Nautilus Environmental

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

- Sample ID: M-001
  Sample Collection Date: June 30, 2017

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

Prepared by: **Nautilus Environmental**

Submitted: **July 13, 2017**

Data Quality Assurance:

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

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Results verified by: Adrienne Cibar

EXECUTIVE SUMMARY

CHRONIC TOXICITY TESTING

CARLSBAD DESALINATION PLANT – JUNE 2017

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

Sampling Date: June 30, 2017

Test Date: June 30, 2017

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

M-001 Effluent Limitation: 16.5 TU_c

Results Summary:

Bioassay Type:	M-001 Effluent Test Results		Effluent Limitation Met? (Yes/No)
Echinoderm Fertilization	NOEC	TU_c	No
	5.0	20	

INTRODUCTION

A 24-hour composite discharge sample was collected in June 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for chronic toxicity monitoring purposes. Due to effects observed in a sample collected and tested for monthly monitoring purposes on June 17, 2016 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 June 30, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The samples were collected on June 30, 2017. 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 the 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:	June 2017
Sample ID:	1. M-001, desalination plant brine effluent 2. ERI Brine 3. Brine Pit 4. Train 4 5. PT Filter EFF 6. M-INF
Sample Collection Date, Time:	1. 6/30/17, 08:00 2. 6/30/17, 08:00 3. 6/30/17, 08:00 4. 6/30/17, 08:00 5. 6/30/17, 08:00 6. 6/30/17, 08:00
Sample Receipt Date, Time:	6/30/17, 11:48
Sampling Method:	24-hour Composite

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	7.72	6.7	6.5	62.6	190	0.03
ERI Brine	7.21	7.3	4.5	68.4	199	<0.02
Brine Pit	8.00	7.0	6.5	30.2[a]	100	0.03
Train 4	7.75	7.0	5.5	70.2	206	<0.02
PT Filter EFF	7.97	7.2	5.5	33.9	107	0.02
M-INF	8.06	7.4	5.5	33.4	114	0.02

[a] Salinity measured in the Brine Pit sample was 30.2 ppt, the value was double checked.

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 control. For the purposes of this round of testing, the M-INF sample was used as control/dilution water for all other samples; Nautilus laboratory seawater collected from the Scripps Institution of Oceanography (SIO) inlet was used as control/dilution water only for the M-INF sample dilution series. 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:	6/30/17, 17:50 through 18:30
Test Organism:	*Strongylocentrotus purpuratus* (purple sea urchin)
Test Organism Source:	Field-collected locally (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 was used to prepare the M-INF dilution series. All other samples were diluted with the M-INF water itself.
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 and M-001 after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP discharges).
Number of Replicates, Organisms per Replicate:	5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined before each test with a preliminary rangefinding test.
Test Chamber Type, Volume per Replicate:	Glass scintillation vial containing 10 mL of test solution
Protocol Used:	EPA/600/R-95/136, 1995 West Coast Marine Chronic
Test Type:	Fertilization; 20-min sperm exposure to effluent followed by a 20-min egg fertilization period
Acceptability Criteria:	Mean fertilization ≥70% in the control, and percent minimum significant difference (PMSD) value <25%
Reference Toxicant Testing:	Copper chloride
Statistical Analysis Software:	CETIS™, version 1.8.7.20

RESULTS

There was a statistically significant decrease in fertilization rate observed in the 6.06, 10, and 15 percent concentrations of the unadjusted M-001 sample compared to the lab control, resulting in a NOEC of 5.0 percent effluent and a TU_c equal to 20. This exceeds the maximum daily permit effluent limitation of 16.5 TU_c. The 6.06 percent concentration (IWC) resulted in a 12 percent effect compared to the lab control, which was not significantly significant using to the TST calculation. The M-001 sample adjusted to 40 ppt prior to dilution preparation resulted in no statistically significant effects in any of the test concentrations and a TU_c less than 6.67.

The Brine Pit sample resulted in statistically significant decreases in fertilization rate for all but the 2.5 percent sample, resulting in a NOEC of 2.5 and a TU_c of 40. The percent effect in the 6.06 percent concentration was 17. The ERI Brine and Train 4 test resulted in a statistically significant decrease in fertilization rate in the 15 percent sample concentration compared to the lab control, resulting in a NOEC of 10 percent effluent and a TU_c equal to 10. The PT Filter and M-INF samples both 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. Two high salinity controls were tested for comparison purposes. One was run at 37.8 ppt with the unadjusted M-001 sample and the other at 39.0 ppt with the Train 4 sample. The high salinity controls for M-001 and Train 4 resulted in fertilization rates of 95.0 and 91.2, respectively. This indicates that decreases in fertilization rates observed in the samples were not likely attributable to elevated salinity.

Statistical results for urchin fertilization toxicity tests are summarized in Table 4, and 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.

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

Sample ID	NOEC (% sample)	LOEC (% sample)	EC_{50} (% sample)	TU_c value (toxic units)	**TST Result (Pass/Fail)**	**Percent Effect at 6.06%**
M-001 (unadjusted)	5.0	6.06	>15	20	Pass	12
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-4.2
ERI Brine	10	15	>15	10	Pass	0.22
Brine Pit	2.5	5.0	13.2	40	Pass	17
Train 4	10	15	>15	10	Pass	6.6
PT Filter EFF	15	>15	>15	<6.67	Pass	0.90
M-INF	15	>15	>15	<6.67	Pass	-0.64

NOEC = No Observed Effect Concentration
LOEC = Lowest Observed Effect Concentration
EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms
TU_c = Chronic Toxic Unit: 100÷NOEC
TST: Pass = sample is non-toxic at the 6.06% IWC according to the TST calculation; Fail = sample is toxic at the 6.06% IWC according to the TST calculation. The TST analysis is not in the existing CDP permit; TST analysis is included here for comparison purposes only.
Percent effect (PE) from control is calculated as: PE= ((mean response in control-mean response in the IWC)/mean response in control) *100. A negative PE results when organism performance in the sample is greater than that in the control.

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

Test Concentration (% Sample)	M-001 Unadjusted Sample		M-001 40 ppt Adjusted[a]	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	34.0	88.0	33.9	86.0
High Salinity Control	37.6	95.0	--	--
2.5	34.5	92.6	34.2	93.2
5.0	35.3	86.8	34.3	93.2
6.06	35.5	77.4*	34.3	89.6
10	36.5	66.6*	34.2	85.4
15	37.8	68.0*	34.9	88.2

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

*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 Purple 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	33.8	90.8	33.9	88.6
2.5	34.8	91.8	33.9	88.4
5.0	35.6	90.8	33.9	73.2*
6.06	36.0	90.6	33.9	73.4*
10	37.3	89.8	33.6	58.6*
15	38.9	81.4*	33.5	36.4*

*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 Purple Urchin Fertilization Testing for the Additional Facility Sample and Influent

Test Concentration (% Sample)	Train 4		Pre-treatment Filtered Effluent		M-INF	
	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	34.0	91.6	33.9	89.0	33.7	93.4
High Salinity Control	39.0	91.2	--	--	--	--
2.5	34.8	86.2	34.0	92.2	33.7	93.8
5.0	35.7	86.2	33.9	89.8	33.8	92.4
6.06	36.1	85.6	34.0	88.2	34.0	94.0
10	37.2	86.4	34.1	87.2	33.9	95.0
15	39.2	60.2*	34.0	90.4	33.9	93.8

*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 the same day as collection and within the appropriate temperature range. All samples were tested within the allowable holding time of 36 hours. 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).

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	39.4	45.1 ± 22.4	24.9

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. 1991. Methods for Aquatic Toxicity Identification Evaluation - Phase I Toxicity Characterization Procedures, 2nd Edition, EPA/600/6-91/003 February 1991.

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

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

Appendix A

Test Data and Statistical Analyses

M-001 Unadjusted

CETIS Summary Report

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

Batch ID:	02-9820-6206	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	~~Natural Seawater~~ M-1NF
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	02-9625-1568	**Code:**	17-0738	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (6.5 °C)	**Station:**	M-001 Unadjusted		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
05-7230-3032	Fertilization Rate	5	6.06	5.505	8.86%	20	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
14-6683-3143	Fertilization Rate	EC25	9.834	7.75	N/A	10.17	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
05-7230-3032	Fertilization Rate	Control Resp	0.88	0.7 - NL	Yes	Passes Acceptability Criteria
14-6683-3143	Fertilization Rate	Control Resp	0.88	0.7 - NL	Yes	Passes Acceptability Criteria
05-7230-3032	Fertilization Rate	PMSD	0.08862	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	0.95	0.9252	0.9748	0.93	0.98	0.008944	0.02	2.11%	0.0%
0	Lab Control	5	0.88	0.8266	0.9334	0.83	0.93	0.01924	0.04301	4.89%	7.37%
2.5		5	0.926	0.8729	0.9791	0.88	0.97	0.01913	0.04278	4.62%	2.53%
5		5	0.868	0.8238	0.9122	0.81	0.9	0.01594	0.03564	4.11%	8.63%
6.06		5	0.774	0.6634	0.8846	0.63	0.86	0.03982	0.08905	11.51%	18.53%
10		5	0.666	0.6042	0.7278	0.6	0.72	0.02227	0.0498	7.48%	29.89%
15		5	0.68	0.5958	0.7642	0.58	0.76	0.03033	0.06782	9.97%	28.42%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	High Salinity Co	0.94	0.93	0.98	0.94	0.96
0	Lab Control	0.86	0.83	0.92	0.86	0.93
2.5		0.88	0.89	0.97	0.97	0.92
5		0.9	0.88	0.86	0.81	0.89
6.06		0.76	0.63	0.79	0.83	0.86
10		0.72	0.68	0.7	0.6	0.63
15		0.76	0.58	0.68	0.72	0.66

@@ AC 7/10/17

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

Analysis ID:	05-7230-3032	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Jul-17 9:47	**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	8.86%	5	6.06	5.505	20

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-1.773	2.362	0.112	8	0.9984	CDF	Non-Significant Effect
		5	0.4291	2.362	0.112	8	0.6774	CDF	Non-Significant Effect
		6.06*	2.973	2.362	0.112	8	0.0136	CDF	Significant Effect
		10*	5.621	2.362	0.112	8	<0.0001	CDF	Significant Effect
		15*	5.288	2.362	0.112	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5102819	0.1020564	5	18.21	<0.0001	Significant Effect
Error	0.1345085	0.00560452	24			
Total	0.6447904		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.801	15.09	0.7307	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9675	0.9031	0.4737	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	0.88	0.8266	0.9334	0.86	0.83	0.93	0.01924	4.89%	0.0%
2.5		5	0.926	0.8729	0.9791	0.92	0.88	0.97	0.01913	4.62%	-5.23%
5		5	0.868	0.8238	0.9122	0.88	0.81	0.9	0.01594	4.11%	1.36%
6.06		5	0.774	0.6634	0.8846	0.79	0.63	0.86	0.03982	11.51%	12.05%
10		5	0.666	0.6042	0.7278	0.68	0.6	0.72	0.02227	7.48%	24.32%
15		5	0.68	0.5958	0.7642	0.68	0.58	0.76	0.03033	9.97%	22.73%

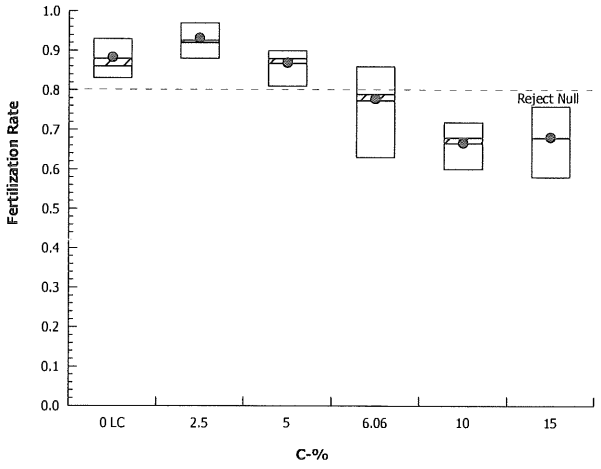
Angular (Corrected) Transformed Summary

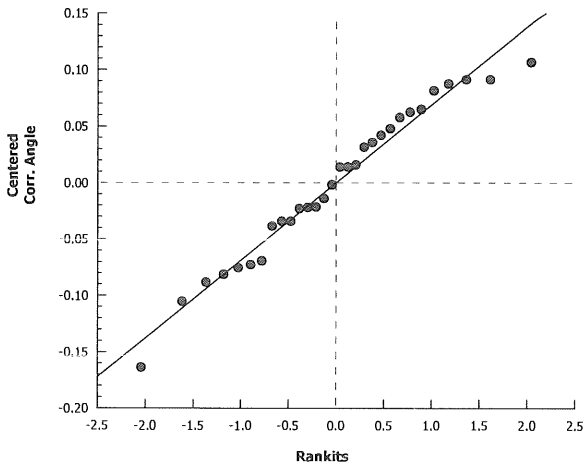
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.221	1.137	1.306	1.187	1.146	1.303	0.03052	5.59%	0.0%
2.5		5	1.305	1.198	1.413	1.284	1.217	1.397	0.03887	6.66%	-6.87%
5		5	1.201	1.138	1.264	1.217	1.12	1.249	0.02276	4.24%	1.66%
6.06		5	1.081	0.9518	1.21	1.095	0.9169	1.187	0.04643	9.61%	11.52%
10		5	0.9554	0.8899	1.021	0.9695	0.8861	1.013	0.02356	5.52%	21.79%
15		5	0.9711	0.8809	1.061	0.9695	0.8657	1.059	0.03247	7.48%	20.5%

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

Analysis ID:	05-7230-3032	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Jul-17 9:47	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1706-S203 | 12-4385-2719

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

Analysis ID:	14-6683-3143	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Jul-17 9:47	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	753277	1000	Yes	Two-Point Interpolation

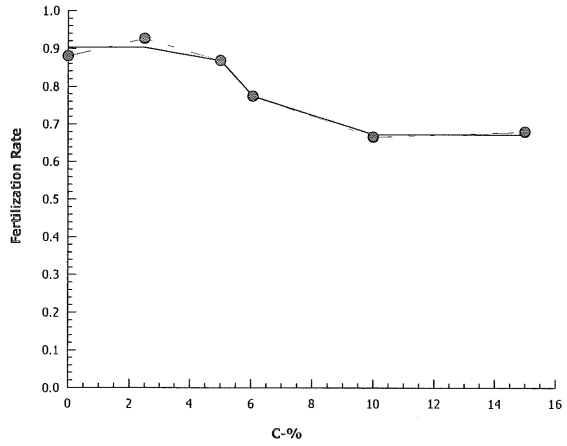
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	9.834	7.75	N/A	10.17	NA	12.9
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	0.88	0.83	0.93	0.01924	0.04301	4.89%	0.0%	440	500
2.5		5	0.926	0.88	0.97	0.01913	0.04278	4.62%	-5.23%	463	500
5		5	0.868	0.81	0.9	0.01594	0.03564	4.11%	1.36%	434	500
6.06		5	0.774	0.63	0.86	0.03982	0.08905	11.51%	12.05%	387	500
10		5	0.666	0.6	0.72	0.02227	0.0498	7.48%	24.32%	333	500
15		5	0.68	0.58	0.76	0.03033	0.06782	9.97%	22.73%	340	500

Graphics



Analyst: AC QA: 7/11/17

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

Analysis ID: 12-0184-5791 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 15:28 **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	5.65%	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*	8.631	1.943	0.088	6	<0.0001	CDF	Non-Significant Effect
		5*	8.831	1.895	0.061	7	<0.0001	CDF	Non-Significant Effect
		6.06*	3.18	2.015	0.104	5	0.0123	CDF	Non-Significant Effect
		10	1.195	1.895	0.062	7	0.1355	CDF	Significant Effect
		15	1.384	1.895	0.075	7	0.1044	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.5102819	0.1020564	5	18 21	<0.0001	Significant Effect
Error	0.1345085	0.00560452	24			
Total	0.6447904		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.801	15.09	0.7307	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9675	0.9031	0.4737	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	0.88	0.8266	0.9334	0.86	0.83	0.93	0.01924	4.89%	0.0%
2.5		5	0.926	0.8729	0.9791	0.92	0.88	0.97	0.01913	4.62%	-5.23%
5		5	0.868	0.8238	0.9122	0.88	0.81	0.9	0.01594	4.11%	1.36%
6.06		5	0.774	0.6634	0.8846	0.79	0.63	0.86	0.03982	11.51%	12.05%
10		5	0.666	0.6042	0.7278	0.68	0.6	0.72	0.02227	7.48%	24.32%
15		5	0.68	0.5958	0.7642	0.68	0.58	0.76	0.03033	9.97%	22.73%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.221	1.137	1.306	1.187	1.146	1.303	0.03052	5.59%	0.0%
2.5		5	1.305	1.198	1.413	1.284	1.217	1.397	0.03887	6.66%	-6.87%
5		5	1.201	1.138	1.264	1.217	1.12	1.249	0.02276	4.24%	1.66%
6.06		5	1.081	0.9518	1.21	1.095	0.9169	1.187	0.04643	9.61%	11.52%
10		5	0.9554	0.8899	1.021	0.9695	0.8861	1.013	0.02356	5.52%	21.79%
15		5	0.9711	0.8809	1.061	0.9695	0.8657	1.059	0.03247	7.48%	20.5%

CETIS Test Data Worksheet

Report Date: 203 29 Jun-17 18:51 (p 1 of 1)
Test Code: 1706-S2241 (B) 12-4385-2719/4A23AFAF

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

Start Date:	30 Jun-17	**Species:**	Strongylocentrotus purpuratus	**Sample Code:**	17- 0738
End Date:	30 Jun-17	**Protocol:**	EPA/600/R-95/136 (1995)	**Sample Source:**	IDE Americas, Inc.
Sample Date:	30 Jun-17	**Material:**	Facility Effluent	**Sample Station:**	M-001 Unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	94	(A) AHS 7/2/17
			32	100	89	
			33	100	81	
			34	100	(A) ~~97~~ 68	
			35	100	97	
			36	100	86	
			37	100	76	
			38	100	83	
			39	100	96	
			40	100	(A) ~~68~~ 94	
			41	100	68	
			42	100	72	
			43	100	86	
			44	100	88	
			45	100	76	
			46	100	98	
			47	100	92	
			48	100	66	
			49	100	79	
			50	100	86	
			51	100	92	
			52	100	63	
			53	100	90	
			54	100	97	
			55	100	86	
			56	100	83	
			57	100	89	
			58	100	88	
			59	100	70	
			60	100	72	
			61	100	58	
			62	100	93	
			63	100	93	
			64	100	60	
			65	100	63	

(A) GH 7/2/17

(B) QIB AC 7/7/17

CETIS Test Data Worksheet

Test Code: 1706-S203 12-4385-2719/4A23AFAF

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

Start Date: 30 Jun-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 17-0738
End Date: 30 Jun-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 30 Jun-17 **Material:** Facility Effluent **Sample Station:** M-001 Unadjusted

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	HS	1	31	100	99	AD 7/1/17
0	HS	2	63			
0	HS	3	46			
0	HS	4	40			
0	HS	5	39			
0	LC	1	50	100	91	
0	LC	2	38			
0	LC	3	51			
0	LC	4	55			
0	LC	5	62			
2.5		1	44			
2.5		2	57			
2.5		3	54			
2.5		4	35			
2.5		5	47			
5		1	53			
5		2	58			
5		3	36			
5		4	33			
5		5	32			
6.06		1	45	100	68	
6.06		2	65			
6.06		3	49			
6.06		4	56			
6.06		5	43			
10		1	42			
10		2	34			
10		3	59			
10		4	64			
10		5	52			
15		1	37			
15		2	61			
15		3	41			
15		4	60			
15		5	48	100	63	

QC: CG

Analyst: EG QA: AC 7/7/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: M-001 (unadjusted) | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17- 0738 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: AO | **Test No:** 1706-S203

Analyst: CH

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.0	7.85	34.0	16.0
High Salinity Control	8.5	7.97	37.6	14.8
2.5	7.9	7.86	34.5	15.9
5.0	7.9	7.86	35.3	15.9
6.06	7.9	7.86	35.5	15.9
10	7.9	7.86	36.5	15.8
15	7.9	7.85	37.8	15.7

Comments: Dilutions made w/ M-INF

QC Check: AC 7/7/17 | **Final Review:** KFP 7/11/17

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

Client: IDE
Sample ID: M-001 unadjusted
Test No.: 1706-S203

Tech initials: AO
Injection Time: 1650

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

(A) 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):	1710	50:1	84,83	16,17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1745	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AO @ 8 6/30/17

QC Check: AC 7/5/17

Final Review: KTP 7/11/17

M-001 40 ppt Adjusted

CETIS Summary Report

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

Batch ID:	13-0866-8077	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	~~Natural Seawater~~ MINF
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	04-9396-0662	**Code:**	17-0738	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (6.5 °C)	**Station:**	M-001 40 ppt		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
14-4818-0451	Fertilization Rate	15	>15	NA	9.08%	< 6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
13-2565-5640	Fertilization Rate	EC25	>15	N/A	N/A	<6.667	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
13-2565-5640	Fertilization Rate	Control Resp	0.86	0.7 - NL	Yes	Passes Acceptability Criteria
14-4818-0451	Fertilization Rate	Control Resp	0.86	0.7 - NL	Yes	Passes Acceptability Criteria
14-4818-0451	Fertilization Rate	PMSD	0.09078	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	0.8111	0.9089	0.83	0.92	0.01761	0.03937	4.58%	0.0%
2.5		5	0.932	0.8769	0.9871	0.86	0.97	0.01985	0.04438	4.76%	-8.37%
5		5	0.932	0.8895	0.9745	0.88	0.97	0.0153	0.03421	3.67%	-8.37%
6.06		5	0.896	0.849	0.943	0.84	0.94	0.01691	0.03782	4.22%	-4.19%
10		5	0.854	0.7947	0.9133	0.8	0.93	0.02135	0.04775	5.59%	0.7%
15		5	0.882	0.8191	0.9449	0.8	0.93	0.02267	0.0507	5.75%	-2.56%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.83	0.88	0.92	0.83	0.84
2.5		0.92	0.86	0.96	0.97	0.95
5		0.88	0.92	0.95	0.94	0.97
6.06		0.94	0.88	0.91	0.84	0.91
10		0.86	0.84	0.84	0.8	0.93
15		0.91	0.9	0.93	0.87	0.8

@ Q18 AC 7/10/17

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

Analysis ID:	14-4818-0451	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 15:43	**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	9.08%	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	-2.825	2.362	0.106	8	1.0000	CDF	Non-Significant Effect
		5	-2.752	2.362	0.106	8	0.9999	CDF	Non-Significant Effect
		6.06	-1.253	2.362	0.106	8	0.9920	CDF	Non-Significant Effect
		10	0.161	2.362	0.106	8	0.7814	CDF	Non-Significant Effect
		15	-0.785	2.362	0.106	8	0.9702	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08480894	0.01696179	5	3.402	0.0183	Significant Effect
Error	0.119664	0.004986	24			
Total	0.204473		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.5809	15.09	0.9889	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.974	0.9031	0.6538	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	0.8111	0.9089	0.84	0.83	0.92	0.01761	4.58%	0.0%
2.5		5	0.932	0.8769	0.9871	0.95	0.86	0.97	0.01985	4.76%	-8.37%
5		5	0.932	0.8895	0.9745	0.94	0.88	0.97	0.0153	3.67%	-8.37%
6.06		5	0.896	0.849	0.943	0.91	0.84	0.94	0.01691	4.22%	-4.19%
10		5	0.854	0.7947	0.9133	0.84	0.8	0.93	0.02135	5.59%	0.7%
15		5	0.882	0.8191	0.9449	0.9	0.8	0.93	0.02267	5.75%	-2.56%

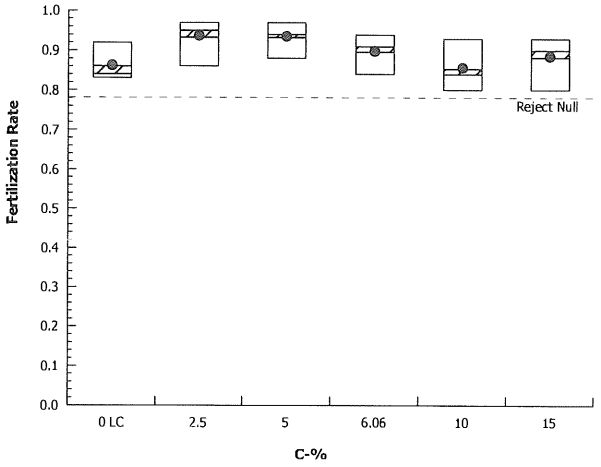
Angular (Corrected) Transformed Summary

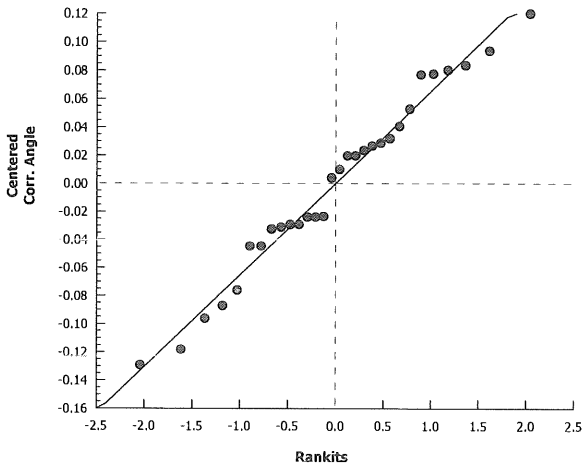
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.19	1.116	1.265	1.159	1.146	1.284	0.02686	5.05%	0.0%
2.5		5	1.317	1.213	1.42	1.345	1.187	1.397	0.03729	6.33%	-10.6%
5		5	1.313	1.23	1.397	1.323	1.217	1.397	0.03017	5.14%	-10.32%
6.06		5	1.246	1.17	1.323	1.266	1.159	1.323	0.02752	4.94%	-4.7%
10		5	1.183	1.093	1.274	1.159	1.107	1.303	0.03264	6.17%	0.6%
15		5	1.225	1.132	1.319	1.249	1.107	1.303	0.03375	6.16%	-2.95%

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

Analysis ID:	14-4818-0451	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 15:43	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





Analyst: AC QA: KFP 7/11/17

CETIS Analytical Report

Test Code: 1706-S204 | 08-6882-6222

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

Analysis ID:	13-2565-5640	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 15:43	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	350119	1000	Yes	Two-Point Interpolation

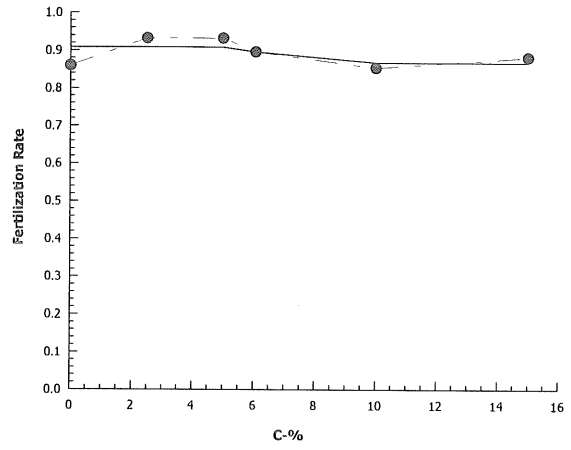
Point Estimates

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	0.86	0.83	0.92	0.01761	0.03937	4.58%	0.0%	430	500
2.5		5	0.932	0.86	0.97	0.01985	0.04438	4.76%	-8.37%	466	500
5		5	0.932	0.88	0.97	0.0153	0.03421	3.67%	-8.37%	466	500
6.06		5	0.896	0.84	0.94	0.01691	0.03782	4.22%	-4.19%	448	500
10		5	0.854	0.8	0.93	0.02135	0.04775	5.59%	0.7%	427	500
15		5	0.882	0.8	0.93	0.02267	0.0507	5.75%	-2.56%	441	500

Graphics



Analyst: AC QA: VFF 7/11/17

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

Analysis ID:	16-7578-2505	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 15:44	**Analysis:**	Parametric Bioequivalence-Two Sample	**Official Results:**	Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	6.34%	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*	9.998	1.943	0.082	6	<0.0001	CDF	Non-Significant Effect
		5*	11.59	1.943	0.070	6	<0.0001	CDF	Non-Significant Effect
		6.06*	10.37	1.895	0.065	7	<0.0001	CDF	Non-Significant Effect
		10*	7.572	1.943	0.075	6	0.0001	CDF	Non-Significant Effect
		15*	8.464	1.943	0.076	6	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08480894	0.01696179	5	3.402	0.0183	Significant Effect
Error	0.119664	0.004986	24			
Total	0.204473		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.5809	15.09	0.9889	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.974	0.9031	0.6538	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	0.8111	0.9089	0.84	0.83	0.92	0.01761	4.58%	0.0%
2.5		5	0.932	0.8769	0.9871	0.95	0.86	0.97	0.01985	4.76%	-8.37%
5		5	0.932	0.8895	0.9745	0.94	0.88	0.97	0.0153	3.67%	-8.37%
6.06		5	0.896	0.849	0.943	0.91	0.84	0.94	0.01691	4.22%	-4.19%
10		5	0.854	0.7947	0.9133	0.84	0.8	0.93	0.02135	5.59%	0.7%
15		5	0.882	0.8191	0.9449	0.9	0.8	0.93	0.02267	5.75%	-2.56%

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	1.116	1.265	1.159	1.146	1.284	0.02686	5.05%	0.0%
2.5		5	1.317	1.213	1.42	1.345	1.187	1.397	0.03729	6.33%	-10.6%
5		5	1.313	1.23	1.397	1.323	1.217	1.397	0.03017	5.14%	-10.32%
6.06		5	1.246	1.17	1.323	1.266	1.159	1.323	0.02752	4.94%	-4.7%
10		5	1.183	1.093	1.274	1.159	1.107	1.303	0.03264	6.17%	0.6%
15		5	1.225	1.132	1.319	1.249	1.107	1.303	0.03375	6.16%	-2.95%

CETIS Test Data Worksheet

Test Code: 170606204 03-8551-4552/16FA7C38

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 0738
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			66	100	93	AHS 7/3/17
			67	100	95	
			68	100	90	
			69	100	91	
			70	100	83	
			71	100	94	
			72	100	97	
			73	100	92	
			74	100	96	
			75	100	86	
			76	100	86	
			77	100	95	
			78	100	84	
			79	100	80	
			80	100	92	
			81	100	84	
			82	100	97	
			83	100	94	
			84	100	84	
			85	100	88	
			86	100	83	
			87	100	93	
			88	100	87	
			89	100	91	
			90	100	84	
			91	100	91	
			92	100	92	
			93	100	88	
			94	100	88	
			95	100	80	

Analyst: HCS QA AC 7/7/17

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:52 (p 1 of 1)
Test Code: MCG-S204 03-8551-4552/16FA7C38

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17-0738
Sample Source: IDE Americas, Inc.
Sample Station: M-001 (40 ppt adj)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	70	100	88	AD 7/1/17
0	LC	2	93			
0	LC	3	92			
0	LC	4	86			
0	LC	5	84			
2.5		1	73			
2.5		2	75			
2.5		3	74			
2.5		4	72			
2.5		5	67			
5		1	85			
5		2	80			
5		3	77			
5		4	83			
5		5	82			
6.06		1	71	100	94	
6.06		2	94			
6.06		3	89			
6.06		4	81			
6.06		5	91			
10		1	76			
10		2	90			
10		3	78			
10		4	95			
10		5	87			
15		1	69			
15		2	68			
15		3	66			
15		4	88			
15		5	79			

QC: CG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: M-001 (40 ppt adjusted) | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17- 0738 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: AD | **Test No:** 1706-S204

Analyst: CH

Concentration %	Initial Readings DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)
Lab Control	8.5	7.95	33.9	15.8
2.5	8.1	7.85	34.2	15.9
5.0	7.9	7.85	34.3	15.9
6.06	7.9	7.86	34.3	16.0
10	8.4	7.98	34.2	15.7
15	8.4	7.97	34.9	15.4

Comments: Dilutions made with M-INF

QC Check: AC 7/7/17 | **Final Review:** KTP 7/11/17

Marine Chronic Bioassay

Brine Dilution Worksheet

Project: IDE

Analyst: AD

Sample ID: M-001 (40 ppt adjusted)

Test Date: 6/30/2017

Test No: 1706-S204

Test Type: Urchin Fertilization

Salinity of Effluent 62.6

Salinity of Seawater 33.5

Date of Brine used: NA

Target Salinity 40.0

Alk. of 40 ppt Adj. Sample: (A) 2+9126 mg/L as CaCO3

	Effluent	Brine Control
Salinity Adjustment Factor: (TS - SE)/(SB - TS) =	**3.48**	**-6.15**

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

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)
100	100	3.48	347.7	448

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

QC Check: AG 7/6/17

Final Review: KFF 7/11/17

(A) AD QA 7/10/17

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

Client: IDE
Sample ID: M-001 40 ppt Adjusted
Test No.: 1706-S204

Tech initials: AP
Injection Time: 1650

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83
Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

(A) 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):	1710	50:1	84, 83	16, 17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1750	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AP QA 6/30/17

QC Check: AC 7/5/17

Final Review: KFP 7/11/17

ERI Brine

CETIS Summary Report

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

Batch ID:	12-4316-0758	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	~~Natural Seawater~~ M-INF
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	18-7927-7034	**Code:**	17-0739	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (4.5 °C)	**Station:**	ERI		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
09-9212-2387	Fertilization Rate	10	15	12.25	6.11%	10	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
12-0743-1815	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
09-9212-2387	Fertilization Rate	Control Resp	0.908	0.7 - NL	Yes	Passes Acceptability Criteria
12-0743-1815	Fertilization Rate	Control Resp	0.908	0.7 - NL	Yes	Passes Acceptability Criteria
09-9212-2387	Fertilization Rate	PMSD	0.06111	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	0.908	0.8944	0.9216	0.89	0.92	0.004898	0.01095	1.21%	0.0%
2.5		5	0.918	0.8941	0.9419	0.89	0.94	0.008602	0.01923	2.1%	-1.1%
5		5	0.908	0.8565	0.9595	0.87	0.97	0.01855	0.04147	4.57%	0.0%
6.06		5	0.906	0.859	0.953	0.85	0.95	0.01691	0.03782	4.17%	0.22%
10		5	0.898	0.8458	0.9502	0.85	0.96	0.01881	0.04207	4.69%	1.1%
15		5	0.814	0.7696	0.8584	0.77	0.85	0.016	0.03578	4.4%	10.35%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.89	0.91	0.91	0.91	0.92
2.5		0.89	0.93	0.94	0.91	0.92
5		0.97	0.89	0.88	0.87	0.93
6.06		0.85	0.95	0.9	0.9	0.93
10		0.85	0.91	0.87	0.96	0.9
15		0.77	0.85	0.85	0.79	0.81

@ QR AC 7/10/17

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

Analysis ID: 09-9212-2387 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 06 Jul-17 14:47 **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	6.11%	10	15	12.25	10

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.5148	2.362	0.086	8	0.9421	CDF	Non-Significant Effect
		5	-0.1989	2.362	0.086	8	0.8854	CDF	Non-Significant Effect
		6.06	-0.02418	2.362	0.086	8	0.8404	CDF	Non-Significant Effect
		10	0.3032	2.362	0.086	8	0.7287	CDF	Non-Significant Effect
		15*	3.738	2.362	0.086	8	0.0022	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08398654	0.01679731	5	5.021	0.0027	Significant Effect
Error	0.08028532	0.003345222	24			
Total	0.1642719		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.431	15.09	0.1340	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9649	0.9031	0.4111	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	0.908	0.8944	0.9216	0.91	0.89	0.92	0.004898	1.21%	0.0%
2.5		5	0.918	0.8941	0.9419	0.92	0.89	0.94	0.008602	2.1%	-1.1%
5		5	0.908	0.8565	0.9595	0.89	0.87	0.97	0.01855	4.57%	0.0%
6.06		5	0.906	0.859	0.953	0.9	0.85	0.95	0.01691	4.17%	0.22%
10		5	0.898	0.8458	0.9502	0.9	0.85	0.96	0.01881	4.69%	1.1%
15		5	0.814	0.7696	0.8584	0.81	0.77	0.85	0.016	4.4%	10.35%

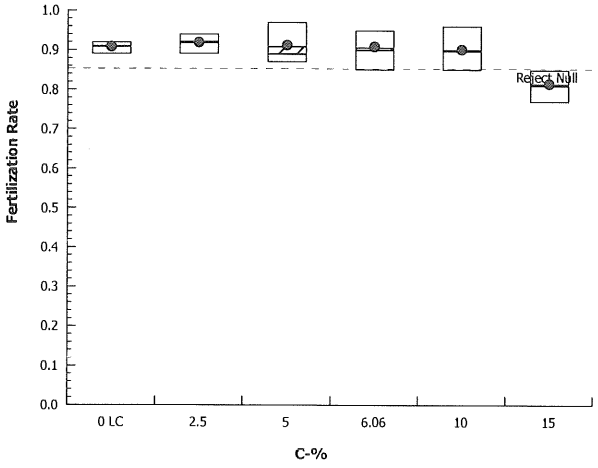
Angular (Corrected) Transformed Summary

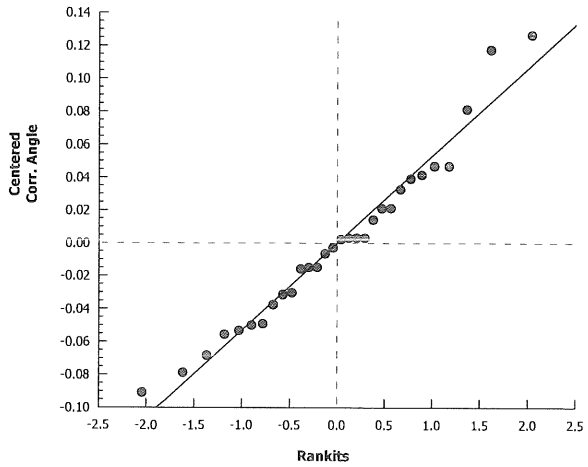
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.263	1.24	1.286	1.266	1.233	1.284	0.00833	1.48%	0.0%
2.5		5	1.282	1.239	1.325	1.284	1.233	1.323	0.01555	2.71%	-1.49%
5		5	1.27	1.17	1.37	1.233	1.202	1.397	0.03604	6.34%	-0.58%
6.06		5	1.264	1.183	1.344	1.249	1.173	1.345	0.02902	5.13%	-0.07%
10		5	1.252	1.158	1.346	1.249	1.173	1.369	0.03371	6.02%	0.88%
15		5	1.126	1.069	1.184	1.12	1.071	1.173	0.02064	4.1%	10.83%

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

Analysis ID:	09-9212-2387	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jul-17 14:47	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	12-0743-1815	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	06 Jul-17 14:47	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	741544	1000	Yes	Two-Point Interpolation

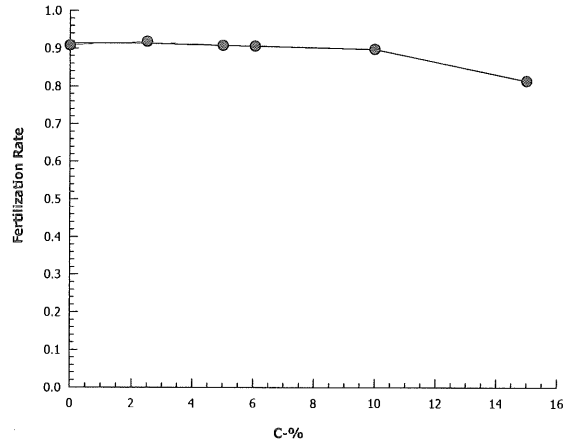
Point Estimates

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	0.908	0.89	0.92	0.004898	0.01095	1.21%	0.0%	454	500
2.5		5	0.918	0.89	0.94	0.008602	0.01923	2.1%	-1.1%	459	500
5		5	0.908	0.87	0.97	0.01855	0.04147	4.57%	0.0%	454	500
6.06		5	0.906	0.85	0.95	0.01691	0.03782	4.17%	0.22%	453	500
10		5	0.898	0.85	0.96	0.01881	0.04207	4.69%	1.1%	449	500
15		5	0.814	0.77	0.85	0.016	0.03578	4.4%	10.35%	407	500

Graphics



CETIS Analytical Report

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

Analysis ID: 02-7313-6603 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 06 Jul-17 14:48 **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	3.08%	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*	19.97	2.015	0.034	5	<0.0001	CDF	Non-Significant Effect
		5*	8.832	2.132	0.078	4	0.0005	CDF	Non-Significant Effect
		6.06*	10.67	2.132	0.063	4	0.0002	CDF	Non-Significant Effect
		10*	8.886	2.132	0.073	4	0.0004	CDF	Non-Significant Effect
		15*	8.302	2.132	0.046	4	0.0006	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.08398654	0.01679731	5	5.021	0.0027	Significant Effect
Error	0.08028532	0.003345222	24			
Total	0.1642719		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	8.431	15.09	0.1340	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9649	0.9031	0.4111	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	0.908	0.8944	0.9216	0.91	0.89	0.92	0.004898	1.21%	0.0%
2.5		5	0.918	0.8941	0.9419	0.92	0.89	0.94	0.008602	2.1%	-1.1%
5		5	0.908	0.8565	0.9595	0.89	0.87	0.97	0.01855	4.57%	0.0%
6.06		5	0.906	0.859	0.953	0.9	0.85	0.95	0.01691	4.17%	0.22%
10		5	0.898	0.8458	0.9502	0.9	0.85	0.96	0.01881	4.69%	1.1%
15		5	0.814	0.7696	0.8584	0.81	0.77	0.85	0.016	4.4%	10.35%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.263	1.24	1.286	1.266	1.233	1.284	0.00833	1.48%	0.0%
2.5		5	1.282	1.239	1.325	1.284	1.233	1.323	0.01555	2.71%	-1.49%
5		5	1.27	1.17	1.37	1.233	1.202	1.397	0.03604	6.34%	-0.58%
6.06		5	1.264	1.183	1.344	1.249	1.173	1.345	0.02902	5.13%	-0.07%
10		5	1.252	1.158	1.346	1.249	1.173	1.369	0.03371	6.02%	0.88%
15		5	1.126	1.069	1.184	1.12	1.071	1.173	0.02064	4.1%	10.83%

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:55 (p 1 of 1)
Test Code: 1706-5207 20-9818-9801/7D0FD5E9

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 0739
Sample Source: IDE Americas, Inc.
Sample Station: ERI

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			126	100	85	7/3/17
			127		90	
			128		89	
			129		81	
			130		89	
			131		93	
			132		94	
			133		79	
			134		77	
			135		91	
			136		91	
			137		90	
			138		87	
			139		91	
			140		93	
			141		91	
			142		87	
			143		96	
			144		88	
			145		85	
			146		97	
			147		92	
			148		91	
			149		89	
			150		92	
			151		95	
			152		85	
			153		93	
			154		85	
			155		90	

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:55 (p 1 of 1)
Test Code: 1706-S207 20-9818-9801/7D0FD5E9

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 8739
Sample Source: IDE Americas, Inc.
Sample Station: ERI

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	128	100	89	AD 7/1/17
0	LC	2	135			
0	LC	3	136			
0	LC	4	139			
0	LC	5	147			
2.5		1	130			
2.5		2	140			
2.5		3	132			
2.5		4	148			
2.5		5	150			
5		1	146			
5		2	149			
5		3	144			
5		4	142			
5		5	131			
6.06		1	145	100	94	
6.06		2	151			
6.06		3	155			
6.06		4	127			
6.06		5	153			
10		1	154			
10		2	141			
10		3	138			
10		4	143			
10		5	137			
15		1	134			
15		2	152			
15		3	126			
15		4	133			
15		5	129			

QC: CG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: ERI | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17- 0739 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: KD | **Test No:** 1706-S207

Analyst: CH

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.0	8.02	33.8	15.0
2.5	8.0	8.02	34.8	14.8
5.0	8.1	8.02	35.6	14.7
6.06	8.0	8.01	36.0	14.8
10	8.1	8.00	37.3	14.7
15	8.1	7.99	38.9	14.6

Comments: Dilutions made with m-INF

QC Check: AC 7/7/17 | **Final Review:** KP 7/11/17

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

Client: IDE
Sample ID: ERI Brine
Test No.: 1706-S207

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Tech initials: AP
Injection Time: 1650

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

(A) 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):	1710	50:1	84,83	16,17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1750	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AP QA 6/30/17

QC Check: AC 7/5/17

Final Review: VAP 7/11/17

Train 4

CETIS Summary Report

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

Batch ID:	06-5489-2972	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	~~Natural Seawater~~ MI - 1 NF
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	18-2923-2635	**Code:**	17-0741	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (5.5°C)	**Station:**	Train 4		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-6168-2472	Fertilization Rate	10	15	12.25	6.24%	10	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
05-4599-9515	Fertilization Rate	EC25	13.35	12.16	N/A	7.489	Linear Interpolation (ICPIN)
		EC50	>15	N/A	N/A	<6.667	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-6168-2472	Fertilization Rate	Control Resp	0.916	0.7 - NL	Yes	Passes Acceptability Criteria
05-4599-9515	Fertilization Rate	Control Resp	0.916	0.7 - NL	Yes	Passes Acceptability Criteria
03-6168-2472	Fertilization Rate	PMSD	0.06236	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	0.912	0.8405	0.9835	0.81	0.95	0.02577	0.05762	6.32%	0.0%
0	Lab Control	5	0.916	0.8825	0.9495	0.89	0.95	0.01208	0.02702	2.95%	-0.44%
2.5		5	0.862	0.8136	0.9104	0.8	0.9	0.01744	0.03899	4.52%	5.48%
5		5	0.862	0.8016	0.9224	0.79	0.91	0.02177	0.04868	5.65%	5.48%
6.06		5	0.856	0.8192	0.8928	0.81	0.89	0.01327	0.02966	3.47%	6.14%
10		5	0.864	0.8398	0.8882	0.84	0.89	0.008718	0.01949	2.26%	5.26%
15		5	0.602	0.4805	0.7235	0.53	0.77	0.04375	0.09783	16.25%	33.99%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	High Salinity Co	0.94	0.93	0.93	0.81	0.95
0	Lab Control	0.9	0.89	0.95	0.9	0.94
2.5		0.8	0.88	0.9	0.88	0.85
5		0.9	0.87	0.84	0.91	0.79
6.06		0.85	0.87	0.86	0.89	0.81
10		0.89	0.85	0.87	0.84	0.87
15		0.54	0.57	0.53	0.6	0.77

(a) Q18 AC 7/10/17

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

Analysis ID:	03-6168-2472	**Endpoint:** Fertilization Rate	**CETIS Version:** CETISv1.8.7
Analyzed:	07 Jul-17 16:12	**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	6.24%	10	15	12.25	10

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	2.186	2.362	0.094	8	0.0704	CDF	Non-Significant Effect
		5	2.148	2.362	0.094	8	0.0758	CDF	Non-Significant Effect
		6.06*	2.43	2.362	0.094	8	0.0436	CDF	Significant Effect
		10	2.158	2.362	0.094	8	0.0742	CDF	Non-Significant Effect
		15*	9.759	2.362	0.094	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.4536385	0.09072769	5	22.81	<0.0001	Significant Effect
Error	0.09546186	0.003977577	24			
Total	0.5491003		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.005	15.09	0.2202	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.951	0.9031	0.1795	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	0.916	0.8825	0.9495	0.9	0.89	0.95	0.01208	2.95%	0.0%
2.5		5	0.862	0.8136	0.9104	0.88	0.8	0.9	0.01744	4.52%	5.9%
5		5	0.862	0.8016	0.9224	0.87	0.79	0.91	0.02177	5.65%	5.9%
6.06		5	0.856	0.8192	0.8928	0.86	0.81	0.89	0.01327	3.47%	6.55%
10		5	0.864	0.8398	0.8882	0.87	0.84	0.89	0.008718	2.26%	5.68%
15		5	0.602	0.4805	0.7235	0.57	0.53	0.77	0.04375	16.25%	34.28%

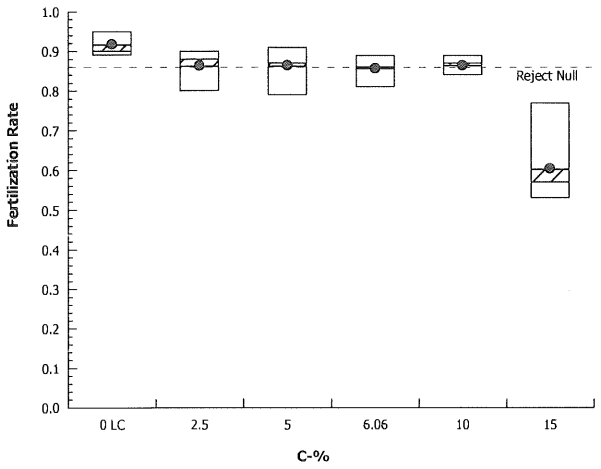
Angular (Corrected) Transformed Summary

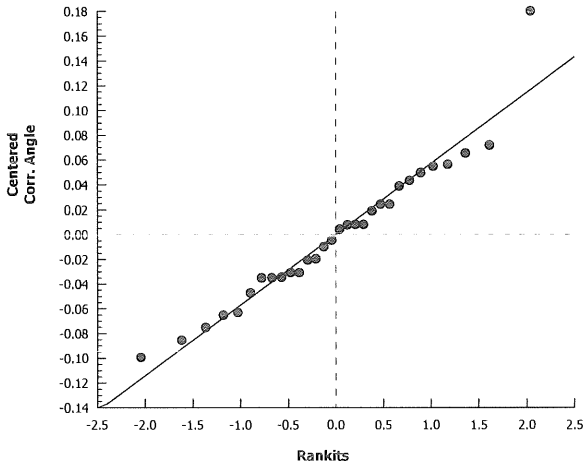
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.28	1.217	1.343	1.249	1.233	1.345	0.02268	3.96%	0.0%
2.5		5	1.193	1.124	1.261	1.217	1.107	1.249	0.02456	4.61%	6.81%
5		5	1.194	1.108	1.281	1.202	1.095	1.266	0.0311	5.82%	6.69%
6.06		5	1.183	1.131	1.235	1.187	1.12	1.233	0.01864	3.52%	7.57%
10		5	1.194	1.158	1.229	1.202	1.159	1.233	0.01278	2.39%	6.73%
15		5	0.8906	0.7611	1.02	0.8556	0.8154	1.071	0.04666	11.72%	30.41%

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

Analysis ID:	03-6168-2472	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 16:12	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1706-S209 | 19-6001-9623

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

Analysis ID:	05-4599-9515	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 16:13	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1804808	1000	Yes	Two-Point Interpolation

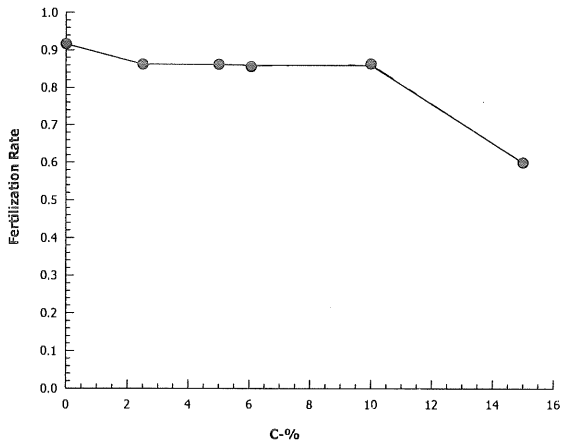
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	13.35	12.16	N/A	7.489	NA	8.225
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	0.916	0.89	0.95	0.01208	0.02702	2.95%	0.0%	458	500
2.5		5	0.862	0.8	0.9	0.01744	0.03899	4.52%	5.9%	431	500
5		5	0.862	0.79	0.91	0.02177	0.04868	5.65%	5.9%	431	500
6.06		5	0.856	0.81	0.89	0.01327	0.02966	3.47%	6.55%	428	500
10		5	0.864	0.84	0.89	0.008718	0.01949	2.26%	5.68%	432	500
15		5	0.602	0.53	0.77	0.04375	0.09783	16.25%	34.28%	300	500

Graphics



Analyst: AC QA: KRP 7/11/17

CETIS Analytical Report

Test Code: 1706-S209 | 19-6001-9623

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

Analysis ID: 04-9502-9462 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 16:58 **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	6.69%	10	15	12.25	10

TST-Welch's t Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5*	7.791	1.895	0.057	7	<0.0001	CDF	Non-Significant Effect
		5*	6.61	1.943	0.069	6	0.0003	CDF	Non-Significant Effect
		6.06*	8.84	1.895	0.048	7	<0.0001	CDF	Non-Significant Effect
		10*	10.99	1.895	0.040	7	<0.0001	CDF	Non-Significant Effect
		15	-1.395	2.015	0.100	5	0.8891	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.4536385	0.09072769	5	22.81	<0.0001	Significant Effect
Error	0.09546186	0.003977577	24			
Total	0.5491003		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	7.005	15.09	0.2202	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.951	0.9031	0.1795	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	0.916	0.8825	0.9495	0.9	0.89	0.95	0.01208	2.95%	0.0%
2.5		5	0.862	0.8136	0.9104	0.88	0.8	0.9	0.01744	4.52%	5.9%
5		5	0.862	0.8016	0.9224	0.87	0.79	0.91	0.02177	5.65%	5.9%
6.06		5	0.856	0.8192	0.8928	0.86	0.81	0.89	0.01327	3.47%	6.55%
10		5	0.864	0.8398	0.8882	0.87	0.84	0.89	0.008718	2.26%	5.68%
15		5	0.602	0.4805	0.7235	0.57	0.53	0.77	0.04375	16.25%	34.28%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.28	1.217	1.343	1.249	1.233	1.345	0.02268	3.96%	0.0%
2.5		5	1.193	1.124	1.261	1.217	1.107	1.249	0.02456	4.61%	6.81%
5		5	1.194	1.108	1.281	1.202	1.095	1.266	0.0311	5.82%	6.69%
6.06		5	1.183	1.131	1.235	1.187	1.12	1.233	0.01864	3.52%	7.57%
10		5	1.194	1.158	1.229	1.202	1.159	1.233	0.01278	2.39%	6.73%
15		5	0.8906	0.7611	1.02	0.8556	0.8154	1.071	0.04666	11.72%	30.41%

Analyst: AC QA: [illegible] 7/11/17

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:57 (p 1 of 1)
Test Code: 1706-S209 19-6001-9623/74D386A7

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

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

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

Sample Code: 17- 0741
Sample Source: IDE Americas, Inc.
Sample Station: Train 2 4 (B)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			216	100	87	CG 7/3/17
			217	100	90	
			218	100	94	
			219	100	85	
			220	100	89.	
			221	100	88	
			222	100	85	
			223	100	86	
			224	100	(A) ~~50~~ 57	
			225	100	87	
			226	100	80	
			227	100	91	
			228	100	77	
			229	100	81	
			230	100	90.	
			231	100	90	
			232	100	54	
			233	100	87	
			234	100	53	CG 7/6/17
			235	100	87	
			236	100	95	
			237	100	60	
			238	100	79	
			239	100	84	
			240	100	89	
			241	100	89	
			242	100	88	
			243	100	90	
			244	100	84	
			245	100	85	

(A) CG Q10 7/3/17

(B) Q18 AC 7/7/17

HSC
A 100 94
B 100 93
C 100 93
D 100 81
E 100 95

Analyst: CG QA: AC 7/7/17

CETIS Test Data Worksheet

Test Code: 1706-S209 19-6001-9623/74D386A7

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

Start Date: 30 Jun-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 17-
End Date: 30 Jun-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** IDE Americas, Inc.
Sample Date: 30 Jun-17 **Material:** Facility Effluent **Sample Station:** Train 2 4

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	243	100	91	AMO 7/1/17
0	LC	2	220			
0	LC	3	236			
0	LC	4	231			
0	LC	5	218			
2.5		1	226			
2.5		2	242			
2.5		3	217			
2.5		4	221			
2.5		5	222			
5		1	230			
5		2	235			
5		3	244			
5		4	227			
5		5	238			
6.06		1	245	100	84	
6.06		2	225			
6.06		3	223			
6.06		4	240			
6.06		5	229			
10		1	241			
10		2	219			
10		3	216			
10		4	239			
10		5	233			
15		1	232			
15		2	224			
15		3	234			
15		4	237			
15		5	228			

QC: CG

AMO QA 6/30/17

Analyst: EG QA: AC 7/7/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: Train 9 4 CH QB 6/30/17 | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17- 0741 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: AD | **Test No:** 1706-S209

Analyst: CH

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.7	7.87	34.0	15.3
2.5	7.9	7.87	34.8	15.2
5.0	8.0	7.87	35.7	15.2
6.06	7.9	7.87	36.1	15.2
10	~~8.0~~ 8.2 (A)	~~7.87~~ 7.88 (A)	~~40.6~~ 39.2 (A)	~~15.2~~ 15.7 (A)
15	8.0	7.88	39.2	15.2
HSC	8.7	7.98	39.0	14.4

HSC = high salinity control

Comments: (A) CH QAS 6/30/17 Dilutions made with M-INF

QC Check: AC 7/7/17 | **Final Review:** KFP 7/11/17

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

Client: IDE
Sample ID: Train 4
Test No.: 1706-5209

Tech initials: AP
Injection Time: 1650

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

egg stock ____ ml
seawater ____ ml

(A)

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):	1710	50:1	84,83	16,17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1745	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AP QA 6/30/17

QC Check: AC 7/5/17

Final Review: KFP 7/11/17

Brine Pit

CETIS Summary Report

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

Batch ID:	16-4465-8123	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	~~Natural Seawater~~ M-INF
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	09-4841-4586	**Code:**	17-0740	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (6.5 °C)	**Station:**	Brine Pit		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
04-9634-7083	Fertilization Rate	2.5	5	3.536	10.4%	40	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
03-0782-7528	Fertilization Rate	EC25	7.896	5.755	9.888	12.66	Linear Interpolation (ICPIN)
		EC50	13.22	11.39	14.98	7.564	

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-0782-7528	Fertilization Rate	Control Resp	0.886	0.7 - NL	Yes	Passes Acceptability Criteria
04-9634-7083	Fertilization Rate	Control Resp	0.886	0.7 - NL	Yes	Passes Acceptability Criteria
04-9634-7083	Fertilization Rate	PMSD	0.1043	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	0.886	0.8154	0.9566	0.81	0.96	0.02542	0.05683	6.42%	0.0%
2.5		5	0.884	0.7968	0.9712	0.81	0.98	0.0314	0.07021	7.94%	0.23%
5		5	0.732	0.6414	0.8226	0.65	0.83	0.03262	0.07294	9.96%	17.38%
6.06		5	0.734	0.6668	0.8012	0.66	0.81	0.02421	0.05413	7.38%	17.16%
10		5	0.586	0.4928	0.6792	0.48	0.67	0.03356	0.07503	12.8%	33.86%
15		5	0.364	0.2577	0.4703	0.22	0.44	0.03829	0.08562	23.52%	58.92%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.87	0.87	0.96	0.92	0.81
2.5		0.98	0.82	0.9	0.91	0.81
5		0.65	0.83	0.67	0.75	0.76
6.06		0.81	0.73	0.66	0.72	0.75
10		0.55	0.64	0.59	0.67	0.48
15		0.44	0.39	0.36	0.22	0.41

@ RIB AC 7/10/17

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

Analysis ID:	04-9634-7083	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Jul-17 9:44	**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	10.4%	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	-0.06711	2.362	0.136	8	0.8524	CDF	Non-Significant Effect
		5*	3.568	2.362	0.136	8	0.0034	CDF	Significant Effect
		6.06*	3.552	2.362	0.136	8	0.0035	CDF	Significant Effect
		10*	6.292	2.362	0.136	8	<0.0001	CDF	Significant Effect
		15*	10.23	2.362	0.136	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.280516	0.2561031	5	30.81	<0.0001	Significant Effect
Error	0.1995245	0.008313522	24			
Total	1.48004		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.025	15.09	0.8457	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9827	0.9031	0.8920	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	0.886	0.8154	0.9566	0.87	0.81	0.96	0.02542	6.42%	0.0%
2.5		5	0.884	0.7968	0.9712	0.9	0.81	0.98	0.0314	7.94%	0.23%
5		5	0.732	0.6414	0.8226	0.75	0.65	0.83	0.03262	9.96%	17.38%
6.06		5	0.734	0.6668	0.8012	0.73	0.66	0.81	0.02421	7.38%	17.16%
10		5	0.586	0.4928	0.6792	0.59	0.48	0.67	0.03356	12.8%	33.86%
15		5	0.364	0.2577	0.4703	0.39	0.22	0.44	0.03829	23.52%	58.92%

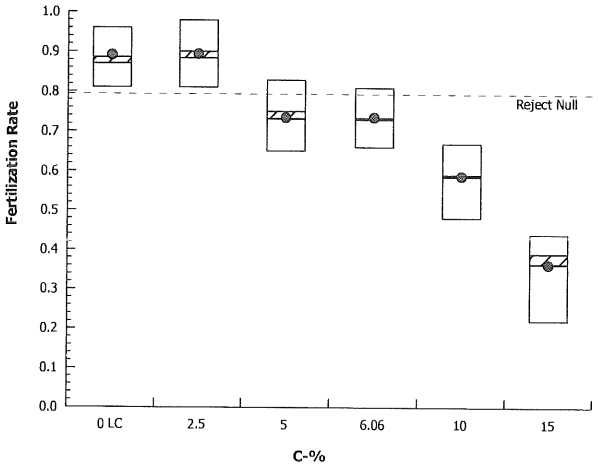
Angular (Corrected) Transformed Summary

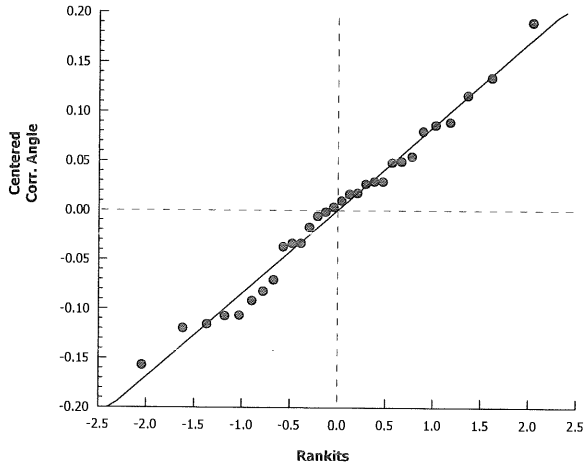
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.235	1.118	1.353	1.202	1.12	1.369	0.04239	7.67%	0.0%
2.5		5	1.239	1.084	1.394	1.249	1.12	1.429	0.05587	10.08%	-0.31%
5		5	1.03	0.9256	1.134	1.047	0.9377	1.146	0.03749	8.14%	16.65%
6.06		5	1.031	0.9537	1.107	1.024	0.9483	1.12	0.0277	6.01%	16.58%
10		5	0.8726	0.7778	0.9674	0.8759	0.7654	0.9589	0.03414	8.75%	29.37%
15		5	0.6453	0.5301	0.7604	0.6745	0.4882	0.7253	0.04147	14.37%	47.77%

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

Analysis ID:	04-9634-7083	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Jul-17 9:44	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	03-0782-7528	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	03 Jul-17 9:44	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1754982	1000	Yes	Two-Point Interpolation

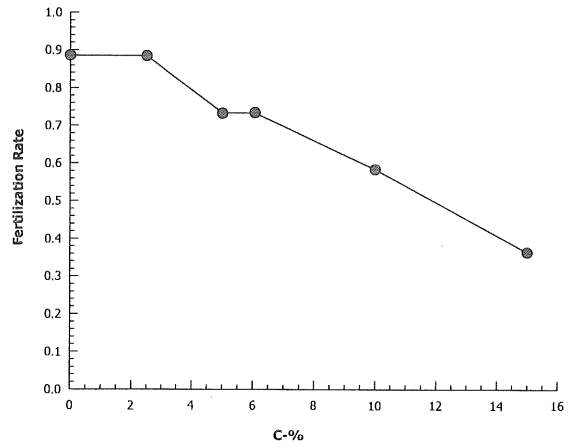
Point Estimates

Level	%	95% LCL	95% UCL	TU	95% LCL	95% UCL
EC25	7.896	5.755	9.888	12.66	10.11	17.38
EC50	13.22	11.39	14.98	7.564	6.676	8.78

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	0.886	0.81	0.96	0.02542	0.05683	6.42%	0.0%	443	500
2.5		5	0.884	0.81	0.98	0.0314	0.07021	7.94%	0.23%	442	500
5		5	0.732	0.65	0.83	0.03262	0.07294	9.96%	17.38%	366	500
6.06		5	0.734	0.66	0.81	0.02421	0.05413	7.38%	17.16%	367	500
10		5	0.586	0.48	0.67	0.03356	0.07503	12.8%	33.86%	292	500
15		5	0.364	0.22	0.44	0.03829	0.08562	23.52%	58.92%	182	500

Graphics



CETIS Analytical Report

Test Code: 1706-S208 | 16-3408-8529

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

Analysis ID: 15-3102-4026 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 15:16 **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	7.12%	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*	4.865	1.943	0.125	6	0.0014	CDF	Non-Significant Effect
		5*	2.098	1.895	0.093	7	0.0371	CDF	Non-Significant Effect
		6.06*	2.466	1.895	0.08	7	0.0215	CDF	Non-Significant Effect
		10	-1.157	1.895	0.088	7	0.8574	CDF	Significant Effect
		15	-5.383	1.895	0.099	7	0.9995	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	1.280516	0.2561031	5	30.81	<0.0001	Significant Effect
Error	0.1995245	0.008313522	24			
Total	1.48004		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	2.025	15.09	0.8457	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9827	0.9031	0.8920	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	0.886	0.8154	0.9566	0.87	0.81	0.96	0.02542	6.42%	0.0%
2.5		5	0.884	0.7968	0.9712	0.9	0.81	0.98	0.0314	7.94%	0.23%
5		5	0.732	0.6414	0.8226	0.75	0.65	0.83	0.03262	9.96%	17.38%
6.06		5	0.734	0.6668	0.8012	0.73	0.66	0.81	0.02421	7.38%	17.16%
10		5	0.586	0.4928	0.6792	0.59	0.48	0.67	0.03356	12.8%	33.86%
15		5	0.364	0.2577	0.4703	0.39	0.22	0.44	0.03829	23.52%	58.92%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.235	1.118	1.353	1.202	1.12	1.369	0.04239	7.67%	0.0%
2.5		5	1.239	1.084	1.394	1.249	1.12	1.429	0.05587	10.08%	-0.31%
5		5	1.03	0.9256	1.134	1.047	0.9377	1.146	0.03749	8.14%	16.65%
6.06		5	1.031	0.9537	1.107	1.024	0.9483	1.12	0.0277	6.01%	16.58%
10		5	0.8726	0.7778	0.9674	0.8759	0.7654	0.9589	0.03414	8.75%	29.37%
15		5	0.6453	0.5301	0.7604	0.6745	0.4882	0.7253	0.04147	14.37%	47.77%

Analyst: AC QA: KMP 7/11/17

CETIS Test Data Worksheet

Test Code: 1706-5208 16-3408-8529/61663651

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

Start Date: 30 Jun-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 17- 0740
End Date: 30 Jun-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 30 Jun-17 | **Material:** Facility Effluent | **Sample Station:** Brine Pit

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			186	100	44	AS 7/2/17
			187	100	76	
			188	100	72	
			189	100	82	
			190	100	67	
			191	100	73	
			192	100	87	
			193	100	81	
			194	100	75	
			195	100	92	
			196	100	39	
			197	100	55	
			198	100	36	
			199	100	22	
			200	100	81	
			201	100	98	
			202	100	67	
			203	100	66	
			204	100	59	
			205	100	48	
			206	100	65	
			207	100	75	
			208	100	83	
			209	100	91	
			210	100	91	
			211	100	64	
			212	100	96	
			213	100	90	
			214	100	81	
			215	100	87	

CETIS Test Data Worksheet

Test Code: 170x-S20816-3408-8529/61663651

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

Start Date: 30 Jun-17 | **Species:** Strongylocentrotus purpuratus | **Sample Code:** 17- 0740
End Date: 30 Jun-17 | **Protocol:** EPA/600/R-95/136 (1995) | **Sample Source:** IDE Americas, Inc.
Sample Date: 30 Jun-17 | **Material:** Facility Effluent | **Sample Station:** Brine Pit

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	215	100	87	AD 7/1/17
0	LC	2	192			
0	LC	3	212			
0	LC	4	195			
0	LC	5	214			
2.5		1	201			
2.5		2	189			
2.5		3	213			
2.5		4	210			
2.5		5	193			
5		1	206			
5		2	208			
5		3	190			
5		4	194			
5		5	187			
6.06		1	200	100	64	
6.06		2	191			
6.06		3	203			
6.06		4	188			
6.06		5	207			
10		1	197			
10		2	211			
10		3	204			
10		4	202			
10		5	205			
15		1	186			
15		2	196			
15		3	198			
15		4	199			
15		5	209			

QC: CG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: Brine Pit | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17- 0740 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: AD | **Test No:** 1706-S208

Analyst: CH

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.0	8.01	33.9	15.0
2.5	8.0	8.03	33.9	14.8
5.0	8.0	8.03	33.9	14.6
6.06	8.0	8.03	33.9	14.8
10	8.0	8.03	33.6	14.7
15	8.0	8.02	33.5	14.7

Comments: Dilutions made with M-ISW

QC Check: AC 7/7/17 | **Final Review:** KFP 7/11/17

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

Client: IOE
Sample ID: Binne Pit
Test No.: 1706-S208

Tech initials: AO
Injection Time: 1650

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

egg stock ____ ml
seawater ____ ml

(A)

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):	1710	50:1	84, 83	16, 17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1750	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AO @ 8 6/30/17

QC Check: AC 7/5/17

Final Review: KTP 7/11/17

PT Filter EFF

CETIS Summary Report

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

Batch ID:	19-0828-4416	**Test Type:**	Fertilization	**Analyst:**	(a)
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	~~Natural Seawater~~ MINE
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	13-1009-4228	**Code:**	17-0742	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Facility Effluent	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (5.5 °C)	**Station:**	Pre-Treatment Filtered		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
21-1002-5944	Fertilization Rate	15	>15	NA	7.01%	< 6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
15-3250-9312	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
15-3250-9312	Fertilization Rate	Control Resp	0.89	0.7 - NL	Yes	Passes Acceptability Criteria
21-1002-5944	Fertilization Rate	Control Resp	0.89	0.7 - NL	Yes	Passes Acceptability Criteria
21-1002-5944	Fertilization Rate	PMSD	0.07012	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	0.89	0.8444	0.9356	0.83	0.92	0.01643	0.03674	4.13%	0.0%
2.5		5	0.922	0.876	0.968	0.88	0.97	0.01655	0.03701	4.01%	-3.6%
5		5	0.898	0.8574	0.9386	0.85	0.93	0.01463	0.03271	3.64%	-0.9%
6.06		5	0.882	0.8255	0.9385	0.81	0.92	0.02035	0.0455	5.16%	0.9%
10		5	0.872	0.826	0.918	0.81	0.9	0.01655	0.03701	4.25%	2.02%
15		5	0.904	0.8554	0.9526	0.85	0.95	0.01749	0.03912	4.33%	-1.57%

Fertilization Rate Detail

C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.83	0.92	0.88	0.91	0.91
2.5		0.91	0.9	0.95	0.97	0.88
5		0.93	0.92	0.88	0.85	0.91
6.06		0.81	0.92	0.89	0.87	0.92
10		0.87	0.81	0.88	0.9	0.9
15		0.95	0.88	0.92	0.92	0.85

(a) QIS AC 7/10/17

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

Analysis ID: 21-1002-5944 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 16:02 **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	7.01%	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	-1.495	2.362	0.093	8	0.9962	CDF	Non-Significant Effect
		5	-0.3254	2.362	0.093	8	0.9117	CDF	Non-Significant Effect
		6.06	0.2868	2.362	0.093	8	0.7351	CDF	Non-Significant Effect
		10	0.7209	2.362	0.093	8	0.5474	CDF	Non-Significant Effect
		15	-0.6304	2.362	0.093	8	0.9561	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02331927	0.004663855	5	1.198	0.3401	Non-Significant Effect
Error	0.09346031	0.00389418	24			
Total	0.1167796		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.7582	15.09	0.9796	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9497	0.9031	0.1659	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	0.89	0.8444	0.9356	0.91	0.83	0.92	0.01643	4.13%	0.0%
2.5		5	0.922	0.876	0.968	0.91	0.88	0.97	0.01655	4.01%	-3.6%
5		5	0.898	0.8574	0.9386	0.91	0.85	0.93	0.01463	3.64%	-0.9%
6.06		5	0.882	0.8255	0.9385	0.89	0.81	0.92	0.02035	5.16%	0.9%
10		5	0.872	0.826	0.918	0.88	0.81	0.9	0.01655	4.25%	2.02%
15		5	0.904	0.8554	0.9526	0.92	0.85	0.95	0.01749	4.33%	-1.57%

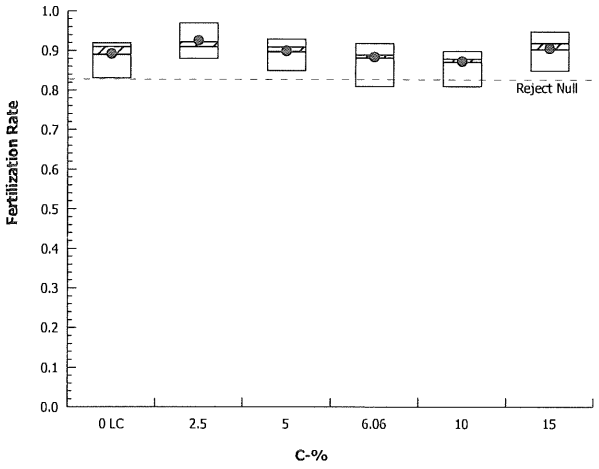
Angular (Corrected) Transformed Summary

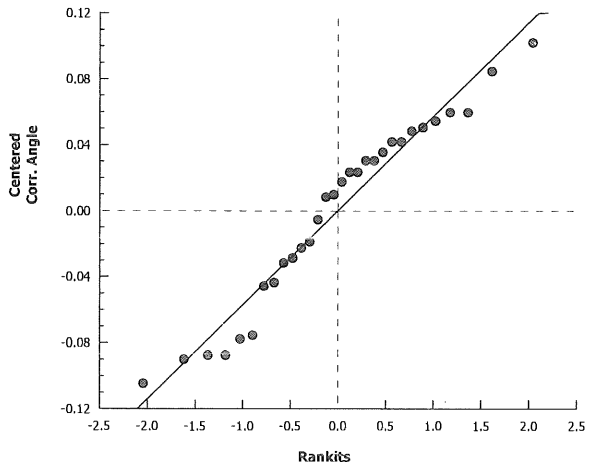
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.236	1.166	1.306	1.266	1.146	1.284	0.02511	4.54%	0.0%
2.5		5	1.295	1.203	1.387	1.266	1.217	1.397	0.03309	5.71%	-4.78%
5		5	1.249	1.183	1.314	1.266	1.173	1.303	0.02369	4.24%	-1.04%
6.06		5	1.225	1.14	1.309	1.233	1.12	1.284	0.03052	5.57%	0.92%
10		5	1.207	1.141	1.273	1.217	1.12	1.249	0.02374	4.4%	2.3%
15		5	1.261	1.178	1.344	1.284	1.173	1.345	0.02985	5.3%	-2.01%

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

Analysis ID:	21-1002-5944	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 16:02	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

Test Code: 1706-S206 | 16-3657-6775

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

Analysis ID:	15-3250-9312	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 16:02	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	130088	1000	Yes	Two-Point Interpolation

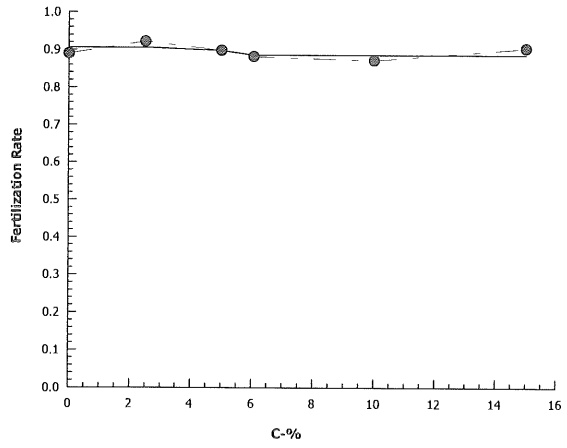
Point Estimates

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	0.89	0.83	0.92	0.01643	0.03674	4.13%	0.0%	445	500
2.5		5	0.922	0.88	0.97	0.01655	0.03701	4.01%	-3.6%	461	500
5		5	0.898	0.85	0.93	0.01463	0.03271	3.64%	-0.9%	449	500
6.06		5	0.882	0.81	0.92	0.02035	0.0455	5.16%	0.9%	441	500
10		5	0.872	0.81	0.9	0.01655	0.03701	4.25%	2.02%	436	500
15		5	0.904	0.85	0.95	0.01749	0.03912	4.33%	-1.57%	452	500

Graphics



Analyst: AC QA: 7/11/17

CETIS Analytical Report

Test Code: 1706-S206 | 16-3657-6775

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

Analysis ID: 15-7034-8634 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 16:03 **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	4.97%	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*	9.665	1.943	0.074	6	<0.0001	CDF	Non-Significant Effect
		5*	10.63	1.895	0.057	7	<0.0001	CDF	Non-Significant Effect
		6.06*	8.298	1.943	0.07	6	<0.0001	CDF	Non-Significant Effect
		10*	9.256	1.895	0.057	7	<0.0001	CDF	Non-Significant Effect
		15*	9.458	1.943	0.069	6	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.02331927	0.004663855	5	1.198	0.3401	Non-Significant Effect
Error	0.09346031	0.00389418	24			
Total	0.1167796		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	0.7582	15.09	0.9796	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9497	0.9031	0.1659	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	0.89	0.8444	0.9356	0.91	0.83	0.92	0.01643	4.13%	0.0%
2.5		5	0.922	0.876	0.968	0.91	0.88	0.97	0.01655	4.01%	-3.6%
5		5	0.898	0.8574	0.9386	0.91	0.85	0.93	0.01463	3.64%	-0.9%
6.06		5	0.882	0.8255	0.9385	0.89	0.81	0.92	0.02035	5.16%	0.9%
10		5	0.872	0.826	0.918	0.88	0.81	0.9	0.01655	4.25%	2.02%
15		5	0.904	0.8554	0.9526	0.92	0.85	0.95	0.01749	4.33%	-1.57%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.236	1.166	1.306	1.266	1.146	1.284	0.02511	4.54%	0.0%
2.5		5	1.295	1.203	1.387	1.266	1.217	1.397	0.03309	5.71%	-4.78%
5		5	1.249	1.183	1.314	1.266	1.173	1.303	0.02369	4.24%	-1.04%
6.06		5	1.225	1.14	1.309	1.233	1.12	1.284	0.03052	5.57%	0.92%
10		5	1.207	1.141	1.273	1.217	1.12	1.249	0.02374	4.4%	2.3%
15		5	1.261	1.178	1.344	1.284	1.173	1.345	0.02985	5.3%	-2.01%

Analyst: AC QA: KMP 7/11/17

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:56 (p 1 of 1)
Test Code: 1706-S206 16-3657-6775/618C2E07

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17-0742
Sample Source: IDE Americas, Inc.
Sample Station: Pre-Treatment Filtered

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			156	100	92	7/3/17 DM
			157		97	
			158		88	
			159		(A) ~~88~~ 88	
			160		92	
			161		91	
			162		87	
			163		88	
			164		91	
			165		92	
			166		93	
			167		92	
			168		95	
			169		91	
			170		83	
			171		90	
			172		85	
			173		87	
			174		89	
			175		90	
			176		90	
			177		95	
			178		88	
			179		81	
			180		(A) ~~84~~ 81	
			181		92	
			182		85	
			183		92	
			184		91	
			185		88	

(A) Q18 DM 07/03/17

Analyst: DM QA: AC 7/7/17

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:56 (p 1 of 1)
Test Code: 1706-S206 16-3657-6775/618C2E07

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Facility Effluent
Sample Code: 17- 0742
Sample Source: IDE Americas, Inc.
Sample Station: Pre-Treatment Filtered

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	170	100	94	AO 7/1/17
0	LC	2	156			
0	LC	3	185			
0	LC	4	164			
0	LC	5	184			
2.5		1	169			
2.5		2	171			
2.5		3	177			
2.5		4	157			
2.5		5	178			
5		1	166			
5		2	160			
5		3	163			
5		4	182			
5		5	161			
6.06		1	179	100	87	
6.06		2	183			
6.06		3	174			
6.06		4	162			
6.06		5	167			
10		1	173			
10		2	180			
10		3	159			
10		4	175			
10		5	176			
15		1	168			
15		2	158			
15		3	165			
15		4	181			
15		5	172			

QC: CG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: Pre-Treatment Filtered | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17-0742 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: AD | **Test No:** 1706-S206

Analyst: CH

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	7.9	8.01	33.9	15.3
2.5	7.9	8.03	34.0	15.4
5.0	8.0	8.02	33.9	15.3
6.06	7.9	8.03	34.0	15.3
10	7.9	8.02	34.1	15.4
15	8.0	7.97	34.0	15.2

Comments: Dilutions made with M-1NF

QC Check: AC 7/7/17 | **Final Review:** KFP 7/11/17

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

Client: IDE
Sample ID: Pre-treatment Filtered
Test No.: 1706-S206

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Tech initials: AP
Injection Time: 1650

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
____ parts seawater

(A) 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):	1710	50:1	84,83	16,17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1750	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AP QA 6/30/17

QC Check: AC 7/5/17

Final Review: KAP 7/11/17

M-INF

CETIS Summary Report

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

Batch ID:	09-2930-1447	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater / Nautilus seawater
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	03-5917-9333	**Code:**	17-0743	**Client:**	IDE
Sample Date:	30 Jun-17 08:00	**Material:**	Receiving Water	**Project:**	Carlsbad Desal Plant
Receive Date:	30 Jun-17 11:48	**Source:**	IDE Americas, Inc.		
Sample Age:	10h (5.5 °C)	**Station:**	M-INF		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
15-4355-0639	Fertilization Rate	15	>15	NA	3.6%	6.667	Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	%	95% LCL	95% UCL	TU	Method
02-7881-1690	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
02-7881-1690	Fertilization Rate	Control Resp	0.934	0.7 - NL	Yes	Passes Acceptability Criteria
15-4355-0639	Fertilization Rate	Control Resp	0.934	0.7 - NL	Yes	Passes Acceptability Criteria
15-4355-0639	Fertilization Rate	PMSD	0.03597	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	0.934	0.9068	0.9612	0.92	0.97	0.009798	0.02191	2.35%	0.0%
2.5		5	0.938	0.9276	0.9484	0.93	0.95	0.003741	0.008366	0.89%	-0.43%
5		5	0.924	0.8941	0.9539	0.89	0.95	0.01077	0.02408	2.61%	1.07%
6.06		5	0.94	0.9168	0.9632	0.92	0.97	0.008366	0.01871	1.99%	-0.64%
10		5	0.95	0.9237	0.9763	0.93	0.98	0.009487	0.02121	2.23%	-1.71%
15		5	0.938	0.9126	0.9634	0.92	0.96	0.009165	0.02049	2.19%	-0.43%

Fertilization Rate Detail

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

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

Analysis ID: 15-4355-0639 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 15:50 **Analysis:** Parametric-Control vs Treatments **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	3.6%	15	>15	NA	6.667

Dunnett Multiple Comparison Test

Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)
Lab Control		2.5	-0.1887	2.362	0.065	8	0.8830	CDF	Non-Significant Effect
		5	0.7422	2.362	0.065	8	0.5376	CDF	Non-Significant Effect
		6.06	-0.4254	2.362	0.065	8	0.9290	CDF	Non-Significant Effect
		10	-1.296	2.362	0.065	8	0.9929	CDF	Non-Significant Effect
		15	-0.2761	2.362	0.065	8	0.9021	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.008165863	0.001633173	5	0.8702	0.5155	Non-Significant Effect
Error	0.04504281	0.001876784	24			
Total	0.05320867		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.976	15.09	0.5529	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9408	0.9031	0.0957	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	0.934	0.9068	0.9612	0.92	0.92	0.97	0.009798	2.35%	0.0%
2.5		5	0.938	0.9276	0.9484	0.94	0.93	0.95	0.003741	0.89%	-0.43%
5		5	0.924	0.8941	0.9539	0.93	0.89	0.95	0.01077	2.61%	1.07%
6.06		5	0.94	0.9168	0.9632	0.94	0.92	0.97	0.008366	1.99%	-0.64%
10		5	0.95	0.9237	0.9763	0.95	0.93	0.98	0.009487	2.23%	-1.71%
15		5	0.938	0.9126	0.9634	0.93	0.92	0.96	0.009165	2.19%	-0.43%

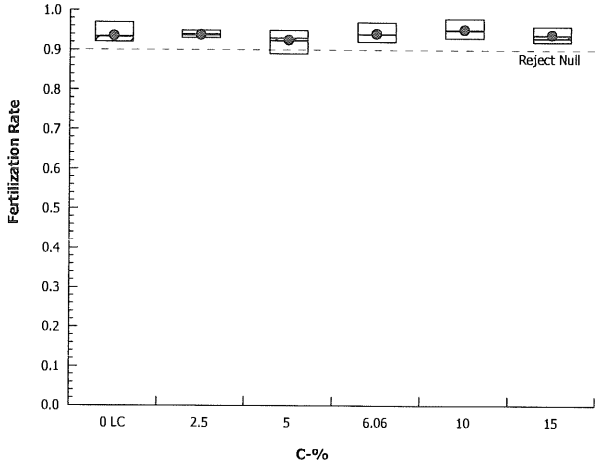
Angular (Corrected) Transformed Summary

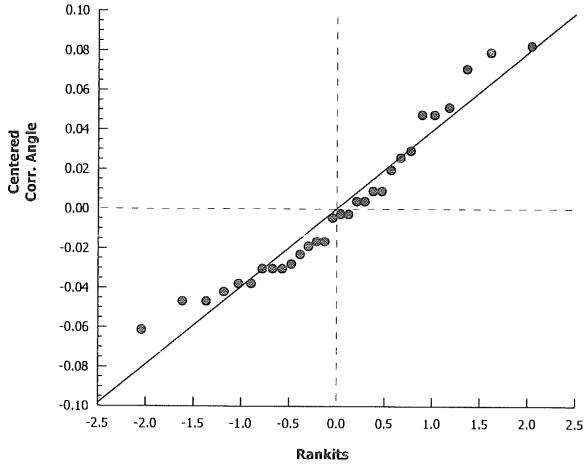
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.314	1.254	1.375	1.284	1.284	1.397	0.02193	3.73%	0.0%
2.5		5	1.32	1.298	1.341	1.323	1.303	1.345	0.007862	1.33%	-0.39%
5		5	1.294	1.238	1.35	1.303	1.233	1.345	0.02013	3.48%	1.55%
6.06		5	1.326	1.273	1.379	1.323	1.284	1.397	0.01911	3.22%	-0.89%
10		5	1.35	1.285	1.415	1.345	1.303	1.429	0.02349	3.89%	-2.7%
15		5	1.322	1.267	1.377	1.303	1.284	1.369	0.01968	3.33%	-0.58%

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

Analysis ID:	15-4355-0639	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 15:50	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	02-7881-1690	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	07 Jul-17 15:50	**Analysis:**	Linear Interpolation (ICPIN)	**Official Results:**	Yes

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1617941	1000	Yes	Two-Point Interpolation

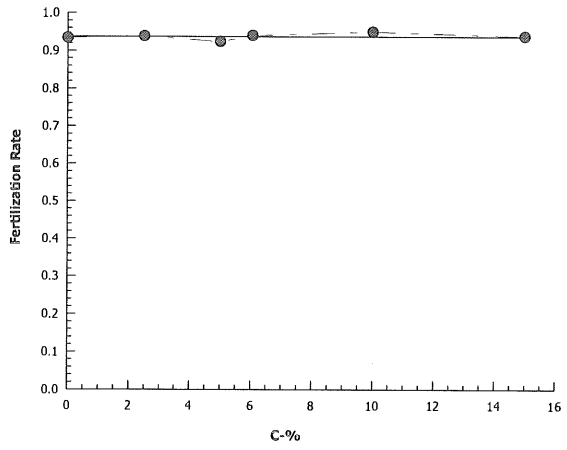
Point Estimates

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	0.934	0.92	0.97	0.009798	0.02191	2.35%	0.0%	467	500
2.5		5	0.938	0.93	0.95	0.003741	0.008366	0.89%	-0.43%	469	500
5		5	0.924	0.89	0.95	0.01077	0.02408	2.61%	1.07%	462	500
6.06		5	0.94	0.92	0.97	0.008366	0.01871	1.99%	-0.64%	470	500
10		5	0.95	0.93	0.98	0.009487	0.02121	2.23%	-1.71%	475	500
15		5	0.938	0.92	0.96	0.009165	0.02049	2.19%	-0.43%	469	500

Graphics



CETIS Analytical Report

TST

Test Code: 1706-S205 | 01-9508-5319

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

Analysis ID: 20-5871-2625 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 07 Jul-17 15:52 **Analysis:** Parametric Bioequivalence-Two Sample **Official Results:** Yes

Data Transform	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C*b < T	NA	NA	0.75	2.59%	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*	18.31	2.015	0.037	5	<0.0001	CDF	Non-Significant Effect
		5*	11.86	1.895	0.049	7	<0.0001	CDF	Non-Significant Effect
		6.06*	13.5	1.895	0.048	7	<0.0001	CDF	Non-Significant Effect
		10*	12.7	1.895	0.054	7	<0.0001	CDF	Non-Significant Effect
		15*	13.11	1.895	0.049	7	<0.0001	CDF	Non-Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.008165863	0.001633173	5	0.8702	0.5155	Non-Significant Effect
Error	0.04504281	0.001876784	24			
Total	0.05320867		29			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.976	15.09	0.5529	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9408	0.9031	0.0957	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	0.934	0.9068	0.9612	0.92	0.92	0.97	0.009798	2.35%	0.0%
2.5		5	0.938	0.9276	0.9484	0.94	0.93	0.95	0.003741	0.89%	-0.43%
5		5	0.924	0.8941	0.9539	0.93	0.89	0.95	0.01077	2.61%	1.07%
6.06		5	0.94	0.9168	0.9632	0.94	0.92	0.97	0.008366	1.99%	-0.64%
10		5	0.95	0.9237	0.9763	0.95	0.93	0.98	0.009487	2.23%	-1.71%
15		5	0.938	0.9126	0.9634	0.93	0.92	0.96	0.009165	2.19%	-0.43%

Angular (Corrected) Transformed Summary

C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.314	1.254	1.375	1.284	1.284	1.397	0.02193	3.73%	0.0%
2.5		5	1.32	1.298	1.341	1.323	1.303	1.345	0.007862	1.33%	-0.39%
5		5	1.294	1.238	1.35	1.303	1.233	1.345	0.02013	3.48%	1.55%
6.06		5	1.326	1.273	1.379	1.323	1.284	1.397	0.01911	3.22%	-0.89%
10		5	1.35	1.285	1.415	1.345	1.303	1.429	0.02349	3.89%	-2.7%
15		5	1.322	1.267	1.377	1.303	1.284	1.369	0.01968	3.33%	-0.58%

Analyst: AC QA: KP 7/11/17

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:53 (p 1 of 1)
Test Code: 1706-S305 01-9508-5319/BA0C407

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

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

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

Sample Code: 17- 0743
Sample Source: IDE Americas, Inc.
Sample Station: (A) ~~IPS~~ M-INF

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			96	100	93	7/3/17
			97	100	94	
			98	100	95	
			99	100	92	
			100	100	93	
			101	100	94	
			102	100	93	
			103	100	89	
			104	100	92	
			105	100	94	
			106	100	94	
			107	100	92	
			108	100	96	
			109	100	95	
			110	100	97	
			111	100	96	
			112	100	97	
			113	100	95	
			114	100	92	
			115	100	93	
			116	100	96	
			117	100	94	
			118	100	92	
			119	100	93	
			120	100	91	
			121	100	92	
			122	100	98	
			123	100	(A) ~~97~~ 93	
			124	100	93	
			125	100	94	

(A) Q18 SG 7/3/17
(B) Q18 AC 7/7/17

CETIS Test Data Worksheet

Report Date: 29 Jun-17 18:53 (p 1 of 1)
Test Code: 170615205 01-9508-5319/BA0C407

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

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

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

Sample Code: 17-0743
Sample Source: IDE Americas, Inc.
Sample Station: ~~IPS~~ M-INF

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	105	100	98	7/1/17 AD
0	LC	2	107			
0	LC	3	110			
0	LC	4	99			
0	LC	5	104			
2.5		1	109			
2.5		2	115			
2.5		3	106			
2.5		4	96			
2.5		5	117			
5		1	103			
5		2	125			
5		3	123			
5		4	120			
5		5	98			
6.06		1	112	100	94	
6.06		2	97			
6.06		3	121			
6.06		4	101			
6.06		5	124			
10		1	122			
10		2	116			
10		3	113			
10		4	119			
10		5	102			
15		1	114			
15		2	111			
15		3	108			
15		4	118			
15		5	100			

QC: CG

@ QI8 AC 7/7/17

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: CH GAB 6/30/17 ~~IPS~~ M-INF | **Start Date/Time:** 6/30/2017 1750

Sample Log No.: 17- 0743 | **End Date/Time:** 6/30/2017 1830

Dilutions made by: KTD | **Test No:** 1706-S205

Analyst: CH

	Initial Readings			
Concentration %	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (°C)**
Lab Control	8.5	7.91	33.7	15.2
2.5	8.6	7.95	33.7	14.6
5.0	8.6	7.97	33.8	14.4
6.06	8.6	7.97	34.0	14.6
10	8.6	7.98	33.9	14.3
15	8.6	7.98	33.9	14.3

Comments: Dilutions made with Nautilus seawater.

QC Check: AC 7/7/17 | **Final Review:** KTP 7/11/17

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

Client: IDE
Sample ID: M-INF
Test No.: 1706-S205

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Tech initials: AP
Injection Time: 1650

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

(A) 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):	1710	50:1	84,83	16,17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1745	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AP @ 1830 6/30/17

QC Check: AC 7/5/17

Final Review: KRP 7/11/17

Appendix B

Sample Receipt Information

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: See below
Test ID No(s).: 1706-S203 to S209

Sample (A, B, C):	M-001	ERI BRINE	BRINE PIT	TRAIN 4
Log-in No. (17-xxxx):	0738	0739	0740	0741
Sample Collection Date & Time:	6/30/17 0800	6/30/17 0800	6/30/17 0800	6/30/17 0800
Sample Receipt Date & Time:	6/30/17 1148	6/30/17 1148	6/30/17 1148	6/30/17 1148
Number of Containers & Container Type:	1 4L cubi	1 4L cubi	1 4L cubi	1 4L cubi
Approx. Total Volume Received (L):	~4L	~4L	~4L	~4L
Check-in Temperature (°C)	6.5	4.5	6.5	5.5
Temperature OK? [1]	(Y) N	(Y) N	(Y) N	(Y) N
DO (mg/L)	6.67	7.3	7.0	7.0
pH (units)	7.72	7.21	8.00	7.75
Conductivity (µS/cm)	—	—	—	—
Salinity (ppt)	62.6	68.4	30.2	70.2
Alkalinity (mg/L) [2]	190	199	100	206
Hardness (mg/L) [2,3]	—	—	—	—
Total Chlorine (mg/L)	0.03	<0.02	0.03	<0.02
Technician Initials	BO	BO	BO	BO/CH

Test Performed: Urchin Fertilization **Control/Dilution Water:** 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 115 Hardness or Salinity: (34 ppt)
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

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

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

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

Additional Comments: _____

Sample Check-In Information

Sample Description:
M-001: colorless, clear, odorless, no debris
ERI BRINE: colorless, clear, odorless, no debris
BRINE PIT: colorless, clear, odorless, no debris
TRAIN 4: colorless, clear, odorless, no debris

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

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

Salinity Adjustment? (Y) N M-001 40ppt adj
Test: M-001 adj Source: Seawater Target ppt: 40
Test: Source: Target ppt:
Test: Source: Target ppt:

pH Adjustment? Y (N)

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

Subsamples for Additional Chemistry Required? Y (N)
NH3 Other _____
Tech Initials A___ B___ C___

QC Check: AC 7/7/17
Final Review: KFP 7/11/17

Nautilus Environmental
4340 Vandever Avenue
San Diego, CA 92120

Client: IDE
Sample ID: See below
Test ID No(s).: 1706-S203 to S209

Sample (A, B, C):	PT FILTER EFF	M-INF		
Log-in No. (17-xxxx):	0742	0743		
Sample Collection Date & Time:	6/30/17 0800	6/30/17 0800		
Sample Receipt Date & Time:	6/30/17 1148	6/30/17 1148		
Number of Containers & Container Type:	1 4L cubi	1 4L cubi		
Approx. Total Volume Received (L):	~4L	~4L		
Check-in Temperature (°C)	5.5	5.5		
Temperature OK? [1]	(Y) N	(Y) N	Y N	Y N
DO (mg/L)	7.2	7.4		
pH (units)	7.97	8.06		
Conductivity (µS/cm)	—	—		
Salinity (ppt)	33.9	33.9		
Alkalinity (mg/L) [2]	107	114		
Hardness (mg/L) [2,3]	—	—		
Total Chlorine (mg/L)	0.02	0.02		
Technician Initials	BO/CM	BO/CM		

Test Performed: Urchin Fert/Emb Control/Dilution Water: 8:2 / (Lab SW) / Lab ART Other: _____
Alkalinity: 115 Hardness or Salinity: 34 ppt
Additional Control? Y N = _____ Alkalinity: _____ Hardness or Salinity: _____

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

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

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

Additional Comments: _____

Sample Check-In Information

Sample Description:
PT Filter eff's colorless, clear, odorless, no debris
M-INF's colorless, clear, odorless, no debris

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

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

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

pH Adjustment? Y (N)

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

Cl_2 Adjustment? Y (N)

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

Sample Aeration? Y (N)

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

Subsamples for Additional Chemistry Required? Y (N)
NH3 Other _____
Tech Initials A___ B___ C___

QC Check: AC 7/7/17
Final Review: KPT 7/11/17

Appendix C

Chain-of-Custody Form

SCREENING / WEEKLY

IDE Technologies

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

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

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

Special instruction: Samples collected during normal plant operation. M-001 is to be run unadjusted and adjusted to 40 ppt for weekly chronic. All other samples are to be run unadjusted. Start: 6/29/17 @ 08:00, End: 6/30/17 @ 08:00. KC

Glass=G Plastic=P

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

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

Sample ID	Date	Time	Sample Type	Preservative ?	Container Type	ANALYSES: Purple Urchin Chronic Fertilization	NOTES:	°C
* M-001 (17- 2188)	6/29-30/17	8:00	24 HR COMP -B	N	4L CUBIE	X	TDS - 56.44 ppt EC - 80.12 mS/cm *	6.5
* ERI BRINE (17-2189)	6/29-30/17	8:00	24 HR COMP -B	N	4L CUBIE	X	TDS - 63.70 ppt EC - 88.20 mS/cm *	4.5
* BRINE PIT (17-2190)	6/29-30/17	8:00	24 HR COMP -B	N	4L CUBIE	X	TDS - 28.71 ppt EC - 45.25 mS/cm *	6.5
* TRAIN 4 (17-2191)	6/29-30/17	8:00	24 HR COMP -B	N	4L CUBIE	X	TDS - 65.52 ppt EC - 90.13 mS/cm *	5.5
* PT FILTER EFF (17-2192)	6/29-30/17	8:00	24 HR COMP -B	N	4L CUBIE	X	TDS - 32.18 ppt EC - 50.17 mS/cm *	5.5
M-INF (17-2193)	6/29-30/17	8:00	24 HR COMP -B	N	4L CUBIE	X	TDS - 32.18 ppt EC - 50.13 mS/cm	5.5

Relinquished By:	Date:	Time:		Received By:	Time:	Sample Condition Upon Receipt:
[signature]	6/30/17	11:00		[signature] 6/30/17	11:00	☐ Iced ☐ Ambient or _____ °C
[signature]	6/30/17	11:48		[signature] 6/30/17	11:48	☐ Iced ☐ Ambient or _____ °C

*To be diluted with M-INF dilution water. KC

Nautilus IDs
17-0738 to -0743

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

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

Batch ID:	06-7011-3891	**Test Type:**	Fertilization	**Analyst:**	
Start Date:	30 Jun-17 17:50	**Protocol:**	EPA/600/R-95/136 (1995)	**Diluent:**	Natural Seawater
Ending Date:	30 Jun-17 18:30	**Species:**	Strongylocentrotus purpuratus	**Brine:**	Not Applicable
Duration:	40m	**Source:**	Pt. Loma	**Age:**	

Sample ID:	17-0246-3500	**Code:**	170630sprt	**Client:**	Internal
Sample Date:	30 Jun-17	**Material:**	Copper chloride	**Project:**	
Receive Date:	30 Jun-17	**Source:**	Reference Toxicant		
Sample Age:	18h	**Station:**	Copper Chloride		

Comparison Summary

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
03-8133-6222	Fertilization Rate	10	20	14.14	6.35%		Dunnett Multiple Comparison Test

Point Estimate Summary

Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method
20-9128-8719	Fertilization Rate	EC50	39.38	37.84	40.98		Trimmed Spearman-Kärber

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
03-8133-6222	Fertilization Rate	Control Resp	0.946	0.7 - NL	Yes	Passes Acceptability Criteria
20-9128-8719	Fertilization Rate	Control Resp	0.946	0.7 - NL	Yes	Passes Acceptability Criteria
03-8133-6222	Fertilization Rate	PMSD	0.06351	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	0.946	0.9148	0.9772	0.91	0.98	0.01122	0.0251	2.65%	0.0%
10		5	0.934	0.9005	0.9675	0.9	0.96	0.01208	0.02702	2.89%	1.27%
20		5	0.822	0.7489	0.8951	0.75	0.89	0.02634	0.05891	7.17%	13.11%
40		5	0.546	0.4032	0.6888	0.44	0.72	0.05144	0.115	21.07%	42.28%
80		5	0.034	0.009796	0.0582	0	0.05	0.008718	0.01949	57.33%	96.41%
160		5	0	0	0	0	0	0	0		100.0%

Fertilization Rate Detail

C-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.95	0.98	0.91	0.95	0.94
10		0.95	0.95	0.91	0.96	0.9
20		0.87	0.75	0.78	0.82	0.89
40		0.45	0.53	0.59	0.72	0.44
80		0.05	0.04	0	0.04	0.04
160		0	0	0	0	0

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

Analysis ID: 03-8133-6222 **Endpoint:** Fertilization Rate **CETIS Version:** CETISv1.8.7
Analyzed: 05 Jul-17 15:40 **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	6.35%	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	0.5336	2.305	0.116	8	0.5887	CDF	Non-Significant Effect
		20*	4.037	2.305	0.116	8	0.0012	CDF	Significant Effect
		40*	10.15	2.305	0.116	8	<0.0001	CDF	Significant Effect
		80*	23.26	2.305	0.116	8	<0.0001	CDF	Significant Effect

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	4.674465	1.168616	4	186	<0.0001	Significant Effect
Error	0.1256386	0.006281928	20			
Total	4.800103		24			

Distributional Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variances	Bartlett Equality of Variance	3.034	13.28	0.5522	Equal Variances
Distribution	Shapiro-Wilk W Normality	0.9701	0.8877	0.6469	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	0.946	0.9148	0.9772	0.95	0.91	0.98	0.01122	2.65%	0.0%
10		5	0.934	0.9005	0.9675	0.95	0.9	0.96	0.01208	2.89%	1.27%
20		5	0.822	0.7489	0.8951	0.82	0.75	0.89	0.02634	7.17%	13.11%
40		5	0.546	0.4032	0.6888	0.53	0.44	0.72	0.05144	21.07%	42.28%
80		5	0.034	0.009796	0.0582	0.04	0	0.05	0.008718	57.33%	96.41%
160		5	0	0	0	0	0	0	0		100.0%

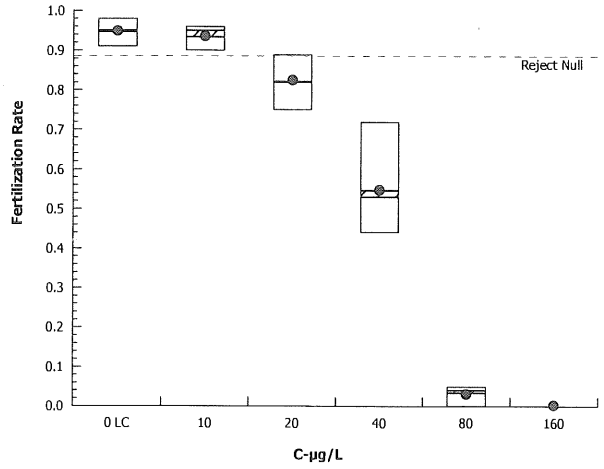
Angular (Corrected) Transformed Summary

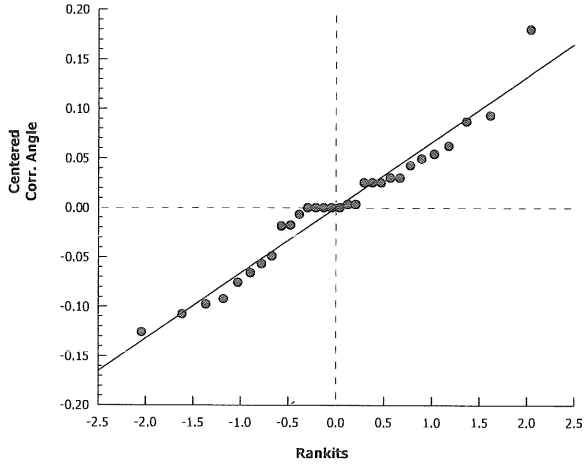
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	1.342	1.269	1.414	1.345	1.266	1.429	0.02615	4.36%	0.0%
10		5	1.315	1.248	1.382	1.345	1.249	1.369	0.02402	4.08%	1.99%
20		5	1.139	1.042	1.236	1.133	1.047	1.233	0.03493	6.85%	15.08%
40		5	0.833	0.6864	0.9796	0.8154	0.7253	1.013	0.05281	14.18%	37.92%
80		5	0.1759	0.08757	0.2643	0.2014	0.05002	0.2255	0.03182	40.45%	86.89%
160		5	0.05002	0.05001	0.05003	0.05002	0.05002	0.05002	0	0.0%	96.27%

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

Analysis ID:	03-8133-6222	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	05 Jul-17 15:40	**Analysis:**	Parametric-Control vs Treatments	**Official Results:**	Yes

Graphics





CETIS Analytical Report

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

Analysis ID:	20-9128-8719	**Endpoint:**	Fertilization Rate	**CETIS Version:**	CETISv1.8.7
Analyzed:	05 Jul-17 15:40	**Analysis:**	Trimmed Spearman-Kärber	**Official Results:**	Yes

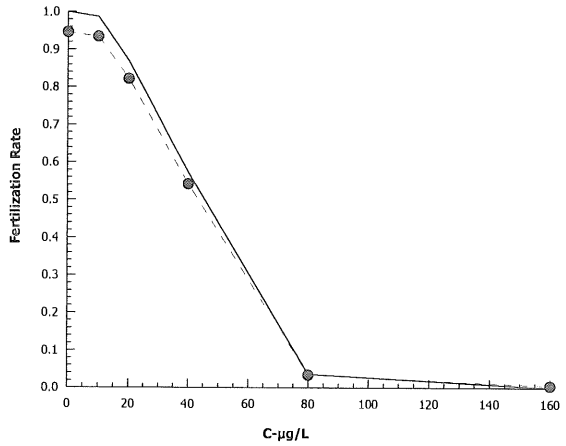
Trimmed Spearman-Kärber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.054	1.27%	1.595	0.008643	39.38	37.84	40.98

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	0.946	0.91	0.98	0.01122	0.0251	2.65%	0.0%	473	500
10		5	0.934	0.9	0.96	0.01208	0.02702	2.89%	1.27%	467	500
20		5	0.822	0.75	0.89	0.02634	0.05891	7.17%	13.11%	411	500
40		5	0.546	0.44	0.72	0.05144	0.115	21.07%	42.28%	271	500
80		5	0.034	0	0.05	0.008718	0.01949	57.33%	96.41%	17	500
160		5	0	0	0	0	0		100.0%	0	500

Graphics

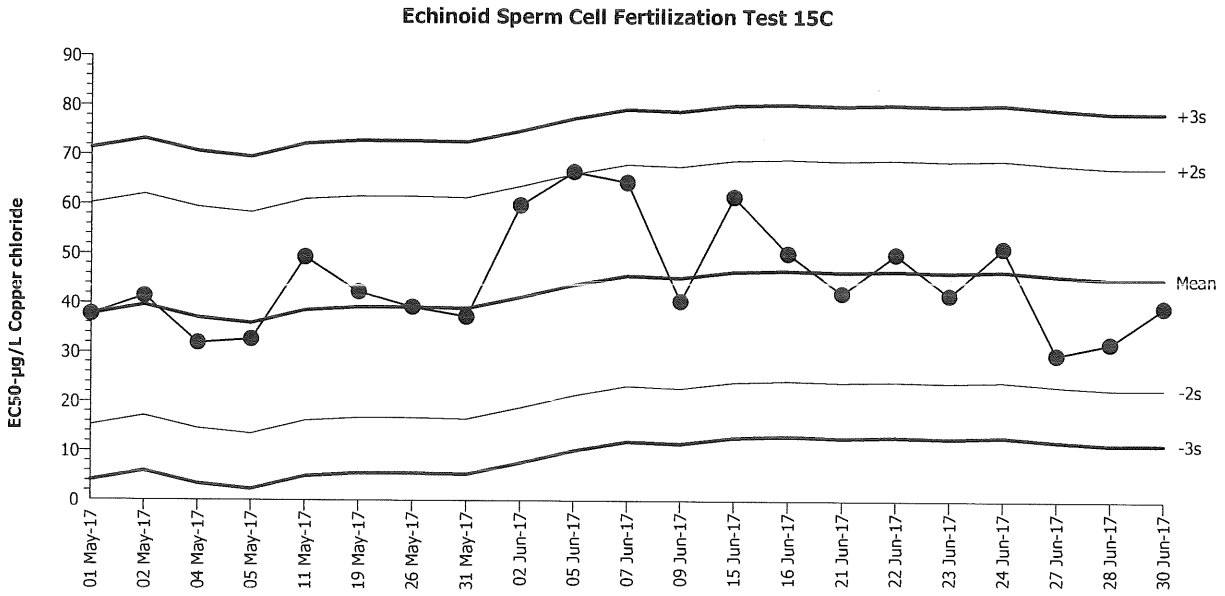


CETIS QC Plot

Report Date: 05 Jul-17 15:41 (1 of 1)

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

Test Type:	Fertilization	**Organism:**	Strongylocentrotus purpuratus (Purpl	**Material:**	Copper chloride
Protocol:	EPA/600/R-95/136 (1995)	**Endpoint:**	Fertilization Rate	**Source:**	Reference Toxicant-REF



Mean:	45.11	**Count:**	20	**-2s Warning Limit:**	22.67	**-3s Action Limit:**	11.45
Sigma:	11.22	**CV:**	24.90%	**+2s Warning Limit:**	67.55	**+3s Action Limit:**	78.77

Quality Control Data

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	May	1	17:50	37.72	-7.386	-0.6583			18-0409-9294	21-4338-2021
2			2	11:56	41.38	-3.729	-0.3324			15-1584-1378	12-5072-1723
3			4	16:15	31.87	-13.24	-1.18			08-0627-0095	01-9095-4567
4			5	15:10	32.62	-12.49	-1.113			16-0368-0463	05-0853-8226
5			11	15:35	49.31	4.197	0.3741			01-5463-5574	13-6197-3009
6			19	16:58	42.3	-2.814	-0.2508			01-3808-3529	10-6921-8357
7			26	16:55	39.23	-5.877	-0.5238			19-8209-6027	02-5970-9183
8			31	15:42	37.28	-7.829	-0.6978			01-0947-8219	10-1735-9410
9		Jun	2	17:00	59.87	14.76	1.315			07-9823-1222	14-2289-5480
10			5	16:33	66.68	21.57	1.922			12-3374-6038	12-3894-8538
11			7	17:28	64.53	19.42	1.731			14-9580-6318	06-4574-2541
12			9	17:15	40.49	-4.625	-0.4122			07-9177-2202	00-5029-9905
13			15	14:30	61.64	16.53	1.473			08-8166-8875	14-1352-0089
14			16	14:30	50.25	5.14	0.4581			02-2217-4481	09-2276-1445
15			21	14:17	42.15	-2.958	-0.2637			20-6379-6831	00-5386-2071
16			22	17:25	50.05	4.935	0.4399			10-9823-5082	04-6220-9409
17			23	16:55	41.8	-3.307	-0.2948			06-0771-4160	11-6079-2504
18			24	13:27	51.33	6.219	0.5543			01-7420-9579	03-5890-9605
19			27	13:13	29.77	-15.34	-1.367			11-6174-9094	14-8592-6950
20			28	14:40	32.02	-13.09	-1.167			06-0030-2581	03-5443-1685
21			30	17:50	39.38	-5.733	-0.511			19-1859-0537	20-9128-8719

CETIS Test Data Worksheet

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

Start Date: 30 Jun-17
End Date: 30 Jun-17
Sample Date: 30 Jun-17
Species: Strongylocentrotus purpuratus
Protocol: EPA/600/R-95/136 (1995)
Material: Copper chloride
Sample Code: 170630sprt
Sample Source: Reference Toxicant
Sample Station: Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
			1	100	96	7/3/17
			2		4	
			3		91	
			4		87	
			5		59	
			6		82	
			7		0	
			8		53	
			9		4	
			10		0	
			11		94	
			12		0	
			13		0	
			14		5	
			15		90	
			16		89	
			17		98	
			18		78	
			19		0	
			20		75	
			21		72	
			22		4	
			23		0	
			24		44	
			25		45	
			26		95	
			27		95	
			28		95	
			29		91	
			30		95	

CETIS Test Data Worksheet

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

Start Date: 30 Jun-17 **Species:** Strongylocentrotus purpuratus **Sample Code:** 170630sprt
End Date: 30 Jun-17 **Protocol:** EPA/600/R-95/136 (1995) **Sample Source:** Reference Toxicant
Sample Date: 30 Jun-17 **Material:** Copper chloride **Sample Station:** Copper Chloride

C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	28	100	93	AD 7/1/17
0	LC	2	17			
0	LC	3	29			
0	LC	4	27			
0	LC	5	11			
10		1	26	100	91	
10		2	30			
10		3	3			
10		4	1			
10		5	15			
20		1	4	100	87	
20		2	20			
20		3	18			
20		4	6			
20		5	16			
40		1	25	100	29	
40		2	8			
40		3	5			
40		4	21			
40		5	24			
80		1	14	100	5	
80		2	22			
80		3	7			
80		4	2			
80		5	9			
160		1	10	100	0	
160		2	13			
160		3	19			
160		4	23			
160		5	12			

QC: CG

Marine Chronic Bioassay | **Water Quality Measurements**

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

Sample ID: $CuCl_2$ | **Start Date/Time:** 6/30/2017 1750

Test No: 170630sprt | **End Date/Time:** 6/30/2017 1830

Dilutions made by: AD

High conc. made (µg/L):	160
Vol. Cu stock added (mL):	7.8
Final Volume (mL):	500
Cu stock concentration (µg/L):	10,200

Analyst: CH

	Initial Readings			
Concentration (µg/L)	**DO (mg/L)**	**pH (units)**	**Salinity (ppt)**	**Temperature (˚C)**
Lab Control	8.4	7.95	33.7	14.8
10	8.3	7.90	33.8	14.4
20	8.3	7.93	33.8	14.4
40	8.1	7.94	33.9	14.5
80	8.2	7.94	33.5	14.5
160	8.2	7.94	33.4	14.3

Comments:

QC Check: AC 7/5/17 | **Final Review:** KTP 7/6/17

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

Client: Internal
Sample ID: CuCl2
Test No.: 170630spr+

Start Date/Time: 6/30/2017 / 1750
End Date/Time: 6/30/2017 / 1830
Species: *S. purpuratus*
Animal Source: Pt. Loma
Date Collected: 6/6/17

Tech initials: AP
Injection Time: 1650

Sperm Absorbance at 400 nm: 1.009 (target range of 0.8 - 1.0 for density of $4x10^6$ sperm/ml)

Eggs Counted: 88, 76, 77, 80, 83 Mean: 80.8 X 50 = 4040 eggs/ml

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

Initial density: 4040 eggs/ml = ____ dilution factor
Final density: 4000 eggs/ml - 1.0 part egg stock
[ ] parts seawater

(A) 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):	1710	50:1	84,83	16,17
Eggs Added (0.5 ml):	1723	100:1	96	4
Test Ended:	1733	—	—	—
		—	—	—

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: 75:1

	Time (B)		Fert.	Unfert.
Sperm Added (100 µl):	1750	QC1	97	3
Eggs Added (0.5 ml):	1810	QC2	94	6
Test Ended:	1830	Egg Control 1	0	100
		Egg Control 2	0	100

Comments: (A) no dilution required
(B) AP @ 8 6/30/17

QC Check: AC 7/5/17

Final Review: KFP 7/6/17

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.