Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID's: M-001, ERI Brine, Train 9
Sample Collection Date: March 9, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **April 10, 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 Cibar

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – MARCH 2017

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

Sampling Date: **March 9, 2017**

Test Date: **March 10, 2017**

Sample IDs: **M-001 Brine Effluent, ERI Brine, and Train 9**

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	Yes
	6.06	16.5	

INTRODUCTION

A 24-hour composite discharge sample was collected in March 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for monthly chronic toxicity monitoring purposes. Due to effects observed in a sample collected and tested for monthly monitoring purposes on June 17, 2016 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). Additional samples collected throughout the facility were also tested for comparison purposes. Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on March 10, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The samples were collected on March 9, 2017. Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the samples were hand delivered to Nautilus the day of sample collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. 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:	March 2017
Sample ID, Material:	1. M-001, desalination plant brine effluent 2. ERI Brine, brine 3. Train 9, brine
Sample Collection Date, Time:	1. 3/9/17, 10:00 2. 3/9/17, 10:00 3. 3/9/17, 10:00
Sample Receipt Date, Time:	3/9/17, 12:07
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	7.92	7.6	4.0	62.0	216	<0.02
ERI Brine	7.46	7.8	3.0	63.4	216	0.03
Train 9	7.45	7.9	4.0	64.4	219	<0.02

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.

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	3/10/17, 14:18 through 14:58
Test Organism:	*Strongylocentrotus purpuratus* (purple sea urchin)
Test Organism Source:	Field-collected locally (off Point Loma in San Diego, CA)
Lab Control/Dilution Water:	Natural seawater (source: Scripps Institution of Oceanography (SIO) inlet), 34±2 parts per thousand (ppt); 20-µm filtered
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control. The same dilution series was also tested with the other samples and M-001 after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP discharges). The 10 percent M-001 dilution was also tested with the pH10/0.45 µm filtration toxicity identification evaluation (TIE) treatment.
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min egg fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25%
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

RESULTS

There was a statistically significant decrease in fertilization rate observed in the 10 and 15 percent concentrations of the unadjusted M-001 sample compared to the lab control, resulting in a NOEC of 6.06 percent effluent and a TU_c equal to 16.5. This meets the maximum daily permit effluent limitation of 16.5 TU_c. The 6.06 percent concentration (IWC) resulted in a 6.1 percent effect compared to the lab control, which was not significantly significant using to the TST calculation. The M-001 sample adjusted to 40 ppt prior to dilution preparation resulted in no statistically significant effects in any of the test concentrations and a TU_c less than 6.67.

The ERI Brine test resulted in a statistically significant decrease in fertilization rate in the 15 percent sample concentration compared to the lab control, resulting in a NOEC of 10 percent effluent and a TU_c equal to 10. The percent effect the 15 percent concentration was 23.6, which was also significant according to the TST. The Train 9 sample test resulted in a NOEC of 5.0 percent sample, and a TU_c value of 20; the 15 percent sample concentration was significant according to the TST. Salinity for all test concentrations and samples was below the NOEC value of 38.5 ppt from previous internal studies at Nautilus.

Statistical results for urchin fertilization toxicity tests are summarized in Table 4, and detailed test results are summarized in Tables 5 and 6. 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	6.1
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	1.4
ERI Brine	10	15	>15	10	Pass	5.7
Train 9	5.0	6.06	>15	20	Pass	11

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
TU_c = Chronic Toxic Unit: 100÷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= ((mean response in control-mean response in the IWC)/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	33.2	88.0	33.2	83.8
2.5	34.2	89.2	33.4	83.8
5.0	35.0	83.3	33.7	82.4
6.06	35.3	82.6	33.9	82.6
10	36.4	62.8*	34.0	83.2
15	37.9	50.6*	34.4	79.0

[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).

Table 6. Detailed Results of Purple Urchin Fertilization Testing for the Additional Facility Samples

Test Concentration (% Sample)	ERI Brine		Train 9	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.1	84.0	33.1	84.4
2.5	34.2	83.2	34.2	75.6
5.0	34.9	79.8	35.0	79.6
6.06	35.4	79.2	35.3	75.2*
10	36.5	76.6	36.6	69.4*
15	38.0	64.2*	38.1	56.4*

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

As part of the ongoing toxicity reduction evaluation and toxicity identification evaluation (TRE/TIE) investigations, the M-001 sample was also tested with the pH 10/filtration TIE treatment. The treatment was performed on the 10 percent sample concentration in order to obtain the highest testable concentration to discern differences between treated and untreated sample while also not having the confounding effect of elevated salinity above the organism tolerance level. Salinity measured in the untreated 10 percent M-001 sample was 36.4 ppt; below the salinity tolerance limit for this organism and test procedure (based on Philips et al. 2012, and internal studies at Nautilus). Results for the sample tested with and without the TIE treatments are presented in Figure 1, and raw datasheets are in Appendix A.

The pH 10/filtration treatment improved fertilization over the untreated baseline sample. This response is consistent with previous M-001 samples tested using this treatment.

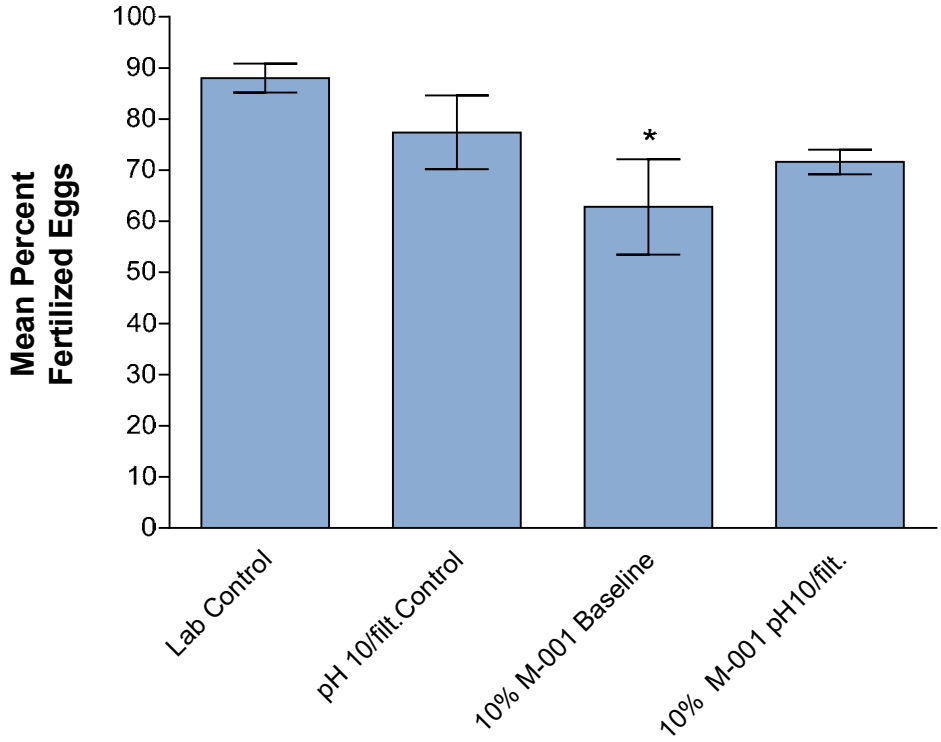


Figure 1. Summary of Urchin Fertilization Results for the M-001 10% Sample with and without the pH10/filtration TIE treatment (Mean ± 1SD). *An asterisk indicates a statistically significant decrease in egg fertilization compared to the lab control using the TST analysis.

QUALITY ASSURANCE

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

Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to evaluate reliability of the results. Additionally, appropriate alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010).

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 7 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration (EC_{50} 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 7. 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	28.7	46.6 ± 37.7	40.4

EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
Historical Mean EC_{50} ± 2 SD = Mean of historical test results plus or minus two standard deviations
CV = Coefficient of Variation

REFERENCES

California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.

Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.

Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20

USEPA. 1991. Methods for Aquatic Toxicity Identification Evaluation - Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.

USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.

USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses

M-001 Unadjusted

CETIS Summary Report

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

Batch ID:	06-2864-0304	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	10 Mar-17 14:18	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	10 Mar-17 14:58	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	15-2497-8726	**Code:**	17-0374	**Client:**	IDE
Sample Date:	09 Mar-17 10:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	09 Mar-17 12:07	**Source:**	IDE Americas, Inc.		
Sample Age:	28h (4 °C)	**Station:**	M-001 (Unadjusted)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
10-2919-3574	Fertilization Rate	6.06	10	7.785	8.14%	16.5	Bonferroni Adj t Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
20-2534-1715	Fertilization Rate	EC25	9.274	7.948	11.62	10.78	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
10-2919-3574	Fertilization Rate	Control Resp	0.88	0.7 - NL	Yes	Passes Acceptability Criteria
20-2534-1715	Fertilization Rate	Control Resp	0.88	0.7 - NL	Yes	Passes Acceptability Criteria
10-2919-3574	Fertilization Rate	PMSD	0.08138	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	0.88	0.8449	0.9151	0.85	0.91	0.01265	0.02828	3.21%	0.0%
2.5		5	0.892	0.8523	0.9317	0.85	0.93	0.01428	0.03194	3.58%	-1.36%
5		4	0.8325	0.7645	0.9005	0.78	0.88	0.02136	0.04272	5.13%	5.4%
6.06		5	0.826	0.7974	0.8546	0.79	0.85	0.0103	0.02302	2.79%	6.14%
10		5	0.628	0.5124	0.7436	0.5	0.71	0.04164	0.09311	14.83%	28.64%
15		5	0.506	0.4076	0.6044	0.38	0.58	0.03544	0.07925	15.66%	42.5%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.91	0.9	0.85	0.89	0.85
2.5		0.93	0.91	0.85	0.9	0.87
5			0.88	0.78	0.85	0.82
6.06		0.83	0.85	0.79	0.82	0.84
10		0.56	0.71	0.7	0.67	0.5
15		0.58	0.48	0.55	0.38	0.54

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

Analysis ID: 10-2919-3574 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 14 Mar-17 17:03 **Analysis:** Parametric-Multiple Comparison **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	8.14%	6.06	10	7.785	16.5

Bonferroni Adj t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.4903	2.5	0.101	8	1.0000	CDF	Non-Significant Effect
		5	1.573	2.5	0.107	7	0.3235	CDF	Non-Significant Effect
		6.06	1.921	2.5	0.101	8	0.1680	CDF	Non-Significant Effect
		10*	7.473	2.5	0.101	8	<0.0001	CDF	Significant Effect
		15*	10.57	2.5	0.101	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.8104195	0.1620839	5	39.64	<0.0001	Significant Effect
Error	0.09404132	0.004088753	23			
Total	0.9044608		28			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.897	15.09	0.3163	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9469	0.9004	0.1518	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	0.88	0.8449	0.9151	0.89	0.85	0.91	0.01265	3.21%	0.0%
2.5		5	0.892	0.8523	0.9317	0.9	0.85	0.93	0.01428	3.58%	-1.36%
5		4	0.8325	0.7645	0.9005	0.835	0.78	0.88	0.02136	5.13%	5.4%
6.06		5	0.826	0.7974	0.8546	0.83	0.79	0.85	0.0103	2.79%	6.14%
10		5	0.628	0.5124	0.7436	0.67	0.5	0.71	0.04164	14.83%	28.64%
15		5	0.506	0.4076	0.6044	0.54	0.38	0.58	0.03544	15.66%	42.5%

