January 22, 2021

Biomonitoring Testing
for
AR0035602

Control No. 251995-1

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
704 Hwy 463 N
Trumann, AR 72472

Prepared by:

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

Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
704 Hwy 463 N
Trumann, AR 72472

Re: Chronic*Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
AR0035602
NPDES Permit No. AFIN 56-00047

Dear Mr. Scotty Jones:

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 12 % effluent, which is above the critical dilution of 9 %. The NOEC for growth occurred at 12 % effluent, which is above the critical dilution of 9 %. **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 12 % effluent, which is above the critical dilution of 9 %. The NOEC for reproduction occurred at 12 % effluent, which is above the critical dilution of 9 %. **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: Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
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ATTN: Ms. Lorre Holt
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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	0.490	PASS
Control Growth CV < or = 40%	14.1	PASS
Growth Minimum Significant Difference 12 to 30%	22.1	PASS
Critical Dilution CV < or = 40%	14.8	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	28.3	PASS
Control CV < or = 40% per Surviving Female	8.66	PASS
Reproduction Minimum Significant Difference 13 to 47%	22.8	PASS
Critical Dilution CV < or = 40%	10.2	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AFIN 56-00047
2. Test Requirements:
   Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
   a. Sampling Point: AR0035602
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.6	6.7	7.7
pH (standard units)	8.1	8.2	8.1
Alkalinity (mg/l as CaCO3)	120	120	120
Hardness (mg/l as CaCO3)	39	40	39
Conductivity (umhos/cm)	450	440	450
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	3.8	<0.1

2. Dilution Water Samples:
   Moderately Hard

Analysis	251741-1
Dissolved oxygen (mg/l)	7.6
pH (standard units)	8.2
Alkalinity (mg/l as CaCO3)	63
Hardness (mg/l as CaCO3)	81
Conductivity (umhos/cm)	320
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:	January 12, 2021 at 1320
Date & Time Test Terminated:	January 19, 2021 at 1505
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:	January 12, 2021 at 1250
Date & Time Test Terminated:	January 18, 2021 at 1445
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Source of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

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

III. Data Analysis

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

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and analyzed with Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC) for Reproduction. Dunnett's Test was used to calculate the PMSD.

IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on December 01, 2020 at 1605 to December 08, 2020 at 1500
The results were as follows: (Control No. 250739-1.)

Survival LC-50: 3774 mg/l
Growth IC-25: 2597 mg/l
Growth PMSD: 6.84

Ceriodaphnia dubia

A chronic reference test was performed on December 01, 2020 at 1630 to December 08, 2020 at 1515
The results were as follows: (Control No. 250739-2.)

Survival LC-50: 1840.7 mg/l
Reproduction IC-25: 991.8 mg/l
Reproduction PMSD: 8.43

V. Organism History

Pimephales promelas (Fathead minnow)

Date: January 12, 2021
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: January 12, 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

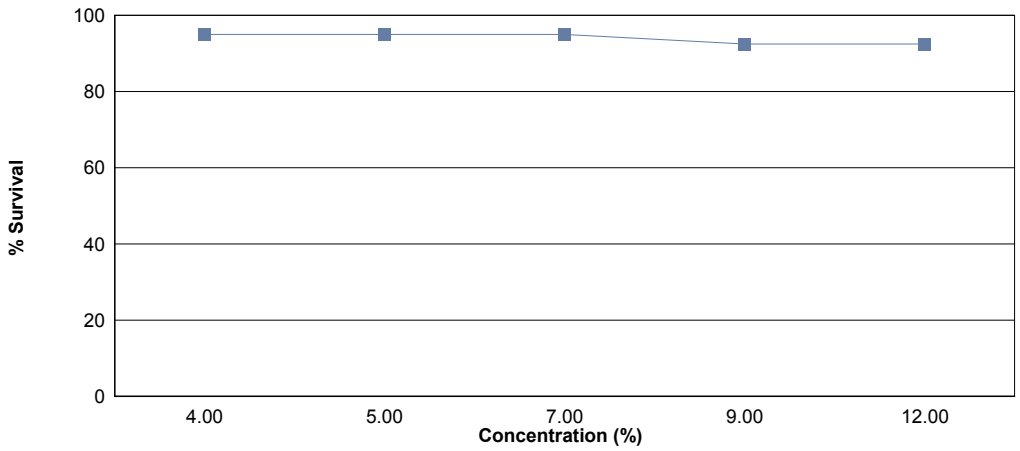
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 9 % effluent.

