Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily & Weekly)
Sample Collection Date: August 22, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **September 7, 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 – AUGUST 2017

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

Sampling Date: August 22, 2017

Test Date: August 23, 2017

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

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type: Urchin Fertilization	Test Date	Effluent Test Results		Effluent Limitation Met? (Yes/No)
		NOEC	TU_c	
	8/23/2017	15	<6.67	Yes

INTRODUCTION

A discharge sample was collected in August 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for weekly accelerated and 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 23, 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 (weekly accelerated and pre-treatment off-spec period)
Monitoring Period:	August 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	8/22/17, 08:00
Sample Receipt Date, Time:	8/23/17, 13:13

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)
8/22/17	7.99	8.5	4.0	33.0	98	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	8/23/17, 16:14 through 16:54
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

No statistically significant decreases in fertilization rate were observed in any effluent concentration tested, compared to the lab control. The NOEC is reported as 15 percent and the TU_c is <6.67, which is less than the maximum effluent limitation of 16.5 for this permit. None of the effluent concentrations were significantly reduced according to the TST analysis. Statistical results are summarized in Table 4, and detailed test results are summarized in Table 5. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and copies of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for M-001 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC_{50} (% sample)	TU_c value (toxic units)	**TST Result (Pass/Fail)**	**Percent Effect at IWC**
M-001	15	>15	>15	<6.67	Pass	-6.1

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	89.0
2.5	92.4
5.0	92.4
6.06	94.4
10	91.4
15	86.4

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 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} **(µg/L Copper)**	Historical Mean EC_{50} ±2 SD **(µg/L Copper)**	CV (%)
8/23/17	41.7	49.4 ± 40.6	41.1

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:	01-8000-9762	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	23 Aug-17 16:14	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	23 Aug-17 16:54	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	11-6603-0963	**Code:**	17-0930	**Client:**	IDE
Sample Date:	22 Aug-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	23 Aug-17 ~~13:10~~ 13:13	**Source:**	IDE Americas, Inc.		
Sample Age:	32h (4 °C)	**Station:**	M-001 (Daily) + Weekly		

RIB AC 9/7/17

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-4860-9621	Fertilization Rate	15	>15	NA	4.98%	≤6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
16-9387-8886	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
02-4860-9621	Fertilization Rate	Control Resp	0.89	0.7 - NL	Yes	Passes Acceptability Criteria
16-9387-8886	Fertilization Rate	Control Resp	0.89	0.7 - NL	Yes	Passes Acceptability Criteria
02-4860-9621	Fertilization Rate	PMSD	0.04984	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	0.89	0.8396	0.9404	0.84	0.93	0.01817	0.04062	4.56%	0.0%
2.5		5	0.924	0.9014	0.9466	0.9	0.95	0.008124	0.01817	1.97%	-3.82%
5		5	0.924	0.9052	0.9428	0.9	0.94	0.006782	0.01517	1.64%	-3.82%
6.06		5	0.944	0.9252	0.9628	0.93	0.97	0.006782	0.01517	1.61%	-6.07%
10		5	0.914	0.8883	0.9397	0.9	0.95	0.009273	0.02074	2.27%	-2.7%
15		5	0.864	0.8109	0.9171	0.8	0.92	0.01913	0.04278	4.95%	2.92%

Fertilization Rate Binomials

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	93/100	93/100	84/100	86/100	89/100
2.5		90/100	95/100	93/100	92/100	92/100
5		93/100	93/100	94/100	92/100	90/100
6.06		94/100	93/100	94/100	94/100	97/100
10		91/100	95/100	91/100	90/100	90/100
15		87/100	87/100	92/100	80/100	86/100

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

Analysis ID: 02-4860-9621 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 25 Aug-17 14:58 **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	4.98%	15	>15	NA	6.667

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.889	2.362	0.070	8	0.9989	CDF	Non-Significant Effect
		5	-1.869	2.362	0.070	8	0.9989	CDF	Non-Significant Effect
		6.06	-3.266	2.362	0.070	8	1.0000	CDF	Non-Significant Effect
		10	-1.283	2.362	0.070	8	0.9927	CDF	Non-Significant Effect
		15	1.369	2.362	0.070	8	0.2690	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0581695	0.0116339	5	5.29	0.0020	Significant Effect
Error	0.05277762	0.002199068	24			
Total	0.1109471		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.316	15.09	0.5048	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9455	0.9031	0.1283	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	0.89	0.8396	0.9404	0.89	0.84	0.93	0.01817	4.56%	0.0%
2.5		5	0.924	0.9014	0.9466	0.92	0.9	0.95	0.008124	1.97%	-3.82%
5		5	0.924	0.9052	0.9428	0.93	0.9	0.94	0.006782	1.64%	-3.82%
6.06		5	0.944	0.9252	0.9628	0.94	0.93	0.97	0.006782	1.61%	-6.07%
10		5	0.914	0.8883	0.9397	0.91	0.9	0.95	0.009273	2.27%	-2.7%
15		5	0.864	0.8109	0.9171	0.87	0.8	0.92	0.01913	4.95%	2.92%

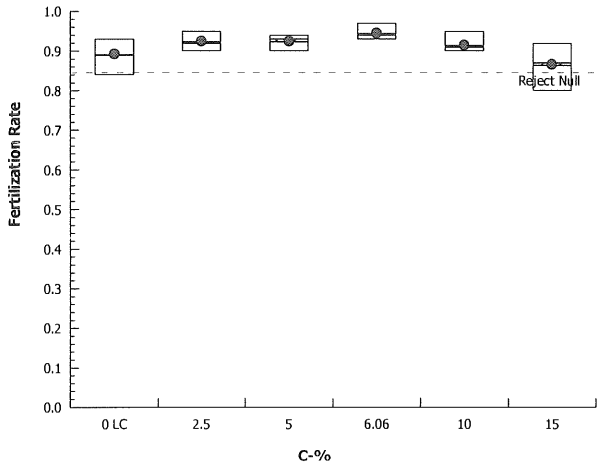
Angular (Corrected) Transformed Summary

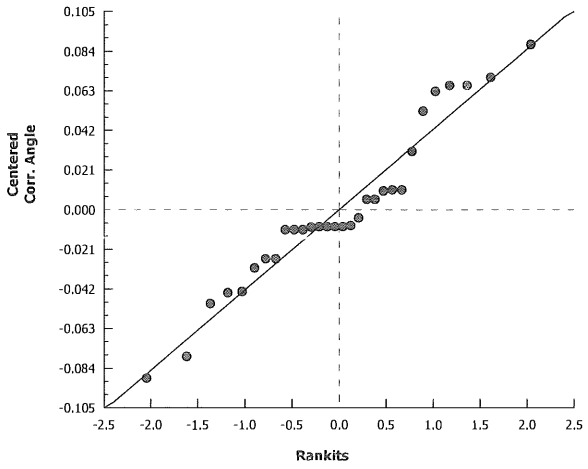
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.237	1.156	1.319	1.233	1.159	1.303	0.02937	5.31%	0.0%
2.5		5	1.293	1.25	1.337	1.284	1.249	1.345	0.01569	2.71%	-4.53%
5		5	1.292	1.258	1.327	1.303	1.249	1.323	0.01251	2.17%	-4.48%
6.06		5	1.334	1.289	1.379	1.323	1.303	1.397	0.01618	2.71%	-7.83%
10		5	1.275	1.225	1.325	1.266	1.249	1.345	0.01795	3.15%	-3.08%
15		5	1.196	1.118	1.274	1.202	1.107	1.284	0.0281	5.25%	3.28%

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

Analysis ID:	02-4860-9621	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Aug-17 14:58	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1708-S206 | 00-5193-4613

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

Analysis ID:	16-9387-8886	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Aug-17 14:58	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	758034	1000	Yes	Two-Point Interpolation

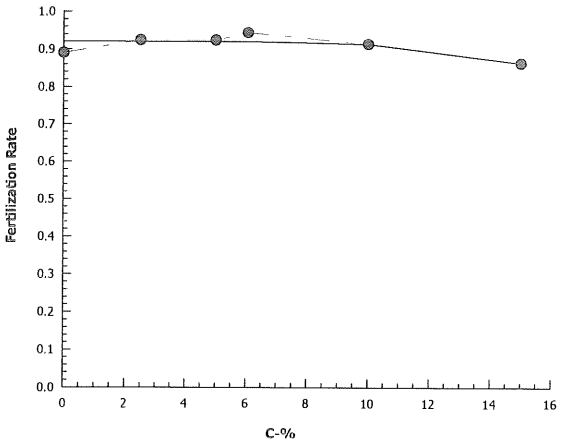
Point Estimates

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	0.89	0.84	0.93	0.01817	0.04062	4.56%	0.0%	445	500
2.5		5	0.924	0.9	0.95	0.008124	0.01817	1.97%	-3.82%	462	500
5		5	0.924	0.9	0.94	0.006782	0.01517	1.64%	-3.82%	462	500
6.06		5	0.944	0.93	0.97	0.006782	0.01517	1.61%	-6.07%	472	500
10		5	0.914	0.9	0.95	0.009273	0.02074	2.27%	-2.7%	457	500
15		5	0.864	0.8	0.92	0.01913	0.04278	4.95%	2.92%	432	500

Graphics



Analyst: _____ QA: AC 9/7/17

CETIS Analytical Report

TST

Test Code: 1708-S206 | 00-5193-4613

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

Analysis ID:	03-6560-2879	**Endpoint:** Fertilization Rate	**CETIS Version:** CETISv1.8.7
Analyzed:	25 Aug-17 14:59	**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	4.79%	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*	13.51	1.895	0.051	7	<0.0001	CDF	Non-Significant Effect
		5*	14.4	1.943	0.049	6	<0.0001	CDF	Non-Significant Effect
		6.06*	14.86	1.895	0.052	7	<0.0001	CDF	Non-Significant Effect
		10*	12.22	1.895	0.054	7	<0.0001	CDF	Non-Significant Effect
		15*	7.525	1.895	0.068	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0581695	0.0116339	5	5.29	0.0020	Significant Effect
Error	0.05277762	0.002199068	24			
Total	0.1109471		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.316	15.09	0.5048	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9455	0.9031	0.1283	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	0.89	0.8396	0.9404	0.89	0.84	0.93	0.01817	4.56%	0.0%
2.5		5	0.924	0.9014	0.9466	0.92	0.9	0.95	0.008124	1.97%	-3.82%
5		5	0.924	0.9052	0.9428	0.93	0.9	0.94	0.006782	1.64%	-3.82%
6.06		5	0.944	0.9252	0.9628	0.94	0.93	0.97	0.006782	1.61%	-6.07%
10		5	0.914	0.8883	0.9397	0.91	0.9	0.95	0.009273	2.27%	-2.7%
15		5	0.864	0.8109	0.9171	0.87	0.8	0.92	0.01913	4.95%	2.92%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.237	1.156	1.319	1.233	1.159	1.303	0.02937	5.31%	0.0%
2.5		5	1.293	1.25	1.337	1.284	1.249	1.345	0.01569	2.71%	-4.53%
5		5	1.292	1.258	1.327	1.303	1.249	1.323	0.01251	2.17%	-4.48%
6.06		5	1.334	1.289	1.379	1.323	1.303	1.397	0.01618	2.71%	-7.83%
10		5	1.275	1.225	1.325	1.266	1.249	1.345	0.01795	3.15%	-3.08%
15		5	1.196	1.118	1.274	1.202	1.107	1.284	0.0281	5.25%	3.28%

Analyst: _____ QA: AC 9/1/17

CETIS Test Data Worksheet

Report Date: 22 Aug-17 15:51 (p 1 of 1)
Test Code: 1708-5206 00-5193-4613/3187595

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

Start Date:	23 Aug-17	**Species:**	Strongylocentrotus purpuratus
End Date:	23 Aug-17	**Protocol:**	EPA/600/R-95/136 (1995)
Sample Date:	22 Aug-17 ~~15:49~~ @	**Material:**	Facility Effluent
Sample Code:	17-0930		
Sample Source:	IDE Americas, Inc.		
Sample Station:	M-001 (Daily) (8/22 Simple)		

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	90	8/24/17
			32	100	89	
			33	100	93	
			34	100	87	
			35	100	95	
			36	100	87	
			37	100	92	
			38	100	91	
			39	100	92	
			40	100	94	
			41	100	94	
			42	100	84	
			43	100	93	
			44	100	92	
			45	100	90	
			46	100	97	
			47	100	93	
			48	100	80	
			49	100	86	
			50	100	91	
			51	100	93	
			52	100	86	
			53	100	95	
			54	100	93	
			55	100	94	
			56	100	92	
			57	100	90	
			58	100	90	
			59	100	93	
			60	100	94	

@ Q18 AC 8/23/17

CETIS Test Data Worksheet

Report Date: 22 Aug-17 15:51 (p 1 of 1)
Test Code: 1708-5206 00-5193-4613/3187595

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

Start Date: 23 Aug-17 | Species: Strongylocentrotus purpuratus | Sample Code: 17-0930
End Date: 23 Aug-17 | Protocol: EPA/600/R-95/136 (1995) | Sample Source: IDE Americas, Inc.
Sample Date: 22 Aug-17 ~~15:49~~ | Material: Facility Effluent | Sample Station: M-001 (Daily) (8/22 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	33	100	84	AD 8/23/17
0	LC	2	51			
0	LC	3	42			
0	LC	4	49			
0	LC	5	32			
2.5		1	31	100	83	
2.5		2	53			
2.5		3	54			
2.5		4	56			
2.5		5	44			
5		1	47	100	92	
5		2	43			
5		3	55			
5		4	37			
5		5	58			
6.06		1	60	100	95	
6.06		2	59			
6.06		3	40			
6.06		4	41			
6.06		5	46			
10		1	38	100	91	
10		2	35			
10		3	50			
10		4	57			
10		5	45			
15		1	36	100	86	
15		2	34			
15		3	39			
15		4	48			
15		5	52			

QC: CG

@@ 13 AC 8/23/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: M-001 Daily (8/22 Sample) | **Start Date/Time:** 8/23/2017 1614

Sample Log No.: 17- 0930 | **End Date/Time:** 8/23/2017 1654

Dilutions made by: AD OBD CG | **Test No:** 1708-S206

Analyst: AD

	Initial Readings			
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.5	8.01	33.6	15.2
2.5	7.6	8.02	33.8	15.8
5.0	7.7	8.02	33.8	15.4
6.06	7.7	8.02	33.8	15.3
10	7.8	8.02	33.8	15.1
15	7.8	8.03	33.9	15.0

Comments:

QC Check: 9/25/17 | **Final Review:** AC 9/7/17

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

Client: (A) ~~External~~ IDE
Sample ID: Ea mooi Daily (8/22)
Test No.: 1708-9206

Start Date/Time: 8/23/2017 / 1614
End Date/Time: 8/23/2017 / 1654
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 8/7/17

Tech initials: CG
Injection Time: 1515

Sperm Absorbance at 400 nm: 0.996 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 73, 82, 74, 80, 86 Mean: 79 X 50 = 3950 eggs/ml

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

Initial density: 3950 eggs/ml = (A) dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

egg stock _____ ml
seawater _____ 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):	1535	50:1	63	37
Eggs Added (0.5 ml):	1555	100:1	88, 82	12, 18
Test Ended:	1605	200:1	95	5
		400:1	100	0

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: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1614	QC1	95	5
Eggs Added (0.5 ml):	1634	QC2	97	3
Test Ended:	1654	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution necessary.
(B) Q18 8/25/17

QC Check: 8/24/17 Final Review: AC 9/7/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Sample Check-In Information

Client: IDE

Tests Performed: Echinoderm Fert/12 others

Project: CDP Compliance

Test ID No.(s): 1708-S206 to S208

	1)	2)	3	4)
Sample ID:	M-001 8/22	M-001 8/23		
Log-in No. (17-xxxx):	0930	0931		
Sample Collection Date & Time:	8/22/17 0800	8/23/17 0800		
Sample Receipt Date & Time:	8/23/17 1310 (B) 313	→		
Number of Containers & Container Type:	1 4L cubi	→		
Approx. Total Volume Received (L):	4L	4L		
Check-in Temp (°C)	4.0	4.0		
Temperature OK? [1]	(Y) N	(Y) N	Y N	Y N
DO (mg/L)	8.5	7.9		
pH (units)	(A) 7.99	8.02		
Conductivity (µS/cm)	—	—		
Salinity (ppt)	33.0	33.0		
Alkalinity (mg/L) [2]	98	100		
Hardness (mg/L) [2, 3]	—	—		
Total Chlorine (mg/L)	<0.02	<0.02		
Technician Initials	TN/AO	TN/AO		

Sample Descriptions:

1) colorless, clear, no odor, no debris
2) colorless, clear, no odor, no debris
3)
4)

COC Complete? (Y) N

Filtration? Y (N)

Pore Size: _____

Organisms or Debris

pH Adjustment? Y (N)

	1	2	3	4	5	6
Initial pH:						
Amount of HCl added:						
Final pH:						

Freshwater Tests:

Control/Dilution Water Source: 8:2 Culligan Other: _____ Alkalinity: _____ Hardness: _____

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

Marine Tests:

Control/Dilution Water Source: (LAB SW) ART SW Other: _____ Alkalinity: 125 Salinity: 33.9

Additional Control? Y (N) = _____ Alkalinity: _____ Salinity: _____

Sample Salted w/ artificial salt? Y N If yes, target ppt and source? _____

Sample salted w/brine? Y N If yes, target ppt? _____

Cl_2 Adjustment? Y (N)

	1	2	3	4	5	6
Initial Free Cl_2:						
STS added:						
Final Free Cl_2:						

Sample Aeration? Y (N)

	1	2	3	4	5	6
Initial D.O.						
Duration & Rate						
Final D.O.						

Notes [1] Temperature for sample must be 0-6°C if received >24 hours past collection time.

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

Additional Comments (A) QLSTN 8/29/17 Tech Error; re-measured 9/7/17

(B) QL8 AC 9/7/17

Subsamples For Additional Chemistry Required? Y (N)

NH3 Other _____

Tech Initials ____

QC Check: by 8/25/17

Final Review: AC 9/7/17

Appendix C

Chain-of-Custody Form

DAILY / WEEKLY

IDE Technologies

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 / Weekly 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 and weekly NPDES requirement. Sample is to be run unadjusted. Start: 8/21/17 @ 8:00, End: 8/22/17 @ 8:00 VH

Glass=G Plastic=P

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

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

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization								NOTES:
M-001 (17- 2710)	8/21-22/2017	8:00-8:00	SW	N	4L CUBIE	X								TDS - 31.71 ppt, EC - 49.36 mS/cm

TEMP Rcvd 4.0

Relinquished By:		Date:	Time:		Received By:						Time:	Sample Condition Upon Receipt:
[signature]		8/23/17	12:10		[signature] 8/23/17							[x] Iced [ ] Ambient or _____ °C
[signature]		8/23/17	13:13		Ashley Dick 8/22/17							[ ] Iced [ ] Ambient or _____ °C

Nautilus ID: 17-0930 1313

RAD 018 8/23/17

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	12-2077-2725	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	23 Aug-17 16:14	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	23 Aug-17 16:54	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	02-2195-0784	**Code:**	170823sprt	**Client:**	Internal
Sample Date:	23 Aug-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	23 Aug-17	**Source:**	Reference Toxicant		
Sample Age:	16h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-1961-1100	Fertilization Rate	<10	10	NA	7.16%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
15-3490-2746	Fertilization Rate	EC50	41.72	39.4	44.17		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-1961-1100	Fertilization Rate	Control Resp	0.958	0.7 - NL	Yes	Passes Acceptability Criteria
15-3490-2746	Fertilization Rate	Control Resp	0.958	0.7 - NL	Yes	Passes Acceptability Criteria
02-1961-1100	Fertilization Rate	PMSD	0.07157	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	0.958	0.9376	0.9784	0.93	0.97	0.007348	0.01643	1.72%	0.0%
10		5	0.838	0.7588	0.9172	0.74	0.89	0.02853	0.0638	7.61%	12.53%
20		5	0.722	0.5457	0.8983	0.52	0.89	0.06351	0.142	19.67%	24.63%
40		5	0.632	0.5684	0.6956	0.57	0.69	0.02289	0.05119	8.1%	34.03%
80		5	0.132	0.07416	0.1898	0.08	0.19	0.02083	0.04658	35.29%	86.22%
160		5	0	0	0	0	0	0	0		100.0%

Fertilization Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	97/100	96/100	96/100	97/100	93/100
10		81/100	86/100	89/100	89/100	74/100
20		82/100	69/100	89/100	69/100	52/100
40		61/100	68/100	61/100	69/100	57/100
80		9/100	14/100	16/100	19/100	8/100
160		0/100	0/100	0/100	0/100	0/100

Analyst: JJ QA: AC 8/30/17

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

Analysis ID: 02-1961-1100 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 25 Aug-17 14:32 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	7.16%	<10	10	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	3.5	2.305	0.135	8	0.0039	CDF	Significant Effect
		20*	5.807	2.305	0.135	8	<0.0001	CDF	Significant Effect
		40*	7.625	2.305	0.135	8	<0.0001	CDF	Significant Effect
		80*	17.03	2.305	0.135	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.815473	0.7038682	4	81.76	<0.0001	Significant Effect
Error	0.172184	0.008609199	20			
Total	2.987657		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	9.095	13.28	0.0588	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9701	0.8877	0.6481	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	0.958	0.9376	0.9784	0.96	0.93	0.97	0.007348	1.72%	0.0%
10		5	0.838	0.7588	0.9172	0.86	0.74	0.89	0.02853	7.61%	12.53%
20		5	0.722	0.5457	0.8983	0.69	0.52	0.89	0.06351	19.67%	24.63%
40		5	0.632	0.5684	0.6956	0.61	0.57	0.69	0.02289	8.1%	34.03%
80		5	0.132	0.07416	0.1898	0.14	0.08	0.19	0.02083	35.29%	86.22%
160		5	0	0	0	0	0	0	0		100.0%

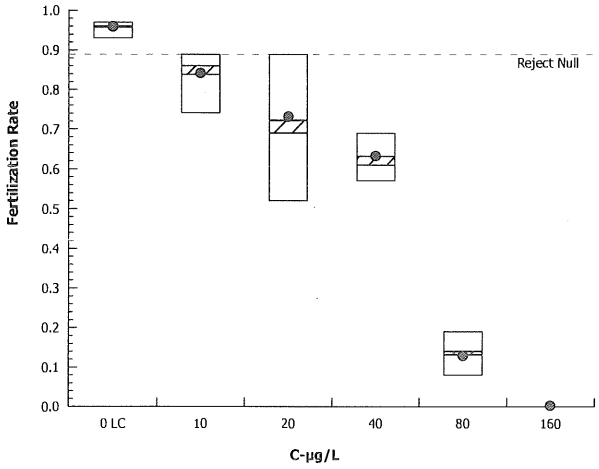
Angular (Corrected) Transformed Summary

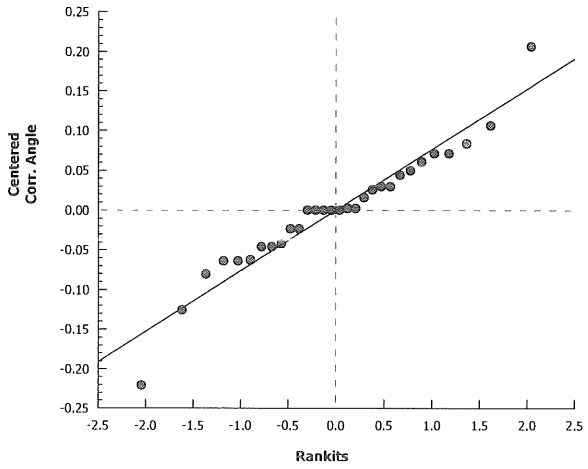
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.367	1.32	1.415	1.369	1.303	1.397	0.01713	2.8%	0.0%
10		5	1.162	1.057	1.266	1.187	1.036	1.233	0.03767	7.25%	15.03%
20		5	1.026	0.8232	1.229	0.9803	0.8054	1.233	0.07313	15.93%	24.93%
40		5	0.9196	0.8535	0.9858	0.8963	0.8556	0.9803	0.02383	5.79%	32.73%
80		5	0.3675	0.2805	0.4545	0.3835	0.2868	0.451	0.03133	19.06%	73.12%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.34%

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

Analysis ID:	02-1961-1100	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Aug-17 14:32	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	15-3490-2746	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	25 Aug-17 14:32	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

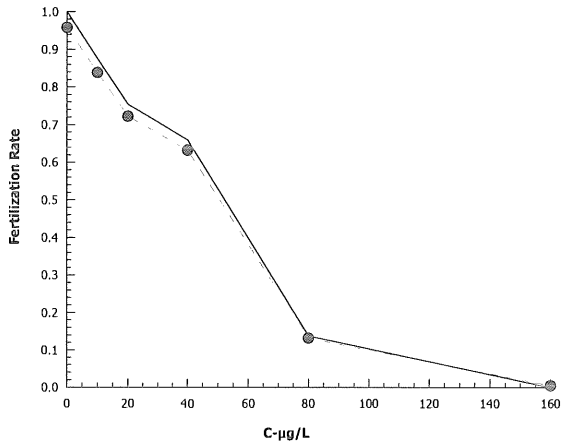
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.042	12.53%	1.62	0.01242	41.72	39.4	44.17

