



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: August 27, 2017

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

Prepared by: Nautilus Environmental

Submitted: September 11, 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

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT — AUGUST 2017

ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: August 27, 2017

Test Date: August 28, 2017

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

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type: Urchin Fertilization	Effluent Test Results		Effluent Limitation Met? (Yes/No)
	NOEC	TU _c	
	6.06	16.5	Yes

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 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 August 28, 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 (pre-treatment off-spec period)
Monitoring Period:	August 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	8/27/17, 08:00
Sample Receipt Date, Time:	8/28/17, 11:50

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 ₃)	Total Chlorine (mg/L)
8/27/17	7.92	8.7	4.5	32.6	111	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	8/28/17, 14:56 through 15:36
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_c) values.

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

RESULTS

A statistically significant decrease in fertilization rate was 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 equal to 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 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)	TU _c value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001	6.06	10	>15	16.5	Pass	2.3

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

EC₅₀ = Concentration expected to cause an adverse effect to 50 percent of the test organisms

TU_c = 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	97.6
2.5	95.6
5.0	97.2
6.06	95.4
10	91.4*
15	84.4*

*An asterisk indicates a statistically significant decrease in fertilization rate compared to the lab control.

QUALITY ASSURANCE

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

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

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC_{50} ($\mu\text{g/L}$ Copper)	Historical Mean $EC_{50} \pm 2$ SD ($\mu\text{g/L}$ Copper)	CV (%)
8/28/17	41.6	51.5 ± 38.8	37.6

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

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: 11 Sep-17 15:19 (p 1 of 1)
Test Code: 1708-S235 | 03-0612-8625

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	19-2756-1831	Test Type:	Fertilization	Analyst:							
Start Date:	28 Aug-17 14:56	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	28 Aug-17 15:36	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	13-3822-2447	Code:	17-0946	Client:	IDE						
Sample Date:	27 Aug-17 08:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	28 Aug-17 11:50	Source:	IDE Americas, Inc.								
Sample Age:	31h (4.5 °C)	Station:	M-001 (Daily)								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
08-6207-7202	Fertilization Rate	6.06	10	7.785	3.34%	16.5	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
01-1300-8025	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					
01-1300-8025	Fertilization Rate	Control Resp	0.976	0.7 - NL	Yes	Passes Acceptability Criteria					
08-6207-7202	Fertilization Rate	Control Resp	0.976	0.7 - NL	Yes	Passes Acceptability Criteria					
08-6207-7202	Fertilization Rate	PMSD	0.0334	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	0.976	0.9593	0.9927	0.96	0.99	0.006	0.01342	1.38%	0.0%
2.5		5	0.956	0.9236	0.9884	0.94	1	0.01166	0.02608	2.73%	2.05%
5		5	0.972	0.9481	0.9959	0.94	0.99	0.008602	0.01924	1.98%	0.41%
6.06		5	0.954	0.9254	0.9826	0.92	0.97	0.0103	0.02302	2.41%	2.25%
10		5	0.914	0.8883	0.9397	0.89	0.94	0.009273	0.02074	2.27%	6.35%
15		5	0.844	0.7785	0.9095	0.77	0.9	0.02358	0.05273	6.25%	13.52%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.96	0.99	0.97	0.99	0.97					
2.5		0.94	0.96	1	0.94	0.94					
5		0.98	0.99	0.97	0.94	0.98					
6.06		0.97	0.92	0.97	0.97	0.94					
10		0.9	0.91	0.93	0.94	0.89					
15		0.87	0.87	0.9	0.77	0.81					
Fertilization Rate Binomials											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	96/100	99/100	97/100	99/100	97/100					
2.5		94/100	96/100	100/100	94/100	94/100					
5		98/100	99/100	97/100	94/100	98/100					
6.06		97/100	92/100	97/100	97/100	94/100					
10		90/100	91/100	93/100	94/100	89/100					
15		87/100	87/100	90/100	77/100	81/100					

CETIS Analytical Report

Report Date: 11 Sep-17 15:19 (p 1 of 2)