The test was initiated on January 12, 2021 at 1320 and continued through January 19, 2021 at 1505. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 12 % effluent
b.) NOEC growth = 12 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.490
4 %	95.0	0.509
5 %	95.0	0.488
7 %	95.0	0.518
9 %	92.5	0.472
12 %	92.5	0.455

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

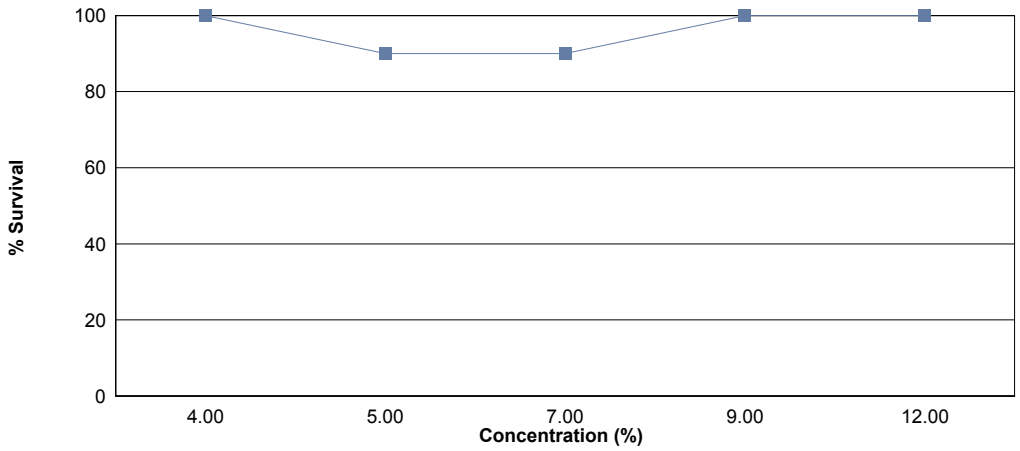
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 9 % effluent.

The test was initiated on January 12, 2021 at 1250 and continued through January 18, 2021 at 1445. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 12 % effluent
b.) NOEC reproduction = 12 % effluent



Summary of the 6-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	28.3
4 %	100	28.2
5 %	90.0	26.0
7 %	90.0	26.5
9 %	100	27.4
12 %	100	27.6

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: January 12, 2021 at 1320
Date and Time Test Terminated: January 19, 2021 at 1505

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
4 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8
5 %	A	8	8	8	8	8	8	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8
7 %	A	8	8	8	7	7	7	7
	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
9 %	A	8	8	8	8	8	8	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	7	7	7
	D	8	8	8	8	8	8	7
	E	8	8	8	8	8	8	8
12 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	7	6
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	7	7	7	7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: January 12, 2021 at 1320
Test Terminated: January 19, 2021 at 1505

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.77267	.77716	0.00449	8	0.561
	B	.76955	.77354	0.00399	8	0.499
	C	.77111	.77474	0.00363	8	0.454
	D	.77111	.77548	0.00437	8	0.546
	E	.77104	.77418	0.00314	8	0.392
4 %	A	.77095	.77633	0.00538	8	0.672
	B	.77955	.78294	0.00339	8	0.424
	C	.76497	.76869	0.00372	8	0.465
	D	.77310	.77663	0.00353	8	0.441
	E	.77476	.77911	0.00435	8	0.544
5 %	A	.76839	.77188	0.00349	8	0.436
	B	.77400	.77781	0.00381	8	0.476
	C	.76981	.77418	0.00437	8	0.546
	D	.77243	.77629	0.00386	8	0.482
	E	.77106	.77508	0.00402	8	0.502
7 %	A	.77929	.78279	0.00350	8	0.438
	B	.77762	.78229	0.00467	8	0.584
	C	.76549	.76884	0.00335	8	0.419
	D	.76951	.77437	0.00486	8	0.608
	E	.77807	.78239	0.00432	8	0.540
9 %	A	.77628	.77953	0.00325	8	0.406
	B	.77448	.77879	0.00431	8	0.539
	C	.76564	.77005	0.00441	8	0.551
	D	.77241	.77568	0.00327	8	0.409
	E	.76858	.77221	0.00363	8	0.454
12 %	A	.76678	.76991	0.00313	8	0.391
	B	.77662	.78025	0.00363	8	0.454
	C	.77365	.77786	0.00421	8	0.526
	D	.77212	.77597	0.00385	8	0.481
	E	.76899	.77236	0.00337	8	0.421

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 12, 2021 at 1250
Date and Time Test Terminated: January 18, 2021 at 1445

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	0	4	5	4	4	3	4	0	32	10	3.20
4	0	0	4	0	10	0	8	0	0	4	26	10	2.60
5	10	8	9	9	0	9	0	10	11	9	75	10	7.50
6	15	16	13	14	18	13	14	15	17	15	150	10	15.0
7													
8													
TOTAL	29	28	26	27	33	26	26	28	32	28	283	10	28.3

Concentration: 4 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	0	0	5	4	5	4	4	0	30	10	3.00
4	0	0	4	5	10	0	8	0	0	3	30	10	3.00
5	10	10	9	10	0	8	0	8	10	7	72	10	7.20
6	18	18	12	15	17	8	16	16	16	14	150	10	15.0
7													
8													
TOTAL	32	32	25	30	32	20	29	28	30	24	282	10	28.2

Concentration: 5 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	5	0	2	6	0	5	4	5	0	31	10	3.10
4	1	0	4	3	9	X	10	0	0	3	30	9	3.33
5	9	9	12	10	0	X	0	10	9	10	69	9	7.67
6	15	15	14	13	17	X	14	14	13	15	130	9	14.4
7													
8													
TOTAL	29	29	30	28	32	0	29	28	27	28	260	10	26.0

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 12, 2021 at 1250
Date and Time Test Terminated: January 18, 2021 at 1445

Concentration: 7 %													
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	X	0	0	0	0	0	9	0.00
3	0	5	3	4	5	X	4	4	4	4	33	9	3.67
4	4	0	2	0	0	X	0	0	0	0	6	9	0.667
5	10	12	8	10	10	X	8	9	10	12	89	9	9.89
6	17	17	16	16	12	X	13	14	16	16	137	9	15.2
7													
8													
TOTAL	31	34	29	30	27	0	25	27	30	32	265	10	26.5

Concentration: 9 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	3	0	4	5	4	0	5	4	4	0	29	10	2.90
4	0	4	0	0	0	4	0	0	0	4	12	10	1.20
5	10	9	10	9	9	7	9	8	10	11	92	10	9.20
6	16	12	15	14	12	11	16	14	17	14	141	10	14.1
7													
8													
TOTAL	29	25	29	28	25	22	30	26	31	29	274	10	27.4

Concentration: 12 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	4	5	4	0	4	4	3	0	32	10	3.20
4	0	0	0	1	0	4	9	9	0	4	27	10	2.70
5	10	13	10	11	9	6	0	0	8	11	78	10	7.80
6	12	17	15	17	15	6	15	15	15	12	139	10	13.9
7													
8													
TOTAL	26	34	29	34	28	16	28	28	26	27	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	4 %	1	1.00000	1.39310
2	4 %	2	1.00000	1.39310
2	4 %	3	0.87500	1.20940
2	4 %	4	0.87500	1.20940
2	4 %	5	1.00000	1.39310
3	5 %	1	0.87500	1.20940
3	5 %	2	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	0.87500	1.20940
3	5 %	5	1.00000	1.39310
4	7 %	1	0.87500	1.20940
4	7 %	2	1.00000	1.39310
4	7 %	3	0.87500	1.20940
4	7 %	4	1.00000	1.39310
4	7 %	5	1.00000	1.39310
5	9 %	1	0.87500	1.20940
5	9 %	2	1.00000	1.39310
5	9 %	3	0.87500	1.20940
5	9 %	4	0.87500	1.20940
5	9 %	5	1.00000	1.39310
6	12 %	1	1.00000	1.39310
6	12 %	2	0.75000	1.04720
6	12 %	3	1.00000	1.39310
6	12 %	4	1.00000	1.39310
6	12 %	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 = 0.2593		
W = 0.8717		
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	4 %	22.50	16.00	5.00	
3	5 %	22.50	16.00	5.00	
4	7 %	22.50	16.00	5.00	
5	9 %	20.00	16.00	5.00	
6	12 %	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 = 0.1267		
W = 0.9557		
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 = 3.734		
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	0.01359	0.002718	0.5149
Within (Error)	24	0.1267	0.005279	
Total	29	0.1403		

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	0.4904	0.4904		
2	4 %	0.5092	0.5092	-0.4091	
3	5 %	0.4884	0.4884	0.04352	
4	7 %	0.5178	0.5178	-0.5963	
5	9 %	0.4718	0.4718	0.4048	
6	12 %	0.4546	0.4546	0.7791	

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	4 %	5	0.1084	22.1	-0.0188
3	5 %	5	0.1084	22.1	0.002
4	7 %	5	0.1084	22.1	-0.0274
5	9 %	5	0.1084	22.1	0.0186
6	12 %	5	0.1084	22.1	0.0358

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Alive	Dead	Total Animals
Control	10	0	10
4 %	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
5 %	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
7 %	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
9 %	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
12 %	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	4 %	10	0	
2	5 %	10	1	
3	7 %	10	1	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.2279		
D* = 1.788		
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	4 %	109.50	75.00	10.00	
3	5 %	114.00	75.00	10.00	
4	7 %	112.00	75.00	10.00	
5	9 %	100.50	75.00	10.00	
6	12 %	105.50	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	42.33	8.467	0.2172
Within (Error)	54	2105	38.98	
Total	59	2147		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2 — No Transformation

Ho:Control<Treatment

Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	28.3	28.3		
2	4 %	28.2	28.2	0.03581	
3	5 %	26	26	0.8237	
4	7 %	26.5	26.5	0.6447	
5	9 %	27.4	27.4	0.3223	
6	12 %	27.6	27.6	0.2507	

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

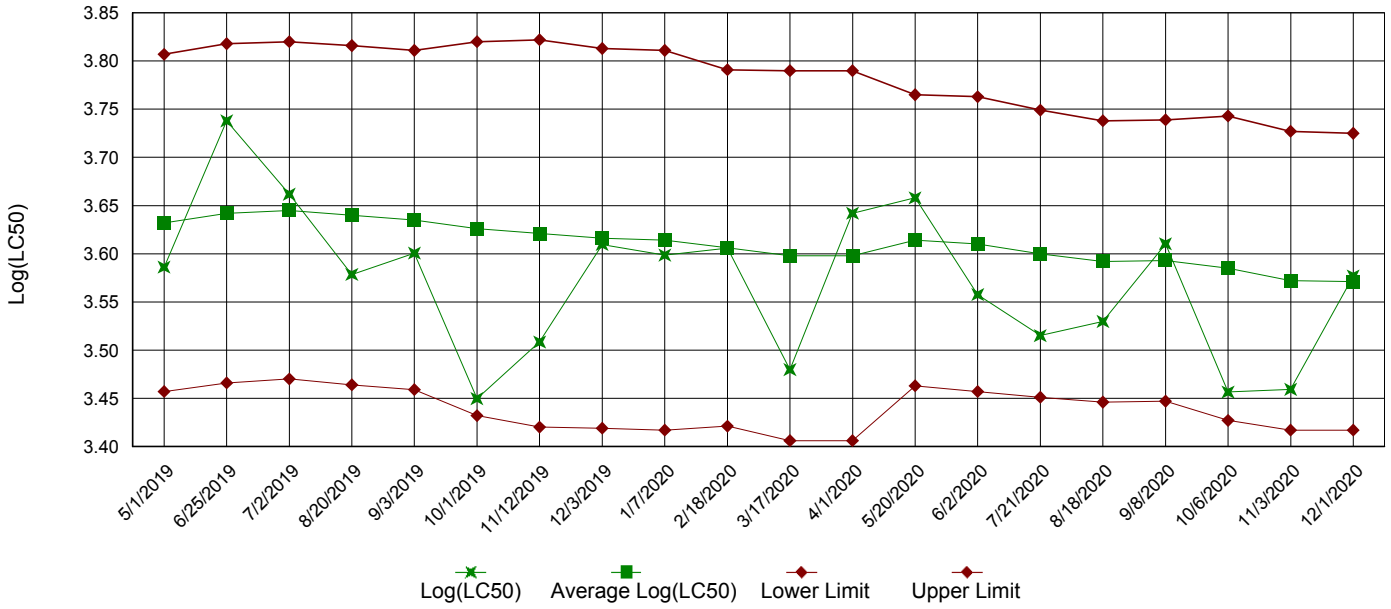
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	4 %	10	6.45	22.8	0.1
3	5 %	10	6.45	22.8	2.3
4	7 %	10	6.45	22.8	1.8
5	9 %	10	6.45	22.8	0.9
6	12 %	10	6.45	22.8	0.7

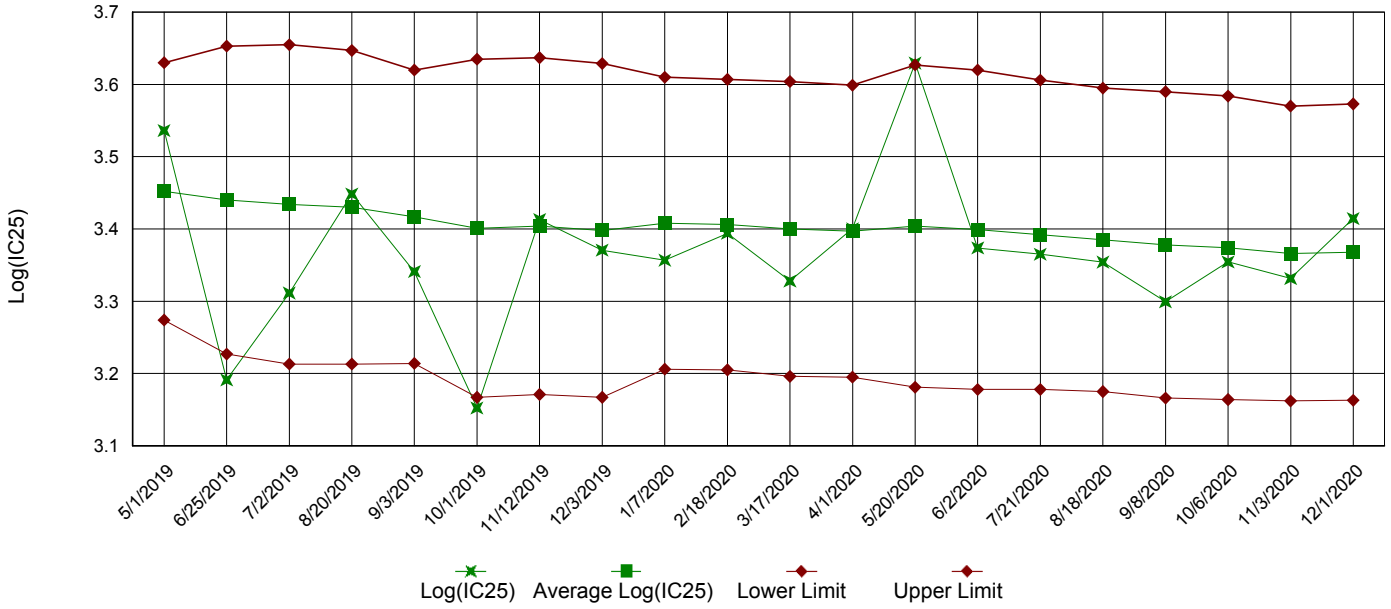
Appendix A3: Test 1000.0

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

LC50 Survival Data



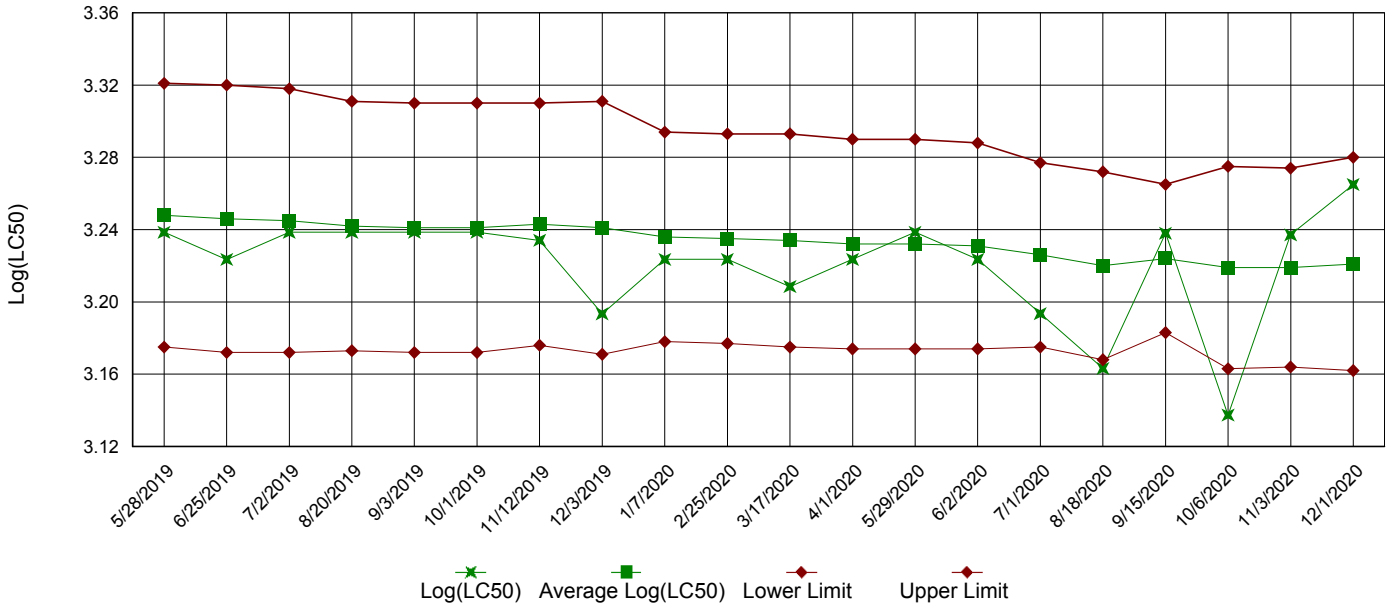
IC25 Growth Data



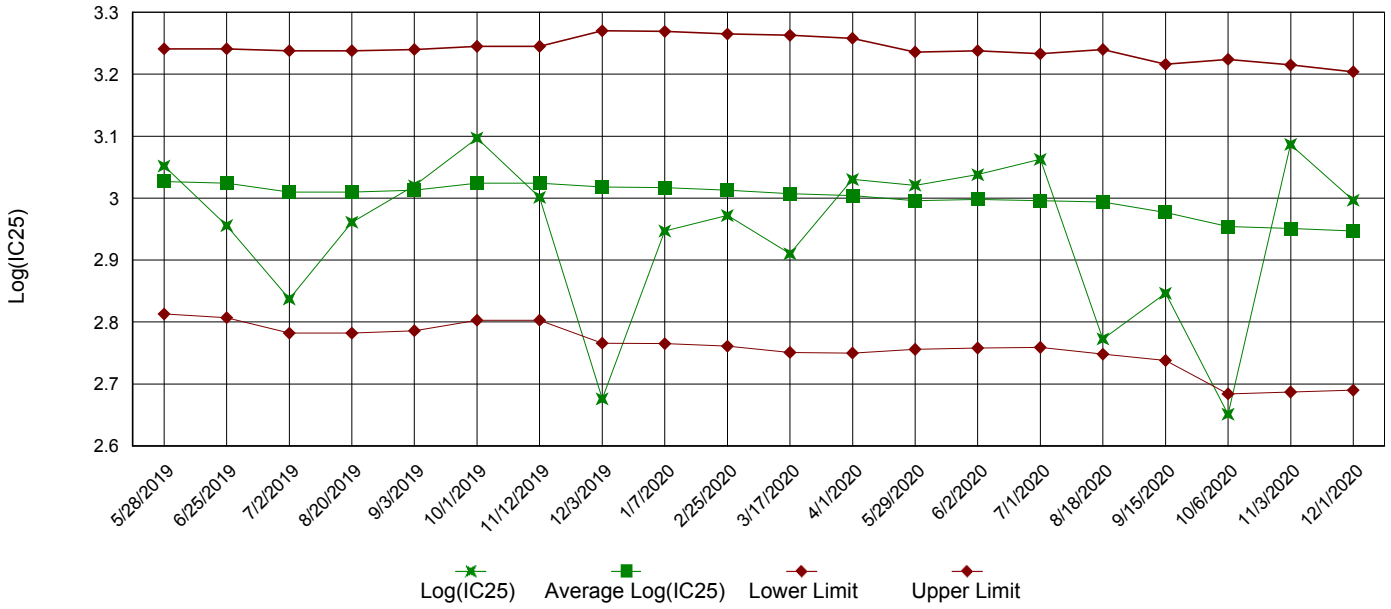
Appendix A3: Test 1002.0

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: Trumann Water and Sewer Commission

NPDES No.: AFIN 56-00047

Date and Time Test Initiated: January 12, 2021 at 1320
Date and Time Test Terminated: January 19, 2021 at 1505
Dilution water used: Moderately Hard

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
4 %	100	100	87.5	87.5	100	100	100	95.0	7.21
5 %	87.5	100	100	87.5	100	100	100	95.0	7.21
7 %	87.5	100	87.5	100	100	97.5	97.5	95.0	7.21
9 %	87.5	100	87.5	87.5	100	100	100	92.5	7.40
12 %	100	75.0	100	100	87.5	100	100	92.5	12.1

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.561	0.499	0.454	0.546	0.392	0.49	14.1
4 %	0.672	0.424	0.465	0.441	0.544	0.509	20.0
5 %	0.436	0.476	0.546	0.482	0.502	0.488	8.22
7 %	0.438	0.584	0.419	0.608	0.540	0.518	16.5
9 %	0.406	0.539	0.551	0.409	0.454	0.472	14.8
12 %	0.391	0.454	0.526	0.481	0.421	0.455	11.5

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: 12 % (TOP6C)

6. LOEC Pimephales Lethality: 12 % (TXP6C)

7. NOEC Pimephales Sublethality: 12 % (TPP6C)

8. LOEC Pimephales Sublethality: 12 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 14.8 (TQP6C)

10. Sublethality for this test: 12 % (51714 or 51714S)

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 310, 343, 356

Test Initiated: DATE: January 12, 2021 TIME: 1320
Test Terminated: DATE: January 19, 2021 TIME: 1505

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	6.9	7.1	6.7	6.1	7.4	7.4
	Final	6.3	6.5	5.4	5.4	6.2	5.2	5.8
pH	Initial	8.2	8.2	8.2	8.2	8.2	8.2	8.2
	Final	7.9	7.9	7.7	7.7	7.6	7.5	7.6

DILUTION 4 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	7.0	6.7	6.4	6.3	7.6	7.3
	Final	6.4	6.6	5.3	5.2	5.9	5.1	5.6
pH	Initial	8.2	8.2	8.2	8.2	8.2	8.2	8.1
	Final	7.9	7.9	7.7	7.6	7.5	7.5	7.6

DILUTION 5 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	6.9	7.0	6.2	6.3	7.6	7.3
	Final	6.4	8.3	5.5	5.4	6.2	5.5	5.5
pH	Initial	8.2	8.2	8.2	8.1	8.2	8.2	8.1
	Final	7.9	7.9	7.8	7.7	7.6	7.5	7.6

DILUTION 7 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	6.6	6.9	6.3	6.2	7.4	6.9
	Final	6.3	6.4	5.6	5.3	6.2	5.7	6.1
pH	Initial	8.2	8.2	8.2	8.1	8.2	8.2	8.1
	Final	8.0	8.0	7.8	7.7	7.6	7.6	7.7

DILUTION 9 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	6.6	7.0	6.3	6.2	7.5	7.2
	Final	6.4	6.3	5.4	5.5	6.2	5.4	6.1
pH	Initial	8.2	8.2	8.2	8.1	8.2	8.2	8.2
	Final	7.9	7.8	7.7	7.8	7.6	7.6	7.7

DILUTION 12 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	6.7	6.8	6.3	6.2	7.6	7.4
	Final	6.5	6.3	5.3	5.5	6.1	5.8	5.6
pH	Initial	8.2	8.2	8.2	8.2	8.2	8.2	8.2
	Final	8.0	7.9	7.8	7.8	7.6	7.6	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID	
120	39	450	<0.05	AR0035602	11-JAN-21
120	40	440	<0.05	AR0035602	13-JAN-21
120	39	450	<0.05	AR0035602	15-JAN-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	81	320	<0.05	251741-1

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Trumann Water and Sewer Commission

NPDES No.: AFIN 56-00047

Date and Time Test Initiated: January 12, 2021 at 1250
Date and Time Test Terminated: January 18, 2021 at 1445
Dilution water used: Moderately Hard

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent 4 %	5 %	7 %	9 %	12 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	90.0	100	100
6 day	100	100	90.0	90.0	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent 4 %	5 %	7 %	9 %	12 %
A	29	32	29	31	29	26
B	28	32	29	34	25	34
C	26	25	30	29	29	29
D	27	30	28	30	28	34
E	33	32	32	27	25	28
F	26	20	0	0	22	16
G	26	29	29	25	30	28
H	28	28	28	27	26	28
I	32	30	27	30	31	26
J	28	24	28	32	29	27
Mean per Adult	28.3	28.2	26.0	26.5	27.4	27.6
Mean per Surviving Adult	28.3	28.2	28.9	29.4	27.4	27.6
CV %	8.66	14.3	5.03	9.47	10.2	18.1

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: 12 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 12 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 12 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 12 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 10.2 (TQP3B)

10. Sublethality for this test: 12 % (51710 or 51710Q)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 310, 343, 356

Test Initiated: DATE: January 12, 2021 TIME: 1250
Test Terminated: DATE: January 18, 2021 TIME: 1445

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	6.9	7.1	6.7	6.1	7.4	7.4
	Final	7.5	7.5	6.6	7.5	7.2	7.4	--
pH	Initial	8.2	8.2	8.2	8.2	8.2	8.2	8.2
	Final	8.6	8.4	8.5	8.3	8.3	8.4	--

DILUTION 4 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	7.0	6.7	6.4	6.3	7.6	7.3
	Final	7.5	6.7	6.5	7.4	6.9	7.5	--
pH	Initial	8.2	8.2	8.2	8.2	8.2	8.2	8.1
	Final	8.6	8.4	8.6	8.3	8.3	8.4	--

DILUTION 5 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	6.9	7.0	6.2	6.3	7.6	7.3
	Final	7.2	6.6	6.4	7.3	6.9	7.5	--
pH	Initial	8.2	8.2	8.2	8.1	8.2	8.2	8.1
	Final	8.6	8.4	8.5	8.3	8.3	8.4	--

DILUTION 7 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.6	6.6	6.9	6.3	6.2	7.4	6.9
	Final	6.7	6.5	6.6	7.3	6.8	7.3	--
pH	Initial	8.2	8.2	8.2	8.1	8.2	8.2	8.1
	Final	8.6	8.4	8.6	8.4	8.3	8.5	--

DILUTION 9 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.7	6.6	7.0	6.3	6.2	7.5	7.2
	Final	6.9	6.7	6.6	7.4	7.1	7.5	--
pH	Initial	8.2	8.2	8.2	8.1	8.2	8.2	8.2
	Final	8.6	8.4	8.6	8.4	8.3	8.4	--

DILUTION 12 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	6.7	6.8	6.3	6.2	7.6	7.4
	Final	6.8	6.7	6.6	7.5	6.9	7.6	--
pH	Initial	8.2	8.2	8.2	8.2	8.2	8.2	8.2
	Final	8.6	8.4	8.6	8.6	8.3	8.5	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID	
120	39	450	<0.05	AR0035602	11-JAN-21
120	40	440	<0.05	AR0035602	13-JAN-21
120	39	450	<0.05	AR0035602	15-JAN-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	81	320	<0.05	251741-1

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: TRUMANN WATER WORKS
Project Reference: AR0035602
Project Manager: Scotty Jones
Sampled By: LORRE Holt

PO No.

Sample Matrix: WATER / SOIL

No of BOTTLES

Analyses Requested: BIOMONITORING-CHRONIC CD+FH

AIC Control No: 25195
AIC Proposal No:
Carrier: FX
Received Temperature °C: 0.2
Remarks

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES
1	AR0035602	1/10/21 8:00 AM - 1/11/21 8:00 AM		V	V		1
		Container Type	P				
		Preservative	ND				

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: LORRE Holt
Phone: 870-483-2882 Fax: 870 483-10525
Report Attention to: LORRE HOLT
Report Address to: 704 HWY 463 N
TRUMANN, AR 72472

Relinquished By	Date/Time	Received By	Date/Time
Lorre Holt	1/11/21 11:20		
Relinquished By:	Date/Time	Received in Lab By: D. BROWN	Date/Time 1-12-21 1015

Comments: TRK# 0201 7824 8078 4904

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Trumann Water Works
e: AR0035602
Scotty Jones
LORRE Holt

PO No.

Sample Matrix: WATER | SOIL

No of BOTTLES

Analyses Requested: Biomonitoring-Chronic CD+FH

AIC Control No: 251995
AIC Proposal No:
Carrier: FX
Received Temperature °C: 0.1

Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	BOTTLES	Remarks
AR0035602	1/12/21 - 1/13/21 8:00 AM - 8:00 AM		V	V		1	
	Container Type	P					
	Preservative	NO					

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

Turn Time Requested: (Please circle)
5 or EXPEDITED IN ____ DAYS
Results requested by: _____
Add AIC contact with questions: LORRE HOLT
870-483-2882 Fax: 870 483-10525
Attention to: LORRE HOLT
Address to: 704 Hwy 463 N
Trumann, AR 72472

Relinquished By:	Date/Time	Received By:	Date/Time
Lorre Holt	1/13/21 10:10 AM		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
			1-14-21 1018

Comments: Fedex 7825 6461 7771

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Trumann Water Works
e: AR0035602
Scotty Jones
LORRE HOLT

PO No.

Sample Matrix

No of BOTTLES

Analyses Requested: Biomonitoring-Chronic CD+FH

AIC Control No: 251995
AIC Proposal No:
Carrier: Fed Ex
Received Temperature °C: 0.1

Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	No of BOTTLES	Remarks
AR0035602	1/14/21 8:00 AM - 1/15/21 8:00 AM		V	V		1	
	Container Type	P					
	Preservative	NO					

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

Time Requested: (Please circle)
5 or EXPEDITED IN ____ DAYS
results requested by: _____
d AIC contact with questions: LORRE HOLT
70-483-2882 Fax: 870 483-6525
ention to: LORRE HOLT
dress to: 704 Hwy 463 N
TRUMANN, AR 72472

Relinquished By:	Date/Time	Received By:	Date/Time
Lorre Holt	1/15/21 12:47 PM		
Relinquished By:	Date/Time	Received in Lab By: Annamarie Stearns	Date/Time 16 Jan 21 0845

Comments: