AMERICAN INTERPLEX CORPORATION
LABORATORIES

Biomonitoring 3rd Qtr
(July, Aug, Sept)

August 9, 2018

Biomonitoring Testing
for
Outfall 001
Benton, AR

Control No. 225442-1

Prepared for:

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

Prepared by:

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AMERICAN INTERPLEX CORPORATION
LABORATORIES

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

John Overbey
Chief Operating Officer

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

Table of Contents

I. Control Acceptance Criteria

II. Outlined Report

III. Data Analysis

IV. Standard Reference Toxicants

V. Organism History

VI. Results Summary
Pimephales promelas (Fathead minnow)
Ceriodaphnia dubia

Appendix A: Raw Data
A1: Test 1000.0
Pimephales promelas (Fathead minnow) Survival and Growth
Test 1002.0
Ceriodaphnia dubia Survival and Reproduction
A2: Statistics
A3: Reference Toxicant

Appendix B: Summary Forms

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.276	PASS
Control Growth CV < or = 40%	9.25	PASS
Growth Minimum Significant Difference 12 to 30%	11.3	BELOW
Critical Dilution CV < or = 40%	7.96	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	20.2	PASS
Control CV < or = 40% per Surviving Female	12.1	PASS
Reproduction Minimum Significant Difference 13 to 47%	16.1	PASS
Critical Dilution CV < or = 40%	16.0	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)	7.8	7.4	7.5
pH (standard units)	7.8	7.8	8.2
Alkalinity (mg/l as CaCO3)	43	48	47
Hardness (mg/l as CaCO3)	41	41	70
Conductivity (umhos/cm)	340	330	220
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	<0.1	0.13

2. Dilution Water Samples:
   Soft

Analysis	225208
Dissolved oxygen (mg/l)	6.8
pH (standard units)	8.0
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	44
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:	July 24, 2018 at 1605
Date & Time Test Terminated:	July 31,2018 at 0855
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:	July 24, 2018 at 1600
Date & Time Test Terminated:	July 30,2018 at 1315
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

4. Source of test organisms: Obtained from in-house cultures
5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms
1. Scientific Name
a. Test 1000.0 *Pimephales promelas*
b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat and following EPA method criteria.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and analyzed with Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC) for Reproduction. Dunnett's Test was used to calculate the PMSD.

IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on July 3, 2018 at 1235 to July 10, 2018 at 0930
The results were as follows: (Control No. 224836-1.)

Survival LC-50: 4478 mg/l
Growth IC-25: 2697 mg/l
Growth PMSD: 8.32

Ceriodaphnia dubia

A chronic reference test was performed on at 1440 to July 10, 2018 at 1130
The results were as follows: (Control No. 224836-2.)

Survival LC-50: 1673 mg/l
Growth IC-25: 1054 mg/l
Growth PMSD: 13.6

V. Organism History

Pimephales promelas (Fathead minnow)

Date: July 24, 2018
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: July 24, 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

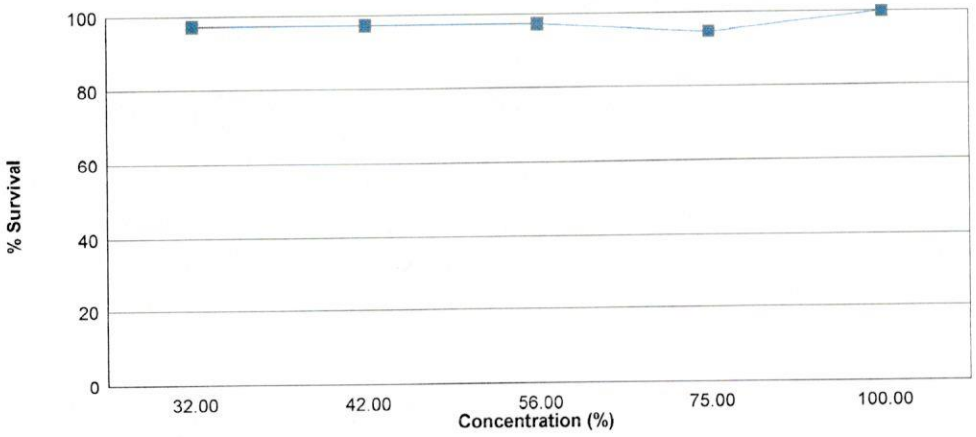
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 July 24, 2018 at 1605 and continued through July 31,2018 at 0855. 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	0.276
32 %	97.5	0.269
42 %	97.5	0.286
56 %	97.5	0.296
75 %	95.0	0.267
100 %	100	0.281

