



August 24, 2020

Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 247628-1

Prepared for:

Mr. Harold Mauldin
City of Hot Springs
320 Davidson Drive
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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:

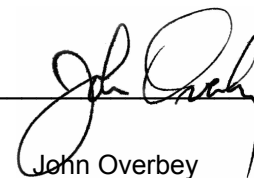
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 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 77 %. **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



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%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.526	PASS
Control Growth CV < or = 40%	5.61	PASS
Growth Minimum Significant Difference 12 to 30%	10.9	BELOW
Critical Dilution CV < or = 40%	9.35	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	24.4	PASS
Control CV < or = 40% per Surviving Female	39.0	PASS
Reproduction Minimum Significant Difference 13 to 47%	35.2	PASS
Critical Dilution CV < or = 40%	20.4	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)	7.7	8.3	8.8
pH (standard units)	7.3	7.5	7.3
Alkalinity (mg/l as CaCO ₃)	32	41	36
Hardness (mg/l as CaCO ₃)	52	43	38
Conductivity (umhos/cm)	280	300	320
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.19	0.22	0.19

2. Dilution Water Samples:

Soft

Analysis	247488-1
Dissolved oxygen (mg/l)	7.1
pH (standard units)	7.8
Alkalinity (mg/l as CaCO ₃)	31
Hardness (mg/l as CaCO ₃)	45
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: August 11, 2020 at 0911
Date & Time Test Terminated: August 18, 2020 at 0840
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: August 11, 2020 at 1110
Date & Time Test Terminated: August 17, 2020 at 1008
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 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 July 21, 2020 at 1450 to July 28, 2020 at 1630

The results were as follows: (Control No. 246513-1.)

Survival LC-50: 3274 mg/l

Growth IC-25: 2318 mg/l

Growth PMSD: 14.9

Ceriodaphnia dubia

A chronic reference test was performed on July 01, 2020 at 1300 to July 08, 2020 at 1400

The results were as follows: (Control No. 246513-2.)

Survival LC-50: 1561.5 mg/l

Reproduction IC-25: 1155 mg/l

Reproduction PMSD: 17.2

V. Organism History

Pimephales promelas (Fathead minnow)

Date: August 11, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: August 11, 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.

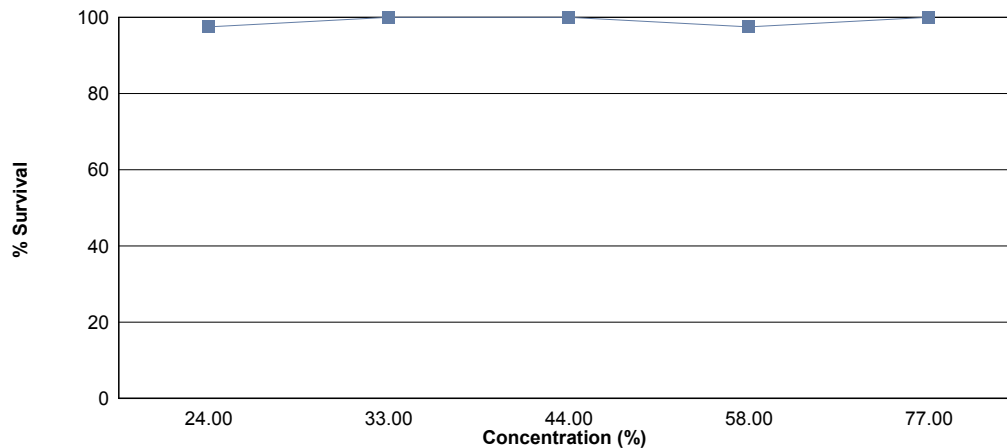
Effluent dilutions for this test were 24 %, 33 %, 44 %, 58 %, 77 % in accordance with the NPDES permit.

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

The test was initiated on August 11, 2020 at 0911 and continued through August 18, 2020 at 0840. 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

(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	100	0.526
24 %	97.5	0.536
33 %	100	0.547
44 %	100	0.547
58 %	97.5	0.516
77 %	100	0.520

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

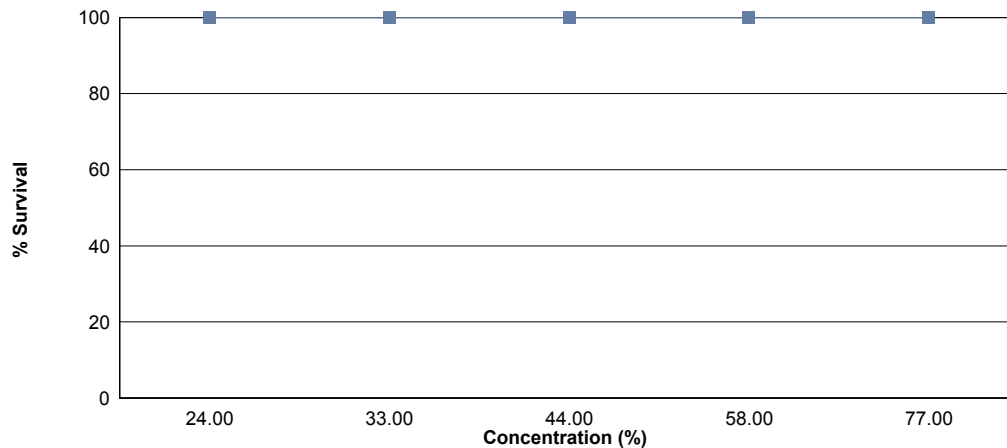
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 August 11, 2020 at 1110 and continued through August 17, 2020 at 1008. 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	24.4
24 %	100	32.5
33 %	100	26.8
44 %	100	32.2
58 %	100	32.1
77 %	100	30.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 11, 2020 at 0911

Date and Time Test Terminated: August 18, 2020 at 0840

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
24 %	A	8	7	7	7	7	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
33 %	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
44 %	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
58 %	A	8	8	8	8	7	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
77 %	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 11, 2020 at 0911

Test Terminated: August 18, 2020 at 0840

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.66215	.66675	0.00460	8	0.575
	B	.66481	.66886	0.00405	8	0.506
	C	.66308	.66731	0.00423	8	0.529
	D	.65737	.66138	0.00401	8	0.501
	E	.65387	.65802	0.00415	8	0.519
24 %	A	.65950	.66348	0.00398	8	0.498
	B	.65288	.65722	0.00434	8	0.542
	C	.65918	.66366	0.00448	8	0.560
	D	.65633	.66075	0.00442	8	0.552
	E	.65353	.65776	0.00423	8	0.529
33 %	A	.65891	.66277	0.00386	8	0.482
	B	.66415	.66851	0.00436	8	0.545
	C	.66287	.66761	0.00474	8	0.592
	D	.67078	.67520	0.00442	8	0.552
	E	.66091	.66543	0.00452	8	0.565
44 %	A	.65641	.66083	0.00442	8	0.552
	B	.65887	.66308	0.00421	8	0.526
	C	.65496	.65941	0.00445	8	0.556
	D	.64937	.65367	0.00430	8	0.538
	E	.65182	.65634	0.00452	8	0.565
58 %	A	.64298	.64684	0.00386	8	0.482
	B	.66954	.67352	0.00398	8	0.498
	C	.66546	.67022	0.00476	8	0.595
	D	.65463	.65845	0.00382	8	0.478
	E	.65781	.66204	0.00423	8	0.529
77 %	A	.66681	.67053	0.00372	8	0.465
	B	.66224	.66601	0.00377	8	0.471
	C	.66534	.66938	0.00404	8	0.505
	D	.65842	.66307	0.00465	8	0.581
	E	.64724	.65186	0.00462	8	0.578

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 11, 2020 at 1110

Date and Time Test Terminated: August 17, 2020 at 1008

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	4	4	0	0	0	4	0	0	0	12	10	1.20	
4	4	2	0	6	6	4	0	3	6	1	32	10	3.20	
5	7	12	12	10	8	8	10	9	9	10	95	10	9.50	
6	0	17	16	15	14	0	17	11	15	0	105	10	10.5	
7														
8														
TOTAL	11	35	32	31	28	12	31	23	30	11	244	10	24.4	

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	6	0	6	6	0	4	0	0	0	22	10	2.20
4	3	0	5	0	0	5	0	4	5	8	30	10	3.00
5	10	13	14	14	12	12	10	8	11	12	116	10	11.6
6	1	23	14	21	15	15	20	15	17	16	157	10	15.7
7													
8													
TOTAL	14	42	33	41	33	32	34	27	33	36	325	10	32.5

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	6	0	0	0	4	3	4	0	0	17	10	1.70
4	3	0	6	3	0	0	0	0	4	3	19	10	1.90
5	8	12	12	10	11	12	12	10	9	13	109	10	10.9
6	0	19	18	0	20	17	17	19	13	0	123	10	12.3
7													
8													
TOTAL	11	37	36	13	31	33	32	33	26	16	268	10	26.8

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 11, 2020 at 1110

Date and Time Test Terminated: August 17, 2020 at 1008

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	6	4	0	5	0	6	5	0	0	26	10	2.60	
4	0	0	1	5	0	4	0	0	4	6	20	10	2.00	
5	5	13	12	11	12	12	11	11	12	14	113	10	11.3	
6	14	20	22	1	17	18	20	16	15	20	163	10	16.3	
7														
8														
TOTAL	19	39	39	17	34	34	37	32	31	40	322	10	32.2	

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	6	0	0	0	0	5	5	0	0	16	10	1.60
4	5	0	5	4	6	6	0	0	4	6	36	10	3.60
5	10	13	12	10	11	13	13	11	11	14	118	10	11.8
6	0	18	18	16	14	17	19	16	17	16	151	10	15.1
7													
8													
TOTAL	15	37	35	30	31	36	37	32	32	36	321	10	32.1

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	5	0	0	4	0	5	5	0	0	19	10	1.90
4	4	1	6	5	0	4	0	0	5	4	29	10	2.90
5	9	13	11	13	11	9	11	9	10	11	107	10	10.7
6	0	25	16	13	13	15	19	17	16	14	148	10	14.8
7													
8													
TOTAL	13	44	33	31	28	28	35	31	31	29	303	10	30.3

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	24 %	1	0.87500	1.20940
2	24 %	2	1.00000	1.39310
2	24 %	3	1.00000	1.39310
2	24 %	4	1.00000	1.39310
2	24 %	5	1.00000	1.39310
3	33 %	1	1.00000	1.39310
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	1.00000	1.39310
4	44 %	2	1.00000	1.39310
4	44 %	3	1.00000	1.39310
4	44 %	4	1.00000	1.39310
4	44 %	5	1.00000	1.39310
5	58 %	1	0.87500	1.20940
5	58 %	2	1.00000	1.39310
5	58 %	3	1.00000	1.39310
5	58 %	4	1.00000	1.39310
5	58 %	5	1.00000	1.39310
6	77 %	1	1.00000	1.39310
6	77 %	2	1.00000	1.39310
6	77 %	3	1.00000	1.39310
6	77 %	4	1.00000	1.39310
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))
<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	24 %	25.00	16.00	5.00	
3	33 %	27.50	16.00	5.00	
4	44 %	27.50	16.00	5.00	
5	58 %	25.00	16.00	5.00	
6	77 %	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 = 0.03547 W = 0.9768 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 7.067 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	0.004545	0.000909	0.615	
Within (Error)	24	0.03547	0.001478		
Total	29	0.04001			
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	0.526	0.526			
2	24 %	0.5362	0.5362	-0.4195		
3	33 %	0.5472	0.5472	-0.8719		
4	44 %	0.5474	0.5474	-0.8801		
5	58 %	0.5164	0.5164	0.3948		
6	77 %	0.52	0.52	0.2468		
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	0.05738	10.9	-0.0102	
3	33 %	5	0.05738	10.9	-0.0212	
4	44 %	5	0.05738	10.9	-0.0214	
5	58 %	5	0.05738	10.9	0.0096	
6	77 %	5	0.05738	10.9	0.006	

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 style="text-align: center;"> D = 0.1859 D* = 1.459 Critical D* = 1.035 (alpha = 0.01, N = 60) </p> <p style="text-align: center;">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 %	136.50	75.00	10.00	
3	33 %	119.50	75.00	10.00	
4	44 %	134.50	75.00	10.00	
5	58 %	137.00	75.00	10.00	
6	77 %	121.50	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	567.1	113.4	1.638	
Within (Error)	54	3739	69.24		
Total	59	4306			
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	24.4	24.4			
2	24 %	32.5	32.5	-2.177		
3	33 %	26.8	26.8	-0.6449		
4	44 %	32.2	32.2	-2.096		
5	58 %	32.1	32.1	-2.069		
6	77 %	30.3	30.3	-1.585		
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	8.596	35.2	-8.1	
3	33 %	10	8.596	35.2	-2.4	
4	44 %	10	8.596	35.2	-7.8	
5	58 %	10	8.596	35.2	-7.7	
6	77 %	10	8.596	35.2	-5.9	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.526	-	
24 %	0.536	-1.90	PASS
33 %	0.547	-3.99	PASS
44 %	0.547	-3.99	PASS
58 %	0.516	1.90	PASS
77 %	0.520	1.14	PASS

Limit = 12

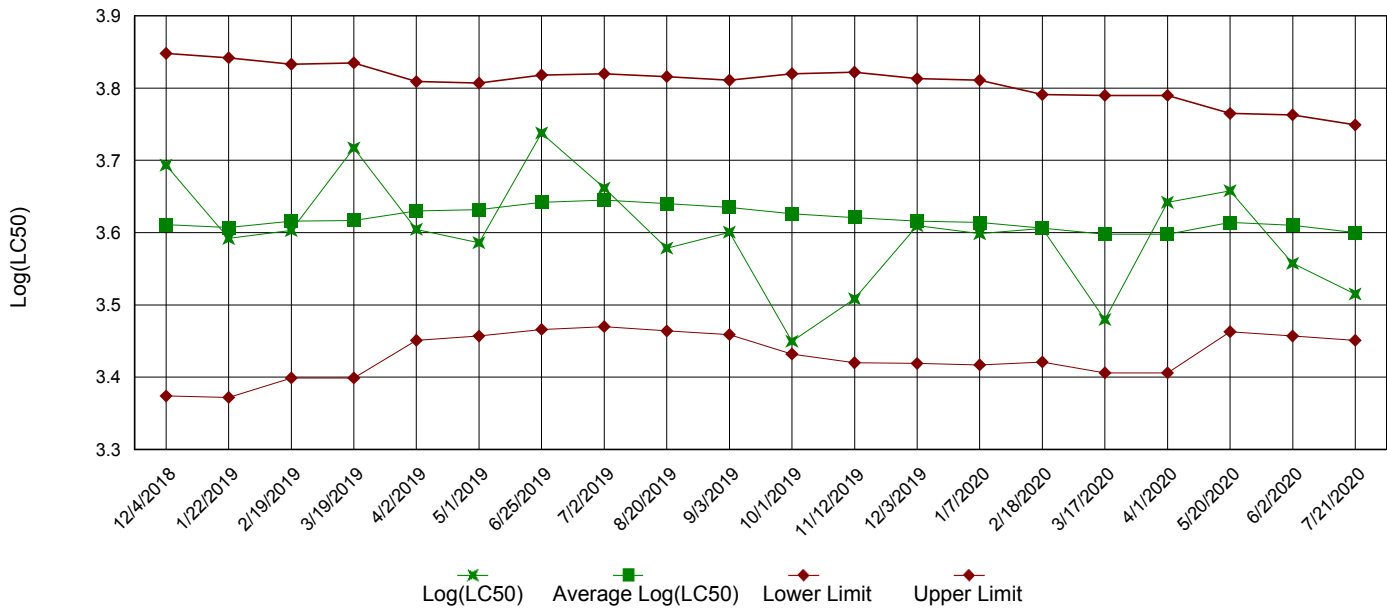
NOEC = 77 %

LOEC = 77 %

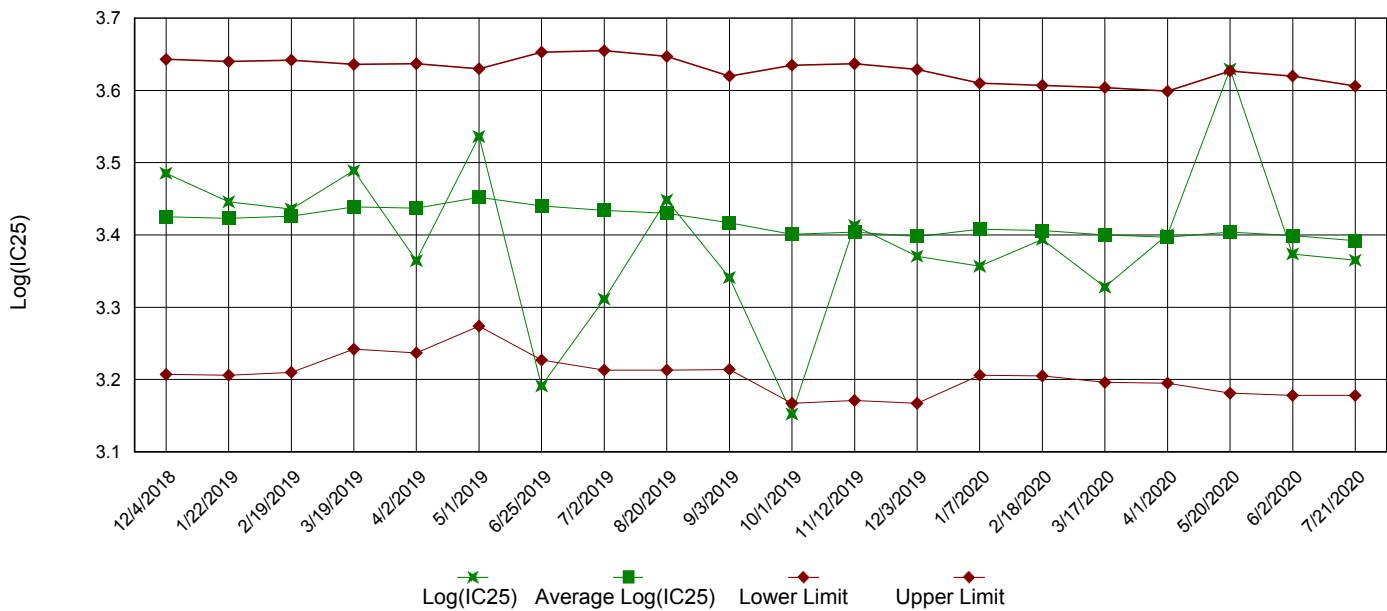
Appendix A3: Test 1000.0

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

LC50 Survival Data

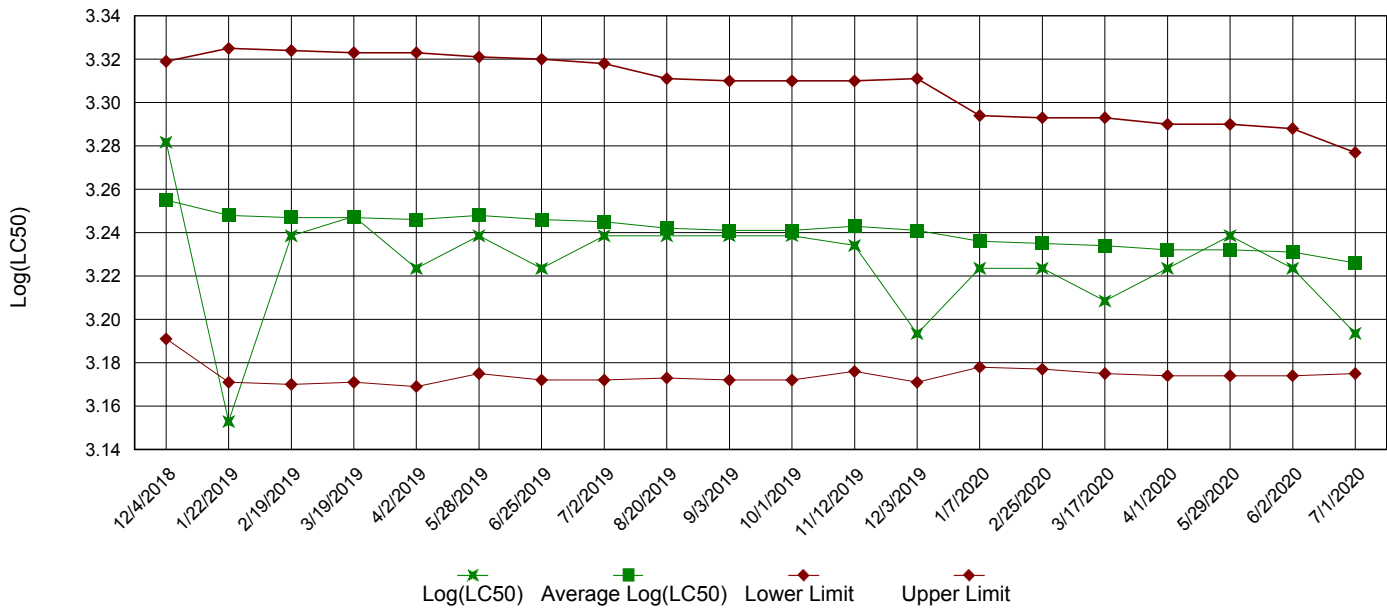


IC25 Growth Data

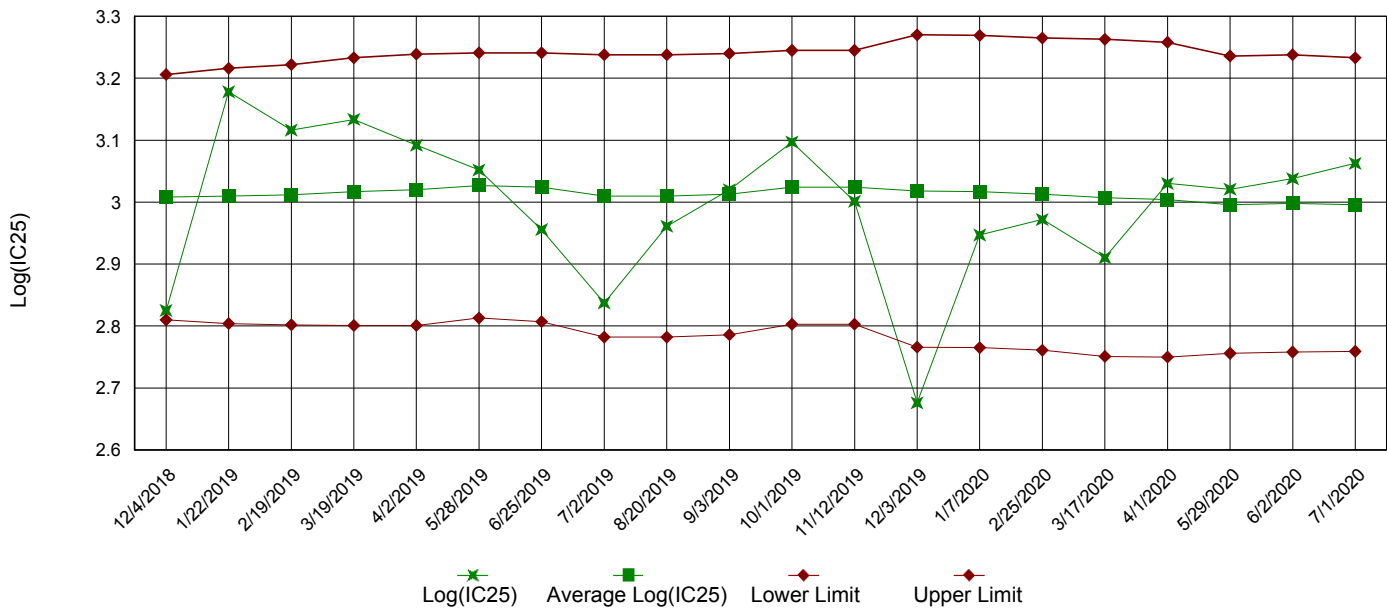


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: August 11, 2020 at 0911

Date and Time Test Terminated: August 18, 2020 at 0840

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	100	100	100	100	0.00
24 %	87.5	100	100	100	100	100	97.5	97.5	5.73
33 %	100	100	100	100	100	100	100	100	0.00
44 %	100	100	100	100	100	100	100	100	0.00
58 %	87.5	100	100	100	100	100	100	97.5	5.73
77 %	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	0.575	0.506	0.529	0.501	0.519	0.526	5.61
24 %	0.498	0.542	0.560	0.552	0.529	0.536	4.53
33 %	0.482	0.545	0.592	0.552	0.565	0.547	7.42
44 %	0.552	0.526	0.556	0.538	0.565	0.547	2.82
58 %	0.482	0.498	0.595	0.478	0.529	0.516	9.35
77 %	0.465	0.471	0.505	0.581	0.578	0.52	10.9

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	(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: 9.35 (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

Test Initiated: DATE: August 11, 2020 TIME: 0911
 Test Terminated: DATE: August 18, 2020 TIME: 0840

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	7.5	7.4	7.4	7.7	7.3	7.4
Final	6.5	5.8	6.5	7.0	6.3	6.7	6.2
pH Initial	7.8	7.8	7.8	7.6	7.7	7.7	7.7
Final	7.7	7.3	7.5	7.5	7.4	7.5	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
24 %							
D.O. Initial	7.0	7.6	7.6	7.2	8.0	7.4	7.6
Final	6.5	5.9	6.3	7.1	6.0	6.4	6.6
pH Initial	7.7	8.0	7.7	7.7	7.7	7.6	7.7
Final	7.6	7.3	7.4	7.4	7.4	7.5	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
33 %							
D.O. Initial	7.3	7.8	7.7	7.4	8.4	7.8	7.7
Final	7.0	6.0	6.2	6.7	5.9	6.4	6.8
pH Initial	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Final	7.6	7.3	7.4	7.5	7.4	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
44 %							
D.O. Initial	7.3	7.5	7.6	7.3	8.1	7.3	7.5
Final	6.6	5.9	6.3	7.1	6.3	6.6	7.5
pH Initial	7.6	7.7	7.6	7.6	7.6	7.6	7.6
Final	7.6	7.4	7.5	7.6	7.5	7.5	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
58 %							
D.O. Initial	7.4	7.5	7.8	7.4	8.0	7.4	7.4
Final	6.5	5.9	6.4	6.8	6.5	6.4	7.4
pH Initial	7.5	7.6	7.6	7.6	7.5	7.6	7.6
Final	7.6	7.3	7.4	7.5	7.4	7.4	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
77 %							
D.O. Initial	7.3	7.6	7.9	7.4	8.2	7.4	7.4
Final	6.8	5.8	6.7	7.2	6.0	6.6	6.6
pH Initial	7.5	7.6	7.6	7.5	7.5	7.5	7.5
Final	7.6	7.4	7.6	7.5	7.4	7.5	7.6

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	52	280	<0.05	Plant Effluent 09-AUG-20
41	43	300	<0.05	Plant Effluent 11-AUG-20
36	38	320	<0.05	Plant Effluent 11-AUG-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	45	170	<0.05	247488-1

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: August 11, 2020 at 1110

Date and Time Test Terminated: August 17, 2020 at 1008

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	11	14	11	19	15	13
B	35	42	37	39	37	44
C	32	33	36	39	35	33
D	31	41	13	17	30	31
E	28	33	31	34	31	28
F	12	32	33	34	36	28
G	31	34	32	37	37	35
H	23	27	33	32	32	31
I	30	33	26	31	32	31
J	11	36	16	40	36	29
Mean per Adult	24.4	32.5	26.8	32.2	32.1	30.3
Mean per Surviving Adult	24.4	32.5	26.8	32.2	32.1	30.3
CV %	39.0	24.1	36.6	25.1	20.4	25.3

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	(58 %)	<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	(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 (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: 39 (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

Test Initiated: DATE: August 11, 2020 TIME: 1110
Test Terminated: DATE: August 17, 2020 TIME: 1008

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	7.5	7.4	7.4	7.7	7.3	7.4
Final	7.3	7.6	7.8	7.9	7.6	7.5	--
pH Initial	7.8	7.8	7.8	7.6	7.7	7.7	7.7
Final	8.2	8.1	8.1	8.2	8.2	8.3	--

DILUTION	DAY						
	1	2	3	4	5	6	7
24 %							
D.O. Initial	7.0	7.6	7.6	7.2	8.0	7.4	7.6
Final	7.4	7.7	7.4	8.5	7.6	8.3	--
pH Initial	7.7	8.0	7.7	7.7	7.7	7.6	7.7
Final	8.3	8.2	8.3	8.3	8.3	8.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
33 %							
D.O. Initial	7.3	7.8	7.7	7.4	8.4	7.8	7.7
Final	8.1	8.1	7.8	8.8	8.1	8.5	--
pH Initial	7.6	7.6	7.6	7.6	7.6	7.6	7.6
Final	8.3	8.3	8.3	8.3	8.4	8.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
44 %							
D.O. Initial	7.3	7.5	7.6	7.3	8.1	7.3	7.5
Final	7.5	7.7	7.5	8.0	7.5	8.0	--
pH Initial	7.6	7.7	7.6	7.6	7.6	7.6	7.6
Final	8.2	8.1	8.2	8.3	8.2	8.3	--

DILUTION	DAY						
	1	2	3	4	5	6	7
58 %							
D.O. Initial	7.4	7.5	7.8	7.4	8.0	7.4	7.4
Final	7.6	7.6	7.5	8.0	7.6	7.7	--
pH Initial	7.5	7.6	7.6	7.6	7.5	7.6	7.6
Final	8.3	8.2	8.3	8.3	8.3	8.5	--

DILUTION	DAY						
	1	2	3	4	5	6	7
77 %							
D.O. Initial	7.3	7.6	7.9	7.4	8.2	7.4	7.4
Final	7.3	7.6	7.7	8.0	7.4	7.5	--
pH Initial	7.5	7.6	7.6	7.5	7.5	7.5	7.5
Final	8.3	8.2	8.3	8.3	8.3	8.4	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	52	280	<0.05	Plant Effluent 09-AUG-20
41	43	300	<0.05	Plant Effluent 11-AUG-20
36	38	320	<0.05	Plant Effluent 11-AUG-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	45	170	<0.05	247488-1



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: City of Hot Springs		P.O. Number <i>2020-622</i>		ANALYSIS REQUESTED		AIC Control Number: <i>277628</i>	
Project Reference: Quarterly Bio-Monitoring		Matrix		Bio-Monitoring		AIC Proposal Number:	
Project Manager: Harold Mauldin		Composite		Water		Carrier:	
Sampled By: <i>SR</i>		Date/Time Collected		Number of Bottles		Received Temperature °C	
AIC Sample Identification		<i>08-07-20 @ COM-210</i>		<i>3</i>		<i>2.0</i>	
NO. Plant Effluent		<input checked="" type="checkbox"/>		<input checked="" type="checkbox"/>		Remarks	
G= Glass P=Plastic NO=None S=Sulfuric Acid pH2 V=VOA Vials		P		NO		Field pH Calibration on @ Buffer	
N=Nitric Acid pH2 H=HCl to pH2 B=NaOH to pH12		Date/Time		Received By:		Date/Time	
T=Sodium Thiosulfate Z=Zinc Acetate A=(NH4)2NH4OH		<i>08-10-20 @ 0745</i>		<i>Bill Mauldin</i>		<i>8-10-2020 @ 0745</i>	
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS		Date/Time		Received By:		Date/Time	
Expedited results requested by:		<i>8-10-2020 @ 0910</i>		<i>[Signature]</i>		<i>8-10-20 @ 0910</i>	
Who should AIC contact with questions:		Relinquished By:		Relinquished By:		Comments	
Amanda Cates		<i>S. Sanders</i>		<i>Bill Mauldin</i>			
Phone: 501-262-1881		Relinquished By:		Relinquished By:			
Fax: 501-262-0339		<i>Bill Mauldin</i>		<i>[Signature]</i>			
Report Attention to: Harold Mauldin		Report Address to: 320 Davidson Drive		Hot Springs, Ar. 71901		E-Mail Address: Hmauldin@cityhs.net	



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: City of Hot Springs		P.O. Number <i>2020-622</i>	
Project Reference: Quarterly Bio-Monitoring		ANALYSIS REQUESTED	
Project Manager: Harold Mauldin		Bio-Monitoring	
Sampled By: <i>SR</i>	AIC	Number of Bottles	
	No.	Water	
Sample Identification	Date/Time Collected	Composite	
Plant Effluent	<i>08-13-20 @ 0937</i>	X	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			
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS		Date/Time	Date/Time
Expedited results requested by: Who should AIC contact with questions: Amanda Cates Phone: 501-262-1881 Fax: 501-262-0339 Report Attention to: Harold Mauldin Report Address to: 320 Davidson Drive Hot Springs, Ar. 71901 E-Mail Address: Hmauldin@cityhs.net		<i>08-14-20 @ 0806</i>	<i>08-14-20 @ 0937</i>
Received By: <i>Bill Mauldin</i>		Received By: <i>Bill Mauldin</i>	Date/Time: <i>08-14-20 @ 0937</i>
Relinquished By: <i>S. Ryders</i>		Relinquished By: <i>Bill Mauldin</i>	Date/Time: <i>08-14-20 @ 0937</i>
Comments			

AIC Control Number: *247628*

AIC Proposal Number:

Carrier:

Received Temperature °c

0.3

Remarks

Field pH Calibration

on _____ @ _____

Buffer

Date/Time

08-14-20 @ 0937

Date/Time

08-14-20 @ 0937



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: City of Hot Springs		P.O. Number <i>2020-622</i>	
Project Reference: Quarterly Bio-Monitoring		Matrix	
Project Manager: Harold Mauldin		Number of Bottles	
Sampled By: <i>SK</i>	Sample Identification	Date/Time Collected	Remarks
AIC No.	Plant Effluent	<i>08-11-20 @ 0900-2400</i>	<i>1.6</i>
G=Glass P=Plastic NO=None S=Sulfuric Acid pH2 V=VOA Vials		Water	Received Temperature °c
N=Nitric Acid pH2 H=HCl to pH2 B=NaOH to pH12		Composite	
T=Sodium Thiosulfate Z=Zinc Acetate A=(NH4)2NH4OH		X	Field pH Calibration on _____ @ _____
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS		X	
Expedited results requested by: Who should AIC contact with questions: Amanda Cates Phone: 501-262-1881 Fax: 501-262-0339 Report Attention to: Harold Mauldin Report Address to: 320 Davidson Drive E-Mail Address: Hmauldin@cityhs.net		Relinquished By: <i>S. Rynders</i>	Received By: <i>Bill Spurr</i>
Date/Time		Date/Time	Date/Time
		<i>08-12-20 @ 0906</i>	<i>8-12-20 @ 0906</i>
Relinquished By: <i>Bill Spurr</i>		Received By: <i>Bill Spurr</i>	Date/Time <i>8-12-20</i>
Comments			