September 10, 2020

Biomonitoring Testing
for
Huntsville

Control No. 248189-1

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

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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	0.630	PASS
Control Growth CV < or = 40%	9.89	PASS
Growth Minimum Significant Difference 12 to 30%	22.0	PASS
Critical Dilution CV < or = 40%	22.9	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	29.7	PASS
Control CV < or = 40% per Surviving Female	13.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	29.6	PASS
Critical Dilution CV < or = 40%	22.9	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)	7.3	7.3	7.4
pH (standard units)	7.6	7.4	7.4
Alkalinity (mg/l as CaCO3)	90	84	60
Hardness (mg/l as CaCO3)	45	50	35
Conductivity (umhos/cm)	450	550	370
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.18	0.88	0.25

2. Dilution Water Samples:
   Moderately Hard

Analysis	247913-1	248063-1	248306-1
Dissolved oxygen (mg/l)	7.2	7.6	7.5
pH (standard units)	8.2	8.1	8.1
Alkalinity (mg/l as CaCO3)	58	60	63
Hardness (mg/l as CaCO3)	88	85	86
Conductivity (umhos/cm)	300	320	330
Residual Chlorine (mg/l)	<0.05	<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:	September 1, 2020 at 1156
Date & Time Test Terminated:	September 08, 2020 at 1155
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:	September 1, 2020 at 1145
Date & Time Test Terminated:	September 07, 2020 at 1302
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. Steel's Many-One Rank test was used to determine the No Observable Effects Concentration (NOEC) for growth. Dunnett's Test was used to calculate the PMSD.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and analyzed with Wilcoxon's Rank Sum with Bonferroni Adjustment 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 August 18, 2020 at 1020 to August 25, 2020 at 0900
The results were as follows: (Control No. 247846-1.)

Survival LC-50: 3386.2 mg/l
Growth IC-25: 2260 mg/l
Growth PMSD: 20.4

Ceriodaphnia dubia

A chronic reference test was performed on August 18, 2020 at 1140 to August 24, 2020 at 1149
The results were as follows: (Control No. 247846-2.)

Survival LC-50: 1456.1 mg/l
Reproduction IC-25: 592.3 mg/l
Reproduction PMSD: 14.8

V. Organism History

Pimephales promelas (Fathead minnow)

Date: September 1, 2020
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: September 1, 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

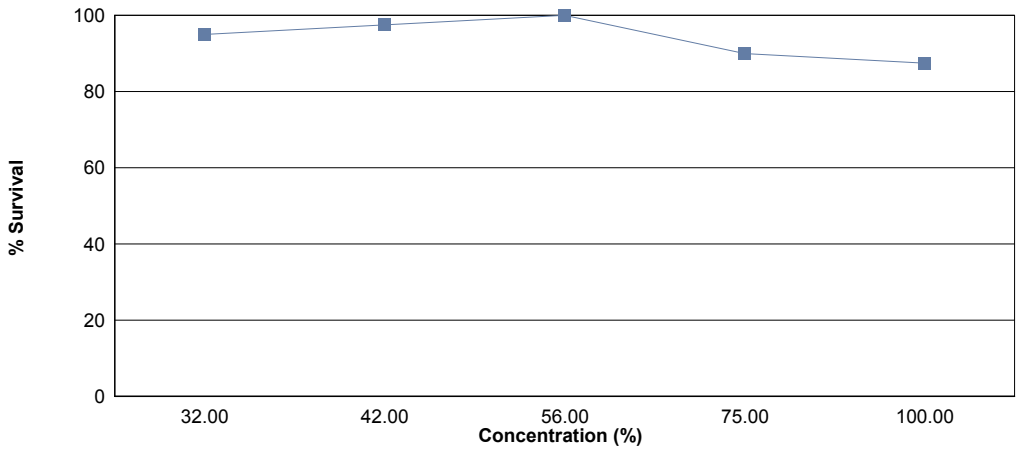
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 September 1, 2020 at 1156 and continued through September 08, 2020 at 1155. 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	0.630
32 %	95.0	0.600
42 %	97.5	0.627
56 %	100	0.676
75 %	90.0	0.604
100 %	87.5	0.605

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

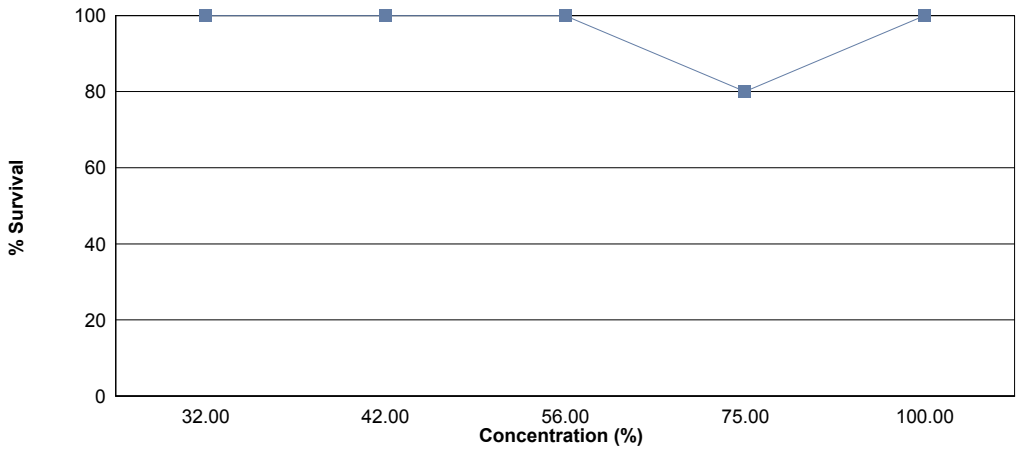
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 September 1, 2020 at 1145 and continued through September 07, 2020 at 1302. 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	29.7
32 %	100	33.8
42 %	100	32.9
56 %	100	29.9
75 %	80.0	27.6
100 %	100	32.3

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: September 1, 2020 at 1156
Date and Time Test Terminated: September 08, 2020 at 1155

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	7	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	7	7	7
42 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	7	7	7	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	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
75 %	A	8	8	8	4	4	4	4
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
100 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	7
	C	8	8	8	7	4	4	4
	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: September 1, 2020 at 1156
Test Terminated: September 08, 2020 at 1155

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65823	.66271	0.00448	8	0.560
	B	.65456	.66007	0.00551	8	0.689
	C	.65737	.66290	0.00553	8	0.691
	D	.65412	.65921	0.00509	8	0.636
	E	.65420	.65878	0.00458	8	0.572
32 %	A	.66002	.66417	0.00415	8	0.519
	B	.65181	.65662	0.00481	8	0.601
	C	.66539	.67071	0.00532	8	0.665
	D	.66026	.66498	0.00472	8	0.590
	E	.65642	.66143	0.00501	8	0.626
42 %	A	.66014	.66545	0.00531	8	0.664
	B	.66097	.66579	0.00482	8	0.602
	C	.66139	.66634	0.00495	8	0.619
	D	.65807	.66284	0.00477	8	0.596
	E	.64838	.65363	0.00525	8	0.656
56 %	A	.64479	.65062	0.00583	8	0.729
	B	.64449	.64920	0.00471	8	0.589
	C	.65191	.65704	0.00513	8	0.641
	D	.65295	.65853	0.00558	8	0.698
	E	.65767	.66346	0.00579	8	0.724
75 %	A	.66446	.66739	0.00293	8	0.366
	B	.65909	.66500	0.00591	8	0.739
	C	.63848	.64360	0.00512	8	0.640
	D	.65928	.66484	0.00556	8	0.695
	E	.66453	.66917	0.00464	8	0.580
100 %	A	.66733	.67243	0.00510	8	0.638
	B	.66456	.66956	0.00500	8	0.625
	C	.65437	.65731	0.00294	8	0.368
	D	.65691	.66273	0.00582	8	0.728
	E	.65233	.65767	0.00534	8	0.668

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 1, 2020 at 1145
Date and Time Test Terminated: September 07, 2020 at 1302

