

August 28, 2018

Biomonitoring Testing
for

Control No. 226081-1

Prepared for:

Mr. Scotty Jones
Trumann Water and Sewer Commission
704 Hwy 463 N
Trumann, AR 72472

Prepared by:

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Trumann Water and Sewer Commission
ATTN: Mr. Scotty Jones
704 Hwy 463 N
Trumann, AR 72472

Re: Chronic *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
NPDES Permit No. AR0035602 AFIN 56-00047

Dear Mr. Scotty Jones:


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 12 % effluent, which is above the critical dilution of 9 %. The NOEC for growth occurred at 12 % effluent, which is above the critical dilution of 9 %. **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 12 % effluent, which is above the critical dilution of 9 %. The NOEC for reproduction occurred at 12 % effluent, which is above the critical dilution of 9 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



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	0.293	PASS
Control Growth CV < or = 40%	11.0	PASS
Growth Minimum Significant Difference 12 to 30%	14.0	PASS
Critical Dilution CV < or = 40%	4.11	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	24.7	PASS
Control CV < or = 40% per Surviving Female	8.33	PASS
Reproduction Minimum Significant Difference 13 to 47%	18.0	PASS
Critical Dilution CV < or = 40%	20.6	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0035602 AFIN 56-00047

2. Test Requirements:

Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:

a. Sampling Point:

b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	7.0	6.0	8.4
pH (standard units)	8.4	8.6	9.1
Alkalinity (mg/l as CaCO ₃)	120	120	110
Hardness (mg/l as CaCO ₃)	36	36	44
Conductivity (umhos/cm)	390	400	410
Residual Chlorine (mg/l)	0.12	0.14	0.12
Ammonia as N (mg/l)	0.33	0.43	0.43

2. Dilution Water Samples:

Moderately Hard

Analysis	226029
Dissolved oxygen (mg/l)	6.1
pH (standard units)	8.2
Alkalinity (mg/l as CaCO ₃)	63
Hardness (mg/l as CaCO ₃)	82
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: August 14, 2018 at 1645
Date & Time Test Terminated: Aug 21, 2018 at 0820
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: August 14, 2018 at 1520
Date & Time Test Terminated: Aug 21, 2018 at 1430
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 Bartlett's test 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 August 1, 2018 at 1545 to Aug 8, 2018 at 0815

The results were as follows: (Control No. 225656-1.)

Survival LC-50: 4221 mg/l

Growth IC-25: 2888 mg/l

Growth PMSD: 17.9

Ceriodaphnia dubia

A chronic reference test was performed on at 1620 to Aug 7, 2018 at 1400

The results were as follows: (Control No. 225656-2.)

Survival LC-50: 1823 mg/l

Growth IC-25: 1219 mg/l

Growth PMSD: 16.2

V. Organism History

Pimephales promelas (Fathead minnow)

Date: August 14, 2018

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: August 14, 2018

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

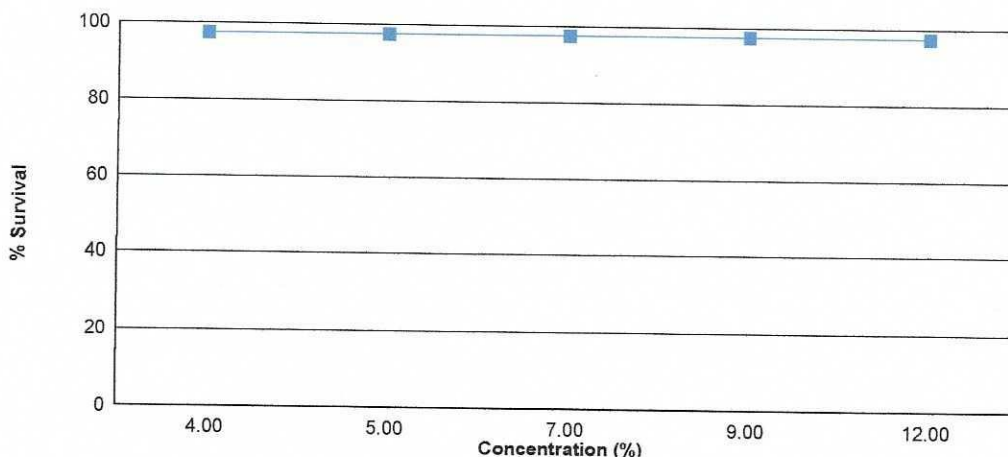
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 9 % effluent.

