Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

- Sample IDs: M-001 (Weekly/Screen), ERI Brine, Brine Pit, Train 4, PT Filter Effluent
  Sample Collection Date: October 11, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **October 27, 2017**

Data Quality Assurance:

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

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Results verified by: Adrienne Cibor

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – OCTOBER 2017

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

Sampling Date: October 11, 2017

Test Date: October 12, 2017

Sample IDs: M-001 Brine Effluent, ERI Brine, Brine Pit, Train 4, and PT Filter EFF

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
	6.06	16.5	

INTRODUCTION

A 24-hour composite discharge sample was collected in October 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for weekly chronic 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). Additional samples collected throughout the facility were also tested for comparison purposes. Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on October 12, 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 samples were hand delivered to Nautilus the day of sample collection. Following arrival at Nautilus, an aliquot of each sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. 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
Sample ID:	1. M-001, brine effluent (screening and weekly accelerated chronic monitoring)[a] 2. ERI Brine 3. Brine Pit 4. PT Filter EFF 5. Train 4
Sample Collection Date, Time:	1. 10/11/17, 08:00 2. 10/11/17, 08:00 3. 10/11/17, 08:00 4. 10/11/17, 08:00 5. 10/11/17, 08:00
Sample Receipt Date, Time:	10/11/17, 12:32
Sampling Method:	24-hour Composite

[a] An M-001 weekly sample was also collected on 10/10/17 and submitted for testing on 10/11/17. However, due to a scheduling miscommunication, that sample was tested out of holding time and therefore results are not reported.

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	7.89	7.4	3.5	59.0	194	<0.02
ERI Brine	7.36	7.6	3.5	68.4	200	<0.02
Brine Pit	7.07	8.3	2.1	22.2	82	0.02
PT Filter EFF	7.18	8.6	4.5	33.9	158	<0.02
Train 4	7.43	6.7	2.8	69.2	210	<0.02

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 lab 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 Pollution 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 the M-001 effluent 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.

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period:	10/12/17, 14:55 through 15:35
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 15 percent concentration of the sample with the highest salinity; 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 other samples. The 40 ppt adjusted M-001 sample was not tested (see QA section).
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

RESULTS

Statistical results for urchin fertilization toxicity tests for all samples are summarized in Table 4. Detailed test results are summarized in Tables 5, 6, and 7. 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.

There was a statistically significant decrease in fertilization rate observed in the 10 and 15 percent test concentrations of the unadjusted M-001 sample compared to the lab control, resulting in a NOEC of 6.06 percent effluent and a TU_c of 16.5 (Table 5). This meets the maximum daily permit effluent limitation of 16.5 TU_c. The 6.06 percent concentration (IWC) resulted in a 2.7 percent effect compared to the lab control, which was not statistically significant according to the TST calculation.

The ERI Brine test did not result in a statistically significant decrease in fertilization rate to any sample concentration tested when compared to the lab control (Table 6). No significant reduction was observed at the 6.06 percent effluent concentration, and the NOEC is reported as 15 percent effluent and the TU_c is less than 6.67. The Brine Pit sample resulted in statistically significant decreases in fertilization rate at the 5, 6.06, 10, and 15 percent concentrations, with a NOEC of 2.5 and a TU_c equal to 40. There was a 21 percent effect in the 6.06 percent Brine Pit sample concentration relative to the lab control, which passes according to the TST.

The PT Filter EFF and Train 4 samples resulted in no statistically significant effects for any of the concentrations tested, resulting in a NOEC of 15 percent and a TU_c less than 6.67 for both samples. A high salinity control was tested to match the salinity of the 15 percent concentration of the Train 4 sample (highest salinity sample) for comparison purposes. The high salinity control resulted in a fertilization rate of 88.4 percent, indicating that decreases in fertilization rate observed in the sample concentrations were not likely attributable to elevated salinity. (Table 7).

Table 4. Statistical Results for Urchin Fertilization Testing

Sample ID	NOEC (% sample)	LOEC (% sample)	EC_{50} (% sample)	TU_c value (toxic units)	**TST Result (Pass/Fail)**	**Percent Effect at 6.06%**
M-001 (unadjusted)	6.06	10	>15	16.5	Pass	2.7
ERI Brine	15	>15	>15	<6.67	Pass	-4.8
Brine Pit	2.5	5	>15	40	Pass	21
PT Filter EFF	15	>15	>15	<6.67	Pass	6.4
Train 4	15	>15	>15	<6.67	Pass	-4.4

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 Urchin Fertilization Testing for the M-001 Sample

Test Concentration (% Sample)	M-001 Unadjusted Sample	
	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.8	90.0
2.5	34.5	91.6
5.0	35.2	86.8
6.06	35.4	87.6
10	36.4	74.6*
15	37.6	75.6*

*An asterisk indicates a statistically significant decrease compared to the lab control using the standard USEPA flowchart statistical method (EPA 1995).

Table 6. Detailed Results of Urchin Fertilization Testing for the ERI Brine and Brine Pit Samples

Test Concentration (% Sample)	ERI Brine		Brine Pit	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.7	87.4	33.6	86.0
2.5	34.7	90.8	33.5	86.0
5.0	35.3	88.8	33.3	74.6*
6.06	35.8	91.6	33.2	68.2*
10	37.0	89.4	32.6	63.6*
15	38.6	88.6	32.0	52.8*

*An asterisk indicates a statistically significant decrease compared to the lab control using the standard USEPA flowchart statistical method (EPA 1995).

Table 7. Detailed Results of Urchin Fertilization Testing for the PT Filter EFF and Train 4 Samples

Test Concentration (% Sample)	PT Filter EFF		Train 4	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.6	84.8	33.7	88.4
High Salinity Control	--	--	39.3	85.8
2.5	33.7	78.6	34.9	89.4
5.0	33.9	81.0	35.7	89.4
6.06	33.9	79.4	36.1	89.6
10	33.9	85.8	37.5	89.2
15	33.8	82.6	39.3	83.8

*An asterisk indicates a statistically significant decrease compared to the lab control using the standard USEPA flowchart statistical method (EPA 1995).

QUALITY ASSURANCE

The samples were received on the same day as collection. All samples with results included in this report were tested on 10/12 within the allowable holding time of 36 hours (see description below for M-001 sample collected on 10/10). 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 alpha levels were used for statistical analyses according to the TST Implementation Document guidelines (USEPA 2010).

An M-001 sample was also collected on 10/10/17 for weekly accelerated unadjusted and 40 ppt adjusted testing, and was submitted for testing on 10/11/17. However, due to a scheduling miscommunication, that sample was tested on 10/12/17 out of holding time and therefore results are not reported. The M-001 sample collected on 10/11/17 with the facility screening samples will serve as the weekly compliance sample tested without 40 ppt adjustment. The M-001 sample has historically been tested following adjustment to 40 ppt for additional information in order 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.

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 8 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration (EC_{50} value) was within two standard deviations (SD) of the historical mean, indicating typical test organism sensitivity to copper. A list of qualifier codes used on bench datasheets can be found in Appendix E.

Table 8. 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	60.2	49.5 ± 30.5	30.9

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. Method Guidance and Recommendations for Whole Effluent Toxicity (WET) Testing (40 CFR Part 136). EPA/821/B-00/004. July 2000.

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

Test Code: 1710-S040 | 12-6199-0123

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

Batch ID:	18-5047-9666	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	12 Oct-17 14:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	12 Oct-17 15:35	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	17-5647-0571	**Code:**	17-1088	**Client:**	IDE
Sample Date:	11 Oct-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	11 Oct-17 12:32	**Source:**	IDE Americas, Inc.		
Sample Age:	31h (3.5 °C)	**Station:**	M-001 Unadjusted		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
13-1944-5963	Fertilization Rate	6.06	10	7.785	4.94%	16.5	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
12-7115-7040	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
12-7115-7040	Fertilization Rate	Control Resp	0.9	0.7 - NL	Yes	Passes Acceptability Criteria
13-1944-5963	Fertilization Rate	Control Resp	0.9	0.7 - NL	Yes	Passes Acceptability Criteria
13-1944-5963	Fertilization Rate	PMSD	0.04937	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	0.9	0.8579	0.9421	0.86	0.94	0.01517	0.03391	3.77%	0.0%
2.5		5	0.916	0.8993	0.9327	0.9	0.93	0.006	0.01342	1.47%	-1.78%
5		5	0.868	0.8303	0.9057	0.82	0.9	0.01356	0.03033	3.49%	3.56%
6.06		5	0.876	0.8371	0.9149	0.83	0.91	0.014	0.0313	3.57%	2.67%
10		5	0.746	0.6782	0.8138	0.69	0.82	0.02441	0.05459	7.32%	17.11%
15		5	0.756	0.7107	0.8013	0.7	0.79	0.01631	0.03647	4.82%	16.0%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.87	0.86	0.94	0.92	0.91
2.5		0.93	0.91	0.91	0.9	0.93
5		0.88	0.9	0.86	0.88	0.82
6.06		0.89	0.83	0.91	0.86	0.89
10		0.82	0.74	0.69	0.7	0.78
15		0.7	0.79	0.74	0.77	0.78

Analyst: JJ QA: AC 10/25/17

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

Analysis ID: 13-1944-5963 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 18 Oct-17 10:15 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.94%	6.06	10	7.785	16.5

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.8222	2.362	0.072	8	0.9730	CDF	Non-Significant Effect
		5	1.713	2.362	0.072	8	0.1618	CDF	Non-Significant Effect
		6.06	1.307	2.362	0.072	8	0.2923	CDF	Non-Significant Effect
		10*	6.864	2.362	0.072	8	<0.0001	CDF	Significant Effect
		15*	6.518	2.362	0.072	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2502022	0.05004044	5	21.78	<0.0001	Significant Effect
Error	0.0551341	0.002297254	24			
Total	0.3053363		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.383	15.09	0.6412	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9482	0.9031	0.1510	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	0.9	0.8579	0.9421	0.91	0.86	0.94	0.01517	3.77%	0.0%
2.5		5	0.916	0.8993	0.9327	0.91	0.9	0.93	0.006	1.47%	-1.78%
5		5	0.868	0.8303	0.9057	0.88	0.82	0.9	0.01356	3.49%	3.56%
6.06		5	0.876	0.8371	0.9149	0.89	0.83	0.91	0.014	3.57%	2.67%
10		5	0.746	0.6782	0.8138	0.74	0.69	0.82	0.02441	7.32%	17.11%
15		5	0.756	0.7107	0.8013	0.77	0.7	0.79	0.01631	4.82%	16.0%

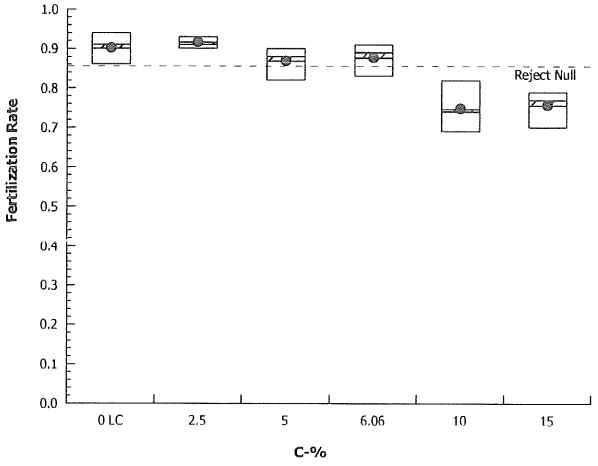
Angular (Corrected) Transformed Summary

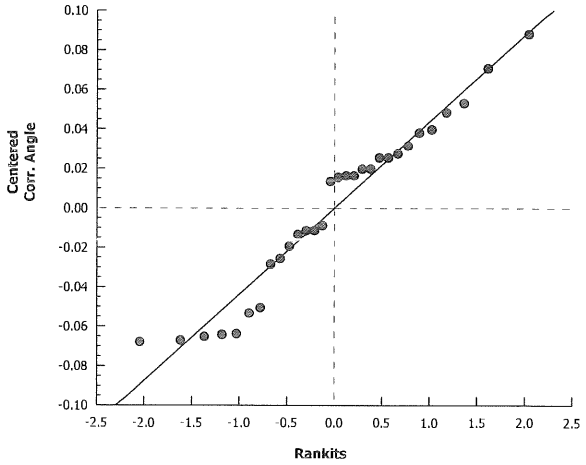
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.253	1.182	1.323	1.266	1.187	1.323	0.0255	4.55%	0.0%
2.5		5	1.277	1.247	1.308	1.266	1.249	1.303	0.01089	1.91%	-1.99%
5		5	1.201	1.146	1.255	1.217	1.133	1.249	0.0196	3.65%	4.15%
6.06		5	1.213	1.155	1.271	1.233	1.146	1.266	0.02095	3.86%	3.16%
10		5	1.044	0.9653	1.124	1.036	0.9803	1.133	0.02852	6.11%	16.61%
15		5	1.055	1.003	1.107	1.071	0.9912	1.095	0.01875	3.98%	15.77%

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

Analysis ID:	13-1944-5963	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Oct-17 10:15	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	12-7115-7040	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-17 8:45	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	616115	1000	Yes	Two-Point Interpolation

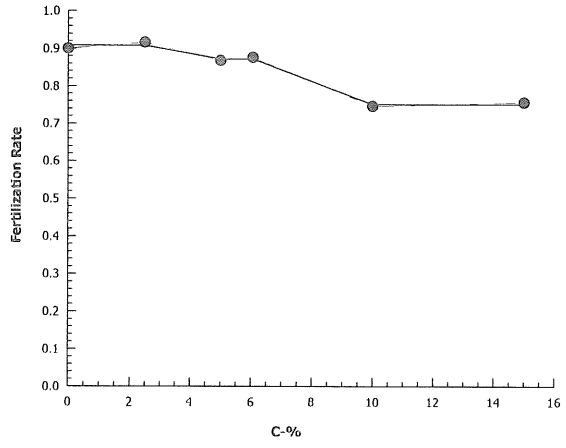
Point Estimates

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	0.9	0.86	0.94	0.01517	0.03391	3.77%	0.0%	450	500
2.5		5	0.916	0.9	0.93	0.006	0.01342	1.47%	-1.78%	458	500
5		5	0.868	0.82	0.9	0.01356	0.03033	3.49%	3.56%	434	500
6.06		5	0.876	0.83	0.91	0.014	0.0313	3.57%	2.67%	438	500
10		5	0.746	0.69	0.82	0.02441	0.05459	7.32%	17.11%	373	500
15		5	0.756	0.7	0.79	0.01631	0.03647	4.82%	16.0%	378	500

Graphics



Analyst: _____ QA: AC 10/25/17

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

Analysis ID: 07-8571-2753 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-17 8:46 **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	3.34%	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*	15.36	1.943	0.043	6	<0.0001	CDF	Non-Significant Effect
		5*	9.539	1.895	0.052	7	<0.0001	CDF	Non-Significant Effect
		6.06*	9.644	1.895	0.054	7	<0.0001	CDF	Non-Significant Effect
		10*	3.06	1.943	0.067	6	0.0111	CDF	Non-Significant Effect
		15*	4.315	1.895	0.051	7	0.0018	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.2502022	0.05004044	5	21.78	<0.0001	Significant Effect
Error	0.0551341	0.002297254	24			
Total	0.3053363		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.383	15.09	0.6412	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9482	0.9031	0.1510	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	0.9	0.8579	0.9421	0.91	0.86	0.94	0.01517	3.77%	0.0%
2.5		5	0.916	0.8993	0.9327	0.91	0.9	0.93	0.006	1.47%	-1.78%
5		5	0.868	0.8303	0.9057	0.88	0.82	0.9	0.01356	3.49%	3.56%
6.06		5	0.876	0.8371	0.9149	0.89	0.83	0.91	0.014	3.57%	2.67%
10		5	0.746	0.6782	0.8138	0.74	0.69	0.82	0.02441	7.32%	17.11%
15		5	0.756	0.7107	0.8013	0.77	0.7	0.79	0.01631	4.82%	16.0%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.253	1.182	1.323	1.266	1.187	1.323	0.0255	4.55%	0.0%
2.5		5	1.277	1.247	1.308	1.266	1.249	1.303	0.01089	1.91%	-1.99%
5		5	1.201	1.146	1.255	1.217	1.133	1.249	0.0196	3.65%	4.15%
6.06		5	1.213	1.155	1.271	1.233	1.146	1.266	0.02095	3.86%	3.16%
10		5	1.044	0.9653	1.124	1.036	0.9803	1.133	0.02852	6.11%	16.61%
15		5	1.055	1.003	1.107	1.071	0.9912	1.095	0.01875	3.98%	15.77%

CETIS Test Data Worksheet

Report Date: 12 Oct-17 08:59 (p 1 of 1)
Test Code: 1711-8048 12-6199-0123/4B3870EB

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

Start Date: 12 Oct-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 17- 1088
End Date: 12 Oct-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 11 Oct-17 | **Material:** Facility Effluent | **Sample Station:** M-001 Unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			96	100	83	DM 10/17/17
			97	100	87	
			98	100	86	
			99	100	93	
			100	100	91	
			101	100	90	
			102	100	82	
			103	100	78	
			104	100	77	
			105	100	70	
			106	100	74	
			107	100	91	
			108	100	89	
			109	100	78	
			110	100	86	
			111	100	74	
			112	100	88	
			113	100	91	
			114	100	91	
			115	100	86	
			116	100	82	
			117	100	79	
			118	100	90	
			119	100	70	
			120	100	94	
			121	100	69	
			122	100	93	
			123	100	89	
			124	100	88	
			125	100	92	

CETIS Test Data Worksheet

Test Code: 1710-S048 12-6199-0123/4B3870EB

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

Start Date:	12 Oct-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:**	17-1088
End Date:	12 Oct-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	IDE Americas, Inc.
Sample Date:	11 Oct-17	**Material:**	Facility Effluent	**Sample Station:**	M-001 ~~Unadjusted~~ (A)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	97			
0	LC	2	98	100	84	BO 10/12/17
0	LC	3	120			
0	LC	4	125			
0	LC	5	107			
2.5		1	99	100	81	BO 10/12/17
2.5		2	100			
2.5		3	113			
2.5		4	118			
2.5		5	122			
5		1	112			
5		2	101	100	86	BO 10/12/17
5		3	110			
5		4	124			
5		5	102			
6.06		1	123			
6.06		2	96			
6.06		3	114			
6.06		4	115			
6.06		5	108	100	83	BO 10/12/17
10		1	116			
10		2	111			
10		3	121	100	67	BO 10/12/17
10		4	119			
10		5	109			
15		1	105			
15		2	117			
15		3	106			
15		4	104	100	48	BO 10/12/17
15		5	103			

(A) CG QB 10/12/17
QC: CG

Analyst: CG QA: AC 10/18/17

Marine Chronic Bioassay

Water Quality Measurements

Client : IDE

Test Species: *S. purpuratus*

Sample ID: M-001

Start Date/Time: 10/12/2017 1455

Sample Log No.: 17- 1088

End Date/Time: 10/12/2017 1535

Dilutions made by: ~~AD~~ (A) CG

Test No: 1710-S040

Analyst: ~~AD~~

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.0	8.11	33.8	15.6
2.5	7.0	8.11	34.5	15.4
5.0	7.0	8.10	35.2	15.1
6.06	7.0	8.10	35.4	15.1
10	7.1	8.09	36.4	15.0
15	7.1	8.07	37.6	14.9

Comments: (A)CG 10/12/17

QC Check: AC 10/18/17

Final Review: KFP 10/26/17

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

Client: IDE
Sample ID: Urndj M-001 (weekly, Screen)
Test No.: 1710-S040

Start Date/Time: 10/12/2017 / 1455
End Date/Time: 10/12/2017 / 1535
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 9/28/17

Tech initials: CG
Injection Time: 1400

Sperm Absorbance at 400 nm: 0.972 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted:	
	80
	71
	72
	75
	80

Mean: (A) 75.72 X 50 = 3860 eggs/ml

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

Initial density:	3860	eggs/ml	=	0.965	dilution factor	egg stock	ml
Final density:	4000	eggs/ml	-	1.0	part egg stock	seawater	ml
				0.035	parts seawater		

(B)

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):	1420	50:1	75	25
Eggs Added (0.5 ml):	1437	100:1	91,95	9,5
Test Ended:	(A) 1457/1447	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):	1455	QC1	93	7
Eggs Added (0.5 ml):	1515	QC2	92	8
Test Ended:	1535	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) CG QA 10/12/17
(B) ~~EG~~ CG QA NO dilution necessary.

QC Check: AC 10/18/17

Final Review: VTP 10/26/17

ERI Brine

CETIS Summary Report

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

Batch ID:	06-9505-8332	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	12 Oct-17 14:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	12 Oct-17 15:35	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	08-8229-4820	**Code:**	17-1089	**Client:**	IDE
Sample Date:	11 Oct-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	11 Oct-17 12:32	**Source:**	IDE Americas, Inc.		
Sample Age:	31h (3.5 °C)	**Station:**	ERI Brine		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-6929-0033	Fertilization Rate	15	>15	NA	6.32%	≤ 6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
16-1381-4962	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
16-1381-4962	Fertilization Rate	Control Resp	0.874	0.7 - NL	Yes	Passes Acceptability Criteria
18-6929-0033	Fertilization Rate	Control Resp	0.874	0.7 - NL	Yes	Passes Acceptability Criteria
18-6929-0033	Fertilization Rate	PMSD	0.06323	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	0.874	0.8552	0.8928	0.86	0.9	0.006782	0.01517	1.74%	0.0%
2.5		5	0.908	0.8858	0.9302	0.89	0.93	0.008	0.01789	1.97%	-3.89%
5		5	0.888	0.8276	0.9484	0.82	0.94	0.02177	0.04868	5.48%	-1.6%
6.06		5	0.916	0.8802	0.9518	0.88	0.95	0.01288	0.02881	3.15%	-4.81%
10		5	0.894	0.8439	0.9441	0.85	0.95	0.01806	0.04037	4.52%	-2.29%
15		5	0.886	0.8618	0.9102	0.86	0.91	0.008718	0.01949	2.2%	-1.37%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9	0.86	0.87	0.87	0.87
2.5		0.91	0.92	0.89	0.89	0.93
5		0.93	0.87	0.88	0.82	0.94
6.06		0.94	0.9	0.95	0.88	0.91
10		0.85	0.95	0.86	0.91	0.9
15		0.9	0.88	0.86	0.91	0.88

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

Analysis ID:	18-6929-0033	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Oct-17 17:52	**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	6.32%	15	>15	NA	6.667

Dunnett Multiple Comparison Test

Control	vs C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control	2.5	-1.687	2.362	0.077	8	0.9979	CDF	Non-Significant Effect
	5	-0.8288	2.362	0.077	8	0.9735	CDF	Non-Significant Effect
	6.06	-2.188	2.362	0.077	8	0.9996	CDF	Non-Significant Effect
	10	-1.09	2.362	0.077	8	0.9871	CDF	Non-Significant Effect
	15	-0.576	2.362	0.077	8	0.9499	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01653912	0.003307824	5	1.231	0.3254	Non-Significant Effect
Error	0.0644832	0.0026868	24			
Total	0.08102231		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.031	15.09	0.1545	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9846	0.9031	0.9295	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	0.874	0.8552	0.8928	0.87	0.86	0.9	0.006782	1.74%	0.0%
2.5		5	0.908	0.8858	0.9302	0.91	0.89	0.93	0.008	1.97%	-3.89%
5		5	0.888	0.8276	0.9484	0.88	0.82	0.94	0.02177	5.48%	-1.6%
6.06		5	0.916	0.8802	0.9518	0.91	0.88	0.95	0.01288	3.15%	-4.81%
10		5	0.894	0.8439	0.9441	0.9	0.85	0.95	0.01806	4.52%	-2.29%
15		5	0.886	0.8618	0.9102	0.88	0.86	0.91	0.008718	2.2%	-1.37%

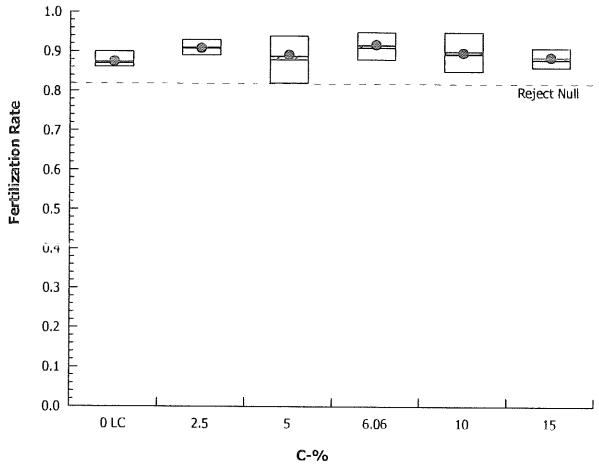
Angular (Corrected) Transformed Summary

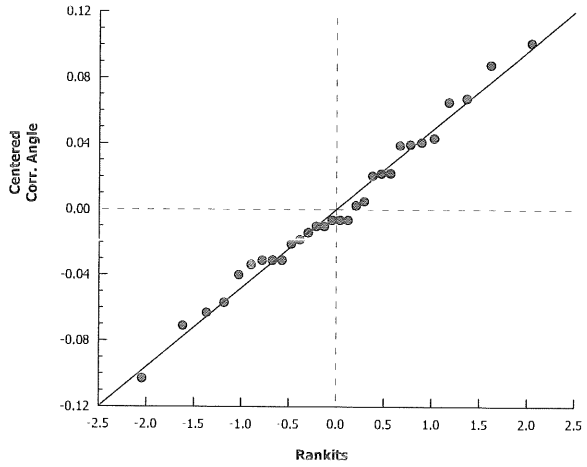
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.208	1.179	1.238	1.202	1.187	1.249	0.01054	1.95%	0.0%
2.5		5	1.264	1.225	1.302	1.266	1.233	1.303	0.01394	2.47%	-4.58%
5		5	1.236	1.139	1.332	1.217	1.133	1.323	0.03487	6.31%	-2.25%
6.06		5	1.28	1.214	1.346	1.266	1.217	1.345	0.02372	4.14%	-5.94%
10		5	1.244	1.159	1.33	1.249	1.173	1.345	0.03083	5.54%	-2.96%
15		5	1.227	1.189	1.266	1.217	1.187	1.266	0.01376	2.51%	-1.56%

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

Analysis ID:	18-6929-0033	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Oct-17 17:52	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	16-1381-4962	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-17 8:56	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	330644	1000	Yes	Two-Point Interpolation

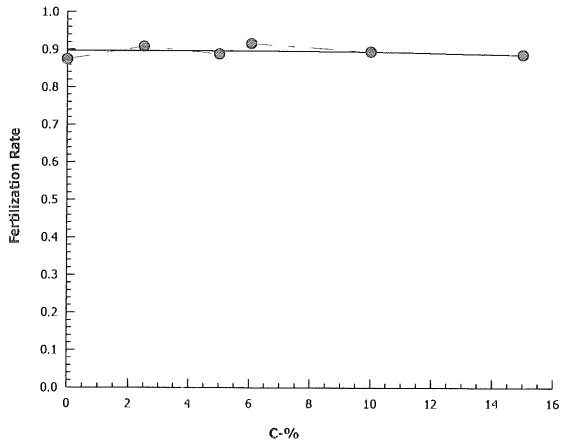
Point Estimates

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	0.874	0.86	0.9	0.006782	0.01517	1.74%	0.0%	437	500
2.5		5	0.908	0.89	0.93	0.008	0.01789	1.97%	-3.89%	454	500
5		5	0.888	0.82	0.94	0.02177	0.04868	5.48%	-1.6%	444	500
6.06		5	0.916	0.88	0.95	0.01288	0.02881	3.15%	-4.81%	458	500
10		5	0.894	0.85	0.95	0.01806	0.04037	4.52%	-2.29%	447	500
15		5	0.886	0.86	0.91	0.008718	0.01949	2.2%	-1.37%	443	500

Graphics



Analyst: _____ QA: 10/25/17

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

Analysis ID: 19-0428-2150 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 19 Oct-17 8:56 **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	2.38%	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*	22.31	1.943	0.031	6	<0.0001	CDF	Non-Significant Effect
		5*	9.209	2.132	0.076	4	0.0004	CDF	Non-Significant Effect
		6.06*	14.95	2.132	0.053	4	<0.0001	CDF	Non-Significant Effect
		10*	10.61	2.132	0.068	4	0.0002	CDF	Non-Significant Effect
		15*	20.22	1.943	0.031	6	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.01653912	0.003307824	5	1.231	0.3254	Non-Significant Effect
Error	0.0644832	0.0026868	24			
Total	0.08102231		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.031	15.09	0.1545	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9846	0.9031	0.9295	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	0.874	0.8552	0.8928	0.87	0.86	0.9	0.006782	1.74%	0.0%
2.5		5	0.908	0.8858	0.9302	0.91	0.89	0.93	0.008	1.97%	-3.89%
5		5	0.888	0.8276	0.9484	0.88	0.82	0.94	0.02177	5.48%	-1.6%
6.06		5	0.916	0.8802	0.9518	0.91	0.88	0.95	0.01288	3.15%	-4.81%
10		5	0.894	0.8439	0.9441	0.9	0.85	0.95	0.01806	4.52%	-2.29%
15		5	0.886	0.8618	0.9102	0.88	0.86	0.91	0.008718	2.2%	-1.37%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.208	1.179	1.238	1.202	1.187	1.249	0.01054	1.95%	0.0%
2.5		5	1.264	1.225	1.302	1.266	1.233	1.303	0.01394	2.47%	-4.58%
5		5	1.236	1.139	1.332	1.217	1.133	1.323	0.03487	6.31%	-2.25%
6.06		5	1.28	1.214	1.346	1.266	1.217	1.345	0.02372	4.14%	-5.94%
10		5	1.244	1.159	1.33	1.249	1.173	1.345	0.03083	5.54%	-2.96%
15		5	1.227	1.189	1.266	1.217	1.187	1.266	0.01376	2.51%	-1.56%

CETIS Test Data Worksheet

Report Date: 12 Oct-17 09:00 (p 1 of 1)
Test Code: 1710-S042 20-7357-5440/7B984010

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

Start Date: 12 Oct-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 17- 1089
End Date: 12 Oct-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 11 Oct-17 | **Material:** Facility Effluent | **Sample Station:** ERI Brine

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			126	100	88	AB 10/13/17
			127	100	92	
			128	100	88	
			129	100	87	
			130	100	88	
			131	100	90	
			132	100	90	
			133	100	91	
			134	100	89	
			135	100	88	
			136	100	85	
			137	100	90	
			138	100	89	
			139	100	91	
			140	100	82	
			141	100	90	
			142	100	91	
			143	100	93	
			144	100	87	
			145	100	86	
			146	100	95	
			147	100	87	
			148	100	94	
			149	100	95	
			150	100	86	
			151	100	94	
			152	100	91	
			153	100	93	
			154	100	87	
			155	100	86	

CETIS Test Data Worksheet

Test Code: 1710-S042-20-7357-5440/7B984010

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

Start Date: 12 Oct-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 17- 1089
End Date: 12 Oct-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 11 Oct-17 **Material:** Facility Effluent **Sample Station:** ERI Brine

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	141			
0	LC	2	150	100	81	10/12/17 RT
0	LC	3	129			
0	LC	4	147			
0	LC	5	154			
2.5		1	152			
2.5		2	127			
2.5		3	138	100	91	10/12/17 RT
2.5		4	134			
2.5		5	153			
5		1	143			
5		2	144	100	82	10/12/17 RT
5		3	135			
5		4	140			
5		5	148			
6.06		1	151			
6.06		2	131			
6.06		3	149			
6.06		4	128	100	87	10/12/17 RT
6.06		5	139			
10		1	136			
10		2	146			
10		3	155			
10		4	133	100	91	10/12/17 RT
10		5	137			
15		1	132			
15		2	126			
15		3	145			
15		4	142			
15		5	130	100	81	10/12/17 RT

QC-CG

Analyst: CG QA: A 10/18/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: ERI Brine | **Start Date/Time:** 10/12/2017 1455

Sample Log No.: 17- 1089 | **End Date/Time:** 10/12/2017 1535

Dilutions made by: CG | **Test No:** 1710-S042

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.0	8.09	33.7	15.9
2.5	6.9	8.07	34.7	15.9
5.0	6.9	8.05	35.3	15.7
6.06	6.9	8.04	35.8	15.8
10	6.9	8.01	37.0	15.7
15	7.0	7.98	38.6	15.4

Comments:

QC Check: AC 10/18/17 | **Final Review:** KTP 10/26/17

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

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

Client: IDE
Sample ID: ERI
Test No.: 1710-S042

Start Date/Time: 10/12/2017 / 1455
End Date/Time: 10/12/2017 / 1535
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 9/28/17

Tech initials: CG
Injection Time: 1400

Sperm Absorbance at 400 nm: 0.972 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 80, 71, 72, 75, 80

Mean: (A) 75.72 X 50 = 3860 eggs/ml

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

Initial density:	3860	eggs/ml	=	0.965	dilution factor	egg stock	ml
Final density:	4000	eggs/ml	-	1.0	part egg stock	seawater	ml
				=0.035	parts seawater		

(B)

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):	1420	50:1	75	25
Eggs Added (0.5 ml):	1437	100:1	91,95	9,5
Test Ended:	(A) 1457/1447	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):	1455	QC1	93	7
Eggs Added (0.5 ml):	1515	QC2	92	8
Test Ended:	1535	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) CG QA 10/12/17
(B) ~~EG~~ CG QA NO dilution necessary.

QC Check: AC 10/18/17

Final Review: KAP 10/26/17

Brine Pit

CETIS Summary Report

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

Batch ID:	16-1156-8204	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	12 Oct-17 14:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	12 Oct-17 15:35	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	10-0214-7825	**Code:**	17-1090	**Client:**	IDE
Sample Date:	11 Oct-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	11 Oct-17 12:32	**Source:**	IDE Americas, Inc.		
Sample Age:	31h (2.1 °C)	**Station:**	Brine Pit		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
21-0672-9863	Fertilization Rate	2.5	5	3.536	8.37%	40	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
03-9807-4100	Fertilization Rate	EC25	9.229	4.522	12.55	10.84	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-9807-4100	Fertilization Rate	Control Resp	0.86	0.7 - NL	Yes	Passes Acceptability Criteria
21-0672-9863	Fertilization Rate	Control Resp	0.86	0.7 - NL	Yes	Passes Acceptability Criteria
21-0672-9863	Fertilization Rate	PMSD	0.08369	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	0.86	0.8066	0.9134	0.8	0.9	0.01924	0.04301	5.0%	0.0%
2.5		5	0.86	0.7762	0.9438	0.79	0.95	0.03017	0.06745	7.84%	0.0%
5		5	0.746	0.6842	0.8078	0.66	0.78	0.02227	0.0498	6.68%	13.26%
6.06		5	0.682	0.6404	0.7236	0.66	0.74	0.01497	0.03347	4.91%	20.7%
10		5	0.636	0.5412	0.7308	0.54	0.71	0.03415	0.07635	12.01%	26.05%
15		5	0.528	0.4997	0.5563	0.5	0.55	0.0102	0.0228	4.32%	38.6%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.89	0.88	0.8	0.83	0.9
2.5		0.79	0.9	0.8	0.95	0.86
5		0.66	0.78	0.75	0.76	0.78
6.06		0.68	0.66	0.74	0.67	0.66
10		0.58	0.54	0.71	0.71	0.64
15		0.55	0.53	0.55	0.5	0.51

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

Analysis ID:	21-0672-9863	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Oct-17 10:16	**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	8.37%	2.5	5	3.536	40

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.153	2.362	0.098	8	0.8746	CDF	Non-Significant Effect
		5*	3.529	2.362	0.098	8	0.0037	CDF	Significant Effect
		6.06*	5.259	2.362	0.098	8	<0.0001	CDF	Significant Effect
		10*	6.405	2.362	0.098	8	<0.0001	CDF	Significant Effect
		15*	9.083	2.362	0.098	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5740837	0.1148167	5	26.67	<0.0001	Significant Effect
Error	0.1033278	0.004305325	24			
Total	0.6774116		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.918	15.09	0.1124	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9688	0.9031	0.5079	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	0.86	0.8066	0.9134	0.88	0.8	0.9	0.01924	5.0%	0.0%
2.5		5	0.86	0.7762	0.9438	0.86	0.79	0.95	0.03017	7.84%	0.0%
5		5	0.746	0.6842	0.8078	0.76	0.66	0.78	0.02227	6.68%	13.26%
6.06		5	0.682	0.6404	0.7236	0.67	0.66	0.74	0.01497	4.91%	20.7%
10		5	0.636	0.5412	0.7308	0.64	0.54	0.71	0.03415	12.01%	26.05%
15		5	0.528	0.4997	0.5563	0.53	0.5	0.55	0.0102	4.32%	38.6%

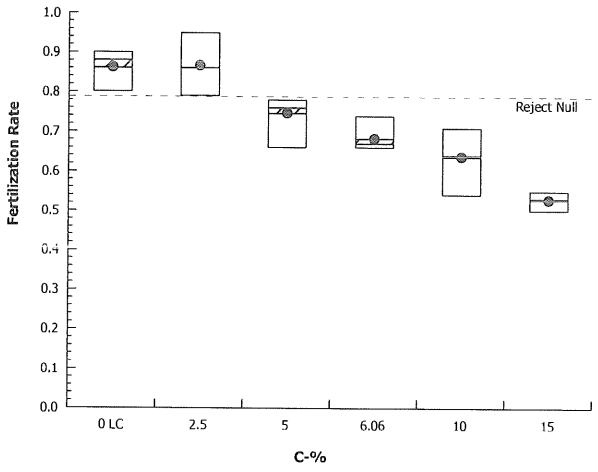
Angular (Corrected) Transformed Summary

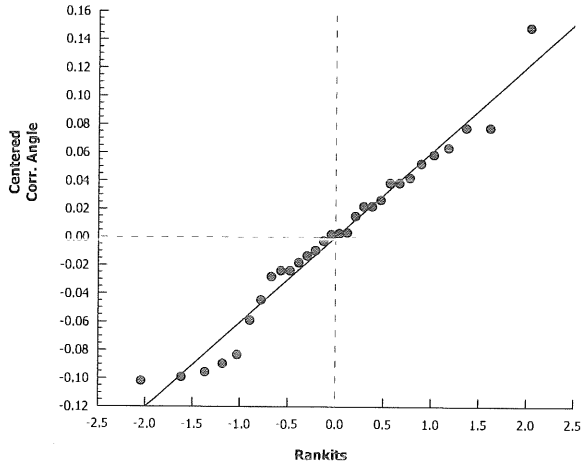
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.19	1.115	1.266	1.217	1.107	1.249	0.02726	5.12%	0.0%
2.5		5	1.197	1.067	1.326	1.187	1.095	1.345	0.04654	8.7%	-0.53%
5		5	1.044	0.9748	1.113	1.059	0.9483	1.083	0.02487	5.33%	12.3%
6.06		5	0.9721	0.9266	1.018	0.9589	0.9483	1.036	0.01638	3.77%	18.33%
10		5	0.9245	0.8258	1.023	0.9273	0.8254	1.002	0.03558	8.61%	22.33%
15		5	0.8134	0.7851	0.8418	0.8154	0.7854	0.8355	0.01022	2.81%	31.66%

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

Analysis ID:	21-0672-9863	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	18 Oct-17 10:16	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: _____ QA: AC 10/25/17

CETIS Analytical Report

Test Code: 1710-S043 | 03-5142-5523

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

Analysis ID:	03-9807-4100	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-17 9:01	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	798217	1000	Yes	Two-Point Interpolation

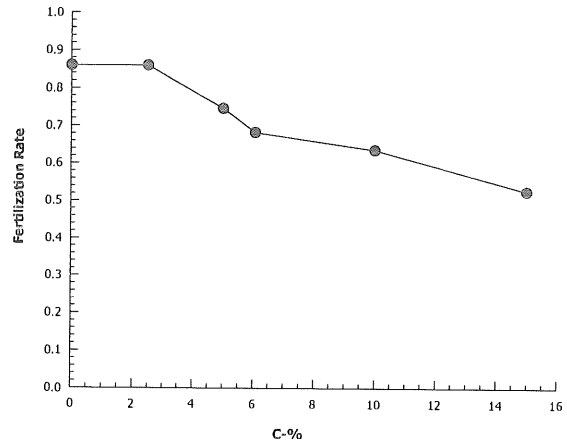
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	9.229	4.522	12.55	10.84	7.966	22.12
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	0.86	0.8	0.9	0.01924	0.04301	5.0%	0.0%	430	500
2.5		5	0.86	0.79	0.95	0.03017	0.06745	7.84%	0.0%	430	500
5		5	0.746	0.66	0.78	0.02227	0.0498	6.68%	13.26%	373	500
6.06		5	0.682	0.66	0.74	0.01497	0.03347	4.91%	20.7%	341	500
10		5	0.636	0.54	0.71	0.03415	0.07635	12.01%	26.05%	318	500
15		5	0.528	0.5	0.55	0.0102	0.0228	4.32%	38.6%	263	500

Graphics



Analyst: _____ QA: AC 10/25/17

CETIS Analytical Report

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

Analysis ID:	14-3394-6605	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	19 Oct-17 9:01	**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	3.62%	6.06	10	7.785	16.5

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	5.979	2.015	0.102	5	0.0009	CDF	Non-Significant Effect
		5*	4.694	1.895	0.061	7	0.0011	CDF	Non-Significant Effect
		6.06*	3.029	1.895	0.05	7	0.0096	CDF	Non-Significant Effect
		10	0.7743	1.943	0.08	6	0.2341	CDF	Significant Effect
		15	-3.471	2.015	0.046	5	0.9911	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5740837	0.1148167	5	26.67	<0.0001	Significant Effect
Error	0.1033278	0.004305325	24			
Total	0.6774116		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.918	15.09	0.1124	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9688	0.9031	0.5079	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	0.86	0.8066	0.9134	0.88	0.8	0.9	0.01924	5.0%	0.0%
2.5		5	0.86	0.7762	0.9438	0.86	0.79	0.95	0.03017	7.84%	0.0%
5		5	0.746	0.6842	0.8078	0.76	0.66	0.78	0.02227	6.68%	13.26%
6.06		5	0.682	0.6404	0.7236	0.67	0.66	0.74	0.01497	4.91%	20.7%
10		5	0.636	0.5412	0.7308	0.64	0.54	0.71	0.03415	12.01%	26.05%
15		5	0.528	0.4997	0.5563	0.53	0.5	0.55	0.0102	4.32%	38.6%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.19	1.115	1.266	1.217	1.107	1.249	0.02726	5.12%	0.0%
2.5		5	1.197	1.067	1.326	1.187	1.095	1.345	0.04654	8.7%	-0.53%
5		5	1.044	0.9748	1.113	1.059	0.9483	1.083	0.02487	5.33%	12.3%
6.06		5	0.9721	0.9266	1.018	0.9589	0.9483	1.036	0.01638	3.77%	18.33%
10		5	0.9245	0.8258	1.023	0.9273	0.8254	1.002	0.03558	8.61%	22.33%
15		5	0.8134	0.7851	0.8418	0.8154	0.7854	0.8355	0.01022	2.81%	31.66%

CETIS Test Data Worksheet

Report Date: 12 Oct-17 09:09 (p 1 of 1)
Test Code: 1710-5043 03-5142-5523/14F253F3

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

Start Date:	12 Oct-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:**	17- 1090
End Date:	12 Oct-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	IDE Americas, Inc.
Sample Date:	11 Oct-17	**Material:**	Facility Effluent	**Sample Station:**	Brine Pit

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			156	100	75	AB 10/17/17
			157	100	71	
			158	100	74	
			159	100	80	
			160	100	90	
			161	100	95	
			162	100	67	
			163	100	66	
			164	100	89	
			165	100	78	
			166	100	55	
			167	100	88	
			168	100	53	
			169	100	78	
			170	100	71	
			171	100	76	
			172	100	66	
			173	100	54	
			174	100	83	
			175	100	66	
			176	100	86	
			177	100	90	
			178	100	55	
			179	100	68	
			180	100	64	
			181	100	79	
			182	100	50	
			183	100	80	
			184	100	51	
			185	100	58	

CETIS Test Data Worksheet

Report Date: 12 Oct-17 09:09 (p 1 of 1)
Test Code: 1710-S043 03-5142-5523/14F253F3

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

Start Date: 12 Oct-17
End Date: 12 Oct-17
Sample Date: 11 Oct-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 1090
Sample Source: IDE Americas, Inc.
Sample Station: Brine Pit

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	164			
0	LC	2	167	100	91	BO 10/12/17
0	LC	3	183			
0	LC	4	174			
0	LC	5	160			
2.5		1	181	100	75	BO 10/12/17
2.5		2	177			
2.5		3	159			
2.5		4	161			
2.5		5	176			
5		1	163			
5		2	165	100	66	BO 10/12/17
5		3	156			
5		4	171			
5		5	169			
6.06		1	179			
6.06		2	175	100	55	BO 10/12/17
6.06		3	158			
6.06		4	162			
6.06		5	172			
10		1	185			
10		2	173			
10		3	157			
10		4	170			
10		5	180	100	45	BO 10/12/17
15		1	166			
15		2	168			
15		3	178			
15		4	182	100	46	BO 10/12/17
15		5	184			

QC·CG

Marine Chronic Bioassay **Water Quality Measurements**

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

Sample ID: Brine Pit **Start Date/Time:** 10/12/2017 1155

Sample Log No.: 17- 1090 **End Date/Time:** 10/12/2017 1535

Dilutions made by: CG **Test No:** 1710-S043

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.1	8.09	33.6	15.7
2.5	7.0	8.09	33.5	15.8
5.0	7.0	8.08	33.3	15.7
6.06	7.1	8.07	33.2	15.5
10	7.1	8.05	32.6	15.5
15	7.1	8.02	32.0	15.3

Comments:

QC Check: AC 10/18/17 **Final Review:** KTP 10/26/17

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

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

Client: IDE
Sample ID: Brine Pit
Test No.: 1710-S043

Start Date/Time: 10/12/2017 / 1455
End Date/Time: 10/12/2017 / 1535
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 9/28/17

Tech initials: CG
Injection Time: 1400

Sperm Absorbance at 400 nm: 0.972 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 80, 71, 72, 75, 80 Mean: (A) 3772 X 50 = 3860 eggs/ml

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

Initial density: 3860 eggs/ml = 0.965 dilution factor (B)
Final density: 4000 eggs/ml - 1.0 part egg stock
~~0.035~~ parts seawater

egg stock _____ ml
seawater _____ 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):	1420	50:1	75	25
Eggs Added (0.5 ml):	1437	100:1	91,95	9,5
Test Ended:	(A) ~~1457~~ 1447	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):	1455	QC1	93	7
Eggs Added (0.5 ml):	1515	QC2	92	8
Test Ended:	1535	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) CG QA 10/12/17
(B) ~~EGG~~ (A) NO dilution necessary.

QC Check: AC 10/18/17

Final Review: KFP 10/26/17

Train 4

CETIS Summary Report

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

Batch ID:	11-8951-2763	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	12 Oct-17 14:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	12 Oct-17 15:35	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	02-1349-7209	**Code:**	17-1091	**Client:**	IDE
Sample Date:	11 Oct-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	11 Oct-17 12:32	**Source:**	IDE Americas, Inc.		
Sample Age:	31h (2.8 °C)	**Station:**	Train 4		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
18-4157-7020	Fertilization Rate	15	>15	NA	4.09%	< 6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
18-7792-7297	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
18-4157-7020	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
18-7792-7297	Fertilization Rate	Control Resp	0.858	0.7 - NL	Yes	Passes Acceptability Criteria
18-4157-7020	Fertilization Rate	PMSD	0.04092	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	0.884	0.8254	0.9426	0.84	0.96	0.02112	0.04722	5.34%	0.0%
0	Lab Control	5	0.858	0.8418	0.8742	0.84	0.87	0.005831	0.01304	1.52%	2.94%
2.5		5	0.894	0.8628	0.9252	0.85	0.91	0.01122	0.0251	2.81%	-1.13%
5		5	0.894	0.8654	0.9226	0.87	0.93	0.0103	0.02302	2.58%	-1.13%
6.06		5	0.896	0.8703	0.9217	0.87	0.92	0.009274	0.02074	2.31%	-1.36%
10		5	0.892	0.8698	0.9142	0.87	0.91	0.008	0.01789	2.01%	-0.91%
15		5	0.838	0.8097	0.8663	0.8	0.86	0.0102	0.0228	2.72%	5.2%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	High Salinity Co	0.84	0.85	0.89	0.96	0.88
0	Lab Control	0.87	0.85	0.87	0.86	0.84
2.5		0.91	0.91	0.9	0.85	0.9
5		0.9	0.93	0.88	0.87	0.89
6.06		0.87	0.88	0.92	0.91	0.9
10		0.88	0.91	0.91	0.87	0.89
15		0.84	0.84	0.8	0.86	0.85

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

Analysis ID:	18-4157-7020	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Oct-17 10:48	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.09%	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	-2.737	2.362	0.048	8	0.9999	CDF	Non-Significant Effect
		5	-2.741	2.362	0.048	8	0.9999	CDF	Non-Significant Effect
		6.06	-2.882	2.362	0.048	8	1.0000	CDF	Non-Significant Effect
		10	-2.547	2.362	0.048	8	0.9999	CDF	Non-Significant Effect
		15	1.344	2.362	0.048	8	0.2781	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03420945	0.00684189	5	6.546	0.0006	Significant Effect
Error	0.02508643	0.001045268	24			
Total	0.05929589		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.289	15.09	0.8079	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9793	0.9031	0.8074	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	0.858	0.8418	0.8742	0.86	0.84	0.87	0.005831	1.52%	0.0%
2.5		5	0.894	0.8628	0.9252	0.9	0.85	0.91	0.01122	2.81%	-4.2%
5		5	0.894	0.8654	0.9226	0.89	0.87	0.93	0.0103	2.58%	-4.2%
6.06		5	0.896	0.8703	0.9217	0.9	0.87	0.92	0.009274	2.31%	-4.43%
10		5	0.892	0.8698	0.9142	0.89	0.87	0.91	0.008	2.01%	-3.96%
15		5	0.838	0.8097	0.8663	0.84	0.8	0.86	0.0102	2.72%	2.33%

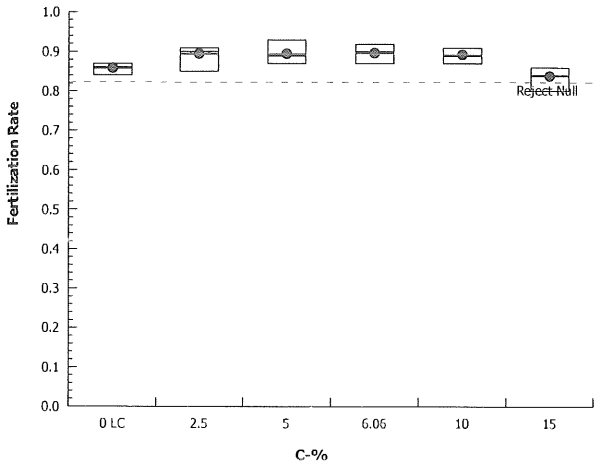
Angular (Corrected) Transformed Summary

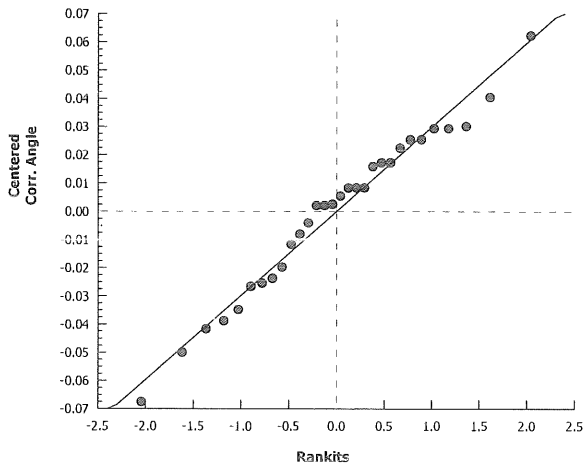
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.185	1.162	1.208	1.187	1.159	1.202	0.008311	1.57%	0.0%
2.5		5	1.241	1.193	1.289	1.249	1.173	1.266	0.01732	3.12%	-4.72%
5		5	1.241	1.192	1.289	1.233	1.202	1.303	0.01744	3.14%	-4.73%
6.06		5	1.244	1.201	1.286	1.249	1.202	1.284	0.01519	2.73%	-4.97%
10		5	1.237	1.201	1.273	1.233	1.202	1.266	0.01292	2.34%	-4.4%
15		5	1.157	1.12	1.195	1.159	1.107	1.187	0.01355	2.62%	2.32%

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

Analysis ID:	18-4157-7020	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Oct-17 10:48	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	18-7792-7297	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Oct-17 10:48	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	312913	1000	Yes	Two-Point Interpolation

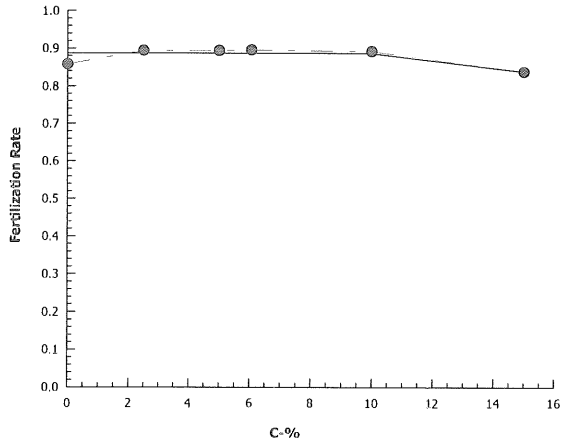
Point Estimates

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	0.858	0.84	0.87	0.005831	0.01304	1.52%	0.0%	429	500
2.5		5	0.894	0.85	0.91	0.01122	0.0251	2.81%	-4.2%	447	500
5		5	0.894	0.87	0.93	0.0103	0.02302	2.58%	-4.2%	447	500
6.06		5	0.896	0.87	0.92	0.009274	0.02074	2.31%	-4.43%	448	500
10		5	0.892	0.87	0.91	0.008	0.01789	2.01%	-3.96%	446	500
15		5	0.838	0.8	0.86	0.0102	0.0228	2.72%	2.33%	419	500

Graphics



Analyst: _____ QA: AC 10/25/17

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

Analysis ID:	09-1943-4577	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	24 Oct-17 10:48	**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	2.49%	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*	19.13	2.015	0.037	5	<0.0001	CDF	Non-Significant Effect
		5*	19.02	2.015	0.037	5	<0.0001	CDF	Non-Significant Effect
		6.06*	21.63	2.015	0.033	5	<0.0001	CDF	Non-Significant Effect
		10*	24.27	2.015	0.029	5	<0.0001	CDF	Non-Significant Effect
		15*	18.01	2.015	0.030	5	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03420945	0.00684189	5	6.546	0.0006	Significant Effect
Error	0.02508643	0.001045268	24			
Total	0.05929589		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.289	15.09	0.8079	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9793	0.9031	0.8074	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	0.858	0.8418	0.8742	0.86	0.84	0.87	0.005831	1.52%	0.0%
2.5		5	0.894	0.8628	0.9252	0.9	0.85	0.91	0.01122	2.81%	-4.2%
5		5	0.894	0.8654	0.9226	0.89	0.87	0.93	0.0103	2.58%	-4.2%
6.06		5	0.896	0.8703	0.9217	0.9	0.87	0.92	0.009274	2.31%	-4.43%
10		5	0.892	0.8698	0.9142	0.89	0.87	0.91	0.008	2.01%	-3.96%
15		5	0.838	0.8097	0.8663	0.84	0.8	0.86	0.0102	2.72%	2.33%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.185	1.162	1.208	1.187	1.159	1.202	0.008311	1.57%	0.0%
2.5		5	1.241	1.193	1.289	1.249	1.173	1.266	0.01732	3.12%	-4.72%
5		5	1.241	1.192	1.289	1.233	1.202	1.303	0.01744	3.14%	-4.73%
6.06		5	1.244	1.201	1.286	1.249	1.202	1.284	0.01519	2.73%	-4.97%
10		5	1.237	1.201	1.273	1.233	1.202	1.266	0.01292	2.34%	-4.4%
15		5	1.157	1.12	1.195	1.159	1.107	1.187	0.01355	2.62%	2.32%

CETIS Test Data Worksheet

Report Date: 12 Oct-17 09:04 (p 1 of 1)
Test Code: 1710-5045 14-2018-0599/54A63C77

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

Start Date: 12 Oct-17
End Date: 12 Oct-17
Sample Date: 11 Oct-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 1091
Sample Source: IDE Americas, Inc.
Sample Station: Train 4

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			216	100	(QIB) ~~60~~ 90	AB 10/16/17 AB 10/16/17
			217	100	88	
			218	100	88	
			219	100	91	
			220	100	85	
			221	100	90	
			~~222~~	100	91	
			223	100	89	
			224	100	85	
			225	100	91	
			226	100	80	
			227	100	84	
			228	100	87	
			229	100	96	
			230	100	92	
			231	100	88	
			232	100	87	
			233	100	89	
			234	100	91	
			235	100	84	
			236	100	85	
			237	100	90	
			238	100	86	
			239	100	87	
			240	100	89	
			241	100	90	
			242	100	87	
			243	100	88	
			244	100	85	
			245	100	87	
			246	100	84	
			247	100	91	
			248	100	84	
			249	100	86	
			250	100	93	

Analyst: AB QA: AC 10/18/17

CETIS Test Data Worksheet

Report Date: 12 Oct-17 09:04 (p 1 of 1)
Test Code: 1710-5045 14-2018-0599/54A63C77

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

Start Date: 12 Oct-17
End Date: 12 Oct-17
Sample Date: 11 Oct-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 1091
Sample Source: IDE Americas, Inc.
Sample Station: Train 4

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	227			
0	HS	2	236			
0	HS	3	223			
0	HS	4	229	100	94	10/12/17 RT
0	HS	5	231			
0	LC	1	239			
0	LC	2	220	100	89	10/12/17 RT
0	LC	3	242			
0	LC	4	238			
0	LC	5	246			
2.5		1	222			
2.5		2	219			
2.5		3	221			
2.5		4	244	100	76	10/12/17 RT
2.5		5	241			
5		1	237			
5		2	250	100	89	10/12/17 RT
5		3	243			
5		4	245			
5		5	240			
6.06		1	232			
6.06		2	218			
6.06		3	230	100	89	10/12/17 RT
6.06		4	234			
6.06		5	216			
10		1	217			
10		2	247	100	89	10/12/17 RT
10		3	225			
10		4	228			
10		5	233			
15		1	248			
15		2	235			
15		3	226			
15		4	249			
15		5	224	100	76	10/12/17 RT

DCG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: Train 4 | **Start Date/Time:** 10/12/2017 1455

Sample Log No.: 17- 1091 | **End Date/Time:** 10/12/2017 1535

Dilutions made by: AD | **Test No:** 1710-S045

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.1	8.11	33.7	15.1
High Salinity Control	7.1	8.06	39.3	15.1
2.5	7.1	8.09	34.9	14.9
5.0	7.0	8.06	35.7	15.0
6.06	7.0	8.05	36.1	15.1
10	7.1	8.02	37.5	15.0
15	7.0	7.97	39.3	15.1

Comments:

QC Check: AC 10/18/17 | **Final Review:** KFP 10/20/17

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

Client: IDE
Sample ID: Train 4
Test No.: 1710-5045

Start Date/Time: 10/12/2017 / 1455
End Date/Time: 10/12/2017 / 1535
Species: *S. purpuratus*
Animal Source: Pt. Loma.
Date Collected: 9/28/17

Tech initials: CG
Injection Time: 1400

Sperm Absorbance at 400 nm: 0.972 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 80, 71, 72, 75, 80
Mean: (A) 75.72 X 50 = 3860 eggs/ml

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

(B)

Initial density:	3860	eggs/ml	=	0.965	dilution factor	egg stock	ml
Final density:	4000	eggs/ml	-	1.0	part egg stock	seawater	ml
				0.035	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):	1420	50:1	75	25
Eggs Added (0.5 ml):	1437	100:1	91,95	9,5
Test Ended:	(A) 1457/1447	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):	1455	QC1	93	7
Eggs Added (0.5 ml):	1515	QC2	92	8
Test Ended:	1535	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) CG QC 10/12/17
(B) ~~EG~~ (A) ND dilution necessary.

QC Check: AC 10/18/17

Final Review: VTP 10/26/17

PT Filter EFF

CETIS Summary Report

Test Code: 1710-S044 | 21-2792-8664

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

Batch ID:	04-8452-6221	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	12 Oct-17 14:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	12 Oct-17 15:35	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	20-2836-5281	**Code:**	17-1092	**Client:**	IDE
Sample Date:	11 Oct-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	11 Oct-17 12:32	**Source:**	IDE Americas, Inc.		
Sample Age:	31h (4.5 °C)	**Station:**	Pre-Treatment Filtered		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-1919-9501	Fertilization Rate	15	>15	NA	7.96%	<6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
12-2955-7712	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
12-2955-7712	Fertilization Rate	Control Resp	0.848	0.7 - NL	Yes	Passes Acceptability Criteria
15-1919-9501	Fertilization Rate	Control Resp	0.848	0.7 - NL	Yes	Passes Acceptability Criteria
15-1919-9501	Fertilization Rate	PMSD	0.07955	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	0.848	0.7786	0.9174	0.75	0.89	0.02498	0.05586	6.59%	0.0%
2.5		5	0.786	0.7138	0.8582	0.7	0.85	0.026	0.05814	7.4%	7.31%
5		5	0.81	0.7611	0.8589	0.77	0.87	0.01761	0.03937	4.86%	4.48%
6.06		5	0.794	0.7167	0.8713	0.72	0.88	0.02786	0.06229	7.85%	6.37%
10		5	0.858	0.8183	0.8977	0.82	0.9	0.01428	0.03194	3.72%	-1.18%
15		5	0.826	0.7974	0.8546	0.79	0.85	0.0103	0.02302	2.79%	2.59%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.87	0.87	0.89	0.86	0.75
2.5		0.7	0.76	0.82	0.85	0.8
5		0.81	0.87	0.77	0.82	0.78
6.06		0.72	0.88	0.82	0.8	0.75
10		0.9	0.88	0.82	0.84	0.85
15		0.85	0.83	0.84	0.79	0.82

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

Analysis ID:	15-1919-9501	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Oct-17 16:43	**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	7.96%	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	2.119	2.362	0.091	8	0.0798	CDF	Non-Significant Effect
		5	1.368	2.362	0.091	8	0.2693	CDF	Non-Significant Effect
		6.06	1.837	2.362	0.091	8	0.1319	CDF	Non-Significant Effect
		10	-0.3117	2.362	0.091	8	0.9091	CDF	Non-Significant Effect
		15	0.8593	2.362	0.091	8	0.4637	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03581528	0.007163056	5	1.931	0.1263	Non-Significant Effect
Error	0.08903499	0.003709791	24			
Total	0.1248503		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.058	15.09	0.5410	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.983	0.9031	0.8990	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	0.848	0.7786	0.9174	0.87	0.75	0.89	0.02498	6.59%	0.0%
2.5		5	0.786	0.7138	0.8582	0.8	0.7	0.85	0.026	7.4%	7.31%
5		5	0.81	0.7611	0.8589	0.81	0.77	0.87	0.01761	4.86%	4.48%
6.06		5	0.794	0.7167	0.8713	0.8	0.72	0.88	0.02786	7.85%	6.37%
10		5	0.858	0.8183	0.8977	0.85	0.82	0.9	0.01428	3.72%	-1.18%
15		5	0.826	0.7974	0.8546	0.83	0.79	0.85	0.0103	2.79%	2.59%

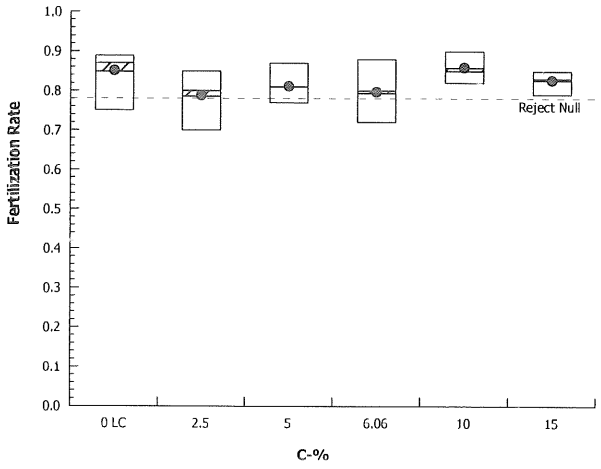
Angular (Corrected) Transformed Summary

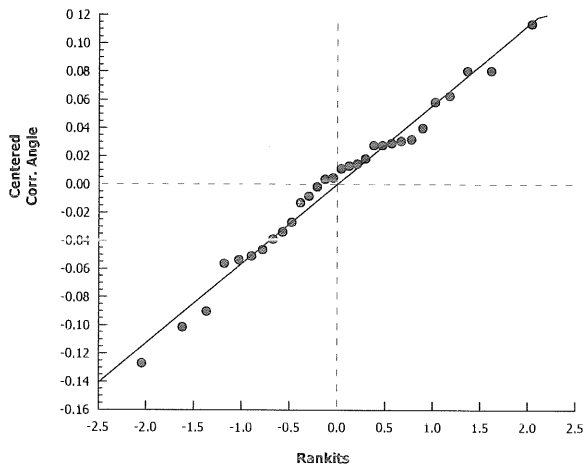
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.174	1.084	1.265	1.202	1.047	1.233	0.03261	6.21%	0.0%
2.5		5	1.093	1.005	1.18	1.107	0.9912	1.173	0.03141	6.43%	6.95%
5		5	1.122	1.057	1.186	1.12	1.071	1.202	0.02313	4.61%	4.49%
6.06		5	1.103	1.005	1.202	1.107	1.013	1.217	0.03541	7.18%	6.03%
10		5	1.186	1.128	1.244	1.173	1.133	1.249	0.02082	3.92%	-1.02%
15		5	1.141	1.104	1.178	1.146	1.095	1.173	0.01341	2.63%	2.82%

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

Analysis ID:	15-1919-9501	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Oct-17 16:43	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	12-2955-7712	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	26 Oct-17 16:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	134713	1000	Yes	Two-Point Interpolation

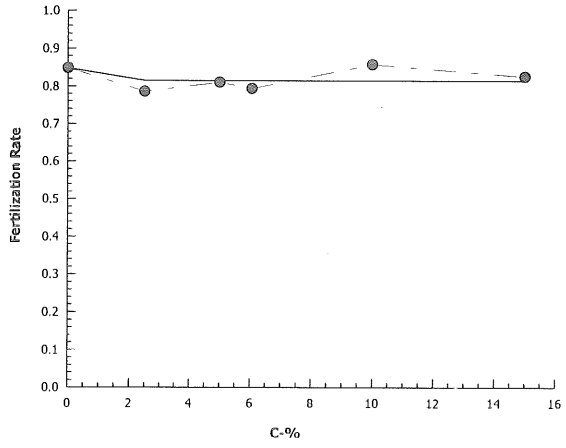
Point Estimates

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	0.848	0.75	0.89	0.02498	0.05586	6.59%	0.0%	424	500
2.5		5	0.786	0.7	0.85	0.026	0.05814	7.4%	7.31%	393	500
5		5	0.81	0.77	0.87	0.01761	0.03937	4.86%	4.48%	405	500
6.06		5	0.794	0.72	0.88	0.02786	0.06229	7.85%	6.37%	397	500
10		5	0.858	0.82	0.9	0.01428	0.03194	3.72%	-1.18%	429	500
15		5	0.826	0.79	0.85	0.0103	0.02302	2.79%	2.59%	413	500

Graphics



CETIS Analytical Report

Test Code: 1710-S044 | 21-2792-8664

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

Analysis ID: 03-7531-4986 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 26 Oct-17 16:43 **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	4.46%	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*	5.324	1.895	0.075	7	0.0005	CDF	Non-Significant Effect
		5*	7.155	1.895	0.064	7	<0.0001	CDF	Non-Significant Effect
		6.06*	5.177	1.895	0.082	7	0.0006	CDF	Non-Significant Effect
		10*	9.515	1.895	0.061	7	<0.0001	CDF	Non-Significant Effect
		15*	9.339	1.943	0.054	6	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.03581528	0.007163056	5	1.931	0.1263	Non-Significant Effect
Error	0.08903499	0.003709791	24			
Total	0.1248503		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	4.058	15.09	0.5410	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.983	0.9031	0.8990	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	0.848	0.7786	0.9174	0.87	0.75	0.89	0.02498	6.59%	0.0%
2.5		5	0.786	0.7138	0.8582	0.8	0.7	0.85	0.026	7.4%	7.31%
5		5	0.81	0.7611	0.8589	0.81	0.77	0.87	0.01761	4.86%	4.48%
6.06		5	0.794	0.7167	0.8713	0.8	0.72	0.88	0.02786	7.85%	6.37%
10		5	0.858	0.8183	0.8977	0.85	0.82	0.9	0.01428	3.72%	-1.18%
15		5	0.826	0.7974	0.8546	0.83	0.79	0.85	0.0103	2.79%	2.59%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.174	1.084	1.265	1.202	1.047	1.233	0.03261	6.21%	0.0%
2.5		5	1.093	1.005	1.18	1.107	0.9912	1.173	0.03141	6.43%	6.95%
5		5	1.122	1.057	1.186	1.12	1.071	1.202	0.02313	4.61%	4.49%
6.06		5	1.103	1.005	1.202	1.107	1.013	1.217	0.03541	7.18%	6.03%
10		5	1.186	1.128	1.244	1.173	1.133	1.249	0.02082	3.92%	-1.02%
15		5	1.141	1.104	1.178	1.146	1.095	1.173	0.01341	2.63%	2.82%

Analyst: AC QA: KPP 10/26/17

CETIS Test Data Worksheet

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

Start Date: 12 Oct-17 14:55 **Species:** Strongylocentrotus purpuratus **Sample Code:** 17-1092
End Date: 12 Oct-17 15:35 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 11 Oct-17 08:00 **Material:** Facility Effluent **Sample Station:** Pre-Treatment Filtered

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			186	100	75	
			187		82	
			188		89	
			189		87	
			190		87	
			191		85	
			192		86	
			193		82	
			194		90	
			195		88	
			196		70	
			197		85	
			198		81	
			199		78	
			200		85	
			201		83	
			202		87	
			203		84	
			204		88	
			205		76	
			206		77	
			207		75	
			208		84	
			209		79	
			210		82	
			211		82	
			212		80	
			213		80	
			214		82	
			215		72	

CETIS Test Data Worksheet

Report Date: 12 Oct-17 09:02 (p 1 of 1)
Test Code: 1710-5044 21-2792-8664/7ED59D58

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: 12 Oct-17
End Date: 12 Oct-17
Sample Date: 11 Oct-17

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

Sample Code: 17- 1092
Sample Source: IDE Americas, Inc.
Sample Station: Pre-Treatment Filtered

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	190			
0	LC	2	189			
0	LC	3	188			
0	LC	4	192			
0	LC	5	207	100	82	10/12/17 RT
2.5		1	196			
2.5		2	205			
2.5		3	210			
2.5		4	200	100	76	10/12/17 RT
2.5		5	212			
5		1	198			
5		2	202			
5		3	206	100	~~53~~ 72	10/12/17 RT
5		4	211			
5		5	199			
6.06		1	215			
6.06		2	195	100	78	10/12/17 RT
6.06		3	214			
6.06		4	213			
6.06		5	186			
10		1	194			
10		2	204	100	82	10/12/17 RT
10		3	187			
10		4	208			
10		5	197			
15		1	191	100	74	10/12/17 RT
15		2	201			
15		3	203			
15		4	209			
15		5	193			

QC: CG (A) QC RT 10/12/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: PT Filt Eff | **Start Date/Time:** 10/12/2017 1455

Sample Log No.: 17-~~4090~~1092 (A) | **End Date/Time:** 10/12/2017 1535

Dilutions made by: AD | **Test No:** 1710-S044

Analyst: AD

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.2	8.09	33.6	15.3
2.5	7.1	8.08	33.7	15.2
5.0	7.1	8.07	33.9	14.9
6.06	7.1	8.06	33.9	15.1
10	7.1	8.04	33.~~8~~9 (A)	15.1
15	7.1	8.00	33.8	15.0

Comments: @Ao 018 10/12/17

QC Check: AC 10/18/17 | **Final Review:** KFP 10/24/17

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

Client: IDE
Sample ID: Pre-treatment filter effluent
Test No.: 1710-S044

Start Date/Time: 10/12/2017 / 1455
End Date/Time: 10/12/2017 / 1535
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 9/28/17

Tech initials: CG
Injection Time: 1400

Sperm Absorbance at 400 nm: 0.972 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 80, 71, 72, 75, 80
Mean: (A) 3772 X 50 = 3860 eggs/ml

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

Initial density:	3860	eggs/ml	=	0.965	dilution factor	egg stock _____	ml
Final density:	4000	eggs/ml	-	1.0	part egg stock	seawater _____	ml
				~~0.035~~	parts seawater		

(B)

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).

Rangefinder Test:	Sperm:Egg Ratio 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):	1420	50:1	75	25
Eggs Added (0.5 ml):	1437	100:1	91,95	9,5
Test Ended:	(A) 1457/1447	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):	1455	QC1	93	7
Eggs Added (0.5 ml):	1515	QC2	92	8
Test Ended:	1535	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) CG QC 10/12/17
(B) ~~EGS~~ NO dilution necessary.

QC Check: AC 10/18/17

Final Review: KTP 10/20/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Sample Check-In Information

Client: IDE

Tests Performed: Urchin Fertilization

Project: CDP Screens

Test ID No.(s): 1710-5040 to 5045 Train 4 (B)

Sample ID:	1) M-001	2) ERI	3) Brine Pit	4) PT Fill	5) ~~RO Train~~	6)
Log-in No. (17-xxxx):	1088	1089	1090	1092	1091	
Sample Collection Date & Time:	10/11/17 0800	10/11/17 0800	10/11/17 0800	10/11/17 0800	10/11/17 0800	
Sample Receipt Date & Time:	10/11/17 1232	10/11/17 1232	10/11/17 1232	10/11/17 1232	10/11/17 1232	
Number of Containers & Container Type:	1 4L cubi	1 4L cubi	1 4L cubi	1 4L cubi	1 4L cubi	
Approx. Total Volume Received (L):	~4L	~4L	~4L	~4L	~4L	
Check-in Temp (°C)	3.5	3.5	2.1	4.5	2.8	
Temperature OK? [1]	(Y) N	(Y) N	(Y) N	(Y) N	(Y) N	Y N
DO (mg/L)	7.46 (A)	7.6	8.3	8.6	6.7	
pH (units)	7.89	7.36	7.07	7.18	7.43	
Conductivity (µS/cm)	—	—	—	—	—	
Salinity (ppt)	~~59.0~~ (C)	~~68.4~~ (C)	22.2	33.9	~~69.2~~ (C)	
Alkalinity (mg/L) [2]	194	200	82	158	210	
Hardness (mg/L) [2,3]	—	—	—	—	—	
Total Chlorine (mg/L)	<0.02	<0.02	0.02	<0.02	<0.02	
Technician Initials	DM/TN/RT	DM/TN/RT	DM/TN/RT	DM/TN/RT	DM/TN/RT	

Sample Descriptions:

1) no color, clear, no odor, no debris
2) no color, clear, no odor, no debris
3) no color, clear, no odor, no debris
4) no color, clear, no odor, no debris
5) no color, clear, no odor, no debris
6) _____

CoC Complete? (Y) N

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

pH Adjustment? Y (N)

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

Freshwater Tests:

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

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

Marine Tests: Urchin Fertilization

Control/Dilution Water Source: (LAB SW) ART SW Other: _____ Alkalinity: 109 Salinity: 34 ppt

Additional Control? (Y) N = High salinity control Alkalinity: NM Salinity: 37.4 ppt (mod) 39.3 (T4)

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

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

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

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

Additional Comments (A) Tuque 10/11/17 (B) PH 4/18 10/11/17 (C) MADE 2:1 DILUTION

NM = not measured

Subsamples For Additional Chemistry Required? Y (N)

NH3 Other _____

Tech Initials _____

QC Check: AD 10/18/17

Final Review: AC 10/25/17

Appendix C

Chain-of-Custody Form

SCREENING + WEEKLY

IDE Technologies

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

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

Project Name: Toxicity Screening NPDES Project Manager: Peter Shen Contact Information: (760) 201-7777

Special instruction: Samples collected during normal plant operationat 48 MGD. Samples are to be run unadjusted. Start: 10/10/17 @ 08:00, End: 10/11/17 @ 08:00. KC OK Heal adj

Glass=G Plastic=P

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

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

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization								NOTES:	Temp
M-001 (17- 3053)	10/10-11/17	8:00	24 HR COMP -B	N	4L CUBIE	X								TDS - 56.51 ppt EC - 79.90 mS/cm	3.5
ERI BRINE (17-3054)	10/10-11/17	8:00	24 HR COMP -B	N	4L CUBIE	X								TDS - 65.13 ppt EC - 89.40 mS/cm	3.5
BRINE PIT (17-3055)	10/10-11/17	8:00	24 HR COMP -B	N	4L CUBIE	X								TDS - 20.60 ppt EC - 33.49 mS/cm	2.1
TRAIN 4 (17-3056)	10/10-11/17	8:00	24 HR COMP -B	N	4L CUBIE	X								TDS - 67.29 ppt EC - 91.86 mS/cm	2.8
PT FILTER EFF (17-3057)	10/10-11/17	8:00	24 HR COMP -B	N	4L CUBIE	X								TDS - 32.57 ppt EC - 50.65 mS/cm	4.5

Relinquished By:	Date:	Time:		Received By:	Time:	Sample Condition Upon Receipt:
Kevin Cagney	10/11/17	1115		[signature] 10/11/17	11:15	☐ Iced ☐ Ambient or _____°C
[signature]	10/11/17	12:32		[signature] 10/11/17	1232	☐ Iced ☐ Ambient or _____°C

10/11/17

Nautilus ID: 17-1088 through 17-1092

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	08-3037-0546	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	12 Oct-17 14:55	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	12 Oct-17 15:35	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	18-9575-2056	**Code:**	171012sprt	**Client:**	Internal
Sample Date:	12 Oct-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	12 Oct-17	**Source:**	Reference Toxicant		
Sample Age:	15h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
16-9454-4286	Fertilization Rate	10	20	14.14	5.43%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
07-1531-2424	Fertilization Rate	EC50	60.18	57.54	62.94		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
07-1531-2424	Fertilization Rate	Control Resp	0.894	0.7 - NL	Yes	Passes Acceptability Criteria
16-9454-4286	Fertilization Rate	Control Resp	0.894	0.7 - NL	Yes	Passes Acceptability Criteria
16-9454-4286	Fertilization Rate	PMSD	0.05427	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	0.894	0.8628	0.9252	0.87	0.92	0.01122	0.0251	2.81%	0.0%
10		5	0.858	0.8476	0.8684	0.85	0.87	0.003741	0.008366	0.98%	4.03%
20		5	0.828	0.7829	0.8731	0.79	0.88	0.01625	0.03633	4.39%	7.38%
40		5	0.73	0.6299	0.8301	0.64	0.82	0.03606	0.08062	11.04%	18.34%
80		5	0.28	0.2509	0.3091	0.26	0.32	0.01049	0.02345	8.38%	68.68%
160		5	0.008	0	0.01839	0	0.02	0.003742	0.008367	104.6%	99.11%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.89	0.92	0.87	0.87	0.92
10		0.87	0.86	0.86	0.85	0.85
20		0.88	0.79	0.81	0.85	0.81
40		0.82	0.65	0.64	0.78	0.76
80		0.32	0.27	0.27	0.28	0.26
160		0	0.02	0.01	0	0.01

Analyst: _____ QA: KTP 10/23/17

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

Analysis ID:	16-9454-4286	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Oct-17 17:45	**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	5.43%	10	20	14.14	

Dunnett Multiple Comparison Test

Control	vs	C-µg/L	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		10	1.797	2.362	0.074	8	0.1411	CDF	Non-Significant Effect
		20*	3.06	2.362	0.074	8	0.0111	CDF	Significant Effect
		40*	6.79	2.362	0.074	8	<0.0001	CDF	Significant Effect
		80*	21.78	2.362	0.074	8	<0.0001	CDF	Significant Effect
		160*	36.72	2.362	0.074	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	5.227365	1.045473	5	424.5	<0.0001	Significant Effect
Error	0.0591016	0.002462567	24			
Total	5.286466		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	13.79	15.09	0.0170	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9826	0.9031	0.8900	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	0.894	0.8628	0.9252	0.89	0.87	0.92	0.01122	2.81%	0.0%
10		5	0.858	0.8476	0.8684	0.86	0.85	0.87	0.003741	0.98%	4.03%
20		5	0.828	0.7829	0.8731	0.81	0.79	0.88	0.01625	4.39%	7.38%
40		5	0.73	0.6299	0.8301	0.76	0.64	0.82	0.03606	11.04%	18.34%
80		5	0.28	0.2509	0.3091	0.27	0.26	0.32	0.01049	8.38%	68.68%
160		5	0.008	0	0.01839	0.01	0	0.02	0.003742	104.6%	99.11%

Angular (Corrected) Transformed Summary

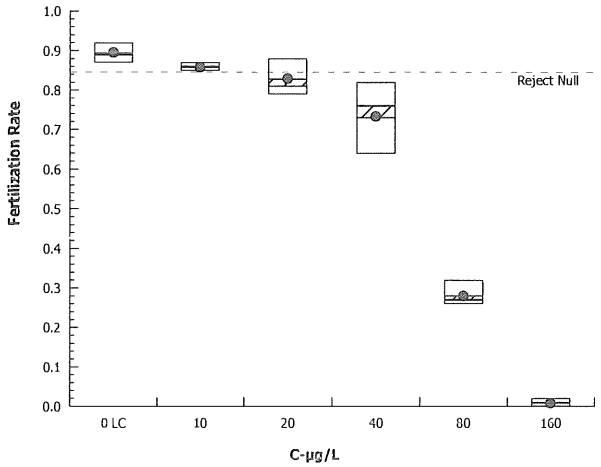
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.241	1.19	1.292	1.233	1.202	1.284	0.01847	3.33%	0.0%
10		5	1.185	1.17	1.199	1.187	1.173	1.202	0.005383	1.02%	4.54%
20		5	1.145	1.084	1.206	1.12	1.095	1.217	0.02211	4.32%	7.74%
40		5	1.028	0.9148	1.141	1.059	0.9273	1.133	0.04072	8.86%	17.17%
80		5	0.5573	0.5253	0.5894	0.5464	0.5351	0.6013	0.01154	4.63%	55.09%
160		5	0.08845	0.04003	0.1369	0.1002	0.05002	0.1419	0.01744	44.09%	92.87%

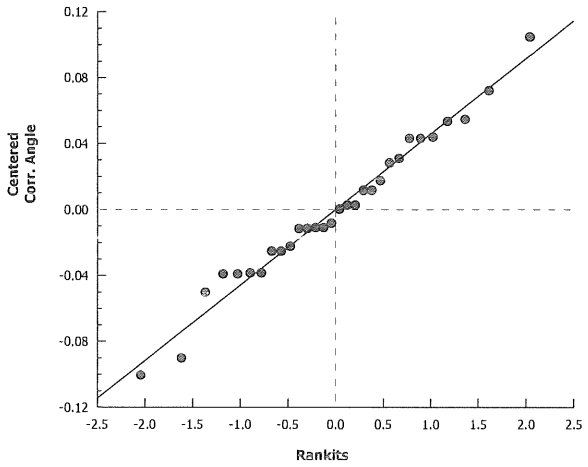
Analyst: JD QA: KTP 10/23/17

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

Analysis ID:	16-9454-4286	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Oct-17 17:45	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	07-1531-2424	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	16 Oct-17 17:45	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

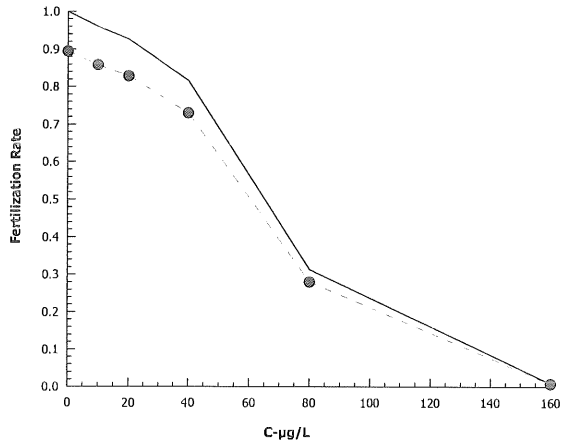
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.106	4.03%	1.779	0.009735	60.18	57.54	62.94

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	0.894	0.87	0.92	0.01122	0.0251	2.81%	0.0%	447	500
10		5	0.858	0.85	0.87	0.003741	0.008366	0.98%	4.03%	429	500
20		5	0.828	0.79	0.88	0.01625	0.03633	4.39%	7.38%	414	500
40		5	0.73	0.64	0.82	0.03606	0.08062	11.04%	18.34%	365	500
80		5	0.28	0.26	0.32	0.01049	0.02345	8.38%	68.68%	140	500
160		5	0.008	0	0.02	0.003742	0.008367	104.6%	99.11%	4	500

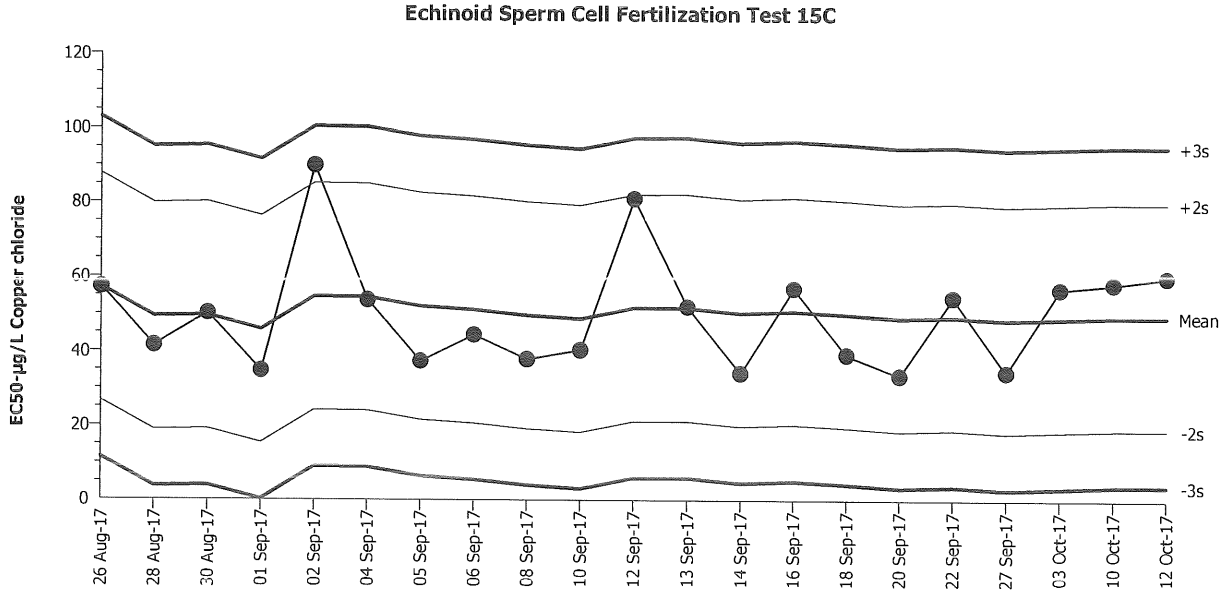
Graphics



Analyst: _____ QA: PRP 10/23/17

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:	49.46	**Count:**	20	**-2s Warning Limit:**	18.94	**-3s Action Limit:**	3.676
Sigma:	15.26	**CV:**	30.90%	**+2s Warning Limit:**	79.98	**+3s Action Limit:**	95.24

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Aug	26	16:00	57.24	7.781	0.5099			10-2039-5656	15-8794-0305
2			28	14:56	41.55	-7.908	-0.5182			08-1525-2751	10-7829-2432
3			30	16:38	50.21	0.7519	0.04927			08-1199-3706	11-0543-3886
4		Sep	1	15:27	34.79	-14.67	-0.9611			13-1244-6646	21-1567-7550
5			2	10:53	89.99	40.53	2.656	(+)		16-4202-9692	18-8681-1855
6			4	16:10	53.77	4.312	0.2826			12-2973-1405	10-6032-1229
7			5	17:07	37.36	-12.1	-0.793			13-1627-7974	14-5447-1160
8			6	17:15	44.41	-5.047	-0.3307			05-5533-8557	16-8161-1582
9			8	15:48	37.91	-11.55	-0.7567			18-6871-7794	04-4479-5076
10			10	14:25	40.4	-9.058	-0.5936			11-6871-9499	08-4248-1228
11			12	15:51	81.07	31.61	2.072	(+)		20-0603-9450	06-1182-7961
12			13	19:07	52.04	2.576	0.1688			01-4575-6189	02-4618-7964
13			14	15:24	34.24	-15.22	-0.9977			11-2846-3680	13-8128-7168
14			16	17:08	56.97	7.51	0.4921			08-9569-1329	19-6375-1112
15			18	15:28	39.21	-10.25	-0.6719			19-2924-5672	02-0031-2532
16			20	16:15	33.62	-15.84	-1.038			00-4454-0074	17-7214-1415
17			22	14:50	54.61	5.149	0.3374			20-3341-5102	16-2759-7635
18			27	15:34	34.46	-15	-0.9827			12-3257-1101	06-9840-2290
19		Oct	3	13:49	56.88	7.419	0.4862			05-1137-7792	06-0895-0170
20			10	15:10	58.36	8.902	0.5833			20-5863-5053	00-1542-1738
21			12	14:55	60.18	10.72	0.7027			05-0863-6526	07-1531-2424

Analyst: _____ QA: PFP 10/23/17

CETIS Test Data Worksheet

Report Date: 12 Oct-17 08:23 (p 1 of 1)
Test Code: 05-0863-6526/171012sprt

Echinoid Sperm Cell Fertilization Test ~~12C~~ 15C (A) — **Nautilus Environmental (CA)**

Start Date: 12 Oct-17
End Date: 12 Oct-17
Sample Date: 12 Oct-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 171012sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	0	AB 10/13/17
			2	100	32	
			3	100	65	
			4	100	87	
			5	100	89	
			6	100	85	
			7	100	85	
			8	100	87	
			9	100	92	
			10	100	81	
			11	100	81	
			12	100	1	
			13	100	86	
			14	100	78	
			15	100	0	
			16	100	79	
			17	100	2	
			18	100	87	
			19	100	26	
			20	100	85	
			21	100	1	
			22	100	86	
			23	100	64	
			24	100	82	
			25	100	92	
			26	100	76	
			27	100	27	
			28	100	88	
			29	100	28	
			30	100	27	

(A) QR18 JB 10/23/17

CETIS Test Data Worksheet

Echinoid Sperm Cell Fertilization Test ~~12C~~ 15C (A) **Nautilus Environmental (CA)**

Start Date: 12 Oct-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 171012sprt
End Date: 12 Oct-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 12 Oct-17 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	5	100	94	CG 10/12/17
0	LC	2	25			
0	LC	3	18			
0	LC	4	8			
0	LC	5	9			
10		1	4			
10		2	22			
10		3	13	100	93	CG 10/12/17
10		4	6			
10		5	20			
20		1	28	100	88	CG 10/12/17
20		2	16			
20		3	11			
20		4	7			
20		5	10			
40		1	24			
40		2	3	100	73	CG 10/12/17
40		3	23			
40		4	14			
40		5	26			
80		1	2			
80		2	27			
80		3	30			
80		4	29	100	27	CG 10/12/17
80		5	19			
160		1	1			
160		2	17			
160		3	12			
160		4	15			
160		5	21	100	0	CG 10/12/17

QC: CG

(A) QA JA 10/23/17

Marine Chronic Bioassay

Water Quality Measurements

Client : Internal

Test Species: *S. purpuratus*

Sample ID: $CuCl_2$

Start Date/Time: 10/12/2017 1455

Test No: 171012sprt

End Date/Time: 10/12/2017 1535

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):	9,600

Analyst: AD

	Initial Readings			
Concentration (µg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	7.2	8.08	33.6	15.5
10	7.1	8.07	33.9	15.4
20	7.1	8.07	33.9	15.3
40	7.1	8.07	33.8	15.4
80	7.1	8.07	33.6	15.3
160	7.1	8.08	33.4	15.4

Comments:

QC Check: AC 10/18/17

Final Review: KTP 10/23/17

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

Client: Internal
Sample ID: CuCl2
Test No.: 171012SPF

Start Date/Time: 10/12/2017 / 1455
End Date/Time: 10/12/2017 / 1535
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 9/28/17

Tech initials: CG
Injection Time: 1400

Sperm Absorbance at 400 nm: 0.972 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 80, 71, 72, 75, 88 Mean: (A) 77.2 X 50 = 3860 eggs/ml

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

Initial density:	3860	eggs/ml	=	0.965	dilution factor	egg stock _____	ml
Final density:	4000	eggs/ml	-	1.0	part egg stock	seawater _____	ml
				0.035	parts seawater		

(B)

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):	1420	50:1	75	25
Eggs Added (0.5 ml):	1437	100:1	91,95	9,5
Test Ended:	(A) ~~1457~~ 1447	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):	1455	QC1	93	7
Eggs Added (0.5 ml):	1515	QC2	92	8
Test Ended:	1535	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) CG QC 10/12/17
(B) ~~EG~~ (A) NO dilution necessary.

QC Check: AC 10/18/17 Final Review: VP 10/23/17

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.