November 19, 2021

Biomonitoring Testing
for
EFF

Control No. 260195-1

Prepared for:

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

Prepared by:

AMERICAN INTERPLEX CORPORATION
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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:

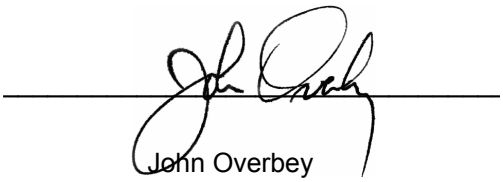
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 test concluded with a statistical significant difference as the 15 % effluent concentration for growth. However, this does not follow a dose-response pattern and is considered an anomaly. **The sample, therefore PASSED both lethal and sublethal effects for the Fathead minnow.**

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	0.383	PASS
Control Growth CV < or = 40%	3.30	PASS
Growth Minimum Significant Difference 12 to 30%	24.4	PASS
Critical Dilution CV < or = 40%	10.2	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	25.1	PASS
Control CV < or = 40% per Surviving Female	35.9	PASS
Reproduction Minimum Significant Difference 13 to 47%	40.3	PASS
Critical Dilution CV < or = 40%	34.7	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: EFF
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.2	7.2	7.5
pH (standard units)	7.3	7.2	7.3
Alkalinity (mg/l as CaCO3)	34	38	41
Hardness (mg/l as CaCO3)	37	42	37
Conductivity (umhos/cm)	370	360	290
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.27	0.15	0.38

2. Dilution Water Samples:
   Soft

Analysis	259972-1	260219-1
Dissolved oxygen (mg/l)	7.2	7.2
pH (standard units)	7.6	7.4
Alkalinity (mg/l as CaCO3)	32	34
Hardness (mg/l as CaCO3)	41	44
Conductivity (umhos/cm)	160	170
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:	November 9, 2021 at 1128
Date & Time Test Terminated:	November 16, 2021 at 0947
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:	November 9, 2021 at 1014
Date & Time Test Terminated:	November 15, 2021 at 1205
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Source of test organisms: In-house culture
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name
   a. Test 1000.0 *Pimephales promelas*
   b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat and following EPA method criteria.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's and Bartlett's test. The survival data was then analyzed using 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. 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 October 05, 2021 at 1504 to October 12, 2021 at 1450
The results were as follows: (Control No. 259170-1.)

Survival LC-50: 3649 mg/l
Growth IC-25: 2291 mg/l
Growth PMSD: 11.7

Ceriodaphnia dubia

A chronic reference test was performed on October 14, 2021 at 1338 to October 21, 2021 at 1255
The results were as follows: (Control No. 259534-2.)

Survival LC-50: 1772 mg/l
Reproduction IC-25: 1278 mg/l
Reproduction PMSD: 10.4

V. Organism History

Pimephales promelas (Fathead minnow)

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

Ceriodaphnia dubia

Date: November 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

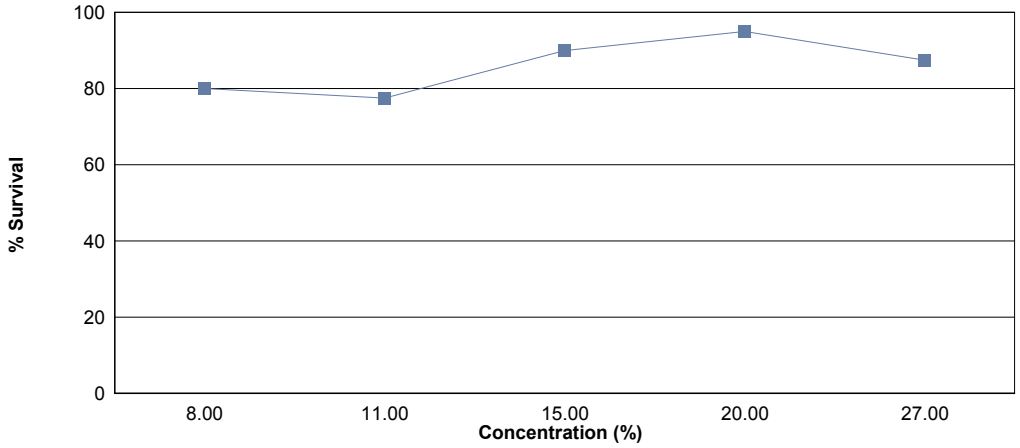
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 November 9, 2021 at 1128 and continued through November 16, 2021 at 0947. 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	100	0.383
8 %	80.0	0.330
11 %	77.5	0.294
15 %	90.0	0.338 *
20 %	95.0	0.354
27 %	87.5	0.365

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

The significant toxicity is not due to true dose response effects, and should be considered an anomaly.

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

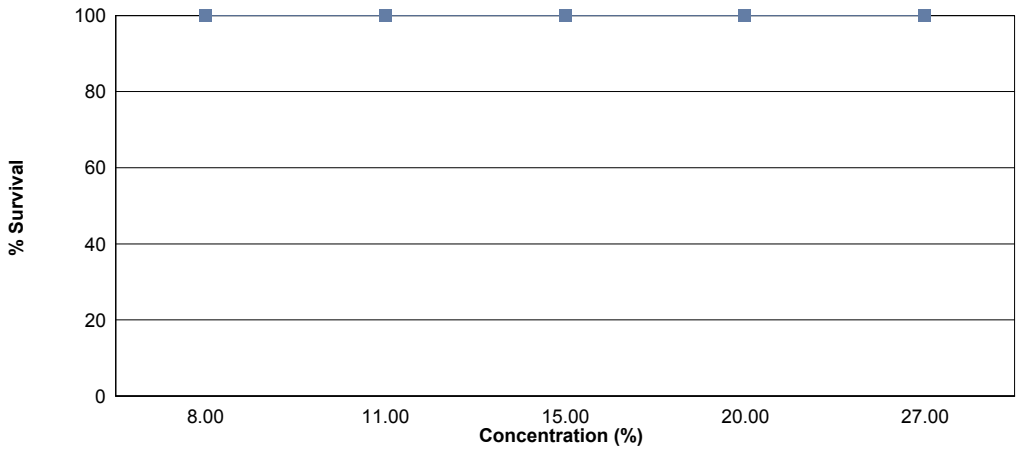
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 November 9, 2021 at 1014 and continued through November 15, 2021 at 1205. 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	25.1
8 %	100	27.4
11 %	100	26.1
15 %	100	26.1
20 %	100	25.9
27 %	100	25.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: November 9, 2021 at 1128
Date and Time Test Terminated: November 16, 2021 at 0947

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	6	5	5
	B	8	8	8	6	6	4	4
	C	8	8	8	8	8	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
11 %	A	8	8	8	8	7	6	6
	B	8	8	8	8	5	2	2
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	7	7
	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	7	7	6	6	6
	D	8	8	8	8	8	7	7
	E	8	8	8	7	7	7	7
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	7	7	7	7
	E	8	8	8	8	7	7	7
27 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	5	5	5	5
	D	8	8	8	8	6	6	6
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: November 9, 2021 at 1128
Test Terminated: November 16, 2021 at 0947

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65627	.65939	0.00312	8	0.390
	B	.66591	.66889	0.00298	8	0.372
	C	.64694	.64991	0.00297	8	0.371
	D	.65895	.66201	0.00306	8	0.382
	E	.65056	.65377	0.00321	8	0.401
8 %	A	.65904	.66147	0.00243	8	0.304
	B	.66041	.66216	0.00175	8	0.219
	C	.65168	.65456	0.00288	8	0.360
	D	.65150	.65435	0.00285	8	0.356
	E	.65851	.66179	0.00328	8	0.410
11 %	A	.66453	.66724	0.00271	8	0.339
	B	.66944	.67004	0.00060	8	0.075
	C	.65307	.65605	0.00298	8	0.372
	D	.65098	.65358	0.00260	8	0.325
	E	.65095	.65383	0.00288	8	0.360
15 %	A	.65005	.65284	0.00279	8	0.349
	B	.67123	.67395	0.00272	8	0.340
	C	.65925	.66213	0.00288	8	0.360
	D	.65644	.65898	0.00254	8	0.318
	E	.64938	.65196	0.00258	8	0.322
20 %	A	.64627	.64957	0.00330	8	0.412
	B	.63961	.64237	0.00276	8	0.345
	C	.65995	.66284	0.00289	8	0.361
	D	.64738	.64995	0.00257	8	0.321
	E	.65349	.65612	0.00263	8	0.329
27 %	A	.63940	.64254	0.00314	8	0.392
	B	.65358	.65673	0.00315	8	0.394
	C	.65655	.65909	0.00254	8	0.318
	D	.66100	.66375	0.00275	8	0.344
	E	.65979	.66279	0.00300	8	0.375

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: November 9, 2021 at 1014
Date and Time Test Terminated: November 15, 2021 at 1205

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	4	0	0	3	0	4	11	10	1.10
4	3	5	4	6	0	4	4	0	5	0	31	10	3.10
5	9	11	10	12	11	10	12	12	13	10	110	10	11.0
6	0	0	0	0	19	15	16	20	14	15	99	10	9.90
7													
8													
TOTAL	12	16	14	18	34	29	32	35	32	29	251	10	25.1

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	4	0	0	4	0	4	12	10	1.20
4	4	6	5	5	0	5	5	0	5	0	35	10	3.50
5	10	9	11	10	11	11	14	14	11	12	113	10	11.3
6	0	0	0	0	18	19	19	22	16	20	114	10	11.4
7													
8													
TOTAL	14	15	16	15	33	35	38	40	32	36	274	10	27.4

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	2	4	0	5	0	4	15	10	1.50
4	4	5	4	6	0	0	5	0	6	0	30	10	3.00
5	11	12	11	10	9	11	11	4	13	10	102	10	10.2
6	0	0	0	0	16	19	18	21	19	21	114	10	11.4
7													
8													
TOTAL	15	17	15	16	27	34	34	30	38	35	261	10	26.1

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: November 9, 2021 at 1014
Date and Time Test Terminated: November 15, 2021 at 1205

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	5	0	0	0	0	0	5	10	0.500
4	4	5	6	3	0	4	6	0	6	5	39	10	3.90
5	7	10	12	11	10	11	11	4	13	11	100	10	10.0
6	17	0	0	0	18	14	20	12	19	17	117	10	11.7
7													
8													
TOTAL	28	15	18	14	33	29	37	16	38	33	261	10	26.1

Concentration: 20 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	4	0	0	4	0	3	11	10	1.10
4	4	6	6	5	0	4	5	0	6	0	36	10	3.60
5	11	10	12	11	11	2	11	10	10	12	100	10	10.0
6	0	0	0	0	21	18	20	18	18	17	112	10	11.2
7													
8													
TOTAL	15	16	18	16	36	24	36	32	34	32	259	10	25.9

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	4	4	0	5	0	3	16	10	1.60
4	4	4	4	6	0	0	6	0	4	0	28	10	2.80
5	10	11	2	0	10	10	13	13	13	6	88	10	8.80
6	0	0	5	9	16	18	21	18	18	20	125	10	12.5
7													
8													
TOTAL	14	15	11	15	30	32	40	36	35	29	257	10	25.7

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	0.62500	0.91174
2	8 %	2	0.50000	0.78540
2	8 %	3	0.87500	1.20940
2	8 %	4	1.00000	1.39310
2	8 %	5	1.00000	1.39310
3	11 %	1	0.75000	1.04720
3	11 %	2	0.25000	0.52360
3	11 %	3	1.00000	1.39310
3	11 %	4	0.87500	1.20940
3	11 %	5	1.00000	1.39310
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	0.75000	1.04720
4	15 %	4	0.87500	1.20940
4	15 %	5	0.87500	1.20940
5	20 %	1	1.00000	1.39310
5	20 %	2	1.00000	1.39310
5	20 %	3	1.00000	1.39310
5	20 %	4	0.87500	1.20940
5	20 %	5	0.87500	1.20940
6	27 %	1	1.00000	1.39310
6	27 %	2	1.00000	1.39310
6	27 %	3	0.62500	0.91174
6	27 %	4	0.75000	1.04720
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 = 1.169
W = 0.9367
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))

Test can not be performed because at least one group has zero variance.
Data FAIL to meet homogeneity of variance assumption.

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 %	20.00	16.00	5.00	
3	11 %	20.00	16.00	5.00	
4	15 %	20.00	16.00	5.00	
5	20 %	22.50	16.00	5.00	
6	27 %	22.50	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 = 0.09379		
W = 0.824		
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 %	20.00	16.00	5.00	
3	11 %	16.50	16.00	5.00	
4	15 %	15.00	16.00	5.00	*
5	20 %	20.00	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

Dunnett's Test for PMSD Calculation

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	0.02387	0.004773	1.221
Within (Error)	24	0.09379	0.003908	
Total	29	0.1177		

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	0.3832	0.3832		
2	8 %	0.3298	0.3298	1.351	
3	11 %	0.2942	0.2942	2.251	
4	15 %	0.3378	0.3378	1.148	
5	20 %	0.3536	0.3536	0.7487	
6	27 %	0.3646	0.3646	0.4704	

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	0.09331	24.4	0.0534
3	11 %	5	0.09331	24.4	0.089
4	15 %	5	0.09331	24.4	0.0454
5	20 %	5	0.09331	24.4	0.0296
6	27 %	5	0.09331	24.4	0.0186

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 = 0.184		
D* = 1.444		
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 %	117.50	75.00	10.00	
3	11 %	111.00	75.00	10.00	
4	15 %	109.50	75.00	10.00	
5	20 %	112.00	75.00	10.00	
6	27 %	108.00	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	28.75	5.75	0.06001
Within (Error)	54	5174	95.81	
Total	59	5203		

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	25.1	25.1		
2	8 %	27.4	27.4	-0.5254	
3	11 %	26.1	26.1	-0.2284	
4	15 %	26.1	26.1	-0.2284	
5	20 %	25.9	25.9	-0.1828	
6	27 %	25.7	25.7	-0.1371	

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

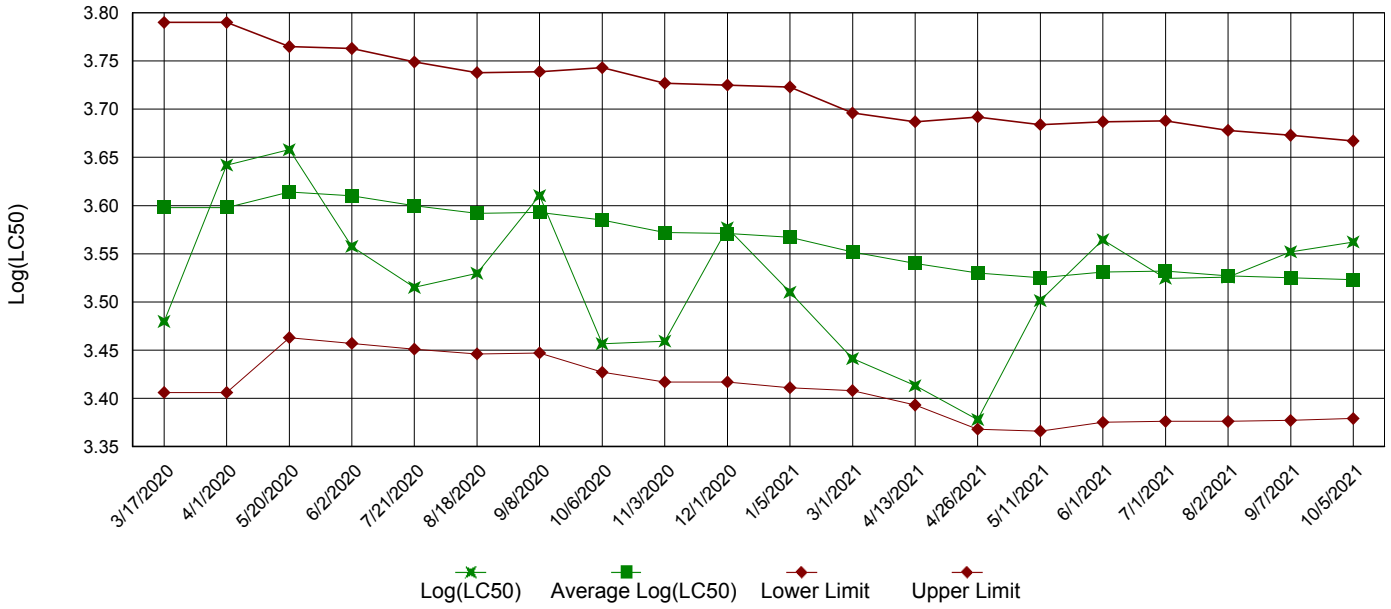
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	8 %	10	10.11	40.3	-2.3
3	11 %	10	10.11	40.3	-1
4	15 %	10	10.11	40.3	-1
5	20 %	10	10.11	40.3	-0.8
6	27 %	10	10.11	40.3	-0.6

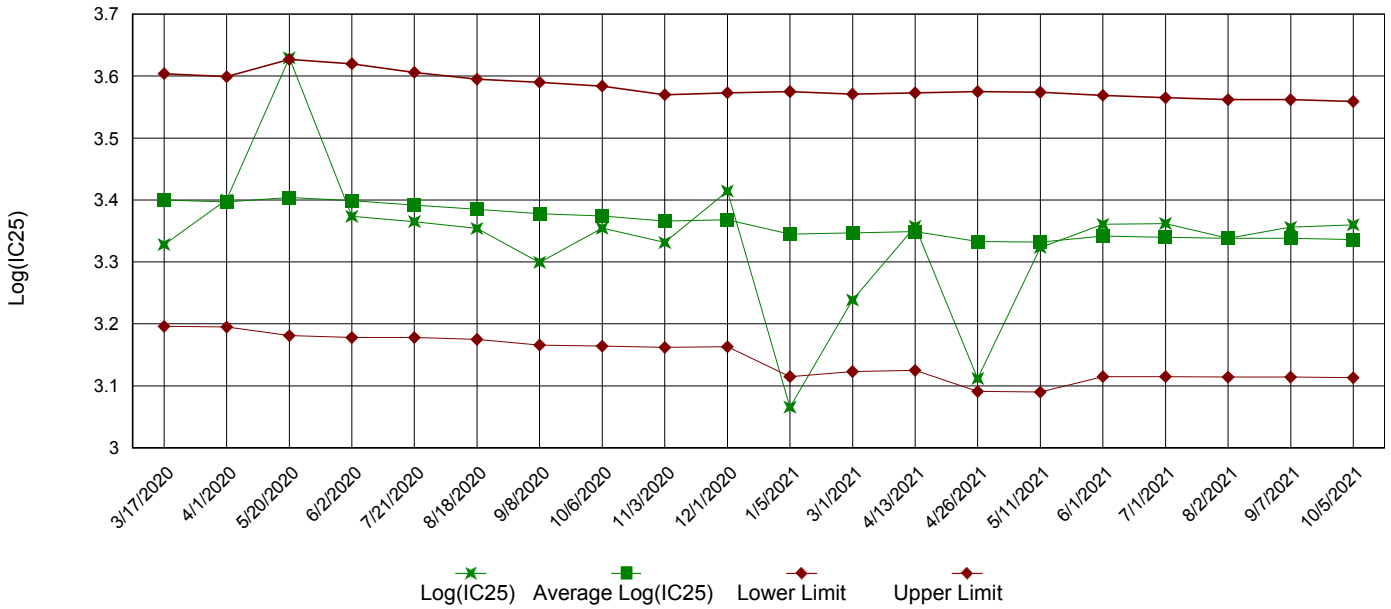
Appendix A3: Test 1000.0

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

LC50 Survival Data



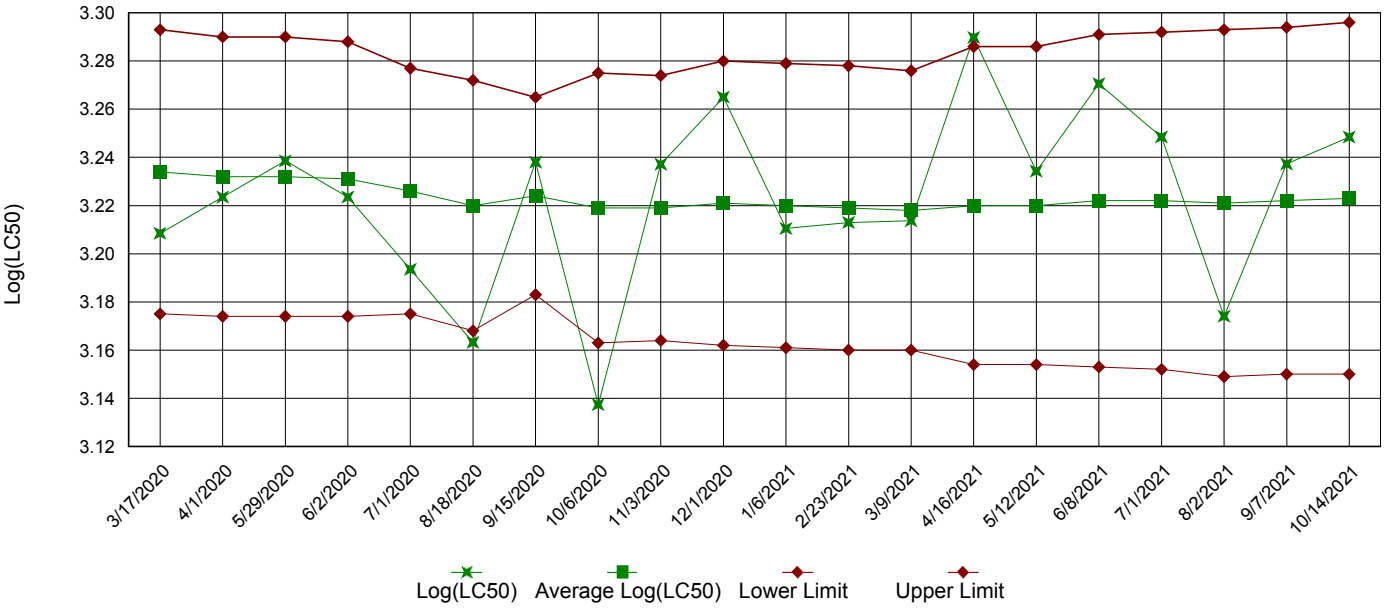
IC25 Growth Data



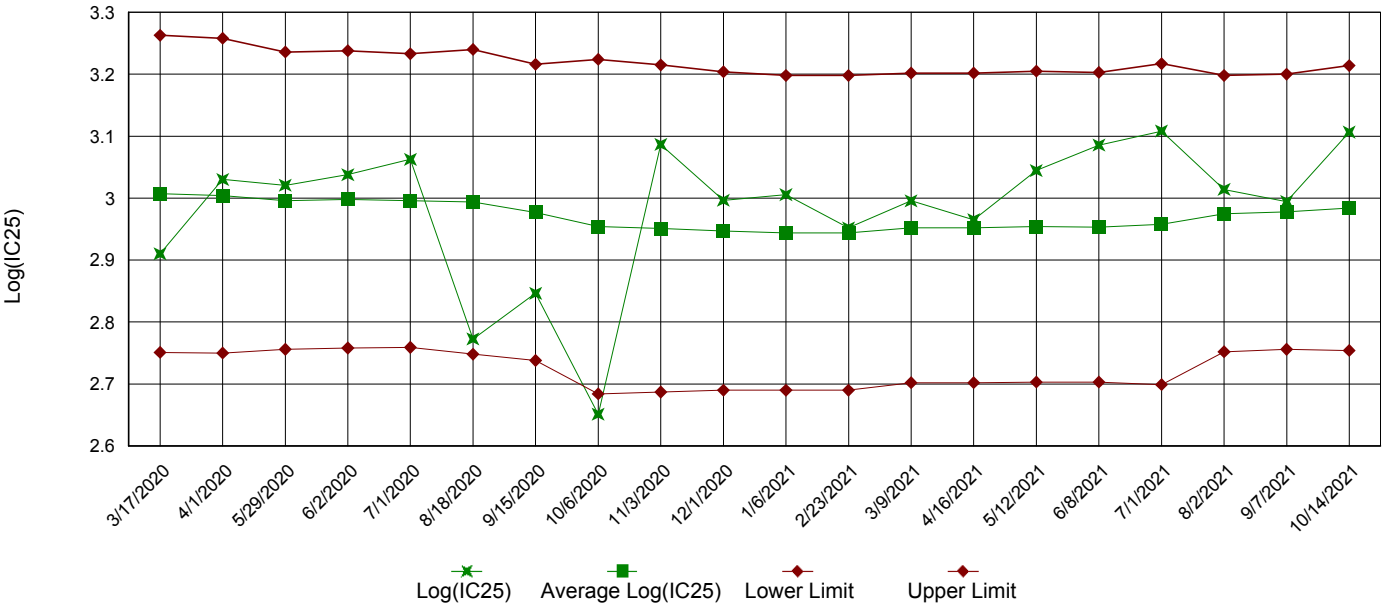
Appendix A3: Test 1002.0

Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0

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

Permittee: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: November 9, 2021 at 1128
Date and Time Test Terminated: November 16, 2021 at 0947
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 %	62.5	50.0	87.5	100	100	100	100	80.0	28.4
11 %	75.0	25.0	100	87.5	100	100	100	77.5	40.2
15 %	100	100	75.0	87.5	87.5	100	100	90.0	11.6
20 %	100	100	100	87.5	87.5	100	100	95.0	7.21
27 %	100	100	62.5	75.0	100	100	100	87.5	20.2

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.390	0.372	0.371	0.382	0.401	0.383	3.30
8 %	0.304	0.219	0.360	0.356	0.410	0.330	22.0
11 %	0.339	0.075	0.372	0.325	0.360	0.294	42.1
15 %	0.349	0.340	0.360	0.318	0.322	0.338	5.26
20 %	0.412	0.345	0.361	0.321	0.329	0.354	10.2
27 %	0.392	0.394	0.318	0.344	0.375	0.365	9.01

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. 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: 10.2 (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: November 9, 2021 TIME: 1128
Test Terminated: DATE: November 16, 2021 TIME: 0947

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.2	7.2	7.2	7.5	7.6	7.4	7.5
	Final	6.3	6.8	7.3	6.7	7.1	7.0	7.1
pH	Initial	7.6	7.5	7.4	7.4	7.5	7.6	7.6
	Final	7.2	7.4	7.5	7.4	7.4	7.3	7.5

DILUTION 8 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.1	6.9	7.1	7.0	7.4	7.3	7.5
	Final	6.0	6.6	7.2	7.1	6.7	6.7	6.6
pH	Initial	7.5	7.4	7.4	7.4	7.3	7.3	7.3
	Final	7.1	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	7.2	7.1	7.2	7.2	7.4	7.4	7.5
	Final	6.1	6.4	7.1	7.1	7.0	6.8	6.6
pH	Initial	7.4	7.4	7.3	7.3	7.3	7.3	7.3
	Final	7.0	7.4	7.5	7.4	7.3	7.3	7.3

DILUTION 15 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.1	6.9	7.2	6.8	7.2	7.4	6.8
	Final	6.1	7.0	7.2	7.2	7.0	7.1	6.7
pH	Initial	7.4	7.5	7.4	7.4	7.3	7.3	7.5
	Final	7.1	7.4	7.5	7.4	7.4	7.4	7.4

DILUTION 20 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.1	6.7	7.3	7.0	7.3	7.4	7.2
	Final	6.0	6.7	7.1	6.8	6.9	7.0	6.5
pH	Initial	7.4	7.4	7.3	7.4	7.3	7.3	7.3
	Final	7.1	7.4	7.4	7.4	7.4	7.4	7.4

DILUTION 27 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.9	6.9	7.2	7.3	7.4	7.4	6.9
	Final	6.0	6.5	7.3	7.0	7.0	6.9	6.6
pH	Initial	7.4	7.3	7.2	7.3	7.3	7.3	7.4
	Final	7.1	7.4	7.5	7.5	7.5	7.4	7.4

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
34	37	370	<0.05	EFF 08-NOV-21
38	42	360	<0.05	EFF 10-NOV-21
41	37	290	<0.05	EFF 12-NOV-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	160	<0.05	259972-1
34	44	170	<0.05	260219-1

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: November 9, 2021 at 1014
Date and Time Test Terminated: November 15, 2021 at 1205
Dilution water used: Soft

PERCENT SURVIVAL

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

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent 8 %	11 %	15 %	20 %	27 %
A	12	14	15	28	15	14
B	16	15	17	15	16	15
C	14	16	15	18	18	11
D	18	15	16	14	16	15
E	34	33	27	33	36	30
F	29	35	34	29	24	32
G	32	38	34	37	36	40
H	35	40	30	16	32	36
I	32	32	38	38	34	35
J	29	36	35	33	32	29
Mean per Adult	25.1	27.4	26.1	26.1	25.9	25.7
Mean per Surviving Adult	25.1	27.4	26.1	26.1	25.9	25.7
CV %	35.9	39.8	36.0	36.2	34.7	42.0

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. 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: 35.9 (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: November 9, 2021 TIME: 1014
Test Terminated: DATE: November 15, 2021 TIME: 1205

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

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

DILUTION 11 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	7.1	7.2	7.2	7.4	7.4	7.5
Final	7.4	7.4	7.5	8.0	7.5	7.4	--
pH Initial	7.4	7.4	7.3	7.3	7.3	7.3	7.3
Final	7.9	7.9	7.8	7.8	7.9	7.8	--

DILUTION 15 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	6.9	7.2	6.8	7.2	7.4	6.8
Final	7.1	7.4	7.5	7.7	7.6	7.5	--
pH Initial	7.4	7.5	7.4	7.4	7.3	7.3	7.5
Final	7.8	7.9	7.9	7.8	7.9	7.8	--

DILUTION 20 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	6.7	7.3	7.0	7.3	7.4	7.2
Final	6.9	7.4	7.3	7.7	7.6	7.6	--
pH Initial	7.4	7.4	7.3	7.4	7.3	7.3	7.3
Final	7.9	7.9	7.9	7.8	8.0	7.8	--

DILUTION 27 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	6.9	7.2	7.3	7.4	7.4	6.9
Final	7.0	7.4	7.5	7.5	7.5	7.4	--
pH Initial	7.4	7.3	7.2	7.3	7.3	7.3	7.4
Final	7.9	7.9	7.9	7.9	8.1	7.9	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
34	37	370	<0.05	EFF 08-NOV-21
38	42	360	<0.05	EFF 10-NOV-21
41	37	290	<0.05	EFF 12-NOV-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	160	<0.05	259972-1
34	44	170	<0.05	260219-1

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Searcy Water Utilities
Project Reference: Bio-Monitoring
Project Manager: Jimmy Smith
Sampled By: Jeremy Cheely

PO No.

SAMPLE MATRIX

NO OF BOTTLES

ANALYSES REQUESTED

AIC CONTROL NO: 260195
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 0.9

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		NO OF BOTTLES	Bio-Monitor	Remarks
1	EFF	11-8-21 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
Brad Anderson	11-8-21 9:45am		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		D. Brown	11-8-21 0945

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.

AIC CONTROL NO: 260195
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 1.8

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL		NO OF BOTTLES	ANALYSES REQUESTED: BIO Monitor
2	EFF	11/02/21 8AM		/	/			1	1
		Container Type						P	P
		Preservative						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:	Date/Time	Received in Lab By:	Date/Time
	11-10-21 9:45AM	D. BROWN	11-10-21 0947

Comments:

5/01

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: SEACCY WATER Utilities
Project Reference: Bio-Monitor
Project Manager: Jimmy SmAH
Sampled By: Brady Anderson

PO No.

AIC CONTROL NO: 210195
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 1.3

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Bio-Monitor	N. Pond	S. Pond
3	EFF	11-12-21 8 am		/	/			1		
X	N. Pond	11-12-21 820 am	/						1	
2	S. Pond	11-12-21 845 am	/							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 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: Brady Anderson	Date/Time 11-12-21 10:13	Received in Lab By:	Date/Time 11-12-21 1013

Comments:

FORM 0060