



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Weekly)
Sample Collection Date: December 19, 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.

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

Results verified by: Adrienne Libor

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT — DECEMBER 2017

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

Sampling Date: December 19, 2017

Test Date: December 20, 2017

Sample ID: M-001

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
	5	20	

INTRODUCTION

A 24-hour composite discharge sample was collected in December 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for weekly accelerated toxicity monitoring purposes. Due to effects observed in a sample collected and tested for monthly monitoring purposes on May 04, 2017 from the CDP discharge monitoring point (M-001), accelerated monitoring was triggered 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 December 20, 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 delivered by courier to Nautilus. 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
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	12/19/17, 8:00
Sample Receipt Date, Time:	12/19/17, 13:45
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	7.83	7.4	2.7	64.5	208	0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	12/20/17, 15:35 through 16:15
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 6.06, 10, and 15 percent concentrations detected in the unadjusted M-001 effluent sample relative to the lab control using the EPA 1995 flowchart statistics. The NOEC is reported as 5 percent effluent and a TU_c equal to 20, which is above the maximum permit effluent limitation of 16.5. A significant decrease was observed at 10 percent effluent concentration tested in the M-001 unadjusted sample using the TST statistical analysis. The high salinity control resulted in 92.8 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 at any percent concentration 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 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)	5	6.06	>15	20	Pass	13
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-3.4

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	33.8	86.6	33.7	88.8
High Salinity Control	38.8	92.8	--	--
2.5	34.6	89.2	34.1	91.6
5.0	35.5	83.0	34.2	89.6
6.06	35.7	75.2*	34.3	91.8
10	36.9	60.0*	34.6	91.0
15	38.9	68.0*	35.0	94.2

* 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 day of collection and was within the appropriate temperature range. The test was 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 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. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ± 2 SD (µg/L Copper)	CV (%)
12/20/17	42.0	46.6 ± 32.9	35.3

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. 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. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. United States Environmental Protection Agency Office of Wastewater Management (EPA-833-R-00-003).
- 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: 28 Dec-17 10:58 (p 1 of 1)
Test Code: 1712-S096 | 07-3883-8140

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	16-3383-7758	Test Type:	Fertilization	Analyst:							
Start Date:	20 Dec-17 15:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	20 Dec-17 16:15	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	07-8752-3429	Code:	17-1301	Client:	IDE						
Sample Date:	19 Dec-17 08:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	19 Dec-17 13:45	Source:	IDE Americas, Inc.								
Sample Age:	32h (2.7 °C)	Station:	M-001 (Unadjusted) <i>Weekly</i>								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
21-4656-0576	Fertilization Rate	5	6.06	5.505	9.47%	20	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
16-7858-3179	Fertilization Rate	EC25	9.323	7.581	N/A	10.73	Linear Interpolation (ICPIN)				
		EC50	>15	N/A	N/A	<6.667					
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
16-7858-3179	Fertilization Rate	Control Resp	0.866	0.7 - NL	Yes	Passes Acceptability Criteria					
21-4656-0576	Fertilization Rate	Control Resp	0.866	0.7 - NL	Yes	Passes Acceptability Criteria					
21-4656-0576	Fertilization Rate	PMSD	0.0947	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	0.928	0.9011	0.9549	0.9	0.96	0.009695	0.02168	2.34%	0.0%
0	Lab Control	5	0.866	0.819	0.913	0.8	0.89	0.01691	0.03782	4.37%	6.68%
2.5		5	0.892	0.8514	0.9326	0.84	0.92	0.01463	0.03271	3.67%	3.88%
5		5	0.83	0.7505	0.9095	0.76	0.91	0.02864	0.06403	7.72%	10.56%
6.06		5	0.752	0.7078	0.7962	0.7	0.8	0.01594	0.03564	4.74%	18.97%
10		5	0.6	0.4626	0.7374	0.44	0.71	0.0495	0.1107	18.45%	35.34%
15		5	0.68	0.5958	0.7642	0.6	0.76	0.03033	0.06782	9.97%	26.72%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	High Salinity Co	0.92	0.93	0.93	0.9	0.96					
0	Lab Control	0.88	0.89	0.8	0.87	0.89					
2.5		0.84	0.91	0.91	0.92	0.88					
5		0.88	0.76	0.82	0.78	0.91					
6.06		0.75	0.7	0.75	0.8	0.76					
10		0.57	0.71	0.58	0.7	0.44					
15		0.72	0.6	0.7	0.76	0.62					

CETIS Analytical Report

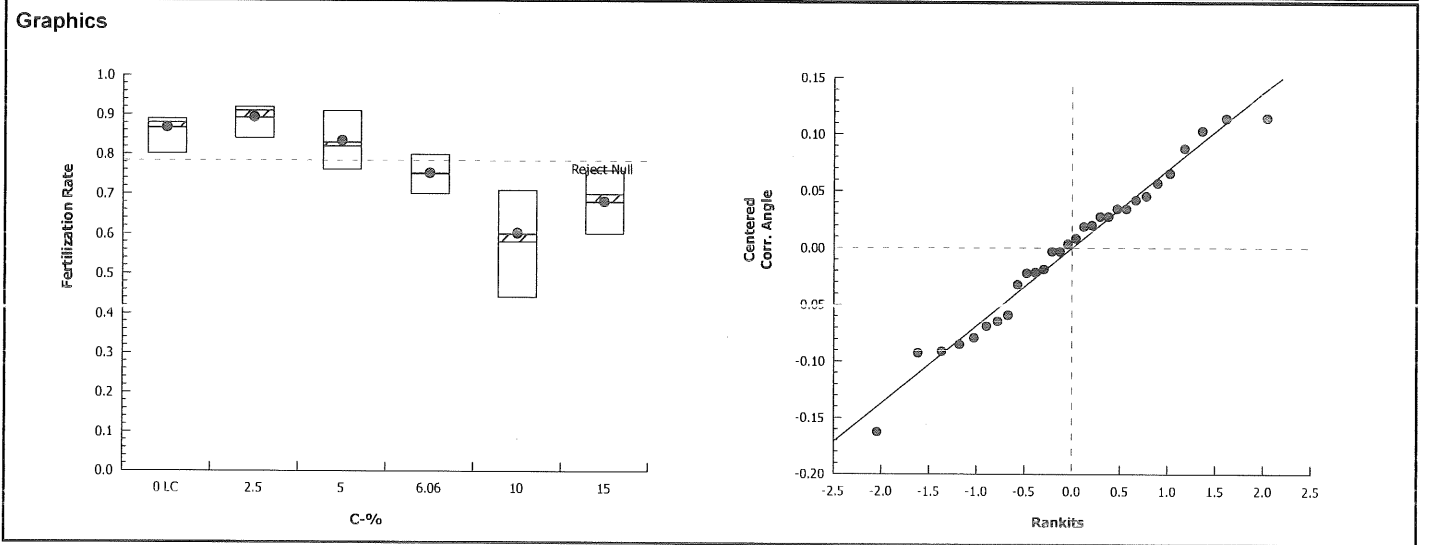
Report Date: 28 Dec-17 10:58 (p 1 of 2)
Test Code: 1712-S096 | 07-3883-8140

