Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: November 16, 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 Cibar

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – NOVEMBER 2017

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

Sampling Date: November 16, 2017

Test Date: November 17, 2017

Sample ID: M-001 (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 November 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 November 17, 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 4º C in the dark until used for testing. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocols described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./Carlsbad Desalination Plant
Sample ID:	M-001 (off-spec period)
Monitoring Period:	November 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	11/16/17, 09:00
Sample Receipt Date, Time:	11/17/17, 11:17

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

Sample Collection Date	pH	DO (mg/L)	Temp (ºC)	Salinity (ppt)	Alkalinity (mg/L as $CaCO_3$)	Total Chlorine (mg/L)
11/16/17	7.70	9.6	2.4	32.2	105	0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	11/17/17, 14:17 through 14:57
Test Organism:	*Strongylocentrotus purpuratus* (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

Statistically significant decreases in fertilization rate were observed at the 10 and 15 percent effluent concentrations tested when compared to the lab control. The NOEC is reported as 6.06 and the TU_c is equal to 16.5, which meets effluent limitation of 16.5 for this permit. Statistically significant decreases were also observed in the 10 and 15 percent effluent concentrations 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	11.7	16.5	Pass	14.1

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
TU_c = Chronic Toxic Unit: 100÷NOEC
TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.
Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) *100. A negative PE results when organism performance in the sample is greater than that in the control.

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

Test Concentration (% Sample)	Mean Percent Fertilization
Lab Control	78.2
2.5	74.4
5.0	68.2
6.06	67.2
10	43.2*
15	31.2*

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

QUALITY ASSURANCE

The sample was received on the day after it was collected and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations reported are deemed reliable. Additionally, appropriate alpha and beta 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} **(µg/L Copper)**	Historical Mean EC_{50} ±2 SD **(µg/L Copper)**	CV (%)
11/17/17	24.0	49.3 ± 30.2	30.6

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

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

Batch ID:	07-5279-4491	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	17 Nov-17 14:17	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	17 Nov-17 14:57	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	
Sample ID:	07-4199-0632	**Code:**	17-1196	**Client:**	IDE
Sample Date:	16 Nov-17 09:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	17 Nov-17 11:17	**Source:**	IDE Americas, Inc.		
Sample Age:	29h (2.4 °C)	**Station:**	M-001 (Daily)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-4406-9614	Fertilization Rate	6.06	10	7.785	17.9%	16.5	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
00-0534-1983	Fertilization Rate	EC25	7.464	6.001	9.654	13.4	Linear Interpolation (ICPIN)
		EC50	11.71	7.781	15.43	8.541	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-0534-1983	Fertilization Rate	Control Resp	0.782	0.7 - NL	Yes	Passes Acceptability Criteria
07-4406-9614	Fertilization Rate	Control Resp	0.782	0.7 - NL	Yes	Passes Acceptability Criteria
07-4406-9614	Fertilization Rate	PMSD	0.1788	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	0.782	0.6825	0.8815	0.68	0.87	0.03583	0.08012	10.25%	0.0%
2.5		5	0.744	0.6166	0.8714	0.64	0.87	0.04589	0.1026	13.79%	4.86%
5		5	0.682	0.6121	0.7519	0.6	0.75	0.02518	0.0563	8.26%	12.79%
6.06		5	0.672	0.6043	0.7397	0.61	0.75	0.02437	0.0545	8.11%	14.07%
10		5	0.432	0.2356	0.6284	0.2	0.57	0.07074	0.1582	36.62%	44.76%
15		5	0.312	0.1998	0.4242	0.17	0.4	0.04042	0.09039	28.97%	60.1%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.8	0.68	0.87	0.84	0.72
2.5		0.64	0.64	0.81	0.87	0.76
5		0.66	0.6	0.71	0.75	0.69
6.06		0.75	0.66	0.7	0.61	0.64
10		0.35	0.57	0.57	0.47	0.2
15		0.4	0.28	0.35	0.36	0.17

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

Analysis ID:	07-4406-9614	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Nov-17 15:56	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	17.9%	6.06	10	7.785	16.5

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.6351	2.362	0.161	8	0.5867	CDF	Non-Significant Effect
		5	1.727	2.362	0.161	8	0.1583	CDF	Non-Significant Effect
		6.06	1.885	2.362	0.161	8	0.1216	CDF	Non-Significant Effect
		10*	5.551	2.362	0.161	8	<0.0001	CDF	Significant Effect
		15*	7.373	2.362	0.161	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.9939312	0.1987862	5	17.19	<0.0001	Significant Effect
Error	0.2775315	0.01156381	24			
Total	1.271463		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.66	15.09	0.3407	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9704	0.9031	0.5492	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	0.782	0.6825	0.8815	0.8	0.68	0.87	0.03583	10.25%	0.0%
2.5		5	0.744	0.6166	0.8714	0.76	0.64	0.87	0.04589	13.79%	4.86%
5		5	0.682	0.6121	0.7519	0.69	0.6	0.75	0.02518	8.26%	12.79%
6.06		5	0.672	0.6043	0.7397	0.66	0.61	0.75	0.02437	8.11%	14.07%
10		5	0.432	0.2356	0.6284	0.47	0.2	0.57	0.07074	36.62%	44.76%
15		5	0.312	0.1998	0.4242	0.35	0.17	0.4	0.04042	28.97%	60.1%

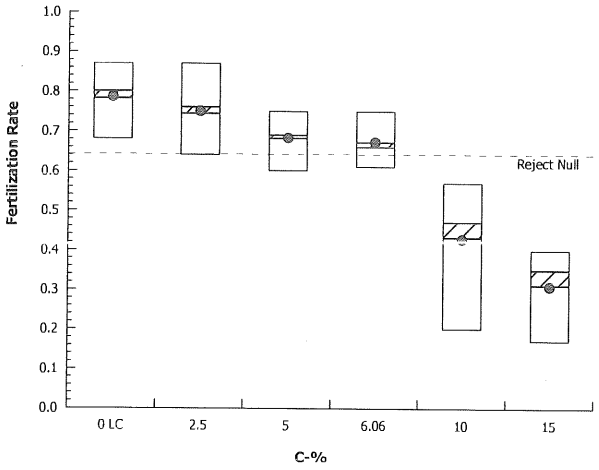
Angular (Corrected) Transformed Summary

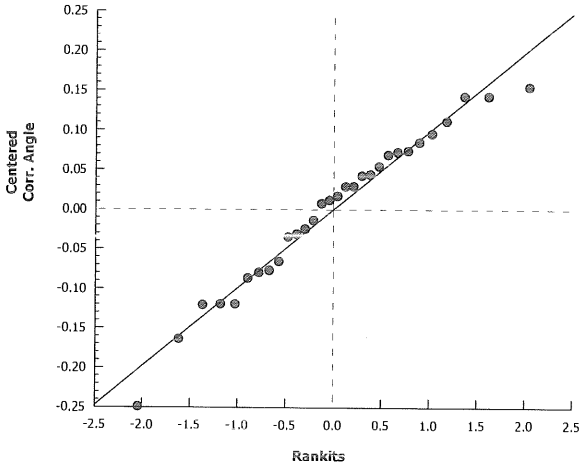
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.09	0.9691	1.211	1.107	0.9695	1.202	0.04361	8.94%	0.0%
2.5		5	1.047	0.8974	1.197	1.059	0.9273	1.202	0.0539	11.51%	3.96%
5		5	0.9728	0.8978	1.048	0.9803	0.8861	1.047	0.02699	6.21%	10.77%
6.06		5	0.962	0.8891	1.035	0.9483	0.8963	1.047	0.02628	6.11%	11.76%
10		5	0.7127	0.5058	0.9196	0.7554	0.4636	0.8556	0.07452	23.38%	34.63%
15		5	0.5888	0.4616	0.7159	0.6331	0.425	0.6847	0.0458	17.4%	46.0%

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

Analysis ID:	07-4406-9614	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Nov-17 15:56	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1711-S105 | 15-2777-7855

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

Analysis ID:	00-0534-1983	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Nov-17 15:56	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2022734	1000	Yes	Two-Point Interpolation

