



May 22, 2020

Biomonitoring Testing
for
EFF

Control No. 245128-1

Prepared for:

Mr. Jimmy Smith
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P.O. Box 1319
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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 NOEC for growth occurred at 27 % effluent, which is above the critical dilution of 20 %. **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%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.433	PASS
Control Growth CV < or = 40%	16.2	PASS
Growth Minimum Significant Difference 12 to 30%	17.0	PASS
Critical Dilution CV < or = 40%	9.48	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	19.5	PASS
Control CV < or = 40% per Surviving Female	30.9	PASS
Reproduction Minimum Significant Difference 13 to 47%	37.9	PASS
Critical Dilution CV < or = 40%	11.3	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)	7.3	7.6	7.2
pH (standard units)	6.4	6.7	6.9
Alkalinity (mg/l as CaCO ₃)	6.9	11	28
Hardness (mg/l as CaCO ₃)	42	38	38
Conductivity (umhos/cm)	270	280	240
Residual Chlorine (mg/l)	<0.05	<0.05	0.050
Ammonia as N (mg/l)	0.36	0.16	0.15

2. Dilution Water Samples:

Soft

Analysis	244938-1
Dissolved oxygen (mg/l)	7.6
pH (standard units)	7.3
Alkalinity (mg/l as CaCO ₃)	32
Hardness (mg/l as CaCO ₃)	41
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: May 12, 2020 at 0924
Date & Time Test Terminated: May 19, 2020 at 0910
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: May 12, 2020 at 1110
Date & Time Test Terminated: May 18, 2020 at 1100
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 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: May 12, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: May 12, 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

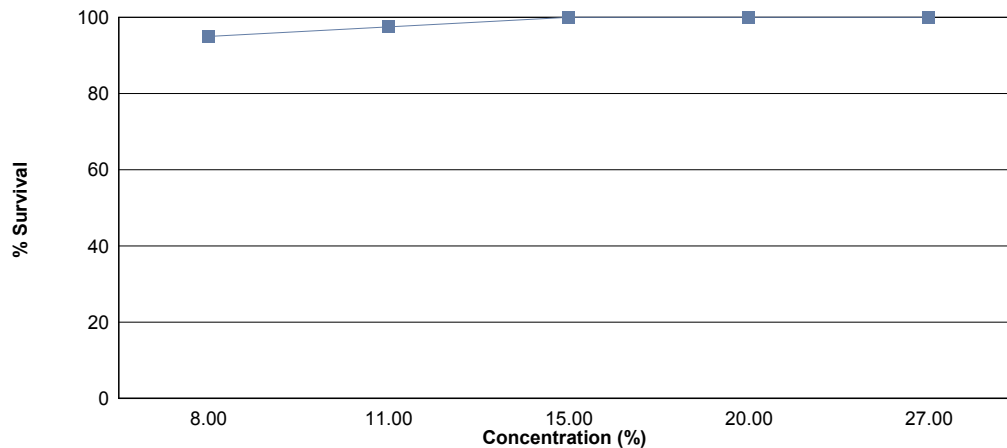
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 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 May 12, 2020 at 0924 and continued through May 19, 2020 at 0910. 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



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.422
8 %	95.0	0.458
11 %	97.5	0.440
15 %	100	0.429
20 %	100	0.486
27 %	100	0.455

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

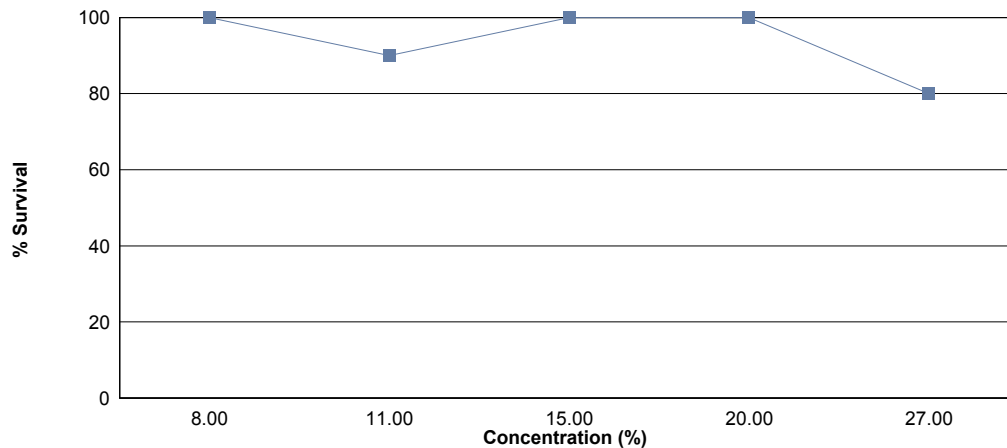
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 May 12, 2020 at 1110 and continued through May 18, 2020 at 1100. 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	19.5
8 %	100	26.8
11 %	90.0	25.1
15 %	100	25.9
20 %	100	28.2
27 %	80.0	24.5

