AMERICAN INTERPLEX CORPORATION
LABORATORIES

Biomonitoring 1st Qtr.
(Jan, Feb, Mar) 2019

February 11, 2019

Biomonitoring Testing
for
Outfall 001
Benton, AR

Control No. 231090-1

Prepared for:

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

Prepared by:

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Little Rock, AR 72204-2322

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

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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	0.322	PASS
Control Growth CV < or = 40%	11.5	PASS
Growth Minimum Significant Difference 12 to 30%	24.8	PASS
Critical Dilution CV < or = 40%	6.15	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	21.2	PASS
Reproduction Minimum Significant Difference 13 to 47%	26.6	PASS
Critical Dilution CV < or = 40%	21.9	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)	9.0	7.7	8.5
pH (standard units)	7.0	7.3	7.0
Alkalinity (mg/l as CaCO3)	32	34	38
Hardness (mg/l as CaCO3)	49	51	60
Conductivity (umhos/cm)	240	230	250
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	<0.1	<0.1	<0.1

2. Dilution Water Samples:
   Soft

Analysis	230915-1
Dissolved oxygen (mg/l)	7.8
pH (standard units)	7.5
Alkalinity (mg/l as CaCO3)	31
Hardness (mg/l as CaCO3)	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:	January 29, 2019 at 1300
Date & Time Test Terminated:	Febuary 5, 2019 at 1305
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:	January 29, 2019 at 1430
Date & Time Test Terminated:	Febuary 4, 2019 at 1238
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 January 22, 2019 at 1305 to January 29, 2019 at 1420
The results were as follows: (Control No. 230903-1.)

Survival LC-50: 3908 mg/l
Growth IC-25: 2792 mg/l
Growth PMSD: 14.5

Ceriodaphnia dubia

A chronic reference test was performed on January 22, 2019 at 1400 to January 28, 2019 at 1405
The results were as follows: (Control No. 230903-2.)

Survival LC-50: 1422 mg/l
Growth IC-25: 1507 mg/l
Growth PMSD: 17

V. Organism History

Pimephales promelas (Fathead minnow)

Date: January 29, 2019
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: January 29, 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

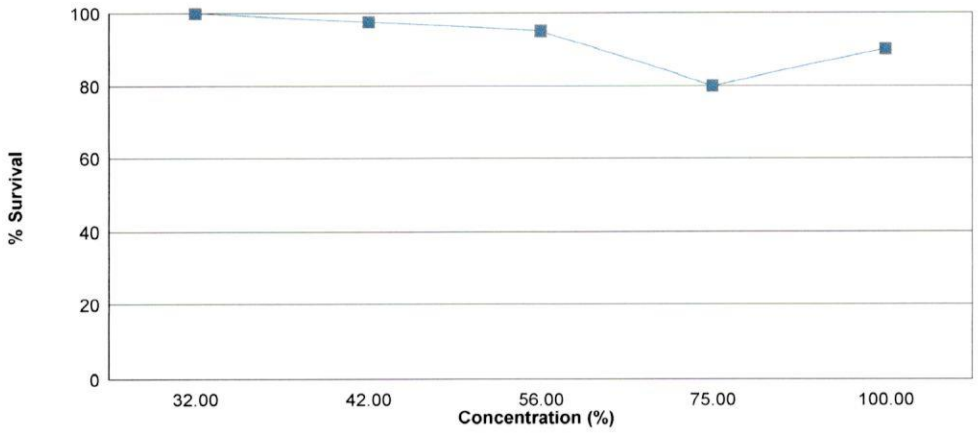
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 January 29, 2019 at 1300 and continued through Febuary 5, 2019 at 1305. 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	0.322
32 %	100	0.351
42 %	97.5	0.361
56 %	95.0	0.290
75 %	80.0	0.297
100 %	90.0	0.378

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

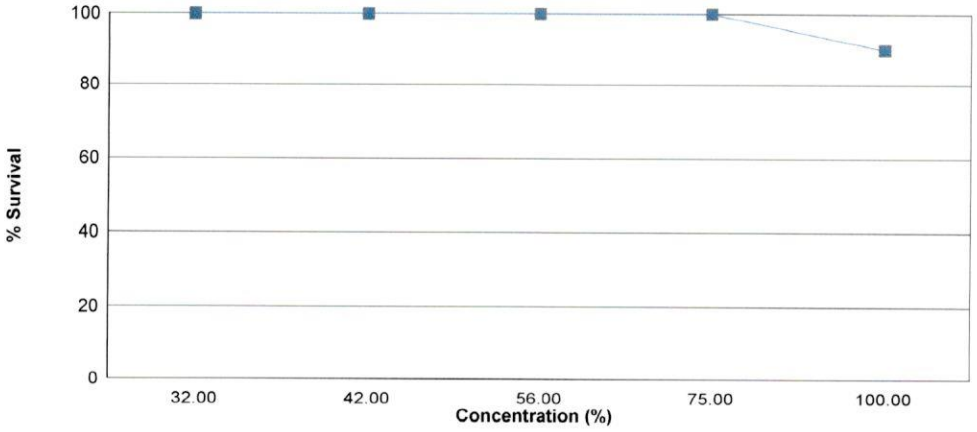
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 January 29, 2019 at 1430 and continued through Febuary 4, 2019 at 1238. 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	22.0
42 %	100	23.2
56 %	100	25.1
75 %	100	24.7
100 %	90.0	23.4

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: January 29, 2019 at 1300
Date and Time Test Terminated: Febuary 5, 2019 at 1305

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	7
	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	7
	D	8	8	8	8	8	7	7
	E	8	8	8	8	8	8	8
75 %	A	8	8	8	7	6	6	6
	B	8	8	8	8	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	3	3
100 %	A	8	8	8	8	8	7	7
	B	8	8	8	8	7	7	7
	C	8	8	8	8	8	7	7
	D	8	8	8	8	7	7	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: January 29, 2019 at 1300
Test Terminated: Febuary 5, 2019 at 1305

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.94109	.94357	0.00248	8	0.310
	B	.93876	.94087	0.00211	8	0.264
	C	.93628	.93901	0.00273	8	0.341
	D	.93417	.93687	0.00270	8	0.338
	E	.93715	.94002	0.00287	8	0.359
32 %	A	.92856	.93107	0.00251	8	0.314
	B	.92595	.92860	0.00265	8	0.331
	C	.92702	.92986	0.00284	8	0.355
	D	.92934	.93229	0.00295	8	0.369
	E	.92555	.92862	0.00307	8	0.384
42 %	A	.93144	.93469	0.00325	8	0.406
	B	.92982	.93209	0.00227	8	0.284
	C	.93259	.93527	0.00268	8	0.335
	D	.93135	.93420	0.00285	8	0.356
	E	.93355	.93696	0.00341	8	0.426
56 %	A	.93233	.93529	0.00296	8	0.370
	B	.93205	.93426	0.00221	8	0.276
	C	.93503	.93731	0.00228	8	0.285
	D	.94182	.94361	0.00179	8	0.224
	E	.93522	.93759	0.00237	8	0.296
75 %	A	.93415	.93685	0.00270	8	0.338
	B	.93083	.93335	0.00252	8	0.315
	C	.92963	.93216	0.00253	8	0.316
	D	.93619	.93922	0.00303	8	0.379
	E	.92553	.92664	0.00111	8	0.139
100 %	A	.92749	.93024	0.00275	8	0.344
	B	.92374	.92676	0.00302	8	0.378
	C	.92552	.92852	0.00300	8	0.375
	D	.92726	.93033	0.00307	8	0.384
	E	.92518	.92845	0.00327	8	0.409

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 29, 2019 at 1430
Date and Time Test Terminated: Febuary 4, 2019 at 1238