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

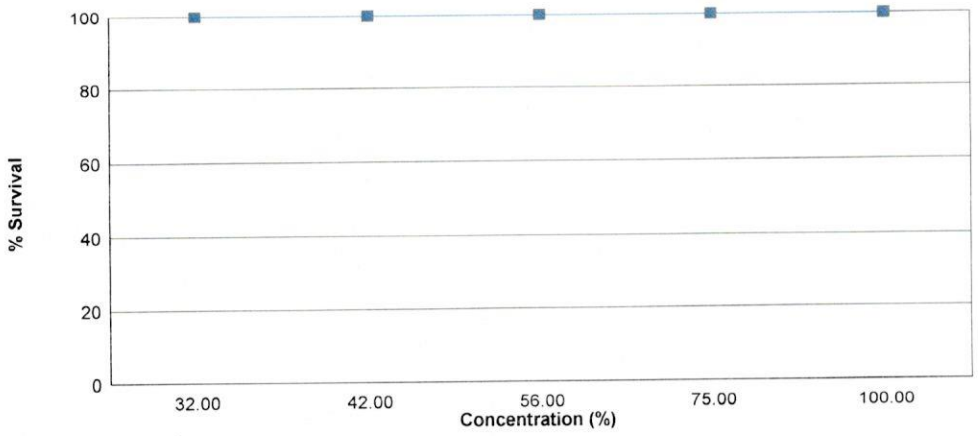
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 July 24, 2018 at 1600 and continued through July 30,2018 at 1315. 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



Summary of the 6-day *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	20.2
32 %	100	22.9
42 %	100	23.1
56 %	100	24.3
75 %	100	26.9
100 %	100	26.0

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: July 24, 2018 at 1605
Date and Time Test Terminated: July 31,2018 at 0855

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	7
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	7
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	7
	E	8	8	8	8	8	8	7
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: July 24, 2018 at 1605
Test Terminated: July 31,2018 at 0855

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.92646	.92887	0.00241	8	0.301
	B	.92204	.92399	0.00195	8	0.244
	C	.93036	.93275	0.00239	8	0.299
	D	.92615	.92841	0.00226	8	0.282
	E	.92687	.92892	0.00205	8	0.256
32 %	A	.93025	.93245	0.00220	8	0.275
	B	.92656	.92882	0.00226	8	0.282
	C	.92609	.92816	0.00207	8	0.259
	D	.92755	.92955	0.00200	8	0.250
	E	.92379	.92604	0.00225	8	0.281
42 %	A	.92739	.92972	0.00233	8	0.291
	B	.92591	.92835	0.00244	8	0.305
	C	.92229	.92457	0.00228	8	0.285
	D	.92465	.92679	0.00214	8	0.268
	E	.92652	.92875	0.00223	8	0.279
56 %	A	.93141	.93396	0.00255	8	0.319
	B	.92874	.93088	0.00214	8	0.268
	C	.93013	.93249	0.00236	8	0.295
	D	.92614	.92865	0.00251	8	0.314
	E	.92695	.92922	0.00227	8	0.284
75 %	A	.92853	.93058	0.00205	8	0.256
	B	.92459	.92676	0.00217	8	0.271
	C	.92301	.92549	0.00248	8	0.310
	D	.92293	.92498	0.00205	8	0.256
	E	.92386	.92581	0.00195	8	0.244
100 %	A	.93058	.93303	0.00245	8	0.306
	B	.93282	.93494	0.00212	8	0.265
	C	.93132	.93374	0.00242	8	0.302
	D	.93044	.93249	0.00205	8	0.256
	E	.92702	.92921	0.00219	8	0.274

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 24, 2018 at 1600
Date and Time Test Terminated: July 30,2018 at 1315

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	4	3	4	4	4	4	5	0	4	2	34	10	3.40
4	0	0	0	0	0	0	0	0	0	1	1	10	0.100
5	7	8	9	8	9	7	10	7	8	8	81	10	8.10
6	8	8	9	10	8	9	9	8	9	8	86	10	8.60
7													
8													
TOTAL	19	19	22	22	21	20	24	15	21	19	202	10	20.2

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	4	3	5	4	4	4	1	4	6	4	39	10	3.90
4	0	1	0	1	0	0	0	0	0	0	2	10	0.200
5	9	8	9	10	8	10	7	10	9	9	89	10	8.90
6	10	11	8	11	11	10	10	9	9	10	99	10	9.90
7													
8													
TOTAL	23	23	22	26	23	24	18	23	24	23	229	10	22.9

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	3	4	4	4	4	4	4	1	4	4	36	10	3.60
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	9	11	10	9	11	8	8	11	10	96	10	9.60
6	9	10	7	9	10	8	10	9	14	13	99	10	9.90
7													
8													
TOTAL	21	23	22	23	23	23	22	18	29	27	231	10	23.1

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: July 24, 2018 at 1600
Date and Time Test Terminated: July 30,2018 at 1315

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	3	5	6	3	4	4	1	0	4	5	35	10	3.50
4	0	0	0	0	0	0	0	6	10	0	16	10	1.60
5	10	11	8	10	10	9	6	0	0	10	74	10	7.40
6	11	13	13	12	13	13	9	10	12	12	118	10	11.8
7													
8													
TOTAL	24	29	27	25	27	26	16	16	26	27	243	10	24.3

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	4	4	5	4	4	4	5	5	5	4	44	10	4.40
4	0	0	0	0	0	0	0	0	11	0	11	10	1.10
5	9	10	11	9	10	11	11	10	0	11	92	10	9.20
6	13	11	13	13	12	11	13	13	11	12	122	10	12.2
7													
8													
TOTAL	26	25	29	26	26	26	29	28	27	27	269	10	26.9

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	5	4	5	5	5	6	4	1	5	4	44	10	4.40
4	0	0	0	0	0	0	0	0	10	0	10	10	1.00
5	9	10	12	12	8	11	13	7	0	10	92	10	9.20
6	9	10	13	13	11	12	13	9	12	12	114	10	11.4
7													
8													
TOTAL	23	24	30	30	24	29	30	17	27	26	260	10	26.0

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	0.87500	1.20940
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	0.87500	1.20940
5	75 %	1	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	1.00000	1.39310
5	75 %	4	0.87500	1.20940
5	75 %	5	0.87500	1.20940
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 = 0.1215		
W = 0.7519		
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 %	25.00	16.00	5.00	
4	56 %	25.00	16.00	5.00	
5	75 %	22.50	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
D = 0.01059		
W = 0.9652		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	
Data PASS normality test (alpha = 0.01).		

Bartlett's Test for Homogeneity of Variance		No Transformation
Calculated B1 statistic = 2.553		
Critical B = 15.086	(alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.		

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.002841	0.0005682	1.286
Within (Error)	24	0.0106	0.0004417	
Total	29	0.01344		

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	0.2764	0.2764		
2	32 %	0.2694	0.2694	0.5266	
3	42 %	0.2856	0.2856	-0.6921	
4	56 %	0.296	0.296	-1.475	
5	75 %	0.2674	0.2674	0.6771	
6	100 %	0.2806	0.2806	-0.316	

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	11.3	0.007
3	42 %	5	0.03137	11.3	-0.0092
4	56 %	5	0.03137	11.3	-0.0196
5	75 %	5	0.03137	11.3	0.009
6	100 %	5	0.03137	11.3	-0.0042

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
D = 0.1613		
D* = 1.266		
Critical D* = 1.035	(alpha = 0.01, N = 60)	
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 %	138.00	75.00	10.00	
3	42 %	134.00	75.00	10.00	
4	56 %	136.50	75.00	10.00	
5	75 %	155.00	75.00	10.00	
6	100 %	144.00	75.00	10.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	289	57.8	5.841
Within (Error)	54	534.4	9.896	
Total	59	823.4		

Critical F = 3.38 (alpha = 0.01, df = 5,54)
2.38 (alpha = 0.05, df = 5,54)

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	20.2	20.2		
2	32 %	22.9	22.9	-1.919	
3	42 %	23.1	23.1	-2.061	
4	56 %	24.3	24.3	-2.914	
5	75 %	26.9	26.9	-4.762	
6	100 %	26	26	-4.123	

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

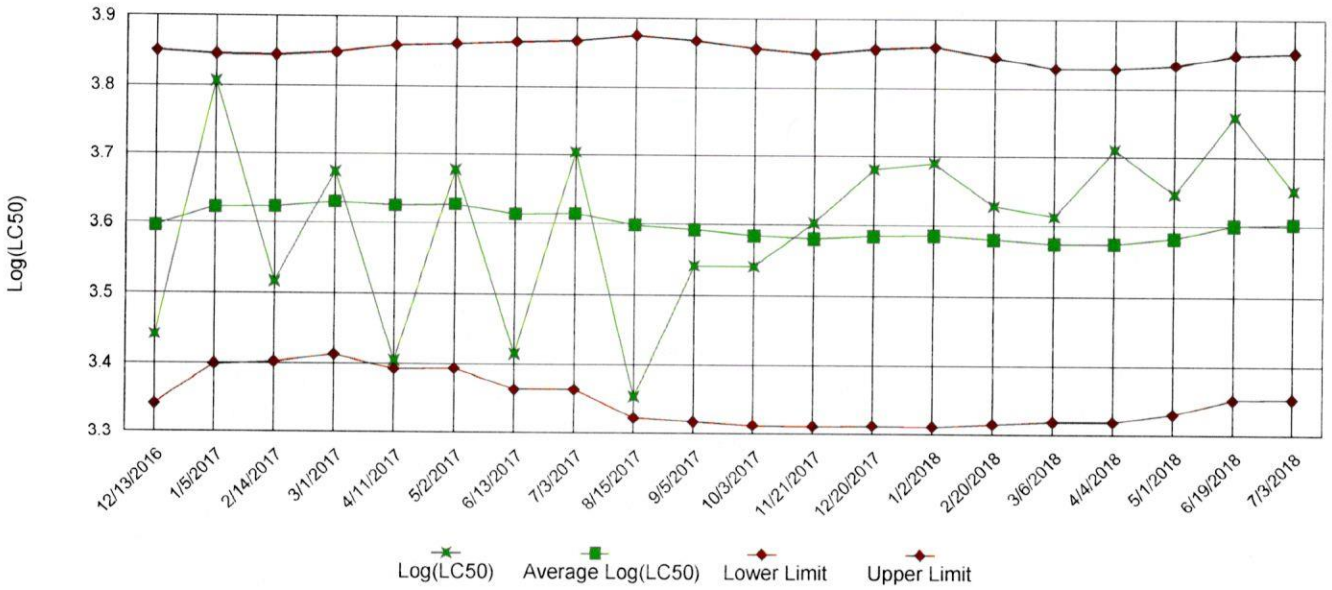
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	32 %	10	3.25	16.1	-2.7
3	42 %	10	3.25	16.1	-2.9
4	56 %	10	3.25	16.1	-4.1
5	75 %	10	3.25	16.1	-6.7
6	100 %	10	3.25	16.1	-5.8

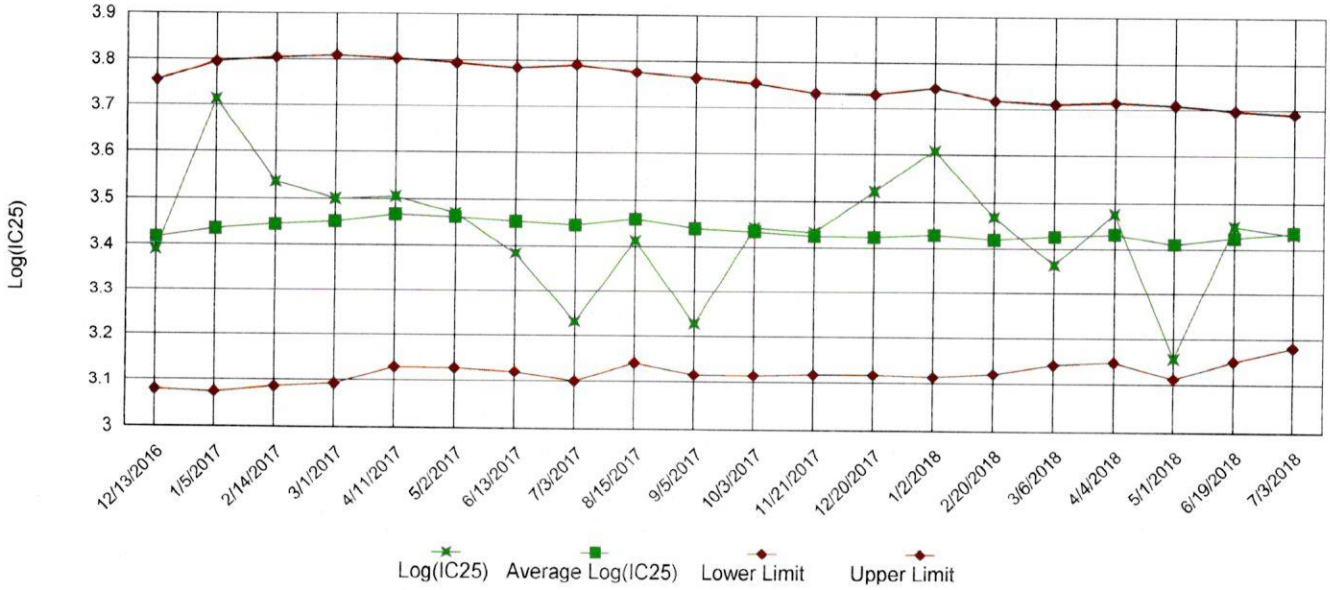
Appendix A3: Test 1000.0

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

LC50 Survival Data



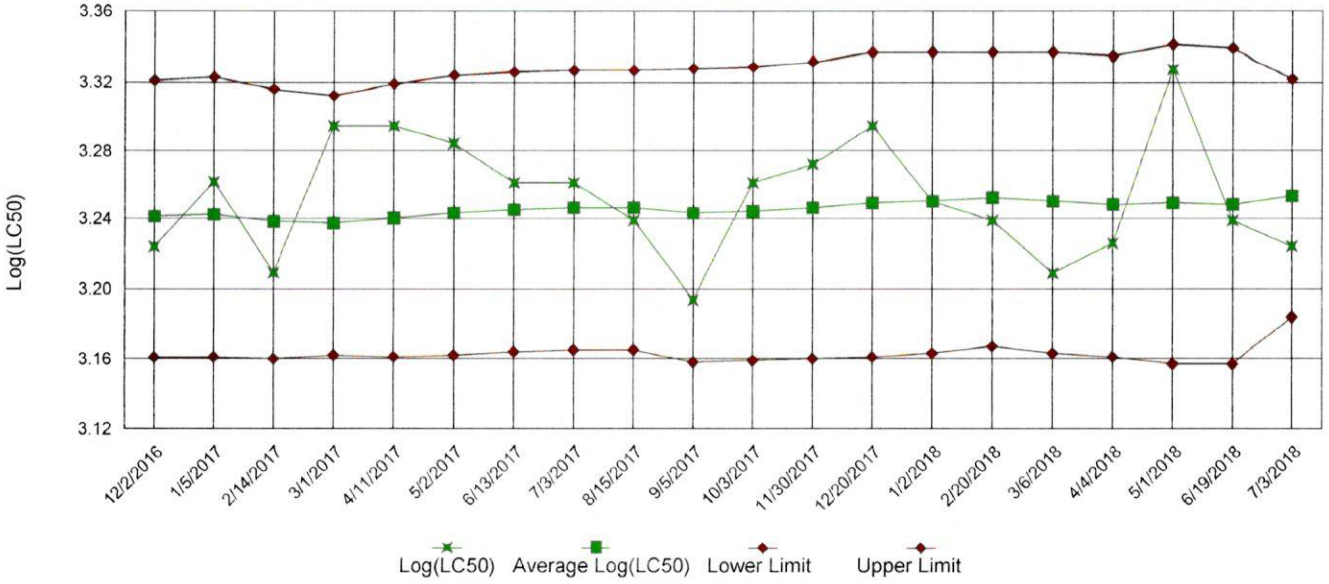
IC25 Growth Data



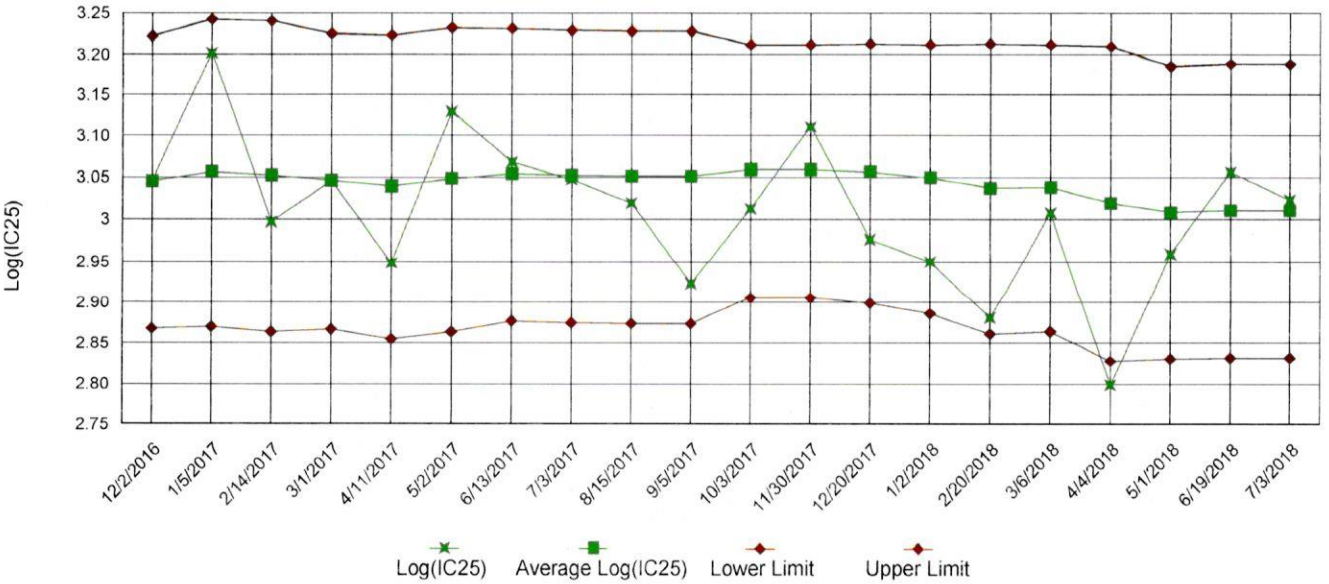
Appendix A3: Test 1002.0

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: July 24, 2018 at 1605
Date and Time Test Terminated: July 31,2018 at 0855
Dilution water used: Soft

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
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	87.5	100	100	97.5	5.73
56 %	100	100	100	100	87.5	100	100	97.5	5.73
75 %	100	100	100	87.5	87.5	100	100	95.0	7.21
100 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.301	0.244	0.299	0.282	0.256	0.276	9.25
32 %	0.275	0.282	0.259	0.250	0.281	0.269	5.28
42 %	0.291	0.305	0.285	0.268	0.279	0.286	4.82
56 %	0.319	0.268	0.295	0.314	0.284	0.296	7.13
75 %	0.256	0.271	0.310	0.256	0.244	0.267	9.60
100 %	0.306	0.265	0.302	0.256	0.274	0.281	7.96

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 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____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 %)	____YES	X NO
b.) 1/2 LOW FLOW DILUTION	(NA)	____YES	____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: 9.25 (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, 322, 329

Test Initiated: DATE: July 24, 2018 TIME: 1605
Test Terminated: DATE: July 31,2018 TIME: 0855

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	6.8	8.2	7.8	8.7	7.7	8.0	8.2
Final	6.9	7.8	7.8	8.3	8.3	7.2	8.0
pH Initial	8.0	8.2	7.9	7.7	8.2	8.3	8.2
Final	8.2	8.0	8.2	7.6	7.6	8.3	7.4

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	7.9	8.1	8.7	7.6	7.8	8.1
Final	6.6	7.8	7.7	8.3	8.2	7.1	8.2
pH Initial	7.9	8.2	7.9	7.8	8.2	8.3	8.2
Final	8.2	7.9	8.1	7.6	7.6	8.2	7.4

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	7.4	7.9	8.6	7.7	7.9	8.1
Final	6.5	8.0	7.6	8.3	8.2	6.9	8.1
pH Initial	7.9	8.2	8.0	7.7	8.2	8.3	8.2
Final	8.2	7.9	8.0	7.6	7.6	8.3	7.4

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	7.5	7.9	8.6	7.7	7.5	8.0
Final	7.0	7.8	7.7	8.4	8.2	5.0	8.2
pH Initial	8.0	8.2	8.0	7.8	8.2	8.3	8.2
Final	8.2	8.0	8.1	7.6	7.7	8.3	7.5

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	7.6	7.9	8.6	7.7	7.3	8.0
Final	6.6	7.8	7.6	8.3	8.2	5.0	8.0
pH Initial	7.9	8.2	8.0	7.8	8.2	8.3	8.3
Final	8.3	8.0	8.1	7.7	7.7	8.3	7.5

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	7.4	8.4	7.5	7.5	8.2
Final	6.5	7.7	7.6	8.3	8.2	7.1	8.1
pH Initial	7.8	8.3	7.8	7.9	8.2	8.4	8.3
Final	8.3	8.0	8.1	7.7	7.7	8.4	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
43	41	340	<0.05	Outfall 001 24-JUL-18
48	41	330	<0.05	Outfall 001 25-JUL-18
47	70	220	<0.05	Outfall 001 27-JUL-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	44	160	<0.05	225208

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: July 24, 2018 at 1600
Date and Time Test Terminated: July 30,2018 at 1315
Dilution water used: Soft

PERCENT SURVIVAL

		Percent Effluent				
Time of Reading	Control	32 %	42 %	56 %	75 %	100 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
6 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

		Percent Effluent				
Replicates	Control	32 %	42 %	56 %	75 %	100 %
A	19	23	21	24	26	23
B	19	23	23	29	25	24
C	22	22	22	27	29	30
D	22	26	23	25	26	30
E	21	23	23	27	26	24
F	20	24	23	26	26	29
G	24	18	22	16	29	30
H	15	23	18	16	28	17
I	21	24	29	26	27	27
J	19	23	27	27	27	26
Mean per Adult	20.2	22.9	23.1	24.3	26.9	26.0
Mean per Surviving Adult	20.2	22.9	23.1	24.3	26.9	26.0
CV %	12.1	8.84	13.1	18.8	5.09	16.0

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

   Is the mean survival significantly different (p=0.05) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION	(100 %)	_____YES	__X__ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	_____YES	_____ 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 %)	_____YES	__X__ NO
b.) 1/2 LOW FLOW DILUTION	(NA)	_____YES	_____ 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: __16__ (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, 322, 329

Test Initiated: DATE: July 24, 2018 TIME: 1600
Test Terminated: DATE: July 30,2018 TIME: 1315

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	6.8	8.2	7.8	8.7	7.7	8.0	8.2
Final	7.0	7.6	8.3	8.0	7.5	7.9	--
pH Initial	8.0	8.2	7.9	7.7	8.2	8.3	8.2
Final	8.5	8.1	8.4	7.9	8.3	8.2	--

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	7.9	8.1	8.7	7.6	7.8	8.1
Final	6.9	7.8	8.2	8.2	6.3	8.0	--
pH Initial	7.9	8.2	7.9	7.8	8.2	8.3	8.2
Final	8.5	8.2	8.4	8.0	8.0	8.5	--

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	7.4	7.9	8.6	7.7	7.9	8.1
Final	7.0	7.8	8.3	8.2	7.5	5.5	--
pH Initial	7.9	8.2	8.0	7.7	8.2	8.3	8.2
Final	8.6	8.2	8.4	8.0	8.9	8.4	--

