



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

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

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

Prepared by: Nautilus Environmental

Submitted: October 2, 2017

Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053). It is also certified by the State of California Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

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Results verified by: Adrienne Libor

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – SEPTEMBER 2017

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

Sampling Date: September 15, 2017

Test Date: September 16, 2017

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

Effluent Limitation: 16.5 TU_c

Results Summary:

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

INTRODUCTION

A discharge sample was collected in September 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 September 16, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

Sample collection and delivery were performed by IDE Americas, Inc. (IDE) personnel. Following arrival at Nautilus, an aliquot of the water sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. The sample was **stored at 4° C in the dark until used for testing**. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocols described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./Carlsbad Desalination Plant
Sample ID:	M-001 (off-spec period)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/15/17, 08:00
Sample Receipt Date, Time:	9/16/17, 14:48

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

Sample Collection Date	pH	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO ₃)	Total Chlorine (mg/L)
9/15/17	7.87	10.6	4.0	35.0	141	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	9/16/17, 17:08 through 17:48
Test Organism:	<i>Strongylocentrotus purpuratus</i> (purple sea urchin)
Test Organism Source:	Field-collected off Point Loma in San Diego, CA
Lab Control/Dilution Water:	Natural seawater (source: Scripps Institution of Oceanography inlet, 34±2 parts per thousand (ppt); 20-µm filtered
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent M-001 sample; lab control
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25.
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in the sample dilution series was compared to that observed in the laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TU_c) values.

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

RESULTS

A statistically significant decrease in the fertilization rate was 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 equal to 16.5, which meets the maximum effluent limitation of 16.5 for this permit. According to the TST analysis no significant difference was observed at any test concentrations when compared to lab control. Statistical results are summarized in Table 4, and detailed test results are summarized in Table 5. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and copies of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)	TU_c value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001	6.06	10	>15	16.5	Pass	0.86

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

TU_c = Chronic Toxic Unit: $100 \div \text{NOEC}$

TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.

Percent effect (PE) from control is calculated as: $PE = ((\text{mean response in control} - \text{mean response in the IWC}) / \text{mean response in control}) * 100$. A negative PE results when organism performance in the sample is greater than that in the control.

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

Test Concentration (% Sample)	Mean Percent Fertilization
Lab Control	92.8
2.5	94.8
5.0	93.6
6.06	92.0
10	85.8*
15	83.2*

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

QUALITY ASSURANCE

The sample was received on the day after collection and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The 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} ($\mu\text{g/L}$ Copper)	Historical Mean $EC_{50} \pm 2$ SD ($\mu\text{g/L}$ Copper)	CV (%)
9/16/17	57.0	49.7 ± 32.9	33.1

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

Historical Mean $EC_{50} \pm 2$ SD = Mean of historical test results plus or minus two standard deviations

CV = Coefficient of Variation

REFERENCES

- California Regional Water Quality Control Board Region 9, San Diego (RWQCB) 2006. Waste Discharge Requirements for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project, Discharge to the Pacific Ocean via the Encina Power Station Discharge Channel. Order No. R9-2006-0065, NPDES No. CA109223. June 2006.
- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Tidepool Scientific Software. 2000-2013. CETIS™ **Comprehensive Environmental Toxicity Information** System Software, Version 1.8.7.20
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.
- USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 20 Sep-17 13:39 (p 1 of 1)
Test Code: 1709-S153 | 13-5595-4448

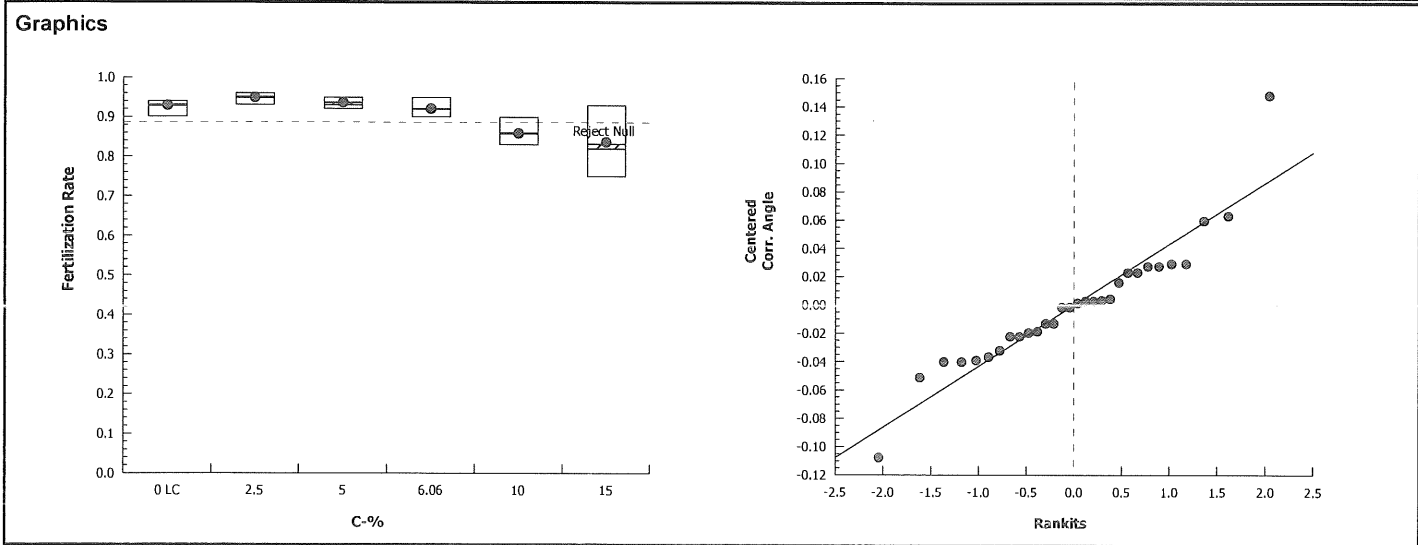
Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	01-9457-6298		Test Type: Fertilization			Analyst:					
Start Date:	16 Sep-17 17:08		Protocol: EPA/600/R-95/136 (1995)			Diluent: Laboratory Seawater					
Ending Date:	16 Sep-17 17:48		Species: Strongylocentrotus purpuratus			Brine: Not Applicable					
Duration:	40m		Source: Pt. Loma			Age:					
Sample ID:	10-1244-0834		Code: 17-1032			Client: IDE					
Sample Date:	15 Sep-17 08:00		Material: Facility Effluent			Project: Carlsbad Desal Plant					
Receive Date:	16 Sep-17 14:48		Source: IDE Americas, Inc.								
Sample Age:	33h (4 °C)		Station: M-001 (Daily)								
Comparison Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
14-3427-0519	Fertilization Rate		6.06	10	7.785	4.47%	16.5	Dunnett Multiple Comparison Test			
Point Estimate Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Method			
19-4024-4669	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		
14-3427-0519	Fertilization Rate		Control Resp		0.928	0.7 - NL		Yes	Passes Acceptability Criteria		
19-4024-4669	Fertilization Rate		Control Resp		0.928	0.7 - NL		Yes	Passes Acceptability Criteria		
14-3427-0519	Fertilization Rate		PMSD		0.04466	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	0.928	0.9076	0.9484	0.9	0.94	0.007348	0.01643	1.77%	0.0%
2.5		5	0.948	0.9318	0.9642	0.93	0.96	0.005831	0.01304	1.38%	-2.16%
5		5	0.936	0.9193	0.9527	0.92	0.95	0.006	0.01342	1.43%	-0.86%
6.06		5	0.92	0.8968	0.9432	0.9	0.95	0.008366	0.01871	2.03%	0.86%
10		5	0.858	0.8214	0.8946	0.83	0.9	0.01319	0.0295	3.44%	7.54%
15		5	0.832	0.7518	0.9122	0.75	0.93	0.02888	0.06458	7.76%	10.34%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.93	0.9	0.93	0.94	0.94					
2.5		0.93	0.94	0.95	0.96	0.96					
5		0.93	0.95	0.95	0.93	0.92					
6.06		0.92	0.91	0.92	0.95	0.9					
10		0.87	0.83	0.83	0.86	0.9					
15		0.82	0.84	0.75	0.82	0.93					

CETIS Analytical Report

Report Date: 20 Sep-17 13:39 (p 1 of 2)
 Test Code: 1709-S153 | 13-5595-4448

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 14-3427-0519		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 20 Sep-17 13:37		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		4.47%	6.06	10	7.785	16.5
Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	-1.349	2.362	0.073	8	0.9940	CDF	Non-Significant Effect		
		5	-0.5099	2.362	0.073	8	0.9415	CDF	Non-Significant Effect		
		6.06	0.4735	2.362	0.073	8	0.6584	CDF	Non-Significant Effect		
		10*	3.696	2.362	0.073	8	0.0025	CDF	Significant Effect		
		15*	4.698	2.362	0.073	8	0.0002	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.1429374		0.02858748		5		11.94	<0.0001	Significant Effect		
Error	0.05745583		0.002393993		24						
Total	0.2003932				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			9.801	15.09	0.0811		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9163	0.9031	0.0216		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	0.928	0.9076	0.9484	0.93	0.9	0.94	0.007348	1.77%	0.0%
2.5		5	0.948	0.9318	0.9642	0.95	0.93	0.96	0.005831	1.38%	-2.16%
5		5	0.936	0.9193	0.9527	0.93	0.92	0.95	0.006	1.43%	-0.86%
6.06		5	0.92	0.8968	0.9432	0.92	0.9	0.95	0.008366	2.03%	0.86%
10		5	0.858	0.8214	0.8946	0.86	0.83	0.9	0.01319	3.44%	7.54%
15		5	0.832	0.7518	0.9122	0.82	0.75	0.93	0.02888	7.76%	10.34%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.3	1.263	1.338	1.303	1.249	1.323	0.01361	2.34%	0.0%
2.5		5	1.342	1.306	1.378	1.345	1.303	1.369	0.01301	2.17%	-3.21%
5		5	1.316	1.282	1.351	1.303	1.284	1.345	0.01239	2.11%	-1.21%
6.06		5	1.286	1.241	1.331	1.284	1.249	1.345	0.01625	2.83%	1.13%
10		5	1.186	1.132	1.24	1.187	1.146	1.249	0.01931	3.64%	8.8%
15		5	1.155	1.04	1.27	1.133	1.047	1.303	0.04156	8.05%	11.18%

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)	
Analysis ID:	14-3427-0519	Endpoint:	Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed:	20 Sep-17 13:37	Analysis:	Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

Report Date: 20 Sep-17 13:39 (p 1 of 1)
Test Code: 1709-S153 | 13-5595-4448

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

Analysis ID: 19-4024-4669	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 20 Sep-17 13:37	Analysis: Linear Interpolation (ICPIN)	Official Results: Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	755455	1000	Yes	Two-Point Interpolation

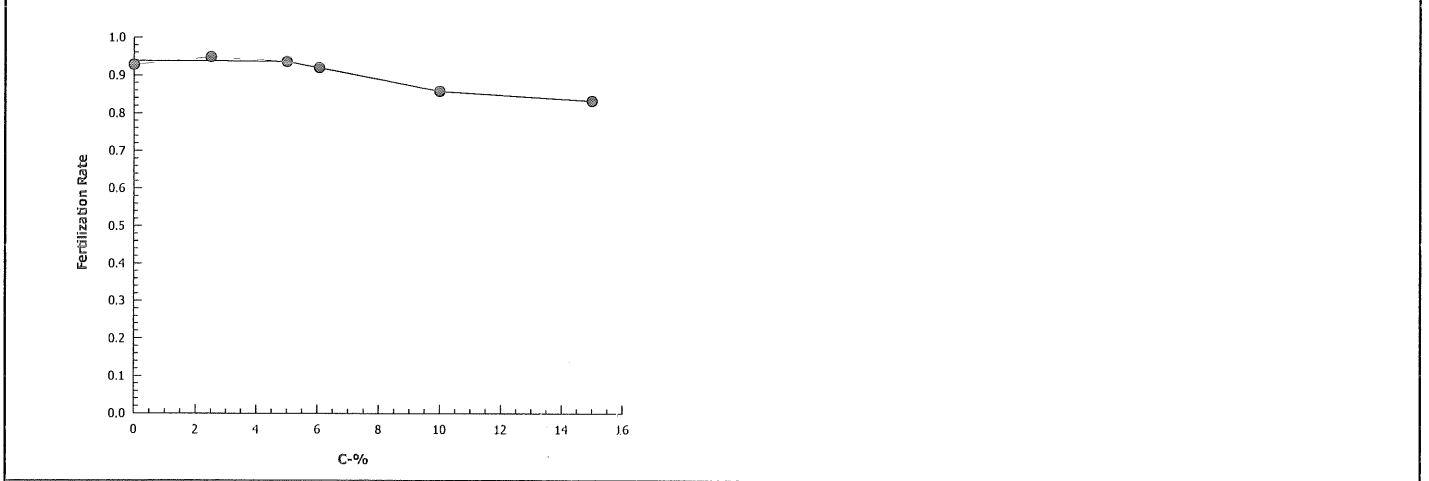
Point Estimates

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	0.928	0.9	0.94	0.007348	0.01643	1.77%	0.0%	464	500
2.5		5	0.948	0.93	0.96	0.005831	0.01304	1.38%	-2.16%	474	500
5		5	0.936	0.92	0.95	0.006	0.01342	1.43%	-0.86%	468	500
6.06		5	0.92	0.9	0.95	0.008366	0.01871	2.03%	0.86%	460	500
10		5	0.858	0.83	0.9	0.01319	0.0295	3.44%	7.54%	429	500
15		5	0.832	0.75	0.93	0.02888	0.06458	7.76%	10.34%	416	500

Graphics



CETIS Analytical Report

TST

Report Date: 20 Sep-17 13:39 (p 1 of 1)

Test Code: 1709-S153 | 13-5595-4448

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 08-9493-1760		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 20 Sep-17 13:38		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	5.73%	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*	22.19	1.895	0.031	7	<0.0001	CDF	Non-Significant Effect		
		5*	21.23	1.895	0.030	7	<0.0001	CDF	Non-Significant Effect		
		6.06*	16.18	1.943	0.037	6	<0.0001	CDF	Non-Significant Effect		
		10*	9.646	1.943	0.042	6	<0.0001	CDF	Non-Significant Effect		
		15*	4.199	2.132	0.091	4	0.0069	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.1429374		0.02858748		5		11.94	<0.0001	Significant Effect		
Error	0.05745583		0.002393993		24						
Total	0.2003932				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			9.801	15.09	0.0811		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9163	0.9031	0.0216		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	0.928	0.9076	0.9484	0.93	0.9	0.94	0.007348	1.77%	0.0%
2.5		5	0.948	0.9318	0.9642	0.95	0.93	0.96	0.005831	1.38%	-2.16%
5		5	0.936	0.9193	0.9527	0.93	0.92	0.95	0.006	1.43%	-0.86%
6.06		5	0.92	0.8968	0.9432	0.92	0.9	0.95	0.008366	2.03%	0.86%
10		5	0.858	0.8214	0.8946	0.86	0.83	0.9	0.01319	3.44%	7.54%
15		5	0.832	0.7518	0.9122	0.82	0.75	0.93	0.02888	7.76%	10.34%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.3	1.263	1.338	1.303	1.249	1.323	0.01361	2.34%	0.0%
2.5		5	1.342	1.306	1.378	1.345	1.303	1.369	0.01301	2.17%	-3.21%
5		5	1.316	1.282	1.351	1.303	1.284	1.345	0.01239	2.11%	-1.21%
6.06		5	1.286	1.241	1.331	1.284	1.249	1.345	0.01625	2.83%	1.13%
10		5	1.186	1.132	1.24	1.187	1.146	1.249	0.01931	3.64%	8.8%
15		5	1.155	1.04	1.27	1.133	1.047	1.303	0.04156	8.05%	11.18%

CETIS Test Data Worksheet

Report Date: 15 Sep-17 16:39 (p 1 of 1)

Test Code: 1709-S153 13-5595-4448/50D23910

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 16 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 16 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 15 Sep-17 Material: Facility Effluent

Sample Code: 17-1032
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (9/15 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	96	9/20/17
			32	100	95	
			33	100	93	
			34	100	93	
			35	100	82	
			36	100	93	
			37	100	91	
			38	100	92	
			39	100	93	
			40	100	94	
			41	100	95	
			42	100	96	
			43	100	90	
			44	100	93	
			45	100	87	
			46	100	83	
			47	100	94	
			48	100	92	
			49	100	82	
			50	100	95	
			51	100	95	
			52	100	93	
			53	100	92	
			54	100	94	
			55	100	90	
			56	100	83	
			57	100	84	
			58	100	90	
			59	100	75	
			60	100	86	

(A) Q18 AC ORSO SG 9/20/17

CETIS Test Data Worksheet

Report Date: 15 Sep-17 16:39 (p 1 of 1)

Test Code: 1709-S/53 13-5595-4448/50D23910

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 16 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 16 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 15 Sep-17 Material: Facility Effluent

Sample Code: 17-1032
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (9/15 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	44			
0	LC	2	43			
0	LC	3	52			
0	LC	4	54			
0	LC	5	47	100	97	AD 9/16/17
2.5		1	39			
2.5		2	40			
2.5		3	32			
2.5		4	42			
2.5		5	31	100	95	
5		1	36			
5		2	50			
5		3	51			
5		4	34			
5		5	38			
6.06		1	53	100	58	AD 9/17
6.06		2	37			
6.06		3	48			
6.06		4	41			
6.06		5	58			
10		1	45			
10		2	56			
10		3	46			
10		4	60			
10		5	55			
15		1	35			
15		2	57			
15		3	59			
15		4	49			
15		5	33			

QC: AD

AD 9/8 9/16/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 Daily 9/15 sample

Start Date/Time: 9/16/2017 1708

Sample Log No.: 17- 1032

End Date/Time: 9/16/2017 1748

Dilutions made by: AD

Test No: 1709-S153

Analyst: RT

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.13	33.5	15.8
2.5	8.4	8.12	33.7	16.0
5.0	8.4	8.11	33.8	15.8
6.06	8.4	8.11	33.9	15.8
10	8.5	8.10	34.0	15.5
15	8.6	8.09	34.1	15.2

Comments:

QC Check: AC 9/20/17

Final Review: 5/10/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDF
 Sample ID: Duke M-001 (9/15 sample)
 Test No.: 1709-S153

Start Date/Time: 9/16/2017 / 1708
 End Date/Time: 9/16/2017 / 1748
 Species: S. purpuratus
 Animal Source: PT-10ma
 Date Collected: 8/22/17

Tech initials: AD
 Injection Time: 1625

Sperm Absorbance at 400 nm: 0.951 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 89 Mean: 94 X 50 = 4800 eggs/ml

97
100
99
95

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

Initial density: 4800 eggs/ml = 1.2 dilution factor egg stock 100 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 20 ml
20 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):	<u>1635</u>	<u>50:1</u>	<u>77</u>	<u>23</u>
Eggs Added (0.5 ml):	<u>1650</u>	<u>100:1</u>	<u>8190</u>	<u>1910</u>
Test Ended:	<u>1700</u>	<u>200:1</u>	<u>96</u>	<u>4</u>

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

Definitive Test Sperm:Egg Ratio Used: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1708</u>	QC1	<u>88</u>	<u>12</u>
Eggs Added (0.5 ml):	<u>1728</u>	QC2	<u>90</u>	<u>10</u>
Test Ended:	<u>1748</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

QC Check: AC 9/20/17

Final Review: 2/10/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Daily M-001 (9/15 sample)
Test ID No(s): 1709-S153

Sample Check-In Information

Sample Description:

A: no color, clear, no odor, no debris

Sample (A, B, C):	<u>A</u>			
Log-in No. (17-xxxx):	<u>1032</u>			
Sample Collection Date & Time:	<u>9/15/17 0800</u>			
Sample Receipt Date & Time:	<u>9/16/17 1948</u>			
Number of Containers & Container Type:	<u>14 Lcdbi</u>			
Approx. Total Volume Received (L):	<u>~4L</u>			
Check-in Temperature (°C)	<u>4.0</u>			
Temperature OK? ¹	<u>(Y) N</u>	<u>Y N</u>	<u>Y N</u>	<u>Y N</u>
DO (mg/L)	<u>10.6</u>			
pH (units)	<u>7.87</u>			
Conductivity (µS/cm)	<u>—</u>			
Salinity (ppt)	<u>35.0</u>			
Alkalinity (mg/L) ²	<u>141</u>			
Hardness (mg/L) ^{2,3}	<u>—</u>			
Total Chlorine (mg/L)	<u>20.62</u>			
Technician Initials	<u>RT</u>			

Test Performed: Urchin Fert. Control/Dilution Water: 8:2 Lab SW / Lab ART Other: —

Alkalinity: 97 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: —

COC Complete (Y/N)?

A Y B — C —

Filtration? Y (N)

Pore Size: —

Organisms — or Debris —

Salinity Adjustment? Y (N)

Test: — Source: — Target ppt: —

Test: — Source: — Target ppt: —

Test: — Source: — Target ppt: —

pH Adjustment? Y (N)

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

Cl₂ Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

Subsamples for Additional Chemistry Required? Y (N)

NH₃ Other —

Tech Initials A — B — C —

QC Check: AC9/20/17

Final Review: 8/10/17

Appendix C

Chain-of-Custody Form



Turn Around Time

Normal: x

RUSH (24 hr):

3 Days:

5 Days:

??? Days

Nautilus ID: 17-103Z

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 22 Sep-17 15:47 (p 1 of 1)
 Test Code: 170916sprt | 08-9569-1329

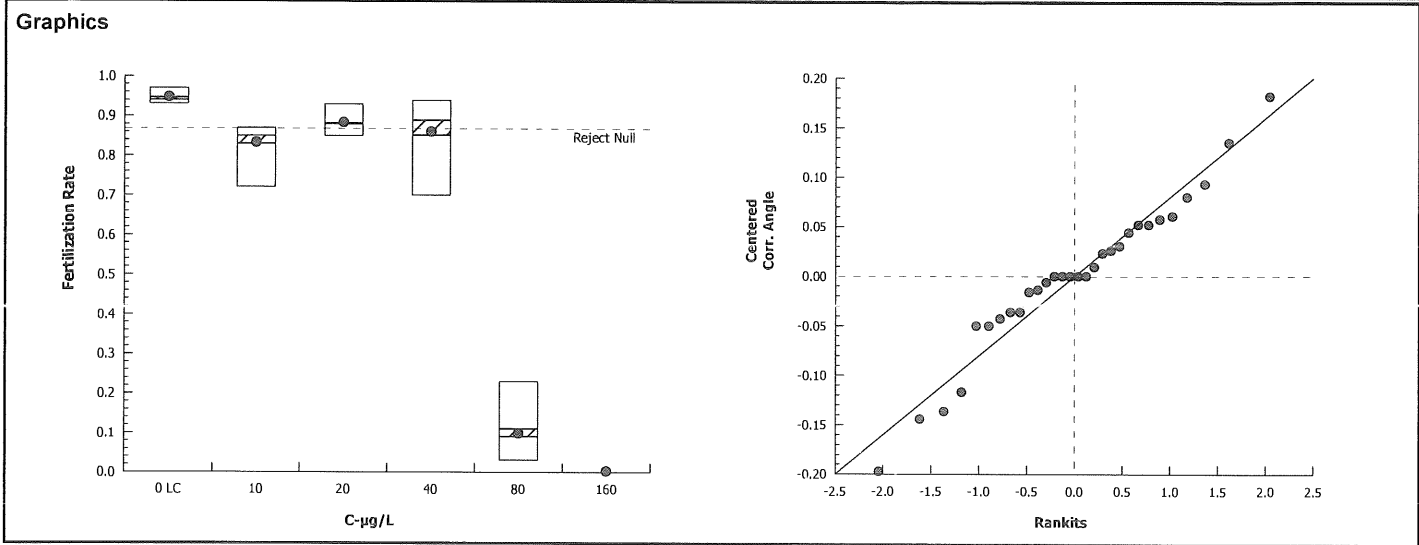
Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	05-4590-7325		Test Type:		Fertilization			Analyst:			
Start Date:	16 Sep-17 17:08		Protocol:		EPA/600/R-95/136 (1995)			Diluent:		Natural Seawater	
Ending Date:	16 Sep-17 17:48		Species:		Strongylocentrotus purpuratus			Brine:		Not Applicable	
Duration:	40m		Source:		Pt. Loma			Age:			
Sample ID:	05-7221-2427		Code:		170916sprt			Client:		Internal	
Sample Date:	16 Sep-17		Material:		Copper chloride			Project:			
Receive Date:	16 Sep-17		Source:		Reference Toxicant						
Sample Age:	17h		Station:		Copper Chloride						
Comparison Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
17-0919-7416	Fertilization Rate		20	40	28.28	8.25%		Dunnett Multiple Comparison Test			
Point Estimate Summary											
Analysis ID	Endpoint		Level	µg/L	95% LCL	95% UCL	TU	Method			
19-6375-1112	Fertilization Rate		EC50	56.97	55.27	58.72		Trimmed Spearman-Kärber			
Test Acceptability											
Analysis ID	Endpoint		Attribute		Test Stat	TAC Limits		Overlap	Decision		
17-0919-7416	Fertilization Rate		Control Resp		0.946	0.7 - NL		Yes	Passes Acceptability Criteria		
19-6375-1112	Fertilization Rate		Control Resp		0.946	0.7 - NL		Yes	Passes Acceptability Criteria		
17-0919-7416	Fertilization Rate		PMSD		0.08246	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	0.946	0.9234	0.9686	0.93	0.97	0.008124	0.01817	1.92%	0.0%
10		5	0.83	0.752	0.908	0.72	0.87	0.02811	0.06285	7.57%	12.26%
20		5	0.882	0.8395	0.9245	0.85	0.93	0.0153	0.03421	3.88%	6.77%
40		5	0.852	0.7357	0.9683	0.7	0.94	0.04188	0.09365	10.99%	9.94%
80		5	0.11	0.005008	0.215	0.03	0.23	0.03782	0.08456	76.87%	88.37%
160		5	0	0	0	0	0	0	0		100.0%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.94	0.93	0.97	0.93	0.96					
10		0.85	0.87	0.87	0.84	0.72					
20		0.85	0.85	0.88	0.93	0.9					
40		0.94	0.7	0.83	0.89	0.9					
80		0.23	0.03	0.04	0.16	0.09					
160		0	0	0	0	0					

CETIS Analytical Report

Report Date: 22 Sep-17 15:47 (p 1 of 2)
Test Code: 170916spt | 08-9569-1329

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 17-0919-7416		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 22 Sep-17 15:44		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		8.25%	20	40	28.28	
Dunnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		10*	3.111	2.305	0.140	8	0.0094	CDF	Significant Effect		
		20	1.908	2.305	0.140	8	0.1034	CDF	Non-Significant Effect		
		40*	2.478	2.305	0.140	8	0.0356	CDF	Significant Effect		
		80*	16.78	2.305	0.140	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	3.388831		0.8472077		4		91.63	<0.0001	Significant Effect		
Error	0.1849151		0.009245756		20						
Total	3.573746				24						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			6.867	13.28	0.1431		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9765	0.8877	0.8076		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	0.946	0.9234	0.9686	0.94	0.93	0.97	0.008124	1.92%	0.0%
10		5	0.83	0.752	0.908	0.85	0.72	0.87	0.02811	7.57%	12.26%
20		5	0.882	0.8395	0.9245	0.88	0.85	0.93	0.0153	3.88%	6.77%
40		5	0.852	0.7357	0.9683	0.89	0.7	0.94	0.04188	10.99%	9.94%
80		5	0.11	0.005008	0.215	0.09	0.03	0.23	0.03782	76.87%	88.37%
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	1.339	1.287	1.391	1.323	1.303	1.397	0.01883	3.14%	0.0%
10		5	1.15	1.052	1.248	1.173	1.013	1.202	0.03516	6.84%	14.13%
20		5	1.223	1.155	1.291	1.217	1.173	1.303	0.0246	4.5%	8.67%
40		5	1.188	1.031	1.346	1.233	0.9912	1.323	0.05682	10.69%	11.25%
80		5	0.3184	0.1467	0.49	0.3047	0.1741	0.5002	0.06182	43.42%	76.23%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.26%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID:	17-0919-7416	Endpoint:	Fertilization Rate
Analized:	22 Sep-17 15:44	Analysis:	Parametric-Control vs Treatments
		CETIS Version:	CETISv1.8.7
		Official Results:	Yes



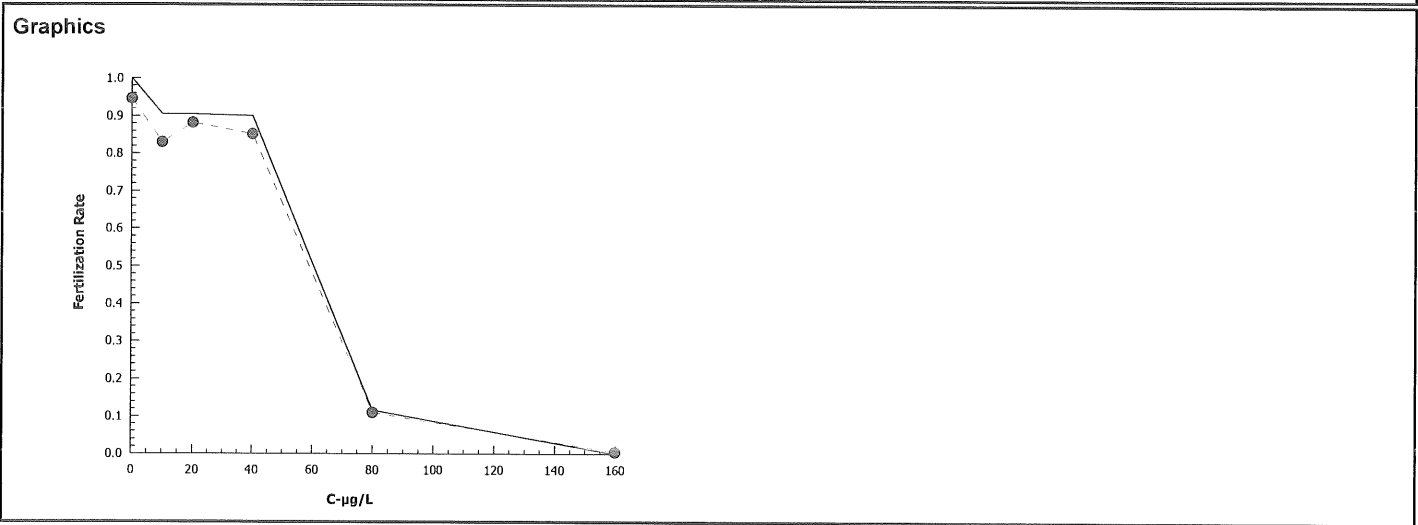
CETIS Analytical Report

Report Date: 22 Sep-17 15:47 (p 1 of 1)
 Test Code: 170916sprt | 08-9569-1329

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Analysis ID:	19-6375-1112	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7		
Analyzed:	22 Sep-17 15:44	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes		

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.054	9.51%	1.756	0.006577	56.97	55.27	58.72

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	0.946	0.93	0.97	0.008124	0.01817	1.92%	0.0%	473	500
10		5	0.83	0.72	0.87	0.02811	0.06285	7.57%	12.26%	415	500
20		5	0.882	0.85	0.93	0.0153	0.03421	3.88%	6.77%	441	500
40		5	0.852	0.7	0.94	0.04188	0.09365	10.99%	9.94%	426	500
80		5	0.11	0.03	0.23	0.03782	0.08456	76.87%	88.37%	55	500
160		5	0	0	0	0	0		100.0%	0	500



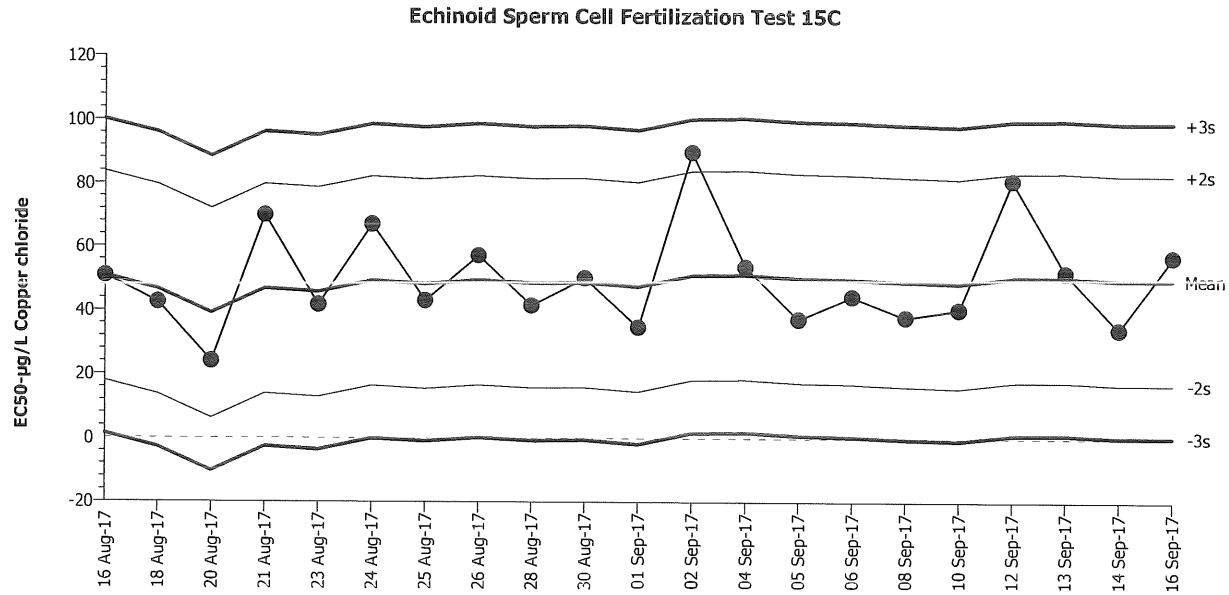
Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Organism: Strongylocentrotus purpuratus (Purpl
Endpoint: Fertilization Rate

Material: Copper chloride
Source: Reference Toxicant-REF



Mean: 49.71

Count: 20

-2s Warning Limit: 16.77

-3s Action Limit: 0.3036

Sigma: 16.47

CV: 33.10%

+2s Warning Limit: 82.65

+3s Action Limit: 99.12

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	16	16:34	50.82	1.107	0.06721			16-3259-1018	06-7497-1035
2			18	14:09	42.53	-7.179	-0.4359			12-6613-4538	02-2322-5589
3			20	14:52	24.05	-25.66	-1.558			06-9655-0092	05-8785-3700
4			21	14:46	69.95	20.24	1.229			08-4756-2919	20-2992-4955
5			23	16:14	41.72	-7.993	-0.4853			02-7595-3678	15-3490-2746
6			24	16:11	67.1	17.39	1.056			04-7651-5518	20-0883-0005
7			25	14:48	43.11	-6.6	-0.4008			06-8816-1100	09-0830-4014
8			26	16:00	57.24	7.531	0.4573			10-2039-5656	15-8794-0305
9			28	14:56	41.55	-8.158	-0.4953			08-1525-2751	10-7829-2432
10			30	16:38	50.21	0.5019	0.03047			08-1199-3706	11-0543-3886
11		Sep	1	15:27	34.79	-14.92	-0.9056			13-1244-6646	21-1567-7550
12			2	10:53	89.99	40.28	2.446	(+)		16-4202-9692	18-8681-1855
13			4	16:10	53.77	4.062	0.2466			12-2973-1405	10-6032-1229
14			5	17:07	37.36	-12.35	-0.7499			13-1627-7974	14-5447-1160
15			6	17:15	44.41	-5.297	-0.3216			05-5533-8557	16-8161-1582
16			8	15:48	37.91	-11.8	-0.7163			18-6871-7794	04-4479-5076
17			10	14:25	40.4	-9.308	-0.5651			11-6871-9499	08-4248-1228
18			12	15:51	81.07	31.36	1.904			20-0603-9450	06-1182-7961
19			13	19:07	52.04	2.326	0.1412			01-4575-6189	02-4618-7964
20			14	15:24	34.24	-15.47	-0.9396			11-2846-3680	13-8128-7168
21			16	17:08	56.97	7.26	0.4408			08-9569-1329	19-6375-1112

CETIS Test Data Worksheet

Report Date: 15 Sep-17 16:37 (p 1 of 1)
 Test Code: 08-9569-1329/170916sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 16 Sep-17 Species: Strongylocentrotus purpuratus Sample Code: 170916sprt
 End Date: 16 Sep-17 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 16 Sep-17 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	93	JS 9/21/17
			2		93	
			3		87	
			4		90	
			5		4	
			6		85	
			7		23	
			8		93	
			9		85	
			10		9	
			11		0	
			12		0	
			13		40	
			14		89	
			15		85	
			16		0	
			17		83	
			18		94	
			19		0	
			20		72	
			21		96	
			22		3	
			23		88	
			24		16	
			25		0	
			26		87	
			27		84	
			28		97	
			29		70	
			30		94	

CETIS Test Data Worksheet

Report Date: 15 Sep-17 16:37 (p 1 of 1)
Test Code: 08-9569-1329/170916sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 16 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 16 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 16 Sep-17 Material: Copper chloride

Sample Code: 170916sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	30			
0	LC	2	2			
0	LC	3	28			
0	LC	4	8			
0	LC	5	21	100	99	AD 9/16/17
10		1	6			
10		2	28			
10		3	3	100	86	
10		4	27			
10		5	20	100	82	
20		1	15			
20		2	9			
20		3	23			
20		4	1			
20		5	4	100	90	
40		1	18			
40		2	29			
40		3	17			
40		4	14	100	90	
40		5	13	100	86	
80		1	7			
80		2	22			
80		3	5			
80		4	24			
80		5	10	100	29	
160		1	16			
160		2	11			
160		3	25			
160		4	19			
160		5	12	100	0	

QC: AD

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 9/16/2017 1708Test No: 170916sprtEnd Date/Time: 9/16/2017 1748Dilutions made by: AD

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: RT

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.6	8.13	33.4	14.8
10	8.5	8.09	33.7	14.8
20	8.4	8.09	33.6	14.7
40	8.4	8.09	33.5	14.6
80	8.4	8.09	33.4	14.6
160	8.4	8.09	33.5	14.6

Comments: _____

QC Check: EG 9/22/17Final Review: AC 9/28/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: Cvch
 Test No.: 170916spt
 Tech initials: AD
 Injection Time: 1625

Start Date/Time: 9/16/2017 11708
 End Date/Time: 9/16/2017 11748
 Species: S. purpuratus
 Animal Source: PT. lorna
 Date Collected: 8/22/17

Sperm Absorbance at 400 nm: 0.951 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 89 Mean: 94 X 50 = 4800 eggs/ml

97
100
99
95

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

Initial density: 4800 eggs/ml = 1.2 dilution factor egg stock 100 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 20 ml
0.20 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							
Range Finder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1635</u>	<u>50:1</u>	<u>77</u>	<u>23</u>
Eggs Added (0.5 ml):	<u>1650</u>	<u>100:1</u>	<u>8190</u>	<u>19110</u>
Test Ended:	<u>1700</u>	<u>200:1</u>	<u>96</u>	<u>4</u>

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

Definitive Test Sperm:Egg Ratio Used: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1708</u>	QC1	<u>88</u>	<u>12</u>
Eggs Added (0.5 ml):	<u>1728</u>	QC2	<u>90</u>	<u>10</u>
Test Ended:	<u>1748</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

QC Check: Eq 9/22/17 Final Review: AC 9/28/17

Appendix E

Qualifier Codes

Glossary of Qualifier Codes:

- Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 - Temperatures out of recommended range; no action taken, test terminated same day
- Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L
- Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 - Test initiated with aeration due to an anticipated drop in D.O.
- Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 - Salinity out of recommended range
- Q8 - Spilled test chamber/ Unable to recover test organism(s)
- Q9 - Inadequate sample volume remaining, 50% renewal performed
- Q10 - Inadequate sample volume remaining, no renewal performed
- Q11 - Sample out of holding time; refer to QA section of report
- Q12 - Replicate(s) not initiated; excluded from data analysis
- Q13 - Survival counts not recorded due to poor visibility or heavy debris
- Q14 - D.O. percent saturation was checked and was $\leq 110\%$
- Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.
- Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.
- Q18 - Incorrect Entry
- Q19 - Illegible Entry
- Q20 - Miscalculation
- Q21 - Other (provide reason in comments section)
- Q22 - Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.
- Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.