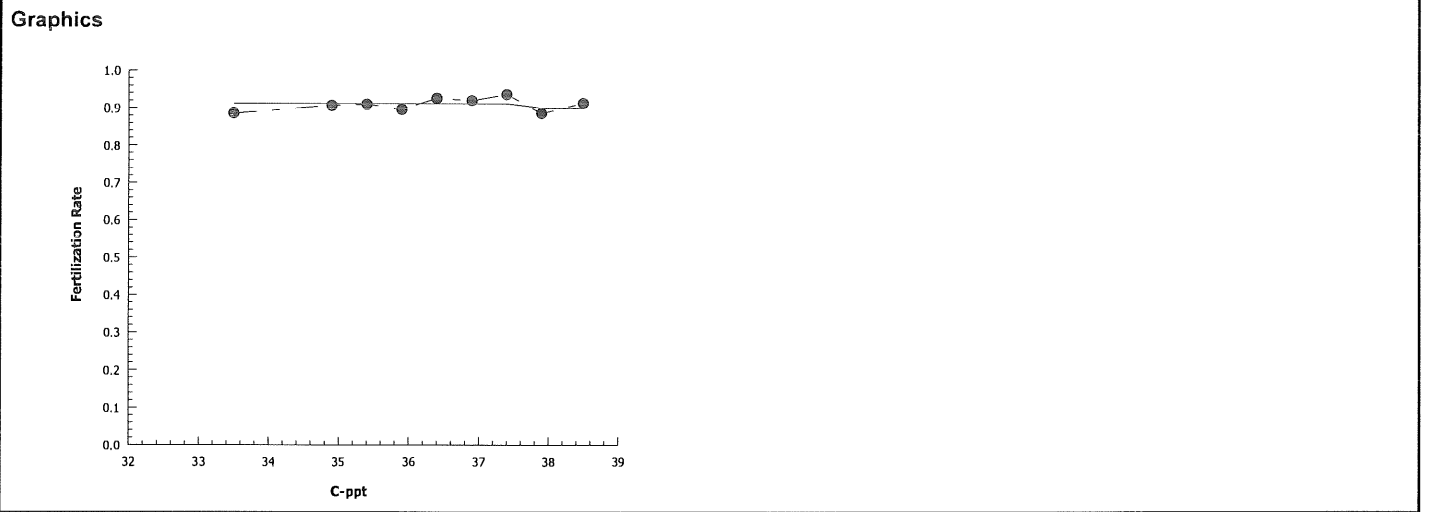
Report Date: 05 Aug-15 09:24 (p 1 of 1)
 Test Code: 1507-S082 | 00-0483-7190

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 18-8484-4421	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 05 Aug-15 9:23	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1008592	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Fertilization Rate Summary			Calculated Variate(A/B)								
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
33.5	Lab Control	5	0.886	0.85	0.92	0.01364	0.0305	3.44%	0.0%	443	500
34.9		5	0.906	0.86	0.93	0.01249	0.02793	3.08%	-2.26%	453	500
35.4		5	0.91	0.85	0.98	0.02739	0.06124	6.73%	-2.71%	455	500
35.9		5	0.896	0.84	0.93	0.01536	0.03435	3.83%	-1.13%	448	500
36.4		5	0.926	0.9	0.96	0.01249	0.02793	3.02%	-4.52%	463	500
36.9		5	0.92	0.9	0.93	0.006324	0.01414	1.54%	-3.84%	460	500
37.4		5	0.936	0.91	0.96	0.008718	0.01949	2.08%	-5.64%	468	500
37.9		5	0.886	0.82	0.92	0.01887	0.04219	4.76%	0.0%	443	500
38.5		5	0.912	0.87	0.95	0.01497	0.03347	3.67%	-2.94%	456	500



CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:15 (p 1 of 2)
 Test Code: 00-0483-7190/49CF46

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)
Start Date: 22 Jul-15	Species: Dendraster excentricus	Sample Code: 1507-S 082
End Date: 22-25 Jul-15	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Poseidon
Sample Date: 22 Jul-15	Material: Brined seawater	Sample Station:

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	90	AC 8/3/15
			2		93	
			3		90	
			4		88	
			5		96	
			6		89	
			7	100	90 93	2 8/4/15
			8	100	91	
			9	100	95	
			10	100	93	
			11		88	
			12		96	
			13		92	
			14		92	
			15		92	
			16		91	
			17		82 91 ⁸ 8/4/15	
			18		82	
			19		94	
			20		92	
			21		90	
			22		89	
			23		93	
			24		91	
			25		86	
			26		97	
			27		89	
			28		90	
			29		93	
			30		95	
			31		91	
			32		89	
			33		92	
			34		93	
			35		93	
			36		92	
			37		98	AC 8/4/15
			38		95	
			39		84	
			40		94	
			41		90	
			42		86	
			43		87	
			44		85	
			45		88	
			46		94	
			47		87	

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:15 (p 2 of 2)

Test Code: 1507-8082 00-0483-7190/49CF46

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
			48	100	91	AC 8/4/15
			49	100	86	
			50	100	85	↓

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:15 (p 1 of 2)
 Test Code: 1507-8082 00-0483-7190/49CF46
 150722015

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Start Date: 22 Jul-15		Species: Dendraster excentricus		Sample Code: ① 1507-8 N/A			
End Date: 22-25 Jul-15		Protocol: EPA/600/R-95/136 (1995)		Sample Source: Poseidon			
Sample Date: ② 22 Jul-15		Material: Brined seawater		Sample Station:			

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	BC	1	45			
0	BC	2	11	100	83	8/7/22/15
0	BC	3	40			
0	BC	4	4			
0	BC	5	19			
0	LC	1	15			
0	LC	2	44			
0	LC	3	31	100	90	8/7/22/15
0	LC	4	42			
0	LC	5	22			
35		1	20			
35		2	3	100	86	8/7/22/15
35		3	29			
35		4	49			
35		5	36			
35.5		1	50			
35.5		2	25	100	77	8/7/22/15
35.5		3	27			
35.5		4	26			
35.5		5	37			
36		1	39	100	78	AC 7/22
36		2	24			
36		3	2			
36		4	8			
36		5	6			
36.5		1	28	100	86	AC 7/22
36.5		2	1			
36.5		3	14			
36.5		4	12			
36.5		5	38			
37		1	34			
37		2	23			
37		3	41			
37		4	10	100	92	AC 7/22
37		5	17			
37.5		1	48	100	90	AC 7/22
37.5		2	35			
37.5		3	30			
37.5		4	5			
37.5		5	7			
38		1	47			
38		2	33			
38		3	18	100	81	8/7/22/15
38		4	13			
38		5	21			
38.5		1	9	100	94	AC 7/22
38.5		2	43			

CETIS Test Data Worksheet

Report Date: 21 Jul-15 17:15 (p 2 of 2)

Test Code: 1507-5082 00-0483-7190/49CF46

C-ppt	Code	Rep	Pos	# Counted	# Fertilized	Notes
38.5		3	16			
38.5		4	46			
38.5		5	32			

RLH

Marine Chronic Bioassay

Water Quality Measurements

Client : Poseidon

Test Species: D. excentricus

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: ~~7/21/2015~~ 7/22/15 1520

Sample Log No.: 15- N/A

End Date/Time: ^{AC 8/18/15} ~~7/21/2015~~ 7/22/15 1600

Dilutions made by: AC

Test No: 1507-S082

Analyst: AD

Concentration ppt	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (C)
Lab Control	8.6	8.09	335	16.0
Brine Control	8.4	8.10	334	16.0
35.0	8.6	8.09	34.9	16.0
35.5	8.7	8.09	35.4	16.0
36.0	8.8	8.09	35.9	15.8
36.5	8.8	8.09	36.4	15.9
37.0	8.8	8.09	36.9	15.9
37.5	8.8	8.09	37.4	15.8
38.0	8.8	8.09	37.9	15.6
38.5	8.8	8.09	38.5	15.6

Comments: High sensitivity salinity meter

QC Check: AC 8/5/15

Final Review: sw 8/11/15

**Giant Kelp
48-hour Germination and Growth**

Test Date: May 12, 2015

CETIS Summary Report

Report Date: 28 May-15 11:45 (p 1 of 2)

Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test **Nautilus Environmental (CA)**

Batch ID: 00-8657-2759	Test Type: Growth-Germination	Analyst:
Start Date: 12 May-15 15:00	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 14 May-15 12:15	Species: Macrocystis pyrifera	Brine: Frozen Seawater
Duration: 45h	Source: La Jolla Cove	Age:

Sample ID: 18-1583-3954	Code: Nautilus Brine	Client: Poseidon
Sample Date: 12 May-15	Material: Natural Seawater	Project: <i>Salinity Tolerance Study</i>
Receive Date: 12 May-15	Source: Poseidon	
Sample Age: 15h	Station: Nautilus Brine	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
01-5183-0033	Germination Rate	38.5	>38.5	NA	8.04%		Dunnett Multiple Comparison Test
12-8384-7212	Mean Length	38.5	>38.5	NA	10.2%		Dunnett Multiple Comparison Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
01-5183-0033	Germination Rate	Control Resp	0.894	0.7 - NL	Yes	Passes Acceptability Criteria
12-8384-7212	Mean Length	Control Resp	14.55	10 - NL	Yes	Passes Acceptability Criteria
01-5183-0033	Germination Rate	PMSD	0.08043	NL - 0.2	No	Passes Acceptability Criteria
12-8384-7212	Mean Length	PMSD	0.1025	NL - 0.2	No	Passes Acceptability Criteria

Germination Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.922	0.8804	0.9636	0.87	0.96	0.01497	0.03347	3.63%	0.0%
0	Lab Control	5	0.894	0.8424	0.9456	0.83	0.94	0.0186	0.04159	4.65%	3.04%
35		5	0.924	0.8893	0.9587	0.89	0.95	0.01249	0.02793	3.02%	-0.22%
35.5		5	0.912	0.8644	0.9596	0.86	0.96	0.01715	0.03834	4.2%	1.09%
36		5	0.91	0.8409	0.9791	0.84	0.96	0.0249	0.05568	6.12%	1.3%
36.5		5	0.942	0.9075	0.9765	0.91	0.97	0.01241	0.02775	2.95%	-2.17%
37		5	0.904	0.8783	0.9297	0.87	0.92	0.009274	0.02074	2.29%	1.95%
37.5		5	0.918	0.8941	0.9419	0.89	0.94	0.008602	0.01924	2.1%	0.43%
38		5	0.938	0.8974	0.9786	0.9	0.98	0.01463	0.03271	3.49%	-1.74%
38.5		5	0.926	0.8588	0.9932	0.84	0.98	0.02421	0.05413	5.85%	-0.43%

Mean Length Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	14.35	12.95	15.75	13.5	16.25	0.5037	1.126	7.85%	0.0%
0	Lab Control	5	14.55	14.15	14.95	14.25	15	0.1458	0.326	2.24%	-1.39%
35		5	15.25	13.4	17.1	14	17.75	0.6661	1.49	9.77%	-6.27%
35.5		5	15.25	13.39	17.11	13.75	17.5	0.6708	1.5	9.84%	-6.27%
36		5	15.85	15.01	16.69	15	16.75	0.3021	0.6755	4.26%	-10.45%
36.5		5	16	15.24	16.76	15.25	16.75	0.2739	0.6124	3.83%	-11.5%
37		5	16	14.82	17.18	14.75	17.25	0.4257	0.952	5.95%	-11.5%
37.5		5	16.35	15.71	16.99	15.75	17	0.2318	0.5184	3.17%	-13.94%
38		5	15.75	15.09	16.41	15	16.25	0.2372	0.5303	3.37%	-9.76%
38.5		5	15.25	13.84	16.66	13.5	16.25	0.5062	1.132	7.42%	-6.27%

CETIS Summary Report

Report Date: 28 May-15 11:45 (p 2 of 2)
 Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test						Nautilus Environmental (CA)
Germination Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	0.92	0.96	0.87	0.94	0.92
0	Lab Control	0.94	0.83	0.89	0.92	0.89
35		0.95	0.93	0.89	0.95	0.9
35.5		0.93	0.92	0.89	0.96	0.86
36		0.96	0.95	0.86	0.94	0.84
36.5		0.94	0.91	0.92	0.97	0.97
37		0.92	0.9	0.92	0.87	0.91
37.5		0.92	0.93	0.89	0.94	0.91
38		0.91	0.98	0.95	0.95	0.9
38.5		0.84	0.95	0.98	0.95	0.91
Mean Length Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Brine Control	14.25	13.5	13.5	14.25	16.25
0	Lab Control	14.25	14.75	15	14.5	14.25
35		14	17.75	14.25	15.25	15
35.5		15.5	13.75	14	15.5	17.5
36		16.75	15	15.75	15.5	16.25
36.5		15.75	15.25	15.75	16.75	16.5
37		16.5	15.5	16	17.25	14.75
37.5		16.75	17	15.75	16	16.25
38		15	16.25	15.5	16.25	15.75
38.5		15.75	16.25	13.5	14.75	16

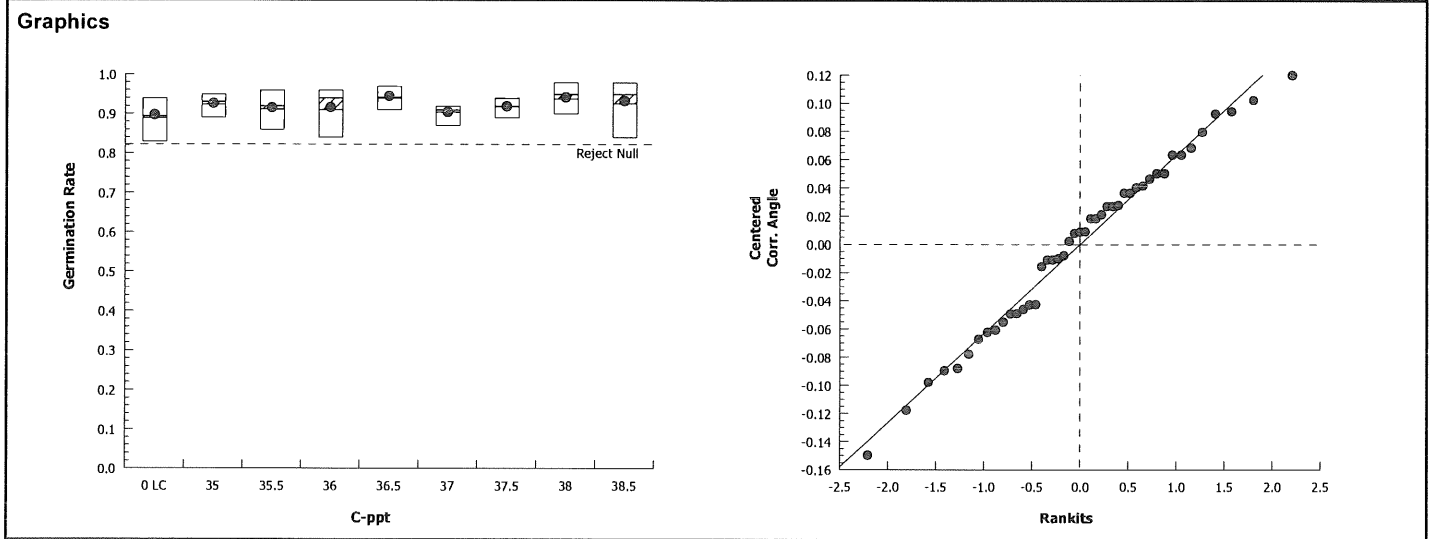
CETIS Analytical Report

Report Date: 28 May-15 11:44 (p 1 of 3)
 Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test										Nautilus Environmental (CA)	
Analysis ID: 01-5183-0033		Endpoint: Germination Rate				CETIS Version: CETISv1.8.7					
Analyzed: 28 May-15 11:43		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	8.04%	38.5	>38.5	NA			
Dunnett Multiple Comparison Test											
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)		
Lab Control		35	-1.174	2.478	0.108	8	0.9957	CDF	Non-Significant Effect		
		35.5	-0.7222	2.478	0.108	8	0.9820	CDF	Non-Significant Effect		
		36	-0.7592	2.478	0.108	8	0.9839	CDF	Non-Significant Effect		
		36.5	-2.05	2.478	0.108	8	0.9998	CDF	Non-Significant Effect		
		37	-0.3043	2.478	0.108	8	0.9443	CDF	Non-Significant Effect		
		37.5	-0.8718	2.478	0.108	8	0.9886	CDF	Non-Significant Effect		
		38	-1.903	2.478	0.108	8	0.9997	CDF	Non-Significant Effect		
		38.5	-1.492	2.478	0.108	8	0.9986	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square	DF	F Stat	P-Value	Decision(α :5%)				
Between	0.03649479		0.004561848	8	0.9543	0.4859	Non-Significant Effect				
Error	0.1720855		0.004780153	36							
Total	0.2085803		44								
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α :1%)					
Variances	Bartlett Equality of Variance		7.736	20.09	0.4597	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.983	0.9308	0.7425	Normal Distribution					
Germination Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.894	0.8424	0.9456	0.89	0.83	0.94	0.0186	4.65%	0.0%
35		5	0.924	0.8893	0.9587	0.93	0.89	0.95	0.01249	3.02%	-3.36%
35.5		5	0.912	0.8644	0.9596	0.92	0.86	0.96	0.01715	4.2%	-2.01%
36		5	0.91	0.8409	0.9791	0.94	0.84	0.96	0.0249	6.12%	-1.79%
36.5		5	0.942	0.9075	0.9765	0.94	0.91	0.97	0.01241	2.95%	-5.37%
37		5	0.904	0.8783	0.9297	0.91	0.87	0.92	0.009273	2.29%	-1.12%
37.5		5	0.918	0.8941	0.9419	0.92	0.89	0.94	0.008602	2.1%	-2.69%
38		5	0.938	0.8974	0.9786	0.95	0.9	0.98	0.01463	3.49%	-4.92%
38.5		5	0.926	0.8588	0.9932	0.95	0.84	0.98	0.02421	5.85%	-3.58%
Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.244	1.161	1.327	1.233	1.146	1.323	0.02983	5.36%	0.0%
35		5	1.295	1.23	1.361	1.303	1.233	1.345	0.02357	4.07%	-4.13%
35.5		5	1.275	1.189	1.361	1.284	1.187	1.369	0.03104	5.44%	-2.54%
36		5	1.277	1.157	1.397	1.323	1.159	1.369	0.04316	7.56%	-2.67%
36.5		5	1.333	1.257	1.41	1.323	1.266	1.397	0.02746	4.61%	-7.21%
37		5	1.257	1.215	1.299	1.266	1.202	1.284	0.01523	2.71%	-1.07%
37.5		5	1.282	1.239	1.325	1.284	1.233	1.323	0.01555	2.71%	-3.07%
38		5	1.327	1.237	1.417	1.345	1.249	1.429	0.03228	5.44%	-6.69%
38.5		5	1.309	1.183	1.435	1.345	1.159	1.429	0.04542	7.76%	-5.25%

Macrocystis Germination and Germ Tube Growth Test Nautilus Environmental (CA)

Analysis ID: 01-5183-0033 Endpoint: Germination Rate CETIS Version: CETISv1.8.7
Analyzed: 28 May-15 11:43 Analysis: Parametric-Control vs Treatments Official Results: Yes



CETIS Analytical Report

Report Date: 28 May-15 11:44 (p 3 of 3)

Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test **Nautilus Environmental (CA)**

Analysis ID: 12-8384-7212 Endpoint: Mean Length CETIS Version: CETISv1.8.7
 Analyzed: 28 May-15 11:43 Analysis: Parametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	10.2%	38.5	>38.5	NA	

Dunnett Multiple Comparison Test

Control	vs C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control	35	-1.164	2.478	1.491	8	0.9956	CDF	Non-Significant Effect
	35.5	-1.164	2.478	1.491	8	0.9956	CDF	Non-Significant Effect
	36	-2.161	2.478	1.491	8	0.9999	CDF	Non-Significant Effect
	36.5	-2.41	2.478	1.491	8	1.0000	CDF	Non-Significant Effect
	37	-2.41	2.478	1.491	8	1.0000	CDF	Non-Significant Effect
	37.5	-2.992	2.478	1.491	8	1.0000	CDF	Non-Significant Effect
	38	-1.995	2.478	1.491	8	0.9998	CDF	Non-Significant Effect
	38.5	-1.164	2.478	1.491	8	0.9956	CDF	Non-Significant Effect

ANOVA Table

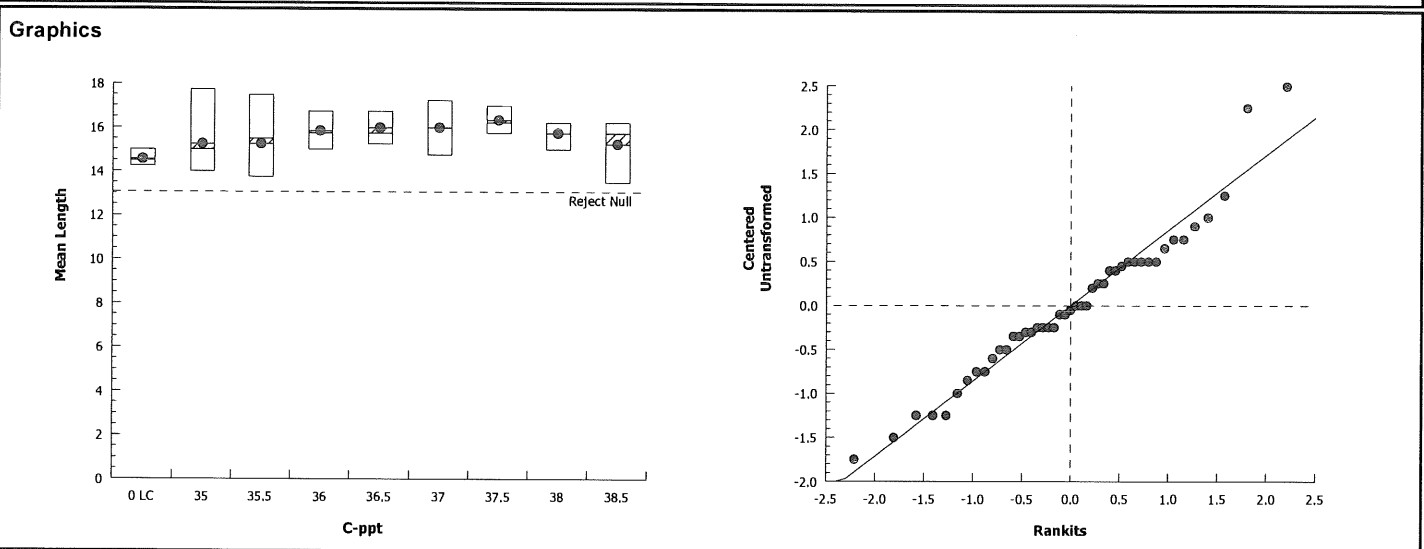
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	12.175	1.521875	8	1.682	0.1367	Non-Significant Effect
Error	32.575	0.9048611	36			
Total	44.75		44			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	14.4	20.09	0.0719	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9623	0.9308	0.1491	Normal Distribution

Mean Length Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	14.55	14.15	14.95	14.5	14.25	15	0.1458	2.24%	0.0%
35		5	15.25	13.4	17.1	15	14	17.75	0.6661	9.77%	-4.81%
35.5		5	15.25	13.39	17.11	15.5	13.75	17.5	0.6708	9.84%	-4.81%
36		5	15.85	15.01	16.69	15.75	15	16.75	0.3021	4.26%	-8.94%
36.5		5	16	15.24	16.76	15.75	15.25	16.75	0.2739	3.83%	-9.97%
37		5	16	14.82	17.18	16	14.75	17.25	0.4257	5.95%	-9.97%
37.5		5	16.35	15.71	16.99	16.25	15.75	17	0.2318	3.17%	-12.37%
38		5	15.75	15.09	16.41	15.75	15	16.25	0.2372	3.37%	-8.25%
38.5		5	15.25	13.84	16.66	15.75	13.5	16.25	0.5062	7.42%	-4.81%



CETIS Analytical Report

Report Date: 28 May-15 11:45 (p 1 of 2)
 Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test Nautilus Environmental (CA)

Analysis ID: 12-5064-8423 Endpoint: Germination Rate CETIS Version: CETISv1.8.7
 Analyzed: 28 May-15 11:43 Analysis: Parametric-Two Sample Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	5.32%	Passes germination rate

Equal Variance t Two-Sample Test

Control	vs Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control	Brine Control	-1.202	1.86	0.076	8	0.8681	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.005960524	0.005960524	1	1.445	0.2638	Non-Significant Effect
Error	0.0330071	0.004125887	8			
Total	0.03896762		9			

Distributional Tests

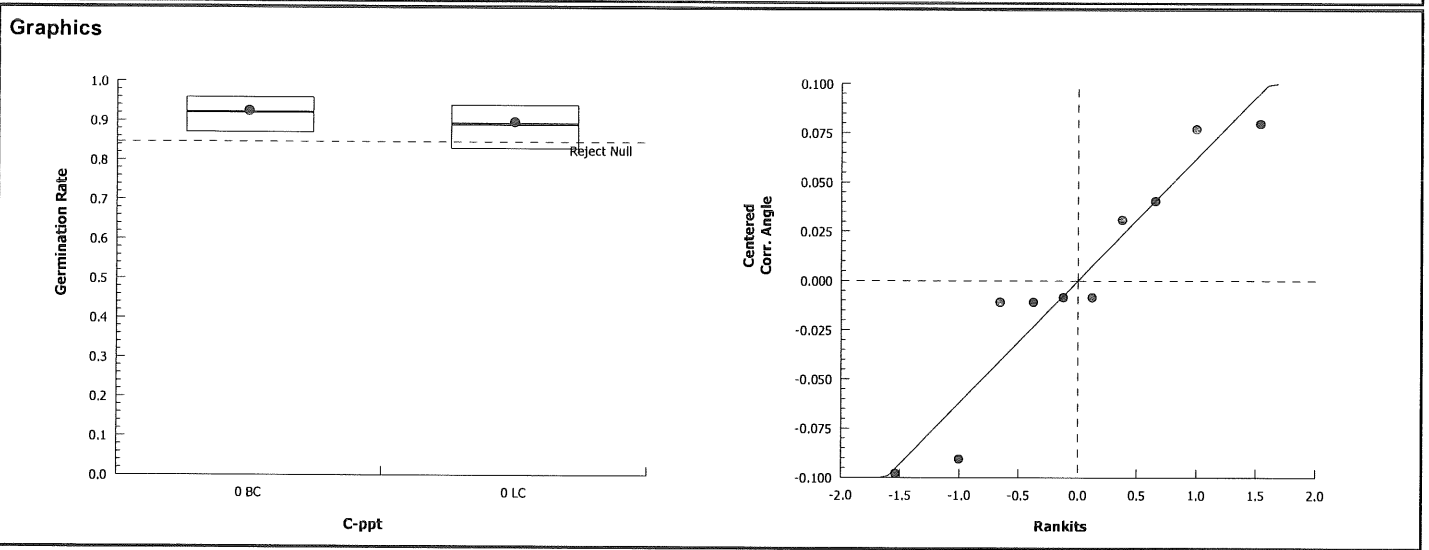
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Variance Ratio F	1.169	23.15	0.8832	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9076	0.7411	0.2647	Normal Distribution

Germination Rate Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.894	0.8424	0.9456	0.89	0.83	0.94	0.0186	4.65%	0.0%
0	Brine Control	5	0.922	0.8804	0.9636	0.92	0.87	0.96	0.01497	3.63%	-3.13%

Angular (Corrected) Transformed Summary

C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.244	1.161	1.327	1.233	1.146	1.323	0.02983	5.36%	0.0%
0	Brine Control	5	1.293	1.216	1.369	1.284	1.202	1.369	0.02758	4.77%	-3.93%



CETIS Analytical Report

Report Date: 28 May-15 11:45 (p 2 of 2)
 Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test				Nautilus Environmental (CA)		
Analysis ID: 00-9063-4448	Endpoint: Mean Length		CETIS Version: CETISv1.8.7			
Analyzed: 28 May-15 11:44	Analysis: Parametric-Two Sample		Official Results: Yes			

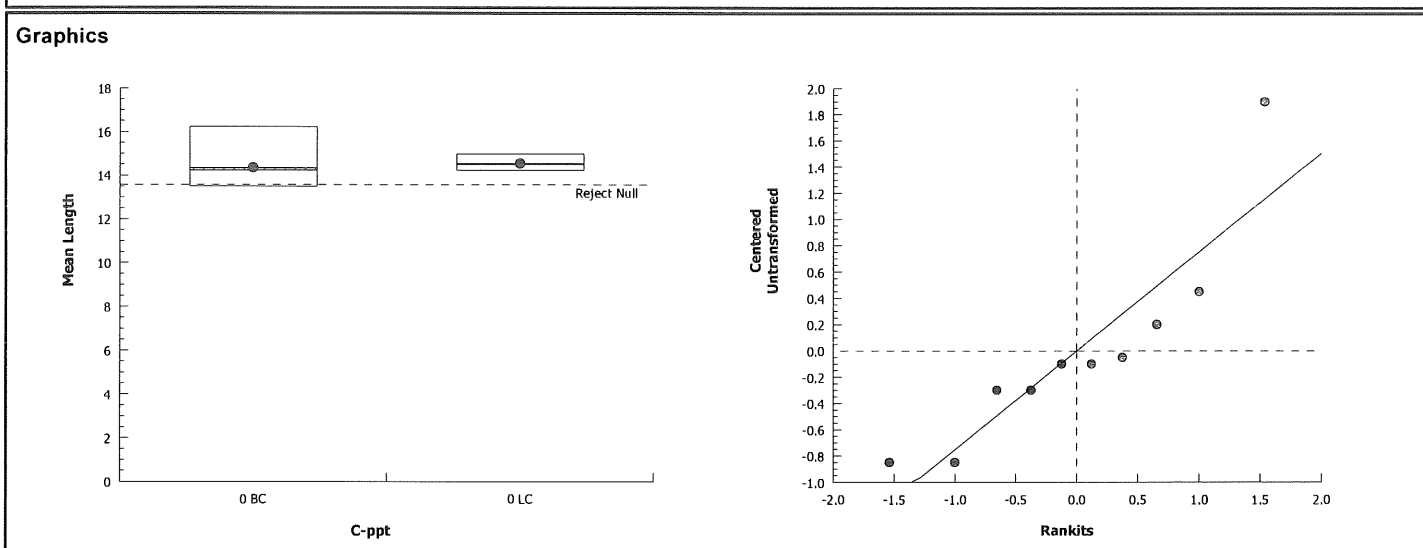
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	6.7%	Passes mean length

Equal Variance t Two-Sample Test								
Control	vs Control	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control	Brine Control	0.3814	1.86	0.975	8	0.3564	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.1	0.1	1	0.1455	0.7128	Non-Significant Effect
Error	5.5	0.6875	8			
Total	5.6		9			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Variance Ratio F	11.94	23.15	0.0340	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.8352	0.7411	0.0386	Normal Distribution

Mean Length Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	14.55	14.15	14.95	14.5	14.25	15	0.1458	2.24%	0.0%
0	Brine Control	5	14.35	12.95	15.75	14.25	13.5	16.25	0.5037	7.85%	1.38%



CETIS Analytical Report

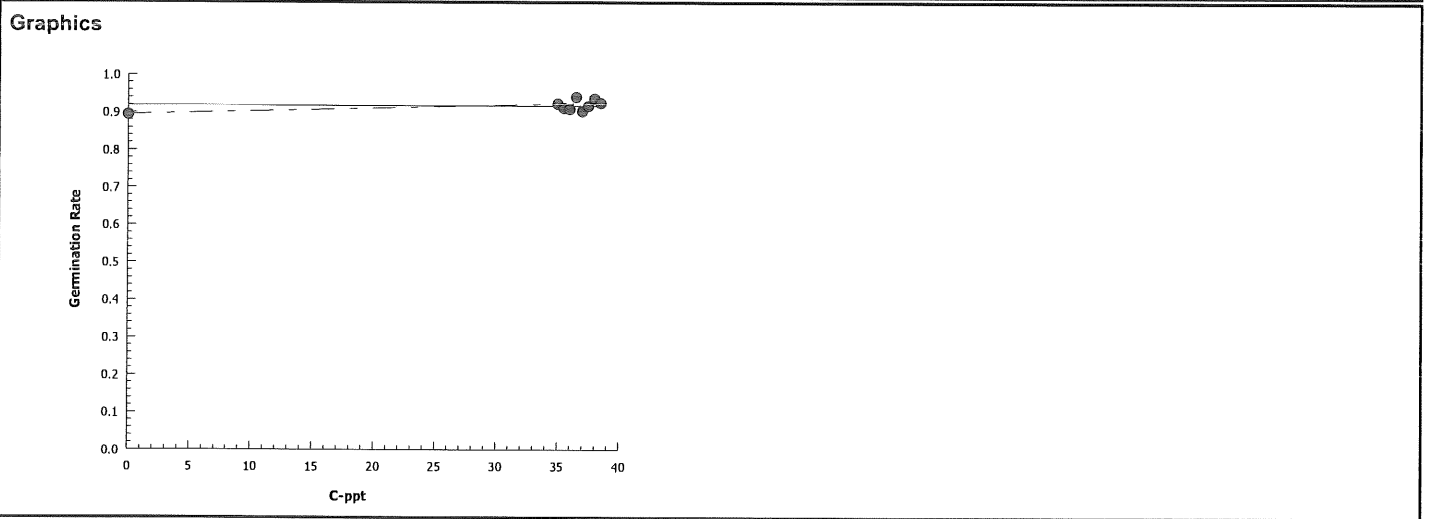
Report Date: 23 Jun-15 11:24 (p 1 of 1)
 Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test			Nautilus Environmental (CA)		
Analysis ID: 00-0530-0673	Endpoint: Germination Rate	CETIS Version: CETISv1.8.7			
Analyzed: 23 Jun-15 11:23	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	778442	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

Germination Rate Summary			Calculated Variate(A/B)									
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
0	Lab Control	5	0.894	0.83	0.94	0.0186	0.04159	4.65%	0.0%	447	500	
35		5	0.924	0.89	0.95	0.01249	0.02793	3.02%	-3.36%	462	500	
35.5		5	0.912	0.86	0.96	0.01715	0.03834	4.2%	-2.01%	456	500	
36		5	0.91	0.84	0.96	0.0249	0.05568	6.12%	-1.79%	455	500	
36.5		5	0.942	0.91	0.97	0.01241	0.02775	2.95%	-5.37%	471	500	
37		5	0.904	0.87	0.92	0.009273	0.02074	2.29%	-1.12%	452	500	
37.5		5	0.918	0.89	0.94	0.008602	0.01923	2.1%	-2.69%	459	500	
38		5	0.938	0.9	0.98	0.01463	0.03271	3.49%	-4.92%	469	500	
38.5		5	0.926	0.84	0.98	0.02421	0.05413	5.85%	-3.58%	463	500	



CETIS Analytical Report

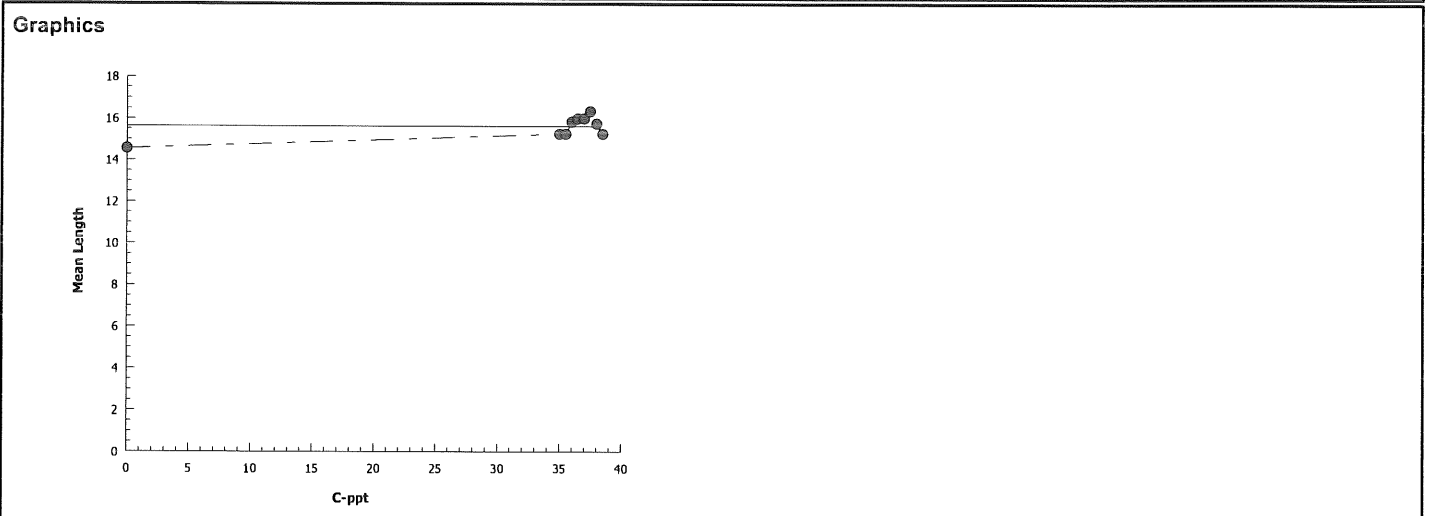
Report Date: 23 Jun-15 11:24 (p 1 of 1)
 Test Code: 1505-S092 | 07-6525-4311

Macrocystis Germination and Germ Tube Growth Test			Nautilus Environmental (CA)
Analysis ID: 04-6246-3634	Endpoint: Mean Length	CETIS Version: CETISv1.8.7	
Analyzed: 23 Jun-15 11:23	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes	

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	944683	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
IC25	>38.5	N/A	N/A
IC50	>38.5	N/A	N/A

Mean Length Summary			Calculated Variate						
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	14.55	14.25	15	0.1458	0.326	2.24%	0.0%
35		5	15.25	14	17.75	0.6661	1.49	9.77%	-4.81%
35.5		5	15.25	13.75	17.5	0.6708	1.5	9.84%	-4.81%
36		5	15.85	15	16.75	0.3021	0.6755	4.26%	-8.94%
36.5		5	16	15.25	16.75	0.2739	0.6124	3.83%	-9.97%
37		5	16	14.75	17.25	0.4257	0.952	5.95%	-9.97%
37.5		5	16.35	15.75	17	0.2318	0.5184	3.17%	-12.37%
38		5	15.75	15	16.25	0.2372	0.5303	3.37%	-8.25%
38.5		5	15.25	13.5	16.25	0.5062	1.132	7.42%	-4.81%



Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental - San Diego

Start Date: 12-May-15

Species: *Macrocystis pyrifera*

Test ID: 1505-S092

End Date: 14-May-15

Protocol: EPA/600/R-95/136 (1995 West Coast Manual)

Sample Source: Poseidon

Sampled: 12-May-15

Sample Station: Nautilus Brine

Random Number	Number Counted	Number Germinated	Tube Length Measurements (micrometer units)										Calibration Factor	Mean Tube Length (µm)
36	100	98	6	7	8	7	7	7	6	8	4	5	2.5	16.25
37	100	95	9	6	8	6	5	5	6	7	8	5	2.5	16.25
38	100	91	6	6	8	6	5	6	6	7	5	6	2.5	15.25
39	100	83	7	6	7	5	4	5	8	5	6	6	2.5	14.75
40	100	92	8	6	8	5	5	6	5	7	7	7	2.5	16.00
41	100	93	8	5	6	6	5	8	5	6	7	6	2.5	15.50
42	100	94	5	5	8	5	7	5	6	5	4	7	2.5	14.25
43	100	95	7	5	7	5	4	7	5	6	5	5	2.5	14.00
44	100	95	5	7	6	7	5	7	5	7	6	7	2.5	15.50
45	100	96	7	5	8	7	6	7	5	7	9	6	2.5	16.75
46	100	96	6	6	6	6	5	8	7	8	4	6	2.5	15.50
47	100	93	7	8	8	7	8	8	6	5	6	5	2.5	17.00
48	100	90	5	6	6	7	5	6	5	6	9	8	2.5	15.75
49	100	98	4	6	5	5	7	7	6	5	5	4	2.5	13.50
50	100	95	7	6	5	5	7	8	7	6	5	5	2.5	15.25
51	100	91	6	7	5	7	6	5	7	7	5	9	2.5	16.00
52	100	92	6	7	5	5	6	8	6	4	4	6	2.5	14.25
53	100	89	5	6	6	8	7	7	7	5	6	6	2.5	15.75
54	100	89	5	8	4	8	5	4	8	5	5	4	2.5	14.00
55	100	94	7	7	8	6	6	8	4	7	5	6	2.5	16.00
56	100	91	7	7	7	8	8	4	8	7	4	5	2.5	16.25
57	100	89	7	4	6	6	4	5	6	6	7	6	2.5	14.25
58	100	96	5	6	5	5	5	5	7	7	4	5	2.5	13.50
59	100	90	6	5	6	7	5	5	6	7	8	7	2.5	15.50
60	100	84	9	7	7	5	5	7	5	6	6	8	2.5	16.25
61	100	93	6	7	7	8	7	7	6	8	8	7	2.5	17.75
62	100	84	6	7	5	8	5	8	7	5	7	5	2.5	15.75
63	100	92	7	6	6	7	8	6	6	5	8	6	2.5	16.25
64	100	87	7	4	4	6	6	5	4	7	6	5	2.5	13.50
65	100	92	5	7	8	7	6	7	4	8	10	4	2.5	16.50
66	100	92	9	7	7	7	7	4	6	5	7	4	2.5	15.75
67	100	91	6	6	6	6	7	7	6	5	4	6	2.5	14.75
68	100	92	7	7	8	6	7	7	6	6	7	6	2.5	16.75
69	100	91	7	6	6	5	5	4	7	7	7	6	2.5	15.00
70	100	97	7	8	4	7	7	9	7	5	6	7	2.5	16.75

QC: BK 5/28/15

Final Review: AC 5/28/15

Analyst BK

Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental - San Diego

Start Date: 12-May-15

Species: *Macrocystis pyrifera*

Test ID: 1505-S092

End Date: 14-May-15

Protocol: EPA/600/R-95/136 (1995 West Coast Manual)

Sample Source: Poseidon

Sampled: 12-May-15

Sample Station: Nautilus Brine

Random Number	Number Counted	Number Germinated	Tube Length Measurements (micrometer units)										Calibration Factor	Mean Tube Length (µm)
71	100	86	6	8	7	7	9	7	9	6	6	5	2.5	17.5
72	100	97	7	7	7	7	8	5	7	6	5	7	2.5	16.5
73	100	92	6	6	3	7	5	3	5	5	8	7	2.5	13.75
74	100	95	7	7	5	7	8	5	6	7	7	6	2.5	16.25
75	100	94	6	5	5	7	7	7	5	7	7	6	2.5	15.5
76	100	89	6	5	5	6	7	5	6	4	6	7	2.5	14.25
77	100	95	4	5	6	8	6	5	6	4	8	7	2.5	14.75
78	100	89	6	7	5	8	5	6	6	5	7	5	2.5	15
79	100	94	5	5	8	7	5	5	6	7	5	4	2.5	14.25
80	100	90	8	8	6	5	5	5	8	5	5	5	2.5	15
81	100	86	7	8	6	8	5	6	6	5	6	6	2.5	15.75
82	100	87	5	9	6	8	7	7	6	7	7	7	2.5	17.25
83	100	95	7	6	5	5	6	7	5	6	7	6	2.5	15
84	100	92	5	6	7	6	5	6	7	6	4	6	2.5	14.5
85	100	94	7	7	5	7	5	6	7	7	5	7	2.5	15.75

QC: BK 5/28/15

Final Review: AC 5/28/15

Analyst BK

Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental - San Diego

Start Date: 12-May-15

Species: *Macrocystis pyrifera*

Test ID: 1535-5092

End Date: 14-May-15

Protocol: EPA/600/R-95/136 (1995 West Coast Manual)

Sample Source: Poseidon

Sampled: 12-May-15

Sample Station: Nautilus Brine

Random Number	Number Counted	Number Germinated	Tube Length Measurements (micrometer units)										Calibration Factor	Mean Tube Length (µm)
36	100	98	6	7	8	7	7	7	6	8	4	5	↑	#DIV/0!
37	100	95	9	6	8	6	5	6	6	7	8	5		#DIV/0!
38	100	91	6	6	8	6	5	6	6	7	5	6		#DIV/0!
39	100	83	7	6	7	5	4	5	8	5	6	6		#DIV/0!
40	100	92	8	6	8	5	5	6	5	7	7	7		#DIV/0!
41	100	93	8	5	6	6	5	5	5	6	7	6		#DIV/0!
42	100	94	5	5	8	5	5	4	5	5	4	7		#DIV/0!
43	100	95	7	5	7	5	4	5	6	6	5	5		#DIV/0!
44	100	95	5	5	6	7	5	7	5	7	6	7		#DIV/0!
45	100	96	7	5	6	6	7	6	5	7	6	6		#DIV/0!
46	100	96	6	6	6	6	6	7	7	8	8	6		#DIV/0!
47	100	93	7	6	8	7	5	6	6	5	6	4		#DIV/0!
48	100	90	5	6	6	5	7	7	6	6	5	9		#DIV/0!
49	100	98	4	6	6	5	5	7	8	7	5	5		#DIV/0!
50	100	95	7	6	5	5	5	7	7	6	5	5		#DIV/0!
51	100	91	6	7	5	5	7	6	8	7	5	5		#DIV/0!
52	100	92	6	6	7	5	7	6	8	4	4	6		#DIV/0!
53	100	89	5	6	6	5	5	7	7	5	6	4		#DIV/0!
54	100	89	5	6	7	5	5	7	8	4	5	5		#DIV/0!
55	100	94	7	7	8	6	8	6	4	7	5	4		#DIV/0!
56	100	91	7	7	7	6	8	4	8	7	4	5	#DIV/0!	
57	100	89	7	4	6	5	7	5	7	6	4	6	#DIV/0!	
58	100	96	5	6	6	5	5	5	7	6	7	4	#DIV/0!	
59	100	90	6	5	6	7	5	5	6	7	8	7	#DIV/0!	
60	100	84	9	7	7	7	5	7	7	5	6	8	#DIV/0!	
61	100	93	6	7	7	7	8	7	6	8	8	7	#DIV/0!	
62	100	84	6	7	5	7	5	8	6	5	7	5	#DIV/0!	
63	100	92	7	6	6	6	8	5	6	5	8	8	#DIV/0!	
64	100	87	7	4	4	6	6	5	6	4	7	6	#DIV/0!	
65	100	92	5	7	8	7	6	7	4	4	8	10	#DIV/0!	
66	100	92	9	7	7	7	7	4	6	5	7	4	#DIV/0!	
67	100	91	6	6	6	6	7	7	6	5	7	4	#DIV/0!	
68	100	92	7	7	6	6	7	7	6	6	7	6	#DIV/0!	
69	100	91	7	6	6	5	5	4	7	7	7	6	#DIV/0!	
70	100	97	7	8	4	7	7	9	7	5	6	7	2.5	#DIV/0!

