



November 30, 2020

Biomonitoring Testing  
for  
Plant Effluent  
City of Hot Springs

Control No. 250354-1

Prepared for:

Mr. Harold Mauldin  
City of Hot Springs  
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Prepared by:

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City of Hot Springs  
ATTN: Mr. Harold Mauldin  
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Re: Chronic 7-Day Renewal *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*  
Plant Effluent - City of Hot Springs  
NPDES Permit No. AR0033880 AFIN#26-00145

Dear Mr. Harold Mauldin:


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 Chief Operating Officer 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 77 % effluent, which is above the critical dilution of 58 %. The NOEC for growth occurred at 77 % effluent, which is above the critical dilution of 58 %. **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 77 % effluent, which is above the critical dilution of 58 %. The NOEC for reproduction occurred at 77 % effluent, which is above the critical dilution of 58 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

**AMERICAN INTERPLEX CORPORATION**

  
\_\_\_\_\_  
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Chief Operating Officer

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I. Control Acceptance Criteria

*Pimephales promelas* (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.468	PASS
Control Growth CV < or = 40%	7.83	PASS
Growth Minimum Significant Difference 12 to 30%	16.6	PASS
Critical Dilution CV < or = 40%	19.2	PASS

*Ceriodaphnia dubia* Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	29.9	PASS
Control CV < or = 40% per Surviving Female	10.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.0	PASS
Critical Dilution CV < or = 40%	22.0	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

B. Source of Effluent/Dilution Water:

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

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.6	8.0	8.1
pH (standard units)	7.3	7.4	7.1
Alkalinity (mg/l as CaCO <sub>3</sub> )	32	36	25
Hardness (mg/l as CaCO <sub>3</sub> )	34	36	44
Conductivity (umhos/cm)	300	320	350
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.13	<0.1	<0.1

2. Dilution Water Samples:  
Soft

Analysis	250311-1	250406-1
Dissolved oxygen (mg/l)	7.5	7.3
pH (standard units)	7.8	7.9
Alkalinity (mg/l as CaCO <sub>3</sub> )	32	34
Hardness (mg/l as CaCO <sub>3</sub> )	41	44
Conductivity (umhos/cm)	170	170
Residual Chlorine (mg/l)	<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: November 17, 2020 at 0950  
Date & Time Test Terminated: November 24, 2020 at 0905  
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 Reproduction Method 1002.0

Date & Time Test Initiated: November 17, 2020 at 1025  
Date & Time Test Terminated: November 23, 2020 at 1110  
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. Source 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 and following EPA method criteria.

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

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

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

#### IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

##### *Pimephales promelas* (Fathead minnow)

A chronic reference test was performed on November 03, 2020 at 1525 to November 10, 2020 at 1545

The results were as follows: (Control No. 249968-1.)

Survival LC-50: 2879 mg/l

Growth IC-25: 2145 mg/l

Growth PMSD: 15.2

##### *Ceriodaphnia dubia*

A chronic reference test was performed on November 03, 2020 at 1520 to November 10, 2020 at 1615

The results were as follows: (Control No. 249968-2.)

Survival LC-50: 1726.4 mg/l

Reproduction IC-25: 1221 mg/l

Reproduction PMSD: 24.6

#### V. Organism History

##### *Pimephales promelas* (Fathead minnow)

Date: November 17, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

##### *Ceriodaphnia dubia*

Date: November 17, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

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

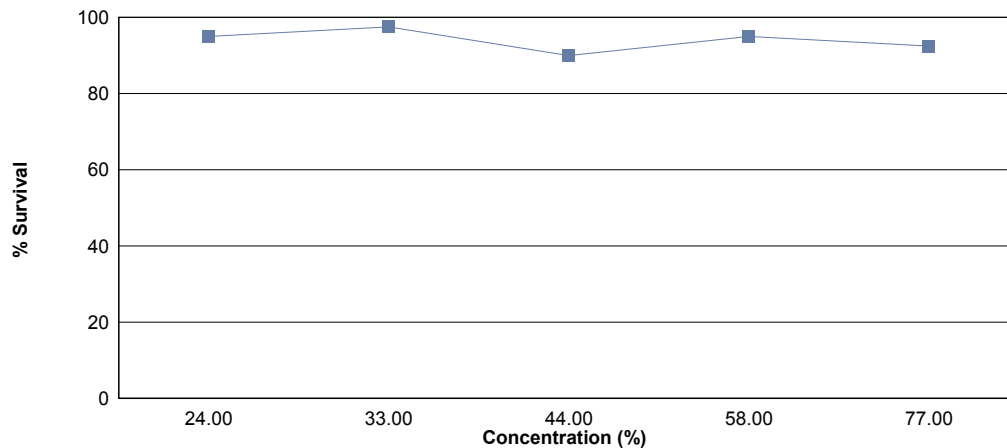
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 24 %, 33 %, 44 %, 58 %, 77 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 58 % effluent.

