



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:

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8600 Kanis Road
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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*
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

A handwritten signature in black ink is written over a horizontal line. Below the line, the name 'John Overbey' and title 'Chief Operating Officer' are printed.

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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 CaCO ₃)	120	120	120
Hardness (mg/l as CaCO ₃)	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 CaCO ₃)	63
Hardness (mg/l as CaCO ₃)	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

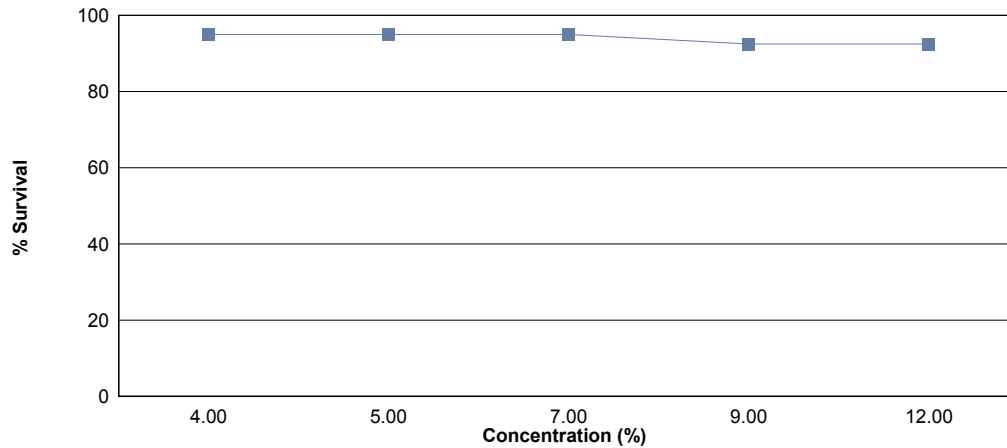
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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

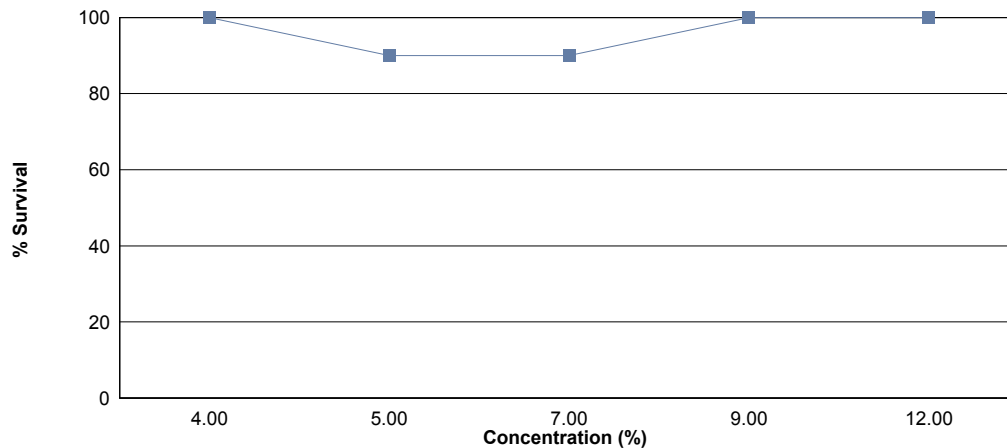
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 <i>Ceriodaphnia dubia</i> 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	0	10	0.00
2	0	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	0	10	0.00
2	0	0	0	0	0	X	0	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
<p>D = 0.1267 W = 0.9557 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 = 3.734 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	0.01359	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
<p style="text-align: center;"> D = 0.2279 D* = 1.788 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 %	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)					

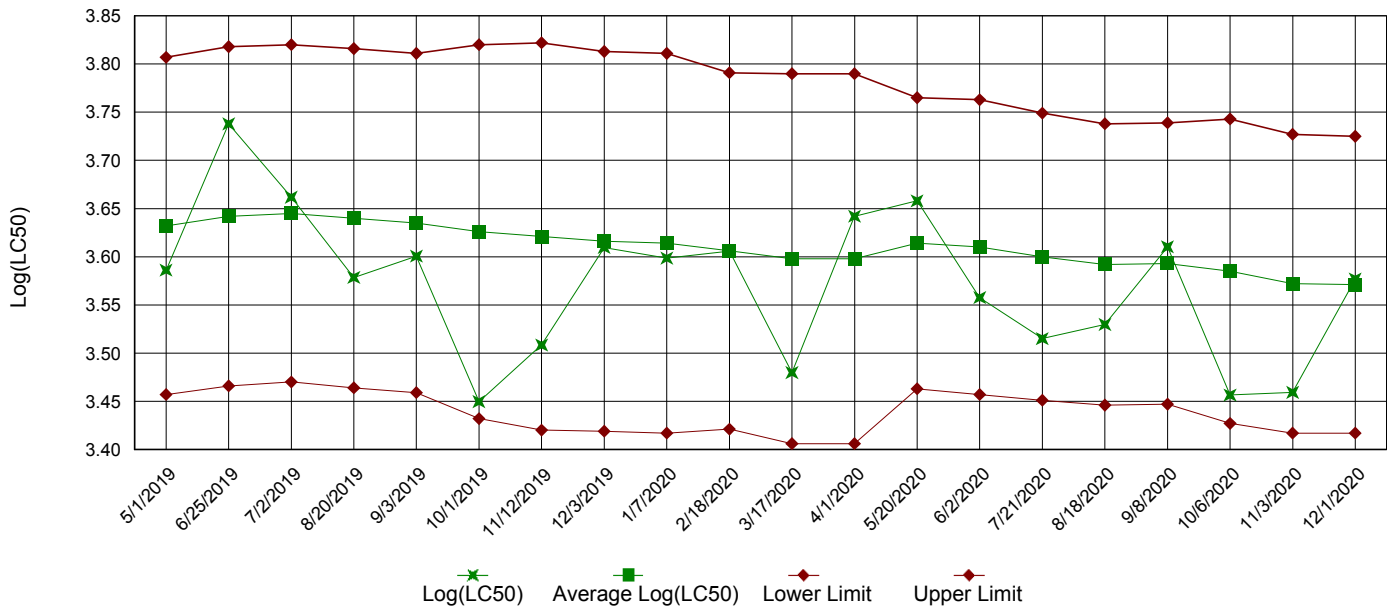
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	28.3	28.3			
2	4 %	28.2	28.2	0.03581		
3	5 %	26	26	0.8237		
4	7 %	26.5	26.5	0.6447		
5	9 %	27.4	27.4	0.3223		
6	12 %	27.6	27.6	0.2507		
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	

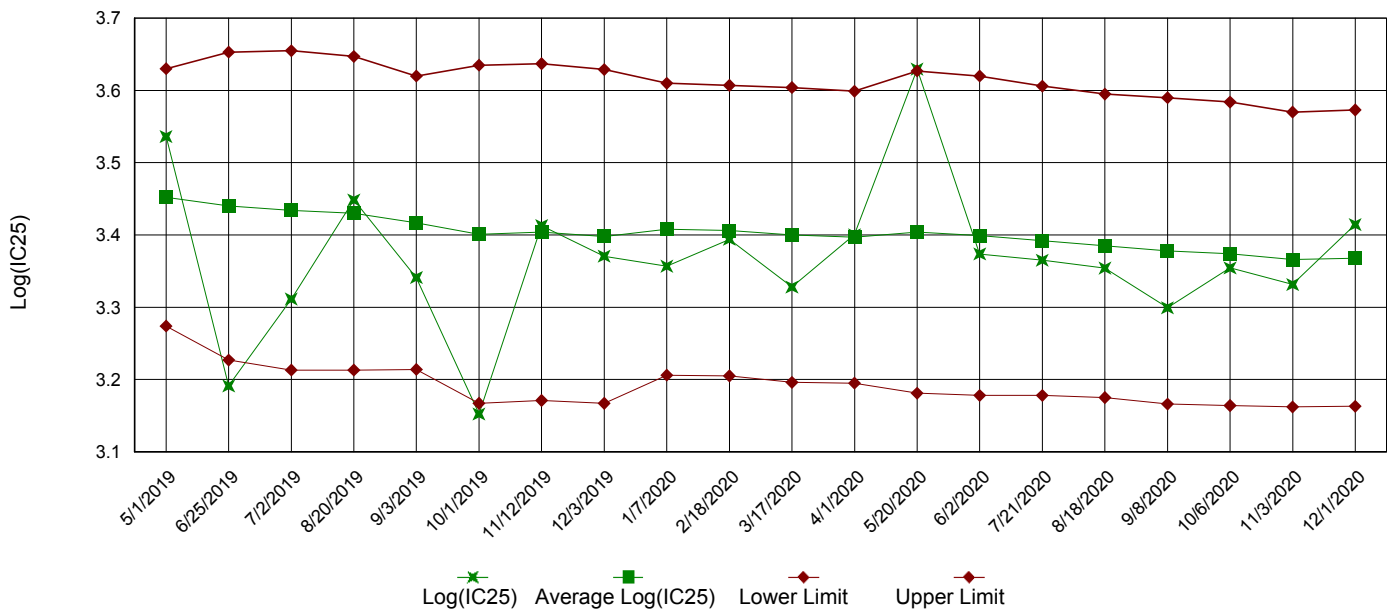
Appendix A3: Test 1000.0

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

LC50 Survival Data

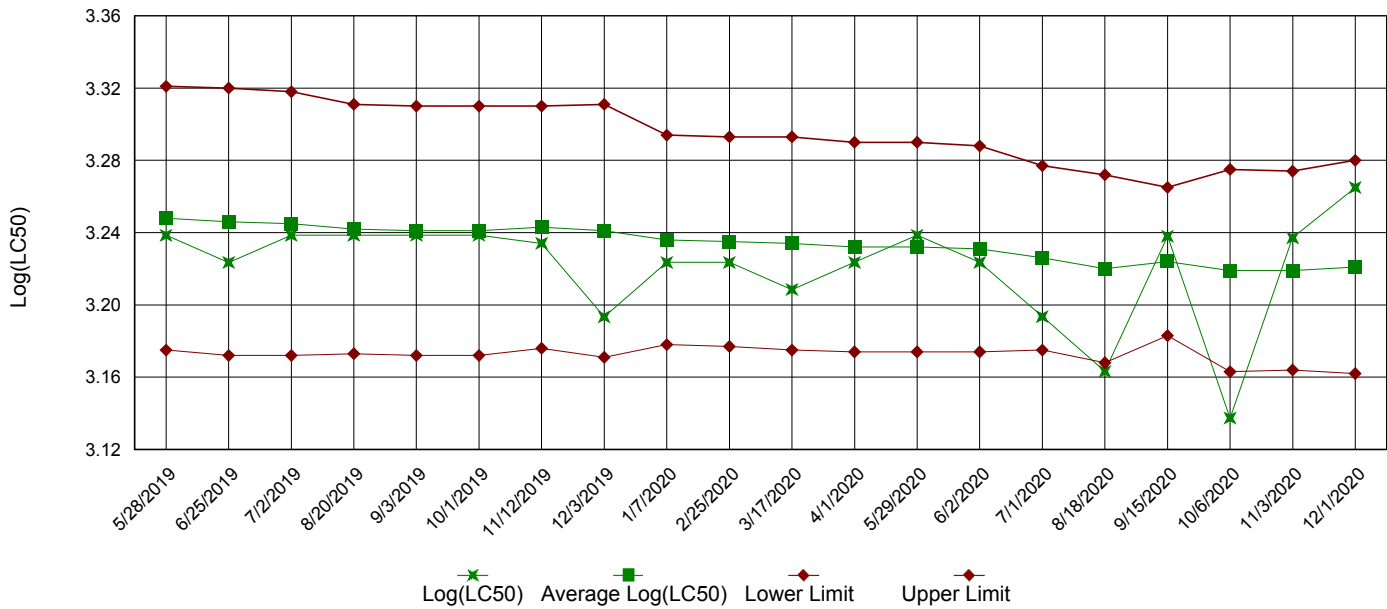


IC25 Growth Data

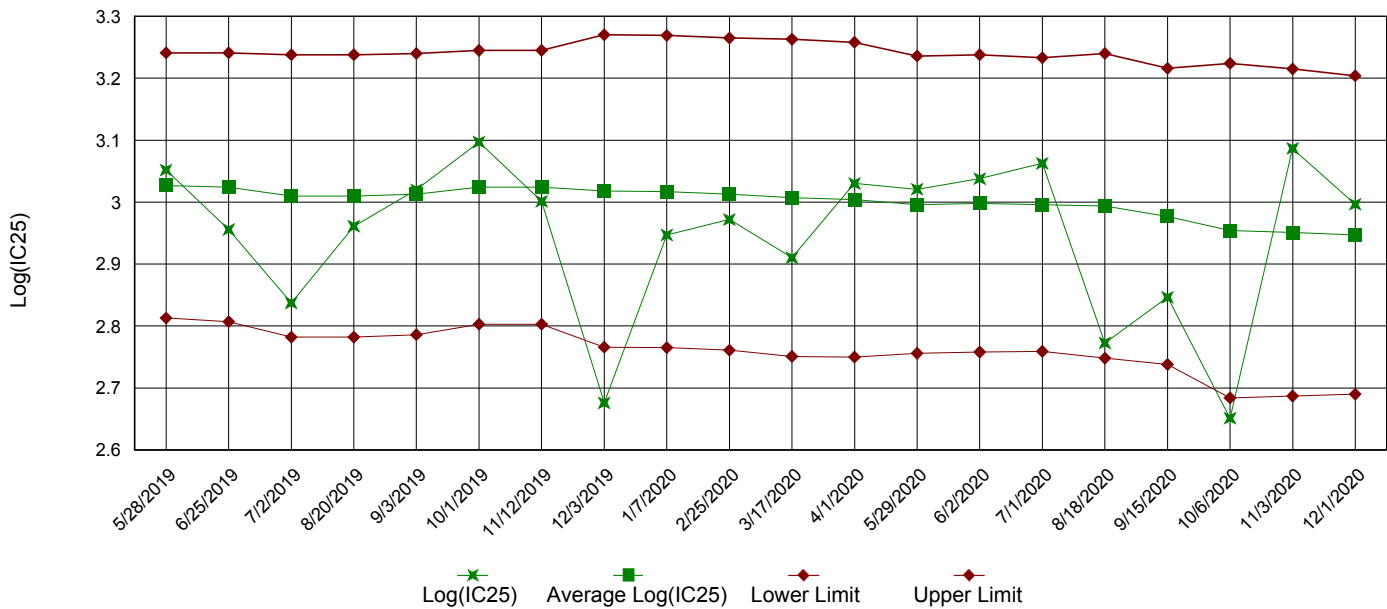


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
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					Mean dry weight, mg	CV%
	A	B	C	D	E		
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	<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: 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	<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: 10.2 (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.: 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	DAY						
	1	2	3	4	5	6	7
Control							
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	DAY						
	1	2	3	4	5	6	7
4 %							
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	DAY						
	1	2	3	4	5	6	7
5 %							
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	DAY						
	1	2	3	4	5	6	7
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	DAY						
	1	2	3	4	5	6	7
9 %							
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	DAY						
	1	2	3	4	5	6	7
12 %							
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



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		Analyses Requested		AIC Control No: <u>251095</u>	
Project Reference: <u>AR0035602</u>		BOTTLES		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		Sample Matrix		Carrier: <u>FX</u>	
Sampled By: <u>LORRE HOLT</u>		WATER		Received Temperature °C	
AIC No. <u>AR0035602</u>		COMPOUND		Remarks	
Date/Time Collected: <u>1/10/21 8:10 AM</u>		GRA B			
Date/Time Collected: <u>1/10/21 8:10 AM</u>		V ✓			
Container Type: <u>P</u>		NO = none		Field pH calibration on _____ @ _____	
Preservative: <u>ND</u>		G = Glass		Buffer: _____	
NO = none		S = Sulfuric acid pH2		T = Sodium Thiosulfate	
Turnaround Time Requested: (Please circle)		V = VOA vials		Z = Zinc acetate	
NORMAL or EXPEDITED IN _____ DAYS		N = Nitric acid pH2		Received By: _____	
Who should AIC contact with questions: <u>LORRE HOLT</u>		Relinquished By: <u>Lor Holt</u>		Date/Time: _____	
Phone: <u>870-483-3832</u> Fax: <u>870-483-10525</u>		Relinquished By: _____		Date/Time: <u>1-12-21</u>	
Report Attention to: <u>LORRE HOLT</u>		Comments: <u>TRM 7824 8078 4904</u>		Received in Lab By: <u>D. BROWN</u>	
Report Address to: <u>Tom Hwy 463 N</u>				Date/Time: <u>10/5</u>	
<u>Trumann, AR 72472</u>					

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. TRUMANN Water Works AR0035202 Scotty Jones LORRE HOLT		Sample Matrix WATER SOIL GRAB COMPS V ✓		No of BOTTLES 1		Analyses Requested Bismuth, Arsenic - Chronic Cd, Pb, Hg		AIC Control No. 251985 AIC Proposal No. Carrier: FX Received Temperature °C 0.1 Remarks	
Sample Identification AR0035202 1/12/01 - 11/3/01 8:00 AM - 8:00 AM		Container Type Plastic Preservative None		Field pH calibration on _____ @ _____ Buffer: _____		Received By: Steve Holt Date/Time: 1/30/01 10:10 AM		Date/Time 1-14-01 10:18	
Time Requested: (Please circle) Expedited in _____ Days results requested by: LORRE HOLT AIC contact with, conditions: LORRE HOLT 870-483-8882 Fax: 870-483-10525 ention to: LORRE HOLT address to: 704 HWY 463 N TRUMANN, AR 72472		Relinquished: By: Steve Holt Date/Time: 1/30/01 10:10 AM		Relinquished: By: Steve Holt Date/Time: 1-14-01 10:18		Comments: Fedex 805 8161 7771		Received in Lab By: Steve Holt Date/Time: 1-14-01 10:18	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. <u>Rymmnn Water Works</u> <u>AR0035602</u> <u>Scotty Jones</u> <u>LORRE HOLT</u>		AIC Control No: <u>251995</u> AIC Proposal No: _____ Carrier: <u>Fed Ex</u> Received Temperature °C: <u>0.1</u>	
Sample Matrix: <u>WATER</u> <u>COMPOST</u> <u>GRA B</u>		Analyses Requested: <u>BIDM @ n: brem - chronic</u> <u>CD + FH</u>	
Date/Time Collected: <u>1/14/12 8:00 AM</u> <u>8:00 AM</u>		No of BOTTLES: <u>1</u>	
Date/Time: _____ Date/Time: _____ Date/Time: _____		Field pH calibration: _____ on _____ @ _____ Buffer: _____	
Container Type: <u>P</u> Preservative: <u>NO</u>		T = Sodium Thiosulfate Z = Zinc acetate	
G = Glass NO = none P = Plastic S = Sulfuric acid pH2		H = HCl to pH2 B = NaOH to pH12	
Time Requested: (Please circle) _____ EXPEDITED IN _____ DAYS		Relinquished: <u>By: LORRE HOLT</u> Date/Time: <u>1/15/12/12:47 PM</u>	
Results requested by: _____ AIC contact with questions: <u>LORRE HOLT</u> <u>704 483-8882</u> Fax: <u>870 483-10525</u>		Received in Lab: _____ By: <u>Annmarie Stearns</u> Date/Time: <u>16 Jan 2012</u> <u>0845</u>	
Attention to: <u>LORRE HOLT</u> Address to: <u>704 HWY 463 N</u> <u>RYMANN, AR 72472</u>		Comments: _____	



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:

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*
Truman
NPDES Permit No. AR0035602 AFIN 56-00047

Dear Mr. Scotty Jones:

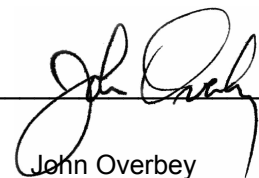
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire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



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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.

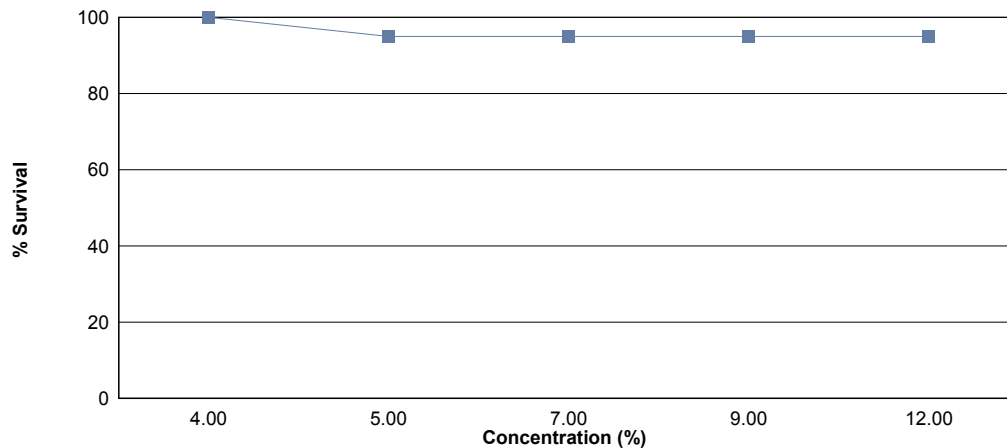
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

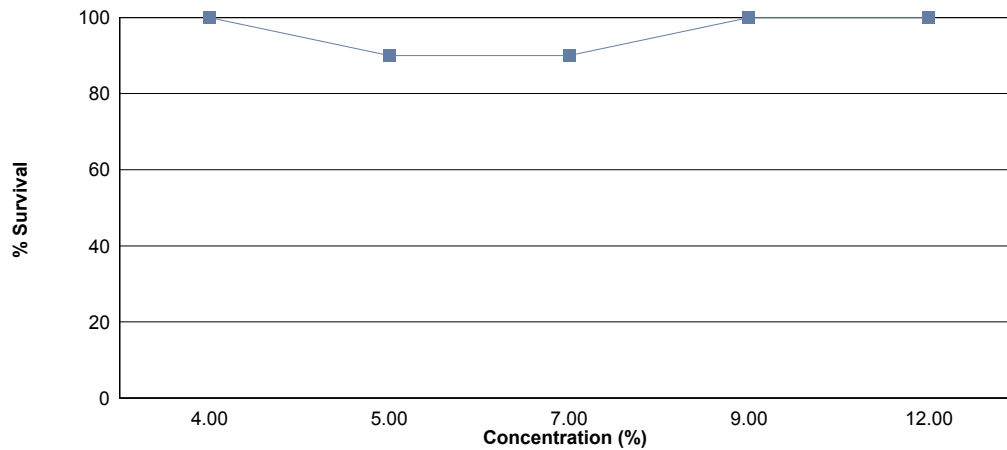
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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



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>D = 0.1776 D* = 1.393 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>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

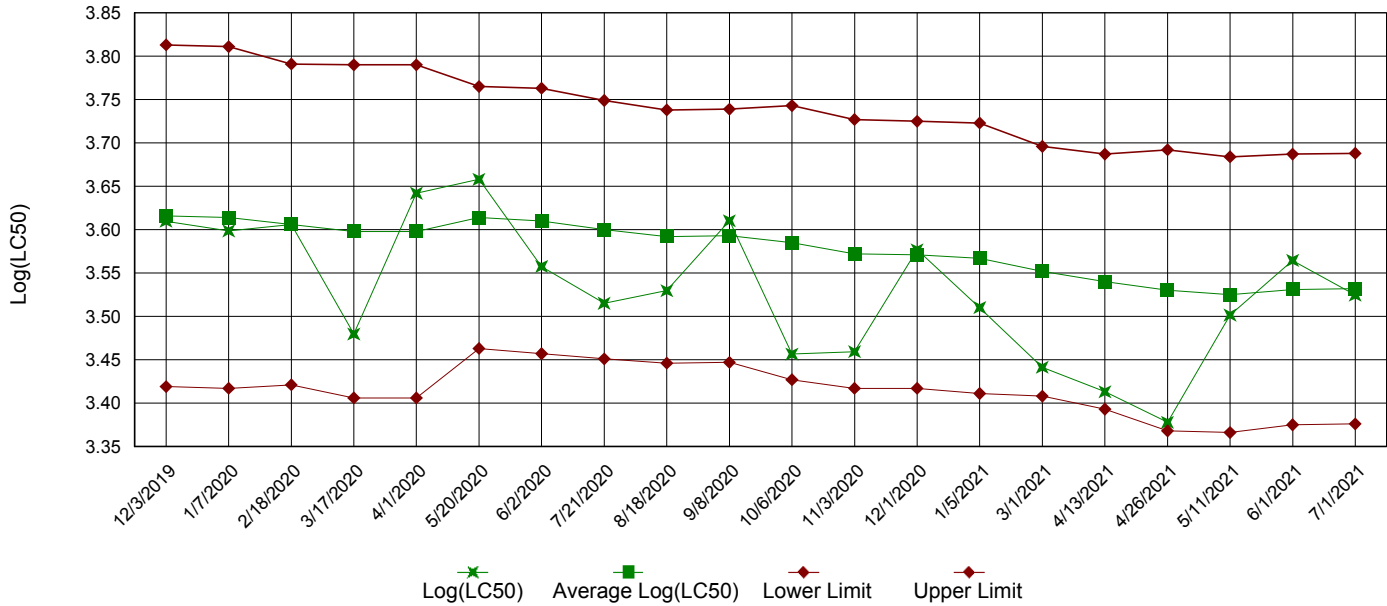
NOEC = 12 %

LOEC = 12 %

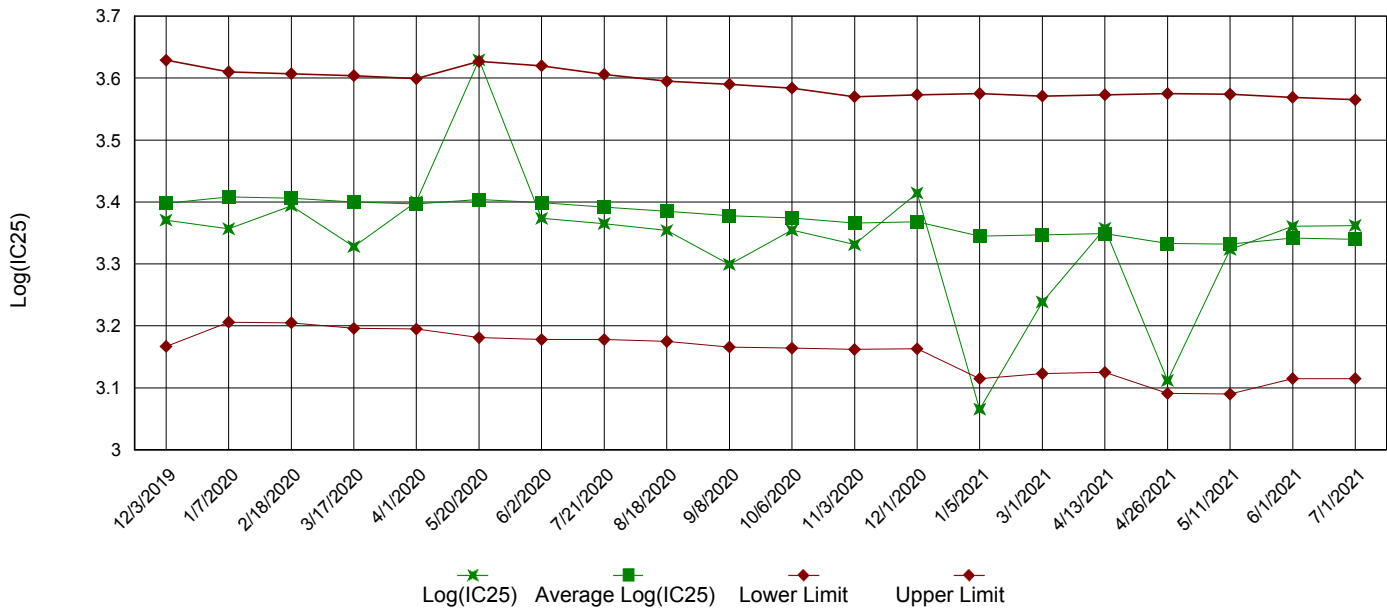
Appendix A3: Test 1000.0

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

LC50 Survival Data

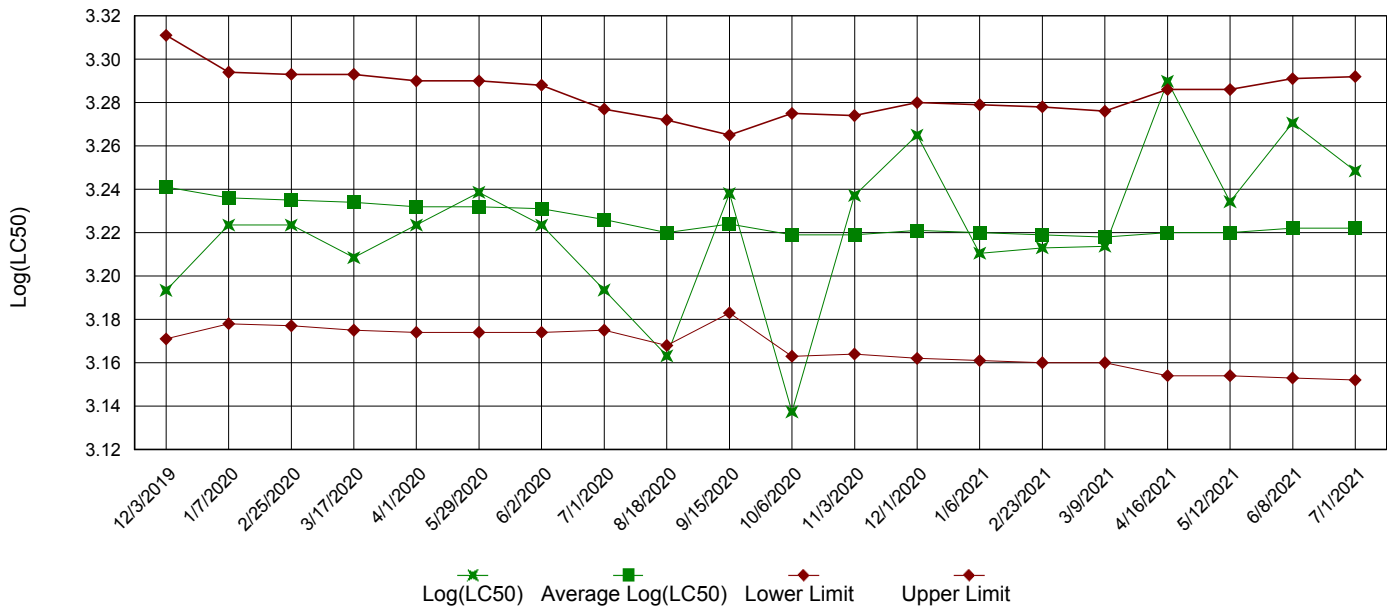


IC25 Growth Data

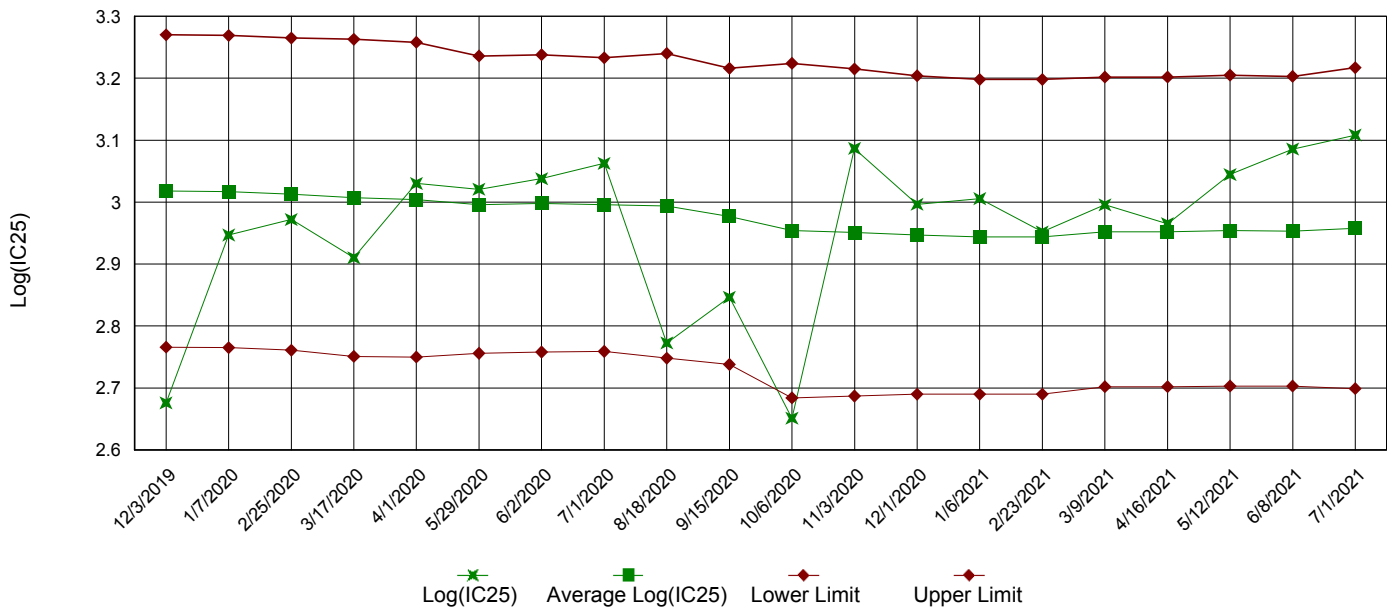


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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		Sample Matrix W A T E R C O M P G R A B V ✓		No of BOTTLES 1		Analyses Requested Bismuth - Chronic Cd + FH		AIC Control No: 257320 AIC Proposal No: Carrier: FX Received Temperature °C 0.1 Remarks	
Identification AR0035602 7/25/02 - 7/26/02 8:00 AM - 8:00 AM		Date/Time Collected 7/25/02 - 7/26/02 8:00 AM - 8:00 AM		Container Type Plastic Preservative NO		Field pH calibration on @ Buffer:		Received T = Sodium Thiosulfate Z = Zinc acetate	
Time Requested: (Please circle) For EXPEDITED IN _____ DAYS results requested by:		Relinquished By: [Signature] / 7/26/02 / 11:05 Relinquished By:		Received By: P. Brown / 7-27-02 / 0923		Comments: TRM# 2818 5195 1306 0201		Date/Time Date/Time	
Address: LORRE HOLT 483 2882 LORRE HOLT 483-16525 LORRE HOLT 483-16525 Hwy 463 N Rumann, AR 72472		Address: LORRE HOLT 483-16525 LORRE HOLT 483-16525 Hwy 463 N Rumann, AR 72472		Address: LORRE HOLT 483-16525 LORRE HOLT 483-16525 Hwy 463 N Rumann, AR 72472		Address: LORRE HOLT 483-16525 LORRE HOLT 483-16525 Hwy 463 N Rumann, AR 72472		Address: LORRE HOLT 483-16525 LORRE HOLT 483-16525 Hwy 463 N Rumann, AR 72472	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PO No. <u>AR0035602</u> Sample Matrix: <u>WATER</u> Container Type: <u>Plastic</u> Preservative: <u>NO</u>		No. of BOTTLES: <u>1</u> Analyses Requested: <u>Bismuth, Bromine - Chronic</u> <u>COU + FH</u>		AIC Control No.: <u>257320</u> AIC Proposal No.: Carrier: <u>Fedev</u> Received Temperature °C: <u>05</u> Remarks:	
Date/Time Collected: <u>7/27/01 7:00 AM</u> Date/Time: <u>8:00 AM</u>		Requiring: <u>H = HCl to pH2</u> <u>B = NaOH to pH12</u>		Received: <u>AM</u> Date/Time: <u>7/28/01 11:10</u>	
Date/Time: <u>7/27/01 7:00 AM</u> Collected: <u>8:00 AM</u>		Requiring: <u>By: [Signature]</u> Date/Time: <u>7/28/01 11:10</u>		Received in Lab: <u>By: [Signature]</u> Date/Time: <u>7-29-01 0930</u>	
Comments: <u>TRUMAN, AR 72472</u>		Comments:		Comments:	

TRK 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 GRADE V ✓		No of BOTTLES 1		Analyses Requested Bismuth, Arsenic - 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 Plastic Preservative None		Field pH calibration on _____ @ _____ Buffer: T = Sodium Thiosulfate Z = Zinc acetate		Date/Time 7/30/01/10:00 Received By: JK3.58		Date/Time 315/19/21 0900	
Relinquished By: Lorre Holt Relinquished Date/Time: 7/30/01/10:00		Comments: 2820 2893 8641		Requisition No: 870 483-0525 Address: 704 Hwy 463 N Trumann, AR 72472		Comments:		Comments:	



November 8, 2021

Biomonitoring Testing
for
AR0035602

Control No. 259819-1

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. AR0035602 AFIN 56-00047

Dear Mr. Scotty Jones:

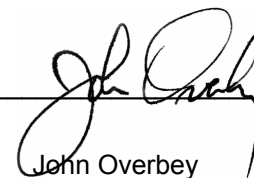
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 <4 %. **The sample PASSED lethal effects, however, FAILED 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
scottypw@gmail.com

Trumann Water and Sewer Commission
ATTN: Ms. Lorre Holt
lorre_holt0201@yahoo.com

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 - Pimephales promelas* (Fathead minnow) Survival and Growth
 - Test 1002.0
 - Ceriodaphnia dubia* Survival and Reproduction
 - A2: Statistics
 - A3: Reference Toxicant
- Appendix B: Summary Forms

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	0.536	PASS
Control Growth CV < or = 40%	4.35	PASS
Growth Minimum Significant Difference 12 to 30%	10.9	BELOW
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	35.8	PASS
Control CV < or = 40% per Surviving Female	6.56	PASS
Reproduction Minimum Significant Difference 13 to 47%	21.7	PASS
Critical Dilution CV < or = 40%	26.8	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: AR0035602
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.4	7.5	8.0
pH (standard units)	8.4	8.5	8.5
Alkalinity (mg/l as CaCO ₃)	130	130	130
Hardness (mg/l as CaCO ₃)	34	33	33
Conductivity (umhos/cm)	510	520	460
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.12	<0.1	0.32

2. Dilution Water Samples:
Moderately Hard

Analysis	259780-1	259815-1
Dissolved oxygen (mg/l)	6.5	7.6
pH (standard units)	7.9	8.0
Alkalinity (mg/l as CaCO ₃)	62	57
Hardness (mg/l as CaCO ₃)	81	81
Conductivity (umhos/cm)	350	360
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: October 26, 2021 at 1505
Date & Time Test Terminated: November 02, 2021 at 1318
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: October 26, 2021 at 1158
Date & Time Test Terminated: November 01, 2021 at 1335
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 October 05, 2021 at 1504 to October 12, 2021 at 1450

The results were as follows: (Control No. 259170-1.)

Survival LC-50: 3649 mg/l

Growth IC-25: 2291 mg/l

Growth PMSD: 11.7

Ceriodaphnia dubia

A chronic reference test was performed on October 14, 2021 at 1338 to October 21, 2021 at 1255

The results were as follows: (Control No. 259534-2.)

Survival LC-50: 1772 mg/l

Reproduction IC-25: 1278 mg/l

Reproduction PMSD: 10.4

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 26, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 26, 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.

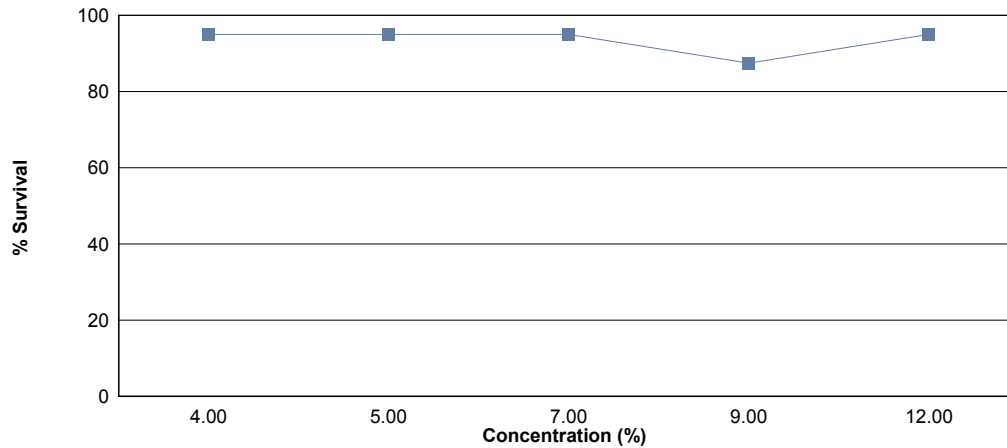
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

The test was initiated on October 26, 2021 at 1505 and continued through November 02, 2021 at 1318. 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 = <4 % effluent

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



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.536
4 %	95.0	0.470 *
5 %	95.0	0.483
7 %	95.0	0.465 *
9 %	87.5	0.453 *
12 %	95.0	0.464 *

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

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

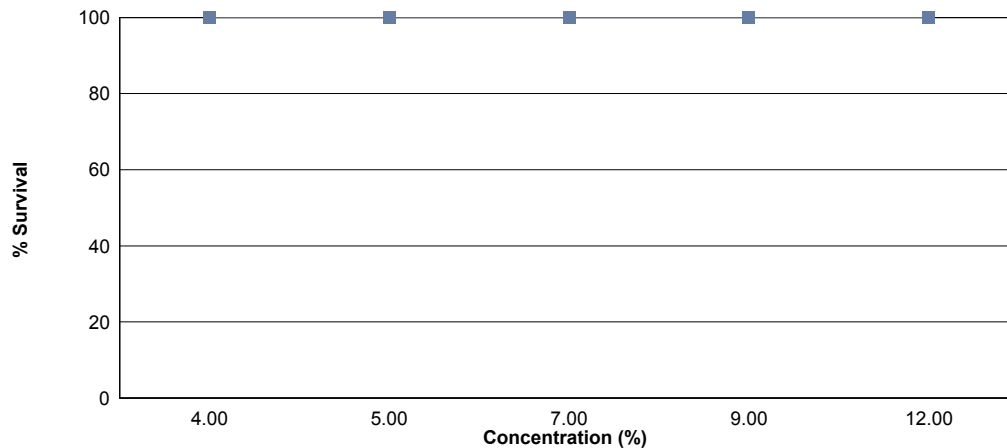
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 October 26, 2021 at 1158 and continued through November 01, 2021 at 1335. 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	35.8
4 %	100	33.7
5 %	100	27.5
7 %	100	31.9
9 %	100	31.1
12 %	100	30.2

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 26, 2021 at 1505

Date and Time Test Terminated: November 02, 2021 at 1318

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

 Test Initiated: October 26, 2021 at 1505
 Test Terminated: November 02, 2021 at 1318

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.66463	.66878	0.00415	8	0.519
	B	.66401	.66827	0.00426	8	0.532
	C	.64860	.65286	0.00426	8	0.532
	D	.65286	.65702	0.00416	8	0.520
	E	.65064	.65525	0.00461	8	0.576
4 %	A	.65749	.66118	0.00369	8	0.461
	B	.65103	.65472	0.00369	8	0.461
	C	.65247	.65603	0.00356	8	0.445
	D	.65936	.66366	0.00430	8	0.538
	E	.65886	.66242	0.00356	8	0.445
5 %	A	.65183	.65547	0.00364	8	0.455
	B	.65428	.65837	0.00409	8	0.511
	C	.65808	.66185	0.00377	8	0.471
	D	.64315	.64753	0.00438	8	0.548
	E	.65722	.66066	0.00344	8	0.430
7 %	A	.65768	.66099	0.00331	8	0.414
	B	.66314	.66664	0.00350	8	0.438
	C	.66594	.66956	0.00362	8	0.452
	D	.65173	.65603	0.00430	8	0.538
	E	.64408	.64794	0.00386	8	0.482
9 %	A	.65591	.65948	0.00357	8	0.446
	B	.66603	.67005	0.00402	8	0.502
	C	.65117	.65454	0.00337	8	0.421
	D	.67451	.67843	0.00392	8	0.490
	E	.65254	.65578	0.00324	8	0.405
12 %	A	.65164	.65532	0.00368	8	0.460
	B	.65870	.66206	0.00336	8	0.420
	C	.66101	.66489	0.00388	8	0.485
	D	.65274	.65670	0.00396	8	0.495
	E	.65797	.66164	0.00367	8	0.459

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 26, 2021 at 1158
Date and Time Test Terminated: November 01, 2021 at 1335

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	5	3	4	5	6	5	5	4	6	47	10	4.70	
5	12	11	12	10	12	10	14	12	11	13	117	10	11.7	
6	19	22	18	18	20	21	19	20	18	19	194	10	19.4	
7														
8														
TOTAL	35	38	33	32	37	37	38	37	33	38	358	10	35.8	

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	0	0	0	0	10	0.00
4	5	4	4	4	4	6	4	5	5	5	46	10	4.60
5	16	11	12	9	13	13	11	9	13	12	119	10	11.9
6	18	21	16	20	19	21	18	19	18	2	172	10	17.2
7													
8													
TOTAL	39	36	32	33	36	40	33	33	36	19	337	10	33.7

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	0	0	0	0	10	0.00
4	4	3	4	3	4	4	5	5	4	6	42	10	4.20
5	12	10	13	9	12	10	12	8	11	15	112	10	11.2
6	0	22	0	19	19	0	21	22	18	0	121	10	12.1
7													
8													
TOTAL	16	35	17	31	35	14	38	35	33	21	275	10	27.5

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 26, 2021 at 1158
Date and Time Test Terminated: November 01, 2021 at 1335

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	0	0	0	0	0	10	0.00
4	6	5	4	2	5	5	6	5	5	5	48	10	4.80	
5	11	10	10	13	12	12	13	14	12	14	121	10	12.1	
6	0	20	19	21	20	17	19	16	18	0	150	10	15.0	
7														
8														
TOTAL	17	35	33	36	37	34	38	35	35	19	319	10	31.9	

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	0	0	0	0	10	0.00
4	4	5	4	4	6	5	4	4	6	3	45	10	4.50
5	11	12	12	13	12	9	10	10	11	13	113	10	11.3
6	19	18	0	18	20	21	19	16	22	0	153	10	15.3
7													
8													
TOTAL	34	35	16	35	38	35	33	30	39	16	311	10	31.1

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	0	0	0	0	10	0.00
4	3	4	6	3	4	6	5	5	4	6	46	10	4.60
5	13	13	14	12	13	13	11	12	11	14	126	10	12.6
6	0	20	0	0	16	18	21	19	20	16	130	10	13.0
7													
8													
TOTAL	16	37	20	15	33	37	37	36	35	36	302	10	30.2

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	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	1.00000	1.39310
2	4 %	4	1.00000	1.39310
2	4 %	5	0.75000	1.04720
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	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	0.75000	1.04720
5	9 %	2	1.00000	1.39310
5	9 %	3	0.75000	1.04720
5	9 %	4	1.00000	1.39310
5	9 %	5	0.87500	1.20940
6	12 %	1	0.87500	1.20940
6	12 %	2	0.87500	1.20940
6	12 %	3	1.00000	1.39310
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))
<p>D = 0.3369 W = 0.8867 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 %	25.00	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
<p>D = 0.0366 W = 0.9342 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 = 2.594 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	0.0222	0.00444	2.911	
Within (Error)	24	0.0366	0.001525		
Total	29	0.0588			
Critical F = 3.9 (alpha = 0.01, df = 5,24) 2.62 (alpha = 0.05, df = 5,24)					
Since F > Critical F 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	0.5358	0.5358			
2	4 %	0.47	0.47	2.664	*	
3	5 %	0.483	0.483	2.138		
4	7 %	0.4648	0.4648	2.875	*	
5	9 %	0.4528	0.4528	3.361	*	
6	12 %	0.4638	0.4638	2.915	*	
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	0.05829	10.9	0.0658		
3	5 %	5	0.05829	10.9	0.0528		
4	7 %	5	0.05829	10.9	0.071		
5	9 %	5	0.05829	10.9	0.083		
6	12 %	5	0.05829	10.9	0.072		

