



June 20, 2019

Biomonitoring Testing
for
EFF

Control No. 235155-1

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:

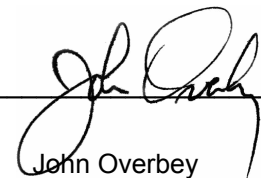
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire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

- I. Control Acceptance Criteria
- II. Outlined Report
- III. Data Analysis
- IV. Standard Reference Toxicants
- V. Organism History
- VI. Results Summary
 - Pimephales promelas* (Fathead minnow)
 - Ceriodaphnia dubia*
- Appendix A: Raw Data
 - A1: Test 1000.0
 - Pimephales promelas* (Fathead minnow) Survival and Growth
 - Test 1002.0
 - Ceriodaphnia dubia* Survival and Reproduction
 - A2: Statistics
 - A3: Reference Toxicant
- Appendix B: Summary Forms

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	0.367	PASS
Control Growth CV < or = 40%	4.44	PASS
Growth Minimum Significant Difference 12 to 30%	18.0	PASS
Critical Dilution CV < or = 40%	9.63	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.1	PASS
Control CV < or = 40% per Surviving Female	32.9	PASS
Reproduction Minimum Significant Difference 13 to 47%	28.3	PASS
Critical Dilution CV < or = 40%	19.8	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)	7.8	8.4	8.2
pH (standard units)	6.6	6.2	6.8
Alkalinity (mg/l as CaCO ₃)	8.5	19	40
Hardness (mg/l as CaCO ₃)	47	45	41
Conductivity (umhos/cm)	240	290	320
Residual Chlorine (mg/l)	0.060	<0.05	0.070
Ammonia as N (mg/l)	0.13	0.18	<0.1

2. Dilution Water Samples:

Soft

Analysis	234982-1
Dissolved oxygen (mg/l)	7.7
pH (standard units)	7.4
Alkalinity (mg/l as CaCO ₃)	31
Hardness (mg/l as CaCO ₃)	40
Conductivity (umhos/cm)	150
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: June 11, 2019 at 1053
Date & Time Test Terminated: June 18, 2019 at 1035
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: June 11, 2019 at 1150
Date & Time Test Terminated: June 17, 2019 at 1144
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Source of test organisms: Obtained from in-house cultures

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

- a. Test 1000.0 *Pimephales promelas*
- b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat and following EPA method criteria.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on May 01, 2019 at 1330 to May 08, 2019 at 1433

The results were as follows: (Control No. 234022-1.)

Survival LC-50: 3855 mg/l

Growth IC-25: 3438 mg/l

Growth PMSD: 16

Ceriodaphnia dubia

A chronic reference test was performed on May 28, 2019 at 1400 to June 04, 2019 at 1413

The results were as follows: (Control No. 234022-2.)

Survival LC-50: 1732 mg/l

Growth IC-25: 1127 mg/l

Growth PMSD: 13.3

V. Organism History

Pimephales promelas (Fathead minnow)

Date: June 11, 2019

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: June 11, 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

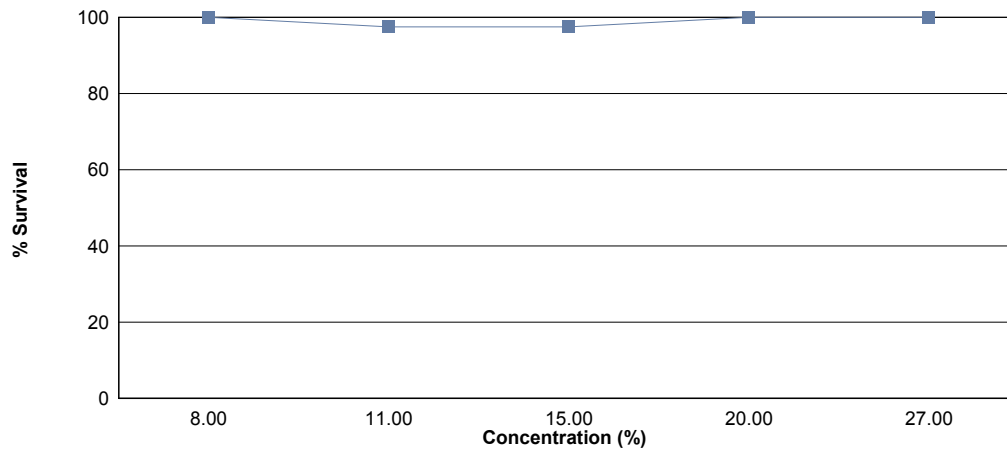
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 June 11, 2019 at 1053 and continued through June 18, 2019 at 1035. 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	100	0.367
8 %	100	0.458
11 %	97.5	0.427
15 %	97.5	0.462
20 %	100	0.459
27 %	100	0.401

