Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

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

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

Prepared by: **Nautilus Environmental**

Submitted: **November 7, 2017**

Data Quality Assurance:

- Nautilus Environmental is accredited in accordance with NELAP by the State of Oregon Environmental Laboratory Accreditation Program (Certificate No. 4053). It is also certified by the State of California Department of Health Services Environmental Laboratory Accreditation Program (Certificate No. 1802) and the State of Washington Department of Ecology (Lab ID C552).
- All data have been reviewed and verified.
- All test results have met minimum test acceptability criteria under their respective EPA protocols, unless otherwise noted in this report.
- All test results have met internal Quality Assurance Program requirements.

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

Results verified by: Adrienne Cibar

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – OCTOBER 2017

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

Sampling Date: October 30, 2017

Test Date: October 31, 2017

Sample ID: M-001 Brine Effluent

M-001
Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	M-001 Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	NOEC	TU_c	No
	5	20	

INTRODUCTION

A 24-hour composite discharge sample was collected in October 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for weekly accelerated toxicity monitoring purposes. Due to effects observed in a sample collected and tested for monthly monitoring purposes on May 04, 2017 from the CDP discharge monitoring point (M-001), accelerated monitoring was triggered according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on October 31, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was couriered to Nautilus the day of sample collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. The sample was stored at 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 protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./ Carlsbad Desalination Plant
Monitoring Period:	October 2017 (weekly accelerated chronic monitoring)
Sample ID, Material:	M-001, desalination plant brine effluent
Sample Collection Date, Time:	10/30/17, 08:00
Sample Receipt Date, Time:	10/30/17, 12:54
Sampling Method:	24-hour Composite

Table 2. Water Quality Measurements upon Sample Receipt

Sample ID	pH	DO (mg/L)	Temp (ºC)	Salinity (ppt)	Alkalinity (mg/L as $CaCO_3$)	Total Chlorine (mg/L)
M-001	7.82	7.3	3.4	60.6	163	<0.02

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	10/31/17, 13:59 through 14:39
Test Organism:	*Strongylocentrotus purpuratus* (purple sea urchin)
Test Organism Source:	Field-collected off Point Loma in San Diego, CA
Lab Control/Dilution Water:	Natural seawater (source: Scripps Institution of Oceanography (SIO) inlet), 34±2 parts per thousand (ppt); 20-µm filtered
Additional Control:	High Salinity Control (HSC) – seawater with Nautilus hypersaline brine added to match the salinity of the 15 percent M-001 effluent concentration; tested to evaluate potential adverse effects due to elevated salinity alone
Test Concentrations:	2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control. The same dilution series was also tested with the sample after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP discharges).
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min egg fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25%
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

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

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies

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

RESULTS

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

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

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

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

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
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	98.8	33.9	98.0
High Salinity Control	37.0	99.0	--	--
2.5	34.5	98.4	34.2	98.0
5.0	35.2	97.8	34.3	98.8
6.06	35.5	96.2*	34.4	99.0
10	36.5	94.4*	34.6	97.4
15	37.1	95.4*	34.9	98.2

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

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

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

Table 6. Reference Toxicant Test Results

Test Species	Endpoint	EC_{50} **(µg/L Copper)**	Historical Mean EC_{50} ±2 SD **(µg/L Copper)**	CV (%)
Purple Urchin	Fertilization	81.4	49.9 ± 30.5	30.6

EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
Historical Mean EC_{50} ± 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:	13-1240-6073	Test Type:	Fertilization	Analyst:	
Start Date:	31 Oct-17 13:59	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Natural Seawater
Ending Date:	31 Oct-17 14:39	Species:	Strongylocentrotus purpuratus	Brine:	Not Applicable
Duration:	40m	Source:	Pt. Loma	Age:	

Sample ID:	08-8002-2042	Code:	17-1128	Client:	IDE
Sample Date:	30 Oct-17 08:00	Material:	Facility Effluent	Project:	Carlsbad Desal Plant
Receive Date:	30 Oct-17 12:54	Source:	IDE Americas, Inc.		
Sample Age:	30h (3.4 °C)	Station:	M-001 Unadjusted		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-9548-5162	Fertilization Rate	5	6.06	5.505	1.8%	20	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
15-0356-3573	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
13-9548-5162	Fertilization Rate	Control Resp	0.988	0.7 - NL	Yes	Passes Acceptability Criteria
15-0356-3573	Fertilization Rate	Control Resp	0.988	0.7 - NL	Yes	Passes Acceptability Criteria
13-9548-5162	Fertilization Rate	PMSD	0.01796	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	0.99	0.9812	0.9988	0.98	1	0.003163	0.007072	0.71%	0.0%
0	Lab Control	5	0.988	0.9824	0.9936	0.98	0.99	0.002001	0.004474	0.45%	0.2%
2.5		5	0.984	0.9729	0.9951	0.97	0.99	0.004	0.008945	0.91%	0.61%
5		5	0.978	0.9676	0.9884	0.97	0.99	0.003741	0.008366	0.86%	1.21%
6.06		5	0.962	0.9381	0.9859	0.93	0.98	0.008602	0.01924	2.0%	2.83%
10		5	0.944	0.9128	0.9752	0.91	0.98	0.01122	0.0251	2.66%	4.65%
15		5	0.954	0.9254	0.9826	0.93	0.98	0.0103	0.02302	2.41%	3.64%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	High Salinity Co	0.98	0.99	0.99	1	0.99
0	Lab Control	0.99	0.99	0.98	0.99	0.99
2.5		0.99	0.99	0.99	0.98	0.97
5		0.98	0.99	0.97	0.97	0.98
6.06		0.98	0.96	0.93	0.97	0.97
10		0.91	0.94	0.98	0.94	0.95
15		0.98	0.96	0.93	0.97	0.93

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

Analysis ID:	13-9548-5162	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:09	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	1.8%	5	6.06	5.505	20

