



Nautilus Environmental

## Aquatic Toxicity Evaluation of a Chemical Product Material: ZF-5

Prepared for: A-Z Comp  
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Submitted: October 20, 2010

### Data Quality Assurance:

- Nautilus Environmental is a state certified laboratory under the California Department of Health Services – Environmental Laboratory Accreditation Program (ELAP), Certificate No. 1802.
- All test results included in this report have met internal Quality Assurance requirements, as well as all minimum acceptability criteria for test controls under the EPA protocol requirements.
- All data have been reviewed and verified.

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Verified by: Steve Carlson Date: 10-20-10

## **INTRODUCTION**

A bioassay test using the Red Abalone *Haliotis rufescens* was performed to determine whether a chemical product would produce any adverse effects on the aquatic environment. The Red Abalone was chosen, as it is one of the more sensitive marine invertebrate test species, and is a good representative of the marine environment. The test looked at the most sensitive life-stage of the abalone, which is the first 48-hours of larval development, and looked at whether the larval shell develops normally or abnormally. The abalone were not exposed directly to the chemical product, but rather were exposed to seawater that was filtered through the chemical product. The primary purpose of this study was to determine whether any toxic substances would leach out, as seawater was filtered through the product. At the end of the 48-hour test exposure, the abalone larvae were examined under a microscope to determine normality. A statistical difference in the percentage of normal versus abnormal larvae, when comparing the sample concentrations to the laboratory control, would indicate toxicity due to the chemical material. Testing was conducted between September 10 and 12, 2010 at the bioassay laboratory of Nautilus Environmental (Nautilus), located in San Diego, California.

## **MATERIALS AND METHODS**

The study was performed in accordance with the EPA protocol "Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms" (EPA/600/R-95/136, August 1995). Testing also followed the general guidelines developed by the EPA's Office of Prevention, Pesticides and Toxic Substances (OPPTS) for conducting aquatic laboratory tests with chemical products to determine ecological effects (OPPTS - Series 850; EPA Publication 712-C-96).

## **TEST MATERIAL**

The chemical product was shipped to the testing laboratory by FedEx delivery service and was received on September 7, 2010. The product was identified as "ZF-5" filtration system. The actual material being tested consisted of a charcoal-grey, fine powder substance, contained within an enclosed chamber. This filter material consisted of HRCM (high reactive carbon mixture), also called a graphene powder. This graphene powder is a pure carbon powder made of breaking graphite at the molecular level. This technology was invented by Dr. Viktor Petrik.

Natural seawater was allowed to filter through this chamber, coming in direct contact or exposure to the graphene powder. Seawater filtered through this material at a rate of approximately one drop every two seconds. The seawater was collected underneath, after filtering through the chamber, and this water was used for preparing all sample concentrations.

#### **LABORATORY CONTROL WATER**

All testing was conducted using natural seawater obtained from the ocean intake system located at the Scripps Institution of Oceanography in San Diego, California. The seawater was transported to the laboratory in a large water truck and held in a re-circulating system with an in-line 20- $\mu$ m fiber filter and a chiller unit. This seawater was used in all phases of testing, including the source of dilution water for all sample preparations. A laboratory control, consisting of just the natural seawater, was also tested and used for quality assurance purposes. All sample material results were analyzed and compared to this laboratory control to determine whether there were any significant differences.

#### **TEST DESIGN AND SAMPLE PREPARATION**

The sample material was a very fine, light weight powder that is insoluble and will float on surface water. Due to the nature of this substance, it was determined to produce a test design that would reflect the volume of the material, rather than the weight. Therefore, concentrations of the material were calculated in equal volume "parts." More specifically, we measured out an equal part of the sample material to an equal part of seawater. The units were measured as one part sample per one million parts seawater, or parts per million (ppm).