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	6	5	5	5	4	3	5	3	3	43	10	4.30
4	0	0	0	0	1	0	1	0	0	8	10	10	1.00
5	12	13	10	12	12	10	7	10	11	0	97	10	9.70
6	17	15	16	16	15	14	11	15	14	14	147	10	14.7
7													
8													
TOTAL	33	34	31	33	33	28	22	30	28	25	297	10	29.7

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	5	6	5	2	5	7	4	0	2	41	10	4.10
4	0	0	0	1	0	0	0	0	0	0	1	10	0.100
5	10	13	12	14	14	13	14	15	11	7	123	10	12.3
6	16	20	18	20	17	14	18	21	14	15	173	10	17.3
7													
8													
TOTAL	31	38	36	40	33	32	39	40	25	24	338	10	33.8

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	5	6	5	6	6	2	4	6	4	3	47	10	4.70
4	0	0	0	0	0	0	1	0	0	0	1	10	0.100
5	10	15	12	12	11	0	10	12	10	12	104	10	10.4
6	19	20	20	19	18	7	17	21	15	21	177	10	17.7
7													
8													
TOTAL	34	41	37	37	35	9	32	39	29	36	329	10	32.9

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: September 1, 2020 at 1145
Date and Time Test Terminated: September 07, 2020 at 1302

Concentration: 56 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	3	1	7	7	4	5	6	6	0	4	43	10	4.30
4	0	0	0	0	0	0	0	0	3	6	9	10	0.900
5	8	14	14	13	11	12	13	12	0	1	98	10	9.80
6	11	13	21	10	16	16	19	19	10	14	149	10	14.9
7													
8													
TOTAL	22	28	42	30	31	33	38	37	13	25	299	10	29.9

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	4	6	7	6	6	0	7	5	2	X	43	9	4.78
4	0	0	LIA	0	0	0	0	0	0	X	0	8	0.00
5	11	12	LIA	12	14	11	13	13	10	X	96	8	12.0
6	12	22	LIA	19	19	13	13	23	16	X	137	8	17.1
7													
8													
TOTAL	27	40	7	37	39	24	33	41	28	0	276	10	27.6

LIA = Lost in Analysis X = Death of Mother

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	5	6	6	5	6	0	0	0	32	10	3.20
4	0	2	0	0	0	0	0	0	0	11	13	10	1.30
5	13	0	15	13	12	11	16	12	7	1	100	10	10.0
6	16	16	19	20	16	14	21	21	17	18	178	10	17.8
7													
8													
TOTAL	33	18	39	39	34	30	43	33	24	30	323	10	32.3

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	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	0.87500	1.20940
3	42 %	1	1.00000	1.39310
3	42 %	2	1.00000	1.39310
3	42 %	3	1.00000	1.39310
3	42 %	4	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	1.00000	1.39310
4	56 %	4	1.00000	1.39310
4	56 %	5	1.00000	1.39310
5	75 %	1	0.50000	0.78540
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	0.50000	0.78540
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 = 0.6407		
W = 0.7494		
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 %	22.50	16.00	5.00	
3	42 %	25.00	16.00	5.00	
4	56 %	27.50	16.00	5.00	
5	75 %	25.00	16.00	5.00	
6	100 %	22.50	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 = 0.2071		
W = 0.8839		
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					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	32 %	24.00	16.00	5.00	
3	42 %	27.00	16.00	5.00	
4	56 %	35.00	16.00	5.00	
5	75 %	30.00	16.00	5.00	
6	100 %	28.00	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Dunnett's Test for PMSD Calculation

ANOVA Table — No Transformation

SOURCE	DF	SS	MS	F
Between	5	0.0204	0.00408	0.4728
Within (Error)	24	0.2071	0.008629	
Total	29	0.2275		

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	0.6296	0.6296		
2	32 %	0.6002	0.6002	0.5004	
3	42 %	0.6274	0.6274	0.03745	
4	56 %	0.6762	0.6762	-0.7932	
5	75 %	0.604	0.604	0.4357	
6	100 %	0.6054	0.6054	0.4119	

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	0.1387	22	0.0294
3	42 %	5	0.1387	22	0.0022
4	56 %	5	0.1387	22	-0.0466
5	75 %	5	0.1387	22	0.0256
6	100 %	5	0.1387	22	0.0242

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 %	8	1	9
Total	18	1	19

Critical Fisher's value (10,9,10) (alpha=0.05) is 5. b value is 8. Since b is greater than 5 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 %	9	1	
5	100 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.1391		
D* = 1.082		
Critical D* = 1.035	(alpha = 0.01, N = 59)	
Data FAIL normality test (alpha = 0.01).		

Wilcoxon's Rank Sum w/ Bonferroni Adjustment					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	Reps	Sig 0.05
1	Control				
2	32 %	126.50	74.00	10	
3	42 %	134.50	74.00	10	
4	56 %	107.50	74.00	10	
5	75 %	98.50	61.00	9	
6	100 %	119.50	74.00	10	
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	160.1	32.01	0.4661
Within (Error)	53	3640	68.68	
Total	58	3801		

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	29.7	29.7		
2	32 %	33.8	33.8	-1.106	
3	42 %	32.9	32.9	-0.8634	
4	56 %	29.9	29.9	-0.05396	
5	75 %	29.889	29.889	-0.04964	
6	100 %	32.3	32.3	-0.7015	

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

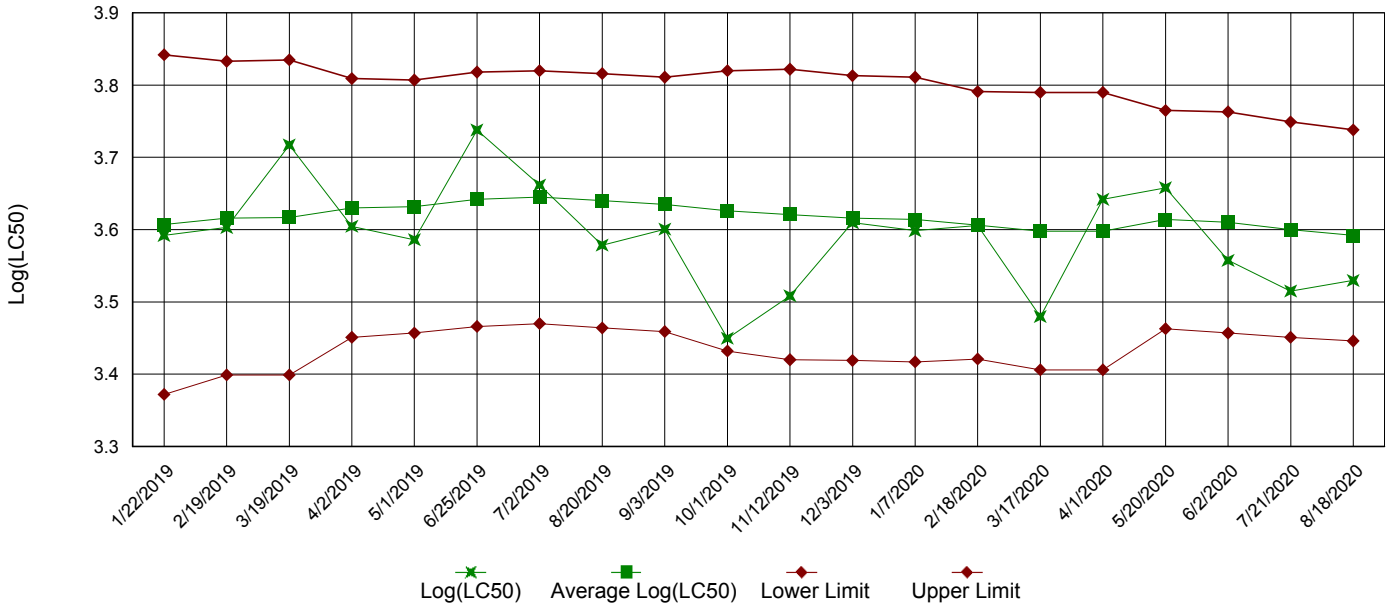
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	32 %	10	8.561	28.8	-4.1
3	42 %	10	8.561	28.8	-3.2
4	56 %	10	8.561	28.8	-0.2
5	75 %	9	8.796	29.6	-0.189
6	100 %	10	8.561	28.8	-2.6

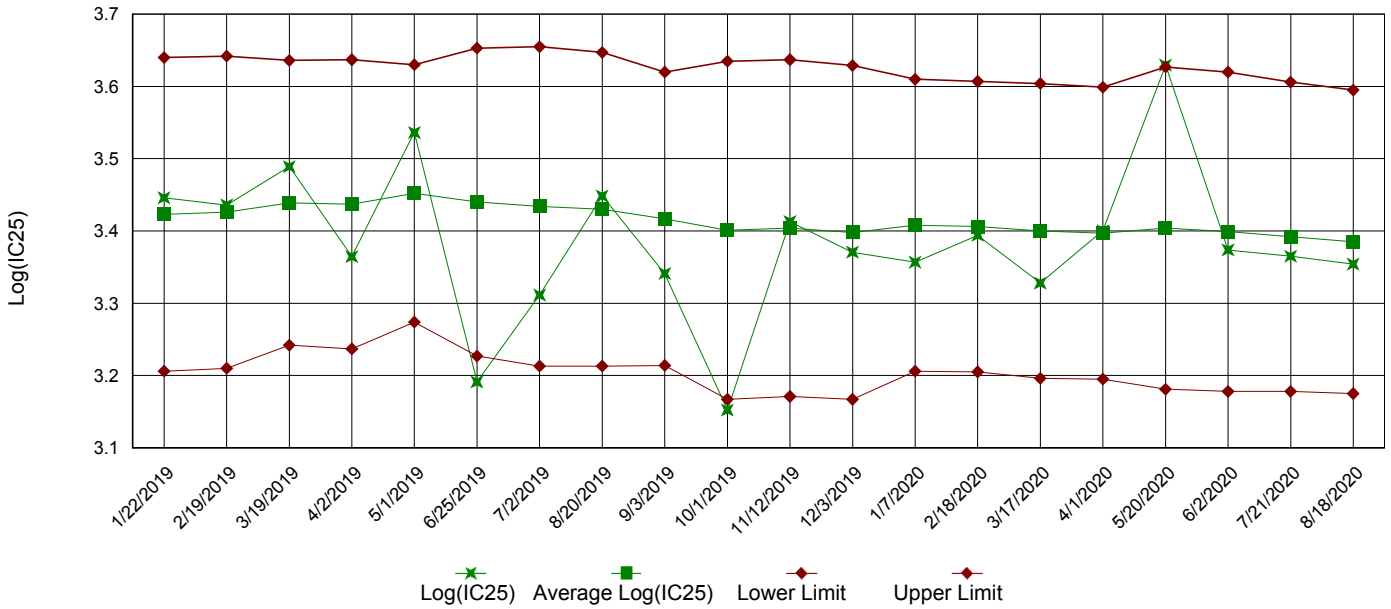
Appendix A3: Test 1000.0

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

LC50 Survival Data



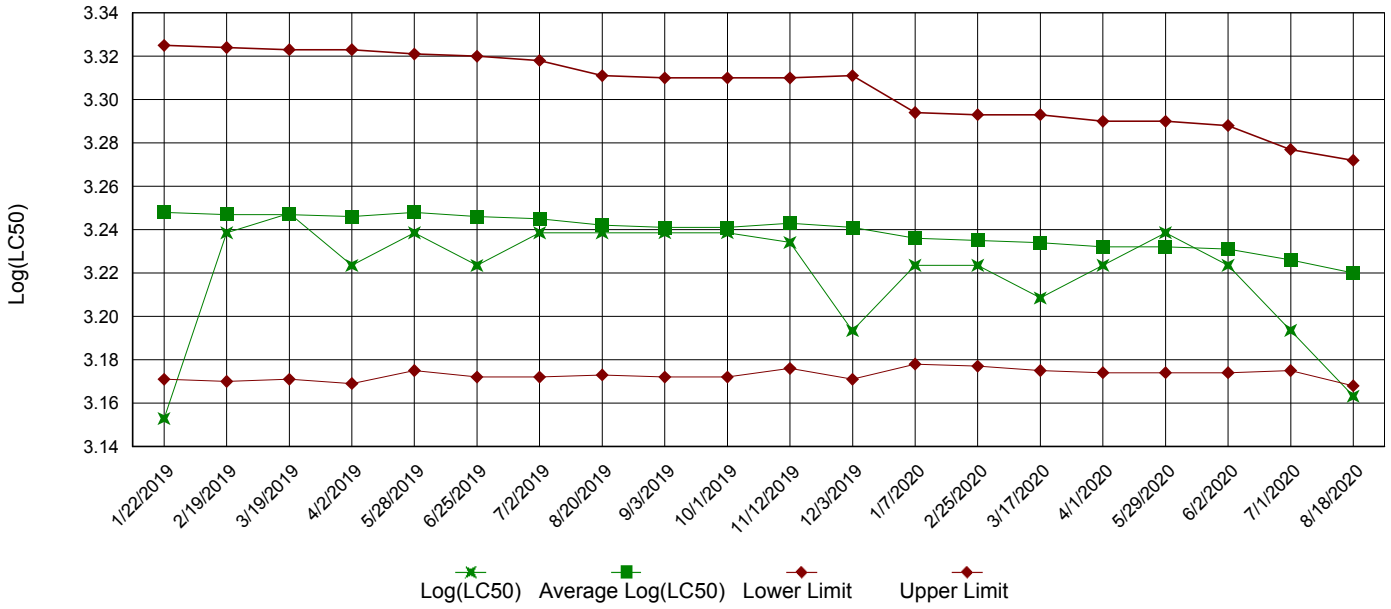
IC25 Growth Data



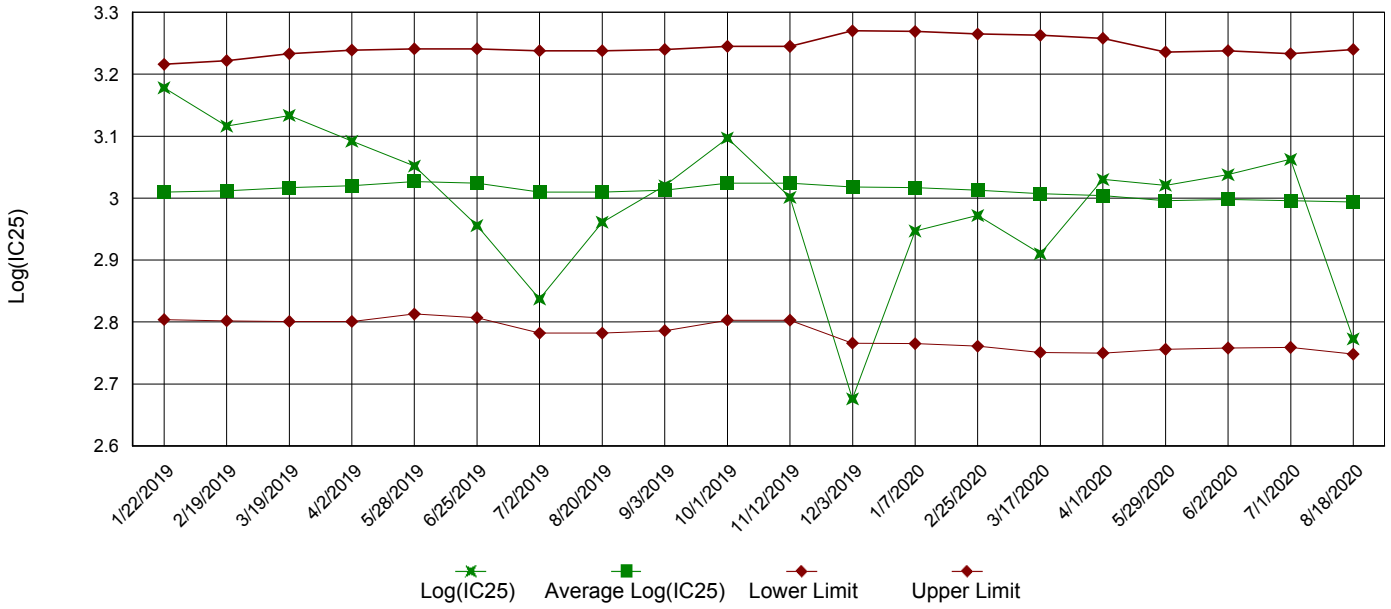
Appendix A3: Test 1002.0

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: September 1, 2020 at 1156
Date and Time Test Terminated: September 08, 2020 at 1155
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 %	87.5	100	100	100	87.5	100	100	95.0	7.21
42 %	100	100	100	87.5	100	100	100	97.5	5.73
56 %	100	100	100	100	100	100	100	100	0.00
75 %	50.0	100	100	100	100	100	100	90.0	24.8
100 %	100	87.5	50.0	100	100	100	100	87.5	24.7

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.560	0.689	0.691	0.636	0.572	0.63	9.89
32 %	0.519	0.601	0.665	0.590	0.626	0.6	8.96
42 %	0.664	0.602	0.619	0.596	0.656	0.627	4.95
56 %	0.729	0.589	0.641	0.698	0.724	0.676	8.87
75 %	0.366	0.739	0.640	0.695	0.580	0.604	24.1
100 %	0.638	0.625	0.368	0.728	0.668	0.605	22.9

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. Steel's Many-One Rank 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: 22.9 (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

Test Initiated: DATE: September 1, 2020 TIME: 1156
Test Terminated: DATE: September 08, 2020 TIME: 1155

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	8.3	7.6	7.6	7.5	7.6	7.1
Final	7.9	6.6	6.9	6.7	6.2	6.4	5.8
pH Initial	8.2	8.1	8.1	8.1	8.1	8.1	8.2
Final	8.1	7.7	7.8	7.8	7.6	7.7	7.6

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	8.2	7.3	7.5	7.8	7.6	7.5
Final	7.9	7.0	6.8	6.4	6.0	6.4	5.8
pH Initial	7.8	8.0	7.7	7.9	7.9	8.0	8.1
Final	8.1	7.9	7.8	7.8	7.7	7.7	7.6

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	8.1	7.3	7.5	7.6	7.7	7.0
Final	8.3	7.0	6.7	7.0	6.1	6.6	6.3
pH Initial	7.8	8.0	7.6	7.8	7.8	8.0	8.1
Final	8.1	7.9	7.8	7.8	7.7	7.7	7.6

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	8.1	7.1	7.5	7.4	7.4	6.9
Final	7.0	6.9	6.6	6.6	6.1	6.2	6.1
pH Initial	7.7	7.9	7.6	7.8	7.7	8.0	8.0
Final	8.1	7.8	7.8	7.8	7.7	7.7	7.6

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	8.1	7.1	7.2	7.3	7.4	7.1
Final	6.9	6.8	6.7	6.6	6.2	6.4	6.2
pH Initial	7.7	7.9	7.5	7.7	7.6	8.0	8.1
Final	8.1	7.9	7.8	7.8	7.7	7.7	7.6

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	8.0	7.3	7.0	7.4	7.6	7.4
Final	6.9	6.8	6.7	6.2	6.0	6.3	5.9
pH Initial	7.6	7.6	7.4	7.5	7.4	8.1	8.0
Final	8.1	7.9	7.9	7.9	7.7	7.7	7.7

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
90	45	450	<0.05	Huntsville #1 31-AUG-20
84	50	550	<0.05	Huntsville #2 02-SEP-20
60	35	370	<0.05	Huntsville #3 04-SEP-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	88	300	<0.05	247913-1
60	85	320	<0.05	248063-1
63	86	330	<0.05	248306-1

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: September 1, 2020 at 1145
Date and Time Test Terminated: September 07, 2020 at 1302
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	80.0	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

		Percent Effluent				
Replicates	Control	32 %	42 %	56 %	75 %	100 %
A	33	31	34	22	27	33
B	34	38	41	28	40	18
C	31	36	37	42	7	39
D	33	40	37	30	37	39
E	33	33	35	31	39	34
F	28	32	9	33	24	30
G	22	39	32	38	33	43
H	30	40	39	37	41	33
I	28	25	29	13	28	24
J	25	24	36	25	0	30
Mean per Adult	29.7	33.8	32.9	29.9	27.6	32.3
Mean per Surviving Adult	29.7	33.8	32.9	29.9	33.6	32.3
CV %	13.3	17.4	27.5	28.4	19.6	22.9

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. Wilcoxon's Rank Sum with Bonferroni Adjustment 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: 22.9 (TQP3B)

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

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: September 1, 2020 TIME: 1145
Test Terminated: DATE: September 07, 2020 TIME: 1302

DILUTION Control	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	8.3	7.6	7.6	7.5	7.6	7.1
Final	8.2	7.3	7.8	8.0	7.8	7.7	--
pH Initial	8.2	8.1	8.1	8.1	8.1	8.1	8.2
Final	8.4	8.3	8.4	8.5	8.4	8.4	--

DILUTION 32 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	8.2	7.3	7.5	7.8	7.6	7.5
Final	8.1	7.6	7.8	7.8	7.6	7.5	--
pH Initial	7.8	8.0	7.7	7.9	7.9	8.0	8.1
Final	8.4	8.4	8.4	8.5	8.4	8.4	--

DILUTION 42 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	8.1	7.3	7.5	7.6	7.7	7.0
Final	8.1	7.6	8.3	8.0	7.6	7.4	--
pH Initial	7.8	8.0	7.6	7.8	7.8	8.0	8.1
Final	8.5	8.4	8.5	8.6	8.4	8.4	--

DILUTION 56 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.2	8.1	7.1	7.5	7.4	7.4	6.9
Final	8.1	7.4	7.9	7.6	7.4	7.6	--
pH Initial	7.7	7.9	7.6	7.8	7.7	8.0	8.0
Final	8.4	8.3	8.4	8.5	8.3	8.3	--

DILUTION 75 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.4	8.1	7.1	7.2	7.3	7.4	7.1
Final	8.1	7.4	7.7	8.0	7.9	7.6	--
pH Initial	7.7	7.9	7.5	7.7	7.6	8.0	8.1
Final	8.4	8.4	8.4	8.6	8.4	8.3	--

DILUTION 100 %	DAY 1	2	3	4	5	6	7
D.O. Initial	7.3	8.0	7.3	7.0	7.4	7.6	7.4
Final	8.0	7.5	7.6	7.8	7.6	7.4	--
pH Initial	7.6	7.6	7.4	7.5	7.4	8.1	8.0
Final	8.4	8.5	8.5	8.6	8.4	8.4	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
90	45	450	<0.05	Huntsville #1 31-AUG-20
84	50	550	<0.05	Huntsville #2 02-SEP-20
60	35	370	<0.05	Huntsville #3 04-SEP-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
58	88	300	<0.05	247913-1
60	85	320	<0.05	248063-1
63	86	330	<0.05	248306-1

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: 248189
AIC PROPOSAL NO:
Carrier: FX
Received on Ice (4°C)? (YES) 0.3 NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SAMPLE MATRIX: SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic	Remarks
1	Huntsville #1	8/30/20 @7:00 8/31/20 @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
[signature]	8/31/20 @8:00		
Relinquished By:	Date/Time	Received in Lab By: D. BROWN	Date/Time 9-1-20 0916

Comments: 8019 4081 1256

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: 248189
AIC PROPOSAL NO:
Carrier: FX
Received on Ice (4°C)? YES 0.5 NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic	Remarks
2	Huntsville #2	9-1-20 @7:00 9-2-20 @5:00		X	X		3	X	
		Container Type	P						
		Preservative	4C						

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

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
[signature]	9-2-20 @8:00		
Relinquished By:	Date/Time	Received in Lab By: D. BROWN	Date/Time 9-3-20 0931

Comments: 8019 408h 1245

5/01

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: 248189
AIC PROPOSAL NO:
Carrier: FX
Received on Ice (4°C)? YES Ol NO

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	SAMPLE MATRIX: WATER	SAMPLE MATRIX: SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Cd & Fh Chronic	Remarks
3	Huntsville #3	9/3/20 @7:00 9/4/20 @5:00		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]	9/4/20 @8:00		
Relinquished By:	Date/Time	Received in Lab By: 56343	Date/Time 5 SEP 20 @910

Comments:
8019 4081 1234

5/01 FORM 2066