



November 6, 2020

Biomonitoring Testing
for
EFF

Control No. 249743-1

Prepared for:

Mr. Jimmy Smith
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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	0.310	PASS
Control Growth CV < or = 40%	12.5	PASS
Growth Minimum Significant Difference 12 to 30%	14.3	PASS
Critical Dilution CV < or = 40%	9.90	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	28.0	PASS
Control CV < or = 40% per Surviving Female	8.91	PASS
Reproduction Minimum Significant Difference 13 to 47%	20.2	PASS
Critical Dilution CV < or = 40%	27.2	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)	7.6	7.6	8.0
pH (standard units)	7.1	7.1	7.2
Alkalinity (mg/l as CaCO ₃)	32	35	22
Hardness (mg/l as CaCO ₃)	43	36	34
Conductivity (umhos/cm)	320	310	160
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.18	<0.1	0.30

2. Dilution Water Samples:

Soft

Analysis	249389-1
Dissolved oxygen (mg/l)	7.8
pH (standard units)	7.9
Alkalinity (mg/l as CaCO ₃)	33
Hardness (mg/l as CaCO ₃)	43
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: October 27, 2020 at 1000
Date & Time Test Terminated: November 03, 2020 at 0900
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: October 27, 2020 at 1040
Date & Time Test Terminated: November 02, 2020 at 1045
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 06, 2020 at 1005 to October 13, 2020 at 1145

The results were as follows: (Control No. 249174-1.)

Survival LC-50: 2861 mg/l

Growth IC-25: 2262 mg/l

Growth PMSD: 24.4

Ceriodaphnia dubia

A chronic reference test was performed on October 06, 2020 at 1120 to October 12, 2020 at 1025

The results were as follows: (Control No. 249174-2.)

Survival LC-50: 1372 mg/l

Reproduction IC-25: 447.8 mg/l

Reproduction PMSD: 15.7

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 27, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 27, 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

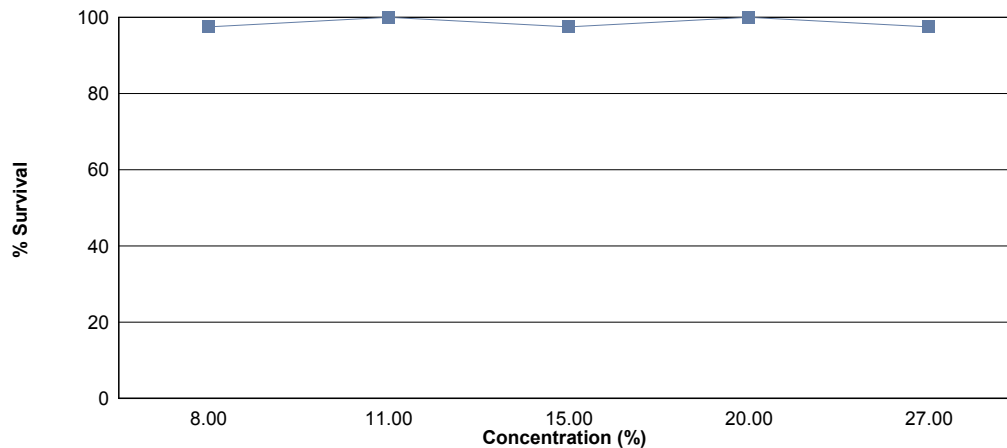
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 October 27, 2020 at 1000 and continued through November 03, 2020 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 growth = 27 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.310
8 %	97.5	0.313
11 %	100	0.278
15 %	97.5	0.274
20 %	100	0.311
27 %	97.5	0.284

