

Bio 2nd Qtr 2019 - 1R
(Apr, May, June)

May 24, 2019

Biomonitoring Testing
for
Outfall 001
Benton, AR

~~Control No. 233987-1R~~

Revised to correct typographical errors on the invalid *C. dubia* test.

Prepared for:

Mr. Jonathon Buff
Benton Utilities
616 West Hazel
Benton, AR 72015

Prepared by:

AMERICAN INTERPLEX CORPORATION
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Benton Utilities
ATTN: Mr. Jonathon Buff
616 West Hazel
Benton, AR 72015

Re: Chronic 7-Day Renewal *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001 - Benton, AR
NPDES Permit No. AR0036498 AFIN# 63-00063

Dear Mr. Jonathon Buff:

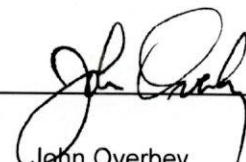
This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire 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 100 % effluent, which is equal to the critical dilution of 100 %. The NOEC for growth occurred at 100 % effluent, which is equal to the critical dilution of 100 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the Fathead minnow test.**

Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: Due to a control failure the test is invalid and will need to be repeated. The data is enclosed for your review.

AMERICAN INTERPLEX CORPORATION



John Overbey
Chief Operating Officer

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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	0.490	PASS
Control Growth CV < or = 40%	5.24	PASS
Growth Minimum Significant Difference 12 to 30%	16.6	PASS
Critical Dilution CV < or = 40%	14.6	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	30.0	FAIL
Control Reproduction > or = 15 per Surviving Female	4.67	FAIL
Control CV < or = 40% per Surviving Female	101	FAIL
Reproduction Minimum Significant Difference 13 to 47%	199	FAIL
Critical Dilution CV < or = 40%	57.5	FAIL

II. Outlined Report

A. Introduction

1. Permit Number: AR0036498 AFIN# 63-00063
2. Test Requirements: Chronic Biomonitoring, Quarterly
Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:

- a. Sampling Point: Outfall 001
- b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.4	8.6	8.1
pH (standard units)	7.2	6.7	6.8
Alkalinity (mg/l as CaCO ₃)	25	23	28
Hardness (mg/l as CaCO ₃)	56	59	48
Conductivity (umhos/cm)	210	210	210
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	0.12	<0.1

2. Dilution Water Samples:

Soft

Analysis	233619-1
Dissolved oxygen (mg/l)	8.5
pH (standard units)	7.6
Alkalinity (mg/l as CaCO ₃)	30
Hardness (mg/l as CaCO ₃)	42
Conductivity (umhos/cm)	160
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: April 30, 2019 at 1550
Date & Time Test Terminated: May 7, 2019 at 1415
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: April 30, 2019 at 1455
Date & Time Test Terminated: May 7, 2019 at 1525
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 Shapiro-Wilk's 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 April 2, 2019 at 1515 to April 9, 2019 at 1450

The results were as follows: (Control No. 233067-1.)

Survival LC-50: 4024 mg/l

Growth IC-25: 2315 mg/l

Growth PMSD: 9.66

Ceriodaphnia dubia

A chronic reference test was performed on April 2, 2019 at 1130 to April 9, 2019 at 1145

The results were as follows: (Control No. 233067-2.)

Survival LC-50: 1673 mg/l

Growth IC-25: 1236 mg/l

Growth PMSD: 26

V. Organism History

Pimephales promelas (Fathead minnow)

Date: April 30, 2019

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: April 30, 2019

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

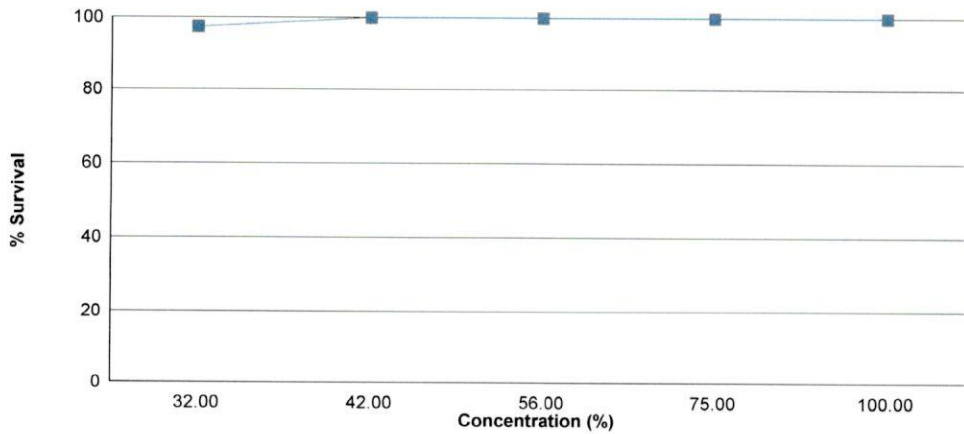
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on April 30, 2019 at 1550 and continued through May 7, 2019 at 1415. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 100 % effluent
- b.) NOEC growth = 100 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.490
32 %	97.5	0.463
42 %	100	0.530
56 %	100	0.489
75 %	100	0.469
100 %	100	0.480

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

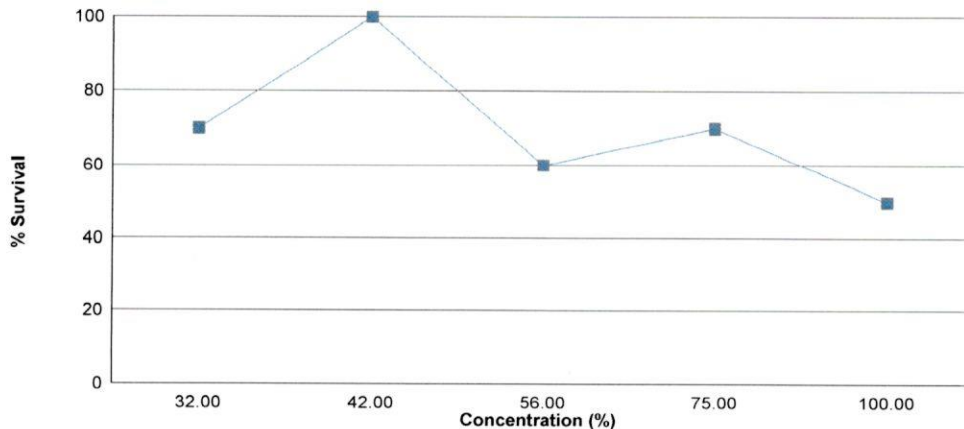
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 100 % effluent.

The test was initiated on April 30, 2019 at 1455 and continued through May 7, 2019 at 1525. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 32 % effluent
- b.) NOEC reproduction = 32 % effluent



Concentration	Percent Survival	Mean Reproduction
Control	30.0	1.60
32 %	70.0	6.70
42 %	100 *	--
56 %	60.0	--
75 %	70.0	--
100 %	50.0	--

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: April 30, 2019 at 1550

Date and Time Test Terminated: May 7, 2019 at 1415

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
32 %	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	7
42 %	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
56 %	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
75 %	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
100 %	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: April 30, 2019 at 1550
Test Terminated: May 7, 2019 at 1415

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92081	.92492	0.00411	8	0.514
	B	.92119	.92528	0.00409	8	0.511
	C	.92043	.92431	0.00388	8	0.485
	D	.91865	.92258	0.00393	8	0.491
	E	.92384	.92744	0.00360	8	0.450
32 %	A	.91992	.92356	0.00364	8	0.455
	B	.91872	.92295	0.00423	8	0.529
	C	.92421	.92817	0.00396	8	0.495
	D	.92891	.93250	0.00359	8	0.449
	E	.92305	.92613	0.00308	8	0.385
42 %	A	.91929	.92322	0.00393	8	0.491
	B	.92058	.92526	0.00468	8	0.585
	C	.92359	.92774	0.00415	8	0.519
	D	.92044	.92447	0.00403	8	0.504
	E	.92572	.93014	0.00442	8	0.552
56 %	A	.92301	.92698	0.00397	8	0.496
	B	.92105	.92453	0.00348	8	0.435
	C	.91968	.92349	0.00381	8	0.476
	D	.92267	.92675	0.00408	8	0.510
	E	.92873	.93296	0.00423	8	0.529
75 %	A	.91640	.91989	0.00349	8	0.436
	B	.91907	.92308	0.00401	8	0.501
	C	.91753	.92111	0.00358	8	0.448
	D	.91855	.92151	0.00296	8	0.370
	E	.92212	.92682	0.00470	8	0.588
100 %	A	.92339	.92724	0.00385	8	0.481
	B	.92016	.92455	0.00439	8	0.549
	C	.92549	.92843	0.00294	8	0.368
	D	.92245	.92667	0.00422	8	0.528
	E	.92370	.92750	0.00380	8	0.475

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 30, 2019 at 1455

Date and Time Test Terminated: May 7, 2019 at 1525

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	1	1	2	1	5	10	0.500	
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
5	0	0	0	0	0	0	7	0	1	0	8	10	0.800	
6	0	X	0	1	0	0	2	0	0	X	3	8	0.375	
7	X	X	X	X	X	X	0	0	0	X	0	3	0.00	
8														
TOTAL	0	0	0	1	0	0	10	1	3	1	16	10	1.60	

Concentration: 32 %														
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	3	2	1	0	0	0	0	6	10	0.600	
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
5	X	0	0	9	7	6	0	0	2	0	24	9	2.67	
6	X	6	0	11	13	0	X	0	7	0	37	8	4.62	
7	X	0	0	X	0	0	X	0	0	0	0	7	0.00	
8														
TOTAL	0	6	0	23	22	7	0	0	9	0	67	10	6.70	

Concentration: 42 %														
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	2	2	0	0	4	10	0.400	
4	0	0	0	0	0	0	0	0	0	1	1	10	0.100	
5	3	0	2	9	2	11	9	9	0	3	48	10	4.80	
6	1	0	10	1	0	13	12	6	8	11	62	10	6.20	
7	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
8														
TOTAL	4	0	12	10	2	24	23	17	8	15	115	10	11.5	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: April 30, 2019 at 1455

Date and Time Test Terminated: May 7, 2019 at 1525

Concentration: 56 %													
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	4	0	0	0	0	4	10	0.400
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	0	0	7	X	0	8	3	8	11	4	41	9	4.56
6	X	0	12	X	11	15	2	11	12	8	71	8	8.88
7	X	0	0	X	0	0	0	X	0	X	0	6	0.00
8													
TOTAL	0	0	19	0	11	27	5	19	23	12	116	10	11.6

Concentration: 75 %													
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	1	0	0	1	0	0	5	0	0	2	9	10	0.900
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	0	0	8	8	0	8	8	5	0	7	44	10	4.40
6	0	4	9	9	X	10	11	8	0	10	61	9	6.78
7	X	0	0	0	X	0	0	X	0	0	0	7	0.00
8													
TOTAL	1	4	17	18	0	18	24	13	0	19	114	10	11.4

Concentration: 100 %													
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	1	0	0	6	0	2	0	9	10	0.900
4	0	0	1	0	0	0	0	0	0	0	1	10	0.100
5	2	0	3	2	6	0	9	0	9	8	39	10	3.90
6	0	X	8	0	10	0	12	X	8	10	48	8	6.00
7	X	X	0	0	0	X	0	X	0	X	0	5	0.00
8													
TOTAL	2	0	12	3	16	0	27	0	19	18	97	10	9.70

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	32 %	1	1.00000	1.39310	
2	32 %	2	1.00000	1.39310	
2	32 %	3	1.00000	1.39310	
2	32 %	4	1.00000	1.39310	
2	32 %	5	0.87500	1.20940	
3	42 %	1	1.00000	1.39310	
3	42 %	2	1.00000	1.39310	
3	42 %	3	1.00000	1.39310	
3	42 %	4	1.00000	1.39310	
3	42 %	5	1.00000	1.39310	
4	56 %	1	1.00000	1.39310	
4	56 %	2	1.00000	1.39310	
4	56 %	3	1.00000	1.39310	
4	56 %	4	1.00000	1.39310	
4	56 %	5	1.00000	1.39310	
5	75 %	1	1.00000	1.39310	
5	75 %	2	1.00000	1.39310	
5	75 %	3	1.00000	1.39310	
5	75 %	4	1.00000	1.39310	
5	75 %	5	1.00000	1.39310	
6	100 %	1	1.00000	1.39310	
6	100 %	2	1.00000	1.39310	
6	100 %	3	1.00000	1.39310	
6	100 %	4	1.00000	1.39310	
6	100 %	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 = 0.027		
W = 0.4161		
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	32 %	25.00	16.00	5.00	
3	42 %	27.50	16.00	5.00	
4	56 %	27.50	16.00	5.00	
5	75 %	27.50	16.00	5.00	
6	100 %	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 = 0.07152 W = 0.9832 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 = 6.469 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	0.01431	0.002862	0.9604	
Within (Error)	24	0.07151	0.00298		
Total	29	0.08582			
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	0.4902	0.4902			
2	32 %	0.4626	0.4626	0.7994		
3	42 %	0.5302	0.5302	-1.159		
4	56 %	0.4892	0.4892	0.02896		
5	75 %	0.4686	0.4686	0.6256		
6	100 %	0.4802	0.4802	0.2896		
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	32 %	5	0.08148	16.6	0.0276		
3	42 %	5	0.08148	16.6	-0.04		
4	56 %	5	0.08148	16.6	0.001		
5	75 %	5	0.08148	16.6	0.0216		
6	100 %	5	0.08148	16.6	0.01		

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Dead	Alive	Total Animals
Control	7	3	10
32 %	3	7	10
Total	10	10	20

Critical Fisher's value (10,10,7) (alpha=0.05) is 2. b value is 3. Since b is greater than 2 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Dead	Alive	Total Animals
Control	7	3	10
42 %	0	10	10
Total	7	13	20

Critical Fisher's value (10,10,7) (alpha=0.05) is 2. b value is 0. Since b is less than or equal to 2 there is A SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Dead	Alive	Total Animals
Control	7	3	10
56 %	4	6	10
Total	11	9	20

Critical Fisher's value (10,10,7) (alpha=0.05) is 2. b value is 4. Since b is greater than 2 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Dead	Alive	Total Animals
Control	7	3	10
75 %	3	7	10
Total	10	10	20

Critical Fisher's value (10,10,7) (alpha=0.05) is 2. b value is 3. Since b is greater than 2 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	Dead	Alive	Total Animals
Control	7	3	10
100 %	5	5	10
Total	12	8	20

Critical Fisher's value (10,10,7) ($\alpha=0.05$) is 2. b value is 5. Since b is greater than 2 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	7	
1	32 %	10	3	
2	42 %	10	0	*
3	56 %	10	4	
4	75 %	10	3	
5	100 %	10	5	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Shapiro - Wilk's Test for Normality		No Transformation
D = 816.5		
W = 0.7994		
Critical W = 0.868	(alpha = 0.01, N = 20)	
Critical W = 0.905	(alpha = 0.05, N = 20)	
Data FAIL normality test (alpha = 0.01).		

Steel's Many-One Rank Test				No Transformation	
Ho: Control < Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	32 %	114.50	82.00	10.00	
Critical values are 1 tailed (k=1)					

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	1	5.505	5.505	0.1053	
Within (Error)	8	418.1	52.26		
Total	9	423.6			
Critical F = 11.26 (alpha = 0.01, df = 1,8)					
5.32 (alpha = 0.05, df = 1,8)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

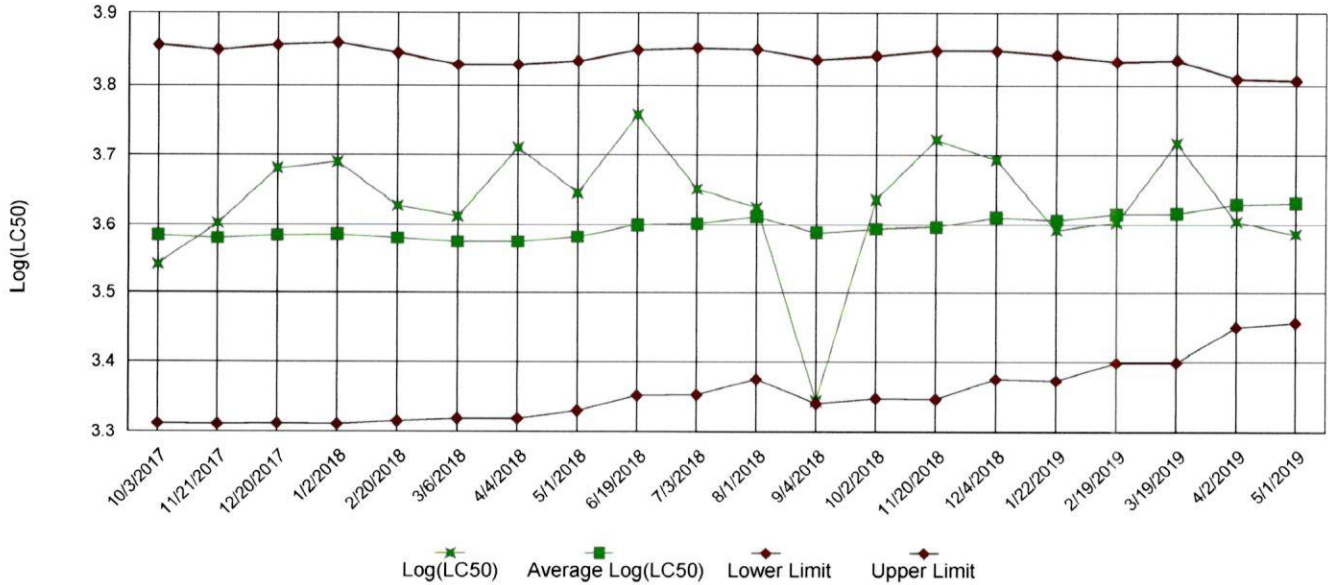
Dunnett's Test - Table 1 of 2					No Transformation	
Ho: Control < Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	4.6667	4.6667			
2	32 %	6.2857	6.2857	-0.3245		
Dunnett's critical value = 1.86 (1 Tailed, alpha = 0.05, df = 1,8)						
WARNING - 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	3				
2	32 %	7	9.279	199	-1.619	

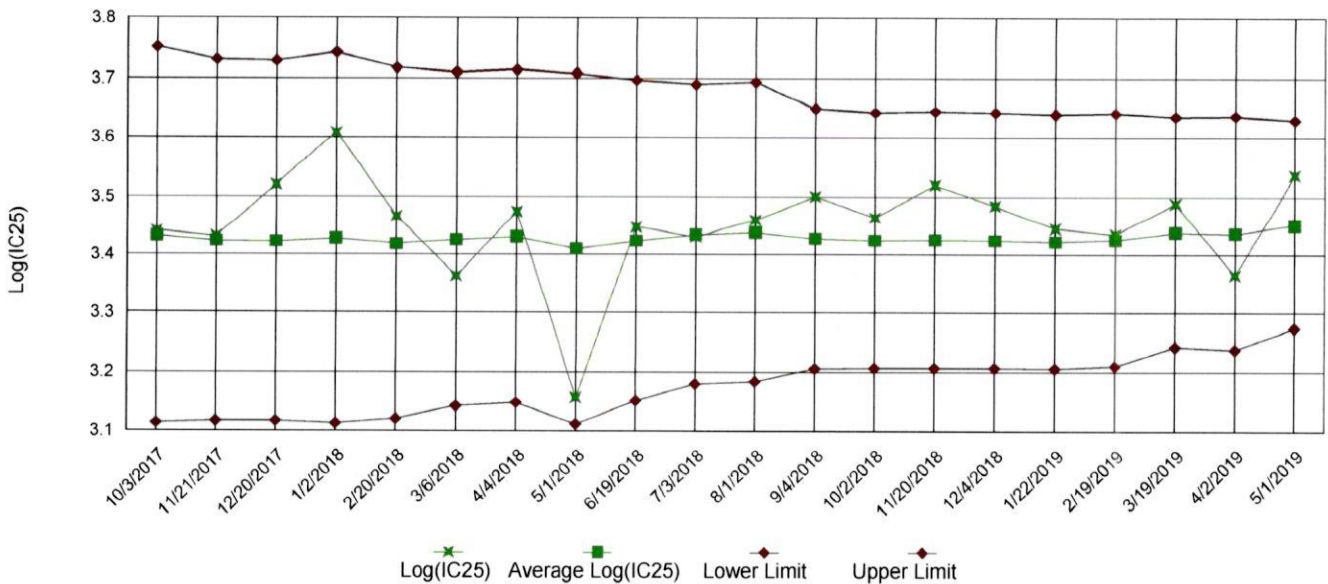
Appendix A3: Test 1000.0

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

LC50 Survival Data

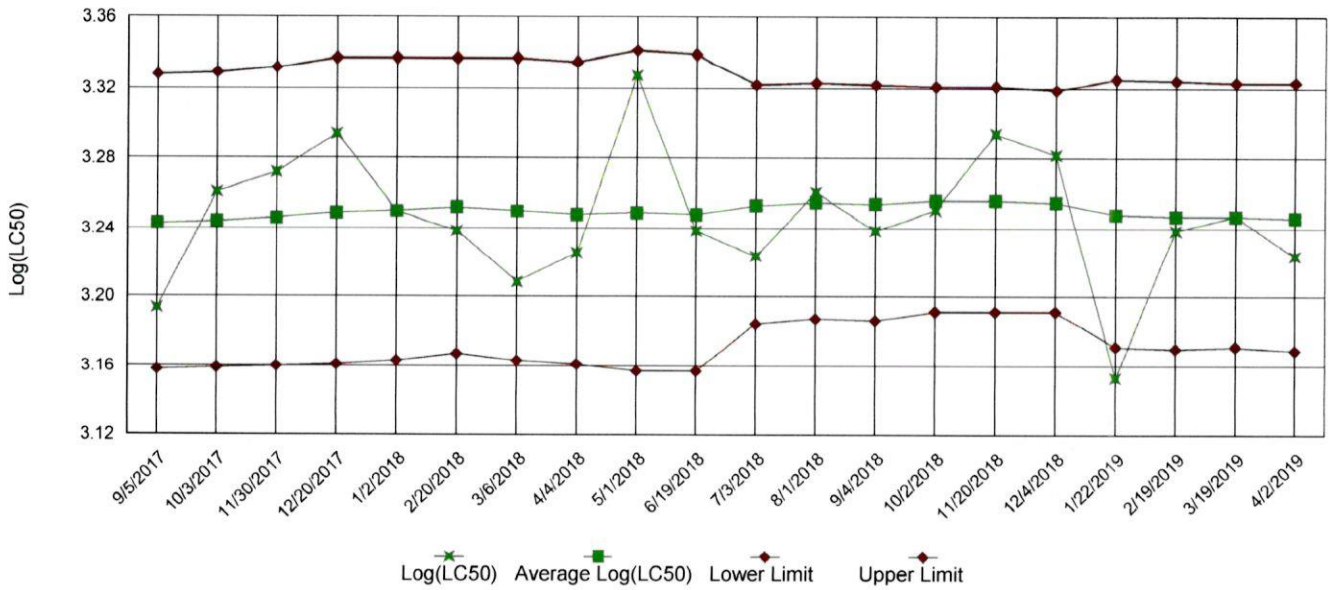


IC25 Growth Data

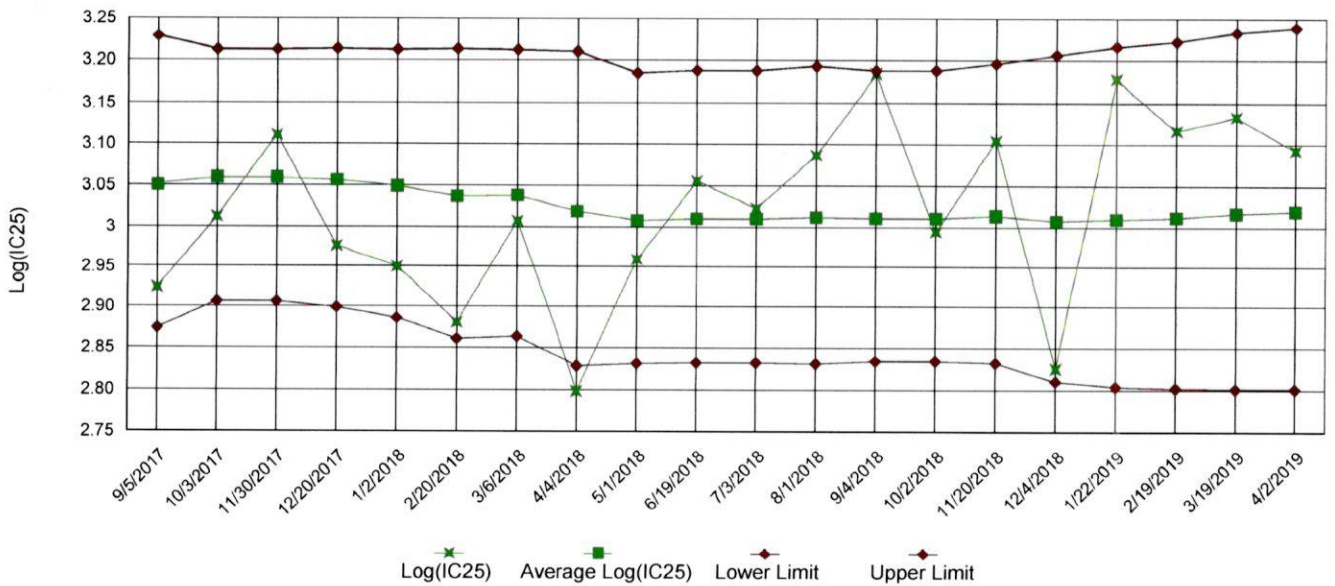


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: Benton Utilities

NPDES No.: AR0036498 AFIN# 63-00063

Date and Time Test Initiated: April 30, 2019 at 1550

Date and Time Test Terminated: May 7, 2019 at 1415

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
32 %	100	100	100	100	87.5	100	100	97.5	5.73
42 %	100	100	100	100	100	100	100	100	0.00
56 %	100	100	100	100	100	100	100	100	0.00
75 %	100	100	100	100	100	100	100	100	0.00
100 %	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	0.514	0.511	0.485	0.491	0.450	0.49	5.24
32 %	0.455	0.529	0.495	0.449	0.385	0.463	11.7
42 %	0.491	0.585	0.519	0.504	0.552	0.53	7.20
56 %	0.496	0.435	0.476	0.510	0.529	0.489	7.35
75 %	0.436	0.501	0.448	0.370	0.588	0.469	17.4
100 %	0.481	0.549	0.368	0.528	0.475	0.48	14.6

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	(100 %)	<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	(100 %)	<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]: | <u>0</u> | (TLP6C) |
| 4. If you answered NO to 2.a) enter [0] otherwise enter [1]: | <u>0</u> | (TGP6C) |
| 5. NOEC <i>Pimephales</i> Lethality: | <u>100 %</u> | (TOP6C) |
| 6. LOEC <i>Pimephales</i> Lethality: | <u>100 %</u> | (TXP6C) |
| 7. NOEC <i>Pimephales</i> Sublethality: | <u>100 %</u> | (TPP6C) |
| 8. LOEC <i>Pimephales</i> Sublethality: | <u>100 %</u> | (TYP6C) |
| 9. Coefficient of variation for <i>Pimephales</i> growth: | <u>14.6</u> | (TQP6C) |

Appendix B: Test 1000.0

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

PERMITTEE: Benton Utilities
NPDES NO.: AR0036498 AFIN# 63-00063
CONTACT: Mr. Jonathon Buff
ANALYST: 280, 310, 343

Test Initiated: DATE: April 30, 2019 TIME: 1550
Test Terminated: DATE: May 7, 2019 TIME: 1415

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.5	7.7	7.9	8.2	7.2	7.5	7.9
Final	6.3	7.1	6.2	5.5	7.0	6.8	5.8
pH Initial	7.6	7.3	7.4	7.6	7.5	7.7	7.6
Final	7.1	7.1	7.0	6.9	7.2	7.1	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	8.3	7.6	8.2	8.2	8.0	7.5	7.8
Final	6.3	7.0	6.3	5.6	6.9	6.7	6.3
pH Initial	7.4	7.2	7.1	7.4	7.3	7.3	7.4
Final	7.0	7.1	7.0	6.9	7.3	7.2	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	8.3	7.5	8.0	9.0	8.3	8.0	8.0
Final	6.4	6.9	6.4	5.3	6.4	6.3	6.1
pH Initial	7.4	7.2	7.1	7.2	7.3	7.3	7.3
Final	7.1	7.0	7.0	6.9	7.2	7.2	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	8.5	7.7	8.0	8.9	8.4	7.8	8.0
Final	6.5	7.1	6.0	5.4	6.5	6.3	6.2
pH Initial	7.3	7.2	7.0	7.1	7.2	7.2	7.2
Final	7.1	7.1	7.0	6.9	7.2	7.2	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
75 %							
D.O. Initial	8.5	7.7	8.2	9.0	8.2	7.4	7.7
Final	6.4	7.1	6.1	5.4	7.2	6.8	6.2
pH Initial	7.2	7.1	6.9	7.0	7.1	7.2	7.1
Final	7.0	7.1	6.9	6.9	7.2	7.2	7.0

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	8.4	7.8	8.6	8.9	8.1	7.5	7.6
Final	6.5	7.0	6.2	5.7	7.5	7.0	6.4
pH Initial	7.2	6.8	6.7	6.8	6.8	6.8	6.8
Final	7.0	7.1	6.9	6.9	7.2	7.2	7.0

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
25	56	210	<0.05	Outfall 001 30-APR-19
23	59	210	<0.05	Outfall 001 01-MAY-19
28	48	210	<0.05	Outfall 001 03-MAY-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
30	42	160	<0.05	233619-1

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

Permittee: Benton Utilities

NPDES No.: AR0036498 AFIN# 63-00063

Date and Time Test Initiated: April 30, 2019 at 1455

Date and Time Test Terminated: May 7, 2019 at 1525

Dilution water used: Soft

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	30.0	70.0	100	60.0	70.0	50.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
A	0	0	4	0	1	2
B	0	6	0	0	4	0
C	0	0	12	19	17	12
D	1	23	10	0	18	3
E	0	22	2	11	0	16
F	0	7	24	27	18	0
G	10	0	23	5	24	27
H	1	0	17	19	13	0
I	3	9	8	23	0	19
J	1	0	15	12	19	18
Mean per Adult	1.60	6.70	11.5	11.6	11.4	9.70
Mean per Surviving Adult	4.67	6.29	11.5	14.2	14.3	15.4
CV %	101	126	72.4	74.8	61.4	57.5

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	(100 %)	<u> X </u> YES	<u> </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	(100 %)	<u> X </u> YES	<u> </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]: | <u> 1 </u> | (TLP3B) |
| 4. If you answered NO to 2.a) enter [0] otherwise enter [1]: | <u> 1 </u> | (TGP3B) |
| 5. NOEC Ceriodaphnia Lethality: | <u> 32 % </u> | (TOP3B) |
| 6. LOEC Ceriodaphnia Lethality: | <u> 42 % </u> | (TXP3B) |
| 7. NOEC Ceriodaphnia Sublethality: | <u> 32 % </u> | (TPP3B) |
| 8. LOEC Ceriodaphnia Sublethality: | <u> 42 % </u> | (TYP3B) |
| 9. Coefficient of variation for Ceriodaphnia Reproduction: | <u> 101 </u> | (TQP3B) |

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

PERMITTEE: Benton Utilities
NPDES NO.: AR0036498 AFIN# 63-00063
CONTACT: Mr. Jonathon Buff
ANALYST: 280, 310, 343

Test Initiated: DATE: April 30, 2019 TIME: 1455
Test Terminated: DATE: May 7, 2019 TIME: 1525

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	8.5	7.7	7.9	8.2	7.2	7.5	7.9
Final	7.7	8.0	8.0	8.2	8.3	8.4	7.7
pH Initial	7.6	7.3	7.4	7.6	7.5	7.7	7.6
Final	7.8	7.9	7.8	7.9	7.9	8.2	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	8.3	7.6	8.2	8.2	8.0	7.5	7.8
Final	7.7	8.0	7.9	8.2	8.0	8.5	8.0
pH Initial	7.4	7.2	7.1	7.4	7.3	7.3	7.4
Final	7.8	7.8	7.8	7.9	7.9	8.1	8.1

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	8.3	7.5	8.0	9.0	8.3	8.0	8.0
Final	7.7	8.0	8.0	7.7	8.6	8.6	8.4
pH Initial	7.4	7.2	7.1	7.2	7.3	7.3	7.3
Final	7.7	7.8	7.9	7.8	7.9	8.1	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	8.5	7.7	8.0	8.9	8.4	7.8	8.0
Final	7.7	8.2	7.8	7.5	8.3	8.5	8.1
pH Initial	7.3	7.2	7.0	7.1	7.2	7.2	7.2
Final	7.7	7.8	7.8	7.8	7.9	8.1	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
75 %							
D.O. Initial	8.5	7.7	8.2	9.0	8.2	7.4	7.7
Final	7.8	8.1	8.2	8.2	7.7	8.4	8.0
pH Initial	7.2	7.1	6.9	7.0	7.1	7.2	7.1
Final	7.7	7.8	7.8	7.8	7.9	8.1	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	8.4	7.8	8.6	8.9	8.1	7.5	7.6
Final	7.7	8.3	8.1	8.5	7.6	7.9	7.5
pH Initial	7.2	6.8	6.7	6.8	6.8	6.8	6.8
Final	7.7	7.9	7.8	7.9	7.9	8.1	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
25	56	210	<0.05	Outfall 001 30-APR-19
23	59	210	<0.05	Outfall 001 01-MAY-19
28	48	210	<0.05	Outfall 001 03-MAY-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
30	42	160	<0.05	233619-1



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Benton Utilities</u>		PO No. <u>100071</u>		NO OF BOTTLES		ANALYSES REQUESTED											
Project Reference: <u>AR003649</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Project Manager: <u>J. Buff</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Sampled By: <u>AK</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Sample Identification: <u>OUTfall001</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Date/Time Collected: <u>5/31/9 0815</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
AIC No. <u>001</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
AIC CONTROL NO. <u>633987</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
AIC PROPOSAL NO. <u>07</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Carrier:		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Received Temperature C		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Remarks		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Field pH calibration on @ Buffer:		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Turnaround Time Requested: (Please circle) <u>NORMAL</u> or EXPEDITED IN ___ DAYS		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Expedited results requested by:		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Who should AIC contact with questions: <u>J. Buff</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Phone: ___ Fax: ___		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Report Attention to:		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Report Address to:		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Email Address: <u>JBuff@Bentonar.org</u>		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
9/2014		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
G Becker@Bentonar.org		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													
Angela F Bentonar.org		Matrix: <u>WATER</u>		Matrix: <u>SOIL</u>													