



Nautilus Environmental

## Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)

Sample Collection Date: September 9, 2017

**Prepared for:** IDE AMERICAS, Inc.  
4590 Carlsbad Boulevard  
Carlsbad, CA 92008

**Prepared by:** Nautilus Environmental

**Submitted:** October 3, 2017

### **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 Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- 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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Results verified by: Adrienne Cibor

## EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING  
CARLSBAD DESALINATION PLANT – SEPTEMBER 2017  
ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: September 9, 2017

Test Date: September 10, 2017

Sample ID: M-001 (off-spec period)

Effluent Limitation: 16.5 TU<sub>c</sub>

### Results Summary:

Bioassay Type: Urchin Fertilization	Effluent Test Results		Effluent Limitation Met? (Yes/No)
	NOEC	TU <sub>c</sub>	
	10	10	Yes

## INTRODUCTION

A discharge sample was collected in September 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for daily chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on September 10, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

## MATERIALS AND METHODS

Sample collection and delivery were performed by IDE Americas, Inc. (IDE) personnel. Following arrival at Nautilus, an aliquot of the water sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. The sample was stored at 4° C in the dark until used for testing. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocols described in USEPA 1995, and the methods are summarized in Table 3.

**Table 1. Sample Information**

Client/Project:	IDE Americas, Inc./Carlsbad Desalination Plant
Sample ID:	M-001 (off-spec period)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/9/17, 08:00
Sample Receipt Date, Time:	9/10/17, 10:15

**Table 2. Water Quality Measurements for the M-001 Sample upon Receipt**

Sample Collection Date	pH	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO <sub>3</sub> )	Total Chlorine (mg/L)
9/9/17	7.90	7.9	5.0	30.8	85	0.11 <sup>a</sup>

<sup>a</sup> Total residual chlorine was measured above 0.1 mg/L in the undiluted sample. However, chlorine measured in the 15 percent concentration was below threshold levels for toxicity and the 6.06 percent concentration was tested with and without sodium thiosulfate (STS) addition to neutralize the chlorine (see Results section for further details).

**Table 3. Echinoderm Fertilization Chronic Bioassay Specifications**

Test Date, Times:	9/10/17, 14:25 through 15:05
Test Organism:	<i>Strongylocentrotus purpuratus</i> (purple sea urchin)
Test Organism Source:	Field-collected off Point Loma in San Diego, CA
Lab Control/Dilution Water:	Natural seawater (source: Scripps Institution of Oceanography inlet, 34±2 parts per thousand (ppt); 20-µm filtered)
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent M-001 sample; lab control. The 6.06 percent concentration was also test with 10 mg/L addition of STS to neutralize chlorine for comparison purposes to the unaltered sample. A method control for 10 mg/L STS addition was also tested.
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
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25.
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in the sample dilution series was compared to that observed in the laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TU<sub>c</sub>) values.

Results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06 percent unadjusted effluent; results are reported as "Pass" if a sample is considered non-toxic at the IWC according to the TST calculation, or "Fail" if considered toxic at the IWC according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis was performed for comparison purposes only.

## RESULTS

A statistically significant decrease in the fertilization rate was observed in the 15 percent effluent concentration compared to the lab control. The NOEC is reported as 10 and the TU<sub>c</sub> is equal to 10, which is below the maximum effluent limitation of 16.5 for this permit. According to the TST analysis no statistically significant effect was observed at any effluent concentration when compared to lab control.

Due to an elevated total residual chlorine level measured in the sample upon receipt (0.11 mg/L), 10 mg/L of STS was added to an aliquot of the M-001 sample to neutralize chlorine, which was then diluted to 6.06 percent for comparison to results in the 6.06 percent unmanipulated sample. Mean fertilization in the STS treated 6.06 percent concentration was 79.6 percent compared to 73.6 percent in the 6.06 percent concentration without STS. Given the standard deviation of both the 6.06 percent unmanipulated sample (9.5%) and the STS-adjusted 6.06 percent concentration (8.0%), there was not a significant difference between the two results. Additionally, prior to test initiation chlorine was measured in the 15 percent concentration of the dilution series that was no de-chlorinated; after dilution the total chlorine in the highest test concentration was 0.03 mg/L, which is not a level likely cause adverse effects to urchin fertilization.

Statistical results are summarized in Table 4, and detailed test results are summarized in Table 5. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and copies of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC <sub>50</sub> (% sample)	TU <sub>c</sub> value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001	10	15	>15	10	Pass	11.8

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

EC<sub>50</sub> = Concentration expected to cause an adverse effect to 50 percent of the test organisms

TU<sub>c</sub> = Chronic Toxic Unit: 100 ÷ NOEC

TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.

Percent effect (PE) from control is calculated as: PE = ((mean response in control - mean response in the IWC) / mean response in control) \* 100. A negative PE results when organism performance in the sample is greater than that in the control.

Table 5. Detailed Results of Purple Urchin Fertilization Testing for the M-001 Sample

Test Concentration (% Sample)	Mean Percent Fertilization
Lab Control	83.4
STS Control	87.6
2.5	86.0
5.0	82.0
6.06	73.6
6.06 w/STS	79.6
10	76.2
15	72.8*

\*An asterisk indicates a statistically significant decrease compared to the lab control.

## QUALITY ASSURANCE

The sample was received on the day after collection and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The lab control met all test acceptability criteria, and the PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations reported are deemed reliable. Additionally, appropriate alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010).

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity met all test acceptability criteria. The median effect ( $EC_{50}$ ) value calculated for this test was within two standard deviations (2SD) of the historical mean for our laboratory, indicating organisms were of typical sensitivity to copper. Results for the reference toxicant test are summarized in Table 6 and presented in full in Appendix D. A list of qualifier codes can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC <sub>50</sub> ( $\mu$ g/L Copper)	Historical Mean EC <sub>50</sub> $\pm$ 2 SD ( $\mu$ g/L Copper)	CV (%)
9/10/17	40.4	49.7 $\pm$ 31.8	32.1

EC<sub>50</sub> = Concentration expected to cause an adverse effect to 50 percent of the test organisms

Historical Mean EC<sub>50</sub>  $\pm$  2 SD = Mean of historical test results plus or minus two standard deviations

CV = Coefficient of Variation

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## REFERENCES

- California Regional Water Quality Control Board Region 9, San Diego (RWQCB) 2006. Waste Discharge Requirements for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project, Discharge to the Pacific Ocean via the Encina Power Station Discharge Channel. Order No. R9-2006-0065, NPDES No. CA109223. June 2006.
- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- 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.
- USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A  
Test Data and Statistical Analyses

# CETIS Summary Report

Report Date:

28 Sep-17 15:01 (p 1 of 1)

Test Code:

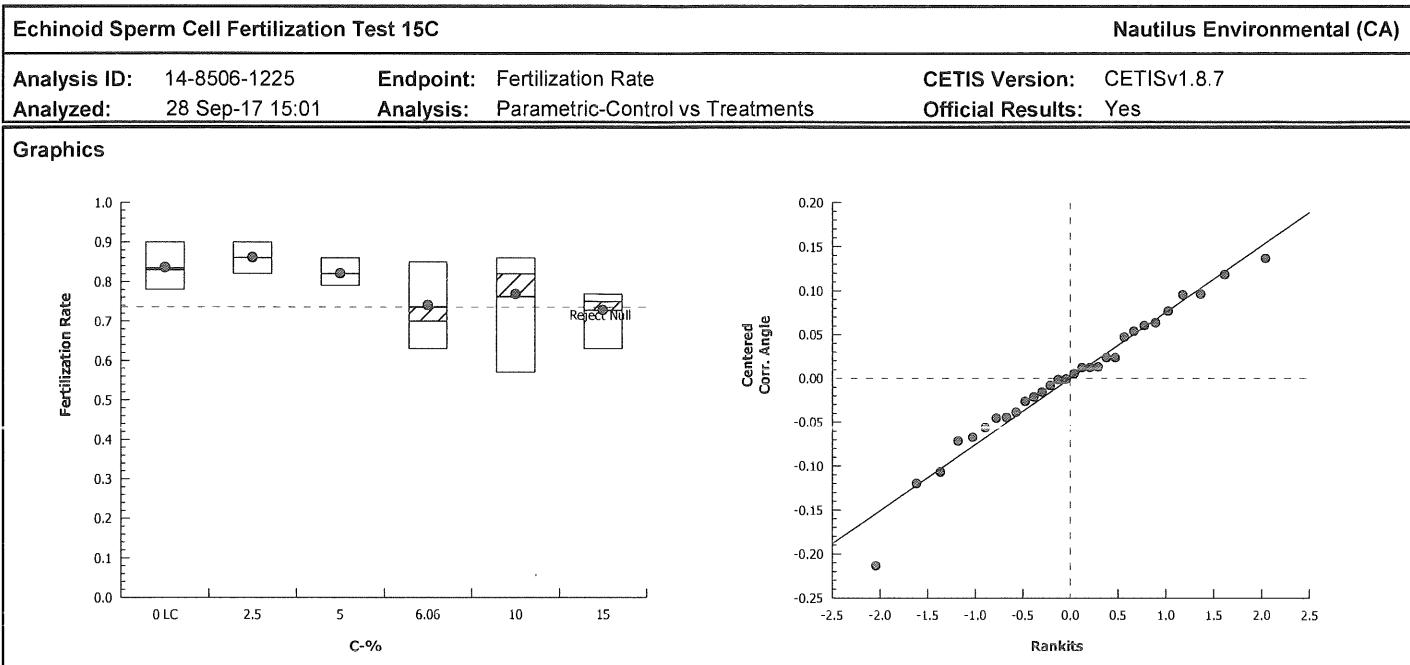
1709-S100 | 11-4903-9690

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)					
Batch ID:	00-1329-4234	Test Type: Fertilization				Analyst:						
Start Date:	10 Sep-17 14:25	Protocol: EPA/600/R-95/136 (1995)				Diluent: Laboratory Seawater						
Ending Date:	10 Sep-17 15:05	Species: Strongylocentrotus purpuratus				Brine: Not Applicable						
Duration:	40m	Source: Pt. Loma				Age:						
Sample ID:	01-4830-3903	Code: 17-0993				Client: IDE						
Sample Date:	09 Sep-17 08:00	Material: Facility Effluent				Project: Carlsbad Desal Plant						
Receive Date:	10 Sep-17 10:15	Source: IDE Americas, Inc.										
Sample Age:	30h (5 °C)	Station: M-001 (Daily)										
<b>Comparison Summary</b>												
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method					
14-8506-1225	Fertilization Rate	10	15	12.25	11.8%	10	Dunnett Multiple Comparison Test					
<b>Point Estimate Summary</b>												
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method					
18-1984-0677	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)					
		EC50	>15	N/A	N/A	<6.667						
<b>Test Acceptability</b>												
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits	Overlap	Decision					
14-8506-1225	Fertilization Rate	Control Resp		0.834	0.7 - NL	Yes	Passes Acceptability Criteria					
18-1984-0677	Fertilization Rate	Control Resp		0.834	0.7 - NL	Yes	Passes Acceptability Criteria					
14-8506-1225	Fertilization Rate	PMSD		0.1176	NL - 0.25	No	Passes Acceptability Criteria					
<b>Fertilization Rate Summary</b>												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect	
0	Lab Control	5	0.834	0.7802	0.8878	0.78	0.9	0.01939	0.04336	5.2%	0.0%	
2.5		5	0.86	0.8238	0.8962	0.82	0.9	0.01304	0.02915	3.39%	-3.12%	
5		5	0.82	0.786	0.854	0.79	0.86	0.01225	0.02739	3.34%	1.68%	
6.06		5	0.736	0.6187	0.8533	0.63	0.85	0.04226	0.0945	12.84%	11.75%	
10		5	0.762	0.6158	0.9082	0.57	0.86	0.05267	0.1178	15.46%	8.63%	
15		5	0.728	0.6586	0.7974	0.63	0.77	0.02498	0.05586	7.67%	12.71%	
<b>Fertilization Rate Detail</b>												
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.83	0.84	0.9	0.78	0.82						
2.5		0.82	0.9	0.85	0.86	0.87						
5		0.79	0.83	0.82	0.86	0.8						
6.06		0.63	0.85	0.68	0.7	0.82						
10		0.83	0.57	0.73	0.86	0.82						
15		0.74	0.75	0.77	0.63	0.75						

# CETIS Analytical Report

Report Date: 28 Sep-17 15:01 (p 1 of 2)  
 Test Code: 1709-S100 | 11-4903-9690

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)				
Analysis ID: 14-8506-1225		Endpoint: Fertilization Rate			CETIS Version: CETISv1.8.7							
Analyzed: 28 Sep-17 15:01		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	11.8%	10	15	12.25	10			
Dunnett Multiple Comparison Test												
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)			
Lab Control	2.5	2.5	-0.6718	2.362	0.123	8	0.9603	CDF	Non-Significant Effect			
	5	5	0.3912	2.362	0.123	8	0.6932	CDF	Non-Significant Effect			
	6.06	6.06	2.254	2.362	0.123	8	0.0618	CDF	Non-Significant Effect			
	10	10	1.629	2.362	0.123	8	0.1845	CDF	Non-Significant Effect			
	15*	15*	2.507	2.362	0.123	8	0.0372	CDF	Significant Effect			
ANOVA Table												
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision( $\alpha$ :5%)			
Between	0.11359		0.022718		5	3.362		0.0192	Significant Effect			
Error	0.1621908		0.006757951		24							
Total	0.2757808				29							
Distributional Tests												
Attribute	Test			Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)					
Variances	Bartlett Equality of Variance			9.515	15.09	0.0902	Equal Variances					
Distribution	Shapiro-Wilk W Normality			0.973	0.9031	0.6247	Normal Distribution					
Fertilization Rate Summary												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	0.834	0.7802	0.8878	0.83	0.78	0.9	0.01939	5.2%	0.0%	
2.5		5	0.86	0.8238	0.8962	0.86	0.82	0.9	0.01304	3.39%	-3.12%	
5		5	0.82	0.786	0.854	0.82	0.79	0.86	0.01225	3.34%	1.68%	
6.06		5	0.736	0.6187	0.8533	0.7	0.63	0.85	0.04226	12.84%	11.75%	
10		5	0.762	0.6158	0.9082	0.82	0.57	0.86	0.05267	15.46%	8.63%	
15		5	0.728	0.6586	0.7974	0.75	0.63	0.77	0.02498	7.67%	12.71%	
Angular (Corrected) Transformed Summary												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.154	1.079	1.229	1.146	1.083	1.249	0.0271	5.25%	0.0%	
2.5		5	1.189	1.136	1.241	1.187	1.133	1.249	0.01898	3.57%	-3.03%	
5		5	1.134	1.089	1.178	1.133	1.095	1.187	0.01619	3.19%	1.76%	
6.06		5	1.037	0.8996	1.174	0.9912	0.9169	1.173	0.04937	10.65%	10.16%	
10		5	1.069	0.9032	1.235	1.133	0.8556	1.187	0.05978	12.5%	7.34%	
15		5	1.024	0.9479	1.099	1.047	0.9169	1.071	0.02725	5.95%	11.3%	