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	0	4	4	4	4	6	4	5	0	35	10	3.50
4	0	5	0	0	0	0	0	0	0	5	10	10	1.00
5	8	8	7	8	7	8	10	9	7	8	80	10	8.00
6	9	0	9	9	10	8	10	12	10	0	77	10	7.70
7													
8													
TOTAL	21	13	20	21	21	20	26	25	22	13	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	5	0	4	4	4	4	4	4	4	0	33	10	3.30
4	0	5	0	0	0	0	0	0	0	5	10	10	1.00
5	9	8	8	9	8	9	9	9	9	6	84	10	8.40
6	11	0	13	10	11	12	12	12	12	0	93	10	9.30
7													
8													
TOTAL	25	13	25	23	23	25	25	25	25	11	220	10	22.0

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	4	0	3	4	4	0	5	4	4	0	28	10	2.80
4	0	6	0	0	0	4	0	0	0	6	16	10	1.60
5	10	10	8	11	8	8	10	9	9	9	92	10	9.20
6	14	0	11	15	11	11	13	11	10	0	96	10	9.60
7													
8													
TOTAL	28	16	22	30	23	23	28	24	23	15	232	10	23.2

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: January 29, 2019 at 1430
Date and Time Test Terminated: Febuary 4, 2019 at 1238

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	5	0	4	4	4	4	5	4	4	0	34	10	3.40
4	0	6	0	0	1	0	0	0	0	5	12	10	1.20
5	9	9	8	9	11	9	12	9	9	12	97	10	9.70
6	15	0	13	13	13	13	13	14	14	0	108	10	10.8
7													
8													
TOTAL	29	15	25	26	29	26	30	27	27	17	251	10	25.1

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	0	4	4	4	4	4	5	4	0	33	10	3.30
4	1	6	0	0	0	0	0	0	0	6	13	10	1.30
5	10	11	9	10	10	11	11	9	10	8	99	10	9.90
6	12	0	15	13	12	10	14	14	12	0	102	10	10.2
7													
8													
TOTAL	27	17	28	27	26	25	29	28	26	14	247	10	24.7

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	4	0	4	4	4	4	6	X	5	0	31	9	3.44
4	0	6	0	0	0	0	0	X	0	7	13	9	1.44
5	11	12	10	11	10	10	11	X	11	8	94	9	10.4
6	13	0	13	14	12	15	15	X	14	0	96	9	10.7
7													
8													
TOTAL	28	18	27	29	26	29	32	0	30	15	234	10	23.4

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	0.87500	1.20940
3	42 %	5	1.00000	1.39310
4	56 %	1	1.00000	1.39310
4	56 %	2	1.00000	1.39310
4	56 %	3	0.87500	1.20940
4	56 %	4	0.87500	1.20940
4	56 %	5	1.00000	1.39310
5	75 %	1	0.75000	1.04720
5	75 %	2	0.87500	1.20940
5	75 %	3	1.00000	1.39310
5	75 %	4	1.00000	1.39310
5	75 %	5	0.37500	0.65906
6	100 %	1	0.87500	1.20940
6	100 %	2	0.87500	1.20940
6	100 %	3	0.87500	1.20940
6	100 %	4	0.87500	1.20940
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 = 0.4673		
W = 0.8218		
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 %	25.00	16.00	5.00	
4	56 %	22.50	16.00	5.00	
5	75 %	20.00	16.00	5.00	
6	100 %	17.50	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 = 0.0688		
W = 0.9334		
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 = 9.176		
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	0.03176	0.006352	2.215
Within (Error)	24	0.06884	0.002868	
Total	29	0.1006		

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	0.3224	0.3224		
2	32 %	0.3506	0.3506	-0.8326	
3	42 %	0.3614	0.3614	-1.151	
4	56 %	0.2902	0.2902	0.9507	
5	75 %	0.2974	0.2974	0.7381	
6	100 %	0.378	0.378	-1.642	

