

# Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Weekly)
Sample Collection Date: October 30, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: November 7, 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.

**California** 4340 Vandever Avenue

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

Results verified by: \_\_\_\_\_\_ Adrienne Cibor

# **EXECUTIVE SUMMARY**

#### CHRONIC TOXICITY TESTING

# CARLSBAD DESALINATION PLANT — OCTOBER 2017 ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: October 30, 2017

Test Date: October 31, 2017

Sample ID: M-001 Brine Effluent

M-001

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

#### Results Summary:

Bioassay Type:	M-001 Efflu Resu		Effluent Limitation Met? (Yes/No)
	NOEC	TUc	NI-
Echinoderm Fertilization	5	20	No

TOXICITY SUMMARY REPORT Client: IDE Americas, Inc.
Test IDs: 1710-128 to S130 Sample Collection Date: October 30, 2017

#### INTRODUCTION

A 24-hour composite discharge sample was collected in October 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for weekly accelerated toxicity monitoring purposes. Due to effects observed in a sample collected and tested for monthly monitoring purposes on May 04, 2017 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). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on October 31, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

#### **MATERIALS AND METHODS**

Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was couriered 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. The sample was stored at 4° C in the dark until used for testing. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

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

Monitoring Period: October 2017 (weekly accelerated chronic monitoring)

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

Sample Collection Date, Time: 10/30/17, 08:00

Sample Receipt Date, Time: 10/30/17, 12:54

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 CaCO₃)	Total Chlorine (mg/L)
M-001	7.82	7.3	3.4	60.6	163	<0.02

TOXICITY SUMMARY REPORT Client: IDE Americas, Inc.
Test IDs: 1710-128 to S130 Sample Collection Date: October 30, 2017

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period: 10/31/17, 13:59 through 14:39

Test Organism: Strongylocentrotus purpuratus (purple sea urchin)

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

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

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

Additional Control: High Salinity Control (HSC) – seawater with Nautilus hypersaline brine

added to match the salinity of the 15 percent M-001 effluent concentration; tested to evaluate potential adverse effects due to

elevated salinity alone

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

control. The same dilution series was also tested with the sample after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP

discharges).

Number of Replicates, Organisms

per Replicate:

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

before each test with a preliminary rangefinding test.

Test Chamber Type, Volume per

Replicate:

Glass scintillation vial containing 10 mL of test solution

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

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

egg fertilization period

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

significant difference (PMSD) value <25%

Reference Toxicant Testing: Copper chloride

Statistical Analysis Software: CETIS™, version 1.8.7.20

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

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant 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

Client: IDE Americas, Inc. Sample Collection Date: October 30, 2017

a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06 percent unadjusted effluent, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST statistical analysis is not in the 2006 CDP permit, the TST results are included for comparison purposes only.

#### **RESULTS**

There was a significant decrease in the fertilization rate in the 6.06, 10, and 15 percent concentrations in the unadjusted M-001 sample relative to the lab control using the EPA 1995 flowchart statistics. The NOEC is reported as 5 percent effluent and a  $TU_c$  equal to 20, which exceeds the maximum permit effluent limitation. The PMSD for this test was very low (1.8 percent), which increases statistical power to detect differences due to low variability within the test. The percent effect between the IWC of 6.06 percent and the lab control was 2.6. None of the concentrations of the M-001 unadjusted sample were significantly reduced from the control using the TST statistical analysis. The 40 ppt adjusted M-001 effluent sample resulted in no significant effects at any concentration tested using both EPA 1995 flowchart statistics the TST analysis. The high salinity control matching salinity in the 15 percent effluent concentration resulted in a mean fertilization rate of 99.0 percent (compared to 98.8 percent in the lab control), suggesting that salinity at this level (up to 37 ppt) was not likely to cause reduced fertilization in this test.

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

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

Sample ID	NOEC (% sample)	LOEC (% sample)	EC <sub>50</sub> (% sample)	TU <sub>c</sub> value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001 (unadjusted)	5	6.06	>15	20	Pass	2.6
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-1.0

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

TUc = Chronic Toxic Unit: 100÷NOEC

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

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

Table 5. Detailed Results of Purple Urchin Fertilization Testing for the M-001 Sample

Test Concentration	M-001 L	Inadjusted Sample	M-001 40 ppt Adjusted <sup>a</sup>			
(% Sample)	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization		
Lab Control	33.9	98.8	33.9	98.0		
High Salinity Control	37.0	99.0				
2.5	34.5	98.4	34.2	98.0		
5.0	35.2	97.8	34.3	98.8		
6.06	35.5	96.2*	34.4	99.0		
10	36.5	94.4*	34.6	97.4		
15	37.1	95.4*	34.9	98.2		

<sup>\*</sup> An asterisk indicates a statistically significant difference when compared to the lab control using EPA 1995 flowchart statistical methods.

#### **QUALITY ASSURANCE**

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

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

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 6 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration ( $EC_{50}$ ) value was just above two standard deviations (SD) of the historical mean, indicating test organisms were less sensitive to copper than typically observed in our laboratory. A list of qualifier codes used on bench datasheets can be found in Appendix E.

Table 6. Reference Toxicant Test Results

Test Species	Endpoint	EC <sub>50</sub> (µg/L Copper)	Historical Mean EC <sub>50</sub> ±2 SD (µg/L Copper)	CV (%)
Purple Urchin	Fertilization	81.4	49.9 ± 30.5	30.6

 $EC_{50}$  = 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

<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 concentrations.

TOXICITY SUMMARY REPORT

Test IDs: 1710-128 to S130

Client: IDE Americas, Inc.

Sample Collection Date: October 30, 2017

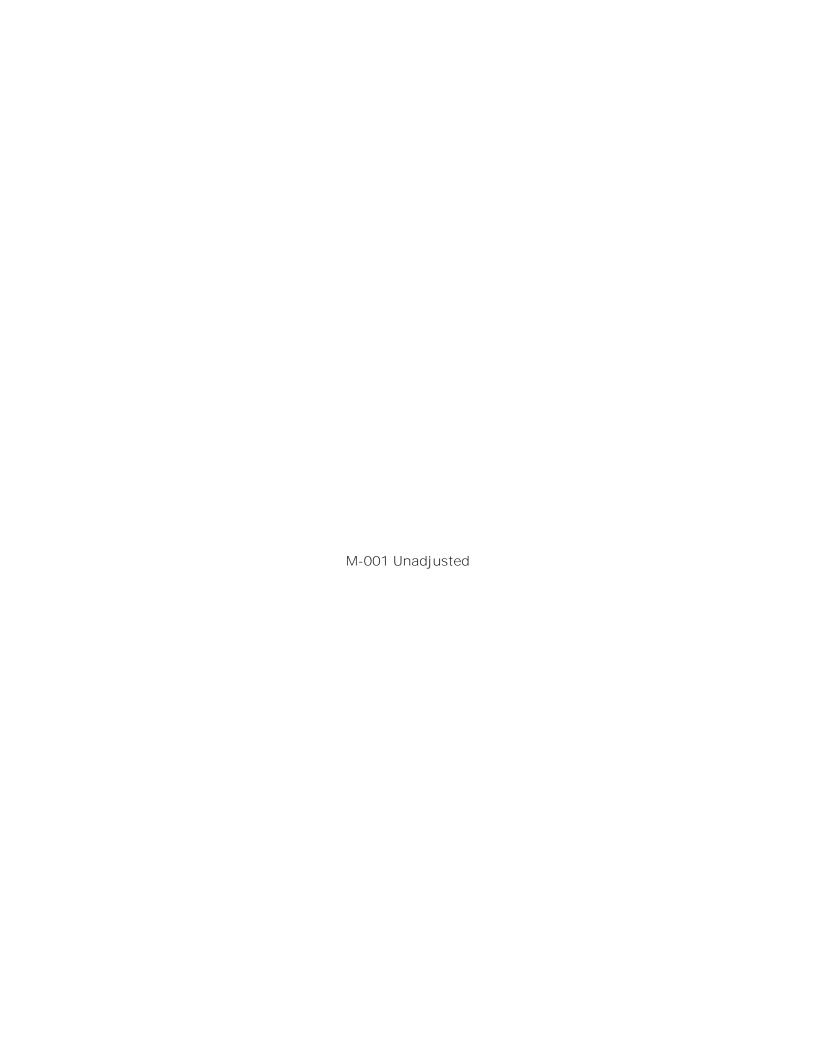
#### REFERENCES

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

- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
- Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.
- USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. United States Environmental Protection Agency Office of Wastewater Management (EPA-833-R-00-003).
- USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses



# **CETIS Summary Report**

Report Date:

01 Nov-17 12:11 (p 1 of 1)

Test Code:

1710-S128 | 09-2205-6048

								Test Code	•	171	0-3120   0	9-2205-6048
Echi noid Spe	rm Cell Fertiliza	tion Test	t 15C							Nautilus	s Environi	mental (CA)
Batc h ID: Start Date: Ending Date: Duration:	13-1240-6073 31 Oct-17 13:5: 31 Oct-17 14:3: 40m	9 <b>P</b> 9 <b>S</b>	est Type: rotocol: pecies: ource:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	, ,	tus		Analyst: Diluent: Brine: Age:		ural Seawate Applicable	er	
-	08-8002-2042 30 Oct-17 08:00 30 Oct-17 12:54 30h (3.4 °C)	0 <b>N</b> 4 <b>S</b>	ode: laterial: ource: tation:	17-1128 Facility Effluent IDE Americas, M-001 Unadjus	Inc.			Client: Project:	IDE Carl	sbad Desal	Plant	
Com parison S	Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Met	hod			
13-9548-5162	Fertilization Rat	te	5	6.06	5.505	1.8%	20	Dun	nett IV	lultiple Com	parison Te	st
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Met	hod			
15-0356-3573	Fertilization Ra	te	EC25 EC50	>15 >15	N/A N/A	N/A N/A	<6.6 <6.6		ar Inte	erpolation (IC	CPIN)	
Test Acceptal	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Ove	rlap	Decision		
13-9548-5162	Fertilization Rat	te	Contro	l Resp	0.988	0.7 - NL		Yes	Yes Passes Acceptability Cr		Criteria	
15-0356-3573	Fertilization Rat	te	Contro	l Resp	0.988	0.7 - NL		Yes		Passes Acceptability Criter		Criteria
13-9548-5162	Fertilization Rat	e	PMSD		0.01796	NL - 0.25		No	****	Passes Ad	cceptability	Criteria
Fertilization R	tate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std	Err	Std Dev	CV%	%Effect
0	High Salinity Co	5	0.99	0.9812	0.9988	0.98	1	0.00	3163	0.007072	0.71%	0.0%
0	Lab Control	5	0.988	0.9824	0.9936	0.98	0.99	0.00	2001	0.004474	0.45%	0.2%
2.5		5	0.984	0.9729	0.9951	0.97	0.99		4	0.008945	0.91%	0.61%
5		5	0.978	0.9676	0.9884	0.97	0.99		3741	0.008366	0.86%	1.21%
6.06		5	0.962	0.9381	0.9859	0.93	0.98		8602	0.01924	2.0%	2.83%
10		5	0.944	0.9128	0.9752	0.91	0.98			0.0251	2.66%	4.65%
15		5	0.954	0.9254	0.9826	0.93	0.98	0.01	03	0.02302	2.41%	3.64%
Fertilization R	tate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
	High Salinity Co		0.99	0.99	1	0.99			_			
•	Lab Control	0.99	0.99	0.98	0.99	0.99						
2.5		0.99	0.99	0.99	0.98	0.97						
5		0.98	0.99	0.97	0.97	0.98						
6.06		0.98	0.96	0.93	0.97	0.97						
10		0.91	0.94	0.98	0.94	0.95						
15		0.98	0.96	0.93	0.97	0.93						

Report Date:

