

August 7, 2015

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
Huntsville, AR

Control No. 192741-1

Prepared for:

Mr. Bill Eoff
Huntsville Water Utilities
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Huntsville, AR 72740

Prepared by:

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Huntsville Water Utilities
ATTN: Mr. Bill Eoff
Post Office Box 430
Huntsville, AR 72740

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001 - Huntsville, AR
NPDES Permit No. AR0022004 AFIN# 44-00018

Dear Mr. Bill Eoff:

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 100 % effluent, which is equal to the critical dilution of 100 %. The NOEC for growth occurred at 0 % effluent, which is below the critical dilution of 100 %. **The sample PASSED lethal effects, however, FAILED 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 100 % effluent, which is equal to the critical dilution of 100 %. The NOEC for reproduction occurred at 100 % effluent, which is equal to the critical dilution of 100 %. **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	0.384	PASS
Control Growth CV < or = 40%	3.05	PASS
Growth Minimum Significant Difference 12 to 30%	14.0	PASS
Critical Dilution CV < or = 40%	12.2	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	25.2	PASS
Control CV < or = 40% per Surviving Female	36.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	41.3	PASS
Critical Dilution CV < or = 40%	36.3	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0022004 AFIN# 44-00018
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 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.5	8.4	8.4
pH (standard units)	8.0	7.7	7.8
Alkalinity (mg/l as CaCO ₃)	150	140	160
Hardness (mg/l as CaCO ₃)	240	230	190
Conductivity (umhos/cm)	1200	1100	1100
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	3.6	2.7	8.0

2. Dilution Water Samples: Synthetic Moderately Hard Water #4236

- a. Dates Prepared: July 17 through July 31, 2015
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.8	8.5	7.8
pH (standard units)	8.5	8.0	7.9
Alkalinity (mg/l as CaCO ₃)	60	58	58
Hardness (mg/l as CaCO ₃)	86	83	83
Conductivity (umhos/cm)	300	330	300
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: July 28, 2015 at 1700
Date & Time Test Terminated: August 4, 2015 at 1530
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: July 28, 2015 at 1430
Date & Time Test Terminated: August 4, 2015 at 1340
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 July 7, 2015 at 1550 to July 14, 2015 at 1410

The results were as follows: (Control No. 192203-1.)

Survival LC-50: 3488 mg/l

Growth IC-25: 2351 mg/l

Growth PMSD: 21

Ceriodaphnia dubia

Chronic reference tests are performed monthly.

A chronic reference test was performed on July 7, 2015 at 1615 to July 14, 2015 at 1415

The results were as follows: (Control No. 192203-2.)

Survival LC-50: 2035 mg/l

Growth IC-25: 1481 mg/l

Growth PMSD: 19.8

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.826
Hardness	EPA 200.7	98.8	0.306
pH	SM 4500-H+ B	100	0.00
Conductivity	EPA 120.1	93.2	0.00

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: July 28, 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: July 28, 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

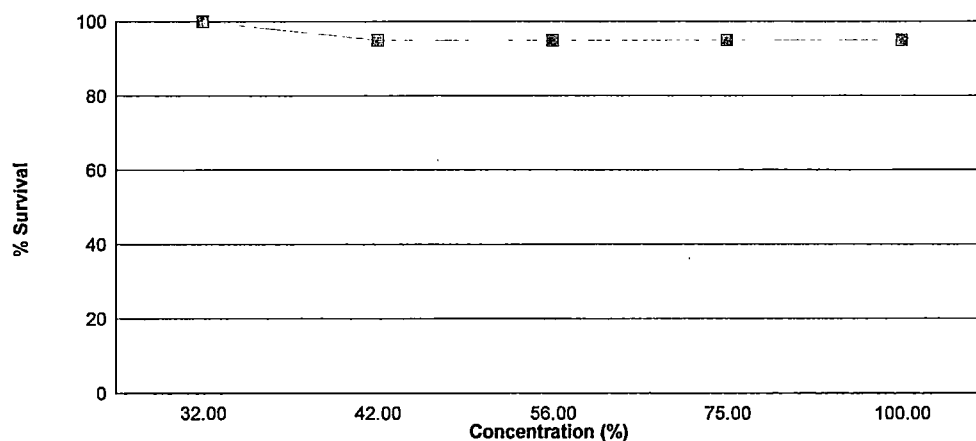
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on July 28, 2015 at 1700 and continued through August 4, 2015 at 1530. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 100 % effluent
- b.) NOEC growth = 0 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.384
32 %	100	0.282 *
42 %	95.0	0.281 *
56 %	95.0	0.284 *
75 %	95.0	0.308 *
100 %	95.0	0.280 *

*Significant difference when compared to the control (p=0.05)

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

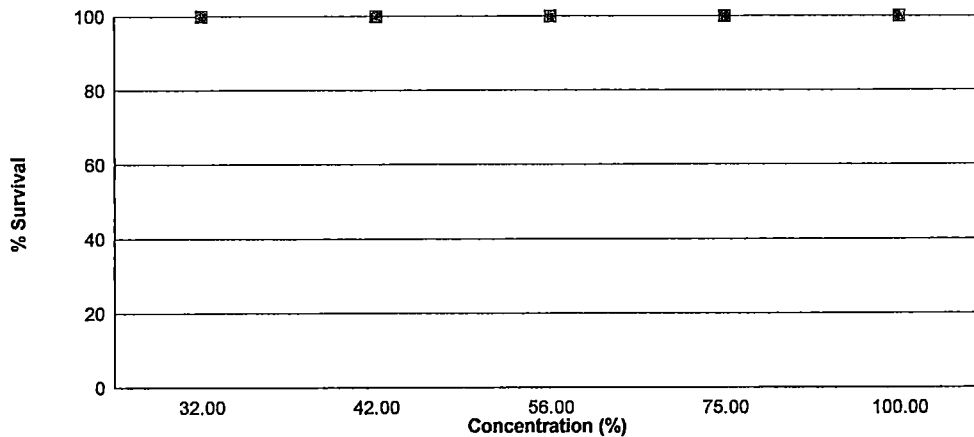
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring with an average of at least 15 young per female.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on July 28, 2015 at 1430 and continued through August 4, 2015 at 1340. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 100 % effluent
- b.) NOEC reproduction = 100 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	25.2
32 %	100	28.3
42 %	100	27.1
56 %	100	29.1
75 %	100	27.8
100 %	100	27.1

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: July 28, 2015 at 1700
Date and Time Test Terminated: August 4, 2015 at 1530

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
32 %	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
42 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	7	6	6
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
56 %	A	8	8	8	8	8	8	8
	B	7	7	7	7	7	7	7
	C	8	8	8	8	8	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
75 %	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	7	7	7	7
	E	8	8	7	7	7	7	7
100 %	A	7	7	7	7	7	7	7
	B	8	8	8	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: July 28, 2015 at 1700
Test Terminated: August 4, 2015 at 1530

Drying Started: August 4, 2015 at 1351
Drying Ended: August 5, 2015 at 1200

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94103	.94409	0.00306	8	0.382
	B	.93995	.94296	0.00301	8	0.376
	C	.93390	.93703	0.00313	8	0.391
	D	.93077	.93398	0.00321	8	0.401
	E	.93284	.93582	0.00298	8	0.372
32 %	A	.93250	.93456	0.00206	8	0.258
	B	.93572	.93824	0.00252	8	0.315
	C	.93553	.93773	0.00220	8	0.275
	D	.93402	.93625	0.00223	8	0.279
	E	.93374	.93600	0.00226	8	0.282
42 %	A	.93758	.93949	0.00191	8	0.239
	B	.94057	.94316	0.00259	8	0.324
	C	.94403	.94606	0.00203	8	0.254
	D	.94414	.94665	0.00251	8	0.314
	E	.94217	.94436	0.00219	8	0.274
56 %	A	.93941	.94210	0.00269	8	0.336
	B	.93403	.93578	0.00175	8	0.219
	C	.93341	.93603	0.00262	8	0.328
	D	.93629	.93853	0.00224	8	0.280
	E	.93382	.93587	0.00205	8	0.256
75 %	A	.93736	.94030	0.00294	8	0.368
	B	.94268	.94513	0.00245	8	0.306
	C	.93843	.94041	0.00198	8	0.248
	D	.93295	.93518	0.00223	8	0.279
	E	.93630	.93901	0.00271	8	0.339
100 %	A	.93242	.93469	0.00227	8	0.284
	B	.93464	.93658	0.00194	8	0.242
	C	.93246	.93452	0.00206	8	0.258
	D	.93319	.93545	0.00226	8	0.282
	E	.93432	.93698	0.00266	8	0.332

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 28, 2015 at 1430

Date and Time Test Terminated: August 4, 2015 at 1340

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	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	4	4	5	3	0	4	3	6	4	5	38	10	3.80
5	10	9	10	8	0	12	11	11	9	12	92	10	9.20
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	11	16	13	13	0	14	13	12	16	14	122	10	12.2
8													
TOTAL	25	29	28	24	0	30	27	29	29	31	252	10	25.2

Concentration: 32 %													
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	4	0	0	0	0	0	0	0	4	10	0.400
4	4	4	0	5	0	4	6	5	4	4	36	10	3.60
5	10	9	11	11	0	10	13	13	10	11	98	10	9.80
6	0	0	14	0	0	0	0	0	0	0	14	10	1.40
7	15	11	0	19	0	18	17	18	15	18	131	10	13.1
8													
TOTAL	29	24	29	35	0	32	36	36	29	33	283	10	28.3

Concentration: 42 %													
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	4	5	4	4	0	5	5	6	5	4	42	10	4.20
5	10	9	10	10	0	12	9	11	11	14	96	10	9.60
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	14	14	15	12	0	17	17	15	12	17	133	10	13.3
8													
TOTAL	28	28	29	26	0	34	31	32	28	35	271	10	27.1

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 28, 2015 at 1430
Date and Time Test Terminated: August 4, 2015 at 1340

Concentration: 56 %														
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	3	0	0	0	0	0	0	0	3	10	0.300	
4	4	4	0	5	0	5	4	5	5	4	36	10	3.60	
5	12	11	8	10	0	11	13	14	10	13	102	10	10.2	
6	0	0	0	18	0	0	0	0	0	0	18	10	1.80	
7	16	19	13	17E	0	16	19	15	15	19	132	10	13.2	
8														
TOTAL	32	34	24	33	0	32	36	34	30	36	291	10	29.1	

E = Excluded fourth brood neonates

Concentration: 75 %													
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	4	6	0	4	0	3	5	5	4	4	35	10	3.50
5	8	12	12	8	0	11	12	12	13	13	101	10	10.1
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	16	15	15	18	0	15	13	17	16	17	142	10	14.2
8													
TOTAL	28	33	27	30	0	29	30	34	33	34	278	10	27.8

Concentration: 100 %													
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	5	0	0	0	0	0	0	0	5	10	0.500
4	3	5	0	4	0	3	5	4	4	4	32	10	3.20
5	10	8	13	13	0	0	13	13	10	9	89	10	8.90
6	0	0	14	0	0	11	0	0	0	0	25	10	2.50
7	13	16	0	15	0	15	17	14	15	15	120	10	12.0
8													
TOTAL	26	29	32	32	0	29	35	31	29	28	271	10	27.1

