

BIOASSAY REPORT

ACUTE TOXICITY TESTS

Conducted August 7 through 11, 2008

**Prepared for
Coating Holdings Ltd
Ripon, Wisconsin**

Prepared by

**S-F ANALYTICAL LABORATORIES
Bioassay Laboratory
2345 South 170th Street
New Berlin, WI 53151**

Lab I.D. No. RG0765

August 2008

Summary

S-F Analytical Laboratories conducted acute toxicity tests on laboratory water exposed to a sample of ORSSS coating provided by Coating Holdings Ltd, Ripon, Wisconsin. The bioassays were conducted from August 7 through 11, 2008, as part of noncompliance toxicity evaluation. *Ceriodaphnia dubia* and fathead minnows were used as the test organisms. The following is a summary of the test results:

Test Media	<u>Acute Toxicity as LC₅₀</u>	
	<i>Ceriodaphnia dubia</i>	Fathead Minnow
ORSSS coating exposed water	>100%	>100%

The results of the tests show that:

- Laboratory control water data were acceptable in all bioassays.
- The ORSSS coating exposed water was not acutely toxic to *Ceriodaphnia dubia* in the 100 percent water concentration. The 48-hour LC₅₀ was greater than 100 percent.
- The ORSSS coating exposed water was not acutely toxic to fathead minnows in the 100 percent water concentration. The 96-hour LC₅₀ was greater than 100 percent.

Introduction

This report presents the results of the laboratory acute toxicity tests conducted by S-F Analytical Laboratories on laboratory water exposed to a sample of ORSSS coating, provided by Coating Holdings Ltd, Ripon, Wisconsin. The bioassays used *Ceriodaphnia dubia* and fathead minnows as the test organisms and were performed from August 7 through 11, 2008, as part of a noncompliance toxicity evaluation.

Methods

All laboratory methods, including organism culture, sample handling, test procedures, and data analyses, were in accordance with the recommendations of the U.S. Environmental Protection Agency (EPA) [1] and the S-F Analytical Bioassay Laboratory Standard Operating Procedures.

Sample Collection and Handling

A photocopy of the sample receipt form is included in Appendix B. One sample of the ORSSS coating was used as follows:

Description	Sample No.	Date Received	Date Tested
ORSSS coating sample	RG0765.01	7/30/08	8/7-9/08

The sample was provided by Coating Holdings Ltd and was shipped to the S-F Analytical Bioassay Laboratory. Upon arrival, the sample was logged in and stored at room temperature for later use.

Test Organisms

All test organisms were cultured at the S-F Analytical Bioassay Laboratory.

Test Procedures

Sample Preparation

Two 4" x 4" sample pieces were submersed in a container of S-F Bioassay laboratory control water and placed on a shaker table (set at 100 oscillations per minute) for 24 hours. The size of the sample per water volume, the exposure period, and agitation on a shaker table were designed to allow potential contaminants to leach into the water. At the end of the exposure period, the sample pieces were removed from the test media and this 100 percent water concentration was tested.

Bioassays

Bioassay test conditions are summarized in Tables 1 and 2.

Physicochemical Monitoring

Total alkalinity and hardness were measured in the laboratory control and the sample exposed water.

Dissolved oxygen (DO), pH, and conductivity were measured initially and daily thereafter in all test solution renewals. DO and pH were measured in one test chamber or composite of each 24-hour old test solution.

Bioassay incubator temperature was electronically monitored and a 24-hour summary of mean values was recorded.

Data Analysis

When appropriate, an LC₅₀ (median lethal concentration) was calculated on each data set using a computer program.

Acute toxicity was defined according to the following EPA criteria:

- Less than 50 percent survival of test organisms at test termination (48 hours for *Ceriodaphnia dubia* and 96 hours for fathead minnows).

Table 1
Summary of Test Conditions for the
***Ceriodaphnia* Acute Bioassay**
Conducted for Coating Holdings Ltd
Ripon, Wisconsin
August 7 through 9, 2008

1.	Test organism	<i>Ceriodaphnia dubia</i> (Crustacea: Cladocera)
2.	Test type	Static renewal
3.	Age of test organisms	Less than 24 hours
4.	Test chamber size	30 mL
5.	Test solution volume	25 mL
6.	Renewal of test solutions	Daily
7.	Number of replicate chambers per solution	4
8.	Number of test organisms per chamber	5
9.	Primary control water	Moderately hard reconstituted laboratory medium
10.	Test media	ORSSS coating- exposed lab control water
11.	Test concentration	100%
12.	Temperature	$20 \pm 1^{\circ}\text{C}$
13.	Feeding regime	None
14.	Aeration	None
15.	Test duration	48 hours
16.	Effects measured/Endpoint	Survival/LC ₅₀
17.	Test acceptability	90% or greater mean survival in the laboratory water control.

