



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily/Weekly)
Sample Collection Date: September 5, 2017

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

Prepared by: Nautilus Environmental

Submitted: September 22, 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 5, 2017

Test Date: September 5, 2017

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

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type: Urchin Fertilization	Effluent Test Results		Effluent Limitation Met? (Yes/No)
	NOEC	TU _c	
	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 and weekly chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on September 5, 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 (weekly, and daily pre-treatment off-spec period)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/5/17, 08:00
Sample Receipt Date, Time:	9/5/17, 12:16

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)
9/5/17	7.90	8.0	3.0	33.7	122	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	9/5/17, 17:07 through 17:47
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 fertilization rate was observed at 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. 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 the 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	3.2

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	94.6
2.5	95.0
5.0	94.2
6.06	91.6
10	88.6*
15	77.4*

*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 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 (%)
9/5/17	37.4	49.5 \pm 34.2	34.5

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: 11 Sep-17 11:12 (p 1 of 1)

Test Code: 1709-S036 | 01-3175-6211

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	12-7452-3096		Test Type:	Fertilization		Analyst:					
Start Date:	05 Sep-17 17:07		Protocol:	EPA/600/R-95/136 (1995)		Diluent:	Laboratory Seawater				
Ending Date:	05 Sep-17 17:47		Species:	Strongylocentrotus purpuratus		Brine:	Not Applicable				
Duration:	40m		Source:	Pt. Loma		Age:					
Sample ID:	13-0420-6614		Code:	17-0974		Client:	IDE				
Sample Date:	05 Sep-17 08:00		Material:	Facility Effluent		Project:	Carlsbad Desal Plant				
Receive Date:	05 Sep-17 12:16		Source:	IDE Americas, Inc.							
Sample Age:	9h (3 °C)		Station:	M-001 (Daily) <i>weekly</i>							
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
20-7682-4076	Fertilization Rate	6.06	10	7.785	4.21%	16.5	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
03-7044-8438	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				
03-7044-8438	Fertilization Rate	Control Resp		0.946	0.7 - NL	Yes	Passes Acceptability Criteria				
20-7682-4076	Fertilization Rate	Control Resp		0.946	0.7 - NL	Yes	Passes Acceptability Criteria				
20-7682-4076	Fertilization Rate	PMSD		0.04214	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	0.946	0.9272	0.9648	0.93	0.97	0.006782	0.01517	1.6%	0.0%
2.5		5	0.95	0.9138	0.9862	0.91	0.98	0.01304	0.02915	3.07%	-0.42%
5		5	0.942	0.9075	0.9765	0.9	0.97	0.01241	0.02775	2.95%	0.42%
6.06		5	0.916	0.869	0.963	0.88	0.96	0.01691	0.03782	4.13%	3.17%
10		5	0.886	0.8536	0.9184	0.84	0.9	0.01166	0.02608	2.94%	6.34%
15		5	0.774	0.7416	0.8064	0.75	0.81	0.01166	0.02608	3.37%	18.18%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.94	0.94	0.95	0.93	0.97					
2.5		0.97	0.96	0.93	0.91	0.98					
5		0.96	0.9	0.95	0.93	0.97					
6.06		0.95	0.88	0.91	0.96	0.88					
10		0.84	0.9	0.9	0.9	0.89					
15		0.75	0.77	0.79	0.81	0.75					

CETIS Analytical Report

Report Date: 11 Sep-17 11:11 (p 1 of 2)

Test Code: 1709-S036 | 01-3175-6211

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 20-7682-4076			Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7			
Analyzed: 11 Sep-17 11:11			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		4.21%	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	-0.434	2.362	0.079	8	0.9304	CDF	Non-Significant Effect		
		5	0.1686	2.362	0.079	8	0.7787	CDF	Non-Significant Effect		
		6.06	1.657	2.362	0.079	8	0.1769	CDF	Non-Significant Effect		
		10*	3.307	2.362	0.079	8	0.0063	CDF	Significant Effect		
		15*	7.854	2.362	0.079	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.275337		0.05506741		5		19.73	<0.0001	Significant Effect		
Error	0.06698934		0.002791223		24						
Total	0.3423264				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			4.208	15.09	0.5198		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9717	0.9031	0.5864		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	0.946	0.9272	0.9648	0.94	0.93	0.97	0.006782	1.6%	0.0%
2.5		5	0.95	0.9138	0.9862	0.96	0.91	0.98	0.01304	3.07%	-0.42%
5		5	0.942	0.9075	0.9765	0.95	0.9	0.97	0.01241	2.95%	0.42%
6.06		5	0.916	0.869	0.963	0.91	0.88	0.96	0.01691	4.13%	3.17%
10		5	0.886	0.8536	0.9184	0.9	0.84	0.9	0.01166	2.94%	6.34%
15		5	0.774	0.7416	0.8064	0.77	0.75	0.81	0.01166	3.37%	18.18%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.338	1.294	1.383	1.323	1.303	1.397	0.01605	2.68%	0.0%
2.5		5	1.353	1.27	1.436	1.369	1.266	1.429	0.02999	4.96%	-1.08%
5		5	1.333	1.261	1.405	1.345	1.249	1.397	0.02597	4.36%	0.42%
6.06		5	1.283	1.194	1.372	1.266	1.217	1.369	0.03189	5.56%	4.14%
10		5	1.228	1.179	1.276	1.249	1.159	1.249	0.01743	3.17%	8.26%
15		5	1.076	1.037	1.115	1.071	1.047	1.12	0.01406	2.92%	19.61%

CETIS Analytical Report

Report Date: 11 Sep-17 11:12 (p 2 of 2)

Test Code: 1709-S036 | 01-3175-6211

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 20-7682-4076

Endpoint: Fertilization Rate

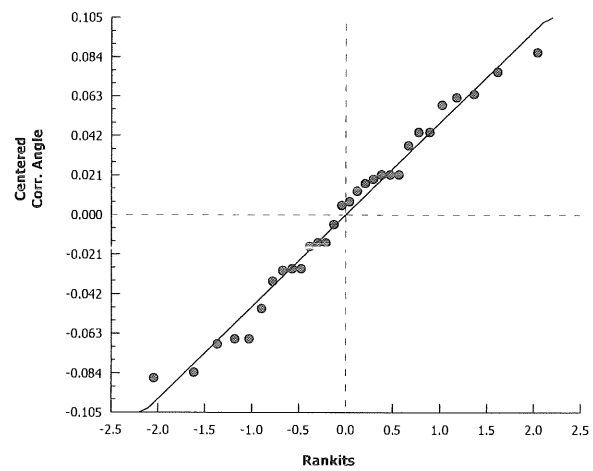
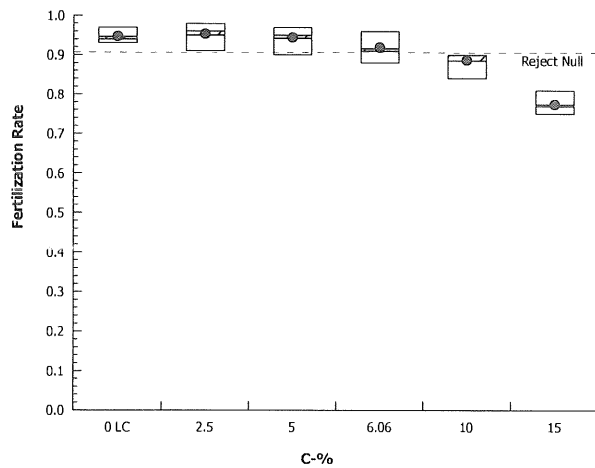
CETIS Version: CETISv1.8.7

Analyzed: 11 Sep-17 11:11

Analysis: Parametric-Control vs Treatments

Official Results: Yes

Graphics



CETIS Analytical Report

Report Date: 11 Sep-17 11:12 (p 1 of 1)

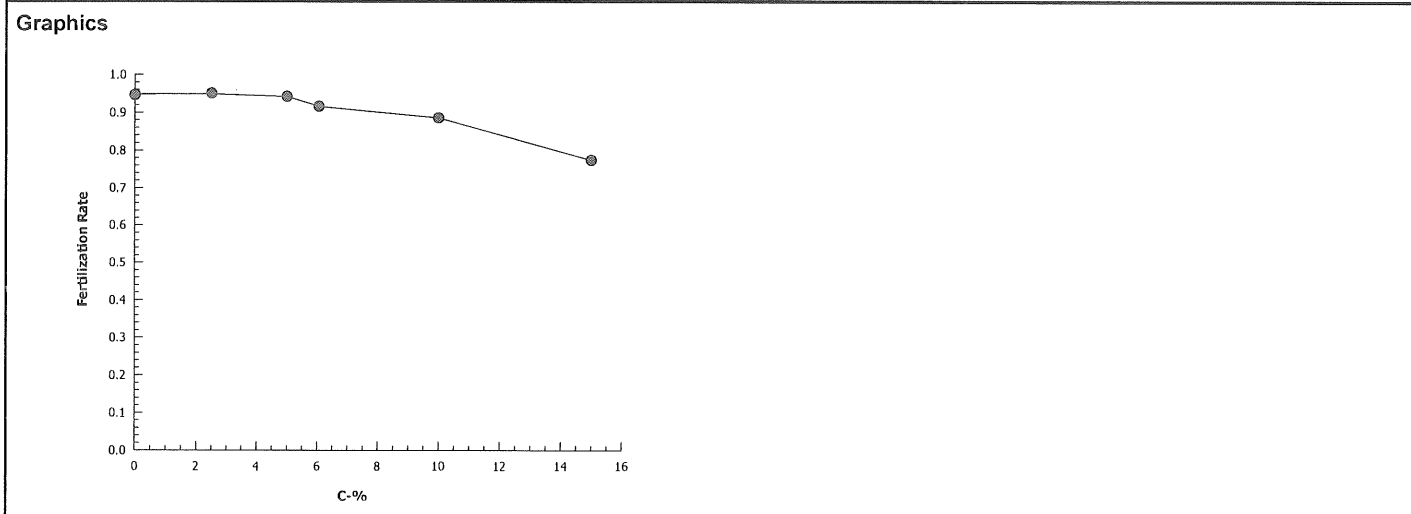
Test Code: 1709-S036 | 01-3175-6211

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	03-7044-8438	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	11 Sep-17 11:11	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1887196	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	0.946	0.93	0.97	0.006782	0.01517	1.6%	0.0%	473	500
2.5		5	0.95	0.91	0.98	0.01304	0.02915	3.07%	-0.42%	475	500
5		5	0.942	0.9	0.97	0.01241	0.02775	2.95%	0.42%	471	500
6.06		5	0.916	0.88	0.96	0.01691	0.03782	4.13%	3.17%	458	500
10		5	0.886	0.84	0.9	0.01166	0.02608	2.94%	6.34%	443	500
15		5	0.774	0.75	0.81	0.01166	0.02608	3.37%	18.18%	387	500



TST

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 05-2467-0604		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 11 Sep-17 11:11		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	1.68%	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*	10.8	2.015	0.065	5	<0.0001	CDF	Non-Significant Effect		
		5*	11.49	2.015	0.058	5	<0.0001	CDF	Non-Significant Effect		
		6.06*	8.193	2.015	0.069	5	0.0002	CDF	Non-Significant Effect		
		10*	10.58	1.895	0.040	7	<0.0001	CDF	Non-Significant Effect		
		15*	3.898	1.895	0.035	7	0.0030	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.275337		0.05506741		5		19.73	<0.0001	Significant Effect		
Error	0.06698934		0.002791223		24						
Total	0.3423264				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			4.208	15.09	0.5198		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9717	0.9031	0.5864		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	0.946	0.9272	0.9648	0.94	0.93	0.97	0.006782	1.6%	0.0%
2.5		5	0.95	0.9138	0.9862	0.96	0.91	0.98	0.01304	3.07%	-0.42%
5		5	0.942	0.9075	0.9765	0.95	0.9	0.97	0.01241	2.95%	0.42%
6.06		5	0.916	0.869	0.963	0.91	0.88	0.96	0.01691	4.13%	3.17%
10		5	0.886	0.8536	0.9184	0.9	0.84	0.9	0.01166	2.94%	6.34%
15		5	0.774	0.7416	0.8064	0.77	0.75	0.81	0.01166	3.37%	18.18%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.338	1.294	1.383	1.323	1.303	1.397	0.01605	2.68%	0.0%
2.5		5	1.353	1.27	1.436	1.369	1.266	1.429	0.02999	4.96%	-1.08%
5		5	1.333	1.261	1.405	1.345	1.249	1.397	0.02597	4.36%	0.42%
6.06		5	1.283	1.194	1.372	1.266	1.217	1.369	0.03189	5.56%	4.14%
10		5	1.228	1.179	1.276	1.249	1.159	1.249	0.01743	3.17%	8.26%
15		5	1.076	1.037	1.115	1.071	1.047	1.12	0.01406	2.92%	19.61%

CETIS Test Data Worksheet

Report Date: 01 Sep-17 09:25 (p 1 of 1)

Test Code: 1709-5086 01-3175-6211/7DA70B3

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 05 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 05 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 05 Sep-17 Material: Facility Effluent

Sample Code: 17-0974
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (9/5 Sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	96	9/8/17
			62		89	
			63		98	
			64		97	
			65		89	
			66		91	
			67		97	
			68		81	
			69		90	
			70		95	
			71		96	
			72		97	
			73		79	
			74		75	
			75		75	
			76		95	
			77		90	
			78		90 90 Q18 RL 9/8/17	
			79		93	
			80		96	
			81		88	
			82		91	
			83		88	
			84		94	
			85		77	
			86		90	
			87		93	
			88		94	
			89		93	
			90		95	

CETIS Test Data Worksheet

Report Date: 01 Sep-17 09:25 (p 1 of 1)

Test Code: 1709-5036 01-3175-6211/7DA70B3

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 05 Sep-17

Species: Strongylocentrotus purpuratus

Sample Code: 17-0974

End Date: 05 Sep-17

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

Sample Source: IDE Americas, Inc.

