



COOPER TIRE & RUBBER COMPANY  
3500 Washington Rd. • Texarkana, AR 71854 • Phone (870) 773-4502

July 11, 2012

Arkansas Department of Environmental Quality  
Water Division - Enforcement Branch  
5301 Northshore Drive  
North Little Rock, Arkansas 72118-5317

RE: Cooper Tire & Rubber Company – AFIN: 46-00005 - NPDES Permit Number AR0038822  
Discharge Number TX1-Q, TX1-B

Dear Sir or Madam:

The above-referenced permit requires Cooper Tire & Rubber Company Texarkana, Arkansas plant to complete routine bio-monitoring sampling at Outfall 001. The permit requires reporting the Lethal No Observed Effluent Concentration (NOEC) for *Daphnia pulex* and *Pimephales promela*. During the sampling events for the reporting period, the facility reported the required survival rate for *D. pulex* and *P. promela* at the 100% concentration level. Enclosed is the Discharge Monitoring Reports for the reporting period as required by the discharge permit AR0038822.

Respectfully,

Scott Cole  
Plant Manager

Encl.



# Bio-Aquatic Testing, Inc.



Ana-Lab  
Cooper Tire & Rubber Co.  
OUTFALL NPDES 001

Client Address:  
3500 WASHINGTON RD.  
TEXARKANA, TX 71844

48 Hr Acute Biomonitoring Report

48543

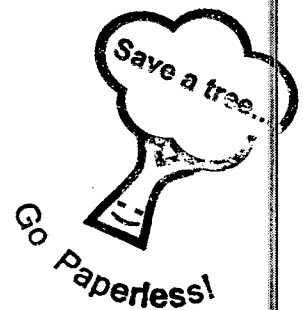
*Daphnia pulex*  
*Pimephales promelas*

April 12, 2012

Approved by:

*Chris Robason*

Chris Robason,  
President



Bio-Aquatic Testing, Inc. • 2501 Mayes Rd. Ste. 100 • Carrollton, Texas • 75006

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Unless otherwise noted in the body of the report, all data reported in this document are in compliance with NELAC standards and apply only to the samples referenced within. This report document may not be edited or reproduced in part or in full by any other entity, unless Bio-Aquatic Testing, Inc. issues written approval.

\*HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST

04/23/12

**BIO-AQUATIC TESTING, INC.**

2501 Mayes Road, Suite 100

Carrollton, Texas 75006

Tel: (972) 242-7750

Fax: (972) 242-7749

**TOXICITY TEST REPORT - 48 Hr Acute**

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Client:	Ana-Lab	Sample:	NPDES 001
Facility:	Cooper Tire & Rubber Co.	Laboratory Number:	48543
Permit No.	AR0038822	Date:	April 12, 2012

*Daphnia pulex* and *Pimephales promelas* passed survival testing requirements.

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**SAMPLE COLLECTION:** Composite effluent samples from Ana-Lab, Cooper Tire & Rubber Co., were received on April 12, 2012 and April 13, 2012. Effluent samples were collected from Outfall NPDES 001 by facility personnel.

The effluent samples were analyzed for total residual chlorine using the Hanna Ion Specific Meter #193711 and contained <0.10 mg/L and <0.10 mg/L, respectively. Effluent and laboratory dilution water pH, temperature, and dissolved oxygen data were collected daily.

**TEST PROCEDURES:**

*Daphnia pulex*

**EPA METHOD: 2021**

The 48 Hr Acute *Daphnia pulex* test was initiated at 16:15 hours on April 12, 2012. Five effluent concentrations of 32%, 42%, 56%, 75%, and 100% were prepared utilizing synthetic water as dilution water. The test was set up with 30mL plastic cups containing 20mL of test solution. Each concentration or control consisted of five replicate cups with eight organisms each, giving a total of 40 (forty) per treatment. The control was conducted concurrently with the test. Test organisms were less than 24-hour old laboratory cultured neonates. Organisms were introduced into the test solutions using a blocking design. Food consisting of a half-milliliter suspension of the green algae, *Selenastrum capricornutum*, and YTC was added to the test solutions each day. The test proceeded for 48 hours. Data on survival was collected daily and water quality parameters were recorded after each 24-hour period. The test was renewed daily with newly prepared solutions. The test ended at 16:00 hours on April 14, 2012. Survival data was statistically ( $p=0.05$ ) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

**SURVIVAL:**

*Daphnia pulex*

The *Daphnia pulex* survival data failed Shapiro Wilk's test for normality at the 0.01 (0.900) alpha level after the arc sine (square root (Y)) transformation. Bartlett's test for homogeneity is sensitive to non-normal data and should not be performed if data fails Shapiro Wilk's test. The non-parametric Steel's Many-One Rank test performed on *Daphnia pulex* survival data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100% Effluent**

**TEST PROCEDURES:**

*Pimephales promelas*

**EPA METHOD: 2000**

The 48 Hr Acute *Pimephales promelas* test was initiated at 17:00 hours on April 12, 2012. Five effluent concentrations of 32%, 42%, 56%, 75%, and 100% were prepared utilizing synthetic water as dilution water. The test was set up with 450mL plastic cups containing 250mL of test solution as test chambers. Each concentration or control consisted of five replicate chambers containing eight organisms each, giving a total of 40 (forty) per treatment. The control was conducted concurrently with the test. Test organisms were laboratory cultured *P. promelas* seven days old, and all larvae used in each test are hatched within 24 hours of each other. The number of surviving larvae and water quality parameters were recorded after each 24 hour period. The test was renewed daily with fresh solutions. Surviving larvae in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for 48 hours. The test ended at 11:25 hours on April 14, 2012. Survival was statistically ( $p=0.05$ ) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

**SURVIVAL:**

*Pimephales promelas*

*Pimephales promelas* survival data failed Shapiro Wilk's test for normality at the 0.01 (0.900) alpha level after the arc sine (square root (Y)) transformation. Bartlett's test for homogeneity is sensitive to non-normal data and should not be performed if data fails Shapiro Wilk's test. The non-parametric Steel's Many-One Rank test performed on *Pimephales promelas* survival data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100% Effluent**

# BIO-AQUATIC TESTING, INC.

## TOXICITY TEST

### 48 Hr Acute *Daphnia pulex*

Client: Ana-Lab Cooper Tire & Rubber Co.

Permit Number: ADPCE

Sample Type: Composite

Receiving Water Name:

AR0038822

Outfall Name: NPDES 001

Lab ID: 48543

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light  
8 hours dark

Begin Date: 4/12/2012

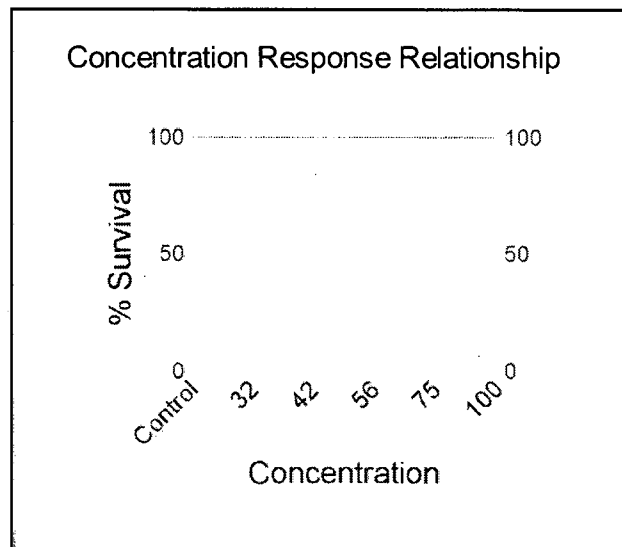
End Date: 4/14/2012

Test Start Time: 16:15

Test End Time: 16:00

### SURVIVAL

Effluent Con. %	Number Of Alive Per Replicate															Avg% Surv.
	4/12					4/13					4/14					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Control	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
32	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
42	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
56	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
75	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
100	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%



BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Pimephales promelas*

Client: Ana-Lab Cooper Tire & Rubber Co.

