May 29, 2020

Biomonitoring Testing
for
Huntsville

Control No. 245344-1

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*
Huntsville
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 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: Huntsville Water Utilities
ATTN: Mr. Bill Eoff
bill9eoff@hotmail.com

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%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.484	PASS
Control Growth CV < or = 40%	11.4	PASS
Growth Minimum Significant Difference 12 to 30%	17.0	PASS
Critical Dilution CV < or = 40%	9.87	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	19.8	PASS
Control CV < or = 40% per Surviving Female	28.5	PASS
Reproduction Minimum Significant Difference 13 to 47%	20.6	PASS
Critical Dilution CV < or = 40%	10.7	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: Huntsville
   b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.3	7.6	7.4
pH (standard units)	7.5	7.2	7.3
Alkalinity (mg/l as CaCO3)	70	82	62
Hardness (mg/l as CaCO3)	65	73	54
Conductivity (umhos/cm)	340	430	380
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.13	0.25	0.31

2. Dilution Water Samples:
   Moderately Hard

Analysis	245175-1	245176-1
Dissolved oxygen (mg/l)	7.2	7.3
pH (standard units)	8.1	7.9
Alkalinity (mg/l as CaCO3)	59	57
Hardness (mg/l as CaCO3)	81	82
Conductivity (umhos/cm)	290	300
Residual Chlorine (mg/l)	<0.05	<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:	May 19, 2020 at 1429
Date & Time Test Terminated:	May 26, 2020 at 1315
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:	May 19, 2020 at 1235
Date & Time Test Terminated:	May 25, 2020 at 1150
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 April 01, 2020 at 0920 to April 08, 2020 at 0920
The results were as follows: (Control No. 243967-1.)

Survival LC-50: 4385 mg/l
Growth IC-25: 2514 mg/l
Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on April 01, 2020 at 1110 to April 07, 2020 at 1118
The results were as follows: (Control No. 243967-2.)

Survival LC-50: 1673.1 mg/l
Reproduction IC-25: 1072 mg/l
Reproduction PMSD: 14.2

V. Organism History

Pimephales promelas (Fathead minnow)

Date: May 19, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: May 19, 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

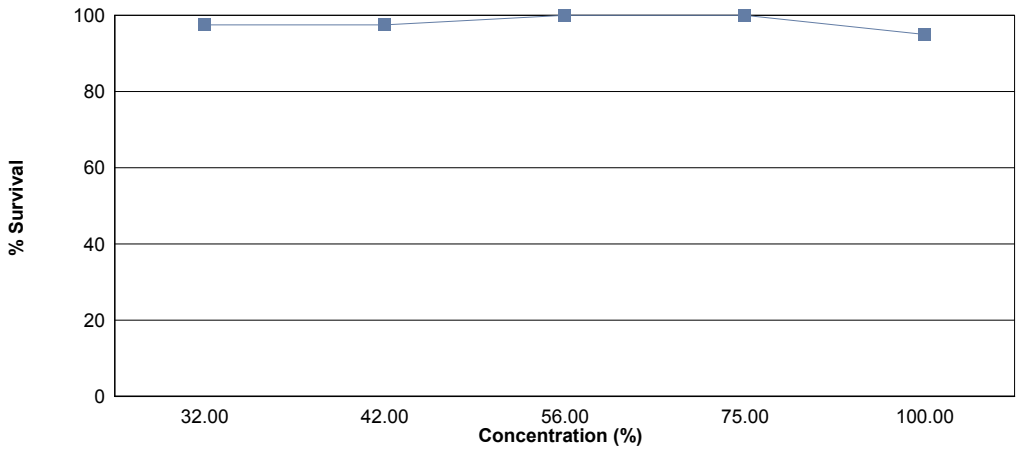
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 May 19, 2020 at 1429 and continued through May 26, 2020 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 growth = 100 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.472
32 %	97.5	0.491
42 %	97.5	0.509
56 %	100	0.515
75 %	100	0.502
100 %	95.0	0.494

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

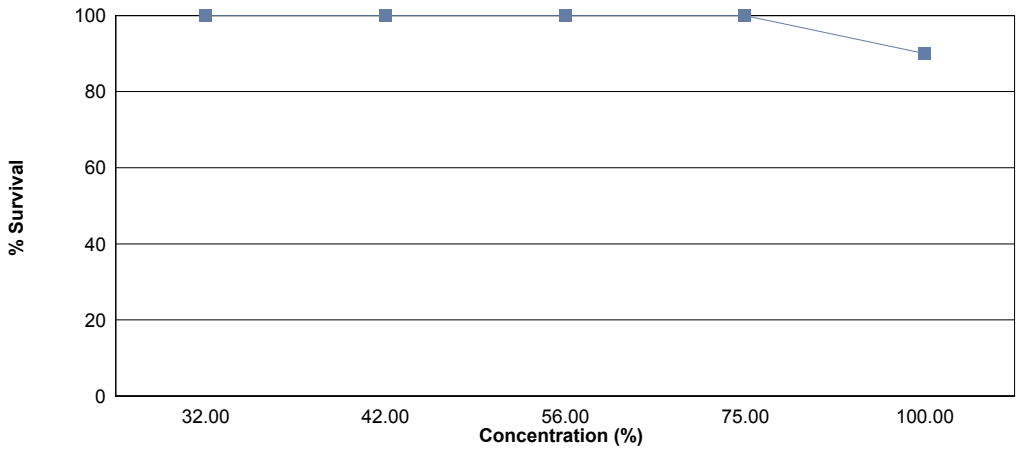
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 May 19, 2020 at 1235 and continued through May 25, 2020 at 1150. 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	19.8
32 %	100	25.5
42 %	100	25.3
56 %	100	25.4
75 %	100	24.1
100 %	90.0	25.0

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: May 19, 2020 at 1429
Date and Time Test Terminated: May 26, 2020 at 1315

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	7	7
	E	8	8	8	8	8	8	8
32 %	A	8	8	8	8	8	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
42 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	7	7	7	7	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	8
75 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
100 %	A	8	8	8	8	8	8	8
	B	8	7	7	7	7	7	7
	C	8	8	8	8	8	7	7
	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: May 19, 2020 at 1429
Test Terminated: May 26, 2020 at 1315

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.67453	.67776	0.00323	8	0.404
	B	.66571	.66938	0.00367	8	0.459
	C	.67471	.67913	0.00442	8	0.552
	D	.67087	.67453	0.00366	8	0.458
	E	.67810	.68200	0.00390	8	0.488
32 %	A	.65614	.65953	0.00339	8	0.424
	B	.67067	.67462	0.00395	8	0.494
	C	.66819	.67272	0.00453	8	0.566
	D	.67132	.67499	0.00367	8	0.459
	E	.66966	.67375	0.00409	8	0.511
42 %	A	.66652	.67027	0.00375	8	0.469
	B	.66746	.67179	0.00433	8	0.541
	C	.67442	.67877	0.00435	8	0.544
	D	.67283	.67711	0.00428	8	0.535
	E	.67114	.67478	0.00364	8	0.455
56 %	A	.67167	.67508	0.00341	8	0.426
	B	.67401	.67825	0.00424	8	0.530
	C	.66899	.67325	0.00426	8	0.532
	D	.67322	.67730	0.00408	8	0.510
	E	.67349	.67812	0.00463	8	0.579
75 %	A	.66869	.67224	0.00355	8	0.444
	B	.67197	.67551	0.00354	8	0.442
	C	.67284	.67762	0.00478	8	0.598
	D	.65858	.66278	0.00420	8	0.525
	E	.66149	.66550	0.00401	8	0.501
100 %	A	.67333	.67747	0.00414	8	0.518
	B	.66848	.67182	0.00334	8	0.418
	C	.67157	.67550	0.00393	8	0.491
	D	.65342	.65736	0.00394	8	0.492
	E	.67213	.67653	0.00440	8	0.550

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 19, 2020 at 1235
Date and Time Test Terminated: May 25, 2020 at 1150

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	3	0	0	0	0	3	10	0.300
4	3	4	4	3	4	0	3	4	2	3	30	10	3.00
5	7	8	8	7	9	10	9	7	7	8	80	10	8.00
6	0	8	11	11	10	13	11	14	7	0	85	10	8.50
7													
8													
TOTAL	10	20	23	21	23	26	23	25	16	11	198	10	19.8

Concentration: 32 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	2	0	3	4	0	4	3	3	27	10	2.70
4	0	0	0	4	0	0	5	0	0	0	9	10	0.900
5	8	9	8	7	8	7	7	9	10	9	82	10	8.20
6	15	11	15	14	13	13	12	11	18	15	137	10	13.7
7													
8													
TOTAL	27	24	25	25	24	24	24	24	31	27	255	10	25.5

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	0	4	3	3	3	3	4	4	3	30	10	3.00
4	0	4	0	0	0	0	0	0	0	0	4	10	0.400
5	8	10	9	7	5	8	10	8	7	7	79	10	7.90
6	14	10	16	13	14	15	7	17	15	19	140	10	14.0
7													
8													
TOTAL	25	24	29	23	22	26	20	29	26	29	253	10	25.3

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: May 19, 2020 at 1235
Date and Time Test Terminated: May 25, 2020 at 1150

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	5	0	4	4	4	3	2	3	35	10	3.50
4	0	0	0	3	0	0	0	0	0	0	3	10	0.300
5	9	8	7	7	5	8	8	5	5	7	69	10	6.90
6	10	18	16	15	13	16	13	17	15	14	147	10	14.7
7													
8													
TOTAL	24	31	28	25	22	28	25	25	22	24	254	10	25.4

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	5	3	4	4	4	3	4	4	4	4	39	10	3.90
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	5	6	7	7	7	8	9	7	7	5	68	10	6.80
6	19	15	18	13	5	12	6	16	15	15	134	10	13.4
7													
8													
TOTAL	29	24	29	24	16	23	19	27	26	24	241	10	24.1

Concentration: 100 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	6	3	3	4	3	3	4	4	3	5	38	10	3.80
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	9	5	8	7	5	9	7	6	8	9X	73	9	8.11
6	17	17	13	14	15	15	13	18	17	X	139	9	15.4
7													
8													
TOTAL	32	25	24	25	23	27	24	28	28	14	250	10	25.0

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	0.87500	1.20940
1	Control	5	1.00000	1.39310
2	32 %	1	0.87500	1.20940
2	32 %	2	1.00000	1.39310
2	32 %	3	1.00000	1.39310
2	32 %	4	1.00000	1.39310
2	32 %	5	1.00000	1.39310
3	42 %	1	1.00000	1.39310
3	42 %	2	1.00000	1.39310
3	42 %	3	1.00000	1.39310
3	42 %	4	1.00000	1.39310
3	42 %	5	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	1.00000	1.39310
5	75 %	1	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	1.00000	1.39310
5	75 %	4	1.00000	1.39310
5	75 %	5	1.00000	1.39310
6	100 %	1	1.00000	1.39310
6	100 %	2	0.87500	1.20940
6	100 %	3	0.87500	1.20940
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 %	27.50	16.00	5.00	
3	42 %	27.50	16.00	5.00	
4	56 %	30.00	16.00	5.00	
5	75 %	30.00	16.00	5.00	
6	100 %	25.00	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 = 0.0694		
W = 0.9675		
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 = 0.6666		
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	0.005832	0.001166	0.4032
Within (Error)	24	0.0694	0.002892	
Total	29	0.07523		

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	0.4722	0.4722		
2	32 %	0.4908	0.4908	-0.5469	
3	42 %	0.5088	0.5088	-1.076	
4	56 %	0.5154	0.5154	-1.27	
5	75 %	0.502	0.502	-0.8762	
6	100 %	0.4938	0.4938	-0.6351	

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	0.08027	17	-0.0186
3	42 %	5	0.08027	17	-0.0366
4	56 %	5	0.08027	17	-0.0432
5	75 %	5	0.08027	17	-0.0298
6	100 %	5	0.08027	17	-0.0216

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 = 0.1446 D* = 1.134 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 %	142.00	75.00	10.00	
3	42 %	135.50	75.00	10.00	
4	56 %	136.50	75.00	10.00	
5	75 %	130.50	75.00	10.00	
6	100 %	136.50	75.00	10.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	243.5	48.7	3.118
Within (Error)	54	843.5	15.62	
Total	59	1087		

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	19.8	19.8		
2	32 %	25.5	25.5	-3.225	
3	42 %	25.3	25.3	-3.112	
4	56 %	25.4	25.4	-3.168	
5	75 %	24.1	24.1	-2.433	
6	100 %	25	25	-2.942	

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

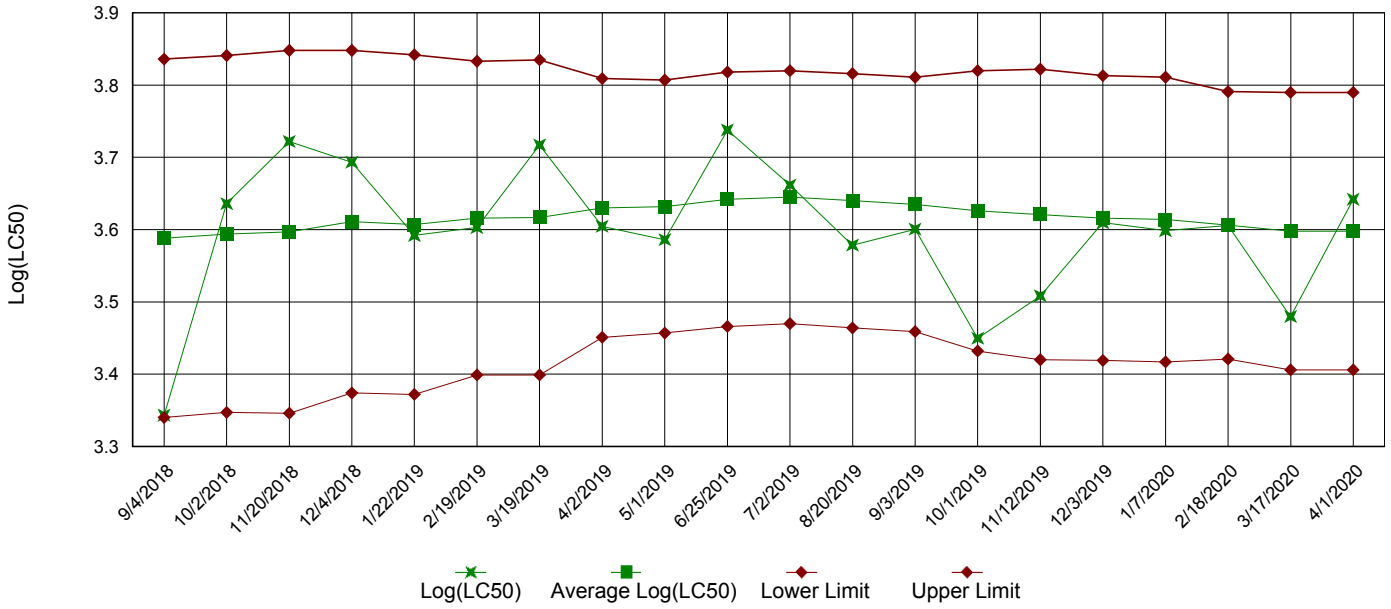
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	32 %	10	4.083	20.6	-5.7
3	42 %	10	4.083	20.6	-5.5
4	56 %	10	4.083	20.6	-5.6
5	75 %	10	4.083	20.6	-4.3
6	100 %	10	4.083	20.6	-5.2

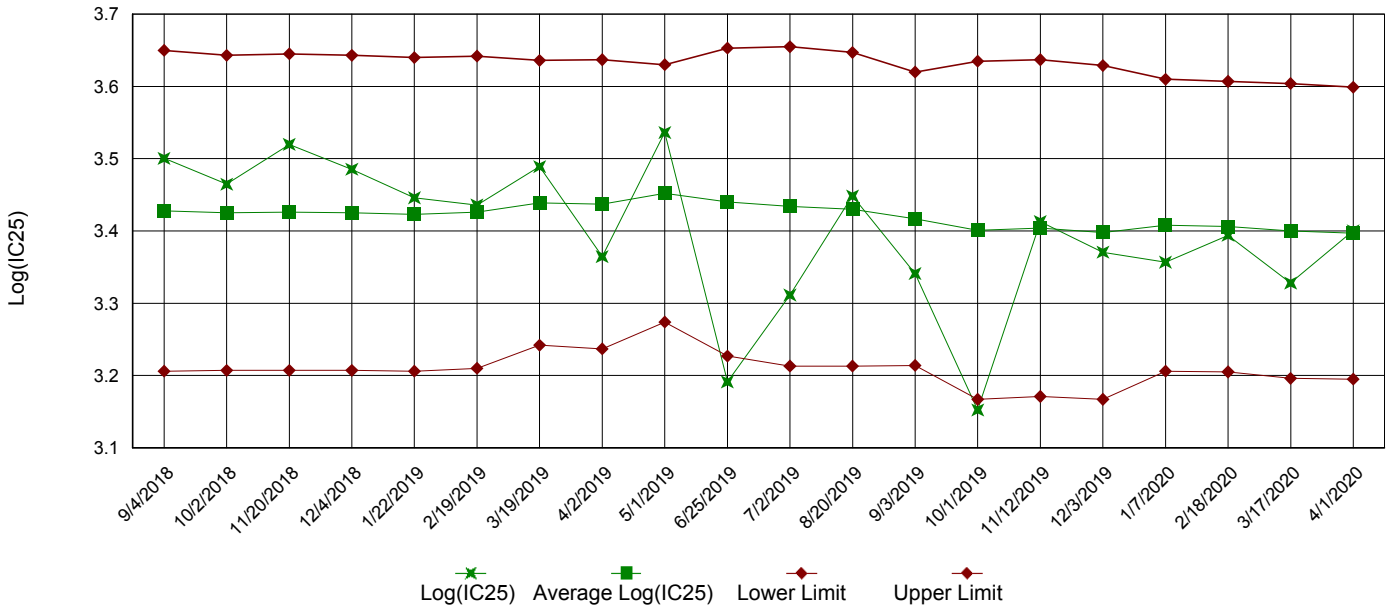
Appendix A3: Test 1000.0

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

LC50 Survival Data



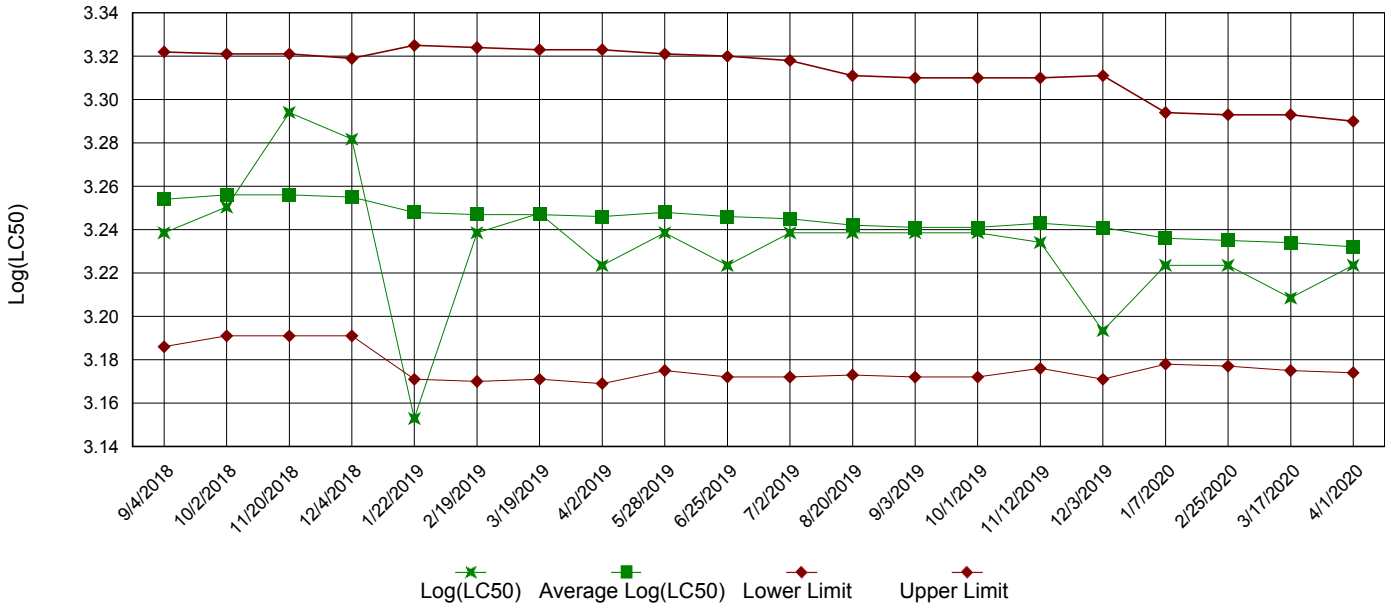
IC25 Growth Data



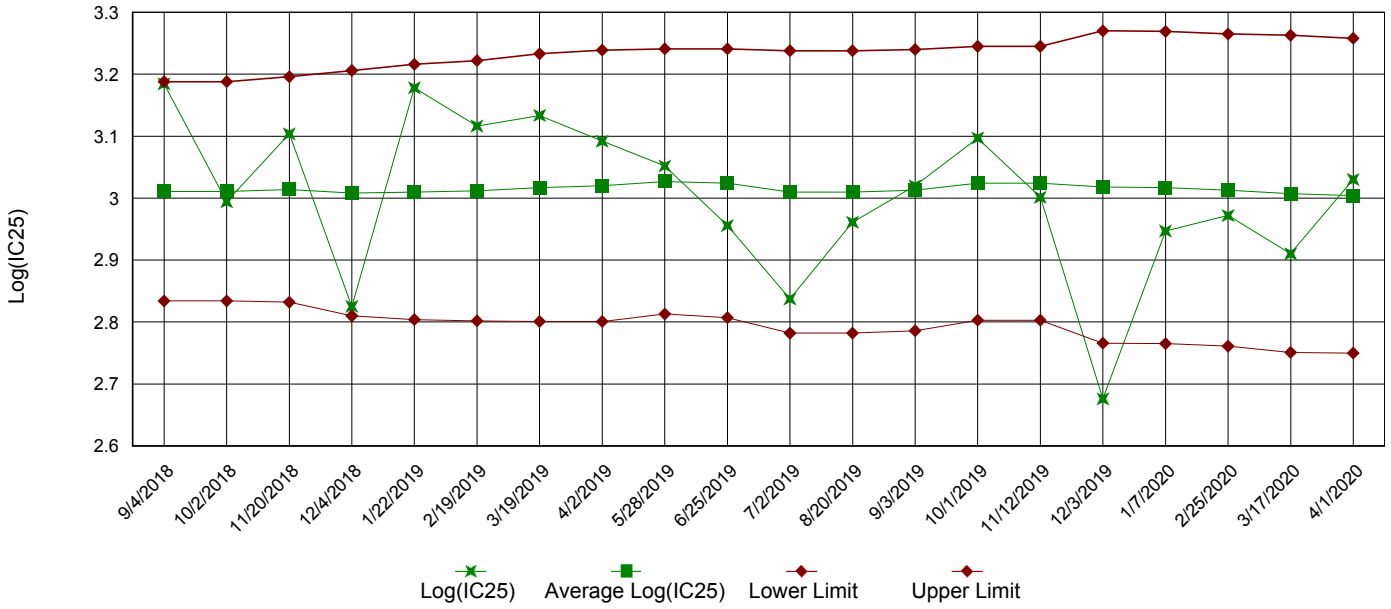
Appendix A3: Test 1002.0

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: May 19, 2020 at 1429
Date and Time Test Terminated: May 26, 2020 at 1315
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	87.5	100	100	100	97.5	5.73
32 %	87.5	100	100	100	100	100	100	97.5	5.73
42 %	100	100	100	100	87.5	100	100	97.5	5.73
56 %	100	100	100	100	100	100	100	100	0.00
75 %	100	100	100	100	100	100	100	100	0.00
100 %	100	87.5	87.5	100	100	100	97.5	95.0	7.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.404	0.459	0.552	0.458	0.488	0.472	11.4
32 %	0.424	0.494	0.566	0.459	0.511	0.491	10.9
42 %	0.469	0.541	0.544	0.535	0.455	0.509	8.48
56 %	0.426	0.530	0.532	0.510	0.579	0.515	10.9
75 %	0.444	0.442	0.598	0.525	0.501	0.502	12.9
100 %	0.518	0.418	0.491	0.492	0.550	0.494	9.87

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: 11.4 (TQP6C)

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

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, 345

Test Initiated: DATE: May 19, 2020 TIME: 1429
Test Terminated: DATE: May 26, 2020 TIME: 1315

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.2	6.9	7.3	6.8	7.2	7.4	7.3
	Final	7.3	6.2	5.8	6.6	6.0	7.2	6.9
pH	Initial	8.1	8.0	7.9	7.9	8.0	8.2	8.0
	Final	8.1	7.6	7.6	7.7	7.9	7.9	8.0

DILUTION 32 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.4	7.2	7.3	6.8	7.4	7.2	7.1
	Final	6.7	5.4	5.4	6.4	6.5	6.9	6.8
pH	Initial	7.8	7.9	7.6	7.7	7.6	8.0	7.7
	Final	8.1	7.6	7.5	7.7	8.0	7.8	7.9

DILUTION 42 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.4	7.2	7.4	7.1	7.4	7.0	7.3
	Final	7.2	5.8	5.6	6.7	6.3	7.0	6.8
pH	Initial	7.7	7.9	7.5	7.6	7.5	7.9	7.6
	Final	8.1	7.6	7.6	7.7	8.0	7.9	8.0

DILUTION 56 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.8	7.2	7.1	7.0	7.1	7.1	7.1
	Final	7.0	5.7	5.8	6.6	6.1	7.0	6.8
pH	Initial	7.6	7.8	7.5	7.6	7.5	7.8	7.6
	Final	8.2	7.6	7.6	7.8	8.1	7.9	8.0

DILUTION 75 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.7	6.9	7.1	6.8	7.1	6.9	7.5
	Final	7.2	5.2	5.6	6.6	6.4	6.8	7.0
pH	Initial	7.5	7.8	7.3	7.5	7.4	7.7	7.5
	Final	8.2	7.6	7.6	7.8	8.1	7.9	8.0

DILUTION 100 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	6.3	6.9	7.6	6.7	7.4	7.3	7.4
	Final	6.8	5.8	5.4	6.4	6.6	6.7	6.7
pH	Initial	7.5	7.5	7.2	7.3	7.3	7.9	7.7
	Final	8.3	7.7	7.6	7.7	8.2	8.0	8.0

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
70	65	340	<0.05	Huntsville #1 18-MAY-20
82	73	430	<0.05	Huntsville #2 20-MAY-20
62	54	380	<0.05	Huntsville #3 22-MAY-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
59	81	290	<0.05	245175-1
57	82	300	<0.05	245176-1

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: May 19, 2020 at 1235
Date and Time Test Terminated: May 25, 2020 at 1150
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	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

		Percent Effluent				
Replicates	Control	32 %	42 %	56 %	75 %	100 %
A	10	27	25	24	29	32
B	20	24	24	31	24	25
C	23	25	29	28	29	24
D	21	25	23	25	24	25
E	23	24	22	22	16	23
F	26	24	26	28	23	27
G	23	24	20	25	19	24
H	25	24	29	25	27	28
I	16	31	26	22	26	28
J	11	27	29	24	24	14
Mean per Adult	19.8	25.5	25.3	25.4	24.1	25.0
Mean per Surviving Adult	19.8	25.5	25.3	25.4	24.1	26.2
CV %	28.5	8.91	12.4	11.2	17.1	10.7

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. 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: 28.5 (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, 345

Test Initiated: DATE: May 19, 2020 TIME: 1235
Test Terminated: DATE: May 25, 2020 TIME: 1150

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	6.9	7.3	6.8	7.2	7.4	7.3
Final	7.4	7.6	6.8	7.3	7.2	7.3	--
pH Initial	8.1	8.0	7.9	7.9	8.0	8.2	8.0
Final	8.4	8.1	8.0	8.4	8.6	8.1	--

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.4	7.2	7.3	6.8	7.4	7.2	7.1
Final	7.6	7.6	6.6	7.3	7.2	7.3	--
pH Initial	7.8	7.9	7.6	7.7	7.6	8.0	7.7
Final	8.5	8.1	8.1	8.3	8.6	8.3	--

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.4	7.2	7.4	7.1	7.4	7.0	7.3
Final	7.6	7.5	6.8	7.5	7.3	7.5	--
pH Initial	7.7	7.9	7.5	7.6	7.5	7.9	7.6
Final	8.5	8.1	8.0	8.3	8.6	8.3	--

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.8	7.2	7.1	7.0	7.1	7.1	7.1
Final	7.3	7.2	6.2	7.0	7.1	7.3	--
pH Initial	7.6	7.8	7.5	7.6	7.5	7.8	7.6
Final	8.5	8.1	8.1	8.3	8.6	8.2	--

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.7	6.9	7.1	6.8	7.1	6.9	7.5
Final	7.5	6.9	6.3	7.3	7.2	7.6	--
pH Initial	7.5	7.8	7.3	7.5	7.4	7.7	7.5
Final	8.6	8.2	8.2	8.4	8.6	8.3	--

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	6.3	6.9	7.6	6.7	7.4	7.3	7.4
Final	7.5	7.3	6.6	7.2	7.3	7.3	--
pH Initial	7.5	7.5	7.2	7.3	7.3	7.9	7.7
Final	8.6	8.1	8.3	8.4	8.5	8.2	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
70	65	340	<0.05	Huntsville #1 18-MAY-20
82	73	430	<0.05	Huntsville #2 20-MAY-20
62	54	380	<0.05	Huntsville #3 22-MAY-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
59	81	290	<0.05	245175-1
57	82	300	<0.05	245176-1

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

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

PO No.

AIC CONTROL NO: 245344
AIC PROPOSAL NO:
Carrier: FX
Received on Ice (4°C)? YES 1.1 NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL		NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic	Remarks
1	Huntsville #1	5-17-20 07:00 - 5-18-20 05:00		X	X			3	X	
		Container Type	P							
		Preservative	4C							

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]	5/18/20 8:01		
Relinquished By:	Date/Time	Received in Lab By: D. BROWN	Date/Time 5-19-20 0856

Comments:

FedEx TRK# 0215 8019 4081 0889

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

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic
2	Huntsville #2	5-19-20 @7:00 5-20-20 @5:00		X	X			3	X
		Container Type	P						
		Preservative	4C						

AIC CONTROL NO: 245344
AIC PROPOSAL NO:
Carrier:
Received on Ice (4°C)? YES 0.5 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 - 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
	5-20-20 @ 8:00a		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
			5/21/20 8:05

Comments: 8019 4081 0878

TRK #8019 4081 0867

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: 245344
AIC PROPOSAL NO:
Carrier: Fedex
Received on Ice (4°C)? YES 0.6 NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic	Remarks
3	Huntsville #3	5-21-20 @ 7:00 5-22-20 @ 5:20		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
	5/22/20 @ 8:00a.		
Relinquished By:	Date/Time	Received in Lab By: CRP345	Date/Time 850 23 May 20

Comments:

5/01