November 20, 2020

Biomonitoring Testing
for
Outfall 001
Huntsville, AR

Control No. 250223-1

Prepared for:

Mr. Bill Eoff
Huntsville Water Utilities
Post Office Box 430
Huntsville, AR 72740

Prepared by:

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

Huntsville Water Utilities
ATTN: Mr. Bill Eoff
Post Office Box 430
Huntsville, AR 72740

Re: Chronic 7-Day Renewal*Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Outfall 001 - Huntsville, AR
NPDES Permit No. AR0022004 AFIN# 44-00018

Dear Mr. Bill Eoff:

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 percent minimum significant difference (PMSD) was below the limit of 12. Following additional calculations provided in the EPA document "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination Systems Program", the NOEC for sublethal effects was calculated to be 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: Huntsville Water Utilities
ATTN: Mr. Bill Eoff
bill9eoff@hotmail.com

Table of Contents

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	0.602	PASS
Control Growth CV < or = 40%	5.89	PASS
Growth Minimum Significant Difference 12 to 30%	9.36	BELOW
Critical Dilution CV < or = 40%	4.40	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	26.3	PASS
Control CV < or = 40% per Surviving Female	15.5	PASS
Reproduction Minimum Significant Difference 13 to 47%	17.6	PASS
Critical Dilution CV < or = 40%	15.7	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0022004 AFIN# 44-00018
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)	7.1	7.3	7.0
pH (standard units)	7.1	7.0	7.2
Alkalinity (mg/l as CaCO3)	56	56	62
Hardness (mg/l as CaCO3)	83	85	26
Conductivity (umhos/cm)	480	460	490
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.29	0.38	<0.1

2. Dilution Water Samples:
   Moderately Hard

Analysis	250034-1
Dissolved oxygen (mg/l)	7.5
pH (standard units)	8.2
Alkalinity (mg/l as CaCO3)	62
Hardness (mg/l as CaCO3)	84
Conductivity (umhos/cm)	290
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:	November 10, 2020 at 1145
Date & Time Test Terminated:	November 17, 2020 at 1130
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:	November 10, 2020 at 1145
Date & Time Test Terminated:	November 16, 2020 at 1331
Type & Volume of Test Chamber:	30 ml disposable beaker
Volume of Sample:	15 ml
Number of Organisms per replicate:	1
Number of Replicates per dilution:	10

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

D. Test Organisms

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

III. Data Analysis

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

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

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

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

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 November 03, 2020 at 1525 to November 10, 2020 at 1545
The results were as follows: (Control No. 249968-1.)

Survival LC-50: 2879 mg/l
Growth IC-25: 2145 mg/l
Growth PMSD: 15.2

Ceriodaphnia dubia

A chronic reference test was performed on November 03, 2020 at 1520 to November 10, 2020 at 1615
The results were as follows: (Control No. 249968-2.)

Survival LC-50: 1726.4 mg/l
Reproduction IC-25: 1221 mg/l
Reproduction PMSD: 24.6

V. Organism History

Pimephales promelas (Fathead minnow)

Date: November 10, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: November 10, 2020
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.

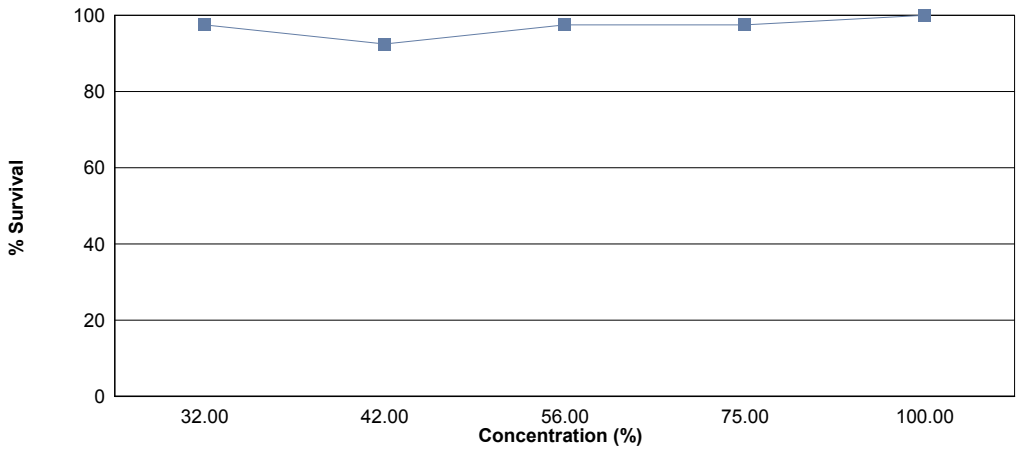
Effluent dilutions for this test were 32 %, 42 %, 56 %, 75 %, 100 % in accordance with the NPDES permit.

