

# **“Practical” Fish Toxicity Test Report**


**Prepared For:**

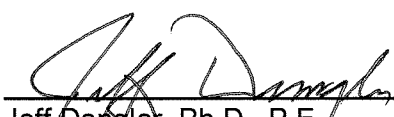
**Car Wash Enterprises  
3977 Leary Way NW  
Seattle, Washington 98107**

**March 22, 2007**

**Prepared By:**

Environmental Partners, Inc.  
295 N.E. Gilman Blvd., Suite 201  
Issaquah, Washington 98027  
(425) 395-0010

  
\_\_\_\_\_  
John Brasino, Ph.D., P.E., L.H.G.  
Principal

  
\_\_\_\_\_  
Jeff Dengler, Ph.D., P.E.  
Senior Engineer

**Project Number: 08404.1**

There is little, if any, reliable data available to assess the storm water loading of a typical curbside car wash event. This study is sponsored by Brown Bear Car Wash to develop a more reliable empirical data set to help evaluate storm water impacts. Brown Bear did not dictate the test procedures or otherwise influence the design or outcome of the study.

## TABLE OF CONTENTS

1.0	TEST DESCRIPTION.....	1
2.0	DISCUSSION OF CAR WASH EFFLUENT FISH TOXICITY TEST .....	2
3.0	DISCUSSION OF SIMULATED EFFLUENT FISH TOXICITY TEST .....	3
4.0	TOXICITY TEST WATER SAMPLES.....	5
5.0	DISCUSSION OF FISH TOXICITY TEST RESULTS .....	6
6.0	PUGET SOUND SETTING.....	8
7.0	TEST RESULT HYPOTHETICAL IMPLICATIONS .....	9
8.0	CONCLUSION .....	10

## TABLES

Table 1 – Car Wash Effluent Fish Toxicity Test Results  
Table 2 – Simulated Effluent Fish Toxicity Test Results  
Table 3 – Fish Toxicity Test Results Summary

## FIGURES

Figure 1 – Overall View of Car Wash Event Location  
Figure 2 – View of Storm Drain and Car Wash Effluent Collection Device  
Figure 3 – View of Typical Car Wash Event Asphalt Surface

## APPENDICES

Appendix A – Laboratory Report – Car Wash Effluent Fish Toxicity Test  
Appendix B – Laboratory Report – Simulated Effluent Fish Toxicity Test  
Appendix C – Hypothetical Implications Calculation Spreadsheet

## 1.0 TEST DESCRIPTION

Two "practical" fish toxicity tests were run. The first test was conducted from August 28 to September 1, 2006 and used effluent water collected from a fund-raiser car wash event at a commercial automotive service location on August 26, 2006. The second test was conducted from November 29 to December 3, 2006 and used a simulated effluent solution containing a consumer car wash detergent. The simulated effluent solution was formulated according to the product label directions with dilution that mimicked a car wash effluent.

The same detergent concentrate was used in water samples for both tests. Juvenile rainbow trout were used in both tests and both tests were conducted according to standard protocols specified in "Methods for Measuring the Acute Toxicity of Effluents and Receiving Waters to Freshwater and Marine Organisms" (EPA-821-R-02-012). The tests were performed by an experienced, certified laboratory.

The tests produced similar results. The first test indicated a percent concentration that was lethal to 50% of the test organisms (LC50) of 3.1%. The second test indicated an LC50 of 3.0%.

There were significant differences in the way the stock water solutions for the two tests were prepared. For the first test, runoff water was collected from the parking lot of an automotive service facility during a fund-raising event. This water ran across approximately 30 feet of asphalt before collection and likely included contact with petroleum hydrocarbons and the grit and grime typically associated with a heavily traveled asphalt lot. Approximately 15 gallons of this water was sampled and delivered "as collected" to the laboratory. Figure 1 presents an overall view of the car wash event location and Figure 2 is a photograph showing a view of the storm drain water collection device. (Note: The youth organization used a car wash kit supplied by King County that prevented the effluent water from entering the storm drain. Effluent water was collected by a storm drain catch basin, shown in the background of Figure 1, and pumped to a sanitary sewer drain, shown in the foreground of Figure 1.)

For the second test, the same detergent concentrate that was used for the car wash event was used by the laboratory to prepare a simulated effluent for testing. This simulated effluent was mixed according to instructions on the product container and was further diluted to simulate addition of rinse water. All water used in the second test was potable.

These tests are termed "practical" fish toxicity tests because the effluent solutions for both were collected or prepared such that each represented the actual runoff water that would be expected to enter into storm water drains and,

eventually, the streams and rivers of Puget Sound. The tests were not run to simply determine the lethal concentration of a pure chemical or to satisfy a discharge permit requirement. As such, the results of these tests represent one piece of evidence that points directly to the impact of wash water from residential driveway or fund-raiser car washes that enters storm drains emptying into water bodies containing threatened and endangered salmon.

## 2.0 DISCUSSION OF CAR WASH EFFLUENT FISH TOXICITY TEST

A 96-hour acute effluent toxicity bioassay test (EPA-821-R-02-012) was performed using juvenile Rainbow Trout (*Oncorhynchus mykiss*) exposed to a standard 0.5 dilution series. The concentration series consisted of 6.25, 12.5, 25, 50, and 100 percent car wash effluent water diluted with potable water. Four replicates of each concentration were run. Potable water was also used to run a laboratory control test.

Prior to test start, dissolved oxygen, pH, conductivity, and temperature of the test waters were measured in each test chamber to ensure parameters were within acceptable limits (prescribed by Environmental Protection Agency (EPA) method guidance). Water quality measurements and survival observations were made daily.

The car wash effluent water caused 100 percent mortality in all concentration steps tested. Complete mortality occurred within 24 hours of test start. Survival of the laboratory control was 100 percent. Results are presented in Table 1 below.

Table 1. Car Wash Effluent Fish Toxicity Test Results			
Test Solution Concentration (%)	Live Organisms at Start of Test	Live Organisms at 96 Hours	Percent Survival
0 (control)	40	40	100
6.25	40	0	0
12.5	40	0	0
25	40	0	0
50	40	0	0
100	40	0	0

The calculated LC50, the concentration of sample that is expected to cause mortality in 50 percent of the select population of organisms, was 3.125 percent due to the complete mortality observed in the lowest concentration tested (6.25 percent) and the 100 percent survival observed in the laboratory control (0 percent). Another measure of toxicity is called Toxic Units (TU = 100/LC50). TU

measurement is typically a specified criterion for discharge monitoring permits. For this case, the Acute Toxic Unit (TUa) result was calculated to be 32, meaning that the tested effluent is 32 times more toxic than an acceptable effluent.

The test was aerated at initiation due to low dissolved oxygen levels (4.3 milligrams per liter (mg/L)) in the received sample car wash water. Dissolved oxygen levels remained within protocol limits for the duration of the test. The results of an associated reference toxicant solution using copper sulfate fell outside the 95% confidence limits of the historical laboratory mean. This indicated that the organisms tested might have been less sensitive to concentrations of copper than typical populations. Since complete mortality was observed in all concentrations of car wash effluent, this reference toxicant deviation had no impact on test results.

Listed below are average test solution physical and chemical data. All parameters were held within acceptable limits during the test period.

Dissolved oxygen:	7.6 mg/L
Temperature:	15.0 +/- 0.1 °C
Conductivity:	0.23 mS/cm
pH:	7.5
Hardness:	99 mg/L (as calcium carbonate)
Alkalinity:	90 mg/L (as calcium carbonate)
Total chlorine:	0 mg/L

(°C = degrees Celsius and mS/cm = milliSiemens per centimeter)

The complete laboratory test report is included in Appendix A.

### **3.0 DISCUSSION OF SIMULATED EFFLUENT FISH TOXICITY TEST**

A 96-hour acute effluent toxicity bioassay test (EPA-821-R-02-012) was performed using juvenile Rainbow Trout (*Oncorhynchus mykiss*) exposed to a concentration series of 0.01, 0.05, 0.1, 0.5, 1, and 10 percent simulated effluent (laboratory-prepared effluent sample) solution diluted with potable water. Four replicates of each concentration were run. Potable water was also used to run a laboratory control test.

Prior to test start, dissolved oxygen, pH, conductivity, and temperature of the test waters were measured in each test chamber to ensure parameters were within acceptable limits (prescribed by EPA method guidance). Water quality measurements and survival observations were made daily.

The simulated effluent solution caused 100 percent mortality in the 10 percent concentration solution and 2.5 percent mortality in the 1 percent concentration solution. All mortality at the 10 percent concentration occurred with 24 hours. Survival rates were 100 percent for all other series concentrations. Survival of the laboratory control was 100 percent. Results are presented in Table 2 below.

<b>Table 2. Simulated Effluent Fish Toxicity Test Results</b>				
<b>Test Solution Concentration (%)</b>	<b>Detergent Concentrate Concentration (ppm)</b>	<b>Live Organisms at Start of Test</b>	<b>Live Organisms at 96 Hours</b>	<b>Percent Survival</b>
0 (control)	0	40	40	100
0.01	0.005	40	40	100
0.05	0.027	40	40	100
0.1	0.053	40	40	100
0.5	0.265	40	40	100
1	0.530	40	39	97.5
10	5.300	40	0	0

The calculated LC50 was 3.046 percent, which equates to a detergent concentrate concentration of approximately 1.6 parts per million (ppm).

The test was aerated at initiation and during its duration due to low dissolved oxygen. Dissolved oxygen levels remained within protocol limits for the duration of the test. The results of an associated reference toxicant solution using copper sulfate fell within the test 95% confidence limits of the historical laboratory mean.

Listed below are average test solution physical and chemical data. All parameters were held within acceptable limits during the test period.

Dissolved oxygen:	10.2 mg/L
Temperature:	11.1 +/- 0.1 °C
Conductivity:	0.32 mS/cm
pH:	8.3
Hardness:	62 mg/L (as calcium carbonate)
Alkalinity:	140 mg/L (as calcium carbonate)
Total chlorine:	0 mg/L

(°C = degrees Celsius and mS/cm = milliSiemens per centimeter)

The complete laboratory test report is included in Appendix B.

#### **4.0 TOXICITY TEST WATER SAMPLES**

The car wash effluent water obtained from the fund-raiser event was a true blind sample and can be considered a typical car wash event effluent. Inquiries were made at local newspapers, schools, service stations, and of individuals who work with youth groups to try to locate a fund-raiser event. The sampler arrived after the event had started and had no input into how the car washing was performed. The location of the event, the type and amount of detergent used, its dilution in a bucket, and the amount of rinse water used was uncontrolled. This car wash event effluent water was used to prepare the dilution series for the first fish toxicity test (i.e., 100, 50, 25, 12.5, and 6.25 percent of the effluent sample).

Cars were washed on an asphalt surface at an oil change service facility. The asphalt condition was typical of a parking lot; its surface had numerous dark spots indicating leaks of petroleum product, as shown in Figure 3. Wash and rinse water that dropped to the asphalt ran about 30 feet across the asphalt to a storm drain grate. The 30-foot traverse was across a driveway of the facility. The event was held on a sunny September day.

The people running the event were using a King County-supplied car wash kit that consisted of an impervious plastic tub, small electric pump, and hose. The plastic tub fit into the storm drain opening and prevented water from going down the drain. It collected the wash water, which was pumped through a hose to an on-site sanitary sewer drain. The car wash effluent water sample was collected from the hose prior to discharge to the sewer. The sample was cooled to 4°C and delivered to the test laboratory the following day.

The simulated effluent solution for the second fish toxicity test used the same detergent that was used during the car wash event. The solution was prepared using directions printed on the product container and was further diluted to simulate the addition of rinse water. All water used in the second test was potable.

