Nautilus Environmental

Chronic Toxicity Test Results of the AEF 330 Polymer Spiking Study for the Carlsbad Desalination Plant

Test Initiation Date: December 15, 2017

Prepared for: **IDE AMERICAS, Inc.**
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Prepared by: **Nautilus Environmental**

Submitted: **January 8, 2018**

Data Quality Assurance:

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

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Results verified by: Adrienne Cibor

INTRODUCTION AND BACKGROUND

In January 2015, the Nautilus Environmental (Nautilus) laboratory in San Diego, California began performing chronic monthly toxicity screening tests of the M-001 effluent sample for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) according to Order No. R9-2006-0065 using the purple urchin (*Strongylocentrotus purpuratus*) fertilization test. In February 2016, a Toxicity Identification Evaluation (TIE) was performed as part of the CDP Toxicity Reduction Evaluation (TRE). This TIE was able to isolate and remove toxicity detected in the baseline sample, to varying degrees with several treatments (solid-phase extraction, 0.45 μm filtration, and pH 10/filtration), all of which have a physical filtration component.

Following recommendations made in the February 2016 TIE progress report (Nautilus, March 2016) and as part of the ongoing TIE and TRE efforts, a series of tests were conducted to evaluate facility process chemicals for the potential to cause adverse effects in the urchin fertilization test. Concentrations tested were provided by plant operators at IDE AMERICAS, Inc. (IDE) to represent a potential range of each product that might be present in the final M-001 effluent sample. Results for the AEF 330 PWG polymer are provided in this report; results for the other products are reported separately.

MATERIALS AND METHODS

IDE personnel collected a subsample of the polymer product labeled AEF 330 PWG in a 100-milliliter (mL) high-density plastic bottle and delivered it to Nautilus on December 7, 2017. The sample was stored in the dark at room temperature at Nautilus until used for testing. The study was performed by adding polymer product into seawater collected from the plant influent location (M-INF). The M-INF sample used for this test was collected on December 7, 2017 and was hand delivered to Nautilus the same day as collection. The M-INF sample was collected in 4-liter (L) low-density polyethylene (LDPE) cubitainers, and was held in insulated ice chests containing wet ice during transport. Appropriate chain-of-custody procedures were followed during all phases of this study.

Immediately upon arrival at Nautilus, an aliquot of the M-INF sample was drawn to measure water quality parameters including pH, dissolved oxygen (DO), salinity, temperature, alkalinity, and total chlorine. The sample was then stored at 4 ± 2°C in the dark until used for testing.

SAMPLE PREPARATION

On the day of test initiation, 1 mL of the AEF 330 PWG was added to 999 mL of deionized water (DI) to create a stock solution of 898 milligrams (mg) per liter based on the chemical specific gravity of 0.898 grams per milliliter (g/mL) measured for the product. The product was soluble in water; therefore, no solvent was used. This solution was allowed to mix for a period of one hour on a magnetic stir plate to ensure complete dissolution of the product. This 898 mg/L stock was used to prepare the spike M-INF samples.

Based on data provided by IDE, five discrete aliquots of the influent sample were tested at concentrations of 0, 0.1, 0.5, 1.0, and 5.0 mg/L of polymer. Spiked solutions were prepared in volumetric flasks and then transferred to glass beakers containing Teflon coated magnetic stir bars; each solution was allowed to mix gently for one hour to create a homogenous mixture. Following mixing, each of these five solutions was then treated as a discrete sample and tested with the same dilution series as the M-001 effluent (lab control, 2.5, 5.0, 6.06, 10, and 15 percent sample). Dilutions of each spiked sample were prepared with standard lab control seawater used at Nautilus, which is natural seawater obtained from Scripps Institution of Oceanography (SIO). In the M-INF dilution series with no polymer added (zero [0] spike), the M-INF sample was also tested undiluted to ensure that no adverse effects to urchin fertilization observed could be attributed to the influent water quality. Since dilutions were prepared with M-INF and SIO seawater, all test concentrations were at ambient seawater salinity.

BIOASSAY TEST METHODS

All testing was performed using procedures published in the U.S. Environmental Protection Agency (USEPA) guidance document: “Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms” (USEPA 1995). All samples included in this report were tested using this same bioassay procedure and test dilution series. A summary of urchin fertilization test methods is summarized in Table 1.

Table 1. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	12/15/17, 15:06 to 15:46
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: SIO inlet), 34 ± 2 parts per thousand (ppt); 20-micrometer (µm) filtered
Sample Spike Concentrations:	0 (no spike), 0.1, 0.5, 1.0, and 5.0 mg/L AEF 330 PWG polymer spiked in M-INF seawater
Dilution Series:	Lab control, 2.5, 5.0, 6.06, 10, and 15 percent of each spiked influent sample
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each bioassay with a preliminary range-finding 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
Randomization:	Each sample was tested with all replicates and control randomized per USEPA protocol
Test Type:	Fertilization; 20-min sperm exposure to sample followed by a 20-min fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25%
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

Statistical analyses were conducted using EPA flowchart specifications as outlined in the test guidance manual (USEPA 1995). Organism performance in each sample dilution series was compared to that observed in its own laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TU_c) values.

For comparison, 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). 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 these tests, the TST analysis was performed comparing each individual concentration to the lab control.

Results and Discussion

There were no statistically significant effects to urchin fertilization observed in the M-INF test with no polymer spiked into the sample, or in the M-INF sample dilutions of M-INF spiked with 0.1, 0.5, and 1.0 mg/L of polymer (Figure 1, a, b, c, and d). The resulting NOEC for these four tests was 15 percent, the highest concentration tested. This resulted in a TU_c value of less than 6.67 for each test, indicating that the polymer product AEF 330 PWG is not likely to cause an adverse effect to urchin fertilization in the effluent concentrations tested, if present at or below 1.0 mg/L in the M-001 effluent.

A statistically significant decrease in urchin egg fertilization was observed in all but the 2.5 percent test concentration of the 5.0 mg/L-spiked sample (Figure 1, e). This translates to a TU_c value of 40 for the 5.0 mg/L spiked M-INF sample. Using the TST calculation, the 10 and 15 percent concentrations of the 5.0 mg/L spiked M-INF were statistically significant.

These results indicate that the polymer product AEF 330 PWG has potential to cause an adverse effect in the urchin fertilization test if present in the final effluent at the higher end of the range of concentrations tested. Further testing of the spiked M-INF at concentrations between 1.0 and 5.0 mg/L would be helpful to determine effective concentrations between these two doses. Additionally, testing of these concentrations of the polymer followed by the pH 10 with filtration treatment could confirm whether the observed effects are removed by the same TIE treatment that has consistently reduced effects observed in previous M-001 effluent samples.

All raw data and statistical analyses are presented in Appendix A. Sample receipt information and chain of custody forms can be found in Appendices B and C, respectively.

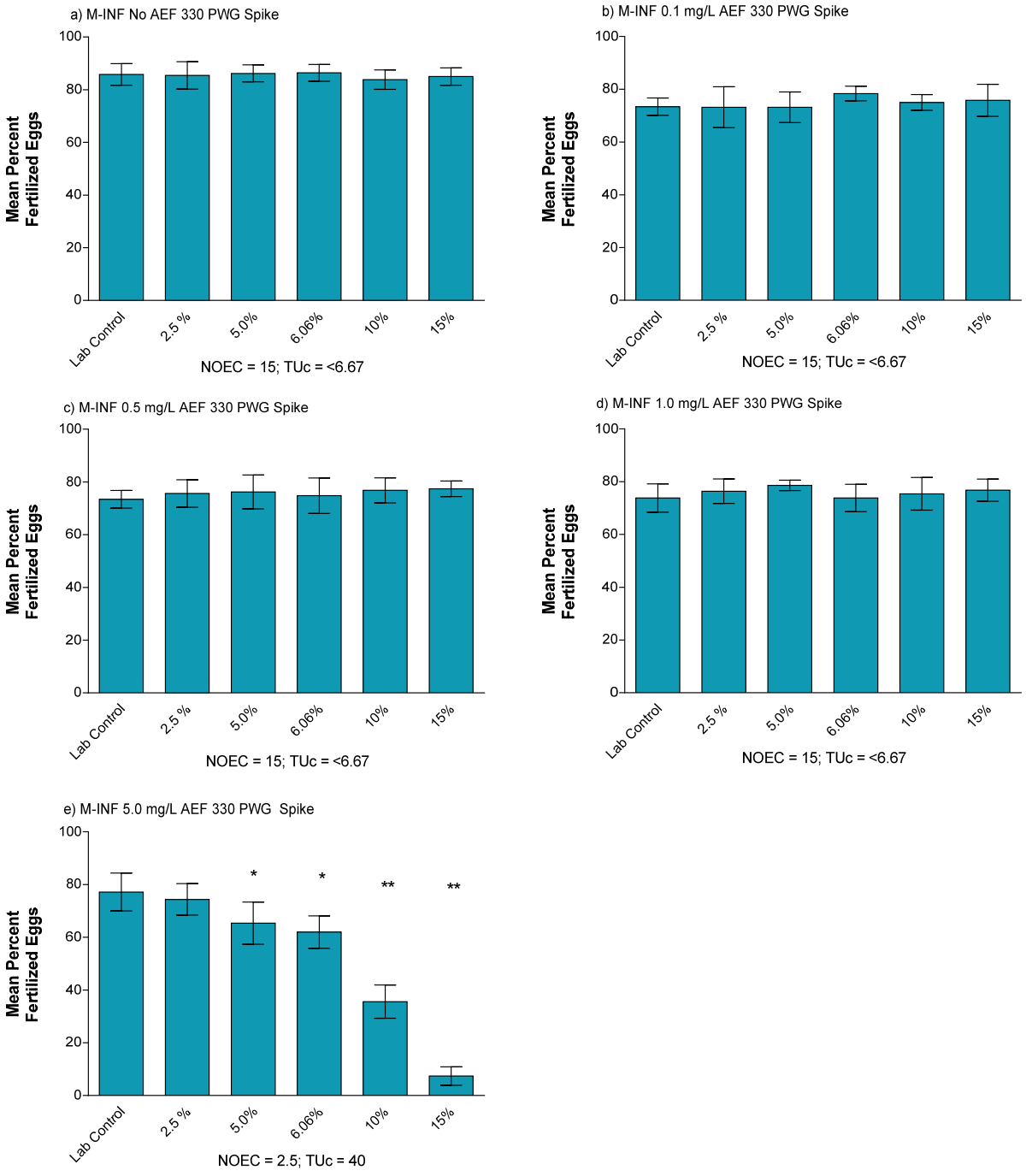


Figure 1. Urchin egg fertilization results of the polymer product AEF 330 PWG spiking study conducted on December 15, 2017 (mean ± standard deviation). *An asterisk indicates a statistically significant decrease relative to the concurrent lab control using EPA 1995 flowchart statistical methods. **Two asterisks indicate a significant reduction in egg fertilization with both the EPA 1995 and the TST statistical methods.

QUALITY ASSURANCE

The laboratory controls all met the minimum acceptability criteria as set by USEPA, as well as all internal QA Program requirements. The PMSD values, which are a measure of test variability, were within the acceptable range. As this was a special study using receiving water, the 36-hour holding time for effluent samples does not apply. Therefore, all test results were deemed valid. Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations and TU_c values are deemed reliable. 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 2 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. Additionally, 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 2. Reference Toxicant Test Results

Test Species	Endpoint	EC_{50} (µg/L Copper)	Historical Mean EC_{50} ±2 SD (µg/L Copper)	CV (%)
Purple Urchin	Egg Fertilization	26.0	47.3 ± 29.2	30.9

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

Nautilus Environmental 2016. Toxicity Identification Evaluation (TIE) Progress Report for the Carlsbad Desalination Plant (CDP) – February through March 2016. March 18, 2016.

Tidepool Scientific Software 2000–2013. CETIS Comprehensive Environmental Toxicity Information System Analysis and Database 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. 1992. Toxicity Identification Evaluation - Characterization of Chronically Toxic Effluents, Phase I. EPA/600/6-91/005F May 1992.

USEPA. 1993. Methods for Aquatic Toxicity Identification Evaluations - Phase II Toxicity Identification Procedures for Samples Exhibiting Acute and Chronic Toxicity. EPA/600/R-92/080 September 1993.

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. 1996. Marine Toxicity Identification Evaluation (TIE) Phase I Guidance Document EPA/600/R-96/054.

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

Appendix A

Raw Data and Statistical Analyses

M-INF Sample, 0 Spike

CETIS Summary Report

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

Batch ID:	04-6233-5140	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 15:06	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 15:46	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	13-1543-2842	**Code:**	AEF 330 PWG 0 17-1249	**Client:**	IDE
Sample Date:	07 Dec-17 10:00	**Material:**	Product Testing	**Project:**	Spiking Study
Receive Date:	07 Dec-17 12:08	**Source:**	IDE Americas, Inc.		
Sample Age:	8d 5h	**Station:**	M-INF 0 Spike		

Sample Note: 0 spike

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-1236-3729	Fertilization Rate	<100	100	NA	4.76%	>1	Equal Variance t Two-Sample Test
15-1068-9584	Fertilization Rate	15	>15	NA	6.98%	<6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
06-8315-5458	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
06-8315-5458	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
15-1068-9584	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
18-1236-3729	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
15-1068-9584	Fertilization Rate	PMSD	0.06985	NL - 0.25	No	Passes Acceptability Criteria
18-1236-3729	Fertilization Rate	PMSD	0.04757	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	0.858	0.8065	0.9095	0.82	0.92	0.01855	0.04147	4.83%	0.0%
2.5		5	0.854	0.7897	0.9183	0.79	0.91	0.02315	0.05177	6.06%	0.47%
5		5	0.862	0.8223	0.9017	0.82	0.9	0.01428	0.03194	3.71%	-0.47%
6.06		5	0.864	0.8242	0.9038	0.84	0.92	0.01435	0.03209	3.72%	-0.7%
10		5	0.838	0.792	0.884	0.78	0.87	0.01655	0.03701	4.42%	2.33%
15		5	0.85	0.8088	0.8912	0.8	0.88	0.01483	0.03317	3.9%	0.93%
100		5	0.794	0.7616	0.8264	0.77	0.83	0.01166	0.02608	3.28%	7.46%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.92	0.88	0.84	0.82	0.83
2.5		0.81	0.79	0.91	0.89	0.87
5		0.88	0.87	0.82	0.84	0.9
6.06		0.85	0.85	0.86	0.92	0.84
10		0.87	0.83	0.87	0.78	0.84
15		0.85	0.8	0.88	0.84	0.88
100		0.81	0.83	0.79	0.77	0.77

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

Analysis ID:	15-1068-9584	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:28	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Sample Note: 0 spike

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	6.98%	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	0.1339	2.362	0.083	8	0.7907	CDF	Non-Significant Effect
		5	-0.1202	2.362	0.083	8	0.8664	CDF	Non-Significant Effect
		6.06	-0.2161	2.362	0.083	8	0.8893	CDF	Non-Significant Effect
		10	0.838	2.362	0.083	8	0.4935	CDF	Non-Significant Effect
		15	0.3709	2.362	0.083	8	0.7016	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00463414	0.0009268279	5	0.3	0.9080	Non-Significant Effect
Error	0.07414252	0.003089271	24			
Total	0.07877666		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.415	15.09	0.9227	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9723	0.9031	0.6026	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	0.858	0.8065	0.9095	0.84	0.82	0.92	0.01855	4.83%	0.0%
2.5		5	0.854	0.7897	0.9183	0.87	0.79	0.91	0.02315	6.06%	0.47%
5		5	0.862	0.8223	0.9017	0.87	0.82	0.9	0.01428	3.71%	-0.47%
6.06		5	0.864	0.8242	0.9038	0.85	0.84	0.92	0.01435	3.72%	-0.7%
10		5	0.838	0.792	0.884	0.84	0.78	0.87	0.01655	4.42%	2.33%
15		5	0.85	0.8088	0.8912	0.85	0.8	0.88	0.01483	3.9%	0.93%

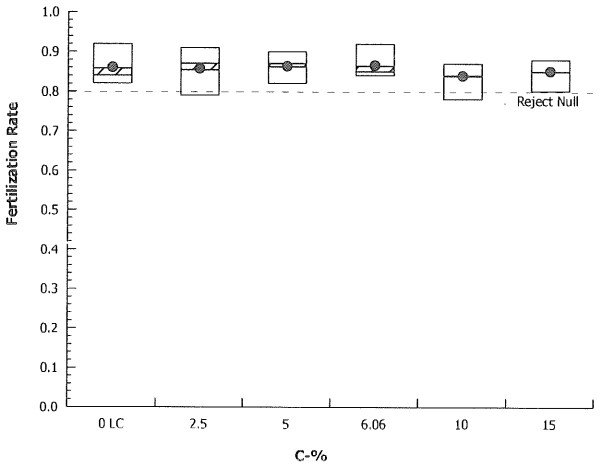
Angular (Corrected) Transformed Summary

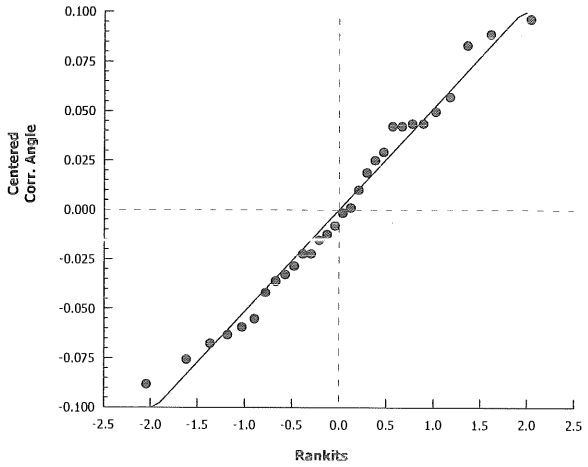
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.188	1.11	1.266	1.159	1.133	1.284	0.02805	5.28%	0.0%
2.5		5	1.183	1.092	1.274	1.202	1.095	1.266	0.0328	6.2%	0.4%
5		5	1.192	1.135	1.249	1.202	1.133	1.249	0.02071	3.88%	-0.36%
6.06		5	1.195	1.133	1.258	1.173	1.159	1.284	0.02261	4.23%	-0.64%
10		5	1.158	1.097	1.219	1.159	1.083	1.202	0.02202	4.25%	2.48%
15		5	1.175	1.118	1.232	1.173	1.107	1.217	0.02048	3.9%	1.1%

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

Analysis ID:	15-1068-9584	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:28	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1712-S044 | 00-7348-8394

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

Analysis ID:	18-1236-3729	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	02 Jan-18 13:45	**Analysis:**	Parametric-Two Sample	**Official Results:**	Yes

Sample Note: 0 spike

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	Test Result
Angular (Corrected)	NA	C > T	NA	NA	4.76%	Fails fertilization rate

Equal Variance t Two-Sample Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100*	2.767	1.86	0.059	8	0.0122	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01911926	0.01911926	1	7.656	0.0244	Significant Effect
Error	0.0199794	0.002497425	8			
Total	0.03909867		9			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	3.709	23.15	0.2323	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9152	0.7411	0.3188	Normal Distribution

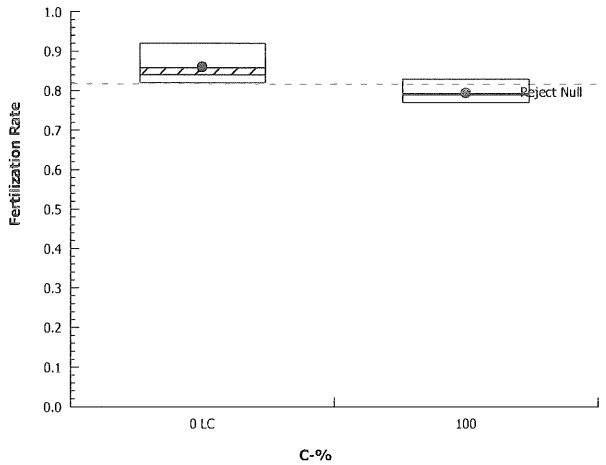
Fertilization Rate Summary

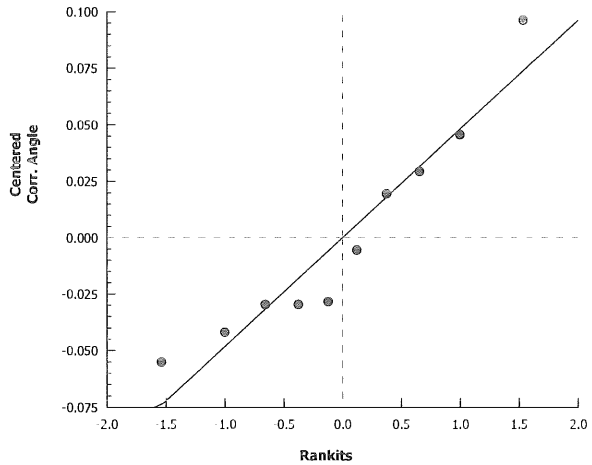
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.858	0.8065	0.9095	0.84	0.82	0.92	0.01855	4.83%	0.0%
100		5	0.794	0.7616	0.8264	0.79	0.77	0.83	0.01166	3.28%	7.46%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.188	1.11	1.266	1.159	1.133	1.284	0.02805	5.28%	0.0%
100		5	1.1	1.06	1.141	1.095	1.071	1.146	0.01457	2.96%	7.36%

Graphics





 Analyst: _____ QA: EG 1/2/18

CETIS Analytical Report

Test Code: 1712-S044 | 00-7348-8394

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

Analysis ID:	06-8315-5458	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:28	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Sample Note: 0 spike

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	45533	1000	Yes	Two-Point Interpolation

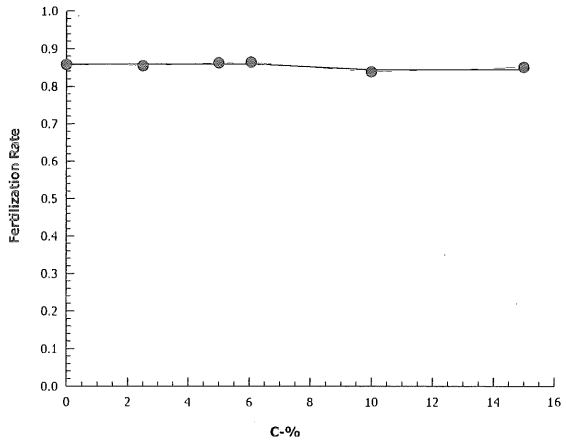
Point Estimates

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	0.858	0.82	0.92	0.01855	0.04147	4.83%	0.0%	429	500
2.5		5	0.854	0.79	0.91	0.02315	0.05177	6.06%	0.47%	427	500
5		5	0.862	0.82	0.9	0.01428	0.03194	3.71%	-0.47%	431	500
6.06		5	0.864	0.84	0.92	0.01435	0.03209	3.72%	-0.7%	432	500
10		5	0.838	0.78	0.87	0.01655	0.03701	4.42%	2.33%	419	500
15		5	0.85	0.8	0.88	0.01483	0.03317	3.9%	0.93%	425	500

Graphics



Analyst: QA: EG 1/2/18

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

Analysis ID:	19-4404-6184	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:28	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Sample Note: 0 spike

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	4.47%	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*	7.499	1.943	0.076	6	0.0001	CDF	Non-Significant Effect
		5*	10.2	1.895	0.056	7	<0.0001	CDF	Non-Significant Effect
		6.06*	9.861	1.895	0.059	7	<0.0001	CDF	Non-Significant Effect
		10*	8.783	1.895	0.058	7	<0.0001	CDF	Non-Significant Effect
		15*	9.669	1.895	0.056	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.00463414	0.0009268279	5	0.3	0.9080	Non-Significant Effect
Error	0.07414252	0.003089271	24			
Total	0.07877666		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.415	15.09	0.9227	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9723	0.9031	0.6026	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	0.858	0.8065	0.9095	0.84	0.82	0.92	0.01855	4.83%	0.0%
2.5		5	0.854	0.7897	0.9183	0.87	0.79	0.91	0.02315	6.06%	0.47%
5		5	0.862	0.8223	0.9017	0.87	0.82	0.9	0.01428	3.71%	-0.47%
6.06		5	0.864	0.8242	0.9038	0.85	0.84	0.92	0.01435	3.72%	-0.7%
10		5	0.838	0.792	0.884	0.84	0.78	0.87	0.01655	4.42%	2.33%
15		5	0.85	0.8088	0.8912	0.85	0.8	0.88	0.01483	3.9%	0.93%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.188	1.11	1.266	1.159	1.133	1.284	0.02805	5.28%	0.0%
2.5		5	1.183	1.092	1.274	1.202	1.095	1.266	0.0328	6.2%	0.4%
5		5	1.192	1.135	1.249	1.202	1.133	1.249	0.02071	3.88%	-0.36%
6.06		5	1.195	1.133	1.258	1.173	1.159	1.284	0.02261	4.23%	-0.64%
10		5	1.158	1.097	1.219	1.159	1.083	1.202	0.02202	4.25%	2.48%
15		5	1.175	1.118	1.232	1.173	1.107	1.217	0.02048	3.9%	1.1%

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

Analysis ID: 19-7334-1766 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 02 Jan-18 13:45 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Sample Note: 0 spike

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	Test Result
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	3.84%	Passes fertilization rate

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		100*	8.187	1.895	0.048	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01911926	0.01911926	1	7.656	0.0244	Significant Effect
Error	0.0199794	0.002497425	8			
Total	0.03909867		9			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Variance Ratio F	3.709	23.15	0.2323	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9152	0.7411	0.3188	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	0.858	0.8065	0.9095	0.84	0.82	0.92	0.01855	4.83%	0.0%
100		5	0.794	0.7616	0.8264	0.79	0.77	0.83	0.01166	3.28%	7.46%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.188	1.11	1.266	1.159	1.133	1.284	0.02805	5.28%	0.0%
100		5	1.1	1.06	1.141	1.095	1.071	1.146	0.01457	2.96%	7.36%

CETIS Test Data Worksheet

Report Date: 14 Dec-17 14:59 (p 1 of 1)
Test Code: 00-7348-8394/461580A 1712-3044

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

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 07 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Product Testing
Sample Code: AEF 330 PWG
Sample Source: IDE Americas, Inc.
Sample Station: M-INF & Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	90	12/19/17
			32	100	87	
			33	100	79	
			34	100	78	
			35	100	89	
			36	100	91	
			37	100	83	
			38	100	89	
			39	100	81	
			40	100	88	
			41	100	92	
			42	100	88	
			43	100	87	
			44	100	82	
			45	100	85	
			46	100	84	
			47	100	82	
			48	100	86	
			49	100	83	
			50	100	80	
			51	100	87	
			52	100	84	
			53	100	85	
			54	100	84	
			55	100	88	
			56	100	85	
			57	100	88	
			58	100	91	
			59	100	92	
			60	100	87	
			100 INF A	100	81	
			100 INF B	100	93	
			100 INF C	100	79	
			100 INF D	100	77	
			100 INF E	100	77	

$\bar{X} = 79.4$

QAP QA 12/22/17

CETIS Test Data Worksheet

Report Date: 14 Dec-17 14:58 (p 1 of 1)
Test Code: 00-7348-8394/461580A 1712-3044

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

Start Date: 15 Dec-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** AEF 330 PWG 5
End Date: 15 Dec-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 07 Dec-17 | **Material:** Product Testing | **Sample Station:** M-INF Q Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	59			
0	LC	2	57			
0	LC	3	52			
0	LC	4	44	100	81	SG 12/18/17
0	LC	5	49			
2.5		1	39			
2.5		2	33			
2.5		3	36	100	86	SG 12/18/17
2.5		4	35			
2.5		5	60			
5		1	42	100	87	SG 12/18/17
5		2	51			
5		3	47			
5		4	58			
5		5	31			
6.06		1	53			
6.06		2	45			
6.06		3	48	100	84	SG 12/18/17
6.06		4	41			
6.06		5	54			
10		1	43			
10		2	37			
10		3	32	100	77	SG 12/18/17
10		4	34			
10		5	46			
15		1	56			
15		2	50			
15		3	40	100	81	SG 12/18/17
15		4	38			
15		5	55			

QC=AC

QA: QB 12/22/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: M-INF (0 Spike) | **Start Date/Time:** 12/15/2017 1506

Sample Log No.: 17- 1249 | **End Date/Time:** 12/15/2017 1546

Dilutions made by: ~~AO OBO EG~~ | **Test No:** 1712-S044

Analyst: CG

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.2	7.99	33.7	15.7
2.5	8.1	8.00	33.8	15.9
5.0	8.2	8.00	33.9	15.7
6.06	8.1	8.00	33.9	15.8
10	8.2	8.00	33.9	15.7
15	8.2	7.98	33.9	15.8
100% M-INF	~~8~~ NM	7.74	33.9	15.8
	EG 6.18 1/2/18			

Comments: NM = Not Measured, Tech Error

QC Check: EG 1/2/18 | **Final Review:** ~~AC~~ 1/2/18

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

Client: IDE
Sample ID: M-INF Ø Spike
Test No.: 1712-S044

Tech initials: EG
Injection Time: 1427

Start Date/Time: 12/15/2017 / 1506
End Date/Time: 12/15/2017 / 1546
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 12/8/17

Sperm Absorbance at 400 nm: 1.061 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 113, 107, 121, 118, 122

Mean: 116.2 X 50 = 5,810 eggs/ml

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

Initial density:	5810 eggs/ml	=	1.4525	dilution factor	egg stock	300 ml
Final density:	4000 eggs/ml	-	1.0	part egg stock	seawater	135.75 ml
			0.4525	parts seawater		

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

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1435	50:1	93/94	7/6
Eggs Added (0.5 ml):	1445	100:1	98	2
Test Ended:	1455	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: 50:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1506	QC1	84	16
Eggs Added (0.5 ml):	1526	QC2	82	18
Test Ended:	1546	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AO 1/22/18

Final Review: EG 1/2/18

M-INF Sample, 0.1 mg/L AEF 330 Polymer Spike

CETIS Summary Report

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

Batch ID:	04-7096-3221	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 15:06	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 15:46	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	
Sample ID:	10-6913-7290	**Code:**	AEF 330 PWG 0.1 17-1247	**Client:**	IDE
Sample Date:	07 Dec-17 10:00	**Material:**	Product Testing	**Project:**	Spiking Study
Receive Date:	07 Dec-17 12:08	**Source:**	IDE Americas, Inc.		
Sample Age:	8d 5h	**Station:**	M-INF 0.1 mg/L spike		

Sample Note: 0.1 mg/L Spike

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
12-7454-2823	Fertilization Rate	15	>15	NA	10.8%	< 6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
13-1247-3580	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
12-7454-2823	Fertilization Rate	Control Resp	0.734	0.7 - NL	Yes	Passes Acceptability Criteria
13-1247-3580	Fertilization Rate	Control Resp	0.734	0.7 - NL	Yes	Passes Acceptability Criteria
12-7454-2823	Fertilization Rate	PMSD	0.1082	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	0.734	0.6932	0.7748	0.69	0.77	0.0147	0.03286	4.48%	0.0%
2.5		5	0.732	0.6357	0.8283	0.61	0.81	0.0347	0.07759	10.6%	0.27%
5		5	0.732	0.6599	0.8041	0.65	0.79	0.02596	0.05805	7.93%	0.27%
6.06		5	0.784	0.7493	0.8187	0.75	0.81	0.01249	0.02793	3.56%	-6.81%
10		5	0.75	0.7127	0.7873	0.72	0.8	0.01342	0.03	4.0%	-2.18%
15		5	0.758	0.6828	0.8332	0.67	0.81	0.02709	0.06058	7.99%	-3.27%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.75	0.77	0.75	0.69	0.71
2.5		0.78	0.61	0.71	0.75	0.81
5		0.74	0.79	0.65	0.7	0.78
6.06		0.81	0.76	0.75	0.81	0.79
10		0.72	0.75	0.74	0.74	0.8
15		0.67	0.72	0.8	0.81	0.79

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

Analysis ID: 12-7454-2823 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 27 Dec-17 9:31 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Sample Note: 0.1 mg/L Spike

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	10.8%	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	-0.00302	2.362	0.087	8	0.8342	CDF	Non-Significant Effect
		5	0.02961	2.362	0.087	8	0.8244	CDF	Non-Significant Effect
		6.06	-1.592	2.362	0.087	8	0.9972	CDF	Non-Significant Effect
		10	-0.4976	2.362	0.087	8	0.9398	CDF	Non-Significant Effect
		15	-0.7951	2.362	0.087	8	0.9710	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01390325	0.002780651	5	0.8212	0.5467	Non-Significant Effect
Error	0.08126665	0.00338611	24			
Total	0.0951699		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.858	15.09	0.3202	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9622	0.9031	0.3520	Normal Distribution

Fertilization Rate Summary

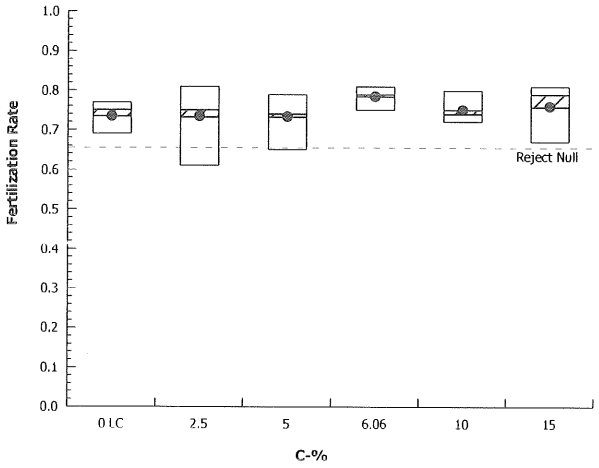
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.734	0.6932	0.7748	0.75	0.69	0.77	0.0147	4.48%	0.0%
2.5		5	0.732	0.6357	0.8283	0.75	0.61	0.81	0.0347	10.6%	0.27%
5		5	0.732	0.6599	0.8041	0.74	0.65	0.79	0.02596	7.93%	0.27%
6.06		5	0.784	0.7493	0.8187	0.79	0.75	0.81	0.01249	3.56%	-6.81%
10		5	0.75	0.7127	0.7873	0.74	0.72	0.8	0.01342	4.0%	-2.18%
15		5	0.758	0.6828	0.8332	0.79	0.67	0.81	0.02709	7.99%	-3.27%

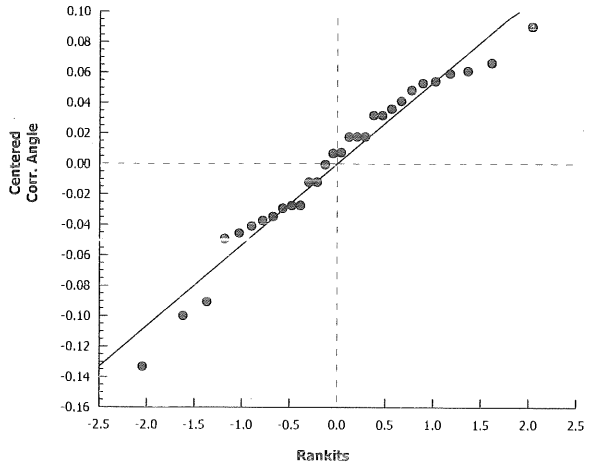
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.029	0.9835	1.075	1.047	0.9803	1.071	0.01656	3.6%	0.0%
2.5		5	1.03	0.9225	1.137	1.047	0.8963	1.12	0.03858	8.38%	-0.01%
5		5	1.028	0.9475	1.109	1.036	0.9377	1.095	0.02915	6.34%	0.11%
6.06		5	1.088	1.046	1.13	1.095	1.047	1.12	0.01513	3.11%	-5.69%
10		5	1.048	1.004	1.092	1.036	1.013	1.107	0.01583	3.38%	-1.78%
15		5	1.059	0.9722	1.145	1.095	0.9589	1.12	0.03116	6.58%	-2.84%

Echinoid Sperm Cell Fertilization Test 15C					Nautilus Environmental (CA)
Analysis ID:	12-7454-2823	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:31	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1712-S045 | 15-0310-3785

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

Analysis ID:	13-1247-3580	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:31	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Sample Note: 0.1 mg/L Spike

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1256196	1000	Yes	Two-Point Interpolation

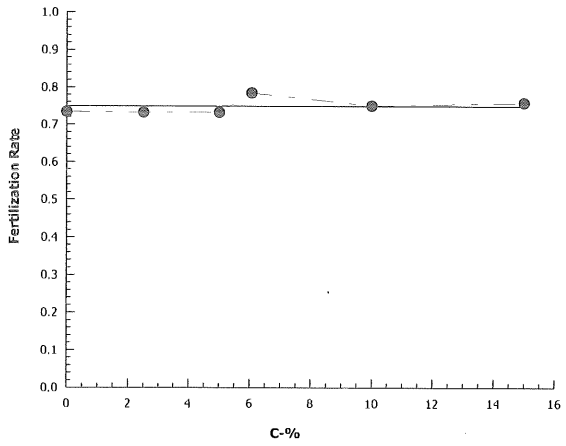
Point Estimates

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	0.734	0.69	0.77	0.0147	0.03286	4.48%	0.0%	367	500
2.5		5	0.732	0.61	0.81	0.0347	0.07759	10.6%	0.27%	366	500
5		5	0.732	0.65	0.79	0.02596	0.05805	7.93%	0.27%	366	500
6.06		5	0.784	0.75	0.81	0.01249	0.02793	3.56%	-6.81%	392	500
10		5	0.75	0.72	0.8	0.01342	0.03	4.0%	-2.18%	375	500
15		5	0.758	0.67	0.81	0.02709	0.06058	7.99%	-3.27%	379	500

Graphics



Analyst: _____ QA: EC 1/2/18

CETIS Analytical Report

TSI

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

Analysis ID:	17-1623-5114	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 9:32	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Sample Note: 0.1 mg/L Spike

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	8.33%	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*	6.353	2.132	0.086	4	0.0016	CDF	Non-Significant Effect
		5*	8.087	2.015	0.064	5	0.0002	CDF	Non-Significant Effect
		6.06*	16.14	1.895	0.037	7	<0.0001	CDF	Non-Significant Effect
		10*	13.7	1.895	0.038	7	<0.0001	CDF	Non-Significant Effect
		15*	8.545	2.015	0.068	5	0.0002	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01390325	0.002780651	5	0.8212	0.5467	Non-Significant Effect
Error	0.08126665	0.00338611	24			
Total	0.0951699		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.858	15.09	0.3202	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9622	0.9031	0.3520	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	0.734	0.6932	0.7748	0.75	0.69	0.77	0.0147	4.48%	0.0%
2.5		5	0.732	0.6357	0.8283	0.75	0.61	0.81	0.0347	10.6%	0.27%
5		5	0.732	0.6599	0.8041	0.74	0.65	0.79	0.02596	7.93%	0.27%
6.06		5	0.784	0.7493	0.8187	0.79	0.75	0.81	0.01249	3.56%	-6.81%
10		5	0.75	0.7127	0.7873	0.74	0.72	0.8	0.01342	4.0%	-2.18%
15		5	0.758	0.6828	0.8332	0.79	0.67	0.81	0.02709	7.99%	-3.27%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.029	0.9835	1.075	1.047	0.9803	1.071	0.01656	3.6%	0.0%
2.5		5	1.03	0.9225	1.137	1.047	0.8963	1.12	0.03858	8.38%	-0.01%
5		5	1.028	0.9475	1.109	1.036	0.9377	1.095	0.02915	6.34%	0.11%
6.06		5	1.088	1.046	1.13	1.095	1.047	1.12	0.01513	3.11%	-5.69%
10		5	1.048	1.004	1.092	1.036	1.013	1.107	0.01583	3.38%	-1.78%
15		5	1.059	0.9722	1.145	1.095	0.9589	1.12	0.03116	6.58%	-2.84%

Analyst: _____ QA: EL 1/2/18

CETIS Test Data Worksheet

Report Date: 14 Dec-17 15:00 (p 1 of 1)
Test Code: 15-0310-3785/~~59978B29~~ 1112-3045

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 07 Dec-17

Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Product Testing

Sample Code: AEF 330 PWG
Sample Source: IDE Americas, Inc.
Sample Station: M-INF 0.1 mg/L Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	74	12/19/17
			62	100	71	
			63	100	69	
			64	100	79	
			65	100	67	
			66	100	77	
			67	100	79	
			68	100	76	
			69	100	75	
			70	100	74	
			71	100	70	
			72	100	79	
			73	100	75	
			74	100	78	
			75	100	71	
			76	100	61	
			77	100	72	
			78	100	75	
			79	100	81	
			80	100	81	
			81	100	65	
			82	100	81	
			83	100	80	
			84	100	74	
			85	100	72	
			86	100	75	
			87	100	75	
			88	100	78	
			89	100	81	
			90	100	80	

Reviewed 12/22/17

CETIS Test Data Worksheet

Report Date: 14 Dec-17 15:00 (p 1 of 1)
Test Code: 15-0310-3785/~~59978B29~~ 1712-5045

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 07 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Product Testing
Sample Code: AEF 330 PWG
Sample Source: IDE Americas, Inc.
Sample Station: M-INF 0.1mg/L Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	73	(A) ~~76~~ 100	76	AES 12/17/17
0	LC	2	66	100	76	AES 12/17/17
0	LC	3	87			
0	LC	4	63			
0	LC	5	62			
2.5		1	88			
2.5		2	76	100	69	AES 12/17/17
2.5		3	75			
2.5		4	69			
2.5		5	82			
5		1	84			
5		2	72			
5		3	81	100	(D) ~~49~~ 73	AES 12/17/17
5		4	71			
5		5	74			
6.06		1	80			
6.06		2	68			
6.06		3	78			
6.06		4	79	(E) ~~85~~ 100	85	AES 12/17/17
6.06		5	64			
10		1	85			
10		2	86			
10		3	70			
10		4	61			
10		5	90	100	69	AES 12/17/17
15		1	65			
15		2	77			
15		3	83			
15		4	89			
15		5	67	100	79	AES 12/17/17

QC: EC

(B) QIR AES 12/17/17

(C) AO QIR 12/22/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE/ CDP Polymer spiking study | **Test Species:** *S. purpuratus*

Sample ID: M-INF (0.1 mg/L AEF 330 PWG polymer) | **Start Date/Time:** 12/15/2017 1506

Sample Log No.: 17- ~~1249~~ 1247 EG 1/2/18 | **End Date/Time:** 12/15/2017 1546

Dilutions made by: ~~AO~~ ~~DO~~ EG | **Test No:** 1712-5045

Analyst: CG

	Initial Readings			
~~Concentration~~ % Sample	~~DO~~ (mg/L)	~~pH~~ (units)	~~Salinity~~ (ppt)	~~Temperature~~ (°C)
Lab Control	8.2	8.03	33.8	15.8
2.5	8.2	8.02	33.8	15.9
5.0	8.2	8.02	33.9	15.8
6.06	8.2	8.01	33.9	15.9
10	8.2	8.00	33.9	15.9
15	8.2	7.99	33.9	15.9

Comments:

QC Check: ~~AO~~ 12/22/17 | **Final Review:** EG 1/2/18

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

Client: IDE
Sample ID: m-INF 0.1 mg/L Spiked AEF330 PWG
Test No.: 1712-5045

Tech initials: EG
Injection Time: 1427

Start Date/Time: 12/15/2017 / 1506
End Date/Time: 12/15/2017 / 1546
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 12/8/17

Sperm Absorbance at 400 nm: 1.061 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 113, 107, 121, 118, 122 Mean: 116.2 X 50 = 5,810 eggs/ml

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

Initial density: 5810 eggs/ml = 1.4525 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.4525 parts seawater

egg stock 300 ml
seawater 135.75 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):	1435	50:1	93/94	7/6
Eggs Added (0.5 ml):	1445	100:1	98	2
Test Ended:	1455	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: 50:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1506	QC1	84	16
Eggs Added (0.5 ml):	1526	QC2	82	18
Test Ended:	1546	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AP 12/22/17

Final Review: EG 1/2/18

M-INF Sample, 0.5 mg/L AEF 330 Polymer Spike

CETIS Summary Report

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

Batch ID:	18-7729-2146	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 15:06	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 15:46	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	14-2322-1328	**Code:**	AEF 330 PWG 0.5 17-1247	**Client:**	IDE
Sample Date:	07 Dec-17 10:00	**Material:**	Product Testing	**Project:**	Spiking Study
Receive Date:	07 Dec-17 12:08	**Source:**	IDE Americas, Inc.		
Sample Age:	8d 5h	**Station:**	M-INF 0.5mg/L spike		

Sample Note: 0.5 mg/L spike

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-9358-4860	Fertilization Rate	15	>15	NA	11.0%	<6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
02-1745-5572	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
02-1745-5572	Fertilization Rate	Control Resp	0.734	0.7 - NL	Yes	Passes Acceptability Criteria
11-9358-4860	Fertilization Rate	Control Resp	0.734	0.7 - NL	Yes	Passes Acceptability Criteria
11-9358-4860	Fertilization Rate	PMSD	0.11	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	0.734	0.6923	0.7757	0.71	0.79	0.01503	0.03362	4.58%	0.0%
2.5		5	0.756	0.6911	0.8209	0.68	0.81	0.02337	0.05225	6.91%	-3.0%
5		5	0.762	0.6823	0.8417	0.67	0.82	0.02871	0.06419	8.42%	-3.82%
6.06		5	0.748	0.6645	0.8315	0.66	0.82	0.03007	0.06723	8.99%	-1.91%
10		5	0.768	0.7088	0.8272	0.69	0.82	0.02131	0.04764	6.2%	-4.63%
15		5	0.774	0.7372	0.8108	0.74	0.81	0.01327	0.02966	3.83%	-5.45%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.74	0.72	0.79	0.71	0.71
2.5		0.8	0.75	0.81	0.68	0.74
5		0.72	0.82	0.67	0.8	0.8
6.06		0.8	0.76	0.7	0.82	0.66
10		0.78	0.78	0.69	0.77	0.82
15		0.76	0.74	0.8	0.76	0.81

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

Analysis ID:	11-9358-4860	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Dec-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	11.0%	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	-0.7021	2.362	0.088	8	0.9632	CDF	Non-Significant Effect
		5	-0.9137	2.362	0.088	8	0.9789	CDF	Non-Significant Effect
		6.06	-0.4813	2.362	0.088	8	0.9374	CDF	Non-Significant Effect
		10	-1.073	2.362	0.088	8	0.9864	CDF	Non-Significant Effect
		15	-1.241	2.362	0.088	8	0.9917	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.007064687	0.001412937	5	0.4031	0.8418	Non-Significant Effect
Error	0.08412815	0.00350534	24			
Total	0.09119283		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.459	15.09	0.6296	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9518	0.9031	0.1883	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	0.734	0.6923	0.7757	0.72	0.71	0.79	0.01503	4.58%	0.0%
2.5		5	0.756	0.6911	0.8209	0.75	0.68	0.81	0.02337	6.91%	-3.0%
5		5	0.762	0.6823	0.8417	0.8	0.67	0.82	0.02871	8.42%	-3.82%
6.06		5	0.748	0.6645	0.8315	0.76	0.66	0.82	0.03007	8.99%	-1.91%
10		5	0.768	0.7088	0.8272	0.78	0.69	0.82	0.02131	6.2%	-4.63%
15		5	0.774	0.7372	0.8108	0.76	0.74	0.81	0.01327	3.83%	-5.45%

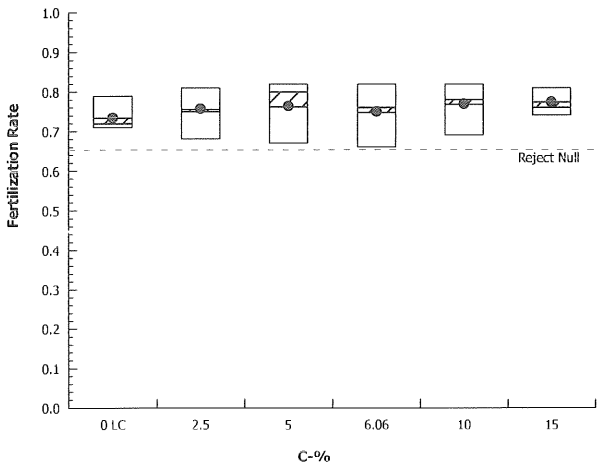
Angular (Corrected) Transformed Summary

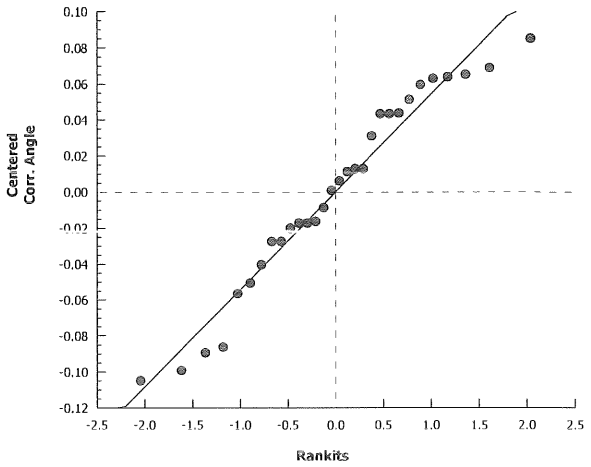
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.03	0.9812	1.078	1.013	1.002	1.095	0.01741	3.78%	0.0%
2.5		5	1.056	0.9807	1.131	1.047	0.9695	1.12	0.02706	5.73%	-2.55%
5		5	1.064	0.9716	1.156	1.107	0.9589	1.133	0.03322	6.98%	-3.32%
6.06		5	1.048	0.9515	1.144	1.059	0.9483	1.133	0.03461	7.39%	-1.75%
10		5	1.07	1.001	1.139	1.083	0.9803	1.133	0.02479	5.18%	-3.9%
15		5	1.076	1.032	1.12	1.059	1.036	1.12	0.01597	3.32%	-4.51%

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

Analysis ID:	11-9358-4860	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Dec-17 15:08	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	02-1745-5572	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Dec-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	1081274	1000	Yes	Two-Point Interpolation

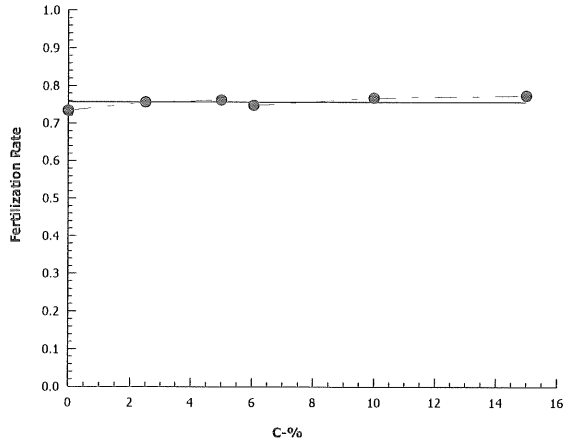
Point Estimates

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	0.734	0.71	0.79	0.01503	0.03362	4.58%	0.0%	367	500
2.5		5	0.756	0.68	0.81	0.02337	0.05225	6.91%	-3.0%	378	500
5		5	0.762	0.67	0.82	0.02871	0.06419	8.42%	-3.82%	381	500
6.06		5	0.748	0.66	0.82	0.03007	0.06723	8.99%	-1.91%	374	500
10		5	0.768	0.69	0.82	0.02131	0.04764	6.2%	-4.63%	384	500
15		5	0.774	0.74	0.81	0.01327	0.02966	3.83%	-5.45%	387	500

Graphics



Analyst: JS/AC QA: AC 12/28/17

CETIS Analytical Report

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

Analysis ID: 19-2393-8919 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 28 Dec-17 15:09 **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	4.71%	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*	9.441	2.015	0.061	5	0.0001	CDF	Non-Significant Effect
		5*	8.17	2.015	0.072	5	0.0002	CDF	Non-Significant Effect
		6.06*	7.445	2.015	0.075	5	0.0003	CDF	Non-Significant Effect
		10*	10.62	1.943	0.054	6	<0.0001	CDF	Non-Significant Effect
		15*	14.73	1.895	0.039	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.007064687	0.001412937	5	0.4031	0.8418	Non-Significant Effect
Error	0.08412815	0.00350534	24			
Total	0.09119283		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.459	15.09	0.6296	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9518	0.9031	0.1883	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	0.734	0.6923	0.7757	0.72	0.71	0.79	0.01503	4.58%	0.0%
2.5		5	0.756	0.6911	0.8209	0.75	0.68	0.81	0.02337	6.91%	-3.0%
5		5	0.762	0.6823	0.8417	0.8	0.67	0.82	0.02871	8.42%	-3.82%
6.06		5	0.748	0.6645	0.8315	0.76	0.66	0.82	0.03007	8.99%	-1.91%
10		5	0.768	0.7088	0.8272	0.78	0.69	0.82	0.02131	6.2%	-4.63%
15		5	0.774	0.7372	0.8108	0.76	0.74	0.81	0.01327	3.83%	-5.45%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.03	0.9812	1.078	1.013	1.002	1.095	0.01741	3.78%	0.0%
2.5		5	1.056	0.9807	1.131	1.047	0.9695	1.12	0.02706	5.73%	-2.55%
5		5	1.064	0.9716	1.156	1.107	0.9589	1.133	0.03322	6.98%	-3.32%
6.06		5	1.048	0.9515	1.144	1.059	0.9483	1.133	0.03461	7.39%	-1.75%
10		5	1.07	1.001	1.139	1.083	0.9803	1.133	0.02479	5.18%	-3.9%
15		5	1.076	1.032	1.12	1.059	1.036	1.12	0.01597	3.32%	-4.51%

CETIS Test Data Worksheet

Report Date: 14 Dec-17 15:02 (p 1 of 1)
Test Code: 12-2045-5598/~~48BEAGAE~~ 1712-5046 (A)

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

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 07 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Product Testing
Sample Code: AEF 330 PWG
Sample Source: IDE Americas, Inc.
Sample Station: M-INF 0.5 mg/L spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			91	100	82	12/19/2017
			92	100	77	
			93	100	80	
			94	100	79	
			95	100	70	
			96	100	81	
			97	100	74	
			98	100	82	
			99	100	80	
			100	100	78	
			101	100	(B) ~~78~~ 68	
			102	100	80	
			103	100	67	
			104	100	(B) ~~67~~ 74	
			105	100	71	
			106	100	76	
			107	100	66	
			108	100	(B) ~~50~~ 78	
			109	100	82	
			110	100	(B) ~~71~~ 74	
			111	100	81	
			112	100	71	
			113	100	80	
			114	100	75	
			115	100	72	
			116	100	72	
			117	100	76	
			118	100	69	
			119	100	76	
			120	100	80	

~~(A) AD~~ Q18 12/22/17

(B) ACS QC Q18 AC 12/28/17

CETIS Test Data Worksheet

Report Date: 14 Dec-17 15:01 (p 1 of 1)
Test Code: 12-2045-5598/~~48BEACAE~~ 1712-5046

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 07 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Product Testing
Sample Code: AEF 330 PWG
Sample Source: IDE Americas, Inc.
Sample Station: M-INF 0.5 mg/L Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	104			
0	LC	2	116			
0	LC	3	94			
0	LC	4	105			
0	LC	5	112	100	81	AES 12/17/17
2.5		1	120			
2.5		2	114			
2.5		3	96			
2.5		4	101	100	77	AES 12/17/17
2.5		5	97			
5		1	115			
5		2	98			
5		3	103	100	71	AES 12/17/17
5		4	99			
5		5	113			
6.06		1	102			
6.06		2	106	100	72	AES 12/17/17
6.06		3	95			
6.06		4	91			
6.06		5	107			
10		1	100	100	74	AES 12/17/17
10		2	108			
10		3	118			
10		4	92			
10		5	109			
15		1	119			
15		2	110			
15		3	93	100	74	AES 12/17/17
15		4	117			
15		5	111			

QC: EG

EPA QA 12/22/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE/ CDP Polymer spiking study

Test Species: *S. purpuratus*

Sample ID: M-INF (0.5 mg/L AEF 330 PWG polymer)

Start Date/Time: 12/15/2017 1506

Sample Log No.: 17-~~1249~~ 1247 EG 1/2/18

End Date/Time: 12/15/2017 1546

Dilutions made by: AD 0930 EG

Test No: 1712-3046

Analyst: CG

	Initial Readings			
Concentration % Sample	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.3	8.02	33.7	16.0
2.5	8.3	8.02	33.9	15.8
5.0	8.3	8.02	33.9	15.9
6.06	8.3	8.02	33.9	15.9
10	8.3	8.01	33.9	15.9
15	8.3	8.00	33.9	16.0

Comments:

QC Check: AD 12/22/17

Final Review: EG 1/2/18

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

Client: IDE
Sample ID: M-INF 0.5 mg/L Spike AEF 330 Rep1
Test No.: 1712-S046

Start Date/Time: 12/15/2017 / 1506
End Date/Time: 12/15/2017 / 1546
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 12/8/17

Tech initials: EG
Injection Time: 1427

Sperm Absorbance at 400 nm: 1.061 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 113, 107, 121, 118, 122

Mean: 116.2 X 50 = 5,810 eggs/ml

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

Initial density: 5810 eggs/ml = 1.4525 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.4525 parts seawater

egg stock 300 ml
seawater 135.75 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):	1435	50:1	93/94	7/6
Eggs Added (0.5 ml):	1445	100:1	98	2
Test Ended:	1455	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: 50:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1506	QC1	84	16
Eggs Added (0.5 ml):	1526	QC2	82	18
Test Ended:	1546	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AO 12/22/17

Final Review: EG 1/2/18

M-INF Sample, 1.0 mg/L AEF 330 Polymer Spike

CETIS Summary Report

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

Batch ID:	15-5862-3717	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 15:06	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 15:46	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	02-5999-3123	**Code:**	AEF 330 PWG 1.0 17-1247	**Client:**	IDE
Sample Date:	07 Dec-17 10:00	**Material:**	Product Testing	**Project:**	Spiking Study
Receive Date:	07 Dec-17 12:08	**Source:**	IDE Americas, Inc.		
Sample Age:	8d 5h	**Station:**	M-INF 1.0 mg/L Spike		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-7163-7108	Fertilization Rate	15	>15	NA	10.1%	6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
07-8955-9937	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
07-8955-9937	Fertilization Rate	Control Resp	0.738	0.7 - NL	Yes	Passes Acceptability Criteria
11-7163-7108	Fertilization Rate	Control Resp	0.738	0.7 - NL	Yes	Passes Acceptability Criteria
11-7163-7108	Fertilization Rate	PMSD	0.1015	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	0.738	0.6715	0.8045	0.67	0.82	0.02396	0.05357	7.26%	0.0%
2.5		5	0.764	0.706	0.822	0.73	0.82	0.02088	0.04669	6.11%	-3.52%
5		5	0.786	0.7618	0.8102	0.76	0.81	0.008718	0.01949	2.48%	-6.5%
6.06		5	0.738	0.6738	0.8022	0.66	0.79	0.02311	0.05167	7.0%	0.0%
10		5	0.754	0.6767	0.8313	0.68	0.84	0.02786	0.06229	8.26%	-2.17%
15		5	0.768	0.7158	0.8202	0.7	0.81	0.01881	0.04207	5.48%	-4.07%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.73	0.82	0.73	0.67	0.74
2.5		0.73	0.81	0.73	0.82	0.73
5		0.78	0.76	0.78	0.8	0.81
6.06		0.79	0.73	0.66	0.78	0.73
10		0.78	0.71	0.76	0.84	0.68
15		0.7	0.76	0.78	0.79	0.81

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

Analysis ID:	11-7163-7108	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Dec-17 16:17	**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	10.1%	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	-0.8447	2.362	0.084	8	0.9746	CDF	Non-Significant Effect
		5	-1.552	2.362	0.084	8	0.9968	CDF	Non-Significant Effect
		6.06	0.009108	2.362	0.084	8	0.8306	CDF	Non-Significant Effect
		10	-0.5439	2.362	0.084	8	0.9459	CDF	Non-Significant Effect
		15	-0.9661	2.362	0.084	8	0.9817	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01141649	0.002283298	5	0.7281	0.6092	Non-Significant Effect
Error	0.0752643	0.003136012	24			
Total	0.08668078		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.149	15.09	0.5281	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9715	0.9031	0.5812	Normal Distribution

Fertilization Rate Summary

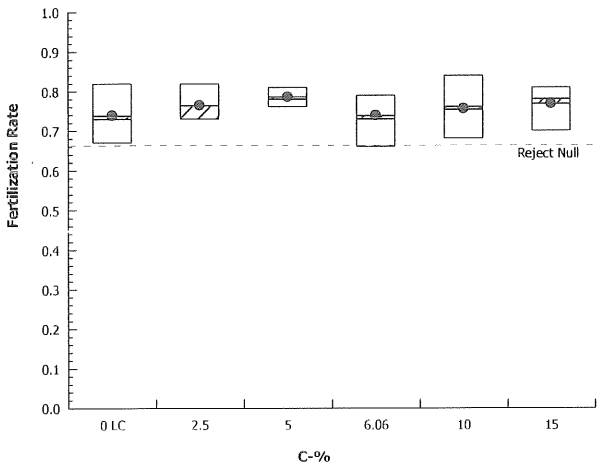
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.738	0.6715	0.8045	0.73	0.67	0.82	0.02396	7.26%	0.0%
2.5		5	0.764	0.706	0.822	0.73	0.73	0.82	0.02088	6.11%	-3.52%
5		5	0.786	0.7618	0.8102	0.78	0.76	0.81	0.008718	2.48%	-6.5%
6.06		5	0.738	0.6738	0.8022	0.73	0.66	0.79	0.02311	7.0%	0.0%
10		5	0.754	0.6767	0.8313	0.76	0.68	0.84	0.02786	8.26%	-2.17%
15		5	0.768	0.7158	0.8202	0.78	0.7	0.81	0.01881	5.48%	-4.07%

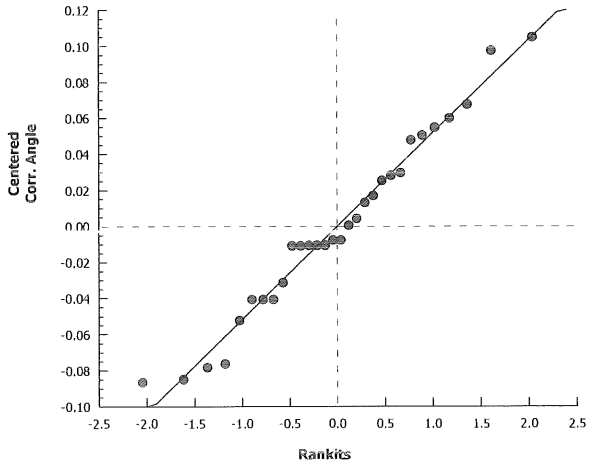
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.035	0.9578	1.113	1.024	0.9589	1.133	0.02789	6.03%	0.0%
2.5		5	1.065	0.9956	1.135	1.024	1.024	1.133	0.02502	5.25%	-2.89%
5		5	1.09	1.061	1.12	1.083	1.059	1.12	0.01063	2.18%	-5.31%
6.06		5	1.035	0.9625	1.107	1.024	0.9483	1.095	0.02606	5.63%	0.03%
10		5	1.054	0.9629	1.146	1.059	0.9695	1.159	0.03298	6.99%	-1.86%
15		5	1.069	1.009	1.13	1.083	0.9912	1.12	0.0219	4.58%	-3.31%

Echinoid Sperm Cell Fertilization Test 15C					Nautilus Environmental (CA)
Analysis ID:	11-7163-7108	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Dec-17 16:17	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1712-S047 | 17-2036-5486

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

Analysis ID:	07-8955-9937	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	28 Dec-17 16:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	378767	1000	Yes	Two-Point Interpolation

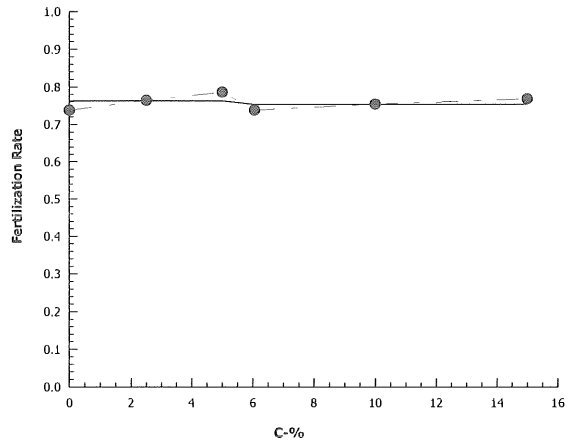
Point Estimates

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	0.738	0.67	0.82	0.02396	0.05357	7.26%	0.0%	369	500
2.5		5	0.764	0.73	0.82	0.02088	0.04669	6.11%	-3.52%	382	500
5		5	0.786	0.76	0.81	0.008718	0.01949	2.48%	-6.5%	393	500
6.06		5	0.738	0.66	0.79	0.02311	0.05167	7.0%	0.0%	369	500
10		5	0.754	0.68	0.84	0.02786	0.06229	8.26%	-2.17%	377	500
15		5	0.768	0.7	0.81	0.01881	0.04207	5.48%	-4.07%	384	500

