October 21, 2022

Biomonitoring Testing
for
Outfall 001

Control No. 269527-1

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*
Outfall 001
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 15 % effluent, which is below the critical dilution of 20 %. **The sample PASSED lethal effects, however, FAILED 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 percent minimum significant difference (PMSD) was below the limit of 13. Following additional calculations provided in the EPA document "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination Systems Program", the NOEC for sublethal effects was calculated to be 27 %. **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
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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	0.448	PASS
Control Growth CV < or = 40%	7.56	PASS
Growth Minimum Significant Difference 12 to 30%	13.9	PASS
Critical Dilution CV < or = 40%	8.47	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	31.0	PASS
Control CV < or = 40% per Surviving Female	10.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	10.8	BELOW
Critical Dilution CV < or = 40%	10.7	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: Outfall 001
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.0	7.1	8.1
pH (standard units)	7.2	7.0	6.8
Alkalinity (mg/l as CaCO3)	41	35	52
Hardness (mg/l as CaCO3)	40	44	41
Conductivity (umhos/cm)	410	380	380
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.25	0.21	0.26

2. Dilution Water Samples:
   Soft

Analysis	269253-1	269314-1
Dissolved oxygen (mg/l)	7.8	8.0
pH (standard units)	6.8	7.0
Alkalinity (mg/l as CaCO3)	32	32
Hardness (mg/l as CaCO3)	45	41
Conductivity (umhos/cm)	160	160
Residual Chlorine (mg/l)	<0.05	<0.05

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

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	October 11, 2022 at 1020
Date & Time Test Terminated:	October 18, 2022 at 0958
Type & Volume of Test Chamber:	500 ml disposable beaker
Volume of Sample:	250 ml
Number of Organisms per replicate:	8
Number of Replicates per dilution:	5

Ceriodaphnia dubia Survival and Reproduction Method 1002.0

Date & Time Test Initiated:	October 11, 2022 at 0945
Date & Time Test Terminated:	October 18, 2022 at 1136
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 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 September 06, 2022 at 1216 to September 13, 2022 at 1223
The results were as follows: (Control No. 268605-1.)

Survival LC-50: 3046.4 mg/l
Growth IC-25: 1754 mg/l
Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on September 06, 2022 at 1135 to September 13, 2022 at 1305
The results were as follows: (Control No. 268605-2.)

Survival LC-50: 1673.7 mg/l
Reproduction IC-25: 1065 mg/l
Reproduction PMSD: 10.6

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 11, 2022
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 11, 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

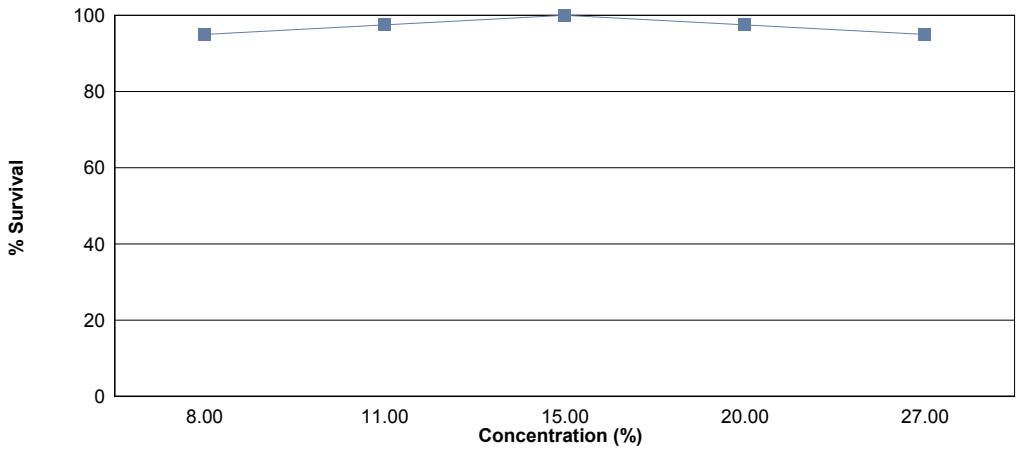
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 October 11, 2022 at 1020 and continued through October 18, 2022 at 0958. 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 = 15 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.437
8 %	95.0	0.446
11 %	97.5	0.439
15 %	100	0.393
20 %	97.5	0.347 *
27 %	95.0	0.323 *

*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.

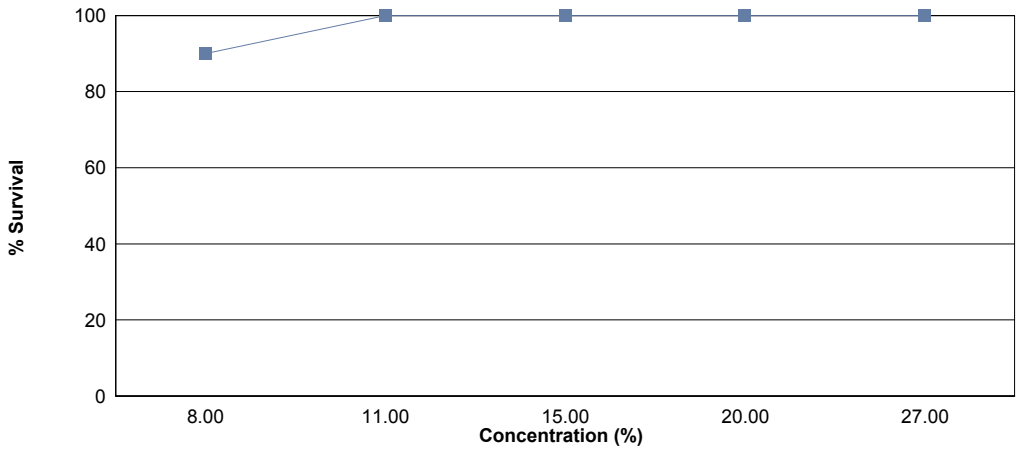
Effluent dilutions for this test were 8 %, 11 %, 15 %, 20 %, 27 % in accordance with the NPDES permit.

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

The test was initiated on October 11, 2022 at 0945 and continued through October 18, 2022 at 1136. 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

(NOEC for sublethal effects was determined by Lower PMSD Bound Test.)



Summary of the 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	31.0
8 %	90.0	29.5
11 %	100	29.6
15 %	100	29.2
20 %	100	29.5
27 %	100	28.6

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 11, 2022 at 1020
Date and Time Test Terminated: October 18, 2022 at 0958

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: October 11, 2022 at 1020
Test Terminated: October 18, 2022 at 0958

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.77091	.77445	0.00354	8	0.442
	B	.76075	.76388	0.00313	8	0.391
	C	.76724	.77061	0.00337	8	0.421
	D	.76921	.77304	0.00383	8	0.479
	E	.77382	.77743	0.00361	8	0.451
8 %	A	.77069	.77410	0.00341	8	0.426
	B	.76345	.76692	0.00347	8	0.434
	C	.76943	.77294	0.00351	8	0.439
	D	.76637	.77015	0.00378	8	0.472
	E	.77188	.77557	0.00369	8	0.461
11 %	A	.76561	.76915	0.00354	8	0.442
	B	.76932	.77276	0.00344	8	0.430
	C	.76588	.76879	0.00291	8	0.364
	D	.76050	.76424	0.00374	8	0.468
	E	.77162	.77555	0.00393	8	0.491
15 %	A	.76282	.76615	0.00333	8	0.416
	B	.77362	.77646	0.00284	8	0.355
	C	.76617	.76903	0.00286	8	0.358
	D	.76815	.77114	0.00299	8	0.374
	E	.77049	.77418	0.00369	8	0.461
20 %	A	.76118	.76378	0.00260	8	0.325
	B	.76050	.76358	0.00308	8	0.385
	C	.76011	.76262	0.00251	8	0.314
	D	.76199	.76493	0.00294	8	0.368
	E	.76736	.77012	0.00276	8	0.345
27 %	A	.77001	.77229	0.00228	8	0.285
	B	.77316	.77574	0.00258	8	0.322
	C	.77044	.77312	0.00268	8	0.335
	D	.77211	.77421	0.00210	8	0.262
	E	.77208	.77536	0.00328	8	0.410

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 11, 2022 at 0945
Date and Time Test Terminated: October 18, 2022 at 1136

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	5	3	4	5	4	5	4	5	5	4	44	10	4.40
5	12	9	11	11	13	12	11	12	10	10	111	10	11.1
6	0	0	0	0	0	0	14	0	16	0	30	10	3.00
7	15	13	19	18	14	15	0	18	0	13	125	10	12.5
8													
TOTAL	32	25	34	34	31	32	29	35	31	27	310	10	31.0

Concentration: 8 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	3	4	5	3	4	5	6	4	4	3	41	10	4.10
5	12	12	10	0	11	10	10	8	13	10	96	10	9.60
6	0	0	0	10	0	0	0	0	15	0	25	10	2.50
7	15	16	7X	14	16	16	17	15	0	17	133	9	14.8
8													
TOTAL	30	32	22	27	31	31	33	27	32	30	295	10	29.5

Concentration: 11 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	5	0	5	10	0.500
4	4	4	4	5	5	4	3	4	0	3	36	10	3.60
5	11	13	11	10	10	9	10	9	10	10	103	10	10.3
6	0	0	0	0	0	0	0	0	17	0	17	10	1.70
7	19	15	14	15	14	14	19	15	0	10	135	10	13.5
8													
TOTAL	34	32	29	30	29	27	32	28	32	23	296	10	29.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 11, 2022 at 0945
Date and Time Test Terminated: October 18, 2022 at 1136

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	3	5	3	6	4	5	3	3	4	40	10	4.00
5	11	12	10	9	10	12	10	7	10	0	91	10	9.10
6	0	0	0	0	0	0	0	0	0	10	10	10	1.00
7	17	14	18	17	17	13	13	15	13	14	151	10	15.1
8													
TOTAL	32	29	33	29	33	29	28	25	26	28	292	10	29.2

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	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	5	4	4	6	3	6	4	3	4	43	10	4.30
5	12	9	8	11	12	9	11	10	8	9	99	10	9.90
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	16	16	17	12	17	16	16	15	17	11	153	10	15.3
8													
TOTAL	32	30	29	27	35	28	33	29	28	24	295	10	29.5

Concentration: 27 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	5	4	5	5	3	5	4	3	3	41	10	4.10
5	10	11	10	9	11	11	10	11	10	7	100	10	10.0
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	14	15	18	14	15	15	15	17	12	10	145	10	14.5
8													
TOTAL	28	31	32	28	31	29	30	32	25	20	286	10	28.6

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

		Transformation of Data		Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	0.87500	1.20940
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	8 %	1	0.87500	1.20940
2	8 %	2	0.87500	1.20940
2	8 %	3	1.00000	1.39310
2	8 %	4	1.00000	1.39310
2	8 %	5	1.00000	1.39310
3	11 %	1	1.00000	1.39310
3	11 %	2	1.00000	1.39310
3	11 %	3	0.87500	1.20940
3	11 %	4	1.00000	1.39310
3	11 %	5	1.00000	1.39310
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	1.00000	1.39310
4	15 %	4	1.00000	1.39310
4	15 %	5	1.00000	1.39310
5	20 %	1	1.00000	1.39310
5	20 %	2	1.00000	1.39310
5	20 %	3	0.87500	1.20940
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	0.87500	1.20940
6	27 %	5	0.87500	1.20940

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality	Transform: Arc Sin(Square Root(Y))
D = 0.162	
W = 0.7738	
Critical W = 0.9	(alpha = 0.01, N = 30)
Critical W = 0.927	(alpha = 0.05, N = 30)
Data FAIL normality test (alpha = 0.01).	

Steel's Many-One Rank Test					Transform: Arc Sin(Square Root(Y))
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	8 %	25.00	16.00	5.00	
3	11 %	27.50	16.00	5.00	
4	15 %	30.00	16.00	5.00	
5	20 %	27.50	16.00	5.00	
6	27 %	25.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.03963		
W = 0.9914		
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		No Transformation
Calculated B1 statistic = 4.848		
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) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.06885	0.01377	8.34
Within (Error)	24	0.03963	0.001651	
Total	29	0.1085		

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	0.4368	0.4368		
2	8 %	0.4464	0.4464	-0.3736	
3	11 %	0.439	0.439	-0.08561	
4	15 %	0.3928	0.3928	1.712	
5	20 %	0.3474	0.3474	3.479	*
6	27 %	0.3228	0.3228	4.436	*

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	0.06065	13.9	-0.0096
3	11 %	5	0.06065	13.9	-0.0022
4	15 %	5	0.06065	13.9	0.044
5	20 %	5	0.06065	13.9	0.0894
6	27 %	5	0.06065	13.9	0.114

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
8 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

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	1	
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 = 0.1156		
D* = 0.907		
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 = 0.8182		
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	31.33	6.267	0.6009
Within (Error)	54	563.4	10.43	
Total	59	594.7		

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	31	31		
2	8 %	29.5	29.5	1.039	
3	11 %	29.6	29.6	0.9693	
4	15 %	29.2	29.2	1.246	
5	20 %	29.5	29.5	1.039	
6	27 %	28.6	28.6	1.662	

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	3.336	10.8	1.5
3	11 %	10	3.336	10.8	1.4
4	15 %	10	3.336	10.8	1.8
5	20 %	10	3.336	10.8	1.5
6	27 %	10	3.336	10.8	2.4

Lower PMSD Bound Test for Ceriodaphnia dubia

Concentration	Reproduction	Relative Difference from Control	Pass/Fail
Control	31.0	-	
8 %	29.5	4.84	PASS
11 %	29.6	4.52	PASS
15 %	29.2	5.81	PASS
20 %	29.5	4.84	PASS
27 %	28.6	7.74	PASS

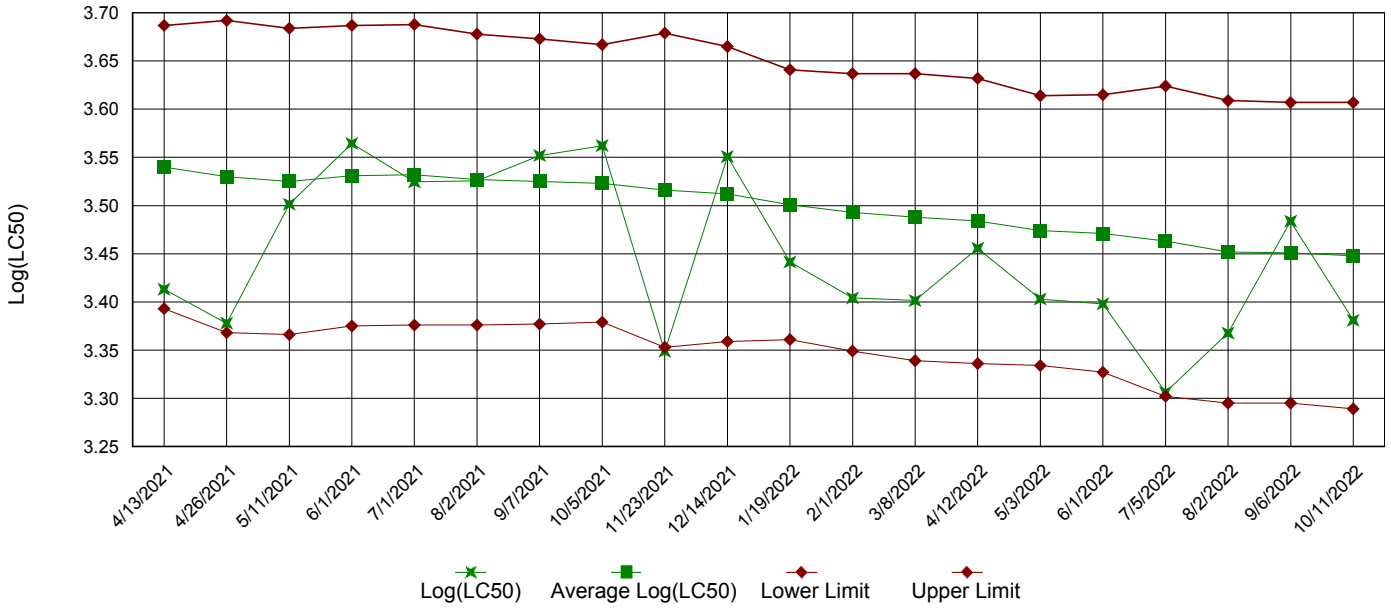
Limit = 13

NOEC = 27 %
LOEC = 27 %

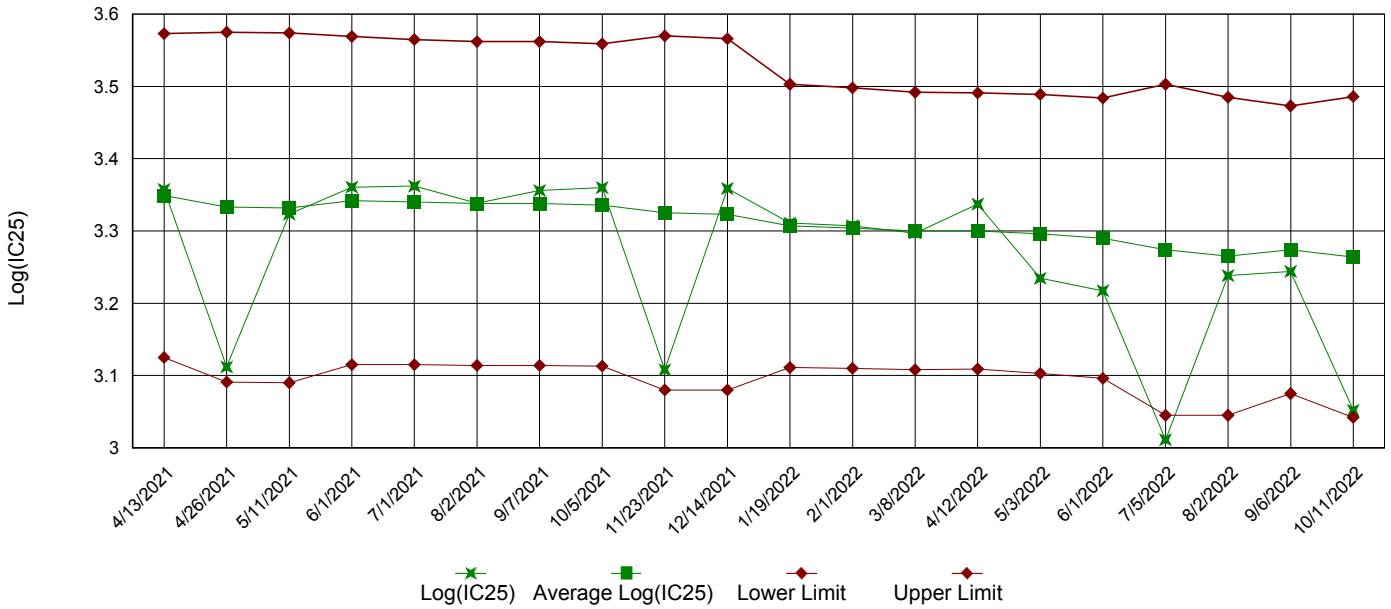
Appendix A3: Test 1000.0

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

LC50 Survival Data



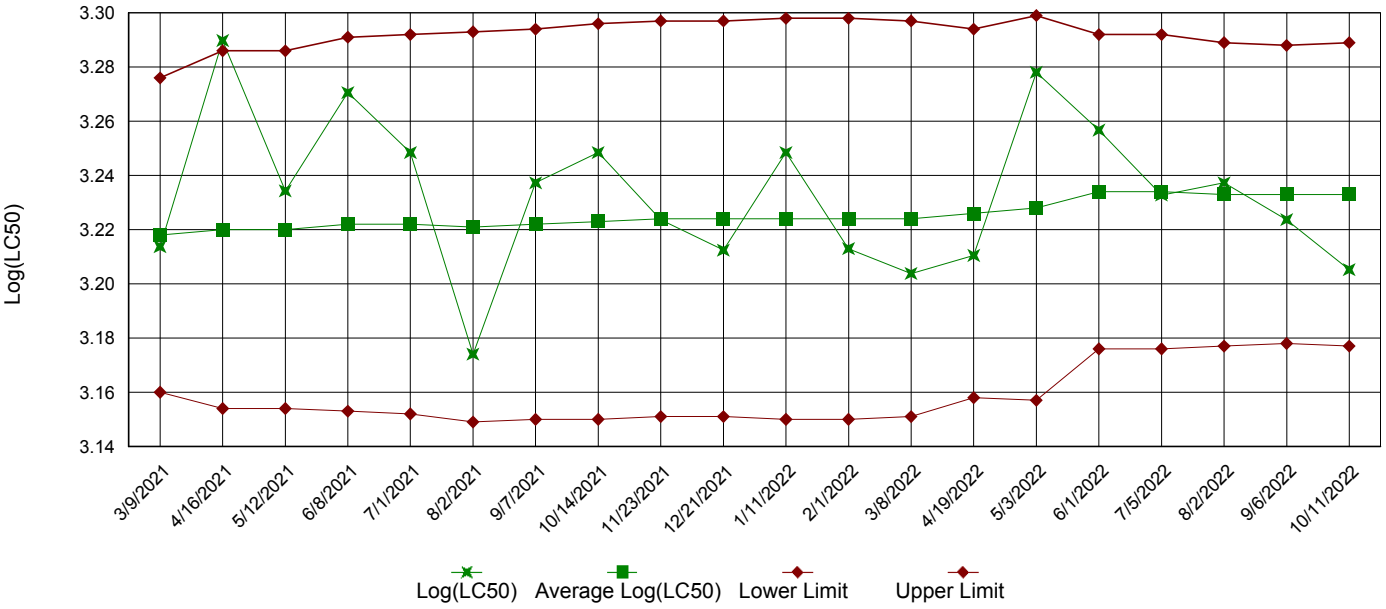
IC25 Growth Data



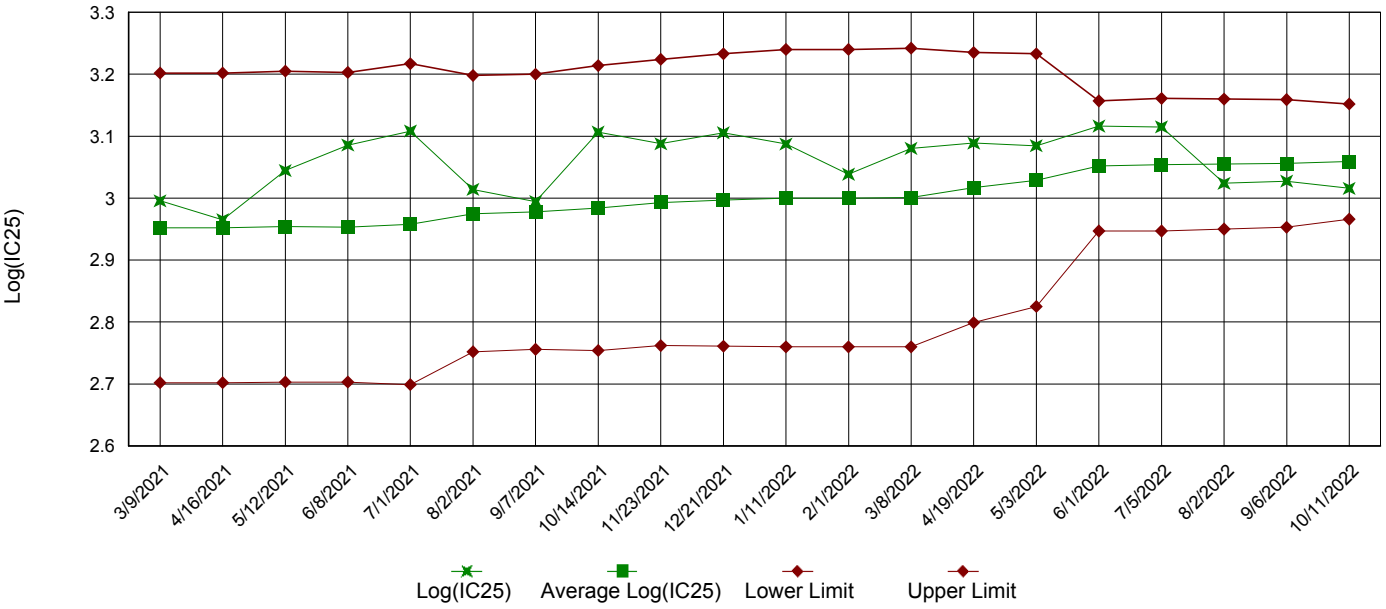
Appendix A3: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0

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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: October 11, 2022 at 1020
Date and Time Test Terminated: October 18, 2022 at 0958
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	100	100	87.5	100	100	100	100	97.5	5.73
8 %	87.5	87.5	100	100	100	100	100	95.0	7.21
11 %	100	100	87.5	100	100	97.5	97.5	97.5	5.73
15 %	100	100	100	100	100	100	100	100	0.00
20 %	100	100	87.5	100	100	100	100	97.5	5.73
27 %	100	100	100	87.5	87.5	100	100	95.0	7.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.442	0.391	0.421	0.479	0.451	0.437	7.56
8 %	0.426	0.434	0.439	0.472	0.461	0.446	4.33
11 %	0.442	0.430	0.364	0.468	0.491	0.439	11.0
15 %	0.416	0.355	0.358	0.374	0.461	0.393	11.5
20 %	0.325	0.385	0.314	0.368	0.345	0.347	8.47
27 %	0.285	0.322	0.335	0.262	0.410	0.323	17.6

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

Appendix B: Test 1000.0

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

1. Steel's Many-One Rank Test:

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

   a.) LOW FLOW OR CRITICAL DILUTION ____YES ___X___ NO
   b.) 1/2 LOW FLOW DILUTION ____YES ____ 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 ___X___YES ____ 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]: 1 (TGP6C)

5. NOEC Pimephales Lethality: 27 % (TOP6C)

6. LOEC Pimephales Lethality: 27 % (TXP6C)

7. NOEC Pimephales Sublethality: 15 % (TPP6C)

8. LOEC Pimephales Sublethality: 20 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 8.47 (TQP6C)

10. Sublethality for this test: 15 % (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: October 11, 2022 TIME: 1020
Test Terminated: DATE: October 18, 2022 TIME: 0958

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	8.1	8.0	7.9	7.8	8.0	8.6
	Final	7.2	7.6	7.1	6.9	7.1	7.8	7.7
pH	Initial	6.8	7.1	7.0	7.3	6.9	7.0	6.7
	Final	7.3	7.5	7.3	7.3	7.4	7.4	6.4

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	8.0	8.1	8.1	7.9	7.8	8.5
	Final	6.8	7.2	6.6	7.2	6.0	6.4	7.4
pH	Initial	7.0	7.2	7.0	7.4	6.9	6.9	6.9
	Final	7.3	7.4	7.1	7.5	7.2	7.0	6.5

DILUTION 11 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.8	8.0	8.0	7.7	7.7	8.4
	Final	6.8	7.1	6.7	6.6	5.9	7.2	7.5
pH	Initial	7.5	7.3	7.1	7.5	6.8	7.0	6.9
	Final	7.4	7.4	7.2	7.2	7.2	7.2	6.6

DILUTION 15 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	7.7	7.9	7.6	7.8	7.6	8.2
	Final	6.6	7.0	6.4	6.6	6.1	7.4	7.8
pH	Initial	7.4	7.4	7.2	7.5	6.9	7.0	7.0
	Final	7.3	7.5	7.2	7.3	7.2	7.3	6.7

DILUTION 20 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.9	7.9	7.9	7.8	7.8	8.3
	Final	6.8	7.2	6.6	6.3	5.9	6.4	7.6
pH	Initial	7.4	7.4	7.3	7.5	7.0	7.0	7.2
	Final	7.3	7.4	7.2	7.2	7.2	7.1	6.7

DILUTION 27 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	7.4	7.9	7.7	7.8	7.6	8.2
	Final	6.7	7.1	6.4	7.0	5.7	6.2	7.4
pH	Initial	7.4	7.5	7.4	7.5	7.0	7.1	7.3
	Final	7.3	7.4	7.2	7.5	7.2	7.1	6.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
41	40	410	<0.05	EFF 10-OCT-22
35	44	380	<0.05	EFF 12-OCT-22
52	41	380	<0.05	EFF 14-OCT-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	45	160	<0.05	269253-1
32	41	160	<0.05	269314-1

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: October 11, 2022 at 0945
Date and Time Test Terminated: October 18, 2022 at 1136
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
7 day	100	90.0	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	8 %	11 %	15 %	20 %	27 %
A	32	30	34	32	32	28
B	25	32	32	29	30	31
C	34	22	29	33	29	32
D	34	27	30	29	27	28
E	31	31	29	33	35	31
F	32	31	27	29	28	29
G	29	33	32	28	33	30
H	35	27	28	25	29	32
I	31	32	32	26	28	25
J	27	30	23	28	24	20
Mean per Adult	31.0	29.5	29.6	29.2	29.5	28.6
Mean per Surviving Adult	31.0	30.3	29.6	29.2	29.5	28.6
CV %	10.3	6.99	10.7	9.39	10.7	13.0

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 _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ 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 _____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: 10.7 (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, 343, 357, 358

Test Initiated: DATE: October 11, 2022 TIME: 0945
Test Terminated: DATE: October 18, 2022 TIME: 1136

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	8.1	8.0	7.9	7.8	8.0	8.6
	Final	7.8	8.1	8.0	7.8	8.1	8.2	8.2
pH	Initial	6.8	7.1	7.0	7.3	6.9	7.0	6.7
	Final	8.1	8.3	8.2	5.5	5.0	7.4	7.3

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.9	8.0	8.1	8.1	7.9	7.8	8.5
	Final	8.0	8.2	8.1	7.8	8.1	8.3	8.2
pH	Initial	7.0	7.2	7.0	7.4	6.9	6.9	6.9
	Final	8.3	8.4	8.2	7.5	7.5	7.5	7.6

DILUTION 11 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.5	7.8	8.0	8.0	7.7	7.7	8.4
	Final	7.8	8.1	8.1	7.6	8.0	8.0	8.1
pH	Initial	7.5	7.3	7.1	7.5	6.8	7.0	6.9
	Final	8.3	8.4	8.2	7.6	7.6	7.7	7.6

DILUTION 15 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	7.7	7.9	7.6	7.8	7.6	8.2
	Final	7.8	8.1	8.1	7.9	7.8	8.3	8.0
pH	Initial	7.4	7.4	7.2	7.5	6.9	7.0	7.0
	Final	8.2	8.3	8.2	7.6	7.7	7.6	7.5

DILUTION 20 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	7.9	7.9	7.9	7.8	7.8	8.3
	Final	8.0	8.3	8.1	7.8	8.0	8.3	8.2
pH	Initial	7.4	7.4	7.3	7.5	7.0	7.0	7.2
	Final	8.3	8.4	8.3	7.6	7.7	7.8	7.7

DILUTION 27 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	7.4	7.9	7.7	7.8	7.6	8.2
	Final	7.8	8.0	8.1	7.8	8.0	8.2	8.0
pH	Initial	7.4	7.5	7.4	7.5	7.0	7.1	7.3
	Final	8.3	8.4	8.3	7.7	7.8	7.9	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
41	40	410	<0.05	EFF 10-OCT-22
35	44	380	<0.05	EFF 12-OCT-22
52	41	380	<0.05	EFF 14-OCT-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	45	160	<0.05	269253-1
32	41	160	<0.05	269314-1

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: SEARCY WATER UTILITIES
Project Reference: BIO-MONITOR
Project Manager: JIMMY SMITH
Sampled By: JEREMY CHEELY

PO No.

NO OF BOTTLES

ANALYSES REQUESTED

AIC CONTROL NO: 269527
AIC PROPOSAL NO:
Carrier:
Received on ice? Yes / No Temp. 0.1 °C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	Bio-Monitor	Remarks
1	EFF	10-10-22 8AM		X	X			1	
		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 A=$(NH_4)_2SO_4$, NH_4OH

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

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By:	Date/Time 10-10-22 10:22AM	Received in Lab By: D. Brown	Date/Time 10.10.22 10:22AM

Comments:

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: SEARCY WATER Utilities
Project Reference: BIO-MONITOR
Project Manager: JIMMY SMITH
Sampled By: JEREMY CLEELY

PO No.

NO OF BOTTLES

MATRIX

ANALYSES REQUESTED

AIC CONTROL NO: 269527
AIC PROPOSAL NO:
Carrier:
Received on ice? Yes No Temp. 0.5 °C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		NO OF BOTTLES	BIO-Monitor	Remarks
2	EFF	10-12-22 8AM		/	/			1	1	
		Container Type							P	Field pH calibration
		Preservative							ND	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 A=$(NH_4)_2SO_4$, NH_4OH

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

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By: Jimmy Smith	Date/Time 10/12/22 9:30 AM	Received in Lab By: D. BROWN	Date/Time 10.12.22 0934AM

Comments:

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: Jeremy Cheely

PO No.

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	Bio-Monitor	CL	Phenolics	HG	Metals
3	EFF	10-13-22 8AM 2PM 10-14-22 8PM 2AM		X	X			1				
	EFF	10-13-22 8AM 2PM 10-14-22 8AM 2AM	X		X				4			
	EFF	10-13-22 8A 2P 10-14-22 8P 2A	X		X					4		
	EFF	10-13-22 8A 2P 10-14-22 8P 2A	X		X						4	
	EFF	10-14-22 8AM		X	X							1
		Container Type						P	P	G	G	P
		Preservative						NO	B	S	NO	N

ANALYSES REQUESTED

AIC CONTROL NO: 269527
AIC PROPOSAL NO:
Carrier:
Received on ice? Yes No Temp. 3.2 °C 1.8
Remarks

AIC 269685

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 A=$(NH_4)_2SO_4$, NH_4OH

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

Relinquished By:	Date/Time	Received By:	Date/Time
Relinquished By:	Date/Time 9:44 AM 10-14-22	Received in Lab By:	Date/Time 10-14-22 0944

Comments:

FORM 0060