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	0.5386	2.362	0.065	8	0.6300	CDF	Non-Significant Effect
		5	1.381	2.362	0.065	8	0.2646	CDF	Non-Significant Effect
		6.06*	3.036	2.362	0.065	8	0.0118	CDF	Significant Effect
		10*	4.55	2.362	0.065	8	0.0003	CDF	Significant Effect
		15*	3.718	2.362	0.065	8	0.0023	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06327607	0.01265521	5	6.719	0.0005	Significant Effect
Error	0.04520147	0.001883394	24			
Total	0.1084775		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.816	15.09	0.3246	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9728	0.9031	0.6181	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	0.988	0.9824	0.9936	0.99	0.98	0.99	0.002001	0.45%	0.0%
2.5		5	0.984	0.9729	0.9951	0.99	0.97	0.99	0.004	0.91%	0.4%
5		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	1.01%
6.06		5	0.962	0.9381	0.9859	0.97	0.93	0.98	0.008602	2.0%	2.63%
10		5	0.944	0.9128	0.9752	0.94	0.91	0.98	0.01122	2.66%	4.45%
15		5	0.954	0.9254	0.9826	0.96	0.93	0.98	0.0103	2.41%	3.44%

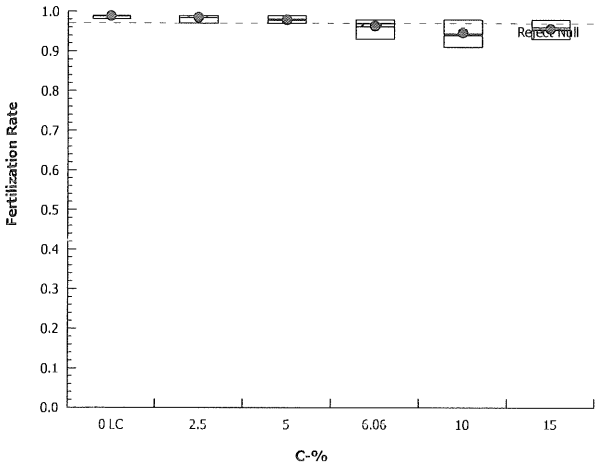
Angular (Corrected) Transformed Summary

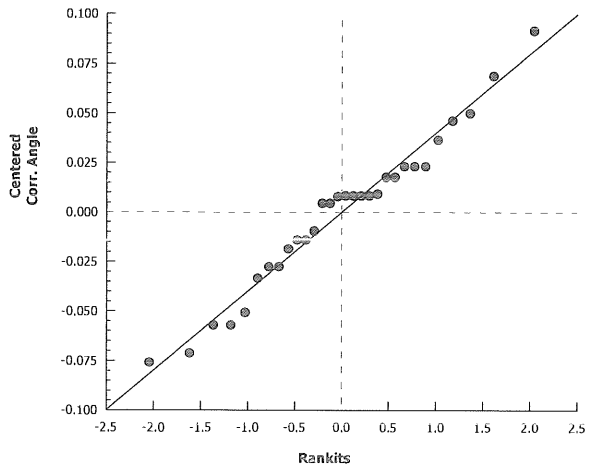
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.462	1.439	1.485	1.471	1.429	1.471	0.008346	1.28%	0.0%
2.5		5	1.447	1.406	1.489	1.471	1.397	1.471	0.01505	2.33%	1.01%
5		5	1.424	1.387	1.462	1.429	1.397	1.471	0.01362	2.14%	2.59%
6.06		5	1.379	1.32	1.438	1.397	1.303	1.429	0.02119	3.44%	5.7%
10		5	1.337	1.264	1.411	1.323	1.266	1.429	0.02638	4.41%	8.54%
15		5	1.36	1.29	1.43	1.369	1.303	1.429	0.02517	4.14%	6.98%

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

Analysis ID:	13-9548-5162	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:09	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

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

Analysis ID:	15-0356-3573	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:11	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1377706	1000	Yes	Two-Point Interpolation

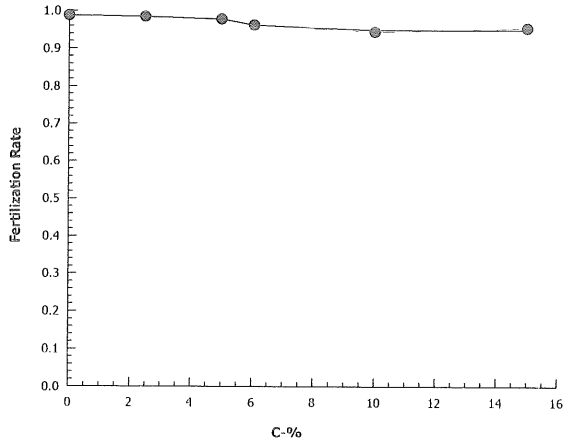
Point Estimates

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

Graphics



Analyst: _____ QA: EG 11/3/17

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

Analysis ID:	14-0750-5451	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:10	**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	1.48%	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*	21.52	2.015	0.033	5	<0.0001	CDF	Non-Significant Effect
		5*	21.86	2.015	0.030	5	<0.0001	CDF	Non-Significant Effect
		6.06*	12.77	2.132	0.047	4	0.0001	CDF	Non-Significant Effect
		10*	8.878	2.132	0.058	4	0.0004	CDF	Non-Significant Effect
		15*	10.16	2.132	0.055	4	0.0003	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.06327607	0.01265521	5	6.719	0.0005	Significant Effect
Error	0.04520147	0.001883394	24			
Total	0.1084775		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	5.816	15.09	0.3246	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9728	0.9031	0.6181	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	0.988	0.9824	0.9936	0.99	0.98	0.99	0.002001	0.45%	0.0%
2.5		5	0.984	0.9729	0.9951	0.99	0.97	0.99	0.004	0.91%	0.4%
5		5	0.978	0.9676	0.9884	0.98	0.97	0.99	0.003741	0.86%	1.01%
6.06		5	0.962	0.9381	0.9859	0.97	0.93	0.98	0.008602	2.0%	2.63%
10		5	0.944	0.9128	0.9752	0.94	0.91	0.98	0.01122	2.66%	4.45%
15		5	0.954	0.9254	0.9826	0.96	0.93	0.98	0.0103	2.41%	3.44%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.462	1.439	1.485	1.471	1.429	1.471	0.008346	1.28%	0.0%
2.5		5	1.447	1.406	1.489	1.471	1.397	1.471	0.01505	2.33%	1.01%
5		5	1.424	1.387	1.462	1.429	1.397	1.471	0.01362	2.14%	2.59%
6.06		5	1.379	1.32	1.438	1.397	1.303	1.429	0.02119	3.44%	5.7%
10		5	1.337	1.264	1.411	1.323	1.266	1.429	0.02638	4.41%	8.54%
15		5	1.36	1.29	1.43	1.369	1.303	1.429	0.02517	4.14%	6.98%

CETIS Test Data Worksheet

Report Date: 27 Oct-17 16:50 (p 1 of 1)
Test Code: 1710-5128 09-2205-6048/1710-S128

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

Start Date: 31 ~~30~~ Oct-17
End Date: 31 ~~30~~ Oct-17
Sample Date: 30 Oct-17

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

Sample Code: 17- 1128
Sample Source: IDE Americas, Inc.
Sample Station: M-001 Unadjusted

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

(A) EG Q.C 11/1/17

CETIS Test Data Worksheet

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

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

Start Date: (A) 31 30 Oct-17 | Species: Strongylocentrotus purpuratus | Sample Code: 17- 1128
End Date: (B) 31 30 Oct-17 | Protocol: EPA/600/R-95/136 (1995) | Sample Source: IDE Americas, Inc.
Sample Date: 30 Oct-17 | Material: Facility Effluent | Sample Station: M-001 Unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	55			
0	HS	2	35	100	99	BO 10/31/17
0	HS	3	52			
0	HS	4	65			
0	HS	5	54			
0	LC	1	58			
0	LC	2	62	100	100	BO 10/31/17
0	LC	3	51			
0	LC	4	32			
0	LC	5	36			
2.5		1	39	100	99	BO 10/31/17
2.5		2	37			
2.5		3	64			
2.5		4	42			
2.5		5	56			
5		1	57	100	98	BO 10/31/17
5		2	41			
5		3	45			
5		4	60			
5		5	48			
6.06		1	44			
6.06		2	49			
6.06		3	46	100	97	BO 10/31/17
6.06		4	53			
6.06		5	40			
10		1	47			
10		2	61	100	93	BO 10/31/17
10		3	31			
10		4	33			
10		5	43			
15		1	59			
15		2	63			
15		3	34	100	97	BO 10/31/17
15		4	38			
15		5	50			

(A) CG 10/31/17

QC: CG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: M-001 (unadjusted) | Start Date/Time: 10/31/2017 1359

Sample Log No.: 17- 1128 | End Date/Time: 10/31/2017 1439

Dilutions made by: CG | Test No: 1710-S128

Analyst: CG

	Initial Readings			
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.02	33.9	15.8
High Salinity Control	8.3	8.01	37.0	15.6
2.5	8.4	8.01	34.5	15.8
5.0	8.2	8.00	35.2	16.0
6.06	8.3	8.00	35.5	15.9
10	8.5	7.99	36.5	15.8
15	8.4	7.98	37.1	15.9

Comments:

QC Check: EG 11/1/17 | Final Review: AC 11/3/17

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

Client: IDE
Sample ID: M-001 unadjusted
Test No.: 1710-S128

Tech initials: CG
Injection Time: 1310

Start Date/Time: 10/31/2017 1359
End Date/Time: 10/31/2017 1439
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 10/10/17

Sperm Absorbance at 400 nm: 1.001 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 93, 94, 89, 96, 91

Mean: 93 X 50 = 4650 eggs/ml

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

Initial density: 4650 eggs/ml = 1.163 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.163 parts seawater

egg stock 100 ml
seawater 16.3 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):	1325	50:1	66	34
Eggs Added (0.5 ml):	1340	100:1	94, 94	6, 6
Test Ended:	1350	200:1	98	2
		—	—	—

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):	1359	QC1	97	3
Eggs Added (0.5 ml):	1419	QC2	98	2
Test Ended:	1439	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: EG 11/1/17

Final Review: A 11/3/17

M-001 40 ppt Adjusted

CETIS Summary Report

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

Batch ID:	12-2186-0218	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	31 Oct-17 13:59	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	31 Oct-17 14:39	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	13-1699-9192	**Code:**	17-1128	**Client:**	IDE
Sample Date:	30 Oct-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Oct-17 12:54	**Source:**	IDE Americas, Inc.		
Sample Age:	30h (3.4 °C)	**Station:**	M-001 (40 ppt adj)		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-1389-4617	Fertilization Rate	15	>15	NA	2.6%	6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
05-3581-8471	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
05-3581-8471	Fertilization Rate	Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria
18-1389-4617	Fertilization Rate	Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria
18-1389-4617	Fertilization Rate	PMSD	0.02597	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	0.98	0.9648	0.9952	0.96	0.99	0.005478	0.01225	1.25%	0.0%
2.5		5	0.98	0.9604	0.9996	0.96	1	0.007071	0.01581	1.61%	0.0%
5		5	0.988	0.9744	1	0.98	1	0.004899	0.01095	1.11%	-0.82%
6.06		5	0.99	0.9812	0.9988	0.98	1	0.003163	0.007072	0.71%	-1.02%
10		5	0.974	0.9552	0.9928	0.96	1	0.006782	0.01517	1.56%	0.61%
15		5	0.982	0.9616	1	0.96	1	0.007349	0.01643	1.67%	-0.2%

Fertilization Rate Detail

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

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

Analysis ID:	18-1389-4617	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:17	**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	2.6%	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	-0.1091	2.362	0.078	8	0.8636	CDF	Non-Significant Effect
		5	-0.9705	2.362	0.078	8	0.9819	CDF	Non-Significant Effect
		6.06	-1.173	2.362	0.078	8	0.9898	CDF	Non-Significant Effect
		10	0.5354	2.362	0.078	8	0.6314	CDF	Non-Significant Effect
		15	-0.3626	2.362	0.078	8	0.9185	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01098375	0.002196749	5	0.8107	0.5536	Non-Significant Effect
Error	0.06503302	0.002709709	24			
Total	0.07601676		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.067	15.09	0.8397	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9635	0.9031	0.3783	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	0.98	0.9648	0.9952	0.98	0.96	0.99	0.005478	1.25%	0.0%
2.5		5	0.98	0.9604	0.9996	0.98	0.96	1	0.007071	1.61%	0.0%
5		5	0.988	0.9744	1	0.98	0.98	1	0.004899	1.11%	-0.82%
6.06		5	0.99	0.9812	0.9988	0.99	0.98	1	0.003163	0.71%	-1.02%
10		5	0.974	0.9552	0.9928	0.97	0.96	1	0.006782	1.56%	0.61%
15		5	0.982	0.9616	1	0.99	0.96	1	0.007349	1.67%	-0.2%

Angular (Corrected) Transformed Summary

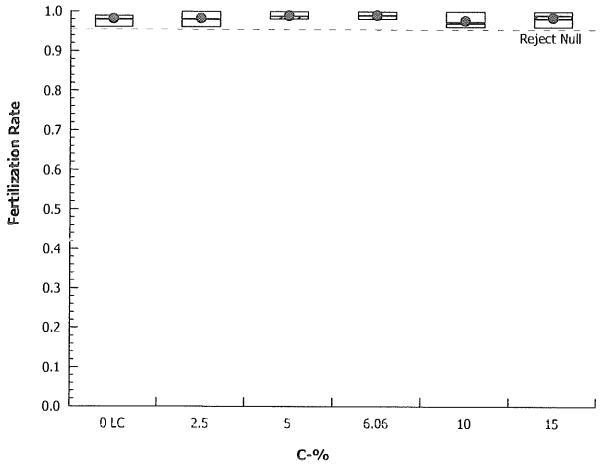
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.434	1.382	1.485	1.429	1.369	1.471	0.01858	2.9%	0.0%
2.5		5	1.437	1.363	1.512	1.429	1.369	1.521	0.02683	4.18%	-0.25%
5		5	1.466	1.403	1.528	1.429	1.429	1.521	0.02251	3.43%	-2.23%
6.06		5	1.472	1.432	1.513	1.471	1.429	1.521	0.01456	2.21%	-2.69%
10		5	1.416	1.342	1.49	1.397	1.369	1.521	0.0267	4.22%	1.23%
15		5	1.446	1.369	1.522	1.471	1.369	1.521	0.02747	4.25%	-0.83%

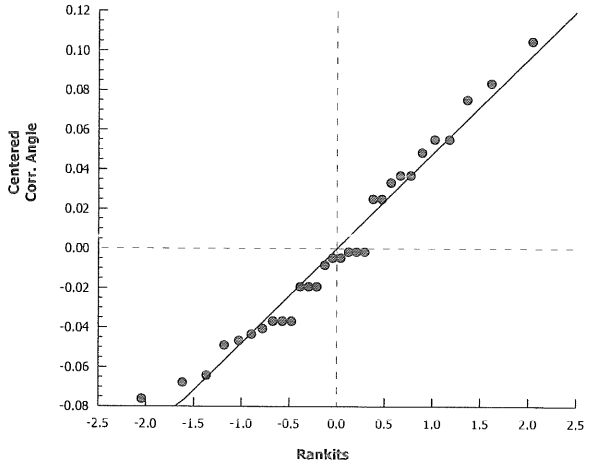
Analyst: _____ QA: EG 11/3/17

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

Analysis ID:	18-1389-4617	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:17	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: _____ QA: EG 11/3/17

CETIS Analytical Report

Test Code: 1710-S129 | 03-9733-9800

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

Analysis ID:	05-3581-8471	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 12:17	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1150329	1000	Yes	Two-Point Interpolation

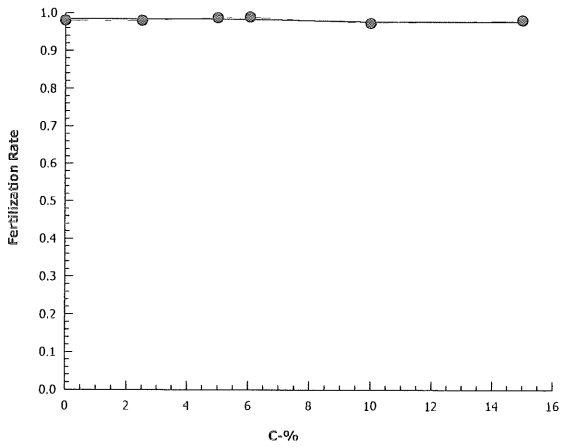
Point Estimates

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

Graphics



Analyst: QA: EL 11/3/17

CETIS Analytical Report

TST

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

Analysis ID: 21-2235-9885 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 01 Nov-17 12:17 **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	1.95%	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*	11.97	1.943	0.059	6	<0.0001	CDF	Non-Significant Effect
		5*	14.75	1.943	0.051	6	<0.0001	CDF	Non-Significant Effect
		6.06*	19.7	1.895	0.038	7	<0.0001	CDF	Non-Significant Effect
		10*	11.31	1.943	0.059	6	<0.0001	CDF	Non-Significant Effect
		15*	12.02	2.015	0.062	5	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01098375	0.002196749	5	0.8107	0.5536	Non-Significant Effect
Error	0.06503302	0.002709709	24			
Total	0.07601676		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.067	15.09	0.8397	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9635	0.9031	0.3783	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	0.98	0.9648	0.9952	0.98	0.96	0.99	0.005478	1.25%	0.0%
2.5		5	0.98	0.9604	0.9996	0.98	0.96	1	0.007071	1.61%	0.0%
5		5	0.988	0.9744	1	0.98	0.98	1	0.004899	1.11%	-0.82%
6.06		5	0.99	0.9812	0.9988	0.99	0.98	1	0.003163	0.71%	-1.02%
10		5	0.974	0.9552	0.9928	0.97	0.96	1	0.006782	1.56%	0.61%
15		5	0.982	0.9616	1	0.99	0.96	1	0.007349	1.67%	-0.2%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.434	1.382	1.485	1.429	1.369	1.471	0.01858	2.9%	0.0%
2.5		5	1.437	1.363	1.512	1.429	1.369	1.521	0.02683	4.18%	-0.25%
5		5	1.466	1.403	1.528	1.429	1.429	1.521	0.02251	3.43%	-2.23%
6.06		5	1.472	1.432	1.513	1.471	1.429	1.521	0.01456	2.21%	-2.69%
10		5	1.416	1.342	1.49	1.397	1.369	1.521	0.0267	4.22%	1.23%
15		5	1.446	1.369	1.522	1.471	1.369	1.521	0.02747	4.25%	-0.83%

Analyst: _____ QA: EL 11/3/17

CETIS Test Data Worksheet

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

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 31 ~~30~~ Oct-17 (A)
End Date: 31 ~~30~~ Oct-17
Sample Date: 30 Oct-17

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

Sample Code: 17-1128
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			66	100	98	11/1/17
			67	100	98	
			68	100	100	
			69	100	100	
			70	100	99	
			71	100	99	
			72	100	97	
			73	100	99	
			74	100	96	
			75	100	99	
			76	100	96	
			77	100	96	
			78	100	98	
			79	100	97	
			80	100	98	
			81	100	100	
			82	100	100	
			83	100	97	
			84	100	98	
			85	100	97	
			86	100	99	
			87	100	98	
			88	100	98	
			89	100	97	
			90	100	97	
			91	100	99	
			92	100	96	
			93	100	100	
			94	100	99	
			95	100	100	

(A) EG Q18 11/1/17

CETIS Test Data Worksheet

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

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

Start Date: (A) ~~30~~ 31 Oct-17
End Date: (A) ~~30~~ 31 Oct-17
Sample Date: 30 Oct-17

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

Sample Code: 17-1128
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (40 ppt adj)

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

QC CG (A) CG 11/8 10/31/17

(B) EG 11/8 11/1/17

Marine Chronic Bioassay | **Water Quality Measurements**

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001 (40 ppt adjusted)

Start Date/Time: 10/31/2017 1359

Sample Log No.: 17- 1128

End Date/Time: 10/31/2017 1439

Dilutions made by: CG

Test No: 1710-S129

Analyst: CG

	Initial Readings			
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	8.03	33.9	15.1
2.5	8.4	8.03	34.2	15.1
5.0	8.4	8.03	34.3	15.1
6.06	8.4	8.04	34.4	15.1
10	8.5	8.03	34.6	15.0
15	8.5	8.03	34.9	15.0

Comments:

QC Check: EG 11/1/17

Final Review: AC 11/3/17

Marine Chronic Bioassay **Brine Dilution Worksheet**

Project: IDE **Analyst:** CG

Sample ID: M-001 (40 ppt adjusted) **Test Date:** 10/31/2017

Test No: 1710-S129 **Test Type:** Urchin Fertilization

Salinity of Effluent 60.6

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

Target Salinity 40.0 **Alk. of 40 ppt Adj. Sample:** 122 mg/L as CaCO3

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**3.17**	**-6.15**

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

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

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

QC Check: EG 11/1/17 **Final Review:** AC 11/3/17

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

Client: IDE
Sample ID: M-001 (40ppt adjusted)
Test No.: 1710-S129

Start Date/Time: 10/31/2017 1359
End Date/Time: 10/31/2017 1439
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 10/10/17

Tech initials: CG
Injection Time: 1310

Sperm Absorbance at 400 nm: 1.001 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 93, 94, 89, 90, 91 Mean: 93 X 50 = 4650 eggs/ml

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

Initial density: 4650 eggs/ml = 1.163 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.163 parts seawater

egg stock 100 ml
seawater 16.3 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):	1325	50:1	66	34
Eggs Added (0.5 ml):	1340	100:1	94, 94	6, 6
Test Ended:	1350	200:1	98	2
		-	-	-

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):	1359	QC1	97	3
Eggs Added (0.5 ml):	1419	QC2	98	2
Test Ended:	1439	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: EG 11/1/17 Final Review: AC 11/3/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

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

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	1128			
Sample Collection Date & Time:	10/30/17 0800			
Sample Receipt Date & Time:	10/30/17 1254			
Number of Containers & Container Type:	1, 4L cubi			
Approx. Total Volume Received (L):	~4			
Check-in Temperature (°C)	3.4			
Temperature OK? [1]	(Y) N	Y N	Y N	Y N
DO (mg/L)	7.3			
pH (units)	7.82			
Conductivity (µS/cm)	–			
Salinity (ppt)	60.6 (@)			
Alkalinity (mg/L) [2]	163			
Hardness (mg/L) [2,3]	–			
Total Chlorine (mg/L)	<0.02			
Technician Initials	CH			

Test Performed: Urchin Fertilization **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 120 Hardness or Salinity: 34 ppt
Additional Control? (Y) N = High Salinity Control Alkalinity: N/A Hardness or Salinity: 37.0 ppt

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: (@) 50% dilution performed to obtain measurement.

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
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: EG 11/1/17
Final Review: AK 11/3/17

Appendix C

Chain-of-Custody Form

WEEKLY

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 Weekly Toxicity_____ Project Manager:____Peter Shen_____ Contact Information:_____(760) 201-7777_____

Special instruction: Sampled during plant operation at 48 MGD via autosampler by a series of grabs collected at one hour intervals. Sample collected to fulfill weekly NPDES requirements. Sample is to be run unadjusted and adjusted to 40 ppt. Start: 10/29/17 @ 08:00, End: 10/30/17 @ 08:00 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- 3206)	10/29-30/17	08:00-08:00	SW	N	4L CUBIE	X								TDS - 56.57 ppt, EC - 79.65 mS/cm

Relinquished By:	Date:	Time:		Received By:	Date:	Time:	Sample Condition Upon Receipt:
[signature]	10/30/17	1200		[signature]	10/30/17	1200	[X] Iced [ ] Ambient or _____ °C
[signature]	10/30/17	1254		[signature]	10/30/2017	1254	[X] Iced [ ] Ambient or 3.4 °C

Nautilus ID: 17-1128

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	19-6668-0859	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	31 Oct-17 13:59	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	31 Oct-17 14:39	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	09-4181-9044	**Code:**	171031sprt	**Client:**	Internal
Sample Date:	31 Oct-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	31 Oct-17	**Source:**	Reference Toxicant		
Sample Age:	14h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
20-6412-1446	Fertilization Rate	40	80	56.57	2.53%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
08-8095-0809	Fertilization Rate	EC50	81.36	78.72	84.09		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
08-8095-0809	Fertilization Rate	Control Resp	0.988	0.7 - NL	Yes	Passes Acceptability Criteria
20-6412-1446	Fertilization Rate	Control Resp	0.988	0.7 - NL	Yes	Passes Acceptability Criteria
20-6412-1446	Fertilization Rate	PMSD	0.02534	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	0.988	0.9776	0.9984	0.98	1	0.003742	0.008367	0.85%	0.0%
10		5	0.97	0.9409	0.9991	0.95	1	0.01049	0.02345	2.42%	1.82%
20		5	0.984	0.9772	0.9908	0.98	0.99	0.00245	0.005479	0.56%	0.4%
40		5	0.97	0.9504	0.9896	0.95	0.99	0.007071	0.01581	1.63%	1.82%
80		5	0.526	0.4406	0.6114	0.43	0.62	0.03076	0.06877	13.08%	46.76%
160		5	0.008	0	0.03021	0	0.04	0.008	0.01789	223.6%	99.19%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	1	0.99	0.98	0.99	0.98
10		0.95	1	0.95	0.99	0.96
20		0.98	0.99	0.98	0.98	0.99
40		0.98	0.95	0.99	0.97	0.96
80		0.55	0.52	0.43	0.62	0.51
160		0	0	0	0	0.04

Analyst: _____ QA KFP 11/2/17

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

Analysis ID:	20-6412-1446	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 10:23	**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	2.53%	40	80	56.57	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10	1.46	2.362	0.087	8	0.2371	CDF	Non-Significant Effect
		20	0.4998	2.362	0.087	8	0.6470	CDF	Non-Significant Effect
		40	1.68	2.362	0.087	8	0.1704	CDF	Non-Significant Effect
		80*	17.75	2.362	0.087	8	<0.0001	CDF	Significant Effect
		160*	37.64	2.362	0.087	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	7.812252	1.56245	5	462.5	<0.0001	Significant Effect
Error	0.08108616	0.00337859	24			
Total	7.893338		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	6.33	15.09	0.2754	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9424	0.9031	0.1055	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	0.988	0.9776	0.9984	0.99	0.98	1	0.003742	0.85%	0.0%
10		5	0.97	0.9409	0.9991	0.96	0.95	1	0.01049	2.42%	1.82%
20		5	0.984	0.9772	0.9908	0.98	0.98	0.99	0.00245	0.56%	0.4%
40		5	0.97	0.9504	0.9896	0.97	0.95	0.99	0.007071	1.63%	1.82%
80		5	0.526	0.4406	0.6114	0.52	0.43	0.62	0.03076	13.08%	46.76%
160		5	0.008	0	0.03021	0	0	0.04	0.008	223.6%	99.19%

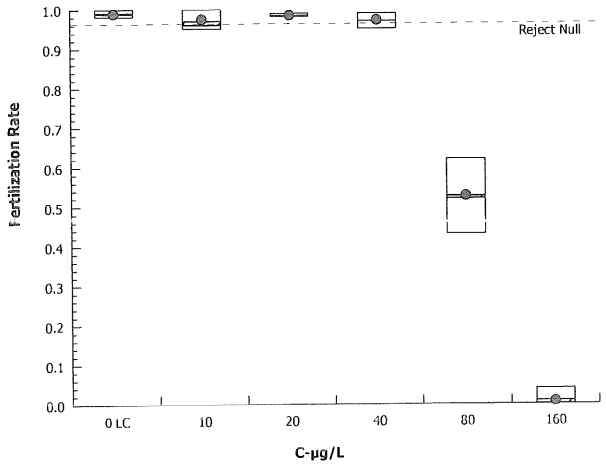
Angular (Corrected) Transformed Summary

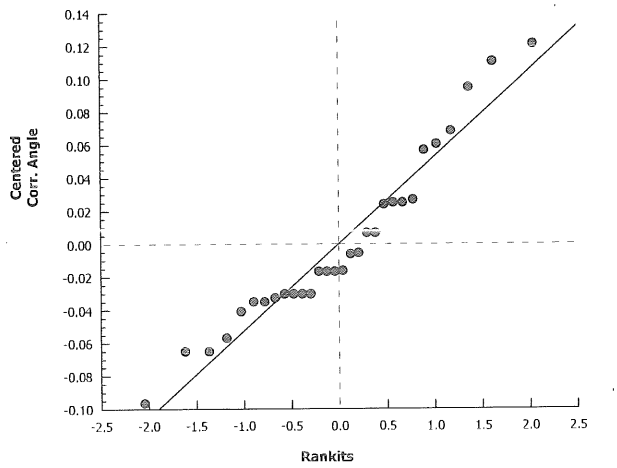
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.464	1.417	1.511	1.471	1.429	1.521	0.01699	2.6%	0.0%
10		5	1.41	1.31	1.51	1.369	1.345	1.521	0.03603	5.71%	3.67%
20		5	1.446	1.417	1.474	1.429	1.429	1.471	0.01022	1.58%	1.26%
40		5	1.402	1.341	1.463	1.397	1.345	1.471	0.02207	3.52%	4.22%
80		5	0.8116	0.7256	0.8976	0.8054	0.7152	0.9066	0.03098	8.54%	44.56%
160		5	0.08029	-0.00375	0.1643	0.05002	0.05002	0.2014	0.03027	84.3%	94.52%

Analyst: _____ QA: KTP 11/2/17

Echinoid Sperm Cell Fertilization Test 15C					Nautilus Environmental (CA)
Analysis ID:	20-6412-1446	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 10:23	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	08-8095-0809	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	01 Nov-17 10:23	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

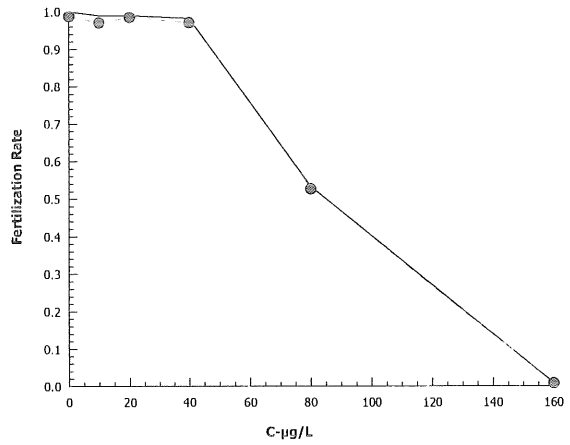
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.012	1.11%	1.91	0.007175	81.36	78.72	84.09

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	0.988	0.98	1	0.003742	0.008367	0.85%	0.0%	494	500
10		5	0.97	0.95	1	0.01049	0.02345	2.42%	1.82%	485	500
20		5	0.984	0.98	0.99	0.00245	0.005479	0.56%	0.4%	492	500
40		5	0.97	0.95	0.99	0.007071	0.01581	1.63%	1.82%	485	500
80		5	0.526	0.43	0.62	0.03076	0.06877	13.08%	46.76%	263	500
160		5	0.008	0	0.04	0.008	0.01789	223.6%	99.19%	4	500

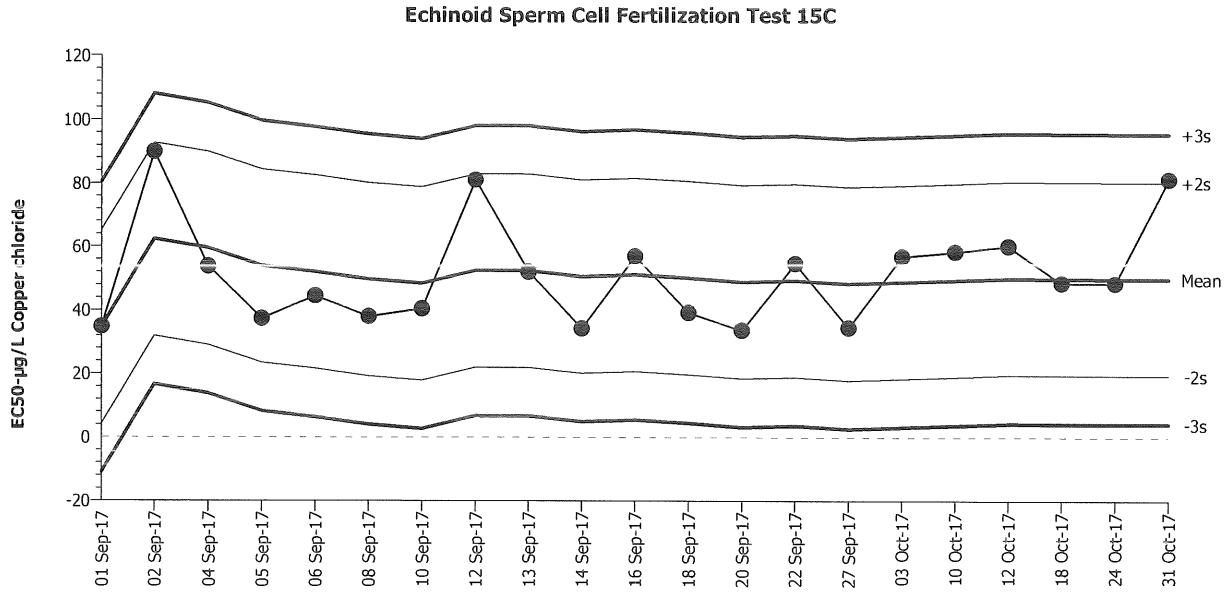
Graphics



Analyst: _____ QA: RFP 11/2/17

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



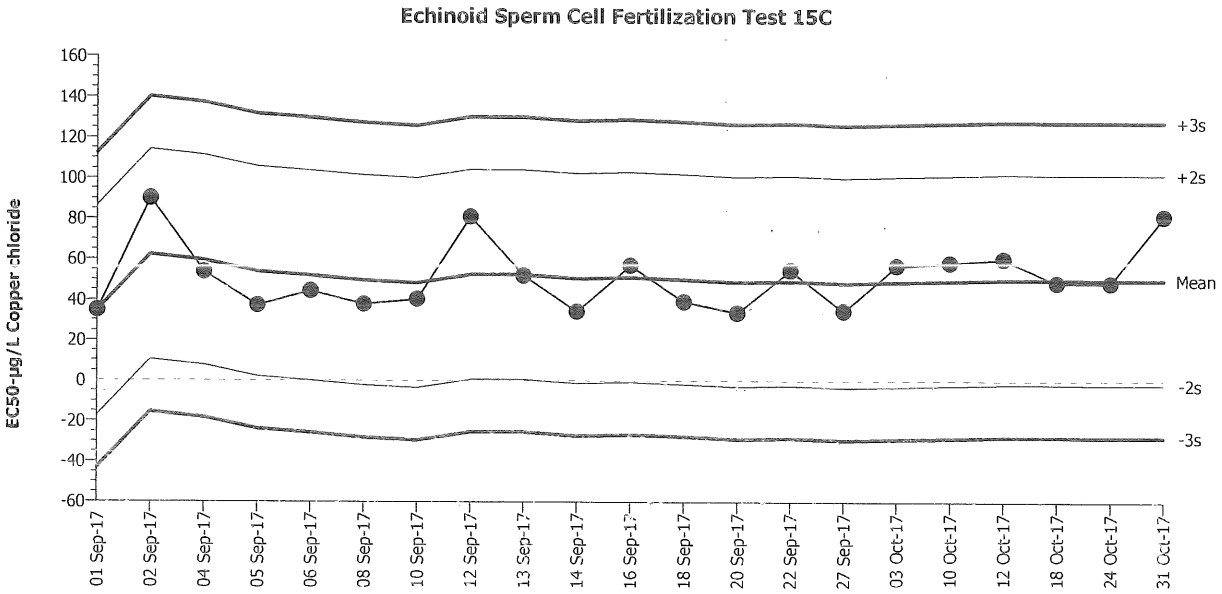
Mean:	49.86	**Count:**	20	**-2s Warning Limit:**	19.38	**-3s Action Limit:**	4.141
Sigma:	15.24	**CV:**	30.60%	**+2s Warning Limit:**	80.34	**+3s Action Limit:**	95.58

Quality Control Data

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

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:	49.86	**Count:**	20	**-2s Warning Limit:**	-1.993	**-3s Action Limit:**	-27.92
Sigma:	25.93	**CV:**	52.00%	**+2s Warning Limit:**	101.7	**+3s Action Limit:**	127.6

Quality Control Data

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

(*) warning and control chart limits recalculated based on the 75th percentile interclaboratory coefficient of variation as defined in EPA-833-R-00-003, for comparison purposes.

CETIS Test Data Worksheet

Test Code: 06-4227-6723/171030sprt (A)

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

Start Date: 31 (A) 30 Oct-17
End Date: 31 (A) 30 Oct-17
Sample Date: 31 (A) 30 Oct-17

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

Sample Code: 171030sprt (A)
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	AB 10/31/17
			2	100	98	
			3	100	0	
			4	100	99	
			5	100	99	
			6	100	0	
			7	100	98	
			8	100	~~47~~ 43	Q18 AB 10/31/17
			9	100	98	
			10	100	62	
			11	100	4	
			12	100	96	
			13	100	99	
			14	100	51	
			15	100	99	
			16	100	99	
			17	100	100	
			18	100	55	
			19	100	97	
			20	100	95	
			21	100	100	
			22	100	98	
			23	100	99	
			24	100	95	
			25	100	98	
			26	100	95	
			27	100	52	
			28	100	98	
			29	100	~~44~~ 0	Q18 AB 11/1/17
			30	100	96	

(A) EG Q18 11/1/17

CETIS Test Data Worksheet

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

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 30 Oct-17
End Date: 30 Oct-17
Sample Date: 30 Oct-17

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

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

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	21			
0	LC	2	13			
0	LC	3	25			
0	LC	4	5			
0	LC	5	7	100	99	CG 10/31/17
10		1	26			
10		2	17			
10		3	24	100	95	CG 10/31/17
10		4	4			
10		5	30			
20		1	9	100	96	CG 10/31/17
20		2	15			
20		3	22	100	98	CG 10/31/17
20		4	2			
20		5	23			
40		1	28			
40		2	20	100	94	CG 10/31/17
40		3	16			
40		4	19	100	94	CG 10/31/17
40		5	12	100	97	CG 10/31/17
80		1	18	100	65	CG 10/31/17
80		2	27			
80		3	8	100	51	CG 10/31/17
80		4	10	100	60	CG 10/31/17
80		5	14			
160		1	3	100	0	CG 10/31/17
160		2	29			
160		3	1			
160		4	6			
160		5	11			

QC: CG (A) CG QC 10/31/17

(B) EG QC 11/1/17

Marine Chronic Bioassay — **Water Quality Measurements**

Client : Internal

Test Species: *S. purpuratus*

Sample ID: $CuCl_2$

Start Date/Time: 10/30/2017 1359

Test No: 171030sprt

End Date/Time: 10/30/2017 1439

Dilutions made by: CG

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	8.3
Final Volume (mL):	500
Cu stock concentration (µg/L):	9600

Analyst: CG

	Initial Readings			
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.6	~~7.~~ 8.00	33.7	15.6
10	8.6	7.99	33.9	15.3
20	8.3	8.00	33.9	15.4
40	8.4	8.00	33.9	15.4
80	8.4	8.00	33.8	15.5
160	8.4	8.01	33.5	15.5

Comments: A CG 0 8 10/31/17 B AP 0 8 10/31/17 C EG 0 8 11/1/17

QC Check: EG 11/1/17

Final Review: KTP 11/2/17

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

Client: Internal
Sample ID: $CuCl_2$
Test No.: 171031sprt

Start Date/Time: 10/31/2017 1359
End Date/Time: 10/31/2017 1439
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 10/10/17

Tech initials: CG
Injection Time: 1310

Sperm Absorbance at 400 nm: 1.001 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 93, 94, 89, 90, 91 Mean: 93 X 50 = 4650 eggs/ml

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

Initial density: 4650 eggs/ml = 1.163 dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
0.163 parts seawater

egg stock 100 ml
seawater 16.3 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):	1325	50:1	66	34
Eggs Added (0.5 ml):	1340	100:1	94, 94	6, 6
Test Ended:	1350	200:1	98	2

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):	1359	QC1	97	3
Eggs Added (0.5 ml):	1419	QC2	98	2
Test Ended:	1439	Egg Control 1	0	100
		Egg Control 2	0	100

Comments:

QC Check: EG 11/1/17 — Final Review: KFP 11/2/17

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.