



October 1, 2020

Biomonitoring Testing
for
Outfall 002A Effl
Heber Springs, AR

Control No. 248580-1

Prepared for:

Mr. Paul Graham
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Prepared by:

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

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

Dear Mr. Paul Graham:

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 10 % effluent, which is above the critical dilution of 8 %. The percent minimum significant difference (PMSD) was below the limit of 12. Following additional calculations provided in the EPA document "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination Systems Program", the NOEC for sublethal effects was calculated to be 10 %. **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 10 % effluent, which is above the critical dilution of 8 %. The NOEC for reproduction occurred at 10 % effluent, which is above the critical dilution of 8 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

A handwritten signature in black ink, appearing to read 'John Overbey', is written over a horizontal line.

John Overbey
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	0.481	PASS
Control Growth CV < or = 40%	5.24	PASS
Growth Minimum Significant Difference 12 to 30%	9.26	BELOW
Critical Dilution CV < or = 40%	7.39	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	27.6	PASS
Control CV < or = 40% per Surviving Female	10.4	PASS
Reproduction Minimum Significant Difference 13 to 47%	19.9	PASS
Critical Dilution CV < or = 40%	16.1	PASS

II. Outlined Report

A. Introduction

1. Permit Number: NPDES Permit AR0022381 AFIN 12-00029
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

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

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.1	7.5	7.7
pH (standard units)	7.2	7.3	7.3
Alkalinity (mg/l as CaCO ₃)	21	20	20
Hardness (mg/l as CaCO ₃)	24	25	25
Conductivity (umhos/cm)	170	160	170
Residual Chlorine (mg/l)	0.070	0.050	<0.05
Ammonia as N (mg/l)	0.50	<0.1	<0.1

2. Dilution Water Samples:
Soft

Analysis	248394-1
Dissolved oxygen (mg/l)	7.7
pH (standard units)	7.8
Alkalinity (mg/l as CaCO ₃)	31
Hardness (mg/l as CaCO ₃)	44
Conductivity (umhos/cm)	170
Residual Chlorine (mg/l)	<0.05

C. Test Methods

1. Test methods used:

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

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: September 15, 2020 at 1255
Date & Time Test Terminated: September 22, 2020 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

Ceriodaphnia dubia Survival and Reproduction Method 1002.0

Date & Time Test Initiated: September 15, 2020 at 1250
Date & Time Test Terminated: September 21, 2020 at 1400
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. 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 Bartlett's test 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 August 18, 2020 at 1020 to August 25, 2020 at 0900

The results were as follows: (Control No. 247846-1.)

Survival LC-50: 3386.2 mg/l

Growth IC-25: 2260 mg/l

Growth PMSD: 20.4

Ceriodaphnia dubia

A chronic reference test was performed on August 18, 2020 at 1140 to August 24, 2020 at 1149

The results were as follows: (Control No. 247846-2.)

Survival LC-50: 1456.1 mg/l

Reproduction IC-25: 592.3 mg/l

Reproduction PMSD: 14.8

V. Organism History

Pimephales promelas (Fathead minnow)

Date: September 15, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: September 15, 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.

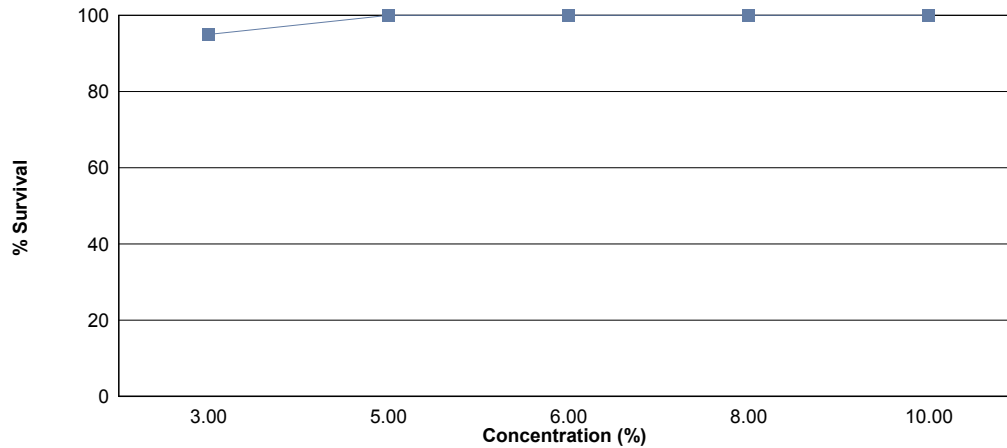
Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 10 % in accordance with the NPDES permit.

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

The test was initiated on September 15, 2020 at 1255 and continued through September 22, 2020 at 1345. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 10 % effluent
- b.) NOEC growth = 10 % effluent

(NOEC for sublethal effects was determined by Lower PMSD Bound Test.)