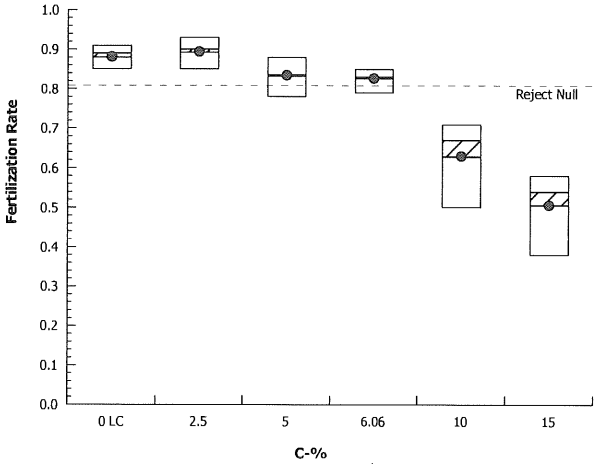
Angular (Corrected) Transformed Summary

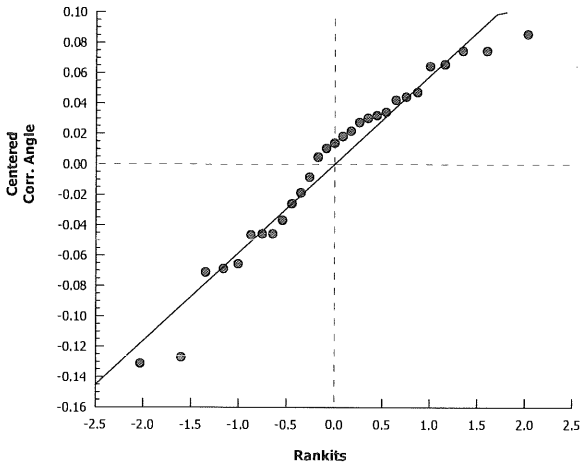
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.219	1.165	1.273	1.233	1.173	1.266	0.0194	3.56%	0.0%
2.5		5	1.239	1.175	1.303	1.249	1.173	1.303	0.02308	4.17%	-1.63%
5		4	1.151	1.06	1.243	1.153	1.083	1.217	0.02868	4.98%	5.54%
6.06		5	1.141	1.104	1.178	1.146	1.095	1.173	0.01341	2.63%	6.38%
10		5	0.9166	0.7973	1.036	0.9589	0.7854	1.002	0.04297	10.48%	24.79%
15		5	0.7913	0.6922	0.8904	0.8254	0.6642	0.8657	0.03569	10.09%	35.08%

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

Analysis ID:	10-2919-3574	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	14 Mar-17 17:03	**Analysis:**	Parametric-Multiple Comparison	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	20-2534-1715	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	14 Mar-17 17:03	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	437372	1000	Yes	Two-Point Interpolation

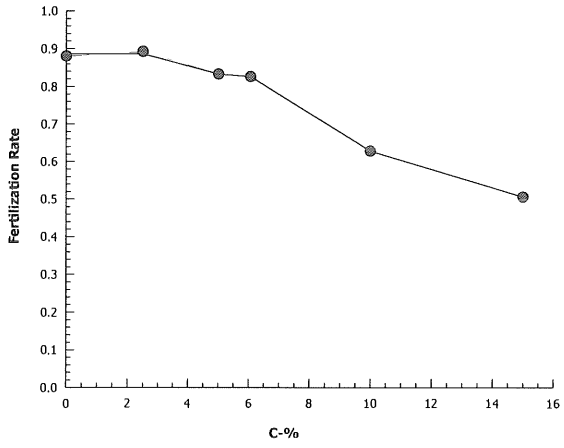
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	9.274	7.948	11.62	10.78	8.609	12.58
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	0.88	0.85	0.91	0.01265	0.02828	3.21%	0.0%	440	500
2.5		5	0.892	0.85	0.93	0.01428	0.03194	3.58%	-1.36%	446	500
5		4	0.8325	0.78	0.88	0.02136	0.04272	5.13%	5.4%	333	400
6.06		5	0.826	0.79	0.85	0.0103	0.02302	2.79%	6.14%	413	500
10		5	0.628	0.5	0.71	0.04164	0.09311	14.83%	28.64%	314	500
15		5	0.506	0.38	0.58	0.03544	0.07925	15.66%	42.5%	253	500

Graphics



CETIS Analytical Report

TST

Report Date: 14 Mar-17 17:07 (p 1 of 1)
Test Code: 1703-S050 | 16-8628-3186

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

Analysis ID: 06-8355-1628 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 14 Mar-17 17:07 **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	6.08%	6.06	10	7.785	16.5

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	11.9	1.943	0.053	6	<0.0001	CDF	Non-Significant Effect
		5*	7.378	2.132	0.069	4	0.0009	CDF	Non-Significant Effect
		6.06*	11.47	1.895	0.037	7	<0.0001	CDF	Non-Significant Effect
		10	0.0552	2.132	0.097	4	0.4793	CDF	Significant Effect
		15	-3.187	2.015	0.078	5	0.9878	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.8104195	0.1620839	5	39.64	<0.0001	Significant Effect
Error	0.09404132	0.004088753	23			
Total	0.9044608		28			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.897	15.09	0.3163	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9469	0.9004	0.1518	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	0.88	0.8449	0.9151	0.89	0.85	0.91	0.01265	3.21%	0.0%
2.5		5	0.892	0.8523	0.9317	0.9	0.85	0.93	0.01428	3.58%	-1.36%
5		4	0.8325	0.7645	0.9005	0.835	0.78	0.88	0.02136	5.13%	5.4%
6.06		5	0.826	0.7974	0.8546	0.83	0.79	0.85	0.0103	2.79%	6.14%
10		5	0.628	0.5124	0.7436	0.67	0.5	0.71	0.04164	14.83%	28.64%
15		5	0.506	0.4076	0.6044	0.54	0.38	0.58	0.03544	15.66%	42.5%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.219	1.165	1.273	1.233	1.173	1.266	0.0194	3.56%	0.0%
2.5		5	1.239	1.175	1.303	1.249	1.173	1.303	0.02308	4.17%	-1.63%
5		4	1.151	1.06	1.243	1.153	1.083	1.217	0.02868	4.98%	5.54%
6.06		5	1.141	1.104	1.178	1.146	1.095	1.173	0.01341	2.63%	6.38%
10		5	0.9166	0.7973	1.036	0.9589	0.7854	1.002	0.04297	10.48%	24.79%
15		5	0.7913	0.6922	0.8904	0.8254	0.6642	0.8657	0.03569	10.09%	35.08%

Outlier Calculation

List potential outlier first in data set.

Data Set		Mean	Std Dev
43.0		75.2	18.38
88.0			
78.0			
85.0			
82.0	Calculated T-value =	1.75	**YES - it is an Outlier**

Critical T-value for 5 replicates is 1.67

If calculated T-value exceeds critical T-value, then data point **is** an outlier.

Entered: AC 3/14/17
QC: JW 4/10/17

CETIS Test Data Worksheet

Report Date: 09 Mar-17 15:23 (p 1 of 1)
Test Code: 1703-5050 16-8628-3186/6482A3B2

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

Start Date: 10 Mar-17
End Date: 10 Mar-17
Sample Date: 09 Mar-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: ~~Seawater~~ Facility Effluent @18 AC 3/14
Sample Code: ~~SAE55426~~ @18 AC 3/14 170374
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Unadjusted)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	78	3/14/17
			32	100	85	
			33	100	83	
			34	100	90	
			35	100	54	
			36	100	90	
			37	100	89	
			38	100	50	
			39	100	85	
			40	100	48	
			41	100	70	
			42	100	91	
			43	100	43	QC = AC 3/14 49/100; rep excluded as statistical outlier.
			44	100	85	
			45	100	87	
			46	100	71	
			47	100	85	
			48	100	91	
			49	100	85	
			50	100	38	
			51	100	93	
			52	100	58	
			53	100	84	
			54	100	56	
			55	100	88	
			56	100	67	
			57	100	79	
			58	100	82	
			59	100	82	
			60	100	55	

pH 10 A	100	85 69 (A)	
pH 10 B	100	70	
pH 10 C	100	71	
pH 10 D	100	73	
pH 10 E	100	75	
pH control A	100	82	
pH control B	100	79	
pH control C	100	83	
pH control D	100	78	
pH control E	100	65	

(A) Q18 3/14/17 DM

			# counted	# fert
SAL	Control	A	100	75
SAL	Control	B	100	79
SAL	Control	C	100	85 (A) 84
SAL	Control	D	100	74
SAL	Control	E	100	71

CETIS Test Data Worksheet

Report Date: 09 Mar-17 15:23 (p 1 of 1)
Test Code: 1703-SDSD 16-8628-3186/6482A3B2

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)
Start Date: 10 Mar-17	**Species:** Strongylocentrotus purpuratus	**Sample Code:** ~~5AE55426~~ QIS AC 3/14 17-0374	
End Date: 10 Mar-17	**Protocol:** EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.	
Sample Date: 09 Mar-17	**Material:** Seawater QIS AC 3/14 Facility Effluent	**Sample Station:** M-001 (Unadjusted)	

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	48			
0	LC	2	34			
0	LC	3	44	100	88	EG 3/10/17
0	LC	4	37			
0	LC	5	49			
2.5		1	51			
2.5		2	42	100	92	EG
2.5		3	39			
2.5		4	36			
2.5		5	45			
5		1	43			
5		2	55	100	83	EG
5		3	31			
5		4	32			
5		5	59			
6.06		1	33			
6.06		2	47			
6.06		3	57	100	79	EG
6.06		4	58			
6.06		5	53			
10		1	54			
10		2	46			
10		3	41	100	68	EG
10		4	56			
10		5	38			
15		1	52			
15		2	40			
15		3	60	100	53	EG
15		4	50			
15		5	35			

QC: EG

Marine Chronic Bioassay **Water Quality Measurements**

Client : IDE **Test Species:** *S. purpuratus*

Sample ID: M-001 (unadjusted) **Start Date/Time:** 3/10/2017 1418

Sample Log No.: 17- 0374 **End Date/Time:** 3/10/2017 1458

Dilutions made by: EG **Test No:** 1703-SO50

Analyst: EG

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.0	8.01	33.2	14.9
2.5	8.0	8.02	34.2	14.8
5.0	8.2	8.03	35.0	14.7
6.06	8.3	8.03	35.3	14.8
10	8.3	8.04	36.4	14.8
15	8.3	8.04	37.9	14.9