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 %	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
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 %	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	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.2096 D* = 1.644 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>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 %	93.50	75.00	10.00	
3	5 %	76.00	75.00	10.00	
4	7 %	88.50	75.00	10.00	
5	9 %	89.00	75.00	10.00	
6	12 %	85.00	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	411	82.2	1.458	
Within (Error)	54	3044	56.37		
Total	59	3455			
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	35.8	35.8			
2	4 %	33.7	33.7	0.6254		
3	5 %	27.5	27.5	2.472	*	
4	7 %	31.9	31.9	1.162		
5	9 %	31.1	31.1	1.4		
6	12 %	30.2	30.2	1.668		
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	7.756	21.7	2.1	
3	5 %	10	7.756	21.7	8.3	
4	7 %	10	7.756	21.7	3.9	
5	9 %	10	7.756	21.7	4.7	
6	12 %	10	7.756	21.7	5.6	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.536	-	
4 %	0.470	12.3	FAIL
5 %	0.483	9.89	PASS
7 %	0.465	13.2	FAIL
9 %	0.453	15.5	FAIL
12 %	0.464	13.4	FAIL

Limit = 12

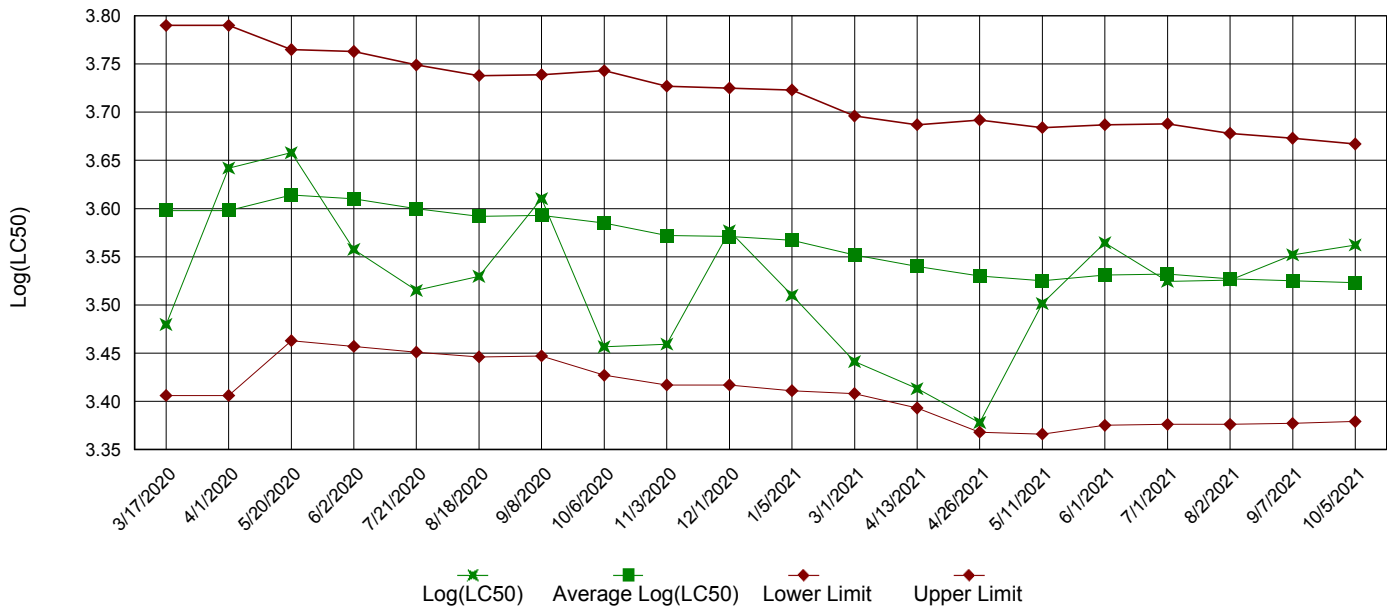
NOEC = <4 %

LOEC = 4 %

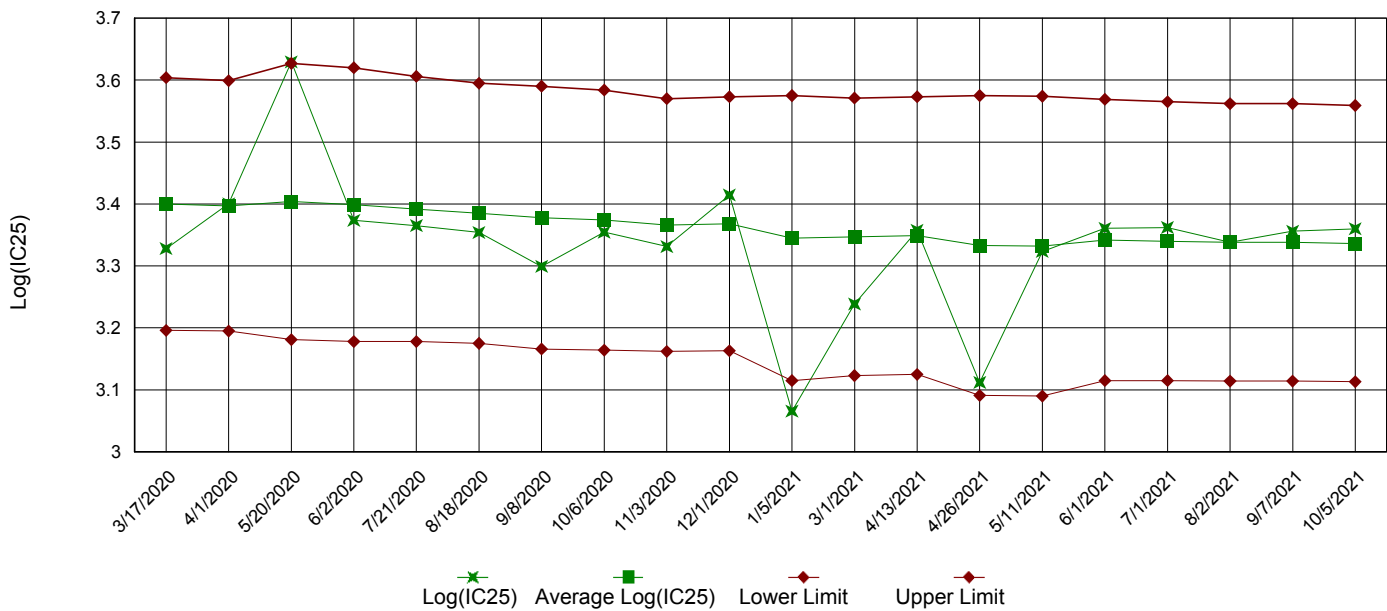
Appendix A3: Test 1000.0

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

LC50 Survival Data

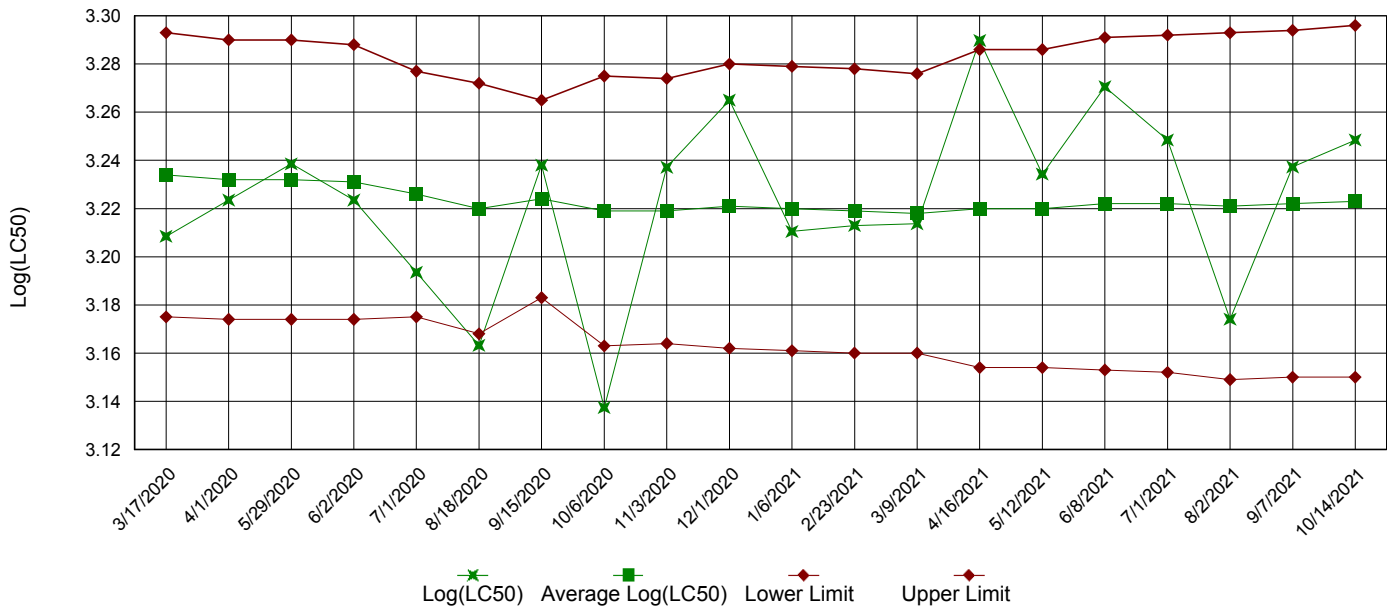


IC25 Growth Data

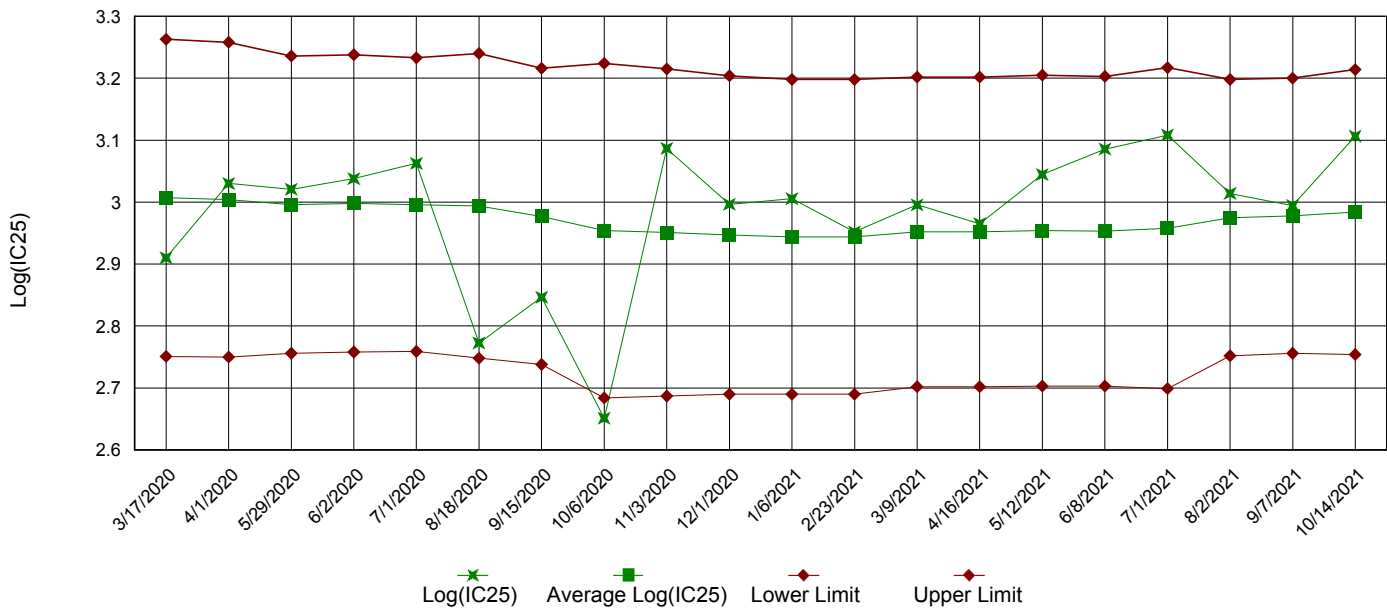


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: October 26, 2021 at 1505

Date and Time Test Terminated: November 02, 2021 at 1318

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	100	100	100	100	100	0.00
4 %	100	100	100	100	75.0	100	100	95.0	11.8
5 %	87.5	100	100	100	87.5	100	100	95.0	7.21
7 %	87.5	100	87.5	100	100	100	100	95.0	7.21
9 %	75.0	100	75.0	100	87.5	97.5	97.5	87.5	14.3
12 %	87.5	87.5	100	100	100	100	100	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	0.519	0.532	0.532	0.520	0.576	0.536	4.35
4 %	0.461	0.461	0.445	0.538	0.445	0.470	8.27
5 %	0.455	0.511	0.471	0.548	0.430	0.483	9.68
7 %	0.414	0.438	0.452	0.538	0.482	0.465	10.3
9 %	0.446	0.502	0.421	0.490	0.405	0.453	9.34
12 %	0.460	0.420	0.485	0.495	0.459	0.464	6.27

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> X </u> YES	<u> </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]: 1 (TGP6C)
5. NOEC *Pimephales* Lethality: 12 % (TOP6C)
6. LOEC *Pimephales* Lethality: 12 % (TXP6C)
7. NOEC *Pimephales* Sublethality: <4 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 4 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 9.34 (TQP6C)
10. Sublethality for this test: <4 % (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: October 26, 2021 TIME: 1505
Test Terminated: DATE: November 02, 2021 TIME: 1318

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	5.5	7.6	6.9	7.2	7.2	7.4
Final	5.0	6.3	6.0	6.5	6.1	6.0	6.8
pH Initial	7.9	8.0	8.0	8.0	8.0	8.1	7.9
Final	7.7	7.6	7.6	7.7	7.6	7.6	7.6

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	5.8	7.5	7.1	7.2	7.3	7.5
Final	5.1	5.8	6.0	6.4	5.9	6.1	6.6
pH Initial	8.0	8.0	8.1	8.0	8.1	8.1	7.9
Final	7.7	7.6	7.6	7.7	7.7	7.6	7.6

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	6.0	7.6	7.3	7.3	7.4	7.5
Final	5.2	5.8	6.0	6.3	5.9	6.2	6.8
pH Initial	8.0	8.0	8.1	8.0	8.0	8.0	7.9
Final	7.6	7.6	7.6	7.7	7.6	7.6	7.6

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.3	6.1	7.6	7.1	7.1	7.1	7.3
Final	5.2	6.1	5.9	6.5	6.3	6.2	6.6
pH Initial	8.1	8.0	8.1	8.0	8.1	8.1	7.9
Final	7.7	7.6	7.7	7.8	7.8	7.6	7.6

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	6.1	7.6	7.0	7.2	7.4	7.4
Final	5.2	6.0	6.2	6.7	6.2	6.4	7.1
pH Initial	8.1	8.0	8.2	8.0	8.1	8.0	8.0
Final	7.7	7.6	7.6	7.8	7.7	7.7	7.8

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.6	6.0	7.6	7.4	7.3	7.4	7.6
Final	5.3	5.8	6.1	6.5	6.1	6.1	6.8
pH Initial	8.1	8.1	8.2	8.1	8.2	8.2	7.9
Final	7.7	7.6	7.7	7.9	7.8	7.7	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	34	510	<0.05	AR0035602 25-OCT-21
130	33	520	<0.05	AR0035602 27-OCT-21
130	33	460	<0.05	AR0035602 29-OCT-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	81	350	<0.05	259780-1
57	81	360	<0.05	259815-1

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: October 26, 2021 at 1158

Date and Time Test Terminated: November 01, 2021 at 1335

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	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	35	39	16	17	34	16
B	38	36	35	35	35	37
C	33	32	17	33	16	20
D	32	33	31	36	35	15
E	37	36	35	37	38	33
F	37	40	14	34	35	37
G	38	33	38	38	33	37
H	37	33	35	35	30	36
I	33	36	33	35	39	35
J	38	19	21	19	16	36
Mean per Adult	35.8	33.7	27.5	31.9	31.1	30.2
Mean per Surviving Adult	35.8	33.7	27.5	31.9	31.1	30.2
CV %	6.56	17.2	34.0	23.4	26.8	30.7

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: 26.8 (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: October 26, 2021 TIME: 1158
Test Terminated: DATE: November 01, 2021 TIME: 1335

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	6.5	5.5	7.6	6.9	7.2	7.2	7.4
Final	5.6	7.6	7.9	7.5	7.8	7.6	--
pH Initial	7.9	8.0	8.0	8.0	8.0	8.1	7.9
Final	8.2	8.3	8.6	8.3	8.6	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	6.5	5.8	7.5	7.1	7.2	7.3	7.5
Final	5.6	7.4	7.8	7.3	7.6	7.6	--
pH Initial	8.0	8.0	8.1	8.0	8.1	8.1	7.9
Final	8.4	8.3	8.7	8.4	8.6	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	6.4	6.0	7.6	7.3	7.3	7.4	7.5
Final	5.7	7.4	7.8	7.5	7.6	7.7	--
pH Initial	8.0	8.0	8.1	8.0	8.0	8.0	7.9
Final	8.5	8.3	8.7	8.4	8.6	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	6.3	6.1	7.6	7.1	7.1	7.1	7.3
Final	5.7	7.4	7.7	7.5	7.7	7.7	--
pH Initial	8.1	8.0	8.1	8.0	8.1	8.1	7.9
Final	8.4	8.3	8.6	8.5	8.6	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	6.4	6.1	7.6	7.0	7.2	7.4	7.4
Final	5.7	7.5	8.0	7.7	7.9	7.8	--
pH Initial	8.1	8.0	8.2	8.0	8.1	8.0	8.0
Final	8.5	8.4	8.7	8.4	8.7	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	6.6	6.0	7.6	7.4	7.3	7.4	7.6
Final	5.7	7.3	8.1	7.4	7.6	7.7	--
pH Initial	8.1	8.1	8.2	8.1	8.2	8.2	7.9
Final	8.5	8.4	8.7	8.5	8.7	8.4	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	34	510	<0.05	AR0035602 25-OCT-21
130	33	520	<0.05	AR0035602 27-OCT-21
130	33	460	<0.05	AR0035602 29-OCT-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	81	350	<0.05	259780-1
57	81	360	<0.05	259815-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: TRUMANN Water Works		Analyses Requested		AIC Control No: 259819	
Project Reference: AR0035602		Sample Matrix		AIC Proposal No:	
Project Manager: SCOTTY JONES				Carrier: FX	
Sampled By: LORRE HOLT		Date/Time Collected: 10/24/21 10:35 AM		Received Temperature °C: 0.2	
AIC No: AR0035602		Date/Time: 8:00 AM - 8:20 AM		Remarks:	
G R A B		C O M P		Field pH calibration on _____ @ _____	
W A T E R L		V		Buffer: _____	
S O I L				T = Sodium Thiosulfate Z = Zinc acetate	
B O T T L E S		1		H = HCl to pH2 B = NaOH to pH12	
PO No.		No of		Date/Time Received By:	
Sample Matrix		B I O T T L E S		Date/Time Received in Lab By:	
W A T E R L				By: D. BROWN	
S O I L				Comments:	
B O T T L E S				By: LORRE HOLT	
G R A B				Relinquished By:	
C O M P				By: _____	
W A T E R L				Date/Time Relinquished:	
S O I L				By: _____	
B O T T L E S				Date/Time Relinquished:	
V				By: _____	
P				Date/Time Relinquished:	
NO				By: _____	
Container Type				Date/Time Relinquished:	
Preservative				By: _____	
P = Plastic				Date/Time Relinquished:	
S = Sulfuric acid pH2				By: _____	
NO = none				Date/Time Relinquished:	
V = VOA vials				By: _____	
N = Nitric acid pH2				Date/Time Relinquished:	
Turnaround Time Requested: (Please circle)				Date/Time Relinquished:	
NORMAL or EXPEDITED IN _____ DAYS				By: _____	
Who should AIC contact with questions: LORRE HOLT				Date/Time Relinquished:	
Phone: 870-483-8832 Fax: 870-483-6525				By: _____	
Report Attention to: LORRE HOLT				Date/Time Relinquished:	
Report Address to: 704 Hwy 463 N				By: _____	
TRUMANN, AR 72472				Date/Time Relinquished:	

TRUMANN 2853 2328 5259

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		PO No.		Analyses Requested		AIC Control No: <u>25982A</u>	
Project Reference: <u>AR0035602</u>		Sample Matrix		BIDM @ n: DRUG-CHRONIC CD + FH		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		WATER				Carrier: <u>Fedley</u>	
Sampled By: <u>LORRE HOLT</u>		COMPS		1		Received Temperature °C	
AIC No. <u>2 AR0035602</u>		GRAB				Remarks	
Date/Time Collected: <u>10/28/01 8:00 AM - 8:00 PM</u>		V ✓				Field pH calibration on _____ @ _____	
Container Type: <u>NO</u>		Preservative: <u>NO</u>				Buffer: _____	
G = Glass NO = none		P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		T = Sodium Thiosulfate Z = Zinc acetate	
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS		Relinquished By: <u>LORE HOLT</u>		Date/Time Relinquished: <u>10/28/01 10:15</u>		Date/Time Received: _____	
Expedited results requested by: _____		Relinquished By: _____		Date/Time Relinquished: _____		Date/Time Received: <u>10-28-01 2:45</u>	
Who should AIC contact with questions: <u>LORRE HOLT</u>		Comments: <u>2854 1330 114</u>		Received in Lab By: <u>[Signature]</u>		Date/Time Received: <u>10-28-01 2:45</u>	
Phone: <u>810-483-2832</u> Fax: <u>810-483-10525</u>		Report Attention to: <u>LORRE HOLT</u>		Report Address to: <u>TRUMANN, AR 72472</u>			

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		PO No.		No of BOTTLES		Analyses Requested		AIC Control No: <u>259819</u>	
Project Reference: <u>AR0035602</u>		Sample Matrix		WATER		BIDMON: BIRM - CHRONIC		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		G R A B		C O M P		CDV FH		Carrier: <u>FedEx</u>	
Sample Identification: <u>3 AR0035602</u>		Date/Time Collected: <u>8/22/01 8:00 AM - 8:05 AM</u>		V ✓				Received Temperature °C: <u>0.1</u>	
By: <u>LORRE HOLT</u>		Container Type: <u>P</u>		Preservative: <u>NO</u>				Remarks:	
AIC No. <u>3</u>		G = Glass NO = none		P = Plastic S = Sulfuric acid pH2				Field pH calibration on @ Buffer:	
Reference: <u>AR0035602</u>		NO = none S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2				T = Sodium Thiosulfate Z = Zinc acetate	
Project: <u>AR0035602</u>		Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN ___ DAYS		Relinquished By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Received By: <u>[Signature]</u>	
Manager: <u>Scotty Jones</u>		Expedited results requested by: <u>LORRE HOLT</u>		By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Received in Lab By: <u>NK358</u>	
Sampled: <u>LORRE HOLT</u>		Who should AIC contact with questions: <u>LORRE HOLT</u>		By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Date/Time: <u>30 Oct 01</u>	
By: <u>LORRE HOLT</u>		Phone: <u>870-483-8832</u> Fax: <u>870-483-16535</u>		By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Date/Time: <u>0840</u>	
AIC No. <u>3</u>		Report Attention to: <u>LORRE HOLT</u>		By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Date/Time: <u>0840</u>	
Reference: <u>AR0035602</u>		Report Address to: <u>704 Hwy 463 N Trumann, AR 72472</u>		By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Date/Time: <u>0840</u>	
Project: <u>AR0035602</u>		Comments:		By: <u>[Signature]</u>		Date/Time: <u>10/29/01 10:47 AM</u>		Date/Time: <u>0840</u>	



January 27, 2022

Biomonitoring Testing
for
AR0035602

Control No. 262168-1

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. 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 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
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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	0.552	PASS
Control Growth CV < or = 40%	5.67	PASS
Growth Minimum Significant Difference 12 to 30%	12.0	PASS
Critical Dilution CV < or = 40%	8.06	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	38.2	PASS
Control CV < or = 40% per Surviving Female	16.0	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.5	PASS
Critical Dilution CV < or = 40%	22.5	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: AR0035602
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.5	7.5	7.9
pH (standard units)	7.9	8.1	8.0
Alkalinity (mg/l as CaCO ₃)	120	120	120
Hardness (mg/l as CaCO ₃)	28	33	33
Conductivity (umhos/cm)	430	440	440
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.88	0.79

2. Dilution Water Samples:
Moderately Hard

Analysis	262038-1
Dissolved oxygen (mg/l)	7.1
pH (standard units)	7.8
Alkalinity (mg/l as CaCO ₃)	62
Hardness (mg/l as CaCO ₃)	83
Conductivity (umhos/cm)	310
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 18, 2022 at 1308
Date & Time Test Terminated: January 25, 2022 at 1259
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 18, 2022 at 1300
Date & Time Test Terminated: January 24, 2022 at 1255
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 December 14, 2021 at 1120 to December 21, 2021 at 1136

The results were as follows: (Control No. 261135-1.)

Survival LC-50: 3555 mg/l

Growth IC-25: 2284 mg/l

Growth PMSD: 10.3

Ceriodaphnia dubia

A chronic reference test was performed on December 14, 2021 at 1114 to December 20, 2021 at 1025

The results were as follows: (Control No. 261135-2.)

Survival LC-50: mg/l

Reproduction IC-25: mg/l

Reproduction PMSD:

V. Organism History

Pimephales promelas (Fathead minnow)

Date: January 18, 2022

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: January 18, 2022

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

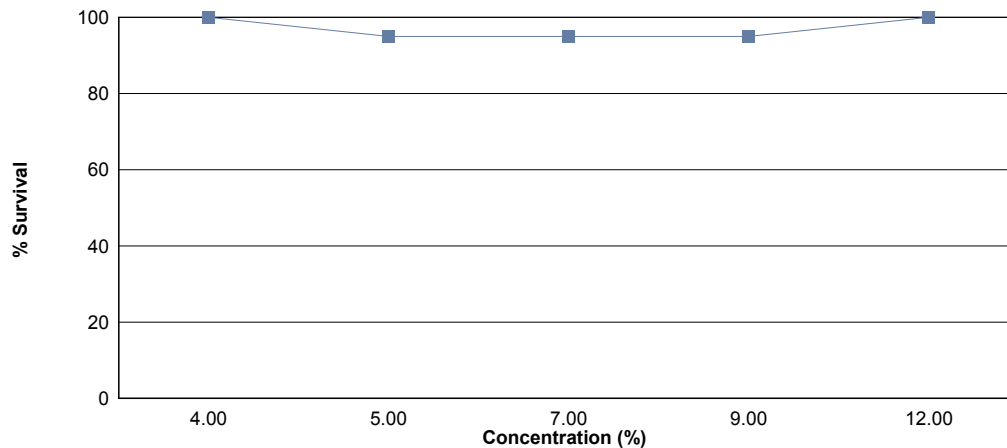
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 18, 2022 at 1308 and continued through January 25, 2022 at 1259. 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	0.552
4 %	100	0.600
5 %	95.0	0.522
7 %	95.0	0.490
9 %	95.0	0.527
12 %	100	0.564

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

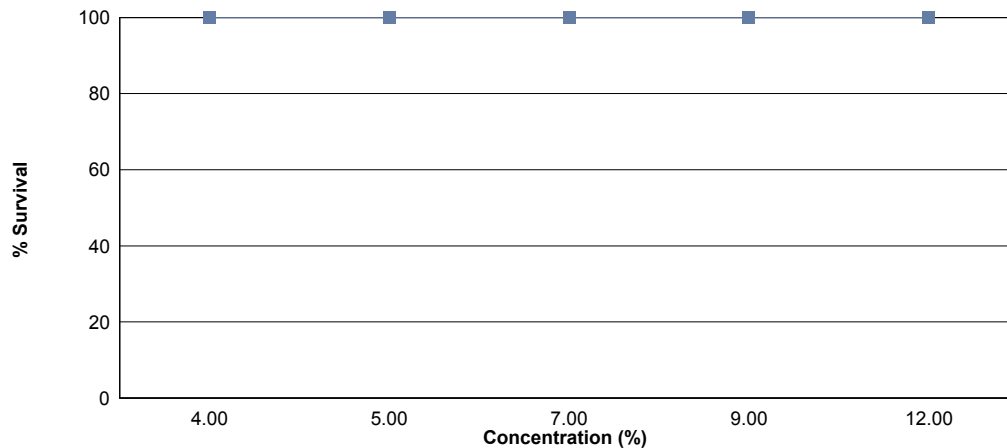
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 18, 2022 at 1300 and continued through January 24, 2022 at 1255. 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	38.2
4 %	100	43.4
5 %	100	36.2
7 %	100	40.2
9 %	100	37.4
12 %	100	38.2

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: January 18, 2022 at 1308

Date and Time Test Terminated: January 25, 2022 at 1259

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	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	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	7	7
	E	8	8	8	8	8	8	8
7 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	7
	C	8	8	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	7	7	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	7	7	7
	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	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: January 18, 2022 at 1308

Test Terminated: January 25, 2022 at 1259

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65738	.66159	0.00421	8	0.526
	B	.65878	.66345	0.00467	8	0.584
	C	.65092	.65517	0.00425	8	0.531
	D	.65695	.66119	0.00424	8	0.530
	E	.67463	.67933	0.00470	8	0.588
4 %	A	.64166	.64666	0.00500	8	0.625
	B	.66339	.66776	0.00437	8	0.546
	C	.65669	.66145	0.00476	8	0.595
	D	.63618	.64098	0.00480	8	0.600
	E	.66675	.67182	0.00507	8	0.634
5 %	A	.66906	.67363	0.00457	8	0.571
	B	.66297	.66753	0.00456	8	0.570
	C	.66377	.66803	0.00426	8	0.532
	D	.66376	.66762	0.00386	8	0.482
	E	.66569	.66934	0.00365	8	0.456
7 %	A	.65256	.65714	0.00458	8	0.572
	B	.66080	.66413	0.00333	8	0.416
	C	.66445	.66828	0.00383	8	0.479
	D	.65354	.65777	0.00423	8	0.529
	E	.66135	.66499	0.00364	8	0.455
9 %	A	.66109	.66509	0.00400	8	0.500
	B	.66594	.67048	0.00454	8	0.568
	C	.65843	.66246	0.00403	8	0.504
	D	.65100	.65489	0.00389	8	0.486
	E	.64266	.64728	0.00462	8	0.578
12 %	A	.66796	.67249	0.00453	8	0.566
	B	.66193	.66620	0.00427	8	0.534
	C	.65503	.65976	0.00473	8	0.591
	D	.66147	.66561	0.00414	8	0.518
	E	.65111	.65599	0.00488	8	0.610

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 18, 2022 at 1300

Date and Time Test Terminated: January 24, 2022 at 1255

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	2	4	5	4	6	5	0	5	6	3	40	10	4.00	
4	0	0	0	0	0	0	2	0	0	4	6	10	0.600	
5	12	13	16	19	13	15	17	13	11	19	148	10	14.8	
6	16	27	25	20	19	20	19	20	22	20E	188	10	18.8	
7														
8														
TOTAL	30	44	46	43	38	40	38	38	39	26	382	10	38.2	

E = Excluded fourth brood neonates

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	5	6	6	0	4	5	0	6	5	6	43	10	4.30
4	0	0	0	6	0	0	6	0	0	0	12	10	1.20
5	16	17	19	16	17	19	17	19	16	15	171	10	17.1
6	20	21	19	21	19	26	18	23	22	19	208	10	20.8
7													
8													
TOTAL	41	44	44	43	40	50	41	48	43	40	434	10	43.4

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	5	6	3	4	4	4	0	6	4	6	42	10	4.20
4	0	0	0	0	0	0	5	0	0	0	5	10	0.500
5	15	17	9	15	17	14	19	11	16	12	145	10	14.5
6	19	20	10	19	20	22	0	20	19	21	170	10	17.0
7													
8													
TOTAL	39	43	22	38	41	40	24	37	39	39	362	10	36.2

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 18, 2022 at 1300

Date and Time Test Terminated: January 24, 2022 at 1255

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	6	6	5	5	6	0	6	6	6	52	10	5.20	
4	0	0	0	0	0	0	5	0	0	0	5	10	0.500	
5	14	16	13	13	14	15	17	17	18	15	152	10	15.2	
6	20	25	24	21	20	20	0	23	22	18	193	10	19.3	
7														
8														
TOTAL	40	47	43	39	39	41	22	46	46	39	402	10	40.2	

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	5	6	5	5	4	4	0	6	0	5	40	10	4.00	
4	0	0	0	0	0	0	4	0	5	0	9	10	0.900	
5	14	16	14	13	14	13	12	15	19	13	143	10	14.3	
6	20	24	21	18	19	18	0	23	20	19	182	10	18.2	
7														
8														
TOTAL	39	46	40	36	37	35	16	44	44	37	374	10	37.4	

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	6	6	6	0	5	6	0	5	4	6	44	10	4.40	
4	0	0	0	0	0	0	3	0	0	0	3	10	0.300	
5	14	19	19	19	15	14	16	15	16	17	164	10	16.4	
6	13	19	21	20	18	24	0	20	21	15	171	10	17.1	
7														
8														
TOTAL	33	44	46	39	38	44	19	40	41	38	382	10	38.2	

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	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	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	1.00000	1.39310
3	5 %	4	0.87500	1.20940
3	5 %	5	1.00000	1.39310
4	7 %	1	1.00000	1.39310
4	7 %	2	0.87500	1.20940
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	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	1.00000	1.39310
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))
<p>D = 0.1215 W = 0.7841 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 %	27.50	16.00	5.00	
3	5 %	22.50	16.00	5.00	
4	7 %	22.50	16.00	5.00	
5	9 %	22.50	16.00	5.00	
6	12 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.04756 W = 0.9577 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 = 2.435 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	0.03614	0.007228	3.647	
Within (Error)	24	0.04756	0.001982		
Total	29	0.0837			
Critical F = 3.9 (alpha = 0.01, df = 5,24) 2.62 (alpha = 0.05, df = 5,24)					
Since F > Critical F 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	0.5518	0.5518			
2	4 %	0.6	0.6	-1.712		
3	5 %	0.5222	0.5222	1.051		
4	7 %	0.4902	0.4902	2.188		
5	9 %	0.5272	0.5272	0.8737		
6	12 %	0.5638	0.5638	-0.4262		
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	0.06645	12	-0.0482	
3	5 %	5	0.06645	12	0.0296	
4	7 %	5	0.06645	12	0.0616	
5	9 %	5	0.06645	12	0.0246	
6	12 %	5	0.06645	12	-0.012	

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 %	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
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 %	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	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.2001 D* = 1.57 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>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 %	134.00	75.00	10.00	
3	5 %	98.00	75.00	10.00	
4	7 %	121.50	75.00	10.00	
5	9 %	101.50	75.00	10.00	
6	12 %	109.50	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	324.5	64.91	1.386	
Within (Error)	54	2529	46.83		
Total	59	2854			
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)					

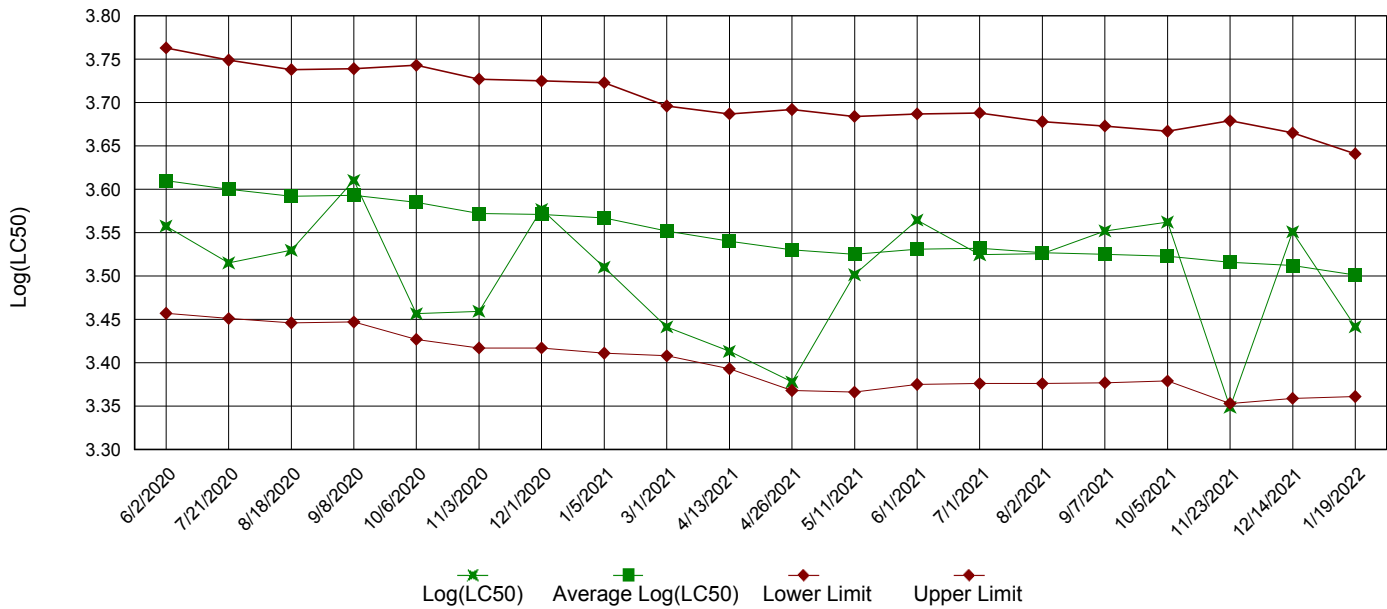
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	38.2	38.2			
2	4 %	43.4	43.4	-1.699		
3	5 %	36.2	36.2	0.6535		
4	7 %	40.2	40.2	-0.6535		
5	9 %	37.4	37.4	0.2614		
6	12 %	38.2	38.2	0		
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	7.07	18.5	-5.2	
3	5 %	10	7.07	18.5	2	
4	7 %	10	7.07	18.5	-2	
5	9 %	10	7.07	18.5	0.8	
6	12 %	10	7.07	18.5	0	

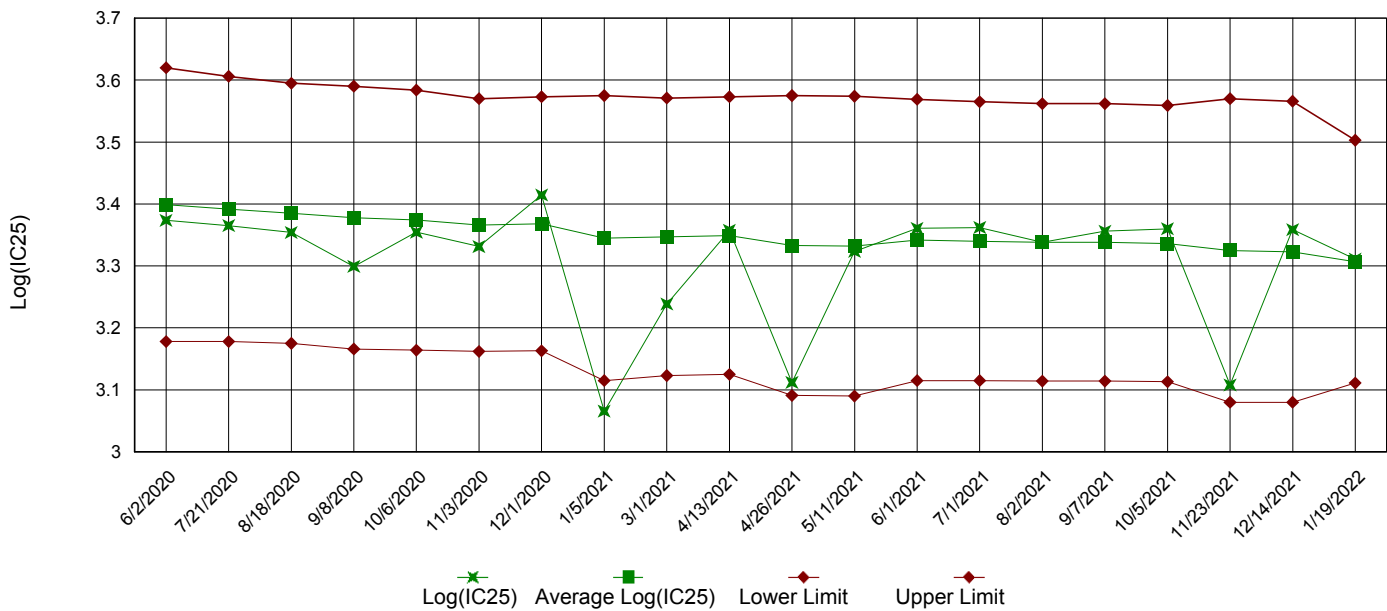
Appendix A3: Test 1000.0

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

LC50 Survival Data

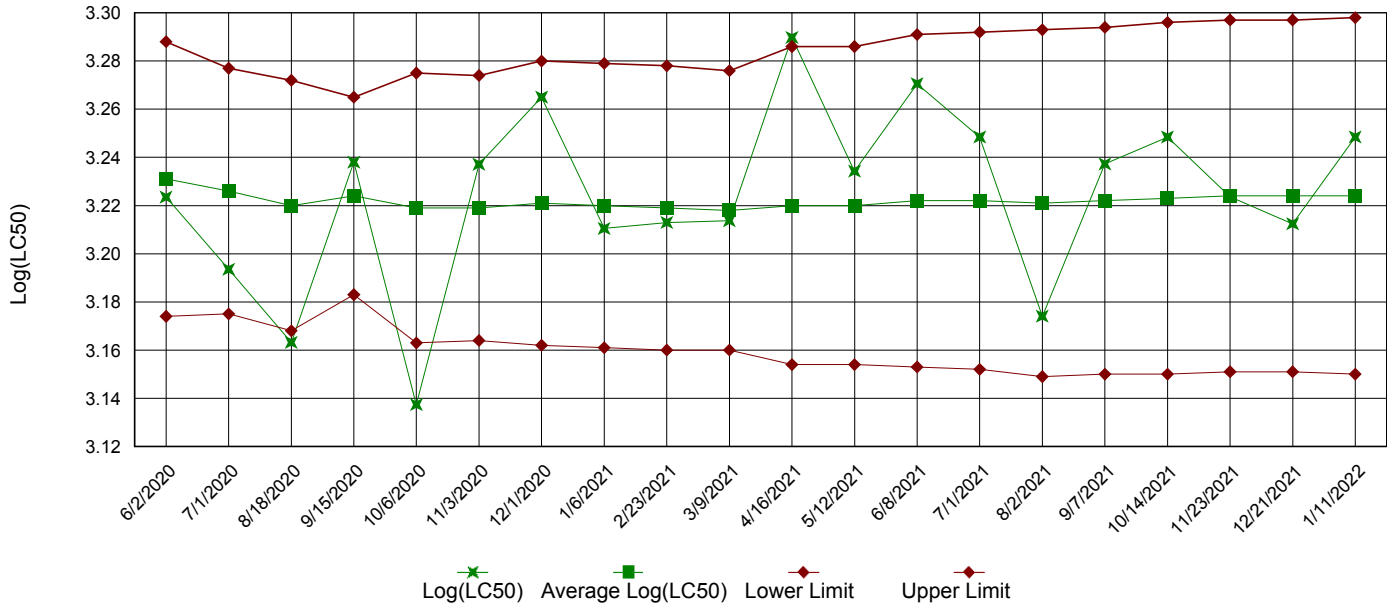


IC25 Growth Data

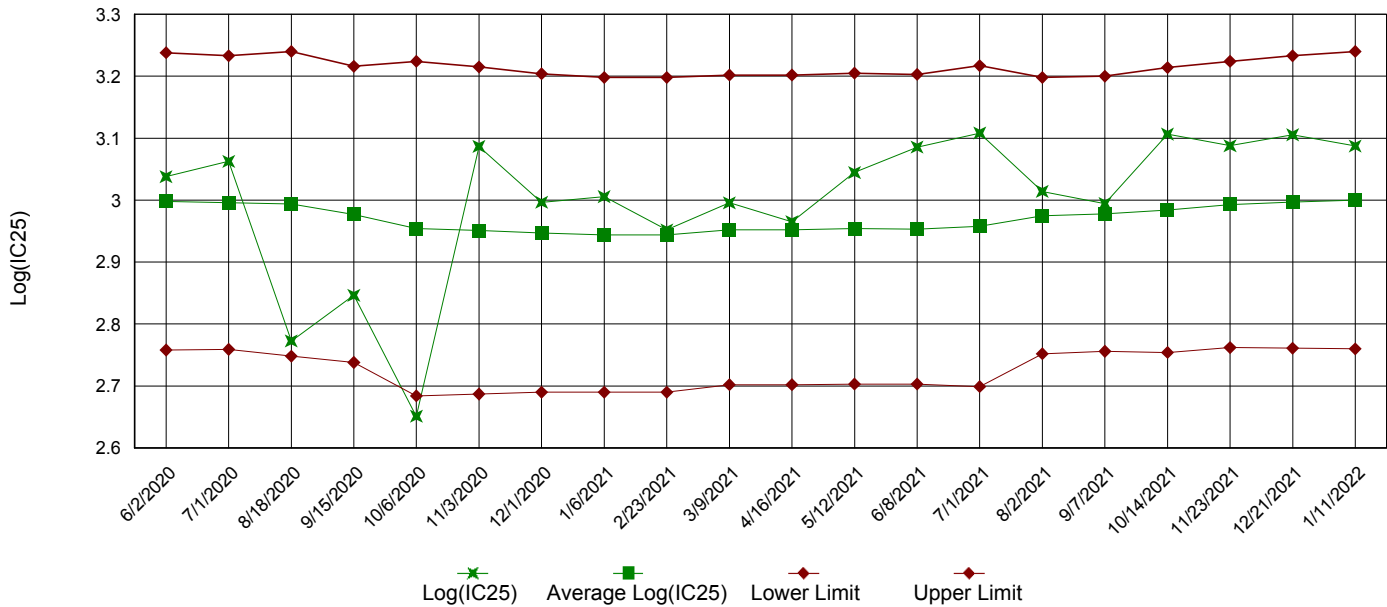


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: January 18, 2022 at 1308

Date and Time Test Terminated: January 25, 2022 at 1259

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

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.526	0.584	0.531	0.530	0.588	0.552	5.67
4 %	0.625	0.546	0.595	0.600	0.634	0.600	5.73
5 %	0.571	0.570	0.532	0.482	0.456	0.522	9.93
7 %	0.572	0.416	0.479	0.529	0.455	0.490	12.5
9 %	0.500	0.568	0.504	0.486	0.578	0.527	8.06
12 %	0.566	0.534	0.591	0.518	0.610	0.564	6.79

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: 8.06 (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: January 18, 2022 TIME: 1308
 Test Terminated: DATE: January 25, 2022 TIME: 1259

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.0	6.9	6.8	7.1	7.6	7.2
Final	6.5	7.0	6.1	7.2	6.5	5.5	6.3
pH Initial	7.8	7.7	7.8	8.0	8.0	7.6	7.6
Final	7.6	7.8	7.5	7.8	7.6	7.5	7.6

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.0	7.2	7.4	6.8	7.7	7.0
Final	6.5	6.6	5.9	7.3	6.5	5.5	7.0
pH Initial	7.9	7.7	7.9	7.9	8.0	7.8	7.7
Final	7.6	7.6	7.5	7.8	7.6	7.5	7.7

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	7.3	7.3	7.6	7.5	7.7	7.2
Final	6.4	6.2	5.9	7.3	6.7	5.6	6.6
pH Initial	7.9	7.7	7.9	7.9	8.0	7.8	7.8
Final	7.6	7.5	7.5	7.8	7.6	7.5	7.6

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.0	7.3	6.8	6.7	7.5	7.5	7.1
Final	6.4	6.1	6.4	7.3	6.5	5.6	6.0
pH Initial	7.9	7.8	8.0	8.0	8.0	7.9	7.8
Final	7.6	7.6	7.6	7.8	7.6	7.5	7.6

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.1	7.2	8.0	6.9	7.5	7.0
Final	6.3	5.8	6.1	7.2	6.5	5.8	6.7
pH Initial	7.9	7.9	8.0	8.0	8.0	7.9	7.8
Final	7.6	7.6	7.7	7.8	7.6	7.6	7.9

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	7.2	7.0	7.4	7.0	7.7	7.2
Final	6.6	5.6	6.2	7.4	6.6	5.7	6.4
pH Initial	7.9	7.9	8.0	7.9	8.1	8.0	8.0
Final	7.6	7.5	7.4	7.9	7.7	7.6	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	28	430	<0.05	AR0035602 17-JAN-22
120	33	440	<0.05	AR0035602 19-JAN-22
120	33	440	<0.05	AR0035602 21-JAN-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	83	310	<0.05	262038-1

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: January 18, 2022 at 1300

Date and Time Test Terminated: January 24, 2022 at 1255

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	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	30	41	39	40	39	33
B	44	44	43	47	46	44
C	46	44	22	43	40	46
D	43	43	38	39	36	39
E	38	40	41	39	37	38
F	40	50	40	41	35	44
G	38	41	24	22	16	19
H	38	48	37	46	44	40
I	39	43	39	46	44	41
J	26	40	39	39	37	38
Mean per Adult	38.2	43.4	36.2	40.2	37.4	38.2
Mean per Surviving Adult	38.2	43.4	36.2	40.2	37.4	38.2
CV %	16.0	7.70	19.8	17.7	22.5	20.2

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: 22.5 (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: January 18, 2022 TIME: 1300
Test Terminated: DATE: January 24, 2022 TIME: 1255

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	7.0	6.9	6.8	7.1	7.6	7.2
Final	7.0	7.4	7.4	7.4	7.3	7.1	--
pH Initial	7.8	7.7	7.8	8.0	8.0	7.6	7.6
Final	7.8	8.1	8.1	8.1	8.0	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	7.2	7.0	7.2	7.4	6.8	7.7	7.0
Final	7.1	7.5	7.7	7.8	7.5	7.0	--
pH Initial	7.9	7.7	7.9	7.9	8.0	7.8	7.7
Final	7.9	8.1	8.1	8.1	8.1	8.2	--

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

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.0	7.3	6.8	6.7	7.5	7.5	7.1
Final	7.3	7.5	7.4	7.1	7.3	7.3	--
pH Initial	7.9	7.8	8.0	8.0	8.0	7.9	7.8
Final	8.1	8.2	8.2	8.1	8.2	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	7.1	7.1	7.2	8.0	6.9	7.5	7.0
Final	7.1	7.8	7.5	7.5	7.3	7.0	--
pH Initial	7.9	7.9	8.0	8.0	8.0	7.9	7.8
Final	8.1	8.2	8.2	8.2	8.1	8.2	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.3	7.2	7.0	7.4	7.0	7.7	7.2
Final	7.1	7.5	7.4	7.5	7.5	7.3	--
pH Initial	7.9	7.9	8.0	7.9	8.1	8.0	8.0
Final	8.1	8.3	8.3	8.3	8.2	8.4	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	28	430	<0.05	AR0035602 17-JAN-22
120	33	440	<0.05	AR0035602 19-JAN-22
120	33	440	<0.05	AR0035602 21-JAN-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	83	310	<0.05	262038-1



LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		AIC Control No: <u>262168</u>	
Project Reference: <u>AR0035602</u>		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		Carrier: <u>FX</u>	
Sampled By: <u>LORRE HOLT</u>		Received Temperature °C: <u>0.3</u>	
AIC Sample Identification: <u>AR0035602</u>		Remarks:	
Date/Time Collected: <u>11/22/11 8:20 AM - 8:20 PM</u>			
Sample Matrix: <u>WATER</u>			
GRA B: <u>✓</u>			
COMP: <u>✓</u>			
SOIL: <u>✓</u>			
NO of BOTTLES: <u>1</u>		Analyses Requested: <u>BIDMON: BROW-CHRONIC CD+FH</u>	
PO No.:			
Container Type: <u>P</u>		Field pH calibration on: <u>@</u>	
Preservative: <u>NO</u>		Buffer:	
G = Glass NO = none		T = Sodium Thiosulfate Z = Zinc acetate	
P = Plastic S = Sulfuric acid pH2		H = HCl to pH2 B = NaOH to pH12	
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN ___ DAYS		Received Date/Time: <u>11/22/11 9:45 AM</u>	
Expedited results requested by:		By: <u>D. BROWN</u>	
Who should AIC contact with questions: <u>LORRE HOLT</u>		Received in Lab Date/Time: <u>1/18/22</u>	
Phone: <u>870-483-3832</u> Fax: <u>870-483-10525</u>		By: <u>D. BROWN</u>	
Report Attention to: <u>LORRE HOLT</u>		Comments:	
Report Address to: <u>704 Hwy 463 N Trumann, AR 72472</u>		<u>2888 1254 6325</u>	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: TRUMANN Water Works		PO No.		No of BOTTLES		Analyses Requested		AIC Control No: 262168	
Project Reference: AR0035202		Sample Matrix		WATER		BIOLOGICAL - CHRONIC		AIC Proposal No:	
Project Manager: Scotty Jones		G R A B		V		CONFH		Carrier: Fedex	
Sampled By: LORRE HOLT		Date/Time Collected		11/19/02 9:20 AM		Remarks		Received Temperature °C	
AIC No. 2 AR0035202		Container Type		P		Field pH calibration		on @	
Preservative		NO = none		S = Sulfuric acid pH2		Buffer:		T = Sodium Thiosulfate	
Turnaround Time Requested: (Please circle)		V = VOA vials		N = Nitric acid pH2		H = HCl to pH2		Z = Zinc acetate	
NORMAL or EXPEDITED IN ___ DAYS		Relinquished By: LORRE HOLT		Date/Time: 11/19/02 9:20 AM		Received By:		Date/Time	
Expedited results requested by:		Relinquished By:		Date/Time		Received in Lab		Date/Time	
Who should AIC contact with questions: LORRE HOLT		By:		Date/Time		By: [Signature]		Date/Time: 1-20-02	
Phone: 810-483-3832 Fax: 810-483-6525		Comments:		Date/Time		Date/Time		Date/Time	
Report Attention to: LORRE HOLT		By:		Date/Time		Date/Time		Date/Time	
Report Address to: TRUMANN, AR 72472		By:		Date/Time		Date/Time		Date/Time	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: TRUMANN Water Works

Project Reference: AR0035202

Project Manager: SCOTTY JONES

Sampled By: LORRE HOLT

AIC No. 3 Sample Identification AR0035202 Date/Time Collected 12/22-12/12/00

Container Type P Preservative NL

Sample Matrix: WATER

Analyses Requested: BIDMONT, BIRMINGHAM - CHRONIC CD + FH

No of BOTTLES: 1

PO No.

AIC Control No.: 262168

AIC Proposal No.:

Carrier: Fed Ex

Received Temperature °C: 0.4

Remarks:

Field pH calibration: on Buffer:

T = Sodium Thiosulfate
Z = Zinc acetate

H = HCl to pH2
B = NaOH to pH12

V = VOA vials
N = Nitric acid pH2

Relinquished By: Lorrie Holt Date/Time: 12/22/00

Relinquished By: Date/Time:

Received By: Date/Time:

Received in Lab By: Date/Time: 22 Jan 02 0850

Comments:

Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN DAYS

Expedited results requested by:

Who should AIC contact with questions: LORRE HOLT

Phone: 870-483-2832 Fax: 870-483-10525

Report Attention to: LORRE HOLT

Report Address to: 104 HWY 463 N TRUMANN, AR 72472



May 10, 2022

Biomonitoring Testing
for
AR0035602

Control No. 264814-1

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:

The statistically significant difference noted at the 5% effluent concentration does not follow a dose response pattern and is considered an anomaly.

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 9 % effluent, which is equal to the critical dilution of 9 %. The NOEC for growth occurred at 9 % effluent, which is equal to 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 percent minimum significant difference (PMSD) was below the limit of 13. 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 *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Chief Operating Officer

A handwritten signature in black ink is written over a horizontal line. Below the signature, the name 'John Overbey' and title 'Chief Operating Officer' are printed in a standard font.

PDF cc: Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
scottypw@gmail.com

Trumann Water and Sewer Commission
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%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.617	PASS
Control Growth CV < or = 40%	10.9	PASS
Growth Minimum Significant Difference 12 to 30%	25.6	PASS
Critical Dilution CV < or = 40%	27.4	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	28.2	PASS
Control CV < or = 40% per Surviving Female	6.21	PASS
Reproduction Minimum Significant Difference 13 to 47%	11.1	BELOW
Critical Dilution CV < or = 40%	13.1	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)	7.9	7.8	7.6
pH (standard units)	7.6	7.7	7.6
Alkalinity (mg/l as CaCO ₃)	83	82	80
Hardness (mg/l as CaCO ₃)	23	25	29
Conductivity (umhos/cm)	390	400	390
Residual Chlorine (mg/l)	<0.05	0.050	0.050
Ammonia as N (mg/l)	0.61	0.43	0.38

2. Dilution Water Samples:
Moderately Hard

Analysis	264725-1
Dissolved oxygen (mg/l)	7.9
pH (standard units)	7.5
Alkalinity (mg/l as CaCO ₃)	60
Hardness (mg/l as CaCO ₃)	81
Conductivity (umhos/cm)	310
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: April 19, 2022 at 1320
Date & Time Test Terminated: April 26, 2022 at 1515
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 19, 2022 at 1220
Date & Time Test Terminated: April 25, 2022 at 1400
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Source of test organisms: In-house culture

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

D. Test Organisms

1. Scientific Name

a. Test 1000.0 *Pimephales promelas*

b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

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

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's and Bartlett's test. The survival data was then analyzed using Dunnett's 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 March 08, 2022 at 1055 to March 15, 2022 at 1000

The results were as follows: (Control No. 263538-1.)

Survival LC-50: 2520 mg/l

Growth IC-25: 1982 mg/l

Growth PMSD: 17.7

Ceriodaphnia dubia

A chronic reference test was performed on March 08, 2022 at 1045 to March 14, 2022 at 1230

The results were as follows: (Control No. 263538-2.)

Survival LC-50: 1598.9 mg/l

Reproduction IC-25: 1203 mg/l

Reproduction PMSD: 24.2

V. Organism History

Pimephales promelas (Fathead minnow)

Date: April 19, 2022

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: April 19, 2022

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

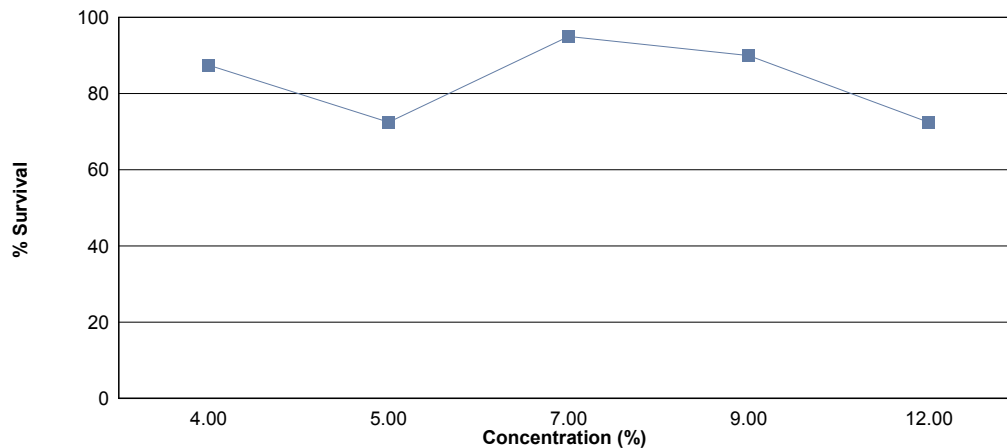
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 19, 2022 at 1320 and continued through April 26, 2022 at 1515. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 9 % effluent
- b.) NOEC growth = 9 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.602
4 %	87.5	0.490
5 %	72.5	0.360 *
7 %	95.0	0.543
9 %	90.0	0.481
12 %	72.5 *	--

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

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

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

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

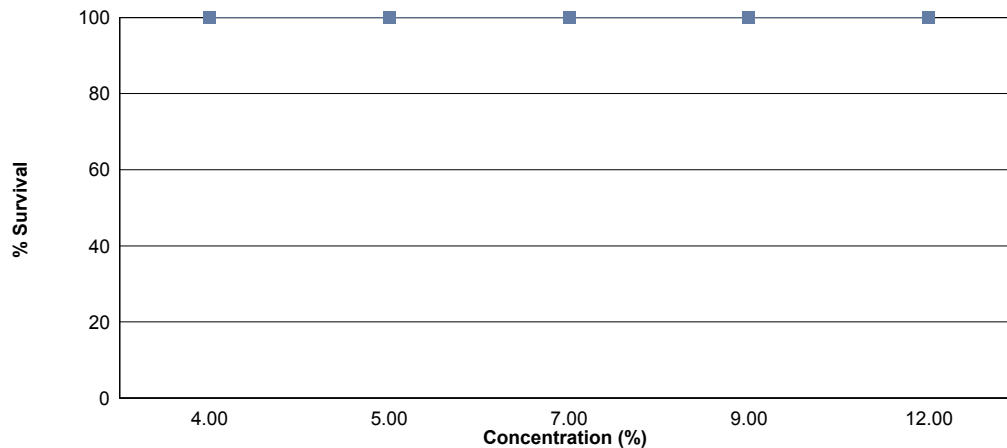
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

The test was initiated on April 19, 2022 at 1220 and continued through April 25, 2022 at 1400. 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

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



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	28.2
4 %	100	29.6
5 %	100	29.1
7 %	100	28.9
9 %	100	30.8
12 %	100	31.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 19, 2022 at 1320

Date and Time Test Terminated: April 26, 2022 at 1515

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: April 19, 2022 at 1320

Test Terminated: April 26, 2022 at 1515

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.73189	.73710	0.00521	8	0.651
	B	.69474	.69909	0.00435	8	0.544
	C	.70741	.71274	0.00533	8	0.666
	D	.70809	.71312	0.00503	8	0.629
	E	.71640	.72057	0.00417	8	0.521
4 %	A	.70846	.71235	0.00389	8	0.486
	B	.71484	.71788	0.00304	8	0.380
	C	.70854	.71254	0.00400	8	0.500
	D	.71605	.72061	0.00456	8	0.570
	E	.72822	.73234	0.00412	8	0.515
5 %	A	.69361	.69711	0.00350	8	0.438
	B	.70178	.70364	0.00186	8	0.232
	C	.71325	.71790	0.00465	8	0.581
	D	.70739	.70927	0.00188	8	0.235
	E	.69375	.69627	0.00252	8	0.315
7 %	A	.70276	.70756	0.00480	8	0.600
	B	.72663	.73100	0.00437	8	0.546
	C	.71709	.72024	0.00315	8	0.394
	D	.71069	.71556	0.00487	8	0.609
	E	.71200	.71653	0.00453	8	0.566
9 %	A	.70667	.71118	0.00451	8	0.564
	B	.72889	.73290	0.00401	8	0.501
	C	.71355	.71814	0.00459	8	0.574
	D	.71174	.71587	0.00413	8	0.516
	E	.70503	.70705	0.00202	8	0.252
12 %	A	.70773	.71073	0.00300	8	0.375
	B	.71678	.71946	0.00268	8	0.335
	C	.70929	.71220	0.00291	8	0.364
	D	.71559	.71893	0.00334	8	0.418
	E	.71960	.72529	0.00569	8	0.711

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 19, 2022 at 1220

Date and Time Test Terminated: April 25, 2022 at 1400

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	4	5	3	0	0	0	0	4	3	3	22	10	2.20	
4	0	0	0	3	4	4	3	0	0	0	14	10	1.40	
5	12	10	10	11	9	10	10	10	10	11	103	10	10.3	
6	16	15	14	14	15	15	14	13	13	14	143	10	14.3	
7														
8														
TOTAL	32	30	27	28	28	29	27	27	26	28	282	10	28.2	

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	4	4	5	3	3	0	3	4	5	5	36	10	3.60
4	0	0	0	0	0	4	0	0	0	0	4	10	0.400
5	10	9	10	11	10	11	9	12	9	10	101	10	10.1
6	16	16	15	17	15	17	15	15	16	13	155	10	15.5
7													
8													
TOTAL	30	29	30	31	28	32	27	31	30	28	296	10	29.6

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	4	0	3	4	0	4	4	5	4	32	10	3.20
4	0	0	4	0	0	4	0	0	0	0	8	10	0.800
5	8	10	10	9	10	11	10	9	10	10	97	10	9.70
6	15	14	16	15	16	15	16	18	16	13	154	10	15.4
7													
8													
TOTAL	27	28	30	27	30	30	30	31	31	27	291	10	29.1

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 19, 2022 at 1220

Date and Time Test Terminated: April 25, 2022 at 1400

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	5	5	4	5	0	0	4	0	4	4	31	10	3.10	
4	0	0	0	0	4	5	0	4	0	0	13	10	1.30	
5	11	10	10	12	12	11	10	0	10	11	97	10	9.70	
6	18	15	17	0	18	14	16	19	16	15	148	10	14.8	
7														
8														
TOTAL	34	30	31	17	34	30	30	23	30	30	289	10	28.9	

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	4	4	6	4	4	5	4	4	4	4	43	10	4.30
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	10	11	11	10	11	11	10	12	10	10	106	10	10.6
6	15	18	17	15	14	19	16	20	17	8	159	10	15.9
7													
8													
TOTAL	29	33	34	29	29	35	30	36	31	22	308	10	30.8

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	5	4	2	0	0	5	4	5	4	33	10	3.30
4	0	0	0	0	3	4	0	0	0	0	7	10	0.700
5	9	10	10	10	11	11	11	10	10	11	103	10	10.3
6	18	17	18	17	16	19	17	15	14	19	170	10	17.0
7													
8													
TOTAL	31	32	32	29	30	34	33	29	29	34	313	10	31.3

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	0.87500	1.20940
2	4 %	1	0.75000	1.04720
2	4 %	2	0.62500	0.91174
2	4 %	3	1.00000	1.39310
2	4 %	4	1.00000	1.39310
2	4 %	5	1.00000	1.39310
3	5 %	1	0.87500	1.20940
3	5 %	2	0.62500	0.91174
3	5 %	3	1.00000	1.39310
3	5 %	4	0.50000	0.78540
3	5 %	5	0.62500	0.91174
4	7 %	1	1.00000	1.39310
4	7 %	2	1.00000	1.39310
4	7 %	3	0.75000	1.04720
4	7 %	4	1.00000	1.39310
4	7 %	5	1.00000	1.39310
5	9 %	1	1.00000	1.39310
5	9 %	2	1.00000	1.39310
5	9 %	3	1.00000	1.39310
5	9 %	4	1.00000	1.39310
5	9 %	5	0.50000	0.78540
6	12 %	1	0.75000	1.04720
6	12 %	2	0.62500	0.91174
6	12 %	3	0.50000	0.78540
6	12 %	4	0.75000	1.04720
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))
<p>D = 1.09 W = 0.9462 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	Transform: Arc Sin(Square Root(Y))
<p>Calculated B1 statistic = 5.264 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) Survival

ANOVA Table			Transform: Arc Sin(Square Root(Y))	
SOURCE	DF	SS	MS	F
Between	5	0.4829	0.09659	2.127
Within (Error)	24	1.09	0.04542	
Total	29	1.573		
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				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	1.3564	0.975		
2	4 %	1.2276	0.875	0.9556	
3	5 %	1.0423	0.725	2.33	
4	7 %	1.3239	0.95	0.2411	
5	9 %	1.2716	0.9	0.6291	
6	12 %	1.0369	0.725	2.37	*
Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24)					

Dunnett's Test - Table 2 of 2				Transform: Arc Sin(Square Root(Y))	
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	0.2125	22.3	0.1
3	5 %	5	0.2125	22.3	0.25
4	7 %	5	0.2125	22.3	0.025
5	9 %	5	0.2125	22.3	0.075
6	12 %	5	0.2125	22.3	0.25

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.2253 W = 0.9493 Critical W = 0.888 (alpha = 0.01, N = 25) Critical W = 0.918 (alpha = 0.05, N = 25)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 3.990 Critical B = 13.277 (alpha = 0.01, df = 4)</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	4	0.1609	0.04022	3.572	
Within (Error)	20	0.2253	0.01126		
Total	24	0.3862			
Critical F = 4.43 (alpha = 0.01, df = 4,20)					
2.87 (alpha = 0.05, df = 4,20)					
Since F > Critical F 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	0.6022	0.6022			
2	4 %	0.4902	0.4902	1.669		
3	5 %	0.3602	0.3602	3.606	*	
4	7 %	0.543	0.543	0.8821		
5	9 %	0.4814	0.4814	1.8		
Dunnett's critical value = 2.3 (1 Tailed, alpha = 0.05, df = 4,20) (Actual df = 4,20)						

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	0.1544	25.6	0.112		
3	5 %	5	0.1544	25.6	0.242		
4	7 %	5	0.1544	25.6	0.0592		
5	9 %	5	0.1544	25.6	0.1208		

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 %	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
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 %	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	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.1481 D* = 1.162 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>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 %	129.00	75.00	10.00	
3	5 %	120.00	75.00	10.00	
4	7 %	126.50	75.00	10.00	
5	9 %	135.00	75.00	10.00	
6	12 %	144.00	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	70.15	14.03	1.523	
Within (Error)	54	497.5	9.213		
Total	59	567.7			
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	28.2	28.2			
2	4 %	29.6	29.6	-1.031		
3	5 %	29.1	29.1	-0.663		
4	7 %	28.9	28.9	-0.5157		
5	9 %	30.8	30.8	-1.915		
6	12 %	31.3	31.3	-2.284		
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	3.136	11.1	-1.4	
3	5 %	10	3.136	11.1	-0.9	
4	7 %	10	3.136	11.1	-0.7	
5	9 %	10	3.136	11.1	-2.6	
6	12 %	10	3.136	11.1	-3.1	

Lower PMSD Bound Test for Ceriodaphnia dubia

Concentration	Reproduction	Relative Difference from Control	Pass/Fail
Control	28.2	-	
4 %	29.6	-4.96	PASS
5 %	29.1	-3.19	PASS
7 %	28.9	-2.48	PASS
9 %	30.8	-9.22	PASS
12 %	31.3	-11.0	PASS

Limit = 13

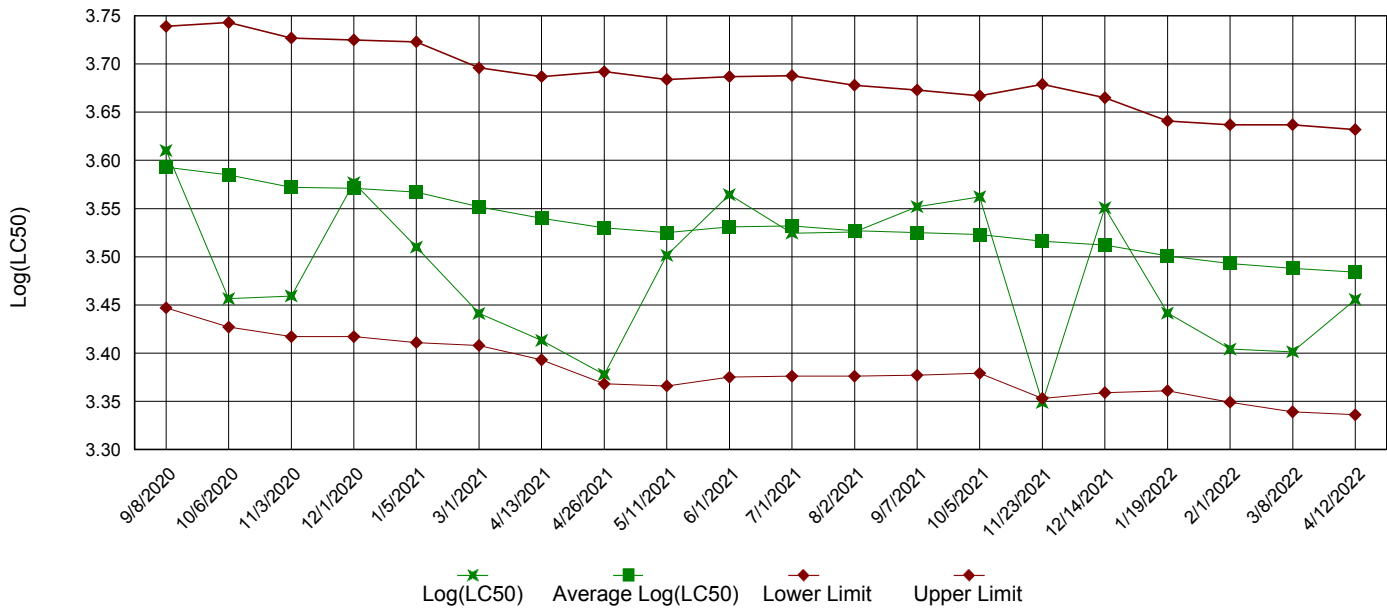
NOEC = 12 %

LOEC = 12 %

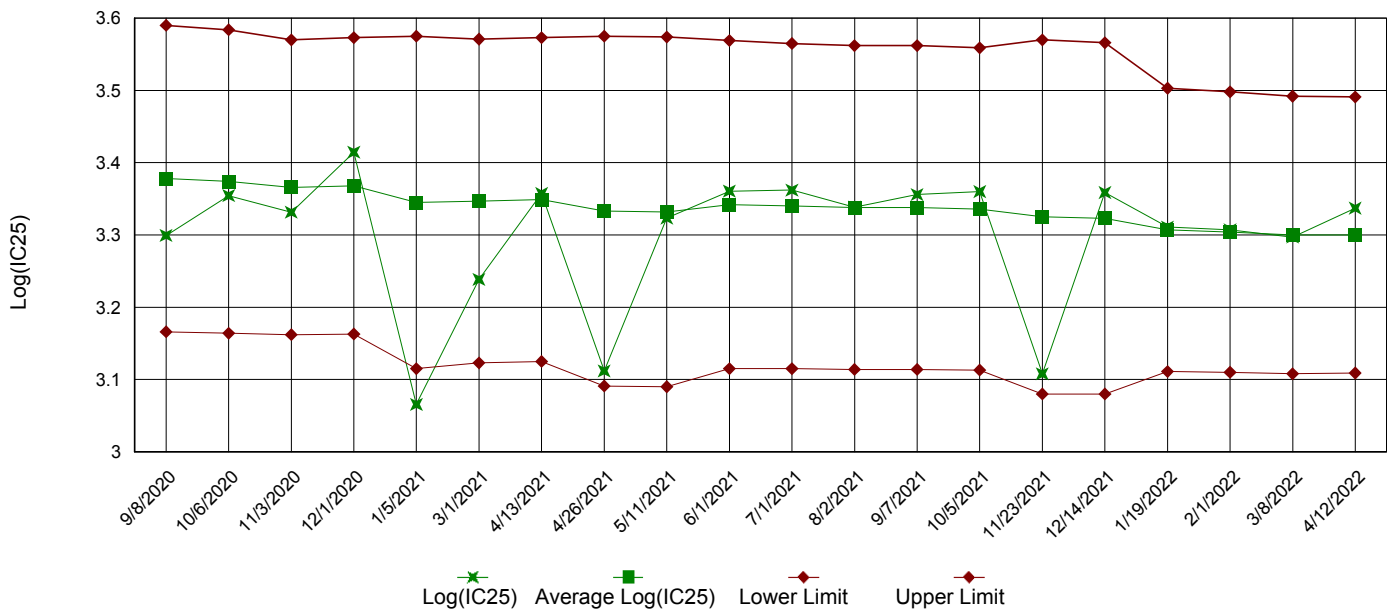
Appendix A3: Test 1000.0

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

LC50 Survival Data

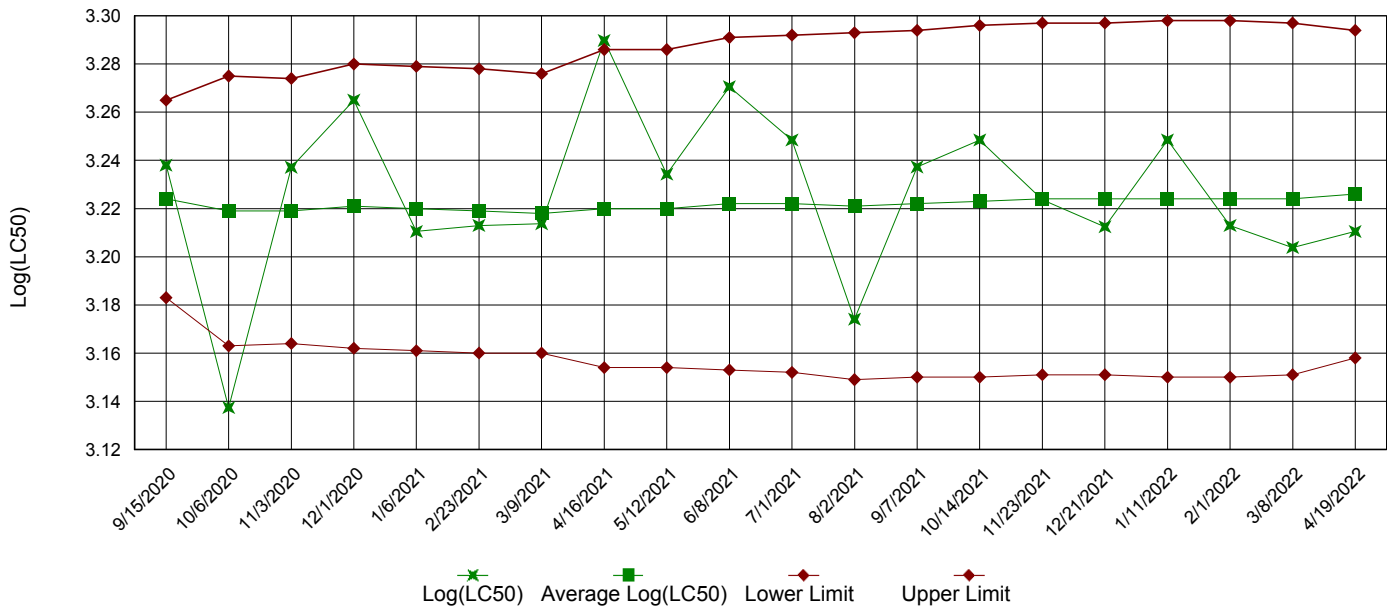


IC25 Growth Data

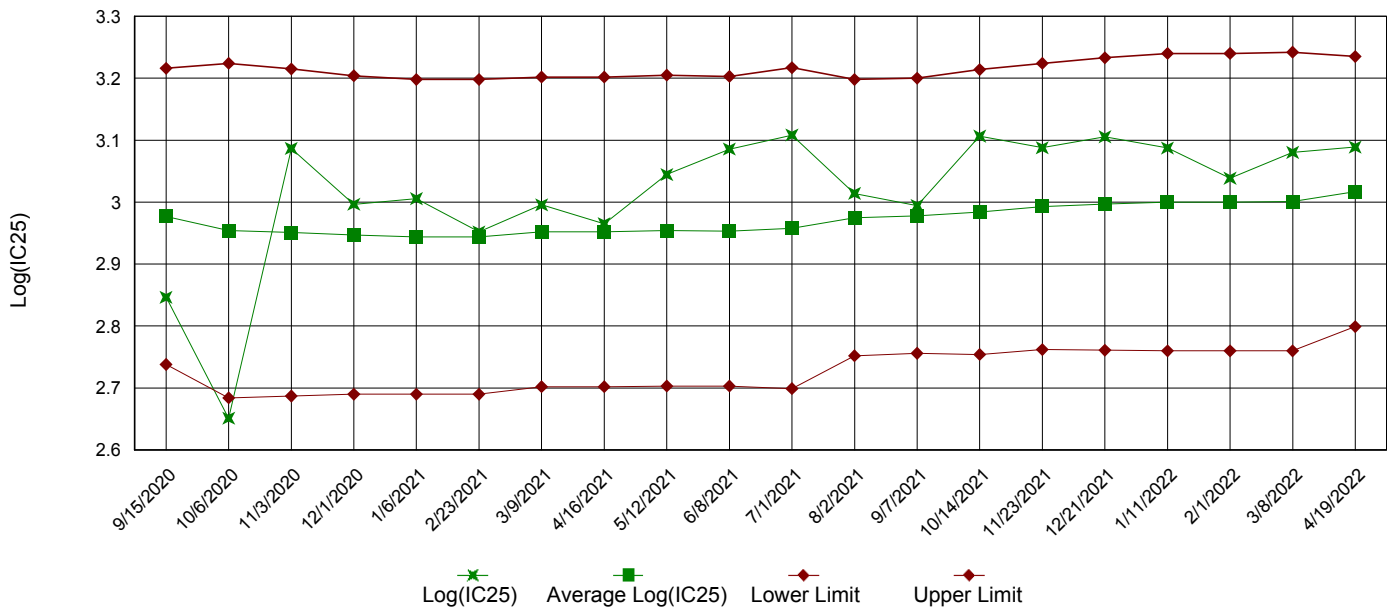


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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.: AFIN 56-00047

Date and Time Test Initiated: April 19, 2022 at 1320

Date and Time Test Terminated: April 26, 2022 at 1515

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	100	87.5	100	100	97.5	5.73
4 %	75.0	62.5	100	100	100	100	100	87.5	20.2
5 %	87.5	62.5	100	50.0	62.5	100	100	72.5	28.3
7 %	100	100	75.0	100	100	100	100	95.0	11.8
9 %	100	100	100	100	50.0	100	100	90.0	24.8
12 %	75.0	62.5	50.0	75.0	100	100	100	72.5	25.6

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.651	0.544	0.666	0.629	0.521	0.602	10.9
4 %	0.486	0.380	0.500	0.570	0.515	0.490	14.1
5 %	0.438	0.232	0.581	0.235	0.315	0.360	41.4
7 %	0.600	0.546	0.394	0.609	0.566	0.543	16.0
9 %	0.564	0.501	0.574	0.516	0.252	0.481	27.4
12 %	0.375	0.335	0.364	0.418	0.711	0.441	35.0

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. Dunnett's 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: 9 % (TOP6C)
6. LOEC *Pimephales* Lethality: 12 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 9 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 9 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 27.4 (TQP6C)
10. Sublethality for this test: 9 % (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, 343, 357, 358

Test Initiated: DATE: April 19, 2022 TIME: 1320
Test Terminated: DATE: April 26, 2022 TIME: 1515

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.9	7.7	7.3	7.8	7.2	7.0	7.4
Final	6.9	6.2	6.1	6.4	6.0	5.6	5.9
pH Initial	7.5	7.5	7.7	7.8	7.8	7.8	7.8
Final	7.5	7.4	7.4	7.5	7.5	7.3	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	8.1	7.8	7.7	8.1	7.3	7.1	7.5
Final	6.9	6.5	6.3	6.3	6.0	5.4	5.6
pH Initial	7.6	7.5	7.8	7.0	7.8	7.8	7.8
Final	7.5	7.5	7.4	7.5	7.4	7.3	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	7.8	7.7	7.7	8.0	7.4	7.3	7.5
Final	7.0	6.5	6.3	6.3	6.2	5.4	6.1
pH Initial	7.7	7.6	7.8	7.8	7.8	7.8	7.8
Final	7.5	7.5	7.5	7.5	7.5	7.3	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.8	7.6	7.2	8.0	7.0	6.8	7.2
Final	6.6	6.3	6.0	6.1	6.0	5.4	5.8
pH Initial	7.8	7.7	7.9	7.9	7.8	7.8	7.9
Final	7.5	7.6	7.6	7.5	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	8.0	7.7	7.7	7.7	7.1	6.9	7.3
Final	6.6	6.4	6.0	6.2	5.8	5.6	5.9
pH Initial	7.8	7.7	7.9	7.9	7.9	7.8	8.0
Final	7.5	7.6	7.6	7.6	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.7	7.7	7.7	8.0	7.4	7.2	7.5
Final	6.6	6.2	6.3	6.3	6.0	5.5	6.1
pH Initial	7.8	7.8	7.8	8.0	7.8	7.8	8.1
Final	7.5	7.5	7.5	7.6	7.4	7.4	7.6

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
83	23	390	<0.05	AR0035602 18-APR-22
82	25	400	0.050	AR0035602 20-APR-22
80	29	390	0.050	AR0035602 20-APR-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
60	81	310	<0.05	264725-1

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: April 19, 2022 at 1220

Date and Time Test Terminated: April 25, 2022 at 1400

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	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	32	30	27	34	29	31
B	30	29	28	30	33	32
C	27	30	30	31	34	32
D	28	31	27	17	29	29
E	28	28	30	34	29	30
F	29	32	30	30	35	34
G	27	27	30	30	30	33
H	27	31	31	23	36	29
I	26	30	31	30	31	29
J	28	28	27	30	22	34
Mean per Adult	28.2	29.6	29.1	28.9	30.8	31.3
Mean per Surviving Adult	28.2	29.6	29.1	28.9	30.8	31.3
CV %	6.21	5.33	5.72	17.8	13.1	6.40

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: 13.1 (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.: AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 343, 357, 358

Test Initiated: DATE: April 19, 2022 TIME: 1220
Test Terminated: DATE: April 25, 2022 TIME: 1400

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.9	7.7	7.3	7.8	7.2	7.0	7.4
Final	7.5	7.4	7.1	--	7.0	7.4	--
pH Initial	7.5	7.5	7.7	7.8	7.8	7.8	7.8
Final	7.8	8.0	7.9	--	7.9	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	8.1	7.8	7.7	8.1	7.3	7.1	7.5
Final	7.4	7.3	7.2	--	7.4	7.5	--
pH Initial	7.6	7.5	7.8	7.0	7.8	7.8	7.8
Final	8.0	8.0	8.0	--	8.0	8.0	--

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

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.8	7.6	7.2	8.0	7.0	6.8	7.2
Final	7.4	7.5	7.1	--	7.1	7.1	--
pH Initial	7.8	7.7	7.9	7.9	7.8	7.8	7.9
Final	8.0	8.0	8.1	--	8.0	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	8.0	7.7	7.7	7.7	7.1	6.9	7.3
Final	7.4	7.5	7.0	--	6.9	7.5	--
pH Initial	7.8	7.7	7.9	7.9	7.9	7.8	8.0
Final	8.0	8.0	8.0	--	8.1	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.7	7.7	7.7	8.0	7.4	7.2	7.5
Final	7.4	7.3	7.2	--	7.1	7.5	--
pH Initial	7.8	7.8	7.8	8.0	7.8	7.8	8.1
Final	8.0	8.1	8.1	--	8.2	8.1	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
83	23	390	<0.05	AR0035602 18-APR-22
82	25	400	0.050	AR0035602 20-APR-22
80	29	390	0.050	AR0035602 20-APR-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
60	81	310	<0.05	264725-1

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Trumann Water Works		AIC Control No: 264814	
Project Reference: AR0035602		AIC Proposal No:	
Project Manager: Scotty Jones		Carrier:	
Sampled By: LORRE HOLT		Received Temperature °C	
AIC No. AR0035602		Remarks	
Date/Time Collected: 4/22/02 8:00 AM			
G R A B V			
C O M P V			
W A T E R L			
S O I L			
Sample Matrix			
No of BOTTLES 1			
PO No.		Analyses Requested	
		BIDMONT, FORM - CHRONIC CD - FA	
Container Type P		Field pH calibration	
Preservative N/D		on _____ @ _____	
G = Glass NO = none S = Sulfuric acid pH2		T = Sodium Thiosulfate Z = Zinc acetate	
V = VOA vials N = Nitric acid pH2		Received Date/Time	
Relinquished By: Scotty Jones		Received By: JK388	
Relinquished Date/Time: 4/22/02 12:46		Received in Lab Date/Time: 23 APR 02 0830	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS		Comments:	
Expedited results requested by:			
Who should AIC contact with questions: LORRE HOLT			
Phone: 870-483-8882 Fax: 870-483-6525			
Report Attention to: LORRE HOLT			
Report Address to: 704 HWY 463 N TRUMANN, AR 72472			

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		AIC Control No: <u>264814</u>	
Project Reference: <u>AR0035602</u>		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		Carrier: <u>FX</u>	
Sampled By: <u>LORRE HOLT</u>		Received Temperature °C: <u>0.6</u>	
AIC Sample Identification: <u>AR0035602</u>		Remarks:	
Date/Time Collected: <u>4/19/02 - 4:00 PM</u>			
Date/Time Collected: <u>8:00 AM - 8:00 AM</u>			
G R A B			
C O M P			
W A T E R L			
S O I L			
V			
No. of BOTTLES			
PO No.		Analyses Requested	
Sample Matrix		<u>BIDMONT FORM - CHRONIC CD + FH</u>	
Container Type: <u>P</u>			
Preservative: <u>NID</u>			
G = Glass			
NO = none			
P = Plastic			
S = Sulfuric acid pH2			
V = VOA vials			
N = Nitric acid pH2			
Turnaround Time Requested: (Please circle)		T = Sodium Thiosulfate	
<u>NORMAL</u> or EXPEDITED IN _____ DAYS		Z = Zinc acetate	
Expedited results requested by:		Date/Time Received	
Who should AIC contact with questions: <u>LORRE HOLT</u>		By: <u>[Signature]</u>	
Phone: <u>870-483-8882</u> Fax: <u>870-483-16525</u>		Date/Time Received in Lab	
Report Attention to: <u>LORRE HOLT</u>		By: <u>D. BROWN</u>	
Report Address to: <u>704 Hwy 463 N</u>		Date/Time	
<u>TRUMANN, AR 72472</u>		<u>0840</u>	
Comments:		<u>2722 2667 9099</u>	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: TRUMANN Water Works			PO No.:			Analyses Requested:			AIC Control No: 764814		
Project Reference: AR0035602			Sample Matrix:			BOTTLES			AIC Proposal No:		
Project Manager: Scotty Jones			WATER			No of			Carrier:		
Sampled By: LORRE HOLT			COMPOST			1			Received Temperature °C		
AIC No: AR0035602			GRA B			✓			Remarks:		
Date/Time Collected: 4/17/22 8:00 AM			V						Field pH calibration on @		
Date/Time: 4/18/22 11:00 AM			V						Buffer:		
Container Type: Plastic			NO						T = Sodium Thiosulfate		
Preservative: NO			NO						Z = Zinc acetate		
G = Glass			NO = none						H = HCl to pH2		
NO = none			S = Sulfuric acid pH2						B = NaOH to pH12		
Turnaround Time Requested: (Please circle)			V = VOA vials						Received		
NORMAL or EXPEDITED IN 3 DAYS			N = Nitric acid pH2						By: [Signature]		
Expedited results requested by:			Relinquished			Date/Time			Date/Time		
Who should AIC contact with questions: LORRE HOLT			By: [Signature]			4/18/22 11:00 AM			Received in Lab		
Phone: 870-483-8832 Fax: 870-483-10525			Relinquished			Date/Time			By: [Signature]		
Report Attention to: LORRE HOLT			By:						Date/Time		
Report Address to: TRUMANN, AR 72472			Comments:						Date/Time		
									Date/Time		

FX: 2721 3029 9295



July 27, 2022

Biomonitoring Testing
for

Control No. 267363-1

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
825 Hwy 463 North
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
825 Hwy 463 North
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:

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.**

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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	0.511	PASS
Control Growth CV < or = 40%	7.76	PASS
Growth Minimum Significant Difference 12 to 30%	19.8	PASS
Critical Dilution CV < or = 40%	9.57	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	30.3	PASS
Control CV < or = 40% per Surviving Female	5.40	PASS
Reproduction Minimum Significant Difference 13 to 47%	14.3	PASS
Critical Dilution CV < or = 40%	11.9	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	Sample 3
Dissolved oxygen (mg/l)	7.8	7.9	7.5
pH (standard units)	8.5	8.4	8.5
Alkalinity (mg/l as CaCO ₃)	130	130	130
Hardness (mg/l as CaCO ₃)	32	29	29
Conductivity (umhos/cm)	420	420	430
Residual Chlorine (mg/l)	<0.05	0.060	0.050
Ammonia as N (mg/l)	0.62	0.62	0.72

2. Dilution Water Samples:

Moderately Hard

Analysis	267121-1	267279-1	267349-1
Dissolved oxygen (mg/l)	8.2	8.1	7.9
pH (standard units)	7.8	7.7	7.8
Alkalinity (mg/l as CaCO ₃)	63	60	60
Hardness (mg/l as CaCO ₃)	84	89	80
Conductivity (umhos/cm)	310	310	320
Residual Chlorine (mg/l)	<0.05	<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 19, 2022 at 1154
Date & Time Test Terminated: July 26, 2022 at 1000
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 19, 2022 at 1200
Date & Time Test Terminated: July 26, 2022 at 1350
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. Steel's Many-One Rank test was used to determine the No Observable Effects Concentration (NOEC) for growth. Dunnett's Test was used to calculate the PMSD.

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

IV. Standard Reference Toxicants

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

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on July 05, 2022 at 1125 to July 12, 2022 at 0952

The results were as follows: (Control No. 266934-1.)

Survival LC-50: 2024 mg/l

Growth IC-25: 1026 mg/l

Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on July 05, 2022 at 1110 to July 12, 2022 at 1303

The results were as follows: (Control No. 266934-2.)

Survival LC-50: 1709 mg/l

Reproduction IC-25: 1303 mg/l

Reproduction PMSD: 15.6

V. Organism History

Pimephales promelas (Fathead minnow)

Date: July 19, 2022

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: July 19, 2022

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

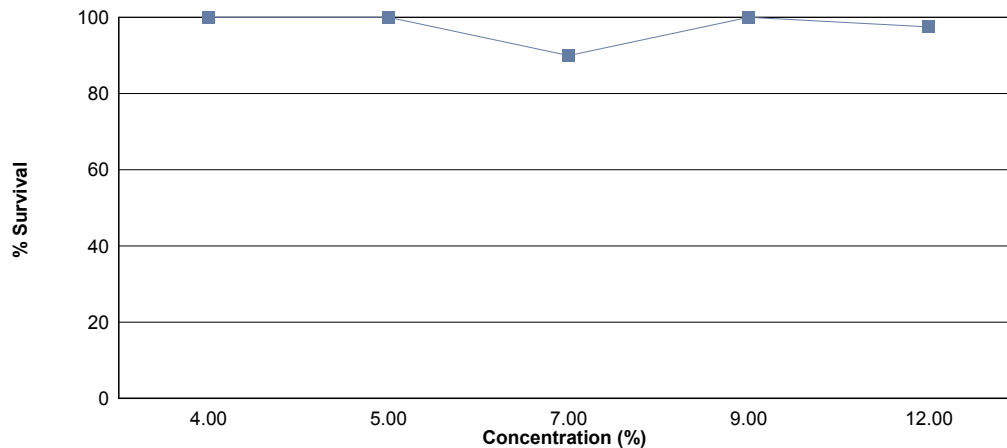
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 19, 2022 at 1154 and continued through July 26, 2022 at 1000. 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	97.5	0.498
4 %	100	0.492
5 %	100	0.463
7 %	90.0	0.470
9 %	100	0.579
12 %	97.5	0.519

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

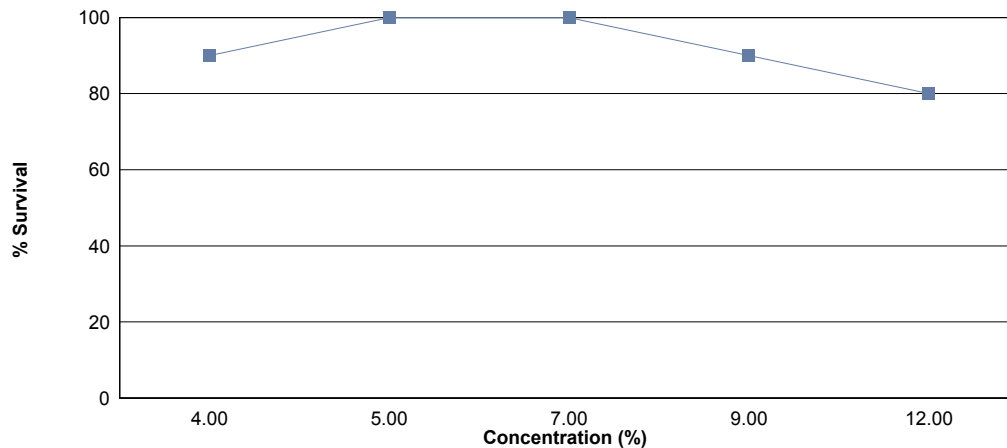
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 19, 2022 at 1200 and continued through July 26, 2022 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 reproduction = 12 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	30.3
4 %	90.0	29.2
5 %	100	29.8
7 %	100	29.8
9 %	90.0	30.1
12 %	80.0	27.8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: July 19, 2022 at 1154

Date and Time Test Terminated: July 26, 2022 at 1000

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: July 19, 2022 at 1154

Test Terminated: July 26, 2022 at 1000

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.67461	.67856	0.00395	8	0.494
	B	.66439	.66883	0.00444	8	0.555
	C	.66524	.66897	0.00373	8	0.466
	D	.66003	.66416	0.00413	8	0.516
	E	.66860	.67229	0.00369	8	0.461
4 %	A	.66422	.66811	0.00389	8	0.486
	B	.67454	.67812	0.00358	8	0.448
	C	.66778	.67128	0.00350	8	0.438
	D	.67723	.68141	0.00418	8	0.522
	E	.67098	.67551	0.00453	8	0.566
5 %	A	.66799	.67159	0.00360	8	0.450
	B	.67419	.67800	0.00381	8	0.476
	C	.67519	.67862	0.00343	8	0.429
	D	.67795	.68181	0.00386	8	0.482
	E	.66645	.67026	0.00381	8	0.476
7 %	A	.66858	.67289	0.00431	8	0.539
	B	.67258	.67462	0.00204	8	0.255
	C	.67171	.67587	0.00416	8	0.520
	D	.67382	.67816	0.00434	8	0.542
	E	.67755	.68150	0.00395	8	0.494
9 %	A	.67044	.67491	0.00447	8	0.559
	B	.66903	.67419	0.00516	8	0.645
	C	.66895	.67316	0.00421	8	0.526
	D	.66979	.67485	0.00506	8	0.632
	E	.66825	.67253	0.00428	8	0.535
12 %	A	.66906	.67340	0.00434	8	0.542
	B	.66878	.67353	0.00475	8	0.594
	C	.67302	.67670	0.00368	8	0.460
	D	.67791	.68221	0.00430	8	0.538
	E	.66367	.66734	0.00367	8	0.459

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 19, 2022 at 1200

Date and Time Test Terminated: July 26, 2022 at 1350

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	3	5	3	5	5	5	4	4	5	4	43	10	4.30	
5	10	10	12	11	12	10	11	11	10	10	107	10	10.7	
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
7	16	17	14	18	14	15	15	14	14	16	153	10	15.3	
8														
TOTAL	29	32	29	34	31	30	30	29	29	30	303	10	30.3	

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	0	0	0	0	10	0.00	
4	5	5	4	5	5	5	4	3	5	5	46	10	4.60	
5	11	10	9X	13	12	10	10	11	12	10	108	9	12.0	
6	0	0	X	0	0	0	0	0	0	0	0	9	0.00	
7	15	15	X	18	15	17	14	14	15	15	138	9	15.3	
8														
TOTAL	31	30	13	36	32	32	28	28	32	30	292	10	29.2	

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	5	3	4	4	6	5	5	5	3	5	45	10	4.50	
5	10	9	13	11	11	10	10	8	9	12	103	10	10.3	
6	0	0	0	0	0	0	0	0	0	12	12	10	1.20	
7	15	15	14	17	19	16	14	14	14	0	138	10	13.8	
8														
TOTAL	30	27	31	32	36	31	29	27	26	29	298	10	29.8	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 19, 2022 at 1200

Date and Time Test Terminated: July 26, 2022 at 1350

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	0	0	0	0	0	10	0.00
4	5	3	3	5	6	5	4	4	5	4	44	10	4.40	
5	11	9	13	12	13	11	9	7	10	11	106	10	10.6	
6	0	0	13	0	0	0	0	0	0	0	13	10	1.30	
7	16	12	0	15	18	15	16	14	15	14	135	10	13.5	
8														
TOTAL	32	24	29	32	37	31	29	25	30	29	298	10	29.8	

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	0	0	0	0	10	0.00
4	5	6	4	5	6	4	5	3	4	5	47	10	4.70
5	11	9	12	13	12	13	10	10	9	11	110	10	11.0
6	0	0	0	0	0	0	0	0	0	0	0	10	0.00
7	10X	14	15	19	14	15	18	15	11	13	144	9	16.0
8													
TOTAL	26	29	31	37	32	32	33	28	24	29	301	10	30.1

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	0	0	0	0	10	0.00
4	4	5	6	4	5	6	4	4	3	5	46	10	4.60
5	0	10	11	12	10	12	10	9	13	10	97	10	9.70
6	5	0	15	13	13	0	0	0	0	14	60	10	6.00
7	10	17X	0	0	0	17	16	12	3X	0	75	8	9.38
8													
TOTAL	19	32	32	29	28	35	30	25	19	29	278	10	27.8

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	0.87500	1.20940
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	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	1.00000	1.39310
3	5 %	5	1.00000	1.39310
4	7 %	1	1.00000	1.39310
4	7 %	2	0.50000	0.78540
4	7 %	3	1.00000	1.39310
4	7 %	4	1.00000	1.39310
4	7 %	5	1.00000	1.39310
5	9 %	1	1.00000	1.39310
5	9 %	2	1.00000	1.39310
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	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))
<p>D = 0.3494 W = 0.6174 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 %	30.00	16.00	5.00	
4	7 %	27.00	16.00	5.00	
5	9 %	30.00	16.00	5.00	
6	12 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
<p>D = 0.1044 W = 0.8763 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					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	4 %	26.00	16.00	5.00	
3	5 %	21.00	16.00	5.00	
4	7 %	29.50	16.00	5.00	
5	9 %	38.00	16.00	5.00	
6	12 %	28.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Dunnett's Test for PMSD Calculation

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.04471	0.008942	2.056	
Within (Error)	24	0.1044	0.00435		
Total	29	0.1491			
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	0.4984	0.4984			
2	4 %	0.492	0.492	0.1534		
3	5 %	0.4626	0.4626	0.8582		
4	7 %	0.47	0.47	0.6808		
5	9 %	0.5794	0.5794	-1.942		
6	12 %	0.5186	0.5186	-0.4843		
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	0.09844	19.8	0.0064	
3	5 %	5	0.09844	19.8	0.0358	
4	7 %	5	0.09844	19.8	0.0284	
5	9 %	5	0.09844	19.8	-0.081	
6	12 %	5	0.09844	19.8	-0.0202	

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 %	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
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 %	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.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
12 %	8	2	10
Total	18	2	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 8. 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	1	
2	5 %	10	0	
3	7 %	10	0	
4	9 %	10	1	
5	12 %	10	2	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p style="text-align: center;"> D = 0.1745 D* = 1.369 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 %	109.00	75.00	10.00	
3	5 %	98.00	75.00	10.00	
4	7 %	101.00	75.00	10.00	
5	9 %	102.50	75.00	10.00	
6	12 %	91.50	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	41.6	8.32	0.4752	
Within (Error)	54	945.4	17.51		
Total	59	987			
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)					

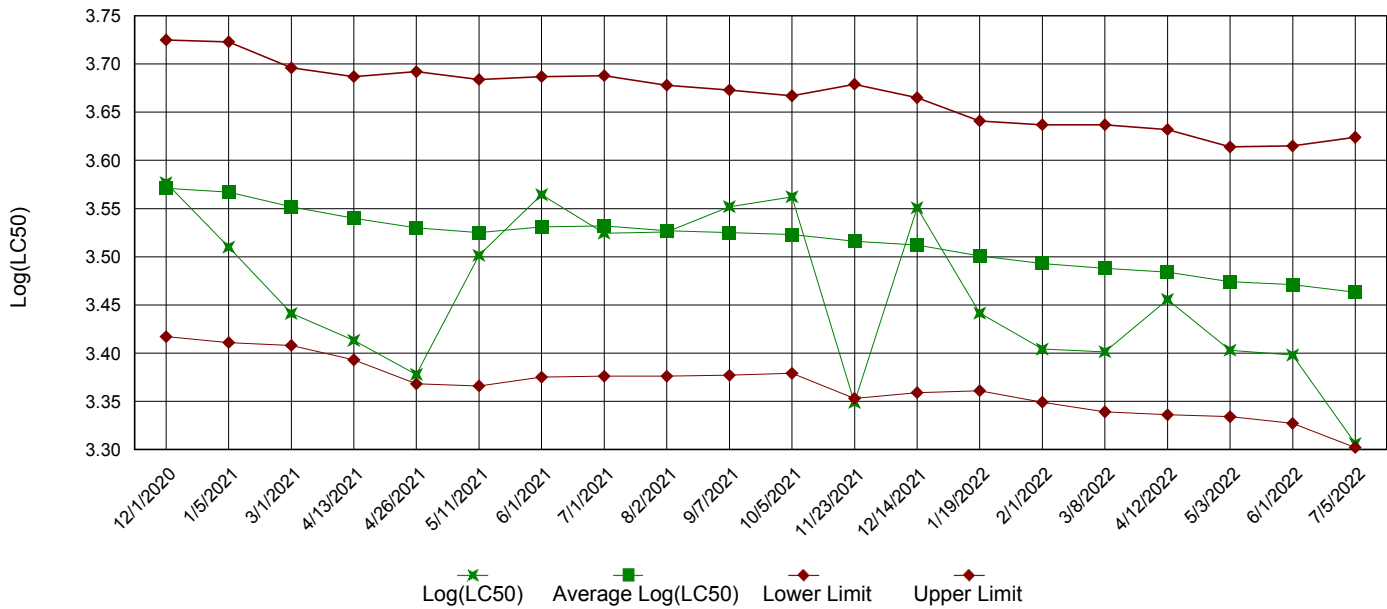
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	30.3	30.3			
2	4 %	29.2	29.2	0.5878		
3	5 %	29.8	29.8	0.2672		
4	7 %	29.8	29.8	0.2672		
5	9 %	30.1	30.1	0.1069		
6	12 %	27.8	27.8	1.336		
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	4.323	14.3	1.1	
3	5 %	10	4.323	14.3	0.5	
4	7 %	10	4.323	14.3	0.5	
5	9 %	10	4.323	14.3	0.2	
6	12 %	10	4.323	14.3	2.5	

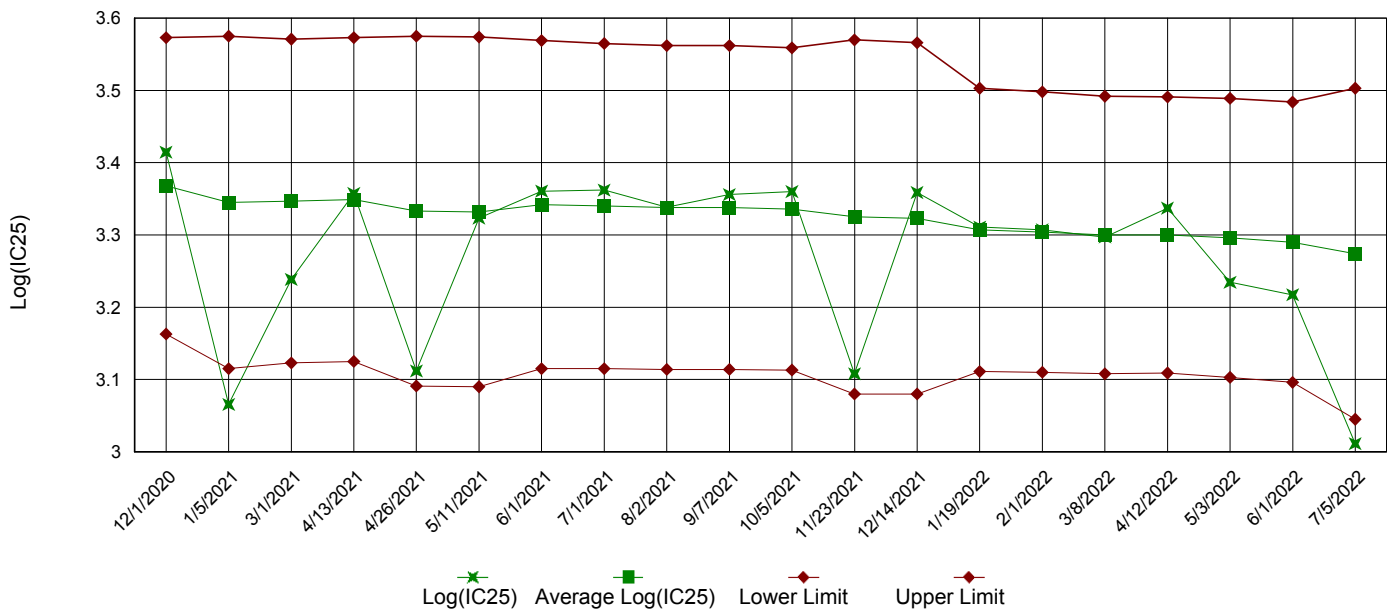
Appendix A3: Test 1000.0

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

LC50 Survival Data

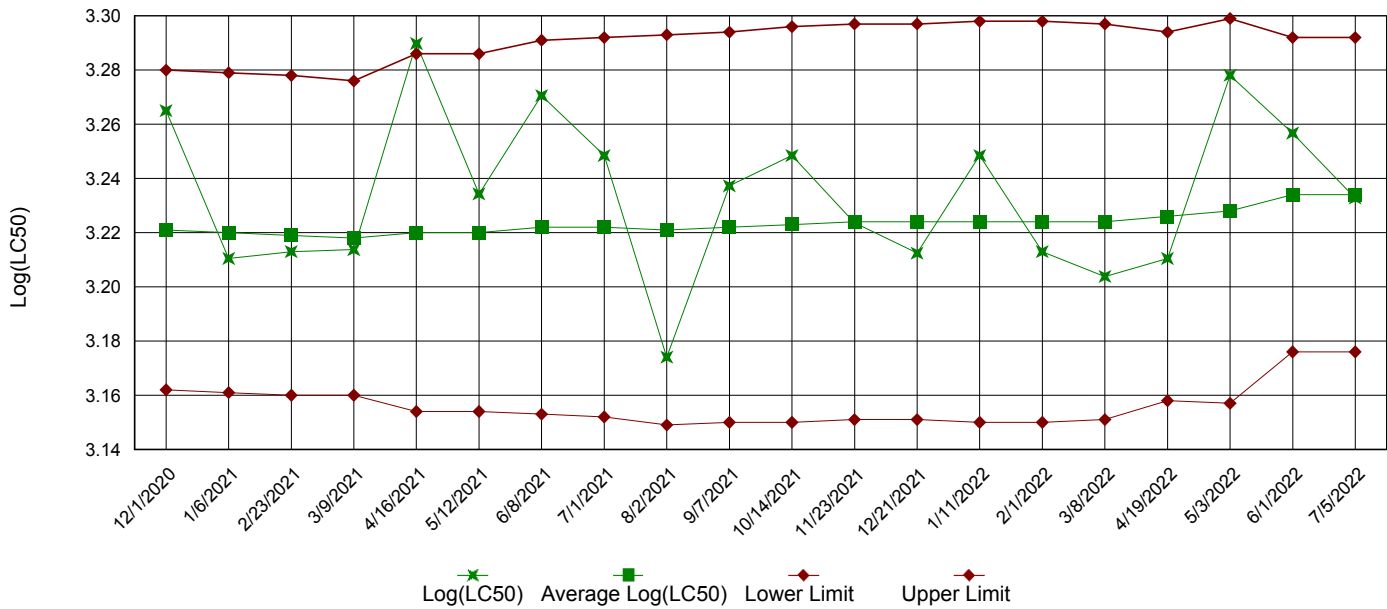


IC25 Growth Data

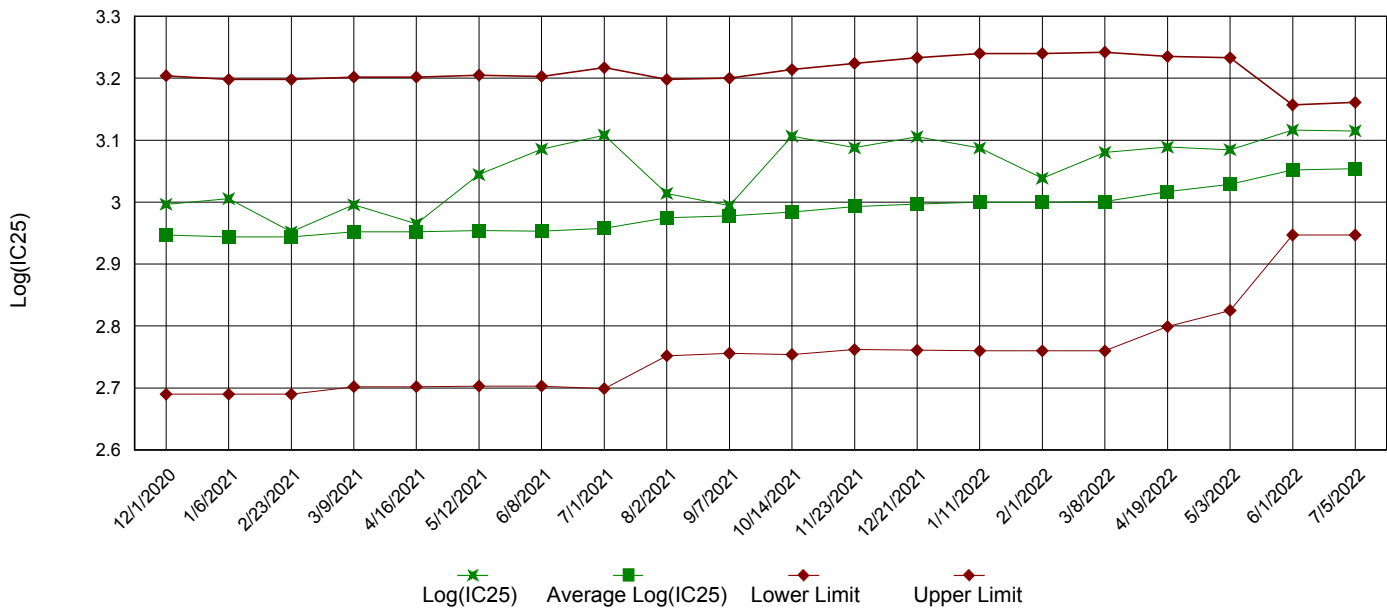


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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 19, 2022 at 1154

Date and Time Test Terminated: July 26, 2022 at 1000

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	100	87.5	100	100	97.5	5.73
4 %	100	100	100	100	100	100	100	100	0.00
5 %	100	100	100	100	100	100	100	100	0.00
7 %	100	50.0	100	100	100	100	100	90.0	24.8
9 %	100	100	100	100	100	100	100	100	0.00
12 %	100	100	100	100	87.5	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	0.494	0.555	0.466	0.516	0.461	0.498	7.76
4 %	0.486	0.448	0.438	0.522	0.566	0.492	10.8
5 %	0.450	0.476	0.429	0.482	0.476	0.463	4.86
7 %	0.539	0.255	0.520	0.542	0.494	0.470	25.9
9 %	0.559	0.645	0.526	0.632	0.535	0.579	9.57
12 %	0.542	0.594	0.460	0.538	0.459	0.519	11.2

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. Steel's Many-One Rank Test:

Is the mean dry weight (growth) significantly different ($p=0.05$) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	<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: 9.57 (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 19, 2022 TIME: 1154
Test Terminated: DATE: July 26, 2022 TIME: 1000

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	7.9	8.1	8.1	7.9	8.1	7.9
Final	6.7	7.4	6.6	6.7	6.2	5.6	6.2
pH Initial	7.8	7.8	7.7	7.8	7.8	7.8	7.7
Final	7.6	7.6	7.4	7.5	7.4	7.3	7.3

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	8.0	8.0	7.8	8.1	7.7
Final	6.7	7.2	6.6	6.5	6.0	5.2	5.7
pH Initial	7.9	7.8	7.8	7.8	7.9	7.8	7.8
Final	7.6	7.7	7.5	7.5	7.4	7.3	7.3

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.1	8.0	8.1	7.8	8.0	7.7
Final	6.8	7.4	6.5	6.3	6.1	5.2	5.5
pH Initial	7.9	7.9	7.8	7.8	7.9	7.9	7.8
Final	7.6	7.7	7.5	7.5	7.4	7.3	7.2

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	8.0	8.1	7.9	8.1	7.8
Final	6.9	7.3	6.7	6.6	6.3	5.6	5.8
pH Initial	7.9	7.9	7.9	7.8	7.9	7.9	7.8
Final	7.6	7.6	7.5	7.5	7.4	7.3	7.3

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	7.9	8.0	7.8	7.9	7.9
Final	6.8	7.2	6.6	6.8	6.1	5.2	5.3
pH Initial	8.0	7.9	7.9	7.9	8.0	7.9	7.8
Final	7.6	7.7	7.5	7.6	7.4	7.3	7.2

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	8.0	8.0	7.9	7.8	7.9	7.8
Final	6.9	7.4	6.6	6.7	6.4	5.4	5.3
pH Initial	8.0	8.0	7.9	7.9	8.0	7.9	7.8
Final	7.6	7.7	7.6	7.6	7.5	7.3	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	32	420	<0.05	AR0035602 18-JUL-22
130	29	420	0.060	AR0035602 20-JUL-22
130	29	430	0.050	AR0035602 22-JUL-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	84	310	<0.05	267121-1
60	89	310	<0.05	267279-1
60	80	320	<0.05	267349-1

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 19, 2022 at 1200

Date and Time Test Terminated: July 26, 2022 at 1350

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
7 day	100	90.0	100	100	90.0	80.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	29	31	30	32	26	19
B	32	30	27	24	29	32
C	29	13	31	29	31	32
D	34	36	32	32	37	29
E	31	32	36	37	32	28
F	30	32	31	31	32	35
G	30	28	29	29	33	30
H	29	28	27	25	28	25
I	29	32	26	30	24	19
J	30	30	29	29	29	29
Mean per Adult	30.3	29.2	29.8	29.8	30.1	27.8
Mean per Surviving Adult	30.3	31.0	29.8	29.8	30.6	28.4
CV %	5.40	7.90	9.85	12.3	11.9	16.8

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: 11.9 (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 19, 2022 TIME: 1200
 Test Terminated: DATE: July 26, 2022 TIME: 1350

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.2	7.9	8.1	8.1	7.9	8.1	7.9
Final	8.0	7.9	8.2	7.7	8.0	8.0	7.7
pH Initial	7.8	7.8	7.7	7.8	7.8	7.8	7.7
Final	8.2	8.1	8.2	8.0	8.0	8.2	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	8.1	8.0	8.0	8.0	7.8	8.1	7.7
Final	7.9	7.8	8.1	7.6	7.8	7.9	7.7
pH Initial	7.9	7.8	7.8	7.8	7.9	7.8	7.8
Final	8.2	8.1	8.2	8.0	8.0	8.2	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	8.1	8.1	8.0	8.1	7.8	8.0	7.7
Final	8.0	7.8	8.1	7.7	8.1	7.8	7.7
pH Initial	7.9	7.9	7.8	7.8	7.9	7.9	7.8
Final	8.2	8.1	8.2	8.0	8.0	8.2	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	8.1	8.0	8.0	8.1	7.9	8.1	7.8
Final	7.9	8.0	8.2	7.7	8.0	8.0	7.6
pH Initial	7.9	7.9	7.9	7.8	7.9	7.9	7.8
Final	8.2	8.1	8.2	8.0	8.1	8.2	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	8.0	7.9	7.9	8.0	7.8	7.9	7.9
Final	8.0	7.8	8.2	7.6	7.9	8.0	7.6
pH Initial	8.0	7.9	7.9	7.9	8.0	7.9	7.8
Final	8.2	8.1	8.2	8.0	8.1	8.3	7.9

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

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	32	420	<0.05	AR0035602 18-JUL-22
130	29	420	0.060	AR0035602 20-JUL-22
130	29	430	0.050	AR0035602 22-JUL-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	84	310	<0.05	267121-1
60	89	310	<0.05	267279-1
60	80	320	<0.05	267349-1

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>TRUMANN Water Works</u>		Analyses Requested		AIC Control No: <u>268363</u>	
Project Reference: <u>AR0035602</u>		BIDMONT - CHRONIC CO + FH		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>				Carrier:	
Sampled By: <u>LORRE HOLT</u>		G R A B		Received Temperature °C	
Date/Time Collected: <u>7/18/02</u>				Remarks	
Sample Identification: <u>AR0035602</u>		W A T E R		Field pH calibration on @	
Date/Time Collected: <u>8:00 AM - 8:00 AM</u>		S O I L		Buffer:	
Sample Matrix: <u>W A T E R</u>		C O M P		T = Sodium Thiosulfate Z = Zinc acetate	
Sample Matrix: <u>V</u>		V O A		H = HCl to pH2 B = NaOH to pH12	
Sample Matrix: <u>V</u>		N		Date/Time Received	
Container Type: <u>P</u>		Relinquished By: <u>LORRE HOLT</u>		Date/Time Relinquished	
Preservative: <u>NO</u>		Relinquished By: <u>LORRE HOLT</u>		Date/Time Relinquished	
G = Glass NO = none S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		Received in Lab By: <u>[Signature]</u>	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS		Comments:		Date/Time Received	
Expedited results requested by:		Who should AIC contact with questions: <u>LORRE HOLT</u>		Date/Time Relinquished	
Phone: <u>810-483-8832</u> Fax: <u>810-483-6525</u>		Report Attention to: <u>LORRE HOLT</u>		Date/Time Relinquished	
Report Address to: <u>104 HWY 463 N</u>		Report Address to: <u>TRUMANN, AR 72472</u>		Date/Time Relinquished	

Fx: 2756 7222 0350



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>TRUMAN WATER WORKS</u>		PO No.		Analyses Requested		AIC Control No: <u>267363</u>	
Project Reference: <u>AR0035602</u>		Sample Matrix		<u>BIDMONT BRM - CHRONIC</u> <u>CD + FH</u>		AIC Proposal No:	
Project Manager: <u>SCOTTY JONES</u>		W A T E R				Carrier: <u>FX</u>	
Sampled By: <u>LORRE HOLT</u>		G R A B		<u>1</u>		Received Temperature °C	
Date/Time Collected: <u>7/20/22 8:00 AM</u>		C O M P				Remarks	
AIC No. <u>2 AR0035602</u>		V				Field pH calibration on @	
Container Type: <u>P</u>		V = VOA vials				Buffer:	
Preservative: <u>NIC</u>		N = Nitric acid pH2				T = Sodium Thiosulfate	
G = Glass		NO = none				Z = Zinc acetate	
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN ___ DAYS		Relinquished		Date/Time		Date/Time	
Expedited results requested by:		By: <u>Lorrey Holt</u>		Date/Time: <u>7/20/22 9:10 AM</u>		By:	
Who should AIC contact with questions: <u>LORRE HOLT</u>		Relinquished		Date/Time		Received in Lab	
Phone: <u>810-483-8832</u> Fax: <u>817-483-1655</u>		By:				By: <u>D. BROWN</u>	
Report Attention to: <u>LORRE HOLT</u>		Comments:				<u>7-21-22</u> <u>0925</u>	
Report Address to: <u>TOM HWY 463 N</u> <u>TRUMAN, AR 72472</u>						<u>2757 7968 6421</u>	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		AIC Control No: <u>267363</u>	
Project Reference: <u>AR0035602</u>		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		Carrier:	
Sampled By: <u>Lorre Holt</u>		Received Temperature °C	
AIC Sample Identification: <u>AR0035602</u>		<u>Oil</u>	
Date/Time Collected: <u>11/12/22</u>		Remarks:	
G R A B		Analyses Requested	
C O M P		<u>Bidmon: long-chronic</u>	
V		<u>CD-FH</u>	
W A T E R			
S O I L			
Sample Matrix			
PO No.			
No of BOTTLES			
V O A vials			
N = Nitric acid pH2			
Sulfuric acid pH2			
P = Plastic			
S = Sulfuric acid pH2			
Container Type			
Preservative			
G = Glass			
NO = none			
Turnaround Time Requested: (Please circle)			
NORMAL or EXPEDITED IN ___ DAYS			
Expedited results requested by:			
Who should AIC contact with questions: <u>LORRE HOLT</u>			
Phone: <u>810-483-3832</u> Fax: <u>810-483-10525</u>			
Report Attention to: <u>LORRE HOLT</u>			
Report Address to: <u>704 Hwy 463 N</u>			
<u>TRUMANN, AR 72472</u>			
Relinquished By: <u>[Signature]</u>		Received By: <u>NK388</u>	
Relinquished Date/Time: <u>7/22/22 9:55</u>		Received Date/Time: <u>2/3/22 12:13</u>	
Comments: <u>Fx: 2758 80025533</u>			



October 27, 2022

Biomonitoring Testing
for

Control No. 269746-1

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
825 Hwy 463 North
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
825 Hwy 463 North
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:

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%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.443	PASS
Control Growth CV < or = 40%	16.1	PASS
Growth Minimum Significant Difference 12 to 30%	20.4	PASS
Critical Dilution CV < or = 40%	19.6	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	26.5	PASS
Control CV < or = 40% per Surviving Female	23.9	PASS
Reproduction Minimum Significant Difference 13 to 47%	29.7	PASS
Critical Dilution CV < or = 40%	36.3	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	Sample 3
Dissolved oxygen (mg/l)	8.0	7.9	8.1
pH (standard units)	8.4	8.6	8.8
Alkalinity (mg/l as CaCO ₃)	140	140	140
Hardness (mg/l as CaCO ₃)	29	29	34
Conductivity (umhos/cm)	470	480	480
Residual Chlorine (mg/l)	0.060	0.050	<0.05
Ammonia as N (mg/l)	0.59	0.55	0.54

2. Dilution Water Samples:
Moderately Hard

Analysis	269638-1	269639-1
Dissolved oxygen (mg/l)	8.3	8.0
pH (standard units)	7.7	7.8
Alkalinity (mg/l as CaCO ₃)	61	61
Hardness (mg/l as CaCO ₃)	82	84
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: October 18, 2022 at 1454
Date & Time Test Terminated: October 25, 2022 at 1330
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: October 18, 2022 at 1405
Date & Time Test Terminated: October 24, 2022 at 1605
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 October 11, 2022 at 0928 to October 18, 2022 at 0903

The results were as follows: (Control No. 269300-1.)

Survival LC-50: 2404 mg/l

Growth IC-25: 1127 mg/l

Growth PMSD: 17.3

Ceriodaphnia dubia

A chronic reference test was performed on October 11, 2022 at 1000 to October 18, 2022 at 1153

The results were as follows: (Control No. 269300-2.)

Survival LC-50: 1604.1 mg/l

Reproduction IC-25: 1037 mg/l

Reproduction PMSD: 15.8

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 18, 2022

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 18, 2022

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

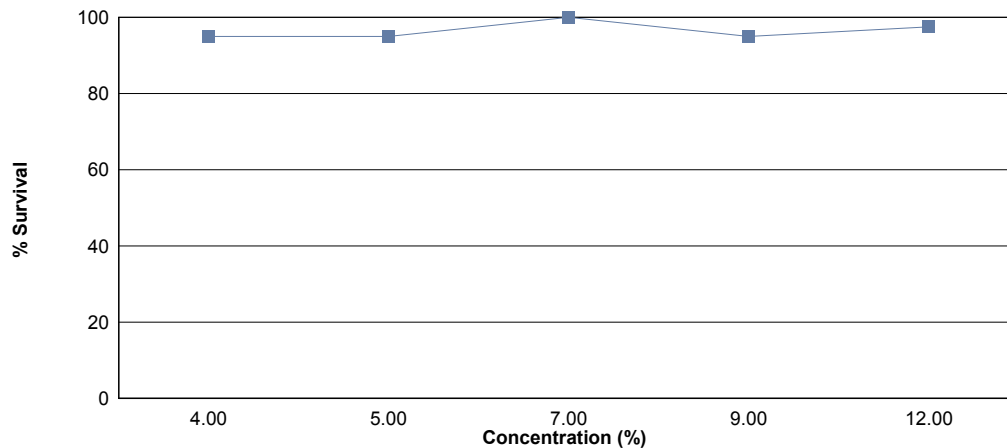
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 October 18, 2022 at 1454 and continued through October 25, 2022 at 1330. 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	97.5	0.432
4 %	95.0	0.386
5 %	95.0	0.366
7 %	100	0.389
9 %	95.0	0.389
12 %	97.5	0.401

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

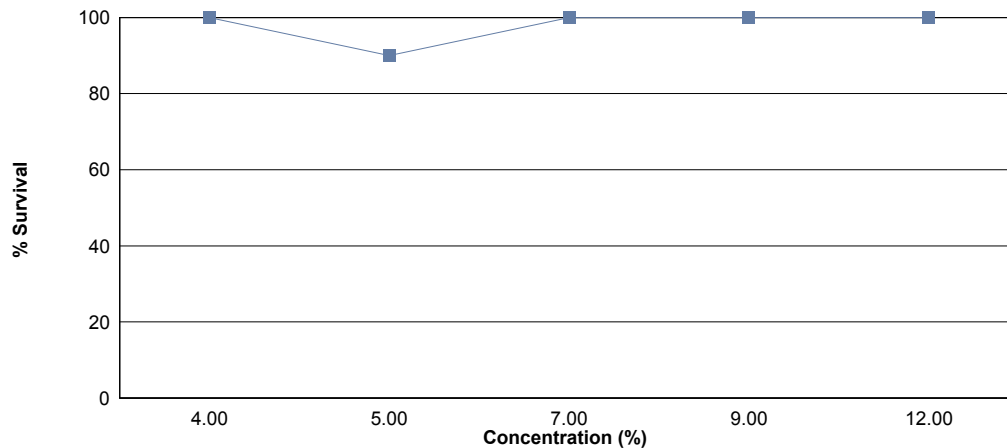
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 October 18, 2022 at 1405 and continued through October 24, 2022 at 1605. 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	26.5
4 %	100	22.8
5 %	90.0	20.5
7 %	100	24.7
9 %	100	21.0
12 %	100	21.6

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 18, 2022 at 1454

Date and Time Test Terminated: October 25, 2022 at 1330

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: October 18, 2022 at 1454

Test Terminated: October 25, 2022 at 1330

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76829	.77255	0.00426	8	0.532
	B	.76536	.76867	0.00331	8	0.414
	C	.77098	.77474	0.00376	8	0.470
	D	.76570	.76859	0.00289	8	0.361
	E	.76887	.77193	0.00306	8	0.382
4 %	A	.76500	.76830	0.00330	8	0.412
	B	.76772	.77065	0.00293	8	0.366
	C	.76402	.76709	0.00307	8	0.384
	D	.76629	.76935	0.00306	8	0.382
	E	.76377	.76684	0.00307	8	0.384
5 %	A	.77013	.77372	0.00359	8	0.449
	B	.76709	.76948	0.00239	8	0.299
	C	.76920	.77247	0.00327	8	0.409
	D	.76495	.76823	0.00328	8	0.410
	E	.76736	.76945	0.00209	8	0.261
7 %	A	.76574	.76880	0.00306	8	0.382
	B	.77530	.77867	0.00337	8	0.421
	C	.77048	.77351	0.00303	8	0.379
	D	.76573	.76844	0.00271	8	0.339
	E	.76454	.76792	0.00338	8	0.422
9 %	A	.77553	.77853	0.00300	8	0.375
	B	.76933	.77291	0.00358	8	0.448
	C	.77136	.77379	0.00243	8	0.304
	D	.76770	.77036	0.00266	8	0.332
	E	.76727	.77114	0.00387	8	0.484
12 %	A	.76732	.77070	0.00338	8	0.422
	B	.76850	.77112	0.00262	8	0.328
	C	.77736	.78085	0.00349	8	0.436
	D	.76137	.76439	0.00302	8	0.378
	E	.77089	.77443	0.00354	8	0.442

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 18, 2022 at 1405

Date and Time Test Terminated: October 24, 2022 at 1605

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	5	4	4	0	0	6	0	0	19	10	1.90	
4	5	4	0	0	0	5	0	0	3	3	20	10	2.00	
5	11	10	12	10	9	8	5	10	10	11	96	10	9.60	
6	0	14	17	16	15	13	10	17	15	13	130	10	13.0	
7														
8														
TOTAL	16	28	34	30	28	26	15	33	28	27	265	10	26.5	

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	5	0	0	0	0	0	5	10	0.500	
4	4	4	3	3	0	5	4	4	3	3	33	10	3.30	
5	12	10	10	11	13	10	9	10	0	10	95	10	9.50	
6	12	0	0	13	15	14	0	14	15	12	95	10	9.50	
7														
8														
TOTAL	28	14	13	27	33	29	13	28	18	25	228	10	22.8	

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	0	0	0	0	10	0.00	
4	3	4	4	3	4	3	3	5	5	3	37	10	3.70	
5	9	12	10	8	0	10	0X	10	10	10	79	9	8.78	
6	0	0	14	11	10	12	X	15	15	12	89	9	9.89	
7														
8														
TOTAL	12	16	28	22	14	25	3	30	30	25	205	10	20.5	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 18, 2022 at 1405

Date and Time Test Terminated: October 24, 2022 at 1605

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	4	0	6	0	0	0	0	0	0	10	10	1.00
4	4	3	0	5	0	4	3	5	5	5	34	10	3.40	
5	10	11	13	10	11	10	0	9	0	9	83	10	8.30	
6	12	0	14	13	13	13	12	15	13	15	120	10	12.0	
7														
8														
TOTAL	26	14	31	28	30	27	15	29	18	29	247	10	24.7	

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	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	1	0	0	1	10	0.100	
4	3	3	4	4	4	5	3	3	3	4	36	10	3.60	
5	10	9	11	11	8	0	9	11	9	12	90	10	9.00	
6	0	0	17	12	0	10	14	13	14	3	83	10	8.30	
7														
8														
TOTAL	13	12	32	27	12	15	26	28	26	19	210	10	21.0	

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	5	0	0	0	0	0	5	10	0.500	
4	4	4	5	4	0	6	3	4	5	3	38	10	3.80	
5	11	11	11	10	11	10	7	10	0	11	92	10	9.20	
6	0	0	15	0	17	14	0	12	11	12	81	10	8.10	
7														
8														
TOTAL	15	15	31	14	33	30	10	26	16	26	216	10	21.6	

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	0.87500	1.20940
2	4 %	1	1.00000	1.39310
2	4 %	2	0.87500	1.20940
2	4 %	3	0.87500	1.20940
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	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	1.00000	1.39310
4	7 %	5	1.00000	1.39310
5	9 %	1	1.00000	1.39310
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	0.87500	1.20940
6	12 %	3	1.00000	1.39310
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))
<p>D = 0.1755 W = 0.7938 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 %	25.00	16.00	5.00	
3	5 %	25.00	16.00	5.00	
4	7 %	30.00	16.00	5.00	
5	9 %	25.00	16.00	5.00	
6	12 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.08374 W = 0.9708 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 = 9.376 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	0.01207	0.002415	0.6922	
Within (Error)	24	0.08374	0.003489		
Total	29	0.09581			
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	0.4318	0.4318			
2	4 %	0.3856	0.3856	1.237		
3	5 %	0.3656	0.3656	1.772		
4	7 %	0.3886	0.3886	1.156		
5	9 %	0.3886	0.3886	1.156		
6	12 %	0.4012	0.4012	0.8191		
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	0.08816	20.4	0.0462		
3	5 %	5	0.08816	20.4	0.0662		
4	7 %	5	0.08816	20.4	0.0432		
5	9 %	5	0.08816	20.4	0.0432		
6	12 %	5	0.08816	20.4	0.0306		

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 %	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 %	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	0	
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 = 0.1547 D* = 1.214 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 %	89.00	75.00	10.00	
3	5 %	83.00	75.00	10.00	
4	7 %	98.50	75.00	10.00	
5	9 %	79.50	75.00	10.00	
6	12 %	86.50	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	272.6	54.51	0.9408	
Within (Error)	54	3129	57.94		
Total	59	3402			
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)					

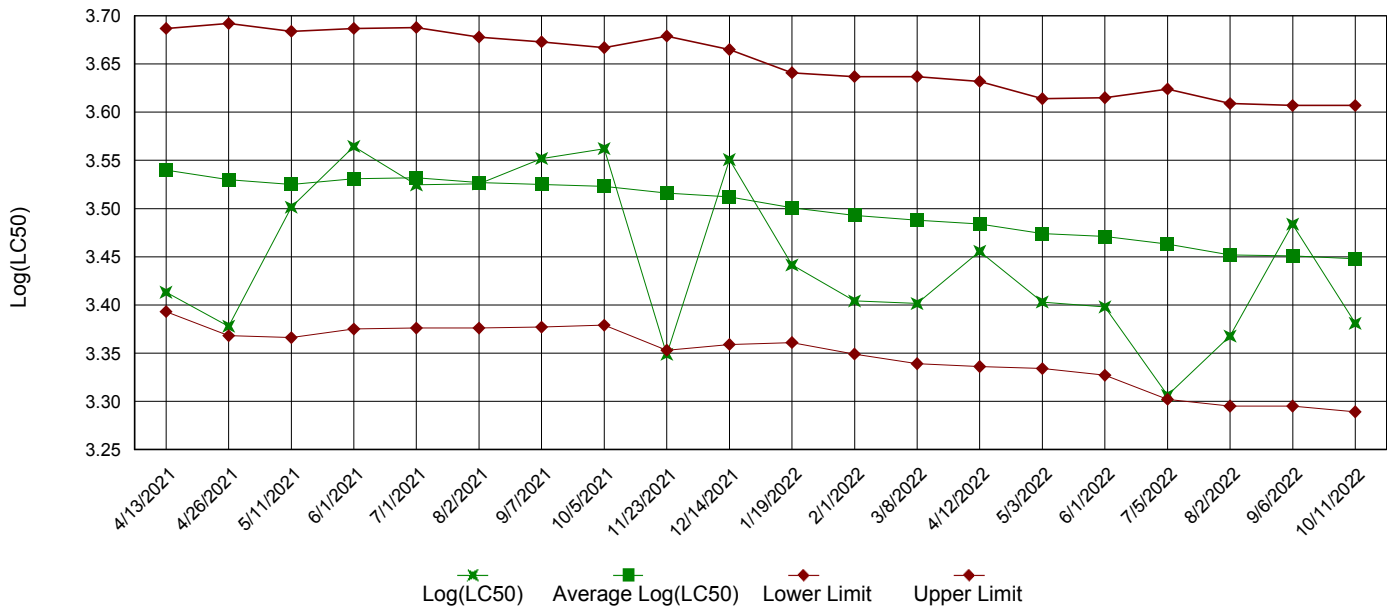
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	26.5	26.5			
2	4 %	22.8	22.8	1.087		
3	5 %	20.5	20.5	1.763		
4	7 %	24.7	24.7	0.5288		
5	9 %	21	21	1.616		
6	12 %	21.6	21.6	1.439		
Dunnett's 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	7.864	29.7	3.7	
3	5 %	10	7.864	29.7	6	
4	7 %	10	7.864	29.7	1.8	
5	9 %	10	7.864	29.7	5.5	
6	12 %	10	7.864	29.7	4.9	

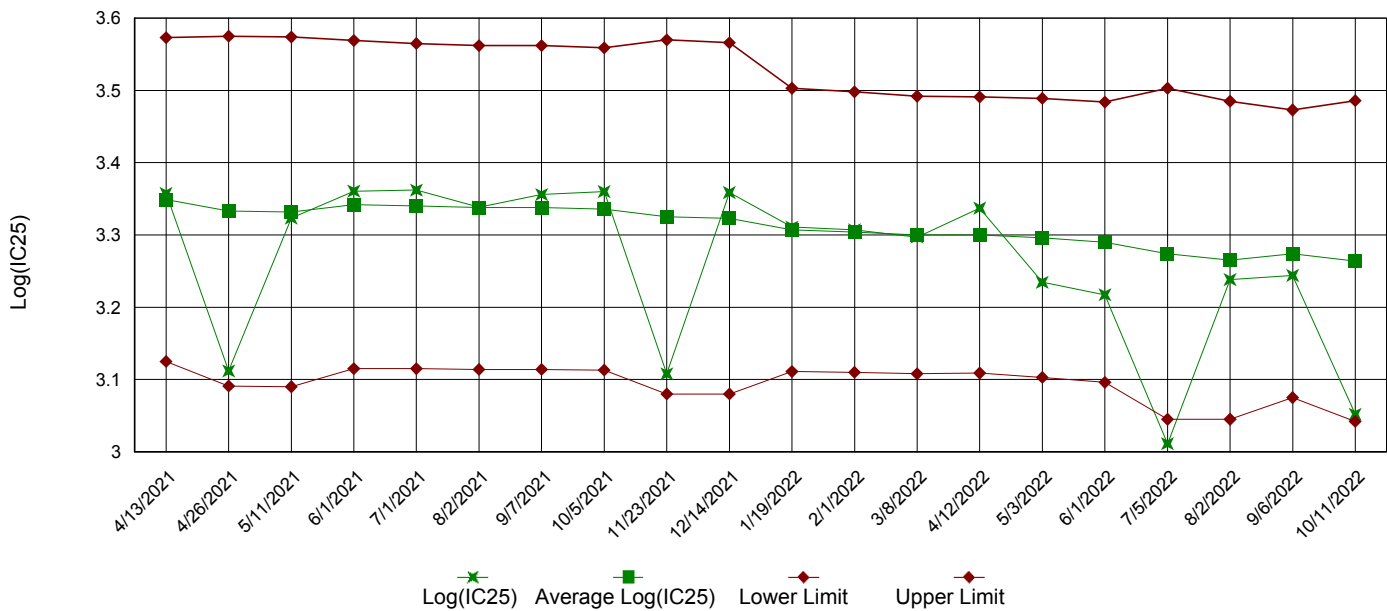
Appendix A3: Test 1000.0

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

LC50 Survival Data

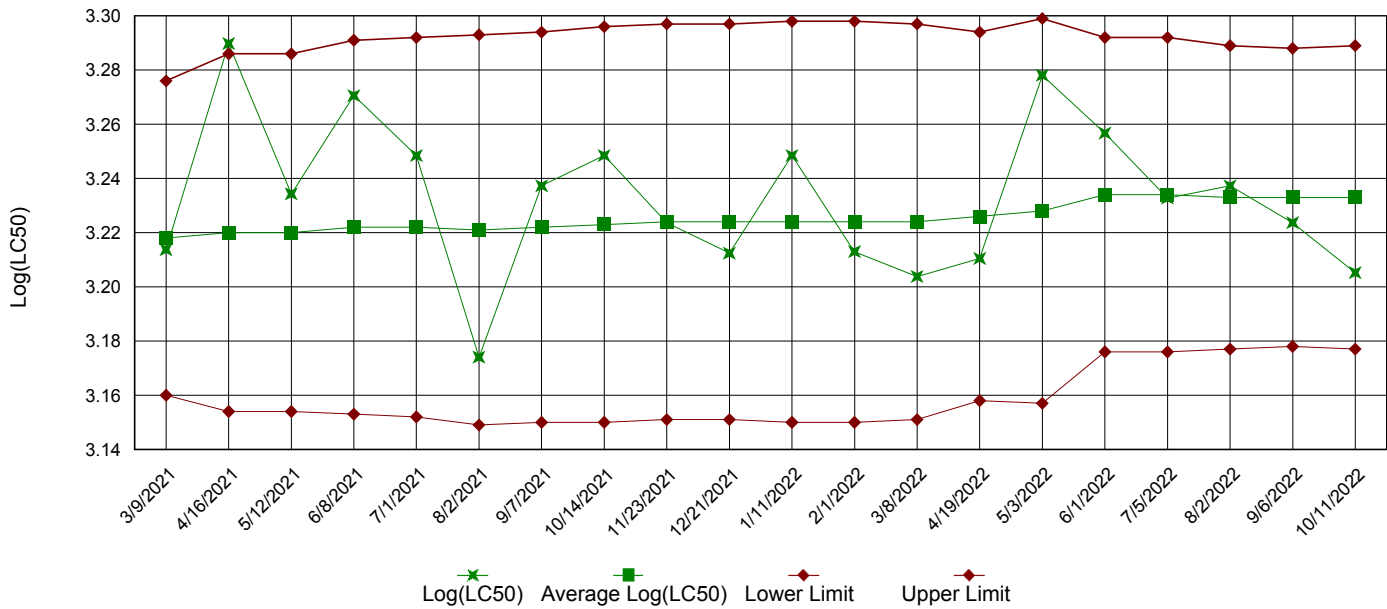


IC25 Growth Data

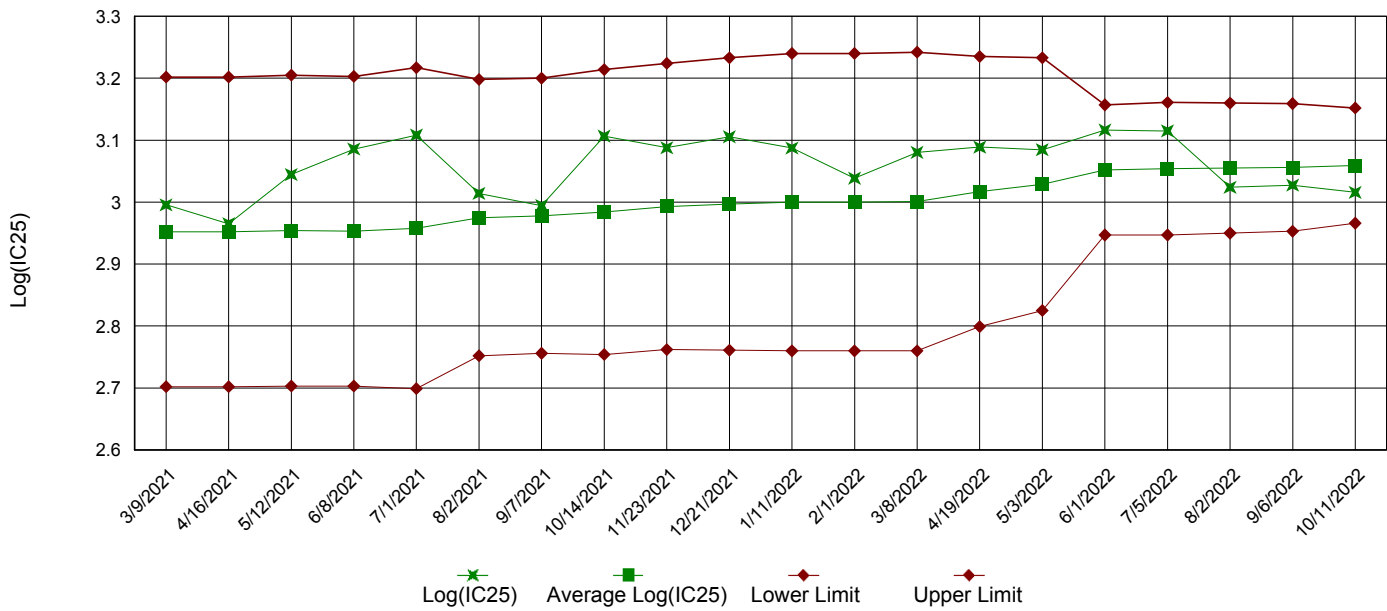


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: October 18, 2022 at 1454

Date and Time Test Terminated: October 25, 2022 at 1330

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	100	87.5	100	97.5	97.5	5.73
4 %	100	87.5	87.5	100	100	100	97.5	95.0	7.21
5 %	100	87.5	100	100	87.5	100	100	95.0	7.21
7 %	100	100	100	100	100	100	100	100	0.00
9 %	100	100	87.5	87.5	100	100	97.5	95.0	7.21
12 %	100	87.5	100	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	0.532	0.414	0.470	0.361	0.382	0.432	16.1
4 %	0.412	0.366	0.384	0.382	0.384	0.386	4.30
5 %	0.449	0.299	0.409	0.410	0.261	0.366	22.1
7 %	0.382	0.421	0.379	0.339	0.422	0.389	8.88
9 %	0.375	0.448	0.304	0.332	0.484	0.389	19.6
12 %	0.422	0.328	0.436	0.378	0.442	0.401	12.0

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: 19.6 (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, 357, 358

Test Initiated: DATE: October 18, 2022 TIME: 1454
Test Terminated: DATE: October 25, 2022 TIME: 1330

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.1	8.0	7.6	7.8	7.8	7.8
Final	7.4	7.1	7.1	6.7	6.1	5.8	6.1
pH Initial	7.7	8.0	7.8	7.9	8.0	8.0	8.0
Final	7.8	7.5	7.6	7.9	7.8	7.2	7.7

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.4	8.4	8.2	7.7	7.9	7.9	7.7
Final	7.4	6.7	6.6	7.0	6.0	5.2	6.0
pH Initial	7.8	8.0	7.9	7.9	8.1	8.1	8.0
Final	7.8	7.5	7.6	8.0	7.7	7.2	7.7

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.5	8.2	7.9	8.0	8.0	7.8
Final	7.2	6.8	6.8	6.6	6.4	5.7	6.3
pH Initial	7.8	8.0	7.9	8.0	8.1	8.1	8.0
Final	7.8	7.6	7.7	7.9	7.9	7.3	7.8

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.5	8.1	7.9	7.8	7.6	7.9
Final	7.2	6.8	6.9	6.6	6.4	5.7	6.1
pH Initial	7.8	8.1	8.0	8.0	8.2	8.2	8.0
Final	7.9	7.6	7.7	7.9	7.8	7.3	7.8

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	8.1	7.6	7.7	7.7	7.9
Final	7.4	6.7	6.9	6.7	6.2	5.6	5.9
pH Initial	7.9	8.1	8.0	8.0	8.2	8.2	8.0
Final	7.9	7.6	7.7	7.9	7.8	7.3	7.8

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.5	8.1	7.7	7.9	7.9	8.0
Final	7.3	6.7	6.7	6.4	6.1	5.6	6.2
pH Initial	7.9	8.1	8.0	8.1	8.2	8.2	8.2
Final	7.9	7.6	7.7	7.9	7.9	7.3	7.9

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
140	29	470	0.060	AR0035602 17-OCT-22
140	29	480	0.050	AR0035602 19-OCT-22
140	34	480	<0.05	AR0035602 21-OCT-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	82	320	<0.05	269638-1
61	84	320	<0.05	269639-1

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: October 18, 2022 at 1405

Date and Time Test Terminated: October 24, 2022 at 1605

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	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	16	28	12	26	13	15
B	28	14	16	14	12	15
C	34	13	28	31	32	31
D	30	27	22	28	27	14
E	28	33	14	30	12	33
F	26	29	25	27	15	30
G	15	13	3	15	26	10
H	33	28	30	29	28	26
I	28	18	30	18	26	16
J	27	25	25	29	19	26
Mean per Adult	26.5	22.8	20.5	24.7	21.0	21.6
Mean per Surviving Adult	26.5	22.8	22.4	24.7	21.0	21.6
CV %	23.9	33.1	30.7	26.2	36.3	39.0

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

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

1. Fisher's Exact Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	<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: 36.3 (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: October 18, 2022 TIME: 1405
Test Terminated: DATE: October 24, 2022 TIME: 1605

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.3	8.1	8.0	7.6	7.8	7.8	7.8
Final	8.0	8.0	7.7	7.9	8.0	7.7	--
pH Initial	7.7	8.0	7.8	7.9	8.0	8.0	8.0
Final	8.2	8.0	8.2	4.6	8.3	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	8.4	8.4	8.2	7.7	7.9	7.9	7.7
Final	8.0	8.0	8.0	5.0	7.9	7.7	--
pH Initial	7.8	8.0	7.9	7.9	8.1	8.1	8.0
Final	8.2	8.1	8.2	8.0	8.3	7.9	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	8.2	8.5	8.2	7.9	8.0	8.0	7.8
Final	8.0	8.0	7.8	7.8	4.3	7.7	--
pH Initial	7.8	8.0	7.9	8.0	8.1	8.1	8.0
Final	8.2	8.1	8.2	8.1	8.5	7.9	--

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

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	8.3	8.2	8.1	7.6	7.7	7.7	7.9
Final	8.1	7.9	8.0	8.0	7.6	7.8	--
pH Initial	7.9	8.1	8.0	8.0	8.2	8.2	8.0
Final	8.2	8.1	8.2	8.2	8.4	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	8.2	8.5	8.1	7.7	7.9	7.9	8.0
Final	8.0	7.8	8.0	7.9	7.9	7.4	--
pH Initial	7.9	8.1	8.0	8.1	8.2	8.2	8.2
Final	8.2	8.2	8.3	8.3	8.5	8.1	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
140	29	470	0.060	AR0035602 17-OCT-22
140	29	480	0.050	AR0035602 19-OCT-22
140	34	480	<0.05	AR0035602 21-OCT-22

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	82	320	<0.05	269638-1
61	84	320	<0.05	269639-1

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 W A T E R C O M P G R A B V		No of BOTTLES 1		Analyses Requested BIDMONITORM-CHRONIC COL-FH		AIC Control No: 2109746 AIC Proposal No: Carrier: FX Received Temperature °C: 0.1 Remarks:	
Date/Time Collected: 12/14/03 - 10:17 AM Date/Time Collected: 8:00 AM - 8:00 PM		Container Type: P Preservative: NO		G = Glass NO = none P = Plastic S = Sulfuric acid pH2		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12 T = Sodium Thiosulfate Z = Zinc acetate	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN ___ DAYS Expedited results requested by:		Relinquished By: AMU Holt Date/Time: 10/17/03 11:00 AM		Relinquished By: BM Date/Time: 10/18/03 0849		Received in Lab Date/Time: 10/18/03 0849		Received in Lab Date/Time: 10/18/03 0849	
Who should AIC contact with questions: LORRE HOLT Phone: 870-483-2882 Fax: 870-485-6525 Report Attention to: LORRE HOLT Report Address to: 925 Hwy 463 N. TRUMANN, AR. 72472		Comments:		Comments: EX: 2799 2924 2598		Comments:		Comments:	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Trumann Water Works Project Reference: AR0035202 Project Manager: Scotty Jones Sampled By: Lorre Holt		PO No. [] Sample Matrix: W A T E R [] S O I L [] G R A B [] C O M P [] Container Type: P Preservative: NO		No of B O T T L E S [] V = VOA vials N = Nitric acid pH2		Analyses Requested BIDMON: BRNG-CHRONIC CD + FH		AIC Control No: 269746 AIC Proposal No: [] Carrier: [] Received Temperature °C: 3.1 Remarks: []	
Sample Identification: AR0035202 Date/Time Collected: 10/18/22 10:24 AM Date/Time Collected: 10/19/22 10:24 AM		Container Type: P Preservative: NO		H = HCl to pH2 B = NaOH to pH12		Received Date/Time: 10/19/22 10:24 AM Received in Lab Date/Time: 10/20/22 0900		Field pH calibration on @ [] Buffer: []	
G = Glass NO = none S = Sulfuric acid pH2		Expedited results requested by: [] Who should AIC contact with questions: LORRE HOLT Phone: 870-483-2882 Fax: 870-483-6525 Report Attention to: LORRE HOLT Report Address to: 925 Hwy 463 N, TRUMANN, AR. 72472		Relinquished By: Lorre Holt Relinquished Date/Time: []		Comments: []		T = Sodium Thiosulfate Z = Zinc acetate	



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u> Project Reference: <u>AR0035602</u> Project Manager: <u>Scotty Jones</u> Sampled By: <u>LORRE HOLT</u>		PO No. _____ Sample Matrix: _____ W A T E R _____ S O I L _____ G R A B _____ C O M P _____ V ✓		No of BOTTLES: <u>1</u>		Analyses Requested: <u>BIDMON: BREQ-CHRONIC CD+FH</u>		AIC Control No.: <u>269746</u> AIC Proposal No.: _____ Carrier: _____ Received Temperature °C: _____ Remarks: _____	
Sample Identification: <u>AR0035602</u> Date/Time Collected: <u>10/22/02</u> Collected By: <u>8:00 AM</u>		Container Type: <u>P</u> Preservative: <u>NO</u>		V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS Expedited results requested by: _____		G = Glass NO = none S = Sulfuric acid pH2		Requisitioned By: <u>LORRE HOLT</u> Date/Time: <u>10/22/02 11:20 AM</u>		Received By: <u>NK358</u> Date/Time: <u>2002 09 20</u>		Field pH calibration on _____ @ _____ Buffer: _____	
Who should AIC contact with questions: <u>LORRE HOLT</u> Phone: <u>870-483-2882</u> Fax: <u>870-485-6525</u> Report Attention to: <u>LORRE HOLT</u> Report Address to: <u>925 Hwy 463 N. TRUMANN, AR. 72472</u>		Comments: _____		Received in Lab: <u>NK358</u>		Date/Time: <u>2002 09 20</u>		Date/Time: _____	



February 7, 2023

Biomonitoring Testing
for

Control No. 272616-1

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
825 Hwy 463 North
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
825 Hwy 463 North
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.

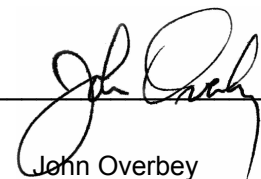
Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

The third sample was not received on the correct day for test renewal. The test was completed with remaining samples previously received as required.

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 5 %. **The sample PASSED lethal effects, however, FAILED 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 percent minimum significant difference (PMSD) was below the limit of 13. 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 *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



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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	0.782	PASS
Control Growth CV < or = 40%	9.58	PASS
Growth Minimum Significant Difference 12 to 30%	11.4	BELOW
Critical Dilution CV < or = 40%	6.22	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	30.0	PASS
Control CV < or = 40% per Surviving Female	3.14	PASS
Reproduction Minimum Significant Difference 13 to 47%	7.05	BELOW
Critical Dilution CV < or = 40%	4.78	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)	8.1	8.5
pH (standard units)	7.8	7.9
Alkalinity (mg/l as CaCO ₃)	150	150
Hardness (mg/l as CaCO ₃)	33	32
Conductivity (umhos/cm)	480	480
Residual Chlorine (mg/l)	<0.05	<0.05
Ammonia as N (mg/l)	7.2	7.4

2. Dilution Water Samples:

Moderately Hard

Analysis	272433-1	272598-1
Dissolved oxygen (mg/l)	7.7	7.8
pH (standard units)	7.9	7.8
Alkalinity (mg/l as CaCO ₃)	62	62
Hardness (mg/l as CaCO ₃)	82	86
Conductivity (umhos/cm)	310	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: January 24, 2023 at 1028
Date & Time Test Terminated: January 31, 2023 at 0900
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 24, 2023 at 1030
Date & Time Test Terminated: January 30, 2023 at 1200
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Source of test organisms: In-house culture

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

D. Test Organisms

1. Scientific Name

a. Test 1000.0 *Pimephales promelas*

b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

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

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

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

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

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 January 03, 2023 at 1015 to January 10, 2023 at 0955

The results were as follows: (Control No. 272003-1.)

Survival LC-50: 2765 mg/l

Growth IC-25: 1412 mg/l

Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on January 03, 2023 at 0931 to January 09, 2023 at 1100

The results were as follows: (Control No. 272003-2.)

Survival LC-50: 1804 mg/l

Reproduction IC-25: 993.2 mg/l

Reproduction PMSD: 17.2

V. Organism History

Pimephales promelas (Fathead minnow)

Date: January 24, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: January 24, 2023

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.

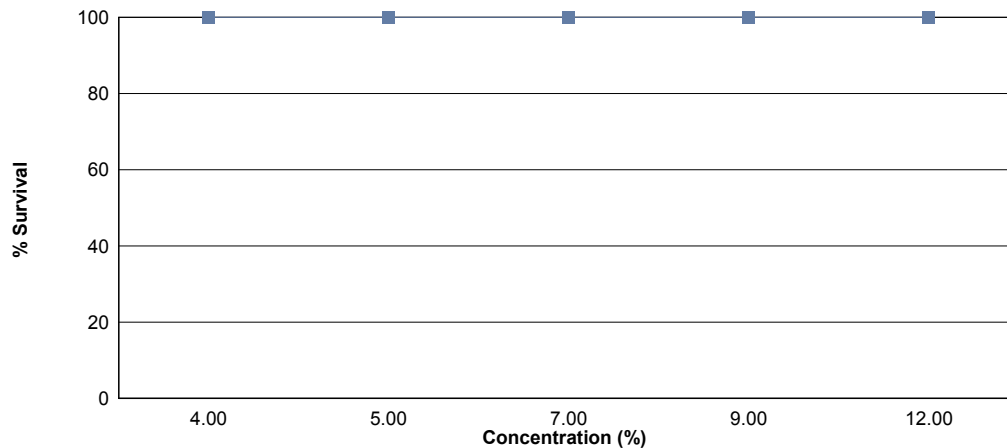
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

The test was initiated on January 24, 2023 at 1028 and continued through January 31, 2023 at 0900. 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 = 5 % 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	0.762
4 %	100	0.685
5 %	100	0.684
7 %	100	0.662 *
9 %	100	0.677
12 %	100	0.645 *

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

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.

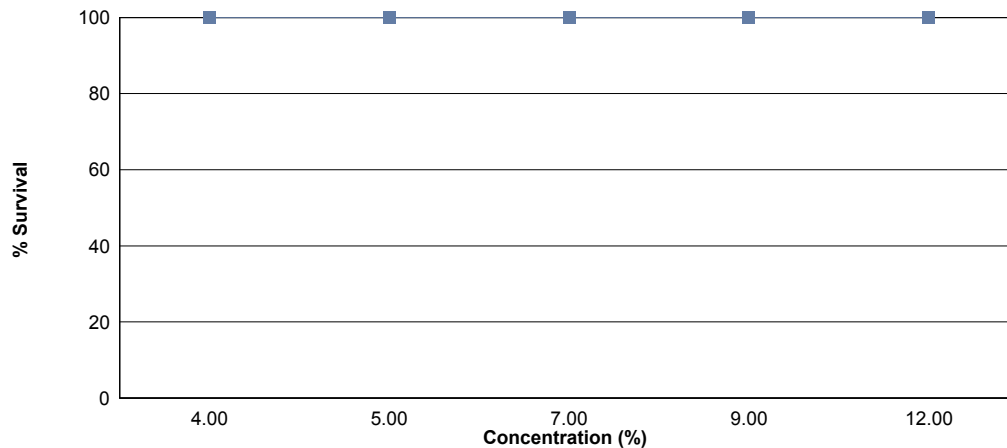
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

The test was initiated on January 24, 2023 at 1030 and continued through January 30, 2023 at 1200. 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

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



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	30.0
4 %	100	28.0
5 %	100	27.9
7 %	100	27.3 *
9 %	100	26.9 *
12 %	100	27.5 *

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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: January 24, 2023 at 1028

Date and Time Test Terminated: January 31, 2023 at 0900

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	7	7	7	7	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
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	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
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	8	8	8	8	8
	E	8	8	8	8	8	8	8
9 %	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
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	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: January 24, 2023 at 1028

Test Terminated: January 31, 2023 at 0900

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76401	.77090	0.00689	8	0.861
	B	.76603	.77137	0.00534	8	0.668
	C	.76512	.77142	0.00630	8	0.788
	D	.76762	.77382	0.00620	8	0.775
	E	.76847	.77422	0.00575	8	0.719
4 %	A	.75917	.76460	0.00543	8	0.679
	B	.76550	.77129	0.00579	8	0.724
	C	.76451	.76964	0.00513	8	0.641
	D	.76338	.76887	0.00549	8	0.686
	E	.77061	.77616	0.00555	8	0.694
5 %	A	.77079	.77652	0.00573	8	0.716
	B	.76522	.77029	0.00507	8	0.634
	C	.76851	.77369	0.00518	8	0.648
	D	.77134	.77607	0.00473	8	0.591
	E	.77483	.78146	0.00663	8	0.829
7 %	A	.76138	.76650	0.00512	8	0.640
	B	.77098	.77662	0.00564	8	0.705
	C	.76591	.77131	0.00540	8	0.675
	D	.76646	.77179	0.00533	8	0.666
	E	.76770	.77271	0.00501	8	0.626
9 %	A	.77144	.77669	0.00525	8	0.656
	B	.77110	.77617	0.00507	8	0.634
	C	.76899	.77442	0.00543	8	0.679
	D	.76614	.77211	0.00597	8	0.746
	E	.76702	.77239	0.00537	8	0.671
12 %	A	.77209	.77697	0.00488	8	0.610
	B	.77238	.77703	0.00465	8	0.581
	C	.76848	.77373	0.00525	8	0.656
	D	.77393	.77921	0.00528	8	0.660
	E	.77145	.77719	0.00574	8	0.718

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 24, 2023 at 1030

Date and Time Test Terminated: January 30, 2023 at 1200

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	4	0	6	0	0	0	5	0	5	20	10	2.00	
4	5	0	4	0	4	6	4	0	5	0	28	10	2.80	
5	11	11	11	12	12	12	10	10	11	11	111	10	11.1	
6	14	15	14	14	13	13	15	15	14	14	141	10	14.1	
7														
8														
TOTAL	30	30	29	32	29	31	29	30	30	30	300	10	30.0	

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	3	0	0	0	4	7	10	0.700
4	4	4	5	4	5	0	3	5	3	0	33	10	3.30
5	13	11	11	10	11	10	10	11	10	10	107	10	10.7
6	15	13	13	12	11	14	13	14	12	16	133	10	13.3
7													
8													
TOTAL	32	28	29	26	27	27	26	30	25	30	280	10	28.0

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	6	0	0	0	0	0	0	0	6	12	10	1.20
4	5	0	5	3	5	5	3	4	4	0	34	10	3.40
5	10	11	11	12	11	12	10	12	10	11	110	10	11.0
6	13	15	13	13	11	13	11	12	9	13	123	10	12.3
7													
8													
TOTAL	28	32	29	28	27	30	24	28	23	30	279	10	27.9

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 24, 2023 at 1030

Date and Time Test Terminated: January 30, 2023 at 1200

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	4	0	0	0	0	0	0	0	0	5	9	10	0.900
4	4	0	6	5	5	0	4	5	5	0	34	10	10	3.40
5	13	10	10	11	11	10	10	12	10	10	107	10	10	10.7
6	13	13	9	12	13	12	13	13	13	12	123	10	10	12.3
7														
8														
TOTAL	30	27	25	28	29	22	27	30	28	27	273	10	10	27.3

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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	4	0	0	0	5	0	4	0	3	16	10	10	1.60
4	5	0	5	4	4	0	4	0	4	0	26	10	10	2.60
5	11	9	10	10	10	10	11	11	10	11	103	10	10	10.3
6	12	13	12	11	12	11	13	14	12	14	124	10	10	12.4
7														
8														
TOTAL	28	26	27	25	26	26	28	29	26	28	269	10	10	26.9

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	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	6	0	4	10	10	10	1.00
4	4	5	4	5	4	4	3	0	5	0	34	10	10	3.40
5	11	10	10	12	10	11	11	12	12	10	109	10	10	10.9
6	11	11	12	13	13	11	12	14	12	13	122	10	10	12.2
7														
8														
TOTAL	26	26	26	30	27	26	26	32	29	27	275	10	10	27.5

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	0.87500	1.20940
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	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	1.00000	1.39310
3	5 %	3	1.00000	1.39310
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	1.00000	1.39310
4	7 %	4	1.00000	1.39310
4	7 %	5	1.00000	1.39310
5	9 %	1	1.00000	1.39310
5	9 %	2	1.00000	1.39310
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	1.00000	1.39310
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))
<p>D = 0.027 W = 0.4161 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 %	30.00	16.00	5.00	
4	7 %	30.00	16.00	5.00	
5	9 %	30.00	16.00	5.00	
6	12 %	30.00	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 = 0.0813 W = 0.9642 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 = 7.541 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	0.04064	0.008129	2.399	
Within (Error)	24	0.0813	0.003388		
Total	29	0.1219			
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	0.7622	0.7622			
2	4 %	0.6848	0.6848	2.103		
3	5 %	0.6836	0.6836	2.135		
4	7 %	0.6624	0.6624	2.711	*	
5	9 %	0.6772	0.6772	2.309		
6	12 %	0.645	0.645	3.184	*	
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	0.08688	11.4	0.0774		
3	5 %	5	0.08688	11.4	0.0786		
4	7 %	5	0.08688	11.4	0.0998		
5	9 %	5	0.08688	11.4	0.085		
6	12 %	5	0.08688	11.4	0.1172		

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 %	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
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 %	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	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.1134 D* = 0.8897 Critical D* = 1.035 (alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 11.74 Critical B = 15.086 (alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	59.33	11.87	2.831	
Within (Error)	54	226.4	4.193		
Total	59	285.7			
Critical F = 3.38 (alpha = 0.01, df = 5,54)					
2.38 (alpha = 0.05, df = 5,54)					
Since F > Critical F 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	30	30			
2	4 %	28	28	2.184		
3	5 %	27.9	27.9	2.293		
4	7 %	27.3	27.3	2.948	*	
5	9 %	26.9	26.9	3.385	*	
6	12 %	27.5	27.5	2.73	*	
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	2.115	7.05	2	
3	5 %	10	2.115	7.05	2.1	
4	7 %	10	2.115	7.05	2.7	
5	9 %	10	2.115	7.05	3.1	
6	12 %	10	2.115	7.05	2.5	

Lower PMSD Bound Test for Ceriodaphnia dubia

Concentration	Reproduction	Relative Difference from Control	Pass/Fail
Control	30.0	-	
4 %	28.0	6.67	PASS
5 %	27.9	7.00	PASS
7 %	27.3	9.00	PASS
9 %	26.9	10.3	PASS
12 %	27.5	8.33	PASS

Limit = 13

NOEC = 12 %

LOEC = 12 %

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.762	-	
4 %	0.685	10.1	PASS
5 %	0.684	10.2	PASS
7 %	0.662	13.1	FAIL
9 %	0.677	11.2	PASS
12 %	0.645	15.4	FAIL

Limit = 12

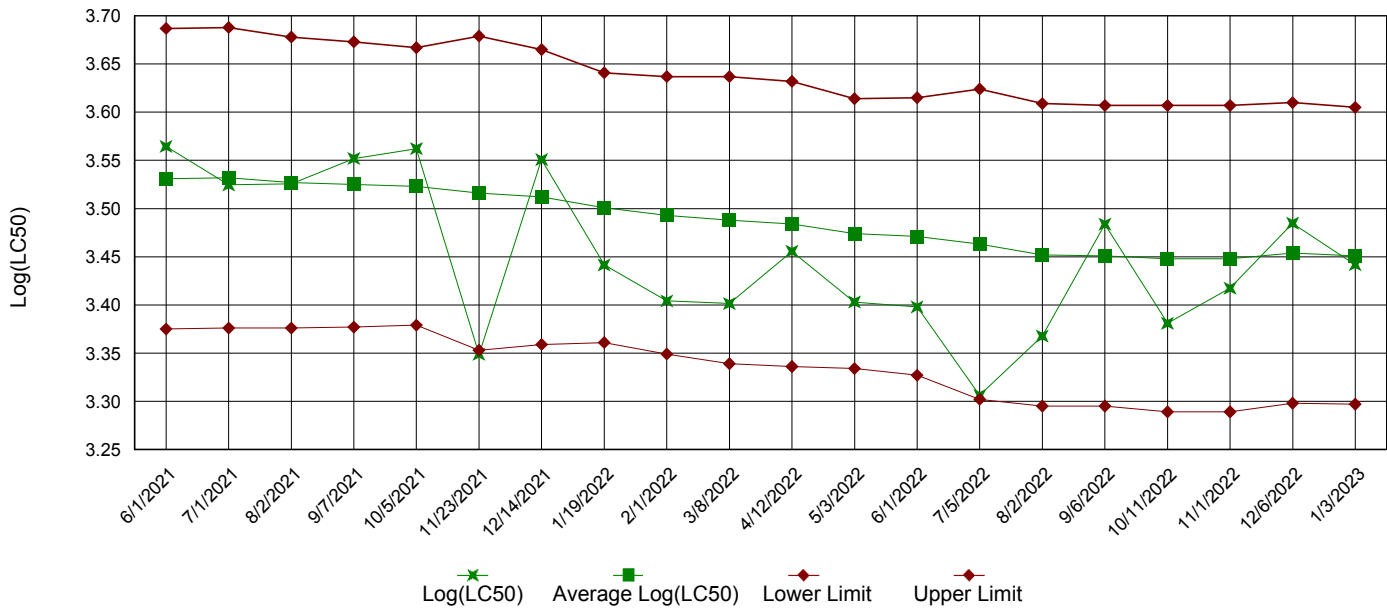
NOEC = 5 %

LOEC = 7 %

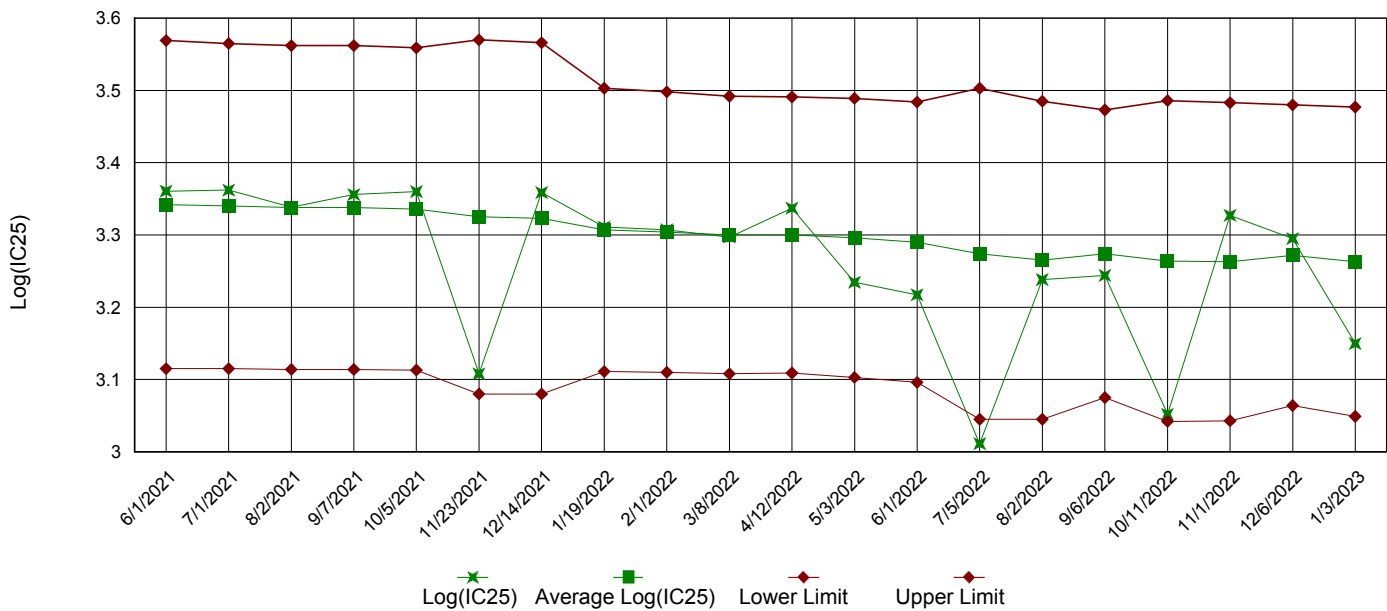
Appendix A3: Test 1000.0

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

LC50 Survival Data

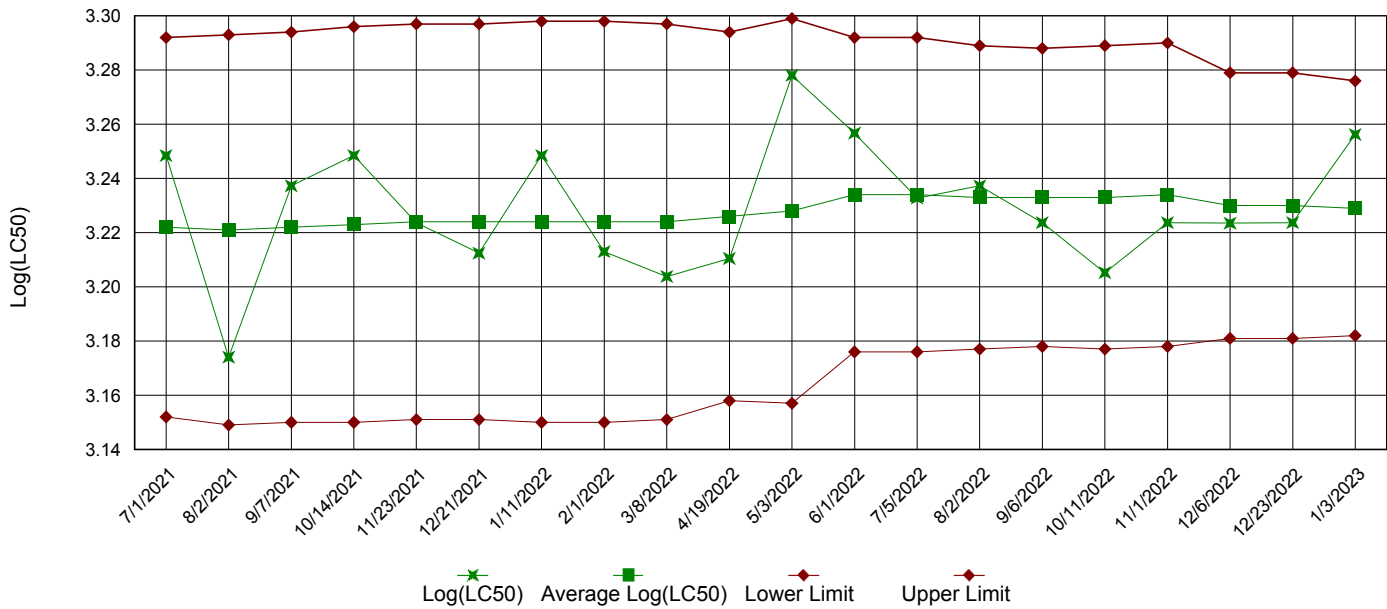


IC25 Growth Data

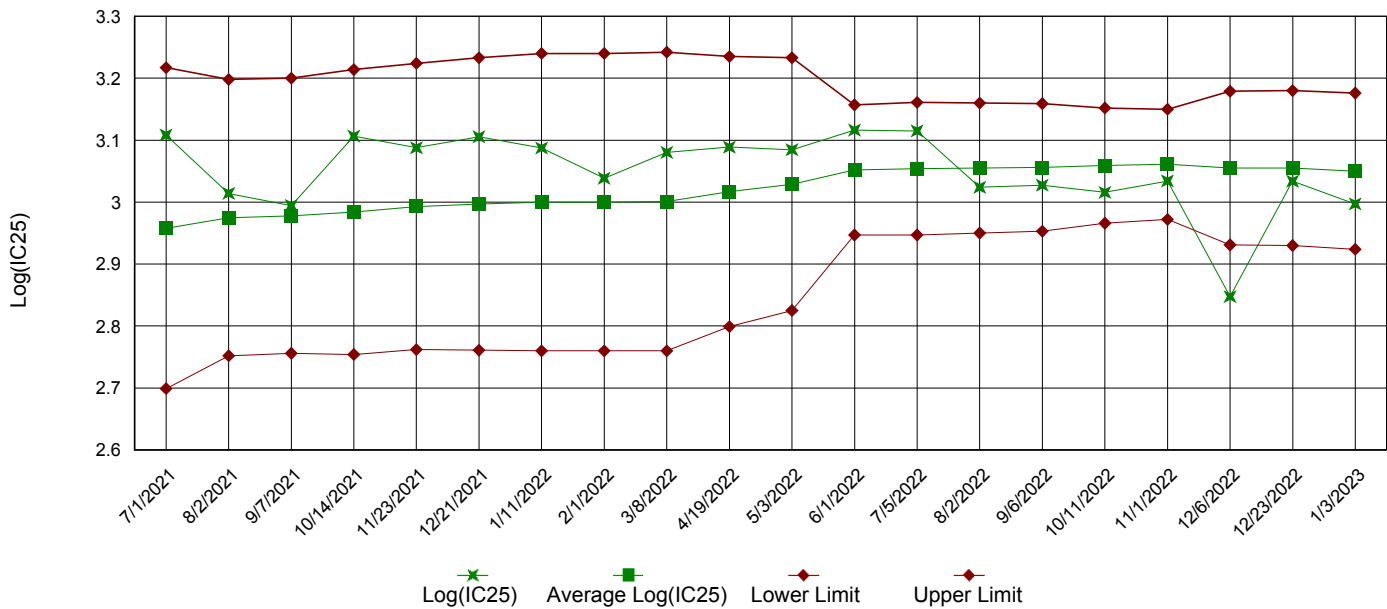


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: January 24, 2023 at 1028

Date and Time Test Terminated: January 31, 2023 at 0900

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

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.861	0.668	0.788	0.775	0.719	0.762	9.58
4 %	0.679	0.724	0.641	0.686	0.694	0.685	4.37
5 %	0.716	0.634	0.648	0.591	0.829	0.684	13.6
7 %	0.640	0.705	0.675	0.666	0.626	0.662	4.66
9 %	0.656	0.634	0.679	0.746	0.671	0.677	6.22
12 %	0.610	0.581	0.656	0.660	0.718	0.645	8.13

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> X </u> YES	<u> </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]: | <u> 0 </u> | (TLP6C) |
| 4. If you answered NO to 2.a) enter [0] otherwise enter [1]: | <u> 1 </u> | (TGP6C) |
| 5. NOEC Pimephales Lethality: | <u> 12 % </u> | (TOP6C) |
| 6. LOEC Pimephales Lethality: | <u> 12 % </u> | (TXP6C) |
| 7. NOEC Pimephales Sublethality: | <u> 5 % </u> | (TPP6C) |
| 8. LOEC Pimephales Sublethality: | <u> 7 % </u> | (TYP6C) |
| 9. Coefficient of variation for Pimephales growth: | <u> 9.58 </u> | (TQP6C) |
| 10. Sublethality for this test: | <u> 5 % </u> | (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: January 24, 2023 TIME: 1028
Test Terminated: DATE: January 31, 2023 TIME: 0900

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.7	7.8	7.8	8.0	8.5	7.7
Final	6.6	7.2	6.1	6.4	6.4	5.6	5.9
pH Initial	7.9	8.0	7.8	7.9	8.0	8.0	7.9
Final	7.6	7.7	7.5	7.6	7.4	7.3	7.4

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.6	7.8	7.9	8.0	8.4	7.6
Final	6.8	7.0	6.3	6.0	6.4	5.8	6.1
pH Initial	7.9	8.0	7.9	7.9	8.0	8.0	8.0
Final	7.7	7.7	7.5	7.6	7.5	7.4	7.4

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.7	7.6	7.9	7.7	8.0	8.3	7.6
Final	6.6	6.9	6.6	6.0	7.0	5.8	5.9
pH Initial	7.9	8.0	7.9	8.0	8.0	8.0	8.0
Final	7.6	7.6	7.5	7.6	7.6	7.4	7.4

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.7	7.8	7.7	8.0	8.1	7.4
Final	6.5	6.7	6.5	6.0	6.4	6.1	5.8
pH Initial	7.9	8.0	8.0	8.0	8.0	8.0	8.0
Final	7.6	7.6	7.5	7.5	7.5	7.5	7.4

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.8	7.7	7.5	8.0	8.3	7.4
Final	6.5	7.0	6.2	6.4	6.6	6.0	5.8
pH Initial	8.0	8.0	7.9	7.9	8.0	8.0	8.0
Final	7.6	7.7	7.5	7.6	7.5	7.5	7.4

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	7.7	7.9	7.8	8.1	8.3	7.7
Final	6.8	6.9	6.4	6.0	6.5	6.0	5.7
pH Initial	8.0	8.1	7.9	7.9	8.1	8.1	8.0
Final	7.7	7.7	7.6	7.6	7.6	7.5	7.4

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
150	33	480	<0.05	AR0035602 23-JAN-23
150	32	480	<0.05	AR0035602 25-JAN-23
NA	NA		NA	AR0035602 27-JAN-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	82	310	<0.05	272433-1
62	86	320	<0.05	272598-1

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: January 24, 2023 at 1030

Date and Time Test Terminated: January 30, 2023 at 1200

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	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	30	32	28	30	28	26
B	30	28	32	27	26	26
C	29	29	29	25	27	26
D	32	26	28	28	25	30
E	29	27	27	29	26	27
F	31	27	30	22	26	26
G	29	26	24	27	28	26
H	30	30	28	30	29	32
I	30	25	23	28	26	29
J	30	30	30	27	28	27
Mean per Adult	30.0	28.0	27.9	27.3	26.9	27.5
Mean per Surviving Adult	30.0	28.0	27.9	27.3	26.9	27.5
CV %	3.14	7.90	9.77	8.81	4.78	7.71

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. Dunnett's 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: 4.78 (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: January 24, 2023 TIME: 1030
Test Terminated: DATE: January 30, 2023 TIME: 1200

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

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

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

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

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	7.6	7.8	7.7	7.5	8.0	8.3	7.4
Final	7.9	7.9	8.0	7.4	8.3	7.8	--
pH Initial	8.0	8.0	7.9	7.9	8.0	8.0	8.0
Final	8.3	8.2	8.2	8.2	8.2	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.6	7.7	7.9	7.8	8.1	8.3	7.7
Final	8.0	7.9	8.0	8.0	8.5	7.8	--
pH Initial	8.0	8.1	7.9	7.9	8.1	8.1	8.0
Final	8.4	8.3	8.3	8.2	8.3	8.2	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
150	33	480	<0.05	AR0035602 23-JAN-23
150	32	480	<0.05	AR0035602 25-JAN-23
NA	NA		NA	AR0035602 27-JAN-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	82	310	<0.05	272433-1
62	86	320	<0.05	272598-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>TRUMANN Water Works</u>		Analyses Requested		AIC Control No: <u>272616</u>	
Project Reference: <u>AR0035602</u>		BOTTLES		AIC Proposal No:	
Project Manager: <u>Scotty Jones</u>		WATER		Carrier: <u>FX</u>	
Sampled By: <u>LORRE HOLT</u>		SOIL		Received Temperature °C	
AIC No. <u>AR0035602</u>		GRA B		Remarks	
Date/Time Collected: <u>1/23/03 - 11:55 AM</u>		COMP			
Date/Time Collected: <u>8:10 AM - 8:00 PM</u>		V			
Container Type: <u>P</u>		V = VOA vials		Field pH calibration	
Preservative: <u>NO</u>		N = Nitric acid pH2		on _____ @ _____	
G = Glass		H = HCl to pH2		Buffer:	
NO = none		B = NaOH to pH12		T = Sodium Thiosulfate	
S = Sulfuric acid pH2		Date/Time: <u>1/23/03/9:40 AM</u>		Z = Zinc acetate	
Turnaround Time Requested: (Please circle)		Relinquished		Received	
NORMAL or EXPEDITED IN _____ DAYS		By: <u>Lorrie Holt</u>		By:	
Expedited results requested by:		Date/Time: <u>1-24-03</u>		Date/Time	
Who should AIC contact with questions: <u>LORRE HOLT</u>		Relinquished		Received in Lab	
Phone: <u>870-483-2882</u> Fax: <u>870-485-6525</u>		By:		By: <u>[Signature]</u>	
Report Attention to: <u>LORRE HOLT</u>		Comments:		Date/Time: <u>0845</u>	
Report Address to: <u>825 HWY 463 N. TRUMANN AR. 72472</u>				Date/Time: <u>0845</u>	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>TRUMANN Water Works</u> Project Reference: <u>AR035202</u> Project Manager: <u>Scotty Jones</u> Sampled By: <u>Lorrie Holt</u>		PO No.: Sample Matrix: W A T E R S O I L V V		No of BOTTLES: <u>1</u>		Analyses Requested: <u>BIDMON, DRUG-CHRONIC</u> <u>CON-FH</u>		AIC Control No.: <u>272616</u> AIC Proposal No.: Carrier: <u>FX</u> Received Temperature °C: <u>21</u> Remarks:	
Sample Identification: <u>AR035202</u> Date/Time Collected: <u>12/13/03</u>		Container Type: <u>P</u> Preservative: <u>NO</u>		Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN ____ DAYS Expedited results requested by:		Relinquished By: <u>[Signature]</u> Relinquished Date/Time: <u>12/23/03 9:20 AM</u>		Received By: <u>[Signature]</u> Received in Lab Date/Time: <u>1-26-23 0840</u>	
G R A B C O M P <u> </u> <u> </u> <u> </u> <u> </u>		G = Glass P = Plastic NO = none S = Sulfuric acid pH2 V = VOA vials N = Nitric acid pH2		H = HCl to pH2 B = NaOH to pH12		T = Sodium Thiosulfate Z = Zinc acetate		Field pH calibration on @ Buffer:	
Who should AIC contact with questions: <u>LORRIE HOLT</u> Phone: <u>870-483-2882</u> Fax: <u>870-485-6525</u> Report Attention to: <u>LORRIE HOLT</u> Report Address to: <u>325 HWY 463 N, TRUMANN, AR 72472</u>		Comments:		Comments:		Comments:		Comments:	

FX: 39384626 0011



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Trumann Water Works		Analyses Requested		AIC Control No: 272616	
Project Reference: AR0035602		BOTTLES		AIC Proposal No:	
Project Manager: Scotty Jones		Sample Matrix		Carrier:	
Sampled By: LORRE HOLT		WATER		Received Temperature °C	
AIC No. 3		SOIL		Remarks	
Sample Identification AR0035602		GRA B		Field pH calibration on @	
Date/Time Collected 10/23/08		COMP		Buffer:	
Collected 8:00 AM - 8:00 PM		V		T = Sodium Thiosulfate	
Container Type P				Z = Zinc acetate	
Preservative NO				H = HCl to pH2	
G = Glass				B = NaOH to pH12	
NO = none				Sulfuric acid pH2	
S = Sulfuric acid pH2				V = VOA vials	
N = Nitric acid pH2				Date/Time	
Turnaround Time Requested: (Please circle)				Date/Time	
(NORMAL) or EXPEDITED IN _____ DAYS				By: AM	
Expedited results requested by:				Date/Time	
Who should AIC contact with questions: LORRE HOLT				Received in Lab	
Phone: 870-483-2882				By: BW	
Fax: 870-483-6525				Date/Time	
Report Attention to: LORRE HOLT				Received in Lab	
Report Address to: 325 Hwy 463 N.				By: [Signature]	
TRUMANN, AR. 72472				Date/Time	
				Comments:	
				EX 3939 3535 2548	

SAMPLE DELIVERED MONDAY

272616-3

FedEx
FO
 FedEx First Overnight®
 151069 REV 3/21

SDR

24
 FO
 09:30
 2548
 06:10
 07:30

X0 LITA
 TRACKING NUMBER: 3939 3535 2548
 SATURDAY 9:30A
 FIRST OVERNIGHT
 AHS
 72204
 LIT
 AR-US

FedEx
 EXPRESS
 451011020127

TO
 ATTN SAMPLE RECEIVING
 AMERICAN INTERPLEX CORPORATION
 8600 KANIS RD
 LITTLE ROCK AR 72204

ORIGIN: JARA (870) 483-2382
 LARGE HOLD
 TRUMANN WATER WORKS
 825 HWY 493 N
 TRUMANN, AR 72472
 UNITED STATES US

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 **ANALYTICAL REPORT****PREPARED FOR**

Attn: Ms. Lorre Holt
Trumann Water and Sewer Commission
825 Hwy 463 North
Trumann, Arkansas 72472

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JOB DESCRIPTION

AR0035602

JOB NUMBER

192-888-1

Job Notes

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Biomonitoring Testing
for

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
825 Hwy 463 North
Trumann, AR 72472

Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
825 Hwy 463 North
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 Eurofins Arkansas. 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.**

Eurofins Arkansas



John Overbey
Chief Operating Officer

PDF cc: Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
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Trumann Water and Sewer Commission
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%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.615	PASS
Control Growth CV < or = 40%	10.7	PASS
Growth Minimum Significant Difference 12 to 30%	12.5	PASS
Critical Dilution CV < or = 40%	9.77	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	24.9	PASS
Control CV < or = 40% per Surviving Female	20.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	23.3	PASS
Critical Dilution CV < or = 40%	28.5	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	Sample 3
Dissolved oxygen (mg/l)	5.7	6.4	6.4
pH (standard units)	7.0	7.6	8.1
Alkalinity (mg/l as CaCO ₃)	62	66	68
Hardness (mg/l as CaCO ₃)	35	37	38
Conductivity (umhos/cm)	400	400	400
Residual Chlorine (mg/l)	0.070	<0.05	<0.05
Ammonia as N (mg/l)	<0.10	0.37	0.16

2. Dilution Water Samples:

Analysis	192-778-A-1	192-778-A-2
Dissolved oxygen (mg/l)	6.5	6.6
pH (standard units)	7.9	8.0
Alkalinity (mg/l as CaCO ₃)	61	60
Hardness (mg/l as CaCO ₃)	97	91
Conductivity (umhos/cm)	310	310
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 18, 2023 at 1156
Date & Time Test Terminated: April 25, 2023 at 1020
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 18, 2023 at 1138
Date & Time Test Terminated: April 24, 2023 at 1319
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 Eurofins'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 March 07, 2023 at 1018 to March 14, 2023 at 1006

The results were as follows: (Control No. 273633-1.)

Survival LC-50: 3208 mg/l

Growth IC-25: 2054 mg/l

Growth PMSD: 8.35

Ceriodaphnia dubia

A chronic reference test was performed on March 07, 2023 at 0947 to March 13, 2023 at 1033

The results were as follows: (Control No. 273633-2.)

Survival LC-50: 1635.8 mg/l

Reproduction IC-25: 1033 mg/l

Reproduction PMSD: 12.3

V. Organism History

Pimephales promelas (Fathead minnow)

Date: April 18, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: April 18, 2023

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

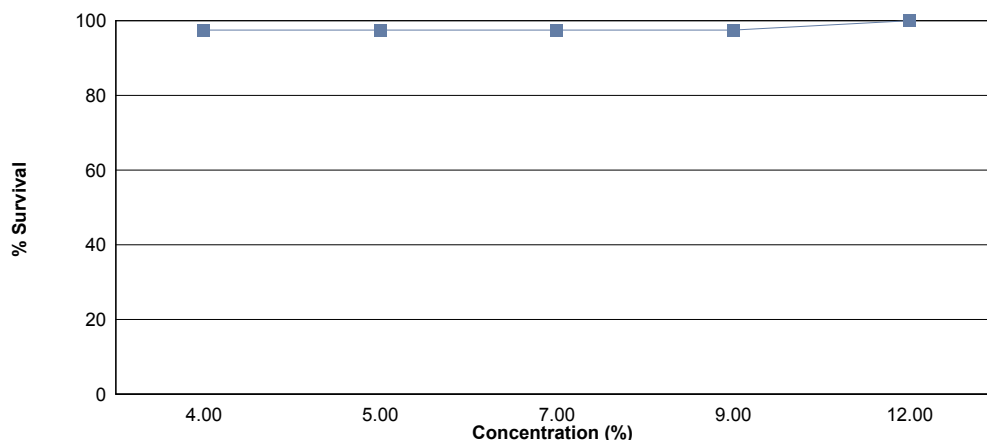
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 18, 2023 at 1156 and continued through April 25, 2023 at 1020. 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	97.5	0.600
4 %	97.5	0.574
5 %	97.5	0.534
7 %	97.5	0.574
9 %	97.5	0.566
12 %	100	0.568

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

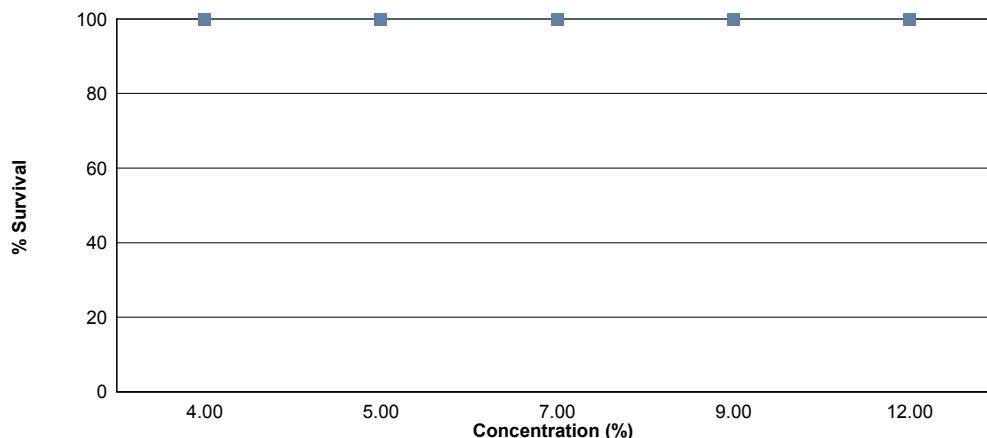
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 18, 2023 at 1138 and continued through April 24, 2023 at 1319. 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



Concentration	Percent Survival	Mean Reproduction
Control	100	24.9
4 %	100	26.0
5 %	100	25.4
7 %	100	26.1
9 %	100	26.0
12 %	100	28.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 18, 2023 at 1156
 Date and Time Test Terminated: April 25, 2023 at 1020

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	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
4 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	7	7	7	7
	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	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	7	7
	E	8	8	8	8	8	8	8
7 %	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	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	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: April 18, 2023 at 1156

Test Terminated: April 25, 2023 at 1020

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.71670	.72137	0.00467	8	0.584
	B	.72032	.72434	0.00402	8	0.502
	C	.69548	.70048	0.00500	8	0.625
	D	.72861	.73402	0.00541	8	0.676
	E	.71381	.71871	0.00490	8	0.612
4 %	A	.71096	.71582	0.00486	8	0.608
	B	.69054	.69470	0.00416	8	0.520
	C	.70117	.70555	0.00438	8	0.548
	D	.72529	.72988	0.00459	8	0.574
	E	.70923	.71420	0.00497	8	0.621
5 %	A	.70625	.71051	0.00426	8	0.532
	B	.70588	.71045	0.00457	8	0.571
	C	.71615	.72037	0.00422	8	0.528
	D	.69347	.69703	0.00356	8	0.445
	E	.71979	.72453	0.00474	8	0.592
7 %	A	.72096	.72563	0.00467	8	0.584
	B	.71593	.72017	0.00424	8	0.530
	C	.69925	.70360	0.00435	8	0.544
	D	.70865	.71373	0.00508	8	0.635
	E	.69310	.69770	0.00460	8	0.575
9 %	A	.72169	.72661	0.00492	8	0.615
	B	.72174	.72564	0.00390	8	0.488
	C	.72507	.72992	0.00485	8	0.606
	D	.71604	.72079	0.00475	8	0.594
	E	.70870	.71293	0.00423	8	0.529
12 %	A	.72902	.73381	0.00479	8	0.599
	B	.71720	.72139	0.00419	8	0.524
	C	.71432	.71889	0.00457	8	0.571
	D	.70768	.71197	0.00429	8	0.536
	E	.69732	.70219	0.00487	8	0.609

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 18, 2023 at 1138

Date and Time Test Terminated: April 24, 2023 at 1319

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	3	0	0	3	10	0.300	
4	3	4	4	3	3	3	5	0	5	4	34	10	3.40	
5	10	10	10	9	9	8	10	9	11	10	96	10	9.60	
6	12	14	13	12	13	0	13	14	13	12	116	10	11.6	
7														
8														
TOTAL	25	28	27	24	25	11	28	26	29	26	249	10	24.9	

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	3	3	0	0	6	10	0.600
4	4	3	4	3	4	3	0	0	4	5	30	10	3.00
5	10	9	9	10	10	9	10	9	11	10	97	10	9.70
6	15	13	14	13	14	0	16	16	13	13	127	10	12.7
7													
8													
TOTAL	29	25	27	26	28	12	29	28	28	28	260	10	26.0

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	3	3	0	0	6	10	0.600
4	3	4	4	4	4	5	0	0	5	5	34	10	3.40
5	10	10	8	10	10	9	10	9	10	11	97	10	9.70
6	14	13	15	16	15	0	16	14	14	0	117	10	11.7
7													
8													
TOTAL	27	27	27	30	29	14	29	26	29	16	254	10	25.4

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 18, 2023 at 1138

Date and Time Test Terminated: April 24, 2023 at 1319

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	3	3	3	0	9	10	0.900	
4	5	4	4	0	4	4	0	0	0	3	24	10	2.40	
5	11	11	10	11	9	11	10	10	11	9	103	10	10.3	
6	15	14	15	16	13	0	13	13	16	10	125	10	12.5	
7														
8														
TOTAL	31	29	29	27	26	15	26	26	30	22	261	10	26.1	

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	3	0	0	3	10	0.300
4	4	5	3	3	4	4	6	0	4	4	37	10	3.70
5	9	11	10	10	12	9	11	10	12	10	104	10	10.4
6	13	15	16	13	15	0	17	10	17	0	116	10	11.6
7													
8													
TOTAL	26	31	29	26	31	13	34	23	33	14	260	10	26.0

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	4	4	0	0	8	10	0.800
4	4	4	4	3	0	5	0	0	6	3	29	10	2.90
5	10	11	11	10	11	10	10	10	11	10	104	10	10.4
6	16	16	18	14	17	0	18	18	16	13	146	10	14.6
7													
8													
TOTAL	30	31	33	27	28	15	32	32	33	26	287	10	28.7

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data				Transform: Arc Sin(Square Root(Y))
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	0.87500	1.20940
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	1.00000	1.39310
2	4 %	5	1.00000	1.39310
3	5 %	1	1.00000	1.39310
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	1.00000	1.39310
4	7 %	2	0.87500	1.20940
4	7 %	3	1.00000	1.39310
4	7 %	4	1.00000	1.39310
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	1.00000	1.39310
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 = 0.135 W = 0.5577 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 %	27.50	16.00	5.00	
3	5 %	27.50	16.00	5.00	
4	7 %	27.50	16.00	5.00	
5	9 %	27.50	16.00	5.00	
6	12 %	30.00	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
D = 0.06056 W = 0.9619 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 = 1.706 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	0.01129	0.002258	0.895	
Within (Error)	24	0.06056	0.002523		
Total	29	0.07185			
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	0.5998	0.5998			
2	4 %	0.5742	0.5742	0.8058		
3	5 %	0.5336	0.5336	2.084		
4	7 %	0.5736	0.5736	0.8247		
5	9 %	0.5664	0.5664	1.051		
6	12 %	0.5678	0.5678	1.007		
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	0.07497	12.5	0.0256	
3	5 %	5	0.07497	12.5	0.0662	
4	7 %	5	0.07497	12.5	0.0262	
5	9 %	5	0.07497	12.5	0.0334	
6	12 %	5	0.07497	12.5	0.032	

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 %	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
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 %	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	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.242 D* = 1.899 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 %	121.50	75.00	10.00	
3	5 %	120.00	75.00	10.00	
4	7 %	117.50	75.00	10.00	
5	9 %	117.50	75.00	10.00	
6	12 %	135.50	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	86.68	17.34	0.5475	
Within (Error)	54	1710	31.67		
Total	59	1797			
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)					

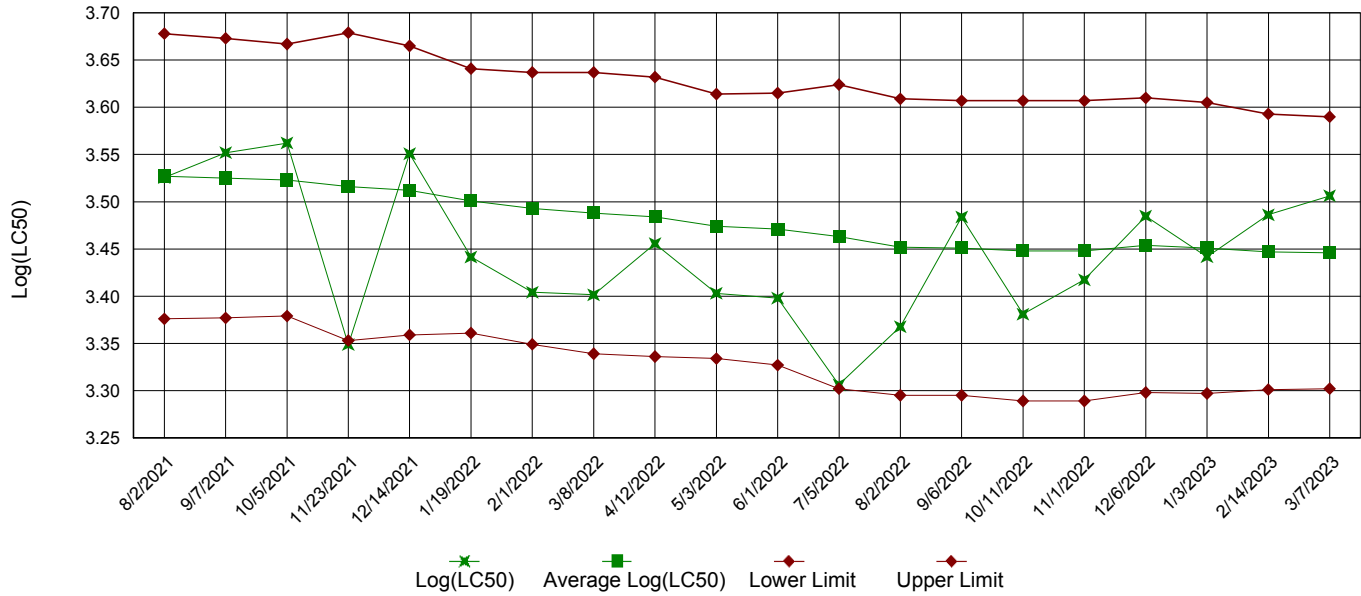
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	24.9	24.9			
2	4 %	26	26	-0.4371		
3	5 %	25.4	25.4	-0.1987		
4	7 %	26.1	26.1	-0.4768		
5	9 %	26	26	-0.4371		
6	12 %	28.7	28.7	-1.51		
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	5.814	23.3	-1.1	
3	5 %	10	5.814	23.3	-0.5	
4	7 %	10	5.814	23.3	-1.2	
5	9 %	10	5.814	23.3	-1.1	
6	12 %	10	5.814	23.3	-3.8	

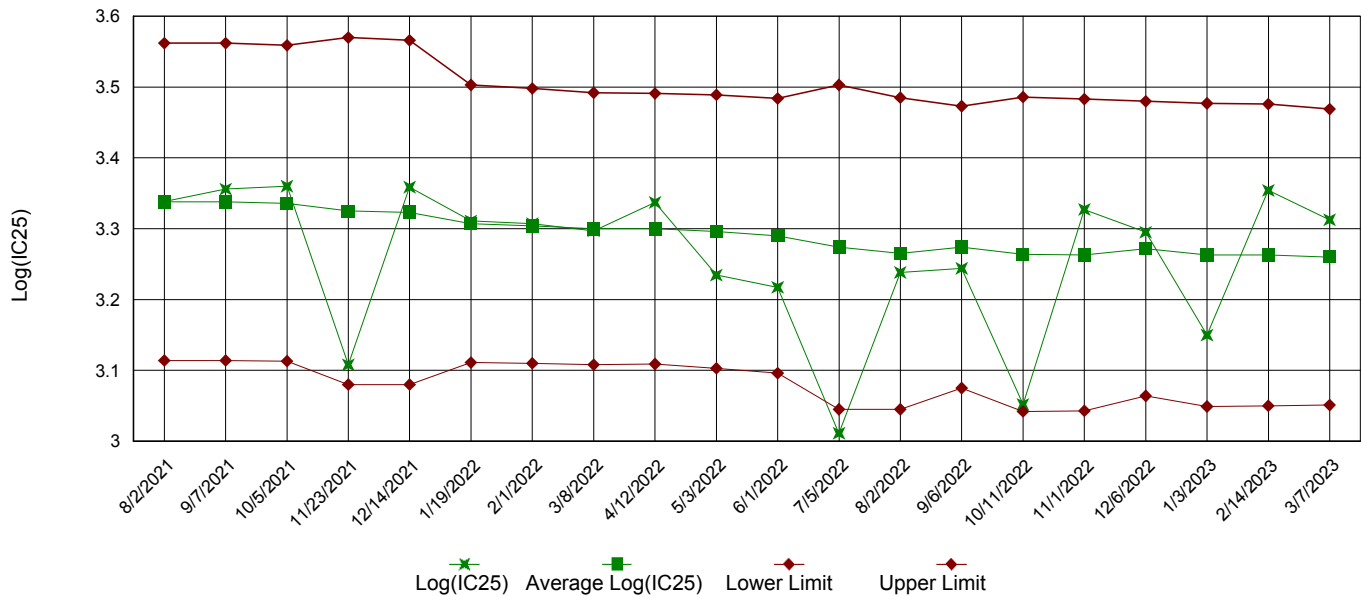
Appendix A3: Test 1000.0

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

LC50 Survival Data

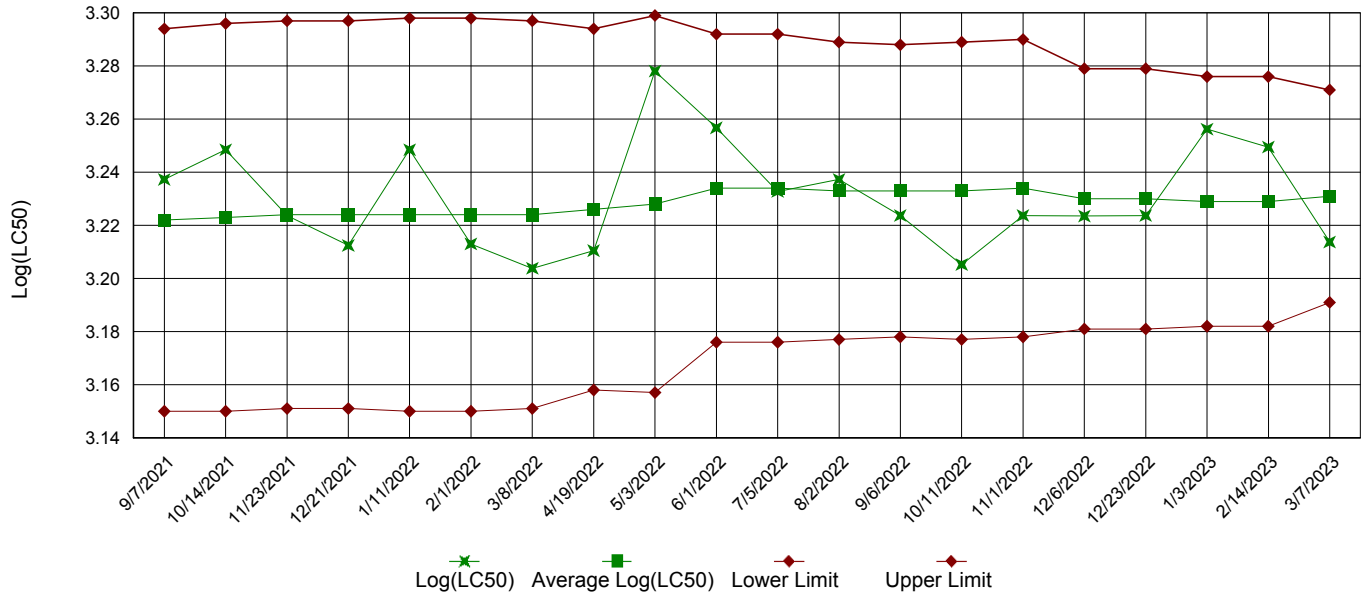


IC25 Growth Data

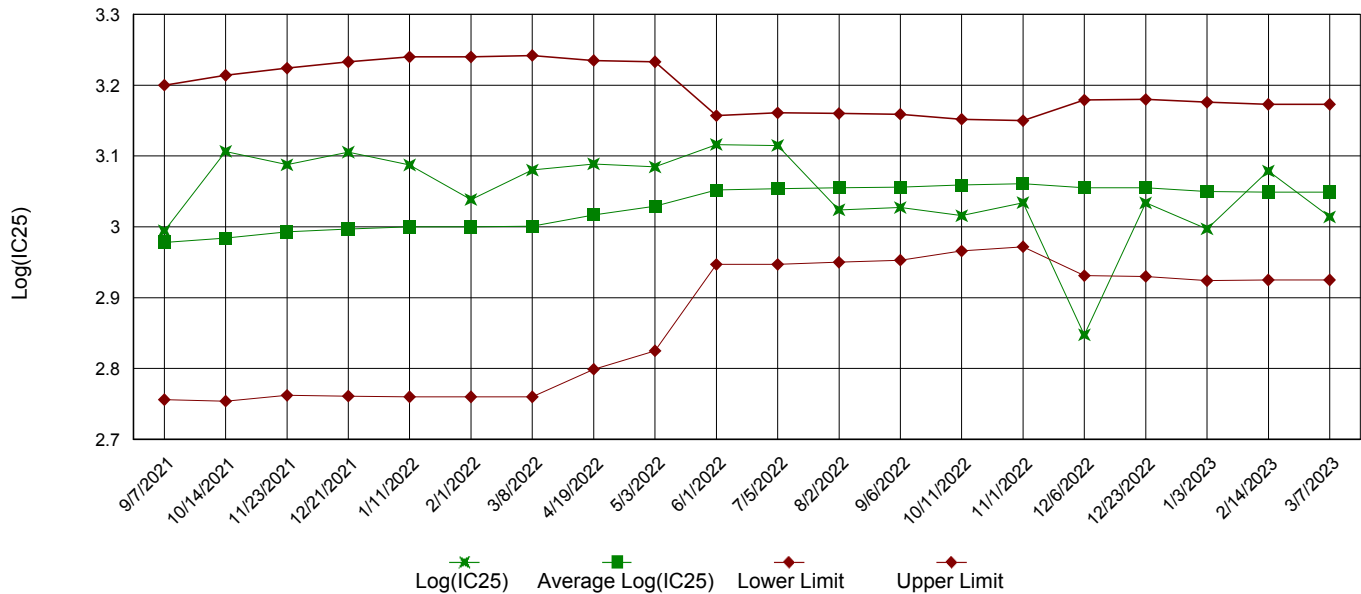


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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 18, 2023 at 1156

Date and Time Test Terminated: April 25, 2023 at 1020

Dilution water used:

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

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.584	0.502	0.625	0.676	0.612	0.600	10.7
4 %	0.608	0.520	0.548	0.574	0.621	0.574	7.26
5 %	0.532	0.571	0.528	0.445	0.592	0.534	10.6
7 %	0.584	0.530	0.544	0.635	0.575	0.574	7.11
9 %	0.615	0.488	0.606	0.594	0.529	0.566	9.77
12 %	0.599	0.524	0.571	0.536	0.609	0.568	6.60

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: 10.7 (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: April 18, 2023 TIME: 1156
 Test Terminated: DATE: April 25, 2023 TIME: 1020

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.5	6.6	6.3	6.2	6.1	5.8
Final	5.4	5.6	6.0	4.8	6.2	5.0	5.5
pH Initial	7.9	8.0	8.0	8.0	7.9	7.9	8.0
Final	7.6	7.6	7.6	7.5	7.8	7.6	7.4

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.4	6.6	6.3	6.2	6.1	5.7
Final	5.6	5.4	6.3	5.2	5.1	5.2	5.7
pH Initial	7.9	7.9	8.0	7.9	7.9	8.0	8.0
Final	7.6	7.5	7.7	7.5	7.5	7.6	7.4

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.3	6.5	6.1	6.2	6.1	5.7
Final	5.6	5.3	5.8	4.7	4.9	4.8	5.4
pH Initial	7.9	7.9	7.9	8.0	7.9	8.0	8.0
Final	7.6	7.4	7.6	7.4	7.5	7.5	7.4

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.4	6.6	6.1	6.3	6.1	5.7
Final	5.5	5.2	6.2	5.8	5.0	4.9	5.5
pH Initial	7.8	7.8	7.9	7.9	7.9	8.0	8.0
Final	7.6	7.4	7.7	7.7	7.5	7.5	7.4

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.6	6.2	6.5	6.2	6.2	6.1	5.7
Final	5.6	5.5	5.8	5.4	5.0	5.2	5.6
pH Initial	7.8	7.8	8.0	7.9	7.9	8.0	8.0
Final	7.6	7.4	7.6	7.6	7.5	7.6	7.4

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.3	6.6	6.3	6.2	6.3	5.7
Final	5.7	5.4	5.8	5.2	5.0	5.0	5.3
pH Initial	7.8	7.8	7.9	7.9	7.9	8.0	8.0
Final	7.6	7.5	7.6	7.5	7.5	7.6	7.4

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	35	400	0.070	AR0035602 17-APR-23
66	37	400	<0.05	AR0035602 19-APR-23
68	38	400	<0.05	AR0035602 21-APR-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	97	310	<0.05	192-778-A-1
60	91	310	<0.05	192-778-A-2

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 18, 2023 at 1138

Date and Time Test Terminated: April 24, 2023 at 1319

Dilution water used:

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	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	25	29	27	31	26	30
B	28	25	27	29	31	31
C	27	27	27	29	29	33
D	24	26	30	27	26	27
E	25	28	29	26	31	28
F	11	12	14	15	13	15
G	28	29	29	26	34	32
H	26	28	26	26	23	32
I	29	28	29	30	33	33
J	26	28	16	22	14	26
Mean per Adult	24.9	26.0	25.4	26.1	26.0	28.7
Mean per Surviving Adult	24.9	26.0	25.4	26.1	26.0	28.7
CV %	20.6	19.5	22.2	17.9	28.5	18.9

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	_____ 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 <i>Ceriodaphnia</i> Lethality: | _____ 12 % (TOP3B) |
| 6. LOEC <i>Ceriodaphnia</i> Lethality: | _____ 12 % (TXP3B) |
| 7. NOEC <i>Ceriodaphnia</i> Sublethality: | _____ 12 % (TPP3B) |
| 8. LOEC <i>Ceriodaphnia</i> Sublethality: | _____ 12 % (TYP3B) |
| 9. Coefficient of variation for <i>Ceriodaphnia</i> Reproduction: | _____ 28.5 (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: April 18, 2023 TIME: 1138
 Test Terminated: DATE: April 24, 2023 TIME: 1319

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.5	6.6	6.3	6.2	6.1	5.8
Final	6.6	6.8	6.7	6.5	6.6	6.0	--
pH Initial	7.9	8.0	8.0	8.0	7.9	7.9	8.0
Final	8.4	8.5	8.6	8.5	8.3	8.2	--

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.4	6.6	6.3	6.2	6.1	5.7
Final	6.5	6.8	6.7	6.2	6.0	6.1	--
pH Initial	7.9	7.9	8.0	7.9	7.9	8.0	8.0
Final	8.4	8.5	8.5	8.4	8.3	8.2	--

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.3	6.5	6.1	6.2	6.1	5.7
Final	6.5	6.7	6.6	6.4	6.2	6.0	--
pH Initial	7.9	7.9	7.9	8.0	7.9	8.0	8.0
Final	8.5	8.5	8.5	8.4	8.3	8.2	--

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.4	6.6	6.1	6.3	6.1	5.7
Final	6.4	6.8	6.6	6.4	6.1	6.0	--
pH Initial	7.8	7.8	7.9	7.9	7.9	8.0	8.0
Final	8.4	8.5	8.5	8.4	8.3	8.2	--

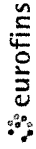
DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.6	6.2	6.5	6.2	6.2	6.1	5.7
Final	6.4	6.8	6.6	6.4	6.0	6.0	--
pH Initial	7.8	7.8	8.0	7.9	7.9	8.0	8.0
Final	8.4	8.6	8.6	8.5	8.3	8.1	--

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	6.3	6.6	6.3	6.2	6.3	5.7
Final	6.5	6.8	6.6	6.5	6.0	6.1	--
pH Initial	7.8	7.8	7.9	7.9	7.9	8.0	8.0
Final	8.4	8.5	8.5	8.5	8.4	8.3	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	35	400	0.070	AR0035602 17-APR-23
66	37	400	<0.05	AR0035602 19-APR-23
68	38	400	<0.05	AR0035602 21-APR-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	97	310	<0.05	192-778-A-1
60	91	310	<0.05	192-778-A-2

Chain of Custody Record



EN 10

FX

Client Information		Sampler: LORE HOLT	Lab PM	COC No: 192-888 COC
Address: 825 Hwy 463 North		Phone: 870-483-2882	E-Mail: lorne-holt@wsw.com	192-162-26 1
City: Trumann		State: AR, 72472	State of Origin	Page: 1 of 1
Phone: 870-2882		Compliance Project: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No	Job #:	
Email: lorne-holt@wsw.com		PO #: 435		
Project Name: FINAL ESCURANT FLUME		Project #:		
Site: FINAL ESCURANT FLUME		SOW #:		

Sample Identification	Sample Date	Sample Time	Sample Type (C=comp, G=grab)	Matrix (W=water, S=solid, O=soil, BT=tissue, A=air)	Field Filtered Sample (Yes or No)	Perform MS/MSD (Yes or No)	1000_FH_1002_CD	Analysis Requested	Preservation Codes	Special Instructions/Note:
AR035L02	*	*	COMP	Water	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>	<input checked="" type="checkbox"/>		A HCL B NaOH C - Zn Acetate D - Nitric Acid E - NaHSO4 F - MeOH G Amchlor H - Ascorbic Acid I - Ice J DI Water K - EDTA L EDA Other:	LIMS: 274544-1 TALS: 888-1
				Water	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>		M - Hexane N None O - AsNaO2 P Na2O4S Q Na2SO3 R - Na2S2O3 S - H2SO4 T TSP Dodecahydrate U - Acetone V MCAA W - pH 4-5 Y - Triana Z other (specify)	* DATE/TIME AS: 4/16-17/23 8-8AM
				Water	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			
				Water	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>			

Possible Hazard Identification
 Non-Hazard Flammable Skin Irritant Poison B Unknown Radiological
 Deliverable Requested I, II, III, IV, Other (specify)

Empty Kit Relinquished by: **Lorne Holt** Date: **4/17/23 9:10 AM** Company: **FWW**

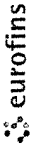
Relinquished by: **Lorne Holt** Date/Time: **4-18-23/0909** Company: **EUR**

Custody Seals Intact: Yes No No
 Cooler Temperature(s) °C and Other Remarks: **0.3**

Sample Disposal (A fee may be assessed if samples are retained longer than 1 month)
 Return To Client Disposal By Lab Archive For _____ Months

Special Instructions/QC Requirements

Chain of Custody Record



Environment Testing

FX

Client Information		Sampler: LORRE HOFF	Lab P#: 192-162-261
Address: Trumann Water and Sewer Commission		Carrier Tracking No(s): 3943 1761	COC No: 192-162-261
City: Trumann		State of Origin: 7625	Page: Page 1 of 1
Slate Zip: AR, 72472		Job #:	
Phone: 870-2882		Analysis Requested	
Email: lorne_boltz@arwater.com		M - Hexane	
Project Name: Final Effluent Flume		N - None	
Site: Final Effluent Flume		O - As/NaO2	
Due Date Requested		P - Na2O4S	
TAT Requested (days)		Q - Na2SO3	
Compliance Project <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		R - Na2SO3	
PO #		S - H2SO4	
IVO #		G - Amchlor	
Project #		T - TSP Dodecahydrate	
SSOW#		U - Acetone	
Sample Date		V - MCAA	
Sample Time		W - pH 4-5	
Sample Type (C=Comp, G=grab)		Y - Trizma	
Preservation Code: Comp		Z - other (specify)	
Matrix (W=water, S=solid, O=wastewat, B=leachate, A=air)		Total Number of containers	
Water		1	
Water		LIMS: 274544-3	
Water		TALS: 888-3	
Special Instructions/Note:		DATE/TIME AS:	
AR035602		4/20/23 - 4/21/23	
8-8		Special Instructions/Note:	
Sample Identification		Return To Client <input type="checkbox"/> Disposal By Lab <input type="checkbox"/> Archive For <input type="checkbox"/> Months	
Possible Hazard Identification		Sample Disposal (A fee may be assessed if samples are retained longer than 1 month)	
Non-Hazard <input type="checkbox"/> Flammable <input type="checkbox"/> Skin Irritant <input type="checkbox"/> Poison B <input type="checkbox"/> Unknown <input type="checkbox"/> Radiological <input type="checkbox"/>		Special Instructions/QC Requirements:	
Deliverable Requested I II III IV, Other (specify)		Empty Kit Relinquished by	
Relinquished by Lorre Hoff		Date	
Relinquished by		Date/Time	
Relinquished by		Date/Time	
Custody Seals Intact: <input type="checkbox"/> Yes <input type="checkbox"/> No		Date/Time	
Custody Seal No		Cooler Temperature(s) °C and Other Remarks:	
0.1		0.14 23 Apr 23	

 **ANALYTICAL REPORT****PREPARED FOR**

Attn: Ms. Lorre Holt
Trumann Water and Sewer Commission
825 Hwy 463 North
Trumann, Arkansas 72472

Generated 8/3/2023 3:23:01 PM

JOB DESCRIPTION

AR0035602

JOB NUMBER

192-3580-1

Job Notes

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Biomonitoring Testing
for
Effluent

Prepared for:

Ms. Lorre Holt
Trumann Water and Sewer Commission
825 Hwy 463 North
Trumann, AR 72472

Trumann Water and Sewer Commission
ATTN: Ms. Lorre Holt
825 Hwy 463 North
Trumann, AR 72472

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

Dear Ms. Lorre Holt:

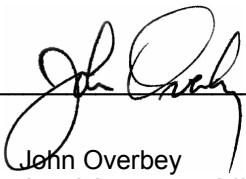
This report is the analytical results and supporting information for the samples submitted to Eurofins Arkansas. The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section 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.**

Eurofins Arkansas



John Overbey
Chief Operating Officer

PDF cc: Trumann Water and Sewer Commission
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Trumann Water and Sewer Commission
ATTN: Ms. Lorre Holt
lorre_holt0201@yahoo.com

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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	95.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.612	PASS
Control Growth CV < or = 40%	10.2	PASS
Growth Minimum Significant Difference 12 to 30%	18.5	PASS
Critical Dilution CV < or = 40%	6.97	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	39.0	PASS
Control CV < or = 40% per Surviving Female	4.68	PASS
Reproduction Minimum Significant Difference 13 to 47%	28.2	PASS
Critical Dilution CV < or = 40%	27.9	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: Effluent
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.8	6.8	6.8
pH (standard units)	7.8	7.9	7.9
Alkalinity (mg/l as CaCO ₃)	120	120	120
Hardness (mg/l as CaCO ₃)	34	35	36
Conductivity (umhos/cm)	420	420	420
Residual Chlorine (mg/l)	0.050	0.060	<0.05
Ammonia as N (mg/l)	1.1	2.6	1.1

2. Dilution Water Samples:

Analysis	192-3238-A-2	192-3445-A-2
Dissolved oxygen (mg/l)	7.1	7.0
pH (standard units)	7.8	8.0
Alkalinity (mg/l as CaCO ₃)	58	60
Hardness (mg/l as CaCO ₃)	85	85
Conductivity (umhos/cm)	310	310
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 18, 2023 at 1309
Date & Time Test Terminated: July 25, 2023 at 1400
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 18, 2023 at 1215
Date & Time Test Terminated: July 24, 2023 at 1410
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 Eurofins'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 June 01, 2023 at 1007 to June 08, 2023 at 0908

The results were as follows: (Control No. 274591-1.)

Survival LC-50: 3141 mg/l

Growth IC-25: 2184 mg/l

Growth PMSD: 15

Ceriodaphnia dubia

A chronic reference test was performed on June 01, 2023 at 1035 to June 08, 2023 at 1222

The results were as follows: (Control No. 274591-2.)

Survival LC-50: 1673 mg/l

Reproduction IC-25: 1097 mg/l

Reproduction PMSD: 16.4

V. Organism History

Pimephales promelas (Fathead minnow)

Date: July 18, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: July 18, 2023

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

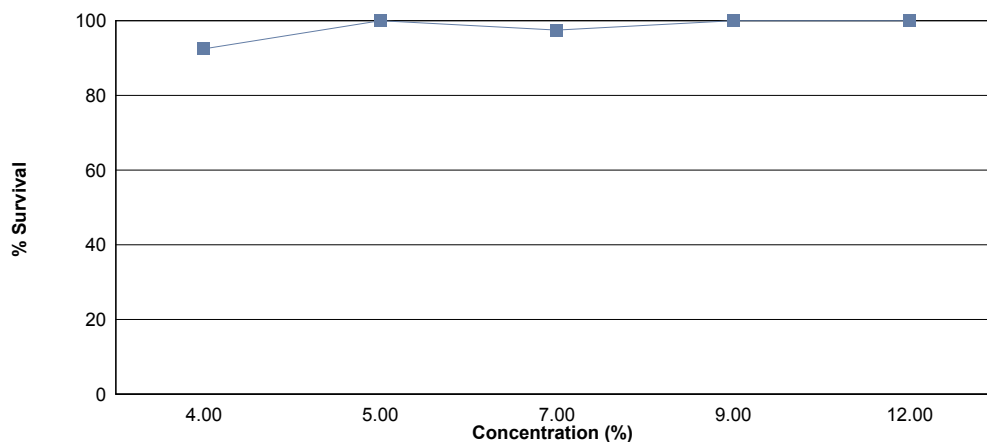
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 18, 2023 at 1309 and continued through July 25, 2023 at 1400. 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	95.0	0.581
4 %	92.5	0.560
5 %	100	0.595
7 %	97.5	0.603
9 %	100	0.656
12 %	100	0.648

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

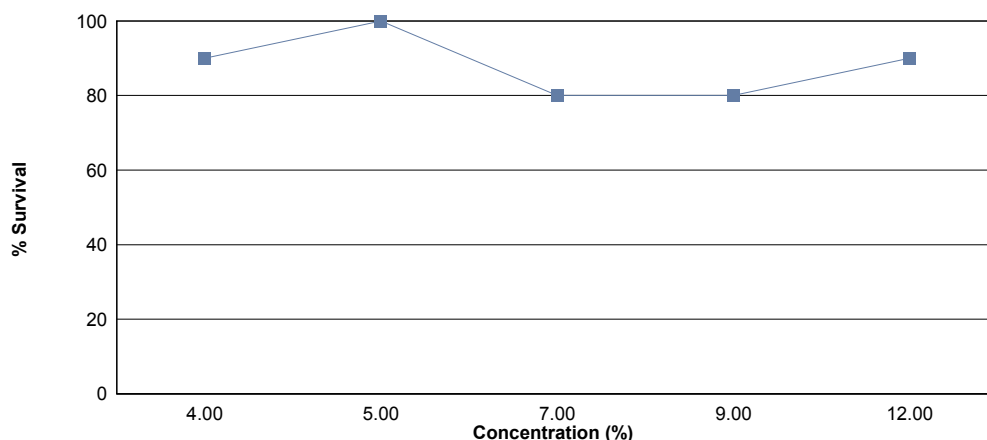
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 18, 2023 at 1215 and continued through July 24, 2023 at 1410. 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	39.0
4 %	90.0	35.5
5 %	100	37.9
7 %	80.0	31.9
9 %	80.0	30.3
12 %	90.0	33.0

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: July 18, 2023 at 1309

Date and Time Test Terminated: July 25, 2023 at 1400

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	7
	D	8	8	8	8	7	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	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	6	6
	E	8	8	8	8	8	8	8
5 %	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
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	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	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
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	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: July 18, 2023 at 1309

Test Terminated: July 25, 2023 at 1400

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.71669	.72189	0.00520	8	0.650
	B	.71402	.71836	0.00434	8	0.542
	C	.72041	.72457	0.00416	8	0.520
	D	.71765	.72209	0.00444	8	0.555
	E	.71224	.71734	0.00510	8	0.638
4 %	A	.70732	.71253	0.00521	8	0.651
	B	.70132	.70594	0.00462	8	0.578
	C	.70777	.71256	0.00479	8	0.599
	D	.72500	.72776	0.00276	8	0.345
	E	.71807	.72307	0.00500	8	0.625
5 %	A	.72338	.72852	0.00514	8	0.642
	B	.71527	.71953	0.00426	8	0.532
	C	.73196	.73636	0.00440	8	0.550
	D	.69542	.70017	0.00475	8	0.594
	E	.72306	.72832	0.00526	8	0.658
7 %	A	.70111	.70550	0.00439	8	0.549
	B	.71607	.72078	0.00471	8	0.589
	C	.72968	.73458	0.00490	8	0.612
	D	.71033	.71486	0.00453	8	0.566
	E	.70919	.71477	0.00558	8	0.698
9 %	A	.73075	.73653	0.00578	8	0.722
	B	.72241	.72742	0.00501	8	0.626
	C	.70128	.70634	0.00506	8	0.632
	D	.70196	.70688	0.00492	8	0.615
	E	.71654	.72202	0.00548	8	0.685
12 %	A	.71411	.71910	0.00499	8	0.624
	B	.71458	.72046	0.00588	8	0.735
	C	.70961	.71464	0.00503	8	0.629
	D	.69858	.70315	0.00457	8	0.571
	E	.70703	.71246	0.00543	8	0.679

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 18, 2023 at 1215

Date and Time Test Terminated: July 24, 2023 at 1410

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	7	6	6	4	6	6	6	7	7	5	60	10	6.00	
4	13	12	10	10	13	14	11	10	12	13	118	10	11.8	
5	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
6	23	22	22	23	18	20	21	22	21	20	212	10	21.2	
7														
8														
TOTAL	43	40	38	37	37	40	38	39	40	38	390	10	39.0	

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	7	7	6	6	6	6	6	5	5	6	60	10	6.00
4	12	14	10	13	11	10	13	13	12	11	119	10	11.9
5	0	0	0	0	0	0	0	0	0	0	0	10	0.00
6	0X	23	23	19	23	24	23	21	20	0	176	9	19.6
7													
8													
TOTAL	19	44	39	38	40	40	42	39	37	17	355	10	35.5

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	7	5	6	4	6	6	6	6	7	7	60	10	6.00
4	10	12	15	14	10	12	10	13	11	10	117	10	11.7
5	0	0	0	0	0	0	0	0	0	0	0	10	0.00
6	23	19	22	21	21	20	19	18	21	18	202	10	20.2
7													
8													
TOTAL	40	36	43	39	37	38	35	37	39	35	379	10	37.9

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 18, 2023 at 1215

Date and Time Test Terminated: July 24, 2023 at 1410

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	7	0	6	5	4	5	7	6	5	6	51	10	5.10	
4	10	0	12	13	12	0	11	12	13	11	94	10	9.40	
5	0	0X	0	0	0	0X	0	0	0	0	0	8	0.00	
6	22	X	22	24	20	X	23	21	22	20	174	8	21.8	
7														
8														
TOTAL	39	0	40	42	36	5	41	39	40	37	319	10	31.9	

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	5	7	6	5	6	6	6	6	7	4	58	10	5.80
4	14	0	13	13	14	10	12	13	0	8	97	10	9.70
5	0	0	0	0	0	0	0	0	0X	0	0	9	0.00
6	18	0X	25	20	19	22	20	24	X	0	148	8	18.5
7													
8													
TOTAL	37	7	44	38	39	38	38	43	7	12	303	10	30.3

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	7	7	6	4	6	5	6	6	6	4	57	10	5.70
4	11	6	12	14	12	13	14	0	14	7	103	10	10.3
5	0	0	0	0	0	0	0	0	0	0	0	10	0.00
6	19	0X	19	21	20	23	18	10	21	19	170	9	18.9
7													
8													
TOTAL	37	13	37	39	38	41	38	16	41	30	330	10	33.0

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	0.87500	1.20940	
1	Control	4	0.87500	1.20940	
1	Control	5	1.00000	1.39310	
2	4 %	1	1.00000	1.39310	
2	4 %	2	0.87500	1.20940	
2	4 %	3	1.00000	1.39310	
2	4 %	4	0.75000	1.04720	
2	4 %	5	1.00000	1.39310	
3	5 %	1	1.00000	1.39310	
3	5 %	2	1.00000	1.39310	
3	5 %	3	1.00000	1.39310	
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	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	1.00000	1.39310	
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	1.00000	1.39310	
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 = 0.1648 W = 0.8385 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 %	26.50	16.00	5.00	
3	5 %	32.50	16.00	5.00	
4	7 %	30.00	16.00	5.00	
5	9 %	32.50	16.00	5.00	
6	12 %	32.50	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 = 0.124 W = 0.9172 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 = 5.355 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	0.03564	0.007129	1.38	
Within (Error)	24	0.124	0.005167		
Total	29	0.1597			
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	0.581	0.581			
2	4 %	0.5596	0.5596	0.4707		
3	5 %	0.5952	0.5952	-0.3123		
4	7 %	0.6028	0.6028	-0.4795		
5	9 %	0.656	0.656	-1.65		
6	12 %	0.6476	0.6476	-1.465		
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	0.1073	18.5	0.0214	
3	5 %	5	0.1073	18.5	-0.0142	
4	7 %	5	0.1073	18.5	-0.0218	
5	9 %	5	0.1073	18.5	-0.075	
6	12 %	5	0.1073	18.5	-0.0666	

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 %	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
7 %	8	2	10
Total	18	2	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6. b value is 8. 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 %	8	2	10
Total	18	2	20

Critical Fisher's value (10,10,10) ($\alpha=0.05$) is 6. b value is 8. 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	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.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	4 %	10	1	
2	5 %	10	0	
3	7 %	10	2	
4	9 %	10	2	
5	12 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.2321 D* = 1.821 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 %	104.50	75.00	10.00	
3	5 %	88.50	75.00	10.00	
4	7 %	100.00	75.00	10.00	
5	9 %	91.50	75.00	10.00	
6	12 %	87.50	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	594	118.8	1.051	
Within (Error)	54	6100	113		
Total	59	6694			
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)					

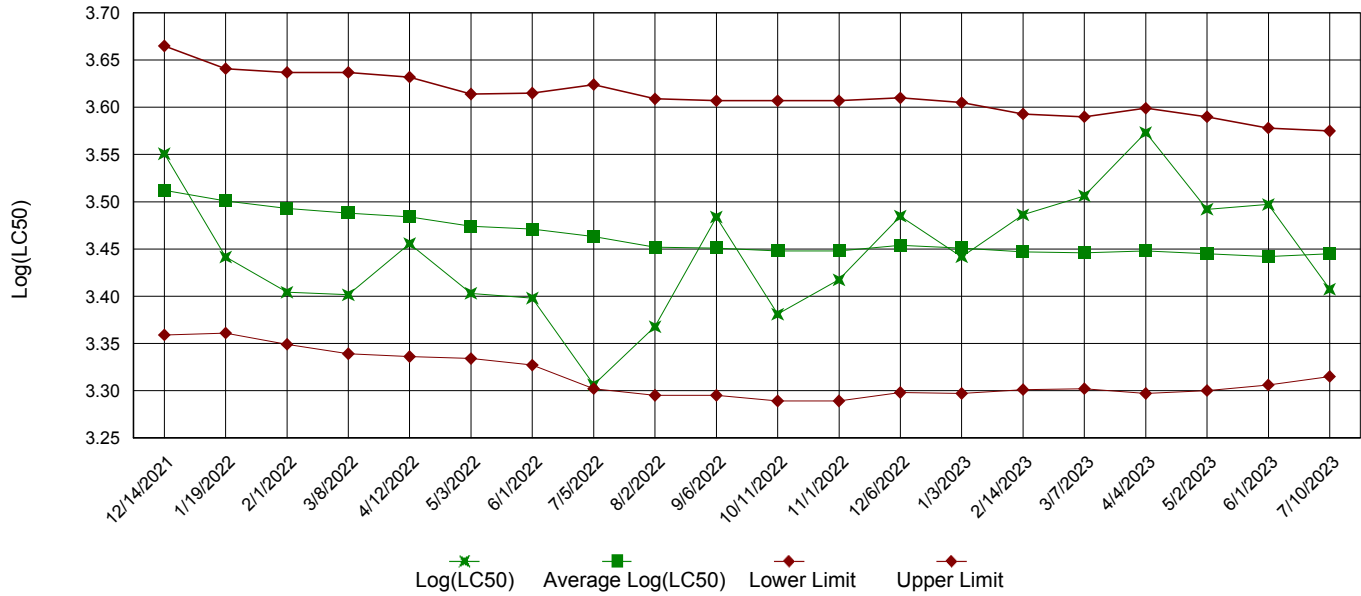
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	39	39			
2	4 %	35.5	35.5	0.7362		
3	5 %	37.9	37.9	0.2314		
4	7 %	31.9	31.9	1.493		
5	9 %	30.3	30.3	1.83		
6	12 %	33	33	1.262		
Dunnett's 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	10.98	28.2	3.5	
3	5 %	10	10.98	28.2	1.1	
4	7 %	10	10.98	28.2	7.1	
5	9 %	10	10.98	28.2	8.7	
6	12 %	10	10.98	28.2	6	

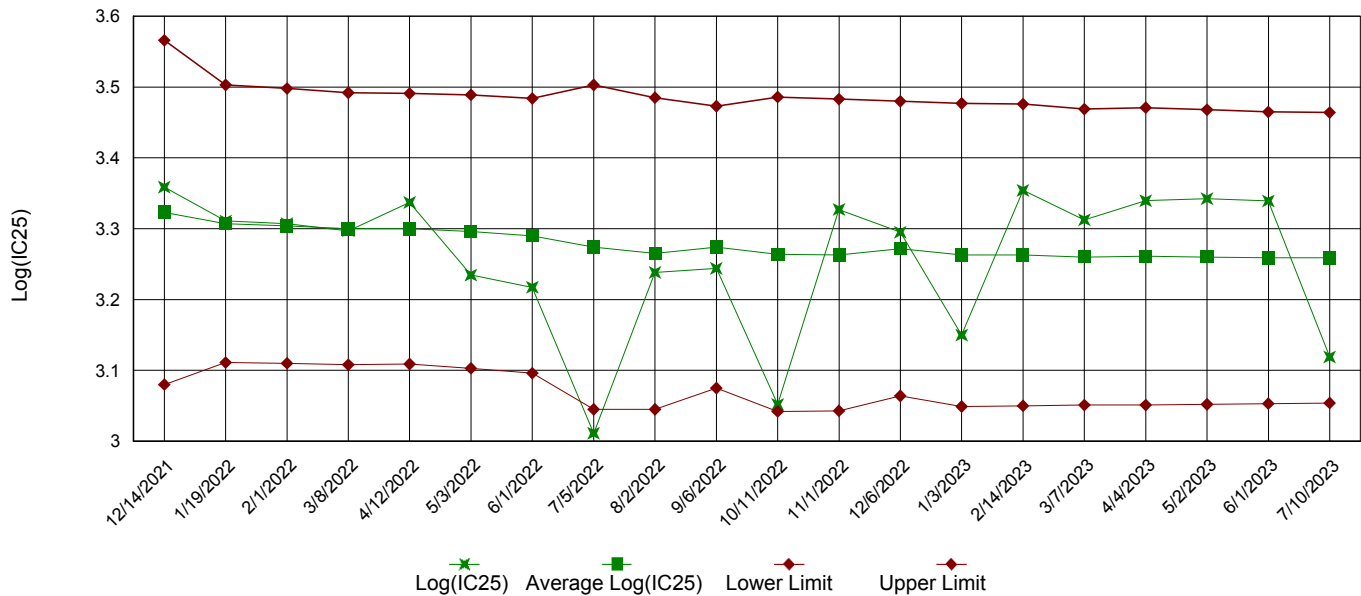
Appendix A3: Test 1000.0

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

LC50 Survival Data

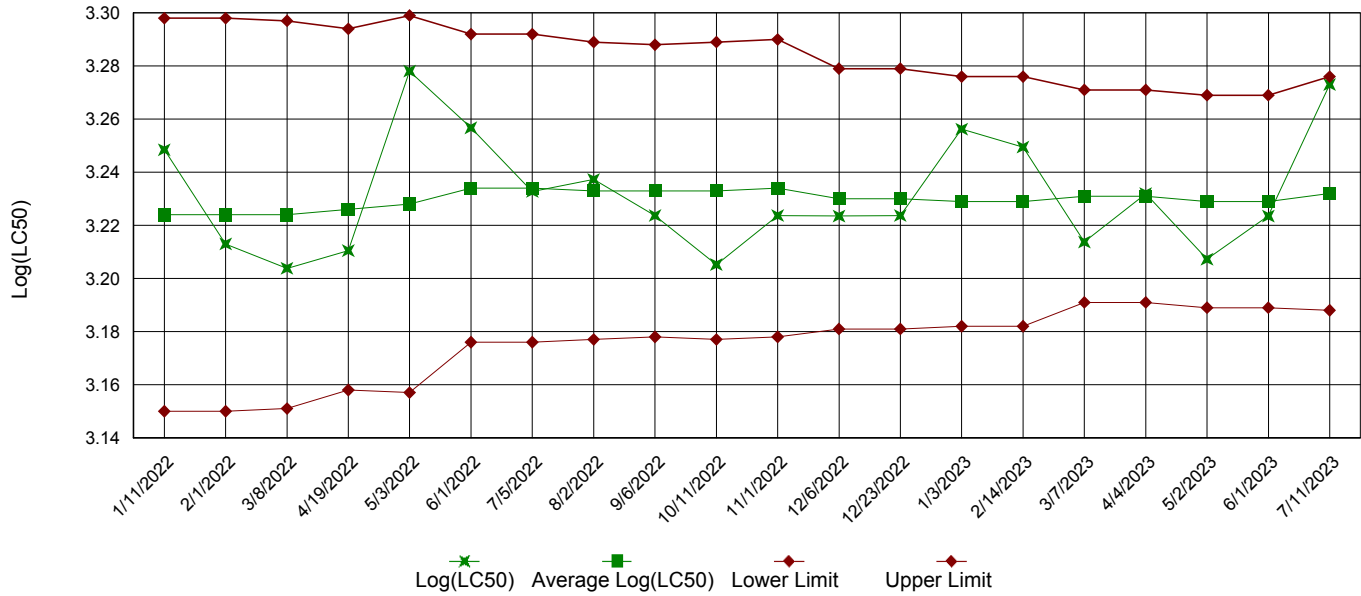


IC25 Growth Data

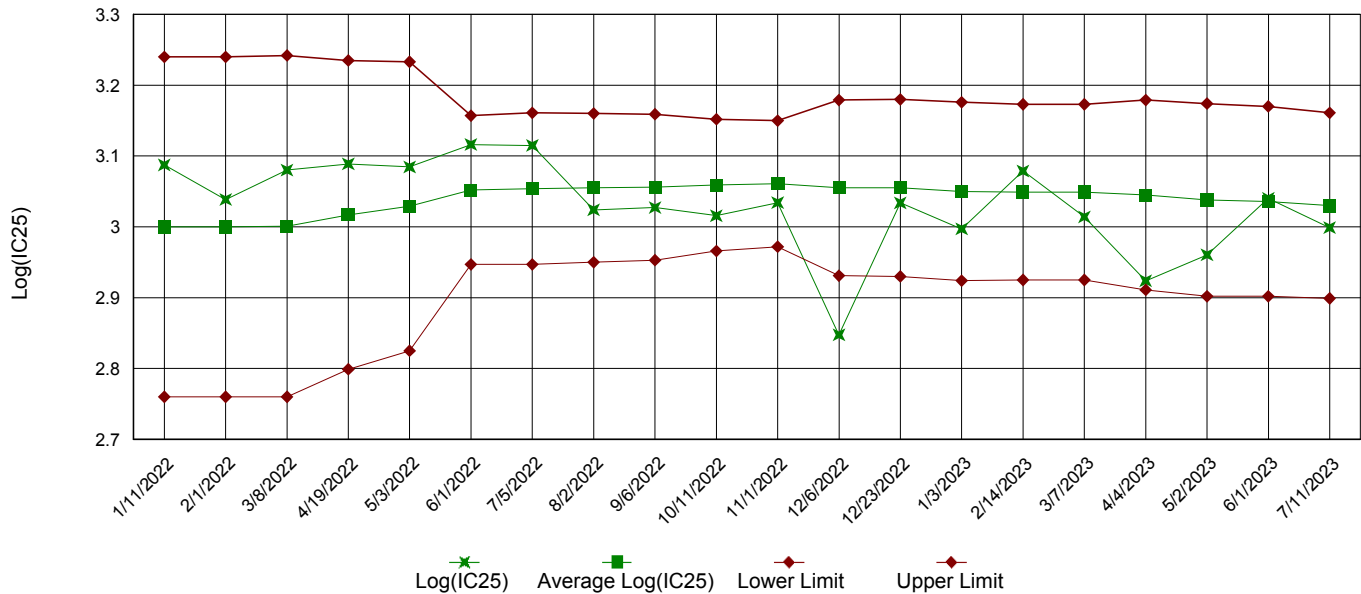


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0
 SUMMARY REPORTING FORMS
 CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
 SURVIVAL AND GROWTH

Permittee: Trumann Water and Sewer Commission

NPDES No.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: July 18, 2023 at 1309

Date and Time Test Terminated: July 25, 2023 at 1400

Dilution water used:

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	87.5	87.5	100	100	100	95.0	7.21
4 %	100	87.5	100	75.0	100	100	100	92.5	12.1
5 %	100	100	100	100	100	100	100	100	0.00
7 %	100	100	100	87.5	100	100	100	97.5	5.73
9 %	100	100	100	100	100	100	100	100	0.00
12 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.650	0.542	0.520	0.555	0.638	0.581	10.2
4 %	0.651	0.578	0.599	0.345	0.625	0.560	22.0
5 %	0.642	0.532	0.550	0.594	0.658	0.595	9.27
7 %	0.549	0.589	0.612	0.566	0.698	0.603	9.67
9 %	0.722	0.626	0.632	0.615	0.685	0.656	6.97
12 %	0.624	0.735	0.629	0.571	0.679	0.648	9.58

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: 10.2 (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: Ms. Lorre Holt
 ANALYST: 280, 343, 357, 358

 Test Initiated: DATE: July 18, 2023 TIME: 1309
 Test Terminated: DATE: July 25, 2023 TIME: 1400

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.1	7.1	7.0	6.9	7.0	7.2	6.6
Final	5.7	5.6	5.8	6.0	5.7	4.8	5.3
pH Initial	7.8	7.8	8.0	7.8	7.9	7.9	8.0
Final	7.6	7.6	7.5	7.6	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	7.2	7.2	7.0	7.1	7.3	7.1	7.0
Final	5.4	5.5	5.6	5.9	5.8	5.0	5.5
pH Initial	7.8	7.8	8.0	7.9	7.9	7.9	7.9
Final	7.6	7.5	7.5	7.6	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	7.2	7.2	7.0	7.2	7.3	7.2	6.9
Final	5.8	5.4	5.6	5.9	6.0	5.2	5.3
pH Initial	7.8	7.9	8.0	7.9	7.9	7.8	7.9
Final	7.6	7.5	7.5	7.6	7.6	7.4	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.2	7.1	7.0	7.0	7.1	7.3	6.7
Final	5.7	5.2	5.5	5.7	5.7	4.9	5.5
pH Initial	7.8	7.9	8.0	7.9	7.9	7.9	8.0
Final	7.6	7.5	7.5	7.6	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	7.1	7.1	7.0	7.1	7.0	7.2	6.6
Final	5.6	5.5	5.2	5.7	5.5	4.9	5.4
pH Initial	7.8	7.8	8.0	7.9	7.9	7.9	8.0
Final	7.6	7.5	7.4	7.5	7.5	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.2	7.1	7.0	7.0	7.4	7.4	6.9
Final	5.6	5.3	5.5	5.7	5.9	5.2	5.2
pH Initial	7.8	7.9	8.0	7.9	8.0	7.9	8.0
Final	7.6	7.5	7.5	7.6	7.6	7.5	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	34	420	0.050	AR0035602 17-JUL-23
120	35	420	0.060	AR0035602 19-JUL-23
120	36	420	<0.05	AR0035602 21-JUL-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	85	310	<0.05	192-3238-A-2
60	85	310	<0.05	192-3445-A-2

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 18, 2023 at 1215

Date and Time Test Terminated: July 24, 2023 at 1410

Dilution water used:

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	90.0	100	80.0	80.0	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	43	19	40	39	37	37
B	40	44	36	0	7	13
C	38	39	43	40	44	37
D	37	38	39	42	38	39
E	37	40	37	36	39	38
F	40	40	38	5	38	41
G	38	42	35	41	38	38
H	39	39	37	39	43	16
I	40	37	39	40	7	41
J	38	17	35	37	12	30
Mean per Adult	39.0	35.5	37.9	31.9	30.3	33.0
Mean per Surviving Adult	39.0	37.3	37.9	39.2	36.1	35.2
CV %	4.68	21.2	6.52	5.05	27.9	22.4

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: 27.9 (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: Ms. Lorre Holt
 ANALYST: 280, 343, 357, 358

Test Initiated: DATE: July 18, 2023 TIME: 1215
 Test Terminated: DATE: July 24, 2023 TIME: 1410

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.1	7.0	6.9	7.0	7.2	6.6
Final	6.7	6.8	7.2	6.4	7.4	6.7	--
pH Initial	7.8	7.8	8.0	7.8	7.9	7.9	8.0
Final	8.1	8.2	8.2	8.1	8.5	8.2	--

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.2	7.0	7.1	7.3	7.1	7.0
Final	6.9	6.7	7.3	6.7	7.3	7.0	--
pH Initial	7.8	7.8	8.0	7.9	7.9	7.9	7.9
Final	8.2	8.3	8.2	8.1	8.5	8.2	--

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.2	7.0	7.2	7.3	7.2	6.9
Final	7.0	6.7	7.3	6.8	7.2	6.9	--
pH Initial	7.8	7.9	8.0	7.9	7.9	7.8	7.9
Final	8.2	8.3	8.2	8.1	8.4	8.2	--

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.1	7.0	7.0	7.1	7.3	6.7
Final	7.0	6.9	7.3	7.0	7.4	6.8	--
pH Initial	7.8	7.9	8.0	7.9	7.9	7.9	8.0
Final	8.2	8.3	8.2	8.2	8.4	8.2	--

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.1	7.0	7.1	7.0	7.2	6.6
Final	6.8	6.8	7.2	6.8	7.4	6.8	--
pH Initial	7.8	7.8	8.0	7.9	7.9	7.9	8.0
Final	8.2	8.3	8.2	8.2	8.5	8.2	--

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.1	7.0	7.0	7.4	7.4	6.9
Final	7.0	6.7	7.3	6.9	7.3	6.6	--
pH Initial	7.8	7.9	8.0	7.9	8.0	7.9	8.0
Final	8.2	8.3	8.2	8.2	8.5	8.3	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	34	420	0.050	AR0035602 17-JUL-23
120	35	420	0.060	AR0035602 19-JUL-23
120	36	420	<0.05	AR0035602 21-JUL-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	85	310	<0.05	192-3238-A-2
60	85	310	<0.05	192-3445-A-2

Chain of Custody Record



Environ



COC No: 192-162-26 1

Page: Page 1 of 1

Job #:

Carrier/Tracking Not(s)
 FedEx

State of Origin

Lab PM
 Lorre Holt

Phone: 870-483-2882

Client Information
 Client Name: Lorre Holt
 PWSID: 435

Company: Trumann Water and Sewer Commission
 Address: 825 Hwy 463 North
 City: Trumann
 State Zip: AR, 72472

Phone: 870-483-2882
 Email: lorre-holt@wsc.yahool.com

Project Name: Final Effluent Flume

Site: Final Effluent Flume

Due Date Requested: [Blank]
 TAT Requested (days): [Blank]
 Compliance Project: Yes No

PO #: [Blank]
 WO #: [Blank]
 Project #: [Blank]
 SSOW#: [Blank]

Sample Identification: AR0035602

Sample Date: 7/13/23
 Sample Time: 8:58 AM
 Sample Type: (C=Comp, G=grab) [Blank]
 Matrix (W=water, S=solid, O=wastewater, BT=tissue, A=Air): Water

Field Filtered Sample (Yes or No): N
 Perform MS/MSD (Yes or No): V

1000_FH_1002_CD

Preservation Codes:
 A - HCl
 B - NaOH
 C - Zn Acetate
 D - Nitric Acid
 E - NaHSO4
 F - MeOH
 G - Amchlor
 H - Ascorbic Acid
 I - Ice
 J - DI Water
 K - EDTA
 L - EDA
 Other: [Blank]

Preservation Codes:
 M - Hexane
 N - None
 O - AsNaO2
 P - Na2OAS
 Q - Na2SO3
 R - Na2SO4
 S - H2SO4
 T - TSP Dodecahydrate
 U - Acetone
 V - MCAA
 W - pH 4-5
 Y - Trizma
 Z - other (specify)

Total Number of containers: 1

LIMS: 274619
 TALS: 3580
 Special Instructions/Note: [Blank]

Possible Hazard Identification
 Non-Hazard
 Flammable
 Skin Irritant
 Poison B
 Unknown
 Radiological

Deliverable Requested I, II, III, IV Other (specify)

Sample Disposal (A fee may be assessed if samples are retained longer than 1 month)
 Return To Client
 Disposal By Lab
 Archive For [Blank] Months

Special Instructions/QC Requirements

Empty Kit Relinquished by: [Blank]

Relinquished by: Lorre Holt
 Date/Time: 7/17/23 / 10:33 AM
 Company: [Blank]

Relinquished by: [Blank]
 Date/Time: [Blank]
 Company: [Blank]

Relinquished by: [Blank]
 Date/Time: [Blank]
 Company: [Blank]

Custody Seals Intact: Yes No
 Custody Seal No: [Blank]

Other Remarks: [Blank]

7813 S302 SW

Client Information
 Client Contact: Ms Lorre Holt
 Company: Trumann Water and Sewer Commission
 Address: 825 Hwy 463 North
 City: Trumann
 State Zip: AR, 72472
 Phone: 870-650-1057 (Tel)
 Email: lorre_holt0201@yahoo.com
 Project Name: Biomonitoring
 Site: FINAL EFFLUENT FLUME

Sampling Information
 Sampler: MORRE HOLT
 Lab PI#: Overbey, John
 E-Mail: john.overbey@et.eurolins.com
 Phone: 870-650-1057
 PMSID: 435

Analysis Requested
 Due Date Requested:
 TAT Requested (days):
 Compliance Project: Yes No
 Purchase Order not required:
 WO #:
 Project #: 19200430
 SSO#:
 Field Filtered Sample (Yes or No)
 PMSID/MSID (Yes or No)
 1000_FH, 1002_CO

Sample Identification
 Sample ID: ARD035602
 Sample Date: 7/19/23
 Sample Time: 8:00 AM
 Sample Type (C=Comp, G=grab): C
 Preservation Code: C
 Matrix (W=water, S=solid, O=wastefall, BT=tissue, A=air): Water
 Water
 Water

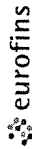
Preservation Codes:
 A - HCL
 B - NaOH
 C - Zn Acetate
 D - Nitric Acid
 E - NaHSO4
 F - MeOH
 G - Amchlor
 H - Ascorbic Acid
 I - Ice
 J - DI Water
 K - EDTA
 L - EDA
 Other:
 M - Hexane
 N - None
 O - Ash/O2
 P - Na2O4S
 Q - Na2SO3
 R - Na2SO3
 S - H2SO4
 T - TSP Dodecahydrate
 U - Acetone
 V - MCAA
 W - pH 4-5
 Y - Trizma
 Z - other (specify)

Special Instructions/Note:
 LIMS: 274619
 TALS: 3580

Chain of Custody
 Relinquished by: Lorre Holt
 Date/Time: 7/19/23 @ 10:30 AM
 Company: Trumann Water and Sewer Commission
 Relinquished by: [Signature]
 Date/Time: 7-20-23 09:55
 Company: [Blank]
 Relinquished by: [Signature]
 Date/Time: [Blank]
 Company: [Blank]
 Custody Seals Intact: Yes No
 Custody Seal No: 03

Disposal Information
 Sample Disposal (A fee may be assessed if samples are retained longer than 1 month)
 Return To Client Disposal By Lab Archive For _____ Months
 Special Instructions/QC Requirements

Chain of Custody Record



78144889 New

Client Information Client Contact: Ms Lorre Holt Company: Trumann Water and Sewer Commission Address: 825 Hwy 463 North City: Trumann State Zip: AR 72472 Phone: 870-650-1057 (Tel) Email: lorre_holt0201@yahoo.com Project Name: Biomonitoring Site: FWA Effluent Flume		Sampler: <u>LORRE HOLT</u> Lab PM: Overbey John Phone: <u>870-650-1057</u> E Mail: john.overbey@et.eurofinsus.com PWSID: <u>435</u>		COC No: 192-1509-206 1 Page: Page 1 of 1 Job #:	
Due Date Requested: _____ TAT Requested (days): _____ Compliance Project: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No PO #: _____ Purchase Order not required: _____ WO #: _____ Project #: 19200430 SSOV#: _____		Analysis Requested: _____ Total Number of Containers: <u>1</u>		Preservation Codes: A - HCL B - NaOH C - Zn Acetate D - Nitric Acid E - NaHSO4 F - MeOH G - Anchlor H - Ascorbic Acid I - Ice J - DI Water K - EDTA L - EDA Other: _____ M - Hexane N - None O - ASN2O2 P - Na2O4S Q - Na2SO3 R - Na2S2O3 S - H2SO4 T - TSP Dodecahydrate U - Acetone V - MCAA W - pH 4-5 Y - Trizma Z - other (specify)	
Sample Identification: <u>AR0035102</u> Sample Date: <u>7/21/23</u> Sample Time: <u>8:30 AM</u> Sample Type (C=Comp, G=grab): <u>C</u> Matrix (W=water, S=solid, O=waste/liq): <u>Water</u> Preservation Code: _____		Field Filtered Sample (Yes or No): <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No Perform MS/MSD (Yes or No): <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No 1000_FH_1002_CD		Special Instructions/Note: <u>LIMS: 274619</u> <u>TALS: 3580</u> <u>Special Instructions/Note</u>	
Possible Hazard Identification <input checked="" type="checkbox"/> Non-Hazard <input type="checkbox"/> Flammable <input type="checkbox"/> Skin Irritant <input type="checkbox"/> Poison B <input type="checkbox"/> Unknown <input type="checkbox"/> Radiological Deliverable Requested I II III IV Other (specify) _____					
Empty Kit Relinquished by _____ Date _____ Time _____ Relinquished by <u>Steve Holt</u> Date <u>7/21/23</u> Time <u>9:30am</u> Company <u>TRUMANN WATER</u> Relinquished by _____ Date _____ Time _____ Company _____ Relinquished by _____ Date _____ Time _____ Company _____					
Sample Disposal (A fee may be assessed if samples are retained longer than 1 month) <input type="checkbox"/> Return To Client <input checked="" type="checkbox"/> Disposal By Lab <input type="checkbox"/> Archive For _____ Months Special Instructions/QC Requirements _____					
Cooler Temperature(s) °C and Other Remarks: <u>9.1</u>					

 **ANALYTICAL REPORT****PREPARED FOR**

Attn: Ms. Lorre Holt
Trumann Water and Sewer Commission
825 Hwy 463 North
Trumann, Arkansas 72472

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JOB DESCRIPTION

Biomonitoring Final Effluent Flume

JOB NUMBER

192-6218-1

Job Notes

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Authorization



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Authorized for release by
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(501)224-5060

Re: Chronic *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
NPDES Permit No. AR0035602 AFIN 56-00047
Control No. 274715-1

This report is the analytical results and supporting information for the samples submitted to Eurofins Arkansas. 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 Laboratory Manager 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. The supporting chemistry data included with this report is intended for accessing the basic water quality of the effluent as required by this test method and is not intended to be utilized for discharge monitoring reports. Test results are summarized below:

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The permit requirement for Survival and Growth is NOEC not less than 9%. The following were concluded from the test:

Survival:	NOEC	LOEC	Growth:	NOEC	LOEC	IC25
	12	>12		12	>12	>12

The sample therefore PASSED the Fathead minnow test.

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The permit requirement for Survival and Reproduction is NOEC not less than 9%. The following were concluded from the test:

Survival:	NOEC	LOEC	Reproduction:	NOEC	LOEC	IC25
	12	>12		12	>12	>12

The sample therefore PASSED the *Ceriodaphnia dubia* test.

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Ceriodaphnia dubia Survival and Reproduction

Ceriodaphnia dubia Chemical Parameters Chart

Appendix (Summary)

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	92.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.688	PASS
Control Growth CV < or = 40% *	10.7	PASS
Growth Minimum Significant Difference 12 to 30%	25.3	PASS
Critical Dilution CV < or = 40% *	24.8	PASS

* EPA Region 6 Requirement

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	35.3	PASS
Control CV < or = 40% per Surviving Female *	8.76	PASS
Reproduction Minimum Significant Difference 13 to 47%	14.0	PASS
Critical Dilution CV < or = 40% *	9.53	PASS

* EPA Region 6 Requirement

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	Sample 3
Dissolved oxygen (mg/l)	8.1	8.5	8.2
pH (standard units)	8.7	8.7	8.7
Alkalinity (mg/l as CaCO3)	130	130	130
Hardness (mg/l as CaCO3)	36	30	30
Conductivity (umhos/cm)	450	460	460
Residual Chlorine (mg/l)	0.050	0.080	<0.05
Ammonia as N (mg/l)	0.35	0.36	0.32

2. Dilution Water Samples:

Analysis	192-6018-A-4	192-6018-A-5	192-6296-A-3
Dissolved oxygen (mg/l)	7.9	8.0	8.0
pH (standard units)	7.9	8.0	8.0
Alkalinity (mg/l as CaCO3)	61	58	61
Hardness (mg/l as CaCO3)	83	85	81
Conductivity (umhos/cm)	320	320	310
Residual Chlorine (mg/l)	<0.05	<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: October 24, 2023 at 1630
Date & Time Test Terminated: October 31, 2023 at 1527
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: October 24, 2023 at 1508
Date & Time Test Terminated: October 30, 2023 at 1640
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 EPA method criteria and CETIS statistical software.

IV. Standard Reference Toxicants

Sodium chloride in synthetic moderately hard water.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on September 05, 2023 at 1530 to September 12, 2023 at 1450
The results were as follows: (Control No. 274660-1.)

Survival LC-50: 2837 mg/l

Growth IC-25: 1943 mg/l

Growth PMSD: 18.9

Ceriodaphnia dubia

A chronic reference test was performed on September 05, 2023 at 1415 to September 11, 2023 at 1345
The results were as follows: (Control No. 274660-2.)

Survival LC-50: mg/l

Reproduction IC-25: mg/l

Reproduction PMSD:

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 24, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 24, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

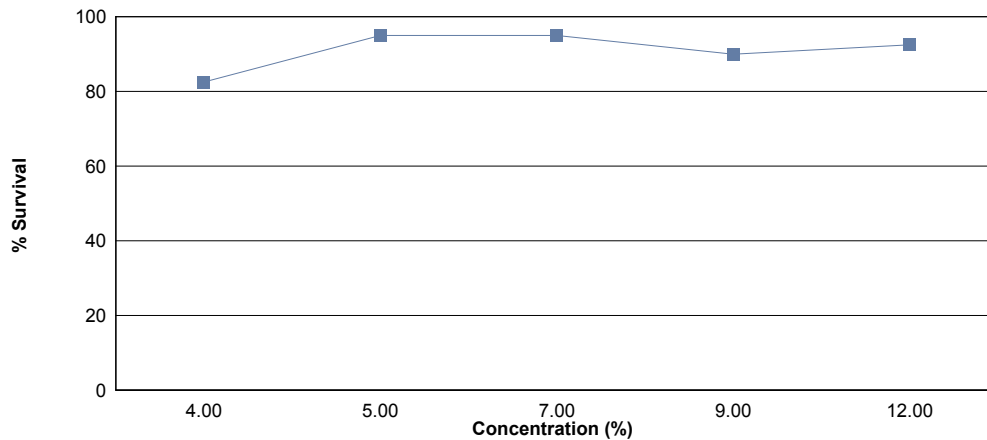
VI. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

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

The test was initiated on October 24, 2023 at 1630 and continued through October 31, 2023 at 1527. 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	92.5	0.636
4 %	82.5	0.616
5 %	95.0	0.657
7 %	95.0	0.770
9 %	90.0	0.691
12 %	92.5	0.693

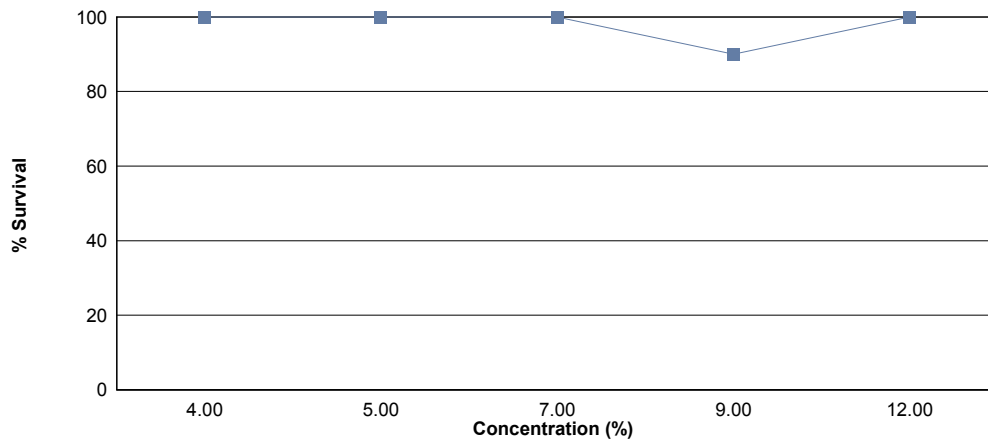
VI. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

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

The test was initiated on October 24, 2023 at 1508 and continued through October 30, 2023 at 1640. 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	35.3
4 %	100	36.8
5 %	100	34.2
7 %	100	37.1
9 %	90.0	31.6
12 %	100	36.4

Appendix (Data): Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 24, 2023 at 1630

Date and Time Test Terminated: October 31, 2023 at 1527

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

Appendix (Data): Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: October 24, 2023 at 1630
 Test Terminated: October 31, 2023 at 1527

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.69562	.70078	0.00516	8	0.645
	B	.70782	.71316	0.00534	8	0.668
	C	.71279	.71703	0.00424	8	0.530
	D	.70616	.71114	0.00498	8	0.622
	E	.69529	.70100	0.00571	8	0.714
4 %	A	.70248	.70801	0.00553	8	0.691
	B	.69882	.70448	0.00566	8	0.708
	C	.70710	.71234	0.00524	8	0.655
	D	.68577	.69100	0.00523	8	0.654
	E	.72173	.72469	0.00296	8	0.370
5 %	A	.71763	.72338	0.00575	8	0.719
	B	.69549	.70076	0.00527	8	0.659
	C	.72253	.72769	0.00516	8	0.645
	D	.71498	.72015	0.00517	8	0.646
	E	.71180	.71672	0.00492	8	0.615
7 %	A	.71162	.71819	0.00657	8	0.821
	B	.69228	.69761	0.00533	8	0.666
	C	.71268	.71853	0.00585	8	0.731
	D	.71815	.72445	0.00630	8	0.788
	E	.71054	.71731	0.00677	8	0.846
9 %	A	.70906	.71465	0.00559	8	0.699
	B	.71265	.71610	0.00345	8	0.431
	C	.68493	.69191	0.00698	8	0.872
	D	.70118	.70767	0.00649	8	0.811
	E	.70267	.70779	0.00512	8	0.640
12 %	A	.72738	.73410	0.00672	8	0.840
	B	.71305	.71854	0.00549	8	0.686
	C	.71998	.72456	0.00458	8	0.572
	D	.70722	.71289	0.00567	8	0.709
	E	.70536	.71064	0.00528	8	0.660

Appendix (Data): Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 24, 2023 at 1508
 Date and Time Test Terminated: October 30, 2023 at 1640

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	7	6	7	5	8	6	6	6	5	4	60	10	6.00	
5	15	10	14	13	13	12	12	11	12	11	123	10	12.3	
6	17	19	18	17	19	14	13	16	17	20	170	10	17.0	
7														
8														
TOTAL	39	35	39	35	40	32	31	33	34	35	353	10	35.3	

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	0	0	0	0	10	0.00	
4	6	5	6	7	6	6	7	7	6	6	62	10	6.20	
5	12	9	13	14	13	13	14	15	14	13	130	10	13.0	
6	19	17	18	21	19	16	15	17	18	16	176	10	17.6	
7														
8														
TOTAL	37	31	37	42	38	35	36	39	38	35	368	10	36.8	

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	0	0	0	0	10	0.00	
4	6	6	5	8	6	5	6	6	5	6	59	10	5.90	
5	12	8	13	13	13	10	11	12	13	11	116	10	11.6	
6	18	16	17	20	18	16	15	19	16	12	167	10	16.7	
7														
8														
TOTAL	36	30	35	41	37	31	32	37	34	29	342	10	34.2	

Appendix (Data): Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 24, 2023 at 1508
 Date and Time Test Terminated: October 30, 2023 at 1640

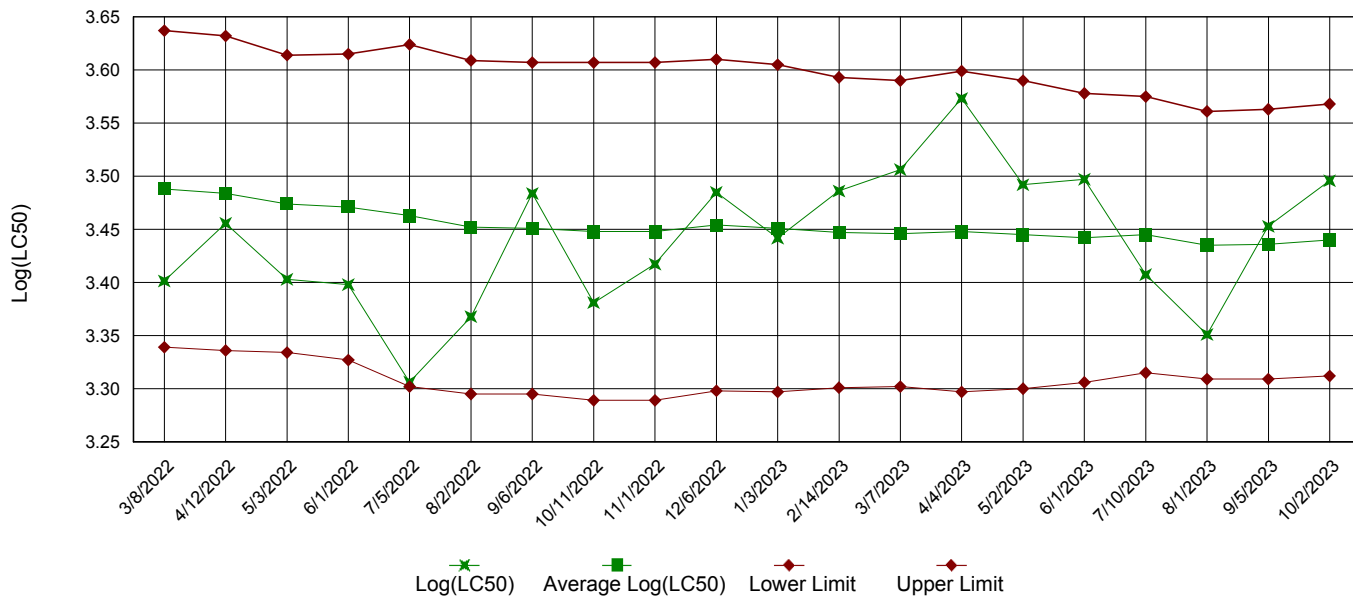
Concentration: 7 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	7	6	7	7	8	7	6	7	6	4	65	10	6.50	
5	12	13	14	13	11	10	14	15	11	14	127	10	12.7	
6	16	18	18	19	18	15	15	21	19	20	179	10	17.9	
7														
8														
TOTAL	35	37	39	39	37	32	35	43	36	38	371	10	37.1	

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	0	0	0	0	10	0.00	
4	5	6X	7	8	7	6	6	6	4	7	62	9	6.89	
5	11	X	14	11	13	10	13	14	12	10	108	9	12.0	
6	15	X	19	15	13	14	18	17	16	19	146	9	16.2	
7														
8														
TOTAL	31	6	40	34	33	30	37	37	32	36	316	10	31.6	

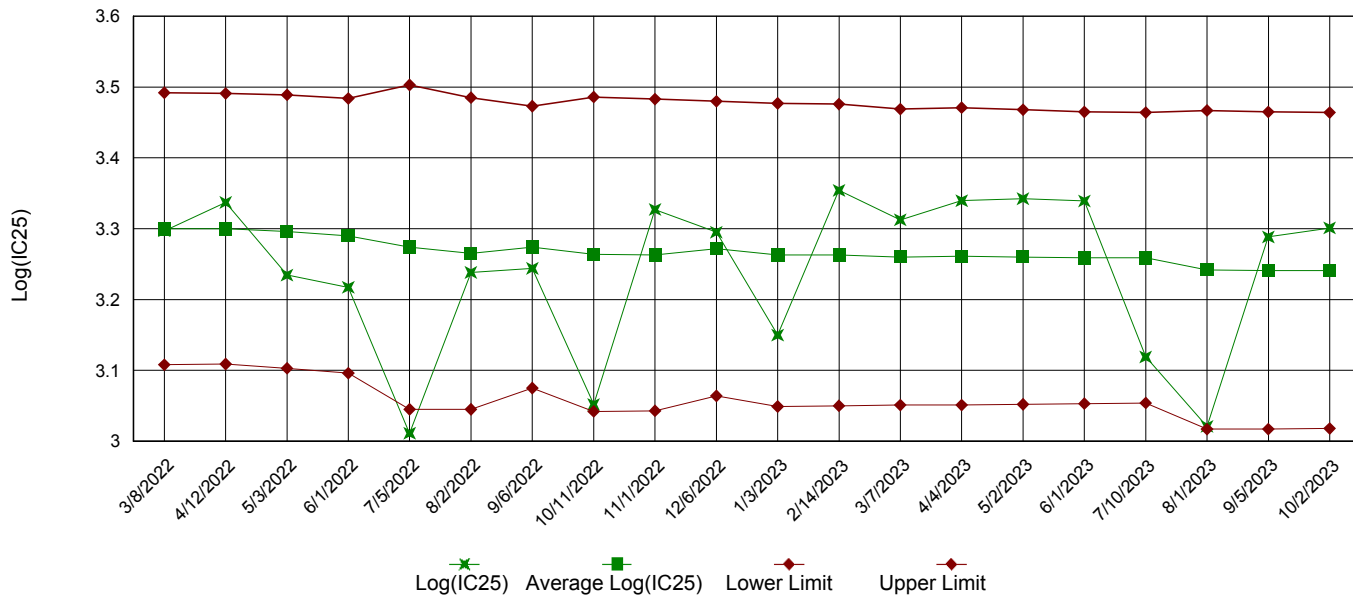
Concentration: 12 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	6	7	6	6	8	6	7	5	6	7	64	10	6.40	
5	12	12	16	14	13	11	12	11	10	11	122	10	12.2	
6	17	20	19	19	17	16	16	18	17	19	178	10	17.8	
7														
8														
TOTAL	35	39	41	39	38	33	35	34	33	37	364	10	36.4	

Appendix (Reference Toxicant): Test 1000.0
Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data

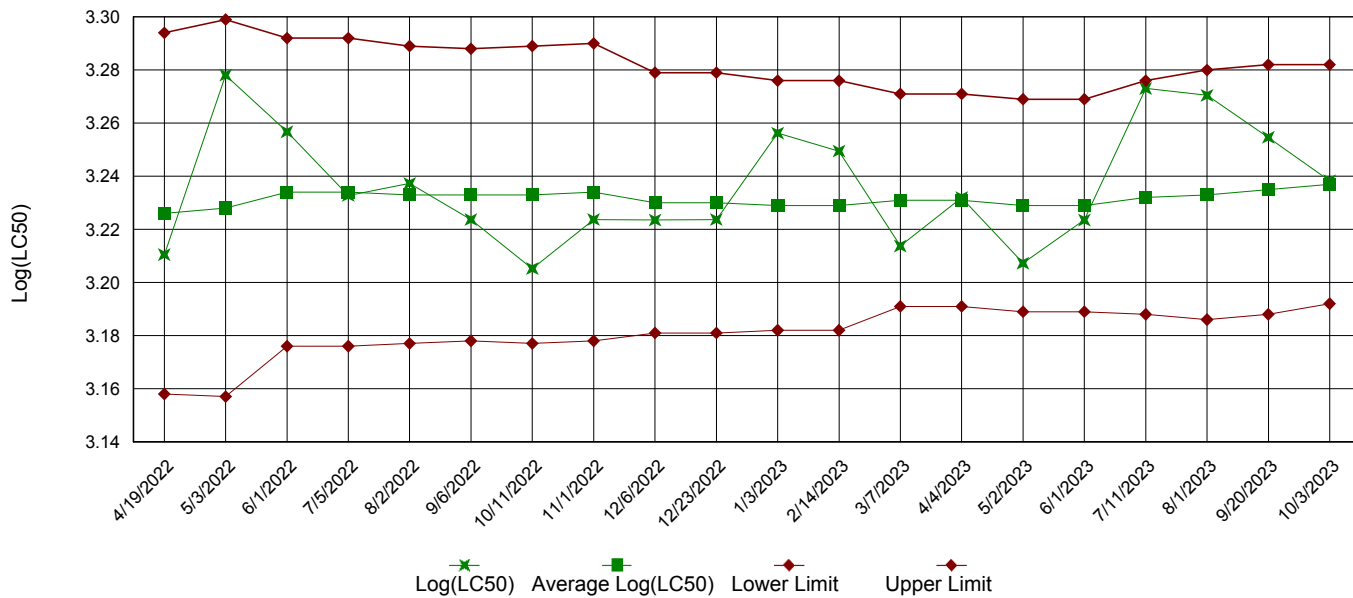


IC25 Growth Data

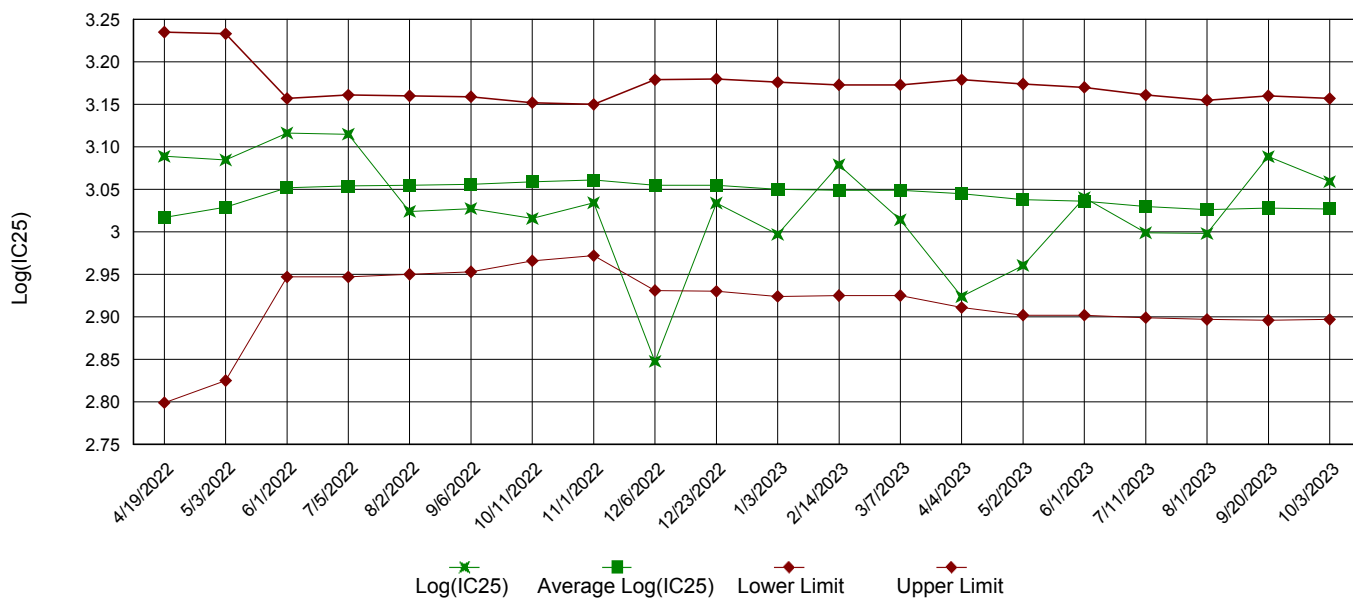


Appendix (Reference Toxicant): Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix (Summary): 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: October 24, 2023 at 1630

Date and Time Test Terminated: October 31, 2023 at 1527

Dilution water used:

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	75.0	100	87.5	100	100	92.5	12.1
4 %	100	87.5	87.5	75.0	62.5	100	90.0	82.5	17.3
5 %	100	87.5	100	100	87.5	100	97.5	95.0	7.21
7 %	100	87.5	87.5	100	100	100	100	95.0	7.21
9 %	87.5	62.5	100	100	100	100	97.5	90.0	18.1
12 %	100	100	75.0	100	87.5	100	97.5	92.5	12.1

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.645	0.668	0.530	0.622	0.714	0.636	10.7
4 %	0.691	0.708	0.655	0.654	0.370	0.616	22.6
5 %	0.719	0.659	0.645	0.646	0.615	0.657	5.84
7 %	0.821	0.666	0.731	0.788	0.846	0.770	9.41
9 %	0.699	0.431	0.872	0.811	0.640	0.691	24.8
12 %	0.840	0.686	0.572	0.709	0.660	0.693	14.0

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix (Summary): 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: 24.8 (TQP6C)
- 10. Sublethality for this test: 12 % (51714 or 51714S)

Appendix (Summary): 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: Ms. Lorre Holt
 ANALYST: GCX6, V6YL, B6YF, QGL9, WK7B

Test Initiated: DATE: October 24, 2023 TIME: 1630
 Test Terminated: DATE: October 31, 2023 TIME: 1527

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	8.0	8.6	8.0	8.3	8.5
Final	6.9	6.8	7.1	6.4	6.8	6.3	6.9
pH Initial	7.9	7.9	8.0	8.0	8.0	7.9	7.9
Final	7.6	7.7	7.6	7.5	7.6	7.4	7.5

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.9	8.0	8.6	8.0	8.4	8.7
Final	7.0	6.9	6.7	6.4	6.3	6.1	6.7
pH Initial	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Final	7.6	7.7	7.6	7.6	7.6	7.4	7.6

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	8.2	8.6	7.7	8.5	8.9
Final	7.0	6.8	6.7	6.7	6.3	6.4	6.6
pH Initial	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Final	7.6	7.7	7.6	7.6	7.6	7.5	7.6

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	8.1	8.0	8.6	8.1	8.3	8.9
Final	7.0	7.1	6.8	6.6	6.4	6.5	6.0
pH Initial	8.0	8.0	8.1	8.0	8.0	8.0	8.0
Final	7.7	7.7	7.6	7.6	7.6	7.5	7.6

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.0	8.0	8.4	8.2	8.3	8.8
Final	7.1	6.6	6.8	6.4	6.2	6.6	6.6
pH Initial	8.0	8.0	8.1	8.0	8.1	8.0	8.0
Final	7.7	7.7	7.7	7.7	7.6	7.5	7.5

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.2	8.6	7.9	8.4	8.8
Final	6.9	6.8	6.8	6.0	6.4	6.4	6.5
pH Initial	8.1	8.1	8.1	8.0	8.1	8.1	8.1
Final	7.7	7.8	7.7	7.6	7.6	7.6	7.6

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	36	450	0.050	AR0035602 23-OCT-23
130	30	460	0.080	AR0035602 25-OCT-23
130	30	460	<0.05	AR0035602 27-OCT-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	83	320	<0.05	192-6018-A-4
58	85	320	<0.05	192-6018-A-5
61	81	310	<0.05	192-6296-A-3

Appendix (Summary): 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: October 24, 2023 at 1508

Date and Time Test Terminated: October 30, 2023 at 1640

Dilution water used:

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	100	100	90.0	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	39	37	36	35	31	35
B	35	31	30	37	6	39
C	39	37	35	39	40	41
D	35	42	41	39	34	39
E	40	38	37	37	33	38
F	32	35	31	32	30	33
G	31	36	32	35	37	35
H	33	39	37	43	37	34
I	34	38	34	36	32	33
J	35	35	29	38	36	37
Mean per Adult	35.3	36.8	34.2	37.1	31.6	36.4
Mean per Surviving Adult	35.3	36.8	34.2	37.1	34.4	36.4
CV %	8.76	7.88	10.9	7.98	9.53	7.68

CV = Coefficient of variation = standard deviation * 100 / mean
 (calculated based on young produced by surviving females)

Appendix (Summary): 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: 9.53 (TQP3B)
- 10. Sublethality for this test: 12 % (51710 or 51710Q)

Appendix (Summary): 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: Ms. Lorre Holt
 ANALYST: GCX6, V6YL, B6YF, QGL9, WK7B

Test Initiated: DATE: October 24, 2023 TIME: 1508
 Test Terminated: DATE: October 30, 2023 TIME: 1640

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	8.0	8.6	8.0	8.3	8.5
Final	8.3	7.4	7.8	7.3	8.2	8.3	--
pH Initial	7.9	7.9	8.0	8.0	8.0	7.9	7.9
Final	8.2	8.1	8.1	8.1	8.1	8.0	--

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.1	7.9	8.0	8.6	8.0	8.4	8.7
Final	8.3	7.8	7.8	7.4	7.9	8.0	--
pH Initial	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Final	8.2	8.1	8.1	8.1	8.1	8.0	--

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	8.2	8.6	7.7	8.5	8.9
Final	8.4	7.8	8.0	7.6	8.1	8.2	--
pH Initial	8.0	8.0	8.0	8.0	8.0	8.0	8.0
Final	8.2	8.1	8.1	8.1	8.2	7.9	--

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.6	8.1	8.0	8.6	8.1	8.3	8.9
Final	8.2	7.7	8.3	7.6	8.3	8.2	--
pH Initial	8.0	8.0	8.1	8.0	8.0	8.0	8.0
Final	8.2	8.1	8.1	8.1	8.2	8.1	--

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.9	8.0	8.0	8.4	8.2	8.3	8.8
Final	8.3	7.0	8.0	7.5	8.2	7.8	--
pH Initial	8.0	8.0	8.1	8.0	8.1	8.0	8.0
Final	8.2	8.1	8.1	8.1	8.2	8.1	--

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.2	8.6	7.9	8.4	8.8
Final	8.3	7.9	8.0	7.5	8.1	8.2	--
pH Initial	8.1	8.1	8.1	8.0	8.1	8.1	8.1
Final	8.2	8.2	8.1	8.1	8.2	8.1	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	36	450	0.050	AR0035602 23-OCT-23
130	30	460	0.080	AR0035602 25-OCT-23
130	30	460	<0.05	AR0035602 27-OCT-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	83	320	<0.05	192-6018-A-4
58	85	320	<0.05	192-6018-A-5
61	81	310	<0.05	192-6296-A-3

CETIS Summary Report

Report Date: 06 Nov-23 14:07 (p 1 of 1)
 Test Code/ID: 274715_FH / 02-5573-1218

1
2
3

Fathead Minnow 7-d Larval Survival and Growth Test

Eurofins Arkansas

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
19-1445-3155	7d Survival Rate	Steel Many-One Rank Sum Test	12	>12	---	20.2%	8.3
13-0036-7706	Mean Dry Biomass-mg	Dunnett Multiple Comparison Test	12	>12	---	25.3%	8.3

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
21-3460-7834	Mean Dry Biomass-mg	Linear Interpolation (ICPIN)	IC25	>12	---	---	<8.3

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
19-1445-3155	7d Survival Rate	Control Resp	0.925	0.8	>>	Yes	Passes Criteria
13-0036-7706	Mean Dry Biomass-mg	Control Resp	0.6358	0.25	>>	Yes	Passes Criteria
21-3460-7834	Mean Dry Biomass-mg	Control Resp	0.6358	0.25	>>	Yes	Passes Criteria
13-0036-7706	Mean Dry Biomass-mg	PMSD	0.2528	0.12	0.3	Yes	Passes Criteria

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.9250	0.7862	1.0640	0.7500	1.0000	0.0500	0.1118	12.09%	0.00%
4		5	0.8250	0.6480	1.0020	0.6250	1.0000	0.0637	0.1425	17.28%	10.81%
5		5	0.9000	0.7300	1.0700	0.7500	1.0000	0.0612	0.1369	15.21%	2.70%
7		5	0.9500	0.8650	1.0350	0.8750	1.0000	0.0306	0.0685	7.21%	-2.70%
9		5	0.9000	0.6976	1.1020	0.6250	1.0000	0.0729	0.1630	18.11%	2.70%
12		5	0.9250	0.7862	1.0640	0.7500	1.0000	0.0500	0.1118	12.09%	0.00%

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	5	0.6358	0.5512	0.7203	0.53	0.7138	0.03044	0.06807	10.71%	0.00%
4		5	0.6155	0.4427	0.7883	0.37	0.7075	0.06225	0.1392	22.61%	3.19%
5		5	0.6568	0.6093	0.7042	0.615	0.7188	0.01709	0.03821	5.82%	-3.30%
7		5	0.7705	0.6805	0.8605	0.6662	0.8463	0.03241	0.07248	9.41%	-21.19%
9		5	0.6907	0.4779	0.9036	0.4312	0.8725	0.07666	0.1714	24.82%	-8.65%
12		5	0.6935	0.5733	0.8137	0.5725	0.84	0.04331	0.09684	13.96%	-9.08%

7d Survival Rate Detail

MD5: EC33DB8E2F89EE26F58D0CA909B8C24C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	0.7500	1.0000	0.8750
4		1.0000	0.8750	0.8750	0.7500	0.6250
5		1.0000	0.7500	1.0000	1.0000	0.7500
7		1.0000	0.8750	0.8750	1.0000	1.0000
9		0.8750	0.6250	1.0000	1.0000	1.0000
12		1.0000	1.0000	0.7500	1.0000	0.8750

Mean Dry Biomass-mg Detail

MD5: 9859C4E4CA67C85F412F27A7C0EFDE8C

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.645	0.6675	0.53	0.6225	0.7138
4		0.6913	0.7075	0.655	0.6537	0.37
5		0.7188	0.6588	0.645	0.6463	0.615
7		0.8213	0.6662	0.7313	0.7875	0.8463
9		0.6988	0.4312	0.8725	0.8112	0.64
12		0.84	0.6862	0.5725	0.7088	0.66

CETIS Analytical Report

Report Date: 06 Nov-23 14:06 (p 1 of 4)
 Test Code/ID: 274715_FH / 02-5573-1218

Fathead Minnow 7-d Larval Survival and Growth Test

Eurofins Arkansas

Analysis ID: 19-1445-3155 Endpoint: 7d Survival Rate CETIS Version: CETIS v2.1.5
 Analyzed: 06 Nov-23 14:03 Analysis: Nonparametric-Control vs Treatments Status Level: 1
 Edit Date: 06 Nov-23 0:00 MD5 Hash: EC33DB8E2F89EE26F58D0CA909B8C24C Editor ID: 004-572-886-9

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Angular (Corrected)	C > T	12	>12	---	8.3	0.1864	20.16%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		4	8	22	16	3	CDF	0.3476	Non-Significant Effect
		5	8	26.5	16	2	CDF	0.7637	Non-Significant Effect
		7	8	28.5	16	2	CDF	0.8883	Non-Significant Effect
		9	8	27	16	2	CDF	0.8003	Non-Significant Effect
		12	8	27.5	16	3	CDF	0.8333	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.925	0.8	>>	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0812855	0.0162571	5	0.5663	0.7249	Non-Significant Effect
Error	0.688995	0.0287081	24			
Total	0.77028		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	2.073	15.09	0.8389	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.8814	0.9031	0.0030	Non-Normal Distribution

7d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.9250	0.7862	1.0000	1.0000	0.7500	1.0000	0.0500	12.09%	0.00%
4		5	0.8250	0.6480	1.0000	0.8750	0.6250	1.0000	0.0637	17.28%	10.81%
5		5	0.9000	0.7300	1.0000	1.0000	0.7500	1.0000	0.0612	15.21%	2.70%
7		5	0.9500	0.8650	1.0000	1.0000	0.8750	1.0000	0.0306	7.21%	-2.70%
9		5	0.9000	0.6976	1.0000	1.0000	0.6250	1.0000	0.0729	18.11%	2.70%
12		5	0.9250	0.7862	1.0000	1.0000	0.7500	1.0000	0.0500	12.09%	0.00%

Angular (Corrected) Transformed Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	1.2870	1.0940	1.4810	1.3930	1.0470	1.3930	0.0697	12.12%	0.00%
4		5	1.1540	0.9274	1.3810	1.2090	0.9117	1.3930	0.0817	15.82%	10.33%
5		5	1.2550	1.0190	1.4900	1.3930	1.0470	1.3930	0.0847	15.10%	2.52%
7		5	1.3200	1.1950	1.4450	1.3930	1.2090	1.3930	0.0450	7.62%	-2.52%
9		5	1.2600	0.9989	1.5210	1.3930	0.9117	1.3930	0.0941	16.69%	2.10%
12		5	1.2870	1.0940	1.4810	1.3930	1.0470	1.3930	0.0697	12.12%	0.00%

7d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.0000	1.0000	0.7500	1.0000	0.8750
4		1.0000	0.8750	0.8750	0.7500	0.6250
5		1.0000	0.7500	1.0000	1.0000	0.7500
7		1.0000	0.8750	0.8750	1.0000	1.0000
9		0.8750	0.6250	1.0000	1.0000	1.0000
12		1.0000	1.0000	0.7500	1.0000	0.8750

Fathead Minnow 7-d Larval Survival and Growth Test

Eurofins Arkansas

Analysis ID: 19-1445-3155 Endpoint: 7d Survival Rate CETIS Version: CETIS v2.1.5
 Analyzed: 06 Nov-23 14:03 Analysis: Nonparametric-Control vs Treatments Status Level: 1
 Edit Date: 06 Nov-23 0:00 MD5 Hash: EC33DB8E2F89EE26F58D0CA909B8C24C Editor ID: 004-572-886-9

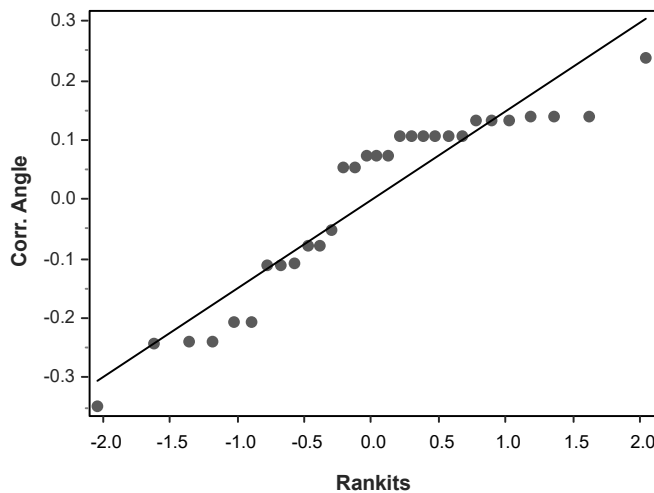
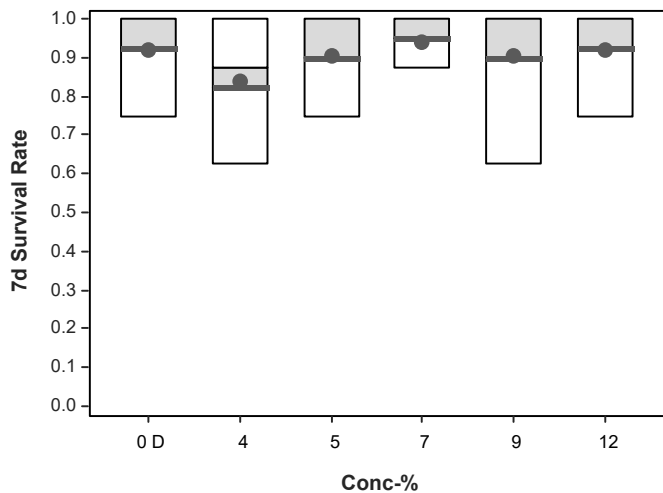
Angular (Corrected) Transformed Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	1.3930	1.3930	1.0470	1.3930	1.2090
4		1.3930	1.2090	1.2090	1.0470	0.9117
5		1.3930	1.0470	1.3930	1.3930	1.0470
7		1.3930	1.2090	1.2090	1.3930	1.3930
9		1.2090	0.9117	1.3930	1.3930	1.3930
12		1.3930	1.3930	1.0470	1.3930	1.2090

7d Survival Rate Binomials

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	8/8	8/8	6/8	8/8	7/8
4		8/8	7/8	7/8	6/8	5/8
5		8/8	6/8	8/8	8/8	6/8
7		8/8	7/8	7/8	8/8	8/8
9		7/8	5/8	8/8	8/8	8/8
12		8/8	8/8	6/8	8/8	7/8

Graphics



Fathead Minnow 7-d Larval Survival and Growth Test

Eurofins Arkansas

Analysis ID: 13-0036-7706	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 06 Nov-23 14:03	Analysis: Parametric-Control vs Treatments	Status Level: 1
Edit Date: 06 Nov-23 0:00	MD5 Hash: 9859C4E4CA67C85F412F27A7C0EFDE8C	Editor ID: 004-572-886-9

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	12	>12	---	8.3	0.1607	25.28%

Dunnett Multiple Comparison Test

Control	vs	Conc-%	df	Test Stat	Critical	MSD	P-Type	P-Value	Decision(α:5%)
Dilution Water		4	8	0.2976	2.362	0.1607	CDF	0.7309	Non-Significant Effect
		5	8	-0.3085	2.362	0.1607	CDF	0.9085	Non-Significant Effect
		7	8	-1.98	2.362	0.1607	CDF	0.9992	Non-Significant Effect
		9	8	-0.8081	2.362	0.1607	CDF	0.9720	Non-Significant Effect
		12	8	-0.8485	2.362	0.1607	CDF	0.9748	Non-Significant Effect

Test Acceptability Criteria

TAC Limits

Attribute	Test Stat	Lower	Upper	Overlap	Decision
Control Resp	0.6358	0.25	>>	Yes	Passes Criteria
PMSD	0.2528	0.12	0.3	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	0.0754848	0.015097	5	1.304	0.2955	Non-Significant Effect
Error	0.277926	0.0115802	24			
Total	0.35341		29			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	9.269	15.09	0.0988	Equal Variances
Distribution	Shapiro-Wilk W Normality Test	0.9349	0.9031	0.0664	Normal Distribution

Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	5	0.6358	0.5512	0.7203	0.645	0.53	0.7138	0.03044	10.71%	0.00%
4		5	0.6155	0.4427	0.7883	0.655	0.37	0.7075	0.06225	22.61%	3.19%
5		5	0.6568	0.6093	0.7042	0.6463	0.615	0.7188	0.01709	5.82%	-3.30%
7		5	0.7705	0.6805	0.8605	0.7875	0.6662	0.8463	0.03241	9.41%	-21.19%
9		5	0.6907	0.4779	0.9036	0.6988	0.4312	0.8725	0.07666	24.82%	-8.65%
12		5	0.6935	0.5733	0.8137	0.6862	0.5725	0.84	0.04331	13.96%	-9.08%

Mean Dry Biomass-mg Detail

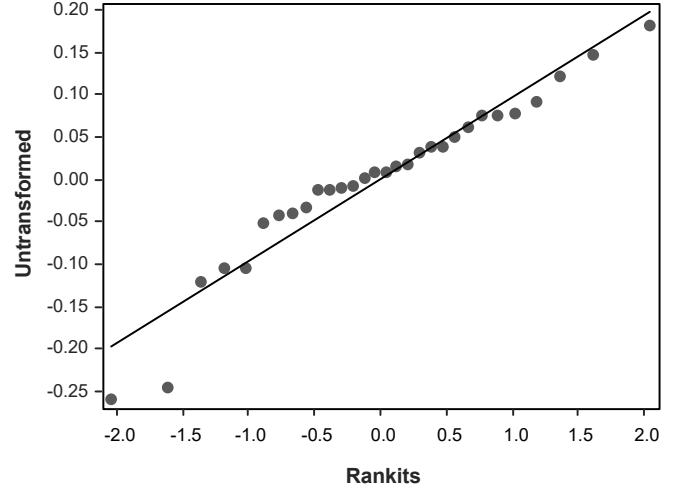
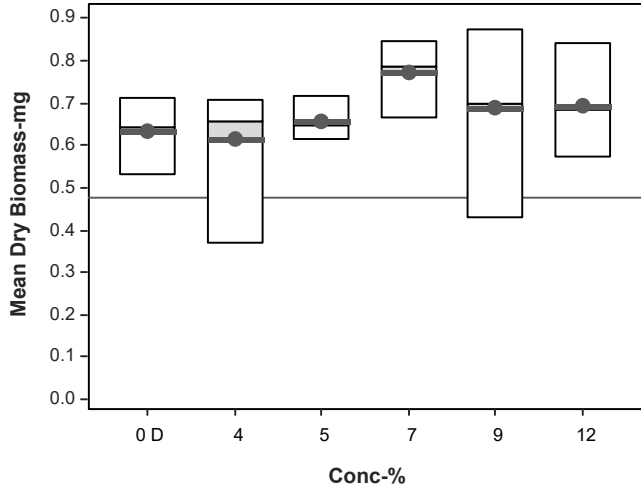
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.645	0.6675	0.53	0.6225	0.7138
4		0.6913	0.7075	0.655	0.6537	0.37
5		0.7188	0.6588	0.645	0.6463	0.615
7		0.8213	0.6662	0.7313	0.7875	0.8463
9		0.6988	0.4312	0.8725	0.8112	0.64
12		0.84	0.6862	0.5725	0.7088	0.66

Fathead Minnow 7-d Larval Survival and Growth Test

Eurofins Arkansas

Analysis ID: 13-0036-7706 Endpoint: Mean Dry Biomass-mg CETIS Version: CETIS v2.1.5
Analyzed: 06 Nov-23 14:03 Analysis: Parametric-Control vs Treatments Status Level: 1
Edit Date: 06 Nov-23 0:00 MD5 Hash: 9859C4E4CA67C85F412F27A7C0EFDE8C Editor ID: 004-572-886-9

Graphics



Fathead Minnow 7-d Larval Survival and Growth Test

Eurofins Arkansas

Analysis ID: 21-3460-7834	Endpoint: Mean Dry Biomass-mg	CETIS Version: CETIS v2.1.5
Analyzed: 06 Nov-23 14:04	Analysis: Linear Interpolation (ICPIN)	Status Level: 1
Edit Date: 06 Nov-23 0:00	MD5 Hash: 9859C4E4CA67C85F412F27A7C0EFDE8C	Editor ID: 004-572-886-9

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	876626	1000	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	0.6358	0.25	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC25	>12	---	---	<8.3	---	---

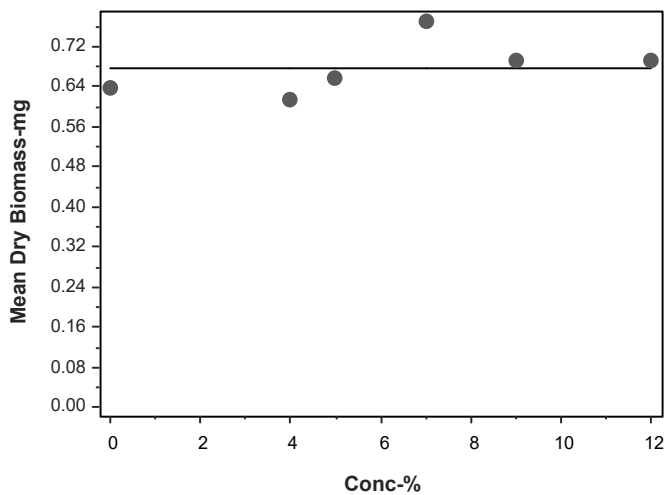
Mean Dry Biomass-mg Summary

Conc-%	Code	Count	Calculated Variate						Isotonic Variate	
			Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	D	5	0.6358	0.645	0.53	0.7138	10.71%	0.00%	0.6771	0.00%
4		5	0.6155	0.655	0.37	0.7075	22.61%	3.19%	0.6771	0.00%
5		5	0.6568	0.6463	0.615	0.7188	5.82%	-3.30%	0.6771	0.00%
7		5	0.7705	0.7875	0.6662	0.8463	9.41%	-21.19%	0.6771	0.00%
9		5	0.6907	0.6988	0.4312	0.8725	24.82%	-8.65%	0.6771	0.00%
12		5	0.6935	0.6862	0.5725	0.84	13.96%	-9.08%	0.6771	0.00%

Mean Dry Biomass-mg Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5
0	D	0.645	0.6675	0.53	0.6225	0.7138
4		0.6913	0.7075	0.655	0.6537	0.37
5		0.7188	0.6588	0.645	0.6463	0.615
7		0.8213	0.6662	0.7313	0.7875	0.8463
9		0.6988	0.4312	0.8725	0.8112	0.64
12		0.84	0.6862	0.5725	0.7088	0.66

Graphics



CETIS Summary Report

Report Date: 06 Nov-23 13:49 (p 1 of 1)
 Test Code/ID: 274715_CD / 09-7725-6681

Ceriodaphnia 7-d Survival and Reproduction Test

Eurofins Arkansas

Multiple Comparison Summary

Analysis ID	Endpoint	Comparison Method	NOEL	LOEL	TOEL	PMSD	TU
03-0111-0338	6d Survival Rate	Fisher Exact/Bonferroni-Holm Test	12	>12	---	---	8.3
18-1052-7296	Reproduction	Steel Many-One Rank Sum Test	12	>12	---	14.0%	8.3

Point Estimate Summary

Analysis ID	Endpoint	Point Estimate Method	Level	%	95% LCL	95% UCL	TU
09-1413-6149	Reproduction	Linear Interpolation (ICPIN)	IC25	>12	---	---	<8.3

Test Acceptability

Analysis ID	Endpoint	Attribute	Test Stat	TAC Limits		Overlap	Decision
				Lower	Upper		
09-1413-6149	Reproduction	Control Resp	35.3	15	>>	Yes	Passes Criteria
18-1052-7296	Reproduction	Control Resp	35.3	15	>>	Yes	Passes Criteria
18-1052-7296	Reproduction	PMSD	0.1396	0.13	0.47	Yes	Passes Criteria

6d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
4		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
5		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
7		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%
9		10	0.9000	0.6738	1.1260	0.0000	1.0000	0.1000	0.3162	35.14%	10.00%
12		10	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.0000	0.00%	0.00%

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Min	Max	Std Err	Std Dev	CV%	%Effect
0	D	10	35.3	33.09	37.51	31	40	0.9781	3.093	8.76%	0.00%
4		10	36.8	34.73	38.87	31	42	0.9165	2.898	7.88%	-4.25%
5		10	34.2	31.53	36.87	29	41	1.181	3.736	10.92%	3.12%
7		10	37.1	34.98	39.22	32	43	0.9363	2.961	7.98%	-5.10%
9		10	31.6	24.8	38.4	6	40	3.008	9.513	30.10%	10.48%
12		10	36.4	34.4	38.4	33	41	0.8844	2.797	7.68%	-3.12%

6d Survival Rate Detail

MD5: 394F06CC8AFBF55B459496DD267D1E3B

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
4		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
5		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
7		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
9		1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

Reproduction Detail

MD5: 05522F7FC9FE47D386D823C80155BC67

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	39	35	39	35	40	32	31	33	34	35
4		37	31	37	42	38	35	36	39	38	35
5		36	30	35	41	37	31	32	37	34	29
7		35	37	39	39	37	32	35	43	36	38
9		31	6	40	34	33	30	37	37	32	36
12		35	39	41	39	38	33	35	34	33	37

Ceriodaphnia 7-d Survival and Reproduction Test

Eurofins Arkansas

Analysis ID: 03-0111-0338 Endpoint: 6d Survival Rate CETIS Version: CETIS v2.1.5
 Analyzed: 06 Nov-23 13:40 Analysis: STP 2xK Contingency Tables Status Level: 1
 Edit Date: 06 Nov-23 0:00 MD5 Hash: 394F06CC8AFBF55B459496DD267D1E3B Editor ID: 004-572-886-9

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units
Untransformed	C > T	12	>12	---	8.3

Fisher Exact/Bonferroni-Holm Test

Control	vs	Conc-%	Test Stat	P-Type	P-Value	Decision(α:5%)
Dilution Water		4	1.0000	Exact	1.0000	Non-Significant Effect
		5	1.0000	Exact	1.0000	Non-Significant Effect
		7	1.0000	Exact	1.0000	Non-Significant Effect
		9	0.5000	Exact	1.0000	Non-Significant Effect
		12	1.0000	Exact	1.0000	Non-Significant Effect

6d Survival Rate Frequencies

Conc-%	Code	NR	R	NR + R	Prop NR	Prop R	%Effect
0	D	10	0	10	1.0000	0.0000	0.00%
4		10	0	10	1.0000	0.0000	0.00%
5		10	0	10	1.0000	0.0000	0.00%
7		10	0	10	1.0000	0.0000	0.00%
9		9	1	10	0.9000	0.1000	10.00%
12		10	0	10	1.0000	0.0000	0.00%

6d Survival Rate Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
4		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
5		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
7		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%
9		10	0.9000	0.6738	1.0000	1.0000	0.0000	1.0000	0.1000	35.14%	10.00%
12		10	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	0.0000	0.00%	0.00%

6d Survival Rate Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
4		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
5		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
7		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
9		1.0000	0.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000
12		1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000	1.0000

6d Survival Rate Binomials

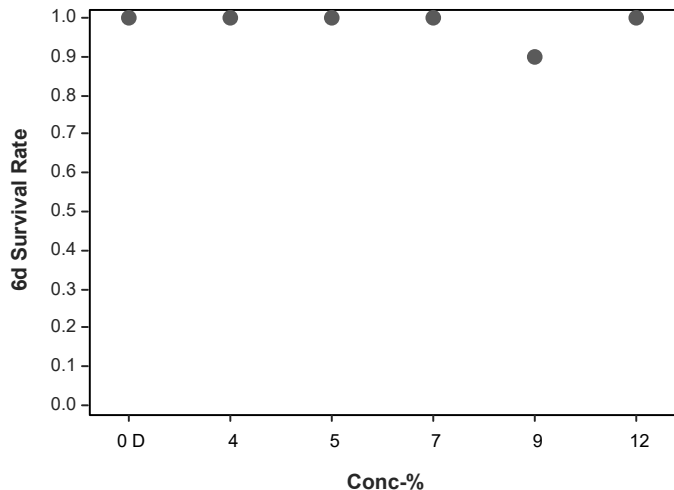
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
4		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
5		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
7		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
9		1/1	0/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1
12		1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1	1/1

Ceriodaphnia 7-d Survival and Reproduction Test

Eurofins Arkansas

Analysis ID: 03-0111-0338 Endpoint: 6d Survival Rate CETIS Version: CETIS v2.1.5
Analyzed: 06 Nov-23 13:40 Analysis: STP 2xK Contingency Tables Status Level: 1
Edit Date: 06 Nov-23 0:00 MD5 Hash: 394F06CC8AFBF55B459496DD267D1E3B Editor ID: 004-572-886-9

Graphics



Ceriodaphnia 7-d Survival and Reproduction Test **Eurofins Arkansas**

Analysis ID: 18-1052-7296	Endpoint: Reproduction	CETIS Version: CETIS v2.1.5
Analyzed: 06 Nov-23 13:40	Analysis: Nonparametric-Control vs Treatments	Status Level: 1
Edit Date: 06 Nov-23 0:00	MD5 Hash: 05522F7FC9FE47D386D823C80155BC67	Editor ID: 004-572-886-9

Data Transform	Alt Hyp	NOEL	LOEL	TOEL	Tox Units	MSDu	PMSD
Untransformed	C > T	12	>12	---	8.3	4.928	13.96%

Steel Many-One Rank Sum Test

Control	vs	Conc-%	df	Test Stat	Critical	Ties	P-Type	P-Value	Decision(α:5%)
Dilution Water		4	18	119.5	75	3	CDF	0.9889	Non-Significant Effect
		5	18	97	75	4	CDF	0.5980	Non-Significant Effect
		7	18	121.5	75	3	CDF	0.9933	Non-Significant Effect
		9	18	93.5	75	5	CDF	0.4745	Non-Significant Effect
		12	18	114.5	75	4	CDF	0.9664	Non-Significant Effect

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	35.3	15	>>	Yes	Passes Criteria
PMSD	0.1396	0.13	0.47	Yes	Passes Criteria

ANOVA Table

Source	Sum Squares	Mean Square	DF	F Stat	P-Value	Decision(α:5%)
Between	215.733	43.1467	5	1.862	0.1163	Non-Significant Effect
Error	1251	23.1667	54			
Total	1466.73		59			

ANOVA Assumptions Tests

Attribute	Test	Test Stat	Critical	P-Value	Decision(α:1%)
Variance	Bartlett Equality of Variance Test	26.75	15.09	6.4E-05	Unequal Variances
Distribution	Shapiro-Wilk W Normality Test	0.7836	0.9459	<1.0E-05	Non-Normal Distribution

Reproduction Summary

Conc-%	Code	Count	Mean	95% LCL	95% UCL	Median	Min	Max	Std Err	CV%	%Effect
0	D	10	35.3	33.09	37.51	35	31	40	0.9781	8.76%	0.00%
4		10	36.8	34.73	38.87	37	31	42	0.9165	7.88%	-4.25%
5		10	34.2	31.53	36.87	34.5	29	41	1.181	10.92%	3.12%
7		10	37.1	34.98	39.22	37	32	43	0.9363	7.98%	-5.10%
9		10	31.6	24.8	38.4	33.5	6	40	3.008	30.10%	10.48%
12		10	36.4	34.4	38.4	36	33	41	0.8844	7.68%	-3.12%

Reproduction Detail

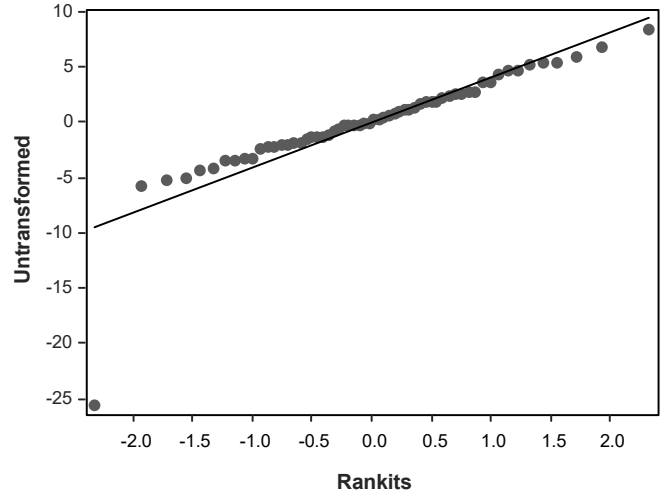
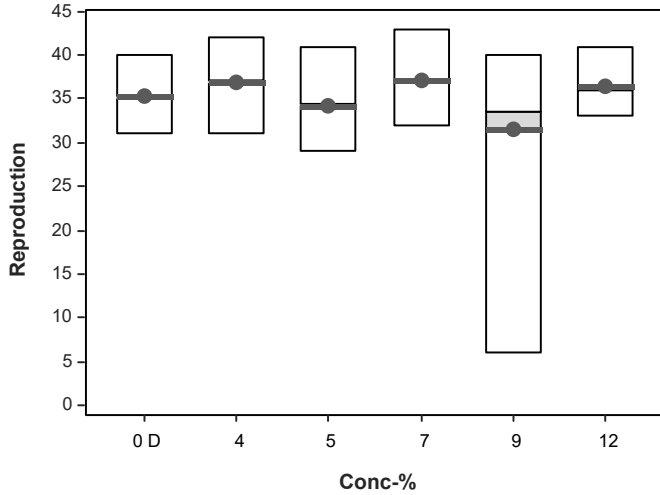
Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	39	35	39	35	40	32	31	33	34	35
4		37	31	37	42	38	35	36	39	38	35
5		36	30	35	41	37	31	32	37	34	29
7		35	37	39	39	37	32	35	43	36	38
9		31	6	40	34	33	30	37	37	32	36
12		35	39	41	39	38	33	35	34	33	37

Ceriodaphnia 7-d Survival and Reproduction Test

Eurofins Arkansas

Analysis ID: 18-1052-7296 Endpoint: Reproduction CETIS Version: CETIS v2.1.5
Analyzed: 06 Nov-23 13:40 Analysis: Nonparametric-Control vs Treatments Status Level: 1
Edit Date: 06 Nov-23 0:00 MD5 Hash: 05522F7FC9FE47D386D823C80155BC67 Editor ID: 004-572-886-9

Graphics



CETIS Analytical Report

Report Date: 06 Nov-23 13:49 (p 1 of 1)
 Test Code/ID: 274715_CD / 09-7725-6681

1
2
3

Ceriodaphnia 7-d Survival and Reproduction Test

Eurofins Arkansas

Analysis ID: 09-1413-6149 Endpoint: Reproduction CETIS Version: CETIS v2.1.5
 Analyzed: 06 Nov-23 13:41 Analysis: Linear Interpolation (ICPIN) Status Level: 1
 Edit Date: 06 Nov-23 0:00 MD5 Hash: 05522F7FC9FE47D386D823C80155BC67 Editor ID: 004-572-886-9

Linear Interpolation Options

X Transform	Y Transform	Seed	Resamples	Exp 95% CL	Method
Linear	Linear	2007593	1000	Yes	Two-Point Interpolation

Test Acceptability Criteria

Attribute	Test Stat	TAC Limits		Overlap	Decision
		Lower	Upper		
Control Resp	35.3	15	>>	Yes	Passes Criteria

Point Estimates

Level	%	95% LCL	95% UCL	Tox Units	95% LCL	95% UCL
IC25	>12	---	---	<8.3	---	---

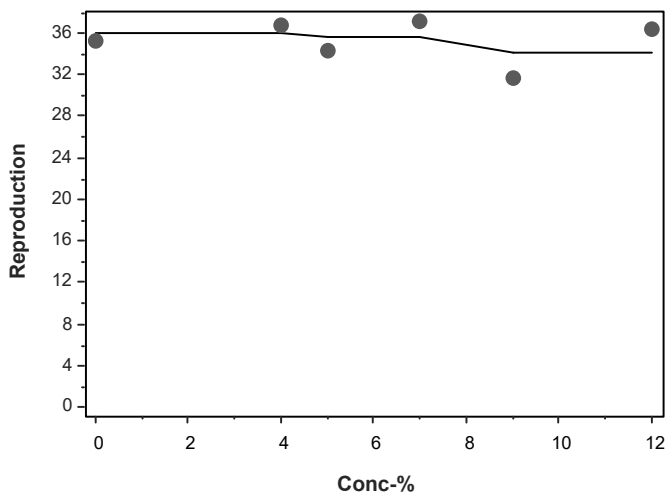
Reproduction Summary

Conc-%	Code	Count	Calculated Variate						Isotonic Variate	
			Mean	Median	Min	Max	CV%	%Effect	Mean	%Effect
0	D	10	35.3	35	31	40	8.76%	0.00%	36.05	0.00%
4		10	36.8	37	31	42	7.88%	-4.25%	36.05	0.00%
5		10	34.2	34.5	29	41	10.92%	3.12%	35.65	1.11%
7		10	37.1	37	32	43	7.98%	-5.10%	35.65	1.11%
9		10	31.6	33.5	6	40	30.10%	10.48%	34	5.69%
12		10	36.4	36	33	41	7.68%	-3.12%	34	5.69%

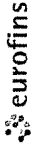
Reproduction Detail

Conc-%	Code	Rep 1	Rep 2	Rep 3	Rep 4	Rep 5	Rep 6	Rep 7	Rep 8	Rep 9	Rep 10
0	D	39	35	39	35	40	32	31	33	34	35
4		37	31	37	42	38	35	36	39	38	35
5		36	30	35	41	37	31	32	37	34	29
7		35	37	39	39	37	32	35	43	36	38
9		31	6	40	34	33	30	37	37	32	36
12		35	39	41	39	38	33	35	34	33	37

Graphics



Chain of Custody Record

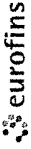


Envi



Client Information Client Name: LORRE HOLT Phone: 870-483-2882 PWSID: 435		Lab PM E-Mail State of Origin: AR		Carrier Tracking No.: 785397550730 COC No: 192-162-26-1 Page 1 of 1 Job #:	
Due Date Requested TAT Requested (days) Compliance Project: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No PO # W/O # Project # SSOW#		Analysis Requested 1000_FH_1002_CD Perform MS/MSD (Yes or No) <input checked="" type="checkbox"/> N Field Filtered Sample (Yes or No) <input checked="" type="checkbox"/> N Total Number of Containers <input checked="" type="checkbox"/> X		Preservation Codes: A - HCL B - NaOH C - Zn Acetate D - Nitric Acid E - NaHSO4 F - MeOH G - Amchlor H - Ascorbic Acid I - Ice J - DI Water K - EDTA L - EDA Other: M - Hexane N - None O - ASNaO2 P - Na2O4S Q - Na2SO3 R - Na2SO3 S - H2SO4 T - TSP Dodecahydrate U - Acetone V - MCAA W - pH 4-5 Y - Trizma Z - other (specify)	
Address: 825 Hwy 463 North City: Trumann State Zip: AR, 72472 Phone: 870-483-2882 Email: lorre-holt@arke.yanac.com Project Name: FINAL ESCULENT FUME Site: AR0035602		Matrix (W=water, S=solid, O=wastewater, A=atmos, Acid) Sample Type (C=Comp, G=grab) Sample Time Sample Date Preservation Code: COMP Matrix: Water Matrix: Water Matrix: Water		Special Instructions/Note: LIMS: 274715 TALS: 6218 DATE/TIME AS: 10/22/23 - 10/23/23 8 AM - 8 AM	
Possible Hazard Identification <input checked="" type="checkbox"/> Non-Hazard <input type="checkbox"/> Flammable <input type="checkbox"/> Skin Irritant <input type="checkbox"/> Poison B <input type="checkbox"/> Unknown <input type="checkbox"/> Radiological Deliverable Requested I II III, IV Other (specify)		Sample Disposal (A fee may be assessed if samples are retained longer than 1 month) <input type="checkbox"/> Return To Client <input checked="" type="checkbox"/> Disposal By Lab <input type="checkbox"/> Archive For _____ Months Special Instructions/QC Requirements		Method of Shipment: Received by: _____ Date/Time: 10/22/23 6:10:00 AM Company: _____ Received by: _____ Date/Time: _____ Company: _____ Received by: D. Blum Date/Time: 10-24-23/0900 Company: _____ Cooler Temperature(s) °C and Other Remarks: 0-4	
Empy Kit Relinquished by Relinquished by: Lorre Holt Date: _____ Relinquished by: _____ Date/Time: 10/22/23 8:08 AM Company: _____ Relinquished by: _____ Date/Time: _____ Company: _____		Custody Seals Intact: <input type="checkbox"/> Yes <input type="checkbox"/> No		Custody Seal No	

Chain of Custody Record



Environment Testing

FXPO

Client Information		Sampler: LORRE HOLT		Lab PM		COC No. 192-162-26 1	
Client Contact: LORRE HOLT		Phone: 870-483-2882		E-Mail: lorre-holt@archieyahnco.com		Page: Page 1 of 1	
Company: Trumann Water and Sewer Commission		Address: 825 Hwy 463 North		City: Trumann		Job #: 10262010365	
State Zip: AR, 72472		Compliance Project: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		PO #: 870-483-2882		Preservation Codes:	
Phone: 870-483-2882		Due Date Requested: 10/26/23		TAT Requested (days): 435		A - HCL B - NaOH C - Zn Acetate D - Nitric Acid E - NaHSO4 F - MeOH G - Amchlor H - Ascorbic Acid I - Ice J - DI Water K - EDTA L - EDA Other:	
Email: lorre-holt@archieyahnco.com		Project #: AR0035602		Sample Date: 10/26/23		M - Hexane N - None O - AsNaO2 P - Na2O4S Q - Na2SO3 R - Na2SO4 S - H2SO4 T - TSP Dodecahydrate U - Acetone V - MCAA W - pH 4-5 Y - Trizma Z - other (specify)	
Site: FINAL Effluent Flume		Sample Type (C=Comp, G=grab): Comp		Sample Time: 2:00 PM		Total Number of containers: 1 Special Instructions/Note:	
Matrix (Water, Solid, or Tissue, A=Air): Water		Field Filtered Sample (Yes or No): <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		Perform MS/MSD (Yes or No): <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No		1000_FH, 1002_CD 10262010365	
Sample Identification: AR0035602		Sample Date: 10/26/23		Sample Time: 2:00 PM		Preservation Code: Water Matrix: Water Sample Type: Water Sample Time: Water	
<input checked="" type="checkbox"/> Non-Hazard <input type="checkbox"/> Flammable <input type="checkbox"/> Skin Irritant <input type="checkbox"/> Poison B <input type="checkbox"/> Unknown <input type="checkbox"/> Radiological <input type="checkbox"/> Deliverable Requested I, II, III, IV Other (specify)		<input type="checkbox"/> Return To Client <input checked="" type="checkbox"/> Disposal By Lab <input type="checkbox"/> Archive For _____ Months		Sample Disposal (A fee may be assessed if samples are retained longer than 1 month)		Special Instructions/QC Requirements	
Empty Kit Relinquished by: LORRE HOLT		Date: 10/25/23		Time: 9:40 AM		Method of Shipment:	
Relinquished by: LORRE HOLT		Date/Time: 10/25/23 9:40 AM		Company:		Received by:	
Relinquished by:		Date/Time:		Company:		Received by:	
Relinquished by:		Date/Time:		Company:		Received by:	
Custody Seals Intact: <input type="checkbox"/> Yes <input type="checkbox"/> No		Custody Seal No:		Cooler Temperature(s) °C and Other Remarks: 0-1		Date/Time: 10/26/23/0855 Company:	

Chain of Custody Record



Environment Testing

FXPO

Client Information Client Name: LORRE HOIT Phone: 870-483-2882 E-Mail: lorre@bolt2000.com		Lab PM: LORRE HOIT Phone: 870-483-2882 E-Mail: lorre@bolt2000.com		CCO No: 192-162-26 1 Page: Page 1 of 1 Job #:	
Company: Trumann Water and Sewer Commission Address: 825 Hwy 463 North City: Trumann State: AR, 72472 Phone: 870-483-2882 Email: lorre@bolt2000.com Project Name: FINAL ESSENT FUME		PWSID: 435 Due Date Requested: TAT Requested (days): Compliance Project: <input checked="" type="checkbox"/> Yes <input type="checkbox"/> No PO #: IVO #: Project #: SSOV#:		Analysis Requested: Preservation Codes: A - HCL B - NaOH C - Zn Acetate D - Nitric Acid E - NaHSO4 F - MeOH G - Amchlor H - Ascorbic Acid I - Ice J - DI Water K - EDTA L - EDA Other:	
Sample Identification: AR0035602		Sample Date: 10/28/23 Sample Time: 8:00 AM Sample Type: Comp Matrix: Water		Field Filtered Sample (Yes or No): <input checked="" type="checkbox"/> Perform MS/MSD (Yes or No): <input checked="" type="checkbox"/> Total Number of Containers: 1 Special Instructions/Note:	
Possible Hazard Identification <input checked="" type="checkbox"/> Non-Hazard <input type="checkbox"/> Flammable <input type="checkbox"/> Skin Irritant <input type="checkbox"/> Poison B <input type="checkbox"/> Unknown <input type="checkbox"/> Radiological					
Deliverable Requested I, II, III, IV Other (specify)					
Empty Kit Relinquished by: LORRE HOIT Date: 10/27/2023 9:45 AM					
Relinquished by: LORRE HOIT Date: 10/27/2023 9:45 AM					
Relinquished by: LORRE HOIT Date: 10/28/23 08:34					
Custody Seals Intact. <input type="checkbox"/> Yes <input type="checkbox"/> No					

