

ENVIRONMENTAL MONITORING DIVISION  
BUREAU OF SANITATION  
CITY OF LOS ANGELES

REFERENCE TOXICANT  
TOXICITY TESTING REPORT

SAMPLE DATE: November 22, 2016

TEST DATE: November 22, 2016

TEST NUMBER: 1611RT2A.U

TEST MATERIAL: Copper Chloride

TEST SPECIES: *Strongylocentrotus purpuratus* PROTOCOL: EPA/600/R-95/136

TEST TYPE: Chronic

RESULT: NOEC < 6.5 µg/L  
IC<sub>25</sub> = 15.5 µg/L

Rea Mara A Crinklaw  
Analyst

Rea Mara A Crinklaw  
Signature

Water Biologist II  
Title

12/15/16  
Date

Kay Yamamoto  
Supervisor

Kay M. Yamamoto  
Signature

Water Biologist III  
Title

12/9/16  
Date

# CETIS Summary Report

Report Date: 05 Dec-16 14:27 (p 1 of 1)  
 Test Code: 1611RT2A.U | 16-5802-5664

Echinoid Fertilization Test				Hyperion Treatment Plant Laboratory							
Job ID:	21-4123-8470	Test Type:	Fertilization	Analyst:	Rea Mara Crinklaw						
Start Date:	22 Nov-16 15:01	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	22 Nov-16 15:41	Species:	Strongylocentrotus purpuratus	Brine:							
Duration:	40m	Source:	David Gutoff	Age:							
Sample ID:	02-9350-2430	Code:	Cu RT	Client:	Watershed Protection Division						
Sample Date:	22 Nov-16 11:37	Material:	Copper chloride	Project:	MS4						
Receive Date:	22 Nov-16 11:37	Source:	Reference Toxicant								
Sample Age:	3h	Station:									
<b>Test Note:</b> Concentration-response relationship shows significant effects at all test concentrations with a sloped curve.											
<b>Comparison Summary</b>											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
15-9126-7905	Fertilization Rate	<6.5	6.5	N/A	5.56%		Dunnett Multiple Comparison Test				
<b>Point Estimate Summary</b>											
Analysis ID	Endpoint	Level	µg/L	95% LCL	95% UCL	TU	Method				
02-1527-6888	Fertilization Rate	EC5	0.7925	0.4976	1.567		Linear Interpolation (ICPIN)				
		EC10	2.213	1.21	5.366						
		EC15	4.76	2.199	19.94						
		EC20	14.44	0.4358	15.63						
		EC25	15.49	11.98	16.65						
		EC40	19.07	17.61	21.56						
EC50	22.53	20.18	25.2								
<b>Test Acceptability</b>											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
02-1527-6888	Fertilization Rate	Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria					
15-9126-7905	Fertilization Rate	Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria					
15-9126-7905	Fertilization Rate	PMSD	0.05558	NL - 0.25	No	Passes Acceptability Criteria					
<b>Fertilization Rate Summary</b>											
Conc-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.98	0.977	0.983	0.97	0.99	0.004083	0.008165	0.83%	0.0%
6.5		4	0.745	0.7081	0.7819	0.62	0.85	0.04941	0.09883	13.27%	23.98%
9.5		4	0.875	0.8533	0.8967	0.79	0.92	0.02901	0.05802	6.63%	10.71%
13.9		4	0.8125	0.7776	0.8474	0.68	0.9	0.04679	0.09359	11.52%	17.09%
20.4		4	0.54	0.518	0.562	0.48	0.62	0.02944	0.05888	10.9%	44.9%
30		4	0.345	0.3351	0.3549	0.32	0.38	0.01323	0.02646	7.67%	64.8%
<b>Fertilization Rate Detail</b>											
Conc-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
0	Dilution Water	0.98	0.97	0.99	0.98						
6.5		0.79	0.72	0.62	0.85						
9.5		0.89	0.79	0.92	0.9						
13.9		0.68	0.84	0.9	0.83						
20.4		0.62	0.54	0.52	0.48						
30		0.35	0.33	0.38	0.32						

# CETIS Analytical Report

Report Date: 05 Dec-16 14:26 (p 1 of 2)  
Test Code: 1611RT2A.U | 16-5802-5664

Echinoid Fertilization Test				Hyperion Treatment Plant Laboratory			
<b>Analysis ID:</b> 15-9126-7905 <b>Analyzed:</b> 26 Nov-16 13:42		<b>Endpoint:</b> Fertilization Rate <b>Analysis:</b> Parametric-Control vs Treatments		<b>CETIS Version:</b> CETISv1.8.1 <b>Official Results:</b> Yes			
<b>Batch ID:</b> 21-4123-8470 <b>Start Date:</b> 22 Nov-16 15:01 <b>Ending Date:</b> 22 Nov-16 15:41 <b>Duration:</b> 40m		<b>Test Type:</b> Fertilization <b>Protocol:</b> EPA/600/R-95/136 (1995) <b>Species:</b> Strongylocentrotus purpuratus <b>Source:</b> David Gutoff		<b>Analyst:</b> Rea Mara Crinklaw <b>Diluent:</b> Laboratory Seawater <b>Brine:</b> <b>Age:</b>			
<b>Sample ID:</b> 02-9350-2430 <b>Sample Date:</b> 22 Nov-16 11:37 <b>Receive Date:</b> 22 Nov-16 11:37 <b>Sample Age:</b> 3h		<b>Code:</b> Cu RT <b>Material:</b> Copper chloride <b>Source:</b> Reference Toxicant <b>Station:</b>		<b>Client:</b> Watershed Protection Division <b>Project:</b> MS4			
<b>Test Note:</b> Concentration-response relationship shows significant effects at all test concentrations with a sloped curve.							
<b>Data Transform</b>	<b>Zeta</b>	<b>Alt Hyp</b>	<b>MC Trials</b>	<b>NOEL</b>	<b>LOEL</b>	<b>TOEL</b>	<b>PMSD</b>
Angular (Corrected)	0	C > T	Not Run	<6.5	6.5	N/A	5.56%
<b>Dunnett Multiple Comparison Test</b>							
<b>Control</b>	<b>vs Conc-µg/L</b>	<b>Test Stat</b>	<b>Critical</b>	<b>DF</b>	<b>MSD</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>
Dilution Water	6.5*	6.759	2.407	6	0.1369	<0.0001	Significant Effect
	9.5*	3.801	2.407	6	0.1369	0.0028	Significant Effect
	13.9*	5.282	2.407	6	0.1369	0.0001	Significant Effect
	20.4*	10.65	2.407	6	0.1369	<0.0001	Significant Effect
	30*	14.13	2.407	6	0.1369	<0.0001	Significant Effect
<b>Test Acceptability Criteria</b>							
<b>Attribute</b>	<b>Test Stat</b>	<b>TAC Limits</b>	<b>Overlap</b>	<b>Decision</b>			
Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria			
PMSD	0.05558	NL - 0.25	No	Passes Acceptability Criteria			
<b>Auxiliary Tests</b>							
<b>Attribute</b>	<b>Test</b>	<b>Test Stat</b>	<b>Critical</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>		
Extreme Value	0	2.268	2.802	0.4025	No Outliers Detected		
<b>ANOVA Table</b>							
<b>Source</b>	<b>Sum Squares</b>	<b>Mean Square</b>	<b>DF</b>	<b>F Stat</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>	
Between	1.631359	0.3262717	5	50.45	<0.0001	Significant Effect	
Error	0.1164188	0.006467711	18				
Total	1.747777	0.3327394	23				
<b>Distributional Tests</b>							
<b>Attribute</b>	<b>Test</b>	<b>Test Stat</b>	<b>Critical</b>	<b>P-Value</b>	<b>Decision(α:1%)</b>		
Variances	Bartlett Equality of Variance	8.428	15.09	0.1342	Equal Variances		
Distribution	Shapiro-Wilk W Normality	0.9516	0.884	0.2928	Normal Distribution		

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Analysis ID: 15-9126-7905      Endpoint: Fertilization Rate  
 Analyzed: 26 Nov-16 13:42      Analysis: Parametric-Control vs Treatments

CETIS Version: CETISv1.8.1  
 Official Results: Yes

## Fertilization Rate Summary

Conc-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.98	0.9769	0.9831	0.97	0.99	0.004083	0.008165	0.83%	0.0%
6.5		4	0.745	0.7074	0.7826	0.62	0.85	0.04941	0.09883	13.27%	23.98%
9.5		4	0.875	0.8529	0.8971	0.79	0.92	0.02901	0.05802	6.63%	10.71%
13.9		4	0.8125	0.7769	0.8481	0.68	0.9	0.04679	0.09359	11.52%	17.09%
20.4		4	0.54	0.5176	0.5624	0.48	0.62	0.02944	0.05888	10.9%	44.9%
30		4	0.345	0.3349	0.3551	0.32	0.38	0.01323	0.02646	7.67%	64.8%

