

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily) Sample Collection Date: September 16, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: October 2, 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 San Diego, California 92120

858.587.7333 fax: 858.587.3961 Results verified by: ______ Adrienne Cibor

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

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

Sampling Date: September 16, 2017

<u>Test Date:</u> September 16, 2017

Sample ID: M-001 off-spec period)

Effluent Limitation: 16.5 TU_c

Results Summary:

	Effluent Te	est Results	Effluent Limitation
Bioassay Type:	NOEC	TUc	Met? (Yes/No)
Urchin Fertilization	10	10	Yes

Test ID: 1709-S154

Client: IDE Americas, Inc.
Sample ID: M-001
Sample Date: September 16, 2017

INTRODUCTION

A discharge sample was collected in September 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for daily chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time 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 September 16, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

Sample collection and delivery were performed by IDE Americas, Inc. (IDE) personnel. Following arrival at Nautilus, an aliquot of the water 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 protocols described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./Carlsbad Desalination Plant
Sample ID:	M-001 (pre-treatment off-spec period)
Monitoring Period:	September 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	9/16/17, 08:00
Sample Receipt Date, Time:	9/16/17, 14:48

Table 2. Water Quality Measurements for the M-001 Sample upon Receipt

Sample Collection	рН	DO	Temp	Salinity	Alkalinity	Total Chlorine
Date		(mg/L)	(°C)	(ppt)	(mg/L as CaCO₃)	(mg/L)
9/16/17	7.96	10.4	2.0	36.2	146	<0.02

Client: IDE Americas, Inc. Test ID: 1709-S154 Sample ID: M-001

Sample Date: September 16, 2017

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times: 9/16/17, 17:08 through 17:48

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 inlet,

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

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

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 fertilization period

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

significant difference (PMSD) value <25.

Copper chloride Reference Toxicant Testing:

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 the sample dilution series was compared to that observed in the laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TUc) values.

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 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; results are reported as "Pass" if a sample is considered non-toxic at the IWC according to the TST calculation, or "Fail" if considered toxic at the IWC according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis was performed for comparison purposes only.

Client: IDE Americas, Inc. Test ID: 1709-S154 Sample ID: M-001

Sample Date: September 16, 2017

RESULTS

A statistically significant decrease in fertilization rate was observed at 15 percent effluent concentration when compared to the lab control. The NOEC is reported as 10 and the TUc is equal to 10, which is below the maximum effluent limitation of 16.5 for this permit. According to the TST analysis, no significant effect was observed at any percent effluent concentration tested when compared to lab control. Statistical results 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 copies 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 I D	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)		TST Result (Pass/Fail)	Percent Effect at IWC
M-001	10	15	>15	10	Pass	3.7

NOEC = No Observed Effect Concentration

LOEC = Lowest Observed Effect Concentration

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

TU_c = Chronic Toxic Unit: 100÷NOEC

TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only. Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) *100. A negative PE results when organism performance in the sample is greater than that in the control.

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

Test Concentration (% Sample)	Mean Percent Fertilization
Lab Control	97.4
2.5	95.4
5.0	97.4
6.06	93.8
10	93.0
15	87.6*

^{*}An asterisk indicates a statistically significant decrease compared to the lab control

Client: IDE Americas, Inc. Test ID: 1709-S154 Sample ID: M-001

Sample Date: September 16, 2017

QUALITY ASSURANCE

The sample was received on the day it was collected and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations reported are deemed reliable. Additionally, appropriate alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010).

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity met all test acceptability criteria. The median effect (EC50) value calculated for this test was within two standard deviations (2SD) of the historical mean for our laboratory, indicating organisms were of typical sensitivity to copper. Results for the reference toxicant test are summarized in Table 6 and presented in full in Appendix D. A list of qualifier codes can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ±2 SD (µg/L Copper)	CV (%)
9/16/17	57.0	49.7 ± 32.9	33.1

 EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms Historical Mean EC₅₀ ± 2 SD = Mean of historical test results plus or minus two standard deviations CV = Coefficient of Variation

TOXICITY SUMMARY REPORT

Client: IDE Americas, Inc. Test ID: 1709-S154 Sample ID: M-001

Sample Date: September 16, 2017

REFERENCES

California Regional Water Quality Control Board Region 9, San Diego (RWQCB) 2006. Waste Discharge Requirements for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project, Discharge to the Pacific Ocean via the Encina Power Station Discharge Channel. Order No. R9-2006-0065, NPDES No. CA109223. June 2006.

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- 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. 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: Test Code: 22 Sep-17 15:40 (p 1 of 1) 1709-S154 | 02-7714-1036

								rest code	•	170	9-3154 0	2-11 14-103
Echinoid Spe	rm Cell Fertiliza	ation Te	st 15C							Nautilus	s Environi	mental (CA)
Batch ID: Start Date: Ending Date: Duration:	20-5820-2462 Test Type: 16 Sep-17 17:08 Protocol: 16 Sep-17 17:48 Species: 40m Source:		Fertilization EPA/600/R-95 Strongylocentr Pt. Loma	Analyst: Diluent: Brine: Age:		oratory Seav	water					
,	14-9041-7850			17-1033 Facility Effluen IDE Americas, M-001 (Daily)				Client: Project:	IDE Carl	Isbad Desal	Plant	
Comparison S	Summary	N	M possession and a second									
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Met	hod			
19-0447-5844			10	15	12.25	4.11%	10	Dun	nett N	lultiple Com	parison Te	st
Point Estimate	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Met	hod			
03-3411-1050	Fertilization Rate EC25			>15 >15	N/A N/A	N/A N/A	<6.66	, , ,				
Test Acceptab	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Ove	rlap	Decision		
03-3411-1050	Fertilization Ra	te	Contro	ol Resp	0.974	0.7 - NL	T Will be constructed	Yes		Passes A	ceptability	Criteria
19-0447-5844	Fertilization Ra			ol Resp	0.974	0.7 - NL		Yes	• • • • • • • • • • • • • • • • • • • •			Criteria
19-0447-5844	Fertilization Ra	te	PMSD		0.04115	NL - 0.25		No	No Passes Acceptability Criteria		Criteria	
Fertilization R	ate Summary											
	Control Type	Count	Mean Mean	95% LCL	95% UCL	Min	Max	Std	Err	Std Dev	CV%	%Effect
	Lab Control	5	0.974	0.9672	0.9808	0.97	0.98	0.00	2449	0.005476	0.56%	0.0%
2.5		5	0.954	0.8967	1	0.89	0.99	0.02	064	0.04615	4.84%	2.05%
5		5	0.974	0.9552	0.9928	0.95	0.99	0.00	6782	0.01517	1.56%	0.0%
6.06		5	0.938	0.9218	0.9542	0.92	0.95	0.00	5831	0.01304	1.39%	3.7%
10		5	0.93	0.8773	0.9827	0.86	0.96	0.01	897	0.04243	4.56%	4.52%
15		5	0.876	0.8244	0.9276	0.84	0.94	0.01	86	0.04159	4.75%	10.06%
Fertilization R	ate Detail											
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.97	0.97	0.98	0.97	0.98						
2.5		0.99	0.99	0.92	0.98	0.89						
5		0.99	0.97	0.95	0.98	0.98						
6.06		0.93	0.95	0.95	0.94	0.92						
10		0.95	0.96	0.86	0.92	0.96						
4												

Analyst: QA: ACQ 28/17

15

0.89

0.94

0.84

0.87

0.84

Report Date: Test Code:

22 Sep-17 15:39 (p 1 of 2)

1709-S154 | 02-7714-1036

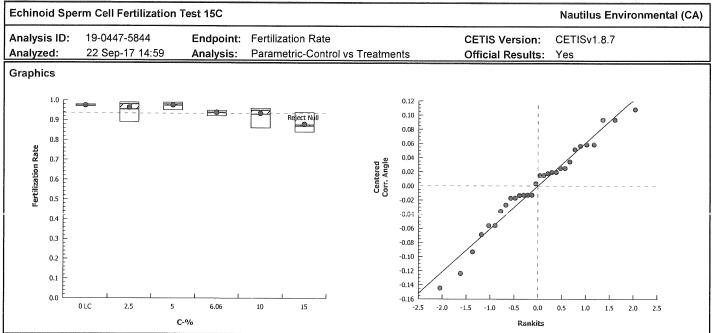
							lest				2-7714-1036
Echinoid Sp	erm Cell Fertiliza	ation Test 1	15C						Nautilus	Environr	nental (CA)
Analysis ID:	19-0447-5844	En	dpoint: Fer	tilization Rat	e		CETI	S Version:	CETISv1.	.8.7	
Analyzed:	22 Sep-17 14:	59 An	alysis: Par	ametric-Con	trol vs Treat	tments	Official Results: Yes				
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cori	rected)	NA	C > T	NA	NA		4.11%	10	15	12.25	10
Dunnett Mul	tiple Compariso	n Test							,		
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(a:5%)		
Lab Control	2.5	· · · · · · · · · · · · · · · · · · ·	0.77	2.362	0.099 8	0.5248	CDF		ificant Effect		
	5		-0.1075	2.362	0.099 8	0.8631	CDF	•	ificant Effect		
	6.06		2.138	2.362	0.099 8	0.0772	CDF	J	ificant Effect		
	10		2.355	2.362	0.099 8	0.0507	CDF	•	ificant Effect		
	15*		4.646	2.362	0.099 8	0.0002	CDF	Significar			
ANOVA Tabl	е										
Source	Sum Squ	iares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(a:5%)		
Between	0.142469	5	0.0284939) .	5	6.517	0.0006	Significar	t Effect		
Error	0.104929	3	0.0043720		24			J			
Total	0.247398	8	V 102 - 102		29	-					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of \	/ariance	13.63	15.09	0.0182	Equal Var	iances			
Distribution	Shapiro-	Wilk W Nor	mality	0.9693	0.9031	0.5191	Normal Distribution				
Fertilization	Rate Summary										
C-%											
	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Control Type Lab Control	Count 5	Mean 0.974	95% LCL 0.9672	95% UCL 0.9808	Median 0.97	Min 0.97	Max 0.98	Std Err 0.002449	CV%	%Effect 0.0%
- The transfer was the - With mission and the											
0		5	0.974	0.9672	0.9808	0.97	0.97	0.98	0.002449	0.56%	0.0%
0 2.5		5 5	0.974 0.954	0.9672 0.8967	0.9808 1	0.97 0.98	0.97 0.89	0.98 0.99	0.002449 0.02064	0.56% 4.84%	0.0% 2.05%
0 2.5 5		5 5 5	0.974 0.954 0.974	0.9672 0.8967 0.9552	0.9808 1 0.9928	0.97 0.98 0.98	0.97 0.89 0.95	0.98 0.99 0.99	0.002449 0.02064 0.006782	0.56% 4.84% 1.56%	0.0% 2.05% 0.0%
0 2.5 5 6.06		5 5 5 5	0.974 0.954 0.974 0.938	0.9672 0.8967 0.9552 0.9218	0.9808 1 0.9928 0.9542	0.97 0.98 0.98 0.94	0.97 0.89 0.95 0.92	0.98 0.99 0.99 0.95	0.002449 0.02064 0.006782 0.005831	0.56% 4.84% 1.56% 1.39%	0.0% 2.05% 0.0% 3.7%
0 2.5 5 6.06 10 15		5 5 5 5 5 5	0.974 0.954 0.974 0.938 0.93 0.876	0.9672 0.8967 0.9552 0.9218 0.8773	0.9808 1 0.9928 0.9542 0.9827	0.97 0.98 0.98 0.94 0.95	0.97 0.89 0.95 0.92 0.86	0.98 0.99 0.99 0.95 0.96	0.002449 0.02064 0.006782 0.005831 0.01897	0.56% 4.84% 1.56% 1.39% 4.56%	0.0% 2.05% 0.0% 3.7% 4.52%
0 2.5 5 6.06 10 15	Lab Control	5 5 5 5 5 5	0.974 0.954 0.974 0.938 0.93 0.876	0.9672 0.8967 0.9552 0.9218 0.8773	0.9808 1 0.9928 0.9542 0.9827	0.97 0.98 0.98 0.94 0.95	0.97 0.89 0.95 0.92 0.86	0.98 0.99 0.99 0.95 0.96	0.002449 0.02064 0.006782 0.005831 0.01897	0.56% 4.84% 1.56% 1.39% 4.56%	0.0% 2.05% 0.0% 3.7% 4.52%
0 2.5 5 6.06 10 15 Angular (Cor C- %	Lab Control	5 5 5 5 5 5 5	0.974 0.954 0.974 0.938 0.93 0.876	0.9672 0.8967 0.9552 0.9218 0.8773 0.8244	0.9808 1 0.9928 0.9542 0.9827 0.9276	0.97 0.98 0.98 0.94 0.95 0.87	0.97 0.89 0.95 0.92 0.86 0.84	0.98 0.99 0.99 0.95 0.96 0.94	0.002449 0.02064 0.006782 0.005831 0.01897 0.0186	0.56% 4.84% 1.56% 1.39% 4.56% 4.75%	0.0% 2.05% 0.0% 3.7% 4.52% 10.06%
0 2.5 5 6.06 10 15 Angular (Cor C- %	Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 5 rmed Sumr	0.974 0.954 0.974 0.938 0.93 0.876	0.9672 0.8967 0.9552 0.9218 0.8773 0.8244	0.9808 1 0.9928 0.9542 0.9827 0.9276	0.97 0.98 0.98 0.94 0.95 0.87	0.97 0.89 0.95 0.92 0.86 0.84	0.98 0.99 0.99 0.95 0.96 0.94	0.002449 0.02064 0.006782 0.005831 0.01897 0.0186	0.56% 4.84% 1.56% 1.39% 4.56% 4.75%	0.0% 2.05% 0.0% 3.7% 4.52% 10.06%
0 2.5 5 6.06 10 15 Angular (Cor	Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 med Sumr Count	0.974 0.954 0.974 0.938 0.93 0.876 mary Mean	0.9672 0.8967 0.9552 0.9218 0.8773 0.8244 95% LCL	0.9808 1 0.9928 0.9542 0.9827 0.9276 95% UCL 1.431	0.97 0.98 0.98 0.94 0.95 0.87 Median 1.397	0.97 0.89 0.95 0.92 0.86 0.84	0.98 0.99 0.99 0.95 0.96 0.94	0.002449 0.02064 0.006782 0.005831 0.01897 0.0186 Std Err 0.007885	0.56% 4.84% 1.56% 1.39% 4.56% 4.75% CV% 1.25%	0.0% 2.05% 0.0% 3.7% 4.52% 10.06% %Effect 0.0%
0 2.5 5 6.06 10 15 Angular (Cor C- % 0 2.5	Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 med Sumr Count 5	0.974 0.954 0.974 0.938 0.93 0.876 Mean 1.41 1.377	0.9672 0.8967 0.9552 0.9218 0.8773 0.8244 95% LCL 1.388 1.239	0.9808 1 0.9928 0.9542 0.9827 0.9276 95% UCL 1.431 1.516	0.97 0.98 0.98 0.94 0.95 0.87 Median 1.397 1.429	0.97 0.89 0.95 0.92 0.86 0.84 Min 1.397 1.233	0.98 0.99 0.99 0.95 0.96 0.94 Max 1.429 1.471	0.002449 0.02064 0.006782 0.005831 0.01897 0.0186 Std Err 0.007885 0.04984	0.56% 4.84% 1.56% 1.39% 4.56% 4.75% CV% 1.25% 8.09%	0.0% 2.05% 0.0% 3.7% 4.52% 10.06% %Effect 0.0% 2.29%
0 2.5 5 6.06 10 15 Angular (Coo C- % 0 2.5 5	Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 5 crmed Sumr Count 5 5	0.974 0.954 0.974 0.938 0.93 0.876 Mean 1.41 1.377	0.9672 0.8967 0.9552 0.9218 0.8773 0.8244 95% LCL 1.388 1.239 1.356	0.9808 1 0.9928 0.9542 0.9827 0.9276 95% UCL 1.431 1.516 1.472	0.97 0.98 0.98 0.94 0.95 0.87 Median 1.397 1.429	0.97 0.89 0.95 0.92 0.86 0.84 Min 1.397 1.233 1.345	0.98 0.99 0.99 0.95 0.96 0.94 Max 1.429 1.471 1.471	0.002449 0.02064 0.006782 0.005831 0.01897 0.0186 Std Err 0.007885 0.04984 0.02082	0.56% 4.84% 1.56% 1.39% 4.56% 4.75% CV% 1.25% 8.09% 3.29%	0.0% 2.05% 0.0% 3.7% 4.52% 10.06% %Effect 0.0% 2.29% -0.32%

Analyst: QA: AC9(28)17

CETIS Analytical Report

Report Date: Test Code: 22 Sep-17 15:40 (p 2 of 2)

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CETIS Analytical Report

Report Date:

22 Sep-17 15:40 (p 1 of 1)

Test Code:

1709-S154 | 02-7714-1036

Echinoid Sperm Cell Fertilization Test 15C

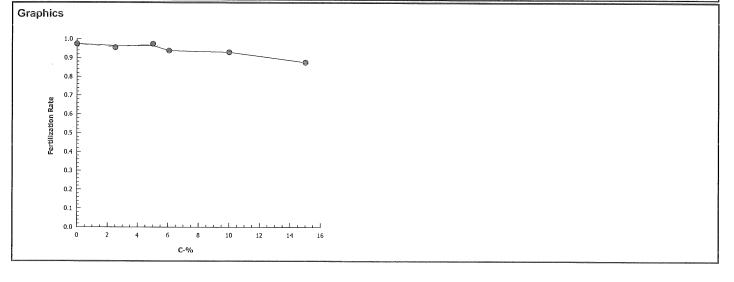
Nautilus Environmental (CA)

Analysis ID: 03-3411-1050 Endpoint: Fertilization Rate CETIS Version: CETISV1.8.7

Analyzed: 22 Sep-17 14:59 Analysis: Linear Interpolation (ICPIN) Official Results: Yes

Linear	Interpola	tion Options						
X Trans	sform	Y Transform	Seed	i k	Resamples	Exp 95% CL	Method	
Linear		Linear	2131	886	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	0.974	0.97	0.98	0.002449	0.005476	0.56%	0.0%	487	500	
2.5		5	0.954	0.89	0.99	0.02064	0.04615	4.84%	2.05%	477	500	
5		5	0.974	0.95	0.99	0.006782	0.01517	1.56%	0.0%	487	500	
6.06		5	0.938	0.92	0.95	0.005831	0.01304	1.39%	3.7%	469	500	
10		5	0.93	0.86	0.96	0.01897	0.04243	4.56%	4.52%	465	500	
15		5	0.876	0.84	0.94	0.0186	0.04159	4.75%	10.06%	438	500	



Report Date: Test Code: 22 Sep-17 15:39 (p 1 of 1) 1709-S154 | 02-7714-1036

							1621	Coue.	170	5-3134 0	2-11 14-103
Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	Environ	mental (CA)
Analysis ID: Analyzed:	20-5573-7571 22 Sep-17 14:		dpoint: Fe alysis: Pa	rtilization Ra rametric Bio		-Two Sampl		IS Version: cial Results:	CETISv1. Yes	8.7	
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	2.54%	15	>15	NA	6.667
TST-Welch's	t Test										
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(α:5%)		
Lab Control	2.5*		6.38	2.132	0.107 4	0.0015	CDF	Non-Signif	icant Effect		
	5*		16.49	2.132	0.046 4	< 0.0001	CDF	•	icant Effect		
	6.06*		19.69	2.015	0.027 5	<0.0001	CDF	-	icant Effect		
	10*		7.22	2.132	0.075 4	0.0010	CDF	•	icant Effect		
	15*		5.113	2.132	0.066 4	0.0035	CDF	_	icant Effect		
ANOVA Tabl	e										
Source	Sum Squ	uares	Mean Sq	uare	DF	F Stat	P-Value	Decision(α:5%)		
Between	0.142469	5	0.028493	9	- 5	6.517	0.0006	Significant	Effect		
Error	0.104929	3	0.004372	055	24	•		•			
Total	0.247398	8		A 2000 A	29	_					
Distributiona	al Tests			377							
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of \	/ariance	13.63	15.09	0.0182	Equal Var	ALTERNATION D'ELLEN			
Distribution	Shapiro-	Wilk W Nor	mality	0.9693	0.9031	0.5191	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	0.974	0.9672	0.9808	0.97	0.97	0.98	0.002449	0.56%	0.0%
2.5		5	0.954	0.8967	1	0.98	0.89	0.99	0.02064	4.84%	2.05%
5		5	0.974	0.9552	0.9928	0.98	0.95	0.99	0.006782	1.56%	0.0%
6.06		5	0.938	0.9218	0.9542	0.94	0.92	0.95	0.005831	1.39%	3.7%
10		5	0.93	0.8773	0.9827	0.95	0.86	0.96	0.01897	4.56%	4.52%
15		5	0.876	0.8244	0.9276	0.87	0.84	0.94	0.0186	4.75%	10.06%
Angular (Cor	rected) Transfor	med Sumr	nary								
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.41	1.388	1.431	1.397	1.397	1.429	0.007885	1.25%	0.0%
2.5		5	1.377	1.239	1.516	1.429	1.233	1.471	0.04984	8.09%	2.29%
5		5	1.414	1.356	1.472	1.429	1.345	1.471	0.02082	3.29%	-0.32%
6.06		5	1.32	1.287	1.353	1.323	1.284	1.345	0.01198	2.03%	6.34%
10		5 5	1.311	1.215	1.407	1.345	1.187	1.369	0.03467	5.91%	6.99%

Analyst: QA: 4 9 / 2 8 / 1 7

CETIS Test Data Worksheet

Report Date:

15 Sep-17 16:40 (p 1 of 1)

Test Code: 1709-5154 02-7714-1036/1084D62C

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: End Date:

16 Sep-17 16 Sep-17 Species: Strongylocentrotus purpuratus Protocol: EPA/600/R-95/136 (1995)

Sample Code: 17- 1033

Sample Source: IDE Americas, Inc.

	e: 16 S		7	Materia	al: Facility Eff	luent Sample Station: M-001 (Daily) (9/10 Sample
C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	99	JS 912017
			62		97	
			63		96	
			64		97	
			65		98	
			66		96	
			67		89 95 95 99	
<u> </u>			68		95	
			69		45	
			70		99	<u> </u>
			71		93	JS 9/21/17
			72		84	
			73		98	
			74		98	
			75		84	
			76 77		98 99	
			78		99	
			79		94	
			80		92	
			81		95	
		-	82		94	
			83		86 97	
			84			
			85		98 89	
			86		97	
			87		92	
	-		88			
			89		87 95	
			90	\downarrow	92	

CETIS Test Data Worksheet

Start Date:

End Date:

Sample Date: 16 Sep-17

Report Date:

15 Sep-17 16:40 (p 1 of 1)

Test Code: 1769-5154 02-7714-1036/1084D62C

Nautilus Environmental (CA)

Echinoid Sperm Cell Fertilization Test 15C 16 Sep-17

16 Sep-17

Species: Strongylocentrotus purpuratus

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

Material: Facility Effluent

Sample Code: 17- 1033 Sample Source: IDE Americas, Inc.

Sample Station: M-001 (Daily) (9/14 Sample)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	62			
0	LC	2	86			
0	LC	3	73			
0	LC	4	64			
0	LC	5	74	100	99	AD 9/16/17
2.5		1	77	1		
2.5		2	61			
2.5		3	79			
2.5		4	84			
2.5		5	67			
5		1	70			
5		2	83			
5		3	68	The second of th		
5		4	65			
5		5	76			
6.06		1	71	100	97	
6.06		2	80			
6.06		3	69			
6.06		4	78			
6.06		5	90			
10		1	89			
10		2	66			
10		3	82			
10		4	87			
10		5	63			
15		1	85			
15		2	75			
15		3	81	_		
15		4	72			
15		5	88			

Marine Chronic Bioassay

Water Quality Measurements

Client:

IDE

Test Species: S. purpuratus

Sample ID:

M-001 Daily 9/16 sample

Start Date/Time: 9/16/2017

Sample Log No.: 17- \033

End Date/Time: 9/16/2017

Dilutions made by:

Test No: 1709-5154

			Analyst:	RT
Concentration	DO (mg/L)	Initial pH (units)	Readings Salinity (ppt)	Temperature (°C)
Lab Control	8.4	8.10	33.3	16.0
2.5	8.4	8.11	33.7	15.3®
5.0	8,4	8.10	33.7	15.3 ®
6.06	8.5	8.11	33.8	153®
10	8.5	8,10	33.9	16.0
15	6.5	8.09	34.2	(5.6

Comments:	R tomporature takon	from sylvagate vial on	tray.
QC Check:	EG 9/22/17	Final Review: AC 9/38/	7

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 Daily (9/16 s a) 17-1033 1709-5		 	End Date Sp	Time: 9/16/2017 / 1708 Time: 9/16/2017 / 174 8 Decies Duman
Tech initials: Injection Time:	AD 11025			Animal S Date Coll	
Sperm Absorbance at 4	400 nm: ().951	(target range of	0.8 - 1.0 for der	nsity of 4x10 ⁶ sper	rm/ml)
Eggs Counted:		an: <u>Φ</u> x :			,
		et counts of 80 eggs er slide for a final den			
Initial density: Final density:	4000 eggs/ml	- <u>1.0</u> par	ition factor t egg stock ts seawater	egg stock seawater	100 ml 20 ml
Prepare the embryo sto existing stock (1 part) a	ock according to the calcund	lated dilution factor		if the dilution factor	or is 2.25, use 100 ml of
			Sperm:Egg F	Ratio	
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 1600 50 40 0.0 10	30		100:1 10 5.0 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 1635 1650 1700	Rangefinder Rat	io: <u>Fert.</u> 11 81,90	Unfert. 23 19:10 4	
this range, choose the r	n-to-egg ratio that results atio closest to 90 percen of reproductive season, s	t unless professiona	reen 80 and 90 p al judgment dicta	percent. If more tl ates consideration	han one concentration is within of other factors (e.g.,
<u>Definitive Test</u>		Sperm:Egg Ratio	Used: <u> S</u> C) <u>, </u>	
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1708 1728 1748	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 90	Unfert. 12 10 100	
Comments:	@ JA 4123/17 Q18				
QC Check:	EG 9/22/17			Final Re	eview:AC_9/38/17_

Appendix B

Sample Receipt Information

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Client:	IDE			,
Sample ID:	Daily	M-001	(9/16	samp(0)
et ID No(e) :	17/9	-C1521		

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1033			
Sample Collection Date & Time:	9/16/17 0600			
Sample Receipt Date & Time:	9/16/17 1448			
Number of Containers & Container Type:	tyl cbi			
Approx. Total Volume Received (L):	14L			
Check-in Temperature (°C)	2.0			
Temperature OK? 1	Y N	Y N	ΥN	Y N
DO (mg/l₋)	10.4			
pH (units)	7,96			
Conductivity (μS/cm)	*Control of the Control of the Contr			
Salinity (ppt)	36.2			
Alkalinity (mg/L) ²	146			
Hardness (mg/L) ^{2, 3}				
Total Chlorine (mg/l_)	<0.0Z			
Technician Initials	RT			

Test Performed:	ardin Fert.	Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
	Additional Control? Y (N)	Alkalinity: 97 Hardness or Salinity: 34 ppt = Alkalinity: Hardness or Salinity:
Test Performed:		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
	Additional Control? Y N	= Alkalinity: Hardness or Salinity:
<u>Test Performed:</u>		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
	Additional Control? Y N	_= Alkalinity: Hardness or Salinity:
Notes:		d be 0-6°C, if received more than 24 hours past collection time. for freshwater samples only, NA = Not Applicable
Additional Comments:		

Sample Check-In Information

A! no adus, clea	1,00 od	C, nodelos	5
COC Complete (Y/N)	?		
A_Y B C			
_~			
Filtration? Y N)		
Pore Size:		_	
Organisms	or	Debris	
Salinity Adjustment?	V/N		
Test:	Source:	Targ	et ppt:
Test:	Source:	-	et ppt: et ppt:
Test:	Source:		et ppt:
pH Adjustment? Y	·	1419	or ppt.
,) A	В	С
Initial pH:			
mount of HCl added:			
Final pH:			
Cl ₂ Adjustment? Y (1
2,	A	В	С
Initial Free Cl ₂ :			
STS added:			
Final Free Cl ₂ :			
• • • • • •	(x)		
Sample Aeration? Y	N A	В	•
Initial D.O.		<u> </u>	C
Duration & Rate			
Final D.O.			
Subsamples for Addi	tional Chen	nistry Require	ed? YN
	T		
Tech Initials A	B	_c	
	QC Ch		-10.1

Appendix C

Chain-of-Custody Form



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

	Silver and the second s	Carrier and the Company of the Compa	Martin - Are to the group grows or proper to 1975	riv.troit.nannuocataine.arnenn	Marcon exploration continues rise exploration continues	Economic anode consular age	on a second contract	anger or many	Day of the Control of	Oth	e(en compositorio de la compositorio	nice Barriell And Ballion March	rrr bays
Project Name: NPDES [Paily Toxicity	Project M	anager:	Peter Sh	nen	Conta	ect Inforn	nation:_	(7	60) 201-	7777_		-	
		ec via autosampler by a Ifill daily NPDES require	_					anges control as	ANA	LYSES				NOTES:
unadjusted. Start: 9/15						Chronic Fertilization								
	Gl	lass=G Plastic=P				nic Fe								
	Yes=Y No=N A	Acid=A Base=B				Chro								
Drinl	king Water=DW Sea	awater=SW Soil=S		Pres										
Sample ID	Date	Time	Sample	Preservative	Container	Purple Urchin								
			Туре	∙-∪ ∪	Туре	Pur		continues regions (C				Married Son Postsock S		
M-001 (17- 2837)	9/15-16/2017	8:00-8:00	SW	N	4L CUBIE	Х								TDS - 35.73 ppt, EC - 55.01 mS/cm
								:				Oddorowania (Common Common Com		

									and the same of th					
Relinquished By:		Date:	Time:		Received By:		p angles and the second	NAVORESKI KERESEN VERSEN	Date:	THE PERSON NAMED IN			Samp	le Condition Upon Receipt:
MAay		9/16/17	1342		Lone#	11 .	44 R	1945	9/16	1713Y	X	Iced		Ambient orOC
Lone 41/ An	ty Riggins	9/16/17	14-18		and,	(Zz			9/6/17			Iced		Ambient or <u>2.0</u> °C
					,	//								

DATLY

Nauhly ID: 17-1033

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date:

22 Sep-17 15:47 (p 1 of 1)

Test Code:

170916sprt | 08-9569-1329

			V					Tool Gode.	•	170	o rospit j o	J-5505-152
Echinoid Spe	erm Cell Fertiliza	ation Test	t 15C							Nautilu	s Environn	nental (CA
Batch ID: Start Date: Ending Date: Duration:	05-4590-7325 16 Sep-17 17:0 16 Sep-17 17:4 40m	08 P 48 S	est Type: rotocol: pecies: ource:	Fertilization EPA/600/R-95, Strongylocentr Pt. Loma	, ,	atus		Analyst: Diluent: Brine: Age:		ural Seawat Applicable	er	
Sample ID: Sample Date: Receive Date Sample Age:	: 16 Sep-17	M S	ode: laterial: ource: tation:	170916sprt Copper chlorid Reference Tox Copper Chlorid	icant		H	Client: Project:	Inte	rnal		
Comparison	Summary											
Analysis ID	Endpoint		NOEL		TOEL	PMSD	TU	Meth				
	Fertilization Ra	ite	20	40	28.28	8.25%		Dunr	nett M	lultiple Com	parison Tes	st
Point Estimat Analysis ID	te Summary Endpoint		Level	μg/L	95% LCL	95% UCL	TU	Meth	nod			
19-6375-1112	Fertilization Ra	te	EC50	56.97	55.27	58.72		Trim	med S	Spearman-k	Kärber	
Test Acceptal	bility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Over	rlap	Decision		
17-0919-7416	Fertilization Ra	te	Contro	l Resp	0.946	0.7 - NL		Yes	***************************************	Passes A	cceptability	Criteria
19-6375-1112	Fertilization Ra	te	Contro	l Resp	0.946	0.7 - NL		Yes			cceptability	
17-0919-7416	Fertilization Ra	te	PMSD		0.08246	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 E	Ξrr	Std Dev	CV%	%Effect
0	Lab Control	5	0.946	0.9234	0.9686	0.93	0.97	0.008	3124	0.01817	1.92%	0.0%
10		5	0.83	0.752	0.908	0.72	0.87	0.028	311	0.06285	7.57%	12.26%
20		5	0.882	0.8395	0.9245	0.85	0.93	0.015	53	0.03421	3.88%	6.77%
40		5	0.852	0.7357	0.9683	0.7	0.94	0.041	188	0.09365	10.99%	9.94%
80		5	0.11	0.005008	0.215	0.03	0.23	0.037	782	0.08456	76.87%	88.37%
160		5	0	0	0	0	0	0		0		100.0%
Fertilization R	Rate Detail											
C-μg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0	Lab Control	0.94	0.93	0.97	0.93	0.96				***************************************		***************************************
10		0.85	0.87	0.87	0.84	0.72						
20		0.85	0.85	0.88	0.93	0.9						
40		0.94	0.7	0.83	0.89	0.9						
80		0.23	0.03	0.04	0.16	0.09						
160		0	0	0	0	0						
-		-	•		•	5						

Analyst: QA: AC9 08/17

Report Date:

22 Sep-17 15:47 (p 1 of 2)

Test Code:

170916sprt | 08-9569-1329

						Test	oouc.		, oop, i oo	3-9569-132
Echinoid Sperm Cell	Fertilization Te	st 15C						Nautilus	Environm	nental (CA
Analysis ID: 17-09	9-7416	Endpoint: F	ertilization Rat	e		CETI	S Version:	CETISv1.	8.7	
Analyzed: 22 Se	p-17 15:44	Analysis: F	arametric-Cor	trol vs Trea	ments	Offic	ial Results	: Yes		
Data Transform	Zeta	Alt Hyp	Trials	Seed	***************************************	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA		8.25%	20	40	28.28	
Dunnett Multiple Co	nparison Test									
Control vs	C-μg/L	Test St	at Critical	MSD DF	P-Value	P-Type	Decision(α:5%)		
Lab Control	10*	3.111	2.305	0.140 8	0.0094	CDF	Significan	t Effect		
	20	1.908	2.305	0.140 8	0.1034	CDF	Non-Signi	ficant Effect		
	10*	2.478	2.305	0.140 8	0.0356	CDF	Significan	t Effect		
	30*	16.78	2.305	0.140 8	<0.0001	CDF	Significan	t Effect		
ANOVA Table					300000000000000000000000000000000000000					
Source S	um Squares	Mean S	quare	DF	F Stat	P-Value	Decision(α:5%)		
Between 3	.388831	0.84720	77	4	91.63	<0.0001	Significan	t Effect		
Error 0	.1849151	0.00924	5756	20						
Total 3	.573746			24						
Distributional Tests										
Attribute	Test		Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett Equality	of Variance	6.867	13.28	0.1431	Equal Var	iances			
Distribution	Shapiro-Wilk W	Normality	0.9765	0.8877	0.8076	Normal Di	stribution			
					0.0070					
Fertilization Rate Su	mmary				0.0070					
Fertilization Rate Su		t Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
	l Type Coun	t Mean 0.946	95% LCL 0.9234	95% UCL 0.9686		Min 0.93	Max 0.97	Std Err 0.008124	CV% 1.92%	%Effect 0.0%
C-µg/L Contro	l Type Coun				Median					
C-μg/L Control	I Type Coun	0.946	0.9234	0.9686	Median 0.94	0.93	0.97	0.008124	1.92%	0.0%
C-μg/L Contro 0 Lab Co	I Type Coun ntrol 5 5	0.946 0.83	0.9234 0.752	0.9686 0.908	Median 0.94 0.85	0.93 0.72	0.97 0.87	0.008124 0.02811	1.92% 7.57%	0.0% 12.26%
C-μg/L Contro 0 Lab Co 10 20	I Type Coun ntrol 5 5 5	0.946 0.83 0.882	0.9234 0.752 0.8395	0.9686 0.908 0.9245	Median 0.94 0.85 0.88	0.93 0.72 0.85	0.97 0.87 0.93	0.008124 0.02811 0.0153	1.92% 7.57% 3.88%	0.0% 12.26% 6.77%
C-µg/L Control 0 Lab Co 10 20 40	I Type Coun ntrol 5 5 5 5	0.946 0.83 0.882 0.852	0.9234 0.752 0.8395 0.7357	0.9686 0.908 0.9245 0.9683	Median 0.94 0.85 0.88 0.89	0.93 0.72 0.85 0.7	0.97 0.87 0.93 0.94	0.008124 0.02811 0.0153 0.04188	1.92% 7.57% 3.88% 10.99%	0.0% 12.26% 6.77% 9.94%
С-µg/L Contro 0 Lab Co 10 20 40 80	I Type Coun ntrol 5 5 5 5 5 5 5	0.946 0.83 0.882 0.852 0.11	0.9234 0.752 0.8395 0.7357 0.005008	0.9686 0.908 0.9245 0.9683 0.215	Median 0.94 0.85 0.88 0.89 0.09	0.93 0.72 0.85 0.7 0.03	0.97 0.87 0.93 0.94 0.23	0.008124 0.02811 0.0153 0.04188 0.03782	1.92% 7.57% 3.88% 10.99%	0.0% 12.26% 6.77% 9.94% 88.37%
С-µg/L Contro 0 Lab Co 10 20 40 80 160	I Type Coun ntrol 5 5 5 5 5 5 5 7	0.946 0.83 0.882 0.852 0.11 0	0.9234 0.752 0.8395 0.7357 0.005008	0.9686 0.908 0.9245 0.9683 0.215	Median 0.94 0.85 0.88 0.89 0.09	0.93 0.72 0.85 0.7 0.03	0.97 0.87 0.93 0.94 0.23	0.008124 0.02811 0.0153 0.04188 0.03782	1.92% 7.57% 3.88% 10.99%	0.0% 12.26% 6.77% 9.94% 88.37%
C-µg/L Control 0 Lab Co 10 20 40 80 160 Angular (Corrected)	I Type Coun ntrol 5 5 5 5 5 5 7 Transformed Su	0.946 0.83 0.882 0.852 0.11 0	0.9234 0.752 0.8395 0.7357 0.005008	0.9686 0.908 0.9245 0.9683 0.215	Median 0.94 0.85 0.88 0.89 0.09	0.93 0.72 0.85 0.7 0.03	0.97 0.87 0.93 0.94 0.23	0.008124 0.02811 0.0153 0.04188 0.03782 0	1.92% 7.57% 3.88% 10.99% 76.87%	0.0% 12.26% 6.77% 9.94% 88.37% 100.0%
C-μg/L Control 0 Lab Co 10 20 40 80 160 Angular (Corrected) C-μg/L Control	I Type Coun ntrol 5 5 5 5 5 5 7 Transformed Su	0.946 0.83 0.882 0.852 0.11 0	0.9234 0.752 0.8395 0.7357 0.005008 0	0.9686 0.908 0.9245 0.9683 0.215 0	Median 0.94 0.85 0.88 0.89 0.09 0	0.93 0.72 0.85 0.7 0.03 0	0.97 0.87 0.93 0.94 0.23 0	0.008124 0.02811 0.0153 0.04188 0.03782 0	1.92% 7.57% 3.88% 10.99% 76.87%	0.0% 12.26% 6.77% 9.94% 88.37% 100.0%
С-µg/L Control 0 Lab Co 10 20 40 80 160 Angular (Corrected) C-µg/L Control 0 Lab Co	Type Coun S 5 5 5 5 5 5 5 5 5	0.946 0.83 0.882 0.852 0.11 0 Immary t Mean 1.339	0.9234 0.752 0.8395 0.7357 0.005008 0 95% LCL	0.9686 0.908 0.9245 0.9683 0.215 0 95% UCL 1.391	Median 0.94 0.85 0.88 0.89 0.09 0	0.93 0.72 0.85 0.7 0.03 0	0.97 0.87 0.93 0.94 0.23 0	0.008124 0.02811 0.0153 0.04188 0.03782 0 Std Err 0.01883	1.92% 7.57% 3.88% 10.99% 76.87% CV% 3.14%	0.0% 12.26% 6.77% 9.94% 88.37% 100.0% %Effect 0.0%
С-µg/L Control 0 Lab Co 10 20 40 80 160 Angular (Corrected) C-µg/L Control 0 Lab Co 10 Control	I Type Coun ntrol 5 5 5 5 5 5 5 Iransformed Su I Type Coun ntrol 5 5	0.946 0.83 0.882 0.852 0.11 0 Immary t Mean 1.339 1.15	0.9234 0.752 0.8395 0.7357 0.005008 0 95% LCL 1.287 1.052	0.9686 0.908 0.9245 0.9683 0.215 0 95% UCL 1.391 1.248	Median 0.94 0.85 0.88 0.89 0.09 0 Median 1.323 1.173	0.93 0.72 0.85 0.7 0.03 0 Min 1.303 1.013	0.97 0.87 0.93 0.94 0.23 0 Max 1.397 1.202	0.008124 0.02811 0.0153 0.04188 0.03782 0 Std Err 0.01883 0.03516	1.92% 7.57% 3.88% 10.99% 76.87% CV% 3.14% 6.84%	0.0% 12.26% 6.77% 9.94% 88.37% 100.0% %Effect 0.0% 14.13%
С-µg/L Control 0 Lab Co 10 20 40 80 160 40 Angular (Corrected) Control 0 Lab Co 10 20	Type Countrol 5	0.946 0.83 0.882 0.852 0.11 0 Immary t Mean 1.339 1.15 1.223	0.9234 0.752 0.8395 0.7357 0.005008 0 95% LCL 1.287 1.052 1.155	0.9686 0.908 0.9245 0.9683 0.215 0 95% UCL 1.391 1.248 1.291	Median 0.94 0.85 0.88 0.89 0.09 0 Median 1.323 1.173 1.217	0.93 0.72 0.85 0.7 0.03 0 Min 1.303 1.013 1.173	0.97 0.87 0.93 0.94 0.23 0 Max 1.397 1.202 1.303	0.008124 0.02811 0.0153 0.04188 0.03782 0 Std Err 0.01883 0.03516 0.0246	1.92% 7.57% 3.88% 10.99% 76.87% CV% 3.14% 6.84% 4.5%	0.0% 12.26% 6.77% 9.94% 88.37% 100.0% %Effect 0.0% 14.13% 8.67%

CETIS Analytical Report

Report Date: Test Code: 22 Sep-17 15:47 (p 2 of 2) 170916sprt | 08-9569-1329

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) Analysis ID: 17-0919-7416 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 22 Sep-17 15:44 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 1.0 F 0.20 0.9 0.15 Reject Null Fertilization Rate 0.10 0.7 0.6 0.5 0.4 -0.05 -0.10 0.2 -0.15 0.1 0.0 -0.20 0 LC 10 20 80 160 -2.5 -2.0 -1.5 -1.0 -0.5 0.0 C-µg/L Rankits

CETIS Analytical Report

Report Date:

22 Sep-17 15:47 (p 1 of 1)

Test Code:

170916sprt | 08-9569-1329

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 19-6375-1112 Analyzed:

22 Sep-17 15:44

Endpoint: Fertilization Rate Analysis:

Trimmed Spearman-Kärber

CETIS Version: Official Results:

CETISv1.8.7

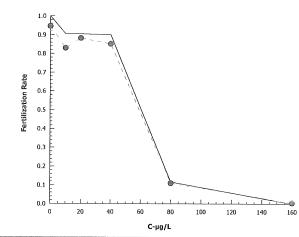
Yes

Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.054	9.51%	1.756	0.006577	56.97	55.27	58.72

Fertilization	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	0.946	0.93	0.97	0.008124	0.01817	1.92%	0.0%	473	500
10		5	0.83	0.72	0.87	0.02811	0.06285	7.57%	12.26%	415	500
20		5	0.882	0.85	0.93	0.0153	0.03421	3.88%	6.77%	441	500
40		5	0.852	0.7	0.94	0.04188	0.09365	10.99%	9.94%	426	500
80		5	0.11	0.03	0.23	0.03782	0.08456	76.87%	88.37%	55	500
160		5	0	0	0	0	0		100.0%	0	500

Graphics



Report Date:

22 Sep-17 15:46 (1 of 1)

Echinoid Sperm Cell Fertilization Test 15C

Mean:

Sigma:

49.71

16.47

Count:

CV:

20

33.10%

Nautilus Environmental (CA)

Test Type: Fertilization

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

Organism: Strongylocentrotus purpuratus (Purpl

Endpoint: Fertilization Rate

Source:

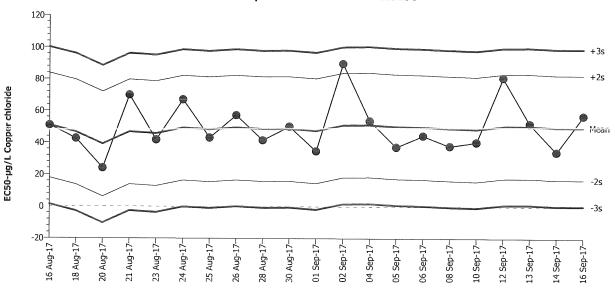
Material: Copper chloride

-3s Action Limit: 0.3036

+3s Action Limit: 99.12

Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



-2s Warning Limit:

+2s Warning Limit:

16.77

82.65

Quality Control Data											
Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	16	16:34	50.82	1.107	0.06721			16-3259-1018	06-7497-1035
2			18	14:09	42.53	-7.179	-0.4359			12-6613-4538	02-2322-5589
3			20	14:52	24.05	-25.66	-1.558			06-9655-0092	05-8785-3700
4			21	14:46	69.95	20.24	1.229			08-4756-2919	20-2992-4955
5			23	16:14	41.72	-7.993	-0.4853			02-7595-3678	15-3490-2746
6			24	16:11	67.1	17.39	1.056			04-7651-5518	20-0883-0005
7			25	14:48	43.11	-6.6	-0.4008			06-8816-1100	09-0830-4014
8			26	16:00	57.24	7.531	0.4573			10-2039-5656	15-8794-0305
9			28	14:56	41.55	-8.158	-0.4953			08-1525-2751	10-7829-2432
10			30	16:38	50.21	0.5019	0.03047			08-1199-3706	11-0543-3886
11		Sep	1	15:27	34.79	-14.92	-0.9056			13-1244-6646	21-1567-7550
12			2	10:53	89.99	40.28	2.446	(+)		16-4202-9692	18-8681-1855
13			4	16:10	53.77	4.062	0.2466			12-2973-1405	10-6032-1229
14			5	17:07	37.36	-12.35	-0.7499			13-1627-7974	14-5447-1160
15			6	17:15	44.41	-5.297	-0.3216			05-5533-8557	16-8161-1582
16			8	15:48	37.91	-11.8	-0.7163			18-6871-7794	04-4479-5076
17			10	14:25	40.4	-9.308	-0.5651			11-6871-9499	08-4248-1228
18			12	15:51	81.07	31.36	1.904			20-0603-9450	06-1182-7961
19			13	19:07	52.04	2.326	0.1412			01-4575-6189	02-4618-7964
20			14	15:24	34.24	-15.47	-0.9396			11-2846-3680	13-8128-7168
21			16	17:08	56.97	7.26	0.4408			08-9569-1329	19-6375-1112

CETIS Test Data Worksheet

Report Date: Test Code:

15 Sep-17 16:37 (p 1 of 1)

08-9569-1329/170916sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: End Date:

16 Sep-17 16 Sep-17

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

Sample Code: Sample Source: Reference Toxicant

170916sprt

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	loride Sample Station: Copper Chloride Notes
			1	100		
			2	100	93	JS 9/21/17
			3		93	
					87	
			4		90	
			5		4	
			6		85	
			7		85 23 93	
			8		93	
			9		85	
			10		9	
			11		Ö	
			12		\cap	
			13		90	
			14		39	
			15		90 89 85	
	T		16		0	
			17		83	
			18			
			19		94	
			20	_	77	
			21		96	
			22		72 96 3	
			23		-	
			24		88 16	
	-		25		7)	
			26			
			27		37	
	+		28		84	
					97	
	-		29 30		70 94	

CETIS Test Data Worksheet

Report Date: Test Code:

15 Sep-17 16:37 (p 1 of 1) 08-9569-1329/170916sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 16 Sep-17 End Date: Sample Date: 16 Sep-17

16 Sep-17

Species: Strongylocentrotus purpuratus

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

Sample Code: Sample Source: Reference Toxicant

170916sprt

Sample Station: Copper Chloride

ample Date					ar. Copper chior	Sample Station: Copper Chloride
C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	30		-	
0	LC	2	2			
0	LC	3	28			
0	LC	4	8			
0	LC	5	21	100	99	AD 9/16/17
10		1	6	1		
10		2	26			
10		3	3	100	84	
10		4	27			
10		5	20	100	82	
20		1	15			
20		2	9			
20		3	23			
20		4	1			
20		5	4	100	90	
40		1	18		•	
40		2	29			
40		3	17			
40		4	14	100	90	
40		5	13	100	86	
80		1	7			
80		2	22			
80		3	5			
80		4	24			
80		5	10	100	29	
160		1	16			
160		2	11			
160		3	25			
160		4	19			
160		5	12	100	0	

OC: AD

Analyst: 400 QA409/28/11

Marine Chronic Bioassay

Water Quality Measurements

@11 /
Client ·

Internal

Test Species: S. purpuratus

Sample ID:

CuCl₂

8 O F Start Date/Time: 9/16/2017

Test No:

170916sprt

End Date/Time: 9/16/2017

Dilutions made by:

High conc. made (μg/L):

160

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

7.8 500 10200

Cu stock concentration (µg/L):

Analyst:

21 Initial Readings DO Concentration рΗ Salinity Temperature (μg/L) (mg/L) (units) (°C) (ppt) Lab Control 8.6 8.13 14.8 33.4 8,5 14.8 10 33.7 8.09 33.6 14.7 20 8.4 8.09 14.6 8.4 40 33.5 8,09 8.4 80 8.09 33.4 14.6 33.5 8.4 8.09 14.6 160

Comments:		
QC Check:	EG 9/22/17	Final Review: AC 9/28/17

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

Marine Chronic Bioassay

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

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Sample ID: Test No.:	Internal CuClz 170916sprt		 	End Date/Tii Spec	me: 9/16/2017 / 1708 me: 9/16/2017 / 1748 iess. Jannary
Tech initials: Injection Time:	77D 11025			Animal Sour	
Sperm Absorbance at 4	00 nm: <u>0.95</u>	(target range of	0.8 - 1.0 for dens	sity of 4x10 ⁶ sperm/	ml)
Eggs Counted:	$\frac{97}{100}$ (ta)	ean: A x street counts of 80 eggs fiter slide for a final den	per vertical pass o	n Sedgwick-	
Initial density: Final density:	4000 eggs/m	- 1.0 par	ution factor t egg stock ts seawater	egg stock	<u>00 </u>
Prepare the embryo sto existing stock (1 part) ar			T. For example, it	f the dilution factor is	s 2.25, use 100 ml of
Rangefinder Test: ml Sperm Stock ml Seawater	50	00:1 1200:1 40 30 10 20	20	atio 00:1 10 5.0 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 1435 1450 1700	Rangefinder Rat	io: <u>Fert.</u> 77 81,90	Unfert. 23 19110 4	
NOTE: Choose a spern this range, choose the ro organism health, stage of	atio closest to 90 perce	ent unless professiona	veen 80 and 90 p al judgment dicta	ercent. If more thar tes consideration of	n one concentration is within fother factors (e.g.,
Definitive Test		Sperm:Egg Ratio	o Used: <u> 50</u>	,)	
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1108 1128 1148	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 90	Unfert. 12 10 100 100	
Comments:					
QC Check:	EG 9/22/	17		Final Revie	ew: AC 9/28/17

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