Based on product label directions, approximately 16 milliliters (mL) of detergent concentrate was mixed with 4 gallons of water to make the wash solution. This wash solution was diluted by a factor of 20 to mimic the addition of rinse water to produce a concentration of approximately 53 parts per million (ppm) that was the simulated effluent solution used to prepare the dilutions series for the second fish toxicity test (i.e., 10, 1, 0.5, 0.1, 0.05, and 0.01 percent of the effluent sample).

An analysis was made of summertime stream flows for several small creeks and streams in King County that flow into Puget Sound, Lake Washington, and Lake Sammamish. Although flows were highly variable depending on stream size and



recent weather, a typical range of summertime flow was about 2 to 10 cubic feet per second (cfs), equivalent to 900 to 4,500 gpm. This range of stream flow rates was compared to an assumed flow of water from two hoses running at 5 gpm each that was assumed to be typical of a fund-raiser car wash event. The ratio of car wash effluent to stream flow was about 1/100 (0.01 or 1%) to 1/1,000 (0.001 or 0.1%).

This analysis was used to bracket the range of the dilution series performed by the laboratory for the second fish toxicity test. Thus, the concentration of the simulated effluent and the dilution series used for this toxicity test represent realistic conditions. Organisms living and swimming in small creeks and streams around northwest lakes and flowing into Puget Sound would likely be exposed to car wash detergent concentrations that were used in both fish toxicity tests reported here.

## 5.0 DISCUSSION OF FISH TOXICITY TEST RESULTS

Table 3 presents a comparison of the LC50 results for the two fish toxicity tests. The two tests were identical in all respects except for the source of the test water. The reported LC50 values are the percent concentrations of the two dilution series at which mortality was estimated for half of the rainbow trout specimens tested.

Table 3. Fish Toxicity Test Results Summary				
Test	Description	LC50	Concentration	Comments
1 <sup>st</sup>	Real car wash event effluent tested	3.125%	Unknown	5-step dilution series, identical to 2 <sup>nd</sup> test in all other respects
2 <sup>nd</sup>	Laboratory-prepared simulated effluent tested	3.046%	1.6 ppm	6-step dilution series, identical to 1 <sup>st</sup> test in all other respects

Because the car wash effluent used in the first toxicity test was generated in an uncontrolled manner it is not possible to make conclusive remarks about the LC50 results of the toxicity test. This is because the amount of detergent and water used was not measured; hence, detergent concentrations in the dilution series were not known. Also, no chemical analyses were performed to determine petroleum hydrocarbon or metals concentrations in the effluent. Nevertheless, the effluent water sample was collected from an actual fund-raising car wash event and the effluent water represented an actual potential impact to a local stream.

On the other hand, the laboratory-prepared simulated effluent solution used in the second fish toxicity test used measured quantities of detergent and water, which allowed exact calculation of detergent concentrations in the dilution series water. Uncertainties associated with this test include lack of exposure to a petroleum-contaminated asphalt parking lot and lack of exposure to grime from a dirty car.

The similarity of LC50 results is unexpected. There is no way to know if this similarity indicates true replicability or is merely coincidental. The common feature between the two tests was the use of the same car wash detergent concentrate. This concentrate is a commercially available product marketed specifically as a car wash detergent. As indicated by the second test results, a detergent concentration of approximately 1.6 ppm is sufficient to kill one-half of a population of juvenile rainbow trout. In the first toxicity test the car wash effluent solution was fatal to all specimens tested within 24 hours down to the minimum dilution tested of 6.25 percent.

Because the simulated effluent solution for the second test was prepared in the laboratory it is reasonable to assume that the fish mortality was due solely to the effect of the chemicals in the car wash concentrate. The most likely chemical that could be found in such a product that would be toxic to fish is a surfactant or mix of surfactants. The exact physiological impact of a surfactant chemical on the fish is unknown in this case. The chemical could be toxic by simple ingestion, could affect the surface chemistry of fish gills and thereby asphyxiate fish, could disrupt or destroy cell membranes, or produce some other lethal effect.

Other research in this area has indicated that detergents as a rule will destroy fish mucus membranes and gills to varying degrees. Natural oils may be washed away affecting oxygen uptake by the gills. The damaged mucus membranes make fish more susceptible to organic chemicals such as petroleum and pesticides and inorganic chemicals found in fertilizers. Thus, smaller concentrations than predicted of these chemicals may become toxic to fish. Some surfactant chemicals in detergents have been shown to break down into more toxic compounds and to mimic natural hormones in fish causing abnormal growth and development, and therefore lowering survival rates.

Material Safety Data Sheets (MSDSs) for the detergent concentrate were obtained but revealed little about the chemical constituents of the product. The MSDS for the product tested listed only the constituents "water" and "surfactant (mixture)." The surfactant was indicated to be at a concentration between 5 and 20 percent. No ecological information was presented in the MSDS. The only precautions listed were to avoid eye contact ("May Cause Eye Irritation"), likely due to a listed pH of 9.

MSDSs for similar car wash products marketed by the same vendor indicated a few chemical compounds. Among those listed for similar products were the following:

- sodium dodecylbenzene sulfonate (CAS 025155-30-0, also known as sodium laurylbenzene sulfonate);
- alcohol ethoxylate, sulfated, sodium salt (CAS 068585-34-2); and
- unsaturated alkyl carboxylic acid diethanolamide (CAS 068155-07-7).

Ecotoxicity information for the first of these chemicals indicates moderate toxicity to fish, high toxicity to nematodes and flatworms, and slight toxicity to crustaceans and zooplankton. The chemical use is listed as microbiocide, adjuvant, fungicide, and insecticide.

## **6.0 PUGET SOUND SETTING**

Puget Sound is home to 3.8 million people, two-thirds of the state's population. By 2020, another 1.4 million people are expected to settle around the Sound. There are approximately 1.8 million people currently living in King County.

Puget Sound is the second largest estuary in the United States. It has 2,300 miles of shoreline. The Puget Sound watershed covers nearly 16,500 square miles and consists of over ten thousand rivers and streams that drain into the Sound. All but a tiny fraction of storm water that falls on developed areas enters storm drains and flows untreated into the Sound.

Over 80% of the surface water flowing into Puget Sound comes from the following major river drainages: Cedar River (Lake Washington), Green/Duwamish, Elwha, Nisqually, Nooksack, Puyallup (White), Skagit, Skokomish, Snohomish, and Stillaguamish. In King County, the major river drainage systems are the White (Puyallup) River, Green/Duwamish River, Cedar River (Lake Washington), Sammamish River, and the Skykomish/Snoqualmie Rivers.

As of 2006, the number of registered vehicles in Washington was approximately 5.6 million. There are approximately 3.7 million vehicles in the Puget Sound area and about 1.7 million of those are in King County.

## 7.0 TEST RESULT HYPOTHETICAL IMPLICATIONS

Assumptions were made and calculations performed for a hypothetical urban or suburban Puget Sound setting in which a small stream is subjected to car wash effluent input. The calculations were done to try to bracket certain parameters that are typical and would be expected to apply in a real life situation. The scenario, which is hypothetical, is presented below. The spreadsheet developed to perform these calculations is presented in Appendix C.

The setting is a small stream watershed that empties into Lake Washington. The stream is about 10 to 20 miles long and during the summer and fall season ranges in flow from about 2 to 20 cubic feet per second (cfs), depending on recent weather. These flows are typical of many small Puget Sound area streams during summer. A time period of 48 hours during a dry August weekend is assumed.

Approximately 100,000 people are assumed to live in the watershed area. Storm drains serving this population feed to the stream. One percent of the cars of the population are washed in driveways during the time period. A consumer car wash detergent is used to wash the cars and 75 gallons of water flows to the storm drain and, subsequently, to the small stream for each car washed.

Calculations indicate that within this watershed approximately 1,000 vehicles will be washed in driveways during the weekend. The 75 gallons of car wash effluent per vehicle will contain 53 parts per million (ppm) of detergent.

A simple "bathtub" calculation was performed in which all the stream flow and all car wash effluent were pooled and the resulting detergent concentration calculated. The calculated detergent concentration ranged from 0.2 ppm to 1.5 ppm for high and low stream flow conditions, respectively. These detergent concentrations are similar to the 1.6 ppm value that was found to be lethal to 50 percent of juvenile rainbow trout tested. Thus, some fish in the stream could be killed and it would be likely that the detergent would wash protective mucus from the gills of some surviving fish. The surviving fish would, thus, be more susceptible to other contaminants that may exist or be introduced into the stream. It is also possible that oxygen uptake necessary for fish survival may be impaired and that other physiological impacts to fish survival may occur. Other freshwater organisms living in the stream would also likely be affected depending on individual species sensitivities.

Minor changes to the assumptions made in the above analysis drive the calculated detergent concentration to much higher values and make significant impacts to fish and other freshwater organisms more likely. For instance, increasing the percentage of cars washed from one percent to 1.5 percent

increases the total amount of detergent flushed to the stream by 50 percent and raises the calculated detergent concentration in the stream to 2.2 ppm for the low flow situation (i.e., 2 cfs). Calculated detergent concentrations skyrocket when the hypothetical stream flow rate is decreased, because dilution by the stream is the most important factor in the calculated detergent concentration.

## **8.0 CONCLUSION**

September and October, when most salmon are returning to Puget Sound area streams to spawn the next generation, typically represents the lowest stream flow time of the year. Although adult fish are found in the streams, they have been severely stressed by the long return migration and are likely more susceptible to deleterious impacts of detergents and pollutants in stream water. A case can be made that during this pivotal time of the year driveway car washing effluent that reaches streams via storm drains is a real detriment to salmon survival.



Figure 1 – Overall View of Car Wash Event Location





Figure 2 – View of Storm Drain and Water Effluent Collection Device





Figure 3 – View of Typical Car Wash Event Asphalt Surface



## **APPENDIX A**

### **Laboratory Report – Car Wash Effluent Fish Toxicity Test**



WESTON SOLUTIONS, INC.  
4729 NE View Dr.  
P.O. Box 216  
Port Gamble, WA 98364  
(360) 297-6903 / (360) 297-6905 FAX  
www.westonsolutions.com

October 4, 2006

Dr. Jeff Dengler  
Environmental Partners, Inc.  
295 NE Gilman Blvd., Suite 201  
Issaquah, Washington 98027

Re: 96-Hour Rainbow Trout Toxicity Testing Results – Car Wash Water

Dear Dr. Dengler:

Enclosed, please find the report for the acute toxicity test performed on one sample of Car Wash effluent, received on the 28<sup>th</sup> of August. Toxicity testing was conducted using juvenile Rainbow Trout between the 28<sup>th</sup> of August and 1<sup>st</sup> of September, 2006. The results of this test are listed in the table below.

Test	Sample ID	Control Survival	100% Test Substance Survival	LC <sub>50</sub>	TUa
Rainbow Trout 96-Hour Survival	Car Wash	100%	0%	3.125%	>32

**Methods:** This testing investigated the survival of juvenile Rainbow Trout exposed to a dilution series of sample Car Wash over a 96-Hour period. The concentration series tested consisted of 6.25, 12.5, 25, 50, and 100 percent test substance diluted with laboratory water. This series is a standard 0.5 dilution used to statistically estimate the level of toxicity an effluent may have on aquatic organisms. The water used for the sample diluent and the Laboratory Control consisted of Evian™ mineral water diluted with deionized water to a hardness of 99 mg/L CaCO<sub>3</sub> (moderately hard water). The exposure chambers utilized for this test were 8-Liter square tubs to which 4-Liters of test solution was added to each. Each concentration was run in replicates of four. Prior to test initiation, dissolved oxygen, pH, conductivity, and temperature was measured in each chamber to ensure parameters were within acceptable limits for the survival of Rainbow Trout. These limits are defined by standardized Environmental Protection Agency (EPA) method guidance and appropriate Weston Solutions standard operating procedures (SOP). Ten juvenile



WESTON SOLUTIONS, INC.  
4729 NE View Dr.  
P.O. Box 216  
Port Gamble, WA 98364  
(360) 297-6903 / (360) 297-6905 FAX  
[www.westonsolutions.com](http://www.westonsolutions.com)

Rainbow Trout were randomly added to each chamber. Water quality measurements and survival observations were then performed daily. Fish were not fed during the course of the test.