Graphics



 Analyst: AC QA: EG 1/2/18

CETIS Analytical Report

Test Code: 1712-S047 | 17-2036-5486

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

Analysis ID: 10-7046-6444 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.7
Analyzed: 28 Dec-17 16:18 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	6.81%	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*	8.853	1.895	0.062	7	<0.0001	CDF	Non-Significant Effect
		5*	13.37	2.015	0.047	5	<0.0001	CDF	Non-Significant Effect
		6.06*	7.735	1.895	0.063	7	<0.0001	CDF	Non-Significant Effect
		10*	7.12	1.943	0.076	6	0.0002	CDF	Non-Significant Effect
		15*	9.676	1.895	0.057	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01141649	0.002283298	5	0.7281	0.6092	Non-Significant Effect
Error	0.0752643	0.003136012	24			
Total	0.08668078		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.149	15.09	0.5281	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9715	0.9031	0.5812	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	0.738	0.6715	0.8045	0.73	0.67	0.82	0.02396	7.26%	0.0%
2.5		5	0.764	0.706	0.822	0.73	0.73	0.82	0.02088	6.11%	-3.52%
5		5	0.786	0.7618	0.8102	0.78	0.76	0.81	0.008718	2.48%	-6.5%
6.06		5	0.738	0.6738	0.8022	0.73	0.66	0.79	0.02311	7.0%	0.0%
10		5	0.754	0.6767	0.8313	0.76	0.68	0.84	0.02786	8.26%	-2.17%
15		5	0.768	0.7158	0.8202	0.78	0.7	0.81	0.01881	5.48%	-4.07%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.035	0.9578	1.113	1.024	0.9589	1.133	0.02789	6.03%	0.0%
2.5		5	1.065	0.9956	1.135	1.024	1.024	1.133	0.02502	5.25%	-2.89%
5		5	1.09	1.061	1.12	1.083	1.059	1.12	0.01063	2.18%	-5.31%
6.06		5	1.035	0.9625	1.107	1.024	0.9483	1.095	0.02606	5.63%	0.03%
10		5	1.054	0.9629	1.146	1.059	0.9695	1.159	0.03298	6.99%	-1.86%
15		5	1.069	1.009	1.13	1.083	0.9912	1.12	0.0219	4.58%	-3.31%

CETIS Test Data Worksheet

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

Start Date: 15 Dec-17 15:06 **Species:** Strongylocentrotus purpuratus **Sample Code:** AEF 330 PWG 1.0
End Date: 15 Dec-17 15:46 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 07 Dec-17 10:00 **Material:** Product Testing **Sample Station:** M-INF 1.0mg/L Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			121	100	80	AC OBO kc 12/19/17
			122		73	
			123		73	
			124		74	
			125		66	
			126		78	
			127		73	
			128		76	
			129		76	
			130		76	
			131		73	
			132		67	
			133		78	
			134		84	
			135		82	
			136		70	
			137		73	
			138		81	
			139		78	
			140		68	
			141		71	
			142		81	
			143		78	
			144		81	
			145		73	
			146		79	
			147		73	
			148		79	
			149		78	
			150		82	

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:20 (p 1 of 1)
Test Code: 17-2036-5486/668AB1AE (AP 1712-5047)

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

Start Date: 15 Dec-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** AEF 330 PWG 1.0
End Date: 15 Dec-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 07 Dec-17 | **Material:** Product Testing | **Sample Station:** M-INF 1.0 mg/L spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	147			
0	LC	2	135			
0	LC	3	131	100	66	AMS 12/17/17
0	LC	4	132			
0	LC	5	124			
2.5		1	123			
2.5		2	142	100	82	AMS 12/17/17
2.5		3	122			
2.5		4	150			
2.5		5	137			
5		1	133			
5		2	128	100	76	AMS 12/17/17
5		3	143			
5		4	121			
5		5	138			
6.06		1	146	100	79	AMS 12/17/17
6.06		2	145			
6.06		3	125			
6.06		4	126			
6.06		5	127			
10		1	149			
10		2	141			
10		3	130			
10		4	134	100	80	AMS 12/15/17
10		5	140			
15		1	136			
15		2	129			
15		3	139			
15		4	148			
15		5	144	100	80	AMS 12/17/17

AC: EG

Reviewed 12/22/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE/ CDP Polymer spiking study

Test Species: *S. purpuratus*

Sample ID: M-INF (1.0 mg/L AEF 330 PWG polymer)

Start Date/Time: 12/15/2017 1506

Sample Log No.: 17- 1247

End Date/Time: 12/15/2017 1546

Dilutions made by: AO JBO EG

Test No: 1712- 3047

Analyst: CG

Concentration % Sample	Initial Readings DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.2	8.03	33.8	15.8
2.5	8.2	8.03	33.9	15.8
5.0	8.3	8.03	33.9	15.9
6.06	8.2	8.03	33.9	15.8
10	8.3	8.01	34.0	15.9
15	8.2	8.01	34.0	15.9

Comments:

QC Check: AO 12/22/17

Final Review: EG 1/2/18

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

Client: IDE
Sample ID: MINF @ 5.0 mg/L Sprite AEF 330 PWG 1.0
Test No.: 1712-SOP47 @

Tech initials: EG
Injection Time: 1427

Start Date/Time: 12/15/2017 / 1506
End Date/Time: 12/15/2017 / 1546
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 12/8/17

Sperm Absorbance at 400 nm: 1.061 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 113, 107, 121, 118, 122

Mean: 116.2 X 50 = 5,810 eggs/ml

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

Initial density: 5810 eggs/ml = 1.4525 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.4525 parts seawater

egg stock 300 ml
seawater 135.75 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):	1435	50:1	93/94	7/6
Eggs Added (0.5 ml):	1445	100:1	98	2
Test Ended:	1455	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: 50:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1506	QC1	86	14
Eggs Added (0.5 ml):	1526	QC2	82	18
Test Ended:	1546	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: ~~AAD~~ Q18 12/22/17 (B) EG Q18 1/2/18

QC Check: AD 12/22/17

Final Review: EG 1/2/18

M-INF Sample, 5.0 mg/L AEF 330 Polymer Spike

CETIS Summary Report

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

Batch ID:	10-1147-0012	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 15:06	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 15:46	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	09-7257-2905	**Code:**	AEF 330 PWG 5.0 17-1247	**Client:**	IDE
Sample Date:	07 Dec-17 10:00	**Material:**	Product Testing	**Project:**	Spiking Study
Receive Date:	07 Dec-17 12:08	**Source:**	IDE Americas, Inc.		
Sample Age:	8d 5h	**Station:**	M-INF 5.0 mg/L spike		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-5121-4867	Fertilization Rate	2.5	5	3.536	12.0%	40	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
11-0658-8786	Fertilization Rate	EC25	6.672	4.981	7.75	14.99	Linear Interpolation (ICPIN)
		EC50	9.552	8.458	10.64	10.47	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-5121-4867	Fertilization Rate	Control Resp	0.772	0.7 - NL	Yes	Passes Acceptability Criteria
11-0658-8786	Fertilization Rate	Control Resp	0.772	0.7 - NL	Yes	Passes Acceptability Criteria
07-5121-4867	Fertilization Rate	PMSD	0.1205	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	0.772	0.6827	0.8613	0.66	0.86	0.03216	0.0719	9.31%	0.0%
2.5		5	0.744	0.6702	0.8178	0.66	0.81	0.02657	0.05941	7.99%	3.63%
5		5	0.654	0.5548	0.7532	0.52	0.71	0.03572	0.07987	12.21%	15.28%
6.06		5	0.62	0.5435	0.6965	0.55	0.69	0.02757	0.06164	9.94%	19.69%
10		5	0.356	0.2777	0.4343	0.25	0.41	0.02821	0.06309	17.72%	53.89%
15		5	0.074	0.03045	0.1175	0.04	0.13	0.01568	0.03507	47.39%	90.41%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.86	0.78	0.66	0.79	0.77
2.5		0.78	0.66	0.81	0.76	0.71
5		0.52	0.64	0.7	0.71	0.7
6.06		0.55	0.65	0.65	0.56	0.69
10		0.39	0.38	0.41	0.25	0.35
15		0.05	0.07	0.08	0.13	0.04

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

Analysis ID:	07-5121-4867	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 16:29	**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	12.0%	2.5	5	3.536	40

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.7503	2.362	0.108	8	0.5338	CDF	Non-Significant Effect
		5*	2.908	2.362	0.108	8	0.0157	CDF	Significant Effect
		6.06*	3.694	2.362	0.108	8	0.0025	CDF	Significant Effect
		10*	9.57	2.362	0.108	8	<0.0001	CDF	Significant Effect
		15*	17.6	2.362	0.108	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.367208	0.4734416	5	90.13	<0.0001	Significant Effect
Error	0.1260669	0.005252788	24			
Total	2.493275		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.6204	15.09	0.9870	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9561	0.9031	0.2448	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	0.772	0.6827	0.8613	0.78	0.66	0.86	0.03216	9.31%	0.0%
2.5		5	0.744	0.6702	0.8178	0.76	0.66	0.81	0.02657	7.99%	3.63%
5		5	0.654	0.5548	0.7532	0.7	0.52	0.71	0.03572	12.21%	15.28%
6.06		5	0.62	0.5435	0.6965	0.65	0.55	0.69	0.02757	9.94%	19.69%
10		5	0.356	0.2777	0.4343	0.38	0.25	0.41	0.02821	17.72%	53.89%
15		5	0.074	0.03045	0.1175	0.07	0.04	0.13	0.01568	47.39%	90.41%

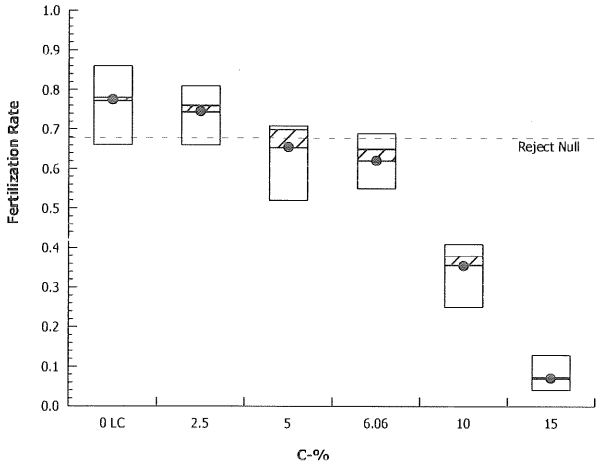
Angular (Corrected) Transformed Summary

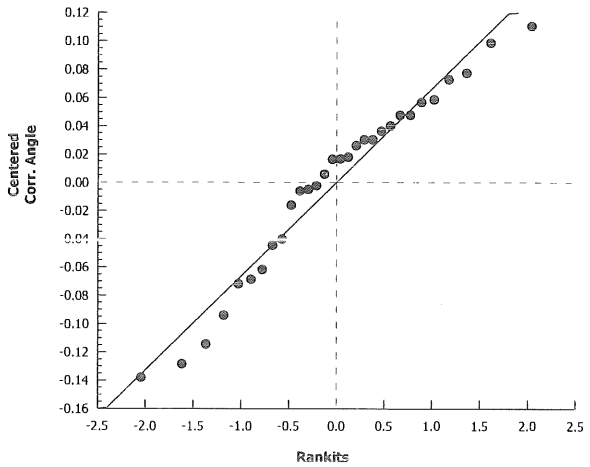
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.077	0.9708	1.183	1.083	0.9483	1.187	0.03816	7.93%	0.0%
2.5		5	1.042	0.9582	1.126	1.059	0.9483	1.12	0.03029	6.5%	3.19%
5		5	0.9434	0.8408	1.046	0.9912	0.8054	1.002	0.03695	8.76%	12.38%
6.06		5	0.9074	0.8285	0.9863	0.9377	0.8355	0.9803	0.02842	7.0%	15.73%
10		5	0.6381	0.5539	0.7222	0.6642	0.5236	0.6949	0.03031	10.62%	40.74%
15		5	0.2701	0.1897	0.3504	0.2678	0.2014	0.3689	0.02894	23.96%	74.92%

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

Analysis ID:	07-5121-4867	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 16:29	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	11-0658-8786	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 16:29	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	252703	1000	Yes	Two-Point Interpolation

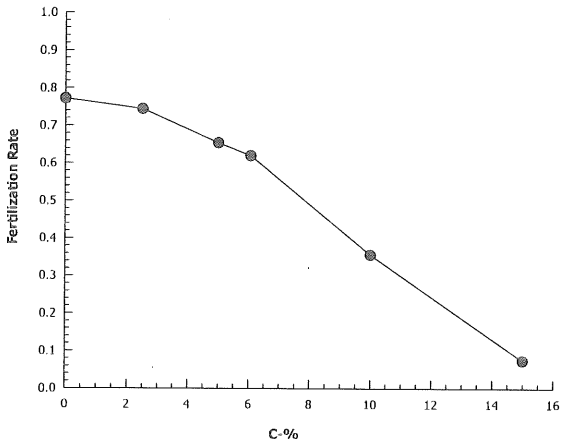
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	6.672	4.981	7.75	14.99	12.9	20.08
EC50	9.552	8.458	10.64	10.47	9.396	11.82

