



April 6, 2020

Biomonitoring Testing
for
Plant Effluent
City of Hot Springs

Control No. 243750-1

Prepared for:

Mr. Harold Mauldin
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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 44 %. **The sample PASSED lethal effects, however, FAILED sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 77 % effluent, which is above the critical dilution of 58 %. The NOEC for reproduction occurred at 77 % effluent, which is above the critical dilution of 58 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



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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	0.525	PASS
Control Growth CV < or = 40%	5.32	PASS
Growth Minimum Significant Difference 12 to 30%	8.31	BELOW
Critical Dilution CV < or = 40%	12.3	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	22.8	PASS
Control CV < or = 40% per Surviving Female	28.5	PASS
Reproduction Minimum Significant Difference 13 to 47%	16.2	PASS
Critical Dilution CV < or = 40%	8.43	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)	7.9	7.9	7.9
pH (standard units)	7.3	7.3	7.1
Alkalinity (mg/l as CaCO ₃)	61	55	50
Hardness (mg/l as CaCO ₃)	31	31	29
Conductivity (umhos/cm)	220	200	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	7.4	6.2

2. Dilution Water Samples:
Soft

Analysis	243360-1
Dissolved oxygen (mg/l)	7.1
pH (standard units)	7.7
Alkalinity (mg/l as CaCO ₃)	33
Hardness (mg/l as CaCO ₃)	42
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: March 24, 2020 at 1000
Date & Time Test Terminated: March 31, 2020 at 0845
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: March 24, 2020 at 1030
Date & Time Test Terminated: March 30, 2020 at 0945
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. Steel's Many-One Rank test was used to determine the No Observable Effects Concentration (NOEC) for growth. Dunnett's Test was used to calculate the PMSD.

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 March 17, 2020 at 1620 to March 24, 2020 at 1445

The results were as follows: (Control No. 243616-1.)

Survival LC-50: 3018 mg/l

Growth IC-25: 2128 mg/l

Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on March 17, 2020 at 1540 to March 24, 2020 at 1615

The results were as follows: (Control No. 243616-2.)

Survival LC-50: 1616.1 mg/l

Reproduction IC-25: 813.4 mg/l

Reproduction PMSD: 17

V. Organism History

Pimephales promelas (Fathead minnow)

Date: March 24, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: March 24, 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.

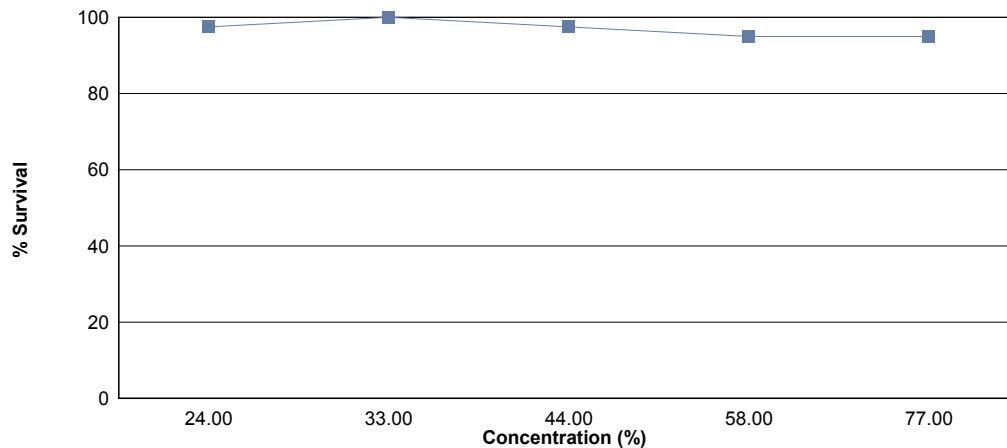
Effluent dilutions for this test were 24 %, 33 %, 44 %, 58 %, 77 % in accordance with the NPDES permit.

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

The test was initiated on March 24, 2020 at 1000 and continued through March 31, 2020 at 0845. 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 = 44 % 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	0.525
24 %	97.5	0.486
33 %	100	0.504
44 %	97.5	0.477 *
58 %	95.0	0.450 *
77 %	95.0	0.424 *

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

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

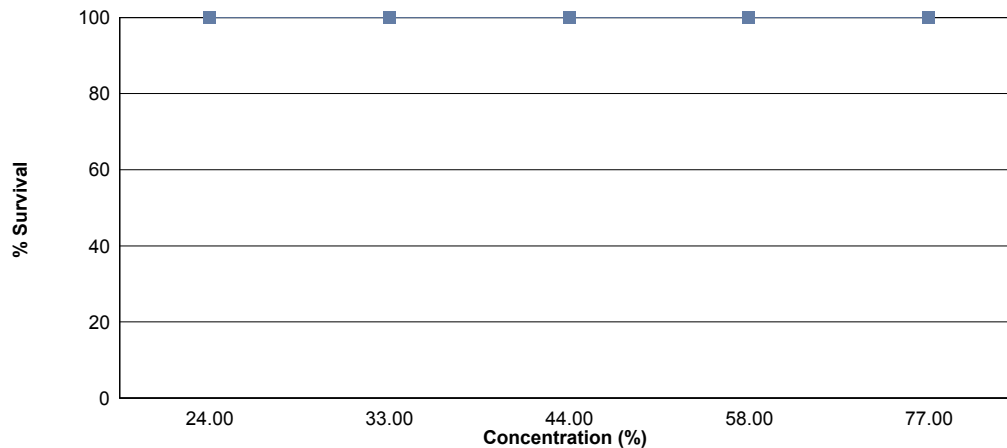
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 March 24, 2020 at 1030 and continued through March 30, 2020 at 0945. 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	22.8
24 %	100	28.9
33 %	100	27.5
44 %	100	28.7
58 %	100	25.9
77 %	100	28.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: March 24, 2020 at 1000

Date and Time Test Terminated: March 31, 2020 at 0845

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	8	8	8
	B	8	8	8	8	8	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
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	7	7
58 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	6	6
	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	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	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: March 24, 2020 at 1000

Test Terminated: March 31, 2020 at 0845

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93645	.94069	0.00424	8	0.530
	B	.92418	.92853	0.00435	8	0.544
	C	.92080	.92525	0.00445	8	0.556
	D	.92419	.92823	0.00404	8	0.505
	E	.92367	.92757	0.00390	8	0.488
24 %	A	.92626	.93012	0.00386	8	0.482
	B	.92578	.92969	0.00391	8	0.489
	C	.92992	.93402	0.00410	8	0.512
	D	.92910	.93281	0.00371	8	0.464
	E	.92825	.93211	0.00386	8	0.482
33 %	A	.92682	.93093	0.00411	8	0.514
	B	.92701	.93098	0.00397	8	0.496
	C	.92818	.93208	0.00390	8	0.488
	D	.93017	.93431	0.00414	8	0.518
	E	.93275	.93679	0.00404	8	0.505
44 %	A	.93018	.93404	0.00386	8	0.482
	B	.92822	.93195	0.00373	8	0.466
	C	.92635	.93034	0.00399	8	0.499
	D	.93153	.93542	0.00389	8	0.486
	E	.92491	.92851	0.00360	8	0.450
58 %	A	.92441	.92833	0.00392	8	0.490
	B	.92496	.92779	0.00283	8	0.354
	C	.92673	.93055	0.00382	8	0.478
	D	.92421	.92784	0.00363	8	0.454
	E	.93033	.93412	0.00379	8	0.474
77 %	A	.93338	.93675	0.00337	8	0.421
	B	.93164	.93530	0.00366	8	0.458
	C	.92508	.92853	0.00345	8	0.431
	D	.92545	.92865	0.00320	8	0.400
	E	.92596	.92926	0.00330	8	0.412

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: March 24, 2020 at 1030

Date and Time Test Terminated: March 30, 2020 at 0945

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	4	3	4	0	4	5	35	10	3.50	
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
5	9	9	10	8	9	8	8	6	7	10	84	10	8.40	
6	13	14	11	14	12	12	11	0	8	14	109	10	10.9	
7														
8														
TOTAL	26	27	24	26	25	23	23	6	19	29	228	10	22.8	

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	5	4	3	4	4	4	4	4	4	40	10	4.00
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	11	10	11	8	10	10	12	11	12	104	10	10.4
6	16	9	14	15	16	14	15	15	14	17	145	10	14.5
7													
8													
TOTAL	29	25	28	29	28	28	29	31	29	33	289	10	28.9

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	4	4	4	3	3	4	4	3	4	3	36	10	3.60
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	8	12	10	10	10	9	10	11	13	13	106	10	10.6
6	12	12	14	12	12	13	14	17	13	14	133	10	13.3
7													
8													
TOTAL	24	28	28	25	25	26	28	31	30	30	275	10	27.5

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: March 24, 2020 at 1030

Date and Time Test Terminated: March 30, 2020 at 0945

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	4	4	5	4	4	3	4	4	5	3	40	10	4.00	
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
5	10	8	10	11	11	9	12	10	10	12	103	10	10.3	
6	13	14	13	15	15	12	16	17	14	15	144	10	14.4	
7														
8														
TOTAL	27	26	28	30	30	24	32	31	29	30	287	10	28.7	

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	5	4	3	4	4	5	4	4	2	3	38	10	3.80
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	10	8	11	10	7	12	10	9	11	97	10	9.70
6	11	13	12	9	13	12	11	15	13	15	124	10	12.4
7													
8													
TOTAL	25	27	23	24	27	24	27	29	24	29	259	10	25.9

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	4	3	4	3	4	5	38	10	3.80
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	13	12	10	9	10	11	13	11	10	108	10	10.8
6	10	14	15	13	11	16	16	15	11	20	141	10	14.1
7													
8													
TOTAL	23	30	31	27	24	29	31	31	26	35	287	10	28.7

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	1.00000	1.39310
2	24 %	2	0.87500	1.20940
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	0.87500	1.20940
5	58 %	1	1.00000	1.39310
5	58 %	2	0.75000	1.04720
5	58 %	3	1.00000	1.39310
5	58 %	4	1.00000	1.39310
5	58 %	5	1.00000	1.39310
6	77 %	1	0.87500	1.20940
6	77 %	2	1.00000	1.39310
6	77 %	3	1.00000	1.39310
6	77 %	4	0.87500	1.20940
6	77 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0.1902 W = 0.7605 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 %	25.00	16.00	5.00	
5	58 %	25.00	16.00	5.00	
6	77 %	22.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
<p>D = 0.0205 W = 0.8974 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					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	24 %	18.00	16.00	5.00	
3	33 %	22.00	16.00	5.00	
4	44 %	16.00	16.00	5.00	*
5	58 %	16.00	16.00	5.00	*
6	77 %	15.00	16.00	5.00	*
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Dunnett's Test for PMSD Calculation

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.03288	0.006577	7.7	
Within (Error)	24	0.0205	0.0008542		
Total	29	0.05338			
Critical F = 3.9 (alpha = 0.01, df = 5,24) 2.62 (alpha = 0.05, df = 5,24)					
Since F > Critical F REJECT Ho: All equal (alpha = 0.05)					

Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	0.5246	0.5246			
2	24 %	0.4858	0.4858	2.099		
3	33 %	0.5042	0.5042	1.104		
4	44 %	0.4766	0.4766	2.597	*	
5	58 %	0.45	0.45	4.036	*	
6	77 %	0.4244	0.4244	5.421	*	
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	0.04362	8.31	0.0388	
3	33 %	5	0.04362	8.31	0.0204	
4	44 %	5	0.04362	8.31	0.048	
5	58 %	5	0.04362	8.31	0.0746	
6	77 %	5	0.04362	8.31	0.1002	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
24 %	10	0	10
Total	20	0	20

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

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
33 %	10	0	10
Total	20	0	20

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

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
44 %	10	0	10
Total	20	0	20

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

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
58 %	10	0	10
Total	20	0	20

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

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
77 %	10	0	10
Total	20	0	20

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

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	24 %	10	0	
2	33 %	10	0	
3	44 %	10	0	
4	58 %	10	0	
5	77 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.1287 D* = 1.01 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 = 20.32 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	24 %	145.50	75.00	10.00	
3	33 %	134.50	75.00	10.00	
4	44 %	143.50	75.00	10.00	
5	58 %	121.50	75.00	10.00	
6	77 %	137.50	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	284.5	56.9	4.452	
Within (Error)	54	690.1	12.78		
Total	59	974.6			
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	22.8	22.8			
2	24 %	28.9	28.9	-3.815		
3	33 %	27.5	27.5	-2.94		
4	44 %	28.7	28.7	-3.69		
5	58 %	25.9	25.9	-1.939		
6	77 %	28.7	28.7	-3.69		
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	3.693	16.2	-6.1	
3	33 %	10	3.693	16.2	-4.7	
4	44 %	10	3.693	16.2	-5.9	
5	58 %	10	3.693	16.2	-3.1	
6	77 %	10	3.693	16.2	-5.9	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.525	-	
24 %	0.486	7.43	PASS
33 %	0.504	4.00	PASS
44 %	0.477	9.14	PASS
58 %	0.450	14.3	FAIL
77 %	0.424	19.2	FAIL

Limit = 12

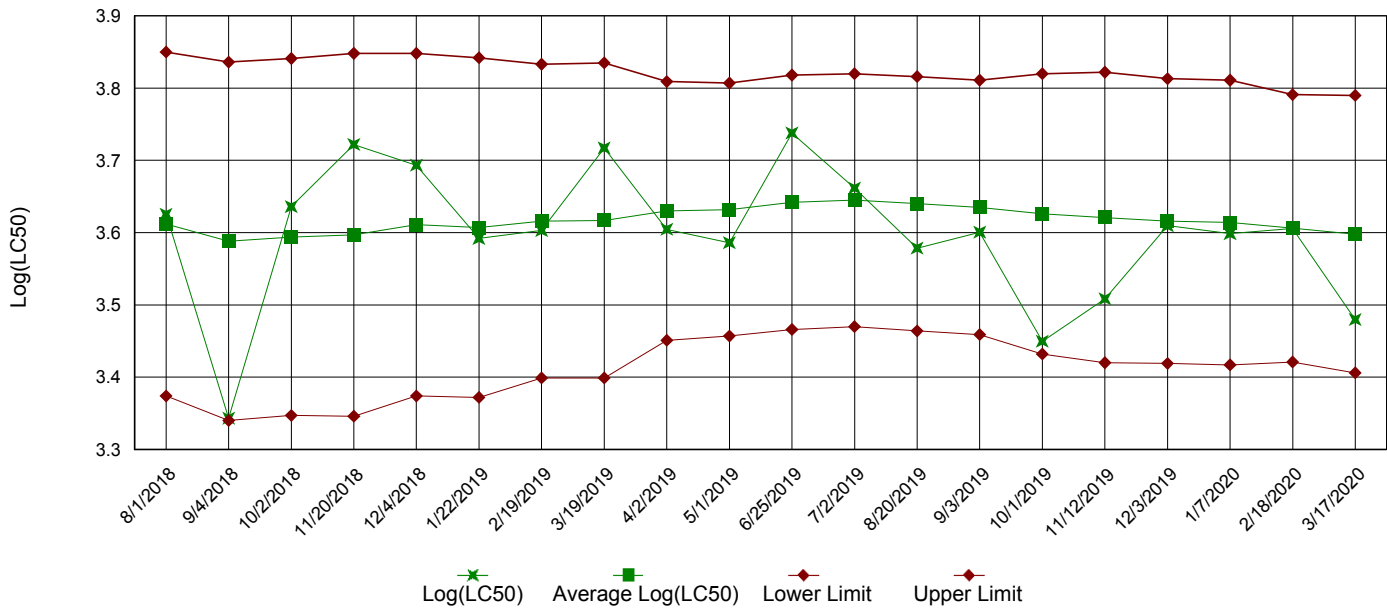
NOEC = 44 %

LOEC = 58 %

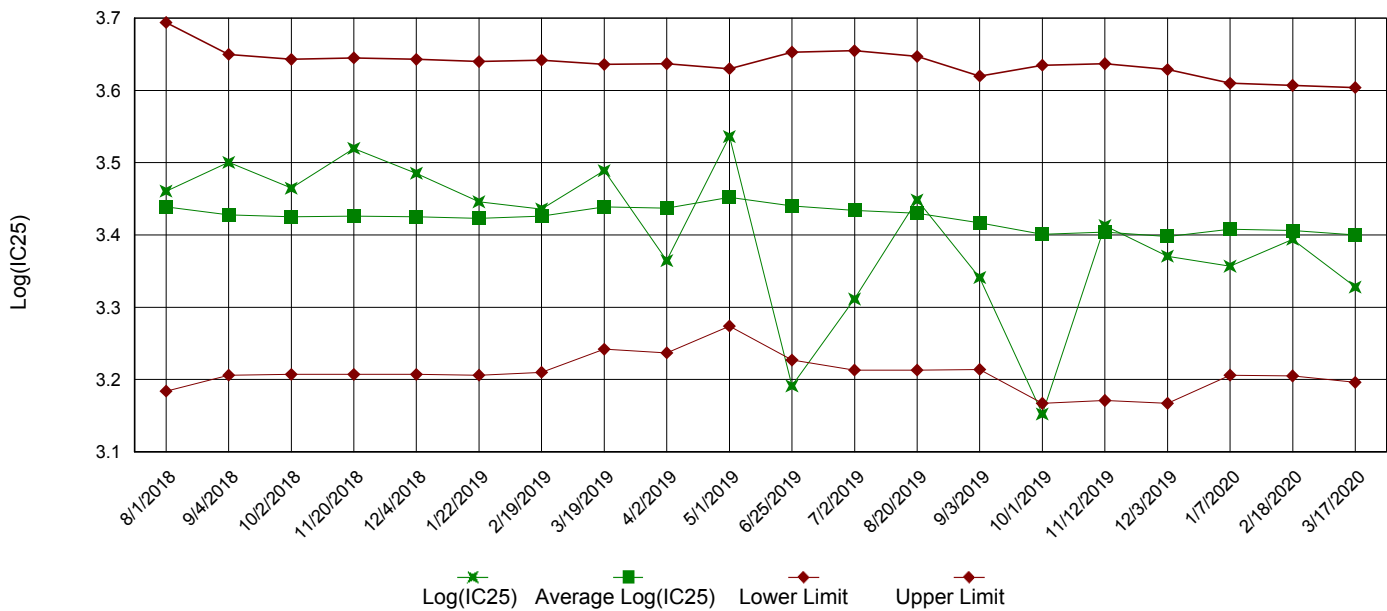
Appendix A3: Test 1000.0

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

LC50 Survival Data

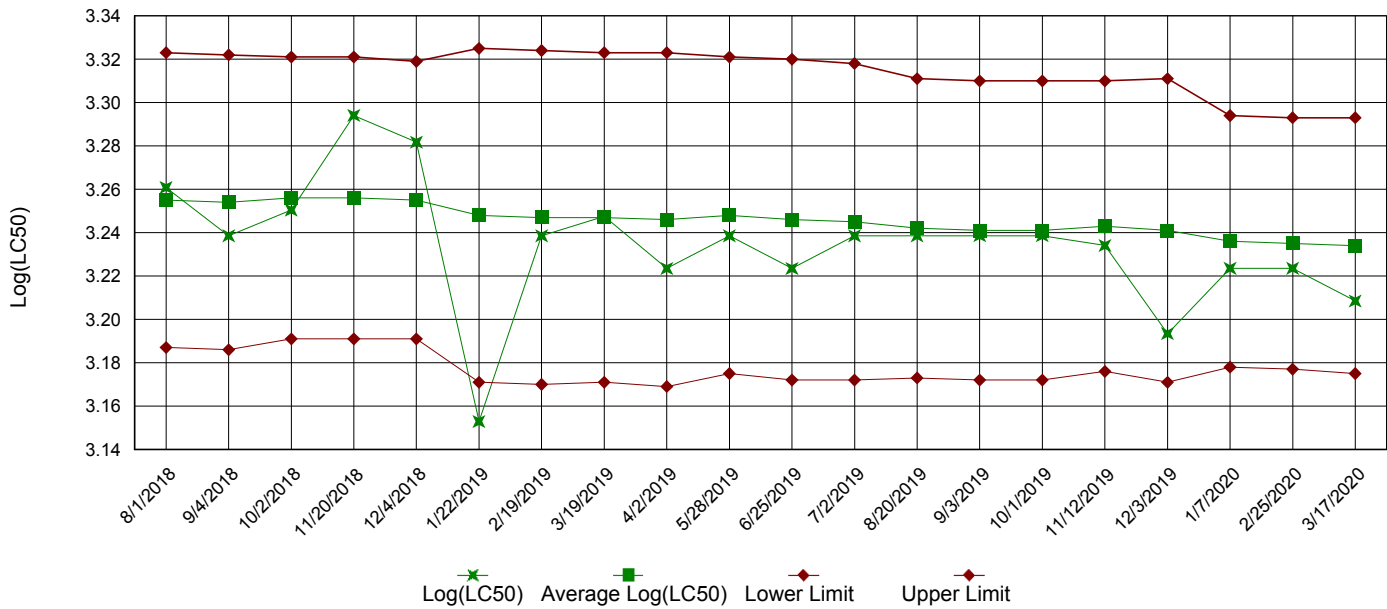


IC25 Growth Data

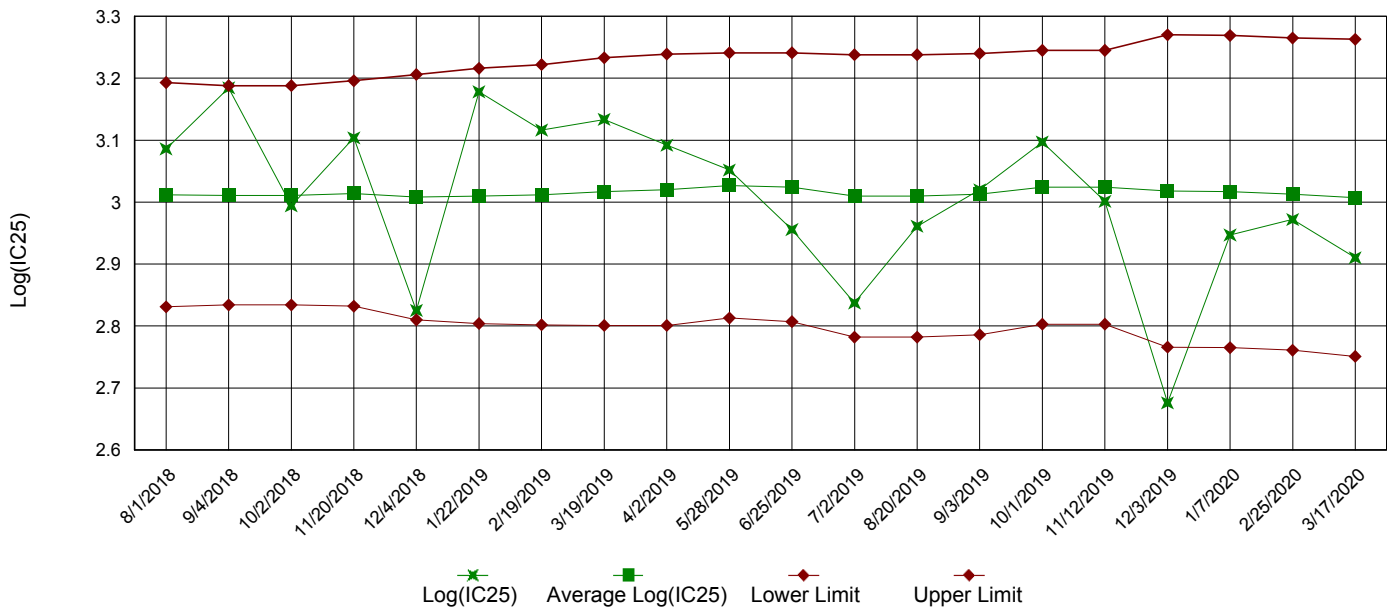


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: March 24, 2020 at 1000

Date and Time Test Terminated: March 31, 2020 at 0845

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 %	100	87.5	100	100	100	100	100	97.5	5.73
33 %	100	100	100	100	100	100	100	100	0.00
44 %	100	100	100	100	87.5	100	100	97.5	5.73
58 %	100	75.0	100	100	100	100	100	95.0	11.8
77 %	87.5	100	100	87.5	100	100	97.5	95.0	7.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.530	0.544	0.556	0.505	0.488	0.525	5.32
24 %	0.482	0.489	0.512	0.464	0.482	0.486	3.57
33 %	0.514	0.496	0.488	0.518	0.505	0.504	2.46
44 %	0.482	0.466	0.499	0.486	0.450	0.477	3.98
58 %	0.490	0.354	0.478	0.454	0.474	0.45	12.3
77 %	0.421	0.458	0.431	0.400	0.412	0.424	5.18

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. Steel's Many-One Rank 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: 44 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 58 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 12.3 (TQP6C)

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: March 24, 2020 TIME: 1000
Test Terminated: DATE: March 31, 2020 TIME: 0845

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	9.1	7.3	7.4	7.4	7.7	7.9
Final	7.6	5.9	6.9	6.4	7.2	6.8	7.3
pH Initial	7.7	7.5	7.6	7.3	7.2	7.5	7.4
Final	7.7	7.2	7.4	7.4	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
24 %							
D.O. Initial	7.2	7.9	7.3	7.2	7.2	7.8	7.8
Final	8.0	7.0	7.0	6.3	7.3	6.9	6.3
pH Initial	7.5	7.4	7.4	7.3	7.2	7.6	7.3
Final	7.8	7.4	7.4	7.4	7.5	7.4	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
33 %							
D.O. Initial	7.7	8.1	7.6	7.6	7.7	7.8	7.5
Final	7.9	5.2	6.8	5.7	7.4	6.5	6.2
pH Initial	7.5	7.5	7.5	7.4	7.3	7.6	7.2
Final	7.8	7.2	7.4	7.3	7.5	7.4	7.3

DILUTION	DAY						
	1	2	3	4	5	6	7
44 %							
D.O. Initial	7.3	8.0	7.4	7.1	7.4	7.5	7.6
Final	7.4	5.0	6.8	6.2	7.4	6.8	6.5
pH Initial	7.5	7.4	7.4	7.3	7.3	7.7	7.2
Final	7.8	7.2	7.4	7.3	7.6	7.5	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
58 %							
D.O. Initial	7.4	8.0	7.4	7.3	7.5	7.6	7.5
Final	7.6	5.0	6.7	6.5	7.2	6.7	6.7
pH Initial	7.5	7.5	7.3	7.2	7.2	7.6	7.2
Final	7.8	7.3	7.4	7.4	7.6	7.5	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
77 %							
D.O. Initial	7.4	7.8	7.9	6.8	7.6	7.6	7.4
Final	7.6	5.4	7.0	6.4	7.3	7.0	6.9
pH Initial	7.4	7.4	7.3	7.2	7.2	7.7	7.2
Final	7.8	7.3	7.5	7.5	7.5	7.5	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	31	220	<0.05	Plant Effluent 22-MAR-20
55	31	200	<0.05	Plant Effluent 24-MAR-20
50	29	180	<0.05	Plant Effluent 26-MAR-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
33	42	170	<0.05	243360-1

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: March 24, 2020 at 1030

Date and Time Test Terminated: March 30, 2020 at 0945

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	26	29	24	27	25	23
B	27	25	28	26	27	30
C	24	28	28	28	23	31
D	26	29	25	30	24	27
E	25	28	25	30	27	24
F	23	28	26	24	24	29
G	23	29	28	32	27	31
H	6	31	31	31	29	31
I	19	29	30	29	24	26
J	29	33	30	30	29	35
Mean per Adult	22.8	28.9	27.5	28.7	25.9	28.7
Mean per Surviving Adult	22.8	28.9	27.5	28.7	25.9	28.7
CV %	28.5	7.19	8.78	8.54	8.43	12.8

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

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

1. Fisher's Exact Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(58 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

2. Steel's Many-One Rank Test:

Is the mean number of young produced per female significantly different ($p=0.05$) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(58 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 77 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 77 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 77 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 77 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 28.5 (TQP3B)

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: March 24, 2020 TIME: 1030
Test Terminated: DATE: March 30, 2020 TIME: 0945

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	9.1	7.3	7.4	7.4	7.7	7.9
Final	8.6	7.0	7.0	7.3	8.0	7.7	--
pH Initial	7.7	7.5	7.6	7.3	7.2	7.5	7.4
Final	8.2	8.0	7.7	7.7	7.6	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
24 %							
D.O. Initial	7.2	7.9	7.3	7.2	7.2	7.8	7.8
Final	8.7	7.4	7.3	7.3	7.9	8.1	--
pH Initial	7.5	7.4	7.4	7.3	7.2	7.6	7.3
Final	8.2	7.9	7.6	7.7	7.6	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
33 %							
D.O. Initial	7.7	8.1	7.6	7.6	7.7	7.8	7.5
Final	8.7	7.3	7.8	7.6	8.0	8.0	--
pH Initial	7.5	7.5	7.5	7.4	7.3	7.6	7.2
Final	8.2	8.0	7.7	7.7	7.6	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
44 %							
D.O. Initial	7.3	8.0	7.4	7.1	7.4	7.5	7.6
Final	8.4	6.8	7.4	7.2	7.9	7.7	--
pH Initial	7.5	7.4	7.4	7.3	7.3	7.7	7.2
Final	8.1	7.8	7.7	7.8	7.7	7.8	--

DILUTION	DAY						
	1	2	3	4	5	6	7
58 %							
D.O. Initial	7.4	8.0	7.4	7.3	7.5	7.6	7.5
Final	8.6	6.6	7.5	7.2	7.9	8.0	--
pH Initial	7.5	7.5	7.3	7.2	7.2	7.6	7.2
Final	8.1	7.8	7.8	7.8	7.6	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
77 %							
D.O. Initial	7.4	7.8	7.9	6.8	7.6	7.6	7.4
Final	8.6	7.2	7.5	7.3	7.9	8.0	--
pH Initial	7.4	7.4	7.3	7.2	7.2	7.7	7.2
Final	8.2	7.9	7.8	7.8	7.6	7.8	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	31	220	<0.05	Plant Effluent 22-MAR-20
55	31	200	<0.05	Plant Effluent 24-MAR-20
50	29	180	<0.05	Plant Effluent 26-MAR-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
33	42	170	<0.05	243360-1



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: City of Hot Springs		P.O. Number 2020-48	
Project Reference: Quarterly Bio-Monitoring		ANALYSIS REQUESTED	
Project Manager: Harold Mauldin		Bio-Monitoring	
Sampled By: <i>AC</i>		Number of Bottles	
AIC No.	Sample Identification	Water	
1	Plant Effluent	Composite	X
			X
Date/Time Collected 3-22-20 @ 0000-2400			
Remarks 0.1			
G= Glass P=Plastic NO=None S=Sulfuric Acid pH2 V=VOA Vials		Field pH Calibration	
N=Nitric Acid pH2 H=HCl to pH2 B=NaOH to pH12		on _____ @ _____	
T=Sodium Thiosulfate Z=Zinc Acetate A=(NH4)2NH4OH		Buffer	
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS	Relinquished By: <i>A. Carby</i>	Date/Time 3-23-20 @ 0750	Received By: <i>Bill Mauldin</i>
Expedited results requested by:	Relinquished By: <i>Bill Mauldin</i>	Date/Time 3-23-20 @ 0950	Received By: <i>Harold Mauldin</i>
Who should AIC contact with questions: Harold Mauldin	Comments		
Phone: 501-262-1125 Ext. 2860			
Fax: 501-262-0339			
Report Attention to: Harold Mauldin			
Report Address to: 320 Davidson Drive			
E-Mail Address: hmauldin@cityhs.net			



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: City of Hot Springs		P.O. Number 2020-48	
Project Reference: Quarterly Bio-Monitoring		Matrix	
Project Manager: Harold Mauldin		Number of Bottles	
Sampled By: AC	Sample Identification	Date/Time Collected	Water
	2	3-24-20 @ 0000 - 2400	3
AIC Control Number: 243750		Bio-Monitoring	
AIC Proposal Number:		Carrier: BILL G.	
Received Temperature °C		Received Temperature °C	
0.2 / 0.6 / 0.5		0.2 / 0.6 / 0.5	
Remarks		Field pH Calibration	
		on _____ @ _____	
		Buffer	
G= Glass P=Plastic NO=None S=Sulfuric Acid pH2 V=VOA Vials		Received By: Bill Hamer	
N=Nitric Acid pH2 H=HCl to pH2 B=NaOH to pH12		Date/Time 3-25-20 @ 0710	
T=Sodium Thiosulfate Z=Zinc Acetate A=(NH4)2NH4OH		Date/Time 3-25-20 @ 0915	
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS		Relinquished By: A. Carter	
Expedited results requested by:		Date/Time 3-25-20 @ 0710	
Who should AIC contact with questions:		Date/Time 3-25-20 @ 0915	
Harold Mauldin		Relinquished By: Bill Hamer	
Phone: 501-262-1125 Ext. 2860		Received By: James Westin	
Fax: 501-262-0339		Date/Time 3-25-20 @ 0915	
Report Attention to: Harold Mauldin		Comments	
Report Address to: 320 Davidson Drive			
E-Mail Address: hmauldin@cityhs.net			



CHAIN OF CUSTODY/ANALYSIS REQUEST FORM

Client: City of Hot Springs		P.O. Number 2020-48	
Project Reference: Quarterly Bio-Monitoring		Matrix	
Project Manager: Harold Mauldin		Number of Bottles	
Sampled By: AC		Water	
AIC No.	Sample Identification	Date/Time Collected	Composite
3	Plant Effluent	3-26-20 @ 0000 - 2400	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		Bio-Monitoring	
Turnaround Time Requested in: (Please Circle) NORMAL or EXPEDITED IN _____ DAYS		Received By: Bill Mauldin	
Expedited results requested by: Who should AIC contact with questions: Harold Mauldin		Date/Time 3/27/2020 @ 0720	
Phone: 501-262-1125 Ext. 2860 Fax: 501-262-0339 Report Attention to: Harold Mauldin Report Address to: 320 Davidson Drive E-Mail Address: hmauldin@cityhs.net		Received By: James Woodrum	
		Date/Time 3/27/2020 @ 0912	
		Comments	
		Carrier: Bill G.	
		Received Temperature °C 0.6° 0.4 0.5.	
		Remarks	
		Field pH Calibration on _____ @ _____ Buffer	