Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily)
Sample Collection Date: December 14, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **January 2, 2018**

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

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – DECEMBER 2017

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

Sampling Date: December 14, 2017

Test Date: December 15, 2017

Sample ID: M-001 (plant off-spec period)

M-001
Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	M-001 Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	NOEC	TU_c	Yes
	10	10	

INTRODUCTION

A discharge sample was collected in December 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) 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 in accordance with 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 December 15, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was couriered to Nautilus the day after sample collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. The sample was stored at 4° C in the dark until used for testing. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./ Carlsbad Desalination Plant
Monitoring Period:	December 2017 (plant off-spec period)
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	12/14/17, 12:30
Sample Receipt Date, Time:	12/15/17, 13:54
Sampling Method:	Grab

Table 2. Water Quality Measurements upon Sample Receipt

Sample ID	pH	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as $CaCO_3$)	Total Chlorine (mg/L)
M-001	7.96	8.3	3.2	61.2	183	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	12/15/17, 18:53 through 19:33
Test Organism:	*Strongylocentrotus purpuratus* (purple sea urchin)
Test Organism Source:	Field-collected off Point Loma in San Diego, CA
Lab Control/Dilution Water:	Natural seawater (source: Scripps Institution of Oceanography (SIO) inlet), 34±2 parts per thousand (ppt); 20-µm filtered
Additional Control:	High Salinity Control (HSC) – seawater with Nautilus hypersaline brine added to match the salinity of the 15 percent M-001 effluent concentration; tested to evaluate potential adverse effects due to elevated salinity alone
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control. The same dilution series was also tested with the sample after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge and CDP discharges).
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min egg fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25%
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

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

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste

concentration (IWC) is 6.06 percent unadjusted effluent, and results are reported as "Pass" if a sample is considered non-toxic according to the TST calculation, or "Fail" if considered toxic according to the TST. As the TST statistical analysis is not in the 2006 CDP permit, the TST results are included for comparison purposes only.

RESULTS

There was a significant decrease in the fertilization rate in the 15 percent effluent concentration in the unadjusted M-001 sample relative to the lab control using the EPA 1995 flowchart statistics. The NOEC is reported as 10 percent effluent and the TU_c is 10, which is below the maximum permit effluent limitation of 16.5 TU_c. No significant decrease was observed at any percent effluent concentration in the M-001 unadjusted sample using the TST statistical analysis. The high salinity control resulted in 97.8 percent mean fertilization indicating that reduced fertilization in the unadjusted sample was not likely due to elevated salinity.

There was no significant decrease in the fertilization rate at any percent effluent concentration in the 40 ppt adjusted M-001 sample relative to the lab control using the EPA 1995 flowchart statistics. No significant decrease was observed at any percent effluent concentration in the M-001 40 ppt adjusted sample using the TST statistical analysis.

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

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

Sample ID	NOEC (% sample)	LOEC (% sample)	EC_{50} (% sample)	TU_c value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001 (unadjusted)	10	15	>15	10	Pass	0.6
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-2.7

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 6.06% IWC according to the TST calculation; Fail = sample is toxic at the 6.06% IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.
Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) *100. A negative PE results when organism performance in the sample is greater than that in the control.

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

Test Concentration (% Sample)	M-001 Unadjusted Sample		M-001 40 ppt Adjusted[a]	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.9	99.0	33.9	95.2
High Salinity Control	37.5	97.8	--	--
2.5	34.6	97.2	34.0	96.2
5.0	35.2	98.8	34.2	97.4
6.06	35.5	98.4	34.3	97.8
10	36.5	96.8	34.6	97.8
15	37.7	92.4*	34.6	97.8

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

[a] For comparison to the M-001 unadjusted sample, the M-001 sample was adjusted with seawater to 40 ppt prior to preparing test concentrations.

QUALITY ASSURANCE

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

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

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 6 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration (EC_{50}) value was slightly above two standard deviations (SD) from the historical mean, indicating organisms may have been less sensitive to copper than typical. A list of qualifier codes used on bench datasheets 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 (%)
12/15/17	76.8	45.7 ± 30.3	33.1

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.

Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.

Tidepool Scientific Software. 2000-2013. CETIS™ Comprehensive Environmental Toxicity Information System Software, Version 1.8.7.20

USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.

USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. United States Environmental Protection Agency Office of Wastewater Management (EPA-833-R-00-003).

USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses

M-001 Unadjusted

CETIS Summary Report

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

Batch ID:	12-3886-8163	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 18:53	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	15 Dec-17 19:33	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	01-7017-4023	**Code:**	17-1294	**Client:**	IDE
Sample Date:	14 Dec-17 12:30	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	15 Dec-17 13:54	**Source:**	IDE Americas, Inc.		
Sample Age:	30h (3.2 °C)	**Station:**	M-001 (Unadjusted) 12/14 Sample		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-0423-4150	Fertilization Rate	10	15	12.25	2.97%	10	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
21-1026-4484	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
14-0423-4150	Fertilization Rate	Control Resp	0.99	0.7 - NL	Yes	Passes Acceptability Criteria
21-1026-4484	Fertilization Rate	Control Resp	0.99	0.7 - NL	Yes	Passes Acceptability Criteria
14-0423-4150	Fertilization Rate	PMSD	0.02971	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	High Salinity Co	5	0.978	0.9541	1	0.95	1	0.008602	0.01924	1.97%	0.0%
0	Lab Control	5	0.99	0.9776	1	0.98	1	0.004472	0.01	1.01%	-1.23%
2.5		5	0.972	0.9411	1	0.94	0.99	0.01114	0.0249	2.56%	0.61%
5		5	0.988	0.9744	1	0.97	1	0.004899	0.01095	1.11%	-1.02%
6.06		5	0.984	0.9598	1	0.95	1	0.008718	0.01949	1.98%	-0.61%
10		5	0.968	0.9518	0.9842	0.95	0.98	0.005831	0.01304	1.35%	1.02%
15		5	0.924	0.8418	1	0.81	0.98	0.0296	0.06618	7.16%	5.52%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	High Salinity Co	0.97	0.99	0.98	0.95	1
0	Lab Control	0.98	1	0.99	1	0.98
2.5		0.99	0.99	0.95	0.94	0.99
5		0.99	0.99	0.99	1	0.97
6.06		0.99	0.99	1	0.95	0.99
10		0.98	0.96	0.97	0.98	0.95
15		0.95	0.93	0.98	0.95	0.81

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

Analysis ID:	14-0423-4150	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:50	**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	2.97%	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	1.327	2.362	0.103	8	0.2846	CDF	Non-Significant Effect
		5	0.1861	2.362	0.103	8	0.7725	CDF	Non-Significant Effect
		6.06	0.4219	2.362	0.103	8	0.6804	CDF	Non-Significant Effect
		10	1.837	2.362	0.103	8	0.1319	CDF	Non-Significant Effect
		15*	3.794	2.362	0.103	8	0.0019	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.09711093	0.01942219	5	4.081	0.0080	Significant Effect
Error	0.1142284	0.004759518	24			
Total	0.2113394		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.816	15.09	0.2347	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9279	0.9031	0.0433	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	0.99	0.9776	1	0.99	0.98	1	0.004472	1.01%	0.0%
2.5		5	0.972	0.9411	1	0.99	0.94	0.99	0.01114	2.56%	1.82%
5		5	0.988	0.9744	1	0.99	0.97	1	0.004899	1.11%	0.2%
6.06		5	0.984	0.9598	1	0.99	0.95	1	0.008718	1.98%	0.61%
10		5	0.968	0.9518	0.9842	0.97	0.95	0.98	0.005831	1.35%	2.22%
15		5	0.924	0.8418	1	0.95	0.81	0.98	0.0296	7.16%	6.67%

Angular (Corrected) Transformed Summary

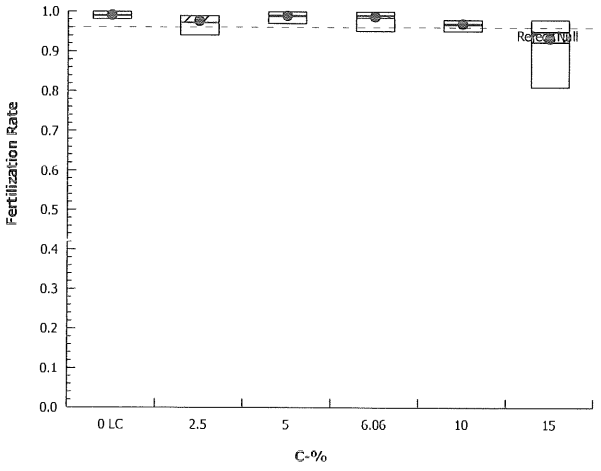
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.474	1.417	1.531	1.471	1.429	1.521	0.02056	3.12%	0.0%
2.5		5	1.416	1.323	1.509	1.471	1.323	1.471	0.03357	5.3%	3.93%
5		5	1.466	1.411	1.521	1.471	1.397	1.521	0.01983	3.03%	0.55%
6.06		5	1.456	1.374	1.537	1.471	1.345	1.521	0.02924	4.49%	1.25%
10		5	1.394	1.348	1.44	1.397	1.345	1.429	0.01646	2.64%	5.44%
15		5	1.308	1.166	1.451	1.345	1.12	1.429	0.05141	8.79%	11.23%

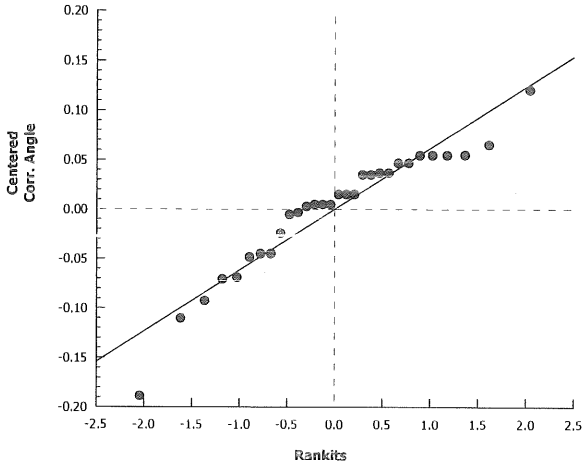
Analyst: _____ QA: EG 12/28/17

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

Analysis ID:	14-0423-4150	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:50	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: _____ QA: EL 12/28/17

CETIS Analytical Report

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

Analysis ID:	21-1026-4484	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1601843	1000	Yes	Two-Point Interpolation

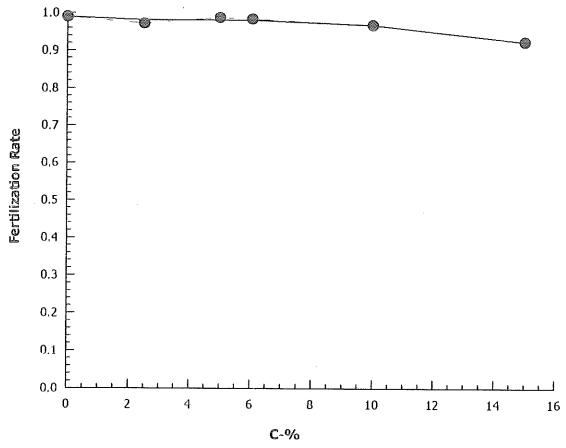
Point Estimates

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	0.99	0.98	1	0.004472	0.01	1.01%	0.0%	495	500
2.5		5	0.972	0.94	0.99	0.01114	0.0249	2.56%	1.82%	486	500
5		5	0.988	0.97	1	0.004899	0.01095	1.11%	0.2%	494	500
6.06		5	0.984	0.95	1	0.008718	0.01949	1.98%	0.61%	492	500
10		5	0.968	0.95	0.98	0.005831	0.01304	1.35%	2.22%	484	500
15		5	0.924	0.81	0.98	0.0296	0.06618	7.16%	6.67%	462	500

Graphics



Analyst: _____ QA: EG 12/28/17

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

Analysis ID:	06-2821-0198	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:50	**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	3.43%	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*	8.407	2.015	0.074	5	0.0002	CDF	Non-Significant Effect
		5*	14.35	1.895	0.048	7	<0.0001	CDF	Non-Significant Effect
		6.06*	10.59	1.943	0.064	6	<0.0001	CDF	Non-Significant Effect
		10*	12.78	1.895	0.043	7	<0.0001	CDF	Non-Significant Effect
		15*	3.782	2.132	0.114	4	0.0097	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.09711093	0.01942219	5	4.081	0.0080	Significant Effect
Error	0.1142284	0.004759518	24			
Total	0.2113394		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.816	15.09	0.2347	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9279	0.9031	0.0433	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	0.99	0.9776	1	0.99	0.98	1	0.004472	1.01%	0.0%
2.5		5	0.972	0.9411	1	0.99	0.94	0.99	0.01114	2.56%	1.82%
5		5	0.988	0.9744	1	0.99	0.97	1	0.004899	1.11%	0.2%
6.06		5	0.984	0.9598	1	0.99	0.95	1	0.008718	1.98%	0.61%
10		5	0.968	0.9518	0.9842	0.97	0 95	0.98	0.005831	1.35%	2.22%
15		5	0.924	0.8418	1	0.95	0.81	0.98	0.0296	7.16%	6.67%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.474	1.417	1.531	1.471	1.429	1.521	0.02056	3.12%	0.0%
2.5		5	1.416	1.323	1.509	1.471	1.323	1.471	0.03357	5.3%	3.93%
5		5	1.466	1.411	1.521	1.471	1.397	1.521	0.01983	3.03%	0.55%
6.06		5	1.456	1.374	1.537	1.471	1.345	1.521	0.02924	4.49%	1.25%
10		5	1.394	1.348	1.44	1.397	1.345	1.429	0.01646	2.64%	5.44%
15		5	1.308	1.166	1.451	1.345	1.12	1.429	0.05141	8.79%	11.23%

Analyst: QA: EG 12/28/17

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:32 (p 1 of 1)
Test Code: 10-2665-4266/~~3D31803A~~ 1712-SG74

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

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 14 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17-1294
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Unadjusted) 12/14 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	100	Read by JC 12/18/17
			2	100	95	
			3	100	99	
			4	100	100	
			5	100	93	
			6	100	100	
			7	100	98	
			8	100	99	
			9	100	99	
			10	100	99	
			11	100	99	
			12	100	98	
			13	100	96	
			14	100	100	
			15	100	99	
			16	100	98	
			17	100	95	
			18	100	99	
			19	100	100	
			20	100	95	
			21	100	98	
			22	100	97	
			23	100	94	
			24	100	95	
			25	100	98	
			26	100	95	
			27	100	99	
			28	100	97	
			29	100	99	
			30	100	81	
			31	100	97	
			32	100	95	
			33	100	98	
			34	100	99	
			35	100	99	

Reviewed QS 12/23/17

Analyst: JC QA: EG 12/28/17

CETIS Test Data Worksheet

Report Date: 22 Dec-17 11:37 (p 1 of 1)
Test Code: 1712-5074 10-2665-4266/3D31803A

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

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 14 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17 – 1294
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (Unadjusted) 12/14 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	31			
0	HS	2	34			
0	HS	3	25			
0	HS	4	26			
0	HS	5	1			
0	LC	1	33			
0	LC	2	19			
0	LC	3	29	100	(A) ~~98~~ 98	BO 12/22/17
0	LC	4	14			
0	LC	5	12			
2.5		1	15			
2.5		2	11			
2.5		3	17			
2.5		4	23			
2.5		5	3			
5		1	9			
5		2	10			
5		3	8			
5		4	6			
5		5	28			
6.06		1	27			
6.06		2	35			
6.06		3	4	100	97	BO 12/22/17
6.06		4	24			
6.06		5	18			
10		1	7			
10		2	13			
10		3	22			
10		4	16			
10		5	32			
15		1	20			
15		2	5			
15		3	21			
15		4	2			
15		5	30			

(A) Q13 BO 12/22/17

Analyst: EG OBO EG 08 ~~EG~~ AC 12/28/17 QA: EG 12/28/17

Marine Chronic Bioassay — **Water Quality Measurements**

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

Sample ID: M-001 (unadjusted) 12/14 Sample — **Start Date/Time:** 12/15/17 1853

Sample Log No.: 17- 1294 — **End Date/Time:** 12/15/17 1933

Dilutions made by: ~~AD~~ OBO ~~PA~~ — **Test No:** 1712-5075 ~~4~~ R

Analyst: AC

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.8	8.08	33.9	14.0
High Salinity Control	8.8	8.04	37.5	14.1
2.5	8.8	8.06	34.6	14.3
5.0	8.8	8.06	35.2	14.2
6.06	8.8	8.06	35.5	14.1
10	8.8	8.05	36.5	14.1
15	8.8	8.04	37.7	14.3

Comments: ~~AD~~ OBO 12/23/17

QC Check: ~~AD~~ OBO 12/23/17 — **Final Review:** EG 12/28/17

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

Client: LDE
Sample ID: M-001 (B) adj, unadjusted 12/14 sample
Test No.: (B) 1712-5075, 1712-5074

Start Date/Time: 12/15/17 1853
End Date/Time: 12/15/17 1933
Species: S. purpuratus
Animal Source: Pt. Loma
Date Collected: 12/8/17

Tech initials: AD OBO PA
Injection Time: 1815

Sperm Absorbance at 400 nm: 0.810 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 79, 75, 78, 78, 90

Mean: 80 X 50 = 4000 eggs/ml

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

Initial density:	4000 eggs/ml	=	1.0	dilution factor	egg stock	(A) ml
Final density:	4000 eggs/ml	-	1.0	part egg stock	seawater	(A) ml
			0	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):	1825	50:1	84	16
Eggs Added (0.5 ml):	1835	100:1	95	5
Test Ended:	1845	100:1	97	3
		200:1	100	100

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):	1853	QC1	99	1
Eggs Added (0.5 ml):	1913	QC2	98	2
Test Ended:	1933	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AD OBO 12/22/17

QC Check: AD 12/23/17 (B)

Final Review: EG 12/28/17

M-001 40 ppt Adjusted

CETIS Summary Report

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

Batch ID:	06-2394-6501	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 18:53	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Laboratory Seawater
Ending Date:	15 Dec-17 19:33	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	14-4153-4684	**Code:**	17-1294	**Client:**	IDE
Sample Date:	14 Dec-17 12:30	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	15 Dec-17 13:54	**Source:**	IDE Americas, Inc.		
Sample Age:	30h (3.2 °C)	**Station:**	M-001 40 ppt 12/14 sample		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
08-1402-9455	Fertilization Rate	15	>15	NA	3.46%	6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
12-5875-4052	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
08-1402-9455	Fertilization Rate	Control Resp	0.952	0.7 - NL	Yes	Passes Acceptability Criteria
12-5875-4052	Fertilization Rate	Control Resp	0.952	0.7 - NL	Yes	Passes Acceptability Criteria
08-1402-9455	Fertilization Rate	PMSD	0.03457	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	0.952	0.9175	0.9865	0.91	0.98	0.01241	0.02775	2.92%	0.0%
2.5		5	0.962	0.9366	0.9874	0.94	0.98	0.009165	0.02049	2.13%	-1.05%
5		5	0.974	0.9552	0.9928	0.95	0.99	0.006782	0.01517	1.56%	-2.31%
6.06		5	0.978	0.9618	0.9942	0.96	0.99	0.005831	0.01304	1.33%	-2.73%
10		5	0.978	0.9676	0.9884	0.97	0.99	0.003741	0.008366	0.86%	-2.73%
15		5	0.978	0.9618	0.9942	0.97	1	0.005831	0.01304	1.33%	-2.73%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.91	0.98	0.96	0.94	0.97
2.5		0.98	0.94	0.97	0.98	0.94
5		0.98	0.98	0.99	0.97	0.95
6.06		0.99	0.99	0.96	0.98	0.97
10		0.98	0.98	0.97	0.99	0.97
15		0.97	0.98	1	0.97	0.97

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

Analysis ID:	08-1402-9455	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:43	**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	3.46%	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	-0.7395	2.362	0.075	8	0.9666	CDF	Non-Significant Effect
		5	-1.812	2.362	0.075	8	0.9986	CDF	Non-Significant Effect
		6.06	-2.23	2.362	0.075	8	0.9997	CDF	Non-Significant Effect
		10	-2.138	2.362	0.075	8	0.9995	CDF	Non-Significant Effect
		15	-2.252	2.362	0.075	8	0.9997	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02203904	0.004407807	5	1.771	0.1572	Non-Significant Effect
Error	0.05974953	0.002489564	24			
Total	0.08178857		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.028	15.09	0.8452	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9739	0.9031	0.6503	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	0.952	0.9175	0.9865	0.96	0.91	0.98	0.01241	2.92%	0.0%
2.5		5	0.962	0.9366	0.9874	0.97	0.94	0.98	0.009165	2.13%	-1.05%
5		5	0.974	0.9552	0.9928	0.98	0.95	0.99	0.006782	1.56%	-2.31%
6.06		5	0.978	0.9618	0.9942	0.98	0.96	0.99	0.005831	1.33%	-2.73%
10		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	-2.73%
15		5	0.978	0.9618	0.9942	0.97	0.97	1	0.005831	1.33%	-2.73%

Angular (Corrected) Transformed Summary

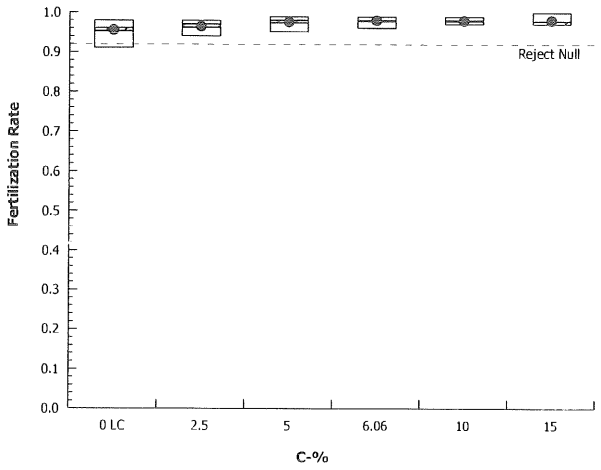
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.357	1.278	1.436	1.369	1.266	1.429	0.02855	4.7%	0.0%
2.5		5	1.38	1.314	1.447	1.397	1.323	1.429	0.02396	3.88%	-1.72%
5		5	1.414	1.356	1.472	1.429	1.345	1.471	0.02082	3.29%	-4.22%
6.06		5	1.427	1.372	1.483	1.429	1.369	1.471	0.02005	3.14%	-5.19%
10		5	1.424	1.387	1.462	1.429	1.397	1.471	0.01362	2.14%	-4.97%
15		5	1.428	1.361	1.495	1.397	1.397	1.521	0.02403	3.76%	-5.24%

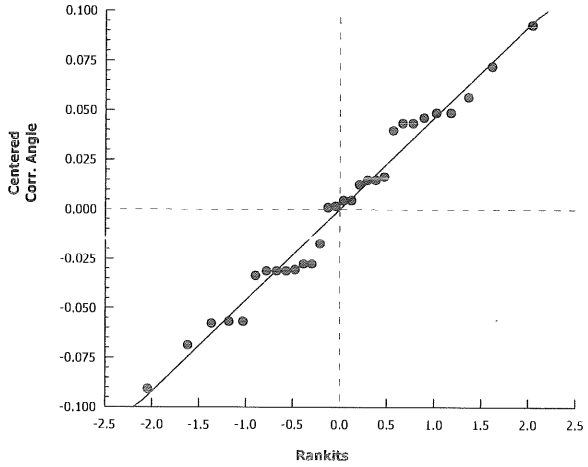
Analyst: _____ QA: EH 12/28/17

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

Analysis ID:	08-1402-9455	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:43	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1712-S075 | 19-9774-3447

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

Analysis ID:	12-5875-4052	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1109086	1000	Yes	Two-Point Interpolation

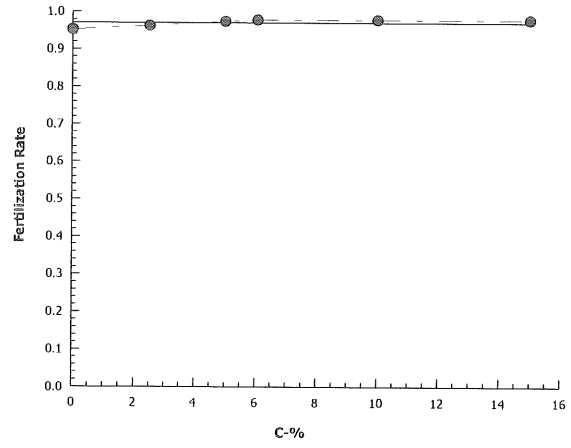
Point Estimates

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	0.952	0.91	0.98	0.01241	0.02775	2.92%	0.0%	476	500
2.5		5	0.962	0.94	0.98	0.009165	0.02049	2.13%	-1.05%	481	500
5		5	0.974	0.95	0.99	0.006782	0.01517	1.56%	-2.31%	487	500
6.06		5	0.978	0.96	0.99	0.005831	0.01304	1.33%	-2.73%	489	500
10		5	0.978	0.97	0.99	0.003741	0.008366	0.86%	-2.73%	489	500
15		5	0.978	0.97	1	0.005831	0.01304	1.33%	-2.73%	489	500

Graphics



Analyst: _____ QA: EG 12/28/17

CETIS Analytical Report

TST

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

Analysis ID:	08-2002-3936	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	22 Dec-17 11:44	**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	2.7%	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*	11.28	1.895	0.061	7	<0.0001	CDF	Non-Significant Effect
		5*	13.27	1.895	0.057	7	<0.0001	CDF	Non-Significant Effect
		6.06*	13.96	1.895	0.056	7	<0.0001	CDF	Non-Significant Effect
		10*	16.03	1.943	0.049	6	<0.0001	CDF	Non-Significant Effect
		15*	12.75	1.895	0.061	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02203904	0.004407807	5	1.771	0.1572	Non-Significant Effect
Error	0.05974953	0.002489564	24			
Total	0.08178857		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.028	15.09	0.8452	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9739	0.9031	0.6503	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	0.952	0.9175	0.9865	0.96	0.91	0.98	0.01241	2.92%	0.0%
2.5		5	0.962	0.9366	0.9874	0.97	0.94	0.98	0.009165	2.13%	-1.05%
5		5	0.974	0.9552	0.9928	0.98	0.95	0.99	0.006782	1.56%	-2.31%
6.06		5	0.978	0.9618	0.9942	0.98	0.96	0.99	0.005831	1.33%	-2.73%
10		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	-2.73%
15		5	0.978	0.9618	0.9942	0.97	0.97	1	0.005831	1.33%	-2.73%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.357	1.278	1.436	1.369	1.266	1.429	0.02855	4.7%	0.0%
2.5		5	1.38	1.314	1.447	1.397	1.323	1.429	0.02396	3.88%	-1.72%
5		5	1.414	1.356	1.472	1.429	1.345	1.471	0.02082	3.29%	-4.22%
6.06		5	1.427	1.372	1.483	1.429	1.369	1.471	0.02005	3.14%	-5.19%
10		5	1.424	1.387	1.462	1.429	1.397	1.471	0.01362	2.14%	-4.97%
15		5	1.428	1.361	1.495	1.397	1.397	1.521	0.02403	3.76%	-5.24%

Analyst: _____ QA: EG 12/28/17

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:34 (p 1 of 1)
Test Code: 19-9774-3447/~~77132557~~ 1748-5075

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 14 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 1294
Sample Source: IDE Americas, Inc.
Sample Station: M-001 40 ppt 12/14 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			36	100	98	Read by JC 12/18/17
			37	100	98	
			38	100	91	
			39	100	97	
			40	100	100	
			41	100	98	
			42	100	97	
			43	100	99	
			44	100	97	
			45	100	97	
			46	100	96	
			47	100	94	
			48	100	94	
			49	100	99	
			50	100	97	
			51	100	98	
			52	100	98	
			53	100	95	
			54	100	98	
			55	100	97	
			56	100	98	
			57	100	94	
			58	100	97	
			59	100	96	
			60	100	97	
			61	100	99	
			62	100	99	
			63	100	98	
			64	100	98	
			65	100	97	

RAD 918 12/23/17

CETIS Test Data Worksheet

Test Code: 1712-5075 19-9774-3447/77132557

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 14 Dec-17

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

Sample Code: 17- 1214
Sample Source: IDE Americas, Inc.
Sample Station: M-001 40 ppt 12/14 sample

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	38			
0	LC	2	64			
0	LC	3	59			
0	LC	4	48	100	98	BO 12/22/17
0	LC	5	39			
2.5		1	36			
2.5		2	47			
2.5		3	60			
2.5		4	41			
2.5		5	57			
5		1	63			
5		2	54			
5		3	62			
5		4	50			
5		5	53			
6.06		1	43			
6.06		2	61			
6.06		3	46			
6.06		4	51			
6.06		5	42	100	Ⓐ98	BO 12/22/17
10		1	52			
10		2	56			
10		3	45			
10		4	49			
10		5	58			
15		1	44			
15		2	37			
15		3	40			
15		4	55			
15		5	65			

Ⓐ@18 BO 12/22/17

Analyst: EG 0BO AC QA: EG 12/28/17

Marine Chronic Bioassay **Water Quality Measurements**

Client : iDE **Test Species:** *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted) (12/14 sample) **Start Date/Time:** 12/15/17 1853

Sample Log No.: 17-1294 **End Date/Time:** 12/15/17 1933

Dilutions made by: AD **Test No:** 1712-3075

Analyst: AC

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.9	8.06	33.9	14.0
2.5	8.9	8.07	34.0	14.0
5.0	8.8	8.06	34.2	14.0
6.06	8.8	8.06	34.3	14.0
10	8.9	8.06	34.6	14.0
15	8.8	8.06	34.6	14.1

Comments:

QC Check: AD 12/23/17 **Final Review:** EM 12/28/17

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

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

Client: IDE (B)
Sample ID: M-001 ~~Ref~~ 40ppt adjusted (12/14 sample)
Test No.: 1712-8073

Start Date/Time: 12/15/17 1853
End Date/Time: 12/15/17 1933
Species: S. purpuratus
Animal Source: Pt. Loma
Date Collected: 12/8/17

Tech initials: AD OBO PA
Injection Time: 1815

Sperm Absorbance at 400 nm: 0.810 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 79, 75, 78, 78, 90
Mean: 80 X 50 = 4000 eggs/ml

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

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

egg stock (A) ml
seawater (A) 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):	1825	50:1	84	16
Eggs Added (0.5 ml):	1835	100:1	95	5
Test Ended:	1845	100:1	97	3
		200:1	100	100

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):	1853	QC1	99	1
Eggs Added (0.5 ml):	1913	QC2	98	2
Test Ended:	1933	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) EG QI8 12/28/17

QC Check: KMS 12/22/17

Final Review: EG 12/28/17

Marine Chronic Bioassay **Brine Dilution Worksheet**

Project: IDE **Analyst:** CG

Sample ID: M-001 (40 ppt adjusted) 12/14/17 sample **Test Date:** 12/15/2017

Test No: 1712-S075 **Test Type:** Urchin Fertilization

Salinity of Effluent 61.2

Salinity of Seawater 33.5 **Date of Brine used:** NA

Target Salinity 40.0 **Alk. of 40 ppt Adj. Sample:** 145 mg/L as CaCO3

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**3.26**	**-6.15**

TS = target salinity
SE = salinity of effluent
SB = salinity of brine

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)
100	100	3.26	326.2	426

Comments: Formula for amount of seawater to dilute sample to 40ppt
Use 40 ppt sample as 100% sample for testing.
NA = not applicable; sample not diluted with Nautilus brine.

QC Check: AD 12/23/17 **Final Review:** EC 12/28/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: M-001
Test ID No(s).: 1712-SOT4 + SOT5

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1294			
Sample Collection Date & Time:	12/14/17 1230			
Sample Receipt Date & Time:	12/15/17 1354			
Number of Containers & Container Type:	1-4L cubi			
Approx. Total Volume Received (L):	~4			
Check-in Temperature (°C)	3.2			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	8.3			
pH (units)	7.96			
Conductivity (µS/cm)	—			
Salinity (ppt)	61.2 (A)			
Alkalinity (mg/L) [2]	183			
Hardness (mg/L) [2,3]	—			
Total Chlorine (mg/L)	<0.02			
Technician Initials	AB/BO			

Test Performed: Urchin Fertilization — Control/Dilution Water: 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 114 Hardness or (Salinity): 34
Additional Control? (Y) N = HSC Alkalinity: 89 Hardness or (Salinity): 37.5

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: (A) Salinity measured by making 1:1 dilution
(B) EG Q18 12/28/17

Sample Check-In Information

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

COC Complete (Y/N)?
A Y B___ C___

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

Salinity Adjustment? (Y) N (B)
Test: Urchin Fertilization Source: Seawater Target ppt: 40
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: AO 12/22/17
Final Review: EG 12/28/17

Appendix C

Chain-of-Custody Form

DAILY

IDE Technologies CDP Laboratories

Page 1 Of _____

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: Sampled during off-spec. Sample collected to fulfill daily NPDES requirement. Sample is to be run adjusted and unadjusted. Sampled 12/14/2017 @ 12:30 VH

Glass=G Plastic=P

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

Drinking Water=DW Seawater=SW Soil=S

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization								NOTES:
M-001 (17- 3526)	12/14/2017	12:30	SW	N	~~1L P~~ 4L Poly	X								TDS - 81.62 ppt, EC - 57.89 mS/cm

Relinquished By:	Date:	Time:		Received By:	Date:	Time:	Sample Condition Upon Receipt:
Kevin Cuny	12/15/17	0958		[signature]	12/15	0959	[X] Iced [ ] Ambient or _____ °C
*				[signature]	12/15/17	1354	[ ] Iced [ ] Ambient or _____ °C

temp = 3.2°C

* not relinquished by courier; tech error

Nautilus ID: 17-1294

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	13-6199-7350	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	15 Dec-17 18:53	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	15 Dec-17 19:33	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	16-5483-0832	**Code:**	171215sprtB	**Client:**	Internal
Sample Date:	15 Dec-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	15 Dec-17	**Source:**	Reference Toxicant		
Sample Age:	19h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
07-7404-7985	Fertilization Rate	40	80	56.57	4.53%		Steel Many-One Rank Sum Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
11-8739-9529	Fertilization Rate	EC50	76.76	73.99	79.63		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-7404-7985	Fertilization Rate	Control Resp	0.976	0.7 - NL	Yes	Passes Acceptability Criteria
11-8739-9529	Fertilization Rate	Control Resp	0.976	0.7 - NL	Yes	Passes Acceptability Criteria
07-7404-7985	Fertilization Rate	PMSD	0.04533	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	0.976	0.9692	0.9828	0.97	0.98	0.002449	0.005476	0.56%	0.0%
10		5	0.938	0.8946	0.9814	0.88	0.97	0.01562	0.03493	3.72%	3.89%
20		5	0.938	0.9059	0.9701	0.91	0.97	0.01158	0.02588	2.76%	3.89%
40		5	0.934	0.8896	0.9784	0.9	0.98	0.016	0.03578	3.83%	4.3%
80		5	0.454	0.2944	0.6136	0.31	0.65	0.0575	0.1286	28.32%	53.48%
160		5	0.004	0	0.0108	0	0.01	0.002449	0.005477	136.9%	99.59%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.97	0.98	0.97	0.98	0.98
10		0.88	0.94	0.94	0.97	0.96
20		0.97	0.92	0.91	0.96	0.93
40		0.9	0.93	0.9	0.96	0.98
80		0.31	0.5	0.4	0.65	0.41
160		0.01	0	0	0.01	0

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

Analysis ID:	07-7404-7985	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 13:34	**Analysis:**	Nonparametric-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	4.53%	40	80	56.57	

Steel Many-One Rank Sum Test

Control	vs	C-µg/L	Test Stat	Critical	Ties	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10*	16	16	1	8	0.0332	Asymp	Significant Effect
		20*	16	16	1	8	0.0332	Asymp	Significant Effect
		40	18.5	16	1	8	0.1075	Asymp	Non-Significant Effect
		80*	15	16	0	8	0.0191	Asymp	Significant Effect
		160*	15	16	0	8	0.0191	Asymp	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.066526	1.413305	5	262.9	<0.0001	Significant Effect
Error	0.1290262	0.005376091	24			
Total	7.195552		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	15.23	15.09	0.0094	Unequal Variances
Distribution	Shapiro-Wilk W Normality	0.9608	0.9031	0.3245	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	0.976	0.9692	0.9828	0.98	0.97	0.98	0.002449	0.56%	0.0%
10		5	0.938	0.8946	0.9814	0.94	0.88	0.97	0.01562	3.72%	3.89%
20		5	0.938	0.9059	0.9701	0.93	0.91	0.97	0.01158	2.76%	3.89%
40		5	0.934	0.8896	0.9784	0.93	0.9	0.98	0.016	3.83%	4.3%
80		5	0.454	0.2944	0.6136	0.41	0.31	0.65	0.0575	28.32%	53.48%
160		5	0.004	0	0.0108	0	0	0.01	0.002449	136.9%	99.59%

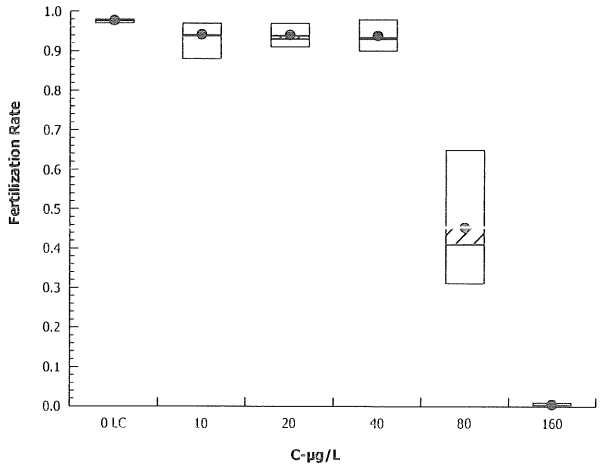
Angular (Corrected) Transformed Summary

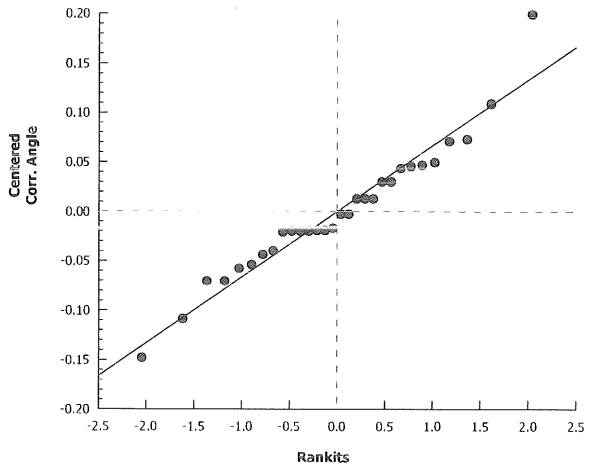
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.416	1.394	1.438	1.429	1.397	1.429	0.007885	1.25%	0.0%
10		5	1.326	1.241	1.411	1.323	1.217	1.397	0.03064	5.17%	6.36%
20		5	1.324	1.254	1.394	1.303	1.266	1.397	0.02524	4.26%	6.51%
40		5	1.32	1.222	1.417	1.303	1.249	1.429	0.03511	5.95%	6.79%
80		5	0.7387	0.5761	0.9013	0.6949	0.5905	0.9377	0.05856	17.73%	47.84%
160		5	0.07008	0.03598	0.1042	0.05002	0.05002	0.1002	0.01228	39.19%	95.05%

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

Analysis ID:	07-7404-7985	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 13:34	**Analysis:**	Nonparametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: JS/AC QA: EG 12/28/17

CETIS Analytical Report

Test Code: 171215sprtB | 02-9159-5360

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

Analysis ID:	11-8739-9529	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	27 Dec-17 13:34	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

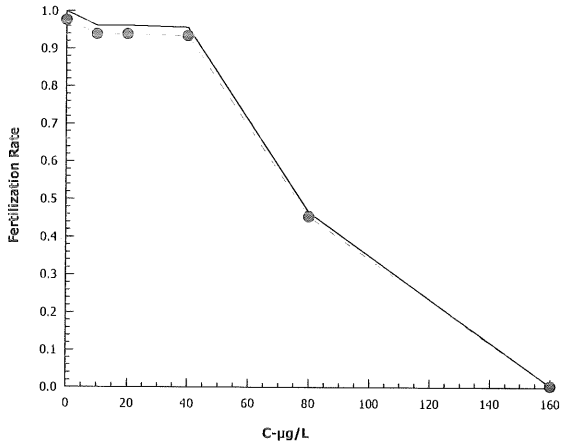
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.024	3.89%	1.885	0.007978	76.76	73.99	79.63

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	0.976	0.97	0.98	0.002449	0.005476	0.56%	0.0%	488	500
10		5	0.938	0.88	0.97	0.01562	0.03493	3.72%	3.89%	469	500
20		5	0.938	0.91	0.97	0.01158	0.02588	2.76%	3.89%	469	500
40		5	0.934	0.9	0.98	0.016	0.03578	3.83%	4.3%	467	500
80		5	0.454	0.31	0.65	0.0575	0.1286	28.32%	53.48%	227	500
160		5	0.004	0	0.01	0.002449	0.005477	136.9%	99.59%	2	500

Graphics



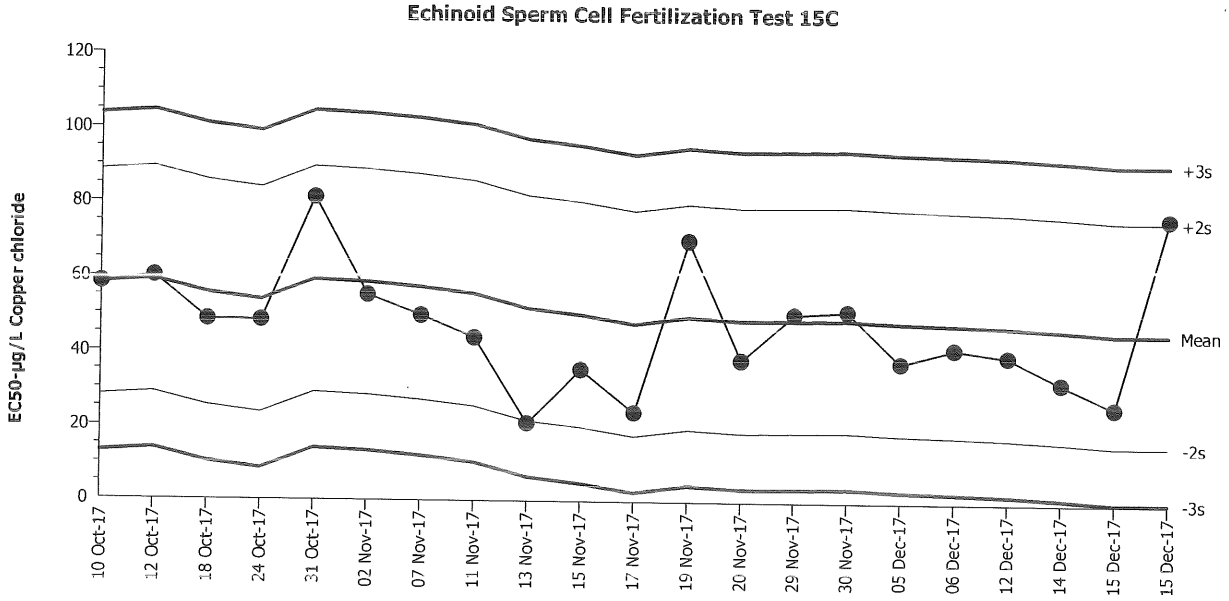
Analyst: JS|AC QA: EG 12/28/17

CETIS QC Plot

Report Date: 28 Dec-17 14:26 (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:	45.71	**Count:**	20	**-2s Warning Limit:**	15.45	**-3s Action Limit:**	0.3225
Sigma:	15.13	**CV:**	33.10%	**+2s Warning Limit:**	75.97	**+3s Action Limit:**	91.1

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Oct	10	15:10	58.36	12.65	0.8362			20-5863-5053	00-1542-1738
2			12	14:55	60.18	14.47	0.9565			05-0863-6526	07-1531-2424
3			18	14:22	48.53	2.82	0.1864			13-0042-6212	05-6771-5532
4			24	13:15	48.41	2.695	0.1781			20-0280-7301	18-5464-1899
5			31	13:59	81.36	35.65	2.356	(+)		06-4227-6723	08-8095-0809
6		Nov	2	12:28	55.32	9.615	0.6355			17-4126-1689	20-0626-8382
7			7	14:30	49.87	4.163	0.2752			10-3521-2857	13-9801-3995
8			11	14:25	43.91	-1.802	-0.1191			14-1655-2339	20-5239-6070
9			13	14:35	20.97	-24.74	-1.635			07-0538-7056	00-9105-4737
10			15	16:09	35.48	-10.23	-0.6759			06-3476-9418	17-5783-9769
11			17	14:17	24.03	-21.68	-1.433			20-8374-1268	00-9691-5869
12			19	10:02	70.21	24.5	1.619			12-1164-1483	20-4501-4622
13			20	15:15	38.26	-7.445	-0.4921			08-0578-7050	18-8950-2431
14			29	15:30	50.6	4.885	0.3229			05-0010-1267	11-1707-1208
15			30	15:28	51.48	5.765	0.3811			09-6334-2928	00-8447-7747
16		Dec	5	16:05	37.64	-8.068	-0.5333			00-4872-5743	06-2243-7863
17			6	15:50	41.57	-4.142	-0.2738			04-9516-7018	18-3148-8943
18			12	12:20	39.55	-6.162	-0.4073			01-8906-4164	02-6832-7767
19			14	15:35	32.51	-13.2	-0.8726			11-6397-1428	17-9802-1610
20			15	15:06	26.01	-19.7	-1.302			06-1613-2535	10-1459-1840
21			15	18:53	76.76	31.05	2.052	(+)		02-9159-5360	11-8739-9529

CETIS Test Data Worksheet

Report Date: 14 Dec-17 17:23 (p 1 of 1)
Test Code: 06-1613-2535/171215sprt

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

Start Date: 15 Dec-17
End Date: 15 Dec-17
Sample Date: 15 Dec-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 171215sprtB
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	31	12/18/17
			2	100	97	
			3	100	1	
			4	100	96	
			5	100	97	
			6	100	65	
			7	100	96	
			8	100	98	
			9	100	96	
			10	100	97	
			11	100	94	
			12	100	93	
			13	100	98	
			14	100	98	
			15	100	90	
			16	100	98	
			17	100	0	
			18	100	93	
			19	100	97	
			20	100	1	
			21	100	40	
			22	100	94	
			23	100	90	
			24	100	0	
			25	100	50	
			26	100	41	
			27	100	0	
			28	100	92	
			29	100	91	
			30	100	88	

CETIS Test Data Worksheet

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

Start Date: 15 Dec-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 171215sprtB
End Date: 15 Dec-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 15 Dec-17 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	10			
0	LC	2	8	100	98	BO 12/22/17
0	LC	3	19			
0	LC	4	14			
0	LC	5	16			
10		1	30			
10		2	11	100	92	BO 12/22/17
10		3	22			
10		4	2			
10		5	7			
20		1	5			
20		2	28			
20		3	29			
20		4	9	100	94	BO 12/22/17
20		5	18			
40		1	15			
40		2	12			
40		3	23			
40		4	4			
40		5	13	100	89	BO 12/22/17
80		1	1			
80		2	25			
80		3	21			
80		4	6	100	60	BO 12/22/17
80		5	26			
160		1	20			
160		2	24			
160		3	27			
160		4	3	100	0	BO 12/22/17
160		5	17			

AC OBO AD

Analyst: AD QA: AC 12/27/17

Marine Chronic Bioassay — **Water Quality Measurements**

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

Sample ID: $CuCl_2$ — **Start Date/Time:** 12/15/17 1853

Test No: 171215sqrt B — **End Date/Time:** 12/15/17 1933

Dilutions made by: AD OBD PA

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10300

Analyst: AC

	Initial Readings			
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	9.0	7.99	33.1	14.5
10	8.8	8.01	33.8	14.4
20	8.8	8.02	33.8	14.2
40	8.8	8.02	33.8	14.2
80	8.8	8.03	33.7	14.2
160	8.7	8.03	33.4	14.3

Comments:

QC Check: AD 12/22/17 — **Final Review:** EG 12/28/17

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

Client: Interval
Sample ID: CuCl2
Test No.: 171215sptB

Start Date/Time: 12/15/17 1853
End Date/Time: 12/15/17 1933
Species: S. purpuratus
Animal Source: PT. Loma
Date Collected: 12/8/17

Tech initials: AD OBO PA
Injection Time: 1815

Sperm Absorbance at 400 nm: 0.810 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 79, 75, 78, 78, 90

Mean: 80 X 50 = 4000 eggs/ml

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

Initial density:	4000	eggs/ml	= 1.0	dilution factor	egg stock	1A ml
Final density:	4000	eggs/ml	- 1.0	part egg stock	seawater	1A ml
			0	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):	1825	50:1	84	16
Eggs Added (0.5 ml):	1835	100:1	95	5
Test Ended:	1845	100:1	97	3
		200:1	100	100

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):	1853	QC1	99	1
Eggs Added (0.5 ml):	1913	QC2	98	2
Test Ended:	1933	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: @No dilution required

QC Check: AO 12/22/17 Final Review: EG 12/28/17

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.