

Biomonitoring 4th Qtr
(Oct, Nov, Dec) 2018

November 5, 2018

Biomonitoring Testing
for
Outfall 001
Benton, AR

Control No. 228097-1

Prepared for:

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

Prepared by:

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



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.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction 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 reproduction 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 *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

A handwritten signature in black ink, appearing to read 'John Overbey', is written over a horizontal line.

John Overbey
Chief Operating Officer

PDF cc: Benton Utilities
ATTN: Mr. Jonathon Buff
jwbuff@bentonar.org

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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	0.274	PASS
Control Growth CV < or = 40%	5.08	PASS
Growth Minimum Significant Difference 12 to 30%	11.5	BELOW
Critical Dilution CV < or = 40%	3.90	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	31.8	PASS
Control CV < or = 40% per Surviving Female	14.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	16.2	PASS
Critical Dilution CV < or = 40%	30.9	PASS

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)	7.4	7.4	7.0
pH (standard units)	7.3	7.2	7.7
Alkalinity (mg/l as CaCO ₃)	35	36	34
Hardness (mg/l as CaCO ₃)	61	58	58
Conductivity (umhos/cm)	240	250	250
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.12	0.11	0.12

2. Dilution Water Samples:
Soft

Analysis	227598
Dissolved oxygen (mg/l)	7.3
pH (standard units)	7.7
Alkalinity (mg/l as CaCO ₃)	32
Hardness (mg/l as CaCO ₃)	41
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: October 23, 2018 at 1435
Date & Time Test Terminated: October 30, 2018 at 1600
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: October 23, 2018 at 1450
Date & Time Test Terminated: October 30, 2018 at 1510
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 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 October 2, 2018 at 1530 to October 09, 2018 at 1340

The results were as follows: (Control No. 227420-1.)

Survival LC-50: 4326 mg/l

Growth IC-25: 2916 mg/l

Growth PMSD: 6.33

Ceriodaphnia dubia

A chronic reference test was performed on at 1640 to October 09, 2018 at 1612

The results were as follows: (Control No. 227420-2.)

Survival LC-50: 1780 mg/l

Growth IC-25: 986.5 mg/l

Growth PMSD: 18.5

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 23, 2018

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 23, 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

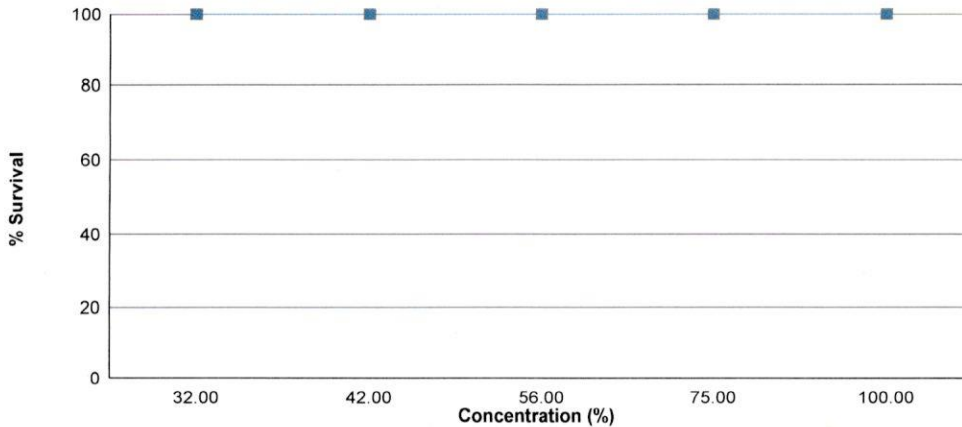
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 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 October 23, 2018 at 1435 and continued through October 30, 2018 at 1600. 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	0.274
32 %	100	0.262
42 %	100	0.287
56 %	100	0.332
75 %	100	0.337
100 %	100	0.328

