



August 5, 2021

Biomonitoring Testing
for
Truman

Control No. 257320-1

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*
Truman
NPDES Permit No. AR0035602 AFIN 56-00047

Dear Mr. Scotty Jones:

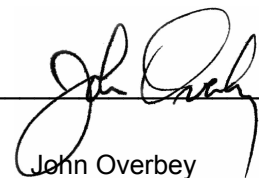
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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:

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 percent minimum significant difference (PMSD) was below the limit of 12. Following additional calculations provided in the EPA document "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination Systems Program", the NOEC for sublethal effects was calculated to be 12 %. **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%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.585	PASS
Control Growth CV < or = 40%	9.05	PASS
Growth Minimum Significant Difference 12 to 30%	10.7	BELOW
Critical Dilution CV < or = 40%	3.84	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	32.3	PASS
Control CV < or = 40% per Surviving Female	18.8	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.6	PASS
Critical Dilution CV < or = 40%	7.86	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: Truman
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.8	7.0	7.6
pH (standard units)	8.0	8.0	7.9
Alkalinity (mg/l as CaCO ₃)	120	130	130
Hardness (mg/l as CaCO ₃)	40	39	38
Conductivity (umhos/cm)	390	400	400
Residual Chlorine (mg/l)	0.070	0.070	0.080
Ammonia as N (mg/l)	0.73	1.0	1.3

2. Dilution Water Samples:
Moderately Hard

Analysis	257131-1	257313-1
Dissolved oxygen (mg/l)	6.9	7.1
pH (standard units)	7.8	7.8
Alkalinity (mg/l as CaCO ₃)	63	63
Hardness (mg/l as CaCO ₃)	80	83
Conductivity (umhos/cm)	290	300
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: July 27, 2021 at 1114
Date & Time Test Terminated: August 03, 2021 at 1040
Type & Volume of Test Chamber: 500 ml disposable beaker
Volume of Sample: 250 ml
Number of Organisms per replicate: 8
Number of Replicates per dilution: 5

Ceriodaphnia dubia Survival and Reproduction Method 1002.0

Date & Time Test Initiated: July 27, 2021 at 1115
Date & Time Test Terminated: August 02, 2021 at 1110
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Source of test organisms: In-house culture

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

a. Test 1000.0 *Pimephales promelas*

b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

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

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

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

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and 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 July 01, 2021 at 1200 to July 08, 2021 at 1005

The results were as follows: (Control No. 256677-1.)

Survival LC-50: 3346 mg/l

Growth IC-25: 2302 mg/l

Growth PMSD: 18.4

Ceriodaphnia dubia

A chronic reference test was performed on July 01, 2021 at 1125 to July 07, 2021 at 1100

The results were as follows: (Control No. 256677-2.)

Survival LC-50: 1772 mg/l

Reproduction IC-25: 1283 mg/l

Reproduction PMSD: 18.3

V. Organism History

Pimephales promelas (Fathead minnow)

Date: July 27, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: July 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.

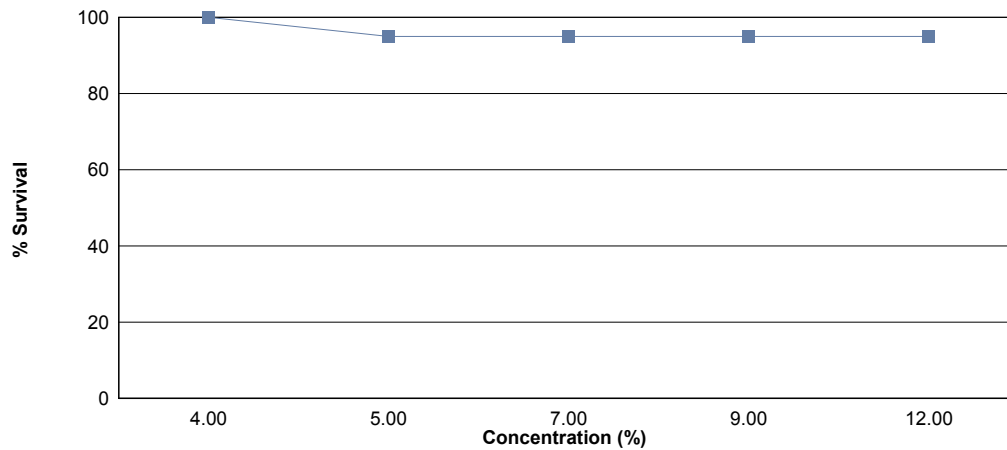
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

The test was initiated on July 27, 2021 at 1114 and continued through August 03, 2021 at 1040. 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

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



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.570
4 %	100	0.575
5 %	95.0	0.537
7 %	95.0	0.520
9 %	95.0	0.533
12 %	95.0	0.530

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

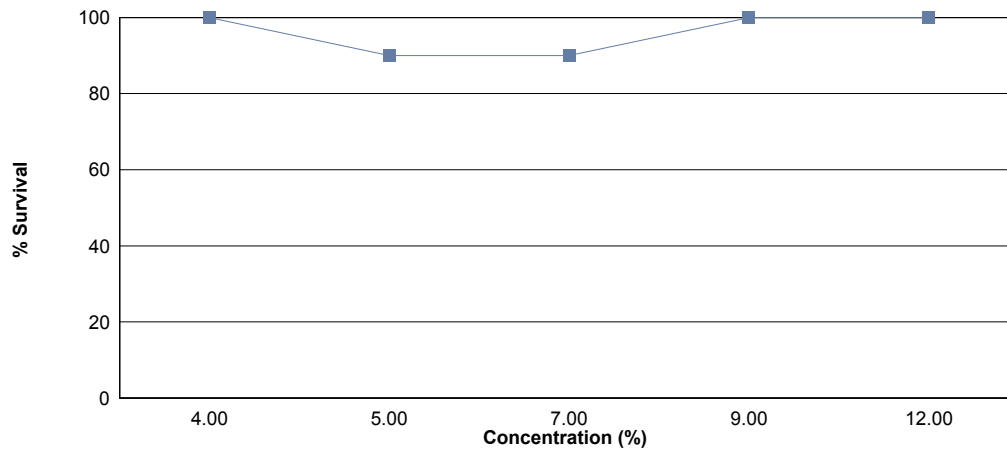
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 July 27, 2021 at 1115 and continued through August 02, 2021 at 1110. 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	32.3
4 %	100	31.1
5 %	90.0	27.1
7 %	90.0	28.3
9 %	100	33.1
12 %	100	31.2

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: July 27, 2021 at 1114

Date and Time Test Terminated: August 03, 2021 at 1040

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: July 27, 2021 at 1114
Test Terminated: August 03, 2021 at 1040

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.66083	.66520	0.00437	8	0.546
	B	.63652	.64072	0.00420	8	0.525
	C	.64221	.64747	0.00526	8	0.658
	D	.66123	.66566	0.00443	8	0.554
	E	.68451	.68903	0.00452	8	0.565
4 %	A	.66646	.67126	0.00480	8	0.600
	B	.65521	.65992	0.00471	8	0.589
	C	.66413	.66847	0.00434	8	0.542
	D	.65938	.66386	0.00448	8	0.560
	E	.66361	.66829	0.00468	8	0.585
5 %	A	.66615	.67074	0.00459	8	0.574
	B	.66882	.67263	0.00381	8	0.476
	C	.66412	.66839	0.00427	8	0.534
	D	.65092	.65583	0.00491	8	0.614
	E	.66360	.66750	0.00390	8	0.488
7 %	A	.65338	.65726	0.00388	8	0.485
	B	.64905	.65329	0.00424	8	0.530
	C	.65182	.65614	0.00432	8	0.540
	D	.66351	.66779	0.00428	8	0.535
	E	.67537	.67944	0.00407	8	0.509
9 %	A	.65604	.66043	0.00439	8	0.549
	B	.65400	.65827	0.00427	8	0.534
	C	.65301	.65740	0.00439	8	0.549
	D	.64524	.64952	0.00428	8	0.535
	E	.65281	.65680	0.00399	8	0.499
12 %	A	.65339	.65798	0.00459	8	0.574
	B	.64991	.65458	0.00467	8	0.584
	C	.65166	.65593	0.00427	8	0.534
	D	.64180	.64554	0.00374	8	0.468
	E	.64708	.65101	0.00393	8	0.491

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 27, 2021 at 1115
Date and Time Test Terminated: August 02, 2021 at 1110

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	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	4	4	4	4	4	7	4	4	3	5	43	10	4.30	
5	10	13	8	10	10	0	12	14	11	12	100	10	10.0	
6	20	19	17	19	17	10	20	19	18	21	180	10	18.0	
7														
8														
TOTAL	34	36	29	33	31	17	36	37	32	38	323	10	32.3	

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	0	0	0	0	0	0	0	6	0	0	6	10	0.600
4	4	4	2	4	4	4	4	0	4	5	35	10	3.50
5	9	11	10	10	11	10	11	12	9	10	103	10	10.3
6	18	16	17	16	16	15	17	21	15	16	167	10	16.7
7													
8													
TOTAL	31	31	29	30	31	29	32	39	28	31	311	10	31.1

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	0	0	0	0	0	0	0	5	0	0	5	10	0.500
4	5	4	4	5	8	4	0	0	0	4	34	10	3.40
5	12	0X	10	10	0	12	8	11	10	12	85	9	9.44
6	16	X	15	16	15	13	16	19	18	19	147	9	16.3
7													
8													
TOTAL	33	4	29	31	23	29	24	35	28	35	271	10	27.1

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 27, 2021 at 1115
Date and Time Test Terminated: August 02, 2021 at 1110

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	0	0	0	0	0	0	0	4	0	0	4	10	0.400	
4	4	4	3	4	3	4	4	0	0	3	29	10	2.90	
5	11	11	11	10	9	14	0	14	12	11	103	10	10.3	
6	18	12	15	15	X	15	16	20	17	19	147	9	16.3	
7														
8														
TOTAL	33	27	29	29	12	33	20	38	29	33	283	10	28.3	

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	0	0	0	0	0	0	0	5	0	0	5	10	0.500
4	4	4	4	4	4	6	5	0	4	4	39	10	3.90
5	9	14	11	11	10	12	11	11	10	11	110	10	11.0
6	19	17	16	19	16	19	14	21	18	18	177	10	17.7
7													
8													
TOTAL	32	35	31	34	30	37	30	37	32	33	331	10	33.1

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	0	0	0	0	0	0	0	4	0	0	4	10	0.400
4	4	5	4	4	4	4	4	0	3	3	35	10	3.50
5	12	14	10	11	11	11	10	11	9	9	108	10	10.8
6	17	19	14	13	15	19	17	19	15	17	165	10	16.5
7													
8													
TOTAL	33	38	28	28	30	34	31	34	27	29	312	10	31.2

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	0.87500	1.20940
1	Control	5	1.00000	1.39310
2	4 %	1	1.00000	1.39310
2	4 %	2	1.00000	1.39310
2	4 %	3	1.00000	1.39310
2	4 %	4	1.00000	1.39310
2	4 %	5	1.00000	1.39310
3	5 %	1	1.00000	1.39310
3	5 %	2	0.87500	1.20940
3	5 %	3	0.87500	1.20940
3	5 %	4	1.00000	1.39310
3	5 %	5	1.00000	1.39310
4	7 %	1	1.00000	1.39310
4	7 %	2	1.00000	1.39310
4	7 %	3	0.87500	1.20940
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	0.87500	1.20940
6	12 %	1	1.00000	1.39310
6	12 %	2	1.00000	1.39310
6	12 %	3	1.00000	1.39310
6	12 %	4	0.87500	1.20940
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))
<p>D = 0.189 W = 0.7736 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

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 %	30.00	16.00	5.00	
3	5 %	25.00	16.00	5.00	
4	7 %	25.00	16.00	5.00	
5	9 %	25.00	16.00	5.00	
6	12 %	25.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.04027 W = 0.9687 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 = 7.618 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	0.01284	0.002568	1.53	
Within (Error)	24	0.04027	0.001678		
Total	29	0.0531			
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	0.5696	0.5696			
2	4 %	0.5752	0.5752	-0.2162		
3	5 %	0.5372	0.5372	1.251		
4	7 %	0.5198	0.5198	1.922		
5	9 %	0.5332	0.5332	1.405		
6	12 %	0.5302	0.5302	1.521		
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	0.06114	10.7	-0.0056	
3	5 %	5	0.06114	10.7	0.0324	
4	7 %	5	0.06114	10.7	0.0498	
5	9 %	5	0.06114	10.7	0.0364	
6	12 %	5	0.06114	10.7	0.0394	

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
<p style="text-align: center;"> D = 0.1776 D* = 1.393 Critical D* = 1.035 (alpha = 0.01, N = 60) </p> <p style="text-align: center;">Data FAIL normality test (alpha = 0.01).</p>	

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 %	84.50	75.00	10.00	
3	5 %	80.00	75.00	10.00	
4	7 %	84.50	75.00	10.00	
5	9 %	101.50	75.00	10.00	
6	12 %	89.00	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	272.5	54.5	1.612	
Within (Error)	54	1826	33.81		
Total	59	2099			
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	32.3	32.3			
2	4 %	31.1	31.1	0.4615		
3	5 %	27.1	27.1	2		
4	7 %	28.3	28.3	1.538		
5	9 %	33.1	33.1	-0.3076		
6	12 %	31.2	31.2	0.423		
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	6.007	18.6	1.2	
3	5 %	10	6.007	18.6	5.2	
4	7 %	10	6.007	18.6	4	
5	9 %	10	6.007	18.6	-0.8	
6	12 %	10	6.007	18.6	1.1	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.570	-	
4 %	0.575	-0.877	PASS
5 %	0.537	5.79	PASS
7 %	0.520	8.77	PASS
9 %	0.533	6.49	PASS
12 %	0.530	7.02	PASS

Limit = 12

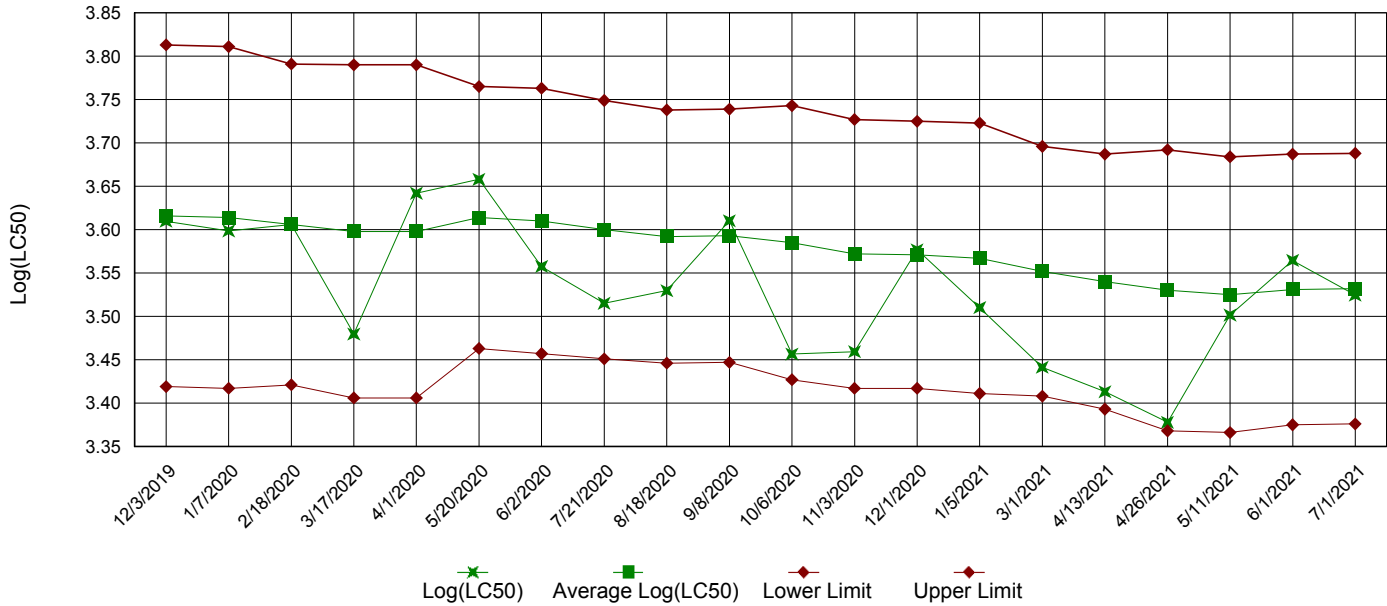
NOEC = 12 %

LOEC = 12 %

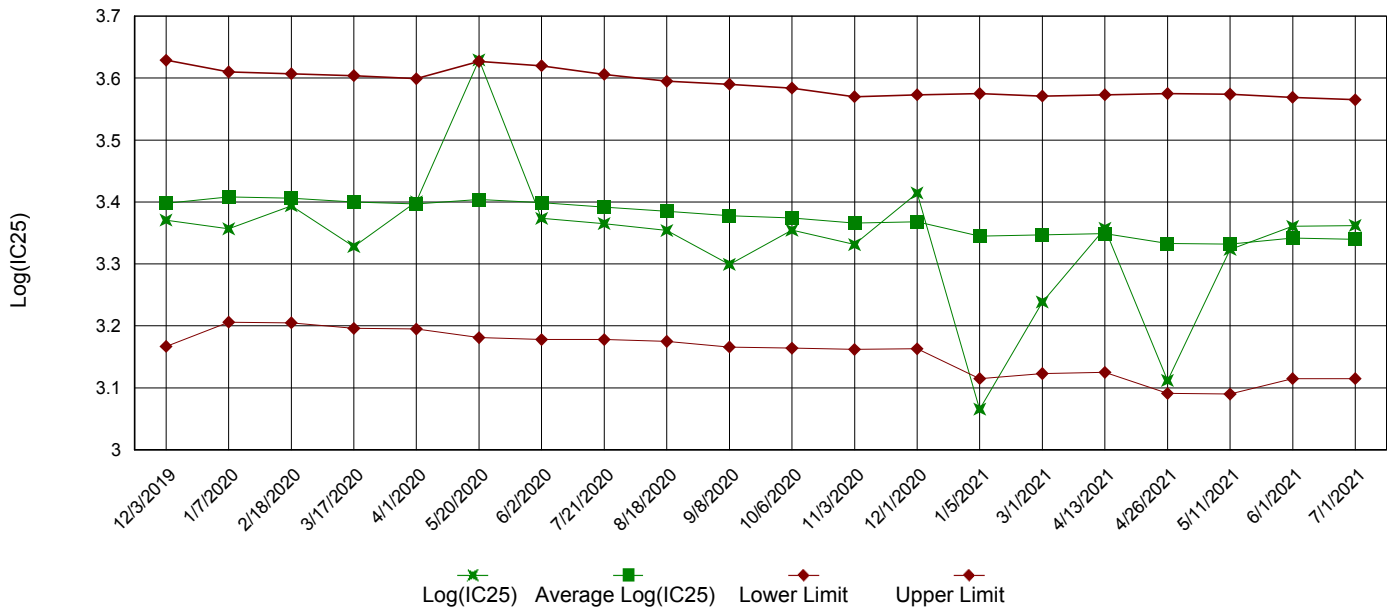
Appendix A3: Test 1000.0

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

LC50 Survival Data

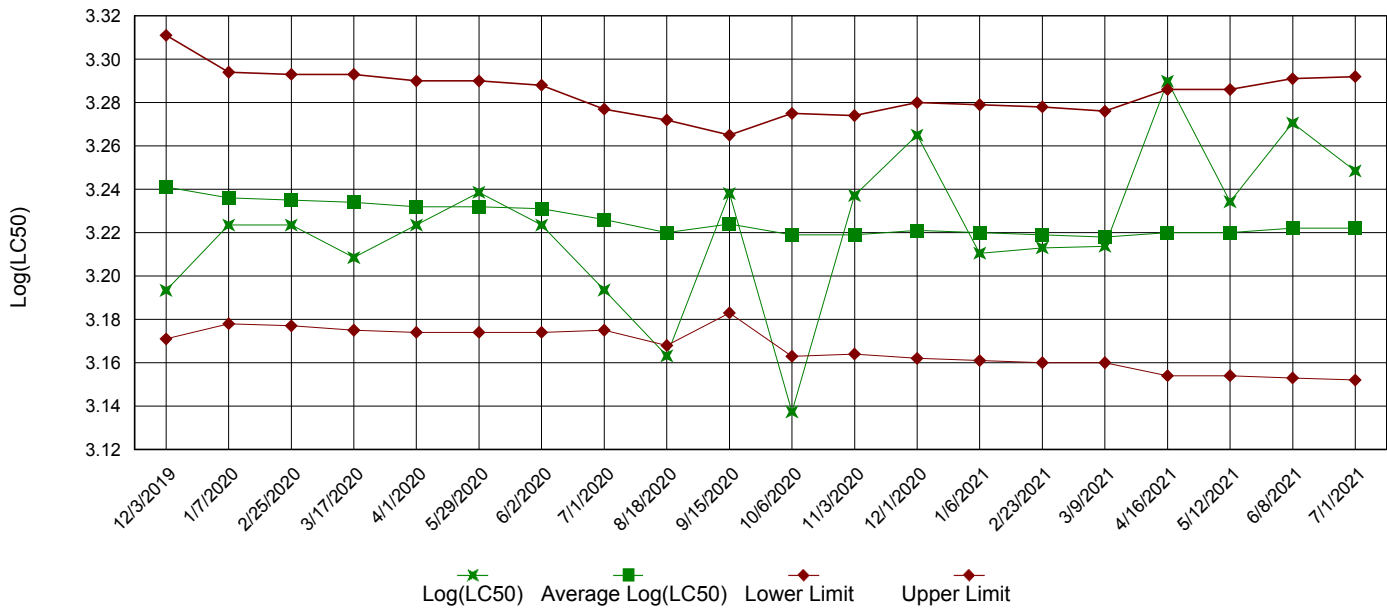


IC25 Growth Data

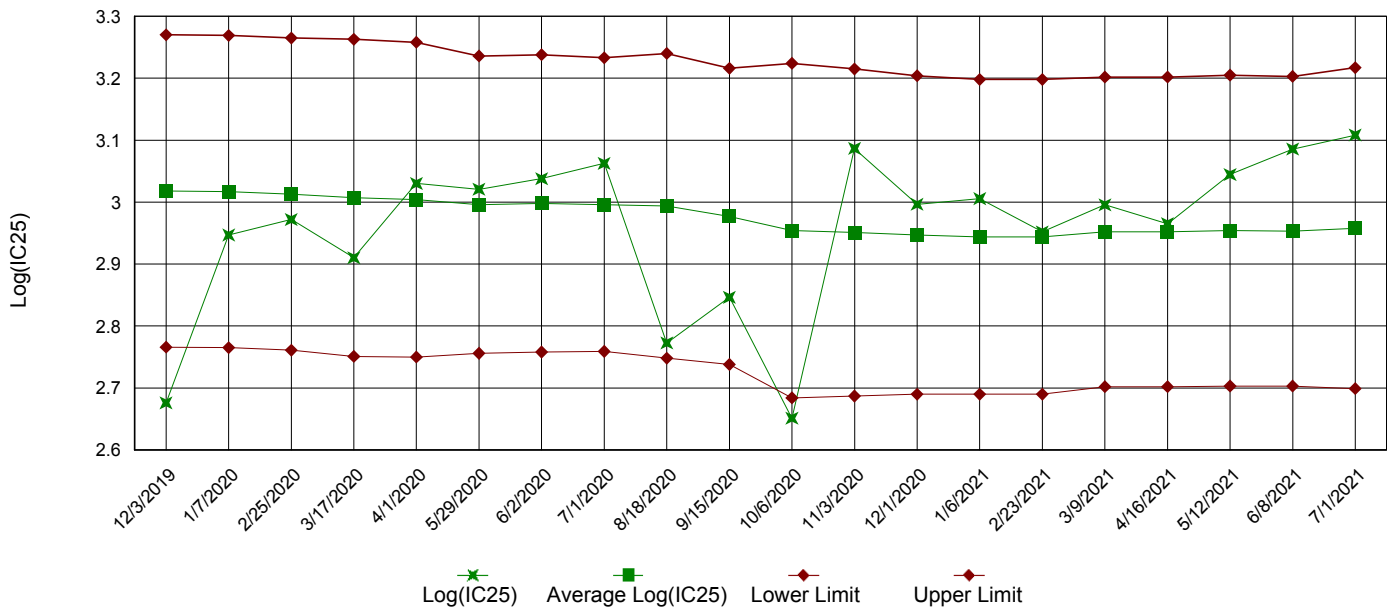


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: July 27, 2021 at 1114

Date and Time Test Terminated: August 03, 2021 at 1040

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	100	100	100	87.5	100	100	100	97.5	5.73
4 %	100	100	100	100	100	100	100	100	0.00
5 %	100	87.5	87.5	100	100	100	100	95.0	7.21
7 %	100	100	87.5	87.5	100	100	100	95.0	7.21
9 %	100	87.5	100	100	87.5	100	100	95.0	7.21
12 %	100	100	100	87.5	87.5	100	97.5	95.0	7.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.546	0.525	0.658	0.554	0.565	0.570	9.05
4 %	0.600	0.589	0.542	0.560	0.585	0.575	4.11
5 %	0.574	0.476	0.534	0.614	0.488	0.537	10.8
7 %	0.485	0.530	0.540	0.535	0.509	0.520	4.38
9 %	0.549	0.534	0.549	0.535	0.499	0.533	3.84
12 %	0.574	0.584	0.534	0.468	0.491	0.530	9.54

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: 9.05 (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, 357, 358

Test Initiated: DATE: July 27, 2021 TIME: 1114
Test Terminated: DATE: August 03, 2021 TIME: 1040

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.9	6.9	7.1	6.8	7.6	7.1	7.2
Final	6.4	6.0	5.8	5.9	6.2	6.2	6.5
pH Initial	7.8	7.8	7.8	7.8	7.9	7.9	7.8
Final	7.6	7.6	7.5	7.4	7.6	7.6	7.5

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.9	7.0	7.2	6.9	7.6	6.9	6.9
Final	6.0	6.2	5.8	5.8	5.9	6.0	6.2
pH Initial	7.9	7.8	7.8	7.8	7.9	7.9	7.8
Final	7.6	7.6	7.5	7.5	7.6	7.5	7.5

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.9	6.8	7.1	6.9	7.3	7.0	7.0
Final	5.8	5.7	6.0	5.7	5.7	6.2	5.9
pH Initial	7.9	7.8	7.9	7.9	7.9	7.9	7.9
Final	7.4	7.4	7.5	7.4	7.5	7.6	7.4

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.8	7.0	7.0	7.0	7.8	7.1	7.2
Final	6.2	6.1	6.1	5.8	6.0	6.2	6.1
pH Initial	7.9	7.9	7.8	7.9	7.9	7.9	7.8
Final	7.6	7.5	7.5	7.5	7.6	7.6	7.4

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.8	7.0	7.0	6.9	7.5	7.2	7.1
Final	6.0	5.7	5.6	5.5	6.0	6.3	6.3
pH Initial	7.9	7.8	7.9	7.9	7.9	7.9	7.9
Final	7.6	7.5	7.4	7.5	7.6	7.6	7.5

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.8	7.0	7.2	6.8	7.5	7.1	7.1
Final	6.0	5.9	6.0	5.9	5.8	6.1	6.1
pH Initial	7.9	7.9	7.9	7.8	7.9	7.9	7.8
Final	7.6	7.5	7.5	7.5	7.6	7.6	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	40	390	0.070	AR0035602 26-JUL-21
130	39	400	0.070	AR0035602 26-JUL-21
130	38	400	0.080	AR0035602 30-JUL-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	80	290	<0.05	257131-1
63	83	300	<0.05	257313-1

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: July 27, 2021 at 1115

Date and Time Test Terminated: August 02, 2021 at 1110

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	100	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	34	31	33	33	32	33
B	36	31	4	27	35	38
C	29	29	29	29	31	28
D	33	30	31	29	34	28
E	31	31	23	12	30	30
F	17	29	29	33	37	34
G	36	32	24	20	30	31
H	37	39	35	38	37	34
I	32	28	28	29	32	27
J	38	31	35	33	33	29
Mean per Adult	32.3	31.1	27.1	28.3	33.1	31.2
Mean per Surviving Adult	32.3	31.1	29.7	30.1	33.1	31.2
CV %	18.8	9.76	14.6	16.7	7.86	11.2

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. 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	<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: 18.8 (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, 357, 358

Test Initiated: DATE: July 27, 2021 TIME: 1115
Test Terminated: DATE: August 02, 2021 TIME: 1110

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	6.9	6.9	7.1	6.8	7.6	7.1	7.2
Final	7.0	6.9	7.1	7.4	7.1	7.4	--
pH Initial	7.8	7.8	7.8	7.8	7.9	7.9	7.8
Final	7.9	8.0	8.1	8.0	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	6.9	7.0	7.2	6.9	7.6	6.9	6.9
Final	7.4	7.1	7.2	7.2	7.3	7.4	--
pH Initial	7.9	7.8	7.8	7.8	7.9	7.9	7.8
Final	8.0	8.0	8.1	8.1	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	6.9	6.8	7.1	6.9	7.3	7.0	7.0
Final	7.5	7.1	7.0	7.2	7.6	7.2	--
pH Initial	7.9	7.8	7.9	7.9	7.9	7.9	7.9
Final	8.0	8.0	8.1	8.1	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	6.8	7.0	7.0	7.0	7.8	7.1	7.2
Final	7.4	7.3	7.0	7.3	7.5	7.1	--
pH Initial	7.9	7.9	7.8	7.9	7.9	7.9	7.8
Final	8.0	8.1	8.1	8.1	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	6.8	7.0	7.0	6.9	7.5	7.2	7.1
Final	7.4	6.9	7.3	7.5	7.6	7.4	--
pH Initial	7.9	7.8	7.9	7.9	7.9	7.9	7.9
Final	8.1	8.1	8.2	8.1	8.2	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	6.8	7.0	7.2	6.8	7.5	7.1	7.1
Final	7.3	7.1	7.4	7.3	7.5	7.4	--
pH Initial	7.9	7.9	7.9	7.8	7.9	7.9	7.8
Final	8.1	8.1	8.2	8.2	8.2	8.1	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	40	390	0.070	AR0035602 26-JUL-21
130	39	400	0.070	AR0035602 26-JUL-21
130	38	400	0.080	AR0035602 30-JUL-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	80	290	<0.05	257131-1
63	83	300	<0.05	257313-1

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. RUMANN WATER WORKS AR0035602 SCOTTY JONES LORRE HOLT		Analyses Requested Bismuth - Chronic Cd + Pb		AIC Control No: 257320 AIC Proposal No: Carrier: FX Received Temperature °C 0.1 Remarks	
Sample Matrix W A T E R G R A B C O M P V ✓		No of BOTTLES 1		Field pH calibration on _____ @ _____ Buffer: _____	
Date/Time Collected 7/26/02 8:00 AM		Relinquished By: [Signature] Date/Time: 7/26/02 11:05		Received By: P. Brown Date/Time: 7-27-02 0923	
Container Type P Preservative NO		Relinquished By: [Signature] Date/Time: 7/26/02 11:05		Received in Lab By: P. Brown	
Comments: TRAK 2818 5195 1306 LORRE HOLT 704 HWY 463 N TRUMANN, AR 72472		Comments: TRAK 2818 5195 1306		Comments: TRAK 2818 5195 1306	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. RUMANN Water Works AR0035602 SCOTTY JONES LORRE HOLT		Sample Matrix WATER SOIL COMPOST GRAB V		No of BOTTLES 1		Analyses Requested Bismuth, Boron - Chronic Cd + FH		AIC Control No. 257320 AIC Proposal No. Carrier: Fedev Received Temperature °C 05 Remarks	
Date/Time Collected 7/27/01 7:00 AM 8:00 AM		Container Type Plastic Preservative None		Field pH calibration on <input checked="" type="checkbox"/> Buffer:		Requiring/Relinquished By: [Signature] Date/Time: 7/28/01 11:10 AM By: [Signature] Date/Time:		Received in Lab By: [Signature] Date/Time: 7-29-01 0930	
Time Requested: (Please circle) EXPEDITED IN _____ DAYS results requested by:		Comments: LORRE HOLT 10483 28882 Fax: 870-483-6525 LORRE HOLT 704 Hwy 463 N TRUMANN, AR 72472		Requiring/Relinquished By: [Signature] Date/Time: 7/28/01 11:10 AM By: [Signature] Date/Time:		Received in Lab By: [Signature] Date/Time: 7-29-01 0930		Comments: ARK 2819 4618 5063 0201	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. RUMANN Water Works AR0035202 Scotty Jones Lorre Holt		Sample Matrix WATER SOIL COMPOST GRAB V ✓		No of BOTTLES 1		Analyses Requested Bismuth, Bromine, Chronic Cd + FH		AIC Control No: 257380 AIC Proposal No: Carrier: Fedex Received Temperature °C 0.1 Remarks	
Time Requested: (Please circle) EXPEDITED IN _____ DAYS results requested by:		Container Type Preservative: P P = Plastic S = Sulfuric acid pH2		Relinquished: Relinquished By: [Signature] Date/Time: 7/30/01/10:00		Field pH calibration on _____ @ _____ Buffer: _____		Date/Time Received in Lab: 315/19/01 Date/Time: 0900	
Time Requested: (Please circle) EXPEDITED IN _____ DAYS results requested by:		Relinquished: Relinquished By: [Signature] Date/Time: 7/30/01/10:00		Receiving: Received in Lab: [Signature] Date/Time: 0900		Comments: 2820 2893 8641		Comments:	

For AIC contact with questions: LORRE HOLT
 10483 28882 Fax: 870 483-0525
 tion to: LORRE HOLT
 104 Hwy 463 N
 TRUMANN, AR 72472