01 Nov-17 12:11 (p 1 of 2)

3		**
Test Code:	1710-S128	09-2205-604
1001 0040.	11 10 0120	00 2200 00

								Coue.			
Echinoid Sp	erm Cell Fertiliza	ation Test	15C						Nautilus	Environ	mental (CA)
Analysis ID: Analyzed:	13-9548-5162 01 Nov-17 12:		<b>dpoint:</b> Fer <b>alysis:</b> Par	tilization Ra ametric-Cor		tments		IS Version: ial Results:	CETISv1. Yes	.8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C > T	NA	NA		1.8%	5	6.06	5.505	20
Dunnett Mul	tiple Compariso	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(	α:5%)		
Lab Control	2.5		0.5386	2.362	0.065 8	0.6300	CDF		icant Effect	***************************************	
	5		1.381	2.362	0.065 8	0.2646	CDF	•	icant Effect		
	6.06*		3.036	2.362	0.065 8	0.0118	CDF	Significant			
	10*		4.55	2.362	0.065 8	0.0003	CDF	Significant			
	15*		3.718	2.362	0.065 8	0.0023	CDF	Significant			
ANOVA Tabl	e										
Source	Sum Squ	ıares	Mean Squ	ıare	DF	F Stat	P-Value	Decision(e	a:5%)		
Between	0.063276	07	0.0126552	:1	5	6.719	0.0005	Significant Effect			
Error	0.045201	47	0.0018833	94	24			3			
Total	0.108477	5			29	_					
Distribution	al Tests								**************************************		
Attribute	Test			Test Stat	Critical	P-Value	Decision(	α:1%)			
Variances	Bartlett E	Equality of \	/ariance	5.816	15.09	0.3246	Foual Var	iancoe			
							Equal Variances Normal Distribution				
Distribution	Shapiro-	Wilk W Nor	mality	0.9728	0.9031	0.6181	•				
	Shapiro- Rate Summary	Wilk W Nor	mality	0.9728	0.9031		•				
		Wilk W Nor	mality Mean	0.9728 95% LCL	0.9031 95% UCL		•		Std Err	CV%	%Effect
Fertilization	Rate Summary					0.6181	Normal Di	stribution	Std Err 0.002001	CV% 0.45%	%Effect
Fertilization C-%	Rate Summary Control Type	Count	Mean	95% LCL	95% UCL	0.6181 Median	Normal Di	stribution Max			
Fertilization C-%	Rate Summary Control Type	Count 5	<b>Mean</b> 0.988	<b>95% LCL</b> 0.9824	<b>95% UCL</b> 0.9936	0.6181 <b>Median</b> 0.99	Min 0.98	Max 0.99	0.002001	0.45%	0.0%
Fertilization C-% 0 2.5	Rate Summary Control Type	Count 5 5	<b>Mean</b> 0.988 0.984	<b>95% LCL</b> 0.9824 0.9729	<b>95% UCL</b> 0.9936 0.9951	0.6181 Median 0.99 0.99	Min 0.98 0.97	Max 0.99 0.99	0.002001 0.004	0.45% 0.91%	0.0% 0.4%
Fertilization C-% 0 2.5	Rate Summary Control Type	Count 5 5 5	Mean 0.988 0.984 0.978	95% LCL 0.9824 0.9729 0.9676	95% UCL 0.9936 0.9951 0.9884	0.6181 Median 0.99 0.99 0.99	Min 0.98 0.97 0.97	Max 0.99 0.99 0.99	0.002001 0.004 0.003741	0.45% 0.91% 0.86%	0.0% 0.4% 1.01%
Fertilization C-% 0 2.5 5 6.06	Rate Summary Control Type	<b>Count</b> 5 5 5 5	Mean 0.988 0.984 0.978 0.962	95% LCL 0.9824 0.9729 0.9676 0.9381	95% UCL 0.9936 0.9951 0.9884 0.9859	Median 0.99 0.99 0.98 0.97	Min 0.98 0.97 0.97 0.93	Max 0.99 0.99 0.99 0.99	0.002001 0.004 0.003741 0.008602	0.45% 0.91% 0.86% 2.0%	0.0% 0.4% 1.01% 2.63%
Fertilization C-% 0 2.5 5 6.06 10	Rate Summary Control Type	Count 5 5 5 5 5 5 5 5 5	Mean 0.988 0.984 0.978 0.962 0.944 0.954	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752	Median 0.99 0.99 0.98 0.97 0.94	Min 0.98 0.97 0.97 0.93 0.91	Max 0.99 0.99 0.99 0.98 0.98	0.002001 0.004 0.003741 0.008602 0.01122	0.45% 0.91% 0.86% 2.0% 2.66%	0.0% 0.4% 1.01% 2.63% 4.45%
Fertilization C-% 0 2.5 5 6.06 10	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 5 5	Mean 0.988 0.984 0.978 0.962 0.944 0.954	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752	Median 0.99 0.99 0.98 0.97 0.94	Min 0.98 0.97 0.97 0.93 0.91	Max 0.99 0.99 0.99 0.98 0.98	0.002001 0.004 0.003741 0.008602 0.01122	0.45% 0.91% 0.86% 2.0% 2.66%	0.0% 0.4% 1.01% 2.63% 4.45%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 7 med Sumr	Mean 0.988 0.984 0.978 0.962 0.944 0.954	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128 0.9254	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752 0.9826	0.6181 Median 0.99 0.99 0.98 0.97 0.94 0.96	Min 0.98 0.97 0.97 0.93 0.91 0.93	Max 0.99 0.99 0.99 0.98 0.98 0.98	0.002001 0.004 0.003741 0.008602 0.01122 0.0103	0.45% 0.91% 0.86% 2.0% 2.66% 2.41%	0.0% 0.4% 1.01% 2.63% 4.45% 3.44%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 5 cmed Sumr	Mean 0.988 0.984 0.978 0.962 0.944 0.954  mary Mean	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128 0.9254	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752 0.9826	0.6181  Median 0.99 0.99 0.98 0.97 0.94 0.96	Min 0.98 0.97 0.97 0.93 0.91 0.93	Max 0.99 0.99 0.99 0.98 0.98 0.98	0.002001 0.004 0.003741 0.008602 0.01122 0.0103 Std Err	0.45% 0.91% 0.86% 2.0% 2.66% 2.41%	0.0% 0.4% 1.01% 2.63% 4.45% 3.44%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 5 cmed Sumr Count 5	Mean 0.988 0.984 0.978 0.962 0.944 0.954  nary Mean 1.462	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128 0.9254 95% LCL 1.439	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752 0.9826 95% UCL 1.485	0.6181  Median 0.99 0.99 0.98 0.97 0.94 0.96  Median 1.471	Min 0.98 0.97 0.97 0.93 0.91 0.93	Max 0.99 0.99 0.99 0.98 0.98 0.98 Max 1.471	0.002001 0.004 0.003741 0.008602 0.01122 0.0103 Std Err 0.008346	0.45% 0.91% 0.86% 2.0% 2.66% 2.41% CV%	0.0% 0.4% 1.01% 2.63% 4.45% 3.44% %Effect 0.0%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 cmed Sumr Count 5	Mean 0.988 0.984 0.978 0.962 0.944 0.954  nary Mean 1.462 1.447	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128 0.9254 95% LCL 1.439 1.406	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752 0.9826 95% UCL 1.485 1.489	0.6181  Median 0.99 0.99 0.98 0.97 0.94 0.96  Median 1.471 1.471	Min 0.98 0.97 0.97 0.93 0.91 0.93 Min 1.429 1.397	Max 0.99 0.99 0.99 0.98 0.98 0.98 Max 1.471 1.471	0.002001 0.004 0.003741 0.008602 0.01122 0.0103 Std Err 0.008346 0.01505 0.01362	0.45% 0.91% 0.86% 2.0% 2.66% 2.41% CV% 1.28% 2.33% 2.14%	0.0% 0.4% 1.01% 2.63% 4.45% 3.44% %Effect 0.0% 1.01% 2.59%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	Rate Summary Control Type Lab Control  rrected) Transfor Control Type	Count 5 5 5 5 5 med Sumr Count 5 5 5	Mean 0.988 0.984 0.978 0.962 0.944 0.954  mary Mean 1.462 1.447 1.424	95% LCL 0.9824 0.9729 0.9676 0.9381 0.9128 0.9254 95% LCL 1.439 1.406 1.387	95% UCL 0.9936 0.9951 0.9884 0.9859 0.9752 0.9826 95% UCL 1.485 1.489 1.462	Median 0.99 0.99 0.98 0.97 0.94 0.96  Median 1.471 1.471 1.429	Min 0.98 0.97 0.97 0.93 0.91 0.93 Min 1.429 1.397 1.397	Max 0.99 0.99 0.99 0.98 0.98 0.98 Max 1.471 1.471 1.471	0.002001 0.004 0.003741 0.008602 0.01122 0.0103 Std Err 0.008346 0.01505	0.45% 0.91% 0.86% 2.0% 2.66% 2.41% CV% 1.28% 2.33%	0.0% 0.4% 1.01% 2.63% 4.45% 3.44% %Effect 0.0% 1.01%

0.4

0.3

0.2

0.1 0.0

0 LC

2.5

6.06

C-%

10

15

Report Date: Test Code:

01 Nov-17 12:11 (p 2 of 2) 1710-S128 | 09-2205-6048

> 1.5 2.0

Rankits

**Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) Analysis ID: 13-9548-5162 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 01 Nov-17 12:09 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 0.100 0.9 0.075 0.8 0.050 Fertilization Rate 0.025 0.6

0.000

-0.025

-0,050

-0.075

-0.100

QA: 65 11/3/17

Report Date:

01 Nov-17 12:11 (p 1 of 1)

Test Code:

1710-S128 | 09-2205-6048

Echi noid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

15-0356-3573 Analysis ID: Analyzed:

01 Nov-17 12:11

Analysis:

Endpoint: Fertilization Rate Linear Interpolation (ICPIN)

**CETIS Version:** Official Results:

CETISv1.8.7 Yes

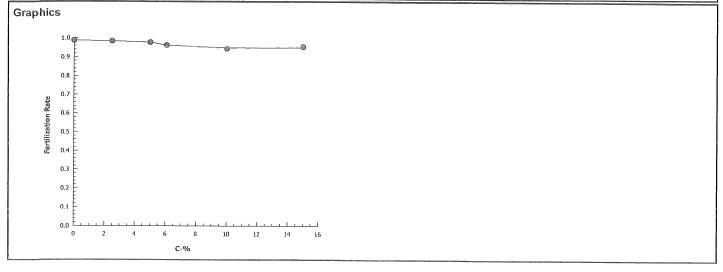
Linear Interpol	lation Options				
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method

Linear 1377706 Linear 1000 Yes Two-Point Interpolation

Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL	
EC25	>15	N/A	N/A	<6.667	NA	NA	
EC5O	>15	N/A	N/A	<6.667	NA	NA	

Fertiliza	tion 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	0.988	0.98	0.99	0.002001	0.004474	0.45%	0.0%	494	500
2.5		5	0.984	0.97	0.99	0.004	0.008945	0.91%	0.4%	492	500
5		5	0.978	0.97	0.99	0.003741	0.008366	0.86%	1.01%	489	500
6.06		5	0.962	0.93	0.98	0.008602	0.01924	2.0%	2.63%	481	500
10		5	0.944	0.91	0.98	0.01122	0.0251	2.66%	4.45%	472	500
15		5	0.954	0.93	0.98	0.0103	0.02302	2.41%	3.44%	477	500



