



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001
Sample Collection Date: January 17, 2017

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

Prepared by: Nautilus Environmental

Submitted: February 7, 2017

Data Quality Assurance:

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

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Adrienne Cabor

EXECUTIVE SUMMARY

MONTHLY CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – JANUARY 2017

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

Sampling Date: January 17, 2017

Test Date: January 18, 2017

Sample ID: M-001 Brine Effluent (weekly accelerated)

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	<u>NOEC</u>	<u>TU_c</u>	No
	2.5	40	

INTRODUCTION

A 24-hour composite discharge sample was collected in January 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) to satisfy monthly monitoring requirements 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 January 18, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The sample was collected on January 17, 2017. Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was hand delivered to Nautilus the day of collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. 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 protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./ Carlsbad Desalination Plant
Monitoring Period:	January 2017 (M-001 monthly monitoring)
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	01/17/17, 09:00
Sample Receipt Date, Time:	01/17/17, 11:53
Sampling Method:	24-hour Composite

Table 2. Water Quality Measurements upon Sample Receipt

Sample ID	pH	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO ₃)	Total Chlorine (mg/L)
M-001	7.89	6.2	2.0	63.0	118	0.06

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	01/18/17, 15:19 through 15:59
Test Organism:	<i>Strongylocentrotus purpuratus</i> (purple sea urchin)
Test Organism Source:	Field-collected locally (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 unadjusted M-001 sample; lab control. The same dilution series was also tested with the M-001 after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP discharges). The 10 percent M-001 dilution was also tested with the pH10/0.45 µm filtration toxicity identification evaluation (TIE) treatment.
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 egg 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 each 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.

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollution 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, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis is for comparison purposes only.

RESULTS

There was a statistically significant decrease in fertilization rate in the 5.0, 6.06, 10, and 15 percent concentrations of the unadjusted M-001 sample compared to the lab control, resulting in a NOEC of 2.5 percent effluent and a TU_c equal to 40. This exceeds the maximum daily permit effluent limitation of 16.5 TU_c . The 6.06 percent concentration (IWC) resulted in a 9.6 percent effect compared to the lab control, which was not significantly significant using to the TST calculation.

The M-001 sample adjusted to 40 ppt prior to dilution preparation resulted in no statistically significant effects in any of the test concentrations and a TU_c less than 6.67. Statistical results for urchin fertilization toxicity tests 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 a copy of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for M-001 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)	TU _c value (toxic units)	TST Result (Pass/Fail)	Percent Effect
M-001 (unadjusted)	2.5	5.0	>15	40	Pass	9.6
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	2.2

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)	M-001 Unadjusted Sample		M-001 40 ppt Adjusted ^a	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.5	87.4	33.4	93.2
2.5	34.3	84.6	33.7	93.2
5.0	35.0	77.4*	33.9	90.2
6.06	35.4	79.0*	34.0	91.2
10	36.5	67.4*	34.3	89.8
15	37.9	65.8*	34.6	89.4

^a For comparison to the M-001 unadjusted sample, the M-001 sample was adjusted with seawater to 40 ppt for this concentration series prior to preparing test concentrations. The 100 percent sample in this series represents 40 ppt adjusted brine effluent.

*An asterisk indicates a statistically significant decrease compared to the lab control using the standard USEPA flowchart statistical method (EPA 1995).

As part of the ongoing toxicity reduction evaluation and toxicity identification evaluation (TRE/TIE) investigations, the M-001 sample was also tested with the pH 10/filtration TIE treatment. The treatment was performed on the 10 percent sample concentration in order to obtain the highest testable concentration to discern differences between treated and untreated sample while also not having the confounding effect of elevated salinity above the organism tolerance level. Salinity measured in the 10 percent M-001 sample was 37.9 ppt; below the salinity tolerance limit for this organism and test procedure (based on Philips et al. 2012, and internal studies at Nautilus). Results for the sample tested with and without the TIE treatments are presented in Figure 1, and raw datasheets are in Appendix A.

The pH 10/filtration treatment improved fertilization over the untreated baseline sample. This response is consistent with previous M-001 samples tested using this treatment. Subsamples of the effluent before and after treatment were submitted to Weck Laboratories for chemical analysis. Those data are pending and will be reported separately.

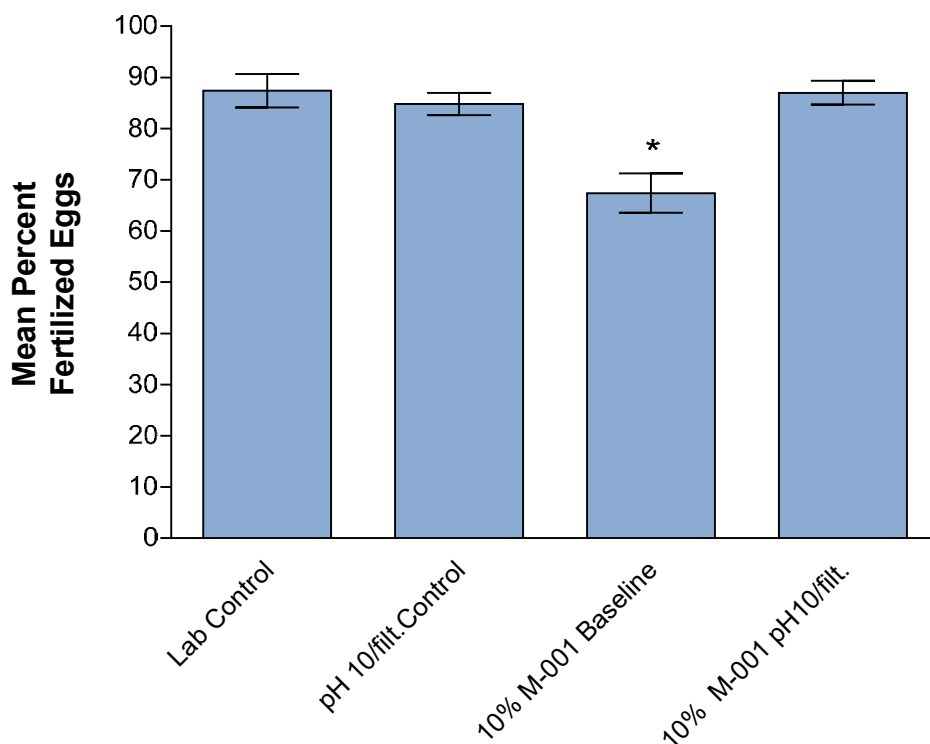


Figure 1. Summary of Urchin Fertilization Results for the M-001 Sample with and without the pH10/filtration TIE treatment (Mean \pm 1SD). *An asterisk indicates a statistically significant decrease compared to the lab control.

QUALITY ASSURANCE

The sample was received on the same day as collection and within the appropriate temperature range. The test was initiated within the allowable holding time of 36 hours. The laboratory controls met the minimum acceptability criteria as set by USEPA. The PMSD values, which are a measure of test variability, were within the acceptable range. Therefore, all test results were deemed valid for reporting purposes.

Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to evaluate reliability of the results. 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 are summarized in Table 6 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. Additionally, the median effect concentration (EC₅₀ value) was within two standard deviations (SD) of the historical mean, indicating typical test organism sensitivity to copper. A list of qualifier codes used on bench datasheets can be found in Appendix E.