Since we were not testing the sample material directly in water, but rather the water that filters through the sample material, we had to calculate the volume of water that flowed through a fixed volume of the powder material. The testing laboratory was informed that each chamber that comes with the ZF-5 filtration system contains 331.1 cubic centimeters of the graphene powder. This volume is equivalent to 331.1 milliliters (ml). Therefore, by pouring 331.1 ml of seawater through the filter, we obtain a 1 to 1 ratio, and the final water collected is at a concentration of 1,000,000 ppm. This became the stock solution used for testing. We diluted this stock by a factor of ten to obtain the highest test concentration, which was 100,000 ppm. We continued diluting by a factor of ten to obtain the final test design. Therefore, the final concentrations used for testing were 100,000 ppm, 10,000 ppm, 1,000 ppm, 100 ppm, 10 ppm, and 1 ppm of the sample. We also tested a 0 ppm, which was seawater not exposed to the filter (lab

control). Each concentration was tested with 5 replicates. Each replicate consisted of a 30-ml glass vial with 20 ml of the sample concentration added. After all the test vials were prepared, they were placed into a holding tray in random order, and placed in an environmentally-controlled test chamber until the test was initiated. See Table 1 for a summary of the test methodology.

**Table 1. Summary of Test Parameters for the Red Abalone Bioassay Test.**

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Test organism	Red Abalone – <i>Haliotis rufescens</i>
Test organism source	The Cultured Abalone (Goleta, CA)
Test organism age at initiation	Single-cell embryo (1 hour post fertilization)
Test duration	48-hours
Test type	Static non-renewal
Feeding	None
Test chamber size	30 ml glass shell vial
Test solution volume	20 ml/ replicate
Number of replicates/ concentration	5
Test concentrations (ppm sample)	100000, 10000, 1000, 100, 10, 1, and 0 (lab control)
Dilution water	Natural seawater
Number of organisms/ replicate	250 to 300
Daily chemistry measurements	Temperature, pH, DO, and salinity
Test temperature	15 ± 1°C
Photoperiod	16 hours light/ 8 hours dark
Aeration	None
Test Protocol	EPA/600/R-95/136
Test endpoint	% normal shell development
Test acceptability criteria	≥ 80% normal shell development in the control

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#### TEST ANIMALS

Bioassay testing was conducted using the Red Abalone, *Haliotis rufescens*. Mature abalone broodstock were obtained from The Cultured Abalone, a commercial mariculture facility located in Goleta, California. The broodstock animals were received six days prior to testing to allow acclimation to laboratory testing conditions. The

animals were separated by sex and held in aerated holding buckets filled with natural seawater and maintained at a test temperature of  $15 \pm 1^\circ\text{C}$ .

On the day of testing, the two buckets with male and female abalone are induced to spawn. The spawning induction process is conducted by adding a small volume of hydrogen peroxide and a Tris buffer solution to each bucket of seawater. The abalone are exposed to the chemicals for a 2.5 hour period, at which point the water is removed and fresh seawater is added to the buckets. The abalone should start spawning within the first hour after the water renewal. The sperm and eggs are then collected and combined to produce fertilized embryos.

Approximately one hour after the fertilization process, the embryos are ready to be added to the test vials and the test initiated. A stock solution is prepared, and approximately 250 to 300 embryos are pipetted into each test vial. At this point, the embryos are still in their one-cell stage. During the course of the 48-hour test, the embryos will become trochophore larvae and then develop into veliger larvae. During the veliger stage, the organisms will swim up into the water column, creating the potential for more physical interaction between the organisms and any toxicants found in the water. For a more detailed description of the testing procedures, please refer to the EPA protocol on the 48-hour Red Abalone Test (EPA/600/R-95/136).

## **RESULTS**

After 48 hours, the test is ended by the addition of formalin to each test vial and capping. This process will preserve the organisms in the vials so they can be examined under a microscope at a later date. Each vial is examined by counting the first 100 organisms observed at 40x magnification. Each organism is scored as normal or abnormal, by examining the abalone's shell development during the first 48 hours of life. A percentage of normal development is determined for each test vial. The final data is then entered into a statistical software program called Comprehensive Environmental Toxicity Information System (CETIS), version 1.7.0.4. The data is analyzed to determine a mean percentage of normal development for each sample concentration. Each sample concentration is then compared to the laboratory control results to determine whether there is a significant difference present, which would indicate toxicity.

Analyzing the final data resulted in 90.2% normal development in the lab control, meeting test acceptability criteria (>80% required). Each of the sample concentrations tested resulted in a range of 89 to 92% normal development, which was not found to be significantly different compared to the lab control. Therefore, the No Observed Effect Concentration (NOEC) was 100,000 ppm (the highest sample concentration tested). The  $\text{EC}_{50}$  (the statistically derived concentration that causes a 50% adverse effect to the

test organisms) was greater than 100,000 ppm. Therefore, we were able to determine that no toxic substances leached out into the water from this filter material, which would cause an adverse effect to the abalone. Final test results can be found in Table 2. Detailed statistical analyses and the raw bench data can be found in Appendix A.

**TABLE 2. Summary of Results:**

Sample ID	Test Concentration (ppm)	Mean Normal Development (%)
ZF-5	Lab Control	90.2
Product Material	1	89.8
	10	89.0
	100	89.8
	1,000	90.8
	10,000	90.7
	100,000	91.4
NOEC = 100,000 ppm	LOEC > 100,000 ppm	EC <sub>50</sub> > 100,000 ppm

NOEC = the highest Concentration that produces No Observed Effect

LOEC = the Lowest Observed Effect Concentration (next concentration higher than the NOEC)

EC<sub>50</sub> = the concentration that causes an adverse effect to 50% of the organisms

### Quality Assurance

The lab control met the test acceptability criterion of 80% or greater normal development. Therefore, all test results are deemed valid. In addition, based on the dose response observed during testing, the calculated effect concentration is deemed reliable.

## **REFERENCES**

- EPA 1995. Short-Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to West Coast Marine and Estuarine Organisms. EPA/600/R-95/136, August 1995.
- Code of Federal Regulations, Title 40. Environmental Test Methods and Guidelines, for testing under the Toxic Substances Control Act. 40 CFR 797 series.
- Office of Prevention, Pesticides, and Toxic Substances (OPPTS), U.S. EPA. Ecological Effects Test Guidelines – Special Considerations for Conducting Aquatic Laboratory Studies. OPPTS 850 series. EPA 712-C-96, April 1996.
- Tidepool Scientific Software, 2001-2002. Comprehensive Environmental Toxicity Information System (CETIS), version 1.7.0.4

**Appendix A**

**Red Abalone Bioassay**

**Test Data and Statistical Analysis**



**CETIS Summary Report**

Report Date: 28 Sep-10 16:51 (p 1 of 1)  
 Test Code: 03-5161-5183/1009-S021B

**Red Abalone Larval Development Test** **Nautilus Environmental (CA)**

Batch ID: 02-6590-4148	Test Type: Development	Analyst:
Start Date: 10 Sep-10 14:45	Protocol: EPA/600/R-95/136 (1995)	Diluent: Natural Seawater
Ending Date: 12 Sep-10 14:30	Species: Haliotis rufescens	Brine: Not Applicable
Duration: 48h	Source: Cultured Abalone	Age:

Sample ID: 01-1776-9755	Code: 100910	Client:
Sample Date: <del>10</del> Sep-10	Material: Filtrate	Project:
Receive Date: <del>10</del> Sep-10 <i>bc</i>	Source: A-Z Comp	
Sample Age: 15h	Station:	

**Comparison Summary**

Analysis ID	Endpoint	NOEL	LOEL	TOEL	PMSD	TU	Method
02-9296-7261	Development Rate	100000	>100000	N/A	5.81%		Dunnett's Multiple Comparison Test

**Test Acceptability**

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits	Overlap	Decision
02-9296-7261	Development Rate	Control Resp	0.902	0.8 - NL	Yes	Result Within Limits
02-9296-7261	Development Rate	PMSD	0.05807	NL - 0.2	No	Result Within Limits

**Development Rate Summary**

Conc-ppm	Control Type	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	Diff%
0	Lab Control	5	0.902	0.8904	0.9136	0.87	0.95	0.01393	0.03114	3.45%	0.0%
1		5	0.8976	0.8937	0.9015	0.88	0.9082	0.004683	0.01047	1.17%	0.48%
10		5	0.89	0.8748	0.9052	0.83	0.94	0.01817	0.04062	4.56%	1.33%
100		5	0.898	0.8842	0.9118	0.87	0.96	0.01655	0.03701	4.12%	0.44%
1000		5	0.908	0.8923	0.9237	0.86	0.96	0.01881	0.04207	4.63%	-0.67%
10000		5	0.9068	0.8978	0.9158	0.88	0.94	0.01074	0.02402	2.65%	-0.53%
100000		5	0.914	0.9072	0.9208	0.89	0.93	0.008124	0.01817	1.99%	-1.33%

**Development Rate Detail**

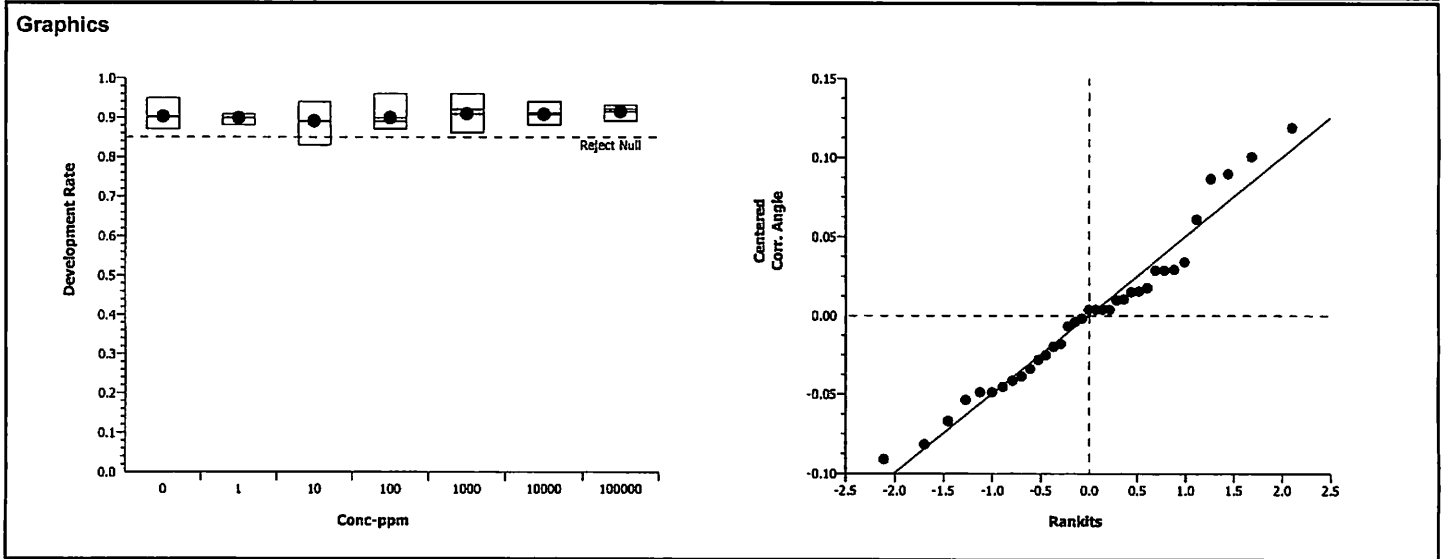
Conc-ppm	Control Type	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	Lab Control	0.9	0.88	0.87	0.95	0.91
1		0.9082	0.9	0.9	0.9	0.88
10		0.91	0.83	0.88	0.89	0.94
100		0.9	0.87	0.96	0.89	0.87
1000		0.87	0.86	0.93	0.92	0.96
10000		0.9167	0.91	0.94	0.88	0.8873
100000		0.93	0.89	0.92	0.9	0.93


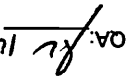
**CETIS Analytical Report**

Report Date: 28 Sep-10 16:51 (p 1 of 2)  
 Test Code: 03-5161-5183/1009-S021B

Red Abalone Larval Development Test								Nautilus Environmental (CA)			
Analysis ID: 02-9296-7261		Endpoint: Development Rate			CETIS Version: CETISv1.7.0						
Analyzed: 28 Sep-10 16:51		Analysis: Parametric-Control vs Treatments			Official Results: Yes						
Sample ID: 01-1776-9755		Code: 100910		Client:							
Sample Date: 10 Sep-10		Material: Filtrate		Project:							
Receive Date: 10 Sep-10		Source: A-Z Comp									
Sample Age: 15h		Station:									
<b>Data Transform</b>	<b>Zeta</b>	<b>Alt Hyp</b>	<b>Monte Carlo</b>	<b>NOEL</b>	<b>LOEL</b>	<b>TOEL</b>	<b>TU</b>	<b>PMSD</b>			
Angular (Corrected)	0	C > T	Not Run	100000	>100000	N/A		5.81%			
<b>Dunnett's Multiple Comparison Test</b>											
<b>Control</b>	<b>vs Conc-ppm</b>	<b>Test Stat</b>	<b>Critical</b>	<b>MSD</b>	<b>P-Value</b>	<b>Decision(5%)</b>					
Lab Control	1	0.3023	2.407	0.08332	0.7596	Non-Significant Effect					
	10	0.5455	2.407	0.08332	0.6608	Non-Significant Effect					
	100	0.1406	2.407	0.08332	0.8156	Non-Significant Effect					
	1000	-0.3832	2.407	0.08332	0.9365	Non-Significant Effect					
	10000	-0.1936	2.407	0.08332	0.9031	Non-Significant Effect					
	100000	-0.5343	2.407	0.08332	0.9559	Non-Significant Effect					
<b>ANOVA Table</b>											
<b>Source</b>	<b>Sum Squares</b>		<b>Mean Square</b>		<b>DF</b>	<b>F Stat</b>	<b>P-Value</b>	<b>Decision(5%)</b>			
Between	0.005249137		0.0008748561		6	0.2921	0.9356	Non-Significant Effect			
Error	0.08385551		0.00299484		28						
Total	0.08910464		0.003869696		34						
<b>ANOVA Assumptions</b>											
<b>Attribute</b>	<b>Test</b>			<b>Test Stat</b>	<b>Critical</b>	<b>P-Value</b>	<b>Decision(1%)</b>				
Variances	Bartlett Equality of Variance			8.833	16.81	0.1832	Equal Variances				
Distribution	Shapiro-Wilk Normality			0.9641		0.3017	Normal Distribution				
<b>Development Rate Summary</b>											
<b>Conc-ppm</b>	<b>Control Type</b>	<b>Count</b>	<b>Mean</b>	<b>95% LCL</b>	<b>95% UCL</b>	<b>Min</b>	<b>Max</b>	<b>Std Err</b>	<b>Std Dev</b>	<b>CV%</b>	<b>Diff%</b>
0	Lab Control	5	0.902	0.8902	0.9138	0.87	0.95	0.01393	0.03114	3.45%	0.0%
1		5	0.8976	0.8936	0.9016	0.88	0.9082	0.004683	0.01047	1.17%	0.48%
10		5	0.89	0.8745	0.9055	0.83	0.94	0.01817	0.04062	4.56%	1.33%
100		5	0.898	0.8839	0.9121	0.87	0.96	0.01655	0.03701	4.12%	0.44%
1000		5	0.908	0.892	0.924	0.86	0.96	0.01881	0.04207	4.63%	-0.67%
10000		5	0.9068	0.8977	0.9159	0.88	0.94	0.01074	0.02402	2.65%	-0.53%
100000		5	0.914	0.9071	0.9209	0.89	0.93	0.008124	0.01817	1.99%	-1.33%
<b>Angular (Corrected) Transformed Summary</b>											
<b>Conc-ppm</b>	<b>Control Type</b>	<b>Count</b>	<b>Mean</b>	<b>95% LCL</b>	<b>95% UCL</b>	<b>Min</b>	<b>Max</b>	<b>Std Err</b>	<b>Std Dev</b>	<b>CV%</b>	<b>Diff%</b>
0	Lab Control	5	1.256	1.235	1.277	1.202	1.345	0.02506	0.05604	4.46%	0.0%
1		5	1.245	1.239	1.252	1.217	1.263	0.007582	0.01695	1.36%	0.83%
10		5	1.237	1.212	1.262	1.146	1.323	0.02918	0.06525	5.28%	1.5%
100		5	1.251	1.225	1.277	1.202	1.369	0.03097	0.06925	5.54%	0.39%
1000		5	1.269	1.241	1.298	1.187	1.369	0.03365	0.07524	5.93%	-1.06%
10000		5	1.263	1.246	1.279	1.217	1.323	0.01894	0.04235	3.35%	-0.53%
100000		5	1.274	1.262	1.287	1.233	1.303	0.01434	0.03206	2.52%	-1.47%

Red Abalone Larval Development Test		Nautilus Environmental (CA)	
Analysis ID: 02-9296-7261	Endpoint: Development Rate	CETIS Version: CETISv1.7.0	Official Results: Yes
Analyzed: 28 Sep-10 16:51	Analysis: Parametric-Control vs Treatments		



Analyst:  OA:  10-5740

Conc-ppm	Code	Rep	Pos	# Counted	# Normal	Notes
206				q6		
207				88		
208				63		
209				89		
210				91	100	
211				87		
212				96		
213				91		
214				87		
215				87		
216				91		
217				89		
218				90		
219				96		
220				90		
221				89		
222				93		
223				92		
224				93		
225				94		
226				90		
227				90		
228				94		
229				86		
230				97		
231				92		
232				88		
233				88		
234				83		
235				90		
236				89		
237				90		
238				94		
239				95		
240				93		

↑

Start Date: 10 Sep-10 Species: *Haliotis rufescens* Sample Code: 10-  
 End Date: 12 Sep-10 Protocol: EPA/600/R-95/136 (1995) Sample Source: A-Z Comp  
 Sample Date: 10 Sep-10 Material: Filtrate Sample Station:

Red Abalone Larval Development Test  
 Nautilus Environmental (CA)  
 Report Date: 27 Sep-10 09:38 (p 1 of 1)  
 Test Code: 03-5161-5183/1009-S021B

**CETIS Test Data Worksheet**

Report Date: 10 Sep-10 11:02 (p 1 of 1)  
 Test Code: 03-5161-5183/1009-S021

Red Abalone Larval Development Test

Nautilus Environmental (CA)

Start Date: 10 Sep-10  
 End Date: 12 Sep-10  
 Sample Date: 10 Sep-10

Species: Haliotis rufescens  
 Protocol: EPA/600/R-95/136 (1995)  
 Material: Filtrate

Sample Code: 10-  
 Sample Source: A-Z Comp  
 Sample Station:

Conc-ppm	Code	Rep	Pos	# Counted	# Normal	Notes
0	LC	1	235			<i>Random # positions</i>
0	LC	2	221			
0	LC	3	215			
0	LC	4	239			
0	LC	5	210			
1		1	209			
1		2	220			
1		3	227			
1		4	218			
1		5	207			
10		1	216			
10		2	234			
10		3	228			
10		4	236			
10		5	238			
100		1	226			
100		2	211			
100		3	212			
100		4	217			
100		5	214			
1000		1	230			
1000		2	229			
1000		3	240			
1000		4	223			
1000		5	219			
10000		1	206			
10000		2	213			
10000		3	225			
10000		4	232			
10000		5	208			
100000		1	224			
100000		2	233			
100000		3	231			
100000		4	237			
100000		5	222			

*QC in v*

**Marine Chronic Bioassay**

**Water Quality Measurements**

Client: AZ Comp  
 Sample ID: ZF-5 Filtration Unit  
 Test ID: 1009-S021

Test Species: Haliotis rufescens  
 Start Date/Time: 9/10/2010 1445  
 End Date/Time: 9/12/2010 1430

Concentration (ppm)	Salinity (ppt)			Temperature (°C)			Dissolved Oxygen (mg/L)			pH (pH units)		
	0	24	48	0	24	48	0	24	48	0	24	48
Lab Control	33.5	33.3	33.3	14.3	15.9	15.1	7.5	7.5	8.7	7.89	8.00	8.05
1	33.2	33.0	33.2	14.3	15.8	15.0	7.6	7.6	8.8	7.87	8.00	8.02
10	33.3	33.1	33.1	14.3	15.8	15.1	7.6	7.6	8.7	7.93	8.00	8.02
100	33.3	33.1	33.4	14.3	15.9	15.1	7.5	7.5	8.8	7.93	8.00	8.04
1,000	33.3	33.2	33.4	14.3	15.9	15.0	7.5	7.5	8.8	7.93	8.00	8.04
10,000	33.4	33.3	33.3	14.3	15.9	15.1	7.5	7.5	8.8	7.93	7.99	8.01
100,000	33.5	33.6	33.9	14.3	15.7	15.0	7.5	7.3	8.6	7.93	8.01	8.04

Technician Initials: \_\_\_\_\_ WQ Readings: 

0	24	48
KL	suu	W

  
 Dilutions made by: 

SL		
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Animal Source/Date Received: The Cultured Abalone, 9/4/10

Comments: 0 hrs: \_\_\_\_\_  
 24 hrs: \_\_\_\_\_  
 48 hrs: \_\_\_\_\_

QC Check: KL 9/28/10

Final Review: sc 10-7-10

Marine Chronic Bioassay

Abalone Embryo-Larval Development

Client: AZ Comp

Test Species: Haliotis rufescens

Sample ID: ZF-5 Filtration Unit

Start Date/Time: 9/10/2010 1445/1500

Test No: 1009-5021

End Date/Time: 9/12/2010 1430

Number of abalone and condition upon receipt/holding:

Males: 3 - good

Females: 3 - good

	#1 / #2	
	Males:	Females:
Tris & peroxide addition time	1015	0945
Spawn time	1310	1320/1330/
Number of spawners	1	2
Condition of spawn (light, moderate, heavy)	Heavy	Mod/Mod
Fertilization time		1340/1350

Embryo counts (per 0.5 ml)		
	Female 1	Female 2
1	128	142
2	154	163
3	137	159
Mean	140	155

QC 194  
QC 196

Time of test Initiation: 1445/1500

Comments: #1: clumpy eggs dark green #2: not as clumpy, lighter green  
Females spawned separately.

QC Check: AC 9/21/10

Final Review: KL 9/28/10

**Appendix B**  
**Chain of Custody Form**  
**And List of Qualifier Codes**







## **Glossary of Qualifier Codes:**

- Q1 - pH out of recommended range; refer to CAR
- Q2 - Temperatures out of recommended range; corrective action taken and recorded in Test Temperature Correction Log
- Q3 - Temperatures out of recommended range; no action taken, test terminated same day
- Q4 - Sample aerated prior to initiation or renewal
- Q5 - Salinity out of recommended range; refer to QA section of report
- Q6 - Spilled test chamber/ Lost test animal
- Q7- Instrumentation Error/Failure; refer to CAR
- Q8 - Inadequate sample volume, 50% renewal performed
- Q9 - Inadequate sample volume, no renewal performed
- Q10 - Sample out of holding time; refer to QA section of report
- Q11 - Refer to QA section of report for explanation
- Q12 - Supplemental information is footnoted
- Q13 - Test initiated with an incorrect number of test organisms
- Q14 - Replicate(s) not initiated
- Q15 - Survival counts not recorded due to poor visibility or heavy debris
- Q16 - Test aerated due to dissolved oxygen levels dropping below 4.0 mg/L
- Q17 - Test initiated with aeration due to an anticipated drop in dissolved oxygen
- Q18 - Airline obstructed or fell out of replicate and replaced, drop in dissolved oxygen occurred
- Q19 - Animals out of appropriate age range at test initiation
- Q20 - Readings not taken, tech error
- Q21 – Organisms in replicate not counted, tech error
- Q22 – Dissolved oxygen above recommended range, but remained within the 100% ±10% saturation requirement