Lab ID: 48543

Permit Number: ADPCE

AR0038822

Test Temperature (oC): 25 ± 1

Sample Type: Composite

Outfall Name: NPDES 001

Photo Period: 16 hours light  
8 hours dark

Receiving Water Name:

Begin Date: 4/12/2012

Test Start Time: 17:00

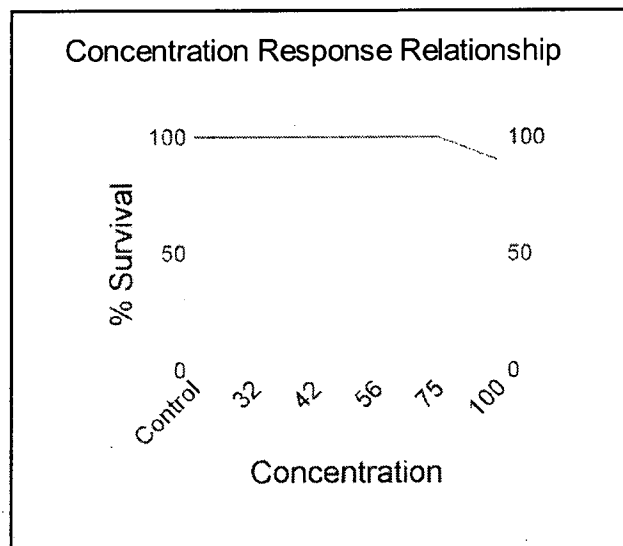
Test End Time: 11:25

End Date: 4/14/2012

SURVIVAL

Effluent Concentration %	Number Of Alive Per Replicate															Avg% Surv.
	4/12					4/13					4/14					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Control	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
32	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
42	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
56	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
75	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
100	8	8	8	8	8	8	8	8	8	7	7	7	8	8	6	90.0%

\* cup spilled



## APPENDIX A

### STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used.

The acute *Mysidopsis bahia* survival data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used.



# Bio-Aquatic Testing, Inc.

48543

2501 Mayes Road, Suite 100  
 Carrollton, TX 75006  
 Tel: 972-242-7750  
 Fax: 972-242-7749

## FRESH WATER TEST SETUP FORM

Client: Ana-Lab

Permit AR0038822

Facility: Cooper Tire & Rubber Co.

Lab Number 48543

Outfall Name: NPDES 001

Number of samples 2

Dilution Water: Synthetic Lab

Receiving Water Name: \_\_\_\_\_

Dechlorinate Sample: No

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	04/12/12	16:00	04/10/12	04/11/12	20:50	20:30
2	04/13/12	15:50	04/11/12	04/12/12	20:50	20:30

<i>Type of Test(s)</i>	
<i>Daphnia pulex</i>	48 Hr Acute
<i>Pimephales promelas</i>	48 Hr Acute

Start Sx # 1 Date: 4/12/2012  
 Renew Sx # 2 Date: 4/13/2012  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_

Controls: Synthetic Lab  
 pH Match: \_\_\_\_\_  
 Hardness Match: moderate

Test Start Date: 4/12/2012 Test End Date: 4/14/2012

Daphnia pulex Test Set Up: 5 Reps & 8 Organisms per Rep

Pimephales Test Set Up: 5 Reps & 8 Organism per Rep

Concentrations: 32 42 56 75 100 % LF % 100

Test Chemistry on these dilutions: 32 42 56 75 100

Samples received by:

<input checked="" type="radio"/> Greyhound	<input type="radio"/> UPS Next Day	<input type="radio"/> Delta Dash	<input type="radio"/> Delta
<input type="radio"/> Pony Express	<input type="radio"/> Client Delivered	<input type="radio"/> Southwest Airlines	<input type="radio"/> DHL
<input type="radio"/> Federal Express	<input type="radio"/> American Airlines	<input type="radio"/> Bio Pick Up	

Other: \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_  
 \_\_\_\_\_

# BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

**Client:** Ana-Lab

**Lab ID:** 48543

**Facility:** Cooper Tire & Rubber Co.

**Outfall:** NPDES 001

**Dilution Water(s):** Synthetic Lab

**Test Date:** April 12, 2012

\*\* 100 %

Effluent Sample #	Received		** Residual Cl <sub>2</sub>	DeChlor (ml/L)	** Ammonia mg/L	Analyst Initials	Initial Salinity	Adjusted Salinity	Temp. Received
	Date	Time							
1	4/12/12	16:00	<0.10	N/A	<0.25	JM	N/A	N/A	5
2	4/13/12	15:50	<0.10	N/A	<0.25	JR	N/A	N/A	5.3

**Chlorine Analysis Method:** Hanna Ion Specific Meter #193711

**Dechlorination Reagent:** Sodium Thiosulfate

Sample #	Received		Hardness (EDTA) As mg/L CaCO <sub>3</sub>		ALKALINITY TO END POINT pH 4.50 +/- 0.05 as mg/L CaCO <sub>3</sub>		Analyst Initials
	Date	Time	CON	100	CON	100	
1	4/12/12	16:00	130.0	96.0	60.0	48.0	PW
2	4/13/12	15:50	130.0	170.0	60.0	147.0	PW

\*\*Hardness taken post zeolite treatment

Date	Lab H <sub>2</sub> O	Sample #	Values are at Highest Dilution		Values are at 100% Dilution		Analyst	Other
			Specific Conductivity as umhos/cm	Salinity (ppt)	Residual Chlorine as mg Cl <sub>2</sub> /L	1 ml 0.02N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> /L		
4/12	Lab H <sub>2</sub> O		428	0.2			DS	
4/13	Lab H <sub>2</sub> O		502	0.3			JL	
4/14	Lab H <sub>2</sub> O						DS	
4/15	Lab H <sub>2</sub> O							
4/16	Lab H <sub>2</sub> O							
4/17	Lab H <sub>2</sub> O							
4/18	Lab H <sub>2</sub> O							
4/12	OUTFALL*	1	194	0.1	<0.10	N/A	DS	
4/13	OUTFALL*	2	701	0.4	<0.10	N/A	JL	
4/14	OUTFALL*						DS	
4/15	OUTFALL*							
4/16	OUTFALL*							
4/17	OUTFALL*							
4/18	OUTFALL*							

\*Conductivity is taken on the highest remaining effluent concentration used for test renewal, not necessarily 100%

# BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

48 Hr Acute

Daphnia pulex

Client: Ana-Lab

Lab ID: 48543

Facility: Cooper Tire & Rubber Co.

Dilution Water(s): Synthetic Lab

Outfall: NPDES 001

Test Begin Date: April 12, 2012

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Control	32	42	56	75	100		
DS	4/12	Start	1	pH	7.8	7.8	7.8	7.8	7.8	7.8		
		25 ± 1		DO (mg/L)	8.3	8.3	8.6	8.9	9.2	9.5		
JL	4/13	24 Hr	1	pH	8.1	8.1	8.1	8.1	8.1	8.1		
		25 ± 1		DO (mg/L)	8.2	8.2	8.3	8.4	8.5	8.5		
DS	4/14	Renew	2	pH	8.2	8.2	8.2	8.2	8.2	8.2		
		48 Hr		DO (mg/L)	8.2	8.2	8.2	8.1	8.1	8.0		
DS	4/14	25 ± 1	2	pH	8.4	8.4	8.4	8.3	8.3	8.3		
		DO (mg/L)		8.1	8.1	8.1	8.1	8.1	8.1			
	4/15	Renew		pH								
		72 Hr		DO (mg/L)								
	4/16	25 ± 1		pH								
		DO (mg/L)										
	4/17	Renew		pH								
		96 Hr		DO (mg/L)								
	4/18	25 ± 1		pH								
		DO (mg/L)										
	4/19	Renew		pH								
		120 Hr		DO (mg/L)								
	4/19	25 ± 1		pH								
		DO (mg/L)										

# BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

48 Hr Acute

Pimephales promelas

Client: Ana-Lab

Lab Number: 48543

Facility: Cooper Tire & Rubber Co.

Dilution Water(s): Synthetic Lab

Outfall: NPDES 001

Test Begin Date: April 12, 2012

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration								
					Control	32	42	56	75	100			
DS	4/12	Start	1	pH	7.8	7.8	7.8	7.8	7.8	7.8			
		25 ± 1		DO (mg/L)	8.3	8.3	8.6	8.9	9.2	9.5			
		24 Hr		pH	7.7	7.7	7.7	7.6	7.6	7.5			
JL	4/13	25 ± 1	1	DO (mg/L)	7.8	7.5	7.3	7.1	7.0	6.8			
		Renew		2	pH	8.2	8.2	8.2	8.2	8.2	8.2		
		DO (mg/L)		8.2	8.2	8.2	8.1	8.1	8.0				
DS	4/14	48 Hr	2	pH	8.2	8.0	8.0	8.0	8.0	7.9			
		25 ± 1		DO (mg/L)	8.1	7.8	7.8	7.7	7.7	7.6			
		Renew			pH								
	4/15	72 Hr		DO (mg/L)									
		25 ± 1		pH									
		Renew			DO (mg/L)								
	4/16	96 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew			pH								
	4/17	120 Hr		DO (mg/L)									
		25 ± 1		pH									
		Renew			DO (mg/L)								
	4/18	144 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew			pH								
	4/19	168 Hr		DO (mg/L)									
		25 ± 1		pH									
					DO (mg/L)								

## Appendix B

*Daphnia pulex*

### BIO-AQUATIC TESTING, INC.

Carrollton, TX

#### REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, cadmium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

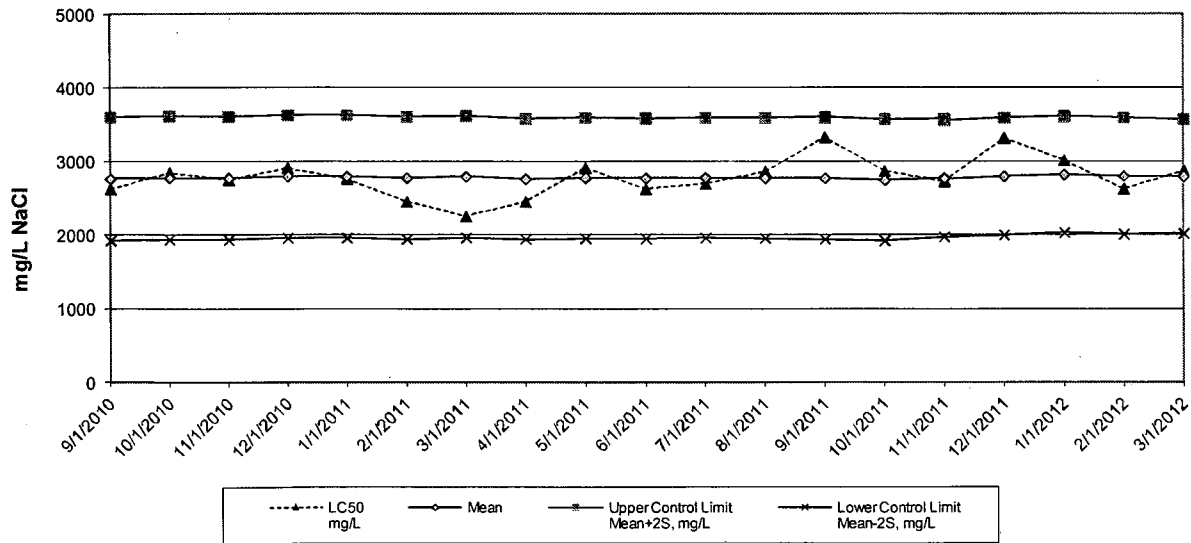
#### ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater
CHEMICAL:	Sodium Chloride
DURATION:	48 Hour Acute
TEST NUMBER:	243
PROJECT NUMBER:	50953
START DATE:	3/28/2012
START TIME:	14:45
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 250 500 1000 2000 3000 4000
NUMBER DEAD PER CONCENTRATION:	0 1 1 2 3 16 40
TEST METHODS:	As listed in EPA-821-R-02-012
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Kärber
LC50:	2871.60 mg/L
95% LOWER CONFIDENCE LIMITS:	2620.66 mg/L
95% UPPER CONFIDENCE LIMITS:	3142.57 mg/L

**REFERENCE TOXICANT STATISTICAL RESULTS: LC<sub>50</sub> AND CONTROL LIMITS  
Daphnia pulex EXPOSED TO SODIUM CHLORIDE, 48 HOUR STATIC RENEWAL**

Test Number	Date	Project Number	Toxicant Lot Number	Statistical Method	LC <sub>50</sub> mg/L	Mean	Twice Standard Deviation 2S	Upper Control Limit Mean+2S, mg/L	Lower Control Limit Mean-2S, mg/L
224	9/7/2010	45724	831604	Trimmed Spearman-Kärber	2625.3	2766.7	837.9	3604.6	1928.9
225	10/1/2010	46169	831604	Trimmed Spearman-Kärber	2848.4	2777.8	836.8	3614.6	1941.0
226	11/23/2010	47181	831604	Trimmed Spearman-Kärber	2751.4	2771.8	837.8	3609.6	1934.0
227	12/28/2010	47698	000520	Trimmed Spearman-Kärber	2912.6	2795.4	836.0	3631.4	1959.4
228	1/26/2011	47833	000520	Trimmed Spearman-Kärber	2765.4	2794.5	835.1	3629.6	1959.4
229	2/24/2011	48034	000520	Trimmed Spearman-Kärber	2449.5	2775.7	831.7	3607.4	1944.0
230	3/23/2011	48122	000520	Trimmed Spearman-Kärber	2255.8	2794.3	827.1	3621.5	1967.2
231	4/26/2011	48254	000520	Trimmed Spearman-Kärber	2452.7	2763.9	821.1	3585.0	1942.8
232	5/26/2011	48342	000520	Trimmed Spearman-Kärber	2913.0	2774.9	821.4	3596.3	1953.5
233	6/27/2011	48478	023007	Trimmed Spearman-Kärber	2625.3	2768.9	819.3	3588.2	1949.7
234	7/27/2011	48937	023007	Trimmed Spearman-Kärber	2694.4	2777.6	816.2	3593.8	1961.4
235	8/2/2011	48959	023007	Trimmed Spearman-Kärber	2862.9	2773.5	816.7	3590.2	1956.9
236	9/7/2011	49154	023007	Trimmed Spearman-Kärber	3329.2	2772.6	828.9	3601.5	1943.8
237	10/5/2011	49219	023007	Trimmed Spearman-Kärber	2868.1	2750.4	825.5	3575.9	1924.8
238	11/8/2011	49503	023007	Trimmed Spearman-Kärber	2729.7	2777.2	799.3	3576.5	1978.0
239	11/29/2011	49797	023007	Trimmed Spearman-Kärber	2741.5	2766.0	791.7	3557.7	1974.2
240	12/27/2011	50692	023007	Trimmed Spearman-Kärber	3317.2	2797.6	796.0	3593.6	2001.6
241	1/18/2012	50765	023007	Trimmed Spearman-Kärber	3015.7	2822.9	791.2	3614.1	2031.8
242	2/22/2012	50855	023007	Trimmed Spearman-Kärber	2625.3	2801.6	790.1	3591.7	2011.5
243	3/28/2012	50950	023007	Trimmed Spearman-Kärber	2871.6	2797.4	776.9	3574.2	2020.5

**Daphnia pulex Acute Control Chart**



## Appendix B

*Pimephales promelas*

### BIO-AQUATIC TESTING, INC.

Carrollton, TX

#### REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, cadmium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