**Results:** The Car Wash effluent caused 100 percent mortality in all treatments tested with complete mortality occurred within 24-Hours of test initiation. Survival in the Laboratory Control was 100 percent. A standard aquatic toxicity test endpoint is the  $LC_{50}$ , which is the concentration of sample that is expected to cause mortality in 50 percent of a select population of organisms. The calculated  $LC_{50}$  for test substance Car Wash was 3.125 percent. Due to the complete mortality observed in the lowest concentration tested (6.25% sample) and the 100 percent survival observed in the Laboratory Control (0 % sample), the  $LC_{50}$  is calculated to be half of the 6.25 percent value (3.125%). Additional testing with a concentration series more closely bracketing the estimated  $LC_{50}$  may provide better resolution on the actual value; however, this test confidently indicates that the  $LC_{50}$  value lies between the 6.25 percent test substance and the Laboratory Control.

Another toxicity test endpoint tool used in compliance monitoring is called Toxic Units, and is used for both chronic and acute testing. In this case, the Acute Toxic Unit (TUa) was calculated to be 32. This value is calculated as being  $100/LC_{50}$ . Many discharge monitoring programs do not allow a TUa of greater than 1 for effluent dischargers. This is usually after taking into consideration the mixing zone concentration as an effluent enters a specific waterbody. A TUa value of 32 indicates that the Car Wash effluent is 32 times more toxic than an acceptable discharged effluent under common EPA National Pollutant Discharge Elimination System (NPDES) permitted discharges.

All testing was performed consistent with our laboratory's quality assurance program. All results are intended to be considered in their entirety, and Weston Solutions is not responsible for use of less than the complete report. Results apply only to the sample tested.

If you have any questions regarding these results, or require additional testing, please call me at (360) 297-6070. Thank you for using the aquatic testing services of Weston Solutions, Inc.

Sincerely,

Brian Hester  
Laboratory Manger

Enclosed: 1 toxicity report, raw data sheets for 1 toxicity report; reference toxicity data sheets, statistical analysis and control chart; sample receipt log; 1 chain of custody

MEC Analytical Systems, Inc.

Analytical Report

Client: Environmental Partners, Inc. Date Received: 28 Aug 06  
 Project: Car Wash Date Test Started: 28 Aug 06  
 Client Sample ID: Car Wash 1, 2, 3 Date Test Ended: 01 Sep 06  
 MEC Test ID: P060828.01a, b, c Matrix: Liquid

96 Hour Acute Effluent Toxicity Bioassay

Weston Testing Protocol No. BIO012  
 EPA-821-R-02-012

Test Organism: *Oncorhynchus mykiss*  
 Age: 22 day(s) old

Test Solution mg/L	Number of Test Organisms at Start of Test	Number of Test Organisms at End of Test	Percent Survival
Control	40	40	100
6.25	40	0	0
12.5	40	0	0
25	40	0	0
50	40	0	0
100	40	0	0

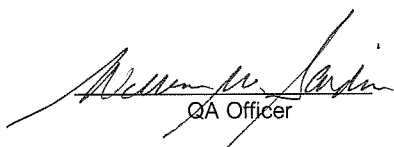
Acute Toxicity Statement for Sample Car Wash 1, 2, 3


Distribution Method	Result	Variance Method	Result
Shapiro-Wilk's Test	Normal; p > 0.01	N/A	Cannot Be Confirmed

Hypothesis Method	LOEC	NOEC	TUa	Point Estimation Method	LC <sub>50</sub>
Steel's Many-One Rank Test	<6.25	6.25	32	Linear Interpolation	3.125

**Acute Toxicity Statement:** Test substance Car Wash expressed a toxic effect on the survival of juvenile Rainbow Trout exposed for 96-hours. Survival in 100 percent test material was 0 percent after 96 hours. The calculated LC<sub>50</sub> of the Car Wash sample was 3.125 percent.

**Protocol Deviations:** The test was aerated initiation due to low dissolved oxygen levels in sample Car Wash at receipt (4.3 mg/L) and continued to test termination. Dissolved oxygen level remained within protocol limits for the duration of the test. The associated reference toxicant LC<sub>50</sub> of 183.26 ppb Cu<sup>2+</sup> falls outside the 95% confidence limits of the historical laboratory mean (68.45± 92.7 ppb Cu<sup>2+</sup>). The results of this test may indicate that the organisms used in these tests may be less sensitive to concentrations of copper as typical populations. This may reduce the ability of the toxicity test to determine toxic effects; however, since complete mortality was observed in all the test treatments of sample Car Wash, this deviation does not impact the significance of the test results.

  
 QA Officer  
 Date: 10-4-06

  
 Approved  
 Date: 10.4.06

**MEC Analytical Systems, Inc.**

**Analytical Report**

Client	Environmental Partners, Inc.	Date Received:	28 Aug 06
Project:	Car Wash	Date Test Started:	28 Aug 06
Client Sample ID:	Car Wash 1, 2, 3	Date Test Ended:	01 Sep 06
MEC Test ID:	P060828.01a, b, c	Matrix:	Liquid

**96 Hour Acute Effluent Toxicity Bioassay**

Weston Testing Protocol No.: BIO012

EPA-821-R-02-012

Test Organism: *Oncorhynchus mykiss*

**Test Solution Physical and Chemical Data**

Analyte:	<b>Alkalinity as CaCO<sub>3</sub></b>	<b>Conductivity</b>	<b>Dissolved Oxygen</b>	<b>Hardness as CaCO<sub>3</sub></b>	<b>pH</b>	<b>Chlorinity</b>
EPA Method:	310.1	120.1	360.1	130.2	150.1	330.5
Method Reporting Limit:	2 mg/L	0.02 mS/cm	1% sat.	5 mg/L	---	0.2 mg/L

Concentration (mg/L)	Hardness (mg/L CaCO <sub>3</sub> )	Alkalinity (mg/L CaCO <sub>3</sub> )
Control	99	90
100	*	*

Total Chlorine (mg/L)			
Concentration (mg/L)	Initial	Renewal	Final
Control	0.0	N/A	N/A
100	*	*	*

\* Test solution too dark for colorimetric analyses.

N/A = Chlorine not present at initiation. Subsequent analyses not required.

Concentration (mg/L)	Statistic	D.O. (% Saturation)	Temp.(°C)	Cond. (mS/cm)	pH
Control	Mean	7.3	14.7	0.19	7.3
	Minimum	6.1	14.2	0.19	6.7
	Maximum	8.3	15.1	0.20	7.9
6.25	Mean	8.3	15.1	0.20	8.0
	Minimum	8.2	14.2	0.20	7.7
	Maximum	8.4	16.0	0.20	8.3
12.5	Mean	7.5	14.6	0.21	7.6
	Minimum	7.3	14.1	0.21	7.5
	Maximum	7.7	15.0	0.21	7.7
25	Mean	7.2	15.1	0.23	7.5
	Minimum	7.2	14.2	0.23	7.4
	Maximum	7.2	16.0	0.23	7.5
50	Mean	6.5	14.6	0.25	7.5
	Minimum	6.5	14.1	0.25	7.5
	Maximum	6.5	15.0	0.26	7.5
100	Mean	6.1	14.6	0.32	7.6
	Minimum	5.2	14.2	0.32	7.5
	Maximum	6.9	15.0	0.33	7.7

# MEC Analytical Systems, Inc.

## Analytical Report

Client:	Environmental Partners, Inc.	Date Received:	28 Aug 06
Project:	Car Wash	Date Test Started:	28 Aug 06
Client Sample ID:	Car Wash 1, 2, 3	Date Test Ended:	01 Sep 06
MEC Test ID:	P060828.01a, b, c	Matrix:	Liquid

### APPENDIX

#### Pertinent Test Data

**TEST:** 96 Hour Acute Effluent Toxicity Bioassay, Weston Testing Protocol BIO012, EPA-821-R-02-012

**LAB CONTROL WATER:** diluted mineral water

Dissolved Oxygen	7.6% Saturation
Temperature	15°C
pH	7.0
Hardness	99 mg/L CaCO <sub>3</sub>
Alkalinity	90 mg/L CaCO <sub>3</sub>

**TEST ORGANISM:** Rainbow Trout, *Oncorhynchus mykiss* Age: 22 day(s) old  
Supplier: Thomas Fish Co.  
Feeding: Fed Tetramin® flake food *ad libitum* daily prior to testing.

**TEST CHAMBER:** 8000-mL containers, 4 replicate controls and 4 replicate samples at concentrations of 6.25, 12.5, 25, 50, and 100 percent test substance, brought to a 4000-mL final volume.

**EXPERIMENTAL DESIGN:**

1. Environmental Partners, Inc. personnel collected a sample at 1210-1230 hours on August 27, 2006. The product sample was delivered by Environmental Partners, Inc. at 0800 hours the following day. Sample temperature upon receipt was 4°C.
2. The temperature of the sample was adjusted to 15 ± 1°C.
3. Ten test organisms were placed in each test container.
4. Test chambers were randomized and held at 15 ± 1 °C for 96 hours with a photoperiod of 16 hours light: 8 hours darkness.
5. Test solution was renewed at 48 hours.

**MORTALITY CRITERIA:** Lack of respiratory movement and lack of reaction to gentle prodding

**ACCEPTIBILITY CRITERIA:** ≥ 90% survival in controls. Evaluation of the concentration-response relationship indicated that the data presented in this report are reliable.

**REFERENCE TOXICITY:** Toxicant: CuSO<sub>4</sub>, Lot No.: 5117-14, Received: 11/30/04, Opened: 12/14/04,  
(Control Chart Included) Expires: 6/2/06.