The test was initiated on November 17, 2020 at 0950 and continued through November 24, 2020 at 0905. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 77 % effluent
- b.) NOEC growth = 77 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.456
24 %	95.0	0.382
33 %	97.5	0.430
44 %	90.0	0.397
58 %	95.0	0.394
77 %	92.5	0.393

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

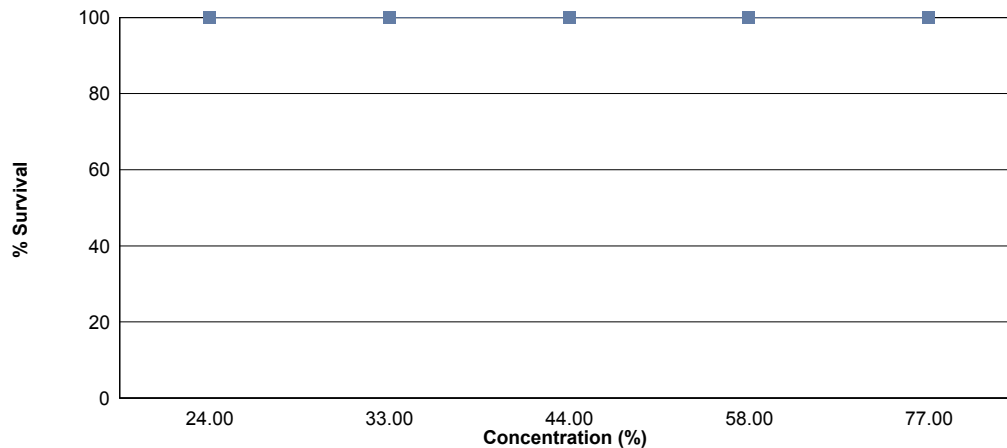
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 24 %, 33 %, 44 %, 58 %, 77 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 58 % effluent.

The test was initiated on November 17, 2020 at 1025 and continued through November 23, 2020 at 1110. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 77 % effluent
- b.) NOEC reproduction = 77 % effluent



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	29.9
24 %	100	31.6
33 %	100	29.9
44 %	100	31.9
58 %	100	30.8
77 %	100	31.6



Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: November 17, 2020 at 0950

Date and Time Test Terminated: November 24, 2020 at 0905

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	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
24 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	7
	C	8	8	8	8	8	8	8
	D	8	8	8	7	7	7	7
	E	8	8	8	8	8	8	8
33 %	A	8	8	8	8	8	8	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
44 %	A	8	8	8	8	8	8	7
	B	8	8	8	7	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	7	7	7	7	7
	E	8	8	8	8	7	7	7
58 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	7	7	7	7	7
	E	8	8	8	8	8	8	8
77 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	6
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	7	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Growth

Test Initiated: November 17, 2020 at 0950

Test Terminated: November 24, 2020 at 0905

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76719	.77047	0.00328	8	0.410
	B	.77049	.77412	0.00363	8	0.454
	C	.77301	.77673	0.00372	8	0.465
	D	.76942	.77348	0.00406	8	0.508
	E	.77382	.77736	0.00354	8	0.442
24 %	A	.77318	.77629	0.00311	8	0.389
	B	.77580	.77882	0.00302	8	0.378
	C	.77666	.77962	0.00296	8	0.370
	D	.77773	.78052	0.00279	8	0.349
	E	.77873	.78211	0.00338	8	0.422
33 %	A	.77240	.77509	0.00269	8	0.336
	B	.77155	.77467	0.00312	8	0.390
	C	.77467	.77833	0.00366	8	0.458
	D	.76994	.77345	0.00351	8	0.439
	E	.77464	.77884	0.00420	8	0.525
44 %	A	.77089	.77441	0.00352	8	0.440
	B	.77164	.77447	0.00283	8	0.354
	C	.77311	.77652	0.00341	8	0.426
	D	.77417	.77715	0.00298	8	0.372
	E	.77276	.77591	0.00315	8	0.394
58 %	A	.77475	.77786	0.00311	8	0.389
	B	.76588	.76804	0.00216	8	0.270
	C	.77790	.78115	0.00325	8	0.406
	D	.77713	.78062	0.00349	8	0.436
	E	.76691	.77065	0.00374	8	0.468
77 %	A	.76865	.77163	0.00298	8	0.372
	B	.77497	.77774	0.00277	8	0.346
	C	.76920	.77229	0.00309	8	0.386
	D	.77680	.78032	0.00352	8	0.440
	E	.76588	.76923	0.00335	8	0.419

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: November 17, 2020 at 1025

Date and Time Test Terminated: November 23, 2020 at 1110

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	5	0	5	10	0.500
4	5	5	5	3	3	5	4	4	0	6	40	10	4.00	
5	11	8	9	9	11	12	12	9	10	11	102	10	10.2	
6	18	16	12	13	13	15	14	18	17	16	152	10	15.2	
7														
8														
TOTAL	34	29	26	25	27	32	30	31	32	33	299	10	29.9	

