



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

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

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

Prepared by: Nautilus Environmental

Submitted: January 2, 2018

Data Quality Assurance:

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

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Results verified by: Adrienne Libor

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – DECEMBER 2017

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

Sampling Date: December 15, 2017

Test Date: December 15, 2017

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

M-001
Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	M-001 Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	NOEC	TU_c	No
	2.5	40	

INTRODUCTION

A discharge sample was collected in December 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for daily chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time in accordance with 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 December 15, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was couriered to Nautilus the day of sample 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. 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 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:	December 2017 (plant off-spec period)
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	12/15/17, 10:30
Sample Receipt Date, Time:	12/15/17, 13:54
Sampling Method:	Grab

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	7.91	8.1	4.0	60.3	209	0.04

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	12/15/17, 18:53 through 19:33
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 (SIO) inlet), 34±2 parts per thousand (ppt); 20-µm filtered
Additional Control:	High Salinity Control (HSC) – seawater with Nautilus hypersaline brine added to match the salinity of the 15 percent M-001 effluent concentration; tested to evaluate potential adverse effects due to elevated salinity alone
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 sample 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 and CDP discharges).
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. 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 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, 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 statistical analysis is not in the 2006 CDP permit, the TST results are included for comparison purposes only.

RESULTS

There was a significant decrease in the fertilization rate in the 5, 6.06, 10, and 15 percent effluent concentrations in the unadjusted M-001 sample relative to the lab control using the EPA 1995 flowchart statistics. The NOEC is reported as 2.5 percent effluent and the TU_c is equal to 40, which is above the maximum permit effluent limitation of 16.5 TU_c . A significant decrease was observed at 15 percent effluent concentration in the M-001 unadjusted sample using the TST statistical analysis. The high salinity control resulted in 98.4 percent mean fertilization indicating that reduced fertilization in the unadjusted sample was not likely due to elevated salinity.

There was no significant decrease in the fertilization rate in any percent effluent concentrations in the 40 ppt adjusted M-001 sample relative to the lab control using the EPA 1995 flowchart statistics. No significant decrease was observed at any percent effluent concentration in the M-001 40 ppt adjusted sample using the TST statistical analysis.

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 at IWC
M-001 (unadjusted)	2.5	5	>15	40	Pass	6.9
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-1.0

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 6.06% IWC according to the TST calculation; Fail = sample is toxic at the 6.06% 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	33.9	98.2	33.9	97.8
High Salinity Control	37.7	98.4	--	--
2.5	34.6	97.0	34.2	98.6
5.0	35.3	94.4*	34.3	98.2
6.06	35.6	91.4*	34.4	98.8
10	36.6	86.4*	34.6	97.8
15	37.9	80.2*	34.9	97.0

* An asterisk indicates a statistically significant difference when compared to the lab control using EPA 1995 flowchart statistical methods.

^a For comparison to the M-001 unadjusted sample, the M-001 sample was adjusted with seawater to 40 ppt prior to preparing test concentrations.

QUALITY ASSURANCE

The sample was received on the same day as collection and was within the appropriate temperature range. The tests were initiated within the 36-hour holding time. 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 threshold effect and 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. The median effect concentration (EC₅₀) value was slightly above two standard deviations (SD) from the historical mean, indicating organisms may have been less sensitive to copper than typical. A list of qualifier codes used on bench datasheets can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ± 2 SD (µg/L Copper)	CV (%)
12/15/17	76.8	45.7 ± 30.3	33.1

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.
- 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

M-001 Unadjusted

CETIS Summary Report

Report Date: 22 Dec-17 12:02 (p 1 of 1)
 Test Code: 1712-S094 | 06-5365-9451

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	19-5407-4607	Test Type:	Fertilization	Analyst:							
Start Date:	15 Dec-17 18:53	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	15 Dec-17 19:33	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	03-2208-3557	Code:	17-1295	Client:	IDE						
Sample Date:	15 Dec-17 10:30	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	15 Dec-17 13:54	Source:	IDE Americas, Inc.								
Sample Age:	8h (4 °C)	Station:	M-001 (Daily Unadjusted)	12/15 sample							
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
11-1561-6682	Fertilization Rate	2.5	5	3.536	2.37%	40	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
12-2289-1557	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)				
		EC50	>15	N/A	N/A	<6.667					
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
11-1561-6682	Fertilization Rate	Control Resp	0.982	0.7 - NL	Yes	Passes Acceptability Criteria					
12-2289-1557	Fertilization Rate	Control Resp	0.982	0.7 - NL	Yes	Passes Acceptability Criteria					
11-1561-6682	Fertilization Rate	PMSD	0.02374	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	High Salinity Co	5	0.984	0.9772	0.9908	0.98	0.99	0.00245	0.005479	0.56%	0.0%
0	Lab Control	5	0.982	0.9684	0.9956	0.97	1	0.004899	0.01095	1.12%	0.2%
2.5		5	0.97	0.9504	0.9896	0.95	0.99	0.007071	0.01581	1.63%	1.42%
5		5	0.944	0.9329	0.9551	0.93	0.95	0.004	0.008944	0.95%	4.07%
6.06		5	0.914	0.8914	0.9366	0.89	0.94	0.008124	0.01817	1.99%	7.11%
10		5	0.864	0.7951	0.9329	0.78	0.93	0.02482	0.0555	6.42%	12.2%
15		5	0.802	0.7528	0.8512	0.74	0.84	0.01772	0.03962	4.94%	18.5%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	High Salinity Co	0.98	0.98	0.98	0.99	0.99					
0	Lab Control	1	0.98	0.98	0.98	0.97					
2.5		0.97	0.98	0.99	0.95	0.96					
5		0.94	0.95	0.93	0.95	0.95					
6.06		0.89	0.91	0.92	0.94	0.91					
10		0.93	0.89	0.85	0.87	0.78					
15		0.84	0.83	0.81	0.74	0.79					

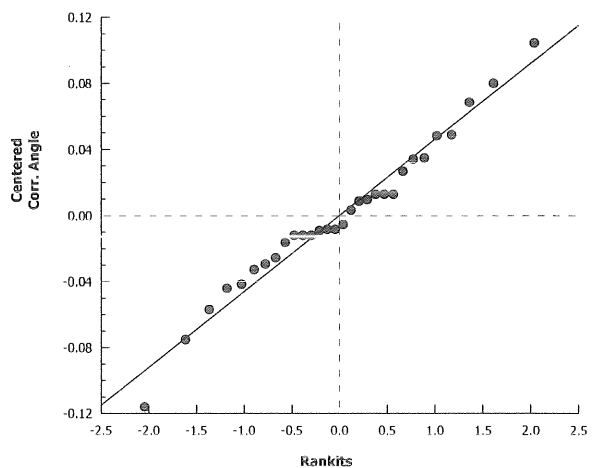
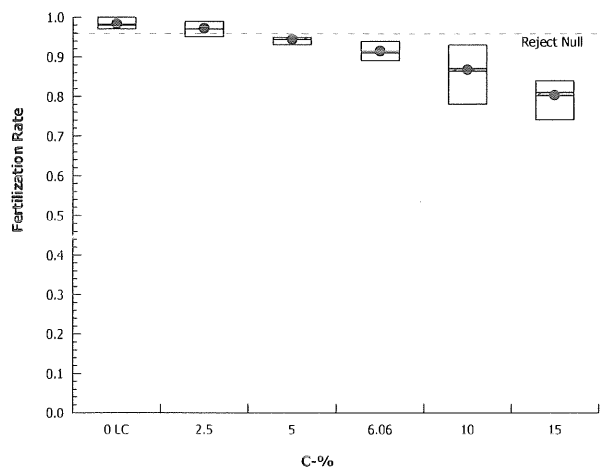
CETIS Analytical Report

