



September 19, 2017

Biomonitoring Testing
for
Plant Effluent
Batesville, AR

Control No. 215928-1

Prepared for:

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

Prepared by:

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

Re: Chronic utilizing *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



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	0.590	PASS
Control Growth CV < or = 40%	9.43	PASS
Growth Minimum Significant Difference 12 to 30%	12.4	PASS
Critical Dilution CV < or = 40%	3.63	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	29.2	PASS
Control CV < or = 40% per Surviving Female	12.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	14.4	PASS
Critical Dilution CV < or = 40%	7.77	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)	8.9	8.9	10
pH (standard units)	8.1	8.2	8.4
Alkalinity (mg/l as CaCO ₃)	110	110	110
Hardness (mg/l as CaCO ₃)	130	130	130
Conductivity (umhos/cm)	680	670	660
Residual Chlorine (mg/l)	0.050	0.050	0.060
Ammonia as N (mg/l)	<0.1	<0.1	<0.1

2. Dilution Water Samples:
Moderately Hard

Analysis	215821
Dissolved oxygen (mg/l)	7.6
pH (standard units)	8.1
Alkalinity (mg/l as CaCO ₃)	58
Hardness (mg/l as CaCO ₃)	82
Conductivity (umhos/cm)	320
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 5, 2017 at 1400
Date & Time Test Terminated: Sep 12, 2017 at 0820
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 5, 2017 at 1415
Date & Time Test Terminated: Sep 12, 2017 at 1330
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 August 15, 2017 at 1515 to Aug 22, 2017 at 0840

The results were as follows: (Control No. 215429-1.)

Survival LC-50: 2250 mg/l

Growth IC-25: 2581 mg/l

Growth PMSD: 6.35

Ceriodaphnia dubia

A chronic reference test was performed on August 15, 2017 at 1540 to Aug 22, 2017 at 1530

The results were as follows: (Control No. 215429-2.)

Survival LC-50: 1732 mg/l

Growth IC-25: 1045 mg/l

Growth PMSD: 22.8

V. Organism History

Pimephales promelas (Fathead minnow)

Date: September 5, 2017

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: September 5, 2017

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

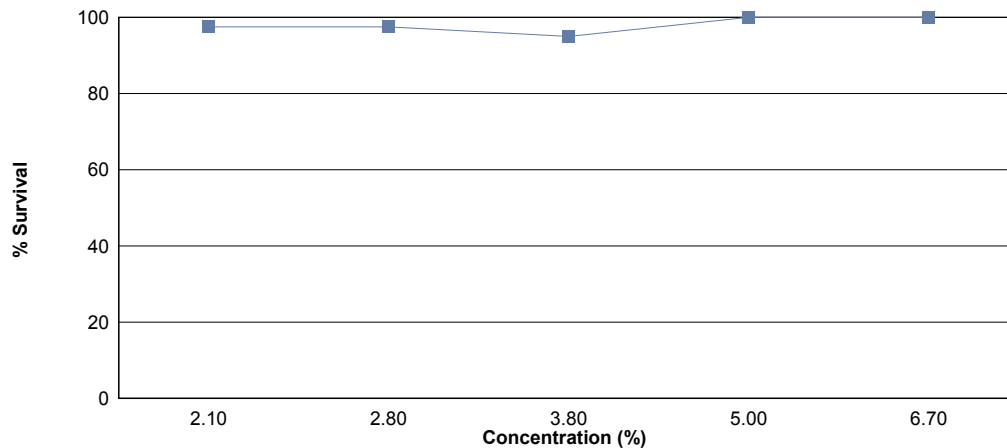
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 5, 2017 at 1400 and continued through Sep 12, 2017 at 0820. 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	0.590
2.1 %	97.5	0.602
2.8 %	97.5	0.597
3.8 %	95.0	0.591
5.0 %	100	0.586
6.7 %	100	0.608

