



March 23, 2022

Biomonitoring Testing  
for  
Eff

Control No. 263772-1

Prepared for:

Mr. Jimmy Smith  
Searcy Water and Sewer System  
P.O. Box 1319  
Searcy, AR 72145

Prepared by:

AMERICAN INTERPLEX CORPORATION  
8600 Kanis Road  
Little Rock, AR 72204-2322

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:

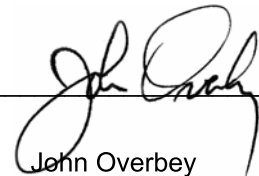
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire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

PDF cc: Searcy Water and Sewer System  
ATTN: Mr. Dan Dawson  
d.dawson@searcywater.org

Searcy Water and Sewer System  
ATTN: Mr. Jimmy Smith  
jsmith67@cablelynx.com

Table of Contents

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

I. Control Acceptance Criteria

*Pimephales promelas* (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.488	PASS
Control Growth CV < or = 40%	12.9	PASS
Growth Minimum Significant Difference 12 to 30%	21.5	PASS
Critical Dilution CV < or = 40%	16.4	PASS

*Ceriodaphnia dubia* Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	28.3	PASS
Control CV < or = 40% per Surviving Female	24.8	PASS
Reproduction Minimum Significant Difference 13 to 47%	24.8	PASS
Critical Dilution CV < or = 40%	19.2	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)	6.8	6.8	6.8
pH (standard units)	6.9	6.8	7.1
Alkalinity (mg/l as CaCO <sub>3</sub> )	18	36	54
Hardness (mg/l as CaCO <sub>3</sub> )	41	38	37
Conductivity (umhos/cm)	260	280	300
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	<0.1	0.16

2. Dilution Water Samples:

Outfall 001

Analysis	263935-1
Dissolved oxygen (mg/l)	7.1
pH (standard units)	6.7
Alkalinity (mg/l as CaCO <sub>3</sub> )	NA
Hardness (mg/l as CaCO <sub>3</sub> )	NA
Conductivity (umhos/cm)	170
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: March 15, 2022 at 0959  
Date & Time Test Terminated: March 22, 2022 at 0915  
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: March 15, 2022 at 1040  
Date & Time Test Terminated: March 21, 2022 at 1228  
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. 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 February 01, 2022 at 1124 to February 08, 2022 at 1130

The results were as follows: (Control No. 262537-1.)

Survival LC-50: 2536 mg/l

Growth IC-25: 2028 mg/l

Growth PMSD: 15.4

##### *Ceriodaphnia dubia*

A chronic reference test was performed on February 01, 2022 at 1433 to February 07, 2022 at 1310

The results were as follows: (Control No. 262537-2.)

Survival LC-50: 1633 mg/l

Reproduction IC-25: 1093 mg/l

Reproduction PMSD: 20.4

#### V. Organism History

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

Date: March 15, 2022

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

##### *Ceriodaphnia dubia*

Date: March 15, 2022

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

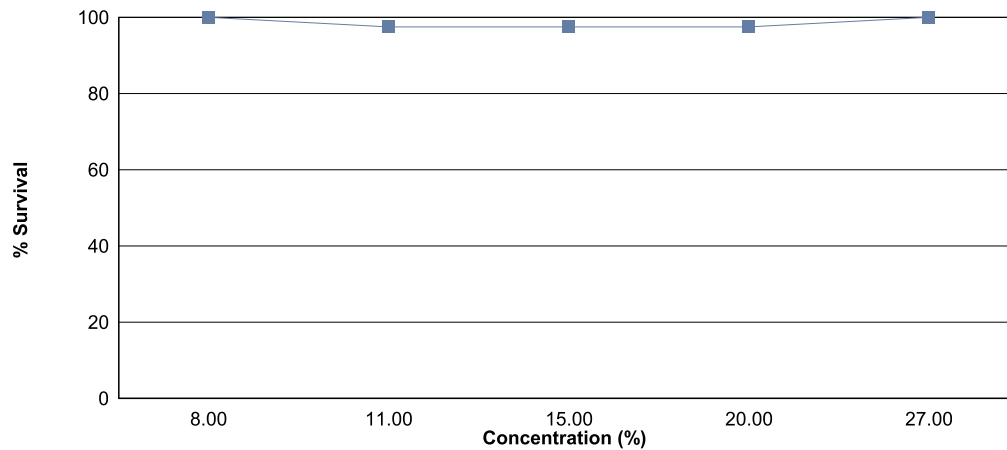
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 March 15, 2022 at 0959 and continued through March 22, 2022 at 0915. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

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



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.476
8 %	100	0.535
11 %	97.5	0.502
15 %	97.5	0.461
20 %	97.5	0.500
27 %	100	0.531

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

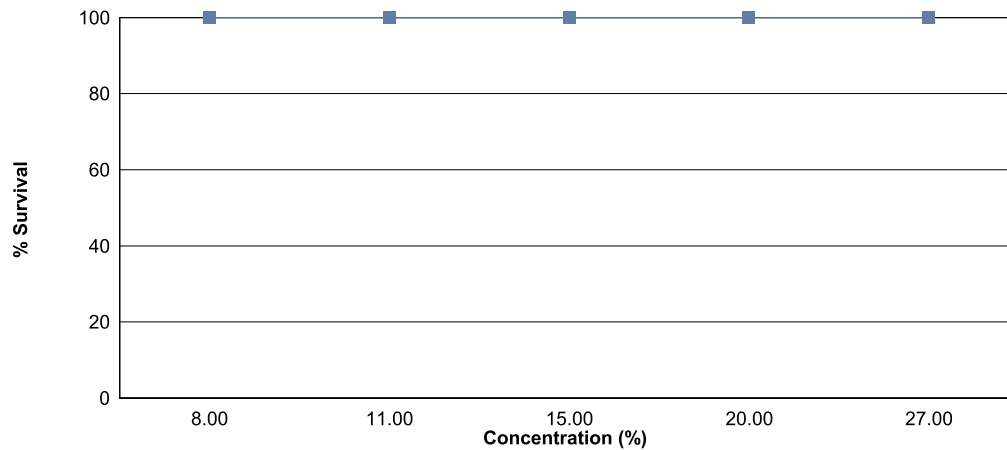
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 March 15, 2022 at 1040 and continued through March 21, 2022 at 1228. 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	28.3
8 %	100	31.0
11 %	100	30.2
15 %	100	30.6
20 %	100	32.4
27 %	100	30.4



Appendix A1: Test 1000.0

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

Date and Time Test Initiated: March 15, 2022 at 0959

Date and Time Test Terminated: March 22, 2022 at 0915

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	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	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
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	7	7	7	7	7	7	7
20 %	A	8	7	7	7	7	7	7
	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: March 15, 2022 at 0959

Test Terminated: March 22, 2022 at 0915

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76932	.77362	0.00430	8	0.538
	B	.77333	.77644	0.00311	8	0.389
	C	.77072	.77423	0.00351	8	0.439
	D	.76963	.77379	0.00416	8	0.520
	E	.77100	.77496	0.00396	8	0.495
8 %	A	.77221	.77617	0.00396	8	0.495
	B	.76735	.77172	0.00437	8	0.546
	C	.76427	.76868	0.00441	8	0.551
	D	.76662	.77067	0.00405	8	0.506
	E	.76188	.76650	0.00462	8	0.578
11 %	A	.76798	.77262	0.00464	8	0.580
	B	.77333	.77627	0.00294	8	0.368
	C	.76360	.76730	0.00370	8	0.462
	D	.77391	.77799	0.00408	8	0.510
	E	.76703	.77175	0.00472	8	0.590
15 %	A	.77027	.77415	0.00388	8	0.485
	B	.76973	.77312	0.00339	8	0.424
	C	.76412	.76814	0.00402	8	0.502
	D	.77085	.77449	0.00364	8	0.455
	E	.76874	.77224	0.00350	8	0.438
20 %	A	.76791	.77084	0.00293	8	0.366
	B	.77530	.77953	0.00423	8	0.529
	C	.77127	.77514	0.00387	8	0.484
	D	.77616	.78075	0.00459	8	0.574
	E	.76900	.77339	0.00439	8	0.549
27 %	A	.76868	.77340	0.00472	8	0.590
	B	.77181	.77575	0.00394	8	0.492
	C	.77464	.77854	0.00390	8	0.488
	D	.77445	.77796	0.00351	8	0.439
	E	.76976	.77492	0.00516	8	0.645

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: March 15, 2022 at 1040

Date and Time Test Terminated: March 21, 2022 at 1228

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	5	6	6	0	0	0	0	0	5	5	27	10	2.70	
4	0	0	0	3	3	0	3	4	0	0	13	10	1.30	
5	11	12	11	12	9	9	10	10	11	11	106	10	10.6	
6	21	16	17	14	0	16	12	13	16	12	137	10	13.7	
7														
8														
TOTAL	37	34	34	29	12	25	25	27	32	28	283	10	28.3	

Concentration: 8 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	5	6	0	0	4	0	0	6	6	32	10	3.20
4	0	0	6	4	6	0	4	3	0	0	23	10	2.30
5	13	11	10	11	9	12	10	11	12	10	109	10	10.9
6	21	18	18	13	0	17	13	14	17	15	146	10	14.6
7													
8													
TOTAL	39	34	40	28	15	33	27	28	35	31	310	10	31.0

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	6	5	4	0	0	5	0	0	6	5	31	10	3.10
4	0	0	0	5	5	0	4	5	0	0	19	10	1.90
5	12	12	13	10	10	10	10	9	13	12	111	10	11.1
6	16	16	18	16	0	16	13	17	16	13	141	10	14.1
7													
8													
TOTAL	34	33	35	31	15	31	27	31	35	30	302	10	30.2

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: March 15, 2022 at 1040

Date and Time Test Terminated: March 21, 2022 at 1228

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	7	6	5	0	0	1	0	0	5	5	29	10	2.90	
4	0	0	0	5	5	0	3	4	0	0	17	10	1.70	
5	13	11	13	10	10	11	12	11	12	12	115	10	11.5	
6	19	20	15	13	0	14	17	15	14	18	145	10	14.5	
7														
8														
TOTAL	39	37	33	28	15	26	32	30	31	35	306	10	30.6	

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	6	6	6	0	0	3	0	0	6	4	31	10	3.10
4	0	0	0	5	7	0	4	4	0	0	20	10	2.00
5	11	10	11	13	10	10	12	11	12	13	113	10	11.3
6	20	14	20	17	0	15	18	18	19	19	160	10	16.0
7													
8													
TOTAL	37	30	37	35	17	28	34	33	37	36	324	10	32.4

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	6	5	4	0	0	4	0	0	6	3	28	10	2.80
4	0	0	0	4	5	0	5	4	0	0	18	10	1.80
5	13	10	11	13	12	10	13	14	12	11	119	10	11.9
6	21	18	18	15	0	18	0	11	21	17	139	10	13.9
7													
8													
TOTAL	40	33	33	32	17	32	18	29	39	31	304	10	30.4

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	1.00000	1.39310
1	Control	2	0.87500	1.20940
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	8 %	1	1.00000	1.39310
2	8 %	2	1.00000	1.39310
2	8 %	3	1.00000	1.39310
2	8 %	4	1.00000	1.39310
2	8 %	5	1.00000	1.39310
3	11 %	1	1.00000	1.39310
3	11 %	2	0.87500	1.20940
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	0.87500	1.20940
5	20 %	2	1.00000	1.39310
5	20 %	3	1.00000	1.39310
5	20 %	4	1.00000	1.39310
5	20 %	5	1.00000	1.39310
6	27 %	1	1.00000	1.39310
6	27 %	2	1.00000	1.39310
6	27 %	3	1.00000	1.39310
6	27 %	4	1.00000	1.39310
6	27 %	5	1.00000	1.39310

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0.108 W = 0.5958 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 %	30.00	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 %	30.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.1126 W = 0.965 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 = 6.419 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	0.02145	0.00429	0.9143	
Within (Error)	24	0.1126	0.004692		
Total	29	0.1341			
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	0.4762	0.4762			
2	8 %	0.5352	0.5352	-1.362		
3	11 %	0.502	0.502	-0.5955		
4	15 %	0.4608	0.4608	0.3555		
5	20 %	0.5004	0.5004	-0.5586		
6	27 %	0.5308	0.5308	-1.26		
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	0.1022	21.5	-0.059	
3	11 %	5	0.1022	21.5	-0.0258	
4	15 %	5	0.1022	21.5	0.0154	
5	20 %	5	0.1022	21.5	-0.0242	
6	27 %	5	0.1022	21.5	-0.0546	



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 = 0.1474 D* = 1.156 Critical D* = 1.035 (alpha = 0.01, N = 60)	
Data FAIL normality test (alpha = 0.01).	

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 %	118.50	75.00	10.00	
3	11 %	116.50	75.00	10.00	
4	15 %	117.50	75.00	10.00	
5	20 %	128.00	75.00	10.00	
6	27 %	115.50	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	88.08	17.62	0.3807	
Within (Error)	54	2499	46.28		
Total	59	2587			
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)					

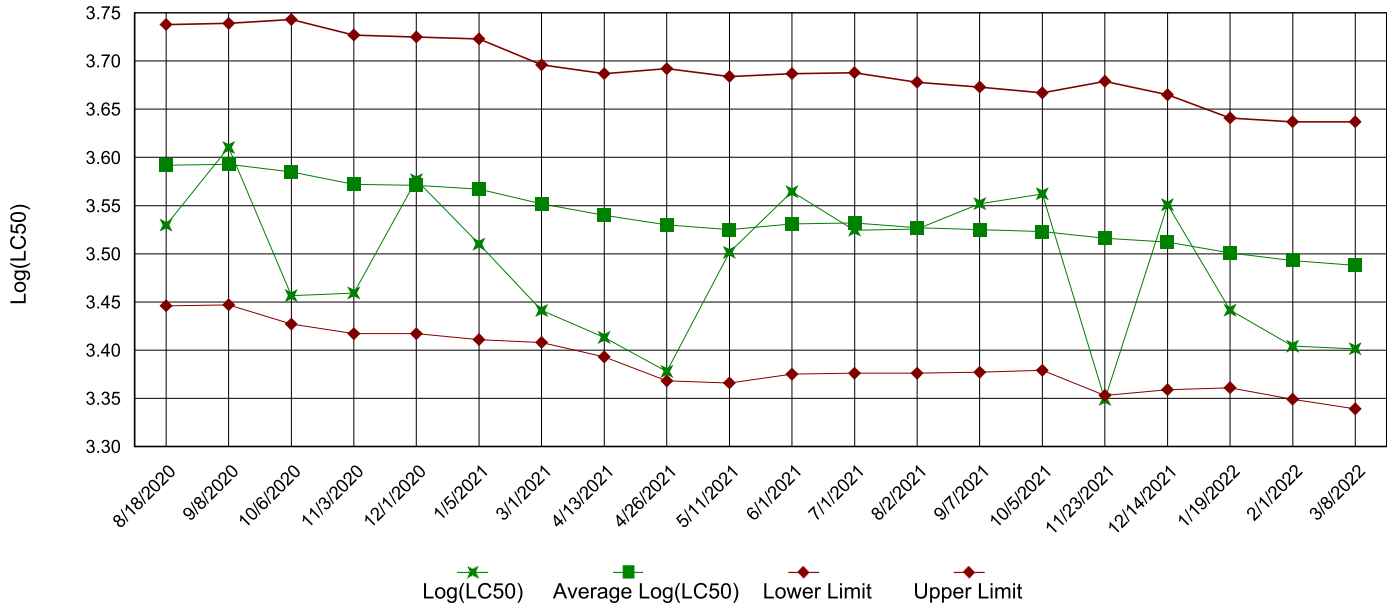
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	28.3	28.3			
2	8 %	31	31	-0.8875		
3	11 %	30.2	30.2	-0.6245		
4	15 %	30.6	30.6	-0.756		
5	20 %	32.4	32.4	-1.348		
6	27 %	30.4	30.4	-0.6903		
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	7.028	24.8	-2.7	
3	11 %	10	7.028	24.8	-1.9	
4	15 %	10	7.028	24.8	-2.3	
5	20 %	10	7.028	24.8	-4.1	
6	27 %	10	7.028	24.8	-2.1	

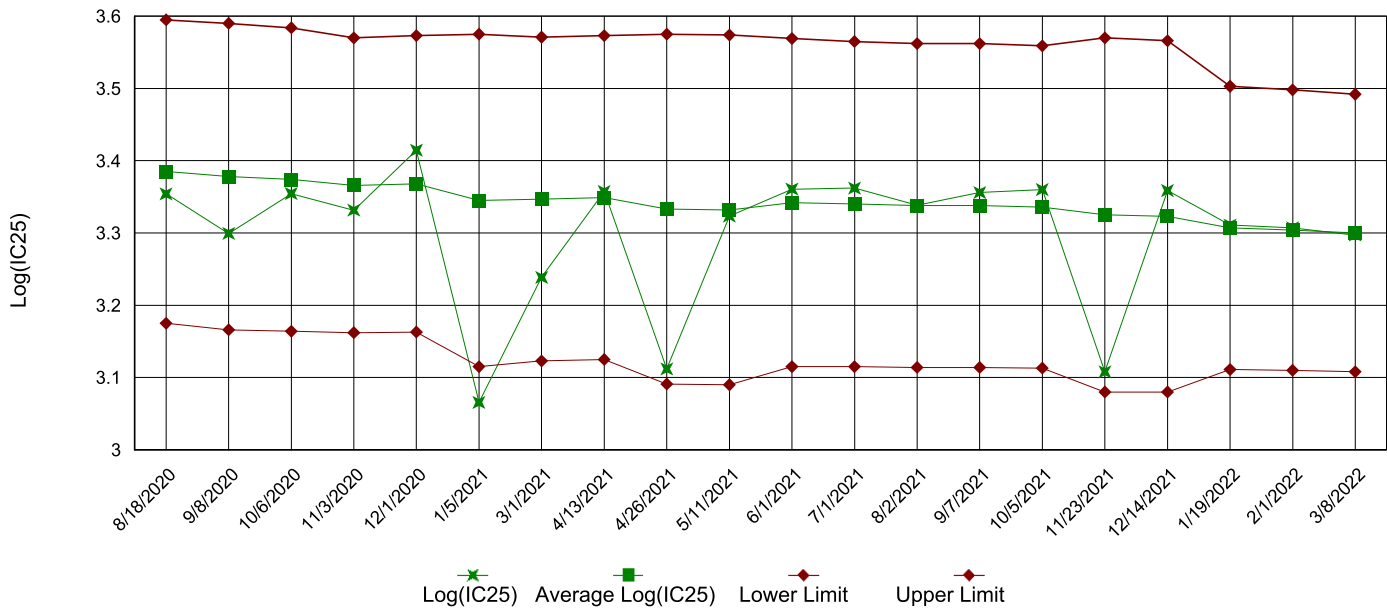
Appendix A3: Test 1000.0

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

LC50 Survival Data

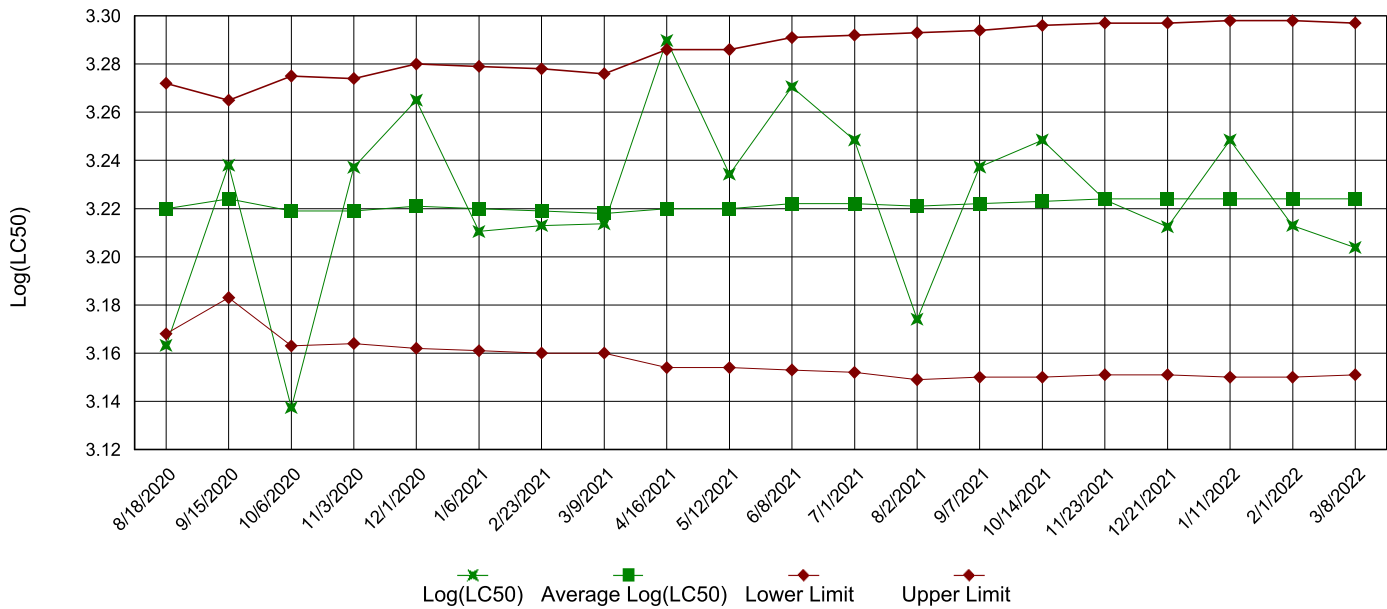


IC25 Growth Data

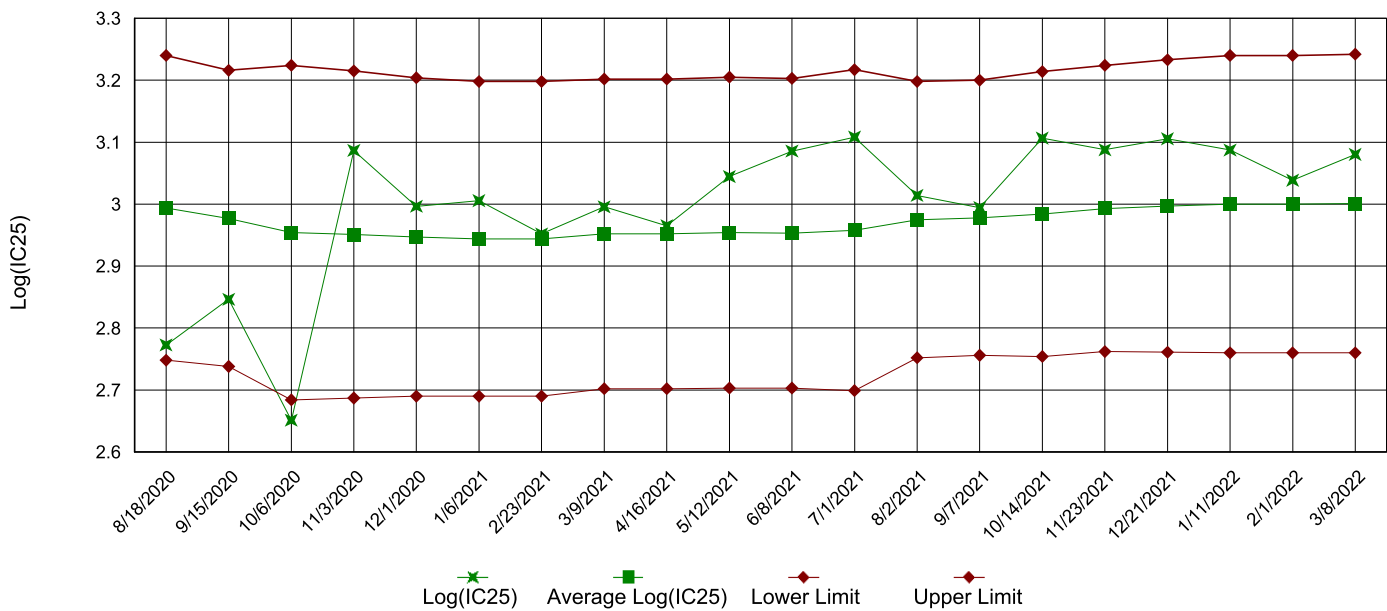


Appendix A3: Test 1002.0  
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: March 15, 2022 at 0959

Date and Time Test Terminated: March 22, 2022 at 0915

Dilution water used: Outfall 001

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	87.5	100	100	100	100	100	97.5	5.73
8 %	100	100	100	100	100	100	100	100	0.00
11 %	100	87.5	100	100	100	100	100	97.5	5.73
15 %	100	100	100	100	87.5	97.5	97.5	97.5	5.73
20 %	87.5	100	100	100	100	100	97.5	97.5	5.73
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	0.538	0.389	0.439	0.520	0.495	0.476	12.9
8 %	0.495	0.546	0.551	0.506	0.578	0.535	6.38
11 %	0.580	0.368	0.462	0.510	0.590	0.502	18.2
15 %	0.485	0.424	0.502	0.455	0.438	0.461	7.02
20 %	0.366	0.529	0.484	0.574	0.549	0.500	16.4
27 %	0.590	0.492	0.488	0.439	0.645	0.531	15.8

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



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

1. Steel's Many-One Rank Test:

Is the mean survival significantly different ( $p=0.05$ ) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	<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 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	<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   (TLP6C)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]:   0   (TGP6C)
5. NOEC *Pimephales* Lethality:   27 %   (TOP6C)
6. LOEC *Pimephales* Lethality:   27 %   (TXP6C)
7. NOEC *Pimephales* Sublethality:   27 %   (TPP6C)
8. LOEC *Pimephales* Sublethality:   27 %   (TYP6C)
9. Coefficient of variation for *Pimephales* growth:   16.4   (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, 343, 357, 358

Test Initiated: DATE: March 15, 2022 TIME: 0959  
Test Terminated: DATE: March 22, 2022 TIME: 0915

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	7.5	7.1	6.3	6.8	6.5	7.8
Final	6.3	6.0	6.5	5.2	5.1	6.0	4.7
pH Initial	6.7	6.9	7.1	6.9	6.9	7.4	6.9
Final	7.3	6.7	7.3	6.9	6.8	6.8	6.2

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	6.9	7.4	7.3	6.2	6.8	6.5	7.4
Final	6.3	6.0	6.4	5.9	5.2	6.1	5.6
pH Initial	6.6	6.7	7.0	7.0	6.8	7.3	6.8
Final	7.2	6.9	7.3	7.1	7.0	6.9	6.4

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	7.1	7.4	7.2	6.2	6.9	6.7	7.5
Final	6.1	6.0	6.4	5.8	5.7	6.2	5.2
pH Initial	6.7	6.6	6.8	6.8	6.8	7.3	6.8
Final	7.2	7.0	7.3	7.0	7.0	7.1	6.3

DILUTION	DAY						
	1	2	3	4	5	6	7
15 %							
D.O. Initial	7.1	7.3	7.0	6.0	6.8	6.6	7.5
Final	6.0	6.0	6.6	5.6	5.2	6.2	5.1
pH Initial	6.8	6.6	6.7	6.8	6.8	7.3	6.8
Final	7.2	7.0	7.3	7.1	7.0	7.0	6.3

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	7.0	7.2	7.0	6.1	6.9	6.6	7.7
Final	6.1	6.0	6.5	5.4	5.1	6.2	5.7
pH Initial	6.8	6.6	6.7	6.8	6.8	7.3	6.9
Final	7.2	7.0	7.2	7.0	7.0	7.1	6.4

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.1	7.2	7.1	5.9	7.2	6.5	7.7
Final	6.1	5.8	6.3	5.6	5.3	6.2	5.6
pH Initial	7.3	6.6	6.7	6.8	6.9	7.3	7.1
Final	7.2	7.0	7.3	7.1	7.0	7.2	6.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
18	41	260	<0.05	Eff 14-MAR-22
36	38	280	<0.05	Eff 16-MAR-22
54	37	300	<0.05	Eff 18-MAR-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
NA	NA	170	<0.05	263935-1

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: March 15, 2022 at 1040

Date and Time Test Terminated: March 21, 2022 at 1228

Dilution water used: Outfall 001

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	37	39	34	39	37	40
B	34	34	33	37	30	33
C	34	40	35	33	37	33
D	29	28	31	28	35	32
E	12	15	15	15	17	17
F	25	33	31	26	28	32
G	25	27	27	32	34	18
H	27	28	31	30	33	29
I	32	35	35	31	37	39
J	28	31	30	35	36	31
Mean per Adult	28.3	31.0	30.2	30.6	32.4	30.4
Mean per Surviving Adult	28.3	31.0	30.2	30.6	32.4	30.4
CV %	24.8	23.2	19.5	22.1	19.2	25.0

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. 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	<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:       24.8 (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, 357, 358

Test Initiated: DATE: March 15, 2022 TIME: 1040  
Test Terminated: DATE: March 21, 2022 TIME: 1228

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	7.5	7.1	6.3	6.8	6.5	7.8
Final	7.2	7.2	7.1	7.0	6.2	6.6	--
pH Initial	6.7	6.9	7.1	6.9	6.9	7.4	6.9
Final	7.8	7.9	7.9	7.6	7.6	7.6	--

DILUTION	DAY						
	1	2	3	4	5	6	7
8 %							
D.O. Initial	6.9	7.4	7.3	6.2	6.8	6.5	7.4
Final	7.1	7.3	7.1	7.1	6.5	6.8	--
pH Initial	6.6	6.7	7.0	7.0	6.8	7.3	6.8
Final	8.0	8.0	7.8	7.7	7.7	7.7	--

DILUTION	DAY						
	1	2	3	4	5	6	7
11 %							
D.O. Initial	7.1	7.4	7.2	6.2	6.9	6.7	7.5
Final	6.8	7.4	7.3	7.5	6.7	7.0	--
pH Initial	6.7	6.6	6.8	6.8	6.8	7.3	6.8
Final	8.0	8.1	7.8	7.7	7.6	7.7	--

DILUTION	DAY						
	1	2	3	4	5	6	7
15 %							
D.O. Initial	7.1	7.3	7.0	6.0	6.8	6.6	7.5
Final	6.9	6.9	7.2	7.1	6.3	6.8	--
pH Initial	6.8	6.6	6.7	6.8	6.8	7.3	6.8
Final	7.9	7.8	7.9	7.8	7.7	7.8	--

DILUTION	DAY						
	1	2	3	4	5	6	7
20 %							
D.O. Initial	7.0	7.2	7.0	6.1	6.9	6.6	7.7
Final	6.9	6.9	7.3	6.9	6.6	6.9	--
pH Initial	6.8	6.6	6.7	6.8	6.8	7.3	6.9
Final	7.9	7.9	7.9	7.8	7.7	7.8	--

DILUTION	DAY						
	1	2	3	4	5	6	7
27 %							
D.O. Initial	7.1	7.2	7.1	5.9	7.2	6.5	7.7
Final	6.9	7.1	7.1	7.0	6.6	6.9	--
pH Initial	7.3	6.6	6.7	6.8	6.9	7.3	7.1
Final	7.9	8.0	7.9	8.0	7.9	7.8	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
18	41	260	<0.05	Eff 14-MAR-22
36	38	280	<0.05	Eff 16-MAR-22
54	37	300	<0.05	Eff 18-MAR-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
NA	NA	170	<0.05	263935-1





CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Seacay Water Utilities		PO No.		ANALYSES REQUESTED		AIC CONTROL NO: Z6377Z	
Project Reference: Bio-Monitoring		MATRIX		NO OF BOTTLES		AIC PROPOSAL NO:	
Project Manager: Jimmy Smith		WATER		S		Carrier:	
Sampled By: Brady Anderson		COMPOUND		NO OF BOTTLES		Received on ice? Yes <input checked="" type="checkbox"/> No <input type="checkbox"/> Temp. °C	
AIC No. 3 EFF		GRA B		S		Remarks	
Date/Time Collected: 3-18-22 8am		/		1		AIC 263880	
Date/Time Collected: 3-18-22 2:45pm		/		1		"	
Date/Time Collected: 3-18-22 2:30pm		/		1		"	
Container Type		P		G		Field pH calibration on @	
Preservative		NO		NO		Buffer:	
G = Glass		V = VOA vials		H = HCl to pH2		T = Sodium Thiosulfate	
NO = none		N = Nitric acid pH2		B = NaOH to pH12		Z = Zinc acetate	
S = Sulfuric acid pH2		Relinquished By:		Date/Time		A=(NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , NH <sub>4</sub> OH	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN ___ DAYS		Relinquished By: <i>Anderson</i>		Date/Time: 3-18-22 10am		Received By: <i>Colt</i>	
Expedited results requested by: _____		Relinquished By: _____		Date/Time: _____		Received in Lab Date/Time: 3-18-22 10am	
Who should AIC contact with questions: _____		Comments: _____		_____		_____	
Contact Phone: _____		_____		_____		_____	
Report Attention to: _____		_____		_____		_____	
Email Address: _____		_____		_____		_____	