



February 26, 2018

Biomonitoring Testing
for
Outfall 001
Batesville, AR

Control No. 220477-1

Prepared for:

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

Prepared by:

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8600 Kanis Road
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Batesville Wastewater Treatment Plant
ATTN: Mr. Eugene Townsley
500 River Bank Road
Batesville, AR 72501

Re: Chronic *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001 - 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

A handwritten signature in black ink is positioned above a horizontal line. Below the line, the name 'John Overbey' and title 'Chief Operating Officer' are printed in a standard font.

PDF cc: Batesville Wastewater Treatment Plant
ATTN: Mr. Eugene Townsley
wwsuper@cityofbatesville.com

Table of Contents

- I. Control Acceptance Criteria
- II. Outlined Report
- III. Data Analysis
- IV. Standard Reference Toxicants
- V. Organism History
- VI. Results Summary
 - Pimephales promelas* (Fathead minnow)
 - Ceriodaphnia dubia*
- Appendix A: Raw Data
 - A1: Test 1000.0
 - Pimephales promelas* (Fathead minnow) Survival and Growth
 - Test 1002.0
 - Ceriodaphnia dubia* Survival and Reproduction
 - A2: Statistics
 - A3: Reference Toxicant
- Appendix B: Summary Forms

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	0.377	PASS
Control Growth CV < or = 40%	9.27	PASS
Growth Minimum Significant Difference 12 to 30%	10.3	BELOW
Critical Dilution CV < or = 40%	6.66	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	22.4	PASS
Control CV < or = 40% per Surviving Female	21.5	PASS
Reproduction Minimum Significant Difference 13 to 47%	27.3	PASS
Critical Dilution CV < or = 40%	18.5	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: Outfall 001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.0	6.1	8.7
pH (standard units)	7.8	7.2	7.9
Alkalinity (mg/l as CaCO ₃)	78	79	79
Hardness (mg/l as CaCO ₃)	150	150	150
Conductivity (umhos/cm)	540	550	540
Residual Chlorine (mg/l)	0.070	<0.05	<0.05
Ammonia as N (mg/l)	0.13	0.21	0.34

2. Dilution Water Samples:
Soft

Analysis	220370
Dissolved oxygen (mg/l)	8.5
pH (standard units)	8.4
Alkalinity (mg/l as CaCO ₃)	32
Hardness (mg/l as CaCO ₃)	47
Conductivity (umhos/cm)	250
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: February 6, 2018 at 1640
Date & Time Test Terminated: 13FEB18 at 0850
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: February 6, 2018 at 1540
Date & Time Test Terminated: 14FEB18 at 1400
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 January 2, 2018 at 1550 to Jan 9, 2018 at 0830

The results were as follows: (Control No. 219494-1.)

Survival LC-50: 4899 mg/l

Growth IC-25: 4052 mg/l

Growth PMSD: 21.2

Ceriodaphnia dubia

A chronic reference test was performed on January 2, 2018 at 1445 to Jan 9, 2018 at 1415

The results were as follows: (Control No. 219494-2.)

Survival LC-50: 1777 mg/l

Growth IC-25: 890.6 mg/l

Growth PMSD: 21.1

V. Organism History

Pimephales promelas (Fathead minnow)

Date: February 6, 2018

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: February 6, 2018

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

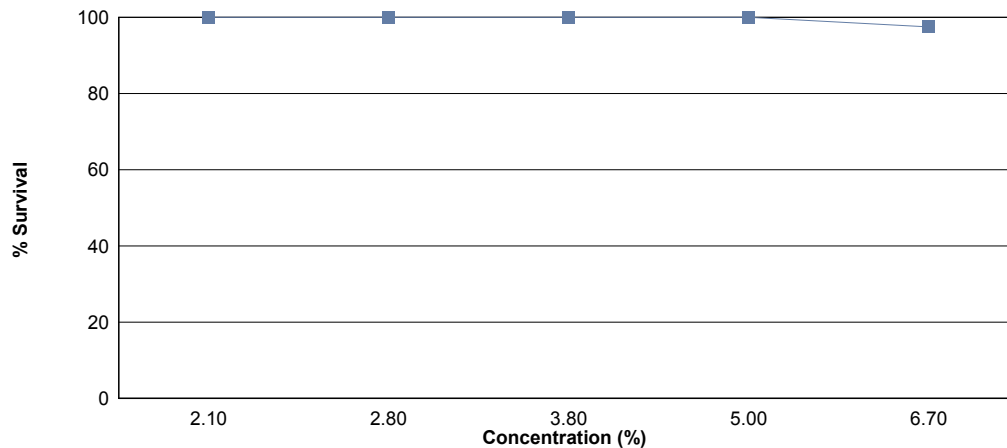
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 February 6, 2018 at 1640 and continued through 13FEB18 at 0850. 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	0.377
2.1 %	100	0.377
2.8 %	100	0.378
3.8 %	100	0.380
5.0 %	100	0.381
6.7 %	97.5	0.381

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

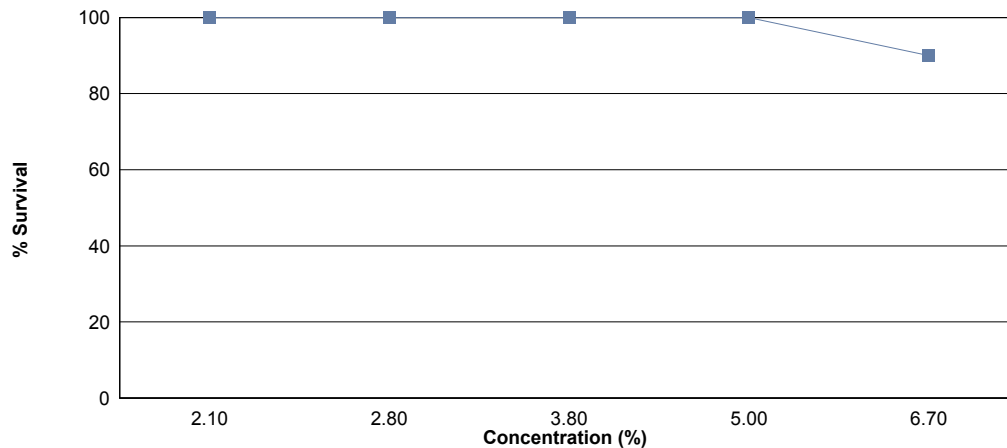
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 February 6, 2018 at 1540 and continued through 14FEB18 at 1400. 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 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	22.4
2.1 %	100	21.7
2.8 %	100	22.4
3.8 %	100	23.5
5.0 %	100	23.5
6.7 %	90.0	20.5

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: February 6, 2018 at 1640

Date and Time Test Terminated: 13FEB18 at 0850

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	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
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	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: February 6, 2018 at 1640

Test Terminated: 13FEB18 at 0850

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93801	.94079	0.00278	8	0.348
	B	.92685	.92967	0.00282	8	0.352
	C	.92513	.92849	0.00336	8	0.420
	D	.93039	.93367	0.00328	8	0.410
	E	.92790	.93074	0.00284	8	0.355
2.1 %	A	.94235	.94530	0.00295	8	0.369
	B	.94307	.94615	0.00308	8	0.385
	C	.93947	.94250	0.00303	8	0.379
	D	.93664	.93987	0.00323	8	0.404
	E	.93972	.94251	0.00279	8	0.349
2.8 %	A	.94228	.94557	0.00329	8	0.411
	B	.93523	.93811	0.00288	8	0.360
	C	.93789	.94092	0.00303	8	0.379
	D	.93583	.93868	0.00285	8	0.356
	E	.93536	.93844	0.00308	8	0.385
3.8 %	A	.93122	.93407	0.00285	8	0.356
	B	.92918	.93228	0.00310	8	0.388
	C	.93995	.94301	0.00306	8	0.382
	D	.93802	.94115	0.00313	8	0.391
	E	.93475	.93781	0.00306	8	0.382
5.0 %	A	.93150	.93455	0.00305	8	0.381
	B	.93590	.93868	0.00278	8	0.348
	C	.93166	.93480	0.00314	8	0.392
	D	.93487	.93782	0.00295	8	0.369
	E	.93060	.93393	0.00333	8	0.416
6.7 %	A	.93579	.93863	0.00284	8	0.355
	B	.93906	.94214	0.00308	8	0.385
	C	.92888	.93217	0.00329	8	0.411
	D	.92879	.93151	0.00272	8	0.340
	E	.93466	.93799	0.00333	8	0.416

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: February 6, 2018 at 1540

Date and Time Test Terminated: 14FEB18 at 1400

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	6	2	7	4	4	4	3	3	4	41	10	4.10	
5	9	0	8	0	0	9	7	8	0	0	41	10	4.10	
6	0	10	0	11	8	0	0	0	7	7	43	10	4.30	
7	13	11	11	14	12	8	8	8	6	8	99	10	9.90	
8														
TOTAL	26	27	21	32	24	21	19	19	16	19	224	10	22.4	

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	4	2	3	4	6	4	4	5	2	4	38	10	3.80	
5	7	0	7	0	0	7	0	8	0	0	29	10	2.90	
6	0	0	0	10	11	0	7	0	6	8	42	10	4.20	
7	15	3	17	18	16	6	8	9	8	8	108	10	10.8	
8														
TOTAL	26	5	27	32	33	17	19	22	16	20	217	10	21.7	

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	3	4	4	4	5	4	4	4	4	5	41	10	4.10	
5	7	0	9	0	0	7	0	9	0	2	34	10	3.40	
6	0	10	0	10	9	0	4	0	3	6	42	10	4.20	
7	15	17	14	16	14	7	7	7	5	5	107	10	10.7	
8														
TOTAL	25	31	27	30	28	18	15	20	12	18	224	10	22.4	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: February 6, 2018 at 1540

Date and Time Test Terminated: 14FEB18 at 1400

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	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	5	6	5	5	5	4	5	0	4	5	44	10	4.40	
5	8	10	8	0	0	9	0	4	0	8	47	10	4.70	
6	0	0	0	12	8	0	9	9	6	0	44	10	4.40	
7	12	14	14	12	14	6	5	6	9	8	100	10	10.0	
8														
TOTAL	25	30	27	29	27	19	19	19	19	21	235	10	23.5	

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
4	4	7	7	6	6	6	4	4	6	4	54	10	5.40	
5	7	0	6	0	0	8	0	9	0	0	30	10	3.00	
6	0	9	0	6	10	0	8	0	6	6	45	10	4.50	
7	14	10	10	13	17	8	8	11	8	7	106	10	10.6	
8														
TOTAL	25	26	23	25	33	22	20	24	20	17	235	10	23.5	

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	0	0	0	0	0	0	X	0	0	0	0	9	0.00	
4	4	6	4	5	4	6	X	0	4	4	37	9	4.11	
5	6	0	8	0	0	7	X	8	0	9	38	9	4.22	
6	0	9	1	5	8	0	X	7	6	0	36	9	4.00	
7	11	13	13	18	14	8	X	0	8	9	94	9	10.4	
8														
TOTAL	21	28	26	28	26	21	0	15	18	22	205	10	20.5	

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	1.00000	1.39310
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	0.87500	1.20940
6	6.7 %	4	1.00000	1.39310
6	6.7 %	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 = 0.027 W = 0.4161 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	2.1 %	27.50	16.00	5.00	
3	2.8 %	27.50	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
<p>D = 0.01632 W = 0.944 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 = 3.890 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	9.507E-05	1.901E-05	0.02796	
Within (Error)	24	0.01632	0.00068		
Total	29	0.01642			
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	0.377	0.377			
2	2.1 %	0.3772	0.3772	-0.01213		
3	2.8 %	0.3782	0.3782	-0.07276		
4	3.8 %	0.3798	0.3798	-0.1698		
5	5 %	0.3812	0.3812	-0.2547		
6	6.7 %	0.3814	0.3814	-0.2668		
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	0.03892	10.3	-0.0002	
3	2.8 %	5	0.03892	10.3	-0.0012	
4	3.8 %	5	0.03892	10.3	-0.0028	
5	5 %	5	0.03892	10.3	-0.0042	
6	6.7 %	5	0.03892	10.3	-0.0044	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
2.1 %	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
2.8 %	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
3.8 %	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
5.0 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
6.7 %	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.

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

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.0873 D* = 0.6849 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 = 7.782 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	64.93	12.99	0.316	
Within (Error)	54	2220	41.11		
Total	59	2285			
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	22.4	22.4			
2	2.1 %	21.7	21.7	0.2441		
3	2.8 %	22.4	22.4	0		
4	3.8 %	23.5	23.5	-0.3836		
5	5 %	23.5	23.5	-0.3836		
6	6.7 %	20.5	20.5	0.6626		
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	6.624	29.6	0.7	
3	2.8 %	10	6.624	29.6	0	
4	3.8 %	10	6.624	29.6	-1.1	
5	5 %	10	6.624	29.6	-1.1	
6	6.7 %	10	6.624	29.6	1.9	

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	24.65	4.93	0.149	
Within (Error)	53	1753	33.08		
Total	58	1778			
Critical F = 3.39 (alpha = 0.01, df = 5,53) 2.39 (alpha = 0.05, df = 5,53)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

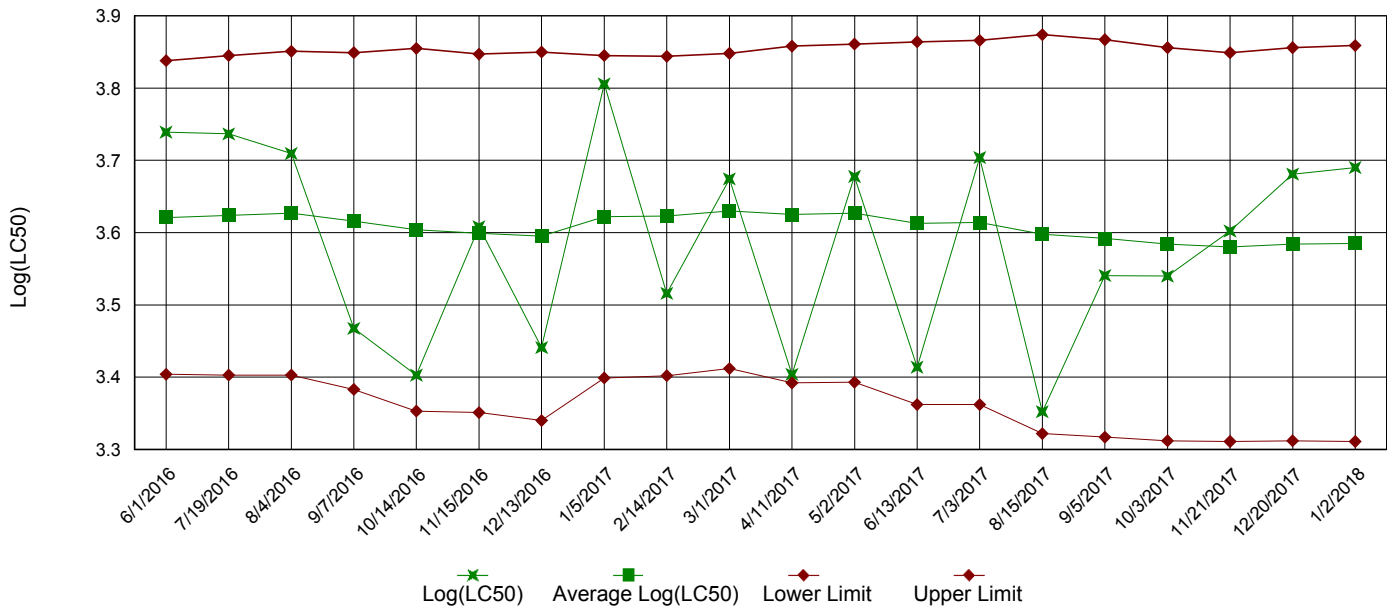
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	22.4	22.4			
2	2.1 %	21.7	21.7	0.2721		
3	2.8 %	22.4	22.4	0		
4	3.8 %	23.5	23.5	-0.4277		
5	5 %	23.5	23.5	-0.4277		
6	6.7 %	22.778	22.778	-0.143		
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	10				
2	2.1 %	10	5.942	26.5	0.7	
3	2.8 %	10	5.942	26.5	0	
4	3.8 %	10	5.942	26.5	-1.1	
5	5 %	10	5.942	26.5	-1.1	
6	6.7 %	9	6.105	27.3	-0.378	

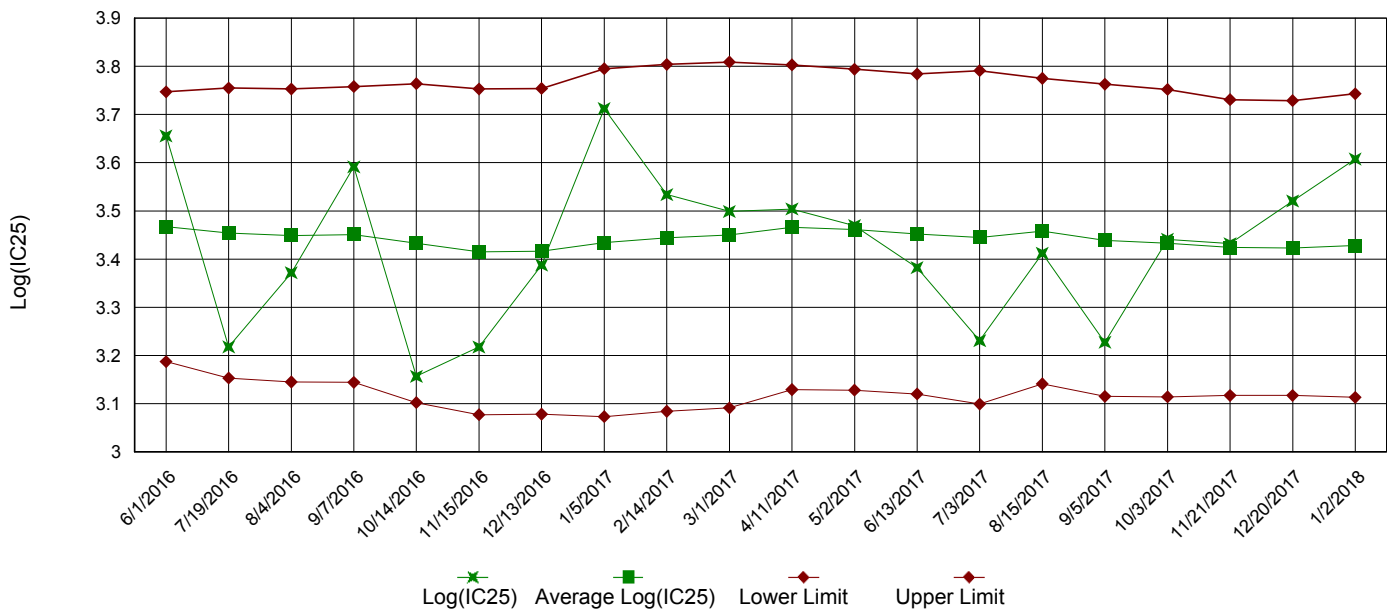
Appendix A3: Test 1000.0

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

LC50 Survival Data

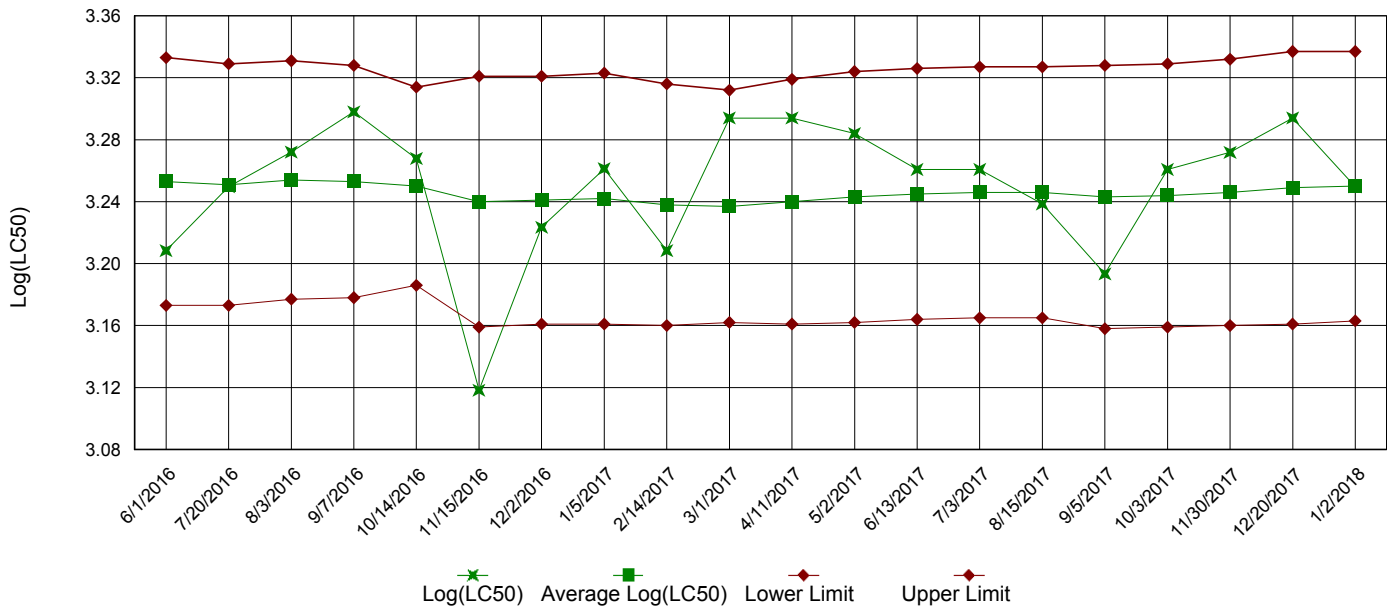


IC25 Growth Data

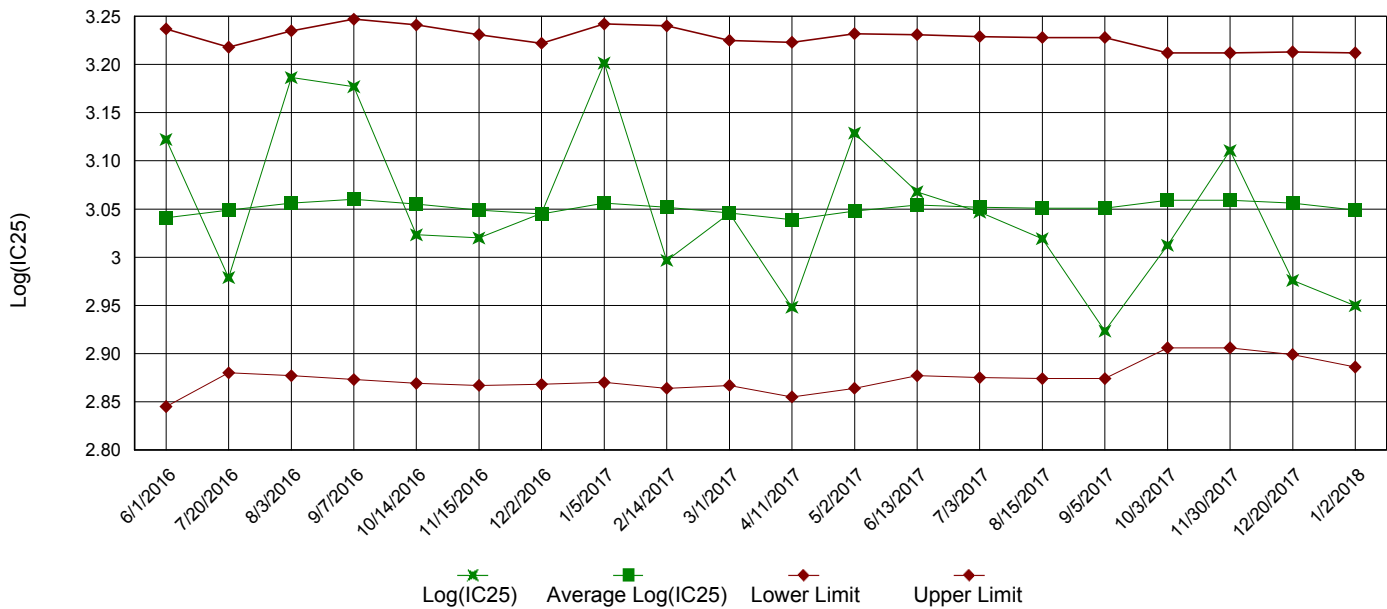


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: February 6, 2018 at 1640

Date and Time Test Terminated: 13FEB18 at 0850

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
2.1 %	100	100	100	100	100	100	100	100	0.00
2.8 %	100	100	100	100	100	100	100	100	0.00
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	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	0.348	0.352	0.420	0.410	0.355	0.377	9.27
2.1 %	0.369	0.385	0.379	0.404	0.349	0.377	5.38
2.8 %	0.411	0.360	0.379	0.356	0.385	0.378	5.83
3.8 %	0.356	0.388	0.382	0.391	0.382	0.38	3.65
5.0 %	0.381	0.348	0.392	0.369	0.416	0.381	6.66
6.7 %	0.355	0.385	0.411	0.340	0.416	0.381	8.79

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 %)	<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	(5.0 %)	<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: 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: 9.27 (TQP6C)

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, 322

 Test Initiated: DATE: February 6, 2018 TIME: 1640
 Test Terminated: DATE: 13FEB18 TIME: 0850

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.5	9.0	8.2	8.0	7.9	7.9	8.1
Final	7.9	8.4	8.2	8.0	7.7	7.5	8.4
pH Initial	8.4	8.1	8.0	8.1	8.2	8.2	7.9
Final	8.3	8.1	7.9	8.0	8.1	7.4	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
2.1 %							
D.O. Initial	8.2	9.0	7.8	7.9	7.5	8.0	8.3
Final	7.7	8.3	7.7	8.1	7.7	7.2	8.4
pH Initial	8.4	8.2	8.0	8.0	8.1	8.2	7.9
Final	8.2	8.1	8.0	8.0	8.1	7.4	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
2.8 %							
D.O. Initial	8.2	8.8	8.2	7.9	8.7	7.8	8.3
Final	7.9	8.3	8.2	8.0	7.7	7.2	8.3
pH Initial	8.4	8.1	8.0	8.0	8.1	8.2	7.9
Final	8.2	8.1	7.9	8.0	8.1	7.4	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
3.8 %							
D.O. Initial	8.2	8.8	8.1	7.9	7.8	7.7	8.2
Final	7.8	8.3	8.0	7.9	8.0	7.4	8.3
pH Initial	8.4	8.1	8.0	8.0	8.1	8.2	7.9
Final	8.2	8.2	7.9	8.0	8.1	7.5	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
5.0 %							
D.O. Initial	8.4	8.7	8.2	8.1	7.9	8.0	8.2
Final	7.9	8.5	8.2	7.8	7.8	7.4	8.3
pH Initial	8.4	8.2	8.0	8.1	8.2	8.2	7.9
Final	8.2	8.2	8.0	8.0	8.1	7.5	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
6.7 %							
D.O. Initial	8.3	8.6	8.1	7.8	7.8	7.9	8.2
Final	7.8	8.1	8.0	7.9	7.9	7.1	8.3
pH Initial	8.4	8.2	8.0	8.1	8.2	8.2	7.8
Final	8.2	8.2	8.0	8.0	8.1	7.6	8.0

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
78	150	540	0.070	Plant Effluent 05-FEB-18
79	150	550	<0.05	Plant Effluent 07-FEB-18
79	150	540	<0.05	Plant Effluent 09-FEB-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	47	250	<0.05	220370

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: February 6, 2018 at 1540

Date and Time Test Terminated: 14FEB18 at 1400

Dilution water used: Soft

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
7 day	100	100	100	100	100	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		2.1 %	2.8 %	3.8 %	5.0 %	6.7 %
A	26	26	25	25	25	21
B	27	5	31	30	26	28
C	21	27	27	27	23	26
D	32	32	30	29	25	28
E	24	33	28	27	33	26
F	21	17	18	19	22	21
G	19	19	15	19	20	0
H	19	22	20	19	24	15
I	16	16	12	19	20	18
J	19	20	18	21	17	22
Mean per Adult	22.4	21.7	22.4	23.5	23.5	20.5
Mean per Surviving Adult	22.4	21.7	22.4	23.5	23.5	22.8
CV %	21.5	38.4	29.7	19.4	18.5	20.0

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 %)	<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 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 %)	<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 (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: 21.5 (TQP3B)

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, 322

 Test Initiated: DATE: February 6, 2018 TIME: 1540
 Test Terminated: DATE: 14FEB18 TIME: 1400

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.5	9.0	8.2	8.0	7.9	7.9	8.1
Final	8.5	8.5	8.1	7.8	7.7	8.2	8.8
pH Initial	8.4	8.1	8.0	8.1	8.2	8.2	7.9
Final	8.0	8.0	8.0	8.4	8.3	7.8	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
2.1 %							
D.O. Initial	8.2	9.0	7.8	7.9	7.5	8.0	8.3
Final	8.4	8.4	7.9	8.0	7.8	8.1	8.7
pH Initial	8.4	8.2	8.0	8.0	8.1	8.2	7.9
Final	8.0	8.0	8.1	8.4	8.3	7.8	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
2.8 %							
D.O. Initial	8.2	8.8	8.2	7.9	8.7	7.8	8.3
Final	8.6	8.4	8.2	8.0	8.1	7.9	8.6
pH Initial	8.4	8.1	8.0	8.0	8.1	8.2	7.9
Final	8.0	8.0	8.1	8.4	8.4	7.8	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
3.8 %							
D.O. Initial	8.2	8.8	8.1	7.9	7.8	7.7	8.2
Final	8.4	8.3	8.0	7.9	8.2	8.2	8.6
pH Initial	8.4	8.1	8.0	8.0	8.1	8.2	7.9
Final	8.0	8.1	8.1	8.5	8.4	7.9	8.1

DILUTION	DAY						
	1	2	3	4	5	6	7
5.0 %							
D.O. Initial	8.4	8.7	8.2	8.1	7.9	8.0	8.2
Final	8.6	8.7	8.2	8.0	8.1	8.1	8.9
pH Initial	8.4	8.2	8.0	8.1	8.2	8.2	7.9
Final	8.1	8.1	8.1	8.6	8.5	7.8	8.1

DILUTION	DAY						
	1	2	3	4	5	6	7
6.7 %							
D.O. Initial	8.3	8.6	8.1	7.8	7.8	7.9	8.2
Final	8.6	8.2	8.0	8.0	8.2	8.2	8.4
pH Initial	8.4	8.2	8.0	8.1	8.2	8.2	7.8
Final	8.2	8.1	8.2	8.7	8.6	7.8	8.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
78	150	540	0.070	Plant Effluent 05-FEB-18
79	150	550	<0.05	Plant Effluent 07-FEB-18
79	150	540	<0.05	Plant Effluent 09-FEB-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	47	250	<0.05	220370

220477

Batesville Wastewater Treatment Plant Chain of Custody

Sampled By: Michael McDANIEL

Date Sampled: 2-5-18

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	2-5-18/2400				C	P	Chronic Biomonitoring	4°C	3

①

COMMENT:

Effluent Flow: 6.05

Chronic Biomonitoring

Relinquished By:

Date/Time:

Relinquished By: Kelly Pool - 2-6-18 - 0700

Received By:

Date/Time:

Received By: Michael McDANIEL 2-6-18/0700

Relinquished By:

Date/Time:

Relinquished By: Michael McDANIEL 2-6-18/0856

Received By:

Date/Time:

Received By: D. Brown 2-6-18/0856 0.1°C

COMMENT:

Batesville Wastewater Treatment Plant
Chain of Custody

Sampled By: Michael McDaniel

Date Sampled: 2-7-18

(2)

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	2-7-18/2400				C	P	Chronic Biomonitoring	4°C	3

COMMENT:

Effluent Flow; 4.06

Chronic Biomonitoring

Relinquished By:

Date/Time:

Holly Seymore

2-8-18/0700

Received By:

Date/Time:

Michael McDaniel

2-8-18/0700

Relinquished By:

Date/Time:

Michael McDaniel

2-8-18/0857

Received By:

Date/Time:

H. Dalindo

2-8-18/0857

COMMENT:

2.0°C

220477

Batesville Wastewater Treatment Plant Chain of Custody

Sampled By: Michael McDaniel

Date Sampled: 2-9-18

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	2-9-18/2400				C	P	Chronic Biomonitoring	4°C	3

3

COMMENT:

Effluent Flow: 6.12

Chronic Biomonitoring

Relinquished By:

Date/Time:

Harry Gynore

2-10-18 / 0700

Received By:

Date/Time:

Michael McDaniel

2-10-18 / 0700

Relinquished By:

Date/Time:

Michael McDaniel

2-10-18 / 0845

Received By:

Date/Time:

Michael McDaniel

2-10-18 / 0845

COMMENT:

0.2°C

G=Grab C=Composite

pH in S.U.

P=Plastic

GL=Glass

Temp/C