Table 2
Summary of Test Conditions for the
Fathead Minnow Acute Bioassay
Conducted for Coating Holdings Ltd
Ripon, Wisconsin
August 7 through 11, 2008

1.	Test organism	<i>Pimephales promelas</i> (Osteichthyes: Cyprinidae)
2.	Test type	Static renewal
3.	Age of test organisms	14 days old
4.	Test chamber size	250 mL
5.	Test solution volume	200 mL
6.	Renewal of test solutions	Daily
7.	Number of replicate chambers per solution	4
8.	Number of test organisms per chamber	5
9.	Primary control water	Moderately hard reconstituted laboratory medium
10.	Test media	ORSSS coating- exposed lab control water
11.	Test concentration	100%
12.	Temperature	$20 \pm 1^{\circ}\text{C}$
13.	Feeding regime	None
14.	Aeration	None, unless DO concentration falls below 40% saturation (then, continuous at a rate not exceeding 100 bubbles per minute)
15.	Test duration	96 hours
16.	Effects measured/Endpoint	Survival/LC ₅₀
17.	Test acceptability	90% or greater mean survival in the laboratory water control

Quality Assurance

Part of the quality assurance and quality control (QA/QC) program at the S-F Analytical Bioassay Laboratory includes the performance of organisms concurrently tested in laboratory media. Tables 1 and 2 present the test acceptability criteria for laboratory control data. The results of the laboratory control tests are listed in Table 3.

In addition, other QA/QC procedures include performing monthly reference toxicant tests using reagent-grade sodium chloride. The results of reference toxicant tests conducted during the past 20 months on the appropriate test organisms are summarized in Appendix C.

Results

Photocopies of laboratory data and computer printouts of the statistical analyses are found in Appendix A. There were no excursions from the protocols and all test conditions were within the limits required by the EPA. The results of the tests are summarized below.

Acute Bioassays

Table 3 presents the results of the acute bioassays. The ORSSS coating exposed water was not acutely toxic to either *Ceriodaphnia dubia* or fathead minnows in the 100 percent water concentration. The LC_{50} analyses could not be mathematically calculated, but the values would be greater than 100 percent for both species.

Laboratory control water data were acceptable in all tests.

Physicochemical Data

All physicochemical parameters measured satisfied the bioassay requirements (see Appendix A).

Table 3
Summary of Results of Acute Bioassays
Conducted for Coating Holdings Ltd
Ripon, Wisconsin
December 4 through 8, 2000

Test Media	Mean Percent Survival	
	<i>Ceriodaphnia dubia</i>	Fathead Minnow
Laboratory Control	100	100
ORSSS coating exposed water 100%	100	90
LC ₅₀	>100%	>100%

Conclusions

The results of the laboratory bioassays conducted August 7 through 11, 2008 on the ORSSS coating sample, provided by Coating Holdings Ltd, and exposed to laboratory water, show the following:

- Laboratory control water data were acceptable in all bioassays.
- The ORSSS coating exposed water was not acutely toxic to *Ceriodaphnia dubia* in the 100 percent water concentration. The 48-hour LC₅₀ was greater than 100 percent.
- The ORSSS coating exposed water was not acutely toxic to fathead minnows in the 100 percent water concentration. The 96-hour LC₅₀ was greater than 100 percent.

Reference

1. US EPA. 2002. *Methods for Measuring the Acute Toxicity of Effluents to Freshwater and Marine Organisms* (Fifth Edition). EPA-821-R-02-012. U.S. Environmental Protection Agency, Office of Water, Washington, DC. 266 p.

APPENDIX A
LABORATORY DATA SHEETS
AND STATISTICAL ANALYSES

SF ANALYTICAL BIOASSAY LABORATORY

CLIENT: Coating Holdings Ltd

TEST DATE: 8/7/08

To the best of our knowledge, the laboratory data reported, is true and accurate.

