

September 17, 2015

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
City of Hot Springs

Control No. 193982-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

AMERICAN INTERPLEX CORPORATION
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City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001 - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

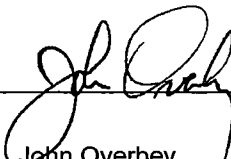
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for reproduction occurred at 87 % effluent, which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
Laboratory Director

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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	0.346	PASS
Control Growth CV < or = 40%	7.84	PASS
Growth Minimum Significant Difference 12 to 30%	17.1	PASS
Critical Dilution CV < or = 40%	9.55	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	25.0	PASS
Control CV < or = 40% per Surviving Female	32.7	PASS
Reproduction Minimum Significant Difference 13 to 47%	22.4	PASS
Critical Dilution CV < or = 40%	10.8	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Methods 1000.0 and 1002.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:

- a. Sampling Point: Outfall 001
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.5	8.0	7.9
pH (standard units)	8.0	7.7	7.8
Alkalinity (mg/l as CaCO ₃)	55	40	36
Hardness (mg/l as CaCO ₃)	64	59	56
Conductivity (umhos/cm)	350	320	370
Residual Chlorine (mg/l)	<0.05	<0.05	0.070
Ammonia as N (mg/l)	0.15	0.14	1.0

2. Dilution Water Samples: Synthetic Soft Water #4249

- a. Dates Prepared: August 26 through September 9, 2015
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	8.0	8.0
pH (standard units)	8.2	8.0	7.8
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	40	40	40
Conductivity (umhos/cm)	160	150	140
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: September 8, 2015 at 1450
Date & Time Test Terminated: September 15, 2015 at 1335
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: September 8, 2015 at 1455
Date & Time Test Terminated: September 15, 2015 at 1620
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. 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.

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 September 1, 2015 at 1630 to September 8, 2015 at 1520

The results were as follows: (Control No. 193794-1.)

Survival LC-50: 4389 mg/l

Growth IC-25: 3757 mg/l

Growth PMSD: 6.75

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on September 1, 2015 at 1500 to September 8, 2015 at 1500

The results were as follows: (Control No. 193794-2.)

Survival LC-50: 1673 mg/l

Growth IC-25: 912 mg/l

Growth PMSD: 17.7

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	99.0	0.457
pH	SM 4500-H+ B	100	0.00
Conductivity	EPA 120.1	100	0.703

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: September 8, 2015

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: September 8, 2015

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

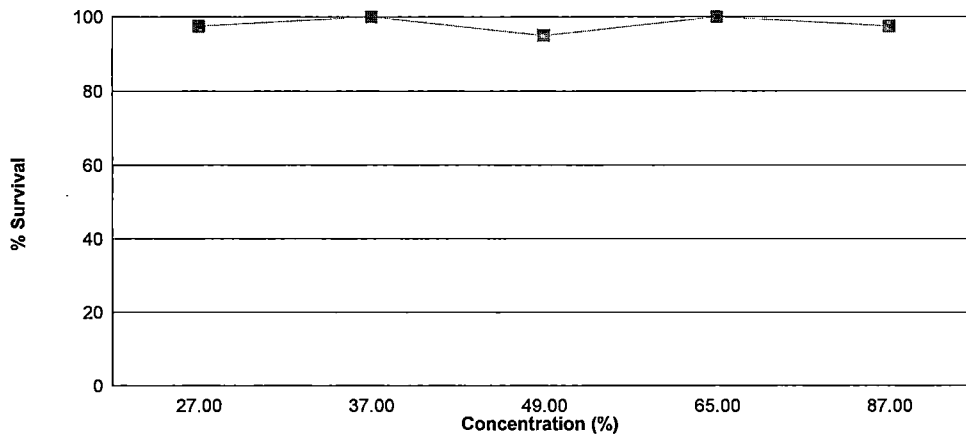
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 8, 2015 at 1450 and continued through September 15, 2015 at 1335. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 87 % effluent
- b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.346
27 %	97.5	0.349
37 %	100	0.381
49 %	95.0	0.366
65 %	100	0.370
87 %	97.5	0.354

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

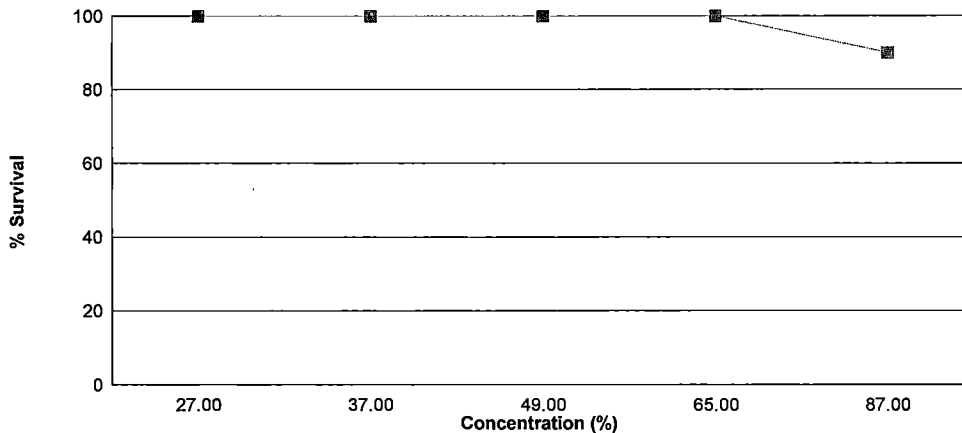
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 8, 2015 at 1455 and continued through September 15, 2015 at 1620. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 87 % effluent
- b.) NOEC reproduction = 87 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	25.0
27 %	100	27.7
37 %	100	28.1
49 %	100	24.9
65 %	100	29.1
87 %	90.0	26.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: September 8, 2015 at 1450
Date and Time Test Terminated: September 15, 2015 at 1335

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
27 %	A	8	8	8	8	8	8	8
	B	7	7	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
37 %	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
49 %	A	8	8	8	7	7	7	7
	B	8	8	8	8	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
65 %	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
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	7	7	7
	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: September 8, 2015 at 1450
Test Terminated: September 15, 2015 at 1335

Drying Started: September 15, 2015 at 1400
Drying Ended: September 16, 2015 at 1047

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94244	.94520	0.00276	8	0.345
	B	.93527	.93818	0.00291	8	0.364
	C	.94187	.94473	0.00286	8	0.358
	D	.93967	.94206	0.00239	8	0.299
	E	.94208	.94498	0.00290	8	0.362
27 %	A	.93637	.93871	0.00234	8	0.292
	B	.94146	.94404	0.00258	8	0.322
	C	.94101	.94409	0.00308	8	0.385
	D	.94271	.94578	0.00307	8	0.384
	E	.94449	.94740	0.00291	8	0.364
37 %	A	.94476	.94769	0.00293	8	0.366
	B	.93946	.94214	0.00268	8	0.335
	C	.94055	.94373	0.00318	8	0.398
	D	.93733	.94084	0.00351	8	0.439
	E	.93958	.94251	0.00293	8	0.366
49 %	A	.94215	.94438	0.00223	8	0.279
	B	.94256	.94512	0.00256	8	0.320
	C	.94349	.94675	0.00326	8	0.408
	D	.94532	.94870	0.00338	8	0.422
	E	.94297	.94619	0.00322	8	0.402
65 %	A	.93912	.94167	0.00255	8	0.319
	B	.93244	.93543	0.00299	8	0.374
	C	.93083	.93405	0.00322	8	0.402
	D	.93851	.94133	0.00282	8	0.352
	E	.93612	.93934	0.00322	8	0.402
87 %	A	.93703	.93972	0.00269	8	0.336
	B	.93780	.94052	0.00272	8	0.340
	C	.94112	.94401	0.00289	8	0.361
	D	.94014	.94304	0.00290	8	0.362
	E	.93569	.93864	0.00295	8	0.369

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 8, 2015 at 1455

Date and Time Test Terminated: September 15, 2015 at 1620

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	3	4	3	4	4	2	0	4	3	1	28	10	2.80	
5	0	8	9	0	0	6	1	0	1	0	25	10	2.50	
6	7	0	1	8	10	3	2	9	10	6	56	10	5.60	
7	14	17	15	16	15	14	0	17	17	16	141	10	14.1	
8														
TOTAL	24	29	28	28	29	25	3	30	31	23	250	10	25.0	

Concentration: 27 %														
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	0	2	1	3	4	0	4	0	1	3	18	10	1.80	
5	6	9	7	1	0	6	0	5	2	1	37	10	3.70	
6	7	0	0	3	9	11	9	10	8	10	67	10	6.70	
7	14	15	19	15	19	18	20	0	17	18	155	10	15.5	
8														
TOTAL	27	26	27	22	32	35	33	15	28	32	277	10	27.7	

Concentration: 37 %														
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	3	3	3	3	4	0	4	2	3	3	28	10	2.80	
5	0	7	7	0	1	6	0	6	0	0	27	10	2.70	
6	6	1	0	7	9	3	8	4	8	10	56	10	5.60	
7	17	19	14	17	18	17	16	19	18	15	170	10	17.0	
8														
TOTAL	26	30	24	27	32	26	28	31	29	28	281	10	28.1	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 8, 2015 at 1455
Date and Time Test Terminated: September 15, 2015 at 1620

