Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: August 31, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **September 11, 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 Cibar

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – AUGUST 2017

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

Sampling Date: August 31, 2017

Test Date: September 1, 2017

Sample ID: M-001 (pre-treatment off-spec period)

Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	Effluent Test Results		Effluent Limitation Met? (Yes/No)
	NOEC	TU_c	
Urchin Fertilization	10	10	Yes

INTRODUCTION

A discharge sample was collected in August 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 1, 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 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:	M-001 (pre-treatment off-spec period)
Monitoring Period:	August 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	8/31/17, 08:00
Sample Receipt Date, Time:	8/31/17, 11:55

Table 2. Water Quality Measurements for the 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)
8/31/17	7.81	8.5	5.0	33.0	100	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times:	9/1/17, 15:27 through 16:07
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
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; 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

A statistically significant decrease in fertilization rate was observed in the 15 percent effluent concentration compared to the lab control. The NOEC is reported as 10 and the TU_c is equal to 10, which is below the maximum effluent limitation of 16.5 for this permit. None of the effluent concentrations were significantly reduced according to the TST analysis. 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 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**
M-001	10	15	>15	10	Pass	-0.43

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	92.0
2.5	94.6
5.0	93.2
6.06	92.4
10	89.4
15	81.2*

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

QUALITY ASSURANCE

The sample was received on the day of collection and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The control met all test acceptability criteria, and 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 (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. Purple Urchin Fertilization Reference Toxicant Test Results

Test Date	EC_{50} **(µg/L Copper)**	Historical Mean EC_{50} ±2 SD **(µg/L Copper)**	CV (%)
9/1/17	34.8	52.0 ± 37.9	36.5

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 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

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

Batch ID:	05-1217-5561	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	01 Sep-17 15:27	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	01 Sep-17 16:07	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	19-2120-2995	**Code:**	17-0961	**Client:**	IDE
Sample Date:	31 Aug-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	31 Aug-17 11:55	**Source:**	IDE Americas, Inc.		
Sample Age:	31h (5 °C)	**Station:**	M-001 (Daily)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-5773-5028	Fertilization Rate	10	15	12.25	4.3%	10	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
11-3625-2660	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-5773-5028	Fertilization Rate	Control Resp	0.92	0.7 - NL	Yes	Passes Acceptability Criteria
11-3625-2660	Fertilization Rate	Control Resp	0.92	0.7 - NL	Yes	Passes Acceptability Criteria
03-5773-5028	Fertilization Rate	PMSD	0.04299	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	0.92	0.8798	0.9602	0.88	0.95	0.01449	0.0324	3.52%	0.0%
2.5		5	0.946	0.9218	0.9702	0.92	0.97	0.008718	0.01949	2.06%	-2.83%
5		5	0.932	0.9136	0.9504	0.91	0.95	0.006633	0.01483	1.59%	-1.3%
6.06		5	0.924	0.8851	0.9629	0.89	0.97	0.014	0.0313	3.39%	-0.43%
10		5	0.894	0.8683	0.9197	0.87	0.92	0.009274	0.02074	2.32%	2.83%
15		5	0.812	0.7714	0.8526	0.77	0.86	0.01463	0.03271	4.03%	11.74%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.88	0.94	0.95	0.89	0.94
2.5		0.97	0.92	0.96	0.94	0.94
5		0.93	0.94	0.95	0.93	0.91
6.06		0.94	0.91	0.89	0.91	0.97
10		0.92	0.89	0.91	0.87	0.88
15		0.82	0.8	0.77	0.86	0.81

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

Analysis ID:	03-5773-5028	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Sep-17 12:51	**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	4.3%	10	15	12.25	10

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.707	2.362	0.071	8	0.9981	CDF	Non-Significant Effect
		5	-0.6627	2.362	0.071	8	0.9594	CDF	Non-Significant Effect
		6.06	-0.2894	2.362	0.071	8	0.9047	CDF	Non-Significant Effect
		10	1.605	2.362	0.071	8	0.1916	CDF	Non-Significant Effect
		15*	5.514	2.362	0.071	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1479219	0.02958438	5	13.24	<0.0001	Significant Effect
Error	0.05361908	0.002234128	24			
Total	0.201541		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.281	15.09	0.6568	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9741	0.9031	0.6549	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	0.92	0.8798	0.9602	0.94	0.88	0.95	0.01449	3.52%	0.0%
2.5		5	0.946	0.9218	0.9702	0.94	0.92	0.97	0.008718	2.06%	-2.83%
5		5	0.932	0.9136	0.9504	0.93	0.91	0.95	0.006633	1.59%	-1.3%
6.06		5	0.924	0.8851	0.9629	0.91	0.89	0.97	0.014	3.39%	-0.43%
10		5	0.894	0.8683	0.9197	0.89	0.87	0.92	0.009274	2.32%	2.83%
15		5	0.812	0.7714	0.8526	0.81	0.77	0.86	0.01463	4.03%	11.74%

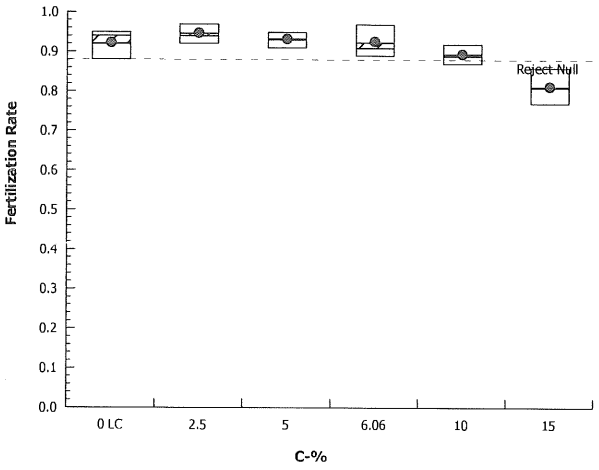
Angular (Corrected) Transformed Summary

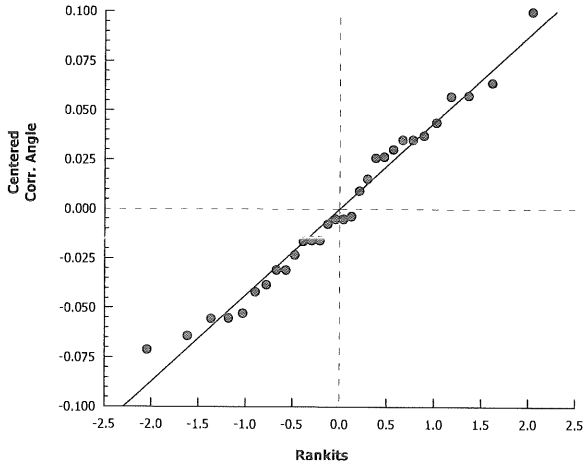
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.288	1.215	1.361	1.323	1.217	1.345	0.02633	4.57%	0.0%
2.5		5	1.339	1.285	1.394	1.323	1.284	1.397	0.01971	3.29%	-3.96%
5		5	1.308	1.272	1.345	1.303	1.266	1.345	0.01309	2.24%	-1.54%
6.06		5	1.297	1.217	1.377	1.266	1.233	1.397	0.02888	4.98%	-0.67%
10		5	1.24	1.198	1.283	1.233	1.202	1.284	0.01525	2.75%	3.72%
15		5	1.123	1.071	1.176	1.12	1.071	1.187	0.01902	3.79%	12.8%

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

Analysis ID:	03-5773-5028	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Sep-17 12:51	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1709-S026 | 03-7375-7124

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

Analysis ID:	11-3625-2660	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Sep-17 12:51	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2011096	1000	Yes	Two-Point Interpolation

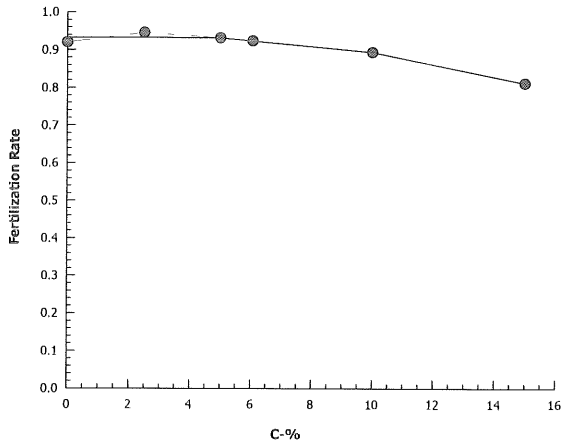
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	>15	N/A	N/A	<6.667	NA	NA
EC50	>15	N/A	N/A	<6.667	NA	NA

Fertilization Rate Summary

			Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	A	B
0	Lab Control	5	0.92	0.88	0.95	0.01449	0.0324	3.52%	0.0%	460	500
2.5		5	0.946	0.92	0.97	0.008718	0.01949	2.06%	-2.83%	473	500
5		5	0.932	0.91	0.95	0.006633	0.01483	1.59%	-1.3%	466	500
6.06		5	0.924	0.89	0.97	0.014	0.0313	3.39%	-0.43%	462	500
10		5	0.894	0.87	0.92	0.009274	0.02074	2.32%	2.83%	447	500
15		5	0.812	0.77	0.86	0.01463	0.03271	4.03%	11.74%	406	500

Graphics



Analyst: _____ QA: AC 9/10/17

CETIS Analytical Report

TST

Report Date: 07 Sep-17 12:52 (p 1 of 1)
Test Code: 1709-S026 | 03-7375-7124

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

Analysis ID: 04-8885-0074 Endpoint: Fertilization Rate CETIS Version: CETISv1.8.7
Analyzed: 07 Sep-17 12:52 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	3.01%	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*	13.37	1.895	0.053	7	<0.0001	CDF	Non-Significant Effect
		5*	14.43	1.943	0.046	6	<0.0001	CDF	Non-Significant Effect
		6.06*	9.455	1.895	0.066	7	<0.0001	CDF	Non-Significant Effect
		10*	10.99	1.895	0.047	7	<0.0001	CDF	Non-Significant Effect
		15*	5.736	1.895	0.052	7	0.0004	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.1479219	0.02958438	5	13.24	<0.0001	Significant Effect
Error	0.05361908	0.002234128	24			
Total	0.201541		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.281	15.09	0.6568	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9741	0.9031	0.6549	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	0.92	0.8798	0.9602	0.94	0.88	0.95	0.01449	3.52%	0.0%
2.5		5	0.946	0.9218	0.9702	0.94	0.92	0.97	0.008718	2.06%	-2.83%
5		5	0.932	0.9136	0.9504	0.93	0.91	0.95	0.006633	1.59%	-1.3%
6.06		5	0.924	0.8851	0.9629	0.91	0.89	0.97	0.014	3.39%	-0.43%
10		5	0.894	0.8683	0.9197	0.89	0.87	0.92	0.009274	2.32%	2.83%
15		5	0.812	0.7714	0.8526	0.81	0.77	0.86	0.01463	4.03%	11.74%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.288	1.215	1.361	1.323	1.217	1.345	0.02633	4.57%	0.0%
2.5		5	1.339	1.285	1.394	1.323	1.284	1.397	0.01971	3.29%	-3.96%
5		5	1.308	1.272	1.345	1.303	1.266	1.345	0.01309	2.24%	-1.54%
6.06		5	1.297	1.217	1.377	1.266	1.233	1.397	0.02888	4.98%	-0.67%
10		5	1.24	1.198	1.283	1.233	1.202	1.284	0.01525	2.75%	3.72%
15		5	1.123	1.071	1.176	1.12	1.071	1.187	0.01902	3.79%	12.8%

Analyst: _____ QA: AC 9/10/17

CETIS Test Data Worksheet

Report Date: 31 Aug-17 12:49 (p 1 of 1)
Test Code: 1709-S026 03-7375-7124/164714C4

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

Start Date: 01 Sep-17
End Date: 01 Sep-17
Sample Date: 31 Aug-17

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

Sample Code: (A) ~~72833B33~~ 17-0961
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) 8/31 Sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	92	9/7/17
			32	100	94	
			33	100	89	
			34	100	94	
			35	100	93	
			36	100	87	
			37	100	97	
			38	100	95	
			39	100	80	
			40	100	91	
			41	100	91	
			42	100	94	
			43	100	95	
			44	100	77	
			45	100	88	
			46	100	86	
			47	100	91	
			48	100	81	
			49	100	96	
			50	100	89	
			51	100	94	
			52	100	91	
			53	100	97	
			54	100	93	
			55	100	94	
			56	100	92	
			57	100	94	
			58	100	88	
			59	100	82	
			60	100	89	

(A) CG QA 8/31/17

CETIS Test Data Worksheet

Test Code: 1709-5026 03-7375-7124/164714C4

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

Start Date: 01 Sep-17
End Date: 01 Sep-17
Sample Date: 31 Aug-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: Ⓐ ~~72833B33~~ 17-0961
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Daily) 8/31 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	45	100	92	BO 9/1/17
0	LC	2	42			
0	LC	3	43			
0	LC	4	33			
0	LC	5	57			
2.5		1	53			
2.5		2	56			
2.5		3	49	100	89	BO 9/1/17
2.5		4	32			
2.5		5	34			
5		1	54			
5		2	51			
5		3	38			
5		4	35			
5		5	41	100	86	BO 9/1/17
6.06		1	55	100	86	BO 9/1/17
6.06		2	40			
6.06		3	50			
6.06		4	52			
6.06		5	37			
10		1	31			
10		2	60			
10		3	47			
10		4	36			
10		5	58	100	79	BO 9/1/17
15		1	59			
15		2	39			
15		3	44	100	69	BO 9/1/17
15		4	46			
15		5	48			

Ⓐ CG 8/31/17

QC: CG

Analyst: CG QA: AC 9/10/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE (sampled 8/31) | **Test Species:** *S. purpuratus*

Sample ID: M-001 (~~unadjusted~~) Daily (A) (8/31 Sample) | **Start Date/Time:** 9/1/2017 1527

Sample Log No.: 17- 0961 | **End Date/Time:** 9/1/2017 1607

Dilutions made by: CG | **Test No:** 1709-5026

Analyst: AO

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.9	8.05	33.7	15.6
2.5	7.8	8.05	33.9	15.6
5.0	7.8	8.05	33.9	15.6
6.06	7.8	8.05	33.9	16.0
10	7.8	8.04	33.9	15.9
15	7.9	8.03	33.9	15.7

Comments: (A) EH @15 8/31/17

QC Check: EH 9/5/17 | **Final Review:** AC 9/10/17

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

Client: IDE
Sample ID: Daily M-001 8/31 sample
Test No.: 1709-5026

Tech initials: CG
Injection Time: 1435

Start Date/Time: 9/1/2017 / 1527
End Date/Time: 9/1/2017 / 1607
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 8/22/17

Sperm Absorbance at 400 nm: 0.908 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 91, 96, 100, 111, 101 Mean: 99.8 X 50 = 4990 eggs/ml

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

Initial density:	4990	eggs/ml	= 1.25	dilution factor	egg stock	100	ml
Final density:	4000	eggs/ml	- 1.0	part egg stock	seawater	0.25	ml
			0.25	parts seawater			

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):	1450	50:1	55	45
Eggs Added (0.5 ml):	1505	100:1	77,81	23,19
Test Ended:	1515	200:1	91,93	9,7
		—	—	—

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: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1527	QC1	84	16
Eggs Added (0.5 ml):	1547	QC2	89	11
Test Ended:	1607	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: EG 9/5/17 Final Review: AC 9/10/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: Daily M-001
Test ID No(s).: ~~1708 5345~~ 1709-5026
9/1 AC 9/1/17

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	0961			
Sample Collection Date & Time:	08/31/17 0800 PDT			
Sample Receipt Date & Time:	08/31/17 1105			
Number of Containers & Container Type:	1, 4L wbi			
Approx. Total Volume Received (L):	4L			
Check-in Temperature (°C)	5.0			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.5			
pH (units)	7.81			
Conductivity (µS/cm)	(H) ~~4410~~ —			
Salinity (ppt)	33.0			
Alkalinity (mg/L) [2]	100			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	<0.02			
Technician Initials	LTP			

Test Performed: Urchin Fertilization — **Control/Dilution Water:** 8:2 / ~~Lab SW~~ / Lab ART **Other:** _____
Alkalinity: 107 Hardness or (Salinity): 34ppt
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART **Other:** _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Test Performed: _____ **Control/Dilution Water:** 8:2 / Lab SW / Lab ART **Other:** _____
Alkalinity: _____ Hardness or Salinity: _____
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

Notes: [1] Temperature of sample should be 0-6°C, if received more than 24 hours past collection time.
[2] mg/L as CaCO3, [3] Measured for freshwater samples only, NA = Not Applicable

Additional Comments: (H) QNS LTP 8/31/17

Sample Check-In Information

Sample Description: A: no color, no odor, clear, no debris

COC Complete (Y/N)? A Y B____ C____

Filtration? Y (N)
Pore Size: _____
Organisms or Debris

Salinity Adjustment? Y (N)
Test: Source: Target ppt:
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

	A	B	C
Initial pH:			
Amount of HCl added:			
Final pH:			

Cl_2 Adjustment? Y (N)

	A	B	C
Initial Free Cl_2:			
STS added:			
Final Free Cl_2:			

Sample Aeration? Y (N)

	A	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			

Subsamples for Additional Chemistry Required? Y (N)
NH3 Other _____
Tech Initials A____ B____ C____

QC Check: AC 9/1/17
Final Review: KS 9/11/17

Appendix C

Chain-of-Custody Form

DAILY

IDE Technologies

CDP laboratory:_____
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: Sampled during pretreatment off-spec via autosampler by a series of grabs collected at one hour intervals. Sample collected to fulfill daily NPDES requirement. Sample is to be run unadjusted. Start: 8/30/17 @ 8:00, End: 8/31/17 @ 8:00 VH

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- 2749)	8/30-31/2017	8:00-8:00	SW	N	4L CUBIE	X								TDS - 31.77 ppt, EC - 49.36 mS/cm

Relinquished By:		Date:	Time:		Received By:					Time:	Sample Condition Upon Receipt:
[signature]		8/31/17	10:55		[signature]				8/31/17	10:55	[x] Iced [ ] Ambient or _____ °C
[signature]		8/31/17	11:55		[signature]				08/31/17	11:55	[✓] Iced [ ] Ambient or 5.0 °C

Nautilus ID: 17-0961

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	12-3939-9797	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	01 Sep-17 15:27	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	01 Sep-17 16:07	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	07-8873-8696	**Code:**	170901sprt	**Client:**	Internal
Sample Date:	01 Sep-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	01 Sep-17	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
11-5239-0309	Fertilization Rate	<10	10	NA	4.77%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
21-1567-7550	Fertilization Rate	EC50	34.79	32.51	37.24		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
11-5239-0309	Fertilization Rate	Control Resp	0.92	0.7 - NL	Yes	Passes Acceptability Criteria
21-1567-7550	Fertilization Rate	Control Resp	0.92	0.7 - NL	Yes	Passes Acceptability Criteria
11-5239-0309	Fertilization Rate	PMSD	0.04775	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	0.92	0.8779	0.9621	0.88	0.96	0.01517	0.03391	3.69%	0.0%
10		5	0.748	0.6851	0.8109	0.7	0.82	0.02267	0.0507	6.78%	18.7%
20		5	0.664	0.6095	0.7185	0.62	0.72	0.01965	0.04393	6.62%	27.83%
40		5	0.472	0.3973	0.5467	0.41	0.55	0.02691	0.06017	12.75%	48.7%
80		5	0.048	0.02412	0.07188	0.02	0.07	0.008602	0.01924	40.07%	94.78%
160		5	0.002	0	0.007553	0	0.01	0.002	0.004472	223.6%	99.78%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.96	0.95	0.88	0.9	0.91
10		0.73	0.7	0.71	0.78	0.82
20		0.63	0.65	0.62	0.7	0.72
40		0.43	0.52	0.45	0.41	0.55
80		0.02	0.07	0.05	0.04	0.06
160		0	0	0	0	0.01

Analyst: _____ QA: 9/7/17

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

Analysis ID: 11-5239-0309 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Sep-17 12:19 **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	4.77%	<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*	7.321	2.362	0.078	8	<0.0001	CDF	Significant Effect
		20*	10.14	2.362	0.078	8	<0.0001	CDF	Significant Effect
		40*	16.04	2.362	0.078	8	<0.0001	CDF	Significant Effect
		80*	32.34	2.362	0.078	8	<0.0001	CDF	Significant Effect
		160*	37.07	2.362	0.078	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.876649	1.17533	5	427.4	<0.0001	Significant Effect
Error	0.06600084	0.002750035	24			
Total	5.94265		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.027	15.09	0.5456	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.943	0.9031	0.1096	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	0.92	0.8779	0.9621	0.91	0.88	0.96	0.01517	3.69%	0.0%
10		5	0.748	0.6851	0.8109	0.73	0.7	0.82	0.02267	6.78%	18.7%
20		5	0.664	0.6095	0.7185	0.65	0.62	0.72	0.01965	6.62%	27.83%
40		5	0.472	0.3973	0.5467	0.45	0.41	0.55	0.02691	12.75%	48.7%
80		5	0.048	0.02412	0.07188	0.05	0.02	0.07	0.008602	40.07%	94.78%
160		5	0.002	0	0.007553	0	0	0.01	0.002	223.6%	99.78%

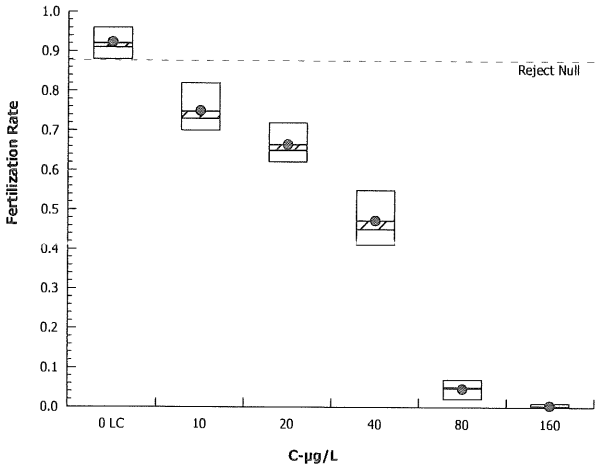
Angular (Corrected) Transformed Summary

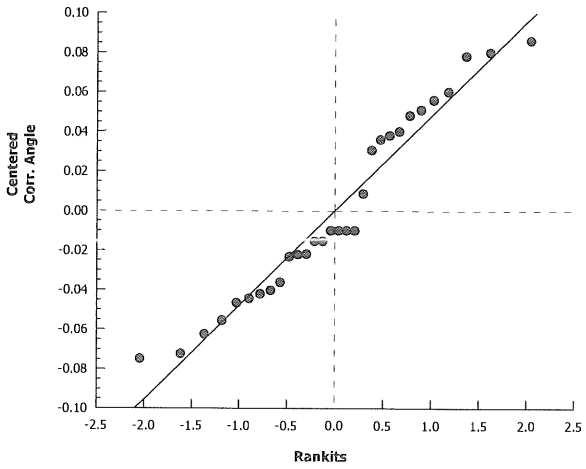
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.289	1.209	1.37	1.266	1.217	1.369	0.0291	5.05%	0.0%
10		5	1.047	0.9725	1.121	1.024	0.9912	1.133	0.02669	5.7%	18.83%
20		5	0.9531	0.895	1.011	0.9377	0.9066	1.013	0.02094	4.91%	26.08%
40		5	0.7573	0.6823	0.8322	0.7353	0.6949	0.8355	0.027	7.97%	41.27%
80		5	0.2168	0.1564	0.2772	0.2255	0.1419	0.2678	0.02175	22.43%	83.19%
160		5	0.06005	0.0322	0.0879	0.05002	0.05002	0.1002	0.01003	37.35%	95.34%

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

Analysis ID:	11-5239-0309	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Sep-17 12:19	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 170901sprt | 13-1244-6646

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

Analysis ID:	21-1567-7550	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Sep-17 12:19	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

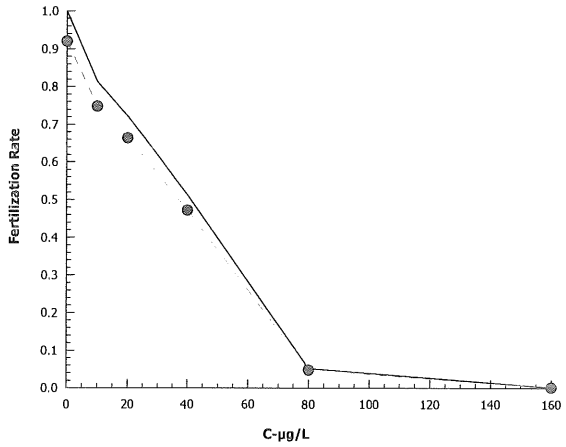
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.08	18.70%	1.542	0.01475	34.79	32.51	37.24

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	0.92	0.88	0.96	0.01517	0.03391	3.69%	0.0%	460	500
10		5	0.748	0.7	0.82	0.02267	0.0507	6.78%	18.7%	374	500
20		5	0.664	0.62	0.72	0.01965	0.04393	6.62%	27.83%	332	500
40		5	0.472	0.41	0.55	0.02691	0.06017	12.75%	48.7%	236	500
80		5	0.048	0.02	0.07	0.008602	0.01924	40.07%	94.78%	24	500
160		5	0.002	0	0.01	0.002	0.004472	223.6%	99.78%	1	500

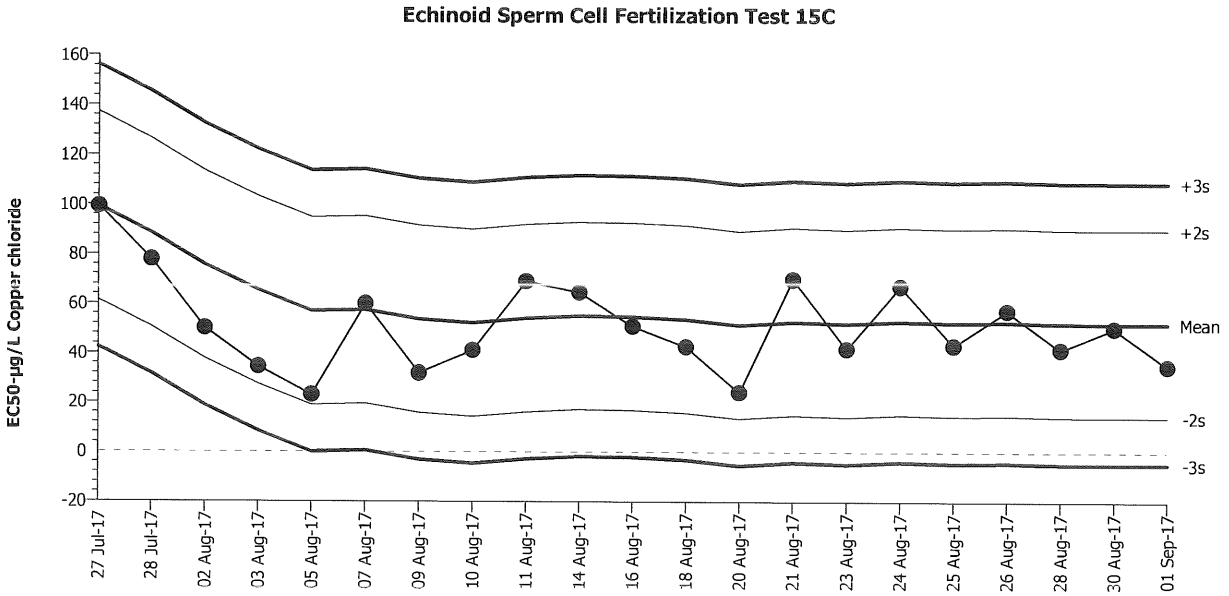
Graphics



Analyst: _____ QA: 9/7/17

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:	51.98	**Count:**	20	**-2s Warning Limit:**	14.04	**-3s Action Limit:**	-4.934
Sigma:	18.97	**CV:**	36.50%	**+2s Warning Limit:**	89.92	**+3s Action Limit:**	108.9

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jul	27	15:55	99.32	47.34	2.496	(+)		02-6715-3770	17-8186-2444
2			28	10:50	77.84	25.86	1.363			21-2559-1280	14-0688-6070
3		Aug	2	15:50	50.06	-1.925	-0.1015			08-9742-2478	08-8646-9232
4			3	0:00	34.43	-17.55	-0.9249			02-7356-2235	20-3051-4002
5			5	19:25	23.07	-28.91	-1.524			11-5994-0488	10-6029-2098
6			7	15:10	59.94	7.959	0.4195			21-2468-7505	14-3489-7019
7			9	17:08	31.92	-20.06	-1.058			13-6999-3036	11-7131-4234
8			10	16:51	41.14	-10.84	-0.5717			00-5471-5288	12-0643-2211
9			11	14:50	69.03	17.05	0.8987			04-5796-5476	07-8184-6783
10			14	14:40	64.51	12.53	0.6603			02-4510-8526	01-5460-0814
11			16	16:34	50.82	-1.163	-0.06131			16-3259-1018	06-7497-1035
12			18	14:09	42.53	-9.449	-0.4981			12-6613-4538	02-2322-5589
13			20	14:52	24.05	-27.93	-1.472			06-9655-0092	05-8785-3700
14			21	14:46	69.95	17.97	0.9472			08-4756-2919	20-2992-4955
15			23	16:14	41.72	-10.26	-0.541			02-7595-3678	15-3490-2746
16			24	16:11	67.1	15.12	0.7972			04-7651-5518	20-0883-0005
17			25	14:48	43.11	-8.87	-0.4676			06-8816-1100	09-0830-4014
18			26	16:00	57.24	5.261	0.2773			10-2039-5656	15-8794-0305
19			28	14:56	41.55	-10.43	-0.5497			08-1525-2751	10-7829-2432
20			30	16:38	50.21	-1.768	-0.09321			08-1199-3706	11-0543-3886
21		Sep	1	15:27	34.79	-17.19	-0.906			13-1244-6646	21-1567-7550

Analyst: JD QA: VS 9/7/17

CETIS Test Data Worksheet

Report Date: 31 Aug-17 12:44 (p 1 of 1)
Test Code: 13-1244-6646/170901sprt

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

Start Date: 01 Sep-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 170901sprt
End Date: 01 Sep-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: ~~31 Aug-17~~ 01 Sept-17 (A) **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	41	9/7/17
			2	100	~~7~~	
			3	100	43	
			4	100	~~0~~	
			5	100	~~0~~	
			6	100	(A) ~~59~~ 45	
			7	100	96	
			8	100	~~0~~	
			9	100	5	
			10	100	55	
			11	100	70	
			12	100	~~70~~	
			13	100	52	
			14	100	73	
			15	100	6	
			16	100	91	
			17	100	~~0~~	
			18	100	4	
			19	100	90	
			20	100	72	
			21	100	82	
			22	100	78	
			23	100	88	
			24	100	71	
			25	100	62	
			26	100	63	
			27	100	95	
			28	100	1	
			29	100	65	
			30	100	2	

(A) CG QR 8/31/17

(A) QR SG 9/7/17

CETIS Test Data Worksheet

Report Date: 31 Aug-17 12:44 (p 1 of 1)
Test Code: 13-1244-6646/170901sprt

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

Start Date: 01 Sep-17
End Date: 01 Sep-17
Sample Date: 31 Aug-17 (A) 01 Sep-17

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

Sample Code: 170901sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	7			
0	LC	2	27	100	99	CG 9/1/17
0	LC	3	23			
0	LC	4	19			
0	LC	5	16			
10		1	14			
10		2	11	100	78	
10		3	24			
10		4	22			
10		5	21			
20		1	26			
20		2	29	100	62	
20		3	25			
20		4	12			
20		5	20			
40		1	3			
40		2	13			
40		3	6			
40		4	1	100	43	
40		5	10			
80		1	30			
80		2	2			
80		3	9	100	9	
80		4	18			
80		5	15			
160		1	4			
160		2	8			
160		3	5			
160		4	17	100	0	
160		5	28			

(A) CG QC 8/31/17

QC: CG

Analyst: EH DBS CG QA: VS 9/7/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: $CuCl_2$ | **Start Date/Time:** 9/1/2017 1527

Test No: 170901sprt | **End Date/Time:** 9/1/2017 1607

Dilutions made by: CG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10 000

Analyst: AD

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.3	7.99	33.5	15.3
10	8.0	8.00	33.8	15.1
20	8.0	8.02	33.8	15.0
40	8.0	8.04	33.8	15.1
80	7.9	8.03	33.6	15.2
160	7.9	8.04	33.3	15.6

Comments:

QC Check: EG 9/5/17 | **Final Review:** MS 9/7/17

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

Client: Internal
Sample ID: CuCl2
Test No.: 170901spf

Start Date/Time: 9/1/2017 / 1527
End Date/Time: 9/1/2017 / 1607
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 8/22/17

Tech initials: CG
Injection Time: 1435

Sperm Absorbance at 400 nm: 0.909 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 91, 96, 100, 111, 101 Mean: 99.8 X 50 = 4990 eggs/ml

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

Initial density: 4990 eggs/ml = 1.25 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.25 parts seawater

egg stock 100 ml
seawater 0.25 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):	1450	50:1	55	45
Eggs Added (0.5 ml):	1505	100:1	77.81	23.19
Test Ended:	1515	200:1	91.93	9.7

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: 150:1

	Time		Fert.	Unfert.
Sperm Added (100 µl):	1527	QC1	84	16
Eggs Added (0.5 ml):	1547	QC2	89	11
Test Ended:	1607	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: EG 9/5/17

Final Review: YB 9/7/17

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.