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	7.5	7.9	8.6	7.7	7.5	8.0
Final	7.0	7.8	8.1	8.2	7.7	8.0	--
pH Initial	8.0	8.2	8.0	7.8	8.2	8.3	8.2
Final	8.5	8.2	8.4	8.1	9.0	8.6	--

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.9	7.6	7.9	8.6	7.7	7.3	8.0
Final	7.0	7.8	8.0	8.2	7.5	8.1	--
pH Initial	7.9	8.2	8.0	7.8	8.2	8.3	8.3
Final	8.5	8.2	8.4	8.1	8.8	8.7	--

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	7.7	7.4	8.4	7.5	7.5	8.2
Final	6.6	7.7	8.0	8.3	7.3	5.7	--
pH Initial	7.8	8.3	7.8	7.9	8.2	8.4	8.3
Final	8.6	8.2	8.4	8.1	8.7	8.7	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID	
43	41	340	<0.05	Outfall 001	24-JUL-18
48	41	330	<0.05	Outfall 001	25-JUL-18
47	70	220	<0.05	Outfall 001	27-JUL-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	44	160	<0.05	225208

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Benton Utilities
Project Reference: AP003649
Project Manager: J. Buff
Sampled By: AF

PO No. 99091

AIC CONTROL NO: 225442
AIC PROPOSAL NO:
Carrier:
Received Temperature C: 1.1°C

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Biomonitoring	ANALYSES REQUESTED: Chronic C.D + ECH	Remarks
1	OUTFALL 001	7/24/18 0810		✓	✓		3	✓	✓	
		Container Type					9			Field pH calibration
		Preservative					No			on _____ @ _____
										Buffer:

G = Glass, NO = none; P = Plastic, S = Sulfuric acid pH2; V = VOA vials, N = Nitric acid pH2; H = HCl to pH2, B = NaOH to pH12; T = Sodium Thiosulfate, Z = Zinc acetate; A=$(NH_4)_2SO_4$, NH_4OH

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

Relinquished By:	Date/Time	Received By:	Date/Time
AF	7/24/18 1107		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		Lisa Hoyt	7-24-18 1107

Comments:

9/2014

FORM 0060

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 1

Client: Benton Utilities
Project Reference: AR003649x
Project Manager: J. Buff
Sampled By: AF
PO No. 99091

AIC CONTROL NO: 225442
AIC PROPOSAL NO:
Carrier: BENTON
Received Temperature C: 0.6

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Biomonitoring	ANALYSES REQUESTED: Chronic CD + FH	Remarks
2	OUTFALL001	7/25/18 0829		✓	✓		3	✓	✓	
		Container Type					8			
		Preservative					No			

Field pH calibration on _____ @ _____ Buffer:

G = Glass, P = Plastic, V = VOA vials, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, H = HCl to pH2, B = NaOH to pH12, Z = Zinc acetate, A=$(NH_4)_2SO_4$, NH_4OH

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

Relinquished By:	Date/Time	Received By:	Date/Time
AF	7/25/18 1036		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		DANNY BROWN	7-25-18 1068

Comments:

9/2014

FORM 0060

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 1

Client: Benton Utilities
Project Reference: AR003649.8
Project Manager: J. Buff
Sampled By: MF

PO No. 99091

AIC CONTROL NO: 225442
AIC PROPOSAL NO:
Carrier: Benton
Received Temperature C: 1.6

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Biomonitoring	ANALYSES REQUESTED: Chronic CD + CH
3	OUTFALL001	7/27/18-0925		✓	✓		3	✓	✓
		Container Type					P		
		Preservative					No		

Remarks

Field pH calibration on _____ @ _____
Buffer:

G = Glass, P = Plastic, V = VOA vials, H = HCl to pH2, T = Sodium Thiosulfate
NO = none, S = Sulfuric acid pH2, N = Nitric acid pH2, B = NaOH to pH12, Z = Zinc acetate, A=$(NH_4)_2SO_4$, NH_4OH

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

Relinquished By:	Date/Time	Received By:	Date/Time
MF	7/27/18 0930	LC	7/27/18 0930
MF	7/27/18 10:37	[illegible]	7/27/18 10:37

Comments:

9/2014

FORM 0060