The test was initiated on August 14, 2018 at 1645 and continued through Aug 21, 2018 at 0820. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 12 % effluent
- b.) NOEC growth = 12 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.293
4 %	97.5	0.293
5 %	97.5	0.292
7 %	97.5	0.276
9 %	97.5	0.285
12 %	97.5	0.290

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

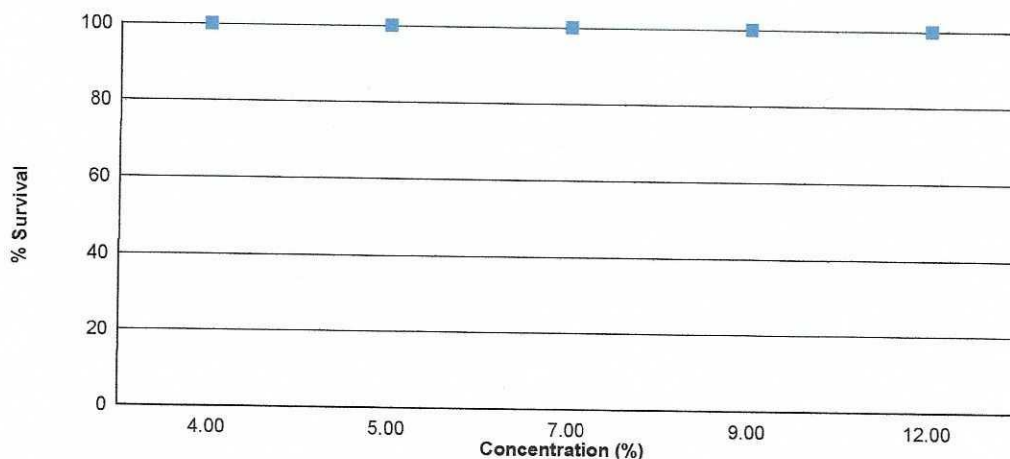
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 4 %, 5 %, 7 %, 9 %, 12 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 9 % effluent.

The test was initiated on August 14, 2018 at 1520 and continued through Aug 21, 2018 at 1430. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 12 % effluent
- b.) NOEC reproduction = 12 % effluent



Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	24.7
4 %	100	24.4
5 %	100	26.8
7 %	100	26.6
9 %	100	24.4
12 %	100	25.4

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 14, 2018 at 1645

Date and Time Test Terminated: Aug 21, 2018 at 0820

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
4 %	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
5 %	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
7 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	7	7	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
9 %	A	8	8	8	8	8	8	8
	B	8	8	7	7	7	7	7
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
12 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	7	7	7	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: August 14, 2018 at 1645

Test Terminated: Aug 21, 2018 at 0820

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.93599	.93878	0.00279	8	0.349
	B	.92593	.92821	0.00228	8	0.285
	C	.92703	.92929	0.00226	8	0.282
	D	.93143	.93357	0.00214	8	0.268
	E	.92617	.92840	0.00223	8	0.279
4 %	A	.93267	.93558	0.00291	8	0.364
	B	.93078	.93306	0.00228	8	0.285
	C	.93032	.93238	0.00206	8	0.258
	D	.92643	.92868	0.00225	8	0.281
	E	.93235	.93455	0.00220	8	0.275
5 %	A	.92838	.93072	0.00234	8	0.292
	B	.92635	.92895	0.00260	8	0.325
	C	.93212	.93424	0.00212	8	0.265
	D	.93048	.93269	0.00221	8	0.276
	E	.92979	.93222	0.00243	8	0.304
7 %	A	.92775	.92988	0.00213	8	0.266
	B	.92545	.92762	0.00217	8	0.271
	C	.93151	.93363	0.00212	8	0.265
	D	.92973	.93215	0.00242	8	0.302
	E	.93015	.93237	0.00222	8	0.278
9 %	A	.93337	.93577	0.00240	8	0.300
	B	.93054	.93273	0.00219	8	0.274
	C	.93451	.93682	0.00231	8	0.289
	D	.93294	.93525	0.00231	8	0.289
	E	.93373	.93591	0.00218	8	0.272
12 %	A	.93125	.93342	0.00217	8	0.271
	B	.92922	.93141	0.00219	8	0.274
	C	.93144	.93358	0.00214	8	0.268
	D	.92781	.93050	0.00269	8	0.336
	E	.92950	.93192	0.00242	8	0.302

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 14, 2018 at 1520

Date and Time Test Terminated: Aug 21, 2018 at 1430

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	0	0	0	0	0	0	10	0.00
4	4	5	3	5	4	5	4	4	5	5	44	10	4.40
5	7	0	0	9	0	7	0	9	0	0	32	10	3.20
6	0	9	8	11	9	1	8	12	7	9	74	10	7.40
7	12	13	12	0	14	9	10	0	15	12	97	10	9.70
8													
TOTAL	23	27	23	25	27	22	22	25	27	26	247	10	24.7

Concentration: 4 %													
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	0	0	0	0	0	0	10	0.00
4	4	4	5	5	4	0	3	4	5	3	37	10	3.70
5	6	0	0	11	9	4	0	9	0	0	39	10	3.90
6	0	11	9	12	5	10	7	0	9	11	74	10	7.40
7	11	16	10	0	9	0	11	12	12	13	94	10	9.40
8													
TOTAL	21	31	24	28	27	14	21	25	26	27	244	10	24.4

Concentration: 5 %													
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	0	0	3	0	0	3	10	0.300
4	4	6	4	5	3	5	4	0	6	6	43	10	4.30
5	8	0	0	10	9	0	8	9	0	0	44	10	4.40
6	0	11	7	14	0	8	0	12	8	9	69	10	6.90
7	12	16	14	0	14	13	13	0	14	13	109	10	10.9
8													
TOTAL	24	33	25	29	26	26	25	24	28	28	268	10	26.8

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 14, 2018 at 1520

Date and Time Test Terminated: Aug 21, 2018 at 1430

Concentration: 7 %													
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	0	0	0	0	0	0	10	0.00
4	6	5	5	4	4	4	6	4	4	6	48	10	4.80
5	7	1	0	9	8	9	0	10	0	0	44	10	4.40
6	0	10	10	12	0	0	7	10	8	9	66	10	6.60
7	13	15	15	0	12	12	13	0	15	13	108	10	10.8
8													
TOTAL	26	31	30	25	24	25	26	24	27	28	266	10	26.6

Concentration: 9 %													
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	0	0	0	0	0	0	10	0.00
4	5	4	5	5	0	0	2	4	5	7	37	10	3.70
5	9	0	0	10	6	6	8	8	1	0	48	10	4.80
6	0	10	10	11	10	11	0	9	7	9	77	10	7.70
7	14	15	12	0	0	0	12	0	16	13	82	10	8.20
8													
TOTAL	28	29	27	26	16	17	22	21	29	29	244	10	24.4

Concentration: 12 %													
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	0	0	0	0	0	0	10	0.00
4	4	6	5	4	2	3	6	3	0	5	38	10	3.80
5	0	0	0	11	0	6	0	9	0	0	26	10	2.60
6	11	10	11	12	17	1	9	10	3	8	92	10	9.20
7	14	16	12	0	15	12	5	0	8	16	98	10	9.80
8													
TOTAL	29	32	28	27	34	22	20	22	11	29	254	10	25.4

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

Transformation of Data			Transform: Arc Sin(Square Root(Y))	
Group	Identification	Rep	Value	Transformed
1	Control	1	1.00000	1.39310
1	Control	2	1.00000	1.39310
1	Control	3	1.00000	1.39310
1	Control	4	1.00000	1.39310
1	Control	5	1.00000	1.39310
2	4 %	1	1.00000	1.39310
2	4 %	2	1.00000	1.39310
2	4 %	3	1.00000	1.39310
2	4 %	4	1.00000	1.39310
2	4 %	5	0.87500	1.20940
3	5 %	1	1.00000	1.39310
3	5 %	2	1.00000	1.39310
3	5 %	3	1.00000	1.39310
3	5 %	4	1.00000	1.39310
3	5 %	5	0.87500	1.20940
4	7 %	1	1.00000	1.39310
4	7 %	2	1.00000	1.39310
4	7 %	3	0.87500	1.20940
4	7 %	4	1.00000	1.39310
4	7 %	5	1.00000	1.39310
5	9 %	1	1.00000	1.39310
5	9 %	2	0.87500	1.20940
5	9 %	3	1.00000	1.39310
5	9 %	4	1.00000	1.39310
5	9 %	5	1.00000	1.39310
6	12 %	1	1.00000	1.39310
6	12 %	2	1.00000	1.39310
6	12 %	3	0.87500	1.20940
6	12 %	4	1.00000	1.39310
6	12 %	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 = 0.135 W = 0.5577 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	4 %	25.00	16.00	5.00	
3	5 %	25.00	16.00	5.00	
4	7 %	25.00	16.00	5.00	
5	9 %	25.00	16.00	5.00	
6	12 %	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
<p>D = 0.01799 W = 0.8722 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					No Transformation
Ho: Control < Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	4 %	26.50	16.00	5.00	
3	5 %	28.00	16.00	5.00	
4	7 %	21.00	16.00	5.00	
5	9 %	29.00	16.00	5.00	
6	12 %	25.50	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 (excluding deaths if applicable)

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.001056	0.0002112	0.2819
Within (Error)	24	0.01798	0.0007492	
Total	29	0.01904		

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	0.2926	0.2926		
2	4 %	0.2926	0.2926	0	
3	5 %	0.2924	0.2924	0.01155	
4	7 %	0.2764	0.2764	0.9358	
5	9 %	0.2848	0.2848	0.4506	
6	12 %	0.2902	0.2902	0.1386	

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	4 %	5	0.04085	14	0
3	5 %	5	0.04085	14	0.0002
4	7 %	5	0.04085	14	0.0162
5	9 %	5	0.04085	14	0.0078
6	12 %	5	0.04085	14	0.0024

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
4 %	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
5 %	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
7 %	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
9 %	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
12 %	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	4 %	10	0	
2	5 %	10	0	
3	7 %	10	0	
4	9 %	10	0	
5	12 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.1228 D* = 0.9635 Critical D* = 1.035 (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 = 18.23 Critical B = 15.086 (alpha = 0.01, df = 5)</p> <p>Data FAIL B1 homogeneity test at 0.01 level.</p>	

Steel's Many-One Rank Test					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	4 %	107.50	75.00	10.00	
3	5 %	126.00	75.00	10.00	
4	7 %	124.50	75.00	10.00	
5	9 %	111.00	75.00	10.00	
6	12 %	115.50	75.00	10.00	
Critical values are 1 tailed (k=5)					

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation (excluding deaths if applicable)

ANOVA Table					No Transformation
SOURCE	DF	SS	MS	F	
Between	5	58.88	11.78	0.633	
Within (Error)	54	1005	18.61		
Total	59	1064			
Critical F = 3.38 (alpha = 0.01, df = 5,54)					
2.38 (alpha = 0.05, df = 5,54)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

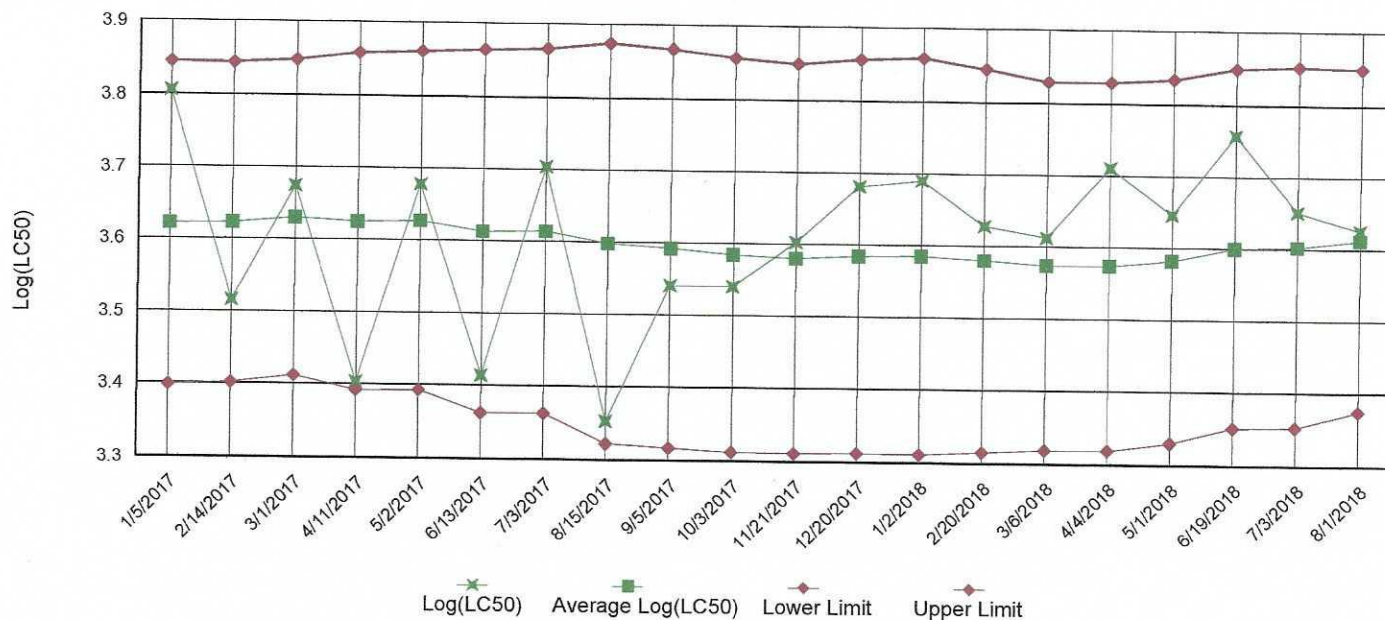
Dunnett's Test - Table 1 of 2					No Transformation
Ho: Control < Treatment					
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05
1	Control	24.7	24.7		
2	4 %	24.4	24.4	0.1555	
3	5 %	26.8	26.8	-1.089	
4	7 %	26.6	26.6	-0.9848	
5	9 %	24.4	24.4	0.1555	
6	12 %	25.4	25.4	-0.3628	
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	4 %	10	4.457	18	0.3
3	5 %	10	4.457	18	-2.1
4	7 %	10	4.457	18	-1.9
5	9 %	10	4.457	18	0.3
6	12 %	10	4.457	18	-0.7

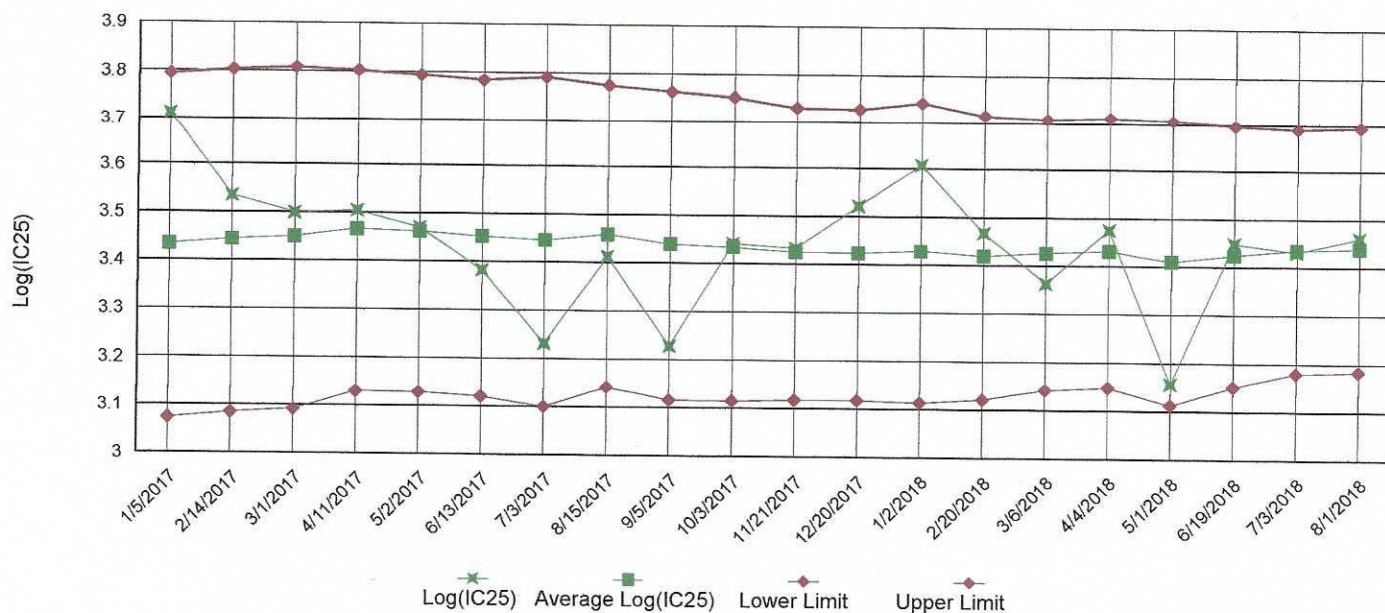
Appendix A3: Test 1000.0

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

LC50 Survival Data

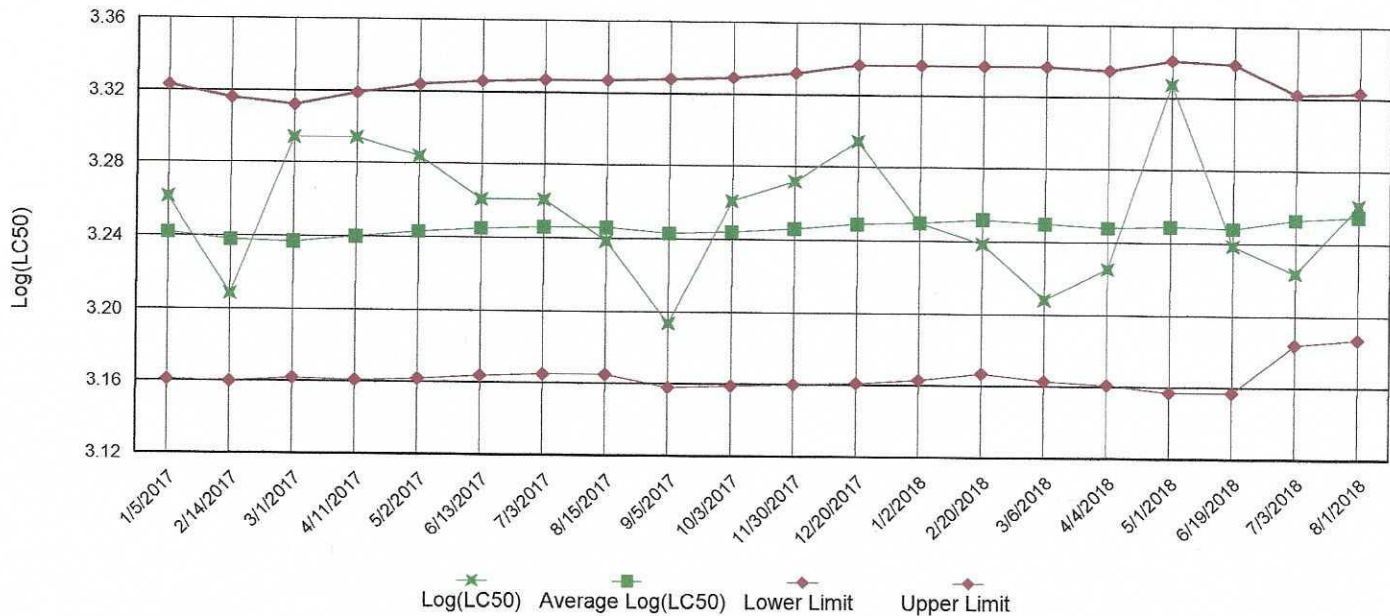


IC25 Growth Data

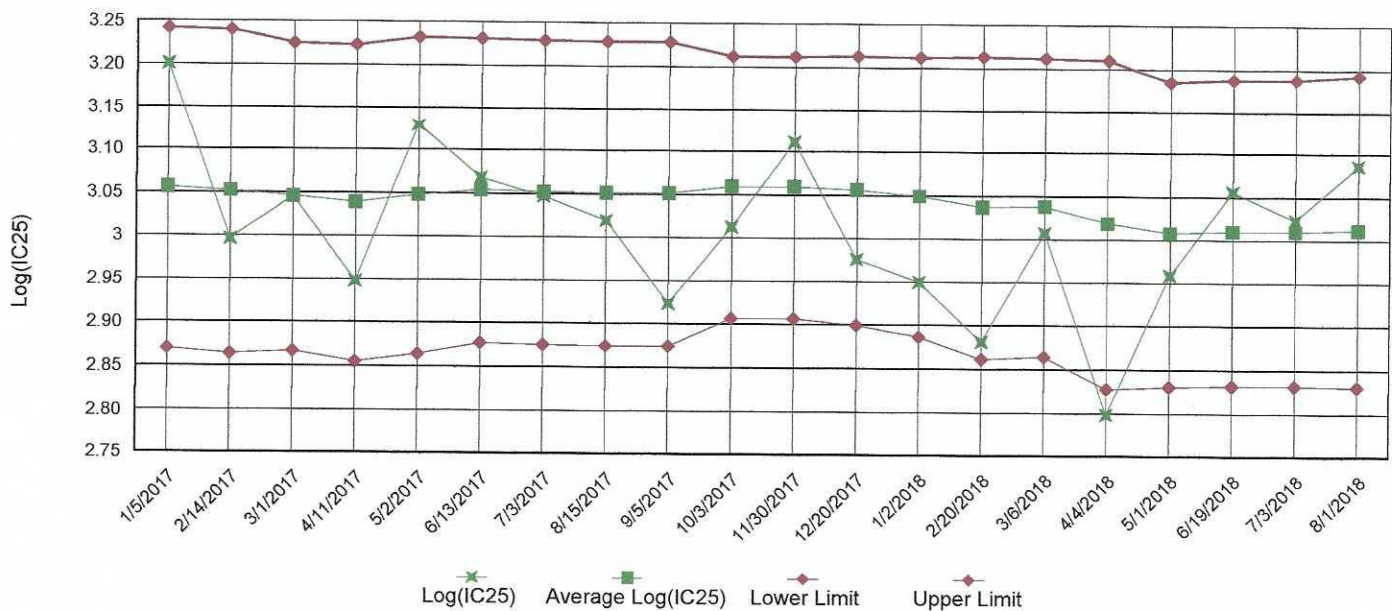


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Trumann Water and Sewer Commission

NPDES No.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: August 14, 2018 at 1645

Date and Time Test Terminated: Aug 21, 2018 at 0820

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
4 %	100	100	100	100	87.5	100	100	97.5	5.73
5 %	100	100	100	100	87.5	100	100	97.5	5.73
7 %	100	100	87.5	100	100	100	100	97.5	5.73
9 %	100	87.5	100	100	100	100	100	97.5	5.73
12 %	100	100	87.5	100	100	100	100	97.5	5.73

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.349	0.285	0.282	0.268	0.279	0.293	11.0
4 %	0.364	0.285	0.258	0.281	0.275	0.293	14.1
5 %	0.292	0.325	0.265	0.276	0.304	0.292	8.05
7 %	0.266	0.271	0.265	0.302	0.278	0.276	5.50
9 %	0.300	0.274	0.289	0.289	0.272	0.285	4.11
12 %	0.271	0.274	0.268	0.336	0.302	0.29	9.99

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	(9 %)	_____ 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	(9 %)	_____ 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: 12 % (TOP6C)

6. LOEC *Pimephales* Lethality: 12 % (TXP6C)

7. NOEC *Pimephales* Sublethality: 12 % (TPP6C)

8. LOEC *Pimephales* Sublethality: 12 % (TYP6C)

9. Coefficient of variation for *Pimephales* growth: 11 (TQP6C)

Appendix B: Test 1000.0

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

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AR0035602 AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 310, 322, 329

Test Initiated: DATE: August 14, 2018 TIME: 1645
Test Terminated: DATE: Aug 21, 2018 TIME: 0820

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	6.1	6.0	5.6	7.2	7.6	7.1	5.7
Final	5.7	5.4	7.3	7.2	7.3	7.6	7.6
pH Initial	8.2	8.2	8.3	8.2	8.5	8.5	8.2
Final	8.2	8.2	8.2	8.7	8.1	8.3	8.1

DILUTION	DAY						
4 %	1	2	3	4	5	6	7
D.O. Initial	6.2	5.8	5.5	6.8	7.6	7.3	5.9
Final	5.9	5.5	7.3	7.8	7.4	7.6	7.5
pH Initial	8.2	8.2	8.3	8.2	8.5	8.5	8.2
Final	8.2	8.2	8.2	8.7	8.1	8.4	8.1

DILUTION	DAY						
5 %	1	2	3	4	5	6	7
D.O. Initial	6.3	5.8	5.7	7.4	7.0	7.1	7.8
Final	5.9	5.6	7.0	7.6	7.2	7.6	7.2
pH Initial	8.2	8.2	8.3	8.1	8.5	8.5	8.2
Final	8.2	8.2	8.3	8.7	8.0	8.4	8.1

DILUTION	DAY						
7 %	1	2	3	4	5	6	7
D.O. Initial	6.2	5.9	5.6	7.0	7.5	7.3	6.3
Final	5.8	5.7	7.2	7.6	7.4	7.7	7.2
pH Initial	8.2	8.2	8.3	8.3	8.5	8.5	8.3
Final	8.2	8.3	8.3	8.7	8.0	8.4	8.0

DILUTION	DAY						
9 %	1	2	3	4	5	6	7
D.O. Initial	6.2	6.0	5.6	7.3	7.5	7.4	6.4
Final	5.8	5.7	7.4	7.6	7.3	7.6	7.7
pH Initial	8.2	8.2	8.3	8.3	8.6	8.5	8.3
Final	8.2	8.3	8.3	8.7	8.1	8.4	7.8

DILUTION	DAY						
12 %	1	2	3	4	5	6	7
D.O. Initial	6.3	5.7	5.5	7.3	7.6	7.4	6.5
Final	5.9	5.6	7.1	7.9	7.6	7.8	7.2
pH Initial	8.2	8.2	8.3	8.4	8.6	8.6	8.4
Final	8.3	8.2	8.3	8.8	8.1	8.5	8.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	36	390	0.12	AR0035602 13-AUG-18
120	36	400	0.14	AR0035602 15-AUG-18
110	44	410	0.12	AR0035602 17-AUG-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	82	310	<0.05	226029

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

Permittee: Trumann Water and Sewer Commission

NPDES No.: AR0035602 AFIN 56-00047

Date and Time Test Initiated: August 14, 2018 at 1520

Date and Time Test Terminated: Aug 21, 2018 at 1430

Dilution water used: Moderately Hard

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		4 %	5 %	7 %	9 %	12 %
A	23	21	24	26	28	29
B	27	31	33	31	29	32
C	23	24	25	30	27	28
D	25	28	29	25	26	27
E	27	27	26	24	16	34
F	22	14	26	25	17	22
G	22	21	25	26	22	20
H	25	25	24	24	21	22
I	27	26	28	27	29	11
J	26	27	28	28	29	29
Mean per Adult	24.7	24.4	26.8	26.6	24.4	25.4
Mean per Surviving Adult	24.7	24.4	26.8	26.6	24.4	25.4
CV %	8.33	19.5	10.4	9.07	20.6	26.7

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	(9 %)	_____ 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	(9 %)	_____ 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: 12 % (TOP3B)

6. LOEC *Ceriodaphnia* Lethality: 12 % (TXP3B)

7. NOEC *Ceriodaphnia* Sublethality: 12 % (TPP3B)

8. LOEC *Ceriodaphnia* Sublethality: 12 % (TYP3B)

9. Coefficient of variation for *Ceriodaphnia* Reproduction: 20.6 (TQP3B)

Appendix B: Test 1002.0

CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Trumann Water and Sewer Commi
NPDES NO.: AR0035602 AFIN 56-00047
CONTACT: Mr. Scotty Jones
ANALYST: 280, 310, 322, 329

Test Initiated: DATE: August 14, 2018 TIME: 1520
Test Terminated: DATE: Aug 21, 2018 TIME: 1430

