March 19, 2021

Biomonitoring Testing
for
EFF

Control No. 253467-1

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

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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	92.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.430	PASS
Control Growth CV < or = 40%	11.5	PASS
Growth Minimum Significant Difference 12 to 30%	25.5	PASS
Critical Dilution CV < or = 40%	32.3	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	20.5	PASS
Control CV < or = 40% per Surviving Female	18.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	20.8	PASS
Critical Dilution CV < or = 40%	9.40	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)	8.0	7.7	7.8
pH (standard units)	7.2	7.1	7.3
Alkalinity (mg/l as CaCO3)	20	34	34
Hardness (mg/l as CaCO3)	30	27	34
Conductivity (umhos/cm)	230	230	240
Residual Chlorine (mg/l)	0.050	<0.05	0.050
Ammonia as N (mg/l)	0.84	<0.1	<0.1

2. Dilution Water Samples:
   Soft

Analysis	253379-1
Dissolved oxygen (mg/l)	7.9
pH (standard units)	7.9
Alkalinity (mg/l as CaCO3)	32
Hardness (mg/l as CaCO3)	43
Conductivity (umhos/cm)	180
Residual Chlorine (mg/l)	<0.05

C. Test Methods
1. Test methods used:
Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	March 9, 2021 at 0915
Date & Time Test Terminated:	March 16, 2021 at 0905
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 9, 2021 at 1115
Date & Time Test Terminated:	March 15, 2021 at 1310
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 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. Steel's Many-One Rank test was used to determine the No Observable Effects Concentration (NOEC) for growth. Dunnett's Test was used to calculate the PMSD.

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 March 01, 2021 at 1415 to March 08, 2021 at 1410
The results were as follows: (Control No. 253029-1.)

Survival LC-50: 2762 mg/l
Growth IC-25: 1732 mg/l
Growth PMSD: 11.1

Ceriodaphnia dubia

A chronic reference test was performed on February 23, 2021 at 1515 to March 02, 2021 at 1500
The results were as follows: (Control No. 253029-2.)

Survival LC-50: 1633 mg/l
Reproduction IC-25: 895.4 mg/l
Reproduction PMSD: 13

V. Organism History

Pimephales promelas (Fathead minnow)

Date: March 9, 2021
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: March 9, 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

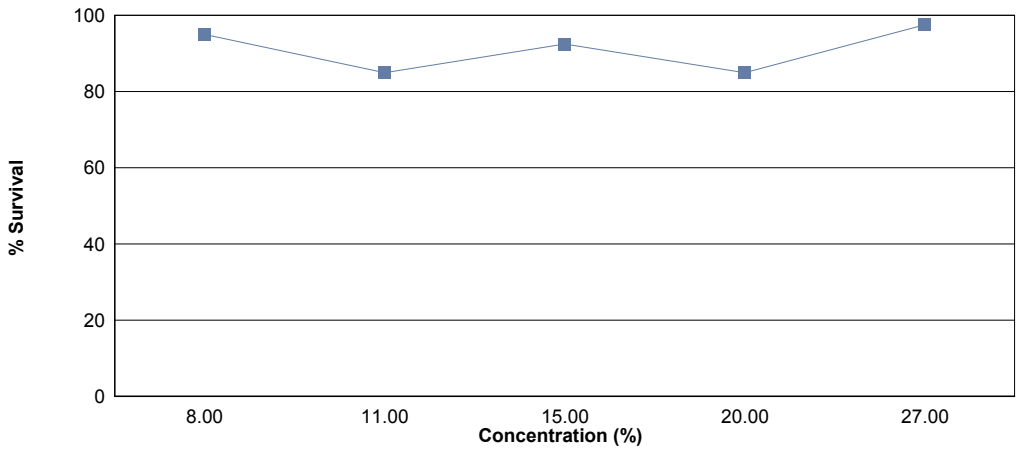
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 March 9, 2021 at 0915 and continued through March 16, 2021 at 0905. 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	92.5	0.398
8 %	95.0	0.408
11 %	85.0	0.291
15 %	92.5	0.372
20 %	85.0	0.346
27 %	97.5	0.405

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

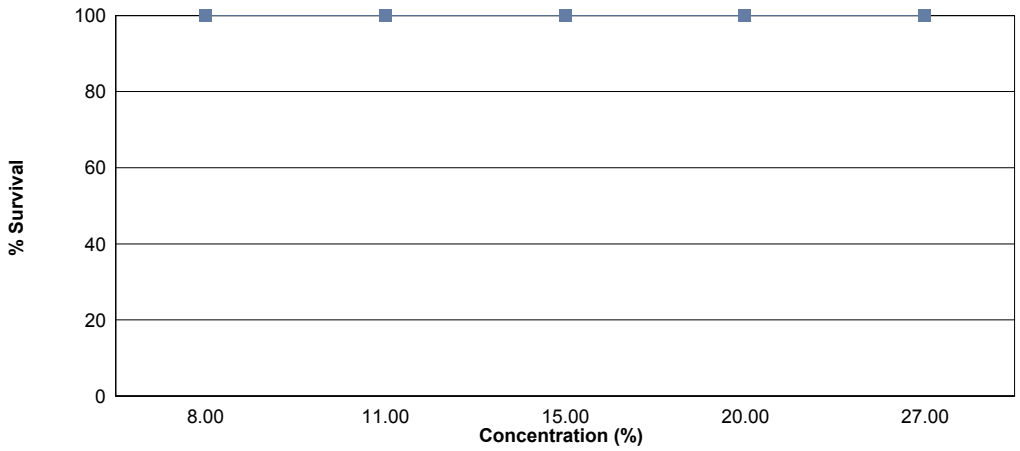
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 9, 2021 at 1115 and continued through March 15, 2021 at 1310. 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 *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	20.5
8 %	100	28.2
11 %	100	29.4
15 %	100	26.0
20 %	100	30.6
27 %	100	28.0

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: March 9, 2021 at 0915
Date and Time Test Terminated: March 16, 2021 at 0905

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	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	7	7	6	6
	E	8	8	8	8	8	8	8
8 %	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	7	7	7
	E	8	8	8	8	8	8	8
11 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	6
	C	8	8	8	8	7	7	6
	D	8	8	8	8	8	7	7
	E	8	8	8	8	8	7	7
15 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	7	7	7
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	7
20 %	A	8	8	8	8	7	6	5
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	7	7
	D	8	8	8	8	7	7	6
	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	7	7	7	7	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: March 9, 2021 at 0915
Test Terminated: March 16, 2021 at 0905

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.71935	.72264	0.00329	8	0.411
	B	.72843	.73168	0.00325	8	0.406
	C	.73069	.73400	0.00331	8	0.414
	D	.72916	.73172	0.00256	8	0.320
	E	.72463	.72816	0.00353	8	0.441
8 %	A	.72518	.72862	0.00344	8	0.430
	B	.72097	.72381	0.00284	8	0.355
	C	.73377	.73716	0.00339	8	0.424
	D	.71824	.72131	0.00307	8	0.384
	E	.72412	.72771	0.00359	8	0.449
11 %	A	.72476	.72791	0.00315	8	0.394
	B	.72943	.73045	0.00102	8	0.128
	C	.72717	.72934	0.00217	8	0.271
	D	.73268	.73527	0.00259	8	0.324
	E	.72401	.72672	0.00271	8	0.339
15 %	A	.72701	.72960	0.00259	8	0.324
	B	.72376	.72694	0.00318	8	0.398
	C	.72676	.72999	0.00323	8	0.404
	D	.72411	.72703	0.00292	8	0.365
	E	.72640	.72934	0.00294	8	0.368
20 %	A	.72425	.72559	0.00134	8	0.168
	B	.72761	.73075	0.00314	8	0.392
	C	.72900	.73202	0.00302	8	0.378
	D	.73086	.73346	0.00260	8	0.325
	E	.73002	.73376	0.00374	8	0.468
27 %	A	.72785	.73085	0.00300	8	0.375
	B	.72742	.73075	0.00333	8	0.416
	C	.72835	.73181	0.00346	8	0.432
	D	.72047	.72357	0.00310	8	0.388
	E	.72122	.72453	0.00331	8	0.414

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: March 9, 2021 at 1115
Date and Time Test Terminated: March 15, 2021 at 1310

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	0	0	0	0	3	3	4	3	3	20	10	2.00
4	0	3	4	4	4	0	0	0	0	0	15	10	1.50
5	9	10	7	8	7	8	7	8	9	8	81	10	8.10
6	11	10	9	0	6	10	9	12	10	12	89	10	8.90
7													
8													
TOTAL	24	23	20	12	17	21	19	24	22	23	205	10	20.5

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	3	0	4	3	4	2	4	4	3	0	27	10	2.70
4	0	4	0	0	0	0	0	0	0	3	7	10	0.700
5	11	10	11	9	12	10	11	12	10	10	106	10	10.6
6	15	13	15	11	16	17	14	15	13	13	142	10	14.2
7													
8													
TOTAL	29	27	30	23	32	29	29	31	26	26	282	10	28.2

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	4	0	3	0	3	3	4	3	4	4	28	10	2.80
4	0	4	0	6	0	0	0	0	0	0	10	10	1.00
5	11	10	10	11	11	12	11	10	12	11	109	10	10.9
6	17	15	17	0	17	15	16	16	18	16	147	10	14.7
7													
8													
TOTAL	32	29	30	17	31	30	31	29	34	31	294	10	29.4

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: March 9, 2021 at 1115
Date and Time Test Terminated: March 15, 2021 at 1310

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	3	0	3	0	0	3	0	3	3	4	19	10	1.90
4	0	2	0	5	4	0	6	0	0	0	17	10	1.70
5	9	9	9	13	9	10	11	9	9	10	98	10	9.80
6	17	14	19	0	13	18	0	13	16	16	126	10	12.6
7													
8													
TOTAL	29	25	31	18	26	31	17	25	28	30	260	10	26.0

Concentration: 20 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	3	4	1	4	4	4	4	4	3	3	34	10	3.40
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	12	9	8	10	12	10	11	10	11	12	105	10	10.5
6	18	17	16	13	19	17	17	17	18	15	167	10	16.7
7													
8													
TOTAL	33	30	25	27	35	31	32	31	32	30	306	10	30.6

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	3	0	3	0	3	5	3	3	3	3	26	10	2.60
4	0	4	0	2	0	0	0	0	0	0	6	10	0.600
5	10	10	7	14	8	11	10	10	10	10	100	10	10.0
6	18	13	17	0	17	20	17	13	18	15	148	10	14.8
7													
8													
TOTAL	31	27	27	16	28	36	30	26	31	28	280	10	28.0

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	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	0.75000	1.04720
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	0.87500	1.20940
2	8 %	5	1.00000	1.39310
3	11 %	1	1.00000	1.39310
3	11 %	2	0.75000	1.04720
3	11 %	3	0.75000	1.04720
3	11 %	4	0.87500	1.20940
3	11 %	5	0.87500	1.20940
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	0.87500	1.20940
4	15 %	4	0.87500	1.20940
4	15 %	5	0.87500	1.20940
5	20 %	1	0.62500	0.91174
5	20 %	2	1.00000	1.39310
5	20 %	3	0.87500	1.20940
5	20 %	4	0.75000	1.04720
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	0.87500	1.20940
6	27 %	5	1.00000	1.39310

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality	Transform: Arc Sin(Square Root(Y))
D = 0.4684	
W = 0.9516	
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 = 4.597	
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	0.1226	0.02451	1.256
Within (Error)	24	0.4684	0.01952	
Total	29	0.5909		

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	1.2872	0.925		
2	8 %	1.3196	0.95	-0.3667	
3	11 %	1.1813	0.85	1.198	
4	15 %	1.2829	0.925	0.04866	
5	20 %	1.1909	0.85	1.09	
6	27 %	1.3564	0.975	-0.7831	

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	0.1449	15.7	-0.025
3	11 %	5	0.1449	15.7	0.075
4	15 %	5	0.1449	15.7	0
5	20 %	5	0.1449	15.7	0.075
6	27 %	5	0.1449	15.7	-0.05

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.1115		
W = 0.8894		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

Steel's Many-One Rank Test					No Transformation
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 %	18.00	16.00	5.00	
4	15 %	20.00	16.00	5.00	
5	20 %	23.00	16.00	5.00	
6	27 %	28.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Dunnett's Test for PMSD Calculation

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	0.05143	0.01029	2.215
Within (Error)	24	0.1115	0.004646	
Total	29	0.163		

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	0.3984	0.3984		
2	8 %	0.4084	0.4084	-0.232	
3	11 %	0.2912	0.2912	2.487	*
4	15 %	0.3718	0.3718	0.617	
5	20 %	0.3462	0.3462	1.211	
6	27 %	0.405	0.405	-0.1531	

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	0.1017	25.5	-0.01
3	11 %	5	0.1017	25.5	0.1072
4	15 %	5	0.1017	25.5	0.0266
5	20 %	5	0.1017	25.5	0.0522
6	27 %	5	0.1017	25.5	-0.0066

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 = 0.168		
D* = 1.318		
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 %	152.00	75.00	10.00	
3	11 %	146.50	75.00	10.00	
4	15 %	138.50	75.00	10.00	
5	20 %	155.00	75.00	10.00	
6	27 %	146.00	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	643.3	128.7	7.562
Within (Error)	54	918.9	17.02	
Total	59	1562		

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	20.5	20.5		
2	8 %	28.2	28.2	-4.173	
3	11 %	29.4	29.4	-4.824	
4	15 %	26	26	-2.981	
5	20 %	30.6	30.6	-5.474	
6	27 %	28	28	-4.065	

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

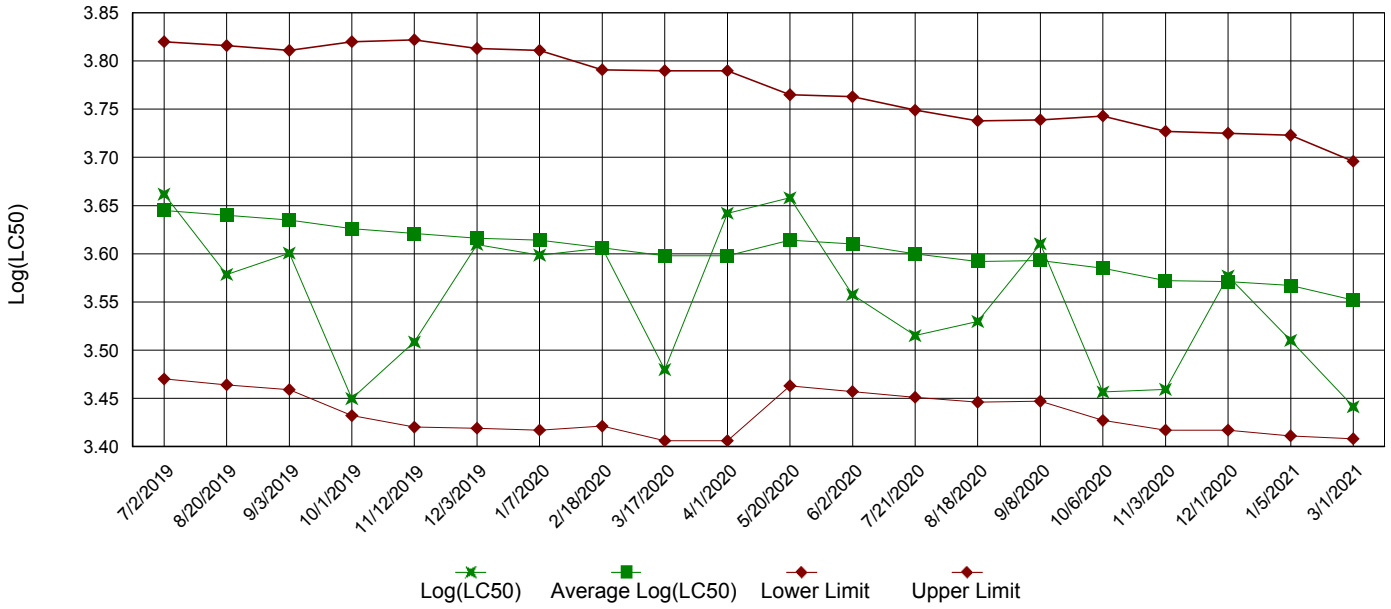
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	8 %	10	4.262	20.8	-7.7
3	11 %	10	4.262	20.8	-8.9
4	15 %	10	4.262	20.8	-5.5
5	20 %	10	4.262	20.8	-10.1
6	27 %	10	4.262	20.8	-7.5

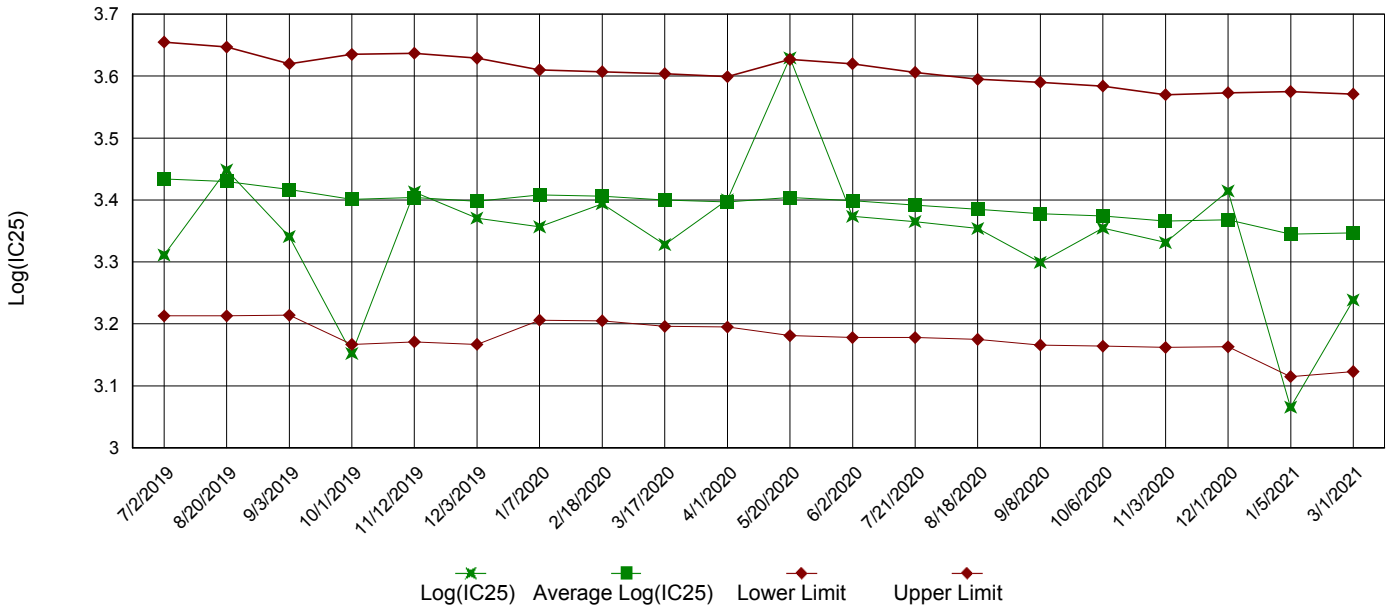
Appendix A3: Test 1000.0

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

LC50 Survival Data



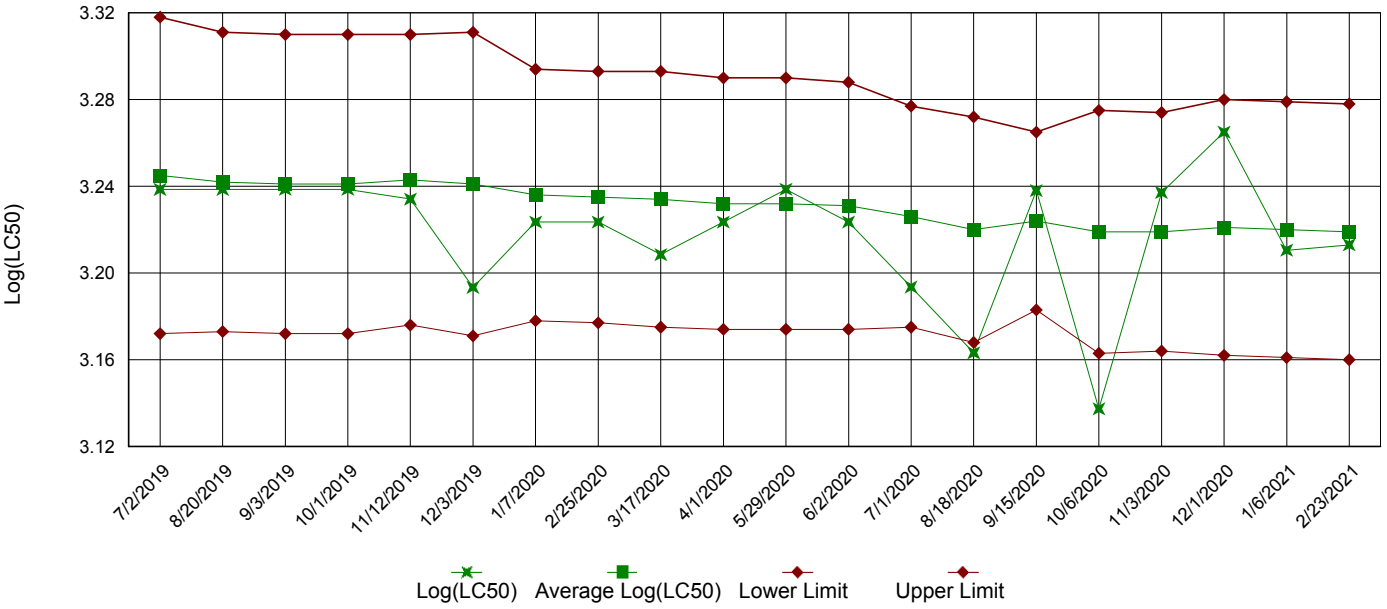
IC25 Growth Data



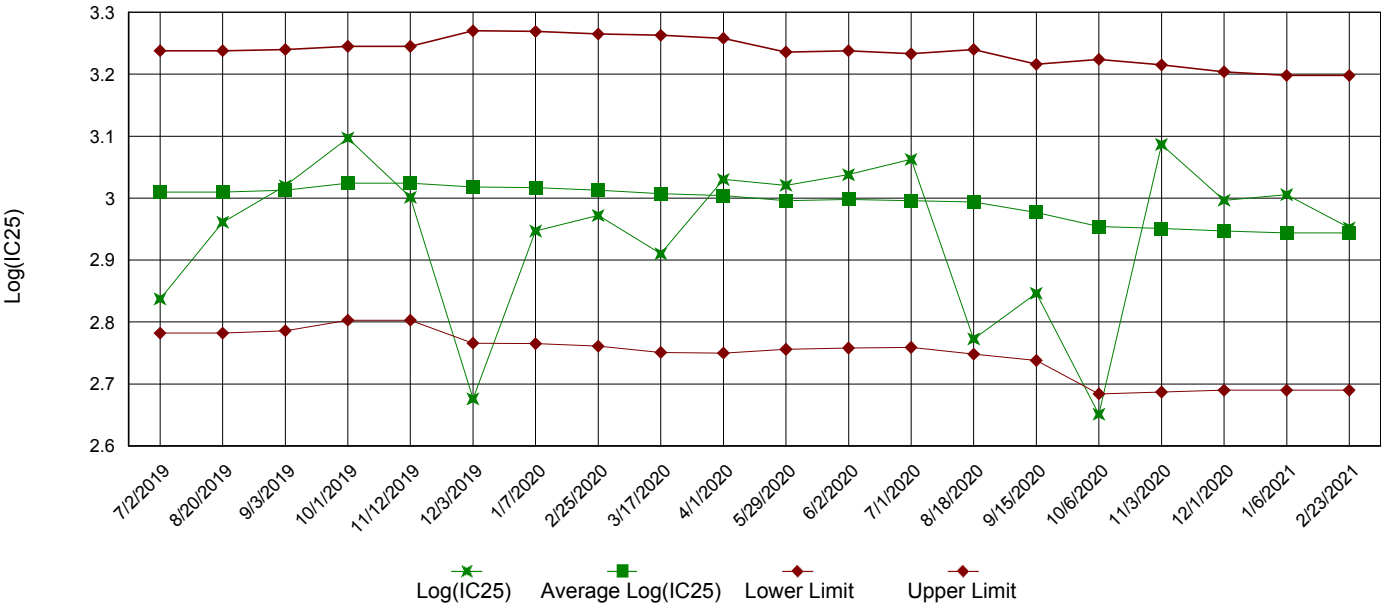
Appendix A3: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: March 9, 2021 at 0915
Date and Time Test Terminated: March 16, 2021 at 0905
Dilution water used: Soft

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	87.5	100	100	75.0	100	100	100	92.5	12.1
8 %	100	87.5	100	87.5	100	100	100	95.0	7.21
11 %	100	75.0	75.0	87.5	87.5	100	100	85.0	12.3
15 %	100	100	87.5	87.5	87.5	100	100	92.5	7.40
20 %	62.5	100	87.5	75.0	100	100	100	85.0	19.2
27 %	100	100	100	87.5	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.411	0.406	0.414	0.320	0.441	0.398	11.5
8 %	0.430	0.355	0.424	0.384	0.449	0.408	9.33
11 %	0.394	0.128	0.271	0.324	0.339	0.291	34.8
15 %	0.324	0.398	0.404	0.365	0.368	0.372	8.58
20 %	0.168	0.392	0.378	0.325	0.468	0.346	32.3
27 %	0.375	0.416	0.432	0.388	0.414	0.405	5.68

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. Dunnett's 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 _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ NO

2. Steel's Many-One Rank 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 _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ 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: 32.3 (TQP6C)

10. Sublethality for this test: 27 % (51714 or 51714S)

AMERICAN INTERPLEX CORPORATION LABORATORIES

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, 356

Test Initiated: DATE: March 9, 2021 TIME: 0915
Test Terminated: DATE: March 16, 2021 TIME: 0905

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.7	7.7	8.0	7.8	7.6	6.9
	Final	7.4	7.0	7.2	6.5	6.8	6.1	6.0
pH	Initial	7.9	7.4	7.7	7.8	7.8	7.8	7.7
	Final	7.6	7.6	7.4	7.4	7.5	7.3	7.4

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.6	7.7	8.0	7.7	7.4	7.0
	Final	7.3	6.2	7.1	6.4	6.7	6.0	6.0
pH	Initial	7.8	7.8	7.7	7.7	7.6	7.6	7.5
	Final	7.6	7.4	7.4	7.4	7.4	7.3	7.4

DILUTION 11 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.6	7.5	8.3	7.8	7.5	7.2
	Final	7.4	6.2	6.9	6.5	6.8	6.2	6.1
pH	Initial	7.7	7.7	7.6	7.6	7.6	7.6	7.8
	Final	7.7	7.4	7.3	7.4	7.4	7.3	7.4

DILUTION 15 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.6	7.4	8.1	7.6	7.6	7.0
	Final	7.1	6.1	7.0	6.5	6.3	5.9	6.1
pH	Initial	7.6	7.6	7.6	7.6	7.6	7.6	7.6
	Final	7.6	7.3	7.4	7.5	7.3	7.3	7.4

DILUTION 20 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.6	7.5	8.2	7.9	7.6	7.1
	Final	7.1	6.2	7.1	6.6	6.7	6.2	6.1
pH	Initial	7.6	7.6	7.6	7.5	7.5	7.5	7.5
	Final	7.6	7.3	7.4	7.5	7.3	7.4	7.4

DILUTION 27 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.5	7.8	8.2	7.7	7.5	7.0
	Final	7.3	6.2	7.1	6.6	6.8	6.2	6.2
pH	Initial	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	Final	7.6	7.3	7.4	7.5	7.4	7.3	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
20	30	230	0.050	EFF 08-MAR-21
34	27	230	<0.05	EFF 10-MAR-21
34	34	240	0.050	EFF 12-MAR-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	43	180	<0.05	253379-1

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 9, 2021 at 1115
Date and Time Test Terminated: March 15, 2021 at 1310
Dilution water used: Soft

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	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

		Percent Effluent				
Replicates	Control	8 %	11 %	15 %	20 %	27 %
A	24	29	32	29	33	31
B	23	27	29	25	30	27
C	20	30	30	31	25	27
D	12	23	17	18	27	16
E	17	32	31	26	35	28
F	21	29	30	31	31	36
G	19	29	31	17	32	30
H	24	31	29	25	31	26
I	22	26	34	28	32	31
J	23	26	31	30	30	28
Mean per Adult	20.5	28.2	29.4	26.0	30.6	28.0
Mean per Surviving Adult	20.5	28.2	29.4	26.0	30.6	28.0
CV %	18.3	9.57	15.6	19.3	9.40	18.3

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 ____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION ____YES ____ 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 ____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION ____YES ____ 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: 18.3 (TQP3B)

10. Lethality for this test: 27 % (51710 or 51710P)

11. Sublethality for this test: 27 % (51710 or 51710Q)

AMERICAN INTERPLEX CORPORATION LABORATORIES

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, 356

Test Initiated: DATE: March 9, 2021 TIME: 1115
Test Terminated: DATE: March 15, 2021 TIME: 1310

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.7	7.7	8.0	7.8	7.6	6.9
	Final	7.8	7.8	8.4	7.7	7.2	7.2	--
pH	Initial	7.9	7.4	7.7	7.8	7.8	7.8	7.7
	Final	8.2	8.2	8.1	8.1	7.9	7.9	--

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.6	7.7	8.0	7.7	7.4	7.0
	Final	7.8	8.0	8.5	7.7	6.9	7.3	--
pH	Initial	7.8	7.8	7.7	7.7	7.6	7.6	7.5
	Final	8.2	8.2	8.2	8.0	7.9	7.9	--

DILUTION 11 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.6	7.5	8.3	7.8	7.5	7.2
	Final	8.0	8.0	8.6	7.8	7.5	7.3	--
pH	Initial	7.7	7.7	7.6	7.6	7.6	7.6	7.8
	Final	8.2	8.3	8.2	8.1	7.8	7.9	--

DILUTION 15 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.6	7.4	8.1	7.6	7.6	7.0
	Final	7.7	7.7	8.4	7.7	6.8	7.0	--
pH	Initial	7.6	7.6	7.6	7.6	7.6	7.6	7.6
	Final	8.1	8.2	8.3	8.1	7.9	7.6	--

DILUTION 20 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.6	7.5	8.2	7.9	7.6	7.1
	Final	7.6	7.8	8.4	7.9	7.3	7.1	--
pH	Initial	7.6	7.6	7.6	7.5	7.5	7.5	7.5
	Final	8.2	8.2	8.3	8.1	7.9	7.9	--

DILUTION 27 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	7.5	7.8	8.2	7.7	7.5	7.0
	Final	7.8	7.9	8.6	7.8	7.4	7.1	--
pH	Initial	7.5	7.5	7.5	7.5	7.5	7.5	7.5
	Final	8.2	8.2	8.3	8.1	7.9	7.9	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
20	30	230	0.050	EFF 08-MAR-21
34	27	230	<0.05	EFF 10-MAR-21
34	34	240	0.050	EFF 12-MAR-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	43	180	<0.05	253379-1

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: SEARCY WATER UTILITIES
Project Reference: BIO-MONITORING
Project Manager: JIMMY SMITH
Sampled By: BRADY ANDERSON

PO No.

SAMPLE MATRIX

NO OF BOTTLES

ANALYSES REQUESTED

AIC CONTROL NO: 253467
AIC PROPOSAL NO:
Carrier: J SMITH
Received Temperature C: 0.2

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES	Bio-Monitor	Remarks
1	EFF	3-8-21 7:45am		J	J		2		
		Container Type						P	Field pH calibration
		Preservative						NO	on _____ @ _____ Buffer:

G = Glass P = Plastic V = VOA vials H = HCl to pH2 T = Sodium Thiosulfate
NO = none S = Sulfuric acid pH2 N = Nitric acid pH2 B = NaOH to pH12 Z = Zinc acetate

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone: Fax
Report Attention to:
Report Address to:

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	3/8/21 10:07am		
Relinquished By:	Date/Time	Received in Lab By: D. BROWN	Date/Time 3-8-21 1007

Comments:

5/01

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Searcy Water Utilities
Project Reference: Bio-Monitoring
Project Manager: Jimmy Smith
Sampled By: Brady Anderson

PO No.

SAMPLE MATRIX

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Bio-Monitor
2	EFF	3-10-21 7:45am	✓	✓	✓		2	2
		Container Type						P
		Preservative						No

AIC CONTROL NO: 253467
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 0.5
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass, NO = none; P = Plastic, 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)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone: Fax
Report Attention to:
Report Address to:

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By:	Date/Time 3-10-21 10:38am	Received in Lab By: D. BROWN	Date/Time 3-10-21 1038

Comments:

5/01

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Scarcy Water Utilities
Project Reference: Bio-Monitoring
Project Manager: Jimmy Smith
Sampled By: Brady Anderson

PO No.

AIC CONTROL NO: 253467
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 0.4,

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SAMPLE MATRIX: SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Bio-Monitor	ANALYSES REQUESTED: N. Pond	ANALYSES REQUESTED: S. Pond
3	EFF	3-12-21 7:45am		✓	✓			2		
	N. Pond	3-11-21 1:15pm	✓						1	
	S. Pond	3-11-21 1pm	✓							1
	S. Pond	3-11-21 1pm	✓							1
	N. Pond	3-11-21 1:15pm	✓						1	
		Container Type						P	G	G
		Preservative						NO	No	No

Remarks

Field pH calibration on _____ @ _____ Buffer:

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z = Zinc acetate

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions:
Phone: Fax
Report Attention to:
Report Address to:

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By: B. Anderson	Date/Time 3-12-21 10:13	Received in Lab By:	Date/Time 3-12-21 1013

Comments:

5/01

FORM 0060