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.469
3 %	95.0	0.464
5 %	100	0.447
6 %	100	0.455
8 %	100	0.495
10 %	100	0.471

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

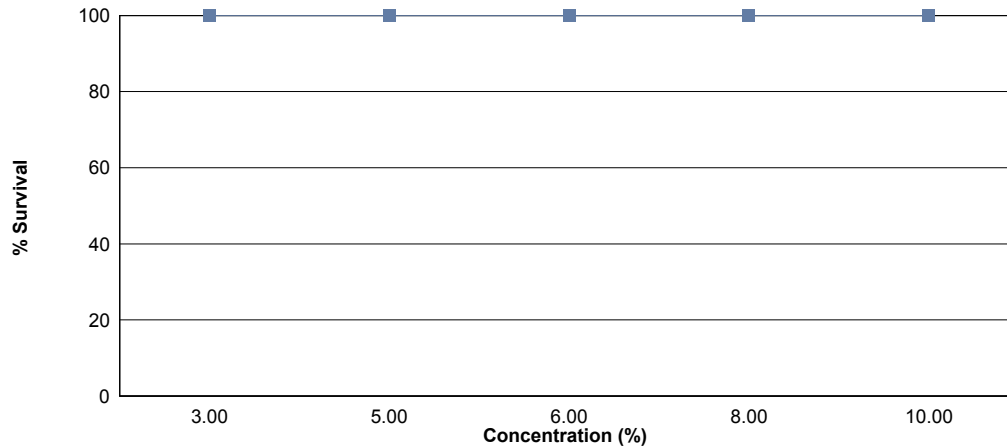
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 3 %, 5 %, 6 %, 8 %, 10 % in accordance with the NPDES permit.

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

The test was initiated on September 15, 2020 at 1250 and continued through September 21, 2020 at 1400. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 10 % effluent
- b.) NOEC reproduction = 10 % effluent



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	27.6
3 %	100	28.9
5 %	100	22.0
6 %	100	26.9
8 %	100	26.2
10 %	100	27.5

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: September 15, 2020 at 1255

Date and Time Test Terminated: September 22, 2020 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	7	7	7	7	7
3 %	A	8	8	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
5 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
6 %	A	8	8	8	8	8	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
8 %	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
10 %	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: September 15, 2020 at 1255

Test Terminated: September 22, 2020 at 1345

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.64784	.65173	0.00389	8	0.486
	B	.65455	.65802	0.00347	8	0.434
	C	.64913	.65275	0.00362	8	0.452
	D	.65884	.66277	0.00393	8	0.491
	E	.64579	.64963	0.00384	8	0.480
3 %	A	.65437	.65800	0.00363	8	0.454
	B	.66208	.66578	0.00370	8	0.462
	C	.65726	.66104	0.00378	8	0.472
	D	.64889	.65247	0.00358	8	0.448
	E	.64889	.65276	0.00387	8	0.484
5 %	A	.65360	.65703	0.00343	8	0.429
	B	.65658	.65982	0.00324	8	0.405
	C	.65060	.65435	0.00375	8	0.469
	D	.64863	.65213	0.00350	8	0.438
	E	.66359	.66756	0.00397	8	0.496
6 %	A	.65798	.66196	0.00398	8	0.498
	B	.66160	.66515	0.00355	8	0.444
	C	.65590	.65926	0.00336	8	0.420
	D	.65949	.66318	0.00369	8	0.461
	E	.66032	.66394	0.00362	8	0.452
8 %	A	.66399	.66782	0.00383	8	0.479
	B	.66037	.66438	0.00401	8	0.501
	C	.65409	.65763	0.00354	8	0.442
	D	.64277	.64692	0.00415	8	0.519
	E	.66333	.66762	0.00429	8	0.536
10 %	A	.65715	.66101	0.00386	8	0.482
	B	.65464	.65856	0.00392	8	0.490
	C	.65423	.65770	0.00347	8	0.434
	D	.65749	.66106	0.00357	8	0.446
	E	.64987	.65388	0.00401	8	0.501

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 15, 2020 at 1250

Date and Time Test Terminated: September 21, 2020 at 1400

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	4	4	3	4	3	3	3	6	6	3	39	10	3.90	
4	0	0	0	0	0	0	0	0	0	9	9	10	0.900	
5	8	8	7	8	8	7	6	9	10	0	71	10	7.10	
6	14	18	16	14	17	15	14	14	16	19	157	10	15.7	
7														
8														
TOTAL	26	30	26	26	28	25	23	29	32	31	276	10	27.6	

Concentration: 3 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	6	4	3	4	4	4	5	5	43	10	4.30
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	8	7	8	8	6	8	9	8	9	10	81	10	8.10
6	20	17	18	16	16	15	13	18	15	17	165	10	16.5
7													
8													
TOTAL	32	28	32	28	25	27	26	30	29	32	289	10	28.9