Test Code: 1708-S235 | 03-0612-8625

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 08-6207-7202		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 11 Sep-17 15:19		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		3.34%	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	1.277	2.362	0.090	8	0.3037	CDF	Non-Significant Effect		
		5	0.2913	2.362	0.090	8	0.7333	CDF	Non-Significant Effect		
		6.06	1.605	2.362	0.090	8	0.1916	CDF	Non-Significant Effect		
		10*	3.821	2.362	0.090	8	0.0018	CDF	Significant Effect		
		15*	6.6	2.362	0.090	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.231007		0.0462014		5		12.66	<0.0001	Significant Effect		
Error	0.08758636		0.003649432		24						
Total	0.3185934				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			3.181	15.09	0.6721	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9614	0.9031	0.3368	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	0.976	0.9593	0.9927	0.97	0.96	0.99	0.006	1.38%	0.0%
2.5		5	0.956	0.9236	0.9884	0.94	0.94	1	0.01166	2.73%	2.05%
5		5	0.972	0.9481	0.9959	0.98	0.94	0.99	0.008602	1.98%	0.41%
6.06		5	0.954	0.9254	0.9826	0.97	0.92	0.97	0.0103	2.41%	2.25%
10		5	0.914	0.8883	0.9397	0.91	0.89	0.94	0.009273	2.27%	6.35%
15		5	0.844	0.7785	0.9095	0.87	0.77	0.9	0.02358	6.25%	13.52%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.421	1.363	1.479	1.397	1.369	1.471	0.02093	3.29%	0.0%
2.5		5	1.372	1.266	1.478	1.323	1.323	1.521	0.03824	6.23%	3.43%
5		5	1.41	1.341	1.478	1.429	1.323	1.471	0.02457	3.9%	0.78%
6.06		5	1.36	1.294	1.425	1.397	1.284	1.397	0.02362	3.89%	4.32%
10		5	1.275	1.228	1.322	1.266	1.233	1.323	0.01682	2.95%	10.27%
15		5	1.169	1.079	1.258	1.202	1.071	1.249	0.03215	6.15%	17.75%

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 08-6207-7202

Endpoint: Fertilization Rate

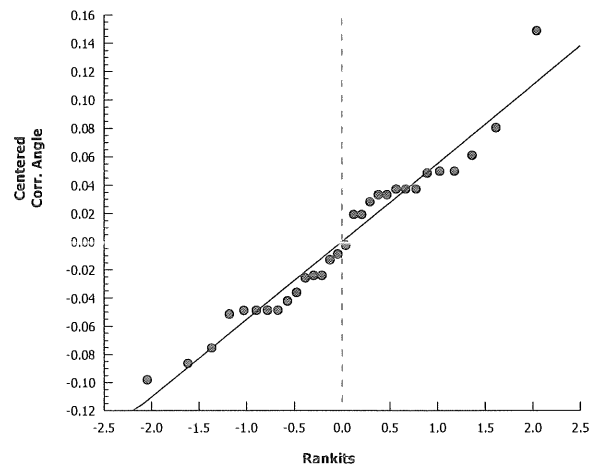
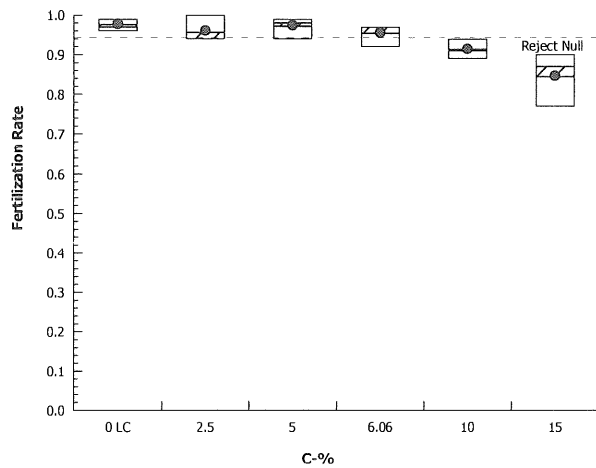
CETIS Version: CETISv1.8.7

Analyzed: 11 Sep-17 15:19

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

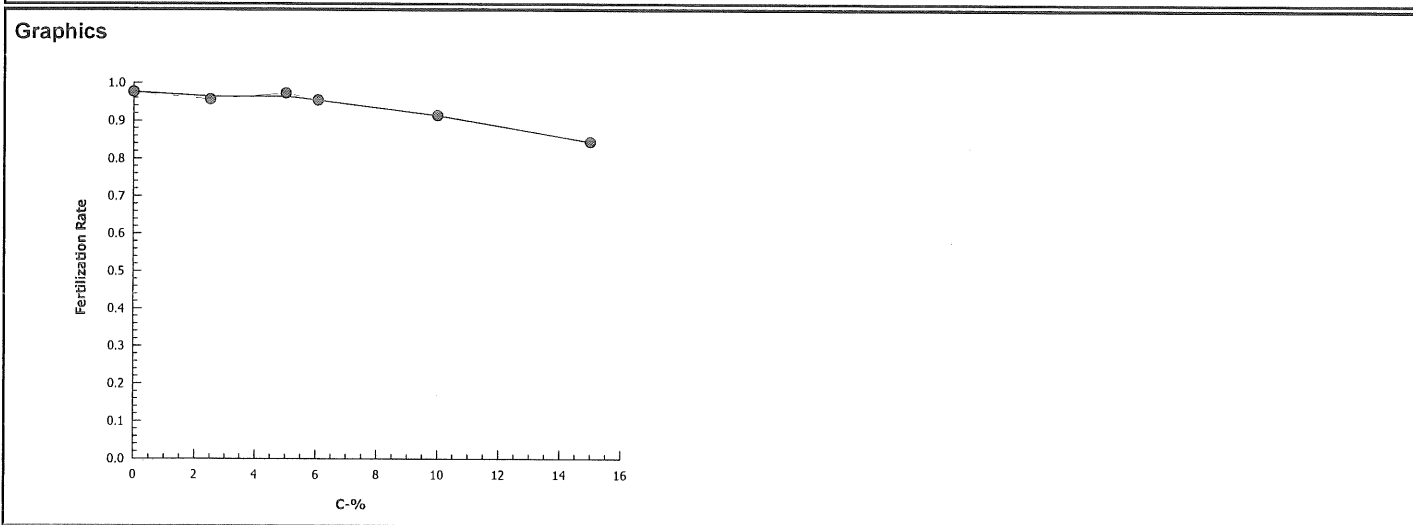
Report Date: 11 Sep-17 15:19 (p 1 of 1)
Test Code: 1708-S235 | 03-0612-8625

