



June 1, 2021

Biomonitoring Testing
for
EFF

Control No. 255486-1

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: Due to a control failure, the test is invalid and will need to be repeated. The data is attached for your review.

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%	72.5	FAIL
Control Growth > or = 0.25 mg per Surviving minnow	0.476	PASS
Control Growth CV < or = 40%	23.2	PASS
Growth Minimum Significant Difference 12 to 30%	39.3	FAIL
Critical Dilution CV < or = 40%	52.4	FAIL

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	18.8	PASS
Control CV < or = 40% per Surviving Female	20.0	PASS
Reproduction Minimum Significant Difference 13 to 47%	30.9	PASS
Critical Dilution CV < or = 40%	33.6	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)	8.6	7.8	7.8
pH (standard units)	6.5	7.0	7.3
Alkalinity (mg/l as CaCO ₃)	9.5	21	22
Hardness (mg/l as CaCO ₃)	34	33	35
Conductivity (umhos/cm)	270	170	200
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	<0.1	<0.1

2. Dilution Water Samples:

Soft

Analysis	255276-1
Dissolved oxygen (mg/l)	8.4
pH (standard units)	7.4
Alkalinity (mg/l as CaCO ₃)	32
Hardness (mg/l as CaCO ₃)	41
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: May 18, 2021 at 1030
Date & Time Test Terminated: May 25, 2021 at 1050
Type & Volume of Test Chamber: 500 ml disposable beaker
Volume of Sample: 250 ml
Number of Organisms per replicate: 8
Number of Replicates per dilution: 5

Ceriodaphnia dubia Survival and Reproduction Method 1002.0

Date & Time Test Initiated: May 18, 2021 at 1050
Date & Time Test Terminated: May 24, 2021 at 1115
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Source of test organisms: In-house culture

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 and Bartlett's test. The survival data was then analyzed using Dunnett's 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 April 26, 2021 at 1538 to May 03, 2021 at 1453

The results were as follows: (Control No. 254918-1.)

Survival LC-50: 2387 mg/l

Growth IC-25: 1294 mg/l

Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on

The results were as follows: (Control No. 254918-2.)

Survival LC-50: mg/l

Reproduction IC-25: mg/l

Reproduction PMSD:

V. Organism History

Pimephales promelas (Fathead minnow)

Date: May 18, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: May 18, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

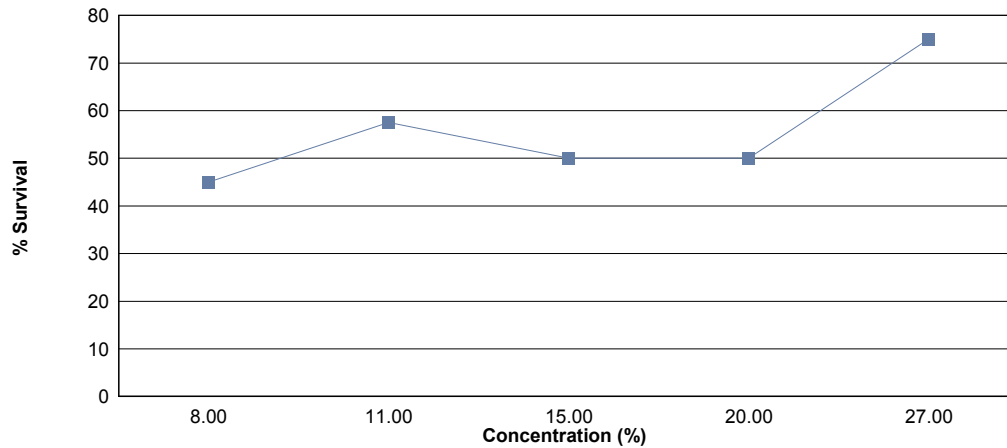
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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

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

The test was initiated on May 18, 2021 at 1030 and continued through May 25, 2021 at 1050. 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 = 0 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	72.5	0.345
8 %	45.0	0.136 *
11 %	57.5	0.229
15 %	50.0	0.150 *
20 %	50.0	0.167 *
27 %	75.0	0.275

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

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

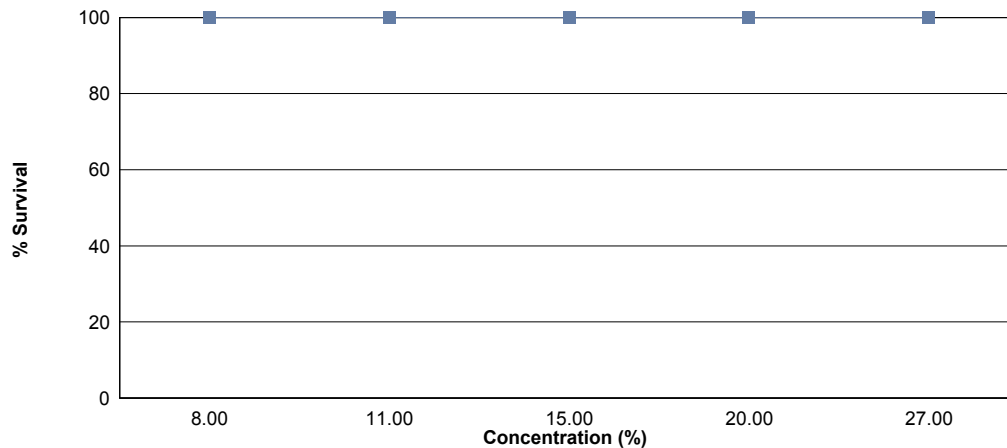
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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

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

The test was initiated on May 18, 2021 at 1050 and continued through May 24, 2021 at 1115. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

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



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	18.8
8 %	100	23.9
11 %	100	24.7
15 %	100	27.1
20 %	100	19.8
27 %	100	25.4

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: May 18, 2021 at 1030

Date and Time Test Terminated: May 25, 2021 at 1050

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	7	7	7	7	7	7	7
	B	7	7	7	7	7	7	7
	C	8	8	6	6	6	6	6
	D	8	8	7	7	6	4	4
	E	8	8	8	8	7	6	5
8 %	A	8	8	8	7	7	6	6
	B	8	8	8	6	6	5	4
	C	8	8	8	6	5	3	1
	D	8	8	7	7	6	5	5
	E	8	7	6	5	5	4	2
11 %	A	8	8	8	8	8	8	8
	B	8	8	7	6	5	5	5
	C	8	7	6	6	5	3	2
	D	8	7	6	6	5	3	3
	E	8	7	6	6	5	5	5
15 %	A	8	8	7	6	6	5	4
	B	8	7	6	5	5	5	5
	C	8	7	7	7	6	4	2
	D	8	8	7	6	6	6	5
	E	8	7	4	4	4	4	4
20 %	A	8	8	7	5	5	5	4
	B	8	8	5	5	5	4	4
	C	8	7	6	5	5	5	4
	D	8	8	8	7	6	5	2
	E	8	8	8	7	7	6	6
27 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	7
	C	8	8	7	6	6	6	6
	D	8	8	7	6	5	4	4
	E	8	7	5	5	5	5	5

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: May 18, 2021 at 1030

Test Terminated: May 25, 2021 at 1050

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65768	.66091	0.00323	8	0.404
	B	.66707	.67003	0.00296	8	0.370
	C	.64869	.65183	0.00314	8	0.392
	D	.64618	.64783	0.00165	8	0.206
	E	.64875	.65156	0.00281	8	0.351
8 %	A	.65177	.65371	0.00194	8	0.242
	B	.66267	.66358	0.00091	8	0.114
	C	.65671	.65717	0.00046	8	0.057
	D	.65142	.65277	0.00135	8	0.169
	E	.66584	.66661	0.00077	8	0.096
11 %	A	.65132	.65473	0.00341	8	0.426
	B	.64653	.64887	0.00234	8	0.292
	C	.65085	.65139	0.00054	8	0.067
	D	.64559	.64656	0.00097	8	0.121
	E	.65508	.65698	0.00190	8	0.238
15 %	A	.65260	.65380	0.00120	8	0.150
	B	.64560	.64758	0.00198	8	0.248
	C	.65215	.65265	0.00050	8	0.062
	D	.67381	.67487	0.00106	8	0.132
	E	.65071	.65199	0.00128	8	0.160
20 %	A	.66581	.66681	0.00100	8	0.125
	B	.66489	.66639	0.00150	8	0.188
	C	.76341	.76464	0.00123	8	0.154
	D	.76887	.76940	0.00053	8	0.066
	E	.77314	.77555	0.00241	8	0.301
27 %	A	.76916	.77205	0.00289	8	0.361
	B	.77084	.77337	0.00253	8	0.316
	C	.76976	.77213	0.00237	8	0.296
	D	.77589	.77723	0.00134	8	0.168
	E	.78084	.78270	0.00186	8	0.232

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 18, 2021 at 1050

Date and Time Test Terminated: May 24, 2021 at 1115

Concentration: Control														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	4	0	0	0	0	0	5	4	6	19	10	1.90	
4	5	0	0	5	5	4	4	0	0	0	23	10	2.30	
5	9	10	8	9	11	7	10	8	5	8	85	10	8.50	
6	0	8	10	10	7	5	0	6	7	8	61	10	6.10	
7														
8														
TOTAL	14	22	18	24	23	16	14	19	16	22	188	10	18.8	

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	4	0	0	0	0	0	5	5	4	18	10	1.80
4	5	0	4	6	6	0	3	0	0	0	24	10	2.40
5	8	9	0	8	6	12	8	9	9	10	79	10	7.90
6	13	14	15	0	12	14	13	12	8	17	118	10	11.8
7													
8													
TOTAL	26	27	19	14	24	26	24	26	22	31	239	10	23.9

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	0	0	0	0	0	4	5	9	10	0.900
4	4	3	0	6	4	3	4	5	0	0	29	10	2.90
5	11	8	9	13	12	12	9	0	8	10	92	10	9.20
6	0	15	11	16	5	16	13	10	13	18	117	10	11.7
7													
8													
TOTAL	15	26	20	35	21	31	26	15	25	33	247	10	24.7

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 18, 2021 at 1050

Date and Time Test Terminated: May 24, 2021 at 1115

Concentration: 15 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	0	0	0	0	0	0	4	0	5	13	10	1.30	
4	0	4	3	5	6	4	5	0	4	0	31	10	3.10	
5	11	9	7	10	6	13	12	10	9	12	99	10	9.90	
6	18	17	15	1	16	18	15	15	0	13	128	10	12.8	
7														
8														
TOTAL	33	30	25	16	28	35	32	29	13	30	271	10	27.1	

Concentration: 20 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	4	0	0	0	0	0	5	6	6	21	10	2.10
4	5	0	3	5	4	0	4	0	0	0	21	10	2.10
5	5	4	9	8	5	0	4	7	10	11	63	10	6.30
6	0	4	13	16	6	14	15	8	7	10	93	10	9.30
7													
8													
TOTAL	10	12	25	29	15	14	23	20	23	27	198	10	19.8

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	0	0	4	0	0	0	0	4	5	5	18	10	1.80
4	6	3	0	6	3	3	4	0	0	0	25	10	2.50
5	9	10	7	8	9	8	10	8	7	8	84	10	8.40
6	13	13	11	16	11	13	10	12	17	11	127	10	12.7
7													
8													
TOTAL	28	26	22	30	23	24	24	24	29	24	254	10	25.4

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	0.87500	1.20940
1	Control	2	0.87500	1.20940
1	Control	3	0.75000	1.04720
1	Control	4	0.50000	0.78540
1	Control	5	0.62500	0.91174
2	8 %	1	0.75000	1.04720
2	8 %	2	0.50000	0.78540
2	8 %	3	0.12500	0.36137
2	8 %	4	0.62500	0.91174
2	8 %	5	0.25000	0.52360
3	11 %	1	1.00000	1.39310
3	11 %	2	0.62500	0.91174
3	11 %	3	0.25000	0.52360
3	11 %	4	0.37500	0.65906
3	11 %	5	0.62500	0.91174
4	15 %	1	0.50000	0.78540
4	15 %	2	0.62500	0.91174
4	15 %	3	0.25000	0.52360
4	15 %	4	0.62500	0.91174
4	15 %	5	0.50000	0.78540
5	20 %	1	0.50000	0.78540
5	20 %	2	0.50000	0.78540
5	20 %	3	0.50000	0.78540
5	20 %	4	0.25000	0.52360
5	20 %	5	0.75000	1.04720
6	27 %	1	1.00000	1.39310
6	27 %	2	0.87500	1.20940
6	27 %	3	0.75000	1.04720
6	27 %	4	0.50000	0.78540
6	27 %	5	0.62500	0.91174

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 1.363		
W = 0.9657		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		Transform: Arc Sin(Square Root(Y))
Calculated B1 statistic = 2.952		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

ANOVA Table			Transform: Arc Sin(Square Root(Y))	
SOURCE	DF	SS	MS	F
Between	5	0.5058	0.1012	1.782
Within (Error)	24	1.363	0.05679	
Total	29	1.868		
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				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	1.0326	0.725		
2	8 %	0.72586	0.45	2.035	
3	11 %	0.87985	0.575	1.013	
4	15 %	0.78358	0.5	1.652	
5	20 %	0.7854	0.5	1.64	
6	27 %	1.0694	0.75	-0.2442	
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2				Transform: Arc Sin(Square Root(Y))	
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	0.3449	46.8	0.275
3	11 %	5	0.3449	46.8	0.15
4	15 %	5	0.3449	46.8	0.225
5	20 %	5	0.3449	46.8	0.225
6	27 %	5	0.3449	46.8	-0.025

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.1982 W = 0.9868 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 3.270 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	0.1666	0.03332	4.035	
Within (Error)	24	0.1982	0.008258		
Total	29	0.3648			
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	0.3446	0.3446			
2	8 %	0.1356	0.1356	3.636	*	
3	11 %	0.2288	0.2288	2.015		
4	15 %	0.1504	0.1504	3.379	*	
5	20 %	0.1668	0.1668	3.094	*	
6	27 %	0.2746	0.2746	1.218		
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	0.1356	39.3	0.209	
3	11 %	5	0.1356	39.3	0.1158	
4	15 %	5	0.1356	39.3	0.1942	
5	20 %	5	0.1356	39.3	0.1778	
6	27 %	5	0.1356	39.3	0.07	

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
D = 0.0913 D* = 0.7163 Critical D* = 1.035 (alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 11.39 Critical B = 15.086 (alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	536.7	107.3	3.404	
Within (Error)	54	1702	31.52		
Total	59	2238			
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)					

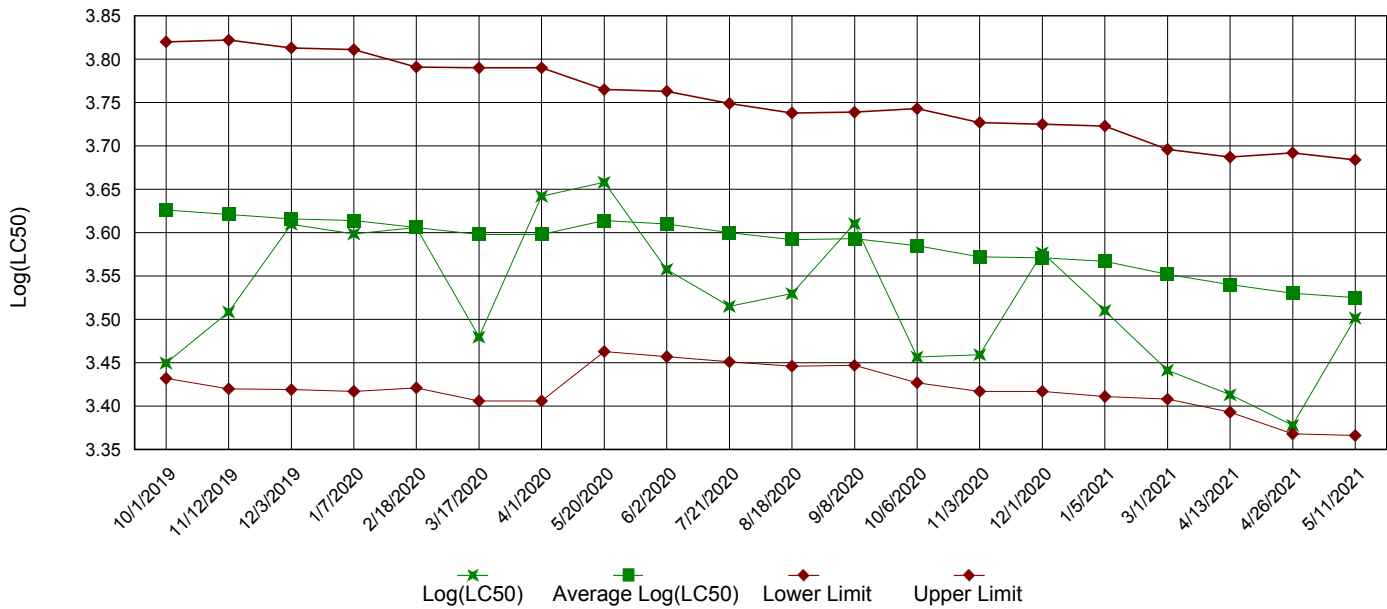
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	18.8	18.8			
2	8 %	23.9	23.9	-2.031		
3	11 %	24.7	24.7	-2.35		
4	15 %	27.1	27.1	-3.306		
5	20 %	19.8	19.8	-0.3983		
6	27 %	25.4	25.4	-2.629		
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	5.8	30.9	-5.1		
3	11 %	10	5.8	30.9	-5.9		
4	15 %	10	5.8	30.9	-8.3		
5	20 %	10	5.8	30.9	-1		
6	27 %	10	5.8	30.9	-6.6		

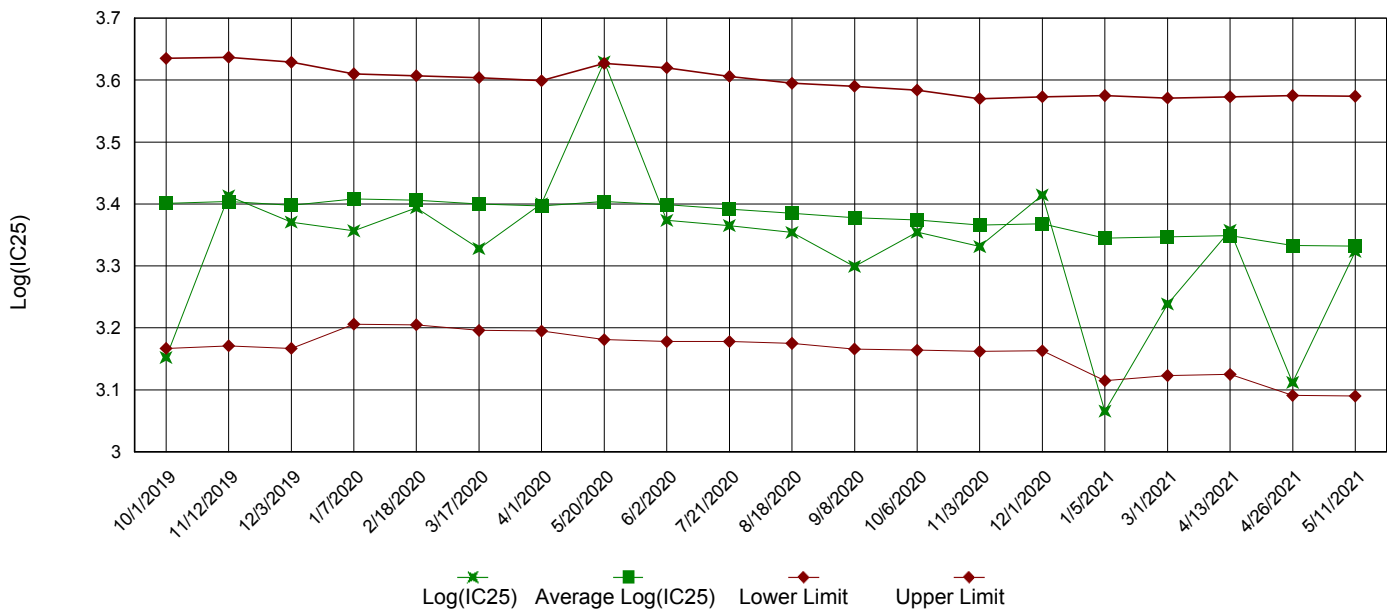
Appendix A3: Test 1000.0

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

LC50 Survival Data

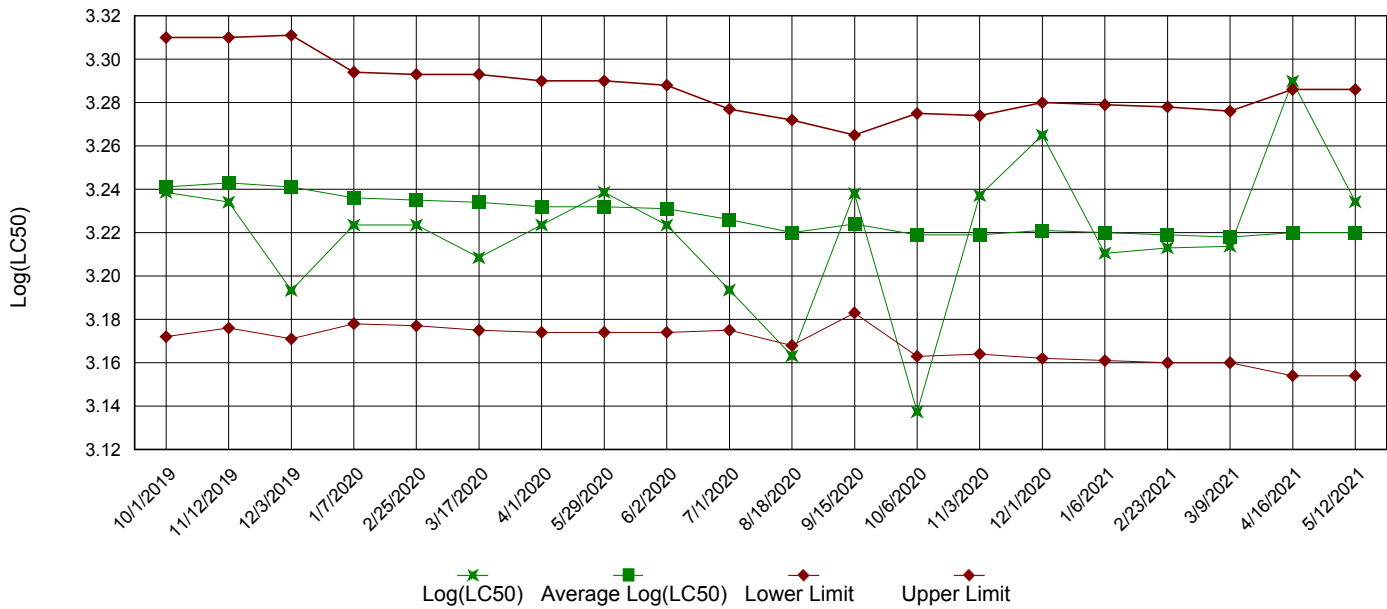


IC25 Growth Data

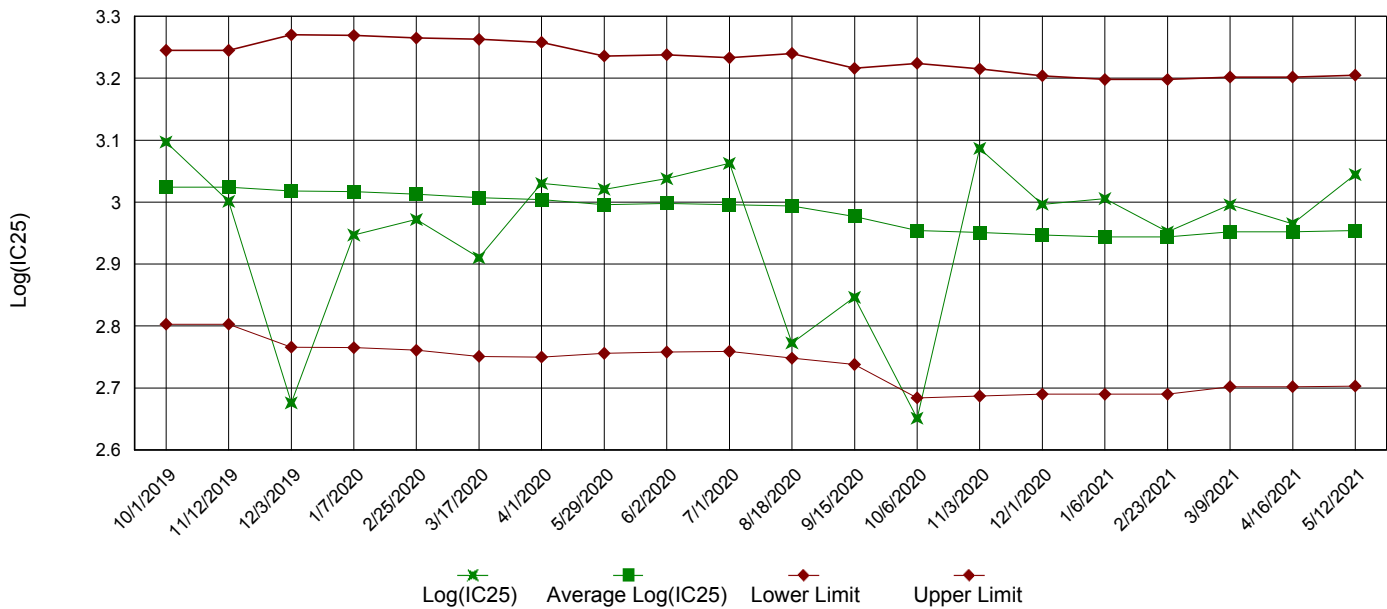


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: May 18, 2021 at 1030

Date and Time Test Terminated: May 25, 2021 at 1050

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	87.5	87.5	75.0	50.0	62.5	95.0	95.0	72.5	22.5
8 %	75.0	50.0	12.5	62.5	25.0	100	97.5	45.0	57.6
11 %	100	62.5	25.0	37.5	62.5	100	92.5	57.5	50.0
15 %	50.0	62.5	25.0	62.5	50.0	100	92.5	50.0	30.6
20 %	50.0	50.0	50.0	25.0	75.0	100	97.5	50.0	35.4
27 %	100	87.5	75.0	50.0	62.5	100	97.5	75.0	26.4

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.404	0.370	0.392	0.206	0.351	0.345	23.2
8 %	0.242	0.114	0.057	0.169	0.096	0.136	53.0
11 %	0.426	0.292	0.067	0.121	0.238	0.229	62.1
15 %	0.150	0.248	0.062	0.132	0.160	0.15	44.3
20 %	0.125	0.188	0.154	0.066	0.301	0.167	52.4
27 %	0.361	0.316	0.296	0.168	0.232	0.275	27.5

CV = Coefficient of variation = standard deviation * 100 / mean

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, 343, 356, 357

Test Initiated: DATE: May 18, 2021 TIME: 1030
Test Terminated: DATE: May 25, 2021 TIME: 1050

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.4	7.2	7.5	7.3	7.2	8.4	7.5
Final	6.9	7.9	5.9	7.0	6.1	5.4	5.8
pH Initial	7.4	7.0	7.5	7.3	7.4	7.6	7.4
Final	7.4	7.9	7.1	7.5	7.2	7.2	7.2

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	7.5	7.7	7.5	7.2	8.5	7.6
Final	6.8	8.0	6.1	6.6	6.2	6.1	5.8
pH Initial	7.3	6.9	7.5	7.4	7.6	7.6	7.6
Final	7.3	8.0	7.2	7.4	7.2	7.3	7.1

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	7.8	7.8	7.5	7.7	8.4	7.2
Final	6.9	7.9	5.8	6.6	6.1	6.3	5.4
pH Initial	7.3	7.0	7.4	7.5	7.6	7.6	7.5
Final	7.3	8.0	7.2	7.5	7.2	7.3	7.1

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.5	7.5	7.8	7.4	7.6	8.3	7.5
Final	7.0	8.1	6.0	6.3	6.2	6.2	5.9
pH Initial	7.3	7.0	7.4	7.4	7.5	7.6	7.5
Final	7.4	8.0	7.2	7.3	7.2	7.4	7.2

DILUTION 20 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.6	7.6	7.7	7.4	7.6	8.4	7.6
Final	7.3	8.1	6.4	6.9	6.4	6.5	6.0
pH Initial	7.3	7.1	7.3	7.5	7.5	7.5	7.5
Final	7.4	8.0	7.3	7.4	7.3	7.4	7.2

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.6	7.4	7.8	7.5	7.7	8.4	7.6
Final	6.6	7.8	6.1	6.6	6.0	6.2	5.7
pH Initial	7.2	7.1	7.3	7.5	7.5	7.5	7.5
Final	7.2	8.1	7.3	7.3	7.2	7.3	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
9.5	34	270	<0.05	EFF 17-MAY-21
21	33	170	<0.05	EFF 19-MAY-21
22	35	200	<0.05	EFF 21-MAY-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	150	<0.05	255276-1

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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: May 18, 2021 at 1050

Date and Time Test Terminated: May 24, 2021 at 1115

Dilution water used: Soft

PERCENT SURVIVAL

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

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		8 %	11 %	15 %	20 %	27 %
A	14	26	15	33	10	28
B	22	27	26	30	12	26
C	18	19	20	25	25	22
D	24	14	35	16	29	30
E	23	24	21	28	15	23
F	16	26	31	35	14	24
G	14	24	26	32	23	24
H	19	26	15	29	20	24
I	16	22	25	13	23	29
J	22	31	33	30	27	24
Mean per Adult	18.8	23.9	24.7	27.1	19.8	25.4
Mean per Surviving Adult	18.8	23.9	24.7	27.1	19.8	25.4
CV %	20.0	19.7	28.4	26.6	33.6	10.7

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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<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: 33.6 (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, 343, 356, 357

Test Initiated: DATE: May 18, 2021 TIME: 1050
Test Terminated: DATE: May 24, 2021 TIME: 1115

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.4	7.2	7.5	7.3	7.2	8.4	7.5
Final	7.9	6.2	7.9	7.7	8.0	8.2	--
pH Initial	7.4	7.0	7.5	7.3	7.4	7.6	7.4
Final	8.1	7.2	8.0	8.2	8.1	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	8.2	7.5	7.7	7.5	7.2	8.5	7.6
Final	8.2	6.2	8.0	8.3	8.3	8.0	--
pH Initial	7.3	6.9	7.5	7.4	7.6	7.6	7.6
Final	8.3	7.2	8.0	8.1	8.3	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	8.2	7.8	7.8	7.5	7.7	8.4	7.2
Final	8.3	5.9	8.2	8.4	8.8	7.9	--
pH Initial	7.3	7.0	7.4	7.5	7.6	7.6	7.5
Final	8.3	7.2	8.1	8.2	8.3	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
15 %							
D.O. Initial	8.5	7.5	7.8	7.4	7.6	8.3	7.5
Final	8.0	6.2	7.9	8.2	8.1	7.8	--
pH Initial	7.3	7.0	7.4	7.4	7.5	7.6	7.5
Final	8.1	7.2	8.0	8.1	8.2	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	8.6	7.6	7.7	7.4	7.6	8.4	7.6
Final	8.0	6.9	8.0	7.8	8.0	8.4	--
pH Initial	7.3	7.1	7.3	7.5	7.5	7.5	7.5
Final	8.1	7.3	8.1	8.2	8.3	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	8.6	7.4	7.8	7.5	7.7	8.4	7.6
Final	8.1	6.7	7.9	8.0	8.3	8.4	--
pH Initial	7.2	7.1	7.3	7.5	7.5	7.5	7.5
Final	8.2	7.2	8.1	8.1	8.2	8.5	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
9.5	34	270	<0.05	EFF 17-MAY-21
21	33	170	<0.05	EFF 19-MAY-21
22	35	200	<0.05	EFF 21-MAY-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	150	<0.05	255276-1



LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>SEARCY WATER UTILITIES</u>		NO OF BOTTLES		ANALYSES REQUESTED												AIC CONTROL NO: <u>255486</u>
Project Reference: <u>BIO-MONITOR</u>		SAMPLE MATRIX														AIC PROPOSAL NO:
Project Manager: <u>JIMMY SMITH</u>		WATER														Carrier:
Sampled By: <u>JEREMY CHEELY</u>		SOIL														Received Temperature C <u>0.3</u>
AIC No. <u>1</u>		GRAVEL														Remarks
Sample Identification <u>EFF</u>		COMPOST														
Date/Time Collected <u>5-17-21 8am</u>		✓														
Container Type																
Preservative																
G = Glass		P = Plastic														Field pH calibration on _____ @ _____ Buffer: _____
NO = none		S = Sulfuric acid pH2														
V = VOA vials		N = Nitric acid pH2														
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS																
Expedited results requested by: _____																
Who should AIC contact with questions: _____																
Phone: _____																
Report Attention to: _____																
Report Address to: _____																
																Received Date/Time
																By: _____
																Received in Lab Date/Time
																By: <u>P. B. Rowland</u> <u>5-17-21 10:38am</u>
																Comments:



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE **3** OF **3**

Client: Searcy Water Utilities				AIC CONTROL NO: 200486				AIC PROPOSAL NO:					
Project Reference: Bio-Monitoring				Carrier:				Received Temperature C					
Project Manager: Jimmy Smith				Remarks:									
Sampled By: Brady Anderson													
AIC No.	Sample Identification	Date/Time Collected	G R A B	C O M P	W A T E R	S O I L	NO OF BOTTLES	ANALYSES REQUESTED				Field pH calibration	
3	EFF	5-21-21 8am		/	✓		1	Bio-Monitor	CD	PHENOLICS	HG	METALS	
	EFF	5-20-21 8am	/				4						
	EFF	5-21-21 8am	/				4						
	EFF	5-20-21 8am	/				4						
	EFF	5-21-21 8am	/				1						
Container Type												on @	
Preservative												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	
Turnaround Time Requested: (Please circle)				Relinquished				Received				Date/Time	
NORMAL or EXPEDITED IN ___ DAYS				By:				By:				Date/Time	
Expedited results requested by: _____				By: <i>[Signature]</i>				Received in Lab				Date/Time	
Who should AIC contact with questions: _____				By: <i>[Signature]</i>				5/21/21 11:09 am				5-21-21 11:09	
Phone: _____				Comments:									
Report Attention to: _____													
Report Address to: _____													