Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: August 25, 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 25, 2017

Test Date: August 25, 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	
	8/25/2017	6.06	16.5	Yes

INTRODUCTION

A discharge sample was collected in August 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for daily chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on August 25, 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:	August 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	8/25/17, 08:00
Sample Receipt Date, Time:	8/25/17, 12:53

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)
8/25/17	8.04	8.1	4.0	32.6	104	0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	8/25/17, 14:48 through 15:28
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 the fertilization rate were observed in the 10 and 15 percent effluent concentrations compared to the lab control. The NOEC is reported as 6.06 and the TU_c is 16.5, which meets the maximum effluent limitation of 16.5 for this permit. None of the effluent concentrations were significantly reduced according to the TST analysis. Statistical results are summarized in Table 4, and detailed test results are summarized in Table 5. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and copies of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for 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	6.06	10	>15	16.5	Pass	6.9

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	86.4
2.5	86.2
5.0	85.6
6.06	80.4
10	75.6*
15	73.6*

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

QUALITY ASSURANCE

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

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

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC_{50} **(µg/L Copper)**	Historical Mean EC_{50} ±2 SD **(µg/L Copper)**	CV (%)
8/25/17	43.1	49.6 ± 40.7	41.0

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:	18-2252-8681	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	25 Aug-17 14:48	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	25 Aug-17 15:28	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	14-5791-7667	**Code:**	17-0943	**Client:**	IDE
Sample Date:	25 Aug-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	25 Aug-17 12:53	**Source:**	IDE Americas, Inc.		
Sample Age:	7h (4 °C)	**Station:**	M-001 (Daily)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-4041-4112	Fertilization Rate	6.06	10	7.785	7.84%	16.5	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
10-4607-4537	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
10-4607-4537	Fertilization Rate	Control Resp	0.864	0.7 - NL	Yes	Passes Acceptability Criteria
17-4041-4112	Fertilization Rate	Control Resp	0.864	0.7 - NL	Yes	Passes Acceptability Criteria
17-4041-4112	Fertilization Rate	PMSD	0.07843	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	0.864	0.8139	0.9141	0.8	0.9	0.01806	0.04037	4.67%	0.0%
2.5		5	0.862	0.7915	0.9325	0.78	0.92	0.02538	0.05675	6.58%	0.23%
5		5	0.856	0.8044	0.9076	0.82	0.92	0.0186	0.04159	4.86%	0.93%
6.06		5	0.804	0.7524	0.8556	0.74	0.85	0.0186	0.04159	5.17%	6.94%
10		5	0.756	0.6899	0.8221	0.68	0.83	0.02379	0.0532	7.04%	12.5%
15		5	0.736	0.6748	0.7972	0.66	0.79	0.02205	0.0493	6.7%	14.81%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.85	0.88	0.89	0.8	0.9
2.5		0.78	0.83	0.9	0.88	0.92
5		0.85	0.82	0.87	0.92	0.82
6.06		0.74	0.83	0.8	0.8	0.85
10		0.76	0.68	0.83	0.75	0.76
15		0.79	0.72	0.76	0.66	0.75

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

Analysis ID:	17-4041-4112	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Aug-17 16:01	**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	7.84%	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	0.002724	2.362	0.093	8	0.8325	CDF	Non-Significant Effect
		5	0.2768	2.362	0.093	8	0.7389	CDF	Non-Significant Effect
		6.06	2.075	2.362	0.093	8	0.0867	CDF	Non-Significant Effect
		10*	3.536	2.362	0.093	8	0.0036	CDF	Significant Effect
		15*	4.133	2.362	0.093	8	0.0008	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.134548	0.02690961	5	6.889	0.0004	Significant Effect
Error	0.09374695	0.003906123	24			
Total	0.228295		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.9858	15.09	0.9637	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9704	0.9031	0.5493	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	0.864	0.8139	0.9141	0.88	0.8	0.9	0.01806	4.67%	0.0%
2.5		5	0.862	0.7915	0.9325	0.88	0.78	0.92	0.02538	6.58%	0.23%
5		5	0.856	0.8044	0.9076	0.85	0.82	0.92	0.0186	4.86%	0.93%
6.06		5	0.804	0.7524	0.8556	0.8	0.74	0.85	0.0186	5.17%	6.94%
10		5	0.756	0.6899	0.8221	0.76	0.68	0.83	0.02379	7.04%	12.5%
15		5	0.736	0.6748	0.7972	0.75	0.66	0.79	0.02205	6.7%	14.81%

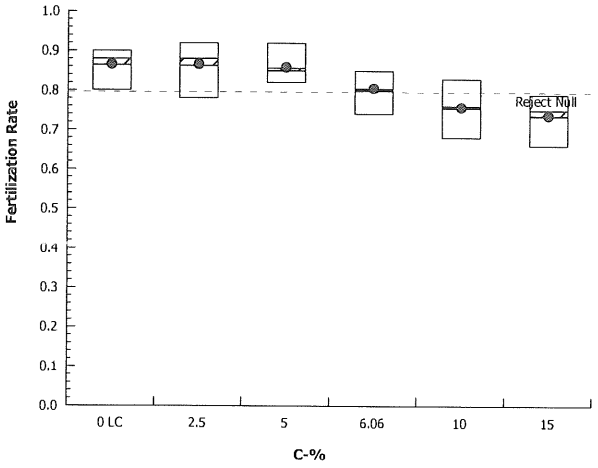
Angular (Corrected) Transformed Summary

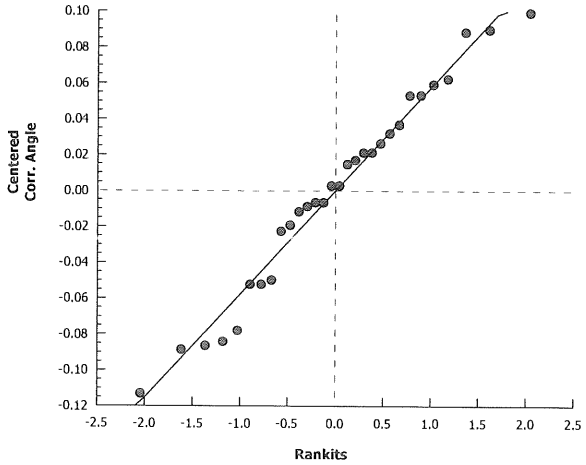
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.196	1.125	1.267	1.217	1.107	1.249	0.02552	4.77%	0.0%
2.5		5	1.196	1.095	1.297	1.217	1.083	1.284	0.03632	6.79%	0.01%
5		5	1.185	1.107	1.263	1.173	1.133	1.284	0.02803	5.29%	0.92%
6.06		5	1.114	1.049	1.178	1.107	1.036	1.173	0.02316	4.65%	6.86%
10		5	1.056	0.9784	1.134	1.059	0.9695	1.146	0.02796	5.92%	11.69%
15		5	1.032	0.9637	1.101	1.047	0.9483	1.095	0.02475	5.36%	13.66%

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

Analysis ID:	17-4041-4112	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Aug-17 16:01	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: JA QA: AC 9/7/17

CETIS Analytical Report

Test Code: 1708-S210 | 19-4640-9870

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

Analysis ID:	10-4607-4537	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Aug-17 16:02	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	871270	1000	Yes	Two-Point Interpolation

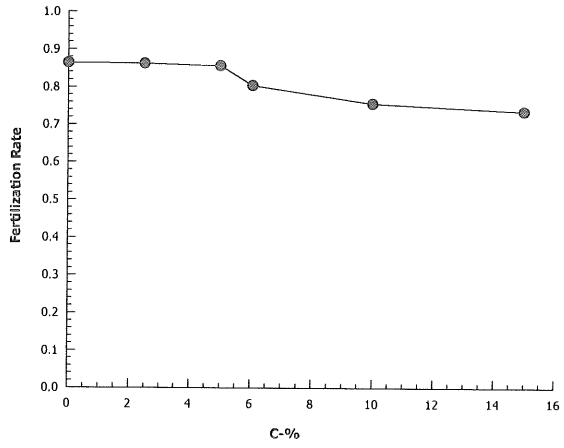
Point Estimates

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	0.864	0.8	0.9	0.01806	0.04037	4.67%	0.0%	432	500
2.5		5	0.862	0.78	0.92	0.02538	0.05675	6.58%	0.23%	431	500
5		5	0.856	0.82	0.92	0.0186	0.04159	4.86%	0.93%	428	500
6.06		5	0.804	0.74	0.85	0.0186	0.04159	5.17%	6.94%	402	500
10		5	0.756	0.68	0.83	0.02379	0.0532	7.04%	12.5%	378	500
15		5	0.736	0.66	0.79	0.02205	0.0493	6.7%	14.81%	368	500

Graphics



Analyst: JJ QA: AC 9/7/17

CETIS Analytical Report

TST

Report Date: 29 Aug-17 16:03 (p 1 of 1)
Test Code: 1708-S210 | 19-4640-9870

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

Analysis ID: 12-9750-1543 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.7
Analyzed: 29 Aug-17 16:02 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	4.75%	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*	7.279	1.943	0.08	6	0.0002	CDF	Non-Significant Effect
		5*	8.485	1.895	0.064	7	<0.0001	CDF	Non-Significant Effect
		6.06*	7.22	1.895	0.057	7	<0.0001	CDF	Non-Significant Effect
		10*	4.698	1.895	0.064	7	0.0011	CDF	Non-Significant Effect
		15*	4.333	1.895	0.059	7	0.0017	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.134548	0.02690961	5	6.889	0.0004	Significant Effect
Error	0.09374695	0.003906123	24			
Total	0.228295		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.9858	15.09	0.9637	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9704	0.9031	0.5493	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	0.864	0.8139	0.9141	0.88	0.8	0.9	0.01806	4.67%	0.0%
2.5		5	0.862	0.7915	0.9325	0.88	0.78	0.92	0.02538	6.58%	0.23%
5		5	0.856	0.8044	0.9076	0.85	0.82	0.92	0.0186	4.86%	0.93%
6.06		5	0.804	0.7524	0.8556	0.8	0.74	0.85	0.0186	5.17%	6.94%
10		5	0.756	0.6899	0.8221	0.76	0.68	0.83	0.02379	7.04%	12.5%
15		5	0.736	0.6748	0.7972	0.75	0.66	0.79	0.02205	6.7%	14.81%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.196	1.125	1.267	1.217	1.107	1.249	0.02552	4.77%	0.0%
2.5		5	1.196	1.095	1.297	1.217	1.083	1.284	0.03632	6.79%	0.01%
5		5	1.185	1.107	1.263	1.173	1.133	1.284	0.02803	5.29%	0.92%
6.06		5	1.114	1.049	1.178	1.107	1.036	1.173	0.02316	4.65%	6.86%
10		5	1.056	0.9784	1.134	1.059	0.9695	1.146	0.02796	5.92%	11.69%
15		5	1.032	0.9637	1.101	1.047	0.9483	1.095	0.02475	5.36%	13.66%

Analyst: JA QA: AC 9/7/17

CETIS Test Data Worksheet

Test Code: 1708-S810 19-4640-9870/7403DB8E

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

Start Date: 25 Aug-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 17-0943
End Date: 25 Aug-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 25 Aug-17 | **Material:** Facility Effluent | **Sample Station:** M-001 (Daily) 8/25 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	75	8/29/17
			62	100	90	
			63	100	76	
			64	100	66	
			65	100	92	
			66	100	82	
			67	100	74	
			68	100	76	
			69	100	83	
			70	100	80	
			71	100	88	
			72	100	90	
			73	100	83	
			74	100	79	
			75	100	72	
			76	100	85	
			77	100	92	
			78	100	80	
			79	100	80	
			80	100	83	
			81	100	78	
			82	100	85	
			83	100	89	
			84	100	82	
			85	100	76	
			86	100	87	
			87	100	68	
			88	100	85	
			89	100	75	
			90	100	88	