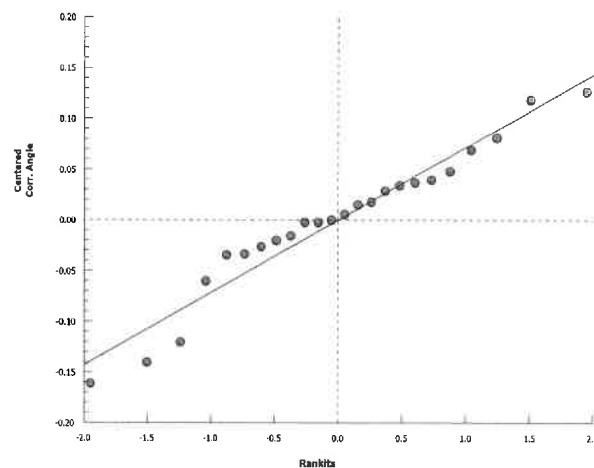
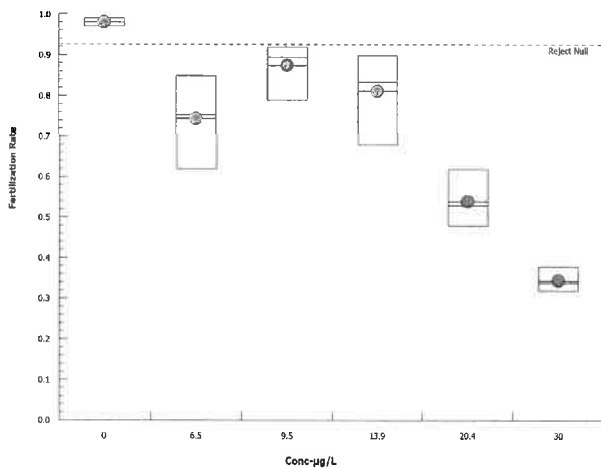
## Angular (Corrected) Transformed Summary

Conc-µg/L	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1.431	1.42	1.443	1.397	1.471	0.01515	0.0303	2.12%	0.0%
6.5		4	1.047	1.004	1.09	0.9066	1.173	0.05704	0.1141	10.9%	26.86%
9.5		4	1.215	1.184	1.247	1.095	1.284	0.04153	0.08306	6.84%	15.1%
13.9		4	1.131	1.086	1.175	0.9695	1.249	0.05847	0.1169	10.34%	20.99%
20.4		4	0.8257	0.8031	0.8483	0.7654	0.9066	0.02971	0.05942	7.2%	42.31%
30		4	0.6276	0.6171	0.6382	0.6013	0.6642	0.01387	0.02774	4.42%	56.15%

## Fertilization Rate Detail

Conc-µg/L	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.98	0.97	0.99	0.98
6.5		0.79	0.72	0.62	0.85
9.5		0.89	0.79	0.92	0.9
13.9		0.68	0.84	0.9	0.83
20.4		0.62	0.54	0.52	0.48
30		0.35	0.33	0.38	0.32

## Graphics



Concentration-response relationship shows significant effects at all test concentrations with a sloped curve.  
 12/5/16 Rc

# CETIS Analytical Report

Report Date: 05 Dec-16 14:27 (p 1 of 2)  
Test Code: 1611RT2A.U | 16-5802-5664

<b>Echinoid Fertilization Test</b>				<b>Hyperion Treatment Plant Laboratory</b>							
<b>Analysis ID:</b> 02-1527-6888		<b>Endpoint:</b> Fertilization Rate		<b>CETIS Version:</b> CETISv1.8.1							
<b>Analyzed:</b> 26 Nov-16 13:42		<b>Analysis:</b> Linear Interpolation (ICPIN)		<b>Official Results:</b> Yes							
<b>Batch ID:</b> 21-4123-8470		<b>Test Type:</b> Fertilization		<b>Analyst:</b> Rea Mara Crinklaw							
<b>Start Date:</b> 22 Nov-16 15:01		<b>Protocol:</b> EPA/600/R-95/136 (1995)		<b>Diluent:</b> Laboratory Seawater							
<b>Ending Date:</b> 22 Nov-16 15:41		<b>Species:</b> Strongylocentrotus purpuratus		<b>Brine:</b>							
<b>Duration:</b> 40m		<b>Source:</b> David Gutoff		<b>Age:</b>							
<b>Sample ID:</b> 02-9350-2430		<b>Code:</b> Cu RT		<b>Client:</b> Watershed Protection Division							
<b>Sample Date:</b> 22 Nov-16 11:37		<b>Material:</b> Copper chloride		<b>Project:</b> MS4							
<b>Receive Date:</b> 22 Nov-16 11:37		<b>Source:</b> Reference Toxicant									
<b>Sample Age:</b> 3h		<b>Station:</b>									
<b>Test Note:</b> Concentration-response relationship shows significant effects at all test concentrations with a sloped curve.											
<b>Linear Interpolation Options</b>											
<b>X Transform</b>	<b>Y Transform</b>	<b>Seed</b>	<b>Resamples</b>	<b>Exp 95% CL</b>	<b>Method</b>						
Log(X+1)	Linear	1.558E+09	200	Yes	Two-Point Interpolation						
<b>Test Acceptability Criteria</b>											
<b>Attribute</b>	<b>Test Stat</b>	<b>TAC Limits</b>	<b>Overlap</b>	<b>Decision</b>							
Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria							
<b>Residual Analysis</b>											
<b>Attribute</b>	<b>Method</b>	<b>Test Stat</b>	<b>Critical</b>	<b>P-Value</b>	<b>Decision(α:5%)</b>						
Extreme Value	Grubbs Extreme Value	2.268	2.802	0.4025	No Outliers Detected						
<b>EC50 Estimates</b>											
<b>Conc</b>	<b>μg/L</b>	<b>95% LCL</b>	<b>95% UCL</b>								
EC5	0.7925	0.4976	1.567								
EC10	2.213	1.21	5.366								
EC15	4.76	2.199	19.94								
EC20	14.44	0.4358	15.63								
EC25	15.49	11.98	16.65								
EC40	19.07	17.61	21.56								
EC50	22.53	20.18	25.2								
<b>Fertilization Rate Summary</b>											
		<b>Calculated Variate(A/B)</b>									
<b>Conc-μg/L</b>	<b>Control Type</b>	<b>Count</b>	<b>Mean</b>	<b>Min</b>	<b>Max</b>	<b>Std Err</b>	<b>Std Dev</b>	<b>CV%</b>	<b>%Effect</b>	<b>A</b>	<b>B</b>
0	Dilution Water	4	0.98	0.97	0.99	0.004083	0.008165	0.83%	0.0%	392	400
6.5		4	0.745	0.62	0.85	0.04941	0.09883	13.27%	23.98%	298	400
9.5		4	0.875	0.79	0.92	0.02901	0.05802	6.63%	10.71%	350	400
13.9		4	0.8125	0.68	0.9	0.04679	0.09359	11.52%	17.09%	325	400
20.4		4	0.54	0.48	0.62	0.02944	0.05888	10.9%	44.9%	216	400
30		4	0.345	0.32	0.38	0.01323	0.02646	7.67%	64.8%	138	400
<b>Fertilization Rate Detail</b>											
<b>Conc-μg/L</b>	<b>Control Type</b>	<b>Rep 1</b>	<b>Rep 2</b>	<b>Rep 3</b>	<b>Rep 4</b>						
0	Dilution Water	0.98	0.97	0.99	0.98						
6.5		0.79	0.72	0.62	0.85						
9.5		0.89	0.79	0.92	0.9						
13.9		0.68	0.84	0.9	0.83						
20.4		0.62	0.54	0.52	0.48						
		0.35	0.33	0.38	0.32						

# CETIS Analytical Report

Report Date: 05 Dec-16 14:27 (p 2 of 2)  
Test Code: 1611RT2A.U | 16-5802-5664

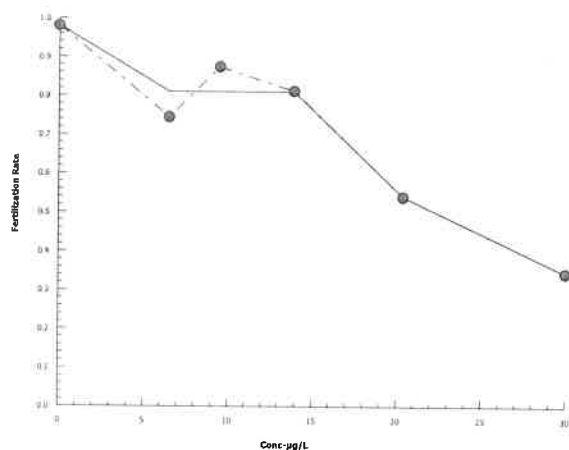
## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Analysis ID: 02-1527-6888  
Analyzed: 26 Nov-16 13:42  
Endpoint: Fertilization Rate  
Analysis: Linear Interpolation (ICPIN)

CETIS Version: CETISv1.8.1  
Official Results: Yes

### Graphics



## Echinoid Fertilization Test

## Hyperion Treatment Plant Laboratory

Test Type: Fertilization

Organism: Strongylocentrotus purpuratus (Purpl

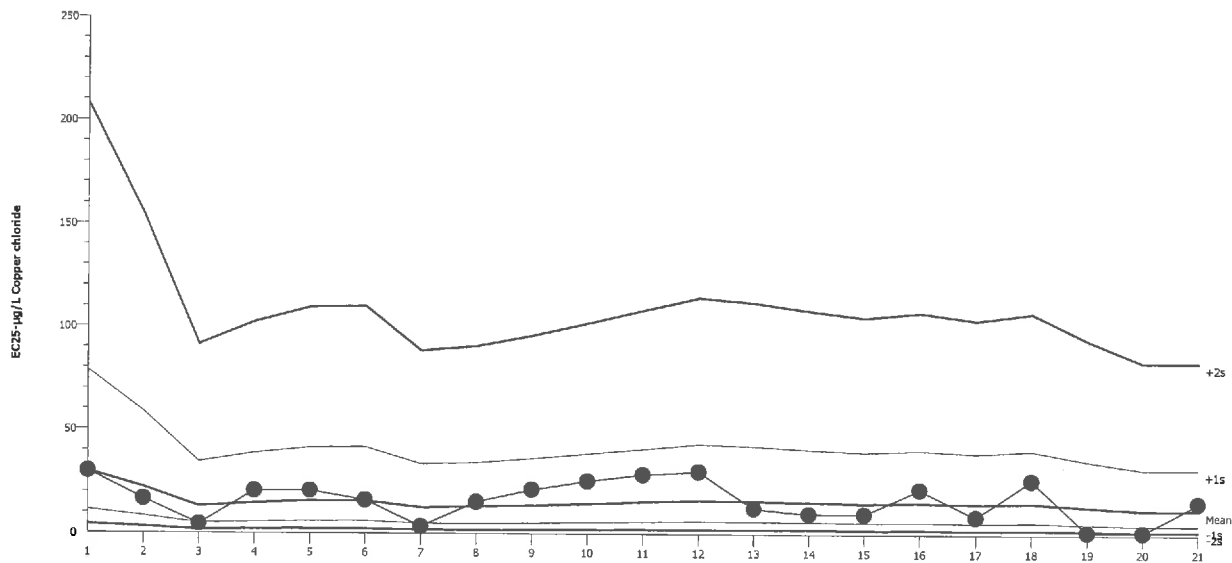
Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF

Echinoid Fertilization Test

Mean: 12  
Sigma: N/ACount: 20  
CV: 164.00%-1s Warning Limit: 4.555  
+1s Warning Limit: 31.65-2s Action Limit: 1.728  
+2s Action Limit: 83.44

## Quality Control Data

Count	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2004	Feb	11	29.92	17.92	0.9425			02-9415-3935	09-5439-6446
2			28	16.64	4.643	0.3374			05-3112-2731	08-9498-1472
3			28	4.569	-7.432	-0.9962			10-2773-6804	07-4530-8211
4			29	20.56	8.561	0.5555			03-1212-4136	13-5407-3332
5			29	20.72	8.717	0.5633			11-7058-6495	01-8633-7201
6		Mar	2	16.21	4.209	0.3102			09-2137-0269	13-7772-0800
7		Apr	18	3.474	-8.527	-1.279	(-)		11-9278-6264	13-4799-4359
8	2005	Jan	5	15.34	3.343	0.2535			11-9712-5110	08-2196-4872
9			19	21.36	9.358	0.5947			12-4585-7379	08-8243-3392
10		Mar	7	25.41	13.41	0.7738			08-1939-8776	00-2976-8574
11			25	28.71	16.71	0.8999			09-9632-4874	00-6080-0300
12			25	30.16	18.16	0.9506			18-5497-6485	04-1608-0162
13	2008	Jun	26	12.56	0.558	0.04688			10-0944-2397	11-0169-5017
14	2010	Aug	25	9.899	-2.102	-0.1987			02-9945-6715	00-8068-9638
15	2011	Dec	1	9.867	-2.134	-0.202			15-6477-9589	14-4147-3826
16	2012	Sep	13	21.75	9.752	0.6136			08-7937-2326	08-7756-3235
17		Oct	23	8.58	-3.421	-0.3462			08-2188-1692	07-5914-6938
18	2013	Sep	6	26.33	14.33	0.8108			16-8889-8873	07-2568-7151
19	2015	Jul	1	1.345	-10.66	-2.258	(-)	(-)	03-4008-6194	06-8546-4015
20	2016	Jan	11	1.215	-10.79	-2.363	(-)	(-)	17-5839-4263	07-4356-3957
21		Nov	22	15.49	3.488	0.2633			16-5802-5664	02-1527-6888

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Test Type: Fertilization

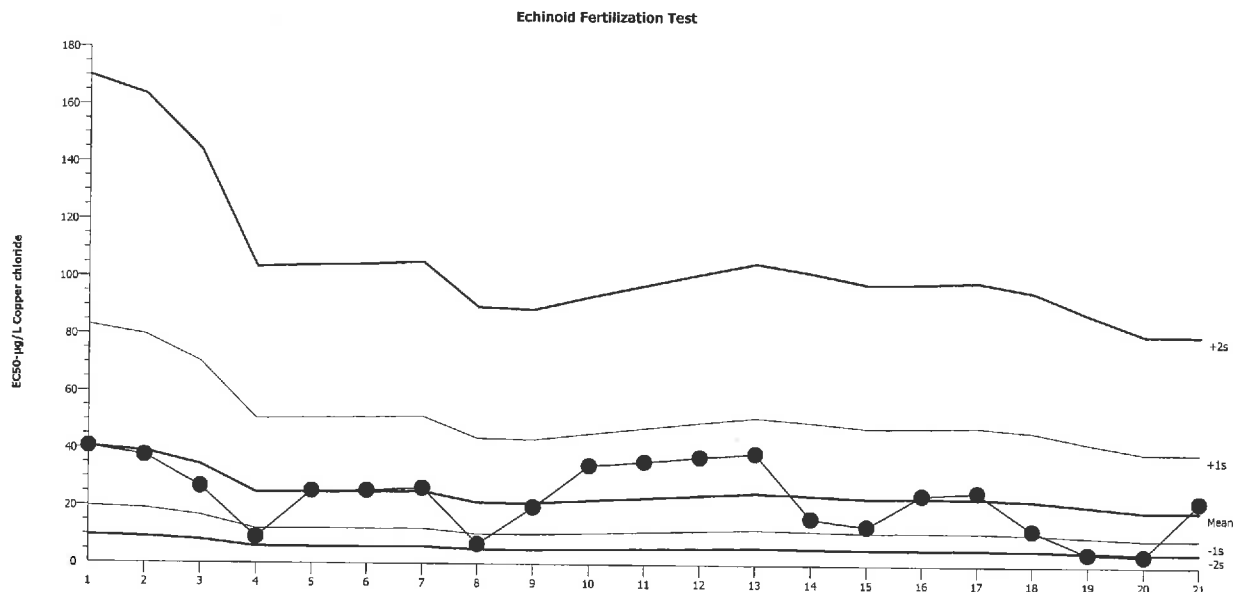
Organism: Strongylocentrotus purpuratus (Purpl

Material: Copper chloride

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

Endpoint: Fertilization Rate

Source: Reference Toxicant-REF



Mean: 19.24

Count: 20

-1s Warning Limit: 9.396

-2s Action Limit: 4.589

Sigma: N/A

CV: 105.00%

+1s Warning Limit: 39.38

+2s Action Limit: 80.62

## Quality Control Data

Count	Year	Month	Day	QC Data	Delta	Sigma	Warning	Action	Test ID	Analysis ID
1	2004	Feb	11	40.57	21.33	1.041	(+)		02-9415-3935	09-5439-6446
2			11	37.54	18.3	0.9328			13-7609-3605	12-8550-1543
3			28	26.8	7.557	0.4624			05-3112-2731	08-9498-1472
4			28	9.139	-10.1	-1.039	(-)		10-2773-6804	07-4530-8211
5			29	25.32	6.077	0.3831			03-1212-4136	13-5407-3332
6			29	25.42	6.181	0.3888			11-7058-6495	01-8633-7201
7		Mar	2	26.36	7.123	0.4396			09-2137-0269	13-7772-0800
8		Apr	18	6.948	-12.29	-1.422	(-)		11-9278-6264	13-4799-4359
9	2005	Jan	5	19.9	0.6645	0.04739			11-9712-5110	08-2196-4872
10			19	34.37	15.13	0.8098			12-4585-7379	08-8243-3392
11		Mar	7	35.79	16.54	0.8661			08-1939-8776	00-2976-8574
12			25	37.52	18.28	0.9322			09-9632-4874	00-6080-0300
13			25	38.87	19.63	0.9813			18-5497-6485	04-1608-0162
14	2008	Jun	26	16.25	-2.989	-0.2356			10-0944-2397	11-0169-5017
15	2010	Aug	25	13.61	-5.634	-0.4835			02-9945-6715	00-8068-9638
16	2011	Dec	1	24.61	5.37	0.3436			15-6477-9589	14-4147-3826
17	2012	Sep	13	25.66	6.424	0.4021			08-7937-2326	08-7756-3235
18		Oct	23	12.49	-6.755	-0.6035			08-2188-1692	07-5914-6938
19	2015	Jul	1	4.499	-14.74	-2.028	(-)	(-)	03-4008-6194	06-8546-4015
20	2016	Jan	11	3.906	-15.33	-2.225	(-)	(-)	17-5839-4263	07-4356-3957
21		Nov	22	22.53	3.293	0.2205			16-5802-5664	02-1527-6888

# CETIS Test Data Worksheet

Report Date: 22 Nov-16 08:08 (p 1 of 1)  
Test Code: 16-5802-5664/1611RT2A.U

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Date: 22 Nov-16 15:01 Species: Strongylocentrotus purpuratus  
End Date: 22 Nov-16 15:41 Protocol: EPA/600/R-95/136 (1995)  
Sample Date: 22 Nov-16 Material: Copper chloride

Sample Code: Cu RT  
Sample Source: Reference Toxicant  
Sample Station:

Conc-µg/L	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	D	1	11	100	98	
0	D	2	31	100	97	
0	D	3	1	100	99	
0	D	4	30	100	98	
6.5		1	32	100	79	72
6.5		2	29	100	72	
6.5		3	22	100	62	
6.5		4	28	100	85	
9.5		1	6	100	89	
9.5		2	21	100	79	
9.5		3	27	100	92	
9.5		4	5	100	90	
13.9		1	4	100	68	
13.9		2	8	100	84	
13.9		3	16	100	90	
13.9		4	13	100	83	
20.4		1	3	100	62	
20.4		2	2	100	54	
20.4		3	7	100	52	
20.4		4	26	100	48	
30		1	24	100	35	
30		2	25	100	33	
30		3	17	100	38	
30		4	19	100	32	

# CETIS Measurement Worksheet

Report Date: 22 Nov-16 08:08 (p 1 of 1)  
 Test Code: 1611RT2A.U | 16-5802-5664

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

rt Date: 22 Nov-16 Species: Strongylocentrotus purpuratus  
 Date: 22 Nov-16 Protocol: EPA/600/R-95/136 (1995)  
 Sample Date: 22 Nov-16 Material: Copper chloride

Sample Code: Cu RT  
 Sample Source: Reference Toxicant  
 Sample Station:

### Dissolved Oxygen-mg/L 11/22

Conc-µg/L	Code	Reading 1
0	D	8.14
6.5		8.03
9.5		8.00
13.9		8.00
20.4		8.02
30		8.01

Measure Time: 1137 *Ac* 11/22/16  
 Instrument ID: #42  
 Analyst: *Ac*

### pH 11/22

Conc-µg/L	Code	Reading 1
0	D	8.00
6.5		8.06
9.5		8.07
13.9		8.08
20.4		8.07
30		8.08

Measure Time: 1137  
 Instrument ID: #4  
 Analyst: *Ac*

### Salinity-ppt 11/22

Conc-µg/L	Code	Reading 1
0	D	33
6.5		33
9.5		33
13.9		33
20.4		33
30		33

Cond Measure Time: 1137 *Ac* 11/22/16  
 Instrument ID: #42 (4)  
 Analyst: *Ac*

### Temperature-°C 11/22

Conc-µg/L	Code	Reading 1
0	D	12.5
6.5		12.4
9.5		12.3
13.9		12.3
20.4		12.4
30		12.2

Measure Time: 1137  
 Instrument ID: #4  
 Analyst: *Ac*

11/23/16

11/24/16

11/25/16

11/26/16

11/27/16

Non-Fert Fertilized Total

Non-Fert Fertilized Total

11/25/16

recount

11/24/16

1	1	99	100
2	<del>57</del>	<del>43</del>	<del>100</del>
3	38	62	100
4	32	68	100
5	10	90	100
6	11	89	100
7	48	52	100
8	16	84	100
9	1	99	100
10	1	99	100
11	0	100	100
12	2	98	100
13	17	83	100
14	1	99	100
15	0	100	100
16	10	90	100
17	62	38	100
18	1	99	100
19	68	32	100
20	1	99	100
21	21	79	100
22	38	62	100
23	1	99	100
24	65	35	100
25	67	33	100
26	52	48	100
27	8	92	100
28	15	85	100
29	28	72	100
30	2	98	100
31	3	97	100
32	21	79	100
33			
ED 34	100	0	100
ED 35	100	0	100
EE-1 36	100	0	100
EE-1 37	100	0	100
EE-2 38	100	0	100
EE-2 39	100	0	100
40			
41			
42			
43			
44			
45			

46			
47	46	54	100
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Figure 2. Sample data sheet for egg and sperm counts. 11/22/16

EGG COUNTS -

Sample	Dilution	Count	Eggs/mL	Undiluted
A	1:100	180/0.100	180/mL x 100	18000
B	1:10	146/0.100	1460/mL x 10	14600
working		183/0.100	1830	

For 100 mL egg suspension at 2,240 eggs/mL use:

100 mL x 2,240 eggs/mL / (counted eggs/mL) = mL of egg stock

224,000 eggs / 14,600 eggs/mL = 15.34 mL

If required stock >100 mL, concentrate egg stock by settling the eggs and decanting off sufficient overlying water to retain:

(\_\_\_\_\_ eggs/mL / 2,240 eggs/mL) x 100 = \_\_\_\_\_ % volume

SPERM COUNTS - used Coulter Counter Multisizer 3

Sample	Dilution	Count	Squares	Sperm/mL
initial "3000:1"				34.81 x 10 <sup>6</sup> /mL
working "1500:1"				18.00 x 10 <sup>6</sup> /mL → FINAL

$$\text{SPERM/mL} = \frac{(\text{DIL. FACT.}) (\text{COUNT}) (4000) (1000)}{(\text{NO. SQUARES COUNTED})}$$

$$\text{ratio} = \frac{(0.100 \text{ mL}) (18.00 \times 10^6 / \text{mL})}{(0.500 \text{ mL}) (1830 \text{ eggs/mL})} = \frac{1967 \text{ sperm}}{1 \text{ egg}}$$

Sperm 15:01-15:04

Eggs 15:21-15:25

Formalin 15:41-15:46

## Trial Fertilization

3000

100%

98%

1500

95%

97%

use "1500:1"

750

90%

93%

Sperm 1334

Eggs 1356

Formalin 1416

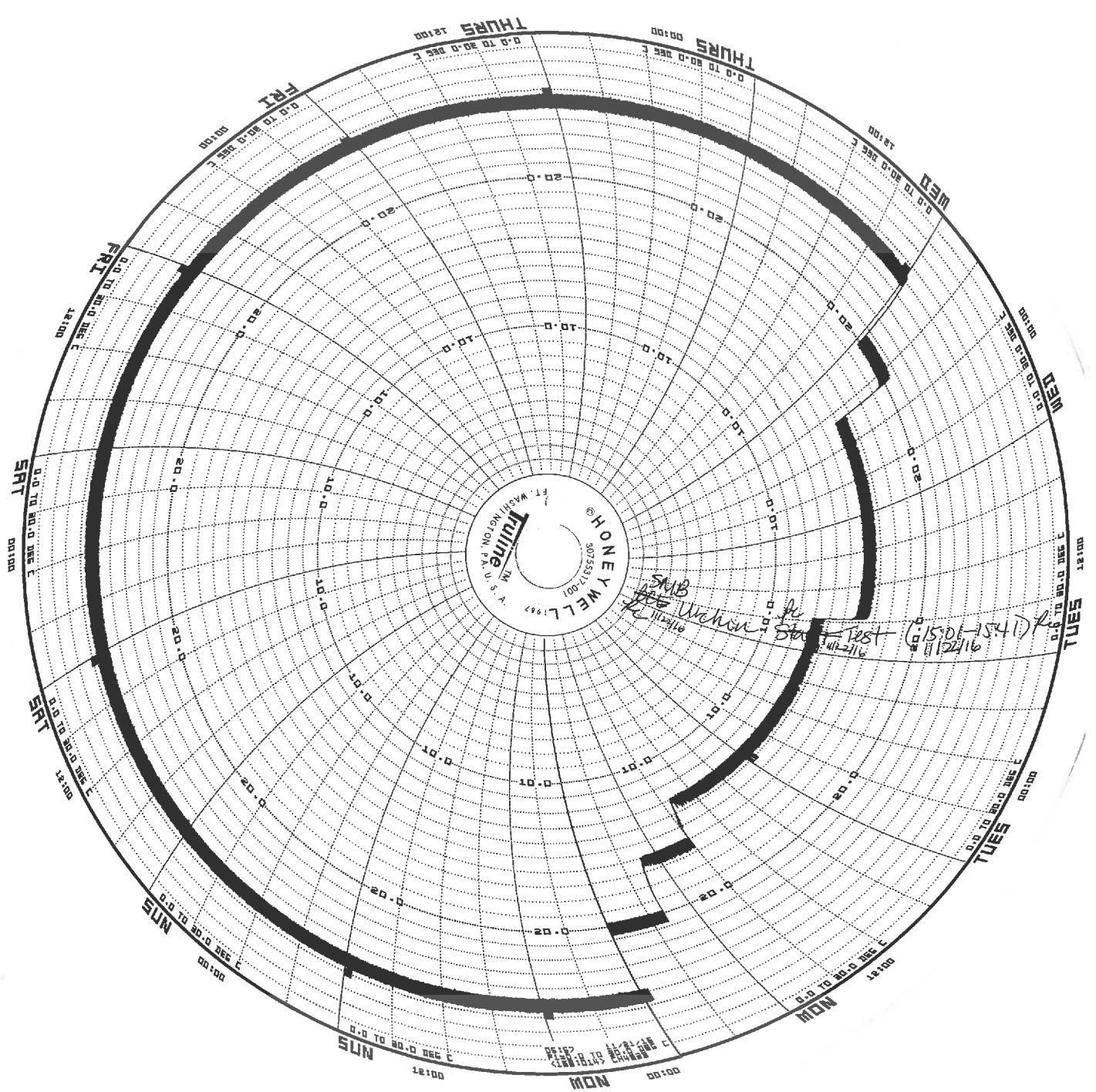
Figure 1. Sample data sheet for spawning record.

11/22/66

Animal No.	Sex	Time		Comments
		Injected	Spawn	
1	M	12:09	12:21	
2	F	12:10	12:12	good quality
3	F	12:11	12:12	good quality
4	F	12:13	12:25	12:25
5	F	12:15	12:16	good quality
6	M	12:16	12:17	good motility
7	M	12:18	12:18	
8	M	12:19	12:34	12:27
9				
10				
11				
12				

Pooled eggs from female nos. 2, 3, 5.

Pooled ( 30 <sup>μ</sup>L ) of sperm each from male nos. 6.



Test: 1611RT 2A.U, 1611072A-B.U

Date: 11/22/16 (15:01-15:41)

\* No HOBO temperature recorder data available

ENVIRONMENTAL MONITORING DIVISION  
BUREAU OF SANITATION  
CITY OF LOS ANGELES

STORMWATER MONITORING PROGRAM  
TOXICITY TESTING REPORT

SAMPLE DATE: November 21, 2016

TEST DATE: November 22, 2016

TEST NUMBER: 1611072A.U

TEST MATERIAL: Station RW-SMB-1

TEST SPECIES: *Strongylocentrotus purpuratus* PROTOCOL: EPA/600/R-95/136

TEST TYPE: Chronic

REFERENCE TOXICANT TEST: 1611RT2A.U

RESULT: Pass, -1.02% Effect

Rea Mara A Crinklaw

Analyst

Rea Mara A Crinklaw  
Signature

Water Biologist II

Title

12/5/16  
Date

Kay Yamamoto

Supervisor

Kay M. Yamamoto  
Signature

Water Biologist III

Title

12/9/16  
Date

# CETIS Summary Report

Report Date: 05 Dec-16 14:48 (p 1 of 1)  
Test Code: 1611072A.U | 06-2289-0124

Echinoid Fertilization Test				Hyperion Treatment Plant Laboratory							
Sh ID:	21-4123-8470	Test Type:	Fertilization	Analyst:	Rea Mara Crinklaw						
Date:	22 Nov-16 15:01	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	22 Nov-16 15:41	Species:	Strongylocentrotus purpuratus	Brine:							
Duration:	40m	Source:	David Gutoff	Age:							
Sample ID:	12-9891-7884	Code:	HT530508	Client:	Watershed Protection Division						
Sample Date:	21 Nov-16 12:02	Material:	Stormwater Monitoring Sample	Project:	MS4						
Receive Date:	21 Nov-16 14:45	Source:	WPD (WATERSHED)								
Sample Age:	27h (13.4 °C)	Station:	RW-SMB-1								
<b>Comparison Summary</b>											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
09-3569-8763	Fertilization Rate	100	>100	N/A	N/A	1	TST-Welch's t Test				
<b>Test Acceptability</b>											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
09-3569-8763	Fertilization Rate	Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria					
<b>Fertilization Rate Summary</b>											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.98	0.977	0.983	0.97	0.99	0.004083	0.008165	0.83%	0.0%
0	Egg/Dilution Wa	2	0	0	0	0	0	0	0		100.0%
0	Egg/Effluent	2	0	0	0	0	0	0	0		100.0%
100		4	0.99	0.9899	0.9901	0.99	0.99	0	0	0.0%	-1.02%
<b>Fertilization Rate Detail</b>											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
	Dilution Water	0.98	0.97	0.99	0.98						
	Egg/Dilution Wa	0	0								
0	Egg/Effluent	0	0								
100		0.99	0.99	0.99	0.99						

# CETIS Analytical Report

Report Date: 05 Dec-16 14:48 (p 1 of 2)  
Test Code: 1611072A.U | 06-2289-0124

Echinoid Fertilization Test				Hyperion Treatment Plant Laboratory							
Analysis ID:	09-3569-8763	Endpoint:	Fertilization Rate	CETIS Version:	CETISv1.8.1						
Analyzed:	05 Dec-16 14:44	Analysis:	Parametric Bioequivalence-Two Sample	Official Results:	Yes						
Batch ID:	21-4123-8470	Test Type:	Fertilization	Analyst:	Rea Mara Crinklaw						
Start Date:	22 Nov-16 15:01	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	22 Nov-16 15:41	Species:	Strongylocentrotus purpuratus	Brine:							
Duration:	40m	Source:	David Gutoff	Age:							
Sample ID:	12-9891-7884	Code:	HT530508	Client:	Watershed Protection Division						
Sample Date:	21 Nov-16 12:02	Material:	Stormwater Monitoring Sample	Project:	MS4						
Receive Date:	21 Nov-16 14:45	Source:	WPD (WATERSHED)								
Sample Age:	27h (13.4 °C)	Station:	RW-SMB-1								
Data Transform	Zeta	Alt Hyp	MC Trials	TST b	Test Result						
Angular (Corrected)	0	C*b > T	Not Run	0.75	Sample passes fertilization rate endpoint						
TST-Welch's t Test											
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value				
Dilution Water		100*	34.95	2.353	3		<0.0001				
							Decision(α:5%)				
							Non-Significant Effect				
Test Acceptability Criteria											
Attribute	Test Stat	TAC Limits	Overlap	Decision							
Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria							
Auxiliary Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)						
Extreme Value	0	1.983	2.127	0.1343	No Outliers Detected						
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.003095853	0.003095853	1	6.743	0.0408	Significant Effect					
Error	0.002754525	0.0004590875	6								
Total	0.005850377	0.00355494	7								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Mod Levene Equality of Variance	2.903	13.75	0.1393	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.7647	0.6451	0.0119	Normal Distribution						
Fertilization Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.98	0.9769	0.9831	0.97	0.99	0.004083	0.008165	0.83%	0.0%
100		4	0.99	0.9899	0.9901	0.99	0.99	0	0	0.0%	-1.02%
Angular (Corrected) Transformed Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1.431	1.42	1.443	1.397	1.471	0.01515	0.0303	2.12%	0.0%
100		4	1.471	1.471	1.471	1.471	1.471	0	0	0.0%	-2.75%

# CETIS Analytical Report

Report Date: 05 Dec-16 14:48 (p 2 of 2)

Test Code: 1611072A.U | 06-2289-0124

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Analysis ID: 09-3569-8763  
Analyzed: 05 Dec-16 14:44

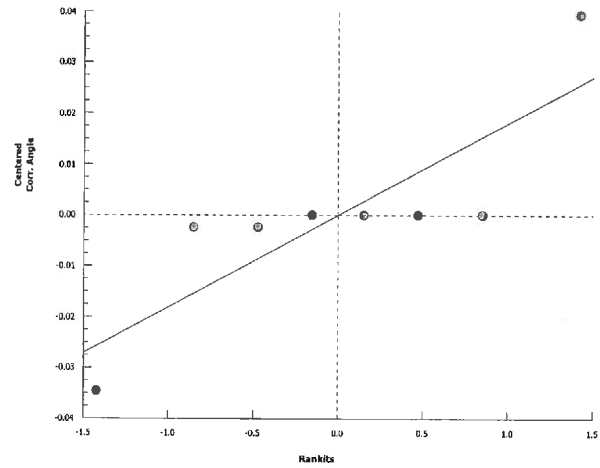
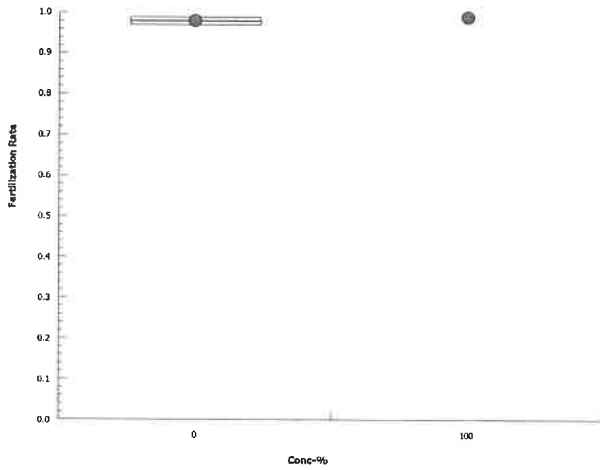
Endpoint: Fertilization Rate  
Analysis: Parametric Bioequivalence-Two Sample

CETIS Version: CETISv1.8.1  
Official Results: Yes

### Fertilization Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.98	0.97	0.99	0.98
100		0.99	0.99	0.99	0.99

### Graphics



# CETIS Test Data Worksheet



Report Date: 22 Nov-16 08:09 (p 1 of 1)  
Test Code: 01-4055-4391/1611072A.U

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Test Date: 22 Nov-16 15:01 Species: Strongylocentrotus purpuratus Sample Code: 8EA043B  
 End Date: 22 Nov-16 15:41 Protocol: EPA/600/R-95/136 (1995) Sample Source: WPD  
 Sample Date: 21 Nov-16 Material: Stormwater Monitoring Sample Sample Station: RW-SMB-1

See 1611 RT2A.U

Conc-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	D	1		100	98	
0	D	2		100	97	
0	D	3		100	99	
0	D	4		100	98	
0	ED	1		100	0	
0	ED	2		100	0	
0	EE	1		100	0	
0	EE	2		100	0	
100		1	14	100	99	
100		2	10	100	99	
100		3	20	100	99	
100		4	18	100	99	

# CETIS Measurement Worksheet

Report Date: 22 Nov-16 08:09 (p 1 of 1)  
Test Code: 1611072A.U | 01-4055-4391

Echinoid Fertilization Test			Hyperion Treatment Plant Laboratory
Start Date: 22 Nov-16	Species: Strongylocentrotus purpuratus	Sample Code: 8EA043B	
Date: 22 Nov-16	Protocol: EPA/600/R-95/136 (1995)	Sample Source: WPD	
Sample Date: 21 Nov-16	Material: Stormwater Monitoring Sample	Sample Station: RW-SMB-1	

Dissolved Oxygen-mg/L 11/22		
Conc-%	Code	Reading 1
0	D	8.14
100		8.20
Measure Time:		1144
Instrument ID:		#2
Analyst:		Re

pH 11/22		
Conc-%	Code	Reading 1
0	D	8.00
100		8.20
Measure Time:		1144
Instrument ID:		#4
Analyst:		Re

Salinity-ppt 11/22		
Conc-%	Code	Reading 1
0	D	33
100		33
Measure Time:		1144
Instrument ID:		#4
Analyst:		Re

Temperature-°C 11/22		
Conc-%	Code	Reading 1
0	D	12.5
100		12.4
Measure Time:		1144
Instrument ID:		#4
Analyst:		Re

11/23/16

11/24/16

11/25/16

11/26/16

11/27/16

Non-Fert Fertilized Total

11/25/16

11/24/16

1	1	99	100
2	57	43	100
3	38	62	100
4	32	68	100
5	10	90	100
6	11	89	100
7	48	52	100
8	16	84	100
9	1	99	100
10	1	99	100
11	0	100	100
12	2	98	100
13	17	83	100
14	1	99	100
15	0	100	100
16	10	90	100
17	62	38	100
18	1	99	100
19	68	32	100
20	1	99	100
21	21	79	100
22	38	62	100
23	1	99	100
24	65	35	100
25	67	33	100
26	52	48	100
27	8	92	100
28	15	85	100
29	28	72	100
30	2	98	100
31	3	97	100
32	21	79	100
33			
ED 34	100	0	100
ED 35	100	0	100
EE-1 36	100	0	100
EE-1 37	100	0	100
EE-2 38	100	0	100
EE-2 39	100	0	100
40			
41			
42			
43			
44			
45			

Non-Fert Fertilized Total

recount

46			
47	46	54	100
48			
49			
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Figure 2. Sample data sheet for egg and sperm counts. 11/22/16

EGG COUNTS -

Sample	Dilution	Count	Eggs/mL	Undiluted
A	1:100	180/0.100	180/mL x 100	18,000
B	1:10	146/0.100	1460/mL x 10	14,600
working		183/0.100	1830	

For 100 mL egg suspension at 2,240 eggs/mL use:

$$100 \text{ mL} \times 2,240 \text{ eggs/mL} / (\text{counted eggs/mL}) = \text{mL of egg stock}$$

$$224,000 \text{ eggs} / 14,600 \text{ eggs/mL} = 15.34 \text{ mL}$$

If required stock >100 mL, concentrate egg stock by settling the eggs and decanting off sufficient overlying water to retain:

$$(\text{_____ eggs/mL} / 2,240 \text{ eggs/mL}) \times 100 = \text{_____ \% volume}$$

SPERM COUNTS - used Coulter Counter Multisizer 3

Sample	Dilution	Count	Squares	Sperm/mL
initial "3000:1"				34.81 x 10 <sup>6</sup> /mL
working "1500:1"				18.00 x 10 <sup>6</sup> /mL → FINAL

$$\text{SPERM/mL} = \frac{(\text{DIL.FACT.}) (\text{COUNT}) (4000) (1000)}{(\text{NO. SQUARES COUNTED})}$$

$$\text{ratio} = \frac{(0.100 \text{ mL}) (18.00 \times 10^6 / \text{mL})}{(0.500 \text{ mL}) (1830 \text{ eggs/mL})} = \frac{1967 \text{ sperm}}{1 \text{ egg}}$$

Sperm 15:01-15:04

Eggs 15:21-15:25

Formalin 15:41-15:46

## Trial Fertilization

3000

100% 98%

1500

95% 97%

use "1500:1"

750

90% 93%

Sperm 1334

Eggs 1356

Formalin 1416

Figure 1. Sample data sheet for spawning record.

11/22/66

Animal No.	Sex	Time		Comments
		Injected	Spawn	
1	M	12:09	12:21	
2	F	12:10	12:12	good quality
3	F	12:11	12:12	good quality
4	F	12:13	12:25	12:25
5	F	12:15	12:16	good quality
6	M	12:16	12:17	good motility
7	M	12:18	12:18	
8	M	12:19	12:34	12:27
9				
10				
11				
12				

Pooled eggs from female nos. 2, 3, 5.

Pooled ( 30 <sup>ml</sup> ) of sperm each from male nos. 6.



ENVIRONMENTAL MONITORING DIVISION  
BUREAU OF SANITATION  
CITY OF LOS ANGELES

STORMWATER MONITORING PROGRAM  
TOXICITY TESTING REPORT

SAMPLE DATE: November 21, 2016

TEST DATE: November 22, 2016

TEST NUMBER: 1611072B.U

TEST MATERIAL: Station RW-SMB-3

TEST SPECIES: *Strongylocentrotus purpuratus* PROTOCOL: EPA/600/R-95/136

TEST TYPE: Chronic

REFERENCE TOXICANT TEST: 1611RT2A.U

RESULT: Pass, -1.53% Effect

Rea Mara A Crinklaw

Analyst

Rea Mara A Crinklaw

Signature

Water Biologist II

Title

12/5/16

Date

Kay Yamamoto

Supervisor

Kay M. Yamamoto

Signature

Water Biologist III

Title

12/9/16

Date

# CETIS Summary Report

Report Date: 05 Dec-16 15:02 (p 1 of 1)  
 Test Code: 1611072B.U | 12-7211-5602

Echinoid Fertilization Test				Hyperion Treatment Plant Laboratory							
Batch ID:	21-4123-8470	Test Type:	Fertilization	Analyst:	Rea Mara Crinklaw						
Start Date:	22 Nov-16 15:01	Protocol:	EPA/600/R-95/136 (1995)	Diluent:	Laboratory Seawater						
Ending Date:	22 Nov-16 15:41	Species:	Strongylocentrotus purpuratus	Brine:							
Duration:	40m	Source:	David Gutoff	Age:							
Sample ID:	06-1214-0088	Code:	HT530509	Client:	Watershed Protection Division						
Sample Date:	21 Nov-16 12:32	Material:	Stormwater Monitoring Sample	Project:	MS4						
Receive Date:	21 Nov-16 14:45	Source:	WPD (WATERSHED)								
Sample Age:	26h (17.1 °C)	Station:	RW-SMB-3								
Comparison Summary											
Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method				
04-5794-9930	Fertilization Rate	100	>100	N/A	N/A	1	TST-Welch's t Test				
Test Acceptability											
Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision					
04-5794-9930	Fertilization Rate	Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria					
Fertilization Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.98	0.977	0.983	0.97	0.99	0.004083	0.008165	0.83%	0.0%
0	Egg/Dilution Wa	2	0	0	0	0	0	0	0		100.0%
0	Egg/Effluent	2	0	0	0	0	0	0	0		100.0%
100		4	0.995	0.9928	0.9972	0.99	1	0.002887	0.005774	0.58%	-1.53%
Fertilization Rate Detail											
Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4						
	Dilution Water	0.98	0.97	0.99	0.98						
	Egg/Dilution Wa	0	0								
0	Egg/Effluent	0	0								
100		0.99	0.99	1	1						

# CETIS Analytical Report

Report Date: 05 Dec-16 15:02 (p 2 of 2)  
 Test Code: 1611072B.U | 12-7211-5602

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

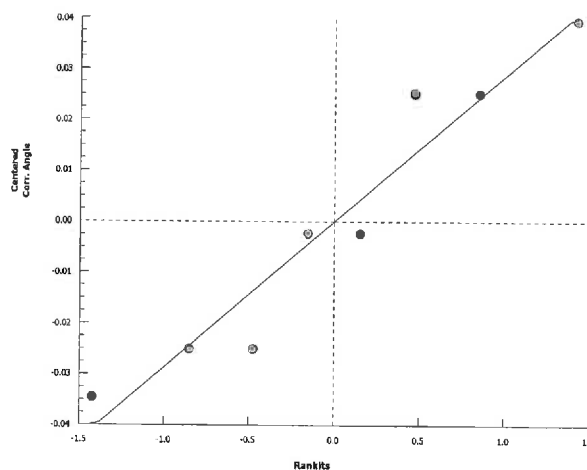
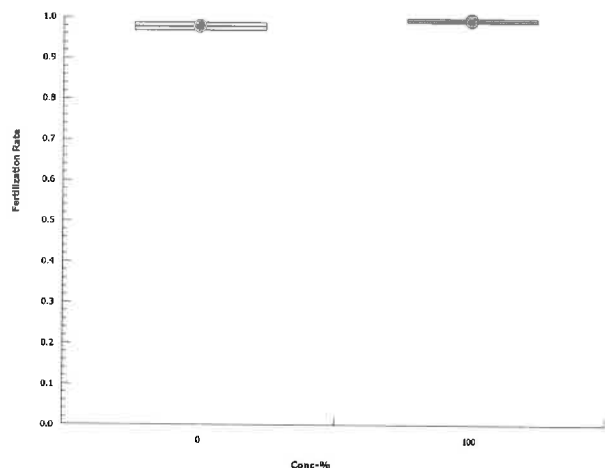
Analysis ID: 04-5794-9930  
 Endpoint: Fertilization Rate  
 Analyzed: 05 Dec-16 15:02  
 Analysis: Parametric Bioequivalence-Two Sample

CETIS Version: CETISv1.8.1  
 Official Results: Yes

### Fertilization Rate Detail

Conc-%	Control Type	Rep 1	Rep 2	Rep 3	Rep 4
0	Dilution Water	0.98	0.97	0.99	0.98
100		0.99	0.99	1	1

### Graphics



# CETIS Analytical Report

Report Date: 05 Dec-16 15:02 (p 1 of 2)

Test Code: 1611072B.U | 12-7211-5602

Echinoid Fertilization Test				Hyperion Treatment Plant Laboratory							
Analysis ID: 04-5794-9930	Endpoint: Fertilization Rate	CETIS Version: CETISv1.8.1									
Analysis Date: 05 Dec-16 15:02	Analysis: Parametric Bioequivalence-Two Sample	Official Results: Yes									
Batch ID: 21-4123-8470	Test Type: Fertilization	Analyst: Rea Mara Crinklaw									
Start Date: 22 Nov-16 15:01	Protocol: EPA/600/R-95/136 (1995)	Diluent: Laboratory Seawater									
Ending Date: 22 Nov-16 15:41	Species: Strongylocentrotus purpuratus	Brine:									
Duration: 40m	Source: David Gutoff	Age:									
Sample ID: 06-1214-0088	Code: HT530509	Client: Watershed Protection Division									
Sample Date: 21 Nov-16 12:32	Material: Stormwater Monitoring Sample	Project: MS4									
Receive Date: 21 Nov-16 14:45	Source: WPD (WATERSHED)										
Sample Age: 26h (17.1 °C)	Station: RW-SMB-3										
Data Transform	Zeta	Alt Hyp	MC Trials	TST b	Test Result						
Angular (Corrected)	0	C*b > T	Not Run	0.75	Sample passes fertilization rate endpoint						
TST-Welch's t Test											
Control	vs	Conc-%	Test Stat	Critical	DF	MSD	P-Value	Decision(α:5%)			
Dilution Water		100*	22.94	2.015	5		<0.0001	Non-Significant Effect			
Test Acceptability Criteria											
Attribute	Test Stat	TAC Limits	Overlap	Decision							
Control Resp	0.98	0.7 - NL	Yes	Passes Acceptability Criteria							
Auxiliary Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:5%)						
Extreme Value	0	1.434	2.127	1.0000	No Outliers Detected						
ANOVA Table											
Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)					
Between	0.008299088	0.008299088	1	9.45	0.0218	Significant Effect					
Error	0.005269198	0.0008781997	6								
Total	0.01356829	0.009177288	7								
Distributional Tests											
Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)						
Variances	Variance Ratio F	1.095	47.47	0.9421	Equal Variances						
Distribution	Shapiro-Wilk W Normality	0.9132	0.6451	0.3769	Normal Distribution						
Fertilization Rate Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	0.98	0.9769	0.9831	0.97	0.99	0.004083	0.008165	0.83%	0.0%
100		4	0.995	0.9928	0.9972	0.99	1	0.002887	0.005774	0.58%	-1.53%
Angular (Corrected) Transformed Summary											
Conc-%	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	Dilution Water	4	1.431	1.42	1.443	1.397	1.471	0.01515	0.0303	2.12%	0.0%
100		4	1.496	1.485	1.507	1.471	1.521	0.01448	0.02895	1.94%	-4.5%

# CETIS Test Data Worksheet

3

Report Date: 22 Nov-16 08:09 (p 1 of 1)  
Test Code: 07-2132-3985/1611072B.U

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Date: 22 Nov-16 15:01 Species: Strongylocentrotus purpuratus  
Date: 22 Nov-16 15:41 Protocol: EPA/600/R-95/136 (1995)  
Sample Date: 21 Nov-16 Material: Stormwater Monitoring Sample

Sample Code: 362C4A61  
Sample Source: WPD  
Sample Station: RW-SMB-3

Conc-%	Code	Rep	Pos	# Counted	# Fertilized	Notes
0	D	1		100	98	
0	D	2		100	97	
0	D	3		100	99	
0	D	4		100	98	
0	ED	1		100	0	
0	ED	2		100	0	
0	EE	1		100	0	
0	EE	2		100	0	
100		1	23	100	99	
100		2	9	100	99	
100		3	12	100	100	
100		4	15	100	100	

STURGEON CONTROLS  
See 1611KT2A.U

## Echinoid Fertilization Test

Hyperion Treatment Plant Laboratory

Start Date: 22 Nov-16      Species: Strongylocentrotus purpuratus      Sample Code: 362C4A61  
 Date: 22 Nov-16      Protocol: EPA/600/R-95/136 (1995)      Sample Source: WPD  
 Sample Date: 21 Nov-16      Material: Stormwater Monitoring Sample      Sample Station: RW-SMB-3

## Dissolved Oxygen-mg/L 11/22

Conc-%	Code	Reading 1
0	D	8.14
100		8.13
Measure Time:		1144
Instrument ID:		#2
Analyst:		Rc

## pH 11/22

Conc-%	Code	Reading 1
0	D	8.00
100		8.19
Measure Time:		1144
Instrument ID:		#4
Analyst:		Rc

## Salinity-ppt 11/22

Conc-%	Code	Reading 1
0	D	33
100		34
Measure Time:		1144
Instrument ID:		#4
Analyst:		Rc

Cond

## Temperature-°C 11/22

Conc-%	Code	Reading 1
0	D	12.5
100		12.4
Measure Time:		1144
Instrument ID:		#4
Analyst:		Rc

11/23/16

11/24/16

11/25/16

11/26/16

11/27/16

Non-Fert Fertilized Total

Non-Fert Fertilized Total

11/27/16

recount

11/27/16

1	1	99	100
2	57	43	100
3	38	62	100
4	32	68	100
5	10	90	100
6	11	89	100
7	48	52	100
8	16	84	100
9	1	99	100
10	1	99	100
11	0	100	100
12	2	98	100
13	17	83	100
14	1	99	100
15	0	100	100
16	10	90	100
17	62	38	100
18	1	99	100
19	68	32	100
20	1	99	100
21	21	79	100
22	38	62	100
23	1	99	100
24	65	35	100
25	67	33	100
26	52	48	100
27	8	92	100
28	15	85	100
29	28	72	100
30	2	98	100
31	3	97	100
32	21	79	100
33			
ED 34	100	0	100
ED 35	100	0	100
EE-1 36	100	0	100
EE-1 37	100	0	100
EE-2 38	100	0	100
EE-2 39	100	0	100
40			
41			
42			
43			
44			
45			

46			
47	46	54	100
48			
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Figure 2. Sample data sheet for egg and sperm counts. 11/22/16

EGG COUNTS -

Sample	Dilution	Count	Eggs/mL	Undiluted
A	1:100	180/0.100	180/mL x 100	18,000
B	1:10	146/0.100	1460/mL x 10	14,600
Working		183/0.100	1830	

For 100 mL egg suspension at 2,240 eggs/mL use:

$$100 \text{ mL} \times 2,240 \text{ eggs/mL} / (\text{counted eggs/mL}) = \text{mL of egg stock}$$

$$224,000 \text{ eggs} / 14,600 \text{ eggs/mL} = 15.34 \text{ mL}$$

If required stock >100 mL, concentrate egg stock by settling the eggs and decanting off sufficient overlying water to retain:

$$(\text{_____ eggs/mL} / 2,240 \text{ eggs/mL}) \times 100 = \text{_____ \% volume}$$

SPERM COUNTS - used Coulter Counter Multisizer 3

Sample	Dilution	Count	Squares	Sperm/mL
initial "3000:1"				34.81 x 10 <sup>6</sup> /mL
working "1500:1"				18.00 x 10 <sup>6</sup> /mL → FINAL

$$\text{SPERM/mL} = \frac{(\text{DIL. FACT.}) (\text{COUNT}) (4000) (1000)}{(\text{NO. SQUARES COUNTED})}$$

$$\text{ratio} = \frac{(0.100 \text{ mL}) (18.00 \times 10^6 / \text{mL})}{(0.500 \text{ mL}) (1830 \text{ eggs/mL})} = \frac{1967 \text{ sperm}}{1 \text{ egg}}$$

Sperm 15:01-15:04

Eggs 15:21-15:25

Formalin 15:41-15:46

## Trial Fertilization

3000      100%    98%

1500      95%    97%    use "1500:1"

750      90%    93%

Sperm 1334

Eggs 1356

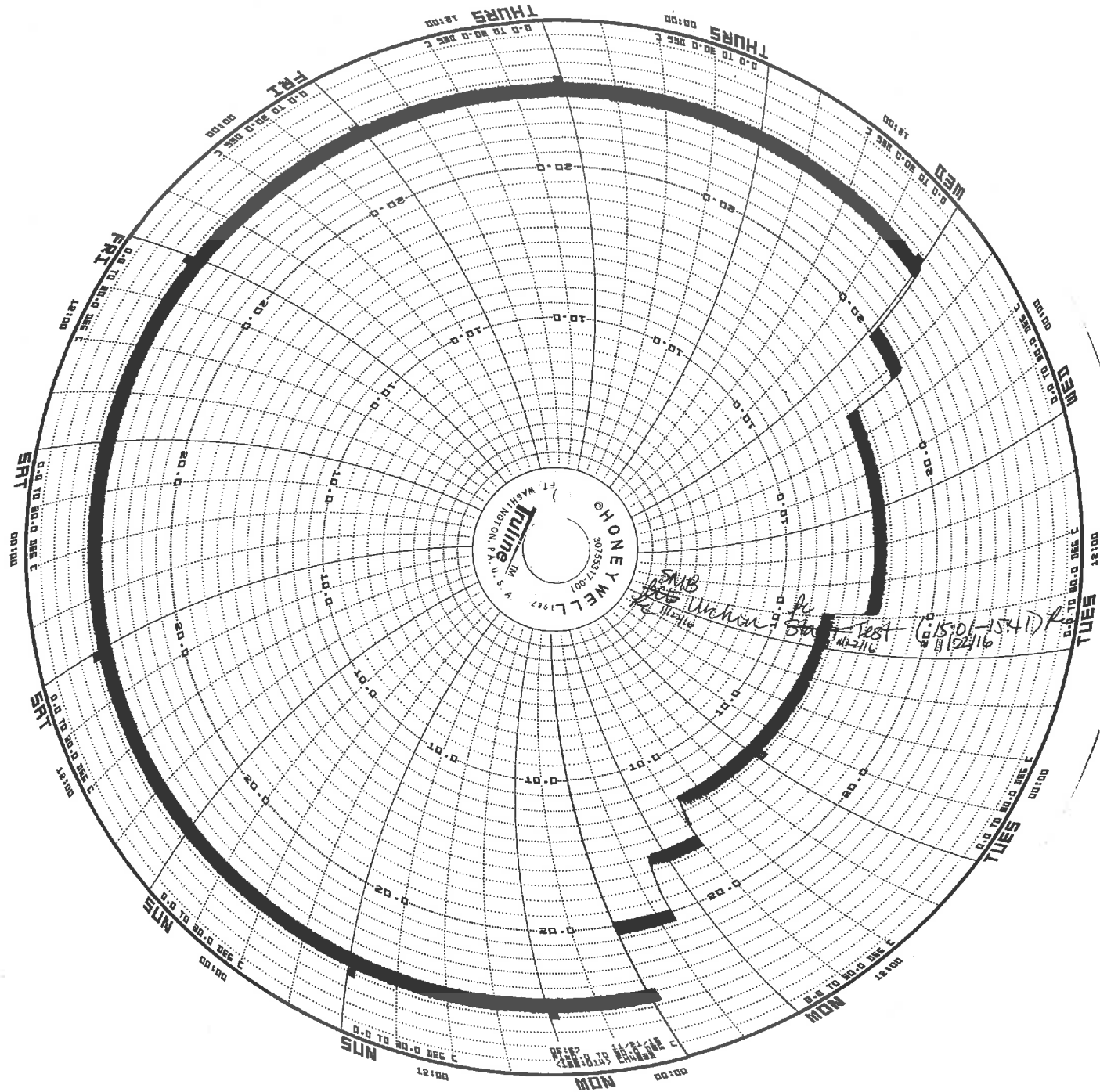
Formalin 1416

Figure 1. Sample data sheet for spawning record. 11/22/66

Animal No.	Sex	Time		2nd Inject. Comments
		Injected	Spawn	
1	M	12:09	12:21	
2	F	12:10	12:12	good quality
3	F	12:11	12:12	good quality
4	F	12:13	12:25	12:25
5	F	12:15	12:16	good quality
6	M	12:16	12:17	good motility
7	M	12:18	12:18	
8	M	12:19	12:34	12:27
9				
10				
11				
12				

Pooled eggs from female nos. 2, 3, 5.

Pooled ( 30 <sup>ml</sup> ) of sperm each from male nos. 6.



Test: 1611RT 2A.U, 1611072A B.U  
 Date: 11/22/16 (15:01-15:41)

\* No HOBO temperature recorder data available