Comments:

QC Check: AC 3/14/17 **Final Review:** JW 4/10/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Marine Chronic Bioassay | **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: IDE
Sample ID: M-001 Unadjusted
Test No.: 1703-S05o

Start Date/Time: 3/10/2017 / 1418
End Date/Time: 3/10/2017 / 1458
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 2/9/17 & 3/6/17

Tech initials: EG
Injection Time: 1340

Sperm Absorbance at 400 nm: 0.855 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 97, 79, 102, 84, 90 Mean: 90.4 X 50 = 4520 eggs/ml

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

Initial density: 4520 eggs/ml = 1.13 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.13 parts seawater

egg stock 200 ml
seawater 26 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							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1350	50:1	67	33
Eggs Added (0.5 ml):	1400	100:1	96/96	4/4
Test Ended:	1410	200:1	100	0
		—	—	—

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):	1418	QC1	92	8
Eggs Added (0.5 ml):	1438	QC2	85	15
Test Ended:	1458	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 3/14/17

Final Review: JW 4/10/17

IDE/ Carlsbad Desalination Plant TIE
Summary of Urchin Fertilization Results
Sample Collection Date: 3/9/17; Test Initiation Date: 3/10/17

Sample ID	Fertilization (%)	Mean Fertilization (%)	Standard Deviation
Lab Control	91 90 85 89 85	88.0	2.8
pH 10/filtration Control	82 79 83 78 65	77.4	7.2
10% Baseline Sample	56 71 70 67 50	62.8	9.3
10% pH 10/filtered Sample	69 70 71 73 75	71.6	2.4

Marine Chronic Bioassay **Water Quality Measurements**

Client : IDE **Test Species:** *S. purpuratus*

Sample ID: M-001 (unadjusted) **Start Date/Time:** 3/10/2017 1418

Sample Log No.: 17- 0374 **End Date/Time:** 3/10/2017 1458

Dilutions made by: EG **Test No:** 1703-SOSO

Analyst: EG

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Salinity Control	7.9	8.06	36.3	15.0
pH10/filt. Control	6.9	8.05	35.7	15.0
pH 10/filt. 10% M-001	6.0	8.14	36.2	15.2

Comments:

QC Check: AC 3/14/17 **Final Review:** JW 4/10/17

TST Summary Sheet

Lab Name	Nautilus	**Client Name**	IDE/CDP
Test ID	LC vs. pH 10 Control	**Test Species**	*S. purpuratus (echinoderm)*
Test Date	3/10/2017	**Test Type**	Chronic
Test Duration	40m	**Endpoint**	Fertilization
Critical Conc.	10%		

Statistic	**Control**	**Critical Concentration**
Percent Mean of Raw Data	0.88	0.78
Mean used in Calcuation (transformed)	1.22	1.08
Variance used in Calcuation (transformed)	0.002	0.007
Standard Deviation of Transformed Data	0.043	0.083
CV of Transformed Data	0.036	0.077
n	5	5

Mean % Effect at Critical Conc.

11.82

Calculated t-value	**Degrees of Freedom**	**Table t-value**	**Percent Difference**
4.1758	5	2.0150	

Results

Pass Sample is Non-toxic

Raw Data

Control Data		**Critical Concentration Data**	
No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)	No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)
100	91	100	82
100	90	100	79
100	85	100	83
100	89	100	79
100	85	100	65

TST Summary Sheet

Lab Name	Nautilus	**Client Name**	IDE/CDP
Test ID	LC vs. pH 10 10% M-001	**Test Species**	*S. purpuratus (echinoderm)*
Test Date	3/10/2017	**Test Type**	Chronic
Test Duration	40m	**Endpoint**	Fertilization
Critical Conc.	10%		

Statistic	**Control**	**Critical Concentration**
Percent Mean of Raw Data	0.88	0.72
Mean used in Calcuation (transformed)	1.22	1.01
Variance used in Calcuation (transformed)	0.002	0.001
Standard Deviation of Transformed Data	0.043	0.027
CV of Transformed Data	0.036	0.027
n	5	5

Mean % Effect at Critical Conc.

18.64

Calculated t-value	**Degrees of Freedom**	**Table t-value**	**Percent Difference**
5.0316	7	1.8946	

Results

Pass Sample is Non-toxic

Raw Data

Control Data		**Critical Concentration Data**	
No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)	No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)
100	91	100	69
100	90	100	70
100	85	100	71
100	89	100	73
100	85	100	75

TST Summary Sheet

Lab Name	Nautilus	**Client Name**	IDE/CDP
Test ID	LC vs. 10% M-001	**Test Species**	*S. purpuratus (echinoderm)*
Test Date	3/10/2017	**Test Type**	Chronic
Test Duration	40m	**Endpoint**	Fertilization
Critical Conc.	10%		

Statistic	**Control**	**Critical Concentration**
Percent Mean of Raw Data	0.88	0.63
Mean used in Calcuation (transformed)	1.22	0.92
Variance used in Calcuation (transformed)	0.002	0.009
Standard Deviation of Transformed Data	0.043	0.096
CV of Transformed Data	0.036	0.105
n	5	5

Mean % Effect at Critical Conc.

28.64

Calculated t-value	**Degrees of Freedom**	**Table t-value**	**Percent Difference**
0.0552	4	2.1318	

Results

Fail	Sample is Toxic

Raw Data

Control Data		**Critical Concentration Data**	
No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)	No. of Organisms Exposed or Counted	Response (Final Count, Weight, Length, etc.)
100	91	100	56
100	90	100	71
100	85	100	70
100	89	100	67
100	85	100	50

M-001 40 ppt Adjusted

CETIS Summary Report

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

Batch ID:	11-8324-5262	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	10 Mar-17 14:18	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	10 Mar-17 14:58	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	03-1402-7483	**Code:**	17-0374	**Client:**	IDE
Sample Date:	09 Mar-17 10:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	09 Mar-17 12:07	**Source:**	IDE Americas, Inc.		
Sample Age:	28h (4 °C)	**Station:**	M-001 (40 ppt adj)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
21-0731-8201	Fertilization Rate	15	>15	NA	6.66%	6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
02-3614-0744	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
02-3614-0744	Fertilization Rate	Control Resp	0.838	0.7 - NL	Yes	Passes Acceptability Criteria
21-0731-8201	Fertilization Rate	Control Resp	0.838	0.7 - NL	Yes	Passes Acceptability Criteria
21-0731-8201	Fertilization Rate	PMSD	0.06657	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	0.838	0.7829	0.8931	0.79	0.89	0.01985	0.04438	5.3%	0.0%
2.5		5	0.838	0.7993	0.8767	0.81	0.88	0.01393	0.03114	3.72%	0.0%
5		5	0.824	0.7534	0.8946	0.76	0.89	0.02542	0.05683	6.9%	1.67%
6.06		5	0.826	0.7988	0.8532	0.8	0.86	0.009798	0.02191	2.65%	1.43%
10		5	0.832	0.7923	0.8717	0.79	0.87	0.01428	0.03194	3.84%	0.72%
15		5	0.79	0.7609	0.8191	0.75	0.81	0.01049	0.02345	2.97%	5.73%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.79	0.89	0.81	0.88	0.82
2.5		0.81	0.81	0.88	0.86	0.83
5		0.76	0.86	0.77	0.84	0.89
6.06		0.82	0.8	0.82	0.86	0.83
10		0.87	0.84	0.81	0.79	0.85
15		0.81	0.75	0.8	0.79	0.8

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

Analysis ID: 21-0731-8201 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 14 Mar-17 17:08 **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	6.66%	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	0.04629	2.362	0.074	8	0.8193	CDF	Non-Significant Effect
		5	0.5621	2.362	0.074	8	0.6195	CDF	Non-Significant Effect
		6.06	0.5825	2.362	0.074	8	0.6104	CDF	Non-Significant Effect
		10	0.3066	2.362	0.074	8	0.7273	CDF	Non-Significant Effect
		15	2.045	2.362	0.074	8	0.0916	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01399879	0.002799758	5	1.137	0.3682	Non-Significant Effect
Error	0.05910699	0.002462791	24			
Total	0.07310577		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.466	15.09	0.3617	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.982	0.9031	0.8750	Normal Distribution

Fertilization Rate Summary

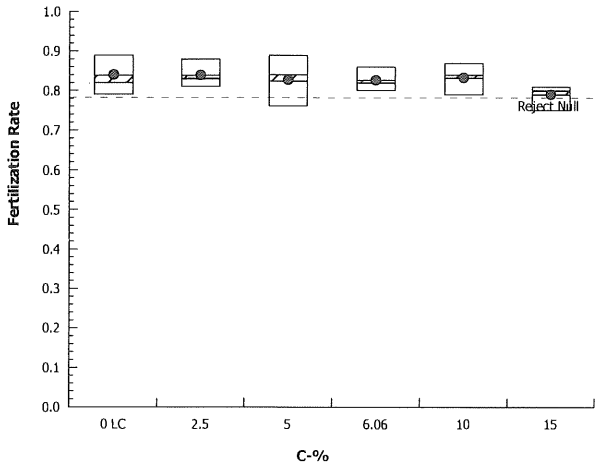
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.838	0.7829	0.8931	0.82	0.79	0.89	0.01985	5.3%	0.0%
2.5		5	0.838	0.7993	0.8767	0.83	0.81	0.88	0.01393	3.72%	0.0%
5		5	0.824	0.7534	0.8946	0.84	0.76	0.89	0.02542	6.9%	1.67%
6.06		5	0.826	0.7988	0.8532	0.82	0.8	0.86	0.009798	2.65%	1.43%
10		5	0.832	0.7923	0.8717	0.84	0.79	0.87	0.01428	3.84%	0.72%
15		5	0.79	0.7609	0.8191	0.8	0.75	0.81	0.01049	2.97%	5.73%

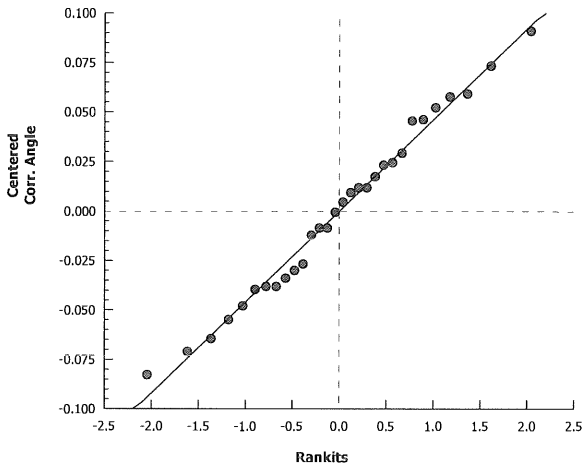
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.159	1.083	1.236	1.133	1.095	1.233	0.02754	5.31%	0.0%
2.5		5	1.158	1.104	1.211	1.146	1.12	1.217	0.01926	3.72%	0.13%
5		5	1.142	1.048	1.235	1.159	1.059	1.233	0.03361	6.58%	1.52%
6.06		5	1.141	1.105	1.178	1.133	1.107	1.187	0.01314	2.57%	1.58%
10		5	1.15	1.097	1.203	1.159	1.095	1.202	0.01908	3.71%	0.83%
15		5	1.095	1.06	1.13	1.107	1.047	1.12	0.01264	2.58%	5.54%