Analyst: RL QA: ACS 8/29/17

CETIS Test Data Worksheet

Report Date: 25 Aug-17 08:59 (p 1 of 1)
Test Code: 1708-5210 19-4640-9870/7403DB8E

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

Start Date: 25 Aug-17
End Date: 25 Aug-17
Sample Date: 25 Aug-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 0943
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	82	100	84	RL 8/25/17
0	LC	2	90			
0	LC	3	83			
0	LC	4	79			
0	LC	5	72			
2.5		1	81	100	90	RL 8/25/17
2.5		2	73			
2.5		3	62			
2.5		4	71			
2.5		5	77			
5		1	76	100	85	RL 8/25/17
5		2	66			
5		3	86			
5		4	65			
5		5	84			
6.06		1	67	100	83	RL 8/25/17
6.06		2	80			
6.06		3	78			
6.06		4	70			
6.06		5	88			
10		1	63	100	91	RL 8/25/17
10		2	87	100	80	RL 8/25/17
10		3	69			
10		4	89			
10		5	68			
15		1	74	100	75	RL 8/25/17
15		2	75			
15		3	85			
15		4	64	100	73	RL 8/25/17
15		5	61			

QC: EG

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (Daily) 8/25 Sample

Start Date/Time: 8/25/2017 1448

Sample Log No.: 17- 0943

End Date/Time: 8/25/2017 1528

Dilutions made by: EG

Test No: 1708-5210

Analyst: CG

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.0	8.12	~~33.5~~ 33 Ⓐ	15.4
2.5	8.0	8.12	33.7	15.5
5.0	7.9	8.12	33.7	15.1
6.06	7.9	8.12	33.7	15.3
10	8.0	8.12	33.7	15.1
15	8.0	8.12	33.7	14.9

Comments: Ⓐ CG QA 8/25/17

QC Check: AC 8/29/17

Final Review: KFP 9/7/17

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

Client: IDE
Sample ID: Daily M-001 8/25 sample
Test No.: 1708-S010

Start Date/Time: 8/25/2017 / 1448
End Date/Time: 8/25/2017 / 1528
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 8/22/17

Tech initials: EG
Injection Time: 1410

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

Eggs Counted: 77, 96, 89, 78, 97

Mean: 87.4 X 50 = 4370 eggs/ml

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

Initial density: 4370 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
____ parts seawater

(A)

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).

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1421	50:1	83/91	17/9
Eggs Added (0.5 ml):	1431	100:1	96/97	4/3
Test Ended:	1441	—	—	—
		—	—	—
		—	—	—

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

Definitive Test

Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1448	QC1	89	11
Eggs Added (0.5 ml):	1508	QC2	91	9
Test Ended:	1528	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) No dilution required

QC Check: AC 8/29/17

Final Review: KFP 9/7/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: JDE
Sample ID: Daily M-001
Test ID No(s).: 1708-5210

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	~~0942~~ 0943			
Sample Collection Date & Time:	8/25/17 0800			
Sample Receipt Date & Time:	8/25/17 1253			
Number of Containers & Container Type:	1-4L Cubi			
Approx. Total Volume Received (L):	~4L			
Check-in Temperature (°C)	4.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.1			
pH (units)	8.04			
Conductivity (µS/cm)	—			
Salinity (ppt)	32.6			
Alkalinity (mg/L) [2]	104			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	0.02			
Technician Initials	TN			

Test Performed: Urchin Fertilization Control/Dilution Water: 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 125 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: (E) QISAC 8/29/17

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: AC 8/29/17
Final Review: KPP 9/7/17

Appendix C

Chain-of-Custody Form

DAILY

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 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: 8/24/17 @ 8:00, End: 8/25/17 @ 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- 2722)	8/24-25/2017	8:00-8:00	SW	N	4L CUBIE	X								TDS - 31.72 ppt, EC - 49.45 mS/cm

RECEIVED TEMP 4.0

Relinquished By:		Date:	Time:		Received By:					Time:	Sample Condition Upon Receipt:
		8/25/17	1200								[X] Iced [ ] Ambient or _____°C
		8/25/17	12:43								[ ] Iced [ ] Ambient or _____°C

NAUTILUS ID: 17-0943

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	11-4051-6467	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	25 Aug-17 14:48	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	25 Aug-17 15:28	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	06-7257-6480	**Code:**	170825sprt	**Client:**	Internal
Sample Date:	25 Aug-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	25 Aug-17	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
17-6926-1872	Fertilization Rate	<10	10	NA	6.39%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
09-0830-4014	Fertilization Rate	EC50	43.11	40.93	45.41		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
09-0830-4014	Fertilization Rate	Control Resp	0.89	0.7 - NL	Yes	Passes Acceptability Criteria
17-6926-1872	Fertilization Rate	Control Resp	0.89	0.7 - NL	Yes	Passes Acceptability Criteria
17-6926-1872	Fertilization Rate	PMSD	0.06386	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	0.89	0.8273	0.9527	0.83	0.93	0.02258	0.0505	5.67%	0.0%
10		5	0.798	0.7344	0.8616	0.75	0.88	0.02289	0.05119	6.41%	10.34%
20		5	0.73	0.6879	0.7721	0.69	0.77	0.01517	0.03391	4.65%	17.98%
40		5	0.58	0.5081	0.6519	0.54	0.68	0.02588	0.05788	9.98%	34.83%
80		5	0.108	0.05726	0.1587	0.08	0.18	0.01828	0.04087	37.84%	87.87%
160		5	0.006	0	0.01711	0	0.02	0.004	0.008944	149.1%	99.33%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.83	0.92	0.93	0.84	0.93
10		0.8	0.88	0.8	0.76	0.75
20		0.77	0.69	0.72	0.71	0.76
40		0.57	0.54	0.57	0.68	0.54
80		0.18	0.1	0.09	0.08	0.09
160		0	0	0.01	0	0.02

Analyst: _____ QA: AC 8/30/17

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

Analysis ID:	17-6926-1872	**Endpoint:** Fertilization Rate	**CETIS Version:** CETISv1.8.7
Analyzed:	29 Aug-17 15:38	**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	6.39%	<10	10	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	3.491	2.362	0.089	8	0.0041	CDF	Significant Effect
		20*	5.679	2.362	0.089	8	<0.0001	CDF	Significant Effect
		40*	9.89	2.362	0.089	8	<0.0001	CDF	Significant Effect
		80*	24.09	2.362	0.089	8	<0.0001	CDF	Significant Effect
		160*	30.8	2.362	0.089	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.394424	1.078885	5	303.9	<0.0001	Significant Effect
Error	0.08521682	0.003550701	24			
Total	5.479641		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.684	15.09	0.7486	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9351	0.9031	0.0671	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	0.89	0.8273	0.9527	0.92	0.83	0.93	0.02258	5.67%	0.0%
10		5	0.798	0.7344	0.8616	0.8	0.75	0.88	0.02289	6.41%	10.34%
20		5	0.73	0.6879	0.7721	0.72	0.69	0.77	0.01517	4.65%	17.98%
40		5	0.58	0.5081	0.6519	0.57	0.54	0.68	0.02588	9.98%	34.83%
80		5	0.108	0.05726	0.1587	0.09	0.08	0.18	0.01828	37.84%	87.87%
160		5	0.006	0	0.01711	0	0	0.02	0.004	149.1%	99.33%

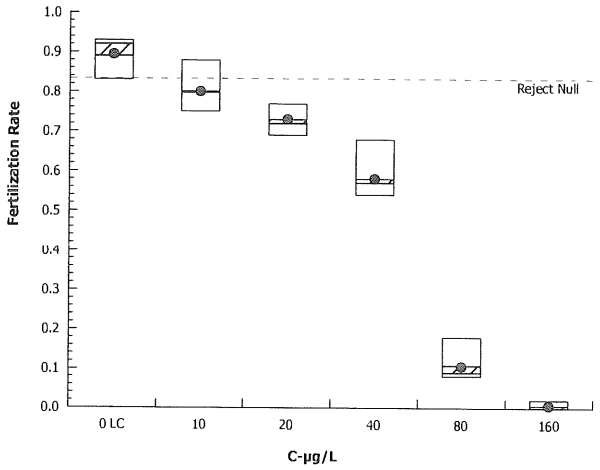
Angular (Corrected) Transformed Summary

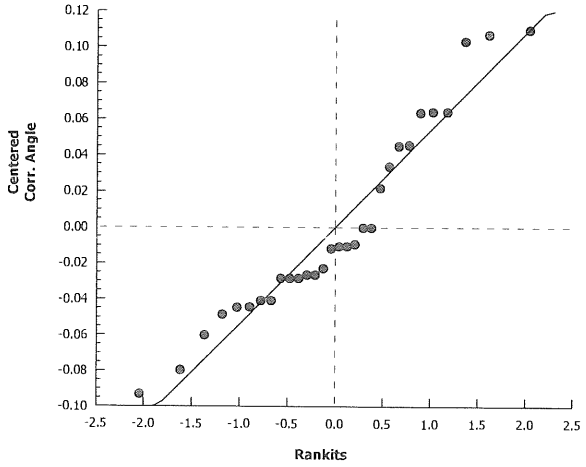
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.239	1.14	1.338	1.284	1.146	1.303	0.03555	6.42%	0.0%
10		5	1.107	1.024	1.191	1.107	1.047	1.217	0.03001	6.06%	10.62%
20		5	1.025	0.9774	1.073	1.013	0.9803	1.071	0.01716	3.74%	17.27%
40		5	0.8663	0.7923	0.9404	0.8556	0.8254	0.9695	0.02667	6.88%	30.08%
80		5	0.3312	0.2554	0.407	0.3047	0.2868	0.4381	0.0273	18.43%	73.27%
160		5	0.07843	0.02677	0.1301	0.05002	0.05002	0.1419	0.0186	53.04%	93.67%

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

Analysis ID:	17-6926-1872	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Aug-17 15:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	09-0830-4014	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	29 Aug-17 15:39	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

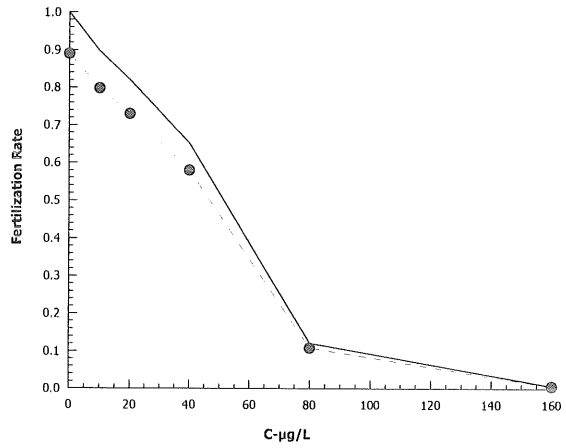
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.11	10.34%	1.635	0.01128	43.11	40.93	45.41

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	0.89	0.83	0.93	0.02258	0.0505	5.67%	0.0%	445	500
10		5	0.798	0.75	0.88	0.02289	0.05119	6.41%	10.34%	399	500
20		5	0.73	0.69	0.77	0.01517	0.03391	4.65%	17.98%	365	500
40		5	0.58	0.54	0.68	0.02588	0.05788	9.98%	34.83%	290	500
80		5	0.108	0.08	0.18	0.01828	0.04087	37.84%	87.87%	54	500
160		5	0.006	0	0.02	0.004	0.008944	149.1%	99.33%	3	500