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)		
Analysis ID: 21-4656-0576		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7								
Analyzed: 28 Dec-17 10:57		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		9.47%	5	6.06	5.505	20
Dunnett Multiple Comparison Test												
Control		vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		2.5		-0.8561	2.362	0.111	8	0.9753	CDF	Non-Significant Effect		
		5		0.9983	2.362	0.111	8	0.4207	CDF	Non-Significant Effect		
		6.06*		3.152	2.362	0.111	8	0.0090	CDF	Significant Effect		
		10*		6.609	2.362	0.111	8	<0.0001	CDF	Significant Effect		
		15*		4.838	2.362	0.111	8	0.0001	CDF	Significant Effect		
ANOVA Table												
Source		Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between		0.4688833		0.09377667		5		17.01	<0.0001	Significant Effect		
Error		0.13228		0.005511665		24						
Total		0.6011633				29						
Distributional Tests												
Attribute		Test		Test Stat	Critical	P-Value		Decision(α:1%)				
Variances		Bartlett Equality of Variance		5.327	15.09	0.3773		Equal Variances				
Distribution		Shapiro-Wilk W Normality		0.9754	0.9031	0.6958		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	0.866	0.819	0.913	0.88	0.8	0.89	0.01691	4.37%	0.0%	
2.5		5	0.892	0.8514	0.9326	0.91	0.84	0.92	0.01463	3.67%	-3.0%	
5		5	0.83	0.7505	0.9095	0.82	0.76	0.91	0.02864	7.72%	4.16%	
6.06		5	0.752	0.7078	0.7962	0.75	0.7	0.8	0.01594	4.74%	13.16%	
10		5	0.6	0.4626	0.7374	0.58	0.44	0.71	0.0495	18.45%	30.72%	
15		5	0.68	0.5958	0.7642	0.7	0.6	0.76	0.03033	9.97%	21.48%	
Angular (Corrected) Transformed Summary												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.198	1.133	1.264	1.217	1.107	1.233	0.0235	4.39%	0.0%	
2.5		5	1.239	1.175	1.302	1.266	1.159	1.284	0.02273	4.1%	-3.35%	
5		5	1.151	1.042	1.261	1.133	1.059	1.266	0.03945	7.66%	3.91%	
6.06		5	1.05	0.999	1.102	1.047	0.9912	1.107	0.01847	3.93%	12.35%	
10		5	0.888	0.7468	1.029	0.8657	0.7253	1.002	0.05084	12.8%	25.9%	
15		5	0.9712	0.8806	1.062	0.9912	0.8861	1.059	0.0326	7.51%	18.96%	

CETIS Analytical Report

Report Date: 28 Dec-17 10:58 (p 2 of 2)
Test Code: 1712-S096 | 07-3883-8140

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)	
Analysis ID:	21-4656-0576	Endpoint:	Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed:	28 Dec-17 10:57	Analysis:	Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

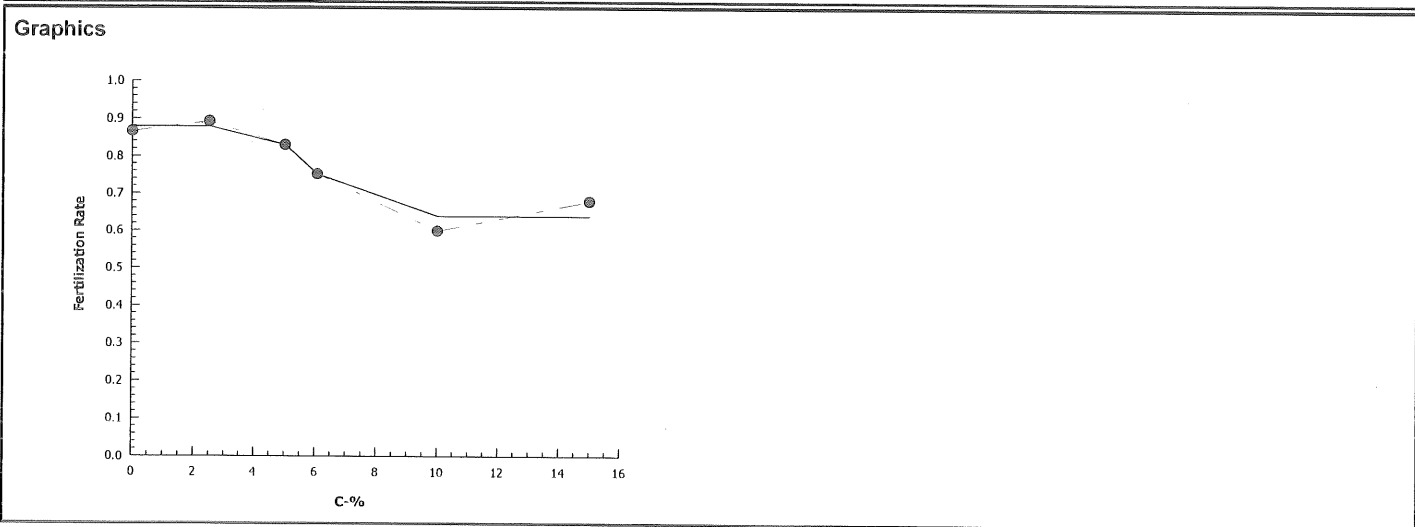
Report Date: 28 Dec-17 10:58 (p 1 of 1)
Test Code: 1712-S096 | 07-3883-8140

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	16-7858-3179	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	28 Dec-17 10:57	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	975989	1000	Yes	Two-Point Interpolation

Point Estimates						
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	9.323	7.581	N/A	10.73	NA	13.19
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	0.866	0.8	0.89	0.01691	0.03782	4.37%	0.0%	433	500
2.5		5	0.892	0.84	0.92	0.01463	0.03271	3.67%	-3.0%	446	500
5		5	0.83	0.76	0.91	0.02864	0.06403	7.72%	4.16%	415	500
6.06		5	0.752	0.7	0.8	0.01594	0.03564	4.74%	13.16%	376	500
10		5	0.6	0.44	0.71	0.0495	0.1107	18.45%	30.72%	300	500
15		5	0.68	0.6	0.76	0.03033	0.06782	9.97%	21.48%	340	500



CETIS Analytical Report

TST

Report Date: 28 Dec-17 10:58 (p 1 of 1)
Test Code: 1712-S096 | 07-3883-8140

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 08-6447-4297		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 28 Dec-17 10:58		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	5.88%	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*	11.81	1.895	0.054	7	<0.0001	CDF	Non-Significant Effect		
		5*	5.849	2.015	0.087	5	0.0010	CDF	Non-Significant Effect		
		6.06*	5.937	1.895	0.048	7	0.0003	CDF	Non-Significant Effect		
		10	-0.2	2.132	0.115	4	0.5744	CDF	Significant Effect		
		15*	1.954	1.943	0.072	6	0.0492	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.4688833		0.09377667		5		17.01	<0.0001	Significant Effect		
Error	0.13228		0.005511665		24						
Total	0.6011633				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			5.327	15.09	0.3773		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9754	0.9031	0.6958		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	0.866	0.819	0.913	0.88	0.8	0.89	0.01691	4.37%	0.0%
2.5		5	0.892	0.8514	0.9326	0.91	0.84	0.92	0.01463	3.67%	-3.0%
5		5	0.83	0.7505	0.9095	0.82	0.76	0.91	0.02864	7.72%	4.16%
6.06		5	0.752	0.7078	0.7962	0.75	0.7	0.8	0.01594	4.74%	13.16%
10		5	0.6	0.4626	0.7374	0.58	0.44	0.71	0.0495	18.45%	30.72%
15		5	0.68	0.5958	0.7642	0.7	0.6	0.76	0.03033	9.97%	21.48%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.198	1.133	1.264	1.217	1.107	1.233	0.0235	4.39%	0.0%
2.5		5	1.239	1.175	1.302	1.266	1.159	1.284	0.02273	4.1%	-3.35%
5		5	1.151	1.042	1.261	1.133	1.059	1.266	0.03945	7.66%	3.91%
6.06		5	1.05	0.999	1.102	1.047	0.9912	1.107	0.01847	3.93%	12.35%
10		5	0.888	0.7468	1.029	0.8657	0.7253	1.002	0.05084	12.8%	25.9%
15		5	0.9712	0.8806	1.062	0.9912	0.8861	1.059	0.0326	7.51%	18.96%

