Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: November 15, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **November 29, 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 15, 2017**

Test Date: **November 15, 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	
	5	20	No

INTRODUCTION

A discharge sample was collected in November 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for daily and weekly 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 15, 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 (pre-treatment off-spec period)
Monitoring Period:	November 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	11/15/17, 09:00
Sample Receipt Date, Time:	11/15/17, 13:43

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)
11/15/17	7.92	9.1	3.5	34.0	130	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	11/15/17, 16:09 through 16:49
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 6.06, 10, and 15 percent effluent concentrations tested when compared to the lab control. The NOEC is reported as 5 and the TU_c is equal to 20, which is above the maximum effluent limitation of 16.5 for this permit. No statistically significant decreases were observed in any of the 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	5	6.06	>15	20	Pass	9.5

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	88.4
2.5	88.2
5.0	85.6
6.06	80.0*
10	80.4*
15	76.6*

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

QUALITY ASSURANCE

The sample was received on the same day 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 less than two standard deviations (2SD) of the historical mean for our laboratory, indicating organisms were more sensitive to copper than typical. 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 (%)
11/15/17	35.5	49.4 ± 30.0	30.3

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

Test Code: 1711-S100 | 19-4490-5136

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

Batch ID:	01-6848-5082	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Nov-17 16:09	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	15 Nov-17 16:49	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	04-4131-0805	**Code:**	17-1184	**Client:**	IDE
Sample Date:	15 Nov-17 09:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	15 Nov-17 13:43	**Source:**	IDE Americas, Inc.		
Sample Age:	7h (3.5 °C)	**Station:**	M-001 (Daily)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-6467-2366	Fertilization Rate	5	6.06	5.505	6.53%	20	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
04-5621-6871	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
04-5621-6871	Fertilization Rate	Control Resp	0.884	0.7 - NL	Yes	Passes Acceptability Criteria
11-6467-2366	Fertilization Rate	Control Resp	0.884	0.7 - NL	Yes	Passes Acceptability Criteria
11-6467-2366	Fertilization Rate	PMSD	0.06535	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	0.884	0.8472	0.9208	0.85	0.93	0.01327	0.02966	3.36%	0.0%
2.5		5	0.882	0.8222	0.9418	0.82	0.95	0.02154	0.04817	5.46%	0.23%
5		5	0.856	0.809	0.903	0.83	0.92	0.01691	0.03782	4.42%	3.17%
6.06		5	0.8	0.7737	0.8263	0.77	0.82	0.009487	0.02121	2.65%	9.5%
10		5	0.804	0.7661	0.8419	0.76	0.84	0.01364	0.0305	3.79%	9.05%
15		5	0.766	0.6907	0.8413	0.68	0.84	0.02713	0.06066	7.92%	13.35%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.93	0.87	0.85	0.88	0.89
2.5		0.88	0.9	0.86	0.95	0.82
5		0.84	0.83	0.92	0.86	0.83
6.06		0.82	0.82	0.79	0.77	0.8
10		0.82	0.76	0.79	0.81	0.84
15		0.68	0.84	0.8	0.77	0.74

Analyst: _____ QA: AC 11/28/17

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

Analysis ID: 11-6467-2366 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 20 Nov-17 11:04 **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	6.53%	5	6.06	5.505	20

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.0194	2.362	0.085	8	0.8390	CDF	Non-Significant Effect
		5	1.146	2.362	0.085	8	0.3567	CDF	Non-Significant Effect
		6.06*	3.288	2.362	0.085	8	0.0066	CDF	Significant Effect
		10*	3.135	2.362	0.085	8	0.0094	CDF	Significant Effect
		15*	4.378	2.362	0.085	8	0.0005	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1121047	0.02242094	5	6.964	0.0004	Significant Effect
Error	0.07727107	0.003219628	24			
Total	0.1893758		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.271	15.09	0.3837	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9642	0.9031	0.3950	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	0.884	0.8472	0.9208	0.88	0.85	0.93	0.01327	3.36%	0.0%
2.5		5	0.882	0.8222	0.9418	0.88	0.82	0.95	0.02154	5.46%	0.23%
5		5	0.856	0.809	0.903	0.84	0.83	0.92	0.01691	4.42%	3.17%
6.06		5	0.8	0.7737	0.8263	0.8	0.77	0.82	0.009487	2.65%	9.5%
10		5	0.804	0.7661	0.8419	0.81	0.76	0.84	0.01364	3.79%	9.05%
15		5	0.766	0.6907	0.8413	0.77	0.68	0.84	0.02713	7.92%	13.35%

