

## 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.

4590 Carlsbad Boulevard

Carlsbad, CA 92008

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.

California

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

Results verified by: \_\_\_ advience abor

#### 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  $4 \pm 2^{\circ}$ 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

Natural seawater (source: SIO inlet), 34 ± 2 parts per thousand (ppt); 20-Lab Control/Dilution Water:

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

per Replicate:

Number of Replicates, Organisms 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

Each sample was tested with all replicates and control randomized per Randomization:

USEPA protocol

Fertilization; 20-min sperm exposure to sample followed by a 20-min Test Type:

fertilization period

Mean fertilization ≥70% in the control, and percent minimum significant Acceptability Criteria:

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<sub>c</sub>) 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.

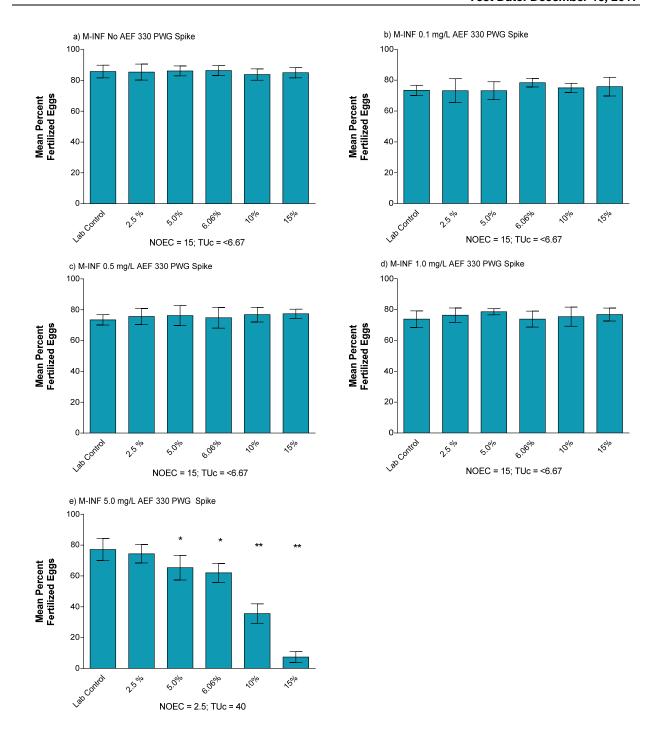
#### **Results and Discussion**

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<sub>c</sub> 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<sub>c</sub> 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	Endpoint EC <sub>50</sub> H (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  $EC_{50} \pm 2$  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** 



## **CETIS Summary Report**

**Report Date:** 02 Jan-18 13:46 (p 1 of 1)

Report Date.	02 Jan-10 13.40 (p 1 01
Test Code:	1712-S044   00-7348-83

								est Code:		17	12-3044   00	J-1340-0394
Echinoid Spe	rm Cell Fertiliza	tion Te	st 15C						N	autilu	s Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	04-6233-5140 15 Dec-17 15:0 15 Dec-17 15:4 40m	)6  6	Test Type: Protocol: Species: Source:	Fertilization EPA/600/R-99 Strongylocent Pt. Loma	, ,	tus	Analyst: Diluent: Natural Seawate Brine: Not Applicable Age:			er		
	13-1543-2842 07 Dec-17 10:0 : 07 Dec-17 12:0 8d 5h	)0 )8	Code: Material: Source: Station:	AEF 330 PWo Product Testi IDE Americas M-INF	ng	9		Client: Project:	IDE Spiking S	Study		
Sample Note:	0 spike		400040000000000000000000000000000000000									
Comparison S	Summary	diomanni omi diomani										
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meth	nod			
18-1236-3729	Fertilization Ra		<100	100	NA	4.76%	>1				-Sample Te	
15-1068-9584	Fertilization Ra	te	15	>15	NA	6.98%	₹6.667	Dunr	nett Multipl	e Com	parison Te	st
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	_ TU	Meth	nod			
06-8315-5458	Fertilization Ra	te	EC25 EC50		N/A N/A	N/A N/A	<6.66 <6.66		ar Interpola	ation (I	CPIN)	
Test Acceptat	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Lim	nits	Ove	rlap De	cision	ı	
06-8315-5458	Fertilization Ra	te	Contr	ol Resp	0.858	0.7 - NL		Yes	Pas	sses A	cceptability	Criteria
15-1068-9584	Fertilization Ra			ol Resp	0.858	0.7 - NL		Yes			cceptability	
18-1236-3729 15-1068-9584	Fertilization Ra		Contre PMSE	ol Resp	0.858 0.06985	0.7 - NL NL - 0.25		Yes No			cceptability	
	Fertilization Ra Fertilization Ra		PMSE		0.00963	NL - 0.25		No			cceptability	
Fertilization R	Rate Summary											
C-%	Control Type	Coun	t Mean	95% LC	L 95% UCL	Min	Max	Std i	Err Sto	l Dev	CV%	%Effect
0	Lab Control	5	0.858	0.8065	0.9095	0.82	0.92	0.01	855 0.0	4147	4.83%	0.0%
2.5		5	0.854	0.7897	0.9183	0.79	0.91	0.02	315 0.0	5177	6.06%	0.47%
5		5	0.862		0.9017	0.82	0.9	0.01		3194	3.71%	-0.47%
6.06		5	0.864		0.9038	0.84	0.92	0.01		3209	3.72%	-0.7%
10		5	0.838		0.884	0.78	0.87	0.01		3701	4.42%	2.33%
15 100		5 5	0.85 0.794	0.8088 0.7616	0.8912 0.8264	0.8 0.77	0.88 0.83	0.01- 0.01		3317 2608	3.9% 3.28%	0.93% 7.46%
		J	0.734	0,7010	0.0204	0.11	0.03	0.01	100 0.0	2000	J.ZU /0	1,70/0
Fertilization R		_										
C-%	Control Type	Rep 1	<del></del>		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						

Report Date:

27 Dec-17 09:28 (p 1 of 2)

Test Code:

1712-S044 | 00-7348-8394

	Manager and access to the Article Co.										
Echinoid Sp	erm Cell Fertiliza	ation Test	15C						Nautilus	Environr	mental (CA)
Analysis ID: Analyzed:	15-1068-9584 27 Dec-17 9:2			tilization Rat ametric-Cor		tments		S Version		8.7	
Sample Note	e: 0 spike										
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Cori	rected)	NA	C > T	NA	NA		6.98%	15	>15	NA	6.667
Dunnett Mul	tiple Compariso	n 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-Sign	ificant Effect		
	5		-0.1202	2.362	0.083 8	0.8664	CDF	Non-Sign	ificant Effect		
	6.06		-0.2161	2.362	0.083 8	0.8893	CDF	Non-Sign	ificant Effect		
	10		0.838	2.362	0.083 8	0.4935	CDF	Non-Sign	ificant Effect		
	15		0.3709	2.362	0.083 8	0.7016	CDF	Non-Sign	ificant Effect		
ANOVA Tabl	е										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	ι(α:5%)		
Between	0.004634	14	0.0009268	3279	5	0.3	0.9080	Non-Sign	ificant Effect		
Error	0.07414252 0.003089271 24										
Total	0.078776	66			29						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(	(a:1%)			
Variances	Bartlett E	Equality of	Variance	1.415	15.09	0.9227	Equal Var	iances		,	
Distribution		Wilk W No		0.9723	0.9031	0.6026	Normal D	stribution			
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 (Co	rrected) Transfor	med Sum	ımary								
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%

Report Date: Test Code: 27 Dec-17 09:28 (p 2 of 2) 1712-S044 | 00-7348-8394

**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 1.0 F 0.100 0.9 0.075 Fertilization Rate 0.050 0.7 0.025 0.5 0.000 0.4 -0.025 0.3 -0.050 0.2 0.1 0.0 0 LC 10 15 -1.5 0.0 -2.5 -2.0 -1.0 -0.5 0.5 1.0 1.5 2.0 C-% Rankits

Report Date:

02 Jan-18 13:46 (p 1 of 1) 1712-S044 I 00-7348-8394

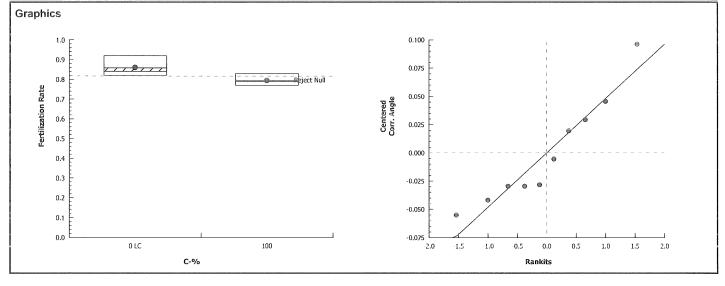
Test Code:

							Test	Code:	1/12-S044   00-/348-8394
Echinoid Spe	rm C	ell Fertilization T	est 15C						Nautilus Environmental (CA)
Analysis ID:		1236-3729	•	ertilization Ra				S Version:	CETISv1.8.7
Analyzed:	02 .	Jan-18 13:45	Analysis: F	Parametric-Two	Sample		Offic	ial Results:	Yes
Sample Note:	0 sp	oike							
Data Transfor	m	Zeta	Alt Hyp	Trials	Seed		PMSD	Test Resul	t
Angular (Corre	cted)	NA	C > T	NA	NA		4.76%	Fails fertiliz	ation rate
Equal Variance	e t T	wo-Sample Test							
Control	vs	C-%	Test St	at Critical	MSD DF	P-Value	P-Type	Decision(a	:5%)
Lab Control		100*	2.767	1.86	0.059 8	0.0122	CDF	Significant	Effect
ANOVA Table									
Source		Sum Squares	Mean S	quare	DF	F Stat	P-Value	Decision(a	::5%)
Between		0.01911926	0.01911	926	1	7.656	0.0244	Significant	Effect
Error		0.0199794	0.00249	7425	8				
Total		0.03909867			9				
Distributional	Test	s							
Attribute		Test		Test Stat	Critical	P-Value	Decision(	α:1%)	
			_	0.700	00.45	0.0000			

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 f	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 (Core	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%		



**Echinoid Sperm Cell Fertilization Test 15C** 

Report Date:

27 Dec-17 09:28 (p 1 of 1)

Test Code:

1712-S044 | 00-7348-8394

Nautilus Environmental (CA)

06-8315-5458 Analysis ID: Analyzed: 27 Dec-17 9:28

Endpoint: Fertilization Rate Analysis:

Linear Interpolation (ICPIN)

**CETIS Version:** Official Results: Yes

CETISv1.8.7

Sample Note: 0 spike

l	Linear	Interpo	lation	Options	
ı					

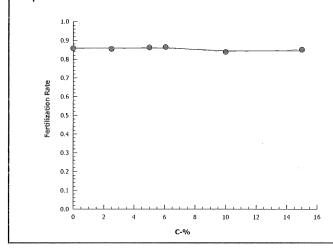
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method	
Linear	Linear	45533	1000	Yes	Two-Point Interpolation	

#### 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

Fertilizati	ion Rate Summary		Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
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



