

# Chronic Toxicity Test Results for the Carlsbad Desalination Plant

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

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: April 10, 2017

#### **Data Quality Assurance:**

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

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Results verified by:

# **EXECUTIVE SUMMARY**

# CHRONIC TOXICITY TESTING CARLSBAD DESALINATION PLANT – MARCH 2017 ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: March 9, 2017

Test Date: March 10, 2017

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

M-001

**Effluent Limitation:** 16.5 TU<sub>c</sub>

#### **Results Summary:**

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

#### INTRODUCTION

A 24-hour composite discharge sample was collected in March 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for monthly chronic toxicity monitoring purposes. Due to effects observed in a sample collected and tested for monthly monitoring purposes on June 17, 2016 from the CDP discharge monitoring point (M-001), accelerated monitoring was triggered according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Additional samples collected throughout the facility were also tested for comparison purposes. Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on March 10, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

Client: IDE Americas, Inc.

Sample Collection Date: March 9, 2017

#### **MATERIALS AND METHODS**

The samples were collected on March 9, 2017. Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the samples were hand delivered to Nautilus the day of sample collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocol described in USEPA 1995, and the methods are summarized in Table 3.

#### **Table 1. Sample Information**

Client/Project: IDE Americas, Inc./ Carlsbad Desalination Plant

Monitoring Period: March 2017

Sample ID, Material: 1. M-001, desalination plant brine effluent

ERI Brine, brine
 Train 9, brine

Sample Collection Date, Time: 1. 3/9/17, 10:00

3/9/17, 10:00
 3/9/17, 10:00

Sample Receipt Date, Time: 3/9/17, 12:07

Sampling Method: 24-hour Composite

**Table 2. Water Quality Measurements upon Sample Receipt** 

Sample ID	рН	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO <sub>3</sub> )	Total Chlorine (mg/L)
M-001	7.92	7.6	4.0	62.0	216	<0.02
ERI Brine	7.46	7.8	3.0	63.4	216	0.03
Train 9	7.45	7.9	4.0	64.4	219	<0.02

Client: IDE Americas, Inc.

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

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollution Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA, 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB, 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06 percent unadjusted effluent, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST statistical analysis is not in the 2006 CDP permit, the TST results are included for comparison purposes only.

TOXICITY SUMMARY REPORT Client: IDE Americas, Inc.
Test ID: 1703-S050 to S054 Sample Collection Date: March 9, 2017

#### **Table 3. Echinoderm Fertilization Chronic Bioassay Specifications**

Test Period: 3/10/17, 14:18 through 14:58

Test Organism: Strongylocentrotus purpuratus (purple sea urchin)

Test Organism Source: Field-collected locally (off Point Loma in San Diego, CA)

Lab Control/Dilution Water: Natural seawater (source: Scripps Institution of Oceanography (SIO) inlet),

34±2 parts per thousand (ppt); 20-µm filtered

Test Concentrations: 2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control.

The same dilution series was also tested with the other samples and M-001 after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP discharges). The 10 percent M-001 dilution was also tested with the pH10/0.45  $\mu m$  filtration toxicity identification

evaluation (TIE) treatment.

Number of Replicates, Organisms

per Replicate:

5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined

before each test with a preliminary rangefinding test.

Test Chamber Type, Volume per

Replicate:

Glass scintillation vial containing 10 mL of test solution

Protocol Used: EPA/600/R-95/136, 1995 West Coast Marine Chronic

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

fertilization period

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

difference (PMSD) value <25%

Reference Toxicant Testing: Copper chloride

Statistical Analysis Software: CETIS™, version 1.8.7.20

#### **RESULTS**

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

Client: IDE Americas, Inc.

Sample Collection Date: March 9, 2017

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

Statistical results for urchin fertilization toxicity tests are summarized in Table 4, and detailed test results are summarized in Tables 5 and 6. Raw test data and full statistical analyses can be found in Appendix A. Sample receipt information and a copy of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for M-001 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC <sub>50</sub> (% sample)	TU <sub>c</sub> value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001 (unadjusted)	6.06	10	>15	16.5	Pass	6.1
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	1.4
ERI Brine	10	15	>15	10	Pass	5.7
Train 9	5.0	6.06	>15	20	Pass	11

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

 $EC_{50}$  = Concentration expected to cause an adverse effect to 50 percent of the test organisms

Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) \*100. A negative PE results when organism performance in the sample is greater than that in the control.

TU<sub>c</sub> = Chronic Toxic Unit: 100÷NOEC

TST: Pass = sample is non-toxic at the 6.06% IWC according to the TST calculation; Fail = sample is toxic at the 6.06% IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.

Test Concentration	M-001 l	Jnadjusted Sample	M-001	. 40 ppt Adjusted <sup>a</sup>
(% Sample)	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.2	88.0	33.2	83.8
2.5	34.2	89.2	33.4	83.8
5.0	35.0	83.3	33.7	82.4
6.06	35.3	82.6	33.9	82.6
10	36.4	62.8*	34.0	83.2
15	37.9	50.6*	34.4	79.0

Client: IDE Americas, Inc.

Sample Collection Date: March 9, 2017

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

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

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

<sup>&</sup>lt;sup>a</sup> For comparison to the M-001 unadjusted sample, the M-001 sample was adjusted with seawater to 40 ppt prior to preparing test

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

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

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

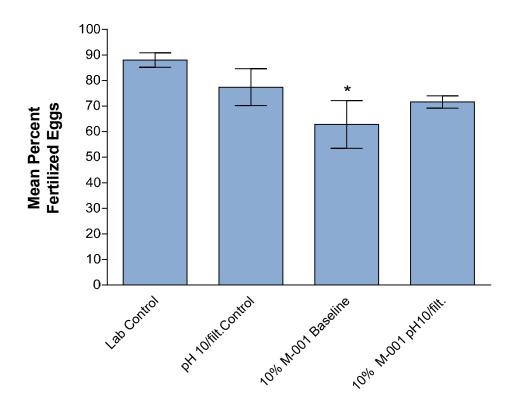


Figure 1. Summary of Urchin Fertilization Results for the M-001 10% Sample with and without the pH10/filtration TIE treatment (Mean  $\pm$  1SD). \*An asterisk indicates a statistically significant decrease in egg fertilization compared to the lab control using the TST analysis.

#### **QUALITY ASSURANCE**

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

Client: IDE Americas, Inc.

Sample Collection Date: March 9, 2017

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

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 7 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration ( $EC_{50}$  value) was within two standard deviations (SD) of the historical mean, indicating typical test organism sensitivity to copper. A list of qualifier codes used on bench datasheets can be found in Appendix E.

**Table 7. Reference Toxicant Test Results** 

Test Species	Endpoint	EC <sub>50</sub> (μg/L Copper)	Historical Mean EC <sub>50</sub> ±2 SD (μg/L Copper)	CV (%)
Purple Urchin	Fertilization	28.7	46.6 ± 37.7	40.4

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

#### REFERENCES

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

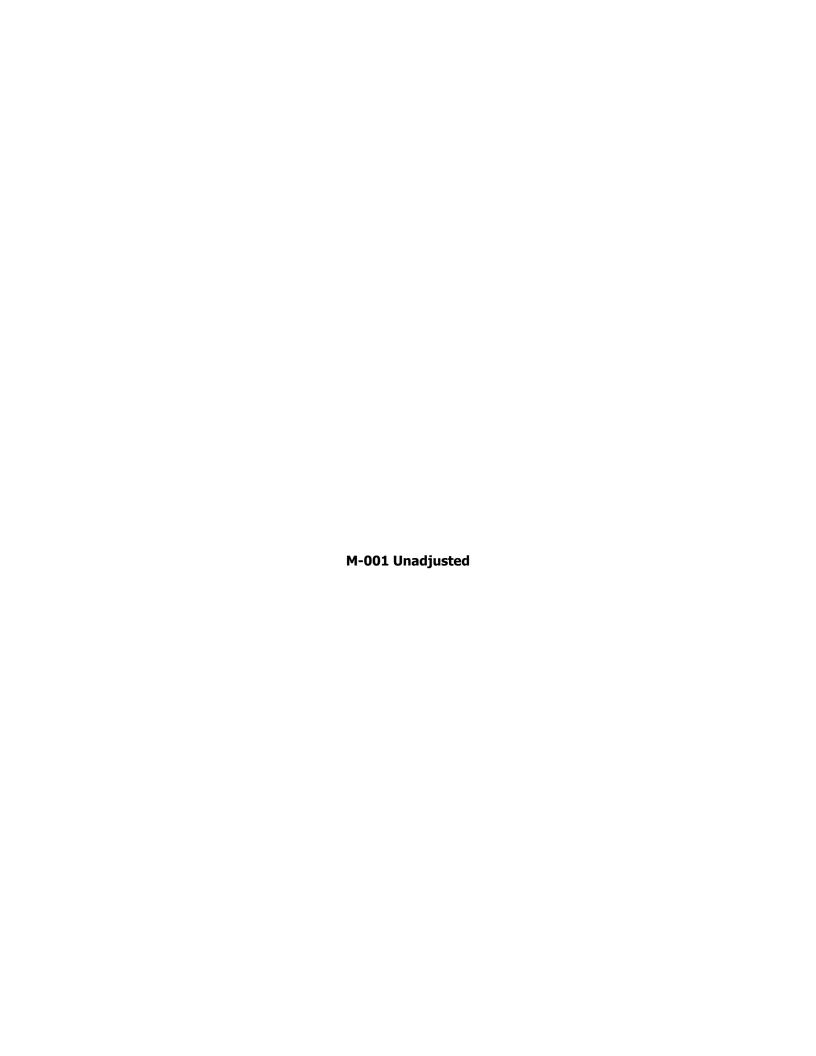
Client: IDE Americas, Inc.

Sample Collection Date: March 9, 2017

- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
- Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20
- USEPA. 1991. Methods for Aquatic Toxicity Identification Evaluation Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.
- USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

# Appendix A

**Test Data and Statistical Analyses** 



# **CETIS Summary Report**

Report Date:

14 Mar-17 17:07 (p 1 of 1)

Test Code:

1703-S050 | 16-8628-3186

		A-1000 (100 (100 (100 (100 (100 (100 (100	*		a training of the same			rest Code:		17	03-8050   1	0-0020-310
Echinoid Spe	erm Cell Fertiliz	ation T	est 15C					and the second		Nautilu	ıs Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	06-2864-0304 10 Mar-17 14: 10 Mar-17 14: 40m		Test Type: Protocol: Species: Source:	Fertilization EPA/600/R-95 Strongylocentr Pt. Loma	, ,	itus		Analyst: Diluent: Brine: Age:		ural Seawal Applicable	ter	
-	15-2497-8726 09 Mar-17 10:0 : 09 Mar-17 12:0 28h (4°C)		Code: Material: Source: Station:	17-0374 Facility Effluer IDE Americas, M-001 (Unadju	Inc.			Client: Project:	IDE Car	: Isbad Desa	l Plant	
Comparison	Summary						***************************************					
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meth	nod			
10-2919-3574	Fertilization Ra	ate	6.06	10	7.785	8.14%	16.5			ni Adj t Test		***************************************
Point Estimat	e Summary				######################################							
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Meth	nod			
20-2534-1715	Fertilization Ra	ate	EC25 EC50	9.274 >15	7.948 N/A	11.62 N/A	10.7 <6.6	8 Linea		erpolation (I	CPIN)	
Test Acceptat	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Ovei	rlap	Decision		
10-2919-3574	Fertilization Ra	ite	Contro	ol Resp	0.88	0.7 - NL		Yes		Passes A	cceptability	Criteria
20-2534-1715	Fertilization Ra	ite	Contro	ol Resp	0.88	0.7 - NL		Yes		Passes A	cceptability	Criteria
10-2919-3574	Fertilization Ra	ite	PMSE	)	0.08138	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	Rate Summary											
C-%	Control Type	Cou	nt Mean	95% LCL	95% UCL	Min	Max	Std I	≣rr	Std Dev	CV%	%Effect
0	Lab Control	5	0.88	0.8449	0.9151	0.85	0.91	0.012	265	0.02828	3.21%	0.0%
2.5		5	0.892	0.8523	0.9317	0.85	0.93	0.014	128	0.03194	3.58%	-1.36%
5		4	0.832	0.7645	0.9005	0.78	0.88	0.02	136	0.04272	5.13%	5.4%
6.06		5	0.826	0.7974	0.8546	0.79	0.85	0.010	03	0.02302	2.79%	6.14%
10		5	0.628	0.5124	0.7436	0.5	0.71	0.047	164	0.09311	14.83%	28.64%
15		5	0.506	0.4076	0.6044	0.38	0.58	0.035	544	0.07925	15.66%	42.5%
Fertilization R	late Detail											
C-%	Control Type	Rep	1 Rep 2	Rep 3	Rep 4	Rep 5						
	Lab Control	0.91	0.9	0.85	0.89	0.85						
2.5		0.93	0.91	0.85	0.9	0.87						
5			0.88	0.78	0.85	0.82						
6.06		0.83	0.85	0.79	0.82	0.84						
10		0.56	0.71	0.7	0.67	0.5						
15		0.58	0.48	0.55	0.38	0.54						

Report Date:

14 Mar-17 17:07 (p 1 of 2)

Test Code:

1703-S050 | 16-8628-3186

	- Annual Control of the Control of t						lest				6-8628-318
Echinoid Sp	erm Cell Fertiliz	ation Test	: 15C						Nautilus	s Environi	mental (CA)
Analysis ID: Analyzed:	10-2919-3574 14 Mar-17 17:		ndpoint: Fer nalysis: Par	tilization Ra ametric-Mu		arison		IS Version: cial Results		.8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Cor	rected)	NA	C > T	NA	NA		8.14%	6.06	10	7.785	16.5
Bonferroni A	\dj t Test										
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5		-0.4903	2.5	0.101 8	1.0000	CDF		ificant Effect		
	5		1.573	2.5	0.107 7	0.3235	CDF	_	ificant Effect		
	6.06		1.921	2.5	0.101 8	0.1680	CDF	_	ificant Effect		
	10*		7.473	2.5	0.101 8	<0.0001	CDF	Significan			
	15*		10.57	2.5	0.101 8	<0.0001	CDF	Significan			
ANOVA Tabl	е									——————————————————————————————————————	
Source	Sum Sqı	ıares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.810419	5	0.1620839	)	5	39.64	<0.0001	Significan	t Effect		**************************************
Error	0.094041	32	0.0040887	'53	23			•			
Total	0.904460	8			28						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(	(α:1%)			
Attribute Variances		Equality of	Variance	Test Stat 5.897	Critical 15.09	<b>P-Value</b> 0.3163	Decision( Equal Var	·			
	Bartlett E	Equality of Wilk W No				**		iances			
Variances Distribution	Bartlett E			5.897	15.09	0.3163	Equal Var	iances			
Variances Distribution Fertilization	Bartlett E Shapiro-			5.897	15.09	0.3163	Equal Var	iances	Std Err	CV%	%Effect
Variances Distribution  Fertilization C-% 0	Bartlett E Shapiro- Rate Summary	Count 5	Mean 0.88	5.897 0.9469	15.09 0.9004	0.3163 0.1518	Equal Var Normal Di	iances stribution	<b>Std Err</b> 0.01265	CV% 3.21%	%Effect 0.0%
Variances Distribution  Fertilization  C-% 0 2.5	Bartlett E Shapiro- Rate Summary Control Type	Wilk W No	ormality <b>M</b> ean	5.897 0.9469 <b>95% LCL</b>	15.09 0.9004 95% UCL	0.3163 0.1518 Median	Equal Var Normal Di	iances stribution			
Variances Distribution  Fertilization C-% 0 2.5 5	Bartlett E Shapiro- Rate Summary Control Type	Count 5 5 4	Mean 0.88	5.897 0.9469 <b>95% LCL</b> 0.8449	15.09 0.9004 <b>95% UCL</b> 0.9151	0.3163 0.1518 <b>Median</b> 0.89	Equal Var Normal Di Min 0.85	iances stribution Max 0.91	0.01265	3.21%	0.0%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06	Bartlett E Shapiro- Rate Summary Control Type	Count 5 5	Mean 0.88 0.892	5.897 0.9469 <b>95% LCL</b> 0.8449 0.8523	15.09 0.9004 <b>95% UCL</b> 0.9151 0.9317	0.3163 0.1518 Median 0.89 0.9	Equal Var Normal Di Min 0.85 0.85	Max 0.91 0.93	0.01265 0.01428	3.21% 3.58%	0.0% -1.36%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10	Bartlett E Shapiro- Rate Summary Control Type	Count 5 5 4 5 5 5	Mean 0.88 0.892 0.8325	5.897 0.9469 <b>95% LCL</b> 0.8449 0.8523 0.7645	15.09 0.9004 <b>95% UCL</b> 0.9151 0.9317 0.9005	0.3163 0.1518 <b>Median</b> 0.89 0.9 0.835 0.83 0.67	Equal Var Normal Di Min 0.85 0.85 0.78	Max 0.91 0.93 0.88	0.01265 0.01428 0.02136	3.21% 3.58% 5.13%	0.0% -1.36% 5.4%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10	Bartlett E Shapiro- Rate Summary Control Type	Count 5 5 4 5	Mean 0.88 0.892 0.8325 0.826	5.897 0.9469 <b>95% LCL</b> 0.8449 0.8523 0.7645 0.7974	15.09 0.9004 <b>95% UCL</b> 0.9151 0.9317 0.9005 0.8546	0.3163 0.1518 <b>Median</b> 0.89 0.9 0.835 0.83	Equal Var Normal Di Min 0.85 0.85 0.78 0.79	Max 0.91 0.93 0.88 0.85	0.01265 0.01428 0.02136 0.0103	3.21% 3.58% 5.13% 2.79%	0.0% -1.36% 5.4% 6.14%
Variances Distribution  Fertilization  C-%  0  2.5  5  6.06  10  15	Bartlett E Shapiro- Rate Summary Control Type	Count 5 5 4 5 5 5	Mean 0.88 0.892 0.8325 0.826 0.628 0.506	5.897 0.9469 <b>95% LCL</b> 0.8449 0.8523 0.7645 0.7974 0.5124	95% UCL 0.9151 0.9005 0.8546 0.7436	0.3163 0.1518 <b>Median</b> 0.89 0.9 0.835 0.83 0.67	Equal Var Normal Di Min 0.85 0.85 0.78 0.79	Max 0.91 0.93 0.88 0.85 0.71	0.01265 0.01428 0.02136 0.0103 0.04164	3.21% 3.58% 5.13% 2.79% 14.83%	0.0% -1.36% 5.4% 6.14% 28.64%
Variances Distribution  Fertilization  C-% 0 2.5 5 6.06 10 15  Angular (Cor	Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 4 5 5 5 cmed Sum	Mean 0.88 0.892 0.8325 0.826 0.628 0.506	5.897 0.9469 <b>95% LCL</b> 0.8449 0.8523 0.7645 0.7974 0.5124	95% UCL 0.9151 0.9005 0.8546 0.7436	0.3163 0.1518 <b>Median</b> 0.89 0.9 0.835 0.83 0.67	Equal Var Normal Di Min 0.85 0.85 0.78 0.79	Max 0.91 0.93 0.88 0.85 0.71	0.01265 0.01428 0.02136 0.0103 0.04164	3.21% 3.58% 5.13% 2.79% 14.83%	0.0% -1.36% 5.4% 6.14% 28.64%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor C-% 0	Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 4 5 5 5 7 med Sum	Mean  0.88  0.892  0.8325  0.826  0.628  0.506	5.897 0.9469 <b>95% LCL</b> 0.8449 0.8523 0.7645 0.7974 0.5124 0.4076	95% UCL 0.9151 0.9005 0.8546 0.7436 0.6044	0.3163 0.1518 <b>Median</b> 0.89 0.9 0.835 0.83 0.67 0.54	Min 0.85 0.85 0.78 0.79 0.5 0.38	Max 0.91 0.93 0.88 0.85 0.71 0.58	0.01265 0.01428 0.02136 0.0103 0.04164 0.03544	3.21% 3.58% 5.13% 2.79% 14.83% 15.66%	0.0% -1.36% 5.4% 6.14% 28.64% 42.5%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 4 5 5 5 cmed Sum	Mean  0.88  0.892  0.8325  0.826  0.628  0.506  mary  Mean	95% LCL 0.8449 0.8523 0.7645 0.7974 0.5124 0.4076	95% UCL 0.9151 0.9005 0.8546 0.7436 0.6044 95% UCL	0.3163 0.1518 Median 0.89 0.9 0.835 0.67 0.54	Equal Var Normal Di Min 0.85 0.85 0.78 0.79 0.5 0.38	Max 0.91 0.93 0.88 0.85 0.71 0.58	0.01265 0.01428 0.02136 0.0103 0.04164 0.03544 Std Err	3.21% 3.58% 5.13% 2.79% 14.83% 15.66%	0.0% -1.36% 5.4% 6.14% 28.64% 42.5%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 4 5 5 cmed Sum Count 5 5 4	Mean  0.88  0.892  0.8325  0.826  0.628  0.506  mary  Mean  1.219	5.897 0.9469 95% LCL 0.8449 0.8523 0.7645 0.7974 0.5124 0.4076 95% LCL 1.165	95% UCL 0.9151 0.9005 0.8546 0.7436 0.6044 95% UCL 1.273	0.3163 0.1518 Median 0.89 0.9 0.835 0.67 0.54 Median 1.233	Min 0.85 0.85 0.78 0.79 0.5 0.38  Min 1.173	Max 0.91 0.93 0.88 0.85 0.71 0.58	0.01265 0.01428 0.02136 0.0103 0.04164 0.03544 Std Err 0.0194	3.21% 3.58% 5.13% 2.79% 14.83% 15.66% CV% 3.56%	0.0% -1.36% 5.4% 6.14% 28.64% 42.5% <b>%Effect</b> 0.0%
Variances Distribution  Fertilization C-% 0 2.5 5 6.06 10 15  Angular (Cor C-% 0 2.5 5	Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 4 5 5 med Sum Count 5 5	Mean  0.88  0.892  0.8325  0.826  0.628  0.506  mary  Mean  1.219  1.239	95% LCL 0.8449 0.8523 0.7645 0.7974 0.5124 0.4076 95% LCL 1.165 1.175	95% UCL 0.9151 0.9005 0.8546 0.7436 0.6044 95% UCL 1.273 1.303	0.3163 0.1518 Median 0.89 0.9 0.835 0.67 0.54 Median 1.233 1.249	Min 0.85 0.85 0.78 0.79 0.5 0.38  Min 1.173	Max 0.91 0.93 0.88 0.85 0.71 0.58 Max 1.266 1.303	0.01265 0.01428 0.02136 0.0103 0.04164 0.03544 Std Err 0.0194 0.02308	3.21% 3.58% 5.13% 2.79% 14.83% 15.66% CV% 3.56% 4.17%	0.0% -1.36% 5.4% 6.14% 28.64% 42.5% %Effect 0.0% -1.63%
Variances Distribution  Fertilization  C-%  0  2.5  5  6.06  10  15	Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 4 5 5 cmed Sum Count 5 5 4	Mean  0.88  0.892  0.8325  0.826  0.628  0.506  mary  Mean  1.219  1.239  1.151	95% LCL 0.8449 0.8523 0.7645 0.7974 0.5124 0.4076 95% LCL 1.165 1.175 1.06	95% UCL 0.9151 0.9005 0.8546 0.7436 0.6044 95% UCL 1.273 1.303 1.243	0.3163 0.1518 Median 0.89 0.9 0.835 0.67 0.54 Median 1.233 1.249 1.153	Min 0.85 0.85 0.78 0.79 0.5 0.38  Min 1.173 1.173 1.083	Max 0.91 0.93 0.88 0.85 0.71 0.58  Max 1.266 1.303 1.217	0.01265 0.01428 0.02136 0.0103 0.04164 0.03544 Std Err 0.0194 0.02308 0.02868	3.21% 3.58% 5.13% 2.79% 14.83% 15.66% CV% 3.56% 4.17% 4.98%	0.0% -1.36% 5.4% 6.14% 28.64% 42.5% %Effect 0.0% -1.63% 5.54%

Report Date: Test Code:

14 Mar-17 17:07 (p 2 of 2)

1703-S050 | 16-8628-3186

**Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) 10-2919-3574 Analysis ID: Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 14 Mar-17 17:03 Analysis: Parametric-Multiple Comparison Official Results: Yes Graphics 1.0 F 0.10 0.08 0.9 0,06 Reject Null 0.04 0.02 0.00 0.6 -0.02 0.5 -0.04 0.4 -0.06 -0.08 0.3 -0.10 0.2 -0.12 0.1 -0,14 0.0 6.06 10 15 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 1.0 1.5 2.0 C-% Rankits

Report Date:

14 Mar-17 17:07 (p 1 of 1)

Test Code:

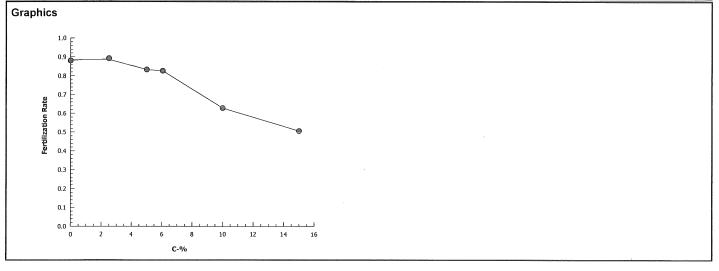
1703-S050 | 16-8628-3186

Echinoid Spe	rm Cell Fertilization	Test 15C			Nautilus Environmental (CA)
Analysis ID:	20-2534-1715	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	14 Mar-17 17:03	Analysis:	Linear Interpolation (ICPIN)	Official Results:	Yes

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

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



TST

Report Date:

14 Mar-17 17:07 (p 1 of 1)

OE 110 AII	ary tiour itop	011		750				Test Code:		1703-S050   16-8628-3186		
Echinoid Sp	erm Cell Fertiliz	ation Tes	t 15C						Nautilu	s Environr	nental (CA)	
Analysis ID: Analyzed:	06-8355-1628 14 Mar-17 17:		•	tilization Ratametric Bioe		-Two Sampl		IS Version:	CETISv1 : Yes	.8.7		
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	6.08%	6.06	10	7.785	16.5	
TST-Welch's	t Test											
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(	α:5%)			
Lab Control	2.5*		11.9	1.943	0.053 6	<0.0001	CDF	Non-Signi	ficant Effect		HARLAN	
	5*		7.378	2.132	0.069 4	0.0009	CDF	Non-Signi	ficant Effect			
	6.06*		11.47	1.895	0.037 7	<0.0001	CDF	Non-Signi	ficant Effect			
	10		0.0552	2.132	0.097 4	0.4793	CDF	Significant	t Effect			
	15		-3.187	2.015	0.078 5	0.9878	CDF	Significant	Effect			
ANOVA Tabl	e									T-M-124 (Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud-12-Aud		
Source	Sum Sqւ	ıares	Mean Squ	ıare	DF	F Stat	P-Value	Decision(	α:5%)			
Between	0.810419	5	0.1620839	)	5	39.64	<0.0001	Significant	Effect			
Error	0.094041	32	0.0040887	'53	23							
Total	0.904460	8			28							
Distribution	al Tests											
Attribute	Test			Test Stat	Critical	P-Value	Decision(	(α:1%)				
Variances	Bartlett E	Equality of	f Variance	5.897	15.09	0.3163	Equal Var	iances				
Distribution	Shapiro-	Wilk W N	ormality	0.9469	0.9004	0.1518	Normal Di	stribution				
Fertilization	Rate Summary					ASSERTATION OF THE PROPERTY OF						
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	0.88	0.8449	0.9151	0.89	0.85	0.91	0.01265	3.21%	0.0%	
2.5		5	0.892	0.8523	0.9317	0.9	0.85	0.93	0.01428	3.58%	-1.36%	
5		4	0.8325	0.7645	0.9005	0.835	0.78	0.88	0.02136	5.13%	5.4%	
6.06		5	0.826	0.7974	0.8546	0.83	0.79	0.85	0.0103	2.79%	6.14%	
10		5	0.628	0.5124	0.7436	0.67	0.5	0.71	0.04164	14.83%	28.64%	
15		5	0.506	0.4076	0.6044	0.54	0.38	0.58	0.03544	15.66%	42.5%	
Angular (Co	rrected) Transfor	rmed Sun	nmary		Manage de la constante de la c				480-			
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect	
0	Lab Control	5	1.219	1.165	1.273	1.233	1.173	1.266	0.0194	3.56%	0.0%	
2.5		5	1.239	1.175	1.303	1.249	1.173	1.303	0.02308	4.17%	-1.63%	
5		4	1.151	1.06	1.243	1.153	1.083	1.217	0.02868	4.98%	5.54%	
5 6.06		4 5	1.151 1.141	1.06 1.104	1.243 1.178	1.153 1.146	1.083 1.095	1.217 1.173	0.02868 0.01341	4.98% 2.63%	5.54% 6.38%	

# **Outlier Calculation**

List potential outlier first in data set.

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

Critical T-value for 5 replicates is 1.67

If calculated T-value exceeds critical T-value, then data point  $\underline{\textbf{is}}$  an outlier.

Entered: AC 3/14/17 OC: 20 4/10/14

# **CETIS Test Data Worksheet**

Report Date:

09 Mar-17 15:23 (p 1 of 1)

Test Code: 1703-505016-8628-3186/6482A3B2

Echinoid Sp									6.10	A 2 13 1 2 1		(CA)
Start Date: End Date:				Species: Strongylocentrotus purpuratus Protocol: EPA/600/R-95/136 (1995)				Sample Code: 5AE55426 170374 Sample Source: IDE Americas, Inc.				
Sample Date	: 09 /	Mar-17	7			Facility Effluer		Sample Stati				
C-%	Code	Rep	Pos	# Counted	# Fertilized		- (	Notes				
			31	100	78	3/14/17						
			32 :	100	35							
			33	100	83							
			34	100	90							
			35	100	54							
			36	(00)	90							
			37	100	39							
			38	100	50							
			39	100	85							
			40	100	48							
			41	100	70							
			42	100	91		, râ l	-				
			43 44	100	43	DC = AC 3/14	4410	Direp e	xc C	uded a	1 Statistica	00
			45	100	85							
MANAGE TO THE PARTY OF THE PART			46	100	-87 -71							
			47	100	. 35							
			48	100	91							
			49	100	85							
			50	100	38							
			51	100	93							
			52	100	58							
			53	100	34							
			54	100	56							
			55	100	88							
			56	100	67							
			57	601	79							
			58	100	82							
			59 60	100	82							
			00	100	55					B rounted	A fert	
		pH 1	A O.	100	35 69 Ø	1	SAL	Confeel	A	100	75	
	f:	H 1	OB	100	70		SAL	(control	(3	100	74	
3/14/17	,	) I† 1:		(00	71			(ontrol		100	82 D 34	
-,,,,	,				73	S	AL			100	74	
	P	4	10 D	£00		\$	AL	101100)				
	p	, 4	10 E	100	75	Š	AL	(ontrol	E	100	71	
ic.	1 con	trol	A	(00)	28							
•	l con			100	79							
	(00			100	33							
6 1	, 00	, ,	, n	100	78							
1.3	600	atro 1	1,/		65							

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

Report Date:

09 Mar-17 15:23 (p 1 of 1)

Test Code:) 703-S050 16-8628-3186/6482A3B2

Echinoid Spe	Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA)							
Start Date: End Date: Sample Date	10 N	Mar-17 Mar-17 Mar-17	7	Protoc	Species: Strongylocentrotus purpuratus  Protocol: EPA/600/R-95/136 (1995)  Material: Seawater Facility Efficient		Sample Code: 155426 17-0374  Sample Source: IDE Americas, Inc.  Sample Station: M-001 (Unadjusted)	
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes	
0	LC	1	48					
0	LC	2	34					
0	LC	3	44	100	88	EG 3/10/17		
0	LC	4	37	(30	00		*	
0	LC	5	49					
2.5		1	51				782.00000,0000000000000000000000000000000	
2.5		2	42	100	92	EG		
2.5		3	39	,				
2.5		4	36			1 177-6 1000-700-7 1-7-010 A ALADA A A		
2.5		5	45					
5		1	43					
5		2	55	100	83	EC		
5		3	31					
5		4	32					
5		5	59					
6.06		1	33					
6.06		2	47					
6.06		3	57	100	79	EG		
6.06		4	58		, ,			
6.06		5	53					
10		1	54	-				
10		2	46					
10		3	41	100	68	EG		
10		4	56		41 4 4 4			
10		5	38					
15		1	52		X2-2	V-11-		
15		2	40			***************************************		
15		3	60	100	53	EG		
15		4	50					
15		5	35					

QC- EG

# Marine Chronic Bioassay

#### **Water Quality Measurements**

Client :	IDE	Test Species: S. purpuratus
Sample ID:	M-001 (unadjusted)	Start Date/Time: 3/10/2017 1418
Sample Log No.:	17-0374	End Date/Time: 3/10/2017 1458
Dilutions made by:	EG	Test No: 1703-5050

			Analyst:	EG
		1		
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.0	8.61	33.2	14.9
2.5	8,6	8.62	34.2	14.8
5.0	8.2	8.63	35.0	14.7
6.06	8.3	8.63	35.3	14.8
10	8.3	8,04	36.4	14.8
15	83	8.04	379	14.9

Comments:		
QC Check:	AC3/14/17	Final Review: ১০০ ৭ ৮   ١٦

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

#### Marine Chronic Bioassay

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

	2					
Client: Sample ID: Test No.:	1DE M-00) Unad 1703-S05	justed	,		: 3/10/2017 / : S. purpurat	1458
Tech initials: Injection Time:	EG 1340			Animal Source Date Collected		+ 3/6/17
Sperm Absorbance at 4	00 nm: <u>0.855</u>	(target range of 0.8 -	- 1.0 for density of 4	x10 <sup>6</sup> sperm/ml)		
Eggs Counted:	Mea	an: <u>90,4</u> X 50 =	= <u>4520</u> eg	gs/ml		
	102 (targe	et counts of 80 eggs per v er slide for a final density c		vick-		
Initial density: Final density:	4000 eggs/ml	= 1.13 dilution - 1.0 part egg 0.13 parts se	stock sea	g stock 20 awater 2	<del></del> ····	
Prepare the embryo stoo existing stock (1 part) ar	ck according to the calcu nd 125 ml of dilution wate	lated dilution factor. Foer (1.25 parts).	r example, if the dil	ution factor is 2.	25, use 100	ml of
		<u>s</u>	perm:Egg Ratio			
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1     1600       50     40       0.0     10	30 2	0:1     400:1       0     10       0     40	200:1 5.0 45	2.5	<b>50:1</b> 1.25 48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1356 1400 1410	Rangefinder Ratio:	Fert. Unfo	ert.		
NOTE: Choose a sperm this range, choose the organism health, stage of	ratio closest to 90 per	cent unless profession	80 and 90 percent. al judgment dictate	If more than or es consideration	ne concentrat n of other fa	tion is within actors (e.g.,
<u>Definitive Test</u>		Sperm:Egg Ratio Use	ed: 100:			
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1418 1438	QC1 QC2 Egg Control 1 Egg Control 2	Fert. Unfe 92 85 0 10	ert. — — ©O		
Comments:						
				<del></del>		· · · · · · · · · · · · · · · · · · ·

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QC Check:

# IDE/ Carlsbad Desalination Plant TIE Summary of Urchin Fertilization Results

Sample Collection Date: 3/9/17; Test Initiation Date: 3/10/17

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

#### Marine Chronic Bioassay

#### **Water Quality Measurements**

	-				-			
Client :	IDE		Test Species: S. purpuratus					
Sample ID:	M-001 (unadjusted)	)	Start Date/Time: 3/10/2017 1418					
Sample Log No.:	17-0374		-	End Date/Time:	3/10/2017 145	58		
Dilutions made by:	Ely		_	Test No:	1703-SOS	50		
				Analyst:	EG			
	ſ		Initial I	Readings				
	Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)			
	Salinity Control	7.9	8.06	36.3	15,0			
	pH10/filt. Control	6.9	8,05	35,7	15.0			
	pH 10/filt. 10% M- 001	6.0	8.14	36.2	15.2			

Comments:		
QC Check:	AC 3/14/17	Final Review: 🗸 ખ નાિગામ

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

# **TST Summary Sheet**

Lab Name Nautilus Client Name IDE/CDP

**Test ID** LC vs. pH 10 Control **Test Species** S. purpuratus (echinoderm)

Test Date 3/10/2017 Test Type Chronic

**Test Duration** 40m **Endpoint** Fertilization

Critical Conc. 10%

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

#### Mean % Effect at Critical Conc.

11.82

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

#### Results

Pass Sample is Non-toxic

#### **Raw Data**

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

# **TST Summary Sheet**

Lab Name Nautilus Client Name IDE/CDP

**Test ID** LC vs. pH 10 10% M-001 **Test Species** S. purpuratus (echinoderm)

Test Date 3/10/2017 Test Type Chronic

Test Duration 40m Endpoint Fertilization

Critical Conc. 10%

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

#### Mean % Effect at Critical Conc.

18.64

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

#### Results

Pass Sample is Non-toxic

#### **Raw Data**

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

# **TST Summary Sheet**

Lab Name Nautilus Client Name IDE/CDP

**Test ID** LC vs. 10% M-001 **Test Species** S. purpuratus (echinoderm)

Test Date 3/10/2017 Test Type Chronic

**Test Duration** 40m **Endpoint** Fertilization

Critical Conc. 10%

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

#### Mean % Effect at Critical Conc.

28.64

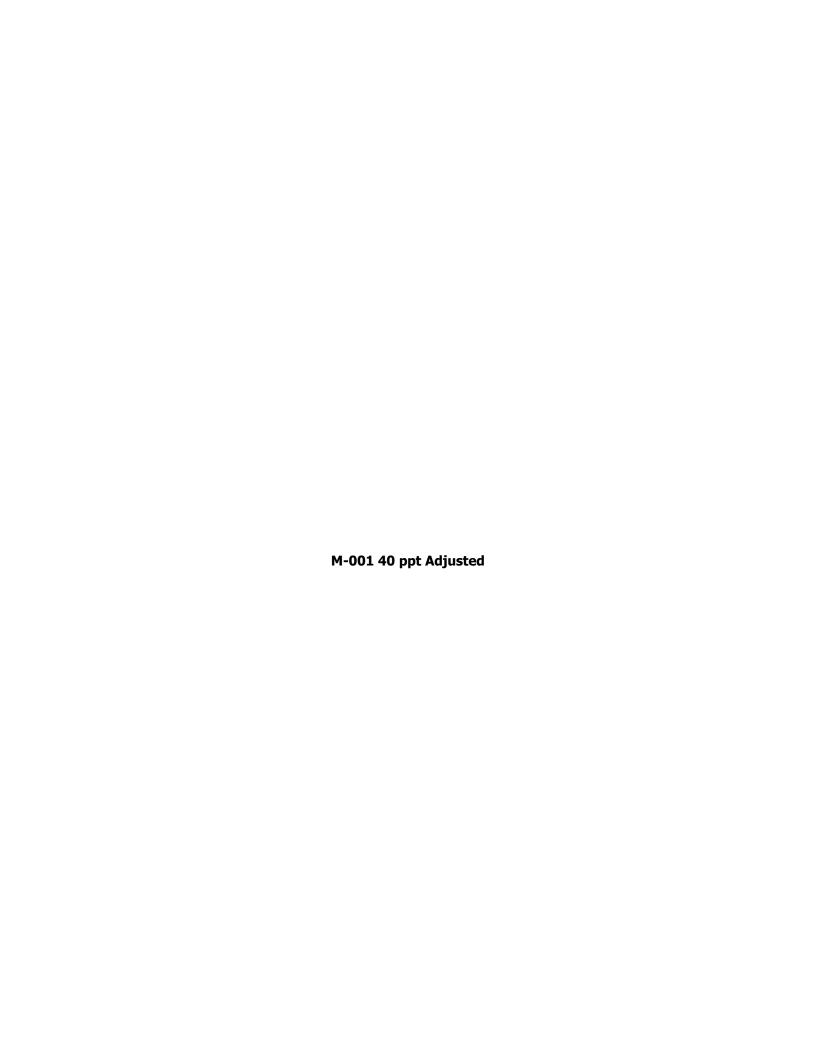
Calculated t-value	Degrees of Freedom	Table t-value	Percent Difference
0.0552	4	2.1318	

#### Results

Fail Sample is Toxic

#### **Raw Data**

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



# **CETIS Summary Report**

Report Date:

14 Mar-17 17:08 (p 1 of 1)

Test Code:

1703-S051 | 00-3723-6090

								rest Code:		170	J3-5051   C	0-3/23-609
Echinoid Spe	rm Cell Fertiliza	tion Te	st 15C				wo by contract of the contract			Nautilu	s Environi	mental (CA
Batch ID: Start Date: Ending Date: Duration:	11-8324-5262 10 Mar-17 14:1 10 Mar-17 14:5 40m	8	Test Type: Protocol: Species: Source:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	` ,	atus		Analyst: Diluent: Brine: Age:		ural Seawat Applicable	er	
•	03-1402-7483 09 Mar-17 10:0 09 Mar-17 12:0 28h (4 °C)	0   7 :	Code: Material: Source: Station:	17-0374 Facility Effluen IDE Americas, M-001 (40 ppt	Inc.			Client: Project:	IDE Carl	sbad Desal	Plant	
Comparison S	Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meth	od			
21-0731-8201	Fertilization Ra	te	15	>15	NA	6.66% ∠	€ 6.66	7 Dunr	nett N	lultiple Com	parison Te	st
Point Estimate	e Summary						***************************************				HALLOW THE STATE OF THE STATE O	
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Meth	od			
02-3614-0744	Fertilization Rat	te	EC25 EC50	>15 >15	N/A N/A	N/A N/A	<6.66		ar Inte	erpolation (I	CPIN)	
Test Acceptab	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Over	lap	Decision		
02-3614-0744	Fertilization Rat	:e	Contro	ol Resp	0.838	0.7 - NL		Yes		Passes A	cceptability	Criteria
21-0731-8201	Fertilization Rat	:e	Contro	ol Resp	0.838	0.7 - NL		Yes		Passes A	cceptability	Criteria
21-0731-8201	Fertilization Rat	e	PMSD		0.06657	NL - 0.25		No	******	Passes A	cceptability	Criteria
Fertilization R	ate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std E	Err	Std Dev	CV%	%Effect
	Lab Control	5	0.838	0.7829	0.8931	0.79	0.89	0.019	985	0.04438	5.3%	0.0%
2.5		5	0.838	0.7993	0.8767	0.81	0.88	0.013	393	0.03114	3.72%	0.0%
5		5	0.824	0.7534	0.8946	0.76	0.89	0.025	542	0.05683	6.9%	1.67%
6.06		5	0.826	. 0.7988	0.8532	0.8	0.86	0.009	798	0.02191	2.65%	1.43%
10		5	0.832	0.7923	0.8717	0.79	0.87	0.014	28	0.03194	3.84%	0.72%
15		5	0.79	0.7609	0.8191	0.75	0.81	0.010	)49	0.02345	2.97%	5.73%
Fertilization R	ate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
	Lab Control	0.79	0.89	0.81	0.88	0.82						
2.5		0.81	0.81	0.88	0.86	0.83						
5		0.76	0.86	0.77	0.84	0.89					*	
6.06		0.82	0.8	0.82	0.86	0.83						
10		0.87	0.84	0.81	0.79	0.85						

15

0.81

0.75

0.8

0.79

8.0

Report Date:

14 Mar-17 17:08 (p 1 of 2)

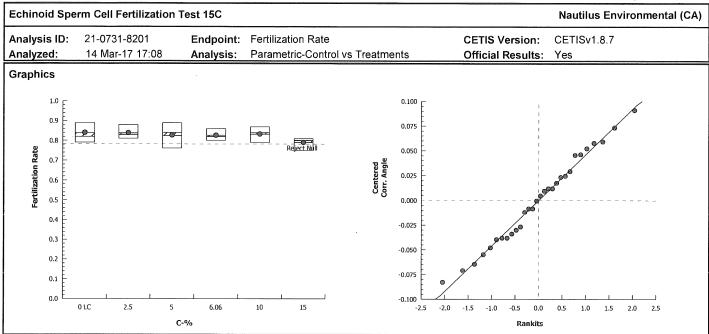
Test Code:

1703-S051 | 00-3723-6090

											10-3723-608
Echinoid Sp	erm Cell Fertiliz	ation Test 1	15C						Nautilus	Environ	mental (CA
Analysis ID:	21-0731-8201	En	dpoint: Fer	tilization Rat	e		CET	S Version	: CETISv1.	8.7	
Analyzed:	14 Mar-17 17:	08 <b>An</b>	<b>alysis:</b> Par	ametric-Con	trol vs Trea	tments	Offic	ial Results	s: Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	rected)	NA	C > T	NA	NA		6.66%	15	>15	NA	∠ 6.667
Dunnett Mul	tiple Compariso	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	ι(α:5%)		
Lab Control	2.5		0.04629	2.362	0.074 8	0.8193	CDF	······································	ificant Effect		
	5		0.5621	2.362	0.074 8	0.6195	CDF		ificant Effect		
	6.06		0.5825	2.362	0.074 8	0.6104	CDF	-	ificant Effect		
	10		0.3066	2.362	0.074 8	0.7273	CDF	•	ificant Effect		
	15		2.045	2.362	0.074 8	0.0916	CDF	_	ificant Effect		
ANOVA Table	e				THE PERSON NAMED IN COLUMN TWO IS NOT THE PERSON NAMED IN COLUMN TWO IS NAM			_			
Source	Sum Squ	uares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	ι(α:5%)		
Between	0.013998	79	0.0027997		5	1.137	0.3682		ificant Effect		
Error	0.059106	.99	0.0024627	'91	24						
Total	0.073105				29	_					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(	α:1%)			
Variances	Bartlett F	Equality of V	/ariance	5.466	15.09	0.3617	Equal Var				U 2000 (100) (1000 (1000 (1000 (100) (1000 (1000 (100) (1000 (1000 (100) (1000 (100) (1000 (100) (1000 (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000 (100) (1000) (1000 (100) (1000) (1
Distribution		Wilk W Nor		0.982	0.9031	0.8750	Normal Di				
Manuscon and Control of Control o							Production of the Control of the Con				
Fertilization	Rate Summary										
Fertilization C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
C-%	•	Count 5	<b>Mean</b> 0.838	<b>95% LCL</b> 0.7829	<b>95% UCL</b> 0.8931	Median 0.82	<b>Min</b> 0.79	<b>Max</b> 0.89	<b>Std Err</b> 0.01985	<b>CV%</b> 5.3%	%Effect
<b>C-%</b>	Control Type										
<b>C-</b> % 0 2.5	Control Type	5	0.838	0.7829	0.8931	0.82	0.79	0.89	0.01985	5.3%	0.0%
<b>C-%</b> 0 2.5 5	Control Type	5 5	0.838 0.838	0.7829 0.7993	0.8931 0.8767	0.82 0.83	0.79 0.81	0.89 0.88	0.01985 0.01393	5.3% 3.72%	0.0% 0.0%
<b>C-%</b> 0 2.5 5 6.06	Control Type	5 5 5	0.838 0.838 0.824	0.7829 0.7993 0.7534	0.8931 0.8767 0.8946	0.82 0.83 0.84	0.79 0.81 0.76	0.89 0.88 0.89	0.01985 0.01393 0.02542	5.3% 3.72% 6.9%	0.0% 0.0% 1.67%
C-% 0 2.5 5 6.06	Control Type	5 5 5 5	0.838 0.838 0.824 0.826	0.7829 0.7993 0.7534 0.7988	0.8931 0.8767 0.8946 0.8532	0.82 0.83 0.84 0.82	0.79 0.81 0.76 0.8	0.89 0.88 0.89 0.86	0.01985 0.01393 0.02542 0.009798	5.3% 3.72% 6.9% 2.65%	0.0% 0.0% 1.67% 1.43%
C-% 0 2.5 5 6.06 10	Control Type	5 5 5 5 5 5	0.838 0.838 0.824 0.826 0.832 0.79	0.7829 0.7993 0.7534 0.7988 0.7923	0.8931 0.8767 0.8946 0.8532 0.8717	0.82 0.83 0.84 0.82 0.84	0.79 0.81 0.76 0.8 0.79	0.89 0.88 0.89 0.86 0.87	0.01985 0.01393 0.02542 0.009798 0.01428	5.3% 3.72% 6.9% 2.65% 3.84%	0.0% 0.0% 1.67% 1.43% 0.72%
C-% 0 2.5 5 6.06 10 15 Angular (Cor	Control Type Lab Control	5 5 5 5 5 5	0.838 0.838 0.824 0.826 0.832 0.79	0.7829 0.7993 0.7534 0.7988 0.7923	0.8931 0.8767 0.8946 0.8532 0.8717	0.82 0.83 0.84 0.82 0.84	0.79 0.81 0.76 0.8 0.79	0.89 0.88 0.89 0.86 0.87	0.01985 0.01393 0.02542 0.009798 0.01428	5.3% 3.72% 6.9% 2.65% 3.84%	0.0% 0.0% 1.67% 1.43% 0.72%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0	Control Type Lab Control	5 5 5 5 5 5 5	0.838 0.838 0.824 0.826 0.832 0.79	0.7829 0.7993 0.7534 0.7988 0.7923 0.7609	0.8931 0.8767 0.8946 0.8532 0.8717 0.8191	0.82 0.83 0.84 0.82 0.84 0.8	0.79 0.81 0.76 0.8 0.79 0.75	0.89 0.88 0.89 0.86 0.87 0.81	0.01985 0.01393 0.02542 0.009798 0.01428 0.01049	5.3% 3.72% 6.9% 2.65% 3.84% 2.97%	0.0% 0.0% 1.67% 1.43% 0.72% 5.73%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0	Control Type Lab Control  rrected) Transfor Control Type	5 5 5 5 5 5 5 rmed Sumn	0.838 0.838 0.824 0.826 0.832 0.79	0.7829 0.7993 0.7534 0.7988 0.7923 0.7609	0.8931 0.8767 0.8946 0.8532 0.8717 0.8191	0.82 0.83 0.84 0.82 0.84 0.8	0.79 0.81 0.76 0.8 0.79 0.75	0.89 0.88 0.89 0.86 0.87 0.81	0.01985 0.01393 0.02542 0.009798 0.01428 0.01049	5.3% 3.72% 6.9% 2.65% 3.84% 2.97%	0.0% 0.0% 1.67% 1.43% 0.72% 5.73%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Control Type Lab Control  rrected) Transfor Control Type	5 5 5 5 5 5 rmed Sumn Count	0.838 0.838 0.824 0.826 0.832 0.79 <b>Mean</b> 1.159	0.7829 0.7993 0.7534 0.7988 0.7923 0.7609 <b>95% LCL</b> 1.083	0.8931 0.8767 0.8946 0.8532 0.8717 0.8191 95% UCL 1.236	0.82 0.83 0.84 0.82 0.84 0.8 <b>Median</b>	0.79 0.81 0.76 0.8 0.79 0.75 <b>Min</b> 1.095	0.89 0.88 0.89 0.86 0.87 0.81	0.01985 0.01393 0.02542 0.009798 0.01428 0.01049 Std Err 0.02754	5.3% 3.72% 6.9% 2.65% 3.84% 2.97% CV% 5.31%	0.0% 0.0% 1.67% 1.43% 0.72% 5.73% %Effect 0.0%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	Control Type Lab Control  rrected) Transfor Control Type	5 5 5 5 5 5 5 <b>crmed Sumn</b> <b>Count</b> 5	0.838 0.838 0.824 0.826 0.832 0.79 <b>Mean</b> 1.159 1.158	0.7829 0.7993 0.7534 0.7988 0.7923 0.7609 95% LCL 1.083 1.104	0.8931 0.8767 0.8946 0.8532 0.8717 0.8191 95% UCL 1.236 1.211	0.82 0.83 0.84 0.82 0.84 0.8 Median 1.133 1.146	0.79 0.81 0.76 0.8 0.79 0.75 <b>Min</b> 1.095 1.12	0.89 0.88 0.89 0.86 0.87 0.81 Max 1.233 1.217	0.01985 0.01393 0.02542 0.009798 0.01428 0.01049 Std Err 0.02754 0.01926	5.3% 3.72% 6.9% 2.65% 3.84% 2.97% CV% 5.31% 3.72%	0.0% 0.0% 1.67% 1.43% 0.72% 5.73% %Effect 0.0% 0.13%
C-% 0 2.5 5 6.06 10 15	Control Type Lab Control  rrected) Transfor Control Type	5 5 5 5 5 5 <b>5</b> <b>Count</b> 5 5	0.838 0.838 0.824 0.826 0.832 0.79 Mean 1.159 1.158 1.142	0.7829 0.7993 0.7534 0.7988 0.7923 0.7609 95% LCL 1.083 1.104 1.048	0.8931 0.8767 0.8946 0.8532 0.8717 0.8191 95% UCL 1.236 1.211 1.235	0.82 0.83 0.84 0.82 0.84 0.8 Median 1.133 1.146 1.159	0.79 0.81 0.76 0.8 0.79 0.75 <b>Min</b> 1.095 1.12 1.059	0.89 0.88 0.89 0.86 0.87 0.81 Max 1.233 1.217 1.233	0.01985 0.01393 0.02542 0.009798 0.01428 0.01049 Std Err 0.02754 0.01926 0.03361	5.3% 3.72% 6.9% 2.65% 3.84% 2.97% CV% 5.31% 3.72% 6.58%	0.0% 0.0% 1.67% 1.43% 0.72% 5.73% <b>%Effect</b> 0.0% 0.13% 1.52%

Report Date: Test Code: 14 Mar-17 17:08 (p 2 of 2)

1703-S051 | 00-3723-6090



Report Date:

14 Mar-17 17:08 (p 1 of 1)

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

Echinoid Sperm Cell Fertilization Test 15C

Analysis ID: 02-3614-0744

Endpoint: Fertilization Rate

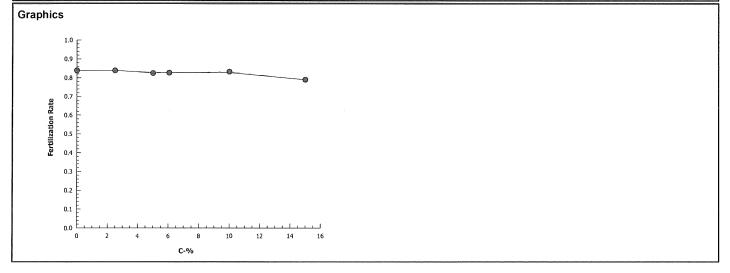
CETIS Version: CETISv1.8.7

Analyzed: 14 Mar-17 17:08 Analysis: Linear Interpolation (ICPIN) Official Results: Yes

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

Point E	stimates					
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	Α	В
0	Lab Control	5	0.838	0.79	0.89	0.01985	0.04438	5.3%	0.0%	419	500
2.5		5	0.838	0.81	0.88	0.01393	0.03114	3.72%	0.0%	419	500
5	,	5	0.824	0.76	0.89	0.02542	0.05683	6.9%	1.67%	412	500
6.06		5	0.826	8.0	0.86	0.009798	0.02191	2.65%	1.43%	413	500
10		5	0.832	0.79	0.87	0.01428	0.03194	3.84%	0.72%	416	500
15		5	0.79	0.75	0.81	0.01049	0.02345	2.97%	5.73%	395	500



Report Date:

14 Mar-17 17:09 (p 1 of 1)

CE 115 An	аіушсаі кер	ort		7	55			ort Date:			:09 (p 1 of 1
Echinoid Sperm Cell Fertilization Test 15C				1	resi	Test Code:		1703-S051   00-3723-6090			
Echinoid Sp	erm Cell Fertiliz	ation Te	st 15C		10.5				Nautilus	s Environ	mental (CA
Analysis ID: Analyzed:	16-1688-5552 14 Mar-17 17:		•	Fertilization Ra Parametric Bio		-Two Samp		IS Version		.8.7	
Data Transfo	orm	Zeta	Alt Hy	p Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cori	rected)	NA	C*b <	T NA	NA	0.75	4.04%	15	>15	NA	< 6.667
TST-Welch's	t Test										
Control	vs C-%		Test S	tat Critical	MSD DE	P-Value	P-Type	Decision	ι(α:5%)		
Lab Control	2.5*		10.21	1.895	0.054 7	<0.0001	CDF	Non-Sign	ificant Effect		
	5*		6.9	1.943	0.077 6	0.0002	CDF	Non-Sign	ificant Effect		
	6.06*		11.09	1.943	0.048 6	<0.0001	CDF	_	ificant Effect		
l	10*		9.966	1.895	0.053 7	<0.0001	CDF	_	ificant Effect		
	15*		9.32	1.943	0.047 6	<0.0001	CDF	_	ificant Effect		
ANOVA Tabl	е				The state of the s					T-100	
Source	Sum Squ	ares	Mean	Square	DF	F Stat	P-Value	Decision	ι(α:5%)		
Between	0.013998	79	0.0027	99758	5	1.137	0.3682	Non-Sign	ificant Effect		***
Error	0.059106	99	0.0024	62791	24						
Total	0.073105	77			29						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett Equality of Variance			5.466	15.09	0.3617	Equal Variances				
Distribution	Shapiro-Wilk W Normality			0.982	0.9031	0.8750	Normal Distribution				
Fertilization	Rate Summary			4.00							
C-%	Control Type	Count	: Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.838	0.7829	0.8931	0.82	0.79	0.89	0.01985	5.3%	0.0%
2.5		5	0.838	0.7993	0.8767	0.83	0.81	0.88	0.01393	3.72%	0.0%
5		5	0.824	0.7534	0.8946	0.84	0.76	0.89	0.02542	6.9%	1.67%
6.06		5	0.826	0.7988	0.8532	0.82	8.0	0.86	0.009798	2.65%	1.43%
10		5	0.832	0.7923	0.8717	0.84	0.79	0.87	0.01428	3.84%	0.72%
15	WWW.	5	0.79	0.7609	0.8191	0.8	0.75	0.81	0.01049	2.97%	5.73%
Angular (Cor	rected) Transfor	med Su	mmary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.159	1.083	1.236	1.133	1.095	1.233	0.02754	5.31%	0.0%
2.5		5	1.158	1.104	1.211	1.146	1.12	1.217	0.01926	3.72%	0.13%
5		5	1.142	1.048	1.235	1.159	1.059	1.233	0.03361	6.58%	1.52%
6.06		5	1.141	1.105	1.178	1.133	1.107	1.187	0.01314	2.57%	1.58%
10		5	1.15	1.097	1.203	1.159	1.095	1.202	0.01908	3.71%	0.83%
15		5	1.095	1.06	1.13	1.107	1.047	1.12	0.01264	2.58%	5.54%

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

Report Date:

09 Mar-17 15:24 (p 1 of 1)

Test Code: 1703-SOS( 00-3723-6090/2382D7A

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 15	C			Nautilus Environmental (CA)
Start Date: End Date: Sample Date	10 1	Mar-17 Mar-17 Mar-17	7			entrotus purpuratus R-95/136 (1995) Iluent	Sample Code: 421 Sample Source: IDE Sample Station: M-	E Americas, Inc.
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes	
			61	100	36	3/13/17		
			62	100	30			
			63	100	81			
			64	100	36 80			
			65	100				
			66	100	77			
			67	100	81			
			68 69	100	76			
			70	100	87		•	
			71	100	86 82			
			72	160	D 7 7 9			
			73	100	B5379			***************************************
			74	100	81			
111111111111111111111111111111111111111			75	100	85			***************************************
			76	100	89			
			77	106	83			
			78	(00	81			AND THE PROPERTY OF THE PROPER
			79	100	88		,	
			80	(00	32			
			81	100	88			
			82	(00)	34 33			
			83	100			V VVIIV-1044444444	
			84	100	81			
			85	100	79			
			86	100	30			
	-		87	100	84			
			88 89	(00)	37			
			90	A) 100 100	17			
	l		90	100	75			

<sup>(</sup>B) PM Q18 3/13/17 (B) Q18 AC 3/14/17 re-count

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

Report Date:

09 Mar-17 15:24 (p 1 of 1)

Test Code: 1763-5051 00-3723-6090/2382D7A

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 150	2		Nautilus Environmental (CA)
Start Date: End Date: Sample Date	10 ľ	Mar-17 Mar-17 Mar-17	7			centrotus purpuratus R-95/136 (1995) fluent	Sample Code: 12B7ADDB 17~0374  Sample Source: IDE Americas, Inc.  Sample Station: M-001 (40 ppt adj)
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes
0	LC	1	72				
0	LC	2	76	100	93	EG 3 10/17	
0	LC	3	67	, ,		10111	
0	LC	4	81				
0	LC	5	88				
2.5		1	78			A CONTRACTOR OF THE CONTRACTOR	
2.5		2	84				
2.5		3	79		4.00.10.11.11		
2.5		4	61	100	90	EG	
2.5		5	77				
5		1	68				
5		2	64				
5		3	66				
5		4	82	160	92	EG	
5	:	5	73				
6.06		1	71			111 = 200000	
6.06		2	62				
6.06		3	80				
6.06		4	70	100	86	EG	
6.06		5	83		<u> </u>		
10		1	69				
10		2	87				
10		3	63				
10		4	85	100	85	ECI	
10		5	75				
15		1	74				
15		2	90				
15		3	65				
15		4	89				
15		5	86	100	36	EC;	

QC-EG

#### Marine Chronic Bioassay

#### **Water Quality Measurements**

A1:4	
Client	

IDE

**Test Species:** S. purpuratus

Sample ID:

M-001 (40 ppt adjusted)

Start Date/Time: 3/10/2017 기식 \ 영

Sample Log No.: 17- 0374

1458 End Date/Time: 3/10/2017

Dilutions made by: EC

Test No: 1703-S051

			Analyst:	AD
			eadings	
Concentration	DO (************************************	pH	Salinity	Temperature
%	(mg/L)	(units)	(ppt)	(°C)
Lab Control	8.2	8.04	33.2	15.1
2.5	8.5	806	33.4	15.0
5.0	85	805	33.7	15.0
6.06	8-5	705	33.9	14.8
10	8.5	85	34.0	148
15	8.5	8.05	34.4	14.7

No al all	
46 71)417	Final Review: Jw 4/10/17
	AC 3/14/17

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

### **Brine Dilution Worksheet**

Project:	IDE		_	Analyst:	EG	- Control of the Cont			
Sample ID:	M-001 (40 ppt adjuste	ed)	Test Date: 3/10/2017						
Test No:	1703-50	51	_	Test Type:	Urchin Fertilization				
Salinity of Effl	luent	62.0	_						
Salinity of Sea	awater	33.5	Date of Brine used: NA		NA	_			
Target Salinity	y	40.0	Alkalinity of B	Brine Control:	NA	_ mg/L as CaCO3			
		<u>Effluent</u>	Brine Control						
Salinity Adjus - SE)/(SB - TS TS = target	·	3.38	-6.15						

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)	
100	100	3.38	338.5	438	

Comments:

Formula for amount of seawater to dilute sample to 40ppt

Use 40 ppt sample as 100% sample for testing.

NA = not applicable; sample not diluted with Nautilus brine.

QC Check: AL 3/14/17

SE = salinity of effluent SB = salinity of brine

Final Review: \_\_\_ 4/10/17

# **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.: Tech initials: Injection Time:	Start Date/Time  M-001 40 ppt adjusted End Date/Time  1703-5051 Species  Animal Source  1340	e: 3/10/2017 / 145% s: S. purpuratus e: Pt. Loma
Sperm Absorbance at 4	Onm:(target range of 0.8 - 1.0 for density of 4x10 <sup>6</sup> sperm/ml	N
Eggs Counted:	Mean: 90,4 X 50 = 4520 eggs/ml  102 (target counts of 80 eggs per vertical pass on Sedgwick-Rafter slide for a final density of 4000 eggs/ml)	
Initial density: Final density:		ml L6ml
Prepare the embryo stoc existing stock (1 part) an	according to the calculated dilution factor. For example, if the dilution factor is 2 125 ml of dilution water (1.25 parts).	2.25, use 100 ml of
	Sperm:Egg Ratio	
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1         1600:1         1200:1         800:1         400:1         200:1           50         40         30         20         10         5.0           0.0         10         20         30         40         45	100:1     50:1       2.5     1.25       47.5     48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time         Rangefinder Ratio:         Fert.         Unfert.           1350         501         67         33           1400         1001         96/96         4/4           1410         2001         100         0	
this range, choose the	o-egg ratio that results in fertilization between 80 and 90 percent. If more than o atio closest to 90 percent unless professional judgment dictates consideration reproductive season, site conditions).	one concentration is within on of other factors (e.g.,
<u>Definitive Test</u>	Sperm:Egg Ratio Used: 100:	
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time         Fert.         Unfert.           1418         QC1         92         8           1438         QC2         85         (5'           1458         Egg Control 1         0         100           Egg Control 2         0         100	
Comments:		
QC Check:	AC 3/14/17 Final Review	· 120 4 4 112

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



# **CETIS Summary Report**

Report Date: Test Code: 23 Mar-17 15:08 (p 1 of 1)

1703-S052 | 11-7976-1138

									lest	Code:		1/0	3-8052   1	1-7976-113
Echinoid Spe	rm Cell Fertiliza	tion Tes	t 15C									Nautilus	s Environn	nental (CA
Batch ID: Start Date: Ending Date: Duration:	03-4833-4906 10 Mar-17 14:1 10 Mar-17 14:5 40m				/R-95/ centro	` '	•			ent: e:	Natural Seawater Not Applicable			
	01-9856-0996 09 Mar-17 10:0 : 09 Mar-17 12:0 28h (3°C)	0 IV 7 S	ode: laterial: ource: tation:	17-0373 Facility E IDE Ame ERI					Clier Proje		IDE Carl	sbad Desal	Plant	
Comparison S	Summary				akeetaaki uu oo ayaa kaasa									
Analysis ID	Endpoint		NOEL	. LOI	ΞL	TOEL	PMSD	TU		Meth	nod			
08-9050-2609	Fertilization Rat	е	10	15		12.25	9.79%	10		Dunr	nett M	lultiple Com	parison Te	st
Point Estimat	e Summary					A								
Analysis ID	Endpoint		Level	%		95% LCL	95% UCL	TU		Meth	nod			
07-0099-4507	Fertilization Ra	e	EC25 EC50			N/A N/A	N/A N/A	<6.6 <6.6		Linea	ar Inte	erpolation (I	CPIN)	
Test Acceptal	oility													
Analysis ID	Endpoint		Attrib	ute		Test Stat	TAC Limi	its		Ovei	rlap	Decision		
07-0099-4507	Fertilization Rat	e	Contr	ol Resp		0.84	0.7 - NL		Yes		Passes A	cceptability	Criteria	
08-9050-2609	Fertilization Rat	e	Contr	ol Resp		0.84	0.7 - NL			Yes		Passes A	cceptability	Criteria
08-9050-2609	Fertilization Rat	e	PMSE	) 		0.09794	NL - 0.25			No		Passes A	cceptability	Criteria
Fertilization F	Rate Summary													
C-%	Control Type	Count	Mean	95%	6 LCL	95% UCL	Min	Max	(	Std I	Err	Std Dev	CV%	%Effec
0	Lab Control	5	0.84	0.75	591	0.9209	0.77	0.91	1	0.02	915	0.06519	7.76%	0.0%
2.5		5	0.832	0.77	798	0.8842	0.77	0.88	3	0.01	881	0.04207	5.06%	0.95%
5		5	0.798	0.70	001	0.8959	0.67	0.86	3	0.03	527	0.07887	9.88%	5.0%
6.06		5	0.792	0.74	136	0.8404	0.73	0.83	3	0.01	744	0.03899	4.92%	5.71%
10		5	0.766			0.8523	0.68	0.85		0.03		0.0695	9.07%	8.81%
15		5	0.642	0.58	303	0.7037	0.58	0.71	1	0.02	223	0.0497	7.74%	23.57%
Fertilization F	Rate Detail													
C-%	Control Type	Rep 1	Rep 2	? Rep	3	Rep 4	Rep 5							
0	Lab Control	0.91	0.77	0.78	3	0.84	0.9							
2.5		0.86	0.88	0.82	2	0.77	0.83							
5		0.86	0.78	0.82	2	0.67	0.86							
6.06		0.83	0.78	0.73	3	0.81	0.81							
10		0.71	0.8	0.68	3	0.85	0.79							
15		0.61	0.65	0.7	1	0.66	0.58							

Report Date: Test Code: 23 Mar-17 15:08 (p 1 of 2)

	VI	,
1703-S052	111-7976-1	138

Analyzed: 23 Mar-17 15:08 Analysis: Parametric-Control vs Treatments Official Results:  Data Transform Zeta Alt Hyp Trials Seed PMSD NOEL L	Nautilus CETISv1.8 Yes LOEL		nental (CA)	
Analyzed:         23 Mar-17 15:08         Analysis:         Parametric-Control vs Treatments         Official Results:           Data Transform         Zeta         Alt Hyp         Trials         Seed         PMSD         NOEL         L           Angular (Corrected)         NA         C > T         NA         NA         9.79%         10         1	Yes	 ३ ७		
Data Transform         Zeta         Alt Hyp         Trials         Seed         PMSD         NOEL         L           Angular (Corrected)         NA         C > T         NA         NA         9.79%         10         1		١. د		
Angular (Corrected) NA C > T NA NA 9.79% 10 1	-OEL			
		TOEL	TU	
Dunnett Multiple Comparison Test	15	12.25	10	
Balliote matapie companion rock				
Control vs C-% Test Stat Critical MSD DF P-Value P-Type Decision(q:	5%)			
Lab Control 2.5 0.3205 2.362 0.109 8 0.7219 CDF Non-Significa	ant Effect			
5 1.205 2.362 0.109 8 0.3324 CDF Non-Significa	ant Effect			
6.06 1.448 2.362 0.109 8 0.2412 CDF Non-Significa	ant Effect			
10 2.077 2.362 0.109 8 0.0863 CDF Non-Significa	ant Effect			
15* 5.086 2.362 0.109 8 <0.0001 CDF Significant E	ffect			
ANOVA Table				
Source Sum Squares Mean Square DF F Stat P-Value Decision(α:	5%)			
Between 0.1791018 0.03582037 5 6.682 0.0005 Significant E	ffect			
Error 0.1286484 0.005360348 24				
Total 0.3077502 29				
Distributional Tests				
Attribute Test Test Stat Critical P-Value Decision(α:1%)				
Variances Bartlett Equality of Variance 3.304 15.09 0.6532 Equal Variances				
Distribution Shapiro-Wilk W Normality 0.9643 0.9031 0.3971 Normal Distribution	Normal Distribution			
Fertilization Rate Summary	A A			
C-% Control Type Count Mean 95% LCL 95% UCL Median Min Max S	Std Err	CV%	%Effect	
0 Lab Control 5 0.84 0.7591 0.9209 0.84 0.77 0.91 0	0.02915	7.76%	0.0%	
2.5 5 0.832 0.7798 0.8842 0.83 0.77 0.88 0	0.01881	5.06%	0.95%	
5 0.798 0.7001 0.8959 0.82 0.67 0.86 0	0.03527	9.88%	5.0%	
6.06 5 0.792 0.7436 0.8404 0.81 0.73 0.83 0	0.01744	4.92%	5.71%	
	0.03108	9.07%	8.81%	
		7.74%	23.57%	
10 5 0.766 0.6797 0.8523 0.79 0.68 0.85 0	0.02223		****	
10 5 0.766 0.6797 0.8523 0.79 0.68 0.85 0	0.02223			
10 5 0.766 0.6797 0.8523 0.79 0.68 0.85 0.15 5 0.642 0.5803 0.7037 0.65 0.58 0.71 0.00 0.00 0.00 0.00 0.00 0.00 0.00	Std Err	CV%	%Effect	
10       5       0.766       0.6797       0.8523       0.79       0.68       0.85       0.61         15       5       0.642       0.5803       0.7037       0.65       0.58       0.71       0.71         Angular (Corrected) Transformed Summary         C-%       Control Type       Count       Mean       95% LCL       95% UCL       Median       Min       Max       5		<b>CV</b> %	%Effect	
10       5       0.766       0.6797       0.8523       0.79       0.68       0.85       0.61         15       5       0.642       0.5803       0.7037       0.65       0.58       0.71       0.71         Angular (Corrected) Transformed Summary         C-%       Control Type       Count       Mean       95% LCL       95% UCL       Median       Min       Max       5         0       Lab Control       5       1.166       1.053       1.278       1.159       1.071       1.266       0	Std Err			
10         5         0.766         0.6797         0.8523         0.79         0.68         0.85         0           15         5         0.642         0.5803         0.7037         0.65         0.58         0.71         0           Angular (Corrected) Transformed Summary           C-%         Control Type         Count         Mean         95% LCL         95% UCL         Median         Min         Max         S           0         Lab Control         5         1.166         1.053         1.278         1.159         1.071         1.266         0           2.5         5         1.151         1.081         1.22         1.146         1.071         1.217         0	<b>Std Err</b> 0.04063	7.8%	0.0%	
10         5         0.766         0.6797         0.8523         0.79         0.68         0.85         0           15         5         0.642         0.5803         0.7037         0.65         0.58         0.71         0           Angular (Corrected) Transforwed Summary           C-%         Control Type         Count         Mean         95% LCL         95% UCL         Median         Min         Max         S           0         Lab Control         5         1.166         1.053         1.278         1.159         1.071         1.266         0           2.5         5         1.151         1.081         1.22         1.146         1.071         1.217         0           5         1.11         0.9918         1.228         1.133         0.9589         1.187         0	Std Err 0.04063 0.02501	7.8% 4.86%	0.0% 1.27%	
10	Std Err 0.04063 0.02501 0.04246	7.8% 4.86% 8.56%	0.0% 1.27% 4.79%	

Report Date: Test Code: 23 Mar-17 15:08 (p 2 of 2) 1703-S052 | 11-7976-1138

**Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) CETISv1.8.7 08-9050-2609 Analysis ID: Endpoint: Fertilization Rate **CETIS Version:** Parametric-Control vs Treatments Analyzed: 23 Mar-17 15:08 Analysis: Official Results: Yes Graphics 1,0 0.12 0.10 0.9 0.08 0.06 Fertilization Rate 0.04 0,02 0.6 0.00 -0.02 0.5 -0.04 0.4 -0.06 -0.08 -0.10 0.2 -0.12 0.1 -0.14 0,0 -0.16 -2.5 1.0 C-% Rankits

Report Date:

23 Mar-17 15:08 (p 1 of 1)

Test Code:

1703-S052 | 11-7976-1138

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Analysis ID: Analyzed:

07-0099-4507 23 Mar-17 15:08

Analysis:

Endpoint: Fertilization Rate

Linear Interpolation (ICPIN)

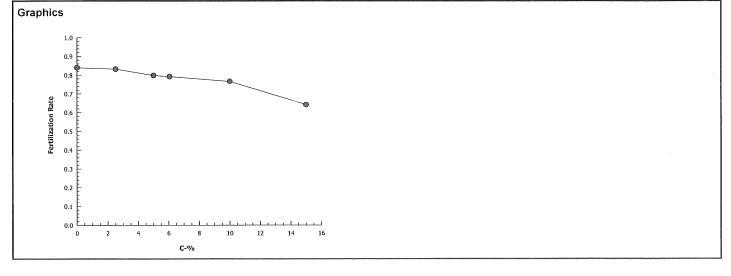
CETIS Version: CETISv1.8.7

Official	Results:	Yes

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

Point E	stimates					
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

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



TST

Report Date:

23 Mar-17 15:30 (p 1 of 1)

1703-S052 | 11-7976-1138

	,			TST	-		<b>Test Code:</b> 1703-S052   11-79			1-7976-1138	
Echinoid Spe	erm Cell Fertiliza	ation Test	15C	·					Nautilus	Environr	nental (CA)
Analysis ID: Analyzed:	14-8409-5217 23 Mar-17 15:0		dpoint: Fer alysis: Par	tilization Rat ametric Bioe		Two Samp		S Version		.8.7	
										TOEL	TU
Data Transfo Angular (Corr		Zeta NA	Alt Hyp C*b < T	Trials NA	Seed NA	<b>TST b</b> 0.75	PMSD 6.14%	NOEL 10	LOEL 15	12.25	10
							0.1170				
TST-Welch's									0/\		
Control	vs C-%		Test Stat			P-Value	P-Type	Decision	· · · · · · · · · · · · · · · · · · ·		
Lab Control	2.5*		7.015	1.895	0.075 7	0.0001	CDF	•	ificant Effect		
	5*		4.508	1.895	0.099 7	0.0014	CDF	_	ificant Effect		
	6.06*		6.054	1.895	0.070 7	0.0003	CDF	•	ificant Effect		
	10*		4.075	1.895	0.091 7	0.0024	CDF	•	ificant Effect		
	15		1.457	1.895	0.073 7	0.0942	CDF	Significar	nt Effect		
ANOVA Tabl	е										
Source	Sum Squ	iares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.179101	8	0.0358203	37	5	6.682	0.0005	Significar	nt Effect		
Error	0.128648	4	0.0053603	348	24	_					
Total	0.307750	2			29				w.=		
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	Equality of \	/ariance	3.304	15.09	0.6532	Equal Variances				
Distribution	Shapiro-	Wilk W Nor	mality	0.9643	0.9031	0.3971	Normal Distribution				
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.84	0.7591	0.9209	0.84	0.77	0.91	0.02915	7.76%	0.0%
2.5		5	0.832	0.7798	0.8842	0.83	0.77	0.88	0.01881	5.06%	0.95%
5		5	0.798	0.7001	0.8959	0.82	0.67	0.86	0.03527	9.88%	5.0%
6.06		5	0.792	0.7436	0.8404	0.81	0.73	0.83	0.01744	4.92%	5.71%
10		5	0.766	0.6797	0.8523	0.79	0.68	0.85	0.03108	9.07%	8.81%
15		5	0.642	0.5803	0.7037	0.65	0.58	0.71	0.02223	7.74%	23.57%
Angular (Co	rrected) Transfo	rmed Sumi	mary						11.00		
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.166	1.053	1.278	1.159	1.071	1.266	0.04063	7.8%	0.0%
2.5		5	1.151	1.081	1.22	1.146	1.071	1.217	0.02501	4.86%	1.27%
5		5	1.11	0.9918	1.228	1.133	0.9589	1.187	0.04246	8.56%	4.79%
6.06		5	1.098	1.04	1.157	1.12	1.024	1.146	0.02108	4.29%	5.75%
10		5	1.069	0.9667	1.172	1.095	0.9695	1.173	0.03696	7.73%	8.25%
15		5	0.93	0.8653	0.9947	0.9377	0.8657	1.002	0.0233	5.6%	20.2%

### **CETIS Test Data Worksheet**

Report Date:

09 Mar-17 15:24 (p 1 of 1)

Test Code: \703-505211-7976-1138/4651B9F2

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 150	:		Nautilus Environmental (CA)				
Start Date: End Date: Sample Date	10 1	Vlar-17 Vlar-17 Vlar-17	7			centrotus purpuratus R-95/136 (1995) luent	Sample Code: BD5CCE4 17-03-13 Sample Source: IDE Americas, Inc. Sample Station: ERI				
C-%	Code	Rep	Pos	# Counted	# Fertilized		No	otes			
			91	100	81		3/1	7/17			
			92	100	68		- 1				
			93	100	83 85						
			94	100	85						
			95	100	66						
			96	100	88						
			97	/00	58						
			98	/00	84						
			99	100	80						
			100	100	90						
			101	100	83						
			102	100	82						
			103	/00	79						
			104	/00	71						
			105 106	/00	86						
			107	/00	78						
			108	160	77 71						
			109	100	77						
			110	100							
			111	00/	65 73						
			112	100	67		***************************************				
			113	100	67. 86						
			114	100	86						
			115	100	78		- Approximate Communication Co				
			116	100	81						
			117	100	78						
			118	/00	82						
			119	100	91						
			120	100	61		۲				

### **CETIS Test Data Worksheet**

Report Date:

09 Mar-17 15:24 (p 1 of 1)

Test Code: 1703-5052 11-7976-1138/4651B9F2

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 150				Nautilus Environmental (
Start Date: End Date: Sample Date	10 [	Mar-17 Mar-17 Mar-17	7			centrotus purpi R-95/136 (1999 fluent		ample Code: BD6CCE4 17-0373 ample Source: IDE Americas, Inc. ample Station: ERI
C-%	Code	Rep	Pos	# Counted	# Fertilized			Notes
0	LC	1	119	100	89	DM	3/10/17	
0	LC	2	109			V		
0	LC	3	106					
0	LC	4	98					
0	LC	5	100					
2.5		1	105	100	71	DM	3/10/17	
2.5		2	96	100	85			
2.5		3	102					
2.5		4	107					
2,5		5	101					
5		1	113	(60	31	DM	3/10/17	
5		2	115					
5		3	118		The Total			
5		4	112		1 11 0 0 0 0 0 0 0 0			
5		5	114					
6.06		1	93	100	S(	DM	3/10/17	
6.06		2	117				·	
6.06		3	111					
6.06		4	91					
6.06		5	116					
10		1	104	100	73	DM	3/10/17	
10		2	99				-	
10		3	92					
10		4	94					
10		5	103					
15		1	120	(00	59	DM	3/10/17	
15		2	110			1		
15		3	108					
15		4	95					
15		5	97					

QC: Eg

## **Water Quality Measurements**

IDE

Test Species: S. purpuratus

Sample ID:

ERI .

Start Date/Time: 3/10/2017 1418

Sample Log No.:

<u>17-</u> 0373

End Date/Time: 3/10/2017 1458

Dilutions made by: \_\_\_\_\_\_

Test No: \_\_1703~SOS2\_\_\_\_\_\_

			Analyst:	AD
		Initial R	eadings	
Concentration	DO	рН	Salinity	Temperature
%	(mg/L)	(units)	(ppt)	(°C)
Lab Control	8.4	8.5	33.1	14.9
2.5	8.4	8.05B	34.2	14.8
5.0	8.5	10.8	349	145
6.06	85	8.00	35.4	14.7
10	85	7.98	36.5	14.7
15	85	7.95	38.0	14.6
	-			

Ca	m	m	Δ	n	te	

RAS OH 3/10/17

QC Check:

AC3/14/17

Final Review: VB3 3

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

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

			Ecimioa	eiin əbenn-ce	i Leimisamon Aaor	ASHEEL
Client:	IDE			Start Date/	ime: 3/10/2017 / (	418
Sample ID:	ERI Brine		_			
Test No.:	1703-8052	<del></del>	_		cies: <i>S. purpuratus</i>	158
	1100 3030				urce: Pt. Loma	
Tech initials:	EG				oted: 2/9/12 + 3	11/12
Injection Time:	1340			Date Colle	sied. DIMP + )	10[17]
injoodon riino.						
Sperm Absorbance at 4	00 nm: <u>0.855</u>	(target range of (	).8 - 1.0 for dens	ity of 4x10 <sup>6</sup> sperm	/ml)	
Eggs Counted:		ean: <u>90,4</u> x s	io = <u>4520</u>	eggs/ml		
	79	1 1 100				
•	- Raf	get counts of 80 eggs p ter slide for a final dens	er vertical pass or	1 Sedgwick-		
	84	ter since for a fillar dells	ity of 4000 eggs/ff	")		
	90					
Initial density:	4520 eggs/ml	= 1,13 dilut	on factor		260 mi	
Final density:	4000 eggs/ml		egg stock	-33 -13-11		
i mai denoity.	+000 eggs/iiii		egg stock s seawater	seawater	<u>26</u> ml	
		0 A 7 parts	seawater			
Prepare the embryo store existing stock (1 part) are	ck according to the calc nd 125 ml of dilution wat	ulated dilution factor. ter (1.25 parts).	For example, if	the dilution factor	is 2.25, use 100 ml of	
			Sperm:Egg Ra	<u>itio</u>		
Rangefinder Test:	<u>2000:1</u> <u>160</u>		800:1 400		<u>100:1</u> 50:1	
ml Sperm Stock ml Seawater	50 4 0.0 10			0 5.0	2.5 1.25	
IIII Ocawalci	0.0	0 20	30 4	0 45	47.5 48.75	i
	Time	Rangefinder Ratio	: Fert.	Unfert.		
Sperm Added (100 µl):	1356	501	67	33		
Eggs Added (0.5 ml):	1400	100:1	actor.	4/4		
Test Ended:	1410	200:1	96/0/10	1/-1		
rest Ended.	1110		166	0		
		### COSE ≥	oreign			
NOTE: Choose a sperm this range, choose the organism health, stage of	ratio closest to 90 pe	rcent unless profess	en 80 and 90 pe ional judgment	ercent. If more that dictates consider	n one concentration is ation of other factors	s within s (e.g.,
Definitive Test		Sperm:Egg Ratio	Used:100	: 1		
	Time	And the state of t	Fert.	Unfert.		
Sperm Added (100 μl):	1418	QC1	92	Official.		
	1438		000	15'		
Eggs Added (0.5 ml):	1458	QC2	87	12		
Test Ended:	1730	Egg Control 1	<u> </u>	100		
		Egg Control 2		100		
Comments:						

QC Check:

AC3/14/17

Final Review: 10830317

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



## **CETIS Summary Report**

Report Date:

23 Mar-17 15:22 (p 1 of 1)

**Test Code:** 1703-S053 | 16-3100-1862

								Test Code:		1/0	3-S053   16	-3100-186
Echinoid Spe	rm Cell Fertiliza	tion Tes	st 15C							Nautilus	Environm	ental (CA)
Batch ID: Start Date: Ending Date: Duration:	21-2695-8916 10 Mar-17 14:1 10 Mar-17 14:5 40m	8 8	Fest Type: Protocol: Species: Source:	EPA/600/R-95/136 (1995) Strongylocentrotus purpuratus				Analyst: Diluent: Brine: Age:		ural Seawate Applicable	er	
							Client: Project:	IDE Carl	sbad Desal	Plant		
Comparison S	Summary						***************************************					
Analysis ID	Endpoint	· · · · · · · · · · · · · · · · · · ·	NOEL	LOEL	TOEL	PMSD	TU	Meth	od			
18-8044-1127				6.06	5.505	9.51%	20	Dunr	nett M	lultiple Com	parison Tes	st
Point Estimate	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Meth	od			
17-4714-8490	Fertilization Ra	te	EC25 EC50		8.713 N/A	15.76 N/A	8.1 <6.6		ar Inte	erpolation (I	CPIN)	
Test Acceptab	oility					59A.5.W. 1974   1	er ennemente en et districté					
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Over	rlap	Decision		
17-4714-8490	Fertilization Ra	te	Contr	ol Resp	0.844	0.7 - NL		Yes		Passes A	cceptability	Criteria
18-8044-1127	Fertilization Ra	te	Contr	ol Resp	0.844	0.7 - NL		Yes		Passes A	cceptability	Criteria
18-8044-1127	Fertilization Ra	te	PMS	)	0.09511	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	Rate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std I	Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.844	0.7803	0.9077	0.76	0.9	0.022	293	0.05128	6.08%	0.0%
2.5		5	0.756	0.6662	0.8458	0.64	0.83	0.032	234	0.07232	9.57%	10.43%
5		5	0.796		0.8446	0.75	0.84			0.03912	4.91%	5.69%
6.06		5	0.752		0.8378	0.64	0.82			0.06907	9.18%	10.9%
10		5	0.694		0.7821	0.58	0.77			0.07092	10.22%	17.77%
15		5	0.564	0.4882	0.6398	0.5	0.66	0.02	731	0.06107	10.83%	33.18%
Fertilization R	Rate Detail											
Fertilization R C-%	Rate Detail Control Type	Rep 1	Rep 2	2 Rep 3	Rep 4	Rep 5	***************************************			-		
		Rep 1	Rep 2	0.85	0.85	0.9					*****	
C-% 0 2.5	Control Type		·	<del></del>						All And Annual Control of the Contro	<del></del>	
<b>C</b> -%	Control Type	0.86	0.76	0.85	0.85	0.9				-		
C-% 0 2.5	Control Type	0.86 0.64	0.76 0.75	0.85 0.76	0.85 0.8	0.9 0.83						
C-% 0 2.5 5	Control Type	0.86 0.64 0.82	0.76 0.75 0.84	0.85 0.76 0.81	0.85 0.8 0.75	0.9 0.83 0.76						

Report Date:

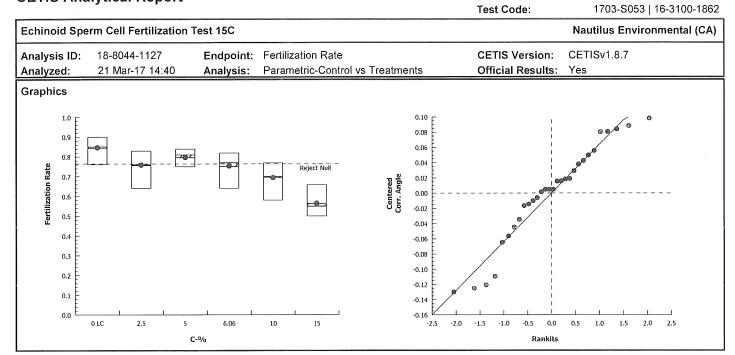
23 Mar-17 15:22 (p 1 of 2)

		,
1703-S053	16-3100-1	862

	,						Test	Code:	170	3-8053   16	-3100-1862
Echinoid Spe	erm Cell Fertiliza	ition Test	15C						Nautilus	s Environn	nental (CA)
Analysis ID: Analyzed:	18-8044-1127 21 Mar-17 14:4		idpoint: Feri		e itrol vs Treat	tments		IS Version:		.8.7	
Data Transfo	rm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	ected)	NA	C > T	NA	NA		9.51%	5	6.06	5.505	20
Dunnett Mult	tiple Compariso	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(a:5%)		
Lab Control	2.5*		2.495	2.362	0.105 8	0.0381	CDF	Significar	t Effect		
	5		1.455	2.362	0.105 8	0.2389	CDF	Non-Sign	ificant Effect		
	6.06*		2.609	2.362	0.105 8	0.0301	CDF	Significar	nt Effect		
	10*		4.087	2.362	0.105 8	0.0009	CDF	Significar	nt Effect		
	15*		7.152	2.362	0.105 8	<0.0001	CDF	Significar	nt Effect		
ANOVA Table	е										
Source	Sum Squ	ares	Mean Squ	are	DF	F Stat	P-Value	Decision	(a:5%)		
Between	0.299008	1	0.0598016	2	5	12.09	<0.0001	Significar	nt Effect		
Error	0.1187604 0.00			48	24						
Total	0.4177685				29						
Distributiona	I Tests					,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,					
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett E	quality of \	Variance	1.255	15.09	0.9395	Equal Var	iances			
Distribution	Shapiro-	Wilk W No	rmality	0.9403	0.9031	0.0925	Normal Distribution				
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.844	0.7803	0.9077	0.85	0.76	0.9	0.02293	6.08%	0.0%
2.5		5	0.756	0.6662	0.8458	0.76	0.64	0.83	0.03234	9.57%	10.43%
5		5	0.796	0.7474	0.8446	0.81	0.75	0.84	0.01749	4.91%	5.69%
6.06		5	0.752	0.6662	0.8378	0.77	0.64	0.82	0.03089	9.18%	10.9%
10		5	0.694	0.6059	0.7821	0.7	0.58	0.77	0.03172	10.22%	17.77%
15		5	0.564	0.4882	0.6398	0.55	0.5	0.66	0.02731	10.83%	33.18%
Angular (Cor	rected) Transfor	med Sum	mary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.168	1.083	1.254	1.173	1.059	1.249	0.03075	5.89%	0.0%
2.5		5	1.057	0.9546	1.16	1.059	0.9273	1.146	0.03696	7.82%	9.5%
5		5	1.104	1.043	1.164	1.12	1.047	1.159	0.02167	4.39%	5.54%
6.06		5	1.052	0.955	1.149	1.071	0.9273	1.133	0.035	7.44%	9.94%
10		5	0.9864	0.892	1.081	0.9912	0.8657	1.071	0.03403	7.71%	15.56%
15		5	0.8501	0.7728	0.9273	0.8355	0.7854	0.9483	0.02782	7.32%	27.24%

Report Date:

23 Mar-17 15:22 (p 2 of 2)



Report Date: Test Code:

23 Mar-17 15:22 (p 1 of 1)

1703-S053 | 16-3100-1862

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Analysis ID: Analyzed:

>15

EC50

17-4714-8490 23 Mar-17 15:22

N/A

Endpoint: Fertilization Rate Analysis:

<6.667

NA

Linear Interpolation (ICPIN)

**CETIS Version:** Official Results: Yes

CETISv1.8.7

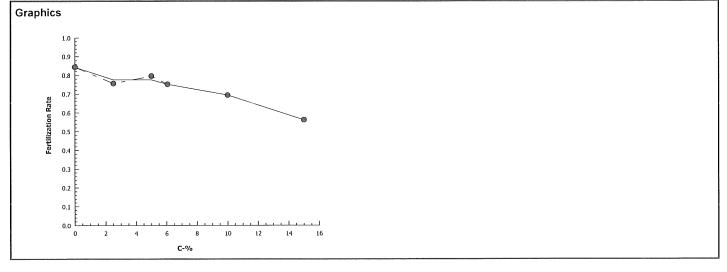
Linear Interpolation Options										
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method .					
Linear	Linear	1370398	1000	Yes	Two-Point Interpolation					

NΑ

Point E	stimates					
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	12.35	8.713	15.76	8.1	6.346	11.48

N/A

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



TST

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

	•			TS	\		Test	Code:	170	3-S053   16	-3100-1862
Echinoid Spe	erm Cell Fertiliza	ation Test 1	15C						Nautilus	Environm	ental (CA)
Analysis ID:	07-7155-2601	En	dpoint: Fer	tilization Rat	е		CET	S Version:	CETISv1	.8.7	
Analyzed:	23 Mar-17 15:2			ametric Bioe		Two Samp	le <b>Offic</b>	ial Results	: Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	ected)	NA	C*b < T	NA	NA	0.75	5.91%	10	15	12.25	10
TST-Welch's	t Test										
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5*		4.156	1.943	0.085 6	0.0030	CDF	Non-Sign	ificant Effect		
	5*		7.184	1.895	0.06 7	<0.0001	CDF	Non-Sign	ificant Effect		
	6.06*		4.199	1.943	0.081 6	0.0028	CDF	Non-Sign	ificant Effect		
	10*		2.682	1.895	0.078 7	0.0157	CDF	Non-Sign	ificant Effect		
	15		-0.7234	1.895	0.068 7	0.7536	CDF	Significar	nt Effect		
ANOVA Table	е										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.299008	1	0.0598016	62	5	12.09	<0.0001	Significar	nt Effect		
Error	0.118760	4	0.0049483	348	24	_					
Total	0.417768	5			29						
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			· · · · · · · · · · · · · · · · · · ·
Variances	Bartlett E	Equality of \	/ariance	1.255	15.09	0.9395	Equal Var	iances			
Distribution	Shapiro-	Wilk W Nor	mality	0.9403	0.9031	0.0925	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	0.844	0.7803	0.9077	0.85	0.76	0.9	0.02293	6.08%	0.0%
2.5		5	0.756	0.6662	0.8458	0.76	0.64	0.83	0.03234	9.57%	10.43%
5		5	0.796	0.7474	0.8446	0.81	0.75	0.84	0.01749	4.91%	5.69%
6.06		5	0.752	0.6662	0.8378	0.77	0.64	0.82	0.03089	9.18%	10.9%
10		5	0.694	0.6059	0.7821	0.7	0.58	0.77	0.03172	10.22%	17.77%
15		5	0.564	0.4882	0.6398	0.55	0.5	0.66	0.02731	10.83%	33.18%
Angular (Co	rrected) Transfoi	rmed Sumi	mary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.168	1.083	1.254	1.173	1.059	1.249	0.03075	5.89%	0.0%
2.5		5	1.057	0.9546	1.16	1.059	0.9273	1.146	0.03696	7.82%	9.5%
5		5	1.104	1.043	1.164	1.12	1.047	1.159	0.02167	4.39%	5.54%
6.06		5	1.052	0.955	1.149	1.071	0.9273	1.133	0.035	7.44%	9.94%
10		5	0.9864	0.892	1.081	0.9912	0.8657	1.071	0.03403	7.71%	15.56%
15		5	0.8501	0.7728	0.9273	0.8355	0.7854	0.9483	0.02782	7.32%	27.24%

Analyst: VB QA: ACUIS 1

### **CETIS Test Data Worksheet**

Report Date:

09 Mar-17 15:25 (p 1 of 1)

Test Code: 1763 -505316-3100-1862/61371D06

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 150	;			Nautilus Environmental (C
Start Date: End Date: Sample Date	10 Mar-17 10 Mar-17 :: 09 Mar-17		Protoco	Species: Strongylocentrotus purpuratus Protocol: EPA/600/R-95/136 (1995) Material: Facility Effluent		Sample Code: 40664A3D 17-037 Sample Source: IDE Americas, Inc. Sample Station: Train 9		
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes	
			121	100	81	3/16/17		
			122	(00)	90			
			123	100	70			
			124	100	83			
			125	100	55			
			126	100	79			
			127	100	80			
			128	(00	82			
			129	100	75			
			130	(00)	86			
			131	(00)	66			
			132	100	84			
			133	100	77			
			134	100	35			
			135	100	64			
			136	(00)	64			
			137 138	100	58			
			139	100	82			
			140	601	74			
			141	100	76			
			142	001	53 76			
			143	60)	77			
			144	100	58			1477
			145	100	5 8 75			
			146		35 			A074311
			147	100	-05 76			
			148	100	73			
			149	100	69		9079839Passe 83.1.1 - 4	11 and 12 Administration .
			150	100	50			

### **CETIS Test Data Worksheet**

Report Date:

09 Mar-17 15:25 (p 1 of 1)

Test Code: 1703-505316-3100-1862/61371D06

Echinoid Sperm Cell Fertilization Test 15C					С	Nautilus Environmental (CA)											
Start Date: 10 Mar-17 End Date: 10 Mar-17 Sample Date: 09 Mar-17		10 Mar-17		10 Mar-17		10 Mar-17		10 Mar-17		10 Mar-17		10 Mar-17				ocentrotus purpuratus /R-95/136 (1995) ffluent	Sample Code: (a) 10664A3D 0375 Sample Source: IDE Americas, Inc. Sample Station: Train 9
C-%	Code	Rep	Pos	# Counted	# Fertilized		Notes										
0	LC	1	130	100	87												
0	LC	2	140														
0	LC	3	146														
0	LC	4	134														
0	LC	5	122														
2.5		1	136	(00)	67												
2.5		2	145														
2.5		3	142														
2.5		4	127														
2.5		5	124														
5		1	138	(00	73												
5		2	132														
5		3	121		·												
5		4	129														
5		5	147														
6.06		1	128	100	82	AC 3/11/17 AC 3/11/17											
6.06		2	126	100	79	A63/11/17											
6.06		3	135		`	.,											
6.06		4	139														
6.06		5	143														
10		1	149	100	72	163/11/7											
10		2	123														
10		3	144														
10		4	133		Serve Serve												
10		5	148														
15		1	141														
15		2	150				,										
15		3	131														
15		4	125														
15		5	137	100	52	A6 3/11/17											

QC:EN QQ18AC ZIN/M

**Water Quality Measurements** 

C1:	
Client	Ξ

IDE

Test Species: S. purpuratus

Sample ID:

Train 9

1418 Start Date/Time: 3/10/2017

Sample Log No.:

17- 0375

End Date/Time: 3/10/2017

Dilutions made by:

Test No:

	Analyst: Analyst:							
<u> </u>	Initial Readings							
Concentration	DO	рН	Salinity	Temperature				
%	(mg/L)	(units)	(ppt)	(°C)				
Lab Control	8.3	8.04	33.1	15.4				
2.5	8.3	8.03	34.2	14.9				
5.0	8.4	8.01	35.0	14.8				
6.06	8.4	8.00	35.3	14.8				
10	85	7.98	36.6	14.8				
15	8.5	7.94	38.1	14.7				

Comments:		
QC Check:	AC 314/17	Final Review: 483 2111

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:	10E Train 9 1703-506 1340	3				Start Date/Tir End Date/Tir Speci Animal Sour Date Collect	me: 3/10/201 ies: <i>S. purpu</i> rce: Pt. Loma	7 / 1458 iratus	
Sperm Absorbance at 40	00 nm:	355 <u> </u> (t	arget range o	f 0.8 - 1.0 fo	r density of	4x10 <sup>6</sup> sperm/ı	ml)		
Eggs Counted:	97 79 102 84 90	(target cou	<u>「Gの, リ</u> X unts of 80 eggs e for a final der	per vertical p	pass on Sedg	gs/ml wick-			
Initial density: Final density:		gs/ml gs/ml	- 1.0 pa	ution factor rt egg stock rts seawater	se	g stock2 awater	-00 ml 26 ml		
Prepare the embryo stoc existing stock (1 part) an	k according to the d 125 ml of dilutio	e calculated on water (1.)	dilution facto 25 parts).	r. For exam	ple, if the di	lution factor is	s 2.25, use 1	00 ml of	
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 50 0.0	1600:1 40 10	1200:1 30 20	<u>Sperm:E</u> <u>800:1</u> 20 30	gg Ratio 400:1 10 40	200:1 5.0 45	100:1 2.5 47.5	50:1 1.25 48.75	

			Sperm:	:Egg Ratio			
2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
50	40	30	20	10	5.0	2.5	1.25
0.0	10	20	30	40	45	47.5	48.75
Time [356] 1466 [1466]	<u>Ra</u> 	100:1	<u>C</u> 90	3 60 4	3/4		
	50 0.0 Time 1356	50 40 0.0 10 Time Ra	50 40 30 0.0 10 20  Time Rangefinder Ra	2000:1   1600:1   1200:1   800:1	50   40   30   20   10   10   10   20   30   40   40   40   10   10   10   10   1	2000:1   1600:1   1200:1   800:1   400:1   200:1     50	$\begin{array}{c ccccccccccccccccccccccccccccccccccc$

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).

QC Check:

AC3/14/17

Final Review: VB3PV17

Appendix B

**Sample Receipt Information** 

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Client:	IDE
Sample ID:	ERI, M-001, Train 9
et ID No(e) :	1702-8017 + CAEZ

Sample (A, B, C):	ERI Brine	M-001	Train 9	
Log-in No. (17-xxxx):	0373	0374	0375	
Sample Collection Date & Time:	3/9/17 1000	3/9/17 1000	3/9/17-1000	
Sample Receipt Date & Time:	3/9/17 1207	3/9/17 1207	3/9/17 1207	
Number of Containers & Container Type:	1,4 Lcubi	2,4Lcub;	1, Ylcubi	
Approx. Total Volume Received (L):	46	~6 L	44	
Check-in Temperature (°C)	3.0	4.0	4.0	
Temperature OK? 1	(Y) N	(Y) N	Ý N	. Y N
DO (mg/L)	7.8	7.6	7.9	
pH (units)	7.46	7.92	7.45	
Conductivity (µS/cm)	_	<del>-</del>		:
Salinity (ppt)	634	62.0	64.4	
Alkalinity (mg/L) <sup>2</sup>	216	216	219	
Hardness (mg/L) <sup>2, 3</sup>	-		_	
Total Chlorine (mg/L)	Ø-03	60.02	60.02	
Technician Initials	EG	毛气	Eh	

Test Performed:	Wrzhin Fert- Additional Control? Y N	Control/Dilution Water: 8:2 Lab SW / Lab ART Other:
Test Performed:		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
		Alkalinity: Hardness or Salinity:
	Additional Control? Y N	= Alkalinity: Hardness or Salinity:
Test Performed:		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
		Alkalinity: Hardness or Salinity:
	Additional Control? Y N	= Alkalinity: Hardness or Salinity:
Notes:	<sup>1</sup> Temperature of sample should	be 0-6°C, if received more than 24 hours past collection time.
	<sup>2</sup> mg/L as CaCO3, <sup>3</sup> Measured	for freshwater samples only, NA = Not Applicable
<u>-</u> ^		
Additional Comments:		
		· · ·

## Sample Check-In Information

Sample Description  ERI: colorless, cle  Mosi: colorless, c  Train 9: colorless, c	lar, no	edor, n	o dekino
COC Complete (Y/N	1)?		
ABC			
Filtration? Y N	i)		
Pore Size:	/		
Organisms	or	Debris	
Salinity Adjustment Test: Urchin Fert-	Source: S	AC 3/14/1 W Targ	et ppt: 40
	Source:	_	et ppt:
Test:	Source:	Targ	et ppt:
pH Adjustment? Y	(N)		
	A	В	С
Initial ph	1:		
Amount of HCI added	d:		
Final ph	1:		
Cl <sub>2</sub> Adjustment? Y	(N) A	В	С
Initial Free Cl <sub>2</sub> :			
STS added:			
Final Free Cl <sub>2</sub> ;			
Sample Aeration? Y	~	7	_
— Initial D.O.	A	В	С
Duration & Rate			
Final D.O.	·		
Subsamples for Add	er PH10+	reatine	ed? Y N N N N N N N N N N N N N N N N N N N
Continuous and the same of the	00.0	ck: <u>AL3</u>	Andlin
Address of the second of the s			
	Final Revie	س رہے :ws	- 4/10/17

**Appendix C** 

**Chain-of-Custody Form** 

	DE Technologies	
--	--------------------	--

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

	en un resident de la companie de la		COLUMN CO	11-2000-01-000-0-0-0-0-0-0-0-0-0-0-0-0-0	Market B. Comment of the Comment of	The other control of the second				. Oth	e1	and the second s	rrr Days
Project Name: Toxicity Screening			roject Manager: <u>Peter</u>		Contact Informat	ion:	(760) 2	01-7777		_			
Special instruction: Samples collect M-001 sample is also to be treated									ANAL	rses			NOTES:
requirements. VH 3/9/2017					<b>∞</b>	tilizatior							
		Glass=G Plastic	=P	and the second of the second of the second		ıic Feı							
	Yes=Y No=N	Acid=A Base=B		_		Chror							
Drinkin	g Water=DW Seaw	ater=SW Soil=S Brine	=B	Pres		chin (	@						
Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	Purple Urchin Chronic Fertilizatior	Acute (					00	
ERI Brine (17- 1227)	3/08-09/17	10:00	24 HR COMP -B	N	4L CUBIE	Х						3,€	TDS - 61.49 ppm EC - 85.32 mS/cm
M-001 (17- 1228)	3/08-09/17	10:00	24 HR COMP -B	N	4L CUBIE (1 L extra)	Х	x					4.0	TDS - 60.26 ppm EC - 84.02 mS/cm
TRAIN 9 (17-1229)	3/08-09/17	10:00	24 HR COMP -B	N	4L CUBIE	Х	-						TDS - 62.16 ppm EC - 86.12 mS/cm
													-
Relinquished By:		Date:	Time:		Received By:			4		Time:		Sam	ple Condition Upon Receipt:
Votans		3/9/17	1100			3	19/	17	1/	ŬĆ	lce	d	Ambient or°C
		3/9/11	12:07		algher	Ĭ)	F	V	1	207	lce	d	Ambient or0C
Acula text.	- 1 0 :	5 A B			Many	> /	D.º		-0	37	3 4	710	7-0375

@ Acute test not required analysis cancelled per client enicio.

## Appendix D

Reference Toxicant Test Data and Statistical Analyses

## **CETIS Summary Report**

Report Date:

21 Mar-17 14:38 (p 1 of 1)

Test Code:

170310sprt | 05-2038-2100

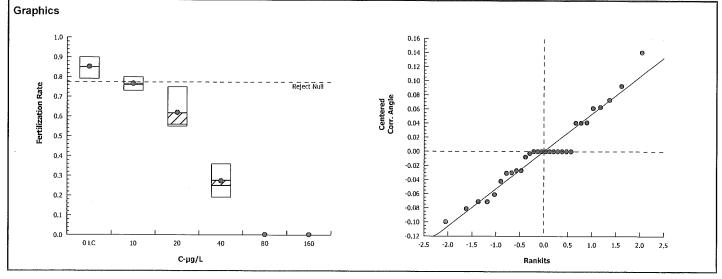
								rest Code:	•	170	o rospit   Ut	5-2038-2100
Echinoid Spe	erm Cell Fertiliza	ation Test	15C	mauri - coaquino de la coaquino de l	4					Nautilu	s Environm	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	11-3277-5320 10 Mar-17 14:1 10 Mar-17 14:5 40m	18 Pr 58 Sp	est Type: otocol: pecies: purce:	Fertilization EPA/600/R-95, Strongylocentr Pt. Loma		tus		Analyst: Diluent: Brine: Age:		ıral Seawat Applicable	er	
Sample ID: Sample Date: Receive Date Sample Age:	: 10 Mar-17	Ma So	ode: aterial: ource: ation:	170310sprt Copper chlorid Reference Tox Copper Chlorid	icant			Client: Project:	Inter	nal		
Comparison	Summary			10 70701 10 300								
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meth	nod			
05-1387-0350	Fertilization Ra	te	<10	10	NA	8.97%		Duni	nett M	ultiple Com	parison Tes	st
Point Estimat	e Summary									***************************************		
Analysis ID	Endpoint		Level	μg/L	95% LCL	95% UCL	TU	Meth	nod			
05-9725-9024	Fertilization Ra	te	EC50	28.71	27.31	30.19		Trim	med S	Spearman-k	(ärber	· · · · · · · · · · · · · · · · · · ·
Test Acceptal	oility					,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,						
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	ts	Ovei	rlap	Decision		
05-1387-0350	Fertilization Ra	te	Contro	ol Resp	0.85	0.7 - NL		Yes		Passes A	cceptability	Criteria
05-9725-9024	Fertilization Ra			ol Resp	0.85	0.7 - NL		Yes			cceptability	
05-1387-0350	Fertilization Ra	te	PMSD		0.08965	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	Rate Summary											
C-μg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std I	Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.85	0.7966	0.9034	0.79	0.9	0.019		0.04301	5.06%	0.0%
10		5	0.766	0.7252	0.8068	0.73	8.0	0.014		0.03286	4.29%	9.88%
20		5	0.618	0.5037	0.7323	0.55	0.75			0.09203	14.89%	27.29%
40 80		5 5	0.276 0	0.1911 0	0.3609 0	0.19	0.36		)59	0.06841	24.79%	67.53%
160		5	0	0	0	0 0	0 0	0 0		0		100.0% 100.0%
Fertilization R	late Detail							100	· · · · · · · · · · · · · · · · · · ·			
C-μg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.88	0.85	0.79	0.83	0.9				· · · · · · · · · · · · · · · · · · ·		
10		0.76	8.0	0.73	0.8	0.74						
20		0.55	0.55	0.68	0.56	0.75						
40		0.19	0.33	0.25	0.25	0.36						
40		•										
80		0	0	0	0	0						

Report Date: Test Code:

21 Mar-17 14:38 (p 1 of 1)

170310sprt | 05-2038-2100

										TO STATE OF THE PARTY OF THE PA	
Echinoid Sp	erm Cell Fertiliza	ation Test 1	15C						Nautilu	s Environr	nental (CA)
Analysis ID:	05-1387-0350	En	dpoint: Fer	tilization Ra	te		CET	IS Version:	CETISv1	1.8.7	
Analyzed:	21 Mar-17 14:	38 <b>A</b> na	alysis: Pa	arametric-Control vs Treatments				ial Results			
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	rected)	NA	C > T	NA	NA		8.97%	<10	10	NA	
Dunnett Mul	tiple Compariso	n Test	······································				HIMOON			TO THE STATE OF TH	
Control	vs C-μg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	10*		2.415	2.227	0.101 8	0.0352	CDF	Significan	·		
	20*		5.949	2.227	0.101 8	<0.0001	CDF	Significan			
	40*		13.81	2.227	0.101 8	<0.0001	CDF	Significan			
ANOVA Table	е	7,001	HIGOOGGAAAAAA							1921180	
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	1.117273		0.3724242	2	3	72.7	<0.0001	Significan	t Effect		
Error	0.081961	66	0.0051226	604	16			•			
	4.400004				19	_					
Total	1.199234										
Total Distributiona									101		
				Test Stat	Critical	P-Value	Decision	(α:1%)			225
Distributiona	al Tests Test	Equality of V	'ariance	Test Stat 2.908		P-Value 0.4060	Decision( Equal Var				
Distributiona Attribute	al Tests Test Bartlett E	Equality of V Wilk W Nori			<b>Critical</b> 11.34 0.866		Decision( Equal Var Normal Di	iances			
Distributional Attribute Variances Distribution	al Tests Test Bartlett E			2.908	11.34	0.4060	Equal Var	iances			
Distributiona Attribute Variances Distribution	al Tests Test Bartlett E Shapiro-			2.908	11.34	0.4060	Equal Var	iances	Std Err	CV%	%Effect
Distributiona Attribute Variances Distribution	Tests Test Bartlett E Shapiro-	Wilk W Nori	mality	2.908 0.9555	11.34 0.866	0.4060 0.4584	Equal Var Normal Di	iances stribution	Std Err 0.01924		
Distributional Attribute Variances Distribution Fertilization	al Tests Test Bartlett E Shapiro- Rate Summary Control Type	Wilk W Nori	mality Mean	2.908 0.9555 <b>95% LCL</b>	11.34 0.866 95% UCL	0.4060 0.4584 Median	Equal Var Normal Di	iances stribution Max		CV% 5.06% 4.29%	0.0%
Distributional Attribute Variances Distribution Fertilization C-µg/L 0	al Tests Test Bartlett E Shapiro- Rate Summary Control Type	Wilk W Norn  Count  5	Mean 0.85	2.908 0.9555 <b>95% LCL</b> 0.7966	11.34 0.866 95% UCL 0.9034	0.4060 0.4584 <b>Median</b> 0.85	Equal Var Normal Di Min 0.79	iances stribution Max 0.9	0.01924 0.0147	5.06% 4.29%	0.0% 9.88%
Distributional Attribute Variances Distribution Fertilization C-µg/L 0 10	al Tests Test Bartlett E Shapiro- Rate Summary Control Type	Count 5 5	Mean 0.85 0.766	2.908 0.9555 <b>95% LCL</b> 0.7966 0.7252	11.34 0.866 95% UCL 0.9034 0.8068	0.4060 0.4584 <b>Median</b> 0.85 0.76	Equal Var Normal Di Min 0.79 0.73	Max 0.9 0.8	0.01924	5.06% 4.29% 14.89%	0.0% 9.88% 27.29%
Distributional Attribute Variances Distribution Fertilization C-µg/L 0 10 20	al Tests Test Bartlett E Shapiro- Rate Summary Control Type	Count 5 5 5	Mean 0.85 0.766 0.618	2.908 0.9555 <b>95% LCL</b> 0.7966 0.7252 0.5037	95% UCL 0.9034 0.8068 0.7323	0.4060 0.4584 Median 0.85 0.76 0.56	Min 0.79 0.73 0.55	Max 0.9 0.8 0.75 0.36	0.01924 0.0147 0.04116	5.06% 4.29%	0.0% 9.88% 27.29% 67.53%
Distributional Attribute Variances Distribution  Fertilization  C-µg/L  0  10  20  40	al Tests Test Bartlett E Shapiro- Rate Summary Control Type	Count 5 5 5 5	Mean 0.85 0.766 0.618 0.276	2.908 0.9555 <b>95% LCL</b> 0.7966 0.7252 0.5037 0.1911	11.34 0.866 95% UCL 0.9034 0.8068 0.7323 0.3609	0.4060 0.4584 Median 0.85 0.76 0.56 0.25	Min 0.79 0.73 0.55 0.19	Max 0.9 0.8 0.75	0.01924 0.0147 0.04116 0.03059	5.06% 4.29% 14.89%	0.0% 9.88% 27.29%
Distributional Attribute Variances Distribution  Fertilization  C-µg/L  0  10  20  40  80  160	al Tests Test Bartlett E Shapiro- Rate Summary Control Type	Count  5 5 5 5 5 5	Mean 0.85 0.766 0.618 0.276 0	2.908 0.9555 <b>95% LCL</b> 0.7966 0.7252 0.5037 0.1911 0	95% UCL 0.9034 0.8068 0.7323 0.3609 0	0.4060 0.4584 Median 0.85 0.76 0.56 0.25	Min 0.79 0.73 0.55 0.19	Max 0.9 0.8 0.75 0.36	0.01924 0.0147 0.04116 0.03059 0	5.06% 4.29% 14.89%	0.0% 9.88% 27.29% 67.53% 100.0%
Distributional Attribute Variances Distribution  Fertilization  C-µg/L  0  10  20  40  80  160	Tests Test Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count  5 5 5 5 5 5	Mean 0.85 0.766 0.618 0.276 0	2.908 0.9555 <b>95% LCL</b> 0.7966 0.7252 0.5037 0.1911 0	95% UCL 0.9034 0.8068 0.7323 0.3609 0	0.4060 0.4584 Median 0.85 0.76 0.56 0.25	Min 0.79 0.73 0.55 0.19	Max 0.9 0.8 0.75 0.36	0.01924 0.0147 0.04116 0.03059 0	5.06% 4.29% 14.89%	0.0% 9.88% 27.29% 67.53% 100.0%
Distributiona Attribute Variances Distribution  Fertilization C-µg/L 0 10 20 40 80 160  Angular (Cor	Tests Test Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 med Summ	Mean 0.85 0.766 0.618 0.276 0	2.908 0.9555 <b>95% LCL</b> 0.7966 0.7252 0.5037 0.1911 0	95% UCL 0.9034 0.8068 0.7323 0.3609 0	0.4060 0.4584 Median 0.85 0.76 0.56 0.25 0	Min 0.79 0.73 0.55 0.19 0	Max 0.9 0.8 0.75 0.36 0	0.01924 0.0147 0.04116 0.03059 0	5.06% 4.29% 14.89% 24.79%	0.0% 9.88% 27.29% 67.53% 100.0%
Distributional Attribute Variances Distribution  Fertilization  C-µg/L  0  10  20  40  80  160  Angular (Cor	Tests Test Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count  5 5 5 5 5 5 med Summ	Mean 0.85 0.766 0.618 0.276 0 0	2.908 0.9555 95% LCL 0.7966 0.7252 0.5037 0.1911 0 0	95% UCL 0.9034 0.8068 0.7323 0.3609 0	0.4060 0.4584 Median 0.85 0.76 0.56 0.25 0	Equal Var Normal Di Min 0.79 0.73 0.55 0.19 0	Max 0.9 0.8 0.75 0.36 0	0.01924 0.0147 0.04116 0.03059 0 0	5.06% 4.29% 14.89% 24.79% CV% 5.13%	0.0% 9.88% 27.29% 67.53% 100.0% 100.0%
Distributiona Attribute Variances Distribution  Fertilization  C-µg/L  0  10  20  40  80  160  Angular (Cor C-µg/L  0	Tests Test Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 5 5 med Summ Count 5	Mean  0.85  0.766  0.618  0.276  0  0  mary  Mean  1.176	2.908 0.9555 95% LCL 0.7966 0.7252 0.5037 0.1911 0 0	95% UCL 0.9034 0.8068 0.7323 0.3609 0 0	0.4060 0.4584 Median 0.85 0.76 0.56 0.25 0 0	Min 0.79 0.73 0.55 0.19 0 0	Max 0.9 0.8 0.75 0.36 0 0	0.01924 0.0147 0.04116 0.03059 0 0 Std Err 0.02696	5.06% 4.29% 14.89% 24.79%	0.0% 9.88% 27.29% 67.53% 100.0% 100.0% %Effect 0.0%
Distributiona Attribute Variances Distribution  Fertilization  C-µg/L  0  10  20  40  80  160  Angular (Cor C-µg/L  0  10	Tests Test Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count  5 5 5 5 5 med Summ Count 5 5 5	Mean 0.85 0.766 0.618 0.276 0 0  mary Mean 1.176 1.067	2.908 0.9555 95% LCL 0.7966 0.7252 0.5037 0.1911 0 0 95% LCL 1.101 1.018	95% UCL 0.9034 0.8068 0.7323 0.3609 0 0 95% UCL 1.251 1.115	0.4060 0.4584 Median 0.85 0.76 0.56 0.25 0 0 Median 1.173 1.059	Min 0.79 0.73 0.55 0.19 0 Min 1.095 1.024	Max 0.9 0.8 0.75 0.36 0 0	0.01924 0.0147 0.04116 0.03059 0 0 Std Err 0.02696 0.01744	5.06% 4.29% 14.89% 24.79% CV% 5.13% 3.66%	0.0% 9.88% 27.29% 67.53% 100.0% 100.0% %Effect 0.0% 9.3%
Distributiona Attribute Variances Distribution  Fertilization 10 20 40 80 160  Angular (Cor C-µg/L 0 10 20 40 20 40 80 160  Angular (20 40 80 160  Angular (20 40 80 10 20 40 80 10 20 40 80 10 20	Tests Test Bartlett E Shapiro- Rate Summary Control Type Lab Control	Count  5 5 5 5 5 med Summ Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.85 0.766 0.618 0.276 0 0  mary Mean 1.176 1.067 0.9066	2.908 0.9555 95% LCL 0.7966 0.7252 0.5037 0.1911 0 0 95% LCL 1.101 1.018 0.7863	95% UCL 0.9034 - 0.8068 0.7323 0.3609 0 0 95% UCL 1.251 1.115 1.027	0.4060 0.4584 Median 0.85 0.76 0.56 0.25 0 0 Median 1.173 1.059 0.8455	Min 0.79 0.73 0.55 0.19 0 0 Min 1.095 1.024 0.8355	Max 0.9 0.8 0.75 0.36 0 0  Max 1.249 1.107 1.047	0.01924 0.0147 0.04116 0.03059 0 0 Std Err 0.02696 0.01744 0.04334	5.06% 4.29% 14.89% 24.79% CV% 5.13% 3.66% 10.69%	0.0% 9.88% 27.29% 67.53% 100.0% 100.0% %Effect 0.0% 9.3% 22.9%



Report Date: Test Code:

21 Mar-17 14:38 (p 1 of 1)

170310sprt | 05-2038-2100

**Echinoid Sperm Cell Fertilization Test 15C** 

Nautilus Environmental (CA)

Analysis ID: Analyzed:

05-9725-9024 21 Mar-17 14:38

Endpoint: Fertilization Rate

**CETIS Version:** 

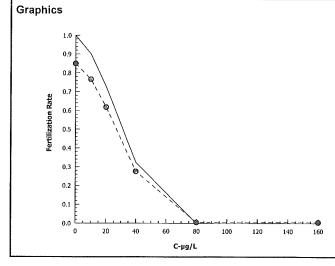
CETISv1.8.7

Analysis: Trimmed Spearman-Kärber Official Results: Yes

Trimmed Spearman-Karber Estimates	5
-----------------------------------	---

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

Fertilizati	on Rate Summary										
C-μg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.85	0.79	0.9	0.01924	0.04301	5.06%	0.0%	425	500
10		5	0.766	0.73	8.0	0.0147	0.03286	4.29%	9.88%	383	500
20		5	0.618	0.55	0.75	0.04116	0.09203	14.89%	27.29%	309	500
40		5	0.276	0.19	0.36	0.03059	0.06841	24.79%	67.53%	138	500
80		5	0	0	0	0	. 0		100.0%	0	500
160		5	0	0	0	0	0		100.0%	0	500



Report Date:

26 Mar-17 11:52 ( 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

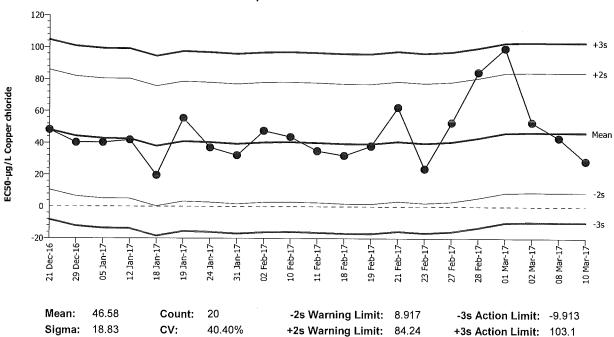
Endpoint: Fertilization Rate

Material: Copper chloride

Source: Reference Toxicant-REF

+3s Action Limit: 103.1

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



84.24

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

## **CETIS Test Data Worksheet**

Report Date:

09 Mar-17 15:29 (p 1 of 1)

Test Code:

05-2038-2100/170310sprt

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 150				Nautilus Environmental (CA		
Start Date: End Date: Sample Date	10 <b>N</b>	Mar-17 Mar-17 Mar-17	7		s: Strongylocer ol: EPA/600/R-9 il: Copper chlor	· · · · · · · · · · · · · · · · · · ·		170310sprt Reference Toxicant Copper Chloride		
C-µg/L	Code	Rep	Pos	# Counted	# Fertilized		Notes			
			1	(00)	0	3/17/17				
			2	100	73					
			3	100	80					
			4	100	33					
			5	100	19					
			6	[00]	68			-		
			7	100	56					
			8	100	0					
			9	100	25					
			10	100	Q					
			11	100	0					
			12	(00)	0					
			13	100	88					
	-		14 15	(00	25					
			16	100	88.00					
			17	100	79					
			18	100	85 74					
			19	100	36					
			20	100 100						
			21	100	76 83					
			22	160	55			11 100 00000000000000000000000000000000		
			23	100	0					
			24	160	0					
			25	100	Ŏ					
			26	(00)	55					
			27	100	0					
			28	100	75					
			29	100	90					
			30	100	80					

@ Q18 PM 3/17/17

### **CETIS Test Data Worksheet**

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

Echinoid Sp	erm C	ell Fe	rtiliza	tion Test 150	3				Nautilus Environmental (CA)
Start Date: 10 Mar-17 End Date: 10 Mar-17 Sample Date: 10 Mar-17					s: Strongyloo ol: EPA/600/l al: Copper ch			Sample Code: Sample Source: Sample Station:	170310sprt Reference Toxicant Copper Chloride
C-µg/L	Code	Rep	Pos	# Counted	# Fertilized			Notes	
. 0	LC	1	13	100	94	EG	3/10/7		
0	LC	2	17		,				
0	LC	3	16						
0	LC	4	21		11-11-20-0			200000000000000000000000000000000000000	
0	LC	5	29						
10		1	20						
10		2	30						
10		3	2						
10		4	3	100	90	EC			
10		5	18		1	,			
20		1	22						
20		2	26						
20		3	6						
20		4	7	100	55	EG			
20		5	28			1			
40		1	5						
40		2	4						
40		3	14						
40		4	9	100	36	EG			
40		5	19		1 10				
80		1	25						
80		2	24						
80		3	10						
80		4	23						
80		5	27	100	1	EG			
160		1	15						
160		2	8						
160		3	12	(00	0	EG			
160		4	1						
160		5	11		~				

ac. Eg

**Water Quality Measurements** 

_					
С	Ιi	0	n	٠	
v	и	c		L	

Internal

Test Species: S. purpuratus

Sample ID:

CuCl<sub>2</sub>

Start Date/Time: 3/10/2017

1418

Test No:

170310sprt

End Date/Time: 3/10/2017

1458

Dilutions made by:

Fa

High conc. made (µg/L):

160

8.2 Vol. Cu stock added (mL): Final Volume (mL):

500

9,800

Cu stock concentration (μg/L):

Analyst:

AD Initial Readings Concentration DO Salinity рΗ Temperature (μ**g/L**) (mg/L) (units) (ppt) (°C) Lab Control 8.04 10 8.00 20 60.8 40 7.98. 80 8.4 8.01 160 8.02 33,0 14.6

Comments	:

QC Check:

AC 314/17

Final Review: <u>1639117</u>

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

Walling Officials Bloa	ssay	Echinoderin Sperm-Cell	rertilization worksneet
Client: Sample ID: Test No.:	Internal Cullz 170310 Sprt	End Date/Tir	me: 3/10/2017 / 1418 me: 3/10/2017 / 1458 ies: <i>S. purpuratus</i>
Tech initials: Injection Time:	1340	Animal Sour Date Collect	rce: Pt. Loma ted: 2 17 17 4 3 6 17
Sperm Absorbance at 40	00 nm: <u>0 855</u> (target r	ange of 0.8 - 1.0 for density of 4x10 <sup>6</sup> sperm/r	ml)
Eggs Counted:	102 (target counts of	Y X 50 = <u>4570</u> eggs/ml 80 eggs per vertical pass on Sedgwick- final density of 4000 eggs/ml)	
Initial density: Final density:			26 0 ml 26 ml

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

	Sperm:Egg Ratio							
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1
ml Sperm Stock	50	40	30	20	10	5.0	2.5	1.25
ml Seawater	0.0	10	20	30	40	45	47.5	48.75
	Time	Ra	ngefinder Ra	tio: Fe	rt. Unf	ert.		
Sperm Added (100 µl):	1356		50:1	6	<del>7</del> 33	<u> </u>		
Eggs Added (0.5 ml):	1400	-	100:1	96	196 41	4		
Test Ended:	1410		200:1		0 0			
			distribution 20			4		

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).

<u>Definitive Test</u>	Sperm:Egg Ratio Used: 100
Time Sperm Added (100 μl): 14 % Eggs Added (0.5 ml): 14 3 % Test Ended: 145 %	Fert.   Unfert.
Comments:	

QC Check:

Appendix E

Qualifier Codes



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

- Q1 Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 Temperatures out of recommended range; no action taken, test terminated same day
- Q3 Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L
- Q4 Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 Test initiated with aeration due to an anticipated drop in D.O.
- Q6 Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 Salinity out of recommended range
- Q8 Spilled test chamber/ Unable to recover test organism(s)
- Q9 Inadequate sample volume remaining, 50% renewal performed
- Q10 Inadequate sample volume remaining, no renewal performed
- Q11 Sample out of holding time; refer to QA section of report
- Q12 Replicate(s) not initiated; excluded from data analysis
- Q13 Survival counts not recorded due to poor visibility or heavy debris
- Q14 D.O. percent saturation was checked and was ≤ 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 organisms received at a <u>temperature</u> greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 Test organisms received at <u>salinity</u> greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Updated: 6/30/15