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	3	4	3	4	4	2	0	4	3	1	28	10	2.80	
5	0	8	9	0	0	6	1	0	1	0	25	10	2.50	
6	7	0	1	8	10	3	2	9	10	6	56	10	5.60	
7	14	17	15	16	15	14	0	17	17	16	141	10	14.1	
8														
TOTAL	24	29	28	28	29	25	3	30	31	23	250	10	25.0	

Concentration: 27 %														
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	0	2	1	3	4	0	4	0	1	3	18	10	1.80	
5	6	9	7	1	0	6	0	5	2	1	37	10	3.70	
6	7	0	0	3	9	11	9	10	8	10	67	10	6.70	
7	14	15	19	15	19	18	20	0	17	18	155	10	15.5	
8														
TOTAL	27	26	27	22	32	35	33	15	28	32	277	10	27.7	

Concentration: 37 %														
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	3	3	3	3	4	0	4	2	3	3	28	10	2.80	
5	0	7	7	0	1	6	0	6	0	0	27	10	2.70	
6	6	1	0	7	9	3	8	4	8	10	56	10	5.60	
7	17	19	14	17	18	17	16	19	18	15	170	10	17.0	
8														
TOTAL	26	30	24	27	32	26	28	31	29	28	281	10	28.1	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 8, 2015 at 1455
Date and Time Test Terminated: September 15, 2015 at 1620

Concentration: 49 %														
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	4	2	3	0	4	4	3	3	3	3	29	10	2.90	
5	0	8	9	5	0	4	0	0	6	0	32	10	3.20	
6	8	0	0	7	9	0	7	9	0	8	48	10	4.80	
7	16	15	17	9	15	0	17	19	15	17	140	10	14.0	
8														
TOTAL	28	25	29	21	28	8	27	31	24	28	249	10	24.9	

Concentration: 65 %														
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	0	1	3	0	4	4	4	3	2	4	25	10	2.50	
5	5	6	8	4	0	8	0	0	0	0	31	10	3.10	
6	13	0	0	11	9	1	9	8	9	8	68	10	6.80	
7	18	19	18	9	17	15	18	18	18	17	167	10	16.7	
8														
TOTAL	36	26	29	24	30	28	31	29	29	29	291	10	29.1	

Concentration: 87 %														
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	X	0	0	0	0	0	9	0.00	
4	0	4	3	4	5	X	0	4	4	4	28	9	3.11	
5	5	8	11	0	1	X	4	0	0	0	29	9	3.22	
6	10	0	0	8	7	X	8	9	9	7	58	9	6.44	
7	17	15	14	19	17	X	17	17	14	18	148	9	16.4	
8														
TOTAL	32	27	28	31	30	0	29	30	27	29	263	10	26.3	

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	27 %	1	1.00000	1.39310
2	27 %	2	0.87500	1.20940
2	27 %	3	1.00000	1.39310
2	27 %	4	1.00000	1.39310
2	27 %	5	1.00000	1.39310
3	37 %	1	1.00000	1.39310
3	37 %	2	1.00000	1.39310
3	37 %	3	1.00000	1.39310
3	37 %	4	1.00000	1.39310
3	37 %	5	1.00000	1.39310
4	49 %	1	0.87500	1.20940
4	49 %	2	0.87500	1.20940
4	49 %	3	1.00000	1.39310
4	49 %	4	1.00000	1.39310
4	49 %	5	1.00000	1.39310
5	65 %	1	1.00000	1.39310
5	65 %	2	1.00000	1.39310
5	65 %	3	1.00000	1.39310
5	65 %	4	1.00000	1.39310
5	65 %	5	1.00000	1.39310
6	87 %	1	1.00000	1.39310
6	87 %	2	1.00000	1.39310
6	87 %	3	0.87500	1.20940
6	87 %	4	1.00000	1.39310
6	87 %	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 = 0.09449 W = 0.7601 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	27 %	25.00	16.00	5.00	
3	37 %	27.50	16.00	5.00	
4	49 %	22.50	16.00	5.00	
5	65 %	27.50	16.00	5.00	
6	87 %	25.00	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 = 0.0376 W = 0.9584 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 = 7.234 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	0.004615	0.000923	0.589
Within (Error)	24	0.0376	0.001567	
Total	29	0.04222		
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	0.3456	0.3456		
2	27 %	0.3494	0.3494	-0.1518	
3	37 %	0.3808	0.3808	-1.406	
4	49 %	0.3662	0.3662	-0.8228	
5	65 %	0.3698	0.3698	-0.9666	
6	87 %	0.3536	0.3536	-0.3195	
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	27 %	5	0.05908	17.1	-0.0038
3	37 %	5	0.05908	17.1	-0.0352
4	49 %	5	0.05908	17.1	-0.0206
5	65 %	5	0.05908	17.1	-0.0242
6	87 %	5	0.05908	17.1	-0.008

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
27 %	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
37 %	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
49 %	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
65 %	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
87 %	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	27 %	10	0	
2	37 %	10	0	
3	49 %	10	0	
4	65 %	10	0	
5	87 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.2169 D* = 1.702 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data FAIL normality test (alpha = 0.01).</p>	

Steel's Many-One Rank Test				No Transformation	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	27 %	114.00	75.00	10.00	
3	37 %	114.50	75.00	10.00	
4	49 %	98.50	75.00	10.00	
5	65 %	122.50	75.00	10.00	
6	87 %	118.50	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	184.2	36.84	1.326	
Within (Error)	53	1473	27.79		
Total	58	1657			
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	25	25			
2	27 %	27.7	27.7	-1.145		
3	37 %	28.1	28.1	-1.315		
4	49 %	24.9	24.9	0.04242		
5	65 %	29.1	29.1	-1.739		
6	87 %	29.222	29.222	-1.743		
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	27 %	10	5.446	21.8	-2.7	
3	37 %	10	5.446	21.8	-3.1	
4	49 %	10	5.446	21.8	0.1	
5	65 %	10	5.446	21.8	-4.1	
6	87 %	9	5.595	22.4	-4.222	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 8, 2015 at 1323
Date and Time Test Terminated: September 15, 2015 at 1620

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.8	8.0	7.8	8.0	8.3	7.9
	Final *1	7.7	7.4	7.6	7.6	8.1	7.5	8.7
	Final *2	8.2	7.8	8.1	8.2	8.3	8.0	8.6
pH, units	Initial	8.2	7.7	8.0	7.9	7.8	8.0	7.6
	Final *1	7.7	8.0	8.1	7.9	8.2	7.8	7.4
	Final *2	8.1	8.4	8.5	8.4	8.4	7.9	8.0
Alkalinity, mg CaCO ₃ /l		31	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l		40	NA	40	NA	40	NA	NA
Conductivity, umhos/cm		160	150	150	140	140	140	140
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.9	8.0	7.9	8.2	8.3	8.0
	Final *1	7.0	7.0	7.7	7.7	8.2	7.6	8.4
	Final *2	8.1	8.1	8.1	8.2	8.2	8.0	8.6
pH, units	Initial	8.1	7.7	7.9	7.8	7.8	8.0	7.6
	Final *1	7.7	7.9	8.1	7.9	8.2	7.9	7.4
	Final *2	8.2	8.4	8.5	8.4	8.4	8.0	8.1

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.1	7.6	8.0	7.8	7.8	8.2	8.3
	Final *1	7.4	7.2	7.8	7.7	8.0	7.6	8.3
	Final *2	8.3	8.1	8.2	8.2	8.1	7.8	8.6
pH, units	Initial	8.1	7.7	7.9	7.8	7.8	8.0	7.6
	Final *1	7.8	8.0	8.1	7.9	8.2	7.8	7.4
	Final *2	8.2	8.4	8.5	8.4	8.4	7.9	8.2

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 8, 2015 at 1323
Date and Time Test Terminated: September 15, 2015 at 1620

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.6	7.8	8.0	7.9	8.0	8.5	8.1
	Final *1	7.6	7.0	7.7	7.6	8.0	4.4	8.2
	Final *2	8.3	8.1	8.2	8.2	8.1	7.9	8.7
pH, units	Initial	8.1	7.7	7.8	7.8	7.8	8.0	7.7
	Final *1	7.8	8.0	8.1	8.0	8.3	7.7	7.4
	Final *2	8.2	8.5	8.5	8.4	8.4	7.9	8.2

Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.9	7.5	8.1	7.8	7.9	8.2	8.0
	Final *1	7.5	7.0	7.7	7.6	7.9	7.5	8.1
	Final *2	8.2	8.0	8.2	8.2	8.1	8.1	8.7
pH, units	Initial	8.0	7.7	7.8	7.8	7.8	8.0	7.6
	Final *1	7.9	8.0	8.2	8.0	8.3	7.9	7.5
	Final *2	8.2	8.5	8.6	8.4	8.4	7.9	8.2
Alkalinity, mg CaCO ₃ /l	56	NA	39	NA	34	NA	NA	NA
Hardness, mg CaCO ₃ /l	55	NA	52	NA	50	NA	NA	NA
Conductivity, umhos/cm	280	280	250	240	280	290	280	280
Res. Chlorine, mg/l	<0.05	NA	<0.05	NA	<0.05	NA	NA	NA

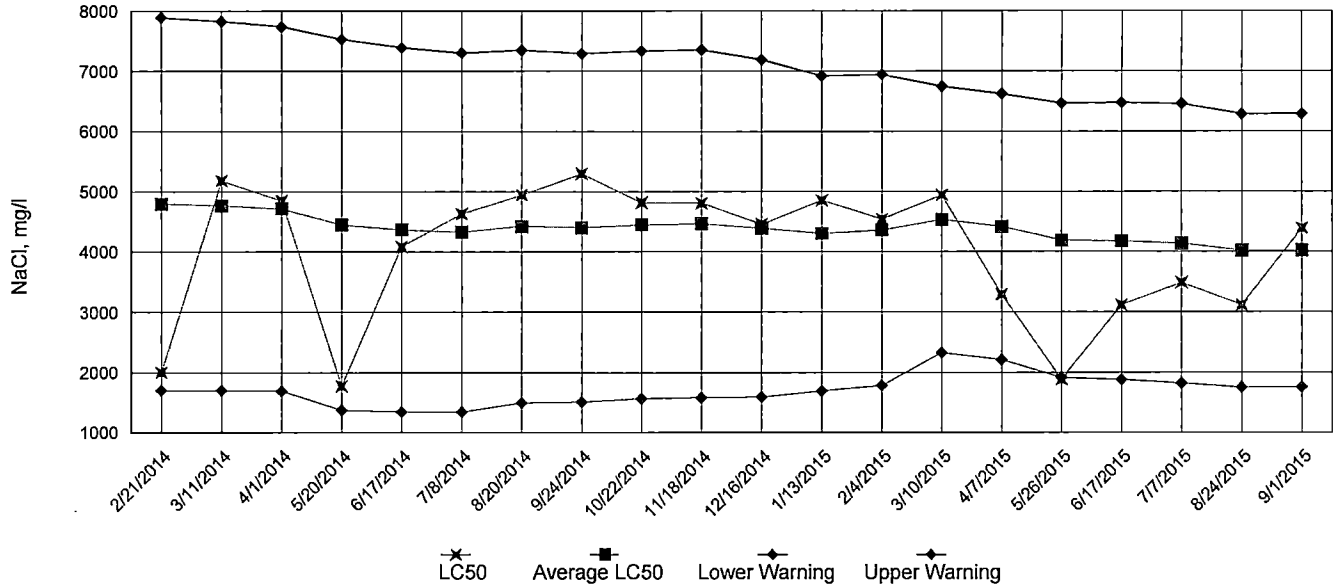
Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.2	7.7	8.2	7.8	8.1	8.3	8.0
	Final *1	7.1	6.7	7.7	7.5	9.3	7.7	8.0
	Final *2	7.9	8.1	8.1	8.2	8.0	8.0	8.7
pH, units	Initial	8.0	7.7	7.7	7.7	7.8	8.0	7.6
	Final *1	7.8	8.0	8.2	8.0	8.4	8.0	7.5
	Final *2	8.2	8.6	8.6	8.5	8.4	8.0	8.4

*1 = data from the *Pimephales promelas* (Fathead Minnow) test *2 = data from the *Ceriodaphnia dubia* test

