



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:

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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. AR0035602 AFIN 56-00047

Dear Mr. Scotty Jones:

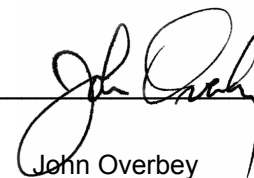
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire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

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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	0.536	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.

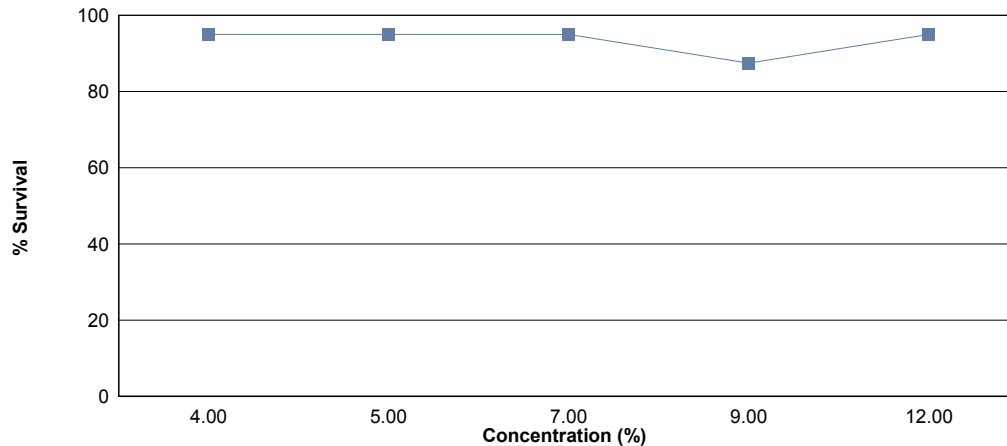
Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

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

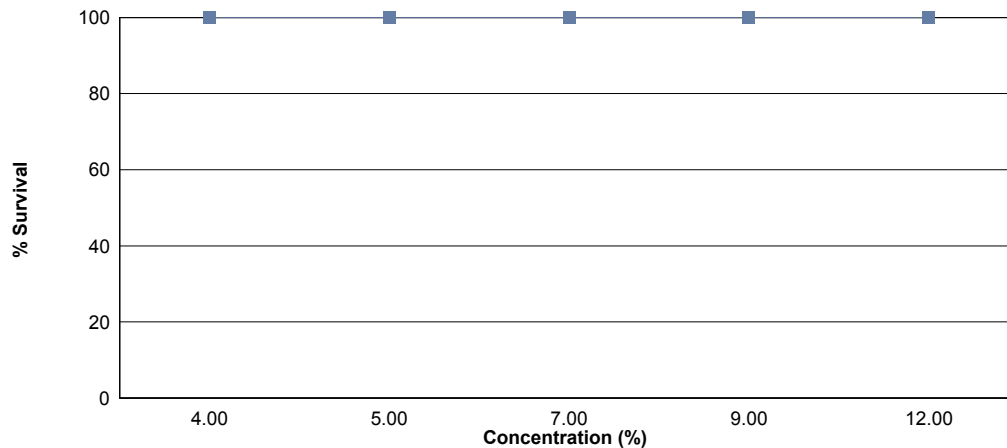
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 style="text-align: center;"> D = 0.2096 D* = 1.644 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 %	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

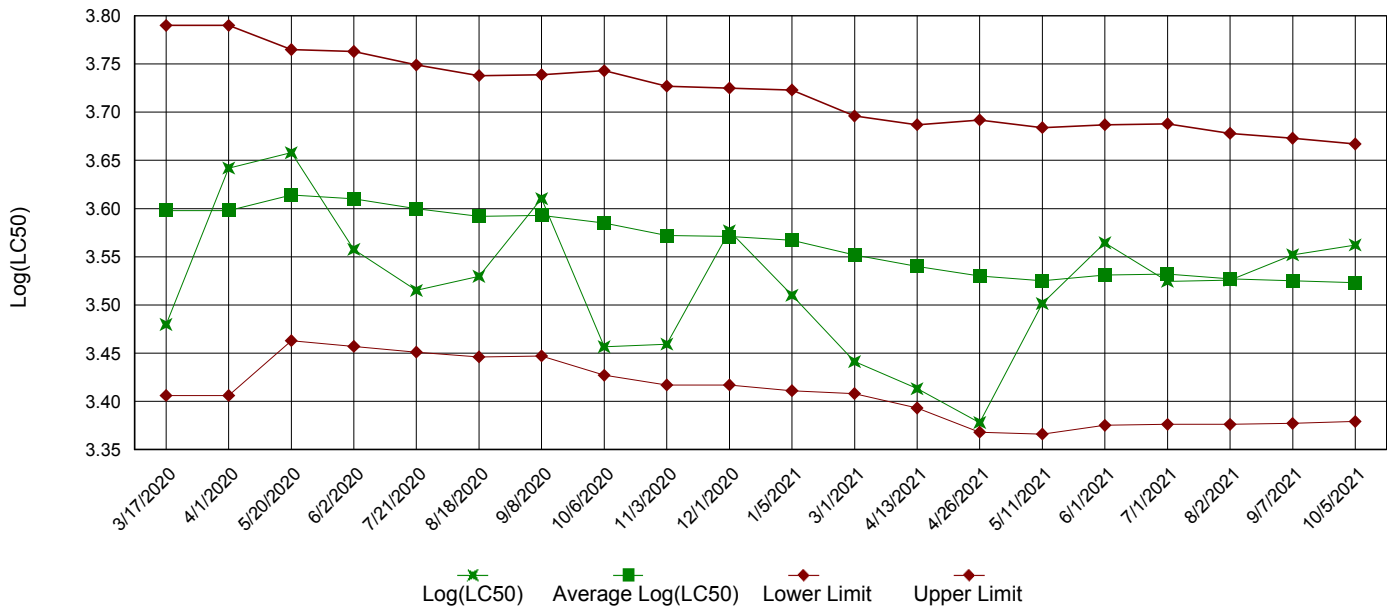
NOEC = <4 %

LOEC = 4 %

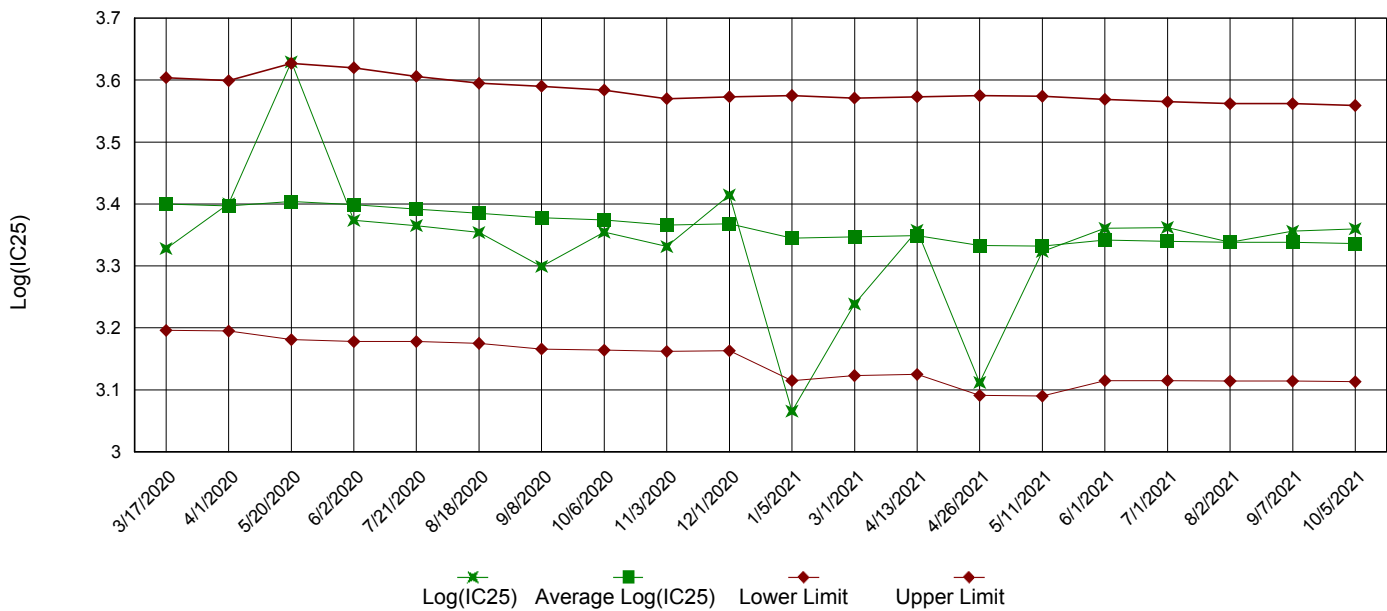
Appendix A3: Test 1000.0

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

LC50 Survival Data

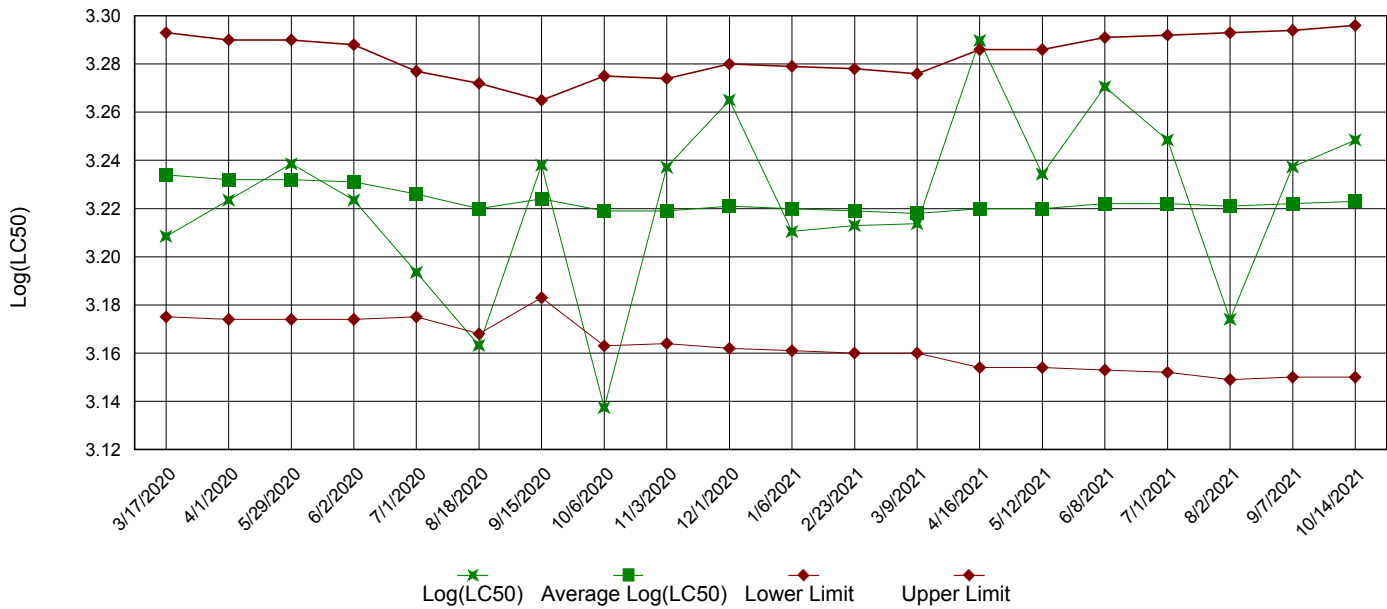


IC25 Growth Data

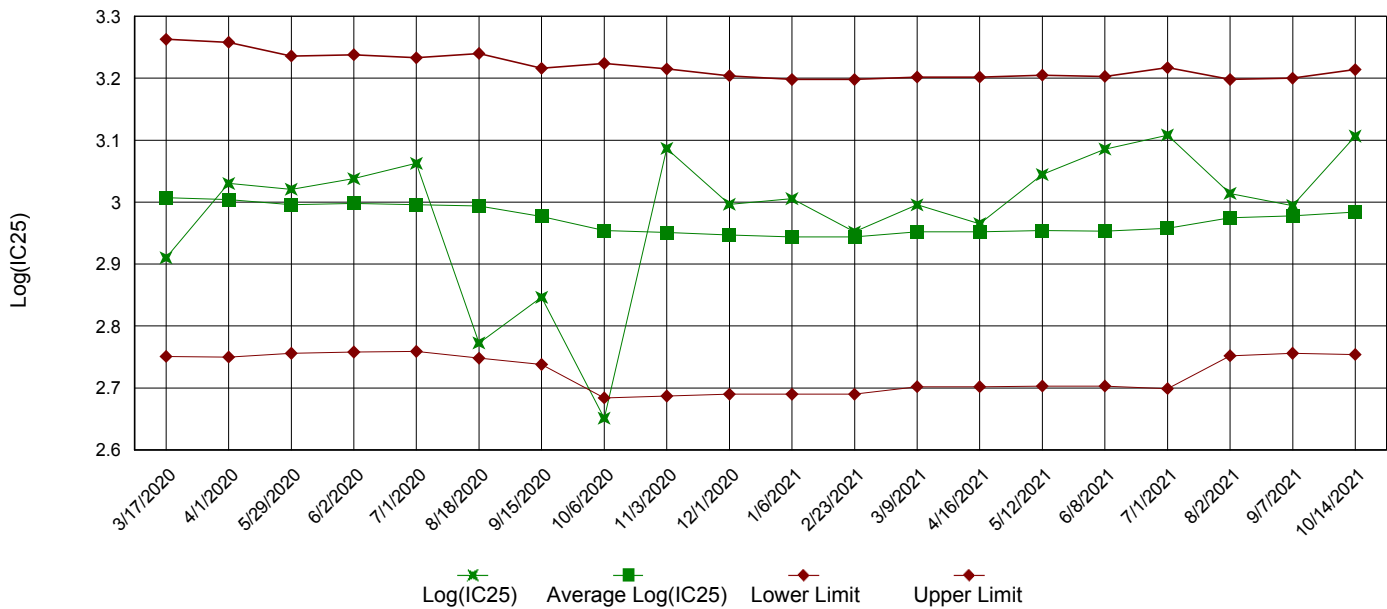


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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		WATER		Carrier: FX	
Sampled By: LORRE HOLT		COMPOUND		Received Temperature °C	
AIC No. AR0035602		GRA B		0.2	
Date/Time Collected: 10/24/01 - 10:35 AM		V ✓		Remarks	
Date/Time Collected: 8:00 AM - 8:20 AM					
Container Type: NO					
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)		Date/Time Received		Date/Time	
NORMAL or EXPEDITED IN ___ DAYS		10/25/01 12:10		10-26-01	
Expedited results requested by:		By: LORRE HOLT		By: D. BROWN	
Who should AIC contact with questions: LORRE HOLT		Date/Time Relinquished		Date/Time	
Phone: 810-483-8832 Fax: 810-483-10525		By:		10-26-01 0930	
Report Attention to: LORRE HOLT		Comments:			
Report Address to: 704 Hwy 463 N					
TRUMANN, AR 72472					

TRM 2853 2328 5259
0201

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>Fedex</u>	
Sampled By: <u>LORRE HOLT</u>		COMPS		1		Received Temperature °C <u>0</u>	
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 <u>②</u>	
Container Type: <u>NO</u>		PRESERVATIVE: <u>NO</u>				Buffer:	
G = Glass NO = none		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) <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: <u>LORRE HOLT</u>		Date/Time Relinquished		Date/Time Received	
Who should AIC contact with questions: <u>LORRE HOLT</u>		By: <u>LORRE HOLT</u>		Received in Lab		Date/Time	
Phone: <u>810-483-2832</u> Fax: <u>810-483-10525</u>		Comments: <u>2854 1330 114</u>		By: <u>[Signature]</u>		Date/Time	
Report Attention to: <u>LORRE HOLT</u>						Date/Time	
Report Address to: <u>TRUMANN, AR 72472</u>						Date/Time	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Trumann Water Works		PO No.		No of BOTTLES		Analyses Requested		AIC Control No: 259819	
Project Reference: AR0035602		Sample Matrix		WATER		BIDMONT, BIRMINGHAM - CHRONIC CDV FH		AIC Proposal No:	
Project Manager: Scotty Jones		G R A B		C O M P				Carrier: FedEx	
Sample Identification: 3 AR0035602		Date/Time Collected: 8/21/02 8:00 AM - 8:00 PM		Date/Time Relinquished: 10/22/10 10:47 AM				Received Temperature °C: 0.1	
By: LORRE HOLT		Container Type: P		Date/Time Relinquished: 10/22/10 10:47 AM				Remarks:	
AIC No. 3		Preservative: N/D		By: Lorreholt				Field pH calibration on @ Buffer:	
Reference: AR0035602		NO = none		By: Lorreholt				T = Sodium Thiosulfate	
Manager: Scotty Jones		S = Sulfuric acid pH2		By: Lorreholt				Z = Zinc acetate	
Sampled: LORRE HOLT		V = VOA vials		By: Lorreholt				Received in Lab By: NK358	
By: LORRE HOLT		N = Nitric acid pH2		By: Lorreholt				Date/Time: 30 Oct 21 0840	
AIC No. 3		Relinquished		By: Lorreholt					
Reference: AR0035602		Comments:		By: Lorreholt					
Manager: Scotty Jones		Time Requested: (Please circle)		By: Lorreholt					
Sampled: LORRE HOLT		NORMAL or EXPEDITED IN _____ DAYS		By: Lorreholt					
By: LORRE HOLT		Expedited results requested by:		By: Lorreholt					
AIC No. 3		Who should AIC contact with questions: LORRE HOLT		By: Lorreholt					
Reference: AR0035602		Phone: 870-483-8832		By: Lorreholt					
Manager: Scotty Jones		Fax: 870-483-1653		By: Lorreholt					
Sampled: LORRE HOLT		Report Attention to: LORRE HOLT		By: Lorreholt					
By: LORRE HOLT		Report Address to: 704 Hwy 463 N Trumann, AR 72472		By: Lorreholt					