CETIS Test Data Worksheet

Report Date: 19 Dec-17 14:06 (p 1 of 1)
 Test Code: 1712-5696 07-3883-8140/2C09C67C

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Dec-17 Species: Strongylocentrotus purpuratus
 End Date: 20 Dec-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 19 Dec-17 Material: Facility Effluent

Sample Code: 17-1307 1301
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	75	12/21/17
			62	100	70	
			63	100	88	
			64	100	91	
			65	100	72	
			66	100	70	
			67	100	76	
			68	100	89	
			69	100	90	
			70	100	91	
			71	100	71	
			72	100	91	
			73	100	76	
			74	100	80	
			75	100	87	
			76	100	96	
			77	100	78	
			78	100	60	
			79	100	58	
			80	100	75	
			81	100	92	
			82	100	82	
			83	100	93	
			84	100	76	
			85	100	57	
			86	100	92	
			87	100	80	
			88	100	88	
			89	100	84	
			90	100	70	
			91	100	62	
			92	100	89	
			93	100	44	
			94	100	93	
			95	100	88	

EG Q18 12/28/17

CETIS Test Data Worksheet

Report Date: 19 Dec-17 14:05 (p 1 of 1)
 Test Code: 17-5096 07-3883-8140/2C09C67C

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Dec-17 Species: Strongylocentrotus purpuratus
 End Date: 20 Dec-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 19 Dec-17 Material: Facility Effluent

Sample Code: 17-1301
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	86			
0	HS	2	83			
0	HS	3	94			
0	HS	4	69			
0	HS	5	76			
0	LC	1	95	100	92	AD 12/20/17
0	LC	2	86			
0	LC	3	74			
0	LC	4	75			
0	LC	5	92			
2.5		1	89			
2.5		2	70			
2.5		3	72			
2.5		4	81			
2.5		5	63			
5		1	88			
5		2	84			
5		3	82			
5		4	77			
5		5	64			
6.06		1	61	100	77	AD 12/20/17
6.06		2	90	↓	68	↓
6.06		3	80		82	
6.06		4	87		75	
6.06		5	73	↓		↓
10		1	85			
10		2	71			
10		3	79			
10		4	66			
10		5	93			
15		1	65			
15		2	78			
15		3	62			
15		4	67			
15		5	91			

QC: CG

AD 12/20/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (unadjusted)

Start Date/Time: 12/20/2017 1535

Sample Log No.: 17- 1301

End Date/Time: 12/20/2017 1615

Dilutions made by: CG

Test No: 1712-S096

Analyst: PH

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.2	8.03	39.8	15.6
High Salinity Control	8.2	8.05	38.8	15.4
2.5	8.1	8.04	34.6	15.8
5.0	8.1	8.04	35.5	15.6
6.06	8.1	8.04	35.7	15.5
10	8.2	8.03	36.9	15.6
15	8.2	8.01	38.9	15.6

Comments:

QC Check:

EG 12/28/17

Final Review:

AC 1/2/18

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-601 unadjusted
 Test No.: 1712-S096
 Tech initials: AD
 Injection Time: 1455

Start Date/Time: 12/20/2017 / 1535
 End Date/Time: 12/20/2017 / 1615
 Species: *S. purpuraceus*
 Animal Source: PT. 10mg
 Date Collected: 12/18/17

Sperm Absorbance at 400 nm: 0.908 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 85 Mean: 84 X 50 = 4200 eggs/ml
89

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

83
83
80

Initial density: 4200 eggs/ml = 1.05 dilution factor
 Final density: 4000 eggs/ml - 1.0 part egg stock
(B) parts seawater

egg stock ml
 seawater ml

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

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

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1507</u>	<u>50:1</u>	<u>86</u>	<u>14</u>
Eggs Added (0.5 ml):	<u>1517</u>	<u>100:1</u>	<u>100</u>	<u>0</u>
Test Ended:	<u>1527</u>	<u>100:1</u>	<u>100</u>	<u>0</u>
		<u>—</u>	<u>—</u>	<u>—</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test

Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1535</u>	QC1	<u>96</u>	<u>4</u>
Eggs Added (0.5 ml):	<u>1555</u>	QC2	<u>97</u>	<u>3</u>
Test Ended:	<u>1615</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(B) AD 12/20/17 No dilution required

(B) AD 12/20/17

QC Check:

EQ 12/28/17

Final Review: AC 1/2/18

M-001 40 ppt Adjusted

CETIS Summary Report

Report Date: 28 Dec-17 10:46 (p 1 of 1)
Test Code: 1712-S097 | 05-3431-4355

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Batch ID:	08-3763-8869	Test Type:	Fertilization	Analyst:							
Start Date:	20 Dec-17 15:35	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	20 Dec-17 16:15	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	03-8048-2221	Code:	17-1301	Client:	IDE						
Sample Date:	19 Dec-17 08:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	19 Dec-17 13:45	Source:	IDE Americas, Inc.								
Sample Age:	32h (2.7 °C)	Station:	M-001 (Daily 40ppt) <i>weekly</i>								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
12-4730-3721	Fertilization Rate	15	>15	NA	9.52%	<6.667	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
20-9758-9338	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					
12-4730-3721	Fertilization Rate	Control Resp	0.888	0.7 - NL	Yes	Passes Acceptability Criteria					
20-9758-9338	Fertilization Rate	Control Resp	0.888	0.7 - NL	Yes	Passes Acceptability Criteria					
12-4730-3721	Fertilization Rate	PMSD	0.09515	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	0.888	0.8388	0.9372	0.83	0.94	0.01772	0.03962	4.46%	0.0%
2.5		5	0.916	0.8952	0.9368	0.89	0.93	0.007483	0.01673	1.83%	-3.15%
5		5	0.896	0.8162	0.9758	0.79	0.94	0.02874	0.06427	7.17%	-0.9%
6.06		5	0.918	0.835	1	0.81	0.98	0.0299	0.06686	7.28%	-3.38%
10		5	0.91	0.8437	0.9763	0.82	0.95	0.02387	0.05339	5.87%	-2.48%
15		5	0.942	0.9033	0.9807	0.89	0.97	0.01393	0.03114	3.31%	-6.08%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.94	0.83	0.89	0.88	0.9					
2.5		0.89	0.93	0.91	0.93	0.92					
5		0.94	0.94	0.93	0.79	0.88					
6.06		0.9	0.95	0.81	0.98	0.95					
10		0.95	0.95	0.82	0.92	0.91					
15		0.95	0.97	0.96	0.89	0.94					

