



November 27, 2019

Biomonitoring Testing  
for  
Eff

Control No. 239937-1

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  
P.O. Box 1319  
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:

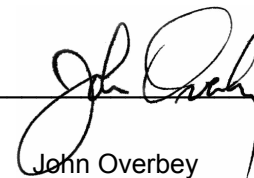
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.40	PASS
Control Growth CV < or = 40%	7.0	PASS
Growth Minimum Significant Difference 12 to 30%	16	PASS
Critical Dilution CV < or = 40%	8.9	PASS

*Ceriodaphnia dubia* Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	21	PASS
Control CV < or = 40% per Surviving Female	9.9	PASS
Reproduction Minimum Significant Difference 13 to 47%	37	PASS
Critical Dilution CV < or = 40%	10	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)	7.4	8.0	8.3
pH (standard units)	6.4	6.3	6.8
Alkalinity (mg/l as CaCO <sub>3</sub> )	5.2	12	17
Hardness (mg/l as CaCO <sub>3</sub> )	44	42	44
Conductivity (umhos/cm)	280	310	330
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.30	0.18	0.24

2. Dilution Water Samples:

Soft

Analysis	239589-1
Dissolved oxygen (mg/l)	7.4
pH (standard units)	7.8
Alkalinity (mg/l as CaCO <sub>3</sub> )	29
Hardness (mg/l as CaCO <sub>3</sub> )	44
Conductivity (umhos/cm)	180
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: November 19, 2019 at 1055  
Date & Time Test Terminated: November 26, 2019 at 0930  
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: November 19, 2019 at 1000  
Date & Time Test Terminated: November 25, 2019 at 0900  
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 October 01, 2019 at 1210 to October 08, 2019 at 1250

The results were as follows: (Control No. 238548-1.)

Survival LC-50: 2816 mg/l

Growth IC-25: 1420 mg/l

Growth PMSD: 0

##### *Ceriodaphnia dubia*

A chronic reference test was performed on October 01, 2019 at 1100 to October 07, 2019 at 1050

The results were as follows: (Control No. 238548-2.)

Survival LC-50: 1732.1 mg/l

Reproduction IC-25: 1250 mg/l

Reproduction PMSD: 20.9

#### V. Organism History

##### *Pimephales promelas* (Fathead minnow)

Date: November 19, 2019

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

##### *Ceriodaphnia dubia*

Date: November 19, 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

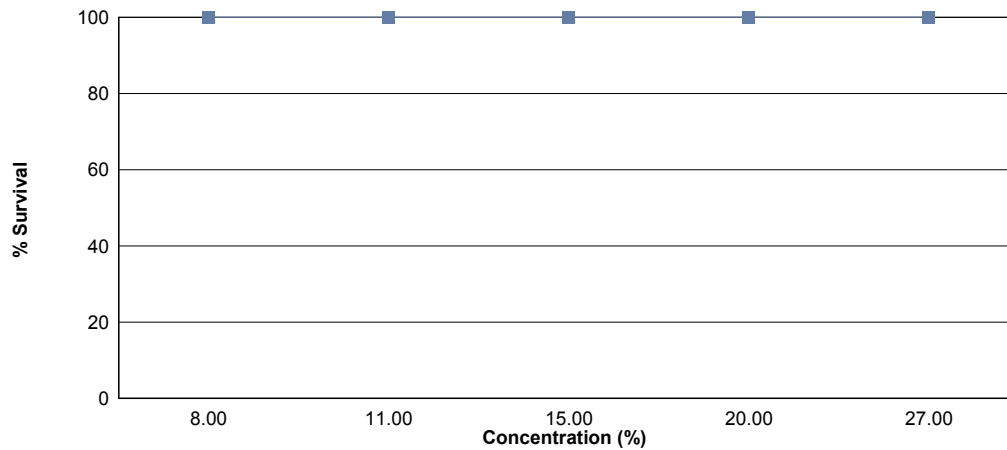
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 November 19, 2019 at 1055 and continued through November 26, 2019 at 0930. 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	0.396
8 %	100	0.380
11 %	100	0.362
15 %	100	0.362
20 %	100	0.402
27 %	100	0.339