Echinoid Sperm Cell Fertilization Test 15C					Nautilus Environmental (CA)
Analysis ID:	21-0731-8201	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	14 Mar-17 17:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1703-S051 | 00-3723-6090

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

Analysis ID:	02-3614-0744	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	14 Mar-17 17:08	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	610813	1000	Yes	Two-Point Interpolation

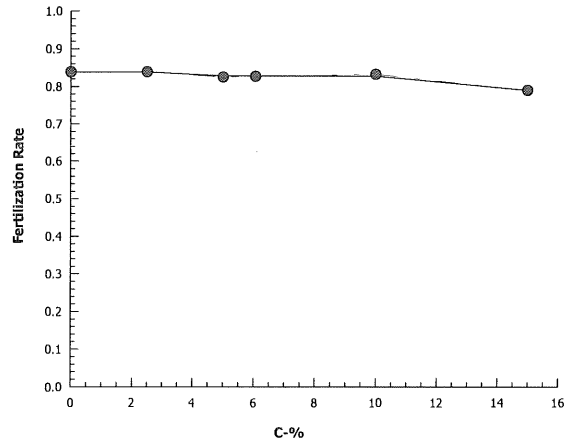
Point Estimates

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	0.838	0.79	0.89	0.01985	0.04438	5.3%	0.0%	419	500
2.5		5	0.838	0.81	0.88	0.01393	0.03114	3.72%	0.0%	419	500
5		5	0.824	0.76	0.89	0.02542	0.05683	6.9%	1.67%	412	500
6.06		5	0.826	0.8	0.86	0.009798	0.02191	2.65%	1.43%	413	500
10		5	0.832	0.79	0.87	0.01428	0.03194	3.84%	0.72%	416	500
15		5	0.79	0.75	0.81	0.01049	0.02345	2.97%	5.73%	395	500

Graphics



 Analyst: AC QA: sw 4/10/17

CETIS Analytical Report

TST

Report Date: 14 Mar-17 17:09 (p 1 of 1)
Test Code: 1703-S051 | 00-3723-6090

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

Analysis ID: 16-1688-5552 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 14 Mar-17 17:09 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	4.04%	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*	10.21	1.895	0.054	7	<0.0001	CDF	Non-Significant Effect
		5*	6.9	1.943	0.077	6	0.0002	CDF	Non-Significant Effect
		6.06*	11.09	1.943	0.048	6	<0.0001	CDF	Non-Significant Effect
		10*	9.966	1.895	0.053	7	<0.0001	CDF	Non-Significant Effect
		15*	9.32	1.943	0.047	6	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01399879	0.002799758	5	1.137	0.3682	Non-Significant Effect
Error	0.05910699	0.002462791	24			
Total	0.07310577		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.466	15.09	0.3617	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.982	0.9031	0.8750	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	0.838	0.7829	0.8931	0.82	0.79	0.89	0.01985	5.3%	0.0%
2.5		5	0.838	0.7993	0.8767	0.83	0.81	0.88	0.01393	3.72%	0.0%
5		5	0.824	0.7534	0.8946	0.84	0.76	0.89	0.02542	6.9%	1.67%
6.06		5	0.826	0.7988	0.8532	0.82	0.8	0.86	0.009798	2.65%	1.43%
10		5	0.832	0.7923	0.8717	0.84	0.79	0.87	0.01428	3.84%	0.72%
15		5	0.79	0.7609	0.8191	0.8	0.75	0.81	0.01049	2.97%	5.73%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.159	1.083	1.236	1.133	1.095	1.233	0.02754	5.31%	0.0%
2.5		5	1.158	1.104	1.211	1.146	1.12	1.217	0.01926	3.72%	0.13%
5		5	1.142	1.048	1.235	1.159	1.059	1.233	0.03361	6.58%	1.52%
6.06		5	1.141	1.105	1.178	1.133	1.107	1.187	0.01314	2.57%	1.58%
10		5	1.15	1.097	1.203	1.159	1.095	1.202	0.01908	3.71%	0.83%
15		5	1.095	1.06	1.13	1.107	1.047	1.12	0.01264	2.58%	5.54%

CETIS Test Data Worksheet

Report Date: 09 Mar-17 15:24 (p 1 of 1)
Test Code: 1703-S051 00-3723-6090/2382D7A

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)
Start Date:	10 Mar-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:** Q18 AC 3/14 ~~12B7ADDB~~ 17-0374
End Date:	10 Mar-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.
Sample Date:	09 Mar-17	**Material:**	Facility Effluent	**Sample Station:** M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	86	3/13/17
			62	100	80	
			63	100	81	
			64	100	86	
			65	100	80	
			66	100	77	
			67	100	81	
			68	100	76	
			69	100	87	
			70	100	86	
			71	100	82	
			72	100	(B) ~~58~~ 79	
			73	100	89	
			74	100	81	
			75	100	85	
			76	100	89	
			77	106	83	
			78	100	81	
			79	100	88	
			80	100	82	
			81	100	88	
			82	100	84	
			83	100	83	
			84	100	81	
			85	100	79	
			86	100	80	
			87	106	84	
			88	100	82	
			89	(A) ~~10~~ 100	79	
			90	100	75	

(A) PM Q18 3/13/17
(B) Q18 AC 3/14/17 re-count

CETIS Test Data Worksheet

Test Code: 1703-S051 00-3723-6090/2382D7A

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)
Start Date:	10 Mar-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:** Q18 AC 3/14 ~~12B7ADDB~~ 17-0374
End Date:	10 Mar-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.
Sample Date:	09 Mar-17	**Material:**	Facility Effluent	**Sample Station:** M-001 (40 ppt adj)

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

QC: EG

Analyst: AC QA: AC 3/14/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE | **Test Species:** *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted) | **Start Date/Time:** 3/10/2017 1418

Sample Log No.: 17- 0374 | **End Date/Time:** 3/10/2017 1458

Dilutions made by: EC | **Test No:** 1703-5051

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.2	8.04	33.2	15.1
2.5	8.5	8.06	33.4	15.0
5.0	8.5	8.05	33.7	15.0
6.06	8.5	8.05	33.9	14.8
10	8.5	8.05	34.0	14.8
15	8.5	8.05	34.4	14.7

Comments:

QC Check: AC 3/14/17 | **Final Review:** JW 4/10/17

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: IDE **Analyst:** EG

Sample ID: M-001 (40 ppt adjusted) **Test Date:** 3/10/2017

Test No: 1703-S051 **Test Type:** Urchin Fertilization

Salinity of Effluent 62.0

Salinity of Seawater 33.5 **Date of Brine used:** NA

Target Salinity 40.0 **Alkalinity of Brine Control:** NA mg/L as CaCO3

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**3.38**	**-6.15**

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	3.38	338.5	438

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: AC 3/14/17 **Final Review:** JW 4/10/17

Marine Chronic Bioassay | **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: IDE
Sample ID: M-001 40 ppt adjusted
Test No.: 1703-SO51

Start Date/Time: 3/10/2017 / 1418
End Date/Time: 3/10/2017 / 1458
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 2/9/17 & 3/6/17

Tech initials: EH
Injection Time: 1340

Sperm Absorbance at 400 nm: 0.855 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 97, 79, 102, 84, 90 Mean: 90.4 X 50 = 4520 eggs/ml

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

Initial density: 4520 eggs/ml = 1.13 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.13 parts seawater

egg stock 200 ml
seawater 26 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							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1350	50:1	67	33
Eggs Added (0.5 ml):	1400	100:1	96/96	4/4
Test Ended:	1410	200:1	100	0
		—	—	—

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):	1418	QC1	92	8
Eggs Added (0.5 ml):	1438	QC2	85	15
Test Ended:	1458	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 3/14/17

Final Review: JW 4/10/17

ERI Brine

CETIS Summary Report

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

Batch ID:	03-4833-4906	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	10 Mar-17 14:18	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	10 Mar-17 14:58	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	01-9856-0996	**Code:**	17-0373	**Client:**	IDE
Sample Date:	09 Mar-17 10:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	09 Mar-17 12:07	**Source:**	IDE Americas, Inc.		
Sample Age:	28h (3 °C)	**Station:**	ERI		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-9050-2609	Fertilization Rate	10	15	12.25	9.79%	10	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-0099-4507	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
07-0099-4507	Fertilization Rate	Control Resp	0.84	0.7 - NL	Yes	Passes Acceptability Criteria
08-9050-2609	Fertilization Rate	Control Resp	0.84	0.7 - NL	Yes	Passes Acceptability Criteria
08-9050-2609	Fertilization Rate	PMSD	0.09794	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	0.84	0.7591	0.9209	0.77	0.91	0.02915	0.06519	7.76%	0.0%
2.5		5	0.832	0.7798	0.8842	0.77	0.88	0.01881	0.04207	5.06%	0.95%
5		5	0.798	0.7001	0.8959	0.67	0.86	0.03527	0.07887	9.88%	5.0%
6.06		5	0.792	0.7436	0.8404	0.73	0.83	0.01744	0.03899	4.92%	5.71%
10		5	0.766	0.6797	0.8523	0.68	0.85	0.03108	0.0695	9.07%	8.81%
15		5	0.642	0.5803	0.7037	0.58	0.71	0.02223	0.0497	7.74%	23.57%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.91	0.77	0.78	0.84	0.9
2.5		0.86	0.88	0.82	0.77	0.83
5		0.86	0.78	0.82	0.67	0.86
6.06		0.83	0.78	0.73	0.81	0.81
10		0.71	0.8	0.68	0.85	0.79
15		0.61	0.65	0.71	0.66	0.58

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

Analysis ID:	08-9050-2609	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Mar-17 15:08	**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	9.79%	10	15	12.25	10

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.3205	2.362	0.109	8	0.7219	CDF	Non-Significant Effect
		5	1.205	2.362	0.109	8	0.3324	CDF	Non-Significant Effect
		6.06	1.448	2.362	0.109	8	0.2412	CDF	Non-Significant Effect
		10	2.077	2.362	0.109	8	0.0863	CDF	Non-Significant Effect
		15*	5.086	2.362	0.109	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1791018	0.03582037	5	6.682	0.0005	Significant Effect
Error	0.1286484	0.005360348	24			
Total	0.3077502		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.304	15.09	0.6532	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9643	0.9031	0.3971	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	0.84	0.7591	0.9209	0.84	0.77	0.91	0.02915	7.76%	0.0%
2.5		5	0.832	0.7798	0.8842	0.83	0.77	0.88	0.01881	5.06%	0.95%
5		5	0.798	0.7001	0.8959	0.82	0.67	0.86	0.03527	9.88%	5.0%
6.06		5	0.792	0.7436	0.8404	0.81	0.73	0.83	0.01744	4.92%	5.71%
10		5	0.766	0.6797	0.8523	0.79	0.68	0.85	0.03108	9.07%	8.81%
15		5	0.642	0.5803	0.7037	0.65	0.58	0.71	0.02223	7.74%	23.57%

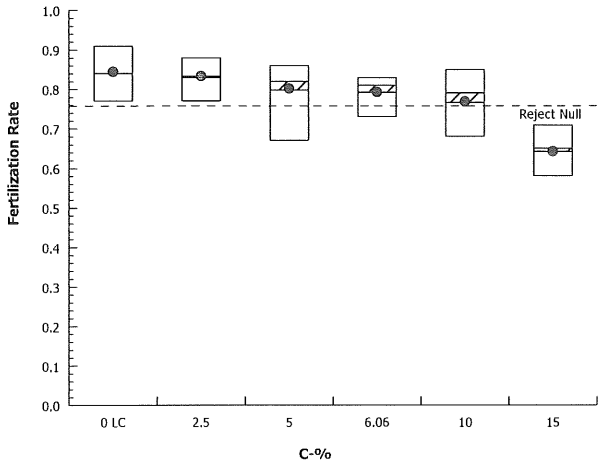
Angular (Corrected) Transformed Summary

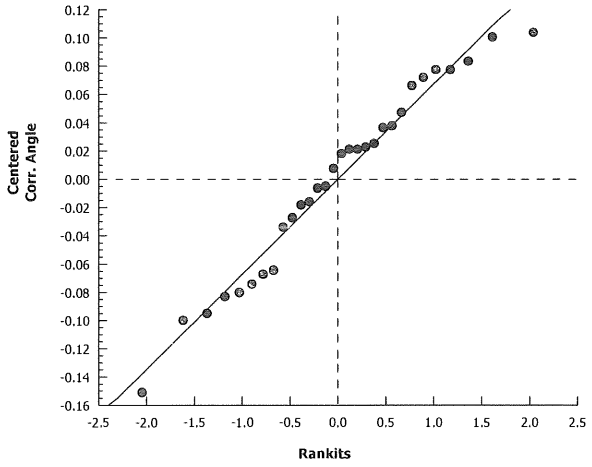
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.166	1.053	1.278	1.159	1.071	1.266	0.04063	7.8%	0.0%
2.5		5	1.151	1.081	1.22	1.146	1.071	1.217	0.02501	4.86%	1.27%
5		5	1.11	0.9918	1.228	1.133	0.9589	1.187	0.04246	8.56%	4.79%
6.06		5	1.098	1.04	1.157	1.12	1.024	1.146	0.02108	4.29%	5.75%
10		5	1.069	0.9667	1.172	1.095	0.9695	1.173	0.03696	7.73%	8.25%
15		5	0.93	0.8653	0.9947	0.9377	0.8657	1.002	0.0233	5.6%	20.2%

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

Analysis ID:	08-9050-2609	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Mar-17 15:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1703-S052 | 11-7976-1138

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

Analysis ID:	07-0099-4507	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Mar-17 15:08	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	657134	1000	Yes	Two-Point Interpolation

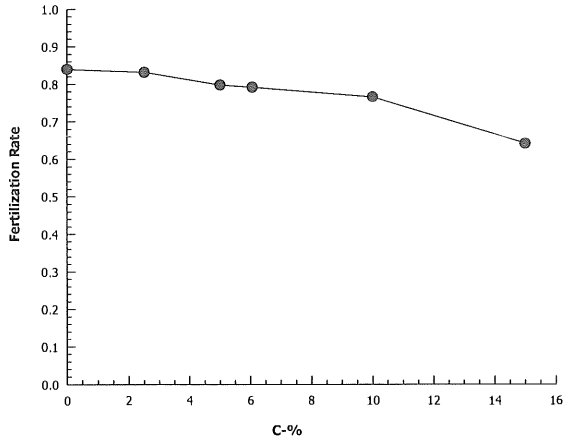
Point Estimates

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	0.84	0.77	0.91	0.02915	0.06519	7.76%	0.0%	420	500
2.5		5	0.832	0.77	0.88	0.01881	0.04207	5.06%	0.95%	416	500
5		5	0.798	0.67	0.86	0.03527	0.07887	9.88%	5.0%	399	500
6.06		5	0.792	0.73	0.83	0.01744	0.03899	4.92%	5.71%	396	500
10		5	0.766	0.68	0.85	0.03108	0.0695	9.07%	8.81%	383	500
15		5	0.642	0.58	0.71	0.02223	0.0497	7.74%	23.57%	321	500

Graphics



Analyst: KS QA: AC 4/13/17

CETIS Analytical Report

TST

Test Code: 1703-S052 | 11-7976-1138

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

Analysis ID:	14-8409-5217	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Mar-17 15:08	**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	6.14%	10	15	12.25	10

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	7.015	1.895	0.075	7	0.0001	CDF	Non-Significant Effect
		5*	4.508	1.895	0.099	7	0.0014	CDF	Non-Significant Effect
		6.06*	6.054	1.895	0.070	7	0.0003	CDF	Non-Significant Effect
		10*	4.075	1.895	0.091	7	0.0024	CDF	Non-Significant Effect
		15	1.457	1.895	0.073	7	0.0942	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1791018	0.03582037	5	6.682	0.0005	Significant Effect
Error	0.1286484	0.005360348	24			
Total	0.3077502		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.304	15.09	0.6532	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9643	0.9031	0.3971	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	0.84	0.7591	0.9209	0.84	0.77	0.91	0.02915	7.76%	0.0%
2.5		5	0.832	0.7798	0.8842	0.83	0.77	0.88	0.01881	5.06%	0.95%
5		5	0.798	0.7001	0.8959	0.82	0.67	0.86	0.03527	9.88%	5.0%
6.06		5	0.792	0.7436	0.8404	0.81	0.73	0.83	0.01744	4.92%	5.71%
10		5	0.766	0.6797	0.8523	0.79	0.68	0.85	0.03108	9.07%	8.81%
15		5	0.642	0.5803	0.7037	0.65	0.58	0.71	0.02223	7.74%	23.57%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.166	1.053	1.278	1.159	1.071	1.266	0.04063	7.8%	0.0%
2.5		5	1.151	1.081	1.22	1.146	1.071	1.217	0.02501	4.86%	1.27%
5		5	1.11	0.9918	1.228	1.133	0.9589	1.187	0.04246	8.56%	4.79%
6.06		5	1.098	1.04	1.157	1.12	1.024	1.146	0.02108	4.29%	5.75%
10		5	1.069	0.9667	1.172	1.095	0.9695	1.173	0.03696	7.73%	8.25%
15		5	0.93	0.8653	0.9947	0.9377	0.8657	1.002	0.0233	5.6%	20.2%

Analyst: KB QA: ACH 3/17

CETIS Test Data Worksheet

Report Date: 09 Mar-17 15:24 (p 1 of 1)
Test Code: 1703-5052-11-7976-1138/4651B9F2

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)
Start Date:	10 Mar-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:** ~~BD5CCE4~~ 17-0373 QIBKB3/23/17
End Date:	10 Mar-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.
Sample Date:	09 Mar-17	**Material:**	Facility Effluent	**Sample Station:** ERI

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			91	100	81	3/17/17
			92	100	68	
			93	100	83	
			94	100	85	
			95	100	66	
			96	100	88	
			97	100	58	
			98	100	84	
			99	100	80	
			100	100	90	
			101	100	83	
			102	100	82	
			103	100	79	
			104	100	71	
			105	100	86	
			106	100	78	
			107	100	77	
			108	100	71	
			109	100	77	
			110	100	65	
			111	100	73	
			112	100	67	
			113	100	86	
			114	100	86	
			115	100	78	
			116	100	81	
			117	100	78	
			118	100	82	
			119	100	91	
			120	100	61	

CETIS Test Data Worksheet

Test Code: 1703-S052 11-7976-1138/4651B9F2

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

Start Date: 10 Mar-17
End Date: 10 Mar-17
Sample Date: 09 Mar-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: ~~BD5CCE4~~ 17-0373 @ 6K33/23/17
Sample Source: IDE Americas, Inc.
Sample Station: ERI

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	119	100	89	DM 3/10/17
0	LC	2	109			
0	LC	3	106			
0	LC	4	98			
0	LC	5	100			
2.5		1	105	100	71	DM 3/10/17
2.5		2	96	100	85	
2.5		3	102			
2.5		4	107			
2.5		5	101			
5		1	113	100	81	DM 3/10/17
5		2	115			
5		3	118			
5		4	112			
5		5	114			
6.06		1	93	100	81	DM 3/10/17
6.06		2	117			
6.06		3	111			
6.06		4	91			
6.06		5	116			
10		1	104	100	73	DM 3/10/17
10		2	99			
10		3	92			
10		4	94			
10		5	103			
15		1	120	100	59	DM 3/10/17
15		2	110			
15		3	108			
15		4	95			
15		5	97			

QC: EG

Analyst: AO QA: AC 3/14/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE | **Test Species:** *S. purpuratus*

Sample ID: ERI | **Start Date/Time:** 3/10/2017 1418

Sample Log No.: 17- 0373 | **End Date/Time:** 3/10/2017 1458

Dilutions made by: EG | **Test No:** 1703-SO52

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.4	8.05	33.1	14.9
2.5	8.4	8.05 3 A	34.2	14.8
5.0	8.5	8.01	34.9	14.5
6.06	8.5	8.00	35.4	14.7
10	8.5	7.98	36.5	14.7
15	8.5	7.95	38.0	14.6

Comments: ~~BAS~~ OH 3/10/17

QC Check: AC 3/14/17 | **Final Review:** KB 3/23/17

Marine Chronic Bioassay | **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: IDE
Sample ID: ERH Brine
Test No.: 1703-S052

Start Date/Time: 3/10/2017 / 1418
End Date/Time: 3/10/2017 / 1458
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 2/9/17 & 3/6/17

Tech initials: EH
Injection Time: 1340

Sperm Absorbance at 400 nm: 0.855 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 97, 79, 102, 84, 90

Mean: 90.4 X 50 = 4520 eggs/ml

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

Initial density: 4520 eggs/ml = 1.13 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.13 parts seawater

egg stock 200 ml
seawater 26 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							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1350	50:1	67	33
Eggs Added (0.5 ml):	1400	100:1	96/96	4/4
Test Ended:	1410	200:1	100	0
		—	—	—

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):	1418	QC1	92	8
Eggs Added (0.5 ml):	1438	QC2	85	15
Test Ended:	1458	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 3/14/17

Final Review: KB 3/23/17

Train 9

CETIS Summary Report

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

Batch ID:	21-2695-8916	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	10 Mar-17 14:18	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	10 Mar-17 14:58	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	02-7513-9133	**Code:**	17-0375	**Client:**	IDE
Sample Date:	09 Mar-17 10:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	09 Mar-17 12:07	**Source:**	IDE Americas, Inc.		
Sample Age:	28h (4 °C)	**Station:**	Train 9		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-8044-1127	Fertilization Rate	5	6.06	5.505	9.51%	20	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
17-4714-8490	Fertilization Rate	EC25	12.35	8.713	15.76	8.1	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
17-4714-8490	Fertilization Rate	Control Resp	0.844	0.7 - NL	Yes	Passes Acceptability Criteria
18-8044-1127	Fertilization Rate	Control Resp	0.844	0.7 - NL	Yes	Passes Acceptability Criteria
18-8044-1127	Fertilization Rate	PMSD	0.09511	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	0.844	0.7803	0.9077	0.76	0.9	0.02293	0.05128	6.08%	0.0%
2.5		5	0.756	0.6662	0.8458	0.64	0.83	0.03234	0.07232	9.57%	10.43%
5		5	0.796	0.7474	0.8446	0.75	0.84	0.01749	0.03912	4.91%	5.69%
6.06		5	0.752	0.6662	0.8378	0.64	0.82	0.03089	0.06907	9.18%	10.9%
10		5	0.694	0.6059	0.7821	0.58	0.77	0.03172	0.07092	10.22%	17.77%
15		5	0.564	0.4882	0.6398	0.5	0.66	0.02731	0.06107	10.83%	33.18%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.86	0.76	0.85	0.85	0.9
2.5		0.64	0.75	0.76	0.8	0.83
5		0.82	0.84	0.81	0.75	0.76
6.06		0.82	0.79	0.64	0.74	0.77
10		0.69	0.7	0.58	0.77	0.73
15		0.53	0.5	0.66	0.55	0.58

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

Analysis ID:	18-8044-1127	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Mar-17 14:40	**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	9.51%	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*	2.495	2.362	0.105	8	0.0381	CDF	Significant Effect
		5	1.455	2.362	0.105	8	0.2389	CDF	Non-Significant Effect
		6.06*	2.609	2.362	0.105	8	0.0301	CDF	Significant Effect
		10*	4.087	2.362	0.105	8	0.0009	CDF	Significant Effect
		15*	7.152	2.362	0.105	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2990081	0.05980162	5	12.09	<0.0001	Significant Effect
Error	0.1187604	0.004948348	24			
Total	0.4177685		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.255	15.09	0.9395	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9403	0.9031	0.0925	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	0.844	0.7803	0.9077	0.85	0.76	0.9	0.02293	6.08%	0.0%
2.5		5	0.756	0.6662	0.8458	0.76	0.64	0.83	0.03234	9.57%	10.43%
5		5	0.796	0.7474	0.8446	0.81	0.75	0.84	0.01749	4.91%	5.69%
6.06		5	0.752	0.6662	0.8378	0.77	0.64	0.82	0.03089	9.18%	10.9%
10		5	0.694	0.6059	0.7821	0.7	0.58	0.77	0.03172	10.22%	17.77%
15		5	0.564	0.4882	0.6398	0.55	0.5	0.66	0.02731	10.83%	33.18%

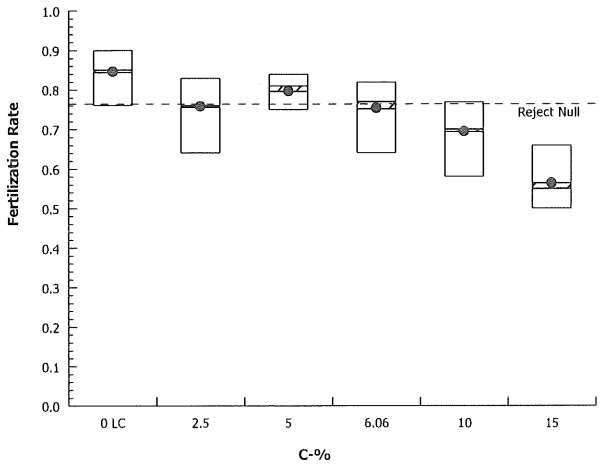
Angular (Corrected) Transformed Summary

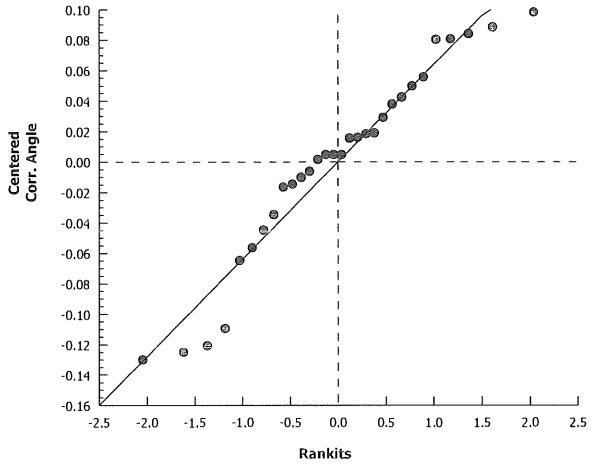
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.168	1.083	1.254	1.173	1.059	1.249	0.03075	5.89%	0.0%
2.5		5	1.057	0.9546	1.16	1.059	0.9273	1.146	0.03696	7.82%	9.5%
5		5	1.104	1.043	1.164	1.12	1.047	1.159	0.02167	4.39%	5.54%
6.06		5	1.052	0.955	1.149	1.071	0.9273	1.133	0.035	7.44%	9.94%
10		5	0.9864	0.892	1.081	0.9912	0.8657	1.071	0.03403	7.71%	15.56%
15		5	0.8501	0.7728	0.9273	0.8355	0.7854	0.9483	0.02782	7.32%	27.24%

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

Analysis ID:	18-8044-1127	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Mar-17 14:40	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	17-4714-8490	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	23 Mar-17 15:22	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1370398	1000	Yes	Two-Point Interpolation

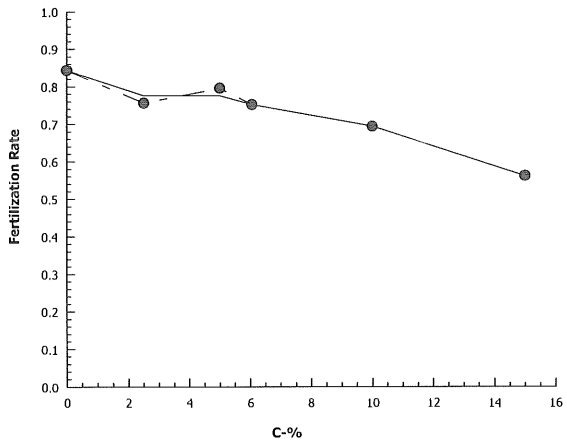
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	12.35	8.713	15.76	8.1	6.346	11.48
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	0.844	0.76	0.9	0.02293	0.05128	6.08%	0.0%	422	500
2.5		5	0.756	0.64	0.83	0.03234	0.07232	9.57%	10.43%	378	500
5		5	0.796	0.75	0.84	0.01749	0.03912	4.91%	5.69%	398	500
6.06		5	0.752	0.64	0.82	0.03089	0.06907	9.18%	10.9%	376	500
10		5	0.694	0.58	0.77	0.03172	0.07092	10.22%	17.77%	347	500
15		5	0.564	0.5	0.66	0.02731	0.06107	10.83%	33.18%	281	500

Graphics



CETIS Analytical Report

TST

Report Date: 23 Mar-17 15:22 (p 1 of 1)
Test Code: 1703-S053 | 16-3100-1862

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

Analysis ID: 07-7155-2601 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 23 Mar-17 15:21 **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	5.91%	10	15	12.25	10

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	4.156	1.943	0.085	6	0.0030	CDF	Non-Significant Effect
		5*	7.184	1.895	0.06	7	<0.0001	CDF	Non-Significant Effect
		6.06*	4.199	1.943	0.081	6	0.0028	CDF	Non-Significant Effect
		10*	2.682	1.895	0.078	7	0.0157	CDF	Non-Significant Effect
		15	-0.7234	1.895	0.068	7	0.7536	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2990081	0.05980162	5	12.09	<0.0001	Significant Effect
Error	0.1187604	0.004948348	24			
Total	0.4177685		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.255	15.09	0.9395	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9403	0.9031	0.0925	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	0.844	0.7803	0.9077	0.85	0.76	0.9	0.02293	6.08%	0.0%
2.5		5	0.756	0.6662	0.8458	0.76	0.64	0.83	0.03234	9.57%	10.43%
5		5	0.796	0.7474	0.8446	0.81	0.75	0.84	0.01749	4.91%	5.69%
6.06		5	0.752	0.6662	0.8378	0.77	0.64	0.82	0.03089	9.18%	10.9%
10		5	0.694	0.6059	0.7821	0.7	0.58	0.77	0.03172	10.22%	17.77%
15		5	0.564	0.4882	0.6398	0.55	0.5	0.66	0.02731	10.83%	33.18%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.168	1.083	1.254	1.173	1.059	1.249	0.03075	5.89%	0.0%
2.5		5	1.057	0.9546	1.16	1.059	0.9273	1.146	0.03696	7.82%	9.5%
5		5	1.104	1.043	1.164	1.12	1.047	1.159	0.02167	4.39%	5.54%
6.06		5	1.052	0.955	1.149	1.071	0.9273	1.133	0.035	7.44%	9.94%
10		5	0.9864	0.892	1.081	0.9912	0.8657	1.071	0.03403	7.71%	15.56%
15		5	0.8501	0.7728	0.9273	0.8355	0.7854	0.9483	0.02782	7.32%	27.24%

CETIS Test Data Worksheet

Test Code: 1703-3053 16-3100-1862/61371D06

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)
Start Date: 10 Mar-17	**Species:** Strongylocentrotus purpuratus	**Sample Code:** ~~10664A3D~~ 17-0375	
End Date: 10 Mar-17	**Protocol:** EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.	
Sample Date: 09 Mar-17	**Material:** Facility Effluent	**Sample Station:** Train 9	

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			121	100	81	3/16/17
			122	100	90	
			123	100	70	
			124	100	83	
			125	100	55	
			126	100	79	
			127	100	80	
			128	100	82	
			129	100	75	
			130	100	86	
			131	100	66	
			132	100	84	
			133	100	77	
			134	100	85	
			135	100	64	
			136	100	64	
			137	100	58	
			138	100	82	
			139	100	74	
			140	100	76	
			141	100	53	
			142	100	76	
			143	100	77	
			144	100	58	
			145	100	75	
			146	100	85	
			147	100	76	
			148	100	73	
			149	100	69	
			150	100	50	

CETIS Test Data Worksheet

Test Code: 1703-SOS3 16-3100-1862/61371D06

Echinoid Sperm Cell Fertilization Test 15C				Nautilus Environmental (CA)
Start Date:	10 Mar-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:** @~~10664A3D~~ 0375
End Date:	10 Mar-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.
Sample Date:	09 Mar-17	**Material:**	Facility Effluent	**Sample Station:** Train 9

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	130	100	87	
0	LC	2	140			
0	LC	3	146			
0	LC	4	134			
0	LC	5	122			
2.5		1	136	100	67	
2.5		2	145			
2.5		3	142			
2.5		4	127			
2.5		5	124			
5		1	138	100	73	
5		2	132			
5		3	121			
5		4	129			
5		5	147			
6.06		1	128	100	82	AG 3/11/17
6.06		2	126	100	79	AG 3/11/17
6.06		3	135			
6.06		4	139			
6.06		5	143			
10		1	149	100	72	AG 3/11/17
10		2	123			
10		3	144			
10		4	133			
10		5	148			
15		1	141			
15		2	150			
15		3	131			
15		4	125			
15		5	137	100	52	AG 3/11/17

QC: E01

@ Q18 AC 3/14/17

Analyst: AG QA: AC 3/14/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE | **Test Species:** *S. purpuratus*

Sample ID: Train 9 | **Start Date/Time:** 3/10/2017 1418

Sample Log No.: 17- 0375 | **End Date/Time:** 3/10/2017 1458

Dilutions made by: EH | **Test No:** 1703-S053

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.3	8.04	33.1	15.4
2.5	8.3	8.03	34.2	14.9
5.0	8.4	8.01	35.0	14.8
6.06	8.4	8.00	35.3	14.8
10	8.5	7.98	36.6	14.8
15	8.5	7.94	38.1	14.7

Comments:

QC Check: AC 3/14/17 | **Final Review:** KB 3/21/17

Marine Chronic Bioassay | **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: IDE
Sample ID: Train 9
Test No.: 1703-S053

Start Date/Time: 3/10/2017 / 1418
End Date/Time: 3/10/2017 / 1458
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 2/9/17 & 3/6/17

Tech initials: EH
Injection Time: 1340

Sperm Absorbance at 400 nm: 0.855 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 97, 79, 102, 84, 90 Mean: 90.4 X 50 = 4520 eggs/ml

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

Initial density: 4520 eggs/ml = 1.13 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.13 parts seawater

egg stock 200 ml
seawater 26 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							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1350	50:1	67	33
Eggs Added (0.5 ml):	1400	100:1	96/96	4/4
Test Ended:	1410	200:1	100	0
		—	—	—

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):	1418	QC1	92	8
Eggs Added (0.5 ml):	1438	QC2	85	15
Test Ended:	1458	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 3/14/17

Final Review: KB 3/21/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: ERI, M-001, Train 9
Test ID No(s).: 1703-S050 to S053

Sample (A, B, C):	ERI Brine	M-001	Train 9	
Log-in No. (17-xxxx):	0373	0374	0375	
Sample Collection Date & Time:	3/9/17 1000	3/9/17 1000	3/9/17 1000	
Sample Receipt Date & Time:	3/9/17 1207	3/9/17 1207	3/9/17 1207	
Number of Containers & Container Type:	1, 4L cubi	2, 4L cubi	1, 4L cubi	
Approx. Total Volume Received (L):	4L	~6 L	4L	
Check-in Temperature (°C)	3.0	4.0	4.0	
Temperature OK? [1]	(Y) N	(Y) N	(Y) N	Y N
DO (mg/L)	7.8	7.6	7.9	
pH (units)	7.46	7.92	7.45	
Conductivity (µS/cm)	—	—	—	
Salinity (ppt)	63.4	62.0	64.4	
Alkalinity (mg/L) [2]	216	216	219	
Hardness (mg/L) [2, 3]	—	—	—	
Total Chlorine (mg/L)	0.03	<0.02	<0.02	
Technician Initials	EG	EG	EG	

Test Performed: Urchin Fert- **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 121 Hardness or (Salinity): 34 ppt
Additional Control? Y (N) = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART Other: _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as $CaCO_3$, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: _____

Sample Check-In Information

Sample Description:
ERI: colorless, clear, no odor, no debris
M001: colorless, clear, no odor, no debris
Train 9: colorless, clear, no odor, no debris

COC Complete (Y/N)?
A Y B____ C____

Filtration? Y (N)
Pore Size: _____
Organisms or Debris

Salinity Adjustment? (Y) N 418 AC 3/14/17
Test: Urchin fert- Source: SW 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:			

Cl_2 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 pH10 treatment M-001
Tech Initials A____ B____ C____

QC Check: AC 3/14/17
Final Review: [illegible] 4/10/17

Appendix C

Chain-of-Custody Form

IDE Technologies

CDP laoratory:_____
Entahlpy Laboratory:____
WECK Laboratory:_____
Nautilus:____X_____
AIM:_____
Other:_____

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

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

Special instruction: Samples collected during plant operation at 40-40 MGD. M-001 is to be run undiluted and 40 ppt adjusted. Undiluted M-001 sample is also to be treated with pH adjustment/filtration. Sample collected to fulfill monthly chronic ~~and quarterly acute~~ @ requirements. VH 3/9/2017

Glass=G Plastic=P

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

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

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization	Acute @						OC	NOTES:
ERI Brine (17- 1227)	3/08-09/17	10:00	24 HR COMP -B	N	4L CUBIE	X							3.0	TDS - 61.49 ppm EC - 85.32 mS/cm
M-001 (17- 1228)	3/08-09/17	10:00	24 HR COMP -B	N	4L CUBIE (1 L extra)	X	X						4.0	TDS - 60.26 ppm EC - 84.02 mS/cm
TRAIN 9 (17-1229)	3/08-09/17	10:00	24 HR COMP -B	N	4L CUBIE	X							4.0	TDS - 62.16 ppm EC - 86.12 mS/cm

Relinquished By:	Date:	Time:		Received By:	Time:	Sample Condition Upon Receipt:
[signature]	3/9/17	1100		[signature] 3/9/17	1100	☐ Iced ☐ Ambient or _____ °C
[signature]	3/9/17	12:07		[signature]	1207	☐ Iced ☐ Ambient or _____ °C

Nautilus ID: 17-0373 to 17-0375

@ Acute test not required, analysis canceled per client email.

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	11-3277-5320	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	10 Mar-17 14:18	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	10 Mar-17 14:58	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	16-9325-7535	**Code:**	170310sprt	**Client:**	Internal
Sample Date:	10 Mar-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	10 Mar-17	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-1387-0350	Fertilization Rate	<10	10	NA	8.97%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
05-9725-9024	Fertilization Rate	EC50	28.71	27.31	30.19		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
05-1387-0350	Fertilization Rate	Control Resp	0.85	0.7 - NL	Yes	Passes Acceptability Criteria
05-9725-9024	Fertilization Rate	Control Resp	0.85	0.7 - NL	Yes	Passes Acceptability Criteria
05-1387-0350	Fertilization Rate	PMSD	0.08965	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	0.85	0.7966	0.9034	0.79	0.9	0.01924	0.04301	5.06%	0.0%
10		5	0.766	0.7252	0.8068	0.73	0.8	0.0147	0.03286	4.29%	9.88%
20		5	0.618	0.5037	0.7323	0.55	0.75	0.04116	0.09203	14.89%	27.29%
40		5	0.276	0.1911	0.3609	0.19	0.36	0.03059	0.06841	24.79%	67.53%
80		5	0	0	0	0	0	0	0		100.0%
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	0.88	0.85	0.79	0.83	0.9
10		0.76	0.8	0.73	0.8	0.74
20		0.55	0.55	0.68	0.56	0.75
40		0.19	0.33	0.25	0.25	0.36
80		0	0	0	0	0
160		0	0	0	0	0

CETIS Analytical Report

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

Analysis ID:	05-1387-0350	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Mar-17 14:38	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	8.97%	<10	10	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	2.415	2.227	0.101	8	0.0352	CDF	Significant Effect
		20*	5.949	2.227	0.101	8	<0.0001	CDF	Significant Effect
		40*	13.81	2.227	0.101	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.117273	0.3724242	3	72.7	<0.0001	Significant Effect
Error	0.08196166	0.005122604	16			
Total	1.199234		19			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.908	11.34	0.4060	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9555	0.866	0.4584	Normal Distribution

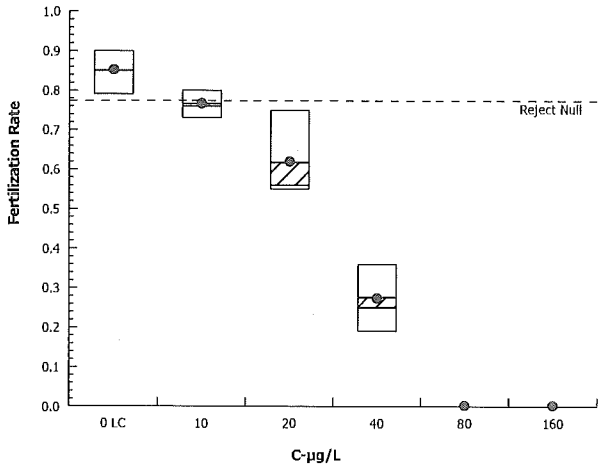
Fertilization Rate Summary

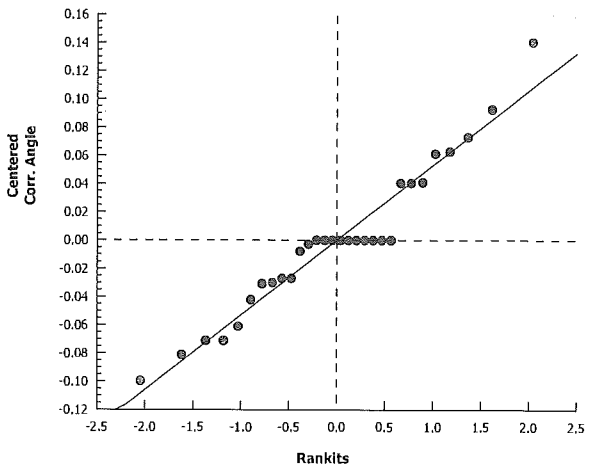
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.85	0.7966	0.9034	0.85	0.79	0.9	0.01924	5.06%	0.0%
10		5	0.766	0.7252	0.8068	0.76	0.73	0.8	0.0147	4.29%	9.88%
20		5	0.618	0.5037	0.7323	0.56	0.55	0.75	0.04116	14.89%	27.29%
40		5	0.276	0.1911	0.3609	0.25	0.19	0.36	0.03059	24.79%	67.53%
80		5	0	0	0	0	0	0	0		100.0%
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	1.176	1.101	1.251	1.173	1.095	1.249	0.02696	5.13%	0.0%
10		5	1.067	1.018	1.115	1.059	1.024	1.107	0.01744	3.66%	9.3%
20		5	0.9066	0.7863	1.027	0.8455	0.8355	1.047	0.04334	10.69%	22.9%
40		5	0.5507	0.455	0.6464	0.5236	0.451	0.6435	0.03447	14.0%	53.17%
80		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.75%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	95.75%

Graphics





CETIS Analytical Report

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

Analysis ID:	05-9725-9024	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	21 Mar-17 14:38	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

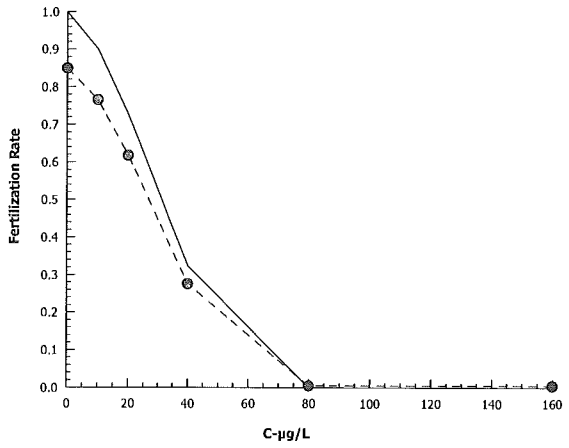
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.15	9.88%	1.458	0.01088	28.71	27.31	30.19

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	0.85	0.79	0.9	0.01924	0.04301	5.06%	0.0%	425	500
10		5	0.766	0.73	0.8	0.0147	0.03286	4.29%	9.88%	383	500
20		5	0.618	0.55	0.75	0.04116	0.09203	14.89%	27.29%	309	500
40		5	0.276	0.19	0.36	0.03059	0.06841	24.79%	67.53%	138	500
80		5	0	0	0	0	0		100.0%	0	500
160		5	0	0	0	0	0		100.0%	0	500

Graphics

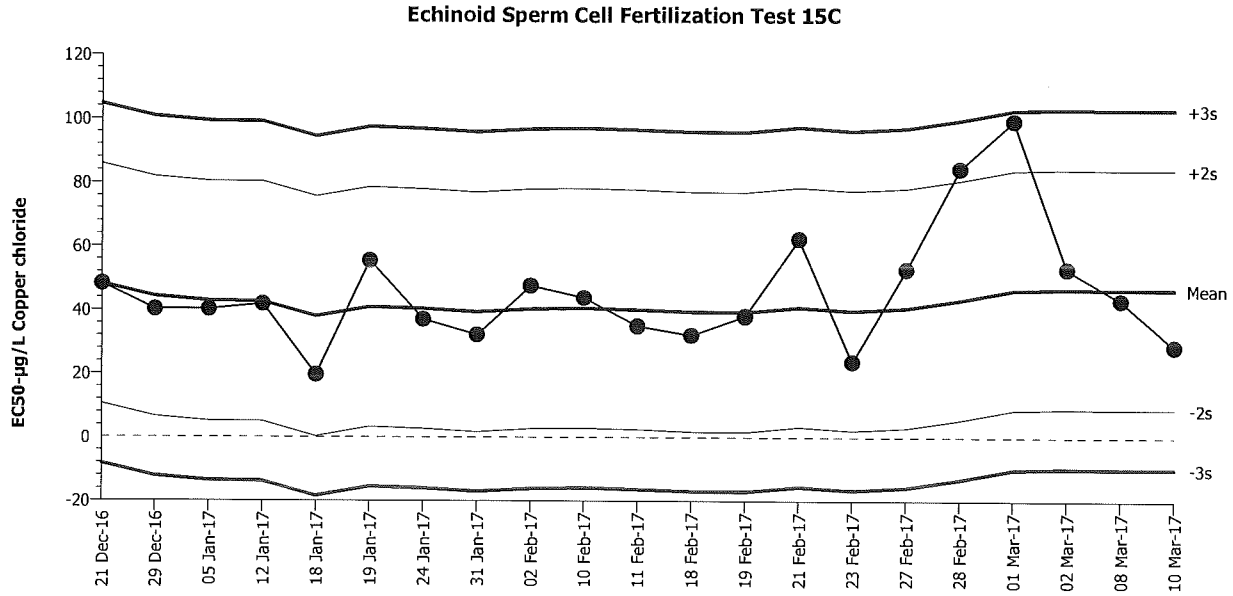


Analyst: KB QA: KTP 4/6/17

CETIS QC Plot

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



Mean:	46.58	**Count:**	20	**-2s Warning Limit:**	8.917	**-3s Action Limit:**	-9.913
Sigma:	18.83	**CV:**	40.40%	**+2s Warning Limit:**	84.24	**+3s Action Limit:**	103.1

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2016	Dec	21	12:12	48.26	1.684	0.08942			14-5051-2365	16-1479-8388
2			29	16:22	40.16	-6.417	-0.3408			17-0784-9661	08-0208-3856
3	2017	Jan	5	14:34	40.21	-6.368	-0.3382			04-1406-8806	15-3393-3643
4			12	17:54	41.95	-4.631	-0.246			14-8351-4083	12-3796-8723
5			18	15:19	19.65	-26.93	-1.43			08-8914-3626	00-6318-6085
6			19	18:19	55.59	9.012	0.4786			09-5789-7052	01-7604-4546
7			24	15:37	37.1	-9.484	-0.5036			18-0430-6783	19-8873-5804
8			31	15:00	32.26	-14.32	-0.7605			14-3391-7268	12-6240-4784
9		Feb	2	16:50	47.66	1.082	0.05748			10-8641-2413	14-8698-1832
10			10	15:05	43.85	-2.733	-0.1451			18-1100-4857	17-9587-0468
11			11	13:32	35.02	-11.56	-0.6137			21-3415-8415	20-8117-2853
12			18	14:43	32.15	-14.43	-0.7662			15-8602-9109	03-0004-4079
13			19	16:00	38.18	-8.404	-0.4463			04-9561-8356	16-1145-1366
14			21	11:42	62.44	15.86	0.8423			15-6576-1294	19-2980-3814
15			23	14:42	23.77	-22.81	-1.211			07-0628-7264	20-4334-6940
16			27	16:05	52.9	6.319	0.3356			10-1635-1121	14-4530-4128
17			28	18:00	84.51	37.93	2.014	(+)		09-8043-1931	05-2317-8363
18		Mar	1	15:45	99.56	52.98	2.814	(+)		17-5791-9592	08-2085-2833
19			2	15:26	53.09	6.514	0.3459			20-3729-5626	20-9062-5332
20			8	12:50	43.2	-3.376	-0.1793			10-8438-6969	12-4014-5220
21			10	14:18	28.71	-17.87	-0.9489			05-2038-2100	05-9725-9024

Analyst: KB QA: KFP 4/6/17

CETIS Test Data Worksheet

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

Start Date:	10 Mar-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:**	170310sprt
End Date:	10 Mar-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	Reference Toxicant
Sample Date:	10 Mar-17	**Material:**	Copper chloride	**Sample Station:**	Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	3/17/17
			2	100	73	
			3	100	80	
			4	100	33	
			5	100	19	
			6	100	68	
			7	100	56	
			8	100	0	
			9	100	25	
			10	100	0	
			11	100	0	
			12	100	0	
			13	100	88	
			14	100	25	
			15	100	~~88~~ 80 Ⓐ	
			16	100	79	
			17	100	85	
			18	100	74	
			19	100	36	
			20	100	76	
			21	100	83	
			22	100	55	
			23	100	0	
			24	100	0	
			25	100	0	
			26	100	55	
			27	100	0	
			28	100	75	
			29	100	90	
			30	100	80	

Ⓐ Q18 DM 3/17/17

CETIS Test Data Worksheet

Report Date: 09 Mar-17 15:29 (p 1 of 1)
Test Code: 05-2038-2100/170310sprt

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

Start Date: 10 Mar-17
End Date: 10 Mar-17
Sample Date: 10 Mar-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 170310sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	13	100	94	EG 3/10/17
0	LC	2	17			
0	LC	3	16			
0	LC	4	21			
0	LC	5	29			
10		1	20			
10		2	30			
10		3	2			
10		4	3	100	90	EG
10		5	18			
20		1	22			
20		2	26			
20		3	6			
20		4	7	100	55	EG
20		5	28			
40		1	5			
40		2	4			
40		3	14			
40		4	9	100	36	EG
40		5	19			
80		1	25			
80		2	24			
80		3	10			
80		4	23			
80		5	27	100	1	EG
160		1	15			
160		2	8			
160		3	12	100	0	EG
160		4	1			
160		5	11			

QC: EG

Marine Chronic Bioassay | **Water Quality Measurements**

Client : Internal | **Test Species:** *S. purpuratus*

Sample ID: $CuCl_2$ | **Start Date/Time:** 3/10/2017 1418

Test No: 170310sprt | **End Date/Time:** 3/10/2017 1458

Dilutions made by: EG

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

Analyst: AD

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.3	8.04	33.0	14.8
10	8.2	8.00	33.4	14.8
20	8.2	8.00	33.4	14.6
40	83	7.98	33.3	14.6
80	8.4	8.01	33.2	14.7
160	8.4	8.02	33.0	14.6

Comments:

QC Check: AC 3/14/17 | **Final Review:** KB 3/21/17

Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

Marine Chronic Bioassay **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Internal
Sample ID: CuCl2
Test No.: 170310 SPF

Tech initials: EG
Injection Time: 1340

Start Date/Time: 3/10/2017 / 1418
End Date/Time: 3/10/2017 / 1458
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 2/9/17 & 3/6/17

Sperm Absorbance at 400 nm: 0.855 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 97, 79, 102, 84, 90 Mean: 90.4 X 50 = 4520 eggs/ml

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

Initial density: 4520 eggs/ml = 1.13 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.13 parts seawater

egg stock 200 ml
seawater 26 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							
Rangefinder Test:	**2000:1**	**1600:1**	**1200:1**	**800:1**	**400:1**	**200:1**	**100:1**	**50:1**
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1350	50:1	67	33
Eggs Added (0.5 ml):	1400	100:1	96/96	4/4
Test Ended:	1410	200:1	100	0
		—	—	—

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):	1418	QC1	92	8
Eggs Added (0.5 ml):	1438	QC2	85	15
Test Ended:	1458	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 3/14/17 Final Review: KB 3/21/17

Appendix E

Qualifier Codes

Nautilus Environmental

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 ≤ 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.