Table 6. Reference Toxicant Test Results

Test Species	Endpoint	EC₅₀ (µg/L Copper)	Historical Mean EC₅₀ ± 2 SD (µg/L Copper)	CV (%)
Purple Urchin	Fertilization	19.7	39.3 ± 21.4	27.2

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

Historical Mean EC₅₀ ± 2 SD = Mean of historical test results plus or minus two standard deviations

CV = Coefficient of Variation

REFERENCES

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
- Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20
- USEPA. 1991. Methods for Aquatic Toxicity Identification Evaluation - Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.
- 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

M-001 Unadjusted

CETIS Summary Report

Report Date: 26 Jan-17 12:10 (p 1 of 1)
 Test Code: 1701-S139 | 20-4257-1769

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	03-4943-9750		Test Type:	Fertilization			Analyst:				
Start Date:	18 Jan-17 15:19		Protocol:	EPA/600/R-95/136 (1995)			Diluent:	Natural Seawater			
Ending Date:	18 Jan-17 15:59		Species:	Strongylocentrotus purpuratus			Brine:	Not Applicable			
Duration:	40m		Source:	Pt. Loma			Age:				
Sample ID:	12-5258-2404		Code:	17-0059			Client:	IDE			
Sample Date:	17 Jan-17 09:00		Material:	Facility Effluent			Project:	Carlsbad Desal Plant			
Receive Date:	17 Jan-17 11:53		Source:	IDE Americas, Inc.							
Sample Age:	30h (2 °C)		Station:	M-001 (Unadjusted)							
Comparison Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
05-8210-1401	Fertilization Rate		2.5	5	3.536	5.06%	40	Dunnett Multiple Comparison Test			
Point Estimate Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Method			
08-4886-5148	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		
05-8210-1401	Fertilization Rate		Control Resp		0.874	0.7 - NL		Yes	Passes Acceptability Criteria		
08-4886-5148	Fertilization Rate		Control Resp		0.874	0.7 - NL		Yes	Passes Acceptability Criteria		
05-8210-1401	Fertilization Rate		PMSD		0.05056	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	0.874	0.8332	0.9148	0.84	0.91	0.0147	0.03286	3.76%	0.0%
2.5		5	0.846	0.7959	0.8961	0.78	0.89	0.01806	0.04037	4.77%	3.2%
5		5	0.774	0.7305	0.8175	0.73	0.81	0.01568	0.03507	4.53%	11.44%
6.06		5	0.79	0.7609	0.8191	0.77	0.82	0.01049	0.02345	2.97%	9.61%
10		5	0.674	0.6262	0.7218	0.63	0.72	0.0172	0.03847	5.71%	22.88%
15		5	0.658	0.6129	0.7031	0.61	0.71	0.01625	0.03633	5.52%	24.71%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.88	0.9	0.91	0.84	0.84					
2.5		0.78	0.86	0.85	0.89	0.85					
5		0.81	0.81	0.76	0.76	0.73					
6.06		0.77	0.81	0.82	0.78	0.77					
10		0.7	0.63	0.72	0.68	0.64					
15		0.65	0.67	0.61	0.71	0.65					

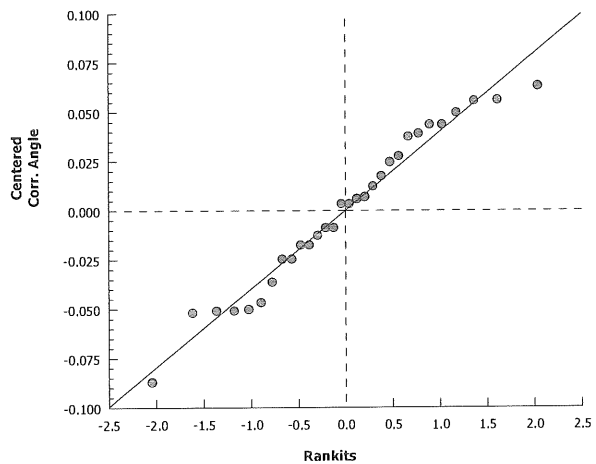
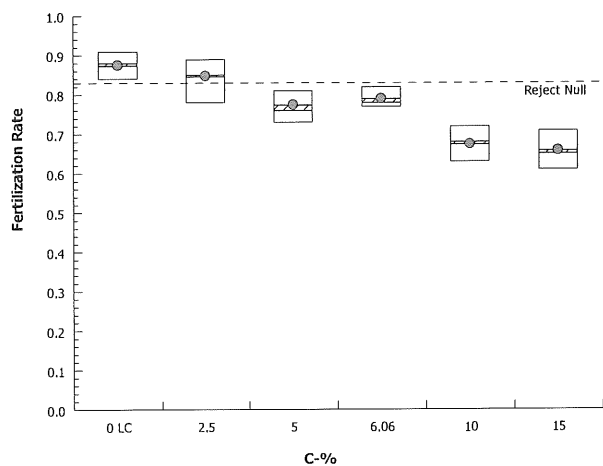
CETIS Analytical Report

Report Date: 26 Jan-17 12:10 (p 1 of 2)
Test Code: 1701-S139 | 20-4257-1769

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)		
Analysis ID: 05-8210-1401			Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 26 Jan-17 12:09			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			5.06%	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	1.477	2.362	0.065	8	0.2316	CDF	Non-Significant Effect			
		5*	4.893	2.362	0.065	8	0.0001	CDF	Significant Effect			
		6.06*	4.201	2.362	0.065	8	0.0007	CDF	Significant Effect			
		10*	9.013	2.362	0.065	8	<0.0001	CDF	Significant Effect			
		15*	9.637	2.362	0.065	8	<0.0001	CDF	Significant Effect			
ANOVA Table												
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)			
Between	0.2826978		0.05653956		5		30.23	<0.0001	Significant Effect			
Error	0.04489008		0.00187042		24							
Total	0.3275879				29							
Distributional Tests												
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)				
Variances	Bartlett Equality of Variance			1.632	15.09	0.8974		Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9647	0.9031	0.4062		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	0.874	0.8332	0.9148	0.88	0.84	0.91	0.0147	3.76%	0.0%	
2.5		5	0.846	0.7959	0.8961	0.85	0.78	0.89	0.01806	4.77%	3.2%	
5		5	0.774	0.7305	0.8175	0.76	0.73	0.81	0.01568	4.53%	11.44%	
6.06		5	0.79	0.7609	0.8191	0.78	0.77	0.82	0.01049	2.97%	9.61%	
10		5	0.674	0.6262	0.7218	0.68	0.63	0.72	0.0172	5.71%	22.88%	
15		5	0.658	0.6129	0.7031	0.65	0.61	0.71	0.01625	5.52%	24.71%	
Angular (Corrected) Transformed Summary												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.21	1.148	1.272	1.217	1.159	1.266	0.02221	4.1%	0.0%	
2.5		5	1.17	1.102	1.237	1.173	1.083	1.233	0.02439	4.66%	3.34%	
5		5	1.076	1.024	1.129	1.059	1.024	1.12	0.01882	3.91%	11.06%	
6.06		5	1.095	1.059	1.131	1.083	1.071	1.133	0.01299	2.65%	9.5%	
10		5	0.9636	0.9126	1.015	0.9695	0.9169	1.013	0.01837	4.26%	20.37%	
15		5	0.9466	0.8988	0.9943	0.9377	0.8963	1.002	0.0172	4.06%	21.78%	

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 05-8210-1401	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 26 Jan-17 12:09	Analysis: Parametric-Control vs Treatments	Official Results: Yes	

Graphics



CETIS Analytical Report

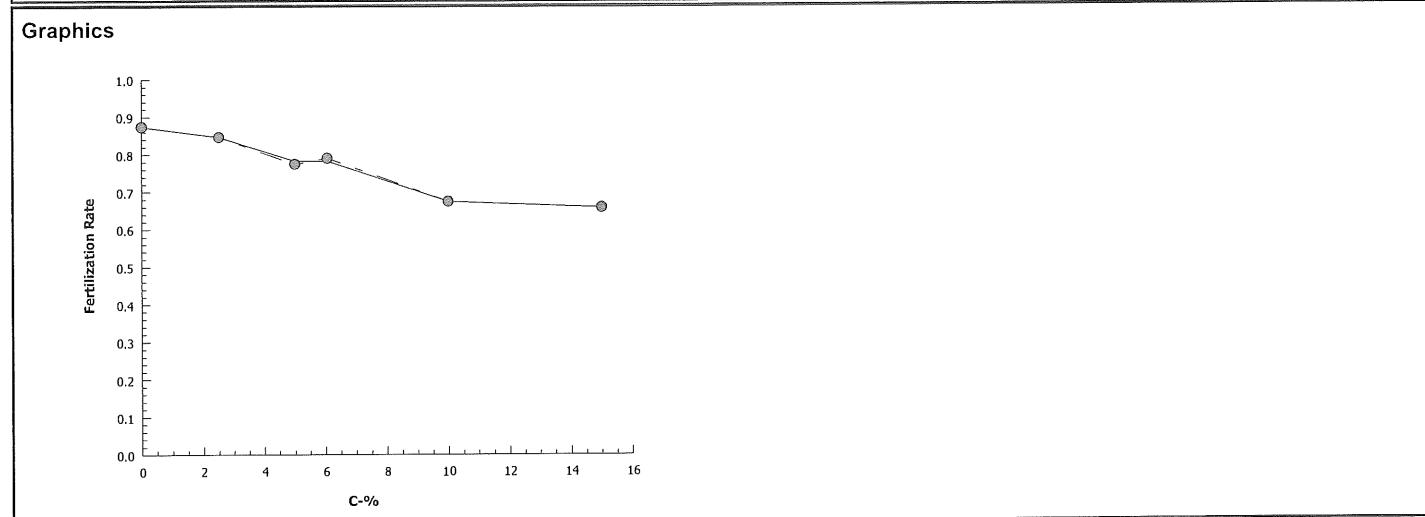
Report Date: 26 Jan-17 12:10 (p 1 of 1)
Test Code: 1701-S139 | 20-4257-1769

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID:	08-4886-5148	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	26 Jan-17 12:09	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	263305	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	0.874	0.84	0.91	0.0147	0.03286	3.76%	0.0%	437	500
2.5		5	0.846	0.78	0.89	0.01806	0.04037	4.77%	3.2%	423	500
5		5	0.774	0.73	0.81	0.01568	0.03507	4.53%	11.44%	387	500
6.06		5	0.79	0.77	0.82	0.01049	0.02345	2.97%	9.61%	395	500
10		5	0.674	0.63	0.72	0.0172	0.03847	5.71%	22.88%	337	500
15		5	0.658	0.61	0.71	0.01625	0.03633	5.52%	24.71%	329	500



CETIS Analytical Report

TST

Report Date: 26 Jan-17 12:10 (p 1 of 1)
Test Code: 1701-S139 | 20-4257-1769

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 13-9351-3806		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 26 Jan-17 12:09		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	3.43%	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*	8.876	1.895	0.056	7	<0.0001	CDF	Non-Significant Effect		
		5*	6.712	1.895	0.048	7	0.0001	CDF	Non-Significant Effect		
		6.06*	8.883	1.895	0.040	7	<0.0001	CDF	Non-Significant Effect		
		10*	2.258	1.895	0.047	7	0.0293	CDF	Non-Significant Effect		
		15	1.626	1.895	0.045	7	0.0740	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.2826978		0.05653956		5		30.23	<0.0001	Significant Effect		
Error	0.04489008		0.00187042		24						
Total	0.3275879				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			1.632	15.09	0.8974		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9647	0.9031	0.4062		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	0.874	0.8332	0.9148	0.88	0.84	0.91	0.0147	3.76%	0.0%
2.5		5	0.846	0.7959	0.8961	0.85	0.78	0.89	0.01806	4.77%	3.2%
5		5	0.774	0.7305	0.8175	0.76	0.73	0.81	0.01568	4.53%	11.44%
6.06		5	0.79	0.7609	0.8191	0.78	0.77	0.82	0.01049	2.97%	9.61%
10		5	0.674	0.6262	0.7218	0.68	0.63	0.72	0.0172	5.71%	22.88%
15		5	0.658	0.6129	0.7031	0.65	0.61	0.71	0.01625	5.52%	24.71%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.21	1.148	1.272	1.217	1.159	1.266	0.02221	4.1%	0.0%
2.5		5	1.17	1.102	1.237	1.173	1.083	1.233	0.02439	4.66%	3.34%
5		5	1.076	1.024	1.129	1.059	1.024	1.12	0.01882	3.91%	11.06%
6.06		5	1.095	1.059	1.131	1.083	1.071	1.133	0.01299	2.65%	9.5%
10		5	0.9636	0.9126	1.015	0.9695	0.9169	1.013	0.01837	4.26%	20.37%
15		5	0.9466	0.8988	0.9943	0.9377	0.8963	1.002	0.0172	4.06%	21.78%

CETIS Test Data Worksheet

Report Date: 16 Jan-17 11:39 (p 1 of 1)

Test Code: 1701-339 20-4257-1769/79BF2BF9

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 18 Jan-17 Species: Strongylocentrotus purpuratus
 End Date: 18 Jan-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 18 Jan-17 Material: Facility Effluent

Sample Code: 4AA8E404 17-0059
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	82	1/19/17
			62	100	71	
			63	100	81	
			64	100	91	
			65	100	65	
			66	100	81	
			67	100	70	
			68	100	85	
			69	100	89	
			70	100	86	
			71	100	76	
			72	100	77	
			73	100	63	
			74	100	65	
			75	100	77	
			76	100	73	
			77	100	81	
			78	100	85	
			79	100	90	
			80	100	68	
			81	100	88	
			82	100	72	
			83	100	78	
			84	100	64	
			85	100	84	
			86	100	61	
			87	100	67	
			88	100	76	
			89	100	84	
			90	100	78	

⑤ Q18 SG 1/19/17

⑤ Q18 FB 1/25/17

CETIS Test Data Worksheet

Report Date: 16 Jan-17 11:39 (p 1 of 1)
 Test Code: 1701-S139 20-4257-1769/79BF2BF9

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 18 Jan-17 Species: Strongylocentrotus purpuratus
 End Date: 18 Jan-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 18 Jan-17 Material: Facility Effluent

Sample Code: 4AA8E404 17-0059
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	81			
0	LC	2	79			
0	LC	3	64	100	94	EG 1/18/17
0	LC	4	89			
0	LC	5	85			
2.5		1	83	100	74	AC 1/18/17
2.5		2	70			
2.5		3	68			
2.5		4	69			
2.5		5	78			
5		1	63	100	83	AC 1/18/17
5		2	66			
5		3	88			
5		4	71			
5		5	76			
6.06		1	75			
6.06		2	77	100	86	EG
6.06		3	61			
6.06		4	90			
6.06		5	72			
10		1	67	100	74	EG
10		2	73	100	73	EG
10		3	82	100	71	EG
10		4	80	100	79	EG
10		5	84	100	66	EG
15		1	74			
15		2	87			
15		3	86			
15		4	62			
15		5	65			

QCEA

Q18EB1/25/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (unadjusted)

Start Date/Time: 1/18/2017 1519

Sample Log No.: 17-0059

End Date/Time: 1/18/2017 1559

Dilutions made by: EG

Test No: 1701-S139

Analyst:

RH

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.07	33.5	14.9
2.5	8.4	8.07	34.3	15.0
5.0	8.4	8.07	35.0	15.0
6.06	8.4	8.07	35.4	15.0
10	8.4	8.07	36.5	15.0
15	8.4	8.07	37.9	15.2

Comments:

QC Check: KB/25/17

Final Review: AC 2/7/17

Echinoderm Bioassay

Fertilization

Client: IDE/ CDP

Test Species: *S. purpuratus*

Project ID: M-001 pH 10/filt.

Start Date/Time: 1/18/2017 1519

Test No.: 1701-51396
Q18AC 2/7/17

End Date/Time: 1/18/2017 1559

[illegible]

QC Check: KB 1/25/17

Final Review: AC 2/7/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE/ CDP

Test Species: *S. purpuratus*

Sample ID: M-001

Start Date/Time: 1/18/2017 1519

Sample Log No.: 17- 0059

End Date/Time: 1/18/2017 1559

Dilutions made by: AC, EG

Test No: 1701-81396

Analyst:

RH

Concentration 10% Treatment	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
pH10/filt. Control	6.2	8.17	33.4	15.4
pH 10/filt. 10% M-001	6.1	8.06	36.1	15.3

Comments:

QC Check:

AC 2/7/17

Final Review:

WY 2/7/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-001 (unadj.)
 Test No.: 1701-S139

Start Date/Time: 1/18/2017 / 1519
 End Date/Time: 1/18/2017 / 1559
 Species: S. purpuratus
 Animal Source: Pl. Loma
 Date Collected: 12/20/16

Tech initials: EL
 Injection Time: 1440

Sperm Absorbance at 400 nm: 0.854 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 85 Mean: 80.6 $\times 50 =$ 4030 eggs/ml

76
93
73
76

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

Initial density: 4030 eggs/ml = (A) dilution factor egg stock (A) ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater (A) ml
(A) 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>1448</u>	<u>50:1</u>	<u>78</u>	<u>22</u>
Eggs Added (0.5 ml):	<u>1458</u>	<u>100:1</u>	<u>94/94</u>	<u>6/6</u>
Test Ended:	<u>1508</u>	<u>200:1</u>	<u>99</u>	<u>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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1519</u>	QC1	<u>91</u>	<u>9</u>
Eggs Added (0.5 ml):	<u>1539</u>	QC2	<u>92</u>	<u>8</u>
Test Ended:	<u>1559</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) No Dilution Needed

QC Check:

EB 1/25/17

Final Review:

AC 2/7/17

M-001 40 ppt Adjusted

CETIS Summary Report

Report Date: 26 Jan-17 12:06 (p 1 of 1)
 Test Code: 1701-S140 | 14-4621-9388

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Batch ID: 12-7726-4590		Test Type: Fertilization				Analyst:					
Start Date: 18 Jan-17 15:19		Protocol: EPA/600/R-95/136 (1995)				Diluent: Natural Seawater					
Ending Date: 18 Jan-17 15:59		Species: Strongylocentrotus purpuratus				Brine: Not Applicable					
Duration: 40m		Source: Pt. Loma				Age:					
Sample ID: 02-9180-6582		Code: 17-0059				Client: IDE					
Sample Date: 17 Jan-17 09:00		Material: Facility Effluent				Project: Carlsbad Desal Plant					
Receive Date: 17 Jan-17 11:53		Source: IDE Americas, Inc.									
Sample Age: 30h (2 °C)		Station: M-001 (40 ppt adj)									
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
13-0410-7296	Fertilization Rate	15	>15	NA	4.28%	< 6.667	Dunnett Multiple Comparison Test				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits		Overlap	Decision			
13-0410-7296	Fertilization Rate	Control Resp		0.932	0.7 - NL		Yes	Passes Acceptability Criteria			
13-0410-7296	Fertilization Rate	PMSD		0.04281	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	0.932	0.8999	0.9641	0.89	0.96	0.01158	0.02588	2.78%	0.0%
2.5		5	0.932	0.9024	0.9616	0.91	0.97	0.01068	0.02387	2.56%	0.0%
5		5	0.902	0.856	0.948	0.85	0.95	0.01655	0.03701	4.1%	3.22%
6.06		5	0.912	0.8958	0.9282	0.9	0.93	0.005831	0.01304	1.43%	2.15%
10		5	0.898	0.8635	0.9325	0.85	0.92	0.01241	0.02775	3.09%	3.65%
15		5	0.894	0.8505	0.9375	0.84	0.93	0.01568	0.03507	3.92%	4.08%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.93	0.94	0.96	0.89	0.94					
2.5		0.92	0.94	0.92	0.91	0.97					
5		0.92	0.85	0.89	0.9	0.95					
6.06		0.9	0.92	0.93	0.9	0.91					
10		0.85	0.91	0.91	0.9	0.92					
15		0.93	0.89	0.84	0.92	0.89					

CETIS Analytical Report

Report Date: 26 Jan-17 12:06 (p 1 of 2)
 Test Code: 1701-S140 | 14-4621-9388

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 13-0410-7296		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 26 Jan-17 12:06		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		4.28%	15	>15	NA	6.667
Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5	-0.01504	2.362	0.074	8	0.8377	CDF	Non-Significant Effect		
		5	1.702	2.362	0.074	8	0.1646	CDF	Non-Significant Effect		
		6.06	1.276	2.362	0.074	8	0.3042	CDF	Non-Significant Effect		
		10	1.993	2.362	0.074	8	0.1005	CDF	Non-Significant Effect		
		15	2.162	2.362	0.074	8	0.0737	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.02338734		0.004677468		5		1.892	0.1333	Non-Significant Effect		
Error	0.05934447		0.002472686		24						
Total	0.08273181				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			3.447	15.09	0.6314	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9652	0.9031	0.4169	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	0.932	0.8999	0.9641	0.94	0.89	0.96	0.01158	2.78%	0.0%
2.5		5	0.932	0.9024	0.9616	0.92	0.91	0.97	0.01068	2.56%	0.0%
5		5	0.902	0.856	0.948	0.9	0.85	0.95	0.01655	4.1%	3.22%
6.06		5	0.912	0.8958	0.9282	0.91	0.9	0.93	0.005831	1.43%	2.15%
10		5	0.898	0.8635	0.9325	0.91	0.85	0.92	0.01241	3.09%	3.65%
15		5	0.894	0.8505	0.9375	0.89	0.84	0.93	0.01568	3.92%	4.08%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.31	1.249	1.372	1.323	1.233	1.369	0.02226	3.8%	0.0%
2.5		5	1.311	1.246	1.376	1.284	1.266	1.397	0.02342	4.0%	-0.04%
5		5	1.257	1.178	1.336	1.249	1.173	1.345	0.02848	5.07%	4.09%
6.06		5	1.27	1.241	1.299	1.266	1.249	1.303	0.01044	1.84%	3.06%
10		5	1.248	1.194	1.302	1.266	1.173	1.284	0.01945	3.49%	4.78%
15		5	1.242	1.173	1.312	1.233	1.159	1.303	0.02501	4.5%	5.19%

CETIS Analytical Report

Report Date: 26 Jan-17 12:06 (p 2 of 2)
 Test Code: 1701-S140 | 14-4621-9388

Echinoid Sperm Cell Fertilization Test 15C

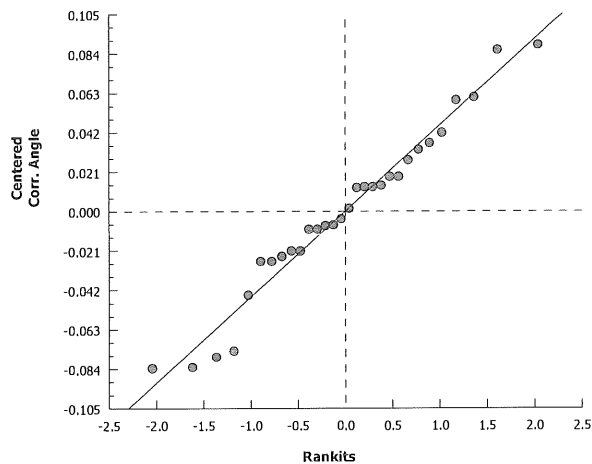
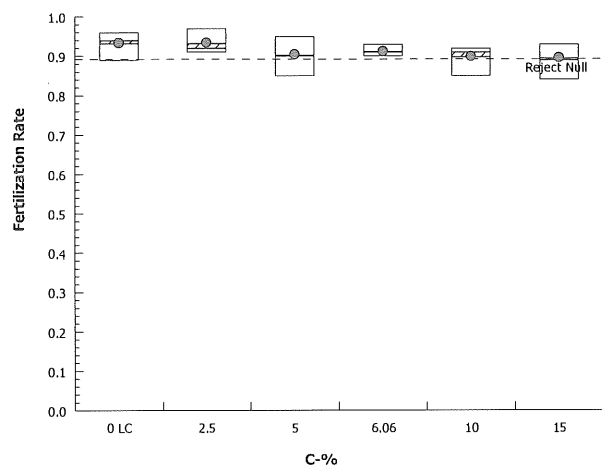
Nautilus Environmental (CA)

Analysis ID: 13-0410-7296
 Analyzed: 26 Jan-17 12:06

Endpoint: Fertilization Rate
 Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics



CETIS Analytical Report

TST

Report Date: 26 Jan-17 12:06 (p 1 of 1)
Test Code: 1701-S140 | 14-4621-9388

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 01-9496-1705		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 26 Jan-17 12:06		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	3.25%	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*	11.41	1.895	0.054	7	<0.0001	CDF	Non-Significant Effect		
		5*	8.303	1.943	0.064	6	<0.0001	CDF	Non-Significant Effect		
		6.06*	14.6	1.943	0.038	6	<0.0001	CDF	Non-Significant Effect		
		10*	10.34	1.895	0.049	7	<0.0001	CDF	Non-Significant Effect		
		15*	8.634	1.943	0.058	6	<0.0001	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α :5%)		
Between	0.02338734		0.004677468		5		1.892	0.1333	Non-Significant Effect		
Error	0.05934447		0.002472686		24						
Total	0.08273181				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α :1%)			
Variances	Bartlett Equality of Variance			3.447	15.09	0.6314		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9652	0.9031	0.4169		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	0.932	0.8999	0.9641	0.94	0.89	0.96	0.01158	2.78%	0.0%
2.5		5	0.932	0.9024	0.9616	0.92	0.91	0.97	0.01068	2.56%	0.0%
5		5	0.902	0.856	0.948	0.9	0.85	0.95	0.01655	4.1%	3.22%
6.06		5	0.912	0.8958	0.9282	0.91	0.9	0.93	0.005831	1.43%	2.15%
10		5	0.898	0.8635	0.9325	0.91	0.85	0.92	0.01241	3.09%	3.65%
15		5	0.894	0.8505	0.9375	0.89	0.84	0.93	0.01568	3.92%	4.08%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.31	1.249	1.372	1.323	1.233	1.369	0.02226	3.8%	0.0%
2.5		5	1.311	1.246	1.376	1.284	1.266	1.397	0.02342	4.0%	-0.04%
5		5	1.257	1.178	1.336	1.249	1.173	1.345	0.02848	5.07%	4.09%
6.06		5	1.27	1.241	1.299	1.266	1.249	1.303	0.01044	1.84%	3.06%
10		5	1.248	1.194	1.302	1.266	1.173	1.284	0.01945	3.49%	4.78%
15		5	1.242	1.173	1.312	1.233	1.159	1.303	0.02501	4.5%	5.19%

CETIS Test Data Worksheet

Report Date: 16 Jan-17 11:37 (p 1 of 1)

Test Code: 1701-5140 14-4621-9388/56338E7C

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 18 Jan-17 Species: Strongylocentrotus purpuratus
End Date: 18 Jan-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 18 Jan-17 Material: Facility Effluent

Sample Code: 11049D76 17-0059
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	92	1/19/17
			32	100	93	
			33	100	95	
			34	100	89	
			35	100	96	
			36	100	84	
			37	100	93	
			38	100	92	
			39	100	90	
			40	100	90	
			41	100	93	
			42	100	91	
			43	100	94	
			44	100	94	
			45	100	90	
			46	100	89	
			47	100	91	
			48	100	89	
			49	100	92	
			50	100	85	
			51	100	91	
			52	100	94	
			53	100	91	
			54	100	92	
			55	100	92	
			56	100	96	
			57	100	89	
			58	100	97	
			59	100	85	
			60	100	92	

Ⓢ Q1B KB1/25/17

CETIS Test Data Worksheet

Report Date: 16 Jan-17 11:37 (p 1 of 1)
 Test Code: 1701-540 14-4621-9388/56338E7C

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 18 Jan-17 Species: Strongylocentrotus purpuratus
 End Date: 18 Jan-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 18 Jan-17 Material: Facility Effluent

Sample Code: 11649076 17-0059
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	41	100	96	EG 1/18/17
0	LC	2	44			
0	LC	3	35			
0	LC	4	34			
0	LC	5	43			
2.5		1	54			
2.5		2	52			
2.5		3	49	100	90	EG
2.5		4	42			
2.5		5	58			
5		1	31			
5		2	59			
5		3	57	100	90	EG
5		4	40			
5		5	33			
6.06		1	45			
6.06		2	38	100	95	EG
6.06		3	32			
6.06		4	56			
6.06		5	53			
10		1	50			
10		2	51			
10		3	47	100	94	EG
10		4	39			
10		5	60			
15		1	37			
15		2	46			
15		3	36	100	84	EG
15		4	55			
15		5	48			

QC EG

ⓐ QLB KB 1/25/17

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: IDEAnalyst: EGSample ID: M-001 (40 ppt adjusted)Test Date: 1/18/2017Test No: 1701-S140Test Type: Urchin FertilizationSalinity of Effluent 63.0Salinity of Seawater 33.5Date of Brine used: NATarget Salinity 40.0Alkalinity of Brine Control: NA mg/L as CaCO₃

	<u>Effluent</u>	<u>Brine Control</u>
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	<u>3.54</u>	<u>-6.15</u>

TS = target salinity

SE = salinity of effluent

SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)
100	100	3.54	353.8	454

Comments: Formula for amount of seawater to dilute sample to 40ppt
Use 40 ppt sample as 100% sample for testing.
NA = not applicable; sample not diluted with Nautilus brine.

QC Check: KB1/25/17Final Review: AC 2/7/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted)

Start Date/Time: 1/18/2017 1519

Sample Log No.: 17-0059

End Date/Time: 1/18/2017 1559

Dilutions made by: EG

Test No: 1701-S140

Analyst:

RH

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.4	8.06	33.4	14.8
2.5	8.4	8.06	33.7	14.6
5.0	8.4	8.07	33.9	14.5
6.06	8.4	8.07	34.0	14.8
10	8.4	8.07	34.3	14.7
15	8.4	8.07	34.6	14.6

Comments:

QC Check: KB 1/25/17

Final Review: AC 2/7/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-001 (40 ppt adj.)
 Test No.: 1701-8140

Start Date/Time: 1/18/2017 / 1519
 End Date/Time: 1/18/2017 / 1559
 Species: S. purpuratus
 Animal Source: Pl. Loma
 Date Collected: 12/20/16

Tech initials: EL
 Injection Time: 1440

Sperm Absorbance at 400 nm: 0.854 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 85 Mean: 80.6 $\times 50 =$ 4030 eggs/ml

76
93
73
76

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

Initial density: 4030 eggs/ml = (A) dilution factor egg stock (A) ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater (A) ml
(A) 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>1448</u>	<u>50:1</u>	<u>78</u>	<u>22</u>
Eggs Added (0.5 ml):	<u>1458</u>	<u>100:1</u>	<u>94/94</u>	<u>6/6</u>
Test Ended:	<u>1508</u>	<u>200:1</u>	<u>99</u>	<u>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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1519</u>	QC1	<u>91</u>	<u>9</u>
Eggs Added (0.5 ml):	<u>1539</u>	QC2	<u>92</u>	<u>8</u>
Test Ended:	<u>1559</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) No Dilution Needed

QC Check:

KB 1/25/17

Final Review:

AC 2/7/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Monthly M-001
Test ID No(s): 1701-S139+5140

Sample Check-In Information

Sample Description:

A: Colorless, clear, odorless, no debris

Sample (A, B, C):	(A)			
Log-in No. (17-xxxx):	0059			
Sample Collection Date & Time:	1/17/17 0900			
Sample Receipt Date & Time:	1/17/17 1153			
Number of Containers & Container Type:	2 4L Cubes			
Approx. Total Volume Received (L):	~5L			
Check-in Temperature (°C)	2.0			
Temperature OK? ¹	(Y) N	Y N	Y N	Y N
DO (mg/L)	6.2			
pH (units)	7.89			
Conductivity (µS/cm)	—			
Salinity (ppt)	63.0			
Alkalinity (mg/L) ²	118			
Hardness (mg/L) ^{2,3}	—			
Total Chlorine (mg/L)	0.06			
Technician Initials	MM			

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

Alkalinity: 114 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: @ a subsample was treated with pH 10/filtration
TIE treatment. See report for details

COC Complete (Y/N)?

A Y B _____ C _____

Filtration? Y (N)

Pore Size: _____

Organisms _____ or _____ Debris

Salinity Adjustment? Y (N) ²

Test: _____ Source: _____ Target ppt: _____

Test: _____ Source: _____ Target ppt: _____

Test: _____ Source: _____ Target ppt: _____

pH Adjustment? Y (N) ²

	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: KB 1/26/17

Final Review: AC 2/7/17

Appendix C

Chain-of-Custody Form



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

NOTES:

Naut. lvs IO: ~~17-0060~~^{MM}_{Q18} V17/17
17-0059

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 26 Jan-17 12:01 (p 1 of 1)

Test Code: 170118sprt | 08-8914-3626

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	11-9574-7644	Test Type:	Fertilization	Analyst:							
Start Date:	18 Jan-17 15:19	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	18 Jan-17 15:59	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	08-7123-1506	Code:	170118sprt	Client:	Internal						
Sample Date:	18 Jan-17	Material:	Copper chloride	Project:							
Receive Date:	18 Jan-17	Source:	Reference Toxicant								
Sample Age:	15h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
12-8205-2347	Fertilization Rate	<10	10	NA	7.61%	Steel Many-One Rank Sum Test					
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
00-6318-6085	Fertilization Rate	EC50	19.65	18.07	21.37	Trimmed Spearman-Kärber					
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
00-6318-6085	Fertilization Rate	Control Resp	0.918	0.7 - NL	Yes	Passes Acceptability Criteria					
12-8205-2347	Fertilization Rate	Control Resp	0.918	0.7 - NL	Yes	Passes Acceptability Criteria					
12-8205-2347	Fertilization Rate	PMSD	0.07613	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	0.918	0.8738	0.9622	0.87	0.97	0.01594	0.03564	3.88%	0.0%
10		5	0.632	0.5975	0.6665	0.59	0.66	0.01241	0.02775	4.39%	31.15%
20		5	0.496	0.3972	0.5948	0.39	0.6	0.03558	0.07956	16.04%	45.97%
40		5	0.124	0.01273	0.2353	0.03	0.26	0.04007	0.08961	72.27%	86.49%
80		5	0.002	0	0.007553	0	0.01	0.002	0.004472	223.6%	99.78%
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	0.87	0.92	0.97	0.92	0.91					
10		0.64	0.66	0.62	0.59	0.65					
20		0.39	0.6	0.45	0.52	0.52					
40		0.07	0.1	0.16	0.03	0.26					
80		0	0.01	0	0	0					
160		0	0	0	0	0					

CETIS Analytical Report

Report Date: 26 Jan-17 12:01 (p 1 of 2)
 Test Code: 170118sprt | 08-8914-3626

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 12-8205-2347		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 26 Jan-17 12:01		Analysis: Nonparametric-Control vs Treatments					Official Results: Yes				
Data Transform		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C > T	NA	NA		7.61%	<10	10	NA	
Steel Many-One Rank Sum Test											
Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		10*	15	17	0	8	0.0158	Asymp	Significant Effect		
		20*	15	17	0	8	0.0158	Asymp	Significant Effect		
		40*	15	17	0	8	0.0158	Asymp	Significant Effect		
		80*	15	17	0	8	0.0158	Asymp	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	4.6696		1.1674		4		184	<0.0001	Significant Effect		
Error	0.1269165		0.006345825		20						
Total	4.796517				24						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			13.53	13.28	0.0090		Unequal Variances			
Distribution	Shapiro-Wilk W Normality			0.9511	0.8877	0.2655		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	0.918	0.8738	0.9622	0.92	0.87	0.97	0.01594	3.88%	0.0%
10		5	0.632	0.5975	0.6665	0.64	0.59	0.66	0.01241	4.39%	31.15%
20		5	0.496	0.3972	0.5948	0.52	0.39	0.6	0.03558	16.04%	45.97%
40		5	0.124	0.01273	0.2353	0.1	0.03	0.26	0.04007	72.27%	86.49%
80		5	0.002	0	0.007553	0	0	0.01	0.002	223.6%	99.78%
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	1.287	1.199	1.374	1.284	1.202	1.397	0.03141	5.46%	0.0%
10		5	0.9192	0.8836	0.9548	0.9273	0.8759	0.9483	0.01282	3.12%	28.56%
20		5	0.7813	0.6819	0.8808	0.8054	0.6745	0.8861	0.03582	10.25%	39.27%
40		5	0.342	0.1706	0.5134	0.3218	0.1741	0.5351	0.06174	40.36%	73.41%
80		5	0.06005	0.0322	0.0879	0.05002	0.05002	0.1002	0.01003	37.35%	95.33%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.11%

