

September 3, 2019

Biomonitoring Testing
for
EFF

Control No. 237296-1

Prepared for:

Mr. Jimmy Smith
Searcy Water and Sewer System
P.O. Box 1319
Searcy, AR 72145

Prepared by:

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Searcy Water and Sewer System
ATTN: Mr. Jimmy Smith
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Searcy, AR 72145

Re: Chronic 7-Day Renewal *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
EFF
NPDES Permit No. AR0021601 AFIN# 73-00055

Dear Mr. Jimmy Smith:

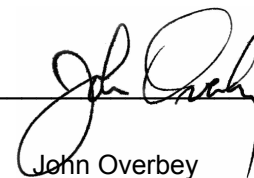
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 27 % effluent, which is above the critical dilution of 20 %. 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 27 %. **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 27 % effluent, which is above the critical dilution of 20 %. The NOEC for reproduction occurred at 27 % effluent, which is above the sub-lethal limit of 20 %. **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%	98	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.64	PASS
Control Growth CV < or = 40%	3.0	PASS
Growth Minimum Significant Difference 12 to 30%	11	BELOW
Critical Dilution CV < or = 40%	10	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	37	PASS
Control CV < or = 40% per Surviving Female	6.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	15	PASS
Critical Dilution CV < or = 40%	15	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0021601 AFIN# 73-00055
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: EFF
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.3	6.3	6.3
pH (standard units)	7.2	7.1	7.2
Alkalinity (mg/l as CaCO ₃)	26	28	38
Hardness (mg/l as CaCO ₃)	41	41	40
Conductivity (umhos/cm)	340	340	350
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.11	0.18	0.13

2. Dilution Water Samples:

Soft

Analysis	236900-1
Dissolved oxygen (mg/l)	6.8
pH (standard units)	7.6
Alkalinity (mg/l as CaCO ₃)	31
Hardness (mg/l as CaCO ₃)	46
Conductivity (umhos/cm)	160
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 20, 2019 at 1105
Date & Time Test Terminated: August 27, 2019 at 0945
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 20, 2019 at 1000
Date & Time Test Terminated: August 26, 2019 at 1115
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 02, 2019 at 1205 to July 09, 2019 at 1200

The results were as follows: (Control No. 235781-1.)

Survival LC-50: 3075 mg/l

Growth IC-25: 2048 mg/l

Growth PMSD: 7.71

Ceriodaphnia dubia

A chronic reference test was performed on July 02, 2019 at 1450 to July 08, 2019 at 1400

The results were as follows: (Control No. 235781-2.)

Survival LC-50: 1732 mg/l

Growth IC-25: 687.5 mg/l

Growth PMSD: 20

V. Organism History

Pimephales promelas (Fathead minnow)

Date: August 20, 2019

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: August 20, 2019

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.

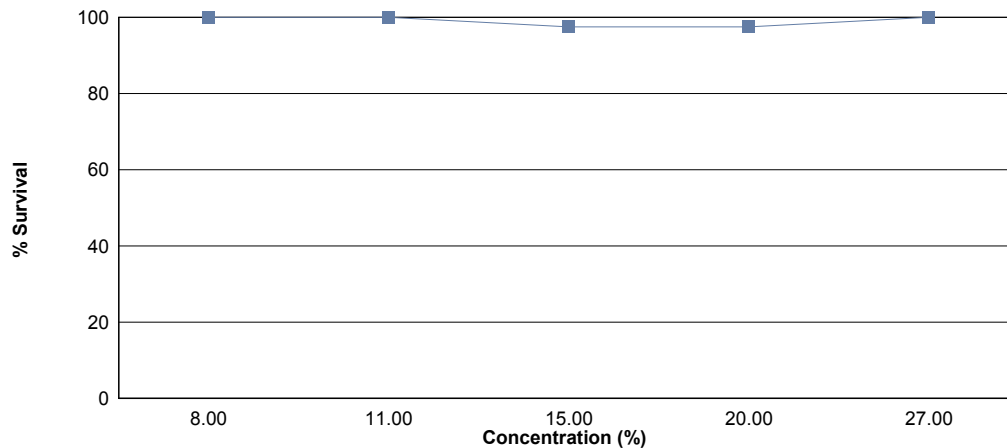
Effluent dilutions for this test were 8 %, 11 %, 15 %, 20 %, 27 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 20 % effluent.

The test was initiated on August 20, 2019 at 1105 and continued through August 27, 2019 at 0945. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 27 % effluent
- b.) NOEC growth = 27 % effluent

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



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.620
8 %	100	0.613
11 %	100	0.656
15 %	97.5	0.559
20 %	97.5	0.544 *
27 %	100	0.556

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

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

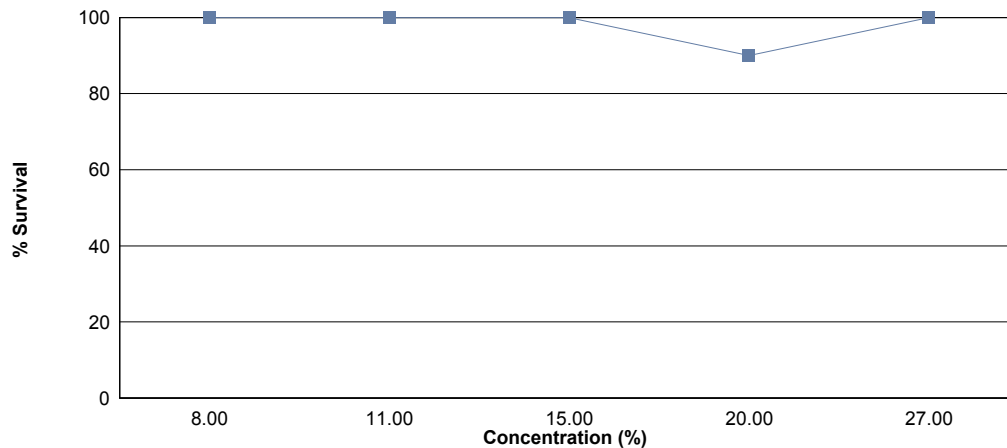
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 8 %, 11 %, 15 %, 20 %, 27 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 20 % effluent.

The test was initiated on August 20, 2019 at 1000 and continued through August 26, 2019 at 1115. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 27 % effluent
- b.) NOEC reproduction = 27 % effluent



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	36.7
8 %	100	37.0
11 %	100	35.8
15 %	100	33.4
20 %	90.0	32.0
27 %	100	35.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 20, 2019 at 1105

Date and Time Test Terminated: August 27, 2019 at 0945

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	7	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
11 %	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
15 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
20 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
27 %	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 20, 2019 at 1105

Test Terminated: August 27, 2019 at 0945

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92992	.93494	0.00502	8	0.628
	B	.92651	.93144	0.00493	8	0.616
	C	.93100	.93584	0.00484	8	0.605
	D	.92989	.93471	0.00482	8	0.602
	E	.92826	.93344	0.00518	8	0.648
8 %	A	.92313	.92779	0.00466	8	0.582
	B	.92414	.92925	0.00511	8	0.639
	C	.92800	.93314	0.00514	8	0.642
	D	.92456	.92951	0.00495	8	0.619
	E	.92683	.93148	0.00465	8	0.581
11 %	A	.92405	.92885	0.00480	8	0.600
	B	.92559	.93076	0.00517	8	0.646
	C	.92380	.92929	0.00549	8	0.686
	D	.92345	.92902	0.00557	8	0.696
	E	.92214	.92735	0.00521	8	0.651
15 %	A	.92365	.92823	0.00458	8	0.572
	B	.92289	.92732	0.00443	8	0.554
	C	.92456	.92861	0.00405	8	0.506
	D	.92498	.92956	0.00458	8	0.572
	E	.92758	.93229	0.00471	8	0.589
20 %	A	.92570	.93030	0.00460	8	0.575
	B	.92792	.93238	0.00446	8	0.558
	C	.92800	.93161	0.00361	8	0.451
	D	.92224	.92654	0.00430	8	0.538
	E	.92043	.92523	0.00480	8	0.600
27 %	A	.92117	.92557	0.00440	8	0.550
	B	.91971	.92314	0.00343	8	0.429
	C	.92035	.92476	0.00441	8	0.551
	D	.91917	.92423	0.00506	8	0.632
	E	.92009	.92502	0.00493	8	0.616

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 20, 2019 at 1000

Date and Time Test Terminated: August 26, 2019 at 1115

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	5	5	4	4	6	5	5	4	4	4	46	10	4.60	
4	0	0	13	0	11	0	11	0	13	0	48	10	4.80	
5	14	14	0	13	0	11	0	13	0	11	76	10	7.60	
6	21	22	19	20	18	19	20	21	18	19	197	10	19.7	
7														
8														
TOTAL	40	41	36	37	35	35	36	38	35	34	367	10	36.7	

Concentration: 8 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	3	4	4	4	5	0	5	5	6	4	40	10	4.00	
4	0	0	0	0	10	0	13	0	13	11	47	10	4.70	
5	11	14	14	14	1	12	0	14	0	0	80	10	8.00	
6	18	23	25	15	20	19	17	22	23	21	203	10	20.3	
7														
8														
TOTAL	32	41	43	33	36	31	35	41	42	36	370	10	37.0	

Concentration: 11 %														
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	6	5	2	4	5	2	1	2	4	5	36	10	3.60	
4	9	0	0	0	11	0	8	8	13	0	49	10	4.90	
5	0	13	12	11	0	14	0	0	0	13	63	10	6.30	
6	22	23	21	23	20	22	20	17	21	21	210	10	21.0	
7														
8														
TOTAL	37	41	35	38	36	38	29	27	38	39	358	10	35.8	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 20, 2019 at 1000

Date and Time Test Terminated: August 26, 2019 at 1115

Concentration: 15 %														
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	5	6	0	6	6	1	0	4	5	0	33	10	3.30	
4	12	1	0	0	12	0	12	0	0	0	37	10	3.70	
5	0	0	13	14	0	13	0	12	1	13	66	10	6.60	
6	20	21	20	23	21	22	19	20	9	23	198	10	19.8	
7														
8														
TOTAL	37	28	33	43	39	36	31	36	15	36	334	10	33.4	

Concentration: 20 %													
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	7	X	5	5	0	1	5	5	4	32	9	3.56
4	0	0	X	0	10	0	10	0	11	0	31	9	3.44
5	14	13	X	7	0	11	0	10	0	12	67	9	7.44
6	22	21	X	23	21	21	13	22	25	22	190	9	21.1
7													
8													
TOTAL	36	41	0	35	36	32	24	37	41	38	320	10	32.0

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	2	8	5	7	0	2	0	5	5	3	37	10	3.70
4	0	0	0	0	11	0	11	0	8	0	30	10	3.00
5	15	11	6	9	0	12	0	13	0	12	78	10	7.80
6	23	23	14	21	23	24	20	21	21	22	212	10	21.2
7													
8													
TOTAL	40	42	25	37	34	38	31	39	34	37	357	10	35.7

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	0.87500	1.20940
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	8 %	1	1.00000	1.39310
2	8 %	2	1.00000	1.39310
2	8 %	3	1.00000	1.39310
2	8 %	4	1.00000	1.39310
2	8 %	5	1.00000	1.39310
3	11 %	1	1.00000	1.39310
3	11 %	2	1.00000	1.39310
3	11 %	3	1.00000	1.39310
3	11 %	4	1.00000	1.39310
3	11 %	5	1.00000	1.39310
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	0.87500	1.20940
4	15 %	4	1.00000	1.39310
4	15 %	5	1.00000	1.39310
5	20 %	1	1.00000	1.39310
5	20 %	2	1.00000	1.39310
5	20 %	3	0.87500	1.20940
5	20 %	4	1.00000	1.39310
5	20 %	5	1.00000	1.39310
6	27 %	1	1.00000	1.39310
6	27 %	2	1.00000	1.39310
6	27 %	3	1.00000	1.39310
6	27 %	4	1.00000	1.39310
6	27 %	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 = 0.08099 W = 0.5968 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	8 %	30.00	16.00	5.00	
3	11 %	30.00	16.00	5.00	
4	15 %	27.50	16.00	5.00	
5	20 %	27.50	16.00	5.00	
6	27 %	30.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.05332 W = 0.9339 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 = 9.258 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	0.04985	0.00997	4.485	
Within (Error)	24	0.05335	0.002223		
Total	29	0.1032			
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	0.6198	0.6198			
2	8 %	0.6126	0.6126	0.2415		
3	11 %	0.6558	0.6558	-1.207		
4	15 %	0.5586	0.5586	2.052		
5	20 %	0.5444	0.5444	2.529	*	
6	27 %	0.5556	0.5556	2.153		
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	8 %	5	0.07037	11.4	0.0072	
3	11 %	5	0.07037	11.4	-0.036	
4	15 %	5	0.07037	11.4	0.0612	
5	20 %	5	0.07037	11.4	0.0754	
6	27 %	5	0.07037	11.4	0.0642	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

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

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

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
11 %	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
15 %	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
20 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. 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
27 %	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	8 %	10	0	
2	11 %	10	0	
3	15 %	10	0	
4	20 %	10	1	
5	27 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p style="text-align: center;"> D = 0.1641 D* = 1.287 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	8 %	106.50	75.00	10.00	
3	11 %	109.00	75.00	10.00	
4	15 %	94.50	75.00	10.00	
5	20 %	100.50	75.00	10.00	
6	27 %	103.00	75.00	10.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	80.09	16.02	0.6212	
Within (Error)	53	1367	25.79		
Total	58	1447			
Critical F = 3.39 (alpha = 0.01, df = 5,53) 2.39 (alpha = 0.05, df = 5,53)					
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	36.7	36.7			
2	8 %	37	37	-0.1321		
3	11 %	35.8	35.8	0.3963		
4	15 %	33.4	33.4	1.453		
5	20 %	35.556	35.556	0.4903		
6	27 %	35.7	35.7	0.4403		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53) WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.						

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	8 %	10	5.246	14.3	-0.3	
3	11 %	10	5.246	14.3	0.9	
4	15 %	10	5.246	14.3	3.3	
5	20 %	9	5.39	14.7	1.144	
6	27 %	10	5.246	14.3	1	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.620	-	
8 %	0.613	1.1	PASS
11 %	0.656	-5.8	PASS
15 %	0.559	9.8	PASS
20 %	0.544	12	PASS
27 %	0.556	10	PASS

Limit = 12

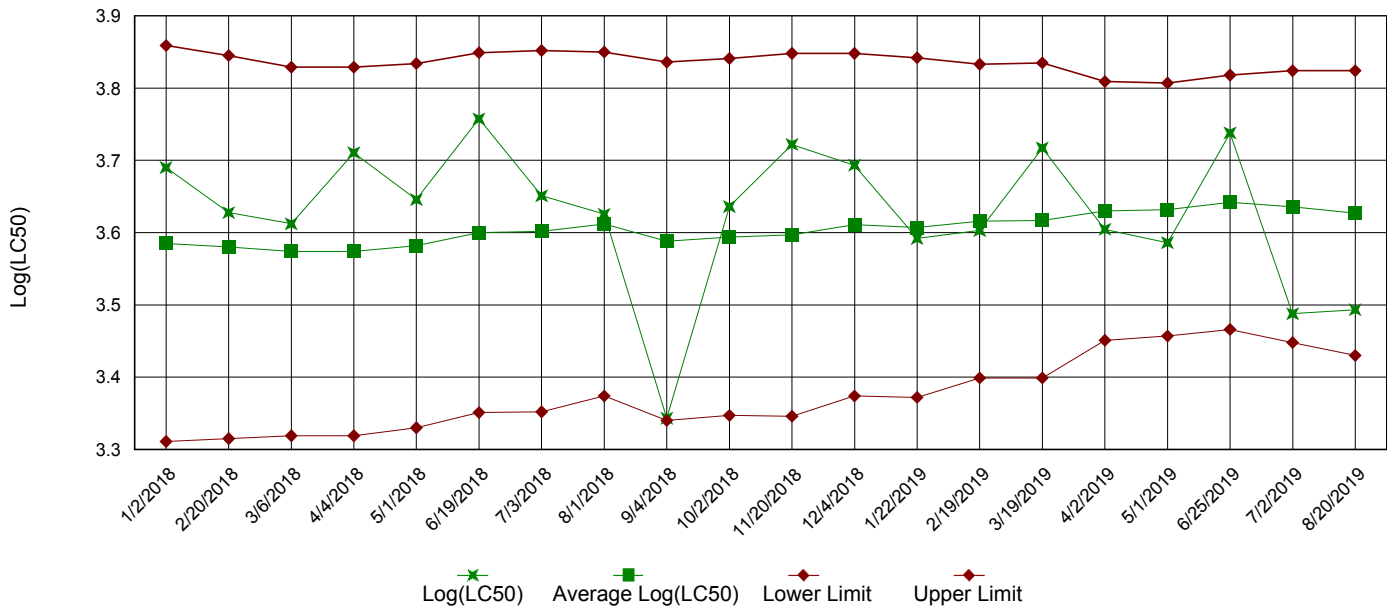
NOEC = 27 %

LOEC = 27 %

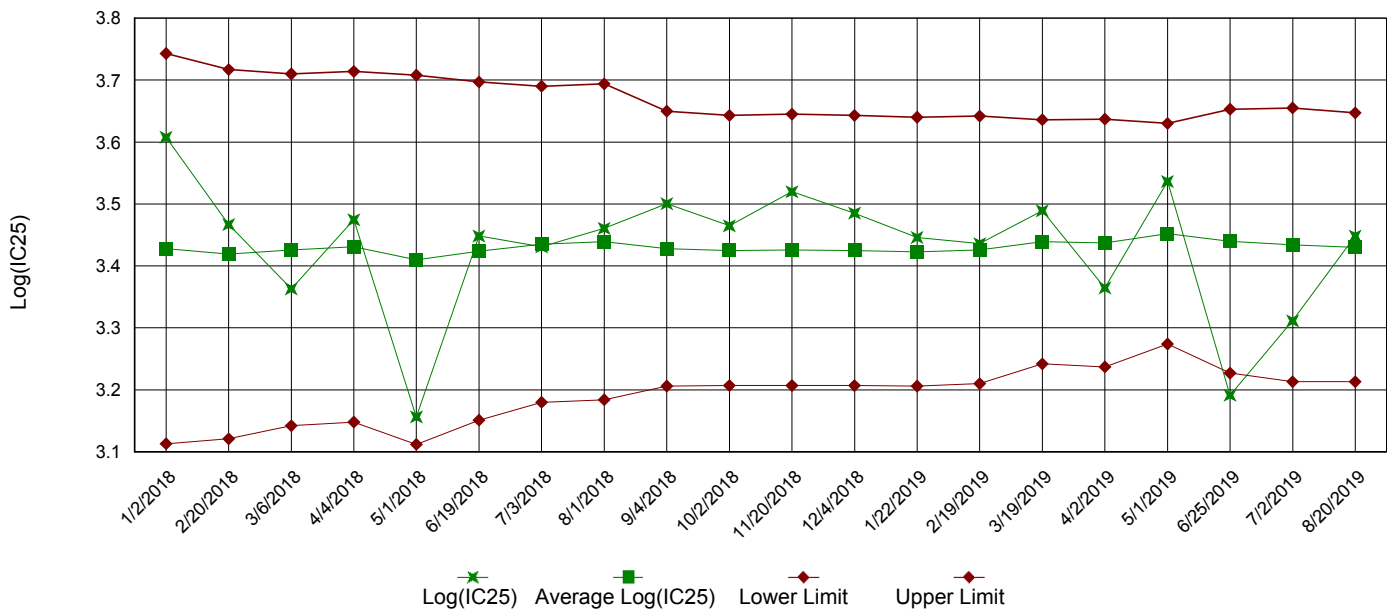
Appendix A3: Test 1000.0

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

LC50 Survival Data

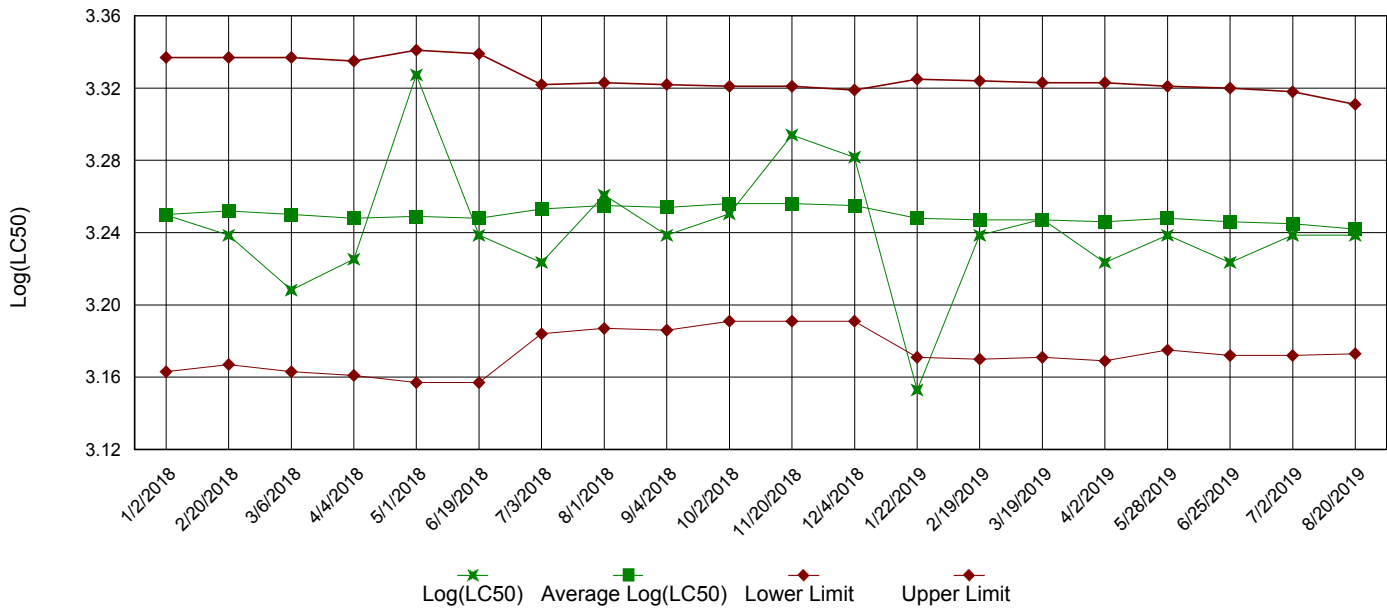


IC25 Growth Data

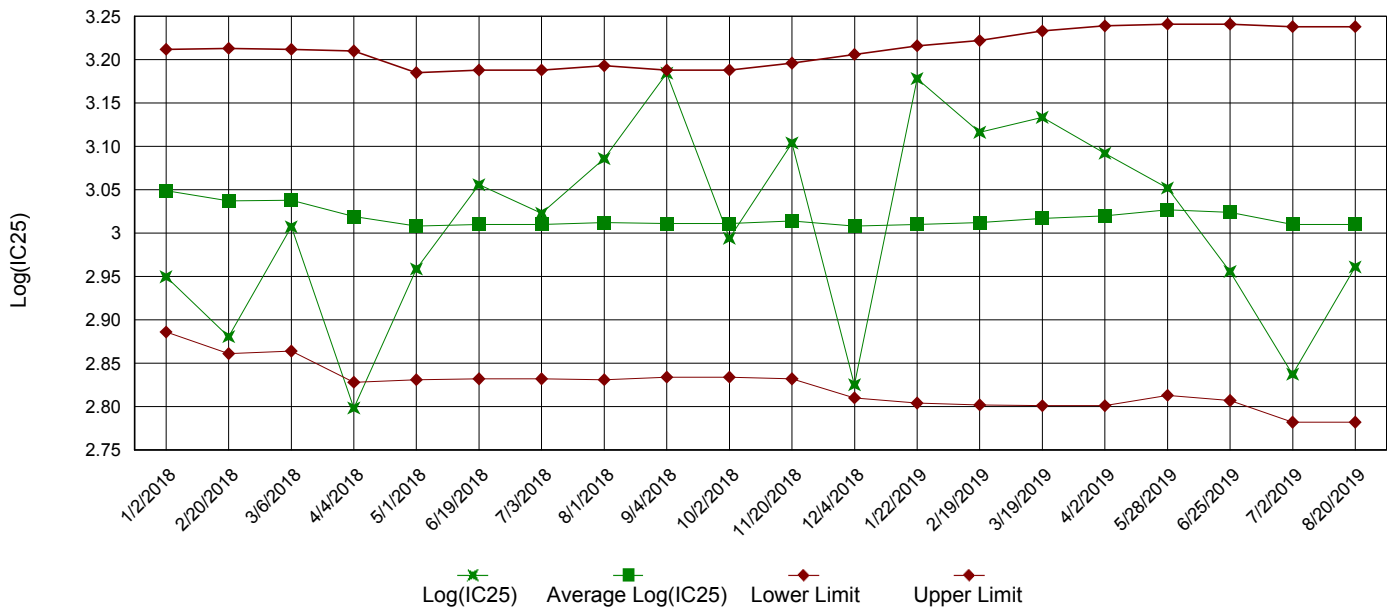


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: August 20, 2019 at 1105

Date and Time Test Terminated: August 27, 2019 at 0945

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	87.5	100	100	100	100	97.5	5.73
8 %	100	100	100	100	100	100	100	100	0.00
11 %	100	100	100	100	100	100	100	100	0.00
15 %	100	100	87.5	100	100	100	100	97.5	5.73
20 %	100	100	87.5	100	100	100	100	97.5	5.73
27 %	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	0.628	0.616	0.605	0.602	0.648	0.62	3.03
8 %	0.582	0.639	0.642	0.619	0.581	0.613	4.85
11 %	0.600	0.646	0.686	0.696	0.651	0.656	5.79
15 %	0.572	0.554	0.506	0.572	0.589	0.559	5.71
20 %	0.575	0.558	0.451	0.538	0.600	0.544	10.5
27 %	0.550	0.429	0.551	0.632	0.616	0.556	14.4

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	(20 %)	<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	(20 %)	<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: 27 % (TOP6C)
6. LOEC *Pimephales* Lethality: 27 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 27 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 27 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 10.5 (TQP6C)

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

PERMITTEE: Searcy Water and Sewer System
NPDES NO.: AR0021601 AFIN# 73-00055
CONTACT: Mr. Jimmy Smith
ANALYST: 280, 310, 343

Test Initiated: DATE: August 20, 2019 TIME: 1105
Test Terminated: DATE: August 27, 2019 TIME: 0945

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.8	6.6	6.0	6.6	6.1	6.4	6.2
Final	5.0	5.1	4.8	5.3	5.2	6.6	5.5
pH Initial	7.6	7.0	7.5	7.5	7.4	7.5	7.1
Final	7.2	7.1	7.1	7.2	7.3	7.1	7.1

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.6	6.5	6.1	6.4	6.4	6.3	6.4
Final	5.2	5.2	4.5	4.4	4.3	5.8	6.0
pH Initial	7.5	7.0	7.4	7.4	7.4	7.5	7.1
Final	7.1	7.1	7.0	7.1	7.1	7.0	7.3

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.6	6.6	6.3	6.4	6.6	6.3	6.5
Final	5.2	4.9	4.9	4.7	4.4	5.9	5.8
pH Initial	7.5	7.0	7.4	7.4	7.4	7.4	7.1
Final	7.2	7.2	7.1	7.1	7.2	7.0	7.2

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	6.3	6.1	6.2	6.6	6.2	6.3
Final	5.4	5.5	5.1	5.0	5.0	5.8	5.6
pH Initial	7.5	7.1	7.4	7.4	7.3	7.4	7.1
Final	7.2	7.2	7.2	7.2	7.3	7.1	7.2

DILUTION 20 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.4	6.2	6.6	6.3	6.4	6.4
Final	5.4	5.4	4.8	5.3	4.9	5.8	5.7
pH Initial	7.4	7.1	7.4	7.4	7.3	7.4	7.0
Final	7.2	7.1	7.1	7.2	7.2	7.1	7.2

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.6	6.0	6.1	6.4	6.1	6.4
Final	5.2	4.9	4.9	4.8	4.8	5.7	5.6
pH Initial	7.4	7.1	7.3	7.4	7.3	7.4	7.1
Final	7.2	7.2	7.2	7.2	7.3	7.1	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
26	41	340	<0.05	Eff 19-AUG-19
28	41	340	<0.05	Eff 21-AUG-19
38	40	350	<0.05	Eff 23-AUG-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	46	160	<0.05	236900-1

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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: August 20, 2019 at 1000

Date and Time Test Terminated: August 26, 2019 at 1115

Dilution water used: Soft

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		8 %	11 %	15 %	20 %	27 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
6 day	100	100	100	100	90.0	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		8 %	11 %	15 %	20 %	27 %
A	40	32	37	37	36	40
B	41	41	41	28	41	42
C	36	43	35	33	0	25
D	37	33	38	43	35	37
E	35	36	36	39	36	34
F	35	31	38	36	32	38
G	36	35	29	31	24	31
H	38	41	27	36	37	39
I	35	42	38	15	41	34
J	34	36	39	36	38	37
Mean per Adult	36.7	37.0	35.8	33.4	32.0	35.7
Mean per Surviving Adult	36.7	37.0	35.8	33.4	35.6	35.7
CV %	6.30	12.0	12.4	23.0	14.6	13.9

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	(20 %)	<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	(20 %)	<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: 27 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 27 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 27 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 27 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 14.6 (TQP3B)
10. Lethality for this test: 27 % (22414 or 51710)
11. Sublethality for this test: 15 % (22414 or 51710)

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

PERMITTEE: Searcy Water and Sewer System
NPDES NO.: AR0021601 AFIN# 73-00055
CONTACT: Mr. Jimmy Smith
ANALYST: 280, 310, 343

Test Initiated: DATE: August 20, 2019 TIME: 1000
Test Terminated: DATE: August 26, 2019 TIME: 1115

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	6.8	6.6	6.0	6.6	6.1	6.4	6.2
Final	5.6	6.1	6.5	7.2	7.1	6.3	--
pH Initial	7.6	7.0	7.5	7.5	7.4	7.5	7.1
Final	7.4	7.6	7.9	8.2	8.1	7.3	--

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	6.6	6.5	6.1	6.4	6.4	6.3	6.4
Final	5.7	6.3	6.0	7.3	7.0	6.3	--
pH Initial	7.5	7.0	7.4	7.4	7.4	7.5	7.1
Final	7.6	7.7	7.9	8.5	8.2	7.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	6.6	6.6	6.3	6.4	6.6	6.3	6.5
Final	5.8	6.6	6.6	7.1	6.9	6.2	--
pH Initial	7.5	7.0	7.4	7.4	7.4	7.4	7.1
Final	7.5	7.8	8.0	8.4	8.2	7.5	--

DILUTION	DAY						
	1	2	3	4	5	6	7
15 %							
D.O. Initial	6.4	6.3	6.1	6.2	6.6	6.2	6.3
Final	6.0	6.3	6.5	7.4	7.0	6.4	--
pH Initial	7.5	7.1	7.4	7.4	7.3	7.4	7.1
Final	7.6	7.8	7.9	8.4	8.3	7.6	--

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	6.5	6.4	6.2	6.6	6.3	6.4	6.4
Final	5.5	6.5	6.7	7.3	7.1	6.3	--
pH Initial	7.4	7.1	7.4	7.4	7.3	7.4	7.0
Final	7.5	7.8	7.8	8.4	8.3	7.6	--

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	6.5	6.6	6.0	6.1	6.4	6.1	6.4
Final	6.0	6.4	6.3	7.0	6.6	6.4	--
pH Initial	7.4	7.1	7.3	7.4	7.3	7.4	7.1
Final	7.5	7.9	7.9	8.4	8.3	7.6	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
26	41	340	<0.05	Eff 19-AUG-19
28	41	340	<0.05	Eff 21-AUG-19
38	40	350	<0.05	Eff 23-AUG-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	46	160	<0.05	236900-1



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

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Searcy Water Utilities
Project Reference: 3rd Atk Bio + Table III
Project Manager: Jimmy Smith
Sampled By: SC, MP, BH, TH, BA, GH

AIC No.	Sample Identification	Date/Time Collected	GRA	B	COMP	SAMPLE MATRIX					NO OF BOTTLES	ANALYSES REQUESTED	AIC CONTROL NO.	AIC PROPOSAL NO.	Carrier	Received on ice (4°C)? YES NO	Remarks
						W	A	S	O	I							
3	Eff	8/22 to 8/23/19 Sam to Sam	✓	✓	✓	✓	✓	✓	✓	2	Bio-Monitors	Metals	Metals	Phenolics	HG	8/22/19	237453
	Eff	8/21/19 8:00am	✓	✓	✓	✓	✓	✓	✓							AIC 237453-1	
	Eff	8/22 to 8:23/19 4 MC 8, 2, 8, 2	✓	✓	✓	✓	✓	✓	✓		H						237453-2
	Eff	" "	✓	✓	✓	✓	✓	✓	✓			H					
	Eff	" "	✓	✓	✓	✓	✓	✓	✓								

Container Type: _____
Preservative: _____
G = Glass P = Plastic V = VOA vials
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2

Reinquinshed By: _____
Relinquished Date/Time: _____
H = HCl to pH2 B = NaOH to pH12

Field pH calibration: _____
on _____ @ _____
Buffer: _____

Received By: _____
Received Date/Time: _____
T = Sodium Thiosulfate
Z = Zinc acetate

Relinquished By: _____
Relinquished Date/Time: _____
Date/Time: 8-23-19

Reinquinshed By: _____
Reinquinshed Date/Time: 10:22
By: AC341

Who should AIC contact with questions: _____
Phone: 501-268-1679 **Fax:** _____
Report Attention to: _____
Report Address to: _____

Comments: Grab Comp, Bio "Grabbed Enough to Fill Jug" JB



8600 Kanis Road
 Little Rock, AR 72204-2322
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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 1

Client: **SEARCY WATER UTILITIES**
 Project: **BID-MONITORING**
 Reference: **TIMMY SMITH**
 Project Manager: **JEREMY CHEELY**
 Sampled By: **JEREMY CHEELY**
 AIC No.: **1**
 Sample Identification: **EFF**
 Date/Time Collected: **8-19-19 7:45am**

PO No. _____
 SAMPLE MATRIX: W A T E R S O I L
 G R A B C O M P
 NO OF BOTTLES: **2**

ANALYSES REQUESTED: **PHENOL**

Carrier: _____
 Received on ice (4°C)? **YES**
 Remarks: _____

Field pH calibration on _____ @ _____
 Buffer: _____

G = Glass P = Plastic V = VOA vials
 NO = none S = Sulfuric acid pH2 N = Nitric acid pH2
 H = HCl to pH2 B = NaOH to pH12
 T = Sodium Thiosulfate Z = Zinc acetate

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN _____ DAYS
 Expedited results requested by: _____

Who should AIC contact with questions: _____
 Phone: _____ Fax: _____
 Report Attention to: _____
 Report Address to: _____

Relinquished By: **Jeff Smith** Date/Time: **8-25am 8-19-19**
 Relinquished By: **Jeff Smith** Date/Time: **8-19-19 10:15am**

Received By: **Jeff Smith** Date/Time: **8-19-19 8:25am**
 Received By: **ALM** Date/Time: **8-19-19 10:15**

Comments: _____



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

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>SEARCY Water Utilities</u>	AIC CONTROL NO: <u>131796</u>	PAGE _____ OF _____
Project Reference: <u>Bio-Monitor</u>	AIC PROPOSAL NO:	
Project Manager: <u>Timmy Smith</u>	Carrier:	
Sampled By: <u>Jelamy Cheek</u>	Received on top (4°C)? YES <input checked="checked" type="checkbox"/> NO <input type="checkbox"/>	
AIC No. <u>2 EFF</u>	Remarks	
PO No.	NO OF BOTTLES	
SAMPLE MATRIX		
W A T E R L	S O I L	
G R A B	C O M P L	
Date/Time Collected <u>8-21-19 7:55am</u>		
Container Type <u>P</u>	Preservative <u>NO</u>	
G = Glass V = VOA vials	P = Plastic	
NO = none	S = Sulfuric acid pH2	H = HCl to pH2
	N = Nitric acid pH2	B = NaOH to pH12
T = Sodium Thiosulfate	Z = Zinc acetate	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS	Relinquished By: <u>JJC</u>	Received Date/Time
Expedited results requested by:	Date/Time <u>8-21-19 7:45 AM</u>	By:
Who should AIC contact with questions:	Date/Time	Received in Lab
Phone: _____	By:	By: <u>AC341</u>
Report Attention to: _____	Comments:	Date/Time <u>8-21-19</u>
Report Address to: _____		By: <u>0957</u>