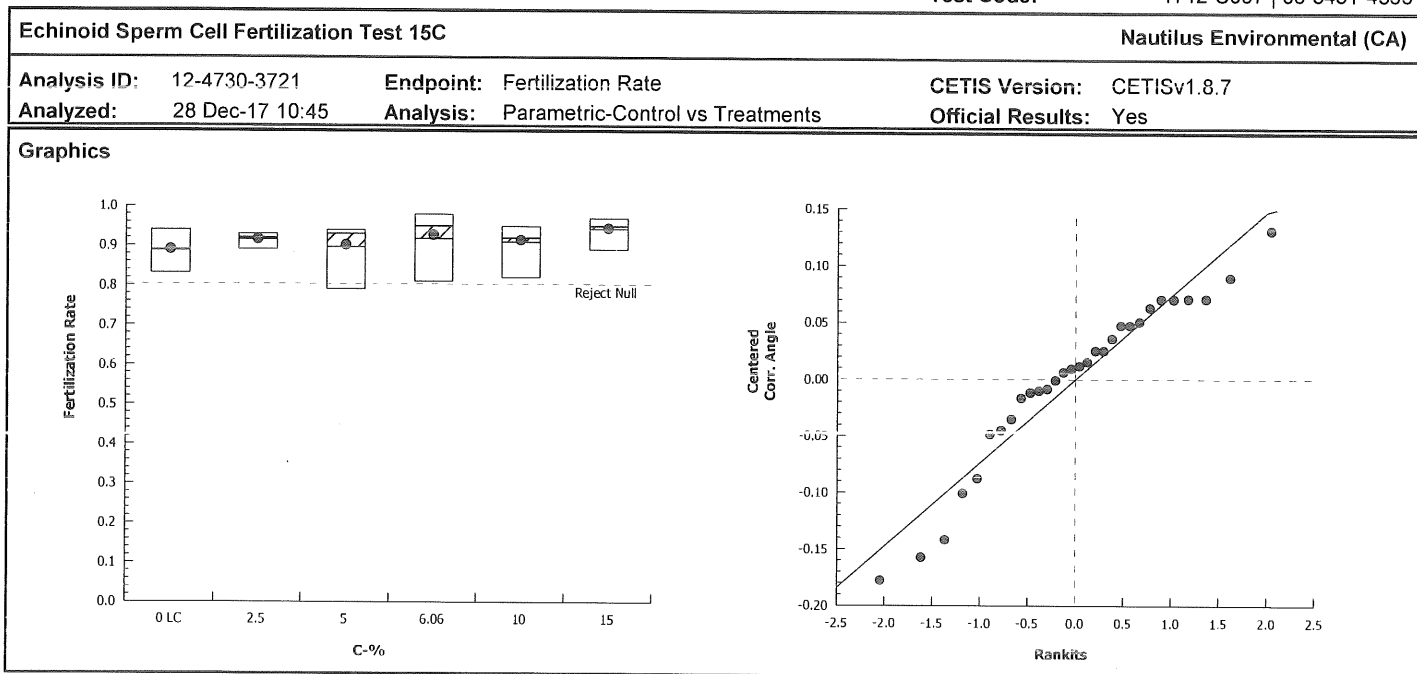
CETIS Analytical Report

Report Date: 28 Dec-17 10:46 (p 1 of 2)
 Test Code: 1712-S097 | 05-3431-4355

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 12-4730-3721		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 28 Dec-17 10:45		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		9.52%	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	-0.8552	2.362	0.122	8	0.9753	CDF	Non-Significant Effect		
		5	-0.362	2.362	0.122	8	0.9184	CDF	Non-Significant Effect		
		6.06	-1.24	2.362	0.122	8	0.9916	CDF	Non-Significant Effect		
		10	-0.7949	2.362	0.122	8	0.9710	CDF	Non-Significant Effect		
		15	-1.933	2.362	0.122	8	0.9991	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.03054784		0.006109567		5	0.9151	0.4880	Non-Significant Effect			
Error	0.1602329		0.00667637		24						
Total	0.1907807				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			6.675	15.09	0.2459	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9362	0.9031	0.0721	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	0.888	0.8388	0.9372	0.89	0.83	0.94	0.01772	4.46%	0.0%
2.5		5	0.916	0.8952	0.9368	0.92	0.89	0.93	0.007483	1.83%	-3.15%
5		5	0.896	0.8162	0.9758	0.93	0.79	0.94	0.02874	7.17%	-0.9%
6.06		5	0.918	0.835	1	0.95	0.81	0.98	0.0299	7.28%	-3.38%
10		5	0.91	0.8437	0.9763	0.92	0.82	0.95	0.02387	5.87%	-2.48%
15		5	0.942	0.9033	0.9807	0.95	0.89	0.97	0.01393	3.31%	-6.08%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.234	1.154	1.313	1.233	1.146	1.323	0.02852	5.17%	0.0%
2.5		5	1.278	1.241	1.314	1.284	1.233	1.303	0.01319	2.31%	-3.58%
5		5	1.252	1.13	1.374	1.303	1.095	1.323	0.044	7.86%	-1.52%
6.06		5	1.298	1.151	1.444	1.345	1.12	1.429	0.05281	9.1%	-5.19%
10		5	1.275	1.167	1.383	1.284	1.133	1.345	0.03893	6.83%	-3.33%
15		5	1.333	1.256	1.411	1.345	1.233	1.397	0.028	4.7%	-8.1%

CETIS Analytical Report

Report Date: 28 Dec-17 10:46 (p 2 of 2)
Test Code: 1712-S097 | 05-3431-4355



CETIS Analytical Report

Report Date: 28 Dec-17 10:46 (p 1 of 1)
Test Code: 1712-S097 | 05-3431-4355

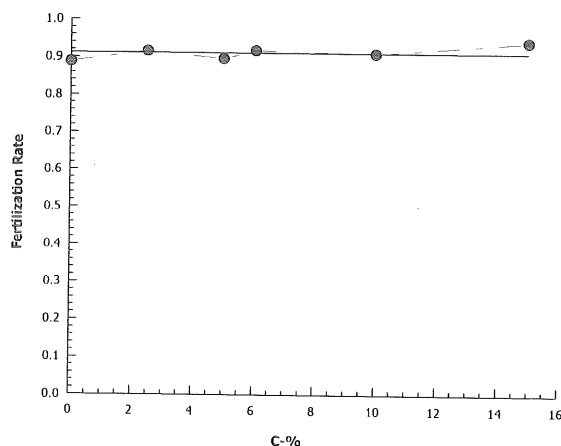
Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)		
Analysis ID:	20-9758-9338	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	28 Dec-17 10:45	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	342569	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	0.888	0.83	0.94	0.01772	0.03962	4.46%	0.0%	444	500
2.5		5	0.916	0.89	0.93	0.007483	0.01673	1.83%	-3.15%	458	500
5		5	0.896	0.79	0.94	0.02874	0.06427	7.17%	-0.9%	448	500
6.06		5	0.918	0.81	0.98	0.0299	0.06686	7.28%	-3.38%	459	500
10		5	0.91	0.82	0.95	0.02387	0.05339	5.87%	-2.48%	455	500
15		5	0.942	0.89	0.97	0.01393	0.03114	3.31%	-6.08%	471	500