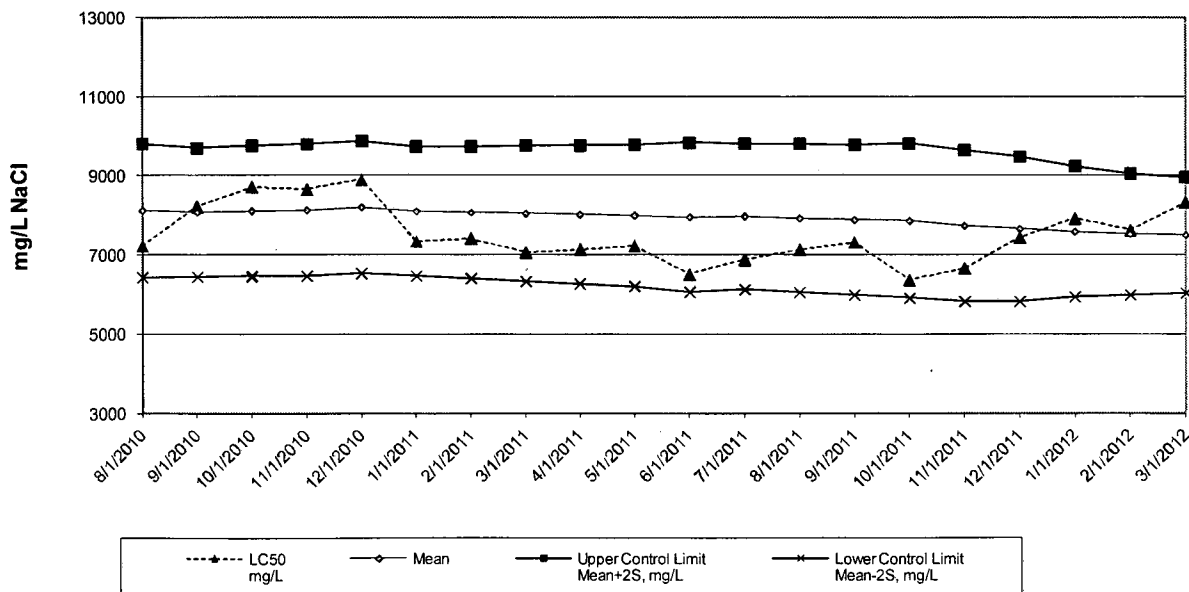
#### ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater
CHEMICAL:	Sodium Chloride
DURATION:	48 Hour Acute
TEST NUMBER:	242
PROJECT NUMBER:	50953
START DATE:	3/28/2012
START TIME:	16:55
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 2000 4000 6000 8000 10000 12000
NUMBER DEAD PER CONCENTRATION:	0 0 0 0 13 38 40
TEST METHODS:	As listed in EPA-821-R-02-012
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Kärber
LC50:	8315.66 mg/L
95% LOWER CONFIDENCE LIMITS:	7986.98 mg/L
95% UPPER CONFIDENCE LIMITS:	8657.87 mg/L

**REFERENCE TOXICANT STATISTICAL RESULTS: LC<sub>50</sub> AND CONTROL LIMITS  
Pimephales promelas EXPOSED TO SODIUM CHLORIDE, 48 HOUR STATIC RENEWAL**

Test Number	Date	Project Number	Toxicant Lot Number	Statistical Method	LC <sub>50</sub> mg/L	Mean	Twice Standard Deviation 2S	Upper Control Limit Mean+2S, mg/L	Lower Control Limit Mean-2S, mg/L
223	8/24/2010	45667	831604	Trimmed Spearman-Kärber	7225.2	8119.3	1689.3	9808.6	6430.0
224	9/8/2010	45733	831604	Trimmed Spearman-Kärber	8215.2	8075.5	1627.4	9702.9	6448.0
225	10/27/2010	46455	831604	Trimmed Spearman-Kärber	8711.7	8109.8	1651.7	9761.6	6458.1
226	11/23/2010	47175	831604	Trimmed Spearman-Kärber	8647.5	8129.0	1668.1	9797.1	6460.9
227	12/29/2010	47696	000520	Trimmed Spearman-Kärber	8897.3	8199.9	1672.3	9872.2	6527.6
228	1/25/2011	47835	000520	Trimmed Spearman-Kärber	7334.5	8103.3	1635.7	9739.0	6467.7
229	2/25/2011	48048	000520	Trimmed Spearman-Kärber	7412.8	8072.0	1664.6	9736.6	6407.5
230	3/23/2011	48121	000520	Trimmed Spearman-Kärber	7059.1	8045.1	1713.5	9758.6	6331.6
231	4/26/2011	48255	000520	Trimmed Spearman-Kärber	7138.7	8022.2	1750.6	9772.8	6271.6
232	5/26/2011	48339	000520	Trimmed Spearman-Kärber	7221.9	7990.7	1785.8	9776.5	6204.9
233	6/29/2011	48483	023007	Trimmed Spearman-Kärber	6503.2	7950.1	1884.6	9834.7	6065.6
234	7/27/2011	48944	023007	Trimmed Spearman-Kärber	6874.8	7967.4	1835.4	9802.8	6132.0
235	8/30/2011	49054	023007	Trimmed Spearman-Kärber	7133.5	7926.0	1872.9	9799.0	6053.1
236	9/29/2011	49213	023007	Trimmed Spearman-Kärber	7315.3	7885.9	1890.0	9775.9	5996.0
237	10/26/2011	49501	023007	Trimmed Spearman-Kärber	6368.1	7863.6	1952.9	9816.4	5910.7
238	11/28/2011	49800	023007	Trimmed Spearman-Kärber	6652.4	7732.7	1906.3	9639.0	5826.4
239	12/28/2011	50693	023007	Trimmed Spearman-Kärber	7435.6	7657.2	1821.9	9479.2	5835.3
240	1/18/2012	50766	023007	Trimmed Spearman-Kärber	7918.8	7585.2	1643.7	9228.8	5941.5
241	2/23/2012	50858	023007	Trimmed Spearman-Kärber	7625.5	7523.3	1530.3	9053.6	5993.0
242	3/28/2012	50953	023007	Trimmed Spearman-Kärber	8315.7	7500.3	1463.6	8963.9	6036.7

**Fathead Minnow Acute Control Chart**





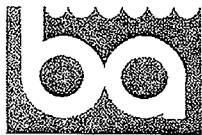
## APPENDIX C

### LITERATURE REFERENCES

- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.
- U.S.E.P.A., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.
- Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J.

# **CHAIN-OF-CUSTODY SHEETS**

## Appendix D



**BIO-AQUATIC TESTING, INC.**  
 2501 MAYES RD., STE. 100  
 CARROLLTON, TX 75006  
 PH: 972-242-7750 FAX: 972-242-7749

**CHAIN OF CUSTODY**



Lab Id : **48543**

Please Review & Complete Sections A, B, C, & D.

Sample No: **48543** -

Check Sample No. :  First,  Second, or  Third.

P.O. No:

Client: Ana-Lab  
 Facility: Cooper Tire & Rubber Co.  
 Permit No: AR0038822  
 Outfall: NPDES 001  
 Client Contact: ROY WHITE  
 Client Phone: 903-984-0551

**B** Use below to make changes, if different from the Scheduled Test(s) in "A":

Freshwater Species					Saltwater Species	
<i>C. dubia</i> (water flea)	<i>D. pulex</i> (water flea)	<i>D. magna</i> (water flea)	<i>P. promelas</i> (minnow)	<i>Selenastrum</i> (green algae)	<i>M. beryllina</i> (minnow)	<i>Mysidopsis</i> (shrimp)
<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input checked="" type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input checked="" type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour

**A** REVIEW SCHEDULED TEST(S):

48 Hr Acute	Daphnia pulex
48 Hr Acute	Pimephales promelas

To Ship the 1st Sample on: 4/9/2012

Concentration: 32 42 56 75 100

Notes: 2nd Quarter Pulex  
 Bi-Monthly Fathead  
 Has WET Limit.

(For TX) Setup separate 24hr Acute Test?

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date		Sample Time (military)		Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
		From	To	From	To			
1 <u>Outfall 001</u>	<u>E</u>	<u>4/10/12</u>	<u>4/11/12</u>	<u>2050</u>	<u>2030</u>	<u>Comp</u>	<u>Charles D. Allen</u> <u>Charles Allen</u>	<u>1</u>
2								
3								

Relinquished By:	Date	Time	Received By:	Date	Time
1 <u>Charles D. Allen</u>	<u>4/12/12</u>	<u>0720</u>	<u>[Signature]</u>	<u>4.12.12</u>	<u>1700</u> <u>1600</u>
2					
3					

**Bio-Aquatic Sample Login**  
**ANALYTICAL TESTING REQUIRED**

BAT sample personnel:  Yes  No  
 Check for Ammonia:  Yes  No  
 Dechlorinate Sample:  Yes  No  
 Dilution Water:  Receiving Stream  
 Synthetic Lab

Date: 4.12.12 Time: 1700 1600 By: [Signature]  
 Temperature: 5 (C) Int. Salinity: ppt Adj. Salinity: ppt  
 Chlorine: 6.1 mg/l Ammonia: 10.25 Other:  
 pH: 7.3 DO: 9.6 mg/l Condition: Good



**BIO-AQUATIC TESTING, INC.**  
 2501 MAYES RD., STE. 100  
 CARROLLTON, TX 75006  
 PH: 972-242-7750 FAX: 972-242-7749

### CHAIN OF CUSTODY

Bio Only:  
No Sample Left

Lab Id : **48543**

Please Review & Complete Sections A, B, C, & D.

Sample No: **48543** -

Check Sample No. : \_\_\_ First,  Second, or \_\_\_ Third.

P.O. No: \_\_\_\_\_

Client: Ana-Lab  
 Facility: Cooper Tire & Rubber Co.  
 Permit No: AR0038822  
 Outfall: NPDES 001  
 Client Contact: ROY WHITE  
 Client Phone: 903-984-0551

**B** Use below to make changes, if different from the Scheduled Test(s) in "A":

Freshwater Species					Saltwater Species	
<i>C. dubia</i> (water flea)	<i>D. pulex</i> (water flea)	<i>D. magna</i> (water flea)	<i>P. promelas</i> (minnow)	<i>Selenastrum</i> (green algae)	<i>M. beryllina</i> (minnow)	<i>Mysidopsis</i> (shrimp)
<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input checked="" type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input checked="" type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour

**A** REVIEW SCHEDULED TEST(S):

48 Hr Acute	<u>Daphnia pulex</u>
48 Hr Acute	<u>Pimephales promelas</u>

To Ship the  
1st Sample on:  
4/9/2012

Concentration: 32 42 56 75 100

Notes: 2nd Quarter Pulex  
Bi-Monthly Fathead  
Has WET Limit

(For TX) Setup separate 24hr Acute Test?

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date		Sample Time (military)		Grab or Composite	Sampled By: (Sign and Print Name)		Number Of Containers Shipped
		From	To	From	To				
1 <u>Outfall 001</u>	<u>E</u>	<u>4/11/12</u>	<u>4/12/12</u>	<u>2050</u>	<u>2030</u>	<u>Comp</u>	<u>Charles Allen</u>	<u>Charles Allen</u>	<u>1</u>
2									
3									

Relinquished By:	Date	Time	Received By:		Date	Time
1 <u>Charles D. Allen</u>	<u>4/13/12</u>	<u>0730</u>	<u>[Signature]</u>	<u>[Signature]</u>	<u>4-13-12</u>	<u>1550</u>
2						
3						

**Bio-Aquatic Sample Login**

**ANALYTICAL TESTING  
REQUIRED**

BAT sample personnel:  Yes  No

Check for Ammonia:  Yes  No

Dechlorinate Sample:  Yes  No

Dilution Water:  Receiving Stream  
 Synthetic Lab

Date: <u>4-13-12</u>	Time: <u>1550</u>	By: <u>[Signature]</u>
Temperature: <u>5.3</u> (C)	Int. Salinity: _____ ppt	Adj. Salinity: _____ ppt
Chlorine: <u>20.1</u> mg/l	Ammonia: <u>20.2</u>	Other: _____
pH: <u>7.3</u>	DO: <u>9.9</u> mg/l	Condition: <u>Good</u>

# REGULATORY AGENCY TABLES

Appendix E

Table 1 (Sheet 1 of 2 )  
BIOMONITORING REPORT

*Daphnia pulex* SURVIVAL TEST

Permittee: Ana-Lab - Cooper Tire & Rubber Co.  
Permit No.: AR0038822  
Outfall No.: NPDES 001

Dates and times FROM: 4/10/2012 @20:50 TO: 4/11/2012 @ 20:30  
Composites were collected: FROM: 4/11/2012 @20:50 TO: 4/12/2012 @ 20:30

Test Initiation: Time: 16:15 Date: 4/12/2012

Dilution Water Used:  Receiving Water  Synthetic Dilution Water

DATA TABLE FOR SURVIVAL OF *Daphnia pulex*

TIME	REPLICATE	EFFLUENT CONCENTRATION (%)					
		0%	32 %	42 %	56 %	75 %	100 %
24 HOUR	A	100	100	100	100	100	100
	B	100	100	100	100	100	100
	C	100	100	100	100	100	100
	D	100	100	100	100	100	100
	E	100	100	100	100	100	100
48 HOUR	A	100	100	100	100	100	100
	B	100	100	100	100	100	100
	C	100	100	100	100	100	100
	D	100	100	100	100	100	100
	E	100	100	100	100	100	100
MEAN		100	100	100	100	100	100
CV % <sup>1</sup>		0.00	0.00	0.00	0.00	0.00	0.00

<sup>1</sup> Coefficient of Variation = (standard deviation/mean) x 100

?= cannot be calculated due to 100% mortality or lab exception

DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST (as appropriate for Lethality)

Is the mean survival at 48 hours significantly different (p=0.05) than the control's survival for the low flow or critical dilution?

CRITICAL DILUTION ( 100 % ):                      YES                      X                      NO

If you report NO, enter a '0' on the DMR form for Parameter No. TEM3D, other wise enter a '1'.

Enter the percent effluent corresponding to each NOEC below:

NOEC SURVIVAL: 100 % Effluent (Parameter TOM3D)

LOEC SURVIVAL: Q\* % Effluent (Parameter TXM3D)

Q\* refers to a value that is not calculable

Prepared by: Deanne Blake

Approved by: Ch. Robison

Table 1 (Sheet 2 of 2 )  
BIOMONITORING REPORT

*Pimephales promelas* SURVIVAL TEST

Permittee: Ana-Lab - Cooper Tire & Rubber Co.  
Permit No.: AR0038822  
Outfall No.: NPDES.001

Dates and times FROM: 4/10/2012 @ 20:50 TO: 4/11/2012 @ 20:30  
Composites were collected: FROM: 4/11/2012 @ 20:50 TO: 4/12/2012 @ 20:30

Test Initiation: Time: 17:00 Date: 4/12/2012

Dilution Water Used:  Receiving Water  Synthetic Dilution Water

DATA TABLE FOR SURVIVAL OF *Pimephales promelas*

TIME	REPLICATE	EFFLUENT CONCENTRATION (%)					
		0%	32 %	42 %	56 %	75 %	100 %
24 HOUR	A	100	100	100	100	100	100
	B	100	100	100	100	100	100
	C	100	100	100	100	100	100
	D	100	100	100	100	100	100
	E	100	100	100	100	100	87.5
48 HOUR	A	100	100	100	100	100	87.5
	B	100	100	100	100	100	87.5
	C	100	100	100	100	100	100
	D	100	100	100	100	100	100
	E	100	100	100	100	100	75
MEAN		100	100	100	100	100	90
CV % <sup>1</sup>		0.00	0.00	0.00	0.00	0.00	11.62

<sup>1</sup> Coefficient of Variation = (standard deviation/mean) x 100

?= cannot be calculated due to 100% mortality or lab exception

DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST (as appropriate for Lethality)  
Is the mean survival at 48 hours significantly different (p=0.05) than the control's survival for the low flow or critical dilution?

CRITICAL DILUTION ( 100 % ): \_\_\_\_\_ YES \_\_\_\_\_ X \_\_\_\_\_ NO

If you report NO, enter a '0' on the DMR form for Parameter No. TEM6C, other wise enter a '1'.

Enter the percent effluent corresponding to each NOEC below:

NOEC SURVIVAL: 100 % Effluent (Parameter TOM6C)

LOEC SURVIVAL: Q\* % Effluent (Parameter TXM6C)

Q\* refers to a value that is not calculable

Prepared by: Deanna Blake

Approved by: Chi Roberson



**Bio-Aquatic Testing, Inc.**



Ana-Lab  
Cooper Tire & Rubber Co.  
OUTFALL NPDES 001

48 Hr Acute Biomonitoring Report

48542

*Pimephales promelas*

June 14, 2012

Approved by: Chris Robason  
Chris Robason,  
President



Bio-Aquatic Testing, Inc. • 2501 Mayes Rd. Ste. 100 • Carrollton, Texas • 75006



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REGULATORY AGENCY TABLES	Appendix E

**Unless otherwise noted in the body of the report, all data reported in this document are in compliance with NELAC standards and apply only to the samples referenced within. This report document may not be edited or reproduced in part or in full by any other entity, unless Bio-Aquatic Testing, Inc. issues written approval.**

**\*HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST**

**BIO-AQUATIC TESTING, INC.**

2501 Mayes Road, Suite 100  
Carrollton, Texas 75006  
Tel: (972) 242-7750  
Fax: (972) 242-7749

**TOXICITY TEST REPORT - 48 Hr Acute**

---

Client:	Ana-Lab	Sample:	NPDES 001
Facility:	Cooper Tire & Rubber Co.	Laboratory Number:	48542
Permit No.	AR0038822	Date:	June 14, 2012

*Pimephales promelas* **passed** survival testing requirements.

---

**SAMPLE COLLECTION:** Composite effluent samples from Ana-Lab, Cooper Tire & Rubber Co., were transported to Bio-Aquatic Testing via Greyhound. Samples were received on June 14, 2012 and June 15, 2012. Effluent samples were collected from Outfall NPDES 001 by facility personnel.

The effluent samples were analyzed for total residual chlorine using the Hanna Ion Specific Meter #193711 and contained <0.10 mg/L and <0.10 mg/L, respectively. Effluent and laboratory dilution water pH, temperature, and dissolved oxygen data were collected daily.

**TEST PROCEDURES:**

*Pimephales promelas*

**EPA METHOD: 2000**

The 48 Hr Acute *Pimephales promelas* test was initiated at 11:30 hours on June 14, 2012. Five effluent concentrations of 32%, 42%, 56%, 75%, and 100% were prepared utilizing synthetic water. The test was set up with 450mL plastic cups containing 250mL of test solution as test chambers. Each concentration or control consisted of five replicate chambers containing eight organisms each, giving a total of 40 (forty) per treatment. The control was conducted concurrently with the test. Test organisms were laboratory cultured *P. promelas* ten days old, and all larvae used in each test are hatched within 24 hours of each other. The number of surviving larvae and water quality parameters were recorded after each 24 hour period. The test was renewed daily with fresh solutions. Surviving larvae in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for 48 hours. The test ended at 11:30 hours on June 16, 2012. Survival data was statistically ( $p=0.05$ ) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

**SURVIVAL:**

*Pimephales promelas*

The *Pimephales promelas* survival data failed Shapiro Wilk's test for normality at the 0.01 (0.900) alpha level after the arc sine (square root (Y)) transformation. Bartlett's test for homogeneity is sensitive to non-normal data and should not be performed if data fails Shapiro Wilk's test. The non-parametric Steel's Many-One Rank test performed on *Pimephales promelas* survival data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

**LOEC: Not Calculable (Q)**

**NOEC: 100% Effluent**

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

48 Hr Acute *Pimephales promelas*

Client: Ana- Cooper Tire & Rubber Co.

Permit Number: ADPCE

Sample Type: Composite

Receiving Water Name:

AR0038822

Outfall Name: NPDES 001

Lab ID: 48542

Test Temperature (oC): 25 ± 1

Photo Period: 16 hours light  
8 hours dark

Begin Date: 6/14/2012

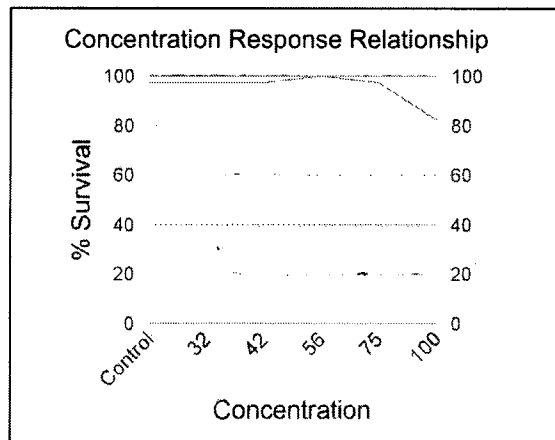
End Date: 6/16/2012

Test Start Time: 11:30 Test End Time: 11:30

SURVIVAL

Effluent Con. %	Number Of Alive Per Replicate															Avg% Surv.
	6/14					6/15					6/16					
	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	
Control	8	8	8	8	8	8	8	8	8	8	8	7	8	8	8	97.5%
32	8	8	8	8	8	8	7	8	8	8	8	7	8	8	8	97.5%
42	8	8	8	8	8	8	8	8	8	8	7	8	8	8	8	97.5%
56	8	8	8	8	8	8	8	8	8	8	8	8	8	8	8	100.0%
75	8	8	8	8	8	8	8	8	8	8	8	8	8	7	8	97.5%
100	8	8	8	8	8	8	7	8	8	8	8	5	5	7	8	82.5%

\* spilled cup



## APPENDIX A

### STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used.

The acute *Mysidopsis bahia* survival data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shipiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used.

48542

### Bio-Aquatic Testing, Inc.

2501 Mayes Road, Suite 100  
Carrollton, TX 75006  
Tel: 972-242-7750  
Fax: 972-242-7749

#### FRESH WATER TEST SETUP FORM

Client: Ana-Lab Permit AR0038822

Facility: Cooper Tire & Rubber Co. Lab Number 48542

Outfall Name: NPDES 001 Number of samples 2

Dilution Water: Synthetic Lab

Receiving Water Name: \_\_\_\_\_

Dechlorinate Sample: No

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	06/14/12	08:30	06/12/12	06/13/12	02:45	02:25
2	06/15/12	08:15	06/13/12	06/14/12	02:45	02:25

**Type of Test(s)**  
Pimephales promelas 48 Hr Acute

Start Sx # 1 Date: 6/14/2012  
 Renew Sx # 2 Date: 6/15/2012  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_  
 Renew Sx # \_\_\_\_\_ Date: \_\_\_\_\_

Controls: Synthetic Lab  
 pH Match: \_\_\_\_\_  
 Hardness Match: moderate

Test Start Date: 6/14/2012 Test End Date: 6/16/2012

Pimephales Test Set Up: 5 Reps & 8 Organisms per Rep

Test Set Up: \_\_\_\_\_

Concentrations: 32 42 56 75 100 % LF % 100

Test Chemistry on these dilutions: 32 42 56 75 100

Samples received by:

<input checked="" type="radio"/> Greyhound	<input type="radio"/> UPS Next Day	<input type="radio"/> Delta Dash	<input type="radio"/> Delta
<input type="radio"/> Pony Express	<input type="radio"/> Client Delivered	<input type="radio"/> Southwest Airlines	<input type="radio"/> DHL
<input type="radio"/> Federal Express	<input type="radio"/> American Airlines	<input type="radio"/> Bio Pick Up	

Other: \_\_\_\_\_  
\_\_\_\_\_  
\_\_\_\_\_

# BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

**Client:** Ana-Lab

**Lab ID:** 48542

**Facility:** Cooper Tire & Rubber Co.

**Outfall:** NPDES 001

**Dilution Water(s):** Synthetic Lab

**Test Date:** June 14, 2012

\*\* 100 %

Effluent Sample #	Received		** Residual Cl <sub>2</sub>	DeChlor (ml/L)	** Ammonia mg/L	Analyst Initials	Initial Salinity	Adjusted Salinity	Temp. Received
	Date	Time							
1	6/14/12	08:30	<0.10	N/A	<0.25	DF	N/A	N/A	3.7
2	6/15/12	08:15	<0.10	N/A	<0.25	DF	N/A	N/A	4.2

**Chlorine Analysis Method:** Hanna Ion Specific Meter #193711      **Dechlorination Reagent:** Sodium Thiosulfate

Sample #	Received		Hardness (EDTA) As mg/L CaCO <sub>3</sub>		ALKALINITY TO END POINT pH 4.50 +/- 0.05 as mg/L CaCO <sub>3</sub>		Analyst Initials
	Date	Time	CON	100	CON	100	
1	6/14/12	08:30	130.0	58.0	55.0	49.0	PW
2	6/15/12	08:15	130.0	158.0	55.0	99.0	PW

\*\*Hardness taken post zeolite treatment

Date	Sample #	Values are at Highest Dilution		Values are at 100% Dilution		Analyst	Other
		Specific Conductivity as umhos/cm	Salinity (ppt)	Residual Chlorine as mg Cl <sub>2</sub> /L	1 ml 0.02N Na <sub>2</sub> S <sub>2</sub> O <sub>3</sub> /L		
6/14	Lab H <sub>2</sub> O	461	0.3			DS	
6/15	Lab H <sub>2</sub> O	466	0.3			JL	
6/16	Lab H <sub>2</sub> O					DS	
6/17	Lab H <sub>2</sub> O						
6/18	Lab H <sub>2</sub> O						
6/19	Lab H <sub>2</sub> O						
6/20	Lab H <sub>2</sub> O						
6/14	OUTFALL*	197	0.1	<0.10	N/A	DS	
6/15	OUTFALL*	397	0.2	<0.10	N/A	JL	
6/16	OUTFALL*					DS	
6/17	OUTFALL*						
6/18	OUTFALL*						
6/19	OUTFALL*						
6/20	OUTFALL*						

\*Conductivity is taken on the highest remaining effluent concentration used for test renewal, not necessarily 100%

# BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

48 Hr Acute

Pimephales promelas

Client: Ana-Lab

Lab ID: 48542

Facility: Cooper Tire & Rubber Co.

Dilution Water(s): Synthetic Lab

Outfall: NPDES 001

Test Begin Date: June 14, 2012

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration							
					Control	32	42	56	75	100		
DS	6/14	Start	1	pH	8.2	8.2	8.2	8.1	8.1	8.0		
		25 ± 1		DO (mg/L)	8.2	8.2	8.2	8.2	8.3	8.3		
JL	6/15	24 Hr	1	pH	7.9	7.9	7.9	7.8	7.7	7.7		
		25 ± 1		DO (mg/L)	7.5	7.5	7.6	7.6	7.6	7.5		
	6/15	Renew	2	pH	7.9	8.0	7.9	7.9	7.8	7.8		
				DO (mg/L)	7.7	8.0	8.1	8.2	8.2	8.3		
DS	6/16	48 Hr	2	pH	7.9	7.9	7.9	7.9	7.9	7.9		
		25 ± 1		DO (mg/L)	7.8	7.8	7.8	7.8	7.7	7.6		
	6/16	Renew		pH								
				DO (mg/L)								
	6/17	72 Hr		pH								
		25 ± 1		DO (mg/L)								
	6/17	Renew		pH								
				DO (mg/L)								
	6/18	96 Hr		pH								
		25 ± 1		DO (mg/L)								
	6/18	Renew		pH								
				DO (mg/L)								
	6/19	120 Hr		pH								
		25 ± 1		DO (mg/L)								
	6/19	Renew		pH								
				DO (mg/L)								
	6/20	144 Hr		pH								
		25 ± 1		DO (mg/L)								
	6/20	Renew		pH								
				DO (mg/L)								
	6/21	168 Hr		pH								
		25 ± 1		DO (mg/L)								



## Appendix B

*Pimephales promelas*

### BIO-AQUATIC TESTING, INC.

Carrollton, TX

#### REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, cadmium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

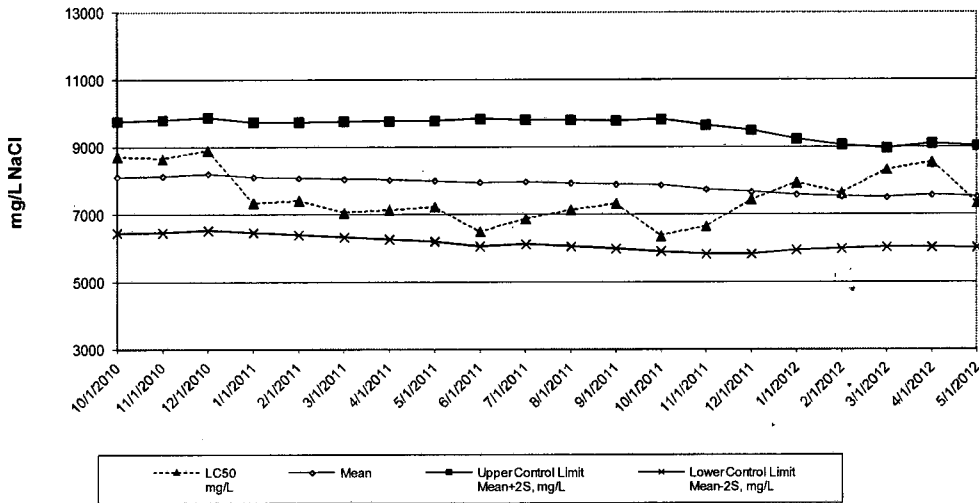
#### ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater
CHEMICAL:	Sodium Chloride
DURATION:	48 Hour Acute
TEST NUMBER:	244
PROJECT NUMBER:	51139
START DATE:	5/29/2012
START TIME:	16:20
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 2000 4000 6000 8000 10000 12000
NUMBER DEAD PER CONCENTRATION:	0 0 0 3 27 40 40
TEST METHODS:	As listed in EPA-821-R-02-012
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Kärber
LC50:	7334.70 mg/L
95% LOWER CONFIDENCE LIMITS:	6993.84 mg/L
95% UPPER CONFIDENCE LIMITS:	7692.17 mg/L

**REFERENCE TOXICANT STATISTICAL RESULTS: LC<sub>50</sub> AND CONTROL LIMITS**  
***Pimephales promelas* EXPOSED TO SODIUM CHLORIDE, 48 HOUR STATIC RENEWAL**

Test Number	Date	Project Number	Toxicant Lot Number	Statistical Method	LC <sub>50</sub> mg/L	Mean	Twice Standard Deviation 2S	Upper Control Limit Mean+2S, mg/L	Lower Control Limit Mean-2S, mg/L
225	10/27/2010	46455	831604	Trimmed Spearman-Kärber	8711.7	8109.8	1651.7	9761.6	6458.1
226	11/23/2010	47175	831604	Trimmed Spearman-Kärber	8647.5	8129.0	1668.1	9797.1	6460.9
227	12/29/2010	47696	000520	Trimmed Spearman-Kärber	8897.3	8199.9	1672.3	9872.2	6527.6
228	1/25/2011	47835	000520	Trimmed Spearman-Kärber	7334.5	8103.3	1635.7	9739.0	6467.7
229	2/25/2011	48048	000520	Trimmed Spearman-Kärber	7412.8	8072.0	1664.6	9736.6	6407.5
230	3/23/2011	48121	000520	Trimmed Spearman-Kärber	7059.1	8045.1	1713.5	9758.6	6331.6
231	4/26/2011	48255	000520	Trimmed Spearman-Kärber	7138.7	8022.2	1750.6	9772.8	6271.6
232	5/26/2011	48339	000520	Trimmed Spearman-Kärber	7221.9	7990.7	1785.8	9776.5	6204.9
233	6/29/2011	48483	023007	Trimmed Spearman-Kärber	6503.2	7950.1	1884.6	9834.7	6065.6
234	7/27/2011	48944	023007	Trimmed Spearman-Kärber	6874.8	7967.4	1835.4	9802.8	6132.0
235	8/30/2011	49054	023007	Trimmed Spearman-Kärber	7133.5	7926.0	1872.9	9799.0	6053.1
236	9/29/2011	49213	023007	Trimmed Spearman-Kärber	7315.3	7885.9	1890.0	9775.9	5996.0
237	10/26/2011	49501	023007	Trimmed Spearman-Kärber	6368.1	7863.6	1952.9	9816.4	5910.7
238	11/23/2011	49800	023007	Trimmed Spearman-Kärber	6652.4	7732.7	1906.3	9639.0	5826.4
239	12/28/2011	50693	023007	Trimmed Spearman-Kärber	7435.6	7657.2	1821.9	9479.2	5835.3
240	1/18/2012	50766	023007	Trimmed Spearman-Kärber	7918.8	7585.2	1643.7	9228.8	5941.5
241	2/23/2012	50858	023007	Trimmed Spearman-Kärber	7625.5	7523.3	1530.3	9053.6	5993.0
242	3/28/2012	50953	023007	Trimmed Spearman-Kärber	8315.7	7500.3	1463.6	8963.9	6036.7
243	4/24/2012	51029	023007	Trimmed Spearman-Kärber	8542.0	7566.2	1528.5	9094.7	6037.7
244	5/29/2012	51139	023007	Trimmed Spearman-Kärber	7334.7	7522.2	1500.3	9022.4	6021.9

**Fathead Minnow Acute Control Chart**



## APPENDIX C

### LITERATURE REFERENCES

- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.
- U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.
- U.S.E.P.A., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.
- Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J.

# **CHAIN-OF-CUSTODY SHEETS**

## Appendix D

Report Date: 06/26/2012 Revision: 0

14 of 17

Bio-Aquatic Lab ID: 48542

**bo** **BIO-AQUATIC TESTING, INC.**  
 2501 MAYES RD., STE. 100  
 CARROLLTON, TX 75006  
 PH: 972-242-7750 FAX: 972-242-7749

**CHAIN OF CUSTODY**  Bio Only, No Sample Left

Please Review & Complete Sections A, B, C, & D. Lab Id: 48543

Check Sample No.:  First,  Second, or  Third. Sample No: 48543

P.O. No: \_\_\_\_\_

Client: Ana-Lab

Facility: Cooper Tire & Rubber Co.

Permit No: AR0038822

Outfall: NPDES 001

Client Contact: ROY WHITE

Client Phone: 903-984-0551

**B. Use below to make changes, if different from the Scheduled Test(s) in "A":**

Freshwater Species				Saltwater Species		
C. dubia (water flea)	D. pulex (water flea)	D. magna (water flea)	P. promelas (minnow)	Selenastrum (green algae)	M. beryllina (minnow)	Mysidopsis (shrimp)
<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input checked="" type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour

**Notes:** 2nd Quarter Pulex  
 Bi-Monthly Fathead  
 Has WET Limit  
TRC 0.01 mg/L

**A. REVIEW SCHEDULED TEST(S):**

48 Hr Acute	<del>Daphnia pulex</del>
48 Hr Acute	Pimephales promelas

To Ship the 1st Sample on: 4/9/2012

Concentration: 32 42 56 75 100

(For TX) Setup separate 24hr Acute Test?

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date		Sample Time (military)		Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
		From	To	From	To			
1 Outfall 001	E	6/12/12	6/13/13	0245	0225	Comp	Charles D. Allen Charles Allen	1
2								
3								

Relinquished By:	Date	Time	Received By:	Date	Time
2					
3					

<b>Bio-Aquatic Sample Login</b> <b>ANALYTICAL TESTING REQUIRED</b>	BAT sample personnel: <input checked="" type="radio"/> Yes <input type="radio"/> No	Date: <u>6-14</u>	Time: <u>0901</u>	By: <u>CA</u>
	Check for Ammonia: <input type="radio"/> Yes <input checked="" type="radio"/> No	Temperature: <u>3.7</u> (C)	Int. Salinity: _____ ppt	Adj. Salinity: _____ ppt
	Dechlorinate Sample: <input type="radio"/> Yes <input checked="" type="radio"/> No	Chlorine: <u>10.1</u> mg/l	Ammonia: <u>10.25</u>	Other: _____
	Dilution Water: <input type="radio"/> Receiving Stream <input checked="" type="radio"/> Synthetic Lab	pH: <u>7.4</u>	DO: <u>9.9</u> mg/l	Condition: <u>Good</u>

Report Date: 06/26/2012 Revision 0

15 of 17

Bio-Aquatic Lab ID: 48542



**BIO-AQUATIC TESTING, INC.**  
 2501 MAYES RD., STE. 100  
 CARROLLTON, TX 75006  
 PH: 972-242-7750 FAX: 972-242-7749

**CHAIN OF CUSTODY**

Bio Only: No Sample Left Lab Id: **48543**

Please Review & Complete Sections A, B, C, & D.

Sample No: **48543-18**

Check Sample No.: \_\_\_ First,  Second, or \_\_\_ Third.

P.O. No: \_\_\_\_\_

Client: **Ana-Lab**  
 Facility: **Cooper Tire & Rubber Co.**  
 Permit No: **AR0038822**  
 Outfall: **NPDES 001**  
 Client Contact: **ROY WHITE**  
 Client Phone: **903-984-0551**

**B. Use below to make changes, if different from the Scheduled Test(s) in "A":**

Freshwater Species					Saltwater Species	
C. dubia (water flea)	D. pulex (water flea)	D. magna (water flea)	P. promelas (minnow)	Selenastrum (green algae)	M. beryllina (minnow)	Mysidopsis (shrimp)
<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input checked="" type="checkbox"/> 24 Hour	<input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour	<input type="checkbox"/> Chronic <input type="checkbox"/> 96 Hour <input type="checkbox"/> 48 Hour <input type="checkbox"/> 24 Hour

**A. REVIEW SCHEDULED TEST(S):**

48 Hr Acute	<del>Daphnia pulex</del>
48 Hr Acute	Pimephales promelas

To Ship the 1st Sample on: **4/9/2012**

Concentration: **32 42 56 75 100**

(For TX ) Setup separate 24hr Acute Test?

Notes: ~~2nd Quarter Pulex~~  
 Bi-Monthly Fathead  
 Has WET Limit

*TRC = 0.02 mg/L*

Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date		Sample Time (military)		Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
		From	To	From	To			
1 Outfall 001	E	6/13/12	6/14/12	0245	0225	Comp	Charles D. Allen Charles Allen	1
2								
3								

Relinquished By:	Date	Time	Received By:	Date	Time
1 Charles D. Allen	6/14/12	6745	<i>[Signature]</i>	6-15-12	0715
2					
3					

**Bio-Aquatic Sample Login**

**ANALYTICAL TESTING REQUIRED**

BAT sample personnel:  Yes  No  
 Check for Ammonia:  Yes  No  
 Dechlorinate Sample:  Yes  No  
 Dilution Water:  Receiving Stream  Synthetic Lab

Date: **6-15** Time: **654<sup>00</sup>** By: *[Signature]*  
 Temperature: **32** (C) Int. Salinity: \_\_\_\_\_ ppt Adj. Salinity: \_\_\_\_\_ ppt  
 Chlorine: **0.1** mg/l Ammonia: **0.26** Other: \_\_\_\_\_  
 pH: **7.7** DO: **9.9** mg/l Condition: **Good**

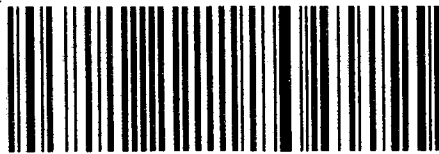
# REGULATORY AGENCY TABLES

## Appendix E





CERTIFIED MAIL™



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**COOPERTIRES**

COOPER TIRE & RUBBER COMPANY  
3500 Washington Road • Texarkana, AR 71854



UNITED STATES POSTAGE



RITNEY BOWES

02 1M

\$ 07.20<sup>0</sup>

0008006971

JUL 17 2012

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AR Dept. of ENVIRONMENTAL Quality  
WATER DIVISION - ENFORCEMENT BRANCH  
5301 Northshore Drive  
North Little Rock, AR  
72118-5317