Concentration: 5 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	2	2	4	4	0	0	2	2	0	0	16	10	1.60
4	0	0	0	0	0	0	0	0	3	8	11	10	1.10
5	6	7	8	8	0	4	7	6	0	0	46	10	4.60
6	16	15	19	19	0	19	11	14	18	16	147	10	14.7
7													
8													
TOTAL	24	24	31	31	0	23	20	22	21	24	220	10	22.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 15, 2020 at 1250

Date and Time Test Terminated: September 21, 2020 at 1400

Concentration: 6 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	3	2	2	4	5	0	0	0	3	23	10	2.30	
4	3	0	0	0	0	0	0	6	5	10	24	10	2.40	
5	8	7	9	8	7	9	7	0	0	0	55	10	5.50	
6	18	19	17	18	19	20	7	13	17	19	167	10	16.7	
7														
8														
TOTAL	33	29	28	28	30	34	14	19	22	32	269	10	26.9	

Concentration: 8 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	2	3	3	4	0	4	4	3	4	4	31	10	3.10
4	0	0	0	0	0	0	0	0	0	5	5	10	0.500
5	6	7	8	8	5	8	9	7	0	0	58	10	5.80
6	18	19	16	19	13	14	17	19	16	17	168	10	16.8
7													
8													
TOTAL	26	29	27	31	18	26	30	29	20	26	262	10	26.2

Concentration: 10 %													
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	5	2	4	4	3	5	0	3	4	4	34	10	3.40
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	8	7	8	8	5	7	5	8	7	8	71	10	7.10
6	17	15	19	20	16	19	13	15	16	20	170	10	17.0
7													
8													
TOTAL	30	24	31	32	24	31	18	26	27	32	275	10	27.5

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data				Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	0.87500	1.20940
2	3 %	1	0.87500	1.20940
2	3 %	2	0.87500	1.20940
2	3 %	3	1.00000	1.39310
2	3 %	4	1.00000	1.39310
2	3 %	5	1.00000	1.39310
3	5 %	1	1.00000	1.39310
3	5 %	2	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	1.00000	1.39310
3	5 %	5	1.00000	1.39310
4	6 %	1	1.00000	1.39310
4	6 %	2	1.00000	1.39310
4	6 %	3	1.00000	1.39310
4	6 %	4	1.00000	1.39310
4	6 %	5	1.00000	1.39310
5	8 %	1	1.00000	1.39310
5	8 %	2	1.00000	1.39310
5	8 %	3	1.00000	1.39310
5	8 %	4	1.00000	1.39310
5	8 %	5	1.00000	1.39310
6	10 %	1	1.00000	1.39310
6	10 %	2	1.00000	1.39310
6	10 %	3	1.00000	1.39310
6	10 %	4	1.00000	1.39310
6	10 %	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	3 %	25.00	16.00	5.00	
3	5 %	30.00	16.00	5.00	
4	6 %	30.00	16.00	5.00	
5	8 %	30.00	16.00	5.00	
6	10 %	30.00	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 = 0.02026 W = 0.9769 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 = 3.372 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	0.006795	0.001359	1.61	
Within (Error)	24	0.02026	0.0008442		
Total	29	0.02705			
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	0.4686	0.4686			
2	3 %	0.464	0.464	0.2503		
3	5 %	0.4474	0.4474	1.154		
4	6 %	0.455	0.455	0.7401		
5	8 %	0.4954	0.4954	-1.458		
6	10 %	0.4706	0.4706	-0.1088		
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)						

Dunnett's Test - Table 2 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control	
1	Control	5				
2	3 %	5	0.04337	9.26	0.0046	
3	5 %	5	0.04337	9.26	0.0212	
4	6 %	5	0.04337	9.26	0.0136	
5	8 %	5	0.04337	9.26	-0.0268	
6	10 %	5	0.04337	9.26	-0.002	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

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

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

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

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

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

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

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

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

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
10 %	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	3 %	10	0	
2	5 %	10	0	
3	6 %	10	0	
4	8 %	10	0	
5	10 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.1316 D* = 1.032 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 18.10 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data FAIL B1 homogeneity test at 0.01 level.</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	3 %	118.50	75.00	10.00	
3	5 %	75.50	75.00	10.00	
4	6 %	109.50	75.00	10.00	
5	8 %	99.50	75.00	10.00	
6	10 %	109.00	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	284.7	56.94	2.012	
Within (Error)	54	1528	28.3		
Total	59	1813			
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	27.6	27.6			
2	3 %	28.9	28.9	-0.5464		
3	5 %	22	22	2.354	*	
4	6 %	26.9	26.9	0.2942		
5	8 %	26.2	26.2	0.5885		
6	10 %	27.5	27.5	0.04203		
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	3 %	10	5.496	19.9	-1.3	
3	5 %	10	5.496	19.9	5.6	
4	6 %	10	5.496	19.9	0.7	
5	8 %	10	5.496	19.9	1.4	
6	10 %	10	5.496	19.9	0.1	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.469	-	
3 %	0.464	1.07	PASS
5 %	0.447	4.69	PASS
6 %	0.455	2.99	PASS
8 %	0.495	-5.54	PASS
10 %	0.471	-0.426	PASS