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

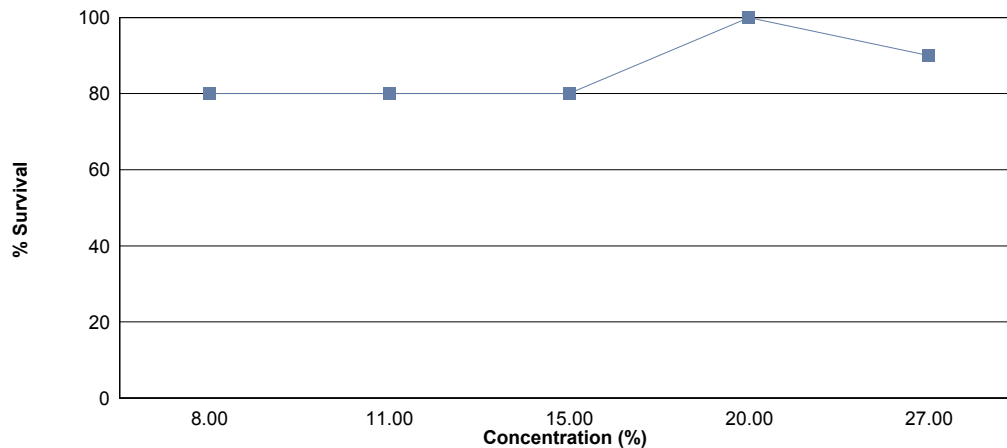
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 November 19, 2019 at 1000 and continued through November 25, 2019 at 0900. 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	21.4
8 %	80.0	20.5
11 %	80.0	19.5
15 %	80.0	19.8
20 %	100	25.8
27 %	90.0	26.1



Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: November 19, 2019 at 1055

Date and Time Test Terminated: November 26, 2019 at 0930

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	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	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: November 19, 2019 at 1055

Test Terminated: November 26, 2019 at 0930

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	1.18984	1.19265	0.00281	8	0.351
	B	1.18295	1.18627	0.00332	8	0.415
	C	1.18383	1.18698	0.00315	8	0.394
	D	1.19837	1.20175	0.00338	8	0.422
	E	1.18992	1.19310	0.00318	8	0.398
8 %	A	1.19642	1.20010	0.00368	8	0.460
	B	1.18677	1.18919	0.00242	8	0.302
	C	1.18960	1.19259	0.00299	8	0.374
	D	1.18369	1.18670	0.00301	8	0.376
	E	1.19157	1.19468	0.00311	8	0.389
11 %	A	1.18699	1.18978	0.00279	8	0.349
	B	1.17960	1.18237	0.00277	8	0.346
	C	1.19105	1.19366	0.00261	8	0.326
	D	1.18948	1.19258	0.00310	8	0.388
	E	1.17860	1.18180	0.00320	8	0.400
15 %	A	1.19183	1.19448	0.00265	8	0.331
	B	1.18310	1.18637	0.00327	8	0.409
	C	1.17683	1.17961	0.00278	8	0.348
	D	1.18434	1.18743	0.00309	8	0.386
	E	1.17613	1.17882	0.00269	8	0.336
20 %	A	1.20936	1.21274	0.00338	8	0.422
	B	1.18950	1.19274	0.00324	8	0.405
	C	1.19545	1.19821	0.00276	8	0.345
	D	1.19859	1.20177	0.00318	8	0.398
	E	1.17648	1.18000	0.00352	8	0.440
27 %	A	1.18448	1.18733	0.00285	8	0.356
	B	1.20117	1.20342	0.00225	8	0.281
	C	1.17682	1.17898	0.00216	8	0.270
	D	1.21103	1.21441	0.00338	8	0.422
	E	1.20282	1.20573	0.00291	8	0.364

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: November 19, 2019 at 1000

Date and Time Test Terminated: November 25, 2019 at 0900

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	3	0	3	3	0	0	2	0	0	11	10	1.10	
4	4	0	4	0	0	4	4	0	4	4	24	10	2.40	
5	7	6	6	7	7	6	7	6	5	8	65	10	6.50	
6	11	12	10	11	11	12	12	10	11	14	114	10	11.4	
7														
8														
TOTAL	22	21	20	21	21	22	23	18	20	26	214	10	21.4	

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	2	0	4	2	0	4	4	0	0	16	10	1.60
4	5	1	4	0	0	4	0	0	4	4	22	10	2.20
5	8	X	8	X	7	9	7	8	8	7	62	8	7.75
6	13	X	15	X	14	14	12	13	11	13	105	8	13.1
7													
8													
TOTAL	26	3	27	4	23	27	23	25	23	24	205	10	20.5

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	X	0	0	2X	0	0	4	0	0	6	8	0.750
4	5	X	3	3	X	5	3	0	4	4	27	8	3.38
5	8	X	7	6	X	8	8	8	7	6	58	8	7.25
6	15	X	15	10	X	14	12	13	11	14	104	8	13.0
7													
8													
TOTAL	28	0	25	19	2	27	23	25	22	24	195	10	19.5

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: November 19, 2019 at 1000

Date and Time Test Terminated: November 25, 2019 at 0900

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	X	0	0	0	0	0	0	9	0.00
3	3	2	5	4X	0	X	3	5	0	4	26	8	3.25	
4	0	0	0	X	0	X	0	0	4	0	4	8	0.500	
5	7	6	8	X	8	X	6	7	8	7	57	8	7.12	
6	12	13	13	X	12	X	15	17	14	15	111	8	13.9	
7														
8														
TOTAL	22	21	26	4	20	0	24	29	26	26	198	10	19.8	

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	3	3	4	3	4	2	3	6	0	3	31	10	3.10
4	0	0	0	0	0	0	0	0	4	0	4	10	0.400
5	8	6	8	7	8	5	6	9	8	7	72	10	7.20
6	14	15	14	16	15	16	14	17	15	15	151	10	15.1
7													
8													
TOTAL	25	24	26	26	27	23	23	32	27	25	258	10	25.8

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	4	4	4	3	4X	4	4	4	0	3	34	9	3.78
4	0	0	0	7	X	0	0	0	3	0	10	9	1.11
5	11	10	10	0	X	10	9	11	10	10	81	9	9.00
6	15	15	14	16	X	17	13	16	14	16	136	9	15.1
7													
8													
TOTAL	30	29	28	26	4	31	26	31	27	29	261	10	26.1

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	1.00000	1.39310
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))
D = 0 W = 0 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)		
Data FAIL normality test (alpha = 0.01).		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	8 %	27.50	16.00	5.00	
3	11 %	27.50	16.00	5.00	
4	15 %	27.50	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 = 0.04519 W = 0.9766 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 = 4.308 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	0.01425	0.00285	1.514	
Within (Error)	24	0.04519	0.001883		
Total	29	0.05944			
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	0.396	0.396			
2	8 %	0.3802	0.3802	0.5757		
3	11 %	0.3618	0.3618	1.246		
4	15 %	0.362	0.362	1.239		
5	20 %	0.402	0.402	-0.2186		
6	27 %	0.3386	0.3386	2.091		
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	0.06477	16.4	0.0158	
3	11 %	5	0.06477	16.4	0.0342	
4	15 %	5	0.06477	16.4	0.034	
5	20 %	5	0.06477	16.4	-0.006	
6	27 %	5	0.06477	16.4	0.0574	



Appendix A2: Statistics

*Ceriodaphnia dubia* Survival

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

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
11 %	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.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
15 %	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.