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

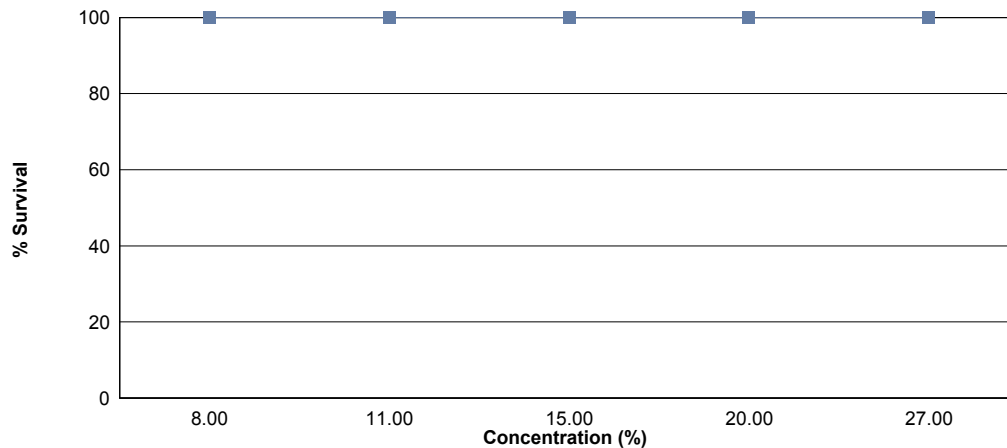
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 October 27, 2020 at 1040 and continued through November 02, 2020 at 1045. 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	28.0
8 %	100	28.4
11 %	100	26.8
15 %	100	27.6
20 %	100	26.4
27 %	100	30.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 27, 2020 at 1000

Date and Time Test Terminated: November 03, 2020 at 0900

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	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
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	7	7	7	7	7
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	7	7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

 Test Initiated: October 27, 2020 at 1000
 Test Terminated: November 03, 2020 at 0900

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76859	.77154	0.00295	8	0.369
	B	.76678	.76887	0.00209	8	0.261
	C	.76263	.76501	0.00238	8	0.298
	D	.76892	.77139	0.00247	8	0.309
	E	.76577	.76829	0.00252	8	0.315
8 %	A	.76933	.77206	0.00273	8	0.341
	B	.77225	.77444	0.00219	8	0.274
	C	.76581	.76841	0.00260	8	0.325
	D	.76514	.76762	0.00248	8	0.310
	E	.76780	.77032	0.00252	8	0.315
11 %	A	.77216	.77444	0.00228	8	0.285
	B	.77056	.77281	0.00225	8	0.281
	C	.76295	.76512	0.00217	8	0.271
	D	.76968	.77182	0.00214	8	0.268
	E	.76518	.76745	0.00227	8	0.284
15 %	A	.77057	.77276	0.00219	8	0.274
	B	.76729	.76962	0.00233	8	0.291
	C	.77087	.77311	0.00224	8	0.280
	D	.76403	.76591	0.00188	8	0.235
	E	.76879	.77109	0.00230	8	0.288
20 %	A	.76868	.77093	0.00225	8	0.281
	B	.76722	.76949	0.00227	8	0.284
	C	.76770	.77031	0.00261	8	0.326
	D	.76517	.76764	0.00247	8	0.309
	E	.76804	.77088	0.00284	8	0.355
27 %	A	.76365	.76605	0.00240	8	0.300
	B	.76906	.77159	0.00253	8	0.316
	C	.76696	.76882	0.00186	8	0.232
	D	.76353	.76611	0.00258	8	0.322
	E	.76752	.76953	0.00201	8	0.251

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 27, 2020 at 1040
Date and Time Test Terminated: November 02, 2020 at 1045

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	0	3	4	4	3	0	0	0	14	10	1.40	
4	3	3	3	0	0	0	0	5	3	3	20	10	2.00	
5	10	9	11	10	8	9	10	11	10	10	98	10	9.80	
6	17	13	16	13	16	15	12	17	15	14	148	10	14.8	
7														
8														
TOTAL	30	25	30	26	28	28	25	33	28	27	280	10	28.0	

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	4	0	3	4	4	3	0	0	0	0	18	10	1.80
4	0	3	0	0	0	0	4	3	2	4	16	10	1.60
5	8	9	11	10	9	9	10	10	9	8	93	10	9.30
6	17	10	16	17	20	7	15	17	20	18	157	10	15.7
7													
8													
TOTAL	29	22	30	31	33	19	29	30	31	30	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	0	0	0	4	4	3	0	3	3	0	17	10	1.70
4	4	3	5	0	0	0	5	0	0	2	19	10	1.90
5	9	10	10	11	9	11	9	5	12	10	96	10	9.60
6	16	0	19	16	17	19	13	17	19	0	136	10	13.6
7													
8													
TOTAL	29	13	34	31	30	33	27	25	34	12	268	10	26.8

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 27, 2020 at 1040
Date and Time Test Terminated: November 02, 2020 at 1045

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	0	0	4	2	3	0	5	4	0	22	10	2.20	
4	0	5	4	0	0	0	3	0	0	2	14	10	1.40	
5	5	10	9	11	9	10	8	12	10	10	94	10	9.40	
6	19	0	16	17	16	18	16	16	15	13	146	10	14.6	
7														
8														
TOTAL	28	15	29	32	27	31	27	33	29	25	276	10	27.6	

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	2	0	0	5	4	0	0	0	0	0	11	10	1.10
4	0	3	3	0	0	3	5	2	4	4	24	10	2.40
5	9	12	9	10	4	11	13	8	13	10	99	10	9.90
6	21	0	20	0	19	11	16	11	15	17	130	10	13.0
7													
8													
TOTAL	32	15	32	15	27	25	34	21	32	31	264	10	26.4

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	4	3	4	0	4	4	0	19	10	1.90
4	4	2	4	0	0	0	4	0	0	4	18	10	1.80
5	11	13	10	11	7	4	12	10	12	9	99	10	9.90
6	19	15	18	18	16	17	16	19	16	13	167	10	16.7
7													
8													
TOTAL	34	30	32	33	26	25	32	33	32	26	303	10	30.3

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	0.87500	1.20940
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	0.87500	1.20940
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	0.87500	1.20940

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0.08099 W = 0.5968 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	8 %	25.00	16.00	5.00	
3	11 %	27.50	16.00	5.00	
4	15 %	25.00	16.00	5.00	
5	20 %	27.50	16.00	5.00	
6	27 %	25.00	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 = 0.02111 W = 0.9702 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 = 8.532 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	0.008438	0.001688	1.919	
Within (Error)	24	0.02111	0.0008796		
Total	29	0.02955			
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	0.3104	0.3104			
2	8 %	0.313	0.313	-0.1386		
3	11 %	0.2778	0.2778	1.738		
4	15 %	0.2736	0.2736	1.962		
5	20 %	0.311	0.311	-0.03199		
6	27 %	0.2842	0.2842	1.397		
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	0.04427	14.3	-0.0026	
3	11 %	5	0.04427	14.3	0.0326	
4	15 %	5	0.04427	14.3	0.0368	
5	20 %	5	0.04427	14.3	-0.0006	
6	27 %	5	0.04427	14.3	0.0262	

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 = 0.1562 D* = 1.225 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 %	120.50	75.00	10.00	
3	11 %	113.00	75.00	10.00	
4	15 %	110.00	75.00	10.00	
5	20 %	105.50	75.00	10.00	
6	27 %	125.00	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	95.68	19.14	0.6408	
Within (Error)	54	1613	29.87		
Total	59	1709			
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)					

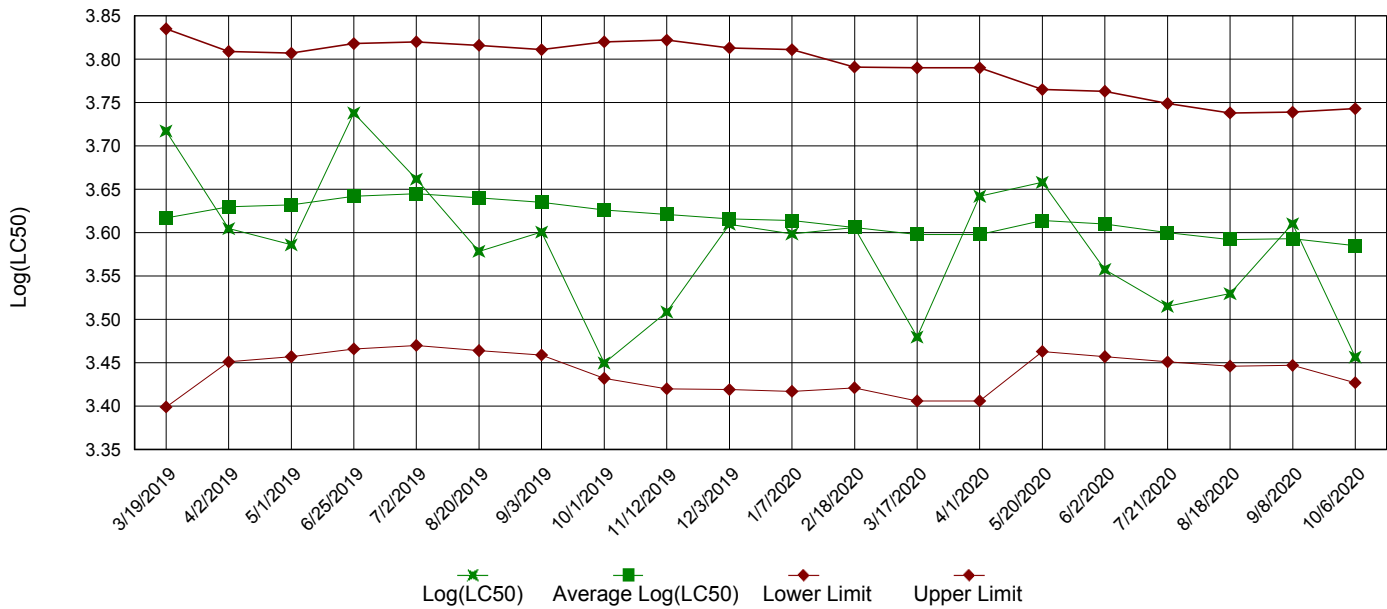
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	28	28			
2	8 %	28.4	28.4	-0.1637		
3	11 %	26.8	26.8	0.491		
4	15 %	27.6	27.6	0.1637		
5	20 %	26.4	26.4	0.6546		
6	27 %	30.3	30.3	-0.941		
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	5.646	20.2	-0.4	
3	11 %	10	5.646	20.2	1.2	
4	15 %	10	5.646	20.2	0.4	
5	20 %	10	5.646	20.2	1.6	
6	27 %	10	5.646	20.2	-2.3	

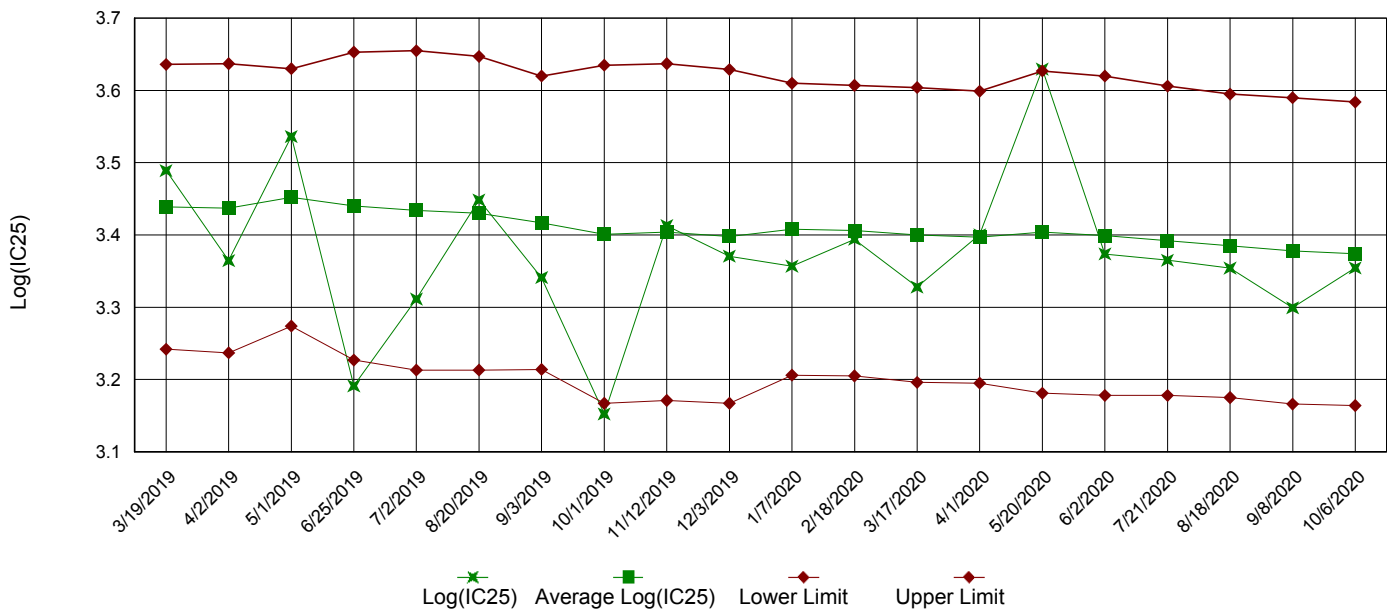
Appendix A3: Test 1000.0

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

LC50 Survival Data

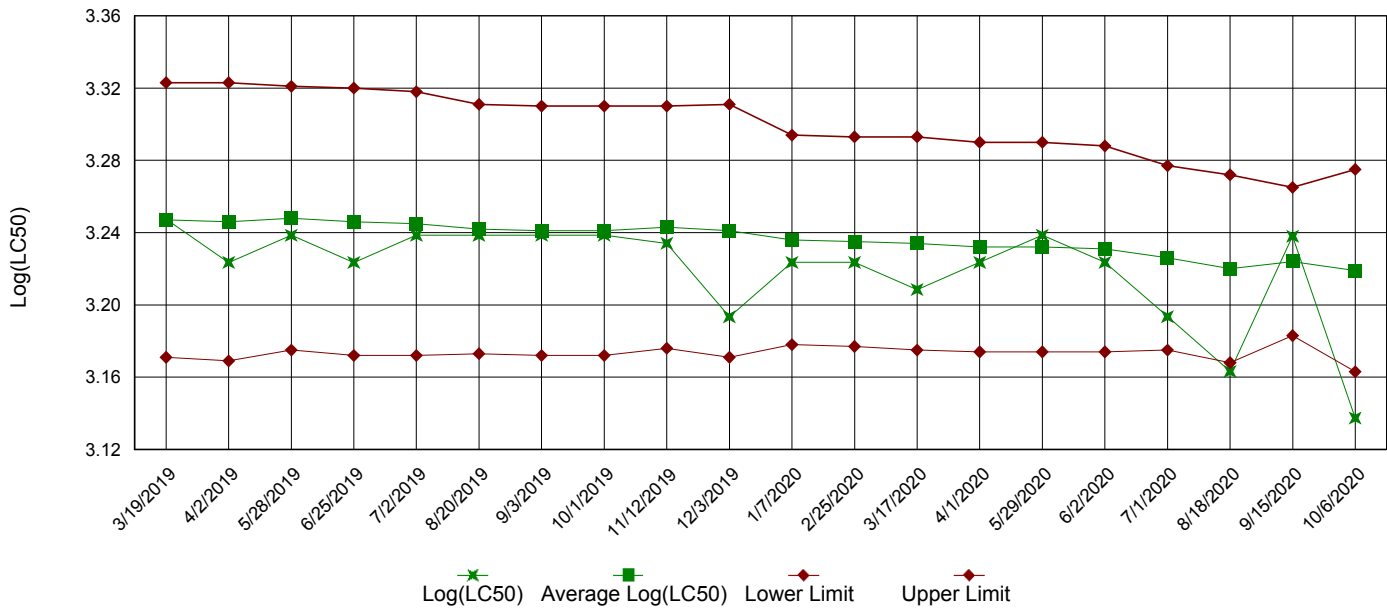


IC25 Growth Data

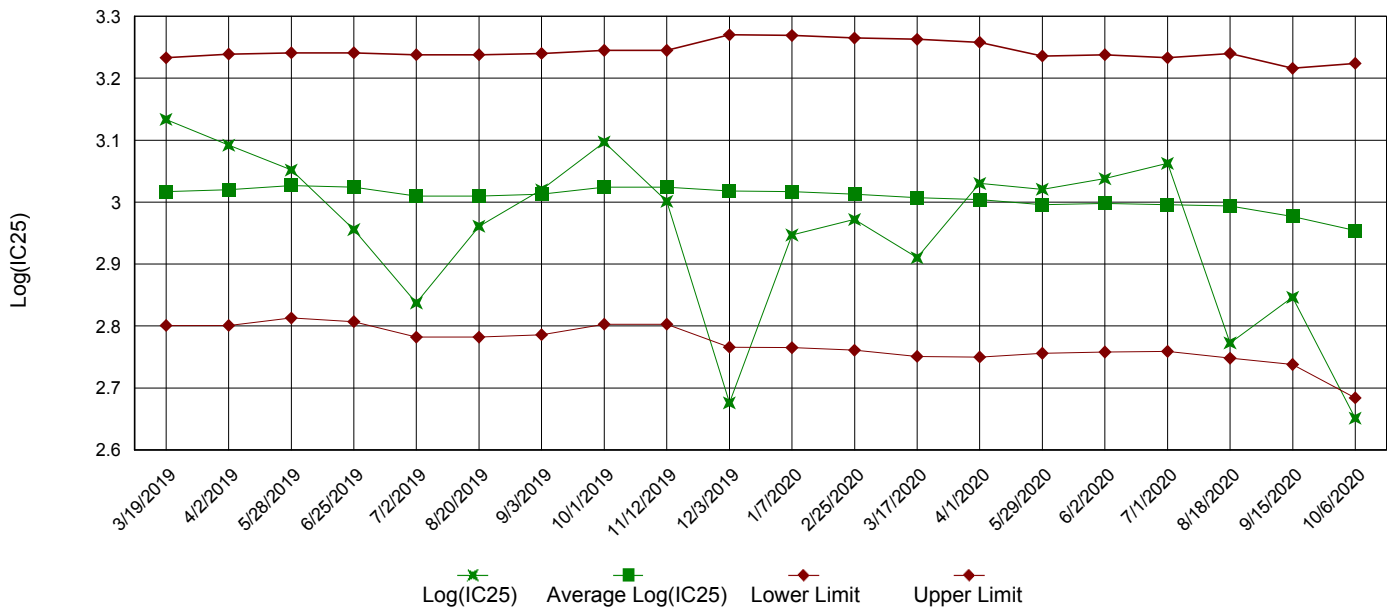


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: October 27, 2020 at 1000

Date and Time Test Terminated: November 03, 2020 at 0900

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	87.5	100	100	100	100	100	97.5	5.73
11 %	100	100	100	100	100	100	100	100	0.00
15 %	100	100	100	100	87.5	100	100	97.5	5.73
20 %	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	87.5	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.369	0.261	0.298	0.309	0.315	0.31	12.5
8 %	0.341	0.274	0.325	0.310	0.315	0.313	7.93
11 %	0.285	0.281	0.271	0.268	0.284	0.278	2.80
15 %	0.274	0.291	0.280	0.235	0.288	0.274	8.26
20 %	0.281	0.284	0.326	0.309	0.355	0.311	9.90
27 %	0.300	0.316	0.232	0.322	0.251	0.284	14.2

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: 12.5 (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

Test Initiated: DATE: October 27, 2020 TIME: 1000
Test Terminated: DATE: November 03, 2020 TIME: 0900

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

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.5	7.7	7.7	7.9	7.6	8.0
Final	7.2	6.9	7.0	6.7	7.2	7.2	7.1
pH Initial	7.8	7.8	7.6	7.6	7.6	7.7	7.7
Final	7.7	7.4	7.4	7.4	7.6	7.6	7.6

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.7	8.0	8.0	7.9	7.4	7.9
Final	7.5	6.7	7.0	6.2	7.3	7.1	7.0
pH Initial	7.7	7.8	7.6	7.6	7.5	7.6	7.7
Final	7.8	7.4	7.4	7.4	7.6	7.6	7.6

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.6	7.8	7.7	7.4	7.9	7.7
Final	7.5	6.6	7.2	6.8	7.5	7.5	7.2
pH Initial	7.7	7.8	7.6	7.6	7.5	7.6	7.7
Final	7.8	7.5	7.4	7.4	7.6	8.1	7.7

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

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.5	7.8	7.8	7.9	7.8	8.1
Final	7.0	7.0	6.8	6.6	7.2	6.9	7.0
pH Initial	7.6	7.7	7.5	7.5	7.4	7.4	7.6
Final	7.7	7.5	7.4	7.4	7.6	7.6	7.6

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	43	320	<0.05	EFF 26-OCT-20
35	36	310	<0.05	EFF 28-OCT-20
22	34	160	<0.05	EFF 30-OCT-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
33	43	160	<0.05	249389-1

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: October 27, 2020 at 1040

Date and Time Test Terminated: November 02, 2020 at 1045

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	30	29	29	28	32	34
B	25	22	13	15	15	30
C	30	30	34	29	32	32
D	26	31	31	32	15	33
E	28	33	30	27	27	26
F	28	19	33	31	25	25
G	25	29	27	27	34	32
H	33	30	25	33	21	33
I	28	31	34	29	32	32
J	27	30	12	25	31	26
Mean per Adult	28.0	28.4	26.8	27.6	26.4	30.3
Mean per Surviving Adult	28.0	28.4	26.8	27.6	26.4	30.3
CV %	8.91	15.4	30.1	18.3	27.2	11.1

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. 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 %)	<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: 27.2 (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

Test Initiated: DATE: October 27, 2020 TIME: 1040
Test Terminated: DATE: November 02, 2020 TIME: 1045

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

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	7.6	7.5	7.7	7.7	7.9	7.6	8.0
Final	7.7	8.1	8.0	7.6	7.8	7.6	--
pH Initial	7.8	7.8	7.6	7.6	7.6	7.7	7.7
Final	8.0	8.3	8.2	8.0	8.0	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	7.1	7.7	8.0	8.0	7.9	7.4	7.9
Final	7.6	8.0	8.1	7.8	8.0	7.8	--
pH Initial	7.7	7.8	7.6	7.6	7.5	7.6	7.7
Final	8.0	8.3	8.2	8.0	8.0	7.9	--

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

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	7.8	7.8	7.7	7.6	7.3	7.9	7.7
Final	7.7	8.0	8.1	7.6	7.8	7.9	--
pH Initial	7.6	7.8	7.5	7.6	7.4	7.5	7.6
Final	8.0	8.3	8.2	8.1	8.0	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.4	7.5	7.8	7.8	7.9	7.8	8.1
Final	7.6	8.1	8.2	7.8	7.8	7.7	--
pH Initial	7.6	7.7	7.5	7.5	7.4	7.4	7.6
Final	8.0	8.2	8.2	8.1	8.1	8.0	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	43	320	<0.05	EFF 26-OCT-20
35	36	310	<0.05	EFF 28-OCT-20
22	34	160	<0.05	EFF 30-OCT-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
33	43	160	<0.05	249389-1