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

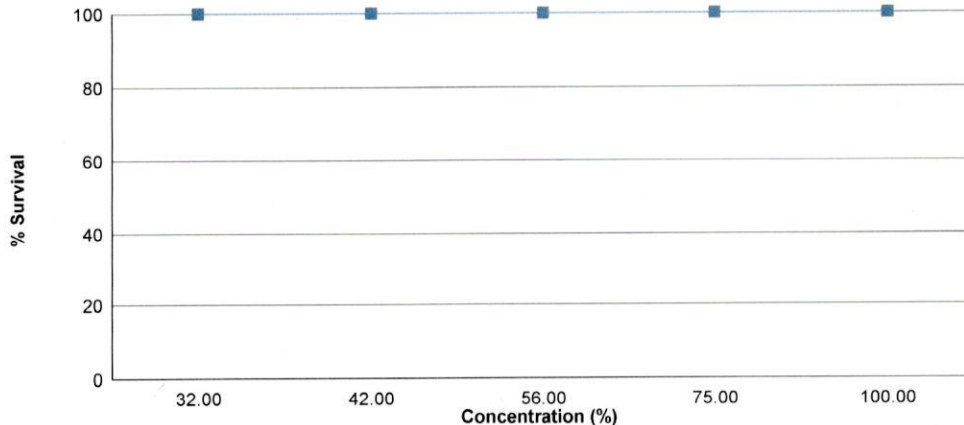
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 October 23, 2018 at 1450 and continued through October 30, 2018 at 1510. 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 reproduction = 100 % effluent



Concentration	Percent Survival	Mean Reproduction
Control	100	31.8
32 %	100	32.2
42 %	100	33.8
56 %	100	32.7
75 %	100	34.1
100 %	100	30.8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 23, 2018 at 1435

Date and Time Test Terminated: October 30, 2018 at 1600

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	8
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: October 23, 2018 at 1435

Test Terminated: October 30, 2018 at 1600

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92603	.92821	0.00218	8	0.272
	B	.92951	.93177	0.00226	8	0.282
	C	.93642	.93858	0.00216	8	0.270
	D	.93383	.93586	0.00203	8	0.254
	E	.93589	.93822	0.00233	8	0.291
32 %	A	.93542	.93738	0.00196	8	0.245
	B	.93698	.93905	0.00207	8	0.259
	C	.94334	.94554	0.00220	8	0.275
	D	.92732	.92933	0.00201	8	0.251
	E	.93219	.93441	0.00222	8	0.278
42 %	A	.92738	.92944	0.00206	8	0.258
	B	.92983	.93218	0.00235	8	0.294
	C	.93134	.93341	0.00207	8	0.259
	D	.92787	.93019	0.00232	8	0.290
	E	.92957	.93223	0.00266	8	0.332
56 %	A	.93158	.93434	0.00276	8	0.345
	B	.92758	.93039	0.00281	8	0.351
	C	.93236	.93482	0.00246	8	0.308
	D	.93152	.93388	0.00236	8	0.295
	E	.93609	.93897	0.00288	8	0.360
75 %	A	.92906	.93171	0.00265	8	0.331
	B	.93293	.93544	0.00251	8	0.314
	C	.93036	.93303	0.00267	8	0.334
	D	.92896	.93167	0.00271	8	0.339
	E	.93161	.93453	0.00292	8	0.365
100 %	A	.92749	.92997	0.00248	8	0.310
	B	.93013	.93272	0.00259	8	0.324
	C	.93165	.93438	0.00273	8	0.341
	D	.92762	.93023	0.00261	8	0.326
	E	.93118	.93390	0.00272	8	0.340

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 23, 2018 at 1450

Date and Time Test Terminated: October 30, 2018 at 1510

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	5	0	4	0	0	5	4	6	0	24	10	2.40
4	6	0	5	0	4	6	0	0	0	5	26	10	2.60
5	11	14	10	10	12	9	10	10	11	4	101	10	10.1
6	0	0	16	3	13	1	19	12	0	6	70	10	7.00
7	18	18	0	12	6	14	0	0	20	9	97	10	9.70
8													
TOTAL	35	37	31	29	35	30	34	26	37	24	318	10	31.8

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	6	0	0	5	5	6	0	22	10	2.20
4	6	4	6	0	4	4	0	0	1	5	30	10	3.00
5	12	14	10	11	10	11	11	10	8	11	108	10	10.8
6	0	0	0	0	0	1	0	12	0	15	28	10	2.80
7	17	15	15	16	15	17	15	3	16	5	134	10	13.4
8													
TOTAL	35	33	31	33	29	33	31	30	31	36	322	10	32.2

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	5	0	0	7	6	6	0	24	10	2.40
4	6	7	4	0	4	4	0	0	0	5	30	10	3.00
5	9	12	13	9	9	8	11	10	10	10	101	10	10.1
6	1	0	0	16	0	0	0	14	0	0	31	10	3.10
7	18	18	16	0	20	19	21	2	17	21	152	10	15.2
8													
TOTAL	34	37	33	30	33	31	39	32	33	36	338	10	33.8

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 23, 2018 at 1450

Date and Time Test Terminated: October 30, 2018 at 1510

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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	6	0	0	3	4	6	0	19	10	1.90	
4	6	6	6	0	6	3	0	0	0	6	33	10	3.30	
5	12	13	10	9	11	3	12	10	11	12	103	10	10.3	
6	2	0	0	0	0	5	0	14	18	0	39	10	3.90	
7	18	12	13	18	18	15	19	0	0	20	133	10	13.3	
8														
TOTAL	38	31	29	33	35	26	34	28	35	38	327	10	32.7	

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	0	0	0	5	0	0	4	5	7	0	21	10	2.10	
4	6	6	6	0	6	5	0	0	0	6	35	10	3.50	
5	13	10	9	12	11	11	12	12	9	13	112	10	11.2	
6	0	2	0	3	0	0	0	17	0	17	39	10	3.90	
7	18	16	15	19	16	14	19	0	17	0	134	10	13.4	
8														
TOTAL	37	34	30	39	33	30	35	34	33	36	341	10	34.1	

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	5	0	6	0	0	5	4	5	5	30	10	3.00	
4	4	0	4	0	5	4	0	0	0	0	17	10	1.70	
5	14	16	11	10	13	13	12	13	11	5	118	10	11.8	
6	0	1	0	0	0	2	0	0	0	0	3	10	0.300	
7	19	20	18	12	14	0	20	13	23	1	140	10	14.0	
8														
TOTAL	37	42	33	28	32	19	37	30	39	11	308	10	30.8	

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	1.00000	1.39310	
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		
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	32 %	27.50	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 = 0.0106 W = 0.9824 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 = 5.334 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	0.02714	0.005428	12.29	
Within (Error)	24	0.0106	0.0004417		
Total	29	0.03774			
Critical F = 3.9 (alpha = 0.01, df = 5,24)					
2.62 (alpha = 0.05, df = 5,24)					
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	0.2738	0.2738			
2	32 %	0.2616	0.2616	0.9178		
3	42 %	0.2866	0.2866	-0.963		
4	56 %	0.3318	0.3318	-4.363		
5	75 %	0.3366	0.3366	-4.725		
6	100 %	0.3282	0.3282	-4.093		
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	0.03137	11.5	0.0122		
3	42 %	5	0.03137	11.5	-0.0128		
4	56 %	5	0.03137	11.5	-0.058		
5	75 %	5	0.03137	11.5	-0.0628		
6	100 %	5	0.03137	11.5	-0.0544		

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
32 %	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
42 %	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
56 %	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
75 %	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
100 %	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	32 %	10	0	
2	42 %	10	0	
3	56 %	10	0	
4	75 %	10	0	
5	100 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.102 D* = 0.8003 Critical D* = 1.035 (alpha = 0.01, N = 60)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 27.52 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data FAIL B1 homogeneity test at 0.01 level.</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	32 %	104.50	75.00	10.00	
3	42 %	115.50	75.00	10.00	
4	56 %	110.00	75.00	10.00	
5	75 %	117.00	75.00	10.00	
6	100 %	108.50	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	77.33	15.47	0.6235	
Within (Error)	54	1340	24.81		
Total	59	1417			
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)					

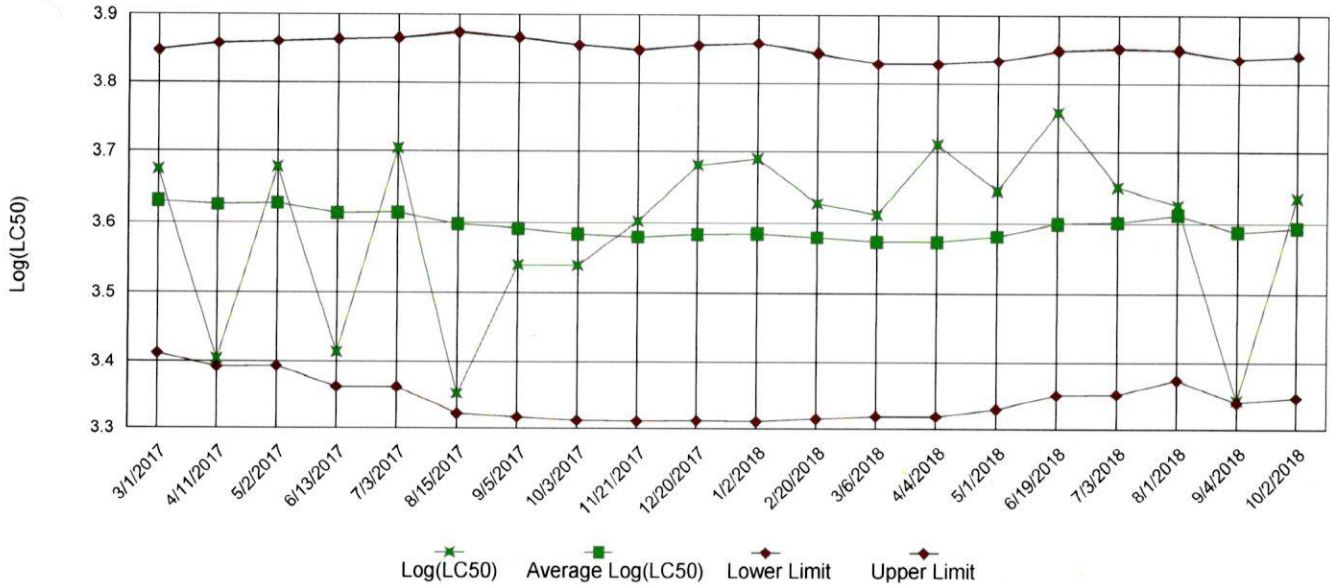
Dunnett's Test - Table 1 of 2					No Transformation	
Ho: Control < Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	31.8	31.8			
2	32 %	32.2	32.2	-0.1796		
3	42 %	33.8	33.8	-0.8978		
4	56 %	32.7	32.7	-0.404		
5	75 %	34.1	34.1	-1.033		
6	100 %	30.8	30.8	0.4489		
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	32 %	10	5.146	16.2	-0.4		
3	42 %	10	5.146	16.2	-2		
4	56 %	10	5.146	16.2	-0.9		
5	75 %	10	5.146	16.2	-2.3		
6	100 %	10	5.146	16.2	1		

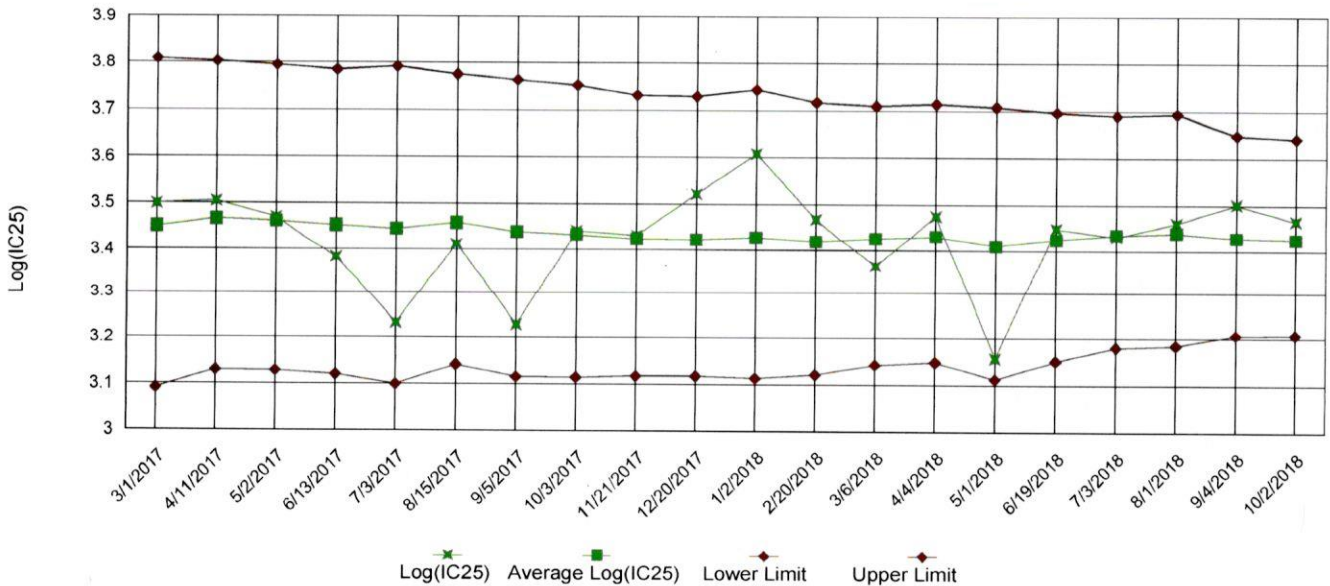
Appendix A3: Test 1000.0

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

LC50 Survival Data

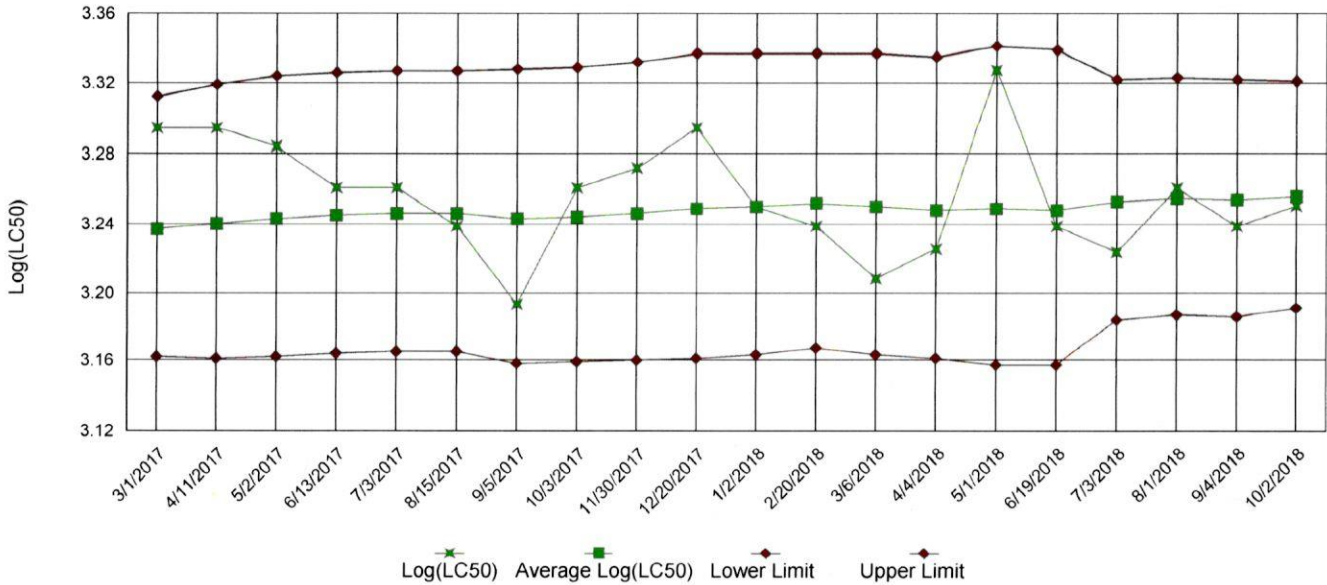


IC25 Growth Data

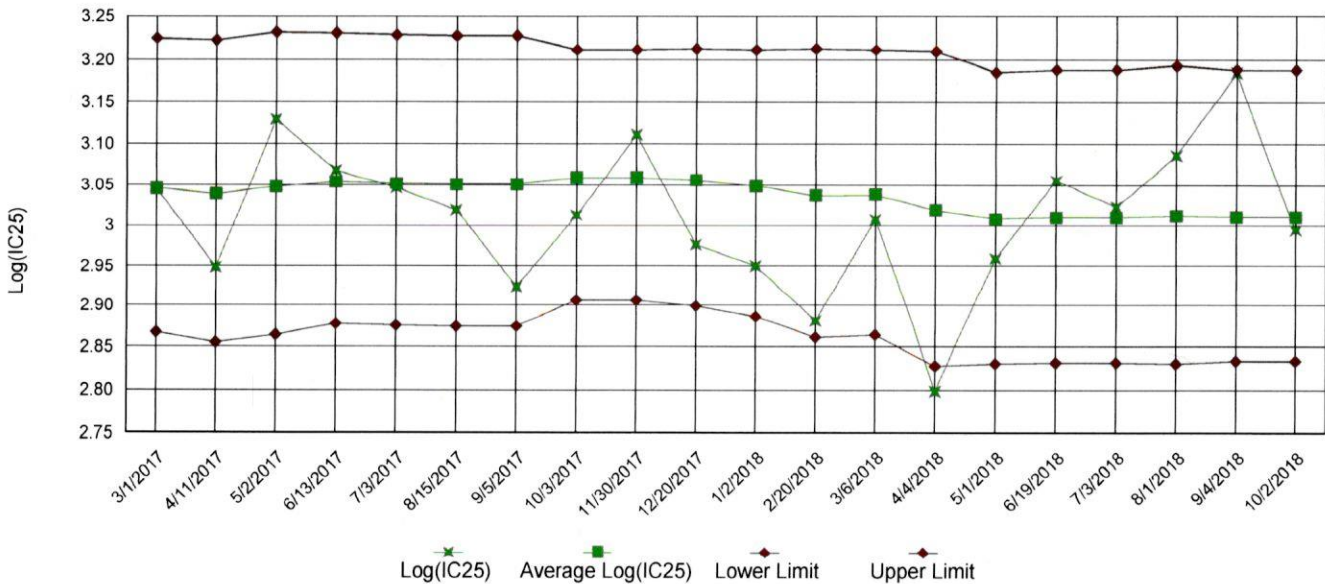


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

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: October 23, 2018 at 1435

Date and Time Test Terminated: October 30, 2018 at 1600

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	100	100	100	100	0.00
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	0.272	0.282	0.270	0.254	0.291	0.274	5.08
32 %	0.245	0.259	0.275	0.251	0.278	0.262	5.55
42 %	0.258	0.294	0.259	0.290	0.332	0.287	10.6
56 %	0.345	0.351	0.308	0.295	0.360	0.332	8.60
75 %	0.331	0.314	0.334	0.339	0.365	0.337	5.48
100 %	0.310	0.324	0.341	0.326	0.340	0.328	3.90

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 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> 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 %)	<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 (TLP6C)

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

5. NOEC Pimephales Lethality: 100 % (TOP6C)

6. LOEC Pimephales Lethality: 100 % (TXP6C)

7. NOEC Pimephales Sublethality: 100 % (TPP6C)

8. LOEC Pimephales Sublethality: 100 % (TYP6C)

9. Coefficient of variation for Pimephales growth: 5.08 (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, 329

Test Initiated: DATE: October 23, 2018 TIME: 1435
Test Terminated: DATE: October 30, 2018 TIME: 1600

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	6.9	7.1	6.9	7.2	7.2	6.5
Final	6.4	6.6	6.6	7.0	7.3	6.9	6.5
pH Initial	7.7	7.5	7.6	7.8	7.7	7.8	7.6
Final	7.4	7.0	7.3	7.6	7.7	7.9	7.8

DILUTION 32 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.1	7.1	7.2	7.0	7.1	7.4	7.0
Final	6.4	6.8	6.8	6.9	7.2	6.8	6.4
pH Initial	7.4	7.4	7.4	7.6	7.7	7.8	7.7
Final	7.4	7.1	7.4	7.6	7.7	7.8	7.8

DILUTION 42 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.2	7.2	7.2	7.2	7.2	7.4	6.8
Final	6.6	6.8	6.4	7.0	7.2	6.8	6.4
pH Initial	7.5	7.5	7.4	7.6	7.8	7.8	7.8
Final	7.4	7.2	7.3	7.7	7.7	7.8	7.8

DILUTION 56 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	7.2	6.9	7.1	6.9	7.2	6.7
Final	6.4	6.7	6.5	6.9	7.2	6.6	6.6
pH Initial	7.4	7.4	7.4	7.5	7.7	7.8	7.8
Final	7.4	7.3	7.4	7.7	7.7	7.8	7.8

DILUTION 75 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.3	7.2	7.2	7.0	7.0	7.2	6.8
Final	6.4	6.4	6.8	7.0	7.2	6.6	6.7
pH Initial	7.3	7.4	7.3	7.4	7.7	7.7	7.7
Final	7.4	7.2	7.5	7.7	7.7	7.8	7.8

DILUTION 100 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.4	7.2	7.4	7.0	7.0	7.2	6.8
Final	6.6	6.7	6.6	7.0	7.2	6.7	6.6
pH Initial	7.3	7.3	7.2	7.2	7.7	7.7	7.8
Final	7.5	7.3	7.4	7.7	7.7	7.8	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
35	61	240	<0.05	Outfall 001 23-OCT-18
36	58	250	<0.05	Outfall 001 24-OCT-18
34	58	250	<0.05	Outfall 001 26-OCT-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	160	<0.05	227598

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: October 23, 2018 at 1450

Date and Time Test Terminated: October 30, 2018 at 1510

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	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
A	35	35	34	38	37	37
B	37	33	37	31	34	42
C	31	31	33	29	30	33
D	29	33	30	33	39	28
E	35	29	33	35	33	32
F	30	33	31	26	30	19
G	34	31	39	34	35	37
H	26	30	32	28	34	30
I	37	31	33	35	33	39
J	24	36	36	38	36	11
Mean per Adult	31.8	32.2	33.8	32.7	34.1	30.8
Mean per Surviving Adult	31.8	32.2	33.8	32.7	34.1	30.8
CV %	14.3	6.84	8.23	12.6	8.35	30.9

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	(100 %)	<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	(100 %)	<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: 100 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 100 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 100 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 100 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 30.9 (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, 329

Test Initiated: DATE: October 23, 2018 TIME: 1450
Test Terminated: DATE: October 30, 2018 TIME: 1510

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.3	6.9	7.1	6.9	7.2	7.2	6.5
Final	6.9	6.6	7.3	6.7	7.1	6.6	6.8
pH Initial	7.7	7.5	7.6	7.8	7.7	7.8	7.6
Final	7.6	7.7	8.1	7.7	7.6	7.8	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	7.1	7.1	7.2	7.0	7.1	7.4	7.0
Final	7.2	6.9	7.4	7.0	7.4	6.6	6.5
pH Initial	7.4	7.4	7.4	7.6	7.7	7.8	7.7
Final	7.7	7.8	8.2	7.8	7.7	7.7	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	7.2	7.2	7.2	7.2	7.2	7.4	6.8
Final	7.1	6.9	7.4	7.1	7.5	6.6	6.5
pH Initial	7.5	7.5	7.4	7.6	7.8	7.8	7.8
Final	7.7	7.8	8.1	7.9	7.9	7.7	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	7.3	7.2	6.9	7.1	6.9	7.2	6.7
Final	7.1	6.8	7.2	7.1	7.4	6.5	6.7
pH Initial	7.4	7.4	7.4	7.5	7.7	7.8	7.8
Final	7.8	7.8	8.1	7.9	7.9	7.7	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
75 %							
D.O. Initial	7.3	7.2	7.2	7.0	7.0	7.2	6.8
Final	7.2	6.9	7.4	7.2	7.4	6.6	6.9
pH Initial	7.3	7.4	7.3	7.4	7.7	7.7	7.7
Final	7.8	7.8	8.0	7.9	8.0	7.7	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	7.4	7.2	7.4	7.0	7.0	7.2	6.8
Final	7.2	6.9	7.4	7.3	7.6	6.6	6.5
pH Initial	7.3	7.3	7.2	7.2	7.7	7.7	7.8
Final	7.8	7.8	8.0	8.1	8.1	7.7	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
35	61	240	<0.05	Outfall 001 23-OCT-18
36	58	250	<0.05	Outfall 001 24-OCT-18
34	58	250	<0.05	Outfall 001 26-OCT-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	41	160	<0.05	227598



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

Client: Benton Utilities
 Project: AL0036498
 Reference: AL0036498
 Project Manager: J. Buff
 Sampled By: AK

PO No. 99532
 NO OF BOTTLES 3

MATRIX
 W A T E R L
 G R A B
 C O M P
 V

Sample Identification: OSTFA11001
 Date/Time Collected: 10-23-18 0925

Carrier: AIC
 Received Temperature C: 0.2
 Remarks:

Field pH calibration on _____ @ _____
 Buffer: _____

NO OF BOTTLES: 3
 MATRIX: WATER L
 G R A B: V
 C O M P: V

Container Type: _____
 Preservative: _____
 G = Glass NO = none P = Plastic S = Sulfuric acid pH2
 V = VOA vials N = Nitric acid pH2

Turnaround Time Requested: (Please circle) _____
 NORMAL or EXPEDITED IN _____ DAYS
 Expedited results requested by: _____
 Who should AIC contact with questions: _____
 Phone: _____ Fax: _____
 Report Attention to: _____
 Report Address to: _____

Relinquished By: AK Date/Time: 10/23/18 0925
 Relinquished By: AK Date/Time: 10-23-18 1135

Received By: AK Date/Time: 10-23-18 0925
 Received in Lab By: AK Date/Time: 10-23-18 11:35

Comments: _____

Field pH calibration on _____ @ _____
 Buffer: _____
 T = Sodium Thiosulfate
 Z = Zinc acetate
 A = (NH₄)₂SO₄, NH₄OH

Email Address: JwBuff@Bentonar.org
 9/2014



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE **3** OF **3**

AIC No. **109991899** AIC PROPOSAL NO. **2280917**

Client: **Benton Utilities**

Project Reference: **AL003649x**

Project Manager: **J. Buff**

Sampled By: **AK**

AIC No. **00TF11001** Date/Time Collected **10/24/18 0811**

MATRIX: **WATER**

NO OF BOTTLES **3**

NO OF ANALYSES REQUESTED

Container Type: **Plastic**

Preservative: **P = Plastic, S = Sulfuric acid pH2**

Turnaround Time Requested: **(Please circle) NORMAL or EXPEDITED IN ___ DAYS**

Expedited results requested by: _____

Who should AIC contact with questions: _____

Phone: _____ Fax: _____

Report Attention to: _____

Report Address to: _____

Email Address: **JwBuff@Bentonar.org**

9/2014

AIC No.	Sample Identification	Date/Time Collected	GRA B	COM P	WATER	SOIL	NO OF BOTTLES	NO OF ANALYSES REQUESTED	Remarks	Received Temperature C	Carrier	Received		Relinquished		Date/Time	
												By:	Date/Time	By:	Date/Time	By:	Date/Time
							3		Monitoring	0.2		By: gfb	10/26/18	By: AK	10/26/18	By: gfb	10:51

Field pH calibration on _____ @ _____ Buffer: _____

T = Sodium Thiosulfate
Z = Zinc acetate
A = (NH₄)₂SO₄, NH₄OH

H = HCl to pH2
B = NaOH to pH12

V = VOA vials
N = Nitric acid pH2

Comments: _____