



November 3, 2020

Biomonitoring Testing
for
East Effluent

Control No. 249566-1

Prepared for:

Ms. Whitney Young
City Water & Light of Jonesboro
5205 Ingels Road
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Prepared by:

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City Water & Light of Jonesboro
ATTN: Ms. Whitney Young
5205 Ingels Road
Jonesboro, AR 72401

Re: *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
East Effluent
NPDES Permit No. AR0043401 AFIN16-00936

Dear Ms. Whitney Young:

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 80 % effluent, which is equal to the sub-lethal limit of 80 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION

A handwritten signature in black ink, appearing to read 'John Overbey', is written over a horizontal line.

John Overbey
Chief Operating Officer



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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	0.404	PASS
Control Growth CV < or = 40%	9.66	PASS
Growth Minimum Significant Difference 12 to 30%	11.5	BELOW
Critical Dilution CV < or = 40%	4.36	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	30.0	PASS
Control CV < or = 40% per Surviving Female	16.1	PASS
Reproduction Minimum Significant Difference 13 to 47%	29.1	PASS
Critical Dilution CV < or = 40%	39.3	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0043401 AFIN16-00936
2. Test Requirements: Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
 - a. Sampling Point: East Effluent
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.7	8.2	7.3
pH (standard units)	8.0	7.8	8.3
Alkalinity (mg/l as CaCO ₃)	140	110	140
Hardness (mg/l as CaCO ₃)	120	120	120
Conductivity (umhos/cm)	770	670	650
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.24	0.17	<0.1

2. Dilution Water Samples:
Moderately Hard,

Analysis	249330-1
Dissolved oxygen (mg/l)	7.2
pH (standard units)	8.2
Alkalinity (mg/l as CaCO ₃)	62
Hardness (mg/l as CaCO ₃)	85
Conductivity (umhos/cm)	310
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: October 20, 2020 at 1020
Date & Time Test Terminated: October 27, 2020 at 0935
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: October 20, 2020 at 1120
Date & Time Test Terminated: October 26, 2020 at 1151
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 October 06, 2020 at 1005 to October 13, 2020 at 1145

The results were as follows: (Control No. 249174-1.)

Survival LC-50: 2861 mg/l

Growth IC-25: 2262 mg/l

Growth PMSD: 24.4

Ceriodaphnia dubia

A chronic reference test was performed on October 06, 2020 at 1120 to October 12, 2020 at 1025

The results were as follows: (Control No. 249174-2.)

Survival LC-50: 1372 mg/l

Reproduction IC-25: 447.8 mg/l

Reproduction PMSD: 15.7

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 20, 2020

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 20, 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.

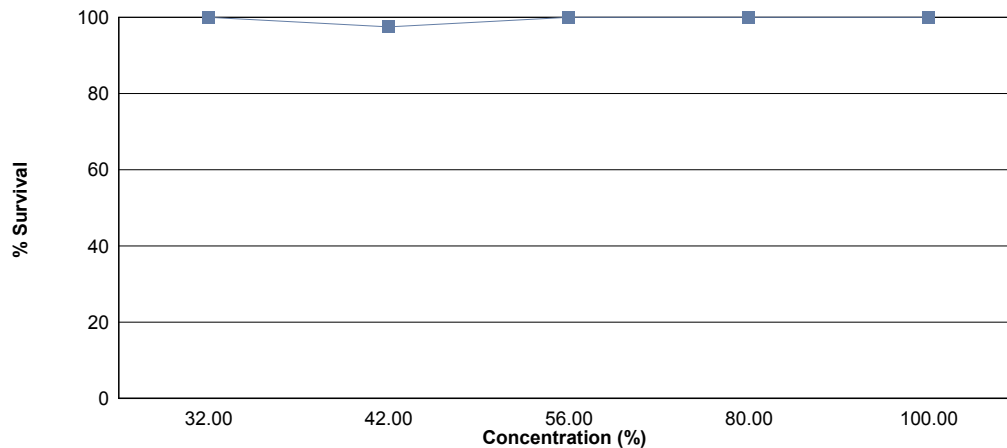
Effluent dilutions for this test were 32 %, 42 %, 56 %, 80 %, 100 % in accordance with the NPDES permit.

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

The test was initiated on October 20, 2020 at 1020 and continued through October 27, 2020 at 0935. 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	0.404
32 %	100	0.439
42 %	97.5	0.398
56 %	100	0.430
80 %	100	0.425
100 %	100	0.441

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

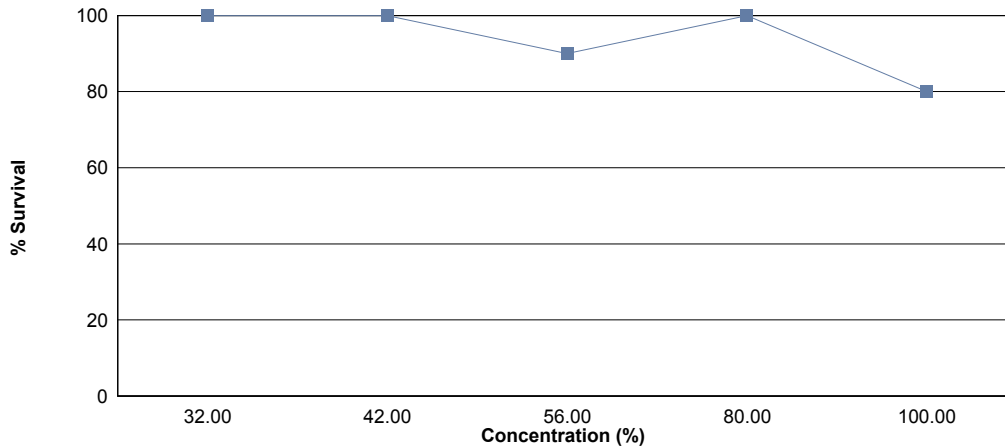
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 32 %, 42 %, 56 %, 80 %, 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 October 20, 2020 at 1120 and continued through October 26, 2020 at 1151. 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 = 80 % effluent



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	30.0
32 %	100	29.3
42 %	100	27.6
56 %	90.0	24.2
80 %	100	28.2
100 %	80.0	18.1 *

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 20, 2020 at 1020

Date and Time Test Terminated: October 27, 2020 at 0935

Concentration	Replicate	Number of Survivors						
		Day 1	Day 2	Day 3	Day 4	Day 5	Day 6	Day 7
Control	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
32 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
42 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	7
56 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
80 %	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	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: October 20, 2020 at 1020

Test Terminated: October 27, 2020 at 0935

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65392	.65754	0.00362	8	0.452
	B	.64811	.65143	0.00332	8	0.415
	C	.66418	.66754	0.00336	8	0.420
	D	.65548	.65828	0.00280	8	0.350
	E	.65706	.66012	0.00306	8	0.382
32 %	A	.66635	.66991	0.00356	8	0.445
	B	.65737	.66046	0.00309	8	0.386
	C	.65645	.66006	0.00361	8	0.451
	D	.66265	.66652	0.00387	8	0.484
	E	.66522	.66865	0.00343	8	0.429
42 %	A	.65974	.66277	0.00303	8	0.379
	B	.64899	.65234	0.00335	8	0.419
	C	.64710	.65003	0.00293	8	0.366
	D	.64804	.65157	0.00353	8	0.441
	E	.65698	.66006	0.00308	8	0.385
56 %	A	.64245	.64561	0.00316	8	0.395
	B	.65849	.66205	0.00356	8	0.445
	C	.65883	.66235	0.00352	8	0.440
	D	.66233	.66543	0.00310	8	0.388
	E	.66142	.66526	0.00384	8	0.480
80 %	A	.65558	.65883	0.00325	8	0.406
	B	.66556	.66888	0.00332	8	0.415
	C	.65435	.65774	0.00339	8	0.424
	D	.65970	.66321	0.00351	8	0.439
	E	.65707	.66059	0.00352	8	0.440
100 %	A	.64953	.65300	0.00347	8	0.434
	B	.65507	.65871	0.00364	8	0.455
	C	.65370	.65706	0.00336	8	0.420
	D	.66237	.66579	0.00342	8	0.428
	E	.66325	.66698	0.00373	8	0.466

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 20, 2020 at 1120

Date and Time Test Terminated: October 26, 2020 at 1151

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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	0	4	4	4	3	4	4	5	4	37	10	3.70	
4	0	4	0	0	0	0	0	0	11	0	15	10	1.50	
5	11	0	10	8	9	8	9	12	0	12	79	10	7.90	
6	19	14	17	17	16	17	18	18	15	18	169	10	16.9	
7														
8														
TOTAL	35	18	31	29	29	28	31	34	31	34	300	10	30.0	

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	0	6	0	4	3	3	5	4	5	34	10	3.40	
4	0	4	0	5	0	0	0	0	0	0	9	10	0.900	
5	11	0	10	10	5	7	10	12	7	13	85	10	8.50	
6	20	16	14	20	19	8	10	18	20	20	165	10	16.5	
7														
8														
TOTAL	35	20	30	35	28	18	23	35	31	38	293	10	29.3	

Concentration: 42 %														
Day	Replicate										No. of Young	No. of Adults	Young per Adult	
	1	2	3	4	5	6	7	8	9	10				
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00	
3	4	0	4	3	5	0	5	4	6	4	35	10	3.50	
4	0	3	0	0	0	2	0	0	9	0	14	10	1.40	
5	10	0	13	11	12	0	10	6	0	10	72	10	7.20	
6	13	3	18	10	20	15	20	15	20	21	155	10	15.5	
7														
8														
TOTAL	27	6	35	24	37	17	35	25	35	35	276	10	27.6	

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 20, 2020 at 1120

Date and Time Test Terminated: October 26, 2020 at 1151

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	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	5	3	0	0	2	4	6	3	23	10	2.30	
4	5	4	0	0	2	3	0	0	0	0	14	10	1.40	
5	0	0	12	11	5	0	11	6	10	4	59	10	5.90	
6	14	14	20	16	2X	18	14	17	14	17	146	9	16.2	
7														
8														
TOTAL	19	18	37	30	9	21	27	27	30	24	242	10	24.2	

Concentration: 80 %													
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	0	5	4	2	2	2	3	0	5	29	10	2.90
4	0	5	0	0	0	0	0	0	0	0	5	10	0.500
5	11	0	7	10	13	13	0	10	0	13	77	10	7.70
6	22	13	19	21	20	1	21	20	19	15	171	10	17.1
7													
8													
TOTAL	39	18	31	35	35	16	23	33	19	33	282	10	28.2

Concentration: 100 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	0	4	0	2	0	3	5	3	0	22	10	2.20
4	0	4	0	4	X	3	0	0	X	0	11	8	1.38
5	11	0	12	11	X	12	11	8	X	7	72	8	9.00
6	17	7	18	12	X	0	3	10	X	9	76	8	9.50
7													
8													
TOTAL	33	11	34	27	2	15	17	23	3	16	181	10	18.1

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	32 %	1	1.00000	1.39310
2	32 %	2	1.00000	1.39310
2	32 %	3	1.00000	1.39310
2	32 %	4	1.00000	1.39310
2	32 %	5	1.00000	1.39310
3	42 %	1	1.00000	1.39310
3	42 %	2	1.00000	1.39310
3	42 %	3	1.00000	1.39310
3	42 %	4	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	80 %	1	1.00000	1.39310
5	80 %	2	1.00000	1.39310
5	80 %	3	1.00000	1.39310
5	80 %	4	1.00000	1.39310
5	80 %	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))
<p>D = 0.027 W = 0.4161 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data FAIL normality test (alpha = 0.01).</p>		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))	
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	32 %	27.50	16.00	5.00	
3	42 %	25.00	16.00	5.00	
4	56 %	27.50	16.00	5.00	
5	80 %	27.50	16.00	5.00	
6	100 %	27.50	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
<p>D = 0.02321 W = 0.9642 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 4.766 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.008027	0.001605	1.66	
Within (Error)	24	0.02321	0.0009671		
Total	29	0.03124			
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	0.4038	0.4038			
2	32 %	0.439	0.439	-1.79		
3	42 %	0.398	0.398	0.2949		
4	56 %	0.4296	0.4296	-1.312		
5	80 %	0.4248	0.4248	-1.068		
6	100 %	0.4406	0.4406	-1.871		
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	0.04642	11.5	-0.0352		
3	42 %	5	0.04642	11.5	0.0058		
4	56 %	5	0.04642	11.5	-0.0258		
5	80 %	5	0.04642	11.5	-0.021		
6	100 %	5	0.04642	11.5	-0.0368		

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 %	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.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
80 %	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 %	8	2	10
Total	18	2	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 8. 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	1	
4	80 %	10	0	
5	100 %	10	2	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.0891 D* = 0.6991 Critical D* = 1.035</p> <p style="text-align: right;">(alpha = 0.01, N = 60)</p> <p>Data PASS normality test (alpha = 0.01).</p>	

Bartlett's Test for Homogeneity of Variance	No Transformation
<p>Calculated B1 statistic = 6.786 Critical B = 15.086</p> <p style="text-align: right;">(alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p>	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	996.1	199.2	2.786	
Within (Error)	54	3861	71.5		
Total	59	4857			
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	30	30			
2	32 %	29.3	29.3	0.1851		
3	42 %	27.6	27.6	0.6347		
4	56 %	24.2	24.2	1.534		
5	80 %	28.2	28.2	0.476		
6	100 %	18.1	18.1	3.147	*	
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	8.735	29.1	0.7	
3	42 %	10	8.735	29.1	2.4	
4	56 %	10	8.735	29.1	5.8	
5	80 %	10	8.735	29.1	1.8	
6	100 %	10	8.735	29.1	11.9	

Lower PMSD Bound Test for Pimephales promelas

Concentration	Growth	Relative Difference from Control	Pass/Fail
Control	0.404	-	
32 %	0.439	-8.66	PASS
42 %	0.398	1.49	PASS
56 %	0.430	-6.44	PASS
80 %	0.425	-5.20	PASS
100 %	0.441	-9.16	PASS