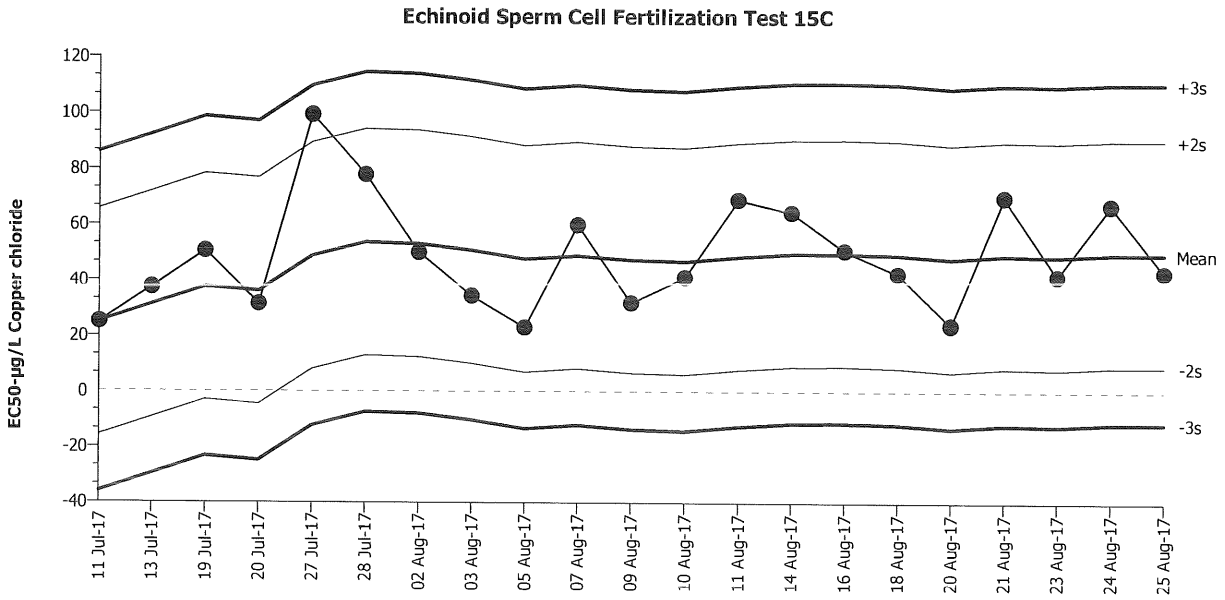
Graphics



Analyst: _____ QA: 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:	49.6	**Count:**	20	**-2s Warning Limit:**	8.924	**-3s Action Limit:**	-11.42
Sigma:	20.34	**CV:**	41.00%	**+2s Warning Limit:**	90.28	**+3s Action Limit:**	110.6

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jul	11	11:33	25.05	-24.55	-1.207			09-0588-2471	00-1661-1655
2			13	15:20	37.5	-12.1	-0.595			05-9787-5418	02-0541-0147
3			19	16:28	50.59	0.9889	0.04862			03-3446-7266	15-7259-8466
4			20	18:10	31.52	-18.08	-0.8889			17-7484-2488	03-0485-5429
5			27	15:55	99.32	49.72	2.445	(+)		02-6715-3770	17-8186-2444
6			28	10:50	77.84	28.24	1.389			21-2559-1280	14-0688-6070
7		Aug	2	15:50	50.06	0.4551	0.02237			08-9742-2478	08-8646-9232
8			3	0:00	34.43	-15.17	-0.7456			02-7356-2235	20-3051-4002
9			5	19:25	23.07	-26.53	-1.304			11-5994-0488	10-6029-2098
10			7	15:10	59.94	10.34	0.5083			21-2468-7505	14-3489-7019
11			9	17:08	31.92	-17.68	-0.8695			13-6999-3036	11-7131-4234
12			10	16:51	41.14	-8.464	-0.4161			00-5471-5288	12-0643-2211
13			11	14:50	69.03	19.43	0.9552			04-5796-5476	07-8184-6783
14			14	14:40	64.51	14.91	0.7328			02-4510-8526	01-5460-0814
15			16	16:34	50.82	1.217	0.05983			16-3259-1018	06-7497-1035
16			18	14:09	42.53	-7.069	-0.3475			12-6613-4538	02-2322-5589
17			20	14:52	24.05	-25.55	-1.256			06-9655-0092	05-8785-3700
18			21	14:46	69.95	20.35	1			08-4756-2919	20-2992-4955
19			23	16:14	41.72	-7.883	-0.3875			02-7595-3678	15-3490-2746
20			24	16:11	67.1	17.5	0.8605			04-7651-5518	20-0883-0005
21			25	14:48	43.11	-6.49	-0.3191			06-8816-1100	09-0830-4014

CETIS Test Data Worksheet

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

Start Date: 25 Aug-17
End Date: 25 Aug-17
Sample Date: 25 Aug-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 170825sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	80	8/28/17
			2	100	0	
			3	100	9	
			4	100	75	
			5	100	0	
			6	100	76	
			7	100	80	
			8	100	18	
			9	100	57	
			10	100	10	
			11	100	68	
			12	100	88	
			13	100	2	
			14	100	84	
			15	100	77	
			16	100	69	
			17	100	83	
			18	100	71	
			19	100	8	
			20	100	93	
			21	100	54	
			22	100	72	
			23	100	0	
			24	100	54	
			25	100	93	
			26	100	57	
			27	100	92	
			28	100	1	
			29	100	76	
			30	100	9	

CETIS Test Data Worksheet

Report Date: 25 Aug-17 08:58 (p 1 of 1)
Test Code: 06-8816-1100/170825sprt

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

Start Date: 25 Aug-17
End Date: 25 Aug-17
Sample Date: 25 Aug-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 170825sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	17			
0	LC	2	27			
0	LC	3	20	100	88	EG 8/25/17
0	LC	4	14			
0	LC	5	25			
10		1	7	100	71	EG
10		2	12			
10		3	1			
10		4	29			
10		5	4			
20		1	15	100	76	EG
20		2	16			
20		3	22			
20		4	18			
20		5	6			
40		1	9	100	58	EG
40		2	24			
40		3	26			
40		4	11			
40		5	21			
80		1	8	100	14	EG
80		2	10			
80		3	30			
80		4	19			
80		5	3			
160		1	23	100	0	EG
160		2	5			
160		3	28			
160		4	2			
160		5	13			

QC: EG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: $CuCl_2$ | **Start Date/Time:** 8/25/2017 1448

Test No: 170825sprt | **End Date/Time:** 8/25/2017 1528

Dilutions made by: EG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,200

Analyst: CG

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.1	8.08	33.4	15.4
10	7.8	8.05	33.6	15.3
20	7.8	8.07	33.6	15.2
40	7.8	8.07	33.5	15.5
80	7.7	8.06	33.5	15.6
160	7.7	8.06	33.3	15.4

Comments:

QC Check: AC 8/29/17 | **Final Review:** KAP 8/30/17

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

Client: Internal
Sample ID: Cu 12
Test No.: 170825spr4

Start Date/Time: 8/25/2017 / 1445
End Date/Time: 8/25/2017 / 1528
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 8/22/17

Tech initials: EG
Injection Time: 1410

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

Eggs Counted: 77, 96, 89, 78, 97 Mean: 87.4 X 50 = 4370 eggs/ml

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

(A)

Initial density: 4370 eggs/ml = ____ dilution factor egg stock ____ ml
Final density: 4000 eggs/ml - 1.0 part egg stock seawater ____ ml
____ 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):	1421	50:1	83/91	17/9
Eggs Added (0.5 ml):	1431	100:1	96/97	4/3
Test Ended:	1441	—	—	—
		—	—	—

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

Definitive Test Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1448	QC1	89	11
Eggs Added (0.5 ml):	1503	QC2	91	9
Test Ended:	1528	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) No Dilution Required

QC Check: AC 8/29/17 Final Review: VFP 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.