**CETIS Analytical Report**Report Date: 28 Sep-17 15:01 (p 2 of 2)  
Test Code: 1709-S100 | 11-4903-9690

# CETIS Analytical Report

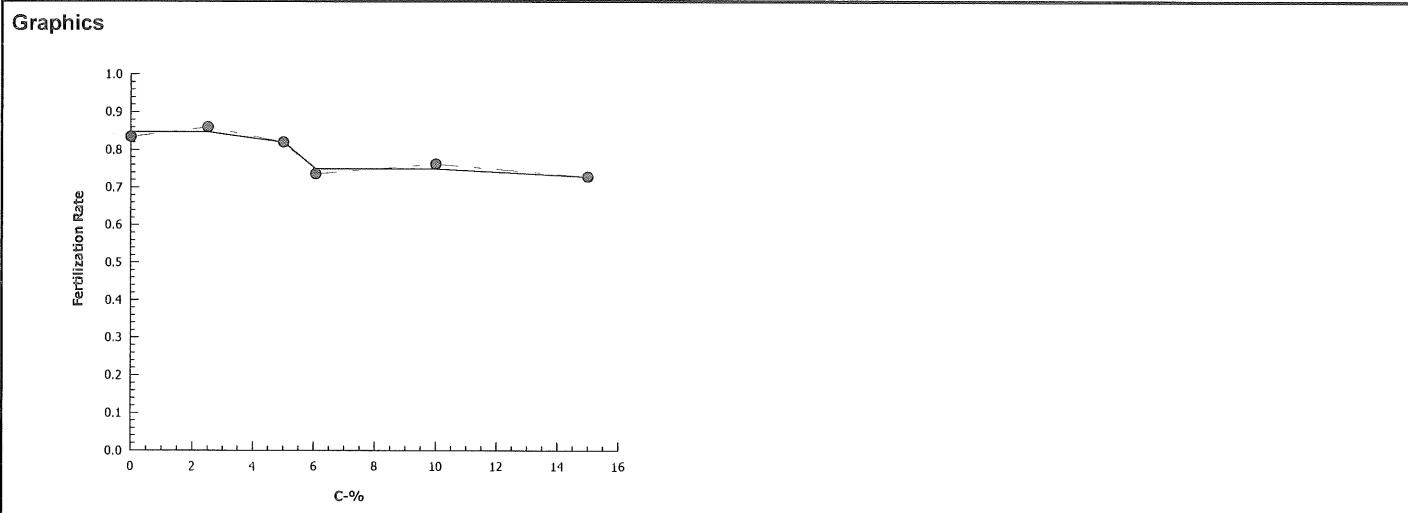
Report Date: 28 Sep-17 15:01 (p 1 of 1)  
Test Code: 1709-S100 | 11-4903-9690

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	18-1984-0677	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	28 Sep-17 15:01	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2125623	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>15	N/A	N/A	<6.667	NA	NA
EC50	>15	N/A	N/A	<6.667	NA	NA

Fertilization Rate Summary			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.834	0.78	0.9	0.01939	0.04336	5.2%	0.0%	417	500
2.5		5	0.86	0.82	0.9	0.01304	0.02915	3.39%	-3.12%	430	500
5		5	0.82	0.79	0.86	0.01225	0.02739	3.34%	1.68%	410	500
6.06		5	0.736	0.63	0.85	0.04226	0.0945	12.84%	11.75%	368	500
10		5	0.762	0.57	0.86	0.05267	0.1178	15.46%	8.63%	381	500
15		5	0.728	0.63	0.77	0.02498	0.05586	7.67%	12.71%	364	500



# CETIS Analytical Report

Report Date: 28 Sep-17 15:02 (p 1 of 1)  
 Test Code: 1709-S100 | 11-4903-9690

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)		
Analysis ID: 02-0039-8567			Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 28 Sep-17 15:01			Analysis: Parametric Bioequivalence-Two Sample				Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	5.79%	15	>15	NA	6.667		
<b>TST-Welch's t Test</b>												
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)			
Lab Control	2.5*	11.63	1.895	0.053	7	<0.0001	CDF	Non-Significant Effect				
	5*	10.32	1.895	0.049	7	<0.0001	CDF	Non-Significant Effect				
	6.06*	3.208	2.015	0.108	5	0.0119	CDF	Non-Significant Effect				
	10*	3.227	2.132	0.135	4	0.0160	CDF	Non-Significant Effect				
	15*	4.651	1.895	0.064	7	0.0012	CDF	Non-Significant Effect				
<b>ANOVA Table</b>												
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision( $\alpha$ :5%)			
Between	0.11359		0.022718		5	3.362		0.0192	Significant Effect			
Error	0.1621908		0.006757951		24							
Total	0.2757808				29							
<b>Distributional Tests</b>												
Attribute	Test		Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)						
Variances	Bartlett Equality of Variance		9.515	15.09	0.0902	Equal Variances						
Distribution	Shapiro-Wilk W Normality		0.973	0.9031	0.6247	Normal Distribution						
<b>Fertilization Rate Summary</b>												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	0.834	0.7802	0.8878	0.83	0.78	0.9	0.01939	5.2%	0.0%	
2.5		5	0.86	0.8238	0.8962	0.86	0.82	0.9	0.01304	3.39%	-3.12%	
5		5	0.82	0.786	0.854	0.82	0.79	0.86	0.01225	3.34%	1.68%	
6.06		5	0.736	0.6187	0.8533	0.7	0.63	0.85	0.04226	12.84%	11.75%	
10		5	0.762	0.6158	0.9082	0.82	0.57	0.86	0.05267	15.46%	8.63%	
15		5	0.728	0.6586	0.7974	0.75	0.63	0.77	0.02498	7.67%	12.71%	
<b>Angular (Corrected) Transformed Summary</b>												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.154	1.079	1.229	1.146	1.083	1.249	0.0271	5.25%	0.0%	
2.5		5	1.189	1.136	1.241	1.187	1.133	1.249	0.01898	3.57%	-3.03%	
5		5	1.134	1.089	1.178	1.133	1.095	1.187	0.01619	3.19%	1.76%	
6.06		5	1.037	0.8996	1.174	0.9912	0.9169	1.173	0.04937	10.65%	10.16%	
10		5	1.069	0.9032	1.235	1.133	0.8556	1.187	0.05978	12.5%	7.34%	
15		5	1.024	0.9479	1.099	1.047	0.9169	1.071	0.02725	5.95%	11.3%	

# CETIS Summary Report

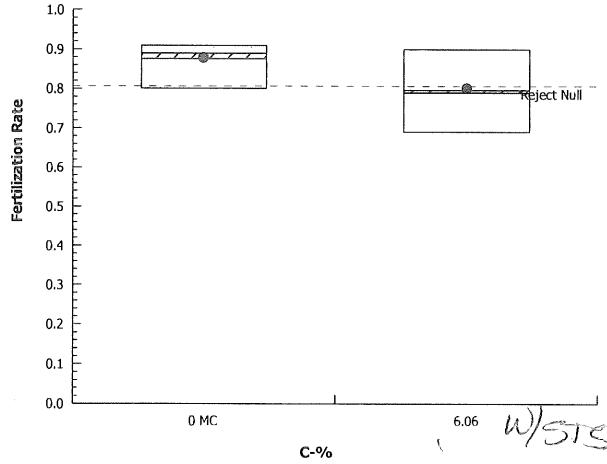
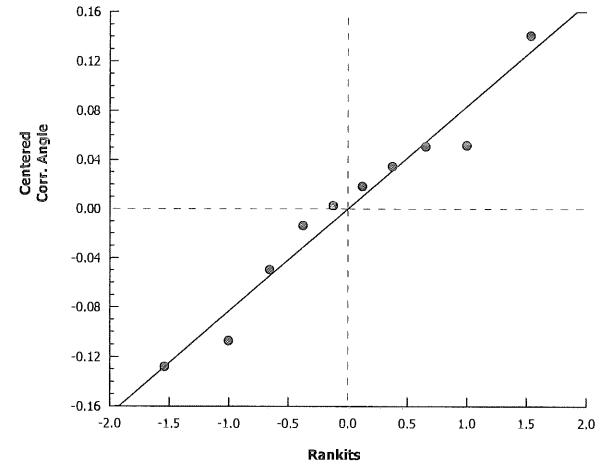
Report Date: 12 Sep-17 13:34 (p 1 of 1)

Test Code: 1709-S100B | 05-8925-2837

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)					
Batch ID:	08-7887-1403	Test Type: Fertilization				Analyst:						
Start Date:	10 Sep-17 14:25	Protocol: EPA/600/R-95/136 (1995)				Diluent: Laboratory Seawater						
Ending Date:	10 Sep-17 15:05	Species: Strongylocentrotus purpuratus				Brine: Not Applicable						
Duration:	40m	Source: Pt. Loma				Age:						
Sample ID:	18-1822-5237	Code: 17-0993				Client: IDE						
Sample Date:	09 Sep-17 08:00	Material: Facility Effluent				Project: Carlsbad Desal Plant						
Receive Date:	10 Sep-17 10:15	Source: IDE Americas, Inc.										
Sample Age:	30h (5 °C)	Station: M-001 (Daily)										
<b>Comparison Summary</b>												
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method					
17-5065-5361	Fertilization Rate	<6.06	6.06	NA	7.96%	>16.5	Equal Variance t Two-Sample Test					
10-1746-8585	Fertilization Rate	6.06	>6.06	NA	8.11%	16.5	TST-Welch's t Test					
<b>Test Acceptability</b>												
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits	Overlap	Decision					
10-1746-8585	Fertilization Rate	Control Resp		0.876	0.7 - NL	Yes	Passes Acceptability Criteria					
17-5065-5361	Fertilization Rate	Control Resp		0.876	0.7 - NL	Yes	Passes Acceptability Criteria					
10-1746-8585	Fertilization Rate	PMSD		0.08108	NL - 0.25	No	Passes Acceptability Criteria					
17-5065-5361	Fertilization Rate	PMSD		0.07961	NL - 0.25	No	Passes Acceptability Criteria					
<b>Fertilization Rate Summary</b>												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect	
0	Method Control	5	0.876	0.8215	0.9305	0.8	0.91	0.01965	0.04393	5.02%	0.0%	
6.06	STS	5	0.796	0.6972	0.8948	0.69	0.9	0.03558	0.07956	10.0%	9.13%	
<b>Fertilization Rate Detail</b>												
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Method Control	0.9	0.88	0.8	0.89	0.91						
6.06		0.69	0.79	0.9	0.76	0.84						

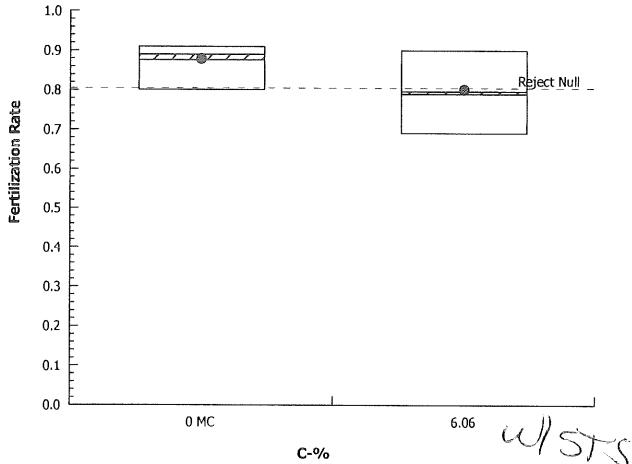
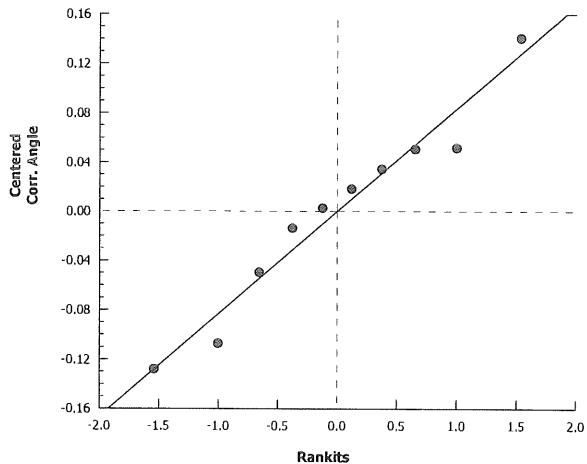
# CETIS Analytical Report

Report Date: 12 Sep-17 13:34 (p 1 of 2)  
 Test Code: 1709-S100B | 05-8925-2837

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 17-5065-5361 Analyzed: 12 Sep-17 13:33			Endpoint: Fertilization Rate Analysis: Parametric-Two Sample			CETIS Version: CETISv1.8.7 Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result					
Angular (Corrected)	NA	C > T	NA	NA	7.96%	Fails fertilization rate					
<b>Equal Variance t Two-Sample Test</b>											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)		
Method Control		6.06*	1.983	1.86	0.099	8	0.0414	CDF	Significant Effect		
<b>ANOVA Table</b>											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision( $\alpha$ :5%)			
Between	0.02807689		0.02807689		1	3.931	0.0827	Non-Significant Effect			
Error	0.05714379		0.007142974		8						
Total	0.08522068				9						
<b>Distributional Tests</b>											
Attribute	Test		Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)					
Variances	Variance Ratio F		2.635	23.15	0.3708	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9629	0.7411	0.8182	Normal Distribution					
<b>Fertilization Rate Summary</b>											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Method Control	5	0.876	0.8215	0.9305	0.89	0.8	0.91	0.01965	5.02%	0.0%
6.06		5	0.796	0.6972	0.8948	0.79	0.69	0.9	0.03558	10.0%	9.13%
<b>Angular (Corrected) Transformed Summary</b>											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Method Control	5	1.214	1.137	1.292	1.233	1.107	1.266	0.02804	5.16%	0.0%
6.06		5	1.108	0.9821	1.235	1.095	0.9803	1.249	0.04551	9.18%	8.73%
<b>Graphics</b>											
											
											

## CETIS Analytical Report

Report Date: 12 Sep-17 13:34 (p 2 of 2)  
 Test Code: 1709-S100B | 05-8925-2837

Echinoid Sperm Cell Fertilization Test 15C <i>TOT</i>							Nautilus Environmental (CA)				
Analysis ID: 10-1746-8585 Analyzed: 12 Sep-17 13:33		Endpoint: Fertilization Rate Analysis: Parametric Bioequivalence-Two Sample			CETIS Version: CETISv1.8.7 Official Results: Yes						
Data Transform		Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	Test Result			
Angular (Corrected)		NA	C*b < T	NA	NA	0.75	8.11%	Passes fertilization rate			
<b>TST-Welch's t Test</b>											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)		
Method Control		6.06*	3.942	2.015	0.101	5	0.0055	CDF	Non-Significant Effect		
<b>ANOVA Table</b>											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision( $\alpha$ :5%)			
Between	0.02807689		0.02807689		1	3.931	0.0827	Non-Significant Effect			
Error	0.05714379		0.007142974		8						
Total	0.08522068				9						
<b>Distributional Tests</b>											
Attribute	Test		Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)					
Variances	Variance Ratio F		2.635	23.15	0.3708	Equal Variances					
Distribution	Shapiro-Wilk W Normality		0.9629	0.7411	0.8182	Normal Distribution					
<b>Fertilization Rate Summary</b>											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Method Control	5	0.876	0.8215	0.9305	0.89	0.8	0.91	0.01965	5.02%	0.0%
6.06		5	0.796	0.6972	0.8948	0.79	0.69	0.9	0.03558	10.0%	9.13%
<b>Angular (Corrected) Transformed Summary</b>											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Method Control	5	1.214	1.137	1.292	1.233	1.107	1.266	0.02804	5.16%	0.0%
6.06		5	1.108	0.9821	1.235	1.095	0.9803	1.249	0.04551	9.18%	8.73%
<b>Graphics</b>											
 <p>Handwritten note: <i>WSTS</i></p>											
											