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	32 %	1	1.00000	1.39310
2	32 %	2	1.00000	1.39310
2	32 %	3	1.00000	1.39310
2	32 %	4	1.00000	1.39310
2	32 %	5	1.00000	1.39310
3	42 %	1	1.00000	1.39310
3	42 %	2	1.00000	1.39310
3	42 %	3	0.75000	1.04720
3	42 %	4	1.00000	1.39310
3	42 %	5	1.00000	1.39310
4	56 %	1	1.00000	1.39310
4	56 %	2	0.87500	1.20940
4	56 %	3	0.87500	1.20940
4	56 %	4	1.00000	1.39310
4	56 %	5	1.00000	1.39310
5	75 %	1	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	1.00000	1.39310
5	75 %	4	0.87500	1.20940
5	75 %	5	0.87500	1.20940
6	100 %	1	0.87500	1.20940
6	100 %	2	0.87500	1.20940
6	100 %	3	1.00000	1.39310
6	100 %	4	1.00000	1.39310
6	100 %	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 = 0.2172 W = 0.7822 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	32 %	27.50	16.00	5.00	
3	42 %	25.00	16.00	5.00	
4	56 %	22.50	16.00	5.00	
5	75 %	22.50	16.00	5.00	
6	100 %	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
D = 0.03107 W = 0.9638 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 8.088 Critical B = 15.086 (alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.0425	0.0085	6.564	
Within (Error)	24	0.03108	0.001295		
Total	29	0.07358			
Critical F = 3.9 (alpha = 0.01, df = 5,24)					
2.62 (alpha = 0.05, df = 5,24)					
Since F > Critical F 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	0.3844	0.3844			
2	32 %	0.2818	0.2818	4.508	*	
3	42 %	0.281	0.281	4.543	*	
4	56 %	0.2838	0.2838	4.42	*	
5	75 %	0.308	0.308	3.357	*	
6	100 %	0.2796	0.2796	4.605	*	
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	32 %	5	0.05371	14	0.1026		
3	42 %	5	0.05371	14	0.1034		
4	56 %	5	0.05371	14	0.1006		
5	75 %	5	0.05371	14	0.0764		
6	100 %	5	0.05371	14	0.1048		

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
32 %	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
42 %	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
56 %	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
75 %	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
100 %	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.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	32 %	10	0	
2	42 %	10	0	
3	56 %	10	0	
4	75 %	10	0	
5	100 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.3189 D* = 2.502 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	32 %	126.50	75.00	10.00	
3	42 %	118.00	75.00	10.00	
4	56 %	135.50	75.00	10.00	
5	75 %	127.00	75.00	10.00	
6	100 %	122.00	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	88.73	17.75	0.1744	
Within (Error)	54	5496	101.8		
Total	59	5585			
Critical F = 3.38 (alpha = 0.01, df = 5,54)					
2.38 (alpha = 0.05, df = 5,54)					
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	25.2	25.2			
2	32 %	28.3	28.3	-0.687		
3	42 %	27.1	27.1	-0.4211		
4	56 %	29.1	29.1	-0.8643		
5	75 %	27.8	27.8	-0.5762		
6	100 %	27.1	27.1	-0.4211		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)						

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	32 %	10	10.42	41.3	-3.1	
3	42 %	10	10.42	41.3	-1.9	
4	56 %	10	10.42	41.3	-3.9	
5	75 %	10	10.42	41.3	-2.6	
6	100 %	10	10.42	41.3	-1.9	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: July 28, 2015 at 1001
Date and Time Test Terminated: August 4, 2015 at 1530

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.8	7.2	8.5	7.7	7.8	7.6	7.4
	Final *1	7.5	8.6	7.3	7.2	7.4	7.7	6.6
	Final *2	7.6	7.8	7.7	7.9	7.8	8.1	8.1
pH, units	Initial	8.5	7.9	8.0	8.2	7.9	8.0	7.9
	Final *1	7.9	7.7	7.8	8.1	8.1	7.9	7.9
	Final *2	8.2	8.3	8.6	8.4	8.5	8.0	8.0
Alkalinity, mg CaCO ₃ /l		60	NA	58	NA	58	NA	NA
Hardness, mg CaCO ₃ /l		86	NA	83	NA	83	NA	NA
Conductivity, umhos/cm		300	310	330	260	300	330	270
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 32 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.1	8.5	7.6	7.6	7.6	7.1
	Final *1	7.2	8.6	7.4	7.2	8.1	6.4	6.6
	Final *2	7.5	7.8	7.6	8.6	7.9	8.1	8.0
pH, units	Initial	7.9	7.9	7.8	8.2	7.9	8.2	8.0
	Final *1	8.0	7.8	8.0	8.3	8.2	7.9	7.9
	Final *2	8.4	8.4	8.8	8.5	8.7	8.2	8.2

Effluent Conc.: 42 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.5	7.3	7.6	7.6	7.0	7.6	7.3
	Final *1	7.2	8.5	7.6	6.9	7.7	7.1	6.6
	Final *2	7.5	7.8	7.8	8.5	7.5	8.0	8.0
pH, units	Initial	8.6	7.9	7.8	8.3	7.9	8.3	8.0
	Final *1	8.1	7.8	8.0	8.3	8.3	7.9	8.0
	Final *2	8.4	8.5	8.8	8.5	8.7	8.2	8.2

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: July 28, 2015 at 1001
Date and Time Test Terminated: August 4, 2015 at 1530

Effluent Conc.: 56 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.4	7.2	7.5	7.6	7.5	7.6	6.9
	Final *1	7.1	8.6	7.5	7.3	7.7	7.0	7.2
	Final *2	7.1	7.8	7.6	8.5	7.4	7.8	7.9
pH, units	Initial	8.6	7.9	7.8	8.3	8.0	8.4	8.0
	Final *1	8.1	7.9	8.1	8.4	8.3	8.1	8.1
	Final *2	8.5	8.6	8.9	8.6	8.8	8.3	8.3

Effluent Conc.: 75 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.4	8.5	7.5	7.3	7.5	7.0
	Final *1	7.2	8.3	7.2	7.1	7.5	6.6	6.8
	Final *2	7.5	7.7	7.7	8.5	7.3	7.9	8.0
pH, units	Initial	8.6	8.0	7.7	8.3	7.9	8.4	8.1
	Final *1	8.2	8.0	8.1	8.4	8.4	8.0	8.1
	Final *2	8.5	8.7	8.9	8.6	8.9	8.4	8.4

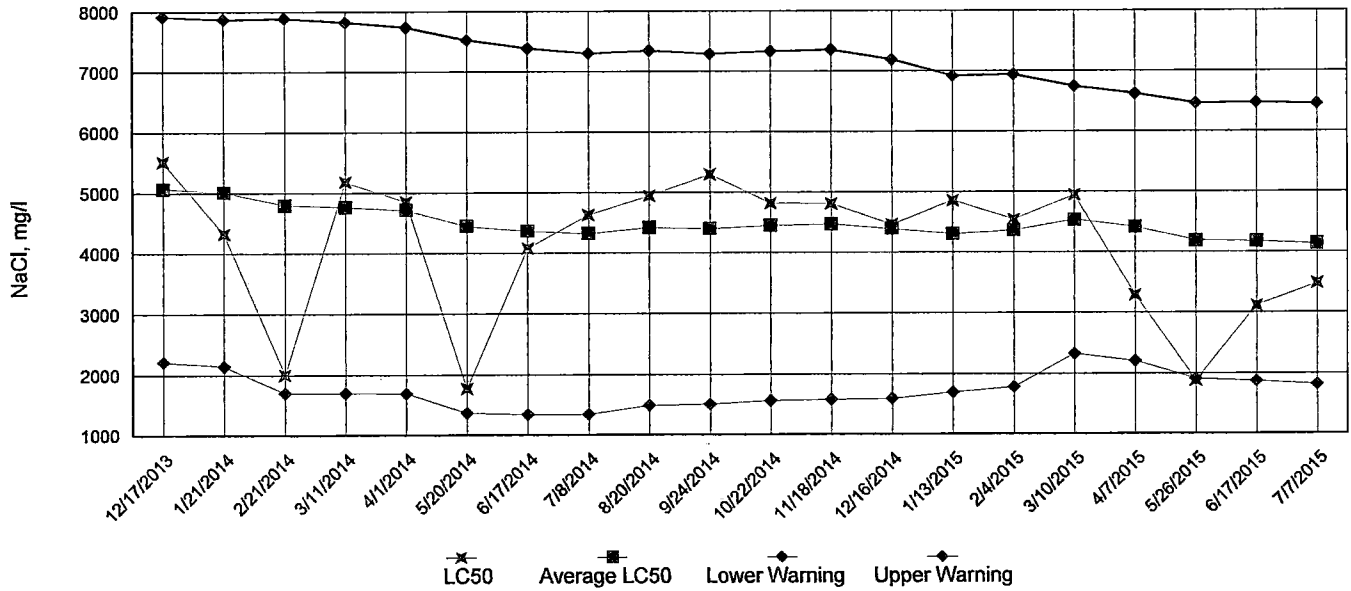
Effluent Conc.: 100 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.5	7.1	8.4	7.6	8.4	7.6	6.8
	Final *1	7.2	8.6	7.4	6.8	7.6	8.5	6.8
	Final *2	7.5	7.7	7.6	8.4	7.4	8.0	8.0
pH, units	Initial	8.0	7.7	7.7	8.3	7.8	8.4	8.1
	Final *1	8.2	8.0	8.2	8.4	8.4	8.0	8.1
	Final *2	8.5	8.8	9.0	8.6	9.0	8.4	8.4
Alkalinity, mg CaCO ₃ /l		150	NA	140	NA	160	NA	NA
Hardness, mg CaCO ₃ /l		240	NA	230	NA	190	NA	NA
Conductivity, umhos/cm		1200	1200	1100	1200	1100	1100	1200
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

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

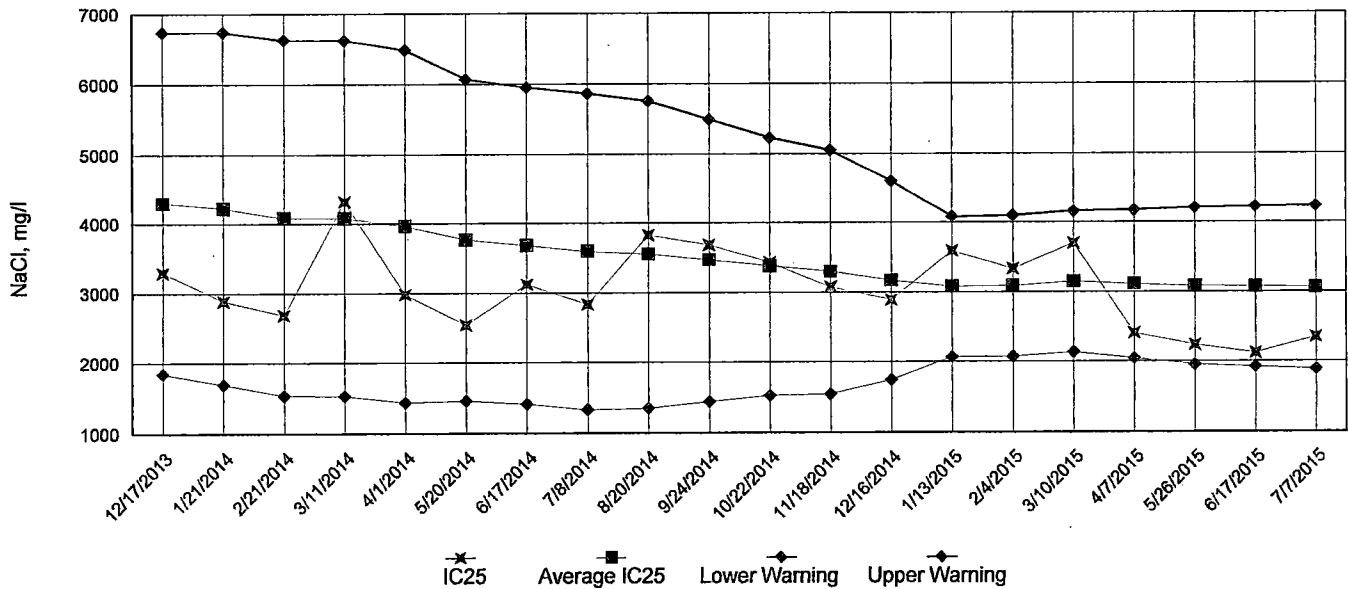
Appendix A4: Test 1000.0

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

LC50 Survival Data



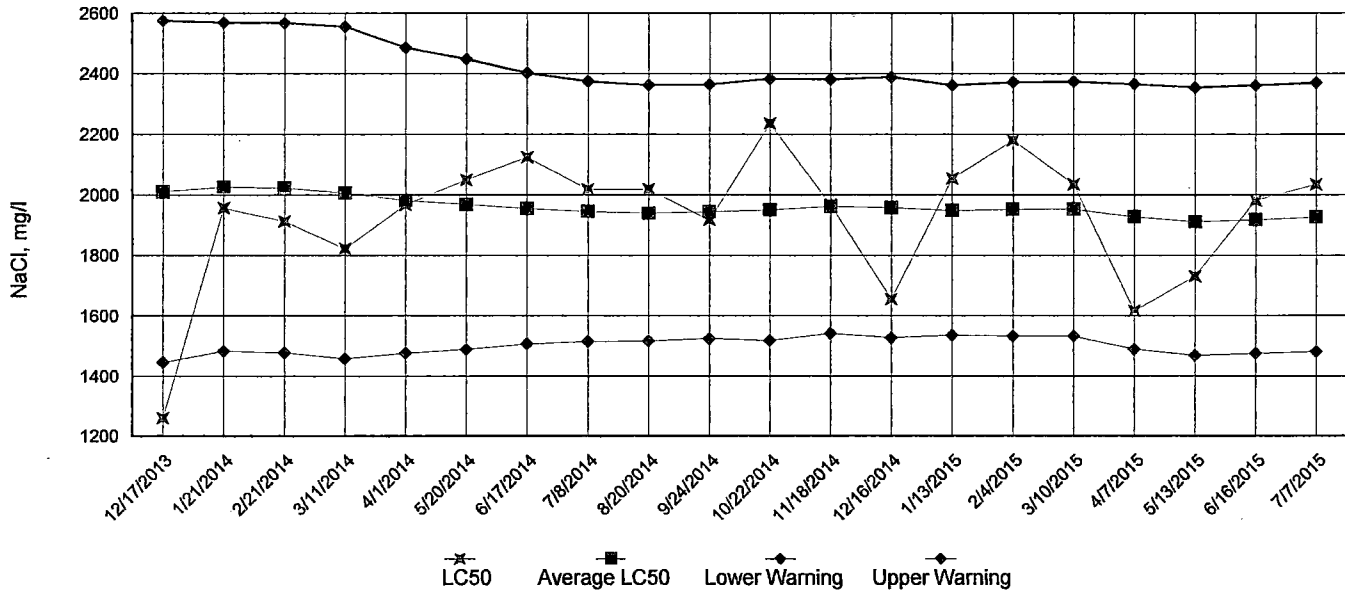
IC25 Growth Data



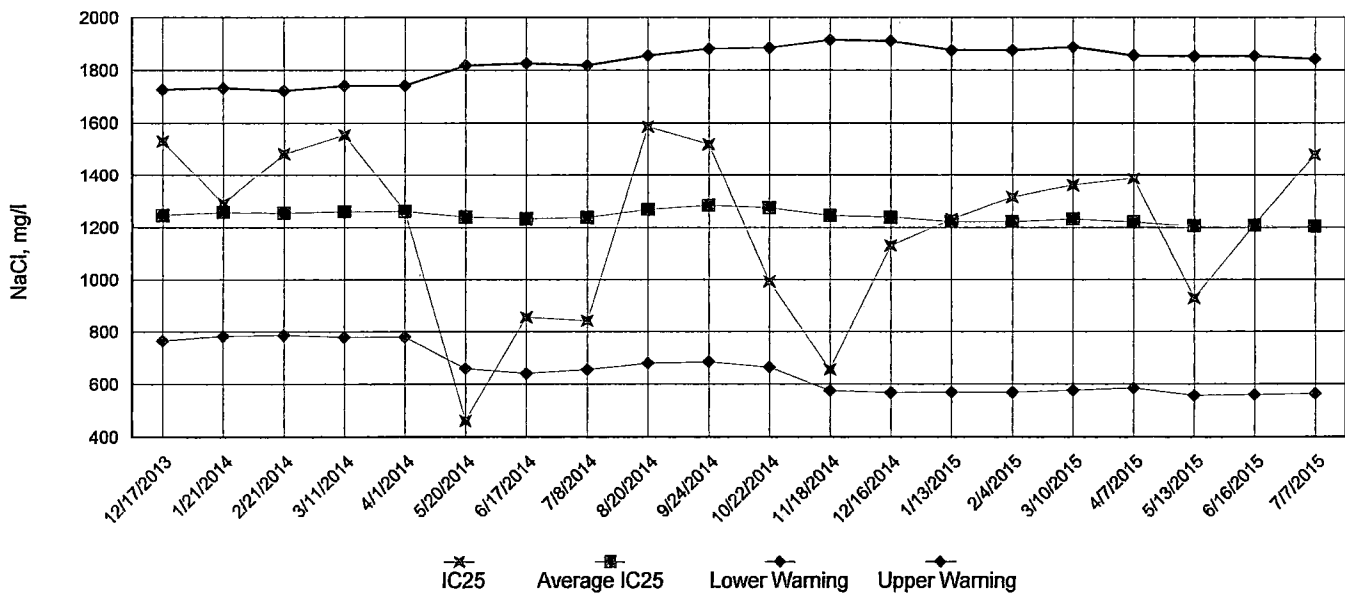
Appendix A4: Test 1002.0

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: Huntsville Water Utilities

NPDES No.: AR0022004 AFIN# 44-00018

Date and Time Test Initiated: July 28, 2015 at 1700

Date and Time Test Terminated: August 4, 2015 at 1530

Dilution water used: Synthetic Moderately Hard Water #4236

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
32 %	100	100	100	100	100	100	100	100	0.00
42 %	100	100	75.0	100	100	100	100	95.0	11.8
56 %	100	87.5	87.5	100	100	97.5	97.5	95.0	7.21
75 %	100	100	100	87.5	87.5	100	100	95.0	7.21
100 %	87.5	87.5	100	100	100	97.5	97.5	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	0.382	0.376	0.391	0.401	0.372	0.384	3.05
32 %	0.258	0.315	0.275	0.279	0.282	0.282	7.36
42 %	0.239	0.324	0.254	0.314	0.274	0.281	13.2
56 %	0.336	0.219	0.328	0.280	0.256	0.284	17.3
75 %	0.368	0.306	0.248	0.279	0.339	0.308	15.4
100 %	0.284	0.242	0.258	0.282	0.332	0.28	12.2

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	(100 %)	<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	(100 %)	<u> X </u> YES	<u> </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]: 1 (TGP6C)
5. NOEC *Pimephales* Lethality: 100 % (TOP6C)
6. LOEC *Pimephales* Lethality: 100 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 0 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 0 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 12.2 (TQP6C)

Appendix B: Test 1000.0

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

PERMITTEE: Huntsville Water Utilities
NPDES NO.: AR0022004 AFIN# 44-00018
CONTACT: Mr. Bill Eoff
ANALYST: 280, 304, 310, 314

SAMPLE No. 1 COLLECTED ending: DATE: July 27, 2015 TIME: 0500
SAMPLE No. 2 COLLECTED ending: DATE: July 29, 2015 TIME: 0500
SAMPLE No. 3 COLLECTED ending: DATE: July 31, 2015 TIME: 0500
Test Initiated: DATE: July 28, 2015 TIME: 1700
Test Terminated: DATE: August 4, 2015 TIME: 1530

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.2	8.5	7.7	7.8	7.6	7.4
Final	7.5	8.6	7.3	7.2	7.4	7.7	6.6
pH Initial	8.5	7.9	8.0	8.2	7.9	8.0	7.9
Final	7.9	7.7	7.8	8.1	8.1	7.9	7.9
Alkalinity	60	NA	58	NA	58	NA	NA
Hardness	86	NA	83	NA	83	NA	NA
Conductivity	300	310	330	260	300	330	270
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 32 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	7.1	8.5	7.6	7.6	7.6	7.1
Final	7.2	8.6	7.4	7.2	8.1	6.4	6.6
pH Initial	7.9	7.9	7.8	8.2	7.9	8.2	8.0
Final	8.0	7.8	8.0	8.3	8.2	7.9	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	620	590	550	540	540	560	550
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 42 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.3	7.6	7.6	7.0	7.6	7.3
Final	7.2	8.5	7.6	6.9	7.7	7.1	6.6
pH Initial	8.6	7.9	7.8	8.3	7.9	8.3	8.0
Final	8.1	7.8	8.0	8.3	8.3	7.9	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	650	690	640	630	630	640	640
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 56 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.2	7.5	7.6	7.5	7.6	6.9
Final	7.1	8.6	7.5	7.3	7.7	7.0	7.2
pH Initial	8.6	7.9	7.8	8.3	8.0	8.4	8.0
Final	8.1	7.9	8.1	8.4	8.3	8.1	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	790	810	770	770	750	760	770
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 75 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.5	7.5	7.3	7.5	7.0
Final	7.2	8.3	7.2	7.1	7.5	6.6	6.8
pH Initial	8.6	8.0	7.7	8.3	7.9	8.4	8.1
Final	8.2	8.0	8.1	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	980	980	930	940	900	910	940
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 100 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.1	8.4	7.6	8.4	7.6	6.8
Final	7.2	8.6	7.4	6.8	7.6	8.5	6.8
pH Initial	8.0	7.7	7.7	8.3	7.8	8.4	8.1
Final	8.2	8.0	8.2	8.4	8.4	8.0	8.1
Alkalinity	150	NA	140	NA	160	NA	NA
Hardness	240	NA	230	NA	190	NA	NA
Conductivity	1200	1200	1100	1200	1100	1100	1200
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

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

Permittee: Huntsville Water Utilities

NPDES No.: AR0022004 AFIN# 44-00018

Date and Time Test Initiated: July 28, 2015 at 1430

Date and Time Test Terminated: August 4, 2015 at 1340

Dilution water used: Synthetic Moderately Hard Water #4236

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
A	25	29	28	32	28	26
B	29	24	28	34	33	29
C	28	29	29	24	27	32
D	24	35	26	33	30	32
E	0	0	0	0	0	0
F	30	32	34	32	29	29
G	27	36	31	36	30	35
H	29	36	32	34	34	31
I	29	29	28	30	33	29
J	31	33	35	36	34	28
Mean per Adult	25.2	28.3	27.1	29.1	27.8	27.1
Mean per Surviving Adult	25.2	28.3	27.1	29.1	27.8	27.1
CV %	36.2	37.6	36.7	37.1	36.3	36.3

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	(100 %)	<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	(100 %)	<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: 100 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 100 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 100 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 100 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 36.3 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Huntsville Water Utilities SAMPLE No. 1 COLLECTED ending: DATE: July 27, 2015 TIME: 0500
 NPDES NO.: AR0022004 AFIN# 44-00018 SAMPLE No. 2 COLLECTED ending: DATE: July 29, 2015 TIME: 0500
 CONTACT: Mr. Bill Eoff SAMPLE No. 3 COLLECTED ending: DATE: July 31, 2015 TIME: 0500
 ANALYST: 280, 304, 310, 314 Test Initiated: DATE: July 28, 2015 TIME: 1430
 Test Terminated: DATE: August 4, 2015 TIME: 1340

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.2	8.5	7.7	7.8	7.6	7.4
Final	7.6	7.8	7.7	7.9	7.8	8.1	8.1
pH Initial	8.5	7.9	8.0	8.2	7.9	8.0	7.9
Final	8.2	8.3	8.6	8.4	8.5	8.0	8.0
Alkalinity	60	NA	58	NA	58	NA	NA
Hardness	86	NA	83	NA	83	NA	NA
Conductivity	300	310	330	260	300	330	270
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 32 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	7.1	8.5	7.6	7.6	7.6	7.1
Final	7.5	7.8	7.6	8.6	7.9	8.1	8.0
pH Initial	7.9	7.9	7.8	8.2	7.9	8.2	8.0
Final	8.4	8.4	8.8	8.5	8.7	8.2	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	620	590	550	540	540	560	550
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 42 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.3	7.6	7.6	7.0	7.6	7.3
Final	7.5	7.8	7.8	8.5	7.5	8.0	8.0
pH Initial	8.6	7.9	7.8	8.3	7.9	8.3	8.0
Final	8.4	8.5	8.8	8.5	8.7	8.2	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	650	690	640	630	630	640	640
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 56 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.2	7.5	7.6	7.5	7.6	6.9
Final	7.1	7.8	7.6	8.5	7.4	7.8	7.9
pH Initial	8.6	7.9	7.8	8.3	8.0	8.4	8.0
Final	8.5	8.6	8.9	8.6	8.8	8.3	8.3
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	790	810	770	770	750	760	770
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 75 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	8.5	7.5	7.3	7.5	7.0
Final	7.5	7.7	7.7	8.5	7.3	7.9	8.0
pH Initial	8.6	8.0	7.7	8.3	7.9	8.4	8.1
Final	8.5	8.7	8.9	8.6	8.9	8.4	8.4
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	980	980	930	940	900	910	940
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 100 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.1	8.4	7.6	8.4	7.6	6.8
Final	7.5	7.7	7.6	8.4	7.4	8.0	8.0
pH Initial	8.0	7.7	7.7	8.3	7.8	8.4	8.1
Final	8.5	8.8	9.0	8.6	9.0	8.4	8.4
Alkalinity	150	NA	140	NA	160	NA	NA
Hardness	240	NA	230	NA	190	NA	NA
Conductivity	1200	1200	1100	1200	1100	1100	1200
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA



8600 Kanis Road
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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE OF

Client: <u>Huntsville Water Utilities</u>			PO No.		NO OF BOTTLES	Cd & Pb Chronic	ANALYSES REQUESTED										AIC CONTROL NO: <u>192741</u>			
Project Reference: <u>Bio Monitoring</u>			SAMPLE MATRIX				WATER	SOIL											AIC PROPOSAL NO:	
Project Manager: <u>Bill Eoff</u>			GRA B	COMP	X	X			3	X										
Sampled By: <u>Bill Eoff</u>																				
AIC No.	Sample Identification	Date/Time Collected											Remarks							
<u>1</u>	<u>Huntsville#1</u>	<u>7/26/15 @ 7:00</u> <u>7/27/15 @ 5:00</u>																		
													Field pH calibration on _____ @ _____							
Container Type <u>p</u>													Buffer:							
Preservative <u>4C</u>																				
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																				
Turnaround Time Requested: (Please circle) - NORMAL or EXPEDITED IN _____ DAYS			Relinquished By: <u>Bm</u>		Date/Time <u>7/27/15 @ 8:00</u>		Received By:						Date/Time							
Expedited results requested by: _____			Relinquished By:		Date/Time		Received in Lab By: <u>John DeG</u>						Date/Time <u>7/28/15</u> <u>08:00</u>							
Who should AIC contact with questions: <u>Bill Eoff</u>													Comments:							
Phone: <u>(479) - 738 - 2085</u> Fax: <u>(479) - 738 - 1285</u>													<u>FedEx # 801940810010</u>							
Report Attention to: <u>Bill Eoff</u>																				
Report Address to: <u>Bill Eoff</u> <u>Huntsville Water Utilities</u> <u>P.O. Box 430</u>																				



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: Huntsville Water Utilities			PO No.		NO OF BOTTLES	Cd & Pb Chronic	ANALYSES REQUESTED										AIC CONTROL NO: 192741							
Project Reference: Bio Monitoring			SAMPLE MATRIX				AIC PROPOSAL NO:												Carrier:					
Project Manager: Bill Eoff			GRA	COMP	WATER	SOIL	BOTTLES	Cd	Pb	Chronic											Received on Ice (4°C)?			
Sampled By: Bill Eoff																								
AIC No.	Sample Identification	Date/Time Collected																				Remarks	0.1°C	
2	Huntsville#2	7/28/15 @ 7:00 7/29/15 @ 5:00		X	X			3	X															
																				Field pH calibration				
																				on _____ @ _____				
																				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				
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS										Relinquished By: <i>BM</i>					Date/Time: 7/29/15 @ 8:00					Received By:				
Expedited results requested by: _____										Relinquished By:					Date/Time:					Received in Lab By: <i>D. Brown</i>				
Who should AIC contact with questions: Bill Eoff																				Date/Time: 7-30-15				
Phone: (479) - 738 - 208 Fax: (479) - 738 - 1285																				0830				
Report Attention to: Bill Eoff																								
Report Address to: Bill Eoff																								
Huntsville Water Utilities																								
P.O. Box 430																								
Comments:																				8019 4081 0021				



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE OF

Client: <u>Huntsville Water Utilities</u>			PO No.		NO OF BOTTLES	Cd & Pb Chronic	ANALYSES REQUESTED										AIC CONTROL NO: <u>101741</u>							
Project: <u>Bio Monitoring</u>			SAMPLE MATRIX				WATER	SOIL											AIC PROPOSAL NO:					
Manager: <u>Bill Eoff</u>			G R A B	C O M P	A T E R	S O I L			3	X											Carrier: <u>FedEx</u>			
Sampled By: <u>Bill Eoff</u>							Date/Time Collected														Received on Ice (4°C)? <u>YES</u> 2.2° NO			
AIC No.	Sample Identification	Date/Time Collected																						Remarks
<u>3</u>	<u>Huntsville #3</u>	<u>7/31/15 @ 7:00</u> <u>7/31/15 @ 5:00</u>		<u>X</u>	<u>X</u>																			
Field pH calibration on _____ @ _____ 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

Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS		Relinquished By: <u>BM</u>	Date/Time: <u>7/31/15 @ 8:00</u>	Received By:	Date/Time:
Expedited results requested by: _____		Relinquished By:	Date/Time:	Received in Lab By: <u>Shawn Worm</u>	Date/Time: <u>8-1-15 (0840)</u>
Who should AIC contact with questions: <u>Bill Eoff</u>		Comments:			
Phone: <u>(479) - 738 - 208</u> Fax: <u>(479) - 738 - 1285</u>					
Report Attention to: <u>Bill Eoff</u>					
Report Address to: <u>Bill Eoff</u> <u>Huntsville Water Utilities</u> <u>P.O. Box 430</u>					

September 3, 2015

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
Huntsville, AR

Control No. 193598-1

Prepared for:

Mr. Bill Eoff
Huntsville Water Utilities
Post Office Box 430
Huntsville, AR 72740

Prepared by:

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



Huntsville Water Utilities
ATTN: Mr. Bill Eoff
Post Office Box 430
Huntsville, AR 72740

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - Huntsville, AR
NPDES Permit No. AR0022004 AFIN# 44-00018

Dear Mr. Bill Eoff:

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 100 % effluent, which is equal to the critical dilution of 100 %. Any statistical difference with sublethal effects cannot be considered toxic due to the minimum significant difference (PMSD) calculated result being below the lower PMSD bounds. **The sample, therefore PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Laboratory Director

A handwritten signature in black ink is written over a horizontal line. Below the line, the name 'John Overbey' and title 'Laboratory Director' are printed.

PDF cc: Huntsville Water Utilities
ATTN: Mr. Bill Eoff
bill9eoff@hotmail.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	0.270	PASS
Control Growth CV < or = 40%	7.32	PASS
Growth Minimum Significant Difference 12 to 30%	11.3	BELOW
Critical Dilution CV < or = 40%	8.15	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0022004 AFIN# 44-00018
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 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.6	7.5	7.8
pH (standard units)	8.2	8.2	8.0
Alkalinity (mg/l as CaCO ₃)	130	120	120
Hardness (mg/l as CaCO ₃)	250	230	210
Conductivity (umhos/cm)	1300	1100	1100
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.44	1.2	2.7

2. Dilution Water Samples: Synthetic Moderate Hard Water #4247

- a. Dates Prepared: August 21 through September 4, 2015
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	8.0	7.9
pH (standard units)	8.5	8.3	8.2
Alkalinity (mg/l as CaCO ₃)	58	58	58
Hardness (mg/l as CaCO ₃)	80	80	80
Conductivity (umhos/cm)	270	270	260
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: August 25, 2015 at 1250
Date & Time Test Terminated: September 1, 2015 at 1345
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 August 24, 2015 at 1700 to August 31, 2015 at 1510

The results were as follows: (Control No. 193554-1.)

Survival LC-50: 3114 mg/l

Growth IC-25: 1530 mg/l

Growth PMSD: 16.9

V. Chemical Analysis/Quality Control

Parameter	Method	% Recovery	Relative % Difference
Alkalinity	SM 2320 B	NA	0.00
Hardness	EPA 200.7	96.1	1.11
pH	SM 4500-H+ B	101	0.134
Conductivity	EPA 120.1	107	0.639

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: August 25, 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

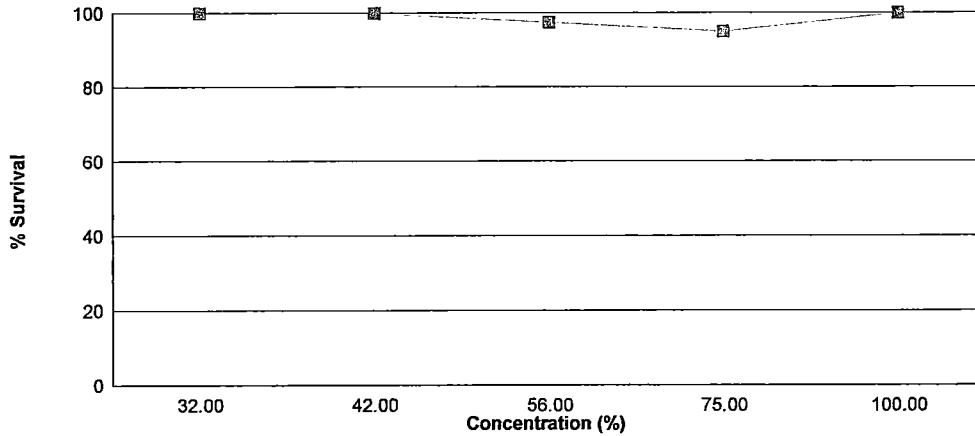
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on August 25, 2015 at 1250 and continued through September 1, 2015 at 1345. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 100 % effluent
- b.) NOEC growth = 100 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.270
32 %	100	0.249
42 %	100	0.270
56 %	97.5	0.269
75 %	95.0	0.243
100 %	100	0.263

