Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: Daily M-001
Sample Collection Date: April 16, 2017

Prepared for: **IDE AMERICAS, Inc.**
4590 Carlsbad Boulevard
Carlsbad, CA 92008

Prepared by: **Nautilus Environmental**

Submitted: **May 5, 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.
- All test results have met internal Quality Assurance Program requirements.

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Results verified by: Adrienne Cibor

EXECUTIVE SUMMARY

DAILY CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – APRIL 2017

ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: **April 16, 2017**

Test Date: **April 17, 2017**

Sample ID: **Daily M-001 Effluent (Shutdown Period)**

Effluent Limitation: **16.5 TU_c**

Results Summary:

Bioassay Type: Urchin Fertilization	Test Date	Effluent Test Results		Effluent Limitation Met? (Yes/No)
		NOEC	TU_c	
	4/17/2017	15	<6.67	Yes

INTRODUCTION

A discharge sample was collected in April 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a time of plant shut down. Daily 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 April 17, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The composite sample was collected on April 16, 2017. 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 4º 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: Daily M-001

Monitoring Period: April 2017 (shutdown period)

Sample Material: Facility Effluent

Sampling Method: Composite

Sample Collection Date, Time: 4/16/17, 08:00

Sample Receipt Date, Time: 4/17/17, 12:28

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

Sample Collection Date	pH	DO (mg/L)	Temp (ºC)	Salinity (ppt)	Alkalinity (mg/L as $CaCO_3$)	Total Chlorine (mg/L)
4/16/17	7.53	8.2	2.0	34.2	120	< 0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	4/17/17, 17:03 through 17:43
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
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent unadjusted 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
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 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 (TU_c) 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, and 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.

RESULTS

There was no statistically significant decrease in the fertilization rate in any of the sample concentrations tested compared to that in the lab control. Therefore, the NOEC is reported as 15 percent effluent and a TU_c of less than 6.67, which is below the permit limit of 16.5. Additionally, none of the effluent concentrations tested were statistically significant using to the TST calculation. 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 Daily M-001 Purple Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC_{50} (% sample)	TU_c value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
Daily M-001	15	>15	>15	<6.67	Pass	0.85

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 Daily M-001 Sample

Test Concentration (% Effluent)	Mean Percent Fertilization
Lab Control	94.0
2.5	91.6
5.0	94.4
6.06	93.2
10	91.0
15	90.4

QUALITY ASSURANCE

The sample was received on the day after collection 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 the dose-response relationship was reviewed to ensure the reliability of the data. Based on the dose response 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 (EC_{50}) 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 (%)
4/17/17	51.7	55.5 ± 44.6	40.1

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

REFERENCES

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

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

Batch ID:	14-8040-3535	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	17 Apr-17 17:03	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	17 Apr-17 17:43	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	17-0562-6790	**Code:**	17-0467	**Client:**	IDE
Sample Date:	16 Apr-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	17 Apr-17 12:28	**Source:**	IDE Americas, Inc.		
Sample Age:	33h (2 °C)	**Station:**	M-001 (Daily)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-8538-5865	Fertilization Rate	15	>15	NA	2.41%	6.667	TST-Welch's t Test
02-8829-6941	Fertilization Rate	15	>15	NA	4.65%	6.667	Dunnett Multiple Comparison Test

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-8829-6941	Fertilization Rate	Control Resp	0.94	0.7 - NL	Yes	Passes Acceptability Criteria
14-8538-5865	Fertilization Rate	Control Resp	0.94	0.7 - NL	Yes	Passes Acceptability Criteria
02-8829-6941	Fertilization Rate	PMSD	0.04646	NL - 0.25	No	Passes Acceptability Criteria
14-8538-5865	Fertilization Rate	PMSD	0.02415	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.94	0.9038	0.9762	0.9	0.98	0.01304	0.02915	3.1%	0.0%
2.5		5	0.916	0.8771	0.9549	0.87	0.95	0.014	0.0313	3.42%	2.55%
5		5	0.944	0.9154	0.9726	0.92	0.97	0.0103	0.02302	2.44%	-0.43%
6.06		5	0.932	0.8933	0.9707	0.89	0.96	0.01393	0.03114	3.34%	0.85%
10		5	0.91	0.8635	0.9565	0.88	0.97	0.01673	0.03742	4.11%	3.19%
15		5	0.904	0.8814	0.9266	0.88	0.93	0.008124	0.01817	2.01%	3.83%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.98	0.94	0.9	0.95	0.93
2.5		0.87	0.93	0.95	0.9	0.93
5		0.95	0.92	0.96	0.97	0.92
6.06		0.89	0.96	0.91	0.94	0.96
10		0.88	0.92	0.9	0.97	0.88
15		0.9	0.88	0.93	0.91	0.9

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

Analysis ID:	02-8829-6941	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 May-17 18:07	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.65%	15	>15	NA	6.667

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	1.343	2.362	0.087	8	0.2785	CDF	Non-Significant Effect
		5	-0.1626	2.362	0.087	8	0.8769	CDF	Non-Significant Effect
		6.06	0.4811	2.362	0.087	8	0.6552	CDF	Non-Significant Effect
		10	1.552	2.362	0.087	8	0.2075	CDF	Non-Significant Effect
		15	1.985	2.362	0.087	8	0.1021	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02646728	0.005293456	5	1.563	0.2085	Non-Significant Effect
Error	0.08130507	0.003387711	24			
Total	0.1077724		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.746	15.09	0.7391	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9673	0.9031	0.4672	Normal Distribution

Fertilization Rate Summary

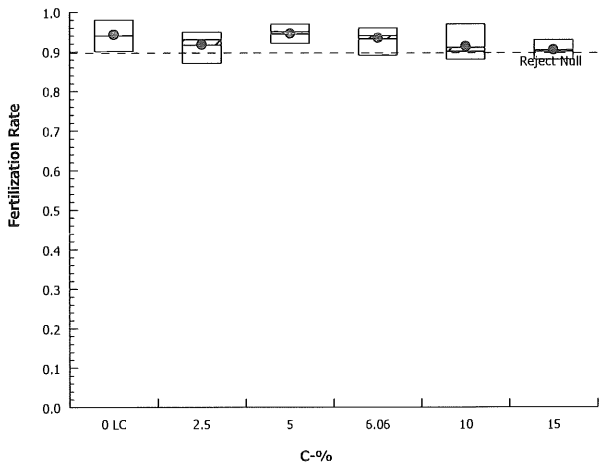
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.94	0.9038	0.9762	0.94	0.9	0.98	0.01304	3.1%	0.0%
2.5		5	0.916	0.8771	0.9549	0.93	0.87	0.95	0.014	3.42%	2.55%
5		5	0.944	0.9154	0.9726	0.95	0.92	0.97	0.0103	2.44%	-0.43%
6.06		5	0.932	0.8933	0.9707	0.94	0.89	0.96	0.01393	3.34%	0.85%
10		5	0.91	0.8635	0.9565	0.9	0.88	0.97	0.01673	4.11%	3.19%
15		5	0.904	0.8814	0.9266	0.9	0.88	0.93	0.008124	2.01%	3.83%

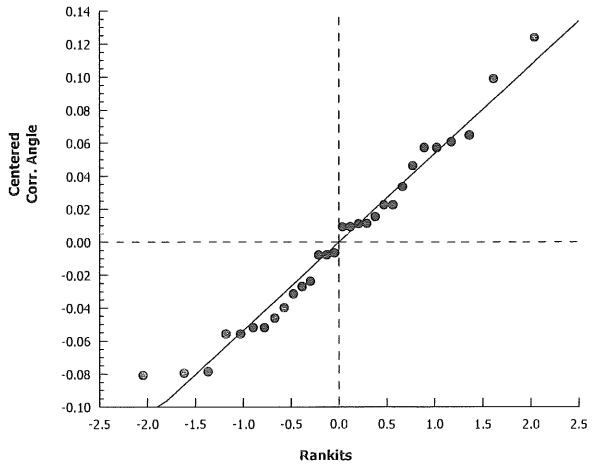
Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.33	1.248	1.412	1.323	1.249	1.429	0.02944	4.95%	0.0%
2.5		5	1.28	1.211	1.35	1.303	1.202	1.345	0.02487	4.34%	3.72%
5		5	1.336	1.273	1.399	1.345	1.284	1.397	0.02268	3.8%	-0.45%
6.06		5	1.312	1.236	1.389	1.323	1.233	1.369	0.02749	4.69%	1.33%
10		5	1.273	1.18	1.365	1.249	1.217	1.397	0.03336	5.86%	4.3%
15		5	1.257	1.218	1.296	1.249	1.217	1.303	0.01401	2.49%	5.49%

Echinoid Sperm Cell Fertilization Test 15C					Nautilus Environmental (CA)
Analysis ID:	02-8829-6941	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 May-17 18:07	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





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

Analysis ID: 14-8538-5865 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 03 May-17 18:07 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	2.41%	15	>15	NA	6.667

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	8.509	1.895	0.063	7	<0.0001	CDF	Non-Significant Effect
		5*	10.69	1.895	0.06	7	<0.0001	CDF	Non-Significant Effect
		6.06*	8.926	1.895	0.067	7	<0.0001	CDF	Non-Significant Effect
		10*	6.882	1.943	0.078	6	0.0002	CDF	Non-Significant Effect
		15*	9.92	1.943	0.051	6	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02646728	0.005293456	5	1.563	0.2085	Non-Significant Effect
Error	0.08130507	0.003387711	24			
Total	0.1077724		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.746	15.09	0.7391	Equal Variances
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Fertilization Rate Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.94	0.9038	0.9762	0.94	0.9	0.98	0.01304	3.1%	0.0%
2.5		5	0.916	0.8771	0.9549	0.93	0.87	0.95	0.014	3.42%	2.55%
5		5	0.944	0.9154	0.9726	0.95	0.92	0.97	0.0103	2.44%	-0.43%
6.06		5	0.932	0.8933	0.9707	0.94	0.89	0.96	0.01393	3.34%	0.85%
10		5	0.91	0.8635	0.9565	0.9	0.88	0.97	0.01673	4.11%	3.19%
15		5	0.904	0.8814	0.9266	0.9	0.88	0.93	0.008124	2.01%	3.83%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.33	1.248	1.412	1.323	1.249	1.429	0.02944	4.95%	0.0%
2.5		5	1.28	1.211	1.35	1.303	1.202	1.345	0.02487	4.34%	3.72%
5		5	1.336	1.273	1.399	1.345	1.284	1.397	0.02268	3.8%	-0.45%
6.06		5	1.312	1.236	1.389	1.323	1.233	1.369	0.02749	4.69%	1.33%
10		5	1.273	1.18	1.365	1.249	1.217	1.397	0.03336	5.86%	4.3%
15		5	1.257	1.218	1.296	1.249	1.217	1.303	0.01401	2.49%	5.49%

CETIS Test Data Worksheet

Report Date: 17 Apr-17 12:47 (p 1 of 1)
Test Code: 1704-5085 02-9246-1285/116E9AE5

Echinoid Sperm Cell Fertilization Test 15C			Nautilus Environmental (CA)
Start Date: 17 Apr-17	**Species:** Strongylocentrotus purpuratus	**Sample Code:** ~~65A9CCA6~~ 17-0467	
End Date: 17 Apr-17	**Protocol:** EPA/600/R-95/136 (1995)	**Sample Source:** IDE Americas, Inc.	
Sample Date: 16 Apr-17	**Material:** Facility Effluent	**Sample Station:** M-001 ~~Unadjusted~~ Dark	

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	96	AG 5/3/17
			32	100	95	
			33	100	93	
			34	100	98	
			35	100	92	
			36	100	96	
			37	100	96	
			38	100	97	
			39	100	90	
			40	100	93	
			41	100	94	
			42	100	91	
			43	100	89	
			44	100	93	
			45	100	90	
			46	100	94	
			47	100	88	
			48	100	90	
			49	100	88	
			50	100	95	
			51	100	91	
			52	100	93	
			53	100	95	
			54	100	88	
			55	100	87	
			56	100	92	
			57	100	92	
			58	100	97	
			59	100	90	
			60	100	96	

Seen 5/3/17

CETIS Test Data Worksheet

Report Date: 17 Apr-17 12:48 (p 1 of 1) (A)
Test Code: 02-9246-1285/~~116E9AE5~~ 1704-5085

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

Start Date: 17 Apr-17
End Date: 17 Apr-17
Sample Date: 16 Apr-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: (A) ~~65A9CCA6~~ 17-0467
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (A) ~~Unadjusted~~ Daily

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	34	100	96	AD 4/17/17
0	LC	2	46			
0	LC	3	48			
0	LC	4	32			
0	LC	5	52			
2.5		1	55	100	93	
2.5		2	40			
2.5		3	50			
2.5		4	37			
2.5		5	44			
5		1	53	100	93	
5		2	57			
5		3	31			
5		4	38			
5		5	56			
6.06		1	43	100	93	
6.06		2	60			
6.06		3	51			
6.06		4	41			
6.06		5	36			
10		1	47			
10		2	35			
10		3	39			
10		4	58			
10		5	49	100	94	
15		1	59			
15		2	54			
15		3	33			
15		4	42			
15		5	45	100	94	

QC = RH

(A) EG Q18 5/3/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: Daily M-001 | **Start Date/Time:** 4/17/2017 1703

Sample Log No.: 17- 0467 | **End Date/Time:** 4/17/2017 1743

Dilutions made by: AGOBOAD | **Test No:** 1704-5085

Analyst: AG

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.9	7.91 ~~33~~ AG 4/17/17	33.5	15.2
2.5	8.2	7.90	33.7	15.5
5.0	8.3	7.90	33.8	15.4
6.06	8.4	7.90	33.8	15.3
10	8.5	7.90	33.7	15.1
15	8.2	7.86	33.5	15.2

Comments:

QC Check: EG 5/3/17 | **Final Review:** 5/3/17

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

Client: IDE
Sample ID: M-001
Test No.: 1704-5085

Start Date/Time: 4/17/2017 / 1703
End Date/Time: 4/17/2017 / 1743
Species: S. purpuratus
Animal Source: PL 'Iona
Date Collected: 3/27/17

Tech initials: AD
Injection Time: 1625

Sperm Absorbance at 400 nm: 0.877 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 92, 103, 90, 90, 111

Mean: 97.2 X 50 = 4860 eggs/ml

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

Initial density: 4860 eggs/ml = 1.22 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
.22 parts seawater

egg stock 100 ml
seawater 22 ml

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

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1634	50:1	79	21
Eggs Added (0.5 ml):	1648	100:1	92,96	8,4
Test Ended:	1658	—	—	—
		—	—	—

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test

Sperm:Egg Ratio Used: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1703	QC1	90	10
Eggs Added (0.5 ml):	1723	QC2	97	3
Test Ended:	1743	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: EG 5/3/17

Final Review: 5/3/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Sample Check-In Information

Client: IDE

Tests Performed: Urchin Fertilization

Project: Daily M-001

Test ID No.(s): 1704-5085 to -5086

Sample ID:	1) Daily M-001	2)	3	4)
Log-in No. (17-xxxx):	0467			
Sample Collection Date & Time:	4/16/17 0800			
Sample Receipt Date & Time:	4/17/17 1228			
Number of Containers & Container Type:	2, 4L wbi			
Approx. Total Volume Received (L):	~6 L			
Check-in Temp (°C)	2.0			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.2			
pH (units)	7.53			
Conductivity (µS/cm)	–			
Salinity (ppt)	34.2			
Alkalinity (mg/L) [2]	120			
Hardness (mg/L) [2, 3]	–			
Total Chlorine (mg/L)	<0.02			
Technician Initials	AG			

Sample Descriptions:

1) colorless, clear, odorless, light debris
2)
3)
4)

COC Complete? (Y) N

Filtration? Y (N)

Pore Size: _____

Organisms or Debris

pH Adjustment? Y (N)

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

Freshwater Tests:

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

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

Marine Tests: Urchin Fertilization

Control/Dilution Water Source: (LAB SW) ART SW Other: _____ Alkalinity: 108 Salinity: 34 ppt

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

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

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

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

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

Additional Comments _____

Subsamples For Additional Chemistry Required? Y (N)

NH3 Other _____

Tech Initials _____

QC Check: CH 4/17/17

Final Review: 4/5/17

Appendix C

Chain-of-Custody Form

IDE Technologies

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

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

Project Name:____NPDES Daily Toxicity_____Project Manager:____Peter Shen_____Contact Information:____(760) 201-7777_____

Special instruction: 48 hour composite sample collected via autosampler by a series of consecutive grabs at 1 hr intervals during plant off-spec (pretreatment only). Sample collected to fulfill daily NPDES requirements. Sample is to be run unadjusted. Start: 4/14/17 @ 08:00, End: 4/16/17 @ 08:00. KC

Glass=G Plastic=P

Yes=Y No=N Acid=A Base=B

Drinking Water=DW Seawater=SW Soil=S Brine=B

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization								NOTES:
M-001 (17- 1456)	4/14-16/17	8:00	48 HR COMP -B	N	2 X 4L CUBIE	X								TDS - 32.06 ppt EC - 49.89 mS/cm

Relinquished By:	Date:	Time:		Received By:	Time:	Sample Condition Upon Receipt:
Kevin Cheung	4/17/17	1000		[signature]	4/17/17 1000	☐ Iced ☐ Ambient or _____ °C
[signature]	4/17/17	1228		[signature]	4/17/17 1228	☐ Iced ☐ Ambient or 2.0 °C

Nautilus ID: 17-0467

2.0 @ receipt temp

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	12-9208-3902	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	17 Apr-17 17:03	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	17 Apr-17 17:43	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	00-7581-5056	**Code:**	170417sprt	**Client:**	Internal
Sample Date:	17 Apr-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	17 Apr-17	**Source:**	Reference Toxicant		
Sample Age:	17h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-2520-4213	Fertilization Rate	<10	10	NA	4.15%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
11-5239-6650	Fertilization Rate	EC50	51.69	49.33	54.17		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
11-5239-6650	Fertilization Rate	Control Resp	0.956	0.7 - NL	Yes	Passes Acceptability Criteria
14-2520-4213	Fertilization Rate	Control Resp	0.956	0.7 - NL	Yes	Passes Acceptability Criteria
14-2520-4213	Fertilization Rate	PMSD	0.04152	NL - 0.25	No	Passes Acceptability Criteria

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.956	0.9334	0.9786	0.94	0.98	0.008124	0.01817	1.9%	0.0%
10		5	0.84	0.7961	0.8839	0.79	0.88	0.01581	0.03536	4.21%	12.13%
20		5	0.85	0.8027	0.8973	0.8	0.9	0.01703	0.03808	4.48%	11.09%
40		5	0.692	0.6148	0.7692	0.64	0.79	0.02782	0.06221	8.99%	27.62%
80		5	0.192	0.1221	0.2619	0.13	0.26	0.02518	0.0563	29.32%	79.92%
160		5	0	0	0	0	0	0	0		100.0%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.98	0.97	0.94	0.95	0.94
10		0.88	0.86	0.79	0.82	0.85
20		0.9	0.83	0.85	0.87	0.8
40		0.71	0.64	0.79	0.64	0.68
80		0.14	0.13	0.23	0.2	0.26
160		0	0	0	0	0

Fertilization Rate Binomials

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	98/100	97/100	94/100	95/100	94/100
10		88/100	86/100	79/100	82/100	85/100
20		90/100	83/100	85/100	87/100	80/100
40		71/100	64/100	79/100	64/100	68/100
80		14/100	13/100	23/100	20/100	26/100
160		0/100	0/100	0/100	0/100	0/100

CETIS Analytical Report

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

Analysis ID:	14-2520-4213	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Apr-17 9:26	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes
Batch ID:	12-9208-3902	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	17 Apr-17 17:03	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	17 Apr-17 17:43	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.15%	<10	10	NA	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	5.415	2.305	0.086	8	<0.0001	CDF	Significant Effect
		20*	5.029	2.305	0.086	8	0.0001	CDF	Significant Effect
		40*	10.14	2.305	0.086	8	<0.0001	CDF	Significant Effect
		80*	24.41	2.305	0.086	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	2.438415	0.6096036	4	174.3	<0.0001	Significant Effect
Error	0.06996133	0.003498067	20			
Total	2.508376		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	1.201	13.28	0.8779	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9603	0.8877	0.4196	Normal Distribution

Fertilization Rate Summary

C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.956	0.9334	0.9786	0.95	0.94	0.98	0.008124	1.9%	0.0%
10		5	0.84	0.7961	0.8839	0.85	0.79	0.88	0.01581	4.21%	12.13%
20		5	0.85	0.8027	0.8973	0.85	0.8	0.9	0.01703	4.48%	11.09%
40		5	0.692	0.6148	0.7692	0.68	0.64	0.79	0.02782	8.99%	27.62%
80		5	0.192	0.1221	0.2619	0.2	0.13	0.26	0.02518	29.32%	79.92%

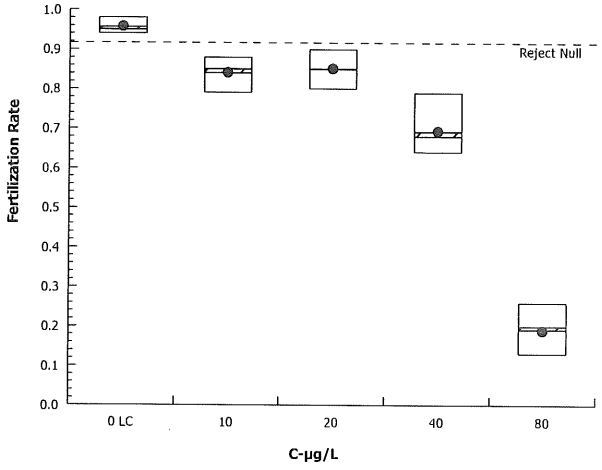
Angular (Corrected) Transformed Summary

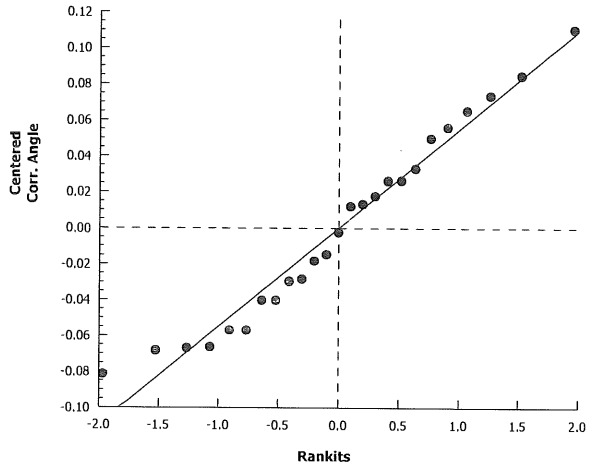
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.364	1.305	1.422	1.345	1.323	1.429	0.02114	3.47%	0.0%
10		5	1.161	1.102	1.22	1.173	1.095	1.217	0.02141	4.12%	14.85%
20		5	1.175	1.108	1.242	1.173	1.107	1.249	0.02415	4.6%	13.8%
40		5	0.9842	0.8981	1.07	0.9695	0.9273	1.095	0.03101	7.05%	27.82%
80		5	0.4503	0.3604	0.5401	0.4636	0.3689	0.5351	0.03236	16.07%	66.98%

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

Analysis ID:	14-2520-4213	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Apr-17 9:26	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	11-5239-6650	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Apr-17 9:26	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes
Batch ID:	12-9208-3902	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	17 Apr-17 17:03	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	17 Apr-17 17:43	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

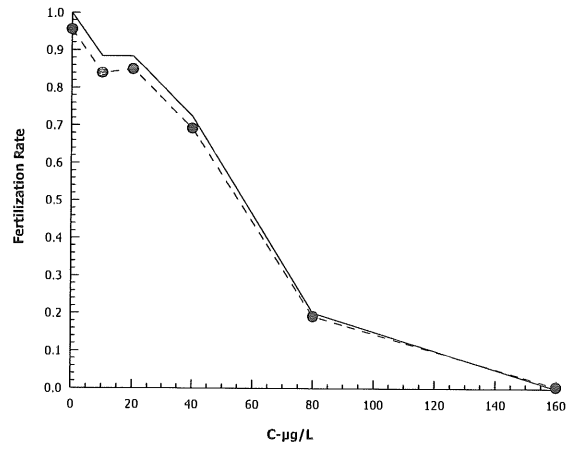
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.044	11.61%	1.713	0.01018	51.69	49.33	54.17

Fertilization Rate Summary

			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.956	0.94	0.98	0.008124	0.01817	1.9%	0.0%	478	500
10		5	0.84	0.79	0.88	0.01581	0.03536	4.21%	12.13%	420	500
20		5	0.85	0.8	0.9	0.01703	0.03808	4.48%	11.09%	425	500
40		5	0.692	0.64	0.79	0.02782	0.06221	8.99%	27.62%	346	500
80		5	0.192	0.13	0.26	0.02518	0.0563	29.32%	79.92%	96	500
160		5	0	0	0	0	0		100.0%	0	500

Graphics

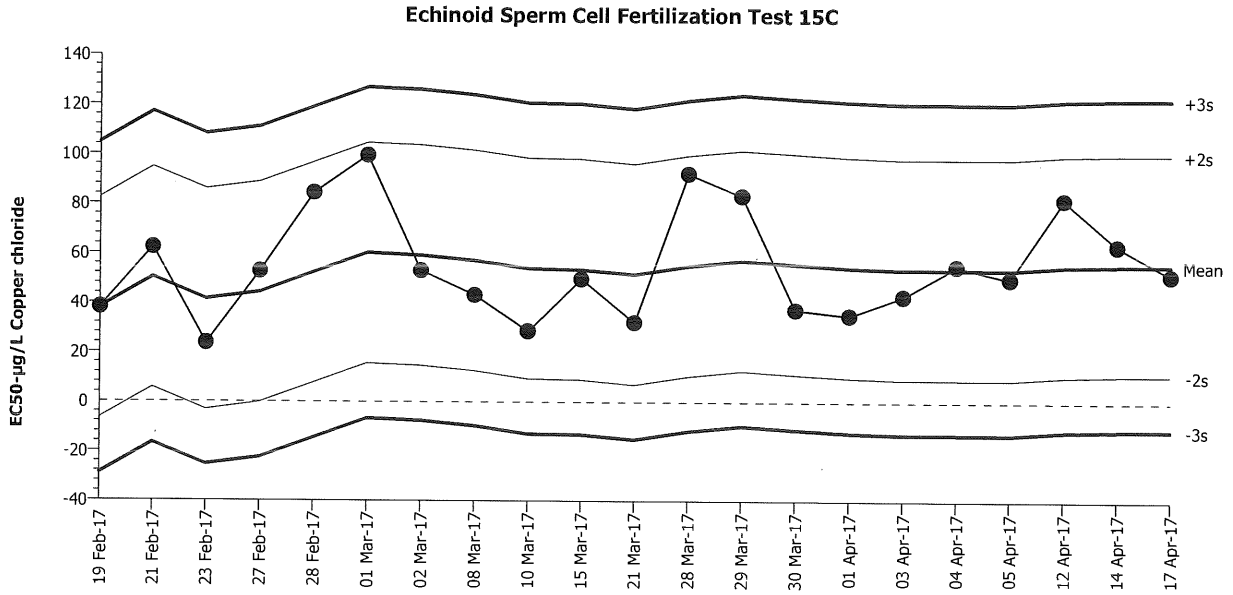


CETIS QC Plot

Report Date: 26 Apr-17 09:27 (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



Mean:	55.53	**Count:**	20	**-2s Warning Limit:**	10.97	**-3s Action Limit:**	-11.31
Sigma:	22.28	**CV:**	40.10%	**+2s Warning Limit:**	100.1	**+3s Action Limit:**	122.4

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Feb	19	16:00	38.18	-17.35	-0.7789			04-9561-8356	16-1145-1366
2			21	11:42	62.44	6.91	0.3101			15-6576-1294	19-2980-3814
3			23	14:42	23.77	-31.76	-1.425			07-0628-7264	20-4334-6940
4			27	16:05	52.9	-2.631	-0.1181			10-1635-1121	14-4530-4128
5			28	18:00	84.51	28.98	1.301			09-8043-1931	05-2317-8363
6		Mar	1	15:45	99.56	44.03	1.976			17-5791-9592	08-2085-2833
7			2	15:26	53.09	-2.436	-0.1093			20-3729-5626	20-9062-5332
8			8	12:50	43.2	-12.33	-0.5532			10-8438-6969	12-4014-5220
9			10	14:18	28.71	-26.82	-1.204			05-2038-2100	05-9725-9024
10			15	15:10	49.84	-5.694	-0.2556			06-7728-1272	00-9516-8529
11			21	11:03	32.23	-23.3	-1.046			15-4686-0543	08-4903-8267
12			28	15:45	92.34	36.81	1.652			19-7829-0165	07-5461-2916
13			29	11:50	83.41	27.88	1.251			05-8182-1994	00-0525-0185
14			30	16:20	37.46	-18.07	-0.8111			04-2787-9157	21-1957-9518
15		Apr	1	11:49	35.15	-20.38	-0.9149			01-0869-3000	14-7307-9005
16			3	17:15	42.93	-12.6	-0.5654			11-7488-2003	08-5368-9216
17			4	12:13	55.13	-0.4028	-0.01808			02-3186-4899	18-3488-7750
18			5	14:33	50.05	-5.479	-0.2459			12-8691-4512	16-6546-2933
19			12	16:30	82.12	26.59	1.193			04-6122-9881	08-0232-5024
20			14	15:34	63.58	8.054	0.3615			06-7326-1133	06-6067-9028
21			17	17:03	51.69	-3.836	-0.1722			13-4494-7236	11-5239-6650

CETIS Test Data Worksheet

Report Date: 17 Apr-17 12:47 (p 1 of 1)
Test Code: 13-4494-7236/170417sprt

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

Start Date: 17 Apr-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 170417sprt
End Date: 17 Apr-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 17 Apr-17 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	95	TN 4/19/17
			2	100	20	
			3	100	85	
			4	100	87	
			5	100	0	
			6	100	14	
			7	100	26	
			8	100	86	
			9	100	64	
			10	100	0	
			11	100	0	TN 4/21/17
			12	100	79	
			13	100	90	
			14	100	88	
			15	100	79	
			16	100	0	
			17	100	94	
			18	100	13	
			19	100	82	
			20	100	80	
			21	100	97	
			22	100	83	
			23	100	71	
			24	100	0	
			25	100	23	
			26	100	98	
			27	100	64	
			28	100	85	
			29	100	94	
			30	100	68	

CETIS Test Data Worksheet

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

Start Date: 17 Apr-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 170417sprt
End Date: 17 Apr-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** Reference Toxicant
Sample Date: 17 Apr-17 | **Material:** Copper chloride | **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	26	100	94	AD 4/17/17
0	LC	2	21			
0	LC	3	17			
0	LC	4	1			
0	LC	5	29			
10		1	14			
10		2	8	100	88	
10		3	12			
10		4	19			
10		5	28			
20		1	13	100	93	
20		2	22			
20		3	3			
20		4	4			
20		5	20			
40		1	23	100	79	
40		2	27			
40		3	15			
40		4	9			
40		5	30			
80		1	6	100	5	
80		2	18			
80		3	25			
80		4	2			
80		5	7			
160		1	11	100	0	
160		2	24			
160		3	10			
160		4	16			
160		5	5			

QC: AD

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: $CuCl_2$ | **Start Date/Time:** 4/17/2017 1703

Test No: 170417sprt | **End Date/Time:** 4/17/2017 1743

Dilutions made by: AG OBO AD

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10200

Analyst: AG

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.3	7.90	33.5	14.9
10	8.3	7.85	33.7	15.3
20	8.2	7.84	33.8	15.4
40	8.1	7.84	33.7	15.5
80	8.1	7.86	33.7	15.6
160	8.1	7.87	33.5	15.6

Comments:

QC Check: AC 4/26/17 | **Final Review:** 5/3/17

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

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

Client: Internal
Sample ID: CuCl2
Test No.: 170417 Sprt

Start Date/Time: 4/17/2017 / 1703
End Date/Time: 4/17/2017 / 1743
Species: S. purpuratus
Animal Source: Pt. Loma
Date Collected: 3/27/17

Tech initials: AD
Injection Time: 1625

Sperm Absorbance at 400 nm: 0.877 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 92, 103, 90, 90, 111

Mean: 97.2 X 50 = 4860 eggs/ml

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

Initial density: 4860 eggs/ml = 1.22 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
.22 parts seawater

egg stock 100 ml
seawater 22 ml

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

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

	Time	Rangefinder Ratio:	Fert.	Unfert.
Sperm Added (100 µl):	1634	50:1	79	21
Eggs Added (0.5 ml):	1648	100:1	92.96	8.4
Test Ended:	1658	—	—	—
		—	—	—

NOTE: Choose a sperm-to-egg ratio that results in fertilization between 80 and 90 percent. If more than one concentration is within this range, choose the ratio closest to 90 percent unless professional judgment dictates consideration of other factors (e.g., organism health, stage of reproductive season, site conditions).

Definitive Test

Sperm:Egg Ratio Used: 100:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1703	QC1	90	10
Eggs Added (0.5 ml):	1723	QC2	97	3
Test Ended:	1743	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: AC 4/25/17

Final Review: 5/3/17

Appendix E

Qualifier Codes

Nautilus Environmental

Glossary of Qualifier Codes:

Q1 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log

Q2 - Temperatures out of recommended range; no action taken, test terminated same day

Q3 - Sample aerated prior to initiation or renewal due to dissolved oxygen (D.O.) levels below 6.0 mg/L

Q4 - Test aerated; D.O. levels dropped below 4.0 mg/L

Q5 - Test initiated with aeration due to an anticipated drop in D.O.

Q6 - Airline obstructed or fell out of replicate and replaced; drop in D.O. occurred

Q7 - Salinity out of recommended range

Q8 - Spilled test chamber/ Unable to recover test organism(s)

Q9 - Inadequate sample volume remaining, 50% renewal performed

Q10 - Inadequate sample volume remaining, no renewal performed

Q11 - Sample out of holding time; refer to QA section of report

Q12 - Replicate(s) not initiated; excluded from data analysis

Q13 - Survival counts not recorded due to poor visibility or heavy debris

Q14 - D.O. percent saturation was checked and was ≤ 110%

Q15 - Did not meet minimum test acceptability criteria. Refer to QA section of report.

Q16 - Percent minimum significant difference (PMSD) was below the lower bound limit for acceptability. This indicates that statistics may be over-sensitive in detecting a difference from the control due to low variability in the data set.

Q17 - Percent minimum significant difference (PMSD) was above the upper bound limit for acceptability. This indicates that statistics may be under-sensitive in detecting a difference from the control due to high variability in the data set.

Q18 - Incorrect Entry

Q19 - Illegible Entry

Q20 - Miscalculation

Q21 - Other (provide reason in comments section)

Q22 - Greater than 10% mortality observed upon receipt and/or in holding prior to test initiation. Organisms acclimated to test conditions at Nautilus and ultimately deemed fit to use for testing.

Q23 - Test organisms received at a temperature greater than 3°C outside the recommended test temperature range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.

Q24 - Test organisms received at salinity greater than 3 ppt outside of the recommended test salinity range. However, due to age-specific protocol requirements and/or sample holding time constraints, the organisms were used to initiate tests upon the day of arrival. Organisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.