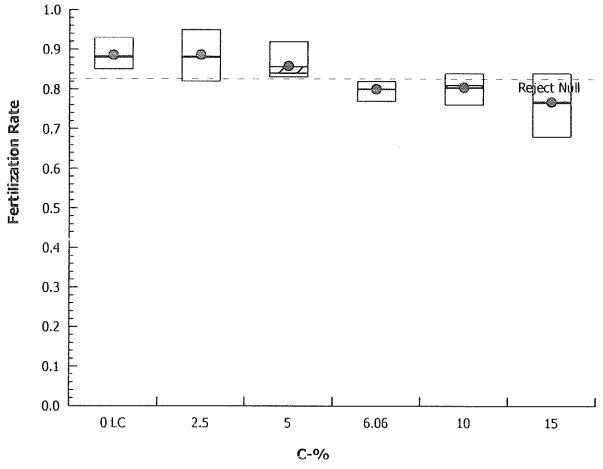
Angular (Corrected) Transformed Summary

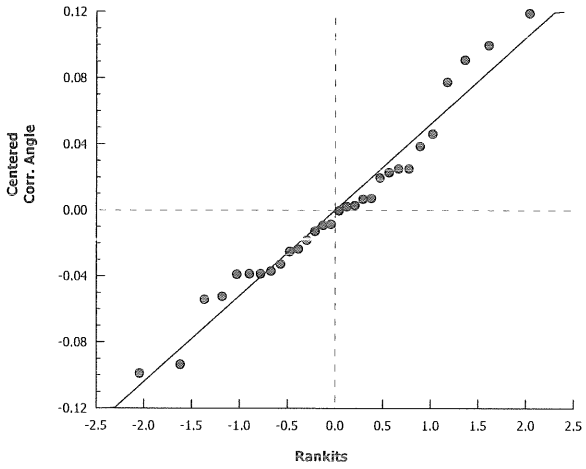
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.226	1.165	1.286	1.217	1.173	1.303	0.02172	3.96%	0.0%
2.5		5	1.226	1.128	1.325	1.217	1.133	1.345	0.03539	6.45%	-0.06%
5		5	1.184	1.112	1.257	1.159	1.146	1.284	0.02603	4.91%	3.36%
6.06		5	1.108	1.075	1.14	1.107	1.071	1.133	0.01181	2.38%	9.63%
10		5	1.113	1.066	1.161	1.12	1.059	1.159	0.01709	3.43%	9.18%
15		5	1.068	0.9793	1.158	1.071	0.9695	1.159	0.0321	6.72%	12.82%

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

Analysis ID:	11-6467-2366	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 11:04	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: _____ QA: 11/28/17

CETIS Analytical Report

Test Code: 1711-S100 | 19-4490-5136

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

Analysis ID:	04-5621-6871	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 11:04	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1908351	1000	Yes	Two-Point Interpolation

