



December 18, 2014
Control No. 185529-1
Page 1 of 19

December 18, 2014

Test Results of
Fourth Quarter
Chronic
Biomonitoring Testing
for
Outfall 002
Heber Springs, AR

Control No. 185529-1

Prepared for:

Mr. Kent Latch
Heber Springs Water & Sewer
1108 West Front Street
Heber Springs, AR 72543

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322



December 18, 2014
Control No. 185529-1
Page 2 of 19

Heber Springs Water & Sewer
ATTN: Mr. Kent Latch
1108 West Front Street
Heber Springs, AR 72543

Re: Chronic utilizing *Pimephales promelas* (Fathead minnow)
Outfall 002 - Heber Springs, AR
NPDES Permit No. NPDES Permit AR0022381 AFIN 12-00029

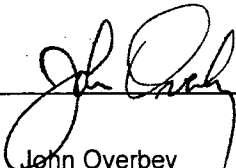
Dear Mr. Kent Latch:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 11 % effluent, which is above the critical dilution of 8 %. The NOEC for growth occurred at 11 % effluent, which is above the critical dilution of 8 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
Laboratory Director

PDF cc: Heber Springs Water & Sewer
ATTN: Mr. Kent Latch
kent@heberspringswater.com

Table of Contents

I. Control Acceptance Criteria

II. Outlined Report

III. Data Analysis

IV. Standard Reference Toxicants

V. Chemical Analysis/Quality Control

VI. Organism History

VII. Results Summary

Pimephales promelas (Fathead minnow)

Appendix A: Raw Data

A1: Test 1000.0

Pimephales promelas (Fathead minnow) Survival and Growth

A2: Statistics

A3: Water Chemistry

A4: Reference Toxicant

Appendix B: Chains of Custody

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.256	PASS
Control Growth CV < or = 40%	9.83	PASS
Growth Minimum Significant Difference 12 to 30%	17.8	PASS
Critical Dilution CV < or = 40%	10.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: NPDES Permit AR0022381 AFIN 12-00029
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Method 1000.0
3. Receiving Stream: White River Basin

B. Source of Effluent/Dilution Water

1. Effluent Samples:

- a. Sampling Point: Outfall 002
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.1	8.5	8.6
pH (standard units)	7.0	6.9	7.2
Alkalinity (mg/l as CaCO ₃)	57	55	50
Hardness (mg/l as CaCO ₃)	41	40	38
Conductivity (umhos/cm)	290	300	300
Residual Chlorine (mg/l)	0.070	<0.05	<0.05
Ammonia as N (mg/l)	9.5	10	10

2. Dilution Water Samples: Synthetic Soft Water #4166

- a. Dates Prepared: December 4 through December 18, 2014
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	8.5	8.8
pH (standard units)	7.3	7.3	7.4
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	48	48	48
Conductivity (umhos/cm)	160	160	160
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods

1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Method 1000.0, Fathead Minnow Survival and Growth.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	December 9, 2014 at 1520
Date & Time Test Terminated:	December 16, 2014 at 1500
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

4. Acclimation of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name
 - a. Test 1000.0 *Pimephales promelas*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on November 18, 2014 at 1600 to November 25, 2014 at 1415

The results were as follows: (Control No. 184773-1.)

Survival LC-50: 4807 mg/l

Growth IC-25: 3075 mg/l

Growth PMSD: 13.5

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	101	1.50
pH	SM 4500-H+ B	101	0.268
Conductivity	EPA 120.1	104	2.65

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: December 9, 2014

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

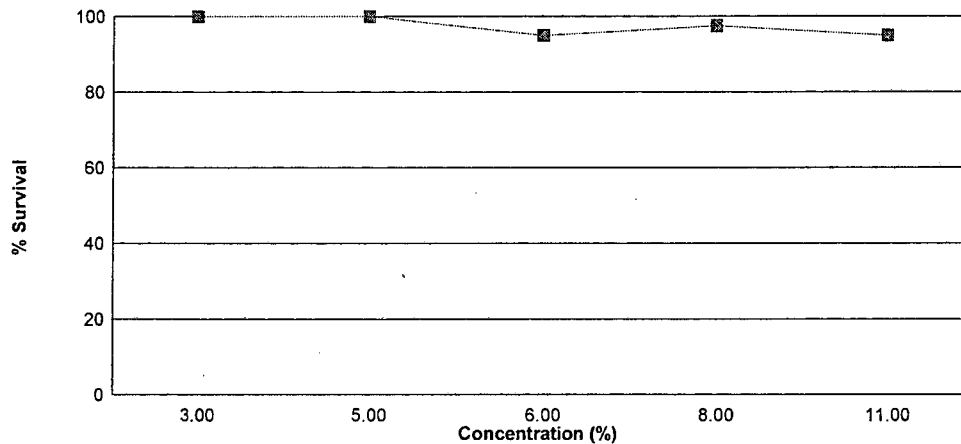
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 11 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 8 % effluent.

The test was initiated on December 9, 2014 at 1520 and continued through December 16, 2014 at 1500. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 11 % effluent
- b.) NOEC growth = 11 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.256
3 %	100	0.222
5 %	100	0.244
6 %	95.0	0.251
8 %	97.5	0.250
11 %	95.0	0.222

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

 Date and Time Test Initiated: December 9, 2014 at 1520
 Date and Time Test Terminated: December 16, 2014 at 1500

Concentration Replicate		Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
3 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
5 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
6 %	A	8	8	8	8	8	7	7
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
11 %	A	8	8	8	8	8	7	7
	B	8	8	7	7	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: December 9, 2014 at 1520
Test Terminated: December 16, 2014 at 1500

Drying Started: December 15, 2014 at 1100
Drying Ended: December 16, 2014 at 1053

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93538	.93742	0.00204	8	0.255
	B	.93142	.93314	0.00172	8	0.215
	C	.93132	.93338	0.00206	8	0.258
	D	.93302	.93525	0.00223	8	0.279
	E	.93798	.94017	0.00219	8	0.274
3 %	A	.93784	.93990	0.00206	8	0.258
	B	.93096	.93265	0.00169	8	0.211
	C	.93005	.93156	0.00151	8	0.189
	D	.93262	.93464	0.00202	8	0.252
	E	.93326	.93486	0.00160	8	0.200
5 %	A	.93848	.94070	0.00222	8	0.278
	B	.93930	.94100	0.00170	8	0.212
	C	.93926	.94160	0.00234	8	0.292
	D	.93746	.93906	0.00160	8	0.200
	E	.93208	.93398	0.00190	8	0.238
6 %	A	.92901	.93065	0.00164	8	0.205
	B	.93715	.93894	0.00179	8	0.224
	C	.93684	.93902	0.00218	8	0.272
	D	.93423	.93646	0.00223	8	0.279
	E	.93537	.93758	0.00221	8	0.276
8 %	A	.93675	.93882	0.00207	8	0.259
	B	.93259	.93427	0.00168	8	0.210
	C	.93261	.93460	0.00199	8	0.249
	D	.93407	.93607	0.00200	8	0.250
	E	.93430	.93657	0.00227	8	0.284
11 %	A	.93053	.93221	0.00168	8	0.210
	B	.92971	.93130	0.00159	8	0.199
	C	.92619	.92814	0.00195	8	0.244
	D	.92711	.92879	0.00168	8	0.210
	E	.92901	.93098	0.00197	8	0.246

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	3 %	1	1.00000	1.39310
2	3 %	2	1.00000	1.39310
2	3 %	3	1.00000	1.39310
2	3 %	4	1.00000	1.39310
2	3 %	5	1.00000	1.39310
3	5 %	1	1.00000	1.39310
3	5 %	2	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	1.00000	1.39310
3	5 %	5	1.00000	1.39310
4	6 %	1	0.87500	1.20940
4	6 %	2	0.87500	1.20940
4	6 %	3	1.00000	1.39310
4	6 %	4	1.00000	1.39310
4	6 %	5	1.00000	1.39310
5	8 %	1	1.00000	1.39310
5	8 %	2	0.87500	1.20940
5	8 %	3	1.00000	1.39310
5	8 %	4	1.00000	1.39310
5	8 %	5	1.00000	1.39310
6	11 %	1	0.87500	1.20940
6	11 %	2	0.87500	1.20940
6	11 %	3	1.00000	1.39310
6	11 %	4	1.00000	1.39310
6	11 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0.108 W = 0.8113 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Steel's Many-One Rank Test			Transform: Arc Sin(Square Root(Y))		
Ho: Control < Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	3 %	27.50	16.00	5.00	
3	5 %	27.50	16.00	5.00	
4	6 %	22.50	16.00	5.00	
5	8 %	25.00	16.00	5.00	
6	11 %	22.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.02229 W = 0.9413 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 1.828 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.00581	0.001162	1.251
Within (Error)	24	0.02229	0.0009288	
Total	29	0.0281		
Critical F = 3.9 (alpha = 0.01, df = 5,24)				
2.62 (alpha = 0.05, df = 5,24)				
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)				

Dunnett's Test - Table 1 of 2					No Transformation
Ho: Control < Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	0.2562	0.2562		
2	3 %	0.222	0.222	1.774	
3	5 %	0.244	0.244	0.6329	
4	6 %	0.2512	0.2512	0.2594	
5	8 %	0.2504	0.2504	0.3009	
6	11 %	0.2218	0.2218	1.785	
Dunnett's critical value = 2.36. (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2						No Transformation
Ho: Control < Treatment						
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control	
1	Control	5				
2	3 %	5	0.04549	17.8	0.0342	
3	5 %	5	0.04549	17.8	0.0122	
4	6 %	5	0.04549	17.8	0.005	
5	8 %	5	0.04549	17.8	0.0058	
6	11 %	5	0.04549	17.8	0.0344	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

 Date and Time Test Initiated: December 9, 2014 at 1418
 Date and Time Test Terminated: December 16, 2014 at 1500

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	8.8	8.5	8.4	8.8	8.5	8.5
	Final	7.4	8.5	7.5	7.4	8.1	7.5	7.7
pH, units	Initial	7.3	7.2	7.3	7.3	7.4	7.6	7.4
	Final	7.3	8.2	7.4	7.4	7.6	7.3	7.4
Alkalinity, mg CaCO ₃ /l		31	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l		48	NA	48	NA	48	NA	NA
Conductivity, umhos/cm		160	150	160	160	160	180	160
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 3 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	8.7	8.6	8.5	8.6	8.4	8.4
	Final	7.0	8.6	7.4	7.2	8.1	7.7	7.5
pH, units	Initial	7.2	7.2	7.2	7.2	7.3	7.5	7.3
	Final	7.2	6.9	7.2	7.4	7.5	7.3	7.4

Effluent Conc.: 5 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	8.8	8.6	8.3	8.7	8.5	8.4
	Final	7.1	8.5	7.4	7.2	8.1	7.5	7.8
pH, units	Initial	7.2	7.2	7.2	7.3	7.3	7.5	7.3
	Final	7.3	6.8	7.3	7.4	7.5	7.3	7.4

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: December 9, 2014 at 1418
Date and Time Test Terminated: December 16, 2014 at 1500

Effluent Conc.: 6 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.2	8.7	8.5	7.5	8.6	8.4	8.5
	Final	7.0	8.5	7.3	7.1	8.1	7.4	7.8
pH, units	Initial	7.2	7.2	7.1	7.2	7.3	7.5	7.4
	Final	7.2	6.8	7.2	7.4	7.5	7.4	7.4

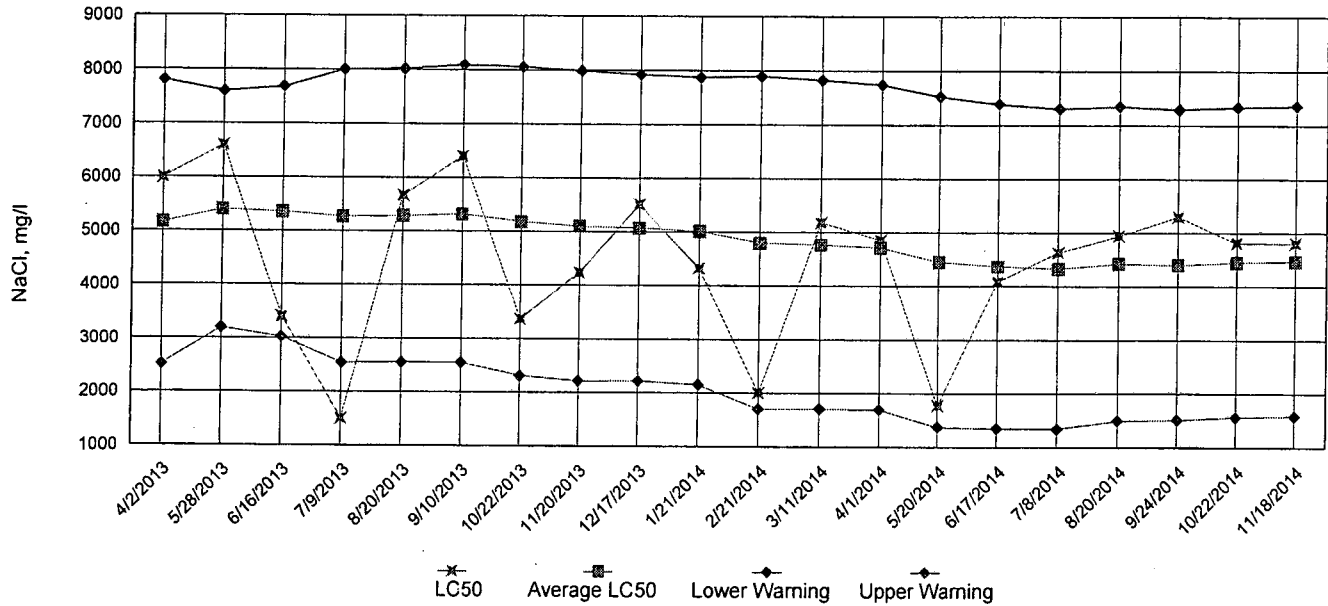
Effluent Conc.: 8 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	8.9	8.7	7.8	8.8	8.2	8.5
	Final	7.3	8.4	7.5	7.3	8.2	7.5	7.2
pH, units	Initial	7.2	7.2	7.1	7.2	7.3	7.4	7.3
	Final	7.4	6.8	7.3	7.4	7.5	7.4	7.4
Alkalinity, mg CaCO ₃ /l		33	NA	35	NA	33	NA	NA
Hardness, mg CaCO ₃ /l		50	NA	48	NA	48	NA	NA
Conductivity, umhos/cm		170	160	170	180	170	190	160
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 11 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	8.8	8.5	8.8	8.6	8.4	8.4
	Final	6.8	8.4	7.5	7.4	8.0	7.7	7.8
pH, units	Initial	7.2	7.2	7.1	7.2	7.3	7.4	7.3
	Final	7.2	6.8	7.4	7.4	7.5	7.4	7.4

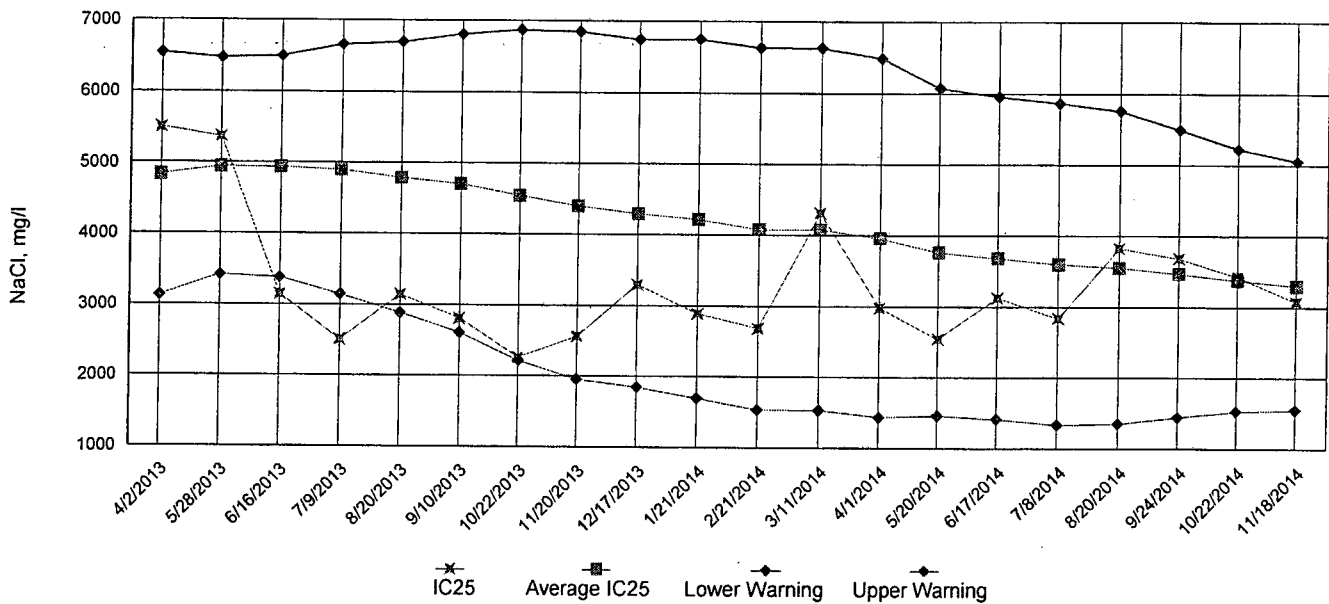
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data



IC25 Growth Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: Heber Springs Water & Sewer

NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: December 9, 2014 at 1520

Date and Time Test Terminated: December 16, 2014 at 1500

Dilution water used: Synthetic Soft Water #4166

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	100	100	100	100	100	100	100	0.00
3 %	100	100	100	100	100	100	100	100	0.00
5 %	100	100	100	100	100	100	100	100	0.00
6 %	87.5	87.5	100	100	100	100	100	95.0	7.21
8 %	100	87.5	100	100	100	100	100	97.5	5.73
11 %	87.5	87.5	100	100	100	100	100	95.0	7.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.255	0.215	0.258	0.279	0.274	0.256	9.83
3 %	0.258	0.211	0.189	0.252	0.200	0.222	14.0
5 %	0.278	0.212	0.292	0.200	0.238	0.244	16.5
6 %	0.205	0.224	0.272	0.279	0.276	0.251	13.6
8 %	0.259	0.210	0.249	0.250	0.284	0.25	10.6
11 %	0.210	0.199	0.244	0.210	0.246	0.222	9.77