Report Date: 22 Dec-17 11:58 (p 1 of 2)
 Test Code: 1712-S094 | 06-5365-9451

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 11-1561-6682		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 22 Dec-17 11:57		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		2.37%	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	1.222	2.362	0.075	8	0.3256	CDF	Non-Significant Effect		
		5*	3.426	2.362	0.075	8	0.0047	CDF	Significant Effect		
		6.06*	5.259	2.362	0.075	8	<0.0001	CDF	Significant Effect		
		10*	7.655	2.362	0.075	8	<0.0001	CDF	Significant Effect		
		15*	10.42	2.362	0.075	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.3883677		0.07767354		5		31.05	<0.0001	Significant Effect		
Error	0.06004647		0.002501936		24						
Total	0.4484141				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			7.336	15.09	0.1968		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9806	0.9031	0.8416		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	0.982	0.9684	0.9956	0.98	0.97	1	0.004899	1.12%	0.0%
2.5		5	0.97	0.9504	0.9896	0.97	0.95	0.99	0.007071	1.63%	1.22%
5		5	0.944	0.9329	0.9551	0.95	0.93	0.95	0.004	0.95%	3.87%
6.06		5	0.914	0.8914	0.9366	0.91	0.89	0.94	0.008124	1.99%	6.93%
10		5	0.864	0.7951	0.9329	0.87	0.78	0.93	0.02482	6.42%	12.02%
15		5	0.802	0.7528	0.8512	0.81	0.74	0.84	0.01772	4.94%	18.33%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.441	1.383	1.499	1.429	1.397	1.521	0.02093	3.25%	0.0%
2.5		5	1.402	1.341	1.463	1.397	1.345	1.471	0.02207	3.52%	2.68%
5		5	1.332	1.309	1.356	1.345	1.303	1.345	0.008492	1.43%	7.52%
6.06		5	1.274	1.233	1.315	1.266	1.233	1.323	0.01477	2.59%	11.55%
10		5	1.199	1.098	1.299	1.202	1.083	1.303	0.03619	6.75%	16.81%
15		5	1.111	1.05	1.172	1.12	1.036	1.159	0.02185	4.4%	22.89%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 11-1561-6682	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 22 Dec-17 11:57	Analysis: Parametric-Control vs Treatments	Official Results: Yes	

Graphics



CETIS Analytical Report

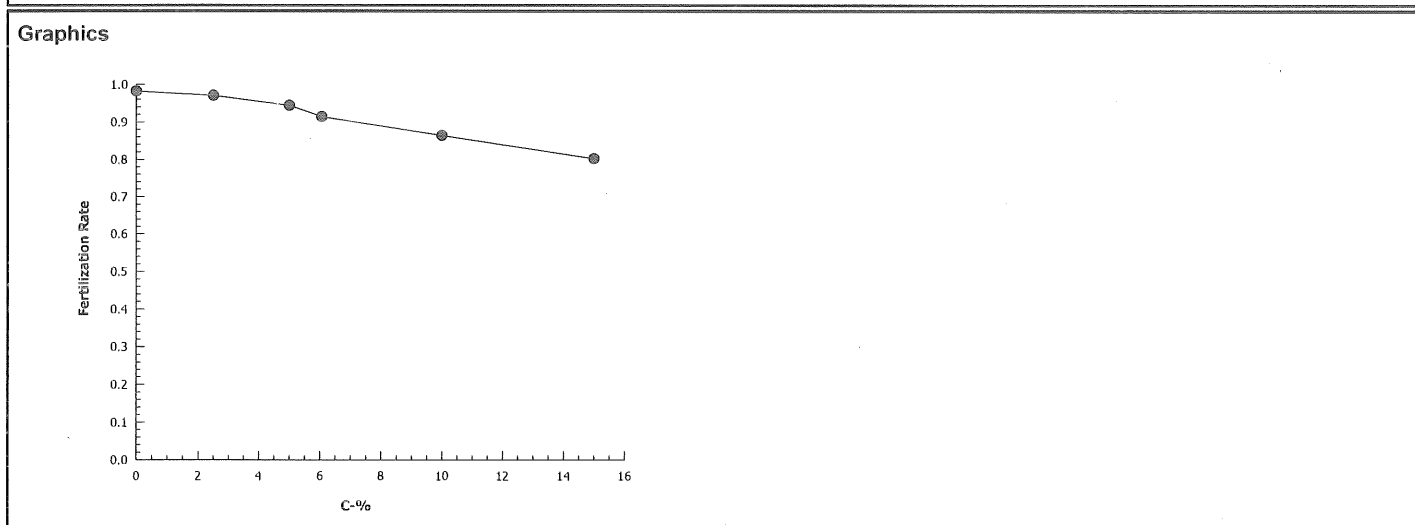
Report Date: 22 Dec-17 11:58 (p 1 of 1)
 Test Code: 1712-S094 | 06-5365-9451

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	12-2289-1557	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	22 Dec-17 11:57	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1028130	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	0.982	0.97	1	0.004899	0.01095	1.12%	0.0%	491	500
2.5		5	0.97	0.95	0.99	0.007071	0.01581	1.63%	1.22%	485	500
5		5	0.944	0.93	0.95	0.004	0.008944	0.95%	3.87%	472	500
6.06		5	0.914	0.89	0.94	0.008124	0.01817	1.99%	6.93%	457	500
10		5	0.864	0.78	0.93	0.02482	0.0555	6.42%	12.02%	432	500
15		5	0.802	0.74	0.84	0.01772	0.03962	4.94%	18.33%	401	500