Report and Data:

Reviewed by:

Tina Diacoy Date: 8-28-08

_____ Date: _____

Approved by:

Jim Stark Date: 8/28/08

ACUTE TEST CHEMICAL DATA*

CLIENT: Coating Holdings LtdTEST ORGANISM: Ceriodaphnia dubia Fathead minnow Daphnia magnaSAMPLE DESCRIPTION: EFFLUENT DRSSS coated sampleSAMPLE No.: RH0129/2 RG0765.02TEST START DATE: 8-7-08TEST END DATE: 8-11-08

INITIAL MEASUREMENTS

CONC. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HRS)				COMMENTS
			0	24	48	72	
1	Lab	DO	8.4	8.4	8.4	8.4	
		pH	8.0	8.0	8.1	8.1	
		COND	0.28	0.27	0.25	0.26	
2	100%	DO	8.5	8.3	8.5	8.5	
		pH	7.6	7.6	7.6	7.6	
		COND	0.33	0.34	0.33	0.32	
3		DO					
		pH					
		COND					
4		DO					
		pH					
		COND					
DATE			8-7	8-8	8-9	8-10-08	
TIME			1545	0945	0930	1013	
SAMPLE No.			1				
DETERMINED BY			TO	TO	TO	JD	

FINAL MEASUREMENTS

CONC. NO.	TEST SOLN	PARAMETER	EXPOSURE PERIOD (HRS)				COMMENTS
			24	48	72	96	
1	Cerio Lab	DO	8.1	8.1			
		pH	8.0	8.0			
2	100%	DO	8.1	8.1			
		pH	7.7	7.6			
3	FISH Lab	DO	8.0	8.0	8.0	8.0	
		pH	7.9	7.9	7.9	7.9	
4	100%	DO	8.0	7.9	8.0	8.0	
		pH	7.6	7.6	7.5	7.5	
DATE			8-8	8-9	8-10-08	8-11	
TIME			1635	1618	1655	1703	
DETERMINED BY			TO	TO	JD	TO	

*DO = Dissolved Oxygen (mg/L)

COND = Conductivity (mmho)

ACUTE BIOASSAY SURVIVAL DATA

(1 - 7 Treatments)

CLIENT: Coatings Holdings Ltd
 TEST ORGANISM: Ceriodaphnia dubia Fathead minnow Other _____
 SAMPLE DESCRIPTION: EFFLUENT DRSSS coated sample
 SAMPLE No.: R40129/B R60765.02 CODE: _____
 TEST START DATE: 8-7-08 TIME: 1605 TEST END DATE: 8-11-08 TIME: 1700

TREAT. NO.	TEST SOLN	REP	FATALITIES PER EXPOSURE PERIOD (Hrs)				TOTAL FATALITIES	MEAN SURVIVAL
			24	48	72	96		
1	cerio Lab	A	0	0			0	100%
		B	0	0				
		C	0	0				
		D	0	0				
2	100%	A	0	0			0	100%
		B	0	0				
		C	0	0				
		D	0	0				
3		A						
		B						
		C						
		D						
4		A						
		B						
		C						
		D						
5	FISH Lab	A	0	0	0	0	0	100%
		B	0	0	0	0		
		C	0	0	0	0		
		D	0	0	0	0		
6	100%	A	0	0	0	0	0	90%
		B	0	0	0	1		
		C	0	0	0	1		
		D	0	0	0	0		
7		A						
		B						
		C						
		D						
DATE			8-8	8-9	8-10-08	8-11		
TIME			1630	1615	1646	1700		
DETERMINED BY:			TD	TD	JD	TD		

COMMENTS:

h

Summary of Test Conditions

Client: Coating Holdings Ltd Sample No. RG-0765-02
RH0129 3

Acute Test X Test Start Date: 8-7-08

Species	Age	Lot No.	48-Hr.*		96-Hr.*	
			NR	R	DR	48Hr R
<i>Ceriodaphnia dubia</i>	<u>X</u>	<u>< 24Hr.</u>	<u>1426</u>	<u>X</u>		
Fathead minnow	<u>X</u>	<u>14dys</u>	<u>8050-2</u>		<u>X</u>	
<i>Daphnia magna</i>		<u>< 24Hr.</u>				

*NR=Nonrenewal R=Renewal DR=Daily renewal

Control Water Lab Media X Identification /No. mH Recon RW 08.69
 Lab Media _____
 Receiving Water _____

Dilution Water Lab Media _____ Receiving Water _____

Chronic Test _____ Test Start Date: _____

Species	Age	Lot No.
<i>Ceriodaphnia dubia</i>	<u>< 24Hr.</u>	
Fathead minnow	<u>< 24Hr.</u>	
Algae		

Control Water Lab Media _____ Identification /No. _____
 Lab Media _____
 Receiving Water _____

Dilution Water Lab Media _____ Receiving Water _____

Analysts: T Draeger, J Doepke, J Stark

Comments/Special Conditions:

PHYSICO-CHEMICAL DATA SUMMARY

CLIENT Coating Holdings Ltd
 TEST DATE 8/7/02 SUMMARIZED BY J. Stark

LABORATORY CONTROL		CONTROL I.D.		
		Rw0869		
TOTAL ALKALINITY mg/L CaCO ₃	54			
HARDNESS mg/L CaCO ₃	100			

SAMPLE DESCRIPTION		SAMPLE NO.		
ORSSS exposed water		RG-0765.02		
TOTAL ALKALINITY mg/L CaCO ₃	60			
HARDNESS mg/L CaCO ₃	95			
TOTAL RESIDUAL CHLORINE mg/L	NA			
TOTAL AMMONIA mg/L	NA			

SAMPLE DESCRIPTION		SAMPLE NO.		
TOTAL ALKALINITY mg/L CaCO ₃				
HARDNESS mg/L CaCO ₃				
TOTAL RESIDUAL CHLORINE mg/L				
TOTAL AMMONIA mg/L				

SAMPLE DESCRIPTION		SAMPLE NO.		
TOTAL ALKALINITY mg/L CaCO ₃				
HARDNESS mg/L CaCO ₃				
TOTAL RESIDUAL CHLORINE mg/L				
TOTAL AMMONIA mg/L				

NA = Not Analyzed * = Duplicate for QA

TEST TEMPERATURE °C (24 HOUR AVERAGE)	ACUTE						
	DATE	8-7	8-8	8-9	8-10		8-11
	TEMP	19.8	19.9	20.3	19.8		20.0
CHRONIC							
DATE							
TEMP							

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APPENDIX B
CHAIN-OF-CUSTODY FORMS

BIOASSAY SAMPLE RECEIPT CHARACTERIZATION

CLIENT Coating Holdings Ltd

DATE RECD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS
7/30/08	RG0765.01 Plastic coated sample ORWAM	NA				B

SIEVED DECHLORINATE CONTAINER TYPE (G/P) USE: IMMEDIATE STORE (4 C)
 APPEARANCE: CLEAR CLOUDY SOLIDS COLOR Ambient
 RECEIVED ON ICE ALIQUOT FOR: HARDNESS ALKALINITY
 COMMENTS:
 (A) These are plastic-coated samples to be used for noncompliance toxicity evaluation. No chemical analyses required. No chain of custody came with sample.

DATE RECD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS
7/30/08	RG0765.02 Plastic coated sample ORSSS	NA				B

SIEVED DECHLORINATE CONTAINER TYPE (G/P) USE: IMMEDIATE STORE (4 C)
 APPEARANCE: CLEAR CLOUDY SOLIDS COLOR
 RECEIVED ON ICE ALIQUOT FOR: HARDNESS ALKALINITY
 COMMENTS: (A)

DATE RECD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS

SIEVED DECHLORINATE CONTAINER TYPE (G/P) USE: IMMEDIATE STORE (4 C)
 APPEARANCE: CLEAR CLOUDY SOLIDS COLOR
 RECEIVED ON ICE ALIQUOT FOR: HARDNESS ALKALINITY
 COMMENTS:

DATE RECD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS

SIEVED DECHLORINATE CONTAINER TYPE (G/P) USE: IMMEDIATE STORE (4 C)
 APPEARANCE: CLEAR CLOUDY SOLIDS COLOR
 RECEIVED ON ICE ALIQUOT FOR: HARDNESS ALKALINITY
 COMMENTS:

DATE RECD	SAMPLE NO. DESCRIPTION	TEMP (C)	DO (mg/L)	pH	COND (mmho)	INITIALS

SIEVED DECHLORINATE CONTAINER TYPE (G/P) USE: IMMEDIATE STORE (4 C)
 APPEARANCE: CLEAR CLOUDY SOLIDS COLOR
 RECEIVED ON ICE ALIQUOT FOR: HARDNESS ALKALINITY
 COMMENTS:

B

APPENDIX C
REFERENCE TOXICANT DATA

Table 1. Reference Toxicant Summary

Laboratory: S-F Analytical Laboratories, Inc., New Berlin, WI
 Test Type: Acute
 Organism: *Ceriodaphnia dubia*
 Toxicant: Sodium Chloride
 Response: Mean % Survival
 Calculation: LC50
 Reporting Period: December 06 through July 08

Test No.	Test Date	Test LC50 (g/L)	12 Month Control Limits			In or Out of Control	Comments
			Mean LC50	Mean +2 S.D.	Mean -2 S.D.		
211	12/20/06	2.26	2.20	2.86	1.55	IN	
212	1/24/07	1.70	2.16	2.86	1.45	IN	
213	2/28/07	1.78	2.11	2.84	1.39	IN	
214	3/28/07	1.86	2.07	2.79	1.36	IN	
215	4/25/07	2.34	2.03	2.59	1.47	IN	
216	5/30/07	1.91	2.00	2.54	1.46	IN	
217	6/27/07	1.77	1.99	2.54	1.44	IN	
218	7/31/07	2.45	2.00	2.58	1.42	IN	
219	8/29/07	1.86	1.96	2.49	1.43	IN	
220	9/26/07	2.45	2.01	2.60	1.42	IN	
221	10/24/07	1.86	2.01	2.59	1.43	IN	
222	11/28/07	2.24	2.01	2.59	1.43	IN	
223	12/12/07	1.78	2.02	2.58	1.46	IN	
224	1/30/08	1.82	1.99	2.54	1.43	IN	
225	2/6/08	2.45	2.04	2.62	1.47	IN	
226	2/14/08	2.09	2.07	2.62	1.51	IN	
227	3/5/08	2.29	2.10	2.66	1.55	IN	
228	3/12/08	1.78	2.06	2.62	1.50	IN	SF Labs moved to a new location
229	3/19/08	2.62	2.11	2.74	1.48	IN	
230	3/26/08	2.34	2.16	2.76	1.55	IN	
231	4/2/08	1.82	2.11	2.71	1.50	IN	
232	4/30/08	2.04	2.12	2.71	1.53	IN	
233	5/28/08	1.89	2.08	2.65	1.51	IN	
234	6/25/08	1.99	2.09	2.64	1.53	IN	
235	7/30/08	2.34	2.10	2.66	1.53	IN	

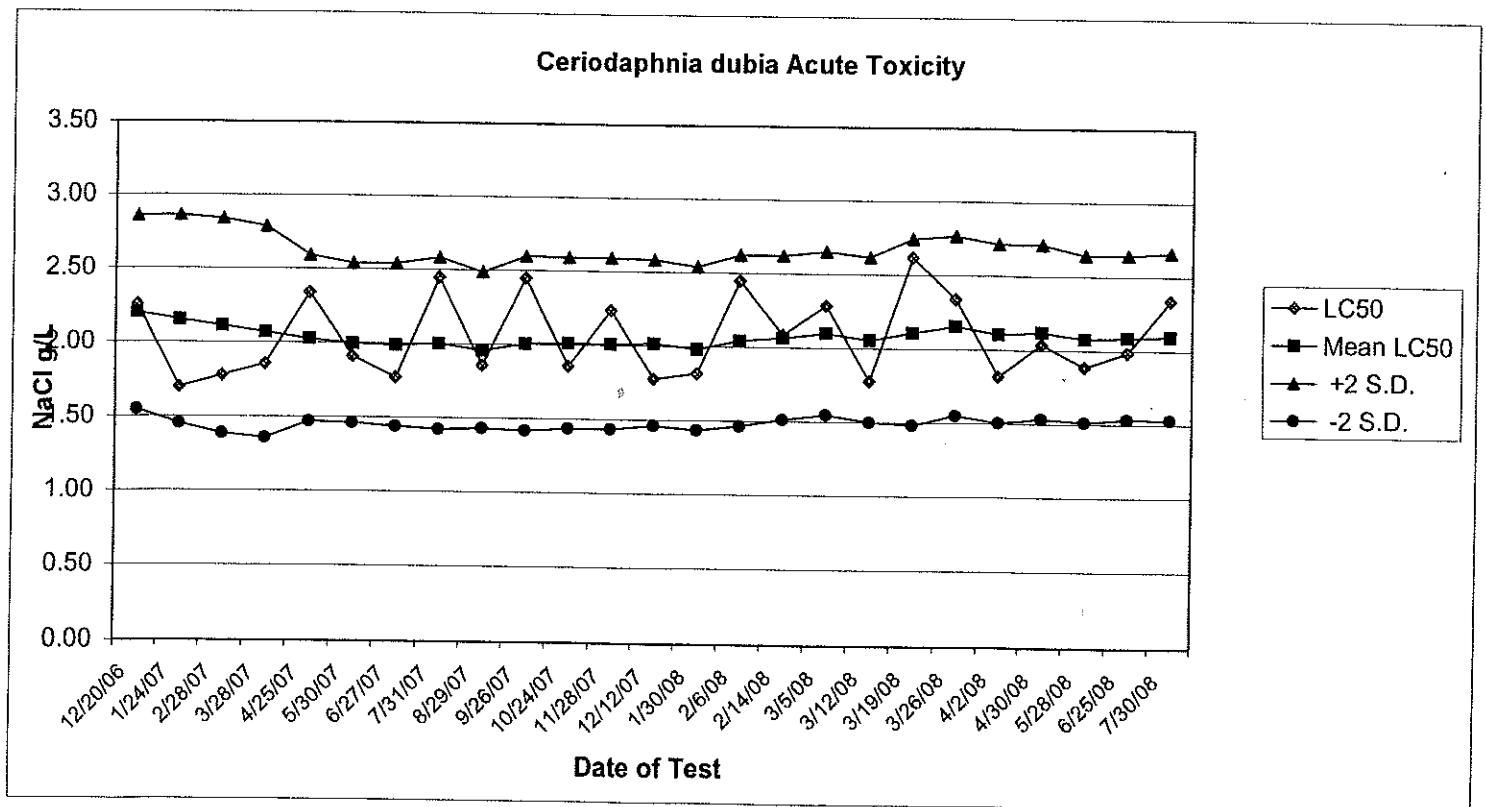


Table 3. Reference Toxicant Summary

Laboratory: S-F Analytical Laboratories, Inc., New Berlin, WI
Test Type: Acute
Organism: Fathead minnow (*Pimephales promelas*)
Test Duration: 96 Hours
Toxicant: Sodium Chloride
Response: Mean % Survival
Calculation: LC50
Reporting Period: January 07 through August 08

Test No	Test Date	Test LC50 (g/L)	12 Month Control Limits			In or Out of Control	Comments
			Mean LC50	Mean +2 S.D.	Mean -2 S.D.		
207	1/3/07	5.68	5.97	8.07	3.88	IN	
208	2/6/07	5.68	5.88	7.91	3.85	IN	
209	3/6/07	5.50	5.89	7.91	3.87	IN	
210	4/3/07	5.95	5.98	7.89	4.07	IN	
211	5/1/07	6.77	6.01	7.96	4.06	IN	
212	6/5/07	6.53	6.16	7.96	4.36	IN	
213	7/10/07	6.32	6.28	7.89	4.67	IN	
214	8/7/07	6.69	6.25	7.80	4.69	IN	
215	9/4/07	6.20	6.38	7.58	5.18	IN	
216	10/4/07	6.69	6.35	7.48	5.21	IN	
217	11/6/07	5.48	6.25	7.46	5.03	IN	
218	12/11/07	5.77	6.11	7.08	5.13	IN	
219	1/8/08	6.50	6.17	7.13	5.21	IN	
220	2/5/08	5.59	6.17	7.14	5.19	IN	
221	3/6/08	5.80	6.19	7.11	5.27	IN	
222	3/11/08	6.61	6.25	7.18	5.31	IN	SF Labs moved to a new location
223	3/19/08	5.95	6.18	7.06	5.29	IN	
224	3/25/08	4.94	6.05	7.15	4.94	OUT	
225	4/1/08	5.03	5.94	7.17	4.71	IN	
226	4/15/08	6.13	6.07	7.45	4.69	IN	
227	5/6/08	6.77	6.16	7.43	4.88	IN	
228	6/10/08	6.06	6.12	7.33	4.90	IN	
229	7/10/08	7.39	6.15	7.45	4.84	IN	
230	8/5/08	6.53	6.11	7.30	4.91	IN	

Acceptable out-of-control data is 5%; Last time data was out of control was 10/1/03 (test # 168) =1 time in 57 tests (= 1.8%). Therefore, no corrective action needed.