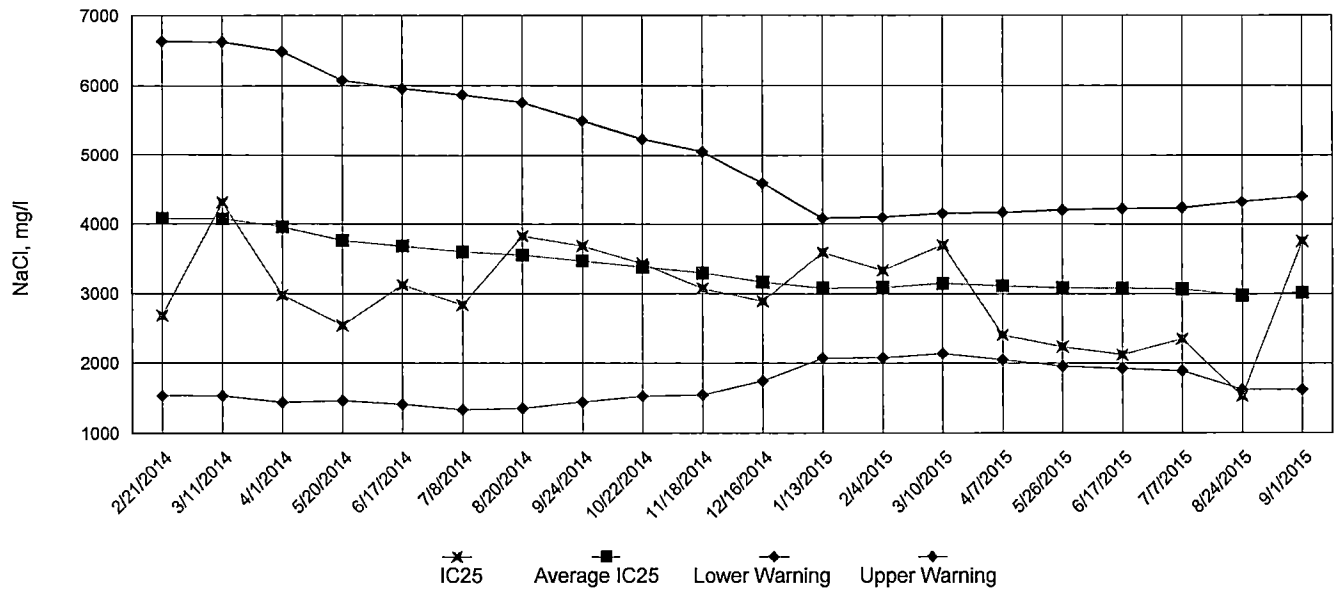
Appendix A4: Test 1000.0

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

LC50 Survival Data

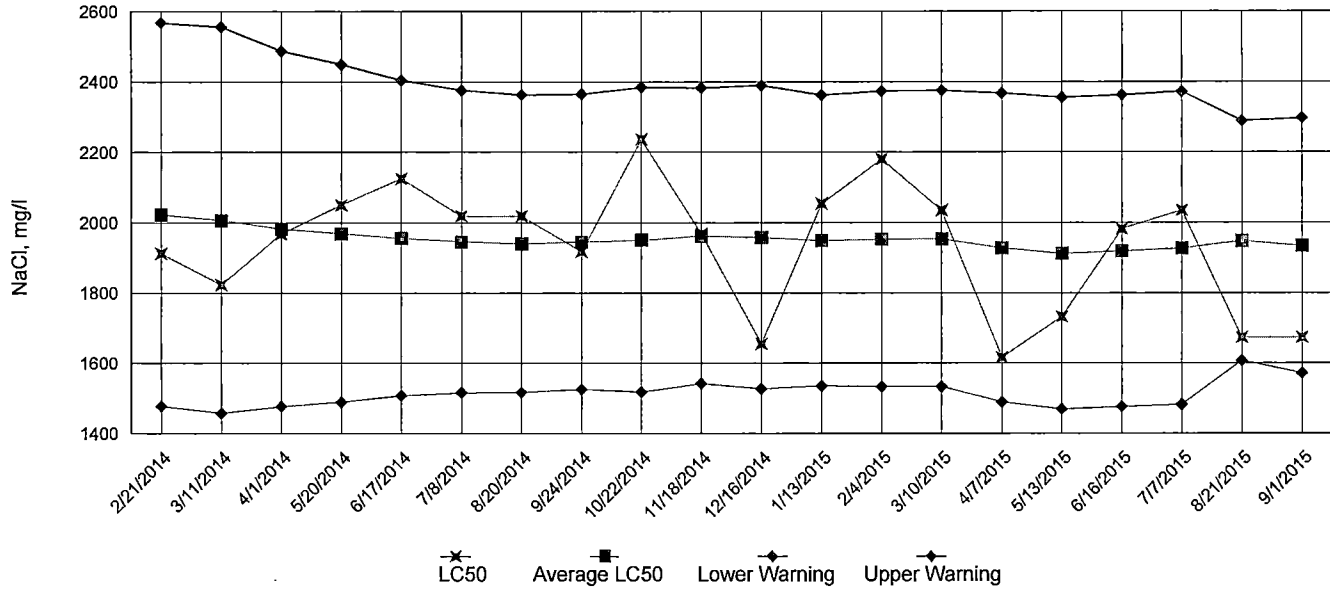


IC25 Growth Data

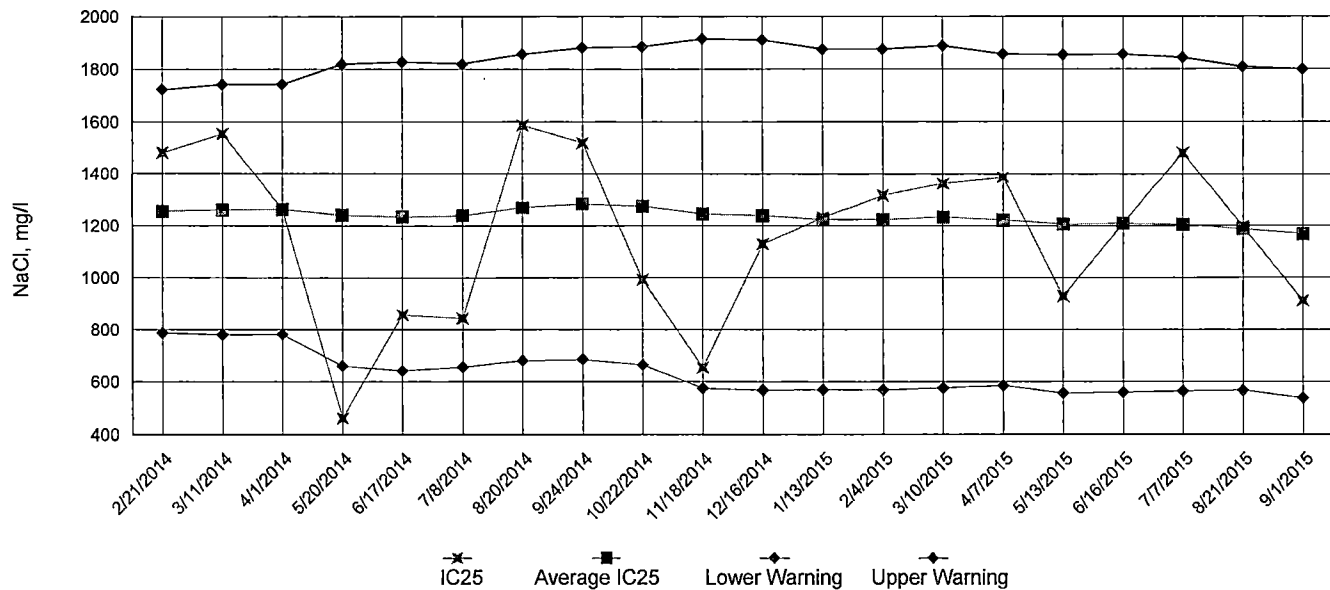


Appendix A4: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0

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

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 8, 2015 at 1450

Date and Time Test Terminated: September 15, 2015 at 1335

Dilution water used: Synthetic Soft Water #4249

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
27 %	100	87.5	100	100	100	97.5	97.5	97.5	5.73
37 %	100	100	100	100	100	100	100	100	0.00
49 %	87.5	87.5	100	100	100	100	100	95.0	7.21
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	87.5	100	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.345	0.364	0.358	0.299	0.362	0.346	7.84
27 %	0.292	0.322	0.385	0.384	0.364	0.349	11.7
37 %	0.366	0.335	0.398	0.439	0.366	0.381	10.4
49 %	0.279	0.320	0.408	0.422	0.402	0.366	17.2
65 %	0.319	0.374	0.402	0.352	0.402	0.37	9.55
87 %	0.336	0.340	0.361	0.362	0.369	0.354	4.14

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	(65 %)	<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	(65 %)	<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: 87 % (TOP6C)
6. LOEC *Pimephales* Lethality: 87 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 87 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 87 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 9.55 (TQP6C)

Appendix B: Test 1000.0

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

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 304, 310, 314

2400
2400
2400

Test Initiated: DATE: September 8, 2015 TIME: 1450
Test Terminated: DATE: September 15, 2015 TIME: 1335

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.8	8.0	7.8	8.0	8.3	7.9
Final	7.7	7.4	7.6	7.6	8.1	7.5	8.7
pH Initial	8.2	7.7	8.0	7.9	7.8	8.0	7.6
Final	7.7	8.0	8.1	7.9	8.2	7.8	7.4
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	40	NA	40	NA	40	NA	NA
Conductivity	160	150	150	140	140	140	140
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.0	7.9	8.2	8.3	8.0
Final	7.0	7.0	7.7	7.7	8.2	7.6	8.4
pH Initial	8.1	7.7	7.9	7.8	7.8	8.0	7.6
Final	7.7	7.9	8.1	7.9	8.2	7.9	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	180	170	200	200	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.6	8.0	7.8	7.8	8.2	8.3
Final	7.4	7.2	7.8	7.7	8.0	7.6	8.3
pH Initial	8.1	7.7	7.9	7.8	7.8	8.0	7.6
Final	7.8	8.0	8.1	7.9	8.2	7.8	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	210	200	190	220	230	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.8	8.0	7.9	8.0	8.5	8.1
Final	7.6	7.0	7.7	7.6	8.0	4.4	8.2
pH Initial	8.1	7.7	7.8	7.8	7.8	8.0	7.7
Final	7.8	8.0	8.1	8.0	8.3	7.7	7.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	240	220	210	180	180	170
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.5	8.1	7.8	7.9	8.2	8.0
Final	7.5	7.0	7.7	7.6	7.9	7.5	8.1
pH Initial	8.0	7.7	7.8	7.8	7.8	8.0	7.6
Final	7.9	8.0	8.2	8.0	8.3	7.9	7.5
Alkalinity	56	NA	39	NA	34	NA	NA
Hardness	55	NA	52	NA	50	NA	NA
Conductivity	280	280	250	240	280	290	280
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	7.7	8.2	7.8	8.1	8.3	8.0
Final	7.1	6.7	7.7	7.5	9.3	7.7	8.0
pH Initial	8.0	7.7	7.7	7.7	7.8	8.0	7.6
Final	7.8	8.0	8.2	8.0	8.4	8.0	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	320	320	290	280	340	340	340
Chlorine	NA	NA	NA	NA	NA	NA	NA

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

Permittee: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 8, 2015 at 1455

Date and Time Test Terminated: September 15, 2015 at 1620

Dilution water used: Synthetic Soft Water #4249

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		27 %	37 %	49 %	65 %	87 %
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				
		27 %	37 %	49 %	65 %	87 %
A	24	27	26	28	36	32
B	29	26	30	25	26	27
C	28	27	24	29	29	28
D	28	22	27	21	24	31
E	29	32	32	28	30	30
F	25	35	26	8	28	0
G	3	33	28	27	31	29
H	30	15	31	31	29	30
I	31	28	29	24	29	27
J	23	32	28	28	29	29
Mean per Adult	25.0	27.7	28.1	24.9	29.1	26.3
Mean per Surviving Adult	25.0	27.7	28.1	24.9	29.1	29.2
CV %	32.7	21.4	8.79	26.4	10.8	5.87

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	(65 %)	<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	(65 %)	<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: 87 % (TOP3B)
- 6. LOEC *Ceriodaphnia* Lethality: 87 % (TXP3B)
- 7. NOEC *Ceriodaphnia* Sublethality: 87 % (TPP3B)
- 8. LOEC *Ceriodaphnia* Sublethality: 87 % (TYP3B)
- 9. Coefficient of variation for *Ceriodaphnia* Reproduction: 32.7 (TQP3B)

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 304, 310, 314

2400
2400
2400

Test Initiated: DATE: September 8, 2015 TIME: 1455
Test Terminated: DATE: September 15, 2015 TIME: 1620

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.8	8.0	7.8	8.0	8.3	7.9
Final	8.2	7.8	8.1	8.2	8.3	8.0	8.6
pH Initial	8.2	7.7	8.0	7.9	7.8	8.0	7.6
Final	8.1	8.4	8.5	8.4	8.4	7.9	8.0
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	40	NA	40	NA	40	NA	NA
Conductivity	160	150	150	140	140	140	140
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.0	7.9	8.2	8.3	8.0
Final	8.1	8.1	8.1	8.2	8.2	8.0	8.6
pH Initial	8.1	7.7	7.9	7.8	7.8	8.0	7.6
Final	8.2	8.4	8.5	8.4	8.4	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	200	190	180	170	200	200	200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.6	8.0	7.8	7.8	8.2	8.3
Final	8.3	8.1	8.2	8.2	8.1	7.8	8.6
pH Initial	8.1	7.7	7.9	7.8	7.8	8.0	7.6
Final	8.2	8.4	8.5	8.4	8.4	7.9	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	210	200	190	220	230	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.8	8.0	7.9	8.0	8.5	8.1
Final	8.3	8.1	8.2	8.2	8.1	7.9	8.7
pH Initial	8.1	7.7	7.8	7.8	7.8	8.0	7.7
Final	8.2	8.5	8.5	8.4	8.4	7.9	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	240	220	210	180	180	170
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.5	8.1	7.8	7.9	8.2	8.0
Final	8.2	8.0	8.2	8.2	8.1	8.1	8.7
pH Initial	8.0	7.7	7.8	7.8	7.8	8.0	7.6
Final	8.2	8.5	8.6	8.4	8.4	7.9	8.2
Alkalinity	56	NA	39	NA	34	NA	NA
Hardness	55	NA	52	NA	50	NA	NA
Conductivity	280	280	250	240	280	290	280
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	7.7	8.2	7.8	8.1	8.3	8.0
Final	7.9	8.1	8.1	8.2	8.0	8.0	8.7
pH Initial	8.0	7.7	7.7	7.7	7.8	8.0	7.6
Final	8.2	8.6	8.6	8.5	8.4	8.0	8.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	320	320	290	280	340	340	340
Chlorine	NA	NA	NA	NA	NA	NA	NA