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

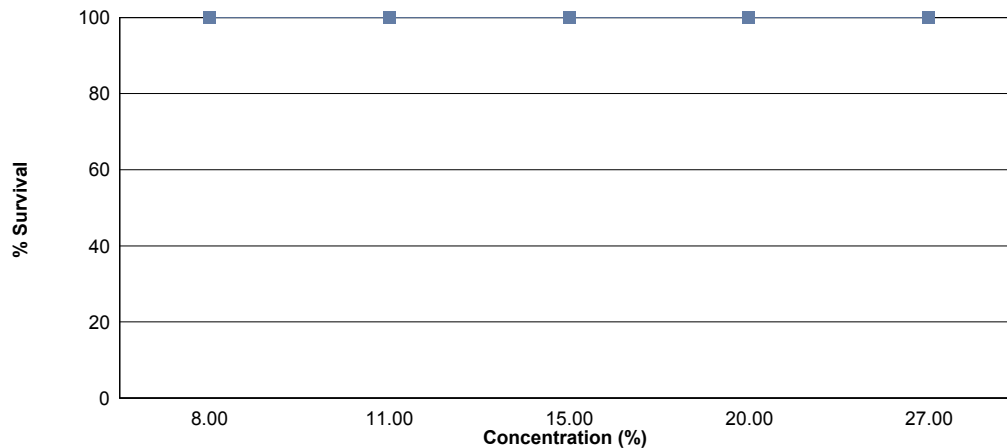
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 June 11, 2019 at 1150 and continued through June 17, 2019 at 1144. 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



Concentration	Percent Survival	Mean Reproduction
Control	100	18.1
8 %	100	28.4
11 %	100	28.6
15 %	100	27.2
20 %	100	27.3
27 %	100	23.1

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: June 11, 2019 at 1053

Date and Time Test Terminated: June 18, 2019 at 1035

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
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	7	7	7	7	7
15 %	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
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: June 11, 2019 at 1053

Test Terminated: June 18, 2019 at 1035

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92149	.92440	0.00291	8	0.364
	B	.92402	.92678	0.00276	8	0.345
	C	.93059	.93346	0.00287	8	0.359
	D	.92313	.92622	0.00309	8	0.386
	E	.91807	.92110	0.00303	8	0.379
8 %	A	.92424	.92714	0.00290	8	0.362
	B	.93654	.94027	0.00373	8	0.466
	C	.93919	.94341	0.00422	8	0.528
	D	.93623	.94006	0.00383	8	0.479
	E	.92873	.93237	0.00364	8	0.455
11 %	A	.92058	.92392	0.00334	8	0.418
	B	.91493	.91875	0.00382	8	0.478
	C	.92105	.92437	0.00332	8	0.415
	D	.92302	.92652	0.00350	8	0.438
	E	.92367	.92674	0.00307	8	0.384
15 %	A	.92123	.92507	0.00384	8	0.480
	B	.92541	.92832	0.00291	8	0.364
	C	.92392	.92781	0.00389	8	0.486
	D	.92595	.92990	0.00395	8	0.494
	E	.91770	.92157	0.00387	8	0.484
20 %	A	.92165	.92520	0.00355	8	0.444
	B	.91943	.92304	0.00361	8	0.451
	C	.92033	.92405	0.00372	8	0.465
	D	.93131	.93457	0.00326	8	0.408
	E	.92298	.92721	0.00423	8	0.529
27 %	A	.93925	.94200	0.00275	8	0.344
	B	.93344	.93688	0.00344	8	0.430
	C	.92556	.92894	0.00338	8	0.422
	D	.92476	.92825	0.00349	8	0.436
	E	.92502	.92799	0.00297	8	0.371

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2019 at 1150

Date and Time Test Terminated: June 17, 2019 at 1144

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	4	0	0	4	0	0	5	4	25	10	2.50
4	0	0	0	4	4	0	3	3	0	0	14	10	1.40
5	8	9	9	8	7	6	8	8	9	8	80	10	8.00
6	12	10	10	0	0	11	0	0	9	10	62	10	6.20
7													
8													
TOTAL	24	23	23	12	11	21	11	11	23	22	181	10	18.1

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	5	0	4	4	2	5	5	3	5	2	35	10	3.50
4	0	6	0	1	0	0	0	0	0	0	7	10	0.700
5	12	12	9	14	15	10	13	13	10	9	117	10	11.7
6	16	0	14	17	12	12	14	14	13	13	125	10	12.5
7													
8													
TOTAL	33	18	27	36	29	27	32	30	28	24	284	10	28.4

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	5	4	0	3	5	4	3	6	5	4	39	10	3.90
4	0	0	0	0	1	0	0	0	0	0	1	10	0.100
5	13	10	11	7	11	11	10	14	13	3	103	10	10.3
6	17	16	15	11	15	14	13	16	15	11	143	10	14.3
7													
8													
TOTAL	35	30	26	21	32	29	26	36	33	18	286	10	28.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2019 at 1150

Date and Time Test Terminated: June 17, 2019 at 1144

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	4	5	4	4	6	2	0	4	5	2	36	10	3.60	
4	0	8	0	0	0	0	5	1	0	0	14	10	1.40	
5	11	0	9	10	7	11	10	8	11	10	87	10	8.70	
6	17	16	14	13	13	12	11	16	11	12	135	10	13.5	
7														
8														
TOTAL	32	29	27	27	26	25	26	29	27	24	272	10	27.2	

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	5	5	1	6	0	4	3	5	5	4	38	10	3.80
4	0	0	0	0	4	0	0	0	0	0	4	10	0.400
5	10	11	10	12	7	10	11	9	9	10	99	10	9.90
6	17	15	12	16	4	16	12	12	14	14	132	10	13.2
7													
8													
TOTAL	32	31	23	34	15	30	26	26	28	28	273	10	27.3

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	5	3	5	4	3	3	6	5	5	0	39	10	3.90
4	0	1	0	0	0	0	0	0	0	5	6	10	0.600
5	7	7	9	8	9	7	7	10	8	9	81	10	8.10
6	14	13	13	11	10	12	8	15	9	0	105	10	10.5
7													
8													
TOTAL	26	24	27	23	22	22	21	30	22	14	231	10	23.1

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	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	0.87500	1.20940
4	15 %	1	1.00000	1.39310
4	15 %	2	0.87500	1.20940
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 = 0.05399 W = 0.5466 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	8 %	27.50	16.00	5.00	
3	11 %	25.00	16.00	5.00	
4	15 %	25.00	16.00	5.00	
5	20 %	27.50	16.00	5.00	
6	27 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.04698 W = 0.9436 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 = 5.829 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	0.03767	0.007534	3.848	
Within (Error)	24	0.04698	0.001958		
Total	29	0.08465			
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	0.3666	0.3666			
2	8 %	0.458	0.458	-3.266		
3	11 %	0.4266	0.4266	-2.144		
4	15 %	0.4616	0.4616	-3.395		
5	20 %	0.4594	0.4594	-3.316		
6	27 %	0.4006	0.4006	-1.215		
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	0.06605	18	-0.0914	
3	11 %	5	0.06605	18	-0.06	
4	15 %	5	0.06605	18	-0.095	
5	20 %	5	0.06605	18	-0.0928	
6	27 %	5	0.06605	18	-0.034	

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 %	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 %	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	0	
5	27 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.1192 D* = 0.9352 Critical D* = 1.035</p> <p style="text-align: right;">(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 = 8.215 Critical B = 15.086</p> <p style="text-align: right;">(alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	846.6	169.3	6.874	
Within (Error)	54	1330	24.63		
Total	59	2177			
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)					

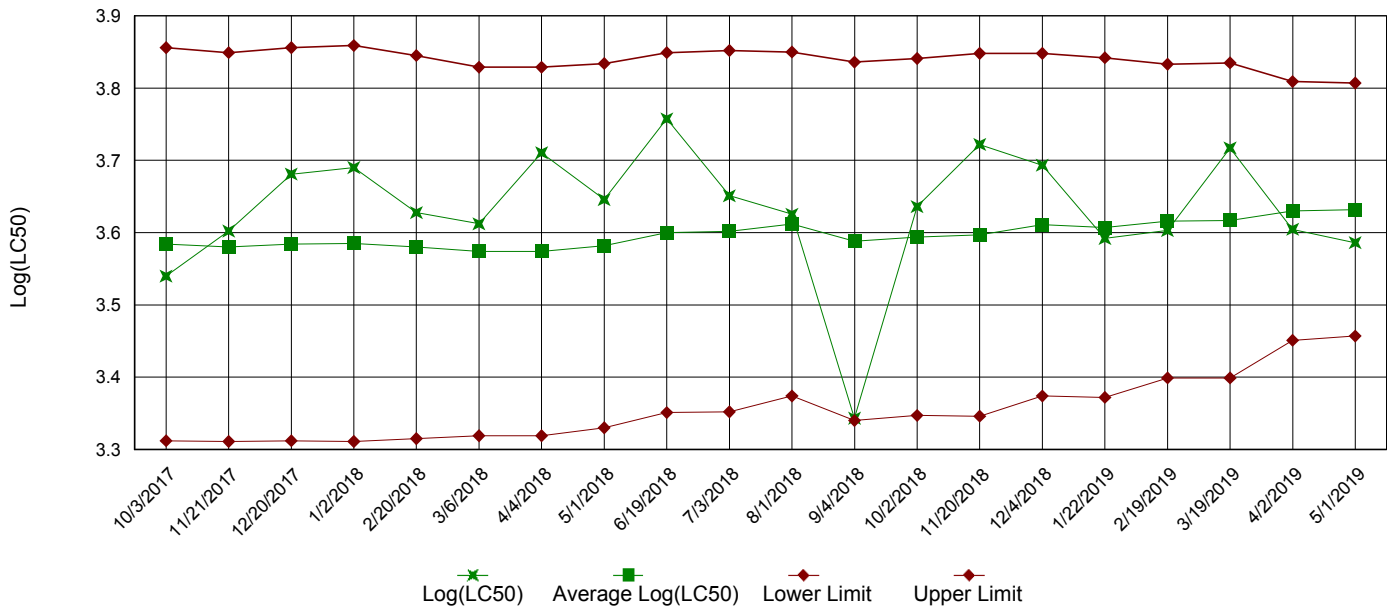
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	18.1	18.1			
2	8 %	28.4	28.4	-4.641		
3	11 %	28.6	28.6	-4.731		
4	15 %	27.2	27.2	-4.1		
5	20 %	27.3	27.3	-4.145		
6	27 %	23.1	23.1	-2.253		
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	5.127	28.3	-10.3	
3	11 %	10	5.127	28.3	-10.5	
4	15 %	10	5.127	28.3	-9.1	
5	20 %	10	5.127	28.3	-9.2	
6	27 %	10	5.127	28.3	-5	

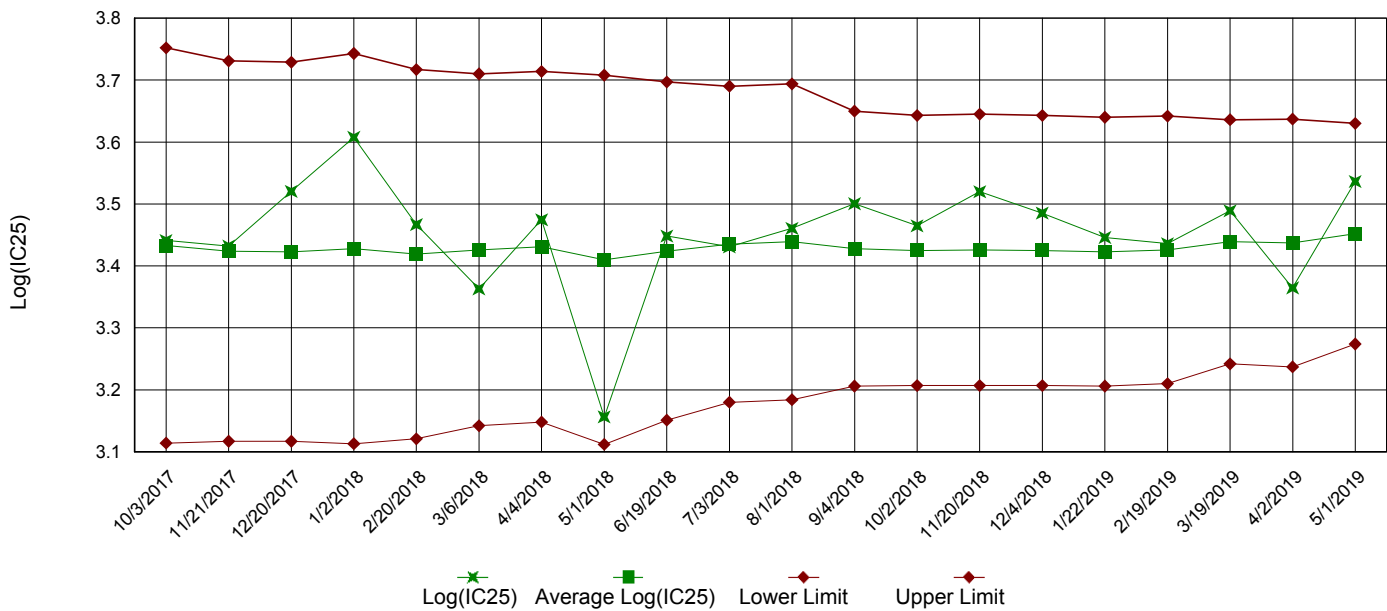
Appendix A3: Test 1000.0

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

LC50 Survival Data

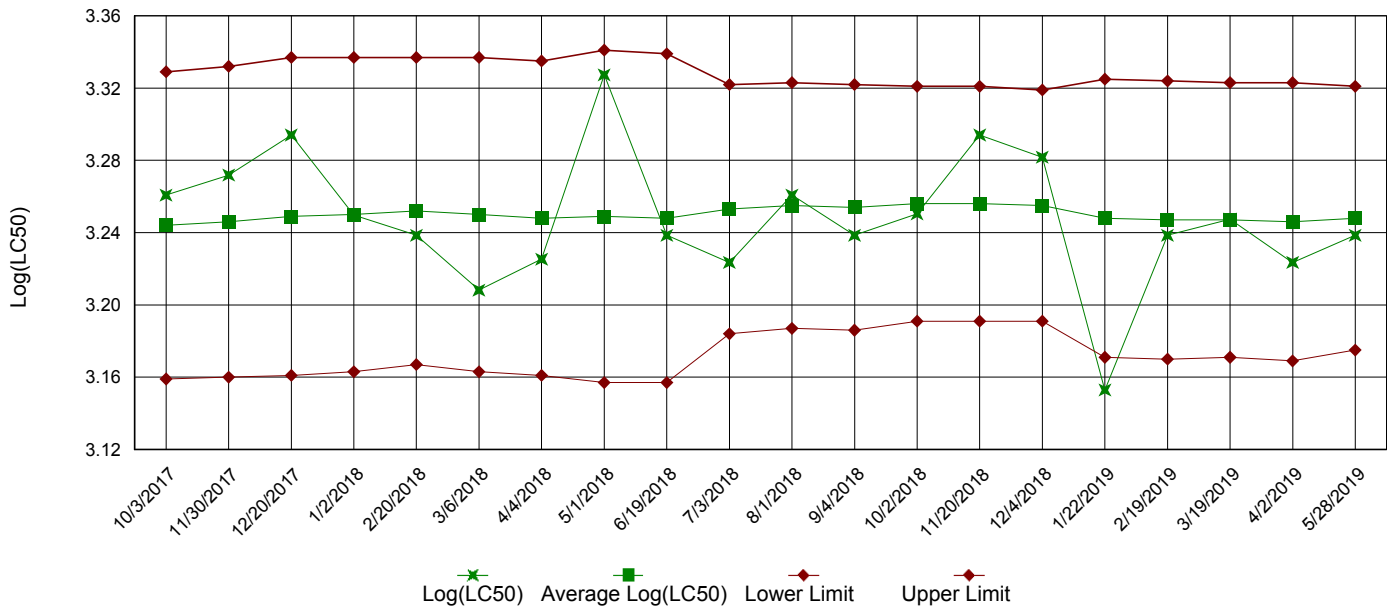


IC25 Growth Data

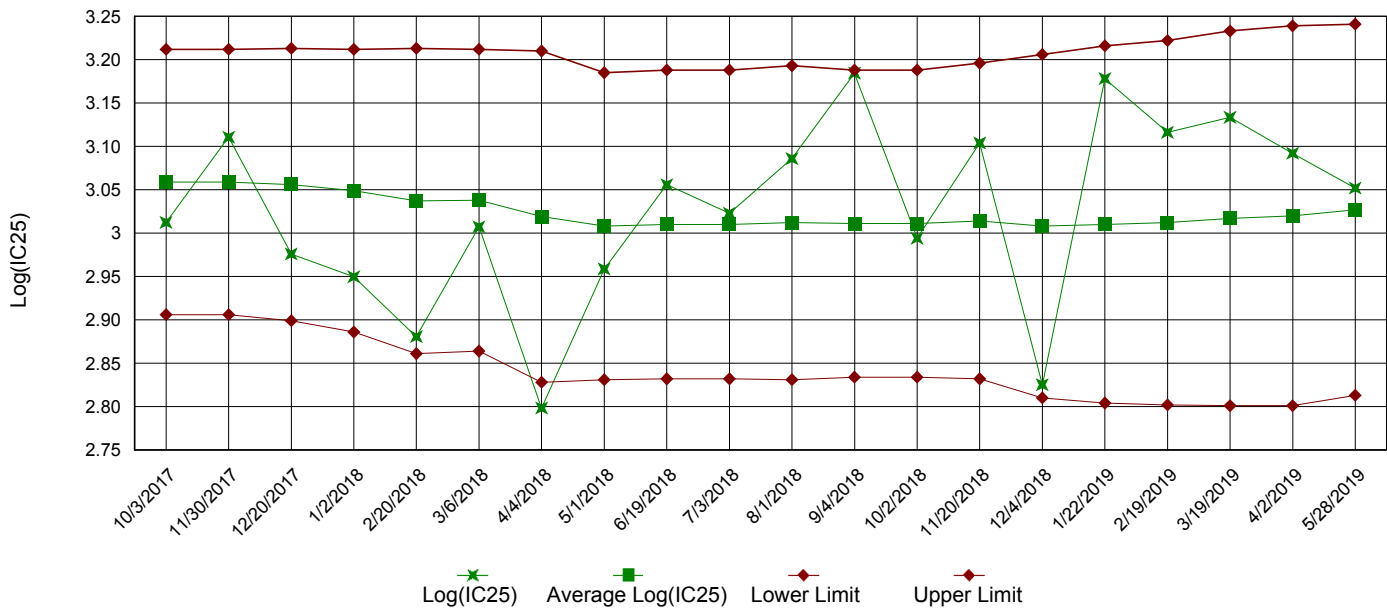


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: June 11, 2019 at 1053

Date and Time Test Terminated: June 18, 2019 at 1035

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
8 %	100	100	100	100	100	100	100	100	0.00
11 %	100	100	100	100	87.5	100	100	97.5	5.73
15 %	100	87.5	100	100	100	100	100	97.5	5.73
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	0.364	0.345	0.359	0.386	0.379	0.367	4.44
8 %	0.362	0.466	0.528	0.479	0.455	0.458	13.2
11 %	0.418	0.478	0.415	0.438	0.384	0.427	8.12
15 %	0.480	0.364	0.486	0.494	0.484	0.462	11.9
20 %	0.444	0.451	0.465	0.408	0.529	0.459	9.63
27 %	0.344	0.430	0.422	0.436	0.371	0.401	10.2

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: 9.63 (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: June 11, 2019 TIME: 1053
 Test Terminated: DATE: June 18, 2019 TIME: 1035

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.6	8.6	8.4	7.8	7.6	8.1
Final	6.2	5.8	5.8	6.7	7.3	6.2	6.4
pH Initial	7.4	7.4	6.7	7.0	7.1	6.9	6.9
Final	7.2	6.9	6.9	7.3	7.4	7.0	7.0

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	7.6	8.2	7.8	7.5	7.6	8.0
Final	6.1	5.9	6.4	6.3	7.4	6.0	6.5
pH Initial	7.4	7.3	6.7	7.0	7.1	7.1	7.0
Final	7.1	6.8	6.9	7.2	7.4	7.0	7.0

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.7	8.4	7.9	8.3	7.8	8.0
Final	5.7	5.7	6.5	6.3	7.4	6.0	6.5
pH Initial	7.3	7.4	6.8	7.0	7.1	7.1	7.1
Final	7.0	6.8	6.9	7.1	7.3	7.0	7.0

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.3	8.3	7.7	7.9	7.6	7.5
Final	5.9	6.0	6.1	6.4	7.1	5.4	6.4
pH Initial	7.2	7.4	6.7	7.0	7.1	7.2	7.1
Final	7.0	6.8	6.9	7.2	7.3	6.9	7.0

DILUTION 20 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	8.0	7.8	7.5	7.5	7.7
Final	6.0	5.7	6.5	6.7	7.0	5.5	6.3
pH Initial	7.2	7.4	6.7	7.0	7.1	7.2	7.1
Final	7.1	6.8	7.0	7.2	7.3	7.0	7.0

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.8	8.3	7.9	7.7	7.5	7.9
Final	5.9	6.3	6.5	6.6	7.2	5.7	6.4
pH Initial	7.2	7.3	6.7	7.0	7.0	7.2	7.1
Final	7.1	6.8	7.0	7.2	7.3	7.0	7.0

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
8.5	47	240	0.060	EFF 10-JUN-19
19	45	290	<0.05	EFF 12-JUN-19
40	41	320	0.070	EFF 14-JUN-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	40	150	<0.05	234982-1

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: June 11, 2019 at 1150

Date and Time Test Terminated: June 17, 2019 at 1144

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	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		8 %	11 %	15 %	20 %	27 %
A	24	33	35	32	32	26
B	23	18	30	29	31	24
C	23	27	26	27	23	27
D	12	36	21	27	34	23
E	11	29	32	26	15	22
F	21	27	29	25	30	22
G	11	32	26	26	26	21
H	11	30	36	29	26	30
I	23	28	33	27	28	22
J	22	24	18	24	28	14
Mean per Adult	18.1	28.4	28.6	27.2	27.3	23.1
Mean per Surviving Adult	18.1	28.4	28.6	27.2	27.3	23.1
CV %	32.9	17.7	20.6	8.45	19.8	18.4

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

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)
5. NOEC Ceriodaphnia Lethality: 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: 32.9 (TQP3B)
10. Lethality for this test: 27 % (22414 or 51710)
11. Sublethality for this test: 27 % (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: June 11, 2019 TIME: 1150
Test Terminated: DATE: June 17, 2019 TIME: 1144

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.7	7.6	8.6	8.4	7.8	7.6	8.1
Final	7.8	8.2	7.8	7.8	7.4	7.9	8.2
pH Initial	7.4	7.4	6.7	7.0	7.1	6.9	6.9
Final	7.7	7.5	7.7	7.7	7.7	7.6	7.6

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	7.9	7.6	8.2	7.8	7.5	7.6	8.0
Final	7.8	8.1	8.2	7.8	7.2	7.9	8.2
pH Initial	7.4	7.3	6.7	7.0	7.1	7.1	7.0
Final	7.8	7.6	7.8	7.7	7.7	7.6	7.6

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	7.5	7.7	8.4	7.9	8.3	7.8	8.0
Final	8.0	8.2	7.6	7.5	7.2	7.7	8.2
pH Initial	7.3	7.4	6.8	7.0	7.1	7.1	7.1
Final	7.8	7.5	7.7	7.7	7.8	7.6	7.6

DILUTION	DAY						
	1	2	3	4	5	6	7
15 %							
D.O. Initial	7.6	7.3	8.3	7.7	7.9	7.6	7.5
Final	8.0	7.8	8.0	7.5	7.1	7.6	8.2
pH Initial	7.2	7.4	6.7	7.0	7.1	7.2	7.1
Final	7.8	7.4	7.8	7.8	7.8	7.6	7.6

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	7.8	7.7	8.0	7.8	7.5	7.5	7.7
Final	7.8	8.1	8.2	7.4	6.8	7.6	7.7
pH Initial	7.2	7.4	6.7	7.0	7.1	7.2	7.1
Final	7.8	7.4	7.7	7.7	7.8	7.6	7.6

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.7	7.8	8.3	7.9	7.7	7.5	7.9
Final	7.9	7.8	8.1	7.7	7.3	7.6	7.8
pH Initial	7.2	7.3	6.7	7.0	7.0	7.2	7.1
Final	7.8	7.4	7.8	7.8	7.9	7.6	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
8.5	47	240	0.060	EFF 10-JUN-19
19	45	290	<0.05	EFF 12-JUN-19
40	41	320	0.070	EFF 14-JUN-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	40	150	<0.05	234982-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: <u>Sassy Waters</u>		AIC CONTROL NO: <u>235155</u>	
Project Reference: <u>Biomonitoring</u>		AIC PROPOSAL NO:	
Project Manager: <u>Jimmy Smith</u>		Carrier:	
Sampled By: <u>Marshall Price</u>		Received Temperature C <u>1.4</u>	
AIC No. <u>2</u>		Remarks	
Sample Identification: <u>ESF</u>			
Date/Time Collected: <u>6/11/19 7:45am</u>			
Date/Time Collected: <u>6/12/19 7:45</u>			
Container Type		Field pH calibration	
Preservative		on _____ @ _____	
G = Glass NO = none P = Plastic S = Sulfuric acid pH2		T = Sodium Thiosulfate Z = Zinc acetate A = (NH4)2, NH4OH	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS		Received Date/Time	
Expedited results requested by: _____		By: _____	
Who should AIC contact with questions: Phone: _____ Fax: _____		Received in Lab Date/Time <u>6-12-19</u>	
Report Attention to: _____		By: <u>D. Brewer</u>	
Report Address to: _____		Comments:	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: SEARCY WATER UTILITIES		AIC CONTROL NO: 23533	
Project Reference: Bio-Monitor		AIC PROPOSAL NO:	
Project Manager: JIMMY SMITH		Carrier:	
Sampled by: JEREMY CHEELY, MARSHALL PACE		Received Temperature C: 21.04	
IC No.		Remarks	
EFF			
EFF			
EFF			
EFF			
EFF			

PO No.	NO OF BOTTLES	ANALYSES REQUESTED				Field pH calibration on @	Buffer:
		BiO-Monitor	Metals	CN	PHENOLICS		
	2						
	1						
	4						
	4						
	4						

Container Type	Preservative	NO = none	G = Glass	S = Sulfuric acid pH2	V = VOA vials	H = HCl to pH2	T = Sodium Thiosulfate
							Z = Zinc acetate
							A = (NH4)2SO4, NH4OH

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By: <i>[Signature]</i>	6-14-19 11:21am	Received In Lab By: ALBY	Date/Time: 06-14-19

Comments:

Urgency 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: _____

Mail Address: _____