Sample Date: 05 Sep-17

Material: Facility Effluent

Sample Station: M-001 (Daily) (9/5 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	84			
0	LC	2	88			
0	LC	3	70			
0	LC	4	79			
0	LC	5	64	100	96	AD 9/5/17
2.5		1	72			
2.5		2	80			
2.5		3	87			
2.5		4	66			
2.5		5	63	100	96	
5		1	61			
5		2	78			
5		3	90			
5		4	89			
5		5	67	100	97	
6.06		1	76			
6.06		2	81			
6.06		3	82			
6.06		4	71			
6.06		5	83	100	90	
10		1	65			
10		2	69			
10		3	86			
10		4	77			
10		5	62	100	90	
15		1	75			
15		2	85			
15		3	73			
15		4	68			
15		5	74	100	79	

QC-CG

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 Daily (9/5 sample)

Start Date/Time: 9/5/2017 1707

Sample Log No.: 17-0974

End Date/Time: 9/5/2017 1747

Dilutions made by: AD

Test No: 1709-3036

Analyst: AD

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.4	8.07	33.4	14.7
2.5	8.3	8.06	33.7	15.4
5.0	8.3	8.06	33.7	15.4
6.06	8.4	8.06	33.7	15.4
10	8.5	8.06	33.6	15.1
15	8.5	8.06	33.7	15.0

Comments:

QC Check: AC 9/10/17

Final Review: 6 9/21/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: Daily M-001 (9/5 sample)
 Test No.: 1709-S

Start Date/Time: 9/5/2017 1707
 End Date/Time: 9/5/2017 1747
 Species: S. purpuratus
 Animal Source: Pt. Loma
 Date Collected: 8/22/17

Tech initials: GA/AD
 Injection Time: 1610

Sperm Absorbance at 400 nm: 0.923 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 116 Mean: 115.6 X 50 = 5780 eggs/ml

105
102
129
126

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

Initial density: 5780 eggs/ml = 1.445 dilution factor
 Final density: 4000 eggs/ml - 1.0 part egg stock
0.445 parts seawater

egg stock 0.22 100 ml
 seawater 0.445 ml

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
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 µl):	<u>1625</u>	<u>50:1</u>	<u>41</u>	<u>59</u>
Eggs Added (0.5 ml):	<u>1644</u>	<u>100:1</u>	<u>88, 88</u>	<u>12, 12</u>
Test Ended:		<u>200:1</u>	<u>96, 96</u>	<u>24, 11</u>
		<u>400:1</u>	<u>106</u>	<u>0</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>1707</u>	QC1	<u>72, 96</u>	<u>28, 9</u>
Eggs Added (0.5 ml):	<u>1727</u>	QC2	<u>96</u>	<u>4</u>
Test Ended:	<u>1747</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: ACG 089/5/17 BAD 018 9/5/17

QC Check: AC 9/10/17 Final Review: 9/14/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Project: CDP Compliance
Test ID No(s): 1709-5036

Sample Check-In Information

Sample Description:

A colorless, clear, odorless, light debris.

Sample ID:	Daily M-001 (9/5 sample)		
Log-in No. (17-xxxx):	0974		
Sample Collection Date & Time:	9/5/17 0800		
Sample Receipt Date & Time:	9/5/17 1216		
Number of Containers & Container Type:	1 4-L		
Approx. Total Volume Received (L):	24L		
Check-in Temperature (°C)	3.0		
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
DO (mg/L)	8.0		
pH (units)	7.90		
Conductivity (µS/cm)	—		
Salinity (ppt)	33.7		
Alkalinity (mg/L) ²	122		
Hardness (mg/L) ^{2,3}	—		
Total Chlorine (mg/L)	20.02		
Technician Initials	CG		

Test Performed: Urchin Fertilization **Control/Dilution Water:** Lab seawater
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 ☒ B ☐ C ☐

Filtration? Y ☒ N ☐

Fore 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: Aca/11/17

Final Review: 8/9/17

Appendix C

Chain-of-Custody Form



DAILY / WEEKLY

Turn Around Time
 Normal: _____ X _____
 RUSH (24 hr): _____
 3 Days: _____
 5 Days: _____
 ??? Days _____

Special instruction: Sampled during pretreatment off-spec via autosampler by a series of grabs collected at one hour intervals. Sample collected to fulfill daily and weekly NPDES requirement. Sample is to be run unadjusted. Start: 9/4/17 @ 8:00, End: 9/5/17 @ 8:00 VH

ANALYSES

NOTES:

Glass=G Plastic=P

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

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

[illegible]

9/5/17

Nautilus ID# 17-0974

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 11 Sep-17 10:41 (p 1 of 1)
Test Code: 170905sprt | 13-1627-7974

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Batch ID: 12-2217-0251		Test Type: Fertilization				Analyst:					
Start Date: 05 Sep-17 17:07		Protocol: EPA/600/R-95/136 (1995)				Diluent: Natural Seawater					
Ending Date: 05 Sep-17 17:47		Species: Strongylocentrotus purpuratus				Brine: Not Applicable					
Duration: 40m		Source: Pt. Loma				Age:					
Sample ID: 14-2874-8177		Code: 170905sprt				Client: Internal					
Sample Date: 05 Sep-17		Material: Copper chloride				Project:					
Receive Date: 05 Sep-17		Source: Reference Toxicant									
Sample Age: 17h		Station: Copper Chloride									
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
18-7744-8472	Fertilization Rate	<10	10	NA	5.09%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
14-5447-1160	Fertilization Rate	EC50	37.36	35.71	39.09		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits		Overlap	Decision			
14-5447-1160	Fertilization Rate	Control Resp		0.962	0.7 - NL		Yes	Passes Acceptability Criteria			
18-7744-8472	Fertilization Rate	Control Resp		0.962	0.7 - NL		Yes	Passes Acceptability Criteria			
18-7744-8472	Fertilization Rate	PMSD		0.05094	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	0.962	0.9484	0.9756	0.95	0.98	0.004899	0.01096	1.14%	0.0%
10		5	0.898	0.8538	0.9422	0.86	0.94	0.01594	0.03564	3.97%	6.65%
20		5	0.81	0.6887	0.9313	0.7	0.9	0.0437	0.09772	12.06%	15.8%
40		5	0.506	0.4435	0.5685	0.46	0.59	0.02249	0.0503	9.94%	47.4%
80		5	0.022	0.003583	0.04042	0	0.04	0.006633	0.01483	67.42%	97.71%
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	0.95	0.96	0.98	0.96	0.96					
10		0.94	0.87	0.93	0.86	0.89					
20		0.9	0.85	0.89	0.71	0.7					
40		0.59	0.48	0.46	0.51	0.49					
80		0.02	0.03	0.04	0.02	0					
160		0	0	0	0	0					

CETIS Analytical Report

Report Date: 11 Sep-17 10:37 (p 1 of 2)
 Test Code: 170905sprt | 13-1627-7974

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 18-7744-8472		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 11 Sep-17 10:36		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		5.09%	<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*	2.781	2.305	0.105	8	0.0191	CDF	Significant Effect		
		20*	5.412	2.305	0.105	8	<0.0001	CDF	Significant Effect		
		40*	12.83	2.305	0.105	8	<0.0001	CDF	Significant Effect		
		80*	27.07	2.305	0.105	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source		Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)	
Between		4.90729		1.226822		4		235.9	<0.0001	Significant Effect	
Error		0.1040318		0.005201589		20					
Total		5.011321				24					
Distributional Tests											
Attribute		Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances		Bartlett Equality of Variance			7.686	13.28	0.1038	Equal Variances			
Distribution		Shapiro-Wilk W Normality			0.9744	0.8877	0.7571	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	0.962	0.9484	0.9756	0.96	0.95	0.98	0.004899	1.14%	0.0%
10		5	0.898	0.8538	0.9422	0.89	0.86	0.94	0.01594	3.97%	6.65%
20		5	0.81	0.6887	0.9313	0.85	0.7	0.9	0.0437	12.06%	15.8%
40		5	0.506	0.4435	0.5685	0.49	0.46	0.59	0.02249	9.94%	47.4%
80		5	0.022	0.003583	0.04042	0.02	0	0.04	0.006633	67.42%	97.71%
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	1.376	1.338	1.415	1.369	1.345	1.429	0.01391	2.26%	0.0%
10		5	1.25	1.174	1.325	1.233	1.187	1.323	0.02714	4.86%	9.21%
20		5	1.13	0.9748	1.284	1.173	0.9912	1.249	0.05577	11.04%	17.93%
40		5	0.7915	0.7288	0.8542	0.7754	0.7454	0.8759	0.02259	6.38%	42.5%
80		5	0.1419	0.07103	0.2127	0.1419	0.05002	0.2014	0.02551	40.21%	89.69%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.37%

CETIS Analytical Report

Report Date: 11 Sep-17 10:37 (p 2 of 2)
 Test Code: 170905sprt | 13-1627-7974

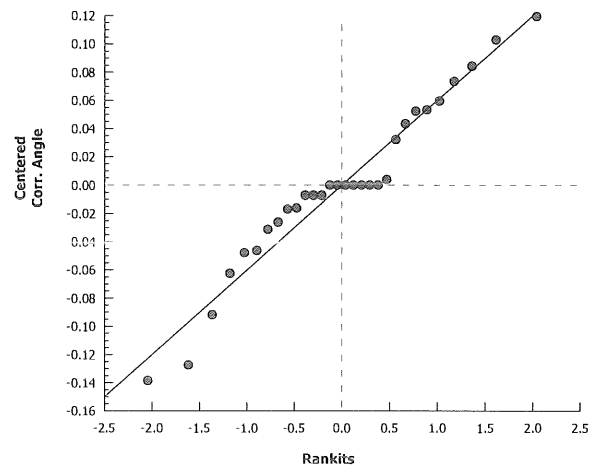
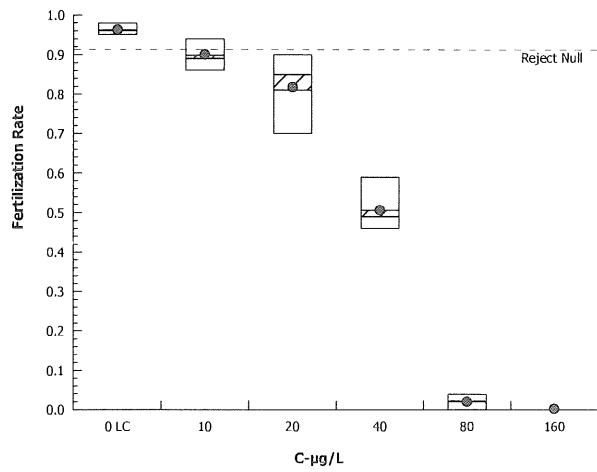
Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 18-7744-8472 Endpoint: Fertilization Rate
 Analyzed: 11 Sep-17 10:36 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics



CETIS Analytical Report

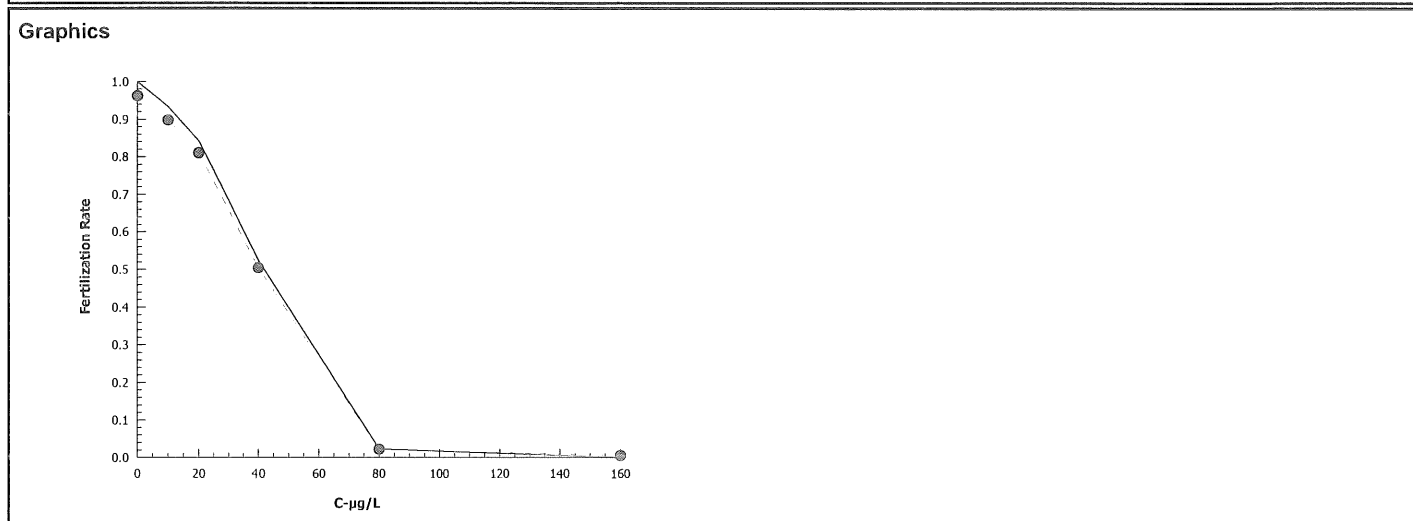
Report Date: 11 Sep-17 10:37 (p 1 of 1)

Test Code: 170905sprt | 13-1627-7974

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID:	14-5447-1160	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7						
Analyzed:	11 Sep-17 10:37	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes						

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.038	6.65%	1.572	0.00982	37.36	35.71	39.09

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	0.962	0.95	0.98	0.004899	0.01096	1.14%	0.0%	481	500
10		5	0.898	0.86	0.94	0.01594	0.03564	3.97%	6.65%	449	500
20		5	0.81	0.7	0.9	0.0437	0.09772	12.06%	15.8%	405	500
40		5	0.506	0.46	0.59	0.02249	0.0503	9.94%	47.4%	252	500
80		5	0.022	0	0.04	0.006633	0.01483	67.42%	97.71%	11	500
160		5	0	0	0	0	0		100.0%	0	500



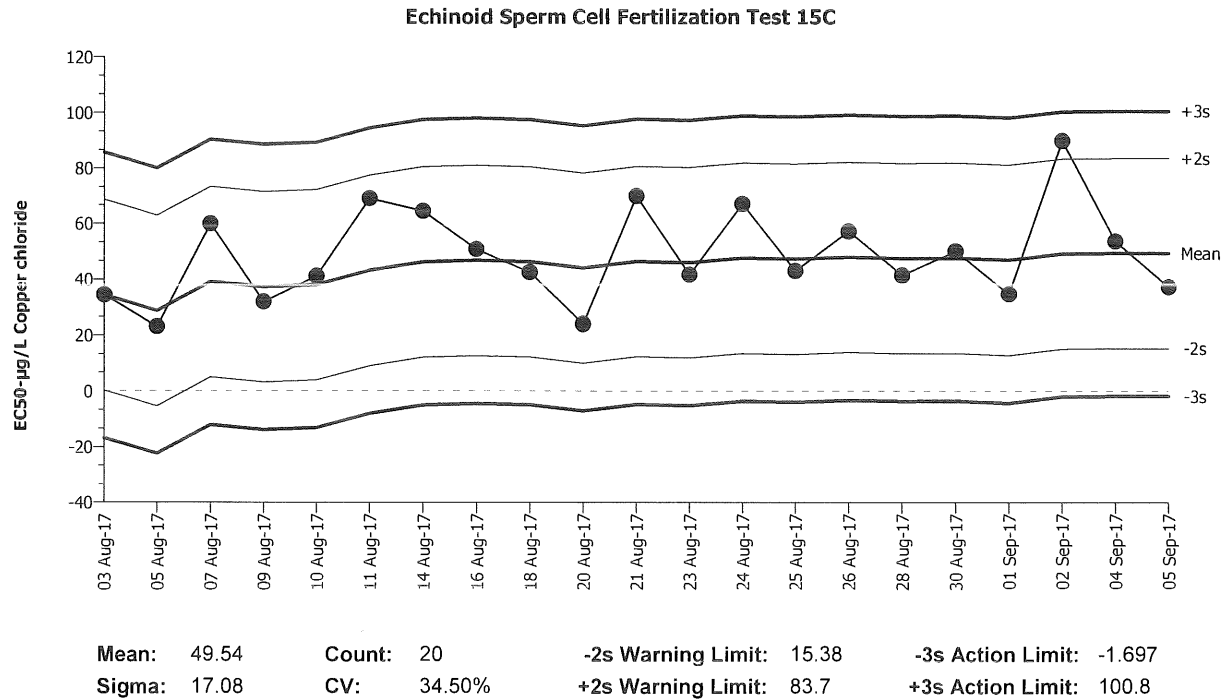
Echinoid Sperm Cell Fertilization Test 15C

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



Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	3	0:00	34.43	-15.11	-0.8844			02-7356-2235	20-3051-4002
2			5	19:25	23.07	-26.47	-1.55			11-5994-0488	10-6029-2098
3			7	15:10	59.94	10.4	0.6088			21-2468-7505	14-3489-7019
4			9	17:08	31.92	-17.62	-1.032			13-6999-3036	11-7131-4234
5			10	16:51	41.14	-8.404	-0.4921			00-5471-5288	12-0643-2211
6			11	14:50	69.03	19.49	1.141			04-5796-5476	07-8184-6783
7			14	14:40	64.51	14.97	0.8762			02-4510-8526	01-5460-0814
8			16	16:34	50.82	1.277	0.07476			16-3259-1018	06-7497-1035
9			18	14:09	42.53	-7.009	-0.4104			12-6613-4538	02-2322-5589
10			20	14:52	24.05	-25.49	-1.492			06-9655-0092	05-8785-3700
11			21	14:46	69.95	20.41	1.195			08-4756-2919	20-2992-4955
12			23	16:14	41.72	-7.823	-0.458			02-7595-3678	15-3490-2746
13			24	16:11	67.1	17.56	1.028			04-7651-5518	20-0883-0005
14			25	14:48	43.11	-6.43	-0.3765			06-8816-1100	09-0830-4014
15			26	16:00	57.24	7.701	0.4509			10-2039-5656	15-8794-0305
16			28	14:56	41.55	-7.988	-0.4677			08-1525-2751	10-7829-2432
17			30	16:38	50.21	0.6719	0.03934			08-1199-3706	11-0543-3886
18		Sep	1	15:27	34.79	-14.75	-0.8633			13-1244-6646	21-1567-7550
19			2	10:53	89.99	40.45	2.369	(+)		16-4202-9692	18-8681-1855
20			4	16:10	53.77	4.232	0.2478			12-2973-1405	10-6032-1229
21			5	0:00	37.36	-12.18	-0.7132			13-1627-7974	14-5447-1160

CETIS Test Data Worksheet

Report Date: 01 Sep-17 09:24 (p 1 of 1)
Test Code: 13-1627-7974/170905sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 05 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 05 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 05 Sep-17 Material: Copper chlorideSample Code: 170905sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	49	9/8/17
			2		46	
			3		90	
			4		93	
			5		87	
			6		70	
			7		96	
			8		51	
			9		0	
			10		89	
			11		0	
			12		8 3	Q18 RL 9/8/17
			13		0	
			14		95	
			15		89	
			16		2 2	Q18 RL 9/8/17
			17		0	
			18		96	
			19		48	
			20		0	
			21		4	
			22		94	
			23		2	
			24		0	
			25		59	
			26		86	
			27		71	
			28		98	
			29		96	
			30		85	

CETIS Test Data Worksheet

Report Date: 01 Sep-17 09:24 (p 1 of 1)

Test Code: 13-1627-7974/170905sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 05 Sep-17

Species: Strongylocentrotus purpuratus

Sample Code: 170905sprt

End Date: 05 Sep-17

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

Sample Source: Reference Toxicant

Sample Date: 05 Sep-17

Material: Copper chloride

Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	14			
0	LC	2	29			
0	LC	3	28			
0	LC	4	7			
0	LC	5	18	100	97	AD 9/5/17
10		1	22			
10		2	5			
10		3	4			
10		4	26			
10		5	10	100	92	
20		1	3			
20		2	30			
20		3	15			
20		4	27			
20		5	6	100	59	
40		1	25			
40		2	19			
40		3	2			
40		4	8			
40		5	1	100	30	
80		1	23			
80		2	12			
80		3	21			
80		4	16			
80		5	13	100	0	
160		1	11			
160		2	24			
160		3	17			
160		4	20			
160		5	9	100	0	

AC-06

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: *S. purpuratus*Sample ID: CuCl₂Start Date/Time: 9/5/2017 1707Test No: 170905sprtEnd Date/Time: 9/5/2017 1747Dilutions 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):	10200

Analyst: AD

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.2	8.04	33.4	16.0
10	8.0	8.02	33.6	15.6
20	7.9	8.01	33.6	15.8
40	7.9	8.01	33.5	15.9
80	7.9	8.02	33.3	15.8
160	8.0	8.02	33.2	15.6

Comments: _____

QC Check: AC 9/11/17Final Review: KTP 9/20/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: 7170905sprt
 Test No.: 5 'cnc12

Start Date/Time: 9/5/2017 1 1707
 End Date/Time: 9/5/2017 1 1747
 Species: S. purpuratus
 Animal Source: Pf. Loma
 Date Collected: 3/22/17

Tech initials: CG/AD
 Injection Time: 1610

Sperm Absorbance at 400 nm: 0.923 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 116 Mean: 115.6 X 50 = 5780 eggs/ml

105
102
124
126

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

Initial density: 5780 eggs/ml
 Final density: 4000 eggs/ml

= 1.445 dilution factor
 - 1.0 part egg stock
0.45 parts seawater

egg stock 100 ml
 seawater 50.45 ml

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

	Sperm:Egg Ratio							
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>1625</u>	<u>50:1</u>	<u>41</u>	<u>59</u>
Eggs Added (0.5 ml):	<u>1644</u>	<u>100:1</u>	<u>88, 88</u>	<u>12, 12</u>
Test Ended:	<u>1654</u>	<u>200:1</u>	<u>98, 86, 99</u>	<u>2, 1</u>
		<u>400:1</u>	<u>100</u>	<u>0</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	QC1	QC2	Egg Control 1	Egg Control 2	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1707</u>					<u>72</u>	<u>28</u>
Eggs Added (0.5 ml):	<u>1727</u>					<u>96</u>	<u>4</u>
Test Ended:	<u>1747</u>					<u>0</u>	<u>100</u>
						<u>0</u>	<u>100</u>

Comments:

ACG 029/5/17 BAD 018 9/5/17

QC Check:

AC 9/11/17

Final Review:

KP 012/17

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.