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(8 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

2. Dunnett's Test:

Is the mean dry weight (growth) significantly different ($p=0.05$) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(8 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP6C)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP6C)

5. NOEC Pimephales Lethality: 11 % (TOP6C)

6. LOEC Pimephales Lethality: 11 % (TXP6C)

7. NOEC Pimephales Sublethality: 11 % (TPP6C)

8. LOEC Pimephales Sublethality: 11 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 10.6 (TQP6C)

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: Heber Springs Water & Sewer SAMPLE No. 1 COLLECTED ending: DATE: December 9, 2014 TIME: 0800
 NPDES NO.: NPDES Permit AR0022381 AFIN SAMPLE No. 2 COLLECTED ending: DATE: December 11, 2014 TIME: 0800
 CONTACT: Mr. Kent Latch SAMPLE No. 3 COLLECTED ending: DATE: December 13, 2014 TIME: 0800
 ANALYST: 280, 304, 310 Test Initiated: DATE: December 9, 2014 TIME: 1520
 Test Terminated: DATE: December 16, 2014 TIME: 1500

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	8.8	8.5	8.4	8.8	8.5	8.5
Final	7.4	8.5	7.5	7.4	8.1	7.5	7.7
pH Initial	7.3	7.2	7.3	7.3	7.4	7.6	7.4
Final	7.3	8.2	7.4	7.4	7.6	7.3	7.4
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	48	NA	48	NA	48	NA	NA
Conductivity	160	150	160	160	160	180	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 3 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.7	8.6	8.5	8.6	8.4	8.4
Final	7.0	8.6	7.4	7.2	8.1	7.7	7.5
pH Initial	7.2	7.2	7.2	7.2	7.3	7.5	7.3
Final	7.2	6.9	7.2	7.4	7.5	7.3	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	160	160	160	160	160	180	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	8.8	8.6	8.3	8.7	8.5	8.4
Final	7.1	8.5	7.4	7.2	8.1	7.5	7.8
pH Initial	7.2	7.2	7.2	7.3	7.3	7.5	7.3
Final	7.3	6.8	7.3	7.4	7.5	7.3	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	160	160	160	170	160	180	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 6 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.7	8.5	7.5	8.6	8.4	8.5
Final	7.0	8.5	7.3	7.1	8.1	7.4	7.8
pH Initial	7.2	7.2	7.1	7.2	7.3	7.5	7.4
Final	7.2	6.8	7.2	7.4	7.5	7.4	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	160	160	160	180	160	180	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.9	8.7	7.8	8.8	8.2	8.5
Final	7.3	8.4	7.5	7.3	8.2	7.5	7.2
pH Initial	7.2	7.2	7.1	7.2	7.3	7.4	7.3
Final	7.4	6.8	7.3	7.4	7.5	7.4	7.4
Alkalinity	33	NA	35	NA	33	NA	NA
Hardness	50	NA	48	NA	48	NA	NA
Conductivity	170	160	170	180	170	190	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	8.8	8.5	8.8	8.6	8.4	8.4
Final	6.8	8.4	7.5	7.4	8.0	7.7	7.8
pH Initial	7.2	7.2	7.1	7.2	7.3	7.4	7.3
Final	7.2	6.8	7.4	7.4	7.5	7.4	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	170	160	170	170	180	180	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

Client: <u>Heber Springs Water</u>			PO No. <u>14841</u>		NO OF BOTTLES	ANALYSES REQUESTED <u>4TH QUARTER CHRONIC BIOMONITORING</u>						AIC CONTROL NO: <u>185529</u>	
Project <u>4th QUARTER</u>			SAMPLE MATRIX									AIC PROPOSAL NO:	
Reference: <u>CHRONIC BIOMONITORING</u>			WATER		3	4TH QUARTER CHRONIC BIOMONITORING						Carrier: <u>HEBER SPRING WATER</u>	
Project Manager: <u>KENT LATCH</u>			SOIL									Received on Ice (4 C)? <u>YES</u> 0.4°C NO	
Sampled By: <u>S.O. & JORY MASSEY</u>			✓									Remarks	
AIC No	Sample Identification	Date/Time Collected	GRAB	COMP							Field pH calibration		
	<u>OUTFALL EFFL 11.5.W.W.T.P.</u>	<u>12-9-14 8AM TO 12-9-14 8AM</u>		<u>24 HR</u>							on _____ @ _____		
Container Type									Buffer:				
Preservative													
Symbol references: G = Glass NO = none S = Sulfuric acid pH2 P = Plastic V = VOA vials B = NaOH to pH12 Z = Zinc acetate N = Nitric acid pH2													
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN <u>7</u> DAYS Expedited results requested by: <u>KENT LATCH</u> Who should AIC contact with questions: <u>SAM QUERRY</u> Phone: <u>501-362-3422</u> Fax: <u>501-362-3338</u> Report Attention to: <u>KENT LATCH</u> Report Address to: <u>HEBER SPRINGS WATER DEPT.</u> <u>1108 WEST FRONT ST.</u> <u>HEBER SPRINGS, AR. 72543</u>						Relinquished By: <u>Jory Massey</u>		Date/Time: <u>12-9-14 1:00 PM</u>		Received By:		Date/Time:	
						Relinquished By:		Date/Time:		Received in Lab By: <u>Kyle Hester</u>		Date/Time: <u>12-9-14 1:00</u>	
Comments:													

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Heber Springs Water & Wastewater Dept.</u>			PO No. <u>14841</u>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>185529</u>				
Project: <u>4th Quarter</u>			Reference: <u>Chronic Bio monitoring</u>			4th Qtr. Chronic Bio monitoring										AIC PROPOSAL NO:				
Project Manager: <u>Kent Latch</u>			MATRIX													Carrier: <u>Heber Springs Water</u>				
Sampled By: <u>S.O. & Joey Massey</u>			G	C											W	S				
AIC No.	Sample Identification	Date/Time Collected	R	O	A	O														
<u>2</u>	<u>outfall 002A steel. H.S. WWT</u>					<input checked="" type="checkbox"/>														
Container Type			Preservative												Field pH calibration					
G = Glass NO = none			P = Plastic S = Sulfuric acid pH2																	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN <u>7</u> DAYS			V = VOA vials N = Nitric acid pH2																	
Expedited results requested by: <u>Kent Latch</u>			H = HCl to pH2 B = NaOH to pH12																	
Who should AIC contact with questions: Phone: <u>362-3422</u> Fax: <u>501-362-3338</u>			Relinquished By: <u>Tom Stanford</u>		Date/Time <u>12-10-14</u> <u>8:21 AM</u>		Received By: <u>Tom Stanford</u>		Date/Time <u>12-10-14</u> <u>8:21 AM</u>											
Report Attention to: <u>Kent Latch</u>			Relinquished By: <u>Tom Stanford</u>		Date/Time <u>12-10-14</u> <u>11:20 AM</u>		Received in Lab By: <u>Tom Stanford</u>		Date/Time <u>12-10-14</u> <u>11:20</u>											
Report Address to: <u>Heber Springs Water</u> <u>1108 W. Front St.</u>													Comments:							
Email Address: <u>Heber Springs, AR 72543</u>																				

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Heber Springs Water & WASTEWATER Dept.			PO No. 14841		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 185529			
Project: 4TH QUARTER			Reference: CHRONIC BIONOWRITING			4TH QTR CHRONIC BIONOWRITING										AIC PROPOSAL NO.:			
Project Manager: KONT LATCH			MATRIX													Carrier: Heber Springs Water			
Sampled By: S.O. JOEY MASSEY			G	C	W	S											Received Temperature C: 09°C		
AIC No.	Sample Identification	Date/Time Collected	A	M	A	O											Remarks		
			B	P	T	I													
3	OUTFALL 003A SPT. DISHWTR.	12-11-14 8AM TO 12-12-14 8AM		24 HR	✓		3												
Container Type													Field pH calibration on _____ @ _____						
Preservative													Buffer:						
G = Glass NO = none			P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate		A = (NH ₄) ₂ SO ₄ , NH ₄ OH								
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN 7 DAYS					Relinquished By: Jam Query		Date/Time: 12-12-14 8:30 AM		Received By: Tom Starfad		Date/Time: 12-12-14 8:30 AM								
Expedited results requested by: KONT LATCH					Relinquished By: Tom Starfad		Date/Time: 12-12-14 10:45 AM		Received in Lab By: Jimmy D		Date/Time: 12/12/14 1045								
Who should AIC contact with questions: Phone: 501-3422 Fax: 501-362-3330					Comments:														
Report Attention to: KONT LATCH																			
Report Address to: Heber Springs Water 1108 W. FRONT ST.																			
Email Address: Heber Springs, AR. 72543																			



December 12, 2014
Control No. 185164-1
Page 1 of 31

December 12, 2014

Test Results of
Fourth Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 002
Heber Springs, AR

Control No. 185164-1

Prepared for:

Mr. Kent Latch
Heber Springs Water & Sewer
1108 West Front Street
Heber Springs, AR 72543

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322



Heber Springs Water & Sewer
ATTN: Mr. Kent Latch
1108 West Front Street
Heber Springs, AR 72543

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 002 - Heber Springs, AR
NPDES Permit No. NPDES Permit AR0022381 AFIN 12-00029

Dear Mr. Kent Latch:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the laboratory director or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: **The test will need to be repeated due to a growth control failure. The data is attached for your review.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 11 % effluent, which is above the critical dilution of 8 %. The NOEC for reproduction occurred at 11 % effluent, which is above the critical dilution of 8 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: Heber Springs Water & Sewer
ATTN: Mr. Kent Latch
kent@heberspringswater.com

Table of Contents

- I. Control Acceptance Criteria
- II. Outlined Report
- III. Data Analysis
- IV. Standard Reference Toxicants
- V. Chemical Analysis/Quality Control
- VI. Organism History

VII. Results Summary

Pimephales promelas (Fathead minnow)
Ceriodaphnia dubia

Appendix A: Raw Data

A1: Test 1000.0

Pimephales promelas (Fathead minnow) Survival and Growth

Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

A2: Statistics

A3: Water Chemistry

A4: Reference Toxicant

Appendix B: Chains of Custody

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	77.5	FAIL
Control Growth > or = 0.25 mg per Surviving minnow	0.276	PASS
Control Growth CV < or = 40%	12.2	PASS
Growth Minimum Significant Difference 12 to 30%	34.6	FAIL
Critical Dilution CV < or = 40%	28.8	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	29.2	PASS
Control CV < or = 40% per Surviving Female	21.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	15.0	PASS
Critical Dilution CV < or = 40%	15.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: NPDES Permit AR0022381 AFIN 12-00029
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Methods 1000.0 and 1002.0
3. Receiving Stream: White River Basin

B. Source of Effluent/Dilution Water

1. Effluent Samples:
 - a. Sampling Point: Outfall 002
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.3	7.8	8.8
pH (standard units)	7.0	6.9	7.4
Alkalinity (mg/l as CaCO ₃)	42	40	38
Hardness (mg/l as CaCO ₃)	34	42	41
Conductivity (umhos/cm)	310	290	270
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	6.9	6.5	7.0

2. Dilution Water Samples: Synthetic Soft Water #4162

- a. Dates Prepared: November 26 through December 10, 2014
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.8	8.3	8.4
pH (standard units)	7.4	7.3	7.8
Alkalinity (mg/l as CaCO ₃)	30	30	30
Hardness (mg/l as CaCO ₃)	47	47	48
Conductivity (umhos/cm)	200	170	150
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods

1. Test methods used:

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: December 2, 2014 at 1530
Date & Time Test Terminated: December 9, 2014 at 1400
Type & Volume of Test Chamber: 500 ml disposable beaker
Volume of Sample: 250 ml
Number of Organisms per replicate: 8
Number of Replicates per dilution: 5

Ceriodaphnia dubia Survival and Growth Method 1002.0

Date & Time Test Initiated: December 2, 2014 at 1520
Date & Time Test Terminated: December 9, 2014 at 1555
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Acclimation of test organisms: Obtained from in-house cultures

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

a. Test 1000.0 *Pimephales promelas*

b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's and Bartlett's test. The survival data was then analyzed using Dunnett's Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and analyzed with Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC) for Reproduction. Dunnett's Test was used to calculate the PMSD.

IV. Standard Reference Toxicants

American Interplex Corporation has an ongoing test organism culturing program. The sensitivity of the offspring is determined by performing a standard reference toxicant test with each effluent test. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

Chronic reference tests are performed monthly.

A chronic reference test was performed on November 18, 2014 at 1600 to November 25, 2014 at 1415

The results were as follows: (Control No. 184773-1.)

Survival LC-50: 4807 mg/l

Growth IC-25: 3075 mg/l

Growth PMSD: 13.5

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on November 18, 2014 at 1720 to November 24, 2014 at 1635

The results were as follows: (Control No. 184773-2.)

Survival LC-50: 1968 mg/l

Growth IC-25: 654.8 mg/l

Growth PMSD: 22.7

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	99.5	6.44
pH	SM 4500-H+ B	101	0.539
Conductivity	EPA 120.1	104	1.30

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: December 2, 2014

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: December 2, 2014

Age: <24 hours

Source: In-house culture

Water Chemistry Record:

Alkalinity: 57-64 mg/l

Hardness: 80-100 mg/l

Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

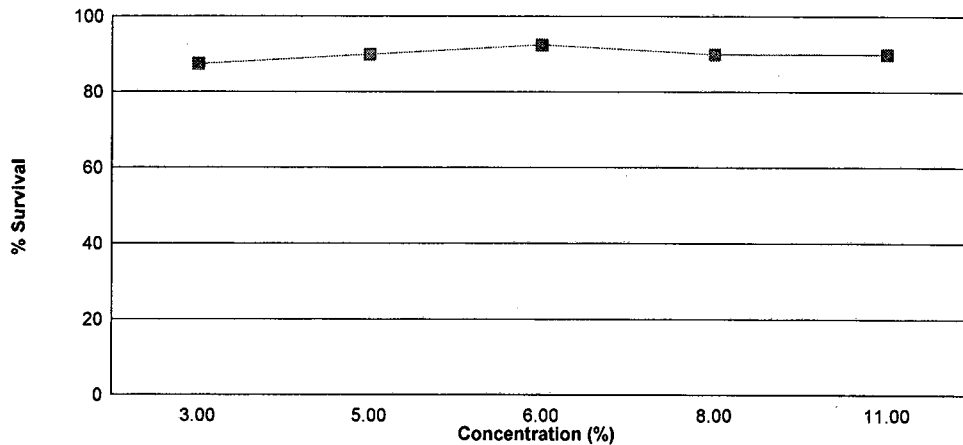
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 11 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 8 % effluent.

The test was initiated on December 2, 2014 at 1530 and continued through December 9, 2014 at 1400. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 11 % effluent
- b.) NOEC growth = 11 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	77.5	0.214
3 %	87.5	0.235
5 %	90.0	0.242
6 %	92.5	0.266
8 %	90.0	0.247
11 %	90.0	0.252

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

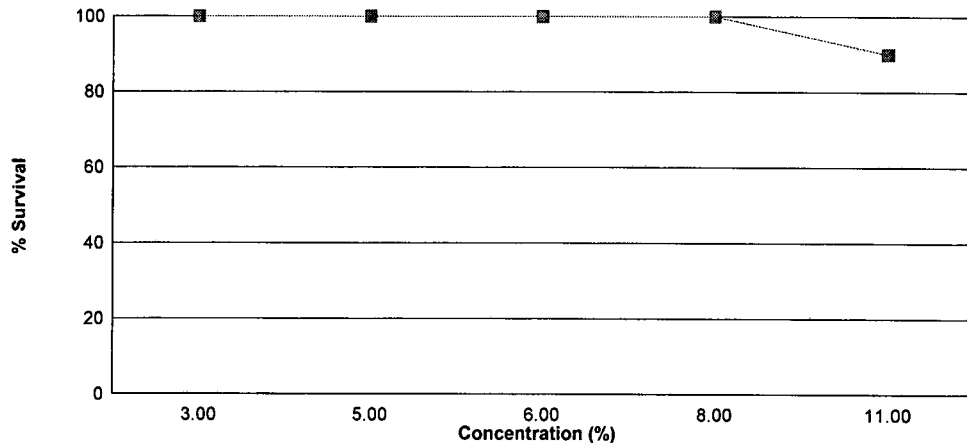
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 11 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 8 % effluent.

The test was initiated on December 2, 2014 at 1520 and continued through December 9, 2014 at 1555. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 11 % effluent
- b.) NOEC reproduction = 11 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	29.2
3 %	100	33.0
5 %	100	33.7
6 %	100	31.4
8 %	100	32.8
11 %	90.0	30.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: December 2, 2014 at 1530

Date and Time Test Terminated: December 9, 2014 at 1400

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	7	5	5	5	5	5
	B	8	8	8	8	8	8	8
	C	8	8	6	6	6	6	6
	D	6	6	5	5	5	5	5
	E	8	8	7	7	7	7	7
3 %	A	7	7	7	7	7	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	7	7	7	7	7	6	6
	E	7	7	7	7	7	6	6
5 %	A	8	8	8	8	8	7	6
	B	8	8	8	8	8	8	8
	C	8	8	7	7	7	7	7
	D	8	8	8	8	8	7	7
	E	8	8	8	8	8	8	8
6 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	7	7	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	7	7	7	7	6
8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	7	7	7	7	7	7
	D	8	7	6	6	6	6	6
	E	7	7	7	7	7	7	7
11 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	6
	C	7	7	7	7	7	7	7
	D	7	7	7	7	7	7	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

 Test Initiated: December 2, 2014 at 1530
 Test Terminated: December 9, 2014 at 1400

 Drying Started: December 8, 2014 at 1142
 Drying Ended: December 10, 2014 at 1148

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94291	.94479	0.00188	8	0.235
	B	.94064	.94259	0.00195	8	0.244
	C	.94076	.94234	0.00158	8	0.198
	D	.93927	.94071	0.00144	8	0.180
	E	.94149	.94321	0.00172	8	0.215
3 %	A	.94423	.94605	0.00182	8	0.228
	B	.94168	.94386	0.00218	8	0.272
	C	.94314	.94506	0.00192	8	0.240
	D	.94633	.94789	0.00156	8	0.195
	E	.94397	.94587	0.00190	8	0.238
5 %	A	.94678	.94867	0.00189	8	0.236
	B	.93787	.94059	0.00272	8	0.340
	C	.94117	.94219	0.00102	8	0.128
	D	.94113	.94288	0.00175	8	0.219
	E	.94118	.94347	0.00229	8	0.286
6 %	A	.94211	.94431	0.00220	8	0.275
	B	.93849	.94045	0.00196	8	0.245
	C	.93978	.94214	0.00236	8	0.295
	D	.94045	.94263	0.00218	8	0.272
	E	.94098	.94291	0.00193	8	0.241
8 %	A	.94524	.94808	0.00284	8	0.355
	B	.94269	.94493	0.00224	8	0.280
	C	.94684	.94849	0.00165	8	0.206
	D	.94702	.94843	0.00141	8	0.176
	E	.94348	.94523	0.00175	8	0.219
11 %	A	.94528	.94758	0.00230	8	0.288
	B	.94302	.94460	0.00158	8	0.198
	C	.94099	.94326	0.00227	8	0.284
	D	.94262	.94442	0.00180	8	0.225
	E	.94110	.94322	0.00212	8	0.265

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: December 2, 2014 at 1520

Date and Time Test Terminated: December 9, 2014 at 1555

Concentration: Control														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	7	8	8	4	7	6	5	5	7	63	10	6.30	
5	14	12	11	12	0	13	9	12	11	13	107	10	10.7	
6	13	0	14	0	10	12	11	0	0	0	60	10	6.00	
7	0	0	0	16	4	0	0	15	14	13	62	10	6.20	
8														
TOTAL	33	19	33	36	18	32	26	32	30	33	292	10	29.2	

Concentration: 3 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	6	6	7	8	6	7	7	6	7	7	67	10	6.70	
5	12	13	12	11	10	11	13	12	12	12	118	10	11.8	
6	0	14	16	0	0	14	12	0	12	2	70	10	7.00	
7	14	0	0	15	14	0	0	15	1	16	75	10	7.50	
8														
TOTAL	32	33	35	34	30	32	32	34	31	37	330	10	33.0	

Concentration: 5 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	6	6	7	7	8	7	7	6	6	4	64	10	6.40	
5	14	13	15	13	12	12	10	11	12	12	124	10	12.4	
6	14	0	15	0	16	14	14	0	0	1	74	10	7.40	
7	0	16	0	13	0	0	0	13	16	17	75	10	7.50	
8														
TOTAL	34	35	37	33	36	33	31	30	34	34	337	10	33.7	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: December 2, 2014 at 1520

Date and Time Test Terminated: December 9, 2014 at 1555

Concentration: 6 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	6	6	7	7	6	7	7	4	7	63	10	6.30	
5	12	13	14	12	10	11	9	10	13	13	117	10	11.7	
6	13	0	15	0	1	0	11	0	15	0	55	10	5.50	
7	0	16	0	14	3	14	0	15	0	17	79	10	7.90	
8														
TOTAL	31	35	35	33	21	31	27	32	32	37	314	10	31.4	

Concentration: 8 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	6	6	7	6	7	7	8	7	7	6	67	10	6.70	
5	15	13	14	11	12	14	12	9	12	11	123	10	12.3	
6	0	13	17	2	12	5	13	0	0	2	64	10	6.40	
7	17	0	0	5	0	0	0	14	18	20	74	10	7.40	
8														
TOTAL	38	32	38	24	31	26	33	30	37	39	328	10	32.8	

Concentration: 11 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	6	6	4	6	7	6	7	5	7	6	60	10	6.00	
5	11	14	X	13	12	12	14	11	13	13	113	9	12.6	
6	0	15	X	2	0	0	15	0	0	0	32	9	3.56	
7	17	0	X	14	16	14	0	13	15	13	102	9	11.3	
8														
TOTAL	34	35	4	35	35	32	36	29	35	32	307	10	30.7	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	0.62500	0.91174
1	Control	2	1.00000	1.39310
1	Control	3	0.75000	1.04720
1	Control	4	0.62500	0.91174
1	Control	5	0.87500	1.20940
2	3 %	1	0.87500	1.20940
2	3 %	2	1.00000	1.39310
2	3 %	3	1.00000	1.39310
2	3 %	4	0.75000	1.04720
2	3 %	5	0.75000	1.04720
3	5 %	1	0.75000	1.04720
3	5 %	2	1.00000	1.39310
3	5 %	3	0.87500	1.20940
3	5 %	4	0.87500	1.20940
3	5 %	5	1.00000	1.39310
4	6 %	1	1.00000	1.39310
4	6 %	2	1.00000	1.39310
4	6 %	3	0.87500	1.20940
4	6 %	4	1.00000	1.39310
4	6 %	5	0.75000	1.04720
5	8 %	1	1.00000	1.39310
5	8 %	2	1.00000	1.39310
5	8 %	3	0.87500	1.20940
5	8 %	4	0.75000	1.04720
5	8 %	5	0.87500	1.20940
6	11 %	1	1.00000	1.39310
6	11 %	2	0.75000	1.04720
6	11 %	3	0.87500	1.20940
6	11 %	4	0.87500	1.20940
6	11 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality	Transform: Arc Sin(Square Root(Y))
<p>D = 0.6446 W = 0.9118 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	Transform: Arc Sin(Square Root(Y))
<p>Calculated B1 statistic = 0.7751 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

ANOVA Table			Transform: Arc Sin(Square Root(Y))	
SOURCE	DF	SS	MS	F
Between	5	0.1143	0.02286	0.8514
Within (Error)	24	0.6445	0.02685	
Total	29	0.7588		
Critical F = 3.9 (alpha = 0.01, df = 5,24)				
2.62 (alpha = 0.05, df = 5,24)				
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)				

Dunnett's Test - Table 1 of 2				Transform: Arc Sin(Square Root(Y))	
Ho: Control < Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	1.0946	0.775		
2	3 %	1.218	0.875	-1.191	
3	5 %	1.2504	0.9	-1.503	
4	6 %	1.2872	0.925	-1.858	
5	8 %	1.2504	0.9	-1.503	
6	11 %	1.2504	0.9	-1.503	
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2				Transform: Arc Sin(Square Root(Y))	
Ho: Control < Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	3 %	5	0.2254	28.5	-0.1
3	5 %	5	0.2254	28.5	-0.125
4	6 %	5	0.2254	28.5	-0.15
5	8 %	5	0.2254	28.5	-0.125
6	11 %	5	0.2254	28.5	-0.125

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.05932 W = 0.9693 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 10.46 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.007492	0.001498	0.606	
Within (Error)	24	0.05932	0.002472		
Total	29	0.06681			
Critical F = 3.9 (alpha = 0.01, df = 5,24)					
2.62 (alpha = 0.05, df = 5,24)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation	
Ho: Control < Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	0.2144	0.2144			
2	3 %	0.2346	0.2346	-0.6424		
3	5 %	0.2418	0.2418	-0.8714		
4	6 %	0.2656	0.2656	-1.628		
5	8 %	0.2472	0.2472	-1.043		
6	11 %	0.252	0.252	-1.196		
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)						

Dunnett's Test - Table 2 of 2						No Transformation	
Ho: Control < Treatment							
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control		
1	Control	5					
2	3 %	5	0.07421	34.6	-0.0202		
3	5 %	5	0.07421	34.6	-0.0274		
4	6 %	5	0.07421	34.6	-0.0512		
5	8 %	5	0.07421	34.6	-0.0328		
6	11 %	5	0.07421	34.6	-0.0376		

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
3 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
5 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
6 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
8 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
11 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	3 %	10	0	
2	5 %	10	0	
3	6 %	10	0	
4	8 %	10	0	
5	11 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.1747 D* = 1.371 Critical D* = 1.035 (alpha = 0.01, N = 60)	
Data FAIL normality test (alpha = 0.01).	

Steel's Many-One Rank Test					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	3 %	122.00	75.00	10.00	
3	5 %	133.00	75.00	10.00	
4	6 %	113.50	75.00	10.00	
5	8 %	119.50	75.00	10.00	
6	11 %	122.50	75.00	10.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	148	29.6	1.747	
Within (Error)	53	898	16.94		
Total	58	1046			
Critical F = 3.39 (alpha = 0.01, df = 5,53)					
2.39 (alpha = 0.05, df = 5,53)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation	
Ho: Control < Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	29.2	29.2			
2	3 %	33	33	-2.064		
3	5 %	33.7	33.7	-2.445		
4	6 %	31.4	31.4	-1.195		
5	8 %	32.8	32.8	-1.956		
6	11 %	33.667	33.667	-2.362		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53)						
WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.						

Dunnett's Test - Table 2 of 2					No Transformation	
Ho: Control < Treatment						
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control	
1	Control	10				
2	3 %	10	4.252	14.6	-3.8	
3	5 %	10	4.252	14.6	-4.5	
4	6 %	10	4.252	14.6	-2.2	
5	8 %	10	4.252	14.6	-3.6	
6	11 %	9	4.368	15	-4.467	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: December 2, 2014 at 1319

Date and Time Test Terminated: December 9, 2014 at 1555

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.8	8.3	8.6	8.4	8.2	8.7
	Final *1	7.6	7.4	7.6	7.9	8.0	7.7	7.5
	Final *2	7.8	7.9	8.3	8.9	6.9	8.1	8.2
pH, units	Initial	7.4	7.5	7.3	7.8	7.8	7.5	7.3
	Final *1	7.4	7.2	7.6	7.6	7.6	7.3	7.3
	Final *2	7.6	7.4	7.9	7.9	7.9	7.3	7.4
Alkalinity, mg CaCO ₃ /l		30	NA	30	NA	30	NA	NA
Hardness, mg CaCO ₃ /l		47	NA	47	NA	48	NA	NA
Conductivity, umhos/cm		200	180	170	140	150	190	160
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 3 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.7	8.1	8.6	8.4	8.1	8.6
	Final *1	7.6	7.3	7.3	7.6	8.0	7.8	7.6
	Final *2	7.8	7.9	8.2	8.7	7.3	8.2	8.2
pH, units	Initial	7.3	7.5	7.3	7.7	7.7	7.5	7.3
	Final *1	7.4	7.2	7.4	7.6	7.6	7.4	7.3
	Final *2	7.6	7.4	7.8	7.8	7.8	7.3	7.3

Effluent Conc.: 5 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.8	8.2	8.6	8.4	8.0	8.6
	Final *1	7.8	7.6	7.2	7.6	7.9	7.7	7.6
	Final *2	8.1	7.8	8.3	8.6	7.3	8.2	8.1
pH, units	Initial	7.3	7.5	7.3	7.7	7.7	7.6	7.3
	Final *1	7.4	7.2	7.4	7.6	7.6	7.3	7.3
	Final *2	7.6	7.4	7.8	7.8	7.9	7.4	7.2

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: December 2, 2014 at 1319

Date and Time Test Terminated: December 9, 2014 at 1555

Effluent Conc.: 6 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.7	8.2	8.6	8.5	8.1	8.7
	Final *1	7.7	7.5	7.3	7.8	8.2	7.6	7.6
	Final *2	8.0	8.0	8.3	8.7	7.3	8.4	8.0
pH, units	Initial	7.4	7.5	7.3	7.7	7.8	7.5	7.4
	Final *1	7.4	7.2	7.4	7.6	7.6	7.3	7.4
	Final *2	7.6	7.3	7.8	7.8	7.8	7.3	7.2

Effluent Conc.: 8 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.7	8.3	8.6	8.5	8.1	8.7
	Final *1	7.6	7.5	6.9	7.8	7.7	7.6	7.6
	Final *2	8.1	8.0	8.3	8.8	7.2	8.4	8.0
pH, units	Initial	7.3	7.4	7.3	7.7	7.8	7.5	7.3
	Final *1	7.4	7.2	7.2	7.6	7.6	7.3	7.3
	Final *2	7.6	7.3	7.8	7.8	7.8	7.4	7.2
Alkalinity, mg CaCO ₃ /l	35	NA	32	NA	33	NA	NA	NA
Hardness, mg CaCO ₃ /l	43	NA	48	NA	49	NA	NA	NA
Conductivity, umhos/cm	190	180	170	150	160	190	170	170
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

Effluent Conc.: 11 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.4	8.3	8.6	8.3	8.0	8.7
	Final *1	7.6	7.5	7.1	7.5	7.9	7.6	7.7
	Final *2	7.9	7.7	8.3	8.7	8.0	8.2	7.9
pH, units	Initial	7.3	7.4	7.2	7.7	7.7	7.6	7.3
	Final *1	7.4	7.2	7.3	7.6	7.7	7.3	7.3
	Final *2	7.5	7.3	7.8	7.8	7.8	7.4	7.2

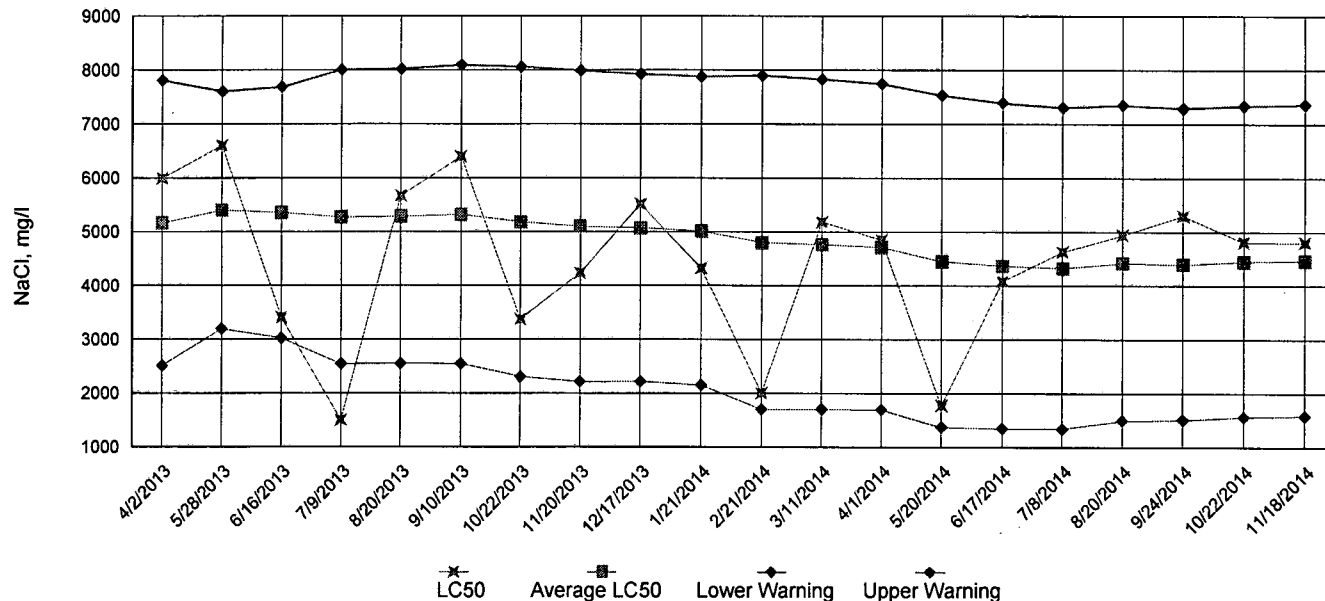
*1 = data from the *Pimephales promelas* (Fathead Minnow) test

*2 = data from the *Ceriodaphnia dubia* test

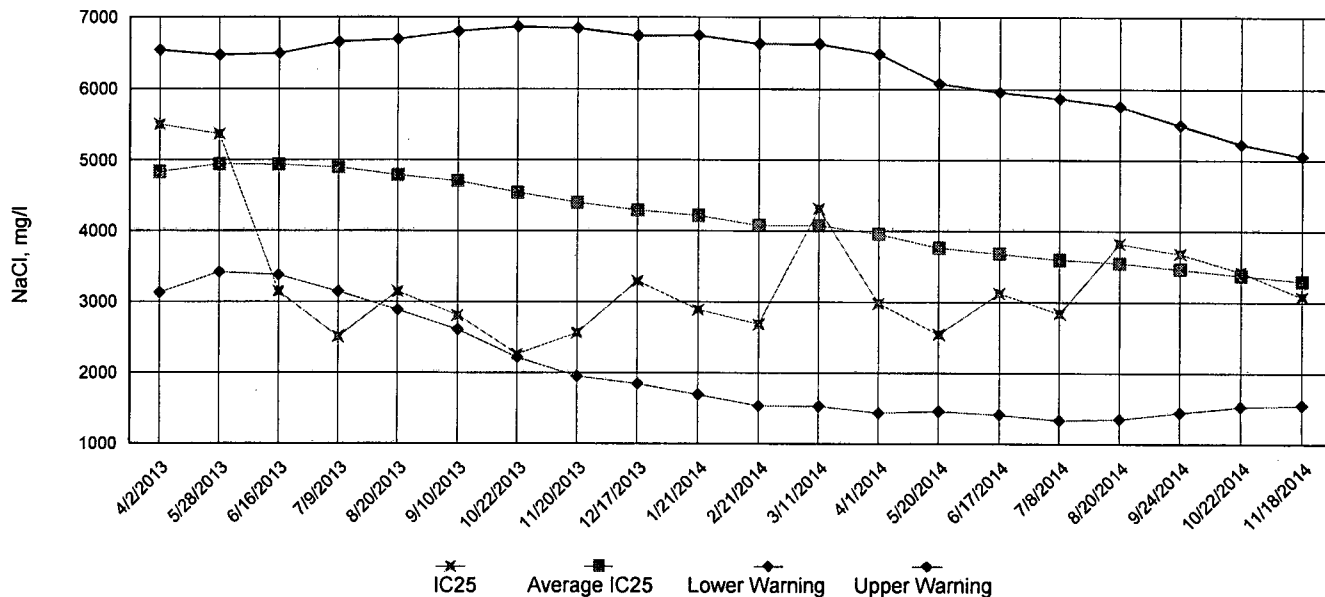
Appendix A4: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data

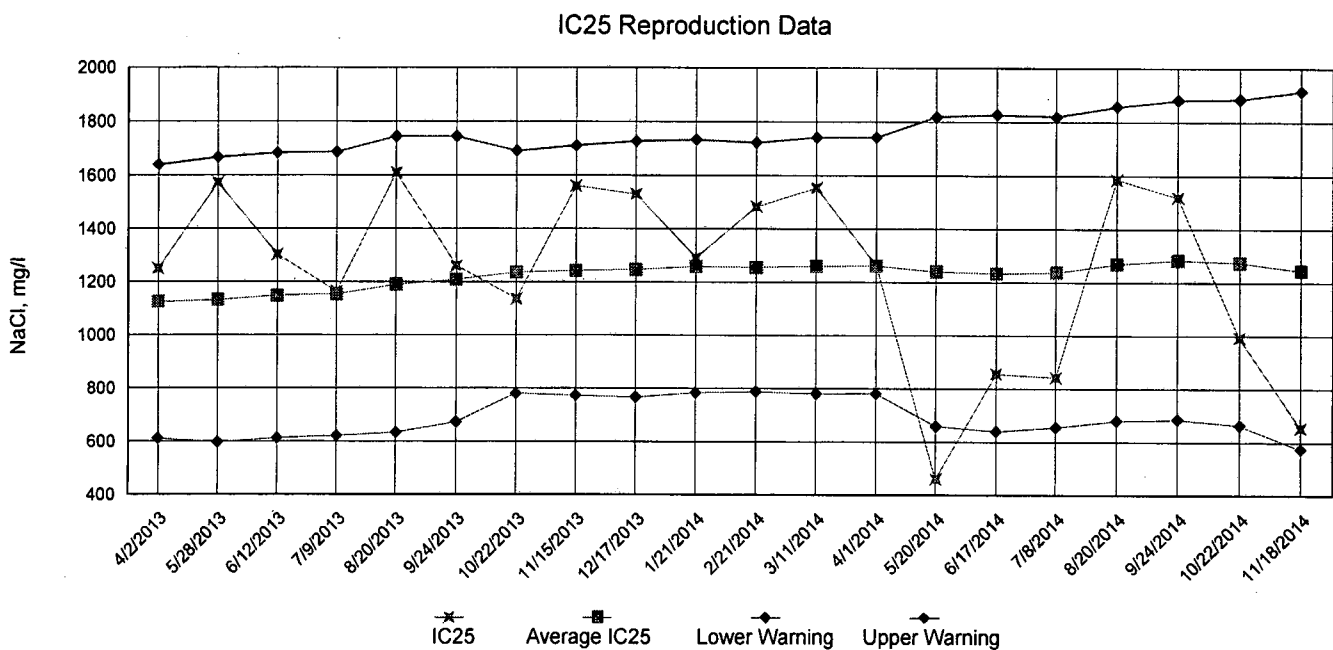
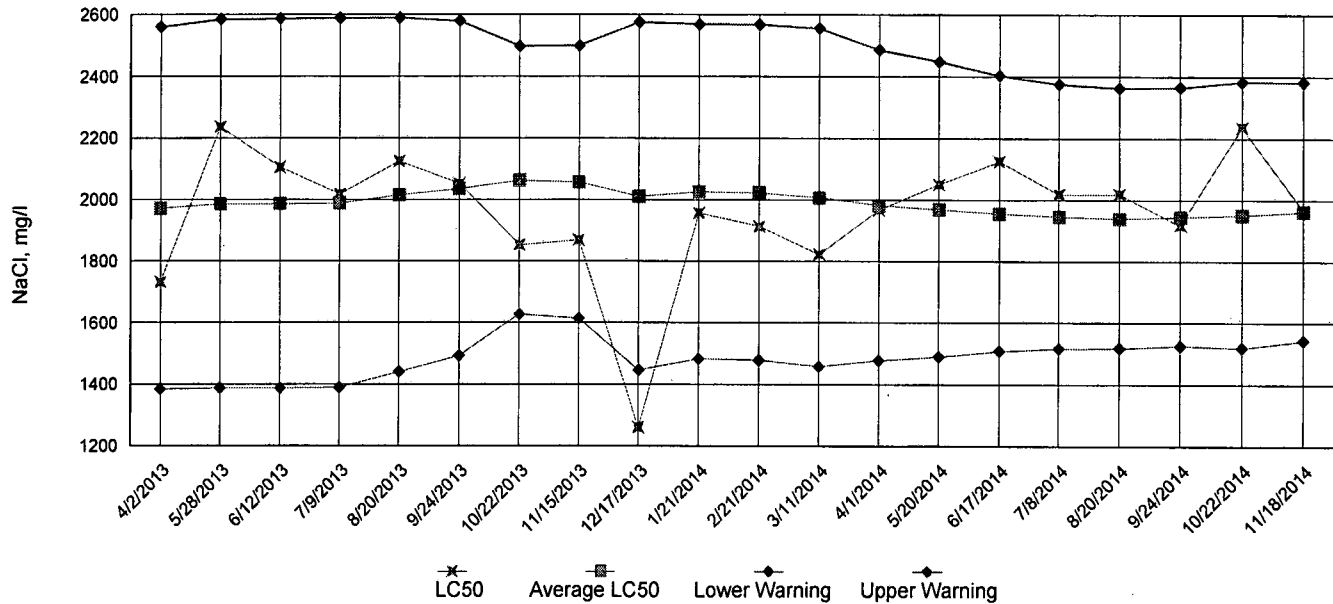


IC25 Growth Data



Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



Appendix B: Test 1000.0

 SUMMARY REPORTING FORMS
 CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
 SURVIVAL AND GROWTH

 Permittee: Heber Springs Water & Sewer

 NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: December 2, 2014 at 1530

Date and Time Test Terminated: December 9, 2014 at 1400

Dilution water used: Synthetic Soft Water #4162

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	62.5	100	75.0	62.5	87.5	95.0	92.5	77.5	21.0
3 %	87.5	100	100	75.0	75.0	92.5	92.5	87.5	14.3
5 %	75.0	100	87.5	87.5	100	100	100	90.0	11.6
6 %	100	100	87.5	100	75.0	100	100	92.5	12.1
8 %	100	100	87.5	75.0	87.5	97.5	92.5	90.0	11.6
11 %	100	75.0	87.5	87.5	100	95.0	95.0	90.0	11.6

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.235	0.244	0.198	0.180	0.215	0.214	12.2
3 %	0.228	0.272	0.240	0.195	0.238	0.235	11.8
5 %	0.236	0.340	0.128	0.219	0.286	0.242	32.8
6 %	0.275	0.245	0.295	0.272	0.241	0.266	8.47
8 %	0.355	0.280	0.206	0.176	0.219	0.247	28.8
11 %	0.288	0.198	0.284	0.225	0.265	0.252	15.5

 $CV = \text{Coefficient of variation} = \text{standard deviation} * 100 / \text{mean}$

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM

Pimephales promelas (Fathead minnow)

CHEMICAL PARAMETERS CHART

PERMITTEE: Heber Springs Water & Sewer SAMPLE No. 1 COLLECTED ending: DATE: December 2, 2014 TIME: 0800
 NPDES NO.: NPDES Permit AR0022381 AFIN SAMPLE No. 2 COLLECTED ending: DATE: December 3, 2014 TIME: 0800
 CONTACT: Mr. Kent Latch SAMPLE No. 3 COLLECTED ending: DATE: December 5, 2014 TIME: 0800
 ANALYST: 280, 304, 310 Test Initiated: DATE: December 2, 2014 TIME: 1530
 Test Terminated: DATE: December 9, 2014 TIME: 1400

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	8.3	8.6	8.4	8.2	8.7
Final	7.6	7.4	7.6	7.9	8.0	7.7	7.5
pH Initial	7.4	7.5	7.3	7.8	7.8	7.5	7.3
Final	7.4	7.2	7.6	7.6	7.6	7.3	7.3
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	47	NA	47	NA	48	NA	NA
Conductivity	200	180	170	140	150	190	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 3 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.7	8.1	8.6	8.4	8.1	8.6
Final	7.6	7.3	7.3	7.6	8.0	7.8	7.6
pH Initial	7.3	7.5	7.3	7.7	7.7	7.5	7.3
Final	7.4	7.2	7.4	7.6	7.6	7.4	7.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	190	190	170	170	210	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	8.2	8.6	8.4	8.0	8.6
Final	7.8	7.6	7.2	7.6	7.9	7.7	7.6
pH Initial	7.3	7.5	7.3	7.7	7.7	7.6	7.3
Final	7.4	7.2	7.4	7.6	7.6	7.3	7.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	180	160	170	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 6 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	8.2	8.6	8.5	8.1	8.7
Final	7.7	7.5	7.3	7.8	8.2	7.6	7.6
pH Initial	7.4	7.5	7.3	7.7	7.8	7.5	7.4
Final	7.4	7.2	7.4	7.6	7.6	7.3	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	170	150	150	190	170
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.7	8.3	8.6	8.5	8.1	8.7
Final	7.6	7.5	6.9	7.8	7.7	7.6	7.6
pH Initial	7.3	7.4	7.3	7.7	7.8	7.5	7.3
Final	7.4	7.2	7.2	7.6	7.6	7.3	7.3
Alkalinity	35	NA	32	NA	33	NA	NA
Hardness	43	NA	48	NA	49	NA	NA
Conductivity	190	180	170	150	160	190	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.4	8.3	8.6	8.3	8.0	8.7
Final	7.6	7.5	7.1	7.5	7.9	7.6	7.7
pH Initial	7.3	7.4	7.2	7.7	7.7	7.6	7.3
Final	7.4	7.2	7.3	7.6	7.7	7.3	7.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	180	160	160	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Heber Springs Water & Sewer

NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: December 2, 2014 at 1520

Date and Time Test Terminated: December 9, 2014 at 1555

Dilution water used: Synthetic Soft Water #4162

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		3 %	5 %	6 %	8 %	11 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		3 %	5 %	6 %	8 %	11 %
A	33	32	34	31	38	34
B	19	33	35	35	32	35
C	33	35	37	35	38	4
D	36	34	33	33	24	35
E	18	30	36	21	31	35
F	32	32	33	31	26	32
G	26	32	31	27	33	36
H	32	34	30	32	30	29
I	30	31	34	32	37	35
J	33	37	34	37	39	32
Mean per Adult	29.2	33.0	33.7	31.4	32.8	30.7
Mean per Surviving Adult	29.2	33.0	33.7	31.4	32.8	33.7
CV %	21.2	6.23	6.26	14.6	15.9	6.64

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(8 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

2. Steel's Many-One Rank Test:

Is the mean number of young produced per female significantly different ($p=0.05$) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(8 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 11 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 11 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 11 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 11 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 21.2 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Heber Springs Water & Sewer SAMPLE No. 1 COLLECTED ending: DATE: December 2, 2014 TIME: 0800
 NPDES NO.: NPDES Permit AR0022381 AFIN SAMPLE No. 2 COLLECTED ending: DATE: December 3, 2014 TIME: 0800
 CONTACT: Mr. Kent Latch SAMPLE No. 3 COLLECTED ending: DATE: December 5, 2014 TIME: 0800
 ANALYST: 280, 304, 310 Test Initiated: DATE: December 2, 2014 TIME: 1520
 Test Terminated: DATE: December 9, 2014 TIME: 1555

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	8.3	8.6	8.4	8.2	8.7
Final	7.8	7.9	8.3	8.9	6.9	8.1	8.2
pH Initial	7.4	7.5	7.3	7.8	7.8	7.5	7.3
Final	7.6	7.4	7.9	7.9	7.9	7.3	7.4
Alkalinity	30	NA	30	NA	30	NA	NA
Hardness	47	NA	47	NA	48	NA	NA
Conductivity	200	180	170	140	150	190	160
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 3 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.7	8.1	8.6	8.4	8.1	8.6
Final	7.8	7.9	8.2	8.7	7.3	8.2	8.2
pH Initial	7.3	7.5	7.3	7.7	7.7	7.5	7.3
Final	7.6	7.4	7.8	7.8	7.8	7.3	7.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	190	190	190	170	170	210	160
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	8.2	8.6	8.4	8.0	8.6
Final	8.1	7.8	8.3	8.6	7.3	8.2	8.1
pH Initial	7.3	7.5	7.3	7.7	7.7	7.6	7.3
Final	7.6	7.4	7.8	7.8	7.9	7.4	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	180	160	170	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 6 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	8.2	8.6	8.5	8.1	8.7
Final	8.0	8.0	8.3	8.7	7.3	8.4	8.0
pH Initial	7.4	7.5	7.3	7.7	7.8	7.5	7.4
Final	7.6	7.3	7.8	7.8	7.8	7.3	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	170	150	150	190	170
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.7	8.3	8.6	8.5	8.1	8.7
Final	8.1	8.0	8.3	8.8	7.2	8.4	8.0
pH Initial	7.3	7.4	7.3	7.7	7.8	7.5	7.3
Final	7.6	7.3	7.8	7.8	7.8	7.4	7.2
Alkalinity	35	NA	32	NA	33	NA	NA
Hardness	43	NA	48	NA	49	NA	NA
Conductivity	190	180	170	150	160	190	170
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.4	8.3	8.6	8.3	8.0	8.7
Final	7.9	7.7	8.3	8.7	8.0	8.2	7.9
pH Initial	7.3	7.4	7.2	7.7	7.7	7.6	7.3
Final	7.5	7.3	7.8	7.8	7.8	7.4	7.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	180	160	160	200	180
Chlorine	NA	NA	NA	NA	NA	NA	NA

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Heber Springs Water</u>			PO No. <u>14841</u>		NO OF BOTTLES	ANALYSES REQUESTED <u>4th QUARTER</u> <u>BIO MONITORING</u>										AIC CONTROL NO: <u>185164</u>	
Project: <u>4th QUARTER</u>			Reference: <u>BIO MONITORING</u>													MATRIX	
Project Manager: <u>KONT LATCH</u>			Sampled By: <u>S.O. & Troy Massey</u>		WATER		SOIL		Carrier: <u>Heber Springs Water Dept.</u>		Received Temperature C: <u>1.1°C</u>						
AIC No.	Sample Identification	Date/Time Collected	GRA B	COMP	WATER	SOIL	BOTTLES		Remarks		Field pH calibration on _____ @ _____ Buffer: _____						
	<u>OUTFALL CO2A EFFL.</u>	<u>12-1-14 SAM TO 12-2-14 SAM</u>		<u>24</u>	<u>✓</u>		<u>3</u>										
Container Type			Preservative		G = Glass NO = none		P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate A=(NH ₄) ₂ SO ₄ , NH ₄ OH				
Turnaround Time Requested: (Please circle) <u>7</u> DAYS NORMAL or EXPEDITED IN <u>7</u> DAYS					Relinquished By: <u>Sam Querry</u>		Date/Time <u>12-2-14 11:33 A.M.</u>		Received By:		Date/Time						
Expedited results requested by: <u>KONT LATCH</u>					Relinquished By:		Date/Time		Received in Lab By: <u>Troy Williams</u>		Date/Time <u>12-2-14 11:33</u>						
Who should AIC contact with questions: <u>SAM QUERRY</u>					Comments:												
Phone: <u>501-362-3000</u> Fax: <u>501-362-3335</u>																	
Report Attention to: <u>KONT LATCH</u>																	
Report Address to: <u>Heber Springs Water 1108 W. FRONT ST - Heber Springs AR. 72543</u>																	
Email Address:																	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Heber Springs Water</u>			PO No. <u>14841</u>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>185164</u>		
Project: <u>4th Quarter</u>			Reference: <u>Bio monitoring</u>													4th Quarter Bio Monitoring		
Project Manager: <u>Kent Latch</u>			Sampled By: <u>S.O. & Saxy Messer</u>			MATRIX		3										
AIC No.	Sample Identification	Date/Time Collected	G R A B	C O M P	W A T E R	S O I L	24 ✓											Received Temperature C <u>0.5 °C</u>
<u>2</u>	<u>Outfall 002A EFFL</u>	<u>12-2-14 8:00am 12-3-14 8:00am</u>		<u>24</u>	<u>✓</u>												Remarks	
Container Type			Preservative		Buffer:										Buffer:			
G = Glass NO = none			P = Plastic S = Sulfuric acid pH2												V = VOA vials N = Nitric acid pH2			
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN <u>2</u> DAYS			Expedited results requested by: <u>Kent Latch</u>		T = Sodium Thiosulfate Z = Zinc acetate A = (NH ₄) ₂ SO ₄ , NH ₄ OH													
Who should AIC contact with questions: <u>SAM Quarry</u>			Phone: <u>501-362-3400</u> Fax: <u>501-362-3338</u>												Date/Time <u>12-3-14 8 AM</u>			
Report Attention to: <u>Kent Latch</u>			Report Address to: <u>Heber Springs Water 1108 West Front St.</u>		Date/Time <u>12-3-14 10:30 AM</u>													
Email Address: <u>Heber Springs AR 72543</u>			Comments:												Date/Time <u>12/3/14 1030</u>			

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Heber Springs Water</u>			PO No. <u>14841</u>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>185164</u>		
Project: <u>4th Quarter</u>			Reference: <u>Bio Monitoring</u>			<u>4th Quarter Bio Monitoring</u>										AIC PROPOSAL NO:		
Project Manager: <u>Kent Latch</u>			MATRIX													Remarks		
Sampled By: <u>S.O. & Joey Massey</u>			G R A B	C O M P	W A T E R	S O I L											Carrier: <u>Heber Springs Water Dept.</u>	
AIC No.	Sample Identification	Date/Time Collected															Received Temperature C <u>0.8</u>	
<u>3</u>	<u>Outfall 002A EFL</u>	<u>12-4-14 8:00 AM 12-5-14 8:00 AM</u>	<u>24</u>	<u>✓</u>	<u>3</u>													
Container Type													Field pH calibration					
Preservative													on _____ @ _____					
G = Glass NO = none			P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate		A = (NH ₄) ₂ SO ₄ , NH ₄ OH		Buffer:					
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN <u>7</u> DAYS					Relinquished By: <u>Joey Massey</u>		Date/Time: <u>12-5-14 10:46 AM</u>		Received By:		Date/Time:							
Expedited results requested by: <u>Kent Latch</u>					Relinquished By: <u>[Signature]</u>		Date/Time:		Received in Lab By: <u>TROY WILLIAMS</u>		Date/Time: <u>12-5-14</u>							
Who should AIC contact with questions: <u>SAM QUERRY</u>					Comments:													
Phone: <u>362-3422</u> Fax: <u>501-362-3398</u>																		
Report Attention to: <u>Kent Latch</u>																		
Report Address to: <u>Heber Springs Water 1108 W. Front. St.</u>																		
Email Address: <u>Heber Springs AR 72543</u>																		



Heber Springs Water & Sewer Dept.
1108 W. Front St.
Heber Springs, AR 72543
PH: 501-362-5501 FAX: 501-362-3338

TO:

ADEQ
5301 Northshore Drive
North Little Rock, AR 72118-5317

