



Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001
Sample Collection Date: June 2, 2017

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

Prepared by: Nautilus Environmental

Submitted: July 3, 2017

Data Quality Assurance:

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

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

Results verified by: Adrienne Libor

EXECUTIVE SUMMARY

MONTHLY CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT — JUNE 2017

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

Sampling Date: June 2, 2017

Test Date: June 2, 2017

Sample IDs: M-001 Brine Effluent

M-001
Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	M-001 Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	<u>NOEC</u>	<u>TU_c</u>	Yes
	6.06	16.5	

INTRODUCTION

A 24-hour composite discharge sample was collected in June 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for monthly chronic toxicity monitoring purposes 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 June 2, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The sample was collected on June 2, 2017. Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was hand delivered to Nautilus the day after 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:	June 2017 (monthly chronic monitoring)
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	6/2/17, 09:00
Sample Receipt Date, Time:	6/2/17, 12:04
Sampling Method:	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	7.96	7.7	5.0	63.8	192	0.03

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	6/2/17, 17:00 through 17:40
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 15 percent concentration of the sample with the highest salinity; 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 (EPS) 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 Pollution Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA, 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB, 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06

percent unadjusted effluent, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST statistical analysis is not in the 2006 CDP permit, the TST results are included for comparison purposes only.

RESULTS

There was a statistically significant decrease in the fertilization rate of the 10 and 15 percent test concentrations of the unadjusted M-001 sample relative to the lab control. Therefore, the NOEC is reported as 6.06 percent effluent and a TU_c equal to 16.5, which meets the maximum permit effluent limitation. The high salinity control scored higher than the lab control, indicating that effects observed in the 10 and 15 percent concentrations of the unadjusted M-001 sample were not likely due to elevated salinity.

The 40 ppt adjusted M-001 effluent sample resulted in no significant effects in any test concentration using the EPA 1995 flowchart statistics. Additionally, none of the test concentrations in the M-001 unadjusted or 40 ppt adjusted sample were significantly reduced from control 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)	6.06	10	>15	16.5	Pass	1.7
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-0.21

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	33.3	93.2	33.3	93.6
High Salinity Control	38.2	95.8	--	--
2.5	34.6	94.6	33.6	94.2
5.0	35.2	92.4	34.0	92.8
6.06	35.6	91.6	34.1	93.8
10	36.9	86.6*	34.4	94.6
15	38.2	87.2*	34.7	94.4

^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.

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

QUALITY ASSURANCE

The sample was received the same day as collection within the appropriate temperature range, and was tested 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. Reference Toxicant Test Results

Test Species	Endpoint	EC ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ± 2 SD (µg/L Copper)	CV (%)
Purple Urchin	Fertilization	59.9	43.5 ± 25.9	29.7

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

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

CV = Coefficient of Variation

REFERENCES

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
- Tidepool Scientific Software. 2000-2013. **CETIS™ Comprehensive Environmental Toxicity Information System** Software, Version 1.8.7.20
- USEPA. 1991. Methods for Aquatic Toxicity Identification Evaluation - Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.
- USEPA. 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: 03 Jul-17 11:01 (p 1 of 1)
Test Code: 1706-S024 | 06-0628-3887

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	12-3331-6812	Test Type:	Fertilization	Analyst:							
Start Date:	02 Jun-17 17:00	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	02 Jun-17 17:40	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	17-9108-9139	Code:	17-0648	Client:	IDE						
Sample Date:	02 Jun-17 09:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	02 Jun-17 12:04	Source:	IDE Americas, Inc.								
Sample Age:	8h (5 °C)	Station:	M-001 Unadjusted								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
06-9344-9627	Fertilization Rate	6.06	10	7.785	5.62%	16.5	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
00-4013-9584	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					
00-4013-9584	Fertilization Rate	Control Resp	0.932	0.7 - NL	Yes	Passes Acceptability Criteria					
06-9344-9627	Fertilization Rate	Control Resp	0.932	0.7 - NL	Yes	Passes Acceptability Criteria					
06-9344-9627	Fertilization Rate	PMSD	0.05615	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	0.958	0.9341	0.9819	0.94	0.99	0.008602	0.01924	2.01%	0.0%
0	Lab Control	5	0.932	0.8798	0.9842	0.87	0.98	0.01881	0.04207	4.51%	2.71%
2.5		5	0.946	0.9272	0.9648	0.93	0.96	0.006782	0.01517	1.6%	1.25%
5		5	0.924	0.8823	0.9657	0.9	0.97	0.01503	0.03362	3.64%	3.55%
6.06		5	0.916	0.8594	0.9726	0.87	0.97	0.0204	0.04561	4.98%	4.38%
10		5	0.866	0.8107	0.9213	0.82	0.92	0.0199	0.0445	5.14%	9.6%
15		5	0.872	0.8354	0.9086	0.85	0.92	0.01319	0.0295	3.38%	8.98%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	High Salinity Co	0.95	0.96	0.94	0.99	0.95					
0	Lab Control	0.87	0.98	0.96	0.93	0.92					
2.5		0.96	0.96	0.93	0.93	0.95					
5		0.9	0.9	0.97	0.95	0.9					
6.06		0.87	0.97	0.87	0.95	0.92					
10		0.82	0.88	0.82	0.92	0.89					
15		0.86	0.85	0.85	0.92	0.88					

CETIS Analytical Report

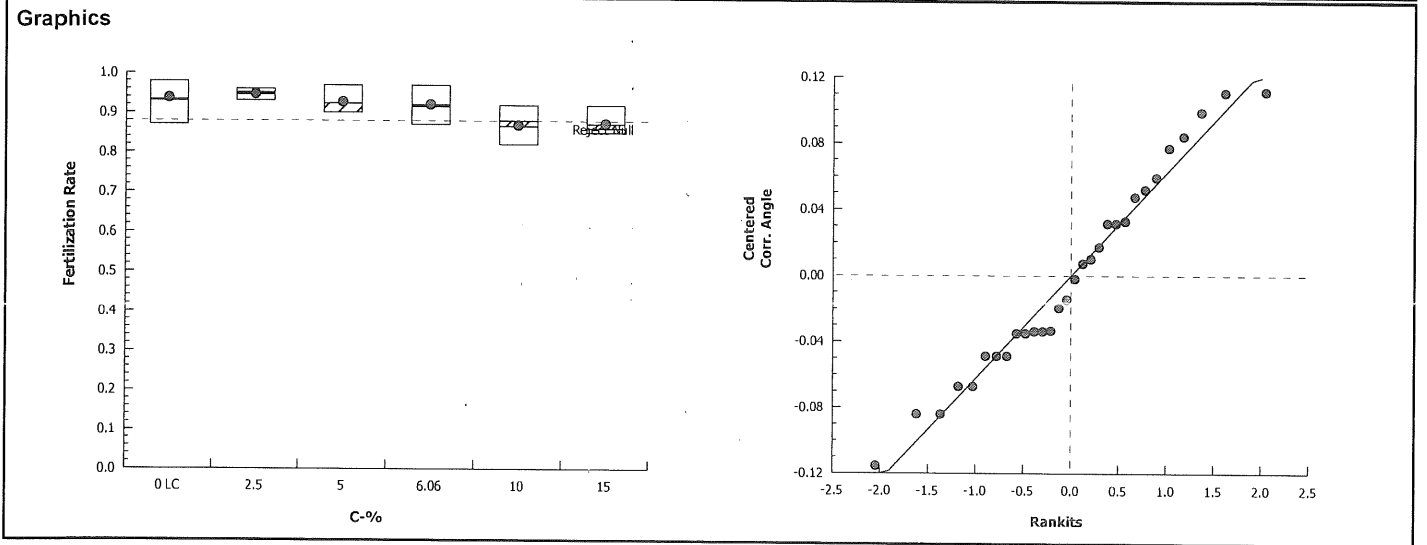
Report Date: 03 Jul-17 11:01 (p 1 of 2)
 Test Code: 1706-S024 | 06-0628-3887