QC: BL 5/28/15

Final Review: AC 5/28/15

Analyst: KFP

Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental - San Diego

Start Date: 12-May-15

Species: *Macrocystis pyrifera*

Test ID: 1505-5092

End Date: 14-May-15

Protocol: EPA/600/R-95/136 (1995 West Coast Manual)

Sample Source: Poseidon

Sampled: 12-May-15

Sample Station: Nautilus Drive

Random Number	Number Counted	Number Germinated	Tube Length Measurements (micrometer units)										Calibration Factor	Mean Tube Length (µm)	
71	100	86	6	8	7	7	9	7	9	6	6	6	5	↑	#DIV/0!
72	100	97	7	7	7	7	8	5	7	5	5	5	#DIV/0!		
73	100	92	6	6	3	7	5	3	5	7	5	7	#DIV/0!		
74	100	95	6	6	5	5	8	5	6	7	7	6	#DIV/0!		
75	100	94	6	5	5	7	7	5	5	7	7	6	#DIV/0!		
76	100	89	4	5	5	6	7	5	5	4	6	7	#DIV/0!		
77	100	95	4	5	6	8	6	5	6	4	8	7	#DIV/0!		
78	100	89	5	5	5	7	5	6	6	5	7	5	#DIV/0!		
79	100	94	5	5	8	7	5	5	6	7	5	4	#DIV/0!		
80	100	90	8	8	6	5	9	5	8	5	5	5	#DIV/0!		
81	100	86	7	8	6	5	9	7	6	5	6	6	#DIV/0!		
82	100	87	7	9	6	8	9	7	6	7	7	6	#DIV/0!		
83	100	95	7	6	5	5	6	7	5	6	7	6	#DIV/0!		
84	100	92	7	6	7	6	5	7	7	6	4	6	#DIV/0!		
85	100	94	7	7	5	7	5	6	7	7	5	7	2.5		

QC: BK 5/28/15

Final Review: AC 5/28/15

Analyst: KFP

Marine Chronic Bioassay

Water Quality Measurements

Client : Poseidon

Test Species: Macrocyctis pyrifera

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: 5/6/2015 5/12/15 1500

Sample Log No.: 45 (A) Nautilus Brine

End Date/Time: 5/8/2015 5/14/15 1215
x 130215 5/11/15

Test No.: 1505-5092

Dilutions made by: BK

Analyst: BK

Analyst: JW

Salinity (ppt)	Initial Readings				Final Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.05	33.0	15.5	7.7	7.97	33.4	15.6
Brine Control	8.0	7.95	34.0	15.7	7.7	7.97	34.3	15.6
35.0	8.2	8.00	35.0	15.3	7.6	7.99	35.5	15.6
35.5	8.4	8.01	35.5	15.2	7.6	8.01	36.0	15.6
36.0	8.4	8.03	36.0	15.1	7.7	8.02	36.6	15.6
36.5	8.5	8.03	36.6	15.2	7.6	8.03	37.2	15.6
37.0	8.5	8.02	37.0	15.2	7.7	8.03	37.5	15.6
37.5	8.4	8.02	37.5	15.2	7.6	8.02	38.1	15.6
38.0	8.5	8.01	38.1	15.4	7.5	8.03	38.6	15.6
38.5	8.5	8.00	38.5	15.5	7.6	8.04	39.1	15.6

Comments: Each Session 5 salinity meter used.
Ⓟ BK Q16 5/28/15

QC Check: BK 5/28/15

Final Review: AC 5/28/15

CETIS Test Data Worksheet

Report Date: 11 May-15 16:03 (p 1 of 2)
 Test Code: A-07-6525-4344/1505-5092

Macrocystis Germination and Germ Tube Growth Test				Nautilus Environmental (CA)			
Start Date: 12 May-15	Species: Macrocystis pyrifera	Sample Code: A-1504- Nautilus Brine					
End Date: 14 May-15	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Poseidon					
Sample Date: 12 May-15	Material: Natural Seawater	Sample Station: Nautilus Brine					

C-ppt	Code	Rep	Pos	# Counted	# Germinated	Mean Length	CalFactor	Notes
0	BC	1	52	100			1	
0	BC	2	58	100			1	
0	BC	3	64	100			1	
0	BC	4	42	100			1	
0	BC	5	63	100			1	
0	LC	1	79	100			1	
0	LC	2	39	100			1	
0	LC	3	78	100			1	
0	LC	4	84	100			1	
0	LC	5	57	100			1	
35		1	43	100			1	
35		2	61	100			1	
35		3	76	100			1	
35		4	50	100			1	
35		5	80	100			1	
35.5		1	41	100			1	
35.5		2	73	100			1	
35.5		3	54	100			1	
35.5		4	46	100			1	
35.5		5	71	100			1	
36		1	45	100			1	
36		2	83	100			1	
36		3	81	100			1	
36		4	75	100			1	
36		5	60	100			1	
36.5		1	85	100			1	
36.5		2	38	100			1	
36.5		3	66	100			1	
36.5		4	70	100			1	
36.5		5	72	100			1	
37		1	65	100			1	
37		2	59	100			1	
37		3	40	100			1	
37		4	82	100			1	
37		5	67	100			1	
37.5		1	68	100			1	
37.5		2	47	100			1	
37.5		3	53	100			1	
37.5		4	55	100			1	
37.5		5	56	100			1	
38		1	69	100			1	
38		2	36	100			1	
38		3	44	100			1	
38		4	37	100			1	
38		5	48	100			1	
38.5		1	62	100			1	
38.5		2	74	100			1	

CETIS Test Data Worksheet

Report Date: 11 May-15 16:03 (p 2 of 2)
Test Code: 07-6525-4314/1505-5092

C-ppt	Code	Rep	Pos	# Counted	# Germinated	Mean Length	CalFactor	Notes
38.5		3	49	100			1	
38.5		4	77	100			1	
38.5		5	51	100			1	

QC-BK

Ⓐ Q15 BK 5/28/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: BK

Sample ID: frozen seawater

Test Date: 5/12/2015

Test No: 1505-5092

Test Type: M. pyrifera

Salinity of Seawater 33.1

Salinity of Brine 89.7

Date of Brine used: 5/8/2015

Test Dilution Volume 250

Alkalinity of Brine Control: 118 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	250
35.0	96.6	241.6	0.03	8.4	250
35.5	95.8	239.4	0.04	10.6	250
36.0	94.9	237.2	0.05	12.8	250
36.5	94.0	235.0	0.06	15.0	250
37.0	93.1	232.8	0.07	17.2	250
37.5	92.2	230.6	0.08	19.4	250
38.0	91.3	228.4	0.09	21.6	250
38.5	90.5	226.1	0.11	23.9	250

DI Volume

Brine Control	40.8	0.58	23.9	250
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Total Brine Volume Required (ml): **152.8**

QC Check: BK 5/28/15

Final Review: AC 5/28/15

Marine Chronic Bioassay

Kelp Spore Germination & Growth Worksheet

Client: Poseidon
 Test No.: 1505-5092
 Tech. Initials: BK

Start Date/Time: 5/12/2015 / 1500
 End Date/Time: 5/14/2015 / 1215
 Test Species: Macrocystis pyrifera

Date Collected: 5/12/15
 Kelp Collectors: DK
 Collection Location: La Jolla Cove

Time of Initial Rinsing and Dessication: 5/12/15 0930
 Time of Rinsing and Transfer to Release Beaker: 5/12/15 1400
 Conditions of Zoospore Density and Motility: Poor density and poor motility
 Time of Blade Removal From Release Beaker: 5/12/15 1450

Density Counts (target = 90): 58 64 67 56 70 Mean: 63
 Mean 63 * 10,000 = 630,000 spores/ml (density of spore release)

If spore release = 900,000 spores/ml: Inoculate with 0.25 ml

If spore release > 900,000 spores/ml: Calculate a dilution factor, x, create a new spore stock of 900,000 spores/ml and inoculate with 0.25 ml.

To calculate the dilution factor: No Dilution Necessary
 Density of spore release — * $\frac{0.25 \text{ ml}}{1 \text{ container}}$ = $\frac{\text{— spores}}{225,000 \text{ spores}}$ = — (x) $\frac{\text{—}}{-1.0}$ dil. factor
 — part(s) seawater

If spore release < 900,000 spores/ml: The volume added should not exceed 0.5 ml. (This volume exceeds the EPA and MBP required volume of no greater than 1% of the total test solution volume. However, it may sometimes be necessary to exceed the 0.3 ml requirement in order to achieve the desired spore density.)

Time of inoculation: 1500 Amount inoculated: 0.5 ml

Location in Environmental Chamber (All replicates in each test must be on the same shelf; do not split up tests among shelves):

Shelf number	Measured Light Intensity Range (must be between 160 and 240 ft-c)	Random Number Range
1	175 - 231	86 - 115
2	184 - 226	116 - 158
3	180 - 237	36 - 85
<u>(4) 49</u>	178 - 228	151 - 185
5		
6		
Timers Checked? <input checked="" type="checkbox"/>	Should be on 16:8 light:dark cycle	initials: <u>BK</u>

24-hour germination check	
QC dish #	% germ.
<u>3</u>	<u>92</u>

Comments: _____

QC Check: BK 5/28/15

Final Review: AC 5/28/15

**Pacific Topsmelt
7-day Survival and Growth**

Test Date: May 5, 2015

CETIS Summary Report

Report Date: 26 Jun-15 09:20 (p 1 of 2)
 Test Code: 1505-S091 | 11-2970-9154

Pacific Topsmelt 7-d Survival and Growth Test **Nautilus Environmental (CA)**

Batch ID: 05-8756-2032	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 05 May-15 13:50	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 12 May-15 11:30	Species: Atherinops affinis	Brine: Frozen Seawater
Duration: 6d 22h	Source: Aquatic Biosystems, CO	Age: 15 d

Sample ID: 02-3838-7973	Code: Nautilus Brine	Client: Poseidon
Sample Date: 05 May-15	Material: Natural Seawater	Project:
Receive Date: 05 May-15	Source: Poseidon	
Sample Age: 14h	Station: Nautilus Brine	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
06-8392-5997	7d Survival Rate	38.5	>38.5	NA	18.6%		Steel Many-One Rank Sum Test
08-3759-0266	Mean Dry Biomass-mg	38.5	>38.5	NA	19.6%		Dunnett Multiple Comparison Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	ppt	95% LCL	95% UCL	TU	Method
09-2555-2009	7d Survival Rate	EC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		EC50	>38.5	N/A	N/A		
10-9737-7367	Mean Dry Biomass-mg	IC25	>38.5	N/A	N/A		Linear Interpolation (ICPIN)
		IC50	>38.5	N/A	N/A		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
06-8392-5997	7d Survival Rate	Control Resp	0.88	0.8 - NL	Yes	Passes Acceptability Criteria	
09-2555-2009	7d Survival Rate	Control Resp	0.88	0.8 - NL	Yes	Passes Acceptability Criteria	
08-3759-0266	Mean Dry Biomass-mg	Control Resp	1.161	0.85 - NL	Yes	Passes Acceptability Criteria	
10-9737-7367	Mean Dry Biomass-mg	Control Resp	1.161	0.85 - NL	Yes	Passes Acceptability Criteria	
06-8392-5997	7d Survival Rate	PMSD	0.1858	NL - 0.25	No	Passes Acceptability Criteria	
08-3759-0266	Mean Dry Biomass-mg	PMSD	0.1956	NL - 0.5	No	Passes Acceptability Criteria	

7d Survival Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	0.0%
0	Lab Control	5	0.88	0.6579	1	0.6	1	0.08	0.1789	20.33%	8.33%
35		5	1	1	1	1	1	0	0	0.0%	-4.17%
35.5		5	1	1	1	1	1	0	0	0.0%	-4.17%
36		5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	0.0%
36.5		5	1	1	1	1	1	0	0	0.0%	-4.17%
37		5	0.92	0.6979	1	0.6	1	0.08	0.1789	19.44%	4.17%
37.5		5	0.92	0.784	1	0.8	1	0.04899	0.1095	11.91%	4.17%
38		5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	0.0%
38.5		5	0.92	0.784	1	0.8	1	0.04899	0.1095	11.91%	4.17%

Mean Dry Biomass-mg Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Brine Control	5	1.078	0.9036	1.252	0.922	1.262	0.06266	0.1401	13.0%	0.0%
0	Lab Control	5	1.161	1.031	1.29	1.066	1.334	0.04669	0.1044	9.0%	-7.72%
35		5	1.041	0.9807	1.101	0.962	1.074	0.02165	0.04841	4.65%	3.42%
35.5		5	1.106	1.049	1.162	1.064	1.172	0.02043	0.04568	4.13%	-2.6%
36		5	1.139	0.8754	1.403	0.97	1.504	0.09502	0.2125	18.65%	-5.72%
36.5		5	1.199	0.9086	1.489	0.978	1.492	0.1045	0.2338	19.5%	-11.25%
37		5	0.898	0.7274	1.069	0.756	1.066	0.06145	0.1374	15.3%	16.67%
37.5		5	1.076	0.9217	1.23	0.916	1.224	0.05557	0.1243	11.55%	0.15%
38		5	1.099	0.9125	1.285	0.926	1.274	0.06711	0.1501	13.66%	-1.97%
38.5		5	1.07	0.9083	1.232	0.856	1.212	0.05838	0.1305	12.19%	0.67%

CETIS Summary Report

Report Date: 26 Jun-15 09:20 (p 2 of 2)
 Test Code: 1505-S091 | 11-2970-9154

Pacific Topsmelt 7-d Survival and Growth Test							Nautilus Environmental (CA)
7d Survival Rate Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	1	0.8	1	1	1	
0	Lab Control	0.8	1	1	1	0.6	
35		1	1	1	1	1	
35.5		1	1	1	1	1	
36		0.8	1	1	1	1	
36.5		1	1	1	1	1	
37		1	1	0.6	1	1	
37.5		1	0.8	0.8	1	1	
38		1	1	0.8	1	1	
38.5		1	1	1	0.8	0.8	
Mean Dry Biomass-mg Detail							
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Brine Control	1.17	0.922	1.262	1.064	0.97	
0	Lab Control	1.066	1.334	1.112	1.116	1.176	
35		1.068	0.962	1.026	1.074	1.074	
35.5		1.172	1.064	1.09	1.07	1.132	
36		1.012	1.504	1.118	0.97	1.092	
36.5		0.978	1.404	1.02	1.492	1.1	
37		1.066	0.774	0.756	1.006	0.888	
37.5		1.16	0.992	0.916	1.224	1.088	
38		1.238	0.926	1.036	1.274	1.02	
38.5		1.09	1.084	1.212	1.11	0.856	

CETIS Analytical Report

Report Date: 26 Jun-15 09:19 (p 1 of 4)
 Test Code: 1505-S091 | 11-2970-9154

Pacific Topsmelt 7-d Survival and Growth Test						Nautilus Environmental (CA)			
Analysis ID: 06-8392-5997		Endpoint: 7d Survival Rate			CETIS Version: CETISv1.8.7				
Analyzed: 23 Jun-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	18.6%	38.5	>38.5	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		35	32.5	16	1	8	0.9941	Asymp	Non-Significant Effect
		35.5	32.5	16	1	8	0.9941	Asymp	Non-Significant Effect
		36	30.5	16	2	8	0.9771	Asymp	Non-Significant Effect
		36.5	32.5	16	1	8	0.9941	Asymp	Non-Significant Effect
		37	29.5	16	2	8	0.9588	Asymp	Non-Significant Effect
		37.5	28.5	16	2	8	0.9304	Asymp	Non-Significant Effect
		38	30.5	16	2	8	0.9771	Asymp	Non-Significant Effect
		38.5	28.5	16	2	8	0.9304	Asymp	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.1043122	0.01303902	8	0.8296	0.5825	Non-Significant Effect
Error	0.5658488	0.01571802	36			
Total	0.670161		44			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Mod Levene Equality of Variance	0.8755	3.256	0.5489	Equal Variances
Variances	Levene Equality of Variance	5.86	3.052	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8352	0.9308	<0.0001	Non-normal Distribution

7d Survival Rate Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.88	0.6579	1	1	0.6	1	0.08	20.33%	0.0%
35		5	1	1	1	1	1	1	0	0.0%	-13.64%
35.5		5	1	1	1	1	1	1	0	0.0%	-13.64%
36		5	0.96	0.8489	1	1	0.8	1	0.04	9.32%	-9.09%
36.5		5	1	1	1	1	1	1	0	0.0%	-13.64%
37		5	0.92	0.6979	1	1	0.6	1	0.08	19.44%	-4.55%
37.5		5	0.92	0.784	1	1	0.8	1	0.04899	11.91%	-4.55%
38		5	0.96	0.8489	1	1	0.8	1	0.04	9.32%	-9.09%
38.5		5	0.92	0.784	1	1	0.8	1	0.04899	11.91%	-4.55%

Angular (Corrected) Transformed Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.206	0.9496	1.462	1.345	0.8861	1.345	0.09228	17.11%	0.0%
35		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-11.57%
35.5		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-11.57%
36		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	-7.62%
36.5		5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	-11.57%
37		5	1.253	0.9984	1.508	1.345	0.8861	1.345	0.09184	16.38%	-3.95%
37.5		5	1.25	1.088	1.412	1.345	1.107	1.345	0.05833	10.43%	-3.67%
38		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	-7.62%
38.5		5	1.25	1.088	1.412	1.345	1.107	1.345	0.05833	10.43%	-3.67%

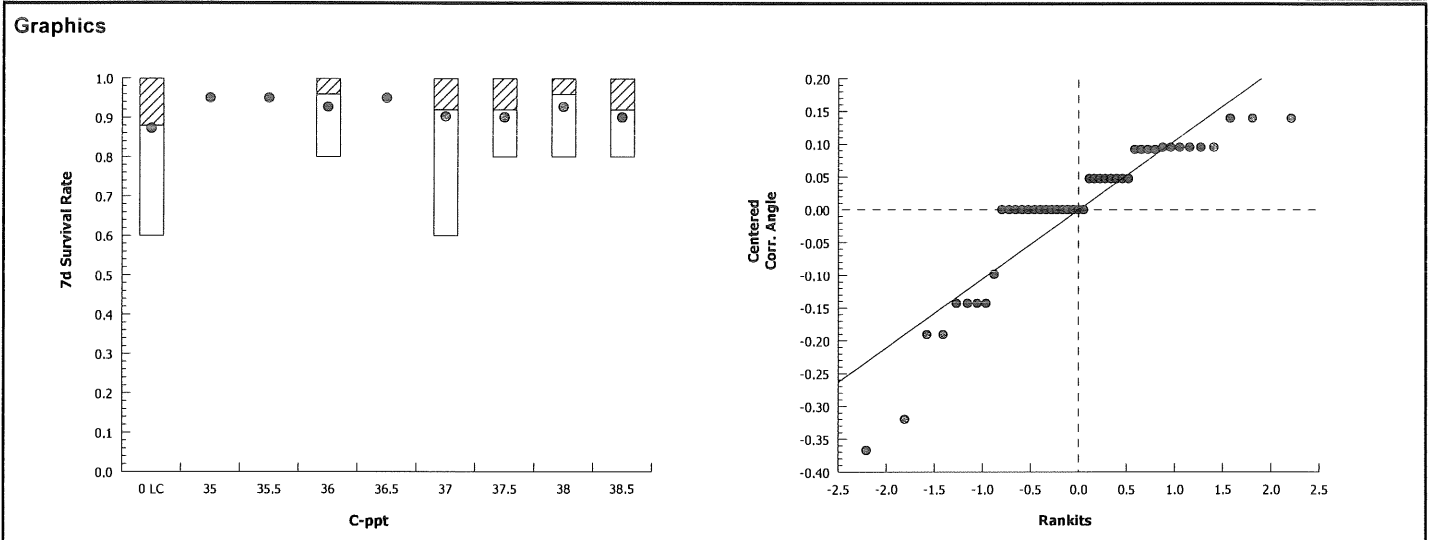
CETIS Analytical Report

Report Date: 26 Jun-15 09:20 (p 2 of 4)
 Test Code: 1505-S091 | 11-2970-9154

Pacific Topsmelt 7-d Survival and Growth Test			Nautilus Environmental (CA)		
Analysis ID: 06-8392-5997	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 23 Jun-15 11:19	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes			

7d Survival Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8	1	1	1	0.6
35		1	1	1	1	1
35.5		1	1	1	1	1
36		0.8	1	1	1	1
36.5		1	1	1	1	1
37		1	1	0.6	1	1
37.5		1	0.8	0.8	1	1
38		1	1	0.8	1	1
38.5		1	1	1	0.8	0.8

Angular (Corrected) Transformed Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1.107	1.345	1.345	1.345	0.8861
35		1.345	1.345	1.345	1.345	1.345
35.5		1.345	1.345	1.345	1.345	1.345
36		1.107	1.345	1.345	1.345	1.345
36.5		1.345	1.345	1.345	1.345	1.345
37		1.345	1.345	0.8861	1.345	1.345
37.5		1.345	1.107	1.345	1.345	1.345
38		1.345	1.345	1.107	1.345	1.345
38.5		1.345	1.345	1.345	1.107	1.107



CETIS Analytical Report

Report Date: 26 Jun-15 09:20 (p 3 of 4)

Test Code: 1505-S091 | 11-2970-9154

Pacific Topsmelt 7-d Survival and Growth Test						Nautilus Environmental (CA)				
Analysis ID: 08-3759-0266		Endpoint: Mean Dry Biomass-mg			CETIS Version: CETISv1.8.7					
Analyzed: 23 Jun-15 11:20		Analysis: Parametric-Control vs Treatments			Official Results: Yes					

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	19.6%	38.5	>38.5	NA	

Dunnett Multiple Comparison Test									
Control	vs	C-ppt	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α :5%)
Lab Control		35	1.309	2.478	0.227	8	0.3608	CDF	Non-Significant Effect
		35.5	0.6023	2.478	0.227	8	0.6864	CDF	Non-Significant Effect
		36	0.2357	2.478	0.227	8	0.8244	CDF	Non-Significant Effect
		36.5	-0.4146	2.478	0.227	8	0.9579	CDF	Non-Significant Effect
		37*	2.867	2.478	0.227	8	0.0208	CDF	Significant Effect
		37.5	0.9253	2.478	0.227	8	0.5386	CDF	Non-Significant Effect
		38	0.6765	2.478	0.227	8	0.6539	CDF	Non-Significant Effect
		38.5	0.9864	2.478	0.227	8	0.5096	CDF	Non-Significant Effect

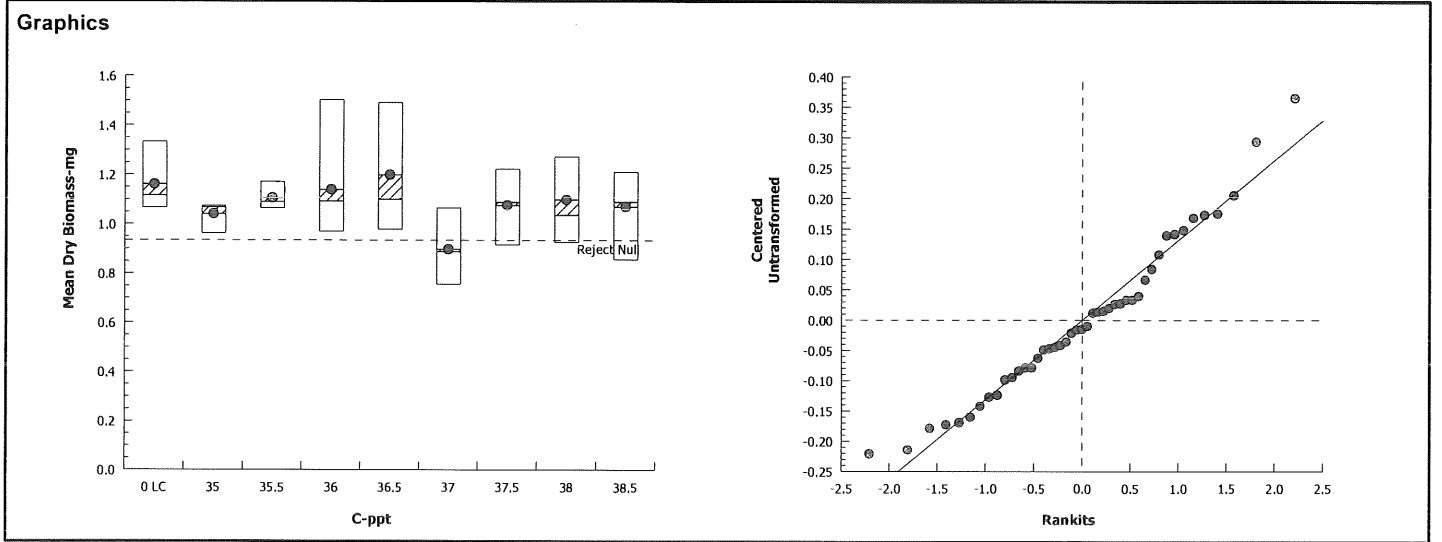
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α :5%)
Between	0.2970223	0.03712779	8	1.768	0.1162	Non-Significant Effect
Error	0.7559742	0.02099928	36			
Total	1.052997		44			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α :1%)
Variances	Bartlett Equality of Variance	14.6	20.09	0.0673	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9685	0.9308	0.2559	Normal Distribution

Mean Dry Biomass-mg Summary											
C-ppt	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.161	1.031	1.29	1.116	1.066	1.334	0.04669	9.0%	0.0%
35		5	1.041	0.9807	1.101	1.068	0.962	1.074	0.02165	4.65%	10.34%
35.5		5	1.106	1.049	1.162	1.09	1.064	1.172	0.02043	4.13%	4.76%
36		5	1.139	0.8754	1.403	1.092	0.97	1.504	0.09502	18.65%	1.86%
36.5		5	1.199	0.9086	1.489	1.1	0.978	1.492	0.1045	19.5%	-3.27%
37		5	0.898	0.7274	1.069	0.888	0.756	1.066	0.06145	15.3%	22.64%
37.5		5	1.076	0.9217	1.23	1.088	0.916	1.224	0.05557	11.55%	7.31%
38		5	1.099	0.9125	1.285	1.036	0.926	1.274	0.06711	13.66%	5.34%
38.5		5	1.07	0.9083	1.232	1.09	0.856	1.212	0.05838	12.19%	7.79%

Mean Dry Biomass-mg Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1.066	1.334	1.112	1.116	1.176
35		1.068	0.962	1.026	1.074	1.074
35.5		1.172	1.064	1.09	1.07	1.132
36		1.012	1.504	1.118	0.97	1.092
36.5		0.978	1.404	1.02	1.492	1.1
37		1.066	0.774	0.756	1.006	0.888
37.5		1.16	0.992	0.916	1.224	1.088
38		1.238	0.926	1.036	1.274	1.02
38.5		1.09	1.084	1.212	1.11	0.856

Pacific Topsmelt 7-d Survival and Growth Test		Nautilus Environmental (CA)	
Analysis ID: 08-3759-0266	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7	Official Results: Yes
Analyzed: 23 Jun-15 11:20	Analysis: Parametric-Control vs Treatments		



CETIS Analytical Report

Report Date: 26 Jun-15 09:20 (p 1 of 2)
 Test Code: 1505-S091 | 11-2970-9154

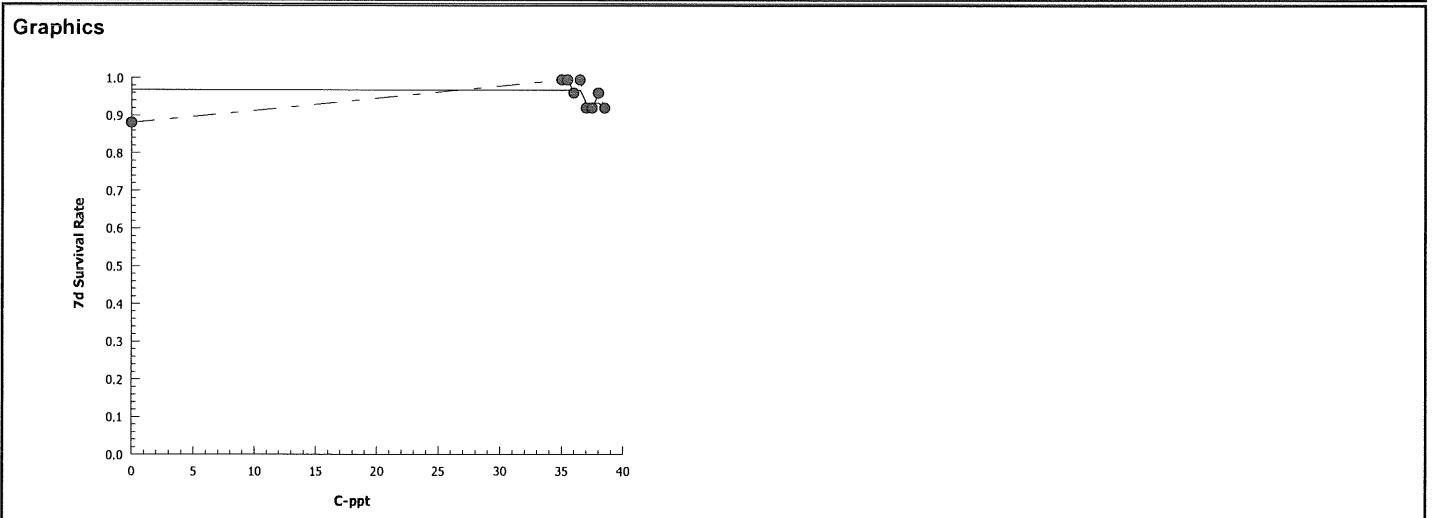
Pacific Topsmelt 7-d Survival and Growth Test			Nautilus Environmental (CA)		
Analysis ID: 09-2555-2009	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 23 Jun-15 11:19	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	196443	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
EC25	>38.5	N/A	N/A
EC50	>38.5	N/A	N/A

7d Survival Rate Summary		Calculated Variate(A/B)									
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.88	0.6	1	0.08	0.1789	20.33%	0.0%	22	25
35		5	1	1	1	0	0	0.0%	-13.64%	25	25
35.5		5	1	1	1	0	0	0.0%	-13.64%	25	25
36		5	0.96	0.8	1	0.04	0.08944	9.32%	-9.09%	24	25
36.5		5	1	1	1	0	0	0.0%	-13.64%	25	25
37		5	0.92	0.6	1	0.08	0.1789	19.44%	-4.55%	23	25
37.5		5	0.92	0.8	1	0.04899	0.1095	11.91%	-4.55%	23	25
38		5	0.96	0.8	1	0.04	0.08944	9.32%	-9.09%	24	25
38.5		5	0.92	0.8	1	0.04899	0.1095	11.91%	-4.55%	23	25

7d Survival Rate Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8	1	1	1	0.6
35		1	1	1	1	1
35.5		1	1	1	1	1
36		0.8	1	1	1	1
36.5		1	1	1	1	1
37		1	1	0.6	1	1
37.5		1	0.8	0.8	1	1
38		1	1	0.8	1	1
38.5		1	1	1	0.8	0.8



CETIS Analytical Report

Report Date: 26 Jun-15 09:20 (p 2 of 2)
 Test Code: 1505-S091 | 11-2970-9154

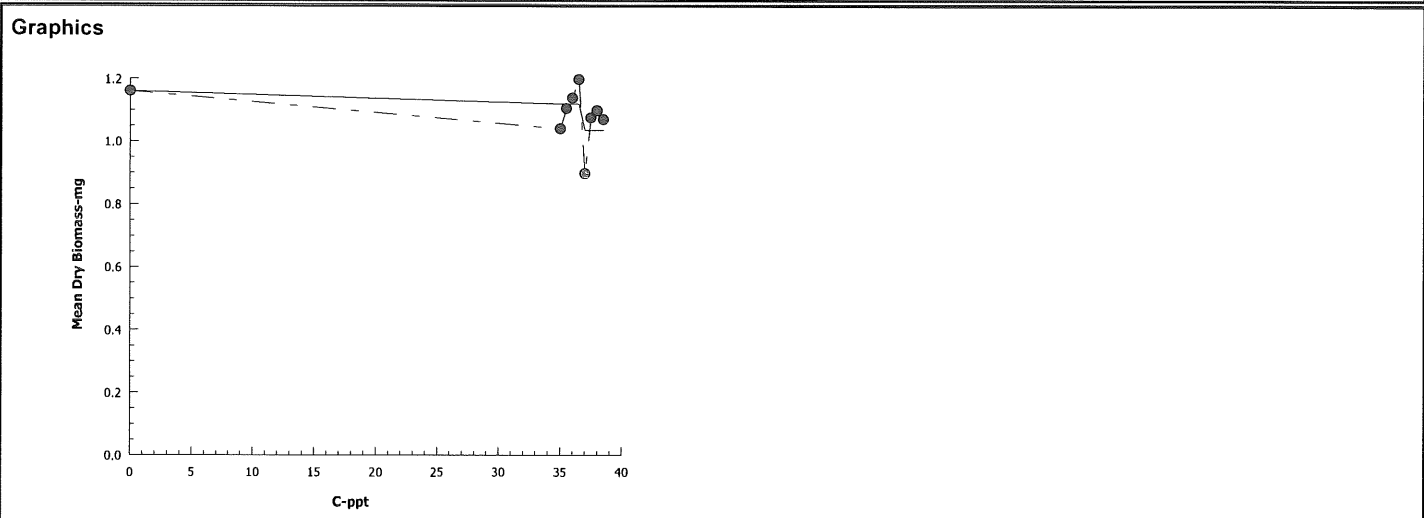
Pacific Topsmelt 7-d Survival and Growth Test			Nautilus Environmental (CA)		
Analysis ID: 10-9737-7367	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7			
Analyzed: 23 Jun-15 11:20	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1863856	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	ppt	95% LCL	95% UCL
IC25	>38.5	N/A	N/A
IC50	>38.5	N/A	N/A

Mean Dry Biomass-mg Summary			Calculated Variate						
C-ppt	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	1.161	1.066	1.334	0.04669	0.1044	9.0%	0.0%
35		5	1.041	0.962	1.074	0.02165	0.04841	4.65%	10.34%
35.5		5	1.106	1.064	1.172	0.02043	0.04568	4.13%	4.76%
36		5	1.139	0.97	1.504	0.09502	0.2125	18.65%	1.86%
36.5		5	1.199	0.978	1.492	0.1045	0.2338	19.5%	-3.27%
37		5	0.898	0.756	1.066	0.06145	0.1374	15.3%	22.64%
37.5		5	1.076	0.916	1.224	0.05557	0.1243	11.55%	7.31%
38		5	1.099	0.926	1.274	0.06711	0.1501	13.66%	5.34%
38.5		5	1.07	0.856	1.212	0.05838	0.1305	12.19%	7.79%

Mean Dry Biomass-mg Detail						
C-ppt	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1.066	1.334	1.112	1.116	1.176
35		1.068	0.962	1.026	1.074	1.074
35.5		1.172	1.064	1.09	1.07	1.132
36		1.012	1.504	1.118	0.97	1.092
36.5		0.978	1.404	1.02	1.492	1.1
37		1.066	0.774	0.756	1.006	0.888
37.5		1.16	0.992	0.916	1.224	1.088
38		1.238	0.926	1.036	1.274	1.02
38.5		1.09	1.084	1.212	1.11	0.856



Marine Chronic Bioassay

Larval Fish Survival

Client: Poseidon

Test Species: A. affinis

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: 5/5/2015 1350

Test No.: 1505-5091

End Date/Time: 5/12/2015 1130

Conc. (<u> </u> ppt)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival	
			0	1	2	3	4	5	6	7		
Lab Control	a	55	5	4	4	4	4	4	4	4	4	80
	b	32	5	5	5	5	5	5	5	5	5	100
	c	58	5	5	5	5	5	5	5	5	5	100
	d	34	5	5	5	5	5	5	5	5	5	100
	e	35	5	3	3	3	3	3	3	3	3	60
Brine Control	a	72	5	5	5	5	5	5	5	5	5	100
	b	48	5	4	4	4	4	4	4	4	4	80
	c	38	5	5	5	5	5	5	5	5	5	100
	d	63	5	5	5	5	5	5	5	5	5	100
	e	46	5	5	5	5	5	5	5	5	5	100
35.0	a	39	5	5	5	5	5	5	5	5	5	100
	b	78	5	5	5	5	5	5	5	5	5	100
	c	74	5	5	5	5	5	5	5	5	5	100
	d	51	5	5	5	5	5	5	5	5	5	100
	e	64	5	5	5	5	5	5	5	5	5	100
35.5	a	71	5	5	5	5	5	5	5	5	5	100
	b	66	5	5	5	5	5	5	5	5	5	100
	c	47	5	5	5	5	5	5	5	5	5	100
	d	40	5	5	5	5	5	5	5	5	5	100
	e	56	5	5	5	5	5	5	5	5	5	100
36.0	a	73	5	4	4	4	4	4	4	4	4	80
	b	43	5	5	5	5	5	5	5	5	5	100
	c	68	5	5	5	5	5	5	5	5	5	100
	d	60	5	5	5	5	5	5	5	5	5	100
	e	61	5	5	5	5	5	5	5	5	5	100
36.5	a	36	5	5	5	5	5	5	5	5	5	100
	b	59	5	5	5	5	5	5	5	5	5	100
	c	54	5	5	5	5	5	5	5	5	5	100
	d	49	5	5	5	5	5	5	5	5	5	100
	e	41	5	5	5	5	5	5	5	5	5	100

Rand # QC: CH Tech Initials: EG CH NH MA Mit BK CH CH
 Initial Count QC: AD Time: 1350 1145 1405 1230 1500 1210 1315 1205

Time Fed (day):	0	1	2	3	4	5	6
morning:	-	0915	0820	0930	0825	0830	+0830
evening:	1540	1530	1655	1530	1730	1545	+1700

Drying Oven Info
 Tare wt. Initials/Date: 582 5/12/15
 Date/Time in: 5/12/15 1305
 Date/Time out: 5/13/15 1550
 Temp (°C): 60.0
 QC Check: VB 5/12/15
 Final Review: ACC 2/6/15

Comments: _____

Marine Chronic Bioassay

Larval Fish Survival

Client: Poseidon

Test Species: A. affinis

Sample ID: Nautilus brine (frozen seawater)

Start Date/Time: 5/5/2015 1350

Test No.: 1505-5091

End Date/Time: 5/12/2015 1130

Conc. (ppt)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival	
			0	1	2	3	4	5	6	7		
37.0	a	76	5	5	5	5	5	5	5	5	5	100
	b	67	5	5	5	5	5	5	5	5	5	100
	c	69	5	3	3	3	3	3	3	3	3	60
	d	33	5	5	5	5	5	5	5	5	5	100
	e	80	5	5	5	5	5	5	5	5	5	100
37.5	a	77	5	5	5	5	5	5	5	5	5	100
	b	65	5	4	4	4	4	4	4	4	4	80
	c	52	5	5	5	5	5	5	5	4	4	80
	d	31	5	5	5	5	5	5	5	5	5	100
	e	57	5	5	5	5	5	5	5	5	5	100
38.0	a	45	5	5	5	5	5	5	5	5	5	100
	b	75	5	5	5	5	5	5	5	5	5	100
	c	50	5	4	4	4	4	4	4	4	4	80
	d	37	5	5	5	5	5	5	5	5	5	100
	e	62	5	5	5	5	5	5	5	5	5	100
38.5	a	44	5	5	5	5	5	5	5	5	5	100
	b	53	5	5	5	5	5	5	5	5	5	100
	c	70	5	5	5	5	5	5	5	5	5	100
	d	42	5	4	4	4	4	4	4	4	4	80
	e	79	5	4	4	4	4	4	4	4	4	80
	a		5									
	b		5									
	c		5									
	d		5									
	e		5									
	a		5									
	b		5									
	c		5									
	d		5									
	e		5									

Rand # QC: CH Tech Initials: EG CH NH NH NH BK CH CH
 Initial Count QC: AD Time: 1350 1145 1405 1230 1600 1216 1315 1205

Time Fed (day): 0 1 2 3 4 5 6
 morning: - 0815 0820 0930 0825 0830 0830
 evening: 1540 1530 1655 1530 1730 1545 + 1700
CH 5/12/15 G18

Drying Oven Info
 Tare wt. Initials/Date: 362 5/12/15
 Date/Time in: 5/12/15 1305
 Date/Time out: 5/13/15 1550
 Temp (°C): 60.0
 QC Check: KB 5/21/15
 Final Review: AL 4/26/15

Comments: ⓐ CH 5/6/15 G18

Marine Chronic Bioassay

Larval Fish Weights

Client: Poseidon

Test Species: A. affinis

Sample ID: Nautilus Brine (frozen seawater)

Start Date/Time: 5/5/2015 1350

Test No.: 1505-S091

End Date/Time: 5/12/2015 1130

Conc. (<u> </u> ppt <u> </u>)	Rep.	pan weight (mg)	pan + fish weight (mg)	total organism weight (mg)
	a	21.68	27.01	5.33
Lab Control	b	22.59	29.26	6.67
	c	22.72	28.28	5.56
	d	22.6	28.18	5.58
	e	22.36	28.24	5.88
	a	24.24	30.09	5.85
Brine Control	b	23.31	27.92	4.61
	c	22.81	29.12	6.31
	d	22.46	27.78	5.32
	e	23.63	28.48	4.85
	a	23.73	29.07	5.34
35	b	23.51	28.32	4.81
	c	22.74	27.87	5.13
	d	22.95	28.32	5.37
	e	23.36	28.73	5.37
	a	22.75	28.61	5.86
35.5	b	23.62	28.94	5.32
	c	23.86	29.31	5.45
	d	22.63	27.98	5.35
	e	22.49	28.15	5.66
	a	22.72	27.78	5.06
36	b	22.12	29.64	7.52
	c	23.36	28.95	5.59
	d	22.81	27.66	4.85
	e	21.42	26.88	5.46
	a	22.74	27.63	4.89
36.5	b	20.67	27.69	7.02
	c	21.05	26.15	5.1
	d	21.37	28.83	7.46
	e	22.98	28.48	5.5
	a	24.04	29.37	5.33
37	b	24.47	28.34	3.87
	c	24.94	28.72	3.78
	d	24.67	29.7	5.03
	e	24.93	29.37	4.44

Tech Initials: SG VCR
 Date/Time: 5/12/15 0945 5/13/2015 1550

QC Check: KB 5/21/15

Final Review: AC 5/20/15

Marine Chronic Bioassay

Larval Fish Weights

Client: Poseidon

Test Species: A. affinis

Sample ID: Nautilus Brine (frozen seawater)

Start Date/Time: 5/5/2015 1350

Test No.: 1505-S091

End Date/Time: 5/12/2015 1130

Conc. (<u> </u> ppt <u> </u>)	Rep.	pan weight (mg)	pan + fish weight (mg)	total organism weight (mg)
	a	22.06	27.86	5.8
37.5	b	21.35	26.31	4.96
	c	21.37	25.95	4.58
	d	22.73	28.85	6.12
	e	23.93	29.37	5.44
	a	22.03	28.22	6.19
38	b	22.42	27.05	4.63
	c	21.14	26.32	5.18
	d	20.03	26.4	6.37
	e	23.5	28.6	5.1
	a	22.99	28.44	5.45
38.5	b	22.89	28.31	5.42
	c	21.26	27.32	6.06
	d	22.28	27.83	5.55
	e	22.11	26.39	4.28
	a			0
	b			0
	c			0
	d			0
	e			0
	a			0
	b			0
	c			0
	d			0
	e			0
	a			0
	b			0
	c			0
	d			0
	e			0
	a			0
	b			0
	c			0
	d			0
	e			0

Tech Initials:	SG	VCR
Date/Time:	5/12/15 0945	5/13/2015 1550

QC Check: ICB 5/21/15

Final Review: AC 6/26/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon

Sample ID: Nautilus brine (frozen seawater)

Test No: 1505-3091

Test Species: *A. affinis*

Start Date/Time: 5/15/2015 1350

End Date/Time: 5/12/2015 1130

Concentration	Lab Control								
	Day	0	1	2	3	4	5	6	7
	Initial								
pH	8.06	7.85	7.78	7.74	8.00	7.83	7.94		
DO (mg/L)	7.7	7.2	6.8	7.6	6.9	7.4	8.0		
Salinity (ppt)	33.3	33.4	33.4	33.4	33.1	33.4	33.4		
Temp (°C)	19.0	19.9	20.0	19.4	20.2	20.1	20.2		
	Final								
pH		7.94	7.81	7.70	7.79	7.88	7.80	7.7	
DO (mg/L)		6.8	6.0	6.0	6.5	6.6	7.3	6.4	
Salinity (ppt)		33.0	33.4	33.5	33.6	33.3	33.3	33.4	
Temp (°C)		19.8	20.0	19.8	19.8	19.8	19.7	20.1	

Concentration	35.5 ppt								
	Day	0	1	2	3	4	5	6	7
	Initial								
pH	8.02	7.86	7.92	8.01	7.99	7.75	7.94		
DO (mg/L)	7.4	7.1	6.7	7.6	7.0	7.4	8.1		
Salinity (ppt)	35.5	35.5	35.5	35.5	35.5	35.5	35.5		
Temp (°C)	19.3	20.0	20.0	19.4	20.1	19.7	20.0		
	Final								
pH		7.93	7.83	7.73	7.77	7.89	7.82	7.84	
DO (mg/L)		6.7	5.8	5.6	5.9	6.1	7.0	6.4	
Salinity (ppt)		36.0	35.6	35.7	35.5	35.4	35.7	35.7	
Temp (°C)		19.4	20.2	19.9	20.1	19.9	19.6	19.5	

Concentration	Brine Control								
	Day	0	1	2	3	4	5	6	7
	Initial								
pH	8.03	7.88	7.95	8.00	8.00	7.91	7.95		
DO (mg/L)	7.5	7.2	6.8	7.6	6.9	7.6	8.1		
Salinity (ppt)	33.3	33.4	33.4	33.4	33.4	33.4	33.4		
Temp (°C)	20.3	20.2	20.4	19.6	20.3	20.0	20.6		
	Final								
pH		7.91	7.78	7.71	7.73	7.84	7.80	7.79	
DO (mg/L)		6.8	6.0	5.8	5.9	6.0	7.2	6.2	
Salinity (ppt)		33.8	33.4	33.6	33.6	33.4	34.1	33.6	
Temp (°C)		19.3	20.0	19.8	19.9	19.7	19.8	19.7	

Concentration	36.0 ppt								
	Day	0	1	2	3	4	5	6	7
	Initial								
pH	8.02	7.88	7.92	8.01	7.99	7.96	7.93		
DO (mg/L)	7.5	7.0	6.8	7.6	7.0	7.5	8.1		
Salinity (ppt)	36.0	36.0	36.0	36.0	36.0	36.0	36.0		
Temp (°C)	19.4	20.0	20.0	19.4	20.1	19.7	20.3		
	Final								
pH		7.91	7.83	7.76	7.81	7.90	7.83	7.84	
DO (mg/L)		6.5	6.4	6.0	6.3	6.3	7.4	6.7	
Salinity (ppt)		36.3	36.1	36.2	36.1	36.0	36.4	36.2	
Temp (°C)		19.2	19.9	19.7	19.7	19.7	19.5	19.6	

Concentration	35.0 ppt								
	Day	0	1	2	3	4	5	6	7
	Initial								
pH	8.02	7.86	7.97	8.00	8.00	7.94	7.94		
DO (mg/L)	7.5	7.2	6.8	7.6	7.1	7.5	8.1		
Salinity (ppt)	35.0	35.0	35.0	35.0	35.0	35.0	35.0		
Temp (°C)	19.4	20.0	20.1	19.5	20.2	19.8	20.5		
	Final								
pH		7.95	7.81	7.75	7.78	7.89	7.81	7.81	
DO (mg/L)		6.0	5.9	5.9	6.1	6.1	7.1	6.2	
Salinity (ppt)		35.2	35.0	35.2	35.0	34.9	35.1	35.1	
Temp (°C)		19.8	19.9	19.7	20.0	19.8	19.7	19.9	

Concentration	36.5 ppt								
	Day	0	1	2	3	4	5	6	7
	Initial								
pH	8.00	7.88	7.93	8.01	7.99	7.97	7.93		
DO (mg/L)	7.4	7.1	6.8	7.6	6.9	7.4	8.1		
Salinity (ppt)	36.5	36.5	36.5	36.5	36.5	36.5	36.5		
Temp (°C)	19.4	20.0	19.9	19.4	20.1	19.6	20.3		
	Final								
pH		7.95	7.84	7.74	7.79	7.88	7.82	7.79	
DO (mg/L)		6.4	5.8	5.6	5.8	6.0	7.0	6.1	
Salinity (ppt)		36.4	36.5	36.6	36.5	36.5	36.6	36.7	
Temp (°C)		19.8	20.1	19.8	20.2	19.8	19.9	19.8	

Animal Source/Date Received: ABS/ 5/1/15

Animal Age at Initiation: 15d

Sample Log-in Numbers: A: N/A C: N/A
 B: N/A

	0	1	2	3	4	5	6	7
Analysts: Initial:	CH	CH	CH	NH	NH	CH	KB	
Final:		ALB	KB	NH	KB	CH	KB	AC
Dilutions made by:	CH	CH	CH	NH	NH	NH	CH	
Sample Used (A, B, C):	Brine	Brine	Brine	Brine	Brine	Brine	Brine	

Comments: * Hach sension 5 used for all water quality readings (salinity)

QC Check: KB 5/21/15

Final Review: AC 6/20/15

Marine Chronic Bioassay

Water Quality Measurements

Client: Poseidon

Sample ID: Nautilus brine (frozen seawater)

Test No: 1505-5091

Test Species: *A. affinis*

Start Date/Time: 5/5/2015 1350

End Date/Time: 5/12/2015 1120

Concentration	37.0 ppt							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.00	7.88	7.93	8.01	7.98	7.97	7.91	
DO (mg/L)	7.4	7.1	6.8	7.6	6.9	7.3	7.9	
Salinity (ppt)	37.0	37.0	37.0	37.0	37.0	37.0	37.0	
Temp (°C)	19.5	20.1	19.9	19.5	20.2	19.6	20.3	
Final:								
pH		7.95	7.84	7.76	7.79	7.90	7.82	7.81
DO (mg/L)		6.7	6.0	5.6	5.7	5.9	6.3	6.4
Salinity (ppt)		37.2	37.2	37.2	37.0	37.0	37.1	37.2
Temp (°C)		19.4	19.9	19.9	19.9	19.8	19.6	19.7

Concentration	38.5 ppt							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	7.98	7.90	7.92	8.00	7.98	7.99	7.91	
DO (mg/L)	7.4	7.3	6.9	7.6	7.0	7.5	7.9	
Salinity (ppt)	38.5	38.5	38.5	38.5	38.5	38.5	38.5	
Temp (°C)	19.5	19.8	19.9	19.1	19.2	19.5	20.3	
Final:								
pH		7.94	7.86	7.79	7.81	7.92	7.85	7.84
DO (mg/L)		6.5	5.8	5.6	5.9	6.0	6.8	6.4
Salinity (ppt)		38.7	38.5	38.7	38.5	38.6	38.0	38.7
Temp (°C)		19.4	19.9	19.7	19.8	19.7	19.7	19.8

Concentration	37.5 ppt							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	7.99	7.88	7.92	8.00	7.98	7.97	7.92	
DO (mg/L)	7.3	7.1	6.8	7.5	6.9	7.4	8.1	
Salinity (ppt)	37.5	37.5	37.5	37.5	37.5	37.5	37.5	
Temp (°C)	19.4	20.1	20.0	19.5	20.2	19.7	20.3	
Final:								
pH		7.95	7.84	7.77	7.79	7.90	7.80	7.80
DO (mg/L)		6.7	5.9	5.6	5.8	6.0	6.3	6.0
Salinity (ppt)		37.7	37.5	37.6	37.5	37.5	38.0	37.7
Temp (°C)		19.2	20.0	19.8	19.7	19.7	19.8	19.9

Concentration								
Day	0	1	2	3	4	5	6	7
Initial:								
pH								
DO (mg/L)								
Salinity (ppt)								
Temp (°C)								
Final:								
pH								
DO (mg/L)								
Salinity (ppt)								
Temp (°C)								

Concentration	38.0 ppt							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.00	7.90	7.93	8.01	7.98	7.99	7.89	
DO (mg/L)	7.4	7.3	6.8	7.6	6.9	7.4	8.1	
Salinity (ppt)	38.0	38.0	38.0	38.0	38.0	38.0	38.0	
Temp (°C)	19.5	19.9	19.9	19.5	20.2	19.6	20.3	
Final:								
pH		7.93	7.85	7.77	7.80	7.93	7.81	7.84
DO (mg/L)		6.7	5.8	5.7	6.0	5.8	6.7	6.4
Salinity (ppt)		38.3	38.0	38.2	38.0	38.0	38.1	38.1
Temp (°C)		19.6	20.1	19.9	20.0	19.8	19.7	19.9

Concentration								
Day	0	1	2	3	4	5	6	7
Initial:								
pH								
DO (mg/L)								
Salinity (ppt)								
Temp (°C)								
Final:								
pH								
DO (mg/L)								
Salinity (ppt)								
Temp (°C)								

Animal Source/Date Received: ABS / 5/11/15

Animal Age at Initiation: 15d

Sample Log-in Numbers: A: N/A C: N/A
B: N/A

	0	1	2	3	4	5	6	7
Analysts: Initial:	CH	CH	CH	NH	NH	CH	KB	
Final:		ALB	KB	NH	KB	CH	KB	AB
Dilutions made by:	CH	CH	CH	NH	NH	NH	CH	
Sample Used (A, B, C):	brine	brine	brine	brine	brine	brine	brine	

Comments: ⊗ Hach Sension 5 used for all water quality readings (salinity)

QC Check: KB 5/21/15

Final Review: AC 6/26/15

Initiation

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: CH

Sample ID: frozen seawater

Test Date: 5/5/2015

Test No: 1505-3091

Test Type: Chronic Topsmelt

Salinity of Seawater 33.3

Salinity of Brine 86.7

Date of Brine used: composited on 5/4/15 using brine from: 4/2/15 - 4/24/15

Test Dilution Volume 1250

Alkalinity of Brine Control: 12.8 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	1250
35.0	96.8	1210.2	0.03	39.8	1250
35.5	95.9	1198.5	0.04	51.5	1250
36.0	94.9	1186.8	0.05	63.2	1250
36.5	94.0	1175.1	0.06	74.9	1250
37.0	93.1	1163.4	0.07	86.6	1250
37.5	92.1	1151.7	0.09	98.3	1250
38.0	91.2	1140.0	0.10	110.0	1250
38.5	90.3	1128.3	0.11	121.7	1250

0.099485019
 0.128745318
 0.158005618
 0.187265918
 0.216526217
 0.245786517
 0.275046816
 0.304307116
 KB KB
 5/2/15

DI Volume				
Brine Control	195.2	0.62	121.7	1250

Total Brine Volume Required (ml): 646.1

QC Check: KB 5/2/15

Final Review: AC 6/26/15

5/6/15 renewal
through 5/11/15

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: Poseidon

Analyst: CH

Sample ID: frozen seawater

Test Date: 5/5/2015

Test No: 15055091

Test Type: Chronic Topsmelt

Salinity of Seawater 33.4

Salinity of Brine 86.7

Date of Brine used: composited on 5/4/15 using brine from: 4/2/15 - 4/24/15

Test Dilution Volume 1000

Alkalinity of Brine Control: 122 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 1000	NA	NA	1000
35.0	97.0	970.0	0.03	30.0	1000
35.5	96.1	960.6	0.04	39.4	1000
36.0	95.1	951.2	0.05	48.8	1000
36.5	94.2	941.8	0.06	58.2	1000
37.0	93.2	932.5	0.07	67.5	1000
37.5	92.3	923.1	0.08	76.9	1000
38.0	91.4	913.7	0.09	86.3	1000
38.5	90.4	904.3	0.11	95.7	1000

~~0.075046904
0.098499062
0.12195122
0.145403377
0.168855535
0.192307692
0.21575985
0.239212008~~

Q10 KB
5/21/15

DI Volume

Brine Control	152.7	0.63	95.7	1000
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Total Brine Volume Required (ml): **502.8**

QC Check: KB 5/21/15

Final Review: AC 6/20/15

APPENDIX C

Glossary of Lab Qualifier Codes

Glossary of Qualifier Codes:

- Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 - Temperatures out of recommended range; no action taken, test terminated same day
- Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L
- Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 - Test initiated with aeration due to an anticipated drop in D.O.
- Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 - Salinity out of recommended range
- Q8 - Spilled test chamber/ Unable to recover test organism(s)
- Q9 - Inadequate sample volume remaining, 50% renewal performed
- Q10 - Inadequate sample volume remaining, no renewal performed
- Q11 - Sample out of holding time; refer to QA section of report
- Q12 - Replicate(s) not initiated; excluded from data analysis
- Q13 - Survival counts not recorded due to poor visibility or heavy debris
- Q14 - D.O. percent saturation was checked and was $\leq 110\%$
- Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.
- Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.
- Q18 - Incorrect Entry
- Q19 - Illegible Entry
- Q20 - Miscalculation
- Q21 - Other (provide reason in comments section)
- Q22 - Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.
- Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

APPENDIX D

**Reference Toxicant
Test Data and Statistical Summaries**

Red Abalone

CETIS Summary Report

Report Date: 16 Dec-14 18:07 (p 1 of 1)
 Test Code: 141210hrrt | 18-3651-9027

Red Abalone Larval Development Test							Nautilus Environmental (CA)				
Batch ID:	00-7981-3930	Test Type:	Development			Analyst:					
Start Date:	10 Dec-14 15:30	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Natural Seawater				
Ending Date:	12 Dec-14 17:00	Species:	Haliotis rufescens			Brine:	Not Applicable				
Duration:	50h	Source:	American Abalone			Age:					
Sample ID:	19-1318-9698	Code:	141210hrrt			Client:	Internal				
Sample Date:	10 Dec-14	Material:	Zinc sulfate			Project:					
Receive Date:	10 Dec-14	Source:	Reference Toxicant								
Sample Age:	16h	Station:	Zinc sulfate								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
20-3619-3450	Development Rate	18	32	24	4.79%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
05-5260-7606	Development Rate	EC50	40.8	40.06	41.55		Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits	Overlap	Decision				
05-5260-7606	Development Rate	Control Resp		0.946	0.8 - NL	Yes	Passes Acceptability Criteria				
20-3619-3450	Development Rate	Control Resp		0.946	0.8 - NL	Yes	Passes Acceptability Criteria				
20-3619-3450	Development Rate	NOEL		18	NL - 56	No	Passes Acceptability Criteria				
20-3619-3450	Development Rate	PMSD		0.04793	NL - 0.2	No	Passes Acceptability Criteria				
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.946	0.9366	0.9554	0.92	0.97	0.01122	0.0251	2.65%	0.0%
10		5	0.964	0.9583	0.9697	0.95	0.99	0.006782	0.01517	1.57%	-1.9%
18		5	0.952	0.9459	0.9581	0.94	0.97	0.007348	0.01643	1.73%	-0.63%
32		5	0.86	0.8423	0.8777	0.79	0.92	0.02121	0.04743	5.52%	9.09%
56		5	0.036	0.02492	0.04708	0	0.08	0.01327	0.02966	82.4%	96.19%
100		5	0	0	0	0	0	0	0		100.0%
Development Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.97	0.92	0.92	0.95	0.97					
10		0.96	0.96	0.95	0.99	0.96					
18		0.97	0.94	0.97	0.94	0.94					
32		0.85	0.86	0.92	0.88	0.79					
56		0	0.02	0.04	0.08	0.04					
100		0	0	0	0	0					

CETIS Analytical Report

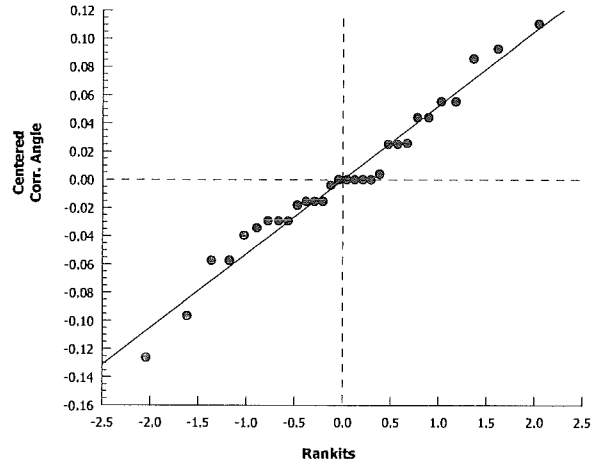
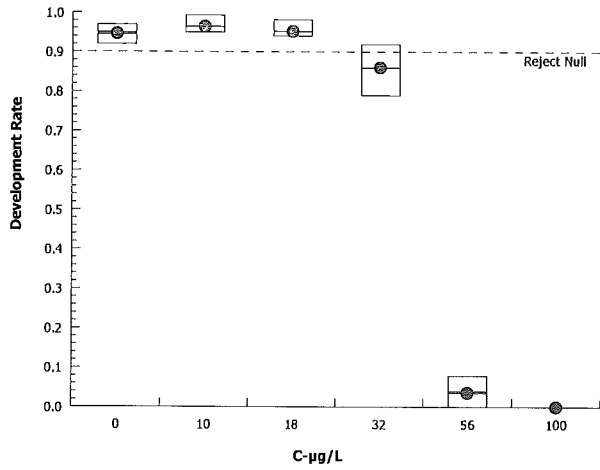
Report Date: 16 Dec-14 18:06 (p 1 of 2)
 Test Code: 141210hrrt | 18-3651-9027

Red Abalone Larval Development Test								Nautilus Environmental (CA)			
Analysis ID: 20-3619-3450		Endpoint: Development Rate			CETIS Version: CETISv1.8.4						
Analyzed: 16 Dec-14 18:06		Analysis: Parametric-Control vs Treatments			Official Results: Yes						
Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU	PMSD		
Angular (Corrected)	NA	C > T	NA	NA	18	32	24		4.79%		
Dunnett Multiple Comparison Test											
Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)			
Lab Control	10	-1.099	2.305	0.091	8	0.9814	CDF	Non-Significant Effect			
	18	-0.2861	2.305	0.091	8	0.8795	CDF	Non-Significant Effect			
	32*	3.792	2.305	0.091	8	0.0020	CDF	Significant Effect			
	56*	29.44	2.305	0.091	8	<0.0001	CDF	Significant Effect			
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	5.321269	1.330317	4	339.7	<0.0001	Significant Effect					
Error	0.07833021	0.00391651	20								
Total	5.399599		24								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Bartlett Equality of Variance	2.625	13.28	0.6224	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.9785	0.8877	0.8533	Normal Distribution						
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.946	0.9148	0.9772	0.95	0.92	0.97	0.01122	2.65%	0.0%
10		5	0.964	0.9452	0.9828	0.96	0.95	0.99	0.006783	1.57%	-1.9%
18		5	0.952	0.9316	0.9724	0.94	0.94	0.97	0.007348	1.73%	-0.63%
32		5	0.86	0.8011	0.9189	0.86	0.79	0.92	0.02121	5.52%	9.09%
56		5	0.036	0	0.07283	0.04	0	0.08	0.01327	82.4%	96.19%
100		5	0	0	0	0	0	0	0		100.0%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.341	1.271	1.411	1.345	1.284	1.397	0.02521	4.2%	0.0%
10		5	1.385	1.324	1.446	1.369	1.345	1.471	0.02195	3.54%	-3.24%
18		5	1.353	1.303	1.403	1.323	1.323	1.397	0.01798	2.97%	-0.84%
32		5	1.191	1.106	1.277	1.187	1.095	1.284	0.03077	5.78%	11.19%
56		5	0.1763	0.0677	0.2849	0.2014	0.05002	0.2868	0.03911	49.61%	86.86%
100		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.27%

Red Abalone Larval Development Test Nautilus Environmental (CA)

Analysis ID: 20-3619-3450 Endpoint: Development Rate CETIS Version: CETISv1.8.4
Analyzed: 16 Dec-14 18:06 Analysis: Parametric-Control vs Treatments Official Results: Yes

Graphics



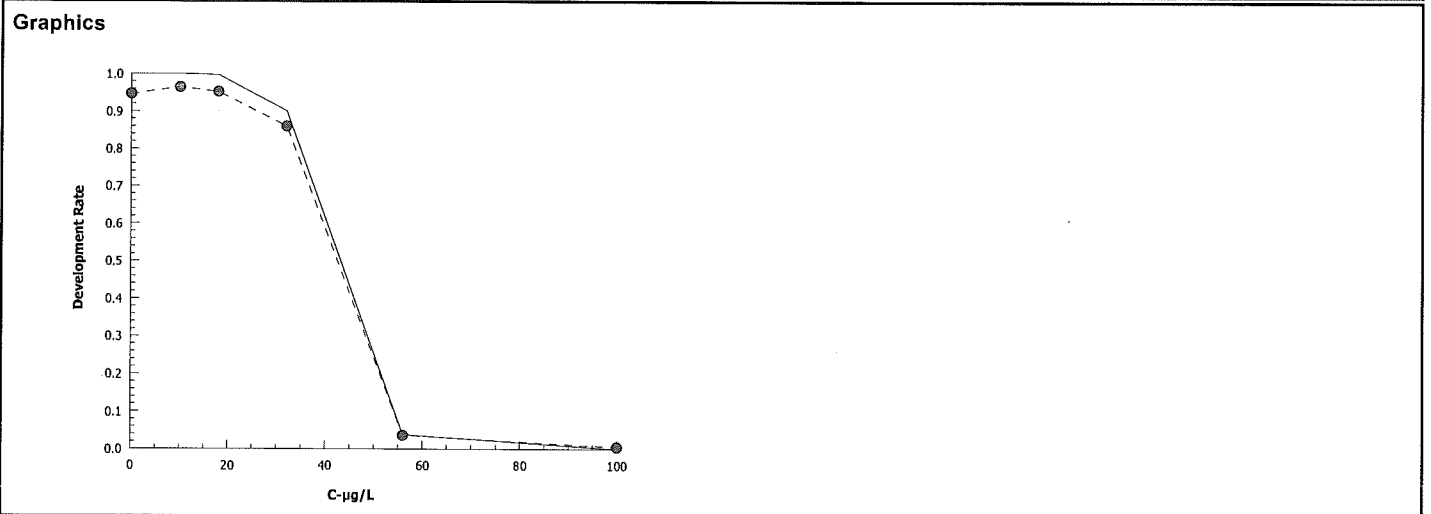
CETIS Analytical Report

Report Date: 16 Dec-14 18:07 (p 1 of 1)
 Test Code: 141210hrrt | 18-3651-9027

Red Abalone Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 05-5260-7606	Endpoint: Development Rate	CETIS Version: CETISv1.8.4			
Analyzed: 16 Dec-14 18:06	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes			

Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.054	0.00%	1.611	0.003965	40.8	40.06	41.55

Development Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.946	0.92	0.97	0.01122	0.0251	2.65%	0.0%	473	500
10		5	0.964	0.95	0.99	0.006783	0.01517	1.57%	-1.9%	482	500
18		5	0.952	0.94	0.97	0.007348	0.01643	1.73%	-0.63%	476	500
32		5	0.86	0.79	0.92	0.02121	0.04743	5.52%	9.09%	430	500
56		5	0.036	0	0.08	0.01327	0.02966	82.4%	96.19%	18	500
100		5	0	0	0	0	0		100.0%	0	500



Red Abalone Larval Development Test

Nautilus Environmental (CA)

Test Type: Development

Organism: Haliotis rufescens (Red Abalone)

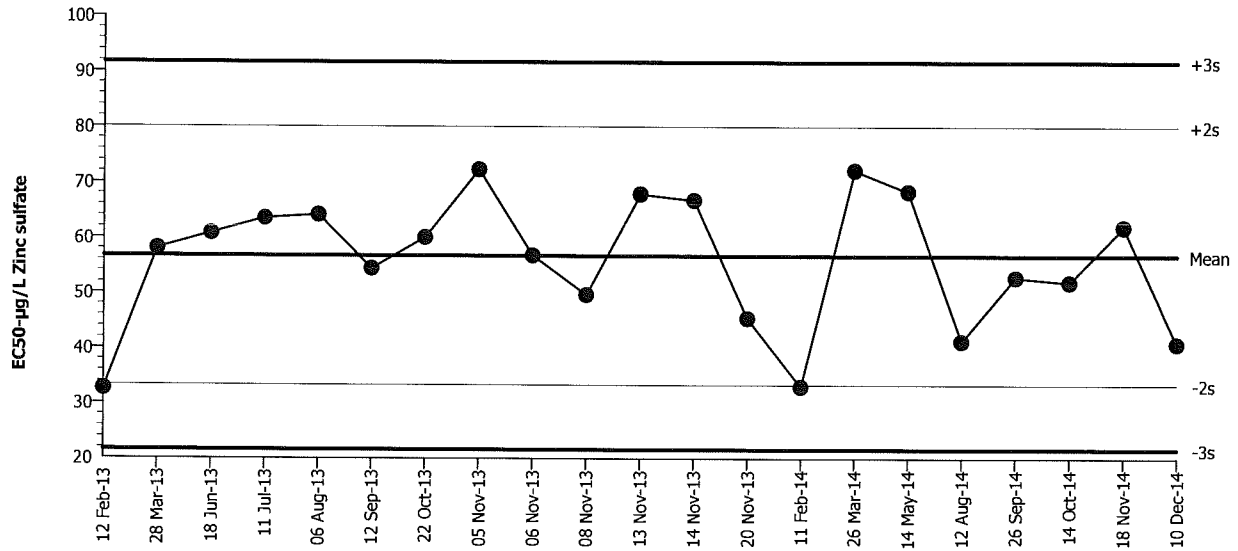
Material: Zinc sulfate

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Development Rate

Source: Reference Toxicant-REF

Red Abalone Larval Development Test



Mean: 56.66 Count: 20 -2s Warning Limit: 33.28 -3s Action Limit: 21.59
 Sigma: 11.69 CV: 20.60% +2s Warning Limit: 80.04 +3s Action Limit: 91.73

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Feb	12	32.63	-24.03	-2.056	(-)		12-6287-3770	13-5482-5100
2		Mar	28	58.01	1.351	0.1156			02-1898-0832	19-4308-5407
3		Jun	18	60.76	4.101	0.3509			15-0077-4900	00-2560-4372
4		Jul	11	63.48	6.821	0.5835			20-1873-1666	04-6373-4064
5		Aug	6	64.09	7.426	0.6352			07-0122-1486	08-7879-6283
6		Sep	12	54.37	-2.289	-0.1958			01-8875-4392	00-0984-0680
7		Oct	22	59.96	3.297	0.282			00-1950-7526	03-4814-7235
8		Nov	5	72.27	15.61	1.336			13-0598-2106	06-6008-6070
9			6	56.71	0.04992	0.00427			17-8546-9636	17-3071-8592
10			8	49.64	-7.018	-0.6004			20-4825-5447	15-3343-8191
11			13	67.88	11.22	0.9595			01-9285-3290	05-2114-7000
12			14	66.73	10.07	0.8613			12-4955-9047	05-7865-8140
13			20	45.42	-11.24	-0.9611			15-8538-2252	14-5629-7331
14	2014	Feb	11	33.01	-23.65	-2.023	(-)		00-8191-4476	07-3868-3337
15		Mar	26	72.1	15.44	1.321			11-0783-9458	08-4579-6000
16		May	14	68.33	11.67	0.9985			14-0092-0578	07-8756-4120
17		Aug	12	41.25	-15.41	-1.318			09-7316-9900	19-6875-2864
18		Sep	26	52.78	-3.877	-0.3316			12-0077-1970	07-6392-1596
19		Oct	14	51.91	-4.753	-0.4066			01-1692-6353	05-8596-4968
20		Nov	18	61.92	5.259	0.4499			12-7477-5365	16-0305-1770
21		Dec	10	40.8	-15.86	-1.357			18-3651-9027	05-5260-7606

CETIS Test Data Worksheet

Report Date: 09 Dec-14 16:07 (p 1 of 1)
 Test Code: 18-3651-9027/141210hrrt

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Start Date: 10 Dec-14 1530 Species: Haliotis rufescens Sample Code: 141210hrrt
 End Date: 12 Dec-14 1700 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 10 Dec-14 — Material: Zinc sulfate Sample Station: Zinc sulfate

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	0	
			2		0	
			3		0	
			4		94	
			5		95	
			6		96	
			7		94	
			8		92	
			9		0	
			10		0	
			11		92	
			12		0	
			13		2	
			14		94	
			15		4	
			16		86	
			17		88 4 914 12/16/14	
			18		6	
			19		88	
			20		97	
			21		96	
			22		97	
			23		85	
			24		92	
			25		97	
			26		96	
			27		79	
			28		95	
			29		97	
			30		99	

CETIS Test Data Worksheet

Report Date: 09 Dec-14 16:07 (p 1 of 1)
 Test Code: 18-3651-9027/141210hrrt

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Start Date: 10 Dec-14 1530 Species: Haliotis rufescens Sample Code: 141210hrrt
 End Date: 12 Dec-14 1700 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 10 Dec-14 Material: Zinc sulfate Sample Station: Zinc sulfate

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	25	100	94	AC 12/12/14
0	LC	2	24			
0	LC	3	8			
0	LC	4	28			
0	LC	5	29			
10		1	26	100	95	AC 12/12/14
10		2	21			
10		3	5			
10		4	30			
10		5	6			
18		1	22	100	99	AC 12/12/14
18		2	7			
18		3	20			
18		4	14			
18		5	4			
32		1	23	94	79	AC 12/12/14
32		2	16			
32		3	11			
32		4	19			
32		5	27			
56		1	1	100	0	AC 12/12/14
56		2	13			
56		3	15			
56		4	18			
56		5	17			
100		1	10	100	0	AC 12/12/14
100		2	3			
100		3	2			
100		4	12			
100		5	9			

QC-VCR

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: ZnSO₄
 Test ID: 141210hrrt

Test Species: Haliotis rufescens
 Start Date/Time: 12/10/2014 1530
 End Date/Time: 12/12/2014 1700

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	Ⓐ 0	24	48	0	24	48	0	24	48
Lab Control	32.9	33.0	32.9	15.5	15.3	14.9	8.1	8.0	8.0	8.05	7.98	7.97
10	32.8	33.0	33.0	15.5	15.1	14.8	8.0	7.9	7.9	8.04	7.98	7.98
18	32.8	32.7	33.0	15.5	14.9	14.9	8.0	8.0	7.9	8.04	7.99	7.98
32	32.8	32.9	33.0	15.5	15.0	14.8	8.0	8.0	7.9	8.04	7.99	7.98
56	32.6	32.7	32.9	15.5	14.9	14.8	8.0	8.0	7.9	8.04	7.99	7.98
100	32.5	32.7	32.7	15.5	14.9	14.9	8.0	8.0	7.9	8.04	7.99	7.97

Technician Initials: _____
 WQ Readings:

0	24	48
VCR/AC	NH	NH

 Dilutions made by:

0	24	48
VCR/AC	-	-

Dilution calcs. (final volume 500 mL):

Conc.	10	18	32	56	100
Vol. Zn stock (mL):	0.53	0.95	1.7	3.0	5.3

Zn Stock Concentration (µg/L): 9,480

Comments: 0 hrs: Ⓐ Temperature taken from a sumgate vial on test trial.
 24 hrs: _____
 48 hrs: _____

QC Check: 12/16/14

Final Review: VB/12/15

Marine Chronic Bioassay

Abalone Embryo-Larval Development

Client: Internal

Test Species: Haliotis rufescens

Sample ID: ZnSO4 Reference Forwent

Start Date/Time: 12/10/2014 15:30

Test No.: 141210 hart

End Date/Time: 12/12/2014 17:00

Animal Source/Date Received: American Abalone/12/09/14

Number of abalone and condition upon receipt/holding:

Males: 4 / Good condition

Females: 4 / Good condition

	Males:	Females:
Tris & peroxide addition time	1100	1030
Spawn time	1330	1400
Number of spawners	4	1
Condition of spawn (light, moderate, heavy)	Heavy	moderate
Fertilization time	1435	

Embryo counts (per 0.5 ml)	
1	121
2	131
3	145
Mean	132.3

Time of test Initiation: 1530

48 hr. QC 97%^{1/2}

Technician Initials: [Signature]

Comments: _____

QC Check: [Signature] 12/16/14

Final Review: [Signature] 1/14/15

CETIS Summary Report

Report Date: 29 May-15 09:59 (p 1 of 1)
 Test Code: 150520hrdv | 08-9621-2840

Red Abalone Larval Development Test							Nautilus Environmental (CA)				
Batch ID:	08-7453-2163	Test Type:	Development			Analyst:					
Start Date:	20 May-15 14:15	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Natural Seawater				
Ending Date:	22 May-15 15:55	Species:	Haliotis rufescens			Brine:	Not Applicable				
Duration:	50h	Source:	American Abalone			Age:					
Sample ID:	19-7969-0228	Code:	150520hrdv			Client:	Internal				
Sample Date:	20 May-15	Material:	Zinc sulfate			Project:					
Receive Date:	20 May-15	Source:	Reference Toxicant								
Sample Age:	14h	Station:	Zinc sulfate								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
10-6271-9277	Development Rate	32	56	42.33	2.58%		Dunnnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
00-2924-1270	Development Rate	EC50	55.54	54.11	57.01		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
00-2924-1270	Development Rate	Control Resp	0.988	0.8 - NL	Yes	Passes Acceptability Criteria					
10-6271-9277	Development Rate	Control Resp	0.988	0.8 - NL	Yes	Passes Acceptability Criteria					
10-6271-9277	Development Rate	NOEL	32	NL - 56	No	Passes Acceptability Criteria					
10-6271-9277	Development Rate	PMSD	0.02582	NL - 0.2	No	Passes Acceptability Criteria					
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.988	0.9744	1	0.97	1	0.004899	0.01095	1.11%	0.0%
10		5	0.986	0.9672	1	0.96	1	0.006782	0.01517	1.54%	0.2%
18		5	0.982	0.9716	0.9924	0.97	0.99	0.003742	0.008367	0.85%	0.61%
32		5	0.984	0.9673	1	0.97	1	0.006	0.01342	1.36%	0.4%
56		5	0.478	0.354	0.602	0.36	0.57	0.04465	0.09985	20.89%	51.62%
100		5	0	0	0	0	0	0	0		100.0%
Development Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	1	0.99	0.99	0.97	0.99					
10		0.99	0.99	1	0.99	0.96					
18		0.97	0.99	0.98	0.98	0.99					
32		0.97	0.99	1	0.99	0.97					
56		0.38	0.57	0.55	0.53	0.36					
100		0	0	0	0	0					

CETIS Analytical Report

Report Date: 29 May-15 09:58 (p 1 of 1)
 Test Code: 150520hrdv | 08-9621-2840

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Analysis ID: 10-6271-9277 Endpoint: Development Rate CETIS Version: CETISv1.8.7
 Analyzed: 29 May-15 9:58 Analysis: Parametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	2.58%	32	56	42.33	

Dunnett Multiple Comparison Test

Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	10	0.1397	2.305	0.09	8	0.7516	CDF	Non-Significant Effect
	18	0.6843	2.305	0.09	8	0.5207	CDF	Non-Significant Effect
	32	0.3786	2.305	0.09	8	0.6564	CDF	Non-Significant Effect
	56*	18	2.305	0.09	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.913564	0.478391	4	125.5	<0.0001	Significant Effect
Error	0.07624391	0.003812195	20			
Total	1.989808		24			

Distributional Tests

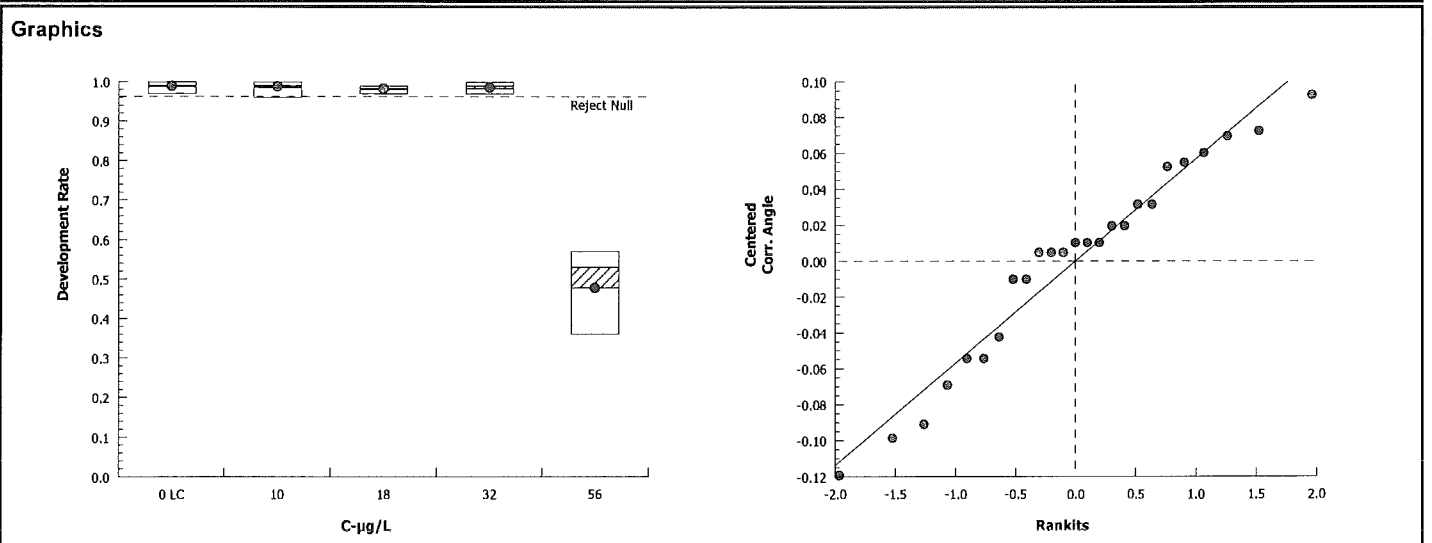
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.534	13.28	0.2367	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.954	0.8877	0.3081	Normal Distribution

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.988	0.9744	1	0.99	0.97	1	0.004899	1.11%	0.0%
10		5	0.986	0.9672	1	0.99	0.96	1	0.006783	1.54%	0.2%
18		5	0.982	0.9716	0.9924	0.98	0.97	0.99	0.003742	0.85%	0.61%
32		5	0.984	0.9673	1	0.99	0.97	1	0.006	1.36%	0.4%
56		5	0.478	0.354	0.602	0.53	0.36	0.57	0.04465	20.89%	51.62%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.466	1.411	1.521	1.471	1.397	1.521	0.01983	3.03%	0.0%
10		5	1.46	1.392	1.529	1.471	1.369	1.521	0.02473	3.79%	0.37%
18		5	1.439	1.4	1.478	1.429	1.397	1.471	0.01413	2.2%	1.82%
32		5	1.451	1.384	1.518	1.471	1.397	1.521	0.02401	3.7%	1.01%
56		5	0.7628	0.6377	0.888	0.8154	0.6435	0.8556	0.04507	13.21%	47.96%



CETIS Analytical Report

Report Date: 29 May-15 09:59 (p 1 of 1)

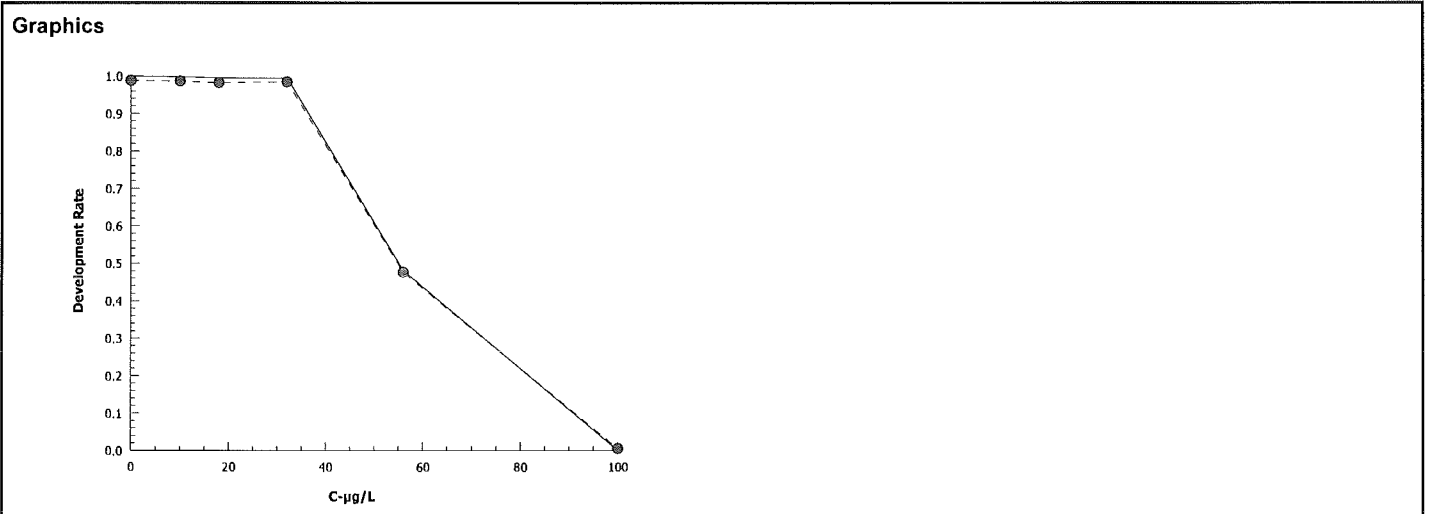
Test Code: 150520hrdv | 08-9621-2840

Red Abalone Larval Development Test **Nautilus Environmental (CA)**

Analysis ID: 00-2924-1270 Endpoint: Development Rate CETIS Version: CETISv1.8.7
 Analyzed: 29 May-15 9:58 Analysis: Trimmed Spearman-Kärber Official Results: Yes

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.012	0.20%	1.745	0.005671	55.54	54.11	57.01

Development Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.988	0.97	1	0.004899	0.01095	1.11%	0.0%	494	500
10		5	0.986	0.96	1	0.006783	0.01517	1.54%	0.2%	493	500
18		5	0.982	0.97	0.99	0.003742	0.008367	0.85%	0.61%	491	500
32		5	0.984	0.97	1	0.006	0.01342	1.36%	0.4%	492	500
56		5	0.478	0.36	0.57	0.04465	0.09985	20.89%	51.62%	238	500
100		5	0	0	0	0	0		100.0%	0	500



Red Abalone Larval Development Test

Nautilus Environmental (CA)

Test Type: Development

Organism: Haliotis rufescens (Red Abalone)

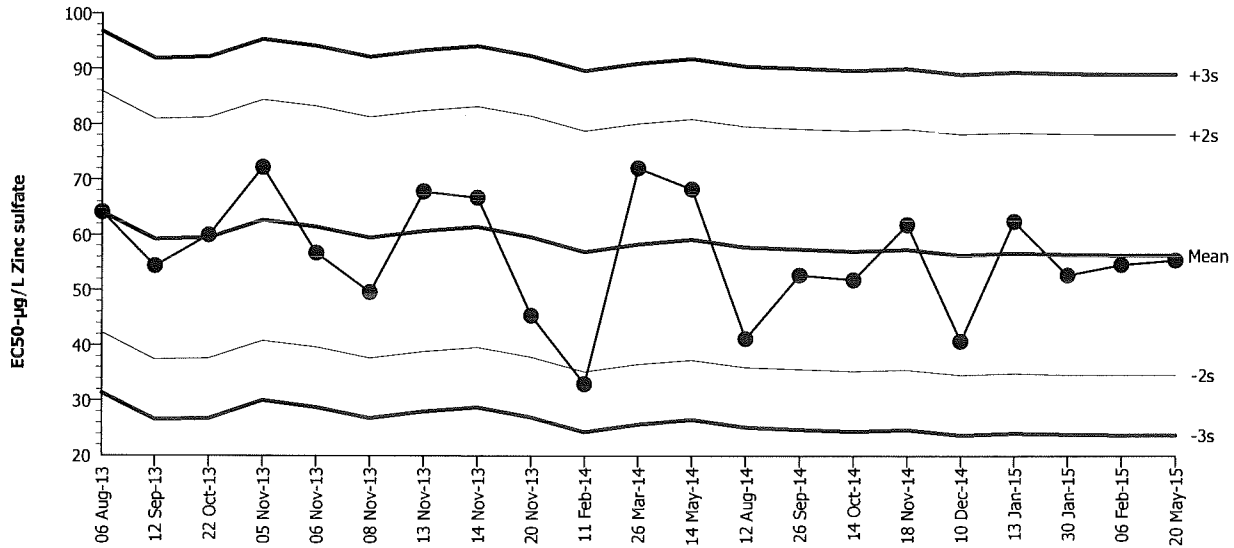
Material: Zinc sulfate

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Development Rate

Source: Reference Toxicant-REF

Red Abalone Larval Development Test



Mean: 56.46 Count: 20 -2s Warning Limit: 34.66 -3s Action Limit: 23.76
 Sigma: 10.9 CV: 19.30% +2s Warning Limit: 78.26 +3s Action Limit: 89.16

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2013	Aug	6	15:35	64.09	7.626	0.6996			07-0122-1486	08-7879-6283
2		Sep	12	14:40	54.37	-2.089	-0.1917			01-8875-4392	00-0984-0680
3		Oct	22	14:50	59.96	3.497	0.3208			00-1950-7526	03-4814-7235
4		Nov	5	14:25	72.27	15.81	1.451			13-0598-2106	06-6008-6070
5			6	15:30	56.71	0.2499	0.02293			17-8546-9636	17-3071-8592
6			8	0:00	49.64	-6.818	-0.6255			20-4825-5447	15-3343-8191
7			13	16:00	67.88	11.42	1.047			01-9285-3290	05-2114-7000
8			14	14:50	66.73	10.27	0.9421			12-4955-9047	05-7865-8140
9			20	15:15	45.42	-11.04	-1.012			15-8538-2252	14-5629-7331
10	2014	Feb	11	15:00	33.01	-23.45	-2.151	(-)		00-8191-4476	07-3868-3337
11		Mar	26	15:25	72.1	15.64	1.435			11-0783-9458	08-4579-6000
12		May	14	15:35	68.33	11.87	1.089			14-0092-0578	07-8756-4120
13		Aug	12	15:35	41.25	-15.21	-1.396			09-7316-9900	19-6875-2864
14		Sep	26	16:10	52.78	-3.677	-0.3373			12-0077-1970	07-6392-1596
15		Oct	14	15:30	51.91	-4.553	-0.4177			01-1692-6353	05-8596-4968
16		Nov	18	15:05	61.92	5.459	0.5008			12-7477-5365	16-0305-1770
17		Dec	10	15:30	40.8	-15.66	-1.436			18-3651-9027	05-5260-7606
18	2015	Jan	13	14:48	62.52	6.058	0.5558			11-7205-2664	10-2598-5960
19			30	15:22	52.83	-3.632	-0.3332			06-2409-9903	03-0729-5027
20		Feb	6	13:33	54.69	-1.771	-0.1625			19-4508-4987	07-8543-5535
21		May	20	14:15	55.54	-0.923	-0.08468			08-9621-2840	00-2924-1270

CETIS Test Data Worksheet

Report Date: 19 May-15 15:21 (p 1 of 1)
 Test Code: 08-9621-2840/150520hrdv

Red Abalone Larval Development Test			Nautilus Environmental (CA)		
Start Date: 20 May-15	Species: Haliotis rufescens	Sample Code: 150520hrdv			
End Date: 22 May-15	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Reference Toxicant			
Sample Date: 20 May-15	Material: Zinc sulfate	Sample Station: Zinc sulfate			

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	96	
			2	100	53	61 QC=AC
			3	100	0	
			4	100	99	
			5	100	99	
			6	100	0	0 QC=AC
			7	100	97	
			8	100	99	
			9	100	97	
			10	100	99	
			11	100	99	
			12	100	99	
			13	100	38	34 QC=AC
			14	100	57	
			15	100	99	
			16	100	99	
			17	100	36	
			18	100	97	
			19	100	99	
			20	100	0	
			21	100	100	
			22	100	100	
			23	100	97	
			24	100	98	
			25	100	98	
			26	100	100	99 QC=AC
			27	100	0	
			28	100	0	
			29	100	55	
			30	100	99	

CETIS Test Data Worksheet

Report Date: 19 May-15 15:20 (p 1 of 1)
 Test Code: 08-9621-2840/150520hrdv

Red Abalone Larval Development Test				Nautilus Environmental (CA)		
Start Date: 20 May-15	Species: Haliotis rufescens	Sample Code: 150520hrdv				
End Date: 22 May-15	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Reference Toxicant				
Sample Date: 20 May-15	Material: Zinc sulfate	Sample Station: Zinc sulfate				

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	21	100	98	
0	LC	2	11			
0	LC	3	30			
0	LC	4	23			
0	LC	5	4			
10		1	10			
10		2	8			
10		3	26			
10		4	16			
10		5	1			
18		1	18			
18		2	12			
18		3	24			
18		4	25			
18		5	15			
32		1	9			
32		2	5			
32		3	22			
32		4	19			
32		5	7			
56		1	13			
56		2	14			
56		3	29			
56		4	2			
56		5	17	100	36	
100		1	27			
100		2	20			
100		3	6			
100		4	3	100	0	
100		5	28	100 no out smus		

QC: AB

100
no out
smus

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: ZnSO₄
 Test ID: 150520hrdv

Test Species: Haliotis rufescens
 Start Date/Time: 5/20/2015 1415
 End Date/Time: 5/22/2015 1555

Concentration (µg/L)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	33.3	33.3	33.6	15.7	15.4	15.5	8.4	8.3	8.3	8.06	8.06	8.06
10	33.3	33.3	33.8	15.7	15.1	15.0	8.3	8.3	8.3	8.05	8.07	8.07
18	33.3	33.4	33.8	15.7	15.0	15.1	8.3	8.3	8.3	8.06	8.08	8.08
32	33.3	33.3	33.8	15.7	15.0	15.0	8.3	8.3	8.4	8.06	8.08	8.08
56	33.2	33.3	33.7	15.7	15.1	15.1	8.3	8.3	8.4	8.06	8.07	8.08
100	33.1	33.2	33.6	15.7	15.2	15.0	8.2	8.3	8.4	8.07	8.08	8.08

Technician Initials: _____
 WQ Readings:

	0	24	48
AC	AC	AC	AD

 Dilutions made by:

AC		
----	--	--

Dilution calcs. (final volume 500 mL):

Conc.	10	18	32	56	100
Vol. Zn stock (mL):	0.50	0.90	1.6	2.8	5.0

Zn Stock Concentration (µg/L): 10,000

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

QC Check: AC 5/29/15 Final Review: KFP 6/11/15

Marine Chronic Bioassay

Abalone Embryo-Larval Development

Client: Internal

Test Species: Haliotis rufescens

Sample ID: Zn SO₄

Start Date/Time: 5/20/2015 1415

Test No.: 150520 hrdlv

End Date/Time: 5/22/2015 1555

Animal Source/Date Received: American Abalone/ 5-14-15

Number of abalone and condition upon receipt/holding:

Males: 4, spawned 5/19/15 in holding

Females: 4

	Males:	Females:
Tris & peroxide addition time	1055	1030
Spawn time	1312	1314
Number of spawners	3	2-4
Condition of spawn (light, moderate, heavy)	moderate	heavy
Fertilization time	1325	

Embryo counts (per 0.5 ml)	
1	124
2	160
3	292
Mean	192

Time of test Initiation: 1415

48 hr. QC 97%

Technician Initials: AC

Comments: _____

QC Check: AC 5/21/15

Final Review: KFP 6/11/15

Urchin Development

CETIS Summary Report

Report Date: 17 Nov-14 14:05 (p 1 of 1)
 Test Code: 141030spdv | 16-2563-7748

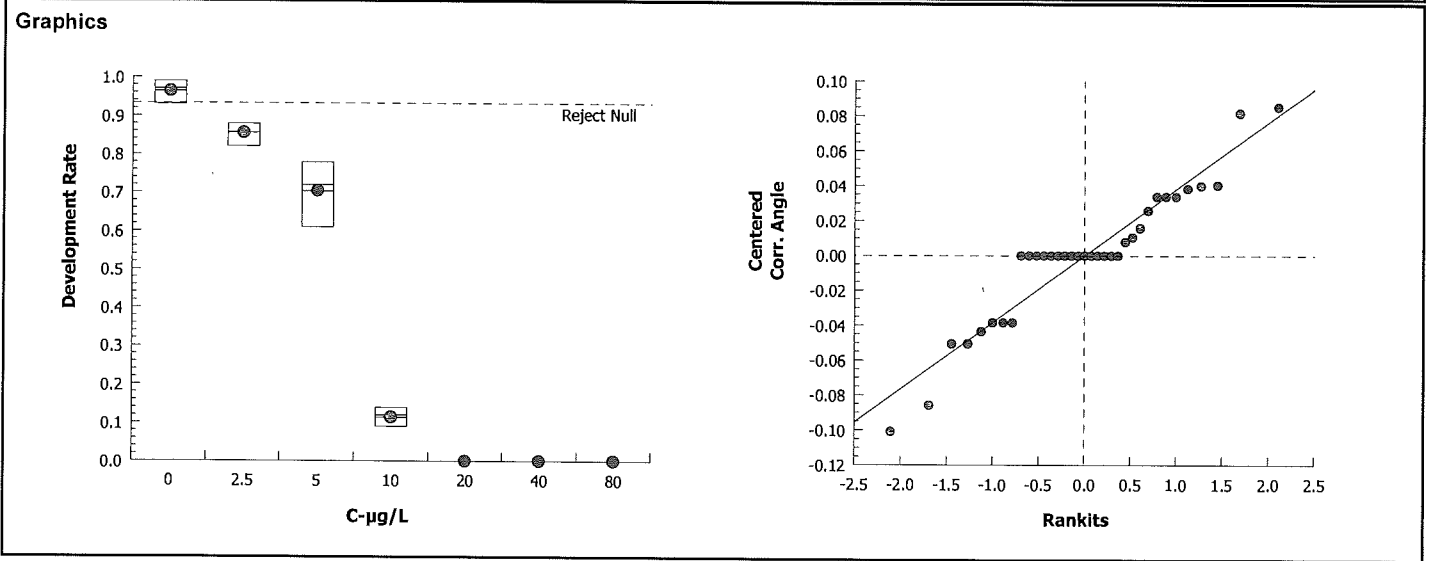
Echinoid Embryo-Larval Development Test							Nautilus Environmental (CA)				
Batch ID:	14-1104-4410	Test Type:	Development	Analyst:							
Start Date:	30 Oct-14 15:00	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	02 Nov-14 17:00	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	74h	Source:	Pt. Loma	Age:							
Sample ID:	20-7882-8985	Code:	141030spdv	Client:	Internal						
Sample Date:	30 Oct-14	Material:	Copper chloride	Project:							
Receive Date:	30 Oct-14	Source:	Reference Toxicant								
Sample Age:	15h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
17-9042-1561	Development Rate	<2.5	2.5	NA	3.24%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
00-5600-3113	Development Rate	EC50	6.16	5.917	6.413		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
00-5600-3113	Development Rate	Control Resp	0.964	0.8 - NL	Yes	Passes Acceptability Criteria					
17-9042-1561	Development Rate	Control Resp	0.964	0.8 - NL	Yes	Passes Acceptability Criteria					
17-9042-1561	Development Rate	PMSD	0.03243	NL - 0.25	No	Passes Acceptability Criteria					
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.964	0.955	0.973	0.93	0.99	0.01077	0.02408	2.5%	0.0%
2.5		5	0.856	0.8437	0.8683	0.82	0.88	0.0147	0.03286	3.84%	11.2%
5		5	0.704	0.6794	0.7286	0.61	0.78	0.02943	0.0658	9.35%	26.97%
10		5	0.114	0.1054	0.1226	0.09	0.14	0.0103	0.02302	20.19%	88.17%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%
Development Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.93	0.98	0.99	0.97	0.95					
2.5		0.88	0.88	0.82	0.82	0.88					
5		0.78	0.61	0.72	0.67	0.74					
10		0.09	0.12	0.13	0.09	0.14					
20		0	0	0	0	0					
40		0	0	0	0	0					
80		0	0	0	0	0					

CETIS Analytical Report

Report Date: 17 Nov-14 11:26 (p 1 of 2)
 Test Code: 141030spdv | 16-2563-7748

Echinoid Embryo-Larval Development Test										Nautilus Environmental (CA)	
Analysis ID: 17-9042-1561		Endpoint: Development Rate			CETIS Version: CETISv1.8.4						
Analyzed: 10 Nov-14 10:51		Analysis: Parametric-Control vs Treatments			Official Results: Yes						
Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU	PMSD		
Angular (Corrected)	NA	C > T	NA	NA	<2.5	2.5	NA		3.24%		
Dunnnett Multiple Comparison Test											
Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)			
Lab Control	2.5*	5.692	2.227	0.080	8	<0.0001	CDF	Significant Effect			
	5*	10.84	2.227	0.080	8	<0.0001	CDF	Significant Effect			
	10*	28.95	2.227	0.080	8	<0.0001	CDF	Significant Effect			
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	3.07234	1.024113	3	313.9	<0.0001	Significant Effect					
Error	0.05220649	0.003262905	16								
Total	3.124547		19								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Bartlett Equality of Variance	2.003	11.34	0.5718	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.9359	0.866	0.2004	Normal Distribution						
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.964	0.9341	0.9939	0.97	0.93	0.99	0.01077	2.5%	0.0%
2.5		5	0.856	0.8152	0.8968	0.88	0.82	0.88	0.0147	3.84%	11.2%
5		5	0.704	0.6223	0.7857	0.72	0.61	0.78	0.02943	9.35%	26.97%
10		5	0.114	0.08541	0.1426	0.12	0.09	0.14	0.0103	20.19%	88.17%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.389	1.307	1.471	1.397	1.303	1.471	0.02968	4.78%	0.0%
2.5		5	1.183	1.126	1.241	1.217	1.133	1.217	0.02068	3.91%	14.8%
5		5	0.9973	0.9081	1.087	1.013	0.8963	1.083	0.03216	7.21%	28.19%
10		5	0.3431	0.2976	0.3885	0.3537	0.3047	0.3835	0.01637	10.67%	75.3%
20		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.4%
40		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.4%
80		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.4%

Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)
Analysis ID: 17-9042-1561	Endpoint: Development Rate	CETIS Version: CETISv1.8.4
Analyzed: 10 Nov-14 10:51	Analysis: Parametric-Control vs Treatments	Official Results: Yes



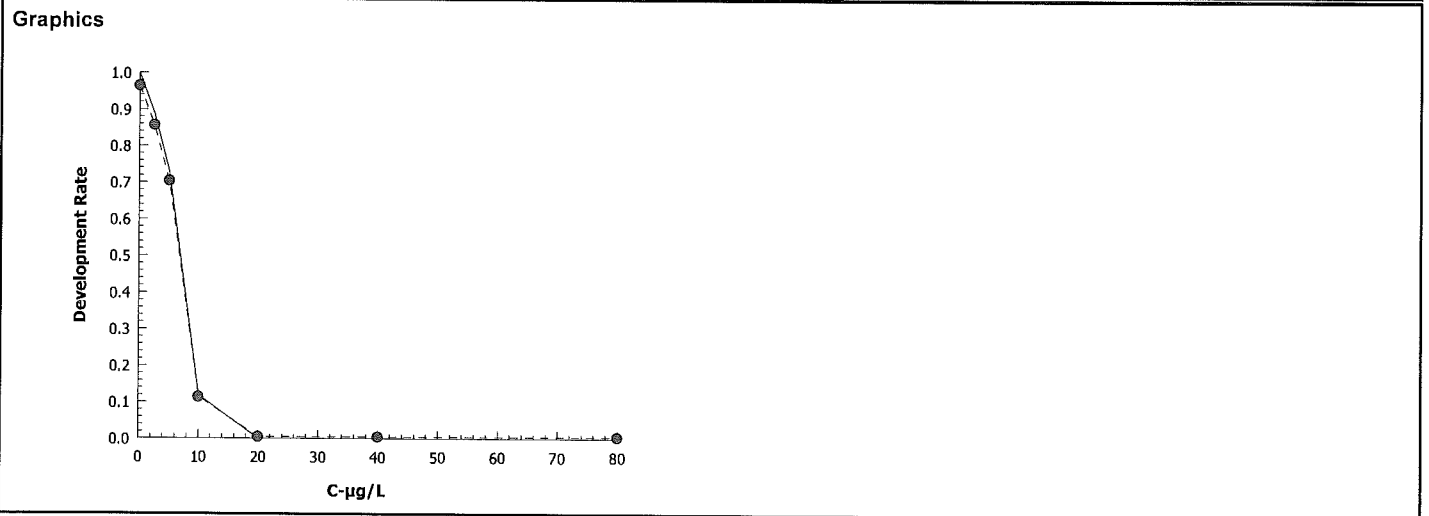
CETIS Analytical Report

Report Date: 17 Nov-14 11:26 (p 1 of 1)
 Test Code: 141030spd | 16-2563-7748

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 00-5600-3113	Endpoint: Development Rate	CETIS Version: CETISv1.8.4			
Analyzed: 10 Nov-14 10:52	Analysis: Trimmed Spearman-Kärber	Official Results: Yes			

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.036	11.20%	0.7896	0.008736	6.16	5.917	6.413

Development Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.964	0.93	0.99	0.01077	0.02408	2.5%	0.0%	482	500
2.5		5	0.856	0.82	0.88	0.0147	0.03286	3.84%	11.2%	428	500
5		5	0.704	0.61	0.78	0.02943	0.0658	9.35%	26.97%	352	500
10		5	0.114	0.09	0.14	0.0103	0.02302	20.19%	88.17%	57	500
20		5	0	0	0	0	0		100.0%	0	500
40		5	0	0	0	0	0		100.0%	0	500
80		5	0	0	0	0	0		100.0%	0	500



Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Test Type: Development

Organism: Strongylocentrotus purpuratus (Purpl

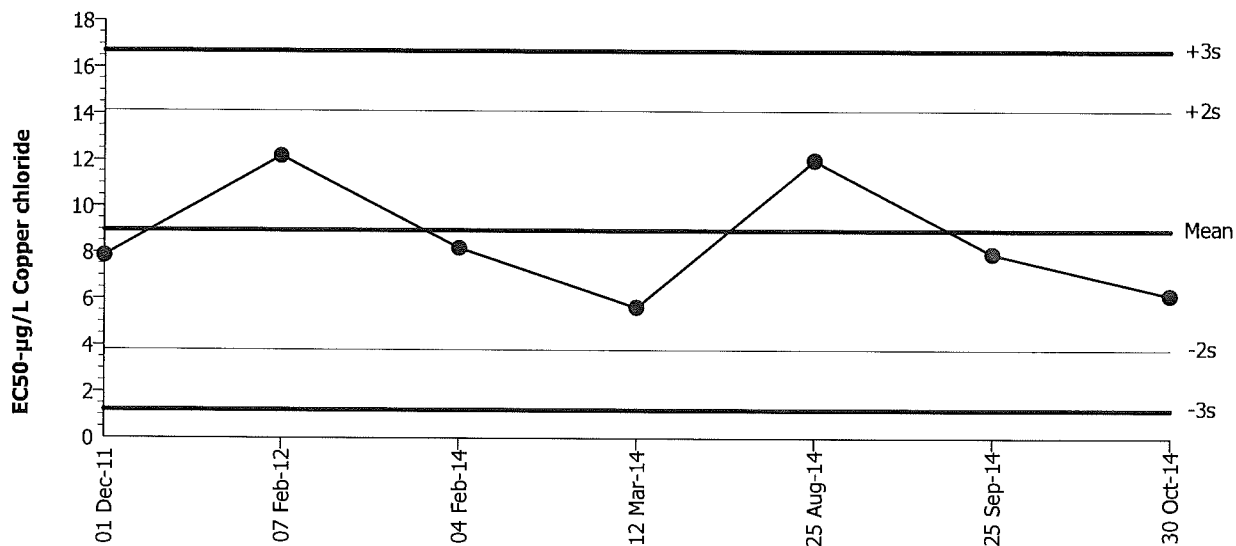
Material: Copper chloride

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Development Rate

Source: Reference Toxicant-REF

Echinoid Embryo-Larval Development Test



Mean: 8.967 Count: 6 -2s Warning Limit: 3.801 -3s Action Limit: 1.218
 Sigma: 2.583 CV: 28.80% +2s Warning Limit: 14.13 +3s Action Limit: 16.72

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Dec	1	7.852	-1.115	-0.4316			07-8281-3338	17-7265-1210
2	2012	Feb	7	12.17	3.203	1.24			12-7990-2055	07-7286-1647
3	2014		4	8.195	-0.7721	-0.2989			14-5835-0600	07-6515-7453
4		Mar	12	5.64	-3.327	-1.288			16-4219-4884	02-2584-6206
5		Aug	25	11.99	3.028	1.172			11-2159-2788	17-1870-3217
6		Sep	25	7.95	-1.017	-0.3938			09-4928-6784	08-7961-1534
7		Oct	30	6.16	-2.807	-1.087			16-2563-7748	00-5600-3113

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:04 (p 1 of 1)
 Test Code: 16-2563-7748/141030spdv

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Start Date: 30 Oct-14	Species: Strongylocentrotus purpuratus	Sample Code: 141030spdv			
End Date: 02 Nov-14	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Reference Toxicant			
Sample Date: 30 Oct-14	Material: Copper chloride	Sample Station: Copper Chloride			

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	0	
			2	100	0	
			3	100	0 SA 11/5/14 95 Q18 → 9	
			4	100	97	
			5	100	93	
			6	100	0	
			7	100	0	
			8	100	0	
			9	100	98	
			10	100	88	
			11	100	72	
			12	100	0	
			13	100	74	
			14	100	0	
			15	100	82	
			16	100	67	
			17	100	61	
			18	100	0	
			19	100	0	
			20	100	0	
			21	100	0	
			22	100	99	
			23	100	14	
			24	100	78	
			25	100	0	
			26	100	0	
			27	100	82	
			28	100	0	
			29	100	95	
			30	100	12	
			31	100	0	
			32	100	9	
			33	100	88	
			34	100	13	
			35	100	80	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 16:04 (p 1 of 1)
 Test Code: 16-2563-7748/141030spdv

Echinoid Embryo-Larval Development Test				Nautilus Environmental (CA)			
Start Date:	30 Oct-14	Species:	Strongylocentrotus purpuratus	Sample Code:	141030spdv		
End Date:	02 Nov-14	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant		
Sample Date:	30 Oct-14	Material:	Copper chloride	Sample Station:	Copper Chloride		

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	5			
0	LC	2	9			
0	LC	3	22	100	99	
0	LC	4	4			
0	LC	5	29			
2.5		1	10			
2.5		2	35			
2.5		3	15			
2.5		4	27			
2.5		5	33			
5		1	24			
5		2	17			
5		3	11	100	78	
5		4	16			
5		5	13			
10		1	3	100	25	
10		2	30			
10		3	34			
10		4	32			
10		5	23			
20		1	6			
20		2	26			
20		3	2	100	0	
20		4	18			
20		5	28			
40		1	7			
40		2	8			
40		3	31			
40		4	14			
40		5	19			
80		1	21			
80		2	12			
80		3	25			
80		4	20	100	0	
80		5	1			

OC-Ac

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: CuCl₂
 Test No.: 141030spdv

Test Species: S. purpuratus
 Start Date/Time: 10/30/2014 15:00
 End Date/Time: 11/3/2014 17:00

Concentration ($\mu\text{g/L}$)	Salinity (ppt)					Temperature ($^{\circ}\text{C}$)					Dissolved Oxygen (mg/L)					pH (pH units)				
	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	33.5	33.3	33.2	32.9		14.5	15.7	15.8	15.2		8.8	7.7	7.9	7.7		8.00	8.01	8.06	7.95	
2.5	33.6	33.6	33.4	33.4		14.5	15.5	15.4	15.1		8.7	7.7	7.9	7.9		8.02	8.01	8.05	7.91	
5	33.6	33.6	33.5	33.4		14.6	15.2	15.2	15.2		8.7	8.1	7.8	7.5		8.01	8.02	8.06	7.94	
10	33.5	33.7	33.7	33.0		14.6	15.2	15.0	15.3		8.6	7.8	7.8	7.5		8.00	8.02	8.07	7.94	
20	33.6	33.6	33.7	33.8		14.6	15.3	15.0	15.0		8.7	8.0	7.9	7.7		8.01	8.01	8.07	7.94	
40	33.5	33.6	33.6	33.6		14.6	15.3	14.8	15.0		8.7	8.0	8.0	7.7		8.03	8.03	8.07	7.95	
80	33.4	33.4	33.5	33.6		14.7	15.3	14.8	15.0		8.7	8.1	8.1	7.6		8.04	8.02	8.08	7.95	

Technician Initials: _____
 WQ Readings:

0	24	48	72	96
5	AL	KFP	AW	/

 Dilutions made by:

AL				
----	--	--	--	--

High conc. made ($\mu\text{g/L}$):	80
Vol. Cu stock added (mL):	4.4
Final Volume (mL):	500
Cu stock concentration ($\mu\text{g/L}$):	9050

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____
 72 hrs: _____

QC Check: KB 11/6/14 Final Review: AC 11/18/14

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Internal
 Sample ID: CuCl2
 Test No.: 141030spdv
 Tech initials: PA
 Injection Time: 1430

Start Date/Time: 10/30/2014 175:00
 End Date/Time: 11/3/2014 1714 1700
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 10/6/14

Sperm Absorbance at 400 nm: 0.823 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 18
24
20
25
24
 Mean: 22.2 X 50 = 1110 eggs/ml
 (target counts of ^{20x}25 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 1110 eggs/ml = 1.1 dilution factor
 Final density: 1000 eggs/ml - 1.0 part egg stock
0.11 parts seawater
 egg stock 260 ml
 seawater 222 ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Volume of Sperm stock needed to fertilize eggs:

Egg Stock (mL) = 225
 Sperm Stock (μL) = 225
 Egg/Sperm Ratio = 1ml:1μl
 Fertilization Time: 1445

Embryo Stock Fertilization Checks:

	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1455</u>	<u>100</u>	<u>0</u>	<u>100</u>
20 minutes (2nd fert. If needed)	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>

Test Initiation Time: 1500 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>97</u>	<u>3</u>	<u>97</u>
End of test QC check	<u>97</u>	<u>3</u>	<u>97</u>

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test up to 96-hrs (ASTM 1999).

QC Check: KB 11/6/14 Final Review: AC 11/18/14

CETIS Summary Report

Report Date: 05 Aug-15 17:32 (p 1 of 1)
 Test Code: 150722spd | 11-3114-3362

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Batch ID: 00-0073-5664	Test Type: Development	Analyst:
Start Date: 22 Jul-15 16:10	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 25 Jul-15 16:10	Species: Strongylocentrotus purpuratus	Brine: Not Applicable
Duration: 72h	Source: Pt. Loma	Age:

Sample ID: 14-5151-8338	Code: 150722spd	Client: Internal
Sample Date: 22 Jul-15	Material: Copper chloride	Project:
Receive Date: 22 Jul-15	Source: Reference Toxicant	
Sample Age: 16h	Station: Copper Chloride	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-4881-4252	Development Rate	<2.5	2.5	NA	1.1%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
14-3278-2605	Development Rate	EC50	10.41	10.05	10.78		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-4881-4252	Development Rate	Control Resp	0.988	0.8 - NL	Yes	Passes Acceptability Criteria
14-3278-2605	Development Rate	Control Resp	0.988	0.8 - NL	Yes	Passes Acceptability Criteria
08-4881-4252	Development Rate	PMSD	0.01102	NL - 0.25	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.988	0.9776	0.9984	0.98	1	0.003742	0.008367	0.85%	0.0%
2.5		5	0.97	0.9612	0.9788	0.96	0.98	0.003162	0.007071	0.73%	1.82%
5		5	0.93	0.9212	0.9388	0.92	0.94	0.003162	0.007071	0.76%	5.87%
10		5	0.594	0.5523	0.6357	0.55	0.64	0.01503	0.03362	5.66%	39.88%
20		5	0.008	0	0.01839	0	0.02	0.003742	0.008367	104.6%	99.19%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

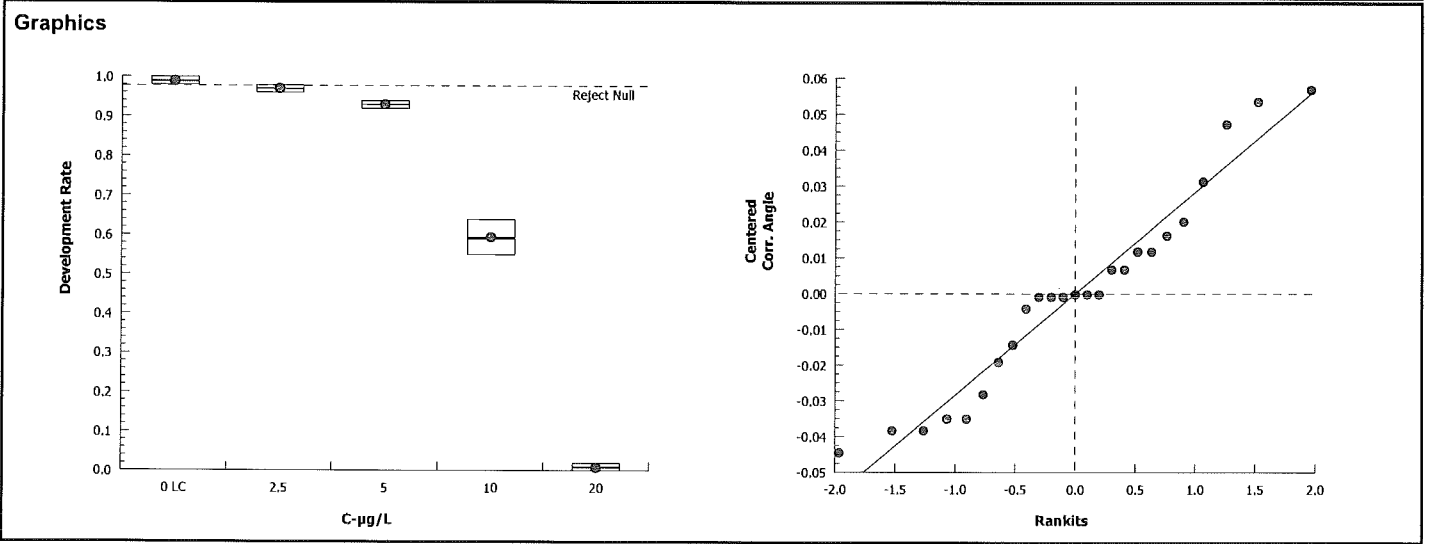
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.99	0.98	0.98	0.99	1
2.5		0.97	0.98	0.97	0.97	0.96
5		0.93	0.94	0.93	0.92	0.93
10		0.55	0.59	0.58	0.61	0.64
20		0.01	0	0.02	0	0.01
40		0	0	0	0	0
80		0	0	0	0	0

CETIS Analytical Report

Report Date: 05 Aug-15 17:31 (p 1 of 2)
 Test Code: 150722spdv | 11-3114-3362

Echinoid Embryo-Larval Development Test										Nautilus Environmental (CA)	
Analysis ID: 08-4881-4252		Endpoint: Development Rate			CETIS Version: CETISv1.8.7						
Analyzed: 05 Aug-15 17:31		Analysis: Parametric-Control vs Treatments			Official Results: Yes						
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	1.1%	<2.5	2.5	NA			
Dunnnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5*	3.39	2.305	0.045	8	0.0051	CDF	Significant Effect		
		5*	8.218	2.305	0.045	8	<0.0001	CDF	Significant Effect		
		10*	29.86	2.305	0.045	8	<0.0001	CDF	Significant Effect		
		20*	70.35	2.305	0.045	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	6.535633		1.633908		4	1710	<0.0001	Significant Effect			
Error	0.0191122		0.0009556102		20						
Total	6.554745				24						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		4.658	13.28	0.3242	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9459	0.8877	0.2026	Normal Distribution					
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.988	0.9776	0.9984	0.99	0.98	1	0.003742	0.85%	0.0%
2.5		5	0.97	0.9612	0.9788	0.97	0.96	0.98	0.003162	0.73%	1.82%
5		5	0.93	0.9212	0.9388	0.93	0.92	0.94	0.003162	0.76%	5.87%
10		5	0.594	0.5523	0.6357	0.59	0.55	0.64	0.01503	5.66%	39.88%
20		5	0.008	0	0.01839	0.01	0	0.02	0.003742	104.6%	99.19%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.464	1.417	1.511	1.471	1.429	1.521	0.01699	2.6%	0.0%
2.5		5	1.398	1.372	1.424	1.397	1.369	1.429	0.009421	1.51%	4.53%
5		5	1.303	1.286	1.321	1.303	1.284	1.323	0.006214	1.07%	10.98%
10		5	0.8801	0.8376	0.9227	0.8759	0.8355	0.9273	0.01534	3.9%	39.88%
20		5	0.08845	0.04003	0.1369	0.1002	0.05002	0.1419	0.01744	44.09%	93.96%
Development Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.99	0.98	0.98	0.99	1					
2.5		0.97	0.98	0.97	0.97	0.96					
5		0.93	0.94	0.93	0.92	0.93					
10		0.55	0.59	0.58	0.61	0.64					
20		0.01	0	0.02	0	0.01					
Angular (Corrected) Transformed Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	1.471	1.429	1.429	1.471	1.521					
2.5		1.397	1.429	1.397	1.397	1.369					
5		1.303	1.323	1.303	1.284	1.303					
10		0.8355	0.8759	0.8657	0.8963	0.9273					
20		0.1002	0.05002	0.1419	0.05002	0.1002					

Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)
Analysis ID: 08-4881-4252	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 05 Aug-15 17:31	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

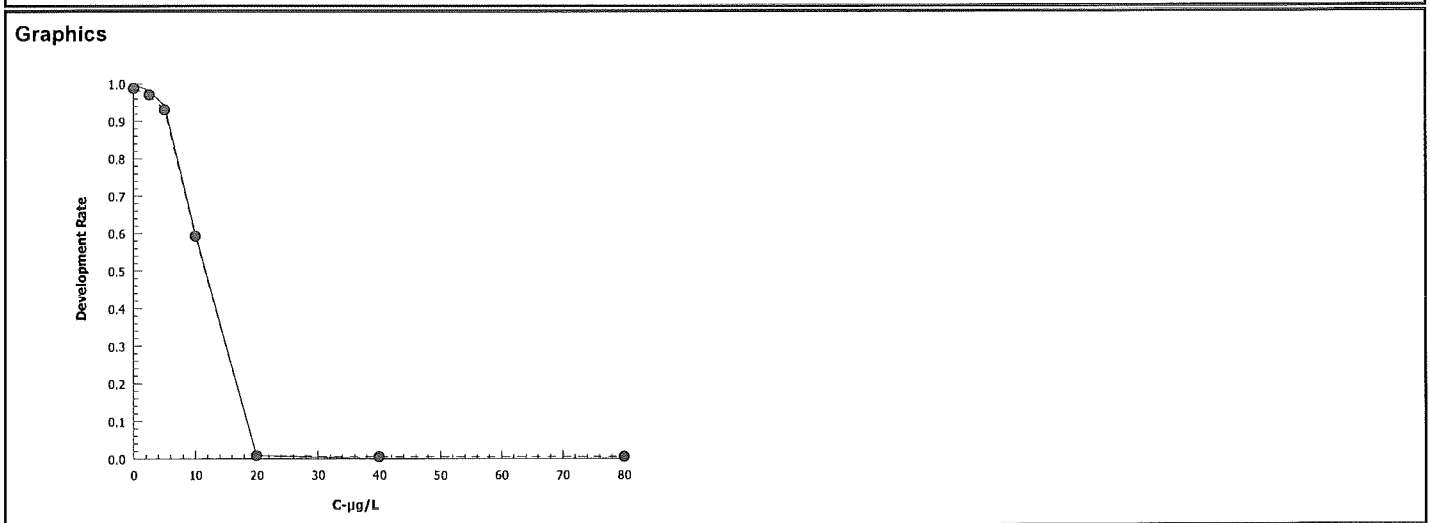
Report Date: 05 Aug-15 17:32 (p 1 of 1)
 Test Code: 150722spdv | 11-3114-3362

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 14-3278-2605	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 05 Aug-15 17:31	Analysis: Trimmed Spearman-Kärber	Official Results: Yes			

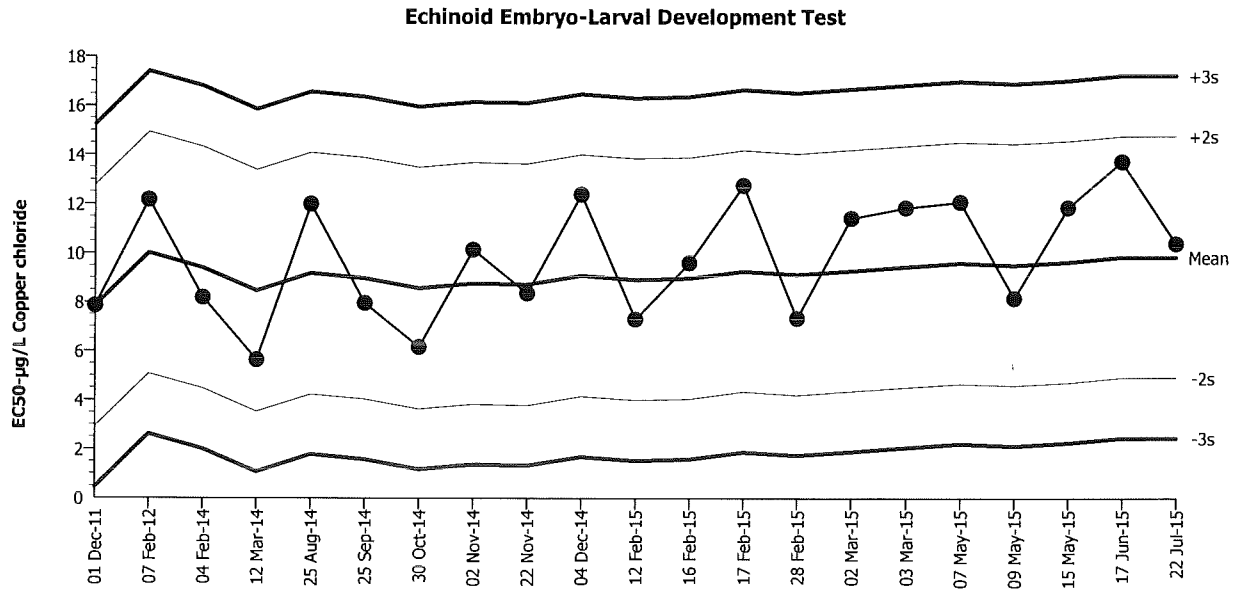
Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.012	1.82%	1.017	0.007669	10.41	10.05	10.78

Development Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.988	0.98	1	0.003742	0.008367	0.85%	0.0%	494	500
2.5		5	0.97	0.96	0.98	0.003162	0.00707	0.73%	1.82%	485	500
5		5	0.93	0.92	0.94	0.003162	0.00707	0.76%	5.87%	465	500
10		5	0.594	0.55	0.64	0.01503	0.03362	5.66%	39.88%	296	500
20		5	0.008	0	0.02	0.003742	0.008367	104.6%	99.19%	4	500
40		5	0	0	0	0	0		100.0%	0	500
80		5	0	0	0	0	0		100.0%	0	500

Development Rate Detail						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.99	0.98	0.98	0.99	1
2.5		0.97	0.98	0.97	0.97	0.96
5		0.93	0.94	0.93	0.92	0.93
10		0.55	0.59	0.58	0.61	0.64
20		0.01	0	0.02	0	0.01
40		0	0	0	0	0
80		0	0	0	0	0



Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)	
Test Type: Development	Organism: Strongylocentrotus purpuratus (Purpl	Material: Copper chloride	
Protocol: EPA/600/R-95/136 (1995)	Endpoint: Development Rate	Source: Reference Toxicant-REF	



Mean: 9.853 **Count:** 20 **-2s Warning Limit:** 4.923 **-3s Action Limit:** 2.458
Sigma: 2.465 **CV:** 25.00% **+2s Warning Limit:** 14.78 **+3s Action Limit:** 17.25

Quality Control Data											
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2011	Dec	1	17:40	7.852	-2.001	-0.8117			07-8281-3338	17-7265-1210
2	2012	Feb	7	15:45	12.17	2.317	0.94			12-7990-2055	07-7286-1647
3	2014		4	14:15	8.195	-1.658	-0.6727			14-5835-0600	07-6515-7453
4		Mar	12	18:20	5.64	-4.213	-1.709			16-4219-4884	02-2584-6206
5		Aug	25	19:10	11.99	2.142	0.8689			11-2159-2788	17-1870-3217
6		Sep	25	17:30	7.95	-1.903	-0.7721			09-4928-6784	08-7961-1534
7		Oct	30	15:00	6.16	-3.693	-1.498			16-2563-7748	00-5600-3113
8		Nov	2	17:00	10.14	0.2904	0.1178			05-9121-9644	01-7691-0405
9			22	16:45	8.358	-1.495	-0.6066			06-8410-0954	18-0830-4230
10		Dec	4	14:10	12.4	2.547	1.033			15-8916-5237	11-1209-5739
11	2015	Feb	12	14:25	7.308	-2.545	-1.033			21-1011-3319	03-9708-7225
12			16	18:25	9.603	-0.2503	-0.1015			12-8378-8021	11-4706-6613
13			17	15:12	12.77	2.912	1.181			12-8622-3584	03-8986-1540
14			28	15:00	7.345	-2.508	-1.018			04-6319-9803	10-6603-8112
15		Mar	2	18:50	11.43	1.572	0.6378			14-8443-1027	00-7020-5756
16			3	19:43	11.86	2.008	0.8145			06-2006-0576	21-3581-4807
17		May	7	15:55	12.1	2.243	0.9098			18-5128-4001	14-3953-8938
18			9	14:30	8.171	-1.682	-0.6822			08-8892-0966	06-5858-7490
19			15	16:37	11.87	2.017	0.8183			06-5352-8837	20-0018-4829
20		Jun	17	16:40	13.75	3.895	1.58			10-5901-9632	03-4943-7748
21		Jul	22	16:10	10.41	0.5562	0.2257			11-3114-3362	14-3278-2605

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:57 (p 1 of 1)
 Test Code: 11-3114-3362/150722spdv

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Start Date: 22 Jul-15 Species: Strongylocentrotus purpuratus Sample Code: 150722spdv
 End Date: 25 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	97	AG 8/5/15 ↓
			2	100	98	
			3	100	0	
			4	100	97	
			5	100	55	
			6	100	0	
			7	100	2	
			8	100	0	
			9	100	1	
			10	100	93	
			11	100	0	
			12	100	59	
			13	100	92	
			14	100	0	
			15	100	0	
			16	100	99	
			17	100	1	
			18	100	0	
			19	100	64	
			20	100	97	
			21	100	96	
			22	100	0	
			23	100	93	
			24	100	58	
			25	100	93	
			26	100	94	
			27	100	0	
			28	100	99	
			29	100	0	
			30	100	100	
			31	100	98	
			32	100	0	
			33	100	61	
			34	100	0	
			35	100	98	

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:57 (p 1 of 1)
 Test Code: 11-3114-3362/150722spdv

Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Start Date: 22 Jul-15 Species: Strongylocentrotus purpuratus Sample Code: 150722spdv
 End Date: 25 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	16	100	100	AC 7/28/15
0	LC	2	35			
0	LC	3	2			
0	LC	4	28			
0	LC	5	30			
2.5		1	20	100	97	AC 7/28/15
2.5		2	31			
2.5		3	4			
2.5		4	1			
2.5		5	21			
5		1	10	100	95	AC 7/28/15
5		2	26			
5		3	23			
5		4	13			
5		5	25			
10		1	5	100	49	AC 7/28/15
10		2	12			
10		3	24			
10		4	33			
10		5	19			
20		1	17	100	0	AC 7/28/15
20		2	22			
20		3	7			
20		4	15			
20		5	9			
40		1	18	100	0	
40		2	11			
40		3	8			
40		4	3			
40		5	14			
80		1	32	100	0	
80		2	34			
80		3	29			
80		4	27			
80		5	6			

0.05

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal

Sample ID: CuCl₂

Test No.: 150722spdv

Test Species: S. purpuratus

Start Date/Time: 7/22/2015 1610

End Date/Time: 7/25/2015 1610

Concentration (<u> </u> µg/L <u> </u>)	Salinity (ppt)				Temperature (°C)				Dissolved Oxygen (mg/L)				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	33.3	33.4	33.3	33.2	14.9	14.8	14.7	15.5	8.9	8.0	7.9	8.2	8.09	8.07	8.05	8.03
2.5	33.4	33.5	33.5	33.4	14.6	14.5	14.5	15.0	8.8	8.1	8.0	8.4	8.08	8.07	8.06	8.05
5	33.5	33.6	33.6	33.5	14.8	14.4	14.4	14.8	8.8	8.2	8.0	8.4	8.08	8.06	8.06	8.05
10	33.5	33.5	33.6	33.5	14.5	14.3	14.3	14.9	8.8	8.2	8.1	8.4	8.08	8.07	8.06	8.05
20	33.5	33.6	33.6	33.5	14.9	14.6	14.5	15.1	8.7	8.2	8.0	8.3	8.08	8.07	8.07	8.06
40	33.4	33.4	33.5	33.4	14.8	14.5	14.4	15.1	8.7	8.3	8.1	8.4	8.09	8.08	8.07	8.06
80	33.3	33.3	33.4	33.3	14.7	14.4	14.3	15.1	8.7	8.3	8.1	8.4	8.09	8.07	8.07	8.06

Technician Initials: _____

WQ Readings:

0	24	48	72
AD	EG	CH	AD
Dilutions made by: AC			

High conc. made (µg/L):	80
Vol. Cu stock added (mL):	4.5
Final Volume (mL):	500
Cu stock concentration (µg/L):	8,850

Comments: 0 hrs: Hach sensor's salinity meter

24 hrs: _____

48 hrs: _____

72 hrs: _____

QC Check: AC 8/3/15

Final Review: KB 8/6/15

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Internal
 Sample ID: CUC12
 Test No.: 150722spdu

Start Date/Time: 7/22/15 1610
 End Date/Time: 7/25/15 1610
 Species: S. purpuratus
 Date Collected: 7/21/15

Tech initials: PA/A
 Injection Time: 1525

Sperm Absorbance at 400 nm: 0.935 ^{AL 018 7/22} (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 28 Mean: 31.2 $\times 50 =$ 1560 eggs/ml
41
29 (target counts of 20 eggs per vertical pass on Sedgwick-Rafter
30 slide for a final density of 1000 eggs/ml)
26

Initial density: 1560 eggs/ml = 1.6 dilution factor
 Final density: 1000 eggs/ml = 1.0 stock
 egg stock 50 ml ^{AL 018 7/22}
 seawater 30 ml _{0.6 sw}

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Add 100 μ L sperm stock per 100mL of egg stock. For example, if you have 60mL of egg stock, add 60 μ L sperm stock.

Embryo Stock Fertilization Checks (Initiate test only when fertilization is $\geq 90\%$):

	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1605</u>	<u>98</u>	<u>2</u>	<u>98</u>
20 minutes (2nd fert. If needed)	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>

Fertilization Time: 1555

Test Initiation Time: 1610 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>99</u>	<u>1</u>	<u>99</u>
End of test QC check	<u>—</u>	<u>—</u>	<u>—</u>

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test to 96-hrs (ASTM 1999).

QC Check: ACB/3/15 Final Review: KB 8/6/15

Sand Dollar Development

CETIS Summary Report

Report Date: 07 Aug-15 10:27 (p 1 of 1)
 Test Code: 141030dedv | 14-4718-7348

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Batch ID: 00-8568-0963	Test Type: Development	Analyst:
Start Date: 30 Oct-14 16:20	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 02 Nov-14 17:20	Species: Dendraster excentricus	Brine: Not Applicable
Duration: 73h	Source: Mission Bay	Age:

Sample ID: 02-2472-0325	Code: 141030dedv	Client: Internal
Sample Date: 30 Oct-14	Material: Copper chloride	Project:
Receive Date: 30 Oct-14	Source: Reference Toxicant	
Sample Age: 16h	Station: Copper Chloride	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-8842-6412	Development Rate	10	>10	NA	5.16%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
06-3337-3291	Development Rate	EC50	13.95	13.81	14.09		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
06-3337-3291	Development Rate	Control Resp	0.93	0.8 - NL	Yes	Passes Acceptability Criteria
07-8842-6412	Development Rate	Control Resp	0.93	0.8 - NL	Yes	Passes Acceptability Criteria
07-8842-6412	Development Rate	PMSD	0.05164	NL - 0.25	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.93	0.8983	0.9617	0.9	0.96	0.0114	0.0255	2.74%	0.0%
2.5		5	0.918	0.8884	0.9476	0.89	0.95	0.01068	0.02387	2.6%	1.29%
5		5	0.928	0.8788	0.9772	0.88	0.98	0.01772	0.03962	4.27%	0.22%
10		5	0.908	0.8664	0.9496	0.87	0.95	0.01497	0.03347	3.69%	2.37%
20		5	0	0	0	0	0	0	0		100.0%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.91	0.93	0.96	0.9	0.95
2.5		0.89	0.95	0.93	0.9	0.92
5		0.9	0.95	0.88	0.98	0.93
10		0.87	0.93	0.95	0.88	0.91
20		0	0	0	0	0
40		0	0	0	0	0
80		0	0	0	0	0

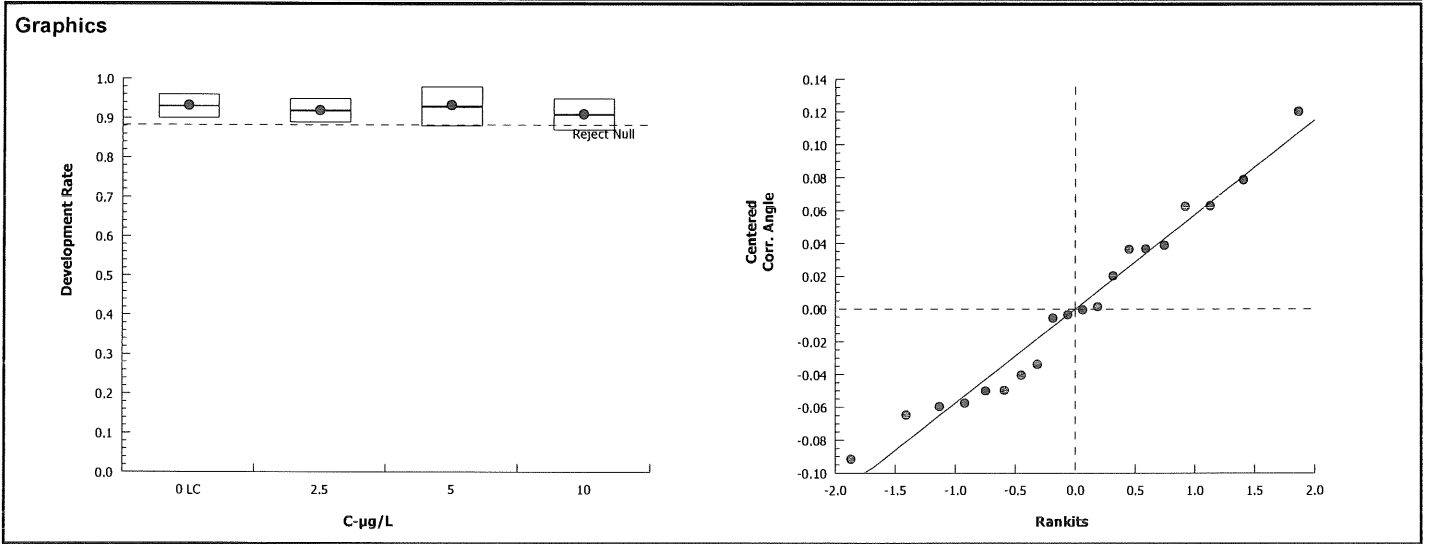
CETIS Analytical Report

Report Date: 07 Aug-15 10:27 (p 1 of 2)

Test Code: 141030dedv | 14-4718-7348

Echinoid Embryo-Larval Development Test										Nautilus Environmental (CA)	
Analysis ID: 07-8842-6412		Endpoint: Development Rate				CETIS Version: CETISv1.8.7					
Analyzed: 07 Aug-15 10:27		Analysis: Parametric-Control vs Treatments				Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	5.16%	10	>10	NA			
Dunnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	0.6118	2.227	0.086	8	0.4953	CDF	Non-Significant Effect		
		5	-0.05363	2.227	0.086	8	0.7688	CDF	Non-Significant Effect		
		10	1.028	2.227	0.086	8	0.3211	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square	DF	F Stat	P-Value	Decision(α:5%)				
Between	0.006063994		0.002021331	3	0.5363	0.6641	Non-Significant Effect				
Error	0.06030232		0.003768895	16							
Total	0.06636631			19							
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		1.669	11.34	0.6439	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9646	0.866	0.6394	Normal Distribution					
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.93	0.8983	0.9617	0.93	0.9	0.96	0.0114	2.74%	0.0%
2.5		5	0.918	0.8884	0.9476	0.92	0.89	0.95	0.01068	2.6%	1.29%
5		5	0.928	0.8788	0.9772	0.93	0.88	0.98	0.01772	4.27%	0.22%
10		5	0.908	0.8664	0.9496	0.91	0.87	0.95	0.01497	3.69%	2.37%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.307	1.243	1.37	1.303	1.249	1.369	0.02281	3.9%	0.0%
2.5		5	1.283	1.227	1.338	1.284	1.233	1.345	0.01995	3.48%	1.82%
5		5	1.309	1.205	1.412	1.303	1.217	1.429	0.03727	6.37%	-0.16%
10		5	1.267	1.193	1.341	1.266	1.202	1.345	0.0266	4.7%	3.05%
Development Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.91	0.93	0.96	0.9	0.95					
2.5		0.89	0.95	0.93	0.9	0.92					
5		0.9	0.95	0.88	0.98	0.93					
10		0.87	0.93	0.95	0.88	0.91					
Angular (Corrected) Transformed Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	1.266	1.303	1.369	1.249	1.345					
2.5		1.233	1.345	1.303	1.249	1.284					
5		1.249	1.345	1.217	1.429	1.303					
10		1.202	1.303	1.345	1.217	1.266					

Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)	
Analysis ID: 07-8842-6412	Endpoint: Development Rate	CETIS Version: CETISv1.8.7	
Analyzed: 07 Aug-15 10:27	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

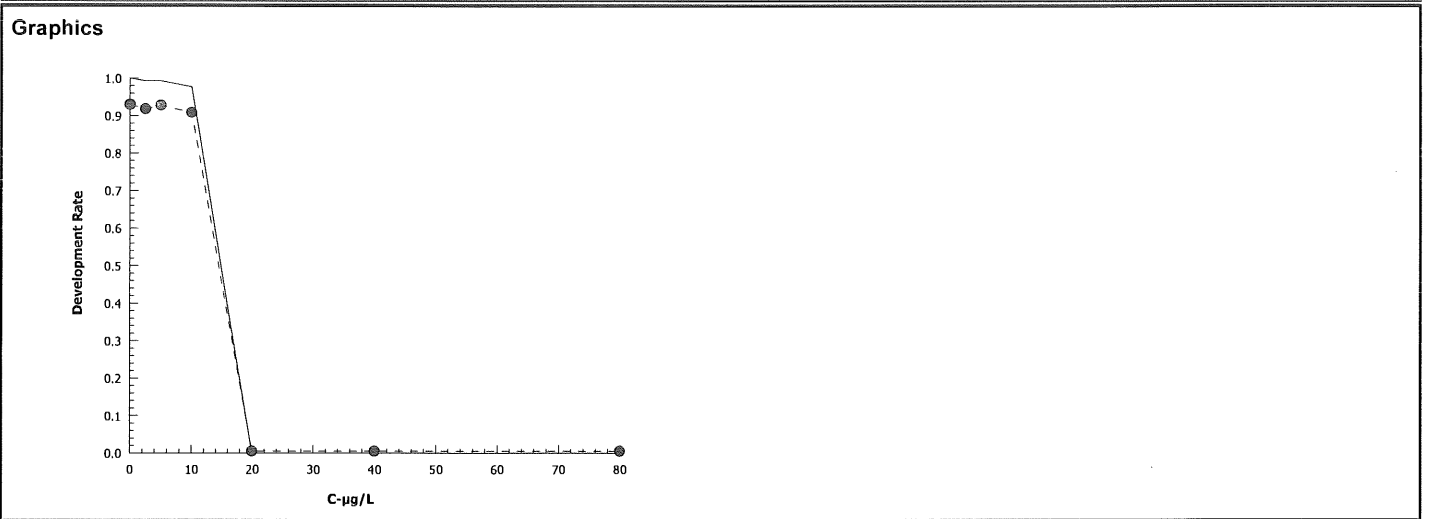
Report Date: 07 Aug-15 10:27 (p 1 of 1)
 Test Code: 141030dedv | 14-4718-7348

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 06-3337-3291	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 07 Aug-15 10:27	Analysis: Trimmed Spearman-Kärber	Official Results: Yes			

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.07	0.75%	1.144	0.00216	13.95	13.81	14.09

Development Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.93	0.9	0.96	0.0114	0.02549	2.74%	0.0%	465	500
2.5		5	0.918	0.89	0.95	0.01068	0.02387	2.6%	1.29%	459	500
5		5	0.928	0.88	0.98	0.01772	0.03962	4.27%	0.22%	464	500
10		5	0.908	0.87	0.95	0.01497	0.03347	3.69%	2.37%	454	500
20		5	0	0	0	0	0		100.0%	0	500
40		5	0	0	0	0	0		100.0%	0	500
80		5	0	0	0	0	0		100.0%	0	500

Development Rate Detail						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.91	0.93	0.96	0.9	0.95
2.5		0.89	0.95	0.93	0.9	0.92
5		0.9	0.95	0.88	0.98	0.93
10		0.87	0.93	0.95	0.88	0.91
20		0	0	0	0	0
40		0	0	0	0	0
80		0	0	0	0	0

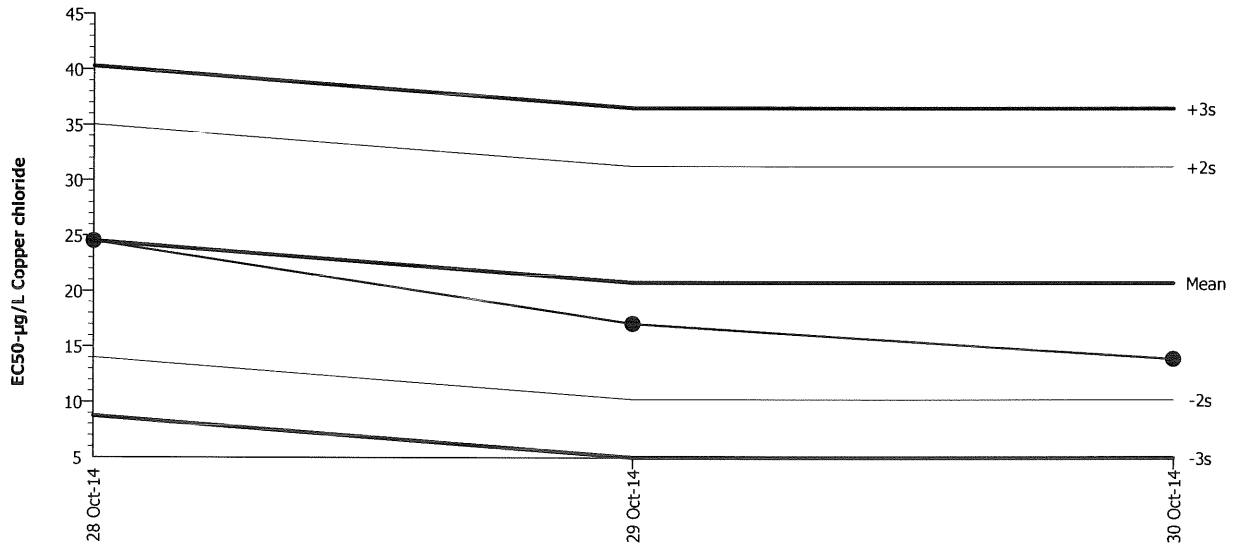


Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Test Type: Development Organism: Dendraster excentricus (Sand Dollar) Material: Copper chloride
 Protocol: EPA/600/R-95/136 (1995) Endpoint: Development Rate Source: Reference Toxicant-REF

Echinoid Embryo-Larval Development Test



Mean: 20.79 Count: 2 -2s Warning Limit: 10.27 -3s Action Limit: 5.015
 Sigma: 5.259 CV: 25.30% +2s Warning Limit: 31.31 +3s Action Limit: 36.57

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Oct	28	12:35	24.51	3.721	0.7075			19-0774-1141	02-3565-1966
2			29	15:45	17.07	-3.717	-0.7068			05-2123-6408	20-1447-5780
3			30	16:20	13.95	-6.844	-1.301			14-4718-7348	06-3337-3291

CETIS Test Data Worksheet

Report Date: 28 Oct-14 15:51 (p 1 of 1)
 Test Code: 14-4718-7348/141030dedv

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Start Date: 30 Oct-14 Species: Dendraster excentricus Sample Code: 141030dedv
 End Date: 02 Nov-14 ^{AC 018 9/15} Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 30 Oct-14 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	91 ^{SA}	11/19/14 Q18 Ø
			2	100	Ø	
			3	100	88 ^{SA}	11/19/14 Q18 Ø
			4		Ø	
			5		Ø	
			6		90	
			7		Ø	
			8		98	
			9		93	
			10		Ø	
			11		88	
			12		Ø	
			13		96	
			14		95	
			15		90	
			16		89	
			17		91	
			18		95	SA Q18
			19		90	11/19/14 Ø
			20		90	
			21		Ø	
			22		Ø	
			23		93	
			24		Ø	
			25		Ø	
			26		95	
			27		Ø	
			28		Ø	
			29		87	
			30		93	
			31		95	
			32		91	
			33		92	
			34		93	
			35		88	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 15:51 (p 1 of 1)
 Test Code: 14-4718-7348/141030dedv

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Start Date:	30 Oct-14	Species:	Dendraster excentricus	Sample Code:	141030dedv
End Date:	03 Nov-14	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant
Sample Date:	30 Oct-14	Material:	Copper chloride	Sample Station:	Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	17			
0	LC	2	30			
0	LC	3	13			
0	LC	4	20			
0	LC	5	26			
2.5		1	16			
2.5		2	18			
2.5		3	23			
2.5		4	15			
2.5		5	33			
5		1	6			
5		2	14			
5		3	11			
5		4	8			
5		5	9			
10		1	29			
10		2	34			
10		3	31			
10		4	35			
10		5	32			
20		1	1			
20		2	28			
20		3	19			
20		4	3			
20		5	7			
40		1	25			
40		2	10			
40		3	4			
40		4	27			
40		5	22			
80		1	12			
80		2	5			
80		3	21			
80		4	2			
80		5	24			

QC=AC

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: CuCl₂
 Test No.: 141030dedv

Test Species: D. excentricus
 Start Date/Time: 10/30/2014 1620
 End Date/Time: 11/3/2014 1720

⁰⁴⁸
~~11/2/14~~

Concentration ($\mu\text{g/L}$)	Salinity (ppt)					Temperature ($^{\circ}\text{C}$)					Dissolved Oxygen (mg/L)					pH (pH units)				
	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96	0	24	48	72	96
Lab Control	33.5	33.5	33.5	33.4		14.5	15.6	15.4	15.0		8.8	8.1	7.8	7.7		8.00	7.99	8.04	7.94	
2.5	33.6	33.6	33.6	33.7		14.5	15.6	15.0	15.0		8.7	8.1	7.9	7.9		8.02	7.94	8.05	7.97	
5	33.6	33.6	33.7	33.7		14.6	15.6	14.9	15.0		8.7	8.1	8.0	7.9		8.01	8.01	8.07	7.97	
10	33.5	33.6	33.7	33.7		14.6	15.8	14.8	15.1		8.6	8.1	8.0	7.9		8.00	8.00	8.04	7.97	
20	33.6	33.6	33.6	33.7		14.6	15.8	15.0	14.9		8.7	8.2	7.9	7.9		8.01	8.00	8.12	7.96	
40	33.5	33.5	33.5	33.6		14.6	15.9	14.9	14.9		8.7	8.2	8.0	7.9		8.03	8.00	8.11	7.97	
80	33.4	33.3	33.3	33.4		14.7	15.7	14.9	15.0		8.7	8.1	8.0	7.9		8.04	8.00	8.11	7.97	

Technician Initials: _____
 WQ Readings:

0	24	48	72	96
PA	AG	KFP	AW	N/A

 Dilutions made by:

AC				
----	--	--	--	--

High conc. made ($\mu\text{g/L}$):

80

 Vol. Cu stock added (mL):

4.4

 Final Volume (mL):

500

 Cu stock concentration ($\mu\text{g/L}$):

9050

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____
 72 hrs: _____

QC Check: AC 8/7/15

Final Review: YS 8/7/15

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Internal
 Sample ID: CyC12
 Test No.: 141030dodv

Start Date/Time: 10/30/2014 11:20
 End Date/Time: 11/3/2014 14:17:20
 Species: D. excentricus
 Animal Source: Mission Bay
 Date Collected: 10/24/14

Tech initials: AC
 Injection Time: 15:15

Sperm Absorbance at 400 nm: 0.744 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 22
19
21
17
26
 Mean: 21 X 50 = 1050 eggs/ml
 (target counts of ²⁵ eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 1050 eggs/ml = 1.05 dilution factor
 Final density: 1000 eggs/ml - 1.0 part egg stock
0.05 parts seawater
 egg stock 100 ml
 seawater ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Volume of Sperm stock needed to fertilize eggs:

Egg Stock (mL) = 100
 Sperm Stock (μ L) = 100
 Egg/Sperm Ratio = 1ml:1ul

Fertilization Time: 1555

Embryo Stock Fertilization Checks:	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1605</u>	<u>98</u>	<u>2</u>	<u>98</u>
20 minutes (2nd fert. If needed)	_____	_____	_____	_____

Test Initiation Time: 1620 Embryo Stock Added: 0.25 ml

Test Termination:	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>97</u>	<u>3</u>	<u>97</u>
End of test QC check	_____	_____	_____

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test up to 96-hrs (ASTM 1999).

QC Check: AC 8/7/15 Final Review: AS 8/7/15

CETIS Summary Report

Report Date: 05 Aug-15 17:33 (p 1 of 1)

Test Code: 150722dedv | 13-7165-0540

Echinoid Embryo-Larval Development Test **Nautilus Environmental (CA)**

Batch ID: 07-5278-8592	Test Type: Development	Analyst:
Start Date: 22 Jul-15 15:10	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 25 Jul-15 15:40	Species: Dendroaster excentricus	Brine: Not Applicable
Duration: 73h	Source: Mission Bay	Age:

Sample ID: 11-6268-5099	Code: 150722dedv	Client: Internal
Sample Date: 22 Jul-15	Material: Copper chloride	Project:
Receive Date: 25 Jul-15	Source: Reference Toxicant	
Sample Age: 15h	Station: Copper Chloride	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-0931-8852	Development Rate	10	20	14.14	10.2%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
17-1529-7817	Development Rate	EC50	14.59	14.4	14.78		Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
04-0931-8852	Development Rate	Control Resp	0.92	0.8 - NL	Yes	Passes Acceptability Criteria
17-1529-7817	Development Rate	Control Resp	0.92	0.8 - NL	Yes	Passes Acceptability Criteria
04-0931-8852	Development Rate	PMSD	0.1019	NL - 0.25	No	Passes Acceptability Criteria

Development Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.92	0.846	0.994	0.83	0.99	0.02665	0.05958	6.48%	0.0%
2.5		5	0.93	0.853	1	0.84	0.98	0.02775	0.06205	6.67%	-1.09%
5		5	0.908	0.7881	1	0.74	0.98	0.04317	0.09654	10.63%	1.3%
10		5	0.97	0.9524	0.9876	0.95	0.98	0.006325	0.01414	1.46%	-5.44%
20		5	0.042	0.001384	0.08262	0	0.09	0.01463	0.03271	77.88%	95.43%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%

Development Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.94	0.94	0.9	0.83	0.99
2.5		0.97	0.84	0.98	0.89	0.97
5		0.98	0.92	0.74	0.96	0.94
10		0.98	0.95	0.96	0.98	0.98
20		0.04	0.05	0	0.09	0.03
40		0	0	0	0	0
80		0	0	0	0	0

CETIS Analytical Report

Report Date: 05 Aug-15 17:33 (p 1 of 2)

Test Code: 150722dedv | 13-7165-0540

Echinoid Embryo-Larval Development Test						Nautilus Environmental (CA)					
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Analysis ID: 04-0931-8852	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 05 Aug-15 17:27	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	10.2%	10	20	14.14	

Dunnnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.2915	2.305	0.162	8	0.8808	CDF	Non-Significant Effect
		5	0.2017	2.305	0.162	8	0.7283	CDF	Non-Significant Effect
		10	-1.396	2.305	0.162	8	0.9918	CDF	Non-Significant Effect
		20*	15.85	2.305	0.162	8	<0.0001	CDF	Significant Effect

ANOVA Table							
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)	
Between	5.211512	1.302878	4	106	<0.0001	Significant Effect	
Error	0.2457891	0.01228945	20				
Total	5.457301		24				

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	5.39	13.28	0.2496	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9527	0.8877	0.2881	Normal Distribution	

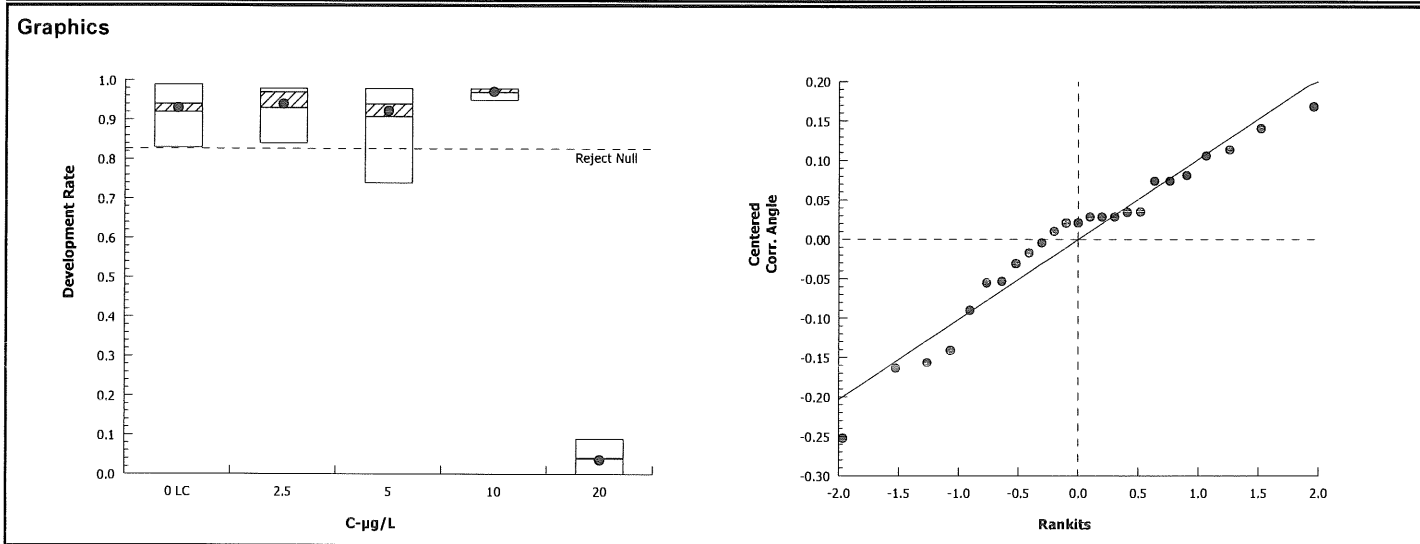
Development Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.92	0.846	0.994	0.94	0.83	0.99	0.02665	6.48%	0.0%
2.5		5	0.93	0.853	1	0.97	0.84	0.98	0.02775	6.67%	-1.09%
5		5	0.908	0.7881	1	0.94	0.74	0.98	0.04317	10.63%	1.3%
10		5	0.97	0.9524	0.9876	0.98	0.95	0.98	0.006325	1.46%	-5.44%
20		5	0.042	0.001384	0.08262	0.04	0	0.09	0.01463	77.88%	95.43%

Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.302	1.155	1.45	1.323	1.146	1.471	0.05317	9.13%	0.0%
2.5		5	1.323	1.175	1.471	1.397	1.159	1.429	0.0534	9.03%	-1.57%
5		5	1.288	1.101	1.476	1.323	1.036	1.429	0.0676	11.73%	1.09%
10		5	1.4	1.35	1.45	1.429	1.345	1.429	0.01794	2.86%	-7.51%
20		5	0.1911	0.07601	0.3063	0.2014	0.05002	0.3047	0.04146	48.51%	85.32%

Development Rate Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	0.94	0.94	0.9	0.83	0.99	
2.5		0.97	0.84	0.98	0.89	0.97	
5		0.98	0.92	0.74	0.96	0.94	
10		0.98	0.95	0.96	0.98	0.98	
20		0.04	0.05	0	0.09	0.03	

Angular (Corrected) Transformed Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	1.323	1.323	1.249	1.146	1.471	
2.5		1.397	1.159	1.429	1.233	1.397	
5		1.429	1.284	1.036	1.369	1.323	
10		1.429	1.345	1.369	1.429	1.429	
20		0.2014	0.2255	0.05002	0.3047	0.1741	

Echinoid Embryo-Larval Development Test		Nautilus Environmental (CA)
Analysis ID: 04-0931-8852	Endpoint: Development Rate	CETIS Version: CETISv1.8.7
Analyzed: 05 Aug-15 17:27	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

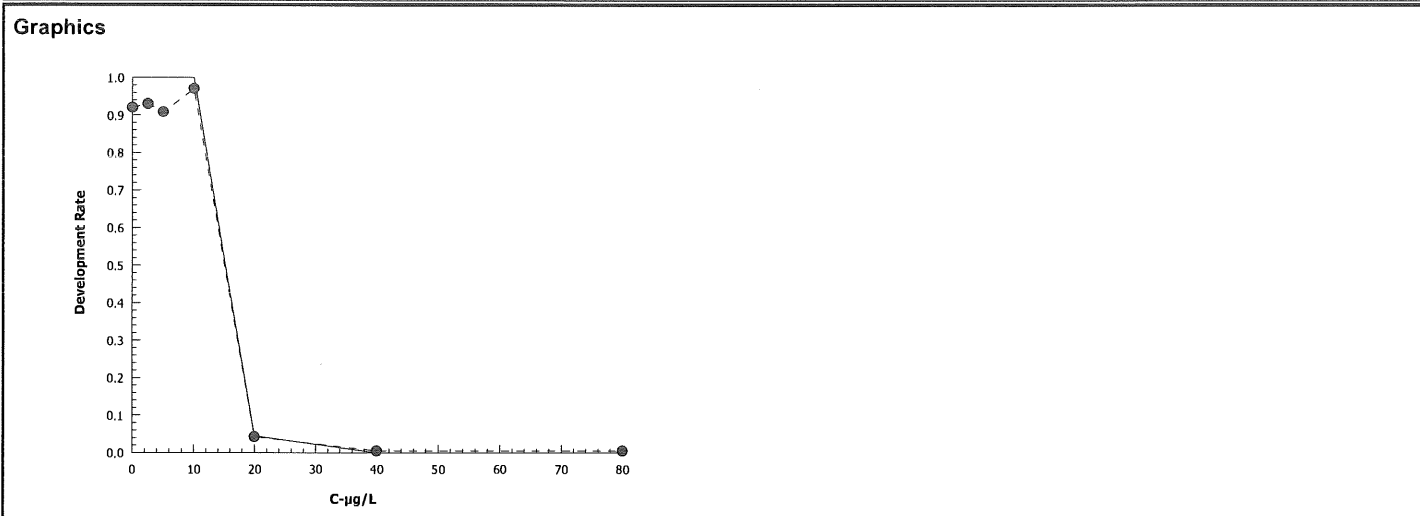
Report Date: 05 Aug-15 17:33 (p 1 of 1)
 Test Code: 150722dedv | 13-7165-0540

Echinoid Embryo-Larval Development Test			Nautilus Environmental (CA)		
Analysis ID: 17-1529-7817	Endpoint: Development Rate	CETIS Version: CETISv1.8.7			
Analyzed: 05 Aug-15 17:32	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes			

Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.08	0.00%	1.164	0.002793	14.59	14.4	14.78

Development Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.92	0.83	0.99	0.02665	0.05958	6.48%	0.0%	460	500
2.5		5	0.93	0.84	0.98	0.02775	0.06205	6.67%	-1.09%	465	500
5		5	0.908	0.74	0.98	0.04317	0.09654	10.63%	1.3%	454	500
10		5	0.97	0.95	0.98	0.006325	0.01414	1.46%	-5.44%	485	500
20		5	0.042	0	0.09	0.01463	0.03271	77.88%	95.43%	21	500
40		5	0	0	0	0	0		100.0%	0	500
80		5	0	0	0	0	0		100.0%	0	500

Development Rate Detail						
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.94	0.94	0.9	0.83	0.99
2.5		0.97	0.84	0.98	0.89	0.97
5		0.98	0.92	0.74	0.96	0.94
10		0.98	0.95	0.96	0.98	0.98
20		0.04	0.05	0	0.09	0.03
40		0	0	0	0	0
80		0	0	0	0	0



Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Test Type: Development

Organism: Dendraster excentricus (Sand Dollar)

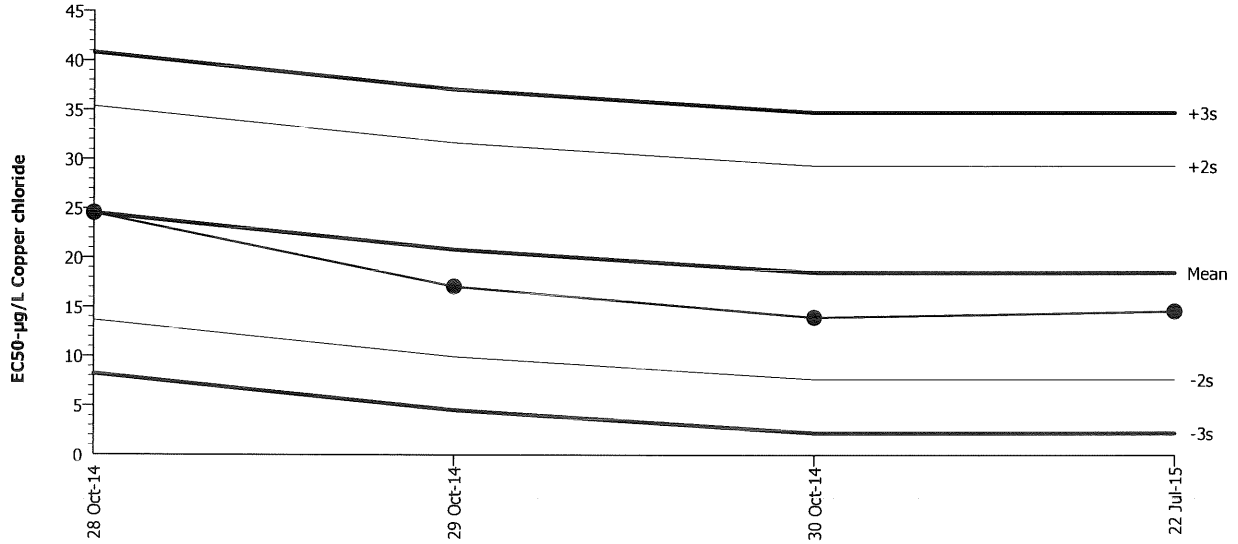
Material: Copper chloride

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Development Rate

Source: Reference Toxicant-REF

Echinoid Embryo-Larval Development Test



Mean: 18.51 Count: 3 -2s Warning Limit: 7.656 -3s Action Limit: 2.229
 Sigma: 5.427 CV: 29.30% +2s Warning Limit: 29.36 +3s Action Limit: 34.79

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Oct	28	12:35	24.51	6.001	1.106			19-0774-1141	02-3565-1966
2			29	15:45	17.07	-1.437	-0.2648			05-2123-6408	20-1447-5780
3			30	16:20	13.95	-4.564	-0.841			14-4718-7348	06-3337-3291
4	2015	Jul	22	15:10	14.59	-3.919	-0.7222			13-7165-0540	17-1529-7817

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:55 (p 1 of 1)
 Test Code: 13-7165-0540/150722dedv

Echinoid Embryo-Larval Development Test

Nautilus Environmental (CA)

Start Date: 22 Jul-15 Species: Dendraster excentricus Sample Code: 150722dedv
 End Date: 25 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	17	100	98	AC 7/28/15
0	LC	2	30			
0	LC	3	13			
0	LC	4	20			
0	LC	5	26			
2.5		1	16	100	95	AC 7/28/15
2.5		2	18			
2.5		3	23			
2.5		4	15			
2.5		5	33			
5		1	6	100	96	AC 7/28/15
5		2	14			
5		3	11			
5		4	8			
5		5	9			
10		1	29	100	94	AC 7/28/15
10		2	34			
10		3	31			
10		4	35			
10		5	32			
20		1	1	100	23	AC 7/28/15
20		2	28			
20		3	19			
20		4	3			
20		5	7			
40		1	25	100	0	AC 7/28/15
40		2	10			
40		3	4			
40		4	27			
40		5	22			
80		1	12	100	0	AC 7/28/15
80		2	5			
80		3	21			
80		4	2			
80		5	24			

QC: 8

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:55 (p 1 of 1)
 Test Code: 13-7165-0540/150722dedv

Echinoid Embryo-Larval Development Test Nautilus Environmental (CA)

Start Date: 22 Jul-15 Species: Dendraster excentricus Sample Code: 150722dedv
 End Date: 25 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Normal	Notes
			1	100	4	CH 7/30/15
			2	100	0	
			3	100	9	
			4	100	0	
			5	100	0	
			6	100	98	
			7	100	96	CH 7/30/15
			8	100	96	
			9	100	94	
			10	100	0	
			11	100	74	
			12	100	0	
			13	100	90	
			14	100	92	
			15	100	89	
			16	100	97	
			17	100	94	
			18	100	84	
			19	100	0	
			20	100	83	
			21	100	0	CH 8/5/15
			22	100	0	
			23	100	98	
			24	100	0	
			25	100	0	
			26	100	99	
			27	100	0	
			28	100	95	CH 8/5/15
			29	100	98	
			30	100	94	
			31	100	96	
			32	100	98	
			33	100	97	
			34	100	95	
			35	100	98	

Marine Chronic Bioassay

Water Quality Measurements

Client: Internal
 Sample ID: CuCl₂
 Test No.: 150722dedv

Test Species: D. excentricus
 Start Date/Time: 7/22/2015 1510
 End Date/Time: 7/25/2015 1540

Concentration (<u> </u> µg/L)	Salinity (ppt)				Temperature (°C)				Dissolved Oxygen (mg/L)				pH (pH units)			
	0	24	48	72	0	24	48	72	0	24	48	72	0	24	48	72
Lab Control	33.3	33.4	33.3	33.2	14.9	14.8	14.7	15.5	8.9	8.0	7.9	8.2	8.09	8.07	8.05	8.03
2.5	33.4	33.5	33.5	33.4	14.6	14.5	14.5	15.0	8.8	8.1	8.0	8.4	8.08	8.07	8.06	8.05
5	33.5	33.6	33.6	33.5	14.8	14.4	14.4	14.8	8.8	8.2	8.0	8.4	8.08	8.06	8.06	8.05
10	33.5	33.5	33.6	33.5	14.5	14.3	14.3	14.9	8.8	8.2	8.1	8.4	8.08	8.07	8.06	8.05
20	33.5	33.6	33.6	33.5	14.9	14.6	14.5	15.1	8.7	8.2	8.0	8.3	8.08	8.07	8.07	8.06
40	33.4	33.4	33.5	33.4	14.8	14.5	14.4	15.1	8.7	8.3	8.1	8.4	8.09	8.08	8.07	8.06
80	33.3	33.3	33.4	33.3	14.7	14.4	14.3	15.1	8.7	8.3	8.1	8.4	8.09	8.07	8.07	8.06

Technician Initials: _____
 WQ Readings:

0	24	48	72
AD	EG	CH	AD

 Dilutions made by:

AC			
----	--	--	--

High conc. made (µg/L):	80
Vol. Cu stock added (mL):	4.5
Final Volume (mL):	500
Cu stock concentration (µg/L):	8,800

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____
 72 hrs: _____

QC Check: AC 8/3/15

Final Review: 8/8/15

Marine Chronic Bioassay

Echinoderm Larval Development Worksheet

Client: Internal
 Sample ID: cu012
 Test No.: 150722 ~~dedv~~ AC 018 8/13/15
 Tech initials: AC
 Injection Time: 1430

Start Date/Time: 7/23/15 1510
 End Date/Time: 7/26/15 1540
 Species: D. excentricus
 Date Collected: 7/17/15

Sperm Absorbance at 400 nm: 1.0 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 16
27
20
18
17
 Mean: 19.6 X 50 = 980 eggs/ml
 (target counts of 20 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 1000 eggs/ml)

Initial density: 980 eggs/ml = 0.98 dilution factor egg stock 100 ml
 Final density: 1000 eggs/ml seawater — ml

Prepare the egg stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Add 100 μ L sperm stock per 100mL of egg stock. For example, if you have 60mL of egg stock, add 60 μ L sperm stock.

Embryo Stock Fertilization Checks (Initiate test only when fertilization is $\geq 90\%$):

	Time	No. Fert.	No. Unfert.	%
10 minutes (1st fert.)	<u>1502</u>	<u>47</u>	<u>3</u>	<u>97</u>
20 minutes (2nd fert. If needed)	<u>—</u>	<u>—</u>	<u>—</u>	<u>—</u>

Fertilization Time: 1452

Test Initiation Time: 1510 Embryo Stock Added: 0.25 ml

Test Termination:

	No. Normal	No. Abnormal	% Normal
72-hour QC check ^a	<u>100</u>	<u>0</u>	<u>100</u>
End of test QC check	<u>—</u>	<u>—</u>	<u>—</u>

Comments: ^a If the embryo development does not meet the mean test acceptability criterion of 80% normally developed, continue the test to 96-hrs (ASTM 1999).

QC Check: AC 8/15/15

Final Review: AC 8/17/15

Urchin Fertilization

CETIS Summary Report

Report Date: 10 Nov-14 10:32 (p 1 of 1)
 Test Code: 141030sprt | 00-9558-0596

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Batch ID: 13-0452-0527	Test Type: Fertilization	Analyst:
Start Date: 30 Oct-14 15:05	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 30 Oct-14 15:45	Species: Strongylocentrotus purpuratus	Brine: Not Applicable
Duration: 40m	Source: Pt. Loma	Age:

Sample ID: 01-9546-4967	Code: 141030sprt	Client: Internal
Sample Date: 30 Oct-14	Material: Copper chloride	Project:
Receive Date: 30 Oct-14	Source: Reference Toxicant	
Sample Age: 15h	Station: Copper Chloride	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
01-4265-2489	Fertilization Rate	<10	10	NA	10.0%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
15-2024-8469	Fertilization Rate	EC50	19.02	17.82	20.3		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
01-4265-2489	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
15-2024-8469	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
01-4265-2489	Fertilization Rate	PMSD	0.1004	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.858	0.8387	0.8773	0.8	0.92	0.02311	0.05167	6.02%	0.0%
10		5	0.636	0.6055	0.6665	0.57	0.73	0.03655	0.08173	12.85%	25.87%
20		5	0.446	0.4238	0.4682	0.35	0.5	0.02657	0.05941	13.32%	48.02%
40		5	0.06	0.04706	0.07294	0	0.09	0.01549	0.03464	57.74%	93.01%
80		5	0	0	0	0	0	0	0		100.0%
160		5	0	0	0	0	0	0	0		100.0%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.81	0.92	0.87	0.89	0.8
10		0.73	0.59	0.72	0.57	0.57
20		0.35	0.5	0.48	0.47	0.43
40		0.09	0.07	0	0.07	0.07
80		0	0	0	0	0
160		0	0	0	0	0

CETIS Analytical Report

Report Date: 10 Nov-14 10:32 (p 1 of 1)
 Test Code: 141030sprt | 00-9558-0596

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA)

Analysis ID: 01-4265-2489 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.4
 Analyzed: 10 Nov-14 10:32 Analysis: Parametric-Control vs Treatments Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	NA	C > T	NA	NA	<10	10	NA		10.0%

Dunnett Multiple Comparison Test

Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	10*	5.058	2.227	0.116	8	0.0002	CDF	Significant Effect
	20*	8.774	2.227	0.116	8	<0.0001	CDF	Significant Effect
	40*	18.33	2.227	0.116	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.455379	0.8184596	3	120	<0.0001	Significant Effect
Error	0.1091171	0.006819817	16			
Total	2.564496		19			

Distributional Tests

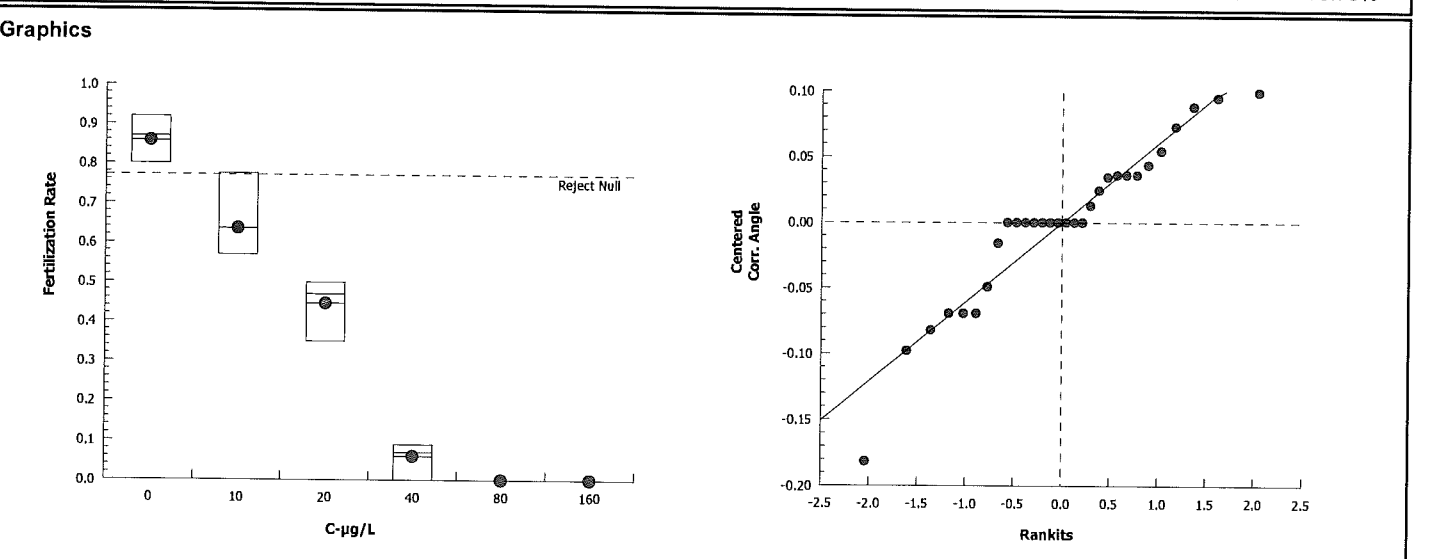
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.059	11.34	0.7869	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9249	0.866	0.1230	Normal Distribution

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.858	0.7938	0.9222	0.87	0.8	0.92	0.02311	6.02%	0.0%
10		5	0.636	0.5345	0.7375	0.59	0.57	0.73	0.03655	12.85%	25.87%
20		5	0.446	0.3722	0.5198	0.47	0.35	0.5	0.02657	13.32%	48.02%
40		5	0.06	0.01699	0.103	0.07	0	0.09	0.01549	57.74%	93.01%
80		5	0	0	0	0	0	0	0	100.0%	100.0%
160		5	0	0	0	0	0	0	0	100.0%	100.0%

Angular (Corrected) Transformed Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.189	1.096	1.282	1.202	1.107	1.284	0.03362	6.32%	0.0%
10		5	0.9249	0.818	1.032	0.8759	0.8556	1.024	0.03853	9.32%	22.22%
20		5	0.7309	0.6559	0.8058	0.7554	0.6331	0.7854	0.027	8.26%	38.54%
40		5	0.2316	0.104	0.3592	0.2678	0.05002	0.3047	0.04595	44.37%	80.52%
80		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.79%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.79%



CETIS Analytical Report

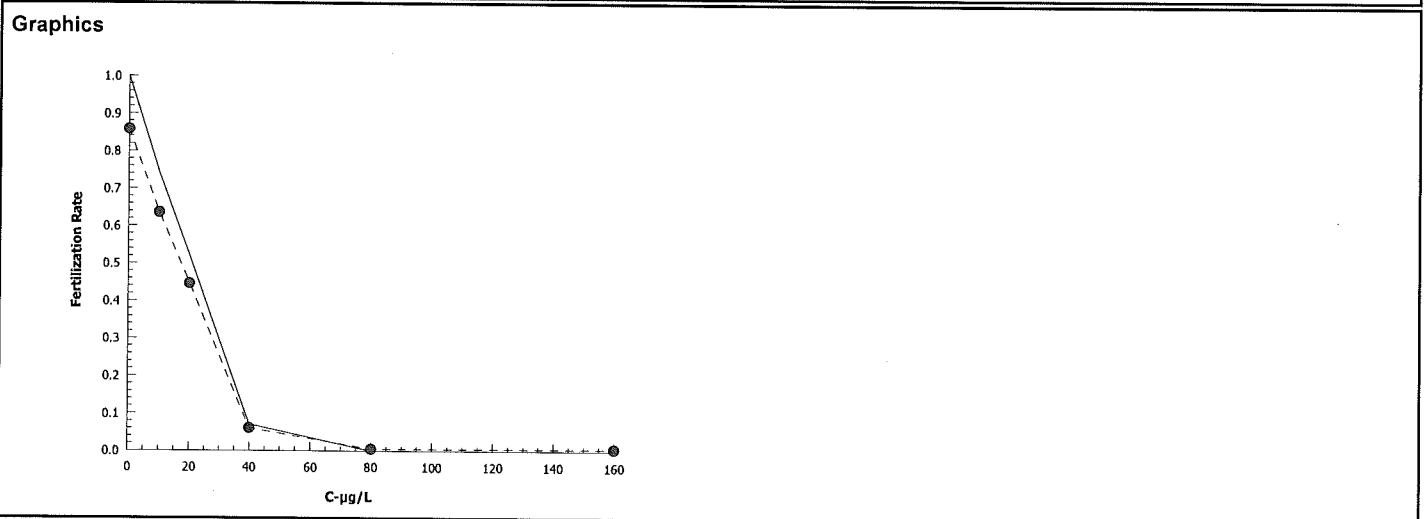
Report Date: 10 Nov-14 10:32 (p 1 of 1)
 Test Code: 141030sprt | 00-9558-0596

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA)

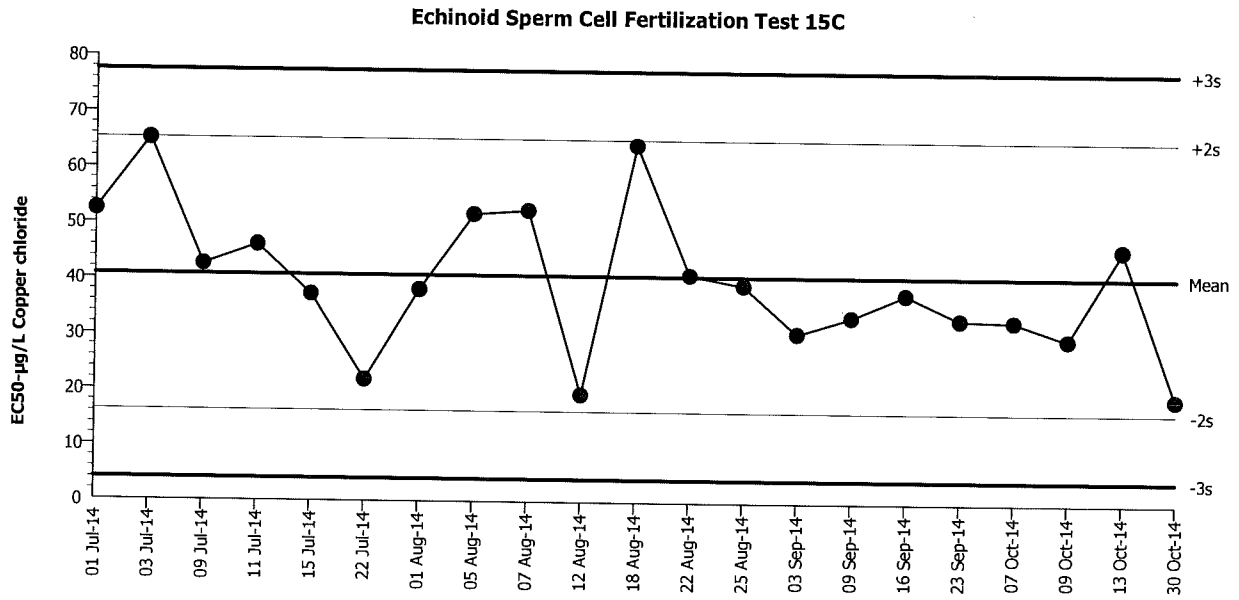
Analysis ID: 15-2024-8469 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.4
 Analyzed: 10 Nov-14 10:32 Analysis: Trimmed Spearman-Kärber Official Results: Yes

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.142	25.87%	1.279	0.01415	19.02	17.82	20.3

Fertilization Rate Summary			Calculated Variate(A/B)									
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
0	Lab Control	5	0.858	0.8	0.92	0.02311	0.05167	6.02%	0.0%	429	500	
10		5	0.636	0.57	0.73	0.03655	0.08173	12.85%	25.87%	317	500	
20		5	0.446	0.35	0.5	0.02657	0.05941	13.32%	48.02%	223	500	
40		5	0.06	0	0.09	0.01549	0.03464	57.74%	93.01%	30	500	
80		5	0	0	0	0	0		100.0%	0	500	
160		5	0	0	0	0	0		100.0%	0	500	



Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Test Type: Fertilization	Organism: Strongylocentrotus purpuratus (Purpl	Material: Copper chloride	
Protocol: EPA/600/R-95/136 (1995)	Endpoint: Fertilization Rate	Source: Reference Toxicant-REF	



Mean: 40.82 **Count:** 20 **-2s Warning Limit:** 16.32 **-3s Action Limit:** 4.07
Sigma: 12.25 **CV:** 30.00% **+2s Warning Limit:** 65.32 **+3s Action Limit:** 77.57

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Jul	1	52.45	11.63	0.9492			03-1633-9040	04-6714-9615
2			3	65.26	24.44	1.995			16-7036-5031	17-9179-4003
3			9	42.65	1.832	0.1496			16-1504-3840	07-5767-6661
4			11	46.17	5.351	0.4368			10-5811-3549	06-0498-7042
5			15	37.29	-3.529	-0.2881			13-2908-2037	08-6489-1251
6			22	21.9	-18.92	-1.545			12-6705-4933	01-8116-2108
7		Aug	1	38.18	-2.641	-0.2156			14-6400-5139	20-5348-7458
8			5	51.86	11.04	0.9013			13-0687-0962	02-1578-6969
9			7	52.56	11.74	0.958			00-5563-1682	00-5377-5202
10			12	19.39	-21.43	-1.749			14-9442-2864	07-1676-7162
11			18	64.39	23.57	1.924			16-6489-0031	15-5233-2184
12			22	41.11	0.2913	0.02378			02-4639-6440	00-1755-8985
13			25	39.27	-1.554	-0.1269			17-8206-5737	15-9942-8515
14		Sep	3	30.64	-10.18	-0.831			15-0487-5875	08-0190-3799
15			9	33.59	-7.226	-0.5899			20-3803-1763	15-4983-2355
16			16	37.75	-3.069	-0.2505			01-5049-2617	15-5471-0022
17			23	33.24	-7.576	-0.6184			02-0667-4002	20-3091-9441
18		Oct	7	32.97	-7.845	-0.6404			14-5637-2531	13-7332-7338
19			9	29.68	-11.14	-0.9091			13-5792-5239	01-7979-4948
20			13	45.96	5.136	0.4193			11-7429-5626	04-4094-9558
21			30	19.02	-21.8	-1.78			00-9558-0596	15-2024-8469

CETIS Test Data Worksheet

Report Date: 28 Oct-14 15:59 (p 1 of 1)
 Test Code: 00-9558-0596/141030sprt

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 30 Oct-14 Species: Strongylocentrotus purpuratus Sample Code: 141030sprt
 End Date: 30 Oct-14 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 30 Oct-14 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes	
			1	100	0	10/31/14	
			2	100	48		
			3	100	0		
			4	100	0		
			5	100	7		
			6	100	0		
			7	100	81		
			8	100	92		
			9	100	89		
			10	100	57		QC: 56, KB 11/6/14
			11	100	0		
			12	100	47		
			13	100	0		
			14	100	0		
			15	100	35		
			16	100	0		
			17	100	43		
			18	100	7		
			19	100	73		
			20	100	87		
			21	100	0		
			22	100	72		
			23	100	0		
			24	100	0		
			25	100	7		
			26	100	59		
			27	100	57		
			28	100	56 72 80		
			29	100	56 85.9		
			30	100	50		

CETIS Test Data Worksheet

Report Date: 28 Oct-14 15:59 (p 1 of 1)
 Test Code: 00-9558-0596/141030sprt

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Start Date:	30 Oct-14	Species:	Strongylocentrotus purpuratus	Sample Code:	141030sprt		
End Date:	30 Oct-14	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant		
Sample Date:	30 Oct-14	Material:	Copper chloride	Sample Station:	Copper Chloride		

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	7			
0	LC	2	8	100	81	
0	LC	3	20			
0	LC	4	9			
0	LC	5	28			
10		1	19			
10		2	26			
10		3	22			
10		4	27			
10		5	10	100	78	
20		1	15			
20		2	30			
20		3	2	100	48	
20		4	12			
20		5	17			
40		1	29			
40		2	25			
40		3	24	100	4	
40		4	5			
40		5	18			
80		1	1			
80		2	4			
80		3	11	100	0	
80		4	21			
80		5	14			
160		1	16			
160		2	6			
160		3	3	100	0	
160		4	13			
160		5	23			

QC=

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: S. purpuratus

Sample ID: CuCl₂

Start Date/Time: 10/30/2014 13:05 15:05

Test No: 141030sprt

End Date/Time: 10/30/2014 13:45 15:45

Dilutions made by: AC

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	9050

Analyst: [Signature]

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (C)
Lab Control	8.6	8.00	33.5	14.5
10	8.6	8.00	33.5	14.6
20	8.7	8.01	33.6	14.6
40	8.7	8.03	33.5	14.6
80	8.7	8.04	33.4	14.6
160	8.7	8.05	33.2	14.7

Comments: _____

QC Check: KB 11/10/14

Final Review: AC 11/18/14

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: CuCl2
 Test No.: 141030spnt
 Tech initials: S/AC
 Injection Time: 1430

Start Date/Time: 10/30/2014 / AC 1505 1505
 End Date/Time: 10/30/2014 / AC 1545 1545
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 10/29/14

Sperm Absorbance at 400 nm: 0.823 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 58 Mean: 63.4 X 50 = 3170 eggs/ml

107
624
57
71

(target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)

Initial density: 3170 eggs/ml
 Final density: 4000 eggs/ml

$$= \frac{0.79}{0.63} \text{ dilution factor}$$

$$= \frac{1.0}{0.37} \text{ part egg stock}$$

$$\text{parts seawater}$$

egg stock 100 ml
 seawater — ml
 No dilution required.

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Rangefinder Test:	Sperm:Egg Ratio							
	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1440</u>	<u>100:1</u>	<u>73</u>	<u>27</u>
Eggs Added (0.5 ml):	<u>1450</u>	<u>150:1</u>	<u>92</u>	<u>8</u>
Test Ended:	<u>1500</u>	<u>150:1</u>	<u>91</u>	<u>9</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test

Sperm:Egg Ratio Used: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1305 1505</u>	QC1	<u>90</u>	<u>10</u>
Eggs Added (0.5 ml):	<u>1325 1525</u>	QC2	<u>84</u>	<u>16</u>
Test Ended:	<u>1345 1545</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

QC Check:

CB 11/10/14

Final Review:

AC 11/8/14

CETIS Summary Report

Report Date: 05 Aug-15 10:59 (p 1 of 1)
Test Code: 150722sprt | 00-0842-3841

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	07-0709-1109	Test Type:	Fertilization	Analyst:							
Start Date:	22 Jul-15 16:17	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	22 Jul-15 16:57	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	06-8412-4767	Code:	150722sprt	Client:	Internal						
Sample Date:	22 Jul-15	Material:	Copper chloride	Project:							
Receive Date:	22 Jul-15	Source:	Reference Toxicant								
Sample Age:	16h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
21-1097-1868	Fertilization Rate	<10	10	NA	4.04%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
01-2722-3396	Fertilization Rate	EC50	35.94	33.88	38.13		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits	Overlap	Decision				
01-2722-3396	Fertilization Rate	Control Resp		0.962	0.7 - NL	Yes	Passes Acceptability Criteria				
21-1097-1868	Fertilization Rate	Control Resp		0.962	0.7 - NL	Yes	Passes Acceptability Criteria				
21-1097-1868	Fertilization Rate	PMSD		0.04035	NL - 0.25	No	Passes Acceptability Criteria				
Fertilization Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.962	0.9436	0.9804	0.94	0.98	0.006633	0.01483	1.54%	0.0%
10		5	0.814	0.7462	0.8818	0.74	0.87	0.02441	0.05459	6.71%	15.38%
20		5	0.75	0.6905	0.8095	0.7	0.8	0.02145	0.04796	6.39%	22.04%
40		5	0.492	0.3812	0.6028	0.39	0.61	0.03992	0.08927	18.15%	48.86%
80		5	0.008	0.002447	0.01355	0	0.01	0.002	0.004472	55.9%	99.17%
160		5	0	0	0	0	0	0	0		100.0%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.94	0.97	0.96	0.98	0.96					
10		0.74	0.82	0.86	0.87	0.78					
20		0.8	0.79	0.76	0.7	0.7					
40		0.39	0.61	0.54	0.42	0.5					
80		0.01	0.01	0.01	0	0.01					
160		0	0	0	0	0					

CETIS Analytical Report

Report Date: 05 Aug-15 10:59 (p 1 of 2)
 Test Code: 150722sprt | 00-0842-3841

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
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Analysis ID: 21-1097-1868	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 05 Aug-15 10:59	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.04%	<10	10	NA	

Dunnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	6.563	2.305	0.088	8	<0.0001	CDF	Significant Effect
		20*	8.653	2.305	0.088	8	<0.0001	CDF	Significant Effect
		40*	15.79	2.305	0.088	8	<0.0001	CDF	Significant Effect
		80*	33.86	2.305	0.088	8	<0.0001	CDF	Significant Effect

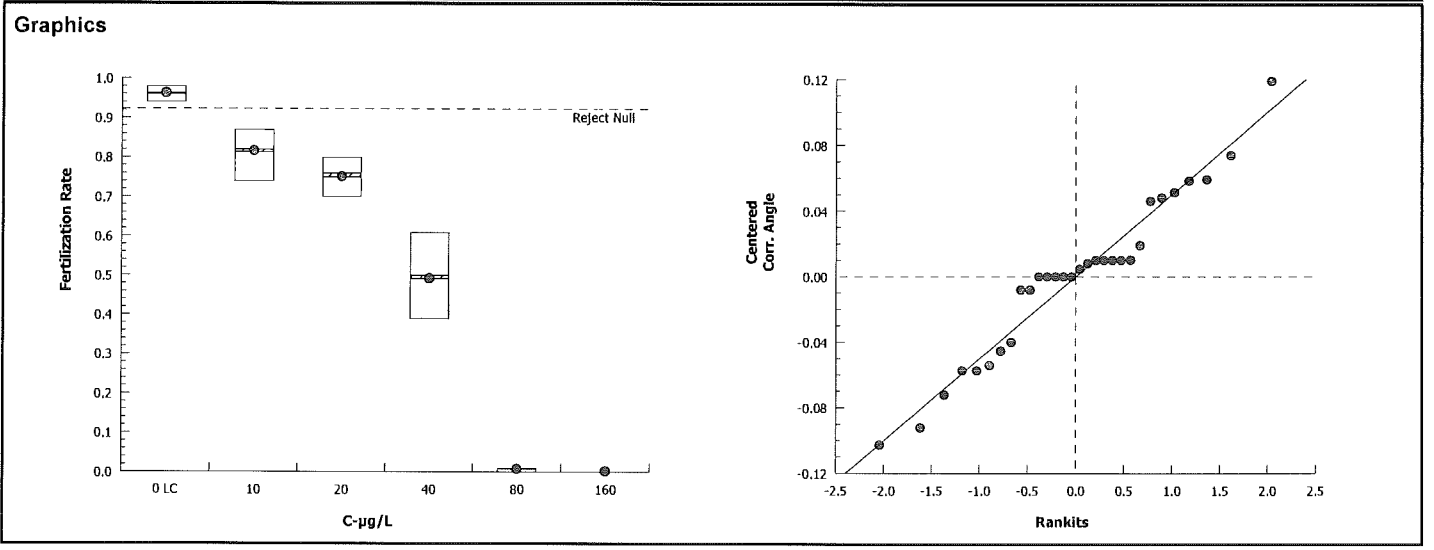
ANOVA Table							
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)	
Between	4.859248	1.214812	4	336.2	<0.0001	Significant Effect	
Error	0.07226564	0.003613282	20				
Total	4.931514		24				

Distributional Tests						
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)	
Variances	Bartlett Equality of Variance	6.887	13.28	0.1420	Equal Variances	
Distribution	Shapiro-Wilk W Normality	0.9687	0.8877	0.6133	Normal Distribution	

Fertilization Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.962	0.9436	0.9804	0.96	0.94	0.98	0.006633	1.54%	0.0%
10		5	0.814	0.7462	0.8818	0.82	0.74	0.87	0.02441	6.71%	15.38%
20		5	0.75	0.6905	0.8095	0.76	0.7	0.8	0.02145	6.39%	22.04%
40		5	0.492	0.3812	0.6028	0.5	0.39	0.61	0.03992	18.15%	48.86%
80		5	0.008	0.002447	0.01355	0.01	0	0.01	0.002	55.9%	99.17%
160		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.378	1.329	1.426	1.369	1.323	1.429	0.01743	2.83%	0.0%
10		5	1.128	1.041	1.215	1.133	1.036	1.202	0.03129	6.2%	18.11%
20		5	1.049	0.9799	1.117	1.059	0.9912	1.107	0.02476	5.28%	23.88%
40		5	0.7773	0.6657	0.889	0.7854	0.6745	0.8963	0.04021	11.57%	43.57%
80		5	0.09014	0.06229	0.118	0.1002	0.05002	0.1002	0.01003	24.88%	93.46%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.37%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 21-1097-1868	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 05 Aug-15 10:59	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



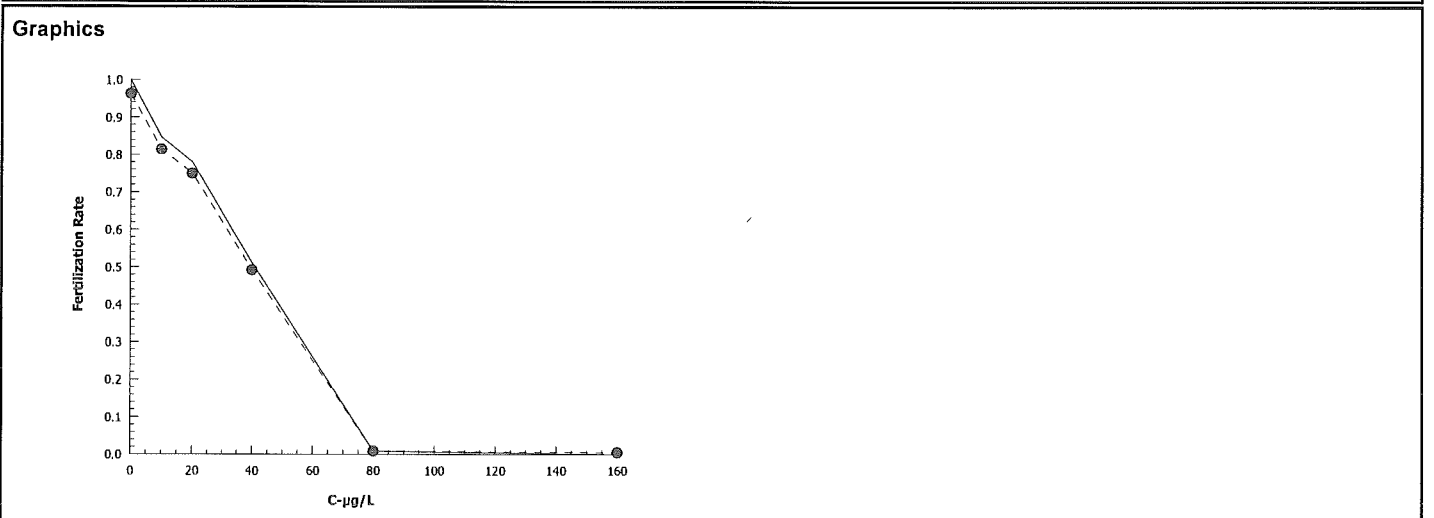
CETIS Analytical Report

Report Date: 05 Aug-15 10:59 (p 1 of 1)
 Test Code: 150722sprt | 00-0842-3841

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 01-2722-3396	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 05 Aug-15 10:59	Analysis: Trimmed Spearman-Kärber	Official Results: Yes			

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.038	15.38%	1.556	0.01281	35.94	33.88	38.13

Fertilization Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.962	0.94	0.98	0.006633	0.01483	1.54%	0.0%	481	500
10		5	0.814	0.74	0.87	0.02441	0.05459	6.71%	15.38%	407	500
20		5	0.75	0.7	0.8	0.02145	0.04796	6.39%	22.04%	375	500
40		5	0.492	0.39	0.61	0.03992	0.08927	18.15%	48.86%	246	500
80		5	0.008	0	0.01	0.002	0.004472	55.9%	99.17%	4	500
160		5	0	0	0	0	0		100.0%	0	500



Echinoid Sperm Cell Fertilization Test 15C

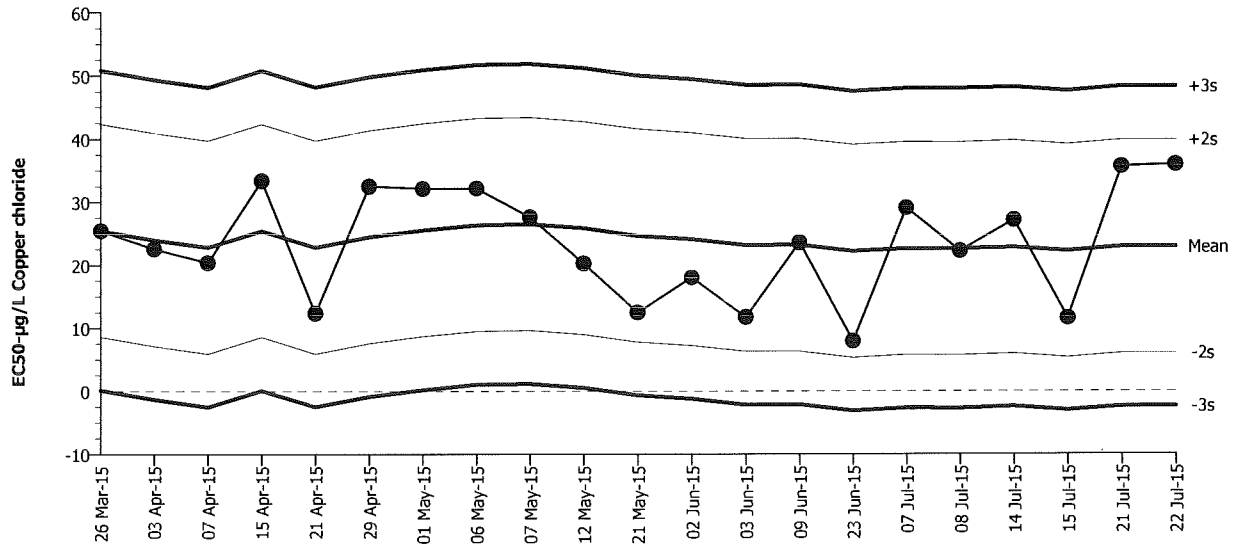
Nautilus Environmental (CA)

Test Type: Fertilization
 Protocol: EPA/600/R-95/136 (1995)

Organism: Strongylocentrotus purpuratus (Purpl)
 Endpoint: Fertilization Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



Mean: 22.93 Count: 20 -2s Warning Limit: 6.036 -3s Action Limit: -2.409
 Sigma: 8.445 CV: 36.80% +2s Warning Limit: 39.82 +3s Action Limit: 48.26

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2015	Mar	26	14:14	25.45	2.524	0.2989			18-4459-4447	20-9665-3202
2		Apr	3	14:01	22.56	-0.3686	-0.04365			07-8763-8572	13-9279-9843
3			7	13:45	20.36	-2.572	-0.3046			14-7381-4605	00-4113-7336
4			15	12:21	33.41	10.48	1.242			18-4629-1325	01-3982-8535
5			21	14:45	12.37	-10.56	-1.25			04-6687-0700	00-9564-1182
6			29	13:12	32.5	9.574	1.134			00-7279-3461	06-1313-6314
7		May	1	14:45	32.13	9.201	1.09			08-3887-8784	07-0987-3806
8			6	16:10	32.21	9.284	1.099			07-9821-3669	18-2350-8328
9			7	15:45	27.64	4.712	0.558			05-8209-5022	06-2905-6100
10			12	14:42	20.26	-2.668	-0.3159			20-1583-4383	17-4186-2489
11			21	15:38	12.5	-10.43	-1.236			03-1600-9362	13-2344-9592
12		Jun	2	14:10	18.03	-4.896	-0.5797			03-8226-7406	18-7623-4531
13			3	15:00	11.77	-11.16	-1.322			01-6843-4438	09-0678-9638
14			9	15:50	23.6	0.6746	0.07988			01-7239-4208	17-5901-6261
15			23	16:10	7.931	-15	-1.776			10-4967-2005	14-2593-5380
16		Jul	7	14:33	29.07	6.143	0.7274			18-8004-9621	11-4682-8456
17			8	14:38	22.27	-0.6565	-0.07774			00-9935-8934	02-7693-7150
18			14	15:00	27.18	4.252	0.5035			17-8977-4567	05-9270-4497
19			15	14:45	11.62	-11.31	-1.34			03-6006-9976	15-4075-1359
20			21	10:41	35.64	12.71	1.505			15-5568-4316	12-3562-8162
21			22	16:17	35.94	13.01	1.541			00-0842-3841	01-2722-3396

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:43 (p 1 of 1)
 Test Code: 00-0842-3841/150722sprt

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 22 Jul-15 Species: Strongylocentrotus purpuratus Sample Code: 150722sprt
 End Date: 22 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	98	7/31/15
			2	100	1	
			3	100	74	
			4	100	0	
			5	100	0	
			6	100	78	
			7	100	79	
			8	100	96	
			9	100	50	
			10	100	80	
			11	100	70	
			12	100	42	
			13	100	97	
			14	100	70	
			15	100	0	
			16	100	76	
			17	100	87	
			18	100	82	
			19	100	54	
			20	100	1	
			21	100	86	
			22	100	61	
			23	100	1	
			24	100	0	
			25	100	0	
			26	100	0	
			27	100	94	
			28	100	1	
			29	100	39	
			30	100	96	

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:43 (p 1 of 1)
 Test Code: 00-0842-3841/150722sprt

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 22 Jul-15 Species: Strongylocentrotus purpuratus Sample Code: 150722sprt
 End Date: 22 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	27			
0	LC	2	13	100	96	g 7/22/15
0	LC	3	30			
0	LC	4	1			
0	LC	5	8			
10		1	3			
10		2	18			
10		3	21	100	95	
10		4	17			
10		5	6			
20		1	10			
20		2	7	100	87	
20		3	16			
20		4	14			
20		5	11			
40		1	29			
40		2	22			
40		3	19	100	38	
40		4	12			
40		5	9			
80		1	20			
80		2	23			
80		3	2	100	0	
80		4	26			
80		5	28			
160		1	5			
160		2	24			
160		3	25			
160		4	4	100	0	
160		5	15			

QC 1.7

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: S. purpuratus

Sample ID: CuCl₂

Start Date/Time: 7/22/2015 1617

Test No: 150722^{3P}_{cert}
KB 8/14/15

End Date/Time: 7/22/2015 1657

Dilutions made by: AC

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	9.0
Final Volume (mL):	500
Cu stock concentration (µg/L):	8,800

Analyst: AD

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (C)
Lab Control	8.9	8.09	33.3	14.9
10	8.8	8.08	33.5	14.5
20	8.7	8.08	33.5	14.9
40	8.7	8.09	33.4	14.8
80	8.7	8.09	33.3	14.7
160	8.8	8.10	33.1	14.8

Comments: _____

QC Check: KB 8/14/15

Final Review: VCR 8/16/15

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: CUC12
 Test No.: 150722spt
 Tech initials: Y
 Injection Time: 1525

Start Date/Time: 7/22/15, 1617
 End Date/Time: 7/22/15, 1657
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 7/9/15

Sperm Absorbance at 400 nm: 0.935 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 87 Mean: 81.8 x 50 = 4090 eggs/ml
82
80 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter
79 slide for a final density of 4000 eggs/ml)
81

Initial density: 4090 eggs/ml = 1.02 dilution factor egg stock 150 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater ml
0.02 parts seawater

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Rangefinder Test:	Sperm:Egg Ratio							
	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1538</u>	<u>100:1</u>	<u>63</u>	<u>37</u>
Eggs Added (0.5 ml):	<u>1553</u>	<u>200:1</u>	<u>84</u>	<u>16</u>
Test Ended:	<u>1603</u>	<u>400:1</u>	<u>97</u>	<u>3</u>
		<u>400:1</u>	<u>95</u>	<u>5</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 350:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1617</u>	QC1	<u>91</u>	<u>9</u>
Eggs Added (0.5 ml):	<u>1637</u>	QC2	<u>93</u>	<u>7</u>
Test Ended:	<u>1657</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

QC Check: KB8/4/15 Final Review: VCR 8/6/15

Sand Dollar Fertilization

CETIS Summary Report

Report Date: 10 Nov-14 10:46 (p 1 of 1)
 Test Code: 141030dert | 15-5280-7209

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	08-7037-7425	Test Type:	Fertilization	Analyst:							
Start Date:	30 Oct-14 16:10	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	30 Oct-14 16:50	Species:	Dendraster excentricus	Brine:	Not Applicable						
Duration:	40m	Source:	Mission Bay	Age:							
Sample ID:	04-7273-8124	Code:	141030dert	Client:	Internal						
Sample Date:	30 Oct-14	Material:	Copper chloride	Project:							
Receive Date:	30 Oct-14	Source:	Reference Toxicant								
Sample Age:	16h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
19-1439-3601	Fertilization Rate	10	20	14.14	3.85%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
15-6995-0711	Fertilization Rate	EC50	26.22	25.25	27.23		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
15-6995-0711	Fertilization Rate	Control Resp	0.962	0.7 - NL	Yes	Passes Acceptability Criteria					
19-1439-3601	Fertilization Rate	Control Resp	0.962	0.7 - NL	Yes	Passes Acceptability Criteria					
19-1439-3601	Fertilization Rate	PMSD	0.03849	NL - 0.25	No	Passes Acceptability Criteria					
Fertilization Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.962	0.9523	0.9717	0.92	0.99	0.01158	0.02588	2.69%	0.0%
2.5		5	0.984	0.9807	0.9873	0.97	0.99	0.004	0.008945	0.91%	-2.29%
5		5	0.986	0.984	0.988	0.98	0.99	0.00245	0.005479	0.56%	-2.5%
10		5	0.964	0.9506	0.9774	0.91	1	0.016	0.03578	3.71%	-0.21%
20		5	0.704	0.6838	0.7242	0.67	0.8	0.02421	0.05413	7.69%	26.82%
40		5	0.172	0.1572	0.1868	0.12	0.21	0.01772	0.03962	23.04%	82.12%
80		5	0.008	0.004876	0.01112	0	0.02	0.003742	0.008367	104.6%	99.17%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.97	0.96	0.92	0.97	0.99					
2.5		0.98	0.99	0.97	0.99	0.99					
5		0.98	0.99	0.98	0.99	0.99					
10		1	0.91	0.95	0.97	0.99					
20		0.8	0.68	0.68	0.69	0.67					
40		0.12	0.21	0.19	0.14	0.2					
80		0.01	0.02	0	0	0.01					

CETIS Analytical Report

Report Date: 10 Nov-14 10:46 (p 1 of 2)
 Test Code: 141030dert | 15-5280-7209

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
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Analysis ID: 19-1439-3601	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.4
Analyzed: 10 Nov-14 10:46	Analysis: Parametric-Control vs Treatments	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	NOEL	LOEL	TOEL	TU	PMSD
Angular (Corrected)	NA	C > T	NA	NA	10	20	14.14		3.85%

Dunnnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.709	2.407	0.090	8	0.9988	CDF	Non-Significant Effect
		5	-1.88	2.407	0.090	8	0.9993	CDF	Non-Significant Effect
		10	-0.4377	2.407	0.090	8	0.9442	CDF	Non-Significant Effect
		20*	10.32	2.407	0.090	8	<0.0001	CDF	Significant Effect
		40*	25.58	2.407	0.090	8	<0.0001	CDF	Significant Effect
		80*	34.58	2.407	0.090	8	<0.0001	CDF	Significant Effect

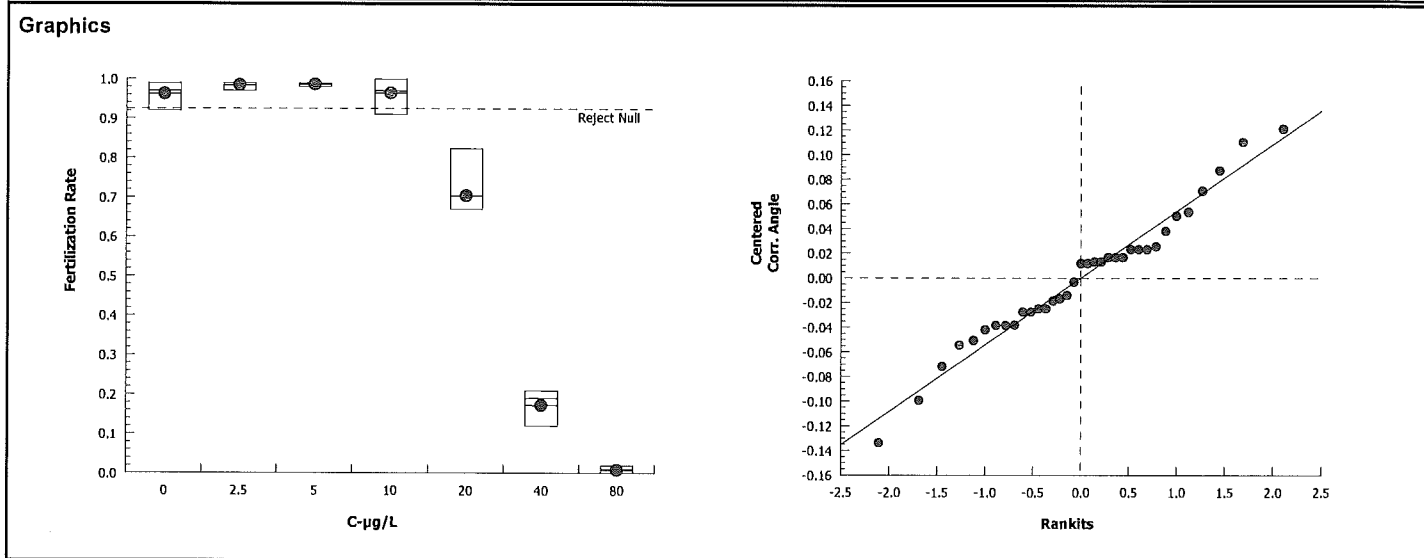
ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	9.343492	1.557249	6	444.1	<0.0001	Significant Effect
Error	0.09819294	0.00350689	28			
Total	9.441685		34			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.709	16.81	0.1374	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9771	0.9146	0.6641	Normal Distribution

Fertilization Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.962	0.9299	0.9941	0.97	0.92	0.99	0.01158	2.69%	0.0%
2.5		5	0.984	0.9729	0.9951	0.99	0.97	0.99	0.004	0.91%	-2.29%
5		5	0.986	0.9792	0.9928	0.99	0.98	0.99	0.00245	0.56%	-2.5%
10		5	0.964	0.9196	1	0.97	0.91	1	0.016	3.71%	-0.21%
20		5	0.704	0.6368	0.7712	0.68	0.67	0.8	0.02421	7.69%	26.82%
40		5	0.172	0.1228	0.2212	0.19	0.12	0.21	0.01772	23.04%	82.12%
80		5	0.008	0	0.01839	0.01	0	0.02	0.003742	104.6%	99.17%

Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.384	1.3	1.467	1.397	1.284	1.471	0.03003	4.85%	0.0%
2.5		5	1.447	1.406	1.489	1.471	1.397	1.471	0.01505	2.33%	-4.63%
5		5	1.454	1.426	1.482	1.471	1.429	1.471	0.01022	1.57%	-5.09%
10		5	1.4	1.275	1.525	1.397	1.266	1.521	0.045	7.19%	-1.19%
20		5	0.9971	0.9201	1.074	0.9695	0.9589	1.107	0.02773	6.22%	27.93%
40		5	0.4256	0.3588	0.4924	0.451	0.3537	0.476	0.02406	12.64%	69.24%
80		5	0.08845	0.04003	0.1369	0.1002	0.05002	0.1419	0.01744	44.09%	93.61%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 19-1439-3601	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.4	
Analyzed: 10 Nov-14 10:46	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



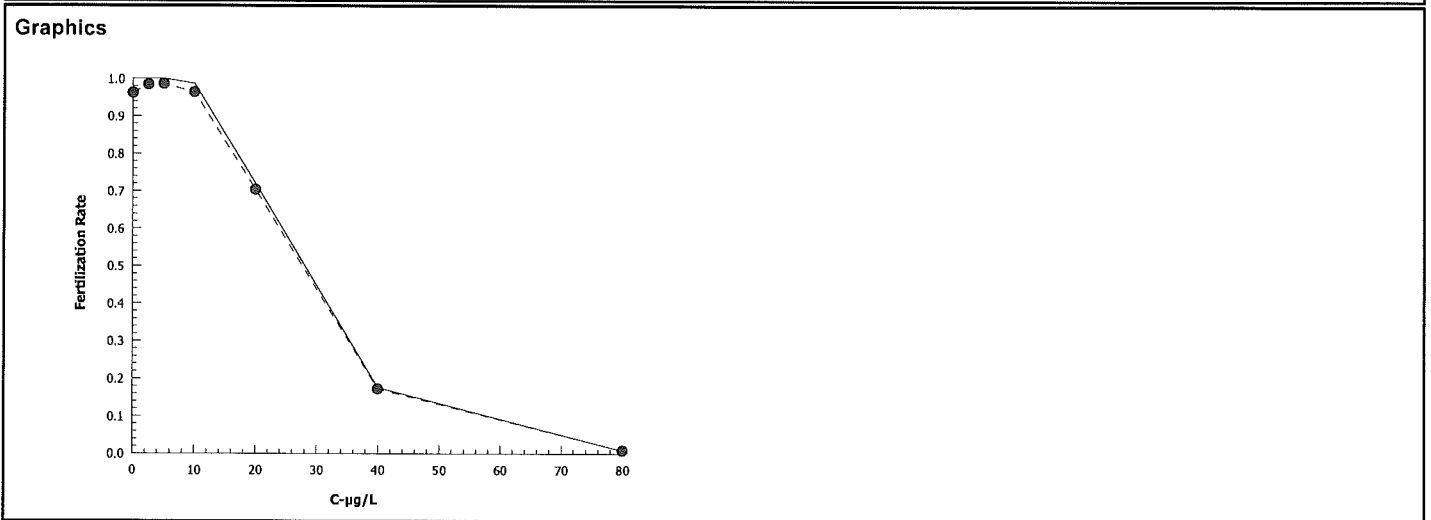
CETIS Analytical Report

Report Date: 10 Nov-14 10:46 (p 1 of 1)
 Test Code: 141030dert | 15-5280-7209

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 15-6995-0711	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.4			
Analyzed: 10 Nov-14 10:46	Analysis: Trimmed Spearman-Kärber	Official Results: Yes			

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.038	0.82%	1.419	0.008184	26.22	25.25	27.23

Fertilization Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.962	0.92	0.99	0.01158	0.02588	2.69%	0.0%	481	500
2.5		5	0.984	0.97	0.99	0.004	0.008945	0.91%	-2.29%	492	500
5		5	0.986	0.98	0.99	0.00245	0.005479	0.56%	-2.5%	493	500
10		5	0.964	0.91	1	0.016	0.03578	3.71%	-0.21%	482	500
20		5	0.704	0.67	0.8	0.02421	0.05413	7.69%	26.82%	352	500
40		5	0.172	0.12	0.21	0.01772	0.03962	23.04%	82.12%	86	500
80		5	0.008	0	0.02	0.003742	0.008367	104.6%	99.17%	4	500



Echinoid Sperm Cell Fertilization Test 15C

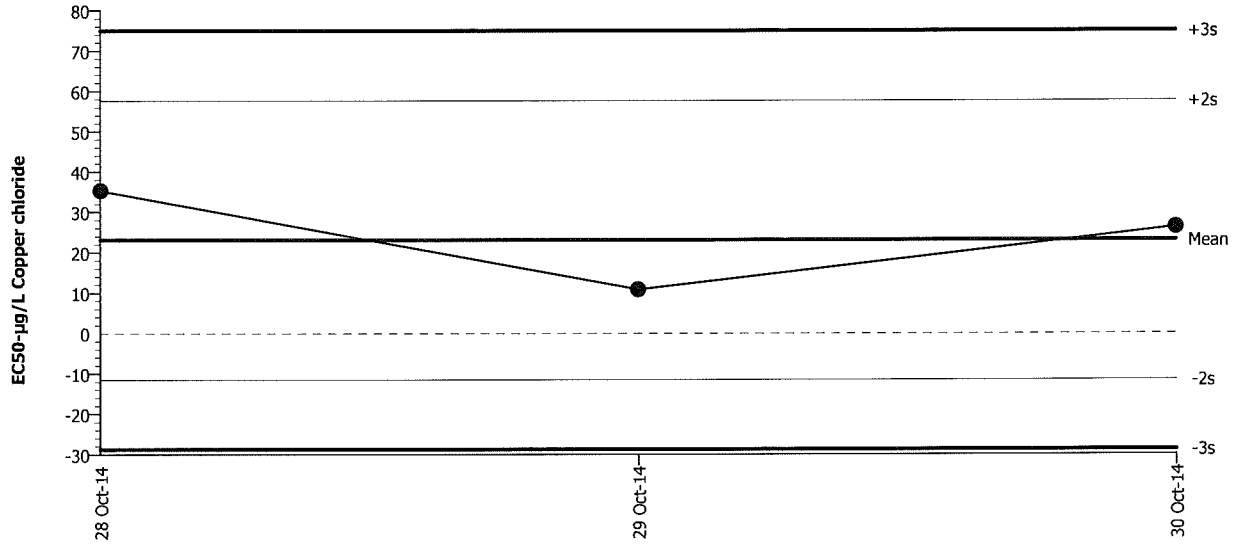
Nautilus Environmental (CA)

Test Type: Fertilization
 Protocol: EPA/600/R-95/136 (1995)

Organism: Dendraster excentricus (Sand Dollar)
 Endpoint: Fertilization Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



Mean: 23.07 Count: 2 -2s Warning Limit: -11.49 -3s Action Limit: -28.77
 Sigma: 17.28 CV: 74.90% +2s Warning Limit: 57.63 +3s Action Limit: 74.91

Quality Control Data

Point	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Oct	28	35.29	12.22	0.7071			13-6517-2086	18-6351-1063
2			29	10.85	-12.22	-0.7069			08-2984-3114	08-2412-4232
3			30	26.22	3.151	0.1823			15-5280-7209	15-6995-0711

CETIS Test Data Worksheet

Report Date: 28 Oct-14 15:56 (p 1 of 1)
 Test Code: 15-5280-7209/141030dert

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)
Start Date:	30 Oct-14	Species:	Dendraster excentricus
End Date:	30 Oct-14	Protocol:	EPA/600/R-95/136 (1995)
Sample Date:	30 Oct-14	Material:	Copper chloride
		Sample Code:	141030dert
		Sample Source:	Reference Toxicant
		Sample Station:	Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	1	11/7/14
			2	100	100	
			3	100	20	
			4	100	21	QC check: 19, KB 11/10/14
			5	100	69	QC check: 72, KB 11/10/14
			6	100	91	
			7	100	2	
			8	100	1	
			9	100	97	
			10	100	80	
			11	100	0	
			12	100	99	
			13	100	99	
			14	100	12	
			15	100	99	
			16	100	98	
			17	100	0	
			18	100	67	
			19	100	99	
			20	100	19	
			21	100	68	
			22	100	95	
			23	100	99	
			24	100	98	
			25	100	99	
			26	100	97	
			27	100	97	
			28	100	68	
			29	100	99	
			30	100	14	
			31	100	99	
			32	100	97	
			33	100	96	
			34	100	92	
			35	100	98	

CETIS Test Data Worksheet

Report Date: 28 Oct-14 15:56 (p 1 of 1)
 Test Code: 15-5280-7209/141030dert

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 30 Oct-14 Species: Dendraster excentricus Sample Code: 141030dert
 End Date: 30 Oct-14 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 30 Oct-14 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	9	100	97	
0	LC	2	33			
0	LC	3	34			
0	LC	4	32			
0	LC	5	23			
2.5		1	35	100	97	
2.5		2	15			
2.5		3	27			
2.5		4	13			
2.5		5	25			
5		1	16	100	100	
5		2	29			
5		3	24			
5		4	19			
5		5	12			
10		1	2	100	92	
10		2	6			
10		3	22			
10		4	26			
10		5	31			
20		1	10	100	72	
20		2	28			
20		3	21			
20		4	5			
20		5	18			
40		1	14	100	10	
40		2	4			
40		3	20			
40		4	30			
40		5	3			
80		1	1			
80		2	7			
80		3	11	100	1	
80		4	17			
80		5	8			

QC/AC

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: D. excentricus

Sample ID: CuCl₂

Start Date/Time: 10/30/2014 16:10

Test No: 141030dert

End Date/Time: 10/30/2014 16:50

Dilutions made by: AC

High conc. made (µg/L):	80
Vol. Cu stock added (mL):	4.4
Final Volume (mL):	500
Cu stock concentration (µg/L):	9050

Analyst: LS

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.02	33.4	14.8
2.5	8.7	8.03	33.4	14.5
5	8.8	8.04	33.4	14.4
10	8.8	8.06	33.5	14.4
20	8.8	8.06	33.5	14.4
40	8.8	8.07	33.4	14.3
80	8.9	8.08	33.3	14.4

Comments: _____

QC Check: KB 11/10/14

Final Review: AC 11/18/14

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: 141030 derf
 Test No.: CuCl2

Start Date/Time: 10/30/2014 / 16:10
 End Date/Time: 10/30/2014 / 16:50
 Species: D. excentricus
 Animal Source: Mission Bay
 Date Collected: 10/24/14

Tech initials: AC
 Injection Time: 15:15

Sperm Absorbance at 400 nm: 0.744 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 37 Mean: 43.8 X 50 = 2190 eggs/ml
41
34 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter
43 slide for a final density of 4000 eggs/ml)
64

Initial density: 2190 eggs/ml = 0.55 dilution factor egg stock 2 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 2 ml
0.45 parts seawater 45 ml poured at per 100 after settling

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Rangefinder Test:	Sperm:Egg Ratio								
	2000:1	1600:1	1200:1	800:1	400:1	200:1	150:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	3.75	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	46.25	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>15:35</u>	<u>50</u>	<u>67</u>	<u>33</u>
Eggs Added (0.5 ml):	<u>15:50</u>	<u>100</u>	<u>90</u>	<u>10</u>
Test Ended:	<u>16:00</u>	<u>150</u>	<u>96</u>	<u>4</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>16:10</u>	QC1	<u>98</u>	<u>2</u>
Eggs Added (0.5 ml):	<u>16:30</u>	QC2	<u>97</u>	<u>3</u>
Test Ended:	<u>16:50</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

QC Check: KB 11/10/14 Final Review: AC 11/18/14

CETIS Summary Report

Report Date: 05 Aug-15 09:29 (p 1 of 1)
 Test Code: 150722dert | 10-2804-3517

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Batch ID: 06-2792-8877	Test Type: Fertilization	Analyst:
Start Date: 22 Jul-15 15:20	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 22 Jul-15 16:00	Species: Dendroaster excentricus	Brine: Not Applicable
Duration: 40m	Source: Mission Bay	Age:

Sample ID: 10-5741-2857	Code: 150722dert	Client: Internal
Sample Date: 22 Jul-15	Material: Copper chloride	Project:
Receive Date: 22 Jul-15	Source: Reference Toxicant	
Sample Age: 15h	Station: Copper Chloride	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-8653-0565	Fertilization Rate	<10	10	NA	8.25%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
10-4495-5773	Fertilization Rate	EC50	16.78	16.14	17.45		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
10-4495-5773	Fertilization Rate	Control Resp	0.874	0.7 - NL	Yes	Passes Acceptability Criteria
14-8653-0565	Fertilization Rate	Control Resp	0.874	0.7 - NL	Yes	Passes Acceptability Criteria
14-8653-0565	Fertilization Rate	PMSD	0.08252	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.874	0.8332	0.9148	0.83	0.92	0.0147	0.03286	3.76%	0.0%
10		5	0.768	0.6745	0.8615	0.65	0.84	0.03367	0.0753	9.81%	12.13%
20		5	0.304	0.2092	0.3988	0.21	0.41	0.03415	0.07635	25.12%	65.22%
40		5	0	0	0	0	0	0	0		100.0%
80		5	0	0	0	0	0	0	0		100.0%
160		5	0	0	0	0	0	0	0		100.0%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.92	0.88	0.88	0.83	0.86
10		0.74	0.8	0.84	0.81	0.65
20		0.3	0.34	0.26	0.21	0.41
40		0	0	0	0	0
80		0	0	0	0	0
160		0	0	0	0	0

CETIS Analytical Report

Report Date: 05 Aug-15 09:29 (p 1 of 1)
 Test Code: 150722dert | 10-2804-3517

Echinoid Sperm Cell Fertilization Test 15C					Nautilus Environmental (CA)				
Analysis ID: 14-8653-0565	Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7				
Analyzed: 05 Aug-15 9:28	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	8.25%	<10	10	NA	

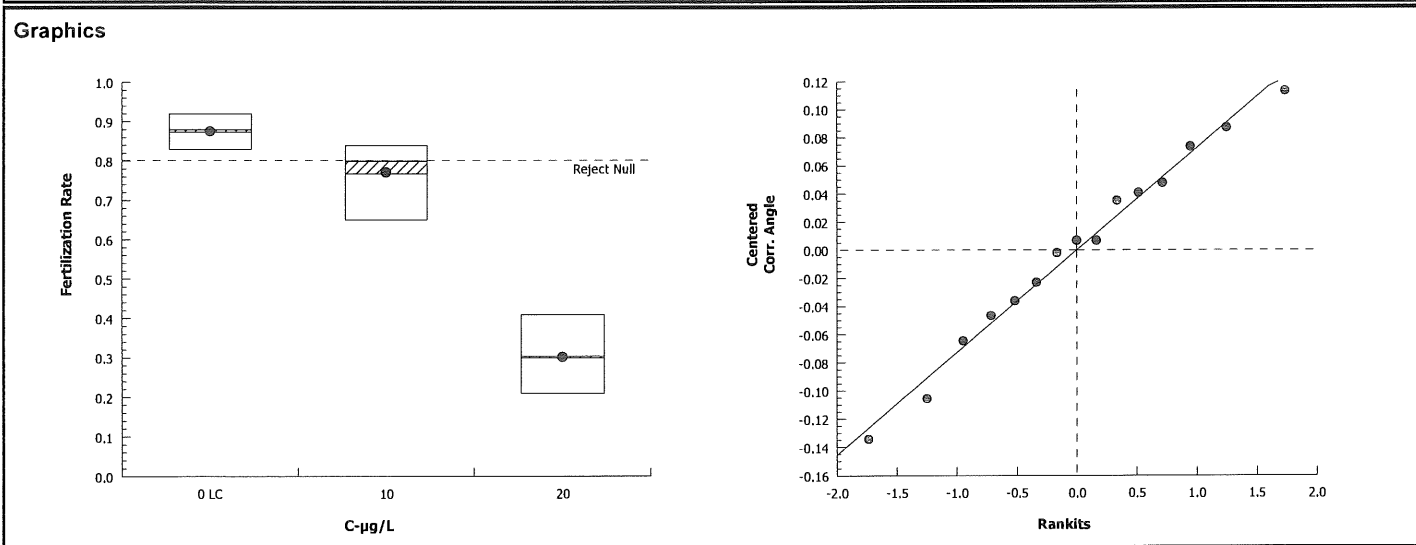
Dunnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	2.894	2.108	0.101	8	0.0123	CDF	Significant Effect
		20*	13.15	2.108	0.101	8	<0.0001	CDF	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.091132	0.5455661	2	95.55	<0.0001	Significant Effect
Error	0.06851911	0.005709926	12			
Total	1.159651		14			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.147	9.21	0.5636	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.982	0.8328	0.9812	Normal Distribution

Fertilization Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.874	0.8332	0.9148	0.88	0.83	0.92	0.0147	3.76%	0.0%
10		5	0.768	0.6745	0.8615	0.8	0.65	0.84	0.03367	9.81%	12.13%
20		5	0.304	0.2092	0.3988	0.3	0.21	0.41	0.03415	25.12%	65.22%

Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.21	1.147	1.273	1.217	1.146	1.284	0.0226	4.18%	0.0%
10		5	1.072	0.9636	1.18	1.107	0.9377	1.159	0.03904	8.14%	11.43%
20		5	0.5816	0.4781	0.6852	0.5796	0.476	0.6949	0.0373	14.34%	51.94%



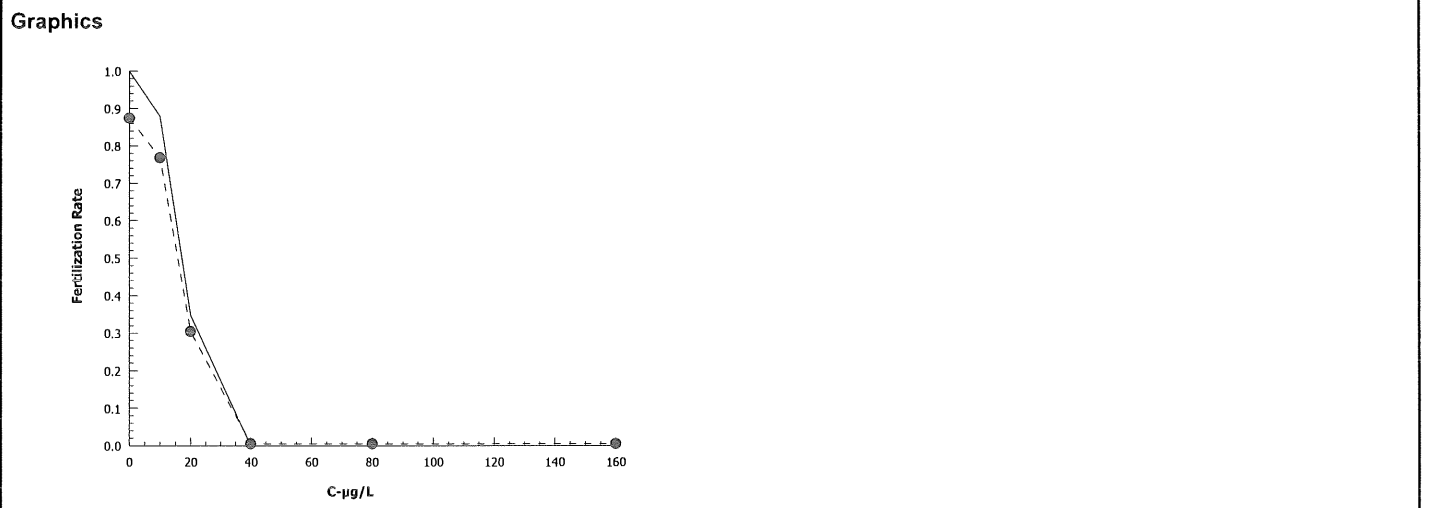
CETIS Analytical Report

Report Date: 05 Aug-15 09:29 (p 1 of 1)
 Test Code: 150722dert | 10-2804-3517

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID: 10-4495-5773	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7			
Analyzed: 05 Aug-15 9:28	Analysis: Trimmed Spearman-Kärber	Official Results: Yes			

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.126	12.13%	1.225	0.008463	16.78	16.14	17.45

Fertilization Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.874	0.83	0.92	0.0147	0.03286	3.76%	0.0%	437	500
10		5	0.768	0.65	0.84	0.03367	0.0753	9.81%	12.13%	384	500
20		5	0.304	0.21	0.41	0.03415	0.07635	25.12%	65.22%	152	500
40		5	0	0	0	0	0		100.0%	0	500
80		5	0	0	0	0	0		100.0%	0	500
160		5	0	0	0	0	0		100.0%	0	500



Echinoid Sperm Cell Fertilization Test 15C

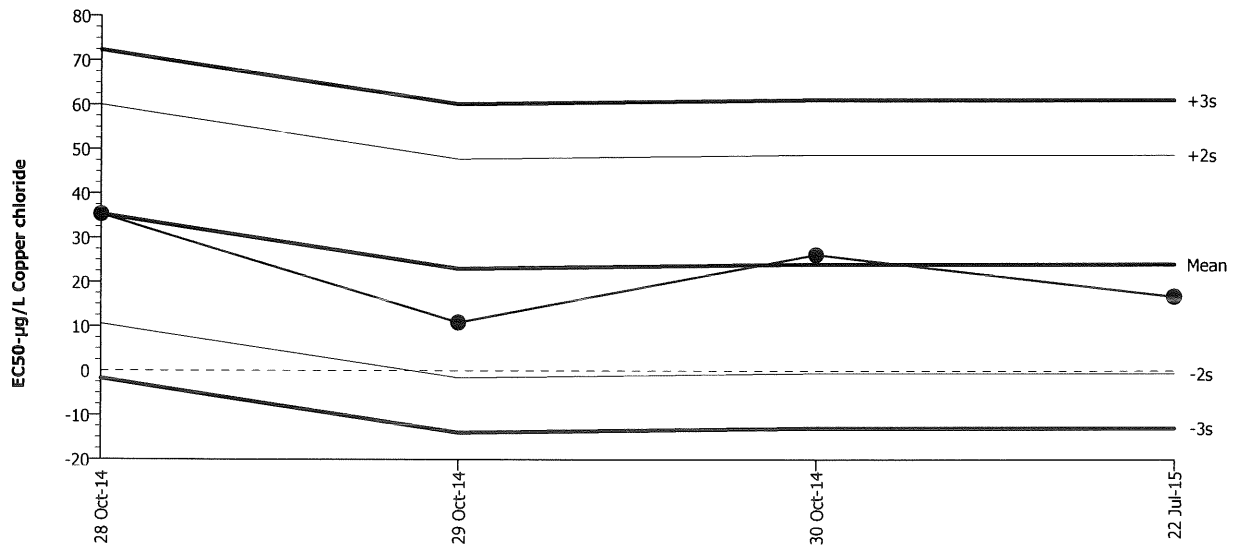
Nautilus Environmental (CA)

Test Type: Fertilization
 Protocol: EPA/600/R-95/136 (1995)

Organism: Dendraster excentricus (Sand Dollar)
 Endpoint: Fertilization Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



Mean: 24.12 Count: 3 -2s Warning Limit: -0.5786 -3s Action Limit: -12.93
 Sigma: 12.35 CV: 51.20% +2s Warning Limit: 48.82 +3s Action Limit: 61.17

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Oct	28	13:00	35.29	11.17	0.9043			13-6517-2086	18-6351-1063
2			29	15:45	10.85	-13.27	-1.074			08-2984-3114	08-2412-4232
3			30	16:10	26.22	2.101	0.1701			15-5280-7209	15-6995-0711
4	2015	Jul	22	15:20	16.78	-7.338	-0.5941			10-2804-3517	10-4495-5773

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:52 (p 1 of 1)
 Test Code: 10-2804-3517/150722dert

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 22 Jul-15 Species: Dendraster excentricus Sample Code: 150722dert
 End Date: 22 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	88	
			2	100	26	
			3	100	0	
			4	100	74	
			5	100	21	
			6	100	0	
			7	100	0	
			8	100	65	R 16 B5 6/4/15
			9	100	88	AC 8/4/15
			10	100	0	
			11	100	83	
			12	100	0	
			13	100	0	
			14	100	0	
			15	100	0	
			16	100	84	
			17	100	0	
			18	100	30	
			19	100	0	
			20	100	80	
			21	100	0	
			22	100	86	
			23	100	0	
			24	100	92	
			25	100	81	
			26	100	0	
			27	100	41	
			28	100	0	
			29	100	0	
			30	100	34	

CETIS Test Data Worksheet

Report Date: 21 Jul-15 10:52 (p 1 of 1)
 Test Code: 10-2804-3517/150722dert

Echinoid Sperm Cell Fertilization Test 15C **Nautilus Environmental (CA)**

Start Date: 22 Jul-15 Species: Dendraster excentricus Sample Code: 150722dert
 End Date: 22 Jul-15 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 22 Jul-15 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	24	100	89	AC 7/22/15
0	LC	2	9			
0	LC	3	1			
0	LC	4	11			
0	LC	5	22			
10		1	4	100	89	AC 7/22/15
10		2	20			
10		3	16			
10		4	25			
10		5	8			
20		1	18	100	37	AC 7/22/15
20		2	30			
20		3	2			
20		4	5			
20		5	27			
40		1	17	100	0	AC 7/22/15
40		2	29			
40		3	6			
40		4	12			
40		5	10			
80		1	19	100	0	AC 7/22/15
80		2	14			
80		3	7			
80		4	23			
80		5	3			
160		1	26	100	0	AC 7/22/15
160		2	28			
160		3	13			
160		4	15			
160		5	21			

QC: y

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: D. excentricus

Sample ID: CuCl₂

Start Date/Time: 7/22/2015 1520

Test No: 150722^{olert}dert
AD Q18 7/21/15

End Date/Time: 7/22/2015 1600

Dilutions made by: AC

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	9.0
Final Volume (mL):	500
Cu stock concentration (µg/L):	8,850

Analyst: AD

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.9	8.09	33.3	14.9
10	8.8	8.08	33.5	14.5
20	8.7	8.08	33.5	14.9
40	8.7	8.09	33.4	14.8
80	8.7	8.09	33.3	14.7
160	8.8	8.10	33.1	14.8

Comments: _____

QC Check: AC 8/5/15

Final Review: YS 8/7/15

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: cu012
 Test No.: 150722 ~~detd~~ AC @ 1403/15
 def+
 Tech initials: AC
 Injection Time: 1430

Start Date/Time: 7/22/15, 1520
 End Date/Time: 7/22/15, 1600
 Species: D. excentricus
 Animal Source: Mission Bay
 Date Collected: 7/17/2015

Sperm Absorbance at 400 nm: 1.0 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 68 Mean: 63.8 X 50 = 3190 eggs/ml
69
54
58
70
 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)

Initial density: 3190 eggs/ml = 0.8 dilution factor egg stock ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater ml
 - parts seawater no dilution

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

Rangefinder Test:	Sperm:Egg Ratio							
	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1445</u>	<u>50</u>	<u>86</u>	<u>14</u>
Eggs Added (0.5 ml):	<u>1500</u>	<u>100</u>	<u>94</u>	<u>6</u>
Test Ended:	<u>1510</u>	<u>-</u>	<u>-</u>	<u>-</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test Sperm:Egg Ratio Used: 100

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1520</u>	QC1	<u>97</u>	<u>3</u>
Eggs Added (0.5 ml):	<u>1540</u>	QC2	<u>87</u>	<u>13</u>
Test Ended:	<u>1600</u>	Egg Control 1	<u>0</u>	<u>-</u>
		Egg Control 2	<u>0</u>	<u>-</u>

Comments: _____

QC Check: AC 8/5/15 Final Review: 8/7/15

Giant Kelp

CETIS Summary Report

Report Date: 02 Jun-15 17:34 (p 1 of 2)

Test Code: 150513mprt | 08-7733-4797

Macrocystis Germination and Germ Tube Growth Test Nautilus Environmental (CA)

Batch ID: 18-3814-7553	Test Type: Growth-Germination	Analyst:
Start Date: 13 May-15 12:20	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater
Ending Date: 15 May-15 11:15	Species: Macrocystis pyrifera	Brine: Not Applicable
Duration: 47h	Source: La Jolla Cove	Age:

Sample ID: 17-2247-4247	Code: 150513mprt	Client: Internal
Sample Date: 13 May-15	Material: Copper chloride	Project:
Receive Date: 13 May-15	Source: Reference Toxicant	
Sample Age: 12h	Station: Copper Chloride	

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-4945-1639	Germination Rate	10	32	17.89	7.2%		Bonferroni Adj t Test
02-9211-9979	Mean Length	10	32	17.89	8.57%		Bonferroni Adj t Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
07-4758-4339	Germination Rate	EC50	118.1	111.6	124.9		Spearman-Kärber
14-5916-3987	Mean Length	IC25	82.9	61.05	114.7		Linear Interpolation (ICPIN)
		IC50	280.1	249	300.2		

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-4758-4339	Germination Rate	Control Resp	0.816	0.7 - NL	Yes	Passes Acceptability Criteria
12-4945-1639	Germination Rate	Control Resp	0.816	0.7 - NL	Yes	Passes Acceptability Criteria
02-9211-9979	Mean Length	Control Resp	12.1	10 - NL	Yes	Passes Acceptability Criteria
14-5916-3987	Mean Length	Control Resp	12.1	10 - NL	Yes	Passes Acceptability Criteria
02-9211-9979	Mean Length	NOEL	10	NL - 35	No	Passes Acceptability Criteria
12-4945-1639	Germination Rate	PMSD	0.07205	NL - 0.2	No	Passes Acceptability Criteria
02-9211-9979	Mean Length	PMSD	0.08566	NL - 0.2	No	Passes Acceptability Criteria

Germination Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.816	0.7802	0.8518	0.78	0.84	0.01288	0.02881	3.53%	0.0%
10		5	0.818	0.7911	0.8449	0.8	0.85	0.009695	0.02168	2.65%	-0.25%
32		5	0.75	0.7222	0.7778	0.72	0.78	0.01	0.02236	2.98%	8.09%
100		4	0.5625	0.4607	0.6643	0.49	0.63	0.03198	0.06397	11.37%	31.07%
180		5	0.286	0.2561	0.3159	0.26	0.32	0.01077	0.02408	8.42%	64.95%
320		5	0.046	0.005195	0.08681	0.02	0.1	0.0147	0.03286	71.44%	94.36%
560		5	0	0	0	0	0	0	0		100.0%

Mean Length Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	12.1	11.49	12.71	11.5	12.75	0.2179	0.4873	4.03%	0.0%
10		5	12.05	11.19	12.91	11.25	13	0.3102	0.6937	5.76%	0.41%
32		5	10.6	9.885	11.31	10	11.5	0.2574	0.5755	5.43%	12.4%
100		4	8.563	7.467	9.658	7.75	9.25	0.3442	0.6884	8.04%	29.24%
180		5	8.15	6.751	9.549	7.25	9.5	0.5037	1.126	13.82%	32.64%
320		5	5.213	4.803	5.622	5	5.75	0.1474	0.3295	6.32%	56.92%
560		5	5	5	5	5	5	0	0	0.0%	58.68%

CETIS Summary Report

Report Date: 02 Jun-15 17:34 (p 2 of 2)
 Test Code: 150513mprt | 08-7733-4797

Macrocystis Germination and Germ Tube Growth Test							Nautilus Environmental (CA)
Germination Rate Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	0.79	0.83	0.84	0.78	0.84	
10		0.83	0.8	0.81	0.85	0.8	
32		0.72	0.75	0.74	0.78	0.76	
100		0.63	0.53		0.49	0.6	
180		0.28	0.26	0.32	0.27	0.3	
320		0.05	0.02	0.04	0.1	0.02	
560		0	0	0	0	0	
Mean Length Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	12.25	11.75	12.75	11.5	12.25	
10		12.5	11.75	13	11.75	11.25	
32		10.25	10.75	10.5	11.5	10	
100		9	8.25		7.75	9.25	
180		7.25	9.5	7.25	9.25	7.5	
320		5	5	5.313	5.75	5	
560		5	5	5	5	5	

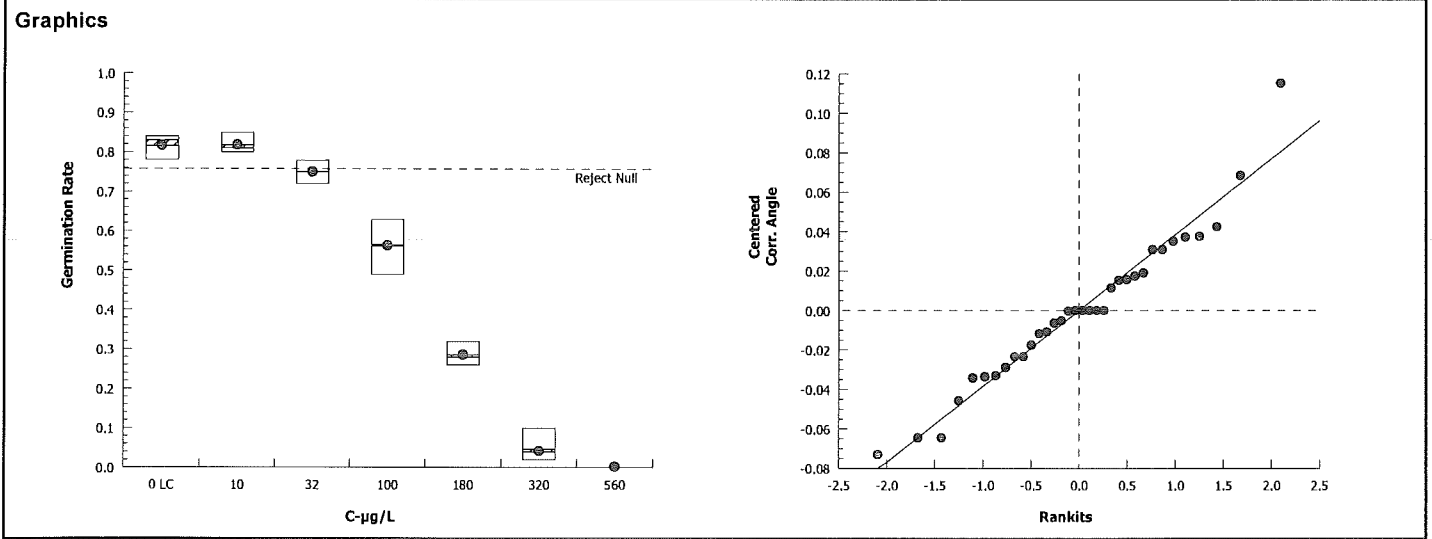
CETIS Analytical Report

Report Date: 02 Jun-15 17:34 (p 1 of 3)
 Test Code: 150513mprt | 08-7733-4797

Macrocystis Germination and Germ Tube Growth Test										Nautilus Environmental (CA)	
Analysis ID: 12-4945-1639		Endpoint: Germination Rate				CETIS Version: CETISv1.8.7					
Analyzed: 02 Jun-15 17:30		Analysis: Parametric-Multiple Comparison				Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	7.2%	10	32	17.89			
Bonferroni Adj t Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		10	-0.07729	2.5	0.073	8	1.0000	CDF	Non-Significant Effect		
		32*	2.777	2.5	0.073	8	0.0268	CDF	Significant Effect		
		100*	9.064	2.5	0.077	7	<0.0001	CDF	Significant Effect		
		180*	19.38	2.5	0.073	8	<0.0001	CDF	Significant Effect		
		320*	31.66	2.5	0.073	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	3.429466		0.6858933		5	323.7	<0.0001	Significant Effect			
Error	0.04873844		0.002119063		23						
Total	3.478205				28						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance		7.947	15.09	0.1592	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9691	0.9004	0.5364	Normal Distribution					
Germination Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.816	0.7802	0.8518	0.83	0.78	0.84	0.01288	3.53%	0.0%
10		5	0.818	0.7911	0.8449	0.81	0.8	0.85	0.009695	2.65%	-0.25%
32		5	0.75	0.7222	0.7778	0.75	0.72	0.78	0.01	2.98%	8.09%
100		4	0.5625	0.4607	0.6643	0.565	0.49	0.63	0.03198	11.37%	31.07%
180		5	0.286	0.2561	0.3159	0.28	0.26	0.32	0.01077	8.42%	64.95%
320		5	0.046	0.005195	0.08681	0.04	0.02	0.1	0.0147	71.44%	94.36%
560		5	0	0	0	0	0	0	0		100.0%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.128	1.083	1.174	1.146	1.083	1.159	0.01649	3.27%	0.0%
10		5	1.131	1.095	1.166	1.12	1.107	1.173	0.01276	2.52%	-0.2%
32		5	1.048	1.015	1.08	1.047	1.013	1.083	0.01156	2.47%	7.16%
100		4	0.8485	0.7456	0.9513	0.8507	0.7754	0.9169	0.03231	7.62%	24.81%
180		5	0.564	0.531	0.597	0.5576	0.5351	0.6013	0.01188	4.71%	50.02%
320		5	0.2065	0.1144	0.2986	0.2014	0.1419	0.3218	0.03318	35.93%	81.7%
560		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.57%

Macrocystis Germination and Germ Tube Growth Test Nautilus Environmental (CA)

Analysis ID: 12-4945-1639 Endpoint: Germination Rate CETIS Version: CETISv1.8.7
Analyzed: 02 Jun-15 17:30 Analysis: Parametric-Multiple Comparison Official Results: Yes



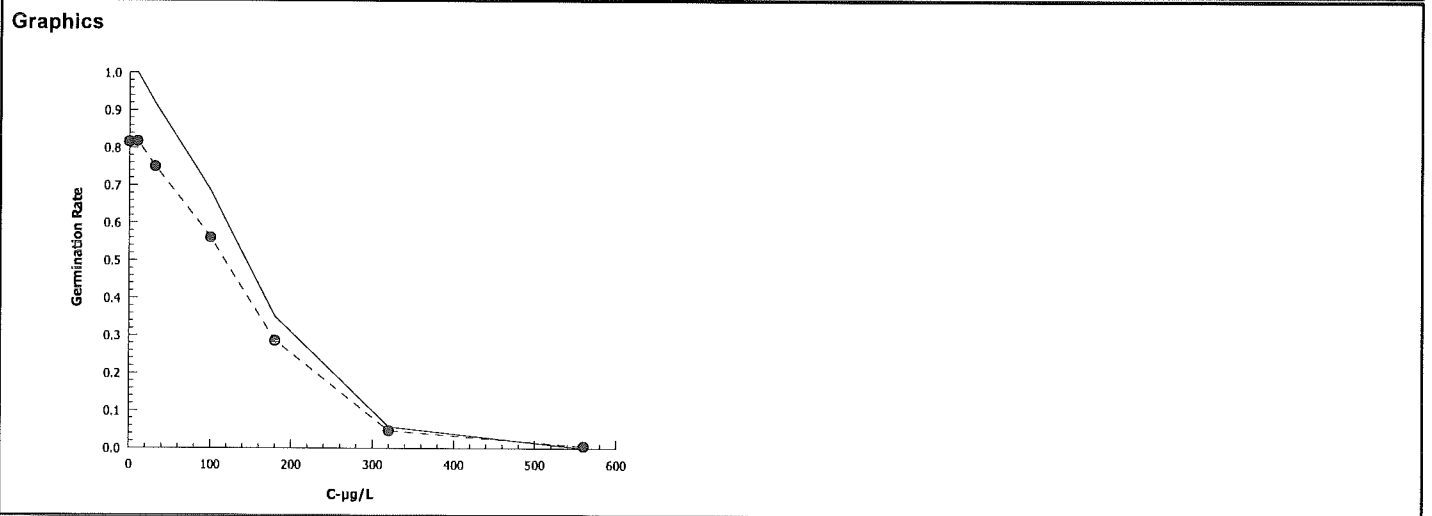
CETIS Analytical Report

Report Date: 02 Jun-15 17:34 (p 1 of 1)
 Test Code: 150513mprt | 08-7733-4797

Macrocystis Germination and Germ Tube Growth Test			Nautilus Environmental (CA)		
Analysis ID: 07-4758-4339	Endpoint: Germination Rate	CETIS Version: CETISv1.8.7			
Analyzed: 02 Jun-15 17:30	Analysis: Untrimmed Spearman-Kärber	Official Results: Yes			

Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.184	0.00%	2.072	0.01219	118.1	111.6	124.9

Germination Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.816	0.78	0.84	0.01288	0.02881	3.53%	0.0%	408	500
10		5	0.818	0.8	0.85	0.009695	0.02168	2.65%	-0.25%	409	500
32		5	0.75	0.72	0.78	0.01	0.02236	2.98%	8.09%	375	500
100		4	0.5625	0.49	0.63	0.03198	0.06397	11.37%	31.07%	224	400
180		5	0.286	0.26	0.32	0.01077	0.02408	8.42%	64.95%	143	500
320		5	0.046	0.02	0.1	0.0147	0.03286	71.44%	94.36%	23	500
560		5	0	0	0	0	0		100.0%	0	500



CETIS Analytical Report

Report Date: 02 Jun-15 17:34 (p 3 of 3)

Test Code: 150513mprt | 08-7733-4797

Macrocystis Germination and Germ Tube Growth Test Nautilus Environmental (CA)

Analysis ID: 02-9211-9979	Endpoint: Mean Length	CETIS Version: CETISv1.8.7
Analyzed: 02 Jun-15 17:31	Analysis: Parametric-Multiple Comparison	Official Results: Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Untransformed	NA	C > T	NA	NA	8.57%	10	32	17.89	

Bonferroni Adj t Test

Control	vs C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	10	0.1231	2.552	1.037	8	1.0000	CDF	Non-Significant Effect
	32*	3.694	2.552	1.037	8	0.0030	CDF	Significant Effect
	100*	8.213	2.552	1.099	7	<0.0001	CDF	Significant Effect
	180*	9.727	2.552	1.037	8	<0.0001	CDF	Significant Effect
	320*	16.96	2.552	1.037	8	<0.0001	CDF	Significant Effect
	560*	17.48	2.552	1.037	8	<0.0001	CDF	Significant Effect

ANOVA Table

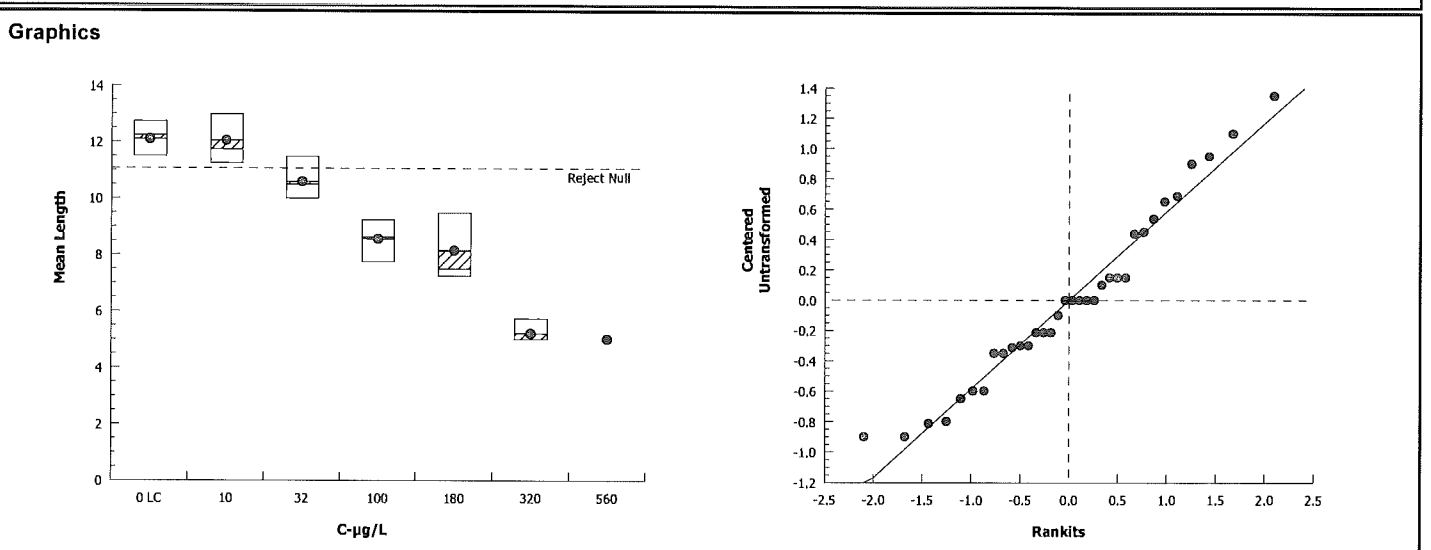
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	262.3404	43.7234	6	106.1	<0.0001	Significant Effect
Error	11.13125	0.4122685	27			
Total	273.4716		33			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	1.917	3.812	0.1251	Equal Variances
Variances	Levene Equality of Variance	7.891	3.558	<0.0001	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9618	0.9125	0.2741	Normal Distribution

Mean Length Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	12.1	11.49	12.71	12.25	11.5	12.75	0.2179	4.03%	0.0%
10		5	12.05	11.19	12.91	11.75	11.25	13	0.3102	5.76%	0.41%
32		5	10.6	9.885	11.31	10.5	10	11.5	0.2574	5.43%	12.4%
100		4	8.563	7.467	9.658	8.625	7.75	9.25	0.3442	8.04%	29.24%
180		5	8.15	6.751	9.549	7.5	7.25	9.5	0.5037	13.82%	32.64%
320		5	5.213	4.803	5.622	5	5	5.75	0.1474	6.32%	56.92%
560		5	5	5	5	5	5	5	0	0.0%	58.68%



CETIS Analytical Report

Report Date: 02 Jun-15 17:34 (p 1 of 1)

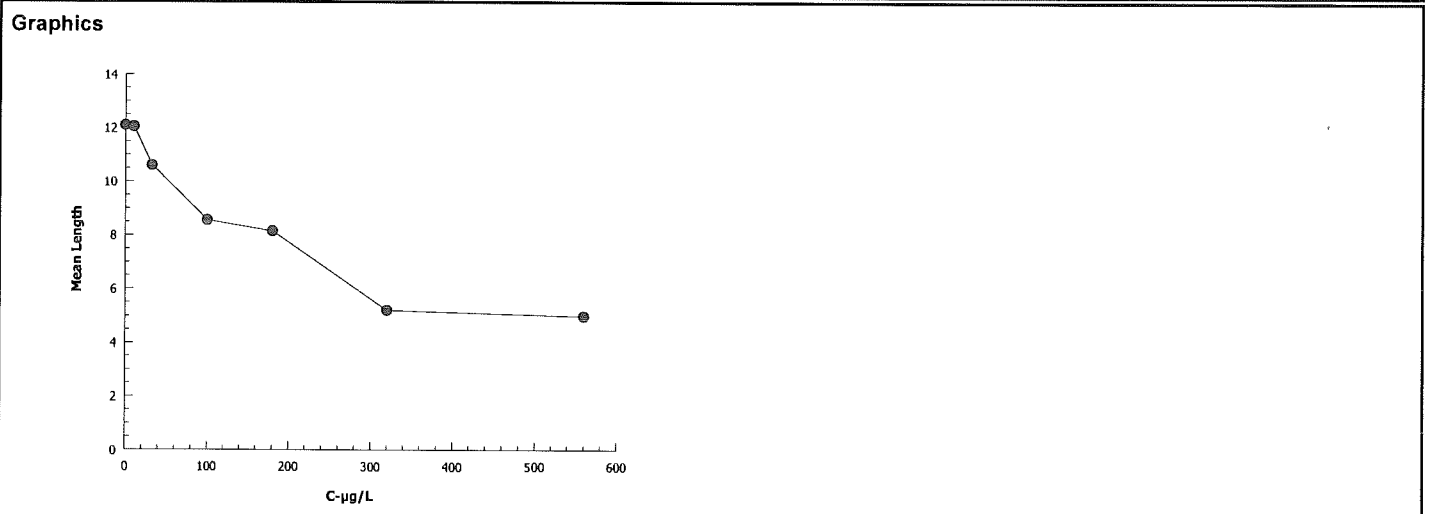
Test Code: 150513mprt | 08-7733-4797

Macrocystis Germination and Germ Tube Growth Test			Nautilus Environmental (CA)		
Analysis ID: 14-5916-3987	Endpoint: Mean Length	CETIS Version: CETISv1.8.7			
Analyzed: 02 Jun-15 17:31	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	985217	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
IC25	82.9	61.05	114.7
IC50	280.1	249	300.2

Mean Length Summary			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	12.1	11.5	12.75	0.2179	0.4873	4.03%	0.0%
10		5	12.05	11.25	13	0.3102	0.6937	5.76%	0.41%
32		5	10.6	10	11.5	0.2574	0.5755	5.43%	12.4%
100		4	8.563	7.75	9.25	0.3442	0.6884	8.04%	29.24%
180		5	8.15	7.25	9.5	0.5037	1.126	13.82%	32.64%
320		5	5.213	5	5.75	0.1474	0.3295	6.32%	56.92%
560		5	5	5	5	0	0	0.0%	58.68%



Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental (CA)

Test Type: Growth-Germination

Organism: Macrocystis pyrifera (Giant Kelp)

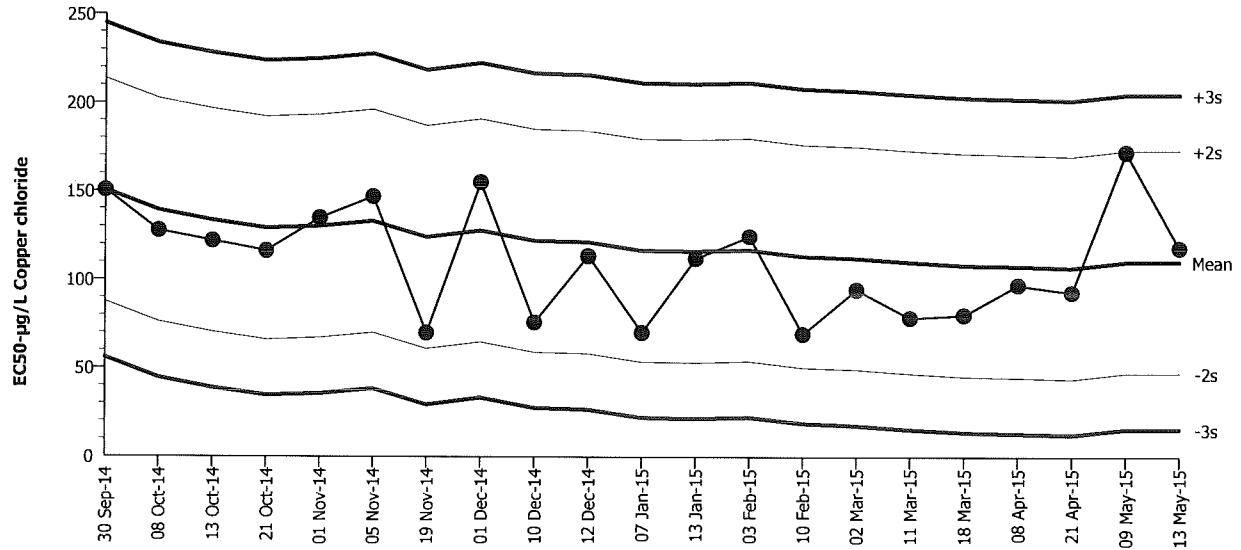
Material: Copper chloride

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Germination Rate

Source: Reference Toxicant-REF

Macrocystis Germination and Germ Tube Growth Test

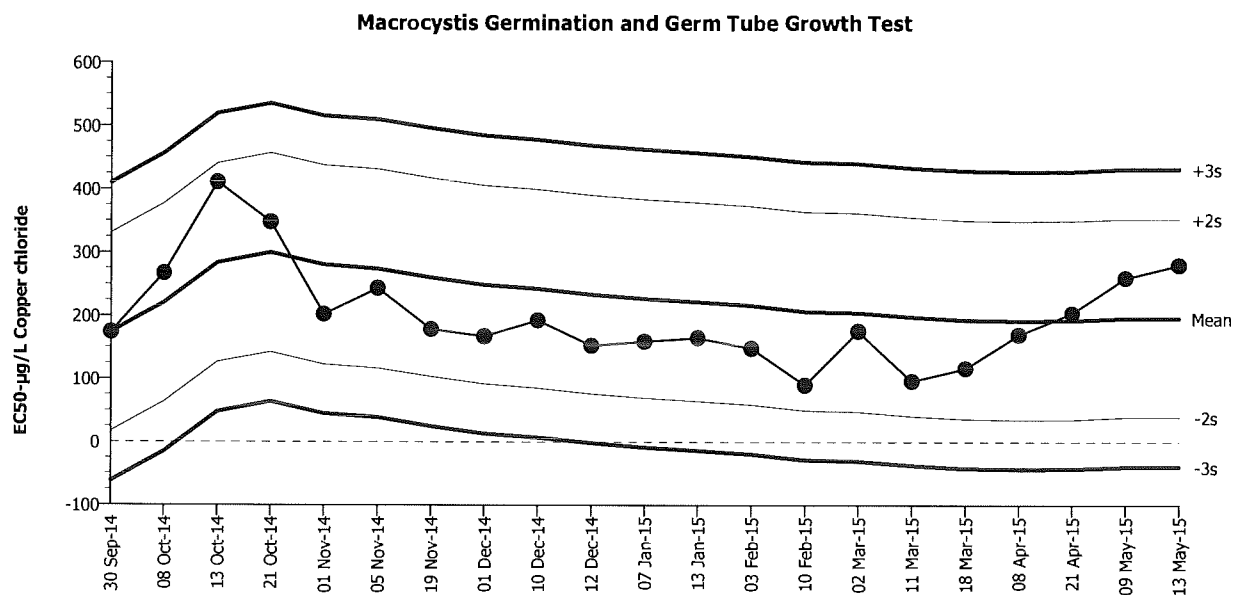


Mean: 110.2 Count: 20 -2s Warning Limit: 47.15 -3s Action Limit: 15.61
 Sigma: 31.54 CV: 28.60% +2s Warning Limit: 173.3 +3s Action Limit: 204.9

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Sep	30	12:15	150.5	40.33	1.279			13-9787-7056	02-8487-1458
2		Oct	8	11:40	127.8	17.6	0.558			15-0209-2295	12-7369-3569
3			13	15:00	121.9	11.71	0.3714			07-9089-8433	21-1969-7792
4			21	11:45	116.2	5.985	0.1898			05-9690-1778	07-6459-5729
5		Nov	1	17:30	134.9	24.67	0.7821			01-6548-8876	19-8891-4456
6			5	12:10	147.1	36.91	1.17			16-4808-9223	03-9213-0972
7			19	11:45	69.84	-40.36	-1.28			19-5595-5174	10-2118-3209
8		Dec	1	14:25	155.2	44.97	1.426			00-5019-0818	01-5801-5093
9			10	11:45	75.77	-34.43	-1.091			20-1153-5143	06-3379-4906
10			12	16:00	113.5	3.325	0.1054			11-8986-9857	15-5507-8419
11	2015	Jan	7	11:50	70.09	-40.11	-1.272			05-1839-9516	16-0872-3144
12			13	15:20	112.1	1.942	0.06156			19-5672-8241	21-3328-2708
13		Feb	3	14:10	124.6	14.4	0.4565			02-0093-1321	09-6992-3779
14			10	13:45	69.37	-40.83	-1.295			15-5769-6471	18-0756-1200
15		Mar	2	19:25	94.56	-15.64	-0.4958			02-8587-0344	12-3520-6702
16			11	11:40	78.5	-31.7	-1.005			16-3863-7280	09-2265-0470
17			18	12:00	80.1	-30.1	-0.9544			00-4348-8755	17-4019-8643
18		Apr	8	13:20	97.16	-13.04	-0.4134			16-4963-4427	03-3491-3881
19			21	16:15	93.03	-17.17	-0.5445			05-9446-7141	00-0365-1165
20		May	9	14:25	172.4	62.22	1.973			11-5639-4173	20-3923-9025
21			13	12:20	118.1	7.857	0.2491			08-7733-4797	07-4758-4339

Macrocystis Germination and Germ Tube Growth Test		Nautilus Environmental (CA)	
Test Type: Growth-Germination	Organism: Macrocystis pyrifera (Giant Kelp)	Material: Copper chloride	
Protocol: EPA/600/R-95/136 (1995)	Endpoint: Mean Length	Source: Reference Toxicant-REF	



Mean: 196.1 **Count:** 20 **-2s Warning Limit:** 39.03 **-3s Action Limit:** -39.52
Sigma: 78.55 **CV:** 40.10% **+2s Warning Limit:** 353.2 **+3s Action Limit:** 431.8

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Sep	30	12:15	173.8	-22.25	-0.2833			13-9787-7056	19-7873-0878
2		Oct	8	11:40	266.9	70.78	0.9011			15-0209-2295	14-5837-8655
3			13	15:00	410.8	214.7	2.734	(+)		07-9089-8433	18-0074-3690
4			21	11:45	348	151.9	1.934			05-9690-1778	02-4326-8088
5		Nov	1	17:30	202.1	6.005	0.07645			01-6548-8876	12-7526-4191
6			5	12:10	243.4	47.34	0.6026			16-4808-9223	00-4645-3717
7			19	11:45	178.4	-17.7	-0.2253			19-5595-5174	15-0041-2395
8		Dec	1	14:25	167.1	-29.02	-0.3695			00-5019-0818	01-4688-9594
9			10	11:45	192.7	-3.373	-0.04294			20-1153-5143	19-2658-2480
10			12	16:00	152.8	-43.27	-0.5509			11-8986-9857	14-7967-2457
11	2015	Jan	7	11:50	159.3	-36.79	-0.4684			05-1839-9516	20-5481-8709
12			13	15:20	165.1	-30.96	-0.3941			19-5672-8241	11-2312-3644
13		Feb	3	14:10	148.5	-47.62	-0.6062			02-0093-1321	00-8294-2622
14			10	13:45	90.23	-105.9	-1.348			15-5769-6471	18-5702-3882
15		Mar	2	19:25	175.9	-20.24	-0.2576			02-8587-0344	07-8323-3127
16			11	11:40	96.64	-99.46	-1.266			16-3863-7280	04-6998-1152
17			18	12:00	116.8	-79.35	-1.01			00-4348-8755	09-8096-6426
18		Apr	8	13:20	170	-26.14	-0.3328			16-4963-4427	08-2168-6694
19			21	16:15	204.1	7.963	0.1014			05-9446-7141	17-6422-9986
20		May	9	14:25	260	63.9	0.8135			11-5639-4173	11-0369-2164
21			13	12:20	280.1	83.99	1.069			08-7733-4797	14-5916-3987

Macrocyctis Germination and Germ Tube Growth Test

Nautilus Environmental (CA)

Test Type: Growth-Germination

Organism: Macrocyctis pyrifera (Giant Kelp)

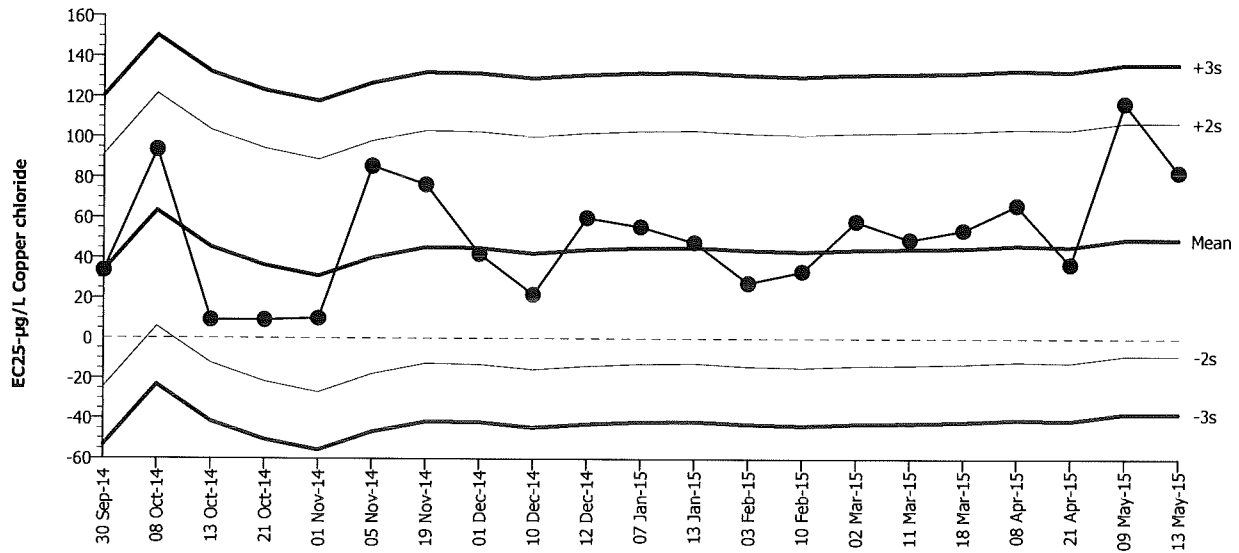
Material: Copper chloride

Protocol: EPA/600/R-95/136 (1995)

Endpoint: Mean Length

Source: Reference Toxicant-REF

Macrocyctis Germination and Germ Tube Growth Test



Mean: 49.42 Count: 20 -2s Warning Limit: -8.499 -3s Action Limit: -37.46
 Sigma: 28.96 CV: 58.60% +2s Warning Limit: 107.3 +3s Action Limit: 136.3

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Sep	30	12:15	33.48	-15.94	-0.5505			13-9787-7056	19-7873-0878
2		Oct	8	11:40	93.66	44.24	1.528			15-0209-2295	14-5837-8655
3			13	15:00	9.074	-40.35	-1.393			07-9089-8433	18-0074-3690
4			21	11:45	9.019	-40.4	-1.395			05-9690-1778	02-4326-8088
5		Nov	1	17:30	9.855	-39.56	-1.366			01-6548-8876	12-7526-4191
6			5	12:10	85.74	36.32	1.254			16-4808-9223	00-4645-3717
7			19	11:45	76.63	27.21	0.9394			19-5595-5174	15-0041-2395
8		Dec	1	14:25	42.05	-7.375	-0.2546			00-5019-0818	01-4688-9594
9			10	11:45	21.81	-27.61	-0.9535			20-1153-5143	19-2658-2480
10			12	16:00	60.17	10.75	0.3713			11-8986-9857	14-7967-2457
11	2015	Jan	7	11:50	55.64	6.221	0.2148			05-1839-9516	20-5481-8709
12			13	15:20	47.84	-1.579	-0.05453			19-5672-8241	11-2312-3644
13		Feb	3	14:10	27.6	-21.82	-0.7535			02-0093-1321	00-8294-2622
14			10	13:45	33.37	-16.05	-0.5543			15-5769-6471	18-5702-3882
15		Mar	2	19:25	58.41	8.991	0.3105			02-8587-0344	07-8323-3127
16			11	11:40	49.36	-0.06467	-0.00223			16-3863-7280	04-6998-1152
17			18	12:00	54.03	4.608	0.1591			00-4348-8755	09-8096-6426
18		Apr	8	13:20	66.52	17.1	0.5903			16-4963-4427	08-2168-6694
19			21	16:15	37.04	-12.38	-0.4276			05-9446-7141	17-6422-9986
20		May	9	14:25	117.1	67.72	2.338	(+)		11-5639-4173	11-0369-2164
21			13	12:20	82.9	33.48	1.156			08-7733-4797	14-5916-3987

Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental - San Diego

Start Date: 13-May-15

Species: *Macrocystis pyrifera*

Test ID: 150513mprt

End Date: 15-May-15

Protocol: EPA/600/R-95/136 (1995 West Coast Manual)

Sample Source: Internal

Sampled: 13-May-15

Sample Station: CuCl₂

Random Number	Number Counted	Number Germinated	Tube Length Measurements (micrometer units)										Calibration Factor	Mean Tube Length (µm)
1	100	75	4	4	5	5	4	5	5	4	4	3	2.5	10.75
2	*													
3	100	32	3	2	3	5	2	2	3	3	3	3	2.5	7.25
4	100	49	4	4	3	3	4	3	2	3	2	3	2.5	7.75
5	100	10	2	2	2	2	2	4	2	3	2	2	2.5	5.75
6	100	63	5	3	3	4	2	3	3	4	5	4	2.5	9.00
7	100	80	4	6	5	6	4	5	5	4	4	4	2.5	11.75
8	100	78	5	5	6	4	5	3	4	5	5	4	2.5	11.50
9	100	4	2	2	3	2	2	2	2	2			2.5	5.31
10	100	83	6	4	5	6	5	5	5	5	5	4	2.5	12.50
11	100	74	5	4	5	5	4	4	4	3	3	5	2.5	10.50
12	100	28	2	2	4	3	3	3	2	4	4	2	2.5	7.25
13	100	2	2	2	2	2	2	2					2.5	5.00
14	100	81	4	4	5	7	5	5	7	6	5	4	2.5	13.00
15	100	78	5	5	5	4	5	6	3	4	5	4	2.5	11.50
16	100	0	2	2									2.5	5.00
17	100	84	5	3	6	5	3	5	6	6	5	5	2.5	12.25
18	100	0	2	2									2.5	5.00
19	100	30	2	2	6	3	3	3	2	2	5	2	2.5	7.50
20	100	85	5	4	4	5	6	5	5	5	5	3	2.5	11.75
21	100	76	3	4	3	4	5	4	4	4	5	4	2.5	10.00
22	100	53	3	3	5	2	4	3	3	4	4	2	2.5	8.25
23	100	0	2	2									2.5	5.00
24	100	60	5	4	4	3	3	3	4	3	5	3	2.5	9.25
25	100	79	5	6	5	4	4	5	5	5	5	5	2.5	12.25
26	100	0	2	2	2								2.5	5.00
27	100	2	2	2	2	2							2.5	5.00
28	100	0	2	2									2.5	5.00
29	100	83	5	3	4	6	5	6	5	4	4	5	2.5	11.75
30	100	26	4	3	5	4	3	3	5	3	3	5	2.5	9.50
31	100	80	3	7	5	6	4	3	4	5	4	4	2.5	11.25
32	100	27	3	4	5	4	3	4	3	3	4	4	2.5	9.25
33	100	5	2	2	2	2							2.5	5.00
34	100	72	4	4	3	3	5	4	5	4	5	4	2.5	10.25
35	100	84	6	6	6	5	4	5	4	5	5	5	2.5	12.75

Technician Note: If there are any germinated spores in the replicate, scan the slide to measure 10 lengths if possible (regardless of number germinated).

* Slide not inoculated, technician error.

QC Check: BK 6/2/15

Final Review: [Signature] 6/4/15

Analyst: BK

Macrocystis Germination and Germ Tube Growth Test

Nautilus Environmental - San Diego

Start Date: 13-May-15

Species: *Macrocystis pyrifera*

Test ID: 150513mprt

End Date: 15-May-15

Protocol: EPA/600/R-95/136 (1995 West Coast Manual)

Sample Source: Internal

Sampled: 13-May-15

Sample Station: CuCl₂

Random Number	Number Counted	Number Germinated	Tube Length Measurements (micrometer units)										Calibration Factor	Mean Tube Length (µm)
1	100	75	4	4	5	5	4	5	5	4	4	3	2.5	#DIV/0!
2	100	⊗	---											
3	100	32	3	2	3	5	2	2	3	3	3	3		#DIV/0!
4	100	49	4	4	3	3	4	3	2	3	2	3		#DIV/0!
5	100	10	2	2	2	2	2	4	2	3	2	2		#DIV/0!
6	100	⊗ 83 63	5	3	3	4	2	3	3	4	5	4		#DIV/0!
7	100	80	4	6	5	6	4	5	5	4	4	4		#DIV/0!
8	100	78	5	5	6	4	5	3	4	5	5	4		#DIV/0!
9	100	4	2	2	3	2	2	2	2	2				#DIV/0!
10	100	83	6	4	5	6	5	5	5	5	5	4		#DIV/0!
11	100	74	5	4	5	5	4	4	4	3	3	5		#DIV/0!
12	100	⊗ 75 28	2	2	4	3	3	3	2	4	4	2		#DIV/0!
13	100	2	2	2	2	2	2	2						#DIV/0!
14	100	81	4	4	5	7	5	5	7	6	5	4		#DIV/0!
15	100	78	5	5	5	4	5	6	3	4	5	4		#DIV/0!
16	100	0	2	2										#DIV/0!
17	100	84	5	3	6	5	3	5	6	6	5	5		#DIV/0!
18	100	0	2	2										#DIV/0!
19	100	30	2	2	6	3	3	3	2	2	5	2		#DIV/0!
20	100	85	5	4	4	5	6	5	5	5	5	3		#DIV/0!
21	100	76	3	4	3	4	5	4	4	4	5	4		#DIV/0!
22	100	53	3	3	5	2	4	3	3	4	4	2		#DIV/0!
23	100	0	2	2										#DIV/0!
24	100	60	5	4	4	3	3	3	4	3	5	3		#DIV/0!
25	100	79	5	6	5	4	4	5	5	5	5	5		#DIV/0!
26	100	0	2	2	2									#DIV/0!
27	100	2	2	2	2	2								#DIV/0!
28	100	0	2	2										#DIV/0!
29	100	83	5	3	4	6	5	6	5	4	4	5		#DIV/0!
30	100	26	4	3	5	4	3	3	5	3	3	5		#DIV/0!
31	100	80	3	7	5	6	4	3	4	5	4	4		#DIV/0!
32	100	27	3	4	5	4	3	4	3	3	4	4		#DIV/0!
33	100	5	2	2	2	2								#DIV/0!
34	100	72	4	4	3	3	5	4	5	4	5	4		#DIV/0!
35	100	84	6	6	6	5	4	5	4	5	5	5		#DIV/0!

Technician Note: If there are any germinated spores in the replicate, scan the slide to measure 10 lengths if possible (regardless of number germinated).

QC Check: BT 6/2/15

Final Review: JE 6/4/15

Analyst: VCR

⊗ 018 VCR 5/15/15

⊗ Slide not inoculated, technician error.

CETIS Test Data Worksheet

Report Date: 12 May-15 10:55 (p 1 of 1)
 Test Code: 08-7733-4797/150513mprt

Macrocystis Germination and Germ Tube Growth Test				Nautilus Environmental (CA)			
Start Date: 13 May-15	Species: Macrocystis pyrifera	Sample Code: 150513mprt					
End Date: 15 May-15	Protocol: EPA/600/R-95/136 (1995)	Sample Source: Reference Toxicant					
Sample Date: 13 May-15	Material: Copper chloride	Sample Station: Copper Chloride					

C-µg/L	Code	Rep	Pos	# Counted	# Germinated	Mean Length	CalFactor	Notes
0	LC	1	25	100			1	
0	LC	2	29	100			1	
0	LC	3	35	100			1	
0	LC	4	8	100			1	
0	LC	5	17	100			1	
10		1	10	100			1	
10		2	7	100			1	
10		3	14	100			1	
10		4	20	100			1	
10		5	31	100			1	
32		1	34	100			1	
32		2	1	100			1	
32		3	11	100			1	
32		4	15	100			1	
32		5	21	100			1	
100		1	6	100			1	
100		2	22	100			1	
100		3	2	100			1	
100		4	4	100			1	
100		5	24	100			1	
180		1	12	100			1	
180		2	30	100			1	
180		3	3	100			1	
180		4	32	100			1	
180		5	19	100			1	
320		1	33	100			1	
320		2	13	100			1	
320		3	9	100			1	
320		4	5	100			1	
320		5	27	100			1	
560		1	26	100			1	
560		2	28	100			1	
560		3	23	100			1	
560		4	16	100			1	
560		5	18	100			1	

QC: AB

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: Macrocystis pyrifera

Sample ID: CuCl₂

Start Date/Time: 5/13/2015 1220

Test No.: 150513mprt

End Date/Time: 5/15/2015 1115

Dilutions made by: AG

Dilution calcs. (final volume 250 mL):

Conc. µg/L	10	32	100	180	320	560
Vol. Cu stock added (mL) :	2.6	0.9	2.9	5.2	9.3	1.6
Cu Stock Conc. (µg/L)	959	8,630	8,630	8,630	8,630	86,900

Analyst: AG

Analyst: SC

Concentration (µg/L)	Initial Readings				Final Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.2	8.03	33.1	15.6	7.4	7.96	33.2	15.1
10	8.0	8.06	33.0	15.5	7.4	7.96	33.2	15.1
32	7.9	8.06	33.2	15.6	7.4	7.96	33.4	15.1
100	7.9	8.07	33.1	15.5	7.4	7.96	33.1	15.1
180	7.9	8.07	32.9	15.7	7.4	7.96	32.9	15.1
320	7.9	8.09	32.5	15.7	7.4	7.95	32.5	15.1
560	7.9	8.06	33.2	15.8	7.4	7.96	32.5	15.1

Comments: _____

QC Check: BL 6/2/15

Final Review: SC 6/4/15

Marine Chronic Bioassay

Kelp Spore Germination & Growth Worksheet

Client: Internal
 Test No.: 150513mprt
 Tech. Initials: AG

Start Date/Time: 5/13/2015 1:20
 End Date/Time: 5/15/2015 1:15
 Test Species: Macrocystis pyrifera

Date Collected: 5/12/15
 Kelp Collectors: OG
 Collection Location: La Jolla Cove

Time of Initial Rinsing and Dessication: 5/12/15 1645
 Time of Rinsing and Transfer to Release Beaker: 5/13/15 1155
 Conditions of Zoospore Density and Motility: High Density & Motility
 Time of Blade Removal From Release Beaker: 5/13/15 1210

Density Counts (target = 90): 128 130 132 135 134 Mean: 131.8

Mean ^{AG} 131.8 * 10,000 = 1,318,000 spores/ml (density of spore release)
 AG
 5/13/15

If spore release = 900,000 spores/ml: Inoculate with 0.25 ml

If spore release > 900,000 spores/ml: Calculate a dilution factor, x, create a new spore stock of 900,000 spores/ml and inoculate with 0.25 ml.

To calculate the dilution factor:

Density of spore release 1,318,000 * $\frac{0.25 \text{ ml}}{1 \text{ container}}$ = $\frac{329,500 \text{ spores}}{225,000 \text{ spores}}$ = 1.46 (x) 1.46 dil. factor
 - 1.0 part spore stock 75 ml
.46 part(s) seawater 34.5 ml

If spore release < 900,000 spores/ml: The volume added should not exceed 0.5 ml. (This volume exceeds the EPA and MBP required volume of no greater than 1% of the total test solution volume. However, it may sometimes be necessary to exceed the 0.3 ml requirement in order to achieve the desired spore density.)

Time of inoculation: 1220 Amount inoculated: 0.25 mL

Location in Environmental Chamber (All replicates in each test must be on the same shelf; do not split up tests among shelves):

Shelf number	Measured Light Intensity Range (must be between 160 and 240 ft-c)	Random Number Range
1		
2		
3		
REFIX (4)	164 - 239	1 - 35
5	161 - 224	186 - 225
6	179 - 226	226 - 265
Timers Checked? <input checked="" type="checkbox"/>	Should be on 16:8 light:dark cycle	initials: <u>AG</u>

24-hour germination check	
QC dish #	% germ.
3	81

Comments: _____

QC Check: BL 6/2/15 Final Review: SC 6/4/15

Pacific Topsmelt

CETIS Summary Report

Report Date: 01 Jun-15 09:05 (p 1 of 2)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test **Nautilus Environmental (CA)**

Batch ID: 11-9577-9322	Test Type: Growth-Survival (7d)	Analyst:
Start Date: 05 May-15 14:15	Protocol: EPA/600/R-95/136 (1995)	Diluent: Diluted Natural Seawater
Ending Date: 12 May-15 11:00	Species: Atherinops affinis	Brine: Not Applicable
Duration: 6d 21h	Source: Aquatic Biosystems, CO	Age: 15 d

Sample ID: 17-1594-3816	Code: 150505aart	Client: Internal
Sample Date: 05 May-15	Material: Copper chloride	Project:
Receive Date: 05 May-15	Source: Reference Toxicant	
Sample Age: 14h	Station: Copper Chloride	

Comparison Summary							
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-7845-5090	7d Survival Rate	37.5	75	53.03	20.5%		Dunnett Multiple Comparison Test
03-0930-3044	96h Survival Rate	37.5	75	53.03	19.6%		Dunnett Multiple Comparison Test
06-6513-3526	Mean Dry Biomass-mg	37.5	>37.5	NA	12.9%		Equal Variance t Two-Sample Test

Point Estimate Summary							
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
13-6031-1332	7d Survival Rate	EC50	62.73	54.69	71.95		Trimmed Spearman-Kärber
02-8524-0195	96h Survival Rate	EC50	69.03	58.59	81.34		Trimmed Spearman-Kärber
13-1227-8396	Mean Dry Biomass-mg	IC25	49.96	40.85	61.5		Linear Interpolation (ICPIN)
		IC50	63.51	53.28	90.73		

Test Acceptability							
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision	
13-6031-1332	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria	
15-7845-5090	7d Survival Rate	Control Resp	1	0.8 - NL	Yes	Passes Acceptability Criteria	
06-6513-3526	Mean Dry Biomass-mg	Control Resp	1.069	0.85 - NL	Yes	Passes Acceptability Criteria	
13-1227-8396	Mean Dry Biomass-mg	Control Resp	1.069	0.85 - NL	Yes	Passes Acceptability Criteria	
15-7845-5090	7d Survival Rate	PMSD	0.2054	NL - 0.25	No	Passes Acceptability Criteria	
06-6513-3526	Mean Dry Biomass-mg	PMSD	0.1287	NL - 0.5	No	Passes Acceptability Criteria	

7d Survival Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	1	1	1	1	1	0	0	0.0%	0.0%
37.5		5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	4.0%
75		5	0.28	0	0.6132	0	0.6	0.12	0.2683	95.83%	72.0%
150		5	0	0	0	0	0	0	0		100.0%
300		5	0	0	0	0	0	0	0		100.0%
600		5	0	0	0	0	0	0	0		100.0%

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	5	1	1	1	1	1	0	0	0.0%	0.0%
37.5		5	0.96	0.8489	1	0.8	1	0.04	0.08944	9.32%	4.0%
75		5	0.36	0.03621	0.6838	0	0.6	0.1166	0.2608	72.44%	64.0%
150		5	0.08	0	0.216	0	0.2	0.04899	0.1095	136.9%	92.0%
300		5	0	0	0	0	0	0	0		100.0%
600		5	0	0	0	0	0	0	0		100.0%

Mean Dry Biomass-mg Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	1.069	0.9786	1.159	0.99	1.162	0.03249	0.07264	6.8%	0.0%
37.5		5	1.047	0.8626	1.232	0.804	1.19	0.06647	0.1486	14.19%	2.02%
75		5	0.308	-0.1115	0.7275	0	0.818	0.1511	0.3378	109.7%	71.18%
150		5	0	0	0	0	0	0	0		100.0%
300		5	0	0	0	0	0	0	0		100.0%
600		5	0	0	0	0	0	0	0		100.0%

CETIS Summary Report

Report Date: 01 Jun-15 09:05 (p 2 of 2)

Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test							Nautilus Environmental (CA)
7d Survival Rate Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	1	1	1	1	1	
37.5		1	1	1	0.8	1	
75		0	0.4	0	0.6	0.4	
150		0	0	0	0	0	
300		0	0	0	0	0	
600		0	0	0	0	0	
96h Survival Rate Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	1	1	1	1	1	
37.5		1	1	1	0.8	1	
75		0.2	0.4	0	0.6	0.6	
150		0	0	0	0.2	0.2	
300		0	0	0	0	0	
600		0	0	0	0	0	
Mean Dry Biomass-mg Detail							
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	
0	Lab Control	1.032	0.99	1.128	1.032	1.162	
37.5		1.074	1.032	1.19	0.804	1.136	
75		0	0.384	0	0.818	0.338	
150		0	0	0	0	0	
300		0	0	0	0	0	
600		0	0	0	0	0	

CETIS Analytical Report

Report Date: 01 Jun-15 09:04 (p 1 of 4)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test						Nautilus Environmental (CA)			
Analysis ID:	15-7845-5090	Endpoint:	7d Survival Rate	CETIS Version:	CETISv1.8.7				
Analyzed:	01 Jun-15 9:04	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes				
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	20.5%	37.5	75	53.03	

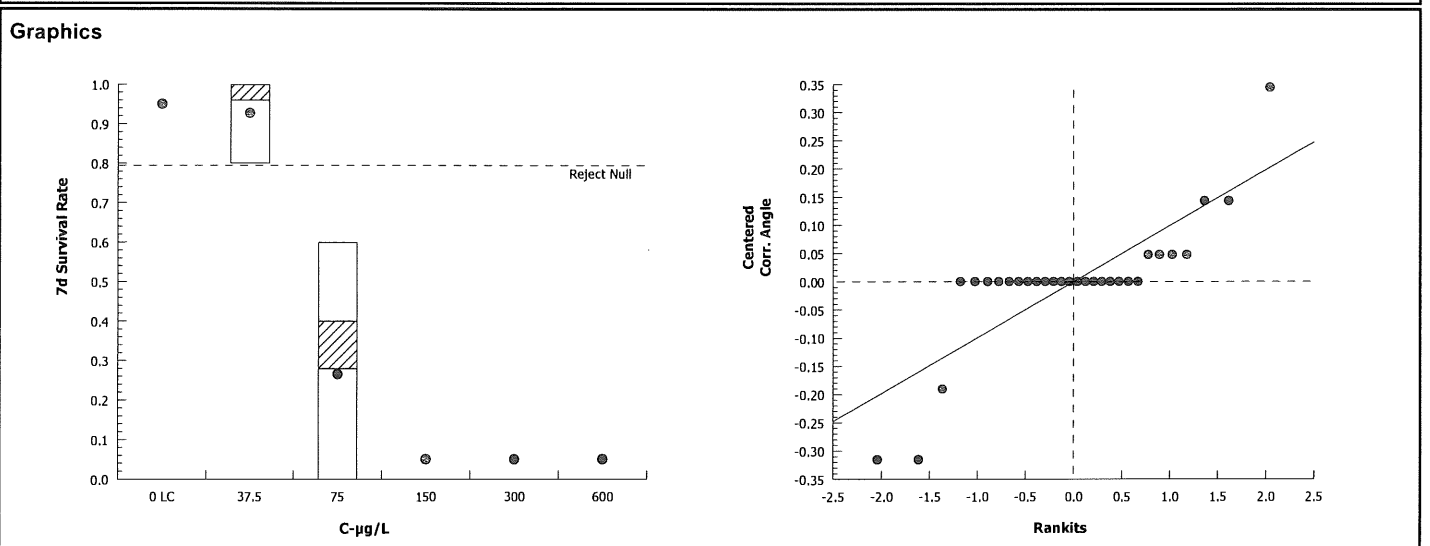
Dunnnett Multiple Comparison Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		37.5	0.41	2.108	0.245	8	0.4965	CDF	Non-Significant Effect
		75*	6.921	2.108	0.245	8	<0.0001	CDF	Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.034508	1.017254	2	30.15	<0.0001	Significant Effect
Error	0.4048181	0.03373484	12			
Total	2.439326		14			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Mod Levene Equality of Variance	4.092	8.022	0.0545	Equal Variances
Variances	Levene Equality of Variance	17.78	6.927	0.0003	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.8802	0.8328	0.0478	Normal Distribution

7d Survival Rate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1	1	1	1	1	1	0	0.0%	0.0%
37.5		5	0.96	0.8489	1	1	0.8	1	0.04	9.32%	4.0%
75		5	0.28	0	0.6132	0.4	0	0.6	0.12	95.83%	72.0%
150		5	0	0	0	0	0	0	0		100.0%
300		5	0	0	0	0	0	0	0		100.0%
600		5	0	0	0	0	0	0	0		100.0%

Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
37.5		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	3.54%
75		5	0.5413	0.1691	0.9135	0.6847	0.2255	0.8861	0.1341	55.38%	59.76%
150		5	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
300		5	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
600		5	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

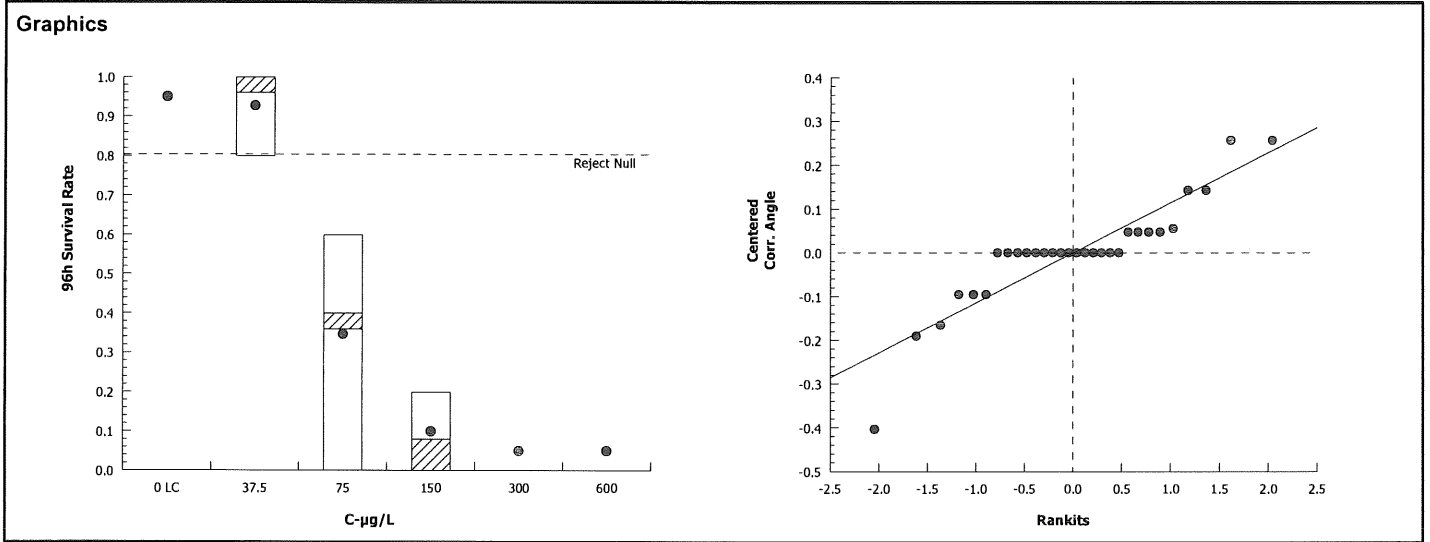


CETIS Analytical Report

Report Date: 01 Jun-15 09:05 (p 2 of 4)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test										Nautilus Environmental (CA)	
Analysis ID: 03-0930-3044		Endpoint: 96h Survival Rate			CETIS Version: CETISv1.8.7						
Analyzed: 01 Jun-15 9:04		Analysis: Parametric-Control vs Treatments			Official Results: Yes						
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C > T	NA	NA	19.6%	37.5	75	53.03			
Dunnnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		37.5	0.4547	2.227	0.233	8	0.5647	CDF	Non-Significant Effect		
		75*	6.837	2.227	0.233	8	<0.0001	CDF	Significant Effect		
		150*	9.781	2.227	0.233	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	3.826172		1.275391		3	46.5	<0.0001	Significant Effect			
Error	0.4388404		0.02742752		16						
Total	4.265012				19						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Mod Levene Equality of Variance		4.418	5.953	0.0260	Equal Variances					
Variances	Levene Equality of Variance		8.445	5.292	0.0014	Unequal Variances					
Distribution	Shapiro-Wilk W Normality		0.9329	0.866	0.1757	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	5	1	1	1	1	1	1	0	0.0%	0.0%
37.5		5	0.96	0.8489	1	1	0.8	1	0.04	9.32%	4.0%
75		5	0.36	0.03621	0.6838	0.4	0	0.6	0.1166	72.44%	64.0%
150		5	0.08	0	0.216	0	0	0.2	0.04899	136.9%	92.0%
300		5	0	0	0	0	0	0	0		100.0%
600		5	0	0	0	0	0	0	0		100.0%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.345	1.345	1.346	1.345	1.345	1.345	0	0.0%	0.0%
37.5		5	1.298	1.165	1.43	1.345	1.107	1.345	0.04763	8.21%	3.54%
75		5	0.6292	0.275	0.9834	0.6847	0.2255	0.8861	0.1276	45.33%	53.23%
150		5	0.3208	0.1588	0.4827	0.2255	0.2255	0.4636	0.05833	40.66%	76.16%
300		5	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%
600		5	0.2255	0.2255	0.2256	0.2255	0.2255	0.2255	0	0.0%	83.24%

Pacific Topsmelt 7-d Survival and Growth Test		Nautilus Environmental (CA)	
Analysis ID: 03-0930-3044	Endpoint: 96h Survival Rate	CETIS Version: CETISv1.8.7	
Analyzed: 01 Jun-15 9:04	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

Report Date: 01 Jun-15 09:05 (p 4 of 4)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test						Nautilus Environmental (CA)	
Analysis ID:	06-6513-3526	Endpoint:	Mean Dry Biomass-mg	CETIS Version:	CETISv1.8.7		
Analyzed:	01 Jun-15 9:04	Analysis:	Parametric-Two Sample	Official Results:	Yes		

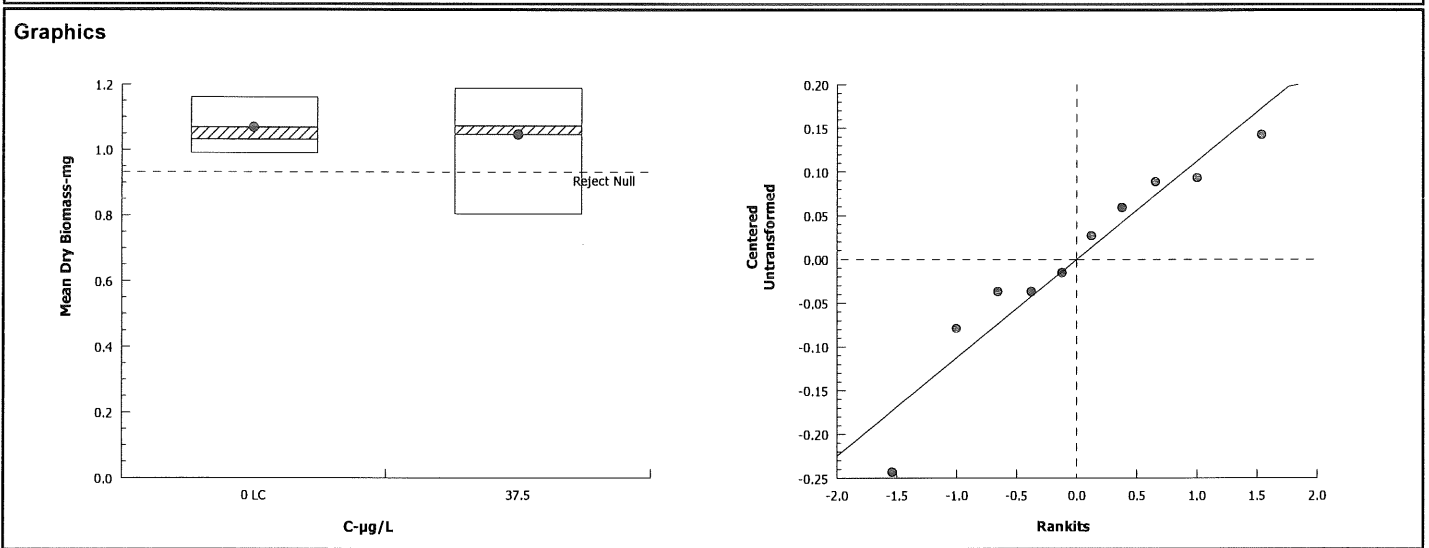
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Untransformed	NA	C > T	NA	NA	12.9%	Passes mean dry biomass-mg

Equal Variance t Two-Sample Test									
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		37.5	0.2919	1.86	0.138	8	0.3889	CDF	Non-Significant Effect

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.001166405	0.001166405	1	0.08523	0.7778	Non-Significant Effect
Error	0.1094818	0.01368523	8			
Total	0.1106482		9			

Distributional Tests					
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	4.187	23.15	0.1944	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9262	0.7411	0.4112	Normal Distribution

Mean Dry Biomass-mg Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.069	0.9786	1.159	1.032	0.99	1.162	0.03249	6.8%	0.0%
37.5		5	1.047	0.8626	1.232	1.074	0.804	1.19	0.06647	14.19%	2.02%



CETIS Analytical Report

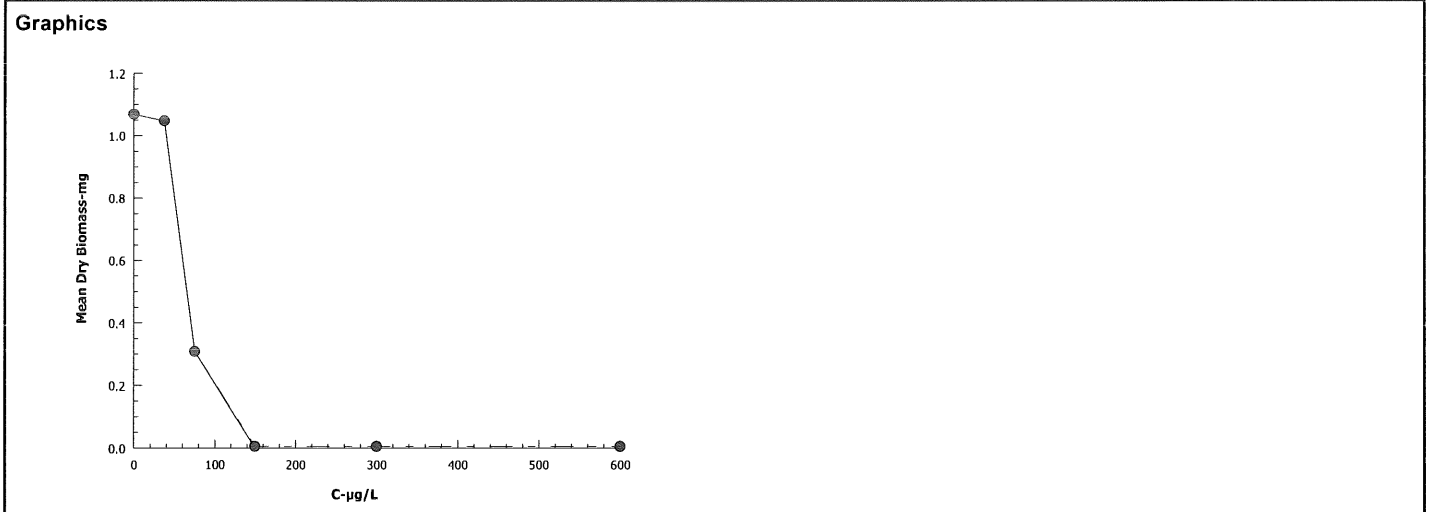
Report Date: 01 Jun-15 09:05 (p 1 of 1)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test			Nautilus Environmental (CA)		
Analysis ID: 13-1227-8396	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETISv1.8.7			
Analyzed: 01 Jun-15 9:04	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes			

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	416493	1000	Yes	Two-Point Interpolation

Point Estimates			
Level	µg/L	95% LCL	95% UCL
IC25	49.96	40.85	61.5
IC50	63.51	53.28	90.73

Mean Dry Biomass-mg Summary			Calculated Variate						
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	1.069	0.99	1.162	0.03249	0.07264	6.8%	0.0%
37.5		5	1.047	0.804	1.19	0.06647	0.1486	14.19%	2.02%
75		5	0.308	0	0.818	0.1511	0.3378	109.7%	71.18%
150		5	0	0	0	0	0		100.0%
300		5	0	0	0	0	0		100.0%
600		5	0	0	0	0	0		100.0%



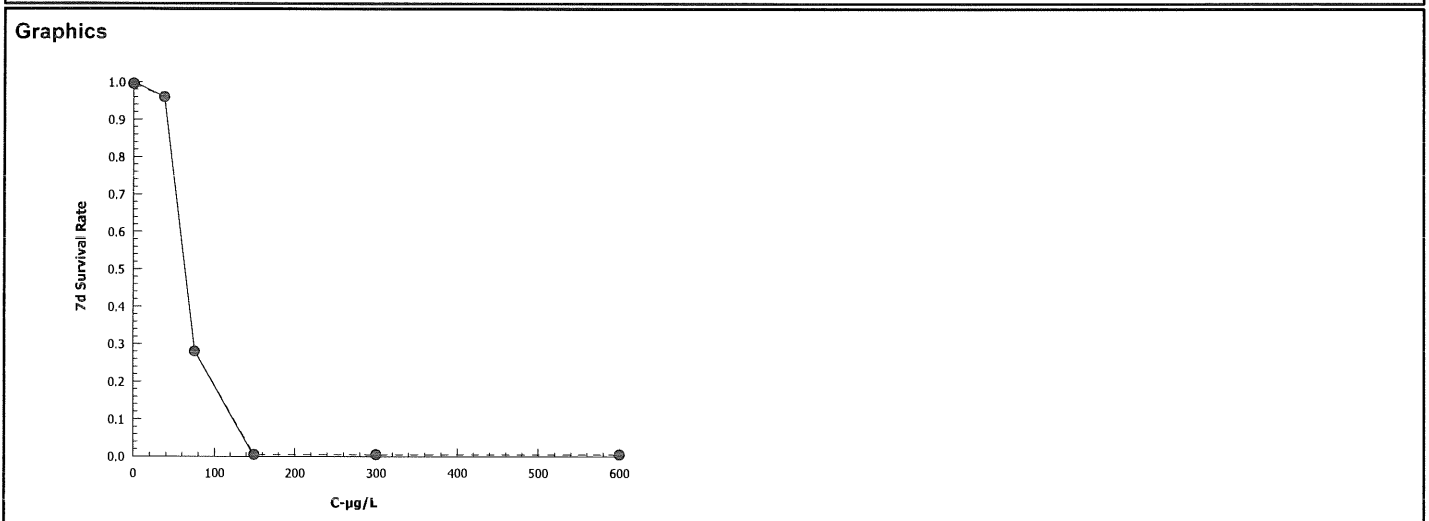
CETIS Analytical Report

Report Date: 01 Jun-15 09:05 (p 1 of 2)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test			Nautilus Environmental (CA)		
Analysis ID: 13-6031-1332	Endpoint: 7d Survival Rate	CETIS Version: CETISv1.8.7			
Analyzed: 01 Jun-15 9:04	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	4.00%	1.797	0.02978	62.73	54.69	71.95

7d Survival Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	1	1	1	0	0	0.0%	0.0%	25	25
37.5		5	0.96	0.8	1	0.04	0.08944	9.32%	4.0%	24	25
75		5	0.28	0	0.6	0.12	0.2683	95.83%	72.0%	7	25
150		5	0	0	0	0	0		100.0%	0	25
300		5	0	0	0	0	0		100.0%	0	25
600		5	0	0	0	0	0		100.0%	0	25



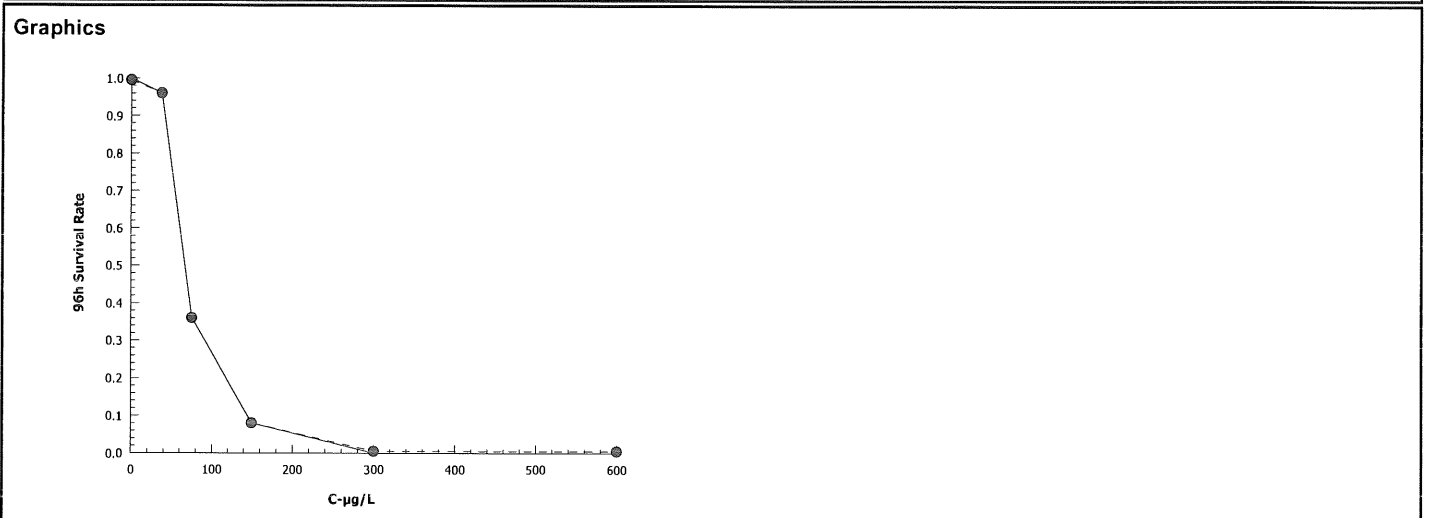
CETIS Analytical Report

Report Date: 01 Jun-15 09:05 (p 2 of 2)
 Test Code: 150505aart | 20-1963-0951

Pacific Topsmelt 7-d Survival and Growth Test				Nautilus Environmental (CA)			
Analysis ID: 02-8524-0195	Endpoint: 96h Survival Rate			CETIS Version: CETISv1.8.7			
Analyzed: 01 Jun-15 9:04	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	4.00%	1.839	0.03562	69.03	58.59	81.34

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	5	1	1	1	0	0	0.0%	0.0%	25	25	
37.5		5	0.96	0.8	1	0.04	0.08944	9.32%	4.0%	24	25	
75		5	0.36	0	0.6	0.1166	0.2608	72.44%	64.0%	9	25	
150		5	0.08	0	0.2	0.04899	0.1095	136.9%	92.0%	2	25	
300		5	0	0	0	0	0		100.0%	0	25	
600		5	0	0	0	0	0		100.0%	0	25	



Pacific Topsmelt 7-d Survival and Growth Test

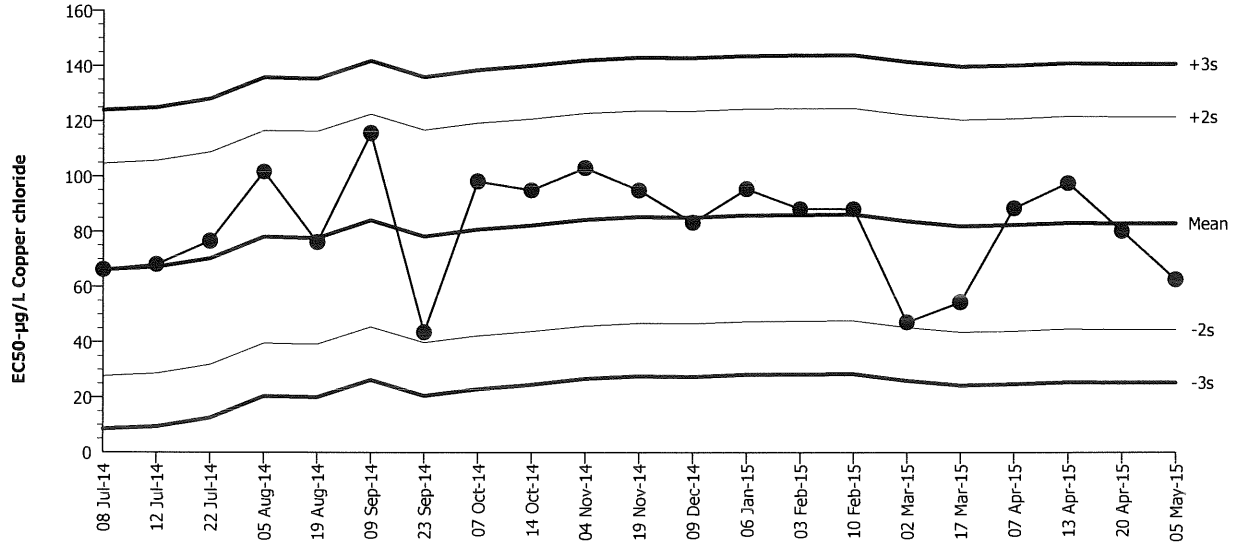
Nautilus Environmental (CA)

Test Type: Growth-Survival (7d)
 Protocol: EPA/600/R-95/136 (1995)

Organism: Atherinops affinis (Topsmelt)
 Endpoint: 7d Survival Rate

Material: Copper chloride
 Source: Reference Toxicant-REF

Pacific Topsmelt 7-d Survival and Growth Test

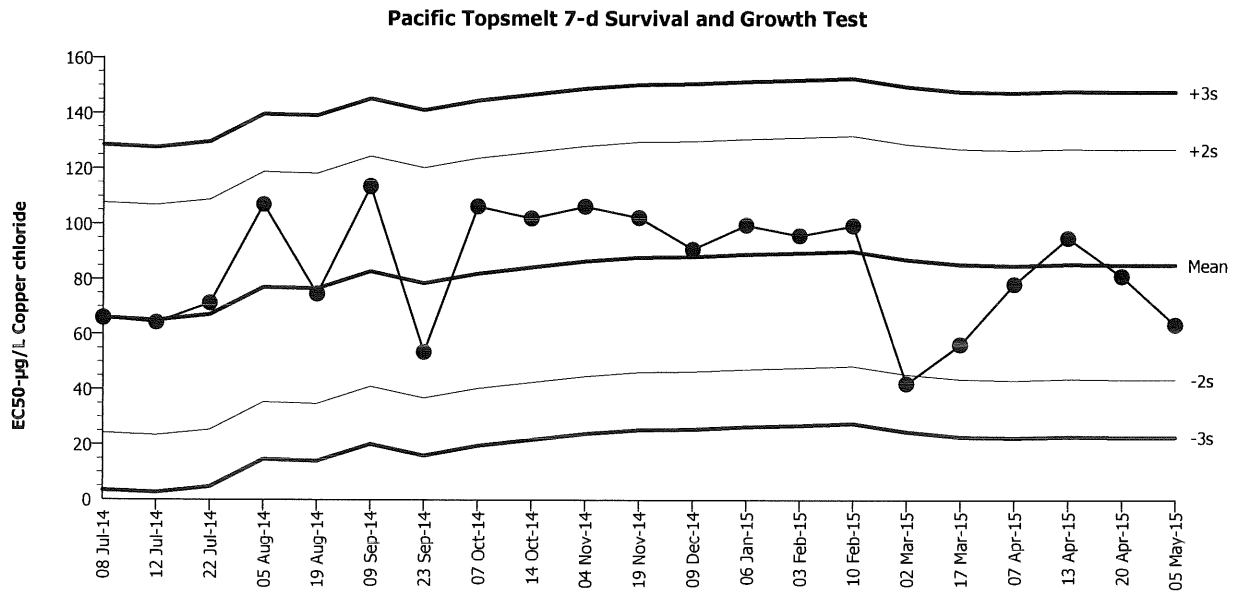


Mean: 83.14 Count: 20 -2s Warning Limit: 44.64 -3s Action Limit: 25.39
 Sigma: 19.25 CV: 23.20% +2s Warning Limit: 121.6 +3s Action Limit: 140.9

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Jul	8	12:15	66.2	-16.94	-0.8799			11-1291-5570	01-3275-8518
2			12	15:30	68.06	-15.08	-0.7832			19-5679-7209	09-1848-2586
3			22	12:10	76.61	-6.532	-0.3393			08-9694-1010	04-9281-6630
4		Aug	5	12:30	101.7	18.52	0.962			02-3572-4318	04-1355-8832
5			19	12:50	76.14	-7.001	-0.3637			10-2220-5806	14-8107-1507
6		Sep	9	13:15	115.7	32.53	1.69			00-2260-7679	08-1265-9953
7			23	10:00	43.5	-39.64	-2.059	(-)		10-2715-0848	08-7935-5641
8		Oct	7	14:55	98.2	15.06	0.7825			03-4335-1097	08-4043-4062
9			14	14:55	94.93	11.79	0.6126			03-4389-3229	08-0313-6046
10		Nov	4	17:00	103.2	20.03	1.04			07-3336-0499	03-9882-1644
11			19	15:35	94.93	11.79	0.6126			01-4951-3716	16-3767-9666
12		Dec	9	13:30	83.34	0.2032	0.01056			18-0939-8222	20-7541-1411
13	2015	Jan	6	11:00	95.52	12.38	0.6429			09-8025-9732	14-3733-1272
14		Feb	3	14:00	88.25	5.109	0.2654			21-1065-0393	01-8543-8572
15			10	14:30	88.25	5.109	0.2654			02-7871-6683	00-0287-3888
16		Mar	2	15:30	47.25	-35.89	-1.865			16-8982-2124	03-5342-0079
17			17	10:15	54.52	-28.62	-1.487			18-0061-0968	11-1706-8798
18		Apr	7	12:35	88.59	5.445	0.2829			19-9342-6452	01-8109-7656
19			13	14:10	97.6	14.46	0.7512			21-1811-2763	06-4686-3592
20			20	13:50	80.38	-2.757	-0.1432			00-6349-8324	16-3484-4333
21		May	5	14:15	62.73	-20.41	-1.06			20-1963-0951	13-6031-1332

Pacific Topsmelt 7-d Survival and Growth Test		Nautilus Environmental (CA)	
Test Type: Growth-Survival (7d)	Organism: Atherinops affinis (Topsmelt)	Material: Copper chloride	
Protocol: EPA/600/R-95/136 (1995)	Endpoint: Mean Dry Biomass-mg	Source: Reference Toxicant-REF	



Mean: 85.3 **Count:** 20 **-2s Warning Limit:** 43.6 **-3s Action Limit:** 22.75
Sigma: 20.85 **CV:** 24.40% **+2s Warning Limit:** 127 **+3s Action Limit:** 147.9

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Jul	8	12:15	65.94	-19.36	-0.9283			11-1291-5570	09-7900-1840
2			12	15:30	64.2	-21.1	-1.012			19-5679-7209	11-2284-5616
3			22	12:10	71.33	-13.97	-0.6701			08-9694-1010	11-8500-0101
4		Aug	5	12:30	107	21.7	1.041			02-3572-4318	06-4448-6794
5			19	12:50	74.65	-10.65	-0.5109			10-2220-5806	20-0116-6202
6		Sep	9	13:15	113.5	28.24	1.354			00-2260-7679	20-3452-2478
7			23	10:00	53.65	-31.65	-1.518			10-2715-0848	08-1906-9903
8		Oct	7	14:55	106.4	21.1	1.012			03-4335-1097	09-3471-6201
9			14	14:55	102.1	16.81	0.806			03-4389-3229	04-1492-4013
10		Nov	4	17:00	106.3	21.03	1.009			07-3336-0499	19-0008-4150
11			19	15:35	102.3	17	0.8156			01-4951-3716	05-1986-4487
12		Dec	9	13:30	90.88	5.578	0.2675			18-0939-8222	21-2156-2542
13	2015	Jan	6	11:00	99.63	14.33	0.6873			09-8025-9732	20-8969-8695
14		Feb	3	14:00	95.81	10.51	0.504			21-1065-0393	08-1230-3729
15			10	14:30	99.42	14.12	0.6771			02-7871-6683	01-6233-9739
16		Mar	2	15:30	42.15	-43.15	-2.07	(-)		16-8982-2124	13-6300-7842
17			17	10:15	56.41	-28.89	-1.386			18-0061-0968	00-1953-5316
18		Apr	7	12:35	78.22	-7.085	-0.3398			19-9342-6452	09-2081-8015
19			13	14:10	94.96	9.66	0.4633			21-1811-2763	05-5570-3525
20			20	13:50	81.16	-4.137	-0.1984			00-6349-8324	17-2159-2892
21		May	5	14:15	63.51	-21.79	-1.045			20-1963-0951	13-1227-8396

Pacific Topsmelt 7-d Survival and Growth Test

Nautilus Environmental (CA)

Test Type: Growth-Survival (7d)

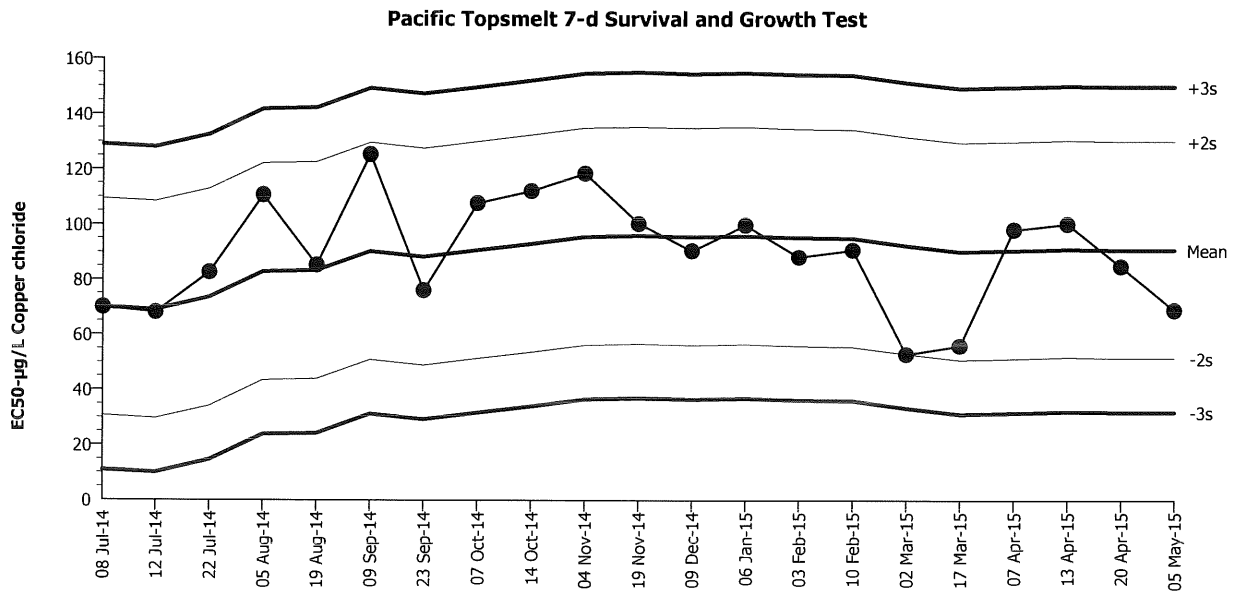
Organism: Atherinops affinis (Topsmelt)

Material: Copper chloride

Protocol: EPA/600/R-95/136 (1995)

Endpoint: 96h Survival Rate

Source: Reference Toxicant-REF



Mean: 90.94 Count: 20 -2s Warning Limit: 51.58 -3s Action Limit: 31.9
 Sigma: 19.68 CV: 21.60% +2s Warning Limit: 130.3 +3s Action Limit: 150

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2014	Jul	8	12:15	69.98	-20.96	-1.065			11-1291-5570	12-3485-8625
2			12	15:30	68.06	-22.88	-1.162			19-5679-7209	14-7190-8017
3			22	12:10	82.64	-8.297	-0.4216			08-9694-1010	03-0721-9902
4		Aug	5	12:30	110.7	19.72	1.002			02-3572-4318	17-8338-0224
5			19	12:50	85.25	-5.692	-0.2892			10-2220-5806	03-5586-7088
6		Sep	9	13:15	125.4	34.44	1.75			00-2260-7679	03-6498-2670
7			23	10:00	76.05	-14.89	-0.7568			10-2715-0848	05-4744-6984
8		Oct	7	14:55	107.7	16.77	0.8523			03-4335-1097	14-3670-0847
9			14	14:55	112.1	21.17	1.076			03-4389-3229	02-1869-3649
10		Nov	4	17:00	118.5	27.57	1.401			07-3336-0499	04-3369-9002
11			19	15:35	100.3	9.405	0.4779			01-4951-3716	01-3053-1585
12		Dec	9	13:30	90.54	-0.3953	-0.02009			18-0939-8222	16-6086-0113
13	2015	Jan	6	11:00	99.86	8.922	0.4533			09-8025-9732	16-4836-2900
14		Feb	3	14:00	88.25	-2.691	-0.1367			21-1065-0393	09-4908-9330
15			10	14:30	90.78	-0.1583	-0.00805			02-7871-6683	04-3347-7000
16		Mar	2	15:30	53.03	-37.91	-1.926			16-8982-2124	18-8086-0569
17			17	10:15	56.06	-34.88	-1.773			18-0061-0968	11-1889-0336
18		Apr	7	12:35	98.3	7.365	0.3742			19-9342-6452	04-7127-1266
19			13	14:10	100.3	9.405	0.4779			21-1811-2763	09-5535-4961
20			20	13:50	84.97	-5.974	-0.3035			00-6349-8324	11-3417-4341
21		May	5	14:15	69.03	-21.91	-1.113			20-1963-0951	02-8524-0195

Marine Chronic Bioassay

Larval Fish Survival

Client: Internal

Test Species: A. affinis

Sample ID: CuCl₂

Start Date/Time: 5/4/2015 5/5/15 1415

Test No.: 150504aart

End Date/Time: 5/11/2015 5/12/15 1100

Conc. (µg/L)	Rep.	Rand #	Test Day / No. Organisms Alive								Percent Survival		
			0	1	2	3	4	5	6	7			
Lab Control	a	3	5	5	5	5	5	5	5	5	5	100	
	b	9	5	5	5	5	5	5	5	5	5	100	
	c	1	5	5	5	5	5	5	5	5	5	100	
	d	7	5	5	5	5	5	5	5	5	5	100	
	e	6	5	5	5	5	5	5	5	5	5	100	
37.5	a	27	5	5	5	5	5	5	5	5	5	100	
	b	21	5	5	5	5	5	5	5	5	5	100	
	c	2	5	5	5	5	5	5	5	5	5	100	
	d	22	5	4	4	4	4	4	4	4	4	80	
	e	30	5	5	5	5	5	5	5	5	5	100	
75	a	17	5	4	3	1	1	1	0	-	-	0	
	b	20	5	4	2	2	2	2	2	2	2	40	
	c	16	5	5	2	1	0	-	-	-	-	0	
	d	26	5	4	3	3	3	3	3	3	3	60	
	e	29	5	5	3	3	3	3	3	3	2	40	
150	a	15	5	4	3	1	0	-	-	-	-	0	
	b	25	5	4	0	-	-	-	-	-	-	0	
	c	13	5	3	2	0	-	-	-	-	-	0	
	d	24	5	3	2	1	1	0	-	-	-	0	
	e	8	5	4	3	1	1	0	-	-	-	0	
300	a	19	5	0								0	
	b	28	5	0								0	
	c	12	5	0	All Dead								0
	d	10	5	0	All Dead								0
	e	23	5	0	All Dead								0
600	a	11	5	0								0	
	b	14	5	0								0	
	c	5	5	0	All Dead								0
	d	4	5	0	All Dead								0
	e	18	5	0	All Dead								0

Rand # QC: EG

Tech Initials

EG BK NH NH VCR BK 1450 1100

Initial Count QC: AD

Time

1415 1000 1100 1203 1430 1200 CH AD

Time Fed (day):	0	1	2	3	4	5	6
morning:	-	0815	0820	0930	0825	0830	0830
evening:	1540	1530	1655	1520	1130	1545	1700

Drying Oven Info

Tare wt. Initials/Date: 56 5/12/15 0935

Date/Time in: 5/12/15 1335

Date/Time out: 5/13/15 1430

Temp (°C): 60.0

QC Check: KBS/2/1/15

Final Review: AC 6/5/15

Comments: @Q18 VCR 5/11/15

@Q18 BK 5/10/15

Marine Chronic Bioassay

Larval Fish Weights

Client: Internal Test Species: A. affinis
 Sample ID: CuCl₂ Start Date/Time: 5/5/2015 1415
 Test No.: 150505aart End Date/Time: 5/12/2015 1100

Conc. (<u> </u> µg/L <u> </u>)	Rep.	pan weight (mg)	pan + fish weight (mg)	total organism weight (mg)
Lab Control	a	22.87	28.03	5.16
	b	23.07	28.02	4.95
	c	20.23	25.87	5.64
	d	21.65	26.81	5.16
	e	24.4	30.21	5.81
37.5	a	21.07	26.44	5.37
	b	22.23	27.39	5.16
	c	20.3	26.25	5.95
	d	19.86	23.88	4.02
	e	23.07	28.75	5.68
75	a	0	0	0
	b	22.21	24.13	1.92
	c	0	0	0
	d	22.02	26.11	4.09
	e	22.86	24.55	1.69
150	a	0	0	0
	b	0	0	0
	c	0	0	0
	d	0	0	0
	e	0	0	0
300	a	0	0	0
	b	0	0	0
	c	0	0	0
	d	0	0	0
	e	0	0	0
600	a	0	0	0
	b	0	0	0
	c	0	0	0
	d	0	0	0
	e	0	0	0
	a			
	b			
	c			
	d			
	e			

Tech Initials:	SG	SG
Date/Time:	5/12/15 0935	5/13/15 1430

QC Check: KBS/2/15

Final Review: AC 6/5/15

Client: Internal
 Sample ID: CuCl₂
 Test No: 150504aart
5
ACQ18/6-5-15

Test Species: A. affinis
 Start Date/Time: 5/4/2015 5/5/15 1415
 End Date/Time: 5/11/2015 5/12/15 1100

Concentration	Lab Control							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.06	8.07	8.04	7.76	8.01	8.14	7.82	
DO (mg/L)	7.9	7.8	7.2	6.6	7.7	7.6	7.3	
Salinity (ppt)	30.2	30.4	30.5	30.4	30.0	29.8	29.8	
Temp (°C)	19.1	20.1	19.9	20.0	19.5	19.3	20.2	
Final:								
pH		7.88	7.78	7.74	7.69	7.81	7.75	7.78
DO (mg/L)		6.7	5.6	6.5	6.4	6.9	7.1	6.5
Temp (°C)		20.1	20.1	19.8	20.1	20.2	20.1	20.2

Concentration	150 µg/L							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.06	8.06	8.04	7.96	8.02	8.14		
DO (mg/L)	7.9	7.9	7.2	6.7	8.1	7.8		
Salinity (ppt)	30.2	30.3	30.4	30.4	29.4	29.9		
Temp (°C)	19.1	20.1	20.0	19.6	19.3	19.3		
Final:								
pH		7.94	7.81	7.82	7.85	7.87	7.82	
DO (mg/L)		6.9	6.1	6.0	7.0	6.9	7.3	
Temp (°C)		20.1	20.2	19.9	20.2	20.1	20.0	

Concentration	37.5 µg/L							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.07	8.08	8.03	7.96	8.02	8.14	7.81	
DO (mg/L)	7.9	7.8	7.2	6.6	7.9	7.7	7.2	
Salinity (ppt)	30.2	30.4	30.5	30.4	30.1	29.9	30.0	
Temp (°C)	19.1	20.1	20.0	19.9	19.4	19.3	20.2	
Final:								
pH		7.90	7.81	7.60	7.72	7.81	7.80	7.81
DO (mg/L)		6.7	6.0	5.9	6.5	6.2	7.3	6.4
Temp (°C)		20.0	20.1	19.7	20.1	20.1	20.1	20.5

Concentration	300 µg/L							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.06	8.06						
DO (mg/L)	7.9	7.9						
Salinity (ppt)	30.2	30.3						
Temp (°C)	19.0	20.1						
Final:								
pH		7.96						
DO (mg/L)		7.0						
Temp (°C)		20.2						

Concentration	75 µg/L							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.07	8.06	8.02	7.96	8.02	8.14	7.89	
DO (mg/L)	7.9	7.7	7.2	6.6	8.0	7.8	7.5	
Salinity (ppt)	30.2	30.3	30.5	30.4	30.0	29.9	30.1	
Temp (°C)	19.1	20.1	20.0	19.9	19.4	19.3	19.9	
Final:								
pH		7.88	7.82	7.83	7.81	7.91	7.82	7.89
DO (mg/L)		6.2	6.2	6.0	6.8	6.5	7.2	7.0
Temp (°C)		20.3	20.0	19.7	20.0	20.1	20.0	20.5

Concentration	600 µg/L							
Day	0	1	2	3	4	5	6	7
Initial:								
pH	8.04	8.05						
DO (mg/L)	7.9	7.9						
Salinity (ppt)	30.1	30.2						
Temp (°C)	19.0	20.2						
Final:								
pH		7.94						
DO (mg/L)		7.2						
Temp (°C)		20.1						

Animal Source/Date Received: ABS / 5/1/15
 Animal Age at Initiation: 15d
 Cu Stock Concentration (µg/L): 86,900

	0	1	2	3	4	5	6	7
Analysts: Initial:	NH	ALB	KB	NH	YS	CH	KB	
Final:		ALB	KB	NH	YS	CH	KB	AG
Dilutions made by:	REG	EG	EG	ALB	EG	NH	EG	
High conc. made (µg/L):	600	600	150	150	150	150	75	
Vol. Cu stock added (mL):	17.3	17.3	4.325	4.325	4.325	4.325	2.163	

Added to Final Volume = 2500 mL

Comments: @Q18 ver 5/14/15
710% mortality was observed in the organisms while in holding at Nautilus.
 QC Check: KB 5/21/15
 Final Review: AC 6/5/15