May 11, 2020

Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 244735-1

Prepared for:

Mr. Harold Mauldin
City of Hot Springs
320 Davidson Drive
Hot Springs, AR 71901

Prepared by:

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

City of Hot Springs
ATTN: Mr. Harold Mauldin
320 Davidson Drive
Hot Springs, AR 71901

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

John Overbey
Chief Operating Officer

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

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

City of Hot Springs
ATTN: Mr. Harold Mauldin
wwlab@cityhs.net

City of Hot Springs
ATTN: Mr. Gordon Yates
gyates@cityhs.net

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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	0.487	PASS
Control Growth CV < or = 40%	5.34	PASS
Growth Minimum Significant Difference 12 to 30%	14.9	PASS
Critical Dilution CV < or = 40%	11.2	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.5	PASS
Control CV < or = 40% per Surviving Female	9.88	PASS
Reproduction Minimum Significant Difference 13 to 47%	13.0	PASS
Critical Dilution CV < or = 40%	10.1	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)	7.1	8.1	7.2
pH (standard units)	6.6	6.9	6.8
Alkalinity (mg/l as CaCO3)	21	23	23
Hardness (mg/l as CaCO3)	28	32	28
Conductivity (umhos/cm)	170	250	190
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.19	0.12

2. Dilution Water Samples:
   244379-1

Analysis		
Dissolved oxygen (mg/l)	7.5	7.0
pH (standard units)	7.4	7.4
Alkalinity (mg/l as CaCO3)	32	31
Hardness (mg/l as CaCO3)	43	42
Conductivity (umhos/cm)	170	180
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:	April 28, 2020 at 1005
Date & Time Test Terminated:	May 05, 2020 at 0900
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:	April 28, 2020 at 1100
Date & Time Test Terminated:	May 04, 2020 at 1000
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 Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

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 April 01, 2020 at 0920 to April 08, 2020 at 0920
The results were as follows: (Control No. 243967-1.)

Survival LC-50: 4385 mg/l
Growth IC-25: 2514 mg/l
Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on April 01, 2020 at 1110 to April 07, 2020 at 1118
The results were as follows: (Control No. 243967-2.)

Survival LC-50: 1673.1 mg/l
Reproduction IC-25: 1072 mg/l
Reproduction PMSD: 14.2

V. Organism History

Pimephales promelas (Fathead minnow)

Date: April 28, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: April 28, 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

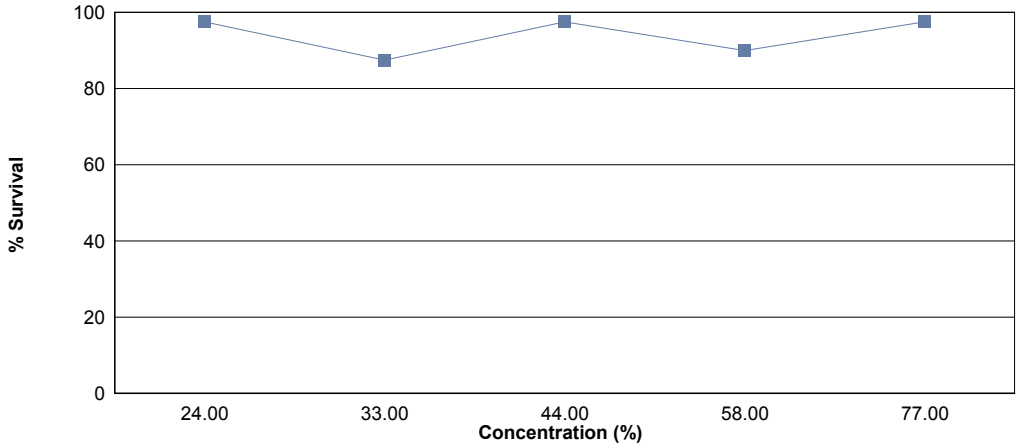
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 April 28, 2020 at 1005 and continued through May 05, 2020 at 0900. 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	100	0.487
24 %	97.5	0.438
33 %	87.5	0.436
44 %	97.5	0.518
58 %	90.0	0.439
77 %	97.5	0.492

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

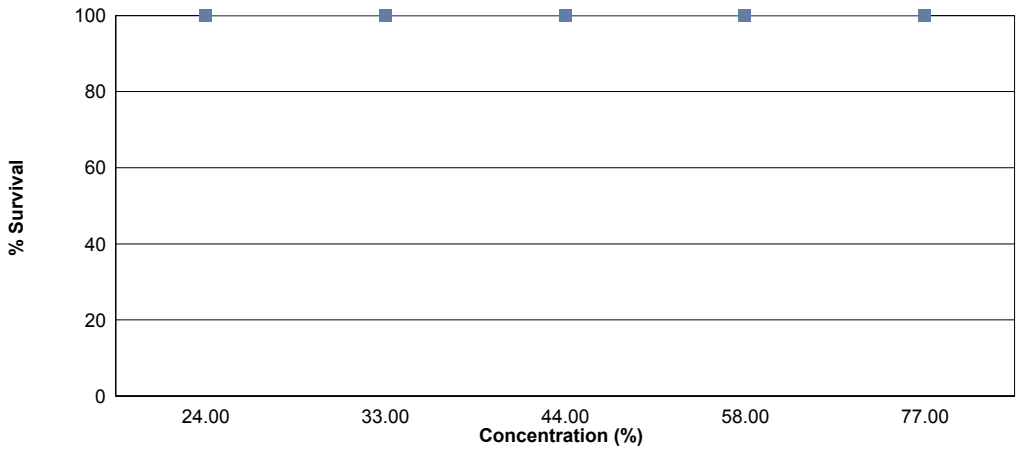
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 April 28, 2020 at 1100 and continued through May 04, 2020 at 1000. 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 *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.5
24 %	100	28.1
33 %	100	28.9
44 %	100	29.4
58 %	100	28.1
77 %	100	28.2

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 28, 2020 at 1005
Date and Time Test Terminated: May 05, 2020 at 0900

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: April 28, 2020 at 1005
Test Terminated: May 05, 2020 at 0900

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92864	.93271	0.00407	8	0.509
	B	.92883	.93261	0.00378	8	0.472
	C	.92415	.92774	0.00359	8	0.449
	D	.92719	.93120	0.00401	8	0.501
	E	.93259	.93664	0.00405	8	0.506
24 %	A	.92840	.93155	0.00315	8	0.394
	B	.93036	.93376	0.00340	8	0.425
	C	.93065	.93431	0.00366	8	0.458
	D	.92688	.93061	0.00373	8	0.466
	E	.92168	.92527	0.00359	8	0.449
33 %	A	.92326	.92595	0.00269	8	0.336
	B	.92143	.92521	0.00378	8	0.472
	C	.91984	.92389	0.00405	8	0.506
	D	.91953	.92292	0.00339	8	0.424
	E	.92333	.92688	0.00355	8	0.444
44 %	A	.92357	.92758	0.00401	8	0.501
	B	.92914	.93301	0.00387	8	0.484
	C	.92349	.92811	0.00462	8	0.578
	D	.92865	.93282	0.00417	8	0.521
	E	.92566	.92971	0.00405	8	0.506
58 %	A	.92083	.92498	0.00415	8	0.519
	B	.91914	.92257	0.00343	8	0.429
	C	.92939	.93255	0.00316	8	0.395
	D	.92808	.93131	0.00323	8	0.404
	E	.92837	.93196	0.00359	8	0.449
77 %	A	.92754	.93209	0.00455	8	0.569
	B	.92606	.92971	0.00365	8	0.456
	C	.92669	.92985	0.00316	8	0.395
	D	.93006	.93404	0.00398	8	0.498
	E	.92662	.93097	0.00435	8	0.544

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 28, 2020 at 1100
Date and Time Test Terminated: May 04, 2020 at 1000

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	0	4	4	4	5	4	4	4	4	37	10	3.70
4	0	2	0	0	0	0	0	0	0	0	2	10	0.200
5	10	9	9	9	8	10	8	8	8	9	88	10	8.80
6	10	8	9	11	11	13	10	12	12	12	108	10	10.8
7													
8													
TOTAL	24	19	22	24	23	28	22	24	24	25	235	10	23.5

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	4	4	4	4	0	6	4	3	4	5	38	10	3.80
4	0	0	0	0	6	0	0	0	0	0	6	10	0.600
5	8	8	10	11	10	10	11	11	9	10	98	10	9.80
6	15	13	12	14	11	15	18	15	12	14	139	10	13.9
7													
8													
TOTAL	27	25	26	29	27	31	33	29	25	29	281	10	28.1

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	5	0	6	6	4	4	5	5	5	4	44	10	4.40
4	0	4	0	0	0	0	0	0	0	0	4	10	0.400
5	10	9	10	9	9	10	10	9	9	0	85	10	8.50
6	14	15	16	18	17	15	16	16	15	14	156	10	15.6
7													
8													
TOTAL	29	28	32	33	30	29	31	30	29	18	289	10	28.9

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 28, 2020 at 1100
Date and Time Test Terminated: May 04, 2020 at 1000

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	5	4	4	3	4	5	5	4	4	43	10	4.30
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	10	10	10	10	10	11	11	10	10	101	10	10.1
6	13	14	17	17	16	13	16	15	15	14	150	10	15.0
7													
8													
TOTAL	27	29	31	31	29	27	32	31	29	28	294	10	29.4

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	4	4	6	5	4	4	5	6	3	5	46	10	4.60
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	8	9	10	10	10	10	10	11	7	10	95	10	9.50
6	14	13	11	15	17	14	14	16	13	13	140	10	14.0
7													
8													
TOTAL	26	26	27	30	31	28	29	33	23	28	281	10	28.1

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	4	3	4	4	6	5	4	4	4	3	41	10	4.10
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	7	10	9	11	11	11	10	10	8	96	10	9.60
6	11	16	14	14	16	14	19	14	15	12	145	10	14.5
7													
8													
TOTAL	24	26	28	27	33	30	34	28	29	23	282	10	28.2

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	0.75000	1.04720
3	33 %	2	1.00000	1.39310
3	33 %	3	1.00000	1.39310
3	33 %	4	0.87500	1.20940
3	33 %	5	0.75000	1.04720
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	0.87500	1.20940
5	58 %	1	0.87500	1.20940
5	58 %	2	1.00000	1.39310
5	58 %	3	0.87500	1.20940
5	58 %	4	1.00000	1.39310
5	58 %	5	0.75000	1.04720
6	77 %	1	1.00000	1.39310
6	77 %	2	1.00000	1.39310
6	77 %	3	0.87500	1.20940
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))
D = 0.2861		
W = 0.8821		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

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 %	20.00	16.00	5.00	
4	44 %	25.00	16.00	5.00	
5	58 %	20.00	16.00	5.00	
6	77 %	25.00	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.05685		
W = 0.9626		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 5.764		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.03087	0.006173	2.606
Within (Error)	24	0.05685	0.002369	
Total	29	0.08772		

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	0.4874	0.4874		
2	24 %	0.4384	0.4384	1.592	
3	33 %	0.4364	0.4364	1.657	
4	44 %	0.518	0.518	-0.9941	
5	58 %	0.4392	0.4392	1.566	
6	77 %	0.4924	0.4924	-0.1624	

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	0.07265	14.9	0.049
3	33 %	5	0.07265	14.9	0.051
4	44 %	5	0.07265	14.9	-0.0306
5	58 %	5	0.07265	14.9	0.0482
6	77 %	5	0.07265	14.9	-0.005

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
D = 0.0814		
D* = 0.6386		
Critical D* = 1.035	(alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.427		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	225.4	45.08	5.123
Within (Error)	54	475.2	8.8	
Total	59	700.6		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2					No Transformation
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	23.5	23.5		
2	24 %	28.1	28.1	-3.467	
3	33 %	28.9	28.9	-4.07	
4	44 %	29.4	29.4	-4.447	
5	58 %	28.1	28.1	-3.467	
6	77 %	28.2	28.2	-3.543	

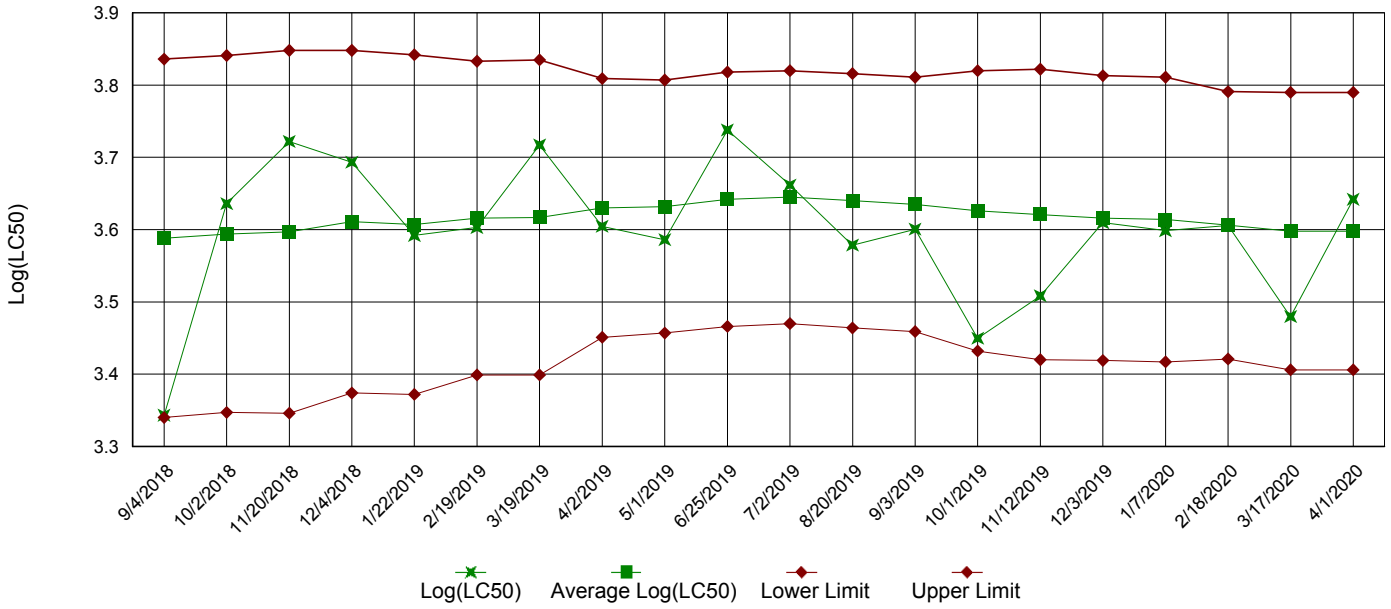
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)

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	3.065	13	-4.6
3	33 %	10	3.065	13	-5.4
4	44 %	10	3.065	13	-5.9
5	58 %	10	3.065	13	-4.6
6	77 %	10	3.065	13	-4.7

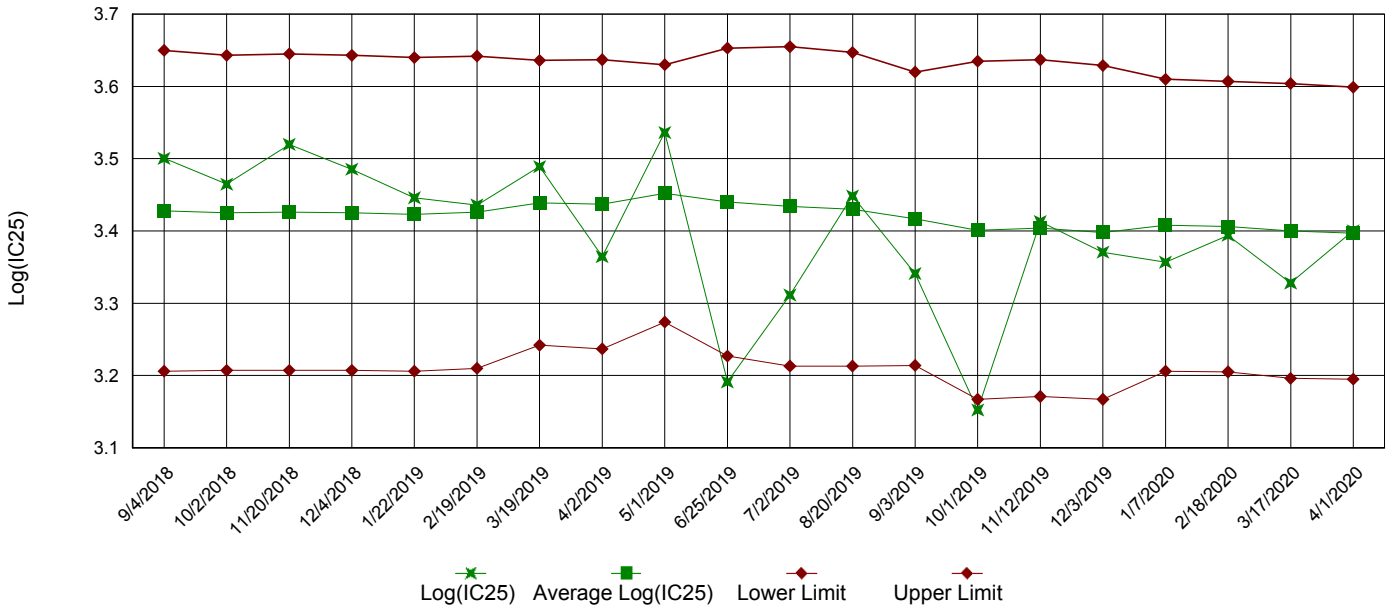
Appendix A3: Test 1000.0

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

LC50 Survival Data



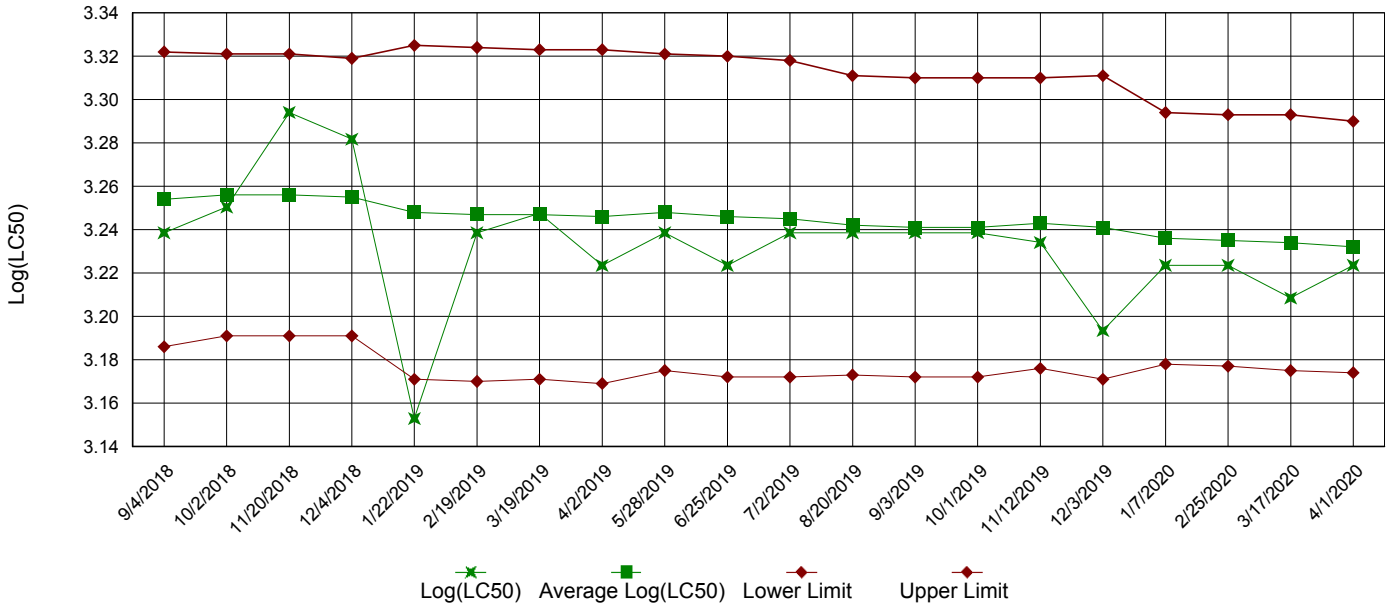
IC25 Growth Data



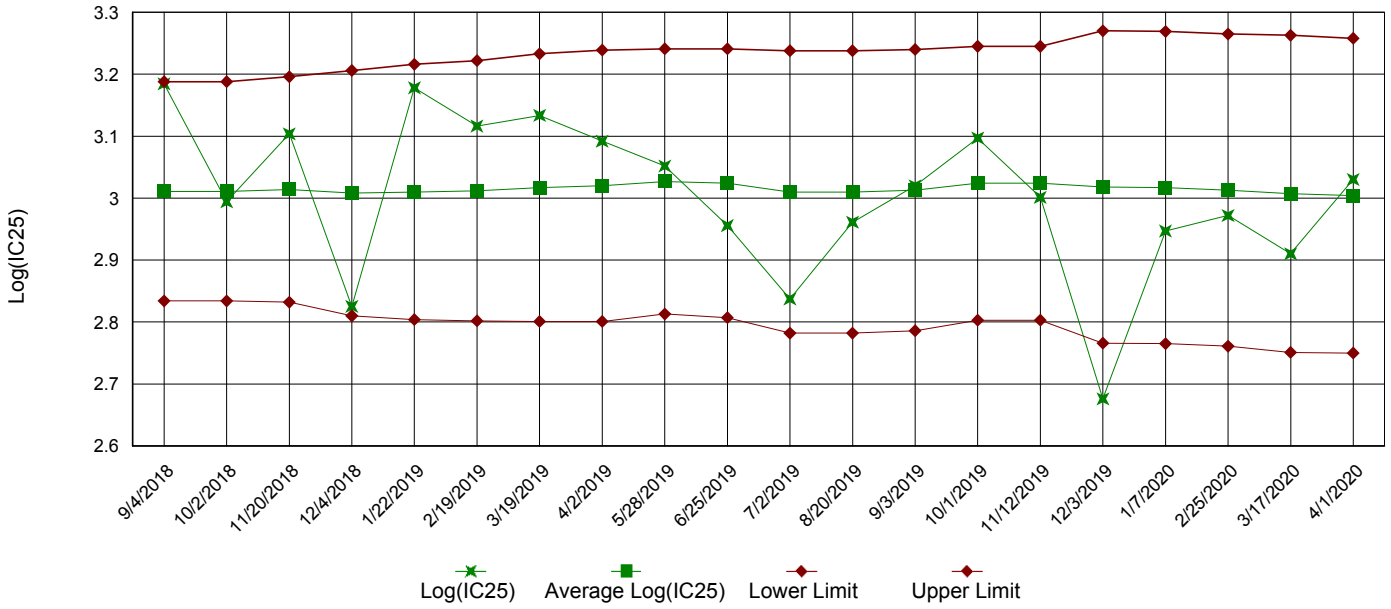
Appendix A3: Test 1002.0

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: April 28, 2020 at 1005
Date and Time Test Terminated: May 05, 2020 at 0900
Dilution water used: 244379-1

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
24 %	87.5	100	100	100	100	100	100	97.5	5.73
33 %	75.0	100	100	87.5	75.0	100	97.5	87.5	14.3
44 %	100	100	100	100	87.5	100	100	97.5	5.73
58 %	87.5	100	87.5	100	75.0	100	100	90.0	11.6
77 %	100	100	87.5	100	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.509	0.472	0.449	0.501	0.506	0.487	5.34
24 %	0.394	0.425	0.458	0.466	0.449	0.438	6.66
33 %	0.336	0.472	0.506	0.424	0.444	0.436	14.7
44 %	0.501	0.484	0.578	0.521	0.506	0.518	6.96
58 %	0.519	0.429	0.395	0.404	0.449	0.439	11.2
77 %	0.569	0.456	0.395	0.498	0.544	0.492	14.1

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 %) ____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION (NA) ____YES ____ 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 %) ____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION (NA) ____YES ____ 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: 11.2 (TQP6C)

10. Sublethality for this test: 77 % (51714 or 51714S)

AMERICAN INTERPLEX CORPORATION LABORATORIES

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, 345

Test Initiated: DATE: April 28, 2020 TIME: 1005
Test Terminated: DATE: May 05, 2020 TIME: 0900

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.8	7.0	7.1	7.2	6.9	7.4
	Final	6.6	5.7	6.1	6.2	6.0	6.1	5.9
pH	Initial	7.4	7.5	7.4	7.2	7.3	7.2	7.3
	Final	7.4	7.2	7.4	7.4	7.3	7.3	7.2

DILUTION 24 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.9	7.4	7.1	7.6	6.9	7.6
	Final	6.4	6.1	5.3	6.7	5.8	5.9	6.2
pH	Initial	7.2	7.3	7.4	7.2	7.3	7.0	7.3
	Final	7.4	7.2	7.2	7.3	7.2	7.3	7.2

DILUTION 33 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.0	6.8	6.9	6.8	7.1	6.6	7.5
	Final	6.6	6.1	5.8	5.9	5.6	5.9	6.4
pH	Initial	7.1	7.1	7.2	7.1	7.1	7.0	7.2
	Final	7.3	7.1	7.2	7.2	7.2	7.2	7.2

DILUTION 44 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.4	7.5	7.2	7.5	6.9	7.0
	Final	6.2	5.8	5.7	6.0	5.7	5.7	6.0
pH	Initial	7.1	7.2	7.2	7.1	7.2	7.0	7.2
	Final	7.4	7.1	7.1	7.2	7.2	7.2	7.2

DILUTION 58 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.2	7.4	7.2	7.6	7.1	7.2
	Final	6.2	5.7	5.9	6.2	5.8	5.6	6.4
pH	Initial	7.0	7.2	7.1	7.0	7.1	7.0	7.2
	Final	7.4	7.1	7.2	7.2	7.2	7.2	7.4

DILUTION 77 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.2	7.4	6.3	7.8	6.7	7.0
	Final	6.4	5.8	5.8	6.7	5.9	6.1	5.8
pH	Initial	6.9	7.1	7.1	7.0	7.0	6.9	7.1
	Final	7.4	7.1	7.1	7.3	7.1	7.2	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
21	28	170	<0.05	Plant Effluent 26-APR-20
23	32	250	<0.05	Plant Effluent 28-APR-20
23	28	190	<0.05	Plant Effluent 30-APR-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	43	170	<0.05	
31	42	180	<0.05	

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: April 28, 2020 at 1100
Date and Time Test Terminated: May 04, 2020 at 1000
Dilution water used: 244379-1

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	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

		Percent Effluent				
Replicates	Control	24 %	33 %	44 %	58 %	77 %
A	24	27	29	27	26	24
B	19	25	28	29	26	26
C	22	26	32	31	27	28
D	24	29	33	31	30	27
E	23	27	30	29	31	33
F	28	31	29	27	28	30
G	22	33	31	32	29	34
H	24	29	30	31	33	28
I	24	25	29	29	23	29
J	25	29	18	28	28	23
Mean per Adult	23.5	28.1	28.9	29.4	28.1	28.2
Mean per Surviving Adult	23.5	28.1	28.9	29.4	28.1	28.2
CV %	9.88	9.26	14.3	6.04	10.1	12.5

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 %)	YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	YES	NO

2. Dunnett's 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 %)	YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	YES	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: 10.1 (TQP3B)

10. Sublethality for this test: 77 % (51710 or 51710Q)

AMERICAN INTERPLEX CORPORATION LABORATORIES

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, 345

Test Initiated: DATE: April 28, 2020 TIME: 1100
Test Terminated: DATE: May 04, 2020 TIME: 1000

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.8	7.0	7.1	7.2	6.9	7.4
	Final	7.2	7.5	7.4	7.6	7.4	7.6	--
pH	Initial	7.4	7.5	7.4	7.2	7.3	7.2	7.3
	Final	7.8	7.8	7.8	7.9	7.6	7.8	--

DILUTION 24 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.9	7.4	7.1	7.6	6.9	7.6
	Final	7.4	7.4	7.9	7.3	7.1	7.8	--
pH	Initial	7.2	7.3	7.4	7.2	7.3	7.0	7.3
	Final	7.8	7.9	7.9	8.0	7.6	7.8	--

DILUTION 33 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.0	6.8	6.9	6.8	7.1	6.6	7.5
	Final	7.4	7.5	7.1	7.8	7.1	7.9	--
pH	Initial	7.1	7.1	7.2	7.1	7.1	7.0	7.2
	Final	7.8	7.8	7.9	7.9	7.6	7.8	--

DILUTION 44 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.4	7.5	7.2	7.5	6.9	7.0
	Final	7.2	7.3	7.8	7.6	7.1	7.7	--
pH	Initial	7.1	7.2	7.2	7.1	7.2	7.0	7.2
	Final	7.8	7.8	7.9	7.8	7.6	7.8	--

DILUTION 58 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.2	7.4	7.2	7.6	7.1	7.2
	Final	7.2	7.1	7.8	7.6	7.2	7.7	--
pH	Initial	7.0	7.2	7.1	7.0	7.1	7.0	7.2
	Final	7.8	7.8	7.8	7.8	7.6	7.8	--

DILUTION 77 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.2	7.4	6.3	7.8	6.7	7.0
	Final	7.1	6.8	7.6	7.7	7.0	7.8	--
pH	Initial	6.9	7.1	7.1	7.0	7.0	6.9	7.1
	Final	7.8	7.8	7.8	7.8	7.6	7.8	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
21	28	170	<0.05	Plant Effluent 26-APR-20
23	32	250	<0.05	Plant Effluent 28-APR-20
23	28	190	<0.05	Plant Effluent 30-APR-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	43	170	<0.05	
31	42	180	<0.05	

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: **City of Hot Springs**

P.O. Number: 2020-459

Project Reference: **Quarterly Bio-Monitoring**

Project Manager: **Harold Mauldin**

Sampled By: SR

ANALYSIS REQUESTED

AIC Control Number: 244735

AIC Proposal Number:

Carrier: BILL G.

Received Temperature °c: 1.3 / 1.7 / 3.3

AIC No.	Sample Identification	Date/Time Collected	Matrix: Composite	Matrix: Water	Number of Bottles	Bio- Monitoring	
1	**Plant Effluent**	04-26-20 0000-2400	X	X	3	X	

Remarks

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
		NO

Field pH Calibration
on_____@_____
Buffer

Turnaround Time Requested in: (Please Circle)
NORMAL or EXPEDITED IN _____DAYS
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**

Relinquished By:	Date/Time	Received By:	Date/Time
S. Ryders	04-27-20 0807	Bill Garner	4-27-2020 @ 0807
Bill Garner	4-27-2020 @ 0935	D. BROWN	4-27-20 0935

Comments

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: **City of Hot Springs**

P.O. Number: 2020-459

Project Reference: **Quarterly Bio-Monitoring**

Project Manager: **Harold Mauldin**

Sampled By: SK

AIC Control Number: 244735

AIC Proposal Number:

Carrier:

Received Temperature °c: 1.5/1.9/4.4

AIC No.	Sample Identification	Date/Time Collected	Matrix: Composite	Matrix: Water	Number of Bottles	ANALYSIS REQUESTED: Bio- Monitoring	
2	**Plant Effluent**	4-28-20 0000-2400	X	X	3	X	

Remarks

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	
		NO	

Field pH Calibration
on_____@_____
Buffer

Turnaround Time Requested in: (Please Circle)
NORMAL or EXPEDITED IN _____DAYS
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**

Relinquished By:	Date/Time	Received By:	Date/Time
S. Ryders	4-29-20 0755	B. Mauldin	4-29-2020 @ 0755
B. Mauldin	4-29-2020 @0947	D. BROWN	4-29-20 0947

Comments

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: **City of Hot Springs**

Project Reference: **Quarterly Bio-Monitoring**

Project Manager: **Harold Mauldin**

Sampled By: SR

P.O. Number: 2020-459

AIC Control Number: 244735

AIC Proposal Number:

Carrier:

Received Temperature °c: 1.1, 0.2, 0.6

AIC No.	Sample Identification	Date/Time Collected	Matrix: Composite	Matrix: Water	Number of Bottles	ANALYSIS REQUESTED: Bio- Monitoring		Remarks
3	**Plant Effluent**	4-30-20 0000-2400	X	X	3	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	
	NO	

Field pH Calibration
on _____ @ _____
Buffer

Turnaround Time Requested in: (Please Circle)
NORMAL or EXPEDITED IN _____ DAYS
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**

Relinquished By:	Date/Time	Received By:	Date/Time
S. Ryders SR	5-1-20 ~~5-1-20~~ 0819	B. Garner	5-1-2020 @ 0819
B. Garner	5-1-2020 @ 0953	DANNY BROWN	5-1-20 0953

Comments