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	0.958	0.93	0.97	0.007348	0.01643	1.72%	0.0%	479	500
10		5	0.838	0.74	0.89	0.02853	0.0638	7.61%	12.53%	419	500
20		5	0.722	0.52	0.89	0.06351	0.142	19.67%	24.63%	361	500
40		5	0.632	0.57	0.69	0.02289	0.05119	8.1%	34.03%	316	500
80		5	0.132	0.08	0.19	0.02083	0.04658	35.29%	86.22%	66	500
160		5	0	0	0	0	0		100.0%	0	500

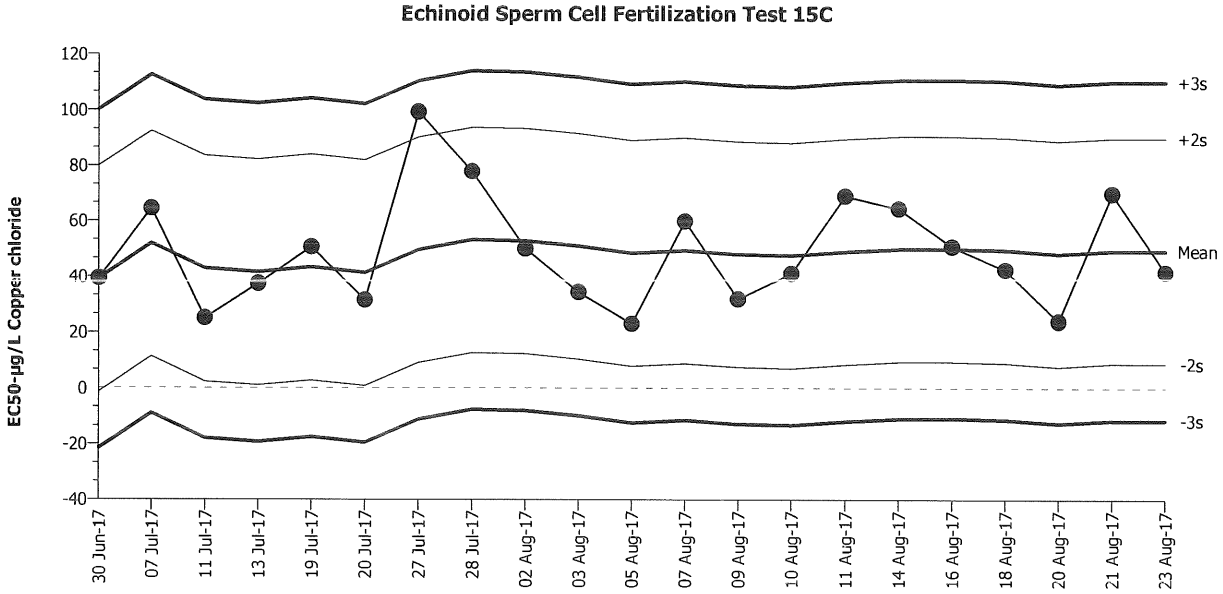
Graphics



Analyst: _____ QA: AC 8/30/17

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:	49.36	**Count:**	20	**-2s Warning Limit:**	8.796	**-3s Action Limit:**	-11.48
Sigma:	20.28	**CV:**	41.10%	**+2s Warning Limit:**	89.92	**+3s Action Limit:**	110.2

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jun	30	17:50	39.38	-9.983	-0.4923			19-1859-0537	20-9128-8719
2		Jul	7	17:35	64.49	15.13	0.746			20-4636-3738	10-9356-2953
3			11	11:33	25.05	-24.31	-1.198			09-0588-2471	00-1661-1655
4			13	15:20	37.5	-11.86	-0.585			05-9787-5418	02-0541-0147
5			19	16:28	50.59	1.229	0.0606			03-3446-7266	15-7259-8466
6			20	18:10	31.52	-17.84	-0.8797			17-7484-2488	03-0485-5429
7			27	15:55	99.32	49.96	2.464	(+)		02-6715-3770	17-8186-2444
8			28	10:50	77.84	28.48	1.404			21-2559-1280	14-0688-6070
9		Aug	2	15:50	50.06	0.6951	0.03428			08-9742-2478	08-8646-9232
10			3	0:00	34.43	-14.93	-0.736			02-7356-2235	20-3051-4002
11			5	19:25	23.07	-26.29	-1.296			11-5994-0488	10-6029-2098
12			7	15:10	59.94	10.58	0.5216			21-2468-7505	14-3489-7019
13			9	17:08	31.92	-17.44	-0.8602			13-6999-3036	11-7131-4234
14			10	16:51	41.14	-8.224	-0.4055			00-5471-5288	12-0643-2211
15			11	14:50	69.03	19.67	0.9699			04-5796-5476	07-8184-6783
16			14	14:40	64.51	15.15	0.7468			02-4510-8526	01-5460-0814
17			16	16:34	50.82	1.457	0.07184			16-3259-1018	06-7497-1035
18			18	14:09	42.53	-6.829	-0.3367			12-6613-4538	02-2322-5589
19			20	14:52	24.05	-25.31	-1.248			06-9655-0092	05-8785-3700
20			21	14:46	69.95	20.59	1.015			08-4756-2919	20-2992-4955
21			23	16:14	41.72	-7.643	-0.3769			02-7595-3678	15-3490-2746

CETIS Test Data Worksheet

Report Date: 22 Aug-17 15:45 (p 1 of 1)
Test Code: 02-7595-3678/170823sprt

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

Start Date:	23 Aug-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:**	170823sprt
End Date:	23 Aug-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Reference Toxicant
Sample Date:	23 Aug-17	**Material:**	Copper chloride	**Sample Station:**	Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	8/24/17
			2	100	69	
			3	100	86	
			4	100	14	
			5	100	68	
			6	100	89	
			7	100	97	
			8	100	81	
			9	100	89	
			10	100	69	
			11	100	61	
			12	100	0	
			13	100	0	
			14	100	0	
			15	100	9	
			16	100	~~25~~ 16	Q18 RL 8/24/17
			17	100	93	
			18	100	97	
			19	100	8	
			20	100	96	
			21	100	96	
			22	100	61	
			23	100	69	
			24	100	74	
			25	100	89	
			26	100	52	
			27	100	0	
			28	100	57	
			29	100	19	
			30	100	82	

CETIS Test Data Worksheet

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

Start Date: 23 Aug-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 170823sprt
End Date: 23 Aug-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 23 Aug-17 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	7			
0	LC	2	21			
0	LC	3	20			
0	LC	4	18	100	97	CG 8/23/17
0	LC	5	17			
10		1	8			
10		2	3			
10		3	25			
10		4	6	100	89	
10		5	24			
20		1	30			
20		2	23			
20		3	9	100	88	
20		4	10			
20		5	26			
40		1	11			
40		2	5			
40		3	22	100	62	
40		4	2			
40		5	28			
80		1	15			
80		2	4	100	23	
80		3	16			
80		4	29			
80		5	19			
160		1	13			
160		2	27	100	0	
160		3	14			
160		4	1			
160		5	12			

QC CG

Analyst: CG QA: AC 8/30/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: $CuCl_2$ | **Start Date/Time:** 8/23/2017 1614

Test No: 170823Spvt | **End Date/Time:** 8/23/2017 1654

Dilutions 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):	10200

Analyst: AD

	Initial Readings			
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.4	7.95	33.5	15.0
10	7.4	7.97	33.7	14.9
20	7.4	7.97	33.7	14.9
40	7.4	7.99	33.7	14.9
80	7.5	7.98	33.6	14.9
160	7.5	7.99	33.3	15.0

Comments:

QC Check: 8/25/17 | **Final Review:** AC 8/30/17

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

Client: Internal
Sample ID: $CuCl_2$
Test No.: 170823sprt

Tech initials: CG
Injection Time: 1515

Start Date/Time: 8/23/2017 / 1614
End Date/Time: 8/23/2017 / 1654
Species: *S. purpuratus*
Animal Source: Pt Loma
Date Collected: 8/7/17

Sperm Absorbance at 400 nm: 0.998 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 73, 82, 74, 80, 86 Mean: 79 X 50 = 3950 eggs/ml

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

Initial density: 3950 eggs/ml = (A) dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

egg stock _____ ml
seawater _____ 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):	1535	50:1	63	37
Eggs Added (0.5 ml):	1555	100:1	88, 82	12, 18
Test Ended:	1605	200:1	95	5
		400:1	100	0

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: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1614	QC1	95	5
Eggs Added (0.5 ml):	1634	QC2	97	3
Test Ended:	1654	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution necessary.

QC Check: 8/25/17

Final Review: AC 8/30/17

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.