



May 5, 2021

Biomonitoring Testing
for

Control No. 254899-1

Prepared for:

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

Prepared by:

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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*
NPDES Permit No. AR0035602 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:

The third effluent sample was not received on Saturday, as required. The test was continued with the second effluent sample submitted. Native Daphnia were observed in this sample during testing.

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

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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	85.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.638	PASS
Control Growth CV < or = 40%	13.6	PASS
Growth Minimum Significant Difference 12 to 30%	15.3	PASS
Critical Dilution CV < or = 40%	9.34	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	32.2	PASS
Control CV < or = 40% per Surviving Female	8.26	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.1	PASS
Critical Dilution CV < or = 40%	17.2	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0035602 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:
- b. Chemical Data:

Analysis	Sample 1	Sample 2
Dissolved oxygen (mg/l)	7.5	7.2
pH (standard units)	7.6	7.7
Alkalinity (mg/l as CaCO ₃)	72	76
Hardness (mg/l as CaCO ₃)	30	32
Conductivity (umhos/cm)	390	400
Residual Chlorine (mg/l)	<0.05	<0.05
Ammonia as N (mg/l)	0.42	0.29

2. Dilution Water Samples:

Moderately Hard

Analysis	254666-1	254929-1
Dissolved oxygen (mg/l)	7.4	7.6
pH (standard units)	7.9	8.1
Alkalinity (mg/l as CaCO ₃)	62	64
Hardness (mg/l as CaCO ₃)	85	83
Conductivity (umhos/cm)	320	320
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: April 27, 2021 at 1317
Date & Time Test Terminated: May 04, 2021 at 1350
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: April 27, 2021 at 1225
Date & Time Test Terminated: May 03, 2021 at 1340
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 Wilcoxon's Rank Sum with Bonferroni Adjustment 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 April 13, 2021 at 1120 to April 20, 2021 at 1105

The results were as follows: (Control No. 254505-1.)

Survival LC-50: 2589 mg/l

Growth IC-25: 2277 mg/l

Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on April 16, 2021 at 1203 to April 22, 2021 at 1035

The results were as follows: (Control No. 254505-2.)

Survival LC-50: 1948.9 mg/l

Reproduction IC-25: 922.9 mg/l

Reproduction PMSD: 13.9

V. Organism History

Pimephales promelas (Fathead minnow)

Date: April 27, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: April 27, 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

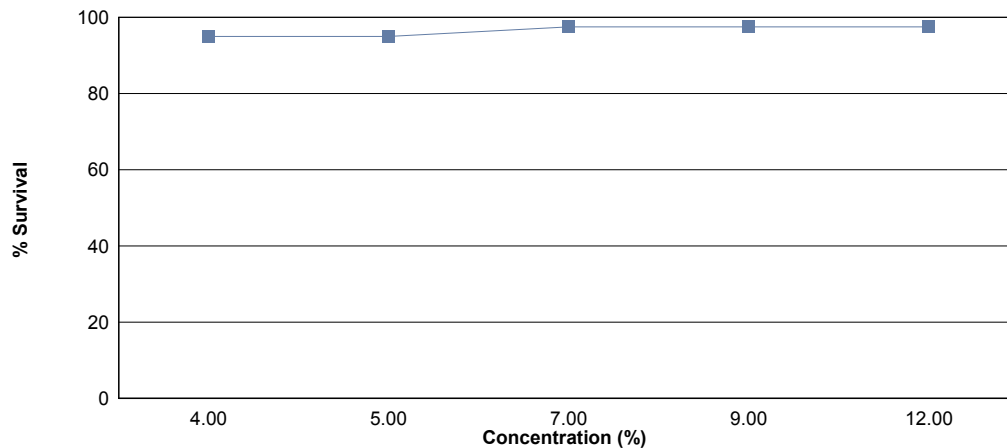
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 April 27, 2021 at 1317 and continued through May 04, 2021 at 1350. 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	85.0	0.542
4 %	95.0	0.517
5 %	95.0	0.511
7 %	97.5	0.561
9 %	97.5	0.537
12 %	97.5	0.533