Graphics



CETIS Analytical Report

TST

Report Date: 28 Dec-17 10:46 (p 1 of 1)
Test Code: 1712-S097 | 05-3431-4355

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 13-3300-8116		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 28 Dec-17 10:46		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	4.79%	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*	14.03	1.943	0.049	6	<0.0001	CDF	Non-Significant Effect		
		5*	6.686	2.015	0.099	5	0.0006	CDF	Non-Significant Effect		
		6.06*	6.537	2.015	0.115	5	0.0006	CDF	Non-Significant Effect		
		10*	7.868	1.943	0.086	6	0.0001	CDF	Non-Significant Effect		
		15*	11.59	1.995	0.067	7	<0.0001	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.03054784		0.006109567		5	0.9151	0.4880	Non-Significant Effect			
Error	0.1602329		0.00667637		24						
Total	0.1907807				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			6.675	15.09	0.2459	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9362	0.9031	0.0721	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	0.888	0.8388	0.9372	0.89	0.83	0.94	0.01772	4.46%	0.0%
2.5		5	0.916	0.8952	0.9368	0.92	0.89	0.93	0.007483	1.83%	-3.15%
5		5	0.896	0.8162	0.9758	0.93	0.79	0.94	0.02874	7.17%	-0.9%
6.06		5	0.918	0.835	1	0.95	0.81	0.98	0.0299	7.28%	-3.38%
10		5	0.91	0.8437	0.9763	0.92	0.82	0.95	0.02387	5.87%	-2.48%
15		5	0.942	0.9033	0.9807	0.95	0.89	0.97	0.01393	3.31%	-6.08%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.234	1.154	1.313	1.233	1.146	1.323	0.02852	5.17%	0.0%
2.5		5	1.278	1.241	1.314	1.284	1.233	1.303	0.01319	2.31%	-3.58%
5		5	1.252	1.13	1.374	1.303	1.095	1.323	0.044	7.86%	-1.52%
6.06		5	1.298	1.151	1.444	1.345	1.12	1.429	0.05281	9.1%	-5.19%
10		5	1.275	1.167	1.383	1.284	1.133	1.345	0.03893	6.83%	-3.33%
15		5	1.333	1.256	1.411	1.345	1.233	1.397	0.028	4.7%	-8.1%

CETIS Test Data Worksheet

Report Date: 19 Dec-17 14:02 (p 1 of 1)

Test Code: 1712-5097 05-3431-4355/1FD8FD73

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Dec-17 Species: Strongylocentrotus purpuratus

Sample Code: 17-1301

End Date: 20 Dec-17 Protocol: EPA/600/R-95/136 (1995)

Sample Source: IDE Americas, Inc.

Sample Date: 19 Dec-17 Material: Facility Effluent

Sample Station: M-001 (Daily 40ppt)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	98	12/21/17
			32	100	93	
			33	100	95	
			34	100	94	
			35	100	92	
			36	100	94	
			37	100	95	
			38	100	95	
			39	100	93	
			40	100	89	
			41	100	95	
			42	100	94	
			43	100	94	
			44	100	Ⓐ 91 97	
			45	100	91	
			46	100	95	
			47	100	93	
			48	100	91	
			49	100	90	
			50	100	92	
			51	100	89	
			52	100	88	
			53	100	89	
			54	100	90	
			55	100	83	
			56	100	88	
			57	100	96	
			58	100	79	
			59	100	81	
			60	100	82	

Ⓐ - Q18 12/21/17 KC

CETIS Test Data Worksheet

Report Date: 19 Dec-17 14:08 (p 1 of 1)
 Test Code: 1712-507705-3431-4355/1FD8FD73

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Dec-17 Species: Strongylocentrotus purpuratus
 End Date: 20 Dec-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 19 Dec-17 Material: Facility Effluent

Sample Code: 17-1301
 Sample Source: IDE Americas, Inc.
 Sample Station: M-001 (Daily 40ppt)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	43			
0	LC	2	55			
0	LC	3	53			
0	LC	4	56			
0	LC	5	54			
2.5		1	51			
2.5		2	33			
2.5		3	48			
2.5		4	32			
2.5		5	50			
5		1	34			
5		2	36			
5		3	47			
5		4	58			
5		5	52			
6.06		1	49	100	92	As 12/20/17
6.06		2	33			
6.06		3	59			
6.06		4	31			
6.06		5	37			
10		1	41			
10		2	46			
10		3	60			
10		4	35			
10		5	45			
15		1	38			
15		2	44			
15		3	57			
15		4	40			
15		5	42			

Q6C9

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted)

Start Date/Time: 12/20/2017 1535

Sample Log No.: 17- 1301

End Date/Time: 12/20/2017 1615

Dilutions made by: CG

Test No: 1712-5097

Analyst:

AD

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.3	8.03	33.1	15.1
2.5	8.2	8.04	34.1	14.6
5.0	8.2	8.04	34.2	14.5
6.06	8.2	8.04	34.3	15.1
10	8.2	8.05	34.6	15.1
15	8.2	8.04	35.0	15.1

Comments:

QC Check:

EG 12/28/17

Final Review:

AC 1/2/18

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: IDEAnalyst: CGSample ID: M-001 (40 ppt adjusted)Test Date: 12/20/2017Test No: ~~1706-S085~~ 1702-S097Test Type: Urchin Fertilization218 g 12/20/17Salinity of Effluent 64.5Salinity of Seawater 33.5Date of Brine used: NATarget Salinity 40.0Alk. of 40 ppt Adj. Sample: 148 mg/L as CaCO₃

	<u>Effluent</u>	<u>Brine Control</u>
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	<u>3.77</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	3.77	376.9	477

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: EG 12/28/17Final Review: AL 1/4/18

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-001 (40ppt adjusted)
 Test No.: 1712-5097

Start Date/Time: 12/20/2017 / 1535
 End Date/Time: 12/20/2017 / 1615
 Species: *S. purpuraceus*
 Animal Source: Pt. Loma
 Date Collected: 12/18/17

Tech initials: AD
 Injection Time: 1455

Sperm Absorbance at 400 nm: 0.908 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 85 Mean: 84 X 50 = 4200 eggs/ml
89

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

83
83
80

Initial density: 4200 eggs/ml = 1.05 dilution factor egg stock ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater ml
(B) 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>1507</u>	<u>50:1</u>	<u>86</u>	<u>14</u>
Eggs Added (0.5 ml):	<u>1517</u>	<u>100:1</u>	<u>100</u>	<u>0</u>
Test Ended:	<u>1527</u>	<u>100:1</u>	<u>100</u>	<u>0</u>
		<u>-</u>	<u>-</u>	<u>-</u>

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test

Sperm:Egg Ratio Used: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1535</u>	QC1	<u>96</u>	<u>4</u>
Eggs Added (0.5 ml):	<u>1555</u>	QC2	<u>97</u>	<u>3</u>
Test Ended:	<u>1615</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(B) 1712-5097 12/20/17. No dilution required.
(B) 1712-5097 12/20/17

QC Check:

EG 12/28/17

Final Review:

AC 1/2/18

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: M-001 (17-3567)
Test ID No(s): 1712-5096 to 5098

Sample Check-In Information

Sample Description:

A: Clear, no color, no odor, no debris

Sample (A, B, C):	<u>A</u>			
Log-in No. (17-xxxx):	<u>1301</u>			
Sample Collection Date & Time:	<u>12/19/17 0800</u>			
Sample Receipt Date & Time:	<u>12/19/17 1345</u>			
Number of Containers & Container Type:	<u>1, 4L cubi</u>			
Approx. Total Volume Received (L):	<u>~4L</u>			
Check-in Temperature (°C)	<u>2.7</u>			
Temperature OK? ¹	<u>(Y) N</u>	<u>Y N</u>	<u>Y N</u>	<u>Y N</u>
DO (mg/L)	<u>7.4</u>			
pH (units)	<u>7.83</u>			
Conductivity (µS/cm)	<u>—</u>			
Salinity (ppt)	<u>64.5 (A)</u>			
Alkalinity (mg/L) ²	<u>208</u>			
Hardness (mg/L) ^{2,3}	<u>—</u>			
Total Chlorine (mg/L)	<u>0.02</u>			
Technician Initials	<u>BO</u>			

COC Complete (Y/N)?