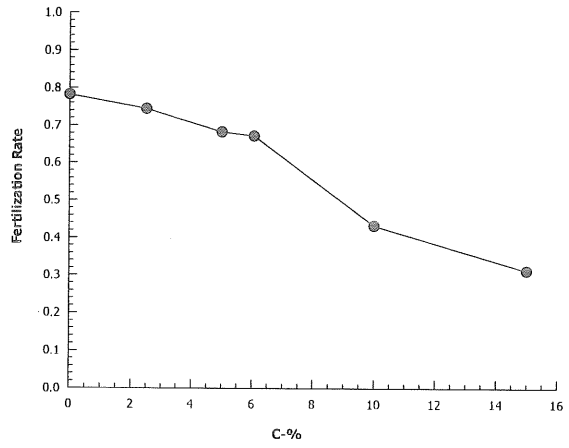
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	7.464	6.001	9.654	13.4	10.36	16.67
EC50	11.71	7.781	15.43	8.541	6.48	12.85

Fertiiization 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	0.782	0.68	0.87	0.03583	0.08012	10.25%	0.0%	391	500
2.5		5	0.744	0.64	0.87	0.04589	0.1026	13.79%	4.86%	372	500
5		5	0.682	0.6	0.75	0.02518	0.0563	8.26%	12.79%	341	500
6.06		5	0.672	0.61	0.75	0.02437	0.0545	8.11%	14.07%	336	500
10		5	0.432	0.2	0.57	0.07074	0.1582	36.62%	44.76%	216	500
15		5	0.312	0.17	0.4	0.04042	0.09039	28.97%	60.1%	156	500

Graphics



Analyst: _____ QA: AC 11/30/17

CETIS Analytical Report

TST

Report Date: 27 Nov-17 15:56 (p 1 of 1)
Test Code: 1711-S105 | 15-2777-7855

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

Analysis ID:	00-6410-5930	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Nov-17 15:56	**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	11.4%	6.06	10	7.785	16.5

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	3.638	1.943	0.123	6	0.0054	CDF	Non-Significant Effect
		5*	3.658	1.895	0.080	7	0.0040	CDF	Non-Significant Effect
		6.06*	3.441	1.895	0.08	7	0.0054	CDF	Non-Significant Effect
		10	-1.29	2.015	0.164	5	0.8733	CDF	Significant Effect
		15	-4.067	1.895	0.107	7	0.9976	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.9939312	0.1987862	5	17.19	<0.0001	Significant Effect
Error	0.2775315	0.01156381	24			
Total	1.271463		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.66	15.09	0.3407	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9704	0.9031	0.5492	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	0.782	0.6825	0.8815	0.8	0.68	0.87	0.03583	10.25%	0.0%
2.5		5	0.744	0.6166	0.8714	0.76	0.64	0.87	0.04589	13.79%	4.86%
5		5	0.682	0.6121	0.7519	0.69	0.6	0.75	0.02518	8.26%	12.79%
6.06		5	0.672	0.6043	0.7397	0.66	0.61	0.75	0.02437	8.11%	14.07%
10		5	0.432	0.2356	0.6284	0.47	0.2	0.57	0.07074	36.62%	44.76%
15		5	0.312	0.1998	0.4242	0.35	0.17	0.4	0.04042	28.97%	60.1%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.09	0.9691	1.211	1.107	0.9695	1.202	0.04361	8.94%	0.0%
2.5		5	1.047	0.8974	1.197	1.059	0.9273	1.202	0.0539	11.51%	3.96%
5		5	0.9728	0.8978	1.048	0.9803	0.8861	1.047	0.02699	6.21%	10.77%
6.06		5	0.962	0.8891	1.035	0.9483	0.8963	1.047	0.02628	6.11%	11.76%
10		5	0.7127	0.5058	0.9196	0.7554	0.4636	0.8556	0.07452	23.38%	34.63%
15		5	0.5888	0.4616	0.7159	0.6331	0.425	0.6847	0.0458	17.4%	46.0%

Analyst: _____ QA: 11/30/17

CETIS Test Data Worksheet

Report Date: 16 Nov-17 10:53 (p 1 of 1)
Test Code: 15-2777-7855/~~5B100A3F~~ 1711-5105

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

Start Date: 17 Nov-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 17- 1196
End Date: 17 Nov-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 16 Nov-17 | **Material:** Facility Effluent | **Sample Station:** M-001 (Daily) (11/16 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	57	Read JC 11/20/17
			32	100	17	
			33	100	75	
			34	100	66	
			35	100	35	
			36	100	81	
			37	100	(B) ~~50~~ 69	
			38	100	47	
			39	100	36	
			40	100	70	
			41	100	76	
			42	100	84	
			43	100	66	
			44	100	57	
			45	100	58	
			46	100	40	
			47	100	(B) ~~32~~ 64	
			48	100	87	
			49	100	70	
			50	100	(B) ~~45~~ 60	
			51	100	71	
			52	100	87	
			53	100	75	
			~~54~~	100	(B) ~~56~~ 61	
			55	100	64	
			56	100	80	
			57	100	78	
			58	100	35	
			59	100	72	
			60	100	64	

~~READ~~ Q18 11/27/17

(B) Q18 AE 11/27/17. JC swirl & re-count.

CETIS Test Data Worksheet

Report Date: 16 Nov-17 10:53 (p 1 of 1)
Test Code: 15-2777-7855/5B100A3F (A 1711-3105)

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

Start Date: 17 Nov-17
End Date: 17 Nov-17
Sample Date: 16 Nov-17

Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent

Sample Code: 17- 11960
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (11/16/17 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	56	100	84	RT 11/17/17
0	LC	2	45	100	75	
0	LC	3	52	100	79	
0	LC	4	42	100	69	
0	LC	5	59	100	81	
2.5		1	55			
2.5		2	60			
2.5		3	36			
2.5		4	48	100	78	RT 11/17/17
2.5		5	41			
5		1	43			
5		2	50			
5		3	51			
5		4	33	100	65	RT 11/17/17
5		5	37			
6.06		1	53			
6.06		2	34			
6.06		3	40			
6.06		4	54	100	60	RT 11/17/17
6.06		5	47			
10		1	35			
10		2	31			
10		3	44			
10		4	38			
10		5	49	100	39	RT 11/17/17
15		1	46			
15		2	57			
15		3	58			
15		4	39	100	30	RT 11/17/17
15		5	32			

QC-CG

EAD @ 8 11/27/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE | **Test Species:** *S. purpuratus*

Sample ID: M-001 Daily (11/16 sample) | **Start Date/Time:** 11/17/2017 1417

Sample Log No.: 17- 1196 | **End Date/Time:** 11/17/2017 1457

Dilutions made by: CG | **Test No:** 1711-S105

Analyst: CG

	Initial Readings			
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.0	8.01	33.9	16.0
2.5	8.0	8.01	34.0	15.8
5.0	8.0	8.00	33.9	15.9
6.06	8.0	8.00	33.9	16.0
10	8.0	7.99	33.8	15.8
15	8.0	7.98	33.7	15.6

Comments: (B) CG 11/17/17

QC Check: AM 11/27/17 | **Final Review:** AC 11/30/17

Marine Chronic Bioassay **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: IDE
Sample ID: M-001 Daily (11/16 sample)
Test No.: 1711-S105

Start Date/Time: 11/17/17 / 1417
End Date/Time: 11/17/17 / 1457
Species: S. purpuratus
Animal Source: Pt. Loma
Date Collected: 9/28/17, 10/16/17

Tech initials: CG
Injection Time: 1325

Sperm Absorbance at 400 nm: 0.942 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 71, 72, 70, 78, 77 Mean: 73.6 X 50 = 3680 eggs/ml

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

Initial density:	3680 eggs/ml	=	0.92	dilution factor	egg stock	(A) ml
Final density:	4000 eggs/ml	-	1.0	part egg stock	seawater	(A) ml
		+	0.08	parts seawater		

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

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1341	50:1	73	27
Eggs Added (0.5 ml):	1354	100:1	80.75	20.25
Test Ended:	(B) 1404	200:1	92.93	8.7
		—	—	—

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: 200:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1417	QC1	85	15
Eggs Added (0.5 ml):	1437	QC2	86	14
Test Ended:	1457	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) NO dilution necessary (B) CG QC 11/17/17

QC Check: AC 11/27/17 Final Review: AC 11/30/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Daily M-001 (11/16 sample)
Test ID No(s).: 1711-S105

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1196			
Sample Collection Date & Time:	11/16/17 0900			
Sample Receipt Date & Time:	11/17/17 1117			
Number of Containers & Container Type:	1-4L cubi			
Approx. Total Volume Received (L):	~4L			
Check-in Temperature (°C)	2.4			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.6			
pH (units)	7.70			
Conductivity (µS/cm)	—			
Salinity (ppt)	32.2			
Alkalinity (mg/L) [2]	105			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	AB			

Test Performed: Urchin Fert. **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 109 Hardness or Salinity: 34ppt
Additional Control? Y (N) = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: [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: _____

Sample Check-In Information

Sample Description:
(A) No color, clear, no odor, no debris

COC Complete (Y/N)?
A Y B___ C___

Filtration? Y (N)
Pore Size: _____
Organisms or Debris

Salinity Adjustment? Y (N)
Test: Source: Target ppt:
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

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

Cl_2 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: EG 11/21/17
Final Review: AC 11/30/17

Appendix C

Chain-of-Custody Form

DAILY

IDE Technologies CDP Laboratories

Page 1 of _____

CDP laoratory:_____
Entahlpy Laboratory:___
WECK Laboratory:_____
Nautilus:___x_____
AIM:_____
Other:_____

Turn Around Time
Normal:___x_____
RUSH (24 hr):_____
3 Days:_____
5 Days:_____
??? Days_____

Project Name:___NPDES Daily Toxicity___ Project Manager:___Peter Shen___ Contact Information:___(760) 201-7777___

Special instruction: Sampled during off-spec via autosampler by a series of grabs collected at one hour intervals. Sample collected to fulfill daily NPDES requirement. Sample is to be run unadjusted. Start: 11/15/17 @ 09:00, End: 11/16/17 @ 09:00 KC

Glass=G Plastic=P

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

Drinking Water=DW Seawater=SW Soil=S

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization								NOTES:
M-001 (17- 3305)	11/15-16/17	09:00-09:00	SW	N	4L CUBIE	X								TDS - 30.36 ppt, EC - 47.43 mS/cm

Receiving Temp. (°C) 2.4

Relinquished By:	Date:	Time:		Received By:	Date:	Time:	Sample Condition Upon Receipt:
[signature]	11/17/17	1030		[signature]	11-17	1038	[X] Iced [ ] Ambient or _____ °C
[signature]	11/17/17	1117		[signature]	11/17/17 1117		[ ] Iced [ ] Ambient or _____ °C

@ 13 AB 11/17/17

Nautilus ID: 17-1196

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	14-0774-0141	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	17 Nov-17 14:17	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	17 Nov-17 14:57	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	17-1170-1480	**Code:**	171117sprt	**Client:**	Internal
Sample Date:	17 Nov-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	17 Nov-17	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-4784-0781	Fertilization Rate	<10	10	NA	10.1%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
00-9691-5869	Fertilization Rate	EC50	24.03	20.15	28.65		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
00-9691-5869	Fertilization Rate	Control Resp	0.826	0.7 - NL	Yes	Passes Acceptability Criteria
18-4784-0781	Fertilization Rate	Control Resp	0.826	0.7 - NL	Yes	Passes Acceptability Criteria
18-4784-0781	Fertilization Rate	PMSD	0.1013	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	0.826	0.779	0.873	0.79	0.87	0.01691	0.03782	4.58%	0.0%
10		5	0.502	0.4014	0.6026	0.43	0.64	0.03625	0.08106	16.15%	39.23%
20		5	0.456	0.3584	0.5536	0.36	0.57	0.03516	0.07861	17.24%	44.79%
40		5	0.332	0.2943	0.3697	0.29	0.36	0.01356	0.03033	9.14%	59.81%
80		5	0.044	0.004151	0.08385	0	0.08	0.01435	0.03209	72.94%	94.67%
160		5	0	0	0	0	0	0	0		100.0%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.79	0.79	0.86	0.87	0.82
10		0.49	0.43	0.49	0.46	0.64
20		0.49	0.43	0.36	0.43	0.57
40		0.35	0.35	0.36	0.31	0.29
80		0.04	0.03	0.08	0	0.07
160		0	0	0	0	0

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

Analysis ID:	18-4784-0781	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 10:48	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.1%	<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*	7.865	2.305	0.104	8	<0.0001	CDF	Significant Effect
		20*	8.902	2.305	0.104	8	<0.0001	CDF	Significant Effect
		40*	11.72	2.305	0.104	8	<0.0001	CDF	Significant Effect
		80*	20.99	2.305	0.104	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.33185	0.5829624	4	114.7	<0.0001	Significant Effect
Error	0.1016093	0.005080464	20			
Total	2.433459		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.452	13.28	0.3482	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9823	0.8877	0.9269	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	0.826	0.779	0.873	0.82	0.79	0.87	0.01691	4.58%	0.0%
10		5	0.502	0.4014	0.6026	0.49	0.43	0.64	0.03625	16.15%	39.23%
20		5	0.456	0.3584	0.5536	0.43	0.36	0.57	0.03516	17.24%	44.79%
40		5	0.332	0.2943	0.3697	0.35	0.29	0.36	0.01356	9.14%	59.81%
80		5	0.044	0.004151	0.08385	0.04	0	0.08	0.01435	72.94%	94.67%
160		5	0	0	0	0	0	0	0		100.0%