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	0.07993	24.8	-0.0282
3	42 %	5	0.07993	24.8	-0.039
4	56 %	5	0.07993	24.8	0.0322
5	75 %	5	0.07993	24.8	0.025
6	100 %	5	0.07993	24.8	-0.0556

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 %	9	1	10
Total	19	1	20

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

Summary of Fisher's Exact Test

Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	32 %	10	0	
2	42 %	10	0	
3	56 %	10	0	
4	75 %	10	0	
5	100 %	10	1	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.2547 D* = 1.998 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 %	123.00	75.00	10.00	
3	42 %	128.50	75.00	10.00	
4	56 %	136.50	75.00	10.00	
5	75 %	136.50	75.00	10.00	
6	100 %	128.50	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	228.5	45.7	1.787
Within (Error)	53	1356	25.58	
Total	58	1585		

Critical F = 3.39 (alpha = 0.01, df = 5,53)
2.39 (alpha = 0.05, df = 5,53)

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	20.2	20.2		
2	32 %	22	22	-0.7958	
3	42 %	23.2	23.2	-1.326	
4	56 %	25.1	25.1	-2.166	
5	75 %	24.7	24.7	-1.99	
6	100 %	26	26	-2.496	

Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,53)
WARNING - Unequal replicate sizes. Critical values assuming equal replicate sizes have been used.

Dunnett's Test - Table 2 of 2 — No Transformation

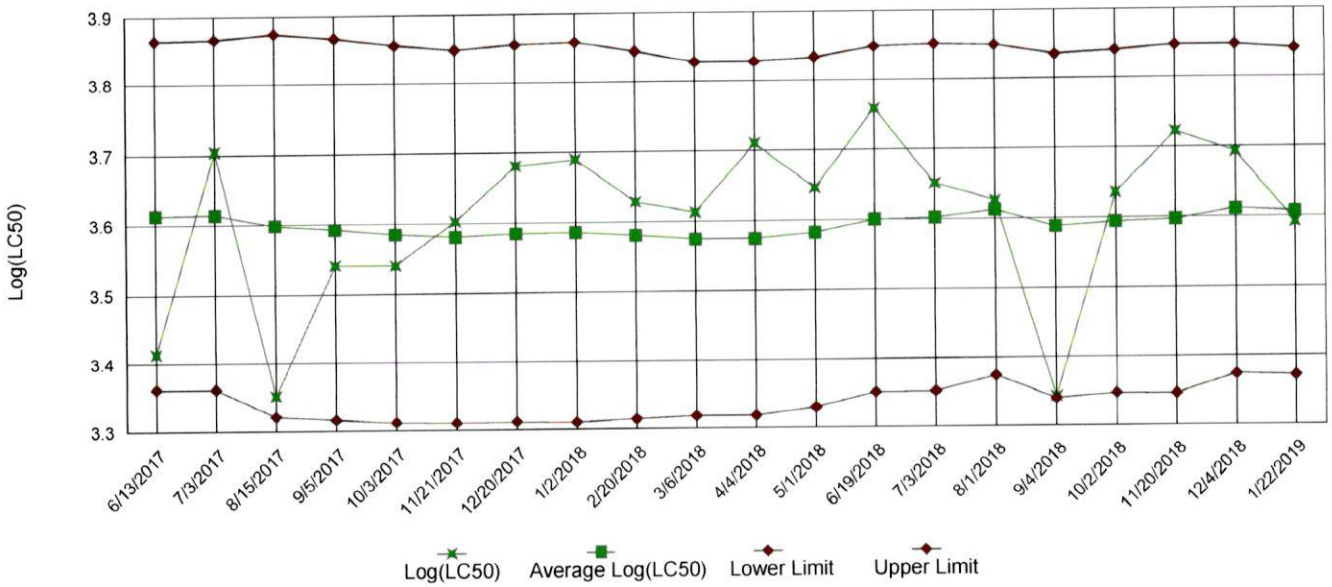
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	32 %	10	5.225	25.9	-1.8
3	42 %	10	5.225	25.9	-3
4	56 %	10	5.225	25.9	-4.9
5	75 %	10	5.225	25.9	-4.5
6	100 %	9	5.368	26.6	-5.8