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

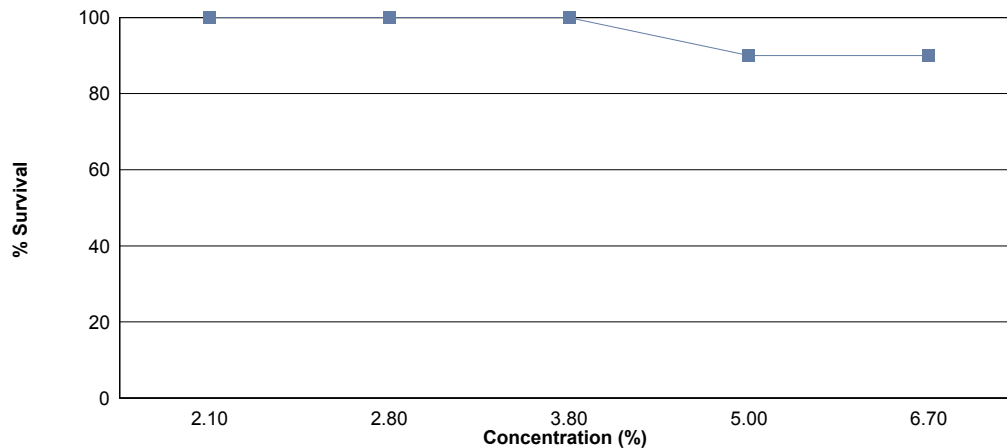
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 5, 2017 at 1415 and continued through Sep 12, 2017 at 1330. 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



Concentration	Percent Survival	Mean Reproduction
Control	100	29.2
2.1 %	100	35.7
2.8 %	100	32.9
3.8 %	100	34.1
5.0 %	90.0	33.6
6.7 %	90.0	31.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: September 5, 2017 at 1400

Date and Time Test Terminated: Sep 12, 2017 at 0820

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	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
2.8 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
3.8 %	A	8	8	8	8	8	8	7
	B	8	8	8	8	8	8	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
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 5, 2017 at 1400

Test Terminated: Sep 12, 2017 at 0820

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93301	.93721	0.00420	8	0.525
	B	.93711	.94232	0.00521	8	0.651
	C	.93329	.93786	0.00457	8	0.571
	D	.93633	.94080	0.00447	8	0.559
	E	.93615	.94132	0.00517	8	0.646
2.1 %	A	.93550	.94042	0.00492	8	0.615
	B	.93665	.94157	0.00492	8	0.615
	C	.93378	.93910	0.00532	8	0.665
	D	.93073	.93479	0.00406	8	0.508
	E	.93429	.93915	0.00486	8	0.608
2.8 %	A	.93001	.93458	0.00457	8	0.571
	B	.92882	.93358	0.00476	8	0.595
	C	.92981	.93455	0.00474	8	0.592
	D	.92893	.93411	0.00518	8	0.648
	E	.92680	.93145	0.00465	8	0.581
3.8 %	A	.93472	.93912	0.00440	8	0.550
	B	.93520	.93940	0.00420	8	0.525
	C	.93586	.94100	0.00514	8	0.642
	D	.92269	.92713	0.00444	8	0.555
	E	.93403	.93951	0.00548	8	0.685
5.0 %	A	.93075	.93572	0.00497	8	0.621
	B	.93664	.94135	0.00471	8	0.589
	C	.93443	.93899	0.00456	8	0.570
	D	.93308	.93775	0.00467	8	0.584
	E	.93374	.93828	0.00454	8	0.568
6.7 %	A	.93128	.93575	0.00447	8	0.559
	B	.92954	.93472	0.00518	8	0.648
	C	.93115	.93610	0.00495	8	0.619
	D	.92738	.93191	0.00453	8	0.566
	E	.93003	.93523	0.00520	8	0.650

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 5, 2017 at 1415

Date and Time Test Terminated: Sep 12, 2017 at 1330

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	6	5	6	7	6	5	5	5	3	5	53	10	5.30	
5	9	0	12	8	11	10	9	0	11	10	80	10	8.00	
6	0	0	0	0	0	13	15	13	0	14	55	10	5.50	
7	15	16	16	14	15	0	1	14	13	0	104	10	10.4	
8														
TOTAL	30	21	34	29	32	28	30	32	27	29	292	10	29.2	