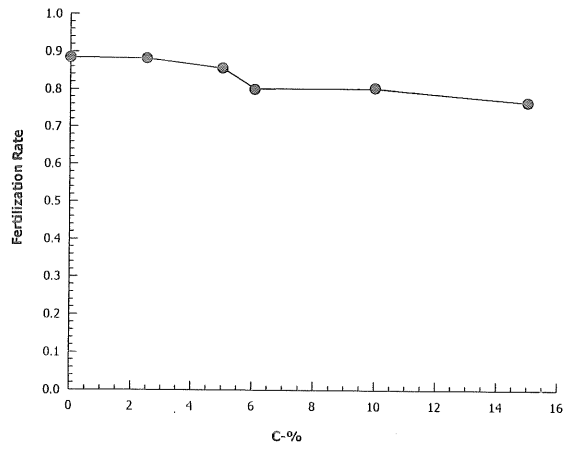
Point Estimates

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	0.884	0.85	0.93	0.01327	0.02966	3.36%	0.0%	442	500
2.5		5	0.882	0.82	0.95	0.02154	0.04817	5.46%	0.23%	441	500
5		5	0.856	0.83	0.92	0.01691	0.03782	4.42%	3.17%	428	500
6.06		5	0.8	0.77	0.82	0.009487	0.02121	2.65%	9.5%	400	500
10		5	0.804	0.76	0.84	0.01364	0.0305	3.79%	9.05%	402	500
15		5	0.766	0.68	0.84	0.02713	0.06066	7.92%	13.35%	383	500

Graphics



Analyst: _____ QA: 11/28/17

CETIS Analytical Report

TST

Test Code: 1711-S100 | 19-4490-5136

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

Analysis ID:	18-6010-1403	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 11:04	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	5.5%	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*	7.882	2.015	0.079	5	0.0003	CDF	Non-Significant Effect
		5*	8.64	1.943	0.06	6	<0.0001	CDF	Non-Significant Effect
		6.06*	9.364	1.895	0.038	7	<0.0001	CDF	Non-Significant Effect
		10*	8.212	1.895	0.045	7	<0.0001	CDF	Non-Significant Effect
		15*	4.147	2.015	0.073	5	0.0045	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1121047	0.02242094	5	6.964	0.0004	Significant Effect
Error	0.07727107	0.003219628	24			
Total	0.1893758		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.271	15.09	0.3837	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9642	0.9031	0.3950	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	0.884	0.8472	0.9208	0.88	0.85	0.93	0.01327	3.36%	0.0%
2.5		5	0.882	0.8222	0.9418	0.88	0.82	0.95	0.02154	5.46%	0.23%
5		5	0.856	0.809	0.903	0.84	0.83	0.92	0.01691	4.42%	3.17%
6.06		5	0.8	0.7737	0.8263	0.8	0.77	0.82	0.009487	2.65%	9.5%
10		5	0.804	0.7661	0.8419	0.81	0.76	0.84	0.01364	3.79%	9.05%
15		5	0.766	0.6907	0.8413	0.77	0.68	0.84	0.02713	7.92%	13.35%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.226	1.165	1.286	1.217	1.173	1.303	0.02172	3.96%	0.0%
2.5		5	1.226	1.128	1.325	1.217	1.133	1.345	0.03539	6.45%	-0.06%
5		5	1.184	1.112	1.257	1.159	1.146	1.284	0.02603	4.91%	3.36%
6.06		5	1.108	1.075	1.14	1.107	1.071	1.133	0.01181	2.38%	9.63%
10		5	1.113	1.066	1.161	1.12	1.059	1.159	0.01709	3.43%	9.18%
15		5	1.068	0.9793	1.158	1.071	0.9695	1.159	0.0321	6.72%	12.82%

Analyst: JH QA: AK 11/28/17

CETIS Test Data Worksheet

Test Code: 1711-5100 19-4490-5136/73ECE5B0

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

Start Date: 15 Nov-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 17- 1184
End Date: 15 Nov-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 15 Nov-17 **Material:** Facility Effluent **Sample Station:** M-001 (Daily) (11/15 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	84	11/16/17
			62	100	88	
			63	100	74	
			64	100	82	
			65	100	96	
			66	100	92	
			67	100	87	
			68	100	93	
			69	100	95	
			70	100	89	
			71	100	81	
			72	100	82	
			73	100	68	
			74	100	77	
			75	100	80	
			76	100	85	
			77	100	84	
			78	100	79	
			79	100	88	
			80	100	79	
			81	100	77	
			82	100	76	
			83	100	83	
			84	100	80	
			85	100	84	
			86	100	86	
			87	100	82	
			88	100	90	
			89	100	83	
			90	100	82	

CETIS Test Data Worksheet

Test Code: 1711-5100 19-4490-5136/73ECE5B0

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

Start Date: 15 Nov-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 17-1184
End Date: 15 Nov-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 15 Nov-17 **Material:** Facility Effluent **Sample Station:** M-001 (Daily) (11/15 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	68			
0	LC	2	67	100	85	CH 11/15/17
0	LC	3	76			
0	LC	4	79			
0	LC	5	70	100	88	SG 11/16/17
2.5		1	62			
2.5		2	83	100	88	SG 11/16/17
2.5		3	86			
2.5		4	69			
2.5		5	87			
5		1	77			
5		2	83			
5		3	66	100	88	SG 11/16/17
5		4	65			
5		5	89			
6.06		1	90	100	80	SG 11/16/17
6.06		2	64			
6.06		3	80	100	84	CH 11/15/17
6.06		4	81			
6.06		5	75			
10		1	72			
10		2	82	100	79	SG 11/16/17
10		3	78			
10		4	71			
10		5	61			
15		1	73			
15		2	85			
15		3	84	100	73	SG 11/16/17
15		4	74			
15		5	63			

QC'd.

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: M-001 Daily (11/15 sample) | **Start Date/Time:** 11/15/2017 1609

Sample Log No.: 17- 1184 | **End Date/Time:** 11/15/2017 1649

Dilutions made by: EG | **Test No:** 1711-5100

Analyst: EG

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	9.0	8.04	33.5	14.9
2.5	8.7	8.01	33.7	15.3
5.0	8.8	8.02	33.8	15.1
6.06	8.9	8.02	33.8	15.0
10	8.9	8.01	33.8	14.9
15	8.9	8.01	33.8	14.8

Comments:

QC Check: EG 11/17/17 | **Final Review:** NC 11/28/17

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

Client: IDE
Sample ID: Daily M-001 - 11/16 sample
Test No.: 1711 - S100

Start Date/Time: 11/15/17 @ ~~11/8/2017~~ / 1609
End Date/Time: ~~11/8/2017~~ 11/13/17 / 1649
Species: S. purpuratus
Animal Source: PT. Loma.
Date Collected: 11/6/17

Tech initials: CG
Injection Time: 1525

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

Eggs Counted: 72, 79, (B) ~~87~~82, 77, 73 Mean: 76.6 X 50 = 3830 eggs/ml

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

Initial density: 3830 eggs/ml = 0.958 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock - 0.04 parts seawater

egg stock (C) ml
seawater (C) 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):	1539	50:1	80	20
Eggs Added (0.5 ml):	1551	100:1	94.96	6.4
Test Ended:	1601	200:1	99	1
		—	—	—

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):	1609	QC1	~~92~~	8
Eggs Added (0.5 ml):	1629	QC2	~~89~~	11
Test Ended:	1649	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) DI8 ACS 11/13/17 (B) CG 11/15/17 (C) No dilution necessary

QC Check: EG 11/17/17 Final Review: _____

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Daily M-051 (11/15 Sample)
Test ID No(s).: 1711-5100

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1184			
Sample Collection Date & Time:	11/15/17 0900			
Sample Receipt Date & Time:	11/15/17 1343			
Number of Containers & Container Type:	1 4L cube			
Approx. Total Volume Received (L):	~4L			
Check-in Temperature (°C)	3.5			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	9.1			
pH (units)	7.92			
Conductivity (µS/cm)	—			
Salinity (ppt)	34.0			
Alkalinity (mg/L) [2]	—			
Hardness (mg/L) [2,3]	130			
Total Chlorine (mg/L)	<0.02			
Technician Initials	~~3.5~~ RAH			

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: @ CIS HCS 11/15/17

Sample Check-In Information

Sample Description:
A = Colorless, Clear, Odorless, 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 11/20/17
Final Review: AC 11/28/17

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 pretreatment 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: 11/14/17 @ 09:00, End: 11/15/17 @ 09:00 VH

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- 3293)	11/14-15/17	9:00-9:00	SW	N	4L CUBIE	X								TDS - 32.23 ppt, EC - 50.15 mS/cm

Relinquished By:	Date:	Time:		Received By:	Date:	Time:	Sample Condition Upon Receipt:
Kevin Cuny	11/15/17	1145			11/15/17	11:45	[X] Iced [ ] Ambient or _____ °C
	11/15-17	1342			11/15/17	1342	[ ] Iced [ ] Ambient or 35 °C

Received: Nautilus 11/15/17 1343.

Nautilus ID = 17-1184

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	13-3003-4485	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Nov-17 16:09	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Nov-17 16:49	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	19-4348-6462	**Code:**	171115sprt	**Client:**	Internal
Sample Date:	15 Nov-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	15 Nov-17	**Source:**	Reference Toxicant		
Sample Age:	16h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-5406-6787	Fertilization Rate	20	40	28.28	6.9%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
17-5783-9769	Fertilization Rate	EC50	35.48	34.17	36.85		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
13-5406-6787	Fertilization Rate	Control Resp	0.916	0.7 - NL	Yes	Passes Acceptability Criteria
17-5783-9769	Fertilization Rate	Control Resp	0.916	0.7 - NL	Yes	Passes Acceptability Criteria
13-5406-6787	Fertilization Rate	PMSD	0.06899	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	0.916	0.9092	0.9228	0.91	0.92	0.002449	0.005476	0.6%	0.0%
10		5	0.894	0.8654	0.9226	0.87	0.92	0.0103	0.02302	2.58%	2.4%
20		5	0.83	0.7444	0.9156	0.76	0.93	0.03082	0.06892	8.3%	9.39%
40		5	0.378	0.2371	0.5189	0.29	0.53	0.05073	0.1134	30.01%	58.73%
80		5	0.008	0	0.01839	0	0.02	0.003742	0.008367	104.6%	99.13%
160		5	0.002	0	0.007553	0	0.01	0.002	0.004472	223.6%	99.78%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.91	0.92	0.92	0.92	0.91
10		0.9	0.87	0.92	0.87	0.91
20		0.93	0.85	0.84	0.77	0.76
40		0.53	0.47	0.3	0.3	0.29
80		0	0.01	0	0.01	0.02
160		0	0.01	0	0	0

Analyst: _____ QA: A(11/27/17

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

Analysis ID: 13-5406-6787 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 20 Nov-17 10:42 **Analysis:** Nonparametric-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	6.9%	20	40	28.28	

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10	19.5	16	2	8	0.1589	Asymp	Non-Significant Effect
		20	20	16	0	8	0.1899	Asymp	Non-Significant Effect
		40*	15	16	0	8	0.0191	Asymp	Significant Effect
		80*	15	16	0	8	0.0191	Asymp	Significant Effect
		160*	15	16	0	8	0.0191	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	8.012068	1.602414	5	358.8	<0.0001	Significant Effect
Error	0.1071979	0.004466581	24			
Total	8.119266		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	23.22	15.09	0.0003	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9156	0.9031	0.0206	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	0.916	0.9092	0.9228	0.92	0.91	0.92	0.002449	0.6%	0.0%
10		5	0.894	0.8654	0.9226	0.9	0.87	0.92	0.0103	2.58%	2.4%
20		5	0.83	0.7444	0.9156	0.84	0.76	0.93	0.03082	8.3%	9.39%
40		5	0.378	0.2371	0.5189	0.3	0.29	0.53	0.05073	30.01%	58.73%
80		5	0.008	0	0.01839	0.01	0	0.02	0.003742	104.6%	99.13%
160		5	0.002	0	0.007553	0	0	0.01	0.002	223.6%	99.78%

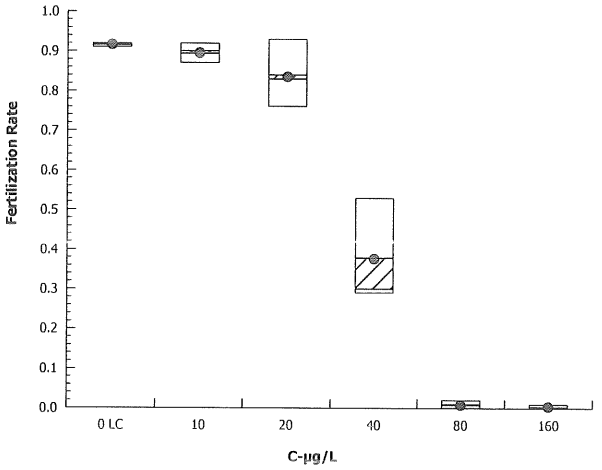
Angular (Corrected) Transformed Summary

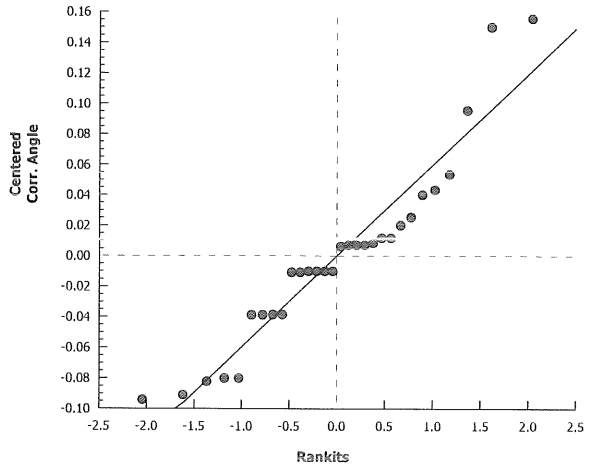
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.277	1.265	1.289	1.284	1.266	1.284	0.004392	0.77%	0.0%
10		5	1.241	1.194	1.287	1.249	1.202	1.284	0.01673	3.02%	2.84%
20		5	1.153	1.031	1.275	1.159	1.059	1.303	0.04394	8.52%	9.7%
40		5	0.6598	0.5148	0.8047	0.5796	0.5687	0.8154	0.0522	17.69%	48.33%
80		5	0.08845	0.04003	0.1369	0.1002	0.05002	0.1419	0.01744	44.09%	93.07%
160		5	0.06005	0.0322	0.0879	0.05002	0.05002	0.1002	0.01003	37.35%	95.3%

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

Analysis ID:	13-5406-6787	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 10:42	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: _____ QA: 11/21/17

CETIS Analytical Report

Test Code: 171115sprt | 06-3476-9418

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

Analysis ID:	17-5783-9769	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	20 Nov-17 10:43	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

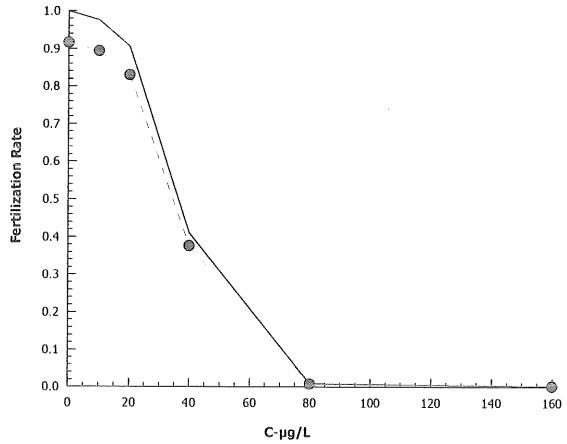
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.084	2.40%	1.55	0.008183	35.48	34.17	36.85

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	0.916	0.91	0.92	0.002449	0.005476	0.6%	0.0%	458	500
10		5	0.894	0.87	0.92	0.0103	0.02302	2.58%	2.4%	447	500
20		5	0.83	0.76	0.93	0.03082	0.06892	8.3%	9.39%	415	500
40		5	0.378	0.29	0.53	0.05073	0.1134	30.01%	58.73%	188	500
80		5	0.008	0	0.02	0.003742	0.008367	104.6%	99.13%	4	500
160		5	0.002	0	0.01	0.002	0.004472	223.6%	99.78%	1	500