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: May 12, 2020 at 0924

Date and Time Test Terminated: May 19, 2020 at 0910

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	7	7	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
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	7	7	7	7	7
	E	8	8	8	8	8	7	7
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	7	7
15 %	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
20 %	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
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: May 12, 2020 at 0924

Test Terminated: May 19, 2020 at 0910

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.66893	.67306	0.00413	8	0.516
	B	.66828	.67137	0.00309	8	0.386
	C	.67038	.67325	0.00287	8	0.359
	D	.67556	.67857	0.00301	8	0.376
	E	.66898	.67275	0.00377	8	0.471
8 %	A	.67712	.68109	0.00397	8	0.496
	B	.66736	.67087	0.00351	8	0.439
	C	.67056	.67429	0.00373	8	0.466
	D	.66439	.66776	0.00337	8	0.421
	E	.66997	.67373	0.00376	8	0.470
11 %	A	.67083	.67441	0.00358	8	0.448
	B	.67287	.67657	0.00370	8	0.462
	C	.67002	.67387	0.00385	8	0.481
	D	.67032	.67366	0.00334	8	0.418
	E	.66031	.66344	0.00313	8	0.391
15 %	A	.66721	.67096	0.00375	8	0.469
	B	.67443	.67746	0.00303	8	0.379
	C	.67133	.67459	0.00326	8	0.408
	D	.67574	.67889	0.00315	8	0.394
	E	.66641	.67036	0.00395	8	0.494
20 %	A	.66590	.66992	0.00402	8	0.502
	B	.66537	.66912	0.00375	8	0.469
	C	.66421	.66811	0.00390	8	0.488
	D	.66629	.66967	0.00338	8	0.422
	E	.66907	.67345	0.00438	8	0.548
27 %	A	.66526	.66893	0.00367	8	0.459
	B	.66820	.67154	0.00334	8	0.418
	C	.66887	.67251	0.00364	8	0.455
	D	.67086	.67414	0.00328	8	0.410
	E	.66707	.67134	0.00427	8	0.534

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 12, 2020 at 1110

Date and Time Test Terminated: May 18, 2020 at 1100

Concentration: Control														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	3	3	3	0	0	3	0	4	16	10	1.60	
4	2	4	0	0	0	0	4	0	0	0	10	10	1.00	
5	7	5	8	9	8	7	6	8	7	8	73	10	7.30	
6	16	0	0	16	11	9	11	11	11	11	96	10	9.60	
7														
8														
TOTAL	25	9	11	28	22	16	21	22	18	23	195	10	19.5	

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	0	0	0	3	0	5	0	0	0	3	11	10	1.10
4	0	5	4	0	2	0	4	5	2	0	22	10	2.20
5	6	8	9	9	10	9	10	9	8	8	86	10	8.60
6	11	14	17	17	13	15	16	15	15	16	149	10	14.9
7													
8													
TOTAL	17	27	30	29	25	29	30	29	25	27	268	10	26.8

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	0	0	0	4	4	0	0	4	2	4	18	10	1.80
4	X	2	2	0	0	2	3	0	0	0	9	9	1.00
5	X	8	9	8	10	9	13	9	11	11	88	9	9.78
6	X	13	16	17	17	16	13	13	16	15	136	9	15.1
7													
8													
TOTAL	0	23	27	29	31	27	29	26	29	30	251	10	25.1

