

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 Sample Collection Date: June 2, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: July 3, 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.
- o All test results have met internal Quality Assurance Program requirements.

California 4340 Vandever Avenue San Diego, California 92120

858.587.7333 fax: 858.587.3961 Results verified by: ______ Adrienne Cibor

EXECUTIVE SUMMARY

MONTHLY CHRONIC TOXICITY TESTING CARLSBAD DESALINATION PLANT — JUNE 2017 ORDER NO. R9-2006-0065; NPDES NO. CA0109223

Sampling Date: June 2, 2017

Test Date: June 2, 2017

Sample I Ds: M-001 Brine Effluent

M-001

Effluent Limitation: 16.5 TUc

Results Summary:

Bioassay Type:	M-001 Efflu Resu		Effluent Limitation Met? (Yes/No)
	<u>NOEC</u>	<u>TU</u> c	\/
Echinoderm Fertilization	6.06	16.5	Yes

TOXICITY SUMMARY REPORT Client: IDE Americas, Inc.
Test IDs: 1706-S024 to S025 Sample Collection Date: June 2, 2017

INTRODUCTION

A 24-hour composite discharge sample was collected in June 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) for monthly chronic toxicity monitoring purposes according to the permit that was adopted in 2006 (Order No. R9-2006-0065). Bioassay testing was conducted at the Nautilus Environmental (Nautilus) laboratory in San Diego, California on June 2, 2017 using the purple urchin (*Strongylocentrotus purpuratus*) chronic fertilization test.

MATERIALS AND METHODS

The sample was collected on June 2, 2017. Sample collection was performed by IDE Americas, Inc. (IDE) personnel, and the sample was hand delivered to Nautilus the day after sample collection. Following arrival at Nautilus, an aliquot of the sample was poured off and the following water quality parameters were measured: pH, dissolved oxygen (DO), temperature, salinity, alkalinity, and total chlorine. The sample was stored at 4° C in the dark until used for testing. A summary of the sample collection and receipt information is provided in Table 1, and water quality parameters measured upon receipt at Nautilus are presented in Table 2. Testing was conducted in accordance with the protocol described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project: IDE Americas, Inc./ Carlsbad Desalination Plant

Monitoring Period: June 2017 (monthly chronic monitoring)

Sample ID, Material: M-001, desalination plant brine effluent

Sample Collection Date, Time: 6/2/17, 09:00

Sample Receipt Date, Time: 6/2/17, 12:04

Sampling Method: Composite

Table 2. Water Quality Measurements upon Sample Receipt

Sample ID	рН	DO (mg/L)	Temp (°C)	Salinity (ppt)	Alkalinity (mg/L as CaCO₃)	Total Chlorine (mg/L)
M-001	7.96	7.7	5.0	63.8	192	0.03

TOXICITY SUMMARY REPORT Client: IDE Americas, Inc.
Test IDs: 1706-S024 to S025 Sample Collection Date: June 2, 2017

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Period: 6/2/17, 17:00 through 17:40

Test Organism: Strongylocentrotus purpuratus (purple sea urchin)

Test Organism Source: Field-collected off Point Loma in San Diego, CA

Lab Control/Dilution Water: Natural seawater (source: Scripps Institution of Oceanography (SIO) inlet),

34±2 parts per thousand (ppt); 20-µm filtered

Additional Control: High Salinity Control (HSC) – seawater with Nautilus hypersaline brine

added to match the 15 percent concentration of the sample with the highest salinity; tested to evaluate potential adverse effects due to

elevated salinity alone.

Test Concentrations: 2.5, 5.0, 6.06, 10, and 15 percent unadjusted M-001 sample; lab control.

The same dilution series was also tested with the sample after adjustment to 40 ppt per request from Poseidon. This adjustment was performed to replicate sample adjustment allowable in the permit for acute testing to reflect maximum salinity concentrations in the effluent prior to discharge to the ocean (i.e., the maximum daily average salinity concentration limit for the combined Encina Power Station Discharge (EPS) and CDP

discharges).

Number of Replicates, Organisms

per Replicate:

5 replicates, 2000 eggs per replicate. Sperm to egg ratio determined

before each test with a preliminary rangefinding test.

Test Chamber Type, Volume per

Replicate:

Glass scintillation vial containing 10 mL of test solution

Protocol Used: EPA/600/R-95/136, 1995 West Coast Marine Chronic

Test Type: Fertilization; 20-min sperm exposure to effluent followed by a 20-min egg

fertilization period

Acceptability Criteria: Mean fertilization ≥70% in the control, and percent minimum significant

difference (PMSD) value < 25%

Reference Toxicant Testing: Copper chloride

Statistical Analysis Software: CETIS™, version 1.8.7.20

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

In addition to EPA flowchart statistical methods, the results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National 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 this sample, the in-stream waste concentration (IWC) is 6.06

Client: IDE Americas, Inc. Sample Collection Date: June 2, 2017

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

RESULTS

There was a statistically significant decrease in the fertilization rate of the 10 and 15 percent test concentrations of the unadjusted M-001 sample relative to the lab control. Therefore, the NOEC is reported as 6.06 percent effluent and a TU_c equal to 16.5, which meets the maximum permit effluent limitation. The high salinity control scored higher than the lab control, indicating that effects observed in the 10 and 15 percent concentrations of the unadjusted M-001 sample were not likely due to elevated salinity.

The 40 ppt adjusted M-001 effluent sample resulted in no significant effects in any test concentration using the EPA 1995 flowchart statistics. Additionally, none of the test concentrations in the M-001 unadjusted or 40 ppt adjusted sample were significantly reduced from control using the TST statistical analysis.

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

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

Sample ID	NOEC (% sample)	LOEC (% sample)	EC ₅₀ (% sample)	TU _c value (toxic units)	TST Result (Pass/Fail)	Percent Effect at IWC
M-001 (unadjusted)	6.06	10	>15	16.5	Pass	1.7
M-001 (40 ppt adjusted)	15	>15	>15	<6.67	Pass	-0.21

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.

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

Test Concentration	M-001 L	Jnadjusted Sample	M-001	40 ppt Adjusted ^a
(% Sample)	Salinity (ppt)	Mean Percent Fertilization	Salinity (ppt)	Mean Percent Fertilization
Lab Control	33.3	93.2	33.3	93.6
High Salinity Control	38.2	95.8		
2.5	34.6	94.6	33.6	94.2
5.0	35.2	92.4	34.0	92.8
6.06	35.6	91.6	34.1	93.8
10	36.9	86.6*	34.4	94.6
15	38.2	87.2*	34.7	94.4

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

QUALITY ASSURANCE

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

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

Results for the concurrent reference toxicant test used to monitor laboratory performance and test organism sensitivity are summarized in Table 6 and presented in full in Appendix D. The reference toxicant test met all test acceptability criteria. The median effect concentration (EC_{50} value) was 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 6. Reference Toxicant Test Results

Test Species	Endpoint	EC ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ±2 SD (µg/L Copper)	CV (%)
Purple Urchin	Fertilization	59.9	43.5 ± 25.9	29.7

 EC_{50} = Concentration expected to cause an adverse effect to 50 percent of the test organisms Historical Mean $EC_{50} \pm 2$ SD = Mean of historical test results plus or minus two standard deviations CV = Coefficient of Variation

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

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Sample Collection Date: June 2, 2017

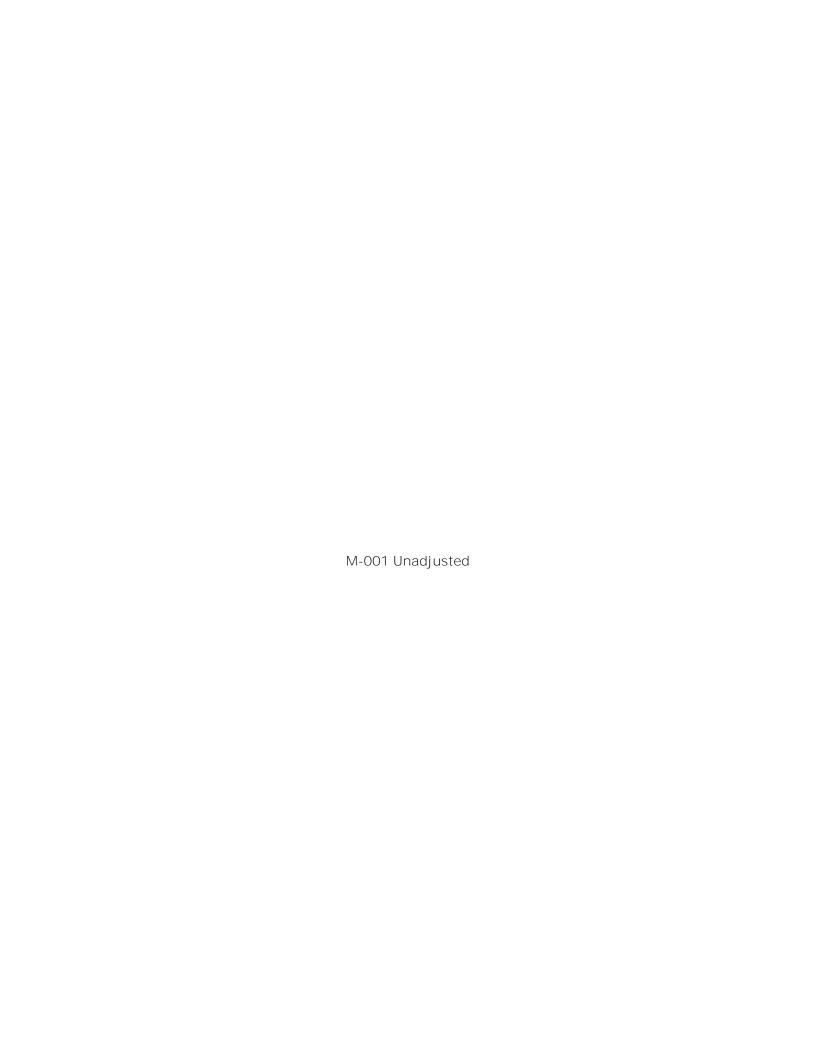
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- Phillips, B.M., B.S. Anderson, K. Siegler, J.P. Voorhees, S. Katz, L. Jennings and R.S. Tjeerdema. 2012. Hyper-Saline Toxicity Thresholds for Nine California Ocean Plan Toxicity Test Protocols. Final Report. University of California, Davis, Department of Environmental Toxicology at Granite Canyon.
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- USEPA. 2000. Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications Under the National Pollutant Discharge Elimination System. United States Environmental Protection Agency Office of Wastewater Management (EPA-833-R-00-003).
- USEPA. 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document. EPA/833/R-10/003. June 2010.

Appendix A

Test Data and Statistical Analyses



CETIS Summary Report

Report Date:

03 Jul-17 11:01 (p 1 of 1)

Test Code:

1706-S024 | 06-0628-3887

Echinoid Speri	m Cell Fertilizat	tion Test 1	5C					1001 0000	•		***************************************	
Echinola Speri	in Centrertinza	ion rest i		3						Nautilu	s Environi	mental (CA)
Start Date: Ending Date:	12-3331-6812 02 Jun-17 17:00 02 Jun-17 17:40 40m	Pro Spe	tocol: cies:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma		tus		Analyst: Diluent: Brine: Age:		ural Seawat Applicable	er	
	17-9108-9139	Cod		17-0648				Client:	IDE			
· -	02 Jun-17 09:00			Facility Effluent				Project:	Carl	sbad Desal	Plant	
	02 Jun-17 12:04			IDE Americas, I								
Sample Age:	8n (5 C)	Stat	ion:	M-001 Unadjust	ied .							
Comparison Su	ummary											
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	ΤU	Met	nod			
06-9344-9627	Fertilization Rat	e	6.06	10	7.785	5.62%	16.5	Dun	nett M	lultiple Com	parison Te	st
Point Estimate	Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Met	nod			
00-4013-9584	Fertilization Rat	е	EC25	>15	N/A	N/A	<6.6	67 Line	ar Inte	erpolation (I	CPIN)	
			EC50	>15	N/A	N/A	<6.6	67		·	,	
Test Acceptabi	ility			A 200 Maria (190 Maria								
Analysis ID	Endpoint		Attribu	te	Test Stat	TAC Limi	ts	Ove	rlap	Decision		
00-4013-9584	Fertilization Rate	е	Contro	Resp	0.932	0.7 - NL		Yes		Passes A	cceptability	Criteria
06-9344-9627	Fertilization Rate	9	Contro	Resp	0.932	0.7 - NL		Yes		Passes A	cceptability	Criteria
06-9344-9627	Fertilization Rate	9	PMSD		0.05615	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization Ra	ite Summary											
C-% C	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std	Err	Std Dev	CV%	%Effect
0 F	High Salinity Co	5	0.958	0.9341	0.9819	0.94	0.99	0.00	8602	0.01924	2.01%	0.0%
0 L	₋ab Control	5	0.932	0.8798	0.9842	0.87	0.98	0.01	881	0.04207	4.51%	2.71%
2.5		5	0.946	0.9272	0.9648	0.93	0.96	0.00	6782	0.01517	1.6%	1.25%
5		5	0.924	0.8823	0.9657	0.9	0.97	0.01	503	0.03362	3.64%	3.55%
6.06		5	0.916	0.8594	0.9726	0.87	0.97	0.02	04	0.04561	4.98%	4.38%
10		5	0.866	0.8107	0.9213	0.82	0.92	0.01	99 .	0.0445	5.14%	9.6%
15		5	0.872	0.8354	0.9086	0.85	0.92	0.01	319	0.0295	3.38%	8.98%
Fertilization Ra	te Detail											
	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0 H	High Salinity Co	0.95	0.96	0.94	0.99	0.95						
0 L	ab Control	0.87	0.98	0.96	0.93	0.92						
2.5		0.00	0.00	0.93	0.93	0.95						
2.0		0.96	0.96	0.55	0.00	0.00						
5		0.96	0.96	0.97	0.95							
				0.97	0.95	0.9						
5		0.9	0.9									

Report Date:

03 Jul-17 11:01 (p 1 of 2)

Test Code:

1706-S024 | 06-0628-3887

							1631	Code:		0-3024 0	6-0628-3887
Echinoid Sp	erm Cell Fertiliza	ation Test 1	15C	-					Nautilus	Environ	mental (CA)
Analysis ID:	06-9344-9627	En	dpoint: Fer	tilization Raf	te		CET	S Version	: CETISv1	.8.7	
Analyzed:	03 Jul-17 11:0	1 An	alysis: Par	ametric-Con	trol vs Trea	tments	Offic	ial Results	s: Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C > T	NA	NA:		5.62%	6.06	10	7.785	16.5
Dunnett Mul	tiple Compariso	n Test				·.				1000	
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5		-0.4815	2.362	0.101 8	0.9375	CDF	Non-Sign	ificant Effect		
	5		0.4596	2.362	0.101 8	0.6644	CDF	J	ificant Effect		
	6.06		0.7369	2.362	0.101 8	0.5400	CDF	Non-Siar	ificant Effect		
	10*		2.753	2.362	0.101 8	0.0221	CDF	Significar			
	15*		2.587	2.362	0.101 8	0.0315	CDF	Significa			
ANOVA Tabl	e										
Source	Sum Squ	ıares	Mean Squ	iare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.083519	89	0.0167039	8	5	3.659	0.0133	Significar			XXXX
Error	0.109567	3	0.0045653	04	24			Ū			
Total	0.193087	2			29	_					
Distribution	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of V	'ariance	4.216	15.09	0.5188	Equal Var	iances		VIII.0.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.1.	
Distribution	Shapiro-	Wilk W Nori	mality	0.967	0.9031	0.4597	Normal Di	stribution			
Fertilization	Rate Summary										
C-%	Control Type	Count									
•			Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	Mean 0.932	95% LCL 0.8798	95% UCL 0.9842	Median 0.93	Min 0.87	Max 0.98	Std Err 0.01881	CV% 4.51%	%Effect 0.0%
0 2.5	Lab Control										***************************************
_	Lab Control	5	0.932	0.8798	0.9842	0.93	0.87	0.98	0.01881	4.51%	0.0%
2.5	Lab Control	5 5	0.932 0.946	0.8798 0.9272	0.9842 0.9648	0.93 0.95	0.87 0.93	0.98 0.96	0.01881 0.006782	4.51% 1.6%	0.0% -1.5%
2.5 5	Lab Control	5 5 5	0.932 0.946 0.924	0.8798 0.9272 0.8823	0.9842 0.9648 0.9657	0.93 0.95 0.9	0.87 0.93 0.9	0.98 0.96 0.97	0.01881 0.006782 0.01503	4.51% 1.6% 3.64%	0.0% -1.5% 0.86%
2.5 5 6.06	Lab Control	5 5 5 5	0.932 0.946 0.924 0.916	0.8798 0.9272 0.8823 0.8594	0.9842 0.9648 0.9657 0.9726	0.93 0.95 0.9 0.92	0.87 0.93 0.9 0.87	0.98 0.96 0.97 0.97	0.01881 0.006782 0.01503 0.0204	4.51% 1.6% 3.64% 4.98%	0.0% -1.5% 0.86% 1.72%
2.5 5 6.06 10 15	Lab Control	5 5 5 5 5 5	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107	0.9842 0.9648 0.9657 0.9726 0.9213	0.93 0.95 0.9 0.92 0.88	0.87 0.93 0.9 0.87 0.82	0.98 0.96 0.97 0.97	0.01881 0.006782 0.01503 0.0204 0.0199	4.51% 1.6% 3.64% 4.98% 5.14%	0.0% -1.5% 0.86% 1.72% 7.08%
2.5 5 6.06 10 15		5 5 5 5 5 5	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107	0.9842 0.9648 0.9657 0.9726 0.9213	0.93 0.95 0.9 0.92 0.88	0.87 0.93 0.9 0.87 0.82	0.98 0.96 0.97 0.97	0.01881 0.006782 0.01503 0.0204 0.0199	4.51% 1.6% 3.64% 4.98% 5.14%	0.0% -1.5% 0.86% 1.72% 7.08%
2.5 5 6.06 10 15 Angular (Co	rrected) Transfor	5 5 5 5 5 5 5	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086	0.93 0.95 0.9 0.92 0.88 0.86	0.87 0.93 0.9 0.87 0.82 0.85	0.98 0.96 0.97 0.97 0.92 0.92	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319	4.51% 1.6% 3.64% 4.98% 5.14% 3.38%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44%
2.5 5 6.06 10 15 Angular (Cor C-%	rrected) Transfor Control Type	5 5 5 5 5 5 rmed Sumn	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086	0.93 0.95 0.9 0.92 0.88 0.86	0.87 0.93 0.9 0.87 0.82 0.85	0.98 0.96 0.97 0.97 0.92 0.92	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319	4.51% 1.6% 3.64% 4.98% 5.14% 3.38%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44%
2.5 5 6.06 10 15 Angular (Coo C-% 0 2.5	rrected) Transfor Control Type	5 5 5 5 5 5 rmed Sumn Count	0.932 0.946 0.924 0.916 0.866 0.872 mary Mean 1.317	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354 95% LCL	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086 95% UCL 1.425	0.93 0.95 0.9 0.92 0.88 0.86 Median 1.303	0.87 0.93 0.9 0.87 0.82 0.85 Min 1.202	0.98 0.96 0.97 0.97 0.92 0.92 Max 1.429	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319 Std Err 0.0386	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0%
2.5 5 6.06 10 15 Angular (Cor C- %	rrected) Transfor Control Type	5 5 5 5 5 5 7med Summ Count	0.932 0.946 0.924 0.916 0.866 0.872 mary Mean 1.317 1.338	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354 95% LCL 1.21 1.297	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086 95% UCL 1.425 1.38	0.93 0.95 0.9 0.92 0.88 0.86 Median 1.303 1.345	0.87 0.93 0.9 0.87 0.82 0.85 Min 1.202 1.303	0.98 0.96 0.97 0.97 0.92 0.92 Max 1.429 1.369	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319 Std Err 0.0386 0.01496	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55% 2.5%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0% -1.56%
2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	rrected) Transfor Control Type	5 5 5 5 5 5 5 Count 5 5	0.932 0.946 0.924 0.916 0.866 0.872 Mean 1.317 1.338 1.298	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354 95% LCL 1.21 1.297 1.212	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086 95% UCL 1.425 1.38 1.384	0.93 0.95 0.9 0.92 0.88 0.86 Median 1.303 1.345 1.249	0.87 0.93 0.9 0.87 0.82 0.85 Min 1.202 1.303 1.249	0.98 0.96 0.97 0.97 0.92 0.92 Max 1.429 1.369 1.397	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319 Std Err 0.0386 0.01496 0.03096	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55% 2.5% 5.33%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0% -1.56% 1.49%

0.5

0.3 0.2 0.1 0.0

0 LC

2.5

6.06

C-%

10

15

Report Date: Test Code:

03 Jul-17 11:01 (p 2 of 2) 1706-S024 | 06-0628-3887

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) Analysis ID: 06-9344-9627 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 03 Jul-17 11:01 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 1.0 F 0.12 0.08 0.8 Fertilization Rate 0.04 0.6

-0.04

-0.12

-2.5 -2.0

1.0 1.5 2.0

Rankits

Echinoid Sperm Cell Fertilization Test 15C

Report Date:

03 Jul-17 11:01 (p 1 of 1)

Test Code:

1706-S024 | 06-0628-3887

Nautilus Environmental (CA)

Analysis ID: 00-4013-9584 Analyzed: 03 Jul-17 11:01

Endpoint: Fertilization Rate Analysis:

Linear Interpolation (ICPIN)

CETIS Version: Official Results: Yes

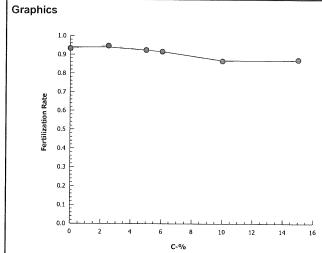
CETISv1.8.7

Linear Interpola	tion Options				
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1880569	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	

Fertilizat	tion Rate Summary		Calculated Variate(A/B)								
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.932	0.87	0.98	0.01881	0.04207	4.51%	0.0%	466	500
2.5		5	0.946	0.93	0.96	0.006782	0.01517	1.6%	-1.5%	473	500
5		5	0.924	0.9	0.97	0.01503	0.03362	3.64%	0.86%	462	500
6.06		5	0.916	0.87	0.97	0.0204	0.04561	4.98%	1.72%	458	500
10		5	0.866	0.82	0.92	0.0199	0.0445	5.14%	7.08%	433	500
15		5	0.872	0.85	0.92	0.01319	0.0295	3.38%	6.44%	436	500



Report Date:

03 Jul-17 11:02 (p 1 of 1)

Test Code:	1706	3-S0	24	06-0628-38	2
	 		-		
	 	_			

							lest	Code:	170	6-8024 0	6-0628-388
Echinoid Sp	erm Cell Fertiliz	ation Test	15C	TST					Nautilus	s Environ	mental (CA)
Analysis ID:	14-3170-3772	En	dpoint: Fer	tilization Ra	te		CET	IS Version:	CETISv1	.8.7	
Analyzed:	03 Jul-17 11:0)1 A n	alysis: Pa	rametric Bio	equivalence	-Two Samp	le Offic	cial Results	: Yes		
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Cor	rected)	NA	C*b < T	NA	NA	0.75	3.38%	15	>15	NA	6.667
TST-Welch's	t Test										
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5*		10.74	2.015	0.066 5	<0.0001	CDF	Non-Signi	ficant Effect		
	5*		7.308	1.895	0.080 7	<0.0001	CDF		ificant Effect		
	6.06*		6.167	1.895	0.092 7	0.0002	CDF	Non-Signi	ficant Effect		
	10*		5.116	1.895	0.078 7	0.0007	CDF	-	ficant Effect		
	15*		6.13	1.895	0.068 7	0.0002	CDF	_	ficant Effect		
ANOVA Tabl	е										
Source	Sum Squ	ares .	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.083519	89	0.0167039	98	5	3.659	0.0133	Significan			, , , , , , , , , , , , , , , , , , ,
Error	0.109567	3	0.0045653	804	24			- 3			
Total	0.193087	2			29	_					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of √	/ariance	4.216	15.09	0.5188	Equal Var				
Distribution	Shapiro-	Wilk W Nor	mality	0.967	0.9031	0.4597	Normal Di				
Eartilization	Data Summanı										
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
	-	Count 5	Mean 0.932	95% LCL 0.8798	95% UCL 0.9842	Median 0.93	Min 0.87	Max 0.98	Std Err 0.01881	CV% 4.51%	
C-%	Control Type				···						0.0%
C-% 0 2.5 5	Control Type	5	0.932	0.8798	0.9842	0.93	0.87	0.98	0.01881	4.51%	
C- % 0 2.5	Control Type	5 5	0.932 0.946	0.8798 0.9272	0.9842 0.9648	0.93 0.95	0.87 0.93	0.98 0.96	0.01881 0.006782	4.51% 1.6% 3.64%	0.0% -1.5% 0.86%
C-% 0 2.5 5	Control Type	5 5 5	0.932 0.946 0.924	0.8798 0.9272 0.8823	0.9842 0.9648 0.9657	0.93 0.95 0.9	0.87 0.93 0.9	0.98 0.96 0.97	0.01881 0.006782 0.01503	4.51% 1.6%	0.0% -1.5% 0.86% 1.72%
C-% 0 2.5 5 6.06	Control Type	5 5 5 5	0.932 0.946 0.924 0.916	0.8798 0.9272 0.8823 0.8594	0.9842 0.9648 0.9657 0.9726	0.93 0.95 0.9 0.92	0.87 0.93 0.9 0.87	0.98 0.96 0.97 0.97	0.01881 0.006782 0.01503 0.0204	4.51% 1.6% 3.64% 4.98%	0.0% -1.5% 0.86%
C-% 0 2.5 5 6.06 10 15	Control Type	5 5 5 5 5 5	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107	0.9842 0.9648 0.9657 0.9726 0.9213	0.93 0.95 0.9 0.92 0.88	0.87 0.93 0.9 0.87 0.82	0.98 0.96 0.97 0.97 0.92	0.01881 0.006782 0.01503 0.0204 0.0199	4.51% 1.6% 3.64% 4.98% 5.14%	0.0% -1.5% 0.86% 1.72% 7.08%
C-% 0 2.5 5 6.06 10 15	Control Type Lab Control	5 5 5 5 5 5	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107	0.9842 0.9648 0.9657 0.9726 0.9213	0.93 0.95 0.9 0.92 0.88	0.87 0.93 0.9 0.87 0.82	0.98 0.96 0.97 0.97 0.92	0.01881 0.006782 0.01503 0.0204 0.0199	4.51% 1.6% 3.64% 4.98% 5.14%	0.0% -1.5% 0.86% 1.72% 7.08%
C-% 0 2.5 5 6.06 10 15 Angular (Cor	Control Type Lab Control	5 5 5 5 5 5 5	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086	0.93 0.95 0.9 0.92 0.88 0.86	0.87 0.93 0.9 0.87 0.82 0.85	0.98 0.96 0.97 0.97 0.92 0.92	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319	4.51% 1.6% 3.64% 4.98% 5.14% 3.38%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44%
C-% 0 2.5 5 6.06 10 15 Angular (Cor	Control Type Lab Control rected) Transfor Control Type	5 5 5 5 5 5 5 7med Sumn	0.932 0.946 0.924 0.916 0.866 0.872	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086	0.93 0.95 0.9 0.92 0.88 0.86	0.87 0.93 0.9 0.87 0.82 0.85	0.98 0.96 0.97 0.97 0.92 0.92	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0	Control Type Lab Control rected) Transfor Control Type	5 5 5 5 5 5 5 Crmed Sumn Count	0.932 0.946 0.924 0.916 0.866 0.872 mary Mean 1.317	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354 95% LCL	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086 95% UCL 1.425	0.93 0.95 0.9 0.92 0.88 0.86 Median	0.87 0.93 0.9 0.87 0.82 0.85 Min 1.202 1.303	0.98 0.96 0.97 0.97 0.92 0.92 Max 1.429	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319 Std Err 0.0386 0.01496	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55% 2.5%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0% -1.56%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Control Type Lab Control rected) Transfor Control Type	5 5 5 5 5 5 7med Sumn Count 5	0.932 0.946 0.924 0.916 0.866 0.872 Mean 1.317 1.338	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354 95% LCL 1.21 1.297	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086 95% UCL 1.425 1.38	0.93 0.95 0.9 0.92 0.88 0.86 Median 1.303 1.345	0.87 0.93 0.9 0.87 0.82 0.85 Min 1.202	0.98 0.96 0.97 0.97 0.92 0.92 Max 1.429 1.369 1.397	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319 Std Err 0.0386 0.01496 0.03096	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55% 2.5% 5.33%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0% -1.56% 1.49%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	Control Type Lab Control rected) Transfor Control Type	5 5 5 5 5 5 med Sumn Count 5 5	0.932 0.946 0.924 0.916 0.866 0.872 mary Mean 1.317 1.338 1.298	0.8798 0.9272 0.8823 0.8594 0.8107 0.8354 95% LCL 1.21 1.297 1.212	0.9842 0.9648 0.9657 0.9726 0.9213 0.9086 95% UCL 1.425 1.38 1.384	0.93 0.95 0.9 0.92 0.88 0.86 Median 1.303 1.345 1.249	0.87 0.93 0.9 0.87 0.82 0.85 Min 1.202 1.303 1.249	0.98 0.96 0.97 0.97 0.92 0.92 Max 1.429 1.369	0.01881 0.006782 0.01503 0.0204 0.0199 0.01319 Std Err 0.0386 0.01496	4.51% 1.6% 3.64% 4.98% 5.14% 3.38% CV% 6.55% 2.5%	0.0% -1.5% 0.86% 1.72% 7.08% 6.44% %Effect 0.0% -1.56%

Report Date:

02 Jun-17 09:18 (p 1 of 1) 06-0628-3887/1706-S024

Test Code:

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	02 Jun-17	Species:	Strongylocentrotus purpuratus	Sample Code:	17-0648
End Date:⊘⊃	_ 02 Jun-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	IDE Americas, Inc.
Sample Date:	01 Jun-17	Material:	Facility Effluent	Sample Station:	M-001 Unadjusted

ample Da	0	Juli-17		Materia	II. Facility Ellius	Sample Station. W-001 Onaujusteu
C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			31	100	85	BO, 6/6/17
			32	100	86	
			33	100	88	
			34	106	97	
			35	100	89	
			36	100	92	
			37	100	82	
			38	00/	93	
			39	100	96	
			40	100	92	
			41	100	9.5	
			42	100	87	
			43	100	82	
			44	100	93 87	
			45	100	87	
			46	100	90	
			47	100	92	
			48	100	95	
			49	100	88 95	
			50	100	95	
			51	100	90	
			52	100	93	
			53	100	87	
	-		54	100	90	
			55	100	96	
			56	100	92	
			57	100	96	
			58	100	98	
			59	100		
			60	100	85	V

@Q18 ACK 7/3/17

CETIS Test Data Worksheet

Report Date: Test Code:

02 Jun-17 09:18 (p 1 of 1) 06-0628-3887/1706-S024

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	02 Jun-17	Species:	Strongylocentrotus purpuratus	Sample Code:	
End Date: 🕥	.02 Jun-17	Protocol:	EPA/600/R-95/136 (1995)	•	IDE Americas, Inc.
End Date: () Sample Date:	ຽ∢ Jun-17	Material:	Facility Effluent	Sample Station:	M-001 Unadjusted

Sample Date	≥ 00€ .	lun-17		Materia	al: Facility Et	
Sample Dat C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	42	100	95	A6 6/2/17
0	LC	2	58			
0	LC	3	39			
0	LC	4	44			
0	LC	5	56			
2.5		1	57	100	92	A6 6/2/17
2.5		2	55		-	
2.5		3	38			
2.5		4	52			
2.5		5	48			
5		1	46	100	97	A6 6/2/17
5		2	54			
5		3	59			
5		4	41			
5		5	51			
6.06		1	45	100	96	AC 6/2/17
6.06		2	34			
6.06		3	53			
6.06		4	50			
6.06		5	40			
10		1	37	100	90	166/2117
10		2	49			
10		3	43			
10		4	36			
10		5	35			. 1
15		1	32	100	a5	A6 6/2119
15		2	31			
15		3	60			
15		4	47			
15		5	33			

HSC A a8/100 A6 6/2/17
B
C
D
E

@01847/3/17

Water Quality Measurements

Client :	IDE	Test Species: S. purpuratus				
Sample ID:	M-001 (unadjusted)	Start Date/Time: 6/2/2017 \700				
Sample Log No.:	17- 0648	End Date/Time: 6/2/2017 ¹ づけの				

Dilutions made by: Test No: 1706-5024

			Analyst:	AO							
	Initial Readings										
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)							
Lab Control	7.9	8.01	33.6	15.6							
2.5	8.1	8.07	34.6	15.8							
5.0	8-1	806	35.2	15.8							
6.06	8.1	8.06	35.6	15.6							
10	8.1	8.00	36.9	15.5							
15	8.0	8.05	38.2	15.7							
HSC	8.0	8.08	38.2	15.8							

