



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: September 21, 2017

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

Prepared by: Nautilus Environmental

Submitted: October 6, 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 21, 2017

Test Date: September 22, 2017

Sample ID: M-001 (plant by-pass period)

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type: Urchin Fertilization	Effluent Test Results		Effluent Limitation Met? (Yes/No)
	NOEC	TU _c	
	<2.5	>40	No

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 plant by-pass. 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 22, 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 (plant by-pass period)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/21/17, 08:00
Sample Receipt Date, Time:	9/22/17, 12:55

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)
9/21/17	7.48	9.6	2.5	32.0	136	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	9/22/17, 14:50 through 15:30
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 all effluent concentrations tested relative to the lab control. The NOEC is reported as less than 2.5 and the TU_c is greater than 40, which is above the maximum effluent limitation of 16.5 for this permit. According to the TST analysis, a significant effect was observed at 15 percent effluent concentration 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	<2.5	2.5	>15	>40	Pass	6.6

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	93.4
2.5	86.6*
5.0	85.6*
6.06	87.2*
10	84.8*
15	75.0*

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

QUALITY ASSURANCE

The sample was received the day after it was collected and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations reported are deemed reliable. Additionally, appropriate alpha 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 (%)
9/20/17	33.6	49.9 ± 33.3	33.4

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: 29 Sep-17 08:30 (p 1 of 1)
 Test Code: 1709-S207 | 09-4269-1276

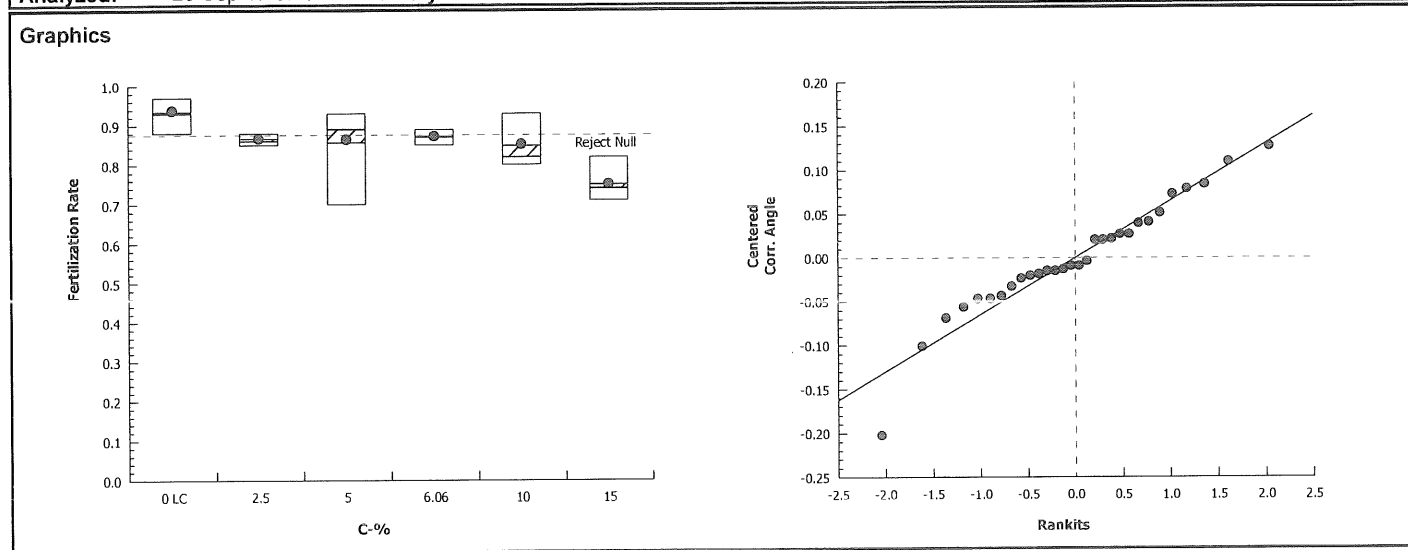
Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	08-8008-4079	Test Type:	Fertilization	Analyst:							
Start Date:	22 Sep-17 14:50	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	22 Sep-17 15:30	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	21-3223-4699	Code:	17-1053	Client:	IDE						
Sample Date:	21 Sep-17 08:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	22 Sep-17 12:55	Source:	IDE Americas, Inc.								
Sample Age:	31h (2.5 °C)	Station:	M-001 (Daily)								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
16-2812-1508	Fertilization Rate	<2.5	2.5	NA	6.23%	>40	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
11-5982-7793	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					
11-5982-7793	Fertilization Rate	Control Resp	0.934	0.7 - NL	Yes	Passes Acceptability Criteria					
16-2812-1508	Fertilization Rate	Control Resp	0.934	0.7 - NL	Yes	Passes Acceptability Criteria					
16-2812-1508	Fertilization Rate	PMSD	0.06234	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	0.934	0.8905	0.9775	0.88	0.97	0.01568	0.03507	3.76%	0.0%
2.5		5	0.866	0.8493	0.8827	0.85	0.88	0.006	0.01342	1.55%	7.28%
5		5	0.856	0.7417	0.9703	0.7	0.93	0.04118	0.09209	10.76%	8.35%
6.06		5	0.872	0.8498	0.8942	0.85	0.89	0.008	0.01789	2.05%	6.64%
10		5	0.848	0.7792	0.9168	0.8	0.93	0.02478	0.05541	6.53%	9.21%
15		5	0.75	0.6924	0.8076	0.71	0.82	0.02074	0.04637	6.18%	19.7%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.96	0.97	0.93	0.93	0.88					
2.5		0.86	0.85	0.88	0.88	0.86					
5		0.89	0.91	0.85	0.7	0.93					
6.06		0.89	0.89	0.85	0.86	0.87					
10		0.81	0.82	0.88	0.8	0.93					
15		0.71	0.74	0.77	0.71	0.82					

CETIS Analytical Report

Report Date: 29 Sep-17 08:30 (p 1 of 2)
Test Code: 1709-S207 | 09-4269-1276

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 16-2812-1508		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 29 Sep-17 8:29		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		6.23%	<2.5	2.5	NA	>40	
Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5*	2.675	2.362	0.107	8	0.0261	CDF	Significant Effect		
		5*	2.745	2.362	0.107	8	0.0225	CDF	Significant Effect		
		6.06*	2.473	2.362	0.107	8	0.0399	CDF	Significant Effect		
		10*	3.125	2.362	0.107	8	0.0096	CDF	Significant Effect		
		15*	5.928	2.362	0.107	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.1840878		0.03681755		5		7.142	0.0003	Significant Effect		
Error	0.1237248		0.005155201		24						
Total	0.3078126				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			13.79	15.09	0.0170		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9519	0.9031	0.1903		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	0.934	0.8905	0.9775	0.93	0.88	0.97	0.01568	3.76%	0.0%
2.5		5	0.866	0.8493	0.8827	0.86	0.85	0.88	0.006	1.55%	7.28%
5		5	0.856	0.7417	0.9703	0.89	0.7	0.93	0.04118	10.76%	8.35%
6.06		5	0.872	0.8498	0.8942	0.87	0.85	0.89	0.008	2.05%	6.64%
10		5	0.848	0.7792	0.9168	0.82	0.8	0.93	0.02478	6.53%	9.21%
15		5	0.75	0.6924	0.8076	0.74	0.71	0.82	0.02074	6.18%	19.7%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.318	1.231	1.405	1.303	1.217	1.397	0.03121	5.3%	0.0%
2.5		5	1.196	1.172	1.221	1.187	1.173	1.217	0.008837	1.65%	9.22%
5		5	1.193	1.041	1.346	1.233	0.9912	1.303	0.05485	10.28%	9.46%
6.06		5	1.206	1.172	1.239	1.202	1.173	1.233	0.01199	2.23%	8.52%
10		5	1.176	1.073	1.279	1.133	1.107	1.303	0.03715	7.06%	10.77%
15		5	1.049	0.9805	1.117	1.036	1.002	1.133	0.02453	5.23%	20.43%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 16-2812-1508	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 29 Sep-17 8:29	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

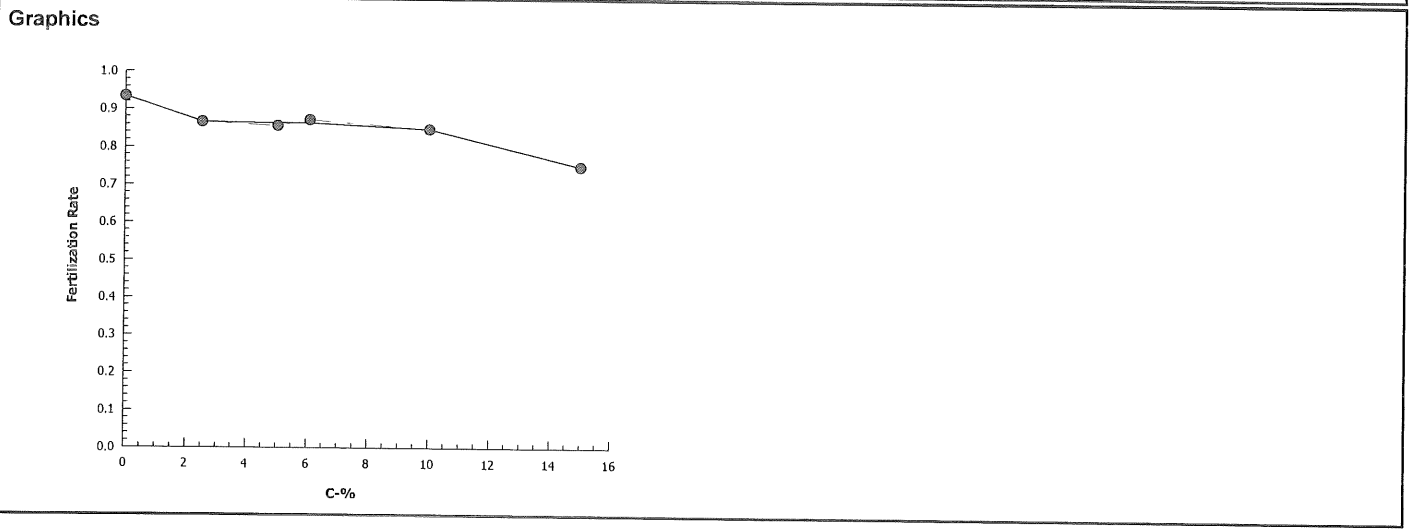
Report Date: 29 Sep-17 08:30 (p 1 of 1)
Test Code: 1709-S207 | 09-4269-1276

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID:	11-5982-7793	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	29 Sep-17 8:29	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	464304	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	0.934	0.88	0.97	0.01568	0.03507	3.76%	0.0%	467	500	
2.5		5	0.866	0.85	0.88	0.006	0.01342	1.55%	7.28%	433	500	
5		5	0.856	0.7	0.93	0.04118	0.09209	10.76%	8.35%	428	500	
6.06		5	0.872	0.85	0.89	0.008	0.01789	2.05%	6.64%	436	500	
10		5	0.848	0.8	0.93	0.02478	0.05541	6.53%	9.21%	424	500	
15		5	0.75	0.71	0.82	0.02074	0.04637	6.18%	19.7%	375	500	



CETIS Analytical Report

TST

Report Date: 29 Sep-17 08:30 (p 1 of 1)

Test Code: 1709-S207 | 09-4269-1276

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 20-5304-3124		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 29 Sep-17 8:29		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	3.35%	10	15	12.25	10
TST-Welch's t Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5*	8.312	2.015	0.050	5	0.0002	CDF	Non-Significant Effect		
		5*	3.435	2.015	0.120	5	0.0093	CDF	Non-Significant Effect		
		6.06*	8.257	2.015	0.053	5	0.0002	CDF	Non-Significant Effect		
		10*	4.271	1.943	0.085	6	0.0026	CDF	Non-Significant Effect		
		15	1.777	1.895	0.064	7	0.0594	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.1840878		0.03681755		5		7.142	0.0003	Significant Effect		
Error	0.1237248		0.005155201		24						
Total	0.3078126				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			13.79	15.09	0.0170		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9519	0.9031	0.1903		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	0.934	0.8905	0.9775	0.93	0.88	0.97	0.01568	3.76%	0.0%
2.5		5	0.866	0.8493	0.8827	0.86	0.85	0.88	0.006	1.55%	7.28%
5		5	0.856	0.7417	0.9703	0.89	0.7	0.93	0.04118	10.76%	8.35%
6.06		5	0.872	0.8498	0.8942	0.87	0.85	0.89	0.008	2.05%	6.64%
10		5	0.848	0.7792	0.9168	0.82	0.8	0.93	0.02478	6.53%	9.21%
15		5	0.75	0.6924	0.8076	0.74	0.71	0.82	0.02074	6.18%	19.7%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.318	1.231	1.405	1.303	1.217	1.397	0.03121	5.3%	0.0%
2.5		5	1.196	1.172	1.221	1.187	1.173	1.217	0.008837	1.65%	9.22%
5		5	1.193	1.041	1.346	1.233	0.9912	1.303	0.05485	10.28%	9.46%
6.06		5	1.206	1.172	1.239	1.202	1.173	1.233	0.01199	2.23%	8.52%
10		5	1.176	1.073	1.279	1.133	1.107	1.303	0.03715	7.06%	10.77%
15		5	1.049	0.9805	1.117	1.036	1.002	1.133	0.02453	5.23%	20.43%

CETIS Test Data Worksheet

Report Date: 21 Sep-17 18:05 (p 1 of 1)

Test Code: 1709-809 09-4269-1276/383053CC

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 22 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 22 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 21 Sep-17 Material: Facility Effluent

Sample Code: 17-1053
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (9/21 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	88	JA 9/28/17
			32		86	
			33		97	
			34		77	
			35		71	
			36		74	
			37		93	
			38		86	
			39		81	
			40		93	
			41		88	
			42		91	
			43		85	
			44		89	
			45		89	
			46		96	
			47		80	
			48		87	
			49		71	
			50		70	
			51		93	
			52		85	
			53		89	
			54		88	
			55		82	
			56		82	
			57		83 88	
			58		93	
			59		85	
			60		86	

JA 9/28/17

CETIS Test Data Worksheet

Report Date: 21 Sep-17 18:05 (p 1 of 1)
 Test Code: 1709-S207 09-4269-1276/383053CC

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 22 Sep-17 Species: Strongylocentrotus purpuratus
 End Date: 22 Sep-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 21 Sep-17 Material: Facility Effluent

Sample Code: 17- 1053
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Daily) (9/21 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	46			
0	LC	2	33			
0	LC	3	58			
0	LC	4	40	100	90	9/22/17 RT
0	LC	5	54			
2.5		1	38			
2.5		2	52			
2.5		3	31			
2.5		4	41	100	89	9/22/17 RT
2.5		5	32			
5		1	45			
5		2	42	100	85	9/22/17 RT
5		3	43			
5		4	50			
5		5	37			
6.06		1	53	100	82	9/22/17 RT
6.06		2	44			
6.06		3	59			
6.06		4	60			
6.06		5	48			
10		1	39			
10		2	55			
10		3	57			
10		4	47	100	81	9/22/17 RT
10		5	51			
15		1	35			
15		2	36			
15		3	34	100	77	9/22/17 RT
15		4	49			
15		5	56			

QC: 16

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (9/21 Sample)

Start Date/Time: 9/22/2017 1450

Sample Log No.: 17- 1053

End Date/Time: 9/22/2017 ~~1510~~ 1530

Dilutions made by: CG

Test No: 1709-8207

Analyst:

CG

Concentration %	initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.6	8.09	33.4	14.3 (A)
2.5	7.5	8.07	33.3	14.3
5.0	7.8	8.07	33.4	14.3
6.06	7.9	8.08	33.6	14.3
10	8.0	8.08	33.5	14.3
15	8.2	8.06	33.5	14.3 ✓

Comments:

(A) Temperature taken from surrogate vial. (B) AO at 9/22/17

QC Check:

AC 9/28/17

Final Review:

8/10/2017

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: Daily M-001 (9/21 sample)
 Test No.: 1709-S207

Start Date/Time: 9/22/2017 / 1450
 End Date/Time: 9/22/2017 / 1530
 Species: S. purpuratus
 Animal Source: Pe. Loma
 Date Collected: 9/5/17

Tech initials: CG
 Injection Time: 1400

Sperm Absorbance at 400 nm: 0.920 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 95 Mean: 93.0 $\times 50 =$ 4690 eggs/ml

92
95
99
88

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

Initial density: 4690 eggs/ml = 1.17 dilution factor egg stock 100 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 17 ml
0.17 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>1418</u>	<u>50:1</u>	<u>63</u>	<u>37</u>
Eggs Added (0.5 ml):	<u>1432</u>	<u>100:1</u>	<u>91.92</u>	<u>9.8</u>
Test Ended:	<u>1442</u>	<u>100:200:1</u>	<u>98</u>	<u>2</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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1450</u>	QC1	<u>90</u>	<u>10</u>
Eggs Added (0.5 ml):	<u>1510</u>	QC2	<u>95</u>	<u>5</u>
Test Ended:	<u>1530</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

ACG 6/29/17

QC Check:

AC 9/28/17

Final Review:

WS 10/2/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Daily M-001 (9/21 sample)
Test ID No(s): 1709-S207

Sample Check-In Information

Sample Description:

A: no color, clear, odorless, no debris

Sample (A, B, C):	M-001			
Log-in No. (17-):xxx:	1053			
Sample Collection Date & Time:	9/21/17 0800			
Sample Receipt Date & Time:	9/22/17 1255			
Number of Containers & Container Type:	14L wbi			
Approx. Total Volume Received (L):	4L			
Check-in Temperature (°C)	2.5			
Temperature OK? ¹	<input checked="" type="radio"/> Y <input type="radio"/> N	<input type="radio"/> Y <input type="radio"/> N	<input type="radio"/> Y <input type="radio"/> N	<input type="radio"/> Y <input type="radio"/> N
DO (mg/L)	9.6			
pH (units)	7.48			
Conductivity (µS/cm)	—			
Salinity (ppt)	32.0			
Alkalinity (mg/L) ²	136			
Hardness (mg/L) ^{2,3}	—			
Total Chlorine (mg/L)	20.02			
Technician Initials	BO/DM			

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

Alkalinity: 97 Hardness or Salinity: 34 ppt

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 ☐ N C ☐ N

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: —

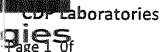
Tech Initials A — B — C —

QC Check: AC 9/28/17

Final Review: BW 10/2/17

Appendix C

Chain-of-Custody Form



Other: _____

??? Days

Nautilus 1P: 17-1053

$$T_{\text{emp}} = 2.5^\circ\text{C}$$

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 05 Oct-17 10:29 (p 1 of 1)
Test Code: 170922sprt | 20-3341-5102

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	06-1631-0929		Test Type:	Fertilization		Analyst:					
Start Date:	22 Sep-17 14:50		Protocol:	EPA/600/R-95/136 (1995)		Diluent:	Natural Seawater				
Ending Date:	22 Sep-17 15:30		Species:	Strongylocentrotus purpuratus		Brine:	Not Applicable				
Duration:	40m		Source:	Pt. Loma		Age:					
Sample ID:	06-3759-5975		Code:	170922sprt		Client:	Internal				
Sample Date:	22 Sep-17		Material:	Copper chloride		Project:					
Receive Date:	22 Sep-17		Source:	Reference Toxicant							
Sample Age:	15h		Station:	Copper Chloride							
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
10-5962-0866	Fertilization Rate	<10	10	NA	15.2%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
00-7052-6214	Fertilization Rate	EC50	54.61	51.52	57.88		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits		Overlap	Decision			
00-7052-6214	Fertilization Rate	Control Resp		0.888	0.7 - NL		Yes	Passes Acceptability Criteria			
10-5962-0866	Fertilization Rate	Control Resp		0.888	0.7 - NL		Yes	Passes Acceptability Criteria			
10-5962-0866	Fertilization Rate	PMSD		0.1515	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	0.888	0.8308	0.9452	0.83	0.93	0.02059	0.04604	5.19%	0.0%
10		5	0.69	0.5918	0.7882	0.58	0.79	0.03536	0.07906	11.46%	22.3%
20		5	0.772	0.6547	0.8893	0.65	0.85	0.04224	0.09445	12.23%	13.06%
40		5	0.638	0.4982	0.7778	0.52	0.75	0.05034	0.1126	17.64%	28.15%
80		5	0.25	0.05784	0.4422	0.05	0.4	0.06921	0.1548	61.9%	71.85%
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	0.93	0.83	0.85	0.9	0.93					
10		0.71	0.65	0.72	0.58	0.79					
20		0.85	0.84	0.65	0.69	0.83					
40		0.75	0.52	0.64	0.75	0.53					
80		0.05	0.12	0.4	0.35	0.33					
160		0	0	0	0	0					

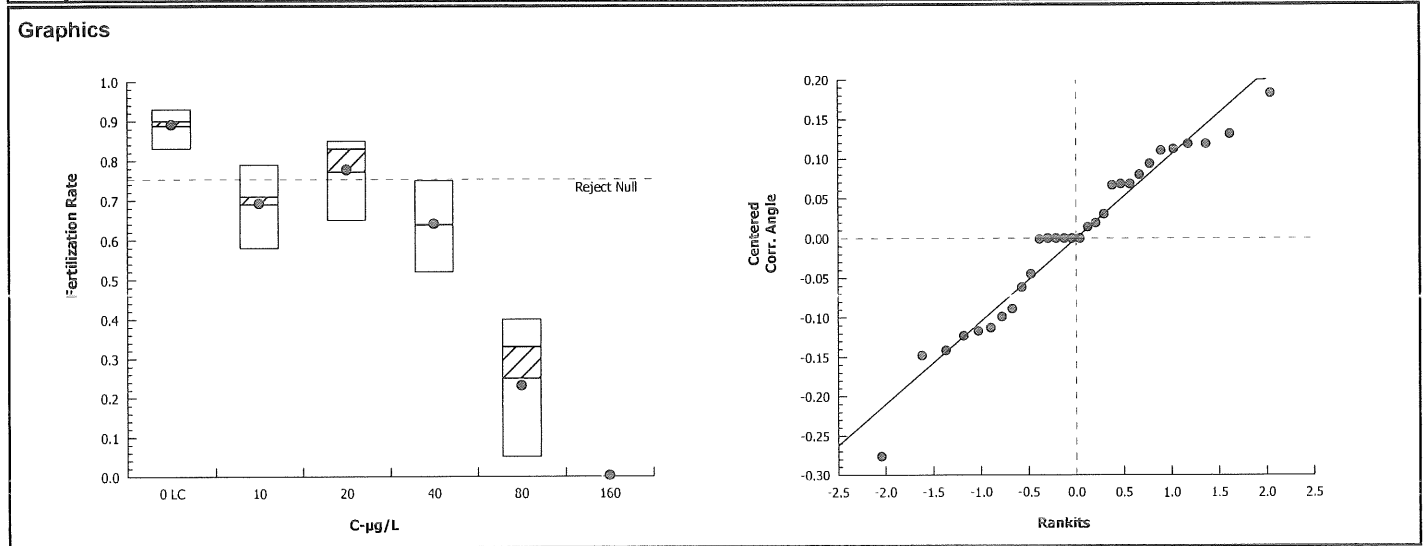
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CETIS Analytical Report

Report Date: 05 Oct-17 10:28 (p 1 of 2)
Test Code: 170922sprt | 20-3341-5102

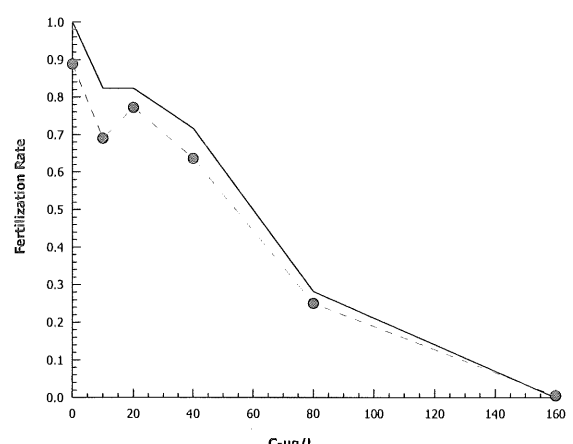
Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 10-5962-0866		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 05 Oct-17 10:28		Analysis: Parametric-Control vs Treatments					Official Results: Yes				
Data Transform		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C > T	NA	NA		15.2%	<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*	3.164	2.305	0.184	8	0.0084	CDF	Significant Effect		
		20	1.952	2.305	0.184	8	0.0957	CDF	Non-Significant Effect		
		40*	3.844	2.305	0.184	8	0.0018	CDF	Significant Effect		
		80*	9.199	2.305	0.184	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	1.500668		0.375167		4		23.64	<0.0001	Significant Effect		
Error	0.3174572		0.01587286		20						
Total	1.818125				24						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			4.733	13.28	0.3158	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9437	0.8877	0.1799	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	0.888	0.8308	0.9452	0.9	0.83	0.93	0.02059	5.19%	0.0%
10		5	0.69	0.5918	0.7882	0.71	0.58	0.79	0.03536	11.46%	22.3%
20		5	0.772	0.6547	0.8893	0.83	0.65	0.85	0.04224	12.23%	13.06%
40		5	0.638	0.4982	0.7778	0.64	0.52	0.75	0.05034	17.64%	28.15%
80		5	0.25	0.05784	0.4422	0.33	0.05	0.4	0.06921	61.9%	71.85%
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	1.235	1.144	1.325	1.249	1.146	1.303	0.03259	5.9%	0.0%
10		5	0.9827	0.876	1.089	1.002	0.8657	1.095	0.03845	8.75%	20.42%
20		5	1.079	0.9412	1.217	1.146	0.9377	1.173	0.04973	10.3%	12.6%
40		5	0.9285	0.7814	1.076	0.9273	0.8054	1.047	0.05297	12.76%	24.81%
80		5	0.5018	0.2526	0.751	0.6119	0.2255	0.6847	0.08974	39.99%	59.36%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.95%

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	10-5962-0866	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	05 Oct-17 10:28	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes



CETIS Analytical Report

Report Date: 05 Oct-17 10:29 (p 1 of 1)
Test Code: 170922spt | 20-3341-5102

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID: 00-7052-6214		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 05 Oct-17 10:28		Analysis: Trimmed Spearman-Kärber		Official Results: Yes							
Trimmed Spearman-Kärber Estimates											
Threshold Option		Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL			
Control Threshold		0.112	17.68%	1.737	0.01265	54.61	51.52	57.88			
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	0.888	0.83	0.93	0.02059	0.04604	5.19%	0.0%	444	500
10		5	0.69	0.58	0.79	0.03536	0.07906	11.46%	22.3%	345	500
20		5	0.772	0.65	0.85	0.04224	0.09445	12.23%	13.06%	386	500
40		5	0.638	0.52	0.75	0.05034	0.1126	17.64%	28.15%	318	500
80		5	0.25	0.05	0.4	0.06921	0.1548	61.9%	71.85%	125	500
160		5	0	0	0	0	0		100.0%	0	500
Graphics											
											

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

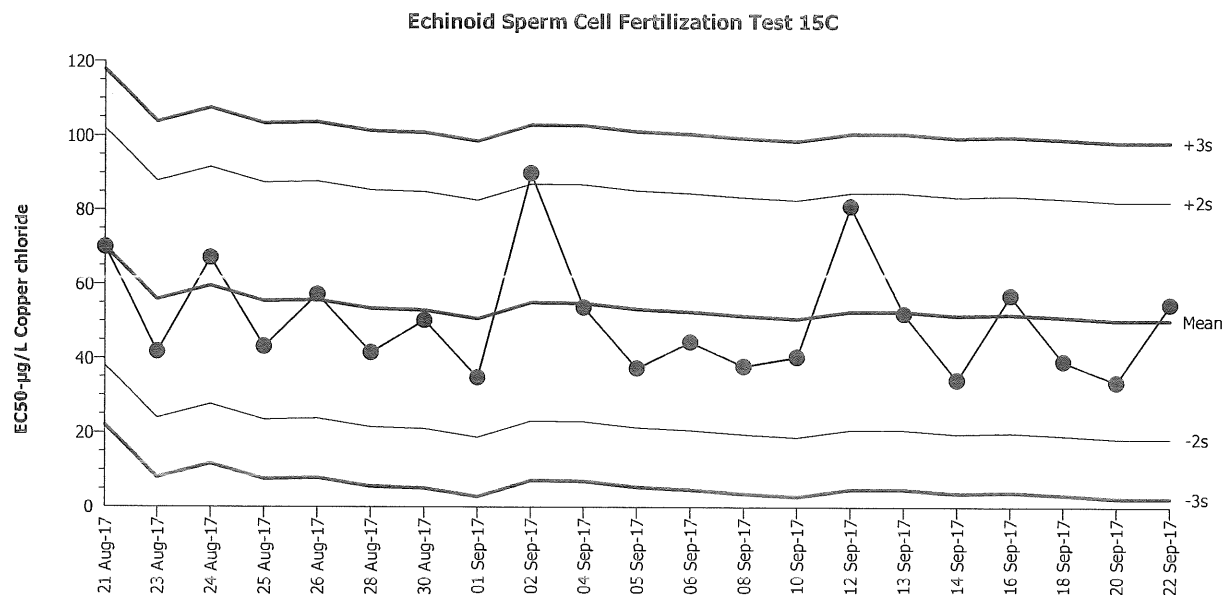
Organism: Strongylocentrotus purpuratus (Purpl

Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF



Mean: 50.33

Count: 20

-2s Warning Limit: 18.37

-3s Action Limit: 2.394

Sigma: 15.98

CV: 31.80%

+2s Warning Limit: 82.29

+3s Action Limit: 98.27

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	21	14:46	69.95	19.62	1.228			08-4756-2919	20-2992-4955
2			23	16:14	41.72	-8.613	-0.539			02-7595-3678	15-3490-2746
3			24	16:11	67.1	16.77	1.05			04-7651-5518	20-0883-0005
4			25	14:48	43.11	-7.22	-0.4518			06-8816-1100	09-0830-4014
5			26	16:00	57.24	6.911	0.4325			10-2039-5656	15-8794-0305
6			28	14:56	41.55	-8.778	-0.5493			08-1525-2751	10-7829-2432
7			30	16:38	50.21	-0.1181	-0.00739			08-1199-3706	11-0543-3886
8		Sep	1	15:27	34.79	-15.54	-0.9722			13-1244-6646	21-1567-7550
9			2	10:53	89.99	39.66	2.482	(+)		16-4202-9692	18-8681-1855
10			4	16:10	53.77	3.442	0.2154			12-2973-1405	10-6032-1229
11			5	17:07	37.36	-12.97	-0.8117			13-1627-7974	14-5447-1160
12			6	17:15	44.41	-5.917	-0.3703			05-5533-8557	16-8161-1582
13			8	15:48	37.91	-12.42	-0.777			18-6871-7794	04-4479-5076
14			10	14:25	40.4	-9.928	-0.6213			11-6871-9499	08-4248-1228
15			12	15:51	81.07	30.74	1.924			20-0603-9450	06-1182-7961
16			13	19:07	52.04	1.706	0.1068			01-4575-6189	02-4618-7964
17			14	15:24	34.24	-16.09	-1.007			11-2846-3680	13-8128-7168
18			16	17:08	56.97	6.64	0.4155			08-9569-1329	19-6375-1112
19			18	15:28	39.21	-11.12	-0.6961			19-2924-5672	02-0031-2532
20			20	16:15	33.62	-16.71	-1.045			00-4454-0074	17-7214-1415
21			22	14:50	54.61	4.279	0.2677			20-3341-5102	16-2759-7635

CETIS Test Data Worksheet

Report Date: 21 Sep-17 18:06 (p 1 of 1)
 Test Code: 20-3341-5102/170922sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 22 Sep-17 Species: Strongylocentrotus purpuratus
 End Date: 22 Sep-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 22 Sep-17 Material: Copper chloride

Sample Code: 170922sprt
 Sample Source: Reference Toxicant
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	65	J 9/28/17
			2		93	
			3		83	
			4		58	
			5		72	
			6		85	
			7		64	
			8		75	
			9		0	
			10		0	
			11		84	
			12		0	
			13		71	
			14		79	
			15		40	
			16		33	
			17		35	
			18		64	
			19		5	
			20		85	
			21		52	
			22		65	
			23		83	
			24		0	
			25		90	
			26		75	
			27		53	
			28		12	
			29		0	
			30		93	

CETIS Test Data Worksheet

Report Date: 21 Sep-17 18:06 (p 1 of 1)
Test Code: 20-3341-5102/170922sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 22 Sep-17 Species: Strongylocentrotus purpuratus Sample Code: 170922sprt
End Date: 22 Sep-17 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
Sample Date: 22 Sep-17 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	30	100	91	DM 9/22/17
0	LC	2	23			
0	LC	3	20			
0	LC	4	25			
0	LC	5	2			
10		1	13	100	83	DM 9/22/17
10		2	22			
10		3	5			
10		4	4			
10		5	14			
20		1	6	100	71	DM 9/22/17
20		2	11			
20		3	1			
20		4	7			
20		5	3			
40		1	8	100	72	DM 9/22/17
40		2	21			
40		3	18	100	49	CG 9/22/17
40		4	26			
40		5	27			
80		1	19	100	12	DM 9/22/17
80		2	28			
80		3	15			
80		4	17			
80		5	16			
160		1	29	100	0	DM 9/22/17
160		2	24			
160		3	10			
160		4	12			
160		5	9			

QC: CG

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 9/22/2017 1450Test No: 170922sprtEnd Date/Time: 9/22/2017 1530Dilutions 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):	10,200

Analyst:

CG

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.08	33.4	15.5
10	8.5	8.08	33.4	15.2
20	8.6	8.09	33.5	15.0
40	8.5	8.09	33.5	15.2
80	8.5	8.10	33.4	15.1
160	8.6	8.12	33.3 ^{33.0} (A)	15.0

Comments:

(A) CG 09/28/17

QC Check:

AC 9/28/17

Final Review:

EG 10/3/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Intermed
 Sample ID: CuCl2
 Test No.: 10922SPR
 Tech initials: CG
 Injection Time: 1400

Start Date/Time: 9/22/2017 / 1450
 End Date/Time: 9/22/2017 / 1530
 Species: S. purpuratus
 Animal Source: Pt. Loma
 Date Collected: 9/5/17

Sperm Absorbance at 400 nm: 0.920 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 95 Mean: 93.8 X 50 = 4690 eggs/ml

92
95
99
88

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

Initial density: 4690 eggs/ml = 1.17 dilution factor egg stock 100 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 17 ml
0.17 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>1418</u>	<u>50:1</u>	<u>63</u>	<u>37</u>
Eggs Added (0.5 ml):	<u>1432</u>	<u>100:1</u>	<u>91.92</u>	<u>9.8</u>
Test Ended:	<u>1442</u>	<u>0.920:200:1</u>	<u>98</u>	<u>2</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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1450</u>	QC1	<u>90</u>	<u>10</u>
Eggs Added (0.5 ml):	<u>1510</u>	QC2	<u>95</u>	<u>5</u>
Test Ended:	<u>1530</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

ACG 9/22/17

QC Check:

AC 9/28/17

Final Review: EG 10/3/17

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.