



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily/Weekly)
Sample Collection Date: September 14, 2017

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

Prepared by: Nautilus Environmental

Submitted: October 5, 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.

California
4340 Vandever Avenue
San Diego, California 92120
858.587.7333
fax: 858.587.3961

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 14, 2017

Test Date: September 14, 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	
	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 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 14, 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 & weekly sample)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/14/17, 08:00
Sample Receipt Date, Time:	9/14/17, 12:52

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)
9/14/17	7.73	8.7	4.5	32.9	111	0.03

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	9/14/17, 15:24 through 16:04
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 in the 5, 6.06, 10, and 15 percent effluent concentrations compared to the lab control. The NOEC is reported as 2.5 and the TU_c is equal to 40, which is above the maximum effluent limitation of 16.5 for this permit. According to the TST analysis no significant effect was observed at any of the effluent concentrations tested 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	5	>15	40	Pass	6.1

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	92.2
2.5	90.8
5.0	84.0*
6.06	86.6*
10	75.0*
15	74.0*

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

QUALITY ASSURANCE

The sample was received on the day 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 (%)
9/14/17	34.2	51.2 ± 32.7	31.9

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: 19 Sep-17 15:17 (p 1 of 1)
Test Code: 1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	02-9308-3550	Test Type:	Fertilization	Analyst:							
Start Date:	14 Sep-17 15:24	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	14 Sep-17 16:04	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	20-6418-9155	Code:	17-1020	Client:	IDE						
Sample Date:	14 Sep-17 08:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	14 Sep-17 12:52	Source:	IDE Americas, Inc.								
Sample Age:	7h (4.5 °C)	Station:	M-001 (Daily) <i>Weekly</i>								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
18-9449-2802	Fertilization Rate	2.5	5	3.536	3.78%	40	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
09-1999-1776	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					
09-1999-1776	Fertilization Rate	Control Resp	0.922	0.7 - NL	Yes	Passes Acceptability Criteria					
18-9449-2802	Fertilization Rate	Control Resp	0.922	0.7 - NL	Yes	Passes Acceptability Criteria					
18-9449-2802	Fertilization Rate	PMSD	0.0378	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	0.922	0.9058	0.9382	0.91	0.94	0.005831	0.01304	1.41%	0.0%
2.5		5	0.908	0.8918	0.9242	0.89	0.92	0.005831	0.01304	1.44%	1.52%
5		5	0.84	0.7761	0.9039	0.76	0.89	0.02302	0.05148	6.13%	8.89%
6.06		5	0.866	0.8452	0.8868	0.85	0.89	0.007483	0.01673	1.93%	6.07%
10		5	0.75	0.7376	0.7624	0.74	0.76	0.004473	0.01	1.33%	18.66%
15		5	0.74	0.6798	0.8002	0.7	0.81	0.02168	0.04848	6.55%	19.74%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.93	0.94	0.91	0.92	0.91					
2.5		0.91	0.89	0.9	0.92	0.92					
5		0.88	0.89	0.84	0.83	0.76					
6.06		0.85	0.87	0.87	0.89	0.85					
10		0.76	0.74	0.75	0.76	0.74					
15		0.7	0.72	0.77	0.7	0.81					

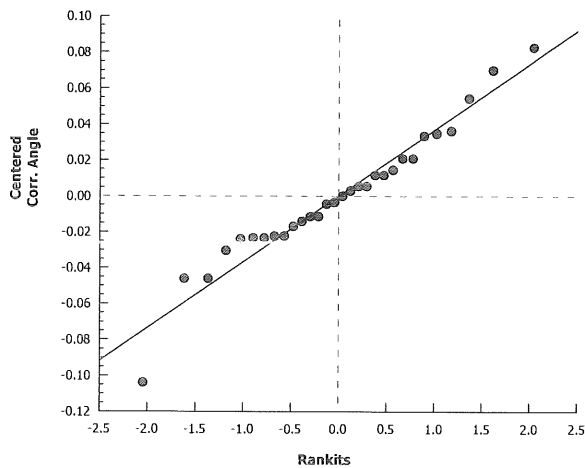
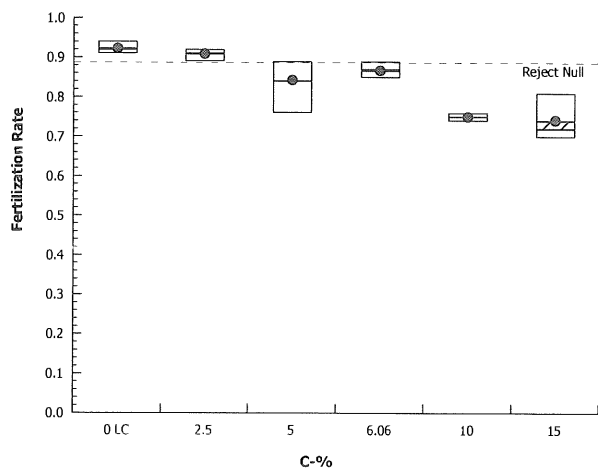
CETIS Analytical Report

Report Date: 19 Sep-17 15:17 (p 1 of 2)
Test Code: 1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 18-9449-2802		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 19 Sep-17 15:15		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		3.78%	2.5	5	3.536	40	
Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	0.9916	2.362	0.060	8	0.4237	CDF	Non-Significant Effect		
		5*	4.924	2.362	0.060	8	0.0001	CDF	Significant Effect		
		6.06*	3.6	2.362	0.060	8	0.0031	CDF	Significant Effect		
		10*	9.445	2.362	0.060	8	<0.0001	CDF	Significant Effect		
		15*	9.84	2.362	0.060	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.2804822		0.05609643		5		34.39	<0.0001	Significant Effect		
Error	0.03915038		0.001631266		24						
Total	0.3196326				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			14.22	15.09	0.0143		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9625	0.9031	0.3580		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	0.922	0.9058	0.9382	0.92	0.91	0.94	0.005831	1.41%	0.0%
2.5		5	0.908	0.8918	0.9242	0.91	0.89	0.92	0.005831	1.44%	1.52%
5		5	0.84	0.7761	0.9039	0.84	0.76	0.89	0.02302	6.13%	8.89%
6.06		5	0.866	0.8452	0.8868	0.87	0.85	0.89	0.007483	1.93%	6.07%
10		5	0.75	0.7376	0.7624	0.75	0.74	0.76	0.004473	1.33%	18.66%
15		5	0.74	0.6798	0.8002	0.72	0.7	0.81	0.02168	6.55%	19.74%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.289	1.258	1.319	1.284	1.266	1.323	0.01106	1.92%	0.0%
2.5		5	1.263	1.235	1.291	1.266	1.233	1.284	0.01001	1.77%	1.97%
5		5	1.163	1.077	1.248	1.159	1.059	1.233	0.03078	5.92%	9.76%
6.06		5	1.197	1.166	1.227	1.202	1.173	1.233	0.01111	2.08%	7.14%
10		5	1.047	1.033	1.062	1.047	1.036	1.059	0.005164	1.1%	18.72%
15		5	1.037	0.9671	1.107	1.013	0.9912	1.12	0.02525	5.44%	19.51%

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)	
Analysis ID:	18-9449-2802	Endpoint:	Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed:	19 Sep-17 15:15	Analysis:	Parametric-Control vs Treatments	Official Results: Yes

Graphics



CETIS Analytical Report

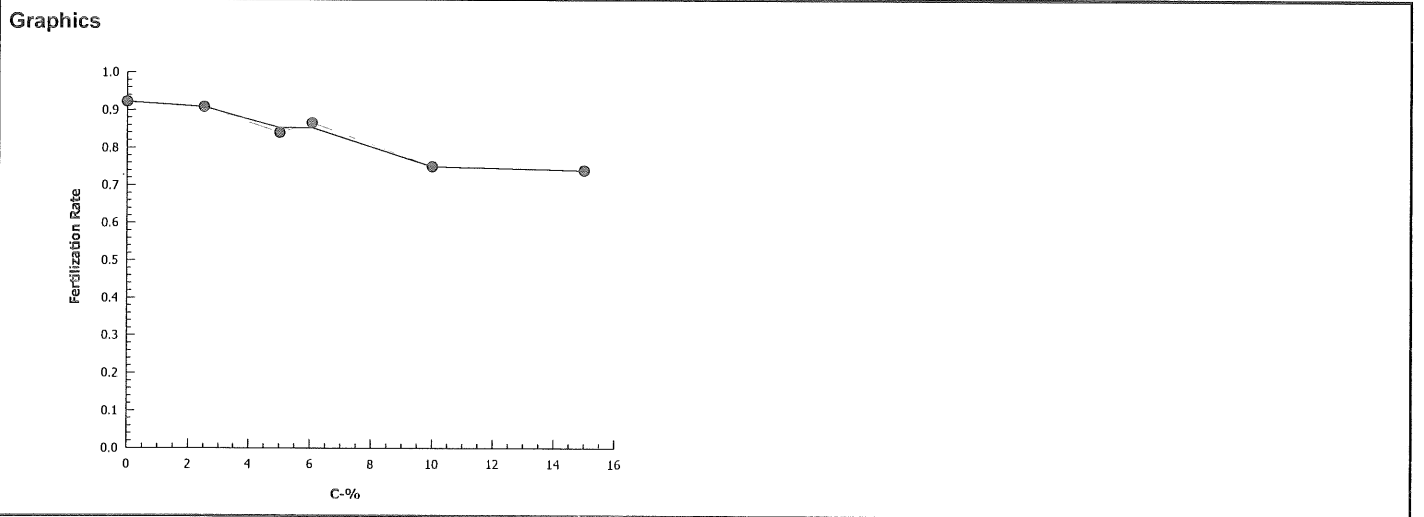
Report Date: 19 Sep-17 15:17 (p 1 of 1)
Test Code: 1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	09-1999-1776	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	19 Sep-17 15:16	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	421281	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	0.922	0.91	0.94	0.005831	0.01304	1.41%	0.0%	461	500
2.5		5	0.908	0.89	0.92	0.005831	0.01304	1.44%	1.52%	454	500
5		5	0.84	0.76	0.89	0.02302	0.05148	6.13%	8.89%	420	500
6.06		5	0.866	0.85	0.89	0.007483	0.01673	1.93%	6.07%	433	500
10		5	0.75	0.74	0.76	0.004473	0.01	1.33%	18.66%	375	500
15		5	0.74	0.7	0.81	0.02168	0.04848	6.55%	19.74%	370	500



CETIS Analytical Report

TST

Report Date: 19 Sep-17 15:17 (p 1 of 1)

Test Code: 1709-S129 | 04-7331-4478

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 04-2701-4770		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 19 Sep-17 15:17		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	3.53%	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*	22.82	1.895	0.025	7	<0.0001	CDF	Non-Significant Effect		
		5*	6.16	2.132	0.068	4	0.0018	CDF	Non-Significant Effect		
		6.06*	16.6	1.895	0.026	7	<0.0001	CDF	Non-Significant Effect		
		10*	8.275	1.943	0.019	6	<0.0001	CDF	Non-Significant Effect		
		15*	2.663	2.132	0.057	4	0.0281	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.2804822		0.05609643		5		34.39	<0.0001	Significant Effect		
Error	0.03915038		0.001631266		24						
Total	0.3196326				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			14.22	15.09	0.0143		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9625	0.9031	0.3580		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	0.922	0.9058	0.9382	0.92	0.91	0.94	0.005831	1.41%	0.0%
2.5		5	0.908	0.8918	0.9242	0.91	0.89	0.92	0.005831	1.44%	1.52%
5		5	0.84	0.7761	0.9039	0.84	0.76	0.89	0.02302	6.13%	8.89%
6.06		5	0.866	0.8452	0.8868	0.87	0.85	0.89	0.007483	1.93%	6.07%
10		5	0.75	0.7376	0.7624	0.75	0.74	0.76	0.004473	1.33%	18.66%
15		5	0.74	0.6798	0.8002	0.72	0.7	0.81	0.02168	6.55%	19.74%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.289	1.258	1.319	1.284	1.266	1.323	0.01106	1.92%	0.0%
2.5		5	1.263	1.235	1.291	1.266	1.233	1.284	0.01001	1.77%	1.97%
5		5	1.163	1.077	1.248	1.159	1.059	1.233	0.03078	5.92%	9.76%
6.06		5	1.197	1.166	1.227	1.202	1.173	1.233	0.01111	2.08%	7.14%
10		5	1.047	1.033	1.062	1.047	1.036	1.059	0.005164	1.1%	18.72%
15		5	1.037	0.9671	1.107	1.013	0.9912	1.12	0.02525	5.44%	19.51%

CETIS Test Data Worksheet

Report Date: 14 Sep-17 10:40 (p 1 of 1)
 Test Code: 1709-5129 04-7331-4478/1C3634AE

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 14 Sep-17 Species: Strongylocentrotus purpuratus
 End Date: 14 Sep-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 14 Sep-17 Material: Facility Effluent

Sample Code: 17- 1020
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Daily) (9/14 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	74	9/19/17
			62	100	77	
			63	100	89	
			64	100	76	
			65	100	92	
			66	100	76	
			67	100	74 87	
			68	100	94	
			69	100	76	
			70	100	70	
			71	100	90	
			72	100	88	
			73	100	85	
			74	100	89	
			75	100	91	
			76	100	81	
			77	100	70	
			78	100	79	
			79	100	75	
			80	100	92	
			81	100	92	
			82	100	91	
			83	100	83	
			84	100	91	
			85	100	93	
			86	100	89	
			87	100	72	
			88	100	87	
			89	100	85	
			90	100	84	

Ⓐ Q18 9/19/17 SG

CETIS Test Data Worksheet

Report Date: 14 Sep-17 10:40 (p 1 of 1)

Test Code: 1709-5129 04-7331-4478/1C3634AE

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 14 Sep-17 Species: Strongylocentrotus purpuratus
End Date: 14 Sep-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 14 Sep-17 Material: Facility Effluent

Sample Code: 17- 1020
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) (9/14 sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	85	100	94	RH 9/14/17
0	LC	2	68			
0	LC	3	84			
0	LC	4	65			
0	LC	5	82			
2.5		1	75	100	93	RH 9/14/17
2.5		2	86			
2.5		3	71			
2.5		4	81			
2.5		5	80			
5		1	72	100	93	RH 9/14/17
5		2	74			
5		3	90			
5		4	83			
5		5	64			
6.06		1	73	100	74	RH 9/14/17
6.06		2	88			
6.06		3	67			
6.06		4	63			
6.06		5	89			
10		1	69	100	73	RH 9/14/17
10		2	61			
10		3	79			
10		4	66			
10		5	78			
15		1	77	100	65	RH 9/14/17
15		2	87			
15		3	62			
15		4	70			
15		5	76			

QC: CG

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (9/14 sample)

Start Date/Time: 9/14/2017 1524

Sample Log No.: 17- 1090

End Date/Time: 9/14/2017 1604

Dilutions made by: CG

Test No: 1709-S129

Analyst: CG

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.12	33.5	15.2
2.5	8.5	8.11	33.7	15.5
5.0	8.6	8.10	33.6	15.1
6.06	8.5	8.10	33.7	① 15.5 15.1
10	8.5	8.09	33.6	15.0
15	8.6	8.08	33.6	14.9

Comments:

① CG Q18 9/14/17

QC Check:

AC 9/15/17

Final Review:

18 10/5/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client:

IDE

Sample ID:

Daily + weekly M-001 (9/14 sample)

Test No.:

1709-S129

Start Date/Time: 9/14/2017 / 1524

End Date/Time: 9/14/2017 / 1604

Species: *S. purpuratus*

Animal Source: Pt. Loma

Date Collected: 9/15/17

Tech initials:

CG

Injection Time:

1430

Sperm Absorbance at 400 nm: 0.988 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted:

78

Mean: 443 X 50 = 4430 eggs/ml

88

95

89

93

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

Initial density:

4430

eggs/ml

= 1.12 dilution factor

egg stock 100 ml

Final density:

4000

eggs/ml

- 1.0 part egg stock

seawater 12 ml

0.12 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):	1451	50:1	75.74	25.26
Eggs Added (0.5 ml):	1505	100:1	96.95	4.5
Test Ended:	1515	—	—	—

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):	1524	QC1	88.94	12
Eggs Added (0.5 ml):	1544	QC2	92	8
Test Ended:	1604	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

AC 9/15/17

QC Check:

AC 9/15/17

Final Review: 9/15/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: 106
Sample ID: +Daily M-001 (9/14 sample)
Test ID No(s): 1709-S129

Sample Check-In Information

Sample Description:

A: colorless, clear, odorless, no debris

Sample (A, B, C):	<u>A</u>			
Log-in No. (17-xxxx):	<u>1020</u>			
Sample Collection Date & Time:	<u>9/13/17 8:00</u>			
Sample Receipt Date & Time:	<u>9/14/17 12:52</u>			
Number of Containers & Container Type:	<u>1-4L CUB</u>			
Approx. Total Volume Received (L):	<u>~4L</u>			
Check-in Temperature (°C)	<u>54.7 4.5</u>			
Temperature OK?	<u>(Y) N</u>	Y N	Y N	Y N
DO (mg/L)	<u>8.7</u>			
pH (units)	<u>7.73</u>			
Conductivity (µS/cm)	<u>—</u>			
Salinity (ppt)	<u>32.9</u>			
Alkalinity (mg/L) ²	<u>111</u>			
Hardness (mg/L) ^{2,3}	<u>—</u>			
Total Chlorine (mg/L)	<u>0.03</u>			
Technician Initials	<u>TN</u>			

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

Alkalinity: 136 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: (A) TN 8/9/17 (B) CA 2/18 9/14/17

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: AC 9/15/17

Final Review: W 1/5/17

Appendix C

Chain-of-Custody Form



CDP Laboratory: _____
 Entahlpy Laboratory: _____
 WECK Laboratory: _____
 Nautilus: _____x _____
 AIM: _____
 Other: _____

Turn Around Time

Normal: x

RUSH (24 hr):

3 Days:

5 Days:

??? Days

Project Name: NPDES Daily/Weekly Toxicity Project Manager: Peter Shen Contact Information: (760) 201-7777

Special instruction: Sampled during 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: 9/13/17 @ 8:00, End: 9/14/17 @ 8:00 VH

ANALYSES

NOTES:

Glass=G Plastic=P

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

Drinking Water=DW Seawater=SW Soil=S

[illegible]

TDS - 31.44 ppt, EC - 48.96 mS/cm

RECEIPT
TEMP
4.5

Relinquished By:

Date:

Time:

~~Received By:~~

Date: Time:

Sample Condition Upon Receipt:

<input checked="" type="checkbox"/>	Iced	<input type="checkbox"/>	Ambient or _____ °C
<input type="checkbox"/>	Iced	<input type="checkbox"/>	Ambient or <u>3.0</u> °C

NATHAN (D. 17-1020)

Appendix D

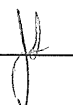
Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 15 Sep-17 14:05 (p 1 of 1)

Test Code: 170914sprt | 11-2846-3680

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	05-8526-3091		Test Type:	Fertilization			Analyst:				
Start Date:	14 Sep-17 15:24		Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Natural Seawater			
Ending Date:	14 Sep-17 16:04		Species:	Strongylocentrotus purpuratus			Brine:	Not Applicable			
Duration:	40m		Source:	Pt. Loma			Age:				
Sample ID:	01-9012-4513		Code:	170914sprt			Client:	Internal			
Sample Date:	14 Sep-17		Material:	Copper chloride			Project:				
Receive Date:	14 Sep-17		Source:	Reference Toxicant							
Sample Age:	15h		Station:	Copper Chloride							
Comparison Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
11-0635-3440	Fertilization Rate		<10	10	NA	8.57%		Dunnett Multiple Comparison Test			
Point Estimate Summary											
Analysis ID	Endpoint		Level	µg/L	95% LCL	95% UCL	TU	Method			
13-8128-7168	Fertilization Rate		EC50	34.24	32.37	36.2		Trimmed Spearman-Kärber			
Test Acceptability											
Analysis ID	Endpoint		Attribute		Test Stat	TAC Limits		Overlap	Decision		
11-0635-3440	Fertilization Rate		Control Resp		0.898	0.7 - NL		Yes	Passes Acceptability Criteria		
13-8128-7168	Fertilization Rate		Control Resp		0.898	0.7 - NL		Yes	Passes Acceptability Criteria		
11-0635-3440	Fertilization Rate		PMSD		0.08572	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	0.898	0.8388	0.9572	0.83	0.94	0.02131	0.04764	5.31%	0.0%
10		5	0.802	0.7273	0.8767	0.74	0.9	0.02691	0.06017	7.5%	10.69%
20		5	0.618	0.4666	0.7694	0.45	0.75	0.05453	0.1219	19.73%	31.18%
40		5	0.484	0.4123	0.5557	0.42	0.55	0.02581	0.05771	11.92%	46.1%
80		5	0.076	0.05717	0.09483	0.06	0.1	0.006782	0.01517	19.95%	91.54%
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	0.94	0.91	0.87	0.83					
10		0.78	0.78	0.74	0.81	0.9					
20		0.61	0.56	0.72	0.75	0.45					
40		0.55	0.46	0.42	0.54	0.45					
80		0.07	0.07	0.1	0.06	0.08					
160		0	0	0	0	0					



8/29/17

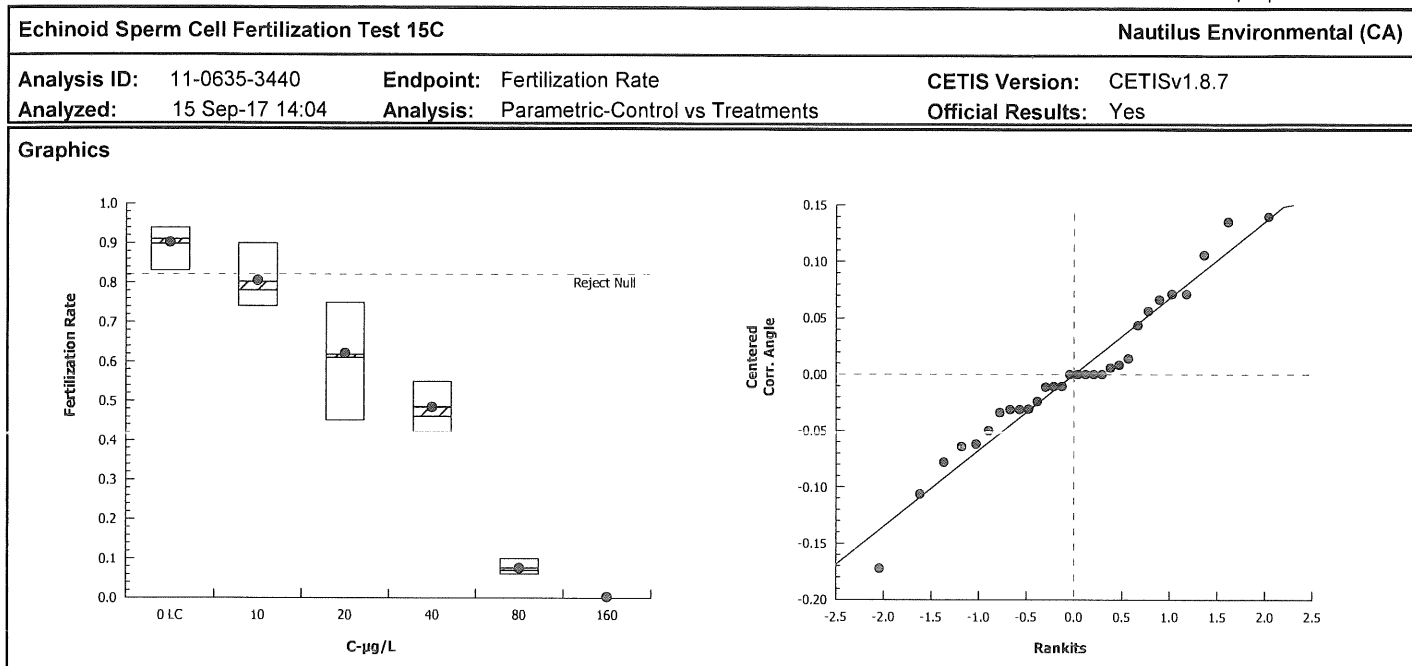
CETIS Analytical Report

Report Date: 15 Sep-17 14:05 (p 1 of 2)
Test Code: 170914spt | 11-2846-3680

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 11-0635-3440		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 15 Sep-17 14:04		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		8.57%	<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*	2.696	2.305	0.118	8	0.0228	CDF	Significant Effect		
		20*	6.723	2.305	0.118	8	<0.0001	CDF	Significant Effect		
		40*	9.419	2.305	0.118	8	<0.0001	CDF	Significant Effect		
		80*	19	2.305	0.118	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	2.834971		0.7087428		4		107.9	<0.0001	Significant Effect		
Error	0.131348		0.006567398		20						
Total	2.966319				24						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			7.22	13.28	0.1247		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9747	0.8877	0.7637		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	0.898	0.8388	0.9572	0.91	0.83	0.94	0.02131	5.31%	0.0%
10		5	0.802	0.7273	0.8767	0.78	0.74	0.9	0.02691	7.5%	10.69%
20		5	0.618	0.4666	0.7694	0.61	0.45	0.75	0.05453	19.73%	31.18%
40		5	0.484	0.4123	0.5557	0.46	0.42	0.55	0.02581	11.92%	46.1%
80		5	0.076	0.05717	0.09483	0.07	0.06	0.1	0.006782	19.95%	91.54%
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	1.252	1.156	1.349	1.266	1.146	1.323	0.03475	6.21%	0.0%
10		5	1.114	1.013	1.215	1.083	1.036	1.249	0.03631	7.29%	11.03%
20		5	0.9075	0.7501	1.065	0.8963	0.7353	1.047	0.0567	13.97%	27.52%
40		5	0.7693	0.6975	0.8412	0.7454	0.7051	0.8355	0.02587	7.52%	38.56%
80		5	0.2783	0.2436	0.313	0.2678	0.2475	0.3218	0.01251	10.05%	77.77%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.01%

CETIS Analytical Report

Report Date: 15 Sep-17 14:05 (p 2 of 2)
Test Code: 170914spt | 11-2846-3680



CETIS Analytical Report

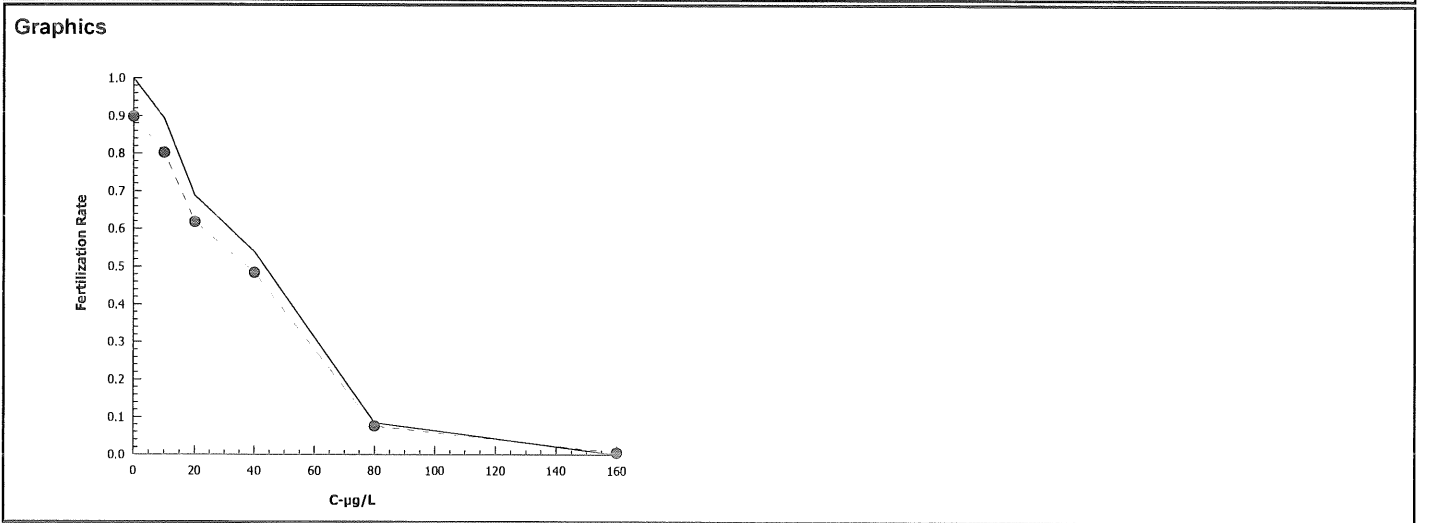
Report Date: 15 Sep-17 14:05 (p 1 of 1)
Test Code: 170914spt | 11-2846-3680

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

Analysis ID: 13-8128-7168	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 15 Sep-17 14:04	Analysis: Trimmed Spearman-Kärber	Official Results: Yes

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.102	10.69%	1.534	0.01213	34.24	32.37	36.2

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	0.898	0.83	0.94	0.02131	0.04764	5.31%	0.0%	449	500
10		5	0.802	0.74	0.9	0.02691	0.06017	7.5%	10.69%	401	500
20		5	0.618	0.45	0.75	0.05453	0.1219	19.73%	31.18%	309	500
40		5	0.484	0.42	0.55	0.02581	0.05771	11.92%	46.1%	242	500
80		5	0.076	0.06	0.1	0.006782	0.01517	19.95%	91.54%	38	500
160		5	0	0	0	0	0		100.0%	0	500



Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

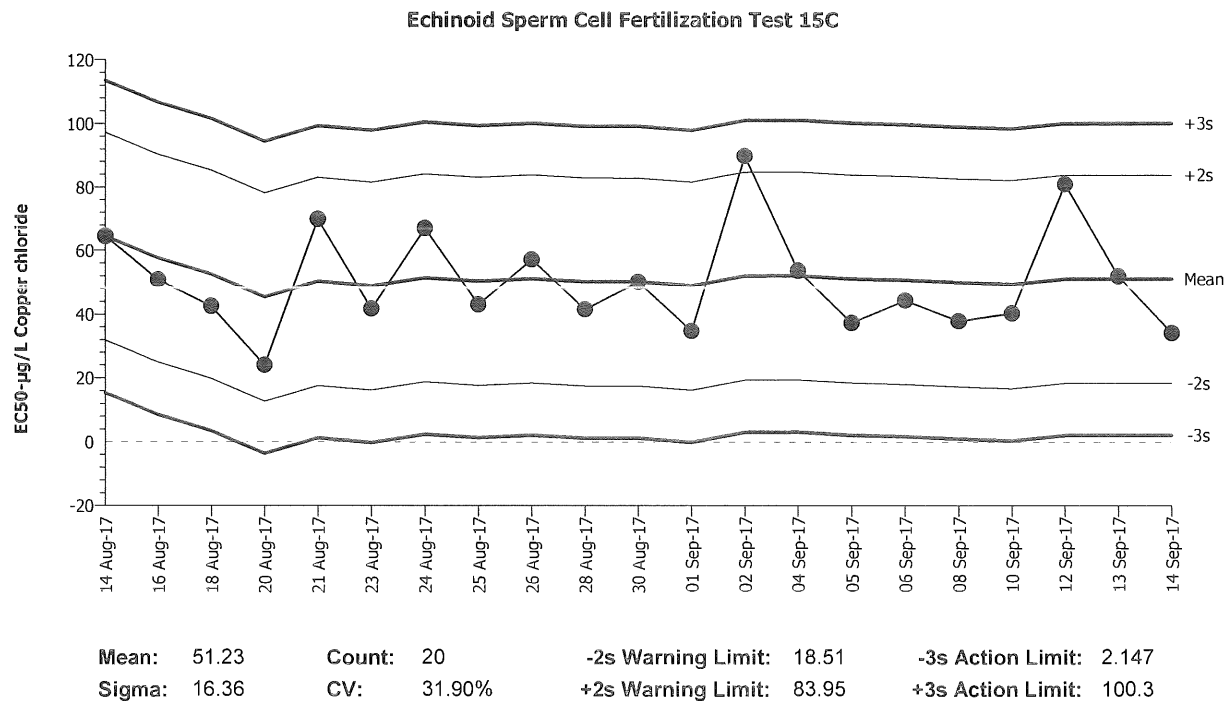
Organism: Strongylocentrotus purpuratus (Purpl

Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF



Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	14	14:40	64.51	13.28	0.8115			02-4510-8526	01-5460-0814
2			16	16:34	50.82	-0.4131	-0.02525			16-3259-1018	06-7497-1035
3			18	14:09	42.53	-8.699	-0.5317			12-6613-4538	02-2322-5589
4			20	14:52	24.05	-27.18	-1.661			06-9655-0092	05-8785-3700
5			21	14:46	69.95	18.72	1.144			08-4756-2919	20-2992-4955
6			23	16:14	41.72	-9.513	-0.5815			02-7595-3678	15-3490-2746
7			24	16:11	67.1	15.87	0.9702			04-7651-5518	20-0883-0005
8			25	14:48	43.11	-8.12	-0.4964			06-8816-1100	09-0830-4014
9			26	16:00	57.24	6.011	0.3674			10-2039-5656	15-8794-0305
10			28	14:56	41.55	-9.678	-0.5916			08-1525-2751	10-7829-2432
11			30	16:38	50.21	-1.018	-0.06223			08-1199-3706	11-0543-3886
12		Sep	1	15:27	34.79	-16.44	-1.005			13-1244-6646	21-1567-7550
13			2	10:53	89.99	38.76	2.369	(+)		16-4202-9692	18-8681-1855
14			4	16:10	53.77	2.542	0.1554			12-2973-1405	10-6032-1229
15			5	17:07	37.36	-13.87	-0.8478			13-1627-7974	14-5447-1160
16			6	17:15	44.41	-6.817	-0.4167			05-5533-8557	16-8161-1582
17			8	15:48	37.91	-13.32	-0.814			18-6871-7794	04-4479-5076
18			10	14:25	40.4	-10.83	-0.6618			11-6871-9499	08-4248-1228
19			12	15:51	81.07	29.84	1.824			20-0603-9450	06-1182-7961
20			13	19:07	52.04	0.8063	0.04928			01-4575-6189	02-4618-7964
21			14	15:24	34.24	-16.99	-1.039			11-2846-3680	13-8128-7168

CETIS Test Data Worksheet

Report Date: 14 Sep-17 10:33 (p 1 of 1)
 Test Code: 11-2846-3680/170914sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 14 Sep-17 Species: Strongylocentrotus purpuratus
 End Date: 14 Sep-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 14 Sep-17 Material: Copper chloride

Sample Code: 170914sprt
 Sample Source: Reference Toxicant
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	94	9/15/17
			2		0	
			3		91	
			4		8	
			5		55	
			6		0	
			7		72	
			8		81	
			9		7	
			10		61	
			11		94	
			12		90	
			13		6	
			14		54	
			15		7	
			16		23 46	Q18 9/15/17 JH
			17		56	
			18		42	
			19		78	
			20		45	
			21		45	
			22		0	
			23		0	
			24		83	
			25		74	
			26		0	
			27		87 75	Q18 9/15/17 JH
			28		10	
			29		87	
			30		78	

CETIS Test Data Worksheet

Report Date: 14 Sep-17 10:33 (p 1 of 1)
 Test Code: 11-2846-3680/170914sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 14 Sep-17 Species: Strongylocentrotus purpuratus Sample Code: 170914sprt
 End Date: 14 Sep-17 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 14 Sep-17 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	1			
0	LC	2	11	100	93	CG 9/14/17
0	LC	3	3			
0	LC	4	29			
0	LC	5	24			
10		1	19			
10		2	30			
10		3	25			
10		4	8	100	83	CG 9/14/17
10		5	12			
20		1	10			
20		2	17			
20		3	7			
20		4	27	100	80	CG 9/14/17
20		5	21			
40		1	5			
40		2	16	100	50	CG 9/14/17
40		3	18			
40		4	14			
40		5	20			
80		1	9			
80		2	15			
80		3	28			
80		4	13			
80		5	4	100	26	CG 9/14/17
160		1	6			
160		2	2			
160		3	26			
160		4	22	100	0	CG 9/14/17
160		5	23			

QC: CG

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 9/14/2017 1524Test No: 170914sprtEnd Date/Time: 9/14/2017 1604Dilutions made by: CG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10200

Analyst: CG

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.8	8.08	33.6	15.9
10	8.4	8.06	33.6	15.8
20	8.3	8.06	33.6	15.8
40	8.3	8.06	33.5	15.8
80	8.3	8.07	33.4	15.9
160	8.3	8.07	33.2	15.7

Comments: _____

QC Check: AC 9/15/17Final Review: 9/12/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: CaC12
 Test No.: 170914 Sprt
 Tech initials: CG
 Injection Time: 1430

Start Date/Time: 9/14/2017 / 1524
 End Date/Time: 9/14/2017 / 1604
 Species: S. purpuratus
 Animal Source: Pr. Loma
 Date Collected: 9/5/17

Sperm Absorbance at 400 nm: 0.988 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 78 Mean: 88.6 X 50 = 4430 eggs/ml

88
95
89
93

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

Initial density: 4430 eggs/ml = 1.12 dilution factor egg stock 100 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 12 ml
0.12 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 µl):	<u>1451</u>	<u>50:1</u>	<u>75.74</u>	<u>25.26</u>
Eggs Added (0.5 ml):	<u>1505</u>	<u>100:1</u>	<u>96.95</u>	<u>4.5</u>
Test Ended:	<u>1515</u>	<u>—</u>	<u>—</u>	<u>—</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>1524</u>	QC1	<u>88.9</u>	<u>12</u>
Eggs Added (0.5 ml):	<u>1544</u>	QC2	<u>92</u>	<u>8</u>
Test Ended:	<u>1604</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

AC 9/15/17

QC Check:

AC 9/15/17

Final Review:

9/29/17

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.