96 Hour LC50:	183.26 ppb
Laboratory Mean:	68.45 ppb
Test Date:	8.28/06

Outside 95 % Confidence Limits\*  
\* See protocol deviations

**STUDY DIRECTOR:** B. Hester  
**INVESTIGATORS:** T. Schuh, J. Word, G. Zandpoor, C. Word



Weston Solutions

BIO012C

## 96 Hour Acute Toxicity Test for Rainbow Trout

Client	EPI
Project:	Car Wash
Client Sample ID:	Car Wash 1, 2, 3
Weston Sample ID:	P060828.01a, b, c
Weston Protocol:	BIO 012
Study Director:	3H

Date Received:	8/28/06
Date Test Started:	8/28/06
Date Test Ended:	9.1.06
Matrix:	Liquid
Species:	O. mykiss
Organisms / Chamber:	10

	Conc.	D.O. (mg/L)		Temp (°C)		Cond. (mS/cm)		pH		Hardness (mg/L CaCO <sub>3</sub> )	Alkalinity (mg/L CaCO <sub>3</sub> )
		Meter #		Meter #		Meter #		Meter #			
Day 0 (0 Hours)	Control	1	7.6	1	15	1	0.191	1	7.0	99	90
Date: 8/28/06	6.25		8.4		16		0.198		8.3		
Replicate: 1	12.5		7.7		15		0.207		7.7		
Time: 1700	25		7.2		16		0.232		7.4		
Technician: JW	50		6.5		15		0.253		7.5		
Sample ID: P060828.01A+B	100		5.2		15		0.321		7.5		
24 Hours	Control	5	6.1	5	15.1	1	0.192	5	6.65		
Date: 8/29/06	6.25		8.2		14.2		0.200		7.67		
Replicate: 2	12.5		7.3		14.1		0.210		7.54		
Time: 1510	25		7.2		14.2		0.233		7.5		
Technician: CW	50		6.5		14.1		0.256		7.5		
	100		6.9		14.2		0.325		7.7		
48 Hours	Control	1	8.3	1	14.2	1	0.195	1	7.3	99	90
Date: 8/30/06											
Replicate: 3											
Time: 1700											
Technician: JW											
Sample ID: NA											
72 Hours	Control	1	8.3	1	14.3	1	0.198	1	7.5		
Date: 8/31/06											
Replicate: 4											
Time: 0800											
Technician: N/A											
96 Hours	Control	1	6.4	1	14.8	1	0.199	1	7.9		
Date: 9/1/06											
Replicate:											
Time: 1400											
Technician: JW											

Total chlorine  
mg/L  
0.0

0.0

Start Time:	1600
End Time:	1535
Supplier:	Thomas Fish Co.
Organism Batch:	TFC 5482 Age: 22 days
Hobo Temp. No.:	N/A
Test Location:	Bath 10

Dilution Water Batch: DMW 001		
pH: 7.0	DO: 7.6	Temp: 15
Ref Tox: P-051027.72	Lot No.: 5117-14	
LC50: 183.26	Test Date: 8.28.06	
Lab Mean: 68.45		
Test Acceptability: X ≥ 90% Control Survival		

- ① Very slow aeration initiated Due to low DO on arrival. 8/28/06  
② i.e. 8/29/06 CW ③ Test solution too dark for colorimetric analysis 8.28.06 BH



Weston Solutions

BIO012C

## 96 Hour Acute Toxicity Test for Rainbow Trout

Client:	EPI
Project:	CAR WASH
Client Sample ID:	Car Wash 1, 2, 3
Weston Sample ID:	P060828.01n, b, c
Weston Protocol:	0 BIO 012
Study Director:	Rdt

Date Received:	8/28/06
Date Test Started:	8/28/06
Date Test Ended:	9.1.06
Matrix:	L
Species:	O. mykiss
Organisms/Chamber:	10

Conc.	Rep	24 Hours		48 Hours		72 Hours		96 Hours	
		Date: 8/29/06 Time: 1515		Date: 8/30/06 Time: 62		Date: 8/31/06 Time: 62		Date: 9/1/06 Time: 4	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
6.25	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
12.5	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
25.0	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
50	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
100	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
Initials:		CW		GZ					

Date / Time:		Technician:			
Length (mm)		Weight (g)		Length (mm)	
1)				6)	
2)				7)	
3)				8)	
4)				9)	
5)				10)	
Average Length (mm):			Average Weight (g):		

Total Volume (L) per Replicate:		Total Grams of Fish Flesh per Liter:	
---------------------------------	--	--------------------------------------	--

Note: All fish taken from Control Rep 1 unless otherwise specified.



# Acute Fish Test-96 Hour

Start Date: 8/28/2006 16:00 Test ID: P060828.01a, b, c Sample ID: Car Wash 1, 2, 3  
 End Date: 9/1/2006 15:35 Lab ID: PGL- Port Gamble Laborator Sample Type: DMR-Discharge Monitoring Report  
 Sample Date: Protocol: EPAA 02-EPA Acute Test Species: OM-Oncorhynchus mykiss  
 Comments:

Conc-%	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
6.25	0.0000	0.0000	0.0000	0.0000
12.5	0.0000	0.0000	0.0000	0.0000
25	0.0000	0.0000	0.0000	0.0000
50	0.0000	0.0000	0.0000	0.0000
100	0.0000	0.0000	0.0000	0.0000

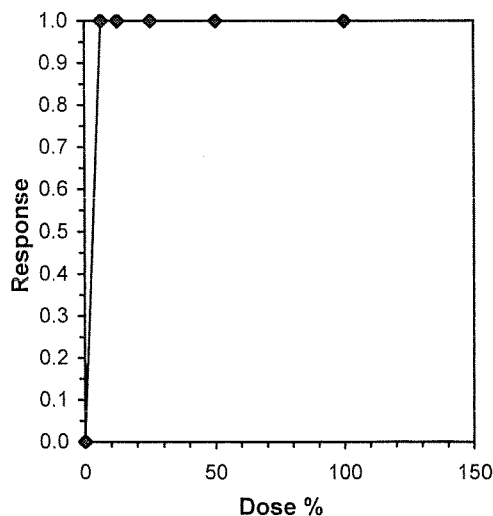
Conc-%	Mean	N-Mean	Transform: Untransformed					Rank Sum	1-Tailed Critical	Isotonic	
			Mean	Min	Max	CV%	N			Mean	N-Mean
Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4			1.0000	1.0000
*6.25	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	10.00	10.00	0.0000	0.0000
*12.5	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	10.00	10.00	0.0000	0.0000
*25	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	10.00	10.00	0.0000	0.0000
*50	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	10.00	10.00	0.0000	0.0000
*100	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4	10.00	10.00	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates normal distribution (p > 0.01)	1	0.884		
Equality of variance cannot be confirmed				
Hypothesis Test (1-tail, 0.05)	NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test	<6.25	6.25		
Treatments vs Control				

## Linear Interpolation (200 Resamples)

Point	%	SD	95% CL(Exp)		Skew
IC05*	0.3125	0.0000	0.3125	0.3125	1.0076
IC10*	0.6250	0.0000	0.6250	0.6250	#DIV/0!
IC15*	0.9375	0.0000	0.9375	0.9375	#DIV/0!
IC20*	1.2500	0.0000	1.2500	1.2500	#DIV/0!
IC25*	1.5625	0.0000	1.5625	1.5625	#DIV/0!
IC40*	2.5000	0.0000	2.5000	2.5000	#DIV/0!
IC50*	3.1250	0.0000	3.1250	3.1250	#DIV/0!

\* indicates IC estimate less than the lowest concentration



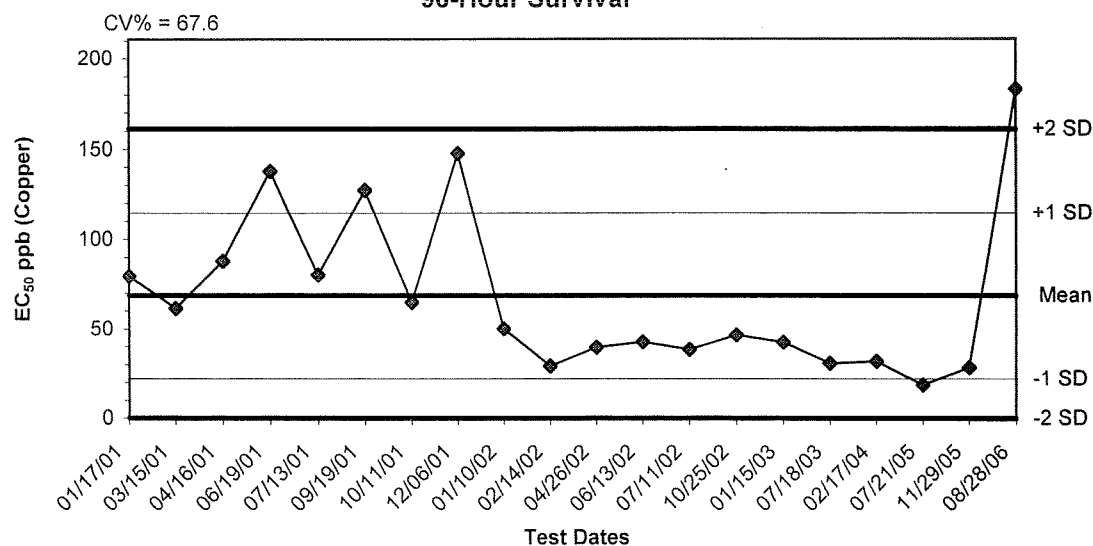
Test: AC-Acute Fish Test  
 Species: OM-Oncorhynchus mykiss  
 Sample ID: Car Wash 1, 2, 3  
 Start Date: 8/28/2006 16:00

Test ID: P060828.01  
 Protocol: EPAA 02-EPA Acute  
 Sample Type: DMR-Discharge Monitoring Report  
 End Date: 9/1/2006 15: Lab ID: PGL- Port Gamble Laboratory

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	Control	10				10	
	2	2	Control	10				10	
	3	3	Control	10				10	
	4	4	Control	10				10	
	5	1	6.250	10				0	
	6	2	6.250	10				0	
	7	3	6.250	10				0	
	8	4	6.250	10				0	
	9	1	12.500	10				0	
	10	2	12.500	10				0	
	11	3	12.500	10				0	
	12	4	12.500	10				0	
	13	1	25.000	10				0	
	14	2	25.000	10				0	
	15	3	25.000	10				0	
	16	4	25.000	10				0	
	17	1	50.000	10				0	
	18	2	50.000	10				0	
	19	3	50.000	10				0	
	20	4	50.000	10				0	
	21	1	100.000	10				0	
	22	2	100.000	10				0	
	23	3	100.000	10				0	
	24	4	100.000	10				0	

Comments:

***Oncorhynchus mykiss* Reference Toxicant Control Chart:  
96-Hour Survival**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
01/17/01	79.4878	68.4472	22.1628	0.0000	114.7315	161.0158
03/15/01	61.4720	68.4472	22.1628	0.0000	114.7315	161.0158
04/16/01	87.9825	68.4472	22.1628	0.0000	114.7315	161.0158
06/19/01	137.7600	68.4472	22.1628	0.0000	114.7315	161.0158
07/13/01	80.1567	68.4472	22.1628	0.0000	114.7315	161.0158
09/19/01	127.2790	68.4472	22.1628	0.0000	114.7315	161.0158
10/11/01	64.7289	68.4472	22.1628	0.0000	114.7315	161.0158
12/06/01	147.8140	68.4472	22.1628	0.0000	114.7315	161.0158
01/10/02	50.1660	68.4472	22.1628	0.0000	114.7315	161.0158
02/14/02	29.1790	68.4472	22.1628	0.0000	114.7315	161.0158
04/26/02	39.7384	68.4472	22.1628	0.0000	114.7315	161.0158
06/13/02	42.6380	68.4472	22.1628	0.0000	114.7315	161.0158
07/11/02	38.3651	68.4472	22.1628	0.0000	114.7315	161.0158
10/25/02	46.5870	68.4472	22.1628	0.0000	114.7315	161.0158
01/15/03	42.5565	68.4472	22.1628	0.0000	114.7315	161.0158
07/18/03	30.7498	68.4472	22.1628	0.0000	114.7315	161.0158
02/17/04	31.8198	68.4472	22.1628	0.0000	114.7315	161.0158
07/21/05	18.7500	68.4472	22.1628	0.0000	114.7315	161.0158
11/29/05	28.4485	68.4472	22.1628	0.0000	114.7315	161.0158
08/28/06	183.2640	68.4472	22.1628	0.0000	114.7315	161.0158