CETIS Analytical Report

TSI

 Report Date: 22 Dec-17 11:58 (p 1 of 1)
 Test Code: 1712-S094 | 06-5365-9451

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 16-9636-1357		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 22 Dec-17 11:57		Analysis: Parametric Bioequivalence-Two Sample					Official Results: Yes				
Data Transform		Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C*b < T	NA	NA	0.75	1.46%	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*	11.87	1.895	0.051	7	<0.0001	CDF	Non-Significant Effect		
		5*	14.11	1.943	0.035	6	<0.0001	CDF	Non-Significant Effect		
		6.06*	8.993	1.895	0.041	7	<0.0001	CDF	Non-Significant Effect		
		10*	2.993	2.015	0.079	5	0.0152	CDF	Non-Significant Effect		
		15	1.131	1.895	0.051	7	0.1476	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.3883677		0.07767354		5		31.05	<0.0001	Significant Effect		
Error	0.06004647		0.002501936		24						
Total	0.4484141				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			7.336	15.09	0.1968		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9806	0.9031	0.8416		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	0.982	0.9684	0.9956	0.98	0.97	1	0.004899	1.12%	0.0%
2.5		5	0.97	0.9504	0.9896	0.97	0.95	0.99	0.007071	1.63%	1.22%
5		5	0.944	0.9329	0.9551	0.95	0.93	0.95	0.004	0.95%	3.87%
6.06		5	0.914	0.8914	0.9366	0.91	0.89	0.94	0.008124	1.99%	6.93%
10		5	0.864	0.7951	0.9329	0.87	0.78	0.93	0.02482	6.42%	12.02%
15		5	0.802	0.7528	0.8512	0.81	0.74	0.84	0.01772	4.94%	18.33%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.441	1.383	1.499	1.429	1.397	1.521	0.02093	3.25%	0.0%
2.5		5	1.402	1.341	1.463	1.397	1.345	1.471	0.02207	3.52%	2.68%
5		5	1.332	1.309	1.356	1.345	1.303	1.345	0.008492	1.43%	7.52%
6.06		5	1.274	1.233	1.315	1.266	1.233	1.323	0.01477	2.59%	11.55%
10		5	1.199	1.098	1.299	1.202	1.083	1.303	0.03619	6.75%	16.81%
15		5	1.111	1.05	1.172	1.12	1.036	1.159	0.02185	4.4%	22.89%

CETIS Test Data Worksheet

Report Date: 15 Dec-17 11:40 (p 1 of 1)
 Test Code: 06-5365-9451/26F60D3B
 1712-5094

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Sample Code: 17- 1295
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Unadjusted) Daily 12/15 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	94	Read by JC 12/18/17
			2	100	95	
			3	100	89	
			4	100	98	
			5	100	85	
			6	100	89	
			7	100	99	
			8	100	87	
			9	100	93	
			10	100	93	
			11	100	95	
			12	100	97	
			13	100	98	
			14	100	91	
			15	100	97	
			16	100	79	
			17	100	98	
			18	100	83	
			19	100	91	
			20	100	94	
			21	100	96	
			22	100	95	
			23	100	98	
			24	100	92	
			25	100	81	
			26	100	99	
			27	100	95	
			28	100	84	
			29	100	98	
			30	100	78	
			31	100	74	
			32	100	99	
			33	100	98	
			34	100	78	
			35	100	100	

AD 018 12/22/17

CETIS Test Data Worksheet

Report Date: 15 Dec-17 11:40 (p 1 of 1)
 Test Code: 17M-504 06-5365-9451/26F60D3B

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Sample Code: 17- 1295
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Unadjusted) 12/15 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	13			
0	HS	2	29			
0	HS	3	23			
0	HS	4	26			
0	HS	5	7			
0	LC	1	35			
0	LC	2	17			
0	LC	3	4	100	96	BO 12/22/17
0	LC	4	34			
0	LC	5	12			
2.5		1	15			
2.5		2	33			
2.5		3	32			
2.5		4	22			
2.5		5	21			
5		1	20			
5		2	2			
5		3	10			
5		4	11			
5		5	27			
6.06		1	6			
6.06		2	14			
6.06		3	24			
6.06		4	1	100	94	BO 12/22/17
6.06		5	19			
10		1	9			
10		2	3			
10		3	5			
10		4	8			
10		5	30			
15		1	28			
15		2	18			
15		3	25			
15		4	31			
15		5	16			

QC: CG

QC: AD

Marine Chronic Bioassay

Water Quality Measurements

Client : IDETest Species: S. purpuratusSample ID: M-001 (unadjusted) 12/15 sampleStart Date/Time: 12/15/17 1853Sample Log No.: 17- 1295End Date/Time: 12/15/17 1933Dilutions made by: ADTest No: 1712-8094

Analyst:

AC

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.8	8.07	33.9	14.0
High Salinity Control	8.9	8.03	37.7	14.0
2.5	8.7	8.06	34.6	14.5
5.0	8.7	8.05	35.3	14.6
6.06	8.6	8.04	35.6	14.5
10	8.7	8.03	36.6	14.4
15	8.7	8.01	37.9	14.5

Comments: _____

QC Check: AD 12/22/17Final Review: EC 12/28/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-001 unadjusted 12/15 sample
 Test No.: 1712-3094
 Tech initials: AD OBO PA
 Injection Time: 1815

Start Date/Time: 12/15/17 1853
 End Date/Time: 12/15/17 1933
 Species: S. purpuratus
 Animal Source: Dr. Lurbe
 Date Collected: 12/18/17

Sperm Absorbance at 400 nm: 0.8140 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 79 Mean: 80 X 50 = 4000 eggs/ml

75
78
78
90

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

Initial density: 4000 eggs/ml = 1.0 dilution factor egg stock 1A ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 1A ml
0 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>1825</u>	<u>50:1</u>	<u>84</u>	<u>16</u>
Eggs Added (0.5 ml):	<u>1835</u>	<u>100:1</u>	<u>95</u>	<u>5</u>
Test Ended:	<u>1845</u>	<u>100:1</u>	<u>97</u>	<u>3</u>
		<u>200:1</u>	<u>100</u>	<u>100</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>1853</u>	QC1	<u>99</u>	<u>1</u>
Eggs Added (0.5 ml):	<u>1913</u>	QC2	<u>98</u>	<u>2</u>
Test Ended:	<u>1933</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

No dilution required

QC Check: MO 12/22/17

Final Review: EE 12/28/17

M-001 40 ppt Adjusted

CETIS Summary Report

Report Date: 22 Dec-17 12:15 (p 1 of 1)

Test Code: 1712-S095 | 18-3208-1749

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)							
Batch ID:	10-9769-7927	Test Type:	Fertilization	Analyst:							
Start Date:	15 Dec-17 18:53	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	15 Dec-17 19:33	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	07-2433-2658	Code:	17-1295	Client:	IDE						
Sample Date:	15 Dec-17 10:30	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	15 Dec-17 13:54	Source:	IDE Americas, Inc.								
Sample Age:	8h (4 °C)	Station:	M-001 (Daily 40ppt)	12/15 sample							
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
00-6042-0415	Fertilization Rate	15	>15	NA	3.35%	6.667	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
17-7722-1389	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			
00-6042-0415	Fertilization Rate	Control Resp		0.978	0.7 - NL		Yes	Passes Acceptability Criteria			
17-7722-1389	Fertilization Rate	Control Resp		0.978	0.7 - NL		Yes	Passes Acceptability Criteria			
00-6042-0415	Fertilization Rate	PMSD		0.03351	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	0.978	0.9526	1	0.96	1	0.009165	0.02049	2.1%	0.0%
2.5		5	0.986	0.9672	1	0.97	1	0.006782	0.01517	1.54%	-0.82%
5		5	0.982	0.9511	1	0.94	1	0.01114	0.0249	2.54%	-0.41%
6.06		5	0.988	0.9676	1	0.97	1	0.007348	0.01643	1.66%	-1.02%
10		5	0.978	0.9676	0.9884	0.97	0.99	0.003741	0.008366	0.86%	0.0%
15		5	0.97	0.9452	0.9948	0.95	1	0.008944	0.02	2.06%	0.82%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	1	0.96	1	0.97	0.96					
2.5		1	0.97	0.99	1	0.97					
5		0.98	1	0.99	1	0.94					
6.06		1	0.97	1	1	0.97					
10		0.97	0.99	0.97	0.98	0.98					
15		0.96	1	0.96	0.98	0.95					

[Signature]

EC 12/28/17

CETIS Analytical Report

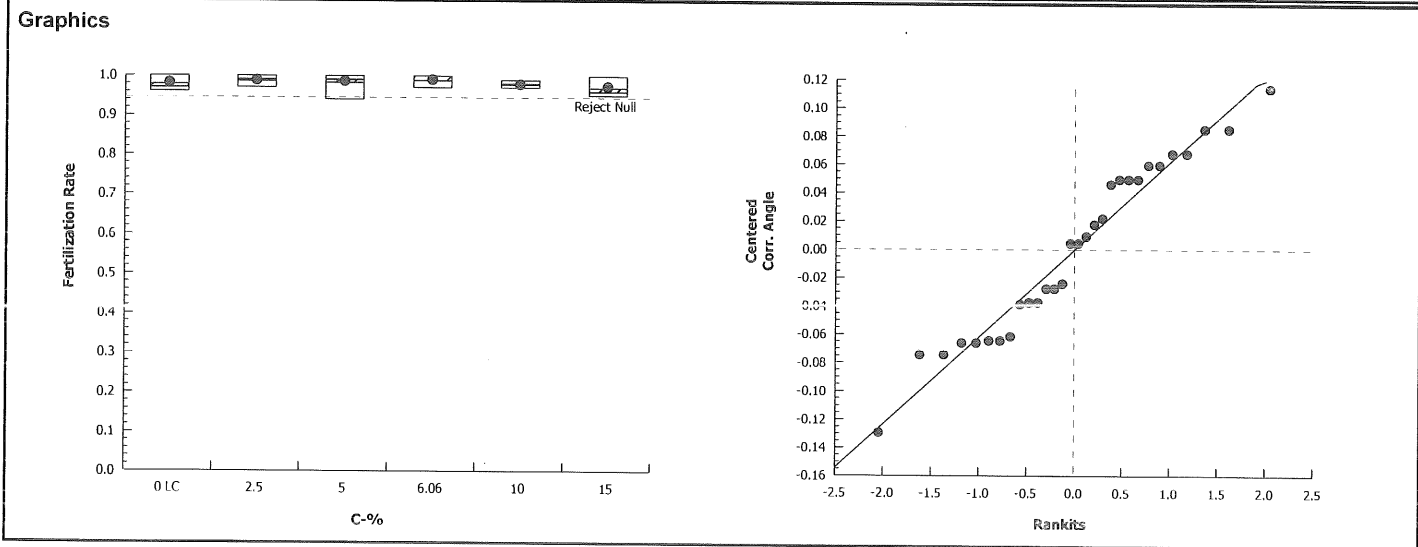
Report Date: 22 Dec-17 12:15 (p 1 of 2)
Test Code: 1712-S095 | 18-3208-1749

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 00-6042-0415		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 22 Dec-17 12:14		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		3.35%	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	-0.6017	2.362	0.101	8	0.9529	CDF	Non-Significant Effect		
		5	-0.4087	2.362	0.101	8	0.9263	CDF	Non-Significant Effect		
		6.06	-0.8365	2.362	0.101	8	0.9740	CDF	Non-Significant Effect		
		10	0.2589	2.362	0.101	8	0.7457	CDF	Non-Significant Effect		
		15	0.6712	2.362	0.101	8	0.5702	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.01464565		0.002929131		5	0.6425	0.6696	Non-Significant Effect			
Error	0.1094092		0.004558718		24						
Total	0.1240549				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			3.468	15.09	0.6282	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.956	0.9031	0.2435	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	0.978	0.9526	1	0.97	0.96	1	0.009165	2.1%	0.0%
2.5		5	0.986	0.9672	1	0.99	0.97	1	0.006782	1.54%	-0.82%
5		5	0.982	0.9511	1	0.99	0.94	1	0.01114	2.54%	-0.41%
6.06		5	0.988	0.9676	1	1	0.97	1	0.007348	1.66%	-1.02%
10		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	0.0%
15		5	0.97	0.9452	0.9948	0.96	0.95	1	0.008944	2.06%	0.82%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.435	1.338	1.533	1.397	1.369	1.521	0.0352	5.48%	0.0%
2.5		5	1.461	1.384	1.538	1.471	1.397	1.521	0.02784	4.26%	-1.79%
5		5	1.453	1.351	1.555	1.471	1.323	1.521	0.03667	5.64%	-1.22%
6.06		5	1.471	1.387	1.556	1.521	1.397	1.521	0.03039	4.62%	-2.49%
10		5	1.424	1.387	1.462	1.429	1.397	1.471	0.01362	2.14%	0.77%
15		5	1.407	1.319	1.495	1.369	1.345	1.521	0.03167	5.03%	2.0%

CETIS Analytical Report

Report Date: 22 Dec-17 12:15 (p 2 of 2)
 Test Code: 1712-S095 | 18-3208-1749

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)	
Analysis ID:	00-6042-0415	Endpoint:	Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed:	22 Dec-17 12:14	Analysis:	Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

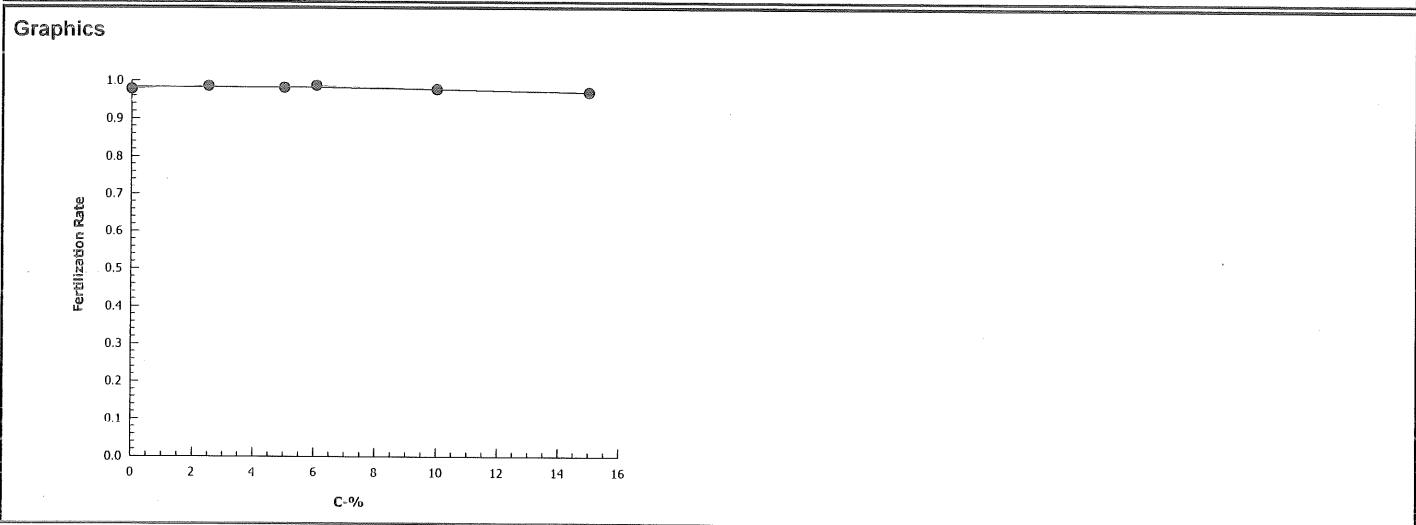
Report Date: 22 Dec-17 12:15 (p 1 of 1)
Test Code: 1712-S095 | 18-3208-1749

