



June 17, 2021

Biomonitoring Testing
for
EFF

Control No. 256024-1

Prepared for:

Mr. Jimmy Smith
Searcy Water and Sewer System
P.O. Box 1319
Searcy, AR 72145

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322



Searcy Water and Sewer System
ATTN: Mr. Jimmy Smith
P.O. Box 1319
Searcy, AR 72145

Re: Chronic 7-Day Renewal *Pimephales promelas* (Fathead minnow)
EFF
NPDES Permit No. AR0021601 AFIN# 73-00055

Dear Mr. Jimmy Smith:

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 27 % effluent, which is above the critical dilution of 20 %. The NOEC for growth occurred at 27 % effluent, which is above the critical dilution of 20 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Chief Operating Officer

A handwritten signature in black ink is written over a horizontal line. The signature is cursive and appears to read 'John Overbey'.

PDF cc: Searcy Water and Sewer System
ATTN: Mr. Dan Dawson
d.dawson@searcywater.org

Searcy Water and Sewer System
ATTN: Mr. Jimmy Smith
jsmith67@cablelynx.com

FTN Associates, Ltd.
ATTN: Mr. Pat Downey
pjd@ftn-assoc.com

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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	0.466	PASS
Control Growth CV < or = 40%	3.33	PASS
Growth Minimum Significant Difference 12 to 30%	12.4	PASS
Critical Dilution CV < or = 40%	10.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0021601 AFIN# 73-00055
2. Test Requirements: Chronic Biomonitoring, Quarterly Test Method 1000.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:

- a. Sampling Point: EFF
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.9	7.2	7.2
pH (standard units)	6.8	6.8	7.5
Alkalinity (mg/l as CaCO ₃)	16	18	31
Hardness (mg/l as CaCO ₃)	35	31	30
Conductivity (umhos/cm)	250	200	180
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.13	<0.1	0.35

2. Dilution Water Samples:

Soft

Analysis	255955-1
Dissolved oxygen (mg/l)	7.0
pH (standard units)	7.5
Alkalinity (mg/l as CaCO ₃)	34
Hardness (mg/l as CaCO ₃)	43
Conductivity (umhos/cm)	160
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 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: June 8, 2021 at 1158
Date & Time Test Terminated: June 15, 2021 at 1045
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 and Bartlett's test. 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 May 11, 2021 at 1705 to May 18, 2021 at 1515

The results were as follows: (Control No. 255332-1.)

Survival LC-50: 3173 mg/l

Growth IC-25: 2106 mg/l

Growth PMSD: 9.54

V. Organism History

Pimephales promelas (Fathead minnow)

Date: June 8, 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

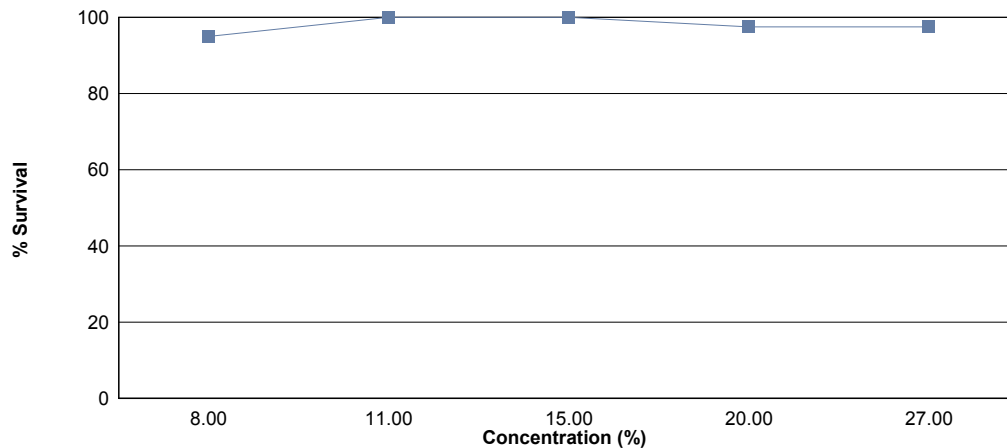
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 8 %, 11 %, 15 %, 20 %, 27 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 20 % effluent.

The test was initiated on June 8, 2021 at 1158 and continued through June 15, 2021 at 1045. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 27 % effluent
- b.) NOEC growth = 27 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.466
8 %	95.0	0.462
11 %	100	0.476
15 %	100	0.481
20 %	97.5	0.471
27 %	97.5	0.497

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: June 8, 2021 at 1158
Date and Time Test Terminated: June 15, 2021 at 1045

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
8 %	A	8	8	8	8	8	8	8
	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	7
11 %	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
15 %	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
20 %	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
27 %	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

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: June 8, 2021 at 1158

Test Terminated: June 15, 2021 at 1045

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.64950	.65327	0.00377	8	0.471
	B	.67603	.67970	0.00367	8	0.459
	C	.66626	.67019	0.00393	8	0.491
	D	.65344	.65708	0.00364	8	0.455
	E	.65485	.65848	0.00363	8	0.454
8 %	A	.65585	.65987	0.00402	8	0.502
	B	.63885	.64282	0.00397	8	0.496
	C	.64363	.64721	0.00358	8	0.448
	D	.65468	.65850	0.00382	8	0.478
	E	.67850	.68158	0.00308	8	0.385
11 %	A	.64591	.64967	0.00376	8	0.470
	B	.65027	.65397	0.00370	8	0.462
	C	.64914	.65288	0.00374	8	0.468
	D	.65637	.66037	0.00400	8	0.500
	E	.66544	.66926	0.00382	8	0.478
15 %	A	.64776	.65168	0.00392	8	0.490
	B	.65539	.65962	0.00423	8	0.529
	C	.65293	.65632	0.00339	8	0.424
	D	.64518	.64928	0.00410	8	0.512
	E	.65133	.65494	0.00361	8	0.451
20 %	A	.66619	.66999	0.00380	8	0.475
	B	.65345	.65767	0.00422	8	0.528
	C	.64672	.65041	0.00369	8	0.461
	D	.64539	.64852	0.00313	8	0.391
	E	.64818	.65218	0.00400	8	0.500
27 %	A	.66556	.66920	0.00364	8	0.455
	B	.65984	.66377	0.00393	8	0.491
	C	.65716	.66145	0.00429	8	0.536
	D	.66653	.67087	0.00434	8	0.542
	E	.67268	.67636	0.00368	8	0.460

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	8 %	1	1.00000	1.39310
2	8 %	2	1.00000	1.39310
2	8 %	3	0.87500	1.20940
2	8 %	4	1.00000	1.39310
2	8 %	5	0.87500	1.20940
3	11 %	1	1.00000	1.39310
3	11 %	2	1.00000	1.39310
3	11 %	3	1.00000	1.39310
3	11 %	4	1.00000	1.39310
3	11 %	5	1.00000	1.39310
4	15 %	1	1.00000	1.39310
4	15 %	2	1.00000	1.39310
4	15 %	3	1.00000	1.39310
4	15 %	4	1.00000	1.39310
4	15 %	5	1.00000	1.39310
5	20 %	1	1.00000	1.39310
5	20 %	2	1.00000	1.39310
5	20 %	3	0.87500	1.20940
5	20 %	4	1.00000	1.39310
5	20 %	5	1.00000	1.39310
6	27 %	1	1.00000	1.39310
6	27 %	2	0.87500	1.20940
6	27 %	3	1.00000	1.39310
6	27 %	4	1.00000	1.39310
6	27 %	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 = 0.09449 W = 0.7601 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	8 %	22.50	16.00	5.00	
3	11 %	27.50	16.00	5.00	
4	15 %	27.50	16.00	5.00	
5	20 %	25.00	16.00	5.00	
6	27 %	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 = 0.03579 W = 0.952 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 = 8.726 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	0.003922	0.0007843	0.526	
Within (Error)	24	0.03579	0.001491		
Total	29	0.03971			
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)					

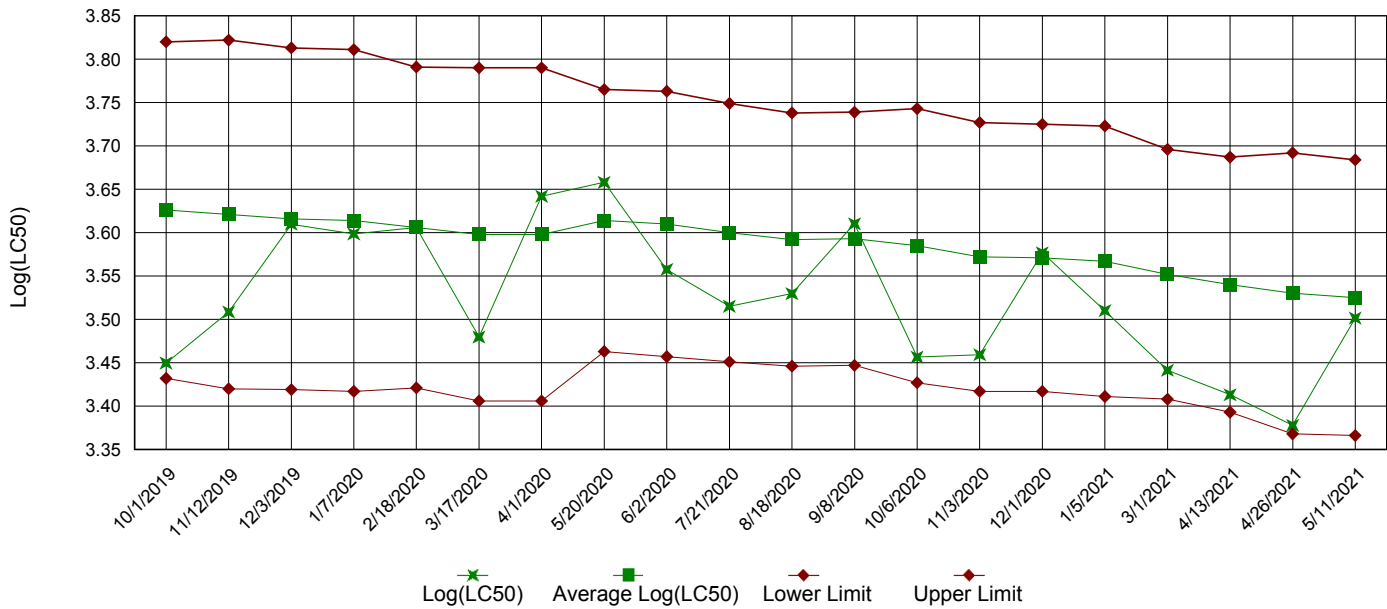
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	0.466	0.466			
2	8 %	0.4618	0.4618	0.172		
3	11 %	0.4756	0.4756	-0.3931		
4	15 %	0.4812	0.4812	-0.6224		
5	20 %	0.471	0.471	-0.2047		
6	27 %	0.4968	0.4968	-1.261		
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	8 %	5	0.05763	12.4	0.0042	
3	11 %	5	0.05763	12.4	-0.0096	
4	15 %	5	0.05763	12.4	-0.0152	
5	20 %	5	0.05763	12.4	-0.005	
6	27 %	5	0.05763	12.4	-0.0308	

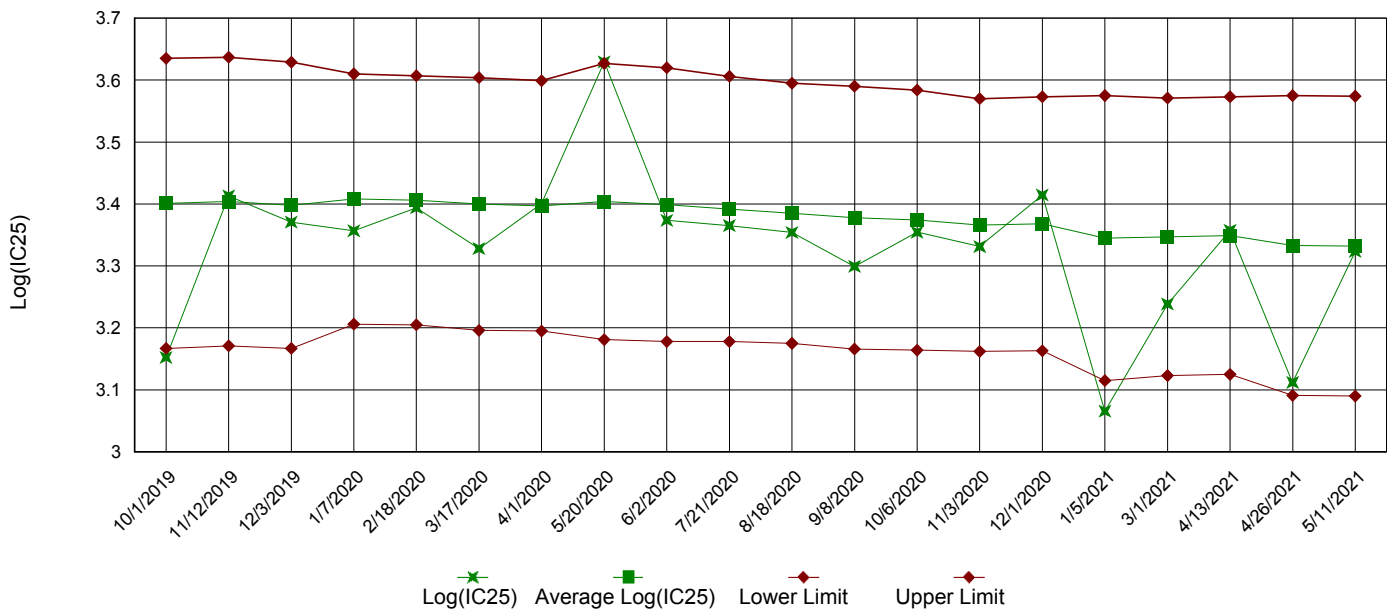
Appendix A3: Test 1000.0

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: Searcy Water and Sewer System

NPDES No.: AR0021601 AFIN# 73-00055

Date and Time Test Initiated: June 8, 2021 at 1158
Date and Time Test Terminated: June 15, 2021 at 1045
Dilution water used: Soft

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
8 %	100	100	87.5	100	87.5	97.5	97.5	95.0	7.21
11 %	100	100	100	100	100	100	100	100	0.00
15 %	100	100	100	100	100	100	100	100	0.00
20 %	100	100	87.5	100	100	100	100	97.5	5.73
27 %	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	0.471	0.459	0.491	0.455	0.454	0.466	3.33
8 %	0.502	0.496	0.448	0.478	0.385	0.462	10.3
11 %	0.470	0.462	0.468	0.500	0.478	0.476	3.11
15 %	0.490	0.529	0.424	0.512	0.451	0.481	9.00
20 %	0.475	0.528	0.461	0.391	0.500	0.471	10.9
27 %	0.455	0.491	0.536	0.542	0.460	0.497	8.25

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: 27 % (TOP6C)
6. LOEC *Pimephales* Lethality: 27 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 27 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 27 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 10.9 (TQP6C)
10. Sublethality for this test: 27 % (51714 or 51714S)

Appendix B: Test 1000.0
 CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
 CHEMICAL PARAMETERS CHART

PERMITTEE: Searcy Water and Sewer System
 NPDES NO.: AR0021601 AFIN# 73-00055
 CONTACT: Mr. Jimmy Smith
 ANALYST: 280, 343, 357, 358

Test Initiated: DATE: June 8, 2021 TIME: 1158
 Test Terminated: DATE: June 15, 2021 TIME: 1045

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.0	7.3	7.4	7.5	7.4	7.4	7.5
Final	7.1	6.3	5.4	6.4	6.2	6.6	6.5
pH Initial	7.5	7.5	7.6	7.5	7.6	7.6	7.6
Final	7.5	7.4	7.2	7.4	7.4	7.4	7.0

DILUTION 8 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.4	7.4	7.3	7.4	7.5	7.2
Final	6.7	6.3	5.5	6.4	5.9	6.4	5.8
pH Initial	7.4	7.4	7.5	7.4	7.5	7.5	7.5
Final	7.4	7.3	7.2	7.3	7.3	7.3	7.0

DILUTION 11 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	6.9	7.2	7.1	7.3	7.3	7.1
Final	6.9	5.9	5.3	6.1	6.0	6.4	6.0
pH Initial	7.4	7.4	7.5	7.5	7.5	7.5	7.5
Final	7.4	7.2	7.2	7.3	7.2	7.4	7.0

DILUTION 15 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.3	6.8	7.3	7.5	7.3	7.2
Final	7.2	6.5	5.8	6.3	6.2	6.7	6.7
pH Initial	7.4	7.5	7.4	7.5	7.5	7.5	7.5
Final	7.4	7.2	7.2	7.3	7.3	7.4	7.2

DILUTION 20 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.4	7.6	7.4	7.2	7.2	7.2
Final	7.2	6.6	5.5	6.2	6.6	6.6	6.9
pH Initial	7.4	7.4	7.4	7.4	7.5	7.4	7.4
Final	7.4	7.3	7.1	7.3	7.4	7.4	7.3

DILUTION 27 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.0	7.4	7.3	7.3	7.4	7.1
Final	6.8	5.7	5.7	6.0	6.0	6.6	6.4
pH Initial	7.3	7.3	7.3	7.4	7.4	7.4	7.4
Final	7.3	7.0	7.1	7.3	7.2	7.4	7.2

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
16	35	250	<0.05	EFF 07-JUN-21
18	31	200	<0.05	EFF 09-JUN-21
31	30	180	<0.05	EFF 11-JUN-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
34	43	160	<0.05	255955-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: SEABY WATER UTILITIES		AIC CONTROL NO: 256024	
Project Reference: BRD-MONITOR		AIC PROPOSAL NO:	
Project Manager: JIMMY SMITH		Carrier:	
Sampled By: JEREMY CHEELY		Received Temperature C 6.8	
AIC Sample Identification		Remarks	
No. 2 EFF			
Date/Time Collected 6-9-21 8AM			
Container Type Preservative		Field pH calibration on _____ @ _____ Buffer:	
G = Glass NO = none P = Plastic S = Sulfuric acid pH2		T = Sodium Thiosulfate Z = Zinc acetate	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS		H = HCl to pH2 B = NaOH to pH12	
Expedited results requested by: _____		V = VOA vials N = Nitric acid pH2	
Who should AIC contact with questions: Phone: _____ Fax _____		Received By: _____ Date/Time _____	
Report Attention to: Report Address to:		Relinquished By: SPK Date/Time 6-9-21 11:45 am	
		Received in Lab By: Patrick Dwyer Date/Time 6-9-21 11:45	
		Comments:	

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 3 OF 3

Client: <u>SEARCY WATER</u>		AIC CONTROL NO: <u>256021</u>	
Project Reference: <u>BIO-MONITOR</u>		AIC PROPOSAL NO:	
Project Manager: <u>Jimmy Smith</u>		Carrier:	
Sampled By: <u>Jimmy Hartsfield</u>		Received Temperature C: <u>24</u>	
AIC No. <u>EFF</u>		Remarks:	
Date/Time Collected: <u>6-11-21 8AM</u>			
Sample Identification: <u>EFF</u>			
Container Type: <u>P</u>		Field pH calibration on _____ @ _____	
Preservative: <u>NO</u>		Buffer: _____	
G = Glass NO = none P = Plastic S = Sulfuric acid pH2		T = Sodium Thiosulfate Z = Zinc acetate	
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS		Received Date/Time: _____	
Expedited results requested by: _____		By: _____	
Who should AIC contact with questions: _____		Received in Lab Date/Time: <u>6-11-21</u>	
Phone: _____		By: <u>[Signature]</u>	
Report Attention to: _____		Date/Time: <u>6-11-21</u>	
Report Address to: _____		Comments: _____	