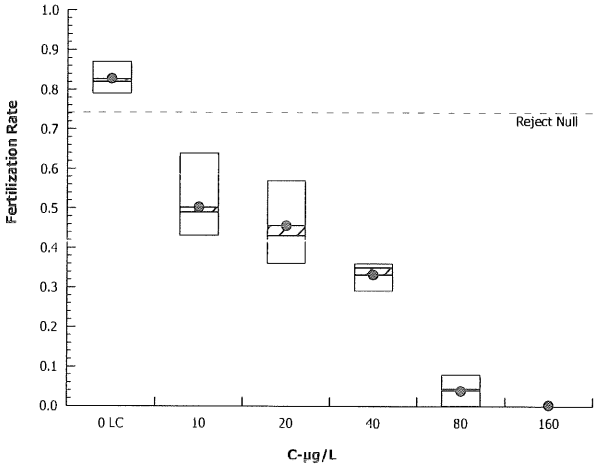
Angular (Corrected) Transformed Summary

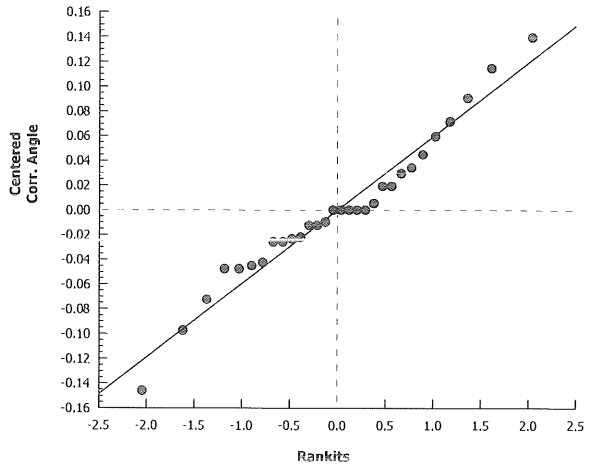
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.142	1.08	1.205	1.133	1.095	1.202	0.02258	4.42%	0.0%
10		5	0.7877	0.686	0.8894	0.7754	0.7152	0.9273	0.03664	10.4%	31.04%
20		5	0.741	0.6425	0.8395	0.7152	0.6435	0.8556	0.03547	10.7%	35.13%
40		5	0.6138	0.5735	0.654	0.6331	0.5687	0.6435	0.0145	5.28%	46.27%
80		5	0.196	0.07951	0.3125	0.2014	0.05002	0.2868	0.04196	47.87%	82.84%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.62%

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

Analysis ID:	18-4784-0781	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 10:48	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: _____ QA: AC 11/27/17

CETIS Analytical Report

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

Analysis ID:	00-9691-5869	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 10:48	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

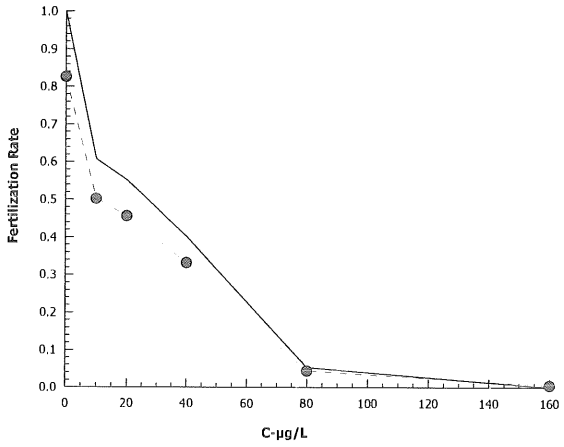
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.174	39.23%	1.381	0.03819	24.03	20.15	28.65

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	0.826	0.79	0.87	0.01691	0.03782	4.58%	0.0%	413	500
10		5	0.502	0.43	0.64	0.03625	0.08106	16.15%	39.23%	251	500
20		5	0.456	0.36	0.57	0.03516	0.07861	17.24%	44.79%	228	500
40		5	0.332	0.29	0.36	0.01356	0.03033	9.14%	59.81%	166	500
80		5	0.044	0	0.08	0.01435	0.03209	72.94%	94.67%	22	500
160		5	0	0	0	0	0		100.0%	0	500

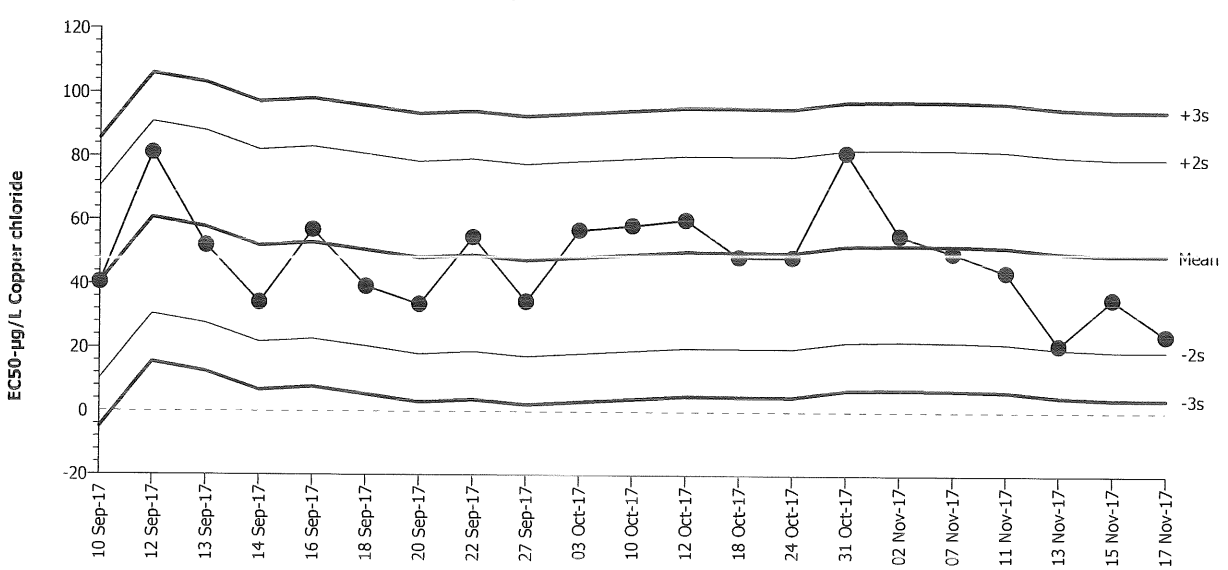
Graphics



Analyst: _____ QA: AE 11/21/17

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:	49.29	**Count:**	20	**-2s Warning Limit:**	19.11	**-3s Action Limit:**	4.025
Sigma:	15.09	**CV:**	30.60%	**+2s Warning Limit:**	79.47	**+3s Action Limit:**	94.56

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Sep	10	14:25	40.4	-8.888	-0.589			11-6871-9499	08-4248-1228
2			12	15:51	81.07	31.78	2.106	(+)		20-0603-9450	06-1182-7961
3			13	19:07	52.04	2.746	0.182			01-4575-6189	02-4618-7964
4			14	15:24	34.24	-15.05	-0.9977			11-2846-3680	13-8128-7168
5			16	17:08	56.97	7.68	0.509			08-9569-1329	19-6375-1112
6			18	15:28	39.21	-10.08	-0.6682			19-2924-5672	02-0031-2532
7			20	16:15	33.62	-15.67	-1.038			00-4454-0074	17-7214-1415
8			22	14:50	54.61	5.319	0.3525			20-3341-5102	16-2759-7635
9			27	15:34	34.46	-14.83	-0.9825			12-3257-1101	06-9840-2290
10		Oct	3	13:49	56.88	7.589	0.5029			05-1137-7792	06-0895-0170
11			10	15:10	58.36	9.072	0.6012			20-5863-5053	00-1542-1738
12			12	14:55	60.18	10.89	0.7218			05-0863-6526	07-1531-2424
13			18	14:22	48.53	-0.7596	-0.05034			13-0042-6212	05-6771-5532
14			24	13:15	48.41	-0.8847	-0.05863			20-0280-7301	18-5464-1899
15			31	13:59	81.36	32.07	2.125	(+)		06-4227-6723	08-8095-0809
16		Nov	2	12:28	55.32	6.035	0.3999			17-4126-1689	20-0626-8382
17			7	14:30	49.87	0.5832	0.03865			10-3521-2857	13-9801-3995
18			11	14:25	43.91	-5.382	-0.3566			14-1655-2339	20-5239-6070
19			13	14:35	20.97	-28.32	-1.877			07-0538-7056	00-9105-4737
20			15	16:09	35.48	-13.81	-0.915			06-3476-9418	17-5783-9769
21			17	14:17	24.03	-25.26	-1.674			20-8374-1268	00-9691-5869

CETIS Test Data Worksheet

Report Date: 16 Nov-17 10:56 (p 1 of 1)
Test Code: 20-8374-1268/171117sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 17 Nov-17
End Date: 17 Nov-17
Sample Date: 17 Nov-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 171117sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	Read by JC 11/20/17
			2	100	29	
			3	100	79	
			4	100	49	
			5	100	0	
			6	100	0	
			7	100	57	
			8	100	86	
			9	100	87	
			10	100	79	
			11	100	35	
			12	100	0	
			13	100	31	
			14	100	82	
			15	100	36	
			16	100	43	
			17	100	4	
			18	100	36	
			19	100	46	
			20	100	49	
			21	100	49	
			22	100	43	
			23	100	0	
			24	100	8	
			25	100	35	
			26	100	3	
			27	100	7	
			28	100	64	
			29	100	0	
			30	100	43	

CETIS Test Data Worksheet

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

Start Date: 17 Nov-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 171117sprt
End Date: 17 Nov-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 17 Nov-17 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	10	100	83	CG 11/17/17
0	LC	2	3	100	88	
0	LC	3	8	100	91	
0	LC	4	9	100	88	
0	LC	5	14	100	86	
10		1	21			
10		2	30	100	~~47~~ 51	
10		3	20			
10		4	19			
10		5	28	100	52	
20		1	4	100	53	
20		2	22			
20		3	18			
20		4	16			
20		5	7	100	52	
40		1	25			
40		2	11	100	35	
40		3	15	100	41	
40		4	13	100	30	
40		5	2			
80		1	17			
80		2	26			
80		3	24	100	7	
80		4	23			
80		5	27			
160		1	6			
160		2	29			
160		3	5			
160		4	1	100	0	
160		5	12			

QC: CG

(A) CG QC 11/17/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : Internal | **Test Species:** *S. purpuratus*

Sample ID: $CuCl_2$ | **Start Date/Time:** 11/17/2017 1417

Test No: 171117sprt | **End Date/Time:** 11/17/2017 1457

Dilutions made by: CG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.3
Final Volume (mL):	500
Cu stock concentration (µg/L):	9600

Analyst: CG

	Initial Readings			
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.0	7.99	33.8 ~~33.7~~ (A)	15.9
10	8.1	7.98	33.8	15.9
20	8.0	7.98	33.8	15.8
40	7.9	7.99	33.8	16.0
80	8.0	7.99	33.6	15.9
160	8.0	7.99	33.5	16.0

Comments: (A) CG QA'd 11/17/17

QC Check: EG 11/20/17 | **Final Review:** AC 11/27/17

Marine Chronic Bioassay | **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Internal
Sample ID: CuCl2
Test No.: 171117spcf

Start Date/Time: 11/17/17 / 1417
End Date/Time: 11/17/17 / 1457
Species: S. purpuratus
Animal Source: Pt. Loma
Date Collected: 9/28/17, 10/26/17

Tech initials: CG
Injection Time: 1325

Sperm Absorbance at 400 nm: 0.942 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 71, 72, 70, 78, 77 Mean: 73.6 X 50 = 3680 eggs/ml

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

Initial density: 3680 eggs/ml = 0.92 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
+ 0.08 parts seawater

egg stock (A) ml
seawater (A) 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).

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1341	50:1	73	27
Eggs Added (0.5 ml):	1354	100:1	80.75	20,25
Test Ended:	(B) 1404	200:1	92,93	8,7

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: 200:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1417	QC1	85	15
Eggs Added (0.5 ml):	1437	QC2	86	14
Test Ended:	1457	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) NO dilution necessary (B) CG QC 11/17/17

QC Check: EG 11/20/17 Final Review: AZ 11/27/17

Appendix E

Qualifier Codes

Nautilus Environmental

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.