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <i>Hot Springs Wastewater</i>			PO No. <i>15-2446</i>		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <i>1932182</i>	
Project Reference: <i>Plant Effluent</i>			MATRIX			<i>Bio-Monitoring</i>										AIC PROPOSAL NO:	
Project Manager: <i>Jim Sorrells</i>			WATER SOIL													Carrier:	
Sampled By: <i>A. Mauldin</i>			G	C	A	S	O	I	L	S	Received Temperature C: <i>0.1</i>						
AIC No.	Sample Identification	Date/Time Collected	B	P							R	L	Remarks				
<i>1</i>	<i>Plant Effluent</i>	<i>9/6/15 0000-2400</i>		<i>X</i>	<i>X</i>												
Container Type			Preservative		Field pH calibration on _____ @ _____ 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 _____ DAYS					Relinquished By: <i>A. Thomas</i>		Date/Time: <i>9-8-15 @ 0900</i>		Received By: <i>A. Mauldin</i>		Date/Time: <i>9/8/15 0900</i>						
Expedited results requested by: _____					Relinquished By: <i>A. Mauldin</i>		Date/Time: <i>9/8/15 @ 1008</i>		Received in Lab By: <i>Danny Brown</i>		Date/Time: <i>9-8-15 1008</i>						
Who should AIC contact with questions: _____					Comments: _____												
Phone: _____ Fax: _____																	
Report Attention to: _____																	
Report Address to: _____																	
Email Address: _____																	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Hot Springs Wastewater</u>			PO No. <u>152446</u>		NO OF B O T T L E S	ANALYSES REQUESTED										AIC CONTROL NO: <u>193982</u>		
Project Reference: <u>PLANT Effluent</u>			MATRIX			Bio-Monitoring										AIC PROPOSAL NO:		
Project Manager: <u>JIM SORRELLS</u>																Carrier: <u>M.S. LR/DEUN</u>		
Sampled By: <u>H MAULPIN</u>			G R A B	C O M P	W A T E R	S O I L											Received Temperature C <u>0</u>	
AIC No.	Sample Identification	Date/Time Collected															Remarks	
<u>2</u>	<u>PLANT Effluent</u>	<u>9/8/15</u> <u>0000-2400</u>	<u>X</u>	<u>X</u>														
													Field pH calibration on _____ @ _____					
													Buffer: _____					
G = Glass P = Plastic NO = none 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 _____ DAYS Expedited results requested by: _____ Who should AIC contact with questions: Phone: _____ Fax: _____ Report Attention to: Report Address to: Email Address:					Relinquished By: <u>H Maulpin</u> Date/Time: <u>9/9/15 @ 11:00</u>		Received By: <u>M. Mann</u> Date/Time: <u>9-9-15 @ 11:00</u>		Relinquished By: <u>M. Mann</u> Date/Time: <u>9-9-15 @ 12:00</u>		Received in Lab By: <u>Raventi</u> Date/Time: <u>9/9/15 12:10</u>		Comments:					

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Hot Springs Wastewater</u>			PO No. <u>15-2446</u>		NO OF BOTTLES	ANALYSES REQUESTED <u>Bio-Monitoring</u>										AIC CONTROL NO: <u>193982</u>	
Project Reference: <u>PLANT Effluent</u>			MATRIX													AIC PROPOSAL NO:	
Project Manager: <u>JIM SORRELLS</u>			WATER			SOIL		Carrier: <u>AS DELIVERY</u>									
Sampled By: <u>H. Mauldin</u>			GRA	COMP	WATER	SOIL	Received Temperature C <u>0.1</u>										
AIC No.	Sample Identification	Date/Time Collected					Remarks										
<u>3</u>	<u>PLANT Effluent</u>	<u>9/10/15 @ 2000-2400</u>	<u>X</u>	<u>X</u>	<u>3</u>	<u>X</u>											
Container Type			Field pH calibration on _____ @ _____														
Preservative			Buffer:														
G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate			NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate A = (NH ₄) ₂ SO ₄ , NH ₄ OH														
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS					Relinquished By: <u>H. Mauldin</u>		Date/Time <u>9/11/15 @ 11:25</u>		Received By: <u>M. Mann</u>		Date/Time <u>9-11-15 @ 11:25</u>						
Expedited results requested by: _____					Relinquished By: <u>M. Mann</u>		Date/Time <u>9-11-15 @ 12:20</u>		Received in Lab By: <u>D. Brun</u>		Date/Time <u>9-11-15 12:20</u>						
Who should AIC contact with questions: Phone: _____ Fax: _____ Report Attention to: Report Address to:					Comments:												
Email Address:																	

September 17, 2015

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for

~~Outfall 001 UV-Treated~~
~~City of Hot Springs~~

Control No. 193983-1

Prepared for:

Mr. James Sorrells
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

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



City of Hot Springs
ATTN: Mr. James Sorrells
320 Davidson Drive
Hot Springs, AR 71901

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 UV Treated - City of Hot Springs
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. James Sorrells:

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:

The sample was UV treated prior to analysis.

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 87 % effluent, which is above the critical dilution of 65 %. The NOEC for growth occurred at 87 % effluent; which is above the critical dilution of 65 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

PDF cc: City of Hot Springs
ATTN: Ms. Jessica Burks
jburks@cityhs.net

City of Hot Springs
ATTN: Mr. Dennis Brunson
dbrunson@cityhs.net

City of Hot Springs
ATTN: Mr. James Sorrells
jsorrells@cityhs.net

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A1: Test 1000.0

Pimephales promelas (Fathead minnow) Survival and Growth

A2: Statistics

A3: Water Chemistry

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Appendix B: Chains of Custody

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	95.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.329	PASS
Control Growth CV < or = 40%	21.1	PASS
Growth Minimum Significant Difference 12 to 30%	26.3	PASS
Critical Dilution CV < or = 40%	13.1	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0033880 AFIN#26-00145
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Method 1000.0
3. Receiving Stream: Lake Catherine

B. Source of Effluent/Dilution Water

1. Effluent Samples:
 - a. Sampling Point: Outfall 001 UV Treated
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.1	8.1	7.9
pH (standard units)	8.0	7.8	8.0
Alkalinity (mg/l as CaCO ₃)	54	42	42
Hardness (mg/l as CaCO ₃)	64	58	50
Conductivity (umhos/cm)	350	320	370
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.15	0.14	1.0

2. Dilution Water Samples: Synthetic Soft Water #4249

- a. Dates Prepared: August 26 through September 9, 2015
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.6	7.7	8.1
pH (standard units)	8.2	7.8	7.9
Alkalinity (mg/l as CaCO ₃)	31	31	31
Hardness (mg/l as CaCO ₃)	40	40	40
Conductivity (umhos/cm)	150	130	130
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: September 8, 2015 at 1620
Date & Time Test Terminated: September 15, 2015 at 1330
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 September 1, 2015 at 1630 to September 8, 2015 at 1520

The results were as follows: (Control No. 193794-1.)

Survival LC-50: 4389 mg/l

Growth IC-25: 3757 mg/l

Growth PMSD: 6.75

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	1.53
Hardness	EPA 200.7	99.0	0.457
pH	SM 4500-H+ B	100	0.00
Conductivity	EPA 120.1	100	0.703

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: September 8, 2015

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

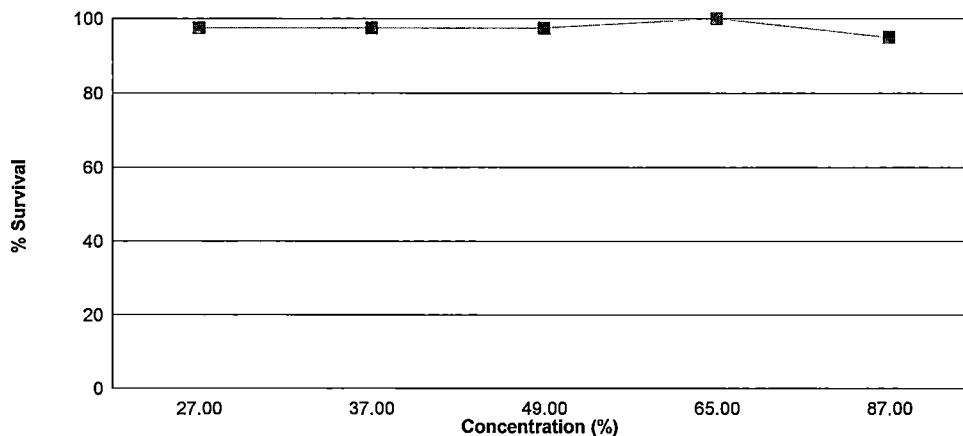
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 27 %, 37 %, 49 %, 65 %, 87 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 65 % effluent.

