



# Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)  
Sample Collection Date: August 11, 2017

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

**Prepared by:** Nautilus Environmental

**Submitted:** September 1, 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 Libor

## INTRODUCTION

A discharge sample was collected in August 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 product water tank overflow 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 August 11, 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 (product water tank overflow)
Monitoring Period:	August 2017
Sample Material:	Facility Effluent
Sampling Method:	Composite
Sample Collection Date, Time:	8/11/17, 06:30
Sample Receipt Date, Time:	8/11/17, 12:27

**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)
8/11/17	7.91	7.0	2.5	64.6	180	<0.02

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

Test Date, Times:	8/11/17, 14:50 through 15:30
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
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

Statistically significant decreases in fertilization rates were observed at the 10 and 15 percent effluent concentrations compared to the lab control. The NOEC is reported as 6.06 and the  $TU_c$  is 16.5, which meets the maximum effluent limitation of 16.5 for this permit. None of the effluent concentrations were significantly reduced according to the TST analysis. 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 M-001 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	6.06	10	>15	16.5	Pass	1.8

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:  $100 \div \text{NOEC}$

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:  $PE = ((\text{mean response in control} - \text{mean response in the IWC}) / \text{mean response in control}) * 100$ . 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	91.0
2.5	89.6
5.0	92.0
6.06	89.4
10	82.2*
15	77.2*

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

## **QUALITY ASSURANCE**

The sample was received on the day of collection and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. 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**

<b>Test Date</b>	<b><math>EC_{50}</math> (<math>\mu\text{g/L}</math> Copper)</b>	<b>Historical Mean <math>EC_{50} \pm 2</math> SD (<math>\mu\text{g/L}</math> Copper)</b>	<b>CV (%)</b>
8/11/17	69.0	$45.7 \pm 37.3$	40.8

$EC_{50}$  = Concentration expected to cause an adverse effect to 50 percent of the test organisms

Historical Mean  $EC_{50} \pm 2$  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: 14 Aug-17 14:01 (p 1 of 1)  
 Test Code: 1708-S112 | 09-9826-9321

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	08-1806-3626	Test Type:	Fertilization	Analyst:							
Start Date:	11 Aug-17 14:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	11 Aug-17 15:30	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	04-6369-0091	Code:	17-0888	Client:	IDE						
Sample Date:	11 Aug-17 06:30	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	11 Aug-17 12:27	Source:	IDE Americas, Inc.								
Sample Age:	8h (2.5 °C)	Station:	M-001 (Daily)								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
00-2162-7214	Fertilization Rate	6.06	10	7.785	7.14%	16.5	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
14-4880-1895	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)				
		EC50	>15	N/A	N/A	<6.667					
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
00-2162-7214	Fertilization Rate	Control Resp	0.91	0.7 - NL	Yes	Passes Acceptability Criteria					
14-4880-1895	Fertilization Rate	Control Resp	0.91	0.7 - NL	Yes	Passes Acceptability Criteria					
00-2162-7214	Fertilization Rate	PMSD	0.07136	NL - 0.25	No	Passes Acceptability Criteria					
Fertilization Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.91	0.8796	0.9404	0.88	0.94	0.01095	0.02449	2.69%	0.0%
2.5		5	0.896	0.8444	0.9476	0.85	0.94	0.0186	0.04159	4.64%	1.54%
5		5	0.92	0.8838	0.9562	0.88	0.96	0.01304	0.02915	3.17%	-1.1%
6.06		5	0.894	0.8439	0.9441	0.83	0.94	0.01806	0.04037	4.52%	1.76%
10		5	0.822	0.7676	0.8764	0.75	0.87	0.0196	0.04382	5.33%	9.67%
15		5	0.772	0.6698	0.8742	0.7	0.89	0.0368	0.08228	10.66%	15.16%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.94	0.9	0.9	0.88	0.93					
2.5		0.94	0.87	0.94	0.88	0.85					
5		0.96	0.93	0.88	0.92	0.91					
6.06		0.9	0.94	0.91	0.83	0.89					
10		0.75	0.83	0.83	0.83	0.87					
15		0.89	0.75	0.7	0.7	0.82					



## CETIS Analytical Report

Report Date: 14 Aug-17 14:01 (p 1 of 2)

Test Code: 1708-S112 | 09-9826-9321

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 00-2162-7214		Endpoint: Fertilization Rate						CETIS Version: CETISv1.8.7			
Analyzed: 14 Aug-17 14:00		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		7.14%	6.06	10	7.785	16.5
Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	0.4756	2.362	0.102	8	0.6575	CDF	Non-Significant Effect		
		5	-0.4543	2.362	0.102	8	0.9335	CDF	Non-Significant Effect		
		6.06	0.5761	2.362	0.102	8	0.6133	CDF	Non-Significant Effect		
		10*	3.031	2.362	0.102	8	0.0119	CDF	Significant Effect		
		15*	4.381	2.362	0.102	8	0.0005	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.1723092		0.03446184		5		7.38	0.0003	Significant Effect		
Error	0.1120704		0.004669598		24						
Total	0.2843795				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			3.378	15.09	0.6419		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9657	0.9031	0.4284		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	0.91	0.8796	0.9404	0.9	0.88	0.94	0.01095	2.69%	0.0%
2.5		5	0.896	0.8444	0.9476	0.88	0.85	0.94	0.0186	4.64%	1.54%
5		5	0.92	0.8838	0.9562	0.92	0.88	0.96	0.01304	3.17%	-1.1%
6.06		5	0.894	0.8439	0.9441	0.9	0.83	0.94	0.01806	4.52%	1.76%
10		5	0.822	0.7676	0.8764	0.83	0.75	0.87	0.0196	5.33%	9.67%
15		5	0.772	0.6698	0.8742	0.75	0.7	0.89	0.0368	10.66%	15.16%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.268	1.214	1.322	1.249	1.217	1.323	0.0195	3.44%	0.0%
2.5		5	1.248	1.16	1.336	1.217	1.173	1.323	0.03165	5.67%	1.62%
5		5	1.288	1.219	1.357	1.284	1.217	1.369	0.02489	4.32%	-1.55%
6.06		5	1.243	1.163	1.323	1.249	1.146	1.323	0.02879	5.18%	1.96%
10		5	1.137	1.068	1.207	1.146	1.047	1.202	0.02501	4.92%	10.33%
15		5	1.079	0.9503	1.208	1.047	0.9912	1.233	0.04634	9.6%	14.93%

# CETIS Analytical Report

Report Date: 14 Aug-17 14:01 (p 2 of 2)

Test Code: 1708-S112 | 09-9826-9321

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 00-2162-7214

Endpoint: Fertilization Rate

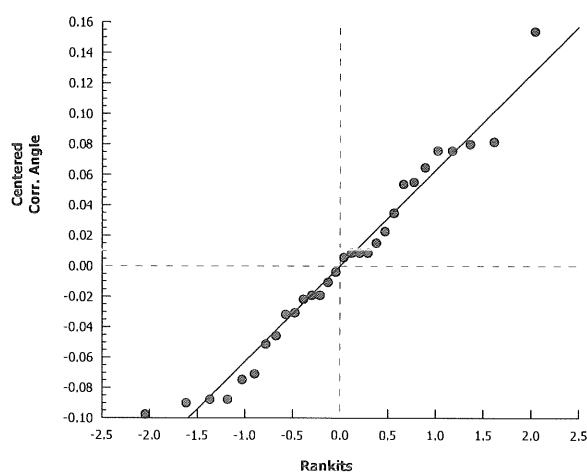
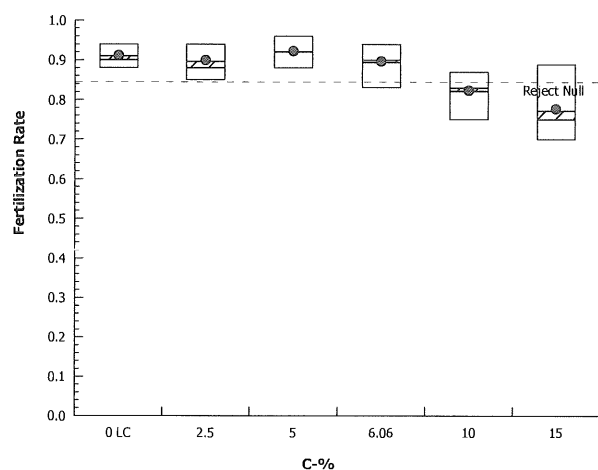
CETIS Version: CETISv1.8.7

Analyzed: 14 Aug-17 14:00

Analysis: Parametric-Control vs Treatments

Official Results: Yes

### Graphics



# CETIS Analytical Report

Report Date: 14 Aug-17 14:01 (p 1 of 1)

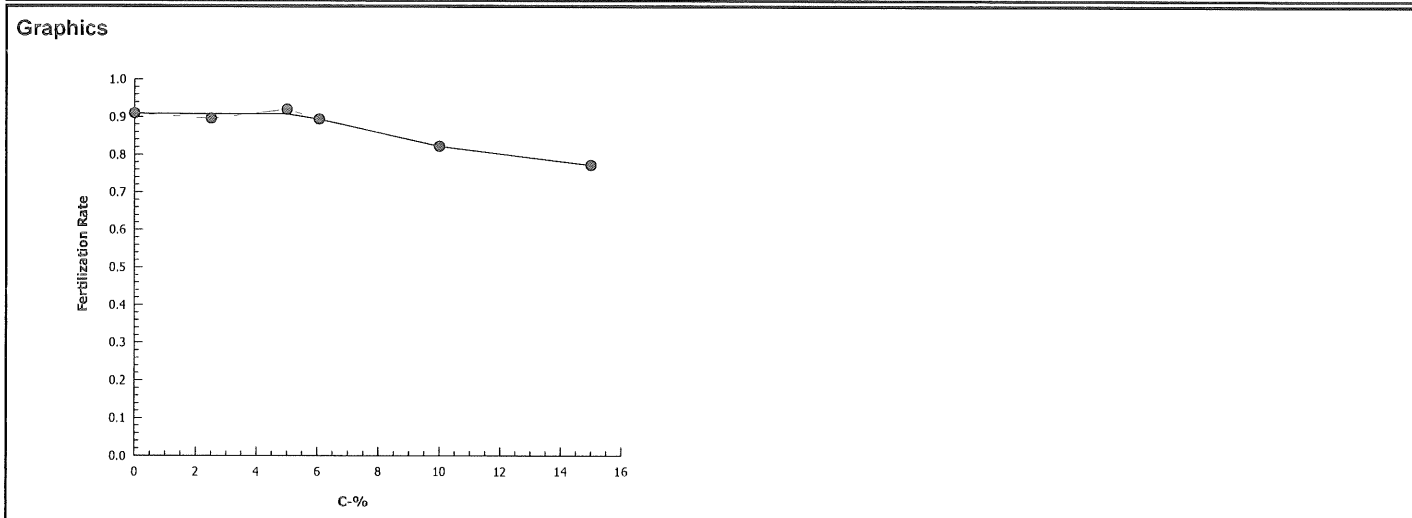
Test Code: 1708-S112 | 09-9826-9321

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Analysis ID:	14-4880-1895	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7		
Analyzed:	14 Aug-17 14:00	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes		

Linear Interpolation Options						
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method	
Linear	Linear	643577	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	0.91	0.88	0.94	0.01095	0.02449	2.69%	0.0%	455	500
2.5		5	0.896	0.85	0.94	0.0186	0.04159	4.64%	1.54%	448	500
5		5	0.92	0.88	0.96	0.01304	0.02915	3.17%	-1.1%	460	500
6.06		5	0.894	0.83	0.94	0.01806	0.04037	4.52%	1.76%	447	500
10		5	0.822	0.75	0.87	0.0196	0.04382	5.33%	9.67%	411	500
15		5	0.772	0.7	0.89	0.0368	0.08228	10.66%	15.16%	386	500



## CETIS Analytical Report

Report Date: 14 Aug-17 14:02 (p 1 of 1)

Test Code: 1708-S112 | 09-9826-9321

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID: 11-1546-6990		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 14 Aug-17 14:01		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	7.26%	15	>15	NA	6.667	
TST-Welch's t Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5*	8.504	2.015	0.070	5	0.0002	CDF	Non-Significant Effect		
		5*	11.67	1.943	0.056	6	<0.0001	CDF	Non-Significant Effect		
		6.06*	9.049	2.015	0.065	5	0.0001	CDF	Non-Significant Effect		
		10*	6.423	1.943	0.056	6	0.0003	CDF	Non-Significant Effect		
		15*	2.629	2.132	0.104	4	0.0291	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.1723092		0.03446184		5		7.38	0.0003	Significant Effect		
Error	0.1120704		0.004669598		24						
Total	0.2843795				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			3.378	15.09	0.6419		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9657	0.9031	0.4284		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	0.91	0.8796	0.9404	0.9	0.88	0.94	0.01095	2.69%	0.0%
2.5		5	0.896	0.8444	0.9476	0.88	0.85	0.94	0.0186	4.64%	1.54%
5		5	0.92	0.8838	0.9562	0.92	0.88	0.96	0.01304	3.17%	-1.1%
6.06		5	0.894	0.8439	0.9441	0.9	0.83	0.94	0.01806	4.52%	1.76%
10		5	0.822	0.7676	0.8764	0.83	0.75	0.87	0.0196	5.33%	9.67%
15		5	0.772	0.6698	0.8742	0.75	0.7	0.89	0.0368	10.66%	15.16%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.268	1.214	1.322	1.249	1.217	1.323	0.0195	3.44%	0.0%
2.5		5	1.248	1.16	1.336	1.217	1.173	1.323	0.03165	5.67%	1.62%
5		5	1.288	1.219	1.357	1.284	1.217	1.369	0.02489	4.32%	-1.55%
6.06		5	1.243	1.163	1.323	1.249	1.146	1.323	0.02879	5.18%	1.96%
10		5	1.137	1.068	1.207	1.146	1.047	1.202	0.02501	4.92%	10.33%
15		5	1.079	0.9503	1.208	1.047	0.9912	1.233	0.04634	9.6%	14.93%

# CETIS Test Data Worksheet

Report Date: 10 Aug-17 12:00 (p 1 of 1)  
 Test Code: 1708-S112 09-9826-9321/3B806189

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 11/10 Aug-17

Species: Strongylocentrotus purpuratus

Sample Code: 17- 0888

End Date: 11/10 Aug-17

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

Sample Source: JDE Americas, Inc.

Sample Date: 11/10 Aug-17

Material: Facility Effluent

Sample Station: M-001 40 ppt

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			91	100	96	8/11/17
			92	100	70	
			93	100	94	
			94	100	82	
			95	100	93	
			96	100	88	
			97	100	89	
			98	100	92	
			99	100	90	
			100	100	94	
			101	100	90	
			102	100	94	
			103	100	91	
			104	100	91	
			105	100	88	
			106	100	83	
			107	100	75	
			108	100	83	
			109	100	85	
			110	100	90	
			111	100	89	
			112	100	94	8/13/17
			113	100	83	
			114	100	87	
			115	100	93	
			116	100	88	
			117	100	87	
			118	100	75	
			119	100	70	
			120	100	83	

BAO 918 8/11/17

# CETIS Test Data Worksheet

Report Date: 10 Aug-17 12:00 (p 1 of 1)

Test Code: 1708-S112 09-9826-9321/3B806189

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 11/10 Aug-17

Species: Strongylocentrotus purpuratus

Sample Code: 17-0888

End Date: 11/10 Aug-17

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

Sample Source: IDE Americas, Inc.

Sample Date: 11/10 Aug-17

Material: Facility Effluent

Sample Station: M-001 40 ppt unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	93	100	96	RL 8/11/17
0	LC	2	101			
0	LC	3	99			
0	LC	4	105			
0	LC	5	115			
2.5		1	112	100	93	8/11/17 RL
2.5		2	114			
2.5		3	100			
2.5		4	116			
2.5		5	109			
5		1	91	100	92	RL 8/11/17
5		2	95			
5		3	96			
5		4	98			
5		5	104			
6.06		1	110	100	88	RL 8/11/17
6.06		2	102			
6.06		3	103			
6.06		4	108			
6.06		5	97			
10		1	118	100	83	RL 8/11/17
10		2	106			
10		3	113			
10		4	120			
10		5	117			
15		1	111	100	87	RL 8/11/17
15		2	107			
15		3	119			
15		4	92			
15		5	94			

QC: AG

QAD C18 8/11/17

QAD AG C18 8/11/17

## Marine Chronic Bioassay

## Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*Sample ID: <sup>Daily unadjusted</sup>  
M-001 (40 ppt adjusted)

Start Date/Time: 8/10/2017 1450

Sample Log No.: 17- 0888

End Date/Time: 8/10/2017 1530

Dilutions made by: AD

Test No: 1708-812

Analyst: AG OBO DM

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.07	<del>16.0</del> 33.8	16.0
2.5	8.5	8.08	34.7	15.9
5.0	8.2	8.08	35.1	15.8
6.06	8.2	8.08	35.5	15.9
10	8.2	8.07	36.4	15.8
15	8.2	8.06	37.7	15.8

Comments: BAD Q18 8/11/17 @ AG Q18 8/11/17 @ DM Q18 8/11/17

QC Check: AC 8/14/17

Final Review: ~~AC~~ 8/31/17

## Marine Chronic Bioassay

## Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE  
 Sample ID: Daily m-001  
 Test No.: 1708-58112  
 Tech initials: AG  
 Injection Time: 1355

Start Date/Time: 8/11/2017 / 1450  
 End Date/Time: 8/11/2017 / 1530  
 Species: S. purpuratus  
 Animal Source: Pilona  
 Date Collected: 7/31/17

Sperm Absorbance at 400 nm: 0.998 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 78 Mean: 83.4 X 50 = 4,170 eggs/ml

86  
83  
84  
86

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

Initial density: 4,170 eggs/ml = (A) dilution factor egg stock 150 ml  
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 0 ml  
(A) 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							
<u>Range Finder Test:</u>	<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	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>1415</u>	<u>200:1, 50:1</u>	<u>70, 68</u>	<u>30, 32</u>
Eggs Added (0.5 ml):	<u>1425</u>	<u>100:1, 100:1</u>	<u>75, 77</u>	<u>25, 23</u>
Test Ended:	<u>1435</u>	<u>200:1, 200:1</u>	<u>87, 89</u>	<u>11, 11</u>
		<u>400:1, 400:1</u>	<u>91, 95</u>	<u>9, 5</u>
		<u>800:1, 800:1</u>	<u>96, 98</u>	<u>4, 2</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: 400:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1450</u>	QC1	<u>90</u>	<u>10</u>
Eggs Added (0.5 ml):	<u>1510</u>	QC2	<u>88</u>	<u>12</u>
Test Ended:	<u>1530</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) No Dilution Required

QC Check:

AC 8/14/17

Final Review:

W 8/31/17



## **Appendix B**

### **Sample Receipt Information**

Nautilus Environmental  
4340 Vandever Avenue  
San Diego, CA 92120

Client: IDE  
Sample ID: Daily M-001  
Test ID No(s): 17080-5112

## Sample Check-In Information

### Sample Description:

A: COLORLESS, CLEAR, ODORLESS, NO DEBRIS

Sample (A, B, C):	<u>A</u>			
Log-in No. (17-xxxx):	<u>0888</u>			
Sample Collection Date & Time:	<u>8/11/17 0630</u>			
Sample Receipt Date & Time:	<u>8/11/17 1227</u>			
Number of Containers & Container Type:	<u>1 - 4L CUBI</u>			
Approx. Total Volume Received (L):	<u>~4L</u>			
Check-in Temperature (°C)	<u>2.5</u>			
Temperature OK? <sup>1</sup>	<u>(Y) N</u>	<u>Y N</u>	<u>Y N</u>	<u>Y N</u>
DO (mg/L)	<u>7.0</u>			
pH (units)	<u>7.91</u>			
Conductivity (µS/cm)	<u>—</u>			
Salinity (ppt)	<u>64.6</u>			
Alkalinity (mg/L) <sup>2</sup>	<u>180</u>			
Hardness (mg/L) <sup>2,3</sup>	<u>—</u>			
Total Chlorine (mg/L)	<u>20.02</u>			
Technician Initials	<u>TN</u>			

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

Alkalinity: 117 Hardness or Salinity: 34ppt  
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: \_\_\_\_\_

### COC Complete (Y/N)?

A Y E \_\_\_\_\_ 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)

	A	B	C
Initial Free Cl <sub>2</sub> :			
STS added:			
Final Free Cl <sub>2</sub> :			

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 8/14/17

Final Review: 8/31/17

## **Appendix C**

### **Chain-of-Custody Form**



**Turn Around Time**

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

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

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

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

??? Days \_\_\_\_\_

Special instruction: Sampled during product water tank overflow 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: 8/10/17 @ 15:15, End: 8/11/17 @ 06:30 KC

NOTES:

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

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

[illegible]

Relinquished By:

Date:

Time:

Received By:

Time:

Sample Condition Upon Receipt:

W. A. J. J.	8/11/17	1037
J. M. J. J. L. M. J. J.	8/11/17	12:27pm

Received by:	Time:	Precondition open/closed:
Sheldon LME50	10:33	<input checked="" type="checkbox"/> Iced <input type="checkbox"/> Ambient or _____ °C
Porter	12:27	<input type="checkbox"/> Iced <input type="checkbox"/> Ambient or 2.5 °C

N/Anticus 10: 17-0888

## **Appendix D**

### **Reference Toxicant Test Data and Statistical Analyses**

## CETIS Summary Report

Report Date: 14 Aug-17 13:44 (p 1 of 1)

Test Code: 170811sprt | 04-5796-5476

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	10-2735-1442	Test Type:	Fertilization	Analyst:							
Start Date:	11 Aug-17 14:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	11 Aug-17 15:30	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	17-3911-4402	Code:	170811sprt	Client:	Internal						
Sample Date:	11 Aug-17	Material:	Copper chloride	Project:							
Receive Date:	11 Aug-17	Source:	Reference Toxicant								
Sample Age:	15h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
16-8916-0308	Fertilization Rate	<10	10	NA	2.79%	Dunnett Multiple Comparison Test					
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
07-8184-6783	Fertilization Rate	EC50	69.03	66.04	72.15	Trimmed Spearman-Kärber					
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
07-8184-6783	Fertilization Rate	Control Resp	0.912	0.7 - NL	Yes	Passes Acceptability Criteria					
16-8916-0308	Fertilization Rate	Control Resp	0.912	0.7 - NL	Yes	Passes Acceptability Criteria					
16-8916-0308	Fertilization Rate	PMSD	0.02789	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	0.912	0.8881	0.9359	0.88	0.93	0.008602	0.01924	2.11%	0.0%
10		5	0.878	0.8676	0.8884	0.87	0.89	0.003742	0.008367	0.95%	3.73%
20		5	0.858	0.8297	0.8863	0.83	0.89	0.0102	0.0228	2.66%	5.92%
40		5	0.778	0.7511	0.8049	0.75	0.8	0.009695	0.02168	2.79%	14.69%
80		5	0.412	0.3652	0.4588	0.37	0.47	0.01685	0.03768	9.15%	54.82%
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	0.88	0.92	0.91	0.93	0.92					
10		0.87	0.88	0.88	0.89	0.87					
20		0.85	0.83	0.87	0.85	0.89					
40		0.75	0.76	0.8	0.79	0.79					
80		0.41	0.39	0.37	0.42	0.47					
160		0	0	0	0	0					

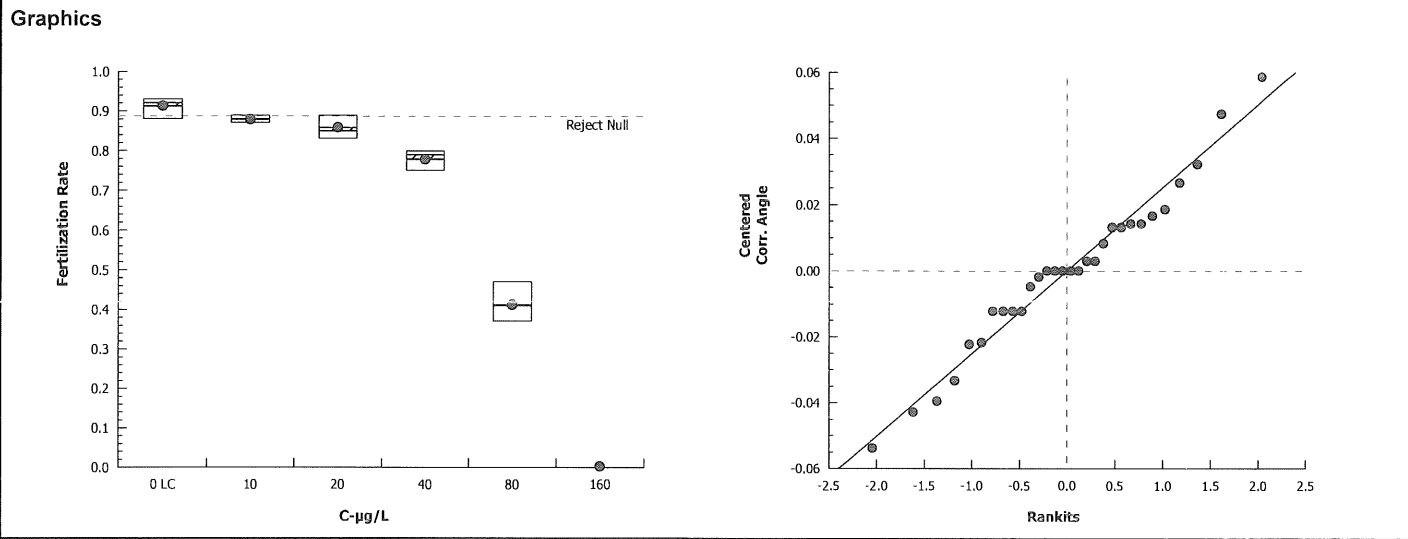
## CETIS Analytical Report

Report Date: 14 Aug-17 13:44 (p 1 of 2)

Test Code: 170811sprt | 04-5796-5476

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 16-8916-0308		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 14 Aug-17 13:42		Analysis: Parametric-Control vs Treatments		Official Results: Yes							
Data Transform		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C > T	NA	NA		2.79%	<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*	2.999	2.305	0.044	8	0.0120	CDF	Significant Effect		
		20*	4.522	2.305	0.044	8	0.0004	CDF	Significant Effect		
		40*	10.06	2.305	0.044	8	<0.0001	CDF	Significant Effect		
		80*	30.36	2.305	0.044	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	1.059735		0.2649338		4		296.3	<0.0001	Significant Effect		
Error	0.01788306		0.000894153		20						
Total	1.077618				24						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			3.975	13.28	0.4094		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9843	0.8877	0.9556		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	0.912	0.8881	0.9359	0.92	0.88	0.93	0.008602	2.11%	0.0%
10		5	0.878	0.8676	0.8884	0.88	0.87	0.89	0.003742	0.95%	3.73%
20		5	0.858	0.8297	0.8863	0.85	0.83	0.89	0.0102	2.66%	5.92%
40		5	0.778	0.7511	0.8049	0.79	0.75	0.8	0.009695	2.79%	14.69%
80		5	0.412	0.3652	0.4588	0.41	0.37	0.47	0.01685	9.15%	54.82%
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	1.271	1.23	1.312	1.284	1.217	1.303	0.01466	2.58%	0.0%
10		5	1.214	1.198	1.23	1.217	1.202	1.233	0.005747	1.06%	4.46%
20		5	1.185	1.144	1.226	1.173	1.146	1.233	0.01481	2.79%	6.73%
40		5	1.081	1.048	1.113	1.095	1.047	1.107	0.01161	2.4%	14.98%
80		5	0.6967	0.6493	0.7442	0.6949	0.6539	0.7554	0.01709	5.49%	45.18%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.06%

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)	
Analysis ID:	16-8916-0308	Endpoint:	Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed:	14 Aug-17 13:42	Analysis:	Parametric-Control vs Treatments	Official Results: Yes





## CETIS Analytical Report

Report Date: 14 Aug-17 13:44 (p 1 of 1)

Test Code: 170811spt | 04-5796-5476

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 07-8184-6783

Endpoint: Fertilization Rate

CETIS Version: CETISv1.8.7

Analyzed: 14 Aug-17 13:43

Analysis: Trimmed Spearman-Kärber

Official Results: Yes

## Trimmed Spearman-Kärber Estimates

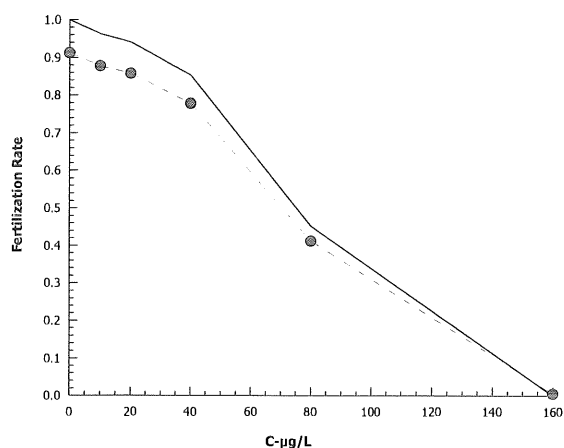
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.088	3.73%	1.839	0.009606	69.03	66.04	72.15

## 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	0.912	0.88	0.93	0.008602	0.01924	2.11%	0.0%	456	500
10		5	0.878	0.87	0.89	0.003742	0.008367	0.95%	3.73%	439	500
20		5	0.858	0.83	0.89	0.0102	0.0228	2.66%	5.92%	429	500
40		5	0.778	0.75	0.8	0.009695	0.02168	2.79%	14.69%	389	500
80		5	0.412	0.37	0.47	0.01685	0.03768	9.15%	54.82%	206	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

Organism: Strongylocentrotus purpuratus (Purpl

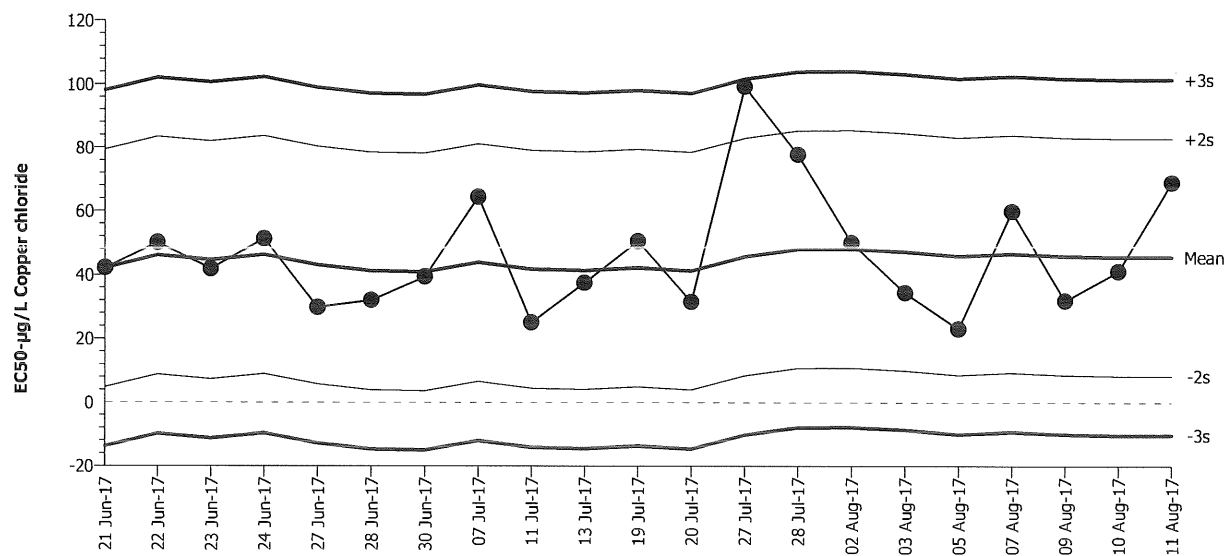
Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF

## Echinoid Sperm Cell Fertilization Test 15C



Mean: 45.67

Count: 20

-2s Warning Limit: 8.368

-3s Action Limit: -10.28

Sigma: 18.65

CV: 40.80%

+2s Warning Limit: 82.97

+3s Action Limit: 101.6

## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jun	21	14:17	42.15	-3.518	-0.1887			20-6379-6831	00-5386-2071
2			22	17:25	50.05	4.375	0.2346			10-9823-5082	04-6220-9409
3			23	16:55	41.8	-3.867	-0.2074			06-0771-4160	11-6079-2504
4			24	13:27	51.33	5.659	0.3034			01-7420-9579	03-5890-9605
5			27	13:13	29.77	-15.9	-0.8526			11-6174-9094	14-8592-6950
6			28	14:40	32.02	-13.65	-0.732			06-0030-2581	03-5443-1685
7			30	17:50	39.38	-6.293	-0.3374			19-1859-0537	20-9128-8719
8		Jul	7	17:35	64.49	18.82	1.009			20-4636-3738	10-9356-2953
9			11	11:33	25.05	-20.62	-1.105			09-0588-2471	00-1661-1655
10			13	15:20	37.5	-8.173	-0.4382			05-9787-5418	02-0541-0147
11			19	16:28	50.59	4.919	0.2637			03-3446-7266	15-7259-8466
12			20	18:10	31.52	-14.15	-0.7587			17-7484-2488	03-0485-5429
13			27	15:55	99.32	53.65	2.877	(+)		02-6715-3770	17-8186-2444
14			28	10:50	77.84	32.17	1.725			21-2559-1280	14-0688-6070
15		Aug	2	15:50	50.06	4.385	0.2351			08-9742-2478	08-8646-9232
16			3	0:00	34.43	-11.24	-0.6025			02-7356-2235	20-3051-4002
17			5	19:25	23.07	-22.6	-1.212			11-5994-0488	10-6029-2098
18			7	15:10	59.94	14.27	0.7651			21-2468-7505	14-3489-7019
19			9	17:08	31.92	-13.75	-0.7375			13-6999-3036	11-7131-4234
20			10	16:51	41.14	-4.534	-0.2431			00-5471-5288	12-0643-2211
21			11	14:50	69.03	23.36	1.253			04-5796-5476	07-8184-6783

