

Nautilus Environmental

## Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily/Weekly)

Sample Collection Date: November 19, 2017

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

**Prepared by:** Nautilus Environmental

**Submitted:** November 30, 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 Cisor

## EXECUTIVE SUMMARY

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

Sampling Date: November 19, 2017

Test Date: November 20, 2017

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

M-001

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

Results Summary:

Bioassay Type:	M-001 Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	NOEC	TU <sub>c</sub>	No
	2.5	40	

## INTRODUCTION

A 24-hour composite discharge sample was collected in November 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for daily and weekly accelerated 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 November 20, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

## MATERIALS AND METHODS

Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was couriered to Nautilus the day of sample collection. Following arrival at Nautilus, an aliquot of the 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 protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./ Carlsbad Desalination Plant
Monitoring Period:	November 2017 (daily/weekly train off-spec period)
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	11/19/17, 07:30
Sample Receipt Date, Time:	11/20/17, 13:00
Sampling Method:	24-hour Composite

Table 2. Water Quality Measurements upon Sample Receipt

Sample ID	pH	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO <sub>3</sub> )	Total Chlorine (mg/L)
M-001	7.79	8.1	4.5	60.8	214	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	11/20/17, 15:15 through 15:55
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 (SIO) inlet), 34±2 parts per thousand (ppt); 20-µm filtered
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control. The same dilution series was also tested with the sample after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge and CDP discharges).
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 egg 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:	<b>CETIS™, version 1.8.7.20</b>

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

**In addition to EPA flowchart statistical methods, the 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, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST statistical analysis is not in the 2006 CDP permit, the TST results are included for comparison purposes only.**

## RESULTS

There was a significant decrease in the fertilization rate in the 5, 6.06, 10, and 15 percent concentrations in the unadjusted M-001 sample relative to the lab control using the EPA 1995 flowchart statistics. The NOEC is reported as 2.5 percent effluent and a TU<sub>c</sub> equal to 40, which exceeds the maximum permit effluent limitation. Significant reductions were observed at 10 and 15 percent effluent concentrations in the M-001 unadjusted sample using the TST statistical analysis.

There were no significant decreases in fertilization rate at any effluent concentration in the 40 ppt adjusted M-001 effluent sample relative to the lab control using the TST or EPA 1995 flowchart statistics.

Statistical results for urchin fertilization toxicity tests 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 a copy 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 (unadjusted)	2.5	5	>15	40	Pass	21
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	2.7

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 6.06% IWC according to the TST calculation; Fail = sample is toxic at the 6.06% 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)	M-001 Unadjusted Sample		M-001 40 ppt Adjusted <sup>a</sup>	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.8	89.2	33.8	88.0
High Salinity Control	37.7	93.4	--	--
2.5	34.5	85.0	34.1	84.6
5.0	35.2	82.8*	34.1	88.4
6.06	35.5	74.2*	34.3	85.6
10	36.5	56.8*	34.5	89.0
15	37.8	61.6*	34.9	84.8

\* An asterisk indicates a statistically significant difference when compared to the lab control using EPA 1995 flowchart statistical methods.

<sup>a</sup> For comparison to the M-001 unadjusted sample, the M-001 sample was adjusted with seawater to 40 ppt prior to preparing test concentrations.

## 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 laboratory controls met the minimum acceptability criteria as set by USEPA. The PMSD values, which are a measure of test variability, were within the acceptable range. Therefore, all test results were deemed valid for reporting purposes.

Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to evaluate reliability of the results. Additionally, appropriate threshold effect and 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 are summarized in Table 6 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration (EC<sub>50</sub> value) was within two standard deviations (SD) of the historical mean, indicating typical test organism sensitivity to copper. A list of qualifier codes used on bench datasheets can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC <sub>50</sub> ( $\mu\text{g/L}$ Copper)	Historical Mean EC <sub>50</sub> $\pm$ 2 SD ( $\mu\text{g/L}$ Copper)	CV (%)
11/20/17	38.3	47.9 $\pm$ 30.0	31.3

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

## REFERENCES

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
- 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. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. United States Environmental Protection Agency Office of Wastewater Management (EPA-833-R-00-003).
- 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

M-001 Unadjusted

## CETIS Summary Report

Report Date: 27 Nov-17 16:28 (p 1 of 1)  
 Test Code: 1711-S119 | 18-0502-1373

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)					
Batch ID:	08-5843-3887	Test Type: Fertilization				Analyst:						
Start Date:	20 Nov-17 15:15	Protocol: EPA/600/R-95/136 (1995)				Diluent: Laboratory Seawater						
Ending Date:	20 Nov-17 15:55	Species: Strongylocentrotus purpuratus				Brine: Not Applicable						
Duration:	40m	Source: Pt. Loma				Age:						
Sample ID:	12-7598-3126	Code: 17-1209				Client: IDE						
Sample Date:	19 Nov-17 07:30	Material: Facility Effluent				Project: Carlsbad Desal Plant						
Receive Date:	20 Nov-17 13:00	Source: IDE Americas, Inc.										
Sample Age:	32h (4.5 °C)	Station: M-001 (Unadjusted) <i>weekly/Daily</i>										
<b>Comparison Summary</b>												
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method					
17-3149-7399	Fertilization Rate	2.5	5	3.536	5.7%	40	Dunnett Multiple Comparison Test					
<b>Point Estimate Summary</b>												
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method					
06-7507-3865	Fertilization Rate	EC25	7.977	6.461	8.973	12.54	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					
06-7507-3865	Fertilization Rate	Control Resp	0.892	0.7 - NL		Yes	Passes Acceptability Criteria					
17-3149-7399	Fertilization Rate	Control Resp	0.892	0.7 - NL		Yes	Passes Acceptability Criteria					
17-3149-7399	Fertilization Rate	PMSD	0.05703	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	High Salinity Co	5	0.934	0.9016	0.9664	0.9	0.97	0.01166	0.02608	2.79%	0.0%	
0	Lab Control	5	0.892	0.8611	0.9229	0.86	0.91	0.01114	0.0249	2.79%	4.5%	
2.5		5	0.85	0.8209	0.8791	0.82	0.87	0.01049	0.02345	2.76%	8.99%	
5		5	0.828	0.7682	0.8878	0.77	0.88	0.02154	0.04817	5.82%	11.35%	
6.06		5	0.742	0.6638	0.8202	0.66	0.83	0.02818	0.06301	8.49%	20.56%	
10		5	0.568	0.4998	0.6362	0.47	0.6	0.02458	0.05495	9.68%	39.19%	
15		5	0.616	0.5848	0.6472	0.58	0.64	0.01122	0.0251	4.08%	34.05%	
<b>Fertilization Rate Detail</b>												
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	High Salinity Co	0.94	0.9	0.94	0.92	0.97						
0	Lab Control	0.91	0.86	0.91	0.91	0.87						
2.5		0.82	0.87	0.87	0.83	0.86						
5		0.79	0.77	0.88	0.83	0.87						
6.06		0.76	0.66	0.75	0.71	0.83						
10		0.47	0.59	0.6	0.59	0.59						
15		0.63	0.58	0.63	0.64	0.6						

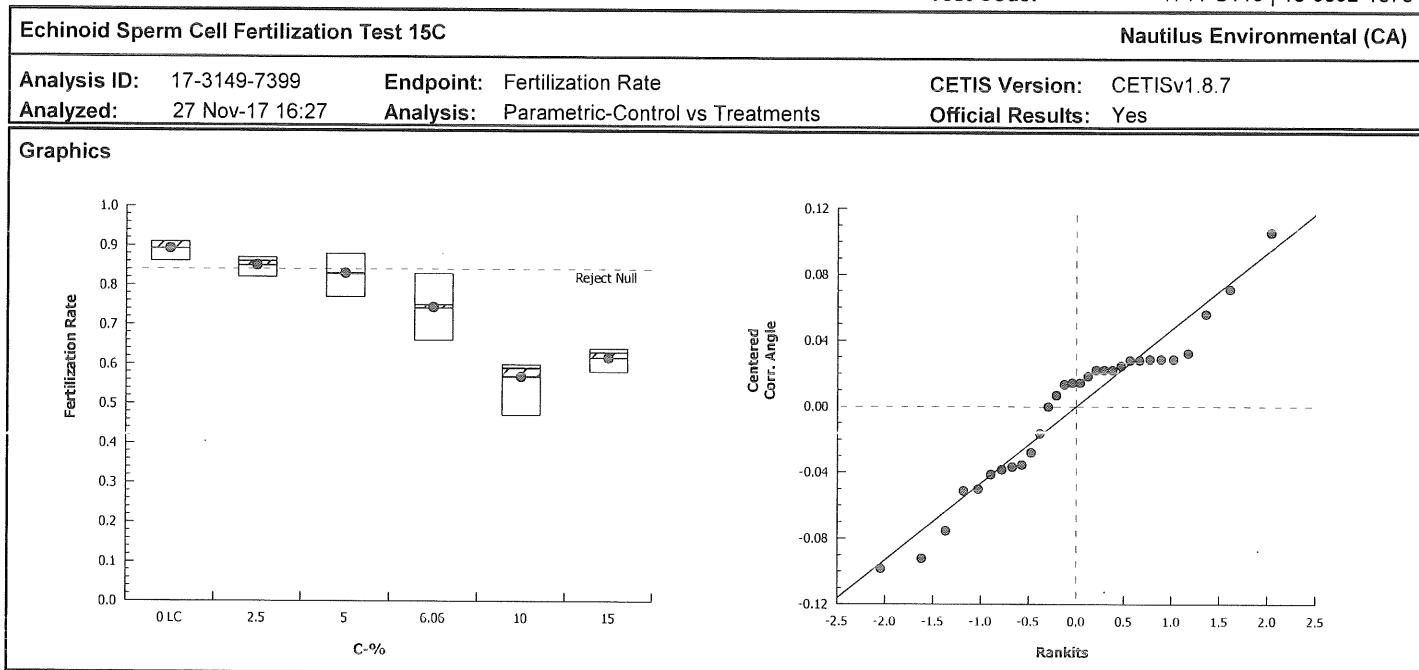
# CETIS Analytical Report

Report Date: 27 Nov-17 16:28 (p 1 of 2)  
 Test Code: 1711-S119 | 18-0502-1373

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)				
Analysis ID: 17-3149-7399			Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7							
Analyzed: 27 Nov-17 16:27			Analysis: Parametric-Control vs Treatments				Official Results: Yes							
Data Transform		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU			
Angular (Corrected)		NA	C > T	NA	NA		5.7%	2.5	5	3.536	40			
Dunnett Multiple Comparison Test														
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision( $\alpha$ :5%)					
Lab Control	2.5		1.958	2.362	0.077	8	0.1070	CDF	Non-Significant Effect					
	5*		2.817	2.362	0.077	8	0.0192	CDF	Significant Effect					
	6.06*		6.069	2.362	0.077	8	<0.0001	CDF	Significant Effect					
	10*		11.82	2.362	0.077	8	<0.0001	CDF	Significant Effect					
	15*		10.31	2.362	0.077	8	<0.0001	CDF	Significant Effect					
ANOVA Table														
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision( $\alpha$ :5%)					
Between	0.5977686		0.1195537		5	45.36		<0.0001	Significant Effect					
Error	0.06326117		0.002635882		24									
Total	0.6610298				29									
Distributional Tests														
Attribute	Test			Test Stat	Critical	P-Value		Decision( $\alpha$ :1%)						
Variances	Bartlett Equality of Variance			5.516	15.09	0.3562		Equal Variances						
Distribution	Shapiro-Wilk W Normality			0.9462	0.9031	0.1340		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	0.892	0.8611	0.9229	0.91	0.86	0.91	0.01114	2.79%	0.0%			
2.5		5	0.85	0.8209	0.8791	0.86	0.82	0.87	0.01049	2.76%	4.71%			
5		5	0.828	0.7682	0.8878	0.83	0.77	0.88	0.02154	5.82%	7.18%			
6.06		5	0.742	0.6638	0.8202	0.75	0.66	0.83	0.02818	8.49%	16.82%			
10		5	0.568	0.4998	0.6362	0.59	0.47	0.6	0.02458	9.68%	36.32%			
15		5	0.616	0.5848	0.6472	0.63	0.58	0.64	0.01122	4.08%	30.94%			
Angular (Corrected) Transformed Summary														
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect			
0	Lab Control	5	1.238	1.188	1.287	1.266	1.187	1.266	0.01766	3.19%	0.0%			
2.5		5	1.174	1.133	1.214	1.187	1.133	1.202	0.01456	2.77%	5.14%			
5		5	1.146	1.066	1.226	1.146	1.071	1.217	0.02871	5.6%	7.39%			
6.06		5	1.04	0.9495	1.131	1.047	0.9483	1.146	0.03275	7.04%	15.92%			
10		5	0.8538	0.7853	0.9224	0.8759	0.7554	0.8861	0.02469	6.47%	31.0%			
15		5	0.9026	0.8706	0.9346	0.9169	0.8657	0.9273	0.01151	2.85%	27.06%			

# CETIS Analytical Report

Report Date: 27 Nov-17 16:28 (p 2 of 2)  
Test Code: 1711-S119 | 18-0502-1373



# CETIS Analytical Report

Report Date: 27 Nov-17 16:28 (p 1 of 1)  
 Test Code: 1711-S119 | 18-0502-1373

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)						
Analysis ID:		06-7507-3865	Endpoint:		Fertilization Rate	CETIS Version:		CETISv1.8.7				
Analyzed:		27 Nov-17 16:27	Analysis:		Linear Interpolation (ICPIN)	Official Results:		Yes				
<b>Linear Interpolation Options</b>												
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method							
Linear	Linear	1731497	1000	Yes	Two-Point Interpolation							
<b>Point Estimates</b>												
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL						
EC25	7.977	6.461	8.973	12.54	11.14	15.48						
EC50	>15	N/A	N/A	<6.667	NA	NA						
<b>Fertilization Rate Summary</b>												
Calculated Variate(A/B)												
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B	
0	Lab Control	5	0.892	0.86	0.91	0.01114	0.0249	2.79%	0.0%	446	500	
2.5		5	0.85	0.82	0.87	0.01049	0.02345	2.76%	4.71%	425	500	
5		5	0.828	0.77	0.88	0.02154	0.04817	5.82%	7.18%	414	500	
6.06		5	0.742	0.66	0.83	0.02818	0.06301	8.49%	16.82%	371	500	
10		5	0.568	0.47	0.6	0.02458	0.05495	9.68%	36.32%	281	500	
15		5	0.616	0.58	0.64	0.01122	0.0251	4.08%	30.94%	308	500	
<b>Graphics</b>												

## CETIS Analytical Report

TST

Report Date:

27 Nov-17 16:28 (p 1 of 1)

Test Code:

1711-S119 | 18-0502-1373

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)				
Analysis ID: 00-8409-1393		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7								
Analyzed: 27 Nov-17 16:28		Analysis: Parametric Bioequivalence-Two Sample				Official Results: Yes								
Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSE	NOEL	LOEL	TOEL	TU				
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	2.29%	6.06	10	7.785	16.5				
<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*	12.48	1.895	0.037	7	<0.0001	CDF	Non-Significant Effect						
	5*	6.892	2.015	0.064	5	0.0005	CDF	Non-Significant Effect						
	6.06*	3.179	2.015	0.071	5	0.0123	CDF	Non-Significant Effect						
	10	-2.652	1.943	0.054	6	0.9810	CDF	Significant Effect						
	15	-1.455	1.895	0.033	7	0.9056	CDF	Significant Effect						
<b>ANOVA Table</b>														
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision( $\alpha$ :5%)					
Between	0.5977686		0.1195537		5	45.36		<0.0001	Significant Effect					
Error	0.06326117		0.002635882		24									
Total	0.6610298				29									
<b>Distributional Tests</b>														
Attribute	Test			Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)							
Variances	Bartlett Equality of Variance			5.516	15.09	0.3562	Equal Variances							
Distribution	Shapiro-Wilk W Normality			0.9462	0.9031	0.1340	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	0.892	0.8611	0.9229	0.91	0.86	0.91	0.01114	2.79%	0.0%			
2.5		5	0.85	0.8209	0.8791	0.86	0.82	0.87	0.01049	2.76%	4.71%			
5		5	0.828	0.7682	0.8878	0.83	0.77	0.88	0.02154	5.82%	7.18%			
6.06		5	0.742	0.6638	0.8202	0.75	0.66	0.83	0.02818	8.49%	16.82%			
10		5	0.568	0.4998	0.6362	0.59	0.47	0.6	0.02458	9.68%	36.32%			
15		5	0.616	0.5848	0.6472	0.63	0.58	0.64	0.01122	4.08%	30.94%			
<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	1.238	1.188	1.287	1.266	1.187	1.266	0.01766	3.19%	0.0%			
2.5		5	1.174	1.133	1.214	1.187	1.133	1.202	0.01456	2.77%	5.14%			
5		5	1.146	1.066	1.226	1.146	1.071	1.217	0.02871	5.6%	7.39%			
6.06		5	1.04	0.9495	1.131	1.047	0.9483	1.146	0.03275	7.04%	15.92%			
10		5	0.8538	0.7853	0.9224	0.8759	0.7554	0.8861	0.02469	6.47%	31.0%			
15		5	0.9026	0.8706	0.9346	0.9169	0.8657	0.9273	0.01151	2.85%	27.06%			

## CETIS Test Data Worksheet

Report Date: 20 Nov-17 13:16 (p 1 of 1)  
 Test Code: 18-0502-1373/1711-S119

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Nov-17 Species: Strongylocentrotus purpuratus  
 End Date: 20 Nov-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 19 Nov-17 Material: Facility Effluent

Sample Code: 17- 1209  
 Sample Source: IDE Americas, Inc.  
 Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	③ 3663	11/21/2017
			32	100	87	
			33	④ 100	66	
			34	100	87	
			35	100	59	
			36	100	97	
			37	100	91	
			38	100	63	
			39	100	60	
			40	100	94	
			41	100	37	
			42	100	86	
			43	100	83	
			44	100	③ 6883	
			45	100	75	
			46	100	92	
			47	100	47	
			48	100	82	
			49	100	91	
			50	100	58	
			51	100	79	
			52	100	87	
			53	100	85	
			54	100	③ 6871	
			55	100	76	
			56	100	90	
			57	100	59	
			58	100	60	
			59	100	86	
			60	100	88	
			61	100	64	
			62	100	77	
			63	100	91	
			64	100	94	
			65	100	59	

(A) - Q19 KC 11/21/17

(B) Q18 J 11/21/17. AD recount.

## CETIS Test Data Worksheet

Report Date: 20 Nov-17 13:16 (p 1 of 1)  
 Test Code: 18-0502-1373/1711-S119

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Nov-17 Species: Strongylocentrotus purpuratus  
 End Date: 20 Nov-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 19 Nov-17 Material: Facility Effluent

Sample Code: 17- 1209  
 Sample Source: IDE Americas, Inc.  
 Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	64	100	96	DM 11/27/17
0	HS	2	56			
0	HS	3	40			
0	HS	4	46			
0	HS	5	36			
0	LC	1	49	100	93	
0	LC	2	59			
0	LC	3	37			
0	LC	4	63			
0	LC	5	32			
2.5		1	48	100	88	
2.5		2	34			
2.5		3	52			
2.5		4	53			
2.5		5	42			
5		1	51	100	84	
5		2	62			
5		3	60			
5		4	44			
5		5	41			
6.06		1	55	100	71	
6.06		2	33			
6.06		3	45			
6.06		4	54			
6.06		5	43			
10		1	47	100	51	
10		2	35			
10		3	58			
10		4	57			
10		5	65			
15		1	31	100	61	✓
15		2	50			
15		3	38			
15		4	61			
15		5	39			

QC: FG

## Marine Chronic Bioassay

## Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (unadjusted)

Start Date/Time: 11/20/2017 1515

Sample Log No.: 17-1209

End Date/Time: 11/20/2017 1555

Dilutions made by: EL obo AC

Test No: 1711-S119

Analyst: RH

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	7.97	33.8	15.2
High Salinity Control	8.5	7.97	37.7	15.0
2.5	8.4	8.00	34.5	15.3
5.0	8.4	8.00	35.2	15.2
6.06	8.4	8.00	35.5	14.8
10	8.5	8.00	36.5	14.6
15	8.4	7.98	37.8	14.5

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

QC Check: No 11/27/17

Final Review: AC 11/30/17

## Marine Chronic Bioassay

## Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDF  
 Sample ID: m-001 (unadjusted)  
 Test No.: 1711-S119

Tech initials: EJ  
 Injection Time: 1438

Sperm Absorbance at 400 nm: 0.930 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 83 Mean: 86.6  $\times 50 =$  4330 eggs/ml

87  
93  
82  
88

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

Initial density: 4330 eggs/ml  
 Final density: 4000 eggs/ml = 1.0825 dilution factor  
 $\frac{1.0}{0.825}$  part egg stock  
 $\frac{0.825}{1.0}$  parts seawater  
 egg stock (A) ml  
 seawater (P) 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	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1445</u>	<u>50:1</u>	<u>84/91</u>	<u>16/9</u>
Eggs Added (0.5 ml):	<u>1455</u>	<u>100:1</u>	<u>95</u>	<u>2</u>
Test Ended:	<u>1505</u>	<u>—</u>	<u>—</u>	<u>—</u>

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

Definitive Test Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1515</u>	QC1	<u>85</u>	<u>15</u>
Eggs Added (0.5 ml):	<u>1535</u>	QC2	<u>91</u>	<u>9</u>
Test Ended:	<u>1555</u>	Egg Control 1	<u>0</u>	<u>100</u>

Comments: (A) EC 11/20/17 No Dilution Required

QC Check: AM 11/27/17

Final Review: AC 11/30/17

M-001 40 ppt Adjusted

## CETIS Summary Report

Report Date:

27 Nov-17 16:10 (p 1 of 1)

Test Code:

1711-S120 | 13-5946-5773

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	19-2558-3247	Test Type:	Fertilization			Analyst:					
Start Date:	20 Nov-17 15:15	Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Natural Seawater				
Ending Date:	20 Nov-17 15:55	Species:	Strongylocentrotus purpuratus			Brine:	Not Applicable				
Duration:	40m	Source:	Pt. Loma			Age:					
Sample ID:	06-8419-9407	Code:	17-1209				Client:	IDE			
Sample Date:	19 Nov-17 07:30	Material:	Brine Effluent				Project:	Carlsbad Desal Plant			
Receive Date:	20 Nov-17 13:00	Source:	IDE Americas, Inc.								
Sample Age:	32h (4.5 °C)	Station:	M-001 (40 ppt adj)								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
02-7249-6664	Fertilization Rate	15	>15	NA	8.01%	< 6.667	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
00-6127-4577	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			
00-6127-4577	Fertilization Rate	Control Resp		0.88	0.7 - NL		Yes	Passes Acceptability Criteria			
02-7249-6664	Fertilization Rate	Control Resp		0.88	0.7 - NL		Yes	Passes Acceptability Criteria			
02-7249-6664	Fertilization Rate	PMSD		0.08015	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	0.88	0.7976	0.9624	0.78	0.95	0.02966	0.06633	7.54%	0.0%
2.5		5	0.846	0.8174	0.8746	0.81	0.87	0.0103	0.02302	2.72%	3.86%
5		5	0.884	0.8362	0.9318	0.85	0.93	0.0172	0.03847	4.35%	-0.45%
6.06		5	0.856	0.8074	0.9046	0.8	0.91	0.01749	0.03912	4.57%	2.73%
10		5	0.89	0.8403	0.9397	0.86	0.96	0.01789	0.04	4.49%	-1.14%
15		5	0.848	0.7728	0.9232	0.78	0.92	0.02709	0.06058	7.14%	3.64%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.91	0.85	0.91	0.78	0.95					
2.5		0.86	0.87	0.81	0.85	0.84					
5		0.85	0.87	0.85	0.93	0.92					
6.06		0.8	0.91	0.86	0.85	0.86					
10		0.88	0.87	0.88	0.86	0.96					
15		0.79	0.78	0.92	0.87	0.88					

Q18 AC 11/30/17

## CETIS Analytical Report

Report Date: 27 Nov-17 16:10 (p 1 of 1)

Test Code: 1711-S120 | 13-5946-5773

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)				
Analysis ID: 02-7249-6664		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7						
Analyzed: 27 Nov-17 15:07		Analysis: Parametric-Control vs Treatments				Official Results: Yes						
Data Transform		Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU		
Angular (Corrected)		NA	C > T	NA	NA	8.01%	15	>15	NA	6.667		
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	1.282	2.362	0.108	8	0.3018	CDF	Non-Significant Effect			
	5	5	-0.00888	2.362	0.108	8	0.8359	CDF	Non-Significant Effect			
	6.06	6.06	0.9322	2.362	0.108	8	0.4504	CDF	Non-Significant Effect			
	10	10	-0.2618	2.362	0.108	8	0.8991	CDF	Non-Significant Effect			
	15	15	1.11	2.362	0.108	8	0.3717	CDF	Non-Significant Effect			
ANOVA Table												
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision( $\alpha$ :5%)			
Between	0.02342007		0.004684013		5	0.9037		0.4949	Non-Significant Effect			
Error	0.1243998		0.005183326		24							
Total	0.1478199				29							
Distributional Tests												
Attribute	Test		Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)						
Variances	Bartlett Equality of Variance		4.915	15.09	0.4263	Equal Variances						
Distribution	Shapiro-Wilk W Normality		0.9848	0.9031	0.9341	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	0.88	0.7976	0.9624	0.91	0.78	0.95	0.02966	7.54%	0.0%	
2.5		5	0.846	0.8174	0.8746	0.85	0.81	0.87	0.0103	2.72%	3.86%	
5		5	0.884	0.8362	0.9318	0.87	0.85	0.93	0.0172	4.35%	-0.45%	
6.06		5	0.856	0.8074	0.9046	0.86	0.8	0.91	0.01749	4.57%	2.73%	
10		5	0.89	0.8403	0.9397	0.88	0.86	0.96	0.01789	4.49%	-1.14%	
15		5	0.848	0.7728	0.9232	0.87	0.78	0.92	0.02709	7.14%	3.64%	
Angular (Corrected) Transformed Summary												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.227	1.101	1.352	1.266	1.083	1.345	0.04517	8.23%	0.0%	
2.5		5	1.168	1.129	1.207	1.173	1.12	1.202	0.01406	2.69%	4.76%	
5		5	1.227	1.15	1.304	1.202	1.173	1.303	0.02782	5.07%	-0.03%	
6.06		5	1.184	1.114	1.254	1.187	1.107	1.266	0.02529	4.78%	3.46%	
10		5	1.239	1.146	1.331	1.217	1.187	1.369	0.03318	5.99%	-0.97%	
15		5	1.176	1.07	1.282	1.202	1.083	1.284	0.03831	7.28%	4.12%	

# CETIS Analytical Report

Report Date: 27 Nov-17 16:10 (p 1 of 1)  
 Test Code: 1711-S120 | 13-5946-5773

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID: 00-6127-4577 Analyzed: 27 Nov-17 15:07		Endpoint: Fertilization Rate Analysis: Linear Interpolation (ICPIN)		CETIS Version: CETISv1.8.7 Official Results: Yes							
<b>Linear Interpolation Options</b>											
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method						
Linear	Linear	607255	1000	Yes	Two-Point Interpolation						
<b>Point Estimates</b>											
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					
<b>Fertilization Rate Summary</b>											
Calculated Variate(A/B)											
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.88	0.78	0.95	0.02966	0.06633	7.54%	0.0%	440	500
2.5		5	0.846	0.81	0.87	0.0103	0.02302	2.72%	3.86%	423	500
5		5	0.884	0.85	0.93	0.0172	0.03847	4.35%	-0.45%	442	500
6.06		5	0.856	0.8	0.91	0.01749	0.03912	4.57%	2.73%	428	500
10		5	0.89	0.86	0.96	0.01789	0.04	4.49%	-1.14%	445	500
15		5	0.848	0.78	0.92	0.02709	0.06058	7.14%	3.64%	424	500

## CETIS Analytical Report

TST

Report Date:

27 Nov-17 16:08 (p 1 of 1)

Test Code:

1711-S120 | 13-5946-5773

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)				
Analysis ID: 05-0295-8155		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7		Official Results: Yes						
Analyzed: 27 Nov-17 15:07		Analysis: Parametric Bioequivalence-Two Sample												
Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU				
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	7.07%	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*		6.77	2.015	0.074	5	0.0005	CDF	Non-Significant Effect					
	5*		7.005	1.895	0.083	7	0.0001	CDF	Non-Significant Effect					
	6.06*		6.25	1.895	0.080	7	0.0002	CDF	Non-Significant Effect					
	10*		6.718	1.895	0.09	7	0.0001	CDF	Non-Significant Effect					
	15*		5.008	1.895	0.097	7	0.0008	CDF	Non-Significant Effect					
<b>ANOVA Table</b>														
Source	Sum Squares		Mean Square		DF	F Stat		P-Value	Decision( $\alpha$ :5%)					
Between	0.02342007		0.004684013		5	0.9037		0.4949	Non-Significant Effect					
Error	0.1243998		0.005183326		24									
Total	0.1478199				29									
<b>Distributional Tests</b>														
Attribute	Test			Test Stat	Critical	P-Value	Decision( $\alpha$ :1%)							
Variances	Bartlett Equality of Variance			4.915	15.09	0.4263	Equal Variances							
Distribution	Shapiro-Wilk W Normality			0.9848	0.9031	0.9341	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	0.88	0.7976	0.9624	0.91	0.78	0.95	0.02966	7.54%	0.0%			
2.5		5	0.846	0.8174	0.8746	0.85	0.81	0.87	0.0103	2.72%	3.86%			
5		5	0.884	0.8362	0.9318	0.87	0.85	0.93	0.0172	4.35%	-0.45%			
6.06		5	0.856	0.8074	0.9046	0.86	0.8	0.91	0.01749	4.57%	2.73%			
10		5	0.89	0.8403	0.9397	0.88	0.86	0.96	0.01789	4.49%	-1.14%			
15		5	0.848	0.7728	0.9232	0.87	0.78	0.92	0.02709	7.14%	3.64%			
<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	1.227	1.101	1.352	1.266	1.083	1.345	0.04517	8.23%	0.0%			
2.5		5	1.168	1.129	1.207	1.173	1.12	1.202	0.01406	2.69%	4.76%			
5		5	1.227	1.15	1.304	1.202	1.173	1.303	0.02782	5.07%	-0.03%			
6.06		5	1.184	1.114	1.254	1.187	1.107	1.266	0.02529	4.78%	3.46%			
10		5	1.239	1.146	1.331	1.217	1.187	1.369	0.03318	5.99%	-0.97%			
15		5	1.176	1.07	1.282	1.202	1.083	1.284	0.03831	7.28%	4.12%			

## CETIS Test Data Worksheet

Report Date:

20 Nov-17 13:18 (p 1 of 1)

Test Code:

13-5946-5773/1711-S120

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Nov-17 Species: Strongylocentrotus purpuratus  
 End Date: 20 Nov-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 19 Nov-17 Material: Brine Effluent

Sample Code: 17-1209

Sample Source: IDE Americas, Inc.

Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			66	100	91	11/21/17
			67	100	86	
			68	100	96	
			69	100	85	
			70	100	92	
			71	100	87	
			72	100	87	
			73	100	88-95	
			74	100	88	
			75	100	93	
			76	100	91	
			77	100	86	
			78	100	79	
			79	100	78	
			80	100	80	
			81	100	85	
			82	100	85	
			83	100	85	
			84	100	85	
			85	100	91	
			86	100	86	
			87	100	92	
			88	100	78	
			89	100	86	
			90	100	88	
			91	100	88	
			92	100	87	
			93	100	87	
			94	100	81	
			95	100	84	

Ⓐ = QIB KC 11/21/17

## CETIS Test Data Worksheet

Report Date:

20 Nov-17 13:18 (p 1 of 1)

Test Code:

13-5946-5773/1711-S120

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Nov-17 Species: Strongylocentrotus purpuratus  
 End Date: 20 Nov-17 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 19 Nov-17 Material: Brine Effluent

Sample Code: 17-1209

Sample Source: IDE Americas, Inc.

Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	66	100	93	PM 11/27/17
0	LC	2	84			
0	LC	3	85			
0	LC	4	79			
0	LC	5	73			
2.5		1	86	100	92	
2.5		2	92			
2.5		3	94			
2.5		4	69			
2.5		5	95			
5		1	81	100	86	
5		2	72			
5		3	83			
5		4	75			
5		5	87			
6.06		1	80	100	88	
6.06		2	76			
6.06		3	77			
6.06		4	82			
6.06		5	67			
10		1	91	100	93	
10		2	93			
10		3	74			
10		4	89			
10		5	68			
15		1	78	100	83	
15		2	88			
15		3	70			
15		4	71			
15		5	90			

QC: EA

## Marine Chronic Bioassay

## Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted)

Start Date/Time: 11/20/2017 1515

Sample Log No.: 17-1209

End Date/Time: 11/20/2017 1555

Dilutions made by: EG opo AC

Test No: 1711-8120

Analyst: DH

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.4	8.02	33.8	14.9
2.5	8.2	8.02	34.1	15.8
5.0	8.3	8.02	34.1	15.2
6.06	8.3	8.02	34.3	15.2
10	8.3	8.02	34.5	15.1
15	8.3	8.01	34.9	15.1

Comments:

QC Check: AD 11/27/17

Final Review: 11/30/17

## Marine Chronic Bioassay

## Brine Dilution Worksheet

Project: IDE

Analyst: EG/AC

Sample ID: M-001 (40 ppt adjusted)

Test Date: 11/20/2017

Test No: 1711-S120

Test Type: Urchin Fertilization

Salinity of Effluent 60.8

Salinity of Seawater 33.5

Date of Brine used: NA

Target Salinity 40.0

Alk. of 40 ppt Adj. Sample: 103 mg/L as CaCO<sub>3</sub>

Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	Effluent	Brine Control
- SE)/(SB - TS) =	3.20	-6.15

TS = target salinity

SE = salinity of effluent

SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)
100	100	3.20	320.0	420

Comments: Formula for amount of seawater to dilute sample to 40ppt  
 Use 40 ppt sample as 100% sample for testing.  
 NA = not applicable; sample not diluted with Nautilus brine.

QC Check: AD 11/27/17

Final Review: AC 11/30/17

## Marine Chronic Bioassay

## Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE  
 Sample ID: M-001 (40 ppt adjusted)  
 Test No.: 1711-5120

Tech initials: EG  
 Injection Time: 1438

Start Date/Time: 11/20/17 / 1515  
 End Date/Time: 11/20/17 / 1555  
 Species: *S. purpuratus*  
 Animal Source: Pt. Loma  
 Date Collected: 11/20/17

Sperm Absorbance at 400 nm: 0.730 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 83 Mean: 86.6 X 50 = 4330 eggs/ml

87

93

82

88

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

Initial density: 4330 eggs/ml = 1.0825 dilution factor  
 Final density: 4000 eggs/ml - 1.0 part egg stock  
 $\frac{0.0825}{0.0825}$  parts seawater      egg stock ml  
 $\frac{A}{A}$  ml 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.
1445	50:1	84/91	16/9
1455	100:1	95	2
1505	-	-	-

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: 75:1

Time	QC1	Fert.	Unfert.
1515	85	15	
1535	91	9	
1555	Egg Control 1	0	100
	Egg Control 2	0	100

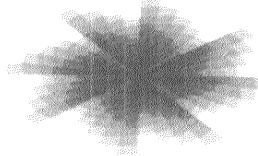
Comments: (A) EC 11/20/17 No Dilution Required

QC Check: (A) 11/27/17

Final Review: (A) 11/30/17

Appendix B  
Sample Receipt Information

## WEEKLY DAILY



**IDE** Technologies Corp Laboratories  
Page 14

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

CDP laboratory: \_\_\_\_\_  
Entahipy Laboratory: \_\_\_\_\_  
WECK Laboratory: \_\_\_\_\_  
Nautilus: \_\_\_ x \_\_\_\_\_  
AIM: \_\_\_\_\_  
Other: \_\_\_\_\_

Turn Around Time

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

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

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

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

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

**Special instruction:** Sampled during train off-spec via autosampler by a series of grabs collected at one hour intervals during plant in production at 38 MGD. Sample collected to fulfill daily NPDES requirement. Sample is to be run adjusted and unadjusted. Start: 11/18/17 @ 08:00, End: 11/19/17 @ 07:30 KC

## ANALYSES

## NOTES:

Glass=G Plastic=P

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

Drinking Water=DW Seawater=SW Soil=S

TDS - 58.44 ppt, EC - 82.50 mS/cm

Relinquished By:	Date:	Time:	Received By:	Date:	Time:	Sample Condition Upon Receipt:		
Karen Gray	11/20/17	1205	J	11/20/17	1205	<input checked="" type="checkbox"/> Iced	<input type="checkbox"/>	Ambient or 4.5 °C
J	11/20/17	1310	Caitlin W	11/20/17	1300	<input checked="" type="checkbox"/> Iced	<input type="checkbox"/>	Ambient or 4.5 °C

Nautilus ID: 17-1209

## Appendix C

### Chain-of-Custody Form

Nautilus Environmental  
4340 Vandever Avenue  
San Diego, CA 92120

Client: IDE

Sample ID: M-001 (weekly/daily)

Test ID No(s.): 1711-5119 to 5121

### Sample Check-In Information

#### Sample Description:

A: no color, clear, no odor, no debris

#### COC Complete (Y/N)?

A  B  C

#### Filtration? Y N

Pore Size: \_\_\_\_\_

Organisms or Debris

#### Salinity Adjustment? Y N Q18 Ac 11/30/17

Test: urine Source: seawater Target ppt: 40

Test: urine Source: seawater Target ppt: 40

Test: urine Source: seawater Target ppt: 40

#### 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: AD 11/27/17

Final Review: AC 11/30/17

#### Sample (A, B, C):

Log-in No. (17-xxxx): 1209

Sample Collection Date & Time: 11/19/17 0730

Sample Receipt Date & Time: 11/20/17 1300

Number of Containers & Container Type: 14L cubi

Approx. Total Volume Received (L): ~4L

Check-in Temperature (°C): 4.5

Temperature OK?  Y  N

DO (mg/L): 8.1

pH (units): 7.79

Conductivity (µS/cm): \_\_\_\_\_

Salinity (ppt): 60.80

Alkalinity (mg/L)<sup>2</sup>: 214

Hardness (mg/L)<sup>2,3</sup>: \_\_\_\_\_

Total Chlorine (mg/L): 0.02

Technician Initials: RT

Test Performed: urinalysis

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

Alkalinity: 132 Hardness or Salinity: 34 ppt

Additional Control?  Y  N

= HSC Alkalinity: 91 Hardness or Salinity: 37.3

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) measured using 1:1 dilution with DI

Appendix D  
Reference Toxicant Test Data and  
Statistical Analyses

## CETIS Summary Report

Report Date:

27 Nov-17 12:33 (p 1 of 1)

Test Code:

171120sprt | 08-0578-7050

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)					
Batch ID:	03-3825-6100	Test Type: Fertilization				Analyst:						
Start Date:	20 Nov-17 15:15	Protocol: EPA/600/R-95/136 (1995)				Diluent: Natural Seawater						
Ending Date:	20 Nov-17 15:55	Species: Strongylocentrotus purpuratus				Brine: Not Applicable						
Duration:	40m	Source: Pt. Loma				Age:						
Sample ID:	08-0224-3944	Code: 171120sprt				Client: Internal						
Sample Date:	20 Nov-17	Material: Copper chloride				Project:						
Receive Date:	20 Nov-17	Source: Reference Toxicant										
Sample Age:	15h	Station: Copper Chloride										
<b>Comparison Summary</b>												
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method					
14-5483-3998	Fertilization Rate	<10	10	NA	7.08%		Dunnett Multiple Comparison Test					
<b>Point Estimate Summary</b>												
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method					
18-8950-2431	Fertilization Rate	EC50	38.26	36.47	40.15		Trimmed Spearman-Kärber					
<b>Test Acceptability</b>												
Analysis ID	Endpoint	Attribute		Test Stat	TAC	Limits	Overlap	Decision				
14-5483-3998	Fertilization Rate	Control Resp		0.892	0.7 - NL		Yes	Passes Acceptability Criteria				
18-8950-2431	Fertilization Rate	Control Resp		0.892	0.7 - NL		Yes	Passes Acceptability Criteria				
14-5483-3998	Fertilization Rate	PMSD		0.07085	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	0.892	0.8478	0.9362	0.84	0.94	0.01594	0.03564	4.0%	0.0%	
10		5	0.802	0.6998	0.9042	0.67	0.88	0.0368	0.08228	10.26%	10.09%	
20		5	0.772	0.6994	0.8446	0.7	0.83	0.02615	0.05848	7.58%	13.45%	
40		5	0.462	0.4178	0.5062	0.43	0.52	0.01594	0.03564	7.71%	48.21%	
80		5	0.014	0	0.03478	0	0.04	0.007483	0.01673	119.5%	98.43%	
160		5	0.002	0	0.007553	0	0.01	0.002	0.004472	223.6%	99.78%	
<b>Fertilization Rate Detail</b>												
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.84	0.94	0.89	0.9	0.89						
10		0.86	0.67	0.79	0.81	0.88						
20		0.83	0.7	0.73	0.77	0.83						
40		0.47	0.43	0.44	0.45	0.52						
80		0	0.01	0.02	0.04	0						
160		0	0	0.01	0	0						

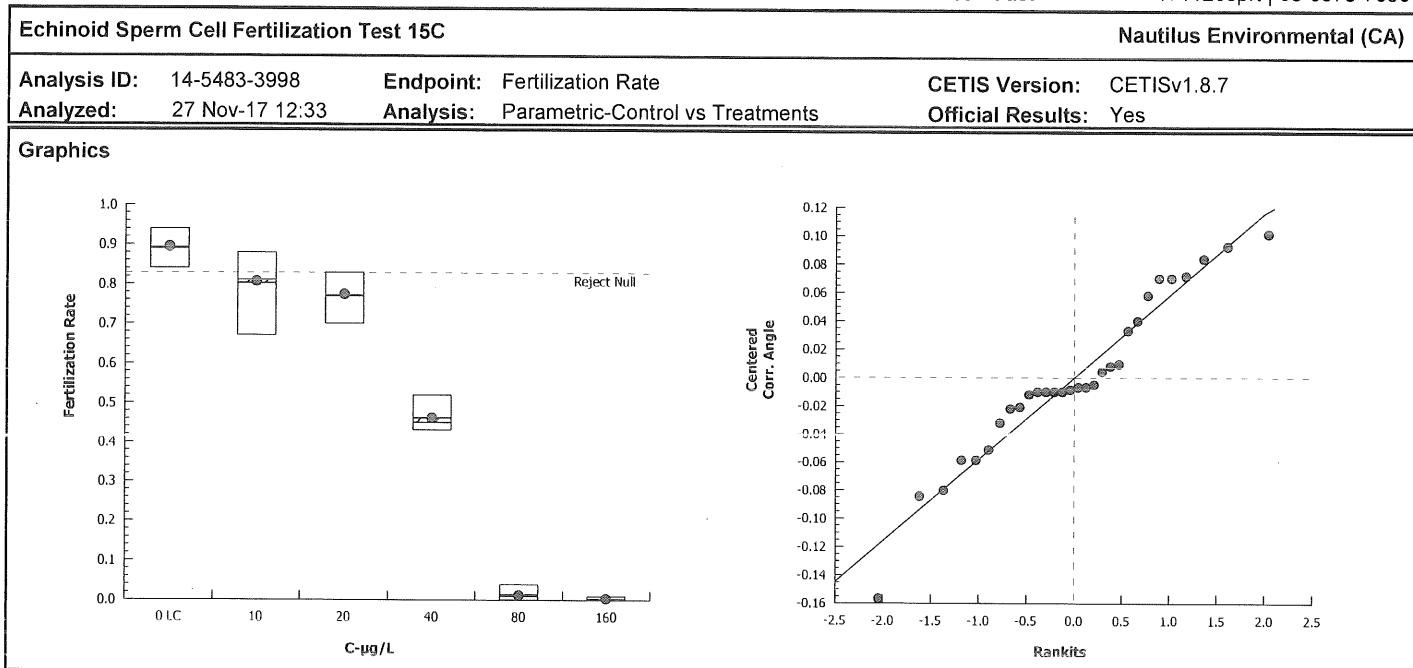
## CETIS Analytical Report

Report Date: 27 Nov-17 12:33 (p 1 of 2)  
 Test Code: 171120sprt | 08-0578-7050

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)							
Analysis ID: 14-5483-3998		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7									
Analyzed: 27 Nov-17 12:33		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	7.08%	<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*	3.073	2.362	0.095	8	0.0108	CDF	Significant Effect							
	20*	4.065	2.362	0.095	8	0.0010	CDF	Significant Effect							
	40*	12.21	2.362	0.095	8	<0.0001	CDF	Significant Effect							
	80*	28.05	2.362	0.095	8	<0.0001	CDF	Significant Effect							
	160*	29.26	2.362	0.095	8	<0.0001	CDF	Significant Effect							
ANOVA Table															
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)							
Between	6.81269		1.362538		5	335.4	<0.0001	Significant Effect							
Error	0.09749699		0.004062374		24										
Total	6.910187				29										
Distributional Tests															
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)								
Variances	Bartlett Equality of Variance			8.371	15.09	0.1369	Equal Variances								
Distribution	Shapiro-Wilk W Normality			0.9526	0.9031	0.1985	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	0.892	0.8478	0.9362	0.89	0.84	0.94	0.01594	4.0%	0.0%				
		5	0.802	0.6998	0.9042	0.81	0.67	0.88	0.0368	10.26%	10.09%				
		5	0.772	0.6994	0.8446	0.77	0.7	0.83	0.02615	7.58%	13.45%				
		5	0.462	0.4178	0.5062	0.45	0.43	0.52	0.01594	7.71%	48.21%				
		5	0.014	0	0.03478	0.01	0	0.04	0.007483	119.5%	98.43%				
		5	0.002	0	0.007553	0	0	0.01	0.002	223.6%	99.78%				
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	1.239	1.167	1.312	1.233	1.159	1.323	0.02612	4.71%	0.0%				
		5	1.116	0.9907	1.24	1.12	0.9589	1.217	0.04497	9.02%	10.0%				
		5	1.076	0.9886	1.163	1.071	0.9912	1.146	0.03133	6.51%	13.22%				
		5	0.7473	0.703	0.7917	0.7353	0.7152	0.8054	0.01597	4.78%	39.71%				
		5	0.1087	0.02859	0.1888	0.1002	0.05002	0.2014	0.02885	59.35%	91.23%				
		5	0.06005	0.0322	0.0879	0.05002	0.05002	0.1002	0.01003	37.35%	95.15%				

# CETIS Analytical Report

Report Date: 27 Nov-17 12:33 (p 2 of 2)  
Test Code: 171120sprt | 08-0578-7050

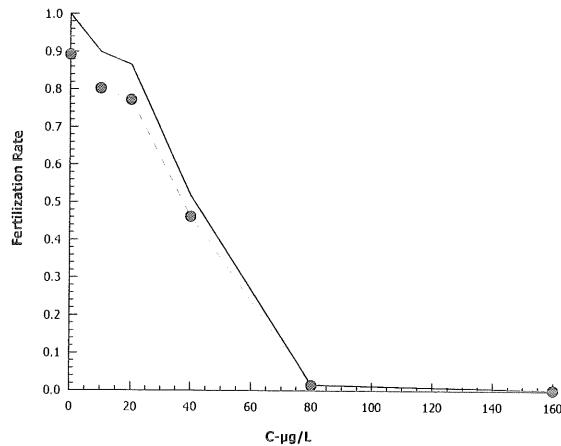


# CETIS Analytical Report

Report Date: 27 Nov-17 12:33 (p 1 of 1)  
 Test Code: 171120sprt | 08-0578-7050

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 18-8950-2431			Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7						
Analyzed: 27 Nov-17 12:33			Analysis: Trimmed Spearman-Kärber		Official Results: Yes						
<b>Trimmed Spearman-Kärber Estimates</b>											
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL				
Control Threshold	0.108	10.09%	1.583	0.01044	38.26	36.47	40.15				
<b>Fertilization Rate Summary</b>					<b>Calculated Variate(A/B)</b>						
C- $\mu$ g/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.892	0.84	0.94	0.01594	0.03564	4.0%	0.0%	446	500
10		5	0.802	0.67	0.88	0.0368	0.08228	10.26%	10.09%	401	500
20		5	0.772	0.7	0.83	0.02615	0.05848	7.58%	13.45%	386	500
40		5	0.462	0.43	0.52	0.01594	0.03564	7.71%	48.21%	231	500
80		5	0.014	0	0.04	0.007483	0.01673	119.5%	98.43%	7	500
160		5	0.002	0	0.01	0.002	0.004472	223.6%	99.78%	1	500

## Graphics



## Echinoid Sperm Cell Fertilization Test 15C

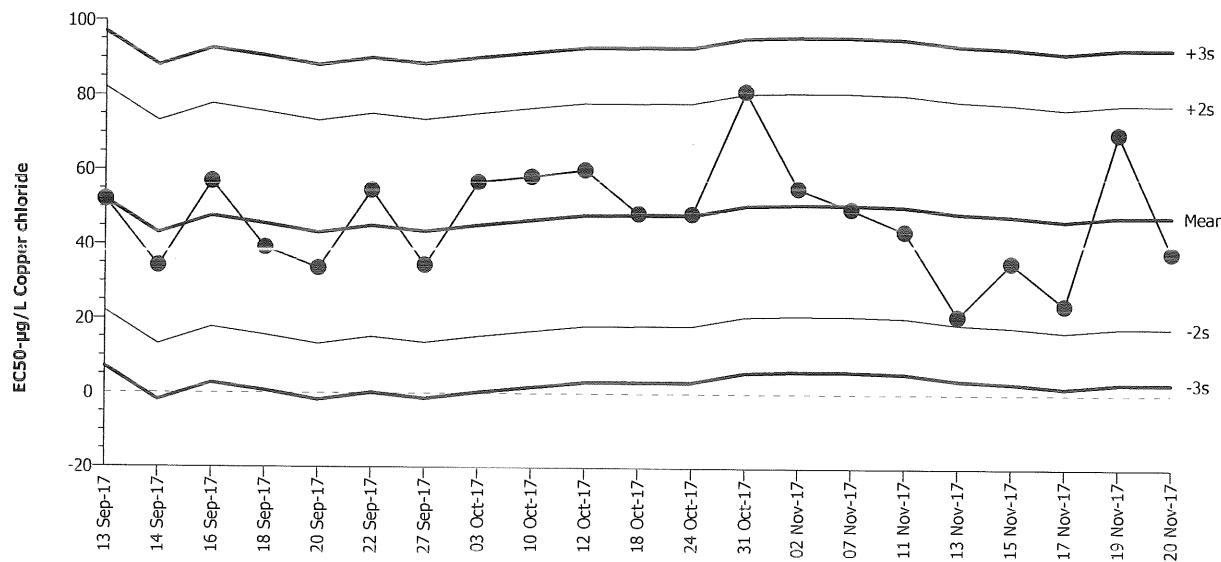
Nautilus Environmental (CA)

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

Organism: Strongylocentrotus purpuratus (Purple)  
 Endpoint: Fertilization Rate

Material: Copper chloride  
 Source: Reference Toxicant-REF

## Echinoid Sperm Cell Fertilization Test 15C



Mean:	47.93	Count:	20	-2s Warning Limit:	17.93	-3s Action Limit:	2.933
Sigma:	15	CV:	31.30%	+2s Warning Limit:	77.93	+3s Action Limit:	92.93

## Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Sep	13	19:07	52.04	4.106	0.2738			01-4575-6189	02-4618-7964
2			14	15:24	34.24	-13.69	-0.913			11-2846-3680	13-8128-7168
3			16	17:08	56.97	9.04	0.6027			08-9569-1329	19-6375-1112
4			18	15:28	39.21	-8.724	-0.5816			19-2924-5672	02-0031-2532
5			20	16:15	33.62	-14.31	-0.9538			00-4454-0074	17-7214-1415
6			22	14:50	54.61	6.679	0.4452			20-3341-5102	16-2759-7635
7			27	15:34	34.46	-13.47	-0.8977			12-3257-1101	06-9840-2290
8	Oct		3	13:49	56.88	8.949	0.5966			05-1137-7792	06-0895-0170
9			10	15:10	58.36	10.43	0.6955			20-5863-5053	00-1542-1738
10			12	14:55	60.18	12.25	0.8168			05-0863-6526	07-1531-2424
11			18	14:22	48.53	0.6004	0.04002			13-0042-6212	05-6771-5532
12			24	13:15	48.41	0.4753	0.03169			20-0280-7301	18-5464-1899
13			31	13:59	81.36	33.43	2.229	(+)		06-4227-6723	08-8095-0809
14	Nov		2	12:28	55.32	7.395	0.493			17-4126-1689	20-0626-8382
15			7	14:30	49.87	1.943	0.1295			10-3521-2857	13-9801-3995
16			11	14:25	43.91	-4.022	-0.2681			14-1655-2339	20-5239-6070
17			13	14:35	20.97	-26.96	-1.797			07-0538-7056	00-9105-4737
18			15	16:09	35.48	-12.45	-0.8298			06-3476-9418	17-5783-9769
19			17	14:17	24.03	-23.9	-1.594			20-8374-1268	00-9691-5869
20			19	10:02	70.21	22.28	1.485			12-1164-1483	20-4501-4622
21			20	15:15	38.26	-9.665	-0.6443			08-0578-7050	18-8950-2431

## CETIS Test Data Worksheet

Report Date: 20 Nov-17 13:17 (p 1 of 1)  
 Test Code: 08-0578-7050/171120sprt

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	20 Nov-17	Species:	Strongylocentrotus purpuratus	Sample Code:	171120sprt	
End Date:	20 Nov-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant	
Sample Date:	20 Nov-17	Material:	Copper chloride	Sample Station:	Copper Chloride	
C- $\mu$ g/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	86	① 86 79 11/21/17
			2	100	86	
			3	100	0	
			4	100	90	
			5	100	2	
			6	100	79	
			7	100	0	
			8	100	67	
			9	100	0	
			10	100	89	
			11	100	0	
			12	100	1	
			13	100	81	
			14	100	83	
			15	100	84	
			16	100	1	
			17	100	89	
			18	100	70	
			19	100	83	
			20	100	88	
			21	100	52	
			22	100	0	
			23	100	95	
			24	100	0	
			25	100	94	
			26	100	47	
			27	100	4	
			28	100	73	
			29	100	43	
			30	100	99	

Ⓐ - Q18 KC 11/21/17

## CETIS Test Data Worksheet

Report Date: 20 Nov-17 13:17 (p 1 of 1)  
 Test Code: 08-0578-7050/171120sprt

## Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	20 Nov-17	Species:	Strongylocentrotus purpuratus	Sample Code:	171120sprt	
End Date:	20 Nov-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant	
Sample Date:	20 Nov-17	Material:	Copper chloride	Sample Station:	Copper Chloride	
C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	15			
0	LC	2	30			
0	LC	3	17			
0	LC	4	4	100	93	EL 11/20/17
0	LC	5	10			
10		1	2			
10		2	6			
10		3	6			
10		4	13			
10		5	20			
20		1	14			
20		2	18			
20		3	28			
20		4	1	100	78	EG
20		5	19			
40		1	26			
40		2	29	100	48	EL
40		3	25			
40		4	23			
40		5	21			
80		1	22			
80		2	12			
80		3	5	100	5	EG
80		4	27			
80		5	11			
160		1	7			
160		2	9			
160		3	16			
160		4	24			
160		5	3			

AC:EG

## Marine Chronic Bioassay

## Water Quality Measurements

Client : Internal

Test Species: S. purpuratusSample ID: CuCl<sub>2</sub>

Start Date/Time: 11/20/2017 1515

Test No: 171120sprt

End Date/Time: 11/20/2017 1555

Dilutions made by: EC 080AC

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.3
Final Volume (mL):	500
Cu stock concentration (µg/L):	9.600

Analyst: RTJ

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.2	8.01	33.8	16.0
10	8.1	7.98	33.9	16.0
20	8.0	7.97	33.9	16.0
40	8.0	7.97	33.8	16.0
80	8.0	7.99	33.7	16.0
160	8.0	8.00	33.4	16.0

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

QC Check: AD 11/27/17

Final Review: AC 11/27/17

## Marine Chronic Bioassay

## Echinoderm Sperm-Cell Fertilization Worksheet

Client: B) HDE Internal  
 Sample ID: BAM 001 CUC 2  
 Test No.: 171120SPRT

Tech initials: E5  
 Injection Time: 1438

Sperm Absorbance at 400 nm: 0.930 (target range of 0.8 - 1.0 for density of  $4 \times 10^6$  sperm/ml)

Eggs Counted: 83 Mean: 86.6  $\times 50 =$  4330 eggs/ml  
87  
93  
82  
88

Initial density: 4330 eggs/ml = 1.0825 dilution factor  
 Final density: 4000 eggs/ml - 1.0 part egg stock  
0.8825 parts seawater      egg stock (A) ml  
 seawater (B) 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	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75
Sperm Added (100 µl):	<u>1445</u>	<u>50:1</u>	<u>84/91</u>	<u>16/19</u>				
Eggs Added (0.5 ml):	<u>1455</u>	<u>100:1</u>	<u>98</u>	<u>2</u>				
Test Ended:	<u>1505</u>	<u>—</u>	<u>—</u>	<u>—</u>				

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

## Definitive Test

Sperm:Egg Ratio Used: 75:1

	Time	Fert.	Unfert.
Sperm Added (100 µl):	<u>1515</u>	<u>85</u>	<u>15</u>
Eggs Added (0.5 ml):	<u>1535</u>	<u>91</u>	<u>9</u>
Test Ended:	<u>1555</u>	<u>0</u>	<u>100</u>
	Egg Control 1	<u>0</u>	<u>100</u>
	Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) EH 11/20/17 No Dilution Required  
(B) No QC 11/27

QC Check:

Ans 11/27/17

Final Review: AC 11/27/17

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.