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

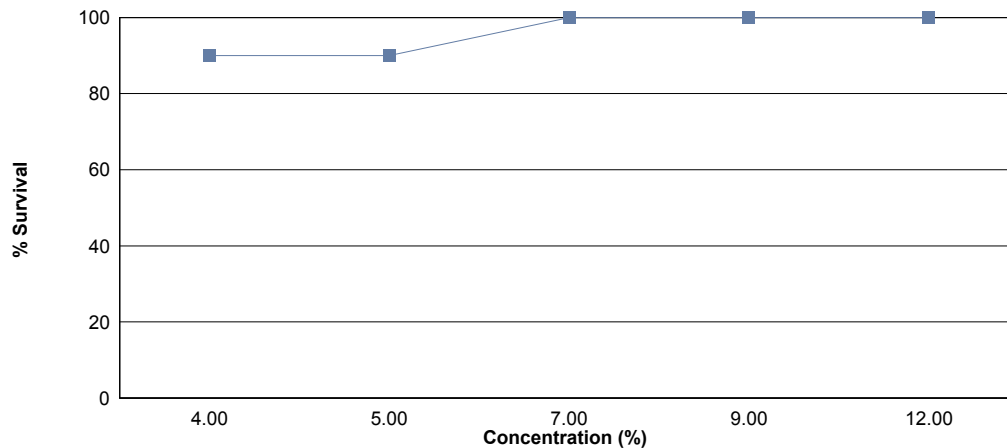
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 April 27, 2021 at 1225 and continued through May 03, 2021 at 1340. 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 <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	32.2
4 %	90.0	29.4
5 %	90.0	30.1
7 %	100	34.2
9 %	100	34.5
12 %	100	33.0

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 27, 2021 at 1317

Date and Time Test Terminated: May 04, 2021 at 1350

Concentration Replicate		Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	6	6	6	5
	B	8	8	8	8	8	8	8
	C	8	8	7	7	7	7	7
	D	8	8	8	7	7	7	6
	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	8
	E	8	8	8	8	7	7	7
5 %	A	8	7	7	7	7	7	7
	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	7
7 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	7	7	7	7	7
	E	8	8	8	8	8	8	8
9 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
12 %	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	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: April 27, 2021 at 1317

Test Terminated: May 04, 2021 at 1350

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76828	.77231	0.00403	8	0.504
	B	.76889	.77399	0.00510	8	0.638
	C	.77203	.77620	0.00417	8	0.521
	D	.76776	.77139	0.00363	8	0.454
	E	.76777	.77253	0.00476	8	0.595
4 %	A	.77399	.77855	0.00456	8	0.570
	B	.77303	.77755	0.00452	8	0.565
	C	.77265	.77620	0.00355	8	0.444
	D	.77053	.77436	0.00383	8	0.479
	E	.76436	.76859	0.00423	8	0.529
5 %	A	.77391	.77807	0.00416	8	0.520
	B	.77750	.78166	0.00416	8	0.520
	C	.77456	.77878	0.00422	8	0.528
	D	.77060	.77424	0.00364	8	0.455
	E	.77006	.77430	0.00424	8	0.530
7 %	A	.77693	.78178	0.00485	8	0.606
	B	.77628	.78041	0.00413	8	0.516
	C	.76502	.76980	0.00478	8	0.598
	D	.77659	.78038	0.00379	8	0.474
	E	.77398	.77887	0.00489	8	0.611
9 %	A	.77194	.77640	0.00446	8	0.558
	B	.77651	.78036	0.00385	8	0.481
	C	.77823	.78277	0.00454	8	0.568
	D	.77384	.77857	0.00473	8	0.591
	E	.77370	.77759	0.00389	8	0.486
12 %	A	.77138	.77582	0.00444	8	0.555
	B	.77273	.77722	0.00449	8	0.561
	C	.77724	.78085	0.00361	8	0.451
	D	.77264	.77675	0.00411	8	0.514
	E	.76747	.77214	0.00467	8	0.584

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 27, 2021 at 1225

Date and Time Test Terminated: May 03, 2021 at 1340

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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	6	6	6	5	5	5	0	6	5	5	49	10	4.90	
4	0	0	0	0	0	0	5	0	0	9	14	10	1.40	
5	13	10	11	12	10	10	12	10	11	0	99	10	9.90	
6	19	16	16	15	13	17	15	17	17	15	160	10	16.0	
7														
8														
TOTAL	38	32	33	32	28	32	32	33	33	29	322	10	32.2	

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	6	6	6	5	4	4	4	6	6	5	52	10	5.20
4	0	0	0	0	0	1	0	0	0	0	1	10	0.100
5	12	12	10	13	10	11	13	12	13	12	118	10	11.8
6	14	13	0X	13	13	15	14	16	13	12	123	9	13.7
7													
8													
TOTAL	32	31	16	31	27	31	31	34	32	29	294	10	29.4

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	6	6	6	6	5	4	5	6	5	5	54	10	5.40
4	0	0	0	0	0	0	0	0	1	14	15	10	1.50
5	12	13	13	13	10	12	13	5LIA	12	4	102	9	11.3
6	18	8	10	14	17	17	16	LIA	14	16	130	9	14.4
7													
8													
TOTAL	36	27	29	33	32	33	34	6	32	39	301	10	30.1

LIA = Lost in Analysis

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 27, 2021 at 1225

Date and Time Test Terminated: May 03, 2021 at 1340

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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	6	7	6	5	5	5	6	6	5	6	57	10	5.70	
4	0	0	0	1	0	0	0	0	0	0	1	10	0.100	
5	14	14	14	12	11	10	13	13	14	12	127	10	12.7	
6	0	20	16	17	15	15	19	19	18	18	157	10	15.7	
7														
8														
TOTAL	20	41	36	35	31	30	38	38	37	36	342	10	34.2	

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	6	7	7	6	4	6	5	6	6	4	57	10	5.70	
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
5	14	14	15	13	10	10	12	10	12	13	123	10	12.3	
6	18	11	21	17	16	18	19	5	20	20	165	10	16.5	
7														
8														
TOTAL	38	32	43	36	30	34	36	21	38	37	345	10	34.5	

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	LIA	0	0	0	0	0	0	9	0.00	
3	5	0	7	6	LIA	4	6	6	5	6	45	9	5.00	
4	0	5	0	0	LIA	0	0	0	0	0	5	9	0.556	
5	15	10	14	12	LIA	10	12	14	14	14	115	9	12.8	
6	13	0	18	20	LIA	11	23	16	14	17	132	9	14.7	
7														
8														
TOTAL	33	15	39	38		25	41	36	33	37	297	9	33.0	

LIA = Lost in Analysis

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	0.62500	0.91174
1	Control	2	1.00000	1.39310
1	Control	3	0.87500	1.20940
1	Control	4	0.75000	1.04720
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	1.00000	1.39310
2	4 %	5	0.87500	1.20940
3	5 %	1	0.87500	1.20940
3	5 %	2	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	1.00000	1.39310
3	5 %	5	0.87500	1.20940
4	7 %	1	1.00000	1.39310
4	7 %	2	1.00000	1.39310
4	7 %	3	1.00000	1.39310
4	7 %	4	0.87500	1.20940
4	7 %	5	1.00000	1.39310
5	9 %	1	1.00000	1.39310
5	9 %	2	0.87500	1.20940
5	9 %	3	1.00000	1.39310
5	9 %	4	1.00000	1.39310
5	9 %	5	1.00000	1.39310
6	12 %	1	1.00000	1.39310
6	12 %	2	1.00000	1.39310
6	12 %	3	0.87500	1.20940
6	12 %	4	1.00000	1.39310
6	12 %	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 = 0.3427 W = 0.8683 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 %	32.00	16.00	5.00	
3	5 %	32.00	16.00	5.00	
4	7 %	33.50	16.00	5.00	
5	9 %	33.50	16.00	5.00	
6	12 %	33.50	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
<p>D = 0.07413 W = 0.9306 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 2.577 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.008151	0.00163	0.5277	
Within (Error)	24	0.07413	0.003089		
Total	29	0.08228			
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	0.5424	0.5424			
2	4 %	0.5174	0.5174	0.7112		
3	5 %	0.5106	0.5106	0.9047		
4	7 %	0.561	0.561	-0.5291		
5	9 %	0.5368	0.5368	0.1593		
6	12 %	0.533	0.533	0.2674		
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	0.08296	15.3	0.025	
3	5 %	5	0.08296	15.3	0.0318	
4	7 %	5	0.08296	15.3	-0.0186	
5	9 %	5	0.08296	15.3	0.0056	
6	12 %	5	0.08296	15.3	0.0094	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
4 %	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
5 %	9	0	9
Total	19	0	19

Critical Fisher's value (10,9,10) ($\alpha=0.05$) is 5. b value is 9. Since b is greater than 5 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 %	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
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 %	9	0	9
Total	19	0	19

Critical Fisher's value (10,9,10) ($\alpha=0.05$) is 5. b value is 9. Since b is greater than 5 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	1	
2	5 %	9	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	9	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
<p>D = 0.199 D* = 1.536 Critical D* = 1.035 (alpha = 0.01, N = 58)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Wilcoxon's Rank Sum w/ Bonferroni Adjustment					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	Reps	Sig 0.05
1	Control				
2	4 %	81.50	74.00	10	
3	5 %	97.50	61.00	9	
4	7 %	124.00	74.00	10	
5	9 %	126.00	74.00	10	
6	12 %	107.50	61.00	9	
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	167.1	33.42	1.108	
Within (Error)	52	1568	30.15		
Total	57	1735			
Critical F = 3.39 (alpha = 0.01, df = 5,52) 2.39 (alpha = 0.05, df = 5,52)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

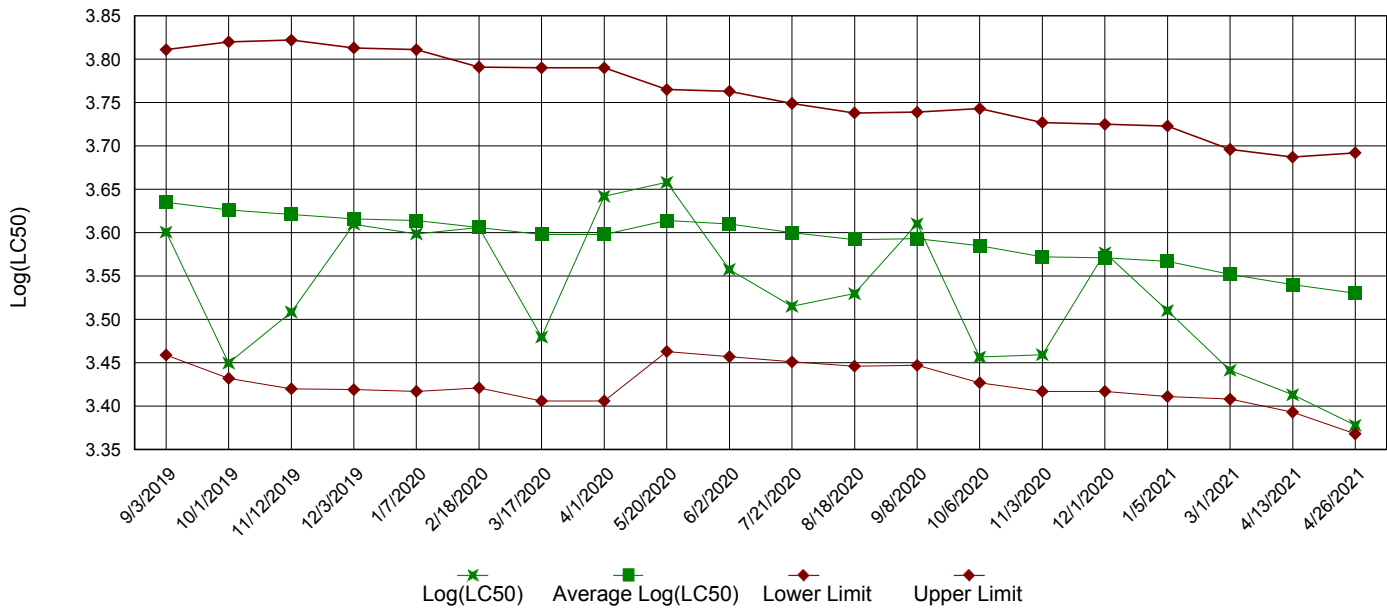
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	32.2	32.2			
2	4 %	29.4	29.4	1.14		
3	5 %	32.778	32.778	-0.2291		
4	7 %	34.2	34.2	-0.8145		
5	9 %	34.5	34.5	-0.9366		
6	12 %	33	33	-0.3171		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,52) WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.						

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	5.672	17.6	2.8	
3	5 %	9	5.828	18.1	-0.578	
4	7 %	10	5.672	17.6	-2	
5	9 %	10	5.672	17.6	-2.3	
6	12 %	9	5.828	18.1	-0.8	

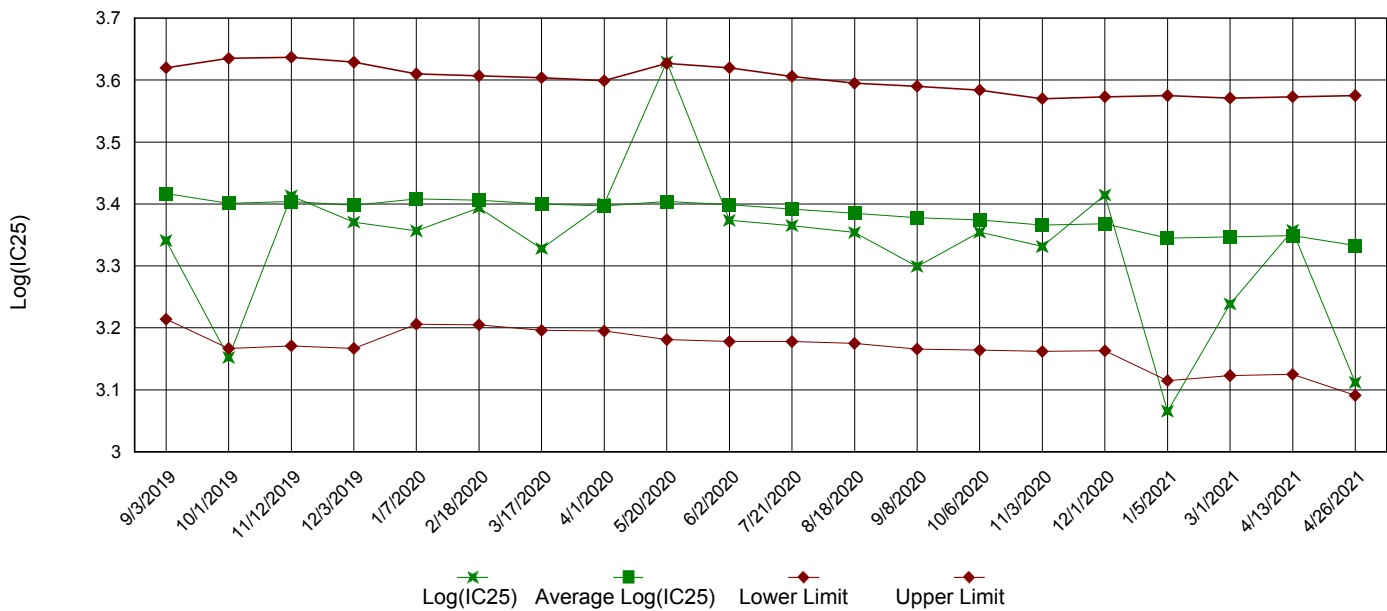
Appendix A3: Test 1000.0

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

LC50 Survival Data

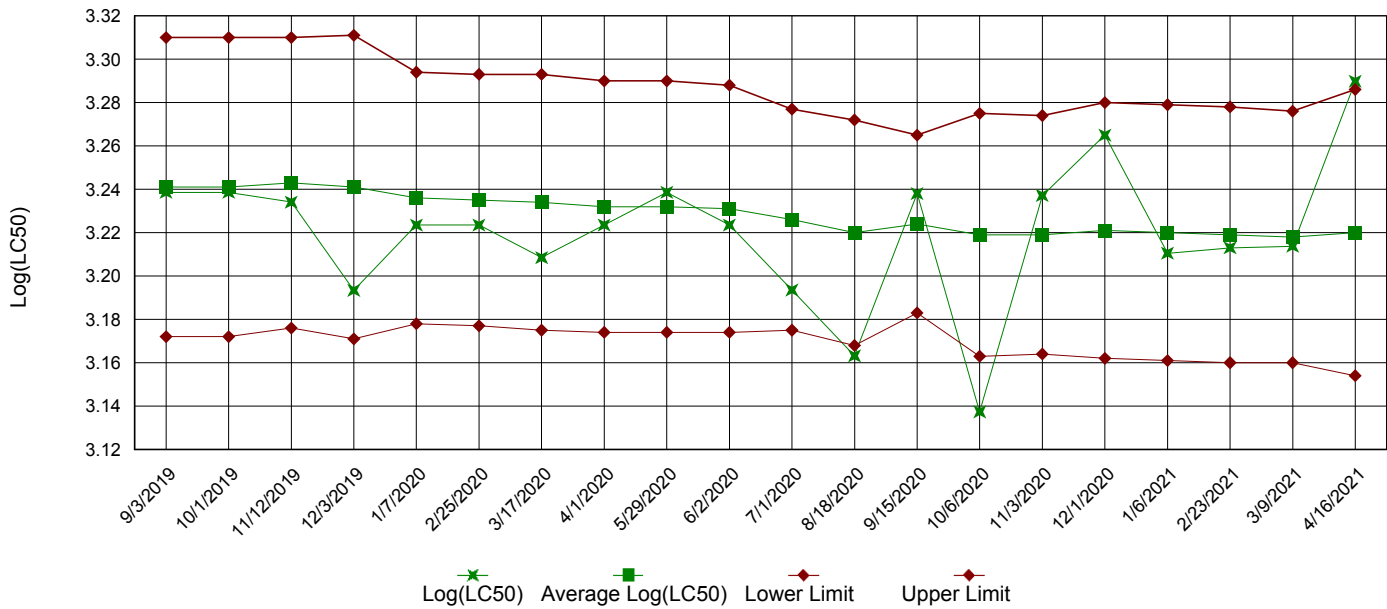


IC25 Growth Data

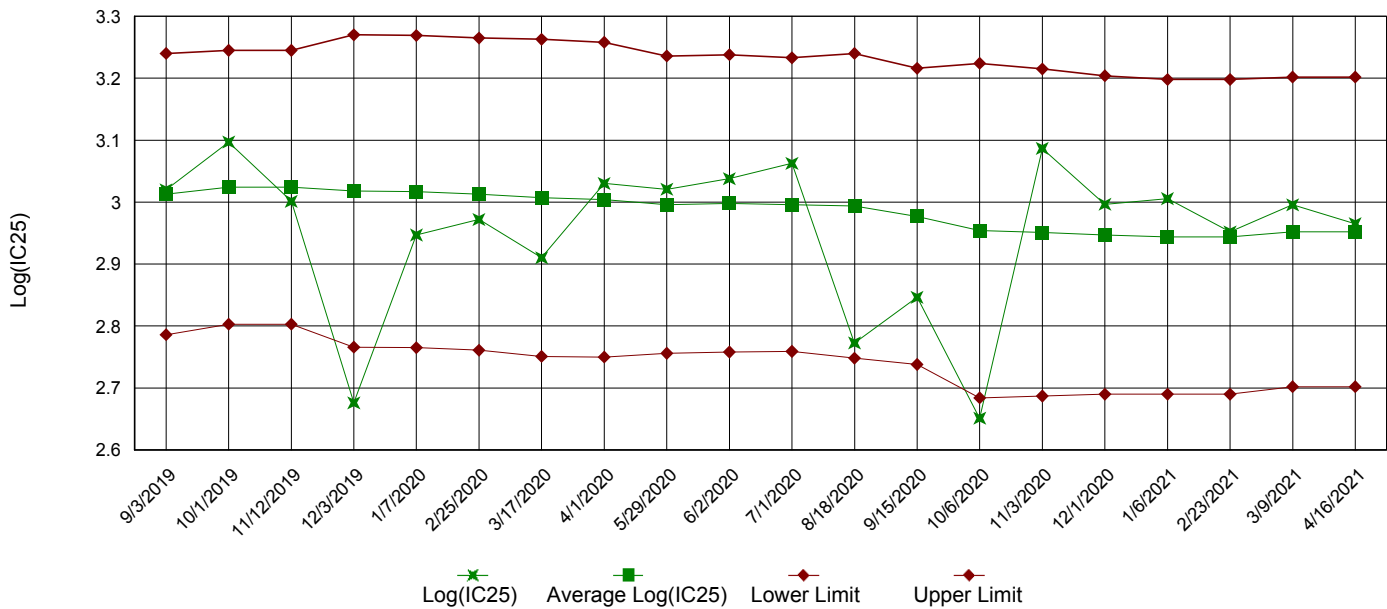


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: April 27, 2021 at 1317

Date and Time Test Terminated: May 04, 2021 at 1350

Dilution water used: Moderately Hard

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	62.5	100	87.5	75.0	100	100	100	85.0	19.2
4 %	100	100	87.5	100	87.5	100	100	95.0	7.21
5 %	87.5	100	100	100	87.5	100	97.5	95.0	7.21
7 %	100	100	100	87.5	100	100	100	97.5	5.73
9 %	100	87.5	100	100	100	100	100	97.5	5.73
12 %	100	100	87.5	100	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.504	0.638	0.521	0.454	0.595	0.542	13.6
4 %	0.570	0.565	0.444	0.479	0.529	0.517	10.6
5 %	0.520	0.520	0.528	0.455	0.530	0.511	6.15
7 %	0.606	0.516	0.598	0.474	0.611	0.561	11.1
9 %	0.558	0.481	0.568	0.591	0.486	0.537	9.34
12 %	0.555	0.561	0.451	0.514	0.584	0.533	9.82

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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<u> </u> YES	<u> </u> 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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<u> </u> YES	<u> </u> 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: 13.6 (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.: AR0035602 AFIN 56-00047
 CONTACT: Mr. Scotty Jones
 ANALYST: 280, 343, 356, 357

Test Initiated: DATE: April 27, 2021 TIME: 1317
 Test Terminated: DATE: May 04, 2021 TIME: 1350

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.4	7.6	7.6	7.6	6.8	7.4
Final	6.7	6.5	6.7	6.7	6.3	5.8	7.0
pH Initial	7.9	7.9	8.1	8.0	8.0	7.9	7.9
Final	7.8	7.8	7.8	7.7	7.8	7.6	7.8

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.7	7.8	7.7	7.6	7.2	7.5
Final	6.7	6.3	6.9	6.6	6.4	5.6	6.6
pH Initial	8.0	8.0	8.1	8.1	8.1	8.0	7.9
Final	7.8	7.6	7.7	7.6	7.7	7.4	7.7

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.5	7.6	7.4	7.8	7.5	7.3	7.6
Final	6.8	6.1	6.6	6.4	6.4	5.7	6.3
pH Initial	8.0	8.0	8.0	8.0	8.1	8.0	7.9
Final	7.8	7.7	7.7	7.6	7.7	7.5	7.6

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.7	7.4	7.7	7.8	6.7	7.4
Final	7.0	6.5	7.0	6.8	5.9	5.4	6.4
pH Initial	7.9	8.0	8.1	8.0	8.1	8.0	7.9
Final	7.8	7.7	7.8	7.7	7.6	7.4	7.6

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.6	7.4	7.7	7.6	6.9	7.5
Final	6.8	6.3	6.9	6.8	6.4	6.1	7.0
pH Initial	7.9	8.0	8.1	8.0	8.1	8.0	7.9
Final	7.8	7.7	7.8	7.7	7.7	7.6	7.7

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.9	7.6	7.7	7.5	7.4	7.4
Final	6.8	6.4	6.8	6.4	6.6	5.8	6.8
pH Initial	7.9	8.0	8.0	8.1	8.0	8.1	7.9
Final	7.9	7.6	7.7	7.6	7.8	7.4	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
72	30	390	<0.05	AR0035602 26-APR-21
76	32	400	<0.05	AR0035602 28-APR-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	85	320	<0.05	254666-1
64	83	320	<0.05	254929-1

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Trumann Water and Sewer Commission

NPDES No.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: April 27, 2021 at 1225

Date and Time Test Terminated: May 03, 2021 at 1340

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	111
48 hour	100	100	100	100	100	100
6 day	100	90.0	90.0	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	38	32	36	20	38	33
B	32	31	27	41	32	15
C	33	16	29	36	43	39
D	32	31	33	35	36	38
E	28	27	32	31	30	
F	32	31	33	30	34	25
G	32	31	34	38	36	41
H	33	34	6	38	21	36
I	33	32	32	37	38	33
J	29	29	39	36	37	37
Mean per Adult	32.2	29.4	30.1	34.2	34.5	33.0
Mean per Surviving Adult	32.2	30.9	32.8	34.2	34.5	33.0
CV %	8.26	6.36	10.8	17.4	17.2	24.9

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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<u> </u> YES	<u> </u> NO

2. Wilcoxon's Rank Sum with Bonferroni Adjustment 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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<u> </u> YES	<u> </u> 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: 17.2 (TQP3B)
10. Sublethality for this test: 12 % (51710 or 51710Q)

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AR0035602 AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 343, 356, 357

Test Initiated: DATE: April 27, 2021 TIME: 1225
Test Terminated: DATE: May 03, 2021 TIME: 1340

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.4	7.4	7.6	7.6	7.6	6.8	7.4
Final	7.5	7.4	7.2	7.3	7.4	7.5	--
pH Initial	7.9	7.9	8.1	8.0	8.0	7.9	7.9
Final	8.3	8.4	8.4	8.0	8.3	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	7.4	7.7	7.8	7.7	7.6	7.2	7.5
Final	7.3	7.6	7.3	7.5	7.8	7.5	--
pH Initial	8.0	8.0	8.1	8.1	8.1	8.0	7.9
Final	8.4	8.5	8.3	8.1	8.4	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	7.5	7.6	7.4	7.8	7.5	7.3	7.6
Final	7.4	7.6	8.1	7.4	7.6	7.7	--
pH Initial	8.0	8.0	8.0	8.0	8.1	8.0	7.9
Final	8.4	8.5	8.4	8.1	8.4	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.4	7.7	7.4	7.7	7.8	6.7	7.4
Final	7.6	7.7	7.6	7.2	7.3	7.5	--
pH Initial	7.9	8.0	8.1	8.0	8.1	8.0	7.9
Final	8.3	8.4	8.4	8.1	8.3	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	7.4	7.6	7.4	7.7	7.6	6.9	7.5
Final	7.6	7.6	7.9	7.3	7.2	7.5	--
pH Initial	7.9	8.0	8.1	8.0	8.1	8.0	7.9
Final	8.3	8.4	8.4	8.2	8.4	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.4	7.9	7.6	7.7	7.5	7.4	7.4
Final	7.6	7.4	8.2	7.4	7.8	7.6	--
pH Initial	7.9	8.0	8.0	8.1	8.0	8.1	7.9
Final	8.4	8.4	8.4	8.2	8.4	8.3	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
72	30	390	<0.05	AR0035602 26-APR-21
76	32	400	<0.05	AR0035602 28-APR-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	85	320	<0.05	254666-1
64	83	320	<0.05	254929-1

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

TRUMANN Water Works AR0035202 Scotty Jones LORRE HOLT		PO No. AR0035202		Analyses Requested BIDM @ n. 10RM - CHRONIC CD + FH		AIC Control No. 254899	
Sample Matrix WATER		No of BOTTLES 1		Carrier: Fedex		AIC Proposal No.:	
Container Type Plastic		Sample Matrix SOIL		Received Temperature °C 0.1		Remarks	
Preservative NIO		WATER		Field pH calibration on @			
NO = none P = Plastic S = Sulfuric acid pH2		GRA B		Buffer:			
Time Requested: (Please circle) EXPEDITED IN _____ DAYS		COM P		T = Sodium Thiosulfate Z = Zinc acetate			
Results requested by: AIC contact with questions: LORRE HOLT 2483 3852 Fax: 870 483-10525		SOIL		Received			
Phone to: LORRE HOLT: 704 HWY 463 N TRUMANN, AR 72472		Relinquished: By: LORRE HOLT		Date/Time 4/24/02 / 1:30 PM			
		Relinquished By:		Date/Time			
				Received in Lab By: Heather Day		Date/Time 4-27-02	
						Comments: 7864 3098 7011	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. TRUMANN Water Works AR0035602 SCOTTY SONES LORRE HOLT		Sample Matrix W A T E R G R A B V		No of BOTTLES 1		Analyses Requested BIRMINGHAM - CHRONIC CD + FH		AIC Control No: 254899 AIC Proposal No: Carrier: FX Received Temperature °C 1.1 Remarks	
Sample Identification AR0035602 4/27/01 8:00 AM 8:00 AM		Date/Time Collected 4/27/01 8:00 AM		Container Type P Preservative NIO		Field pH calibration on @ Buffer:		Received T = Sodium Thiosulfate Z = Zinc acetate	
Time Requested: (Please circle) EXPEDITED IN _____ DAYS		Relinquished By: <i>[Signature]</i> Date/Time: 4/28/01 10:30 AM		Relinquished By: _____ Date/Time: _____		Received in Lab By: P. BROWN Date/Time: 4-29-01 10:16		Comments: 7865 1829 1309	
Results requested by: AIC contact with instructions: LORRE HOLT 2-483-3838 Fax: 870-483-10525 Attention to: LORRE HOLT 704 Hwy 463 N TRUMANN, AR 72472									

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. RUMANN Water Works AR0035602 Scotty Jones LORRE HOLT		Sample Matrix WATER G R A B V		No of BOTTLES 1		Analyses Requested Bismuth - Chronic Cd + Pb		AIC Control No: 254899 AIC Proposal No: Carrier: Fedex Received Temperature °C 10.10 Remarks	
Sample Identification AR0035602 Date/Time Collected 4/28/02 8:00 AM		Container Type Plastic Preservative None		Field pH calibration on @ Buffer: T = Sodium Thiosulfate Z = Zinc acetate		Relinquished By: [Signature] Date/Time: 4/30/02 9:20 AM Received By: [Signature] Date/Time: 5-3-02 0839		Comments: LORRE HOLT 877 483-0525 LORRE HOLT 704 Hwy 463 N RUMANN, AR 72472	

Do Not Lift Using This Tag

ORIGIN ID: JBRA (870) 483-2882
LORRE HOLT
TRUMANN WATER WORKS
204 HIGHWAY 463 N
TRUMANN, AR 72472
UNITED STATES US

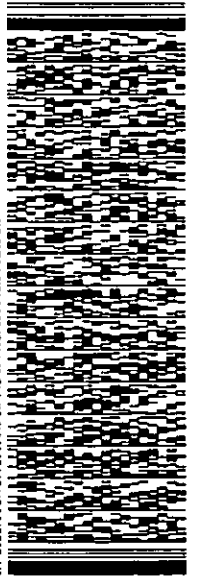
SHIP DATE: 30APR21
ACTWGT: 28.40 LB
CAD: 6394335/SSF2201
DIRS: 17x13x10 IN
BILL CREDIT CARD

**TO SAMPLE RECEIVING
AMERICAN INTERPLEX
8600 KANIS RD**

LITTLE ROCK AR 72204

(501) 224-6080
JNU
P&E

DEPT:

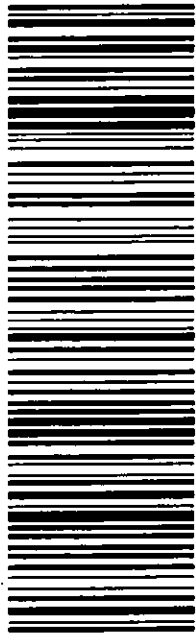


**MON - 03 MAY 10:30A
PRIORITY OVERNIGHT**

TRK# 7866 1175 9926
0201

X2 LITA

72204
AR-US LIT



Part # 156297425-960725EXP 02/22