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 25, 2015 at 1250
Date and Time Test Terminated: September 1, 2015 at 1345

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
32 %	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
42 %	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
56 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	7	7	7	7	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
75 %	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	7	7
	E	8	8	8	8	8	8	8
100 %	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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 25, 2015 at 1250
Test Terminated: September 1, 2015 at 1345

Drying Started: August 31, 2015 at 1020
Drying Ended: September 2, 2015 at 1025

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94324	.94530	0.00206	8	0.258
	B	.93619	.93840	0.00221	8	0.276
	C	.93874	.94089	0.00215	8	0.269
	D	.94667	.94865	0.00198	8	0.248
	E	.94586	.94826	0.00240	8	0.300
32 %	A	.94654	.94834	0.00180	8	0.225
	B	.94760	.94976	0.00216	8	0.270
	C	.94058	.94261	0.00203	8	0.254
	D	.94454	.94658	0.00204	8	0.255
	E	.93993	.94184	0.00191	8	0.239
42 %	A	.93644	.93849	0.00205	8	0.256
	B	.94208	.94420	0.00212	8	0.265
	C	.93616	.93814	0.00198	8	0.248
	D	.93860	.94083	0.00223	8	0.279
	E	.93650	.93890	0.00240	8	0.300
56 %	A	.93829	.94047	0.00218	8	0.272
	B	.93632	.93876	0.00244	8	0.305
	C	.93751	.93934	0.00183	8	0.229
	D	.94277	.94484	0.00207	8	0.259
	E	.94460	.94685	0.00225	8	0.281
75 %	A	.94613	.94801	0.00188	8	0.235
	B	.94291	.94477	0.00186	8	0.232
	C	.94510	.94698	0.00188	8	0.235
	D	.94405	.94607	0.00202	8	0.252
	E	.94596	.94806	0.00210	8	0.262
100 %	A	.94624	.94819	0.00195	8	0.244
	B	.94424	.94645	0.00221	8	0.276
	C	.94556	.94779	0.00223	8	0.279
	D	.94534	.94723	0.00189	8	0.236
	E	.94520	.94745	0.00225	8	0.281

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	32 %	1	1.00000	1.39310
2	32 %	2	1.00000	1.39310
2	32 %	3	1.00000	1.39310
2	32 %	4	1.00000	1.39310
2	32 %	5	1.00000	1.39310
3	42 %	1	1.00000	1.39310
3	42 %	2	1.00000	1.39310
3	42 %	3	1.00000	1.39310
3	42 %	4	1.00000	1.39310
3	42 %	5	1.00000	1.39310
4	56 %	1	1.00000	1.39310
4	56 %	2	1.00000	1.39310
4	56 %	3	0.87500	1.20940
4	56 %	4	1.00000	1.39310
4	56 %	5	1.00000	1.39310
5	75 %	1	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	0.87500	1.20940
5	75 %	4	0.87500	1.20940
5	75 %	5	1.00000	1.39310
6	100 %	1	1.00000	1.39310
6	100 %	2	1.00000	1.39310
6	100 %	3	1.00000	1.39310
6	100 %	4	1.00000	1.39310
6	100 %	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 = 0.06749 W = 0.7138 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	32 %	27.50	16.00	5.00	
3	42 %	27.50	16.00	5.00	
4	56 %	25.00	16.00	5.00	
5	75 %	22.50	16.00	5.00	
6	100 %	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 = 0.01011 W = 0.9828 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 = 2.220 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	0.003503	0.0007006	1.663	
Within (Error)	24	0.01011	0.0004212		
Total	29	0.01361			
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	0.2702	0.2702			
2	32 %	0.2486	0.2486	1.664		
3	42 %	0.2696	0.2696	0.04623		
4	56 %	0.2692	0.2692	0.07704		
5	75 %	0.2432	0.2432	2.08		
6	100 %	0.2632	0.2632	0.5393		
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	32 %	5	0.03063	11.3	0.0216		
3	42 %	5	0.03063	11.3	0.0006		
4	56 %	5	0.03063	11.3	0.001		
5	75 %	5	0.03063	11.3	0.027		
6	100 %	5	0.03063	11.3	0.007		

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 25, 2015 at 0909
Date and Time Test Terminated: September 1, 2015 at 1345

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.0	7.7	8.0	7.7	7.9	8.0	7.9
	Final	8.2	7.8	7.4	7.6	7.3	7.5	7.2
pH, units	Initial	8.5	8.1	8.3	8.0	8.2	8.0	7.9
	Final	7.9	7.9	7.7	7.7	8.1	8.0	7.9
Alkalinity, mg CaCO ₃ /l		58	NA	58	NA	58	NA	NA
Hardness, mg CaCO ₃ /l		80	NA	80	NA	80	NA	NA
Conductivity, umhos/cm		270	280	270	320	260	270	270
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 32 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.6	7.8	7.9	7.5	7.7	7.8	7.6
	Final	8.2	7.2	6.9	7.2	7.6	7.1	7.2
pH, units	Initial	8.3	8.2	8.3	8.0	8.1	8.1	7.8
	Final	8.0	8.0	7.8	7.7	8.3	8.0	7.9

Effluent Conc.: 42 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.5	7.8	7.9	7.0	7.9	7.8	7.7
	Final	8.2	7.4	7.1	7.3	7.2	7.4	7.2
pH, units	Initial	8.3	8.3	8.2	7.9	8.1	8.1	7.9
	Final	8.0	8.1	8.0	7.8	8.3	8.1	8.0

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: August 25, 2015 at 0909
Date and Time Test Terminated: September 1, 2015 at 1345

Effluent Conc.: 56 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.8	7.8	7.5	7.8	7.8	7.8
	Final	9.4	7.2	7.1	7.2	7.5	7.2	7.2
pH, units	Initial	8.3	8.3	8.2	8.0	8.1	8.2	7.9
	Final	7.4	8.1	8.0	7.8	8.3	8.2	8.1

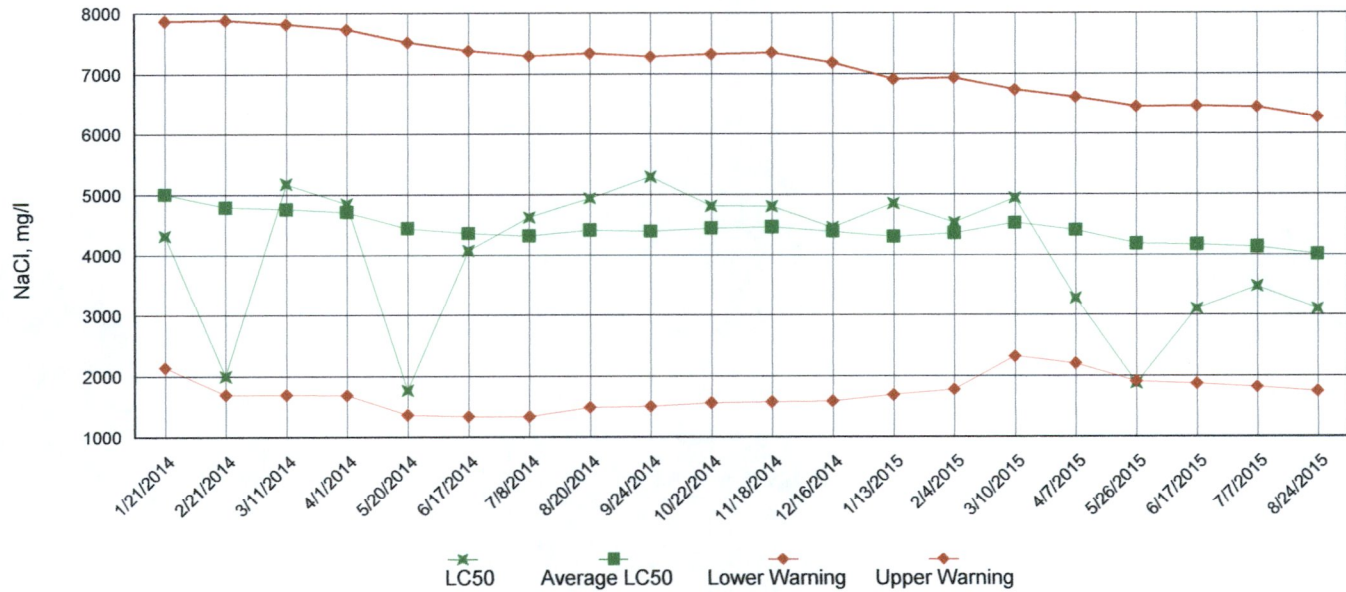
Effluent Conc.: 75 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.7	7.8	7.9	7.1	8.0	7.9	7.8
	Final	9.4	7.5	7.4	7.4	7.4	7.2	7.1
pH, units	Initial	8.2	8.2	8.2	8.0	8.1	8.2	7.9
	Final	8.1	8.2	8.1	8.0	8.4	8.3	8.2

Effluent Conc.: 100 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	7.6	7.7	7.5	7.0	7.8	7.5	7.3
	Final	9.3	7.6	7.1	7.4	7.4	7.4	7.3
pH, units	Initial	8.2	8.1	8.2	7.8	8.0	8.3	7.8
	Final	8.1	8.4	8.2	8.0	8.4	8.3	8.3
Alkalinity, mg CaCO ₃ /l		130	NA	120	NA	120	NA	NA
Hardness, mg CaCO ₃ /l		250	NA	230	NA	210	NA	NA
Conductivity, umhos/cm		1300	1300	1100	1100	1100	1100	1100
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

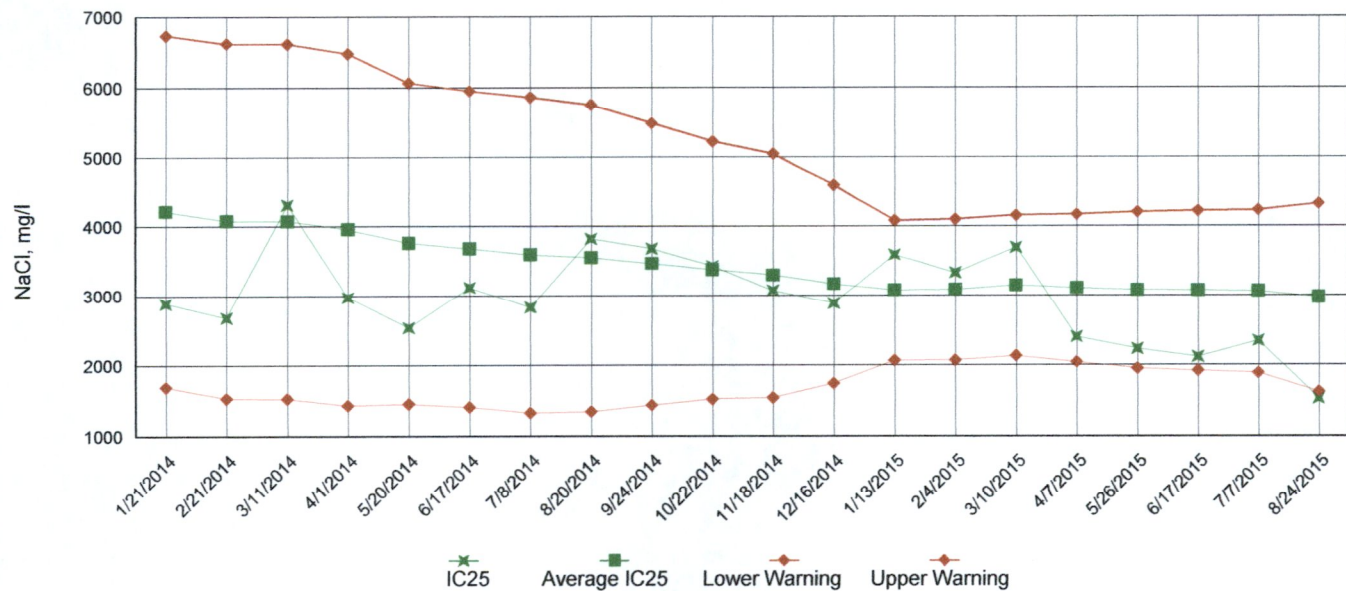
Appendix A4: Test 1000.0

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: Huntsville Water Utilities

NPDES No.: AR0022004 AFIN# 44-00018

Date and Time Test Initiated: August 25, 2015 at 1250

Date and Time Test Terminated: September 1, 2015 at 1345

Dilution water used: Synthetic Moderate Hard Water #4247

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
32 %	100	100	100	100	100	100	100	100	0.00
42 %	100	100	100	100	100	100	100	100	0.00
56 %	100	100	87.5	100	100	97.5	97.5	97.5	5.73
75 %	100	100	87.5	87.5	100	100	100	95.0	7.21
100 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.258	0.276	0.269	0.248	0.300	0.27	7.32
32 %	0.225	0.270	0.254	0.255	0.239	0.249	6.90
42 %	0.256	0.265	0.248	0.279	0.300	0.27	7.61
56 %	0.272	0.305	0.229	0.259	0.281	0.269	10.4
75 %	0.235	0.232	0.235	0.252	0.262	0.243	5.40
100 %	0.244	0.276	0.279	0.236	0.281	0.263	8.15

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	(100 %)	<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	(100 %)	<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: 100 % (TOP6C)
6. LOEC Pimephales Lethality: 100 % (TXP6C)
7. NOEC Pimephales Sublethality: 100 % (TPP6C)
8. LOEC Pimephales Sublethality: 100 % (TYP6C)
9. Coefficient of variation for Pimephales growth: 8.15 (TQP6C)

Appendix B: Test 1000.0

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

PERMITTEE: Huntsville Water Utilities SAMPLE No. 1 COLLECTED ending: DATE: August 24, 2015 TIME: 0500
 NPDES NO.: AR0022004 AFIN# 44-00018 SAMPLE No. 2 COLLECTED ending: DATE: August 26, 2015 TIME: 0500
 CONTACT: Mr. Bill Eoff SAMPLE No. 3 COLLECTED ending: DATE: August 28, 2015 TIME: 0500
 ANALYST: 280, 304, 310, 314 Test Initiated: DATE: August 25, 2015 TIME: 1250
 Test Terminated: DATE: September 1, 2015 TIME: 1345

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.0	7.7	8.0	7.7	7.9	8.0	7.9
Final	8.2	7.8	7.4	7.6	7.3	7.5	7.2
pH Initial	8.5	8.1	8.3	8.0	8.2	8.0	7.9
Final	7.9	7.9	7.7	7.7	8.1	8.0	7.9
Alkalinity	58	NA	58	NA	58	NA	NA
Hardness	80	NA	80	NA	80	NA	NA
Conductivity	270	280	270	320	260	270	270
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	7.6	7.8	7.9	7.5	7.7	7.8	7.6
Final	8.2	7.2	6.9	7.2	7.6	7.1	7.2
pH Initial	8.3	8.2	8.3	8.0	8.1	8.1	7.8
Final	8.0	8.0	7.8	7.7	8.3	8.0	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	570	580	550	580	530	550	540
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	7.5	7.8	7.9	7.0	7.9	7.8	7.7
Final	8.2	7.4	7.1	7.3	7.2	7.4	7.2
pH Initial	8.3	8.3	8.2	7.9	8.1	8.1	7.9
Final	8.0	8.1	8.0	7.8	8.3	8.1	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	670	680	620	660	590	610	610
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	7.7	7.8	7.8	7.5	7.8	7.8	7.8
Final	9.4	7.2	7.1	7.2	7.5	7.2	7.2
pH Initial	8.3	8.3	8.2	8.0	8.1	8.2	7.9
Final	7.4	8.1	8.0	7.8	8.3	8.2	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	810	820	730	780	710	740	720
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
75 %							
D.O. Initial	7.7	7.8	7.9	7.1	8.0	7.9	7.8
Final	9.4	7.5	7.4	7.4	7.4	7.2	7.1
pH Initial	8.2	8.2	8.2	8.0	8.1	8.2	7.9
Final	8.1	8.2	8.1	8.0	8.4	8.3	8.2
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	1000	1000	890	930	870	890	890
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	7.6	7.7	7.5	7.0	7.8	7.5	7.3
Final	9.3	7.6	7.1	7.4	7.4	7.4	7.3
pH Initial	8.2	8.1	8.2	7.8	8.0	8.3	7.8
Final	8.1	8.4	8.2	8.0	8.4	8.3	8.3
Alkalinity	130	NA	120	NA	120	NA	NA
Hardness	250	NA	230	NA	210	NA	NA
Conductivity	1300	1300	1100	1100	1100	1100	1100
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA



8600 Kanis Road
 Little Rock, AR 72204-2322
 (501) 224-5060
 FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

Client: Huntsville Water Utilities			PO No.		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 103598						
Project Reference: Bio Monitoring			SAMPLE MATRIX			Chronic ONLY											AIC PROPOSAL NO:					
Project Manager: Bill Eoff			GRA	COMP	WATER		SOIL	BOTTLES											Carrier: <u>FedEx</u>			
Sampled By: Bill Eoff																Received on Ice (4°C)? (YES) 0.1 NO						
AIC No.	Sample Identification	Date/Time Collected																			Remarks	
	Huntsville#1	8-23-15 @ 7:00 - 8:45 C SWD		X	X			3	X													
																		Field pH calibration on _____ @ _____ 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																						
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS												Relinquished By: <u>BM</u>		Date/Time: <u>8/24/15 @ 8:00</u>		Received By:		Date/Time:				
Expedited results requested by: _____												Relinquished By:		Date/Time:		Received in Lab By: <u>[Signature]</u>		Date/Time: <u>8/25/15 0840</u>				
Who should AIC contact with questions: <u>Bill Eoff</u>												Comments: <u>FedEx # 801940810113</u>										
Phone: <u>(479) - 738 - 208</u> Fax: <u>(479) - 738 - 1285</u>																						
Report Attention to: <u>Bill Eoff</u>																						
Report Address to: <u>Bill Eoff</u> <u>Huntsville Water Utilities</u> <u>P.O. Box 430</u>																						



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: <u>Huntsville Water Utilities</u>			PO No.		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>193598</u>	
Project Reference: <u>Bio Monitoring</u>			SAMPLE MATRIX			Chronic ONLY											AIC PROPOSAL NO:
Project Manager: <u>Bill Eoff</u>			WATER	SOIL	BOTTLES												Carrier: <u>FedX</u>
Sampled By: <u>Bill Eoff</u>						GRAB	COMP										
AIC No.	Sample Identification	Date/Time Collected															
	<u>Huntsville#2</u>	<u>8-25-15 0730-8-26-15 0850</u>			<u>3</u>												
															Field pH calibration		
															on _____ @ _____		
															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																	
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS					Relinquished By: <u>BM</u>		Date/Time: <u>8/26/15 08:00</u>		Received By:			Date/Time:					
Expedited results requested by: _____					Relinquished By:		Date/Time:		Received in Lab By: <u>D. Brown</u>			Date/Time: <u>8-27-15 0810</u>					
Who should AIC contact with questions: <u>Bill Eoff</u>					Comments:										8019 4081 0124		
Phone: <u>(479) - 738 - 208</u> Fax: <u>(479) - 738 - 1285</u>																	
Report Attention to: <u>Bill Eoff</u>																	
Report Address to: <u>Bill Eoff</u> <u>Huntsville Water Utilities</u> <u>P.O. Box 430</u>																	



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 3 OF 3

Client: Huntsville Water Utilities			PO No.		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 193598					
Project Reference: Bio Monitoring			SAMPLE MATRIX			Fh Chronic ONLY													AIC PROPOSAL NO:		
Project Manager: Bill Eoff			G R A B	C O M P	W A T E R		S O I L											Carrier: Fed Ex			
Sampled By: Bill Eoff																		Received on Ice (4°C)? <u>YES</u> 0-8° NO			
AIC No.	Sample Identification	Date/Time Collected															Remarks				
3	Huntsville#3	8-27-15 08:00 8-28-15 05:00		X	X																
			Container Type P														Field pH calibration on @ Buffer:				
			Preservative 4C																		
			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																		
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN ___ DAYS						Relinquished By: <u>BM</u>		Date/Time: <u>8/28/15 08:00</u>		Received By:		Date/Time:									
Expedited results requested by:						Relinquished By:		Date/Time:		Received in Lab By: <u>Shawn Wor</u>		Date/Time: <u>8-29-15 (0800)</u>									
Who should AIC contact with questions: <u>Bill Eoff</u>						Comments:															
Phone: <u>(479) - 738 - 208</u> Fax: <u>(479) - 738 - 1285</u>																					
Report Attention to: <u>Bill Eoff</u>																					
Report Address to: <u>Bill Eoff</u> <u>Huntsville Water Utilities</u> <u>P.O. Box 430</u>																					

September 24, 2015

Test Results of
Third Quarter
Chronic 7-Day Renewal
Biomonitoring Testing
for
Outfall 001
Huntsville, AR

Control No. 194202-1

Prepared for:

Mr. Bill Eoff
Huntsville Water Utilities
Post Office Box 430
Huntsville, AR 72740

Prepared by:

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

Huntsville Water Utilities
ATTN: Mr. Bill Eoff
Post Office Box 430
Huntsville, AR 72740

Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow)
Outfall 001 - Huntsville, AR
NPDES Permit No. AR0022004 AFIN# 44-00018

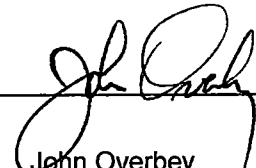
Dear Mr. Bill Eoff:

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 100 % effluent, which is equal to the critical dilution of 100 %. The NOEC for growth occurred at 100 % effluent, which is equal to the critical dilution of 100 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
Laboratory Director

PDF cc: Huntsville Water Utilities
ATTN: Mr. Bill Eoff
bill9eoff@hotmail.com

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- VI. Organism History
- VII. Results Summary
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 - Pimephales promelas* (Fathead minnow) Survival and Growth
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 - 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	0.295	PASS
Control Growth CV < or = 40%	4.14	PASS
Growth Minimum Significant Difference 12 to 30%	15.6	PASS
Critical Dilution CV < or = 40%	11.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0022004 AFIN# 44-00018
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 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.3	6.0	7.4
pH (standard units)	8.1	8.1	7.9
Alkalinity (mg/l as CaCO ₃)	130	140	130
Hardness (mg/l as CaCO ₃)	250	260	230
Conductivity (umhos/cm)	1400	1500	1400
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	1.1	0.46	0.39

2. Dilution Water Samples: Synthetic Moderately Hard Water #4251
 - a. Dates Prepared: September 4 through September 18, 2015
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.7	7.5	7.4
pH (standard units)	8.2	8.0	8.1
Alkalinity (mg/l as CaCO ₃)	62	62	62
Hardness (mg/l as CaCO ₃)	86	86	86
Conductivity (umhos/cm)	270	340	340
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 15, 2015 at 1400
Date & Time Test Terminated:	September 22, 2015 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

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	2.15
Hardness	EPA 200.7	97.4	1.59
pH	SM 4500-H+ B	101	0.134
Conductivity	EPA 120.1	100	5.56

VI. Organism History

Pimephales promelas (Fathead minnow)

Date: September 15, 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

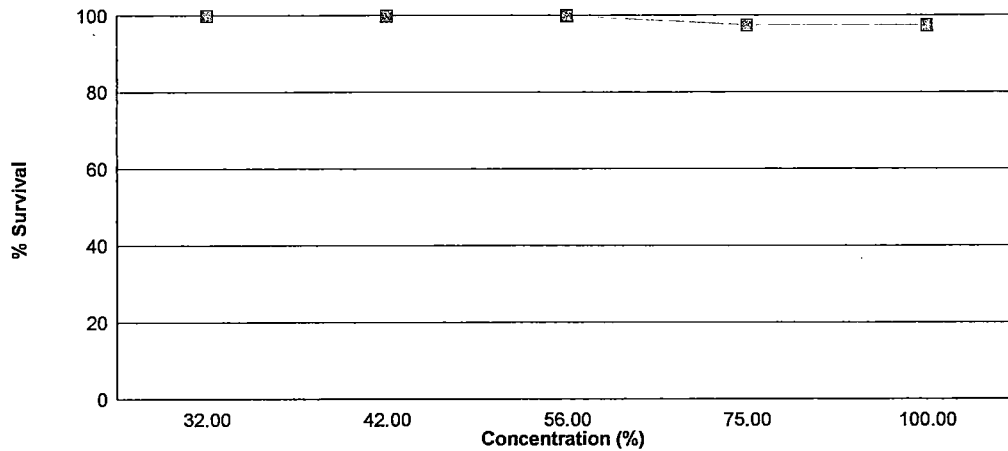
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (increase in weight) of the larvae.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on September 15, 2015 at 1400 and continued through September 22, 2015 at 1400. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 100 % effluent
- b.) NOEC growth = 100 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.295
32 %	100	0.280
42 %	100	0.255
56 %	100	0.276
75 %	97.5	0.286
100 %	97.5	0.312

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: September 15, 2015 at 1400

Date and Time Test Terminated: September 22, 2015 at 1400

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
32 %	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
42 %	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
56 %	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
75 %	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	7	7
100 %	A	8	8	8	8	8	7	7
	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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: September 15, 2015 at 1400
Test Terminated: September 22, 2015 at 1400

Drying Started: September 18, 2015 at 1315
Drying Ended: September 23, 2015 at 1415

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94260	.94508	0.00248	8	0.310
	B	.94097	.94342	0.00245	8	0.306
	C	.94393	.94623	0.00230	8	0.288
	D	.94647	.94875	0.00228	8	0.285
	E	.94403	.94631	0.00228	8	0.285
32 %	A	.94417	.94671	0.00254	8	0.318
	B	.94328	.94531	0.00203	8	0.254
	C	.94366	.94606	0.00240	8	0.300
	D	.94168	.94389	0.00221	8	0.276
	E	.94456	.94659	0.00203	8	0.254
42 %	A	.94562	.94765	0.00203	8	0.254
	B	.94443	.94625	0.00182	8	0.228
	C	.94248	.94447	0.00199	8	0.249
	D	.94527	.94747	0.00220	8	0.275
	E	.94477	.94693	0.00216	8	0.270
56 %	A	.95056	.95293	0.00237	8	0.296
	B	.94087	.94297	0.00210	8	0.262
	C	.94879	.95082	0.00203	8	0.254
	D	.94879	.95109	0.00230	8	0.288
	E	.94553	.94775	0.00222	8	0.278
75 %	A	.94401	.94644	0.00243	8	0.304
	B	.95085	.95338	0.00253	8	0.316
	C	.95105	.95266	0.00161	8	0.201
	D	.94245	.94465	0.00220	8	0.275
	E	.94870	.95137	0.00267	8	0.334
100 %	A	.94581	.94779	0.00198	8	0.248
	B	.94420	.94688	0.00268	8	0.335
	C	.93965	.94220	0.00255	8	0.319
	D	.94120	.94374	0.00254	8	0.318
	E	.94573	.94846	0.00273	8	0.341

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	32 %	1	1.00000	1.39310
2	32 %	2	1.00000	1.39310
2	32 %	3	1.00000	1.39310
2	32 %	4	1.00000	1.39310
2	32 %	5	1.00000	1.39310
3	42 %	1	1.00000	1.39310
3	42 %	2	1.00000	1.39310
3	42 %	3	1.00000	1.39310
3	42 %	4	1.00000	1.39310
3	42 %	5	1.00000	1.39310
4	56 %	1	1.00000	1.39310
4	56 %	2	1.00000	1.39310
4	56 %	3	1.00000	1.39310
4	56 %	4	1.00000	1.39310
4	56 %	5	1.00000	1.39310
5	75 %	1	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	1.00000	1.39310
5	75 %	4	1.00000	1.39310
5	75 %	5	0.87500	1.20940
6	100 %	1	0.87500	1.20940
6	100 %	2	1.00000	1.39310
6	100 %	3	1.00000	1.39310
6	100 %	4	1.00000	1.39310
6	100 %	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 = 0.05399 W = 0.5466 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	32 %	27.50	16.00	5.00	
3	42 %	27.50	16.00	5.00	
4	56 %	27.50	16.00	5.00	
5	75 %	25.00	16.00	5.00	
6	100 %	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 = 0.02285 W = 0.9262 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 = 9.992 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	0.009144	0.001829	1.921	
Within (Error)	24	0.02285	0.0009521		
Total	29	0.03199			
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	0.2948	0.2948			
2	32 %	0.2804	0.2804	0.7379		
3	42 %	0.2552	0.2552	2.029		
4	56 %	0.2756	0.2756	0.9839		
5	75 %	0.286	0.286	0.4509		
6	100 %	0.3122	0.3122	-0.8916		
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	32 %	5	0.04606	15.6	0.0144	
3	42 %	5	0.04606	15.6	0.0396	
4	56 %	5	0.04606	15.6	0.0192	
5	75 %	5	0.04606	15.6	0.0088	
6	100 %	5	0.04606	15.6	-0.0174	

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 15, 2015 at 0935

Date and Time Test Terminated: September 22, 2015 at 1400

Effluent Conc.: Control		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.7	7.8	7.5	8.6	7.4	7.2	7.6
	Final	7.7	7.4	7.4	7.4	7.6	7.6	7.0
pH, units	Initial	8.2	7.8	8.0	8.0	8.1	8.3	8.0
	Final	7.7	7.7	7.7	7.9	8.1	7.8	7.7
Alkalinity, mg CaCO ₃ /l		62	NA	62	NA	62	NA	NA
Hardness, mg CaCO ₃ /l		86	NA	86	NA	86	NA	NA
Conductivity, umhos/cm		270	320	340	320	340	380	300
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

Effluent Conc.: 32 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.7	7.5	8.3	7.3	7.0	7.6
	Final	7.5	7.3	7.1	7.3	7.5	7.3	7.1
pH, units	Initial	8.1	7.8	8.0	7.9	8.0	8.4	8.0
	Final	7.9	7.8	7.9	8.0	8.2	7.9	7.9

Effluent Conc.: 42 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.5	7.6	8.4	7.7	7.4	7.4	8.6
	Final	7.5	7.4	7.2	7.2	7.5	7.2	7.1
pH, units	Initial	8.1	7.8	8.0	8.0	8.0	8.4	8.0
	Final	7.9	7.9	8.0	8.1	8.3	8.0	7.9

Appendix A3: Water Chemistry

Routine Chemical and Physical Data

Date and Time Test Initiated: September 15, 2015 at 0935

Date and Time Test Terminated: September 22, 2015 at 1400

Effluent Conc.: 56 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.4	7.6	8.2	7.4	7.4	7.1	8.6
	Final	7.6	7.2	7.2	7.3	7.5	7.3	7.4
pH, units	Initial	8.2	7.8	8.1	7.9	8.0	8.4	8.0
	Final	8.1	8.0	8.0	8.1	8.3	8.0	8.1

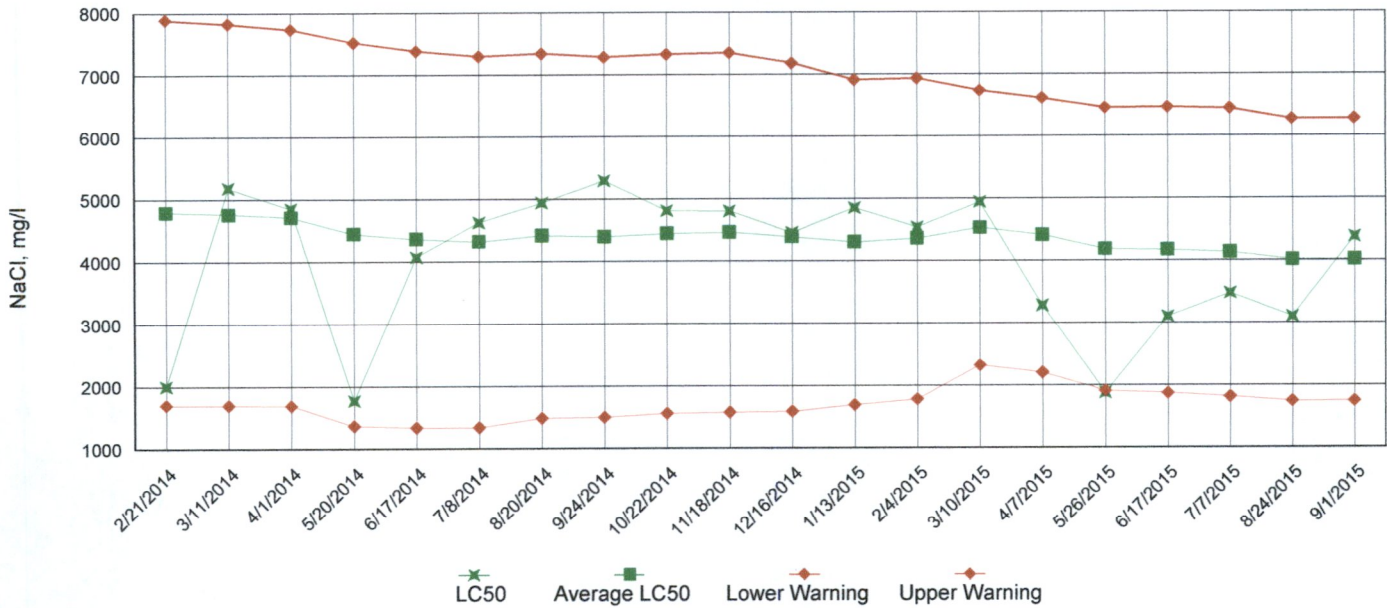
Effluent Conc.: 75 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.4	7.6	8.2	7.4	7.2	7.4	8.6
	Final	7.4	7.2	7.2	7.1	7.4	7.6	6.7
pH, units	Initial	8.2	7.8	8.1	8.0	8.0	8.5	8.0
	Final	8.1	8.0	8.1	8.1	8.4	8.2	8.0

Effluent Conc.: 100 %		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
DO, mg/l	Initial	8.3	7.5	6.0	7.5	7.4	7.4	8.5
	Final	7.6	7.2	7.4	7.2	8.2	7.5	7.0
pH, units	Initial	8.1	7.7	8.1	8.1	7.9	8.5	8.0
	Final	8.2	8.2	8.2	8.2	8.5	8.2	8.2
Alkalinity, mg CaCO ₃ /l		130	NA	140	NA	130	NA	NA
Hardness, mg CaCO ₃ /l		250	NA	260	NA	230	NA	NA
Conductivity, umhos/cm		1400	1400	1500	1500	1400	1500	1400
Res. Chlorine, mg/l		<0.05	NA	<0.05	NA	<0.05	NA	NA

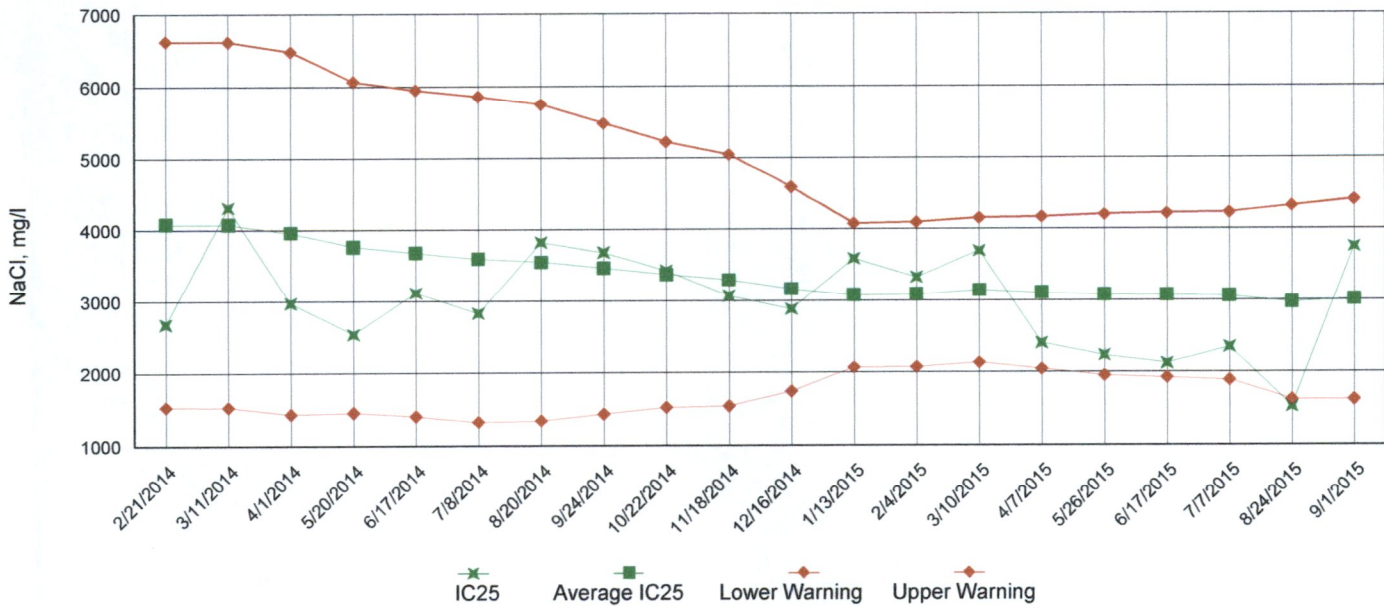
Appendix A4: Test 1000.0

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: Huntsville Water Utilities

NPDES No.: AR0022004 AFIN# 44-00018

Date and Time Test Initiated: September 15, 2015 at 1400

Date and Time Test Terminated: September 22, 2015 at 1400

Dilution water used: Synthetic Moderately Hard Water #4251

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
32 %	100	100	100	100	100	100	100	100	0.00
42 %	100	100	100	100	100	100	100	100	0.00
56 %	100	100	100	100	100	100	100	100	0.00
75 %	100	100	100	100	87.5	100	100	97.5	5.73
100 %	87.5	100	100	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	0.310	0.306	0.288	0.285	0.285	0.295	4.14
32 %	0.318	0.254	0.300	0.276	0.254	0.28	10.1
42 %	0.254	0.228	0.249	0.275	0.270	0.255	7.31
56 %	0.296	0.262	0.254	0.288	0.278	0.276	6.36
75 %	0.304	0.316	0.201	0.275	0.334	0.286	18.2
100 %	0.248	0.335	0.319	0.318	0.341	0.312	11.9

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	(100 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> 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	(100 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> 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: 100 % (TOP6C)
6. LOEC *Pimephales* Lethality: 100 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 100 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 100 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 11.9 (TQP6C)

Appendix B: Test 1000.0

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

PERMITTEE: Huntsville Water Utilities SAMPLE No. 1 COLLECTED ending: DATE: September 14, 2015 TIME: 0500
 NPDES NO.: AR0022004 AFIN# 44-00018 SAMPLE No. 2 COLLECTED ending: DATE: September 16, 2015 TIME: 0500
 CONTACT: Mr. Bill Eoff SAMPLE No. 3 COLLECTED ending: DATE: September 18, 2015 TIME: 0500
 ANALYST: 280, 304, 310, 314 Test Initiated: DATE: September 15, 2015 TIME: 1400
 Test Terminated: DATE: September 22, 2015 TIME: 1400

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.7	7.8	7.5	8.6	7.4	7.2	7.6
Final	7.7	7.4	7.4	7.4	7.6	7.6	7.0
pH Initial	8.2	7.8	8.0	8.0	8.1	8.3	8.0
Final	7.7	7.7	7.7	7.9	8.1	7.8	7.7
Alkalinity	62	NA	62	NA	62	NA	NA
Hardness	86	NA	86	NA	86	NA	NA
Conductivity	270	320	340	320	340	380	300
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA

DILUTION 32 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.5	7.7	7.5	8.3	7.3	7.0	7.6
Final	7.5	7.3	7.1	7.3	7.5	7.3	7.1
pH Initial	8.1	7.8	8.0	7.9	8.0	8.4	8.0
Final	7.9	7.8	7.9	8.0	8.2	7.9	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	610	650	710	690	690	720	680
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 42 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.5	7.6	8.4	7.7	7.4	7.4	8.6
Final	7.5	7.4	7.2	7.2	7.5	7.2	7.1
pH Initial	8.1	7.8	8.0	8.0	8.0	8.4	8.0
Final	7.9	7.9	8.0	8.1	8.3	8.0	7.9
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	720	750	820	810	800	840	790
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 56 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.4	7.6	8.2	7.4	7.4	7.1	8.6
Final	7.6	7.2	7.2	7.3	7.5	7.3	7.4
pH Initial	8.2	7.8	8.1	7.9	8.0	8.4	8.0
Final	8.1	8.0	8.0	8.1	8.3	8.0	8.1
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	870	890	980	970	960	1000	940
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 75 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.4	7.6	8.2	7.4	7.2	7.4	8.6
Final	7.4	7.2	7.2	7.1	7.4	7.6	6.7
pH Initial	8.2	7.8	8.1	8.0	8.0	8.5	8.0
Final	8.1	8.0	8.1	8.1	8.4	8.2	8.0
Alkalinity	NA	NA	NA	NA	NA	NA	NA
Hardness	NA	NA	NA	NA	NA	NA	NA
Conductivity	1100	1100	1200	1200	1200	1200	1200
Chlorine	NA	NA	NA	NA	NA	NA	NA

DILUTION 100 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	7.5	6.0	7.5	7.4	7.4	8.5
Final	7.6	7.2	7.4	7.2	8.2	7.5	7.0
pH Initial	8.1	7.7	8.1	8.1	7.9	8.5	8.0
Final	8.2	8.2	8.2	8.2	8.5	8.2	8.2
Alkalinity	130	NA	140	NA	130	NA	NA
Hardness	250	NA	260	NA	230	NA	NA
Conductivity	1400	1400	1500	1500	1400	1500	1400
Chlorine	<0.05	NA	<0.05	NA	<0.05	NA	NA



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 (501) 224-5060
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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

Client: <u>Huntsville Water Utilities</u>			PO No.		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: <u>194202</u>						
Project Reference: <u>Bio Monitoring</u>			SAMPLE MATRIX			Chronic <u>only</u>													AIC PROPOSAL NO:			
Project Manager: <u>Bill Eoff</u>			WATER	SOIL	3		X												Carrier: <u>Fed X</u>			
Sampled By: <u>Bill Eoff</u>						GRAB		COMP											Received on Ice (4°C)? <u>YES</u> NO			
AIC No.	Sample Identification	Date/Time Collected																		Remarks <u>0.1°</u>		
<u>1</u>	<u>Huntsville#1</u>	<u>9-13-15 9:00</u> <u>9-14-15 5:00</u>		X																		
			Container Type <u>p</u>												Field pH calibration on _____ @ _____							
			Preservative <u>4C</u>												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																			
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS					Relinquished By: <u>[Signature]</u>		Date/Time: <u>9-14-15 8:00</u>		Received By:		Date/Time:											
Expedited results requested by: _____					Relinquished By:		Date/Time:		Received in Lab By: <u>D. Brown</u>		Date/Time: <u>9-15-15 0815</u>											
Who should AIC contact with questions: <u>Bill Eoff</u>					Comments:																	
Phone: <u>(479) - 738 - 208</u> Fax: <u>(479) - 738 - 1285</u>																						
Report Attention to: <u>Bill Eoff</u>																						
Report Address to: <u>Bill Eoff</u> <u>Huntsville Water Utilities</u> <u>P.O. Box 430</u>																						

8019 4081 0157



8600 Karis Road
 Little Rock, AR 72204-2322
 (501) 224-5060
 FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: Huntsville Water Utilities			PO No.		NO OF BOTTLES	ANALYSES REQUESTED										AIC CONTROL NO: 194202			
Project Reference: Bio Monitoring			SAMPLE MATRIX			Fh Chronic Only											AIC PROPOSAL NO:		
Project Manager: Bill Eoff			GRA	COMP	WATER		SOIL	3											Carrier: FedEx
Sampled By: Bill Eoff																Received on Ice (4°C)? YES NO			
AIC No.	Sample Identification	Date/Time Collected																Remarks	
2	Huntsville#2	9-15-15 8:30 9-16-15 5:30		X	X													0.1	
Field-pH calibration on _____ @ _____ Buffer:																			
Container Type p			Preservative 4C																
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							
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS							Relinquished By: <i>BM</i>		Date/Time: 9-16-15 8:00		Received By:		Date/Time:						
Expedited results requested by: _____							Relinquished By:		Date/Time:		Received in Lab By: <i>D. Brown</i>		Date/Time: 9-17-15 0800						
Who should AIC contact with questions: Bill Eoff							Comments:												
Phone: (479) - 738 - 208 Fax: (479) - 738 - 1285																			
Report Attention to: Bill Eoff																			
Report Address to: Bill Eoff Huntsville Water Utilities P.O. Box 430																			

Littleville Water Utilities
Box 430
Littleville, AR 72740

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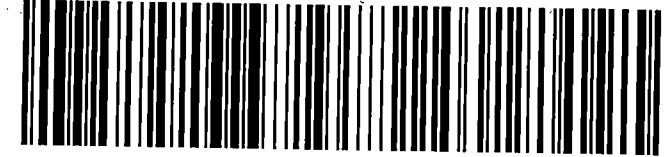
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North Little Rock, AR ~~72118~~ 5317
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