Echinoid Sperm Cell Fertilization Test 15C										Nautilus Environmental (CA)	
Analysis ID: 06-9344-9627		Endpoint: Fertilization Rate					CETIS Version: CETISv1.8.7				
Analyzed: 03 Jul-17 11:01		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		5.62%	6.06	10	7.785	16.5
Dunnett Multiple Comparison Test											
Control		vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)	
Lab Control			2.5	-0.4815	2.362	0.101	8	0.9375	CDF	Non-Significant Effect	
			5	0.4596	2.362	0.101	8	0.6644	CDF	Non-Significant Effect	
			6.06	0.7369	2.362	0.101	8	0.5400	CDF	Non-Significant Effect	
			10*	2.753	2.362	0.101	8	0.0221	CDF	Significant Effect	
			15*	2.587	2.362	0.101	8	0.0315	CDF	Significant Effect	
ANOVA Table											
Source		Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)	
Between		0.08351989		0.01670398		5		3.659	0.0133	Significant Effect	
Error		0.1095673		0.004565304		24					
Total		0.1930872				29					
Distributional Tests											
Attribute		Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances		Bartlett Equality of Variance			4.216	15.09	0.5188	Equal Variances			
Distribution		Shapiro-Wilk W Normality			0.967	0.9031	0.4597	Normal Distribution			
Fertilization Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.932	0.8798	0.9842	0.93	0.87	0.98	0.01881	4.51%	0.0%
2.5		5	0.946	0.9272	0.9648	0.95	0.93	0.96	0.006782	1.6%	-1.5%
5		5	0.924	0.8823	0.9657	0.9	0.9	0.97	0.01503	3.64%	0.86%
6.06		5	0.916	0.8594	0.9726	0.92	0.87	0.97	0.0204	4.98%	1.72%
10		5	0.866	0.8107	0.9213	0.88	0.82	0.92	0.0199	5.14%	7.08%
15		5	0.872	0.8354	0.9086	0.86	0.85	0.92	0.01319	3.38%	6.44%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.317	1.21	1.425	1.303	1.202	1.429	0.0386	6.55%	0.0%
2.5		5	1.338	1.297	1.38	1.345	1.303	1.369	0.01496	2.5%	-1.56%
5		5	1.298	1.212	1.384	1.249	1.249	1.397	0.03096	5.33%	1.49%
6.06		5	1.286	1.179	1.393	1.284	1.202	1.397	0.03867	6.72%	2.39%
10		5	1.2	1.118	1.282	1.217	1.133	1.284	0.02958	5.51%	8.93%
15		5	1.207	1.149	1.265	1.187	1.173	1.284	0.02088	3.87%	8.39%

CETIS Analytical Report

Report Date: 03 Jul-17 11:01 (p 2 of 2)
 Test Code: 1706-S024 | 06-0628-3887

Echinoid Sperm Cell Fertilization Test 15C		Nautilus Environmental (CA)	
Analysis ID: 06-9344-9627	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7	
Analyzed: 03 Jul-17 11:01	Analysis: Parametric-Control vs Treatments	Official Results: Yes	



CETIS Analytical Report

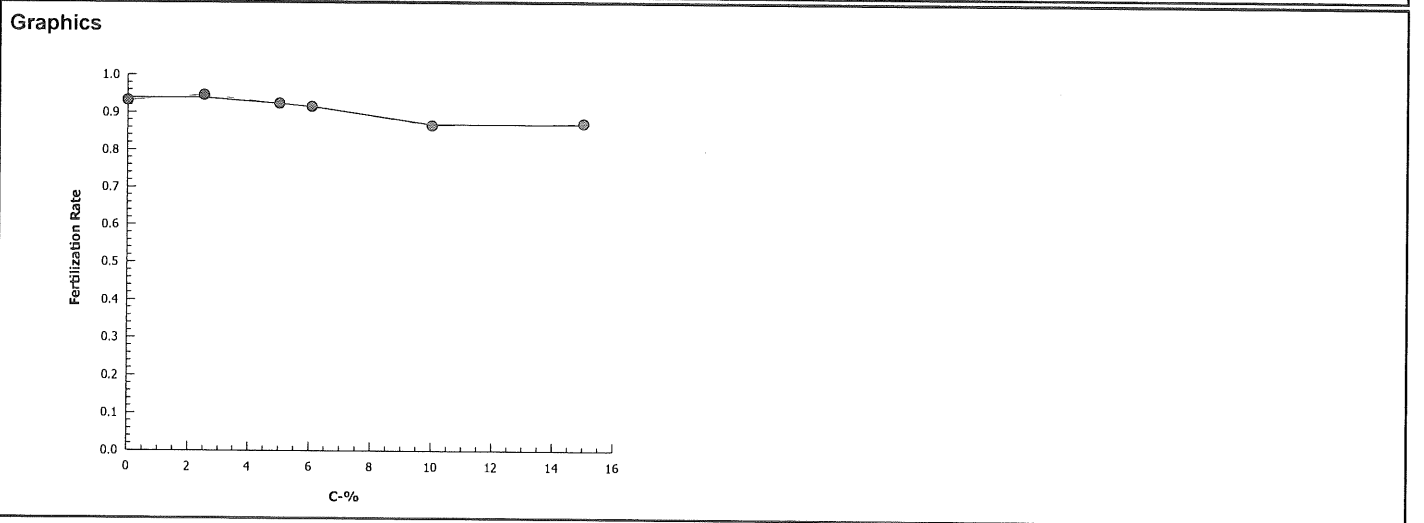
Report Date: 03 Jul-17 11:01 (p 1 of 1)
Test Code: 1706-S024 | 06-0628-3887

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	00-4013-9584	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	03 Jul-17 11:01	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1880569	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	0.932	0.87	0.98	0.01881	0.04207	4.51%	0.0%	466	500
2.5		5	0.946	0.93	0.96	0.006782	0.01517	1.6%	-1.5%	473	500
5		5	0.924	0.9	0.97	0.01503	0.03362	3.64%	0.86%	462	500
6.06		5	0.916	0.87	0.97	0.0204	0.04561	4.98%	1.72%	458	500
10		5	0.866	0.82	0.92	0.0199	0.0445	5.14%	7.08%	433	500
15		5	0.872	0.85	0.92	0.01319	0.0295	3.38%	6.44%	436	500



CETIS Analytical Report

Report Date: 03 Jul-17 11:02 (p 1 of 1)

Test Code: 1706-S024 | 06-0628-3887

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID: 14-3170-3772		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 03 Jul-17 11:01		Analysis: Parametric Bioequivalence-Two Sample				Official Results: Yes					
Data Transform		Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C*b < T	NA	NA	0.75	3.38%	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*	10.74	2.015	0.066	5	<0.0001	CDF	Non-Significant Effect		
		5*	7.308	1.895	0.080	7	<0.0001	CDF	Non-Significant Effect		
		6.06*	6.167	1.895	0.092	7	0.0002	CDF	Non-Significant Effect		
		10*	5.116	1.895	0.078	7	0.0007	CDF	Non-Significant Effect		
		15*	6.13	1.895	0.068	7	0.0002	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF	F Stat	P-Value	Decision(α:5%)			
Between	0.08351989		0.01670398		5	3.659	0.0133	Significant Effect			
Error	0.1095673		0.004565304		24						
Total	0.1930872				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			4.216	15.09	0.5188	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.967	0.9031	0.4597	Normal Distribution				
Fertilization Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.932	0.8798	0.9842	0.93	0.87	0.98	0.01881	4.51%	0.0%
2.5		5	0.946	0.9272	0.9648	0.95	0.93	0.96	0.006782	1.6%	-1.5%
5		5	0.924	0.8823	0.9657	0.9	0.9	0.97	0.01503	3.64%	0.86%
6.06		5	0.916	0.8594	0.9726	0.92	0.87	0.97	0.0204	4.98%	1.72%
10		5	0.866	0.8107	0.9213	0.88	0.82	0.92	0.0199	5.14%	7.08%
15		5	0.872	0.8354	0.9086	0.86	0.85	0.92	0.01319	3.38%	6.44%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.317	1.21	1.425	1.303	1.202	1.429	0.0386	6.55%	0.0%
2.5		5	1.338	1.297	1.38	1.345	1.303	1.369	0.01496	2.5%	-1.56%
5		5	1.298	1.212	1.384	1.249	1.249	1.397	0.03096	5.33%	1.49%
6.06		5	1.286	1.179	1.393	1.284	1.202	1.397	0.03867	6.72%	2.39%
10		5	1.2	1.118	1.282	1.217	1.133	1.284	0.02958	5.51%	8.93%
15		5	1.207	1.149	1.265	1.187	1.173	1.284	0.02088	3.87%	8.39%

CETIS Test Data Worksheet

Report Date: 02 Jun-17 09:18 (p 1 of 1)
Test Code: 06-0628-3887/1706-S024

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus
End Date: 02 Jun-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 01 Jun-17 Material: Facility Effluent

Sample Code: 17- 0648
Sample Source: IDE Americas, Inc.
Sample Station: M-001 Unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	85	BO 6/6/17 ↓
			32	100	86	
			33	100	88	
			34	100	97	
			35	100	89	
			36	100	92	
			37	100	82	
			38	100	93	
			39	100	96	
			40	100	92	
			41	100	95	
			42	100	87	
			43	100	82	
			44	100	93	
			45	100	87	
			46	100	90	
			47	100	92	
			48	100	95	
			49	100	88	
			50	100	95	
			51	100	90	
			52	100	93	
			53	100	87	
			54	100	90	
			55	100	96	
			56	100	92	
			57	100	96	
			58	100	98	
			59	100	97	
			60	100	85	

(#counted, #Fertilized)

HSC-A : (100, 95)

HSC-B : (100, 96)

HSC-C : (100, 94)

HSC-D : (100, 99)

HSC-E : (100, 95)

@Q18 AC 7/3/17

CETIS Test Data Worksheet

Report Date: 02 Jun-17 09:18 (p 1 of 1)
Test Code: 06-0628-3887/1706-S024

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus
End Date: 02 Jun-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 01 Jun-17 Material: Facility Effluent

Sample Code: 17-0648
Sample Source: IDE Americas, Inc.
Sample Station: M-001 Unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	42	100	95	AG 6/2/17
0	LC	2	58			
0	LC	3	39			
0	LC	4	44			
0	LC	5	56			
2.5		1	57	100	92	AG 6/2/17
2.5		2	55			
2.5		3	38			
2.5		4	52			
2.5		5	48			
5		1	46	100	97	AG 6/2/17
5		2	54			
5		3	59			
5		4	41			
5		5	51			
6.06		1	45	100	96	AG 6/2/17
6.06		2	34			
6.06		3	53			
6.06		4	50			
6.06		5	40			
10		1	37	100	90	AG 6/2/17
10		2	49			
10		3	43			
10		4	36			
10		5	35			
15		1	32	100	95	AG 6/2/17
15		2	31			
15		3	60			
15		4	47			
15		5	33			

HSC A 95/100 AG 6/2/17
B
C
D
E

QC: AG

@Q18AC 7/3/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDETest Species: S. purpuratusSample ID: M-001 (unadjusted)Start Date/Time: 6/2/2017 1700Sample Log No.: 17- 0648End Date/Time: 6/2/2017 1740Dilutions made by: ABTest No: 1706-5024Analyst: AO

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.9	8.01	33.6	15.6
2.5	8.1	8.07	34.6	15.8
5.0	8.1	8.06	35.2	15.8
6.06	8.1	8.06	35.6	15.6
10	8.1	8.06	36.9	15.5
15	8.0	8.05	38.2	15.7
HSC	8.0	8.08	38.2	15.8

Comments: _____

QC Check: EG 6/13/17Final Review: AC 7/3/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-001 Unadjusted
 Test No.: 1706-5024
 Tech initials: AB
 Injection Time: 1620

Start Date/Time: 6/2/2017 11700
 End Date/Time: 6/2/2017 11740
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 5/22/17

Sperm Absorbance at 400 nm: 0.980 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 77 Mean: 78 $\times 50 = 3,900$ eggs/ml

84
77
76
76

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

Initial density: 3,900 eggs/ml = (A) dilution factor egg stock 150 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater (A) ml
(A) parts seawater

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

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

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1632</u>	<u>50:1, 50:1</u>	<u>81,70</u>	<u>19,30</u>
Eggs Added (0.5 ml):	<u>1642</u>	<u>100:1, 100:1</u>	<u>96,96</u>	<u>4,4</u>
Test Ended:	<u>1652</u>	<u>200:1, 200:1</u>	<u>100,100</u>	<u>0,0</u>
		<u>400:1, 400:1</u>	<u>100,100</u>	<u>0,0</u>

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

Definitive Test Sperm:Egg Ratio Used: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1700</u>	QC1	<u>98</u>	<u>2</u>
Eggs Added (0.5 ml):	<u>1720</u>	QC2	<u>94</u>	<u>1</u>
Test Ended:	<u>1740</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) No Dilution Required

QC Check:

EG 6/13/17

Final Review:

AC 7/3/17

M-001 40 ppt Adjusted

CETIS Summary Report

Report Date: 03 Jul-17 11:06 (p 1 of 1)
Test Code: 1706-S025 | 09-5761-0582

Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	11-1898-3513	Test Type:	Fertilization	Analyst:							
Start Date:	02 Jun-17 17:00	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater						
Ending Date:	02 Jun-17 17:40	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable						
Duration:	40m	Source:	Pt. Loma	Age:							
Sample ID:	17-1585-4440	Code:	17-0648	Client:	IDE						
Sample Date:	02 Jun-17 09:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant						
Receive Date:	02 Jun-17 12:04	Source:	IDE Americas, Inc.								
Sample Age:	8h (5 °C)	Station:	M-001 40 ppt								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
18-8810-7863	Fertilization Rate	15	>15	NA	4.43%	< 6.667	Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method				
17-2343-3849	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					
17-2343-3849	Fertilization Rate	Control Resp	0.936	0.7 - NL	Yes	Passes Acceptability Criteria					
18-8810-7863	Fertilization Rate	Control Resp	0.936	0.7 - NL	Yes	Passes Acceptability Criteria					
18-8810-7863	Fertilization Rate	PMSD	0.04426	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	0.936	0.8962	0.9758	0.9	0.98	0.01435	0.03209	3.43%	0.0%
2.5		5	0.942	0.9216	0.9624	0.92	0.96	0.007348	0.01643	1.74%	-0.64%
5		5	0.928	0.8773	0.9787	0.86	0.96	0.01828	0.04087	4.4%	0.85%
6.06		5	0.938	0.9059	0.9701	0.9	0.97	0.01158	0.02588	2.76%	-0.21%
10		5	0.946	0.9125	0.9795	0.9	0.97	0.01208	0.02702	2.86%	-1.07%
15		5	0.944	0.9329	0.9551	0.93	0.95	0.004	0.008944	0.95%	-0.85%
Fertilization Rate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.98	0.91	0.94	0.9	0.95					
2.5		0.96	0.95	0.93	0.95	0.92					
5		0.93	0.93	0.96	0.86	0.96					
6.06		0.97	0.93	0.94	0.95	0.9					
10		0.97	0.95	0.9	0.95	0.96					
15		0.95	0.95	0.95	0.93	0.94					

CETIS Analytical Report

Report Date: 03 Jul-17 11:06 (p 1 of 2)
Test Code: 1706-S025 | 09-5761-0582

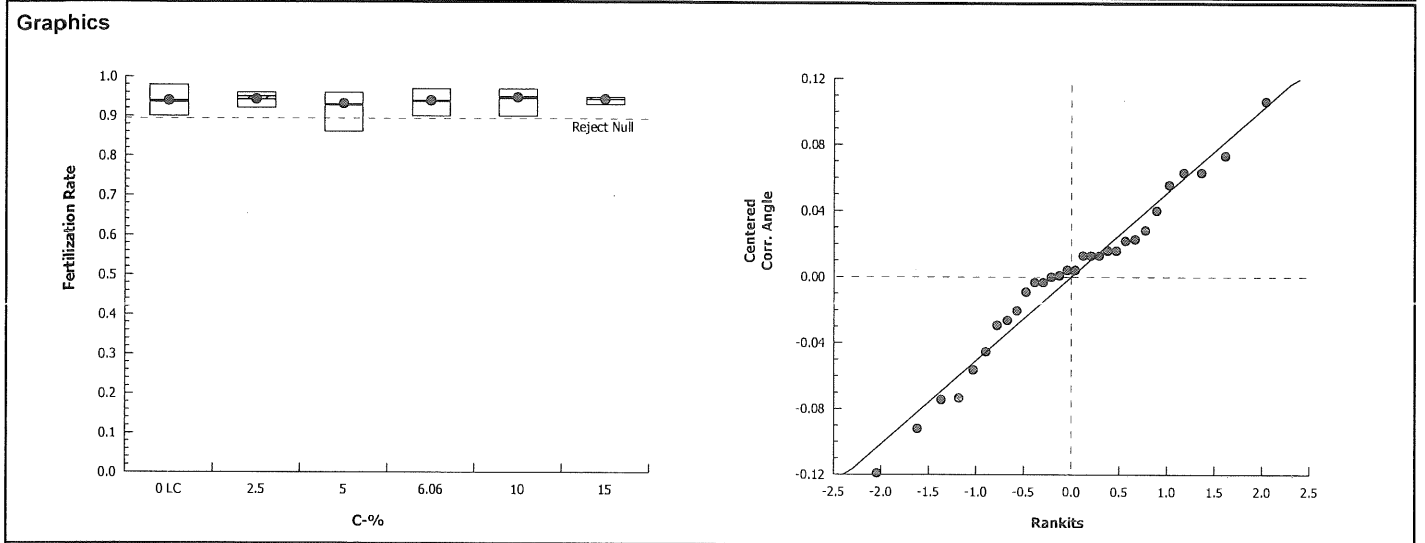
Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Analysis ID: 18-8810-7863		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 03 Jul-17 11:05		Analysis: Parametric-Control vs Treatments		Official Results: Yes							
Data Transform		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C > T	NA	NA		4.43%	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	-0.1972	2.362	0.082	8	0.8850	CDF	Non-Significant Effect		
		5	0.4609	2.362	0.082	8	0.6639	CDF	Non-Significant Effect		
		6.06	-0.02718	2.362	0.082	8	0.8412	CDF	Non-Significant Effect		
		10	-0.5336	2.362	0.082	8	0.9446	CDF	Non-Significant Effect		
		15	-0.284	2.362	0.062	8	0.9036	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.003416797		0.0006833594		5		0.2244	0.9483	Non-Significant Effect		
Error	0.07307683		0.003044868		24						
Total	0.07649363				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett Equality of Variance			7.111	15.09	0.2125	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.9729	0.9031	0.6215	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	0.936	0.8962	0.9758	0.94	0.9	0.98	0.01435	3.43%	0.0%
2.5		5	0.942	0.9216	0.9624	0.95	0.92	0.96	0.007348	1.74%	-0.64%
5		5	0.928	0.8773	0.9787	0.93	0.86	0.96	0.01828	4.4%	0.85%
6.06		5	0.938	0.9059	0.9701	0.94	0.9	0.97	0.01158	2.76%	-0.21%
10		5	0.946	0.9125	0.9795	0.95	0.9	0.97	0.01208	2.86%	-1.07%
15		5	0.944	0.9329	0.9551	0.95	0.93	0.95	0.004	0.95%	-0.85%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.323	1.234	1.411	1.323	1.249	1.429	0.03195	5.4%	0.0%
2.5		5	1.329	1.286	1.373	1.345	1.284	1.369	0.01559	2.62%	-0.52%
5		5	1.306	1.214	1.399	1.303	1.187	1.369	0.03328	5.7%	1.22%
6.06		5	1.323	1.256	1.391	1.323	1.249	1.397	0.02429	4.1%	-0.07%
10		5	1.341	1.272	1.41	1.345	1.249	1.397	0.0249	4.15%	-1.41%
15		5	1.332	1.309	1.356	1.345	1.303	1.345	0.008492	1.43%	-0.75%

CETIS Analytical Report

Report Date: 03 Jul-17 11:06 (p 2 of 2)
Test Code: 1706-S025 | 09-5761-0582

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

Analysis ID: 18-8810-7863	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed: 03 Jul-17 11:05	Analysis: Parametric-Control vs Treatments	Official Results: Yes



CETIS Analytical Report

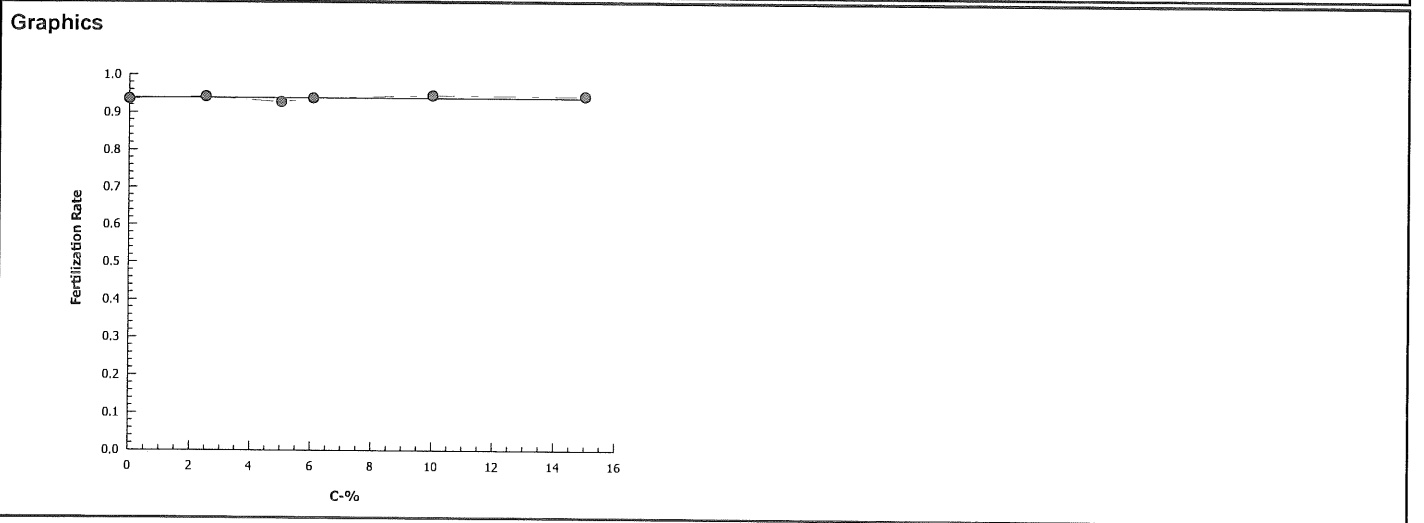
Report Date: 03 Jul-17 11:06 (p 1 of 1)
Test Code: 1706-S025 | 09-5761-0582

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)	
Analysis ID:	17-2343-3849	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	03 Jul-17 11:05	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

Linear Interpolation Options					
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1934435	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	0.936	0.9	0.98	0.01435	0.03209	3.43%	0.0%	468	500
2.5		5	0.942	0.92	0.96	0.007348	0.01643	1.74%	-0.64%	471	500
5		5	0.928	0.86	0.96	0.01828	0.04087	4.4%	0.85%	464	500
6.06		5	0.938	0.9	0.97	0.01158	0.02588	2.76%	-0.21%	469	500
10		5	0.946	0.9	0.97	0.01208	0.02702	2.86%	-1.07%	473	500
15		5	0.944	0.93	0.95	0.004	0.008944	0.95%	-0.85%	472	500



CETIS Analytical Report

Report Date: 03 Jul-17 11:07 (p 1 of 1)
Test Code: 1706-S025 | 09-5761-0582

Echinoid Sperm Cell Fertilization Test 15C						Nautilus Environmental (CA)					
Analysis ID: 08-6389-0595		Endpoint: Fertilization Rate				CETIS Version: CETISv1.8.7					
Analyzed: 03 Jul-17 11:07		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	2.64%	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	1.943	0.056	6	<0.0001	CDF	Non-Significant Effect		
		5*	7.67	1.895	0.078	7	<0.0001	CDF	Non-Significant Effect		
		6.06*	9.719	1.895	0.065	7	<0.0001	CDF	Non-Significant Effect		
		10*	10.11	1.895	0.065	7	<0.0001	CDF	Non-Significant Effect		
		15*	13.39	2.132	0.054	4	<0.0001	CDF	Non-Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	0.003416797		0.0006833594		5		0.2244	0.9483	Non-Significant Effect		
Error	0.07307683		0.003044868		24						
Total	0.07649363				29						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			7.111	15.09	0.2125		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.9729	0.9031	0.6215		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	0.936	0.8962	0.9758	0.94	0.9	0.98	0.01435	3.43%	0.0%
2.5		5	0.942	0.9216	0.9624	0.95	0.92	0.96	0.007348	1.74%	-0.64%
5		5	0.928	0.8773	0.9787	0.93	0.86	0.96	0.01828	4.4%	0.85%
6.06		5	0.938	0.9059	0.9701	0.94	0.9	0.97	0.01158	2.76%	-0.21%
10		5	0.946	0.9125	0.9795	0.95	0.9	0.97	0.01208	2.86%	-1.07%
15		5	0.944	0.9329	0.9551	0.95	0.93	0.95	0.004	0.95%	-0.85%
Angular (Corrected) Transformed Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.323	1.234	1.411	1.323	1.249	1.429	0.03195	5.4%	0.0%
2.5		5	1.329	1.286	1.373	1.345	1.284	1.369	0.01559	2.62%	-0.52%
5		5	1.306	1.214	1.399	1.303	1.187	1.369	0.03328	5.7%	1.22%
6.06		5	1.323	1.256	1.391	1.323	1.249	1.397	0.02429	4.1%	-0.07%
10		5	1.341	1.272	1.41	1.345	1.249	1.397	0.0249	4.15%	-1.41%
15		5	1.332	1.309	1.356	1.345	1.303	1.345	0.008492	1.43%	-0.75%

CETIS Test Data Worksheet

Report Date: 02 Jun-17 09:20 (p 1 of 1)
Test Code: 09-5761-0582/1706-S025

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus
End Date: 02 Jun-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 01 Jun-17 Material: Facility Effluent

Sample Code: 17-0648
Sample Source: IDE Americas, Inc.
Sample Station: M-001 40 ppt

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	92	TN 6/14/17
			62	100	98	
			63	100	95	
			64	100	96	
			65	100	93 930	
			66	100	95	
			67	100	93	
			68	100	96	
			69	100	97	
			70	100	94	
			71	100	95	
			72	100	94	
			73	100	86	
			74	100	94	
			75	100	96	
			76	100	95	
			77	100	93	
			78	100	90	
			79	100	90	
			80	100	95	
			81	100	95	TN 6/15/17
			82	100	96	
			83	100	95	
			84	100	97	
			85	100	93	
			86	100	91	
			87	100	95	
			88	100	90	
			89	100	93	
			90	100	95	

A) Q18 TN 6/14/17

B) Q18 AC 7/3/17

CETIS Test Data Worksheet

Report Date: 02 Jun-17 09:20 (p 1 of 1)
Test Code: 09-5761-0582/1706-S025

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus
End Date: 02 Jun-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 01 Jun-17 Material: Facility Effluent

Sample Code: 17-0648
Sample Source: IDE Americas, Inc.
Sample Station: M-001 40 ppt

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	62	100	94	Ab 6/2/17
0	LC	2	86			
0	LC	3	72			
0	LC	4	88			
0	LC	5	81			
2.5		1	82	100	93	Ab 6/2/17
2.5		2	80			
2.5		3	85			
2.5		4	83			
2.5		5	61			
5		1	67			
5		2	89	100	97	Ab 6/2/17
5		3	64			
5		4	73			
5		5	68			
6.06		1	84	100	98	Ab 6/2/17
6.06		2	77			
6.06		3	74			
6.06		4	66			
6.06		5	79			
10		1	69	100	94	Ab 6/2/17
10		2	71			
10		3	78			
10		4	90			
10		5	75			
15		1	63	100	94	Ab 6/2/17
15		2	76			
15		3	87			
15		4	65			
15		5	70			

@018 AC 7/3/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted)

Start Date/Time: 6/2/2017 1700

Sample Log No.: 17- 0648

End Date/Time: 6/2/2017 1740

Dilutions made by: AG

Test No: 1706-5025

Analyst: AD

Concentration %	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.0	8.01	33.3	14.0
2.5	8.1	8.03	33.6	14.7
5.0	8.1	8.05	34.0	14.4
6.06	8.2	8.07	34.1	14.2
10	8.1	8.09	34.4	14.3
15	8.1	8.09	34.7	14.3
	8.0	8.00		14.0 (B)

Comments: ~~AD 08~~ 6/2/17

QC Check: EG 6/13/17

Final Review: 8/13/17

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: IDEAnalyst: AGSample ID: M-001 (40 ppt adjusted)Test Date: 6/2/2017Test No: 1706-5025Test Type: Urchin FertilizationSalinity of Effluent 63.8Salinity of Seawater 33.5Date of Brine used: NATarget Salinity 40.0Alk. of 40 ppt Adj. Sample: 142 mg/L as CaCO₃

	<u>Effluent</u>	<u>Brine Control</u>
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	<u>3.66</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	3.66	366.2	466

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: EH 6/13/17Final Review: 58 7/13/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: IDE
 Sample ID: M-001 40ppt adjusted
 Test No.: 1706-5025
 Tech initials: AB
 Injection Time: 1620

Start Date/Time: 6/2/2017 / 1700
 End Date/Time: 6/2/2017 / 1740
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 5/22/17

Sperm Absorbance at 400 nm: 0.980 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 77 Mean: 78 $\times 50 = 3,900$ eggs/ml

84
77
76
76

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

Initial density: 3,900 eggs/ml = (A) dilution factor egg stock 150 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater (A) ml
(A) parts seawater

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

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

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 μ l):	<u>1632</u>	<u>50:1, 50:1</u>	<u>81,70</u>	<u>9,30</u>
Eggs Added (0.5 ml):	<u>1642</u>	<u>100:1, 100:1</u>	<u>96,96</u>	<u>4,4</u>
Test Ended:	<u>1652</u>	<u>200:1, 200:1</u>	<u>100, 100</u>	<u>0, 0</u>
		<u>400:1, 400:1</u>	<u>100, 100</u>	<u>0, 0</u>

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

Definitive Test Sperm:Egg Ratio Used: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 μ l):	<u>1700</u>	QC1	<u>98</u>	<u>2</u>
Eggs Added (0.5 ml):	<u>1720</u>	QC2	<u>94</u>	<u>1</u>
Test Ended:	<u>1740</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(B) No Dilution Required

QC Check:

EH 6/13/17

Final Review:

8 7/3/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: M-001
Test ID No(s): 1706-S024 to S026

Sample Check-In Information

Sample Description:

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

Sample (A, B, C):	<u>A</u>			
Log-in No. (17-xxxx):	<u>0648</u>			
Sample Collection Date & Time:	<u>6/2/17 0900</u>			
Sample Receipt Date & Time:	<u>6/2/17 1204</u>			
Number of Containers & Container Type:	<u>1, 4L cubic</u>			
Approx. Total Volume Received (L):	<u>~4L</u>			
Check-in Temperature (°C)	<u>5.0</u>			
Temperature OK? ¹	<u>(Y) N</u>	<u>Y N</u>	<u>Y N</u>	<u>Y N</u>
DO (mg/L)	<u>7.7</u>			
pH (units)	<u>7.96</u>			
Conductivity (µS/cm)	<u>—</u>			
Salinity (ppt)	<u>(A) 45.1 63.8²</u>			
Alkalinity (mg/L) ²	<u>192</u>			
Hardness (mg/L) ^{2,3}	<u>—</u>			
Total Chlorine (mg/L)	<u>0.03</u>			
Technician Initials	<u>DM</u>			

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

Additional Control? (Y) N = ASC Alkalinity: NA Hardness or Salinity: 34 ppt
Q18 AC 713

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

Additional Control? Y N = — Alkalinity: — Hardness or Salinity: —

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

Additional Control? Y N = — Alkalinity: — Hardness or Salinity: —

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

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

Additional Comments: (A) Q18 DM 6/2/17
(A) Salinity half DI and half sample
(A) EQ Q18 6/26/17
DM = not measured, tech error

COC Complete (Y/N)?

A Y B C

Filtration? Y (N)

Pore Size: —

Organisms — or Debris —

Salinity Adjustment? Y (N)

Test: — Source: — Target ppt: —

Test: — Source: — Target ppt: —

Test: — Source: — Target ppt: —

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl₂ Adjustment? Y (N)

	A	B	C
Initial Free Cl ₂ :			
STS added:			
Final Free Cl ₂ :			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Subsamples for Additional Chemistry Required? Y (N)

NH₃ Other —

Tech Initials A B C

QC Check: EG 6/26/17

Final Review: UE 7/3/17

Appendix C

Chain-of-Custody Form



IDE
Technologies

CDP Laboratory: _____
Entalpy Laboratory: _____
WECK Laboratory: _____
Nautilus: X
AIM: _____
Other: _____

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

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

Special instruction: 24 hour composite sample collected via autosampler by a series of consecutive grabs at 6 hr intervals during normal plant operation. Sample collected to fulfill monthly NPDES requirement. Sample is to be run unadjusted and adjusted to 40 ppt. Start: 6/1/17 @ 09:00, End: 6/2/17 @ 09:00. KC

ANALYSES

NOTES:

Glass=G Plastic=P

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

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

Preservative ?

Container

Type

Purple Urchin Chronic Fertilization

X

TDS 61.49 ppt, EC 85.47 mS/cm

Temp
Pool
5.0

Relinquished By:

Date:

Time:

Received By:

Time:

Sample Condition Upon Receipt:

☐ Iced

☐ Ambient or _____ °C

☐ Iced

☐ Ambient or _____ °C

Nautilus 10:17-0648

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date: 12 Jun-17 16:41 (p 1 of 1)

Test Code: 170602sprt | 07-9823-1222

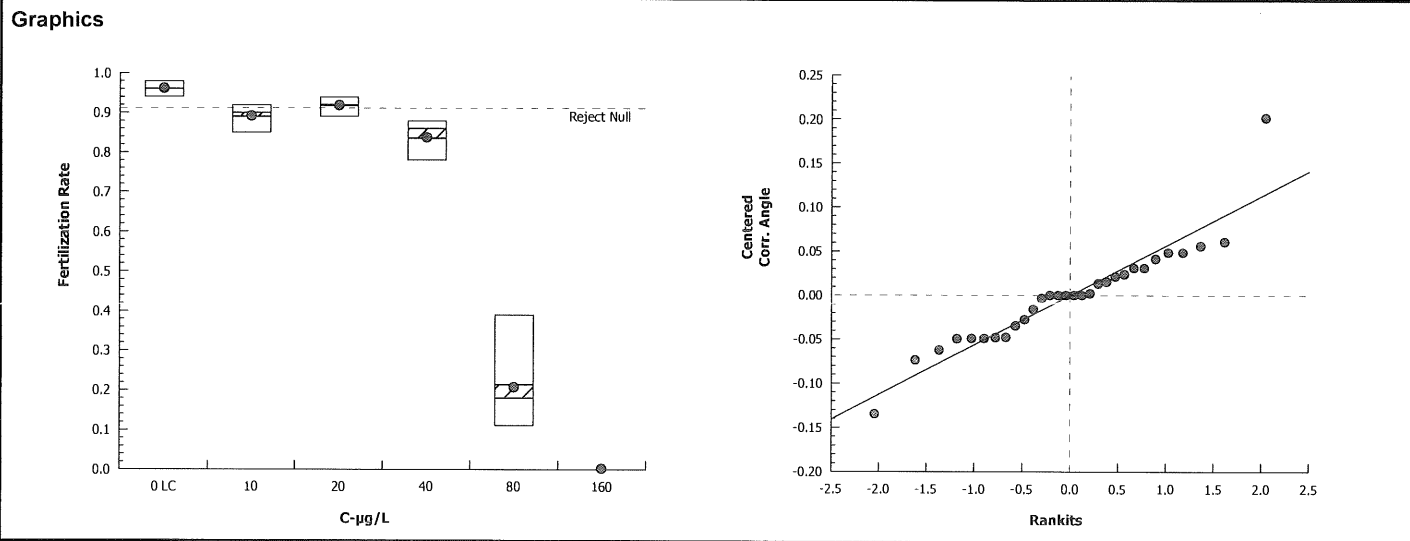
Echinoid Sperm Cell Fertilization Test 15C							Nautilus Environmental (CA)				
Batch ID:	18-6047-4052	Test Type:	Fertilization				Analyst:				
Start Date:	02 Jun-17 17:00	Protocol:	EPA/600/R-95/136 (1995)				Diluent:	Natural Seawater			
Ending Date:	02 Jun-17 17:40	Species:	Strongylocentrotus purpuratus				Brine:	Not Applicable			
Duration:	40m	Source:	Pt. Loma				Age:				
Sample ID:	09-0099-4586	Code:	170602sprt				Client:	Internal			
Sample Date:	02 Jun-17	Material:	Copper chloride				Project:				
Receive Date:	02 Jun-17	Source:	Reference Toxicant								
Sample Age:	17h	Station:	Copper Chloride								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
21-0790-4675	Fertilization Rate	20	40	28.28	4.96%		Dunnett Multiple Comparison Test				
Point Estimate Summary											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
14-2289-5480	Fertilization Rate	EC50	59.87	57.65	62.16		Trimmed Spearman-Kärber				
Test Acceptability											
Analysis ID	Endpoint	Attribute		Test Stat	TAC Limits		Overlap	Decision			
14-2289-5480	Fertilization Rate	Control Resp		0.96	0.7 - NL		Yes	Passes Acceptability Criteria			
21-0790-4675	Fertilization Rate	Control Resp		0.96	0.7 - NL		Yes	Passes Acceptability Criteria			
21-0790-4675	Fertilization Rate	PMSD		0.04963	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	0.96	0.9404	0.9796	0.94	0.98	0.007071	0.01581	1.65%	0.0%
10		5	0.89	0.8488	0.9312	0.85	0.92	0.01483	0.03317	3.73%	7.29%
20		5	0.918	0.8941	0.9419	0.89	0.94	0.008602	0.01923	2.1%	4.38%
40		5	0.836	0.7822	0.8898	0.78	0.88	0.01939	0.04336	5.19%	12.92%
80		5	0.214	0.08242	0.3456	0.11	0.39	0.04739	0.106	49.52%	77.71%
160		5	0	0	0	0	0	0	0		100.0%
Fertilization Rate Detail											
C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5					
0	Lab Control	0.97	0.96	0.98	0.94	0.95					
10		0.85	0.92	0.9	0.86	0.92					
20		0.93	0.92	0.89	0.91	0.94					
40		0.86	0.78	0.88	0.8	0.86					
80		0.11	0.22	0.17	0.39	0.18					
160		0	0	0	0	0					