Report Date: Test Code: 01 Nov-17 12:11 (p 1 of 1) 1710-S128 | 09-2205-6048

							1631	Code:		0-0120   0	9-2205-604
Echinoid Spe	erm Cell Fertiliza	ation Test	15C						Nautilus	Environ	mental (CA
Analysis ID: Analyzed:	14-0750-5451 01 Nov-17 12:		ndpoint: Fer nalysis: Par	tilization Rat		-Two Samp		IS Version:	CETISv1.	8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	rected)	NA	C*b < T	NA	NA	0.75	1.48%	15	>15	NA	6.667
TST-Welch's	t Test	And the control of th									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(	α:5%)		
Lab Control	2.5*	-200	21.52	2.015	0.033 5	<0.0001	CDF	Non-Signi	ficant Effect		
	5*		21.86	2.015	0.030 5	<0.0001	CDF	=	ficant Effect		
	6.06*		12.77	2.132	0.047 4	0.0001	CDF	•	ficant Effect		
	10*		8.878	2.132	0.058 4	0.0004	CDF	_	ficant Effect		
	15*		10.16	2.132	0.055 4	0.0003	CDF		ficant Effect		
ANOVA Table	e										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision(	α:5%)		
Between	0.063276	07	0.0126552	21	5	6.719	0.0005	Significant Effect			
Error	0.045201	47	0.0018833	394	24			J			
Total	0.108477	5			29	_					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(	(α:1%)			
Variances	Bartlett E	quality of	Variance	5.816	15.09	0.3246	Equal Var	iances			
Distribution	Shapiro-	Wilk W No	rmality	0.9728	0.9031	0.6181	Normal Di	stribution			
Fertilization	Rate Summary									A CONTRACTOR OF THE PARTY OF TH	
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.988	0.9824	0.9936	0.99	0.98	0.99	0.002001	0.45%	0.0%
2.5		5	0.984	0.9729	0.9951	0.99	0.97	0.99	0.004	0.91%	0.4%
5		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	1.01%
6.06		5	0.962	0.9381	0.9859	0.97	0.93	0.98	0.008602	2.0%	2.63%
6.06 10		5 5	0.962 0.944	0.9381 0.9128	0.9859 0.9752	0.97 0.94	0.93 0.91	0.98 0.98	0.008602 0.01122	2.0% 2.66%	2.63% 4.45%
10 15	rected) Transfor	5 5	0.944 0.954	0.9128	0.9752	0.94	0.91	0.98	0.01122	2.66%	4.45%
10 15	rected) Transfor Control Type	5 5	0.944 0.954	0.9128	0.9752	0.94	0.91	0.98	0.01122	2.66%	4.45%
10 15 Angular (Cor		5 5 med Sum	0.944 0.954 mary	0.9128 0.9254	0.9752 0.9826	0.94 0.96	0.91 0.93	0.98 0.98	0.01122 0.0103	2.66% 2.41%	4.45% 3.44%
10 15 Angular (Cor C-%	Control Type	5 5 med Sum Count	0.944 0.954 mary Mean	0.9128 0.9254 95% LCL	0.9752 0.9826 <b>95% UCL</b>	0.94 0.96 <b>Median</b>	0.91 0.93 Min	0.98 0.98 Max	0.01122 0.0103 Std Err	2.66% 2.41% CV%	4.45% 3.44% %Effect 0.0%
10 15 Angular (Cor C-%	Control Type	5 5 med Sum Count 5 5	0.944 0.954 mary Mean 1.462 1.447	0.9128 0.9254 95% LCL 1.439	0.9752 0.9826 <b>95% UCL</b> 1.485	0.94 0.96 <b>Median</b> 1.471 1.471	0.91 0.93 <b>Min</b> 1.429 1.397	0.98 0.98 Max 1.471 1.471	0.01122 0.0103 <b>Std Err</b> 0.008346 0.01505	2.66% 2.41% CV% 1.28% 2.33%	4.45% 3.44% %Effect 0.0% 1.01%
10 15 <b>Angular (Cor.</b> C-% 0 2.5	Control Type	5 5 med Sum Count 5	0.944 0.954 mary Mean 1.462	0.9128 0.9254 <b>95% LCL</b> 1.439 1.406	0.9752 0.9826 95% UCL 1.485 1.489 1.462	0.94 0.96 <b>Median</b> 1.471 1.471 1.429	0.91 0.93 Min 1.429 1.397 1.397	0.98 0.98 Max 1.471 1.471 1.471	0.01122 0.0103 <b>Std Err</b> 0.008346 0.01505 0.01362	2.66% 2.41% CV% 1.28% 2.33% 2.14%	4.45% 3.44% %Effect 0.0% 1.01% 2.59%
10 15 <b>Angular (Cor.</b> <b>C-%</b> 0 2.5 5	Control Type	5 5 med Sum Count 5 5 5	0.944 0.954 mary Mean 1.462 1.447 1.424	0.9128 0.9254 <b>95% LCL</b> 1.439 1.406 1.387	0.9752 0.9826 <b>95% UCL</b> 1.485 1.489	0.94 0.96 <b>Median</b> 1.471 1.471	0.91 0.93 <b>Min</b> 1.429 1.397	0.98 0.98 Max 1.471 1.471	0.01122 0.0103 <b>Std Err</b> 0.008346 0.01505	2.66% 2.41% CV% 1.28% 2.33%	4.45% 3.44% %Effect 0.0% 1.01%

Analyst: QA: 124 11/3/17

Report Date:

27 Oct-17 16:50 (p 1 of 1)

Test Code: 1710-5178 09-2205-6048/1710-S128

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 3\ 30 Oct-17 End Date: 3\ 90 Oct-17 Sample Date: 30 Oct-17

Species: Strongylocentrotus purpuratus

Sample Code: 17- 1/28

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

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

C-%			D	40	ALE COL COL	
	Code	Rep			# Fertilized	Notes
			31	100	98	11/1/17
			32	/00	99	
			33	/00	94	
			34	100	93	
			35	100	99	
			36	100	99	
			37	1,00	99	
			38	(00)	97	
			39	/00	99	
			40	100	97	
			41	100	49	
			42	100	98	
			43	100	95	
			44	100	98 97	
			45	100	97	
			46	/00	93	
			47	100	91	
			48	100	98	
			49	100	96	
			50	100	93	
			51	100	98	
			52	100	99	
			53	100	97	
			54	100	99/	
			55	100	98	
			56	160	4,3	
			57	160	9,8	
			58	100	99	
			59	100	98	
			60	100	97	
			61	100	94	
			62	100	99	
			63	100	98	
			64	100	99	
			65	100	160	

( EG Q18 11/1/17

Report Date:

27 Oct-17 16:50 (p 1 of 1) 09-2205-6048/1710-S128

Test Code:

17-1128

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

Start Date: (3) 30 Oct-17

End Date: (3) 30 Oct-17

Sample Date: 30 Oct-17 Species: Strongylocentrotus purpuratus Sample Code: Protocol: EPA/600/R-95/136 (1995) Sample Source: IDE Americas, Inc. Material: Facility Effluent Sample Station: M-001 Unadjusted

C-%		Rep	Pos		# Fertilized	Notes
<u>٠-%</u> 0	HS	Rep 1	55	# Counted	# rerunzea	NOTES
0	HS			,		
		2	35	100	99	BO 10/31/17
0	HS	3	52			
0	HS	4	65			
0	HS	5	54			
0	LC	1	58			
0	LC	2	62	100	100	BO 10/31/17+
0	LC	3	51			
0	LC	4	32			
0	LC	5	36			
2.5		1	39	100	99	BO 10/31/17
2.5		2	37			
2.5		3	64			
2.5		4	42			
2.5		5	56			
5		1	57	100	98	BO 10/31/17
5		2	41			
5		3	45			
5		4	60			
5		5	48			3000
6.06		1	44			
6.06		2	49			
6.06		3	46	100	97	BO 10/31/17
6.06		4	53	.00	, .	50 10/3///
6.06		5	40			
10		1	47			
10		2	61	100	93	Bo 10/31/17
10		3	31	100	12	100 1013111
10		4	33			
10		5	43			
15		1	59			
15	-	2	63			. 100 %
15		3	34	100	97	Po 10/2:117
15		4	38	100	) T	BO 10/31/17
15		5	50			
10		, , , , , , , , , , , , , , , , , , ,		8.611		

(A)(C6/b)(0/31/17)

QC: (G

#### Marine Chronic Bioassay

**Water Quality Measurements** 

Client :	IDE	Test Species: S. purpuratus
Sample ID:	M-001 (unadjusted)	Start Date/Time: 10/31/20/7 1359

Sample Log No.: 17- 1128 End Date/Time: 10/31/2017 14/39

> Analyst: Initial Readings DO Concentration рΗ Salinity Temperature % (mg/L) (units) (ppt) (°C) 8.02 Lab Control 33.9 4.3 8.01 37.0 **High Salinity** Control 8.01 2.5 8.00 16.0 5.0 8.00 6.06 10 7.99 7.98 8.4 15

Comments:			Milliologic material proposation of the state of the stat		V-68000-16000-1000-1000-1000-1000-1000-10
QC Check:	EG 11/1/17	-	Fin	al Review: AC 11/3/17	

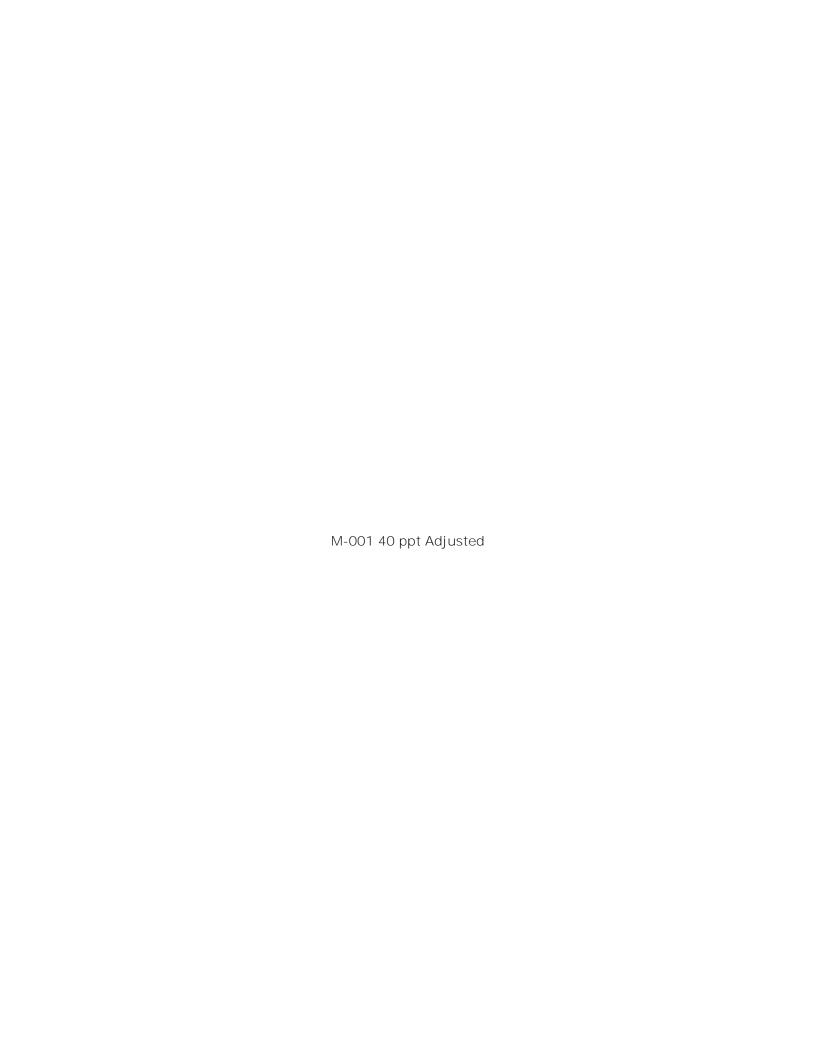
Nautilus Environmental. 4340 Vandever Avenue. San Diego, CA 92120.

# Marine Chronic Bioassay

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

# **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.:	1DE M-001 UN 1710-5128	adjusted		End Date/Ti Spec	ime: 10/31/12017/ 1359 ime: 10/31/2017/ 1439 cies: S. purpuratus
Tech initials: Injection Time:	<u>CG</u> (310			Animal Sou Date Collec	rce: <u>Pt· Lbma</u> ted: <u>tv   10   13</u>
Sperm Absorbance at 40	0 nm: 1,001	(target range of	0.8 - 1.0 for densi	ty of 4x10 <sup>6</sup> sperm/n	nl)
Eggs Counted:		Mean: <u>93</u> X (target counts of 80 eggs Rafter slide for a final den	per vertical pass or		
Initial density: Final density:	4000 eggs.	/ml - 1.0 par	tion factor t egg stock ts seawater	egg stock seawater	100 ml 16-3 ml
Prepare the embryo stock stock (1 part) and 125 ml	k according to the ca of dilution water (1.2	alculated dilution factor. 25 parts).	For example, if th	e dilution factor is 2	2.25, use 100 ml of existing
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 50 0.0	1600:1 1200:1 30 10 20	Sperm: Egg Ra           800:1         400           20         10           30         40	0:1 0 200:1 5.0	100:1     50:1       2.5     1.25       47.5     48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 13.25 13.60	Rangefinder Rati	0: <u>Fert.</u> <u>Ub</u> <u>94</u> 95	Unfert. 34 6,6 2	
NOTE: Choose a sperm- this range, choose the rat health, stage of reproduct	tio closest to 90 perc	ent unless professional	en 80 and 90 pero judgment dictates	cent. If more than of consideration of o	one concentration is within ther factors (e.g., organism
<u>Definitive Test</u>		Sperm:Egg Ratio	Used: 105:1		
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1359 1419 1439	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 97 98 0	Unfert. 3 2 100 100	Δ,
Comments:					·
QC Check:	EG 11/1/17			Final Revie	ew. A (1/3/17



# **CETIS Summary Report**

Report Date:

01 Nov-17 12:17 (p 1 of 1)

Test Code:

1710-S129 | 03-9733-9800

											,	
Echi noid Sper	rm Cell Fertiliza	tion Te	est 15C							Nautilus	Environr	nental (CA
Batc h ID: Start Date: Endi ng Date: Durætion:	12-2186-0218 31 Oct-17 13:5 31 Oct-17 14:3 40m		Test Type: Protocol: Species: Source:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma	, ,	tus		Analyst: Diluent: Brine: Age:		ural Seawate Applicable	ег	
=	13-1699-9192 30 Oct-17 08:0 30 Oct-17 12:5 30h (3.4 °C)		Code: Material: Source: Station:	17-1128 Facility Effluen IDE Americas, M-001 (40 ppt	Inc.			Client: Project:	IDE Carl	sbad Desal	Plant	
Com parison S	Summary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Met	:hod			
18-1389-4617	Fertilization Ra	te	15	>15	NA	2.6%	4 6.66	67 Dur	nett M	lultiple Com	parison Te	st
Point Estimate	e Summary											,
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Met	:hod			
05-3581-8471	Fertilization Ra	te	EC25 EC50		N/A N/A	N/A N/A	<6.6 <6.6		ear Inte	erpolation (IC	CPIN)	
Test Acceptab	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Ove	erlap	Decision		
05-3581-8471 18-1389-4617 18-1389-4617	Fertilization Ra Fertilization Ra Fertilization Ra	te		ol Resp ol Resp )	0.98 0.98 0.02597	0.7 - NL 0.7 - NL NL - 0.25		Yes Yes No		Passes Ad Passes Ad Passes Ad	ceptability	Criteria
Fertifization R	ate Summary										3	
C-%	Control Type	Cour	nt Mean	95% LCL	95% UCL	Min	Max	c Std	Err	Std Dev	CV%	%Effect
	Lab Control	5	0.98	0.9648	0.9952	0.96	0.99	0.00	5478	0.01225	1.25%	0.0%
2.5		5	0.98	0.9604	0.9996	0.96	1		07071	0.01581	1.61%	0.0%
5		5	0.988	0.9744	1	0.98	1		04899	0.01095	1.11%	-0.82%
6.06		5 5	0.99	0.9812	0.9988	0.98	1		03163	0.007072	0.71%	-1.02%
10 15		5	0.974 0.982	0.9552 0.9616	0.9928 1	0.96 0.96	1 1		06782	0.01517	1.56%	0.61%
		3	0.962	0.0010		0.90	I	0.00	)7349	0.01643	1.67%	-0.2%
Fertilization R												
	Control Type	Rep '			Rep 4	Rep 5			***************************************			
	Lab Control	0.99	0.99	0.96	0.98	0.98						
2.5		0.98	1	0.99	0.96	0.97						

5

10

15

6.06

1

1

0.99

0.97

0.98

0.99

0.97

0.99

0.98

0.97

1

1

0.98

0.98

0.97

0.99

1

0.99

0.96

0.96

Report Date: Test Code: 01 Nov-17 12:17 (p 1 of 2)

1710-S129 | 03-9733-9800

							Test			0-S129   0	
Echinoid Sp	erm Cell Fertiliza	ation Test	15C						Nautilus	s Environ	mental (CA
Analysis ID: Analyzed:	18-1389-4617 01 Nov-17 12:		ndpoint: Fer	tilization Ra		tments		IS Version		.8.7	
Data Transfe		Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	**************************************	NA	C > T	NA	NA NA		2.6%	15	>15	NA	6.667
Dunnett Mul	tiple Compariso	n Toet						· · ·			0.007
Control	vs C-%	ii iest	Test Stat	Critical	MCD DE	. D. Value	D. Toma	Danislass	( PD/)		
Lab Control	2.5			Critical 2.362		P-Value	P-Type	Decision	· · · · · · · · · · · · · · · · · · ·	****	
Lab Control	2.5 5		-0.1091		0.078 8	0.8636	CDF	•	ificant Effect		
			-0.9705	2.362	0.078 8	0.9819	CDF	_	ificant Effect		
	6.06		-1.173	2.362	0.078 8	0.9898	CDF	Ü	ificant Effect		
	10		0.5354	2.362	0.078 8	0.6314	CDF	J	ificant Effect		
	15		-0.3626	2.362	0.078 8	0.9185	CDF	Non-Sign	ificant Effect		
ANOVA Tabl	е										
Source	Sum Squ	ıares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.010983	75	0.0021967	<b>'</b> 49	5	0.8107	0.5536	Non-Sign	ificant Effect		
Error	0.065033	02	0.0027097	'09	24						
Total	0.076016	76		100	29	_					
Distribution	al Tests					To the second of					
Attribute	Test			Test Stat	Critical	P-Value	Decision(	α:1%)			
Variances	Bartlett E	quality of	Variance	2.067		0.8397					
Variances Distribution		quality of Wilk W No			15.09 0.9031		Equal Var Normal Di	iances			
Distribution				2.067	15.09	0.8397	Equal Var	iances			
Distribution Fertilization	Shapiro-			2.067	15.09	0.8397	Equal Var	iances	Std Err	CV%	%Effect
Distribution Fertilization C-%	Shapiro-	Wilk W No	rmality	2.067 0.9635	15.09 0.9031	0.8397 0.3783	Equal Var Normal Di	iances stribution	Std Err 0.005478	C <b>V</b> %	
Distribution Fertilization C-% 0	Shapiro- Rate Summary Control Type	Wilk W No	rmality  Mean	2.067 0.9635 95% LCL	15.09 0.9031 <b>95% UCL</b>	0.8397 0.3783 Median	Equal Var Normal Di	iances stribution			0.0%
Distribution Fertilization C-% 0 2.5	Shapiro- Rate Summary Control Type	Wilk W No  Count  5	Mean 0.98	2.067 0.9635 <b>95% LCL</b> 0.9648	15.09 0.9031 <b>95% UCL</b> 0.9952	0.8397 0.3783 <b>Median</b> 0.98	Equal Var Normal Di Min 0.96	iances stribution Max 0.99	0.005478 0.007071	1.25% 1.61%	0.0%
Fertilization C-% 0 2.5	Shapiro- Rate Summary Control Type	Count 5 5	Mean 0.98 0.98	2.067 0.9635 95% LCL 0.9648 0.9604	15.09 0.9031 <b>95% UCL</b> 0.9952 0.9996	0.8397 0.3783 <b>Median</b> 0.98 0.98	Equal Var Normal Di Min 0.96 0.96	iances stribution Max 0.99	0.005478 0.007071 0.004899	1.25% 1.61% 1.11%	0.0% 0.0% -0.82%
Fertilization C-% 0 2.5 5 6.06	Shapiro- Rate Summary Control Type	Count 5 5 5	Mean 0.98 0.98 0.988	2.067 0.9635 <b>95% LCL</b> 0.9648 0.9604 0.9744	15.09 0.9031 <b>95% UCL</b> 0.9952 0.9996 1	0.8397 0.3783 Median 0.98 0.98 0.98 0.99	Min 0.96 0.96 0.98 0.98	Max 0.99 1 1 1	0.005478 0.007071 0.004899 0.003163	1.25% 1.61% 1.11% 0.71%	0.0% 0.0% -0.82% -1.02%
Fertilization C-% 0 2.5 5 6.06	Shapiro- Rate Summary Control Type	Count 5 5 5 5	Mean 0.98 0.98 0.988 0.988 0.99	2.067 0.9635 <b>95% LCL</b> 0.9648 0.9604 0.9744 0.9812	95% UCL 0.9952 0.9996 1 0.9988	0.8397 0.3783 <b>Median</b> 0.98 0.98 0.98	Min 0.96 0.96 0.98	iances stribution  Max  0.99  1	0.005478 0.007071 0.004899	1.25% 1.61% 1.11%	0.0% 0.0% -0.82%
Fertilization C-% 0 2.5 5 6.06 10	Shapiro- Rate Summary Control Type	Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.98 0.98 0.98 0.988 0.99 0.974 0.982	2.067 0.9635 <b>95% LCL</b> 0.9648 0.9604 0.9744 0.9812 0.9552	95% UCL 0.9952 0.9986 1 0.9988 0.9928	0.8397 0.3783 Median 0.98 0.98 0.98 0.99 0.97	Min 0.96 0.98 0.98 0.96	Max 0.99 1 1 1 1	0.005478 0.007071 0.004899 0.003163 0.006782	1.25% 1.61% 1.11% 0.71% 1.56%	0.0% -0.82% -1.02% 0.61%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.98 0.98 0.98 0.988 0.99 0.974 0.982	2.067 0.9635 <b>95% LCL</b> 0.9648 0.9604 0.9744 0.9812 0.9552	95% UCL 0.9952 0.9986 1 0.9988 0.9928	0.8397 0.3783 Median 0.98 0.98 0.98 0.99 0.97	Min 0.96 0.98 0.98 0.96	Max 0.99 1 1 1 1	0.005478 0.007071 0.004899 0.003163 0.006782	1.25% 1.61% 1.11% 0.71% 1.56%	0.0% 0.0% -0.82% -1.02% 0.61%
Distribution  Fertilization  C-%  0  2.5  5  6.06  10  15  Angular (Cor	Shapiro- Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.98 0.98 0.98 0.989 0.974 0.982	2.067 0.9635 95% LCL 0.9648 0.9604 0.9744 0.9812 0.9552 0.9616	95% UCL 0.9952 0.9996 1 0.9988 0.9928 1	0.8397 0.3783 Median 0.98 0.98 0.98 0.99 0.97 0.99	Min 0.96 0.96 0.98 0.98 0.96 0.96	Max 0.99 1 1 1 1 1	0.005478 0.007071 0.004899 0.003163 0.006782 0.007349	1.25% 1.61% 1.11% 0.71% 1.56% 1.67%	0.0% 0.0% -0.82% -1.02% 0.61% -0.2%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor	Shapiro- Rate Summary Control Type Lab Control  rected) Transfor Control Type	Count 5 5 5 5 5 5 cmed Sum	Mean 0.98 0.98 0.98 0.99 0.974 0.982  mary Mean	2.067 0.9635 95% LCL 0.9648 0.9604 0.9744 0.9812 0.9552 0.9616	95% UCL 0.9952 0.9996 1 0.9988 0.9928 1	0.8397 0.3783 Median 0.98 0.98 0.98 0.99 0.97 0.99	Min 0.96 0.96 0.98 0.98 0.96 0.96	Max 0.99 1 1 1 1 1 Max	0.005478 0.007071 0.004899 0.003163 0.006782 0.007349	1.25% 1.61% 1.11% 0.71% 1.56% 1.67%	0.0% 0.0% -0.82% -1.02% 0.61% -0.2%
Distribution  Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Shapiro- Rate Summary Control Type Lab Control  rected) Transfor Control Type	Count 5 5 5 5 5 5 Count Count Count 5	Mean 0.98 0.98 0.98 0.99 0.974 0.982 mary Mean 1.434	2.067 0.9635 95% LCL 0.9648 0.9604 0.9744 0.9812 0.9552 0.9616 95% LCL 1.382	95% UCL 0.9952 0.9996 1 0.9988 0.9928 1 95% UCL 1.485	0.8397 0.3783 Median 0.98 0.98 0.98 0.99 0.97 0.99 Median 1.429	Min 0.96 0.98 0.98 0.96 0.96 0.96 0.96 1.369	Max 0.99 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0.005478 0.007071 0.004899 0.003163 0.006782 0.007349 Std Err 0.01858	1.25% 1.61% 1.11% 0.71% 1.56% 1.67% CV% 2.9%	0.0% 0.0% -0.82% -1.02% 0.61% -0.2% %Effect 0.0%
Fertilization C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	Shapiro- Rate Summary Control Type Lab Control  rected) Transfor Control Type	Count 5 5 5 5 5 Count Count Count 5 5 5 5 5 5 5 5 5 5 5 6 6 6 7 7 8 8 8 8 8 8 8 8 8 8 8 8 8 8 8	Mean 0.98 0.98 0.98 0.99 0.974 0.982  mary Mean 1.434 1.437	2.067 0.9635 95% LCL 0.9648 0.9604 0.9744 0.9812 0.9552 0.9616 95% LCL 1.382 1.363	95% UCL 0.9952 0.9996 1 0.9988 0.9928 1 95% UCL 1.485 1.512	0.8397 0.3783 Median 0.98 0.98 0.99 0.97 0.99 Median 1.429 1.429	Min 0.96 0.96 0.98 0.98 0.96 0.96 0.96 1.369	Max 0.99 1 1 1 1 1 1 1 1 1.471 1.521 1.521	0.005478 0.007071 0.004899 0.003163 0.006782 0.007349 Std Err 0.01858 0.02683 0.02251	1.25% 1.61% 1.11% 0.71% 1.56% 1.67% CV% 2.9% 4.18% 3.43%	0.0% 0.0% -0.82% -1.02% 0.61% -0.2% %Effect 0.0% -0.25% -2.23%
Fertilization C-% 0 2.5 5 6.06 10 15	Shapiro- Rate Summary Control Type Lab Control  rected) Transfor Control Type	Count 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5 5	Mean 0.98 0.98 0.98 0.99 0.974 0.982  mary  Mean 1.434 1.437 1.466	2.067 0.9635 95% LCL 0.9648 0.9604 0.9744 0.9812 0.9552 0.9616 95% LCL 1.382 1.363 1.403	95% UCL 0.9952 0.9996 1 0.9988 0.9928 1 1 95% UCL 1.485 1.512 1.528	0.8397 0.3783 Median 0.98 0.98 0.99 0.97 0.99 Median 1.429 1.429 1.429	Min 0.96 0.96 0.98 0.98 0.96 0.96 0.96 1.369 1.429	Max 0.99 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1 1	0.005478 0.007071 0.004899 0.003163 0.006782 0.007349 Std Err 0.01858 0.02683	1.25% 1.61% 1.11% 0.71% 1.56% 1.67% CV% 2.9% 4.18%	0.0% 0.0% -0.82% -1.02% 0.61% -0.2% %Effect 0.0% -0.25%

Analyst: QA: EC, 11/3/17

C-%

Report Date: Test Code:

01 Nov-17 12:17 (p 2 of 2) 1710-S129 | 03-9733-9800

1.0 1.5 2.0

Rankits

Echi noid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) 18-1389-4617 Analysis ID: Endpoint: Fertilization Rate CETIS Version: CETISv1.8.7 Analyzed: 01 Nov-17 12:17 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 0.12 0.9 0.10 8.0 0.08 0.7 0.06 0.6 0,04 0.02 0.4 0.00 0.3 -0.02 0.2 -0.04 0.1 0.0 -0.08 2.5 0 LC 6.06 10 15

Report Date: Test Code:

01 Nov-17 12:17 (p 1 of 1)

1710-S129 | 03-9733-9800

Echinoid Sperm Cell Fertilization Test 15C

N/A

N/A

Nautilus Environmental (CA)

Analysis ID: 05-3581-8471 Analyzed:

EC50

>15

01 Nov-17 12:17

Endpoint: Fertilization Rate

<6.667

NA

Analysis: Linear Interpolation (ICPIN)

**CETIS Version:** Official Results:

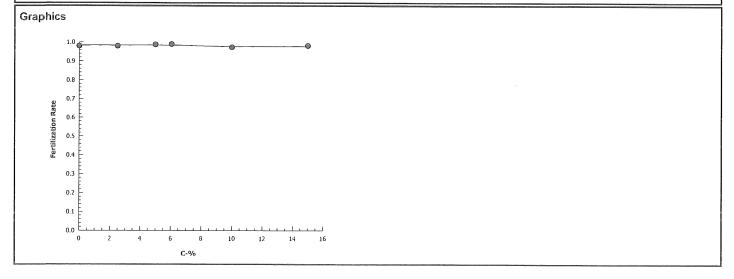
CETISv1.8.7

Yes	

Linear	Interpola	tion Options						
X Trans	sform	Y Transform	Seed	d	Resamples	Exp 95% CL	Method	
Linear		Linear	1150	329	1000	Yes	Two-Point Interpolation	
Point E	stimates							E CONTRACTOR CONTRACTO
Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL		
EC25	>15	N/A	N/A	<6.667	' NA	NA		

NΑ

Fertiliza	tion 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	0.98	0.96	0.99	0.005478	0.01225	1.25%	0.0%	490	500
2.5		5	0.98	0.96	1	0.007071	0.01581	1.61%	0.0%	490	500
5		5	0.988	0.98	1	0.004899	0.01095	1.11%	-0.82%	494	500
6.06		5	0.99	0.98	1	0.003163	0.007072	0.71%	-1.02%	495	500
10		5	0.974	0.96	1	0.006782	0.01517	1.56%	0.61%	487	500
15		5	0.982	0.96	1	0.007349	0.01643	1.67%	-0.2%	491	500



755

Report Date: Test Code: 01 Nov-17 12:18 (p 1 of 1) 1710-S129 | 03-9733-9800

			72							0 0 120   0	00 01 00 000
Echinoid Sp	erm Cell Fertiliz	ation Tes	t 15C						Nautilus	s Environ	mental (CA
Analysis ID:	21-2235-9885	E	ndpoint: F	ertilization Ra	te		CET	'IS Version	: CETISv1	.8.7	
Analyzed:	01 Nov-17 12:	:17 🚜	nalysis: P	arametric Bio	equivalence	-Two Samp	le <b>Off</b> ic	cial Results	s: Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C*b < T	NA	NA	0.75	1.95%	15	>15	NA	6.667
TST-Welch's	t Test										
Control	vs C-%		Test Sta	at Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5*		11.97	1.943	0.059 6	<0.0001	CDF		ificant Effect		
	5*		14.75	1.943	0.051 6	<0.0001	CDF	_	ificant Effect		
	6.06*		19.7	1.895	0.038 7	<0.0001	CDF		ificant Effect		
	10*		11.31	1.943	0.059 6	<0.0001	CDF	Ü	ificant Effect		
ļ	15*		12.02	2.015	0.062 5	<0.0001	CDF	_	ificant Effect		
41101/4 7 11				2.010	0.002	10.0001	ODI	14011-Olgii	meant Enect		
ANOVA Tabl											
Source	Sum Squ		Mean S		DF	F Stat	P-Value	Decision			
Between	0.010983		0.00219		5	0.8107	0.5536	Non-Sign	ificant Effect		
Error	0.065033		0.00270	9709	24	_					
Total	0.076016 	76			29			****			
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision	(α:1%)			
Variances	Bartlett B	Equality of	Variance	2.067	15.09	0.8397	Equal Var	iances		Notice that the second	
Distribution	Shapiro-	Wilk W N	ormality	0.9635	0.9031	0.3783	Normal D	istribution	•		
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.98	0.9648	0.9952	0.98	0.96	0.99	0.005478	1.25%	0.0%
2.5		5	0.98	0.9604	0.9996	0.98	0.96	1	0.007071	1.61%	0.0%
5		5	0.988	0.9744	1	0.98	0.98	1	0.004899	1.11%	-0.82%
6.06		5	0.99	0.9812	0.9988	0.99	0.98	1	0.003163	0.71%	-1.02%
10		5	0.974	0.9552	0.9928	0.97	0.96	1	0.006782	1.56%	0.61%
15		5	0.982	0.9616	1	0.99	0.96	1	0.007349	1.67%	-0.2%
Angular (Cor	rected) Transfor	med Sun	nmary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.434	1.382	1.485	1.429	1.369	1.471	0.01858	2.9%	0.0%
				4.000	1.512	1.429	1.369	1.521	0.02683	4.18%	-0.25%
2.5		5	1.437	1.363	1.512	1.429	1.309	1.521	0.02003	4.1070	-0.2376
<ul><li>2.5</li><li>5</li></ul>		5 5	1.437 1.466	1.363 1.403	1.512	1.429	1.429	1.521	0.02663	3.43%	
											-2.23% -2.69%
5		5	1.466	1.403	1.528	1.429	1.429	1.521	0.02251	3.43%	-2.23%

Analyst: QA: EL 113/17

Report Date:

27 Oct-17 16:51 (p 1 of 1) 03-9733-9800/1710-S129

Test Code:

Nautilus Environmental (CA)

Echimoid Sperm Cell Fertilization Test 15C

Start Date: 31.20 Oct-17 End Date: 31.20 Oct-17

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

Material: Brine Effluent Facility FCCL 1

Sample Code: 17-1128 Sample Source: IDE Americas, Inc.

C-%	Code	Rep	Pos	# Counted	# Fertilized	rent Facility Effluent Sample Station: M-001 (40 ppt adj)  Notes
			66	100	98	11/1/17
			67	100	48	
			68	100	100	
			69	100	100	
			70	100	99	
			71	100	99	
			72	100	94	
			73	100	99	
			74	100	96	
			75	1,00	då	
			76	100	96	
			77	100	36	
			78	100	98	
			79	100	97	
			80	100	98	
	1		81	100	/00	
			82	//00	700	
			83	100	-33-	
			84	100	10	
			85	100	97	
			86	100	99	
		-	87 88	100	- 10	
			89	100	(0)	
			90	100	97	
			91	6	adt	
			92	100	-a\}-	
			93	100	10	
			94	100	990	
			95	00/	100	

(B) EG Q18 11/1/17

Report Date:

27 Oct-17 16:51 (p 1 of 1)

Test Code:

03-9733-9800/1710-S129

#### Echimoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: @730 Oct-17	Species:	Strongylocentrotus purpuratus	Sample Code:	17~1128
End Date: 🔗 3/30 Oct-17		EPA/600/R-95/136 (1995)	Sample Source:	IDE Americas, Inc.
Sample Date: 30 Oct-17	Material:	Brine Effluent Facility Effluent	Sample Station:	M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	86			
0	LC	2	70	100	100	BO (0/3/119
0	LC	3	77	_		(3),
0	LC	4	87			
0	LC	5	67			
2.5		1	84			
2.5		2	81	[00]	99	BO (0/3//17
2.5		3	94	,		
2.5		4	76			
2.5		5	79			
5		1	68			
5		2	66	100	98	BO 10/31/17
5		3	88			
5		4	80			
5		5	93			
6.06		1	91			
6.06		2	73	100	(00)	BO 10/31/17
6.06		3	82			
6.06		4	78			
6.06		5	72			
10		1	69	(00	94	BO 10/31/17
10		2	83			
10		3	90			
10		4	89			
10		5	92			
15		1	85			
15		2	75			
15	-	3	95	100	97	Bo 10131/17
15		4	71			
15		5	74			

6. CG (BCG 611810/3/17

# Marine Chronic Bioassay

Water Quality Measurements

Client :	IDE	Test Species:	S. purpuratus	
Sample ID:	M-001 (40 ppt adjusted)	Start Date/Time:	10/31/2017	1359
Sample Log No.:	17- 1128	End Date/Time:	10/31/2017	1439

			Analy St.	1 CG
			eadings	
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.03	33.9	15.1
2.5	8.4	4.03	34.2	15.1
5.0	8.4	8.03	34.3	15.1
6.06	8.4	8.04	34.4	15-1
10	8.5	8.03	34.6	15.0
15	8.5	8.03	34.9	15.0

Comments:		
QC Check:	EG 11/17	Final Review:

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

#### Magine Chronic Bioassay

#### **Brine Dilution Worksheet**

Profect:	IDE
----------	-----

Analyst: CC1\_\_\_\_\_

Sample ID:

M-001 (40 ppt adjusted)

Test Date: 10/31/2017

Test No:

1710-5129

**Test Type:** Urchin Fertilization

Sali nity of Effluent

60.6

Sali nity of Seawater

33.5

Date of Brine used: NA

40.0

Alk. of 40 ppt Adj. Sample: mg/L as CaCO3

Effluent

Brine Control

Salinity Adjustment Factor: (TS

- SE)/(SB - TS) =

**Target Salinity** 

3.17

-6.15

TS = target salinity

SE = salinity of effluent

SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)
100	100	3.17	316.9	417

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: EG 11/17

Final Review: ACII3/17

# Marine Chronic Bioassay

# **Echinoderm Sperm-Cell Fertilization Worksheet**

Client: Sample ID: Test No.:	DE M-001 (40, 1710-5129	opt adjusted)	- -	Enc		ולן פּצ'ן (3i S. purpuratu	1439 1s
Tech initials:	CG				mal Source: _ te Collected: _	10/10/12	4
Injection Time:	1310						
Sperm Absorbance at 40	00 nm: 1,001	(target range of 0	0.8 - 1.0 for den	sity of 4x10 <sup>6</sup>	sperm/ml)		
Eggs Counted:	93 M	ean: <u>93</u> X 50	0 = 465	C_eggs/m	ıl		
	$\frac{51}{90}$ Ra	rget counts of 80 eggs p fter slide for a final dens	•	•			
	91	0					
Initial density: Final density:	4000 eggs/m		egg stock	egg sto seawat			
Prepare the embryo stoc stock (1 part) and 125 m	k according to the calc of dilution water (1.25	ulated dilution factor. F		the dilution f	actor is 2.25,	use 100 ml	of existing
	•		Sperm:Egg F	<u>Ratio</u>			
Rangefinder Test: ml Sperm Stock ml Seawater	50	00:1     1200:1       40     30       10     20	800:1 <u>4</u> 20 30	00:1 10 40	5.0	2.5	<b>50:1</b> 1.25 48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 13.25 13.40 13.50	Rangefinder Ratio	2: <u>Fert.</u> <u>Ub</u> <u>94</u> 9	Unfert. 34 4 6,6 2			
NOTE: Choose a sperm- this range, choose the ra health, stage of reproduc	tio closest to 90 percer	nt unless professional j	en 80 and 90 pe udgment dictate	ercent. If mo es considera	re than one contion of other f	oncentration actors (e.g.	n is within , organism
Definitive Test		Sperm:Egg Ratio	Used: 100:-				
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1359 1419 1439	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 97 90 0	Unfert. 3 2 100 100			N.
Comments:		The state of the s					
QC Check:	EG 11/17			Fi	nal Review:	AC ula	hin
Nautilus Environmental. 4340		 Diego, CA 92120.		1 1	Harriew	- 1112	<del> '' </del>

Appendix B

Sample Receipt Information

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Client:	IDE
Sample ID:	M-001
Test ID No(s).:	1710-5128 to 5130

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1128			
Sample Collection Date & Time:	10/30/17 0900			
Sample Receipt Date & Time:	10/30/17 1254			
Number of Containers & Container Type:	1,4L aubi			
Approx. Total Volume Received (L):	~ 4			
Check-in Temperature (°C)	3.4			
Temperature OK? <sup>1</sup>	Ŷ N	ΥN	Y N	Y N
DO (mg/L)	7.3			
pH (units)	7.82			
Conductivity (µS/cm)	Quarters.			
Salinity (ppt)	60.60			
Alkalinity (mg/L) <sup>2</sup>	163			
Hardness (mg/L) <sup>2, 3</sup>	, married			
Total Chlorine (mg/L)	0.02			
Technician Initials	CH	313 30 30 30 30 30 30 30 30 30 30 30 30 30		

Test Performed:	Urchin Ferfilization	୦୮ମ ontrol/Dilution Water:	8:2 / Lab SW / Lab /	ART Other:
			Hardness or Salinity: 34	
	Additional Control? Y N =	Migh Solomby Alkalini	ty: NA Hardnes	s or <u>Salinity</u> : <u>37-0 ppt</u>
Test Performed:	С	ontrol/Dilution Water:	8:2 / Lab SW / Lab /	ART Other:
		Alkalinity:	Hardness or Salinity:	
	Additional Control?Y N =	Alkalini	ty: Hardnes	s or Salinity:
Test Performed:	c	ontrol/Dilution Water:	8:2 / Lab SW / Lab	ART Other:
		Alkalinity:	Hardness or Salinity:	
	Additional Control? Y N =	Alkalini	ty: Hardnes	s or Salinity:
Notes:	<sup>1</sup> Temperature of sample should b	e 0-6°C, if received more	than 24 hours past collect	on time.
	<sup>2</sup> mg/L as CaCO3, <sup>3</sup> Measured for	freshwater samples only	NA = Not Applicable	
Additional Comments:	@ 50% dilution	performed.	to obtain me	asvænent.

#### Sample Check-In Information

No color, cla	·····		
COC Complete (Y/N)?	?		
A B C			
Filtration? Y (N)	)		
Filtration? Y (N) Pore Size:			
Organisms	or	— Debris	
Organisms	01	Deniis	
Salinity Adjustment?	(Ý) N		
Test: Wichin		ealu attil Targe	t ppt: 닉이
Test:	Source:	Targe	
Test:	Source:	Targe	t ppt:
pH Adjustment? Y (	N)		
	A	В	С
Initial pH:			
mount of HCI added:			
Final pH:			
Cl <sub>2</sub> Adjustment? Y	Ñ\		-
\	$\subseteq$ A	В	С
Initial Free Cl <sub>2</sub> :			
STS added:			
Final Free Cl <sub>2</sub> :			
	(A)		
Sample Aeration? Y	$\cup$	В	С
Initial D.O.	Α		
Duration & Rate			
Final D.O.			
	•		·
Subsamples for Addi	tional Chei	nistry Require	d? Y (N)
	r		$\sim$
Tech Initials A	В	_ c	
	00.0	neck: <u>EG</u>	11/1/12
	Final Re	1 1	11/1/12

Appendix C

Chain-of-Custody Form

WEEKLY



Market Control for the discount of the second secon	reditions to distinct the Executive Court, \$1,000, \$400 as a concept of a physicist.		1000 1000 1000 1000 1000 1000 1000 100	allo miles in the country of	The state of the s	trois services				Oth	er:			??? Days			
Market Control of the	Veekly Toxicity	Project M		Peter		Co	ntact Info	ormation	:	(760) 20	1-7777	**************************************	and the second s				
		operation at 48 MGD via ple collected to fulfill we							ANA	LYSES				NOTES:			
		ted to 40 ppt. Start: 10/2				Purple Urchin Chronic Fertilization		·									
	_	nic Fe															
	Yes=Y No=N Acid=A Base=B																
Drink	king Water=DW Sea	awater=SW Soil=S		Pres		chin (											
Sample ID	Date	Time	Sample	Preservative	Container	urple Un											
	1.0 (0.0 - 0.1 - 1.0		Type	0	Туре	AND THE PARTY OF T		Maria ya ya marakarin da		the state of	S. College (1. de aproximato) y		Since Date of the later of the				
M-001 (17- 3206)	10/29-30/17	08:00-08:00	SW	N	4L CUBIE	X								TDS - 56.57 ppt, EC - 79.65 mS/cm			
					4												
Relinquished By:		Date:	Time:		Received By:				Date:	Time:			Samp	le Condition Upon Receipt:			
Wayn		10/30/17	1200		Ž			10/-	Zefry	DE	X	Iced		Ambient or0C			
	de la companya de la	10/30/19 /	2.5		JAN	~	— į	0/30/	2017 1	254	X	Iced		Ambient or <u>3.4</u> °C			

CDP laoratory:\_

Nautilus:\_\_\_x\_

Entahlpy Laboratory:\_\_\_\_

WECK Laboratory:\_\_\_

Turn Around Time

3 Days:\_\_\_\_ 5 Days:\_\_\_\_

Normal:\_\_\_x\_

RUSH (24 hr):\_\_\_\_

Nautilus DD: 17-1128

# Appendix D

Reference Toxicant Test Data and Statistical Analyses

# **CETIS Summary Report**

Report Date: Test Code: 01 Nov-17 10:24 (p 1 of 1) 171031sprt | 06-4227-6723

	· · · · · · · · · · · · · · · · · · ·						-	1621.00			oropit   oo	
Echinoid Sper	m Cell Fertilization	on Test 15C								Nautilus	Environme	ental (CA)
Batch ID: Start Date: Ending Date: Duration:	31 Oct-17 13:59			EPA/600/R-95/136 (1995) Strongylocentrotus purpuratus				Analys Diluent Brine: Age:	t: Natui	ral Seawater Applicable		
Sample ID: Sample Date: Receive Date: Sample Age:				1031sprt pper chloride ference Toxic pper Chloride	ant			Client: Projec	Interr t:	nal		
Comparison Summary												
Analysis ID 20-6412-1446	Endpoint Fertilization Rate		OEL )	LOEL 80	<b>TOEL</b> 56.57	PMSD 2.53%	TU		Method Dunnett M	ultiple Comp	arison Test	t
Point Estimate Summary												
Analysis ID	Endpoint	Le	evel	μg/L	95% LCL	95% UCL	TU		Method			211
08-8095-0809	Fertilization Rate	E	C50	81.36	78.72	84.09			Trimmed S	Spearman-K	ärber	
Test Acceptability												
Analysis ID	Endpoint	A	ttribute		Test Stat	TAC Limi	ts		Overlap	Decision		
08-8095-0809	Fertilization Rate	C	ontrol R	Resp	0.988	0.7 - NL			Yes	Passes Ac		
20011	Fertilization Rate		ontrol R	Resp	0.988	0.7 - NL			Yes		ceptability	
20-6412-1446	Fertilization Rate	P P	MSD		0.02534	NL - 0.25			No	Passes Ac	ceptability	
Fertilization F	Rate Summary											
C-µg/L	Control Type	Count M	lean	95% LCL	95% UCL	Min	Ma	Х	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5 0	.988	0.9776	0.9984	0.98	1		0.003742	0.008367	0.85%	0.0%
10		5 0	.97	0.9409	0.9991	0.95	1		0.01049	0.02345	2.42%	1.82%
20		_	.984	0.9772	0.9908	0.98	0.9		0.00245	0.005479	0.56%	0.4%
40			.97	0.9504	0.9896	0.95	0.9		0.007071	0.01581 0.06877	1.63% 13.08%	1.82% 46.76%
80			.526	0.4406	0.6114 0.03021	0.43 0	0.6		0.03076 0.008	0.00877	223.6%	99.19%
160		5 0	.008	0	0.03021	U	0.0	~	0.000	0.01700	220.070	
Fertilization F	Rate Detail											
C-µg/L	Control Type		Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	•	.99	0.98	0.99	0.98						
10				0.95	0.99	0.96						
20			.99	0.98	0.98	0.99						
40			.95	0.99	0.97	0.96						
80			).52	0.43	0.62	0.51						
160		0 0	)	0	0	0.04						

Report Date: Test Code: 01 Nov-17 10:24 (p 1 of 2) 171031sprt | 06-4227-6723

Echinoid Spe	erm Cell Fertilizat	ion Test 1	5C						Nautilus	Environme	ental (CA)
a	20-6412-1446	End	point: Fert	ilization Rate	)		CETIS	S Version:	CETISv1.8	3.7	
Analysis ID: Analyzed:	01 Nov-17 10:2				rol vs Treatr	nents	Offici	al Results:	Yes		
-				Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Data Transfor		Zeta	Alt Hyp	NA NA	NA		2.53%	40	80	56.57	
Angular (Corre	ected)	NA	C > T	IVA	INA		2.5570	10			
Dunnett Mult	iple Comparison	Test									
Control	vs C-μg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(			
Lab Control	10		1.46	2.362	0.087 8	0.2371	CDF	•	icant Effect		
	20		0.4998	2.362	0.087 8	0.6470	CDF	0	icant Effect		
	40		1.68	2.362	0.087 8	0.1704	CDF	Non-Signi	ficant Effect		
	80*		17.75	2.362	0.087 8	<0.0001	CDF	Significant	Effect		
	160*		37.64	2.362	0.087 8	<0.0001	CDF	Significan	Effect		
ANOVA Table	e										
Source	Sum Squ	ares	Mean Squ	ıare	DF	F Stat	P-Value	Decision(	α:5%)		
Between	7.812252		1.56245		5	462.5	<0.0001	Significan	t Effect		
	0.0810861	16	0.0033785	59	24						
Error Total	7.893338				29						
Distributiona				York Chak	Critical	P-Value	Decision	(a·1%)			
Attribute	Test			Test Stat 6.33	15.09	0.2754	Equal Var				
Variances	Bartlett E	iquality of \	Bartlett Equality of Variance				Equal val	ialices			
					0.0004	0.4055	NormalD	ictribution			
Distribution	Shapiro-\	Wilk W Nor		0.9424	0.9031	0.1055	Normal D	istribution			
	Shapiro-\ Rate Summary			0.9424	0.9031	0.1055	Normal D	istribution			
Fertilization	Rate Summary			0.9424 95% LCL	0.9031 95% UCL	0.1055 Median	Normal D  Min	istribution Max	Std Err	CV%	%Effec
Fertilization C-µg/L	Rate Summary Control Type	Wilk W Nor	mality Mean	95% LCL					<b>Std Err</b> 0.003742	CV% 0.85%	0.0%
Fertilization C-µg/L	Rate Summary	Wilk W Nor  Count  5	Mean 0.988	95% LCL 0.9776	95% UCL	Median	Min	Max			
Fertilization C-μg/L 0 10	Rate Summary Control Type	Wilk W Nor  Count  5 5	Mean 0.988 0.97	95% LCL 0.9776 0.9409	<b>95% UCL</b> 0.9984 0.9991	Median	Min 0.98	Max 1	0.003742	0.85%	0.0%
Fertilization C-µg/L 0 10 20	Rate Summary Control Type	Wilk W Nor  Count  5  5  5	Mean 0.988 0.97 0.984	95% LCL 0.9776 0.9409 0.9772	95% UCL 0.9984 0.9991 0.9908	Median 0.99 0.96 0.98	Min 0.98 0.95 0.98	<b>Max</b> 1	0.003742 0.01049	0.85% 2.42%	0.0% 1.82%
Fertilization C-µg/L 0 10 20 40	Rate Summary Control Type	Count  5 5 5 5 5	Mean 0.988 0.97 0.984 0.97	95% LCL 0.9776 0.9409 0.9772 0.9504	95% UCL 0.9984 0.9991 0.9908 0.9896	Median 0.99 0.96 0.98 0.97	Min 0.98 0.95 0.98 0.95	Max 1 1 0.99	0.003742 0.01049 0.00245	0.85% 2.42% 0.56%	0.0% 1.82% 0.4% 1.82%
Fertilization C-µg/L 0 10 20 40 80	Rate Summary Control Type	Count  5 5 5 5 5 5	Mean 0.988 0.97 0.984 0.97 0.526	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114	Median 0.99 0.96 0.98 0.97 0.52	Min 0.98 0.95 0.98	Max 1 1 0.99 0.99	0.003742 0.01049 0.00245 0.007071	0.85% 2.42% 0.56% 1.63%	0.0% 1.82% 0.4%
Fertilization C-µg/L 0 10 20 40 80 160	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5	Mean 0.988 0.97 0.984 0.97 0.526 0.008	95% LCL 0.9776 0.9409 0.9772 0.9504	95% UCL 0.9984 0.9991 0.9908 0.9896	Median 0.99 0.96 0.98 0.97	Min 0.98 0.95 0.98 0.95 0.43	Max 1 1 0.99 0.99 0.62	0.003742 0.01049 0.00245 0.007071 0.03076	0.85% 2.42% 0.56% 1.63% 13.08%	0.0% 1.82% 0.4% 1.82% 46.76%
Fertilization C-µg/L 0 10 20 40 80 160	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 7 7 7 7 7 7 7 7 7 7 7 7 7	Mean 0.988 0.97 0.984 0.97 0.526 0.008	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021	Median 0.99 0.96 0.98 0.97 0.52	Min 0.98 0.95 0.98 0.95 0.43	Max 1 1 0.99 0.99 0.62 0.04	0.003742 0.01049 0.00245 0.007071 0.03076 0.008	0.85% 2.42% 0.56% 1.63% 13.08% 223.6%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19%
Fertilization C-µg/L 0 10 20 40 80 160	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 rmed Sum Count	Mean 0.988 0.97 0.984 0.97 0.526 0.008 mary Mean	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021	Median 0.99 0.96 0.98 0.97 0.52 0	Min 0.98 0.95 0.98 0.95 0.43 0	Max 1 1 0.99 0.99 0.62 0.04	0.003742 0.01049 0.00245 0.007071 0.03076 0.008	0.85% 2.42% 0.56% 1.63% 13.08% 223.6%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19%
Fertilization C-µg/L 0 10 20 40 80 160 Angular (Co	Rate Summary Control Type Lab Control	Count 5 5 5 5 5 5 7 7 7 7 7 7 7 7 7 7 7 7 7	Mean 0.988 0.97 0.984 0.97 0.526 0.008	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0 95% LCL 1.417	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021 95% UCL 1.511	Median 0.99 0.96 0.98 0.97 0.52 0  Median 1.471	Min 0.98 0.95 0.98 0.95 0.43 0	Max 1 1 0.99 0.99 0.62 0.04 Max 1.521	0.003742 0.01049 0.00245 0.007071 0.03076 0.008 Std Err 0.01699	0.85% 2.42% 0.56% 1.63% 13.08% 223.6% CV% 2.6%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19% %Effect 0.0%
Fertilization C-µg/L 0 10 20 40 80 160 Angular (Co	Rate Summary Control Type Lab Control  orrected) Transfo Control Type	Count 5 5 5 5 5 5 rmed Sum Count	Mean 0.988 0.97 0.984 0.97 0.526 0.008 mary Mean	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021 95% UCL 1.511 1.51	Median 0.99 0.96 0.98 0.97 0.52 0  Median 1.471 1.369	Min 0.98 0.95 0.98 0.95 0.43 0 Min 1.429 1.345	Max 1 1 0.99 0.99 0.62 0.04 Max 1.521 1.521	0.003742 0.01049 0.00245 0.007071 0.03076 0.008 Std Err 0.01699 0.03603	0.85% 2.42% 0.56% 1.63% 13.08% 223.6% CV% 2.6% 5.71%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19% %Effect 0.0% 3.67%
Fertilization C-µg/L 0 10 20 40 80 160 Angular (Co	Rate Summary Control Type Lab Control  orrected) Transfo Control Type	Count 5 5 5 5 5 7 med Sum Count 5	Mean 0.988 0.97 0.984 0.97 0.526 0.008 mary Mean 1.464	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0 95% LCL 1.417	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021 95% UCL 1.511 1.51 1.474	Median 0.99 0.96 0.98 0.97 0.52 0  Median 1.471 1.369 1.429	Min 0.98 0.95 0.98 0.95 0.43 0  Min 1.429 1.345 1.429	Max  1 1 0.99 0.99 0.62 0.04  Max 1.521 1.521 1.471	0.003742 0.01049 0.00245 0.007071 0.03076 0.008 Std Err 0.01699 0.03603 0.01022	0.85% 2.42% 0.56% 1.63% 13.08% 223.6% CV% 2.6% 5.71% 1.58%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19% %Effect 0.0% 3.67% 1.26%
Fertilization C-µg/L 0 10 20 40 80 160  Angular (Co C-µg/L 0 10	Rate Summary Control Type Lab Control  orrected) Transfo Control Type	Count 5 5 5 5 5 rmed Sum Count 5 5 5	Mean 0.988 0.97 0.984 0.97 0.526 0.008 mary Mean 1.464 1.41	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0 95% LCL 1.417 1.31	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021 95% UCL 1.511 1.51	Median 0.99 0.96 0.98 0.97 0.52 0  Median 1.471 1.369 1.429 1.397	Min 0.98 0.95 0.98 0.95 0.43 0  Min 1.429 1.345 1.429 1.345	Max  1 1 0.99 0.99 0.62 0.04  Max 1.521 1.521 1.471 1.471	0.003742 0.01049 0.00245 0.007071 0.03076 0.008 Std Err 0.01699 0.03603 0.01022 0.02207	0.85% 2.42% 0.56% 1.63% 13.08% 223.6% CV% 2.6% 5.71% 1.58% 3.52%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19% %Effect 0.0% 3.67% 1.26% 4.22%
Fertilization C-µg/L 0 10 20 40 80 160  Angular (Co C-µg/L 0 10 20	Rate Summary Control Type Lab Control  orrected) Transfo Control Type	Count 5 5 5 5 5 crmed Sum Count 5 5 5 5	Mean  0.988 0.97 0.984 0.97 0.526 0.008  mary  Mean  1.464 1.41 1.446	95% LCL 0.9776 0.9409 0.9772 0.9504 0.4406 0 95% LCL 1.417 1.31 1.417	95% UCL 0.9984 0.9991 0.9908 0.9896 0.6114 0.03021 95% UCL 1.511 1.51 1.474	Median 0.99 0.96 0.98 0.97 0.52 0  Median 1.471 1.369 1.429	Min 0.98 0.95 0.98 0.95 0.43 0  Min 1.429 1.345 1.429	Max  1 1 0.99 0.99 0.62 0.04  Max 1.521 1.521 1.471	0.003742 0.01049 0.00245 0.007071 0.03076 0.008 Std Err 0.01699 0.03603 0.01022	0.85% 2.42% 0.56% 1.63% 13.08% 223.6% CV% 2.6% 5.71% 1.58%	0.0% 1.82% 0.4% 1.82% 46.76% 99.19% %Effect 0.0% 3.67% 1.26%

Report Date: Test Code: 01 Nov-17 10:24 (p 2 of 2) 171031sprt | 06-4227-6723

Nautilus Environmental (CA) Echinoid Sperm Cell Fertilization Test 15C CETISv1.8.7 **CETIS Version:** Endpoint: Fertilization Rate Analysis ID: 20-6412-1446 Parametric-Control vs Treatments Official Results: Yes Analysis: 01 Nov-17 10:23 Analyzed: Graphics 0.14 0.12 0.9 0.10 8.0 0.08 Fertilization Rate 0.7 0.06 0.6 0.04 0.02 0.00 0.4 -0,02 -0.04 0.2 -0.06 0.1 -0.08 0.0 -2.0 -1.5 1.0 1.5 2.0 Rankits C-μg/L

**Echinoid Sperm Cell Fertilization Test 15C** 

Report Date: Test Code:

01 Nov-17 10:24 (p 1 of 1) 171031sprt | 06-4227-6723

est Code: 171031spit

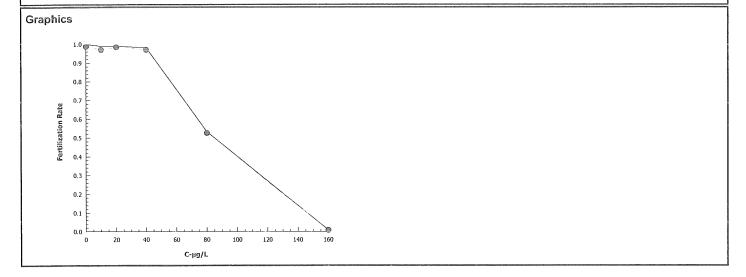
Nautilus Environmental (CA)

CETIS Version: CETISv1.8.7

Analysis ID:08-8095-0809Endpoint:Fertilization RateCETIS Version:CETAnalyzed:01 Nov-17 10:23Analysis:Trimmed Spearman-KärberOfficial Results:Yes

Trimmed Spearman-Kärber Estimates Threshold Option Threshold Mu Sigma EC50 95% LCL 95% UCL Trim 0.007175 81.36 Control Threshold 0.012 1.11% 1.91 78.72 84.09

Fertilizati	on Rate Summary		Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.988	0.98	1	0.003742	0.008367	0.85%	0.0%	494	500
10		5	0.97	0.95	1	0.01049	0.02345	2.42%	1.82%	485	500
20		5	0.984	0.98	0.99	0.00245	0.005479	0.56%	0.4%	492	500
40		5	0.97	0.95	0.99	0.007071	0.01581	1.63%	1.82%	485	500
30		5	0.526	0.43	0.62	0.03076	0.06877	13.08%	46.76%	263	500
160		5	0.008	0	0.04	0.008	0.01789	223.6%	99.19%	4	500



**CETIS QC Plot**Report Date: 01 Nov-17 10:24 ( 1 of 1)

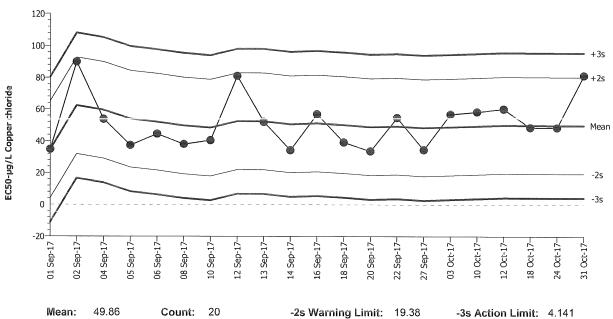
#### Echinoid Sperm Cell Fertilization Test 15C

#### Nautilus Environmental (CA)

Test Type: Fertilization Organism: Strongylocentrotus purpuratus (Purpl Material: Copper chloride

Protocol: EPA/600/R-95/136 (1995) Endpoint: Fertilization Rate Source: Reference Toxicant-REF

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



+2s Warning Limit:

80.34

+3s Action Limit: 95.58

Quali	tu Ca	ntrol	Data
Quan	$w \cup o$	ntroi	Data

Sigma:

15.24

CV:

30.60%

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Sep	1	15:27	34.79	-15.07	-0.9886			13-1244-6646	21-1567-7550
2			2	10:53	89.99	40.13	2.633	(+)		16-4202-9692	18-8681-1855
3			4	16:10	53.77	3.912	0.2567			12-2973-1405	10-6032-1229
4			5	17:07	37.36	-12.5	-0.8203			13-1627-7974	14-5447-1160
5			6	17:15	44.41	-5.447	-0.3574			05-5533-8557	16-8161-1582
6			8	15:48	37.91	-11.95	-0.7839			18-6871-7794	04-4479-5076
7			10	14:25	40.4	-9.458	-0.6206			11-6871-9499	08-4248-1228
8			12	15:51	81.07	31.21	2.048	(+)		20-0603-9450	06-1182-7961
9			13	19:07	52.04	2.176	0.1428			01-4575-6189	02-4618-7964
10			14	15:24	34.24	-15.62	-1.025			11-2846-3680	13-8128-7168
11			16	17:08	56.97	7.11	0.4665			08-9569-1329	19-6375-1112
12			18	15:28	39.21	-10.65	-0.6991			19-2924-5672	02-0031-2532
13			20	16:15	33.62	-16.24	-1.065			00-4454-0074	17-7214-1415
14			22	14:50	54.61	4.749	0.3116			20-3341-5102	16-2759-7635
15			27	15:34	34.46	-15.4	-1.01			12-3257-1101	06-9840-2290
16		Oct	3	13:49	56.88	7.019	0.4606			05-1137-7792	06-0895-0170
17			10	15:10	58.36	8.502	0.5579			20-5863-5053	00-1542-1738
18			12	14:55	60.18	10.32	0.6773			05-0863-6526	07-1531-2424
19			18	14:22	48.53	-1.33	-0.08725			13-0042-6212	05-6771-5532
20			24	13:15	48.41	-1.455	-0.09545			20-0280-7301	18-5464-1899
21			31	13:59	81.36	31.5	2.067	(+)		06-4227-6723	08-8095-0809

Echimoid Sperm Cell Fertilization Test 15C

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

Nautilus Environmental (CA)

Test Type: Fertilization

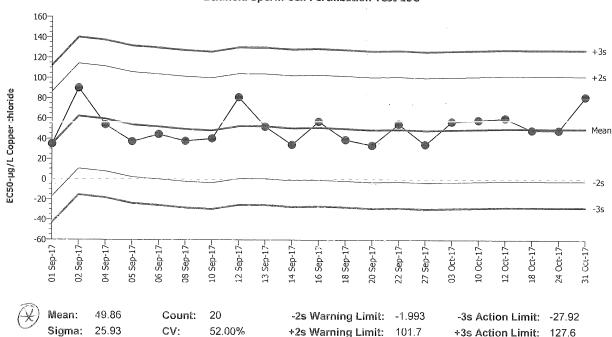
Organism: Strongylocentrotus purpuratus (Purpl

Endpoint: Fertilization Rate

Material: Source:

Copper chloride Reference Toxicant-REF

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



Quality Control Data											
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Sep	1	15:27	34.79	-15.07	-0.5811			13-1244-6646	21-1567-7550
2			2	10:53	89.99	40.13	1.548			16-4202-9692	18-8681-1855
3			4	16:10	53.77	3.912	0.1509			12-2973-1405	10-6032-1229
4			5	17:07	37.36	-12.5	-0.4821			13-1627-7974	14-5447-1160
5			6	17:15	44.41	-5.447	-0.2101			05-5533-8557	16-8161-1582
6			8	15:48	37.91	-11.95	-0.4608			18-6871-7794	04-4479-5076
7			10	14:25	40.4	-9.458	-0.3648			11-6871-9499	08-4248-1228
8			12	15:51	81.07	31.21	1.204			20-0603-9450	06-1182-7961
9			13	19:07	52.04	2.176	0.08394			01-4575-6189	02-4618-7964
10			14	15:24	34.24	-15.62	-0.6026			11-2846-3680	13-8128-7168
11			16	17:08	56.97	7.11	0.2742			08-9569-1329	19-6375-1112
12			18	15:28	39.21	-10.65	-0.4109			19-2924-5672	02-0031-2532
13			20	16:15	33.62	-16.24	-0.6262			00-4454-0074	17-7214-1415
14			22	14:50	54.61	4.749	0.1831			20-3341-5102	16-2759-7635
15			27	15:34	34.46	-15.4	-0.5938	0.0		12-3257-1101	06-9840-2290
16		Oct	3	13:49	56.88	7.019	0.2707			05-1137-7792	06-0895-0170
17			10	15:10	58.36	8.502	0.3279 .			20-5863-5053	00-1542-1738
18			12	14:55	60.18	10.32	0.3981	-		05-0863-6526	07-1531-2424
19			18	14:22	48.53	-1.33	-0.05128			13-0042-6212	05-6771-5532
20			24	13:15	48.41	-1.455	-0.05611			20-0280-7301	18-5464-1899
21			31	13:59	81.36	31.5	1.215			06-4227-6723	08-8095-0809

(4) warning and control chart limits recalculated based on the 15th percentile interclaboratory coefficient of variation as defined in EPA-833-R-00-003, for comparison purposes.

Report Date: Test Code:

27 Oct-17 16:50 (p 1 of 1) 06-4227-6723/171030sprt

Nautilus Environmental (CA)

Echimoid Sperm Cell Fertilization Test 15C

Sample Code: 171030sprt Start Date: 91,30,Oct-17 End Date: 31,30,Oct-17 Sample Date? 36 Oct-17 Species: Strongylocentrotus purpuratus Protocol: EPA/600/R-95/136 (1995) Sample Source: Reference Toxicant Material: Copper chloride Sample Station: Copper Chloride

imple Dat	ا تاور بها	JC(- 1 /		Water	al: Copper ch	onde 3	ample Station: Copper Chloride
C-µg/L	Code	Rep	Pos	# Counted	# Fertilized		Notes
			1	100	D	AB I	0/31/17
			2	100	98		
			3	(00	O		
			4	100	99		
			5	100	99		
			6	100	0		
			7	(00	98		
			8	100	43 43	Q18 AB 10/31/17	
			9	100	98		
			10	100	62		
			11	100	4		
			12	100	96		
			13	100	99		
			14	100	51		
			15	100	99		
			16	100	99		
			17	100	(0b 55		
			19	(00)	53		
			20	100	97 95		
			21				
			22	100	100 98		
			23		99		
			24	100	95		
	-		25	(00	98		
			26	(00	95		
			27	(00	95 52		
			28	100	98		
			29	100	44 0	Q18 AB 11/1/17	
			30	100	96		

(A) EG 018 11/1/17

Report Date: Test Code:

27 Oct-17 16:50 (p 1 of 1) 06-4227-6723/171030sprt

# Nautilus Environmental (CA)

Echinoid Sperm Cell Fertilization Test 15C Start Date 1 31 30 Oct-17 End Date: 33 30 Oct-17 Sample Date: 36 Oct-17

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

Material: Copper chloride

171030sprt Sample Code: Sample Source: Reference Toxicant Sample Station: Copper Chloride

nple Dat C-µg/L	(6)				al: Copper chloride	Sample Station: Copper Chloride
C-μg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	21			
0	LC	2	13			
0	LC	3	25			
0	LC	4	5			
0	LC	5	7	100	99	CG 10/31/17
10		1	26			
10		2	17			
10		3	24	100	95	CG 10/31/17
10		4	4			
10		5	30			
20		1	9	100	96	CG 10/31/17
20		2	15			
20		3	22	100	98	CG 10/31/17
20		4	2			00, 10, 31, 11
20		5	23			
40		1	28			
40		2	20	100	94	CG 10/31/17
40		3	16			
40		4	19	100	94	CG 10/31/17
40		5	12	O0 j	97	CG 10/31/17
80		1	18	(00)	65	CG W/31/17
80		2	27			
80		3	8	100	51	CG 10/31/17
80		4	10	100	60	CG 10/31/17
80		5	14			× VI I I
160		1	3	100	0	CG 16/31/17
160		2	29			
160		3	1			
160		4	6			
160		5	11			

QC: CG (A) CGQ1810/3/17

(B) EG Q18 11/1/17

#### Marine Chronic Bioassay

# **Water Quality Measurements**

Client :	Internal	Test Species: S. purpuratus
Sample ID:	CuCl <sub>2</sub>	Start Date/Time: 10/30/2017 / 359
Test No:	3) 171030sprt	Beg 1439 1439
	(B)	(B) E C

Dilutions made by: 

High conc. made (μg/L): 160

Vol. Cu stock added (mL): 8 3
Final Volume (mL): 500

Cu stock concentration (μg/L):

Analyst:

	Initial Readings									
Concentration (μg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)						
Lab Control	8-6	A7-8.00	33.7	15.6						
10	8.6	7.99	33.9	15.3						
20	8.3	8.00	33.9	15.4						
40	8.4	Š. 00	33.9	15.4						
80	8.4	8.00	33.8	15.5						
160	8.4	8.01	33.5	13.5						

Comments:	BC6Q1810/31/17 BADOLS 10/31/17	@ EG 618 N/17
QC Check:	EG 11/1/7	Final Review: FTP 11/2/17

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

# **Marine Chronic Bioassay**

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

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

	•								
Client:	Interna	Q				Start Date/Tir	ne 10/31/201	71 1359	
Sample ID:	CVCI2						me: 10/31/28		
est No.:	171031501	+					es: S. purpui		
	(1103)501				Animal Source: Pt. Lbma				
ech initials:	CL					Date Collect			
jection Time:	1310								
jection rime.									
perm Absorbance at 40	0 nm: <u>1,00</u>	<u> </u> (t	arget range of	0.8 - 1.0 for	density of 4	x10 <sup>6</sup> sperm/m	nl)		
Eggs Counted:	93	Mean: _	93_x	50 = <u>4</u> (	<u>050</u> eg	gs/ml			
	94								
	(target counts of 80 eggs per vertical pass on Sedgwick-								
	Rafter slide for a final density of 4000 eggs/ml)								
	91								
itial density:	4650 eg	gs/ml	= 1.163  dilution	eg	egg stock00ml				
nal density:	4000 eggs/ml - <u>1.0</u> part egg s					awater	<u>16.3 ml</u>		
	০,1০3 parts seawater								
angefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1	50:1	
Sperm Stock	50	40	30	20	10	5.0	2.5	1.25	
Seawater	0.0	10	20	30	40	45	47.5	48.75	
	771		langefinder De	tio. For	مال	fort			
	Time	<u>r</u>	tangefinder Ra ≲0∵-\	7 5	7.1	<u>fert.</u>			
perm Added (100 µl):	13/3			$\frac{\omega}{\omega}$					
ggs Added (0.5 ml):	1590	_	100-1		717 0	, 0			
est Ended:	1320		100:1						
						33			
OTE: Choose a spermis range, choose the ra	-to-egg ratio that tio closest to 90 p	results in fe ercent unle	rtilization betwees professiona	een 80 and 9 Il judgment o	90 percent. dictates cons	If more than sideration of c	one concentr ther factors (	ation is withir e.g., organisr	
ealth, stage of reproduc	tive season, site	conditions).							
efinitive Test		S	Sperm:Egg Rat	io Used:	05:1				
Cilitate 1 ook					· · · · · · · · · · · · · · · · · · ·				
	Time			Fe	<u>rt. Un</u>	ifert.			
perm Added (100 µl):	1359	C	QC1	9	<u> 1</u>	<u>3_</u>			
ggs Added (0.5 ml):	1419	(	QC2	9	<u>8</u>	2_			
Fest Ended:	1439	E	gg Control 1	<u> </u>	<u> </u>	<u>00</u>			
		E	Egg Control 2		<u> </u>	00			
L									
omments:							THE		
			,						
								1 1	
C Check	EG WIN	17				Final Rev	ew: KTP	11/2/17	

Appendix E

Qualifier Codes



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

- Q1 Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q2 Temperatures out of recommended range; no action taken, test terminated same day
- Q3 Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L
- Q4 Test aerated; D.O. levels dropped below 4.0 mg/L
- Q5 Test initiated with aeration due to an anticipated drop in D.O.
- Q6 Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred
- Q7 Salinity out of recommended range
- Q8 Spilled test chamber/ Unable to recover test organism(s)
- Q9 Inadequate sample volume remaining, 50% renewal performed
- Q10 Inadequate sample volume remaining, no renewal performed
- Q11 Sample out of holding time; refer to QA section of report
- Q12 Replicate(s) not initiated; excluded from data analysis
- Q13 Survival counts not recorded due to poor visibility or heavy debris
- Q14 D.O. percent saturation was checked and was ≤ 110%
- Q15 Did not meet minimum test acceptability criteria. Refer to QA section of report.
- Q16 Percent minimum significant difference (PMSD) was <u>below</u> the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.
- Q17 Percent minimum significant difference (PMSD) was <u>above</u> the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.
- Q18 Incorrect Entry
- Q19 Illegible Entry
- Q20 Miscalculation
- Q21 Other (provide reason in comments section)
- Q22 Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation.
   Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.
- Q23 Test or ganisms r eceived at a <u>temperature</u> greater than 3°C ou tside the r ecommended t est temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. O rganisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 Test organisms received at <u>salinity</u> greater than 3 ppt outside of the recommended test salinity range. H owever, due t o age -specific pr otocol r equirements and/ or s ample ho lding t ime constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Updated: 6/30/15