CETIS Analytical Report

Report Date: 26 Jan-17 12:01 (p 2 of 2)
 Test Code: 170118spt | 08-8914-3626

Echinoid Sperm Cell Fertilization Test 15C

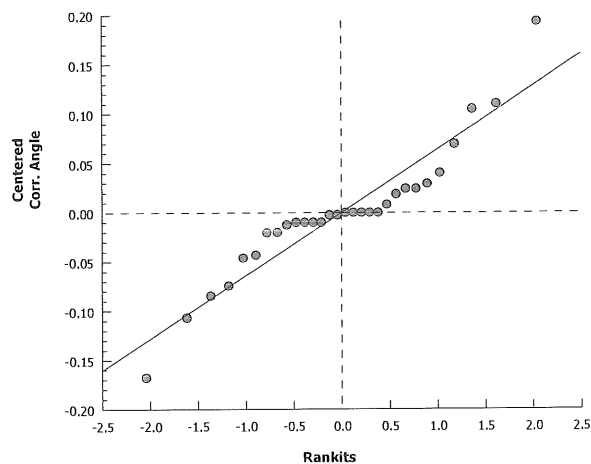
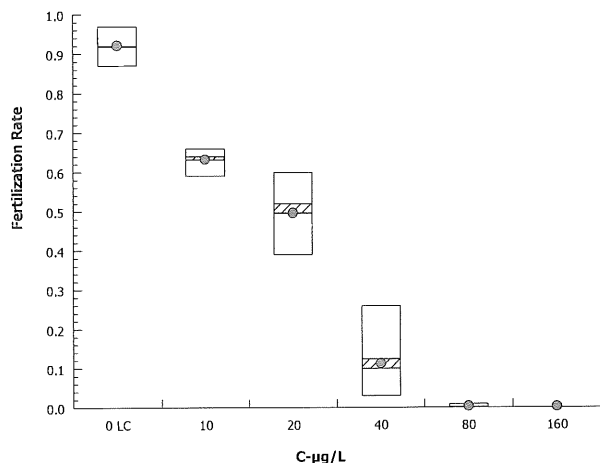
Nautilus Environmental (CA)

Analysis ID: 12-8205-2347
 Analyzed: 26 Jan-17 12:01

Endpoint: Fertilization Rate
 Analysis: Nonparametric-Control vs Treatments

CETIS Version: CETISv1.8.7
 Official Results: Yes

Graphics



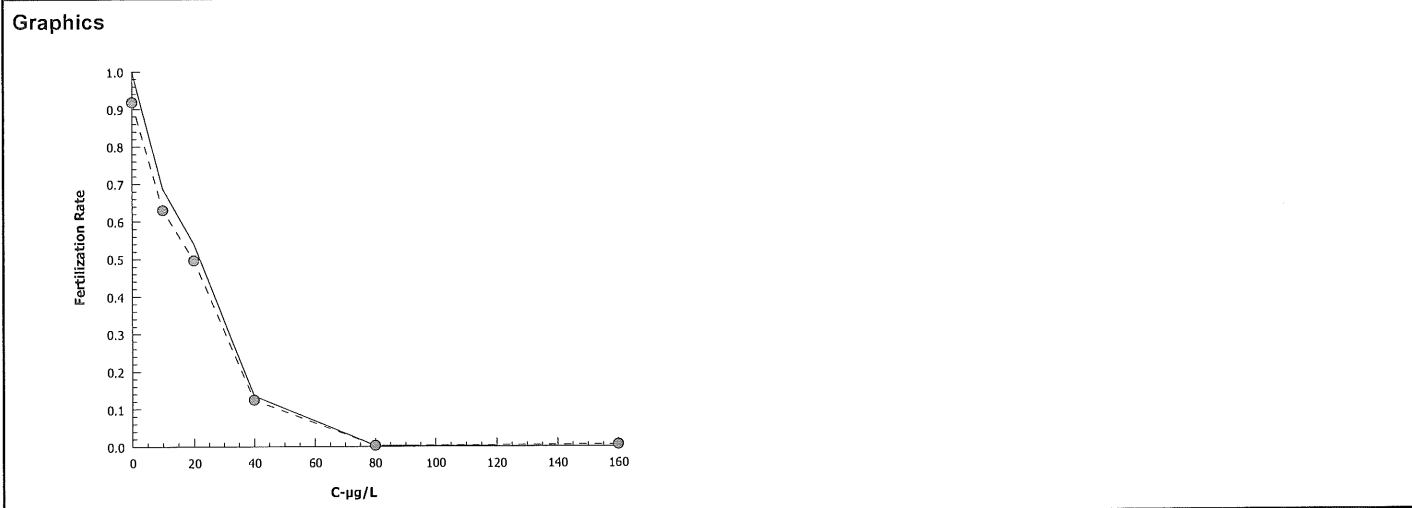
CETIS Analytical Report

Report Date: 26 Jan-17 12:01 (p 1 of 1)
 Test Code: 170118spt | 08-8914-3626

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Analysis ID:	00-6318-6085	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7		
Analyzed:	26 Jan-17 12:01	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes		

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.082	31.15%	1.293	0.01822	19.65	18.07	21.37

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	0.918	0.87	0.97	0.01594	0.03564	3.88%	0.0%	459	500
10		5	0.632	0.59	0.66	0.01241	0.02775	4.39%	31.15%	315	500
20		5	0.496	0.39	0.6	0.03558	0.07956	16.04%	45.97%	248	500
40		5	0.124	0.03	0.26	0.04007	0.08961	72.27%	86.49%	62	500
80		5	0.002	0	0.01	0.002	0.004472	223.6%	99.78%	1	500
160		5	0	0	0	0	0		100.0%	0	500



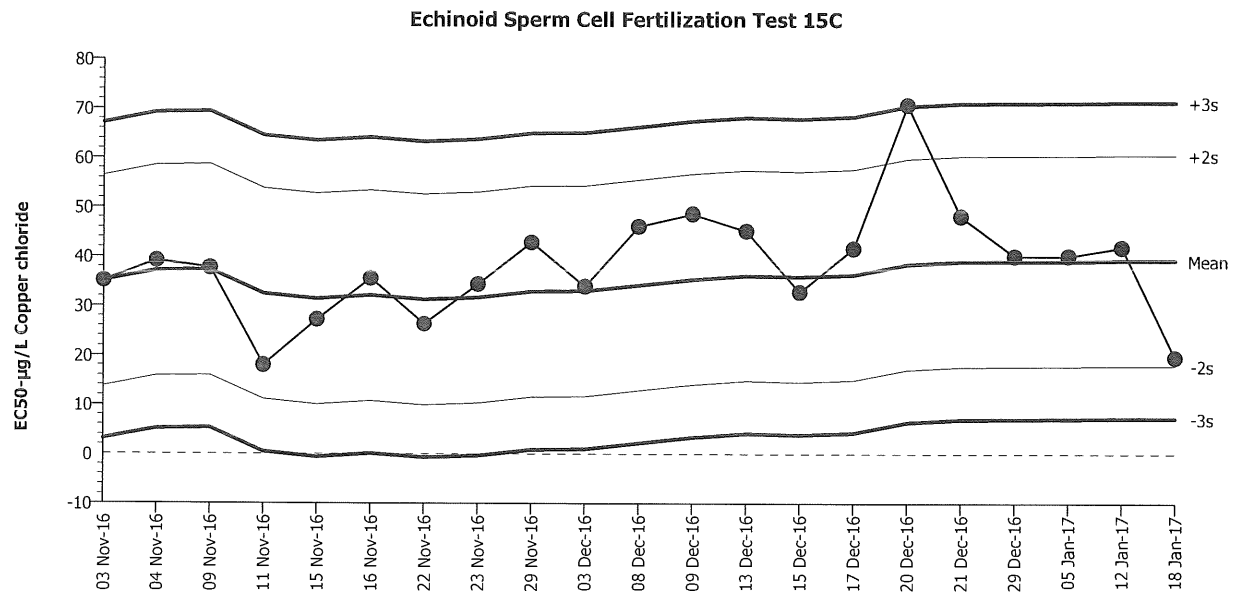
Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Organism: Strongylocentrotus purpuratus (Purpl
Endpoint: Fertilization Rate

Material: Copper chloride
Source: Reference Toxicant-REF



Mean: 39.33

Count: 20

-2s Warning Limit: 17.97

-3s Action Limit: 7.289

Sigma: 10.68

CV: 27.20%

+2s Warning Limit: 60.69

+3s Action Limit: 71.37

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Nov	3	15:45	35.12	-4.215	-0.3946			20-3136-0658	11-0001-2879
2			4	15:36	39.19	-0.1379	-0.01291			20-6041-1964	14-0189-3509
3			9	12:42	37.73	-1.603	-0.1501			14-1208-8602	07-4301-7295
4			11	16:16	17.98	-21.35	-1.999			05-3831-5746	09-0240-4709
5			15	14:41	27.24	-12.09	-1.132			05-8975-3204	09-5350-2298
6			16	15:57	35.58	-3.747	-0.3509			11-0010-5225	08-2681-2077
7			22	17:20	26.36	-12.97	-1.215			15-6301-8591	13-1300-2147
8			23	16:18	34.42	-4.913	-0.46			14-8399-9000	17-7821-1750
9			29	16:03	42.87	3.542	0.3316			21-1597-0375	02-3336-7521
10		Dec	3	15:05	33.99	-5.34	-0.5			02-8144-9736	08-6113-6246
11			8	15:04	46.14	6.811	0.6377			11-3755-6520	02-1834-2654
12			9	16:26	48.68	9.348	0.8753			11-3406-8076	17-4460-9811
13			13	15:11	45.26	5.929	0.5552			10-6683-7365	04-6270-7422
14			15	17:33	32.87	-6.458	-0.6047			01-7454-5472	05-8893-7899
15			17	15:22	41.72	2.388	0.2236			08-9842-8510	01-1488-1013
16			20	15:17	70.85	31.52	2.951	(+)		16-6092-1425	02-2928-0983
17			21	12:12	48.26	8.934	0.8365			14-5051-2365	16-1479-8388
18			29	16:22	40.16	0.8328	0.07798			17-0784-9661	08-0208-3856
19	2017	Jan	5	14:34	40.21	0.8818	0.08257			04-1406-8806	15-3393-3643
20			12	17:54	41.95	2.619	0.2452			14-8351-4083	12-3796-8723
21			18	15:19	19.65	-19.68	-1.842			08-8914-3626	00-6318-6085

CETIS Test Data Worksheet

Report Date: 16 Jan-17 11:35 (p 1 of 1)
Test Code: 08-8914-3626/170118spt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 18 Jan-17 Species: Strongylocentrotus purpuratus
End Date: 18 Jan-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 18 Jan-17 Material: Copper chloride

Sample Code: 170118spt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	1/19/17
			2	100	64	
			3	100	3	
			4	100	16	
			5	100	60	
			6	100	45	
			7	100	39	
			8	100	66	
			9	100	7	
			10	100	87	
			11	100	0	
			12	100	26	
			13	100	0	
			14	100	10	
			15	100	62	
			16	100	0	
			17	100	52	
			18	100	52	
			19	100	0	
			20	100	0	
			21	100	65	
			22	100	0	
			23	100	97	
			24	100	92	
			25	100	1	
			26	100	92	
			27	100	0	
			28	100	91	
			29	100	59	
			30	100	0	

CETIS Test Data Worksheet

Report Date: 16 Jan-17 11:35 (p 1 of 1)
Test Code: 08-8914-3626/170118sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 18 Jan-17 Species: Strongylocentrotus purpuratus
End Date: 18 Jan-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 18 Jan-17 Material: Copper chloride

Sample Code: 170118sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	10	100	43	EG 1/18/17
0	LC	2	24			
0	LC	3	23			
0	LC	4	26			
0	LC	5	28			
10		1	2			
10		2	8	100	72	
10		3	15			
10		4	29			
10		5	21			
20		1	7			
20		2	5	100	43	EG
20		3	6			
20		4	17			
20		5	18			
40		1	9	100	11	EG
40		2	14	X		
40		3	4			
40		4	3			
40		5	12			
80		1	11			
80		2	25	100	0	EG
80		3	19			
80		4	16			
80		5	27			
160		1	20			
160		2	13			
160		3	1	100	0	EG
160		4	30			
160		5	22			

QC: EG

⊗ EG 1/18/17

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 1/18/2017 1519Test No: 170118sprtEnd Date/Time: 1/18/2017 1559Dilutions made by: EG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.2
Final Volume (mL):	500
Cu stock concentration (µg/L):	9800

Analyst: RH

Initial Readings				
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.6	8.02	33.4	14.9
10	8.5	8.00	33.4	14.6
20	8.4	8.01	33.4	14.6
40	8.3	8.03	33.5	14.9
80	8.3	8.04	33.2	14.7
160	8.3	8.04	33.0	14.8

Comments: _____

QC Check: KOB 1/25/17Final Review: KFP 1/26/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: cu C12
 Test No.: 170118sprt

Start Date/Time: 1/18/2017 1 1519
 End Date/Time: 1/18/2017 1 1559
 Species: S. purpuratus
 Animal Source: Pt. Loma
 Date Collected: 12/20/16

Tech initials: EL
 Injection Time: 1440

Sperm Absorbance at 400 nm: 0.854 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 85 Mean: 80.6 X 50 = 4030 eggs/ml

76
93
73
76

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

Initial density: 4030 eggs/ml = (A) dilution factor egg stock (A) ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater (A) ml
(A) 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>1448</u>	<u>50:1</u>	<u>78</u>	<u>22</u>
Eggs Added (0.5 ml):	<u>1458</u>	<u>100:1</u>	<u>94/94</u>	<u>6/6</u>
Test Ended:	<u>1508</u>	<u>200:1</u>	<u>99</u>	<u>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: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1519</u>	QC1	<u>91</u>	<u>9</u>
Eggs Added (0.5 ml):	<u>1539</u>	QC2	<u>92</u>	<u>8</u>
Test Ended:	<u>1559</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) No Dilution Needed

QC Check:

KB 1/25/17

Final Review: KFP 1/26/17

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.