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	0.772	0.66	0.86	0.03216	0.0719	9.31%	0.0%	386	500
2.5		5	0.744	0.66	0.81	0.02657	0.05941	7.99%	3.63%	372	500
5		5	0.654	0.52	0.71	0.03572	0.07987	12.21%	15.28%	327	500
6.06		5	0.62	0.55	0.69	0.02757	0.06164	9.94%	19.69%	310	500
10		5	0.356	0.25	0.41	0.02821	0.06309	17.72%	53.89%	178	500
15		5	0.074	0.04	0.13	0.01568	0.03507	47.39%	90.41%	37	500

Graphics



Analyst: AL QA EC 1/2/18

CETIS Analytical Report

TST

Report Date: 27 Dec-17 16:33 (p 1 of 1)
Test Code: 1712-S048 | 19-1559-0836

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

Analysis ID: 11-4422-2496 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.7
Analyzed: 27 Dec-17 16:33 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	8.33%	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*	5.633	1.895	0.079	7	0.0004	CDF	Non-Significant Effect
		5*	2.907	1.895	0.089	7	0.0114	CDF	Non-Significant Effect
		6.06*	2.475	1.895	0.076	7	0.0213	CDF	Non-Significant Effect
		10	-4.066	1.895	0.079	7	0.9976	CDF	Significant Effect
		15	-13.21	1.895	0.077	7	1.0000	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.367208	0.4734416	5	90.13	<0.0001	Significant Effect
Error	0.1260669	0.005252788	24			
Total	2.493275		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.6204	15.09	0.9870	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9561	0.9031	0.2448	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	0.772	0.6827	0.8613	0.78	0.66	0.86	0.03216	9.31%	0.0%
2.5		5	0.744	0.6702	0.8178	0.76	0.66	0.81	0.02657	7.99%	3.63%
5		5	0.654	0.5548	0.7532	0.7	0.52	0.71	0.03572	12.21%	15.28%
6.06		5	0.62	0.5435	0.6965	0.65	0.55	0.69	0.02757	9.94%	19.69%
10		5	0.356	0.2777	0.4343	0.38	0.25	0.41	0.02821	17.72%	53.89%
15		5	0.074	0.03045	0.1175	0.07	0.04	0.13	0.01568	47.39%	90.41%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.077	0.9708	1.183	1.083	0.9483	1.187	0.03816	7.93%	0.0%
2.5		5	1.042	0.9582	1.126	1.059	0.9483	1.12	0.03029	6.5%	3.19%
5		5	0.9434	0.8408	1.046	0.9912	0.8054	1.002	0.03695	8.76%	12.38%
6.06		5	0.9074	0.8285	0.9863	0.9377	0.8355	0.9803	0.02842	7.0%	15.73%
10		5	0.6381	0.5539	0.7222	0.6642	0.5236	0.6949	0.03031	10.62%	40.74%
15		5	0.2701	0.1897	0.3504	0.2678	0.2014	0.3689	0.02894	23.96%	74.92%

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:22 (p 1 of 1) (A)
Test Code: 19-1559-0836/~~722D98B4~~ 1712-3048

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

Start Date: 15 Dec-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** AEF 330 PWG 5.0
End Date: 15 Dec-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 07 Dec-17 **Material:** Product Testing **Sample Station:** M-INF 5.0 mg/L Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			151	100	60	Read by JC 12/19/17
			152	100	(B) ~~56~~ 71	
			153	100	64	
			154	100	39	
			155	100	52	
			156	100	(B) ~~56~~ 78	
			157	100	~~78~~	
			158	100	65	
			159	100	71	
			160	100	76	
			161	100	5	
			162	100	7	
			163	100	69	
			164	100	86	
			165	100	70	
			166	100	79	
			167	100	8	
			168	100	65	
			169	100	77	
			170	100	25	
			171	100	~~4~~	
			172	100	55	
			173	100	41	
			174	100	13	
			175	100	35	
			176	100	81	
			177	100	~~38~~	
			178	100	66	
			179	100	70	
			180	100	56	

(A) QLS 12/22/17

(B) QLS QCd AC 0130 ACS 12/27/17

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:22 (p 1 of 1)
Test Code: 19-1559-0836/~~722D08B4~~ 1115-5048

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

Start Date: 15 Dec-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** AEF 330 PWG 5.0
End Date: 15 Dec-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 07 Dec-17 | **Material:** Product Testing | **Sample Station:** M-INF 5.0mg/L Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	164	100	89	AES 12/17/17
0	LC	2	157			
0	LC	3	151			
0	LC	4	166			
0	LC	5	169			
2.5		1	156			
2.5		2	178			
2.5		3	176			
2.5		4	160			
2.5		5	152	100	79	AES 12/17/17
5		1	155			
5		2	153			
5		3	165			
5		4	159	100	68	AES 12/17/17
5		5	179			
6.06		1	172			
6.06		2	168	100	66	AES 12/17/17
6.06		3	158			
6.06		4	180			
6.06		5	163			
10		1	154			
10		2	177			
10		3	173	100	51	
10		4	170			
10		5	175			
15		1	161			
15		2	162			
15		3	167			
15		4	174			
15		5	171	100	9	

QC: EM

RAO QIS 12/22/17

Marine Chronic Bioassay **Water Quality Measurements**

Client : IDE/ CDP Polymer spiking study **Test Species:** *S. purpuratus*

Sample ID: M-INF (5.0 mg/L AEF 330 PWG polymer) **Start Date/Time:** 12/15/2017 1506

Sample Log No.: 17- 1247 **End Date/Time:** 12/15/2017 1546

Dilutions made by: AD OBU EG **Test No:** 1712-5048

Analyst: CG

	Initial Readings			
Concentration % Sample	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.3	8.03	33.9	15.5
2.5	8.3	8.04	33.9	15.6
5.0	8.3	8.03	33.9	15.7
6.06	8.3	8.03	33.9	15.7
10	8.3	8.02	33.9	15.7
15	8.3	8.00	33.9	15.7

Comments:

QC Check: AD 12/22/17 **Final Review:** EG 1/2/18

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

Client: IDE
Sample ID: M-INF 5.0mg/L Spike AEF 330 PWG
Test No.: 1712-S04P

Start Date/Time: 12/15/2017 / 1506
End Date/Time: 12/15/2017 / 1546
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 12/8/17

Tech initials: EG
Injection Time: 1427

Sperm Absorbance at 400 nm: 1.061 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 113, 107, 121, 118, 122
Mean: 116.2 X 50 = 5,810 eggs/ml

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

Initial density:	5810	eggs/ml	=	1.4525	dilution factor	egg stock	300 ml
Final density:	4000	eggs/ml	-	1.0	part egg stock	seawater	135.75 ml
				0.4525	parts seawater		

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

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1435	50:1	93/94	7/6
Eggs Added (0.5 ml):	1445	100:1	98	2
Test Ended:	1455	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: 50:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1506	QC1	81	14
Eggs Added (0.5 ml):	1526	QC2	82	18
Test Ended:	1546	Egg Control 1	0	0
		Egg Control 2	0	0

Comments:

QC Check: AO 12/20/17

Final Review: EG 1/2/18

Appendix B

Sample Check-in Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE

Tests Performed: Urchin Fertilization

Project: Spiking Study

Test ID No.(s): 1712-5044 to 5052

AEF 330 prog AC 125 prog

Sample ID:	1) Polymer	2) coagulant	3 Influent Dilution water	4)
Log-in No. (17-xxxx):	1247	1248	1249	
Sample Collection Date & Time:	12/7/17 1000	12/7/17 1000	12/7/17 N/A	
Sample Receipt Date & Time:	12/7/17 1208	12/7/17 1208	12/7/17 ~~N/A~~ 1208 (A)	
Number of Containers & Container Type:	1, 100mL plastic	1, 100mL plastic	4, 4L cubi;	
Approx. Total Volume Received (L):	~100mL	~100mL	~16	
Check-in Temp (°C)	—	—	21.5	
Temperature OK? [1]	— Y N	— Y N	(Y) N	Y N
DO (mg/L)	—	—	7.0	
pH (units)	—	—	7.83	
Conductivity (µS/cm)	—	—	—	
Salinity (ppt)	—	—	33.9	
Alkalinity (mg/L) [2]	—	—	112	
Hardness (mg/L) [2,3]	—	—	—	
Total Chlorine (mg/L)	—	—	0.02	
Technician Initials	CH	CH	CH	

Freshwater Tests:

Control/Dilution Water Source: 8:2 Culligan Other: _____ Alkalinity: _____ Hardness: _____

Additional Control? Y N = _____ Alkalinity: _____ Hardness: _____

Marine Tests:

Control/Dilution Water Source: (LAB SW) ART SW Other: _____ Alkalinity: 116 Salinity: 34ppt

Additional Control? Y (N) = _____ Alkalinity: _____ Salinity: _____

Sample Salted w/ artificial salt? Y (N) If yes, target ppt and source? _____

Sample salted w/brine? Y (N) If yes, target ppt? _____

Notes [1] Temperature for sample must be 0-6°C if received >24 hours past collection time.

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

Additional Comments (A) EG 1/2/18 Q18

QC Check: EG 1/2/18

Sample Check-In Information

Sample Descriptions:

1) light Yellow color, opaque, Mild odor, no debris
2) No color, clear, mild odor, no debris
3) No color, clear, no odor, no debris
4) _____

COC Complete? (Y) N

Filtration? Y (N)

Pore Size: _____

Organisms or Debris

pH Adjustment? Y (N)

	1	2	3	4	5	6
Initial pH:						
Amount of HCl added:						
Final pH:						

Cl_2 Adjustment? Y (N)

	1	2	3	4	5	6
Initial Free Cl_2:						
STS added:						
Final Free Cl_2:						

Sample Aeration? Y (N)

	1	2	3	4	5	6
Initial D.O.						
Duration & Rate						
Final D.O.						

Subsamples For Additional Chemistry Required? Y (N)

NH3 Other _____

Tech Initials _____

Final Review: AC 1/8/18

Appendix C

Chain of Custody Form

SPIKING STUDY

IDE Technologies

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

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

Project Name: Special Toxicity Spiking Study Project Manager: Peter Shen Contact Information: (760) 201-7777

Special instruction: 4 x 4 liter cubies collected at M-INF on 12/7/17 @ 11:00 to be used as dilution water. KC

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							
AEF 330 PWG (17-3470)	12/7/2017	10:00	Polymer Grab	N	100 mL poly	X							
AC 125 PWG (17-3471)	12/7/2017	10:00	Coagulant Grab	N	100 mL poly	X							

Included 4 cube Tanks of M-INF water for dilution purposes collected for KC

NOTES:

Samples received at ambient temperature

Relinquished By:	Date:	Time:	Received By:	Date:	Time:
[illegible]	12/7/17	11:30	[illegible]	12/7/17	[illegible]
[illegible]	12/7/17	12:08 PM	[illegible]	12/7/17	12:08
			[illegible]	12/7/17	1208

Sample Condition Upon Receipt:

☐ Iced ☐ Ambient or _____ °C

☒ Iced ☑ Ambient or 21.5 °C

Nautilus ID's: 17-1247 to 17-1249

Appendix D

Reference Toxicant Test Data

CETIS Summary Report

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

Batch ID:	16-2826-4250	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 15:06	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 15:46	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	12-3187-6232	**Code:**	171215sprtA	**Client:**	Internal
Sample Date:	15 Dec-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	15 Dec-17	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-5068-5136	Fertilization Rate	<10	10	NA	9.77%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
10-1459-1840	Fertilization Rate	EC50	26.01	24.46	27.67		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
10-1459-1840	Fertilization Rate	Control Resp	0.818	0.7 - NL	Yes	Passes Acceptability Criteria
18-5068-5136	Fertilization Rate	Control Resp	0.818	0.7 - NL	Yes	Passes Acceptability Criteria
18-5068-5136	Fertilization Rate	PMSD	0.0977	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	0.818	0.7729	0.8631	0.76	0.86	0.01625	0.03633	4.44%	0.0%
10		5	0.678	0.5909	0.7651	0.57	0.75	0.03137	0.07014	10.35%	17.11%
20		5	0.524	0.4423	0.6057	0.48	0.64	0.02943	0.0658	12.56%	35.94%
40		5	0.26	0.1772	0.3428	0.19	0.36	0.02983	0.06671	25.66%	68.22%
80		5	0.012	0	0.03421	0	0.04	0.008	0.01789	149.1%	98.53%
160		5	0.006	0	0.02266	0	0.03	0.006	0.01342	223.6%	99.27%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.82	0.82	0.76	0.83	0.86
10		0.65	0.71	0.71	0.75	0.57
20		0.5	0.64	0.49	0.48	0.51
40		0.21	0.26	0.36	0.19	0.28
80		0.02	0	0	0.04	0
160		0	0.03	0	0	0

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

Analysis ID:	18-5068-5136	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 16:36	**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	9.77%	<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*	3.919	2.362	0.098	8	0.0014	CDF	Significant Effect
		20*	7.759	2.362	0.098	8	<0.0001	CDF	Significant Effect
		40*	14.44	2.362	0.098	8	<0.0001	CDF	Significant Effect
		80*	24.91	2.362	0.098	8	<0.0001	CDF	Significant Effect
		160*	25.49	2.362	0.098	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.971019	0.9942037	5	231.4	<0.0001	Significant Effect
Error	0.1031157	0.004296489	24			
Total	5.074134		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.204	15.09	0.9445	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9518	0.9031	0.1884	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	0.818	0.7729	0.8631	0.82	0.76	0.86	0.01625	4.44%	0.0%
10		5	0.678	0.5909	0.7651	0.71	0.57	0.75	0.03137	10.35%	17.11%
20		5	0.524	0.4423	0.6057	0.5	0.48	0.64	0.02943	12.56%	35.94%
40		5	0.26	0.1772	0.3428	0.26	0.19	0.36	0.02983	25.66%	68.22%
80		5	0.012	0	0.03421	0	0	0.04	0.008	149.1%	98.53%
160		5	0.006	0	0.02266	0	0	0.03	0.006	223.6%	99.27%

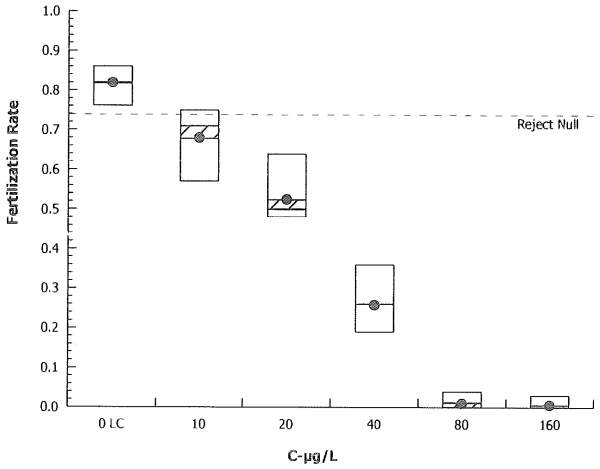
Angular (Corrected) Transformed Summary

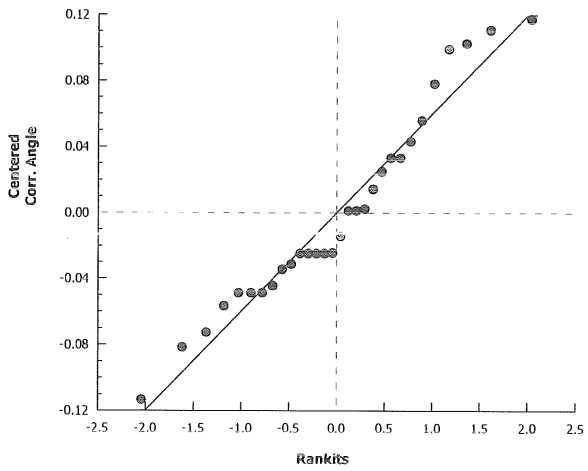
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.131	1.074	1.189	1.133	1.059	1.187	0.02074	4.1%	0.0%
10		5	0.969	0.8766	1.061	1.002	0.8556	1.047	0.03327	7.68%	14.36%
20		5	0.8098	0.727	0.8925	0.7854	0.7654	0.9273	0.0298	8.23%	28.43%
40		5	0.5326	0.4389	0.6264	0.5351	0.451	0.6435	0.03375	14.17%	52.92%
80		5	0.09866	0.01194	0.1854	0.05002	0.05002	0.2014	0.03124	70.79%	91.28%
160		5	0.07483	0.005943	0.1437	0.05002	0.05002	0.1741	0.02481	74.14%	93.39%

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

Analysis ID:	18-5068-5136	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 16:36	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 171215sprtA | 06-1613-2535

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

Analysis ID:	10-1459-1840	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 16:36	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

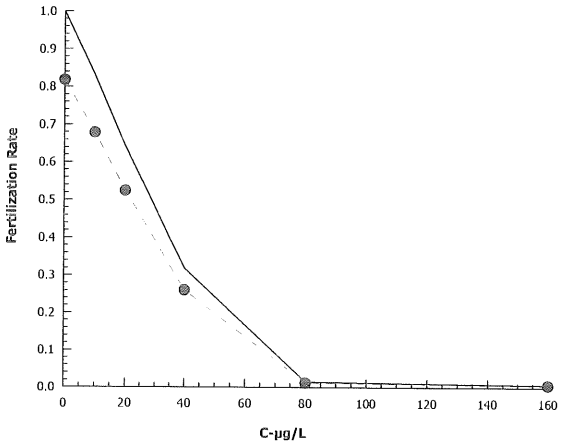
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.182	17.11%	1.415	0.0134	26.01	24.46	27.67

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	0.818	0.76	0.86	0.01625	0.03633	4.44%	0.0%	409	500
10		5	0.678	0.57	0.75	0.03137	0.07014	10.35%	17.11%	339	500
20		5	0.524	0.48	0.64	0.02943	0.0658	12.56%	35.94%	262	500
40		5	0.26	0.19	0.36	0.02983	0.06671	25.66%	68.22%	130	500
80		5	0.012	0	0.04	0.008	0.01789	149.1%	98.53%	6	500
160		5	0.006	0	0.03	0.006	0.01342	223.6%	99.27%	3	500

Graphics

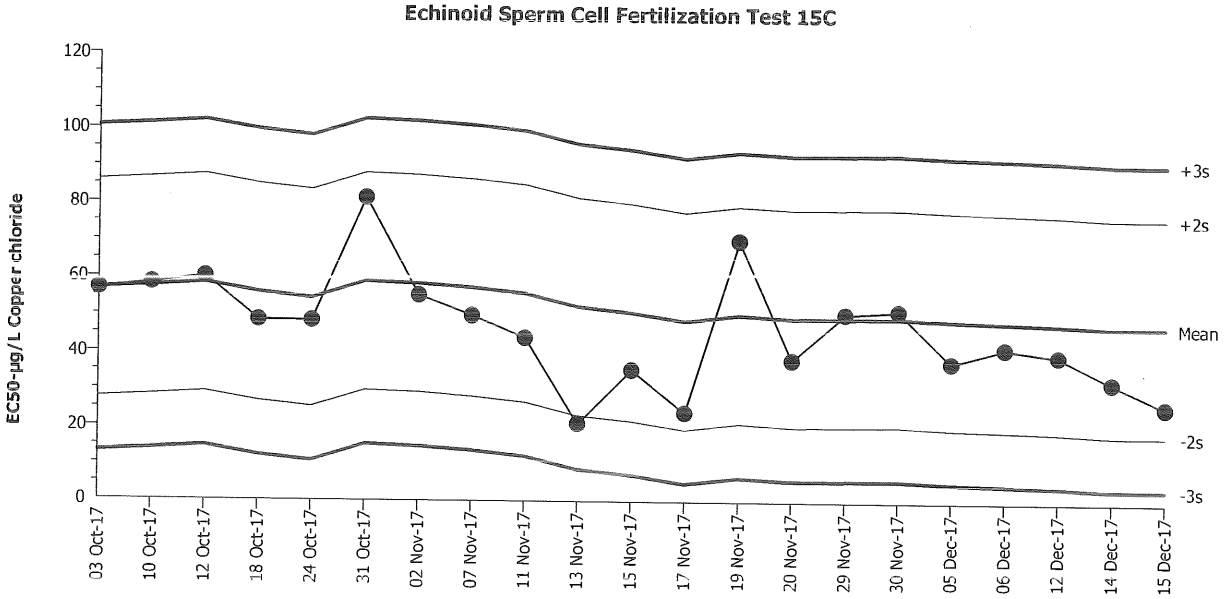


Analyst: EG QA: KTP 1/2/18

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:	47.26	**Count:**	20	**-2s Warning Limit:**	18.1	**-3s Action Limit:**	3.516
Sigma:	14.58	**CV:**	30.90%	**+2s Warning Limit:**	76.42	**+3s Action Limit:**	91

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Oct	3	13:49	56.88	9.619	0.6598			05-1137-7792	06-0895-0170
2			10	15:10	58.36	11.1	0.7614			20-5863-5053	00-1542-1738
3			12	14:55	60.18	12.92	0.8863			05-0863-6526	07-1531-2424
4			18	14:22	48.53	1.27	0.08713			13-0042-6212	05-6771-5532
5			24	13:15	48.41	1.145	0.07855			20-0280-7301	18-5464-1899
6			31	13:59	81.36	34.1	2.339	(+)		06-4227-6723	08-8095-0809
7		Nov	2	12:28	55.32	8.065	0.5531			17-4126-1689	20-0626-8382
8			7	14:30	49.87	2.613	0.1792			10-3521-2857	13-9801-3995
9			11	14:25	43.91	-3.352	-0.2299			14-1655-2339	20-5239-6070
10			13	14:35	20.97	-26.29	-1.803			07-0538-7056	00-9105-4737
11			15	16:09	35.48	-11.78	-0.8077			06-3476-9418	17-5783-9769
12			17	14:17	24.03	-23.23	-1.593			20-8374-1268	00-9691-5869
13			19	10:02	70.21	22.95	1.574			12-1164-1483	20-4501-4622
14			20	15:15	38.26	-8.995	-0.617			08-0578-7050	18-8950-2431
15			29	15:30	50.6	3.335	0.2288			05-0010-1267	11-1707-1208
16			30	15:28	51.48	4.215	0.2891			09-6334-2928	00-8447-7747
17		Dec	5	16:05	37.64	-9.618	-0.6597			00-4872-5743	06-2243-7863
18			6	15:50	41.57	-5.692	-0.3904			04-9516-7018	18-3148-8943
19			12	12:20	39.55	-7.712	-0.529			01-8906-4164	02-6832-7767
20			14	15:35	32.51	-14.75	-1.012			11-6397-1428	17-9802-1610
21			15	15:06	26.01	-21.25	-1.457			06-1613-2535	10-1459-1840

CETIS Test Data Worksheet

Report Date: 20 Dec-17 10:51 (p 1 of 1)
Test Code: 02-9159-5360/171215sprtBA Q18 JP 12/20/17

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

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 15 Dec-17

Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride

Sample Code: 171215sprtBA Q18 JP 12/20/17
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	2	12/21/17
			2	100	75	
			3	100	0	
			4	100	19	
			5	100	50	
			6	100	4	
			7	100	(K) 54 57	
			8	100	82	
			9	100	48	
			10	100	82	
			11	100	71	
			12	100	26	
			13	100	28	
			14	100	(B) 66 83	
			15	100	21	
			16	100	86	
			17	100	0	
			18	100	51	
			19	100	76	
			20	100	0	
			21	100	0	
			22	100	71	
			23	100	36	
			24	100	3	
			25	100	0	
			26	100	0	
			27	100	0	
			28	100	64	
			29	100	49	
			30	100	(E) 57 65	

EG Q18 12/28/17 (B)

(A) JP OBO ACS Q18 12/27/17

(B) JP OBO ACS Q18 12/27/17

(E) EG Q18 12/28/17

Analyst: SG QA: AC 12/27/17

CETIS Test Data Worksheet

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

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

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	10	100	79	BO 12/15/17
0	LC	2	8			
0	LC	3	19	100	78	BO 12/15/17
0	LC	4	14			
0	LC	5	16			
10		1	30			
10		2	11	100	67	BO 12/15/17
10		3	22			
10		4	2			
10		5	7			
20		1	5			
20		2	28			
20		3	29	100	46	BO 12/15/17
20		4	9			
20		5	18			
40		1	15			
40		2	12	100	31	BO 12/15/17
40		3	23			
40		4	4			
40		5	13			
80		1	1	100	2	BO 12/15/17
80		2	25			
80		3	21			
80		4	6			
80		5	26			
160		1	20			
160		2	24			
160		3	27			
160		4	3	100	0	BO 12/15/17
160		5	17			

QC FAC

ReQC 18

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: *S. purpuratus*

Sample ID: $CuCl_2$

Start Date/Time: 12/15/2017 1506

Test No: 171215sprtA

End Date/Time: 12/15/2017 1546

Dilutions made by: EG oBo AC

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.3
Final Volume (mL):	500
Cu stock concentration (µg/L):	7,600

Analyst: CG

	Initial Readings			
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.3	7.95	33.8	15.7
10	8.3	7.92	33.8	15.4
20	8.2	7.94	33.8	15.6
40	8.3	7.95	33.7	15.5
80	8.3	7.95	33.6	15.5
160	8.3	7.95	33.4	15.6

Comments:

QC Check: AC 12/27/17

Final Review: EG 12/29/17

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

Client: Internal
Sample ID: CuCl2
Test No.: 171215Spr7A

Tech initials: EG
Injection Time: 1427

Start Date/Time: 12/15/2017 / 1506
End Date/Time: 12/15/2017 / 1546
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 12/8/17

Sperm Absorbance at 400 nm: 1.061 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 113, 107, 121, 118, 122 Mean: 116.2 X 50 = 5,810 eggs/ml

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

Initial density: 5810 eggs/ml = 1.4525 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.4525 parts seawater

egg stock 300 ml
seawater 135.75 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):	1435	50:1	93/94	7/6
Eggs Added (0.5 ml):	1445	100:1	98	2
Test Ended:	1455	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: 50:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1506	QC1	84	16
Eggs Added (0.5 ml):	1526	QC2	82	18
Test Ended:	1546	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 12/27/17 Final Review: EG 12/29/17

Appendix E

Lab Data 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.