Limit = 12

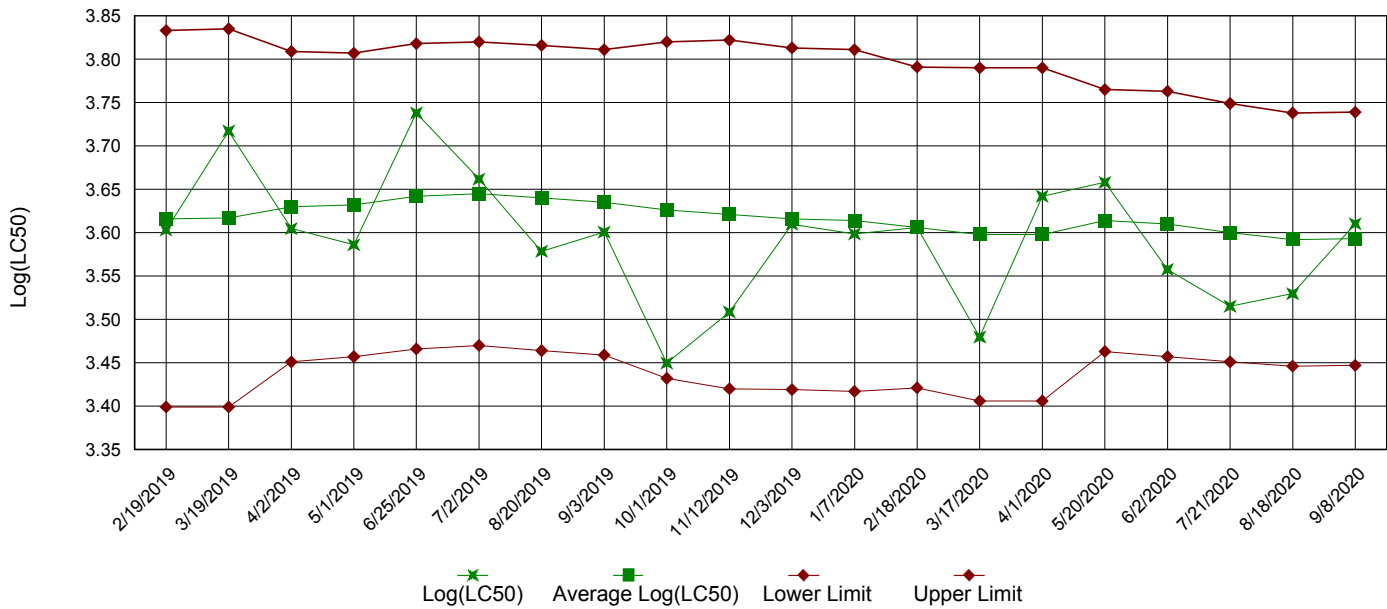
NOEC = 10 %

LOEC = 10 %

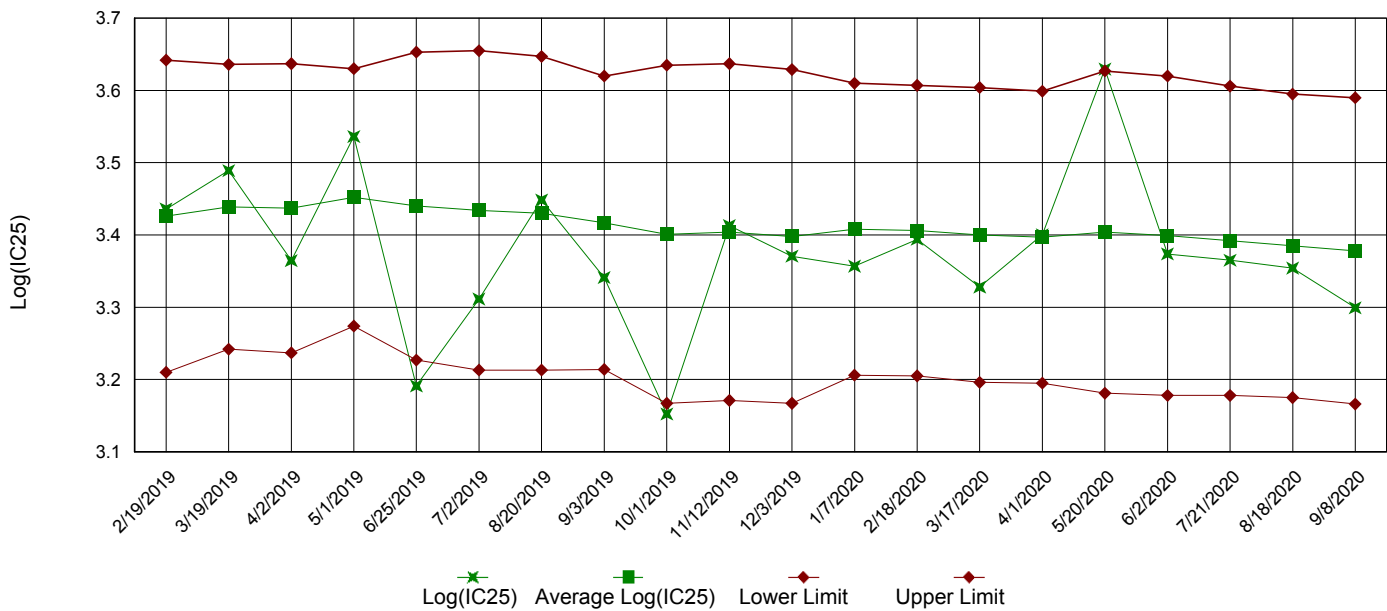
Appendix A3: Test 1000.0

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

LC50 Survival Data

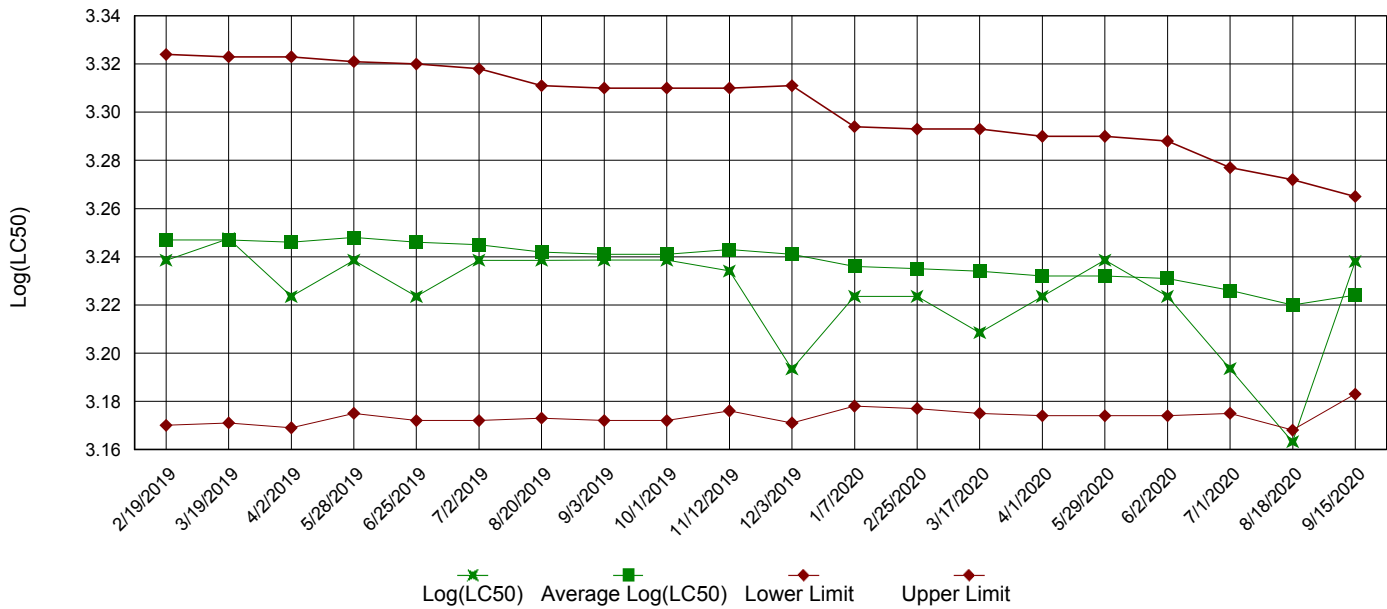


IC25 Growth Data

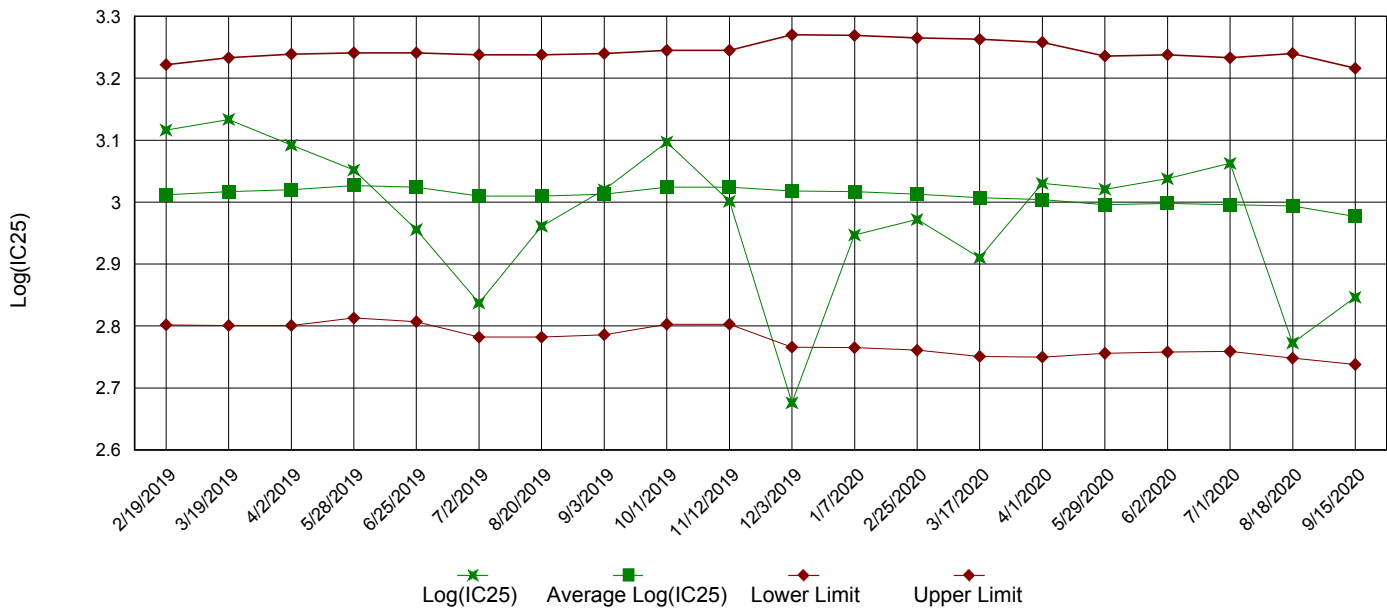


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: Heber Springs Water & Sewer

NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: September 15, 2020 at 1255

Date and Time Test Terminated: September 22, 2020 at 1345

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	100	100	100	100	87.5	100	100	97.5	5.73
3 %	87.5	87.5	100	100	100	100	100	95.0	7.21
5 %	100	100	100	100	100	100	100	100	0.00
6 %	100	100	100	100	100	100	100	100	0.00
8 %	100	100	100	100	100	100	100	100	0.00
10 %	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	0.486	0.434	0.452	0.491	0.480	0.469	5.24
3 %	0.454	0.462	0.472	0.448	0.484	0.464	3.09
5 %	0.429	0.405	0.469	0.438	0.496	0.447	7.94
6 %	0.498	0.444	0.420	0.461	0.452	0.455	6.26
8 %	0.479	0.501	0.442	0.519	0.536	0.495	7.39
10 %	0.482	0.490	0.434	0.446	0.501	0.471	6.17

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

Appendix B: Test 1000.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Steel's Many-One Rank Test:

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

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

2. Dunnett's Test:

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

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

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP6C)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP6C)
5. NOEC *Pimephales* Lethality: 10 % (TOP6C)
6. LOEC *Pimephales* Lethality: 10 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 10 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 10 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 7.39 (TQP6C)
10. Sublethality for this test: 10 % (51714 or 51714S)

Appendix B: Test 1000.0
 CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
 CHEMICAL PARAMETERS CHART

 PERMITTEE: Heber Springs Water & Sewer
 NPDES NO.: NPDES Permit AR0022381 AFIN
 CONTACT: Mr. Paul Graham
 ANALYST: 280, 310, 343

 Test Initiated: DATE: September 15, 2020 TIME: 1255
 Test Terminated: DATE: September 22, 2020 TIME: 1345

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.3	8.0	7.6	7.0	7.6
Final	6.9	6.9	6.8	6.7	7.1	7.1	6.5
pH Initial	7.8	7.8	7.8	7.9	8.0	8.0	7.9
Final	7.6	7.6	7.5	7.6	8.0	7.7	7.6

DILUTION 3 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.4	7.6	7.8	7.1	7.0	7.4
Final	6.5	6.9	6.8	6.7	7.0	6.3	6.3
pH Initial	7.8	7.8	7.8	7.9	7.9	8.0	7.9
Final	7.4	7.6	7.5	7.6	8.0	7.7	7.5

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.3	7.1	7.8	7.1	7.2	7.1
Final	6.5	6.9	6.9	7.1	7.1	6.7	6.6
pH Initial	7.8	7.8	7.8	7.9	7.9	8.0	7.9
Final	7.4	7.6	7.5	7.7	8.0	7.7	7.6

DILUTION 6 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.9	7.4	7.7	7.4	7.2	7.4
Final	6.6	6.8	6.8	6.8	7.1	6.4	6.6
pH Initial	7.8	7.8	7.8	7.9	7.9	8.0	7.9
Final	7.5	7.6	7.6	7.6	8.0	7.7	7.7

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.3	7.2	8.1	7.4	7.2	7.8
Final	6.6	7.0	7.1	6.9	7.3	7.2	6.9
pH Initial	7.8	7.8	7.8	7.8	7.8	8.0	7.9
Final	7.5	7.5	7.5	7.6	8.0	7.7	7.6

DILUTION 10 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.5	7.6	7.9	7.1	7.1	7.5
Final	6.5	6.9	7.1	6.7	7.1	6.7	6.6
pH Initial	7.8	7.7	7.7	7.8	7.9	8.0	7.9
Final	7.4	7.6	7.6	7.6	8.0	7.7	7.6

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
21	24	170	0.070	Outfall 002A Effl. Heber Springs Water 15-SEP-20
20	25	160	0.050	Outfall 002A Effl. Heber Springs Water 16-SEP-20
20	25	170	<0.05	Outfall 002A Effl. Heber Springs Water 18-SEP-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	44	170	<0.05	248394-1

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

Permittee: Heber Springs Water & Sewer

NPDES No.: NPDES Permit AR0022381 AFIN 12-00029

Date and Time Test Initiated: September 15, 2020 at 1250

Date and Time Test Terminated: September 21, 2020 at 1400

Dilution water used: Soft

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		3 %	5 %	6 %	8 %	10 %
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				
		3 %	5 %	6 %	8 %	10 %
A	26	32	24	33	26	30
B	30	28	24	29	29	24
C	26	32	31	28	27	31
D	26	28	31	28	31	32
E	28	25	0	30	18	24
F	25	27	23	34	26	31
G	23	26	20	14	30	18
H	29	30	22	19	29	26
I	32	29	21	22	20	27
J	31	32	24	32	26	32
Mean per Adult	27.6	28.9	22.0	26.9	26.2	27.5
Mean per Surviving Adult	27.6	28.9	22.0	26.9	26.2	27.5
CV %	10.4	8.85	39.0	24.2	16.1	16.6

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	(8 %)	<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	(8 %)	<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: 10 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 10 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 10 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 10 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 16.1 (TQP3B)
10. Sublethality for this test: 10 % (51710 or 51710Q)

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

PERMITTEE: Heber Springs Water & Sewer
NPDES NO.: NPDES Permit AR0022381 AFIN
CONTACT: Mr. Paul Graham
ANALYST: 280, 310, 343

Test Initiated: DATE: September 15, 2020 TIME: 1250
Test Terminated: DATE: September 21, 2020 TIME: 1400

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.7	7.5	7.3	8.0	7.6	7.0	7.6
Final	7.4	7.4	7.8	7.2	7.7	7.4	--
pH Initial	7.8	7.8	7.8	7.9	8.0	8.0	7.9
Final	8.0	8.0	8.0	8.0	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
3 %							
D.O. Initial	7.7	7.4	7.6	7.8	7.1	7.0	7.4
Final	7.4	7.2	7.9	7.4	7.3	7.1	--
pH Initial	7.8	7.8	7.8	7.9	7.9	8.0	7.9
Final	8.0	8.0	8.0	8.1	8.1	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	8.0	7.3	7.1	7.8	7.1	7.2	7.1
Final	7.4	7.1	8.1	7.3	7.4	7.1	--
pH Initial	7.8	7.8	7.8	7.9	7.9	8.0	7.9
Final	8.0	8.0	8.0	8.0	8.2	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
6 %							
D.O. Initial	7.6	7.9	7.4	7.7	7.4	7.2	7.4
Final	7.4	7.2	8.0	7.4	7.6	7.2	--
pH Initial	7.8	7.8	7.8	7.9	7.9	8.0	7.9
Final	8.0	8.0	8.0	8.0	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	7.6	7.3	7.2	8.1	7.4	7.2	7.8
Final	7.7	7.4	8.0	7.3	7.5	7.6	--
pH Initial	7.8	7.8	7.8	7.8	7.8	8.0	7.9
Final	8.0	7.9	8.0	8.0	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
10 %							
D.O. Initial	7.7	7.5	7.6	7.9	7.1	7.1	7.5
Final	7.4	7.3	8.0	7.4	7.2	7.0	--
pH Initial	7.8	7.7	7.7	7.8	7.9	8.0	7.9
Final	8.0	8.0	8.0	8.1	8.3	8.0	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
21	24	170	0.070	Outfall 002A Effl. Heber Springs Water 15-SEP-20
20	25	160	0.050	Outfall 002A Effl. Heber Springs Water 16-SEP-20
20	25	170	<0.05	Outfall 002A Effl. Heber Springs Water 18-SEP-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	44	170	<0.05	248394-1



