



Bio-Aquatic Testing, Inc.



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

48 Hr Acute Biomonitoring Report

48544

Pimephales promelas

August 09, 2012

Approved by: Chris Robason
Chris Robason,
President

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



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*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:	48544
Permit No.	AR0038822	Date:	August 09, 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 August 09, 2012 and August 10, 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 17:10 hours on August 09, 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 11:22 hours on August 11, 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*

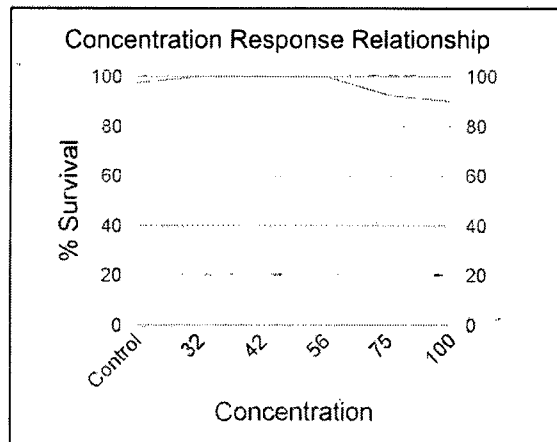
Client: Ana- Cooper Tire & Rubber Co. Lab ID: 48544
 Permit Number: ADPCE AR0038822 Test Temperature (oC): 25 ± 1
 Sample Type: Composite Outfall Name: NPDES 001 Photo Period: 16 hours light
 Receiving Water Name: Begin Date: 8/9/2012 8 hours dark
 End Date: 8/11/2012

Test Start Time: 17:10 Test End Time: 11:22

SURVIVAL

Effluent Con. %	Number Of Alive Per Replicate															Avg% Surv.
	8/9					8/10					8/11					
	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	7	8	97.5%
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	6	7	92.5%
100	8	8	8	8	8	8	8	8	8	8	8	6	7	7	8	90.0%

* 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.

48544

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 48544

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	08/09/12	11:00	08/07/12	08/08/12	17:02	16:40
2	08/10/12	09:40	08/08/12	08/09/12	17:02	16:40

Type of Test(s)	
<u>Pimephales promelas</u>	<u>48 Hr Acute</u>
_____	_____

Start Sx # 1 Date: 8/9/2012
 Renew Sx # 2 Date: 8/10/2012
 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: 8/9/2012 Test End Date: 8/11/2012

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: 48544

Facility: Cooper Tire & Rubber Co.

Outfall: NPDES 001

Dilution Water(s): Synthetic Lab

Test Date: August 9, 2012

** 100 %

Effluent Sample #	Received		** Residual Cl ₂	DeChlor (ml/L)	** Ammonia mg/L	Analyst Initials	Initial Salinity	Adjusted Salinity	Temp. Received
	Date	Time							
1	8/9/12	11:00	<0.10	N/A	<0.25	JM	N/A	N/A	4
2	8/10/12	09:40	<0.10	N/A	<0.25	JR	N/A	N/A	3.9

Chlorine Analysis Method: Hanna Ion Specific Meter #193711

Dechlorination Reagent: Sodium Thiosulfate

Sample #	Received		Hardness (EDTA) As mg/L CaCO ₃		ALKALINITY TO END POINT pH 4.50 +/- 0.05 as mg/L CaCO ₃		Analyst Initials
	Date	Time	CON	100	CON	100	
1	8/9/12	11:00	140.0	56.0	60.0	40.0	PW
2	8/10/12	09:40	140.0	98.0	60.0	78.0	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 ₂ /L	1 ml 0.02N Na ₂ S ₂ O ₃ /L		
8/9	Lab H2O	503	0.3			AH	
8/10	Lab H2O	470	0.3			MH	
8/11	Lab H2O						
8/12	Lab H2O						
8/13	Lab H2O						
8/14	Lab H2O						
8/15	Lab H2O						
8/9	OUTFALL*	222	0.1	<0.10	N/A	AH	
8/10	OUTFALL*	396	0.2	<0.10	N/A	MH	
8/11	OUTFALL*						
8/12	OUTFALL*						
8/13	OUTFALL*						
8/14	OUTFALL*						
8/15	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: 48544

Facility: Cooper Tire & Rubber Co.

