

December 28, 2020

Biomonitoring Testing
for
Outfall 001

Control No. 251213-1

Prepared for:

Mr. Vincent Miles
Pine Bluff Wastewater Utility
1520 South Ohio Street
Pine Bluff, AR 71601-6055

Prepared by:

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



Pine Bluff Wastewater Utility
ATTN: Mr. Vincent Miles
1520 South Ohio Street
Pine Bluff, AR 71601-6055

Re: 7-day chronic *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001
NPDES Permit No. AR 0033316 AFIN 35-00149

Dear Mr. Vincent Miles:

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: Due to the percent minimum significant difference (PMSD) exceeding the upper limit without a statistically significant effect detected, the test is invalid and will need to be repeated.

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.

PDF cc: Pine Bluff Wastewater Utility
ATTN: Mr. Vincent Miles
vincent@pbwastewater.com

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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	0.366	PASS
Control Growth CV < or = 40%	12.6	PASS
Growth Minimum Significant Difference 12 to 30%	15.7	PASS
Critical Dilution CV < or = 40%	5.32	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	22.9	PASS
Control CV < or = 40% per Surviving Female	33.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	48.9	FAIL
Critical Dilution CV < or = 40%	35.9	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR 0033316 AFIN 35-00149
2. Test Requirements: quarterly
Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
 - a. Sampling Point: Outfall 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.4	6.8	6.8
pH (standard units)	7.8	7.8	7.8
Alkalinity (mg/l as CaCO ₃)	130	130	130
Hardness (mg/l as CaCO ₃)	33	31	32
Conductivity (umhos/cm)	640	640	640
Residual Chlorine (mg/l)	<0.05	0.060	0.060
Ammonia as N (mg/l)	1.5	1.4	1.6

2. Dilution Water Samples:
Moderately Hard

Analysis	251103-1	251245-1
Dissolved oxygen (mg/l)	7.2	7.4
pH (standard units)	8.0	8.1
Alkalinity (mg/l as CaCO ₃)	58	62
Hardness (mg/l as CaCO ₃)	83	82
Conductivity (umhos/cm)	310	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: December 15, 2020 at 0915
Date & Time Test Terminated: December 22, 2020 at 0910
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: December 15, 2020 at 1039
Date & Time Test Terminated: December 21, 2020 at 1100
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: December 15, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: December 15, 2020

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

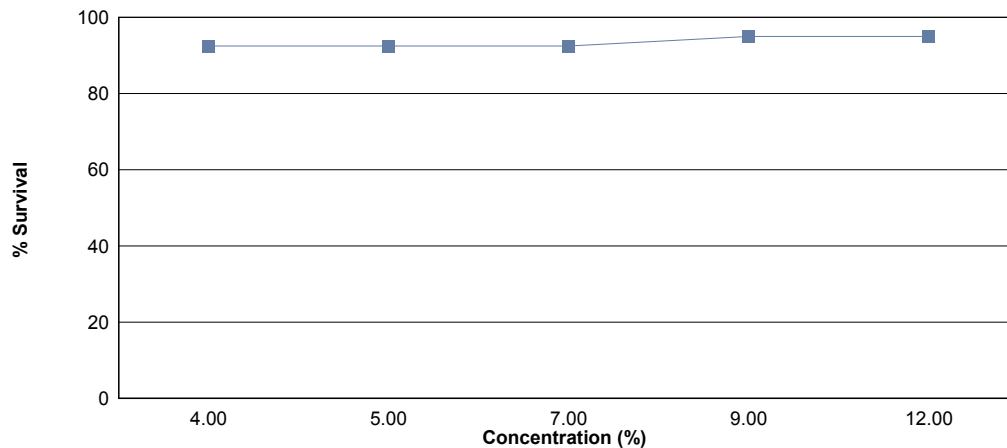
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 15, 2020 at 0915 and continued through December 22, 2020 at 0910. 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	0.357
4 %	92.5	0.317
5 %	92.5	0.331
7 %	92.5	0.304
9 %	95.0	0.317
12 %	95.0	0.306

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

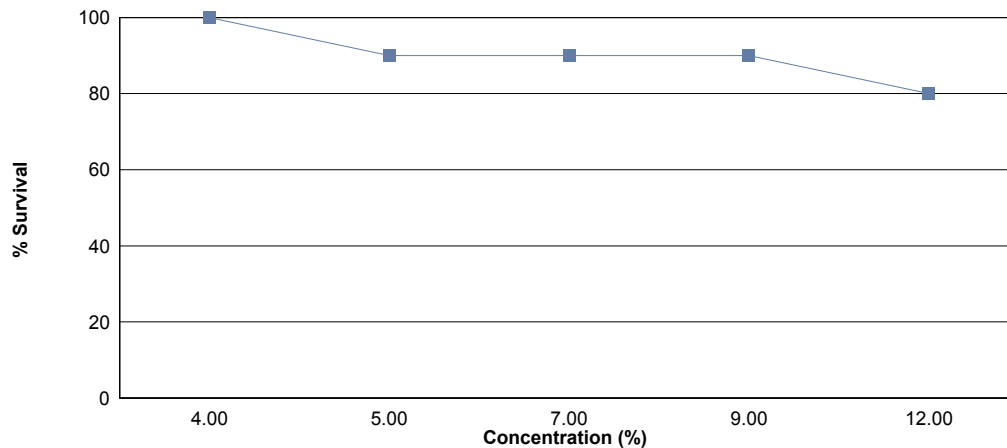
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 December 15, 2020 at 1039 and continued through December 21, 2020 at 1100. 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	22.9
4 %	100	24.0
5 %	90.0	22.2
7 %	90.0	23.4
9 %	90.0	20.3
12 %	80.0	19.2

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: December 15, 2020 at 0915

Date and Time Test Terminated: December 22, 2020 at 0910

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	7	7
	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	7	7
	C	8	8	8	8	8	8	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	7	7
5 %	A	8	8	8	8	8	7	7
	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	7	7
7 %	A	8	8	8	8	7	7	7
	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	7
9 %	A	8	8	8	8	8	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	7	7
12 %	A	8	8	8	8	7	7	7
	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	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: December 15, 2020 at 0915

Test Terminated: December 22, 2020 at 0910

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.77599	.77932	0.00333	8	0.416
	B	.76734	.77006	0.00272	8	0.340
	C	.76949	.77204	0.00255	8	0.319
	D	.77271	.77586	0.00315	8	0.394
	E	.75466	.75720	0.00254	8	0.318
4 %	A	.76012	.76298	0.00286	8	0.358
	B	.75779	.75979	0.00200	8	0.250
	C	.77291	.77506	0.00215	8	0.269
	D	.77610	.77887	0.00277	8	0.346
	E	.77509	.77799	0.00290	8	0.362
5 %	A	.77014	.77262	0.00248	8	0.310
	B	.76896	.77125	0.00229	8	0.286
	C	.77990	.78267	0.00277	8	0.346
	D	.78207	.78475	0.00268	8	0.335
	E	.76891	.77193	0.00302	8	0.378
7 %	A	.77015	.77230	0.00215	8	0.269
	B	.77155	.77383	0.00228	8	0.285
	C	.77359	.77596	0.00237	8	0.296
	D	.77723	.77988	0.00265	8	0.331
	E	.76893	.77165	0.00272	8	0.340
9 %	A	.77318	.77594	0.00276	8	0.345
	B	.76851	.77095	0.00244	8	0.305
	C	.77988	.78234	0.00246	8	0.308
	D	.77323	.77578	0.00255	8	0.319
	E	.77004	.77249	0.00245	8	0.306
12 %	A	.77200	.77410	0.00210	8	0.262
	B	.77021	.77269	0.00248	8	0.310
	C	.77165	.77397	0.00232	8	0.290
	D	.77209	.77458	0.00249	8	0.311
	E	.76785	.77071	0.00286	8	0.358

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: December 15, 2020 at 1039

Date and Time Test Terminated: December 21, 2020 at 1100

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	2	4	4	4	3	37	10	3.70	
5	10	12	11	10	13	10	11	11	12	11	111	10	11.1	
6	13	0	0	0	14	0	15	15	9	15	81	10	8.10	
7														
8														
TOTAL	27	16	15	14	31	12	30	30	25	29	229	10	22.9	

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	0	0	0	0	0	10	0.00
4	4	4	4	4	3	3	3	4	4	4	37	10	3.70
5	9	10	11	8	10	11	12	11	13	12	107	10	10.7
6	15	0	0	0	15	18	16	0	15	17	96	10	9.60
7													
8													
TOTAL	28	14	15	12	28	32	31	15	32	33	240	10	24.0

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	X	0	0	0	0	0	9	0.00
4	4	3	4	4	4	X	4	4	6	4	37	9	4.11
5	11	10	11	12	13	X	13	10	12	10	102	9	11.3
6	15	0	0	0	20	X	0	15	17	16	83	9	9.22
7													
8													
TOTAL	30	13	15	16	37	0	17	29	35	30	222	10	22.2

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: December 15, 2020 at 1039

Date and Time Test Terminated: December 21, 2020 at 1100

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	X	0	0	9	0.00
4	4	3	4	4	4	4	4	3	X	4	34	9	3.78	
5	11	10	9	15	11	10	12	10	X	10	98	9	10.9	
6	12	0	0	0	18	17	17	18	X	20	102	9	11.3	
7														
8														
TOTAL	27	13	13	19	33	31	33	31	0	34	234	10	23.4	

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	X	0	0	9	0.00
3	0	0	0	0	0	0	0	0	X	0	0	9	0.00
4	4	4	0	3	4	4	5	4	X	4	32	9	3.56
5	12	10	7	11	11	0	10	9	X	10	80	9	8.89
6	14	0	10	0	17	9	15	10	X	16	91	9	10.1
7													
8													
TOTAL	30	14	17	14	32	13	30	23	0	30	203	10	20.3

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	X	0	0	0	0	X	0	0	8	0.00
3	0	0	0	X	0	0	0	0	X	0	0	8	0.00
4	1	4	1	X	4	4	4	4	X	4	26	8	3.25
5	5	11	8	X	9	8	10	9	X	10	70	8	8.75
6	0	16	0	X	17	13	15	17	X	18	96	8	12.0
7													
8													
TOTAL	6	31	9	0	30	25	29	30	0	32	192	10	19.2

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	0.87500	1.20940
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
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	0.87500	1.20940
3	5 %	1	0.87500	1.20940
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	0.87500	1.20940
4	7 %	2	0.87500	1.20940
4	7 %	3	1.00000	1.39310
4	7 %	4	1.00000	1.39310
4	7 %	5	0.87500	1.20940
5	9 %	1	0.87500	1.20940
5	9 %	2	1.00000	1.39310
5	9 %	3	1.00000	1.39310
5	9 %	4	1.00000	1.39310
5	9 %	5	0.87500	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 = 0.2295 W = 0.8402 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 %	22.50	16.00	5.00	
3	5 %	22.50	16.00	5.00	
4	7 %	22.50	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 = 0.0341 W = 0.9603 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 = 4.701 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	0.009774	0.001955	1.376	
Within (Error)	24	0.0341	0.001421		
Total	29	0.04388			
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	0.3574	0.3574			
2	4 %	0.317	0.317	1.695		
3	5 %	0.331	0.331	1.107		
4	7 %	0.3042	0.3042	2.231		
5	9 %	0.3166	0.3166	1.711		
6	12 %	0.3062	0.3062	2.148		
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	0.05627	15.7	0.0404		
3	5 %	5	0.05627	15.7	0.0264		
4	7 %	5	0.05627	15.7	0.0532		
5	9 %	5	0.05627	15.7	0.0408		
6	12 %	5	0.05627	15.7	0.0512		

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 %	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	0	
2	5 %	10	1	
3	7 %	10	1	
4	9 %	10	1	
5	12 %	10	2	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.179 D* = 1.404 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 %	113.50	75.00	10.00	
3	5 %	108.50	75.00	10.00	
4	7 %	115.50	75.00	10.00	
5	9 %	101.00	75.00	10.00	
6	12 %	101.50	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	175.4	35.08	0.2986	
Within (Error)	54	6347	117.5		
Total	59	6522			
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)					

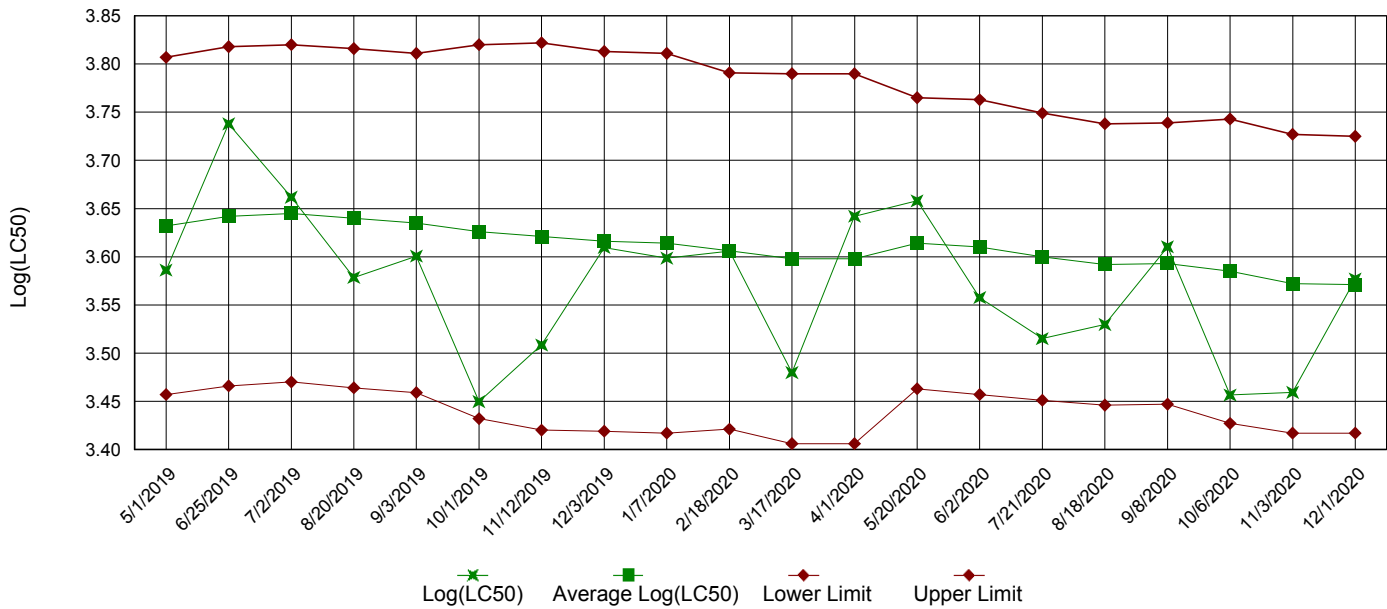
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	22.9	22.9			
2	4 %	24	24	-0.2269		
3	5 %	22.2	22.2	0.1444		
4	7 %	23.4	23.4	-0.1031		
5	9 %	20.3	20.3	0.5363		
6	12 %	19.2	19.2	0.7633		
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	11.2	48.9	-1.1	
3	5 %	10	11.2	48.9	0.7	
4	7 %	10	11.2	48.9	-0.5	
5	9 %	10	11.2	48.9	2.6	
6	12 %	10	11.2	48.9	3.7	

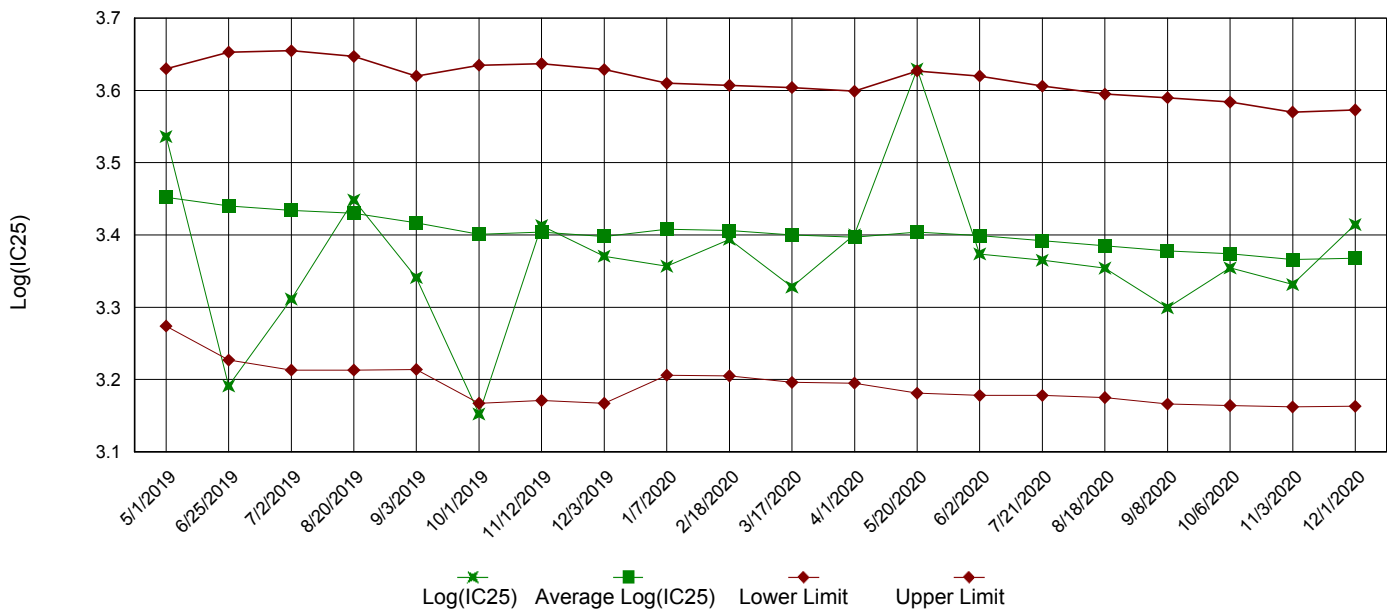
Appendix A3: Test 1000.0

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

LC50 Survival Data

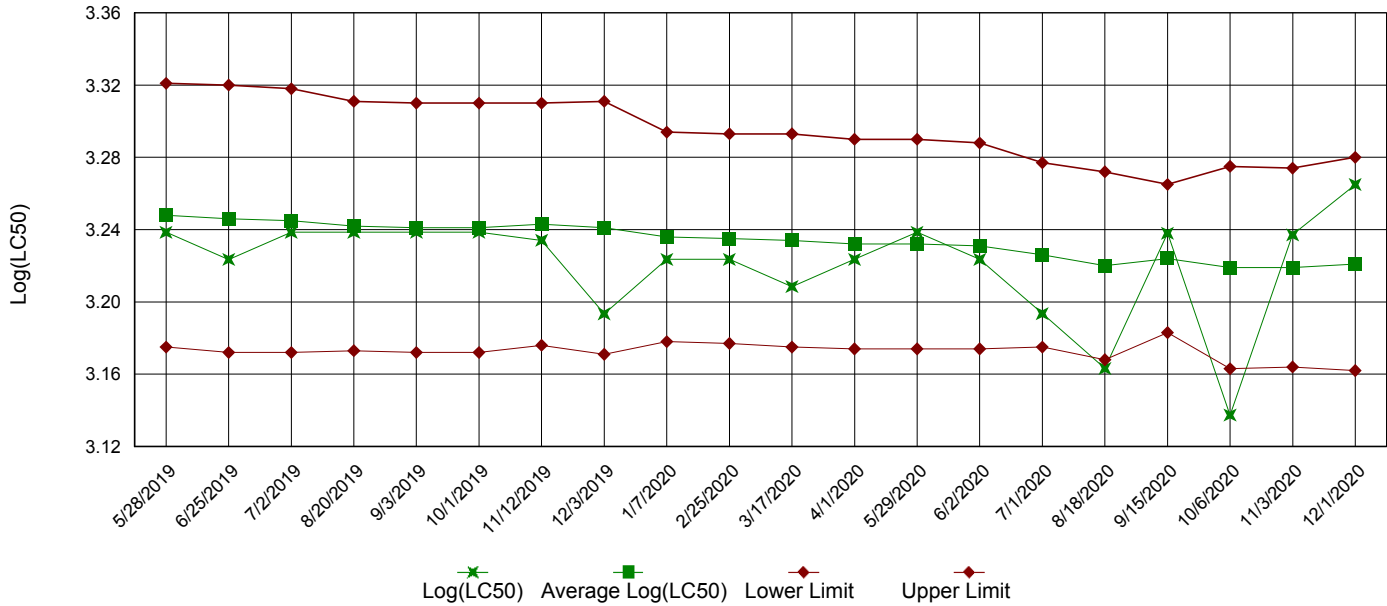


IC25 Growth Data

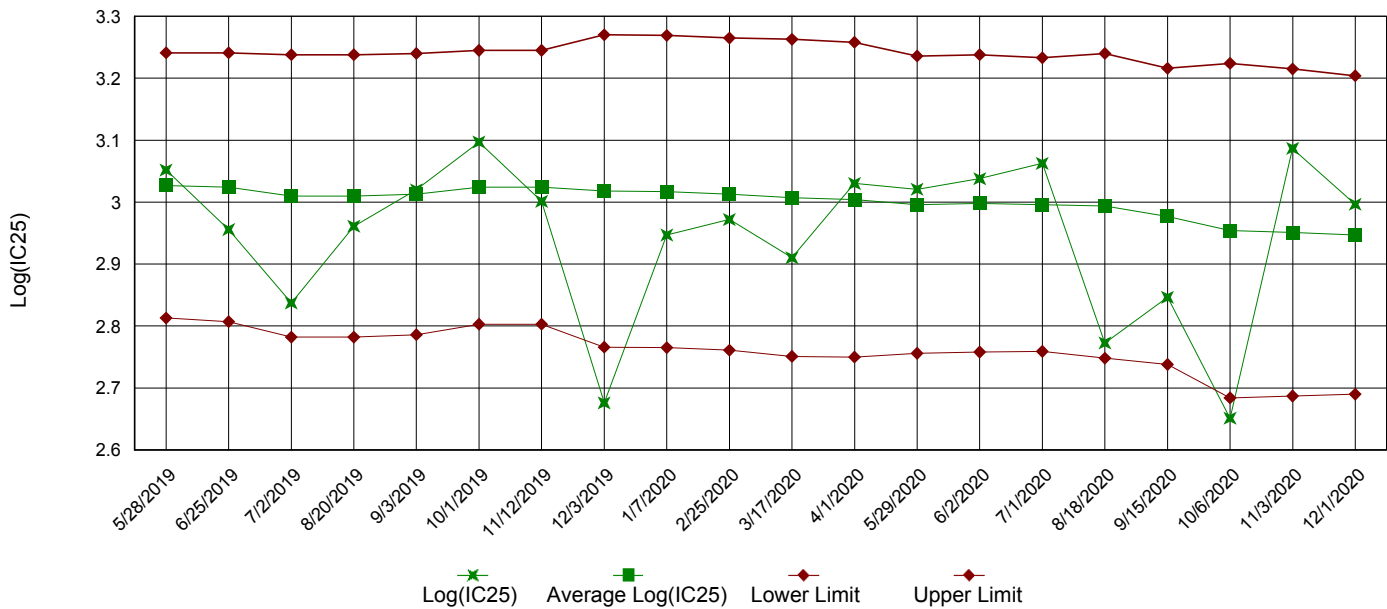


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Pine Bluff Wastewater Utility

NPDES No.: AR 0033316 AFIN 35-00149

Date and Time Test Initiated: December 15, 2020 at 0915

Date and Time Test Terminated: December 22, 2020 at 0910

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	87.5	100	100	100	100	97.5	5.73
4 %	100	87.5	87.5	100	87.5	100	100	92.5	7.40
5 %	87.5	87.5	100	100	87.5	100	100	92.5	7.40
7 %	87.5	87.5	100	100	87.5	100	100	92.5	7.40
9 %	87.5	100	100	100	87.5	100	100	95.0	7.21
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	0.416	0.340	0.319	0.394	0.318	0.357	12.6
4 %	0.358	0.250	0.269	0.346	0.362	0.317	16.8
5 %	0.310	0.286	0.346	0.335	0.378	0.331	10.6
7 %	0.269	0.285	0.296	0.331	0.340	0.304	9.96
9 %	0.345	0.305	0.308	0.319	0.306	0.317	5.32
12 %	0.262	0.310	0.290	0.311	0.358	0.306	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	(9 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> 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	(9 %)	<input type="checkbox"/> YES	<input checked="" type="checkbox"/> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<input type="checkbox"/> YES	<input type="checkbox"/> 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: 12.6 (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: Pine Bluff Wastewater Utility
NPDES NO.: AR 0033316 AFIN 35-00149
CONTACT: Mr. Vincent Miles
ANALYST: 280, 310, 343, 356

Test Initiated: DATE: December 15, 2020 TIME: 0915
Test Terminated: DATE: December 22, 2020 TIME: 0910

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.2	7.6	7.4	7.7	7.7	7.5	7.4
Final	7.5	7.2	8.6	6.9	8.5	7.1	
pH Initial	8.0	8.1	8.1	8.1	8.1	8.1	8.0
Final	8.0	7.7	7.6	7.5	7.5	7.9	

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	7.2	7.5	7.3	7.7	7.5	7.4	7.2
Final	7.8	7.5	6.5	6.7	8.4	7.4	
pH Initial	8.0	8.1	8.1	8.1	8.1	8.0	8.0
Final	8.0	7.8	7.7	7.6	7.7	8.0	

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	7.2	7.5	7.4	8.0	7.5	7.7	7.4
Final	7.7	7.6	6.8	6.8	8.4	7.1	
pH Initial	8.0	8.1	8.1	8.1	8.1	8.0	8.0
Final	8.0	7.9	7.8	7.7	7.7	7.9	

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.1	7.6	7.4	8.0	7.5	7.7	7.4
Final	8.0	8.0	7.0	8.6	8.4	7.2	
pH Initial	8.0	8.1	8.1	8.1	8.1	8.0	8.0
Final	8.1	8.0	7.8	8.0	7.9	7.9	

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	7.1	7.5	7.4	7.9	7.5	7.8	7.4
Final	7.6	7.8	7.0	8.6	8.3	7.2	
pH Initial	8.0	8.0	8.1	8.1	8.1	8.0	8.0
Final	8.1	8.0	7.8	8.2	8.0	7.9	

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.0	7.5	7.3	7.7	7.4	7.7	7.3
Final	7.8	8.0	6.7	6.6	8.3	7.3	
pH Initial	8.0	8.0	8.1	8.0	8.0	8.0	8.0
Final	8.0	8.1	7.8	7.8	8.1	7.9	

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID	
130	33	640	<0.05	Outfall 001	14-DEC-20
130	31	640	0.060	Outfall 001	16-DEC-20
130	32	640	0.060	Outfall 001	18-DEC-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	83	310	<0.05	251103-1
62	82	300	<0.05	251245-1

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

Permittee: Pine Bluff Wastewater Utility

NPDES No.: AR 0033316 AFIN 35-00149

Date and Time Test Initiated: December 15, 2020 at 1039

Date and Time Test Terminated: December 21, 2020 at 1100

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	90.0	80.0
6 day	100	100	90.0	90.0	90.0	80.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	27	28	30	27	30	6
B	16	14	13	13	14	31
C	15	15	15	13	17	9
D	14	12	16	19	14	0
E	31	28	37	33	32	30
F	12	32	0	31	13	25
G	30	31	17	33	30	29
H	30	15	29	31	23	30
I	25	32	35	0	0	0
J	29	33	30	34	30	32
Mean per Adult	22.9	24.0	22.2	23.4	20.3	19.2
Mean per Surviving Adult	22.9	24.0	24.7	26.0	22.6	24.0
CV %	33.6	36.6	37.9	33.3	35.9	43.4

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

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Pine Bluff Wastewater Utility
NPDES NO.: AR 0033316 AFIN 35-00149
CONTACT: Mr. Vincent Miles
ANALYST: 280, 310, 343, 356

Test Initiated: DATE: December 15, 2020 TIME: 1039
Test Terminated: DATE: December 21, 2020 TIME: 1100

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.2	7.6	7.4	7.7	7.7	7.5	7.4
Final	8.0	7.2	8.0	7.9	7.3	7.0	--
pH Initial	8.0	8.1	8.1	8.1	8.1	8.1	8.0
Final	8.5	8.5	8.5	8.4	8.4	8.3	--

DILUTION	DAY						
	1	2	3	4	5	6	7
4 %							
D.O. Initial	7.2	7.5	7.3	7.7	7.5	7.4	7.2
Final	8.0	7.7	8.1	7.7	7.8	7.2	--
pH Initial	8.0	8.1	8.1	8.1	8.1	8.0	8.0
Final	8.6	8.6	8.6	8.5	8.5	8.3	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5 %							
D.O. Initial	7.2	7.5	7.4	8.0	7.5	7.7	7.4
Final	8.3	7.7	8.3	7.9	8.7	7.6	--
pH Initial	8.0	8.1	8.1	8.1	8.1	8.0	8.0
Final	8.6	8.5	8.5	8.5	8.5	8.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
7 %							
D.O. Initial	7.1	7.6	7.4	8.0	7.5	7.7	7.4
Final	8.3	7.7	8.3	8.0	8.7	7.8	--
pH Initial	8.0	8.1	8.1	8.1	8.1	8.0	8.0
Final	8.6	8.6	8.6	8.5	8.5	8.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
9 %							
D.O. Initial	7.1	7.5	7.4	7.9	7.5	7.8	7.4
Final	8.2	7.6	8.1	8.0	8.7	7.6	--
pH Initial	8.0	8.0	8.1	8.1	8.1	8.0	8.0
Final	8.7	8.6	8.6	8.6	8.5	8.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
12 %							
D.O. Initial	7.0	7.5	7.3	7.7	7.4	7.7	7.3
Final	8.2	7.8	8.1	7.6	8.7	7.6	--
pH Initial	8.0	8.0	8.1	8.0	8.0	8.0	8.0
Final	8.7	8.6	8.6	8.6	8.6	8.6	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
130	33	640	<0.05	Outfall 001 14-DEC-20
130	31	640	0.060	Outfall 001 16-DEC-20
130	32	640	0.060	Outfall 001 18-DEC-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	83	310	<0.05	251103-1
62	82	300	<0.05	251245-1



8600 Kanis Road
 Little Rock, AR 72204-2322
 (501) 224-5060
 FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

Start time 12/13/20; 0845 HRS
 end time 12/14/20; 0645 HRS

Client: <u>Fine Bluff Wastewater</u>		PO No. <u>49930</u>		ANALYSES REQUESTED											
Project Reference: <u>Biomonitoring</u>		SAMPLE MATRIX		NO OF BOTTLES											
Project Manager: <u>Vincent Miles</u>		WATER													
Sampled By: <u>Isaiah Solomon</u>		G R A B													
AIC No. <u>001</u>		Date/Time Collected													
		<u>12/13/20 @ 0845</u>													
		<u>12/14/20 @ 0645</u>													
Remarks		Container Type													
		<u>P</u>													
		Preservative													
		<u>N0</u>													
Carrier:		G = Glass													
		NO = none													
Received on <u>12/13/20</u> @ <u>0845</u>		S = Sulfuric acid pH2													
		P = Plastic													
		V = VOA vials													
		T = Sodium Thiosulfate													
		Z = Zinc acetate													
Field pH calibration		Relinquished													
		By: <u>[Signature]</u>													
		Date/Time													
		<u>12-14-20 1118</u>													
		Received													
		By: <u>D. Brown</u>													
		Date/Time													
		<u>12-14-20 1118</u>													
		Received in Lab													
		By: <u>D. Brown</u>													
		Date/Time													
		<u>12-14-20 1118</u>													
Comments:		Tumaround Time Requested: (Please circle)													
		NORMAL or EXPEDITED IN ___ DAYS													
		Expedited results requested by:													
		Who should AIC contact with questions: <u>Vincent Miles</u>													
		Phone: <u>(501) 692-8955</u> Fax: <u>(501) 535-0822</u>													
		Report Attention to: <u>Vincent Miles</u>													
		Report Address to: <u>900 Island Harbor Marina Rd</u>													
		<u>Fine Bluff, AR 71602</u>													



Start time 12/15/20 @ 0845 HRS
 end time 12/16/20 @ 0645 HRS

8600 Kanis Road
 Little Rock, AR 72204-2322
 (501) 224-5060
 FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: Pine Bluff Wastewater		PO No. 49930		NO OF BOTTLES		ANALYSES REQUESTED	
Project Reference: Biomonitoring		SAMPLE MATRIX		BOTTLES			
Project Manager: Vincent Mikes		WATER		O			
Sampled By: Joretta Shaw		G R A B		T			
AIC No. Outfall 001		C O M P		T L E S			
Date/Time Collected: 12/15/20 @ 0845		S O I L					
Date/Time Collected: 12/16/20 @ 0645		R					
Container Type: P		NO					
Preservative: NO							
G = Glass		P = Plastic		V = VOA vials		H = HCl to pH2	
NO = none		S = Sulfuric acid pH2		N = Nitric acid pH2		B = NaOH to pH12	
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN _____ DAYS		Relinquished By: Vincent Mikes 12/16/20 1050		Received By: Vincent Mikes 12/16/20 1050		Date/Time	
Expedited results requested by: _____		Relinquished By: _____		Received in Lab By: Vincent Mikes 12-16-20		Date/Time 1050	
Who should AIC contact with questions: Vincent Mikes		Comments:					
Phone: 870-692-8955 Fax: 870-535-0822							
Report Attention to: Vincent Mikes							
Report Address to: 900 Island Harbor Marina Rd							
Pine Bluff, AR 71602							