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	17-7722-1389	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	22 Dec-17 12:15	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	862174	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	0.978	0.96	1	0.009165	0.02049	2.1%	0.0%	489	500
2.5		5	0.986	0.97	1	0.006782	0.01517	1.54%	-0.82%	493	500
5		5	0.982	0.94	1	0.01114	0.0249	2.54%	-0.41%	491	500
6.06		5	0.988	0.97	1	0.007348	0.01643	1.66%	-1.02%	494	500
10		5	0.978	0.97	0.99	0.003741	0.008366	0.86%	0.0%	489	500
15		5	0.97	0.95	1	0.008944	0.02	2.06%	0.82%	485	500



CETIS Analytical Report

TST

Report Date: 22 Dec-17 12:16 (p 1 of 1)
Test Code: 1712-S095 | 18-3208-1749

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 12-8305-1173		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 22 Dec-17 12:15		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	2.34%	15	>15	NA	6.667
TST-Welch's t Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5*	10.02	1.895	0.073	7	<0.0001	CDF	Non-Significant Effect		
		5*	8.329	1.895	0.086	7	<0.0001	CDF	Non-Significant Effect		
		6.06*	9.802	1.895	0.076	7	<0.0001	CDF	Non-Significant Effect		
		10*	11.71	2.015	0.06	5	<0.0001	CDF	Non-Significant Effect		
		15*	8.009	1.895	0.078	7	<0.0001	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.01464565		0.002929131		5	0.6425	0.6696	Non-Significant Effect			
Error	0.1094092		0.004558718		24						
Total	0.1240549				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			3.468	15.09	0.6282	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.956	0.9031	0.2435	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	0.978	0.9526	1	0.97	0.96	1	0.009165	2.1%	0.0%
2.5		5	0.986	0.9672	1	0.99	0.97	1	0.006782	1.54%	-0.82%
5		5	0.982	0.9511	1	0.99	0.94	1	0.01114	2.54%	-0.41%
6.06		5	0.988	0.9676	1	1	0.97	1	0.007348	1.66%	-1.02%
10		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	0.0%
15		5	0.97	0.9452	0.9948	0.96	0.95	1	0.008944	2.06%	0.82%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.435	1.338	1.533	1.397	1.369	1.521	0.0352	5.48%	0.0%
2.5		5	1.461	1.384	1.538	1.471	1.397	1.521	0.02784	4.26%	-1.79%
5		5	1.453	1.351	1.555	1.471	1.323	1.521	0.03667	5.64%	-1.22%
6.06		5	1.471	1.387	1.556	1.521	1.397	1.521	0.03039	4.62%	-2.49%
10		5	1.424	1.387	1.462	1.429	1.397	1.471	0.01362	2.14%	0.77%
15		5	1.407	1.319	1.495	1.369	1.345	1.521	0.03167	5.03%	2.0%

CETIS Test Data Worksheet

Report Date: 15 Dec-17 12:10 (p 1 of 1)
 Test Code: 18-3208-1749/6D335955
 1712-1095

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Sample Code: 17-1295
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Daily 40ppt) 12/15 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			36	100	100	Read by JC 12/19/17
			37	100	98	
			38	100	97	
			39	100	97	
			40	100	100	
			41	100	95	
			42	100	98	
			43	100	94	
			44	100	97	
			45	100	96	
			46	100	99	
			47	100	100	
			48	100	97	
			49	100	100	
			50	100	99	
			51	100	100	
			52	100	99	
			53	100	96	
			54	100	98	
			55	100	100	
			56	100	96	
			57	100	97	
			58	100	97	
			59	100	100	
			60	100	100	
			61	100	100	
			62	100	98	
			63	100	97	
			64	100	96	
			65	100	100	

Read Q18 12/22/13

CETIS Test Data Worksheet

Report Date: 22 Dec-17 11:36 (p 1 of 1)
 Test Code: 1712-5095 18-3208-1749/6D335955

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

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

Sample Code: 17-1295
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Daily 40ppt) 12/15 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	59			
0	LC	2	45	100	97	Bo 12/22/17
0	LC	3	55			
0	LC	4	39			
0	LC	5	53			
2.5		1	60			
2.5		2	57			
2.5		3	52			
2.5		4	61			
2.5		5	38			
5		1	62			
5		2	40			
5		3	46			
5		4	49			
5		5	43			
6.06		1	65			
6.06		2	48			
6.06		3	51	100	100	Bo 12/22/17
6.06		4	47			
6.06		5	63			
10		1	58			
10		2	50			
10		3	44			
10		4	37			
10		5	42			
15		1	64			
15		2	36			
15		3	56			
15		4	54			
15		5	41			

Marine Chronic Bioassay

Water Quality Measurements

Client : IDETest Species: S. purpuratusSample ID: M-001 (40 ppt adjusted) 12/15 sampleStart Date/Time: 12/15/17 1853Sample Log No.: 17-1295End Date/Time: 12/15/17 1933Dilutions made by: ADTest No: 1712-3095Analyst: AC

Concentration %	initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	9.0	8.08	33.9	14.0
2.5	8.9	8.07	34.2	14.0
5.0	8.9	8.06	34.3	14.0
6.06	8.9	8.07	34.4	14.0
10	8.9	8.07	34.6	14.0
15	8.9	8.07	34.9	14.0

Comments: _____

QC Check: AD 12/22/17Final Review: EG 12/28/17

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: IDE

Analyst: CG

Sample ID: M-001 (40 ppt adjusted) 12/15/17 sample

Test Date: 12/15/2017

Test No: 1712-8095

Test Type: Urchin Fertilization

Salinity of Effluent 60.3

Salinity of Seawater 33.5

Date of Brine used: NA

Target Salinity 40.0

Alk. of 40 ppt Adj. Sample: 141 mg/L as CaCO₃

	<u>Effluent</u>	<u>Brine Control</u>
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	<u>3.12</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	3.12	312.3	412

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: AD 12/22/17

Final Review: EG 12/28/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M001 40ppt adjusted 12/15 sample
 Test No.: 1712-8095
 Tech initials: AD OBO PA
 Injection Time: 1815

Start Date/Time: 12/15/17 1853
 End Date/Time: 12/15/17 1933
 Species: *S. purpuratus*
 Animal Source: Dr. Luthi
 Date Collected: 12/18/17

Sperm Absorbance at 400 nm: 0.810 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 79 Mean: 80 X 50 = 4000 eggs/ml

75
78
78
90

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

Initial density: 4000 eggs/ml = 1.0 dilution factor egg stock 10 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 10 ml
0 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>1825</u>	<u>50:1</u>	<u>84</u>	<u>16</u>
Eggs Added (0.5 ml):	<u>1835</u>	<u>100:1</u>	<u>95</u>	<u>5</u>
Test Ended:	<u>1845</u>	<u>100:1</u>	<u>97</u>	<u>3</u>
		<u>200:1</u>	<u>100</u>	<u>100</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>1853</u>	QC1	<u>99</u>	<u>1</u>
Eggs Added (0.5 ml):	<u>1913</u>	QC2	<u>98</u>	<u>2</u>
Test Ended:	<u>1933</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

no dilution required

QC Check:

AD 12/22/17

Final Review:

EG 12/28/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: 1 DE
Sample ID: Daily M-001 (12/15)
Test ID No(s): 1712-S094 and S095

Sample Check-In Information

Sample Description:

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

COC Complete (Y/N)?

A Y B C

Filtration? Y (N)

Pore Size:
Organisms or Debris

Salinity Adjustment? Y (N)

Test: within fertilization Source: seawater Target ppt: 40

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: AD 12/22/17

Final Review: EG 12/28/17

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1295			
Sample Collection Date & Time:	12/15/17 1030			
Sample Receipt Date & Time:	12/15/17 1354			
Number of Containers & Container Type:	1 - 4L ubi			
Approx. Total Volume Received (L):	4			
Check-in Temperature (°C)	4.0			
Temperature OK? ¹	<u>(Y)</u> N	Y N	Y N	Y N
DO (mg/L)	8.1			
pH (units)	7.91			
Conductivity (µS/cm)	<u> </u>			
Salinity (ppt)	60.3 <u>(8)</u>			
Alkalinity (mg/L) ²	209			
Hardness (mg/L) ^{2,3}	<u> </u>			
Total Chlorine (mg/L)	0.04			
Technician Initials	AB/BO			

Test Performed: Within Fert Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:

Alkalinity: 116 Hardness or Salinity: 34
Additional Control? (Y) N = HSC Alkalinity: 99 Hardness or Salinity: 37.7

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:

Salinity measured by making 1:1 dilution
EG Q19 12/28/17 AB Q18 12/22/17

Appendix C

Chain-of-Custody Form



CDP Laboratories
Page 1 Of

Turn Around Time

Normal: x

RUSH (24 hr):

3 Days:

5 Days:

??? Days

Special instruction: Sampled during off-spec. Sample collected to fulfill daily NPDES requirement. Sample is to be run adjusted and unadjusted. Sampled 12/15/2017 @ 10:30 KC

NOTES:

Drinking Water=DW Seawater=SW Soil=S

[illegible]

TDS - 57.85 ppt, EC - 81.37 mS/cm

$$temp = 4.0^{\circ}C$$

Relinquished By:	Date:	Time:	Received By:	Date:	Time:	Sample Condition Upon Receipt:	
<i>Rayen Helit</i>	12-15-17	1206	<i>Y. Mc...</i>	12/15	12:06	<input checked="" type="checkbox"/> Iced	<input type="checkbox"/> Ambient or <u>4.0</u> °C
<i>[Signature]</i>			<i>[Signature]</i>	12/15/17	1354	<input type="checkbox"/> Iced	<input type="checkbox"/> Ambient or _____ °C

*not relinquished by courier; tech error.

Nautilus ID: 17-1295

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 28 Dec-17 14:26 (p 1 of 1)

Test Code: 171215sprtB | 02-9159-5360

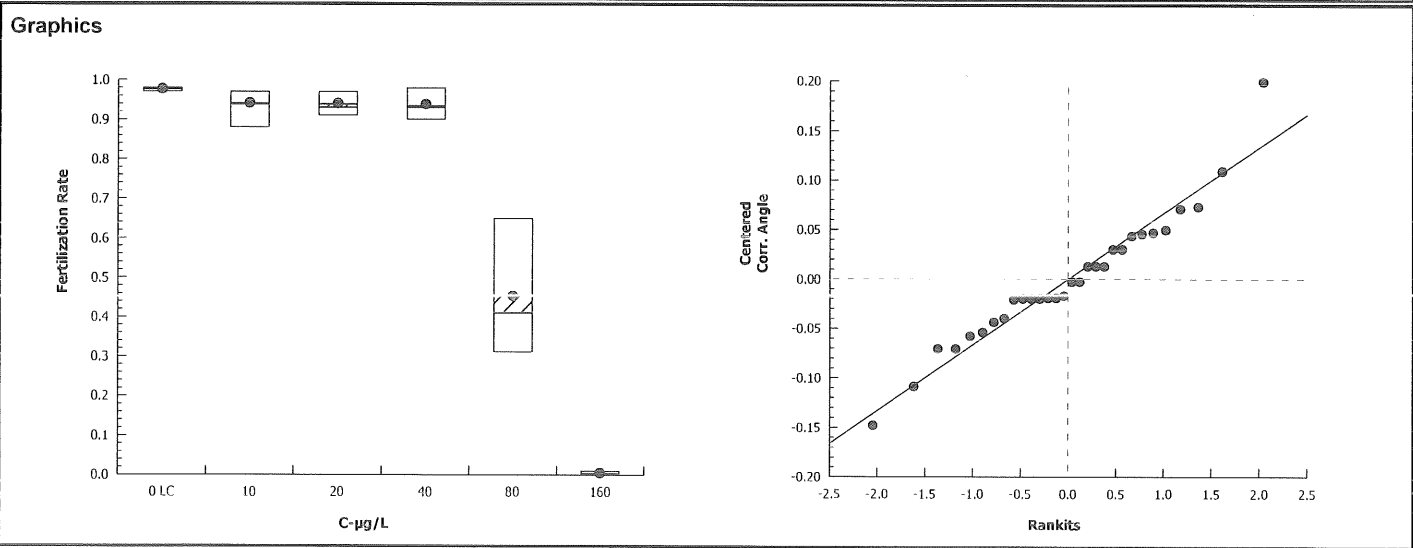
Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	13-6199-7350	Test Type:	Fertilization	Analyst:							
Start Date:	15 Dec-17 18:53	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	15 Dec-17 19:33	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	16-5483-0832	Code:	171215sprtB	Client:	Internal						
Sample Date:	15 Dec-17	Material:	Copper chloride	Project:							
Receive Date:	15 Dec-17	Source:	Reference Toxicant								
Sample Age:	19h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
07-7404-7985	Fertilization Rate	40	80	56.57	4.53%	Steel Many-One Rank Sum Test					
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
11-8739-9529	Fertilization Rate	EC50	76.76	73.99	79.63	Trimmed Spearman-Kärber					
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
07-7404-7985	Fertilization Rate	Control Resp	0.976	0.7 - NL	Yes	Passes Acceptability Criteria					
11-8739-9529	Fertilization Rate	Control Resp	0.976	0.7 - NL	Yes	Passes Acceptability Criteria					
07-7404-7985	Fertilization Rate	PMSD	0.04533	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	0.976	0.9692	0.9828	0.97	0.98	0.002449	0.005476	0.56%	0.0%
10		5	0.938	0.8946	0.9814	0.88	0.97	0.01562	0.03493	3.72%	3.89%
20		5	0.938	0.9059	0.9701	0.91	0.97	0.01158	0.02588	2.76%	3.89%
40		5	0.934	0.8896	0.9784	0.9	0.98	0.016	0.03578	3.83%	4.3%
80		5	0.454	0.2944	0.6136	0.31	0.65	0.0575	0.1286	28.32%	53.48%
160		5	0.004	0	0.0108	0	0.01	0.002449	0.005477	136.9%	99.59%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.97	0.98	0.97	0.98	0.98					
10		0.88	0.94	0.94	0.97	0.96					
20		0.97	0.92	0.91	0.96	0.93					
40		0.9	0.93	0.9	0.96	0.98					
80		0.31	0.5	0.4	0.65	0.41					
160		0.01	0	0	0.01	0					

