

Chronic Toxicity Test Results for the Carlsbad Desalination Plant

❖ Sample ID: M-001 (Daily) Sample Collection Date: August 27, 2017

Prepared for: IDE AMERICAS, Inc.

4590 Carlsbad Boulevard Carlsbad, CA 92008

Prepared by: Nautilus Environmental

Submitted: September 11, 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

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EXECUTIVE SUMMARY

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

Sampling Date: August 27, 2017

Test Date: August 28, 2017

Sample ID: M-001 (pre-treatment off-spec period)

Effluent Limitation: 16.5 TU_c

Results Summary:

	Effluent Tes	st Results	Effluent Limitation
Bioassay Type:	NOEC	TU_c	Met? (Yes/No)
Urchin Fertilization	6.06	16.5	Yes

Client: IDE Americas, Inc. Test ID: 1708-S235

Sample ID: M-001 Sample Date: August 27, 2017

INTRODUCTION

A discharge sample was collected in August 2017 for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project (CDP) permit for daily chronic toxicity monitoring purposes. The discharge sample was collected from the CDP M-001 discharge monitoring point during a period of off-spec plant operation. Chronic toxicity testing for the effluent sample was conducted during this time 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 August 28, 2017 using the purple urchin (Strongylocentrotus purpuratus) chronic fertilization test.

MATERIALS AND METHODS

Sample collection and delivery were performed by IDE Americas, Inc. (IDE) personnel. Following arrival at Nautilus, an aliquot of the water 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 protocols described in USEPA 1995, and the methods are summarized in Table 3.

Table 1. Sample Information

Client/Project:	IDE Americas, Inc./Carlsbad Desalination Plant
Sample ID:	M-001 (pre-treatment off-spec period)
Monitoring Period:	August 2017
Sample Material:	Facility Effluent
Sampling Method:	24hr Composite
Sample Collection Date, Time:	8/27/17, 08:00
Sample Receipt Date, Time:	8/28/17, 11:50

Table 2. Water Quality Measurements for the M-001 Sample upon Receipt

Sample Collection	рН	DO	Temp	Salinity	Alkalinity	Total Chlorine
Date		(mg/L)	(°C)	(ppt)	(mg/L as CaCO₃)	(mg/L)
8/27/17	7.92	8.7	4.5	32.6	111	<0.02

TOXICITY SUMMARY REPORT

Test ID: 1708-S235 Sample ID: M-001

Sample Date: August 27, 2017

Client: IDE Americas, Inc.

Table 3. Echinoderm Fertilization Chronic Bioassay Specifications

Test Date, Times: 8/28/17, 14:56 through 15:36

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 inlet,

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

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

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

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

min fertilization period

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

significant difference (PMSD) value < 25.

Copper chloride Reference Toxicant Testing:

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 the sample dilution series was compared to that observed in the laboratory control exposure. Results were used to calculate the No Observed Effect Concentration (NOEC) and chronic toxic unit (TUc) values.

Results were also analyzed using the USEPA's Test of Significant Toxicity (TST) approach specified in National Pollutant Discharge Elimination System Test of Significant Toxicity Implementation Document (USEPA 2010). Notably, the California State Water Resources Control Board (SWRCB) published a Draft Policy for Toxicity Assessment and Control (SWRCB 2012), which includes the TST as an alternative method to evaluate toxicity data. This approach applies a modified t-test that takes into account both the statistical power of the test and the magnitude of biological effects in determining the presence of a response. For this sample, the in-stream waste concentration (IWC) is 6.06 percent unadjusted effluent; results are reported as "Pass" if a sample is considered non-toxic at the IWC according to the TST calculation, or "Fail" if considered toxic at the IWC according to the TST. As the TST is not included in the CDP permit at this time, the TST analysis was performed for comparison purposes only.

Test ID: 1708-S235

Client: IDE Americas, Inc.
Sample ID: M-001

Sample Date: August 27, 2017

RESULTS

A statistically significant decrease in fertilization rate was observed at the 10 and 15 percent effluent concentrations compared to the lab control. The NOEC is reported as 6.06 and the TU_c is equal to 16.5, which meets the maximum effluent limitation of 16.5 for this permit. None of the effluent concentrations were significantly reduced according to the TST analysis. Statistical results 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 copies of the chain-of-custody form are in Appendices B and C, respectively.

Table 4. Statistical Results for Purple Urchin Fertilization Testing

Sample I D	NOEC	LOEC	EC ₅₀	TU _c value	TST Result	Percent
	(% sample)	(% sample)	(% sample)	(toxic units)	(Pass/Fail)	Effect at IWC
M-001	6.06	10	>15	16.5	Pass	2.3

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

 $TU_c = Chronic Toxic Unit: 100 \div NOEC$

TST: Pass = sample is non-toxic at the IWC according to the TST calculation; Fail = sample is toxic at the 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)	Mean Percent Fertilization
Lab Control	97.6
2.5	95.6
5.0	97.2
6.06	95.4
10	91.4*
15	84.4*

^{*}An asterisk indicates a statistically significant decrease in fertilization rate compared to the lab control.

TOXICITY SUMMARY REPORT

Client: IDE Americas, Inc. Test ID: 1708-S235 Sample ID: M-001

Sample Date: August 27, 2017

QUALITY ASSURANCE

The sample was received on the day after collection and was within the appropriate temperature range. The test was initiated within the 36-hour holding time. The control met all test acceptability criteria, and the PMSD value, which is a measure of test variability, was within the acceptable limits. Statistical analyses followed USEPA flowchart selections and dose-response relationships were reviewed to ensure the reliability of the data. Based on the dose responses observed during testing, the calculated effect concentrations reported are deemed reliable. 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 met all test acceptability criteria. The median effect (EC50) value calculated for this test was within two standard deviations (2SD) of the historical mean for our laboratory, indicating organisms were of typical sensitivity to copper. Results for the reference toxicant test are summarized in Table 6 and presented in full in Appendix D. A list of qualifier codes can be found in Appendix E.

Table 6. Urchin Fertilization Reference Toxicant Test Results

Test Date	EC ₅₀ (µg/L Copper)	Historical Mean EC ₅₀ ±2 SD (μg/L Copper)	CV (%)
8/28/17	41.6	51.5 ± 38.8	37.6

 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

TOXICITY SUMMARY REPORT

Test ID: 1708-S235

Client: IDE Americas, Inc.
Sample ID: M-001

Sample Date: August 27, 2017

REFERENCES

California Regional Water Quality Control Board Region 9, San Diego (RWQCB) 2006. Waste Discharge Requirements for the Poseidon Resources (Channelside) LLC, Carlsbad Desalination Project, Discharge to the Pacific Ocean via the Encina Power Station Discharge Channel. Order No. R9-2006-0065, NPDES No. CA109223. June 2006.

- California State Water Resources Control Board (SWRCB) 2012. Draft Policy for Toxicity Assessment and Control. June 2012. Sacramento, CA.
- Tidepool Scientific Software. 2000-2013. **CETIS™ Co**mprehensive Environmental Toxicity Information System Software, Version 1.8.7.20
- USEPA. 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136.
- USEPA. 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

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Test Code:

1708-S235 | 03-0612-8625

Echinoid Spe	rm Cell Fertiliza	ation Test	15C									mental (CA)
Batch ID: Start Date: Ending Date: Duration:	19-2756-1831 28 Aug-17 14:5 28 Aug-17 15:5 40m	56 P i 36 S i	est Type: rotocol: pecies: purce:	Fertilization EPA/600/R-95/ Strongylocentro Pt. Loma		itus	[E	Analyst: Diluent: Brine: Age:		oratory Sea Applicable		
1	13-3822-2447 27 Aug-17 08:0 : 28 Aug-17 11:5 31h (4.5°C)	00 M 50 S 0	ode: aterial: ource: ation:	17-0946 Facility Effluent IDE Americas, M-001 (Daily)				Client: Project:	IDE Carl	sbad Desal	Plant	
Comparison S	Summary			NATIONAL MARKET NATIONAL MARKE								
Analysis ID	Endpoint		NOEL	. LOEL	TOEL	PMSD	TU	Weth	od			
08-6207-7202	Fertilization Ra	te	6.06	10	7.785	3.34%	16.5	Dunr	ett M	lultiple Com	parison Te	st
Point Estimat	e Summary											
Analysis ID	Endpoint		Level	%	95% LCL	95% UCL	TU	Meth	nod			
01-1300-8025	Fertilization Ra	te	EC25	>15	N/A	N/A	<6.66			erpolation (I	CPIN)	arche Wille August III Antonio agrafiante
			EC50	>15	N/A	N/A	<6.66			,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,,	O,	
Test Acceptab	oility						No. 10 April					
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Limi	ite	Over	dan	Decision		
01-1300-8025	Fertilization Ra	te		ol Resp	0.976	0.7 - NL	1.0	Yes	iap		cceptability	Criteria
08-6207-7202	Fertilization Ra	te		ol Resp	0.976	0.7 - NL		Yes			cceptability	
08-6207-7202	Fertilization Ra	te	PMSD)	0.0334	NL - 0.25		No		Passes A	cceptability	Criteria
Fertilization R	ate Summary											
C-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std E	Err	Std Dev	CV%	%Effect
0	Lab Control	5	0.976	0.9593	0.9927	0.96	0.99	0.006		0.01342	1.38%	0.0%
2.5		5	0.956	0.9236	0.9884	0.94	1	0.011	166	0.02608	2.73%	2.05%
5		5	0.972	0.9481	0.9959	0.94	0.99	0.008	3602	0.01924	1.98%	0.41%
6.06		5	0.954	0.9254	0.9826	0.92	0.97	0.010		0.02302	2.41%	2.25%
10 15		5 5	0.914	0.8883	0.9397	0.89	0.94	0.009		0.02074	2.27%	6.35%
13		3	0.844	0.7785	0.9095	0.77	0.9	0.023	558	0.05273	6.25%	13.52%
Fertilization R	ate Detail											
	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
	Lab Control	0.96	0.99	0.97	0.99	0.97						
2.5		0.94	0.96	1	0.94	0.94						
5		0.98	0.99	0.97	0.94	0.98						
6.06		0.97	0.92	0.97	0.97	0.94						
10		0.9	0.91	0.93	0.94	0.89						
15		0.87	0.87	0.9	0.77	0.81						
Fertilization R	ate Binomials				 -							
C-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
	Lab Control	96/100	99/100	97/100	99/100	97/100						
2.5		94/100	96/100	100/100	94/100	94/100						
5		98/100	99/100	97/100	94/100	98/100						
6.06		97/100	92/100	97/100	97/100	94/100						
10		90/100	91/100		94/100	89/100						
15		87/100	87/100	90/100	77/100	81/100						

Report Date:

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~= 110 /1110	aiyticai Rep	OIL					-	Code:	1708	•	3-0612-8625
Echinoid Spe	erm Cell Fertiliza	ation Test	15C								nental (CA)
Analysis ID: Analyzed:	08-6207-7202 11 Sep-17 15:		ndpoint: Fer nalysis: Par	tilization Rat ametric-Con		tments		S Version:		8.7	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	TU
Angular (Corr	rected)	NA	C > T	NA	NA		3.34%	6.06	10	7.785	16.5
Dunnett Mult	tiple Compariso	n Test									
Control	vs C-%		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(α:5%)		
Lab Control	2.5		1.277	2.362	0.090 8	0.3037	CDF	Non-Signi	ficant Effect		
	5		0.2913	2.362	0.090 8	0.7333	CDF	Non-Signi	ficant Effect		
	6.06		1.605	2.362	0.090 8	0.1916	CDF	Non-Signi	ficant Effect		
	10*		3.821	2.362	0.090 8	0.0018	CDF	Significan	t Effect		
	15*		6.6	2.362	0.090 8	<0.0001	CDF	Significan	t Effect		
ANOVA Table	е										
Source	Sum Squ	iares	Mean Squ	are	DF	F Stat	P-Value	Decision	(α:5%)		
Between	0.231007		0.0462014		5	12.66	<0.0001	Significan	t Effect		
Error	0.087586	36	0.0036494	32	24						
Total	0.318593	4			29	***************************************					
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of	Variance	3.181	15.09	0.6721	Equal Var	iances			
Distribution	Shapiro-	Wilk W No	ormality	0.9614	0.9031	0.3368	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	_									,
	Lab Control	5	0.976	0.9593	0.9927	0.97	0.96	0.99	0.006	1.38%	0.0%
2.5	Lab Control	5 5	0.976 0.956	0.9593 0.9236	0.9927 0.9884	0.97 0.94	0.96 0.94	0.99 1	0.006 0.01166		
2.5 5	Lab Control									1.38%	0.0%
	Cap Control	5 5 5	0.956	0.9236	0.9884	0.94	0.94	1	0.01166	1.38% 2.73% 1.98% 2.41%	0.0% 2.05%
5	Lab Control	5 5	0.956 0.972	0.9236 0.9481	0.9884 0.9959	0.94 0.98	0.94 0.94	1 0.99	0.01166 0.008602	1.38% 2.73% 1.98%	0.0% 2.05% 0.41%
5 6.06	Lab Control	5 5 5	0.956 0.972 0.954	0.9236 0.9481 0.9254	0.9884 0.9959 0.9826	0.94 0.98 0.97	0.94 0.94 0.92	1 0.99 0.97	0.01166 0.008602 0.0103	1.38% 2.73% 1.98% 2.41%	0.0% 2.05% 0.41% 2.25%
5 6.06 10 15	rrected) Transfol	5 5 5 5 5	0.956 0.972 0.954 0.914 0.844	0.9236 0.9481 0.9254 0.8883	0.9884 0.9959 0.9826 0.9397	0.94 0.98 0.97 0.91	0.94 0.94 0.92 0.89	1 0.99 0.97 0.94	0.01166 0.008602 0.0103 0.009273	1.38% 2.73% 1.98% 2.41% 2.27%	0.0% 2.05% 0.41% 2.25% 6.35%
5 6.06 10 15		5 5 5 5 5 rmed Sum Count	0.956 0.972 0.954 0.914 0.844	0.9236 0.9481 0.9254 0.8883	0.9884 0.9959 0.9826 0.9397	0.94 0.98 0.97 0.91	0.94 0.94 0.92 0.89	1 0.99 0.97 0.94	0.01166 0.008602 0.0103 0.009273	1.38% 2.73% 1.98% 2.41% 2.27%	0.0% 2.05% 0.41% 2.25% 6.35%
5 6.06 10 15 Angular (Cor C- %	rrected) Transfol	5 5 5 5 5 rmed Sum	0.956 0.972 0.954 0.914 0.844	0.9236 0.9481 0.9254 0.8883 0.7785	0.9884 0.9959 0.9826 0.9397 0.9095	0.94 0.98 0.97 0.91 0.87	0.94 0.94 0.92 0.89 0.77	1 0.99 0.97 0.94 0.9	0.01166 0.008602 0.0103 0.009273 0.02358	1.38% 2.73% 1.98% 2.41% 2.27% 6.25%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52%
5 6.06 10 15 Angular (Cor C- % 0 2.5	rrected) Transfol Control Type	5 5 5 5 5 med Sum Count 5	0.956 0.972 0.954 0.914 0.844 mary Mean	0.9236 0.9481 0.9254 0.8883 0.7785	0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479 1.478	0.94 0.98 0.97 0.91 0.87	0.94 0.94 0.92 0.89 0.77 Min 1.369 1.323	1 0.99 0.97 0.94 0.9 Max 1.471 1.521	0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093 0.03824	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29% 6.23%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0% 3.43%
5 6.06 10 15 Angular (Cor C- % 0 2.5 5	rrected) Transfol Control Type	5 5 5 5 5 rmed Sum Count 5 5	0.956 0.972 0.954 0.914 0.844 mary Mean 1.421 1.372 1.41	0.9236 0.9481 0.9254 0.8883 0.7785 95% LCL 1.363 1.266 1.341	0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479 1.478 1.478	0.94 0.98 0.97 0.91 0.87 Median 1.397	0.94 0.92 0.89 0.77 Min 1.369 1.323 1.323	1 0.99 0.97 0.94 0.9 Max 1.471 1.521 1.471	0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093 0.03824 0.02457	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29% 6.23% 3.9%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0% 3.43% 0.78%
5 6.06 10 15 Angular (Cor C- % 0 2.5	rrected) Transfol Control Type	5 5 5 5 5 Count 5 5 5	0.956 0.972 0.954 0.914 0.844 mary Mean 1.421 1.372 1.41 1.36	0.9236 0.9481 0.9254 0.8883 0.7785 95% LCL 1.363 1.266	0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479 1.478 1.478 1.425	0.94 0.98 0.97 0.91 0.87 Median 1.397 1.323	0.94 0.94 0.92 0.89 0.77 Min 1.369 1.323 1.323 1.284	1 0.99 0.97 0.94 0.9 Max 1.471 1.521 1.471 1.397	0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093 0.03824	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29% 6.23%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0% 3.43%
5 6.06 10 15 Angular (Cor C- % 0 2.5 5	rrected) Transfol Control Type	5 5 5 5 5 rmed Sum Count 5 5	0.956 0.972 0.954 0.914 0.844 mary Mean 1.421 1.372 1.41	0.9236 0.9481 0.9254 0.8883 0.7785 95% LCL 1.363 1.266 1.341	0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479 1.478 1.478	0.94 0.98 0.97 0.91 0.87 Median 1.397 1.323 1.429	0.94 0.92 0.89 0.77 Min 1.369 1.323 1.323	1 0.99 0.97 0.94 0.9 Max 1.471 1.521 1.471	0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093 0.03824 0.02457	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29% 6.23% 3.9%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0% 3.43% 0.78%

Report Date: Test Code: 11 Sep-17 15:19 (p 2 of 2) 1708-S235 | 03-0612-8625

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) Analysis ID: 08-6207-7202 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 11 Sep-17 15:19 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 0.16 E 1.0 • 0.9 8.0 Fertilization Rate 0.08 0.06 0.6 0.04 0.02 0.5 0.00 -0.02 0.3 -0.04 -0.06 -0.08 0.1 -0.10 0.0 -0.12 0 LC 6.06 2.5 10 15 -1.5 -2.5 -2.0 -1.0 -0.5 0.0 1.0 1.5 2.0 C-% Rankits

Report Date:

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Test Code:

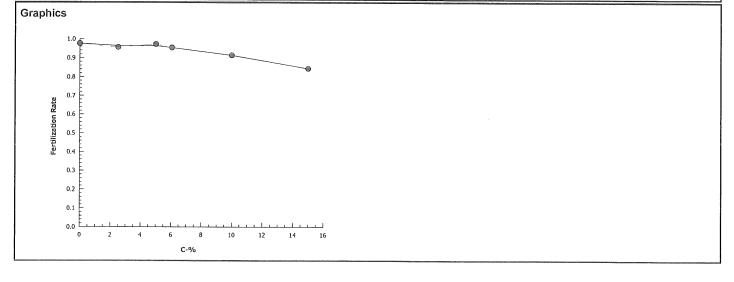
1708-S235 | 03-0612-8625

Echinoid Sperm Cell Fertilization Test 15CNautilus Environmental (CA)Analysis ID:01-1300-8025Endpoint:Fertilization RateCETIS Version:CETISv1.8.7Analyzed:11 Sep-17 15:19Analysis:Linear Interpolation (ICPIN)Official Results:Yes

X TransformY TransformSeedResamplesExp 95% CLMethodLinearLinear14703631000YesTwo-Point Interpolation	Linear Interpola	ation Options				
Linear Linear 1470363 1000 Yes Two-Point Interpolation	X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
	Linear	Linear	1470363	1000	Yes	Two-Point Interpolation

Point	t Estimates					
Leve	I %	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

Fertiliza	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	0.976	0.96	0.99	0.006	0.01342	1.38%	0.0%	488	500
2.5		5	0.956	0.94	1	0.01166	0.02608	2.73%	2.05%	478	500
5		5	0.972	0.94	0.99	0.008602	0.01924	1.98%	0.41%	486	500
6.06		5	0.954	0.92	0.97	0.0103	0.02302	2.41%	2.25%	477	500
10		5	0.914	0.89	0.94	0.009273	0.02074	2.27%	6.35%	457	500
15		5	0.844	0.77	0.9	0.02358	0.05273	6.25%	13.52%	422	500



Report Date:

11 Sep-17 15:20 (p 1 of 1)

Test Code:

1708-S235 | 03-0612-8625

Echinoid Sp	erm Cell Fertiliza	ation Test 1	15C	です					Nautilus	Environ	mental (CA)
Analysis ID:	18-5135-8443	En	dpoint: Fer	tilization Ra	te		CFT	IS Version:	CETISv1	8.7	***************************************
Analyzed:	11 Sep-17 15:		•	ametric Bio		-Two Samp		ial Results:		.0.1	
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed	TST b	PMSD	NOEL	LOEL	TOEL	TU
Angular (Cori	rected)	NA	C*b < T	NA	NA	0.75	2.51%	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*	<u> </u>	7.413	2.015	0.083 5	0.0004	CDF		ficant Effect	/////////////////////////////////////	
	5*		11.8	1.943	0.057 6	<0.0001	CDF		ficant Effect		
	6.06*		10.36	1.943	0.055 6	<0.0001	CDF	-	ficant Effect		
	10*		9.092	1.895	0.044 7	<0.0001	CDF	•	ficant Effect		
	15*		2.88	2.015	0.072 5	0.0173	CDF	_	ficant Effect		
ANOVA Tabl	e										
Source	Sum Squ	ıares	Mean Squ	ıare	DF	F Stat	P-Value	Decision(α:5%)		
Between	0.231007		0.0462014		5	12.66	<0.0001	Significant	Effect		
Error	0.087586	36	0.0036494	132	24			J			
Total	0.318593	4			29						
Distributiona	al Tests									1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0 1 0	
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett E	Equality of V	ariance	3.181	15.09	0.6721	Equal Var	Equal Variances			
Distribution	Shapiro-	Wilk W Nor	mality	0.9614	0.9031	0.3368	Normal Distribution				
Cortilization											
refunzation	Rate Summary										
C-%	Rate Summary Control Type	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
C-%	•	Count 5	Mean 0.976	95% LCL 0.9593	95% UCL 0.9927	Median 0.97	Min 0.96	Max 0.99	Std Err 0.006	CV% 1.38%	%Effect
C-%	Control Type										
C-%	Control Type	5	0.976	0.9593	0.9927	0.97	0.96	0.99	0.006	1.38%	0.0%
C-% 0 2.5	Control Type	5 5	0.976 0.956	0.9593 0.9236	0.9927 0.9884	0.97 0.94	0.96 0.94	0.99 1	0.006 0.01166	1.38% 2.73%	0.0% 2.05%
C-% 0 2.5 5	Control Type	5 5 5	0.976 0.956 0.972	0.9593 0.9236 0.9481	0.9927 0.9884 0.9959	0.97 0.94 0.98	0.96 0.94 0.94	0.99 1 0.99	0.006 0.01166 0.008602	1.38% 2.73% 1.98%	0.0% 2.05% 0.41%
C-% 0 2.5 5 6.06	Control Type	5 5 5 5	0.976 0.956 0.972 0.954	0.9593 0.9236 0.9481 0.9254	0.9927 0.9884 0.9959 0.9826	0.97 0.94 0.98 0.97	0.96 0.94 0.94 0.92	0.99 1 0.99 0.97	0.006 0.01166 0.008602 0.0103	1.38% 2.73% 1.98% 2.41%	0.0% 2.05% 0.41% 2.25%
C-% 0 2.5 5 6.06 10 15	Control Type	5 5 5 5 5 5	0.976 0.956 0.972 0.954 0.914 0.844	0.9593 0.9236 0.9481 0.9254 0.8883	0.9927 0.9884 0.9959 0.9826 0.9397	0.97 0.94 0.98 0.97 0.91	0.96 0.94 0.94 0.92 0.89	0.99 1 0.99 0.97 0.94	0.006 0.01166 0.008602 0.0103 0.009273	1.38% 2.73% 1.98% 2.41% 2.27%	0.0% 2.05% 0.41% 2.25% 6.35%
C-% 0 2.5 5 6.06 10 15	Control Type Lab Control	5 5 5 5 5 5 rmed Summ	0.976 0.956 0.972 0.954 0.914 0.844	0.9593 0.9236 0.9481 0.9254 0.8883	0.9927 0.9884 0.9959 0.9826 0.9397	0.97 0.94 0.98 0.97 0.91	0.96 0.94 0.94 0.92 0.89	0.99 1 0.99 0.97 0.94	0.006 0.01166 0.008602 0.0103 0.009273	1.38% 2.73% 1.98% 2.41% 2.27%	0.0% 2.05% 0.41% 2.25% 6.35%
C-% 0 2.5 5 6.06 10 15 Angular (Cor	Control Type Lab Control	5 5 5 5 5 5 mmed Summ Count	0.976 0.956 0.972 0.954 0.914 0.844	0.9593 0.9236 0.9481 0.9254 0.8883 0.7785	0.9927 0.9884 0.9959 0.9826 0.9397 0.9095	0.97 0.94 0.98 0.97 0.91 0.87	0.96 0.94 0.94 0.92 0.89 0.77	0.99 1 0.99 0.97 0.94 0.9	0.006 0.01166 0.008602 0.0103 0.009273 0.02358	1.38% 2.73% 1.98% 2.41% 2.27% 6.25%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 rmed Summ	0.976 0.956 0.972 0.954 0.914 0.844 mary	0.9593 0.9236 0.9481 0.9254 0.8883 0.7785	0.9927 0.9884 0.9959 0.9826 0.9397 0.9095	0.97 0.94 0.98 0.97 0.91 0.87	0.96 0.94 0.94 0.92 0.89 0.77	0.99 1 0.99 0.97 0.94 0.9	0.006 0.01166 0.008602 0.0103 0.009273 0.02358	1.38% 2.73% 1.98% 2.41% 2.27% 6.25%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 mmed Summ Count	0.976 0.956 0.972 0.954 0.914 0.844 nary Mean 1.421	0.9593 0.9236 0.9481 0.9254 0.8883 0.7785 95% LCL 1.363	0.9927 0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479	0.97 0.94 0.98 0.97 0.91 0.87 Median 1.397	0.96 0.94 0.94 0.92 0.89 0.77	0.99 1 0.99 0.97 0.94 0.9 Max	0.006 0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 med Summ Count 5	0.976 0.956 0.972 0.954 0.914 0.844 hary Mean 1.421 1.372	0.9593 0.9236 0.9481 0.9254 0.8883 0.7785 95% LCL 1.363 1.266	0.9927 0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479 1.478	0.97 0.94 0.98 0.97 0.91 0.87 Median 1.397 1.323	0.96 0.94 0.94 0.92 0.89 0.77 Min 1.369 1.323	0.99 1 0.99 0.97 0.94 0.9 Max 1.471 1.521	0.006 0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093 0.03824	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29% 6.23%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0% 3.43%
C-% 0 2.5 5 6.06 10 15 Angular (Cor C-% 0 2.5 5	Control Type Lab Control rrected) Transfor Control Type	5 5 5 5 5 5 med Summ Count 5 5	0.976 0.956 0.972 0.954 0.914 0.844 hary Mean 1.421 1.372 1.41	0.9593 0.9236 0.9481 0.9254 0.8883 0.7785 95% LCL 1.363 1.266 1.341	0.9927 0.9884 0.9959 0.9826 0.9397 0.9095 95% UCL 1.479 1.478 1.478	0.97 0.94 0.98 0.97 0.91 0.87 Median 1.397 1.323 1.429	0.96 0.94 0.94 0.92 0.89 0.77 Min 1.369 1.323 1.323	0.99 1 0.99 0.97 0.94 0.9 Max 1.471 1.521 1.471	0.006 0.01166 0.008602 0.0103 0.009273 0.02358 Std Err 0.02093 0.03824 0.02457	1.38% 2.73% 1.98% 2.41% 2.27% 6.25% CV% 3.29% 6.23% 3.9%	0.0% 2.05% 0.41% 2.25% 6.35% 13.52% %Effect 0.0% 3.43% 0.78%

CETIS Test Data Worksheet

Report Date:

28 Aug-17 10:20 (p 1 of 1)

Test Code: 7708-5235 03-0612-8625/123F26F1

Echinoid	Sperm	Cell	Fertilization	Test	15C

Nautilus Environmental (CA)

Start Date: End Date:

28 Aug-17 28 Aug-17

Species: Strongylocentrotus purpuratus

Sample Code: 17-0946 Sample Source: IDE Americas, Inc.

Sample Date: 27 Aug-17

Protocol: EPA/600/R-95/136 (1995)

Material: Facility Effluent 8/27 Samuelo Sample Station: M-001 (Daily)

C-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
			61	100	96	8/30/17
			62		94	
			63		87	
			64		94	
			65		97	
			66		92	
			67		\$1	
			68		98	
			69		\$1 98 9490	Q19 RL re-count AC9/11/17
			70		1 99	
			71		94	
			72		97	
			73		91	
			74		87	
			75 76		97	
			76		99	
			78		89 99	
			79		97	
		-	80			
			81		96	
			82		100	
			83		94	
			84		94	
			85		97	
			86		98	
			87		98	
			88		94	
			89			
			90		77	
				61		

Report Date:

28 Aug-17 10:20 (p 1 of 1)

Test Code: \$70\$-5335 03-0612-8625/123F26F1

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Start Date: End Date:

Sample Date: 27 Aug-17

28 Aug-17 28 Aug-17

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

Material: Facility Effluent

Sample Code: 17-0946 Sample Source: IDE Americas, Inc. Sample Station: M-001 (Daily) (Collected 8 | 27/17)

	, cp	1 03	# Counted		Notes			
LC	1	80			Notes			
			100	77	KG 8 25 17			
LC			1000					
			100	99	EL			
				1 1 1 1 1 1 1 1				
		86	160	94	EG			
	3	65						
	4	62						
	5	68						
	1	81	(W	96	EL			
	2	66						
	3	79						
	4	85						
	5	88						
	1	90	100	90	EG			
	2	73						
	3	87						
	4	71						
	5	77						
	1	74	100	75	EG			
	2	63		47				
	3	69						
	4	89						
	5	67						
	LC LC LC	LC 3 LC 4 LC 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 2 3 4 5 1 4 5 1 2 3 4 5 1 4 5 1 2 3 4 5 1 4 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6 6	LC 3 72 LC 4 78 LC 5 75 1 84 2 61 3 82 4 64 5 83 1 86 2 70 3 65 4 62 5 68 1 81 2 66 3 79 4 85 5 88 1 90 2 73 3 87 4 71 5 77 1 74 2 63 3 69 4 89	LC 3 72 LC 4 78 LC 5 75 1 84 (O()) 2 61 3 82 4 64 5 83 1 86 (O()) 2 70 3 65 4 62 5 68 1 81 (O()) 2 66 3 79 4 85 5 88 1 90 (O()) 2 73 3 87 4 71 5 77 1 74 (O()) 2 63 3 69 4 89	LC 2 78 LC 4 78 LC 5 75 1 84 (0) 99 2 61 3 82 4 64 5 83 1 86 (0) 94 2 70 3 65 4 62 5 68 1 81 (0) 96 2 66 3 79 4 85 5 88 1 90 (00 90) 2 73 3 87 4 71 5 77 1 74 (00 96) 2 63 3 69 4 89			

QC:CG

Marine Chronic Bioassay

Water Quality Measurements

Client:

IDE

Test Species: S. purpuratus

Sample ID:

M-001 Daily (8/27/17)

Start Date/Time: 8/28/2017 1456

Sample Log No.: 17- 0946

End Date/Time: 8/28/2017 (536

Dilutions made by: ______CG

Test No: 1708-5235

			Analyst:	EG
CONTRACT AND LINES AND LIN		Initial F	Readings	
Concentration	DO	рН	Salinity	Temperature
%	(mg/L)	(units)	(ppt)	(,C)
Lab Control	7.5	8.14	33.8	15.9®
2.5	7,4	8-13	33.8 33.8 0	15.9
5.0	7,5	8.12	33.7	15.9
6.06	7.5	8.12	33.7	15.9
10	7.5	8.11	33.7	15.9
15	7.5	8.10	33.7	15.9

Co	m	m	a	mil	e •

(A) Eh a19 8/28/17 (B) Temperature recorded from Surrogate vialantray

QC Check:

Final Review: 6 1/11/17

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

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

Client: Sample ID: Test No.:	Deily M-001 (8/27 Sam 1708-S	Start Date/Time: 8/28/2017 / 1456 End Date/Time: 8/28/2017 / 1536 Species: S. purpuratus
Tech initials: Injection Time:	(6)	Animal Source: Pf. Loma. Date Collected: 5 72 17
Sperm Absorbance at 40	00 nm: <u>しりり</u> (target range of	0.8 - 1.0 for density of 4x10 ⁶ sperm/ml)
Eggs Counted:	73 Mean: 70-4 x	50 = 35% eggs/ml
		per vertical pass on Sedgwick- sity of 4000 eggs/ml)
	72	
Initial density: Final density:	4000 eggs/ml - 1.0 pa	ution factor egg stock 200 ml t egg stock seawater — 1214 ml ts seawater

Prepare the embryo stock according to the calculated dilution factor. For example, if the dilution factor is 2.25, use 100 ml of existing stock (1 part) and 125 ml of dilution water (1.25 parts).

				<u>Sperr</u>	n:Egg Rati	<u>o</u>			
Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:	1 2	00:1	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	Ra	ngefinder Ra	tio:	Fert.	Unfert.			
Sperm Added (100 µl):	1418		50:1		75	25			
Eggs Added (0.5 ml):	1436		100.1		89,39	11, 11			
Test Ended:	1448		100.1		99	1			
			400:1		100				

<u>NOTE</u>: 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 U	sed: 100:1		
Sperm Added (100 μl): Eggs Added (0.5 ml): Test Ended:	Time 1456 1516 1536	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 94 0	Unfert. 6 4 100 100	
Comments:	(A)CG (3/6 8/14/17				
QC Check:	A(8/34)7			Final Reviev	N: 559/MM7

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

Appendix B

Sample Receipt Information

Nautilus Environmental 4340 Vandever Avenue San Diego, CA 92120

Client:	1DE Dood Ed Q18 8/25/17
Sample ID:	Daily M-001 8/27 sample
Test ID No(s).:	1708-S235

Sample (A, B, C):	A			
Log-in No. (17-xxxx):	0946			
Sample Collection Date & Time:	8/27/17-0800			
Sample Receipt Date & Time:	8/28/17/150			
Number of Containers & Container Type:	1,4Lcvbi			
Approx. Total Volume Received (L):	3 -			
Check-in Temperature (°C)	4.5			
Temperature OK? ¹	(Ý) N	YN	Y N	Y N
DO (mg/L)	8.7			
pH (units)	7.92			
Conductivity (µS/cm)				
Salinity (ppt)	32.6			
Alkalinity (mg/L) ²	1/1			
Hardness (mg/L) ^{2, 3}	-			
Total Chlorine (mg/L)	40.02			
Technician Initials	EA			

Test Performed:	Additional Control? Y (N)	Control/Dilution Water: 8:2 / Lab SW / Lab ART Other: Alkalinity: Alkalinity: Hardness or Salinity: Hardness or Salinity:
Test Performed:		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
	Additional Control? Y N	= Alkalinity: Hardness or Salinity:
Test Performed:		Control/Dilution Water: 8:2 / Lab SW / Lab ART Other:
	Additional Control? Y N	Alkalinity: Hardness or Salinity: Hardness or Salinity:
Notes:		d 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:		100	Islam
n: co () - co	Jan Jic or	Lac I pe C	<i>[25 16 40]</i>
COC Complete (Y/N) A B C	?		
Filtration? Y N)		
Pore Size:		-	
Organisms	or	Debris	
Salinity Adjustment?	YN		
Test:	Source:	Targe	et ppt:
Test:	Source:	Targo	et ppt:
Test:	Source:	Targe	et ppt:
pH Adjustment? Y(Ŋ		
	A	В	С
Initial pH:			
Amount of HCI added:			
Final pH:			
Cl₂ Adjustment? Y (N)		
	A	В	С
Initial Free Cl ₂ :			
STS added:			
Final Free Cl ₂ :			
Sample Aeration? Y	N		
	Α	В	С
Initial D.O.			
Duration & Rate			
Final D.O.			
Subsamples for Addit		stry Require	d? Y N
Tech Initials A	R	C	
		ck: <u>AC 87 :</u>	28/17
	Final Revie	ew: <u>\</u>	N/17

Appendix C

Chain-of-Custody Form



CDP laoratory:	Turn Around Time
Entahlpy Laboratory:	Normal: X
WECK Laboratory:	RUSH (24 hr):
Nautilus: X	3 Days:
AIM:	5 Days:
Othory	222.0

And the second of the second o	gran - from the constant arms the plan good state about the party of	rom romandari commende de son entren por contra describ			The state of the s			- and the second of the second of	Ot.	ner:	LO VANDON, I DANIE	Discourage of the Control of the Con	??? Days
Project Name: NPDES Daily Tox		Project Manage				50) 201- 77	777_						
Special instruction: Sampled of intervals. Sample collected to	luring pretreatment : fulfill daily NPDES re	off-spec via autosar quirement. Sample	npler by a series of grat is to be run unadiusted	s colled . Start:	ted at one hour 8/26/17 @ 8:00. End:				ANALYSES		Ta minor work view	edati estili jeji (riik ese	NOTES:
8/27/17 @ 8:00 VH		•	•		, ,,	Fertilization							
						ertilli							
		Glass=G Plastic=	P			ic Fe							
	Yes=Y No=N A	Acid=A Base=B		2000 000 M 000 000		Chror							
Drinki	ng Water=DW Seawa	er=SW Soil=S Brine=	3	Pre		hin (
Sample ID	Date	Time	Sample	Preservative	Container	Purple Urchin Chronic							
			Туре	ive ?	Туре	Purpl							
M-001 (17- 2732)	8/26-27/2017	8:00-8:00	SW	N	4L CUBIE	X							TDS - 31.93 ppt, EC - 49.62 mS/cm
													100 - 31.95 ppt, LC - 45.02 m3/tm
											-		
							·			pod na distributo o			
Relinquished By:		Date:	Time:		Received By:	الميسها			Time:			Samp	ele Condition Upon Receipt:
Kent	~~	8/28/17	10:07		James	Action to the second second	me	- AND STREET,	1.007	x	Iced		Ambient or°C
James &	Just	0/78/17	1150		V ULL	- 81	28/1	7	1150	X	Iced	Ш	Ambient or 4.5° C

Nantilus 10: 17-0946

Appendix D

Reference Toxicant Test Data and Statistical Analyses

CETIS Summary Report

Report Date:

30 Aug-17 16:01 (p 1 of 1)

Test Code:

170828sprt | 08-1525-2751

	X								•		or oobit 1 or	1020 270
Echinoid Spe	erm Cell Fertiliza	ition Test	15C							Nautilus	Environn	nental (CA)
Batch ID: Start Date: Ending Date: Duration:	04-3497-0759 28 Aug-17 14:5 28 Aug-17 15:3 40m	56 Pr 36 Sp	est Type: rotocol: pecies: purce:					Analyst: Diluent: Brine: Age:		ural Seawate Applicable	er	
Sample ID: Sample Date: Receive Date Sample Age:	: 28 Aug-17	Mi Sc	ode: aterial: ource: ation:	170828sprt Copper chlorid Reference Tox Copper Chloric	ricant			Client: Project:	Inte	rnal		
Comparison	Summary									90.000		
Analysis ID 07-9256-6029	Endpoint Fertilization Ra	te	NOEL <10	. LOEL	TOEL NA	PMSD 3.39%	TU	Met Dun		lultiple Com	parison Tes	st
Point Estimat	te Summary Endpoint		Level	μg/L	95% LCL	95% UCL	ΤU	Met	hod			
10-7829-2432	Fertilization Ra	te	EC50	41.55	39.24	44		Trim	med :	Spearman-K	ärber	
Test Accepta	bility											2000-200
Analysis ID	Endpoint		Attrib	ute	Test Stat	TAC Lim	its	Ove	rlap	Decision		
07-9256-6029 10-7829-2432 07-9256-6029	Fertilization Ra Fertilization Ra Fertilization Ra	te		ol Resp 0.966 0.7 - NL ol Resp 0.966 0.7 - NL 0 0.03392 NL - 0.25				Yes Yes No		Passes Ad	cceptability cceptability cceptability	Criteria
Fertilization F	Rate Summary								·			
C-μg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std	Err	Std Dev	CV%	%Effect
0 10 20 40 80 160	Lab Control	5 5 5 5 5	0.966 0.856 0.754 0.596 0.156 0.004	0.9434 0.8372 0.7142 0.4783 0.1143	0.9886 0.8748 0.7938 0.7137 0.1977 0.01511	0.95 0.83 0.72 0.46 0.11	0.99 0.87 0.8 0.71 0.2 0.02	0.00 0.01 0.04 0.01	238 503	0.01817 0.01517 0.03209 0.09476 0.03362 0.008944	1.88% 1.77% 4.26% 15.9% 21.55% 223.6%	0.0% 11.39% 21.95% 38.3% 83.85% 99.59%
Fertilization F	Rate Detail											
C-μg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5						
0 10 20 40	Lab Control	0.96 0.87 0.75 0.63	0.95 0.86 0.72 0.63	0.98 0.86 0.77 0.46	0.95 0.86 0.73 0.55	0.99 0.83 0.8 0.71						
80 160		0.2 0	0.17 0.02	0.16 0	0.14 0	0.11 0						
		40770X40										

Analyst: QA: MP91617

Report Date:

30 Aug-17 16:00 (p 1 of 2)

Test Code:

170828sprt | 08-1525-2751

								Code:			3-1525-275
Echinoid Sp	erm Cell Fertiliz	ation Test	15C						Nautilus	Environn	nental (CA)
Analysis ID:	07-9256-6029	En	dpoint: Fe	rtilization Rat	e		CET	S Version:	CETISv1.	.8.7	
Analyzed:	30 Aug-17 16		-					ial Results			
Data Transfo	orm	Zeta	Alt Hyp	Trials	Seed		PMSD	NOEL	LOEL	TOEL	ΤU
Angular (Corr	rected)	NA	C > T	NA	NA		3.39%	<10	10	NA	
Dunnett Mul	tiple Comparisc	n Test									
Control	vs C-µg/L		Test Stat	Critical	MSD DF	P-Value	P-Type	Decision	(a:5%)		
Lab Control	10*		6.014	2.362	0.082 8	<0.0001	CDF	Significan			
	20*		9.72	2.362	0.082 8	<0.0001	CDF	Significan			
	40*		14.56	2.362	0.082 8	<0.0001	CDF	Significan			
	80*		28.28	2.362	0.082 8	<0.0001	CDF	Significan			
	160*		37.9	2.362	0.082 8	<0.0001	CDF	Significan			
			07.0	2.002	0.002 0	-0.0001	ODI	Oigiiiioaii	t Elicot		
ANOVA Tabl									4		
Source	Sum Sq		Mean Sq		DF	F Stat	P-Value	Decision	<u> </u>		
Between	6.265867		1.253173		5	411.1	<0.0001	Significan	t Effect		
Error	0.073154		0.003048	112	24	_					
Total	6.339022)			29		Section 2015 Revision 1997				
Distributiona	al Tests										
Attribute	Test			Test Stat	Critical	P-Value	Decision(α:1%)			
Variances	Bartlett	Equality of ∖	'ariance	8.93	15.09	0.1119	Equal Var	iances			
Distribution	Shapiro	-Wilk W Nor	mality	0.9716	0.9031	0.5836	Normal Di	stribution			
Fertilization	Rate Summary				a California de California				i Ammili wa wa managa ayaa aa aa aa aa aa aa aa aa		
C-μg/L	Control Type	Count	Mean								
	, , , , , , , , , , , , , , , , , , ,		Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	Lab Control	5	0.966	95% LCL 0.9434	95% UCL 0.9886	Median 0.96	Min 0.95	Max 0.99	Std Err 0.008124	CV%	%Effect
0 10										***	
_		5	0.966	0.9434	0.9886	0.96	0.95	0.99	0.008124	1.88%	0.0%
10		5 5	0.966 0.856	0.9434 0.8372	0.9886 0.8748	0.96 0.86	0.95 0.83	0.99 0.87	0.008124 0.006782	1.88% 1.77%	0.0% 11.39%
10 20		5 5 5	0.966 0.856 0.754	0.9434 0.8372 0.7142	0.9886 0.8748 0.7938	0.96 0.86 0.75	0.95 0.83 0.72	0.99 0.87 0.8	0.008124 0.006782 0.01435	1.88% 1.77% 4.26%	0.0% 11.39% 21.95%
10 20 40		5 5 5 5	0.966 0.856 0.754 0.596	0.9434 0.8372 0.7142 0.4783	0.9886 0.8748 0.7938 0.7137	0.96 0.86 0.75 0.63	0.95 0.83 0.72 0.46	0.99 0.87 0.8 0.71	0.008124 0.006782 0.01435 0.04238	1.88% 1.77% 4.26% 15.9%	0.0% 11.39% 21.95% 38.3%
10 20 40 80 160		5 5 5 5 5 5	0.966 0.856 0.754 0.596 0.156 0.004	0.9434 0.8372 0.7142 0.4783 0.1143	0.9886 0.8748 0.7938 0.7137 0.1977	0.96 0.86 0.75 0.63 0.16	0.95 0.83 0.72 0.46 0.11	0.99 0.87 0.8 0.71 0.2	0.008124 0.006782 0.01435 0.04238 0.01503	1.88% 1.77% 4.26% 15.9% 21.55%	0.0% 11.39% 21.95% 38.3% 83.85%
10 20 40 80 160	Lab Control	5 5 5 5 5 5	0.966 0.856 0.754 0.596 0.156 0.004	0.9434 0.8372 0.7142 0.4783 0.1143	0.9886 0.8748 0.7938 0.7137 0.1977	0.96 0.86 0.75 0.63 0.16	0.95 0.83 0.72 0.46 0.11	0.99 0.87 0.8 0.71 0.2	0.008124 0.006782 0.01435 0.04238 0.01503	1.88% 1.77% 4.26% 15.9% 21.55%	0.0% 11.39% 21.95% 38.3% 83.85%
10 20 40 80 160 Angular (Con	Lab Control	5 5 5 5 5 5 5	0.966 0.856 0.754 0.596 0.156 0.004	0.9434 0.8372 0.7142 0.4783 0.1143	0.9886 0.8748 0.7938 0.7137 0.1977 0.01511	0.96 0.86 0.75 0.63 0.16	0.95 0.83 0.72 0.46 0.11	0.99 0.87 0.8 0.71 0.2 0.02	0.008124 0.006782 0.01435 0.04238 0.01503 0.004	1.88% 1.77% 4.26% 15.9% 21.55% 223.6%	0.0% 11.39% 21.95% 38.3% 83.85% 99.59%
10 20 40 80 160 Angular (Cor C-μg/L	Lab Control rrected) Transfo Control Type	5 5 5 5 5 5 rmed Sumr	0.966 0.856 0.754 0.596 0.156 0.004 mary	0.9434 0.8372 0.7142 0.4783 0.1143 0	0.9886 0.8748 0.7938 0.7137 0.1977 0.01511	0.96 0.86 0.75 0.63 0.16 0	0.95 0.83 0.72 0.46 0.11 0	0.99 0.87 0.8 0.71 0.2 0.02	0.008124 0.006782 0.01435 0.04238 0.01503 0.004 Std Err	1.88% 1.77% 4.26% 15.9% 21.55% 223.6%	0.0% 11.39% 21.95% 38.3% 83.85% 99.59%
10 20 40 80 160 Angular (Cor C-µg/L	Lab Control rrected) Transfo Control Type	5 5 5 5 5 5 rmed Summ Count	0.966 0.856 0.754 0.596 0.156 0.004 mary Mean 1.392	0.9434 0.8372 0.7142 0.4783 0.1143 0 95% LCL 1.323	0.9886 0.8748 0.7938 0.7137 0.1977 0.01511 95% UCL 1.461	0.96 0.86 0.75 0.63 0.16 0	0.95 0.83 0.72 0.46 0.11 0	0.99 0.87 0.8 0.71 0.2 0.02	0.008124 0.006782 0.01435 0.04238 0.01503 0.004 Std Err 0.02492	1.88% 1.77% 4.26% 15.9% 21.55% 223.6% CV% 4.0%	0.0% 11.39% 21.95% 38.3% 83.85% 99.59% %Effect 0.0%
10 20 40 80 160 Angular (Cor C-µg/L 0	Lab Control rrected) Transfo Control Type	5 5 5 5 5 5 rmed Summ Count 5	0.966 0.856 0.754 0.596 0.156 0.004 nary Mean 1.392 1.182	0.9434 0.8372 0.7142 0.4783 0.1143 0 95% LCL 1.323 1.156	0.9886 0.8748 0.7938 0.7137 0.1977 0.01511 95% UCL 1.461 1.208	0.96 0.86 0.75 0.63 0.16 0 Median 1.369 1.187	0.95 0.83 0.72 0.46 0.11 0 Min 1.345 1.146	0.99 0.87 0.8 0.71 0.2 0.02 Max 1.471 1.202	0.008124 0.006782 0.01435 0.04238 0.01503 0.004 Std Err 0.02492 0.009464	1.88% 1.77% 4.26% 15.9% 21.55% 223.6% CV% 4.0% 1.79%	0.0% 11.39% 21.95% 38.3% 83.85% 99.59% %Effect 0.0% 15.09%
10 20 40 80 160 Angular (Con C-µg/L 0 10 20	Lab Control rrected) Transfo Control Type	5 5 5 5 5 5 rmed Sum Count 5 5	0.966 0.856 0.754 0.596 0.156 0.004 mary Mean 1.392 1.182 1.053	0.9434 0.8372 0.7142 0.4783 0.1143 0 95% LCL 1.323 1.156 1.006	0.9886 0.8748 0.7938 0.7137 0.1977 0.01511 95% UCL 1.461 1.208 1.099	0.96 0.86 0.75 0.63 0.16 0 Median 1.369 1.187 1.047	0.95 0.83 0.72 0.46 0.11 0 Min 1.345 1.146 1.013	0.99 0.87 0.8 0.71 0.2 0.02 Max 1.471 1.202 1.107	0.008124 0.006782 0.01435 0.04238 0.01503 0.004 Std Err 0.02492 0.009464 0.01685	1.88% 1.77% 4.26% 15.9% 21.55% 223.6% CV% 4.0% 1.79% 3.58%	0.0% 11.39% 21.95% 38.3% 83.85% 99.59% %Effect 0.0% 15.09% 24.38%

0.2

0.1

0.0

0 LC

10

20

C-µg/L

40

80

160

Report Date: Test Code:

-0.5

Rankits

1.0 1.5 2.0

30 Aug-17 16:01 (p 2 of 2) 170828sprt | 08-1525-2751

Echinoid Sperm Cell Fertilization Test 15C Nautilus Environmental (CA) Analysis ID: 07-9256-6029 Endpoint: Fertilization Rate **CETIS Version:** CETISv1.8.7 Analyzed: 30 Aug-17 16:00 Analysis: Parametric-Control vs Treatments Official Results: Yes Graphics 0.12 ____ 0.10 Reject Null 0.9 -0.08 0.8 0.06 ___ Fertilization Rate 0.04 0.7 0.02 0.6 0.00 0.5 -0.02 0.04 0.4 -0.06

> -0.08 -0.10

> -0.12

-0.14

-0.16

-2.5 -2.0 -1.5

Report Date:

30 Aug-17 16:01 (p 1 of 1)

Test Code:

170828sprt | 08-1525-2751

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Analysis ID: 10-7829-2432 Analyzed:

30 Aug-17 16:00

Endpoint: Fertilization Rate Analysis:

Trimmed Spearman-Kärber

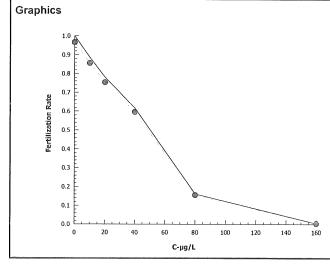
CETIS Version: Official Results: Yes

CETISv1.8.7

Trimmed Spearman-Karber Estimates

Threshold Option	Threshold	Trim	Mu	Sigma	EC50	95% LCL	95% UCL
Control Threshold	0.034	11.39%	1.619	0.01245	41.55	39.24	44

Fertilizati	on 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	0.966	0.95	0.99	0.008124	0.01817	1.88%	0.0%	483	500
10		5	0.856	0.83	0.87	0.006782	0.01517	1.77%	11.39%	428	500
20		5	0.754	0.72	8.0	0.01435	0.03209	4.26%	21.95%	377	500
40		5	0.596	0.46	0.71	0.04238	0.09476	15.9%	38.3%	298	500
80		5	0.156	0.11	0.2	0.01503	0.03362	21.55%	83.85%	78	500
160		5	0.004	0	0.02	0.004	0.008944	223.6%	99.59%	2	500



Analyst: QA: VTP9/6/17

Report Date:

30 Aug-17 16:04 (1 of 1)

Echinoid Sperm Cell Fertilization Test 15C

Nautilus Environmental (CA)

Test Type: Fertilization

Protocol: EPA/600/R-95/136 (1995)

Organism: Strongylocentrotus purpuratus (Purpl

Endpoint: Fertilization Rate

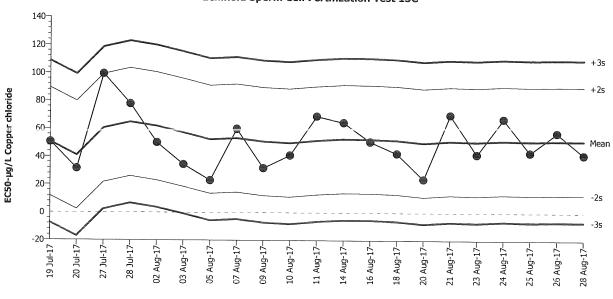
Material:

Source:

Copper chloride

Reference Toxicant-REF





Mean: 51.49 Count: 20 -2s Warning Limit: 12.73 -3s Action Limit: -6.646 Sigma: 19.38 CV: 37.60% +2s Warning Limit: +3s Action Limit: 109.6

Quai	lity	Control	Data
------	------	---------	------

Point	Year	Month	Day	Time	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2017	Jul	19	16:28	50.59	-0.9011	-0.04649			03-3446-7266	15-7259-8466
2			20	18:10	31.52	-19.97	-1.03			17-7484-2488	03-0485-5429
3			27	15:55	99.32	47.83	2.468	(+)		02-6715-3770	17-8186-2444
4			28	10:50	77.84	26.35	1.36			21-2559-1280	14-0688-6070
5		Aug	2	15:50	50.06	-1.435	-0.07404			08-9742-2478	08-8646-9232
6			3	0:00	34.43	-17.06	-0.8801			02-7356-2235	20-3051-4002
7			5	19:25	23.07	-28.42	-1.466			11-5994-0488	10-6029-2098
8			7	15:10	59.94	8.449	0.4359			21-2468-7505	14-3489-7019
9			9	17:08	31.92	-19.57	-1.01			13-6999-3036	11-7131-4234
10			10	16:51	41.14	-10.35	-0.5343			00-5471-5288	12-0643-2211
11			11	14:50	69.03	17.54	0.905			04-5796-5476	07-8184-6783
12			14	14:40	64.51	13.02	0.6716			02-4510-8526	01-5460-0814
13			16	16:34	50.82	-0.6731	-0.03473			16-3259-1018	06-7497-1035
14			18	14:09	42.53	-8.959	-0.4623			12-6613-4538	02-2322-5589
15			20	14:52	24.05	-27.44	-1.416			06-9655-0092	05-8785-3700
16			21	14:46	69.95	18.46	0.9524			08-4756-2919	20-2992-4955
17			23	16:14	41.72	-9.773	-0.5043			02-7595-3678	15-3490-2746
18			24	16:11	67.1	15.61	0.8056			04-7651-5518	20-0883-0005
19			25	14:48	43.11	-8.38	-0.4324			06-8816-1100	09-0830-4014
20			26	16:00	57.24	5.751	0.2967			10-2039-5656	15-8794-0305
21			28	14:56	41.55	-9.938	-0.5128			08-1525-2751	10-7829-2432

CETIS Test Data Worksheet

Report Date:

28 Aug-17 10:11 (p 1 of 1) 08-1525-2751/170828sprt

Test Code:

Nautilus Environmental (CA)

Echinoid Sperm Cell Fertilization Test 15C

28 Aug-17 End Date: 28 Aug-17

Start Date:

Species: Strongylocentrotus purpuratus **Protocol**: EPA/600/R-95/136 (1995)

Sample Code: 170828sprt Sample Source: Reference Toxicant

Sample Date: 28 Aug-17

nple Dat C-µg/L	Code				al: Copper chloride	Sample Station: Copper Chloride
o-pg/L	Code	veh			# Fertilized	Notes
			1	100	96	8/30/17
			2	100	2	
			3	160	Q'	
			4	100	86	
			5	100	2 27 86 75	
			6	100	86	
			7	100	86 55 63	
			8	/@	63	
			9	100	87	
***			10	/00	87 95	
			11	100	i7 99 63	
			12	100	99	
			13	100	63	
			14	100	86	
			15	901	86	
			16	100	16	
			17	/00	46	
			18	100	73	
			19	100	11	
			20	100	20	
			21	/00	77	
			22	100	95	
			23	00	<i>e</i> 4	
			24	1∞	14	
			25	100	Qr .	
			26	100	74	
			27	100	98	
			28	100	83 72	
			29	100	72	
			30	100	0	

Report Date:

28 Aug-17 10:11 (p 1 of 1) 08-1525-2751/170828sprt

Test Code:

Nautilus Environmental (CA)

Echimoid Sperm Cell Fertilization Test 15C

Start Date:28 Aug-17Species:Strongylocentrotus purpuratusSample Code:170828sprtEnd Date:28 Aug-17Protocol:EPA/600/R-95/136 (1995)Sample Source:Reference ToxicantSample Date:28 Aug-17Material:Copper chlorideSample Station:Copper Chloride

-μg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	LC	1	1			
0	LC	2	10	100	98	CG 8/28/7)
0	LC	3	27			
0	LC	4	22			
0	LC	5	12			
10		1	9			
10		2	15			
10		3	4	100	88	
10		4	6			
10		5	28			
20		1	5			
20		2	29	100	79	
20		3	21			
20		4	18			
20		5	14			
40		1	13			
40		2	8			
40		3	17	100	47	
40		4	7			
40		5	26			
80		1	20			
80		2	11	OU	15	
80		3	16			
80		4	24			
80		5	19			
160		1	3			
160		2	2			-
160		3	30			
160		4	23			
160		5	25	100	O	V

QC: CG

Marine Chronic Bioassay

Water Quality Measurements

Client :	Internal	Test Species: S. purpuratus
Sample ID:	CuCl ₂	Start Date/Time: 8/28/2017 1456
Test No:	170828sprt	End Date/Time: 8/28/2017 \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \ \

рΗ

(units)

8.13

8.12

Dilutions made by:

High conc. made (μg/L): 160

Vol. Cu stock added (mL): 38

Final Volume (mL): 500

DO

(mg/L)

Cu stock concentration (µg/L): 10 100

Concentration

(μg/L)

Lab Control

10

20

40

Analyst: EG

Initial Readings
I Salinity Temperature ('C)

33.7 15.1

33.7 15.2

33.6 15.1

80	7.6	8.12	33.4	15.2	
160	7.6	8.13	33.2	15.2	
			1		
A(8)=	30/17		Final Review	: KFP 9/16/17	711-1

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

Comments:

QC Check:

Marine Chronic Bioassay

Echinoderm Sperm-Cell Fertilization Worksheet

						=			
Client: Sample ID:	cucl	mas 2 35 soc	7			End Date	e/Time: 8/28 e/Time: 8/28	3/2017 /	1456 1536
Test No.:	1100	0554	T				pecies: S. p		
		,				Animal S	Source: 🏋	Longo	₹ ,
Tech initials:	(6					Date Co	llected:{	1211	7
Injection Time:	1400								
•									
Sperm Absorbance at 4	00 nm:	014	_(target rang	e of 0.8 - 1.	.0 for density	of 4x10 ⁶ spe	erm/ml)		
Eggs Counted:	73	_ Mear	: 70.4	_X 50 =	3520	_eggs/ml			
	61								
	67		counts of 80 e slide for a fina			•			
		_							
	72	_							
Initial density:	3520	eggs/ml	= 5-88	_dilution fac	ctor	egg stock	200	ml	
Final density:	4000	eaas/ml	- 1.0	part egg s	tock	seawater	- 1724	 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).

parts seawater

	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
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1414 1431	<u>Ra</u>	ngefinder Ra 50:1 lou:1 1ou:1 4po:1		5 2	fert. 5 +11		

<u>NOTE</u>: 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).

<u>Definitive Test</u>		Sperm:Egg Ratio Used: 106:1				
Sperm Added (100 µl): Eggs Added (0.5 ml): Test Ended:	Time 1456 1516 1536	QC1 QC2 Egg Control 1 Egg Control 2	Fert. 94 0 0	Unfert.		
Comments:	(RCGGH8/28/17					
QC Check:	AC8/34/17			Final	Review: PTP 9/6/1	1

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

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