



December 30, 2021

Biomonitoring Testing  
for  
AR0035602

Control No. 261164-1

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)  
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:

**Using best professional judgment, Dunnett's procedure was utilized to evaluate Fathead growth. The statistically significant difference noted at the 7% effluent concentration for growth 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 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.**

**AMERICAN INTERPLEX CORPORATION**

  
\_\_\_\_\_  
John Overbey  
Chief Operating Officer

PDF cc: Trumann Water and Sewer Commission  
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Trumann Water and Sewer Commission  
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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	0.560	PASS
Control Growth CV < or = 40%	6.63	PASS
Growth Minimum Significant Difference 12 to 30%	20.6	PASS
Critical Dilution CV < or = 40%	12.2	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0035602 AFIN 56-00047
2. Test Requirements:  
Test Method 1000.0

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)	6.7	7.0	6.6
pH (standard units)	8.1	8.0	8.0
Alkalinity (mg/l as CaCO <sub>3</sub> )	130	130	130
Hardness (mg/l as CaCO <sub>3</sub> )	31	29	27
Conductivity (umhos/cm)	450	460	460
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	<0.1	6.4

2. Dilution Water Samples:  
Moderately Hard

Analysis	260890-1	261120-1
Dissolved oxygen (mg/l)	6.3	6.5
pH (standard units)	7.9	7.9
Alkalinity (mg/l as CaCO <sub>3</sub> )	61	62
Hardness (mg/l as CaCO <sub>3</sub> )	84	83
Conductivity (umhos/cm)	300	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 Method 1000.0, Fathead Minnow Survival and Growth.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

*Pimephales promelas* (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: December 14, 2021 at 1513

Date & Time Test Terminated: December 21, 2021 at 1500

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

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*

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. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

#### 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 November 23, 2021 at 1330 to November 30, 2021 at 1347

The results were as follows: (Control No. 260579-1.)

Survival LC-50: 2232 mg/l

Growth IC-25: 1282 mg/l

Growth PMSD: 0

#### V. Organism History

##### *Pimephales promelas* (Fathead minnow)

Date: December 14, 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

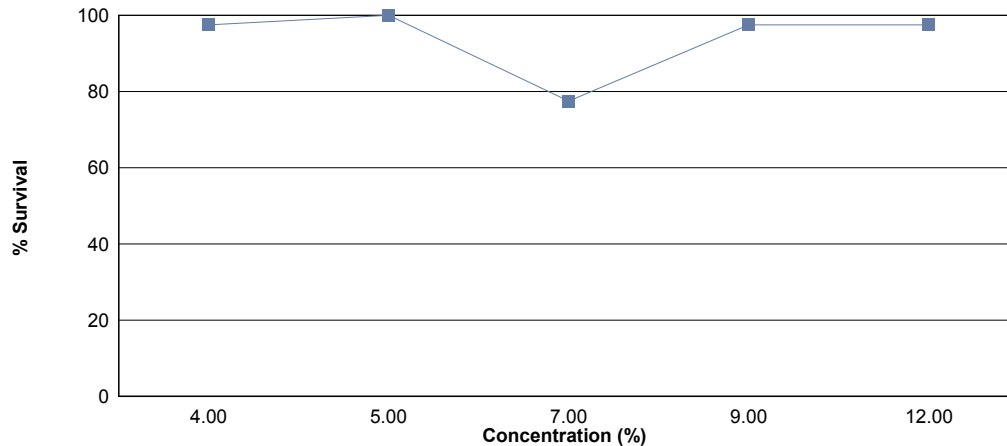
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 December 14, 2021 at 1513 and continued through December 21, 2021 at 1500. 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	0.546
4 %	97.5	0.473
5 %	100	0.490
7 %	77.5	0.379 *
9 %	97.5	0.453
12 %	97.5	0.459

\*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.

## Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: December 14, 2021 at 1513

Date and Time Test Terminated: December 21, 2021 at 1500

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	7	7	7	7
	E	8	8	8	8	8	8	8
4 %	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	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	7	7	7	7	7
	B	8	7	7	7	7	4	3
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	8	6
	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	7	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	7	7	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8



Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Growth

Test Initiated: December 14, 2021 at 1513

Test Terminated: December 21, 2021 at 1500

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.66152	.66576	0.00424	8	0.530
	B	.65139	.65555	0.00416	8	0.520
	C	.65055	.65506	0.00451	8	0.564
	D	.65429	.65841	0.00412	8	0.515
	E	.63942	.64423	0.00481	8	0.601
4 %	A	.65539	.65913	0.00374	8	0.468
	B	.64943	.65328	0.00385	8	0.481
	C	.64473	.64873	0.00400	8	0.500
	D	.65565	.65927	0.00362	8	0.452
	E	.67084	.67455	0.00371	8	0.464
5 %	A	.65096	.65497	0.00401	8	0.501
	B	.65039	.65434	0.00395	8	0.494
	C	.65326	.65727	0.00401	8	0.501
	D	.65592	.65985	0.00393	8	0.491
	E	.65984	.66355	0.00371	8	0.464
7 %	A	.64954	.65335	0.00381	8	0.476
	B	.66570	.66670	0.00100	8	0.125
	C	.65981	.66352	0.00371	8	0.464
	D	.65322	.65594	0.00272	8	0.340
	E	.65659	.66052	0.00393	8	0.491
9 %	A	.65765	.66148	0.00383	8	0.479
	B	.65633	.66043	0.00410	8	0.512
	C	.63772	.64142	0.00370	8	0.462
	D	.64576	.64867	0.00291	8	0.364
	E	.66355	.66715	0.00360	8	0.450
12 %	A	.65556	.65992	0.00436	8	0.545
	B	.65840	.66233	0.00393	8	0.491
	C	.64768	.65063	0.00295	8	0.369
	D	.66198	.66591	0.00393	8	0.491
	E	.65900	.66221	0.00321	8	0.401

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	0.87500	1.20940
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	0.87500	1.20940
4	7 %	2	0.37500	0.65906
4	7 %	3	0.87500	1.20940
4	7 %	4	0.75000	1.04720
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	1.00000	1.39310
5	9 %	5	1.00000	1.39310
6	12 %	1	1.00000	1.39310
6	12 %	2	1.00000	1.39310
6	12 %	3	0.87500	1.20940
6	12 %	4	1.00000	1.39310
6	12 %	5	1.00000	1.39310

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0.415 W = 0.7496 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 %	30.00	16.00	5.00	
4	7 %	19.00	16.00	5.00	
5	9 %	27.50	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 = 0.1358 W = 0.8746 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>	

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.07383	0.01477	2.61	
Within (Error)	24	0.1358	0.005658		
Total	29	0.2096			
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)					

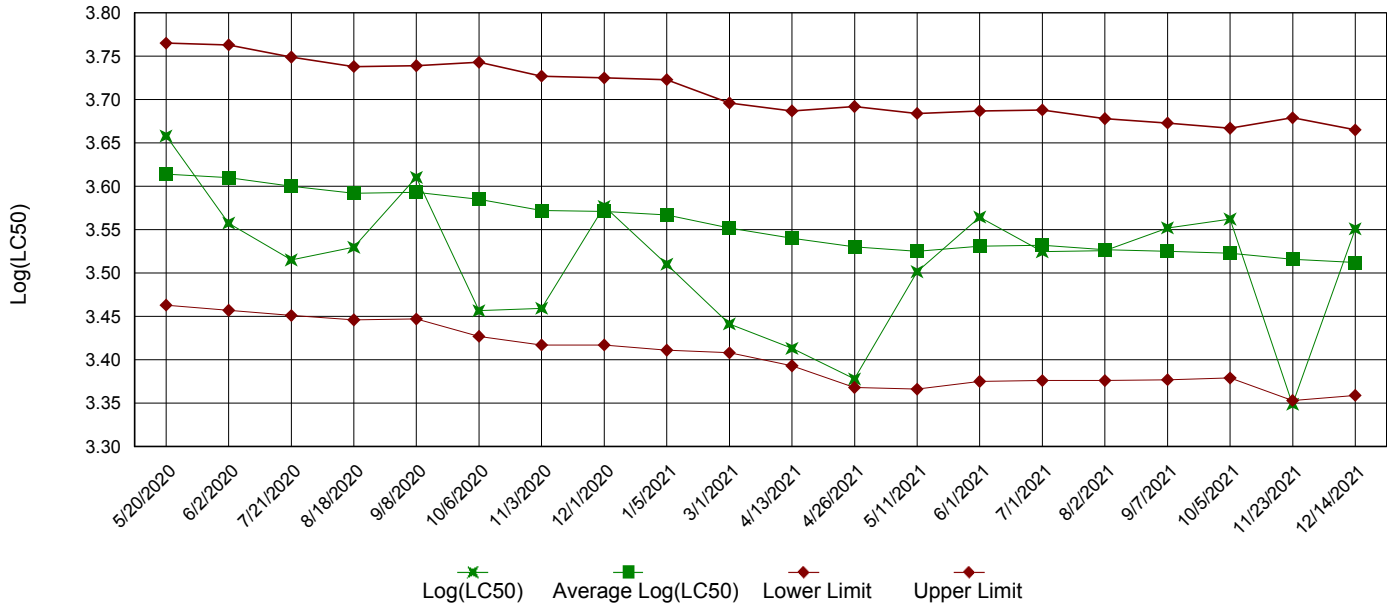
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	0.546	0.546			
2	4 %	0.473	0.473	1.534		
3	5 %	0.4902	0.4902	1.173		
4	7 %	0.3792	0.3792	3.506	*	
5	9 %	0.4534	0.4534	1.946		
6	12 %	0.4594	0.4594	1.82		
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	0.1123	20.6	0.073	
3	5 %	5	0.1123	20.6	0.0558	
4	7 %	5	0.1123	20.6	0.1668	
5	9 %	5	0.1123	20.6	0.0926	
6	12 %	5	0.1123	20.6	0.0866	

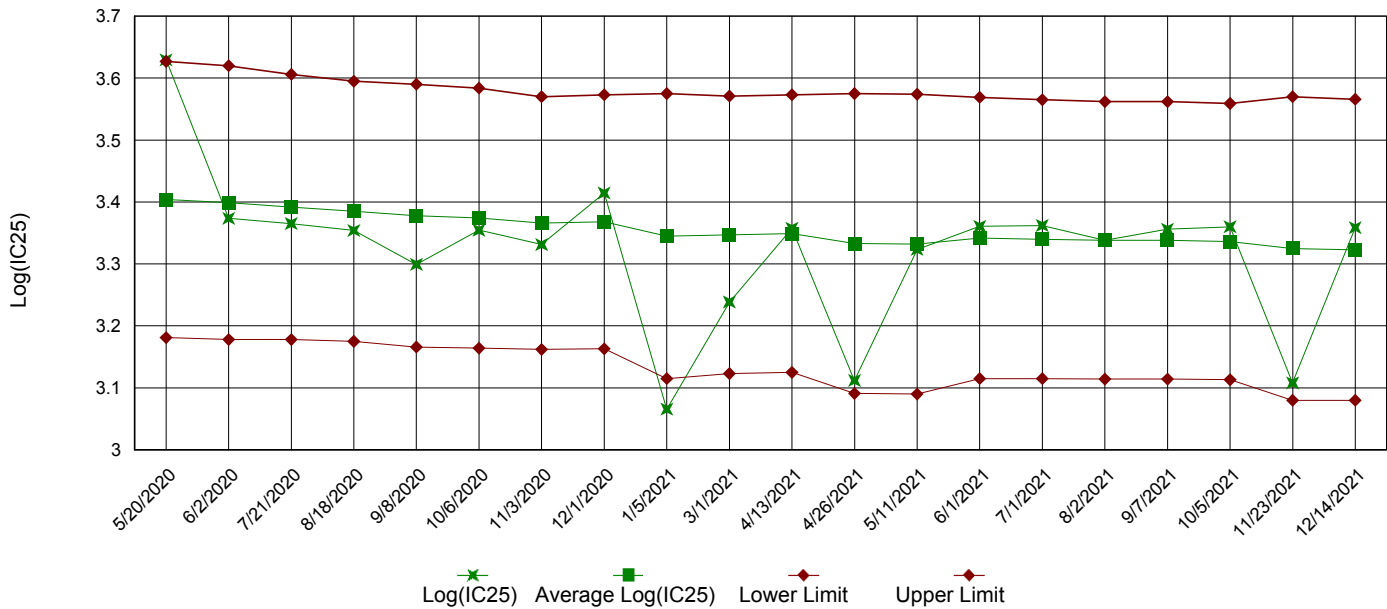
Appendix A3: Test 1000.0

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

LC50 Survival Data



IC25 Growth 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: December 14, 2021 at 1513

Date and Time Test Terminated: December 21, 2021 at 1500

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 %	87.5	100	100	100	100	100	100	97.5	5.73
5 %	100	100	100	100	100	100	100	100	0.00
7 %	87.5	37.5	87.5	75.0	100	100	97.5	77.5	31.0
9 %	100	100	87.5	100	100	100	100	97.5	5.73
12 %	100	100	87.5	100	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.530	0.520	0.564	0.515	0.601	0.546	6.63
4 %	0.468	0.481	0.500	0.452	0.464	0.473	3.87
5 %	0.501	0.494	0.501	0.491	0.464	0.490	3.12
7 %	0.476	0.125	0.464	0.340	0.491	0.379	40.7
9 %	0.479	0.512	0.462	0.364	0.450	0.453	12.2
12 %	0.545	0.491	0.369	0.491	0.401	0.459	15.7

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:   12.2   (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: Mr. Scotty Jones  
 ANALYST: 280, 343, 357, 358

Test Initiated: DATE: December 14, 2021 TIME: 1513  
 Test Terminated: DATE: December 21, 2021 TIME: 1500

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.3	5.8	6.5	6.0	6.5	5.8	5.9
Final	5.7	5.8	5.6	6.0	6.8	6.6	5.9
pH Initial	7.9	7.9	7.9	7.8	8.0	7.8	7.8
Final	7.5	7.6	7.4	7.7	7.6	7.7	7.6

DILUTION 4 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.3	5.9	6.6	5.9	6.6	5.8	5.9
Final	5.5	5.8	6.0	6.0	6.7	6.4	5.7
pH Initial	7.9	7.8	7.9	7.8	8.0	7.8	7.8
Final	7.5	7.6	7.5	7.7	7.6	7.7	7.5

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.3	5.8	6.6	5.9	6.5	5.8	5.9
Final	5.7	5.9	5.9	6.0	6.9	6.3	5.8
pH Initial	7.9	7.9	7.9	7.8	8.0	7.8	7.8
Final	7.5	7.6	7.4	7.7	7.7	7.7	7.6

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.3	5.8	6.7	6.0	6.5	5.9	5.8
Final	5.5	5.6	6.0	6.0	6.7	6.6	5.7
pH Initial	8.0	7.9	8.0	7.8	8.0	7.9	7.8
Final	7.5	7.6	7.5	7.7	7.7	7.7	7.6

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	5.8	6.7	6.0	6.6	6.0	5.8
Final	5.6	5.6	6.0	6.1	6.9	6.5	5.8
pH Initial	8.0	7.8	8.0	7.8	8.0	7.9	7.8
Final	7.6	7.6	7.5	7.7	7.7	7.7	7.6

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.4	5.9	6.7	5.7	6.4	5.9	6.0
Final	5.5	5.4	5.9	5.9	6.8	6.5	5.8
pH Initial	8.0	7.9	8.0	7.8	8.0	7.9	7.8
Final	7.5	7.6	7.5	7.8	7.7	7.8	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	31	450	<0.05	AR0035602 13-DEC-21
130	29	460	<0.05	AR0035602 15-DEC-21
130	27	460	<0.05	AR0035602 17-DEC-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
61	84	300	<0.05	260890-1
62	83	310	<0.05	261120-1