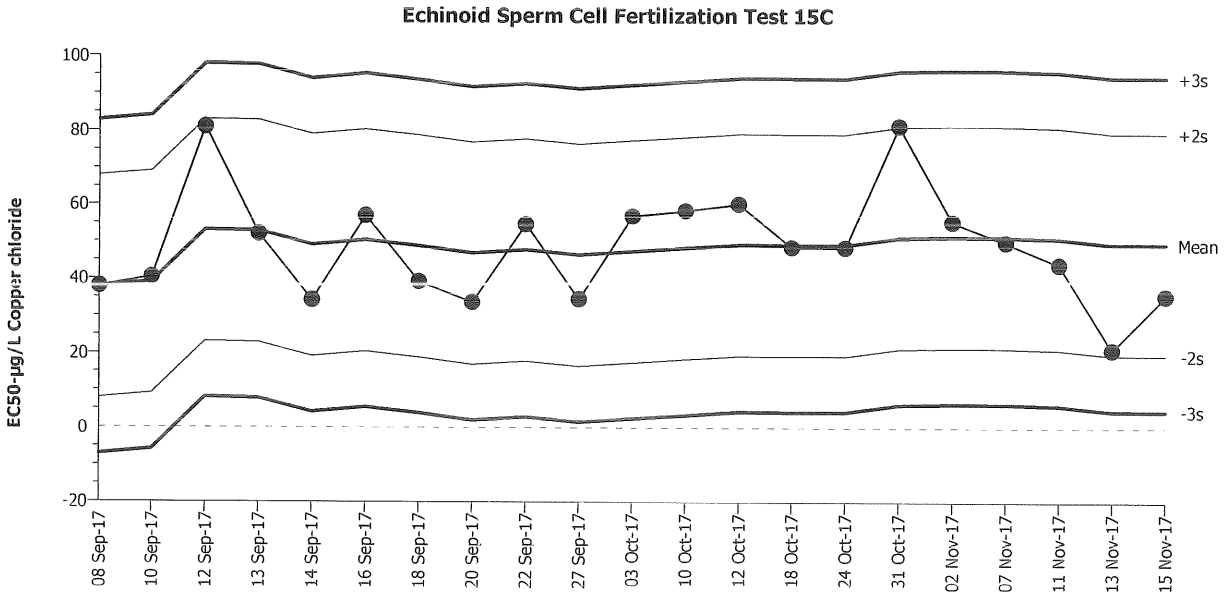
Graphics



Analyst: _____ QA: 11/27/17

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:	49.42	**Count:**	20	**-2s Warning Limit:**	19.46	**-3s Action Limit:**	4.476
Sigma:	14.98	**CV:**	30.30%	**+2s Warning Limit:**	79.38	**+3s Action Limit:**	94.36

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Sep	8	15:48	37.91	-11.51	-0.7682			18-6871-7794	04-4479-5076
2			10	14:25	40.4	-9.018	-0.602			11-6871-9499	08-4248-1228
3			12	15:51	81.07	31.65	2.113	(+)		20-0603-9450	06-1182-7961
4			13	19:07	52.04	2.616	0.1747			01-4575-6189	02-4618-7964
5			14	15:24	34.24	-15.18	-1.014			11-2846-3680	13-8128-7168
6			16	17:08	56.97	7.55	0.504			08-9569-1329	19-6375-1112
7			18	15:28	39.21	-10.21	-0.6818			19-2924-5672	02-0031-2532
8			20	16:15	33.62	-15.8	-1.055			00-4454-0074	17-7214-1415
9			22	14:50	54.61	5.189	0.3464			20-3341-5102	16-2759-7635
10			27	15:34	34.46	-14.96	-0.9983			12-3257-1101	06-9840-2290
11		Oct	3	13:49	56.88	7.459	0.498			05-1137-7792	06-0895-0170
12			10	15:10	58.36	8.942	0.5969			20-5863-5053	00-1542-1738
13			12	14:55	60.18	10.76	0.7185			05-0863-6526	07-1531-2424
14			18	14:22	48.53	-0.8896	-0.05939			13-0042-6212	05-6771-5532
15			24	13:15	48.41	-1.015	-0.06774			20-0280-7301	18-5464-1899
16			31	13:59	81.36	31.94	2.132	(+)		06-4227-6723	08-8095-0809
17		Nov	2	12:28	55.32	5.905	0.3942			17-4126-1689	20-0626-8382
18			7	14:30	49.87	0.4532	0.03025			10-3521-2857	13-9801-3995
19			11	14:25	43.91	-5.512	-0.3679			14-1655-2339	20-5239-6070
20			13	14:35	20.97	-28.45	-1.899			07-0538-7056	00-9105-4737
21			15	16:09	35.48	-13.94	-0.9304			06-3476-9418	17-5783-9769

CETIS Test Data Worksheet

Report Date: 04 Nov-17 14:53 (p 1 of 1)
Test Code: 06-3476-9418/~~171108sprt~~ 171115sprt (B)

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

Start Date: (A) 15 08 Nov-17
End Date: (A) 15 08 Nov-17
Sample Date: 15 08 Nov-17 (A)

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

Sample Code: 171115sprt ~~171108sprt~~ (A)
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	30	11/16/17
			2	100	84	
			3	100	92	
			4	100	77	
			5	100	92	
			6	100	91	
			7	100	29	
			8	100	0	
			9	100	76	
			10	100	0	
			11	100	87	
			12	100	0	
			13	100	93	
			14	100	53	
			15	100	47	
			16	100	0	
			17	100	1	
			18	100	91	
			19	100	85	
			20	100	92	
			21	100	87	
			22	100	1	
			23	100	30	
			24	100	2	
			25	100	0	
			26	100	0	
			27	100	91	
			28	100	90	
			29	100	92	
			30	100	1	

(A) QA Ars 11/13/17

(B) EA QA 11/17/17

CETIS Test Data Worksheet

Report Date: 04 Nov-17 14:53 (p 1 of 1)
Test Code: 06-3476-9418/~~171108spr~~ 171115spr

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

Start Date: ~~08~~ 15 Nov-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** ~~171108spr~~ 171115spr
End Date: ~~08~~ 15 Nov-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: ~~08~~ 15 Nov-17 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	6			
0	LC	2	3			
0	LC	3	29			
0	LC	4	20	100	95	SG 11/16/17
0	LC	5	18			
10		1	28			
10		2	21			
10		3	5	100	91	SG 11/16/17
10		4	11			
10		5	27			
20		1	13			
20		2	19			
20		3	2			
20		4	4	100	85	SG 11/16/17
20		5	9			
40		1	14	100	51	AO 11/15/17
40		2	15			
40		3	23			
40		4	1	100	30	SG 11/16/17
40		5	7			
80		1	8	100	1	SG 11/16/17
80		2	30			
80		3	26			
80		4	17			
80		5	24			
160		1	10			
160		2	22			
160		3	12			
160		4	16	100	0	SG 11/16/17
160		5	25			

QC: CG

(A) QA Acc 11/13/17

(B) EG QA 11/17/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: $CuCl_2$ | **Start Date/Time:** ~~11/8/2017~~ 11/15/17 @ 1609

Test No: ~~171108sprt~~ 171115sprt @ | **End Date/Time:** ~~11/8/2017~~ 11/15/17 @ 1649

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):	9,600

Analyst: CG

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.8	7.99	33.5	15.5
10	8.6	7.99	33.9	15.7
20	8.6	7.98	33.8	15.5
40	8.6	7.98	33.8	15.6
80	8.6	7.98	33.6	15.8
160	8.6	7.99	33.5	15.8

Comments: @ RIB AES 11/13/17

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

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

Client: Internal
Sample ID: CuCl2
Test No.: 171115spT

Start Date/Time: ~~11/8/2017~~ 11/15/17 @ 1609
End Date/Time: ~~11/8/2017~~ 11/15/17 @ 1649
Species: S. purpuratus
Animal Source: Pt. Loma.
Date Collected: 11/10/17

Tech initials: CG
Injection Time: 1525

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

Eggs Counted: 72, 79, (B) ~~87~~ 82, 77, 73 Mean: 76.6 X 50 = 3830 eggs/ml

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

Initial density: 3830 eggs/ml = 0.958 dilution factor
Final density: 4000 eggs/ml
- 1.0 part egg stock
- 0.04 parts seawater

egg stock (C) ml
seawater (E) 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):	1539	50:1	80	20
Eggs Added (0.5 ml):	1551	100:1	94,96	6,4
Test Ended:	1601	200:1	99	1
			—	—

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):	1609	QC1	~~92~~ 92	~~8~~ 8
Eggs Added (0.5 ml):	1629	QC2	~~89~~ 89	11
Test Ended:	1649	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: @ 1618 ACS 11/13/17 (B) EG 11/15/17 (C) No dilution necessary

QC Check: EG 11/17/17 Final Review: AC 11/21/17

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.