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 %	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.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	8 %	10	2	
2	11 %	10	2	
3	15 %	10	2	
4	20 %	10	0	
5	27 %	10	1	

Appendix A2: Statistics

*Ceriodaphnia dubia* Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.2414 D* = 1.894 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 %	128.00	75.00	10.00	
3	11 %	118.50	75.00	10.00	
4	15 %	116.00	75.00	10.00	
5	20 %	148.00	75.00	10.00	
6	27 %	144.00	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	447.5	89.5	1.51	
Within (Error)	54	3200	59.26		
Total	59	3647			
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)					

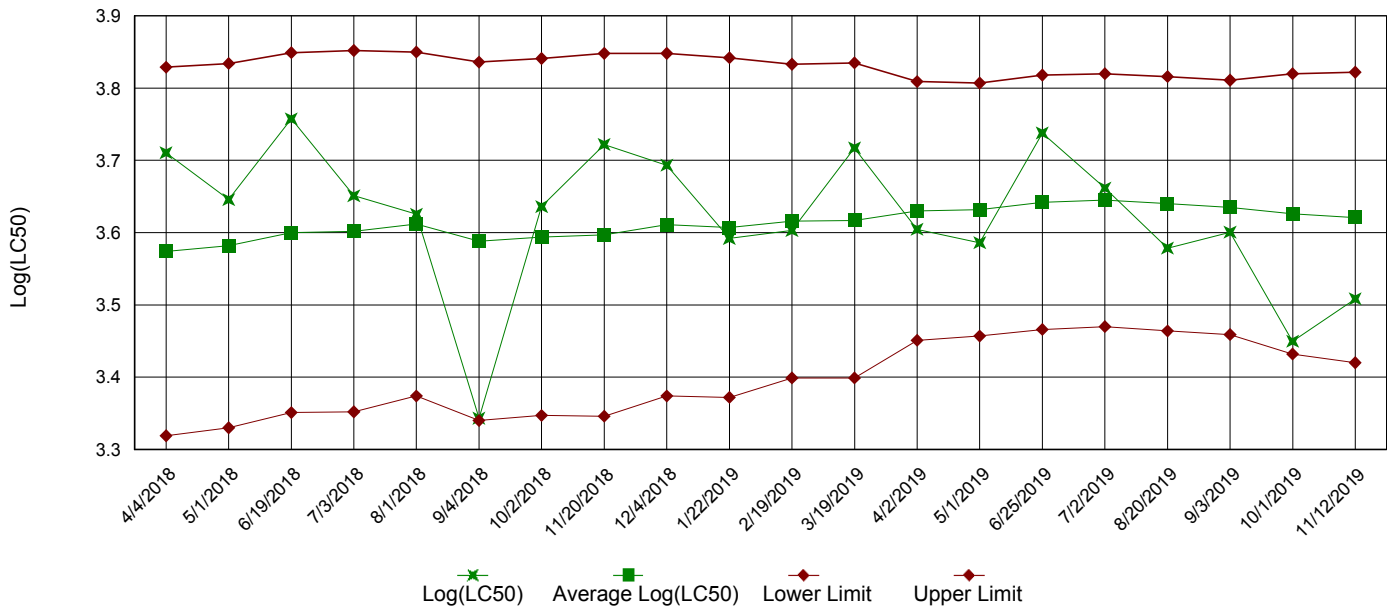
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	21.4	21.4			
2	8 %	20.5	20.5	0.2614		
3	11 %	19.5	19.5	0.5519		
4	15 %	19.8	19.8	0.4648		
5	20 %	25.8	25.8	-1.278		
6	27 %	26.1	26.1	-1.365		
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	7.953	37.2	0.9	
3	11 %	10	7.953	37.2	1.9	
4	15 %	10	7.953	37.2	1.6	
5	20 %	10	7.953	37.2	-4.4	
6	27 %	10	7.953	37.2	-4.7	

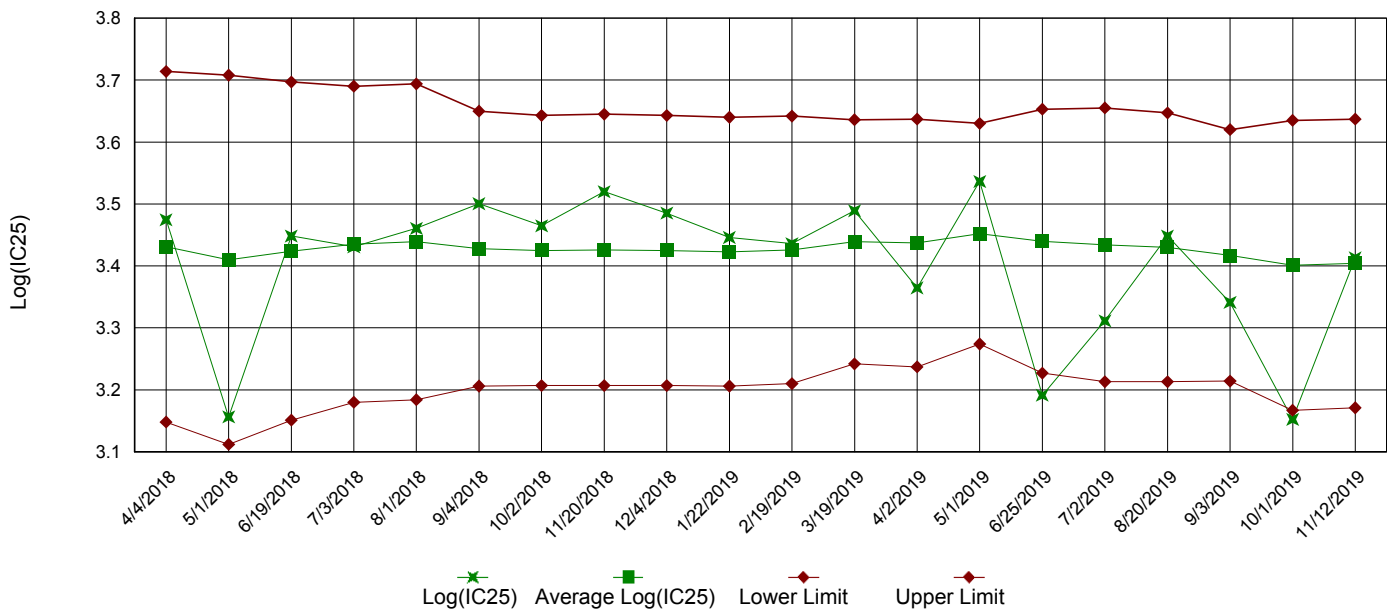
Appendix A3: Test 1000.0

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

LC50 Survival Data

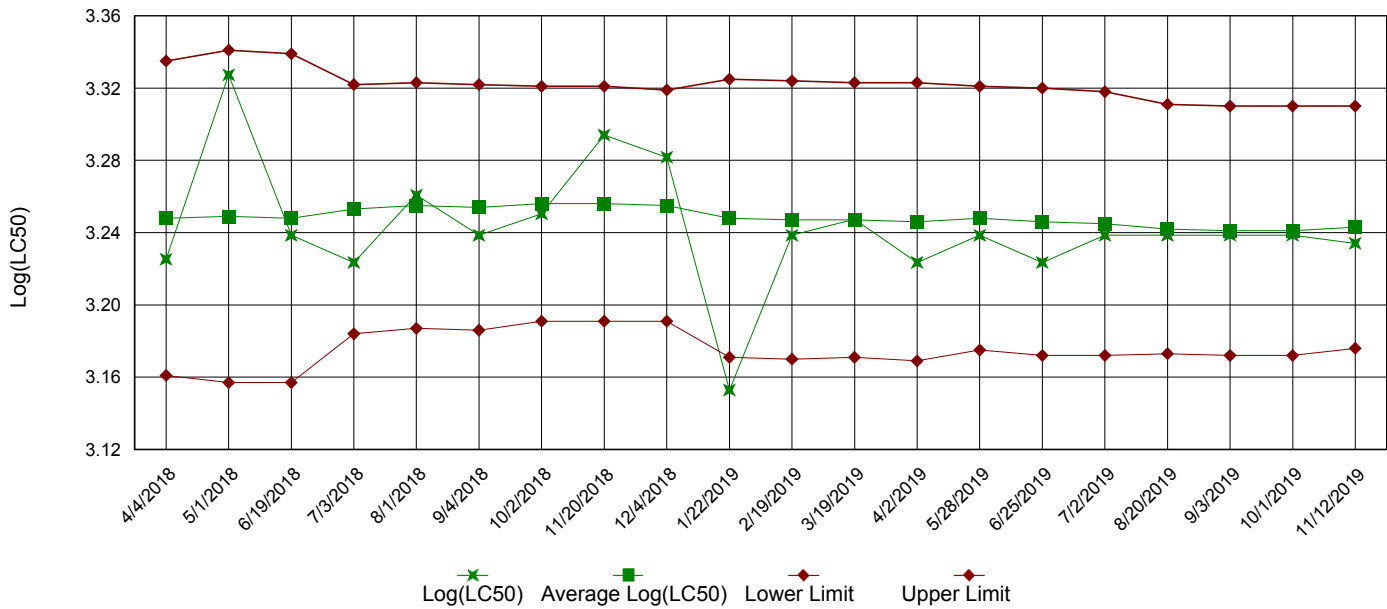


IC25 Growth Data

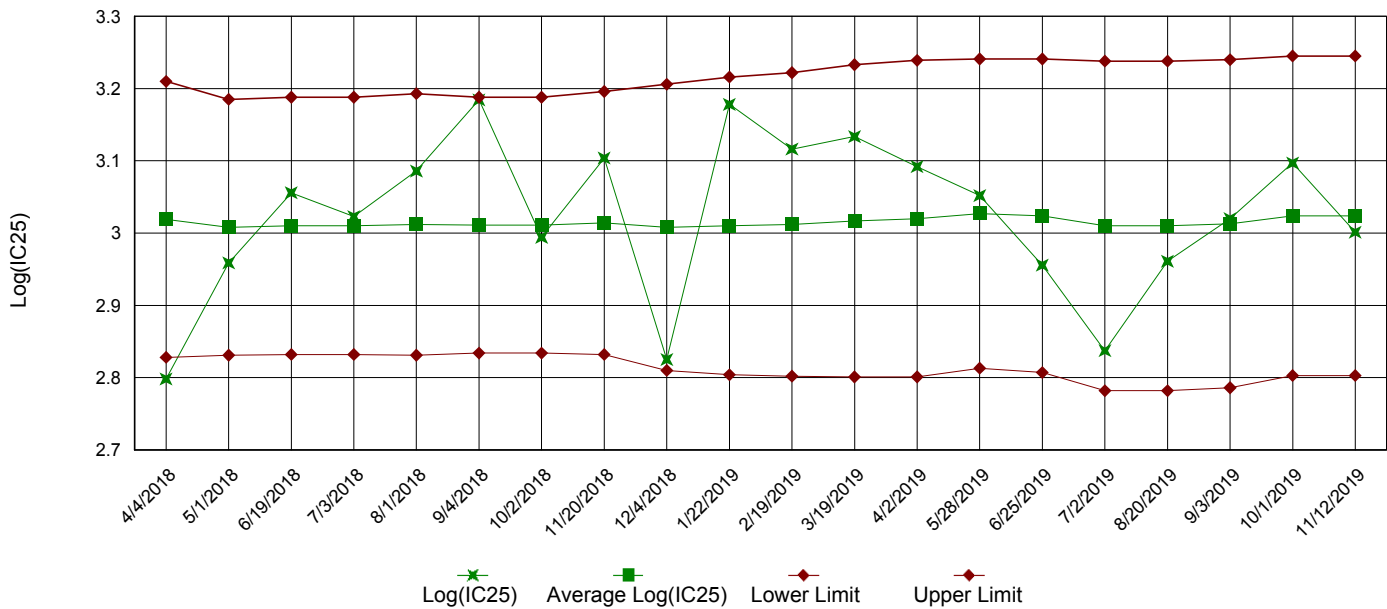


Appendix A3: Test 1002.0  
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: November 19, 2019 at 1055

Date and Time Test Terminated: November 26, 2019 at 0930

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	100	100	100	100	0.00
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	0.351	0.415	0.394	0.422	0.398	0.396	7.00
8 %	0.460	0.302	0.374	0.376	0.389	0.38	14.8
11 %	0.349	0.346	0.326	0.388	0.400	0.362	8.56
15 %	0.331	0.409	0.348	0.386	0.336	0.362	9.39
20 %	0.422	0.405	0.345	0.398	0.440	0.402	8.90
27 %	0.356	0.281	0.270	0.422	0.364	0.339	18.6

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:   8.9   (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, 345

Test Initiated: DATE: November 19, 2019 TIME: 1055  
Test Terminated: DATE: November 26, 2019 TIME: 0930

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.9	7.2	7.6	7.9	7.5	7.3
Final	7.5	6.1	6.8	6.8	7.7	6.3	6.9
pH Initial	7.8	7.5	7.4	7.4	7.8	7.7	7.6
Final	7.7	7.2	7.3	7.4	7.7	7.6	7.2

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.2	7.9	7.5	7.8	7.0	7.6
Final	7.6	6.3	6.8	6.8	7.6	6.4	6.7
pH Initial	7.6	7.5	7.4	7.4	7.5	7.6	7.6
Final	7.5	7.1	7.2	7.3	7.7	7.6	7.2

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.7	7.9	7.8	8.2	7.1	7.7
Final	7.6	6.5	7.2	7.4	7.8	6.7	6.6
pH Initial	7.5	7.5	7.4	7.4	7.4	7.6	7.6
Final	7.5	7.1	7.2	7.4	7.7	7.6	7.1

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.4	7.9	7.9	8.3	7.6	7.5
Final	7.4	6.4	7.2	7.2	7.5	6.2	6.6
pH Initial	7.5	7.5	7.3	7.4	7.4	7.6	7.5
Final	7.5	7.1	7.2	7.3	7.7	7.5	7.2

DILUTION 20 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	7.4	7.6	7.3	8.3	7.6	7.6
Final	7.6	6.2	6.8	7.0	7.7	6.6	6.5
pH Initial	7.4	7.5	7.3	7.3	7.3	7.6	7.5
Final	7.5	7.1	7.2	7.4	7.7	7.6	7.3

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	8.0	8.1	8.4	7.7	7.4
Final	7.5	6.5	7.1	7.5	7.7	6.4	6.6
pH Initial	7.3	7.4	7.2	7.3	7.3	7.6	7.4
Final	7.4	7.0	7.2	7.4	7.7	7.5	7.3

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
5.2	44	280	<0.05	Eff 18-NOV-19
12	42	310	<0.05	Eff 20-NOV-19
17	44	330	<0.05	Eff 22-NOV-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
29	44	180	<0.05	239589-1

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: November 19, 2019 at 1000

Date and Time Test Terminated: November 25, 2019 at 0900

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	90.0	100	100
6 day	100	80.0	80.0	80.0	100	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		8 %	11 %	15 %	20 %	27 %
A	22	26	28	22	25	30
B	21	3	0	21	24	29
C	20	27	25	26	26	28
D	21	4	19	4	26	26
E	21	23	2	20	27	4
F	22	27	27	0	23	31
G	23	23	23	24	23	26
H	18	25	25	29	32	31
I	20	23	22	26	27	27
J	26	24	24	26	25	29
Mean per Adult	21.4	20.5	19.5	19.8	25.8	26.1
Mean per Surviving Adult	21.4	24.8	24.1	24.2	25.8	28.6
CV %	9.90	7.08	11.8	12.6	10.1	6.81

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: 10.1 (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, 345

Test Initiated: DATE: November 19, 2019 TIME: 1000  
Test Terminated: DATE: November 25, 2019 TIME: 0900

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

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	8.0	7.2	7.9	7.5	7.8	7.0	7.6
Final	7.9	8.0	7.7	8.3	7.7	7.1	--
pH Initial	7.6	7.5	7.4	7.4	7.5	7.6	7.6
Final	7.9	7.9	7.7	7.8	7.8	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	7.6	7.7	7.9	7.8	8.2	7.1	7.7
Final	8.0	8.0	7.6	8.0	7.7	7.5	--
pH Initial	7.5	7.5	7.4	7.4	7.4	7.6	7.6
Final	7.9	7.9	7.6	7.8	7.8	7.9	--

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

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	7.3	7.4	7.6	7.3	8.3	7.6	7.6
Final	7.7	7.8	7.3	8.2	7.7	7.7	--
pH Initial	7.4	7.5	7.3	7.3	7.3	7.6	7.5
Final	7.8	7.8	7.6	7.8	7.8	7.8	--

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	8.0	7.8	8.0	8.1	8.4	7.7	7.4
Final	8.0	8.0	7.6	8.3	7.4	7.2	--
pH Initial	7.3	7.4	7.2	7.3	7.3	7.6	7.4
Final	7.8	7.8	7.6	7.8	7.8	7.8	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
5.2	44	280	<0.05	Eff 18-NOV-19
12	42	310	<0.05	Eff 20-NOV-19
17	44	330	<0.05	Eff 22-NOV-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
29	44	180	<0.05	239589-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Seasay Waters</u>		AIC CONTROL NO: <u>239937</u>	
Project Reference: <u>Biomonitoring</u>		AIC PROPOSAL NO:	
Project Manager: <u>Jimmy Smith</u>		Carrier:	
Sampled By: <u>Mark Price</u>		Received Temperature C: <u>6.1</u>	
Remarks:		Remarks:	
AIC No. <u>1</u>		Sample Identification <u>Eff</u>	
Date/Time Collected <u>11-18-19</u>		Date/Time Collected <u>8:00am</u>	
AIC No. <u>1</u>		Sample Identification <u>Eff</u>	
Date/Time Collected <u>11-18-19</u>		Date/Time Collected <u>8:00am</u>	
G R A B		C O M P	
W A T E R		S O I L	
NO OF BOTTLES		ANALYSES REQUESTED	
1		1	
2		2	
Field pH calibration on @		Field pH calibration on @	
Buffer:		Buffer:	
G = Glass		P = Plastic	
NO = none		S = Sulfuric acid pH2	
V = VOA vials		N = Nitric acid pH2	
H = HCl to pH2		B = NaOH to pH12	
T = Sodium Thiosulfate		Z = Zinc acetate	
A = (NH4)2, NH4OH			
Turnaround Time Requested: (Please circle) <u>      </u> DAYS		Received Date/Time	
Expedited results requested by: <u>Boone-Harbors</u>		By: <u>D. Brown</u>	
Who should AIC contact with questions: <u>Boone-Harbors</u>		Received in Lab Date/Time <u>11-18-19</u>	
Phone: <u>      </u> Fax: <u>      </u>		By: <u>D. Brown</u>	
Report Attention to: <u>      </u>		Date/Time <u>11-18-19</u>	
Report Address to: <u>      </u>		Comments: <u>      </u>	





CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 3 OF 1

Client: <u>Sasky Water Utilities</u>		PO No.		ANALYSES REQUESTED										AIC CONTROL NO. <u>690401</u>	
Project Reference: <u>Bio-monitoring</u>		SAMPLE MATRIX												AIC PROPOSAL NO.:	
Project Manager: <u>Jimmy Smith</u>		WATER												Carrier:	
Sampled By: <u>Marshall Price</u>		SOIL												Received Temperature C	
AIC Sample Identification		GRA B												Remarks	
No. <u>Eff</u>		COMP												Field pH calibration on _____ @ _____	
		/												Buffer:	
		/												T = Sodium Thiosulfate	
														Z = Zinc acetate	
														H = HCl to pH2	
														B = NaOH to pH2	
														V = VOA vials	
														N = Nitric acid pH2	
														Relinquished By: _____	
														Date/Time _____	
														Received By: <u>AC341</u>	
														Date/Time <u>11-22-19</u>	
														Relinquished By: _____	
														Date/Time _____	
														Comments:	