TST

Report Date: Test Code: 02 Jan-18 13:45 (p 2 of 2) 1712-S044 | 00-7348-8394

								Code:			
Echinoid Spe	erm Cell Fertiliza	tion Tes	t 15C						Nautilus	Environr	nental (CA)
Analysis ID:	19-4404-6184	Ε	ndpoint: F	ertilization Rat	е		CET	S Version:	CETISv1.	8.7	
Analyzed:	27 Dec-17 9:28		-	arametric Bioe		Two Sample	e Offic	ial Results:	Yes		
Sample Note	: 0 spike										
Data Transfo	rm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corre	ected)	NA	C*b < T	NA	NA	0.75	4.47%	15	>15	NA	6.667
TST-Welch's	t Test										
Control	vs C-%		Test St	at Critical	MSD DF	P-Value	P-Type	Decision(	a:5%)		
Lab Control	2.5*		7.499	1.943	0.076 6	0.0001	CDF	Non-Signif	icant Effect		
ı	5*		10.2	1.895	0.056 7	<0.0001	CDF	Non-Signif	icant Effect		
	6.06*		9.861	1.895	0.059 7	<0.0001	CDF	Non-Signif	icant Effect		
	10*		8.783	1.895	0.058 7	<0.0001	CDF	Non-Signif	icant Effect		
ı	15*		9.669	1.895	0.056 7	<0.0001	CDF	Non-Signif	icant Effect		
ANOVA Table	9										
Source	Sum Squ	ares	Mean S	quare	DF	F Stat	P-Value	Decision(	a:5%)		
Between	0.004634	14	0.00092	68279	5	0.3	0.9080	Non-Signif	icant Effect		
Error	0.074142	52	0.00308	39271	24						
Total	0.0787766	36			29						
Distributiona	ıl Tests										
	nl Tests Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Attribute	Test	equality of	f Variance								
	Test Bartlett E		f Variance ormality	Test Stat 1.415 0.9723	Critical 15.09 0.9031	P-Value 0.9227 0.6026	Decision Equal Var Normal D	riances			
Attribute Variances Distribution	Test Bartlett E Shapiro-			1.415	15.09	0.9227	Equal Var	riances			
Attribute Variances Distribution Fertilization	Test Bartlett E			1.415	15.09	0.9227	Equal Var	riances	Std Err	CV%	%Effect
Attribute Variances Distribution Fertilization	Test  Bartlett E Shapiro-N	Wilk W N	ormality	1.415 0.9723	15.09 0.9031	0.9227 0.6026	Equal Var Normal D	iances istribution	Std Err 0.01855	CV% 4.83%	%Effect
Attribute Variances Distribution Fertilization C-% 0	Test  Bartlett E Shapiro-V  Rate Summary  Control Type	Count	Mean 0.858	1.415 0.9723 95% LCL 0.8065	15.09 0.9031 95% UCL	0.9227 0.6026 Median	Equal Var Normal D	iances istribution Max 0.92			
Attribute Variances Distribution Fertilization C-% 0 2.5	Test  Bartlett E Shapiro-V  Rate Summary  Control Type	Count 5 5	Mean 0.858 0.854	1.415 0.9723 <b>95%</b> LCL	15.09 0.9031 <b>95% UCL</b> 0.9095	0.9227 0.6026 Median 0.84	Equal Var Normal D Min 0.82	Max 0.92 0.91	0.01855	4.83%	0.0%
Attribute Variances Distribution Fertilization C-% 0 2.5 5	Test  Bartlett E Shapiro-V  Rate Summary  Control Type	Count 5 5 5	Mean 0.858	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223	15.09 0.9031 95% UCL 0.9095 0.9183	0.9227 0.6026 <b>Median</b> 0.84 0.87	Equal Var Normal D Min 0.82 0.79	iances istribution Max 0.92	0.01855 0.02315	4.83% 6.06%	0.0% 0.47%
Attribute Variances Distribution Fertilization C-% 0 2.5 5 6.06	Test  Bartlett E Shapiro-V  Rate Summary  Control Type	Count 5 5 5 5	Mean 0.858 0.854 0.862 0.864	1.415 0.9723 95% LCL 0.8065 0.7897	15.09 0.9031 95% UCL 0.9095 0.9183 0.9017	0.9227 0.6026 Median 0.84 0.87 0.87 0.85	Min 0.82 0.79 0.82	Max 0.92 0.91 0.9 0.92	0.01855 0.02315 0.01428	4.83% 6.06% 3.71% 3.72%	0.0% 0.47% -0.47% -0.7%
Attribute Variances Distribution Fertilization C-% 0 2.5 5	Test  Bartlett E Shapiro-V  Rate Summary  Control Type	Count 5 5 5	Mean 0.858 0.854 0.862	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242	15.09 0.9031 95% UCL 0.9095 0.9183 0.9017 0.9038	0.9227 0.6026 Median 0.84 0.87 0.87	Equal Var Normal D Min 0.82 0.79 0.82 0.84	Max 0.92 0.91 0.9	0.01855 0.02315 0.01428 0.01435	4.83% 6.06% 3.71%	0.0% 0.47% -0.47%
Attribute Variances Distribution Fertilization C-% 0 2.5 5 6.06 10 15	Test  Bartlett E Shapiro-V  Rate Summary  Control Type	Count 5 5 5 5 5 5 5 5 5 5	Mean 0.858 0.854 0.862 0.864 0.838 0.85	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884	0.9227 0.6026 Median 0.84 0.87 0.87 0.85 0.84	Min 0.82 0.79 0.82 0.84 0.78	Max 0.92 0.91 0.9 0.92 0.97	0.01855 0.02315 0.01428 0.01435 0.01655	4.83% 6.06% 3.71% 3.72% 4.42%	0.0% 0.47% -0.47% -0.7% 2.33%
Attribute Variances Distribution Fertilization C-% 0 2.5 5 6.06 10 15	Test  Bartlett E Shapiro-I  Rate Summary  Control Type  Lab Control	Count 5 5 5 5 5 5 7 med Sur	0.858 0.854 0.862 0.864 0.838 0.85	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884 0.8912	0.9227 0.6026 Median 0.84 0.87 0.87 0.85 0.84 0.85	Min 0.82 0.79 0.82 0.84 0.78	Max 0.92 0.91 0.9 0.92 0.97	0.01855 0.02315 0.01428 0.01435 0.01655	4.83% 6.06% 3.71% 3.72% 4.42%	0.0% 0.47% -0.47% -0.7% 2.33%
Attribute Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Corr	Test Bartlett E Shapiro-I Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 7 med Sur	0.858 0.854 0.862 0.864 0.838 0.85	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792 0.8088	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884 0.8912	0.9227 0.6026 Median 0.84 0.87 0.87 0.85 0.84 0.85	Min 0.82 0.79 0.82 0.78 0.84 0.78	Max 0.92 0.91 0.9 0.92 0.87 0.88	0.01855 0.02315 0.01428 0.01435 0.01655 0.01483	4.83% 6.06% 3.71% 3.72% 4.42% 3.9%	0.0% 0.47% -0.47% -0.7% 2.33% 0.93%
Attribute Variances Distribution  Fertilization (C-%) 0 2.5 5 6.06 10 15  Angular (Cor C-%)	Test Bartlett E Shapiro-N Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 5 cmed Sur	Mean 0.858 0.854 0.862 0.864 0.838 0.85	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792 0.8088	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884 0.8912	0.9227 0.6026 Median 0.84 0.87 0.87 0.85 0.84 0.85	Min 0.82 0.79 0.82 0.78 0.84 0.78 0.8	Max 0.92 0.91 0.9 0.92 0.87 0.88	0.01855 0.02315 0.01428 0.01435 0.01655 0.01483	4.83% 6.06% 3.71% 3.72% 4.42% 3.9%	0.0% 0.47% -0.47% -0.7% 2.33% 0.93%
Attribute Variances Distribution Fertilization (C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Test Bartlett E Shapiro-N Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 7 med Sur Count 5	Mean 0.858 0.854 0.862 0.864 0.838 0.85  nmary Mean 1.188	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792 0.8088	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884 0.8912 95% UCL 1.266	0.9227 0.6026 Median 0.84 0.87 0.85 0.84 0.85 Median 1.159	Min 0.82 0.79 0.82 0.84 0.78 0.8	Max 0.92 0.91 0.9 0.92 0.87 0.88	0.01855 0.02315 0.01428 0.01435 0.01655 0.01483 Std Err 0.02805	4.83% 6.06% 3.71% 3.72% 4.42% 3.9% CV% 5.28%	0.0% 0.47% -0.47% -0.7% 2.33% 0.93% %Effect
Attribute Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor C-% 0	Test Bartlett E Shapiro-N Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 cmed Sur Count 5 5	Mean 0.858 0.854 0.862 0.864 0.838 0.85 nmary Mean 1.188 1.183	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792 0.8088 95% LCL 1.11 1.092	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884 0.8912 95% UCL 1.266 1.274	0.9227 0.6026 Median 0.84 0.87 0.85 0.84 0.85 Median 1.159 1.202	Min 0.82 0.79 0.82 0.84 0.78 0.8 Min 1.133 1.095	Max 0.92 0.91 0.9 0.92 0.87 0.88	0.01855 0.02315 0.01428 0.01435 0.01655 0.01483 Std Err 0.02805 0.0328	4.83% 6.06% 3.71% 3.72% 4.42% 3.9% CV% 5.28% 6.2%	0.0% 0.47% -0.47% -0.7% 2.33% 0.93% %Effect 0.0% 0.4%
Attribute Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor C-% 0 2.5 5 5	Test Bartlett E Shapiro-N Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 Count Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.858 0.854 0.862 0.864 0.838 0.85 nmary Mean 1.188 1.183 1.192	1.415 0.9723 95% LCL 0.8065 0.7897 0.8223 0.8242 0.792 0.8088 95% LCL 1.11 1.092 1.135	95% UCL 0.9095 0.9183 0.9017 0.9038 0.884 0.8912 95% UCL 1.266 1.274 1.249	0.9227 0.6026 Median 0.84 0.87 0.85 0.84 0.85 Median 1.159 1.202 1.202	Min 0.82 0.79 0.82 0.84 0.78 0.8 Min 1.133 1.095 1.133	Max 0.92 0.91 0.9 0.92 0.87 0.88 Max 1.284 1.266 1.249	0.01855 0.02315 0.01428 0.01435 0.01655 0.01483 Std Err 0.02805 0.0328 0.02071	4.83% 6.06% 3.71% 3.72% 4.42% 3.9% CV% 5.28% 6.2% 3.88%	0.0% 0.47% -0.47% -0.7% 2.33% 0.93% %Effect 0.0% 0.4% -0.36%

TSI

Report Date: Test Code:

02 Jan-18 13:45 (p 1 of 2)

1712-S044 | 00-7348-8394

							Test	Code:	171	2-S044   0i	0-7348-839 <sub>1</sub>
Echinoid Spe	rm Cell Fertiliza	ation Test 1	15C						Nautilus	s Environr	nental (CA
Analysis ID: Analyzed:	19-7334-1766 02 Jan-18 13:4			tilization Rat		·Two Samp		S Version: ial Results:	CETISv1 Yes	.8.7	
Sample Note:	0 spike										
Data Transfor	m	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	Test Resu	ılt		
Angular (Corre	cted)	NA	C*b < T	NA	NA	0.75	3.84%	Passes fe	rtilization ra	te	
TST-Welch's t	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-Signi	ficant Effect		
ANOVA Table		de encontra de encontrato de de una licula contra de del secue de debe								eskeptusekonikinuossa kitautunosoo ohka suurimuun	https://www.com/com/com/com/com/com/com/com/com/com/
Source	Sum Squ	ares	Mean Sq	uare	DF	F Stat	P-Value	Decision(	α:5%)		
Between	0.0191192	26	0.0191192	26	1	7.656	0.0244	Significant	Effect		***************************************
Error	0.0199794	4	0.002497	125	8			-			
Total	0.0390986	67			9						
Distributional	Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(	a:1%)			
Variances	Variance	Ratio F	da su destina hora su rabrido e qui caha di contenent e cu discussor	3.709	23.15	0.2323	Equal Var	iances			
Distribution	Shapiro-\	Wilk W Nor	mality	0.9152	0.7411	0.3188	Normal Di	stribution			
Fertilization R	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 (Corr	ected) Transfor	med Sumn	nary								
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%

Report Date:

14 Dec-17 14:59 (p 1 of 1)

Test Code:

Nautilus Environmental (CA)

**Echinoid Sperm Cell Fertilization Test 15C** 

Start Date:15 Dec-17Species:Strongylocentrotus purpuratusSample Code:AEF 330 PWGEnd Date:15 Dec-17Protocol:EPA/600/R-95/136 (1995)Sample Source:IDE Americas, Inc.Sample Date:07 Dec-17Material:Product TestingSample Station:M-INF X SOURCE

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	90	12/19/17
			32	100	87	12   1   { }
			33	100	79	
			34	100	78	
			35	100	78 89	
			36	100	91	
			37	(00)	83 84	
			38	100	84	
			39	(00)	81	
			40	100	_88	
			41	100	92	
			42	001	88 37 92 85 85	
			43	100	97	
			44	100	82	
			45	100	85	
	_		46	100	89	
			47 48	100	82 86	
			49	100	86	
			50	100	83 80	
			51	100	00	
			52		97 84	
			53	100	0,-	
			54	100	84	
			55	100	88	
			56	100	85	
			57	(00)	88	
			58	ia	81	
			59	106	86 91 92	
			60	100	87	
	1		1			
				100	81	
	10	0 1mf	A		and the second s	
	10	0 11	FB	100	93	
	101	) 184F		100	79	
	100	148	= D = E	[ 100	77	
	100	INF	E	100	M	

QAP 9812/22/17

Analyst: CQA: AO

#### **CETIS Test Data Worksheet**

Report Date:

14 Dec-17 14:58 (p 1 of 1)

Test Code:

Nautilus Environmental (CA)

**Echinoid Sperm Cell Fertilization Test 15C** 

Start Date: End Date:

15 Dec-17 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  $N < n_1 V_2$ 

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			30 12/19/11
2.5		1	39			·
2.5	<u> </u>	2	33			
2.5		3	36	/00	86	56 12/18/17
2.5		4	35			701.2/1
2.5		5	60			
5		1	42	100	87	50 12/18/17
5		2	51	1 2		
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			

BA=018 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

Dilutions made by: Ato OBO E 61

Test No: 1712-S044

			Analyst:	CG							
		Initial Readings									
Concentration %	DO (mg/L)	p									
Lab Control	8.2	7.99	33.7.	(°c) (5.7							
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	G.2	8.00	339.	15.7							
15	8.2	7.98	33.9	15.8							
100% M-INF	& NM	7.74	33.9	15.8							
	1/2/18										

400						
Co	m	m	e	n	ts	:

NM = Not Measured, Tech Error

QC Check:

Final Review:  $\frac{1}{2/18}$ 

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

## Marine Chronic Bioassay

## **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.: Tech initials: Injection Time:	1427 1427	1)(0			End Date/Tir		1 1546
Sperm Absorbance at 4	00 nm:	(target range of	0.8 - 1.0 for d	density of 4	x10 <sup>6</sup> sperm/r	ni)	
Eggs Counted:	107 (targ	et counts of 80 eggs or slide for a final dem	per vertical pa	ss on Sedgw	gs/ml vick-		
Initial density: Final density:	4000 eggs/ml	- <u>1.0</u> par	tion factor t egg stock ts seawater	0.0		35.75 ml	
Prepare the embryo stock (1 part) and 125 m	ck according to the calcu nl of dilution water (1.25 p	lated dilution factor parts).	. For exampl	le, if the dilu	ution factor is	s 2.25, use 100	0 ml of existing
			Sperm:Ec	gg Ratio			
Rangefinder Test: ml Sperm Stock ml Seawater	<b>2000:1 1600 50 40 0.0 10</b>	30	800:1 20 30	400:1 10 40	200:1 5.0 45	2.5 47.5	50:1 1.25 48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1435 1445 1465	Rangefinder Rati	60: Fert. 97 98 100	Unfe 3/94 7, 2	ert. <u>(</u> 		
NOTE: Choose a sperm this range, choose the ra health, stage of reproduc	atio closest to 90 percent	unless professiona	een 80 and 9 al judgment d	00 percent. ictates con	If more than sideration of	one concentr other factors (	ation is within (e.g., organism
Definitive Test	•	Sperm:Egg Ratio	Used:	50:1			
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1506 1926 1946	QC1 QC2 Egg Control 1 Egg Control 2	Fert.	Unfe 1 10 10	ert. 2 3 — 0		4
Comments:					×		
QC Check:	PO 12/22/1				Final Revie	w: Eq 1	2/18

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



## **CETIS Summary Report**

Report Date:

27 Dec-17 09:32 (p 1 of 1)

Test Code:

1712-S045 | 15-0310-3785

Echinoid Spe	rm Cell Fertiliza	tion Tes	t 15C							Nautilus	s Environr	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	04-7096-3221 15 Dec-17 15:0 15 Dec-17 15:4 40m	06 F	est Type: Protocol: Species: Source:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	, ,	tus	C E	nalyst: Diluent: Brine: .ge:		ıral Seawate Applicable	er	
	10-6913-7290 07 Dec-17 10:0 07 Dec-17 12:0 8d 5h	00 <b>N</b> 08 S	Code: //aterial: Source: Station:	AEF 330 PWG Product Testing IDE Americas, M-INF	9	1247 sp:ke		ilient: Project:	IDE Spik	ing Study		
Sample Note:	0.1 mg/L Spike											
Comparison S	Summary								,			
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meth	od			
12-7454-2823	Fertilization Ra	te	15	>15	NA	10.8% <	6.667	Dunr	ett M	Iultiple Com	parison Te	st
Point Estimate	e Summary	200 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1										
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Meth	od			
13-1247-3580	Fertilization Ra	te	EC25 EC50	>15 >15	N/A N/A	N/A N/A	<6.667 <6.667		r Inte	erpolation (I	CPIN)	
Test Acceptab	oility		No the control of the									
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limit	ts	Over	lap	Decision		
12-7454-2823	Fertilization Rat	te	Contro	ol Resp	0.734	0.7 - NL		Yes	···········	Passes A	cceptability	Criteria
13-1247-3580	Fertilization Rat			ol Resp	0.734	0.7 - NL		Yes			cceptability	
12-7454-2823	Fertilization Rat	te	PMSD		0.1082	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	ate Summary											
	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std E	Err	Std Dev	CV%	%Effect
i	Lab Control	5	0.734	0.6932	0.7748	0.69	0.77	0.014		0.03286	4.48%	0.0%
2.5		5	0.732	0.6357	0.8283	0.61	0.81	0.034		0.07759	10.6%	0.27%
5		5	0.732	0.6599	0.8041	0.65	0.79	0.025		0.05805	7.93%	0.27%
6.06 10		5 5	0.784 0.75	0.7493 0.7127	0.8187 0.7873	0.75 <sub>.</sub> 0.72	0.81 0.8	0.012 0.013		0.02793 0.03	3.56% 4.0%	-6.81% -2.18%
15		5	0.758	0.6828	0.7673	0.72	0.81	0.013		0.06058	7.99%	-2.16% -3.27%
Fertilization R	ate Detail		And the same of th									
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	8.0						
15		0.67	0.72	0.8	0.81	0.79						

Analyst: QA: EC 12/18

Report Date:

27 Dec-17 09:32 (p 1 of 2)

Test Code:

1712-S045 | 15-0310-3785

Echinoid Sp	erm Cell Fertiliza	ation Test	15C	**************************************					Nautilus	Environ	mental (CA)
Analysis ID: Analyzed:	12-7454-2823 27 Dec-17 9:3			ertilization Rat arametric-Cor		tments		IS Version		.8.7	
Sample Note	e: 0.1 mg/L Spike	9									
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	***************************************	PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Cor	rected)	NA	C > T	NA	NA		10.8%	15	>15	NA	6.667
Dunnett Mul	tiple Comparisor	n Test									
Control	vs C-%		Test Sta	at Critical	MSD DF	P-Value	P-Type	Decision	n(α:5%)		
Lab Control	2.5		-0.00302	2 2.362	0.087 8	0.8342	CDF	Non-Sigr	nificant Effect		
	5		0.02961	2.362	0.087 8	0.8244	CDF	Non-Sigr	nificant Effect		
	6.06		-1.592	2.362	0.087 8	0.9972	CDF	Non-Sigr	nificant Effect		
	10		-0.4976	2.362	0.087 8	0.9398	CDF	Non-Sigr	nificant Effect		
	15		-0.7951	2.362	0.087 8	0.9710	CDF	Non-Sigr	nificant Effect		
ANOVA Tabl	le										
Source	Sum Squ	ıares	Mean S	quare	DF	F Stat	P-Value	Decision	η(α:5%)		
Between	0.013903	25	0.00278	0651	5	0.8212	0.5467	Non-Sigr	nificant Effect		
Error	0.081266	65	0.00338	611	24						
Total	0.095169	9	WARRANT		29	**************************************			***************************************		
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(a:1%)			
Variances	Bartlett E	Equality of '	Variance	5.858	15.09	0.3202	Equal Var	iances			***************************************
Distribution	Shapiro-	Wilk W No	rmality	0.9622	0.9031	0.3520	Normal Di	stribution			
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	8.0	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 (Co	rrected) Transfor	med Sum	тагу								
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%
4.0		5	1.048	1.004	1.092	1.036	1.013	1.107	0.01583	3.38%	-1.78%
10 15		5	1.059	0.9722	1.145	1.095	0.9589	1.12	0.03116	6.58%	-2.84%

Report Date: Test Code: 27 Dec-17 09:32 (p 2 of 2) 1712-S045 | 15-0310-3785

**Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) Analysis ID: 12-7454-2823 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 27 Dec-17 9:31 Analyzed: Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 1.0 F 0.10 0.08 0.9 0.06 0.8 0.04 Fertilization Rate 0.7 0.02 0.00 0.6 -0.02 0.5 -0.04 0.4 -0.06 -0.08 0.3 0.2 -0.12 0.1 -0.14 0.0 0 LC 2.5 10 15 -2.5 -2.0 -1.0 -1.5 -0.5 0.0 0.5 1.0 1.5 2.0 C-% Rankits

Report Date:

27 Dec-17 09:32 (p 1 of 1)

Test Code:

1712-S045 | 15-0310-3785

Nautilus Environmental (CA)

Analysis ID:

13-1247-3580 27 Dec-17 9:31

**Echinoid Sperm Cell Fertilization Test 15C** 

Endpoint: Fertilization Rate

Analysis:

Linear Interpolation (ICPIN)

**CETIS Version:** Official Results:

CETISv1.8.7

Yes

Sample Note: 0.1 mg/L Spike

Linear	Interpolation	Options
--------	---------------	---------

ı	zinour interpora	aon options				
L	X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
	Linear	Linear	1256196	1000	Yes	Two-Point Interpolation
-		TOWNS OF THE PARTY				

#### Point Estimates

Graphics

Analyzed:

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

Fertilizati	on Rate Summary		Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	В
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	8.0	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

# 1.0 0.9 0.8 0.1

C-%

10

12

Report Date:

27 Dec-17 09:32 (p 1 of 1)

Test Code:

1712-S045 | 15-0310-3785

						<del></del>					0 0010 0700
Echinoid Sp	erm Cell Fertiliza	ation Te	st 15C						Nautilus	Environi	mental (CA)
Analysis ID:	17-1623-5114		Endpoint: Fer	tilization Rat	te		CET	IS Version	: CETISv1	.8.7	
Analyzed:	27 Dec-17 9:3	2	Analysis: Par	ametric Bioe	equivalence	-Two Samp	le <b>Offic</b>	ial Results	s: Yes		
Sample Note	e: 0.1 mg/L Spike	9									-
Data Transfe	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	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	n(α:5%)		
Lab Control	2.5*		6.353	2.132	0.086 4	0.0016	CDF	Non-Sigr	nificant Effect		
	5*		8.087	2.015	0.064 5	0.0002	CDF	Non-Sigr	nificant Effect		
	6.06*		16.14	1.895	0.037 7	<0.0001	CDF	Non-Sigr	nificant Effect		
	10*		13.7	1.895	0.038 7	<0.0001	CDF	Non-Sigr	nificant Effect		
	15*		8.545	2.015	0.068 5	0.0002	CDF	Non-Sigr	nificant Effect		
ANOVA Tabl	е										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	n(α:5%)		
Between	0.013903	25	0.0027806	551	5	0.8212	0.5467	Non-Sigr	nificant Effect		
Error	0.081266	65	0.0033861	1	24						
Total	0.095169	9			29						
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(a:1%)			
Variances	Bartlett E	auality o	of Variance	5.858	15.09	0.3202	Equal Var			<del>//</del>	
Distribution			Vormality	0.9622	0.9031	0.3520	Normal D				
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 (Co	rrected) Transfor	med Su	mmary								
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%

## **CETIS Test Data Worksheet**

Report Date: Test Code:

14 Dec-17 15:00 (p 1 of 1)

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Start Date: 15 Dec-17 End Date:

15 Dec-17

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

Sample Source: IDE Americas, Inc.

Sample Code: AEF 330 PWG

Sample Date: 07 Dec-17

Material: Product Testing

Sample Station: M-INF

111

	te: 0/1				al: Product Te	sting		Sample	Station: M-INF	O.Imgic Spi
C-%	Code	Rep		# Counted	# Fertilized				Notes	0. 7
			61	10D	174	12/19	117			9 90000
			62	100	71	1				
		/	63	100	69					
			64	100	79		2000/74414			
			65	100	67				PARKETTI	
			66	100	77					
			67	100	79					
			68	100	76					
			69	100	75					
			70	100	74				***************************************	
			71 72	100	70					
			73	100	79 75					
			74	100	78					
			75	100						
			76	100	7-1					
			77	100	G1 72					
		-	78	(00)	75					
			79	100 100	81					
			80	,	81					
			81	100 100	65					***************************************
	1		82	(00	81		70174			
			83	100	80					
			84	100	74					
			85	100	72					
			86	(00)	75					
			87	100	75					
			88	100	78					
			89	100	81	B	7			
			90	100	90					

Bpm Q18 12/22/17

#### **CETIS Test Data Worksheet**

Report Date: Test Code: 14 Dec-17 15:00 (p 1 of 1)

15-0310-3785/<del>59978B2</del>9

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Start Date:15 Dec-17Species:Strongylocentrotus purpuratusSample Code:AEF 330 PWGEnd Date:15 Dec-17Protocol:EPA/600/R-95/136 (1995)Sample Source:IDE Americas, Inc.County Protocol:17 Dec-17Protocol:EPA/600/R-95/136 (1995)Sample Source:IDE Americas, Inc.

Sample Date: 07 Dec-17 Material: Product Testing Sample Station: M-INF Original Spike

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes Notes
0	LC	1	73	A76 100	7.6	ACS 12/17/17
0	LC	2	66	100	76 76	ACS 12/17/17
0	LC	3	87		,	
0	LC	4	63			
0	LC	5	62			
2.5		1	88			
2.5		2	76	100	άĩ	ñ(5 12/17/17
2.5		3	75			100
2.5		4	69			
2.5		5	82			
5		1	84			
5		2	72		- VILLEY	
5		3	81	100	044 73	AG 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	£85 100	85	AG 12/17/17
6.06		5	64			
10		1	85		1100000	
10		2	86			
10		3	70			
10		4	61			
10		5	90	100	69	19(5 12/17/17
15		1	65			
15		2	77			
15		3	83			
15		4	89			
15		5	67	100	79	AG 12/17/17

QC: EG

@ Q18 415 12/17/17

@AOQ18 12/12/17

### **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 1500

Sample Log No.:

17- 1247 Eags 1/2/18

End Date/Time: 12/15/2017 154(p

Dilutions made by: AO (NO) E(3

Test No: 1712-5045

			Analyst:	CG						
		Initial Readings								
Concentration	DO	pН	Salinity	Temperature						
% Sample	(mg/L)	(units)	(ppt)	(°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:	A0 12/22/17	Final Review:	EC 1/2/18

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

## Marine Chronic Bioassay

## **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.:	1712-5045	IL SPLIO AF	<u>F</u> 330 PWG	Ε	nd Date/Tim	e: 12/15/2017 e: 12/15/2017 es: S. purpurat e: β\$ . L	1 1546 tus
Tech initials: Injection Time:	- FG 1427			D	ate Collecte		17
Sperm Absorbance at 4	00 nm:	(target range of	0.8 - 1.0 for d	ensity of 4x	10 <sup>6</sup> sperm/m	ıl)	
Eggs Counted:	107 (targe	n: 162 X 5 It counts of 80 eggs p r slide for a final dens	per vertical pas sity of 4000 egg	s on Sedgwid			
Initial density: Final density:	4000 eggs/ml	- <u>1.0</u> part	tion factor egg stock s seawater	egg : seaw		00 ml 5.75 ml	
Prepare the embryo stock (1 part) and 125 m	ck according to the calcul nl of dilution water (1.25 p	ated dilution factor. arts).	For example	e, if the diluti	ion factor is	2.25, use 100	ml of existing
			Sperm:Eg	g Ratio			
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1     1600       50     40       0.0     10	1200:1 30 20	800:1 20 30	400:1 10 40	200:1 5.0 45	2.5 47.5	50:1 1.25 48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1435 1445 1455	Rangefinder Rati	0: <u>Fert.</u> 93 100	Unfer 3/94 7/ 2 0	t. <u>6</u> - -		
this range, choose the ra	n-to-egg ratio that results atio closest to 90 percent ctive season, site conditio	unless professiona	een 80 and 90 I judgment die	D percent. If ctates consid	more than deration of c	one concentra other factors (e	tion is within ∍.g., organism
<u>Definitive Test</u>		Sperm:Egg Ratio	Used:	50:1	_		
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1506 1926 1946	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 84 0 0	Unfert 1 8 100	2		4
Comments:							
OC Charles	A- 10300A					ρ	lalia
QC Check:	1 1/2 1/0/	<u>Y</u> 1		l	Final Review	1: KU !	0112

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



## **CETIS Summary Report**

Report Date:

28 Dec-17 15:09 (p 1 of 1)

Test Code:

1712-S046 | 12-2045-5598

		***************************************						est Code.		111	2-3040   12	2-2043-338
Echinoid Spe	rm Cell Fertiliza	ition Test 1	15C							Nautilus	s Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	18-7729-2146 15 Dec-17 15:0 15 Dec-17 15:4 40m	)6 Pro 16 Spo	otocol: ecies:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	•	tus	E	Analyst: Diluent: Brine: Age:		ıral Seawate Applicable	er	
-	14-2322-1328 07 Dec-17 10:0 : 07 Dec-17 12:0 8d 5h	)8 <b>So</b> i	terial: urce:	AEF 330 PWG Product Testing IDE Americas, M-INF 0.5	9	.i		Client: Project:	IDE Spik	ing Study		
Sample Note:	0.5 mg/L spike											
Comparison §	Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meti	hod			
11-9358-4860		te	15	>15	NA	11.0% .	< 6.667	Dun	nett N	lultiple Com	parison Te	st
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Met	hod			
02-1745-5572	Fertilization Ra	te	EC25 EC50	>15 >15	N/A N/A	N/A N/A	<6.66 <6.66		ar Inte	erpolation (l	CPIN)	
Test Acceptat	oility											
Analysis ID	Endpoint		Attribu	ute	Test Stat	TAC Lim	its	Ove	rlap	Decision		
02-1745-5572	Fertilization Ra	te	Contro	l Resp	0.734	0.7 - NL		Yes		Passes A	cceptability	Criteria
11-9358-4860	Fertilization Ra	te	Contro	l Resp	0.734	0.7 - NL		Yes		Passes A	cceptability	Criteria
11-9358-4860	Fertilization Ra	te	PMSD		0.11	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization F	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	0.01	503	0.03362	4.58%	0.0%
2.5		5	0.756	0.6911	0.8209	0.68	0.81	0.02	337	0.05225	6.91%	-3.0%
5		5	0.762	0.6823	0.8417	0.67	0.82	0.02		0.06419	8.42%	-3.82%
6.06		5	0.748	0.6645	0.8315	0.66	0.82	0.03		0.06723	8.99%	-1.91%
10		5	0.768	0.7088	0.8272	0.69	0.82	0.02		0.04764	6.2%	-4.63%
15		5	0.774	0.7372	0.8108	0.74	0.81	0.01	327	0.02966	3.83%	-5.45%
Fertilization F												
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		8.0	0.75	0.81	0.68	0.74						
5		0.72	0.82	0.67	8.0	8.0						
6.06		8.0	0.76	0.7	0.82	0.66						
10		0.78	0.78	0.69	0.77	0.82						
15		0.76	0.74	8.0	0.76	0.81						

Report Date:

28 Dec-17 15:08 (p 1 of 2)

Test Code:	1712-S046	12-2045-5598

							rest	Coue.	1712	-3040   1.	2-2040-0090
Echinoid Sp	perm Cell Fertiliza	ition Test 1	5C						Nautilus	Environr	nental (CA)
Analysis ID: Analyzed:	11-9358-4860 28 Dec-17 15:0			ertilization Rat arametric-Con		tments		IS Version: ial Results		8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C > T	NA	NA		11.0%	15	>15	NA	6.667
Dunnett Mul	Itiple Comparisor	n Test									
Control	vs C-%		Test Sta	at Critical	MSD DF	P-Value	P-Type	Decision	(a:5%)		
Lab Control	2.5		-0.7021	2.362	0.088 8	0.9632	CDF	Non-Sign	ificant Effect		
	5		-0.9137	2.362	0.088 8	0.9789	CDF		ificant Effect		
	6.06		-0.4813	2.362	0.088 8	0.9374	CDF	Ŭ	ificant Effect		
	10		-1.073	2.362	0.088 8	0.9864	CDF	_	ificant Effect		
	15		-1.241	2.362	0.088 8	0.9917	CDF	_	ificant Effect		
ANOVA Tabl	le										
Source	Sum Squ	ares	Mean S	quare	DF	F Stat	P-Value	Decision	ı(α:5%)		
Between	0,007064	687	0.00141	2937	5	0.4031	0.8418	Non-Sign	ificant Effect		
Error	0.084128		0.00350		24			J			
Total	0.091192				29						
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	Equality of \	/ariance	3.459	15.09	0.6296	Equal Var	iances			
Distribution		Wilk W Nor		0.9518	0.9031	0.1883	Normal D				
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	8.0	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 (Co	rrected) Transfor	med Sumr	nary								
Angular (Co C-%	orrected) Transfor	med Sumr Count	nary Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
•	•		-	<b>95% LCL</b> 0.9812	95% UCL 1.078	Median	Min 1.002	Max 1.095	Std Err 0.01741	CV% 3.78%	%Effect
C-%	Control Type	Count	Mean								
<b>C-%</b>	Control Type	Count 5	Mean 1.03	0.9812	1.078	1.013	1.002	1.095	0.01741	3.78%	0.0%
C-% 0 2.5	Control Type	Count 5 5	Mean 1.03 1.056	0.9812 0.9807	1.078 1.131	1.013 1.047	1.002 0.9695	1.095 1.12	0.01741 0.02706	3.78% 5.73%	0.0% -2.55%
C-% 0 2.5 5	Control Type	5 5 5	1.03 1.056 1.064	0.9812 0.9807 0.9716	1.078 1.131 1.156	1.013 1.047 1.107	1.002 0.9695 0.9589	1.095 1.12 1.133	0.01741 0.02706 0.03322	3.78% 5.73% 6.98%	0.0% -2.55% -3.32%

Report Date: Test Code: 28 Dec-17 15:09 (p 2 of 2) 1712-S046 | 12-2045-5598

**Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) CETISv1.8.7 Analysis ID: 11-9358-4860 Endpoint: Fertilization Rate **CETIS Version:** 28 Dec-17 15:08 Analysis: Parametric-Control vs Treatments Official Results: Yes Analyzed: Graphics 0.10 1.0 0.08 0.9 0,06 0.8 -0-Fertilization Rate 0.04 0.7 Centered Corr. Angle 0.02 Reject Null 0.6 0,00 0.5 -0.02 0.4 -0,04 0.3 -0.06 0.2 -0.08 0.1 -0.10 0.0 -0.12 0 LC 6.06 10 15 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 0.5 1.0 1.5 2.0 C-% Rankits

Report Date: Test Code:

28 Dec-17 15:09 (p 1 of 1)

1712-S046 | 12-2045-5598

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

02-1745-5572 Analysis ID: Analyzed: 28 Dec-17 15:08 Endpoint: Fertilization Rate Analysis:

Linear Interpolation (ICPIN)

CETIS Version: Official Results: Yes

CETISv1.8.7

	Linea	r Interpo	lation	Options
--	-------	-----------	--------	---------

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
	The state of the s			NOTICE AND ADDRESS OF THE PARTY	THE RESERVE OF THE PERSON NAMED IN

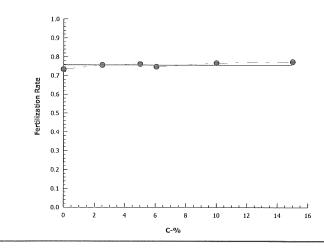
Linear Linear 1000 Two-Point Interpolation 1081274 Yes

#### 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	В
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: 15/AC QA: C13/78/7

000-089-187-4

CETIS™ v1.8.7.20

Report Date:

28 Dec-17 15:10 (p 1 of 1)

Test Code:

1712-S046 | 12-2045-5598

							Test	Code:	171	2-S046   1	2-2045-559
Echinoid Sp	oerm Cell Fertiliz	ation Test	15C	757	and the second s				Nautilus	s Environi	mental (CA)
Analysis ID:			dpoint: Fe					IS Version	: CETISv1	.8.7	OSSET CASIAN CONTRACTOR AND
Analyzed:	28 Dec-17 15:	09 <b>A</b> n	ialysis: Pa	rametric Bio	equivalence-	-Two Sampl	e Offic	ial Results	s: Yes		
Data Transf	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C*b < T	NA	NA	0.75	4.71%	15	>15	NA	6.667
TST-Welch's	s t Test	**************************************									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	ι(α:5%)		
Lab Control	2.5*		9.441	2.015	0.061 5	0.0001	CDF	Non-Sigr	ificant Effect		
	5*		8.17	2.015	0.072 5	0.0002	CDF	Non-Sigr	nificant Effect		
	6.06*		7.445	2.015	0.075 5	0.0003	CDF	Non-Sign	ificant Effect		
	10*		10.62	1.943	0.054 6	< 0.0001	CDF	Non-Sign	ificant Effect		
	15*		14.73	1.695	0.039 7	<0.0001	CDF	Non-Sign	ificant Effect		
ANOVA Tab	le										
Source	Sum Sqւ	ıares	Mean Sq	uare	DF	F Stat	P-Value	Decision	ι(α:5%)		
Between	0.007064	687	0.0014129	937	5	0.4031	0.8418	Non-Sigr	ificant Effect		
Error	0.084128	15	0.003505	34	24						
Total	0.091192	83			29	_					
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(	(α:1%)			
Variances	Bartlett B	Equality of \	/ariance	3.459	15.09	0.6296	Equal Var	iances			
Distribution	Shapiro-	Wilk W No	rmality	0.9518	0.9031	0.1883	Normal Di	stribution			
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	8.0	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 (Co	rrected) Transfoi	med Sumi	mary								
		C	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
C-%	Control Type	Count	Wicaii						······		
<b>C-</b> %	Control Type  Lab Control	5	1.03	0.9812	1.078	1.013	1.002	1.095	0.01741	3.78%	0.0%
				0.9812 0.9807	1.078 1.131	1.013 1.047	1.002 0.9695	1.095 1.12	0.01741 0.02706	3.78% 5.73%	0.0% -2.55%
0		5	1.03								
0 2.5		5 5 5 5	1.03 1.056	0.9807	1.131	1.047	0.9695	1.12	0.02706	5.73%	-2.55%
0 2.5 5		5 5 5	1.03 1.056 1.064	0.9807 0.9716	1.131 1.156	1.047 1.107	0.9695 0.9589	1.12 1.133	0.02706 0.03322	5.73% 6.98%	-2.55% -3.32%

Analyst: JSAC QA: AC12/28/17

#### **CETIS Test Data Worksheet**

Report Date:

14 Dec-17 15:02 (p 1 of 1) 12-2045-5598/48BEAGAE

Test Code:

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Start Date: 15 Dec-17 Species: Strongylocentrotus purpuratus Sample Code: AEF 330 PWG 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

D. 5 mal Sample Station: M-INF

Sample Dat				Mater	ial: Product Te	sting		Sa	mple Statio	n: M-INF	UIS	male	SANK
C-%	Code	Rep	Pos	# Counted	# Fertilized	ı	1		Notes			0,-	-2/ / /
***************************************			91	100	82	12/10	1/2017						
			92	100	77	1							
11111 T 111111			93	100	80								
			94	100	79								
			95	100	70								
			96	(00)	31								
	-		97	100	174		A		18.7				
	-		98	100	82								
	_		99	[00]	80								
			100	(00	78			***************************************					
			101	100	(B) 78 68								
			102	100	80								
			103	(00	67		Paleston -						
	-		104		BOH 74			× 2002 2000					
	-		105	100	41								
			106 107	100	76								
			107	100	660		VA-MAN						
			109		B) 50 78								
			110	100	82								
*******	-		111		B 64 74								
			112	[00]	81								
			113	100	(2.0								V
			114	(00)	80								1000000
			115		72								
***************************************			116	100	72						***************************************		
***************************************			117										
			118	100	69								
			119	100	76								
		~	120	la	30	-	d						
				(W	$\bot$ $\bigcirc$ $\bigcirc$ $\bigcirc$ $\bigcirc$ $\bot$		-						

BHO Q18 12/22/17 (B) ACSQC 918 AC 1928/19

## **CETIS Test Data Worksheet**

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

Echinoid Sperm Cell Fertilization Test 15C

# Nautilus Environmental (CA)

Start Date:	15 Dec-17	Species:	Strongylocentrotus purpuratus	Sample Code:	AEF 33	0 PWG	
End Date:	15 Dec-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	IDE Am	ericas, Inc.	
Sample Date:	07 Dec-17	Material:	Product Testing	Sample Station:	M-INF	1,5 mali	Soile

ibie nan				Marchie	ai. Floudeti	soung	Sample Station. W-INF	11.3 Male X	PIK
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes	<i>y</i>	
0	LC	1	104						
0	LC	2	116						
0	LC	3	94				The second of th		
0	LC	4	105						
0	LC	5	112	100	18	A(5 12/17/17			
2.5		1	120					10110	
2.5		2	114						
2.5		3	96						
2.5		4	101	100	77	415 12/17/17			
2.5		5	97						
5		1	115						
5		2	98						
5		3	103	100	71	A(5 12/17/17			
5		4	99	1					
5		5	113						
6.06		1	102						
6.06		2	106	100	72	ACS 12/17/17			
6.06		3	95						
6.06		4	91						
6.06		5	107						
10		1	100	100	74	146 12/17/17		100 ANA ANA ANA ANA ANA ANA ANA ANA ANA A	
10		2	108		1				
10		3	118				***************************************		
10		4	92						
10		5	109						
15		1	119						
15		2	110			_			
15		3	93	100	74	Ars 12/17/17		4444,4,4,4,4,4,4	
15	and the second second second	4	117		. (		11.100	11 11 11 11 11 11 11 11 11 11 11 11 11	
15		5	111						

QC:EG

PAO 018 12/22/17

Analyst: A QA:

## 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

Sample Log No.:

End Date/Time: 12/15/2017

Dilutions made by: Pro 050 EG

Test No: 1712-5046

			Analyst:	CG
Concentration % Sample	DO (mg/L)	Initial R pH (units)	eadings Salinity	Temperature
Lab Control	(mg/L)	8.02	(ppt) 33.7	(°C)
2.5	4.3	8.02	33.9	15.8
5.0	8-3	4.02	33.9	15,9
6.06	6.3	8.02	33.9	15.9.
10	4.3	8.01	33.9	159
15	4.3	8.00	33.9.	16.0

Comments:				
QC Check:	PO 12/22/17	Final Review:	EG 1/2/18	

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

# **Marine Chronic Bioassay**

# **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.:	1DE M-INF 0.5 n 1712-SO46	rg/L Spile	AEF 330 f _	<sup>₹36</sup> γ Ε	ind Date/Tir Speci	me: 12/15/201 me: 12/15/201 es: <i>S. purpur</i>	7    546 atus
Tech initials: Injection Time:	EG 1427				Animal Sour Pate Collect		Lima 17
Sperm Absorbance at 4	00 nm:	(target range of	0.8 - 1.0 for de	ensity of 4x	10 <sup>6</sup> sperm/r	nl)	
Eggs Counted:	107 121 (targe 118 122	n: <u>                                     </u>	per vertical pass sity of 4000 egg	s on Sedgwi			
Initial density: Final density:	<u>ら多い</u> eggs/ml 4000 eggs/ml	= <u>  ५५२५</u> dilui - <u>1.0</u> part <u>७.५६२५</u> part	egg stock			500 ml 35.75 ml	
Prepare the embryo stoc stock (1 part) and 125 m	ck according to the calculate of dilution water (1.25 p	ated dilution factor. arts).			ion factor is	2.25, use 10	0 ml of existing
			Sperm:Egg	<u>Ratio</u>			
Rangefinder Test:	<u>2000:1</u> <u>1600:</u>		-	400:1	200:1	100:1	50:1_
ml Sperm Stock ml Seawater	50 40 0.0 10	30 20	20 30	10	5.0	2.5	1.25
III Geawatei	0.0	20	30	40	45	47.5	48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1435 1445 1455	Rangefinder Ration 50   100   100   200   1	o: <u>Fert.</u> <u>93</u> <u>100</u>	Unfej 7/2 2 0	<u>t.</u> <u>6</u> -		
this range, choose the ra	n-to-egg ratio that results i atio closest to 90 percent ctive season, site conditio	unless professiona	een 80 and 90 I judgment dic	percent. I states consi	f more than deration of	one concenti other factors	ration is within (e.g., organism
<u>Definitive Test</u>		Sperm:Egg Ratio	Used:	50:1	_		
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1506 1926 1946	QC1 QC2 Egg Control 1 Egg Control 2	Fert.	Unfer 1 & 1 & 1 O	<u>t</u> .		Ą
Comments:							
					***************************************		
QC Check:	11 cels 04				Final Revie	w: FG I	2/18
Nautilus Environmental. 434	0 Vandever Avenue. San Die	– go, CA 92120.					- 1.0



# **CETIS Summary Report**

Report Date:

28 Dec-17 16:18 (p 1 of 1)

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

				MINE AND THE PROPERTY OF THE P				Test C	.oae:	171	2-5047   1	7-2036-5486
Echinoid Spe	rm Cell Fertiliza	tion Test 1	5C							Nautilu	s Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	15-5862-3717 15 Dec-17 15:0 15 Dec-17 15:4 40m	6 Prof 6 Spe	t Type: tocol: cies: rce:	Fertilization EPA/600/R-95/136 (1995) Strongylocentrotus purpuratus Pt. Loma				Analys Diluer Brine: Age:	nt: Na	tural Seawat t Applicable	er	
•	02-5999-3123 07 Dec-17 10:0 07 Dec-17 12:0 8d 5h	8 Sou	le: erial: rce: ion:	AEF 330 PWG Product Testing IDE Americas, M-INF	I	Spike		Client Projec		E iking Study		
Comparison 9	Summary				Ö							
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU		Method			
11-7163-7108	Fertilization Rat	e	15	>15	NA	10.1% <	6.66	7	Dunnett	Multiple Com	parison Te	st
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU		Method			
07-8955-9937	Fertilization Rat	e	EC25 EC50	>15 >15	N/A N/A	N/A N/A	<6.6 <6.6		Linear In	terpolation (I	CPIN)	
Test Acceptat	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limit	ts		Overlap	Decision		
07-8955-9937	Fertilization Rat	:e	Contro	ol Resp	0.738 0.7 - NL				Yes	Passes A	cceptability	Criteria
11-7163-7108	Fertilization Rat	e	Contro	ol Resp	0.738	0.7 - NL			Yes	Passes A	cceptability	Criteria
11-7163-7108	Fertilization Rat	:e	PMSE	)	0.1015	NL - 0.25			No	Passes A	cceptability	Criteria
Fertilization R	tate 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 R	tate Detail					THE PROPERTY OF THE PROPERTY O	MAYAKEN ÇA ORON ÇA					
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	8.0	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						
10		0.1	0.70	0.70	0.13	0.01						

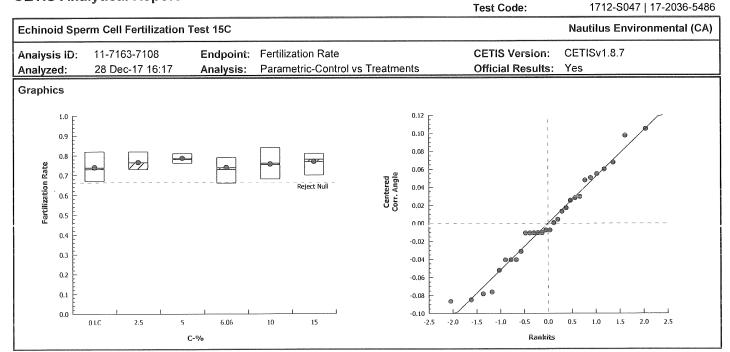
Report Date: Test Code:

28 Dec-17 16:18 (p 1 of 2)

1712-S047 | 17-2036-5486

Description								Test	Code:	171	2-S047   1	7-2036-548
Analyzed:   28 Dec-17 16:17	Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	s Environ	mental (CA
Analyzer   28 Dec-17 16:17   Analysis:   Parametric-Control vs Treatments   Parametric Southout vs	Analysis ID:	11-7163-7108	Er	ndpoint: Fer	tilization Ra	te		CET	IS Version	: CETISv1	.8.7	
Angular (Corrected) NA C > T NA NA C > T NA NA NA C > T NA	Analyzed:	28 Dec-17 16	:17 <b>A</b> r	nalysis: Pa	rametric-Coi	ntrol vs Trea	atments	Offic	cial Result			
Dunnett Multiple   Comparisor   Test   Stat   Critical   MSD   DF   P-Value   P-Type   Decision(a:5%)	Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Control   vs   C-W	Angular (Corr	rected)	NA	C > T	NA	NA		10.1%	15	>15	NA	6.667
Lab Control 2.5	Dunnett Muli	tiple Compariso	n Test									
Lab Control   2.5	Control	vs C-%		Test Stat	Critical	MSD D	P-Value	P-Type	Decision	ı(α:5%)		
	Lab Control	2.5		-0.8447	2.362	0.084 8	0.9746	CDF				
		5		-1.552	2.362	0.084 8	0.9968		•			
10		6.06		0.009108	2.362	0.084 8	0.8306		Ū			
ANOVA Table  Source Sum Square Decision 0.01141649 0.002283298 5 0.7281 0.6092 Non-Significant Effect  For 0.0752643 0.003136012 24  Total 0.08668078 7 29  Distributional Tests  Attribute Test Test Stat Critical P-Value Decision(α:1%)  Variances Bartlett Equality of Variance 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 0.768 0.768 0.7618 0.8012 0.788 0.780 0.788 0.768 0.810 0.768 0.810 0.768 0.820 0.788 0.780 0.7		10		-0.5439	2.362				_			
Source   Sum Square   DF   F Stat   P-Value   Decision(α:5%)		15	EAST-COLOR OF THE COLOR OF THE									
Between	ANOVA Table	e										
From	Source	Sum Sqı	uares	Mean Squ	uare	DF	F Stat	P-Value	Decision	ı(α:5%)		
Error	Between	0.011416	649	0.0022832	298	5	0.7281		Non-Siar	ificant Effect		
Test   State   Critical   P-Value   Decision(a:1%)	Error	0.075264	3	0.0031360	)12	24						
Test   Test   State   Critical   P-Value   Decision(α:1%)	Total	0.086680	78			29						
Variances   Bartlett Equality of Variance   4.149   15.09   0.5281   Equal Variances   Normal Distribution	Distributiona	I Tests										
Distribution   Shapiro-Wilk W Normality   0.9715   0.9031   0.5812   Normal Distribution	Attribute	Test			Test Stat	Critical	P-Value	Decision(	(α:1%)			
Distribution   Shapiro-Wilk W Normality   0.9715   0.9031   0.5812   Normal Distribution	Variances	Bartlett I	Equality of \	/ariance	4.149	15.09	0.5281	Equal Var	iances			
C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max Std Err CV% %Effe 0.0 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° 0.765 0.786 0.7618 0.8102 0.78 0.76 0.81 0.008718 2.48% -6.5% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.066 0.84 0.02786 8.26% -2.17° 0.8313 0.76 0.68 0.84 0.02786 8.26% -2.17° 0.74 0.74 0.74 0.74 0.74 0.74 0.74 0.74	Distribution	Shapiro-	Wilk W No	mality	0.9715	0.9031	0.5812					
Lab Control   5	Fertilization f	Rate Summary										
5 0.764 0.706 0.822 0.73 0.73 0.82 0.02088 6.11% -3.52° 0.73 0.73 0.82 0.02088 6.11% -3.52° 0.736 0.786 0.7618 0.8102 0.78 0.76 0.81 0.008718 2.48% -6.5% 0.066 0.79 0.02311 7.0% 0.0% 0.0% 0.0738 0.6738 0.8022 0.73 0.66 0.79 0.02311 7.0% 0.0% 0.0% 0.0754 0.6767 0.8313 0.76 0.68 0.84 0.02786 8.26% -2.17° 0.15 0.768 0.7158 0.8202 0.78 0.7 0.81 0.01881 5.48% -4.07° 0.000000000000000000000000000000000	C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
5 0.786 0.7618 0.8102 0.78 0.76 0.81 0.008718 2.48% -6.5% 0.606 5 0.738 0.6738 0.8022 0.73 0.66 0.79 0.02311 7.0% 0.0% 0.0% 0.0754 0.6767 0.8313 0.76 0.68 0.84 0.02786 8.26% -2.17 0.754 0.758 0.7158 0.8202 0.78 0.7 0.81 0.01881 5.48% -4.07 0.758 0.759	-	Lab Control	5	0.738	0.6715	0.8045	0.73	0.67	0.82	0.02396	7.26%	0.0%
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	2.5		5	0.764	0.706	0.822	0.73	0.73	0.82	0.02088	6.11%	-3.52%
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	5		5	0.786	0.7618	0.8102	0.78	0.76		0.008718		-6.5%
10	6.06		5	0.738	0.6738	0.8022	0.73	0.66	0.79	0.02311	7.0%	
15	10		5	0.754	0.6767	0.8313	0.76	0.68	0.84	0.02786		-2.17%
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         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	0.768	0.7158	0.8202	0.78	0.7				-4.07%
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% -2.899	Angular (Cori	rected) Transfor	med Sumr	nary								
5 1.065 0.9956 1.135 1.024 1.024 1.133 0.02502 5.25% -2.899 5 1.09 1.061 1.12 1.083 1.059 1.12 0.01063 2.18% -5.319 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% -1.869	C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
2.5     5     1.065     0.9956     1.135     1.024     1.024     1.133     0.02502     5.25%     -2.89°       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°	0	Lab Control	5	1.035	0.9578	1.113	1.024	0.9589	1.133	0.02789	6.03%	0.0%
5 1.09 1.061 1.12 1.083 1.059 1.12 0.01063 2.18% -5.319 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% -1.869	2.5		5	1.065	0.9956	1.135	1.024	1.024				-2.89%
5.06 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% -1.869	5		5	1.09	1.061							-5.31%
10 5 1.054 0.9629 1.146 1.059 0.9695 1.159 0.03298 6.99% -1.86°	6.06		5	1.035	0.9625							
1,00	10		5									-1.86%
5 1.069 1.009 1.13 1.083 0.9912 1.12 0.0219 4.58% -3.319	15		5	1.069	1.009	1.13	1.083					-3.31%

Report Date: Test Code: 28 Dec-17 16:18 (p 2 of 2)



Report Date:

28 Dec-17 16:18 (p 1 of 1)

Test Code:

1712-S047 | 17-2036-5486

CETISv1.8.7

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Analysis ID: 07-8955-9937 Analyzed: 28 Dec-17 16:17

Analysis:

Endpoint: Fertilization Rate Linear Interpolation (ICPIN) **CETIS Version:** Official Results: Yes

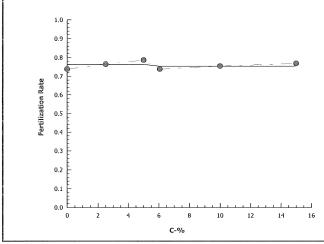
Linear Interpolation Options										
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method					
Linear	Linear	378767	1000	Yes	Two-Point Interpolation					

### Point Estimates

Leve	el %	, 0	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC2	!5 >	15	N/A	N/A	<6.667	NA	NA
EC5	90 >	15	N/A	N/A	<6.667	NA	NA

Fertilizat	tion Rate Summary										
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
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



Report Date:

28 Dec-17 16:18 (p 1 of 1)

Test Code:

1712-S047 | 17-2036-5486

Market		0.00					162	coue.	171	2-3047   1	7-2030-548
Echinoid Sp	oerm Cell Fertiliz	ation Tes	t 15C	151	-				Nautilus	s Environ	mental (CA
Analysis ID:		_	•	Fertilization Ra	-			IS Version		.8.7	
Analyzed:	28 Dec-17 16	:18 🔑	nalysis:	Parametric Bio	equivalence	-Two Samp	le Offi	cial Result	s: Yes		
Data Transfe	orm	Zeta	Alt Hy	p Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C*b < 1	T NA	NA	0.75	6.81%	15	>15	NA	6.667
TST-Welch's	s t Test										
Control	vs C-%		Test St	tat Critical	MSD DF	P-Value	P-Type	Decision	ι(α:5%)		
Lab Control	2.5*		8.853	1.895	0.062 7	<0.0001	CDF		nificant Effect		
	5*		13.37	2.015	0.047 5	<0.0001	CDF		nificant Effect		
	6.06*		7.735	1.895	0.063 7	<0.0001	CDF		ificant Effect		
	10*		7.12	1.943	0.076 6	0.0002	CDF		ificant Effect		
	15*		9.676	1.895	0.057 7	<0.0001	CDF		ificant Effect		
ANOVA Tabl	le										
Source	Sum Squ	ıares	Mean S	Square	DF	F Stat	P-Value	Decision	ι(α:5%)		
Between	0.011416	49	0.00228	33298	5	0.7281	0.6092		ificant Effect		
Error	0.075264	3	0.00313	36012	24				mount Endot		
Total	0.086680	78		•	29	_					
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	'α:1%)			
Variances	Bartlett E	Equality of	Variance	4.149	15.09	0.5281	Equal Var				· · · · · · · · · · · · · · · · · · ·
Distribution	Shapiro-	Wilk W N	ormality	0.9715	0.9031	0.5812	Normal D				
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 (Cor	rrected) Transfor	med Sum	ımary								
C-%		01	Moon	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
	Control Type	Count	Mean	33 /0 LCL							
	Control Type Lab Control	5	1.035	0.9578	1.113	1.024	0.9589	1.133	0.02789	6.03%	0.0%
2.5					1.113 1.135	1.024 1.024	0.9589 1.024	1.133 1.133	0.02789 0.02502		
2.5		5	1.035	0.9578						6.03% 5.25% 2.18%	0.0% -2.89% -5.31%
2.5 5		5 5	1.035 1.065	0.9578 0.9956	1.135	1.024	1.024	1.133	0.02502	5.25%	-2.89% -5.31%
0 2.5 5 6.06 10		5 5 5	1.035 1.065 1.09	0.9578 0.9956 1.061	1.135 1.12	1.024 1.083	1.024 1.059	1.133 1.12	0.02502 0.01063	5.25% 2.18%	-2.89%

Analyst: 4 QA: EC 1/2/18

Report Date: Test Code:

28 Dec-17 16:20 (p 1 of 1) 17-2036-5486/1712-S047

**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 **Protocol**: EPA/600/R-95/136 (1995) End Date: 15 Dec-17 15:46 Sample Source: IDE Americas, Inc.

Sample Date: 07 Dec-17 10:00 Material: Product Testing Sample Station: M-INF & Soil Soil

nple Da	te: U/L	Jec-1	7 10:00		I: Product Tes	iting	Sample	Station: IVI-IIVI	1.0mg/L	SPIK
C-%	Code	Rep	Pos	# Counted	# Fertilized		n	Notes	· J.	-
			121	100	80	AC UBO KC	12/19/17			
			122	1	73					
			123		73					
			124		74					
			125		66					
			126		78					
			127		73					
10			128		76					
			129		76					
			130		16 16					
			131		73					
			132		67					
			133		78					
			134		84 82 70					
			135		82					
			136	and the second	70					
			137		73					
			138	**************************************	81					
			139		78					
			140		68					
			141		7)					
			142		13 81 78 68 -11 81					
			143	Marketing and a second	1 🗡					
			144		81					
			145		73					
			146		79					
			147		73					
			148		79					
			149		78					
			150	V	82					

### **CETIS Test Data Worksheet**

Report Date: Test Code:

14 Dec-17 17:20 (p 1 of 1) 17-2036-5486/668AB1AE

**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 / Dm

nple Dat	e. 0/ L	Jec-17		wateria	al: Product T	esung	Sample Station: M-INF	1,0 mg/c	-SQ).
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes	0'	1
0	LC	1	147		****				
0	LC	2	135						
0	LC	3	131	100	66	AG 12/17/17			
0	LC	4	132						
0	LC	5	124						
2.5		1	123		****				
2.5		2	142	100	82	MS 12/17/17			
2.5		3	122	****		111111		770000000000000000000000000000000000000	
2.5		4	150						
2.5		5	137						·
5		1	133						,
5		2	128	100	76	A16 12/17/17			
5		3	143		7.0				
5		4	121	~~~					
5		5	138						
6.06		1	146	100	79	ALG 12/17/17			
6.06		2	145		- (				
6.06		3	125			A.9.			
6.06		4	126						
6.06		5	127						
10		1	149						
10		2	141						
10		3	130				Adoption and the second		
10		4	134	100	80	ATG 12/15/17			
10		5	140			12 16/1/11			
15		1	136						
15		2	129						
15		3	139						
15		4	148		, U.A				
15		5	144	100	80	A(5 12/17/17			

Blan 018 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

Sample Log No.:

17-1247

End Date/Time: 12/15/2017

Dilutions made by: 100 000 100

Test No: 1712

			Analyst:	CG
Communication			eadings	
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	8.03	33.9	15.8
5.0	8.3	8.03	33.9	15.9
6.06	6.2	8.03	33.9	15.8
10	8.3	8.01	34.0.	15.9
15	8.2	8.01	34.0	159

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

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

Marine Chronic Bioa	issay	Echinoderm Sperm-Cell Fertilization Worksheet
Client: Sample ID:	M-INFOSOMALL SPANO AET.	Start Date/Time: 12/15/2017 / 1506 End Date/Time: 12/15/2017 / 1546
Test No.:	1712-50847 0'	Species: S. purpuratus
Tech initials:	Eh C	Animal Source: Pt. Loma  Date Collected: 12817
Injection Time:	1427	,
Sperm Absorbance at 40	00 nm:(target range of 0.8 - 1.	.0 for density of 4x10 <sup>6</sup> sperm/ml)
Eggs Counted:	$113$ Mean: $162 \times 50 =$	5,810 eggs/ml
	(target counts of 80 eggs per verting Rafter slide for a final density of 4	
	122	
Initial density:	5810 eggs/ml = $1.462%$ dilution fac	ctor egg stock 300 ml
Final density:	4000 eggs/ml - 1.0 part egg st อ.ฯริวฦ parts seaw	tock seawater <u>135.75</u> ml
Prepare the embryo stoc stock (1 part) and 125 ml	k according to the calculated dilution factor. For e l of dilution water (1.25 parts).	xample, if the dilution factor is 2.25, use 100 ml of existing

				Sperm:	Egg Ratio			
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 50 0.0	1600:1 40 10	1200:1 30 20	800:1 20 30	400:1 10 40	200:1 5.0 45	100:1 2.5 47.5	50:1 1.25 48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1435 1445 1455		ngefinder Ra 50 1 100 1 200 1		73/94 7 8 Z			

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).

nealth, stage of reproc	ductive season, site conditi	ions).		
Definitive Test		Sperm:Egg Ratio Used:	50:1	
Sperm Added (100 µl) Eggs Added (0.5 ml): Test Ended:	Time 1506 1926 1946	QC1 QC2 Egg Control 1 Egg Control 2	rt. Unfert.  14 2 18 100 100	<b>X</b> .
Comments:	PAD 018 121	22/17 (BEG 918	1/2/18	
QC Check:	A0 12/22/	L1	Final Review:	in 12/18

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



# **CETIS Summary Report**

Report Date:

27 Dec-17 16:33 (p 1 of 1)

**Test Code:** 1712-S048 | 19-1559-0836

								rest ocac.		1.7	2-00-0   1.	5-1333-003
Echinoid Spe	rm Cell Fertiliza	ation To	est 15C		-					Nautilu	s Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	10-1147-0012 15 Dec-17 15:0 15 Dec-17 15:4 40m		Test Type: Protocol: Species: Source:	Fertilization EPA/600/R-95 Strongylocentr Pt. Loma		itus				al Seawat pplicable	er	
	09-7257-2905 07 Dec-17 10:0 : 07 Dec-17 12:0 8d 5h		Code: Material: Source: Station:	AEF 330 PWG Product Testin IDE Americas, M-INF 5.0	g	1247 Spike			IDE Spikir	ng Study		
Comparison S	Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Metho	nd			
07-5121-4867	Fertilization Ra	te	2.5	5	3.536	12.0%	40			Itiple Com	parison Tes	st
Point Estimate	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Metho	od			
11-0658-8786	Fertilization Ra	te	EC25 EC50	6.672 9.552	4.981 8.458	7.75 10.64	14.99 10.47	Linear		oolation (I	CPIN)	
Test Acceptab	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	ts	Overla	an	Decision		
07-5121-4867	Fertilization Ra	te	Contro	ol Resp	0.772	0.7 - NL		Yes			cceptability	Criteria
11-0658-8786	Fertilization Rat	te	Contro	ol Resp	0.772	0.7 - NL		Yes			cceptability	
07-5121-4867	Fertilization Rat	te	PMSD	)	0.1205	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	ate Summary											
C-%	Control Type	Coun	t Mean	95% LCL	95% UCL	Min	Max	Std Er	rr :	Std Dev	CV%	%Effect
0	Lab Control	5	0.772	0.6827	0.8613	0.66	0.86	0.0321	16	0.0719	9.31%	0.0%
2.5		5	0.744	0.6702	0.8178	0.66	0.81	0.0265	57	0.05941	7.99%	3.63%
5		5	0.654	0.5548	0.7532	0.52	0.71	0.0357	72	0.07987	12.21%	15.28%
6.06		5	0.62	0.5435	0.6965	0.55	0.69	0.0275	57	0.06164	9.94%	19.69%
10		5	0.356	0.2777	0.4343	0.25	0.41	0.0282	21 (	0.06309	17.72%	53.89%
15		5	0.074	0.03045	0.1175	0.04	0.13	0.0156	88	0.03507	47.39%	90.41%
=	néa Datail											
Fertilization R	ate Detail											
	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
C-%		Rep 1	Rep 2	Rep 3	<b>Rep 4</b> 0.79	Rep 5	a) diversion and a second			make o construction and a constr		
C-%	Control Type						330					
C-% 0	Control Type	0.86	0.78	0.66	0.79	0.77	<del></del>					
C-% 0 2.5	Control Type	0.86 0.78	0.78 0.66	0.66 0.81	0.79 0.76	0.77 0.71	330					
C-% 0 2.5 5	Control Type	0.86 0.78 0.52	0.78 0.66 0.64	0.66 0.81 0.7	0.79 0.76 0.71	0.77 0.71 0.7						

Report Date:

27 Dec-17 16:33 (p 1 of 2)

Test Code:

1712-S048 | 19-1559-0836

							rest	Code:	171	2-3040   18	1-1009-003
Echinoid Sp	erm Cell Fertiliza	ation Test	15C						Nautilus	Environn	nental (CA)
Analysis ID: Analyzed:	07-5121-4867 27 Dec-17 16:		dpoint: Fer	tilization Rat		tmonto		IS Version:		.8.7	
						unents		ial Results			
Data Transfo		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA ————	C > T	NA ————————————————————————————————————	NA ————————————————————————————————————		12.0%	2.5	5	3.536	40
Dunnett Mul	Itiple Compariso	n 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-Sign	ificant Effect		
	5*		2.908	2.362	0.108 8	0.0157	CDF	Significan	t Effect		
	6.06*		3.694	2.362	0.108 8	0.0025	CDF	Significan	t Effect		
	10*		9.57	2.362	0.108 8	<0.0001	CDF	Significan	t Effect		
	15*		17.6	2.362	0.106 6	<0.0001	CDF	Significan	it Effect		
ANOVA Tabl	le										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	2.367208		0.4734416	3	5	90.13	<0.0001	Significan	t Effect		
Error	0.126066	9	0.0052527	<b>'</b> 88	24						
Total	2.493275				29	_					
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	α:1%)			
Variances	Bartlett B	quality of \	/ariance	0.6204	15.09	0.9870	Equal Var	iances			
Distribution	Shapiro-	Wilk W Nor	mality	0.9561	0.9031	0.2448	Normal Di	stribution			
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%
	rrected) Transfor	med Sumr	mary								
	rrected) Transfor Control Type	med Sumr Count	nary Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
Angular (Co C-%	•	Count 5	-	<b>95% LCL</b> 0.9708	<b>95% UCL</b> 1.183	Median 1.083	<b>Min</b> 0.9483	<b>Max</b>	<b>Std Err</b> 0.03816	CV% 7.93%	%Effect
Angular (Cod C-%	Control Type	Count	Mean								
Angular (Co	Control Type	Count 5	<b>Mean</b> 1.077	0.9708	1.183	1.083	0.9483	1.187	0.03816	7.93%	0.0%
Angular (Cod C-% 0 2.5	Control Type	Count 5 5	Mean 1.077 1.042	0.9708 0.9582	1.183 1.126	1.083 1.059	0.9483 0.9483	1.187 1.12	0.03816 0.03029	7.93% 6.5%	0.0% 3.19%
Angular (Con C-% 0 2.5 5	Control Type	5 5 5	Mean 1.077 1.042 0.9434	0.9708 0.9582 0.8408	1.183 1.126 1.046	1.083 1.059 0.9912	0.9483 0.9483 0.8054	1.187 1.12 1.002	0.03816 0.03029 0.03695	7.93% 6.5% 8.76%	0.0% 3.19% 12.38%

Report Date: Test Code: 27 Dec-17 16:33 (p 2 of 2) 1712-S048 | 19-1559-0836

**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 1.0 0.12 0.10 0.9 Fertilization Rate 0.04 0.02 0.6 0.00 0.5 0.4 -0.06 0.3 -0,08 -0.10 0.2 -0.12 0.1 -0.14 -0.16 0 LC 2.5 6.06 -2.5 -2.0 -1.5 -0.5 0.0 1.0 C-% Rankits

Report Date:

27 Dec-17 16:33 (p 1 of 1)

Test Code:

1712-S048 | 19-1559-0836

Echinoid Sperm Cell Fertilization Test 15C

Analysis ID: 11-0658-8786 Endpoint: Fertilization Rate
Analyzed: 27 Dec-17 16:29 Analysis: Linear Interpolation (ICPIN)

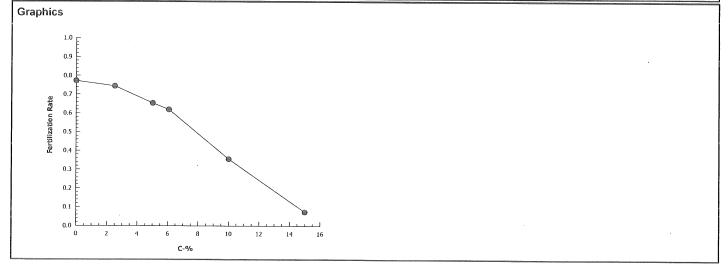
Nautilus Environmental (CA)

CETIS Version: CETISv1.8.7

Official Results: Yes

Linear	Interpola	tion Options						
X Trans	sform	Y Transform	Seed	t	Resamples	Exp 95% CL	Method	
Linear	· · · · · · · · · · · · · · · · · · ·	Linear	2527	'03	1000	Yes	Two-Point Interpolation	min and a second
Point E	stimates							
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		

Fertilizat	tion Rate Summary										
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	В
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





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

				>1			lesi	Code:	171	12-S048   19	9-1559-0836
Echinoid Sp	erm Cell Fertiliz	ation Tes	t 15C						Nautilu	s Environr	nental (CA)
Analysis ID:	11-4422-2496	Е	indpoint: F	ertilization Ra	te		CET	'IS Version	: CETISv1	1.8.7	
Analyzed:	27 Dec-17 16		-	Parametric Bio		-Two Samp		cial Results			
Data Transfo	orm	Zeta	Alt Hy	o Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	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 St	at Critical	MSD DF	P-Value	P-Type	Decision	ı(a:5%)		
Lab Control	2.5*		5.633	1.895	0.079 7	0.0004	CDF	Non-Sign	ificant Effec	t	
	5*		2.907	1.895	0.089 7	0.0114	CDF	Non-Sigr	ificant Effec	t	
	6.06*		2.475	1.895	0.076 7	0.0213	CDF	Non-Sign	ificant Effec	t	
	10		-4.066	1.895	0.079 7	0.9976	CDF	Significa	nt Effect		
	15		-13.21	1.895	0.077 7	1.0000	CDF	Significa	nt Effect		
ANOVA Tabl	е										
Source	Sum Sqı	ıares	Mean S	quare	DF	F Stat	P-Value	Decision	ι(α:5%)		
Between	2.367208		0.47344	16	5	90.13	<0.0001	Significar	nt Effect		
Error	0.126066	0.00525	52788	24		ŭ					
Total	2.493275				29	_					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(a:1%)			
Variances	Bartlett B	Equality of	Variance	0.6204	15.09	0.9870	Equal Variances				
Distribution	Shapiro-	Wilk W N	ormality	0.9561	0.9031	0.2448	Normal D	Normal Distribution			
Fertilization	Rate Summary		A CONTRACTOR OF THE CONTRACTOR								
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 (Cor	rected) Transfor	med Sun	nmary								
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%
			The state of the s								

### **CETIS Test Data Worksheet**

Report Date: Test Code: 14 Dec-17 17:22 (p 1 of 1)

19-1559-0836/7<del>22D98B</del>4 foy 8

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Start Date: 15 End Date: 15

15 Dec-17 15 Dec-17 Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)

Sample Code: AEF 330 PWG 5.0
Sample Source: IDE Americas, Inc.

Sample Date: 07 Dec-17

Material: Product Testing

Sample Station: M-INF 5.0 mgh Spilce

mpie Date					ar. Product restir	9		Sample Station: W-INF 5,	JIMAN SPIN
C-%	Code	Rep	Pos	# Counted	# Fertilized			Notes	
			151	[00]	ido	Read 6	oy JC	12/19/17	
			152	100	856 71		1		
			153	100	64				
			154	(00	39				
			155	100	52 35678				
			156	100	® 56,78				
			157	100	+8	· · · · · · · · · · · · · · · · · · ·			
			158	(00)	65 71				
			159	100	71				
			160	100	76				
			161	100	5				
			162	100	7				
			163	(00)	69				
			164	100	86				
			165	100	70				
			166 167	(00)	+9				
			168	100	8	and an additional design of the second			
			169	100	65 77				
			170	100					
			171	100	25				
			172	100	S5				
			173	100					
			173	100	-41				
			175	100	13				
			176	100	35				
			177	100	81				
Aug 1,			178		38				
			179	<u> </u>	660	***************************************			
			180		70				
	L			100	56				

BP186 080 ACS 12/27/17

Analyst: QA: Abo

### **CETIS Test Data Worksheet**

Report Date:

14 Dec-17 17:22 (p 1 of 1) 19-1559-0836/722D98B4

Test Code:

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Start Date: 15 Dec-17 Species: Strongylocentrotus purpuratus AEF 330 PWG 5.0 Sample Code: 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 MA/1 CONV.

C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes	
0	LC	1	164	100	89	415 12/17/17		
0	LC	2	157					
0	LC	3	151				110000000000000000000000000000000000000	***************************************
0	LC	4	166			7377774111	1770-1770-1770-1770-1770-1770-1770-1770	
0	LC	5	169				7,400	
2.5		1	156				1110707000 111	4/411
2.5		2	178					
2.5		3	176					
2.5		4	160					
2.5		5	152	100	79	A15 12/17/17		
5		1	155				THE STATE OF THE S	
5		2	153					
5		3	165					
5		4	159	100	68	ACS 12/17/17		
5		5	179					
6.06		1	172					
6.06		2	168	100	66	A/S 12/17/17		
6.06		3	158					
6.06		4	180					
6.06		5	163					
10		1	154				774374	
10		2	177					
10		3	173	100	51		V-0	
10		4	170		L.			
10		5	175					
15		1	161					
15		2	162					
15		3	167					
15		4	174					
15		5	171	100	9			

QC. VIN BAOQ18 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)

1506 Start Date/Time: 12/15/2017

Sample Log No.:

17-1247

End Date/Time: 12/15/2017

Analyst:

Dilutions made by: AD OBO ES

Test No: \\\\\>-

				166		
Concentration	DO	Initial R	eadings Salinity	Temperature		
% Sample	(mg/L)	(units)	(ppt)	(°C)		
Lab Control	8.3	8.03	33.9	(5.5		
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	6.02	33.9	15.7		
15	8.3	8.00	33.9	15.7		

0	i Brana	rra	01	nts	
UC	m	m	eı	กเร	

QC Check:

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

## Marine Chronic Bioassay

## **Echinoderm Sperm-Cell Fertilization Worksheet**

Final Review: FC, 1/2/18

	-					
Client: Sample ID: Test No.:	1DE M-INF 5.00	modil spulce AEF	330 PWG E		12/15/2017 / S. purpuratus	1506 1546
Tech initials: Injection Time:	EG 1427			Animal Source: Date Collected:		<u> </u>
Sperm Absorbance at 40	00 nm: 1,061	(target range of 0.8 - 1	.0 for density of 4x	k10 <sup>6</sup> sperm/ml)		
Eggs Counted:	107 (targe	en: X 50 = x 50	tical pass on Sedgw			
	118					
Initial density: Final density:	4000 eggs/ml	= ्रप् <sup>१२२</sup> dilution fa - <u>1.0</u> part egg s <u>७.प६२</u> parts sea	stock sea	stock <u>30</u> water <u>135</u>	0 ml	
	ck according to the calcunt of dilution water (1.25 p	llated dilution factor. For parts).	example, if the dilu	ution factor is 2.	.25, use 100 m	l of existing
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 1600 50 40 0.0 10	1200:1 800 30 20	10	200:1 5.0 45	2.5	50:1 1.25 8.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1435 1445 1455	Rangefinder Ratio:  50   100   200	Fert. Unfr 93/94 7 98 2 100 0	ert.		
this range, choose the ra	n-to-egg ratio that results atio closest to 90 percen active season, site conditi	s in fertilization between 8 t unless professional judg ions).	0 and 90 percent. Iment dictates con	If more than or sideration of ot	ne concentration her factors (e.ç	on is within g., organism
Definitive Test		Sperm:Egg Ratio Use	d: 50:1			
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1506 1926 1946	QC1 QC2 Egg Control 1 Egg Control 2	Fert. Unf	ert. \$ }		8.
Comments:						

Ac 4/20/17 Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

QC Check:

Appendix B

**Sample Check-in Information** 

## Sample Check-In Information

-5-				- 1.1 4=:	
Client:		Tests Performed:	Urchin t	erbilization	Sample Descriptions:
Project: Spiking Stud		Test ID No.(s):	1717-504	14 to 5052	1) light Yellow color, spaque, Mildodor, no debns
1	AEF330 PNG	AC 125 PWG			2) NO color, crear, mild odor no depris
	1) folymer	2) coaquiant	3 Dilution wooter	-4)	3) NO Color, clear, no odor, no depris
Log-in No. (17-xxx):	1247	1248	1249		4)
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 1203	(2/7/17 m/A	108	COC Complete? (Y) N
Number of Containers & Container Type:	1, 100 m L plastic	1, loome plastic	4. 4 wb;		
Approx. Total Volume Received (L):	2100mL	~100mL	~16		
Check-in Temp (°C)	-	**************************************	21.5		Filtration? Y N
Temperature OK? 1	— Y N	Y N	⊗ N	Y N	Pore Size:
DO (mg/L)		_	7.0		Organisms or Debris
pH (units)		_	7.83		•
Conductivity (µS/cm)	_	_			•
Salinity (ppt)		_	33.9		pH Ad∥ustment? Y (N )
Alkalinity (mg/L) <sup>2</sup>	_	_	112		1 2 3 4 5 6
Hardness (mg/L) <sup>2, 3</sup>	-	_	* gamestim.		Initial pH:
Total Chlorine (mg/L)	_	_	0.02		Amount of HC  added:
Technician Initials	CH	ctt	CH		Final pH:
Freshwater Tests:					
Control/Dilution Water Source: 8:2	Culligan Othe	r:	Alkalinity:	Hardness:	Cl <sub>2</sub> Adjustment? Y $(\hat{N})$
Additional Control? Y N	=		Alkalinity:	Hardness:	1 2 3 4 5 6
Marine Tests:					Initial Free Cl <sub>2</sub> .
Control/Dilution Water Source: LAB SW	ART SW Oth	ner:	Alkalinity: 116	Salinity: 34ppt	STS added:
Additional Control? Y N	=			_ Salinity:	Final Free Cl <sub>2</sub> :
Sample Salted w/ artificial salt? Y	If yes, target ppt and	source?			Sample Aeration? Y (N)
Sample salted w/brine? Y (N)	If yes, target ppt?				1 2 3 4 5 6
	,				Initial D.O.
Notes <sup>1</sup> Temperature for sample must	be 0-6°C if received >24	4 hours past collection tin	ne.		Duration & Rate
<sup>2</sup> mg/L as CaCO3, <sup>3</sup> Measu					Final D.O.
		,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	,	
Additional Comments 🕑 Eq. 1 2 18	Q\8				Subsamples For Additional Chemistry Required? Y(N)
					NH3 Other
					Tech Initials
QC Check: E6 1/2/18					Final Review: AC/8/18
as show as					rillal Review.

Appendix C

**Chain of Custody Form** 

SPIKING STUDY



CDP laoratory:	Turn Around Time
Entahlpy Laboratory:	Normal:
WECK Laboratory:	RUSH (24 hr):
Nautilus:X	3 Days:
AIM:	5 Days:
Other:	222 Dave

Project Name: Special Toxicity Spiki	ing Study	Project Manag	er:Peter Shen		_Contact Information:_	(760	0) 201-777	7	The same to be seen a second	20 (100000000000000000000000000000000000	117.	
Special instruction: 4 x 4 liter co	ubies collected at M-I	NF on 12/7/17 @ 11:0	0 to be used as dilution w	ater. KC				ANA	LYSES		troppe provide a sign	NOTES:
	and the state of t					Fertilization						Samples received at ambient temperati
	Yes=Y No=N A	Glass=G Plastic=P				Chronic						at ambient temperati
Drinkin		er=SW Soil=S Brine=E		l ,		in Ch						
Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	Purple Urchin						
AEF 330 PWG (17-3470)	12/7/2017	10:00	Polymer Grab	N	100 mL poly	Х						
AC 125 PWG (17-3471)	12/7/2017	10:00	Coagulant Grab	N	100 mL poly	Х						
molude	alta	alet	Aluer of									
	di Gut	- wa -c Per		<u> </u>								
Relinquished By L.C.	wg-	Date:	Time: 1/30	<u> </u>	Received By:		12/1	7/1/7	Time:	) -/	Samp	ple Condition Upon Receipt:
		2/17	12:08 Juj		Tall	6	H7/ 1,12,	19117	9/4 5/2 1208 /	Iced		Ambient or°C  Ambient or°C

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

Appendix D

**Reference Toxicant Test Data** 

# **CETIS Summary Report**

Report Date:

28 Dec-17 14:21 (p 1 of 1)

Test Code:

171215sprtA | 06-1613-2535

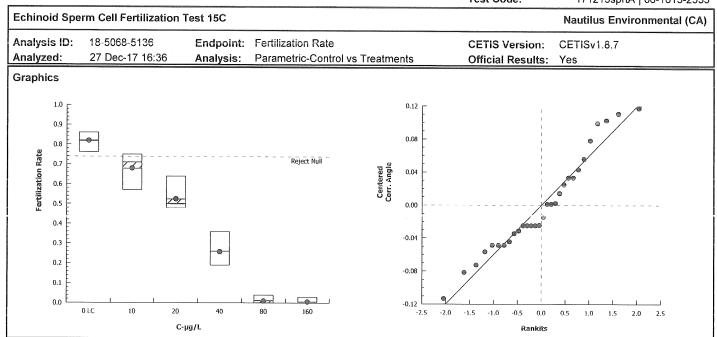
	A 11 F										·		
Echinoid Sper	rm Cell Fertiliza	tion Test 1	SC							Nautilus	Environm	ental (CA)	
Batch ID: Start Date: Ending Date: Duration:	16-2826-4250 15 Dec-17 15:0 15 Dec-17 15:4 40m	6 Prof	ocol: cies:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	Analyst: Diluent: Brine: Age:		ural Seawate Applicable	er					
Sample ID: Sample Date: Receive Date: Sample Age:		Cod Mate Sou Stat	erial: rce:	171215sprtA Copper chloride Reference Toxio Copper Chlorido	cant			Client: Project:					
Comparison S	Summary									occus accessorates de la celadad de la menocio este a filosoficios de la celadad de la celadad de la celadad d			
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Met	hod				
18-5068-5136	8-5136 Fertilization Rate			10	NA	9.77%		Dun	nett M	lultiple Com	parison Tes	t	
Point Estimate	e Summary		-										
Analysis ID	Endpoint		Level	μg/L	95% LCL	95% UCL	TU	Met	hod				
10-1459-1840	Fertilization Rat	e	EC50	26.01	24.46	27.67		Trim	nmed :	Spearman-k	(ärber		
Test Acceptab	oility												
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Ove	rlap	Decision			
10-1459-1840	Fertilization Rat	:e	Contro	Control Resp 0.818 0.7 - NL			Yes	HARACON DANIES AND CONTRA	Passes A	cceptability	Criteria		
18-5068-5136	Fertilization Rat	ie.	Contro	l Resp	0.818	0.7 - NL		Yes		Passes A	cceptability	Criteria	
18-5068-5136	Fertilization Rat	te	PMSD		0.0977	NL - 0.25		No		Passes A	cceptability	Criteria	
Fertilization R	tate Summary												
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	c Std	Err	Std Dev	CV%	%Effect	
0	Lab Control	5	0.818	0.7729	0.8631	0.76	0.86	6 0.01	1625	0.03633	4.44%	0.0%	
10		5	0.678	0.5909	0.7651	0.57	0.75	5 0.03	3137	0.07014	10.35%	17.11%	
20		5	0.524	0.4423	0.6057	0.48	0.64	4 0.02	2943	0.0658	12.56%	35.94%	
40		5	0.26	0.1772	0.3428	0.19	0.36	6 0.02	2983	0.06671	25.66%	68.22%	
80		5	0.012	0	0.03421	0	0.04	4 0.00	80	0.01789	149.1%	98.53%	
160		5	0.006	0	0.02266	0	0.03	3 0.00	)6 	0.01342	223.6%	99.27%	
Fertilization R	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							

Report Date: Test Code:

28 Dec-17 14:21 (p 1 of 2) 171215sprtA | 06-1613-2535

							lest	Code:	1712	і БѕрплА і Об	-1613-2535
Echinoid Sp	erm Cell Fertili	zation Test 1	15C						Nautilus	Environm	ental (CA)
Analysis ID:	18-5068-513	6 En	dpoint: Fer	tilization Rat	e		CETI	S Version:	CETISv1	.8.7	
Analyzed:	27 Dec-17 16		•	ametric-Con		ments	Offic	ial Results	: Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	ected)	NA	C > T	NA	NA		9.77%	<10	10	NA	
Dunnett Mul	tiple Comparis	on Test									
Control	vs C-μg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(a:5%)		
Lab Control	10*		3.919	2.362	0.098 8	0.0014	CDF	Significar	t Effect		
	20*		7.759	2.362	0.098 8	<0.0001	CDF	Significar	t Effect		
	40*		14.44	2.362	0.098 8	<0.0001	CDF	Significar	it Effect		
	80*		24.91	2.362	0.098 8	< 0.0001	CDF	Significar	it Effect		
	160*		25.49	2.362	0.098 8	<0.0001	CDF	Significar	it Effect		**************************************
ANOVA Tabl	е										
Source	Sum Sc	uares	Mean Squ	uare	DF	F Stat	P-Value	Decision	(a:5%)		
Between	4.97101	9	0.9942037	7	5	231.4	<0.0001	Significar	t Effect		
Error	0.10311	57	0.0042964	189	24	_					
Total	5.07413	4			29						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(a:1%)			
Variances	Bartlett	Equality of \	/ariance	1.204	15.09	0.9445	Equal Var	iances			
Distribution	Shapiro	o-Wilk W Nor	mality	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 (Co	rrected) Transf	ormed Sumr	mary								
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%

Report Date: Test Code: 28 Dec-17 14:21 (p 2 of 2) 171215sprtA | 06-1613-2535



Report Date:

28 Dec-17 14:21 (p 1 of 1)

Test Code:

171215sprtA | 06-1613-2535

**Echinoid Sperm Cell Fertilization Test 15C** 

Analysis ID: Analyzed:

10-1459-1840 27 Dec-17 16:36

Endpoint: Fertilization Rate Analysis:

Trimmed Spearman-Kärber

**CETIS Version:** 

Nautilus Environmental (CA)

Official Results:

Yes

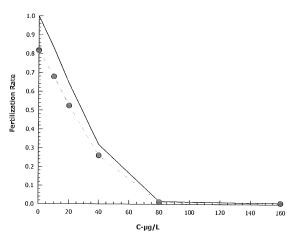
**CETISv1.8.7** 

Trimmed Spearman-Kärber Estim	ates
-------------------------------	------

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

Fertilizati	on Rate Summary		Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
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



Report Date:

28 Dec-17 14:24 (1 of 1)

#### **Echinoid Sperm Cell Fertilization Test 15C**

#### Nautilus Environmental (CA)

Test Type: Fertilization

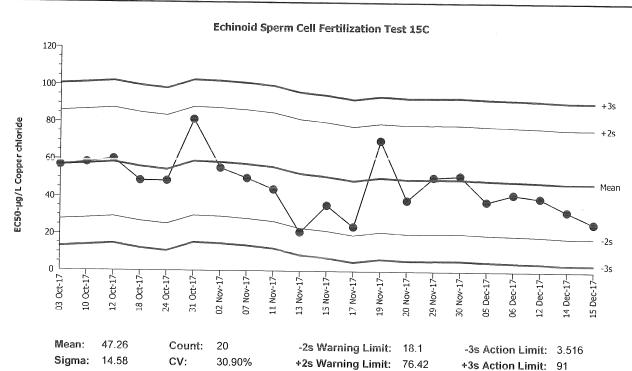
Protocol: EPA/600/R-95/136 (1995)

Organism: Strongylocentrotus purpuratus (Purpl

Endpoint: Fertilization Rate

Material: Copper chloride

Source: Reference Toxicant-REF



Quality Control Data											
Point		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				14:17	24.03	-23.23	-1.593			20-8374-1268	00-9691-5869
13				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				15:30	50.6	3.335	0.2288			05-0010-1267	11-1707-1208
16				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				15:50	41.57	-5.692	-0.3904			04-9516-7018	18-3148-8943
19				12:20	39.55	-7.712	-0.529			01-8906-4164	02-6832-7767
20				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: Test Code:

20 Dec-17 10:51 (p 1 of 1)

02-9159-5360/171215sprt的A 似以

12/20/17

### **Echinoid Sperm Cell Fertilization Test 15C**

Nautilus Environmental (CA)

Start Date:

15 Dec-17 15 Dec-17

Species: Strongylocentrotus purpuratus

Sample Code:

Q18 \$ 12/20/17 171215sprt**ß** 🕰

End Date:

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
V 99			1	100	2	12/21/17-
			2	100	75 8 19	
			3	[60	0	
			4	100	19	
			5	/60	-50	
			6	760	05457	
			7	100	05457	
			8	160	32	
			9	100	48	
			10	100	82	
			11	100	82 48 82 71	
			12	100	26 28 06683 21 86 87 51	
			13	100	28	
			14	100	<b>66</b> 83	
			15	100	21	
			16	/co	86	
			17	100	€.	
			18	100	_51	
			19	100	76	
	-		20	160	-6	
			21	100	6	
	$\perp$		22	100	71	
			23	100	36	
	-		24	100	71 36 3	
	-		25	100	8	
			26	100	8	
			27	100	19,	,
	-		28	100	64	
	-		29 30	100	49	·
			30	100	657 65	·

(9) y 000 ACS Q18 12/27/17
(9) y 000 ACS Q18 12/27/17
(5) EG Q18 12/27/17

Analyst: SGn OA: ACIDIO7/17

### **CETIS Test Data Worksheet**

Report Date: Test Code:

14 Dec-17 17:23 (p 1 of 1) 06-1613-2535/171215sprtA

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

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

15 Dec-17

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

Material: Copper chloride

Sample Code: 171215sprt Å Sample Source: Reference Toxicant Sample Station: Copper Chloride

C-µg/L 0	Ouc				# Eartilized	
	LC	Rep 1	Pos 10	# Counted	# Fertilized	Notes
				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			V V V V
10		4	2			
10		5	7			
20		1	5			
20		2	28			
20		3	29	100	46	BO 17/15/17
20		4	9			
20		5	18			
40		1	15			
40		2	12	100	31	130 17 11 5 117
40		3	23			150 12 1(5)17
40		4	4			
40		5	13			
80		1	1	100	2	BO 12/15/17
80		2	25		00	10 10117
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	100		NO 19111-

OCTAL Redlix

## Marine Chronic Bioassay

**Water Quality Measurements** 

Client :	Internal	Test Species: S. purpuratus
Sample ID:	CuCl <sub>2</sub>	Start Date/Time: 12/15/2017 1506
Test No:	171215sprt.A	End Date/Time: 12/15/2017 ) 546

Dilutions made by: F4 080 AC

High conc. made (μg/L):

Vol. Cu stock added (mL):

Final Volume (mL):

500

Cu stock concentration (µg/L): 7, 600

Analyst:

				(7)	
		Initial R	eadings		
Concentration	DO	рН	Salinity	Temperature	
(μg/L)	(mg/L)	(units)	(ppt)	(°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	83	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

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

# Marine Chronic Bioassay

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

# **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.:	Internal CuCl2 171215	SPAR			;	Start Date/Tim End Date/Tim Specie Animal Source	ne: <u>12/15/201</u> es: <i>S. purpur</i>	7 1 1546			
Tech initials: Injection Time:	1427	- -				Date Collecte	ed: 12(8	17			
Sperm Absorbance at 40	0 nm:	(tar	get range of	0.8 - 1.0 for	density of 4	lx10 <sup>6</sup> sperm/n	nl)				
Eggs Counted:	Mean: $162 \times 50 = 6.80^\circ$ eggs/ml  121 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)										
Initial density: Final density:		rs/ml = rs/ml	- <u>1.0</u> par	ution factor rt egg stock rts seawater	se	J	500 ml 35.75 ml				
Prepare the embryo stoc stock (1 part) and 125 ml			lilution factor	r. For exam <sub>l</sub>	ple, if the dil	ution factor is	2.25, use 10	0 ml of exist	ting		
				Sperm:F	gg Ratio						
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 50 0.0	40 10	1200:1 30 20	800:1 20 30	400:1 10 40	200:1 5.0 45	2.5 47.5	50:1 1.25 48.75			
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1435 1445 1455	Rar	100   200	tio: <u>Fer</u> 98 100	t. <u>Uni</u>	fert.					
NOTE: Choose a sperm- this range, choose the ra health, stage of reproduc	tio closest to 90 p	ercent unles			•						
Definitive Test		Spe	erm:Egg Rati	o Used:	50:1						
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1506 1926 1946			Fer   S2   D   C	t. Un	fert. 4 8 00 00 00					
Comments:											
QC Check:	AC12/27	17				Final Revie	ew: E4	12/29/17	,		

Appendix E

**Lab Data Qualifier Codes** 



#### **Glossary of Qualifier Codes:**

- Q1 Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 Temperatures out of recommended range; no action taken, test terminated same day
- Q3 Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L
- Q4 Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 Test initiated with aeration due to an anticipated drop in D.O.
- Q6 Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 Salinity out of recommended range
- Q8 Spilled test chamber/ Unable to recover test organism(s)
- Q9 Inadequate sample volume remaining, 50% renewal performed
- Q10 Inadequate sample volume remaining, no renewal performed
- Q11 Sample out of holding time; refer to QA section of report
- Q12 Replicate(s) not initiated; excluded from data analysis
- Q13 Survival counts not recorded due to poor visibility or heavy debris
- Q14 D.O. percent saturation was checked and was ≤ 110%
- Q15 Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 Percent minimum significant difference (PMSD) was <u>below</u> 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 <u>above</u> 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 or ganisms r eceived at a <u>temperature</u> greater than 3°C ou tside t he r ecommended t est 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. O rganisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 Test organisms received at <u>salinity</u> greater than 3 ppt outside of the recommended test salinity range. H owever, due t o age -specific pr otocol r equirements and/ or s ample ho lding t ime 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.

Updated: 6/30/15