CETIS Analytical Report

Report Date: 28 Dec-17 14:26 (p 1 of 2)
 Test Code: 171215sprtB | 02-9159-5360

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 07-7404-7985		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 27 Dec-17 13:34		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			4.53%	40	80	56.57	
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*	16	16	1	8	0.0332	Asymp	Significant Effect		
		20*	16	16	1	8	0.0332	Asymp	Significant Effect		
		40	18.5	16	1	8	0.1075	Asymp	Non-Significant Effect		
		80*	15	16	0	8	0.0191	Asymp	Significant Effect		
		160*	15	16	0	8	0.0191	Asymp	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	7.066526		1.413305		5	262.9	<0.0001	Significant Effect			
Error	0.1290262		0.005376091		24						
Total	7.195552				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			15.23	15.09	0.0094	Unequal Variances				
Distribution	Shapiro-Wilk W Normality			0.9608	0.9031	0.3245	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	0.976	0.9692	0.9828	0.98	0.97	0.98	0.002449	0.56%	0.0%
10		5	0.938	0.8946	0.9814	0.94	0.88	0.97	0.01562	3.72%	3.89%
20		5	0.938	0.9059	0.9701	0.93	0.91	0.97	0.01158	2.76%	3.89%
40		5	0.934	0.8896	0.9784	0.93	0.9	0.98	0.016	3.83%	4.3%
80		5	0.454	0.2944	0.6136	0.41	0.31	0.65	0.0575	28.32%	53.48%
160		5	0.004	0	0.0108	0	0	0.01	0.002449	136.9%	99.59%
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	1.416	1.394	1.438	1.429	1.397	1.429	0.007885	1.25%	0.0%
10		5	1.326	1.241	1.411	1.323	1.217	1.397	0.03064	5.17%	6.36%
20		5	1.324	1.254	1.394	1.303	1.266	1.397	0.02524	4.26%	6.51%
40		5	1.32	1.222	1.417	1.303	1.249	1.429	0.03511	5.95%	6.79%
80		5	0.7387	0.5761	0.9013	0.6949	0.5905	0.9377	0.05856	17.73%	47.84%
160		5	0.07008	0.03598	0.1042	0.05002	0.05002	0.1002	0.01228	39.19%	95.05%

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 07-7404-7985	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 27 Dec-17 13:34	Analysis: Nonparametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

Report Date: 28 Dec-17 14:26 (p 1 of 1)
 Test Code: 171215sprtB | 02-9159-5360

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

Analysis ID: 11-8739-9529 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.7
 Analyzed: 27 Dec-17 13:34 Analysis: Trimmed Spearman-Kärber Official Results: Yes

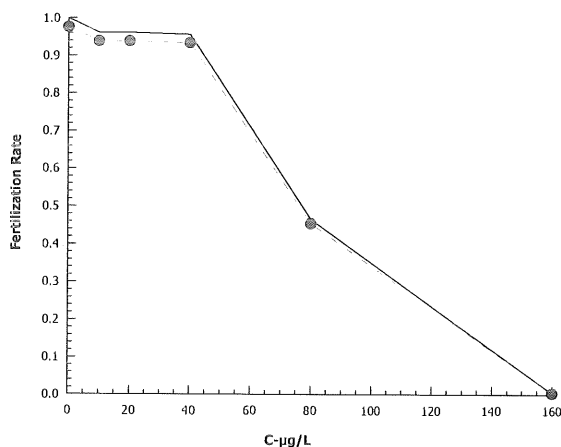
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.024	3.89%	1.885	0.007978	76.76	73.99	79.63

Fertilization Rate Summary

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	0.976	0.97	0.98	0.002449	0.005476	0.56%	0.0%	488	500	
10		5	0.938	0.88	0.97	0.01562	0.03493	3.72%	3.89%	469	500	
20		5	0.938	0.91	0.97	0.01158	0.02588	2.76%	3.89%	469	500	
40		5	0.934	0.9	0.98	0.016	0.03578	3.83%	4.3%	467	500	
80		5	0.454	0.31	0.65	0.0575	0.1286	28.32%	53.48%	227	500	
160		5	0.004	0	0.01	0.002449	0.005477	136.9%	99.59%	2	500	

Graphics



Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

Organism: Strongylocentrotus purpuratus (Purpl

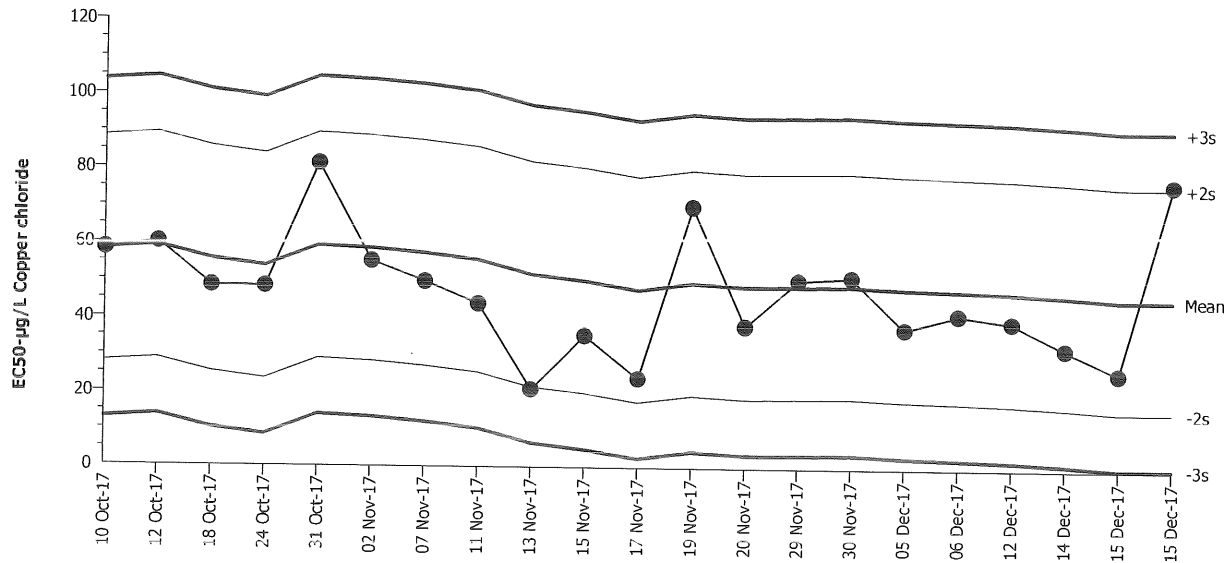
Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



Mean: 45.71

Count: 20

-2s Warning Limit: 15.45

-3s Action Limit: 0.3225

Sigma: 15.13

CV: 33.10%

+2s Warning Limit: 75.97

+3s Action Limit: 91.1

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Oct	10	15:10	58.36	12.65	0.8362			20-5863-5053	00-1542-1738
2			12	14:55	60.18	14.47	0.9565			05-0863-6526	07-1531-2424
3			18	14:22	48.53	2.82	0.1864			13-0042-6212	05-6771-5532
4			24	13:15	48.41	2.695	0.1781			20-0280-7301	18-5464-1899
5			31	13:59	81.36	35.65	2.356	(+)		06-4227-6723	08-8095-0809
6		Nov	2	12:28	55.32	9.615	0.6355			17-4126-1689	20-0626-8382
7			7	14:30	49.87	4.163	0.2752			10-3521-2857	13-9801-3995
8			11	14:25	43.91	-1.802	-0.1191			14-1655-2339	20-5239-6070
9			13	14:35	20.97	-24.74	-1.635			07-0538-7056	00-9105-4737
10			15	16:09	35.48	-10.23	-0.6759			06-3476-9418	17-5783-9769
11			17	14:17	24.03	-21.68	-1.433			20-8374-1268	00-9691-5869
12			19	10:02	70.21	24.5	1.619			12-1164-1483	20-4501-4622
13			20	15:15	38.26	-7.445	-0.4921			08-0578-7050	18-8950-2431
14			29	15:30	50.6	4.885	0.3229			05-0010-1267	11-1707-1208
15			30	15:28	51.48	5.765	0.3811			09-6334-2928	00-8447-7747
16		Dec	5	16:05	37.64	-8.068	-0.5333			00-4872-5743	06-2243-7863
17			6	15:50	41.57	-4.142	-0.2738			04-9516-7018	18-3148-8943
18			12	12:20	39.55	-6.162	-0.4073			01-8906-4164	02-6832-7767
19			14	15:35	32.51	-13.2	-0.8726			11-6397-1428	17-9802-1610
20			15	15:06	26.01	-19.7	-1.302			06-1613-2535	10-1459-1840
21			15	18:53	76.76	31.05	2.052	(+)		02-9159-5360	11-8739-9529

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:23 (p 1 of 1)
Test Code: 06-1613-2535/171215sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17 Species: Strongylocentrotus purpuratus
End Date: 15 Dec-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 15 Dec-17 Material: Copper chlorideSample Code: 171215sprtB
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	31	12/18/17
			2	100	97	
			3	100	1	
			4	100	96	
			5	100	97	
			6	100	65	
			7	100	96	
			8	100	98	
			9	100	96	
			10	100	97	
			11	100	94	
			12	100	93	
			13	100	98	
			14	100	98	
			15	100	90	
			16	100	98	
			17	100	0	
			18	100	93	
			19	100	97	
			20	100	1	
			21	100	40	
			22	100	94	
			23	100	90	
			24	100	0	
			25	100	50	
			26	100	41	
			27	100	0	
			28	100	92	
			29	100	91	
			30	100	88	

CETIS Test Data Worksheet

Report Date: 22 Dec-17 10:37 (p 1 of 1)
 Test Code: 02-9159-5360/171215sprtB

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17 Species: Strongylocentrotus purpuratus
 End Date: 15 Dec-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 15 Dec-17 Material: Copper chloride

Sample Code: 171215sprtB
 Sample Source: Reference Toxicant
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	10			
0	LC	2	8	100	98	BO 12/22/17
0	LC	3	19			
0	LC	4	14			
0	LC	5	16			
10		1	30			
10		2	11	100	92	BO 12/22/17
10		3	22			
10		4	2			
10		5	7			
20		1	5			
20		2	28			
20		3	29			
20		4	9	100	94	BO 12/22/17
20		5	18			
40		1	15			
40		2	12			
40		3	23			
40		4	4			
40		5	13	100	89	BO 12/22/17
80		1	1			
80		2	25			
80		3	21			
80		4	6	100	60	BO 12/22/17
80		5	26			
160		1	20			
160		2	24			
160		3	27			
160		4	3	100	0	BO 12/22/17
160		5	17			

AC OBO An

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 12/15/17 1853Test No: 1712158pt + BEnd Date/Time: 12/15/17 1933Dilutions made by: AD OBO PA

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10300

Analyst:

AC

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	9.0	7.99	33.7	14.5
10	8.8	8.01	33.8	14.4
20	8.8	8.02	33.8	14.2
40	8.8	8.02	33.8	14.2
80	8.8	8.03	33.7	14.2
160	8.7	8.03	33.4	14.3

Comments: _____

QC Check:

AD 12/22/17

Final Review:

EG 12/28/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Intermed
 Sample ID: CUC12
 Test No.: 171215 Spt B
 Tech initials: AD OBO PA
 Injection Time: 1815

Start Date/Time: 12/15/17 1853
 End Date/Time: 12/15/17 1933
 Species: S. purpuratus
 Animal Source: Dr. Luthi
 Date Collected: 12/18/17

Sperm Absorbance at 400 nm: 0.814 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 79 Mean: 80 X 50 = 4000 eggs/ml

75
78
78
90

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

Initial density: 4000 eggs/ml = 1.0 dilution factor egg stock 15 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater 15 ml
0 parts seawater

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

	Sperm:Egg Ratio							
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1825</u>	<u>50:1</u>	<u>84</u>	<u>16</u>
Eggs Added (0.5 ml):	<u>1835</u>	<u>100:1</u>	<u>95</u>	<u>5</u>
Test Ended:	<u>1845</u>	<u>100:1</u>	<u>97</u>	<u>3</u>
		<u>200:1</u>	<u>100</u>	<u>100</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>1853</u>	QC1	<u>99</u>	<u>1</u>
Eggs Added (0.5 ml):	<u>1913</u>	QC2	<u>98</u>	<u>2</u>
Test Ended:	<u>1933</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: No dilution required

QC Check: AD 12/22/17 Final Review: EG 12/28/17

Appendix E
Qualifier Codes

Glossary of Qualifier Codes:

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