Dilution Water(s): Synthetic Lab

Outfall: NPDES 001

Test Begin Date: August 9, 2012

NR indicates that the test is non-renewal.

ANALYST	DATE	TIME	SX#	UNIT	Concentration								
					Control	32	42	56	75	100			
AH	8/9	Start	1	pH	8.2	8.1	8.1	8.0	7.9	7.9			
		25 ± 1		DO (mg/L)	7.6	7.7	7.6	7.5	7.6	7.6			
MH	8/10	24 Hr	1	pH	7.9	7.8	7.8	7.7	7.7	7.7			
		25 ± 1		DO (mg/L)	8.1	7.7	7.7	7.5	7.5	7.3			
		Renew	2	pH	8.1	8.0	8.0	7.9	7.9	7.8			
				DO (mg/L)	7.6	7.7	7.7	7.8	7.8	8.0			
MH	8/11	48 Hr	2	pH	8.1	8.0	8.0	7.9	7.9	7.8			
		25 ± 1		DO (mg/L)	7.8	7.7	7.7	7.6	7.6	7.6			
		Renew		pH									
				DO (mg/L)									
	8/12	72 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew		pH									
				DO (mg/L)									
	8/13	96 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew		pH									
				DO (mg/L)									
	8/14	120 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew		pH									
				DO (mg/L)									
	8/15	144 Hr		pH									
		25 ± 1		DO (mg/L)									
		Renew		pH									
				DO (mg/L)									
	8/16	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, copper chloride, potassium 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.

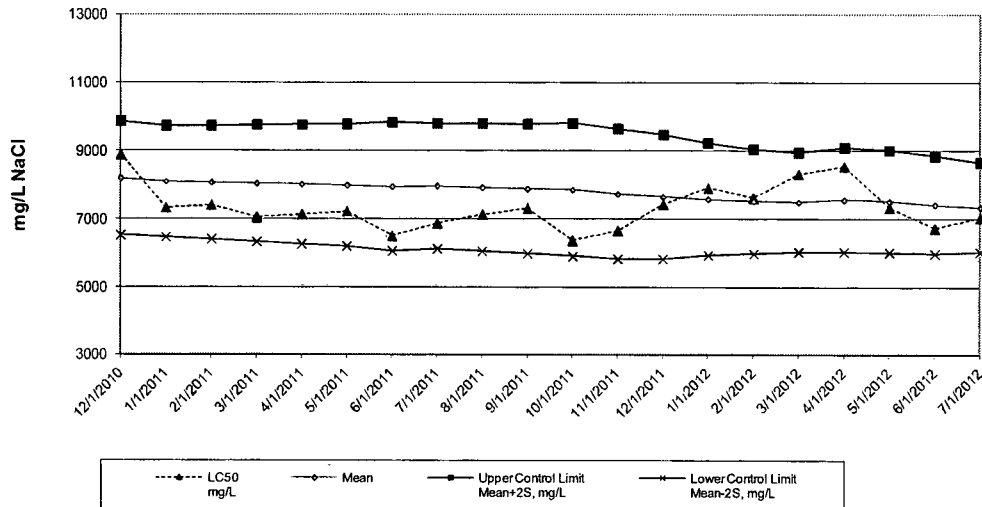
ACUTE REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater
CHEMICAL:	Sodium Chloride
DURATION:	48 Hour Acute
TEST NUMBER:	246
PROJECT NUMBER:	51362
START DATE:	7/31/2012
START TIME:	17:40
TOTAL NUMBER EXPOSED:	40 organisms per concentration
CONCENTRATIONS (mg/L):	CON 2000 4000 6000 8000 10000 12000
NUMBER DEAD PER CONCENTRATION:	0 1 0 1 36 40 40
TEST METHODS:	As listed in EPA-821-R-02-012
STATISTICAL METHODS:	SURVIVAL: Trimmed Spearman-Kärber
LC50:	7048.59 mg/L
95% LOWER CONFIDENCE LIMITS:	6832.38 mg/L
95% UPPER CONFIDENCE LIMITS:	7271.64 mg/L

REFERENCE TOXICANT STATISTICAL RESULTS: LC₅₀ AND CONTROL LIMITS
Pimephales promelas EXPOSED TO SODIUM CHLORIDE, 48 HOUR STATIC RENEWAL

Test Number	Date	Project Number	Toxicant Lot Number	Statistical Method	LC ₅₀ mg/L	Mean	Twice Standard Deviation 2S	Upper Control Limit Mean+2S, mg/L	Lower Control Limit Mean-2S, mg/L
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	11/27/2011	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
245	6/26/2012	51227	134905	Trimmed Spearman-Kärber	6732.0	7423.2	1429.4	8852.5	5993.8
246	7/31/2012	51362	134905	Trimmed Spearman-Kärber	7048.6	7343.2	1315.3	8658.6	6027.9

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



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: **48544**

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

Sample No: **48544**

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:
 Client Phone:

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 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

REVIEW SCHEDULED TEST(S):

48 Hr Acute	Pimephales promelas

To Ship the 1st Sample on: **8/14/2012**

Concentration: 32 42 56 75 100

Notes: Bi-Monthly Fathead, *Artly Pulex*
 Has WET Limit for Fathead Lethal
 Send off total and dissolved zinc for each sample. (BG) **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	8/7/12	8/8/12	1702	1640	Comp	Charles D. Allen Charles Allen	1
2								
3								

Relinquished By:	Date	Time	Received By:	Date	Time

Bio-Aquatic Sample Login

BAT sample personnel: Yes No

Check for Ammonia: Yes No

Dechlorinate Sample: Yes No

Dilution Water: Receiving Synthetic

ANALYTICAL TESTING REQUIRED

Date: **8-9-12** Time: **1100** By: **[Signature]**

Temperature: **4** (C) Int. Salinity: ppt Adj. Salinity: ppt

Chlorine: **20.1** mg/l Ammonia: **6.25** Other:

pH: **8.73** DO: **3.0** 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: **48544**

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

Sample No: **48544**

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: _____

Client Phone: _____

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 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	Pimephales promelas

To Ship the
1st Sample on:
8/14/2012

Concentration: 32 42 56 75 100

Notes: Bi-Monthly Fathead, only pulex

Has WET Limit for Fathead Lethal

Send off total and dissolved zinc for each sample. (BG)

TRC = 0.03 mg/l

(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	8/8/12	8/9/12	1702	1640	Camp	Charles Allen Charles Allen	1
2								
3								

Relinquished By:	Date	Time	Received By:	Date	Time
1 Charles Allen	8/9/12	1720			
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
 Synthetic

Date: <u>8-10-12</u>	Time: <u>1030</u>	By: <u>JA</u>
Temperature: <u>29</u> (C)	Int. Salinity: _____ ppt	Adj. Salinity: _____ ppt
Chlorine: <u>20.1</u> mg/l	Ammonia: <u>20.00</u>	Other: _____
pH: <u>7.6</u>	DO: <u>9.9</u> mg/l	Condition: <u>Good</u>

REGULATORY AGENCY TABLES

Appendix E

Table 1 (Sheet 1 of 1)
BIOMONITORING REPORT

Pimephales promelas SURVIVAL TEST

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

Dates and times FROM: 8/7/2012 @ 17:02 TO: 8/8/2012 @ 16:40
Composites were collected: FROM: 8/8/2012 @ 17:02 TO: 8/9/2012 @ 16:40

Test Initiation: Time: 17:10 Date: 8/9/2012

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	100
48 HOUR	A	100	100	100	100	100	100
	B	100	100	100	100	100	75
	C	100	100	100	100	100	87.5
	D	87.5	100	100	100	75	87.5
	E	100	100	100	100	87.5	100
MEAN		97.5	100	100	100	92.5	90
CV % ¹		5.73	0.00	0.00	0.00	12.09	11.62

¹ 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: Dianne Blake
Report Date: 08/21/2012 Revision: 6 17 of 17

Approved by: Chi Robison
Bio-Aquatic Lab ID: 48544



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