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: <u>Heber Springs Water & Wastewater Dept.</u>		NO OF BOTTLES		ANALYSES REQUESTED	
Project Reference: <u>2nd half bio monitoring</u>		PO No. <u>160239</u>		Chronic Bio Monitoring	
Project Manager: <u>Paul Graham</u>		SAMPLE MATRIX		2nd half	
Sampled By: <u>Joey Messy</u>		WATER			
AIC No. <u>1</u>		G R A B			
Sample Identification: <u>Outfall 002 BEFR</u>		C O M P			
Date/Time Collected: <u>9-14-20 8 AM</u>		S O I L			
Remarks: <u>H.S. WWTP</u>		✓			
Carrier:					
Received on ice (4°C)?					
(YES) <u>0.5</u> NO					
AIC CONTROL NO: <u>248580</u>					
AIC PROPOSAL NO:					
Field pH calibration					
on _____ @ _____					
Buffer:					
G = Glass		V = VOA vials		T = Sodium Thiosulfate	
NO = none		N = Nitric acid pH2		Z = Zinc acetate	
S = Sulfuric acid pH2		H = HCl to pH2			
		B = NaOH to pH12			
Turnaround Time Requested: (Please circle)		Relinquished		Received	
NORMAL or EXPEDITED IN <u>7</u> DAYS		By:		By:	
Expedited results requested by: <u>Paul Graham</u>		Date/Time		Date/Time	
Who should AIC contact with questions: <u>Joey Messy</u>		Relinquished		Received in Lab	
Phone: <u>501-250-3442</u>		By: <u>Joey Messy</u>		By: <u>D. Brown</u>	
Report Attention to: <u>Paul Graham</u>		Date/Time		Date/Time	
Report Address to: <u>Heber Springs Water</u>		By: <u>Joey Messy</u>		By: <u>D. Brown</u>	
<u>1108 West Front St.</u>		Comments:			
<u>Heber Springs AR 72543</u>					

FORM 0060

Samples Every 6 HRS = 4
 2 pm, 8 pm, 2 AM, 8 AM.

EMail: Nora @ Heber Springs Water.com



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

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: Heber Springs Water/Wastewater Dept Project: 2nd half Bio Monitoring Project Manager: Paul Graham		PO No. 16239 SAMPLE MATRIX WATER	NO OF BOTTLES 1	ANALYSES REQUESTED Chronic Bio Monitoring 2nd half	AIC CONTROL NO: 248580 AIC PROPOSAL NO:
Sample Identification: 002A ESCL Date/Time Collected: 9-15-20 8 AM H.S. WWT P	G R A B C O M P 24 PP	W A T E R L S O I L	Carrier: See Received on Ice (4°C)? YES NO	Remarks:	Field pH calibration on @ Buffer:
By: See, Massey Date/Time Collected: 9-15-20 8 AM H.S. WWT P	Container Type: Plastic Preservative: Sulfuric acid pH2	G = Glass 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
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN 7 DAYS Expedited results requested by: Paul Graham	Relinquished By: See M Date/Time: 9-15-20 11:03 AM	Relinquished By: See M Date/Time: 9-15-20 11:03 AM	Received By: See M Date/Time: 9-16-20 11:02	Received By: See M Date/Time: 9-16-20 11:02	Comments:
Who should AIC contact with questions: See Massey Phone: 501-224-3442 Fax: 501-224-3338 Report Attention to: Paul Graham Report Address to: Heber Springs Water 1108 West Front St. Heber Springs, AR 72543	5101 E Mail: Nora@HeberSpringsWater.com				

FORM 0060
 Samples Every 6 HRS = 4
 2pm, 8pm, 2 AM, 6 AM



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 3 OF 4

Client: Heber Springs Water Dept.		PO No. 16239		NO OF BOTTLES		ANALYSES REQUESTED		AIC CONTROL NO: 248580	
Project Reference: 2nd half Bio Monitoring		MATRIX WATER		1		Chronic Bio Monitoring		AIC PROPOSAL NO:	
Project Manager: Paul Graham		G R A B				2nd half		Carrier: Joey	
Sampled By: Soey Massey		C O M P						Received Temperature C	
AIC No. 9-17-20 to 8AM		P = Plastic						Remarks	
Sample Identification: 9-18-20 to 8AM		S = Sulfuric acid pH2						Field pH calibration on @ Buffer:	
Vial No. 1		V = VOA vials							
		N = Nitric acid pH2							
		H = HCl to pH2							
		B = NaOH to pH12							
		T = Sodium Thiosulfate							
		Z = Zinc acetate							
		A = (NH4)2SO4, NH4OH							
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN 7 DAYS		Relinquished By:		Date/Time		Received By:		Date/Time	
Expedited results requested by: Paul Graham		By: Soey Massey		9-18-20 11:04AM		By: D. Brown		9-18-20 11:04AM	
Who should AIC contact with questions: Soey Massey		Reinquired By:		Date/Time		Received in Lab By:		Date/Time	
Phone: 813-250-3442 Fax: 362-3338		By: Soey Massey		9-18-20 11:04AM		By: D. Brown		9-18-20 11:04AM	
Report Attention to: Paul Graham		Comments:							
Report Address to: Heber Springs Water									
1108 West Point St									
Heber Springs AR 72543									
Email Address: Nora@HeberSpringsWater.com									

FORM 0060

Samples Every 6 HRS = 4
2pm, 8pm, 2AM, 8AM

9/2014