Concentration: 24 %														
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	5	5	10	0.500	
4	6	4	6	5	4	5	5	5	3	0	43	10	4.30	
5	13	8	12	9	12	11	13	9	11	6	104	10	10.4	
6	19	13	15	15	15	15	17	20	20	15	164	10	16.4	
7														
8														
TOTAL	38	25	33	29	31	31	35	34	34	26	316	10	31.6	

Concentration: 33 %														
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	3	0	0	5	8	10	0.800	
4	5	6	4	4	4	5	1	5	6	0	40	10	4.00	
5	16	13	12	8	11	13	0	13	12	14	112	10	11.2	
6	0	14	17	17	10	17	8	19	17	20	139	10	13.9	
7														
8														
TOTAL	21	33	33	29	25	35	12	37	35	39	299	10	29.9	

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: November 17, 2020 at 1025

Date and Time Test Terminated: November 23, 2020 at 1110

Concentration: 44 %														
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	5	0	5	10	10	10	1.00
4	6	4	5	4	5	6	5	0	5	0	40	10	10	4.00
5	9	11	12	9	11	12	13	10	11	9	107	10	10	10.7
6	16	13	14	14	17	16	17	19	16	20	162	10	10	16.2
7														
8														
TOTAL	31	28	31	27	33	34	35	34	32	34	319	10	10	31.9

Concentration: 58 %														
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	6	0	0	6	10	10	0.600
4	5	6	0	5	4	6	6	1	5	4	42	10	10	4.20
5	11	11	5	12	12	10	12	13	10	9	105	10	10	10.5
6	13	16	9	17	13	16	20	18	17	16	155	10	10	15.5
7														
8														
TOTAL	29	33	14	34	29	32	38	38	32	29	308	10	10	30.8

Concentration: 77 %														
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	6	6	10	10	0.600
4	5	5	5	4	3	6	5	6	6	0	45	10	10	4.50
5	12	9	10	10	12	14	12	10	6	13	108	10	10	10.8
6	17	13	15	15	18	16	18	18	13	14	157	10	10	15.7
7														
8														
TOTAL	34	27	30	29	33	36	35	34	25	33	316	10	10	31.6

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	0.87500	1.20940
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	24 %	1	1.00000	1.39310
2	24 %	2	0.87500	1.20940
2	24 %	3	1.00000	1.39310
2	24 %	4	0.87500	1.20940
2	24 %	5	1.00000	1.39310
3	33 %	1	0.87500	1.20940
3	33 %	2	1.00000	1.39310
3	33 %	3	1.00000	1.39310
3	33 %	4	1.00000	1.39310
3	33 %	5	1.00000	1.39310
4	44 %	1	0.87500	1.20940
4	44 %	2	0.87500	1.20940
4	44 %	3	1.00000	1.39310
4	44 %	4	0.87500	1.20940
4	44 %	5	0.87500	1.20940
5	58 %	1	1.00000	1.39310
5	58 %	2	0.87500	1.20940
5	58 %	3	1.00000	1.39310
5	58 %	4	0.87500	1.20940
5	58 %	5	1.00000	1.39310
6	77 %	1	1.00000	1.39310
6	77 %	2	0.75000	1.04720
6	77 %	3	1.00000	1.39310
6	77 %	4	0.87500	1.20940
6	77 %	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))
D = 0.2593		
W = 0.9151		
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		Transform: Arc Sin(Square Root(Y))
Calculated B1 statistic = 2.660		
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) Survival

ANOVA Table			Transform: Arc Sin(Square Root(Y))	
SOURCE	DF	SS	MS	F
Between	5	0.04488	0.008976	0.8311
Within (Error)	24	0.2593	0.0108	
Total	29	0.3042		
Critical F = 3.9 (alpha = 0.01, df = 5,24) 2.62 (alpha = 0.05, df = 5,24)				
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)				

Dunnett's Test - Table 1 of 2				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	1.3564	0.975		
2	24 %	1.3196	0.95	0.5599	
3	33 %	1.3564	0.975	0	
4	44 %	1.2461	0.9	1.678	
5	58 %	1.3196	0.95	0.5599	
6	77 %	1.2872	0.925	1.053	
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	5			
2	24 %	5	0.08516	8.92	0.025
3	33 %	5	0.08516	8.92	0
4	44 %	5	0.08516	8.92	0.075
5	58 %	5	0.08516	8.92	0.025
6	77 %	5	0.08516	8.92	0.05

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.06186 W = 0.9736 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 = 6.580 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	0.02001	0.004003	1.553	
Within (Error)	24	0.06186	0.002578		
Total	29	0.08188			
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	0.4558	0.4558			
2	24 %	0.3816	0.3816	2.311		
3	33 %	0.4296	0.4296	0.8159		
4	44 %	0.3972	0.3972	1.825		
5	58 %	0.3938	0.3938	1.931		
6	77 %	0.3926	0.3926	1.968		
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	24 %	5	0.07579	16.6	0.0742	
3	33 %	5	0.07579	16.6	0.0262	
4	44 %	5	0.07579	16.6	0.0586	
5	58 %	5	0.07579	16.6	0.062	
6	77 %	5	0.07579	16.6	0.0632	

Appendix A2: Statistics

*Ceriodaphnia dubia* Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
24 %	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
33 %	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
44 %	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
58 %	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
77 %	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	24 %	10	0	
2	33 %	10	0	
3	44 %	10	0	
4	58 %	10	0	
5	77 %	10	0	

Appendix A2: Statistics

*Ceriodaphnia dubia* Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.1371 D* = 1.076 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	24 %	119.00	75.00	10.00	
3	33 %	116.00	75.00	10.00	
4	44 %	125.50	75.00	10.00	
5	58 %	117.50	75.00	10.00	
6	77 %	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

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	39.75	7.95	0.2938	
Within (Error)	54	1461	27.06		
Total	59	1501			
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)					

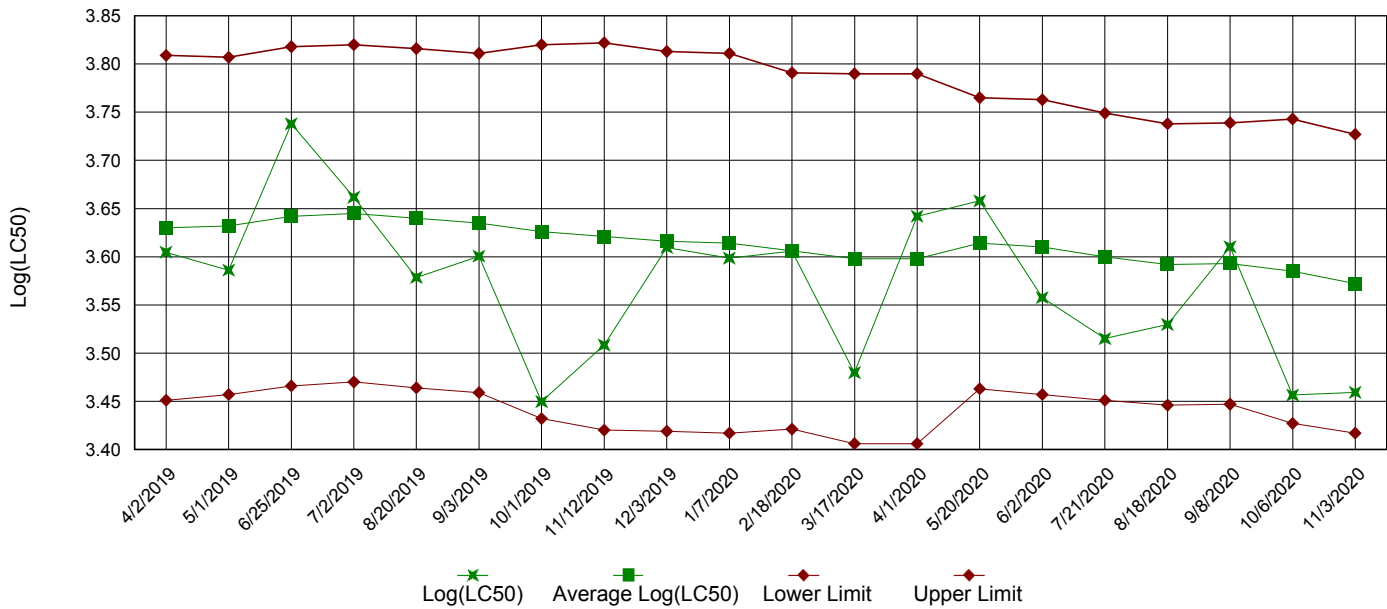
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	29.9	29.9			
2	24 %	31.6	31.6	-0.7308		
3	33 %	29.9	29.9	0		
4	44 %	31.9	31.9	-0.8597		
5	58 %	30.8	30.8	-0.3869		
6	77 %	31.6	31.6	-0.7308		
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	24 %	10	5.374	18	-1.7	
3	33 %	10	5.374	18	0	
4	44 %	10	5.374	18	-2	
5	58 %	10	5.374	18	-0.9	
6	77 %	10	5.374	18	-1.7	

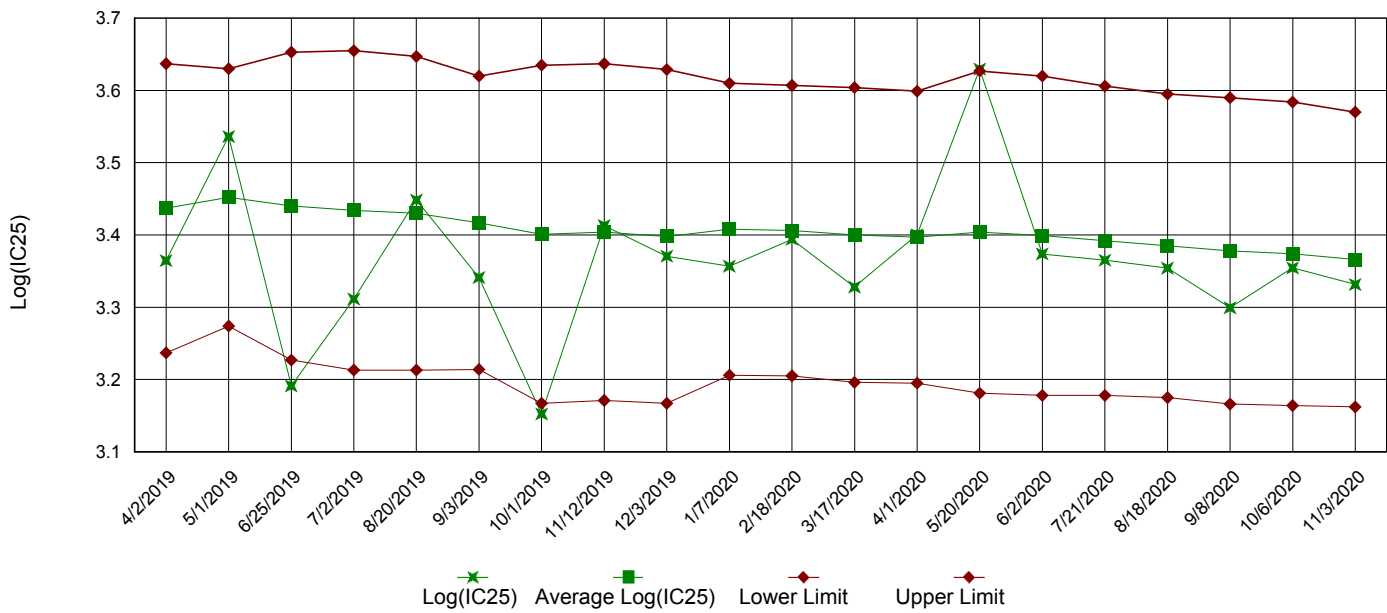
Appendix A3: Test 1000.0

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

LC50 Survival Data

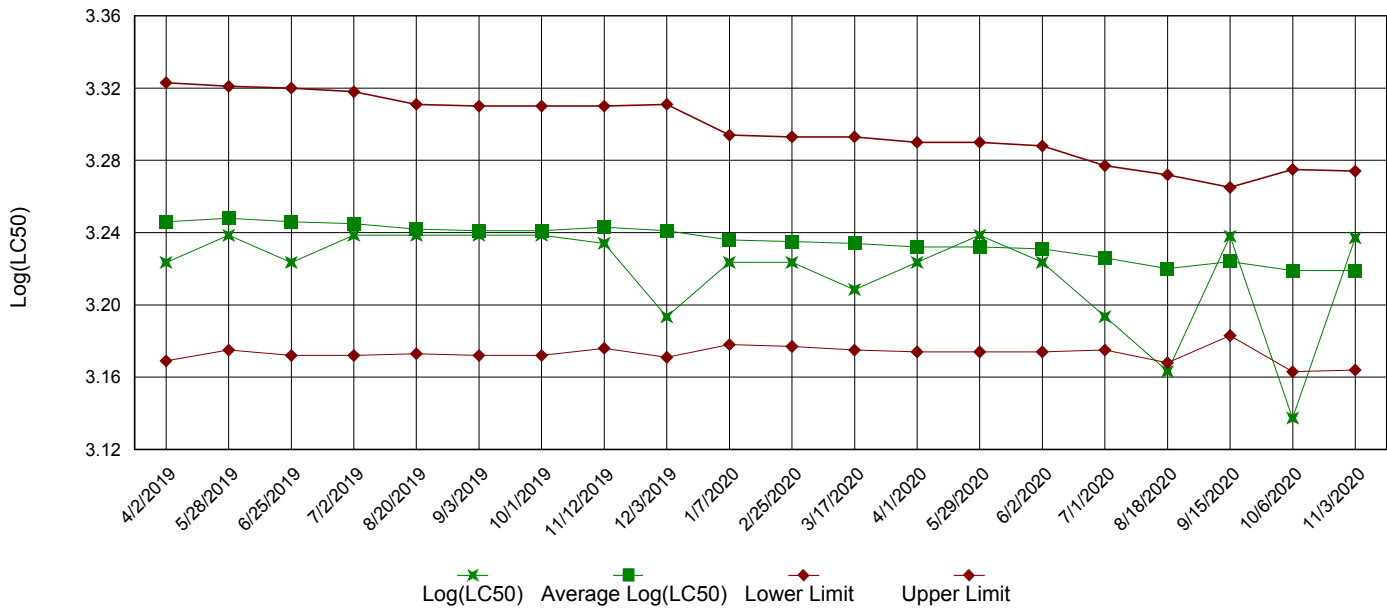


IC25 Growth Data

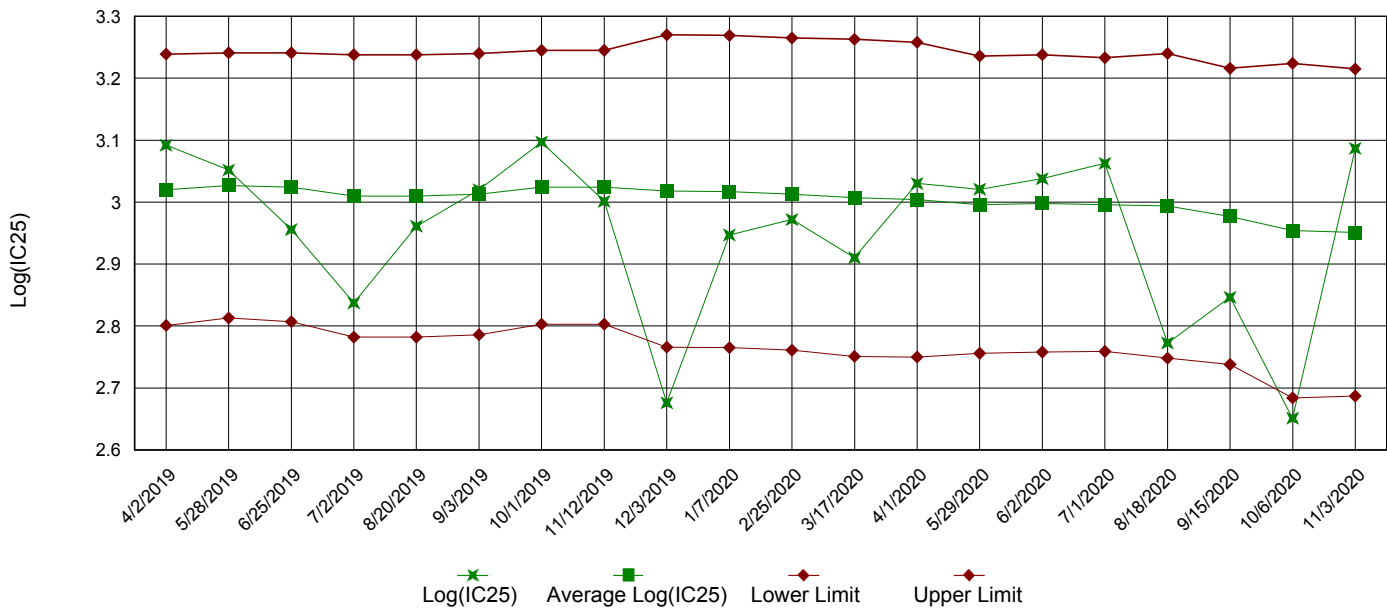


Appendix A3: Test 1002.0  
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: November 17, 2020 at 0950

Date and Time Test Terminated: November 24, 2020 at 0905

Dilution water used: Soft

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	87.5	100	100	100	100	100	100	97.5	5.73
24 %	100	87.5	100	87.5	100	100	100	95.0	7.21
33 %	87.5	100	100	100	100	100	100	97.5	5.73
44 %	87.5	87.5	100	87.5	87.5	100	100	90.0	6.21
58 %	100	87.5	100	87.5	100	100	100	95.0	7.21
77 %	100	75.0	100	87.5	100	100	100	92.5	12.1

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.410	0.454	0.465	0.508	0.442	0.456	7.83
24 %	0.389	0.378	0.370	0.349	0.422	0.382	7.05
33 %	0.336	0.390	0.458	0.439	0.525	0.43	16.6
44 %	0.440	0.354	0.426	0.372	0.394	0.397	9.05
58 %	0.389	0.270	0.406	0.436	0.468	0.394	19.2
77 %	0.372	0.346	0.386	0.440	0.419	0.393	9.52

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. Dunnett's 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	(58 %)	<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	(58 %)	<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:   77 %   (TOP6C)
6. LOEC *Pimephales* Lethality:   77 %   (TXP6C)
7. NOEC *Pimephales* Sublethality:   77 %   (TPP6C)
8. LOEC *Pimephales* Sublethality:   77 %   (TYP6C)
9. Coefficient of variation for *Pimephales* growth:   19.2   (TQP6C)
10. Sublethality for this test:   77 %   (51714 or 51714S)

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. Harold Mauldin  
 ANALYST: 280, 310, 343, 356

 Test Initiated: DATE: November 17, 2020 TIME: 0950  
 Test Terminated: DATE: November 24, 2020 TIME: 0905

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.5	7.3	7.3	7.4	7.6	7.5	7.6
Final	7.2	7.6	6.8	6.7	6.7	7.1	6.2
pH Initial	7.8	7.8	7.9	7.8	7.9	7.8	7.8
Final	7.7	7.9	7.6	7.6	7.6	7.7	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
24 %							
D.O. Initial	7.8	7.3	7.4	7.5	7.6	7.5	7.6
Final	7.5	7.8	7.0	6.6	7.0	6.7	6.2
pH Initial	7.7	7.7	7.7	7.7	7.6	7.6	7.6
Final	7.7	7.8	7.7	7.6	7.7	7.7	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
33 %							
D.O. Initial	7.9	7.3	7.6	7.7	7.9	7.5	7.6
Final	7.1	7.7	7.1	6.7	6.8	7.1	6.5
pH Initial	7.6	7.7	7.7	7.7	7.6	7.6	7.6
Final	7.6	7.8	7.7	7.6	7.6	7.7	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
44 %							
D.O. Initial	8.0	6.9	7.6	7.5	7.8	7.3	7.6
Final	7.0	7.7	6.8	6.6	6.8	7.0	5.8
pH Initial	7.6	7.6	7.7	7.7	7.5	7.6	7.6
Final	7.6	7.7	7.7	7.6	7.6	7.7	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
58 %							
D.O. Initial	8.0	7.2	7.8	7.6	7.9	7.4	7.5
Final	7.2	7.8	6.8	6.6	6.9	7.2	6.1
pH Initial	7.5	7.5	7.6	7.6	7.4	7.4	7.5
Final	7.6	7.6	7.6	7.6	7.7	7.7	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
77 %							
D.O. Initial	8.3	7.2	7.2	7.6	7.8	7.3	7.6
Final	8.1	8.0	7.1	6.2	7.0	7.0	6.5
pH Initial	7.4	7.6	7.6	7.5	7.3	7.4	7.4
Final	7.5	7.6	7.7	7.6	7.7	7.7	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	34	300	<0.05	Plant Effluent 15-NOV-20
36	36	320	<0.05	Plant Effluent 17-NOV-20
25	44	350	<0.05	Plant Effluent 19-NOV-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	170	<0.05	250311-1
34	44	170	<0.05	250406-1

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: November 17, 2020 at 1025

Date and Time Test Terminated: November 23, 2020 at 1110

Dilution water used: Soft

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		24 %	33 %	44 %	58 %	77 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
6 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		24 %	33 %	44 %	58 %	77 %
A	34	38	21	31	29	34
B	29	25	33	28	33	27
C	26	33	33	31	14	30
D	25	29	29	27	34	29
E	27	31	25	33	29	33
F	32	31	35	34	32	36
G	30	35	12	35	38	35
H	31	34	37	34	38	34
I	32	34	35	32	32	25
J	33	26	39	34	29	33
Mean per Adult	29.9	31.6	29.9	31.9	30.8	31.6
Mean per Surviving Adult	29.9	31.6	29.9	31.9	30.8	31.6
CV %	10.3	12.9	28.0	8.42	22.0	11.6

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	(58 %)	<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. 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	(58 %)	<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 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)
5. NOEC *Ceriodaphnia* Lethality: 77 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 77 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 77 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 77 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 22 (TQP3B)
10. Sublethality for this test: 77 % (51710 or 51710Q)

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. Harold Mauldin  
ANALYST: 280, 310, 343, 356

Test Initiated: DATE: November 17, 2020 TIME: 1025  
Test Terminated: DATE: November 23, 2020 TIME: 1110

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.5	7.3	7.3	7.4	7.6	7.5	7.6
Final	7.4	7.6	7.4	7.7	7.7	7.7	--
pH Initial	7.8	7.8	7.9	7.8	7.9	7.8	7.8
Final	8.2	8.0	8.2	8.4	8.2	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
24 %							
D.O. Initial	7.8	7.3	7.4	7.5	7.6	7.5	7.6
Final	7.7	6.8	7.8	7.8	7.4	7.5	--
pH Initial	7.7	7.7	7.7	7.7	7.6	7.6	7.6
Final	8.4	7.9	8.3	8.4	8.2	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
33 %							
D.O. Initial	7.9	7.3	7.6	7.7	7.9	7.5	7.6
Final	7.9	6.6	7.8	7.8	7.5	7.5	--
pH Initial	7.6	7.7	7.7	7.7	7.6	7.6	7.6
Final	8.4	7.8	8.3	8.4	8.1	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
44 %							
D.O. Initial	8.0	6.9	7.6	7.5	7.8	7.3	7.6
Final	7.4	6.7	7.8	7.8	7.7	7.6	--
pH Initial	7.6	7.6	7.7	7.7	7.5	7.6	7.6
Final	8.4	7.8	8.4	8.6	8.1	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
58 %							
D.O. Initial	8.0	7.2	7.8	7.6	7.9	7.4	7.5
Final	7.8	6.9	7.4	7.8	7.6	7.6	--
pH Initial	7.5	7.5	7.6	7.6	7.4	7.4	7.5
Final	8.5	7.9	8.4	8.5	8.0	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
77 %							
D.O. Initial	8.3	7.2	7.2	7.6	7.8	7.3	7.6
Final	7.7	6.9	7.8	7.9	7.8	7.5	--
pH Initial	7.4	7.6	7.6	7.5	7.3	7.4	7.4
Final	8.3	7.9	8.4	8.4	8.0	8.0	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	34	300	<0.05	Plant Effluent 15-NOV-20
36	36	320	<0.05	Plant Effluent 17-NOV-20
25	44	350	<0.05	Plant Effluent 19-NOV-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	170	<0.05	250311-1
34	44	170	<0.05	250406-1