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 12, 2020 at 1110

Date and Time Test Terminated: May 18, 2020 at 1100

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	0	0	0	5	0	2	0	4	0	4	15	10	1.50	
4	5	2	5	0	0	4	0	0	4	0	20	10	2.00	
5	8	7	11	8	8	9	11	10	11	9	92	10	9.20	
6	11	0	15	12	17	15	17	16	15	14	132	10	13.2	
7														
8														
TOTAL	24	9	31	25	25	30	28	30	30	27	259	10	25.9	

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	0	0	3	2	5	6	2	2	4	24	10	2.40
4	4	4	3	0	0	0	0	0	0	0	11	10	1.10
5	9	8	9	9	11	4	9	8	10	10	87	10	8.70
6	15	15	12	14	19	17	18	15	17	18	160	10	16.0
7													
8													
TOTAL	28	27	24	26	32	26	33	25	29	32	282	10	28.2

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	0	0	0	X	4	3	4	5	3	5	24	9	2.67
4	3	3	2	X	0	0	0	0	0	0	8	9	0.889
5	6X	8	10	X	10	9	11	9	11	10	84	8	10.5
6	X	14	19	X	15	18	15	12	18	18	129	8	16.1
7													
8													
TOTAL	9	25	31	0	29	30	30	26	32	33	245	10	24.5

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	0.87500	1.20940
1	Control	3	1.00000	1.39310
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	0.87500	1.20940
2	8 %	5	0.87500	1.20940
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	0.87500	1.20940
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	1.00000	1.39310
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	1.00000	1.39310
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 = 0.09449 W = 0.7601 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 %	25.00	16.00	5.00	
3	11 %	27.50	16.00	5.00	
4	15 %	30.00	16.00	5.00	
5	20 %	30.00	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 = 0.05524 W = 0.9501 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 3.055 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	0.01359	0.002718	1.181	
Within (Error)	24	0.05524	0.002302		
Total	29	0.06883			
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	0.4216	0.4216			
2	8 %	0.4584	0.4584	-1.213		
3	11 %	0.44	0.44	-0.6064		
4	15 %	0.4288	0.4288	-0.2373		
5	20 %	0.4858	0.4858	-2.116		
6	27 %	0.4552	0.4552	-1.107		
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	0.07161	17	-0.0368	
3	11 %	5	0.07161	17	-0.0184	
4	15 %	5	0.07161	17	-0.0072	
5	20 %	5	0.07161	17	-0.0642	
6	27 %	5	0.07161	17	-0.0336	

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 %	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.

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 %	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
27 %	8	2	10
Total	18	2	20

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

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.2078 D* = 1.63 Critical D* = 1.035 (alpha = 0.01, N = 60)</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	8 %	143.00	75.00	10.00	
3	11 %	139.50	75.00	10.00	
4	15 %	139.00	75.00	10.00	
5	20 %	148.00	75.00	10.00	
6	27 %	133.00	75.00	10.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	448	89.6	1.747	
Within (Error)	54	2770	51.3		
Total	59	3218			
Critical F = 3.38 (alpha = 0.01, df = 5,54) 2.38 (alpha = 0.05, df = 5,54)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

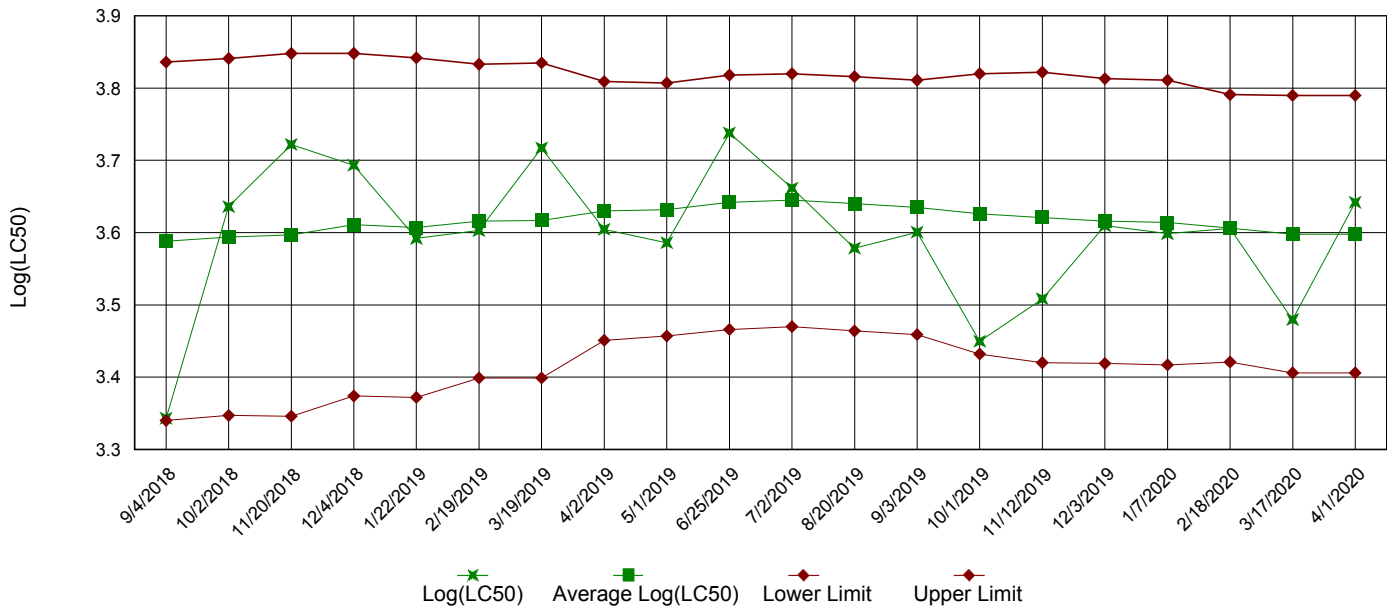
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	19.5	19.5			
2	8 %	26.8	26.8	-2.279		
3	11 %	25.1	25.1	-1.748		
4	15 %	25.9	25.9	-1.998		
5	20 %	28.2	28.2	-2.716		
6	27 %	24.5	24.5	-1.561		
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	8 %	10	7.399	37.9	-7.3	
3	11 %	10	7.399	37.9	-5.6	
4	15 %	10	7.399	37.9	-6.4	
5	20 %	10	7.399	37.9	-8.7	
6	27 %	10	7.399	37.9	-5	

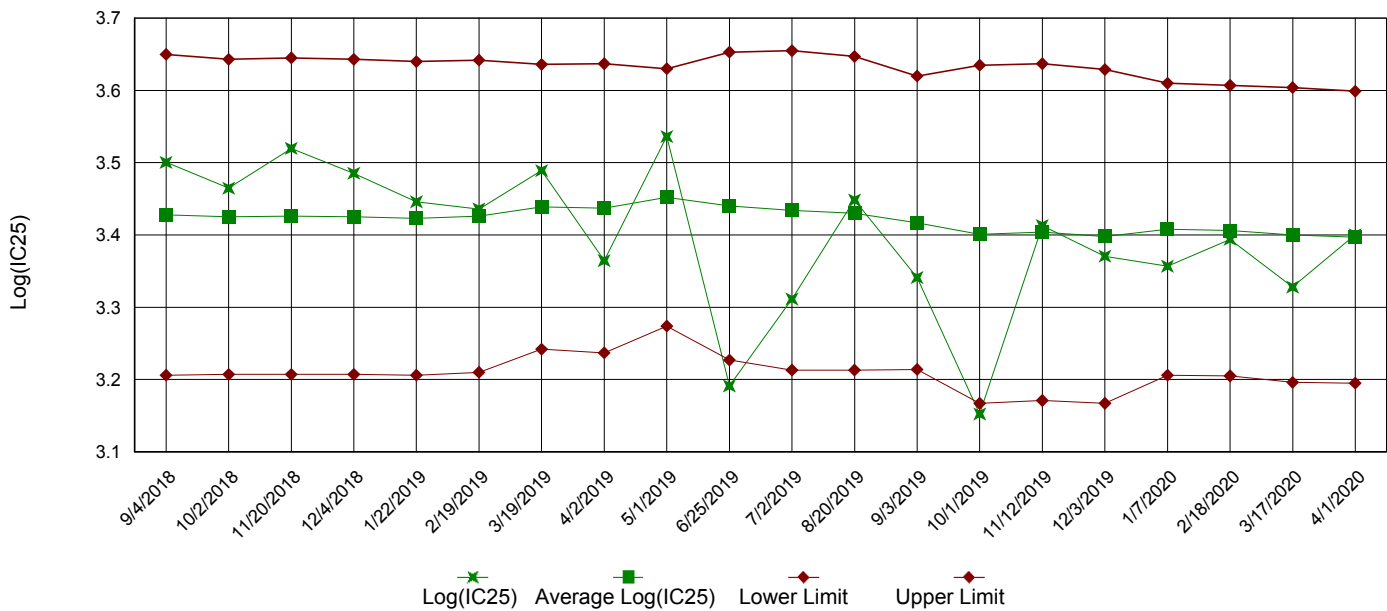
Appendix A3: Test 1000.0

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

LC50 Survival Data

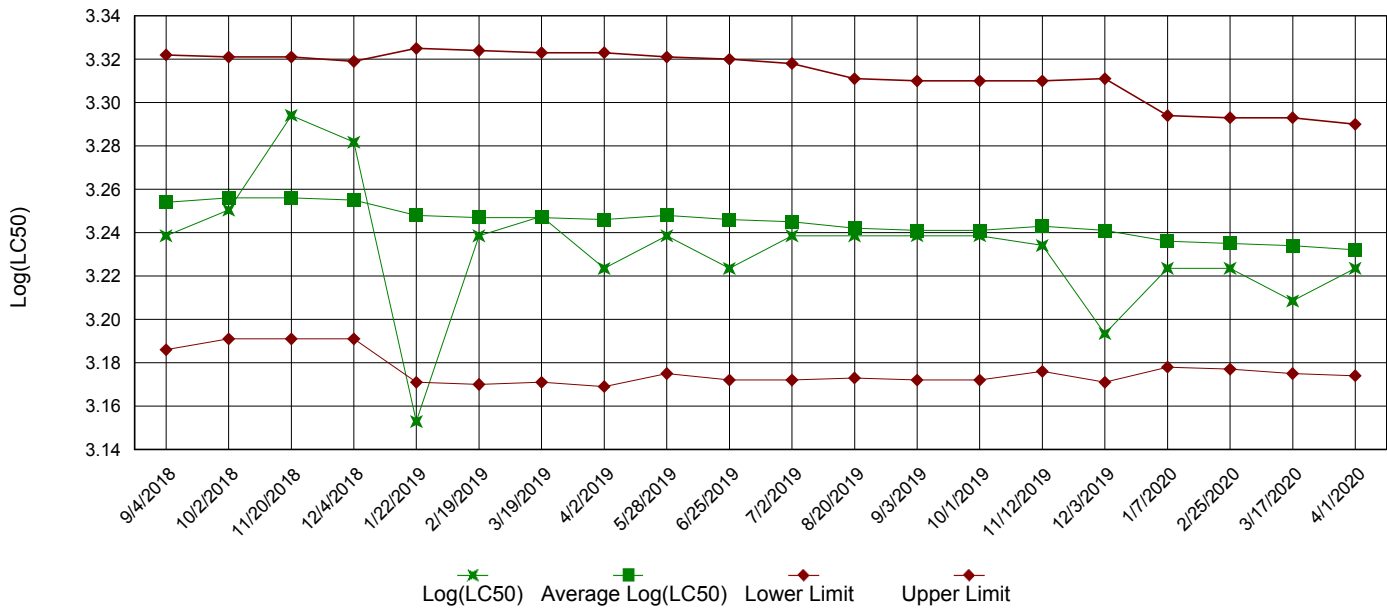


IC25 Growth Data

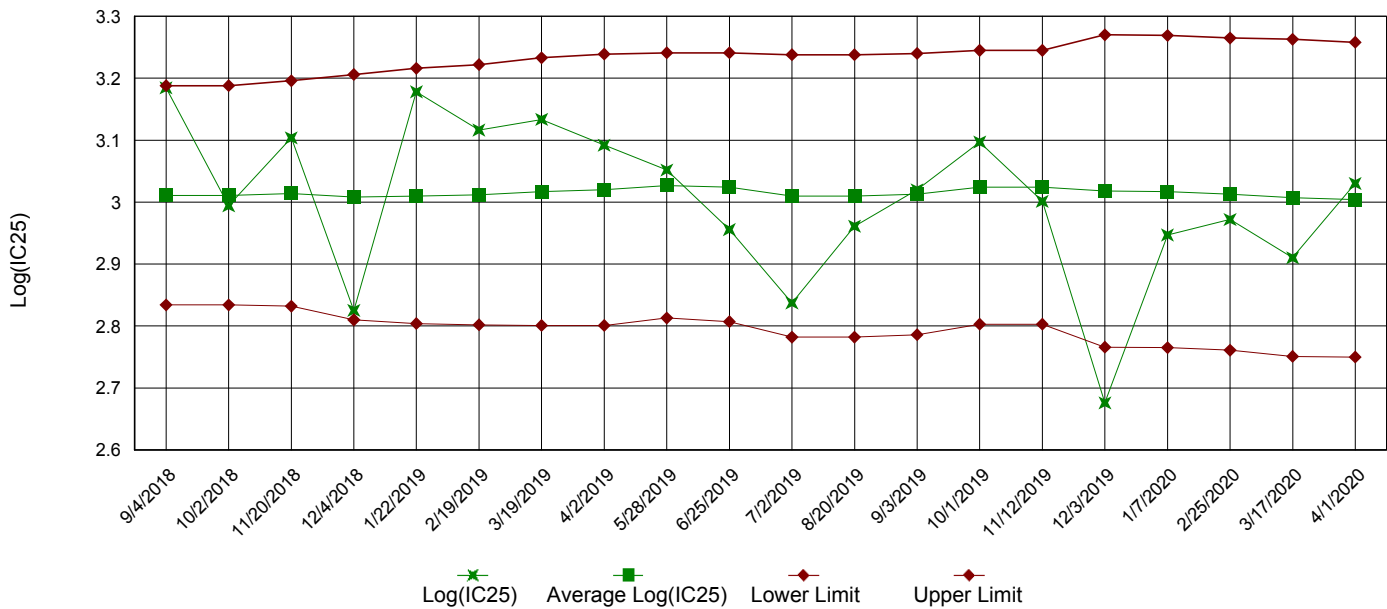


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: May 12, 2020 at 0924

Date and Time Test Terminated: May 19, 2020 at 0910

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	87.5	100	100	100	100	100	97.5	5.73
8 %	100	100	100	87.5	87.5	100	100	95.0	7.21
11 %	100	100	100	100	87.5	100	100	97.5	5.73
15 %	100	100	100	100	100	100	100	100	0.00
20 %	100	100	100	100	100	100	100	100	0.00
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	0.516	0.386	0.359	0.376	0.471	0.422	16.2
8 %	0.496	0.439	0.466	0.421	0.470	0.458	6.34
11 %	0.448	0.462	0.481	0.418	0.391	0.44	8.13
15 %	0.469	0.379	0.408	0.394	0.494	0.429	11.7
20 %	0.502	0.469	0.488	0.422	0.548	0.486	9.48
27 %	0.459	0.418	0.455	0.410	0.534	0.455	10.8

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: 16.2 (TQP6C)
10. Sublethality for this test: 27 % (51714 or 51714S)

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, 345

Test Initiated: DATE: May 12, 2020 TIME: 0924
Test Terminated: DATE: May 19, 2020 TIME: 0910

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.8	7.0	7.0	6.6	8.2	7.2
Final	7.2	6.2	6.9	6.9	6.9	6.4	6.4
pH Initial	7.3	7.5	7.5	7.5	7.7	7.5	7.6
Final	7.5	7.1	7.5	7.5	7.8	7.5	7.6

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	7.1	6.9	6.0	6.9	7.2
Final	6.8	5.8	6.2	6.9	6.8	5.9	6.0
pH Initial	7.3	7.4	7.5	7.4	7.4	7.6	7.8
Final	7.3	7.0	7.4	7.5	7.8	7.5	7.5

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.1	7.5	7.2	6.6	7.2	7.0
Final	6.8	6.0	5.0	6.7	6.6	6.1	5.9
pH Initial	7.3	7.3	7.4	7.3	7.5	7.5	7.5
Final	7.2	7.1	7.2	7.5	7.7	7.4	7.4

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.8	7.0	6.8	6.5	6.9	7.1
Final	6.6	5.1	6.0	6.7	6.7	6.2	6.0
pH Initial	7.3	7.3	7.4	7.3	7.6	7.5	7.5
Final	7.3	7.0	7.2	7.5	7.8	7.5	7.5

DILUTION 20 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	7.8	7.2	6.9	6.4	7.0	6.9
Final	7.2	6.0	6.4	7.2	7.2	6.0	6.3
pH Initial	7.3	7.3	7.4	7.3	7.6	7.5	7.5
Final	7.4	7.1	7.3	7.6	7.8	7.5	7.6

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.7	7.3	7.1	5.8	7.1	7.0
Final	7.1	5.8	5.8	7.2	7.0	6.3	6.1
pH Initial	7.2	7.2	7.3	7.2	7.4	7.4	7.5
Final	7.3	7.0	7.2	7.5	7.7	7.4	7.4

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
6.9	42	270	<0.05	EFF 11-MAY-20
11	38	280	<0.05	EFF 13-MAY-20
28	38	240	0.050	EFF 15-MAY-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	160	<0.05	244938-1

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: May 12, 2020 at 1110

Date and Time Test Terminated: May 18, 2020 at 1100

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	90.0	100	100	80.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		8 %	11 %	15 %	20 %	27 %
A	25	17	0	24	28	9
B	9	27	23	9	27	25
C	11	30	27	31	24	31
D	28	29	29	25	26	0
E	22	25	31	25	32	29
F	16	29	27	30	26	30
G	21	30	29	28	33	30
H	22	29	26	30	25	26
I	18	25	29	30	29	32
J	23	27	30	27	32	33
Mean per Adult	19.5	26.8	25.1	25.9	28.2	24.5
Mean per Surviving Adult	19.5	26.8	27.9	25.9	28.2	29.5
CV %	30.9	14.6	8.68	24.9	11.3	9.42

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: 30.9 (TQP3B)
10. Lethality for this test: 27 % (51710 or 51710P)
11. Sublethality for this test: 27 % (51710 or 51710Q)

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, 345

Test Initiated: DATE: May 12, 2020 TIME: 1110
Test Terminated: DATE: May 18, 2020 TIME: 1100

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.6	7.8	7.0	7.0	6.6	8.2	7.2
Final	7.8	7.2	7.4	7.1	7.9	6.9	--
pH Initial	7.3	7.5	7.5	7.5	7.7	7.5	7.6
Final	8.0	7.9	8.0	8.0	8.0	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	7.8	7.7	7.1	6.9	6.0	6.9	7.2
Final	7.7	6.9	6.5	7.2	7.9	7.0	--
pH Initial	7.3	7.4	7.5	7.4	7.4	7.6	7.8
Final	7.9	7.7	7.7	7.9	8.0	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	7.8	7.1	7.5	7.2	6.6	7.2	7.0
Final	7.8	7.1	6.3	7.2	7.7	7.1	--
pH Initial	7.3	7.3	7.4	7.3	7.5	7.5	7.5
Final	7.9	7.7	7.5	7.8	8.0	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
15 %							
D.O. Initial	7.4	7.8	7.0	6.8	6.5	6.9	7.1
Final	7.8	6.8	6.7	7.1	7.7	6.8	--
pH Initial	7.3	7.3	7.4	7.3	7.6	7.5	7.5
Final	7.8	7.7	7.7	7.8	7.9	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	7.3	7.8	7.2	6.9	6.4	7.0	6.9
Final	7.2	7.1	6.6	7.1	7.7	6.8	--
pH Initial	7.3	7.3	7.4	7.3	7.6	7.5	7.5
Final	7.8	7.7	7.8	7.8	7.9	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.6	7.7	7.3	7.1	5.8	7.1	7.0
Final	7.2	7.3	6.9	7.4	7.6	7.2	--
pH Initial	7.2	7.2	7.3	7.2	7.4	7.4	7.5
Final	7.8	7.7	7.8	7.8	7.9	8.0	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
6.9	42	270	<0.05	EFF 11-MAY-20
11	38	280	<0.05	EFF 13-MAY-20
28	38	240	0.050	EFF 15-MAY-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	160	<0.05	244938-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Searcy Water Utilities</u>		AIC CONTROL NO: <u>245128</u>	
Project Reference: <u>Bio-Monitoring</u>		AIC PROPOSAL NO:	
Project Manager: <u>Jimmy Smith</u>		Carrier:	
Sampled By: <u>Jeremy Cheely</u>		Received Temperature C: <u>1.5</u>	
AIC Sample Identification		Remarks	
No.	1	EFF	5-11-20 8am
G R A B		C O M P	
NO OF BOTTLES		2	
PO No.		ANALYSES REQUESTED	
SAMPLE MATRIX		W A T E R	
S O I L		B I O - M O N I T O R	
Container Type		Field pH calibration	
Preservative		on @	
G = Glass		Buffer:	
NO = none		T = Sodium Thiosulfate	
P = Plastic		Z = Zinc acetate	
S = Sulfuric acid pH2		H = HCl to pH2	
V = VOA vials		B = NaOH to pH12	
N = Nitric acid pH2		Received	
Relinquished		By:	
By:		Date/Time	
Relinquished		Received in Lab	
By: <u>Brady Anderson</u>		By: <u>D. Brown</u>	
Date/Time		Date/Time	
5-11-20 9:30am		5-11-20 9:30	
Comments:		0930	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Searcy Water Utilities</u>		AIC CONTROL NO: <u>245128</u>	
Project Reference: <u>2nd Qtr. Biomonitoring</u>		AIC PROPOSAL NO:	
Project Manager: <u>Jimmy Smith</u>		Carrier:	
Sampled By: <u>BA</u>		Received Temperature C: <u>2.4</u>	
AIC Sample Identification: <u>2 Eff</u>		Remarks:	
Date/Time Collected: <u>5/13/20 8:00am</u>			
G R A B			
C O M P			
NO OF BOTTLES: <u>2</u>			
PO No.		ANALYSES REQUESTED	
SAMPLE MATRIX			
W A T E R			
S O I L			
Container Type		Field pH calibration	
Preservative		on _____ @ _____	
G = Glass		Buffer:	
NO = none		T = Sodium Thiosulfate	
P = Plastic		Z = Zinc acetate	
S = Sulfuric acid pH2		H = HCl to pH2	
V = VOA vials		B = NaOH to pH12	
N = Nitric acid pH2		Received By:	
Turnaround Time Requested: (Please circle)		Date/Time	
NORMAL or EXPEDITED IN _____ DAYS		Received in Lab	
Expedited results requested by _____		By: <u>DANNY BREWSTER</u>	
Who should AIC contact with questions:		Date/Time	
Phone: _____		5-13-20	
Report Attention to: _____		0922	
Report Address to: _____		Comments:	



LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 3 OF 3

Client: Searcy Water Utilities			PO No.		ANALYSES REQUESTED						NO OF BOTTLES	AIC CONTROL NO:			
Project Reference: Bio-Monitor			SAMPLE MATRIX		W	A	S	O	I	L	Bio-Monitor		245128		
Project Manager: Jimmy Smith			G R A B								C	N	H	Phenolics	HG
Sampled By: Brady Anderson + Jeremy Kelly			Date/Time Collected								Received Temperature C		Remarks		
3	EFF		5-14-20	8 AM											
	EFF		5-14-20	8 AM 2P	/										
	EFF		5-15-20	8 AM 2P	/										
	EFF		5-14-20	8 AM 2P	/										
	EFF		5-15-20	8 AM 2P	/										
	EFF		5-14-20	8 AM											

Field pH calibration on _____ @ _____

Buffer: _____

T = Sodium Thiosulfate
Z = Zinc acetate

H = HCl to pH2
B = NaOH to pH12

Relinquished		Received	
By:	Date/Time	By:	Date/Time
By: Brady Anderson	5-15-20 10:43	By: [Signature]	5-15-20 1043

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