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	7	6	4	7	6	6	6	7	6	5	60	10	6.00
5	11	13	13	9	11	12	14	0	11	14	108	10	10.8
6	0	0	0	0	0	18	16	14	0	19	67	10	6.70
7	15	20	18	19	17	0	0	18	15	18E	122	10	12.2
8													
TOTAL	33	39	35	35	34	36	36	39	32	38	357	10	35.7

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	7	0	6	6	6	6	6	8	5	6	56	10	5.60
5	11	3	14	12	9	12	12	0	9	9	91	10	9.10
6	0	8	0	0	0	19	17	13	0	13	70	10	7.00
7	16	9	17	21	16	18E	1	17	15	0	112	10	11.2
8													
TOTAL	34	20	37	39	31	37	36	38	29	28	329	10	32.9

E = Excluded fourth brood neonates

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 5, 2017 at 1415

Date and Time Test Terminated: Sep 12, 2017 at 1330

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	7	5	5	5	6	6	6	5	5	55	10	5.50	
5	14	13	16	10	11	11	14	0	12	11	112	10	11.2	
6	0	0	17	0	0	17	16	13	0	16	79	10	7.90	
7	8	18	0	16	17	0	0	20	16	19E	95	10	9.50	
8														
TOTAL	27	38	38	31	33	34	36	39	33	32	341	10	34.1	

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	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	7	8	6	5	5	6	7	5	6	61	10	6.10
5	12	11	16	13	13	11	11	0	13	12	112	10	11.2
6	0	0	0	0	0	14	17	12	0	17	60	10	6.00
7	18	20	X	19	18	0	0	13	15	0	103	9	11.4
8													
TOTAL	36	38	24	38	36	30	34	32	33	35	336	10	33.6

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	0	0	0	0	0	10	0.00
4	6	7	5	7	6	4	4	6	3	6	54	10	5.40
5	11	8	10	0	11	12	10	0	12	16	90	10	9.00
6	0	0	0	11	0	14	15	12	0	20	72	10	7.20
7	15	X	14	16	19	0	14E	19	18	0	101	9	11.2
8													
TOTAL	32	15	29	34	36	30	29	37	33	42	317	10	31.7

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	0.87500	1.20940
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	0.87500	1.20940
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	0.87500	1.20940
4	3.8 %	2	0.87500	1.20940
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))
<p>D = 0.09449 W = 0.7601 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	2.1 %	25.00	16.00	5.00	
3	2.8 %	25.00	16.00	5.00	
4	3.8 %	22.50	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 = 0.05738 W = 0.9842 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 = 5.894 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	0.001694	0.0003388	0.1417	
Within (Error)	24	0.05738	0.002391		
Total	29	0.05907			
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	0.5904	0.5904			
2	2.1 %	0.6022	0.6022	-0.3816		
3	2.8 %	0.5974	0.5974	-0.2263		
4	3.8 %	0.5914	0.5914	-0.03234		
5	5 %	0.5864	0.5864	0.1293		
6	6.7 %	0.6084	0.6084	-0.582		
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	0.07298	12.4	-0.0118		
3	2.8 %	5	0.07298	12.4	-0.007		
4	3.8 %	5	0.07298	12.4	-0.001		
5	5 %	5	0.07298	12.4	0.004		
6	6.7 %	5	0.07298	12.4	-0.018		

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 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
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	1	
5	6.7 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.1109 D* = 0.8701 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 = 12.39 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	248.9	49.78	2.196	
Within (Error)	54	1224	22.67		
Total	59	1473			
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	29.2	29.2			
2	2.1 %	35.7	35.7	-3.053		
3	2.8 %	32.9	32.9	-1.738		
4	3.8 %	34.1	34.1	-2.301		
5	5 %	33.6	33.6	-2.066		
6	6.7 %	31.7	31.7	-1.174		
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	4.919	16.8	-6.5	
3	2.8 %	10	4.919	16.8	-3.7	
4	3.8 %	10	4.919	16.8	-4.9	
5	5 %	10	4.919	16.8	-4.4	
6	6.7 %	10	4.919	16.8	-2.5	

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	251.1	50.22	3.217	
Within (Error)	52	811.9	15.61		
Total	57	1063			
Critical F = 3.39 (alpha = 0.01, df = 5,52)					
2.39 (alpha = 0.05, df = 5,52)					
Since F > Critical F REJECT Ho: All equal (alpha = 0.05)					

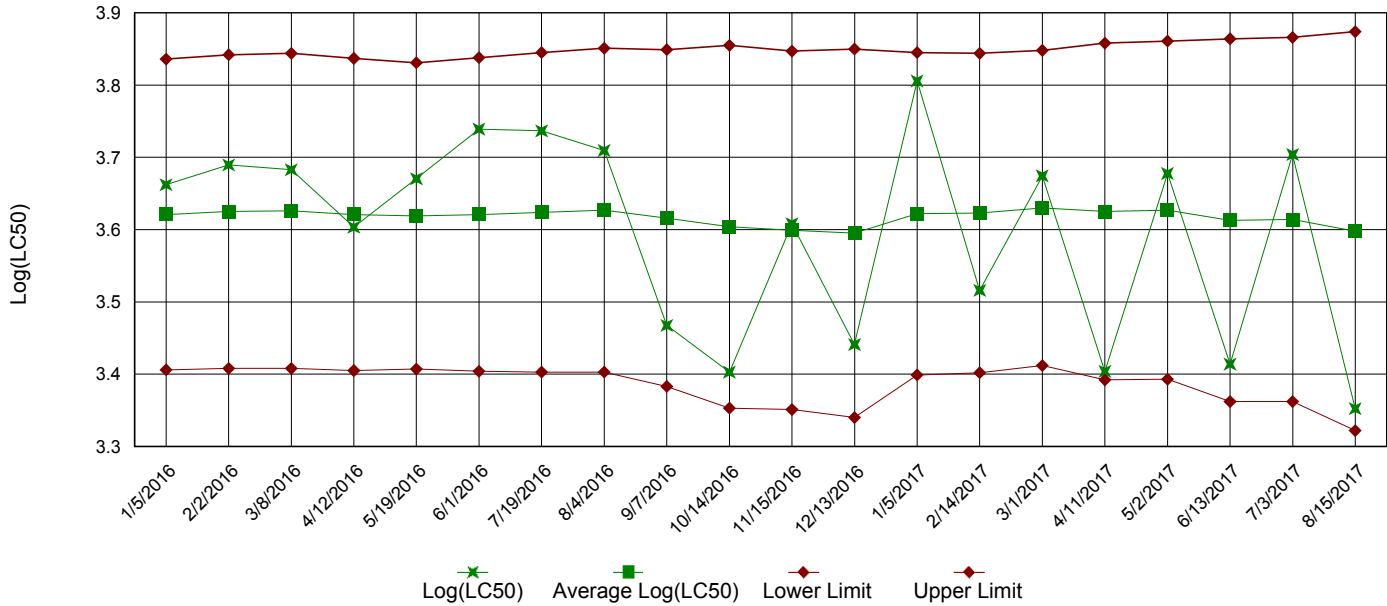
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	29.2	29.2			
2	2.1 %	35.7	35.7	-3.679		
3	2.8 %	32.9	32.9	-2.094		
4	3.8 %	34.1	34.1	-2.773		
5	5 %	34.667	34.667	-3.012		
6	6.7 %	33.556	33.556	-2.4		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,52)						
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	4.082	14	-6.5	
3	2.8 %	10	4.082	14	-3.7	
4	3.8 %	10	4.082	14	-4.9	
5	5 %	9	4.193	14.4	-5.467	
6	6.7 %	9	4.193	14.4	-4.356	