A Y B — C —

Filtration? Y (N)

Pore Size: —

Organisms — or Debris —

Salinity Adjustment? (Y) N

Test: Urchin 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: EG 12/28/17

Final Review: AC 1/2/18

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

Alkalinity: 116 Hardness or Salinity: 34 ppt
Additional Control? (Y) N = HSC Alkalinity: NM Hardness or Salinity: 38.8

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: —

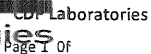
Notes: ¹ Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.

² mg/L as CaCO₃, ³ Measured for freshwater samples only, NA = Not Applicable

Additional Comments: NM = Not Measured, tech error (A) Salinity measured by making 1:1 dilution

Appendix C

Chain-of-Custody Form



CDP Laboratory: _____
 Entalphy Laboratory: _____
 WECK Laboratory: _____
 Nautilus: _____x_____

AIM: _____
 Other: _____

Turn Around Time

Normal: x

RUSH (24 hr):

3 Days:

5 Days:

??? Days

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

Special instruction: Sampled during plant operation at 49 MGD via autosampler by a series of grabs collected at one hour intervals. Sample collected to fulfill weekly NPDES requirements. Sample is to be run adjusted and unadjusted. Start: 12/18/17 @ 08:00, End: 12/19/17 @ 08:00
KC

ANALYSES

NOTES:

Glass=G Plastic=P

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

Drinking Water=DW Seawater=SW Soil=S

[illegible]

TDS - 61.13 ppt, EC - 84.89 mS/cm

Relinquished By:

Date:

Time:

Received By:

Date: Time:

Sample Condition Upon Receipt:

Kevin Gray

12/19/17

1030

15

7/17	1013
------	------

☒ Iced ☐ Ambient or _____ °C

12/19/17

2.145

And type

12/19/17	1345
----------	------

☒ Iced ☐ Ambient or 2.7 °C

Mantillas ID: 17-1301

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 29 Dec-17 10:20 (p 1 of 1)
Test Code: 171220sprt | 10-8152-0796

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	05-7971-9096		Test Type: Fertilization				Analyst:				
Start Date:	20 Dec-17 15:35		Protocol: EPA/600/R-95/136 (1995)				Diluent:		Natural Seawater		
Ending Date:	20 Dec-17 16:15		Species: Strongylocentrotus purpuratus				Brine:		Not Applicable		
Duration:	40m		Source: Pt. Loma				Age:				
Sample ID:	21-4659-3972		Code: 171220sprt				Client:		Internal		
Sample Date:	20 Dec-17		Material: Copper chloride				Project:				
Receive Date:	20 Dec-17		Source: Reference Toxicant								
Sample Age:	16h		Station: Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Method			
01-9785-4182	Fertilization Rate		10	20	14.14	5.86%		Dunnett Multiple Comparison Test			
Point Estimate Summary											
Analysis ID	Endpoint		Level	µg/L	95% LCL	95% UCL	TU	Method			
13-6880-4388	Fertilization Rate		EC50	41.98	40.29	43.74		Trimmed Spearman-Kärber			
Test Acceptability											
Analysis ID	Endpoint		Attribute		Test Stat	TAC Limits		Overlap	Decision		
01-9785-4182	Fertilization Rate		Control Resp		0.94	0.7 - NL		Yes	Passes Acceptability Criteria		
13-6880-4388	Fertilization Rate		Control Resp		0.94	0.7 - NL		Yes	Passes Acceptability Criteria		
01-9785-4182	Fertilization Rate		PMSD		0.05856	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	0.94	0.9083	0.9717	0.9	0.96	0.0114	0.0255	2.71%	0.0%
10		5	0.896	0.8793	0.9127	0.88	0.91	0.006	0.01342	1.5%	4.68%
20		5	0.856	0.8051	0.9069	0.79	0.9	0.01833	0.04099	4.79%	8.94%
40		5	0.562	0.4526	0.6714	0.45	0.69	0.03942	0.08815	15.68%	40.21%
80		5	0.056	0	0.1255	0.01	0.15	0.02502	0.05595	99.9%	94.04%
160		5	0.002	0	0.007553	0	0.01	0.002	0.004472	223.6%	99.79%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.96	0.93	0.9	0.96	0.95					
10		0.88	0.89	0.89	0.91	0.91					
20		0.85	0.79	0.9	0.87	0.87					
40		0.52	0.57	0.58	0.45	0.69					
80		0.15	0.02	0.04	0.06	0.01					
160		0	0.01	0	0	0					

CETIS Analytical Report

Report Date: 29 Dec-17 10:19 (p 1 of 2)
Test Code: 171220spt | 10-8152-0796

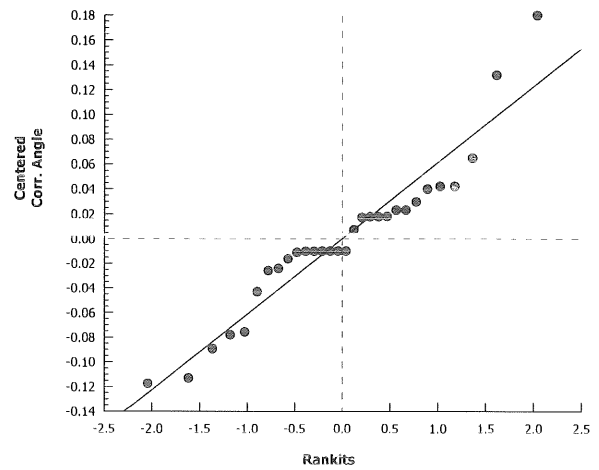
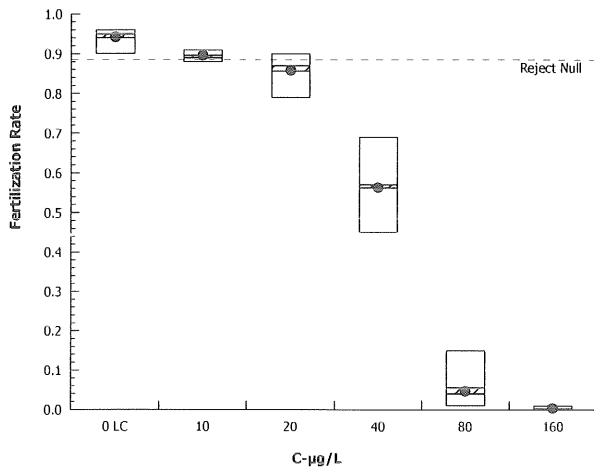
Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)		
Analysis ID: 01-9785-4182			Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 29 Dec-17 10:19			Analysis: Parametric-Control vs Treatments					Official Results: Yes				
Data Transform		Zeta	Alt Hyp	Trials	Seed			PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C > T	NA	NA			5.86%	10	20	14.14	
Dunnett Multiple Comparison Test												
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)			
Lab Control		10	1.943	2.362	0.103	8	0.1100	CDF	Non-Significant Effect			
		20*	3.297	2.362	0.103	8	0.0064	CDF	Significant Effect			
		40*	11.03	2.362	0.103	8	<0.0001	CDF	Significant Effect			
		80*	25.57	2.362	0.103	8	<0.0001	CDF	Significant Effect			
		160*	29.2	2.362	0.103	8	<0.0001	CDF	Significant Effect			
ANOVA Table												
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)			
Between	7.548054		1.509611		5		320.6	<0.0001	Significant Effect			
Error	0.1130065		0.004708604		24							
Total	7.66106				29							
Distributional Tests												
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)				
Variances	Bartlett Equality of Variance			14.13	15.09	0.0148		Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9264	0.9031	0.0394		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	0.94	0.9083	0.9717	0.95	0.9	0.96	0.0114	2.71%	0.0%	
10		5	0.896	0.8793	0.9127	0.89	0.88	0.91	0.006	1.5%	4.68%	
20		5	0.856	0.8051	0.9069	0.87	0.79	0.9	0.01833	4.79%	8.94%	
40		5	0.562	0.4526	0.6714	0.57	0.45	0.69	0.03942	15.68%	40.21%	
80		5	0.056	0	0.1255	0.04	0.01	0.15	0.02502	99.9%	94.04%	
160		5	0.002	0	0.007553	0	0	0.01	0.002	223.6%	99.79%	
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	1.327	1.263	1.391	1.345	1.249	1.369	0.02301	3.88%	0.0%	
10		5	1.243	1.216	1.27	1.233	1.217	1.266	0.009878	1.78%	6.35%	
20		5	1.184	1.113	1.255	1.202	1.095	1.249	0.02545	4.81%	10.78%	
40		5	0.8485	0.7368	0.9602	0.8556	0.7353	0.9803	0.04024	10.61%	36.07%	
80		5	0.2177	0.07464	0.3608	0.2014	0.1002	0.3977	0.05153	52.93%	83.6%	
160		5	0.06005	0.0322	0.0879	0.05002	0.05002	0.1002	0.01003	37.35%	95.48%	

CETIS Analytical Report

Report Date: 29 Dec-17 10:19 (p 2 of 2)
 Test Code: 171220sprt | 10-8152-0796

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 01-9785-4182	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 29 Dec-17 10:19	Analysis: Parametric-Control vs Treatments	Official Results: Yes	

Graphics



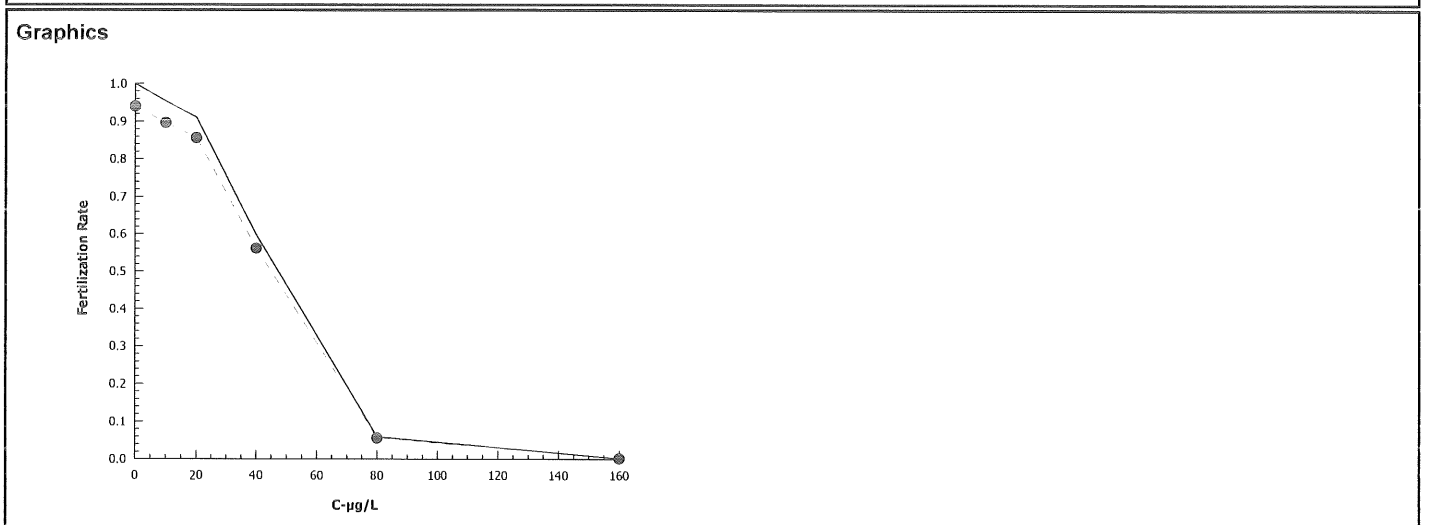
CETIS Analytical Report

Report Date: 29 Dec-17 10:19 (p 1 of 1)
Test Code: 171220sprt | 10-8152-0796

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Analysis ID: 13-6880-4388	Endpoint: Fertilization Rate			CETIS Version: CETISv1.8.7			
Analyzed: 29 Dec-17 10:19	Analysis: Trimmed Spearman-Kärber			Official Results: Yes			

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.06	4.68%	1.623	0.008914	41.98	40.29	43.74

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	0.94	0.9	0.96	0.0114	0.0255	2.71%	0.0%	470	500
10		5	0.896	0.88	0.91	0.006	0.01342	1.5%	4.68%	448	500
20		5	0.856	0.79	0.9	0.01833	0.04099	4.79%	8.94%	428	500
40		5	0.562	0.45	0.69	0.03942	0.08815	15.68%	40.21%	281	500
80		5	0.056	0.01	0.15	0.02502	0.05595	99.9%	94.04%	28	500
160		5	0.002	0	0.01	0.002	0.004472	223.6%	99.79%	1	500



Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

Organism: Strongylocentrotus purpuratus (Purpl

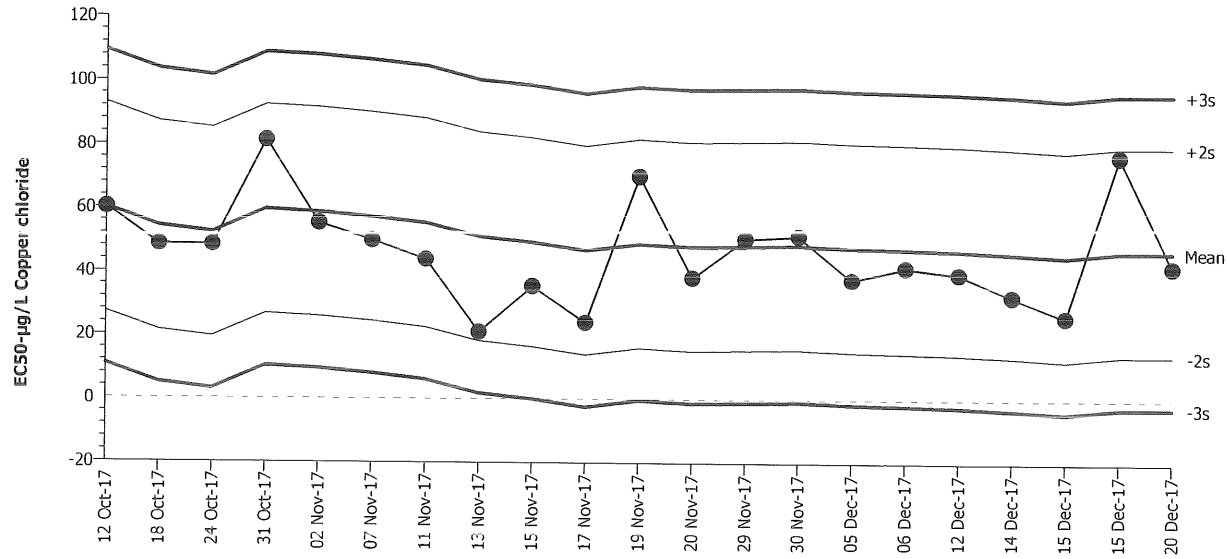
Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



Mean: 46.63

Count: 20

-2s Warning Limit: 13.75

-3s Action Limit: -2.688

Sigma: 16.44

CV: 35.30%

+2s Warning Limit: 79.51

+3s Action Limit: 95.95

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Oct	12	14:55	60.18	13.55	0.8244			05-0863-6526	07-1531-2424
2			18	14:22	48.53	1.9	0.1156			13-0042-6212	05-6771-5532
3			24	13:15	48.41	1.775	0.108			20-0280-7301	18-5464-1899
4			31	13:59	81.36	34.73	2.113	(+)		06-4227-6723	08-8095-0809
5		Nov	2	12:28	55.32	8.695	0.5289			17-4126-1689	20-0626-8382
6			7	14:30	49.87	3.243	0.1973			10-3521-2857	13-9801-3995
7			11	14:25	43.91	-2.722	-0.1656			14-1655-2339	20-5239-6070
8			13	14:35	20.97	-25.66	-1.561			07-0538-7056	00-9105-4737
9			15	16:09	35.48	-11.15	-0.678			06-3476-9418	17-5783-9769
10			17	14:17	24.03	-22.6	-1.375			20-8374-1268	00-9691-5869
11			19	10:02	70.21	23.58	1.434			12-1164-1483	20-4501-4622
12			20	15:15	38.26	-8.365	-0.5088			08-0578-7050	18-8950-2431
13			29	15:30	50.6	3.965	0.2412			05-0010-1267	11-1707-1208
14			30	15:28	51.48	4.845	0.2947			09-6334-2928	00-8447-7747
15		Dec	5	16:05	37.64	-8.988	-0.5467			00-4872-5743	06-2243-7863
16			6	15:50	41.57	-5.062	-0.3079			04-9516-7018	18-3148-8943
17			12	12:20	39.55	-7.082	-0.4308			01-8906-4164	02-6832-7767
18			14	15:35	32.51	-14.12	-0.8591			11-6397-1428	17-9802-1610
19			15	15:06	26.01	-20.62	-1.254			06-1613-2535	10-1459-1840
20			15	18:53	76.76	30.13	1.833			02-9159-5360	11-8739-9529
21			20	15:35	41.98	-4.652	-0.283			10-8152-0796	13-6880-4388

CETIS Test Data Worksheet

Report Date: 19 Dec-17 13:57 (p 1 of 1)
Test Code: 10-8152-0796/171220sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Dec-17 Species: Strongylocentrotus purpuratus
End Date: 20 Dec-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 20 Dec-17 Material: Copper chlorideSample Code: 171220sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	12/21/17 ↓
			2	100	85	
			3	100	90	
			4	100	0	
			5	100	96	
			6	100	2	
			7	100	6	
			8	100	79	
			9	100	0	
			10	100	15	
			11	100	52	
			12	100	45	
			13	100	95	
			14	100	58	
			15	100	1	
			16	100	89	
			17	100	0	
			18	100	69	
			19	100	88	
			20	100	91	
			21	100	91	
			22	100	93	
			23	100	57	
			24	100	96	
			25	100	4	
			26	100	87	
			27	100	89	
			28	100	90	
			29	100	1	
			30	100	87	

CETIS Test Data Worksheet

Report Date: 19 Dec-17 13:57 (p 1 of 1)
 Test Code: 10-8152-0796/171220sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 20 Dec-17 Species: Strongylocentrotus purpuratus Sample Code: 171220sprt
 End Date: 20 Dec-17 Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant
 Sample Date: 20 Dec-17 Material: Copper chloride Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	24	100	95	AD 12/20/17
0	LC	2	22			
0	LC	3	28			
0	LC	4	5			
0	LC	5	13			
10		1	19	100	78	AD 12/20/17
10		2	27			
10		3	16			
10		4	21			
10		5	20			
20		1	2	100	81	AD 12/20/17
20		2	8			
20		3	3			
20		4	30			
20		5	26			
40		1	11	100	43	AD 12/20/17
40		2	23			
40		3	14			
40		4	12			
40		5	18			
80		1	10	100	9	AD 12/20/17
80		2	6			
80		3	25			
80		4	7			
80		5	29			
160		1	1	100	0	EG OBS AD 12/20/17
160		2	15			
160		3	9			
160		4	17			
160		5	4			

QC: AD

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 12/20/2017 1535Test No: 171220sptEnd Date/Time: 12/20/2017 1615Dilutions made by: AD

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

Analyst:

RH

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.6	8.07	33.7	14.1
10	8.6	8.06	33.9	14.0
20	8.4	8.03	34.0	(A) 13.8 14.1
40	8.4	8.03	33.9	14.0
80	8.4	8.00	33.8	14.0
160	8.4	7.99	33.6	14.1

Comments: (A) RHG 12/20/17QC Check: AC 12/27/17Final Review: EG 12/29/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal
 Sample ID: Cu 42
 Test No.: 171220 split

Start Date/Time: 12/20/2017 / 1535
 End Date/Time: 12/20/2017 / 1615
 Species: *S. purpuratus*
 Animal Source: Pt. Loma
 Date Collected: 12/18/17

Tech initials: AD
 Injection Time: 1455

Sperm Absorbance at 400 nm: 0.908 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 85 Mean: 84 X 50 = 4200 eggs/ml
89
83
83
80

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

Initial density: 4200 eggs/ml = 1.05 dilution factor
 Final density: 4000 eggs/ml - 1.0 part egg stock
0.05 parts seawater

egg stock ml
 seawater ml

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

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

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1507</u>	<u>50:1</u>	<u>86</u>	<u>14</u>
Eggs Added (0.5 ml):	<u>1517</u>	<u>100:1</u>	<u>100</u>	<u>0</u>
Test Ended:	<u>1527</u>	<u>100:1</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: 75:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1535</u>	QC1	<u>96</u>	<u>4</u>
Eggs Added (0.5 ml):	<u>1555</u>	QC2	<u>97</u>	<u>3</u>
Test Ended:	<u>1615</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

BAO 018 12/20/17 No dilution required
BAO 018 12/20/17

QC Check:

EG 12/29/17

Final Review: KTP 12/18

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.