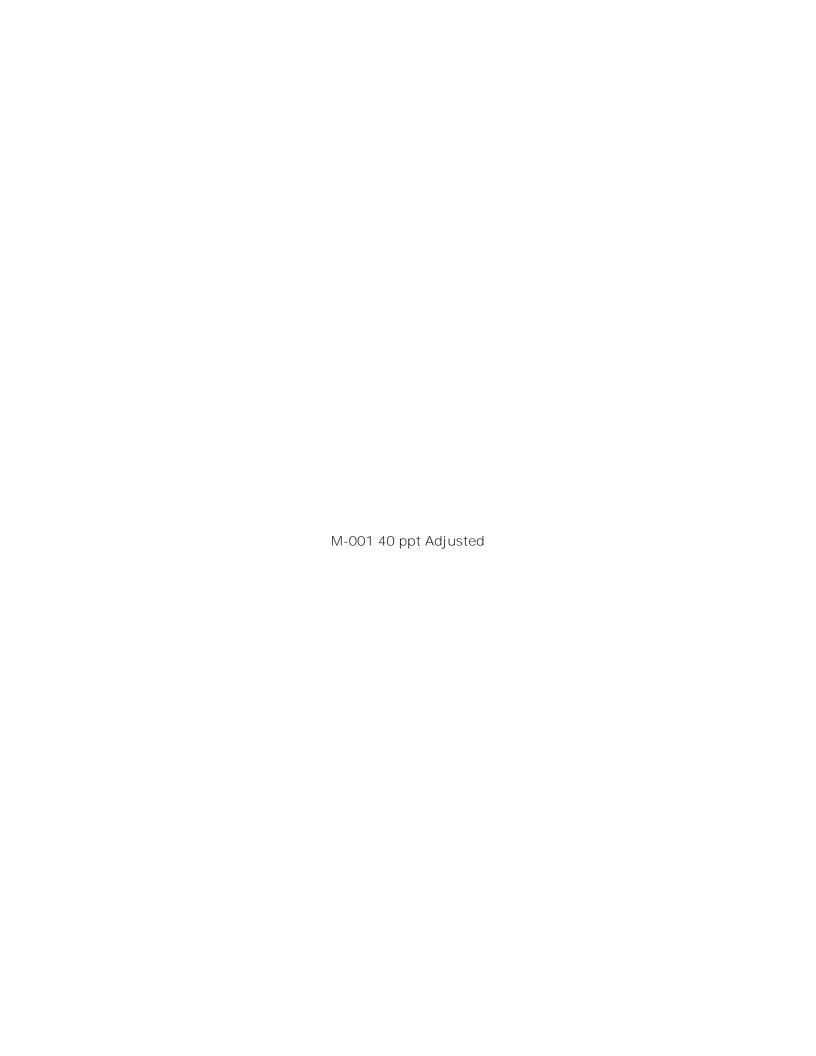
Comments:		
QC Check:	EG 6/13/17	Final Review: AC 7/3/17

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

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

Echinoderm Sperm-Cell Fertilization Worksheet

O".	105						
Client: Sample ID:	M-001 Unadji	1 .				ne: <u>6/2/2017</u>	
Test No.:	1706-5024	onea				ne: 6/2/2017	
	11-0 3001					es: <i>S. purpui</i> ce: Point Lor	
Tech initials:	A6					ce. <u>Foint Lor</u> ed: <i>ろ</i> /22	
Injection Time:	1626				Date Oblicet	cu. <u> </u>	
geetter. v.iiv.e.	.0/20						
Sperm Absorbance at 4		(target range of			10 ⁶ sperm/m	l)	
Eggs Counted:		in: <u>78</u> x 5	50 = 3,90	<u>රට</u> egg	s/ml		
	- 84						
		et counts of 80 eggs i			ick-		
	76 Rafte	r slide for a final dens	sity of 4000 eg	gs/ml)			
	76						
Initial density:	3,400 eggs/ml	= A dilu	tion factor	900	stock \	S∫ ml	
Final density:	4000 eggs/ml		egg stock		water	$\begin{pmatrix} 2 & 0 \\ A \end{pmatrix} \qquad ml$	
,	-99	[[]]	s seawater	300	water _	''''	÷
							•
Prepare the embryo sto	ck according to the calcula	ated dilution factor.	For example,	if the dilution	on factor is 2	.25, use 100	ml of existing
stock (1 part) and 125 n	nl of dilution water (1.25 pa	arts).					
	•						
D			Sperm:Egg				
Rangefinder Test: ml Sperm Stock	2000:1 1600 50 40		<u>800:1</u>	400:1	200:1	100:1	50:1
ml Seawater	50 40 0.0 10	30 20	20 30	10 40	5.0 45	2.5 47.5	1.25 48.75
	•	20	30	40	45	47.5	40.75
	Time	Rangefinder Ratio	o: Fert.	Unfe	rt.		
Sperm Added (100 µl):	1632	50:1.50.1	81,70	19,3	0		
Eggs Added (0.5 ml):	1642	1001/1001	96,91	6 4,1	4		
Test Ended:	1652	2001, 2001	100,1	00 0,0)		
		400.1,400.1	100,11	00 <u>0, (</u>	2		
NOTE: Choose a sperm	n-to-egg ratio that results i	n fertilization betwee	en 80 and 90	percent. If	more than or	ne concentra	tion is within
this range, choose the ra	atio closest to 90 percent u ctive season, site condition	inless professional _.	judgment dict	ates consid	eration of oth	ner factors (e	.g., organism
nealth, stage of reproduc	silve season, site condition	15).					
Definitive Test	•	Sperm:Egg Ratio	Usod: IA	cs: 1			
301111111111111111111111111111111111111	_	operm.egg Ratio	03eu. <u>(</u>	· · · ·			
•	Time		Fert.	Unfe	rt		
Sperm Added (100 µl):	1700	QC1	98	2			
Eggs Added (0.5 ml):	1720	QC2	<u> </u>		_		
Test Ended:	1740	Egg Control 1	Q4 0		- 2		₹ .
		Egg Control 2	0				*,
		_33) ·		
Comments:	DNo Dilotion P	equired					
		U					
	el chala					۸	7/21
QC Check:	EG 6/13/17				Final Review	N: <u>A</u> C	113/17



CETIS Summary Report

Report Date:

03 Jul-17 11:06 (p 1 of 1)

Test Code:

1706-S025 | 09-5761-0582

				The state of the s				Test Code		170	6-5025 0	9-5761-0582
Echinoid Spe	rm Cell Fertiliza	tion Te	st 15C							Nautilus	Environ	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	02 Jun-17 17:00 02 Jun-17 17:40		Test Type: Fertilization Protocol: EPA/600/R-95/136 (1995) Species: Strongylocentrotus purpuratus Source: Pt. Loma				Analyst: Diluent: Brine: Age:		ural Seawate Applicable	er		
· ·	02 Jun-17 09:00 Mater : 02 Jun-17 12:04 Sourc			erial: Facility Effluent rce: IDE Americas, Inc.				Client: Project:				
Comparison S	Summary			, to below								
Analysis ID	Endpoint		NOEL	LOEL	TOEL	PMSD	TU	Meti	hod			
18-8810-7863	Fertilization Ra	te	15	>15	NA	4.43% «	₹ 6.66	7 Duni	nett N	fultiple Com	parison Te	st
Point Estimat	e Summary										TO THE RESERVE OF THE PERSON O	
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Metl	nod			
17-2343-3849	Fertilization Ra	te	EC25 EC50	>15 >15	N/A N/A	N/A N/A	<6.6 <6.6		ar Inte	erpolation (IC	CPIN)	
Test Acceptab	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	its	Ove	rlap	Decision		
17-2343-3849	Fertilization Ra	te	Contro	Control Resp 0.936 0.7 - NL			Yes		Passes Ac	ceptability	Criteria	
18-8810-7863	Fertilization Rat			Control Resp		0.7 - NL		Yes		Passes Ac	ceptability	Criteria
18-8810-7863	Fertilization Rat	te	PMSD)	0.04426 NL - 0.25		****	No		Passes Ac	ceptability	Criteria
Fertilization R	tate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std	Err	Std Dev	CV%	%Effect
	Lab Control	5	0.936	0.8962	0.9758	0.9	0.98		435	0.03209	3.43%	0.0%
2.5		5	0.942	0.9216	0.9624	0.92	0.96	0.00	7348	0.01643	1.74%	-0.64%
5		5	0.928	0.8773	0.9787	0.86	0.96	0.01	828	0.04087	4.4%	0.85%
6.06		5	0.938	0.9059	0.9701	0.9	0.97	0.01	158	0.02588	2.76%	-0.21%
10		5	0.946	0.9125	0.9795	0.9	0.97			0.02702	2.86%	-1.07%
15		5	0.944	0.9329	0.9551	0.93	0.95	0.004	4	0.008944	0.95%	-0.85%
Fertilization R	tate Detail											
**************************************	Control Type	Rep 1	Rep 2		Rep 4	Rep 5				****		· · · · · · · · · · · · · · · · · · ·
	Lab Control	0.98	0.91	0.94	0.9	0.95						-
2.5		0.96	0.95	0.93	0.95	0.92						
5		0.93	0.93	0.96	0.86	0.96						
6.06		0.97	0.93	0.94	0.95	0.9						
10		0.97	0.95	0.9	0.95	0.96						
15		0.95	0.95	0.95	0.93	0.94						

Analyst: AC QA: 57/3/17

Report Date:

03 Jul-17 11:06 (p 1 of 2)

				Test Code:	1706-S025 09-5761-0582
Echinoid Spe	erm Cell Fertilization	Test 15C			Nautilus Environmental (CA)
Analysis ID:	18-8810-7863	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.7
Analyzed:	03 Jul-17 11:05	Analysis:	Parametric-Control vs Treatments	Official Results:	Yes

Analyzou: Go dui 17 11.	- AI	idiyələ. Tal	umetrie e	ona va meatmenta	OIII	ciai itesuit	3. 103		
Data Transform	Zeta	Alt Hyp	Trials	Seed	PMSD	NOEL	LOEL	TOEL	TU
Angular (Corrected)	NA	C > T	NA	NA	4.43%	15	>15	NA	6.667

Dunnett Multip	Dunnett Multiple Comparison Test											
Control	vs	C-%	Test Stat	Critical	MSD	DF	P-Value	P-Type	Decision(α:5%)			
Lab Control		2.5	-0.1972	2.362	0.082	8	0.8850	CDF	Non-Significant Effect			
		5	0.4609	2.362	0.082	8	0.6639	CDF	Non-Significant Effect			
		6.06	-0.02718	2.362	0.082	8	0.8412	CDF	Non-Significant Effect			
		10	-0.5336	2.362	0.082	8	0.9446	CDF	Non-Significant Effect			
		15	-0.284	2.362	0.062	8	0.9036	CDF	Non-Significant Effect			

ANOVA Table						
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.003416797	0.0006833594	5	0.2244	0.9483	Non-Significant Effect
Error	0.07307683	0.003044868	24			
Total	0.07649363		29			

Distributional Tes	Distributional Tests										
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Bartlett Equality of Variance	7.111	15.09	0.2125	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.9729	0.9031	0.6215	Normal Distribution						

Fertilizati	ion Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.936	0.8962	0.9758	0.94	0.9	0.98	0.01435	3.43%	0.0%
2.5		5	0.942	0.9216	0.9624	0.95	0.92	0.96	0.007348	1.74%	-0.64%
5		5	0.928	0.8773	0.9787	0.93	0.86	0.96	0.01828	4.4%	0.85%
6.06		5	0.938	0.9059	0.9701	0.94	0.9	0.97	0.01158	2.76%	-0.21%
10		5	0.946	0.9125	0.9795	0.95	0.9	0.97	0.01208	2.86%	-1.07%
15		5	0.944	0.9329	0.9551	0.95	0.93	0.95	0.004	0.95%	-0.85%

Angular (Angular (Corrected) Transformed Summary												
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect		
0	Lab Control	5	1.323	1.234	1.411	1.323	1.249	1.429	0.03195	5.4%	0.0%		
2.5		5	1.329	1.286	1.373	1.345	1.284	1.369	0.01559	2.62%	-0.52%		
5		5	1.306	1.214	1.399	1.303	1.187	1.369	0.03328	5.7%	1.22%		
6.06		5	1.323	1.256	1.391	1.323	1.249	1.397	0.02429	4.1%	-0.07%		
10		5	1.341	1.272	1.41	1.345	1.249	1.397	0.0249	4.15%	-1.41%		
15		5	1.332	1.309	1.356	1.345	1.303	1.345	0.008492	1.43%	-0.75%		

0.3 0.2

0.1

0 LC

2.5

6.06

C-%

10

15

Report Date:

03 Jul-17 11:06 (p 2 of 2)

1706-S025 | 09-5761-0582

1.0 1.5 2.0

Rankits

Test Code: **Echinoid Sperm Cell Fertilization Test 15C** Nautilus Environmental (CA) Analysis ID: 18-8810-7863 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 03 Jul-17 11:05 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 0.12 0.9 0.08 8.0 Fertilization Rate Centered Corr. Angle 0.04 0.6 0.00 0.4

-0.04

-0.08

-0.12

-2,5 -2.0

Report Date:

03 Jul-17 11:06 (p 1 of 1)

Test Code:

1706-S025 | 09-5761-0582

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 17-2343-3849

03 Jul-17 11:05

Endpoint: Fertilization Rate

Linear Interpolation (ICPIN)

CETIS Version:

CETISv1.8.7

Analyzed: Analysis:

Official Results: Yes

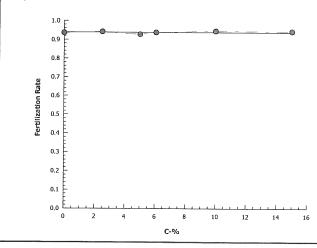
Linear Interpol	ation Options				
X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	1934435	1000	Yes	Two-Point Interpolation
1					

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	NΑ

Fertilizat	tion Rate Summary				Cal	culated Varia	te(A/B)				
C-%	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.936	0.9	0.98	0.01435	0.03209	3.43%	0.0%	468	500
2.5		5	0.942	0.92	0.96	0.007348	0.01643	1.74%	-0.64%	471	500
5		5	0.928	0.86	0.96	0.01828	0.04087	4.4%	0.85%	464	500
6.06		5	0.938	0.9	0.97	0.01158	0.02588	2.76%	-0.21%	469	500
10		5	0.946	0.9	0.97	0.01208	0.02702	2.86%	-1.07%	473	500
15		5	0.944	0.93	0.95	0.004	0.008944	0.95%	-0.85%	472	500

Graphics



Report Date:

03 Jul-17 11:07 (p 1 of 1)

Test Code:

1706-S025 | 09-5761-0582

											19-5/61-05
Echinoid Sp	erm Cell Fertiliz	ation Test	15C	TST	-				Nautilus	s Environ	mental (C
Analysis ID: Analyzed:	08-6389-0595 03 Jul-17 11:0		dpoint: Fer alysis: Par	tilization Ra ametric Bio		-Two Samp		IS Version: cial Results	CETISv1	.8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cor	rected)	NA	C*b < T	NA	NA	0.75	2.64%	15	>15	NA	6.667
TST-Welch's	s t Test								Real Control of the C		
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision(α:5%)		
Lab Control	2.5*	W. W. (1994)	11.81	1.943	0.056 6	<0.0001	CDF		ficant Effect		
	5*		7.67	1.895	0.078 7	<0.0001	CDF	ū	ficant Effect		
	6.06*		9.719	1.895	0.065 7	<0.0001	CDF	•	ficant Effect		
	10*		10.11	1.895	0.065 7	<0.0001	CDF	Ū	ficant Effect		
	15*		13.39	2.132	0.054 4	<0.0001	CDF	•	ficant Effect		
ANOVA Tabl	e										
Source	Sum Squ	uares	Mean Squ	are	DF	F Stat	P-Value	Decision(α:5%)		
Between	0.003416	797	0.0006833	594	5	0.2244	0.9483	· · · · · · · · · · · · · · · · · · ·	ficant Effect		
Error	0.073076	83	0.0030448	68	24						
Total	0.076493	63			29	_					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of \	/ariance	7.111	15.09	0.2125	Equal Var			· · · · · · · · · · · · · · · · · · ·	
Distribution		Wilk W Nor		0.9729	0.9031	0.6215	Normal Di				
Fertilization	Rate Summary										
C-%	Control Type	Count	Mean	95% LCL	95% UCL	8.0 1.					
^					,	Median	Min	Max	Std Err	CV%	%Effect
	Lab Control	5	0.936	0.8962	0.9758	0.94	Min 0.9	Max 0.98	Std Err 0.01435	CV% 3.43%	
	Lab Control	5 5	0.936 0.942		*				0.01435		0.0%
2.5	Lab Control			0.8962	0.9758	0.94	0.9	0.98		3.43%	
2.5 5	Lab Control	5	0.942	0.8962 0.9216	0.9758 0.9624	0.94 0.95	0.9 0.92	0.98 0.96	0.01435 0.007348	3.43% 1.74%	0.0% -0.64% 0.85%
2.5 5 6.06	Lab Control	5 5	0.942 0.928	0.8962 0.9216 0.8773	0.9758 0.9624 0.9787	0.94 0.95 0.93	0.9 0.92 0.86	0.98 0.96 0.96	0.01435 0.007348 0.01828	3.43% 1.74% 4.4%	0.0% -0.64% 0.85% -0.21%
2.5 5 6.06 10	Lab Control	5 5 5	0.942 0.928 0.938	0.8962 0.9216 0.8773 0.9059	0.9758 0.9624 0.9787 0.9701	0.94 0.95 0.93 0.94	0.9 0.92 0.86 0.9	0.98 0.96 0.96 0.97	0.01435 0.007348 0.01828 0.01158	3.43% 1.74% 4.4% 2.76%	0.0% -0.64% 0.85%
2.5 5 6.06 10 15	Lab Control	5 5 5 5	0.942 0.928 0.938 0.946 0.944	0.8962 0.9216 0.8773 0.9059 0.9125	0.9758 0.9624 0.9787 0.9701 0.9795	0.94 0.95 0.93 0.94 0.95	0.9 0.92 0.86 0.9	0.98 0.96 0.96 0.97 0.97	0.01435 0.007348 0.01828 0.01158 0.01208	3.43% 1.74% 4.4% 2.76% 2.86%	-0.64% 0.85% -0.21% -1.07%
2.5 5 6.06 10 15 Angular (Cor	rected) Transfor Control Type	5 5 5 5 5 rmed Sumr	0.942 0.928 0.938 0.946 0.944 mary	0.8962 0.9216 0.8773 0.9059 0.9125	0.9758 0.9624 0.9787 0.9701 0.9795	0.94 0.95 0.93 0.94 0.95	0.9 0.92 0.86 0.9	0.98 0.96 0.96 0.97 0.97	0.01435 0.007348 0.01828 0.01158 0.01208	3.43% 1.74% 4.4% 2.76% 2.86%	0.0% -0.64% 0.85% -0.21% -1.07%
2.5 5 6.06 10 15 Angular (Cor C-%	rected) Transfor	5 5 5 5 5 7med Summ Count	0.942 0.928 0.938 0.946 0.944	0.8962 0.9216 0.8773 0.9059 0.9125 0.9329	0.9758 0.9624 0.9787 0.9701 0.9795 0.9551	0.94 0.95 0.93 0.94 0.95 0.95	0.9 0.92 0.86 0.9 0.9	0.98 0.96 0.96 0.97 0.97	0.01435 0.007348 0.01828 0.01158 0.01208 0.004	3.43% 1.74% 4.4% 2.76% 2.86% 0.95%	0.0% -0.64% 0.85% -0.21% -1.07% -0.85%
2.5 5 5.06 10 15 Angular (Cor C-% 0	rected) Transfor Control Type	5 5 5 5 5 7med Summ Count 5	0.942 0.928 0.938 0.946 0.944 mary	0.8962 0.9216 0.8773 0.9059 0.9125 0.9329	0.9758 0.9624 0.9787 0.9701 0.9795 0.9551	0.94 0.95 0.93 0.94 0.95 0.95	0.9 0.92 0.86 0.9 0.9 0.93	0.98 0.96 0.96 0.97 0.97 0.95	0.01435 0.007348 0.01828 0.01158 0.01208 0.004	3.43% 1.74% 4.4% 2.76% 2.86% 0.95%	0.0% -0.64% 0.85% -0.21% -1.07% -0.85%
2.5 5 6.06 10 15 Angular (Cor C- % 0 2.5	rected) Transfor Control Type	5 5 5 5 5 7med Summ Count 5 5	0.942 0.928 0.938 0.946 0.944 Mean 1.323 1.329 1.306	0.8962 0.9216 0.8773 0.9059 0.9125 0.9329 95% LCL 1.234 1.286 1.214	0.9758 0.9624 0.9787 0.9701 0.9795 0.9551 95% UCL	0.94 0.95 0.93 0.94 0.95 0.95 Median	0.9 0.92 0.86 0.9 0.9 0.93 Min	0.98 0.96 0.96 0.97 0.97 0.95 Max	0.01435 0.007348 0.01828 0.01158 0.01208 0.004 Std Err 0.03195	3.43% 1.74% 4.4% 2.76% 2.86% 0.95% CV% 5.4%	0.0% -0.64% 0.85% -0.21% -1.07% -0.85% %Effect 0.0%
2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5 6.06	rected) Transfor Control Type	5 5 5 5 5 7med Summ Count 5 5 5	0.942 0.928 0.938 0.946 0.944 Mean 1.323 1.329	0.8962 0.9216 0.8773 0.9059 0.9125 0.9329 95% LCL 1.234 1.286	0.9758 0.9624 0.9787 0.9701 0.9795 0.9551 95% UCL 1.411 1.373	0.94 0.95 0.93 0.94 0.95 0.95 Median 1.323 1.345	0.9 0.92 0.86 0.9 0.9 0.93 Min 1.249 1.284	0.98 0.96 0.96 0.97 0.97 0.95 Max 1.429 1.369	0.01435 0.007348 0.01828 0.01158 0.01208 0.004 Std Err 0.03195 0.01559	3.43% 1.74% 4.4% 2.76% 2.86% 0.95% CV% 5.4% 2.62%	0.0% -0.64% 0.85% -0.21% -1.07% -0.85% %Effect 0.0% -0.52%
0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5 6.06 10	rected) Transfor Control Type	5 5 5 5 5 7med Summ Count 5 5	0.942 0.928 0.938 0.946 0.944 Mean 1.323 1.329 1.306	0.8962 0.9216 0.8773 0.9059 0.9125 0.9329 95% LCL 1.234 1.286 1.214	0.9758 0.9624 0.9787 0.9701 0.9795 0.9551 95% UCL 1.411 1.373 1.399	0.94 0.95 0.93 0.94 0.95 0.95 Median 1.323 1.345 1.303	0.9 0.92 0.86 0.9 0.93 Min 1.249 1.284 1.187	0.98 0.96 0.96 0.97 0.97 0.95 Max 1.429 1.369 1.369	0.01435 0.007348 0.01828 0.01158 0.01208 0.004 Std Err 0.03195 0.01559 0.03328	3.43% 1.74% 4.4% 2.76% 2.86% 0.95% CV% 5.4% 2.62% 5.7%	0.0% -0.64% 0.85% -0.21% -1.07% -0.85% %Effect 0.0% -0.52% 1.22%

CETIS Test Data Worksheet

Report Date: Test Code:

02 Jun-17 09:20 (p 1 of 1) 09-5761-0582/1706-S025

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Sample Code: 17-0648 Start Date: 02 Jun-17 Strongylocentrotus purpuratus Species: Sample Source: IDE Americas, Inc. End Date: 02 02 Jun-17 Sample Date: 91 Jun-17 Protocol: EPA/600/R-95/136 (1995) Sample Station: M-001 40 ppt Material: Facility Effluent

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	97 98 95 96 98 930 95 93 96 77	TN 6/14/17
			62	(W)	વર્ષ્ટ્ર	
			63	iw	95	
			64	COJ	96	
			65	100	936 936	
			66	100	95	
			67	100	93	
			68	(00)	96	
			69	100	77	
			70	100	94	
			71	100	9'5	
			72	100 100	94	
			73	100	86	
			74	100	95 94 86 94 96 95	
			75 76	100	96	
_			77	100	95	
			78	100	73	
			79	100	90	
			80	100 120	90 95 95 96 96 95 94 93	
			81	100	95	TN 6/15/17
			82	100	96	10 010/17
			83	/WD	95	
****			84	Cu)	97	
			85	OU	93	
			86	100	91	
			87	100 100	95	
			88	100	90	
			89	100	93	
			90	100	95	

@ Q14726/14/12 100 18 AC 7/3/17

CETIS Test Data Worksheet

Report Date: Test Code: 02 Jun-17 09:20 (p 1 of 1) 09-5761-0582/1706-S025

Nautilus Environmental (CA)

st Code: 09-5761-05

Echinoid Spe	erm Cell Fertiliza				
01 1 0 -1	00 1 47	C	Ctrongula controtus numerostus	0	4

Start Date:02 Jun-17Species:Strongylocentrotus purpuratusSample Code:17- 0648End Date:02 Jun-17Protocol:EPA/600/R-95/136 (1995)Sample Source:IDE Americas, Inc.Sample Date:01 Jun-17Material:Facility EffluentSample Station:M-001 40 ppt

Sample Date	37				al: Facility Ef	fluent Sample Station: M-001 40 ppt
C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	62	100	94	AL61217
0	LC	2	86			
0	LC	3	72			
0	LC	4	88			
0	LC	5	81			
2.5		1	82	100	a3	186612117
2.5		2	80			
2.5		3	85			
2.5		4	83			
2.5		5	61			·
5		1	67			
5		2	89	100	97	Bo 6/2/17
5		3	64			
5		4	73		***************************************	
5		5	68			
6.06		1	84	100	ag	No 6/2/17
6.06		2	77	,		
6.06		3	74			
6.06		4	66			
6.06		5	79			
10		1	69	60)	94	M6 6/2/17
10		2	71	~~~	1	
10		3	78			
10		4	90			
10		5	75			
15		1	63	100	ay	K 612/17
15		2	76			
15		3	87			
15		4	65			
15		5	70			

@018 AC 713/17

Analyst: AC QA: 87/5/17

Water Quality Measurements

01:	_
Client	•
CHELST	

IDE

Test Species: S. purpuratus

Sample ID:

M-001 (40 ppt adjusted)

1700 Start Date/Time: 6/2/2017

Sample Log No.: 17- 0648

End Date/Time: 6/2/2017

Dilutions made by: \bigcirc

Test No: 1706-5025

			Analyst:	AD						
	Initial Readings									
Concentration %	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)						
Lab Control	8.0	801	33.3	14.0						
2.5	8.1	8.03	33.6	14.7						
5.0	8.1	8.05	34.0	14.4						
6.06	8.2	8.0-7	34.1	14.2						
10	8-1	8.09	34.4	14.3						
15	8.1	8.09	34.7	143						
	8-0	8110		14.0						

_			
$C \cap$	mi	nat	nts:

QC Check:

Final Review: _\&\mathbb{V}_3/17

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

Brine Dilution Worksheet

Project:	IDE	. 2000	Analyst: AG	7177.00.0
Sample ID:	M-001 (40 ppt adjuste	ed)	Test Date: 6/2/2017	
Test No:	1706-50	25	Test Type: Urchin Ferti	lization
Salinity of Effl	uent	63.8	_	
Salinity of Sea	awater	33.5	Date of Brine used: NA	
Target Salinity		40.0	Alk. of 40 ppt Adj. Sample: 142	mg/L as CaCO3
		Effluent	Brine Control	
- SE)/(SB - TS) TS = target		3.66	-6.15	

Concentration %	Effluent Volume (ml)	Salinity Adjustment Factor	Seawater Volume (ml)	Final Volume (ml)
100	100	3.66	366.2	466

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: E4 6 13/17

SB = salinity of brine

Final Review: 8 7/3/17

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

Echinoderm Sperm-Cell Fertilization Worksheet

Client:	IDE	Start Da	te/Time: 6/2/2017 / \7 ⊘
Sample ID:	M-001 40ppt	12 / 1	te/Time: 6/2/2017 / 1746
Test No.:	1706-5025		Species: S. purpuratus
	Λ ;		Source: Point Loma
Tech initials:	H6		ollected: 5/22/17
Injection Time:	1626		
,			
Sperm Absorbance at 4		(target range of 0.8 - 1.0 for density of 4x10 ⁶ spe	rm/ml)
Eggs Counted:		n: $\frac{78}{}$ x 50 = $\frac{3,400}{}$ eggs/ml	
	34		
		t counts of 80 eggs per vertical pass on Sedgwick-	
	76 Railei	slide for a final density of 4000 eggs/ml)	
	76		
Initial density:	ろ,400 eggs/ml	= dilution factor egg stock	150 ml
Final density:	4000 eggs/ml	= <u>UV</u> dilution factor egg stock - <u>1.0</u> part egg stock seawater	
Tillar dollorly.	1000 0993/1111	part egg stock seawater	_(A)ml
		parto scawater	
Prepare the embryo stoo	ck according to the calcula	ted dilution factor. For example, if the dilution facto	r is 2.25, use 100 ml of existing
stock (1 part) and 125 m	nl of dilution water (1.25 pa	rts).	
	•		
		Sperm:Egg Ratio	
Rangefinder Test:	2000:1 1600:	1 1200:1 800:1 400:1 200:1	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	Daniel Date	
Crosses Added (100 1).	Time	Rangefinder Ratio: Fert. Unfert.	
Sperm Added (100 µl):	1632	- 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1 - 1	
Eggs Added (0.5 ml):	1642	100% 1001 9696 9,4	
Test Ended:	1652	2001, 2001 100 100 0,0	
		400.1,400.) 100,100 0,0	
NOTE O			
NOTE: Choose a sperm	1-to-egg ratio that results in	fertilization between 80 and 90 percent. If more the	an one concentration is within
health, stage of reproduc	ctive season, site condition	nless professional judgment dictates consideration	of other factors (e.g., organism
· · · · · · · · · · · · · · · · · · ·		0).	
Definitive Test		Sperm:Egg Ratio Used: (001)	
	-		
•	Time	Fert. Unfert.	
Sperm Added (100 µl):	1700	QC1 98 2	
Eggs Added (0.5 ml):	1720	$\begin{array}{ccccc} QC1 & & & & & & & & \\ QC2 & & & & & & & \\ Egg Control 1 & & & & & & \\ \hline \end{array}$	
Test Ended:	1740	Egg Control 1 0 100	,
, , , , , , , , , , , , , , , , , , , ,		Egg Control 2	Landaria
Comments:	BNo Dilotion Re	1100	
2 2	Dr. 1 - 1 1 1 1010 100	-40.00	
	1 1.		~12 I.
QC Check:	EG 6/13/17	_ Final F	Review: <u>*</u> 7/3/17

Appendix B

Sample Receipt Information

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Client:	IDE
Sample ID:	M-001
Test ID No(s).:	1706-SO24 to SO26

Sample (A, B, C):	A			
Log-in No. (17-κκκ);	0648			
Sample Collection Date & Time:	6/2/17 0900			
Sample Receipt Date & Time:	6/2/17 1204			
Number of Containers & Container Type:	1, 41 cubie			
Approx. Total Volume Received (L):	~ 46			
Check-in Temperature (°C)	5.0			
Temperature OK? 1	Ŷ N	Y N	У И	ΥN
DO (mg/L)	7.7			
pH (units)	7.96			
Conductivity (μS/cm)				
Salinity (ppt)	@ 47-1 63.80			
Alkalinity (mg/L) ²	192			
Hardness (mg/L) ^{2, 3}				
Total Chlorine (mg/L)	0,03			
Technician Initials	DM			

Test Performed:	Li Urchin tert. Control/Dilution Water: 8:2	Lab SW Lab ART Other:
	Alkalinity:2Hardr	ness or Salinity: 34ppt
	Alkalinity: 99 12 Hardr Additional Control? (A) A = 17 Alkalinity: N	Hardness or Salinity: 38,200+
	Ø16 AC 713	
Test Performed:		/ Lab SW / Lab ART Other:
	Alkalinity: Hardr	ness or Salinity:
	Additional Control? Y N = Alkalinity:	Hardness or Salinity:
Test Performed:	l: Control/Dilution Water: 8:2	/ Lab SW / Lab ART Other:
	Alkalinity: Hardr	ness 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
	•	,
tional Comments:		nm=not-measured; techemor
	1 Salinity half DI and half sample	,
	C) EG 018 6/26/17	

Sample Check-In Information

Sample Description: A: no (clor, Clenr, no odor, light deba 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			
			···········
	?		
A_Y_BC			
Filtration 2 V N			
	ノ		
	or	— Debris	
	(5)		
	\sim	T	AA-
		-	
		=	• •
	200	rarge	ı ppı.
on Adjustment?	\cup	D	_
Initial nH			
•			
•	A		
oiz riajastinonti i	\smile	R	C
Initial Free Cl ₂ :			
-			
Final Free Cl ₂ :			
Rample Accation? V	(N)		
vample rieration:	\smile	B	C
Initial D.O.			
Duration & Rate			
Final D.O.			
Subsamples for Add	itional Chor	nietn/ Paguiro	45 V (1)
	rional Chei	insuy ivequile	· · · ·
Tech Initials /			
			6/26/17

Appendix C

Chain-of-Custody Form



Project Name: NPDES Daily Toxici	- Principal Color Section (Color Section (Color Section (Color Section (Color Section (Color Section (Color Sec	Marini ya Masaka nga da kana ya masaka kana ya masaka kana ya masaka ka ma								Othe	r:			??? Days
Special instruction: 24 hour compos		Project Manage	er: Peter Shen	(Contact Information:(7	60) 201- 77	77							
plant operation. Sample collected to 6/1/17 @ 09:00, End: 6/2/17 @ 09:0	AN Alutuom ilitini o	DES requirement. Sam	i series of consecutive gra iple is to be run unadjuste	ibs at 6 h ed and ac	r intervals during normal djusted to 40 ppt. Start:				ANAL	YSES			Marin ma	NOTES:
						Chronic Fertilizatior								
		Glass=G Plastic=	:P	Non-galliana, a		ic Fert								
	Yes=Y No=N	Acid=A Base=B			1	hron								
Drinking	Water=DW Seawa	iter=SW Soil=S Brine=	В	Pro		in C								
Sample ID	Date	Time	Sample	Preservative	Container	ple Urchin								
			Туре	e	Туре	Purple								
M-001 (17- 1961) 1	6/1-2/17	9:00	24 HR COMP -B	N	1 x 4L CUBIE	Х								TDS 61.49 ppt, EC -85.47 mS/cm
														iss @n.,) ppc, cc-poc (nis/cm
													7	
												_		
											+	-		
											_			·
											-			
									-		_			
	Contract of the contract of th					to the same of the same post of the		Hu						
telinquished By:	1	AND DESCRIPTION OF THE PROPERTY OF THE PROPERT	Time:		Received By:	and the same of the same of			Т	ime:		Sa	ample	Condition Upon Receipt:
There	~~/	6/2/17	1000	7		5/2	2//	710	المانيان		lo	ed		mbient orOC
		62/11	12:041		(Loh like	A /K	1/2	1/21	201	1	lc	ed	^	mhiant or °C

Markus 10-17-0648

CDP laoratory:_

Nautilus: X

Entahlpy Laboratory:____

WECK Laboratory:_____

Turn Around Time

Normal:__

3 Days:__

5 Days:__

RUSH (24 hr):__

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date:

12 Jun-17 16:41 (p 1 of 1)

Test Code:

170602sprt | 07-9823-1222

Echinoid Spe	rm Cell Fertiliza	tion Test	15C									nental (CA)
Batch ID:	18-6047-4052			Fertilization				Analyst:		Nauulu	2 CHAHOIIII	ieniai (CA)
Start Date:	02 Jun-17 17:0	0 P r	otocol:	EPA/600/R-95/	(136 (1995)			Diluent:	Natu	ıral Seawat	er	
Ending Date:	02 Jun-17 17:4	0 S p	ecies:	Strongylocentre	otus purpura	tus		Brine:	Not.	Applicable		
Duration:	40m	Sc	urce:	Pt. Loma				Age:				
Sample ID:	09-0099-4586	Co	de:	170602sprt				Client:	Inter	nal		
Sample Date:			terial:	Copper chloride				Project:				
Receive Date:			urce:	Reference Tox								
Sample Age:	1/h	St	ation:	Copper Chlorid	e							
Comparison S	Summary											
Analysis ID	Endpoint		NOEL	. LOEL	TOEL	PMSD	TU	Meth	od			
21-0790-4675	Fertilization Ra	te	20	40	28.28	4.96%		Dunr	nett M	ultiple Com	parison Tes	st
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	μg/L	95% LCL	95% UCL	ΤU	Meth	od			
14-2289-5480	Fertilization Ra	te	EC50	59.87	57.65	62.16		Trimi	med S	Spearman-k	(ärber	
Test Acceptat	oility											
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Lim	its	Over	lap	Decision		
14-2289-5480	Fertilization Ra			ol Resp	0.96	0.7 - NL		Yes		Passes A	cceptability	Criteria
21-0790-4675	Fertilization Ra			ol Resp	0.96	0.7 - NL		Yes		Passes A	cceptability	Criteria
21-0790-4675	Fertilization Ra	te	PMSD		0.04963	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	tate Summary											
C-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std E	Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.96	0.9404	0.9796	0.94	0.98	0.007	7071	0.01581	1.65%	0.0%
10		5	0.89	0.8488	0.9312	0.85	0.92	0.014	183	0.03317	3.73%	7.29%
20		5	0.918	0.8941	0.9419	0.89	0.94			0.01923	2.1%	4.38%
40		5	0.836	0.7822	0.8898	0.78	0.88			0.04336	5.19%	12.92%
80 160		5 5	0.214 0	0.08242 0	0.3456 0	0.11 0	0.39 0	0.047 0	′39	0.106 0	49.52%	77.71%
		<u> </u>	<u> </u>	V	J	U	U	U		U		100.0%
Fertilization R												
	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5				····		
İ	Lab Control	0.97	0.96	0.98	0.94	0.95						
10		0.85	0.92	0.9	0.86	0.92						
20		0.93	0.92	0.89	0.91	0.94						
40		0.86	0.78	0.88	0.8	0.86						
80		0.11	0.22	0.17	0.39	0.18						
160		0	0	0	0	0						

Report Date:

12 Jun-17 16:41 (p 1 of 2)

Test Code: 170602sprt | 07-9823-1222

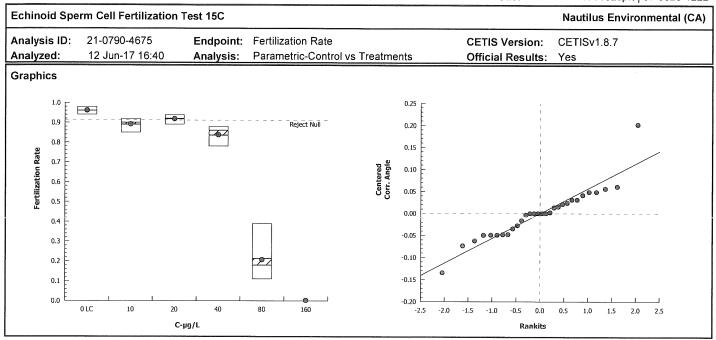
	Al-Maria de la companya de la compa		·				rest	Code:	1700	ouzspit U	7-9823-1222	
Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	s Environn	nental (CA)	
Analysis ID:	21-0790-4675	En	dpoint: Fer	tilization Ra	te		CET	S Version:	CETISv1	.8.7		
Analyzed:	12 Jun-17 16:	40 An	alysis: Par	ametric-Cor	ntrol vs Trea	tments	Offic	ial Results	: Yes			
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU	
Angular (Cori	rected)	NA	C > T	NA	NA		4.96%	20	40	28.28		
Dunnett Mul	tiple Compariso	n Test										
Control	vs C-μg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)			
Lab Control	10*		3.086	2.305	0.103 8	0.0099	CDF	Significan	t Effect			
	20		2.044	2.305	0.103 8	0.0814	CDF	Non-Sign	ficant Effect			
	40*		4.867	2.305	0.103 8	0.0002	CDF	Significan				
	80*		20.24	2.305	0.103 8	<0.0001	CDF	Significan	t Effect			
ANOVA Tabl	е											
Source	Sum Sqւ	ıares	Mean Squ	ıare	DF	F Stat	P-Value	Decision	(α:5%)			
Between	2.611561		0.6528901		4	132	<0.0001	Significan	t Effect			
Error	0.098901	14	0.0049450)57	20							
Total	2.710462				24							
Distributiona	al Tests					· · · · · · · · · · · · · · · · · · ·						
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)				
Variances	Bartlett E	Equality of V	/ariance	8.244	13.28	0.0831	Equal Var	iances				
Distribution	Shapiro-	Wilk W Nor	mality	0.918	0.8877	0.0461	Normal Di	stribution				
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	0.96	0.9404	0.9796	0.96	0.94	0.98	0.007071	1.65%	0.0%	
10		5	0.89	0.8488	0.9312	0.9	0.85	0.92	0.01483	3.73%	7.29%	
20		_										
40		5	0.918	0.8941	0.9419	0.92	0.89	0.94	0.008602	2.1%	4.38%	
		5	0.836	0.7822	0.9419 0.8898	0.92 0.86	0.89 0.78	0.94 0.88	0.008602 0.01939	2.1% 5.19%	12.92%	
80		5 5	0.836 0.214	0.7822 0.08242	0.8898 0.3456	0.86 0.18						
		5	0.836	0.7822	0.8898	0.86	0.78	0.88	0.01939	5.19%	12.92%	
80 160	rected) Transfor	5 5 5	0.836 0.214 0	0.7822 0.08242	0.8898 0.3456	0.86 0.18	0.78 0.11	0.88 0.39	0.01939 0.04739	5.19%	12.92% 77.71%	
80 160	rected) Transfor Control Type	5 5 5	0.836 0.214 0	0.7822 0.08242	0.8898 0.3456	0.86 0.18	0.78 0.11	0.88 0.39	0.01939 0.04739	5.19%	12.92% 77.71%	
80 160 Angular (Cor	ŕ	5 5 5 med Sumn	0.836 0.214 0	0.7822 0.08242 0	0.8898 0.3456 0	0.86 0.18 0	0.78 0.11 0	0.88 0.39 0	0.01939 0.04739 0	5.19% 49.52%	12.92% 77.71% 100.0%	
80 160 Angular (Cor C-μg/L	Control Type	5 5 5 med Sumn Count	0.836 0.214 0 nary Mean	0.7822 0.08242 0	0.8898 0.3456 0 95% UCL	0.86 0.18 0 Median	0.78 0.11 0	0.88 0.39 0	0.01939 0.04739 0	5.19% 49.52% CV%	12.92% 77.71% 100.0%	
80 160 Angular (Cor C-μg/L	Control Type	5 5 5 rmed Sumn Count 5	0.836 0.214 0 mary Mean 1.373	0.7822 0.08242 0 95% LCL 1.321	0.8898 0.3456 0 95% UCL 1.424	0.86 0.18 0 Median 1.369	0.78 0.11 0 Min 1.323	0.88 0.39 0 Max 1.429	0.01939 0.04739 0 Std Err 0.01862	5.19% 49.52% CV% 3.03%	12.92% 77.71% 100.0% %Effect 0.0%	
80 160 Angular (Cor C-μg/L 0 10	Control Type	5 5 5 rmed Sumn Count 5	0.836 0.214 0 mary Mean 1.373 1.236	0.7822 0.08242 0 95% LCL 1.321 1.17	0.8898 0.3456 0 95% UCL 1.424 1.301	0.86 0.18 0 Median 1.369 1.249	0.78 0.11 0 Min 1.323 1.173	0.88 0.39 0 Max 1.429 1.284	0.01939 0.04739 0 Std Err 0.01862 0.02357	5.19% 49.52% CV% 3.03% 4.27%	12.92% 77.71% 100.0% %Effect 0.0% 10.0%	
80 160 Angular (Cor C-μg/L 0 10 20	Control Type	5 5 5 med Sumn Count 5 5 5	0.836 0.214 0 mary Mean 1.373 1.236 1.282	0.7822 0.08242 0 95% LCL 1.321 1.17 1.239	0.8898 0.3456 0 95% UCL 1.424 1.301 1.325	0.86 0.18 0 Median 1.369 1.249 1.284	0.78 0.11 0 Min 1.323 1.173 1.233	0.88 0.39 0 Max 1.429 1.284 1.323	0.01939 0.04739 0 Std Err 0.01862 0.02357 0.01555	5.19% 49.52% CV% 3.03% 4.27% 2.71%	12.92% 77.71% 100.0% %Effect 0.0% 10.0% 6.62%	

Analyst: 1W QA: MPIELIST

Report Date: Test Code:

12 Jun-17 16:41 (p 2 of 2)

170602sprt | 07-9823-1222



Report Date:

12 Jun-17 16:41 (p 1 of 1)

Test Code:

170602sprt | 07-9823-1222

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 14-2289-5480 Analyzed:

12 Jun-17 16:40

Endpoint: Fertilization Rate

Analysis:

Trimmed Spearman-Kärber

CETIS Version:

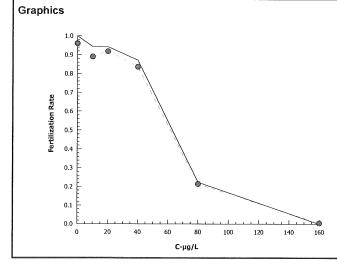
CETISv1.8.7

Official Results: Yes

Trimmed Spearman-Kärber Estimates

Threshold Option Threshold Trim Sigma Mu EC50 95% LCL 95% UCL Control Threshold 0.04 5.83% 1.777 0.008176 59.87 57.65 62.16

Fertilization Rate Summary			Calculated Variate(A/B)								
C-µg/L	Control Type	Count	Mean	Min	Max	Std Err	Std Dev	CV%	%Effect	Α	В
0	Lab Control	5	0.96	0.94	0.98	0.007071	0.01581	1.65%	0.0%	480	500
10		5	0.89	0.85	0.92	0.01483	0.03317	3.73%	7.29%	445	500
20		5	0.918	0.89	0.94	0.008602	0.01923	2.1%	4.38%	459	500
40		5	0.836	0.78	0.88	0.01939	0.04336	5.19%	12.92%	418	500
80		5	0.214	0.11	0.39	0.04739	0.106	49.52%	77.71%	107	500
160		5	0	0	0	0	0		100.0%	0	500



Copper chloride

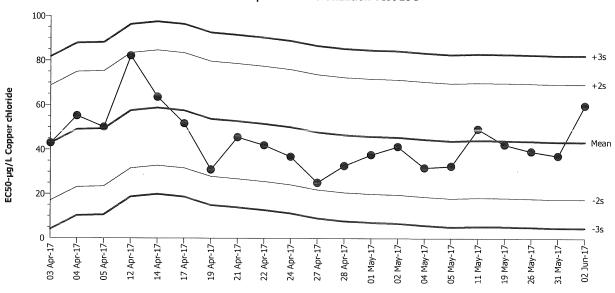
Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization Organism: Strongylocentrotus purpuratus (Purpl Material:

Protocol: EPA/600/R-95/136 (1995) Endpoint: Fertilization Rate Source: Reference Toxicant-REF

Echinoid Sperm Cell Fertilization Test 15C



 Mean:
 43.53
 Count:
 20
 -2s Warning Limit:
 17.65
 -3s Action Limit:
 4.708

 Sigma:
 12.94
 CV:
 29.70%
 +2s Warning Limit:
 69.41
 +3s Action Limit:
 82.35

Qual	ity	Con	trol	Data
------	-----	-----	------	------

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Apr	3	17:15	42.93	-0.5962	-0.04607			11-7488-2003	08-5368-9216
2			4	12:13	55.13	11.6	0.8962			02-3186-4899	18-3488-7750
3			5	14:33	50.05	6.521	0.5039			12-8691-4512	16-6546-2933
4			12	16:30	82.12	38.59	2.982	(+)		04-6122-9881	08-0232-5024
5			14	15:34	63.58	20.05	1.55			06-7326-1133	06-6067-9028
6			17	17:03	51.69	8.164	0.6309			13-4494-7236	11-5239-6650
7			19	15:40	31	-12.53	-0.968			16-6386-1330	20-1211-2894
8			21	12:05	45.55	2.015	0.1557			10-3448-9907	04-0175-0159
9			22	14:33	41.96	-1.568	-0.1212			11-1560-7674	00-7687-9708
10			24	16:05	36.84	-6.692	-0.5171			09-2574-2389	13-4474-8503
11			27	12:19	25.18	-18.35	-1.418			10-3853-1638	12-2181-2382
12			28	15:50	32.81	-10.72	-0.8286			04-2889-8959	08-9738-9274
13		May	1	17:50	37.72	-5.806	-0.4487			18-0409-9294	21-4338-2021
14			2	11:56	41.38	-2.149	-0.1661			15-1584-1378	12-5072-1723
15			4	16:15	31.87	-11.66	-0.9015			08-0627-0095	01-9095-4567
16			5	15:10	32.62	-10.91	-0.8429			16-0368-0463	05-0853-8226
17			11	15:35	49.31	5.777	0.4465			01-5463-5574	13-6197-3009
18			19	16:58	42.3	-1.234	-0.09534			01-3808-3529	10-6921-8357
19			26	16:55	39.23	-4.297	-0.3321			19-8209-6027	02-5970-9183
20			31	15:42	37.28	-6.249	-0.4829			01-0947-8219	10-1735-9410
21		Jun	2	17:00	59.87	16.34	1.262		•	07-9823-1222	14-2289-5480

CETIS Test Data Worksheet

Report Date:

02 Jun-17 09:21 (p 1 of 1)

Test Code:

07-9823-1222/170602sprt

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date:	02 Jun-17	Species:	Strongylocentrotus purpuratus	Sample Code:	170602sprt
End Date:	02 Jun-17	Protocol:	EPA/600/R-95/136 (1995)	Sample Source:	Reference Toxicant
Sample Date:	02 Jun-17	Material:	Copper chloride	Sample Station:	Copper Chloride

		un-17		waterial. Copper Chloride					
C-µg/L	Code	Rep			# Fertilized	Notes			
			1	100	93	6/12/17 BO			
			2	100	Ő				
			3	100	86				
			4	100	22 98				
			5	100	98	·			
			6	100	88				
			7	100	97				
			8	100	0				
			9	100	94				
			10	100	92				
			11	100	91				
			12	100	17				
			13	100	92				
			14	100	90				
			15	100	0				
			16	100	94				
			17	100	0				
			18	100	0				
			19	100					
			20	100	85				
			21	100	96				
			22	100	86				
			23	100	78				
			24	100	18				
			25	100	95				
			26	100	92				
			27	106	44390				
			28	100	89				
			29	100	86				
			30	100	80	\bigvee			

@ Q18 BO 6/12/17

CETIS Test Data Worksheet

Echinoid Sperm Cell Fertilization Test 15C

Report Date:

02 Jun-17 09:21 (p 1 of 1) 07-9823-1222/170602sprt

Test Code:

Nautilus Environmental (CA)

Start Date: 02 Jun-17 Species: Strongylocentrotus purpuratus Sample Code: 170602sprt End Date: Protocol: EPA/600/R-95/136 (1995) 02 Jun-17 Sample Source: Reference Toxicant Sample Date: 02 Jun-17 Material: Copper chloride Sample Station: Copper Chloride

•		: UZ Juli-17		wateri	ai: Copper c	nioride Sample Station: Copper Chloride
C-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	7	100	99	166/2/17
0	LC	2	21			
0	LC	3	5			
0	LC	4	16			
0	LC	5	25			
10		1	20	100	92	16612/17
10		2	26			
10		3	14			
10		4	29			
10		5	13			
20		1	1	100	88	AC 612/17
20		2	10		-	,
20		3	28			
20		4	11			
20		5	9			
40		1	3	100	83	16 6/2117
40		2	23	100	77	Ar 6/2/17
40		3	6	100	92	16 6/2/17 A6 6/2/17 16 6/2/17
40		4	30			No 15/11
40		5	22			
80		1	19	100	19	A6 6/2/17
80		2	4	100	14	
80		3	12	-		
80		4	27			
80		5	24			
160		1	18	100	Ò	P66/2/17
160		2	17			
160		3	8			
160		4	2			
160		5	15			



Water Quality Measurements

Analyst:

Client :	Internal	Test Species: S. purpuratus				
Sample ID:	CuCl ₂	Start Date/Time: 6/2/2017 \7 60				
Test No:	170602sprt	End Date/Time: 6/2/2017 \740				

Dilutions made by:

High conc. made (μg/L): 160

Vol. Cu stock added (mL): 7.8

Final Volume (mL): 500

Cu stock concentration (μg/L):

	,		_	AD				
	Initial Readings							
Concentration (μg/L)	DO (mg/L)	pH (units)	Salinity (ppt)	Temperature (°C)				
Lab Control	8.0	7,99	33.2	15.6				
10	8.0	801	33.7	15.4				
20	8.0	8.02	33.6	15.5				
40	80	8-03	334	15.4				
80	8.0	8-04	33.5	15.5				
160	8.1	8.05	33.3	15.4				

Comments:		
QC Check:	KFP 6/12/17	Final Review: _> ~ 6 12 17

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

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Sample ID: Test No.:	Onternal (UC) 17060251 0 () 170602517	120 uCb		End Date/Time	: S. purpuratus
Tech initials: Injection Time:	A6 1626			Date Collected	
Sperm Absorbance at 4	100 nm: <u>0.990</u>	(target range of 0.8 - 1	.0 for density of 4	1x10 ⁶ sperm/ml)	
Eggs Counted:		n: $\frac{78}{}$ X 50 = et counts of 80 eggs per ver r slide for a final density of	tical pass on Sedo		
Initial density: Final density:	3,400 eggs/ml 4000 eggs/ml	dilution fa - 1.0 part egg s (R) parts sea	stock se	gg stock 150 eawater R	Oml
Prepare the embryo sto stock (1 part) and 125 n	ck according to the calcula nl of dilution water (1.25 pa	ted dilution factor. For exrts).	kample, if the dilu	ition factor is 2.25	5, use 100 ml of existing
Rangefinder Test: ml Sperm Stock ml Seawater	2000:1 1600: 50 40 0.0 10		10	200:1 5.0 45	100:1 2.5 47.5 48.75
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1632 1642 1652	Rangefinder Ratio: 50 / 50 / 100 / 1	Fert. Un 81,70 (9, 16,96 (4, 100,100 (0, 100,100 (0,	0	
this range, choose the r	n-to-egg ratio that results in atio closest to 90 percent u ctive season, site condition	ınless professional judgm	and 90 percent. ent dictates cons	If more than one ideration of othe	concentration is within r factors (e.g., organism
Definitive Test		Sperm:Egg Ratio Used	: <u> </u>		
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1700 1720 1740	QC1 QC2 Egg Control 1 Egg Control 2	98 2	fert.	
Comments:	(BNO Dilotion Party alk 6/12	egoved 11			
QC Check: Nautilus Environmental. 434	VTP 6 [2] 7 10 Vandever Avenue. San Dieg	— go, CA 92120.		Final Review:	Jw 6/12/17

Appendix E

Qualifier Codes



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 <u>below</u> 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 <u>above</u> 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 or ganisms r eceived at a <u>temperature</u> greater than 3°C ou tside the r ecommended t est 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. O rganisms were acclimated to the appropriate test conditions upon receipt and prior to test initiation.
- Q24 Test organisms received at <u>salinity</u> greater than 3 ppt outside of the recommended test salinity range. H owever, due t o age -specific pr otocol r equirements and/ or s ample ho lding t ime 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.

Updated: 6/30/15