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

The test was initiated on November 10, 2020 at 1145 and continued through November 17, 2020 at 1130. 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

(NOEC for sublethal effects was determined by Lower PMSD Bound Test.)



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.602
32 %	97.5	0.600
42 %	92.5	0.565
56 %	97.5	0.583
75 %	97.5	0.608
100 %	100	0.599

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

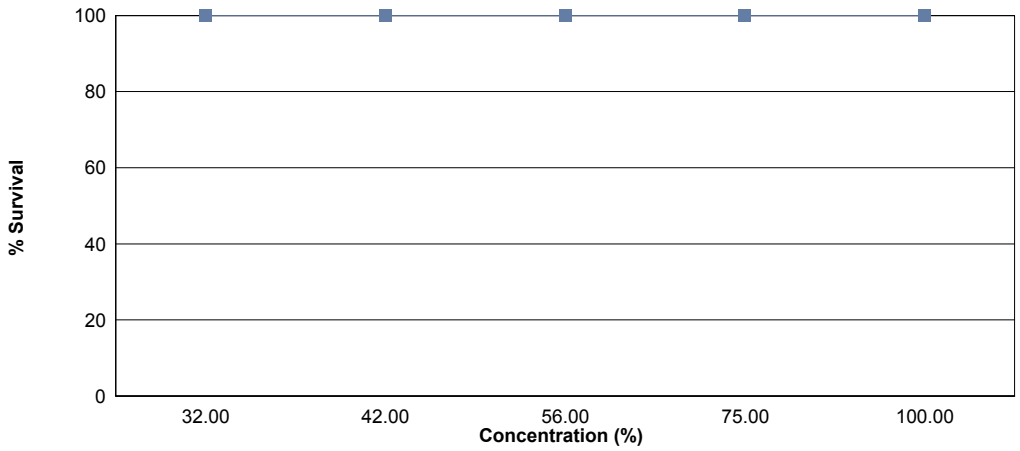
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 November 10, 2020 at 1145 and continued through November 16, 2020 at 1331. 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	26.3
32 %	100	29.4
42 %	100	24.4
56 %	100	24.9
75 %	100	29.4
100 %	100	26.9

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: November 10, 2020 at 1145
Date and Time Test Terminated: November 17, 2020 at 1130

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	7	7	7	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
42 %	A	8	8	8	7	7	7	7
	B	8	8	8	8	8	7	7
	C	8	8	8	7	7	7	7
	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	7	7	7
75 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	7	7	7
	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: November 10, 2020 at 1145
Test Terminated: November 17, 2020 at 1130

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.76578	.77077	0.00499	8	0.624
	B	.76678	.77133	0.00455	8	0.569
	C	.76530	.77026	0.00496	8	0.620
	D	.77404	.77852	0.00448	8	0.560
	E	.77319	.77830	0.00511	8	0.639
32 %	A	.77483	.77984	0.00501	8	0.626
	B	.75508	.76008	0.00500	8	0.625
	C	.76247	.76681	0.00434	8	0.542
	D	.77093	.77573	0.00480	8	0.600
	E	.76607	.77092	0.00485	8	0.606
42 %	A	.76612	.77044	0.00432	8	0.540
	B	.76922	.77354	0.00432	8	0.540
	C	.77366	.77794	0.00428	8	0.535
	D	.76333	.76838	0.00505	8	0.631
	E	.77076	.77541	0.00465	8	0.581
56 %	A	.77330	.77829	0.00499	8	0.624
	B	.77669	.78139	0.00470	8	0.588
	C	.77188	.77683	0.00495	8	0.619
	D	.77253	.77697	0.00444	8	0.555
	E	.77042	.77464	0.00422	8	0.528
75 %	A	.77292	.77822	0.00530	8	0.662
	B	.76495	.76927	0.00432	8	0.540
	C	.77302	.77793	0.00491	8	0.614
	D	.76735	.77238	0.00503	8	0.629
	E	.77160	.77636	0.00476	8	0.595
100 %	A	.75944	.76430	0.00486	8	0.608
	B	.77645	.78104	0.00459	8	0.574
	C	.77411	.77919	0.00508	8	0.635
	D	.77459	.77944	0.00485	8	0.606
	E	.76164	.76622	0.00458	8	0.572

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: November 10, 2020 at 1145
Date and Time Test Terminated: November 16, 2020 at 1331

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	3	0	3	4	4	4	4	6	4	4	36	10	3.60
4	0	3	0	0	0	0	0	0	0	0	3	10	0.300
5	9	6	7	9	9	12	9	8	7	9	85	10	8.50
6	12	11	12	11	16	18	16	15	15	13	139	10	13.9
7													
8													
TOTAL	24	20	22	24	29	34	29	29	26	26	263	10	26.3

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	4	3	4	5	5	3	5	6	6	41	10	4.10
4	4	0	0	0	0	0	0	0	0	0	4	10	0.400
5	11	8	9	10	12	10	11	12	10	9	102	10	10.2
6	10	15	15	17	15	15	17	14	12	17	147	10	14.7
7													
8													
TOTAL	25	27	27	31	32	30	31	31	28	32	294	10	29.4

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	2	5	6	5	4	5	4	5	5	41	10	4.10
4	3	0	0	0	0	0	0	0	0	0	3	10	0.300
5	11	6	9	10	12	11	10	11	7	11	98	10	9.80
6	10	6	12	12	10	11	14	15	9	3	102	10	10.2
7													
8													
TOTAL	24	14	26	28	27	26	29	30	21	19	244	10	24.4

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: November 10, 2020 at 1145
Date and Time Test Terminated: November 16, 2020 at 1331

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	5	4	4	4	4	4	5	4	5	44	10	4.40
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	10	11	10	10	8	11	8	13	11	11	103	10	10.3
6	8	13	1	15	13	5	4	17	18	8	102	10	10.2
7													
8													
TOTAL	23	29	15	29	25	20	16	35	33	24	249	10	24.9

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	3	5	0	3	4	1	5	0	4	4	29	10	2.90
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	11	9	9	11	12	11	10	12	10	12	107	10	10.7
6	15	15	16	15	17	15	12	16	19	18	158	10	15.8
7													
8													
TOTAL	29	29	25	29	33	27	27	28	33	34	294	10	29.4

Concentration: 100 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	4	3	5	0	5	5	1	0	23	10	2.30
4	0	2	0	0	0	1	0	0	0	9	12	10	1.20
5	11	10	3	10	12	8	11	12	11	9	97	10	9.70
6	16	16	10	18	8	15	12	15	17	10	137	10	13.7
7													
8													
TOTAL	27	28	17	31	25	24	28	32	29	28	269	10	26.9

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	0.87500	1.20940
2	32 %	4	1.00000	1.39310
2	32 %	5	1.00000	1.39310
3	42 %	1	0.87500	1.20940
3	42 %	2	0.87500	1.20940
3	42 %	3	0.87500	1.20940
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	0.87500	1.20940
5	75 %	1	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	0.87500	1.20940
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 = 0.1215		
W = 0.8244		
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 %	20.00	16.00	5.00	
4	56 %	25.00	16.00	5.00	
5	75 %	25.00	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 = 0.03427		
W = 0.9686		
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 = 1.229		
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	0.006307	0.001261	0.8831
Within (Error)	24	0.03427	0.001428	
Total	29	0.04057		

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	0.6024	0.6024		
2	32 %	0.5998	0.5998	0.1088	
3	42 %	0.5654	0.5654	1.548	
4	56 %	0.5828	0.5828	0.8201	
5	75 %	0.608	0.608	-0.2343	
6	100 %	0.599	0.599	0.1423	

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	0.0564	9.36	0.0026
3	42 %	5	0.0564	9.36	0.037
4	56 %	5	0.0564	9.36	0.0196
5	75 %	5	0.0564	9.36	-0.0056
6	100 %	5	0.0564	9.36	0.0034

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 = 0.0877 D* = 0.6881 Critical D* = 1.035 (alpha = 0.01, N = 60)	
Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 10.75 Critical B = 15.086 (alpha = 0.01, df = 5)	
Data PASS B1 homogeneity test at 0.01 level.	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	231.1	46.22	2.313
Within (Error)	54	1079	19.98	
Total	59	1310		

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	26.3	26.3		
2	32 %	29.4	29.4	-1.551	
3	42 %	24.4	24.4	0.9505	
4	56 %	24.9	24.9	0.7004	
5	75 %	29.4	29.4	-1.551	
6	100 %	26.9	26.9	-0.3002	

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	4.618	17.6	-3.1
3	42 %	10	4.618	17.6	1.9
4	56 %	10	4.618	17.6	1.4
5	75 %	10	4.618	17.6	-3.1
6	100 %	10	4.618	17.6	-0.6

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.602	-	
32 %	0.600	0.332	PASS
42 %	0.565	6.15	PASS
56 %	0.583	3.16	PASS
75 %	0.608	-0.997	PASS
100 %	0.599	0.498	PASS

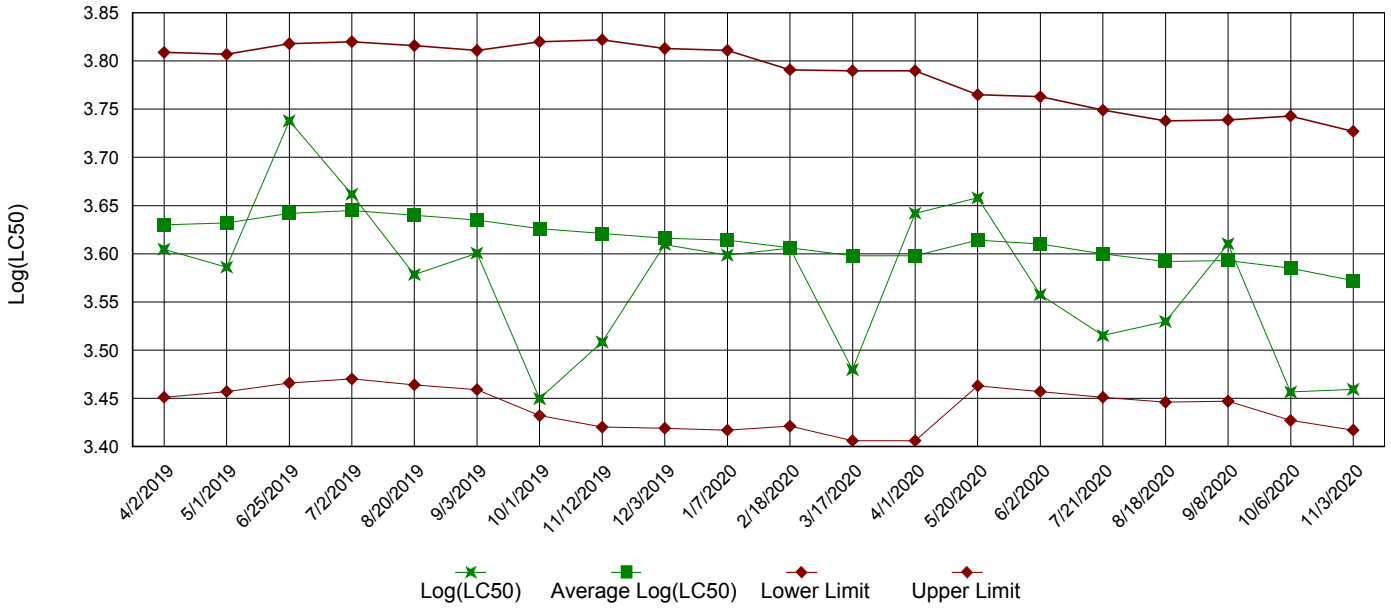
Limit = 12

NOEC = 100 %
LOEC = 100 %

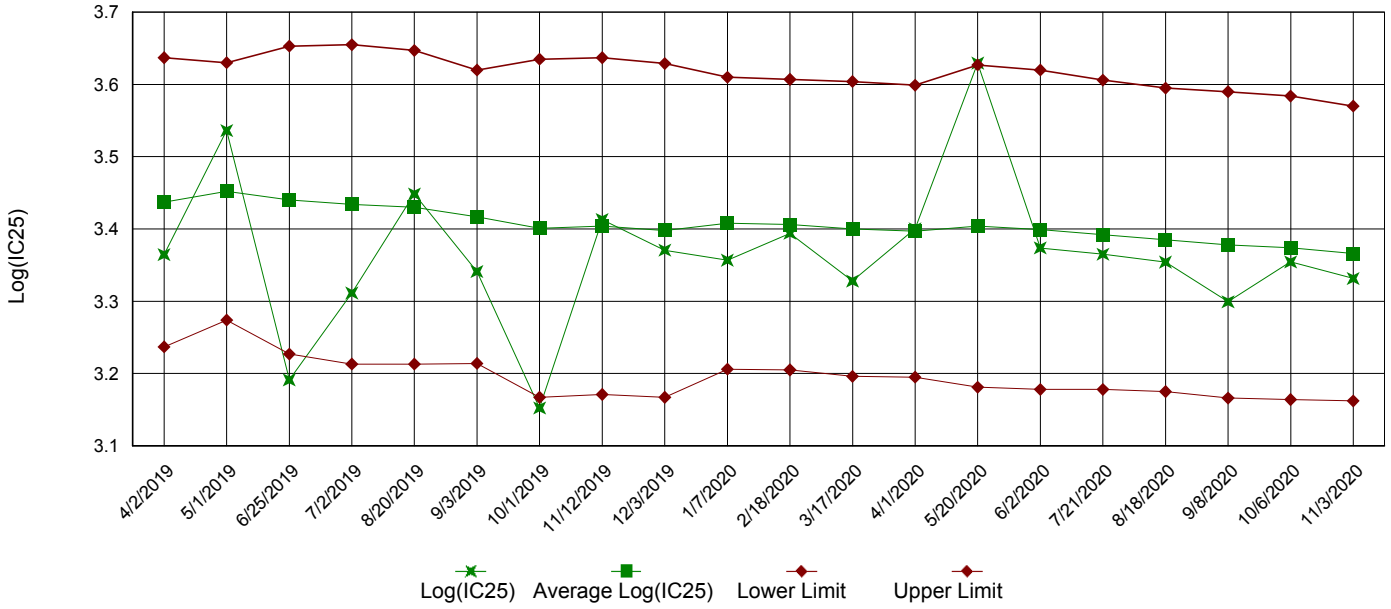
Appendix A3: Test 1000.0

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

LC50 Survival Data



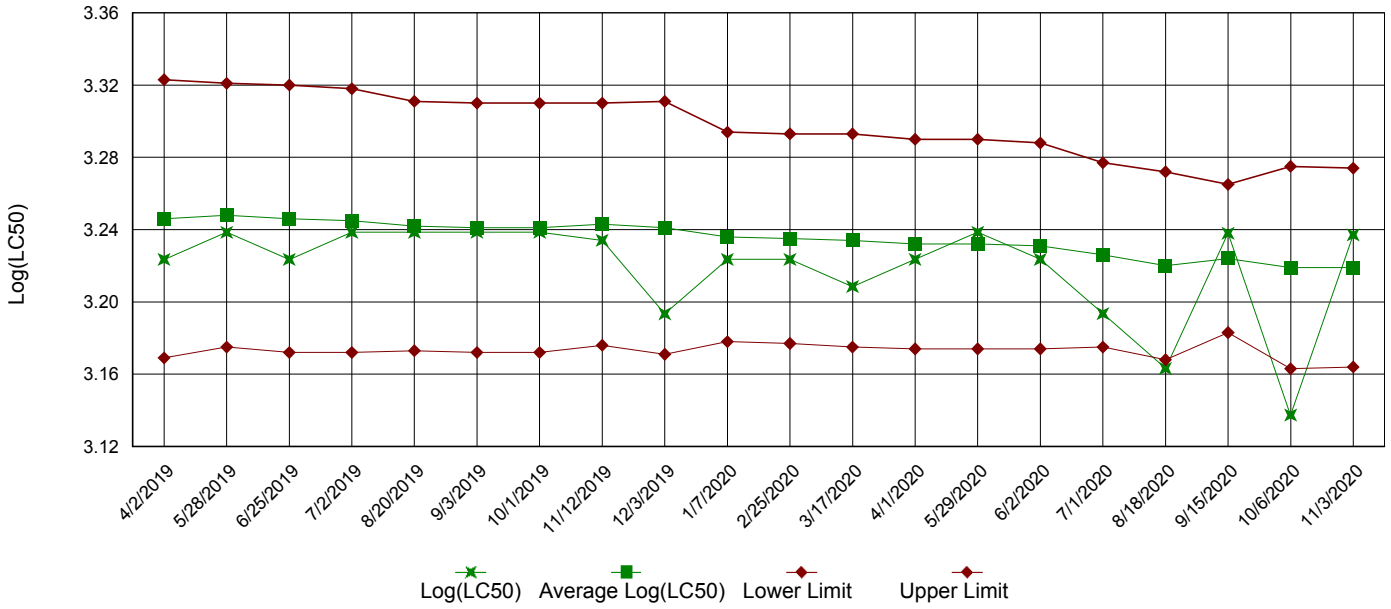
IC25 Growth Data



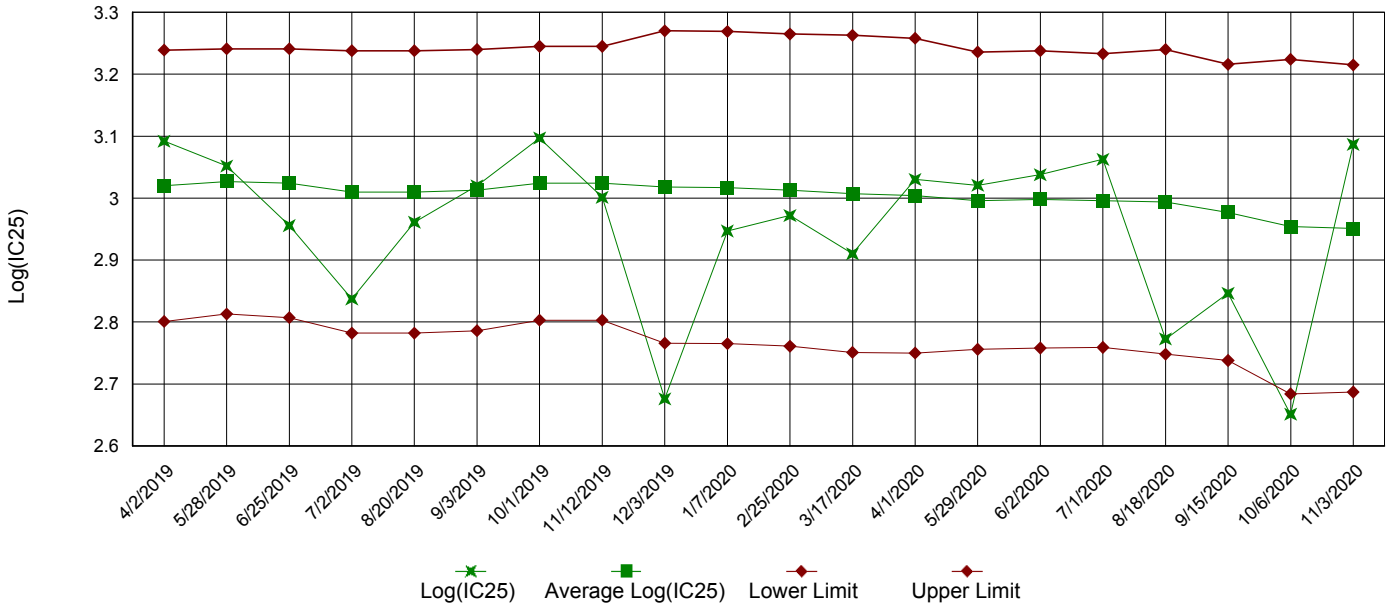
Appendix A3: Test 1002.0

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: Huntsville Water Utilities

NPDES No.: AR0022004 AFIN# 44-00018

Date and Time Test Initiated: November 10, 2020 at 1145
Date and Time Test Terminated: November 17, 2020 at 1130
Dilution water used: Moderately Hard

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	87.5	100	100	100	97.5	97.5	5.73
42 %	87.5	87.5	87.5	100	100	100	100	92.5	7.40
56 %	100	100	100	100	87.5	100	100	97.5	5.73
75 %	100	100	87.5	100	100	100	100	97.5	5.73
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	0.624	0.569	0.620	0.560	0.639	0.602	5.89
32 %	0.626	0.625	0.542	0.600	0.606	0.6	5.72
42 %	0.540	0.540	0.535	0.631	0.581	0.565	7.27
56 %	0.624	0.588	0.619	0.555	0.528	0.583	7.08
75 %	0.662	0.540	0.614	0.629	0.595	0.608	7.44
100 %	0.608	0.574	0.635	0.606	0.572	0.599	4.40

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: 5.89 (TQP6C)

10. Sublethality for this test: 100 % (51714 or 51714S)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1000.0

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

PERMITTEE: Huntsville Water Utilities
NPDES NO.: AR0022004 AFIN# 44-00018
CONTACT: Mr. Bill Eoff
ANALYST: 280, 310, 343

Test Initiated: DATE: November 10, 2020 TIME: 1145
Test Terminated: DATE: November 17, 2020 TIME: 1130

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.5	7.6	7.4	7.8	7.8	7.8	7.7
Final	7.3	7.3	7.2	7.3	7.4	7.5	6.7
pH Initial	8.2	8.2	8.2	8.2	8.2	8.1	8.1
Final	8.0	8.0	8.0	7.9	7.9	7.9	7.8

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	7.4	7.2	7.5	7.6	7.6	7.5
Final	7.1	7.5	7.4	6.7	7.2	6.8	6.9
pH Initial	8.1	8.3	7.7	7.9	7.8	7.8	7.9
Final	8.0	8.0	8.0	7.7	7.9	7.8	7.8

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.5	7.3	7.6	7.4	7.5	7.6	7.7
Final	7.3	7.5	7.4	6.9	7.3	7.3	7.0
pH Initial	8.1	8.1	7.7	7.8	7.7	7.7	7.9
Final	8.0	8.0	7.9	7.8	7.8	7.9	7.8

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	7.6	7.3	7.6	7.5	7.0	7.4
Final	7.2	7.3	7.1	7.0	7.1	7.1	6.6
pH Initial	8.1	8.1	7.5	7.7	7.6	7.6	7.8
Final	8.0	8.0	7.9	7.8	7.8	7.8	7.8

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	7.6	7.3	7.5	7.4	7.4	7.6
Final	7.4	7.0	6.9	7.0	7.2	7.1	6.4
pH Initial	8.0	8.1	7.4	7.6	7.4	7.5	7.7
Final	8.0	8.0	7.8	7.8	7.8	7.8	7.7

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	7.3	7.3	7.4	7.0	7.4	7.4
Final	7.1	7.3	7.0	6.9	7.0	7.0	6.6
pH Initial	7.1	7.6	7.0	7.4	7.2	7.3	7.5
Final	7.9	7.9	7.8	7.7	7.7	7.7	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
56	83	480	<0.05	Huntsville #1 09-NOV-20
56	85	460	<0.05	Huntsville #2 11-NOV-20
62	26	490	<0.05	Huntsville #3 13-NOV-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	84	290	<0.05	250034-1

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Huntsville Water Utilities

NPDES No.: AR0022004 AFIN# 44-00018

Date and Time Test Initiated: November 10, 2020 at 1145
Date and Time Test Terminated: November 16, 2020 at 1331
Dilution water used: Moderately Hard

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	24	25	24	23	29	27
B	20	27	14	29	29	28
C	22	27	26	15	25	17
D	24	31	28	29	29	31
E	29	32	27	25	33	25
F	34	30	26	20	27	24
G	29	31	29	16	27	28
H	29	31	30	35	28	32
I	26	28	21	33	33	29
J	26	32	19	24	34	28
Mean per Adult	26.3	29.4	24.4	24.9	29.4	26.9
Mean per Surviving Adult	26.3	29.4	24.4	24.9	29.4	26.9
CV %	15.5	8.36	20.6	27.0	10.2	15.7

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

Appendix B: Test 1002.0

SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

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

   a.) LOW FLOW OR CRITICAL DILUTION (100 %) _____YES ___X___ NO
   b.) 1/2 LOW FLOW DILUTION (NA) _____YES _____ NO

2. Dunnett's Test:

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

   a.) LOW FLOW OR CRITICAL DILUTION (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: 15.7 (TQP3B)

10. Sublethality for this test: 100 % (51710 or 51710Q)

AMERICAN INTERPLEX CORPORATION LABORATORIES

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Huntsville Water Utilities
NPDES NO.: AR0022004 AFIN# 44-00018
CONTACT: Mr. Bill Eoff
ANALYST: 280, 310, 343

Test Initiated: DATE: November 10, 2020 TIME: 1145
Test Terminated: DATE: November 16, 2020 TIME: 1331

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.5	7.6	7.4	7.8	7.8	7.8	7.7
Final	7.6	7.7	7.8	7.6	7.8	7.2	--
pH Initial	8.2	8.2	8.2	8.2	8.2	8.1	8.1
Final	8.4	8.3	8.6	8.3	8.3	8.2	--

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	7.4	7.2	7.5	7.6	7.6	7.5
Final	7.4	7.6	7.5	7.4	7.6	7.2	--
pH Initial	8.1	8.3	7.7	7.9	7.8	7.8	7.9
Final	8.4	8.4	8.4	8.2	8.3	8.2	--

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.5	7.3	7.6	7.4	7.5	7.6	7.7
Final	7.6	7.7	7.6	7.4	7.7	7.4	--
pH Initial	8.1	8.1	7.7	7.8	7.7	7.7	7.9
Final	8.4	8.3	8.4	8.2	8.2	8.2	--

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	7.6	7.3	7.6	7.5	7.0	7.4
Final	7.6	7.2	7.7	7.4	6.9	7.1	--
pH Initial	8.1	8.1	7.5	7.7	7.6	7.6	7.8
Final	8.4	8.3	8.4	8.2	8.2	8.2	--

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	7.6	7.3	7.5	7.4	7.4	7.6
Final	7.6	7.6	7.7	7.6	7.7	7.2	--
pH Initial	8.0	8.1	7.4	7.6	7.4	7.5	7.7
Final	8.4	8.3	8.3	8.2	8.2	8.2	--

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.1	7.3	7.3	7.4	7.0	7.4	7.4
Final	7.3	7.3	7.4	7.6	7.8	7.3	--
pH Initial	7.1	7.6	7.0	7.4	7.2	7.3	7.5
Final	8.3	8.2	8.4	8.2	8.2	8.2	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
56	83	480	<0.05	Huntsville #1 09-NOV-20
56	85	460	<0.05	Huntsville #2 11-NOV-20
62	26	490	<0.05	Huntsville #3 13-NOV-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	84	290	<0.05	250034-1

AMERICAN INTERPLEX CORPORATION
LABORATORIES

8600 Kanis Road
Little Rock, AR 72204-2322
(501) 224-5060
FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE OF

Client: Huntsville Water Utilities
Project Reference: Bio Monitoring
Project Manager: Bill Eoff
Sampled By: Bill Eoff

PO No.

AIC CONTROL NO: 250223
AIC PROPOSAL NO:
Carrier: FX
Received on Ice (4°C)? (YES) NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic
1	Huntsville #1	11-8-20 @7:00 11-9-20 @5:00		X	X		3	X
		Container Type	P					
		Preservative	4C					

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

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: Bill Eoff
Phone: (479) - 738 - 208 Fax: (479) - 738 - 1285
Report Attention to: Bill Eoff
Report Address to: Bill Eoff
Huntsville Water Utilities
P.O. Box 430

Relinquished By:	Date/Time	Received By:	Date/Time
BM	11/9/20 @8:00		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
			11-10-20 0847

Comments:
FX 8019 4081 1841

AMERICAN INTERPLEX CORPORATION
LABORATORIES

8600 Kanis Road
Little Rock, AR 72204-2322
(501) 224-5060
FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Huntsville Water Utilities
Project Reference: Bio Monitoring
Project Manager: Bill Eoff
Sampled By: Bill Eoff

PO No.

SAMPLE MATRIX: W = WATER, S = SOIL

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES	Cd & Fh Chronic
	Huntsville #2	11-10-20 @7:00 11-11-20 @5:00		X	X		3	X
		Container Type	P					
		Preservative	4C					

AIC CONTROL NO: 250223
AIC PROPOSAL NO:
Carrier: Bill
Received on Ice (4°C)? (YES) 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

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: Bill Eoff
Phone: (479) - 738 - 208 Fax: (479) - 738 - 1285
Report Attention to: Bill Eoff
Report Address to: Bill Eoff
Huntsville Water Utilities
P.O. Box 430

Relinquished By:	Date/Time	Received By:	Date/Time
[signature]	11/12/20 @ 11:30		
Relinquished By:	Date/Time	Received in Lab By: [signature]	Date/Time 11-12-20 1137

Comments:

AMERICAN INTERPLEX CORPORATION
LABORATORIES

8600 Kanis Road
Little Rock, AR 72204-2322
(501) 224-5060
FAX (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Huntsville Water Utilities
Project Reference: Bio Monitoring
Project Manager: Bill Eoff
Sampled By: Bill Eoff

PO No.

AIC CONTROL NO: 250223
AIC PROPOSAL NO:
Carrier: Fed Ex
Received on Ice (4°C)? YES 0.1 NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic	Remarks
	Huntsville #3	11/12/20 @7:00 11/13/20 @5:00		X	X		3	X	
		Container Type	P						Field pH calibration
		Preservative	4C						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

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: Bill Eoff
Phone: (479) - 738 - 2081 Fax: (479) - 738 - 1285
Report Attention to: Bill Eoff
Report Address to: Bill Eoff
Huntsville Water Utilities
P.O. Box 430

Relinquished By:	Date/Time	Received By:	Date/Time
BM	11/13/20 @8:00		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
			14 Nov 20 0835

Comments: