



October 8, 2018

Biomonitoring Testing  
for  
Plant Effluent  
Batesville, AR

Control No. 227257-1

Prepared for:

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

Prepared by:

AMERICAN INTERPLEX CORPORATION  
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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*  
Plant Effluent - Batesville, AR  
NPDES Permit No. NPDES AR0020702 AFIN 32-00044

Dear Mr. Eugene Townsley:

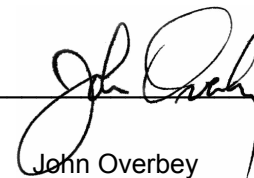
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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**



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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	0.402	PASS
Control Growth CV < or = 40%	13.6	PASS
Growth Minimum Significant Difference 12 to 30%	16.1	PASS
Critical Dilution CV < or = 40%	13.5	PASS

*Ceriodaphnia dubia* Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	23.6	PASS
Control CV < or = 40% per Surviving Female	24.8	PASS
Reproduction Minimum Significant Difference 13 to 47%	29.0	PASS
Critical Dilution CV < or = 40%	26.0	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 Effluent
  - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.3	6.0	6.0
pH (standard units)	8.2	8.6	8.4
Alkalinity (mg/l as CaCO <sub>3</sub> )	160	150	150
Hardness (mg/l as CaCO <sub>3</sub> )	130	140	140
Conductivity (umhos/cm)	680	690	640
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.13	<0.1	<0.1

2. Dilution Water Samples:  
Moderately Hard

Analysis	226930
Dissolved oxygen (mg/l)	6.1
pH (standard units)	8.1
Alkalinity (mg/l as CaCO <sub>3</sub> )	59
Hardness (mg/l as CaCO <sub>3</sub> )	81
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: September 25, 2018 at 1550  
Date & Time Test Terminated: October 02, 2018 at 0830  
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: September 25, 2018 at 1605  
Date & Time Test Terminated: October 01, 2018 at 1605  
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 September 4, 2018 at 1415 to September 11, 2018 at 1540

The results were as follows: (Control No. 226620-1.)

Survival LC-50: 2204 mg/l

Growth IC-25: 3166 mg/l

Growth PMSD: 17.4

##### *Ceriodaphnia dubia*

A chronic reference test was performed on September 4, 2018 at 1410 to September 11, 2018 at 1630

The results were as follows: (Control No. 226620-2.)

Survival LC-50: 1732 mg/l

Growth IC-25: 1529 mg/l

Growth PMSD: 23.3

#### V. Organism History

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

Date: September 25, 2018

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

##### *Ceriodaphnia dubia*

Date: September 25, 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

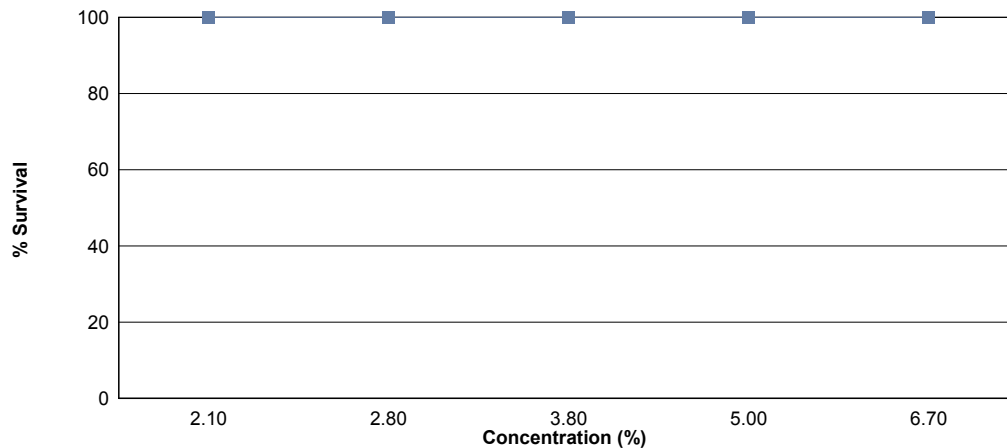
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 September 25, 2018 at 1550 and continued through October 02, 2018 at 0830. 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	0.402
2.1 %	100	0.419
2.8 %	100	0.374
3.8 %	100	0.340
5.0 %	100	0.354
6.7 %	100	0.373

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

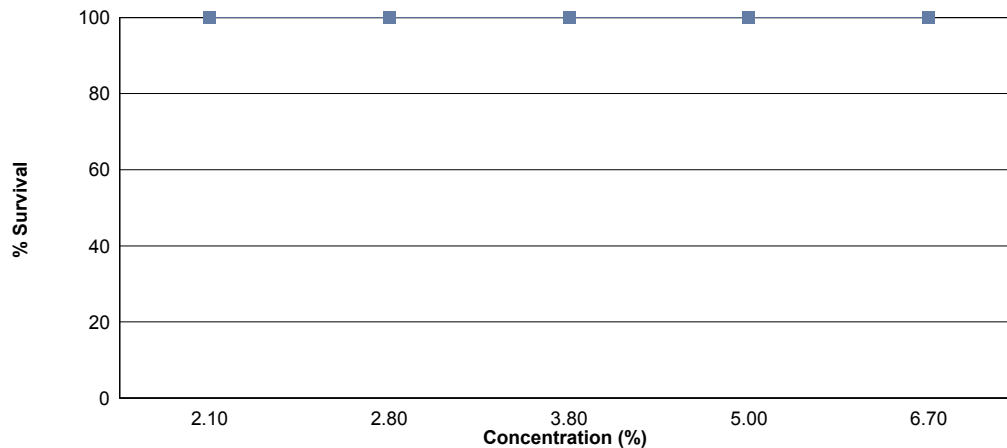
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 September 25, 2018 at 1605 and continued through October 01, 2018 at 1605. 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 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	23.6
2.1 %	100	23.5
2.8 %	100	21.6
3.8 %	100	25.7
5.0 %	100	23.9
6.7 %	100	24.5



Appendix A1: Test 1000.0

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

Date and Time Test Initiated: September 25, 2018 at 1550

Date and Time Test Terminated: October 02, 2018 at 0830

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	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: September 25, 2018 at 1550

Test Terminated: October 02, 2018 at 0830

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93926	.94188	0.00262	8	0.328
	B	.93500	.93804	0.00304	8	0.380
	C	.93690	.94055	0.00365	8	0.456
	D	.93520	.93830	0.00310	8	0.388
	E	.93444	.93809	0.00365	8	0.456
2.1 %	A	.93375	.93692	0.00317	8	0.396
	B	.93049	.93406	0.00357	8	0.446
	C	.93725	.94036	0.00311	8	0.389
	D	.93731	.94063	0.00332	8	0.415
	E	.93950	.94308	0.00358	8	0.448
2.8 %	A	.93628	.93915	0.00287	8	0.359
	B	.93648	.93973	0.00325	8	0.406
	C	.93574	.93893	0.00319	8	0.399
	D	.93734	.94025	0.00291	8	0.364
	E	.93600	.93874	0.00274	8	0.342
3.8 %	A	.93372	.93633	0.00261	8	0.326
	B	.93226	.93536	0.00310	8	0.388
	C	.93485	.93707	0.00222	8	0.278
	D	.93052	.93388	0.00336	8	0.420
	E	.93103	.93335	0.00232	8	0.290
5.0 %	A	.93500	.93785	0.00285	8	0.356
	B	.93260	.93504	0.00244	8	0.305
	C	.92959	.93226	0.00267	8	0.334
	D	.93331	.93677	0.00346	8	0.432
	E	.93161	.93434	0.00273	8	0.341
6.7 %	A	.93197	.93515	0.00318	8	0.398
	B	.93007	.93296	0.00289	8	0.361
	C	.93289	.93569	0.00280	8	0.350
	D	.93202	.93526	0.00324	8	0.405
	E	.93895	.94176	0.00281	8	0.351

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: September 25, 2018 at 1605

Date and Time Test Terminated: October 01, 2018 at 1605

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	4	5	3	0	4	5	5	5	4	4	39	10	3.90	
4	0	0	0	4	6	0	0	0	0	0	10	10	1.00	
5	12	10	10	11	9	10	8	10	10	7	97	10	9.70	
6	13	13	0	0	0	12	11	14	12	15	90	10	9.00	
7														
8														
TOTAL	29	28	13	15	19	27	24	29	26	26	236	10	23.6	

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	4	5	4	6	4	4	2	4	4	4	41	10	4.10
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	8	10	10	8	10	11	4	10	10	9	90	10	9.00
6	12	11	0	14	13	14	0	10	14	16	104	10	10.4
7													
8													
TOTAL	24	26	14	28	27	29	6	24	28	29	235	10	23.5

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	5	5	5	2	3	4	4	4	41	10	4.10
4	0	0	0	0	0	0	0	0	0	3	3	10	0.300
5	9	11	11	10	11	11	9	7	9	0	88	10	8.80
6	16	12	0	0	15	11	0	17	11	2	84	10	8.40
7													
8													
TOTAL	29	28	16	15	31	24	12	28	24	9	216	10	21.6

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: September 25, 2018 at 1605

Date and Time Test Terminated: October 01, 2018 at 1605

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	6	4	5	6	4	4	0	5	4	4	42	10	4.20	
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
5	13	10	11	12	11	10	4	8	9	10	98	10	9.80	
6	12	14	0	14	13	13	8	12	16	15	117	10	11.7	
7														
8														
TOTAL	31	28	16	32	28	27	12	25	29	29	257	10	25.7	

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	5	4	6	6	4	4	5	4	5	43	10	4.30
4	8	0	0	0	0	0	0	0	0	0	8	10	0.800
5	0	10	9	11	10	12	8	11	11	11	93	10	9.30
6	6	10	0	3	13	11	12	13	14	13	95	10	9.50
7													
8													
TOTAL	14	25	13	20	29	27	24	29	29	29	239	10	23.9

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	0	0	5	5	3	2	5	4	33	10	3.30
4	0	0	5	0	0	0	1	0	0	0	6	10	0.600
5	12	12	8	9	9	11	9	7	10	8	95	10	9.50
6	14	12	0	11	14	11	9	13	14	13	111	10	11.1
7													
8													
TOTAL	31	28	13	20	28	27	22	22	29	25	245	10	24.5

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	1.00000	1.39310
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))
D = 0 W = 0 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 %	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 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.04511 W = 0.9549 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 = 5.318 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	0.02132	0.004264	2.268	
Within (Error)	24	0.04511	0.00188		
Total	29	0.06643			
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	0.4016	0.4016			
2	2.1 %	0.4188	0.4188	-0.6272		
3	2.8 %	0.374	0.374	1.006		
4	3.8 %	0.3404	0.3404	2.232		
5	5 %	0.3536	0.3536	1.75		
6	6.7 %	0.373	0.373	1.043		
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	0.06472	16.1	-0.0172	
3	2.8 %	5	0.06472	16.1	0.0276	
4	3.8 %	5	0.06472	16.1	0.0612	
5	5 %	5	0.06472	16.1	0.048	
6	6.7 %	5	0.06472	16.1	0.0286	



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 %	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.

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	0	

Appendix A2: Statistics

*Ceriodaphnia dubia* Reproduction

Kolmogorov Test for Normality	No Transformation
<p style="text-align: center;">           D = 0.2073            D* = 1.626            Critical D* = 1.035                      (alpha = 0.01, N = 60)         </p> <p style="text-align: center;">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	2.1 %	107.50	75.00	10.00	
3	2.8 %	99.50	75.00	10.00	
4	3.8 %	120.50	75.00	10.00	
5	5 %	109.50	75.00	10.00	
6	6.7 %	109.00	75.00	10.00	

Critical values are 1 tailed (k=5)

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	90.8	18.16	0.4125	
Within (Error)	54	2377	44.02		
Total	59	2468			
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)					

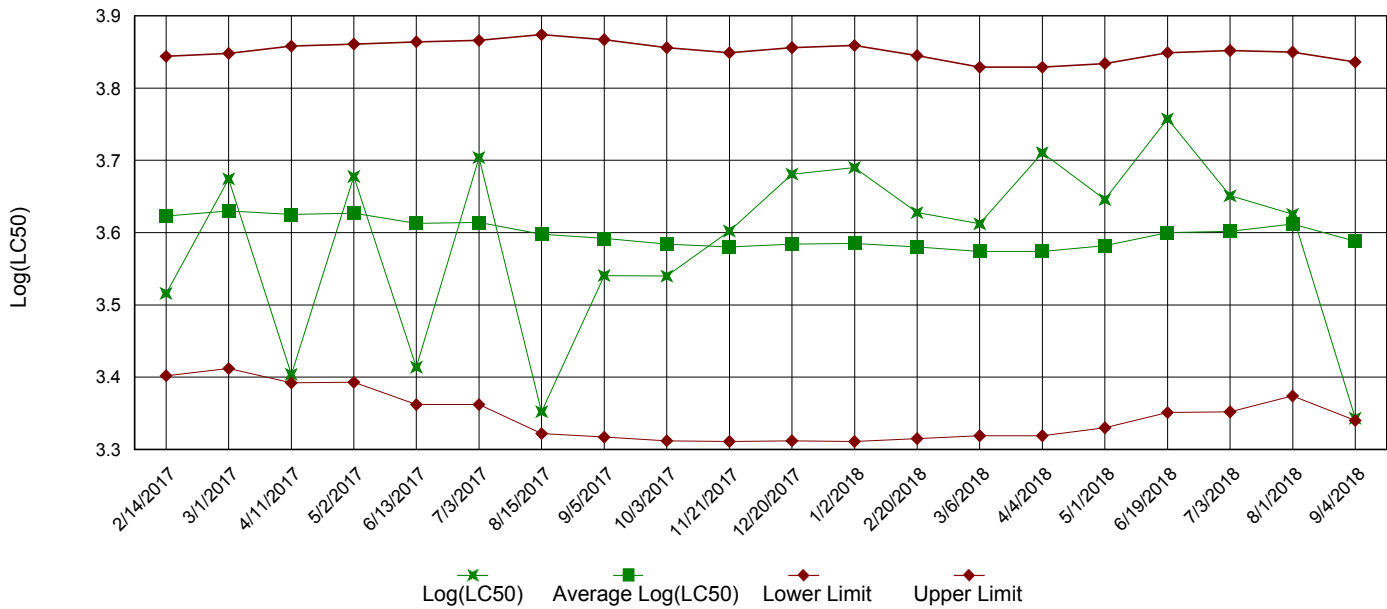
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	23.6	23.6			
2	2.1 %	23.5	23.5	0.0337		
3	2.8 %	21.6	21.6	0.674		
4	3.8 %	25.7	25.7	-0.7077		
5	5 %	23.9	23.9	-0.1011		
6	6.7 %	24.5	24.5	-0.3033		
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	6.854	29	0.1	
3	2.8 %	10	6.854	29	2	
4	3.8 %	10	6.854	29	-2.1	
5	5 %	10	6.854	29	-0.3	
6	6.7 %	10	6.854	29	-0.9	

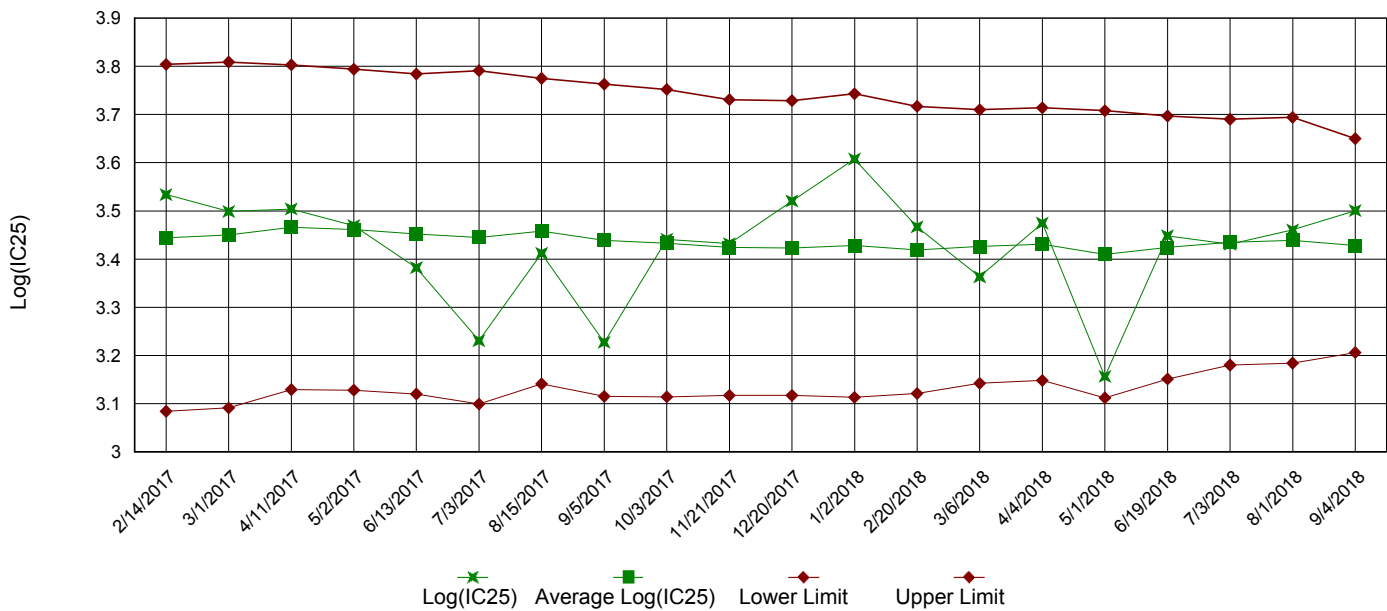
Appendix A3: Test 1000.0

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

LC50 Survival Data

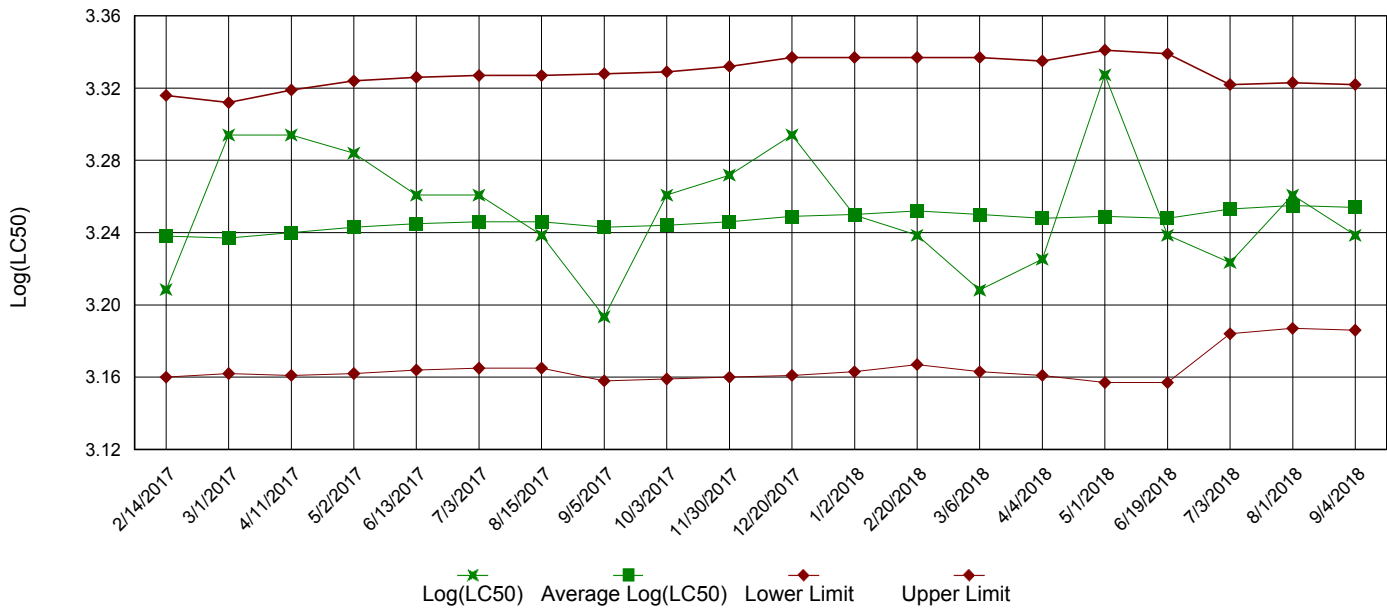


IC25 Growth Data

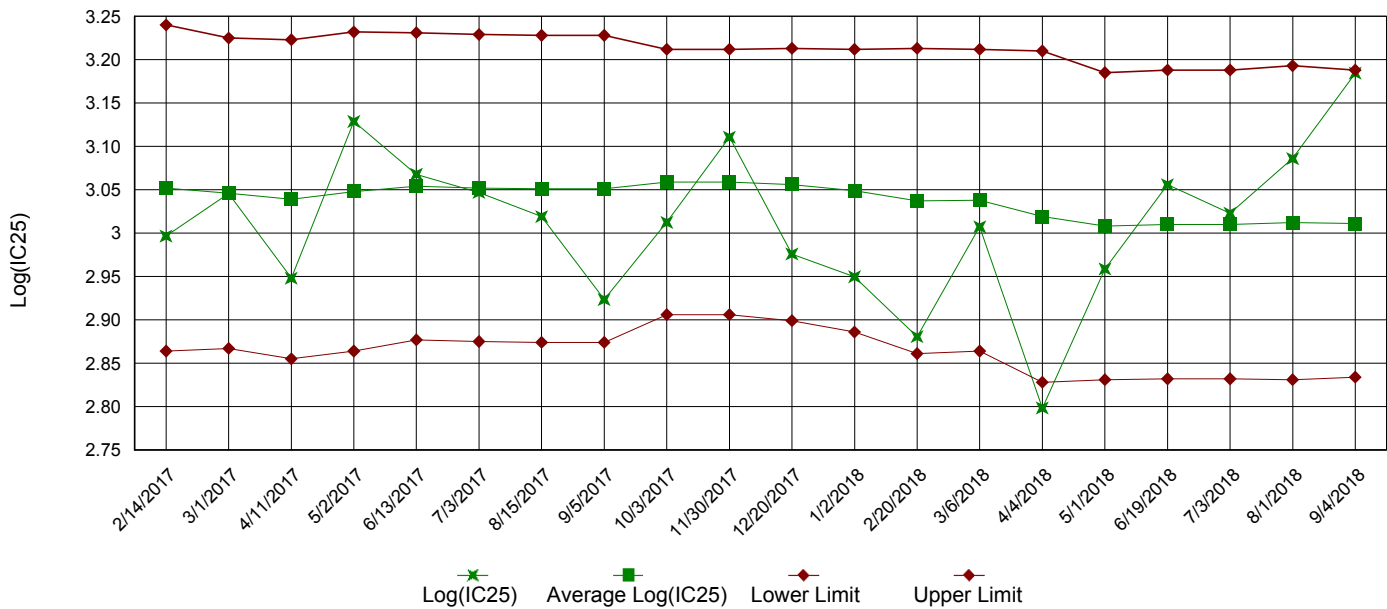


Appendix A3: Test 1002.0  
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: September 25, 2018 at 1550

Date and Time Test Terminated: October 02, 2018 at 0830

Dilution water used: Moderately Hard

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	100	100	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	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.328	0.380	0.456	0.388	0.456	0.402	13.6
2.1 %	0.396	0.446	0.389	0.415	0.448	0.419	6.56
2.8 %	0.359	0.406	0.399	0.364	0.342	0.374	7.32
3.8 %	0.326	0.388	0.278	0.420	0.290	0.34	18.1
5.0 %	0.356	0.305	0.334	0.432	0.341	0.354	13.5
6.7 %	0.398	0.361	0.350	0.405	0.351	0.373	7.10

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: 13.6 (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, 329

Test Initiated: DATE: September 25, 2018 TIME: 1550  
Test Terminated: DATE: October 02, 2018 TIME: 0830

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	6.1	6.3	6.3	6.2	6.0	6.1	7.0
Final	5.9	6.1	6.3	6.0	6.1	6.8	7.6
pH Initial	8.1	8.1	8.2	8.2	8.2	8.3	8.2
Final	7.8	8.3	8.2	8.2	8.2	7.8	8.3

DILUTION	DAY						
	1	2	3	4	5	6	7
2.1 %							
D.O. Initial	6.3	6.2	6.1	6.2	6.1	6.2	7.1
Final	6.0	6.2	6.2	6.1	6.1	6.9	7.6
pH Initial	8.0	8.1	8.2	8.2	8.1	8.2	8.1
Final	7.8	8.2	8.2	8.1	8.2	7.8	8.3

DILUTION	DAY						
	1	2	3	4	5	6	7
2.8 %							
D.O. Initial	6.5	6.3	6.2	6.2	6.1	6.2	7.1
Final	6.3	6.6	6.2	6.2	6.0	6.9	7.6
pH Initial	8.0	8.1	8.2	8.2	8.1	8.2	8.1
Final	8.0	8.2	8.2	8.1	8.2	7.9	8.3

DILUTION	DAY						
	1	2	3	4	5	6	7
3.8 %							
D.O. Initial	6.4	6.4	6.3	6.2	6.1	6.1	7.0
Final	6.5	6.7	6.3	6.3	6.2	7.1	7.7
pH Initial	8.0	8.1	8.2	8.1	8.1	8.2	8.1
Final	8.0	8.2	8.2	8.2	8.2	8.1	8.4

DILUTION	DAY						
	1	2	3	4	5	6	7
5.0 %							
D.O. Initial	6.4	6.5	6.4	6.3	6.2	6.2	7.2
Final	6.7	6.6	6.5	6.1	6.5	7.1	8.0
pH Initial	8.1	8.1	8.2	8.2	8.1	8.2	8.2
Final	8.1	8.3	8.2	8.2	8.3	8.1	8.4

DILUTION	DAY						
	1	2	3	4	5	6	7
6.7 %							
D.O. Initial	6.5	6.3	6.3	6.2	6.2	6.3	7.0
Final	6.5	6.8	6.6	6.3	6.4	7.3	8.0
pH Initial	8.1	8.2	8.2	8.2	8.2	8.3	8.2
Final	8.1	8.3	8.2	8.2	8.3	8.2	8.4

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
160	130	680	<0.05	Plant Effluent 24-SEP-18
150	140	690	<0.05	Plant Effluent 26-SEP-18
150	140	640	<0.05	Plant Effluent 28-SEP-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
59	81	290	<0.05	226930

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: September 25, 2018 at 1605

Date and Time Test Terminated: October 01, 2018 at 1605

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
6 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		2.1 %	2.8 %	3.8 %	5.0 %	6.7 %
A	29	24	29	31	14	31
B	28	26	28	28	25	28
C	13	14	16	16	13	13
D	15	28	15	32	20	20
E	19	27	31	28	29	28
F	27	29	24	27	27	27
G	24	6	12	12	24	22
H	29	24	28	25	29	22
I	26	28	24	29	29	29
J	26	29	9	29	29	25
Mean per Adult	23.6	23.5	21.6	25.7	23.9	24.5
Mean per Surviving Adult	23.6	23.5	21.6	25.7	23.9	24.5
CV %	24.8	32.2	36.6	25.4	26.0	21.9

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 %)	<u>      </u> YES	<u>  X  </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u>      </u> YES	<u>      </u> NO

2. Steel's Many-One Rank 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 %)	<u>      </u> YES	<u>  X  </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u>      </u> YES	<u>      </u> 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:   26   (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, 329

Test Initiated: DATE: September 25, 2018 TIME: 1605  
Test Terminated: DATE: October 01, 2018 TIME: 1605

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	6.1	6.3	6.3	6.2	6.0	6.1	7.0
Final	6.0	6.0	6.4	6.2	5.6	6.7	6.8
pH Initial	8.1	8.1	8.2	8.2	8.2	8.3	8.2
Final	8.2	8.2	8.2	8.2	8.2	8.0	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
2.1 %							
D.O. Initial	6.3	6.2	6.1	6.2	6.1	6.2	7.1
Final	5.9	6.1	6.4	6.2	5.9	6.9	7.3
pH Initial	8.0	8.1	8.2	8.2	8.1	8.2	8.1
Final	8.1	8.2	8.2	8.2	8.3	8.0	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
2.8 %							
D.O. Initial	6.5	6.3	6.2	6.2	6.1	6.2	7.1
Final	6.0	6.1	6.5	6.2	5.9	6.8	7.4
pH Initial	8.0	8.1	8.2	8.2	8.1	8.2	8.1
Final	8.1	8.2	8.2	8.2	8.2	8.0	8.1

DILUTION	DAY						
	1	2	3	4	5	6	7
3.8 %							
D.O. Initial	6.4	6.4	6.3	6.2	6.1	6.1	7.0
Final	6.2	5.9	6.4	6.2	5.8	6.7	7.4
pH Initial	8.0	8.1	8.2	8.1	8.1	8.2	8.1
Final	8.1	8.2	8.2	8.2	8.3	8.0	8.1

DILUTION	DAY						
	1	2	3	4	5	6	7
5.0 %							
D.O. Initial	6.4	6.5	6.4	6.3	6.2	6.2	7.2
Final	6.3	6.1	6.4	6.2	6.0	6.8	7.2
pH Initial	8.1	8.1	8.2	8.2	8.1	8.2	8.2
Final	8.0	8.2	8.2	8.2	8.3	8.1	8.1

DILUTION	DAY						
	1	2	3	4	5	6	7
6.7 %							
D.O. Initial	6.5	6.3	6.3	6.2	6.2	6.3	7.0
Final	5.9	6.0	6.4	6.2	5.9	6.8	7.1
pH Initial	8.1	8.2	8.2	8.2	8.2	8.3	8.2
Final	8.0	8.2	8.2	8.2	8.3	8.1	8.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
160	130	680	<0.05	Plant Effluent 24-SEP-18
150	140	690	<0.05	Plant Effluent 26-SEP-18
150	140	640	<0.05	Plant Effluent 28-SEP-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
59	81	290	<0.05	226930

227257

Batesville Wastewater Treatment Plant  
Chain of Custody

0.6

Sampled By: Michael McDaniel

Date Sampled: 9-24-18

①

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type		P GL	Analysis Required	Preserve	NC
					G	C				
Plant Effluent	9-24-18/2400				C		P	Chronic Biomonitoring	4°C	3

COMMENT:

Effluent Flow: 4.55

Chronic Biomonitoring

Relinquished By:

Date/Time:

Ricky Pool

9-25-18 / 0700

Received By:

Date/Time:

Michael Williams

9-25-18 / 0700

Relinquished By:

Date/Time:

Michael Williams

9-25-18 / 0855

Received By:

Date/Time:

Jory Williams

9-25-18 / 08:55

COMMENT:

0.6

**Batesville Wastewater Treatment Plant**  
Chain of Custody

Sampled By: Michael McDaniel

Date Sampled: 9-26-18

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type		P GL	Analysis Required	Preserve	NC
					G	C				
Plant Effluent	9-26-18/2400					C	P	Chronic Biomonitoring	4°C	3

2

COMMENT:

Effluent Flow: 4.54

Chronic Biomonitoring

Relinquished By:

Date/Time:

Holly Synore

9-27-18/0700

Received By:

Date/Time:

Michael McDaniel

9-27-18/0700

Relinquished By:

Date/Time:

Michael McDaniel

9-27-18/0857

Received By:

Date/Time:

Frank Hays

9-27-18 / 0857

COMMENT:

0.4°C

**Batesville Wastewater Treatment Plant**  
Chain of Custody

Sampled By: Michael McDaniel

Date Sampled: 9-28-18

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type		P GL	Analysis Required	Preserve	NC
					G	C				
Plant Effluent	9-28-18/2400					C	P	Chronic Biomonitoring	4°C	3

③

COMMENT:

Effluent Flow: 4.50

Chronic Biomonitoring

Relinquished By:

Date/Time:

Holly Seymour

9/29/18 - 0600

Received By:

Date/Time:

Michael McDaniel

9-29-18 / 0600

Relinquished By:

Date/Time:

Michael McDaniel

9-29-18 / 0813

Received By:

Date/Time:

54316

29 Sep 18 1.0

COMMENT: