June 25, 2019

Biomonitoring Testing
for
Plant Efluent
Batesville, AR

Control No. 235198-1

Prepared for:

Mr. Eugene Townsley
Batesville Wastewater Treatment Plant
500 River Bank Road
Batesville, AR 72501

Prepared by:

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

Batesville Wastewater Treatment Plant
ATTN: Mr. Eugene Townsley
500 River Bank Road
Batesville, AR 72501

Re: Chronic*Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Plant Efluent - Batesville, AR
NPDES Permit No. NPDES AR0020702 AFIN 32-00044

Dear Mr. Eugene Townsley:

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 6.7 % effluent, which is above the critical dilution of 5.0 %. The NOEC for growth occurred at 6.7 % effluent, which is above the critical dilution of 5.0 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 6.7 % effluent, which is above the critical dilution of 5.0 %. The NOEC for reproduction occurred at 6.7 % effluent, which is above the critical dilution of 5.0 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

John Overbey
Chief Operating Officer

PDF cc: Batesville Wastewater Treatment Plant
ATTN: Mr. Eugene Townsley
wwsuper@cityofbatesville.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	0.468	PASS
Control Growth CV < or = 40%	4.72	PASS
Growth Minimum Significant Difference 12 to 30%	12.6	PASS
Critical Dilution CV < or = 40%	9.36	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	90.0	PASS
Control Reproduction > or = 15 per Surviving Female	22.7	PASS
Control CV < or = 40% per Surviving Female	22.7	PASS
Reproduction Minimum Significant Difference 13 to 47%	16.2	PASS
Critical Dilution CV < or = 40%	10.0	PASS

II. Outlined Report

A. Introduction

1. Permit Number: NPDES AR0020702 AFIN 32-00044
2. Test Requirements:

Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
   a. Sampling Point: Plant Efluent
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.6	8.1	8.2
pH (standard units)	7.6	7.5	7.7
Alkalinity (mg/l as CaCO3)	70	72	73
Hardness (mg/l as CaCO3)	140	140	140
Conductivity (umhos/cm)	600	610	620
Residual Chlorine (mg/l)	0.060	<0.05	<0.05
Ammonia as N (mg/l)	0.29	<0.1	<0.1

2. Dilution Water Samples:
   Moderately Hard

Analysis	234973-1
Dissolved oxygen (mg/l)	8.0
pH (standard units)	7.8
Alkalinity (mg/l as CaCO3)	59
Hardness (mg/l as CaCO3)	84
Conductivity (umhos/cm)	290
Residual Chlorine (mg/l)	<0.05

C. Test Methods

1. Test methods used:
   Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.
2. Endpoint: No Observable Effects Concentration (NOEC)
3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated:	June 11, 2019 at 1215
Date & Time Test Terminated:	June 18, 2019 at 1230
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:	June 11, 2019 at 1440
Date & Time Test Terminated:	June 19, 2019 at 1415
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 Bartlett's test and analyzed with Dunnett's 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 May 01, 2019 at 1330 to May 08, 2019 at 1433
The results were as follows: (Control No. 234022-1.)

Survival LC-50: 3855 mg/l
Growth IC-25: 3438 mg/l
Growth PMSD: 16

Ceriodaphnia dubia

A chronic reference test was performed on May 28, 2019 at 1400 to June 04, 2019 at 1413
The results were as follows: (Control No. 234022-2.)

Survival LC-50: 1732 mg/l
Growth IC-25: 1127 mg/l
Growth PMSD: 13.3

V. Organism History

Pimephales promelas (Fathead minnow)

Date: June 11, 2019
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: June 11, 2019
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

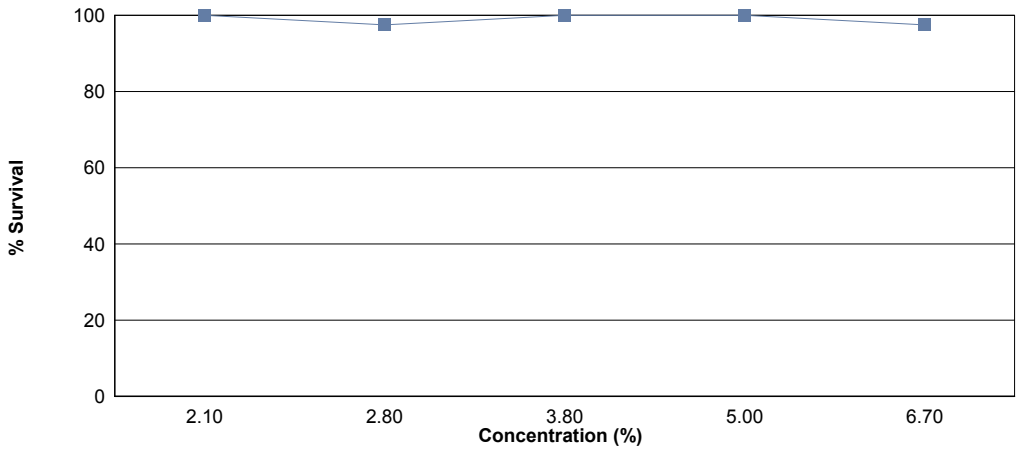
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 2.1 %, 2.8 %, 3.8 %, 5.0 %, 6.7 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 5.0 % effluent.

The test was initiated on June 11, 2019 at 1215 and continued through June 18, 2019 at 1230. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 6.7 % effluent
b.) NOEC growth = 6.7 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.468
2.1 %	100	0.496
2.8 %	97.5	0.469
3.8 %	100	0.478
5.0 %	100	0.506
6.7 %	97.5	0.523

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

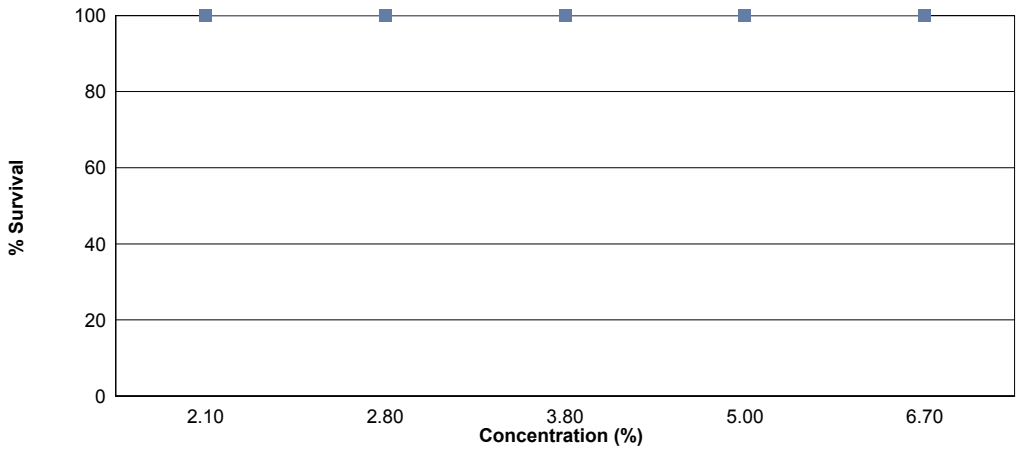
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 2.1 %, 2.8 %, 3.8 %, 5.0 %, 6.7 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 5.0 % effluent.

The test was initiated on June 11, 2019 at 1440 and continued through June 19, 2019 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

a.) NOEC survival = 6.7 % effluent
b.) NOEC reproduction = 6.7 % effluent



Summary of the 8-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	90.0	22.4
2.1 %	100	28.0
2.8 %	100	29.1
3.8 %	100	27.3
5.0 %	100	27.0
6.7 %	100	26.4

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: June 11, 2019 at 1215
Date and Time Test Terminated: June 18, 2019 at 1230

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
2.1 %	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
2.8 %	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
3.8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
5.0 %	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
6.7 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	7	7	7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: June 11, 2019 at 1215
Test Terminated: June 18, 2019 at 1230

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91908	.92281	0.00373	8	0.466
	B	.92868	.93259	0.00391	8	0.489
	C	.91396	.91752	0.00356	8	0.445
	D	.91361	.91719	0.00358	8	0.448
	E	.92016	.92410	0.00394	8	0.492
2.1 %	A	.92062	.92491	0.00429	8	0.536
	B	.92552	.92985	0.00433	8	0.541
	C	.93223	.93615	0.00392	8	0.490
	D	.92784	.93139	0.00355	8	0.444
	E	.92257	.92632	0.00375	8	0.469
2.8 %	A	.92785	.93117	0.00332	8	0.415
	B	.92764	.93132	0.00368	8	0.460
	C	.92811	.93216	0.00405	8	0.506
	D	.93056	.93385	0.00329	8	0.411
	E	.93483	.93927	0.00444	8	0.555
3.8 %	A	.92760	.93119	0.00359	8	0.449
	B	.92066	.92427	0.00361	8	0.451
	C	.91117	.91507	0.00390	8	0.488
	D	.92350	.92742	0.00392	8	0.490
	E	.91871	.92281	0.00410	8	0.512
5.0 %	A	.92017	.92426	0.00409	8	0.511
	B	.91911	.92298	0.00387	8	0.484
	C	.92307	.92776	0.00469	8	0.586
	D	.91868	.92247	0.00379	8	0.474
	E	.91874	.92253	0.00379	8	0.474
6.7 %	A	.91808	.92223	0.00415	8	0.519
	B	.92304	.92745	0.00441	8	0.551
	C	.93342	.93763	0.00421	8	0.526
	D	.92975	.93386	0.00411	8	0.514
	E	.92194	.92598	0.00404	8	0.505

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2019 at 1440
Date and Time Test Terminated: June 19, 2019 at 1415

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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	4	0	0	0	0	0	0	4	8	10	0.800
4	0	0	0	0	0	1	2	0	3	0	6	10	0.600
5	7	7	8	11	9	7	7	8	6	8	78	10	7.80
6	9	13	12	11	11	11	8	0	11	12	98	10	9.80
7	1	0	0	0	0	0	0	10	0	0	11	10	1.10
8	0	10	0	0	0	12E	0	13	X	9E	23	9	2.56
TOTAL	17	30	24	22	20	19	17	31	20	24	224	10	22.4

E = Excluded fourth brood neonates

Concentration: 2.1 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	5	4	0	4	4	4	3	5	6	40	10	4.00
4	0	0	0	6	0	0	0	12	0	0	18	10	1.80
5	9	9	9	10	11	10	8	0	9	12	87	10	8.70
6	15	14	13	11	14	15	12	13	12	16	135	10	13.5
7	0	0	10E	2E	0	0	12E	14E	14E	12E	0	10	0.00
8	16E	12E	0	13E	15E	12E	0	0	0	0	0	10	0.00
TOTAL	29	28	26	27	29	29	24	28	26	34	280	10	28.0

E = Excluded fourth brood neonates

Concentration: 2.8 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	5	4	0	4	4	4	5	4	4	38	10	3.80
4	0	0	0	4	0	0	0	3	0	0	7	10	0.700
5	9	9	12	12	11	11	7	9	8	11	99	10	9.90
6	14	14	13	13	15	12	15	12	16	14	138	10	13.8
7	0	0	13E	0	13E	0	15E	12E	9	0	9	10	0.900
8	17E	15E	0	15E	0	14E	0	0	0	9E	0	10	0.00
TOTAL	27	28	29	29	30	27	26	29	37	29	291	10	29.1

E = Excluded fourth brood neonates

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: June 11, 2019 at 1440
Date and Time Test Terminated: June 19, 2019 at 1415

Concentration: 3.8 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	0	4	0	4	4	4	3	5	5	34	10	3.40
4	0	1	0	4	0	0	0	0	0	0	5	10	0.500
5	10	10	9	9	10	10	8	10	11	12	99	10	9.90
6	13	12	14	11	13	13	12	14	15	17	134	10	13.4
7	0	0	14E	0	15E	0	11E	12E	12E	13E	0	10	0.00
8	14E	15E	0	14E	0	15E	1	0	0	0	1	10	0.100
TOTAL	28	23	27	24	27	27	25	27	31	34	273	10	27.3

E = Excluded fourth brood neonates

Concentration: 5.0 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	5	4	0	4	4	4	4	4	4	38	10	3.80
4	0	0	0	4	0	0	0	8	0	1	13	10	1.30
5	11	10	11	12	7	4	9	0	10	10	84	10	8.40
6	14	14	13	12	13	13	13	15	14	14	135	10	13.5
7	0	0	0	1E	0	0	1E	17E	13E	0	0	10	0.00
8	15E	14E	13E	16E	13E	13E	16E	1E	0	12E	0	10	0.00
TOTAL	30	29	28	28	24	21	26	27	28	29	270	10	27.0

E = Excluded fourth brood neonates

Concentration: 6.7 %													
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	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	4	5	0	0	4	4	4	4	3	33	10	3.30
4	0	0	0	4	4	0	0	10	0	3	21	10	2.10
5	10	11	7	10	4	10	9	0	4	10	75	10	7.50
6	15	14	15	13	11	14	11	14	14	14	135	10	13.5
7	0	12E	1E	0	1E	11E	0	17E	17E	0	0	10	0.00
8	12E	0	13E	14E	16E	0	11E	1E	0	15E	0	10	0.00
TOTAL	30	29	27	27	19	28	24	28	22	30	264	10	26.4

E = Excluded fourth brood neonates

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	2.1 %	1	1.00000	1.39310
2	2.1 %	2	1.00000	1.39310
2	2.1 %	3	1.00000	1.39310
2	2.1 %	4	1.00000	1.39310
2	2.1 %	5	1.00000	1.39310
3	2.8 %	1	0.87500	1.20940
3	2.8 %	2	1.00000	1.39310
3	2.8 %	3	1.00000	1.39310
3	2.8 %	4	1.00000	1.39310
3	2.8 %	5	1.00000	1.39310
4	3.8 %	1	1.00000	1.39310
4	3.8 %	2	1.00000	1.39310
4	3.8 %	3	1.00000	1.39310
4	3.8 %	4	1.00000	1.39310
4	3.8 %	5	1.00000	1.39310
5	5 %	1	1.00000	1.39310
5	5 %	2	1.00000	1.39310
5	5 %	3	1.00000	1.39310
5	5 %	4	1.00000	1.39310
5	5 %	5	1.00000	1.39310
6	6.7 %	1	1.00000	1.39310
6	6.7 %	2	1.00000	1.39310
6	6.7 %	3	1.00000	1.39310
6	6.7 %	4	1.00000	1.39310
6	6.7 %	5	0.87500	1.20940

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
D = 0.05399		
W = 0.5466		
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	2.1 %	27.50	16.00	5.00	
3	2.8 %	25.00	16.00	5.00	
4	3.8 %	27.50	16.00	5.00	
5	5 %	27.50	16.00	5.00	
6	6.7 %	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
D = 0.03731		
W = 0.9569		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 7.826		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.01213	0.002426	1.56
Within (Error)	24	0.03731	0.001555	
Total	29	0.04944		

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	0.468	0.468		
2	2.1 %	0.496	0.496	-1.123	
3	2.8 %	0.4694	0.4694	-0.05613	
4	3.8 %	0.478	0.478	-0.401	
5	5 %	0.5058	0.5058	-1.516	
6	6.7 %	0.523	0.523	-2.205	

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	2.1 %	5	0.05886	12.6	-0.028
3	2.8 %	5	0.05886	12.6	-0.0014
4	3.8 %	5	0.05886	12.6	-0.01
5	5 %	5	0.05886	12.6	-0.0378
6	6.7 %	5	0.05886	12.6	-0.055

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test

Identification	Dead	Alive	Total Animals
Control	1	9	10
2.1 %	0	10	10
Total	1	19	20

Critical Fisher's value (10,10,1) (alpha=0.05) is negative. b value is 0. NO SIGNIFICANT DIFFERENCE.

Fisher's Exact Test

Identification	Dead	Alive	Total Animals
Control	1	9	10
2.8 %	0	10	10
Total	1	19	20

Critical Fisher's value (10,10,1) (alpha=0.05) is negative. b value is 0. NO SIGNIFICANT DIFFERENCE.

Fisher's Exact Test

Identification	Dead	Alive	Total Animals
Control	1	9	10
3.8 %	0	10	10
Total	1	19	20

Critical Fisher's value (10,10,1) (alpha=0.05) is negative. b value is 0. NO SIGNIFICANT DIFFERENCE.

Fisher's Exact Test

Identification	Dead	Alive	Total Animals
Control	1	9	10
5.0 %	0	10	10
Total	1	19	20

Critical Fisher's value (10,10,1) (alpha=0.05) is negative. b value is 0. NO SIGNIFICANT DIFFERENCE.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Dead	Alive	Total Animals
Control	1	9	10
6.7 %	0	10	10
Total	1	19	20

Critical Fisher's value (10,10,1) (alpha=0.05) is negative. b value is 0. NO SIGNIFICANT DIFFERENCE.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	1	
1	2.1 %	10	0	
2	2.8 %	10	0	
3	3.8 %	10	0	
4	5.0 %	10	0	
5	6.7 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.1156 D* = 0.907 Critical D* = 1.035 (alpha = 0.01, N = 60) Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 5.028 Critical B = 15.086 (alpha = 0.01, df = 5) Data PASS B1 homogeneity test at 0.01 level.	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	264.8	52.96	4.443
Within (Error)	54	643.8	11.92	
Total	59	908.6		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

Since F > Critical F REJECT Ho: All equal (alpha = 0.05)

Dunnett's Test - Table 1 of 2 — No Transformation

Ho:Control<Treatment

Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	22.4	22.4		
2	2.1 %	28	28	-3.627	
3	2.8 %	29.1	29.1	-4.339	
4	3.8 %	27.3	27.3	-3.174	
5	5 %	27	27	-2.979	
6	6.7 %	26.4	26.4	-2.591	

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	2.1 %	10	3.567	15.9	-5.6
3	2.8 %	10	3.567	15.9	-6.7
4	3.8 %	10	3.567	15.9	-4.9
5	5 %	10	3.567	15.9	-4.6
6	6.7 %	10	3.567	15.9	-4

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	225.5	45.1	3.749
Within (Error)	53	637.4	12.03	
Total	58	862.9		

Critical F = 3.39 (alpha = 0.01, df = 5,53)
2.39 (alpha = 0.05, df = 5,53)

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	22.667	22.667		
2	2.1 %	28	28	-3.346	
3	2.8 %	29.1	29.1	-4.037	
4	3.8 %	27.3	27.3	-2.907	
5	5 %	27	27	-2.719	
6	6.7 %	26.4	26.4	-2.342	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53)
WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.

Dunnett's Test - Table 2 of 2 — No Transformation

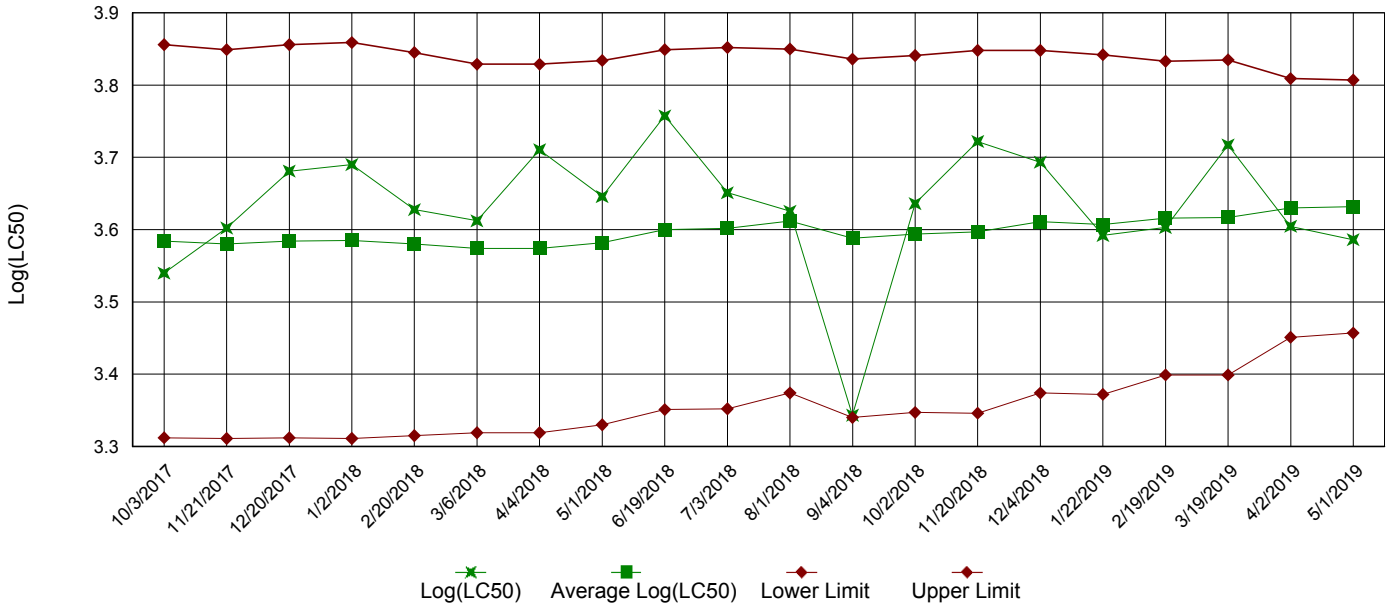
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	9			
2	2.1 %	10	3.681	16.2	-5.333
3	2.8 %	10	3.681	16.2	-6.433
4	3.8 %	10	3.681	16.2	-4.633
5	5 %	10	3.681	16.2	-4.333
6	6.7 %	10	3.681	16.2	-3.733

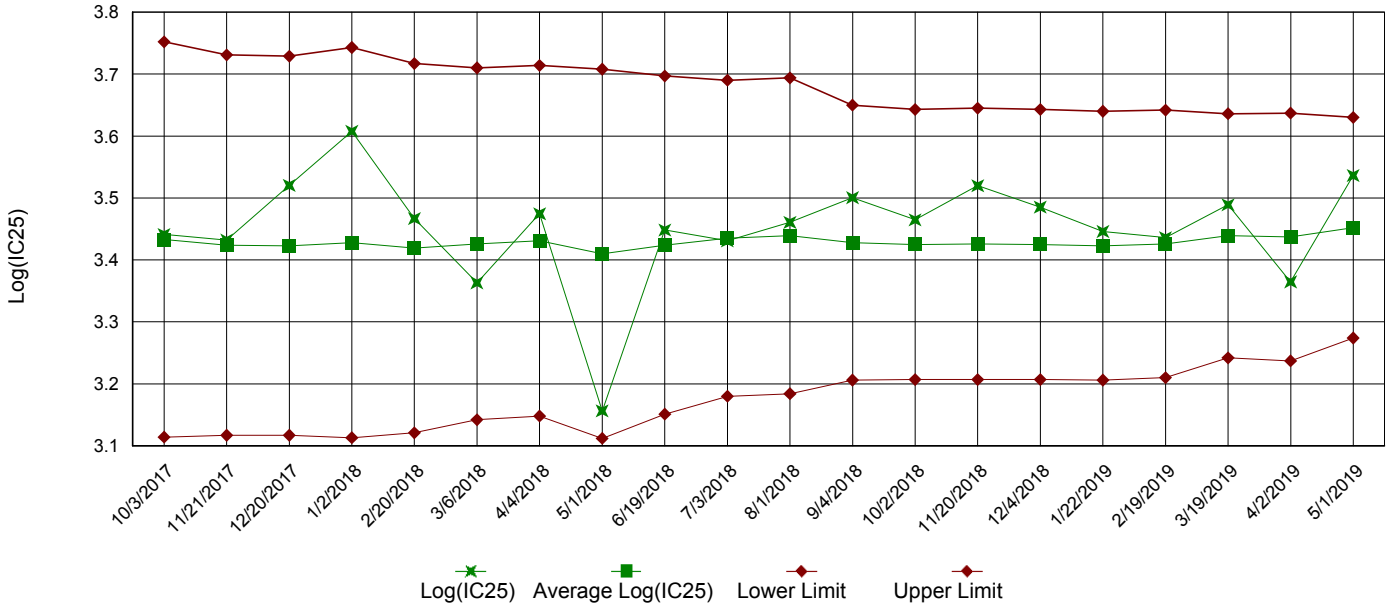
Appendix A3: Test 1000.0

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

LC50 Survival Data



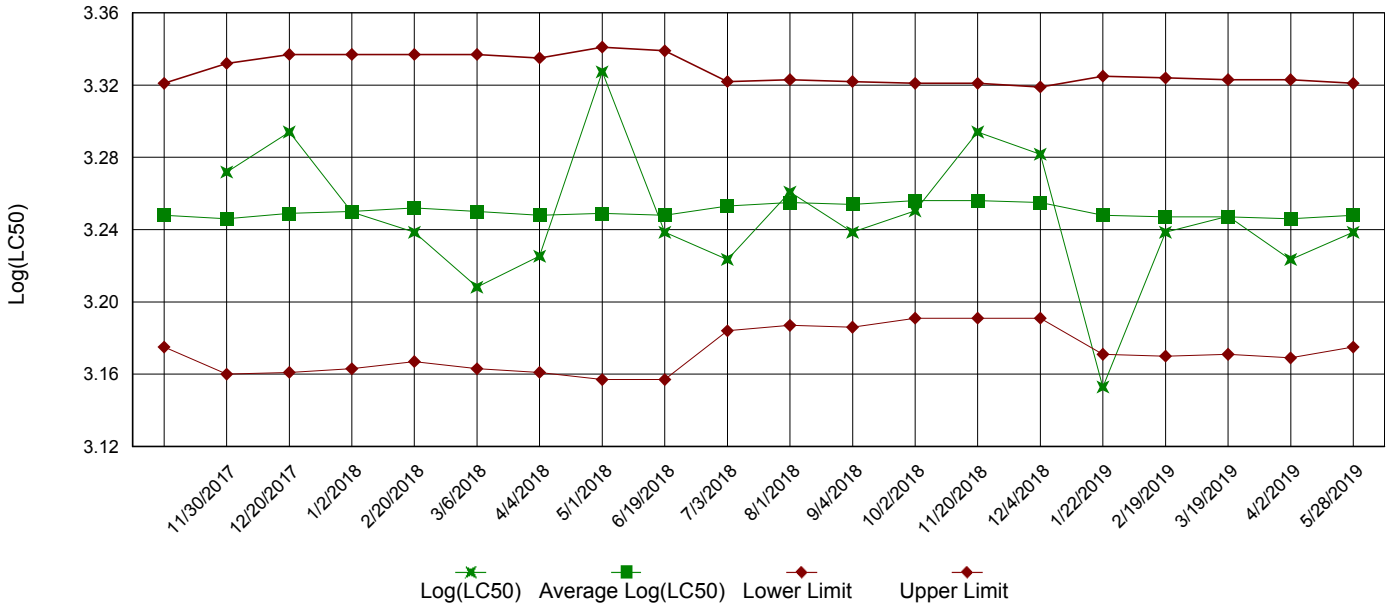
IC25 Growth Data



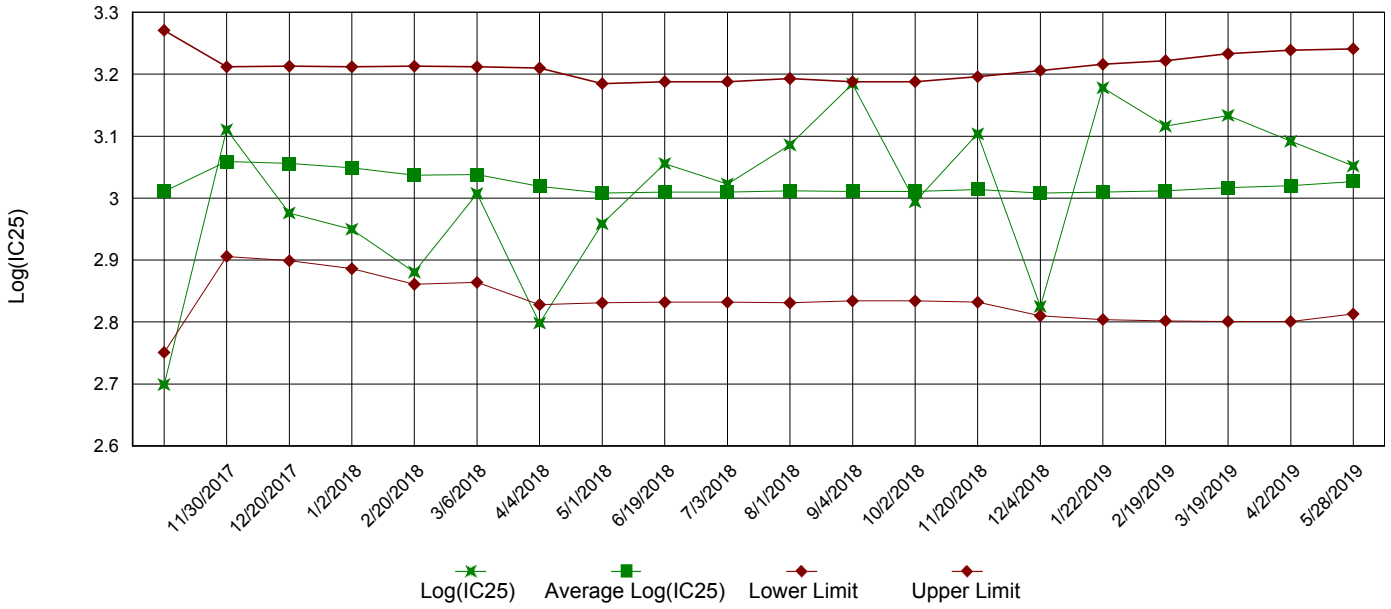
Appendix A3: Test 1002.0

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: Batesville Wastewater Treatment Plant

NPDES No.: NPDES AR0020702 AFIN 32-00044

Date and Time Test Initiated: June 11, 2019 at 1215
Date and Time Test Terminated: June 18, 2019 at 1230
Dilution water used: Moderately Hard

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
2.1 %	100	100	100	100	100	100	100	100	0.00
2.8 %	87.5	100	100	100	100	100	100	97.5	5.73
3.8 %	100	100	100	100	100	100	100	100	0.00
5.0 %	100	100	100	100	100	100	100	100	0.00
6.7 %	100	100	100	100	87.5	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.466	0.489	0.445	0.448	0.492	0.468	4.72
2.1 %	0.536	0.541	0.490	0.444	0.469	0.496	8.49
2.8 %	0.415	0.460	0.506	0.411	0.555	0.469	13.1
3.8 %	0.449	0.451	0.488	0.490	0.512	0.478	5.70
5.0 %	0.511	0.484	0.586	0.474	0.474	0.506	9.36
6.7 %	0.519	0.551	0.526	0.514	0.505	0.523	3.33

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 (5.0 %) ____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION (NA) ____YES ____ NO

2. Dunnett's Test:

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

   a.) LOW FLOW OR CRITICAL DILUTION (5.0 %) ____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION (NA) ____YES ____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP6C)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP6C)

5. NOEC Pimephales Lethality: 6.7 % (TOP6C)

6. LOEC Pimephales Lethality: 6.7 % (TXP6C)

7. NOEC Pimephales Sublethality: 6.7 % (TPP6C)

8. LOEC Pimephales Sublethality: 6.7 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 9.36 (TQP6C)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1000.0

CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: Batesville Wastewater Treatment F
NPDES NO.: NPDES AR0020702 AFIN 32-000
CONTACT: Mr. Eugene Townsley
ANALYST: 280, 310, 343

Test Initiated: DATE: June 11, 2019 TIME: 1215
Test Terminated: DATE: June 18, 2019 TIME: 1230

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	8.3	8.0	7.7	7.6	7.5
Final	6.1	5.9	5.4	7.0	7.3	5.6	6.9
pH Initial	7.8	8.0	7.7	7.9	7.7	7.8	7.8
Final	7.4	7.0	7.2	7.4	7.8	7.3	7.4

DILUTION 2.1 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	8.0	7.8	7.5	7.8	7.6	7.6
Final	5.7	5.6	5.4	6.9	7.1	5.4	6.5
pH Initial	7.8	8.0	7.7	7.8	7.7	7.8	7.8
Final	7.3	7.0	7.2	7.4	7.7	7.3	7.4

DILUTION 2.8 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.2	7.7	8.4	7.4	7.9	7.4	7.3
Final	5.6	5.9	5.5	6.6	7.0	5.2	6.3
pH Initial	7.8	8.0	7.7	7.8	7.8	7.8	7.8
Final	7.3	7.1	7.2	7.5	7.7	7.3	7.4

DILUTION 3.8 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.1	7.6	8.1	7.4	7.8	7.6	7.6
Final	5.9	6.2	5.7	5.8	6.6	5.5	6.6
pH Initial	7.9	8.0	7.7	7.8	7.8	7.8	7.8
Final	7.4	7.1	7.3	7.4	7.6	7.3	7.4

DILUTION 5.0 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	8.0	8.3	7.8	7.3	7.6	7.6
Final	6.0	6.0	5.7	6.5	7.2	5.4	6.7
pH Initial	7.9	8.0	7.7	7.9	7.8	7.8	7.8
Final	7.4	7.1	7.3	7.5	7.8	7.3	7.5

DILUTION 6.7 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	7.3	7.9	7.3	7.6	7.3	7.5
Final	6.2	6.3	5.4	6.3	6.9	6.2	6.6
pH Initial	7.9	8.0	7.7	7.9	7.8	7.8	7.8
Final	7.4	7.2	7.4	7.5	7.7	7.4	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
70	140	600	0.060	Plant Effluent 10-JUN-19
72	140	610	<0.05	Plant Effluent 12-JUN-19
73	140	620	<0.05	Plant Effluent 14-JUN-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
59	84	290	<0.05	234973-1

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Batesville Wastewater Treatment Plant

NPDES No.: NPDES AR0020702 AFIN 32-00044

Date and Time Test Initiated: June 11, 2019 at 1440
Date and Time Test Terminated: June 19, 2019 at 1415
Dilution water used: Moderately Hard

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent 2.1 %	2.8 %	3.8 %	5.0 %	6.7 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
8 day	90.0	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 8 DAYS

Replicates	Control	Percent Effluent 2.1 %	2.8 %	3.8 %	5.0 %	6.7 %
A	17	29	27	28	30	30
B	30	28	28	23	29	29
C	24	26	29	27	28	27
D	22	27	29	24	28	27
E	20	29	30	27	24	19
F	19	29	27	27	21	28
G	17	24	26	25	26	24
H	31	28	29	27	27	28
I	20	26	37	31	28	22
J	24	34	29	34	29	30
Mean per Adult	22.4	28.0	29.1	27.3	27.0	26.4
Mean per Surviving Adult	22.7	28.0	29.1	27.3	27.0	26.4
CV %	22.7	9.52	10.4	11.8	10.0	13.7

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	(5.0 %)	_____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	_____YES	_____NO

2. Dunnett's Test:

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION	(5.0 %)	_____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	_____YES	_____NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 6.7 % (TOP3B)

6. LOEC Ceriodaphnia Lethality: 6.7 % (TXP3B)

7. NOEC Ceriodaphnia Sublethality: 6.7 % (TPP3B)

8. LOEC Ceriodaphnia Sublethality: 6.7 % (TYP3B)

9. Coefficient of variation for Ceriodaphnia Reproduction: 22.7 (TQP3B)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Batesville Wastewater Treatment F
NPDES NO.: NPDES AR0020702 AFIN 32-000
CONTACT: Mr. Eugene Townsley
ANALYST: 280, 310, 343

Test Initiated: DATE: June 11, 2019 TIME: 1440
Test Terminated: DATE: June 19, 2019 TIME: 1415

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	7.8	8.3	8.0	7.7	7.6	7.5
Final	7.4	8.4	8.0	7.2	6.9	7.7	7.5
pH Initial	7.8	8.0	7.7	7.9	7.7	7.8	7.8
Final	8.0	7.8	8.1	7.8	8.1	7.8	7.9

DILUTION 2.1 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	8.0	7.8	7.5	7.8	7.6	7.6
Final	7.4	8.3	7.8	7.0	6.9	7.9	7.6
pH Initial	7.8	8.0	7.7	7.8	7.7	7.8	7.8
Final	8.0	8.0	8.2	7.9	8.1	8.0	8.0

DILUTION 2.8 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.2	7.7	8.4	7.4	7.9	7.4	7.3
Final	7.6	8.1	7.6	7.2	6.7	7.7	7.7
pH Initial	7.8	8.0	7.7	7.8	7.8	7.8	7.8
Final	8.1	8.0	8.1	7.9	8.1	8.0	8.0

DILUTION 3.8 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.1	7.6	8.1	7.4	7.8	7.6	7.6
Final	7.8	8.2	7.8	7.1	6.7	7.6	7.7
pH Initial	7.9	8.0	7.7	7.8	7.8	7.8	7.8
Final	8.1	8.0	8.2	7.9	8.1	8.1	7.9

DILUTION 5.0 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	8.0	8.3	7.8	7.3	7.6	7.6
Final	7.3	8.1	8.0	7.0	7.1	7.4	7.5
pH Initial	7.9	8.0	7.7	7.9	7.8	7.8	7.8
Final	8.0	8.0	8.2	8.0	8.1	8.1	8.0

DILUTION 6.7 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	7.3	7.9	7.3	7.6	7.3	7.5
Final	7.3	8.4	8.2	7.0	6.8	7.6	7.6
pH Initial	7.9	8.0	7.7	7.9	7.8	7.8	7.8
Final	8.0	8.0	8.2	8.1	8.1	8.1	8.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
70	140	600	0.060	Plant Effluent 10-JUN-19
72	140	610	<0.05	Plant Effluent 12-JUN-19
73	140	620	<0.05	Plant Effluent 14-JUN-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
59	84	290	<0.05	234973-1

235198

Batesville Wastewater Treatment Plant
Chain of Custody

Sampled By: Michael McDaniel Date Sampled: 6-10-19

(1)

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Annalyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	6-10-19/2400				C	P	Cronic Biomonitoring	4°C	3

COMMENT: 3.91 MGD

Effluent Flow: ~~17115478~~6

Chronic Biomonitoring

Relinquished By: Jaye Smith Date/Time: 6-11-19/0700

Received By: [signature] Date/Time: 6-11-19/0700

Relinquished By: [signature] Date/Time: 6-11-19/0905

Received By: [signature] Date/Time: 6-11-19 0905

COMMENT: 0.4°C

G=Grab C=Composite pH in S.U. P=Plastic Gl =Glass Temp/C

235198

Batesville Wastewater Treatment Plant
Chain of Custody

Sampled By: Michael McDaniel Date Sampled: 6-12-19

(2)

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Annalyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	6-12-19/2400				C	P	Cronic BioMonitoring	4°C	3

COMMENT:

Effluent Flow; 3.99 Chronic Biomonitoring

Relinquished By: Jay Cousin Date/Time: 6-13-19 /0700

Received By: Michael McDaniel Date/Time: 6-13-19/0700

Relinquished By: Michael McDaniel Date/Time: 6-13-19/0853

Received By: AC341 Date/Time: 06-13-19 /0853

COMMENT:

0.4

G=Grab C=Composite pH in S.U. P=Plastic Gl =Glass Temp/C

235198

Batesville Wastewater Treatment Plant
Chain of Custody

Sampled By: Michael McDaniel Date Sampled: 6-14-19

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Annalyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	6-14-19/2400				C	P	Chronic Biomonitoring	4°C	3

3

COMMENT:

Effluent Flow: 4.05 Chronic Biomonitoring

Relinquished By: Date/Time:

Risley Pool - 6-15-19-0700

Received By: Date/Time:

6-15-19/0700

Relinquished By: Date/Time:

6-15-19/0820

Received By: Date/Time:

15 Jun 19 (0820) 0.6°

COMMENT:

G=Grab C=Composite pH in S.U. P=Plastic Gl=Glass Temp/C