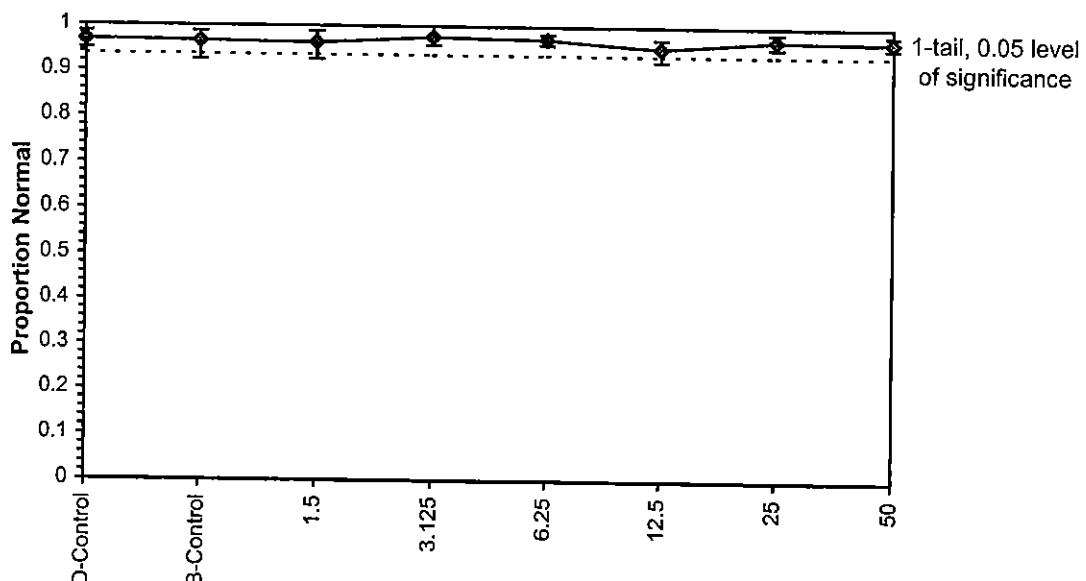


Bivalve Larval Development

Bivalve Larval Survival and Development Test-Proportion Normal										
Start Date:	6/10/03	Test ID:	0306-26NW	Sample ID:	Spirit of Columbia					
End Date:	6/12/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW-Black Water					
Sample Date:	6/9/03	Protocol:	ASTM 94	Test Species:	MS-Mytilis species					
Comments:										
Conc-%	1	2	3	4	5					
D-Control	0.9864	0.9542	0.9767	0.9483	0.9714					
B-Control	0.9871	0.9244	0.9685	0.9767	0.9675					
1.5	0.9591	0.9625	0.9737	0.9258	0.9879					
3.125	1.0000	0.9571	0.9672	0.9821	0.9676					
6.25	0.9624	0.9777	0.9593	0.9829	0.9746					
12.5	0.9234	0.9725	0.9668	0.9600	0.9383					
25	0.9762	0.9537	0.9552	0.9780	0.9856					
50	0.9630	0.9825	0.9550	0.9563	0.9803					
Transform: Arcsin Square Root										
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	1-Tailed Critical	MSD
D-Control	0.9674	1.0027	1.3938	1.3414	1.4540	3.305	5			
B-Control	0.9648	1.0000	1.3897	1.2923	1.4568	4.371	5			
1.5	0.9618	0.9968	1.3812	1.2948	1.4604	4.383	5	0.401	2.409	0.0753
3.125	0.9748	1.0103	1.4228	1.3621	1.5371	4.866	5	-0.931	2.409	0.0753
6.25	0.9714	1.0068	1.4029	1.3678	1.4397	2.171	5	-0.294	2.409	0.0753
12.5	0.9522	0.9869	1.3543	1.2904	1.4043	3.526	5	1.263	2.409	0.0753
25	0.9697	1.0051	1.3999	1.3538	1.4503	3.029	5	-0.196	2.409	0.0753
50	0.9674	1.0027	1.3925	1.3571	1.4382	2.786	5	0.039	2.409	0.0753
Auxiliary Tests										
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)							Statistic	Critical	Skew	Kurt
Bartlett's Test indicates equal variances (p = 0.78)							0.97294	0.91	0.33988	-0.1739
The control means are not significantly different (p = 0.91)							3.23414	16.8119		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	50	>50		2	0.03131	0.03231	0.00223	0.00244	0.5006	6, 28

Dose-Response Plot



Bivalve Larval Survival and Development Test-Proportion Alive						
Start Date:	6/10/03	Test ID:	0306-26NW	Sample ID:	Spirit of Columbia	
End Date:	6/12/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW-Black Water	
Sample Date:	6/9/03	Protocol:	ASTM 94	Test Species:	MS-Mytilis species	
Comments:						

Conc-%	1	2	3	4	5
D-Control	0.9132	0.9917	1.0000	0.9587	1.0000
B-Control	0.9587	0.9298	0.9174	1.0000	1.0000
1.5	0.9091	0.9917	1.0000	0.9463	1.0000
3.125	0.9091	0.9628	1.0000	0.9215	1.0000
6.25	1.0000	1.0000	1.0000	0.9669	0.9752
12.5	0.9711	1.0000	0.9959	1.0000	1.0000
25	1.0000	1.0000	1.0000	0.9380	1.0000
50	1.0000	0.9463	1.0000	1.0000	0.8388

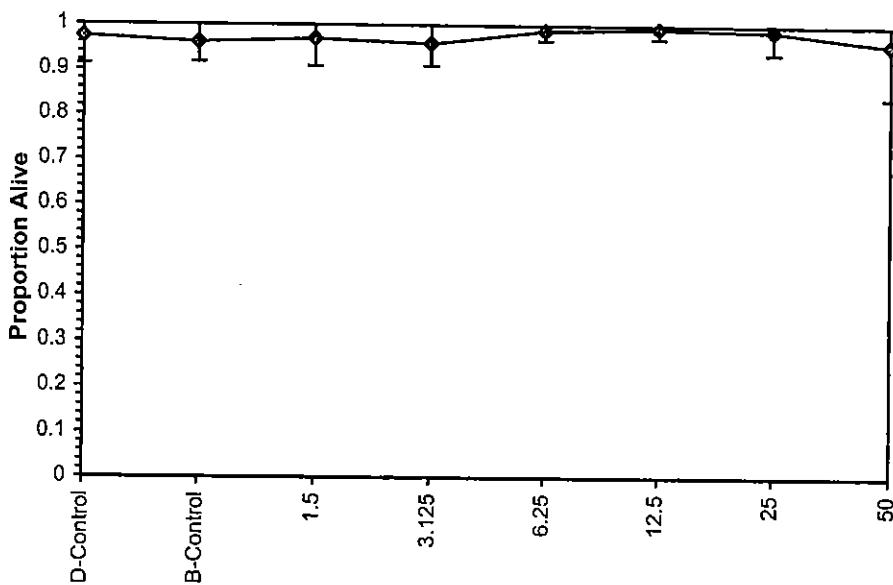
Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%		
D-Control	0.9727	1.0120	1.4390	1.2718	1.5386	8.134	5	
B-Control	0.9612	1.0000	1.4050	1.2792	1.5386	8.972	5	
1.5	0.9694	1.0086	1.4317	1.2645	1.5386	8.705	5	26.50 16.00
3.125	0.9587	0.9974	1.4011	1.2645	1.5386	9.452	5	26.00 16.00
6.25	0.9884	1.0284	1.4833	1.3880	1.5386	5.142	5	31.00 16.00
12.5	0.9934	1.0335	1.5045	1.3999	1.5386	3.994	5	32.00 16.00
25	0.9876	1.0275	1.4948	1.3192	1.5386	6.566	5	32.00 16.00
50	0.9570	0.9957	1.4221	1.1577	1.5386	12.073	5	28.00 16.00

Auxiliary Tests

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p <= 0.01$)	0.90955	0.91	-0.6828	-0.4672
Bartlett's Test indicates equal variances ($p = 0.55$)	4.96767	16.8119		
The control means are not significantly different ($p = 0.67$)	0.44143	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	50	>50		2

Dose-Response Plot



Test: BV-Bivalve Larval Survival and Development Test
 Species: MS-Mytilis species
 Sample ID: Spirit of Columbia #1
 Start Date: 06/10/2003 End Date: 06/12/2003

Test ID: 0306-26NW
 Protocol: ASTM 94
 Sample Type: EFF2-Industrial
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
1						225	208	inc 7/7/03
2						289	276	
3						240	231	
4						233	223	
5						232	220	
6						268	256	
7						243	228	
8						255	248	
9						220	211	
10						247	239	
11						246	259	
12						241252	241	
13						257	251	
14						250	240	
15						247	244	
16						230	224	
17						252	247246	
18						229	212	
19						235	217	
20						246	238	
21						240	229	
22						246	256	
23						232	229	
24						259	247	
25						236	230	
26						244	236	
27						269	263	
28						257	251	
29						221	218	
30						203	199	
31						227	222	
32						223	219	
33						246	236	
34						251241	233	
35						222	215	
36						229	225	
37						277	273	
38						234	230	
39						270	260	
40						245	238	✓

Comments:

Day 0

246

240

235

247

260

224

Mean = 242

St. dev. = 12.2

CV = 5.0%

Test: BV-Bivalve Larval Survival and Development Test
 Species: MS-Mytilis species
 Sample ID: Spirit of Columbia
 Start Date: 6/10/03 End Date: 6/12/03

Test ID: 0306-26NW
 Protocol: ASTM 94
 Sample Type: BW-Black Water
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
29	1	1	D-Control	242	221	221	218	
21	2	2	D-Control	242	240	240	229	
28	3	3	D-Control	242	257	257	251	
5	4	4	D-Control	242	232	232	220	
40	5	5	D-Control	242	245	245	238	
23	6	1	B-Control	242	232	232	229	
1	7	2	B-Control	242	225	225	208	
35	8	3	B-Control	242	222	222	215	
13	9	4	B-Control	242	257	257	251	
20	10	5	B-Control	242	246	246	238	
9	11	1	1.500	242	220	220	211	
3	12	2	1.500	242	240	240	231	
11	13	3	1.500	242	266	266	259	
18	14	4	1.500	242	229	229	212	
15	15	5	1.500	242	247	247	244	
16	16	1	3.125	242	220	220	224	
4	17	2	3.125	242	233	233	223	
26	18	3	3.125	242	244	244	236	
32	19	4	3.125	242	223	223	219	
10	20	5	3.125	242	247	247	239	
22	21	1	6.250	242	266	266	256	
27	22	2	6.250	242	269	269	263	
33	23	3	6.250	242	246	246	236	
38	24	4	6.250	242	234	234	230	
25	25	5	6.250	242	236	236	230	
19	26	1	12.500	242	235	235	217	
8	27	2	12.500	242	255	255	248	
34	28	3	12.500	242	241	241	233	
14	29	4	12.500	242	250	250	240	
7	30	5	12.500	242	243	243	228	
17	31	1	25.000	242	252	252	246	
24	32	2	25.000	242	259	259	247	
6	33	3	25.000	242	268	268	256	
31	34	4	25.000	242	227	227	222	
37	35	5	25.000	242	277	277	273	
39	36	1	50.000	242	270	270	260	
36	37	2	50.000	242	229	229	225	
2	38	3	50.000	242	289	289	276	
12	39	4	50.000	242	252	252	241	
30	40	5	50.000	242	203	203	199	

Comments:

Saltwater Chronic

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Client: Shannon and Wilson
 Sample ID: Spirit of Columbia #1
 Contact:
 Test No.: 0306-2 (eNW)

Start Date & Time: 6/10/03
 End Date & Time: 6/12/03
 Test Organisms: Mytilus species
 Test Protocol:
 Date Received: 6/10/03

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
	0	24	48	0	24	48	0	24	48	0	24	48
Control	8.0	8.8	8.0	8.02	7.88	7.99	29.2	30.1	30.4	16.0	15.0	14.6
brine con.	7.1	9.0	8.7	7.88	7.95	7.94	29.0	30.2	31.0	15.5	14.4	14.3
1.5	7.9	8.7	8.8	7.96	7.95	7.94	29.0	29.6	31.0	16.0	15.2	14.5
.3.125	8.0	8.6	8.5	7.97	7.94	7.97	29.3	30.5	31.0	16.0	15.4	14.6
6.25	8.0	8.9	8.6	7.98	7.95	7.97	29.1	29.9	31.0	16.0	15.0	14.2
12.5	7.9	9.0	8.6	7.98	7.94	7.95	29.2	30.7	31.0	16.0	14.8	14.2
25	8.3	8.7	8.9	7.97	7.93	7.95	29.8	31.0	31.0	15.5	14.6	14.4
50	8.4	8.6	8.1	7.96	7.93	7.97	30.3	31.0	31.0	15.8	15.0	14.2
Conc.	Alkalinity* *(mg/L as CaCO ₃)		Chlorine Resid. (mg/L)			Sample Description: _____						
Control												
Highest conc.						Analyst Initials: <u>NF Sm</u>						

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

Reviewed: KSQA check: XJ

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2-0
 Fife, WA 98424
 (253) 922-4296

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424

Marine Dilution Worksheet

Client: Spirit of Columbia
Sample ID: BW #1
Test No.: 0306-27NW, 0306-26NW
0306-25NW
Brine salinity: 80

Analyst: mm
Test Date: 6/10/03
Test Type: Bivalve and Echinoderm survival

$$\text{Equation for Salinity Adjustment: } VB = VE \frac{30 - SE}{SB - 30}$$

$$\text{Effluent Salinity Adjustment: } \frac{30 - 20}{80 - 30} = \frac{10}{50} = .2$$

$$\text{Brine Control Salinity Adjustment: } \frac{30 - 0}{80 - 30} = \frac{30}{50} = .6$$

Concentration	Effluent Volume (mL)	Salinity Adjustment	Brine Volume (mL)	Seawater Volume (mL)	Total Volume (mL)
Control	NA	NA	NA		250 ml
1.5	3.75	.2	.75		
3.125	7.81	.2	1.56		
6.25	15.6	.2	3.125		
12.5	31.25	.2	6.25		
25	62.5	.2	12.5		
50	125	.2	25		250 ml

DI Volume				
Brine Control	41.7	.6	25	250 ml

Calculations for all concentrations except highest:

1. Effluent Volume = Total Volume X Concentration (%)
2. Brine Volume = Effluent Volume X Salinity Adjustment
3. Seawater Volume = Total Volume - Effluent Volume + Brine Volume

Calculation for highest concentration:

1. Effluent Volume = Total Volume / (1.0 + Salinity Adjustment)
2. Brine Volume = Total Volume - Effluent Volume
3. Concentration = Effluent Volume / Total Volume

Calculation for brine control:

1. Brine Volume = Brine Volume for highest concentration
2. DI Volume = Brine Volume / Brine Control Salinity Adjustment
3. Seawater Volume = Total Volume - DI Volume + Brine Volume

Reviewed/ Date: 10

Bivalve Larval Survival and Development Test-Proportion Alive

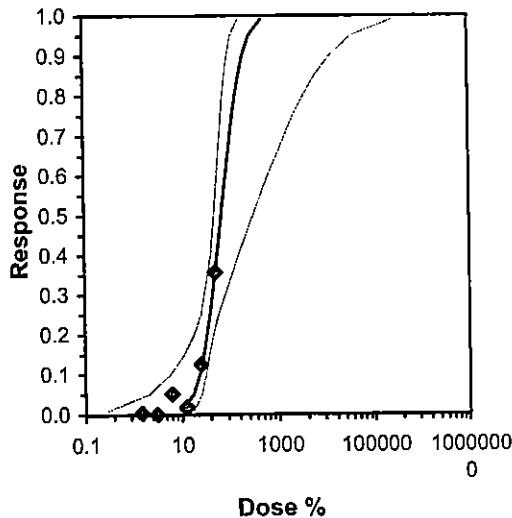
Start Date: 6/10/03 Test ID: 0306-27NW Sample ID: Spirit of Columbia
 End Date: 6/12/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: GW-Gray Water
 Sample Date: 6/9/03 Protocol: ASTM 94 Test Species: MS-Mytilis species
 Comments:

Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	0.9298	0.9339	1.0000
B-Control	1.0000	0.8595	0.8595	1.0000	0.9835
1.5	1.0000	0.8471	1.0000	1.0000	1.0000
3.125	1.0000	1.0000	1.0000	0.9959	0.8843
6.25	1.0000	0.8636	1.0000	0.8017	0.9504
12.5	0.8967	0.9298	0.9711	1.0000	0.9793
25	0.8388	1.0000	0.6364	0.9256	0.8554
50	0.4876	0.6612	0.8182	0.5992	0.5620

Conc-%	Transform: Arcsin Square Root						t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%					
D-Control	0.9727	1.0343	1.4458	1.3025	1.5386	8.791	5			33	1210
B-Control	0.9405	1.0000	1.3785	1.1866	1.5386	13.026	5				
1.5	0.9694	1.0308	1.4647	1.1691	1.5386	11.284	5	-0.185	2.409	0.2459	37 1210
3.125	0.9760	1.0378	1.4692	1.2237	1.5386	9.389	5	-0.229	2.409	0.2459	29 1210
6.25	0.9231	0.9815	1.3451	1.1092	1.5386	14.580	5	0.987	2.409	0.2459	93 1210
12.5	0.9554	1.0158	1.3822	1.2436	1.5386	8.274	5	0.623	2.409	0.2459	54 1210
25	0.8512	0.9051	1.2190	0.9235	1.5386	18.363	5	2.222	2.409	0.2459	180 1210
*50	0.6256	0.6652	0.9171	0.7730	1.1303	14.742	5	5.179	2.409	0.2459	453 1210

Auxiliary Tests			Statistic	Critical	Skew	Kurt				
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)			0.95686	0.91	-0.2881	-0.1959				
Bartlett's Test indicates equal variances (p = 0.84)			2.72711	16.8119						
The control means are not significantly different (p = 0.51)			0.68484	2.30601						
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	25	50	35.3553	4	0.11582	0.11764	0.19673	0.02606	7.0E-05	6, 28

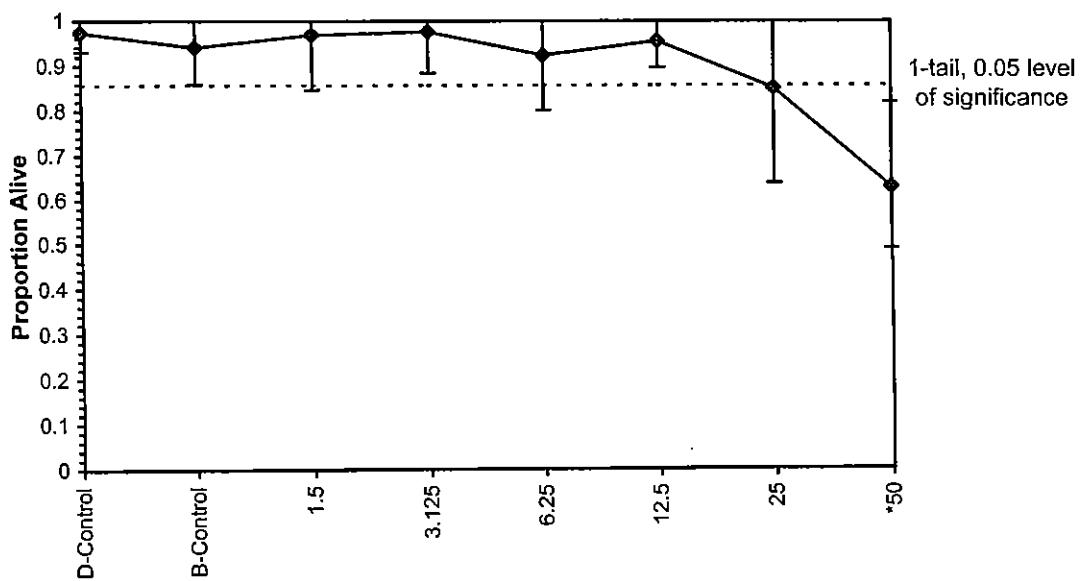
Maximum Likelihood-Probit										
Parameter	Value	SE	95% Fiducial Limits	Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	2.72169	0.69772	0.78451 4.65887	0.02727	57.3941	9.48773	1.0E-11	1.84105	0.36742	10
Intercept	-0.0108	1.10266	-3.0722 3.0507							
TSCR	0.03672	0.0105	0.00757 0.06588							
Point	Probits	%	95% Fiducial Limits							
EC01	2.674	9.68964	0.29725 18.0381							
EC05	3.355	17.2465	2.13597 25.9821							
EC10	3.718	23.4523	5.97556 32.2821							
EC15	3.964	28.8567	11.5997 38.5436							
EC20	4.158	34.027	18.6894 46.6596							
EC25	4.326	39.1951	26.0526 59.3742							
EC40	4.747	55.9715	41.7503 157.038							
EC50	5.000	69.3508	49.5878 315.213							
EC60	5.253	85.9283	57.5958 646.999							
EC75	5.674	122.708	72.4783 2179.23							
EC80	5.842	141.345	79.1474 3539.85							
EC85	6.036	166.67	87.5862 6239.13							
EC90	6.282	205.077	99.3562 12747.4							
EC95	6.645	278.87	119.547 36825.8							
EC99	7.326	496.359	168.55 270350							



Significant heterogeneity detected (p = 1.02E-11)

Bivalve Larval Survival and Development Test-Proportion Alive

Start Date:	6/10/03	Test ID:	0306-27NW	Sample ID:	Spirit of Columbia
End Date:	6/12/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	GW-Gray Water
Sample Date:	6/9/03	Protocol:	ASTM 94	Test Species:	MS-Mytilis species
Comments:					

Dose-Response Plot

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 6/10/03	Test ID: 0306-27NW	Sample ID: Spirit of Columbia
End Date: 6/12/03	Lab ID: WAAEE-AMEC NW Bioassay	Sample Type: GW-Gray Water
Sample Date: 6/9/03	Protocol: ASTM 94	Test Species: MS-Mytilis species
Comments:		

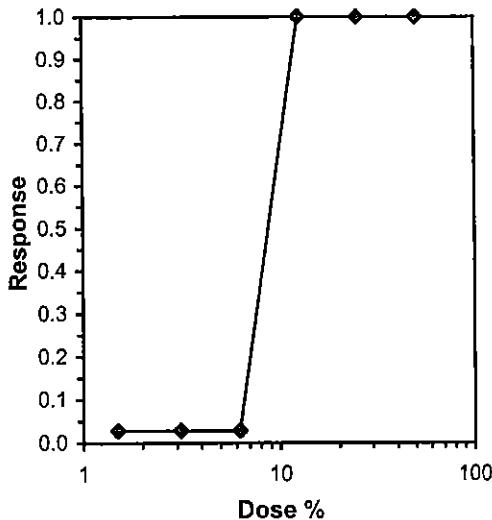
Conc-%	1	2	3	4	5
D-Control	0.9518	0.9672	0.9511	0.9779	0.9377
B-Control	0.9522	0.9615	0.9808	0.9084	0.9286
1.5	0.9187	0.9366	0.9472	0.9271	0.9234
3.125	0.9174	0.9704	0.8984	0.9502	0.9019
6.25	0.9465	0.9761	0.8930	0.9485	0.9000
12.5	0.0000	0.0000	0.0000	0.0000	0.0000
*25	0.0000	0.0000	0.0000	0.0000	0.0000
*50	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%				
D-Control	0.9572	1.0115	1.3652	1.3186	1.4215	2.939	5		52	1201
B-Control	0.9463	1.0000	1.3438	1.2633	1.4317	4.855	5			
*1.5	0.9306	0.9834	1.3049	1.2817	1.3388	1.751	5	16.00	16.00	83 1192
3.125	0.9276	0.9803	1.3043	1.2463	1.3978	5.023	5	20.00	16.00	86 1213
6.25	0.9328	0.9858	1.3163	1.2376	1.4155	5.590	5	21.00	16.00	77 1119
*12.5	0.0000	0.0000	0.0329	0.0321	0.0339	2.262	5	15.00	16.00	1157 1157
*25	0.0000	0.0000	0.0349	0.0311	0.0403	9.666	5	15.00	16.00	1046 1046
*50	0.0000	0.0000	0.0411	0.0355	0.0460	9.513	5	15.00	16.00	757 757

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.92019	0.91	0.51255	1.61117
Bartlett's Test indicates unequal variances ($p = 2.10E-11$)	61.6296	16.8119		
The control means are not significantly different ($p = 0.55$)	0.62575	2.30601		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.25	12.5	8.83883	16

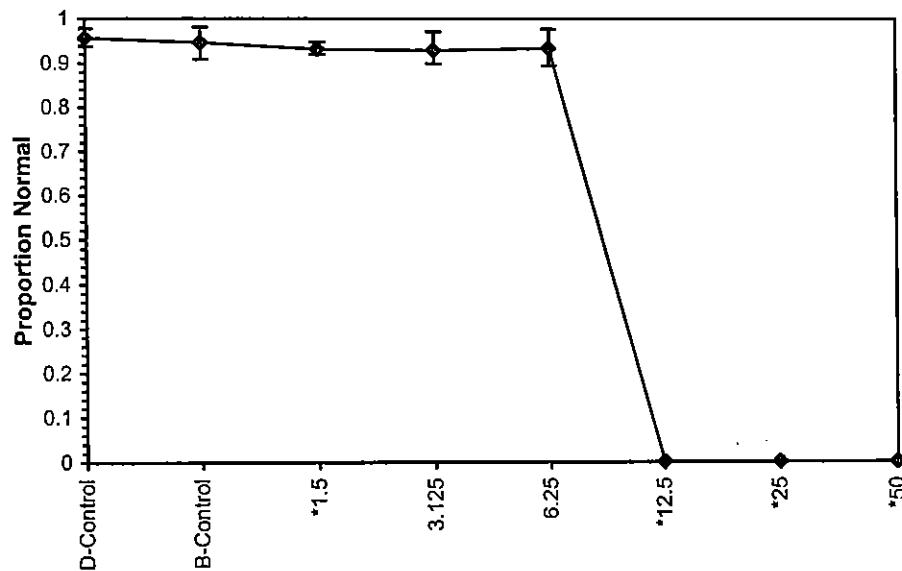
Trimmed Spearman-Karber

Trim Level	EC50	95% CL
0.0%		
5.0%	8.7518	8.7203
10.0%	8.7518	8.7203
20.0%	8.7518	8.7203
Auto-2.8%	8.7495	8.6557
		8.8443



Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 6/10/03 Test ID: 0306-27NW Sample ID: Spirit of Columbia
End Date: 6/12/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: GW-Gray Water
Sample Date: 6/9/03 Protocol: ASTM 94 Test Species: MS-Mytilis species
Comments:

Dose-Response Plot

Test: BV-Bivalve Larval Survival and Development Test
 Species: MS-Mytilis species
 Sample ID: Spirit of Columbia #2
 Start Date: 06/10/2003 End Date: 06/12/2003

Test ID: 0306-27NW
 Protocol: ASTM 94
 Sample Type: EFF2-Industrial
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
41						243	0	6/18/03
42						274	0	
43						194	184	
44						217	0	
45						208	204	
46						237	0	
47						145	0	bivalve larvae difficult to distinguish among heavy debris
48						134	0	"
49						205	192	
50						226	221	↓
51				270	240-45	262	103	6/19/03
52				209	209-103	204	"	
53				249	237			
54				246	221	6/23	7242	
55				154	0			
56				241	229			
57				198	0	6/24	744	
58				247	229			
59				238	221			
60				251	228			
61				243	230			
62				235	0			
63				118	0	bivalve larvae difficult to distinguish among debris		
64				230	207			
65				246	233			
66				242	222			
67				244	236			
68				225	214			
69				208	200			
70				232	259			
71				203	0			
72				243	217			
73				257	241			
74				160	0	Difficult to distinguish among debris		
75				258	0			
76				248	229			
77				246	226			
78				225	0			
79				214	193			
80				207	0			

Comments:

Test: BV-Bivalve Larval Survival and Development Test Species: MS-Mytilis species Sample ID: SPIRIT OF COLUMBIA Start Date: 6/10/03 End Date: 6/12/03				Test ID: 0306-27NW Protocol: ASTM 94 Sample Type: GW-Gray Water Lab ID: WAAEE-AMEC NW Bioassay				
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
53	1	1	D-Control	242	249	249	237	
67	2	2	D-Control	242	244	244	236	
68	3	3	D-Control	242	225	225	214	
50	4	4	D-Control	242	226	226	221	
73	5	5	D-Control	242	257	257	241	
70	6	1	B-Control	242	272	272	259	
69	7	2	B-Control	242	208	208	200	
45	8	3	B-Control	242	208	208	204	
60	9	4	B-Control	242	251	251	228	
59	10	5	B-Control	242	238	238	221	
77	11	1	1.500	242	246	246	226	
49	12	2	1.500	242	205	205	192	
65	13	3	1.500	242	246	246	233	
58	14	4	1.500	242	247	247	229	
76	15	5	1.500	242	248	248	229	
66	16	1	3.125	242	242	242	222	
51	17	2	3.125	242	270	270	262	
54	18	3	3.125	242	246	246	221	
56	19	4	3.125	242	241	241	229	
79	20	5	3.125	242	214	214	193	
61	21	1	6.250	242	243	243	230	
52	22	2	6.250	242	209	209	204	
72	23	3	6.250	242	243	243	217	
43	24	4	6.250	242	194	194	184	
64	25	5	6.250	242	230	230	207	
44	26	1	12.500	242	217	217	0	
78	27	2	12.500	242	225	225	0	
62	28	3	12.500	242	235	235	0	
41	29	4	12.500	242	243	243	0	
46	30	5	12.500	242	237	237	0	
71	31	1	25.000	242	203	203	0	
75	32	2	25.000	242	258	258	0	
55	33	3	25.000	242	154	154	0	
42	34	4	25.000	242	224	224	0	
80	35	5	25.000	242	207	207	0	
63	36	1	50.000	242	118	118	0	
74	37	2	50.000	242	160	160	0	
57	38	3	50.000	242	198	198	0	
47	39	4	50.000	242	145	145	0	
48	40	5	50.000	242	136	136	0	

Comments:

Bivalve Development Bioassay Worksheet

Client: Spirit of Columbia Start Date/Time: 6/10/03 1615
Test No.: C366-27NW, D306-26NW RT061063MS End Date/Time: 6/12/03 1615
Test Species: M. gallo Date Received: 6/10/03

Sample Type: Cruise ship waste water

Test Chamber Type and Sample Volume: 30 ml vial

Spawn Initiation Time: 1230

Number of Spawners: 4 Male 3 Female

Spawn Condition: eg good

Fertilization Time: 1427

Egg Stock Density Calculation:

Eggs Counted (x): 11 10
12 12
8 6
5 4
5 9

Mean 8.2 8.2 Overall Mean: 8.2

$$\text{Mean: } 8.2 \times 42 = 344 \text{ eggs/ml}$$

Stock Dilution Factor

$$\frac{\text{Initial Stock} - 344 \text{ eggs/ml}}{\text{Inoculum Stock} - 200 \text{ eggs/ml}} = 1.72$$

Percent Division Upon Inoculation: 98

Time of Inoculation: 1615

Comments: _____

Reviewed/ Date: MR 6/10/03

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424
(253) 922-4296

Saltwater Chronic

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Client: Shannon and Wilson
 Sample ID: SOC #2 Graywater
 Contact:
 Test No.: D30Q-27NW

Start Date & Time: 6/10/03
 End Date & Time: 6/12/03
 Test Organisms: Mytilus species
 Test Protocol:
 Date Received: 6/10/03

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
	0	24	48	8.00 NF	24	48	0	24	48	0	24	48
control	8.0	8.8	8.0	7.88	7.88	7.87	29.02	30.1	30.4	15.5	15.0	14.6
brine control	7.7	8.7	8.4	7.88	7.93	7.96	29.0	30.9	31.0	15.5	14.6	14.5
1.5	7.9	8.6	8.4	7.93	7.93	7.93	29.0	31.0	31.0	16.0	15.2	14.3
3.125	8.1	8.6	8.7	7.92	7.90	7.91	29.0	30.6	31.0	15.4	15.4	14.0
6.25	7.9	8.3	8.4	7.91	7.87	7.88	29.0	30.7	31.0	15.4	14.8	14.2
12.5	7.9	7.5	7.4	7.84	7.79	7.76	29.0	29.9	31.0	15.0	15.0	14.4
25	7.7	5.4	6.0	7.74	7.59	7.63	29.2	30.1	31.0	16.0	14.5	14.2
50	7.4	3.5	3.8	7.58	7.44	7.9 mg 7.52	30.5	31.0	31.0	15.8	15.2	14.3
Conc.	Alkalinity* *(mg/L as CaCO ₃)	Chlorine Resid. (mg/L)			Sample Description: _____							
Control												
Highest conc.					Analyst Initials: <u>NF Sm</u>							

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

Reviewed: MQA check: M

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2-0
 Fife, WA 98424
 (253) 922-4296

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424

Marine Dilution Worksheet

Client: Spirit of Columbia
Sample ID: CW #2 Graywater
Test No.: 0306-27NW, 0306-29NW
Brine salinity: 80

Analyst: ML
Test Date: 6/11/03
Test Type: Bivalve and Echinoderm species cell

$$\text{Equation for Salinity Adjustment: } VB = VE \frac{30 - SE}{SB - 30}$$

$$\text{Effluent Salinity Adjustment: } \frac{30 - 0}{80 - 30} = \frac{30}{50} = .6$$

$$\text{Brine Control Salinity Adjustment: } \frac{30 - 0}{80 - 30} = \frac{30}{50} = .6$$

Concentration	Effluent Volume (mL)	Salinity Adjustment	Brine Volume (mL)	Seawater Volume (mL)	Total Volume (mL)
Control	NA	NA	NA		250ml
1.5	3.75	.6	2.25		
3.125	7.81	.6	4.7		
6.25	15.6	.6	9.36		
12.5	31.25	.6	18.75		
25	62.5	.6	37.5		
50	125	.6	75		

DI Volume					
Brine Control	125	.6	75		250ml

Calculations for all concentrations except highest:

1. Effluent Volume = Total Volume X Concentration (%)
2. Brine Volume = Effluent Volume X Salinity Adjustment
3. Seawater Volume = Total Volume - Effluent Volume + Brine Volume

Calculation for highest concentration:

1. Effluent Volume = Total Volume / (1.0 + Salinity Adjustment)
2. Brine Volume = Total Volume - Effluent Volume
3. Concentration = Effluent Volume / Total Volume

Calculation for brine control:

1. Brine Volume = Brine Volume for highest concentration
2. DI Volume = Brine Volume / Brine Control Salinity Adjustment
3. Seawater Volume = Total Volume - DI Volume + Brine Volume

Reviewed/ Date: ML

Bivalve Larval Survival and Development Test-Proportion Alive

Start Date:	6/13/03	Test ID:	0306-56NW	Sample ID:	Spirit of Oceanus
End Date:	6/15/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water
Sample Date:	6/12/03	Protocol:	ASTM 87	Test Species:	CG-Crassostrea gigas

Comments:

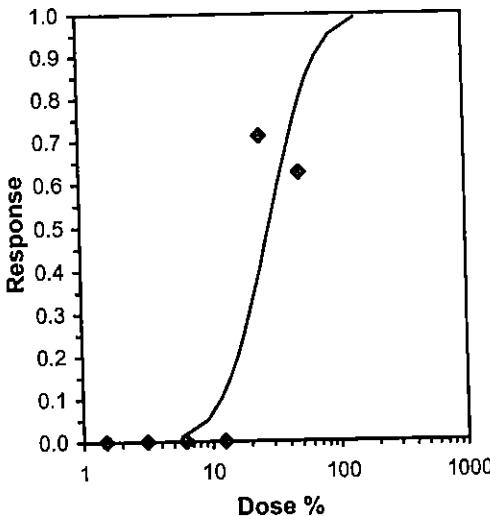
Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	0.6964	1.0000
B-Control	0.7991	1.0000	1.0000	1.0000	1.0000
1.5	1.0000	1.0000	0.8839	1.0000	1.0000
3.125	1.0000	1.0000	1.0000	1.0000	1.0000
6.25	1.0000	1.0000	1.0000	1.0000	1.0000
12.5	1.0000	0.8661	1.0000	1.0000	1.0000
25	0.5357	0.2232	0.2366	0.0893	0.2723
50	0.4866	0.1563	0.2634	0.2991	0.5580

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%				
D-Control	0.9393	0.9786	1.4274	0.9873	1.5374	17.236	5		68	1120
B-Control	0.9598	1.0000	1.4511	1.1060	1.5374	13.294	5			1120
1.5	0.9768	1.0177	1.4745	1.2231	1.5374	9.531	5	28.00	16.00	26
3.125	1.0000	1.0419	1.5374	1.5374	1.5374	0.000	5	30.00	16.00	0
6.25	1.0000	1.0419	1.5374	1.5374	1.5374	0.000	5	30.00	16.00	0
12.5	0.9732	1.0140	1.4691	1.1961	1.5374	10.388	5	28.00	16.00	30
*25	0.2714	0.2828	0.5347	0.3034	0.8211	34.769	5	15.00	16.00	816
*50	0.3527	0.3674	0.6279	0.4064	0.8436	28.343	5	15.00	16.00	725

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.88813	0.91	-1.1217	2.20864
Equality of variance cannot be confirmed				
The control means are not significantly different (p = 0.87)	0.16989	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	12.5	25	17.6777	8

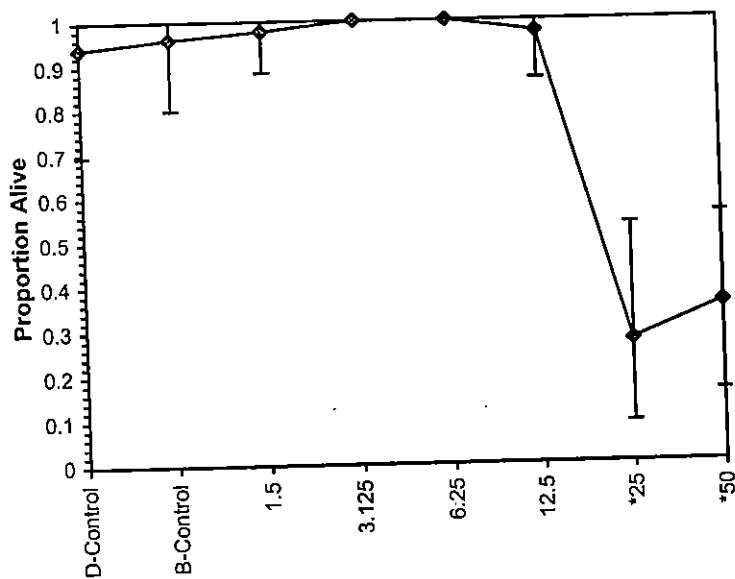
Parameter	Value	SE	Maximum Likelihood-Probit		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			95% Fiducial Limits								
Slope	3.3323	1.42802	-0.6325	7.29713		0.06071	716.499	9.48773	9.3E-154	1.45804	0.30009
Intercept	0.14137	2.05525	-5.5649	5.84767							
TSCR	0.01954	0.0309	-0.0662	0.10533							
Point	Probits	%	95% Fiducial Limits								
EC01	2.674	5.75334									
EC05	3.355	9.21365									
EC10	3.718	11.8428									
EC15	3.964	14.0286									
EC20	4.158	16.05									
EC25	4.326	18.0149									
EC40	4.747	24.0997									
EC50	5.000	28.7105									
EC60	5.253	34.2033									
EC75	5.674	45.7561									
EC80	5.842	51.3576									
EC85	6.036	58.7579									
EC90	6.282	69.6024									
EC95	6.645	89.464									
EC99	7.326	143.272									



Significant heterogeneity detected (p = 9.32E-154)

Bivalve Larval Survival and Development Test-Proportion Alive

Start Date:	6/13/03	Test ID:	0306-56NW	Sample ID:	Spirit of Oceanus
End Date:	6/15/03	Lab ID:	WAAEE-AMEC NW Bioassa	Sample Type:	BW/GW-Combined gray & black water
Sample Date:	6/12/03	Protocol:	ASTM 87	Test Species:	CG-Crassostrea gigas
Comments:					

Dose-Response Plot

Bivalve Larval Survival and Development Test-Proportion Normal									
Start Date:	6/13/03	Test ID:	0306-56NW	Sample ID:	Spirit of Oceanus				
End Date:	6/15/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water				
Sample Date:	6/12/03	Protocol:	ASTM 87	Test Species:	CG-Crassostrea gigas				
Comments:									

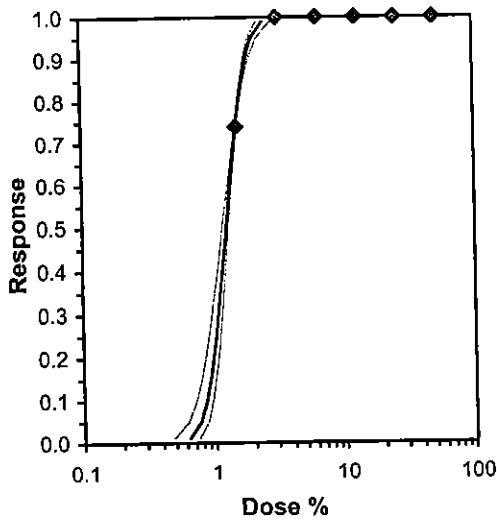
Conc-%	1	2	3	4	5				
D-Control	0.9021	0.8936	0.9409	0.9103	0.9246				
B-Control	0.8939	0.9356	0.8347	0.9309	0.9018				
1.5	0.2025	0.2771	0.1869	0.1702	0.3360				
3.125	0.0000	0.0000	0.0000	0.0000	0.0035				
6.25	0.0000	0.0000	0.0000	0.0000	0.0000				
12.5	0.0000	0.0000	0.0000	0.0000	0.0000				
25	0.0000	0.0000	0.0000	0.0000	0.0000				
50	0.0000	0.0000	0.0000	0.0000	0.0000				

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical	Number Resp	Total Number
			Mean	Min	Max	CV%				
D-Control	0.9143	1.0166	1.2751	1.2386	1.3253	2.700	5		100	1162
B-Control	0.8994	1.0000	1.2524	1.1521	1.3143	5.178	5			
*1.5	0.2345	0.2608	0.5023	0.4253	0.6183	16.177	5	15.00	16.00	897 1177
*3.125	0.0007	0.0008	0.0370	0.0300	0.0593	33.641	5	15.00	16.00	1296 1297
*6.25	0.0000	0.0000	0.0317	0.0301	0.0327	3.263	5	15.00	16.00	1251 1251
*12.5	0.0000	0.0000	0.0333	0.0311	0.0359	5.173	5	15.00	16.00	1135 1135
*25	0.0000	0.0000	0.0723	0.0457	0.1120	33.701	5	15.00	16.00	304 304
*50	0.0000	0.0000	0.0607	0.0447	0.0846	26.180	5	15.00	16.00	395 395

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.86945	0.91	1.08672	4.7946
Bartlett's Test indicates unequal variances (p = 9.95E-11)	58.3031	16.8119		
The control means are not significantly different (p = 0.51)	0.69142	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

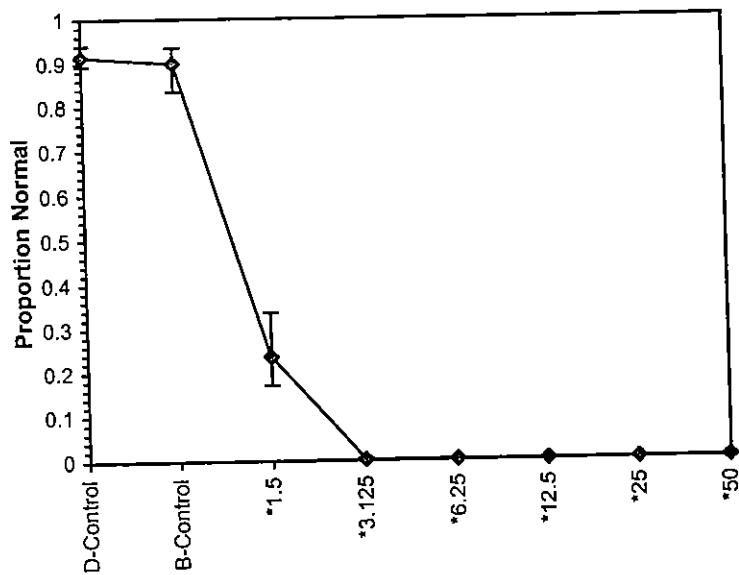
Parameter	Value	SE	Maximum Likelihood-Probit			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			95% Fiducial Limits	Control	Chi-Sq							
Slope	7.83663	0.92802	6.01771 9.65555	0.08606	0.00066	9.48773	1			0.09411	0.12761	3
Intercept	4.26248	0.17447	3.92051 4.60445									
TSCR	0.08606	0.00823	0.06993 0.10218									
Point	Probits	%	95% Fiducial Limits									
EC01	2.674	0.62698	0.47882 0.7421									
EC05	3.355	0.76598	0.62121 0.87341									
EC10	3.718	0.85227	0.71362 0.95277									
EC15	3.964	0.91592	0.78356 1.01043									
EC20	4.158	0.96988	0.84394 1.0588									
EC25	4.326	1.01869	0.89938 1.10222									
EC40	4.747	1.15288	1.05526 1.22025									
EC50	5.000	1.24197	1.16099 1.29814									
EC60	5.253	1.33795	1.27566 1.38277									
EC75	5.674	1.51419	1.47646 1.55191									
EC80	5.842	1.59041	1.55176 1.63812									
EC85	6.036	1.6841	1.6354 1.75427									
EC90	6.282	1.80986	1.74008 1.91986									
EC95	6.645	2.01375	1.9021 2.20092									
EC99	7.326	2.46018	2.24173 2.85156									



Significant heterogeneity detected (p < 0.01)

Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 6/13/03 Test ID: 0306-56NW Sample ID: Spirit of Oceanus
End Date: 6/15/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
Sample Date: 6/12/03 Protocol: ASTM 87 Test Species: CG-Crassostrea gigas
Comments:

Dose-Response Plot

Test: BV-Bivalve Larval Survival and Development Test
 Species: MS Mytilus species *Crassostrea gigas* NF
 Sample ID: Spirit of Oceanus CV
 Start Date: 06/19/2003 End Date: 06/21/2003

Test ID: 0306-56NW
 Protocol: ASTM 94
 Sample Type: EFF2-Industrial
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
81						20	0	everything broken up in 7/8
82						255	212	11
83						253	85	
84						198	57	
85						27	0	
86						67	0	
87						109	0	
88						250	0	
89						179	160	
90						53	0	
91						61	0	
92						234	0	
93						235	212	
94						156	142	
95						285	181	
96						225	0	
97						50	0	
98						237	223	
99						239	0	
100						120	2	
101						244	0	
102						224	202	
103						125	0	
104						257	0	
105						276	0	
106						233	218	
107						227	0	
108						282	252	
109						259	0	
110						245	0	
111						194	0	
112						252	233	
113						35	0	
114						275	256	
115						241	0	
116						248	207	
117						249	69	
118						235	40	
119						242	49	
120						59	0	V

Comments:

Day 0
 #1 236
 #2 228
 #3 230
 #4 236
 #5 211
 #6 206

mean = 224
 st.dev. = 12
 CV = 5.4%

Test: BV-Bivalve Larval Survival and Development Test Species: CG-Crassostrea gigas Sample ID: Spirit of Oceanus Start Date: 6/13/03 End Date: 6/15/03					Test ID: 0306-56NW Protocol: ASTM 87 Sample Type: BW/GW-Combined gray & black water Lab ID: WAAEE-AMEC NW Bioassay			
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
93	1	1	D-Control	224	235	235	212	
108	2	2	D-Control	224	282	282	252	
98	3	3	D-Control	224	237	237	223	
94	4	4	D-Control	224	156	156	142	
112	5	5	D-Control	224	252	252	233	
89	6	1	B-Control	224	179	179	160	
106	7	2	B-Control	224	233	233	218	
116	8	3	B-Control	224	248	248	207	
114	9	4	B-Control	224	275	275	256	
102	10	5	B-Control	224	224	224	202	
119	11	1	1.500	224	242	242	49	
117	12	2	1.500	224	249	249	69	
84	13	3	1.500	224	198	198	37	
118	14	4	1.500	224	235	235	40	
83	15	5	1.500	224	253	253	85	
115	16	1	3.125	224	241	241	0	
85	17	2	3.125	224	277	277	0	
88	18	3	3.125	224	250	250	0	
101	19	4	3.125	224	244	244	0	
95	20	5	3.125	224	285	285	1	
92	21	1	6.250	224	234	234	0	
104	22	2	6.250	224	257	257	0	
99	23	3	6.250	224	239	239	0	
105	24	4	6.250	224	276	276	0	
110	25	5	6.250	224	245	245	0	
109	26	1	12.500	224	259	259	0	
111	27	2	12.500	224	194	194	0	
107	28	3	12.500	224	227	227	0	
82	29	4	12.500	224	230	230	0	
96	30	5	12.500	224	225	225	0	
100	31	1	25.000	224	120	120	0	
97	32	2	25.000	224	50	50	0	
90	33	3	25.000	224	53	53	0	
81	34	4	25.000	224	20	20	0	
91	35	5	25.000	224	61	61	0	
87	36	1	50.000	224	109	109	0	
113	37	2	50.000	224	35	35	0	
120	38	3	50.000	224	59	59	0	
86	39	4	50.000	224	67	67	0	
103	40	5	50.000	224	125	125	0	

Comments:

Saltwater Chronic

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Client: Shannon + Wilson
 Sample ID: Spirit of Oceans
 Contact:
 Test No.: 0306-56 NW

Start Date & Time: 6/13/03 1900
 End Date & Time: 6/15/03 1900
 Test Organisms: CV
 Test Protocol:
 Date Received:

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
Control	7.6	7.8	7.9	7.85	7.93	7.96	29.1	29.5	30.6	15.1	15.5	15.8
B. Control	7.8	8.4	8.6	7.84	7.93	7.99	29.5	29.9	30.3	15.2	15.5	15.9
1.5	7.8	8.3	7.9	7.92	7.94	8.03	28.7	29.3	30.0	15.0	15.5	16.0
3.125	7.9	8.3	8.6	7.93	7.90	8.04	28.9	29.3	30.0	15.0	15.5	16.0
6.25	7.6	8.0	8.0	7.91	7.98	8.12	29.3	29.8	30.5	15.0	15.5	16.6
12.5	7.7	8.0	8.2	7.98	7.94	8.15	29.0	29.7	30.3	15.6	15.5	15.8
25	7.7	8.1	7.9	8.03	8.03	8.12	29.0	29.7	30.5	15.0	15.5	15.9
50	7.9	7.9	8.0	8.05	8.10	8.17	28.7	29.1	30.0	15.0	15.5	15.7
Conc.	Alkalinity* *(mg/L as CaCO ₃)		Chlorine Resid. (mg/L)			Sample Description: _____						
Control												
Highest conc.						Analyst Initials: <u>ML RS</u>						

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2
 Fife, WA 98424
 (253) 922-4296

Reviewed: ML QA check: ML

Bivalve Development Bioassay Worksheet

Client: Shanann + Wilson
Test No.: F0306-56MV
Test Species: Oyster

Start Date/Time: 6/13/03 1900
End Date/Time: 6/15/03 1900
Date Received: 6/13/03

Sample Type: cruise ship

Test Chamber Type and Sample Volume: 30 ml vial

Spawn Initiation Time: 1500

Number of Spawners: 1 Male 1 Female

Spawn Condition: Good

Fertilization Time: 1550

Egg Stock Density Calculation:

Eggs Counted (x):
32 _____
28 _____
36 _____
31 _____
27 _____

Mean 30.8 Overall Mean 30.8

Mean
30.8 X 42 = 1293 eggs/ml (initial stock)

Stock Dilution Factor
Initial Stock 1293 eggs/ml = 6.4
Innoculum Stock 200 eggs/ml

Percent division upon inoculation: 100%

Time of inoculation: 1900

Comments:

Reviewed/ Date: 6/13/03

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424
(253) 922-4296

Bivalve Larval Survival and Development Test-Proportion Alive

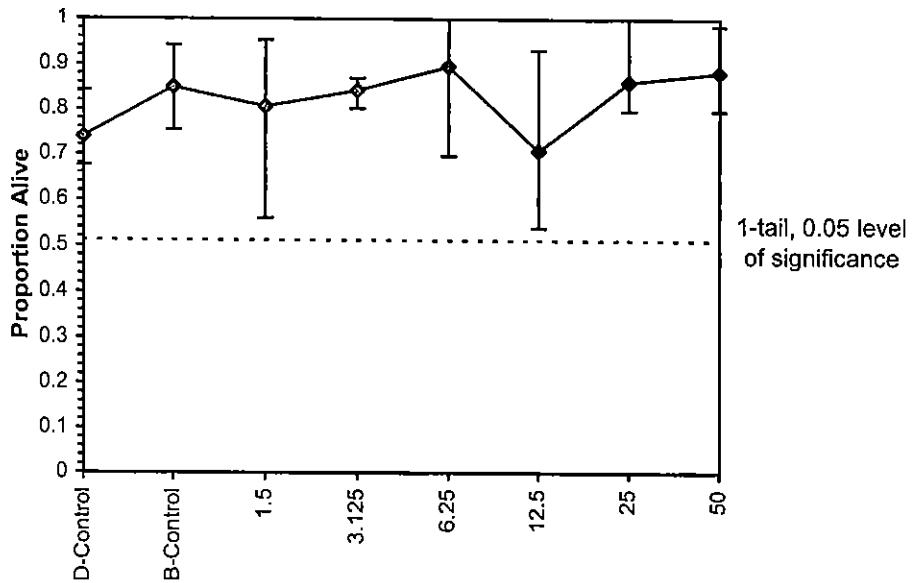
Start Date: 6/17/03 Test ID: 0306-38NW Sample ID: Sun Princess
 End Date: 6/19/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 6/16/03 Protocol: ASTM 94 Test Species: SP-Strongylocentrotus purpuratus
 Comments:

Conc-%	1	2	3	4	5
D-Control	0.7853	0.7120	0.6754	0.6806	0.8429
B-Control	0.9424	0.7539	0.8010	0.9319	0.8168
1.5	0.9529	0.5602	0.8063	0.9110	0.8010
3.125	0.8010	0.8691	0.8691	0.8639	0.8010
6.25	0.6963	0.9372	1.0000	0.9162	0.9267
12.5	0.9319	0.5393	0.6702	0.7068	0.6859
25	1.0000	0.8168	0.8796	0.8063	0.7958
50	0.8377	0.8377	0.9843	0.9476	0.7958

Conc-%	Transform: Arcsin Square Root						1-Tailed			
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.7393	0.8705	1.0383	0.9646	1.1633	8.260	5			
B-Control	0.8492	1.0000	1.1848	1.0517	1.3284	10.528	5			
1.5	0.8063	0.9494	1.1378	0.8458	1.3520	16.988	5	-1.006	2.409	0.2382
3.125	0.8408	0.9901	1.1622	1.1085	1.2006	4.231	5	-1.253	2.409	0.2382
6.25	0.8953	1.0543	1.2826	0.9872	1.5346	15.210	5	-2.470	2.409	0.2382
12.5	0.7068	0.8323	1.0130	0.8247	1.3069	17.542	5	0.256	2.409	0.2382
25	0.8597	1.0123	1.2193	1.1019	1.5346	14.917	5	-1.830	2.409	0.2382
50	0.8806	1.0370	1.2399	1.1019	1.4451	11.771	5	-2.038	2.409	0.2382

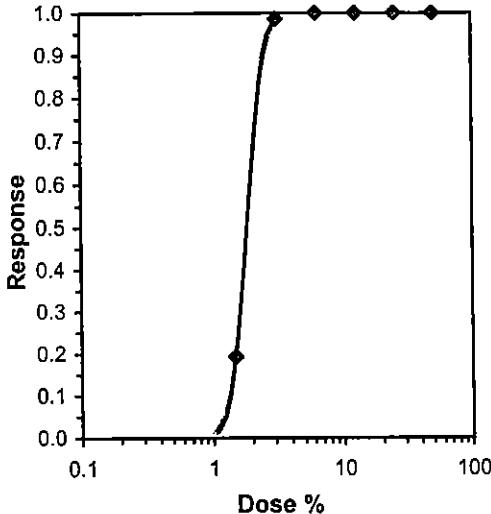
Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95259	0.91	0.33501	0.43497
Bartlett's Test indicates equal variances ($p = 0.23$)	8.1387	16.8119		
The control means are not significantly different ($p = 0.06$)	2.16373	2.30601		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	50	>50		2
			MSDu	MSDp
			MSB	MSE
			F-Prob	df
			0.02445	0.08458
			6, 28	

Dose-Response Plot



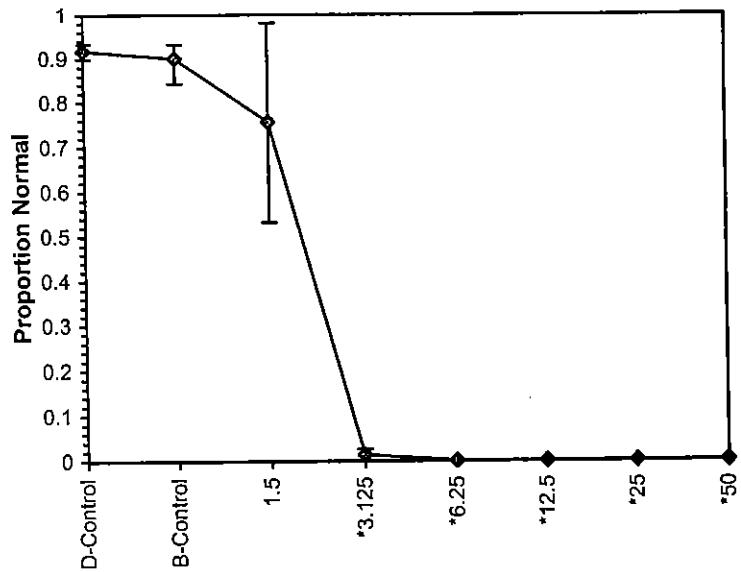
Bivalve Larval Survival and Development Test-Proportion Normal											
Start Date:	6/17/03	Test ID:	0306-38NW	Sample ID:	Sun Princess						
End Date:	6/19/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water						
Sample Date:	6/16/03	Protocol:	ASTM 94	Test Species:	SP-Strongylocentrotus purpuratus						
Comments:											
Conc-%	1	2	3	4	5						
D-Control	0.9333	0.9265	0.8992	0.9000	0.9255						
B-Control	0.9000	0.9028	0.8431	0.9326	0.9231						
1.5	0.7143	0.9813	0.5325	0.7701	0.7843						
3.125	0.0261	0.0181	0.0120	0.0061	0.0000						
6.25	0.0000	0.0000	0.0000	0.0000	0.0000						
12.5	0.0000	0.0000	0.0000	0.0000	0.0000						
25	0.0000	0.0000	0.0000	0.0000	0.0000						
50	0.0000	0.0000	0.0000	0.0000	0.0000						
Transform: Arcsin Square Root											
Conc-%	Mean	N-Mean	Mean	Min	Max	CV%	N	Rank Sum	1-Tailed Critical		
D-Control	0.9169	1.0184	1.2794	1.2478	1.3096	2.259	5				
B-Control	0.9003	1.0000	1.2528	1.1636	1.3081	4.445	5				
1.5	0.7565	0.8403	1.0834	0.8179	1.4336	20.599	5	20.00	16.00		
*3.125	0.0125	0.0138	0.1051	0.0404	0.1624	45.405	5	15.00	16.00		
*6.25	0.0000	0.0000	0.0384	0.0361	0.0434	7.363	5	15.00	16.00		
*12.5	0.0000	0.0000	0.0435	0.0375	0.0493	9.634	5	15.00	16.00		
*25	0.0000	0.0000	0.0391	0.0361	0.0406	4.747	5	15.00	16.00		
*50	0.0000	0.0000	0.0387	0.0365	0.0406	4.511	5	15.00	16.00		
Auxiliary Tests				Statistic	Critical		Number	Total			
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)				0.57455	0.91		58	706			
Bartlett's Test indicates unequal variances (p = 2.77E-21)				109.362	16.8119						
The control means are not significantly different (p = 0.37)				0.94578	2.30601						
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU							
Steel's Many-One Rank Test	1.5	3.125	2.16506	66.6667							
Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	9.66211	0.4421	8.7956 10.5286		0.08215	0.00165	9.48773	1	0.26617	0.1035	3
Intercept	2.42828	0.12396	2.18532 2.67125								
TSCR	0.08215	0.01033	0.0619 0.10241								
Point	Probits	%	95% Fiducial Limits								
EC01	2.674	1.06022	0.99822 1.11645								
EC05	3.355	1.24718	1.18984 1.29953								
EC10	3.718	1.35997	1.30556 1.41023								
EC15	3.964	1.44178	1.38925 1.49093								
EC20	4.158	1.51029	1.45902 1.55893								
EC25	4.326	1.57166	1.52116 1.62027								
EC40	4.747	1.73758	1.68699 1.78867								
EC50	5.000	1.84572	1.793 1.90077								
EC60	5.253	1.96059	1.90375 2.02193								
EC75	5.674	2.16757	2.09908 2.24506								
EC80	5.842	2.25565	2.18085 2.34158								
EC85	6.036	2.36283	2.27948 2.46009								
EC90	6.282	2.50497	2.40904 2.61874								
EC95	6.645	2.73151	2.61322 2.87455								
EC99	7.326	3.21319	3.04044 3.42782								

Significant heterogeneity detected (p < 0.01)



Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 6/17/03 Test ID: 0306-38NW Sample ID: Sun Princess
End Date: 6/19/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
Sample Date: 6/16/03 Protocol: ASTM 94 Test Species: SP-Strongylocentrotus purpuratus
Comments:

Dose-Response Plot

Test: BV-Bivalve Larval Survival and Development Test
 Species: MS-Mytilus species *S. purpuratus* sp
 Sample ID: Spirit of Oceanus Sun Prince
 Start Date: 06/18/2003 End Date: 06/24/2003

Test ID: 0306-32NW
 Protocol: ASTM 94
 Sample Type: OTH-Other sample type
 Lab ID: WAAEE-AMEC NW Bioassay

0306-38NW

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
1						188	0	
2						165	1	
3						144	130	
4						161	149	
5						182	130	
6						160	0	
7						178	166	
8						131	0	
9						192	0	
10						160	0	
11						168	0	
12						153	0	
13						154	82	
14						174	134	
15						129	116	
16						152	0	
17						128	0	
18						150	140	
19						133	0	
20						153	129	
21						103	0	
22						179	0	
23						130	117	
24						192	0	
25						177	0	
26						181	0	
27						107	105	
28						166	3	
29						152	0	
30						136	126	
31						153	120	
32						135	0	
33						66	2	
34						75	0	
35						78	0	
36						153	4	
37						180	162	
38						156	0	
39						156	144	
40						154	0	

Comments:

Day 0
 1 195
 2 180
 3 171
 4 180
 5 198
 6 224

Mean = 191
 St. Dev. = 18.9
 C.V. = 9.9%

ToxCalc 5.0

Test: BV-Bivalve Larval Survival and Development Test Species: SP-Strongylocentrotus purpuratus Sample ID: Sun Princess Start Date: 6/17/03 End Date: 6/19/03						Test ID: 0306-38NW Protocol: ASTM 94 Sample Type: BW/GW-Combined gray & black water Lab ID: WAAEE-AMEC NW Bioassay		
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
18	1	1	D-Control	191	150	150	140	
30	2	2	D-Control	191	136	136	126	
15	3	3	D-Control	191	129	129	116	
23	4	4	D-Control	191	130	130	117	
4	5	5	D-Control	191	161	161	149	
37	6	1	B-Control	191	180	180	162	
3	7	2	B-Control	191	144	144	130	
20	8	3	B-Control	191	153	153	129	
7	9	4	B-Control	191	178	178	166	
39	10	5	B-Control	191	156	156	144	
5	11	1	1.500	191	182	182	130	
27	12	2	1.500	191	107	107	105	
13	13	3	1.500	191	154	154	82	
14	14	4	1.500	191	174	174	134	
31	15	5	1.500	191	153	153	120	
36	16	1	3.125	191	153	153	4	
28	17	2	3.125	191	166	166	3	
33	18	3	3.125	191	166	166	2	
2	19	4	3.125	191	165	165	1	
12	20	5	3.125	191	153	153	0	
19	21	1	6.250	191	133	133	0	
22	22	2	6.250	191	179	179	0	
24	23	3	6.250	191	192	192	0	
34	24	4	6.250	191	175	175	0	
25	25	5	6.250	191	177	177	0	
35	26	1	12.500	191	178	178	0	
21	27	2	12.500	191	103	103	0	
17	28	3	12.500	191	128	128	0	
32	29	4	12.500	191	135	135	0	
8	30	5	12.500	191	131	131	0	
9	31	1	25.000	191	192	192	0	
38	32	2	25.000	191	156	156	0	
11	33	3	25.000	191	168	168	0	
40	34	4	25.000	191	154	154	0	
16	35	5	25.000	191	152	152	0	
6	36	1	50.000	191	160	160	0	
10	37	2	50.000	191	160	160	0	
1	38	3	50.000	191	188	188	0	
26	39	4	50.000	191	181	181	0	
29	40	5	50.000	191	152	152	0	

Comments:

Saltwater Chronic

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Client: Shannon + Wilson
 Sample ID: Sun Princess
 Contact:
 Test No.: 0306-38NW

Start Date & Time: 6/17/03 1800
 End Date & Time: 6/20/03 1750
 Test Organisms: S. purpuras
 Test Protocol:
 Date Received: 6/16/03

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
	0	24	48	0	24	48	0	24	48	0	24	48
con	8.0	8.1	7.9	7.84	7.70	7.86	29.0	29.3	29.9	15.0	15.2	15.5
brine con	7.9	7.7	7.6	7.86	7.84	7.87	29.2	30.7	30.9	15.0	15.2	15.6
1.5	8.0	7.8	7.7	7.87	7.84	7.89	29.0	29.0	29.7	15.0	16.0	19.6
3.125	8.1	8.0	7.6	7.85	7.85	7.88	29.0	29.0	29.7	15.0	15.3	15.8
6.25	8.1	7.9	7.8	7.81	7.87	7.92	29.0	24.5	29.9	15.0	15.3	15.7
12.5	8.3	7.3	7.8	7.75	7.81	7.96	29.0	29.9	30.6	15.0	15.6	15.8
25	8.3	7.6	7.7	7.67	7.75	8.01	29.1	29.8	30.3	15.0	15.7	15.9
50	8.5	7.9	7.7	7.58	7.99	8.10	29.0	29.7	30.8	15.0	15.5	15.9
Conc.	Alkalinity* (mg/L as CaCO ₃)		Chlorine Resid. (mg/L)			Sample Description: _____						
Control						Analyst Initials: <u>M</u> <u>m</u>						
Highest conc.												

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2-0
 Fife, WA 98424
 (253) 922-4296

Reviewed: _____ QA check: _____

Echinoderm
Pineapple Development Bioassay Worksheet

Client: Sharon + Wilson
Test No.: Sun Princess 0306-38 NW
Test Species: ~~Nudibranch species~~
S. purpuratus
Sample Type: 30 ml vial
Start Date/Time: 6/17/03
End Date/Time: 6/19/03
Date Received: 6/17/03
Test Chamber Type and Sample Volume: 30 ml vial

Spawn Initiation Time: 1400

Number of Spawners: 2 Male 3 Female

Spawn Condition: good

Fertilization Time: 1700

Egg Stock Density Calculation:

Eggs Counted (x): 5 3 5 4 4 Mean 4.2 Overall Mean: _____

$$\text{Mean: } 4.2 \times 42 = 176 \text{ eggs/ml}$$

$$\begin{array}{rcl} & 176 & \text{Stock Dilution Factor} \\ \text{Initial Stock - } & 2000 \text{ eggs/ml} & = \\ \text{Inoculum Stock - } & 200 \text{ eggs/ml} & \end{array}$$

Percent Division Upon Inoculation: 95%

Time of Inoculation: 1800

Comments: _____

Reviewed/ Date: BS

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424
(253) 922-4296

Bivalve Larval Survival and Development Test-Proportion Alive

Start Date: 6/19/03 Test ID: 0306-54NW Sample ID: Norwegian Wind
 End Date: 6/21/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 6/18/03 Protocol: ASTM 94 Test Species: CG-Crassostrea gigas
 Comments:

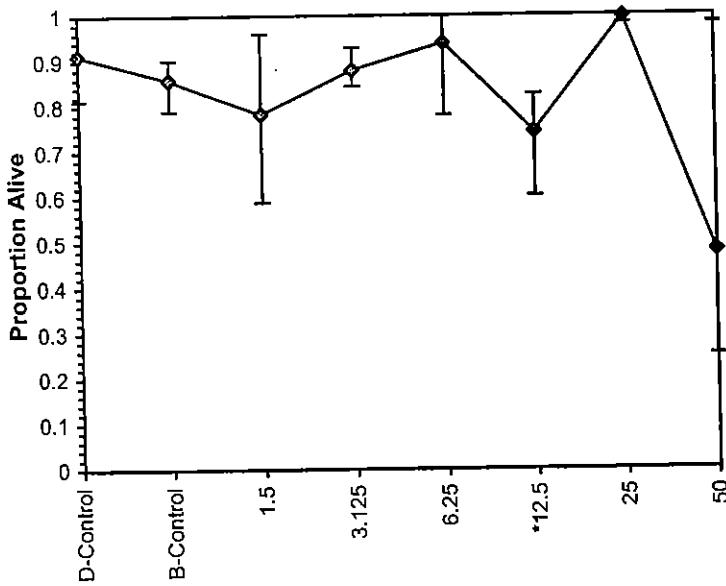
Conc-%	1	2	3	4	5
D-Control	0.8661	0.9107	1.0000	0.9688	0.8125
B-Control	0.7902	0.8661	0.8393	0.9018	0.8929
1.5	0.7589	0.9598	0.5893	0.7455	0.8571
3.125	0.8750	0.8438	0.8616	0.8839	0.9286
6.25	1.0000	0.9911	0.7813	0.9688	0.9509
12.5	0.7768	0.6027	0.7366	0.7723	0.8259
25	1.0000	1.0000	1.0000	1.0000	0.9821
50	0.9821	0.3616	0.5223	0.2813	0.2500

Conc-%	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical
	Mean	N-Mean	Mean	Min	Max	CV%		
D-Control	0.9116	1.0624	1.3034	1.1230	1.5374	12.616	5	
B-Control	0.8580	1.0000	1.1878	1.0950	1.2520	5.347	5	
1.5	0.7821	0.9116	1.1054	0.8752	1.3690	16.610	5	19.00 16.00
3.125	0.8786	1.0239	1.2174	1.1644	1.3002	4.216	5	24.00 16.00
6.25	0.9384	1.0937	1.3676	1.0841	1.5374	12.772	5	30.00 16.00
*12.5	0.7429	0.8658	1.0426	0.8888	1.1404	9.045	5	16.00 16.00
25	0.9964	1.1613	1.5173	1.4368	1.5374	2.966	5	37.00 16.00
50	0.4795	0.5588	0.7945	0.5236	1.4368	47.258	5	19.00 16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p \leq 0.01$)	0.89141	0.91	1.40864	5.08179
Bartlett's Test indicates unequal variances ($p = 1.53E-03$)	21.4317	16.8119		
The control means are not significantly different ($p = 0.18$)	1.46678	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	50	>50	2	

Dose-Response Plot



Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 6/19/03 Test ID: 0306-54NW Sample ID: Norwegian Wind
 End Date: 6/21/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 6/18/03 Protocol: ASTM 94 Test Species: CG-Crassostrea gigas
 Comments:

Conc-%	1	2	3	4	5
D-Control	0.9124	0.9265	0.9254	0.9493	0.8242
B-Control	0.9548	0.9433	0.9628	0.8911	0.9600
1.5	0.8647	0.8791	0.9015	0.6168	0.9271
3.125	0.8980	0.9153	0.9119	0.9192	0.9038
6.25	0.9241	0.8739	0.9486	0.9171	0.9155
12.5	0.3103	0.4296	0.2061	0.6127	0.6973
25	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000	0.0000

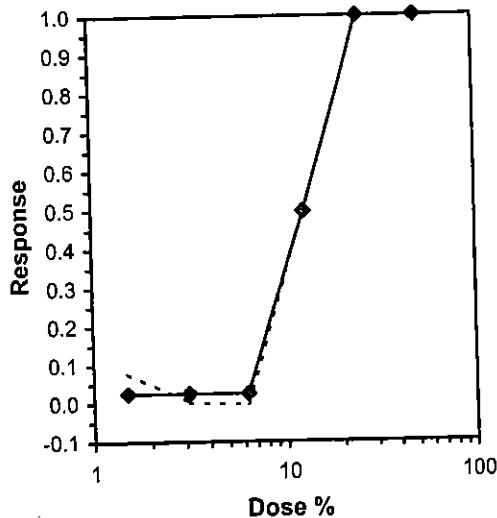
Conc-%	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N		
D-Control	0.9076	0.9630	1.2685	1.1381	1.3437	6.118	5		92 1025
B-Control	0.9424	1.0000	1.3335	1.2345	1.3766	4.356	5		
1.5	0.8378	0.8890	1.1724	0.9033	1.2974	13.260	5	22.00	16.00 140 876
3.125	0.9097	0.9653	1.2658	1.2457	1.2826	1.186	5	22.00	16.00 89 984
6.25	0.9158	0.9718	1.2792	1.2077	1.3420	3.754	5	26.00	16.00 90 1051
*12.5	0.4512	0.4788	0.7328	0.4712	0.9882	29.091	5	15.00	16.00 451 832
*25	0.0000	0.0000	0.0317	0.0293	0.0337	5.540	5	15.00	16.00 1251 1251
*50	0.0000	0.0000	0.0531	0.0337	0.0669	25.221	5	15.00	16.00 537 537

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.84319	0.91	-0.6471	3.32768
Bartlett's Test indicates unequal variances (p = 5.97E-11)	59.3966	16.8119		
The control means are not significantly different (p = 0.17)	1.49917	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	6.25	12.5	8.83883	16

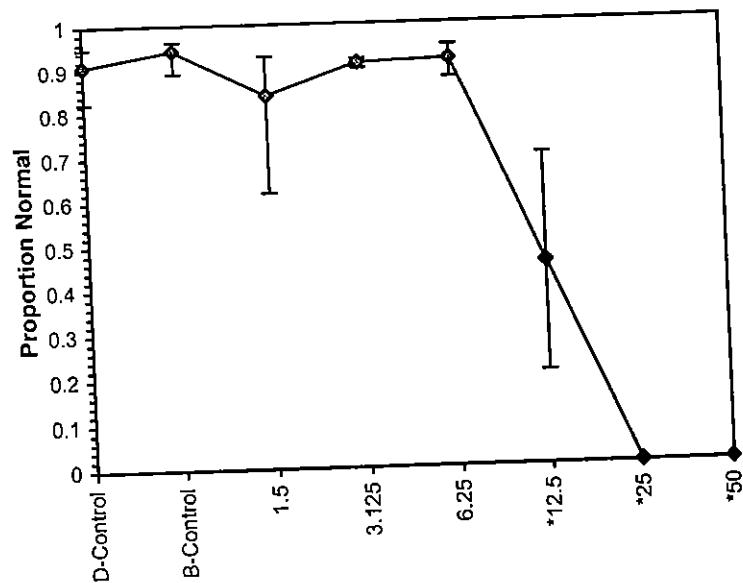
Trimmed Spearman-Karber

Trim Level	EC50	95% CL
0.0%		
5.0%	12.430	12.101 12.767
10.0%	12.444	12.076 12.822
20.0%	12.471	11.986 12.977
Auto-2.4%	12.423	12.110 12.743



Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 6/19/03 Test ID: 0306-54NW Sample ID: Norwegian Wind
End Date: 6/21/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
Sample Date: 6/18/03 Protocol: ASTM 94 Test Species: CG-Crassostrea gigas
Comments:

Dose-Response Plot

Test: BV-Bivalve Larval Survival and Development Test Species: MS-Mytilus species <i>C. gigas</i> Sample ID: ADEC Cruise Ship Norwegian wind Start Date: 06/19/2003 End Date: 06/21/2003				Test ID: 0306-54NW Protocol: ASTM 94 Sample Type: EFF2-Industrial Lab ID: WAAEE-AMEC NW Bioassay				
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
1				228	211	228	211	NE 7/11/03
2				194	177	194	177	
3				204	189	204	189	
4				217	199	217	199	
5				249	0	249	0	↓
6				220	0	220	0	KR 7/11/03
7				215	189	215	189	
8				194	183	194	183	
9				81	0	81	0	
10				217	206	217	206	
11				174	54	174	54	
12				206	189	206	189	
13				292	0	292	0	↓
14				189	173	189	173	SM 7/14/03
15				213	195	213	195	
16				117	0	117	0	
17				224	207	224	207	
18				200	192	200	192	↓
19				173	106	173	106	
20				261	0	261	0	
21				170	147	170	147	
22				222	194	222	194	
23				202	180	202	180	
24				229	0	229	0	
25				175	110	175	110	
26				182	150	182	150	
27				56	0	56	0	
28				196	176	196	176	
29				192	178	192	178	
30				193	176	193	176	
31				165	34	165	34	
32				188	181	188	181	
33				63	0	63	0	
34				167	103	167	103	
35				185	129	185	129	
36				132	119	132	119	
37				177	169	177	169	
38				220	0	220	0	
39				135	58	135	58	
40				98	182	98	182	

Comments:

Day 0

#1	236	Mean = 224
#2	228	St.dev = 12
#3	230	CV = 5.4%
#4	236	
#5	211	
#6	206	

Test: BV-Bivalve Larval Survival and Development Test Species: CG-Crassostrea gigas Sample ID: Norwegian Wind				Test ID: 0306-54NW Protocol: ASTM 94 Sample Type: BW/GW-Combined gray & black water Lab ID: WAAEE-AMEC NW Bioassay				
Start Date: 6/19/03	End Date: 6/21/03							
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
2	1	1	D-Control	224	194	194	177	
3	2	2	D-Control	224	204	204	189	
1	3	3	D-Control	224	228	228	211	
10	4	4	D-Control	224	217	217	206	
26	5	5	D-Control	224	182	182	150	
37	6	1	B-Control	224	177	177	169	
8	7	2	B-Control	224	194	194	183	
32	8	3	B-Control	224	188	188	181	
23	9	4	B-Control	224	202	202	180	
18	10	5	B-Control	224	200	200	192	
21	11	1	1.500	224	170	170	147	
7	12	2	1.500	224	215	215	189	
36	13	3	1.500	224	132	132	119	
34	14	4	1.500	224	167	167	103	
29	15	5	1.500	224	192	192	178	
28	16	1	3.125	224	196	196	176	
14	17	2	3.125	224	189	189	173	
30	18	3	3.125	224	193	193	176	
40	19	4	3.125	224	198	198	182	
12	20	5	3.125	224	208	208	188	
17	21	1	6.250	224	224	224	207	
22	22	2	6.250	224	222	222	194	
25	23	3	6.250	224	175	175	166	
4	24	4	6.250	224	217	217	199	
15	25	5	6.250	224	213	213	195	
11	26	1	12.500	224	174	174	54	
39	27	2	12.500	224	135	135	58	
31	28	3	12.500	224	165	165	34	
19	29	4	12.500	224	173	173	106	
35	30	5	12.500	224	185	185	129	
13	31	1	25.000	224	292	292	0	
20	32	2	25.000	224	261	261	0	
5	33	3	25.000	224	249	249	0	
24	34	4	25.000	224	229	229	0	
38	35	5	25.000	224	220	220	0	
6	36	1	50.000	224	220	220	0	
9	37	2	50.000	224	81	81	0	
16	38	3	50.000	224	117	117	0	
33	39	4	50.000	224	63	63	0	
27	40	5	50.000	224	56	56	0	

Comments:

Saltwater Chronic

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Client: Shannon + Wilson
 Sample ID: Norwegian Wind
 Contact: _____
 Test No.: 0306-54NW

Start Date & Time: 6/19/03
 End Date & Time: 6/21/03
 Test Organisms: Oysters
 Test Protocol: _____
 Date Received: _____

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
	0	24	48	0	24	48	0	24	48	0	24	48
C	7.7	8.0	8.1	8.01	8.00	8.09	28.3	29.2	29.5	15.0	15.0	15.5
Brine C	7.8	8.0	8.2	8.04	7.99	8.06	28.8	29.2	30.3	15.0	15.0	15.8
1.5	7.7	8.0	8.2	8.00	8.03	8.12	29.1	29.4	30.5	15.0	15.0	15.4
3.25	7.8	8.0	7.1	8.04	8.05	8.09	29.1	29.7	30.5	15.0	15.0	15.3
6.25	8.0	7.9	8.3	7.93	8.04	8.09	29.4	29.5	30.4	15.0	15.0	15.3
12.5	8.0	7.9	8.1	7.90	8.03	8.05	29.4	29.8	30.6	15.0	15.0	15.5
25	7.7	7.9	8.0	7.86	7.97	8.11	28.7	29.9	30.1	15.0	15.0	15.3
50.0	7.5	7.9	8.0	7.95	7.98	8.12	28.9	29.4	30.7	15.0	15.0	15.5
Conc.	Alkalinity* *(mg/L as CaCO ₃)		Chlorine Resid. (mg/L)			Sample Description: _____			Analyst Initials: <u>nm</u>			
Control												
Highest conc.												

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

Reviewed: KBQA check: KB

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2-0
 Fife, WA 98424
 (253) 922-4296

Bivalve Larval Survival and Development Test-Proportion Alive									
Start Date:	6/21/03	Test ID:	0306-61NW	Sample ID:					Ryndam
End Date:	6/23/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:					BW/GW-Combined gray & black water
Sample Date:	6/20/03	Protocol:	ASTM 94	Test Species:					CG-Crassostrea gigas
Comments:									

Conc-%	1	2	3	4	5
D-Control	0.9108	0.9484	0.8075	1.0000	0.8592
B-Control	1.0000	0.8967	0.8075	0.9812	0.8967
1.5	0.9202	0.9108	0.9484	0.9014	0.9155
3.125	0.7793	0.7465	0.8263	1.0000	0.7277
6.25	1.0000	0.9859	0.9577	0.6948	0.9296
12.5	1.0000	0.8826	0.9906	0.8498	0.9108
25	0.7793	1.0000	0.7700	0.8028	0.7559
50	0.9671	0.8732	0.8732	0.8920	0.4695

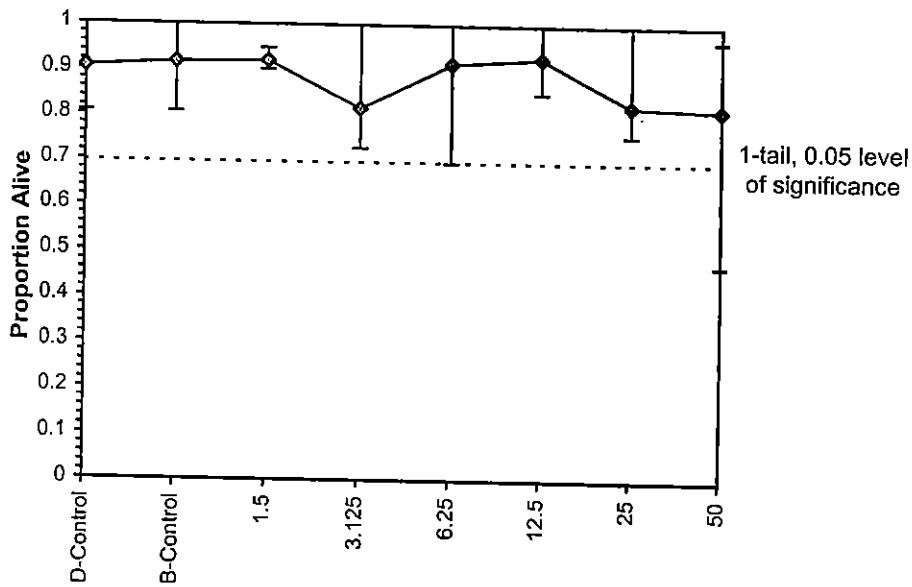
Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				t-Stat	1-Tailed Critical	MSD
			Mean	Min	Max	CV%			
D-Control	0.9052	0.9877	1.2897	1.1166	1.5365	12.551	5		
B-Control	0.9164	1.0000	1.3147	1.1166	1.5365	12.763	5		
1.5	0.9192	1.0031	1.2841	1.2514	1.3415	2.673	5	0.047	2.409
3.125	0.8160	0.8904	1.1648	1.0218	1.5365	18.256	5	1.062	2.409
6.25	0.9136	0.9969	1.3280	0.9855	1.5365	15.887	5	-0.326	2.409
12.5	0.9268	1.0113	1.3343	1.1728	1.5365	12.067	5	-0.380	2.409
25	0.8216	0.8965	1.1707	1.0540	1.5365	17.557	5	1.012	2.409
50	0.8150	0.8893	1.1586	0.7549	1.3885	20.538	5	1.115	2.409

Auxiliary Tests

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.96338	0.91	0.15208	0.65829
Bartlett's Test indicates equal variances ($p = 0.13$)	9.95671	16.8119		
The control means are not significantly different ($p = 0.82$)	0.24057	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	MSDu	MSDp	MSB	MSE	F-Prob	df
Dunnett's Test	50	>50		2	0.20908	0.22652	0.03148	0.03456	0.50156	6, 28

Dose-Response Plot



Bivalve Larval Survival and Development Test-Proportion Normal							
Start Date:	6/21/03	Test ID:	0306-61NW	Sample ID:	Ryndam		
End Date:	6/23/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water		
Sample Date:	6/20/03	Protocol:	ASTM 94	Test Species:	CG-Crassostrea gigas		
Comments:							

Conc-%	1	2	3	4	5
D-Control	0.9742	0.8812	0.7907	0.9227	0.9727
B-Control	0.9820	0.9162	0.7500	0.9665	0.9476
1.5	0.9439	0.9639	0.9010	0.7813	0.9538
3.125	0.8313	0.9811	0.9659	0.9615	0.8129
6.25	0.8505	0.9571	0.8676	0.8851	0.9141
12.5	0.7860	0.8298	0.7204	0.8398	0.7835
25	0.3614	0.2455	0.5061	0.1287	0.4596
50	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%	N		
D-Control	0.9083	0.9954	1.2836	1.0956	1.4096	10.313	5		87 971
B-Control	0.9125	1.0000	1.2974	1.0472	1.4362	11.693	5		
1.5	0.9088	0.9959	1.2801	1.0841	1.3797	9.354	5	26.00	16.00
3.125	0.9106	0.9979	1.2925	1.1235	1.4330	11.240	5	28.00	16.00
6.25	0.8949	0.9807	1.2466	1.1738	1.3623	5.975	5	24.00	16.00
12.5	0.7919	0.8679	1.0990	1.0136	1.1590	5.243	5	17.00	16.00
*25	0.3403	0.3729	0.6134	0.3669	0.7915	28.219	5	15.00	16.00
*50	0.0000	0.0000	0.0389	0.0348	0.0500	16.103	5	15.00	16.00

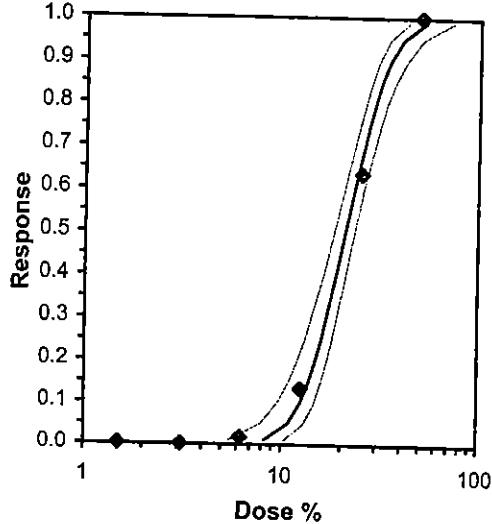
Auxiliary Tests

	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	0.95987	0.91	-0.5568	-0.1353
Bartlett's Test indicates unequal variances (p = 7.15E-04)	23.2551	16.8119		
The control means are not significantly different (p = 0.88)	0.15392	2.30601		

Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	12.5	25	17.6777	8

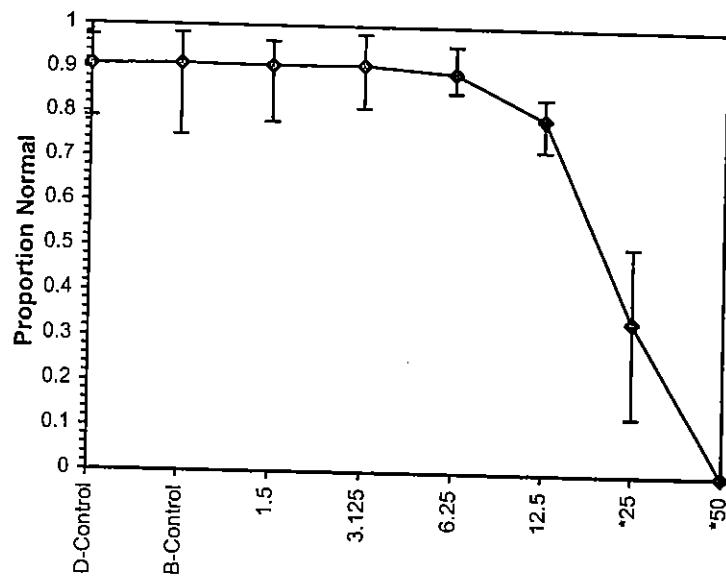
Parameter	Value	SE	Maximum Likelihood-Probit		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			95% Fiducial Limits								
Slope	5.80654	0.56525	4.23714	7.37594							
Intercept	-2.6573	0.77335	-4.8045	-0.5102	0.0896	24.2374	9.48773	7.2E-05	1.31875	0.17222	7
TSCR	0.09476	0.01117	0.06227	0.12725							
Point	Probits	%	95% Fiducial Limits								
EC01	2.674	8.28139	5.5323	10.529							
EC05	3.355	10.851	7.96167	13.1076							
EC10	3.718	12.5325	9.64906	14.7584							
EC15	3.964	13.8119	10.9717	16.0078							
EC20	4.158	14.9212	12.1385	17.0934							
EC25	4.326	15.9436	13.225	18.1011							
EC40	4.747	18.8415	16.3135	21.0399							
EC50	5.000	20.8327	18.3914	23.1801							
EC60	5.253	23.0344	20.5946	25.7108							
EC75	5.674	27.2212	24.4469	31.0553							
EC80	5.842	29.0864	26.0362	33.6422							
EC85	6.036	31.4225	27.9434	37.0316							
EC90	6.282	34.6302	30.4477	41.9159							
EC95	6.645	39.9966	34.431	50.5804							
EC99	7.326	52.407	43.04	72.4924							

Significant heterogeneity detected (p = 7.16E-05)



Start Date: 6/21/03 Test ID: 0306-61NW Sample ID: Ryndam
End Date: 6/23/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
Sample Date: 6/20/03 Protocol: ASTM 94 Test Species: CG-Crassostrea gigas
Comments:

Dose-Response Plot



Test: BV-Bivalve Larval Survival and Development Test Species: CR-Crassostrea virginica 8/9/03 Sample ID: Ryndam Start Date: 06/21/2003					Test ID: 0306-61NW Protocol: ASTM 87 Sample Type: EFF2-Industrial Lab ID: WAAEE-AMEC NW Bioassay			
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
1						100	0	NP 7/22/03 inc 7/22/03
2						180	0	NP 7/23/03
3						198	181	
4						194	189	
5						206	0	
6						159	156	
7						170	170	
8						194	187	
9						234	225	
10						210	201	
11						222	218	
12						209	202	
13						195	186	
14						191	181	
15						196	185	↓
16						183	178	SM 7/23/03
17						188	156	
18						202	178	
19						204	177	
20						190	0	
21						194	152	
22						161	74	
23						191	175	
24						186	0	
25						148	131	
26						202	182	
27						181	152	
28						215	109	
29						164	83	
30						172	129	↓
31						166	138	SM 7/24/03
32				.		192	150	
33						172	136	
34						214	182	
35						155	136	
36						211	152	
37						166	60	
38						171	22	
39						220	203	
40						224	55	↓

Comments:

Day 0

1 219
2 217
3 213
4 206
5 201
6 220

Mean = 213
St. Dev. = 7.7
CV = 3.6%

Test: BV-Bivalve Larval Survival and Development Test
 Species: CG-Crassostrea gigas
 Sample ID: Ryndam
 Start Date: 6/21/03 End Date: 6/23/03

Test ID: 0306-61NW
 Protocol: ASTM 94
 Sample Type: BW/GW-Combined gray & black water
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
4	1	1	D-Control	213	194	194	189	
18	2	2	D-Control	213	202	202	178	
33	3	3	D-Control	213	172	172	136	
39	4	4	D-Control	213	220	220	203	
16	5	5	D-Control	213	183	183	178	
11	6	1	B-Control	213	222	222	218	
23	7	2	B-Control	213	191	191	175	
30	8	3	B-Control	213	172	172	129	
12	9	4	B-Control	213	209	209	202	
14	10	5	B-Control	213	191	191	181	
15	11	1	1.500	213	196	196	185	
8	12	2	1.500	213	194	194	187	
26	13	3	1.500	213	202	202	182	
32	14	4	1.500	213	192	192	150	
13	15	5	1.500	213	195	195	186	
31	16	1	3.125	213	166	166	138	
6	17	2	3.125	213	159	159	156	
7	18	3	3.125	213	176	176	170	
9	19	4	3.125	213	234	234	225	
35	20	5	3.125	213	155	155	126	
34	21	1	6.250	213	214	214	182	
10	22	2	6.250	213	210	210	201	
19	23	3	6.250	213	204	204	177	
25	24	4	6.250	213	148	148	131	
3	25	5	6.250	213	198	198	181	
28	26	1	12.500	213	215	215	169	
17	27	2	12.500	213	188	188	156	
36	28	3	12.500	213	211	211	152	
27	29	4	12.500	213	181	181	152	
21	30	5	12.500	213	194	194	152	
37	31	1	25.000	213	166	166	60	
40	32	2	25.000	213	224	224	55	
29	33	3	25.000	213	164	164	83	
38	34	4	25.000	213	171	171	22	
22	35	5	25.000	213	161	161	74	
5	36	1	50.000	213	206	206	0	
24	37	2	50.000	213	186	186	0	
2	38	3	50.000	213	186	186	0	
20	39	4	50.000	213	190	190	0	
1	40	5	50.000	213	100	100	0	

Comments:

Saltwater Chronic

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Client: Shannon + Wilson
 Sample ID: Ryndam
 Contact:
 Test No.: 0306-61NW

Start Date & Time: 6/21/03 1920
 End Date & Time: 6/23/03 1730
 Test Organisms: oyster
 Test Protocol:
 Date Received: 6/19/03

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
	0	24	48	0	24	48	0	24	48	0	24	48
C	8.0	7.9	7.1	7.8	7.90	8.01	28.0	28.2	30.2	16.4	16.0	15.8
Brine C	8.2	7.1	6.9	7.83	7.85	8.01	28.5	28.7	30.5	15.6	16.0	15.9
1.5	8.2	8.1	7.0	7.85	7.89	8.01	28.0	28.4	30.0	15.1	15.8	15.5
3.125	8.1	8.0	7.3	7.86	7.85	8.04	28.4	28.3	30.5	15.0	15.5	15.5
6.25	8.3	8.0	7.1	7.91	7.93	8.07	28.2	28.1	29.7	15.6	15.9	15.5
12.5	8.4	8.3	7.1	7.95	7.98	8.11	28.0	28.8	29.5	15.1	15.3	16.0
25	8.3	8.3	7.1	8.01	8.04	8.18	28.4	28.5	29.0	15.6	15.3	15.6
50	8.2	8.3	7.0	8.09	8.12	8.26	28.6	28.6	29.7	15.7	15.1	15.7
Conc.	Alkalinity* *(mg/L as CaCO ₃)		Chlorine Resid. (mg/L)		Sample Description:							
Control												
Highest conc.					Analyst Initials: <u>mcg, RL</u>							

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

Reviewed: SL

QA check: V3

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2-0
 Fife, WA 98424
 (253) 922-4296

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424

Marine Dilution Worksheet

Client: Ryn dam
Sample ID: _____
Test No.: 0306-61NW, 0306-62NW
Brine salinity: 67

Analyst: me
Test Date: 6/21/03
Test Type: _____

Equation for Salinity Adjustment: $VB = VE \frac{30 - SE}{SB - 30}$

Effluent Salinity Adjustment: $\frac{30 - 0}{67 - 30} = \frac{30}{37} = .81$

Brine Control Salinity Adjustment:

Concentration	Effluent Volume (ml)	Salinity Adjustment	Brine Volume (ml)	Seawater Volume (ml)	Total Volume (ml)
Control	NA	NA	NA		250ml
1.5	3.75	.81	3.0		1
3.125	7.81	.81	6.3		1
6.25	15.6	.81	12.6		1
12.5	31.25	.81	25.3		1
25	62.5	.81	50.6		1
50	125	.81	101		1

DI Volume					
Brine Control	125	.81	101		250ml

Calculations for all concentrations except highest:

1. Effluent Volume = Total Volume X Concentration (%)
2. Brine Volume = Effluent Volume X Salinity Adjustment
3. Seawater Volume = Total Volume - Effluent Volume + Brine Volume

Calculation for highest concentration:

1. Effluent Volume = Total Volume / (1.0 + Salinity Adjustment)
2. Brine Volume = Total Volume - Effluent Volume
3. Concentration = Effluent Volume / Total Volume

Calcuation for brine control:

1. Brine Volume = Brine Volume for highest concentration
2. DI Volume = Brine Volume / Brine Control Salinity Adjustment
3. Seawater Volume = Total Volume - DI Volume + Brine Volume

Reviewed/ Date: _____

Bivalve Development Bioassay Worksheet

Client: Shannon Wilson Ryndam Start Date/Time: 6/21/03 92C
Test No.: 0306-61 NW End Date/Time: 4/23/03 1730
Test Species: OYSTER Crassostrea virginica KB
gigas Date Received: 6/19/03

Sample Type: Cruise ship

Test Chamber Type and Sample Volume: 20 ml vial

Spawn Initiation Time: 1500

Number of Spawners: Male 5 Female 5

Spawn Condition: good

Fertilization Time: 1545

Egg Stock Density Calculation:

Eggs Counted (x): 66 59
60 56
59

Mean 60 Overall Mean: _____

Mean: 60 \times 42 = 2520 eggs/ml

Initial Stock - 2520 eggs/ml = 12.6
Inoculum Stock - 200 eggs/ml

Percent Division Upon Inoculation: 98%

Time of Inoculation: 1920

Comments: _____

Reviewed/ Date: BS

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424
(253) 922-4296

Bivalve Larval Survival and Development Test-Proportion Alive

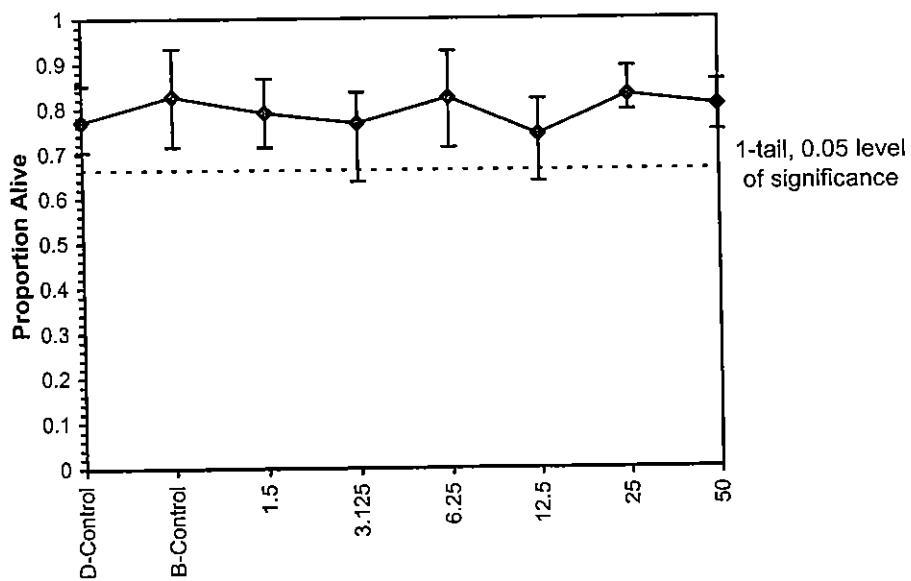
Start Date: 9/13/03 Test ID: 0309-05NW Sample ID: CARNIVAL SPIRIT
 End Date: 9/15/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 9/12/03 Protocol: ASTM 94 Test Species: MS-Mytilis species
 Comments:

Conc-%	1	2	3	4	5
D-Control	0.7041	0.8520	0.8316	0.7602	0.7092
B-Control	0.7143	0.8929	0.8163	0.7755	0.9337
1.5	0.7959	0.8673	0.7959	0.7143	0.7806
3.125	0.6378	0.8367	0.7857	0.8163	0.7602
6.25	0.8367	0.7143	0.7755	0.9286	0.8673
12.5	0.7653	0.8214	0.6378	0.7602	0.7245
25	0.8061	0.8112	0.7959	0.8929	0.8418
50	0.7857	0.8316	0.8622	0.7500	0.8061

Conc-%	Transform: Arcsin Square Root						1-Tailed			
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.7714	0.9333	1.0760	0.9956	1.1760	7.708	5			
B-Control	0.8265	1.0000	1.1519	1.0069	1.3103	10.596	5			
1.5	0.7908	0.9568	1.0985	1.0069	1.1980	6.200	5	-0.448	2.409	0.1210
3.125	0.7673	0.9284	1.0713	0.9250	1.1548	8.360	5	0.094	2.409	0.1210
6.25	0.8245	0.9975	1.1474	1.0069	1.3002	9.808	5	-1.423	2.409	0.1210
12.5	0.7418	0.8975	1.0404	0.9250	1.1345	7.388	5	0.709	2.409	0.1210
25	0.8296	1.0037	1.1475	1.1021	1.2373	4.791	5	-1.424	2.409	0.1210
50	0.8071	0.9765	1.1180	1.0472	1.1905	4.898	5	-0.837	2.409	0.1210

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.97984	0.91	-0.1018	-0.3749
Bartlett's Test indicates equal variances ($p = 0.81$)	3.01192	16.8119		
The control means are not significantly different ($p = 0.28$)	1.15078	2.30601		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	50	>50		2
			MSDu	MSDp
			MSB	MSE
			F-Prob	df

Dose-Response Plot



Bivalve Larval Survival and Development Test-Proportion Normal

Start Date:	9/13/03	Test ID:	0309-05NW	Sample ID:	CARNIVAL SPIRIT
End Date:	9/15/03	Lab ID:	WAAEE-AMEC NW Bioassa	Sample Type:	BW/GW-Combined gray & black water
Sample Date:	9/12/03	Protocol:	ASTM 94	Test Species:	MS-Mytilis species

Comments:

Conc-%	1	2	3	4	5
D-Control	0.7536	0.7605	0.8528	0.7047	0.8273
B-Control	0.8643	0.8571	0.8125	0.8289	0.7486
1.5	0.7436	0.8353	0.8397	0.8429	0.8758
3.125	0.8800	0.8110	0.8117	0.8000	0.7852
6.25	0.8841	0.7857	0.8026	0.8077	0.8353
12.5	0.7733	0.7516	0.8640	0.8255	0.9014
25	0.8038	0.7358	0.7436	0.5371	0.6242
50	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Transform: Arcsin Square Root					Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max				
D-Control	0.7798	0.9483	1.0853	0.9963	1.1770	6.739	5		166 756
B-Control	0.8223	1.0000	1.1379	1.0456	1.1935	5.185	5		
1.5	0.8275	1.0063	1.1451	1.0398	1.2107	5.516	5	33.00	16.00 134 775
3.125	0.8176	0.9943	1.1312	1.0889	1.2171	4.404	5	32.00	16.00 139 752
6.25	0.8231	1.0010	1.1386	1.0895	1.2235	4.623	5	33.00	16.00 142 808
12.5	0.8232	1.0010	1.1416	1.0490	1.2514	7.300	5	32.00	16.00 131 727
25	0.6889	0.8378	0.9833	0.8226	1.1119	11.716	5	20.00	16.00 256 813
*50	0.0000	0.0000	0.0398	0.0385	0.0413	2.670	5	15.00	16.00 791 791

Auxiliary Tests

Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.9818	0.91	-0.1686	0.13363
Bartlett's Test indicates unequal variances ($p = 2.48E-05$)	31.0484	16.8119		
The control means are not significantly different ($p = 0.25$)	1.2535	2.30601		

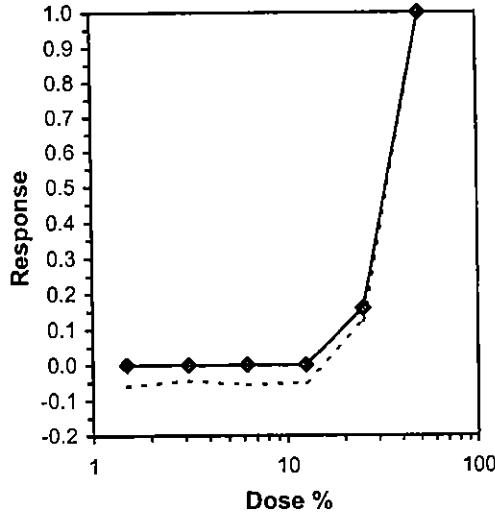
Hypothesis Test (1-tail, 0.05)

NOEC	LOEC	ChV	TU
------	------	-----	----

Steel's Many-One Rank Test	25	50	35.3553	4
----------------------------	----	----	---------	---

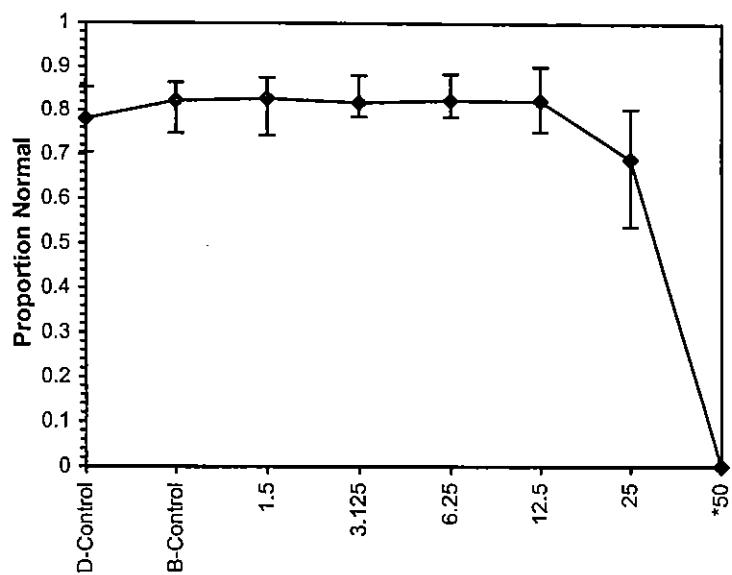
Trimmed Spearman-Karber

Trim Level	EC50	95% CL
0.0%	31.695	31.139 32.262
5.0%	32.381	31.719 33.056
10.0%	32.890	32.031 33.771
20.0%	33.135	32.724 33.551
Auto-0.0%	31.695	31.139 32.262



Bivalve Larval Survival and Development Test-Proportion Normal

Start Date: 9/13/03 Test ID: 0309-05NW Sample ID: CARNIVAL SPIRIT
End Date: 9/15/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
Sample Date: 9/12/03 Protocol: ASTM 94 Test Species: MS-Mytilus species
Comments:

Dose-Response Plot

Test: BV-Bivalve Larval Survival and Development Test
 Species: MS-Mytilis species
 Sample ID: Carnival Spirit ~~REF REP TOX~~
 Start Date: 8/6/03 End Date: 8/8/03 - 9/15/03

Test ID: RT080803MS ~~RT091103MS~~ 0309-DS NW
 Protocol: ASTM 94 BW/GW
 Sample Type: BW/GW-Combined gray & black water Cx CL
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
1						156	116	NE 10/7/03
2						150	116	
3						154	125	
4						164	133	
5						175	94	
6						125	108	
7						164	145	
8						142	128	
9						156	131	
10						175	150	
11						125	110	
12						152	126	
13						152	122	
14						169	0	
15						160	130	
16						147	0	
17						138	104	
18						158	0	
19						139	115	
20						182	147	↓
21						163	0	NE 10/8/03
22						159	117	
23						153	134	
24						149	123	
25						170	142	
26						148	118	
27						170	142	
28						161	121	
29						154	0	
30						163	139	
31						183	137	
32						165	103	
33						167	127	
34						140	110	
35						149	105	
36						160	128	
37						156	116	
38						140	121	
39						158	127	
40						149	117	↓

Comments:

RT Day 0

1 211
 2 212
 3 192
 4 200
 5 205
 6 156

avg = 196
 St Dev = 20.9
 C = 10.7%

Test: BV-Bivalve Larval Survival and Development Test Species: MS-Mytilis species Sample ID: Carnival Spirit Start Date: 9/13/03 End Date: 9/15/03				Test ID: 0309-05NW Protocol: ASTM 94 Sample Type: BW/GW-Combined gray & black water Lab ID: WAAEE-AMEC NW Bioassay				
Pos	ID	Rep	Group	Initial Density	Final Density	Total Counted	Number Normal	Notes
17	1	1	D-Control	196	138	138	104	
33	2	2	D-Control	196	167	167	127	
30	3	3	D-Control	196	163	163	139	
35	4	4	D-Control	196	149	149	105	
19	5	5	D-Control	196	139	139	115	
38	6	1	B-Control	196	140	140	121	
10	7	2	B-Control	196	175	175	150	
15	8	3	B-Control	196	160	160	130	
12	9	4	B-Control	196	152	152	126	
31	10	5	B-Control	196	183	183	137	
1	11	1	1.500	196	156	156	116	
25	12	2	1.500	196	170	170	142	
9	13	3	1.500	196	156	156	131	
26	14	4	1.500	196	140	140	118	
23	15	5	1.500	196	153	153	134	
11	16	1	3.125	196	125	125	110	
4	17	2	3.125	196	164	164	133	
3	18	3	3.125	196	154	154	125	
36	19	4	3.125	196	160	160	128	
40	20	5	3.125	196	149	149	117	
7	21	1	6.250	196	164	164	145	
34	22	2	6.250	196	140	140	110	
13	23	3	6.250	196	152	152	122	
20	24	4	6.250	196	182	182	147	
27	25	5	6.250	196	170	170	142	
2	26	1	12.500	196	150	150	116	
28	27	2	12.500	196	161	161	121	
6	28	3	12.500	196	125	125	108	
24	29	4	12.500	196	149	149	123	
8	30	5	12.500	196	142	142	128	
39	31	1	25.000	196	158	158	127	
22	32	2	25.000	196	159	159	117	
37	33	3	25.000	196	156	156	116	
5	34	4	25.000	196	175	175	94	
32	35	5	25.000	196	165	165	103	
29	36	1	50.000	196	154	154	0	
21	37	2	50.000	196	163	163	0	
14	38	3	50.000	196	169	169	0	
16	39	4	50.000	196	147	147	0	
18	40	5	50.000	196	158	158	0	

Comments:

48 Hour Toxicity Test Data Sheet - Northwest Bioassay Laboratory

Saltwater Chronic

Client: Shannon + wilson
 Sample ID: Carnival spirit
 Contact:
 Test No.: 0309-05NW

Start Date & Time: 9/13/03 1340
 End Date & Time: 9/15/03 1340
 Test Organisms: M. galloprovincialis
 Test Protocol:
 Date Received: 9/12/03

Concentration or Percent	Dissolved Oxygen (mg/L)			pH (units)			Salinity (ppt)			Temperature (°C)		
C	8.3	10.0	8.6	7.87	7.90	7.85	28.0	28.0	28.0	14.7	14.0	14.6
Brine C	8.3	8.9	8.7	7.96	7.91	7.87	28.2	28.3	28.4	14.8	14.0	13.9
1.5	8.3	8.7	8.7	7.90	7.91	7.85	28.3	28.3	28.1	14.4	14.0	14.2
3.125	8.2	8.8	8.5	7.87	7.91	7.90	28.0	28.5	29.0	14.7	14.6	13.8
6.25	8.3	8.8	8.7	7.90	7.91	7.90	28.3	28.7	29.2	14.7	14.0	14.2
12.5	8.4	8.6	8.6	7.88	7.90	7.89	28.0	29.0	29.1	14.9	14.2	14.2
25	8.2	8.1	8.5	7.86	7.87	7.89	28.3	28.3	29.2	14.2	14.1	13.9
50	8.6	7.6	8.4	7.71	7.83	7.87	28.4	29.3	29.7	15.2	14.1	13.9
Conc.	Alkalinity* *(mg/L as CaCO ₃)		Chlorine Resid. (mg/L)			Sample Description:						
Control												
Highest conc.												

Comments: 0 hrs: _____
 24 hrs: _____
 48 hrs: _____

Reviewed: VB

QA check: KJ

AMEC Earth & Environmental
 Northwest Bioassay Laboratory
 5009 Pacific Hwy. E. Suite 2-0
 Fife, WA 98424
 (253) 922-4296

Bivalve Development Bioassay Worksheet

Client: Shannon + Wilson
Test No.: 0309-05NW / RT 09303 MS
Test Species: *U. gulfus provincialis*

Start Date/Time:
End Date/Time:
Date Received:

9/4/03 9/13/03 1340
9/15/03 1340
9/12/03

Sample Type: cruise ship

Test Chamber Type and Sample Volume: 30 ml vial

Spawn Initiation Time: 1000

Number of Spawners: 3 Male 3 Female

Spawn Condition: good

Fertilization Time: 1140

Egg Stock Density Calculation:

Eggs Counted (x):	<u>6</u>	<u>6</u>	
	<u>5</u>	<u>7</u>	
	<u>4</u>	<u>2</u>	
	<u>3</u>	<u>4</u>	
	<u>7</u>	<u>6</u>	
Mean	<u>5</u>	<u>5</u>	Overall Mean <u>5</u>

$$\text{Mean } \underline{5} \times 42 = \underline{210} \text{ eggs/ml (initial stock)}$$

$$\begin{array}{l} \text{Initial Stock } \underline{210} \text{ eggs/ml} \\ \text{Innoculum Stock } \underline{200} \text{ eggs/ml} \end{array} = \text{Stock Dilution Factor } \underline{1.05}$$

Percent division upon inoculation: 9.6%

Time of inoculation: 1340

Comments: _____

Reviewed/ Date: KJS

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424
(253) 922-4296

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424

Marine Dilution Worksheet

Client: Shannon + wilson
Sample ID: Carnival Spirit
Test No.: 0309-05
Brine salinity: 98

Analyst: mm
Test Date: 9/13/03
Test Type: Bivalve development

$$\text{Equation for Salinity Adjustment: } VB = VE \quad \frac{30 - SE}{SB - 30}$$

$$\text{Effluent Salinity Adjustment: } \frac{30 - 0}{98 - 30} = \frac{30}{68} = .44$$

$$\text{Brine Control Salinity Adjustment: } \frac{30 - 0}{98 - 30} = \frac{30}{68} = .44$$

Concentration	Effluent Volume (mL)	Salinity Adjustment	Brine Volume (mL)	Seawater Volume (mL)	Total Volume (mL)
Control	NA	NA	NA	-	250 mL
1.5	3.75	.44	1.65	-	
3.125	7.81	.44	3.44	-	
6.25	15.63	.44	6.88	-	
12.5	31.25	.44	13.75	-	
25	62.5	.44	27.5	-	
50	125	.44	55	-	

DI Volume					
Brine Control	125	.44	55	-	250 mL

Calculations for all concentrations except highest:

1. Effluent Volume = Total Volume X Concentration (%)
2. Brine Volume = Effluent Volume X Salinity Adjustment
3. Seawater Volume = Total Volume - Effluent Volume + Brine Volume

Calculation for highest concentration:

1. Effluent Volume = Total Volume / (1.0 + Salinity Adjustment)
2. Brine Volume = Total Volume - Effluent Volume
3. Concentration = Effluent Volume / Total Volume

Calcuation for brine control:

1. Brine Volume = Brine Volume for highest concentration
2. DI Volume = Brine Volume / Brine Control Salinity Adjustment
3. Seawater Volume = Total Volume - DI Volume + Brine Volume

Reviewed/ Date: 10/10/03

Echinoderm Sperm Cell Fertilization

Sperm Cell Fertilization Test-Proportion Fertilized

Start Date: 6/10/03 Test ID: 0306-28NW Sample ID: Spirit of Columbia
 End Date: 6/10/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW-Black Water
 Sample Date: 6/9/03 Protocol: DL 87-Dinnel/Link Test Species: SP-Strongylocentrotus purpuratus

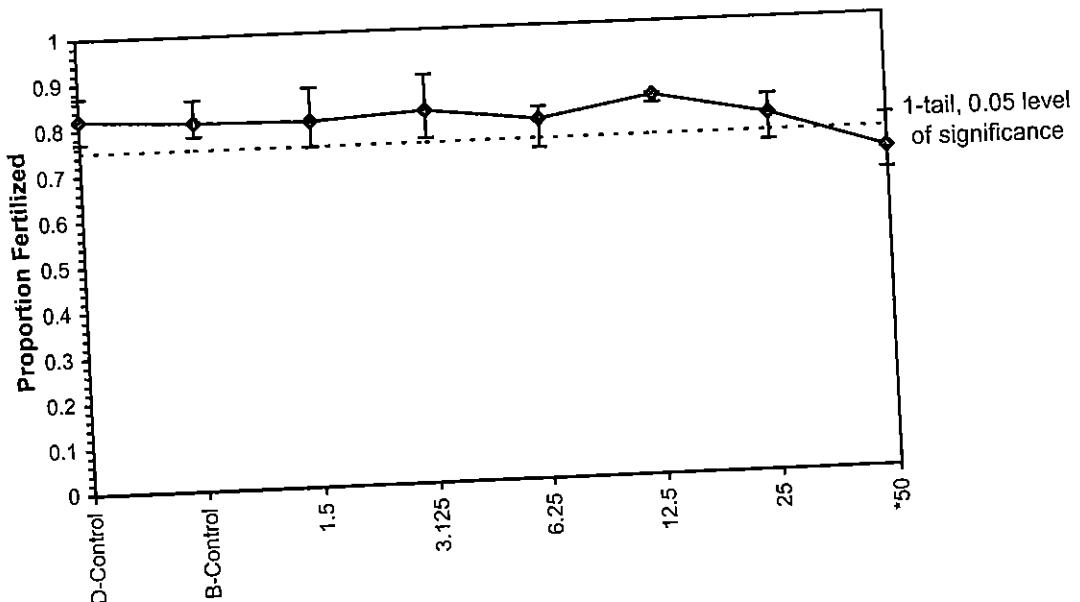
Comments:

Conc-%	1	2	3	4	5
D-Control	0.8500	0.8700	0.8100	0.7700	0.8000
B-Control	0.8200	0.7900	0.8000	0.7800	0.8600
1.5	0.8500	0.7500	0.7500	0.8000	0.8800
3.125	0.8300	0.8300	0.9000	0.7600	0.7800
6.25	0.7300	0.8100	0.8200	0.8000	0.8000
12.5	0.8400	0.8400	0.8400	0.8400	0.8200
25	0.7600	0.7300	0.8300	0.8000	0.8200
50	0.6600	0.7800	0.6800	0.7300	0.6800

Conc-%	Transform: Arcsin Square Root						1-Tailed			
	Mean	N-Mean	Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.8200	1.0123	1.1345	1.0706	1.2019	4.640	5			
B-Control	0.8100	1.0000	1.1209	1.0826	1.1873	3.702	5	0.461	2.409	0.0845
1.5	0.8060	0.9951	1.1183	1.0472	1.2171	6.779	5	-0.054	2.409	0.0845
3.125	0.8200	1.0123	1.1364	1.0588	1.2490	6.493	5	1.035	2.409	0.0845
6.25	0.7920	0.9778	1.0982	1.0244	1.1326	3.879	5	-0.554	2.409	0.0845
12.5	0.8360	1.0321	1.1540	1.1326	1.1593	1.032	5	1.162	2.409	0.0845
25	0.7880	0.9728	1.0938	1.0244	1.1458	4.669	5	3.869	2.409	0.0845
*50	0.7060	0.8716	0.9989	0.9483	1.0826	5.470	5			

Auxiliary Tests	Statistic	Critical	Skew	Kurt	
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.95772	0.91	0.28942	-0.4115	
Bartlett's Test indicates equal variances ($p = 0.13$)	9.86603	16.8119			
The control means are not significantly different ($p = 0.66$)	0.45449	2.30601			
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	
Dunnett's Test	25	50	35.3553	4	0.06895 0.08394 0.01322 0.00307 0.00339 6, 28

Dose-Response Plot



Test: SC-Sperm Cell Fertilization Test
 Species: SP-Strongylocentrotus purpuratus
 Sample ID: Spirit of Columbia #1 Blackwater
 Start Date: 06/10/2003 End Date: 06/10/2003

Test ID: 0306-28NW
 Protocol: DL 87-Dinnel/Link
 Sample Type: EFF2-Industrial
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
1				100	80	
2				100	78	
3				100	80	
4				100	87	
5				100	76	
6				100	80	
7				100	73	
8				100	77	
9				100	81	
10				100	82	
11				100	75	
12				100	83	
13				100	84	
14				100	68	
15				100	80	
16				100	81	
17				100	82	
18				100	84	
19				100	78	
20				100	75	
21				100	73	
22				100	82	
23				100	90	
24				100	80	
25				100	66	
26				100	73	
27				100	88	
28				100	84	
29				100	68	
30				100	82	
31				100	85	
32				100	84	
33				100	80	
34				100	79	
35				100	83	
36				100	83	
37				100	78	
38				100	85	
39				100	86	
40				100	76	

Comments:

Reviewed by: VH

Test: SC-Sperm Cell Fertilization Test
 Species: SP-Strongylocentrotus purpuratus
 Sample ID: Spirit of Columbia
 Start Date: 6/10/03 End Date: 6/10/03

Test ID: 0306-28NW
 Protocol: DL 87-Dinnel/Link
 Sample Type: BW-Black Water
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
31	1	1	D-Control	100	85	
4	2	2	D-Control	100	87	
9	3	3	D-Control	100	81	
8	4	4	D-Control	100	77	
3	5	5	D-Control	100	80	
17	6	1	B-Control	100	82	
34	7	2	B-Control	100	79	
6	8	3	B-Control	100	80	
19	9	4	B-Control	100	78	
39	10	5	B-Control	100	86	
38	11	1	1.500	100	85	
20	12	2	1.500	100	75	
11	13	3	1.500	100	75	
24	14	4	1.500	100	80	
27	15	5	1.500	100	88	
35	16	1	3.125	100	83	
36	17	2	3.125	100	83	
23	18	3	3.125	100	90	
5	19	4	3.125	100	76	
37	20	5	3.125	100	78	
26	21	1	6.250	100	73	
16	22	2	6.250	100	81	
22	23	3	6.250	100	82	
15	24	4	6.250	100	80	
1	25	5	6.250	100	80	
28	26	1	12.500	100	84	
32	27	2	12.500	100	84	
13	28	3	12.500	100	84	
18	29	4	12.500	100	82	
30	30	5	12.500	100	82	
40	31	1	25.000	100	76	
21	32	2	25.000	100	73	
12	33	3	25.000	100	83	
33	34	4	25.000	100	80	
10	35	5	25.000	100	82	
25	36	1	50.000	100	66	
2	37	2	50.000	100	78	
14	38	3	50.000	100	68	
7	39	4	50.000	100	73	
29	40	5	50.000	100	68	

Comments:

AMEC Earth & Environmental
Northwest Bioassay Laboratory
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424

Physical and Chemical
Measurements of Test Solutions
Salt Water Bioassays

Client: Shannon + Wilson
Sample ID: SOC #1 BW
Test No: 0306-26NW

Analyst: NF
Test Date: 6/10/03
Test Type: Echinoderm Spermcell
Test Species: S. purpuratus

Concentration	Initial Readings			
	D.O. (mg/l)	pH	Salinity (ppt)	Temp (°C)
Con	8.0	8.02	29.2	13.0
brine con	7.7	7.88	29.0	13.0
1.5	7.9	7.96	29.0	13.0
3.125	8.0	7.97	29.3	13.0
6.25	8.0	7.98	29.1	13.0
12.5	7.9	7.98	29.2	13.0
25	8.3	7.97	29.8	13.0
50	8.4	7.96	30.3	13.0

Sample Description: _____

Echinoderm Source:

AMEC San Diego
Mission Bay, CA

Date Received:

6/10/03

Comments: _____

Reviewed: ml 6/10/03

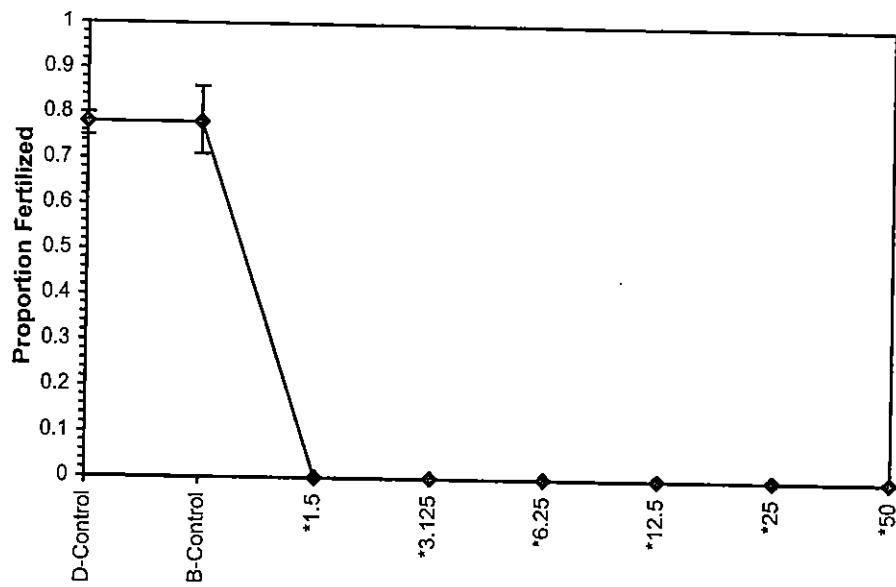
Start Date: 6/10/03 Test ID: 0306-29NW Sample ID: Spirit of Columbia
 End Date: 6/10/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: GW-Gray Water
 Sample Date: 6/9/03 Protocol: DL 87-Dinnel/Link Test Species: SP-Strongylocentrotus purpuratus
 Comments:

Conc-%	1	2	3	4	5
D-Control	0.8000	0.7800	0.7800	0.7500	0.7900
B-Control	0.7400	0.8600	0.7800	0.7100	0.8100
*1.5	0.0000	0.0000	0.0000	0.0000	0.0000
*3.125	0.0000	0.0000	0.0000	0.0000	0.0000
*6.25	0.0000	0.0000	0.0000	0.0000	0.0000
*12.5	0.0000	0.0000	0.0000	0.0000	0.0000
*25	0.0000	0.0000	0.0000	0.0000	0.0000
*50	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root				Rank Sum	1-Tailed Critical
			Mean	Min	Max	CV%		
D-Control	0.7800	1.0000	1.0829	1.0472	1.1071	2.067	5	
B-Control	0.7800	1.0000	1.0855	1.0021	1.1873	6.671	5	
*1.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00 16.00
*3.125	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00 16.00
*6.25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00 16.00
*12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00 16.00
*25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00 16.00
*50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00 16.00

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ($p <= 0.01$)	0.39327	0.91	-2.0225	16.7802
Equality of variance cannot be confirmed				
The control means are not significantly different ($p = 0.94$)	0.07798	2.30601		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

Dose-Response Plot



Test: SC-Sperm Cell Fertilization Test	Test ID: 0306-29NW					
Species: SP-Strongylocentrotus purpuratus	Protocol: DL 87-Dinnel/Link					
Sample ID: Spirit of Columbia #2 Gray water	Sample Type: EFF2-Industrial					
Start Date: 06/10/2003	End Date: 06/10/2003					
	Lab ID: WAAEE-AMEC NW Bioassay					
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
41				100	0	
42				100	0	
43				100	83	
44				100	81	
45				100	0	
46				100	0	
47				100	0	
48				100	0	
49				100	0	
50				100	71	
51				100	80	
52				100	0	
53				100	75	
54				100	0	
55				100	0	
56				100	0	
57				100	74	
58				100	0	
59				100	0	
60				100	0	
61				100	0	
62				100	78	
63				100	0	
64				100	0	
65				100	0	
66				100	0	
67				100	0	
68				100	78	
69				100	0	
70				100	78	
71				100	0	
72				100	79	
73				100	0	
74				100	0	
75				100	0	
76				100	0	
77				100	0	
78				100	0	
79				100	0	
80				100	0	

Comments:

Test: SC-Sperm Cell Fertilization Test
 Species: SP-Strongylocentrotus purpuratus
 Sample ID: Spirit of Columbia
 Start Date: 6/10/03 End Date: 6/10/03

Test ID: 0306-29NW
 Protocol: DL 87-Dinnel/Link
 Sample Type: GW-Gray Water
 Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
51	1	1	D-Control	100	80	
70	2	2	D-Control	100	78	
68	3	3	D-Control	100	78	
53	4	4	D-Control	100	75	
72	5	5	D-Control	100	79	
57	6	1	B-Control	100	74	
43	7	2	B-Control	100	86	
62	8	3	B-Control	100	78	
50	9	4	B-Control	100	71	
44	10	5	B-Control	100	81	
47	11	1	1.500	100	0	
61	12	2	1.500	100	0	
54	13	3	1.500	100	0	
66	14	4	1.500	100	0	
77	15	5	1.500	100	0	
74	16	1	3.125	100	0	
63	17	2	3.125	100	0	
78	18	3	3.125	100	0	
49	19	4	3.125	100	0	
48	20	5	3.125	100	0	
58	21	1	6.250	100	0	
65	22	2	6.250	100	0	
80	23	3	6.250	100	0	
60	24	4	6.250	100	0	
46	25	5	6.250	100	0	
41	26	1	12.500	100	0	
76	27	2	12.500	100	0	
79	28	3	12.500	100	0	
73	29	4	12.500	100	0	
45	30	5	12.500	100	0	
59	31	1	25.000	100	0	
69	32	2	25.000	100	0	
75	33	3	25.000	100	0	
42	34	4	25.000	100	0	
55	35	5	25.000	100	0	
67	36	1	50.000	100	0	
71	37	2	50.000	100	0	
56	38	3	50.000	100	0	
64	39	4	50.000	100	0	
52	40	5	50.000	100	0	

Comments:

AMEC Earth & Environmental
Northwest Bioassay Laboratory
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424

Physical and Chemical
Measurements of Test Solutions
Salt Water Bioassays

Client: Shannon + Wilson
Sample ID: SOC#2 Gray water
Test No: 0306-27NW

Analyst: NF
Test Date: 6/10/03
Test Type: Echinoderm sperm cell
Test Species: S. purpuratus

Concentration (ppm)	Initial Readings				
	TDS	pH	Salinity	Temp	DO
CON	8.0	8.02	29.2	13.0	
Brine CON	7.7	7.88	29.0	13.0	
1.5	7.9	7.93	29.0	13.0	
3.125	8.1	7.92	29.0	13.0	
6.25	7.9	7.91	29.0	13.0	
12.5	7.9	7.84	29.0	13.0	
25	7.7	7.74	29.2	13.0	
50	7.4	7.58	30.5	13.0	

Sample Description: _____

Echinoderm Source: AMEC San Diego
Mission Bay, CA Date Received: 6/10/03

Comments: _____

Reviewed: MW 6/10/03

Echinoderm Egg Fertilization Bioassay Worksheet

Client: Spirit of Columbia
 Test No.: 0306-29NW, 0306-28NW
 Test Species: *S. purpuratus*

Start Date/Time: 6/16/03
 End Date/Time: 6/16/03
 Date Collected: 6/9/03

Injection Time: 1230

Eggs Counted (x): 68 Mean: 61 $\times 42 =$ 2562 eggs/ml
64
57
60
62 2562 eggs/ml = Egg Dilution Factor
2000 eggs/ml 1.28

Sperm Counted (y) or Absorbance at 400nm: 8

2000:1 Sperm Stock = $\frac{y}{4.0 \times 10^6}$ (0.1) = 0 Sperm Dilution Factor

Rangefinder Test:	Sperm to Egg Ratio						
	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1
mLs Sperm Stock	50	40	30	20	10	5	2.5
mLs Seawater Diluent	0	10	20	30	40	45	47.5

Sperm Added:	Time	Rangefinder Ratio:	Fert.	Unfert.	Percent Fert.
			100	0	100%
Eggs Added:	<u>1550</u>	<u>200:1</u>	<u>99</u>	<u>1</u>	<u>99%</u>
Test Ended:	<u>1400</u>	<u>400:1</u>	<u>100</u>	<u>0</u>	<u>100%</u>
		<u>800:1</u>	<u>100</u>	<u>0</u>	<u>100%</u>

Definitive Test	Time	Sperm:Egg Ratio Used:		
		Fert.	Unfert.	Percent Fert.
Sperm Added:	<u>1410</u>	<u>80%</u>	<u>20%</u>	<u>80%</u>
Eggs Added:	<u>1430</u>	<u>78%</u>	<u>22%</u>	<u>78%</u>
Test Ended:	<u>1450</u>	<u>0</u>	<u>100</u>	<u>0%</u>
		<u>0</u>	<u>100</u>	<u>0%</u>

Comments:

AMEC Earth & Environmental
 Northwest Bioassay Lab
 5009 Pacific Hwy. E., Suite 2
 Fife, WA 98424
 (253) 922-4296

Reviewed/ Date: KB

Sperm Cell Fertilization Test-Proportion Fertilized					
Start Date:	6/13/03	Test ID:	0306-33NW	Sample ID:	Spirit of Oceanus
End Date:	6/13/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water
Sample Date:	6/12/03	Protocol:	DL 87-Dinnel/Link	Test Species:	SP-Strongylocentrotus purpuratus
Comments:					
Conc-%	1	2	3	4	5
D-Control	0.9900	0.9800	0.9900	0.9500	0.9700
B-Control	1.0000	0.9100	0.9200	0.9100	0.9100
1.5	0.8200	0.7100	0.7200	0.8800	0.8200
3.125	0.8100	0.7000	0.8200	0.8700	0.8500
6.25	0.2900	0.0900	0.2800	0.2200	0.2900
12.5	0.0200	0.0100	0.0000	0.0300	0.0200
25	0.0000	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0100	0.0000	0.0000	0.0000

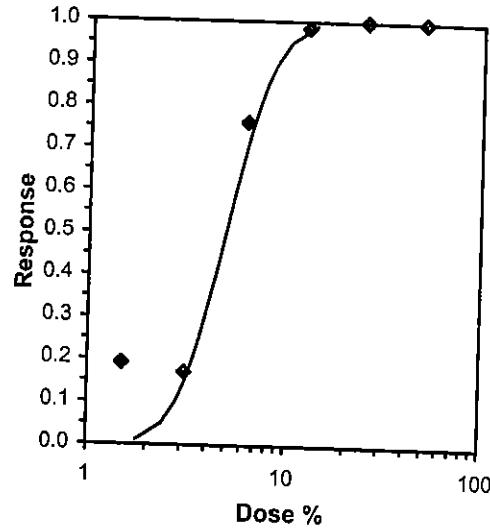
Conc-%	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%				
D-Control	0.9760	1.0495	1.4224	1.3453	1.4706	3.737	5			
B-Control	0.9300	1.0000	1.3206	1.2661	1.5208	8.493	5		12	500
*1.5	0.7900	0.8495	1.0995	1.0021	1.2171	8.254	5	15.00	16.00	105 500
*3.125	0.8100	0.8710	1.1237	0.9912	1.2019	7.205	5	15.00	16.00	95 500
*6.25	0.2340	0.2516	0.4976	0.3047	0.5687	22.695	5	15.00	16.00	383 500
*12.5	0.0160	0.0172	0.1216	0.0500	0.1741	39.352	5	15.00	16.00	492 500
*25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00	16.00	500 500
*50	0.0020	0.0022	0.0601	0.0500	0.1002	37.346	5	15.00	16.00	499 500

Auxiliary Tests

		Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)		0.92576	0.91		
Equality of variance cannot be confirmed			-1.0561	1.84042	
The control means are not significantly different (p = 0.10)					
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU	
Steel's Many-One Rank Test	<1.5	1.5			

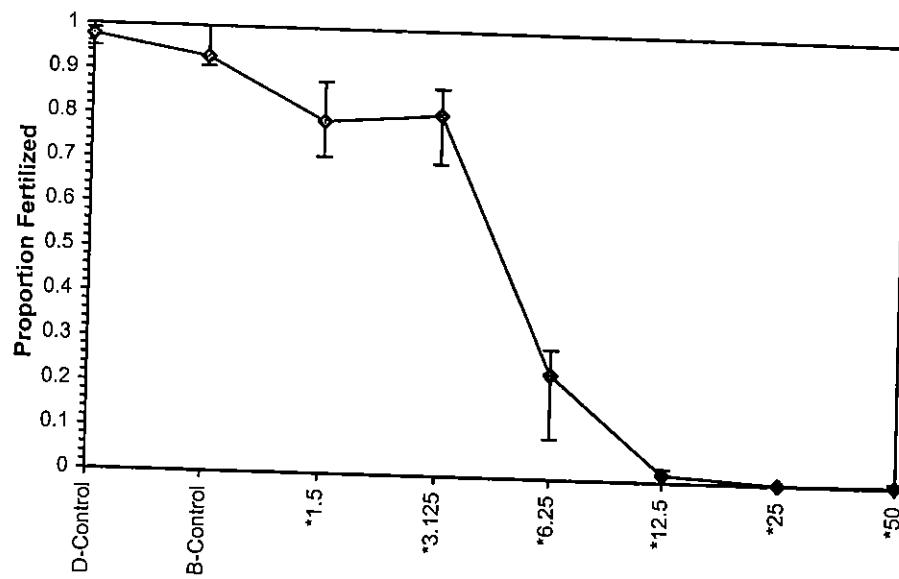
Parameter	Value	SE	Maximum Likelihood-Probit			Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			95% Fiducial Limits	Control	Chi-Sq							
Slope	5.27274	26.4864	-68.265 78.811			0.024	35708.8	9.48773	0.0E+00	0.69646	0.18965	13
Intercept	1.32778	20.4336	-55.405 58.0607									
TSCR	0.10653	0.94508	-2.5174 2.7305									
Point	Probits	%	95% Fiducial Limits									
EC01	2.674	1.79991										
EC05	3.355	2.42382										
EC10	3.718	2.84055										
EC15	3.964	3.16148										
EC20	4.158	3.44221										
EC25	4.326	3.70284										
EC40	4.747	4.45048										
EC50	5.000	4.97113										
EC60	5.253	5.55269										
EC75	5.674	6.67383										
EC80	5.842	7.17914										
EC85	6.036	7.81663										
EC90	6.282	8.69976										
EC95	6.645	10.1955										
EC99	7.326	13.7296										

Significant heterogeneity detected (p = 0.00E+00)



Start Date: 6/13/03 Test ID: 0306-33NW Sample ID: Spirit of Oceanus
 End Date: 6/13/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 6/12/03 Protocol: DL 87-Dinnel/Link Test Species: SP-Strongylocentrotus purpuratus
 Comments:

Dose-Response Plot



Test: SC-Sperm Cell Fertilization Test Species: SP-Strongylocentrotus purpuratus Sample ID: Spirit of Oceanus Start Date: 06/13/2003 End Date: 06/13/2003					Test ID: 0306-33NW Protocol: DL 87-Dinnel/Link Sample Type: OTH-Other sample type Lab ID: WAAEE-AMEC NW Bioassay	
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
1				100	70	NF 7/28/03
2				100	2	
3				100	91	
4				100	97	
5				100	85	
6				100	82	
7				100	71	
8				100	0	
9				100	98	
10				100	0	
11				100+100 NF	91	
12				100	72	
13				100	45	
14				100	82	
15				100	88	
16				100	0	
17				100	1	
18				100	92	
19				100	99	
20				100	81	
21				100	91	
22				100	0	
23				100	82	
24				100	0	
25				100	AT 29	
26				100	28	
27				100	3	
28				100	9	
29				100	0	
30				100	100	
31				100	1	
32				100	99	
33				100	29	
34				100	0	
35				100	0	
36				100	0	
37				100	0	
38				100	87	
39				100	22	
40				100	0	

Comments:

Test: SC-Sperm Cell Fertilization Test						Test ID: 0306-33NW
Species: SP-Strongylocentrotus purpuratus						Protocol: DL 87-Dinnel/Link
Sample ID: Spirit of Oceanus						Sample Type: BW/GW-Combined gray & black water
Start Date: 6/13/03 End Date: 6/13/03						Lab ID: WAAEE-AMEC NW Bioassay
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
33	1	1	D-Control	100	99	
9	2	2	D-Control	100	98	
19	3	3	D-Control	100	99	
13	4	4	D-Control	100	95	
4	5	5	D-Control	100	97	
30	6	1	B-Control	100	100	
21	7	2	B-Control	100	91	
18	8	3	B-Control	100	92	
11	9	4	B-Control	100	91	
3	10	5	B-Control	100	91	
14	11	1	1.500	100	82	
7	12	2	1.500	100	71	
12	13	3	1.500	100	72	
15	14	4	1.500	100	88	
6	15	5	1.500	100	82	
20	16	1	3.125	100	81	
1	17	2	3.125	100	70	
23	18	3	3.125	100	82	
38	19	4	3.125	100	87	
5	20	5	3.125	100	85	
34	21	1	6.250	100	29	
28	22	2	6.250	100	9	
26	23	3	6.250	100	28	
39	24	4	6.250	100	22	
25	25	5	6.250	100	29	
2	26	1	12.500	100	2	
17	27	2	12.500	100	1	
29	28	3	12.500	100	0	
27	29	4	12.500	100	3	
32	30	5	12.500	100	2	
24	31	1	25.000	100	0	
22	32	2	25.000	100	0	
36	33	3	25.000	100	0	
35	34	4	25.000	100	0	
37	35	5	25.000	100	0	
16	36	1	50.000	100	0	
31	37	2	50.000	100	1	
10	38	3	50.000	100	0	
40	39	4	50.000	100	0	
8	40	5	50.000	100	0	

Comments:

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424

Marine Dilution Worksheet

Client: Shannontwilson (spirit of Oceanus)
Sample ID: Spirit of Oceanus
Test No.: 0306 - 32NW, 0306 - 33NW

Analyst: nic
Test Date: 6/13/03
Test Type: Bivalve, sperm cell fertilization

Brine salinity: 80 ppt

Equation for Salinity Adjustment: $VB = VE \frac{30 - SE}{SB - 30}$

Effluent Salinity Adjustment: $\frac{30 - 0}{80 - 30} = \frac{30}{50} = .6$

Brine Control Salinity Adjustment: $\frac{30 - 0}{80 - 30} = \frac{30}{50} = .6$

Concentration	Effluent Volume (mL)	Salinity Adjustment	Brine Volume (mL)	Seawater Volume (mL)	Total Volume (mL)
Control	NA	NA	NA		250ml
1.5	3.75	.6	2.25		
3.125	7.81	.6	4.7		
6.25	15.6	.6	9.36		
12.5	31.25	.6	18.75		
25	62.5	.6	37.5		
50	125	.6	75		

DI Volume					
Brine Control	125	.6	75		250ml

Calculations for all concentrations except highest:

1. Effluent Volume = Total Volume X Concentration (%)
2. Brine Volume = Effluent Volume X Salinity Adjustment
3. Seawater Volume = Total Volume - Effluent Volume + Brine Volume

Calculation for highest concentration:

1. Effluent Volume = Total Volume / (1.0 + Salinity Adjustment)
2. Brine Volume = Total Volume - Effluent Volume
3. Concentration = Effluent Volume / Total Volume

Calculation for brine control:

1. Brine Volume = Brine Volume for highest concentration
2. DI Volume = Brine Volume / Brine Control Salinity Adjustment
3. Seawater Volume = Total Volume - DI Volume + Brine Volume

Reviewed/ Date: 14

Echinoderm Egg Fertilization Bioassay Worksheet

Client: Shannon + wilson
 Test No.: _____
 Test Species: _____

Start Date/Time: 6/13/03
 End Date/Time: _____
 Date Collected: 6/12/03

Injection Time: 1330

Eggs Counted (x): 49 Mean: _____ $\times 42 =$ 1999 eggs/ml
55
43
49
42 2000 eggs/ml = 1
2000 eggs/ml

Sperm Counted (y) or Absorbance at 400nm: 1,02

2000:1 Sperm Stock = y / 4.0 x 10⁶ (0.1) = 0.2 Sperm Dilution Factor

Sperm to Egg Ratio						
<u>Rangefinder Test:</u>	<u>2000:1</u>	<u>1600:1</u>	<u>1200:1</u>	<u>800:1</u>	<u>400:1</u>	<u>200:1</u>
mLs Sperm Stock	50	40	30	20	10	5
mLs Seawater Diluent	0	10	20	30	40	45
						100:1
						2.5
						47.5

Sperm Added:	Time	Rangefinder Ratio:	Fert.	Unfert.	Percent Fert.
	<u>1410</u>	<u>600:1</u>	<u>96%</u>	<u>4</u>	<u>96%</u>
Eggs Added:	<u>1420</u>	<u>1200:1</u>	<u>99</u>	<u>1</u>	<u>99%</u>
Test Ended:	<u>1430</u>				

Definitive Test	Time	Sperm:Egg Ratio Used: <u>1200 : 1</u>		
Sperm Added:	<u>1453</u>	Fert	Unfert.	Percent Fert.
Eggs Added:	<u>1513</u>	<u>98</u>	<u>2</u>	<u>98%</u>
Test Ended:	<u>1533</u>	QC1 QC2 Egg Control 1 Egg Control 2	<u>4</u> <u>1</u> <u>99</u> <u>1</u>	<u>96%</u> <u>1%</u> <u>1%</u> <u>1%</u>

Comments: _____

Reviewed/ Date: VS

AMEC Earth & Environmental
 Northwest Bioassay Lab
 5009 Pacific Hwy. E., Suite 2
 Fife, WA 98424
 (253) 922-4296

AMEC Earth & Environmental
Northwest Bioassay Laboratory
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424

Physical and Chemical
Measurements of Test Solutions
Salt Water Bioassays

Client: Shannon + Wilson
Sample ID: Spirit of Oceanus
Test No: 0306 - 33 NW

Analyst: WV
Test Date: 6/13/03
Test Type: Sperm cell
Test Species: S. purpuratus

Concentration	Initial Readings				Notes
	D.O. (mg/l)	pH	Salinity (PSU)	Temp (°C)	
C	7.7	7.83	28.4	15.1	11.8
Brine C	7.6	28.89	28.7	15.2	11.8
1.5	7.9	7.84	29.0	15.3	11.8
3.125	7.7	7.84	28.9	15.4	11.8
6.25	8.0	7.82	28.8	15.4	11.8
12.5	7.7	7.79	29.3	15.2	11.8
25	7.8	7.76	28.5	15.0	11.8
50	7.9	7.69	30.1	15.5	11.8

Sample Description: Gray water (cruise ship)

Echinoderm Source: AMEC Sandiego Date Received: 6/13/03

Comments: _____

Reviewed: WS

Sperm Cell Fertilization Test-Proportion Fertilized

Start Date: 6/17/03 Test ID: 0306-38NW Sample ID: Sun Princess
 End Date: 6/17/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 6/16/03 Protocol: DL 87-Dinnel/Link Test Species: SP-Strongylocentrotus purpuratus

Comments:

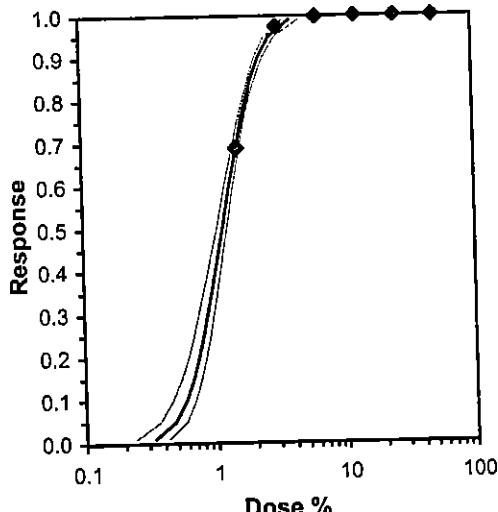
Conc-%	1	2	3	4	5
D-Control	1.0000	1.0000	1.0000	1.0000	1.0000
B-Control	0.9800	0.9100	0.8500	0.9000	0.9600
*1.5	0.3900	0.2600	0.3400	0.1900	0.3700
*3.125	0.0800	0.0100	0.0300	0.0100	0.0000
*6.25	0.0000	0.0000	0.0000	0.0100	0.0000
*12.5	0.0000	0.0000	0.0000	0.0000	0.0000
*25	0.0000	0.0000	0.0000	0.0000	0.0000
*50	0.0000	0.0000	0.0000	0.0000	0.0000

Conc-%	Transform: Arcsin Square Root						Rank Sum	1-Tailed Critical	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%				
D-Control	1.0000	1.0870	1.5208	1.5208	1.5208	0.000	5		0	500
B-Control	0.9200	1.0000	1.2973	1.1731	1.4289	7.828	5			
*1.5	0.3100	0.3370	0.5874	0.4510	0.6745	15.828	5	15.00	16.00	345 500
*3.125	0.0260	0.0283	0.1422	0.0500	0.2868	64.763	5	15.00	16.00	487 500
*6.25	0.0020	0.0022	0.0601	0.0500	0.1002	37.346	5	15.00	16.00	499 500
*12.5	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00	16.00	500 500
*25	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00	16.00	500 500
*50	0.0000	0.0000	0.0500	0.0500	0.0500	0.000	5	15.00	16.00	500 500

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)	0.80112	0.91	0.16378	4.4666
Equality of variance cannot be confirmed				
The control means are significantly different (p = 1.16E-03)	4.92037	2.30601		

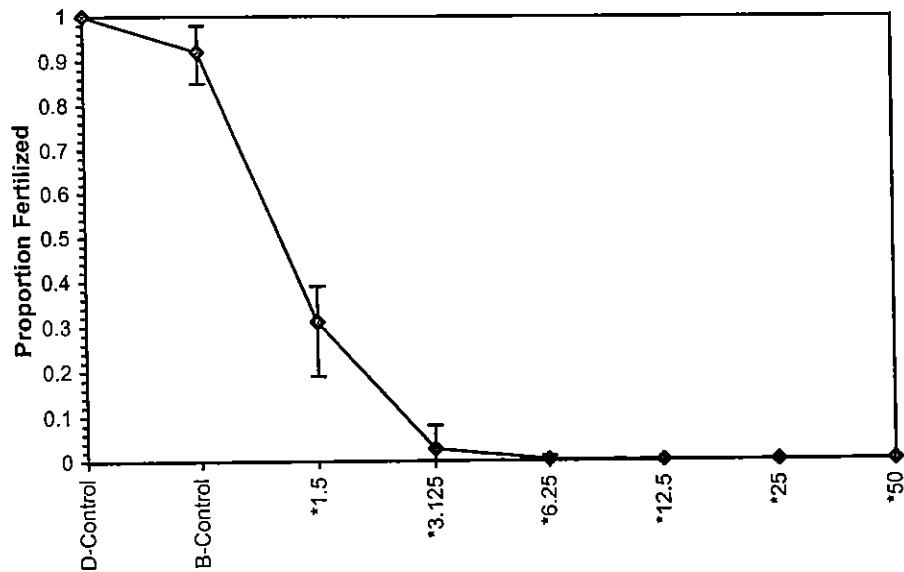
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<1.5	1.5		

Parameter	Value	SE	Maximum Likelihood-Probit		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
			95% Fiducial Limits								
Slope	4.33651	0.36235	3.6263	5.04672	0	1.42673	9.48773	0.84	0.06009	0.2306	3
Intercept	4.73944	0.10454	4.53454	4.94433							
TSCR											
Point	Probits	%	95% Fiducial Limits								
EC01	2.674	0.33391	0.23931	0.42484							
EC05	3.355	0.47949	0.36837	0.58059							
EC10	3.718	0.58151	0.46343	0.68602							
EC15	3.964	0.66235	0.54096	0.76794							
EC20	4.158	0.73453	0.6116	0.84013							
EC25	4.326	0.80269	0.67939	0.90761							
EC40	4.747	1.00384	0.88441	1.10395							
EC50	5.000	1.14838	1.03502	1.24371							
EC60	5.253	1.31374	1.20881	1.40402							
EC75	5.674	1.64295	1.54963	1.73416							
EC80	5.842	1.79541	1.70038	1.8966							
EC85	6.036	1.99108	1.88518	2.11588							
EC90	6.282	2.26784	2.13276	2.44381							
EC95	6.645	2.75036	2.54073	3.04946							
EC99	7.326	3.94951	3.49322	4.66579							



Sperm Cell Fertilization Test-Proportion Fertilized

Start Date:	6/17/03	Test ID:	0306-38NW	Sample ID:	Sun Princess
End Date:	6/17/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water
Sample Date:	6/16/03	Protocol:	DL 87-Dinnel/Link	Test Species:	SP-Strongylocentrotus purpuratus
Comments:					

Dose-Response Plot

Test: SC-Sperm Cell Fertilization Test Species: SP-Strongylocentrotus purpuratus Sample ID: Sun Princess Start Date: 06/17/2003 End Date: 06/17/2003					Test ID: 0306-38NW Protocol: DL 87-Dinnel/Link Sample Type: EFF2-Industrial Lab ID: WAAEE-AMEC NW Bioassay	
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
1				100	1	NF 7/28/03
2				100	0	
3				100	26	
4				100	3	
5				100	0	
6				100	8	
7				100	8	
8				100	0	↓
9				100	0	SM 7/28/03
10				100	0	
11				100	0	
12				100	96	
13				100	0	
14				100	37	
15				100	100	
16				100	0	
17				100	0	
18				100	98	
19				100	91	
20				100	1	
21				100	100	
22				100	0	
23				100	0	
24				100	90	
25				100	0	
26				100	0	
27				100	8	
28				100	0	
29				100	1	
30				100	39	
31				100	0	
32				100	85	
33				100	34	
34				100	100	
35				100	100	
36				100	0	
37				100	0	
38				100	19	
39				100	100	↓
40				100	0	

Comments:

Test: SC-Sperm Cell Fertilization Test Species: SP-Strongylocentrotus purpuratus Sample ID: Sun Princess Start Date: 6/17/03 End Date: 6/17/03					Test ID: 0306-38NW Protocol: DL 87-Dinnel/Link Sample Type: BW/GW-Combined gray & black water Lab ID: WAAEE-AMEC NW Bioassay	
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
21	1	1	D-Control	100	100	
35	2	2	D-Control	100	100	
39	3	3	D-Control	100	100	
34	4	4	D-Control	100	100	
15	5	5	D-Control	100	100	
18	6	1	B-Control	100	98	
19	7	2	B-Control	100	91	
32	8	3	B-Control	100	85	
24	9	4	B-Control	100	90	
12	10	5	B-Control	100	96	
30	11	1	1.500	100	39	
3	12	2	1.500	100	26	
33	13	3	1.500	100	34	
38	14	4	1.500	100	19	
14	15	5	1.500	100	37	
27	16	1	3.125	100	8	
29	17	2	3.125	100	1	
4	18	3	3.125	100	3	
20	19	4	3.125	100	1	
9	20	5	3.125	100	0	
23	21	1	6.250	100	0	
6	22	2	6.250	100	0	
17	23	3	6.250	100	0	
1	24	4	6.250	100	1	
31	25	5	6.250	100	0	
37	26	1	12.500	100	0	
28	27	2	12.500	100	0	
2	28	3	12.500	100	0	
26	29	4	12.500	100	0	
36	30	5	12.500	100	0	
22	31	1	25.000	100	0	
7	32	2	25.000	100	0	
10	33	3	25.000	100	0	
25	34	4	25.000	100	0	
11	35	5	25.000	100	0	
13	36	1	50.000	100	0	
5	37	2	50.000	100	0	
40	38	3	50.000	100	0	
16	39	4	50.000	100	0	
8	40	5	50.000	100	0	

Comments:

AMEC Earth & Environmental
Northwest Bioassay Laboratory
5009 Pacific Hwy. E., Suite 2
Fife, WA 98424

Physical and Chemical
Measurements of Test Solutions
Salt Water Bioassays

Client: Shannon + Wilson
Sample ID: Sun Princess
Test No: 6306-38NW

Analyst: me
Test Date: 6/17/03
Test Type: Speron cell
Test Species: S. purpuratus

Concentration	Initial Readings			
	D.O. (mg/l)	pH	Salinity (ppt)	Temp. (°C)
C	8.0	7.84	29.0	12.0
Brine	7.9	7.82	29.2	12.0
1.5	8.0	7.87	29.0	12.0
3.125	8.1	7.85	29.0	12.0
6.25	8.1	7.81	29.0	12.0
12.5	8.3	7.75	29.0	12.0
25	8.3	7.67	29.1	12.0
50	8.5	7.58	29.0	12.0

Sample Description: _____

Echinoderm Source: Mission Bay Jetty Date Received: 6/17/03

Comments: _____

Reviewed: XH

Sperm Cell Fertilization Test-Proportion Fertilized

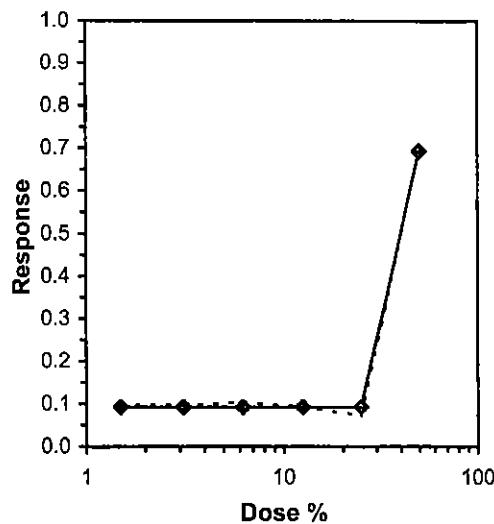
Start Date: 6/19/03 Test ID: 0306-55NW Sample ID: Norwegian Wind
 End Date: 6/19/03 Lab ID: WAAEE-AMEC NW Bioassay Sample Type: BW/GW-Combined gray & black water
 Sample Date: 6/18/03 Protocol: DL 87-Dinnel/Link Test Species: SP-Strongylocentrotus purpuratus
 Comments:

Conc-%	1	2	3	4	5
D-Control	0.9000	0.8300	0.8100	0.8200	0.8000
B-Control	0.7700	0.8600	0.7800	0.7700	0.6700
1.5	0.8000	0.6500	0.8600	0.6600	0.7900
3.125	0.7500	0.7500	0.7600	0.8100	0.6900
6.25	0.6600	0.7900	0.7500	0.7700	0.7600
12.5	0.8100	0.7500	0.7600	0.7600	0.7000
25	0.8600	0.7800	0.8700	0.7400	0.6100
50	0.2000	0.2200	0.2100	0.3800	0.2700

Conc-%	Transform: Arcsin Square Root						t-Stat	1-Tailed Critical	MSD	Number Resp	Total Number
	Mean	N-Mean	Mean	Min	Max	CV%					
D-Control	0.8320	1.0805	1.1509	1.1071	1.2490	4.930	5			84	500
B-Control	0.7700	1.0000	1.0740	0.9589	1.1873	7.534	5				
1.5	0.7520	0.9766	1.0550	0.9377	1.1873	10.268	5	1.890	2.409	0.1222	124
3.125	0.7520	0.9766	1.0507	0.9803	1.1198	4.715	5	1.976	2.409	0.1222	124
6.25	0.7460	0.9688	1.0439	0.9483	1.0948	5.394	5	2.109	2.409	0.1222	127
12.5	0.7560	0.9818	1.0552	0.9912	1.1198	4.333	5	1.887	2.409	0.1222	122
25	0.7720	1.0026	1.0808	0.8963	1.2019	11.528	5	1.382	2.409	0.1222	114
*50	0.2560	0.3325	0.5277	0.4636	0.6642	15.661	5	12.286	2.409	0.1222	372
											500

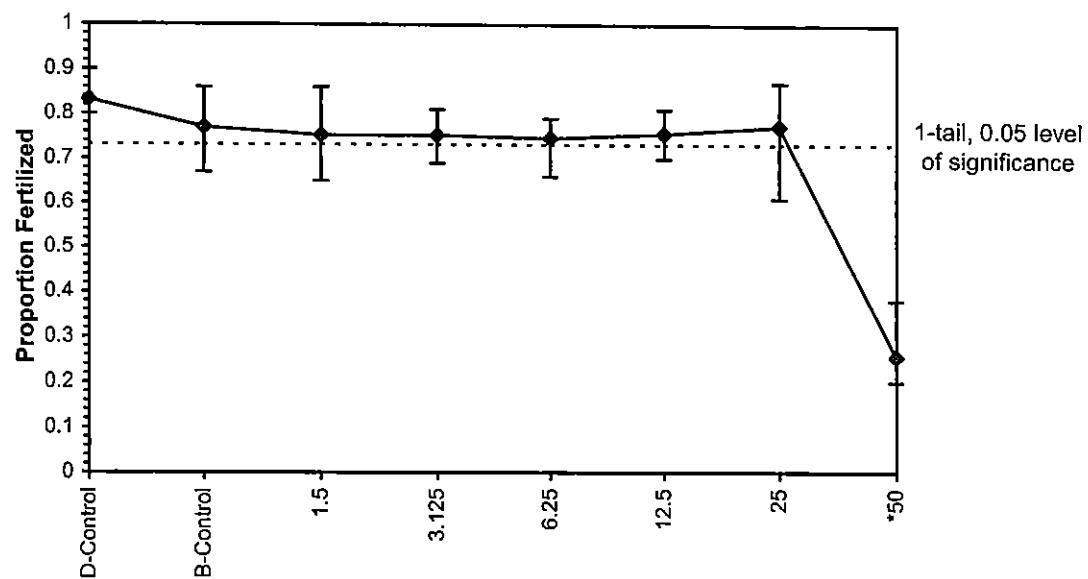
Auxiliary Tests	Statistic	Critical	Skew	Kurt						
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.97827	0.91	-0.1224	0.2001						
Bartlett's Test indicates equal variances ($p = 0.31$)	7.10144	16.8119								
The control means are not significantly different ($p = 0.12$)	1.73968	2.30601								
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU						
Dunnett's Test	25	50	35.3553	4	0.09997	0.11989	0.21895	0.00643	1.3E-11	6, 28

Trimmed Spearman-Karber		
Trim Level	EC50	95% CL
0.0%		
5.0%		
10.0%		
20.0%		
Auto-30.8%	40.046	38.717
		41.422



Sperm Cell Fertilization Test-Proportion Fertilized

Start Date:	6/19/03	Test ID:	0306-55NW	Sample ID:	Norwegian Wind
End Date:	6/19/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water
Sample Date:	6/18/03	Protocol:	DL 87-Dinnel/Link	Test Species:	SP-Strongylocentrotus purpuratus
Comments:					

Dose-Response Plot

Test: SC-Sperm Cell Fertilization Test Species: SP-Strongylocentrotus purpuratus Sample ID: ADEC Cruise Ship <u>Norwegian Wind</u> Start Date: 06/19/2003 End Date: 06/19/2003					Test ID: 0306-55NW Protocol: DL 87-Dinnel/Link Sample Type: EFF2-Industrial Lab ID: WAAEE-AMEC NW Bioassay	
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
1				100	66	at 7/29/03
2				100	78	
3				100	81	
4				100	69	
5				100	86	
6				100	75	
7				100	90	
8				100	84-83-38	
9				100	27	
10				100	78	
11				100	77	
12				100	82	
13				100	76	
14				100	87	
15				100	65	
16				100	75	
17				100	77	
18				100	75	
19				100	20	
20				100	70	
21				100	76	
22				100	77	
23				100	79	
24				100	80	
25				100	76	
26				100	67	
27				100	66	
28				100	75	
29				100	86	
30				100	80	
31				100	21	
32				100	82	
33				100	81	
34				100	83	
35				100	79	
36				100	81	
37				100	74	
38				100	76	
39				100	86	
40				100	61	SV

Comments:

Test: SC-Sperm Cell Fertilization Test Species: SP-Strongylocentrotus purpuratus Sample ID: NORWEGIAN Start Date: 6/19/03 End Date: 6/19/03						Test ID: 0306-55NW Protocol: DL 87-Dinnel/Link Sample Type: BW/GW-Combined gray & black water Lab ID: WAAEE-AMEC NW Bioassay
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
7	1	1	D-Control	100	90	
34	2	2	D-Control	100	83	
36	3	3	D-Control	100	81	
32	4	4	D-Control	100	82	
30	5	5	D-Control	100	80	
11	6	1	B-Control	100	77	
39	7	2	B-Control	100	86	
2	8	3	B-Control	100	78	
17	9	4	B-Control	100	77	
26	10	5	B-Control	100	67	
24	11	1	1.500	100	80	
15	12	2	1.500	100	65	
5	13	3	1.500	100	86	
1	14	4	1.500	100	66	
23	15	5	1.500	100	79	
16	16	1	3.125	100	75	
18	17	2	3.125	100	75	
21	18	3	3.125	100	76	
3	19	4	3.125	100	81	
4	20	5	3.125	100	69	
27	21	1	6.250	100	66	
35	22	2	6.250	100	79	
6	23	3	6.250	100	75	
22	24	4	6.250	100	77	
38	25	5	6.250	100	76	
33	26	1	12.500	100	81	
28	27	2	12.500	100	75	
13	28	3	12.500	100	76	
25	29	4	12.500	100	76	
20	30	5	12.500	100	70	
29	31	1	25.000	100	86	
10	32	2	25.000	100	78	
14	33	3	25.000	100	87	
37	34	4	25.000	100	74	
40	35	5	25.000	100	61	
19	36	1	50.000	100	20	
12	37	2	50.000	100	22	
31	38	3	50.000	100	21	
8	39	4	50.000	100	38	
9	40	5	50.000	100	27	

Comments:

AMEC Earth & Environmental
Northwest Bioassay Laboratory
5009 Pacific Highway E., Suite 2-0
Fife, WA 98424

Physical and Chemical
Measurements of Test Solutions
Salt Water Bioassays

Client: Shannon + wilson
Sample ID: Norwegian wind
Test No: 0306-55NW

Analyst: mr
Test Date: 6/19/03
Test Type: Sperm cell fertilization
Test Species: S. purpuratus

Concentration	Initial Readings			
	D.O. (mg/l)	pH	Salinity (ppt)	Temp. (°C)
C	7.7	8.01	28.3	12.0
Brine C	7.8	8.04	28.8	12.0
1.5	7.7	8.00	29.1	12.0
3.125	7.8	8.04	29.1	12.0
6.25	8.0	7.93	29.4	12.0
12.5	8.0	7.90	29.4	12.0
25	7.7	7.86	28.7	12.0
50	7.5	7.95	28.9	12.0

Sample Description: AMEC San diego
Echinoderm Source: mission bay Jetty Date Received: 6/19/03

Comments: _____
Reviewed: Xb

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy. E. Suite 2-0
Fife, WA 98424

Marine Dilution Worksheet

Client: Shannon + wilson
Sample ID: Norwegian wind
Test No.: _____

Analyst: mc
Test Date: 6/19/03
Test Type: Bivalve, sperm cell fertilization

Brine salinity: 67

Equation for Salinity Adjustment: $VB = VE \frac{30 - SE}{SB - 30}$

Effluent Salinity Adjustment: $\frac{30 - 0}{67 - 30} = \frac{30}{37} = .81$

Brine Control Salinity Adjustment:

Concentration	Effluent Volume (ml)	Salinity Adjustment	Brine Volume (ml)	Seawater Volume (ml)	Total Volume (ml)
Control	NA	NA	NA		250ml
1.5	3.75	.81	3.0		
3.125	7.81	.81	6.3		
6.25	15.6	.81	12.6		
12.5	31.25	.81	25.3		
25	62.5	.81	50.6		
50	125	.81	101		✓

DI Volume					
Brine Control	125	.81	101		250ml

Calculations for all concentrations except highest:

1. Effluent Volume = Total Volume X Concentration (%)
2. Brine Volume = Effluent Volume X Salinity Adjustment
3. Seawater Volume = Total Volume - Effluent Volume + Brine Volume

Calculation for highest concentration:

1. Effluent Volume = Total Volume / (1.0 + Salinity Adjustment)
2. Brine Volume = Total Volume - Effluent Volume
3. Concentration = Effluent Volume / Total Volume

Calculation for brine control:

1. Brine Volume = Brine Volume for highest concentration
2. DI Volume = Brine Volume / Brine Control Salinity Adjustment
3. Seawater Volume = Total Volume - DI Volume + Brine Volume

Reviewed/ Date: KB

Echinoderm Egg Fertilization Bioassay Worksheet

Client: Shawer + wilson
 Test No.: _____
 Test Species: S purpuratus

Start Date/Time: 6/19/03
 End Date/Time: _____
 Date Collected: 6/18/03

Injection Time: 1300

Eggs Counted (x): 33 Mean: 36.2 x 42 = 1520 eggs/ml
37
37
42
32

Egg Dilution Factor

$$\frac{1520 \text{ eggs/ml}}{2000 \text{ eggs/ml}} = .76$$

Sperm Counted (y) or Absorbance at 400nm: 1.65

2000:1 Sperm Stock = $\frac{y}{4.0 \times 10^6} (0.1) = .00$

Sperm Dilution Factor

Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1
mLs Sperm Stock	50	40	30	20	10	5	2.5
mLs Seawater Diluent	0	10	20	30	40	45	47.5

Sperm Added:	Time	Rangefinder Ratio:	Fert.	Unfert.	Percent Fert.
	<u>1320</u>	<u>100:1</u>	<u>88</u>	<u>24</u>	
Eggs Added:	<u>1330</u>	<u>200:1</u>	<u>98</u>	<u>2</u>	<u>98</u>
Test Ended:		<u>400:1</u>	<u>99</u>	<u>1</u>	<u>99%</u>
		<u>800:1</u>			

Definitive Test	Time	Sperm:Egg Ratio Used: <u>400:1</u>		
Sperm Added:	<u>1400</u>	Fert	Unfert.	Percent Fert.
Eggs Added:	<u>1420</u>	<u>48</u>	<u>4</u>	<u>98</u>
Test Ended:	<u>1440</u>	<u>100</u>	<u>0</u>	<u>100</u>
		Egg Control 1	<u>0</u>	<u>0%</u>
		Egg Control 2	<u>0</u>	<u>0%</u>

Comments: _____

AMEC Earth & Environmental
 Northwest Bioassay Lab
 5009 Pacific Hwy. E., Suite 2
 Fife, WA 98424
 (253) 922-4296

Reviewed/ Date: KJS

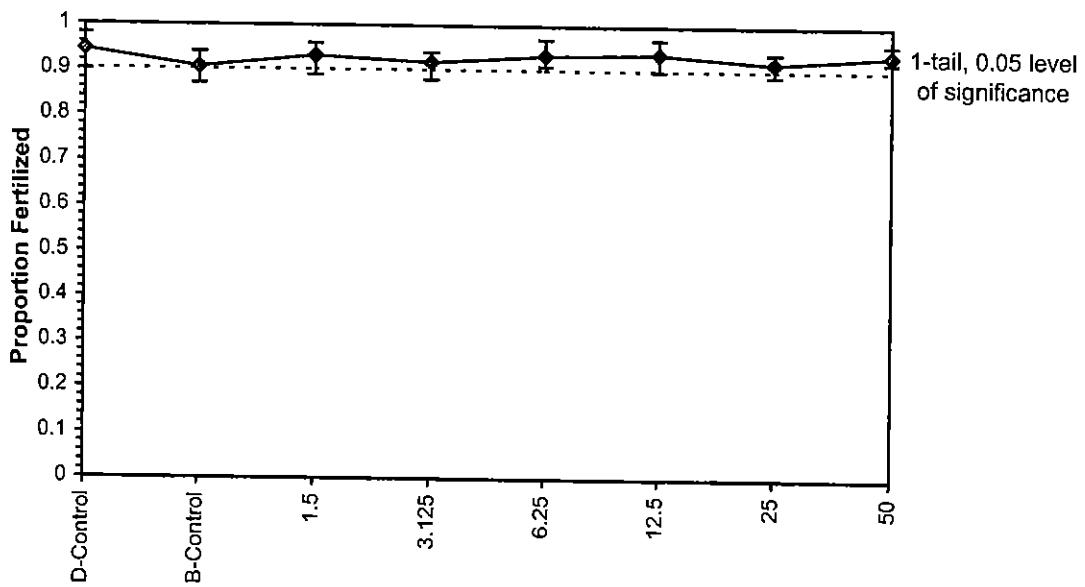
Sperm Cell Fertilization Test-Proportion Fertilized									
Start Date:	6/21/03	Test ID:	0306-62NW	Sample ID:	Ryndam				
End Date:	6/21/03	Lab ID:	WAAEE-AMEC NW Bioassay	Sample Type:	BW/GW-Combined gray & black water				
Sample Date:	6/20/03	Protocol:	DL 87-Dinnel/Link	Test Species:	SP-Strongylocentrotus purpuratus				
Comments:									

Conc-%	1	2	3	4	5
D-Control	0.9700	0.9300	0.9800	0.9400	0.9000
B-Control	0.8700	0.9300	0.9400	0.8800	0.9100
1.5	0.9400	0.9500	0.8900	0.9600	0.9200
3.125	0.9400	0.9300	0.9000	0.8800	0.9400
6.25	0.9300	0.9700	0.9400	0.9100	0.9200
12.5	0.9300	0.9600	0.9700	0.9300	0.9000
25	0.9400	0.8900	0.8900	0.9400	0.9300
50	0.9400	0.9300	0.9200	0.9600	0.9300

Conc-%	Mean	N-Mean	Transform: Arcsin Square Root					1-Tailed		
			Mean	Min	Max	CV%	N	t-Stat	Critical	MSD
D-Control	0.9440	1.0419	1.3402	1.2490	1.4289	5.411	5			
B-Control	0.9060	1.0000	1.2623	1.2019	1.3233	4.171	5			
1.5	0.9320	1.0287	1.3110	1.2327	1.3694	4.107	5	0.871	2.409	0.0808
3.125	0.9180	1.0132	1.2832	1.2171	1.3233	3.729	5	1.700	2.409	0.0808
6.25	0.9340	1.0309	1.3146	1.2661	1.3967	3.848	5	0.762	2.409	0.0808
12.5	0.9380	1.0353	1.3243	1.2490	1.3967	4.443	5	0.475	2.409	0.0808
25	0.9180	1.0132	1.2830	1.2327	1.3233	3.637	5	1.704	2.409	0.0808
50	0.9360	1.0331	1.3166	1.2840	1.3694	2.480	5	0.704	2.409	0.0808

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution ($p > 0.01$)	0.96596	0.91	0.01325	-0.914
Bartlett's Test indicates equal variances ($p = 0.87$)	2.4503	16.8119		
The control means are not significantly different ($p = 0.09$)	1.94401	2.30601		
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Dunnett's Test	50	>50		2
				MSDu
				MSDp
				MSB
				MSE
				F-Prob
				df
				6, 28

Dose-Response Plot



Test: SC-Sperm Cell Fertilization Test Species: SP-Strongylocentrotus purpuratus Sample ID: Ryndam Start Date: 06/21/2003				Test ID: 0306-62NW Protocol: DL 87-Dinnel/Link Sample Type: EFF2-Industrial Lab ID: WAAEE-AMEC NW Bioassay		
Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
1				100	94	{
2				100	93	
3				100	98	
4				100	90	
5				100	89	
6				100	91	
7				100	88	
8				100	99	
9				100	92	
10				100	93	
11				100	93	
12				100	94	
13				100	91	
14				100	93	
15				100	94	
16				100	90	
17				100	74	
18				100	76	
19				100	88	
20				100	94	
21				100	93	
22				100	93	
23				100	76	
24				100	97	
25				100	72	
26				100	97974	
27				100	95	
28				100	94	
29				100	87	
30				100	94	
31				100	96	
32				100	93	
33				100	94	
34				100	89	
35				100	93	
36				100	97	
37				100	94	
38				100	93	
39				100	92	✓
40				100	90	

Comments:

Test: SC-Sperm Cell Fertilization Test
Species: SP-Strongylocentrotus purpuratus
Sample ID: Ryndam
Start Date: 6/21/03 End Date: 6/21/03

Test ID: 0306-62NW
Protocol: DL 87-Dinnel/Link
Sample Type: BW/GW-Combined gray & black water
Lab ID: WAAEE-AMEC NW Bioassay

Pos	ID	Rep	Group	Total Counted	Number Fertilized	Notes
36	1	1	D-Control	100	97	
14	2	2	D-Control	100	93	
3	3	3	D-Control	100	98	
15	4	4	D-Control	100	94	
40	5	5	D-Control	100	90	
29	6	1	B-Control	100	87	
22	7	2	B-Control	100	93	
1	8	3	B-Control	100	94	
7	9	4	B-Control	100	88	
13	10	5	B-Control	100	91	
17	11	1	1.500	100	94	
27	12	2	1.500	100	95	
8	13	3	1.500	100	89	
31	14	4	1.500	100	96	
9	15	5	1.500	100	92	
28	16	1	3.125	100	94	
35	17	2	3.125	100	93	
16	18	3	3.125	100	90	
19	19	4	3.125	100	88	
37	20	5	3.125	100	94	
10	21	1	6.250	100	93	
24	22	2	6.250	100	97	
30	23	3	6.250	100	94	
6	24	4	6.250	100	91	
39	25	5	6.250	100	92	
21	26	1	12.500	100	93	
23	27	2	12.500	100	96	
26	28	3	12.500	100	97	
11	29	4	12.500	100	93	
4	30	5	12.500	100	90	
33	31	1	25.000	100	94	
34	32	2	25.000	100	89	
5	33	3	25.000	100	89	
12	34	4	25.000	100	94	
38	35	5	25.000	100	93	
20	36	1	50.000	100	94	
32	37	2	50.000	100	93	
25	38	3	50.000	100	92	
18	39	4	50.000	100	96	
2	40	5	50.000	100	93	

Comments:

Echinoderm Egg Fertilization Bioassay Worksheet

Client: Shannon + W. SOR Ryndas Start Date/Time: 6/21/03
 Test No.: _____ End Date/Time: 6/21/03
 Test Species: _____ Date Collected: 6/20/03

Injection Time: 1700

Eggs Counted (x): 35 Mean: _____ $\times 42 =$ 1755 eggs/ml
37
42
46
49 $\frac{1755 \text{ eggs/ml}}{2000 \text{ eggs/ml}} =$.877 Egg Dilution Factor

Sperm Counted (y) or Absorbance at 400nm: .989

2000:1 Sperm Stock = $\frac{y}{4.0 \times 10^6} (0.1) =$ 0 Sperm Dilution Factor

Rangefinder Test:	2000:1	1600:1	1200:1	800:1	400:1	200:1	100:1
mLs Sperm Stock	50	40	30	20	10	5	2.5
mLs Seawater Diluent	0	10	20	30	40	45	47.5

Sperm Added:	Time	Rangefinder Ratio:	Fert.	Unfert.	Percent Fert.
	<u>1725</u>	<u>100:1</u>	<u>100</u>	<u>0</u>	<u>100%</u>
Eggs Added:	<u>1734</u>	<u>100:1</u>	<u>100</u>	<u>0</u>	<u>100%</u>
Test Ended:	<u>1744</u>	<u>400:1</u> <u>800:1</u>	<u>100</u>	<u>0</u>	<u>100%</u>

Definitive Test	Time	Sperm:Egg Ratio Used: <u>200:1</u>		
Sperm Added:	<u>1755</u>	Fert.	Unfert.	Percent Fert.
Eggs Added:		<u>97</u>	<u>3</u>	<u>97</u>
Test Ended:		<u>99</u>	<u>1</u>	<u>99</u>
		Egg Control 1	<u>99</u>	<u>1%</u>
		Egg Control 2	<u>0</u>	<u>100</u>

Comments: _____

AMEC Earth & Environmental
 Northwest Bioassay Lab
 5009 Pacific Highway E., Suite 2-0
 Fife, WA 98424
 (253) 922-4296

Reviewed/ Date: 6/21/03

AMEC Earth & Environmental
Northwest Bioassay Lab
5009 Pacific Hwy., E. Suite 2-0
Fife, WA 98424

Physical and Chemical
Measurements of Test Solutions
Salt Water Bioassays

Client: Shannon + wilson
Sample ID: Ryndam
Test No: 0306-b2NW

Analyst: m
Test Date: 6/21/03
Test Type: Sperm cell fertilization
Test Species: S purpuratus

Concentration	Initial Readings			
	D.O. (mg/l)	pH	Salinity (ppt)	Temp (C)
C	8.0	7.79	28.0	12.9
Brine C	8.2	7.83	28.5	12.9
1.5	8.2	7.85	28.0	12.9
3.125	8.1	7.84	28.4	12.7
6.25	8.3	7.91	28.2	12.8
12.5	8.4	7.95	28.6	12.8
25	8.3	8.01	28.4	12.9
50	8.2	8.09	28.6	13.0

Sample Description: Amel

Echinoderm Source: San Diego Date Received: 6/21/03
mission bay Jetty

Comments: _____

Reviewed: ✓