Limit = 12

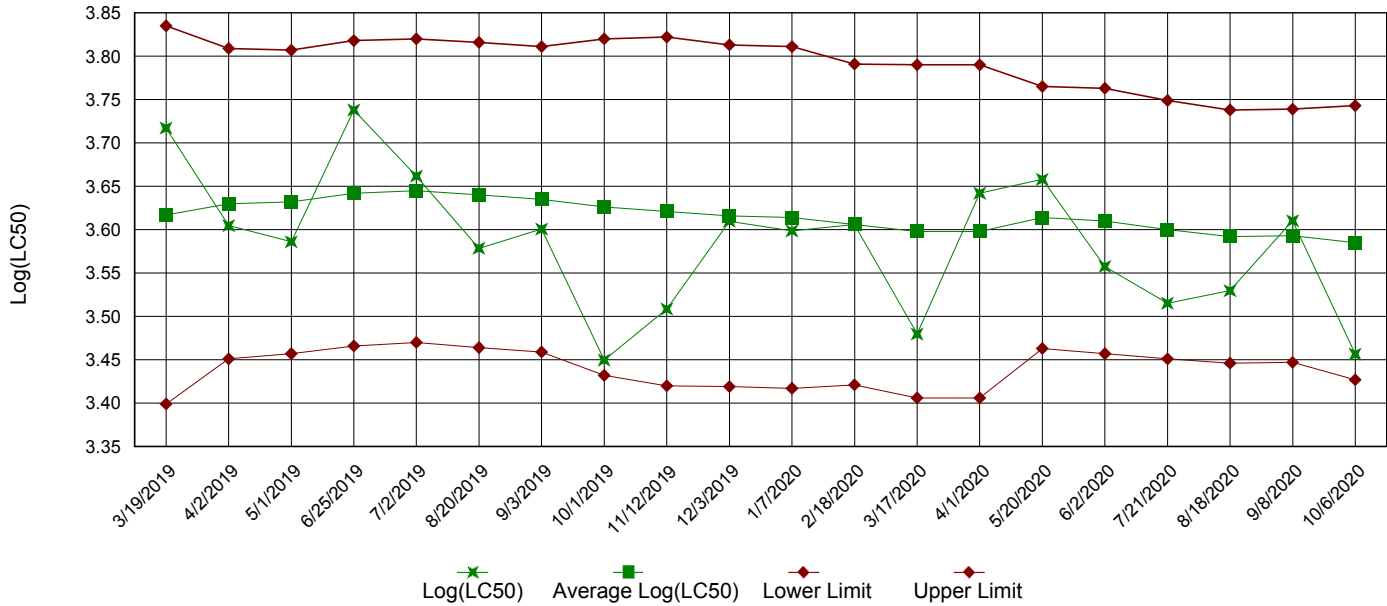
NOEC = 100 %

LOEC = 100 %

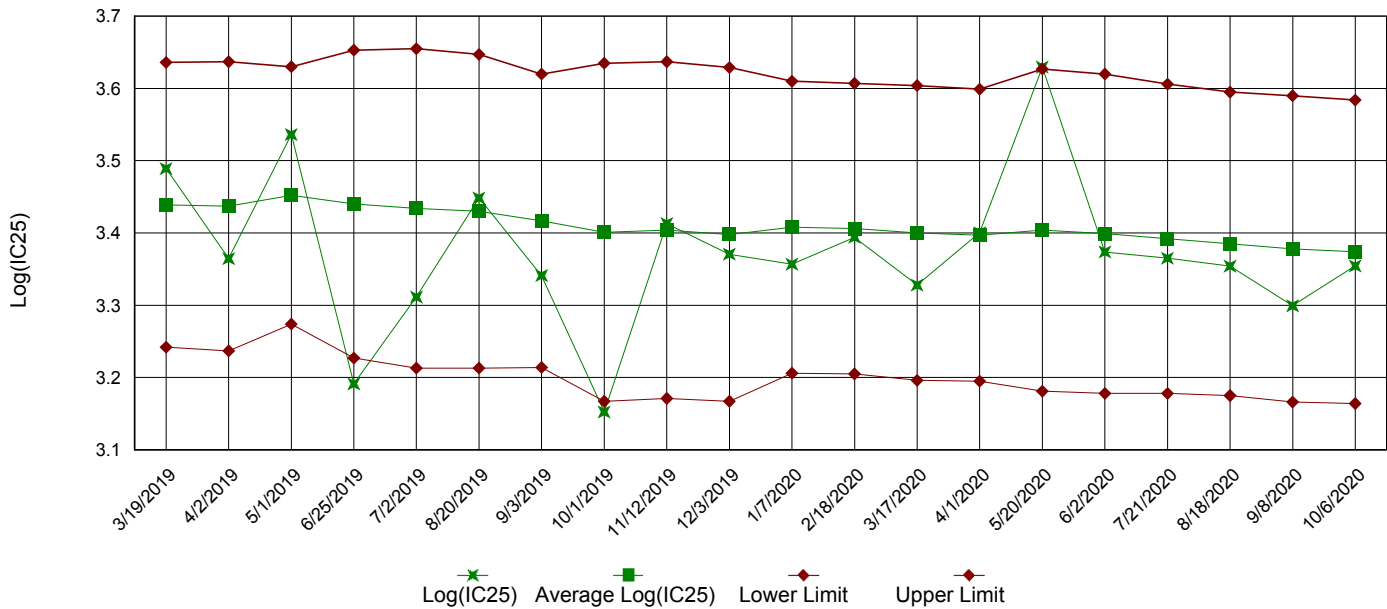
Appendix A3: Test 1000.0

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

LC50 Survival Data

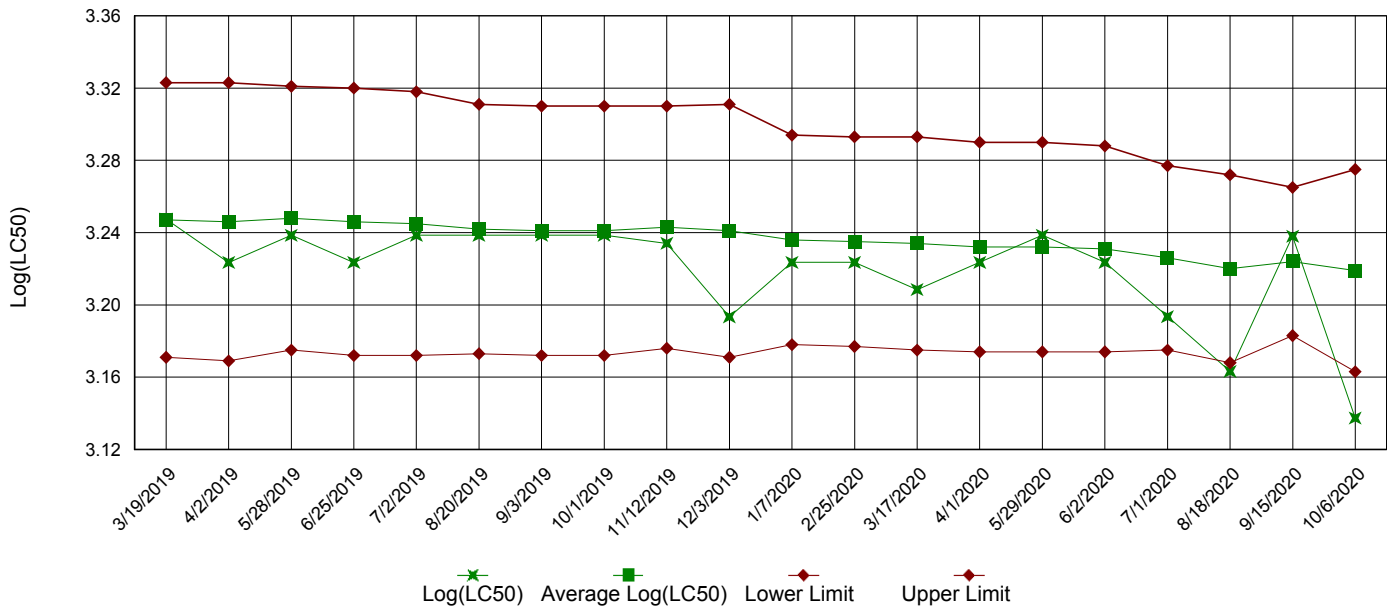


IC25 Growth Data

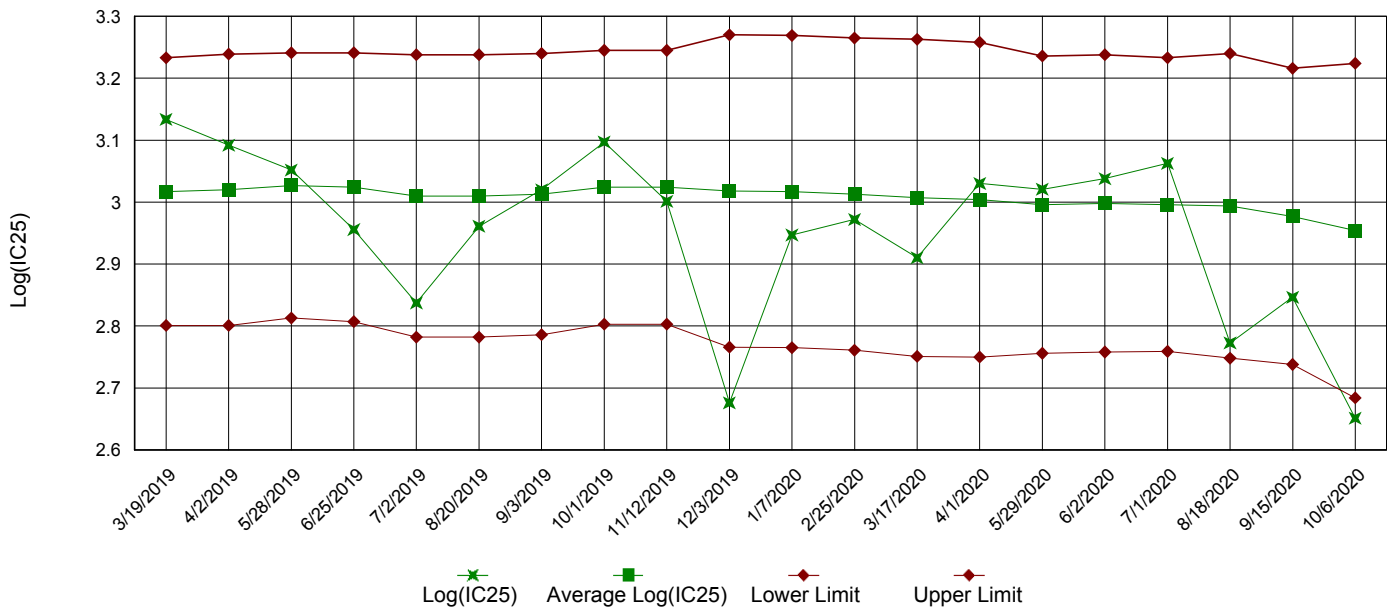


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: City Water & Light of Jonesboro

NPDES No.: AR0043401 AFIN16-00936

Date and Time Test Initiated: October 20, 2020 at 1020

Date and Time Test Terminated: October 27, 2020 at 0935

Dilution water used: Moderately Hard,

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers					Mean percent survival			CV%
	A	B	C	D	E	24 hr	48 hr	7 days	
Control	100	100	100	100	100	100	100	100	0.00
32 %	100	100	100	100	100	100	100	100	0.00
42 %	100	100	100	100	87.5	100	100	97.5	5.73
56 %	100	100	100	100	100	100	100	100	0.00
80 %	100	100	100	100	100	100	100	100	0.00
100 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.452	0.415	0.420	0.350	0.382	0.404	9.66
32 %	0.445	0.386	0.451	0.484	0.429	0.439	8.14
42 %	0.379	0.419	0.366	0.441	0.385	0.398	7.79
56 %	0.395	0.445	0.440	0.388	0.480	0.43	8.87
80 %	0.406	0.415	0.424	0.439	0.440	0.425	3.50
100 %	0.434	0.455	0.420	0.428	0.466	0.441	4.36

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 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> 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 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> NO

- | | | |
|--|----------------|-------------------|
| 3. If you answered NO to 1.a) enter [0] otherwise enter [1]: | <u> 0 </u> | (TLP6C) |
| 4. If you answered NO to 2.a) enter [0] otherwise enter [1]: | <u> 0 </u> | (TGP6C) |
| 5. NOEC Pimephales Lethality: | <u> 100 % </u> | (TOP6C) |
| 6. LOEC Pimephales Lethality: | <u> 100 % </u> | (TXP6C) |
| 7. NOEC Pimephales Sublethality: | <u> 100 % </u> | (TPP6C) |
| 8. LOEC Pimephales Sublethality: | <u> 100 % </u> | (TYP6C) |
| 9. Coefficient of variation for Pimephales growth: | <u> 9.66 </u> | (TQP6C) |
| 10. Lethality for this test: | <u> 100 % </u> | (51714 or 51714R) |
| 11. Sublethality for this test: | <u> 100 % </u> | (51714 or 51714S) |

Appendix B: Test 1000.0
CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
CHEMICAL PARAMETERS CHART

PERMITTEE: City Water & Light of Jonesboro
NPDES NO.: AR0043401 AFIN16-00936
CONTACT: Ms. Whitney Young
ANALYST: 280, 310, 343

Test Initiated: DATE: October 20, 2020 TIME: 1020
Test Terminated: DATE: October 27, 2020 TIME: 0935

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.2	7.4	7.5	7.2	7.1	7.4	7.5
Final	6.8	6.8	7.1	7.7	7.3	6.3	6.5
pH Initial	8.2	8.1	8.2	8.1	8.1	8.1	8.2
Final	7.9	7.9	8.0	8.1	8.1	7.8	7.8

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	7.6	7.2	7.8	7.3	7.2	7.2	7.4
Final	6.6	6.8	6.9	7.2	7.1	6.7	6.8
pH Initial	8.1	8.1	8.0	8.0	8.2	8.2	8.1
Final	8.0	8.1	8.1	8.2	8.2	7.9	7.9

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	7.8	7.2	7.9	7.6	7.1	7.5	7.8
Final	6.0	6.6	6.7	7.0	7.3	6.5	6.7
pH Initial	8.1	8.1	8.0	8.0	8.2	8.2	8.1
Final	7.9	8.0	8.1	8.2	8.3	7.9	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	7.6	7.1	7.6	7.3	7.4	7.3	7.2
Final	6.2	6.8	7.1	7.4	7.1	7.1	6.5
pH Initial	8.0	8.1	7.9	7.9	8.3	8.3	8.1
Final	8.0	8.1	8.2	8.3	8.3	8.2	8.0

DILUTION	DAY						
	1	2	3	4	5	6	7
80 %							
D.O. Initial	7.8	7.3	7.7	7.4	7.1	6.9	7.2
Final	6.2	6.9	7.1	7.4	7.4	6.6	7.2
pH Initial	8.0	8.1	7.8	7.8	8.3	8.4	8.1
Final	8.0	8.2	8.2	8.3	8.4	8.1	8.2

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	7.7	7.3	8.2	7.4	7.3	7.4	7.7
Final	6.3	6.8	6.8	7.3	7.1	7.0	7.2
pH Initial	8.0	8.2	7.8	7.9	8.3	8.4	8.1
Final	8.2	8.3	8.3	8.4	8.4	8.2	8.3

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
140	120	770	<0.05	East Effluent 19-OCT-20
110	120	670	<0.05	East Effluent 21-OCT-20
140	120	650	<0.05	East Effluent 23-OCT-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	85	310	<0.05	249330-1

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: City Water & Light of Jonesboro

NPDES No.: AR0043401 AFIN16-00936

Date and Time Test Initiated: October 20, 2020 at 1120

Date and Time Test Terminated: October 26, 2020 at 1151

Dilution water used: Moderately Hard,

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		32 %	42 %	56 %	80 %	100 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
6 day	100	100	100	90.0	100	80.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		32 %	42 %	56 %	80 %	100 %
A	35	35	27	19	39	33
B	18	20	6	18	18	11
C	31	30	35	37	31	34
D	29	35	24	30	35	27
E	29	28	37	9	35	2
F	28	18	17	21	16	15
G	31	23	35	27	23	17
H	34	35	25	27	33	23
I	31	31	35	30	19	3
J	34	38	35	24	33	16
Mean per Adult	30.0	29.3	27.6	24.2	28.2	18.1
Mean per Surviving Adult	30.0	29.3	27.6	25.9	28.2	22.0
CV %	16.1	23.6	36.4	23.5	29.6	39.3

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 %)	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> 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 %)	<u> X </u> YES	<u> </u> NO
b.) 1/2 LOW FLOW DILUTION	(NA)	<u> </u> YES	<u> </u> 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]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: 100 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 100 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 80 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 100 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 39.3 (TQP3B)
10. Lethality for this test: 100 % (51710 or 51710P)
11. Sublethality for this test: 80 % (51710 or 51710Q)

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: City Water & Light of Jonesboro
NPDES NO.: AR0043401 AFIN16-00936
CONTACT: Ms. Whitney Young
ANALYST: 280, 310, 343

Test Initiated: DATE: October 20, 2020 TIME: 1120
Test Terminated: DATE: October 26, 2020 TIME: 1151

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.2	7.4	7.5	7.2	7.1	7.4	7.5
Final	7.4	7.5	7.5	7.7	7.5	7.4	7.4
pH Initial	8.2	8.1	8.2	8.1	8.1	8.1	8.2
Final	8.4	8.6	8.5	8.5	8.4	8.4	8.4

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	7.6	7.2	7.8	7.3	7.2	7.2	7.4
Final	7.5	7.9	7.5	7.8	7.6	7.6	7.6
pH Initial	8.1	8.1	8.0	8.0	8.2	8.2	8.1
Final	8.6	8.7	8.6	8.6	8.4	8.6	8.6

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	7.8	7.2	7.9	7.6	7.1	7.5	7.8
Final	7.8	8.0	7.7	8.2	7.7	7.8	7.8
pH Initial	8.1	8.1	8.0	8.0	8.2	8.2	8.1
Final	8.7	8.8	8.6	8.6	8.5	8.6	8.6

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	7.6	7.1	7.6	7.3	7.4	7.3	7.2
Final	7.4	7.7	7.1	7.7	7.4	7.2	7.2
pH Initial	8.0	8.1	7.9	7.9	8.3	8.3	8.1
Final	8.6	8.7	8.6	8.6	8.5	8.6	8.6

DILUTION	DAY						
	1	2	3	4	5	6	7
80 %							
D.O. Initial	7.8	7.3	7.7	7.4	7.1	6.9	7.2
Final	7.2	7.6	7.4	7.7	7.7	7.7	7.7
pH Initial	8.0	8.1	7.8	7.8	8.3	8.4	8.1
Final	8.7	8.8	8.7	8.7	8.6	8.6	8.6

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	7.7	7.3	8.2	7.4	7.3	7.4	7.7
Final	7.5	7.7	7.5	7.8	7.7	7.7	7.7
pH Initial	8.0	8.2	7.8	7.9	8.3	8.4	8.1
Final	8.7	8.8	8.7	8.7	8.7	8.8	8.8

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
140	120	770	<0.05	East Effluent 19-OCT-20
110	120	670	<0.05	East Effluent 21-OCT-20
140	120	650	<0.05	East Effluent 23-OCT-20

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	85	310	<0.05	249330-1

COPY



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: JONESBORO CWL		PO No.:		ANALYSES REQUESTED											
Project Reference: ARMY WET TESTING		SAMPLE MATRIX		BOTTLES											
Project Manager: WHITNEY YOUNG		W A T E R		NO OF											
Sampled By: RS/GC		S O I L		B O T T L E S											
AIC No.		C O M P		NO OF											
Sample Identification		G R A B		B O T T L E S											
Date/Time Collected		X		NO OF											
10/18-19/20		X		NO OF											
10:00-9:00AM		X		NO OF											
10/18-19/20		X		NO OF											
10:00-9:00AM		X		NO OF											
9/22-23/20		X		NO OF											
10-9:00AM		X		NO OF											
9/22-23/20		X		NO OF											
10-9:00AM		X		NO OF											
10/1-2/20		X		NO OF											
8-8AM		X		NO OF											
10/5-6/20		X		NO OF											
10-9AM		X		NO OF											
10/5-6/20		X		NO OF											
10-9AM		X		NO OF											
Container Type				NO OF											
Preservative				NO OF											
G = Glass				NO OF											
NO = none				NO OF											
P = Plastic				NO OF											
S = Sulfuric acid pH2				NO OF											
V = VOA vials				NO OF											
N = Nitric acid pH2				NO OF											
H = HCl to pH2				NO OF											
B = NaOH to pH12				NO OF											
T = Sodium Thiosulfate				NO OF											
Z = Zinc acetate				NO OF											
Turnaround Time Requested: (Please circle)				NO OF											
NORMAL or EXPEDITED IN _____ DAYS				NO OF											
Expedited results requested by:				NO OF											
Who should AIC contact with questions:				NO OF											
Phone:				NO OF											
Report Attention to:				NO OF											
Report Address to:				NO OF											
Relinquished By: Matthew Hammond				NO OF											
Date/Time Relinquished: 10-19-20				NO OF											
Relinquished By: Matthew Hammond				NO OF											
Date/Time Relinquished: 1:22 PM				NO OF											
Received By: [Signature]				NO OF											
Date/Time Received: 10-19-20				NO OF											
Received By: [Signature]				NO OF											
Date/Time Received: 13:22				NO OF											
Comments: * East Effluent: C. dubia parallel study w/ extended UV treatment				NO OF											
* West Effluent: P. promelas w/ UV treatment				NO OF											



COPY

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Jonesboro CWL		PO No.		ANALYSES REQUESTED		AIC CONTROL NO: 249566	
Project Reference: AMY WET		SAMPLE MATRIX		NO OF BOTTLES		AIC PROPOSAL NO:	
Project Manager: Whitney Young		WATER		3		Carrier: CWL	
Sampled By: GC/RS		SOIL		3		Received Temperature C: 8.1	
AIC No. 2		COMPOUND		3		Remarks: * P. promelas w/ UV treatment	
Sample Identification		GRA B					
Date/Time Collected		X					
10/20-21/20		X					
10:00-9:00AM							
10/20-21/20							
10:00-9:00AM							
Container Type							
Preservative							
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:							
Phone:							
Fax:							
Report Attention to:							
Report Address to:							
Relinquished By: <i>[Signature]</i>		Date/Time: 10/21/20 12:44 PM		Received By: <i>[Signature]</i>		Date/Time: 10-21-20 1244	
Relinquished By: <i>[Signature]</i>		Date/Time:		Received in Lab By: D. BROWN		Date/Time:	
Comments: * EAST Effluent ; c. dubia parallel study w/ extended UV treatment							