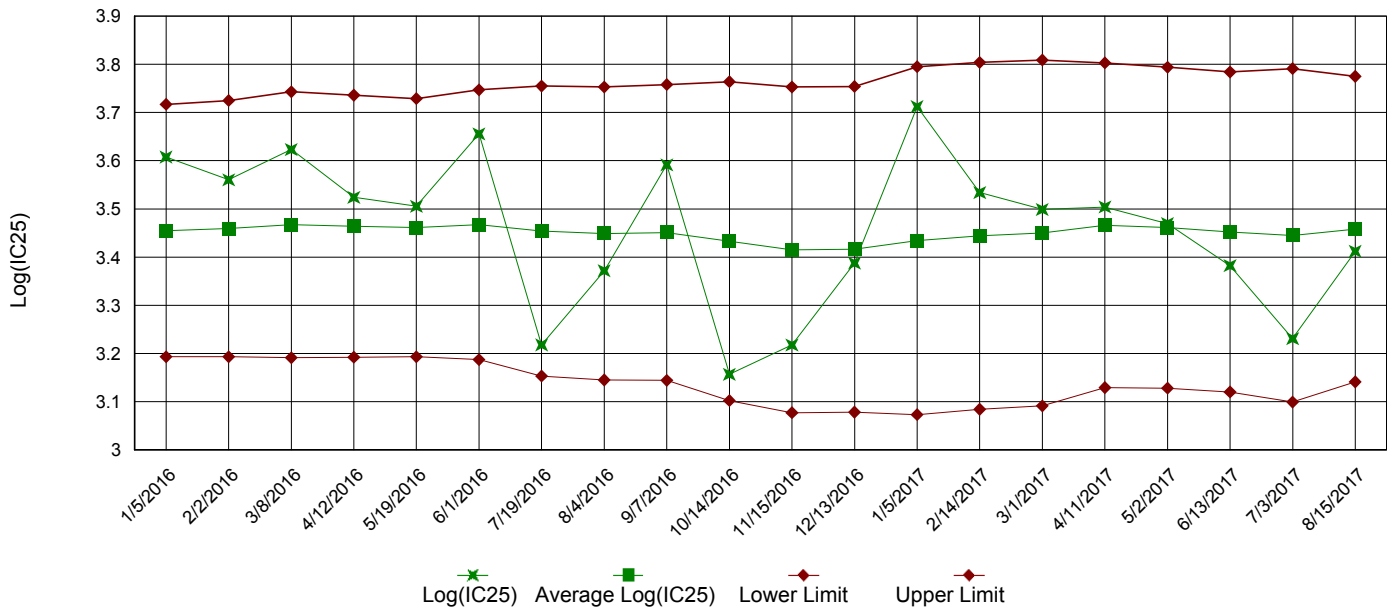
Appendix A3: Test 1000.0

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

LC50 Survival Data

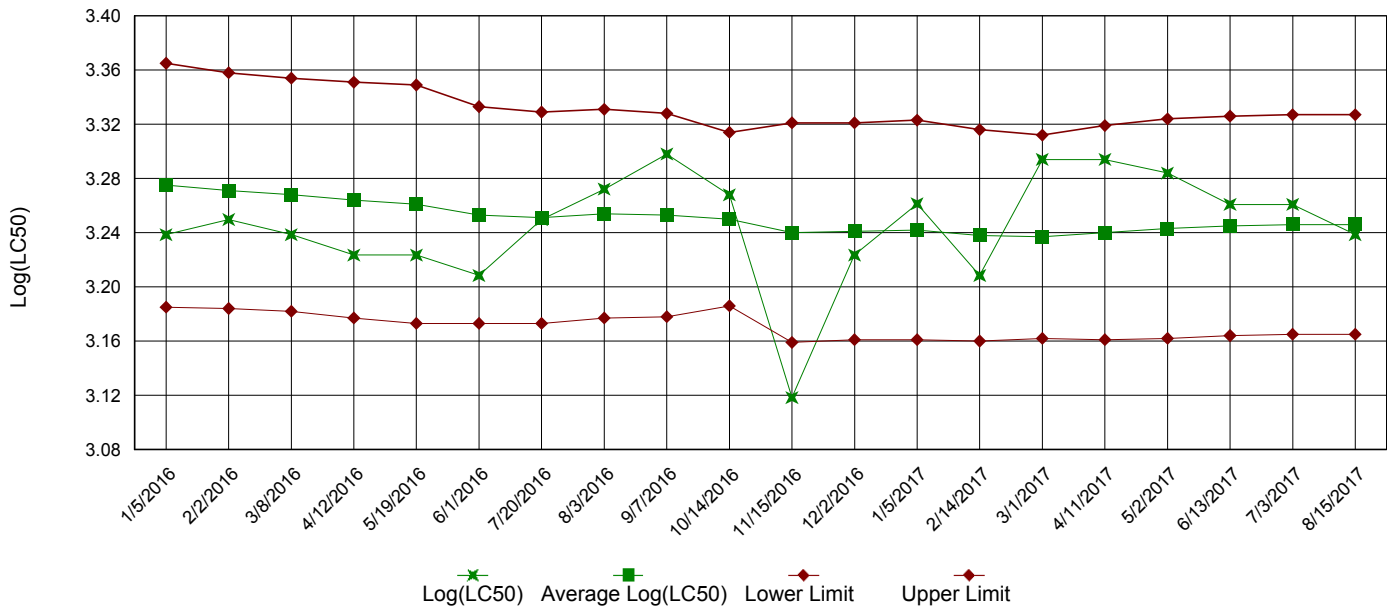


IC25 Growth Data

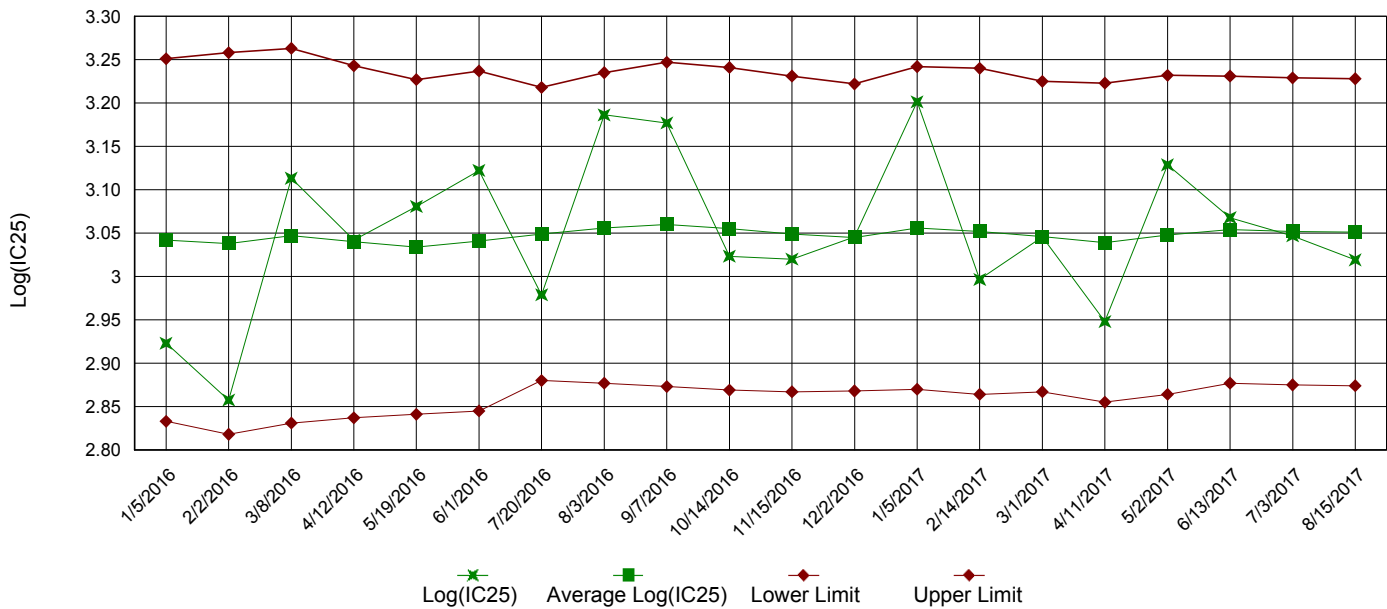


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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 5, 2017 at 1400

Date and Time Test Terminated: Sep 12, 2017 at 0820

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	87.5	100	100	100	100	100	97.5	5.73
2.8 %	100	87.5	100	100	100	100	100	97.5	5.73
3.8 %	87.5	87.5	100	100	100	100	100	95.0	7.21
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	0.525	0.651	0.571	0.559	0.646	0.59	9.43
2.1 %	0.615	0.615	0.665	0.508	0.608	0.602	9.53
2.8 %	0.571	0.595	0.592	0.648	0.581	0.597	4.99
3.8 %	0.550	0.525	0.642	0.555	0.685	0.591	11.6
5.0 %	0.621	0.589	0.570	0.584	0.568	0.586	3.63
6.7 %	0.559	0.648	0.619	0.566	0.650	0.608	7.19

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: 9.43 (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: September 5, 2017 TIME: 1400
Test Terminated: DATE: Sep 12, 2017 TIME: 0820

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.6	8.1	8.0	7.7	8.1	7.9	7.7
Final	7.6	7.0	6.6	8.0	7.6	6.7	5.9
pH Initial	8.1	8.0	8.1	8.0	8.3	7.9	8.1
Final	7.9	7.5	7.5	8.2	8.0	7.4	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
2.1 %							
D.O. Initial	7.8	8.0	8.1	8.0	8.1	8.2	7.8
Final	7.6	6.8	7.1	8.0	8.1	6.4	5.9
pH Initial	8.2	8.0	8.2	7.9	8.3	7.9	8.0
Final	7.9	7.5	7.6	8.3	8.0	7.4	7.4

DILUTION	DAY						
	1	2	3	4	5	6	7
2.8 %							
D.O. Initial	7.4	7.9	8.0	8.0	8.3	8.3	8.0
Final	7.6	7.0	6.9	8.0	8.0	6.6	6.1
pH Initial	8.2	8.0	8.2	7.9	8.3	8.0	8.0
Final	7.9	7.5	7.5	8.2	8.0	7.4	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
3.8 %							
D.O. Initial	7.2	8.1	7.9	8.0	8.0	8.4	8.0
Final	7.8	7.1	6.9	7.9	7.9	6.8	6.2
pH Initial	8.3	8.1	8.2	7.9	8.4	8.0	8.0
Final	7.9	7.5	7.6	8.2	8.0	7.5	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
5.0 %							
D.O. Initial	7.6	7.8	8.0	7.8	8.1	8.1	7.9
Final	7.6	7.2	6.8	8.0	7.8	6.5	5.9
pH Initial	8.2	8.0	8.2	7.9	8.4	8.0	8.0
Final	7.9	7.6	7.6	8.3	8.0	7.5	7.5

DILUTION	DAY						
	1	2	3	4	5	6	7
6.7 %							
D.O. Initial	7.6	8.0	7.8	8.0	8.2	8.2	8.5
Final	7.7	7.1	6.5	7.9	7.9	6.7	6.4
pH Initial	8.3	8.0	8.2	7.9	8.4	8.0	8.0
Final	7.9	7.6	7.6	8.4	8.1	7.5	7.6

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
110	130	680	0.050	Plant Effluent 04-SEP-17
110	130	670	0.050	Plant Effluent 06-SEP-17
110	130	660	0.060	Plant Effluent 08-SEP-17

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	82	320	<0.05	215821

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 5, 2017 at 1415

Date and Time Test Terminated: Sep 12, 2017 at 1330

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
7 day	100	100	100	100	90.0	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	30	33	34	27	36	32
B	21	39	20	38	38	15
C	34	35	37	38	24	29
D	29	35	39	31	38	34
E	32	34	31	33	36	36
F	28	36	37	34	30	30
G	30	36	36	36	34	29
H	32	39	38	39	32	37
I	27	32	29	33	33	33
J	29	38	28	32	35	42
Mean per Adult	29.2	35.7	32.9	34.1	33.6	31.7
Mean per Surviving Adult	29.2	35.7	32.9	34.1	34.7	33.6
CV %	12.2	6.74	18.0	10.9	7.77	12.7

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 %)	<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: 12.2 (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: September 5, 2017 TIME: 1415
Test Terminated: DATE: Sep 12, 2017 TIME: 1330

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.6	8.1	8.0	7.7	8.1	7.9	7.7
Final	8.2	7.8	8.4	8.2	8.3	8.4	--
pH Initial	8.1	8.0	8.1	8.0	8.3	7.9	8.1
Final	8.3	8.3	8.4	8.6	8.5	8.0	--

DILUTION	DAY						
	1	2	3	4	5	6	7
2.1 %							
D.O. Initial	7.8	8.0	8.1	8.0	8.1	8.2	7.8
Final	8.0	7.6	8.3	8.3	8.3	8.3	--
pH Initial	8.2	8.0	8.2	7.9	8.3	7.9	8.0
Final	8.4	8.4	8.4	8.6	8.6	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
2.8 %							
D.O. Initial	7.4	7.9	8.0	8.0	8.3	8.3	8.0
Final	8.0	8.0	8.5	8.3	8.4	8.6	--
pH Initial	8.2	8.0	8.2	7.9	8.3	8.0	8.0
Final	8.4	8.4	8.5	8.7	8.6	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
3.8 %							
D.O. Initial	7.2	8.1	7.9	8.0	8.0	8.4	8.0
Final	8.2	8.2	8.3	8.3	8.4	8.6	--
pH Initial	8.3	8.1	8.2	7.9	8.4	8.0	8.0
Final	8.4	8.4	8.5	8.7	8.6	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
5.0 %							
D.O. Initial	7.6	7.8	8.0	7.8	8.1	8.1	7.9
Final	7.9	8.0	8.3	8.3	8.2	8.6	--
pH Initial	8.2	8.0	8.2	7.9	8.4	8.0	8.0
Final	8.4	8.4	8.5	8.8	8.7	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
6.7 %							
D.O. Initial	7.6	8.0	7.8	8.0	8.2	8.2	8.5
Final	8.3	8.1	8.6	8.4	8.4	8.5	--
pH Initial	8.3	8.0	8.2	7.9	8.4	8.0	8.0
Final	8.4	8.5	8.6	8.8	8.7	8.2	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
110	130	680	0.050	Plant Effluent 04-SEP-17
110	130	670	0.050	Plant Effluent 06-SEP-17
110	130	660	0.060	Plant Effluent 08-SEP-17

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	82	320	<0.05	215821

215928

Batesville Wastewater Treatment Plant Chain of Custody

Sampled By: *Michael McDaniel*

Date Sampled: *9-4-17*

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	9-4-17/2400				C	P	Chronic Biomonitoring	4°C	3

①

COMMENT:

Effluent Flow: *4.47*

Chronic Biomonitoring

Relinquished By:

Date/Time:

Rudolf J. Pool 9-5-17 - 0700

Received By:

Date/Time:

Michael McDaniel 9-5-17 / 0700

Relinquished By:

Date/Time:

Michael McDaniel 9-5-17 / 0905

Received By:

Date/Time:

Lupe Hopt 9-5-17 / 0905

COMMENT:

0,4°C

215928

Batesville Wastewater Treatment Plant Chain of Custody

Sampled By: Michael McDaniel

Date Sampled: 9-6-17

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type G C	P GL	Analysis Required	Preserve	NC
Plant Effluent	9-6-17/2400				C	P	Chronic Biomonitoring	4°C	3

2

COMMENT:

Effluent Flow: 4.52

Chronic Biomonitoring

Relinquished By:

Date/Time:

Holly Seymore

9-7-17/0700

Received By:

Date/Time:

Michael McDaniel

9-7-17/0700

Relinquished By:

Date/Time:

Michael McDaniel

9-7-17/0855

Received By:

Date/Time:

D. Brown

9-7-17/0855

0.7°C

COMMENT:

215928

Batesville Wastewater Treatment Plant Chain of Custody

Sampled By: Michael McDaniel

Date Sampled: 9-8-17

Sample ID	Date/Time Collected	Temp	Grab pH	Time/pH Analyzed	Type G C	P GL	Analysis Required	Preserve	NC
PLANT Effluent	9-8-17/2400				C	P	Chronic Biomonitoring	4°C	3

3

COMMENT:

Effluent Flow: 4.60

Chronic Biomonitoring

Relinquished By:

Date/Time:

Holly Sigmore

9-9-17/0700

Received By:

Date/Time:

Mich McDaniel

9-9-17/0700

Relinquished By:

Date/Time:

Mich McDaniel

9-9-17/0858

Received By:

Date/Time:

[Signature]

9-9-17 / 0858

COMMENT:

0.3°C