September 1, 2021

Biomonitoring Testing
for
EFF

Control No. 258068-1

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 percent minimum significant difference (PMSD) was below the limit of 12. 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 Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 27 % effluent, which is above the critical dilution of 20 %. The NOEC for reproduction occurred at 27 % effluent, which is above the sub-lethal limit of 20 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Chief Operating Officer

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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.579	PASS
Control Growth CV < or = 40%	12.8	PASS
Growth Minimum Significant Difference 12 to 30%	11.6	BELOW
Critical Dilution CV < or = 40%	7.68	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	28.6	PASS
Control CV < or = 40% per Surviving Female	9.64	PASS
Reproduction Minimum Significant Difference 13 to 47%	22.9	PASS
Critical Dilution CV < or = 40%	25.8	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0021601 AFIN# 73-00055
2. Test Requirements: Chronic Biomonitoring, Quarterly
   Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
   a. Sampling Point: EFF
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.1	7.6	7.2
pH (standard units)	7.3	7.2	7.4
Alkalinity (mg/l as CaCO3)	39	45	48
Hardness (mg/l as CaCO3)	34	34	37
Conductivity (umhos/cm)	360	380	400
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.10	<0.1	0.14

2. Dilution Water Samples:
   Soft

Analysis	257863-1
Dissolved oxygen (mg/l)	6.8
pH (standard units)	7.4
Alkalinity (mg/l as CaCO3)	32
Hardness (mg/l as CaCO3)	42
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:	August 24, 2021 at 1009
Date & Time Test Terminated:	August 31, 2021 at 1040
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:	August 24, 2021 at 1100
Date & Time Test Terminated:	August 31, 2021 at 0935
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 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 August 02, 2021 at 1600 to August 09, 2021 at 1430
The results were as follows: (Control No. 257498-1.)

Survival LC-50: 3355 mg/l
Growth IC-25: 2180 mg/l
Growth PMSD: 19.8

Ceriodaphnia dubia

A chronic reference test was performed on August 02, 2021 at 1425 to August 09, 2021 at 1315
The results were as follows: (Control No. 257498-2.)

Survival LC-50: 1492.9 mg/l
Reproduction IC-25: 1033 mg/l
Reproduction PMSD: 19.9

V. Organism History

Pimephales promelas (Fathead minnow)

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

Ceriodaphnia dubia

Date: August 24, 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.

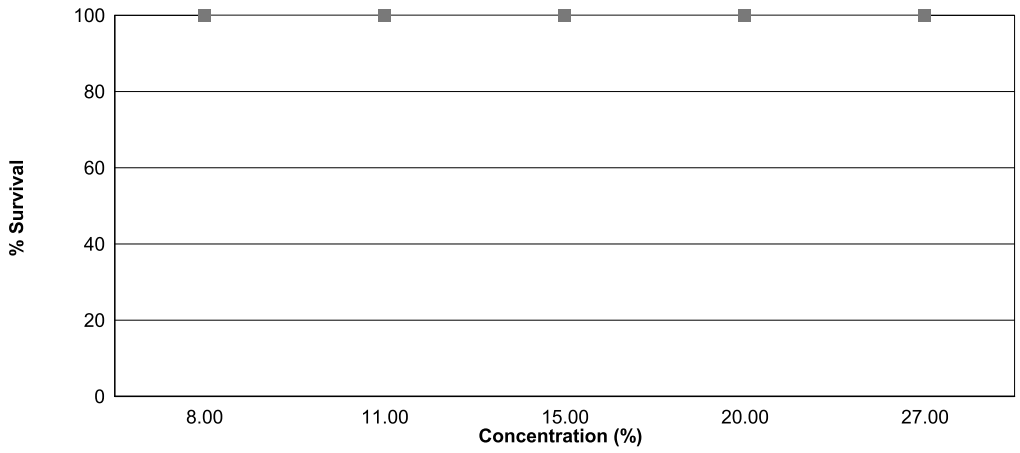
Effluent dilutions for this test were 8 %, 11 %, 15 %, 20 %, 27 % in accordance with the NPDES permit.

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

The test was initiated on August 24, 2021 at 1009 and continued through August 31, 2021 at 1040. 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

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



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.579
8 %	100	0.567
11 %	100	0.554
15 %	100	0.540
20 %	100	0.584
27 %	100	0.625

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

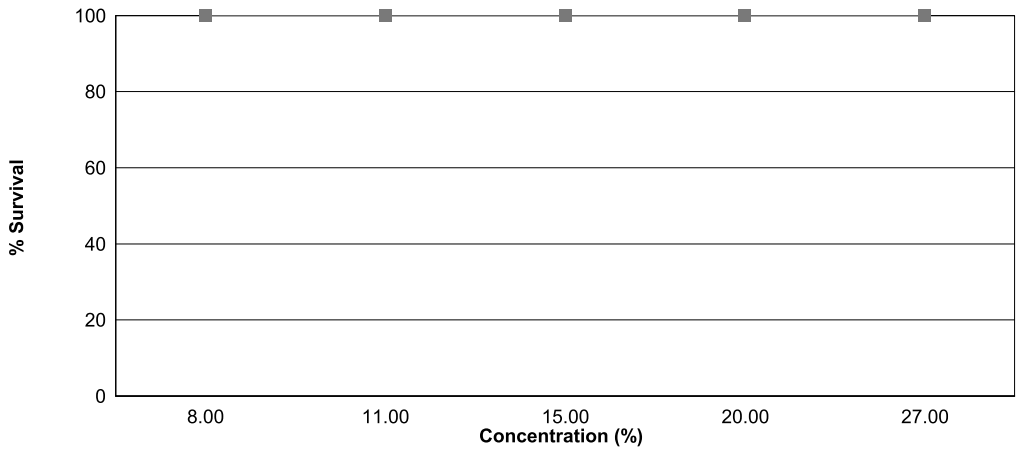
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 August 24, 2021 at 1100 and continued through August 31, 2021 at 0935. 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 7-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	28.6
8 %	100	30.3
11 %	100	29.8
15 %	100	25.0
20 %	100	26.1
27 %	100	27.6

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 24, 2021 at 1009
Date and Time Test Terminated: August 31, 2021 at 1040

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	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	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
15 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	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	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: August 24, 2021 at 1009
Test Terminated: August 31, 2021 at 1040

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.64768	.65222	0.00454	8	0.568
	B	.65061	.65560	0.00499	8	0.624
	C	.66040	.66415	0.00375	8	0.469
	D	.65805	.66338	0.00533	8	0.666
	E	.65764	.66218	0.00454	8	0.568
8 %	A	.66187	.66677	0.00490	8	0.612
	B	.65389	.65819	0.00430	8	0.538
	C	.65117	.65540	0.00423	8	0.529
	D	.64921	.65392	0.00471	8	0.589
	E	.64929	.65383	0.00454	8	0.568
11 %	A	.65114	.65556	0.00442	8	0.552
	B	.64370	.64804	0.00434	8	0.542
	C	.66085	.66506	0.00421	8	0.526
	D	.66013	.66446	0.00433	8	0.541
	E	.64583	.65069	0.00486	8	0.608
15 %	A	.64369	.64813	0.00444	8	0.555
	B	.67219	.67640	0.00421	8	0.526
	C	.66244	.66678	0.00434	8	0.542
	D	.66411	.66856	0.00445	8	0.556
	E	.65869	.66287	0.00418	8	0.522
20 %	A	.64843	.65320	0.00477	8	0.596
	B	.66193	.66673	0.00480	8	0.600
	C	.64824	.65292	0.00468	8	0.585
	D	.65776	.66183	0.00407	8	0.509
	E	.66100	.66603	0.00503	8	0.629
27 %	A	.66045	.66565	0.00520	8	0.650
	B	.65269	.65702	0.00433	8	0.541
	C	.64069	.64587	0.00518	8	0.648
	D	.66761	.67286	0.00525	8	0.656
	E	.66824	.67326	0.00502	8	0.628

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 24, 2021 at 1100
Date and Time Test Terminated: August 31, 2021 at 0935

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	4	5	5	4	3	5	4	3	6	5	44	10	4.40
5	10	0	0	0	13	11	11	9	0	9	63	10	6.30
6	13	10	10	8	16	0	0	14	8	14	93	10	9.30
7	0	15	16	12	0	15	16	0	12	0	86	10	8.60
8													
TOTAL	27	30	31	24	32	31	31	26	26	28	286	10	28.6

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	6	6	4	3	3	3	5	5	5	43	10	4.30
5	9	0	0	0	11	11	12	13	0	11	67	10	6.70
6	14	9	10	11	16	18	10	17	10	0	115	10	11.5
7	0	16	17	18	0	0	0	0	15	12	78	10	7.80
8													
TOTAL	26	31	33	33	30	32	25	35	30	28	303	10	30.3

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	0	0	0	10	0.00
4	3	7	5	4	4	1	4	4	5	5	42	10	4.20
5	10	0	0	0	12	13	11	2	0	13	61	10	6.10
6	0	11	10	1	0	12	0	17	11	15	77	10	7.70
7	20	15	19	15	16	0	18	0	15	0	118	10	11.8
8													
TOTAL	33	33	34	20	32	26	33	23	31	33	298	10	29.8

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 24, 2021 at 1100
Date and Time Test Terminated: August 31, 2021 at 0935

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	0	0	5	6	4	0	0	6	3	0	24	10	2.40
5	11	0	0	0	12	3	4	13	0	5	48	10	4.80
6	15	7	11	0	16	19	0	17	3	0	88	10	8.80
7	0	14	19	6	0	0	21	0	14	16	90	10	9.00
8													
TOTAL	26	21	35	12	32	22	25	36	20	21	250	10	25.0

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	2	5	6	5	0	0	0	4	5	5	32	10	3.20
5	7	0	0	0	8	13	1	6	0	11	46	10	4.60
6	0	1	10	10	0	0	16	0	10	0	47	10	4.70
7	12	10	16	22	18	11	0	17	15	15	136	10	13.6
8													
TOTAL	21	16	32	37	26	24	17	27	30	31	261	10	26.1

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	0	6	6	4	5	0	0	0	5	1	27	10	2.70
5	12	0	0	0	13	7	3	3	0	2	40	10	4.00
6	0	12	11	10	0	0	17	13	13	0	76	10	7.60
7	19	17	21	22	17	17	0	0	14	6	133	10	13.3
8													
TOTAL	31	35	38	36	35	24	20	16	32	9	276	10	27.6

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	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	1.00000	1.39310
3	11 %	3	1.00000	1.39310
3	11 %	4	1.00000	1.39310
3	11 %	5	1.00000	1.39310
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	1.00000	1.39310
4	15 %	4	1.00000	1.39310
4	15 %	5	1.00000	1.39310
5	20 %	1	1.00000	1.39310
5	20 %	2	1.00000	1.39310
5	20 %	3	1.00000	1.39310
5	20 %	4	1.00000	1.39310
5	20 %	5	1.00000	1.39310
6	27 %	1	1.00000	1.39310
6	27 %	2	1.00000	1.39310
6	27 %	3	1.00000	1.39310
6	27 %	4	1.00000	1.39310
6	27 %	5	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		
W = 0		
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 %	27.50	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 %	27.50	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 = 0.04898		
W = 0.9541		
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 = 8.183		
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	0.02137	0.004275	2.095
Within (Error)	24	0.04898	0.002041	
Total	29	0.07036		

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	0.579	0.579		
2	8 %	0.5672	0.5672	0.413	
3	11 %	0.5538	0.5538	0.882	
4	15 %	0.5402	0.5402	1.358	
5	20 %	0.5838	0.5838	-0.168	
6	27 %	0.6246	0.6246	-1.596	

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	0.06743	11.6	0.0118
3	11 %	5	0.06743	11.6	0.0252
4	15 %	5	0.06743	11.6	0.0388
5	20 %	5	0.06743	11.6	-0.0048
6	27 %	5	0.06743	11.6	-0.0456

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 = 0.0811 D* = 0.6363 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 = 18.80 Critical B = 15.086 (alpha = 0.01, df = 5)	
Data FAIL B1 homogeneity test at 0.01 level.	

Steel's Many-One Rank Test					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	8 %	120.50	75.00	10.00	
3	11 %	124.00	75.00	10.00	
4	15 %	87.50	75.00	10.00	
5	20 %	93.50	75.00	10.00	
6	27 %	112.50	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	216	43.2	1.077
Within (Error)	54	2167	40.13	
Total	59	2383		

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	28.6	28.6		
2	8 %	30.3	30.3	-0.6001	
3	11 %	29.8	29.8	-0.4236	
4	15 %	25	25	1.271	
5	20 %	26.1	26.1	0.8825	
6	27 %	27.6	27.6	0.353	

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	6.544	22.9	-1.7
3	11 %	10	6.544	22.9	-1.2
4	15 %	10	6.544	22.9	3.6
5	20 %	10	6.544	22.9	2.5
6	27 %	10	6.544	22.9	1

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.579	-	
8 %	0.567	2.07	PASS
11 %	0.554	4.32	PASS
15 %	0.540	6.74	PASS
20 %	0.584	-0.864	PASS
27 %	0.625	-7.94	PASS

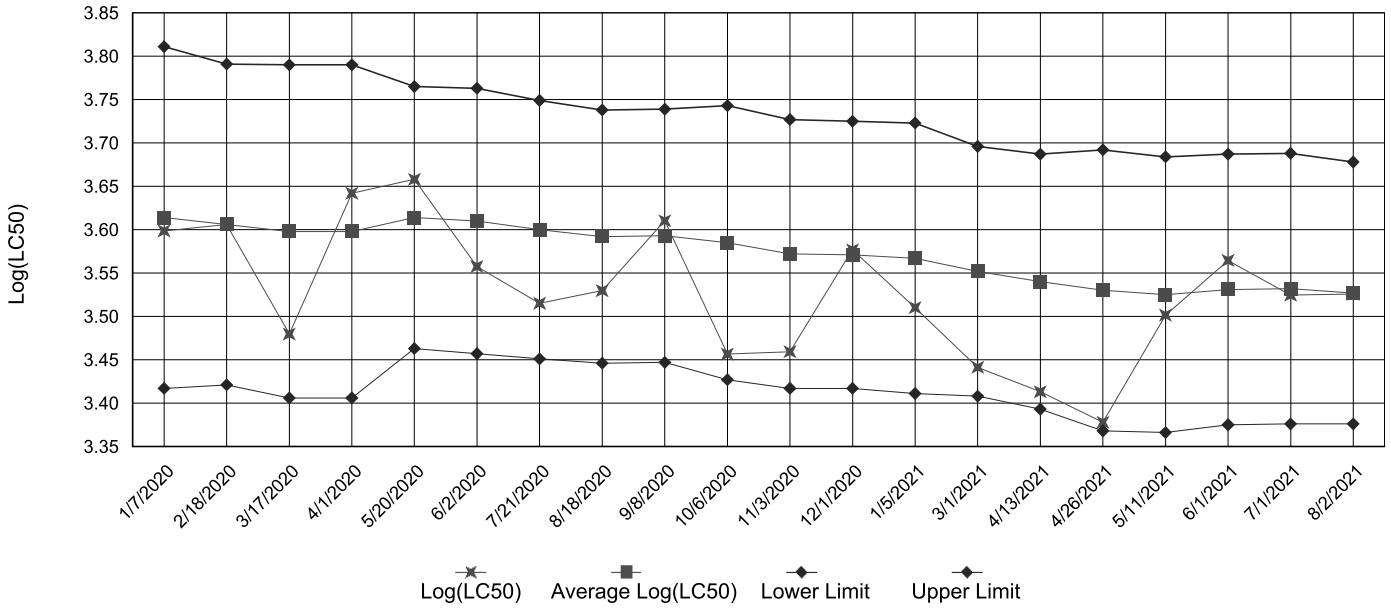
Limit = 12

NOEC = 27 %
LOEC = 27 %

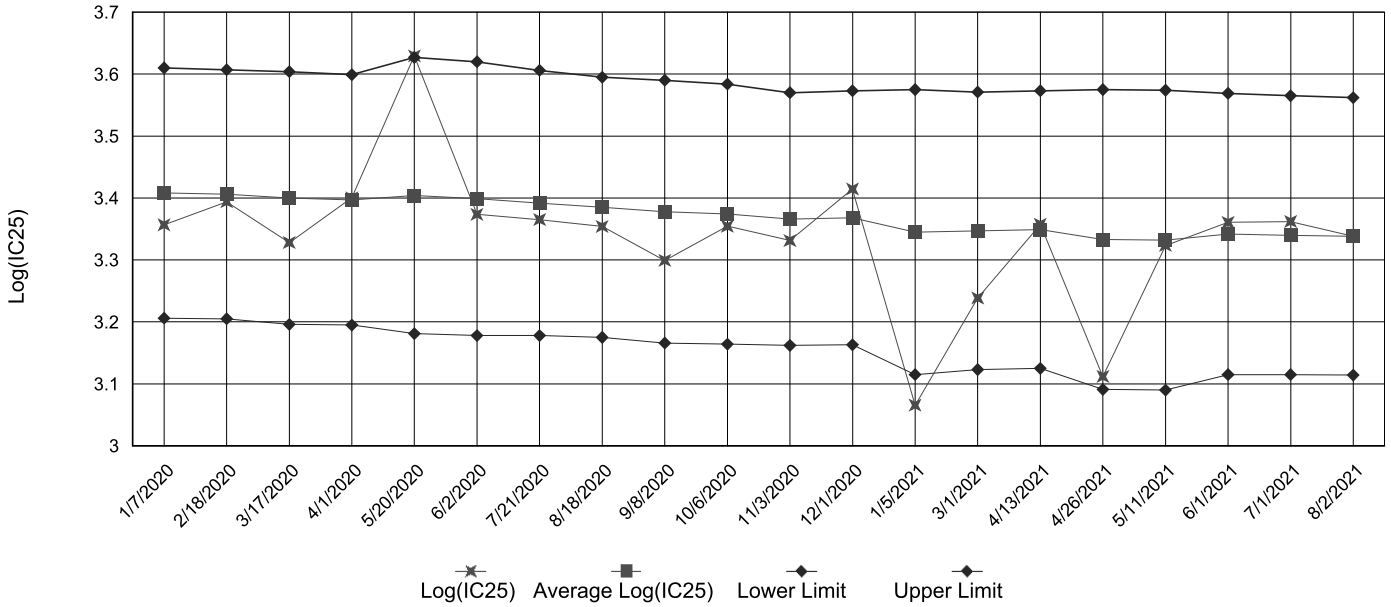
Appendix A3: Test 1000.0

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

LC50 Survival Data



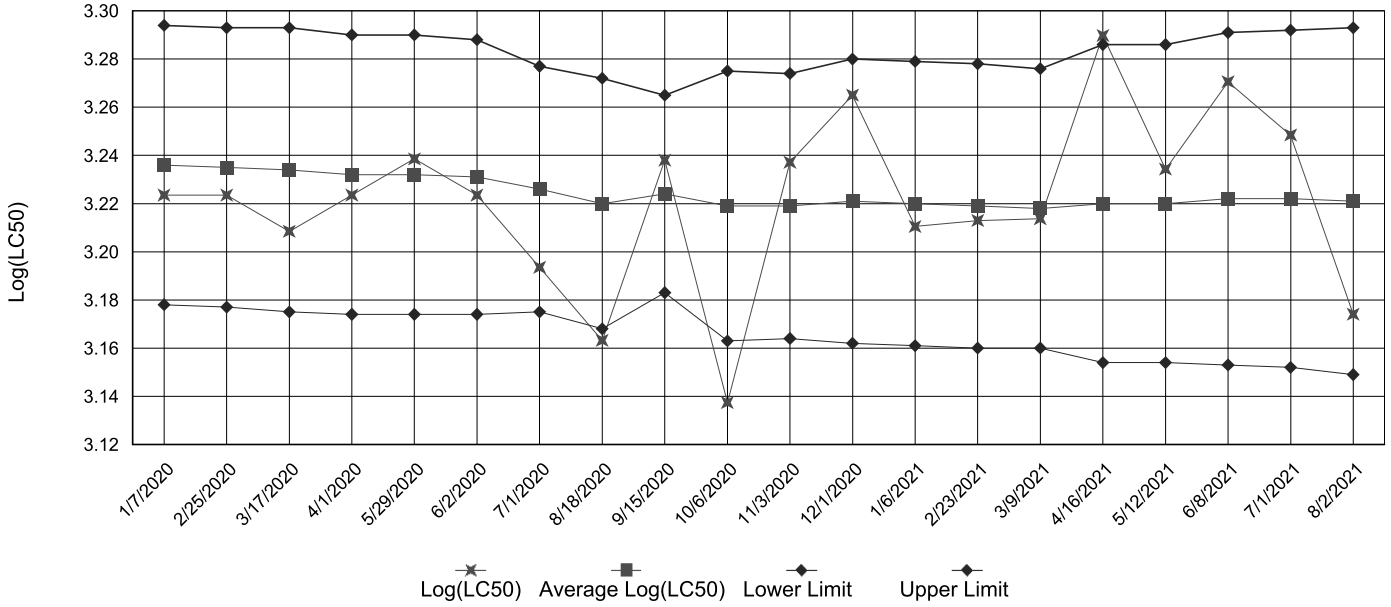
IC25 Growth Data



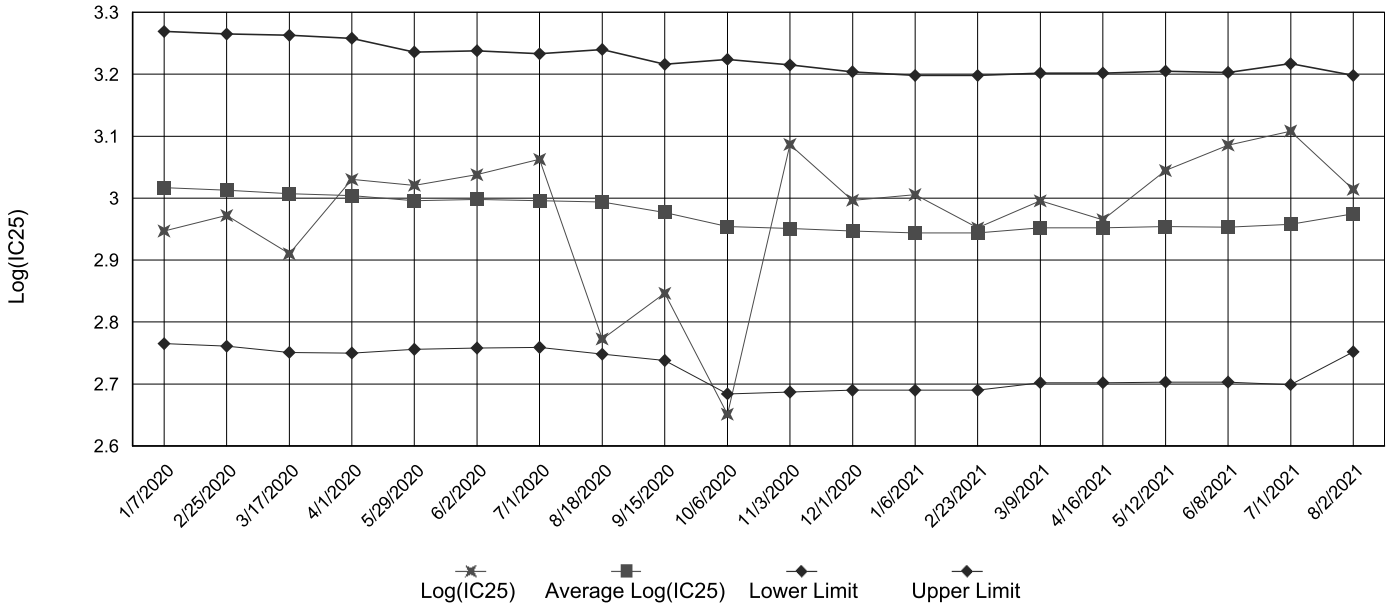
Appendix A3: Test 1002.0

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: August 24, 2021 at 1009
Date and Time Test Terminated: August 31, 2021 at 1040
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	100	100	100	100	100	100	0.00
8 %	100	100	100	100	100	100	100	100	0.00
11 %	100	100	100	100	100	100	100	100	0.00
15 %	100	100	100	100	100	100	100	100	0.00
20 %	100	100	100	100	100	100	100	100	0.00
27 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.568	0.624	0.469	0.666	0.568	0.579	12.8
8 %	0.612	0.538	0.529	0.589	0.568	0.567	6.10
11 %	0.552	0.542	0.526	0.541	0.608	0.554	5.72
15 %	0.555	0.526	0.542	0.556	0.522	0.540	2.93
20 %	0.596	0.600	0.585	0.509	0.629	0.584	7.68
27 %	0.650	0.541	0.648	0.656	0.628	0.625	7.67

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 _____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: 12.8 (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, 343, 357, 358

Test Initiated: DATE: August 24, 2021 TIME: 1009
Test Terminated: DATE: August 31, 2021 TIME: 1040

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.8	7.0	7.1	7.2	7.2	7.4	7.0
	Final	6.2	6.2	6.4	5.9	5.6	6.0	6.2
pH	Initial	7.4	7.3	7.4	7.3	7.5	7.4	7.4
	Final	7.1	7.2	7.3	7.1	7.0	7.1	7.1

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.9	6.7	7.5	7.1	7.1	6.9	7.0
	Final	6.0	6.1	6.4	5.7	6.1	6.1	6.1
pH	Initial	7.4	7.4	7.4	7.4	7.4	7.5	7.3
	Final	7.1	7.2	7.2	7.1	7.1	7.1	7.2

DILUTION 11 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.9	6.8	7.4	6.8	7.4	7.0	7.1
	Final	6.0	6.0	6.4	5.9	6.0	6.2	6.1
pH	Initial	7.3	7.4	7.4	7.4	7.4	7.5	7.4
	Final	7.1	7.1	7.2	7.1	7.1	7.1	7.1

DILUTION 15 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.9	7.0	7.3	7.2	7.1	7.4	7.4
	Final	6.3	6.2	6.4	5.8	6.1	6.3	6.2
pH	Initial	7.4	7.4	7.4	7.4	7.4	7.5	7.4
	Final	7.2	7.2	7.2	7.1	7.1	7.2	7.2

DILUTION 20 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.9	7.1	7.2	7.2	7.2	7.4	7.3
	Final	6.2	6.0	6.3	5.9	6.2	6.1	6.4
pH	Initial	7.4	7.3	7.4	7.3	7.5	7.4	7.4
	Final	7.1	7.1	7.3	7.1	7.2	7.2	7.2

DILUTION 27 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.9	6.6	7.5	7.0	7.2	7.2	6.8
	Final	6.1	6.0	6.3	5.9	5.7	5.8	6.2
pH	Initial	7.3	7.4	7.4	7.4	7.5	7.4	7.4
	Final	7.2	7.1	7.2	7.2	7.1	7.2	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
39	34	360	<0.05	EFF 23-AUG-21
45	34	380	<0.05	EFF 25-AUG-21
48	37	400	<0.05	EFF 27-AUG-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	42	180	<0.05	257863-1

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: August 24, 2021 at 1100
Date and Time Test Terminated: August 31, 2021 at 0935
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	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

		Percent Effluent				
Replicates	Control	8 %	11 %	15 %	20 %	27 %
A	27	26	33	26	21	31
B	30	31	33	21	16	35
C	31	33	34	35	32	38
D	24	33	20	12	37	36
E	32	30	32	32	26	35
F	31	32	26	22	24	24
G	31	25	33	25	17	20
H	26	35	23	36	27	16
I	26	30	31	20	30	32
J	28	28	33	21	31	9
Mean per Adult	28.6	30.3	29.8	25.0	26.1	27.6
Mean per Surviving Adult	28.6	30.3	29.8	25.0	26.1	27.6
CV %	9.64	10.6	16.7	30.0	25.8	35.6

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: 25.8 (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: August 24, 2021 TIME: 1100
Test Terminated: DATE: August 31, 2021 TIME: 0935

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

DILUTION 8 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	6.7	7.5	7.1	7.1	6.9	7.0
Final	7.1	7.4	7.2	7.5	7.3	7.2	7.2
pH Initial	7.4	7.4	7.4	7.4	7.4	7.5	7.3
Final	7.9	7.7	7.7	7.8	7.9	7.7	7.8

DILUTION 11 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	6.8	7.4	6.8	7.4	7.0	7.1
Final	7.0	7.2	7.4	7.6	7.7	7.3	7.3
pH Initial	7.3	7.4	7.4	7.4	7.4	7.5	7.4
Final	7.9	7.7	7.7	7.8	7.8	7.7	7.9

DILUTION 15 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	7.0	7.3	7.2	7.1	7.4	7.4
Final	7.4	7.5	7.1	7.2	7.6	7.5	7.4
pH Initial	7.4	7.4	7.4	7.4	7.4	7.5	7.4
Final	7.9	7.7	7.6	7.8	7.9	7.8	7.9

DILUTION 20 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	7.1	7.2	7.2	7.2	7.4	7.3
Final	7.3	7.4	7.3	7.5	7.4	7.4	7.1
pH Initial	7.4	7.3	7.4	7.3	7.5	7.4	7.4
Final	7.8	7.8	7.8	7.8	8.0	7.8	8.0

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	6.6	7.5	7.0	7.2	7.2	6.8
Final	7.2	7.3	7.3	7.6	7.3	7.0	7.2
pH Initial	7.3	7.4	7.4	7.4	7.5	7.4	7.4
Final	8.0	7.8	7.8	7.9	8.0	7.7	7.9

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
39	34	360	<0.05	EFF 23-AUG-21
45	34	380	<0.05	EFF 25-AUG-21
48	37	400	<0.05	EFF 27-AUG-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	42	180	<0.05	257863-1

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

AIC CONTROL NO: 258068
AIC PROPOSAL NO:
Carrier: J. SMITH
Received Temperature C: 2.8

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		NO OF BOTTLES	ANALYSES REQUESTED: Bio-Monitoring	Remarks
	EFF	8-23-21 8am		✓	✓				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=(NH4)2, NH4OH

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
Jimmy Smith	8/23/21 10:28am		
Relinquished By:	Date/Time	Received in Lab By: D. BROWN	Date/Time 8-23-21 1029

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 CHEELY

PO No.

SAMPLE MATRIX

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES	Bio-Monitor	ANALYSES REQUESTED
2	EFF	8-25-21 8AM		/			1	/	
		Container Type						P	
		Preservative						ND	

AIC CONTROL NO: 258068
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 2.5
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 A=(NH4)2, NH4OH

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: [signature]	Date/Time 8-25-21 10:00 AM	Received in Lab By: D. BROWN	Date/Time 8-25-21 1000

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: Thomas Hartsfield

PO No.

AIC CONTROL NO: 258068
AIC PROPOSAL NO:
Carrier: J. SMITH
Received Temperature C: 1.6

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Bio-Monitor	CN	Phenolics	HG	Metals
3	EFF	8/27/21 8AM		✓	✓			1				
	EFF	8/26/21 8AM 2PM 8/27/21 8AM 2PM		/					4			
	EFF	8/26/21 8AM 2PM 8/27/21 8PM 2PM		/						4		
	EFF	8/26/21 8AM 2PM 8/27/21 8PM 2PM		/							4	
	EFF	8/27/21 8AM		/								1
		Container Type						P	P	G	G	P
		Preservative						NO	B	S	NO	N

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 A=(NH4)2, NH4OH

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
Jimmy Smith	8/27/21 10:30am		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		D. BROWN	8-27-21 1030

Comments:

FORM 0060