## CETIS Test Data Worksheet

Report Date: 10 Aug-17 12:12 (p 1 of 1)

Test Code: 04-5796-5476/170811spt

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 11 Aug-17

Species: Strongylocentrotus purpuratus

Sample Code: 170811spt

End Date: 11 Aug-17

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

Sample Source: Reference Toxicant

Sample Date: 11 Aug-17

Material: Copper chloride

Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	92	
			2		85	
			3		0	
			4		87	
			5		89	
			6		75	
			7		37	
			8		42	
			9		0	
			10		88	
			11		85	
			12		76	
			13		0	
			14		87	
			15		39	
			16		88	
			17		0	
			18		92	
			19		79	
			20		89	
			21		87	
			22		41	
			23		87	
			24		80	
			25		47	
			26		91	
			27		0	
			28		93	
			29		83	
			30	✓	88	

A/B Q18 8/14/17

## CETIS Test Data Worksheet

Report Date: 10 Aug-17 12:12 (p 1 of 1)

Test Code: 04-5796-5476/170811sprt

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 11 Aug-17 Species: Strongylocentrotus purpuratus

Sample Code: 170811sprt

End Date: 11 Aug-17 Protocol: EPA/600/R-95/136 (1995)

Sample Source: Reference Toxicant

Sample Date: 11 Aug-17 Material: Copper chloride

Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	16	100	88	AG 8/11/17
0	LC	2	18			
0	LC	3	26			
0	LC	4	28			
0	LC	5	1			
10		1	23	100	88	
10		2	30			
10		3	10			
10		4	20			
10		5	14			
20		1	2	100	85	
20		2	29			
20		3	21			
20		4	11			
20		5	5			
40		1	6			
40		2	12	100	82	
40		3	24			
40		4	19			
40		5	4			
80		1	22	100	41	
80		2	15			
80		3	7			
80		4	8			
80		5	25			
160		1	17	100	0	
160		2	27			
160		3	3			
160		4	9			
160		5	13			

QC:AL

## Marine Chronic Bioassay

## Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl<sub>2</sub>Start Date/Time: 8/11/2017 1450Test No: 170811sprtEnd Date/Time: 8/11/2017 1530Dilutions made by: Am

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10200

Analyst: AL

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.0	8.08	33.5	15.8
10	8.0	8.07	34.0	15.8
20	8.1	8.07	34.0	15.7
40	8.1	8.08	34.0	15.6
80	8.1	8.07	33.9	15.7
160	8.0	8.07	33.7	15.9

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

QC Check: ALB/14/17Final Review: KFP8/21/17

# Marine Chronic Bioassay

# Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal  
 Sample ID: CuCl2  
 Test No.: 170811sp7  
 Tech initials: AG  
 Injection Time: 1355

Start Date/Time: 8/11/2017 / 1450  
 End Date/Time: 8/11/2017 / 1530  
 Species: S. purpuratus  
 Animal Source: Alamo  
 Date Collected: 7/31/17

Sperm Absorbance at 400 nm: 0.998 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 78 Mean: 83.4 X 50 = 4,170 eggs/ml

86  
83  
84  
86

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

Initial density: 4,170 eggs/ml = (A) dilution factor egg stock 150 ml  
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 0 ml  
(A) 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							
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>1415</u>	<u>200:1, 50:1</u>	<u>70,68</u>	<u>30,32</u>
Eggs Added (0.5 ml):	<u>1425</u>	<u>100:1, 100:1</u>	<u>75,77</u>	<u>25,23</u>
Test Ended:	<u>1435</u>	<u>200:1, 200:1</u>	<u>89,89</u>	<u>11,11</u>
		<u>400:1, 400:1</u>	<u>91,95</u>	<u>9,5</u>
		<u>800:1, 800:1</u>	<u>96,98</u>	<u>4,2</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: 400:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1450</u>	QC1	<u>90</u>	<u>10</u>
Eggs Added (0.5 ml):	<u>1510</u>	QC2	<u>88</u>	<u>12</u>
Test Ended:	<u>1530</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: (A) No Dilution Required

QC Check: AC 8/14/17 Final Review: KFP 8/21/17

## **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  $\leq 110\%$
- 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.