Updated 9/26/06 BH

# Acute Fish Test-96 Hour

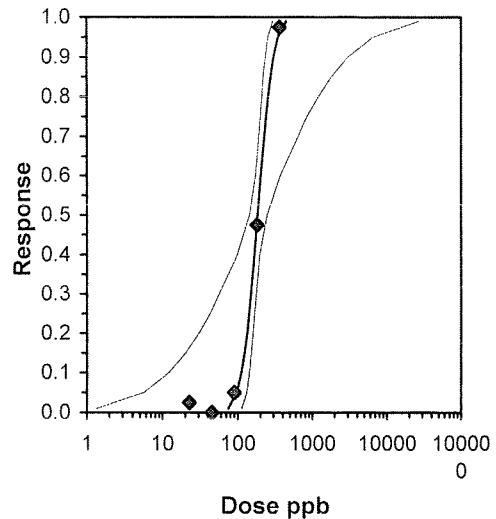
Start Date: 8/28/2006 16:30 Test ID: P051027.72 Sample ID: REF-Ref Toxicant  
End Date: 9/1/2006 14:10 Lab ID: PGL- Port Gamble Laborator Sample Type: CUSO-Copper sulfate  
Sample Date: Protocol: EPAA 02-EPA Acute Test Species: OM-Oncorhynchus mykiss  
Comments:

Conc-ppb	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
22.5	1.0000	1.0000	1.0000	0.9000
45	1.0000	1.0000	1.0000	1.0000
90	1.0000	1.0000	0.8000	1.0000
180	0.5000	0.5000	0.6000	0.5000
360	0.0000	0.0000	0.0000	0.1000

Conc-ppb	Transform: Untransformed							Rank Sum	1-Tailed Critical	Mean	N-Mean
	Mean	N-Mean	Mean	Min	Max	CV%	N				
Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4			1.0000	0.0000
22.5	0.9750	0.9750	0.9750	0.9000	1.0000	5.128	4	16.00	10.00	0.9750	0.0250
45	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4	18.00	10.00	1.0000	0.0000
90	0.9500	0.9500	0.9500	0.8000	1.0000	10.526	4	16.00	10.00	0.9500	0.0500
*180	0.5250	0.5250	0.5250	0.5000	0.6000	9.524	4	10.00	10.00	0.5250	0.4750
*360	0.0250	0.0250	0.0250	0.0000	0.1000	200.000	4	10.00	10.00	0.0250	0.9750

Auxiliary Tests					Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution (p <= 0.01)					0.88152	0.884	-1.1417	3.32127
Equality of variance cannot be confirmed								
Hypothesis Test (1-tail, 0.05)					NOEC	LOEC	ChV	TU
Steel's Many-One Rank Test					90	180	127.279	
Treatments vs Control								

Maximum Likelihood-Probit											
Parameter	Value	SE	95% Fiducial Limits		Control	Chi-Sq	Critical	P-value	Mu	Sigma	Iter
Slope	5.97692	2.49772	1.08139	10.8724	0	0.1671	7.81473	0.98	2.26308	0.16731	6
Intercept	-8.5262	5.64594	-19.592	2.53982							
TSCR											
Point	Probits	ppb	95% Fiducial Limits								
EC01	2.674	74.7919	1.32853	113.327							
EC05	3.355	97.2469	5.63162	131.811							
EC10	3.718	111.856	12.1272	143.285							
EC15	3.964	122.934	20.2964	151.969							
EC20	4.158	132.515	30.4771	159.687							
EC25	4.326	141.328	43.0423	167.213							
EC40	4.747	166.222	98.1868	196.469							
EC50	5.000	183.264	142.671	244.676							
EC60	5.253	202.053	172.153	366.938							
EC75	5.674	237.643	199.869	847.115							
EC80	5.842	253.448	209.04	1197.8							
EC85	6.036	273.201	219.484	1800.02							
EC90	6.282	300.257	232.659	3014.22							
EC95	6.645	345.365	252.805	6493.55							
EC99	7.326	449.054	293.942	27535.3							



Test: AC-Acute Fish Test Species: OM-Oncorhynchus mykiss Sample ID: REF-Ref Toxicant Start Date: 8/28/2006 16:30	Test ID: P051027.72 Protocol: EPAA 02-EPA Acute Sample Type: CUSO-Copper sulfate End Date: 9/1/2006 14: Lab ID: PGL- Port Gamble Laboratory
---	--

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	Control	10				10	
	2	2	Control	10				10	
	3	3	Control	10				10	
	4	4	Control	10				10	
	5	1	22.500	10				10	
	6	2	22.500	10				10	
	7	3	22.500	10				10	
	8	4	22.500	10				9	
	9	1	45.000	10				10	
	10	2	45.000	10				10	
	11	3	45.000	10				10	
	12	4	45.000	10				10	
	13	1	90.000	10				10	
	14	2	90.000	10				10	
	15	3	90.000	10				8	
	16	4	90.000	10				10	
	17	1	180.000	10				5	
	18	2	180.000	10				5	
	19	3	180.000	10				6	
	20	4	180.000	10				5	
	21	1	360.000	10				0	
	22	2	360.000	10				0	
	23	3	360.000	10				0	
	24	4	360.000	10				1	

Comments:



96 Hour Rainbow Trout (with Renewal)  
Reference Toxicant Test

Test ID: P051027.72	Replicates: 4	Study Director: BH	Location: BATH 10							
Dilution Water Batch: DMW 001	Organism Batch: TFC 5482	Associated Test(s): EPL	No. of Organisms: 10							
Toxicant: Copper Sulfate (0.509gCu/LCuSO <sub>4</sub> )	Date Prepared: 8/28/06	Initials: GZ								
Lot Number: 5117-14										
Target Concentrations: 360, 180, 90, 45, 22.5 ppb	Quantity of Stock Toxicant:					Quantity of Diluent:				
	Target	Actual				Target	Actual			
		R1	R2	R3	R4		R1	R2	R3	R4
360 ppb	2.829 mL	2.8283	2.8288	2.82907	2.82970	4 L	4000.0	4000.0	4000.0	4000.0
180 ppb	1.415 mL	1.41500	1.41523	1.41531	1.41500	4 L	4000.0	4000.0	4000.4	4000.0
90 ppb	0.707 mL	0.70692	0.70733	0.70731	0.70731	4 L	4000.0	4000.0	4000.0	4000.0
45 ppb	0.354 mL	0.35422	0.35424	0.35438	0.35432	4 L	4000.0	4000.1	4000.0	4000.0
22.5 ppb	0.177 mL	0.17704	0.17740	0.17691	0.17691	4 L	4000.0	4000.0	4000.0	4000.0

0 Hours

Date:

WQ Time:

Start Time:

Initials:

8/28/06

1910

1630

JW

STOCK

	Control	22.5	45	90	180	360
D.O. (mg/L)	7.591	9.0	8.9	10.1	9.8	10.0
Temperature	15	14	16	15	15	15
Conductivity	0.185	0.184	0.184	0.178	0.175	0.175
pH	7.5	9.0	7.2	7.2	7.2	7.1

Survival Data

24 Hours

Date:

8/29/06

Time:

1550

Initials:

CW

	Control	22.5	45	90	180	360
No. Alive Rep 1	10	10	10	10	10	3(7)
No. Alive Rep 2	10	10	10	10	8(2)	4(6)
No. Alive Rep 3	10	10	10	10	10	3(7)
No. Alive Rep 4	10	10	10	10	8(2)	6(4)

① WC 8/28/06 JW



## 96 Hour Rainbow Trout (with Renewal) Reference Toxicant Test

2.829  
1.415  
0.707  
0.354  
0.177

48 Hour Renewal Information			Date: 8/30/06 Initials: GZ			
Concentration	Toxicant Amount	Diluent Amount				
360 ppb	2.82889	4000.0				
180 ppb	1.41487	4000.0				
90 ppb	0.70721	↓				
45 ppb	0.35409					
22.5 ppb	0.17728					

48 Hour Survival		Date: 8/30/06		Time: 1705		Initials: JZ	
	Control	22.5	45	90	180	360	
No. Alive Rep 1	10	10	10	10	5(5)	0(3)	
No. Alive Rep 2	10	10	10	10	5(3)	0(4)	
No. Alive Rep 3	10	10	10	8(2)	7(3)	0(3)	
No. Alive Rep 4	10	9(1)	10	10	5(3)	1(5)	

72 Hours		Date: 8/31		Time: 1000		Initials: GZ	
	Control	22.5	45	90	180	360	
No. Alive Rep 1	10	10	10	10	5	—	
No. Alive Rep 2	10	10	10	10	5	—	
No. Alive Rep 3	10	10	10	8	7	—	
No. Alive Rep 4	10	9	10	10	5	1	

96 Hours		Date: 7/1/06		WQ Time: 1400		Replicate: 4		Initials: JZ	
STOCK									
	Control	22.5	45	90	180	360			
D.O. (mg/L)	8.2	8.1	7.8	7.8	8.2	8.5			
Temperature	14.6	14.4	14.3	14.5	14.6	14.8			
Conductivity									
pH	8.3	8.2	8.2	8.1	8.0	7.9			

96 Hour Survival Data		End Time: 1410		Initials: JZ		
	Control	22.5	45	90	180	360
No. Alive Rep 1	10	10	10	10	5	—
No. Alive Rep 2	10	10	10	10	5	—
No. Alive Rep 3	10	10	10	8	6(1)	—
No. Alive Rep 4	10	9	10	10	5	1

## **APPENDIX B**

### **Laboratory Report – Simulated Effluent Fish Toxicity Test**





WESTON SOLUTIONS, INC.  
4729 NE View Dr.  
P.O. Box 216  
Port Gamble, WA 98364  
(360) 297-6903 / (360) 297-6905 FAX  
www.westonsolutions.com

December 21, 2006

Dr. Jeff Dengler  
Environmental Partners, Inc.  
295 NE Gilman Blvd., Suite 201  
Issaquah, Washington 98027

Re: 96-Hour Rainbow Trout Toxicity Testing Results – Blue Coral Concentrate

Dear Dr. Dengler:

Enclosed, please find the report for the acute toxicity test performed on the Blue Coral brand car wash detergent, received on the 21<sup>st</sup> of November. Toxicity testing was conducted using juvenile Rainbow Trout between the 29<sup>th</sup> of November and 3<sup>rd</sup> of December, 2006. The results of this test are listed in the table below.

Test	Sample ID	Control Survival	LC <sub>50</sub>
Rainbow Trout 96-Hour Survival	Blue Coral Concentrate	100%	1614.41 µg/L

**Methods:** This testing investigated the survival of juvenile Rainbow Trout exposed to a concentration series of the Blue Coral product over a 96-Hour period. Previous testing with Car Wash effluent (comprised of the same product) resulted in a calculated LC<sub>50</sub> of 3.125 percent. Due to the complete mortality observed in the lowest concentration tested (6.25% sample) and the 100 percent survival observed in the Laboratory Control (0 % sample), the LC<sub>50</sub> was calculated to be half of the 6.25 percent value (3.125%). This additional testing included a concentration series that more closely bracketed the estimated LC<sub>50</sub> in order to provide increased resolution of the actual value. Because additional samples of Car Wash effluent were not available, the preparation of a mock effluent was proposed. This mock effluent was prepared in the laboratory with the Blue Coral product and laboratory water to simulate the Car Wash effluent. The proposed concentrations of mock effluent to be tested were 10, 1, 0.5, 0.1, 0.05, and 0.01 percent effluent. Calculations for the concentration series utilized the prescribed recipe for creating a batch of the Blue Coral wash water and included an estimation of dilution after rinsing<sup>1</sup>. This information was utilized to estimate the actual concentration of Blue Coral



WESTON SOLUTIONS, INC.  
4729 NE View Dr.  
P.O. Box 216  
Port Gamble, WA 98364  
(360) 297-6903 / (360) 297-6905 FAX  
www.westonsolutions.com

product contained in a “mock” effluent. The equivalent concentrations of the proposed mock effluent above were 5300, 530, 265, 53, 26.5, and 5.25µg/L (parts per billion) test substance diluted with laboratory water.

The water used for the sample diluent and the Laboratory Control consisted of carbon filtered tap water with a hardness of 62 mg/L CaCO<sub>3</sub> (slightly hard water). The exposure chambers utilized for this test were 8-Liter square tubs to which 4-Liters of test solution was added to each. Each concentration was run in replicates of four. Prior to test initiation, dissolved oxygen, pH, conductivity, and temperature was measured in each chamber to ensure parameters were within acceptable limits for the survival of Rainbow Trout. These limits are defined by standardized Environmental Protection Agency (EPA) method guidance and appropriate Weston Solutions standard operating procedures (SOP). Ten juvenile Rainbow Trout were randomly added to each chamber. Water quality measurements and survival observations were then performed daily. Fish were not fed during the course of the test.

**Results:** The Blue Coral concentrate caused 100 percent mortality in the highest concentration tested (5300 µg/L). As in the previous study, complete mortality was observed in the first 24 hours of exposure. Survival in the next highest concentration (530 µg/L) was 97.5 percent, with all other treatments, including the laboratory control, having 100 percent survival. The calculated LC<sub>50</sub> for Blue Coral concentrate was 1614.41 µg/L. This value equates to 3.05 percent of mock effluent, which correlates with the Car Wash effluent LC<sub>50</sub> of 3.125 percent.

It is important to note that the mock effluent did not take into consideration the chemicals or particulate matter from the washed cars and roads that make up the Car Wash effluent. The effect of the more complex Car Wash effluent interacting with the soap concentrate may increase or decrease the toxicity of the sample when compared to the soap concentrate alone. One must use caution when directly comparing the results of these two tests

All testing was performed consistent with our laboratory’s quality assurance program. All results are intended to be considered in their entirety, and Weston Solutions is not responsible for use of less than the complete report. Results apply only to the sample tested. If you have any questions regarding these results, or require additional testing, please call me at (360) 297-6070. Thank you for using the aquatic testing services of Weston Solutions, Inc.

Sincerely,

Brian Hester  
Laboratory Manger

Enclosed: 1 toxicity report, raw data sheets for 1 toxicity report; reference toxicity data sheets, statistical analysis and control chart; 1 chain of custody

<sup>1</sup> Email communiqué with Jeff Dengler. 26<sup>th</sup> October 2006.

# Weston Solutions, Inc.

## Analytical Report

Client	Environmental Partners, Inc.	Date Received:	22 Nov 06
Project:	Coral Blue Product Testing	Date Test Started:	29 Nov 06
Client Sample ID:	Blue Coral (concentrate)	Date Test Ended:	03 Dec 06
MEC Test ID:	P061122.01	Matrix:	Liquid

### 96 Hour Acute Effluent Toxicity Bioassay

Weston Testing Protocol No. BIO012  
WDOE WQ-R-95-80

Test Organism: *Oncorhynchus mykiss*  
Age: 16 day(s) old

Test Solution µg/L	Number of Test Organisms at Start of Test	Number of Test Organisms at End of Test	Percent Survival
Control	40	40	100
5.25	40	40	100
26.5	40	40	100
53	40	40	100
265	40	40	100
530	40	39	97.5
5300	40	0	0

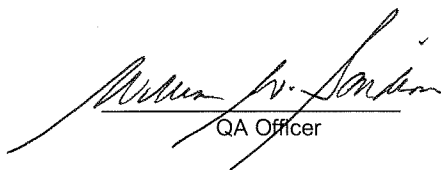
### Acute Toxicity Statement for Sample Blue Coral (concentrate)


Distribution Method	Result	Variance Method	Result
Shapiro-Wilk's Test	Non-Normal; $p \leq 0.01$	N/A	Cannot Be Confirmed

Hypothesis Method	LOEC	NOEC	TUa	Point Estimation Method	LC <sub>50</sub>
Steel's Many-One Rank Test	530 µg/L	5300 µg/L	NA	Trimmed Spearman-Kärber	1614.41 µg/L

**Acute Toxicity Statement:** Test substance Blue Coral expressed a toxic effect on the survival of juvenile Rainbow Trout exposed for 96-hours. Survival in 5300µg/L test material was 0 percent after 96 hours. The calculated LC<sub>50</sub> of the Car Wash sample was 1614.41 percent.

**Protocol Deviations:** The test was aerated at initiation due to low dissolved oxygen levels in previous testing and continued to test termination. Dissolved oxygen level remained within protocol limits for the duration of the test. The associated reference toxicant LC<sub>50</sub> of 112.50 ppb Cu<sup>2+</sup> falls with the 95% confidence limits of the historical laboratory mean (710.10 ± 94.6 ppb Cu<sup>2+</sup>). The results of this test indicate that the organisms used in these tests are relatively as sensitive to concentrations of copper as previous testing populations.

 12/21/06  
QA Officer Date

 12/21/06  
Approved Date

# Weston Solutions, Inc.

## Analytical Report

Client	Environmental Partners, Inc.	Date Received:	22 Nov 06
Project:	Coral Blue Product Testing	Date Test Started:	29 Nov 06
Client Sample ID:	Blue Coral (concentrate)	Date Test Ended:	03 Dec 06
MEC Test ID:	P061122.01	Matrix:	Liquid

### 96 Hour Acute Effluent Toxicity Bioassay

Weston Testing Protocol No.: BIO012  
WDOE WQ-R-95-80

Test Organism: *Oncorhynchus mykiss*

### Test Solution Physical and Chemical Data

Analyte:	Alkalinity as CaCO <sub>3</sub>	Conductivity	Dissolved Oxygen	Hardness as CaCO <sub>3</sub>	pH	Chlorinity
EPA Method:	310.1	120.1	360.1	130.2	150.1	330.5
Method Reporting Limit:	2 mg/L	0.02 mS/cm	1% sat.	5 mg/L	---	0.2 mg/L

Concentration	Hardness (mg/L CaCO <sub>3</sub> )	Alkalinity (mg/L CaCO <sub>3</sub> )
Control / Diluent	62	140

Total Chlorine (mg/L)			
Concentration	Initial	Renewal	Final
Control / Diluent	0.0	N/A	N/A

N/A = Chlorine not present at initiation. Subsequent analyses not required.

Concentration (µg/L)	Statistic	D.O. (% Saturation)	Temp.(°C)	Cond. (mS/cm)	pH
Control	Mean	10.2	11.1	0.32	8.1
	Minimum	9.1	10.6	0.31	7.6
	Maximum	11.5	11.5	0.33	8.9
5.25	Mean	10.2	11.1	0.32	8.3
	Minimum	9.6	10.6	0.31	7.8
	Maximum	11.5	11.5	0.32	8.8
26.5	Mean	10.2	11.1	0.32	8.3
	Minimum	9.2	10.6	0.31	7.8
	Maximum	11.6	11.5	0.32	8.8
53	Mean	10.4	11.1	0.32	8.2
	Minimum	9.4	10.6	0.31	7.8
	Maximum	11.6	11.7	0.32	8.6
265	Mean	10.1	11.1	0.32	8.2
	Minimum	8.5	10.6	0.31	7.9
	Maximum	11.6	11.4	0.32	8.4
530	Mean	10.2	11.1	0.32	8.2
	Minimum	8.5	10.6	0.31	7.9
	Maximum	11.5	11.1	0.32	8.4
5300	Mean	10.8	10.8	0.33	8.7
	Minimum	10.0	10.7	0.33	8.0
	Maximum	11.6	10.8	0.34	9.3

## Weston Solutions, Inc.

### Analytical Report

Client:	Environmental Partners, Inc.	Date Received:	22 Nov 06
Project:	Coral Blue Product Testing	Date Test Started:	29 Nov 06
Client Sample ID:	Blue Coral (concentrate)	Date Test Ended:	03 Dec 06
MEC Test ID:	P061122.01	Matrix:	Liquid

### APPENDIX

#### Pertinent Test Data

**TEST:** 96 Hour Acute Effluent Toxicity Bioassay, Weston Testing Protocol BIO012, WDOE WQ-R-95-80

**LAB CONTROL WATER:** diluted mineral water

Dissolved Oxygen	11.5% Saturation
Temperature	10.6°C
pH	7.8
Hardness	62 mg/L CaCO <sub>3</sub>
Alkalinity	140 mg/L CaCO <sub>3</sub>

**TEST ORGANISM:** Rainbow Trout, *Oncorhynchus mykiss* Age: 16 day(s) old  
Supplier: Thomas Fish Co.  
Feeding: Trout chow granular food *ad libitum* daily prior to testing.

**TEST CHAMBER:** 8000-mL containers, 4 replicate controls and 4 replicate samples at concentrations of 5.25, 25.6, 53, 265, 530, and 5300 µg/L test substance, brought to a 4000-mL final volume.

**EXPERIMENTAL DESIGN:**

1. Environmental Partners, Inc. personnel collected a sample at 1030 hours on November 20, 2006. The product sample was delivered to Weston Solutions, Inc. at 1200 hours the following day.
2. The temperature of the sample was adjusted to  $12 \pm 1^\circ\text{C}$ .
3. Ten test organisms were placed in each test container.
4. Test chambers were randomized and held at  $12 \pm 1^\circ\text{C}$  for 96 hours with a photoperiod of 16 hours light: 8 hours darkness.
5. Test solution was renewed at 48 hours.

**MORTALITY CRITERIA:** Lack of respiratory movement and lack of reaction to gentle prodding

**ACCEPTIBILITY CRITERIA:**  $\geq 90\%$  survival in controls. Evaluation of the concentration-response relationship indicated that the data presented in this report are reliable.

**REFERENCE TOXICITY:** Toxicant: CuSO<sub>4</sub>, Lot No.: 5117-14, Received: 10/27/05, Opened: 11/15/05,  
(Control Chart Included) Expires: 4/28/07.  
96 Hour LC50: 112.5 ppb  
Laboratory Mean: 70.1 ppb  
Test Date: 11/28/2006

**STUDY DIRECTOR:** B. Hester  
**INVESTIGATORS:** T. Schuh, J. Word, G. Zandpoor, C. Word

# Acute Fish Test-96 Hour

Start Date: 11/29/2006 11:30 Test ID: P061122.01 Sample ID: CORAL BLUE  
 End Date: 12/3/2006 12:00 Lab ID: PGL- Port Gamble Laboratory Sample Type: EFF2-Industrial  
 Sample Date: Protocol: WDOE WQ-R95-80 Test Species: OM-Oncorhynchus mykiss  
 Comments:

Conc-ppb	1	2	3	4
Control	1.0000	1.0000	1.0000	1.0000
5.25	1.0000	1.0000	1.0000	1.0000
26.5	1.0000	1.0000	1.0000	1.0000
53	1.0000	1.0000	1.0000	1.0000
265	1.0000	1.0000	1.0000	1.0000
530	1.0000	1.0000	0.9000	1.0000
5300	0.0000	0.0000	0.0000	0.0000

Conc-ppb	Transform: Untransformed							Rank Sum	1-Tailed Critical	Mean	N-Mean
	Mean	N-Mean	Mean	Min	Max	CV%	N				
Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4			1.0000	0.0000
5.25	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4	18.00	10.00	1.0000	0.0000
26.5	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4	18.00	10.00	1.0000	0.0000
53	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4	18.00	10.00	1.0000	0.0000
265	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	4	18.00	10.00	1.0000	0.0000
530	0.9750	0.9750	0.9750	0.9000	1.0000	5.128	4	16.00	10.00	0.9750	0.0250
5300	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	4			0.0000	1.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Shapiro-Wilk's Test indicates non-normal distribution ( $p \leq 0.01$ )	0.46508	0.884	-3.0206	13.9892
Equality of variance cannot be confirmed				

## Hypothesis Test (1-tail, 0.05)

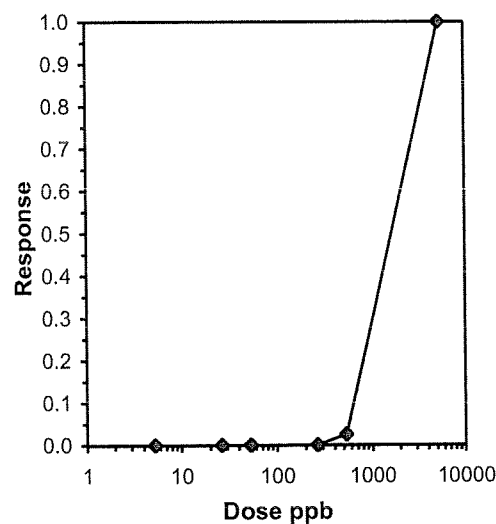
Steel's Many-One Rank Test

Treatments vs Control

NOEC	LOEC	ChV	TU
530	5300	1676.01	

## Trimmed Spearman-Kärber

Trim Level	EC50	95% CL	
0.0%	1614.41	1277.77	2039.74
5.0%	1627.25	1346.91	1965.95
10.0%	1627.25	1346.91	1965.95
20.0%	1627.25	1346.91	1965.95
Auto-0.0%	1614.41	1277.77	2039.74



Test: AC-Acute Fish Test  
 Species: OM-Oncorhynchus mykiss  
 Sample ID: Coral Blue  
 Start Date: 11/29/2006 11:30

Test ID: P061122.01  
 Protocol: WDOE WQ-R95-80  
 Sample Type: EFF2-Industrial  
 Lab ID: PGL- Port Gamble Laboratory

Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	Control	10				10	
	2	2	Control	10				10	
	3	3	Control	10				10	
	4	4	Control	10				10	
	5	1	5.250	10				10	
	6	2	5.250	10				10	
	7	3	5.250	10				10	
	8	4	5.250	10				10	
	9	1	26.500	10				10	
	10	2	26.500	10				10	
	11	3	26.500	10				10	
	12	4	26.500	10				10	
	13	1	53.000	10				10	
	14	2	53.000	10				10	
	15	3	53.000	10				10	
	16	4	53.000	10				10	
	17	1	265.000	10				10	
	18	2	265.000	10				10	
	19	3	265.000	10				10	
	20	4	265.000	10				10	
	21	1	530.000	10				10	
	22	2	530.000	10				10	
	23	3	530.000	10				9	
	24	4	530.000	10				10	
	25	1	5300.000	10				0	
	26	2	5300.000	10				0	
	27	3	5300.000	10				0	
	28	4	5300.000	10				0	

Comments:



96 Hour Acute Toxicity Test for Rainbow Trout

Client	EPI
Project:	Coral blue product testing
Client Sample ID:	BLUECORAL CONC.
Weston Sample ID:	7061122.01
Weston Protocol:	BIO 012C
Study Director:	BH

Date Received:	11.22.06
Date Test Started:	11.29.06
Date Test Ended:	12/3/06
Matrix:	L
Species:	O. mykiss
Organisms / Chamber:	10

	Conc.	D.O. (mg/L)		Temp (°C)		Cond. (mS/cm)		pH		Hardness (mg/L CaCO <sub>3</sub> )	Alkalinity (mg/L CaCO <sub>3</sub> )
		Meter #		Meter #		Meter #		Meter #			
Day 0 (0 Hours)	Control	1	11.5	1	10.6	1	325	1	7.8	62	140
Date: 11.29.06	5.25 ppb		11.5		10.6		323		8.3		
Replicate: 1	26.5		11.6		10.6		322		8.3		
Time: 1100	53		11.6		10.6		323		7.9		
Technician: JW	265		11.6		10.6		323		7.9		
Sample ID:	530		11.5		10.6		324		8.0		
	5300		11.6		10.7		335		8.0		
24 Hours	Control	1	10.0	1	10.8	1	319	1	8.9		
Date: 11.30.06	5.25		10.0		11.3		315		8.8		
Replicate: 2	26.5		10.2		11.0		317		8.8		
Time: 1015	53		10.0		10.9		317		8.6		
Technician: TS	265		10.1		11.2		318		8.5		
	530		10.2		11.1		318		8.4		
	5300		10.0		10.8		331		9.3		
48 Hours	Control	1	10.6	1	11.1	1	307	1	7.6	69	148
Date: 12.1.06	5.25		10.1		11.0		312		8.0		
Replicate: 3	26.5		10.8		10.7		314		8.1		
Time: 1045	53		10.6		11.0		313		8.2		
Technician: TS	265		10.6		11.1		317		8.2		
Sample ID:	530		10.69		11.2		308		8.3		
	5300										
72 Hours	Control	1	9.8	1	11.5	1	310	1	8.5		
Date: 12/2/06	5.25		9.6		11.2		316		8.4		
Replicate: 4	26.5		9.4		11.5		309		8.4		
Time: 1415	53		10.4		11.7		310		8.4		
Technician: JS	265		9.8		11.4		315		8.4		
	530		9.7		11.3		315		8.4		
	5300										
96 Hours	Control	1	9.1	1	11.3	1	318	1	7.7		
Date: 12/3/06	5.25		9.6		11.5		309		7.8		
Replicate: 1	26.5		9.2		11.5		315		7.8		
Time: 1205	53		9.4		11.4		314		7.8		
Technician: TS	265		10.5		11.4		324		7.8		
	530		8.5		11.4		314		7.9		
	5300										

Start Time:	1130
End Time:	1200
Supplier:	Thomas Fish Co.
Organism Batch:	TFC 5207 Age: 16 days old
Hobo Temp. No.:	N/A
Test Location:	Room 1

Dilution Water Batch: CFTW 011		
pH: 7.8	DO: 11.5	Temp: 10.6
Ref Tox: P051027.81	Lot No.: 5117-14	
LC50: 112.50	Test Date: 11.29.06	
Lab Mean:	70.09	
Test Acceptability: $\checkmark$ > 90% Control Survival		

① WC 12.1.06 BH





## 96 Hour Acute Toxicity Test for Rainbow Trout

Client	EPI
Project:	Coral blue product testing
Client Sample ID:	BLUE CORAL CONC.
Weston Sample ID:	P061122.01
Weston Protocol:	BIO 012C
Study Director:	BH

Date Received:	11.29.06
Date Test Started:	11.29.06
Date Test Ended:	12/3/06
Matrix:	L
Species:	O. mykiss
Organisms/Chamber:	10

Conc.	Rep	24 Hours Date: 11.30.06 Time: 1030		48 Hours Date: 12.1.06 Time: 1100		72 Hours Date: 12/2/06 Time: 1420		96 Hours Date: 12/3/06 Time: 1206	
		# Alive	# Dead	# Alive	# Dead	# Alive	# Dead	# Alive	# Dead
Control	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
5.25 ppb	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
26.5	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
53	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
265	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	10	0
	4	10	0	10	0	10	0	10	0
530	1	10	0	10	0	10	0	10	0
	2	10	0	10	0	10	0	10	0
	3	10	0	10	0	10	0	9	1
	4	10	0	10	0	10	0	10	0
5300	1	0	10						
	2	0	10						
	3	0	10						
	4	0	10						
Initials:		TS		TS		TB		TB	

Date / Time:		Technician:	
Length (mm)	Weight (g)	Length (mm)	Weight (g)
1)		6)	
2)		7)	
3)		8)	
4)		9)	
5)		10)	
Average Length (mm):		Average Weight (g):	

Total Volume (L) per Replicate:	Total Grams of Fish Flesh per Liter:
---------------------------------	--------------------------------------

Note: All fish taken from Control Rep 1 unless otherwise specified.

① Not required 12/2/06 BH

① BH



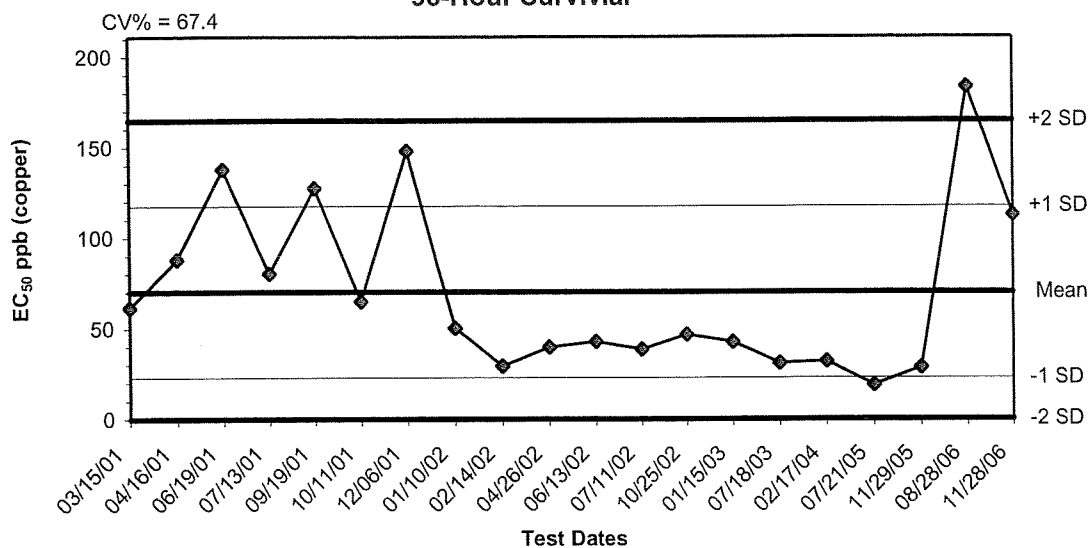
**CHAIN OF CUSTODY**  
**13235**

- ☐ 2433 Infirmary Drive • Calisno, CA 92006 • (607) 501-7176, FAX 435-1355  
☐ 98 Main St., Ste. #428 • Tiburon, CA 94920 • (415) 435-1847, FAX 435-0479  
☐ 1440 Sunsetway, Ste. 908 • Oakland, CA 94612 • (510) 808-0302, FAX 894-9710  
☐ 152 Sunset View Lane • Sequim, WA 98382 • (360) 582-1758, FAX 582-1879  
☒ 4729 NE View Drive • Port Gamble, WA 98364 • (360) 297-6903, FAX 297-6905

DATE 11/25/06 PAGE 7 OF 7

[illegible]

***Oncorhynchus mykiss* Reference Toxicant Control Chart:  
96-Hour Survival**



Dates	Values	Mean	-1 SD	-2 SD	+1 SD	+2 SD
03/15/01	61.4720	70.0978	22.8210	0.0000	117.3746	164.6514
04/16/01	87.9825	70.0978	22.8210	0.0000	117.3746	164.6514
06/19/01	137.7600	70.0978	22.8210	0.0000	117.3746	164.6514
07/13/01	80.1567	70.0978	22.8210	0.0000	117.3746	164.6514
09/19/01	127.2790	70.0978	22.8210	0.0000	117.3746	164.6514
10/11/01	64.7289	70.0978	22.8210	0.0000	117.3746	164.6514
12/06/01	147.8140	70.0978	22.8210	0.0000	117.3746	164.6514
01/10/02	50.1660	70.0978	22.8210	0.0000	117.3746	164.6514
02/14/02	29.1790	70.0978	22.8210	0.0000	117.3746	164.6514
04/26/02	39.7384	70.0978	22.8210	0.0000	117.3746	164.6514
06/13/02	42.6380	70.0978	22.8210	0.0000	117.3746	164.6514
07/11/02	38.3651	70.0978	22.8210	0.0000	117.3746	164.6514
10/25/02	46.5870	70.0978	22.8210	0.0000	117.3746	164.6514
01/15/03	42.5565	70.0978	22.8210	0.0000	117.3746	164.6514
07/18/03	30.7498	70.0978	22.8210	0.0000	117.3746	164.6514
02/17/04	31.8198	70.0978	22.8210	0.0000	117.3746	164.6514
07/21/05	18.7500	70.0978	22.8210	0.0000	117.3746	164.6514
11/29/05	28.4485	70.0978	22.8210	0.0000	117.3746	164.6514
08/28/06	183.2640	70.0978	22.8210	0.0000	117.3746	164.6514
11/28/06	112.5000	70.0978	22.8210	0.0000	117.3746	164.6514

Updated 12/19/06 JW

### Acute Fish Test-96 Hour

Start Date: 11/28/2006 12:30	Test ID: P051027.81	Sample ID: REF-Ref Toxicant
End Date: 12/3/2006 12:02	Lab ID: PGL- Port Gamble Laborator	Sample Type: CUSO-Copper sulfate
Sample Date:	Protocol: WDOE WQ-R95-80	Test Species: OM-Oncorhynchus mykiss
Comments:		

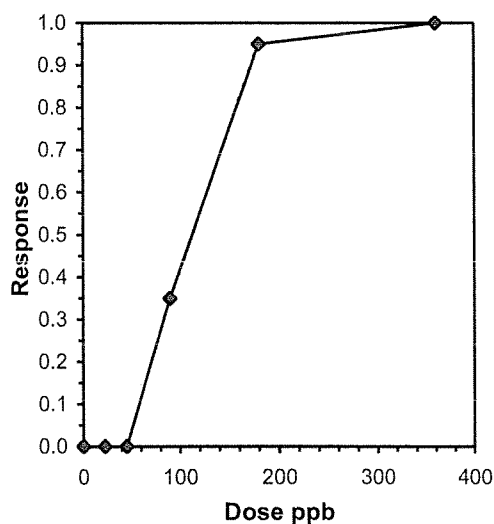
Conc-ppb	1	2
Control	1.0000	1.0000
22.5	1.0000	1.0000
45	1.0000	1.0000
90	0.4000	0.9000
180	0.1000	0.0000
360	0.0000	0.0000

Conc-ppb	Transform: Untransformed							Isotonic	
	Mean	N-Mean	Mean	Min	Max	CV%	N	Mean	N-Mean
Control	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	2	1.0000	1.0000
22.5	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	2	1.0000	1.0000
45	1.0000	1.0000	1.0000	1.0000	1.0000	0.000	2	1.0000	1.0000
90	0.6500	0.6500	0.6500	0.4000	0.9000	54.393	2	0.6500	0.6500
180	0.0500	0.0500	0.0500	0.0000	0.1000	141.421	2	0.0500	0.0500
360	0.0000	0.0000	0.0000	0.0000	0.0000	0.000	2	0.0000	0.0000

Auxiliary Tests	Statistic	Critical	Skew	Kurt
Normality of the data set cannot be confirmed				
Equality of variance cannot be confirmed				

#### Linear Interpolation (200 Resamples)

Point	ppb	SD	95% CL(Exp)		Skew
IC05	51.43	7.60	40.71	115.71	1.0002
IC10	57.86	15.20	36.43	186.43	1.0002
IC15	64.29	15.31	32.14	189.64	0.9107
IC20	70.71	15.50	27.86	192.86	0.7914
IC25	77.14	15.76	23.57	196.07	0.6490
IC40	97.50	16.94	7.50	202.50	0.0616
IC50	112.50	18.24	0.00	202.50	-0.4174



Test: AC-Acute Fish Test					Test ID: 051027.81				
Species: OM-Oncorhynchus mykiss					Protocol: WDOE WQ-R95-80				
Sample ID: REF-Ref Toxicant					Sample Type: CUSO-Copper sulfate				
Start Date: 11/28/2006 12:30					End Date: 12/3/2006 1 Lab ID: PGL- Port Gamble Laboratory				
Pos	ID	Rep	Group	Start	24 Hr	48 Hr	72 Hr	96 Hr	Notes
	1	1	Control	10				10	
	2	2	Control	10				10	
	3	1	22.500	10				10	
	4	2	22.500	10				10	
	5	1	45.000	10				10	
	6	2	45.000	10				10	
	7	1	90.000	10				4	
	8	2	90.000	10				9	
	9	1	180.000	10				1	
	10	2	180.000	10				0	
	11	1	360.000	10				0	
	12	2	360.000	10				0	

Comments:



## 96 Hour Rainbow Trout (with Renewal) Reference Toxicant Test

Test ID: <u>7051027-81</u>		Replicates: 2		Study Director: <u>BH</u>		Location: <u>Room 2</u>	
Dilution Water Batch: <u>CFTW 011</u>		Organism Batch: <u>TFC 5287</u>		Associated Test(s): <u>EPI</u>		No. of Organisms: 10	
Toxicant: Copper Sulfate (0.509gCu/LCuSO <sub>4</sub> )		Date Prepared: <u>11/28/06</u>		Initials: <u>BH</u>			
Lot Number: <u>5117-14</u>							
Target Concentrations:	Quantity of Stock: Target:	Actual:		Quantity of Diluent: Target:	Actual:		
		R1	R2		R1	R2	
360 ppb	2.829 mL	<u>2.829</u>	<u>2.829</u>	4 L	<u>4000.0</u>	<u>4000.0</u>	
180 ppb	1.415 mL	<u>1.415</u>	<u>1.415</u>	4 L	<u>4000.0</u>	<u>4000.0</u>	
90 ppb	0.707 mL	<u>0.707</u>	<u>0.707</u>	4 L	<u>4000.0</u>	<u>4000.0</u>	
45 ppb	0.354 mL	<u>0.354</u>	<u>0.354</u>	4 L	<u>4000.0</u>	<u>4000.0</u>	
22.5 ppb	0.177 mL	<u>0.177</u>	<u>0.177</u>	4 L	<u>4000.0</u>	<u>4000.0</u>	
<b>0 Hours</b> Date: <u>11/29/06</u> WQ Time: <u>1210</u> Start Time: <u>1230</u> Initials: <u>LS</u> <div style="text-align: center;"><b>STOCK</b></div>							
	Control	22.5	45	90	180	360	
D.O. (%)	<u>11.7</u>	<u>11.8</u>	<u>11.6</u>	<u>11.8</u>	<u>11.7</u>	<u>11.8</u>	
Temperature	<u>10.5</u>	<u>10.5</u>	<u>10.5</u>	<u>10.5</u>	<u>10.6</u>	<u>10.6</u>	
Conductivity	<u>317</u>	<u>316</u>	<u>322</u>	<u>318</u>	<u>316</u>	<u>317</u>	
pH	<u>8.0</u>	<u>8.2</u>	<u>8.2</u>	<u>8.1</u>	<u>8.0</u>	<u>8.1</u>	
<b>24 Hours</b> Date: <u>11/30/06</u> Time: <u>1045</u> Initials: <u>TS</u>							
	Control	22.5	45	90	180	360	
No. Alive Rep 1	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	
No. Alive Rep 2	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	<u>10</u>	



## 96 Hour Rainbow Trout (with Renewal) Reference Toxicant Test

<b>48 Hours</b>		Date: 12/1/06		Time: 1035		Initials: TS	
Renewal Information							
Target Concentrations:	Quantity of Stock: Target:	Actual:		Quantity of Diluent: Target:	Actual:		
		R1	R2		R1	R2	
360 ppb	2.829 mL	2.829	+	4 L	4000.0		
180 ppb	1.415 mL	1.415	g	4 L	4000.0	g	
90 ppb	0.707 mL	0.707	BH	4 L	4000.0	BH	
45 ppb	0.354 mL	0.354		4 L	4000.0		
22.5 ppb	0.177 mL	0.177		4 L	4000.0		
	Control	22.5	45	90	180	360	
No. Alive Rep 1	10	10	10	8(2)	3(2) 1	6(4)	
No. Alive Rep 2	10	10	10	9(1)	3(3) 2	3(7) 3	
<b>72 Hours</b>		Date: 12/2/06		Time: 1415		Initials: TS	
	Control	22.5	45	90	180	360	
No. Alive Rep 1	10	10	10	5(3)	3(4)	0(6)	
No. Alive Rep 2	10	10	10	9	1(6)	1(2)	
<b>96 Hours</b>		Date: 12/3/06		WQ Time: 1153		Replicate: 4/1 Initials: TS	
STOCK							
	Control	22.5	45	90	180	360	
D.O. (%)	8.6	8.4	8.9	7.5	9.0	8.7	
Temperature	11.3	11.3	11.3	11.3	11.3	11.3	
Conductivity	309	308	315	308	307	307	
pH	7.1	7.2	7.3	7.4	7.5	7.5	
<b>96 Hour Survival Data</b>		End Time: 1202		Initials: TS			
	Control	22.5	45	90	180	360	
No. Alive Rep 1	10	10	10	4(1)	1(2)	NA	
No. Alive Rep 2	10	10	10	9	0(1)	0(1)	

① WQ 12/1/06 TS correct count = 7(3)      ③ WQ 12/1/06 TS  
 ② WQ 12/1/06 TS correct count = 7(3)      ④ Fail correct count = 3(7)  
 ④ SM 12.1.06 BH ☒ Pass      ☐ Fail

Notes:

## **APPENDIX C**

### **Hypothetical Implications Calculation Spreadsheet**



## Calculation of Vehicle Washing Impact on Small Stream

gray boxes contain independent variables that may be changed for varying assumptions

### Location and Vehicle Facts

100,000 assumed population along a small stream that feeds into Lake Washington

1.00 ratio of vehicles to people (approximately correct according to WA DOT statistics)

100,000 total number of vehicles

### Small Stream Facts

15 length of small stream, miles

18 mean width of stream, feet

range of stream flow rates during August

low flow rate (typical of small Puget Sound area stream)

high flow rate (typical of small Puget Sound area stream)

2 low volumetric flow rate, cubic feet/second

20 high volumetric flow rate, cubic feet/second

898 low flow rate, gallons/minute

8,977 high flow rate, gallons/minute

0.25 mean depth of stream at low flow rate, feet

1.25 mean depth of stream at high flow rate, feet

0.44 low flow velocity, feet/second

0.89 high flow velocity, feet per second

### Overall Car Washing Estimate

48 time period, August weekend with no rain (hours)

1.50 percent of vehicles washed during time period

1,500 total vehicles washed during time period

### Individual Driveway Car Wash Event

5 hose flow rate, gallons/minute

15 time that hose is running, minutes

75 total water to storm drain, gallons

53 detergent concentration to stormdrain, parts per million (ppm)

(Note: detergent concentration derived from car wash product directions)

### Bathtub Calculation

calculate total stream flow and detergent concentration for time period, assuming all water is collected in a tub

low flow rate

high flow rate

345,600 total volume of stream, cubic feet

3,456,000 total volume of stream, cubic feet

15,040 total volume of all car wash water, cubic feet

15,040 total volume of all car wash water, cubic feet

2.2 detergent concentration in total volume of water, ppm

0.2297 detergent concentration in total volume of water, ppm

(Note: fish toxicity test indicated 1.6 ppm of detergent lethal to 50 percent of juvenile rainbow trout)

### Time and Distance Analysis (assume uniform distribution in time and distance)

100 number of car washes per mile of stream

31 number of car washes per hour of time period