## CETIS Analytical Report

Report Date: 03 Oct-17 16:39 (p 1 of 1)  
 Test Code: 1709-S100B | 05-8925-2837

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)			
Analysis ID: 06-2404-4089 Analyzed: 03 Oct-17 16:39	Endpoint: Fertilization Rate Analysis: Parametric-Two Sample				CETIS Version: CETISv1.8.7 Official Results: Yes					
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result				
Angular (Corrected)	NA	C > T	NA	NA	9.23%	Passes fertilization rate				
<b>Equal Variance t Two-Sample Test</b>										
Control	vs C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)		
Lab Control	6.06	0.8578	1.86	0.098	8	0.2080	CDF	Non-Significant Effect		
<b>ANOVA Table</b>										
Source	Sum Squares	Mean Square		DF	F Stat	P-Value	Decision( $\alpha$ :5%)			
Between	0.00516032	0.00516032		1	0.7358	0.4160	Non-Significant Effect			
Error	0.05610806	0.007013508		8						
Total	0.06126839			9						
<b>Distributional Tests</b>										
Attribute	Test	Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)					
Variances	Variance Ratio F	2.821	23.15	0.3392	Equal Variances					
Distribution	Shapiro-Wilk W Normality	0.9757	0.7411	0.9379	Normal Distribution					
<b>Fertilization Rate Summary</b>										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max		
0	Lab Control	5	0.834	0.7802	0.8878	0.83	0.78	0.9		
6.06		5	0.796	0.6972	0.8948	0.79	0.69	0.9		
Std Err							CV%	%Effect		
0							5.2%	0.0%		
6.06							10.0%	4.56%		
<b>Angular (Corrected) Transformed Summary</b>										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max		
0	Lab Control	5	1.154	1.079	1.229	1.146	1.083	1.249		
6.06		5	1.108	0.9821	1.235	1.095	0.9803	1.249		
Std Err							CV%	%Effect		
0							5.25%	0.0%		
6.06							9.18%	3.94%		

## CETIS Test Data Worksheet

Report Date: 10 Sep-17 14:36 (p 1 of 1)  
 Test Code: 109-S108 11-4903-9690/447CF44A

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 10 Sep-17 Species: Strongylocentrotus purpuratus  
 End Date: 10 Sep-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 09 Sep-17 Material: Facility Effluent

Sample Code: 17-0993  
 Sample Source: IDE Americas, Inc.  
 Sample Station: M-001 (Daily)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	85	9/11/17
			62		85	
			63		86	
			64		90	
			65		63	
			66		83	
			67		75	
			68		77	
			69		(a) 67-57	
			70		90	
			71		82	
			72		81	
			73		75	
			74		70	
			75		(a) 69-78 QC 14 ac 9/11/17	
			76		83	
			77		82	
			78		68	
			79		86	
			80		82	
			81		63	
			82		86	
			83		84	
			84		80	
			85		79	
			86		87	
			87		73	
			88		74	
			89		83	
			90	✓	82	

④ Q18 AC 9/28/17  
 CA re-count

## CETIS Test Data Worksheet

Report Date: 10 Sep-17 14:54 (p 1 of 1)  
 Test Code: J709-S108 11-4903-9690/447CF44A

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 10 Sep-17 Species: Strongylocentrotus purpuratus  
 End Date: 10 Sep-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 09 Sep-17 Material: Facility Effluent

Sample Code: 17-C993  
 Sample Source: IDE Americas, Inc.  
 Sample Station: M-001 (Daily) (9/9 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	76	100	83	AC 9/10/17
0	LC	2	83			
0	LC	3	64			
0	LC	4	75			
0	LC	5	71			
2.5		1	72		84	
2.5		2	70			
2.5		3	61			
2.5		4	82			
2.5		5	86			
5		1	85		70	
5		2	89			
5		3	90			
5		4	63			
5		5	84			
6.06		1	81		63	
6.06		2	62			
6.06		3	78			
6.06		4	74			
6.06		5	77			
10		1	66		84	
10		2	69			
10		3	87			
10		4	79			
10		5	80			
15		1	88			
15		2	67			
15		3	68			
15		4	65			
15		5	73			

*Ac 9/9*

STS control A  
 B  
 C  
 D  
 E

STS 100% A  
 B  
 C  
 D  
 E

## Marine Chronic Bioassay

## Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: Daily M-001 (9/9/17 Sample)

Start Date/Time: 9/10/2017 1425

Sample Log No.: 17- 0993

End Date/Time: 9/10/2017 1505

Dilutions made by: 4

Test No: 1709-S100

Analyst: 4

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	6.5	8.07	33.9	15.1
2.5	8.6	8.08	33.4	14.8
5.0	8.6	8.08	33.4	14.8
6.06	8.6	8.09	33.4	14.6
10	8.6	8.09	33.2	14.5
15	8.6	8.09	33.1	14.4

Comments: \_\_\_\_\_

QC Check: AC9/12/17

Final Review: 09/10/2017

## Echinoderm Bioassay

## Fertilization

**Client: IDE**

## **Test Species: *S. purpuratus***

**Project ID:** CDP

Start Date/Time: 9/10/2017 1425

Test No.: 1709~S100 b

End Date/Time: 9/10/2017 1505

QC Check: Ac 9/28/17

Final Review: 4/12/17

## Marine Chronic Bioassay

## Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: Daily M-001 (9/9/17 Sample)

Start Date/Time: 9/10/2017 1425

Sample Log No.: 17-0993

End Date/Time: 9/10/2017 1505

Dilutions made by: *g*

Test No: 1709-S100

Analyst: *g*

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
STS Control (10mg/L)	8.5	8.01	33.1	15.7
6.06 STS (10mg/L)	8.5	8.03	33.2	15.2

Comments: \_\_\_\_\_

QC Check: *AC 9/12/17*Final Review: *g 10/12/17*

## Marine Chronic Bioassay

## Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE  
 Sample ID: Daly M-001 (9/9 sample)  
 Test No.: 1709-S100

Tech initials: JZ  
 Injection Time: 1356

Sperm Absorbance at 400 nm: 0.976 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 71 Mean: 71.6  $\times 50 =$  3830 eggs/ml  
72  
74  
84  
62

Initial density: 3830 eggs/ml. = 0.96 dilution factor  
 Final density: 4000 eggs/ml - 1.0 part egg stock  
         parts seawater      egg stock 150 ml  
                                seawater        ml

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							
	<u>2000:1</u>	<u>1600:1</u>	<u>1200:1</u>	<u>800:1</u>	<u>400:1</u>	<u>200:1</u>	<u>100:1</u>	<u>50:1</u>
ml Sperm Stock	<u>50</u>	<u>40</u>	<u>30</u>	<u>20</u>	<u>10</u>	<u>5.0</u>	<u>2.5</u>	<u>1.25</u>
ml Seawater	<u>0.0</u>	<u>10</u>	<u>20</u>	<u>30</u>	<u>40</u>	<u>45</u>	<u>47.5</u>	<u>48.75</u>

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1357</u>	<u>50:1</u>	<u>72</u>	<u>28</u>
Eggs Added (0.5 ml):	<u>1407</u>	<u>100:1</u>	<u>40</u>	<u>10</u>
Test Ended:	<u>1417</u>	<u>100:1</u>	<u>92</u>	<u>8</u>
		<u>200:1</u>	<u>100</u>	<u>0</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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1415</u>	QC1	<u>94</u>	<u>6</u>
Eggs Added (0.5 ml):	<u>1445</u>	QC2	<u>95</u>	<u>5</u>
Test Ended:	<u>1505</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

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QC Check: AC 9/12/17

Final Review: is 10/2/17

Appendix B  
Sample Receipt Information

Nautilus Environmental  
4340 Vandever Avenue  
San Diego, CA 92120

Client: IDE  
Sample ID: M-001  
Test ID No(s.): 1709-S100 + S101

### Sample Check-In Information

#### Sample Description:

9/9 = Colorless, Odorless, Clear, no debris.  
9/10 = Colorless, Clear, odorless, no debris.

Sample (A, B, C):	A	A		
Log-in No. (17-xxxx):	0993	0994 (R)		
Sample Collection Date & Time:	9/9/17 0800	9/10/17 0800		
Sample Receipt Date & Time:	9/10/17 1015	9/10/17 1015		
Number of Containers & Container Type:	1-4L Cubi	1-4L Cubi		
Approx. Total Volume Received (L):	~4	~4		
Check-in Temperature (°C)	5.0	6.0		
Temperature OK? <sup>1</sup>	(Y) N	(Y) N	Y N	Y N
DO (mg/L)	7.9	7.6		
pH (units)	7.90	7.80		
Conductivity (µS/cm)	—	—		
Salinity (ppt)	30.8	33.1		
Alkalinity (mg/L) <sup>2</sup>	85	91		
Hardness (mg/L) <sup>2,3</sup>	—	—		
Total Chlorine (mg/L)	0.11	20.02		
Technician Initials	RH	RH		

Test Performed: Urchin Fert. Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: \_\_\_\_\_

Alkalinity: 91 Hardness or Salinity: 34 ppt

Additional Control? Y (N) = Alkalinity: \_\_\_\_\_ Hardness or Salinity: \_\_\_\_\_

Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: \_\_\_\_\_

Alkalinity: \_\_\_\_\_ Hardness or Salinity: \_\_\_\_\_

Additional Control? Y N = Alkalinity: \_\_\_\_\_ Hardness or Salinity: \_\_\_\_\_

Test Performed: Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: \_\_\_\_\_

Alkalinity: \_\_\_\_\_ Hardness or Salinity: \_\_\_\_\_

Additional Control? Y N = Alkalinity: \_\_\_\_\_ Hardness or Salinity: \_\_\_\_\_

Notes: <sup>1</sup> Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.

<sup>2</sup> mg/L as CaCO<sub>3</sub>, <sup>3</sup> Measured for freshwater samples only, NA = Not Applicable

Additional Comments: (A) 9/9/17 (B) Sample dechlorinated at rate of 10 mg/L  
(B) Sample tested with & without STS addition to neutralize chlorine  
9/9

COC Complete (Y/N)?

A (Y) B ( ) C ( )

Filtration? Y (N)

Pore Size: \_\_\_\_\_  
Organisms or Debris

Salinity Adjustment? Y (N)

Test: Source: Target ppt:

Test: Source: Target ppt:

Test: Source: Target ppt:

pH Adjustment? Y (N)

A ( ) B ( ) C ( )

Initial pH: \_\_\_\_\_

Amount of HCl added: \_\_\_\_\_

Final pH: \_\_\_\_\_

Cl<sub>2</sub> Adjustment? Y (N)

(B) 9/9 sample A ( ) B ( ) C ( )

Initial Free Cl<sub>2</sub>: 0.07

STS added: (C) 9/9 sample

Final Free Cl<sub>2</sub>: 0.03

Sample Aeration? Y (N)

A ( ) B ( ) C ( )

Initial D.O. \_\_\_\_\_

Duration & Rate \_\_\_\_\_

Final D.O. \_\_\_\_\_

Subsamples for Additional Chemistry Required? Y (N)

NH<sub>3</sub> Other \_\_\_\_\_

Tech Initials A ( ) B ( ) C ( )

QC Check: AC 9/12/17

Final Review: 9/10/17

## Appendix C

### Chain-of-Custody Form



CDP laboratory: \_\_\_\_\_  
Entahlyp Laboratory: \_\_\_\_\_  
WECK Laboratory: \_\_\_\_\_  
Nautilus: \_\_\_\_\_ X \_\_\_\_\_  
AIM: \_\_\_\_\_  
Other: \_\_\_\_\_

**Turn Around Time**

Normal: \_\_\_\_\_ X

RUSH (24 hr): \_\_\_\_\_

3 Days: \_\_\_\_\_

5 Days: \_\_\_\_\_

??? Days

Project Name: NPDES Daily Toxicity Project Manager: Peter Shen Contact Information: (760) 201-7777

**Special instruction:** Sampled during off-spec via autosampler by a series of grabs collected at one hour intervals. Sample collected to fulfill daily NPDES requirement. Sample is to be run unadjusted. Start: 9/8/17 @ 8:00, End: 9/9/17 @ 8:00 VH

## ANALYSES

**NOTES:**

Glass=G Plastic=P

Yes=Y No=N Acid=A Base=B

Drinking Water=DW Seawater=SW Soil=S Brine=B

TDS - 31.88 ppt, EC - 49.59 mS/cm

Relinquished By:	Date:	Time:	Received By:	Time:	Sample Condition Upon Receipt:
W. Payne	9/10/17	0930	Katil Payne	9/10/17 0930	<input checked="" type="checkbox"/> Iced <input type="checkbox"/> Ambient or _____ °C
Katil Payne	9/10/17	1015	Roselle Haggard	9/10/17 1015	<input type="checkbox"/> Iced <input type="checkbox"/> Ambient or <u>50</u> °C

Nautlius ID = 17 - 0993

Appendix D  
Reference Toxicant Test Data and  
Statistical Analyses

## CETIS Summary Report

Report Date:

12 Sep-17 10:46 (p 1 of 1)

Test Code:

170910sprt | 11-6871-9499

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)					
Batch ID:	19-5847-3524	Test Type: Fertilization				Analyst:						
Start Date:	10 Sep-17 14:25	Protocol: EPA/600/R-95/136 (1995)				Diluent: Natural Seawater						
Ending Date:	10 Sep-17 15:05	Species: Strongylocentrotus purpuratus				Brine: Not Applicable						
Duration:	40m	Source: Pt. Loma				Age:						
Sample ID:	06-1457-0997	Code: 170910sprt				Client: Internal						
Sample Date:	10 Sep-17	Material: Copper chloride				Project:						
Receive Date:	10 Sep-17	Source: Reference Toxicant										
Sample Age:	14h	Station: Copper Chloride										
<b>Comparison Summary</b>												
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method					
08-3724-5737	Fertilization Rate	10	20	14.14	10.9%		Dunnett Multiple Comparison Test					
<b>Point Estimate Summary</b>												
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method					
08-4248-1228	Fertilization Rate	EC50	40.4	38.71	42.17		Trimmed Spearman-Kärber					
<b>Test Acceptability</b>												
Analysis ID	Endpoint	Attribute		Test Stat	TAC	Limits	Overlap	Decision				
08-3724-5737	Fertilization Rate	Control Resp		0.876	0.7	- NL	Yes	Passes Acceptability Criteria				
08-4248-1228	Fertilization Rate	Control Resp		0.876	0.7	- NL	Yes	Passes Acceptability Criteria				
08-3724-5737	Fertilization Rate	PMSD		0.1088	NL	- 0.25	No	Passes Acceptability Criteria				
<b>Fertilization Rate Summary</b>												
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect	
0	Lab Control	5	0.876	0.8299	0.9221	0.85	0.93	0.01661	0.03715	4.24%	0.0%	
10		5	0.848	0.7605	0.9355	0.76	0.92	0.03153	0.0705	8.31%	3.2%	
20		5	0.734	0.627	0.841	0.64	0.84	0.03855	0.0862	11.74%	16.21%	
40		5	0.548	0.4253	0.6707	0.39	0.64	0.0442	0.09884	18.04%	37.44%	
80		5	0.05	0.01074	0.08926	0.01	0.09	0.01414	0.03162	63.25%	94.29%	
160		5	0	0	0	0	0	0	0		100.0%	
<b>Fertilization Rate Detail</b>												
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.9	0.93	0.85	0.85	0.85						
10		0.81	0.76	0.83	0.92	0.92						
20		0.81	0.69	0.69	0.64	0.84						
40		0.39	0.52	0.64	0.61	0.58						
80		0.05	0.03	0.07	0.09	0.01						
160		0	0	0	0	0						

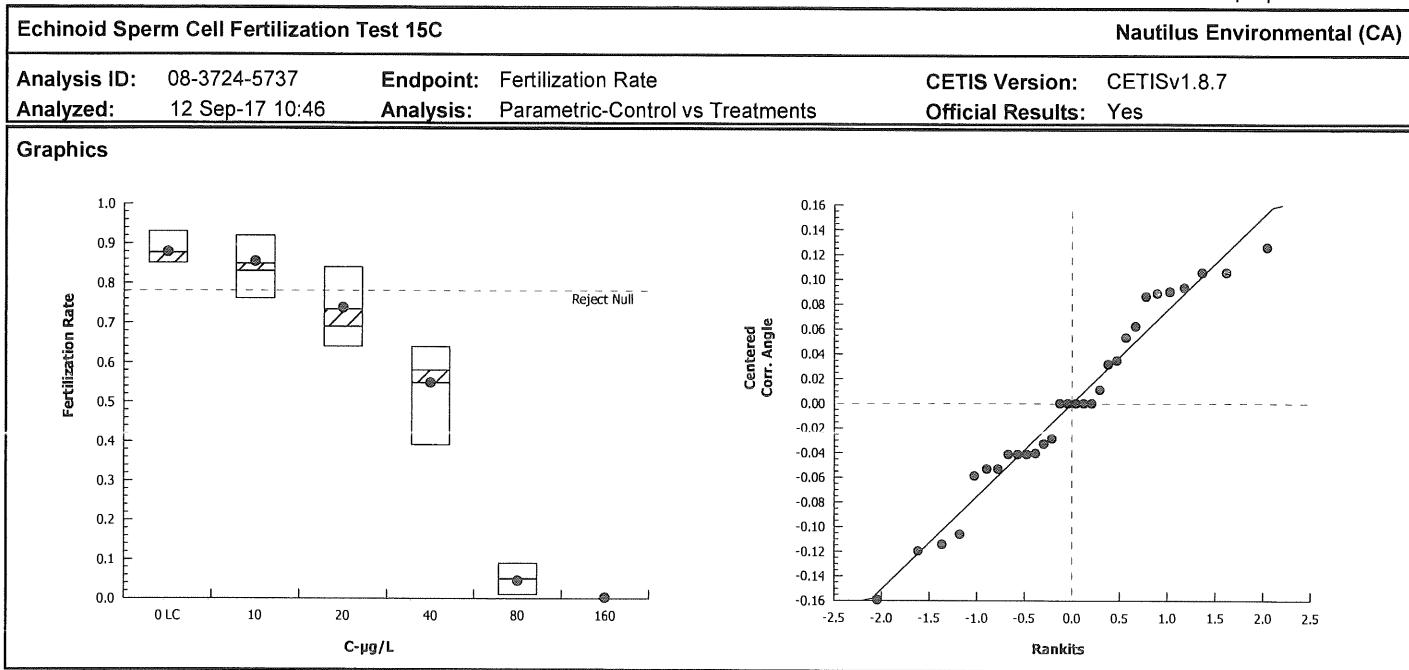
## CETIS Analytical Report

Report Date: 12 Sep-17 10:46 (p 1 of 2)  
 Test Code: 170910sprt | 11-6871-9499

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)				
Analysis ID: 08-3724-5737		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7						
Analyzed: 12 Sep-17 10:46		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	10.9%	10	20	14.14				
<b>Dunnett Multiple Comparison Test</b>												
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)			
Lab Control	10	0.6302	2.305	0.131	8	0.5452	CDF	Non-Significant Effect				
	20*	3.186	2.305	0.131	8	0.0080	CDF	Significant Effect				
	40*	6.701	2.305	0.131	8	<0.0001	CDF	Significant Effect				
	80*	17.61	2.305	0.131	8	<0.0001	CDF	Significant Effect				
<b>ANOVA Table</b>												
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision(α:5%)			
Between	3.341765		0.8354412		4	103.7		<0.0001	Significant Effect			
Error	0.1611481		0.008057405		20							
Total	3.502913				24							
<b>Distributional Tests</b>												
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)					
Variances	Bartlett Equality of Variance			1.335	13.28	0.8554	Equal Variances					
Distribution	Shapiro-Wilk W Normality			0.9389	0.8877	0.1395	Normal Distribution					
<b>Fertilization Rate Summary</b>												
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	0.876	0.8299	0.9221	0.85	0.85	0.93	0.01661	4.24%	0.0%	
10		5	0.848	0.7605	0.9355	0.83	0.76	0.92	0.03153	8.31%	3.2%	
20		5	0.734	0.627	0.841	0.69	0.64	0.84	0.03855	11.74%	16.21%	
40		5	0.548	0.4253	0.6707	0.58	0.39	0.64	0.0442	18.04%	37.44%	
80		5	0.05	0.01074	0.08926	0.05	0.01	0.09	0.01414	63.25%	94.29%	
160		5	0	0	0	0	0	0			100.0%	
<b>Angular (Corrected) Transformed Summary</b>												
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.214	1.14	1.288	1.173	1.173	1.303	0.02662	4.9%	0.0%	
10		5	1.178	1.053	1.304	1.146	1.059	1.284	0.04534	8.6%	2.95%	
20		5	1.033	0.9089	1.158	0.9803	0.9273	1.159	0.04484	9.7%	14.9%	
40		5	0.8338	0.7099	0.9578	0.8657	0.6745	0.9273	0.04464	11.97%	31.33%	
80		5	0.2144	0.1147	0.3142	0.2255	0.1002	0.3047	0.03592	37.45%	82.34%	
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0	0.05002	0	0.0%	

# CETIS Analytical Report

Report Date: 12 Sep-17 10:46 (p 2 of 2)  
Test Code: 170910sprt | 11-6871-9499

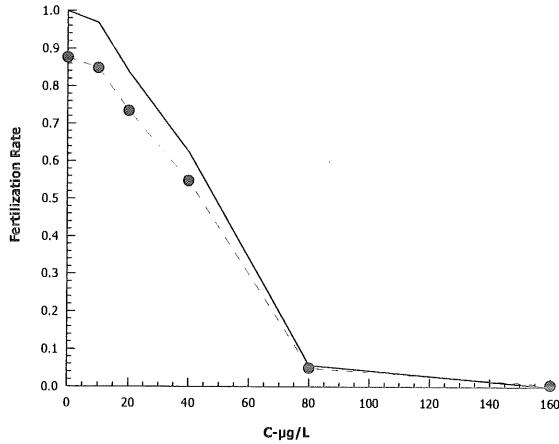


# CETIS Analytical Report

Report Date: 12 Sep-17 10:46 (p 1 of 1)  
Test Code: 170910sprt | 11-6871-9499

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 08-4248-1228			Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7		Official Results: Yes				
Analyzed: 12 Sep-17 10:46			Analysis: Trimmed Spearman-Kärber								
Trimmed Spearman-Kärber Estimates											
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL				
Control Threshold	0.124	3.20%	1.606	0.009277	40.4	38.71	42.17				
Fertilization Rate Summary					Calculated Variate(A/B)						
C- $\mu$ g/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.876	0.85	0.93	0.01661	0.03715	4.24%	0.0%	438	500
10		5	0.848	0.76	0.92	0.03153	0.0705	8.31%	3.2%	424	500
20		5	0.734	0.64	0.84	0.03855	0.0862	11.74%	16.21%	367	500
40		5	0.546	0.39	0.64	0.0442	0.09884	18.04%	37.44%	274	500
80		5	0.05	0.01	0.09	0.01414	0.03162	63.25%	94.29%	25	500
160		5	0	0	0	0	0	100.0%	0	500	

## Graphics



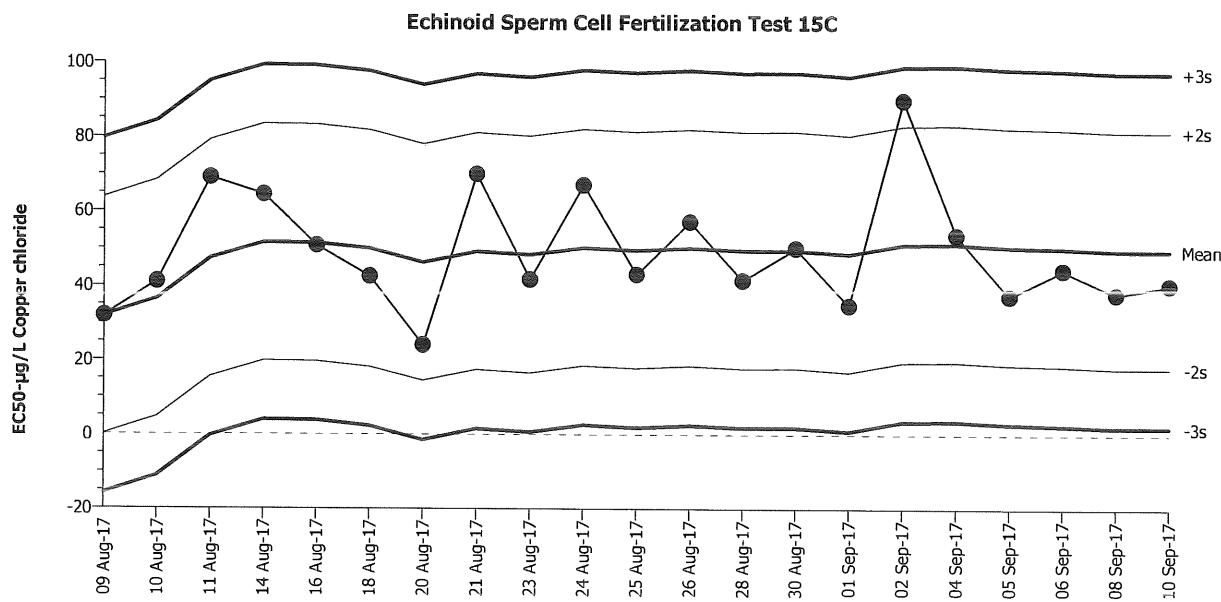
## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Organism: Strongylocentrotus purpuratus (Purple)  
 Endpoint: Fertilization Rate

Material: Copper chloride  
 Source: Reference Toxicant-REF



Mean: 49.66 Count: 20 -2s Warning Limit: 17.82 -3s Action Limit: 1.896  
 Sigma: 15.92 CV: 32.10% +2s Warning Limit: 81.5 +3s Action Limit: 97.42

## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	9	17:08	31.92	-17.74	-1.115			13-6999-3036	11-7131-4234
2			10	16:51	41.14	-8.524	-0.5355			00-5471-5288	12-0643-2211
3			11	14:50	69.03	19.37	1.217			04-5796-5476	07-8184-6783
4			14	14:40	64.51	14.85	0.9325			02-4510-8526	01-5460-0814
5			16	16:34	50.82	1.157	0.07267			16-3259-1018	06-7497-1035
6			18	14:09	42.53	-7.129	-0.4478			12-6613-4538	02-2322-5589
7			20	14:52	24.05	-25.61	-1.609			06-9655-0092	05-8785-3700
8			21	14:46	69.95	20.29	1.274			08-4756-2919	20-2992-4955
9			23	16:14	41.72	-7.943	-0.4989			02-7595-3678	15-3490-2746
10			24	16:11	67.1	17.44	1.096			04-7651-5518	20-0883-0005
11			25	14:48	43.11	-6.55	-0.4115			06-8816-1100	09-0830-4014
12			26	16:00	57.24	7.581	0.4762			10-2039-5656	15-8794-0305
13			28	14:56	41.55	-8.108	-0.5093			08-1525-2751	10-7829-2432
14			30	16:38	50.21	0.5519	0.03466			08-1199-3706	11-0543-3886
15	Sep		1	15:27	34.79	-14.87	-0.9338			13-1244-6646	21-1567-7550
16			2	10:53	89.99	40.33	2.534	(+)		16-4202-9692	18-8681-1855
17			4	16:10	53.77	4.112	0.2583			12-2973-1405	10-6032-1229
18			5	17:07	37.36	-12.3	-0.7727			13-1627-7974	14-5447-1160
19			6	17:15	44.41	-5.247	-0.3296			05-5533-8557	16-8161-1582
20			8	15:48	37.91	-11.75	-0.7379			18-6871-7794	04-4479-5076
21			10	14:25	40.4	-9.258	-0.5815			11-6871-9499	08-4248-1228

## CETIS Test Data Worksheet

Report Date: 07 Sep-17 13:53 (p 1 of 1)  
 Test Code: 11-6871-9499/170910sprt

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 10 Sep-17 Species: Strongylocentrotus purpuratus  
 End Date: 10 Sep-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 10 Sep-17 Material: Copper chloride

Sample Code: 170910sprt  
 Sample Source: Reference Toxicant  
 Sample Station: Copper Chloride

C- $\mu$ g/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	7	9/11/17
			2		83	
			3		0	
			4		93	
			5		85	
			6		0	
			7		64	
			8		90	
			9		89	
			10		0	
			11		64	
			12		69	
			13		5	
			14		0	
			15		3	
			16		52	
			17		9	
			18		39	
			19		92	
			20		0	
			21		85	
			22		76	
			23		85	
			24		61	
			25		81	
			26		69	
			27		1	
			28		58	
			29		81	
			30	✓	92	

## CETIS Test Data Worksheet

Report Date: 07 Sep-17 13:53 (p 1 of 1)  
 Test Code: 11-6871-9499/170910sprt

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 10 Sep-17 Species: Strongylocentrotus purpuratus  
 End Date: 10 Sep-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 10 Sep-17 Material: Copper chloride

Sample Code: 170910sprt  
 Sample Source: Reference Toxicant  
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	8			
0	LC	2	4	100	65	8-9/10/17
0	LC	3	21			
0	LC	4	23			
0	LC	5	5			
10		1	25			
10		2	22			
10		3	2			
10		4	19			
10		5	30	100	92	
20		1	29			
20		2	26			
20		3	12	100	74	
20		4	11			
20		5	9			
40		1	18			
40		2	16			
40		3	7	100	65	
40		4	24			
40		5	28			
80		1	13			
80		2	15			
80		3	1	100	5	
80		4	17			
80		5	27			
160		1	6			
160		2	20			
160		3	3	100	0	
160		4	10			
160		5	14			

QC118

## Marine Chronic Bioassay

## Water Quality Measurements

Client : Internal

Test Species: S. purpuratusSample ID: CuCl<sub>2</sub>Start Date/Time: 9/10/2017 1425

Test No: 170910sprt

End Date/Time: 9/10/2017 1505Dilutions made by: g

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,200

Analyst: g

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.8	8.11	33.5	14.4
10	8.6	8.10	33.5	14.3
20	8.6	8.10	33.4	14.3
40	8.5	8.08	33.3	14.4
80	8.5	8.08	33.3	14.3
160	8.5	8.10	33.1	14.3

Comments: \_\_\_\_\_

QC Check: AC 9/12/17Final Review: KFP 9/20/17

## Marine Chronic Bioassay

## Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal  
 Sample ID: CUC12  
 Test No.: 110910SPRT

Tech initials: SJ  
 Injection Time: 1350

Sperm Absorbance at 400 nm: 0.876 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 71 Mean: 71.6  $\times 50 =$  3830 eggs/ml

72  
74  
84  
82

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

Initial density: 3830 eggs/ml  
 Final density: 4000 eggs/ml = 0.96 dilution factor  
 $\frac{1.0}{\text{part egg stock}} \quad \frac{\text{egg stock}}{\text{seawater}} \quad \frac{150}{\text{ml}} \quad \frac{\text{ml}}{\text{parts seawater}}$

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>1357</u>	<u>50%</u>	<u>72</u>	<u>28</u>				
Eggs Added (0.5 ml):	<u>1407</u>	<u>100%</u>	<u>80</u>	<u>10</u>				
Test Ended:	<u>1417</u>	<u>100%</u>	<u>92</u>	<u>8</u>				
		<u>200%</u>	<u>100</u>	<u>0</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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1425</u>	QC1	<u>99</u>	<u>6</u>
Eggs Added (0.5 ml):	<u>1445</u>	QC2	<u>95</u>	<u>5</u>
Test Ended:	<u>1505</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

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QC Check: AC 9/2/17

Final Review: KFP 9/20/17

## Appendix E

### 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 ≤ 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.