CETIS Analytical Report

Report Date: 12 Jun-17 16:41 (p 1 of 2)
Test Code: 170602sprt | 07-9823-1222

Echinoid Sperm Cell Fertilization Test 15C								Nautilus Environmental (CA)			
Analysis ID: 21-0790-4675		Endpoint: Fertilization Rate		CETIS Version: CETISv1.8.7							
Analyzed: 12 Jun-17 16:40		Analysis: Parametric-Control vs Treatments		Official Results: Yes							
Data Transform		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)		NA	C > T	NA	NA		4.96%	20	40	28.28	
Dunnett Multiple Comparison Test											
Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)		
Lab Control		10*	3.086	2.305	0.103	8	0.0099	CDF	Significant Effect		
		20	2.044	2.305	0.103	8	0.0814	CDF	Non-Significant Effect		
		40*	4.867	2.305	0.103	8	0.0002	CDF	Significant Effect		
		80*	20.24	2.305	0.103	8	<0.0001	CDF	Significant Effect		
ANOVA Table											
Source	Sum Squares		Mean Square		DF		F Stat	P-Value	Decision(α:5%)		
Between	2.611561		0.6528901		4		132	<0.0001	Significant Effect		
Error	0.09890114		0.004945057		20						
Total	2.710462				24						
Distributional Tests											
Attribute	Test			Test Stat	Critical	P-Value		Decision(α:1%)			
Variances	Bartlett Equality of Variance			8.244	13.28	0.0831		Equal Variances			
Distribution	Shapiro-Wilk W Normality			0.918	0.8877	0.0461		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	0.96	0.9404	0.9796	0.96	0.94	0.98	0.007071	1.65%	0.0%
10		5	0.89	0.8488	0.9312	0.9	0.85	0.92	0.01483	3.73%	7.29%
20		5	0.918	0.8941	0.9419	0.92	0.89	0.94	0.008602	2.1%	4.38%
40		5	0.836	0.7822	0.8898	0.86	0.78	0.88	0.01939	5.19%	12.92%
80		5	0.214	0.08242	0.3456	0.18	0.11	0.39	0.04739	49.52%	77.71%
160		5	0	0	0	0	0	0	0		100.0%
Angular (Corrected) Transformed Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.373	1.321	1.424	1.369	1.323	1.429	0.01862	3.03%	0.0%
10		5	1.236	1.17	1.301	1.249	1.173	1.284	0.02357	4.27%	10.0%
20		5	1.282	1.239	1.325	1.284	1.233	1.323	0.01555	2.71%	6.62%
40		5	1.156	1.084	1.228	1.187	1.083	1.217	0.02594	5.02%	15.77%
80		5	0.4728	0.3175	0.6281	0.4381	0.3381	0.6745	0.05593	26.45%	65.56%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.36%

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)	
Analysis ID:	21-0790-4675	Endpoint:	Fertilization Rate	CETIS Version: CETISv1.8.7
Analyzed:	12 Jun-17 16:40	Analysis:	Parametric-Control vs Treatments	Official Results: Yes



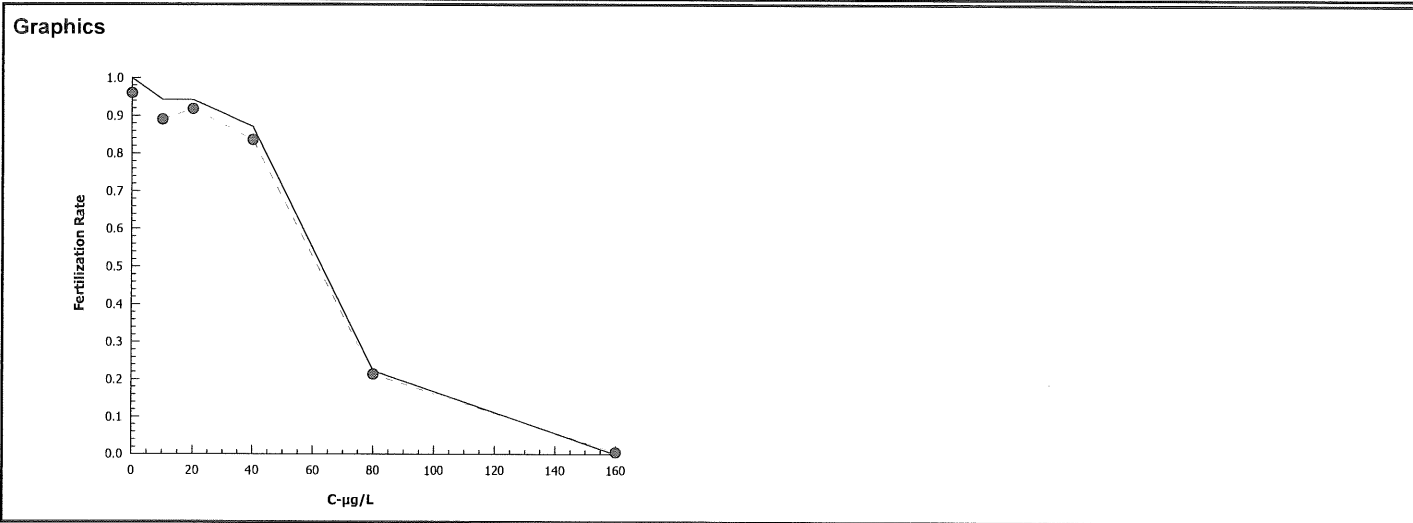
CETIS Analytical Report

Report Date: 12 Jun-17 16:41 (p 1 of 1)
 Test Code: 170602sprt | 07-9823-1222

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)			
Analysis ID:	14-2289-5480	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7		
Analyzed:	12 Jun-17 16:40	Analysis:	Trimmed Spearman-Kärber	Official Results:	Yes		

Trimmed Spearman-Kärber Estimates							
Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.04	5.83%	1.777	0.008176	59.87	57.65	62.16

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	0.96	0.94	0.98	0.007071	0.01581	1.65%	0.0%	480	500
10		5	0.89	0.85	0.92	0.01483	0.03317	3.73%	7.29%	445	500
20		5	0.918	0.89	0.94	0.008602	0.01923	2.1%	4.38%	459	500
40		5	0.836	0.78	0.88	0.01939	0.04336	5.19%	12.92%	418	500
80		5	0.214	0.11	0.39	0.04739	0.106	49.52%	77.71%	107	500
160		5	0	0	0	0	0		100.0%	0	500



Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

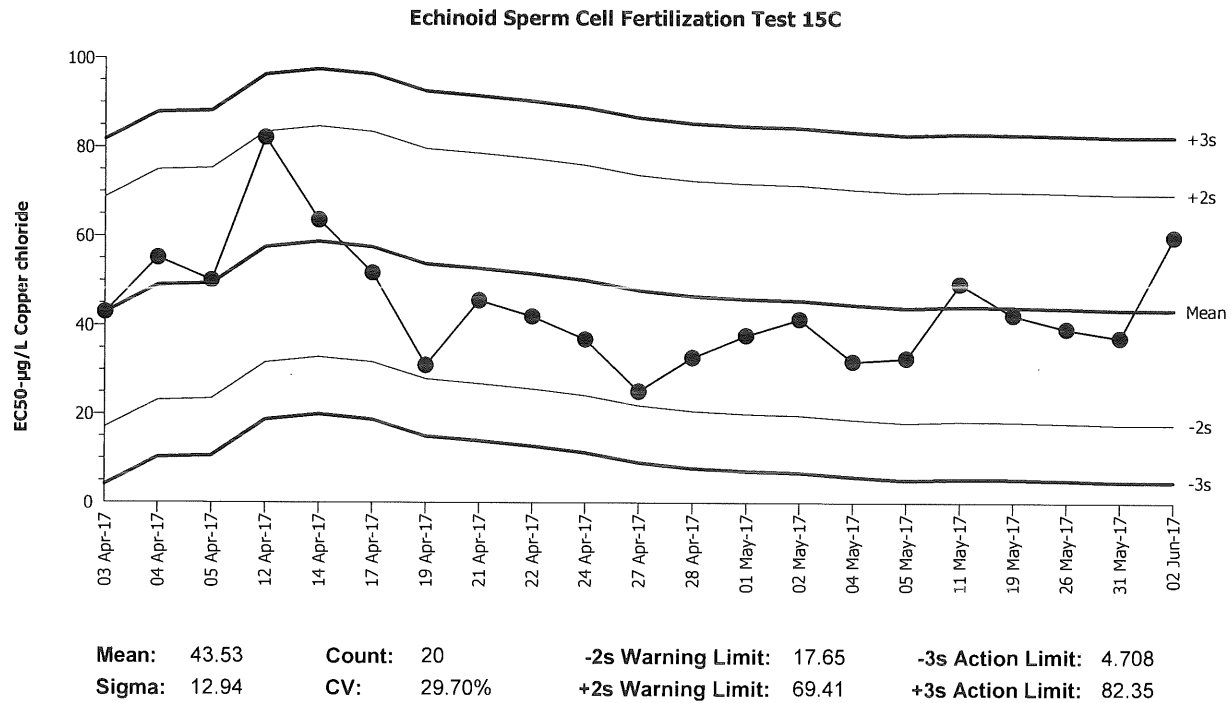
Organism: Strongylocentrotus purpuratus (Purpl

Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF



Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Apr	3	17:15	42.93	-0.5962	-0.04607			11-7488-2003	08-5368-9216
2			4	12:13	55.13	11.6	0.8962			02-3186-4899	18-3488-7750
3			5	14:33	50.05	6.521	0.5039			12-8691-4512	16-6546-2933
4			12	16:30	82.12	38.59	2.982	(+)		04-6122-9881	08-0232-5024
5			14	15:34	63.58	20.05	1.55			06-7326-1133	06-6067-9028
6			17	17:03	51.69	8.164	0.6309			13-4494-7236	11-5239-6650
7			19	15:40	31	-12.53	-0.968			16-6386-1330	20-1211-2894
8			21	12:05	45.55	2.015	0.1557			10-3448-9907	04-0175-0159
9			22	14:33	41.96	-1.568	-0.1212			11-1560-7674	00-7687-9708
10			24	16:05	36.84	-6.692	-0.5171			09-2574-2389	13-4474-8503
11			27	12:19	25.18	-18.35	-1.418			10-3853-1638	12-2181-2382
12			28	15:50	32.81	-10.72	-0.8286			04-2889-8959	08-9738-9274
13		May	1	17:50	37.72	-5.806	-0.4487			18-0409-9294	21-4338-2021
14			2	11:56	41.38	-2.149	-0.1661			15-1584-1378	12-5072-1723
15			4	16:15	31.87	-11.66	-0.9015			08-0627-0095	01-9095-4567
16			5	15:10	32.62	-10.91	-0.8429			16-0368-0463	05-0853-8226
17			11	15:35	49.31	5.777	0.4465			01-5463-5574	13-6197-3009
18			19	16:58	42.3	-1.234	-0.09534			01-3808-3529	10-6921-8357
19			26	16:55	39.23	-4.297	-0.3321			19-8209-6027	02-5970-9183
20			31	15:42	37.28	-6.249	-0.4829			01-0947-8219	10-1735-9410
21		Jun	2	17:00	59.87	16.34	1.262			07-9823-1222	14-2289-5480

CETIS Test Data Worksheet

Report Date: 02 Jun-17 09:21 (p 1 of 1)
 Test Code: 07-9823-1222/170602sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus
 End Date: 02 Jun-17 Protocol: EPA/600/R-95/136 (1995)
 Sample Date: 02 Jun-17 Material: Copper chloride

Sample Code: 170602sprt
 Sample Source: Reference Toxicant
 Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	93	6/12/17 BO ↓
			2	100	0	
			3	100	86	
			4	100	22	
			5	100	98	
			6	100	88	
			7	100	97	
			8	100	0	
			9	100	94	
			10	100	92	
			11	100	91	
			12	100	17	
			13	100	92	
			14	100	90	
			15	100	0	
			16	100	94	
			17	100	0	
			18	100	0	
			19	100	11	
			20	100	85	
			21	100	96	
			22	100	86	
			23	100	78	
			24	100	18	
			25	100	95	
			26	100	92	
			27	100	4739 ^(A)	
			28	100	89	
			29	100	86	
			30	100	80	

Ⓐ Q18 BO 6/12/17

CETIS Test Data Worksheet

Report Date: 02 Jun-17 09:21 (p 1 of 1)
Test Code: 07-9823-1222/170602sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus
End Date: 02 Jun-17 Protocol: EPA/600/R-95/136 (1995)
Sample Date: 02 Jun-17 Material: Copper chloride

Sample Code: 170602sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	7	100	99	AG 6/2/17
0	LC	2	21			
0	LC	3	5			
0	LC	4	16			
0	LC	5	25			
10		1	20	100	92	AG 6/2/17
10		2	26			
10		3	14			
10		4	29			
10		5	13			
20		1	1	100	88	AG 6/2/17
20		2	10			
20		3	28			
20		4	11			
20		5	9			
40		1	3	100	83	AG 6/2/17
40		2	23	100	77	AG 6/2/17
40		3	6	100	82	AG 6/2/17
40		4	30			
40		5	22			
80		1	19	100	19	AG 6/2/17
80		2	4	100	14	
80		3	12			
80		4	27			
80		5	24			
160		1	18	100	0	AG 6/2/17
160		2	17			
160		3	8			
160		4	2			
160		5	15			

QC: AG

Marine Chronic Bioassay

Water Quality Measurements

Client : InternalTest Species: S. purpuratusSample ID: CuCl₂Start Date/Time: 6/2/2017 1700Test No: 170602sptEnd Date/Time: 6/2/2017 1740Dilutions made by: AB

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	12,200

Analyst:

AD

Concentration (µg/L)	Initial Readings			
	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.0	7.99	33.2	15.6
10	8.0	8.01	33.7	15.6
20	8.0	8.02	33.6	15.5
40	8.0	8.03	33.4	15.4
80	8.0	8.04	33.5	15.5
160	8.1	8.05	33.3	15.4

Comments: _____

QC Check: KFP 6/12/17Final Review: JW 6/12/17

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Internal/CuCl₂
 Sample ID: 170602s CuCl₂
 Test No.: 170602spt
 Tech initials: AB
 Injection Time: 1620

Start Date/Time: 6/2/2017 11700
 End Date/Time: 6/2/2017 11740
 Species: S. purpuratus
 Animal Source: Point Loma
 Date Collected: 5/22/17

Sperm Absorbance at 400 nm: 0.980 (target range of 0.8 - 1.0 for density of 4×10^6 sperm/ml)

Eggs Counted: 77 Mean: 78 X 50 = 3,900 eggs/ml

84
77
76
76

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

Initial density: 3,900 eggs/ml = (A) dilution factor egg stock 150 ml
 Final density: 4000 eggs/ml - 1.0 part egg stock seawater (A) ml
(A) parts seawater

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

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

	Time	Range Finder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	<u>1632</u>	<u>50:1, 50:1</u>	<u>81,70</u>	<u>19,30</u>
Eggs Added (0.5 ml):	<u>1642</u>	<u>100:1, 100:1</u>	<u>96,96</u>	<u>4,4</u>
Test Ended:	<u>1652</u>	<u>200:1, 200:1</u>	<u>100,100</u>	<u>0,0</u>
		<u>400:1, 400:1</u>	<u>100,100</u>	<u>0,0</u>

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

Definitive Test

Sperm:Egg Ratio Used: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	<u>1700</u>	QC1	<u>98</u>	<u>2</u>
Eggs Added (0.5 ml):	<u>1720</u>	QC2	<u>94</u>	<u>1</u>
Test Ended:	<u>1740</u>	Egg Control 1	<u>0</u>	<u>100</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments:

(A) No Dilution Required
(B) KFP QR 6/12/17

QC Check:

KFP 6/12/17

Final Review: JW 6/12/17

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.