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	01-1300-8025	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	11 Sep-17 15:19	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1470363	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	0.976	0.96	0.99	0.006	0.01342	1.38%	0.0%	488	500
2.5		5	0.956	0.94	1	0.01166	0.02608	2.73%	2.05%	478	500
5		5	0.972	0.94	0.99	0.008602	0.01924	1.98%	0.41%	486	500
6.06		5	0.954	0.92	0.97	0.0103	0.02302	2.41%	2.25%	477	500
10		5	0.914	0.89	0.94	0.009273	0.02074	2.27%	6.35%	457	500
15		5	0.844	0.77	0.9	0.02358	0.05273	6.25%	13.52%	422	500



CETIS Analytical Report

Report Date: 11 Sep-17 15:20 (p 1 of 1)
 Test Code: 1708-S235 | 03-0612-8625

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID: 18-5135-8443		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 11 Sep-17 15:19		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	2.51%	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*	7.413	2.015	0.083	5	0.0004	CDF	Non-Significant Effect		
		5*	11.8	1.943	0.057	6	<0.0001	CDF	Non-Significant Effect		
		6.06*	10.36	1.943	0.055	6	<0.0001	CDF	Non-Significant Effect		
		10*	9.092	1.895	0.044	7	<0.0001	CDF	Non-Significant Effect		
		15*	2.88	2.015	0.072	5	0.0173	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.231007		0.0462014		5		12.66	<0.0001	Significant Effect		
Error	0.08758636		0.003649432		24						
Total	0.3185934				29						
Distributional Tests											
Attribute	Test		Test Stat	Critical	P-Value		Decision(α:1%)				
Variances	Bartlett Equality of Variance		3.181	15.09	0.6721		Equal Variances				
Distribution	Shapiro-Wilk W Normality		0.9614	0.9031	0.3368		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	0.976	0.9593	0.9927	0.97	0.96	0.99	0.006	1.38%	0.0%
2.5		5	0.956	0.9236	0.9884	0.94	0.94	1	0.01166	2.73%	2.05%
5		5	0.972	0.9481	0.9959	0.98	0.94	0.99	0.008602	1.98%	0.41%
6.06		5	0.954	0.9254	0.9826	0.97	0.92	0.97	0.0103	2.41%	2.25%
10		5	0.914	0.8883	0.9397	0.91	0.89	0.94	0.009273	2.27%	6.35%
15		5	0.844	0.7785	0.9095	0.87	0.77	0.9	0.02358	6.25%	13.52%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.421	1.363	1.479	1.397	1.369	1.471	0.02093	3.29%	0.0%
2.5		5	1.372	1.266	1.478	1.323	1.323	1.521	0.03824	6.23%	3.43%
5		5	1.41	1.341	1.478	1.429	1.323	1.471	0.02457	3.9%	0.78%
6.06		5	1.36	1.294	1.425	1.397	1.284	1.397	0.02362	3.89%	4.32%
10		5	1.275	1.228	1.322	1.266	1.233	1.323	0.01682	2.95%	10.27%
15		5	1.169	1.079	1.258	1.202	1.071	1.249	0.03215	6.15%	17.75%

CETIS Test Data Worksheet

Report Date: 28 Aug-17 10:20 (p 1 of 1)
 Test Code: 1708-5235 03-0612-8625/123F26F1

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 28 Aug-17 Species: Strongylocentrotus purpuratus
 End Date: 28 Aug-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 27 Aug-17 Material: Facility Effluent

Sample Code: 17-0946
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Daily) 8/27 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	96	8/30/17
			62		94	
			63		87	
			64		94	
			65		97	
			66		92	
			67		81	
			68		98	
			69		99	Q19 RL re-count AC 9/11/17
			70		99	
			71		94	
			72		97	
			73		91	
			74		87	
			75		97	
			76		99	
			77		89	
			78		99	
			79		97	
			80		96	
			81		97	
			82		100	
			83		94	
			84		94	
			85		97	
			86		98	
			87		93	
			88		94	
			89		77	
			90		90	

CETIS Test Data Worksheet

Report Date: 28 Aug-17 10:20 (p 1 of 1)

Test Code: 1708-S235 03-0612-8625/123F26F1

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 28 Aug-17 Species: Strongylocentrotus purpuratus
End Date: 28 Aug-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 27 Aug-17 Material: Facility Effluent

Sample Code: 17-0946
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (Collected 8/27/17)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	80	100	94	EG 8/25/17
0	LC	2	76			
0	LC	3	72			
0	LC	4	78			
0	LC	5	75			
2.5		1	84	100	99	EG
2.5		2	61			
2.5		3	82			
2.5		4	64			
2.5		5	83			
5		1	86	100	94	EG
5		2	70			
5		3	65			
5		4	62			
5		5	68			
6.06		1	81	100	96	EG
6.06		2	66			
6.06		3	79			
6.06		4	85			
6.06		5	88			
10		1	90	100	90	EG
10		2	73			
10		3	87			
10		4	71			
10		5	77			
15		1	74	100	75	EG
15		2	63			
15		3	69			
15		4	89			
15		5	67			

QC: CG

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 Daily (8/27/17)

Start Date/Time: 8/28/2017 1456

Sample Log No.: 17-0946

End Date/Time: 8/28/2017 1536

Dilutions made by: CG

Test No: 1708-S235

Analyst:

EG

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.5	8.14	33.8	15.9 (B)
2.5	7.4	8.13	33.8 33.8 (A)	15.9
5.0	7.5	8.12	33.7	15.9
6.06	7.5	8.12	33.7	15.9
10	7.5	8.11	33.7	15.9
15	7.5	8.10	33.7	15.9

Comments:

(A) EG Q19 8/28/17 (B) Temperature recorded from surrogate vial on tray

QC Check:

AC 8/30/17

Final Review:

ls 9/11/17

Echinoderm Sperm-Cell Fertilization Worksheet

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

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE Dredge EQ 18 8/25/17
Sample ID: Daily M-001 8/27 sample
Test ID No(s): 1708-S235

Sample Check-In Information

Sample Description:
A - colorless, clear, no odor, no debris

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	0946			
Sample Collection Date & Time:	8/27/17 0800			
Sample Receipt Date & Time:	8/28/17 1150			
Number of Containers & Container Type:	1, 4L cubi			
Approx. Total Volume Received (L):	3 L			
Check-in Temperature (°C)	4.5			
Temperature OK? ¹	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.7			
pH (units)	7.92			
Conductivity (µS/cm)	-			
Salinity (ppt)	32.6			
Alkalinity (mg/L) ²	111			
Hardness (mg/L) ^{2,3}	-			
Total Chlorine (mg/L)	20.02			
Technician Initials	EH			

Test Performed: urchin fertilization Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: _____

Alkalinity: 107 Hardness or Salinity: 34 ppt
Additional Control? Y (N) = _____ Alkalinity: _____ Hardness or Salinity: _____

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

Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

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

Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

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

² mg/L as CaCO₃, ³ Measured for freshwater samples only, NA = Not Applicable

Additional Comments: _____

COC Complete (Y/N)?

A Y B _____ C _____

Filtration? Y (N)

Pore Size: _____

Organisms _____ or _____ Debris

Salinity Adjustment? Y (N)

Test:: _____ Source: _____ Target ppt: _____

Test:: _____ Source: _____ Target ppt: _____

Test:: _____ Source: _____ Target ppt: _____

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl₂ Adjustment? Y (N)

	A	B	C
Initial Free Cl ₂ :			
STS added:			
Final Free Cl ₂ :			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Subsamples for Additional Chemistry Required? Y (N)

NH₃ Other _____

Tech Initials A _____ B _____ C _____

QC Check: AL 8/28/17

Final Review: W 9/11/17

Appendix C

Chain-of-Custody Form

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 30 Aug-17 16:01 (p 1 of 1)
Test Code: 170828sprt | 08-1525-2751

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	04-3497-0759		Test Type:			Fertilization		Analyst:			
Start Date:	28 Aug-17 14:56		Protocol:			EPA/600/R-95/136 (1995)		Diluent:		Natural Seawater	
Ending Date:	28 Aug-17 15:36		Species:			Strongylocentrotus purpuratus		Brine:		Not Applicable	
Duration:	40m		Source:			Pt. Loma		Age:			
Sample ID:	14-6644-8283		Code:			170828sprt		Client:		Internal	
Sample Date:	28 Aug-17		Material:			Copper chloride		Project:			
Receive Date:	28 Aug-17		Source:			Reference Toxicant					
Sample Age:	15h		Station:			Copper Chloride					
Comparison Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
07-9256-6029	Fertilization Rate		<10	10	NA	3.39%		Dunnett Multiple Comparison Test			
Point Estimate Summary											
Analysis ID	Endpoint		Level	µg/L	95% LCL	95% UCL	TU	Method			
10-7829-2432	Fertilization Rate		EC50	41.55	39.24	44		Trimmed Spearman-Kärber			
Test Acceptability											
Analysis ID	Endpoint		Attribute		Test Stat	TAC Limits		Overlap	Decision		
07-9256-6029	Fertilization Rate		Control Resp		0.966	0.7 - NL		Yes	Passes Acceptability Criteria		
10-7829-2432	Fertilization Rate		Control Resp		0.966	0.7 - NL		Yes	Passes Acceptability Criteria		
07-9256-6029	Fertilization Rate		PMSD		0.03392	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	0.966	0.9434	0.9886	0.95	0.99	0.008124	0.01817	1.88%	0.0%
10		5	0.856	0.8372	0.8748	0.83	0.87	0.006782	0.01517	1.77%	11.39%
20		5	0.754	0.7142	0.7938	0.72	0.8	0.01435	0.03209	4.26%	21.95%
40		5	0.596	0.4783	0.7137	0.46	0.71	0.04238	0.09476	15.9%	38.3%
80		5	0.156	0.1143	0.1977	0.11	0.2	0.01503	0.03362	21.55%	83.85%
160		5	0.004	0	0.01511	0	0.02	0.004	0.008944	223.6%	99.59%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.96	0.95	0.98	0.95	0.99					
10		0.87	0.86	0.86	0.86	0.83					
20		0.75	0.72	0.77	0.73	0.8					
40		0.63	0.63	0.46	0.55	0.71					
80		0.2	0.17	0.16	0.14	0.11					
160		0	0.02	0	0	0					

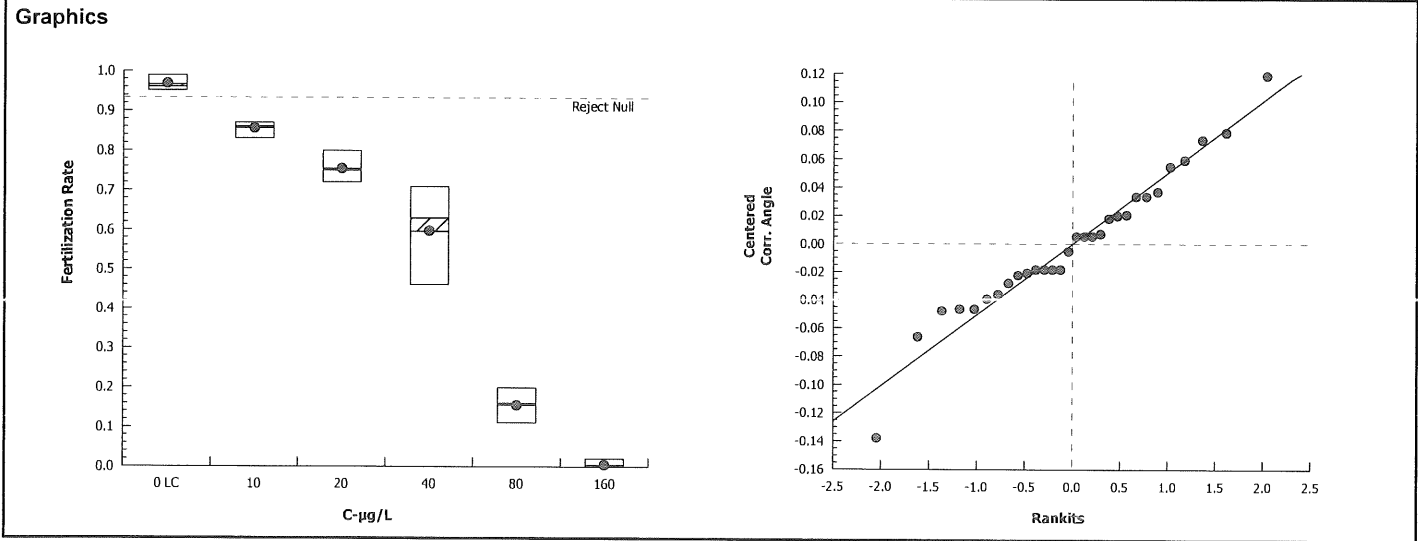
CETIS Analytical Report

Report Date: 30 Aug-17 16:00 (p 1 of 2)

Test Code: 170828sprt | 08-1525-2751

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 07-9256-6029		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 30 Aug-17 16:00		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		3.39%	<10	10	NA	
Dunnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		10*	6.014	2.362	0.082	8	<0.0001	CDF	Significant Effect		
		20*	9.72	2.362	0.082	8	<0.0001	CDF	Significant Effect		
		40*	14.56	2.362	0.082	8	<0.0001	CDF	Significant Effect		
		80*	28.28	2.362	0.082	8	<0.0001	CDF	Significant Effect		
		160*	37.9	2.362	0.082	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	6.265867		1.253173		5		411.1	<0.0001	Significant Effect		
Error	0.07315469		0.003048112		24						
Total	6.339022				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			8.93	15.09	0.1119		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9716	0.9031	0.5836		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	0.966	0.9434	0.9886	0.96	0.95	0.99	0.008124	1.88%	0.0%
10		5	0.856	0.8372	0.8748	0.86	0.83	0.87	0.006782	1.77%	11.39%
20		5	0.754	0.7142	0.7938	0.75	0.72	0.8	0.01435	4.26%	21.95%
40		5	0.596	0.4783	0.7137	0.63	0.46	0.71	0.04238	15.9%	38.3%
80		5	0.156	0.1143	0.1977	0.16	0.11	0.2	0.01503	21.55%	83.85%
160		5	0.004	0	0.01511	0	0	0.02	0.004	223.6%	99.59%
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	1.392	1.323	1.461	1.369	1.345	1.471	0.02492	4.0%	0.0%
10		5	1.182	1.156	1.208	1.187	1.146	1.202	0.009464	1.79%	15.09%
20		5	1.053	1.006	1.099	1.047	1.013	1.107	0.01685	3.58%	24.38%
40		5	0.8834	0.7628	1.004	0.9169	0.7454	1.002	0.04341	10.99%	36.54%
80		5	0.4043	0.346	0.4627	0.4115	0.3381	0.4636	0.021	11.61%	70.95%
160		5	0.0684	0.01738	0.1194	0.05002	0.05002	0.1419	0.01838	60.07%	95.09%

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID:	07-9256-6029	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	30 Aug-17 16:00	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes



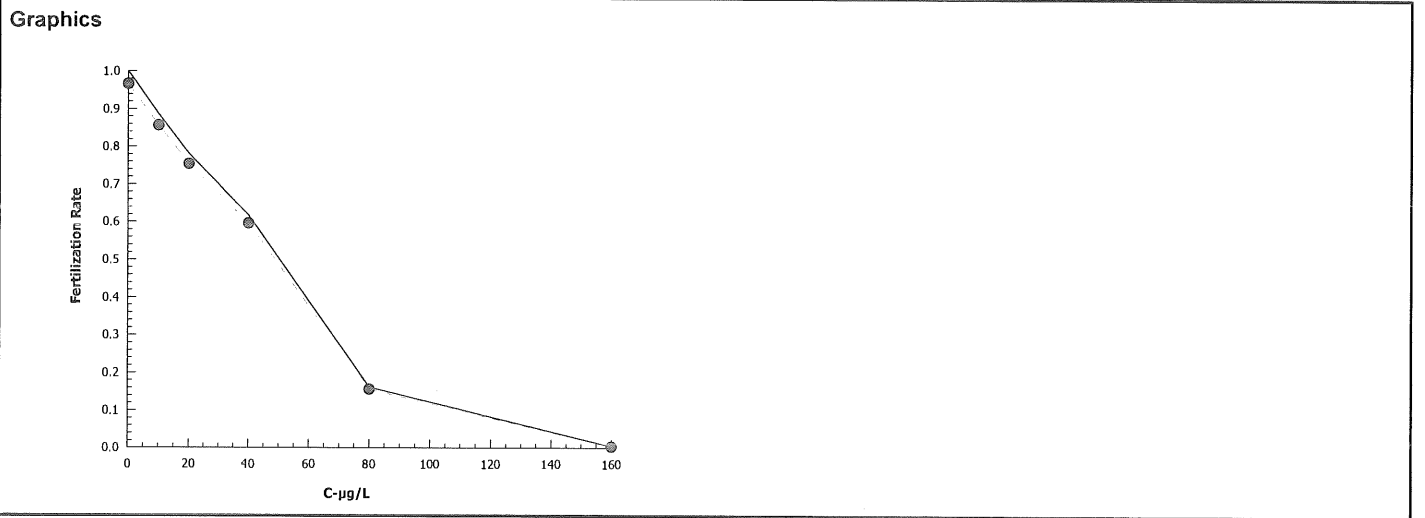
CETIS Analytical Report

Report Date: 30 Aug-17 16:01 (p 1 of 1)
 Test Code: 170828spt | 08-1525-2751

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Analysis ID:	10-7829-2432	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7		
Analyzed:	30 Aug-17 16:00	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes		

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.034	11.39%	1.619	0.01245	41.55	39.24	44

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	0.966	0.95	0.99	0.008124	0.01817	1.88%	0.0%	483	500
10		5	0.856	0.83	0.87	0.006782	0.01517	1.77%	11.39%	428	500
20		5	0.754	0.72	0.8	0.01435	0.03209	4.26%	21.95%	377	500
40		5	0.596	0.46	0.71	0.04238	0.09476	15.9%	38.3%	298	500
80		5	0.156	0.11	0.2	0.01503	0.03362	21.55%	83.85%	78	500
160		5	0.004	0	0.02	0.004	0.008944	223.6%	99.59%	2	500



Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

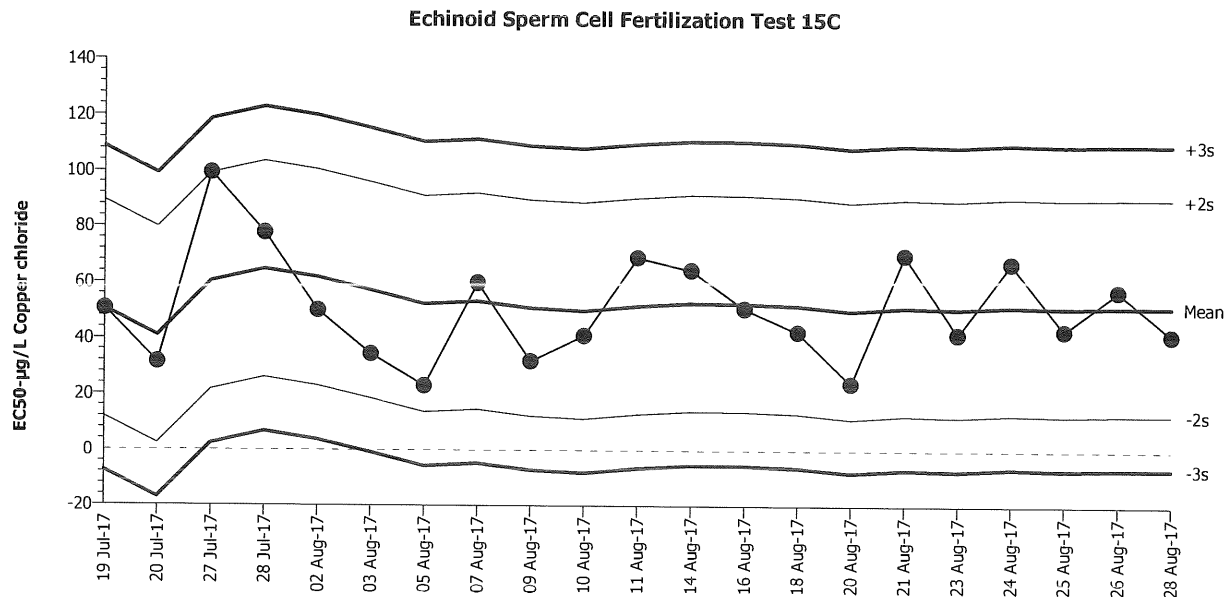
Organism: Strongylocentrotus purpuratus (Purpl

Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF



Mean: 51.49

Count: 20

-2s Warning Limit: 12.73

-3s Action Limit: -6.646

Sigma: 19.38

CV: 37.60%

+2s Warning Limit: 90.25

+3s Action Limit: 109.6

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jul	19	16:28	50.59	-0.9011	-0.04649			03-3446-7266	15-7259-8466
2			20	18:10	31.52	-19.97	-1.03			17-7484-2488	03-0485-5429
3			27	15:55	99.32	47.83	2.468	(+)		02-6715-3770	17-8186-2444
4			28	10:50	77.84	26.35	1.36			21-2559-1280	14-0688-6070
5		Aug	2	15:50	50.06	-1.435	-0.07404			08-9742-2478	08-8646-9232
6			3	0:00	34.43	-17.06	-0.8801			02-7356-2235	20-3051-4002
7			5	19:25	23.07	-28.42	-1.466			11-5994-0488	10-6029-2098
8			7	15:10	59.94	8.449	0.4359			21-2468-7505	14-3489-7019
9			9	17:08	31.92	-19.57	-1.01			13-6999-3036	11-7131-4234
10			10	16:51	41.14	-10.35	-0.5343			00-5471-5288	12-0643-2211
11			11	14:50	69.03	17.54	0.905			04-5796-5476	07-8184-6783
12			14	14:40	64.51	13.02	0.6716			02-4510-8526	01-5460-0814
13			16	16:34	50.82	-0.6731	-0.03473			16-3259-1018	06-7497-1035
14			18	14:09	42.53	-8.959	-0.4623			12-6613-4538	02-2322-5589
15			20	14:52	24.05	-27.44	-1.416			06-9655-0092	05-8785-3700
16			21	14:46	69.95	18.46	0.9524			08-4756-2919	20-2992-4955
17			23	16:14	41.72	-9.773	-0.5043			02-7595-3678	15-3490-2746
18			24	16:11	67.1	15.61	0.8056			04-7651-5518	20-0883-0005
19			25	14:48	43.11	-8.38	-0.4324			06-8816-1100	09-0830-4014
20			26	16:00	57.24	5.751	0.2967			10-2039-5656	15-8794-0305
21			28	14:56	41.55	-9.938	-0.5128			08-1525-2751	10-7829-2432

CETIS Test Data Worksheet

Report Date: 28 Aug-17 10:11 (p 1 of 1)
 Test Code: 08-1525-2751/170828sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 28 Aug-17 Species: Strongylocentrotus purpuratus
 End Date: 28 Aug-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 28 Aug-17 Material: Copper chloride

Sample Code: 170828sprt
 Sample Source: Reference Toxicant
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	96	8/30/17
			2	100	2	
			3	100	0	
			4	100	86	
			5	100	75	
			6	100	86	
			7	100	55	
			8	100	63	
			9	100	87	
			10	100	95	
			11	100	17	
			12	100	99	
			13	100	63	
			14	100	80	
			15	100	86	
			16	100	16	
			17	100	46	
			18	100	73	
			19	100	11	
			20	100	20	
			21	100	77	
			22	100	95	
			23	100	0	
			24	100	14	
			25	100	0	
			26	100	71	
			27	100	98	
			28	100	83	
			29	100	72	
			30	100	0	

CETIS Test Data Worksheet

Report Date: 28 Aug-17 10:11 (p 1 of 1)
 Test Code: 08-1525-2751/170828sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 28 Aug-17 Species: Strongylocentrotus purpuratus
 End Date: 28 Aug-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 28 Aug-17 Material: Copper chloride

Sample Code: 170828sprt
 Sample Source: Reference Toxicant
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	1			
0	LC	2	10	100	98	CG 8/28/17
0	LC	3	27			
0	LC	4	22			
0	LC	5	12			
10		1	9			
10		2	15			
10		3	4	100	88	
10		4	6			
10		5	28			
20		1	5			
20		2	29	100	79	
20		3	21			
20		4	18			
20		5	14			
40		1	13			
40		2	8			
40		3	17	100	47	
40		4	7			
40		5	26			
80		1	20			
80		2	11	100	15	
80		3	16			
80		4	24			
80		5	19			
160		1	3			
160		2	2			
160		3	30			
160		4	23			
160		5	25	100	0	

QC: CG

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 8/28/2017 1456Test No: 170828sprtEnd Date/Time: 8/28/2017 1536Dilutions made by: CG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10160

Analyst:

EG

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.8	8.13	33.7	15.1
10	7.5	8.12	33.7	15.2
20	7.6	8.13	33.6	15.1
40	7.5	8.12	33.6	15.3
80	7.6	8.12	33.4	15.2
160	7.6	8.13	33.2	15.2

Comments:

QC Check:

AC 8/30/17Final Review: KFP 9/6/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: Cu C12
 Test No.: 170828 sprt
 Tech initials: CG
 Injection Time: 1400

Start Date/Time: 8/28/2017 / 1456
 End Date/Time: 8/28/2017 / 1536
 Species: S. purpuratus
 Animal Source: Pl. Loma
 Date Collected: 8/22/17

Sperm Absorbance at 400 nm: 0.014 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 73 Mean: 70.4 X 50 = 3520 eggs/ml

61
67
79
72

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

Initial density: 3520 eggs/ml = 0.88 dilution factor
 Final density: 4000 eggs/ml - 1.0 part egg stock
-0.12 parts seawater
 egg stock 200 ml
 seawater -1224 ml
(A)

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>1418</u>	<u>50:1</u>	<u>35</u>	<u>25</u>
Eggs Added (0.5 ml):	<u>1438</u>	<u>100:1</u>	<u>89.89</u>	<u>11.11</u>
Test Ended:	<u>1448</u>	<u>100:1</u>	<u>99</u>	<u>1</u>
		<u>400:1</u>	<u>100</u>	<u>0</u>

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

Definitive Test Sperm:Egg Ratio Used: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1456</u>	QC1	<u>94</u>	<u>6</u>
Eggs Added (0.5 ml):	<u>1516</u>	QC2	<u>96</u>	<u>4</u>
Test Ended:	<u>1536</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: ACG 8/28/17

QC Check: AC 8/30/17

Final Review: YTP 9/6/17

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.