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: <b>City of Hot Springs</b>		P.O. Number <b>2020-249</b>	
Project Reference: <b>Quarterly Bio-Monitoring</b>		Matrix Composite	
Project Manager: <b>Harold Mauldin</b>		Number of Bottles 3	
Sampled By: <i>Amanda Cates</i>		Water	
AIC	Sample Identification	Date/Time Collected	
No.	<b>Plant Effluent</b>	<b>11-15-20 @ 0000-2400</b>	
Remarks			
AIC Control Number: <b>250354</b>		Received Temperature °c <b>05.0.1.0.1</b>	
AIC Proposal Number:		Carrier:	
ANALYSIS REQUESTED Bio-Monitoring		Field pH Calibration on _____ @ _____	
Relinquished By: <i>A. Cates</i>		Date/Time <b>11-15-20 @ 0845</b>	Received By: <i>B. Samuel</i>
Relinquished By: <i>B. Samuel</i>		Date/Time <b>11-16-20 @ 1010</b>	Received By: <i>[Signature]</i>
Turnaround Time Requested in: (Please Circle) _____ DAYS			
Expedited results requested by:			
Who should AIC contact with questions: <b>Amanda Cates</b> Phone: <b>501-262-1881</b> Fax: <b>501-262-0339</b> Report Attention to: <b>Harold Mauldin</b> Report Address to: <b>320 Davidson Drive</b> Hot Springs, Ar. 71901 E-Mail Address: <b>Hmauldin@cityhs.net</b>			
Comments <b>0.5, 0.1, 0.1</b>			

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=[NH4]2NH4OH



# CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: <b>City of Hot Springs</b>		P.O. Number <b>2020-849</b>	
Project Reference: <b>Quarterly Bio-Monitoring</b>		Matrix	
Project Manager: <b>Harold Mauldin</b>		Number of Bottles	
Sampled By: <i>AC</i>		Water	
AIC Sample Identification		Composite	
Date/Time Collected		X	
<b>Plant Effluent</b>		X	
Date/Time Collected		3	
<b>11-17-20 @ 0000-2400</b>		Remarks	
G= Glass P=Plastic NO=None S=Sulfuric Acid pH2 V=VOA Vials		Field pH Calibration	
N=Nitric Acid pH2 H=HCl to pH2 B=NaOH to pH12		on _____ @ _____	
T=Sodium Thiosulfate Z=Zinc Acetate A=(NH4)2NH4OH		Buffer	
Turnaround Time Requested in: (Please Circle) _____ DAYS		Date/Time	
NORMAL or EXPEDITED		<b>11-18-20 @ 0908</b>	
Expedited results requested by:		Received By: <i>B. Sanner</i>	
Who should AIC contact with questions:		Date/Time	
Amanda Cates		<b>11-18-20 @ 0918</b>	
Phone: <b>501-262-1881</b>		Date/Time	
Fax: <b>501-262-0339</b>		<b>11-18-20 @ 1015</b>	
Report Attention to: <b>Harold Mauldin</b>		Received By: <i>Lucy Hays</i>	
Report Address to: <b>320 Davidson Drive</b>		Date/Time	
E-Mail Address: <b>Hmauldin@cityhs.net</b>		<b>1015</b>	
Comments			





CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: <b>City of Hot Springs</b>		P.O. Number <b>2020-849</b>	
Project Reference: <b>Quarterly Bio-Monitoring</b>		Matrix	
Project Manager: <b>Harold Mauldin</b>		Number of Bottles	
Sampled By: <i>AC</i>		Water	
AIC Sample Identification		Composite	
Date/Time Collected <b>11-19-20 @ 0000-2400</b>		X	
Plant Effluent		X	
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=(NH4)2NH4OH		P	
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS		NO	
Expedited results requested by: Who should AIC contact with questions: <b>Amanda Cates</b> Phone: <b>501-262-1881</b> Fax: <b>501-262-0339</b> Report Attention to: <b>Harold Mauldin</b> Report Address to: <b>320 Davidson Drive</b> Hot Springs, Ar. 71901 E-Mail Address: <b>Hmauldin@cityhs.net</b>		Field pH Calibration on _____ @ _____ Buffer _____	
Relinquished By: <i>A. Cates</i>		Received By: <i>B. Mauldin</i>	
Date/Time Relinquished: <b>11-20-20 @ 0740</b>		Date/Time Received: <b>11-20-20 @ 0740</b>	
Relinquished By: <i>B. Mauldin</i>		Received By: <i>Greg Hoyt</i>	
Date/Time Relinquished: <b>11-20-20 @ 0936</b>		Date/Time Received: <b>11-20-20 @ 0936</b>	
Comments <b>0.1°C / 0.2°C / 0.1°C</b>			