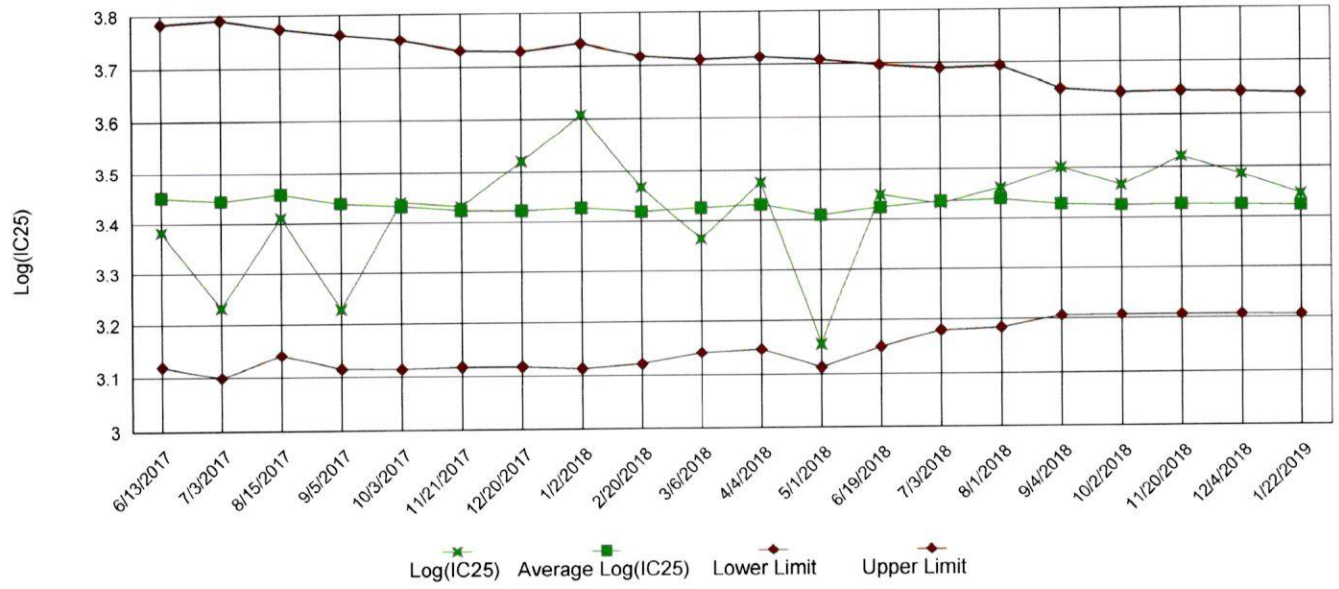
Appendix A3: Test 1000.0

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

LC50 Survival Data



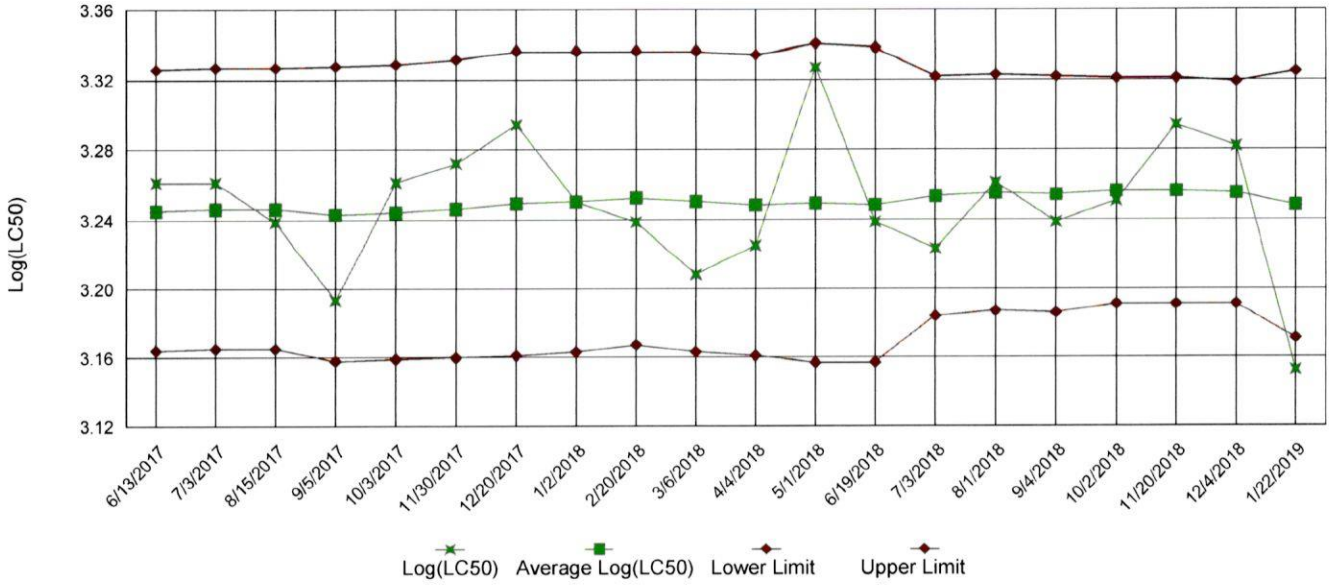
IC25 Growth Data



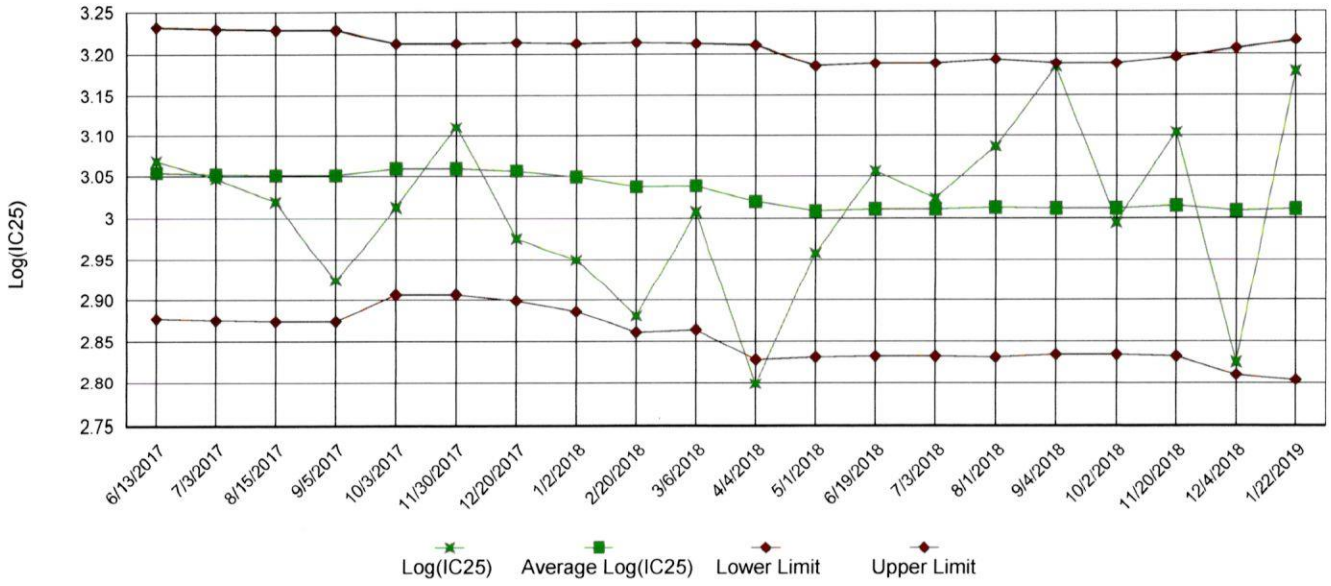
Appendix A3: Test 1002.0

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: January 29, 2019 at 1300
Date and Time Test Terminated: Febuary 5, 2019 at 1305
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	100	100	100	100	0.00
42 %	100	100	100	87.5	100	100	100	97.5	5.73
56 %	100	100	87.5	87.5	100	100	100	95.0	7.21
75 %	75.0	87.5	100	100	37.5	100	100	80.0	32.4
100 %	87.5	87.5	87.5	87.5	100	100	100	90.0	6.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.310	0.264	0.341	0.338	0.359	0.322	11.5
32 %	0.314	0.331	0.355	0.369	0.384	0.351	8.06
42 %	0.406	0.284	0.335	0.356	0.426	0.361	15.7
56 %	0.370	0.276	0.285	0.224	0.296	0.29	18.1
75 %	0.338	0.315	0.316	0.379	0.139	0.297	31.0
100 %	0.344	0.378	0.375	0.384	0.409	0.378	6.15

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: 11.5 (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: January 29, 2019 TIME: 1300
Test Terminated: DATE: Febuary 5, 2019 TIME: 1305

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	8.0	7.9	7.8	7.5	7.6	7.2
Final	8.0	7.0	7.1	7.7	7.3	7.0	6.8
pH Initial	7.5	7.4	7.5	7.5	7.6	7.6	7.5
Final	7.5	7.4	7.4	7.6	7.6	7.5	7.4

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.9	8.1	8.2	8.0	7.8	7.6	7.3
Final	8.0	7.1	7.1	7.8	7.4	7.1	6.8
pH Initial	7.3	7.4	7.5	7.4	7.4	7.4	7.4
Final	7.5	7.3	7.3	7.5	7.6	7.5	7.4

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	8.6	8.1	8.1	7.9	7.7	7.4
Final	8.2	7.2	7.2	7.9	7.4	7.1	7.0
pH Initial	7.3	7.4	7.5	7.4	7.4	7.4	7.3
Final	7.5	7.3	7.3	7.5	7.6	7.5	7.4

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.2	8.4	8.0	7.9	7.7	7.7	7.4
Final	8.0	6.9	6.9	7.6	7.2	7.0	6.7
pH Initial	7.2	7.4	7.5	7.4	7.4	7.4	7.3
Final	7.5	7.4	7.4	7.5	7.6	7.5	7.4

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	7.9	7.8	7.8	7.6	7.3
Final	8.0	6.9	7.0	7.6	7.4	7.0	6.7
pH Initial	7.2	7.4	7.4	7.3	7.3	7.4	7.3
Final	7.5	7.4	7.4	7.5	7.6	7.6	7.4

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	9.0	8.3	7.7	8.0	8.5	8.0	7.6
Final	8.1	7.0	6.8	8.0	7.2	6.8	6.8
pH Initial	7.0	7.2	7.3	7.1	7.0	7.1	7.1
Final	7.5	7.4	7.4	7.4	7.6	7.5	7.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID	
32	49	240	<0.05	Outfall 001	29-JAN-19
34	51	230	<0.05	Outfall 001	30-JAN-19
38	60	250	<0.05	Outfall 001	01-FEB-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	41	160	<0.05	230915-1

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: January 29, 2019 at 1430
Date and Time Test Terminated: Febuary 4, 2019 at 1238
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	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

		Percent Effluent				
Replicates	Control	32 %	42 %	56 %	75 %	100 %
A	21	25	28	29	27	28
B	13	13	16	15	17	18
C	20	25	22	25	28	27
D	21	23	30	26	27	29
E	21	23	23	29	26	26
F	20	25	23	26	25	29
G	26	25	28	30	29	32
H	25	25	24	27	28	0
I	22	25	23	27	26	30
J	13	11	15	17	14	15
Mean per Adult	20.2	22.0	23.2	25.1	24.7	23.4
Mean per Surviving Adult	20.2	22.0	23.2	25.1	24.7	26.0
CV %	21.2	24.3	21.0	20.2	20.4	21.9

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

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(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: 21.9 (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: January 29, 2019 TIME: 1430
Test Terminated: DATE: Febuary 4, 2019 TIME: 1238

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.8	8.0	7.9	7.8	7.5	7.6	7.2
Final	8.1	7.9	8.0	8.1	7.8	7.6	7.5
pH Initial	7.5	7.4	7.5	7.5	7.6	7.6	7.5
Final	8.1	7.9	7.9	8.0	8.1	7.9	7.8

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

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.0	8.6	8.1	8.1	7.9	7.7	7.4
Final	8.4	8.0	8.2	8.0	7.6	7.8	7.8
pH Initial	7.3	7.4	7.5	7.4	7.4	7.4	7.3
Final	8.0	8.0	7.9	8.1	8.0	7.8	7.7

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.2	8.4	8.0	7.9	7.7	7.7	7.4
Final	8.2	8.2	7.9	8.0	7.8	7.5	7.5
pH Initial	7.2	7.4	7.5	7.4	7.4	7.4	7.3
Final	8.0	7.9	7.9	8.0	8.0	7.9	7.8

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	7.9	7.8	7.8	7.6	7.3
Final	8.2	8.0	8.0	8.1	7.8	7.6	7.6
pH Initial	7.2	7.4	7.4	7.3	7.3	7.4	7.3
Final	8.0	8.0	8.0	8.1	8.0	7.9	7.8

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	9.0	8.3	7.7	8.0	8.5	8.0	7.6
Final	8.4	7.9	8.0	8.1	7.7	7.7	7.4
pH Initial	7.0	7.2	7.3	7.1	7.0	7.1	7.1
Final	8.0	8.0	8.0	8.1	8.1	8.0	7.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	49	240	<0.05	Outfall 001 29-JAN-19
34	51	230	<0.05	Outfall 001 30-JAN-19
38	60	250	<0.05	Outfall 001 01-FEB-19

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	41	160	<0.05	230915-1

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Benton Utilities
Project Reference: AR003649x
Project Manager: J. Buff
Sampled By: AF

PO No. 100071

AIC CONTROL NO: 231090
AIC PROPOSAL NO:
Carrier: J. BUFF
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 + EH	Remarks
1	OUTFALL 001	1/29/19 0815		✓	✓		3	✓	✓	
		Container Type						P		
		Preservative						No		

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
AF	1/29/19 0830	GB	1/29/19 0834
GB	1/29/19 0918	Received in Lab By: D. Brown	1-29-19 0918

Comments:

9/2014

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Benton Utilities
Project Reference: AR003649x
Project Manager: J. Buff
Sampled By: A.F.

PO No. 100071

AIC CONTROL NO: 231090
AIC PROPOSAL NO:
Carrier:
Received Temperature 6.7

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Biomonitoring	ANALYSES REQUESTED: Chronic C.D + FH	Remarks
2	OUTFALL001	1/30/19 0820		✓	✓		3	✓	✓	
		Container Type						P		
		Preservative						No		

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
AF	1/30/19 0857		1/30/19 0857
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		ALS#1	01/30/19 0935

Comments:

9/2014

FORM 0060

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Benton Utilities
Project Reference: AR003649x
Project Manager: J. Buff
Sampled By: AF

PO No. 100071

AIC CONTROL NO: 231090
AIC PROPOSAL NO:
Carrier:
Received Temperature: 0.7

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX: WATER	MATRIX: SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Biomonitoring	ANALYSES REQUESTED: Chronic CS + FH	Remarks
3	OUTFALL001	2/1/19 0807		✓	✓		3	✓	✓	
		Container Type						P		
		Preservative						No		

Field pH calibration 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	2/1/19 0910	JB	2/1/19 0910
JB	2/1/19 0949	AC341	01/02/19 0949

Comments:

9/2014

FORM 0060