The test was initiated on September 8, 2015 at 1620 and continued through September 15, 2015 at 1330. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 87 % effluent
- b.) NOEC growth = 87 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	95.0	0.313
27 %	97.5	0.359
37 %	97.5	0.320
49 %	97.5	0.322
65 %	100	0.340
87 %	95.0	0.315

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: September 8, 2015 at 1620

Date and Time Test Terminated: September 15, 2015 at 1330

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	7	7	7	7	7	6	6
27 %	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	7	7	7
37 %	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	8	8	8	8	8
49 %	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	7
	E	8	8	8	8	8	8	8
65 %	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
87 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	6
	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: September 8, 2015 at 1620
Test Terminated: September 15, 2015 at 1330

Drying Started: September 14, 2015 at 1530
Drying Ended: September 16, 2015 at 1542

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94286	.94572	0.00286	8	0.358
	B	.93944	.94224	0.00280	8	0.350
	C	.93656	.93898	0.00242	8	0.302
	D	.94215	.94498	0.00283	8	0.354
	E	.93750	.93912	0.00162	8	0.202
27 %	A	.94574	.94814	0.00240	8	0.300
	B	.94884	.95157	0.00273	8	0.341
	C	.94451	.94818	0.00367	8	0.459
	D	.94307	.94609	0.00302	8	0.378
	E	.94563	.94817	0.00254	8	0.318
37 %	A	.94038	.94277	0.00239	8	0.299
	B	.94750	.94999	0.00249	8	0.311
	C	.94270	.94470	0.00200	8	0.250
	D	.94295	.94587	0.00292	8	0.365
	E	.93709	.94008	0.00299	8	0.374
49 %	A	.94284	.94539	0.00255	8	0.319
	B	.93816	.94084	0.00268	8	0.335
	C	.94525	.94796	0.00271	8	0.339
	D	.94035	.94265	0.00230	8	0.288
	E	.93645	.93909	0.00264	8	0.330
65 %	A	.93465	.93734	0.00269	8	0.336
	B	.93702	.93921	0.00219	8	0.274
	C	.93778	.94097	0.00319	8	0.399
	D	.93992	.94265	0.00273	8	0.341
	E	.93394	.93672	0.00278	8	0.348
87 %	A	.94296	.94506	0.00210	8	0.262
	B	.94541	.94799	0.00258	8	0.322
	C	.94542	.94723	0.00181	8	0.226
	D	.93755	.94063	0.00308	8	0.385
	E	.94127	.94429	0.00302	8	0.378

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	0.75000	1.04720	
2	27 %	1	1.00000	1.39310	
2	27 %	2	1.00000	1.39310	
2	27 %	3	1.00000	1.39310	
2	27 %	4	1.00000	1.39310	
2	27 %	5	0.87500	1.20940	
3	37 %	1	1.00000	1.39310	
3	37 %	2	1.00000	1.39310	
3	37 %	3	0.87500	1.20940	
3	37 %	4	1.00000	1.39310	
3	37 %	5	1.00000	1.39310	
4	49 %	1	1.00000	1.39310	
4	49 %	2	1.00000	1.39310	
4	49 %	3	1.00000	1.39310	
4	49 %	4	0.87500	1.20940	
4	49 %	5	1.00000	1.39310	
5	65 %	1	1.00000	1.39310	
5	65 %	2	1.00000	1.39310	
5	65 %	3	1.00000	1.39310	
5	65 %	4	1.00000	1.39310	
5	65 %	5	1.00000	1.39310	
6	87 %	1	1.00000	1.39310	
6	87 %	2	1.00000	1.39310	
6	87 %	3	0.75000	1.04720	
6	87 %	4	1.00000	1.39310	
6	87 %	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 = 0.2724 W = 0.6663 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	27 %	28.00	16.00	5.00	
3	37 %	28.00	16.00	5.00	
4	49 %	28.00	16.00	5.00	
5	65 %	30.00	16.00	5.00	
6	87 %	27.50	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 = 0.07298 W = 0.9844 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 = 5.309 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	0.008037	0.001607	0.5286	
Within (Error)	24	0.07297	0.00304		
Total	29	0.08101			
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	0.3132	0.3132			
2	27 %	0.3592	0.3592	-1.319		
3	37 %	0.3198	0.3198	-0.1893		
4	49 %	0.3222	0.3222	-0.2581		
5	65 %	0.3396	0.3396	-0.7571		
6	87 %	0.3146	0.3146	-0.04015		
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	27 %	5	0.0823	26.3	-0.046	
3	37 %	5	0.0823	26.3	-0.0066	
4	49 %	5	0.0823	26.3	-0.009	
5	65 %	5	0.0823	26.3	-0.0264	
6	87 %	5	0.0823	26.3	-0.0014	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 8, 2015 at 1323

Date and Time Test Terminated: September 15, 2015 at 1330

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.6	7.7	7.7	7.9	8.1	8.3	8.1
	Final	7.8	7.4	7.6	7.5	8.1	7.5	8.0
pH, units	Initial	8.2	7.7	7.8	7.7	7.9	7.8	7.8
	Final	7.8	8.0	7.9	7.8	8.2	7.8	7.5
Alkalinity, mg CaCO ₃ /l		31	NA	31	NA	31	NA	NA
Hardness, mg CaCO ₃ /l		40	NA	40	NA	40	NA	NA
Conductivity, umhos/cm		150	130	130	120	130	140	140
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 27 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.9	8.0	7.9	8.0	8.4	8.8
	Final	7.7	7.2	7.5	7.6	8.1	8.8	8.0
pH, units	Initial	8.2	7.7	7.8	7.6	7.9	7.8	7.7
	Final	7.8	8.0	8.0	7.9	8.2	8.1	7.5

Effluent Conc.: 37 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.8	7.8	7.9	7.9	8.4	7.8
	Final	7.8	7.0	7.6	7.5	8.0	7.9	8.0
pH, units	Initial	8.2	7.6	7.8	7.6	7.9	7.8	7.6
	Final	7.9	8.0	8.1	7.9	8.2	7.9	7.5

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 8, 2015 at 1323
Date and Time Test Terminated: September 15, 2015 at 1330

Effluent Conc.: 49 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.6	7.7	7.7	8.1	8.5	7.9
	Final	7.7	6.9	7.7	7.5	8.1	7.8	8.0
pH, units	Initial	8.2	7.6	7.8	7.6	7.9	7.9	7.7
	Final	7.8	8.0	8.1	7.9	8.2	7.9	7.5

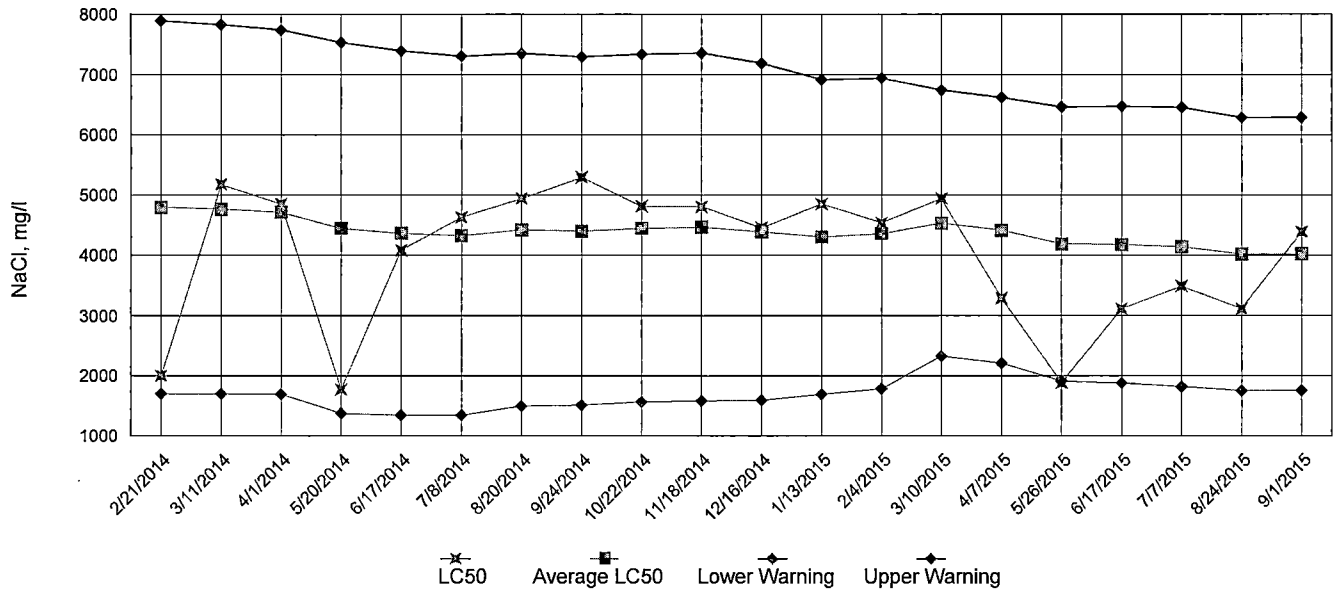
Effluent Conc.: 65 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.4	7.7	7.8	8.1	8.7	8.0
	Final	7.7	7.1	7.6	7.6	8.2	7.8	8.0
pH, units	Initial	8.1	7.6	7.8	7.5	7.9	7.9	7.6
	Final	7.9	8.0	8.1	7.9	8.3	7.9	7.6
Alkalinity, mg CaCO ₃ /l		47	NA	41	NA	35	NA	NA
Hardness, mg CaCO ₃ /l		61	NA	53	NA	56	NA	NA
Conductivity, umhos/cm		280	320	250	240	280	300	280
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 87 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.7	7.9	7.8	7.9	8.6	8.0
	Final	7.6	7.4	7.5	7.7	8.1	7.6	8.0
pH, units	Initial	8.1	7.6	7.7	7.5	7.9	7.9	7.6
	Final	7.9	8.1	8.2	8.0	8.3	8.0	7.6

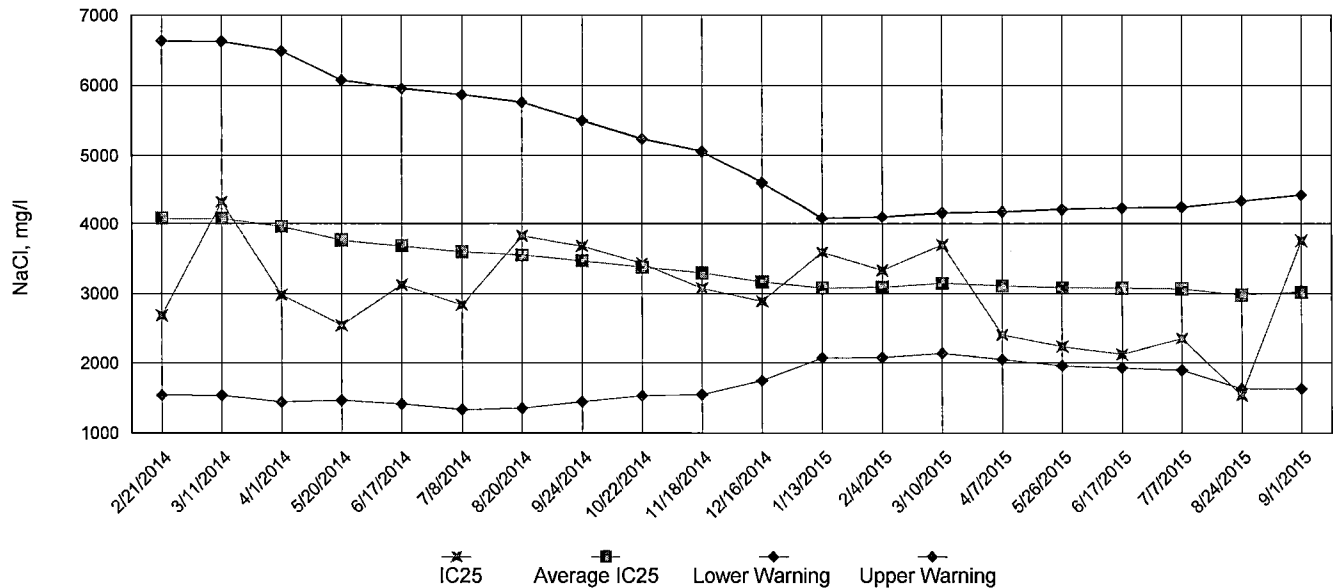
Appendix A4: Test 1000.0

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: City of Hot Springs

NPDES No.: AR0033880 AFIN#26-00145

Date and Time Test Initiated: September 8, 2015 at 1620

Date and Time Test Terminated: September 15, 2015 at 1330

Dilution water used: Synthetic Soft Water #4249

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	75.0	97.5	97.5	95.0	11.8
27 %	100	100	100	100	87.5	100	100	97.5	5.73
37 %	100	100	87.5	100	100	100	100	97.5	5.73
49 %	100	100	100	87.5	100	100	100	97.5	5.73
65 %	100	100	100	100	100	100	100	100	0.00
87 %	100	100	75.0	100	100	100	100	95.0	11.8

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.358	0.350	0.302	0.354	0.202	0.313	21.1
27 %	0.300	0.341	0.459	0.378	0.318	0.359	17.5
37 %	0.299	0.311	0.250	0.365	0.374	0.32	15.9
49 %	0.319	0.335	0.339	0.288	0.330	0.322	6.37
65 %	0.336	0.274	0.399	0.341	0.348	0.34	13.1
87 %	0.262	0.322	0.226	0.385	0.378	0.315	22.3

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	(65 %)	<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	(65 %)	<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: 87 % (TOP6C)

6. LOEC Pimephales Lethality: 87 % (TXP6C)

7. NOEC Pimephales Sublethality: 87 % (TPP6C)

8. LOEC Pimephales Sublethality: 87 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 21.1 (TQP6C)

Appendix B: Test 1000.0

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

PERMITTEE: City of Hot Springs
NPDES NO.: AR0033880 AFIN#26-00145
CONTACT: Mr. James Sorrells
ANALYST: 280, 304, 310, 314

2400
2400
2400

Test Initiated: DATE: September 8, 2015 TIME: 1620
Test Terminated: DATE: September 15, 2015 TIME: 1330

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.7	7.7	7.9	8.1	8.3	8.1
Final	7.8	7.4	7.6	7.5	8.1	7.5	8.0
pH Initial	8.2	7.7	7.8	7.7	7.9	7.8	7.8
Final	7.8	8.0	7.9	7.8	8.2	7.8	7.5
Alkalinity	31	NA	31	NA	31	NA	NA
Hardness	40	NA	40	NA	40	NA	NA
Conductivity	150	130	130	120	130	140	140
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.9	8.0	7.9	8.0	8.4	8.8
Final	7.7	7.2	7.5	7.6	8.1	8.8	8.0
pH Initial	8.2	7.7	7.8	7.6	7.9	7.8	7.7
Final	7.8	8.0	8.0	7.9	8.2	8.1	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	210	200	180	180	200	210	190
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 37 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.8	7.8	7.9	7.9	8.4	7.8
Final	7.8	7.0	7.6	7.5	8.0	7.9	8.0
pH Initial	8.2	7.6	7.8	7.6	7.9	7.8	7.6
Final	7.9	8.0	8.1	7.9	8.2	7.9	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	220	220	200	190	220	230	220
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 49 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.6	7.7	7.7	8.1	8.5	7.9
Final	7.7	6.9	7.7	7.5	8.1	7.8	8.0
pH Initial	8.2	7.6	7.8	7.6	7.9	7.9	7.7
Final	7.8	8.0	8.1	7.9	8.2	7.9	7.5
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	250	240	220	210	240	260	240
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 65 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.4	7.7	7.8	8.1	8.7	8.0
Final	7.7	7.1	7.6	7.6	8.2	7.8	8.0
pH Initial	8.1	7.6	7.8	7.5	7.9	7.9	7.6
Final	7.9	8.0	8.1	7.9	8.3	7.9	7.6
Alkalinity	47	NA	41	NA	35	NA	NA
Hardness	61	NA	53	NA	56	NA	NA
Conductivity	280	320	250	240	280	300	280
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 87 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	7.9	7.8	7.9	8.6	8.0
Final	7.6	7.4	7.5	7.7	8.1	7.6	8.0
pH Initial	8.1	7.6	7.7	7.5	7.9	7.9	7.6
Final	7.9	8.1	8.2	8.0	8.3	8.0	7.6
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	320	270	300	280	340	360	340
Chlorine	NA	NA	NA	NA	NA	NA	NA



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Hot Springs Wastewater			PO No. 15-2844		NO OF BOTTLES	ANALYSES REQUESTED BIO-MONITORING Pimephales promelas (Fat Head Minnow) ONLY										AIC CONTROL NO: 193983	
Project Reference: PLANT Effluent			MATRIX													AIC PROPOSAL NO:	
Project Manager: Jim Sorrells			WATER SOIL													Carrier:	
Sampled By: H Mauldin			G R A M B P													Received Temperature C 0.1	
AIC No.	Sample Identification	Date/Time Collected														Remarks	
1	PLANT Effluent	9/8/15 @ 0000-2400	X	X													
														Field pH calibration on _____ @ _____			
														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 _____ DAYS						Relinquished By: A. Thompson		Date/Time 9-8-15 @ 0900		Received By: H Mauldin		Date/Time 9/8/15 @ 0900					
Expedited results requested by: _____						Relinquished By: H Mauldin		Date/Time 9/8/15 @ 1008		Received in Lab By: Danny Brown		Date/Time 9-8-15 1008					
Who should AIC contact with questions: Phone: _____ Fax: _____						Comments:											
Report Attention to: Report Address to:																	
Email Address:																	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Hot Springs Wastewater			PO No. 15-2844		NO OF BOTTLES	ANALYSES REQUESTED Bio Monitoring Pimephales promelas (Fathead Minnow eggs)										AIC CONTROL NO: 193983			
Project Reference: PLANT Effluent			MATRIX													AIC PROPOSAL NO:			
Project Manager: JIM SORRELLS			WATER SOIL													Carrier: HS/LL DELIVERY			
Sampled By: H MAULDIN			G	C	NO OF BOTTLES											Received Temperature C 0c			
AIC No.	Sample Identification	Date/Time Collected	A	S												Remarks			
2	PLANT Effluent	9/8/15 @ 2:20-2:40	X	X	3														
																Field pH calibration on _____ @ _____			
			Container Type													Buffer:			
			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) NORMAL or EXPEDITED IN _____ DAYS						Relinquished By: H Mauldin		Date/Time 9/9/15 @ 1:00		Received By: M. Mann		Date/Time 9-9-15 @ 11:00							
Expedited results requested by: _____						Relinquished By: M. Mann		Date/Time 9-9-15 @ 12:10		Received in Lab By: Frankie Hui		Date/Time 9/9/15 12:10							
Who should AIC contact with questions: _____						Comments: UV Method													
Phone: _____ Fax: _____																			
Report Attention to: _____																			
Report Address to: _____																			
Email Address: _____																			



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Hot Springs Wastewater			PO No. 15-2844		NO OF B O T T L E S	ANALYSES REQUESTED										AIC CONTROL NO: 193983	
Project Reference: PLANT EFFLUENT						Bio Monitoring Pimephales promelas (Fathead Minnow only)										AIC PROPOSAL NO:	
Project Manager: JIM SORRELLS			MATRIX														
Sampled By: H MAULDIN			G R A B	C O M P		W A T E R	S O I L	Received Temperature C: 17.5 DELIVERY		Remarks							
AIC No.	Sample Identification	Date/Time Collected															
3	PLANT EFFLUENT	9/10/15 @ 0000-2400	X	X			3	X									
Container Type							P		Field pH calibration on _____ @ _____								
Preservative							NO		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 _____ DAYS				Relinquished By: A. Madt		Date/Time: 9/11/15 @ 11:25		Received By: M. Mann		Date/Time: 9-11-15 @ 11:25							
Expedited results requested by: _____				Relinquished By: M. Mann		Date/Time: 9-11-15 @ 12:20		Received in Lab By: D. Brown		Date/Time: 9-11-15 1220							
Who should AIC contact with questions: Phone: _____ Fax: _____				Comments: UV													
Report Attention to: Report Address to:																	
Email Address:																	

Sanitary Sewer Overflow (SSO) Monthly Report

Facility Name: Hot Springs Wastewater NPDES Permit No.: AR0033880 Monitoring Period (Month/Year) September / 2015

No Sanitary Sewer Overflows This Monitoring Period

Summary Report Code Descriptions				
Cause(s) of SSO	SSO Impact	Action(s) Taken	Ultimate Discharge Location	
CO -Construction	D -Debris	NEAH -No Evidence Adverse Health/Environmental Impact		CR -Creek/Stream/River (specify)
E -Equipment Failure	G -Grease	OEHC -Observed or Evidence of Human Contact	EC -Environmental Cleanup	DI -Ditch
HC -Hydro Clean	LF -Line Failure	EFK -Evidence of Fish Kill	HC -Hydro Cleaned	DR -Drop Inlet
R -Rainfall	RG -Roots/Grease		HR -Hand Rodded	GR -Ground Surface
RO -Roots	V -Vandalism		EN -Referred to Engineering	PA -Paved Area
			PN -Public Notification	CB -Contained in Building

Location	Manhole #	Start Date of SSO	End Date of SSO	Estimated Volume (in gallons)	Cause of SSO	Environmental Impact	Action(s) Taken to Address SSO	Discharge Location
2816 Albert Pike Rd.	20" Pressure Sewer Main	08/20/2015	08/20/2015	28,845 Revision for last month SSO	LF	NEAH	HC & EC	CR—Lake Hamilton
421 Lakepark Dr.	Carpenter Dam Pump Station	09/21/2015	09/21/2015	300	P	NEAH	EC	GR-Ground
Lakeland Dr. & Lake Hamilton Dr.	Hot Springs #2 Pump Station	09/26/2015	09/26/2015	1000	E	NEAH	EC	CR—Lake Hamilton

Signature of Cognizant or Ranking Official

Date 10-20-2015

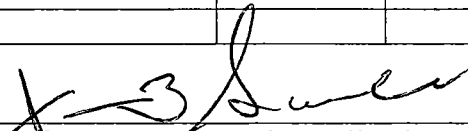
Sanitary Sewer Overflow (SSO) Monthly Report

Facility Name: SWWWTP NPDES Permit No.: AR0050148 Monitoring Period (Month/Year) September / 2015

No Sanitary Sewer Overflows This Monitoring Period

Summary Report Code Descriptions				
Cause(s) of SSO	SSO Impact	Action(s) Taken	Ultimate Discharge Location	
CO -Construction	D -Debris	NEAH -No Evidence Adverse Health/Environmental Impact		CR -Creek/Stream/River (specify)
E -Equipment Failure	G -Grease	OEHC -Observed or Evidence of Human Contact	EC -Environmental Cleanup	DI -Ditch
HC -Hydro Clean	LF -Line Failure	EFK -Evidence of Fish Kill	HC -Hydro Cleaned	DR -Drop Inlet
R -Rainfall	RG -Roots/Grease		HR -Hand Rodded	GR -Ground Surface
RO -Roots	V -Vandalism		EN -Referred to Engineering	PA -Paved Area
			PN -Public Notification	CB -Contained in Building

Location	Manhole #	Start Date of SSO	End Date of SSO	Estimated Volume (in gallons)	Cause of SSO	Environmental Impact	Action(s) Taken to Address SSO	Discharge Location
2460 Marion Anderson Rd.	MH# 13484	09/18/2015	09/18/2015	1500	E	NEAH	HC & EC	CR-Lake Hamilton

Signature of Cognizant or Ranking Official: 

Date: 10-20-2015

I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system, or those persons directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate, and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment of knowing violations.

NON-COMPLIANCE REPORT

Arkansas Department of Environmental Quality
NPDES Enforcement Section
5301 Northshore Drive
North Little Rock, AR 72118

RE: NPDES Permit No: AR0050148 Discharge Number: 001A

Facility: Hot Springs City of WWTP

Address: 365 Winkler Road

City: Hot Springs State: AR Zip: 71913

Contact: James Sorrells Phone: 1-501-262-1125 Ext 10

Date of Non-Compliance	Parameter Exceeded	Quantity or Loading	Quality or Concentration	Permit Limits
September-2015	CBOD	--	5.42 mg/l	5.0 mg/l

We fill this problem was due to:

Our raw influent BOD's have dropped to 80.0mg/l. The F/M's have dropped to well below the normal ranges This is a continued problem starting in august, we have gone through everything in process control assuring that it is not a problem with the SBR itself..

We plan on correcting the problem in this manner:

We are looking every where

Time estimated that it will take to correct problem:

We are still experiencing some of the same issues going into October but it is getting better as of this week

Sincerely,
James B Sorrells



Authorized Signature

10-20-2015

Date

MEMO TO: Richard Healey, ADEQ
Dennis Taylor, AR Dept of Health (ADH)
Bobby Pharr, ENTERGY

FROM: Gary Carnahan, P.E., Hot Springs City Engineer

CC Stephen Youngblood, ADH
Jason Gilkey, ADH
Bill Burrough, Hot Springs City Manager
Scott Bundy, Hot Springs Utility Director
Bobby Harris, Hot Springs Utility Operations Manager

Regarding the force main break yesterday, Scott Bundy has asked me to continue communicating with ADEQ, the Department of Health and Entergy so that you are well informed of the situation and our remediation measures.

Since the repair is now complete, we have had time this morning to review more of the project details. The pipe break occurred while our crews were on the job to repair a leaking bell gasket joint in our "new" 20" PVC force main.

As a first step this morning, we reviewed our standard operating procedures and affirmed that our utility operations staff did follow SOP for the repair. They had the repair pipe and materials on the job, and they had adequate manpower, machinery, and equipment on site for the repair. They had planned for incidental water loss during the repair by having two 2800 gallon pumper trucks on site. They had shut off the Fairwood Lift Station for the anticipated short repair period. In the event of a delay in the repair, they had changed the Fairwood side (up-pipe) valves so the Fairwood LS would pump through the bypass main if necessary. When we installed the new main, we left the old 15" main in place so it could be used as a backup or bypass main if necessary. They had also closed the 20" butterfly valve just 600 feet east (down-pipe) of the repair section. So, they believed that the repair section was isolated from any flow.

We also reviewed the volume of water that was lost during the break and the amount that might have entered the lake. The break occurred at 12:45 pm and the water flowing from the pipe was stopped at 1:10 pm. The water was stopped by closing a 24" valve that is located about 800 feet farther down-pipe. The spill from the break occurred because the 20" butterfly valve closest to the repair section did not fully close. Twenty five minutes passed until we realized that the valve must be partially open and then closed the 24" valve. The 1400 feet of pipe up to the next high point in the line contained 22,845 gallons. Additionally, between the break and the 24" valve there is a pump station that pumps 800 gallons per minute. We checked the pumping records and found that it pumped for ten minutes during the time of the break. Since the 20" valve was partially closed, we are assuming that at least half of that flow travelled down-pipe as normal, but that possibly half of the flow went up-pipe to the break. That would have added an additional 4,000 gallon to the pipe volume, so the estimated volume of the spill was 26,845 gallons.

Wastewater from the broken pipe traveled over that 25 minute period, into the road ditch, across gravel and paved parking areas, and through grassed swales for 600 feet toward a seawall on Lake Hamilton. The wastewater collected in the ditches and low areas along that 600 feet. The standby pumper trucks were able to collect and remove 20,000 gallons (ten loads at 2,000 gallons per load) from those areas

before it entered the lake. Some of the wastewater also soaked into or was trapped by the grass and gravel areas and did not enter the lake. The grass also trapped the debris from the spill. Consequently, we did not see any floating debris or any gray, cloudy, or discolored water in the lake.

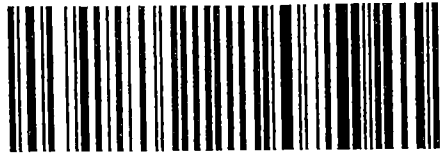
We are now implementing the following actions:

- We have collected lake water samples and are having them analyzed for fecal coliform, e. coli, and dissolved oxygen. If there are negative sample results, we will continue monitoring.
- We will hire an independent emergency clean-up contractor to remove any contaminated soils, gravel, etc and restore surfaces to their previous condition.
- We will review our SOP and amend it to include additional precautions for future main repairs (for example, double valve isolation).
- We retained the broken pipe and will have it tested by an independent lab to see if there is an identifiable cause of the rupture.
- We will continue to maintain open communication with ADEQ, the Department of Health, and Entergy.

Once again we sincerely regret this unexpected event and will continue remediation measures to safeguard public health and the water quality of Lake Hamilton. We will also try to learn as much as possible from this event and use those lessons learned in the future.

City of Hot Springs
Wastewater Treatment Plant
320 Davidson Drive
Hot Springs, Ar 71901

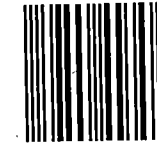
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