DILUTION	DAY						
Control	1	2	3	4	5	6	7
D.O. Initial	6.1	6.0	5.6	7.2	7.6	7.1	5.7
Final	6.4	5.8	7.0	7.4	7.3	7.4	7.3
pH Initial	8.2	8.2	8.3	8.2	8.5	8.5	8.2
Final	8.4	8.4	8.5	8.8	8.1	8.2	8.3

DILUTION	DAY						
4 %	1	2	3	4	5	6	7
D.O. Initial	6.2	5.8	5.5	6.8	7.6	7.3	5.9
Final	6.2	5.9	7.3	7.8	7.3	7.6	7.5
pH Initial	8.2	8.2	8.3	8.2	8.5	8.5	8.2
Final	8.5	8.5	8.5	8.8	8.1	8.3	8.3

DILUTION	DAY						
5 %	1	2	3	4	5	6	7
D.O. Initial	6.3	5.8	5.7	7.4	7.0	7.1	7.8
Final	6.5	5.8	7.1	7.5	7.4	7.6	7.5
pH Initial	8.2	8.2	8.3	8.1	8.5	8.5	8.2
Final	8.4	8.5	8.5	8.8	8.1	8.3	8.3

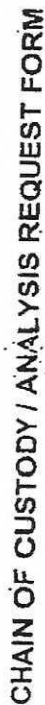
DILUTION	DAY						
7 %	1	2	3	4	5	6	7
D.O. Initial	6.2	5.9	5.6	7.0	7.5	7.3	6.3
Final	6.2	6.0	7.2	7.8	7.5	7.6	7.6
pH Initial	8.2	8.2	8.3	8.3	8.5	8.5	8.3
Final	8.3	8.4	8.5	8.8	8.1	8.4	8.4

DILUTION	DAY						
9 %	1	2	3	4	5	6	7
D.O. Initial	6.2	6.0	5.6	7.3	7.5	7.4	6.4
Final	6.1	6.0	7.1	7.5	7.5	7.7	7.6
pH Initial	8.2	8.2	8.3	8.3	8.6	8.5	8.3
Final	8.3	8.5	8.5	8.8	8.1	8.4	8.4

DILUTION	DAY						
12 %	1	2	3	4	5	6	7
D.O. Initial	6.3	5.7	5.5	7.3	7.6	7.4	6.5
Final	6.1	5.9	7.2	7.9	7.5	7.9	7.7
pH Initial	8.2	8.2	8.3	8.4	8.6	8.6	8.4
Final	8.3	8.5	8.6	8.8	8.1	8.5	8.5

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
120	36	390	0.12	AR0035602 13-AUG-18
120	36	400	0.14	AR0035602 15-AUG-18
110	44	410	0.12	AR0035602 17-AUG-18

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
63	82	310	<0.05	226029

Form 0080 May 2001

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>Trumann Water Works</u>		AIC Control No. <u>226081</u>	
Project Reference: <u>AR0035602</u>		AIC Proposal No. _____	
Project Manager: <u>Scotty Jones</u>		Carrier: <u>LED EX</u>	
Sampled By: <u>LORRE HOLT</u>		Received Temperature °C <u>25</u>	
AIC Sample Identification No. <u>3 AR0035602</u>		Remarks _____	
Date/Time Collected <u>8/14/88 8:40 AM - 8:00 PM</u>			
G R A B C O M P			
W A T E R L			
No of B O T T L E S <u>3</u>			
PO No. _____			
Sample Matrix			
Analyses Requested			
BIDMONT BRNG-CHRONIC CD+FH			
Field pH calibration on _____ @ _____			
Buffer: _____			
T = Sodium Thiosulfate Z = Zinc acetate			
H = HCl to pH2 B = NaOH to pH12			
V = VOA vials N = Nitric acid pH2			
Relinquished By: <u>LORRE HOLT</u>		Received By: _____	
Date/Time Relinquished _____		Date/Time Received <u>8/17/89 9:45 AM</u>	
By: _____		Received in Lab By: _____	
Date/Time _____		Date/Time <u>18 Aug 89</u>	
Comments: _____		<u>5-3 10</u>	
Expedited results requested by: _____			
Who should AIC contact with questions: <u>LORRE HOLT</u>			
Phone: <u>870-483-4882</u> Fax: <u>870-483-6525</u>			
Report Attention to: <u>LORRE HOLT</u>			
Report Address to: <u>704 HWY 4630, TRUMANN, AR 72472</u>			

From: American Interplex interplex@americaninterplex.com
Subject: Control Number:226081 (AR0035602)
Date: Aug 28, 2018 at 4:45:41 PM
To: Ms. Lorre Holt lorre_holt0201@yahoo.com

Dear Ms. Lorre Holt,

Your report for control number 226081 (AR0035602) is enclosed as an attachment to this e-mail.

If you would prefer to receive your reports via our website (<https://clients.americaninterplex.com>), please call our Client Services

Department or myself at [501-224-5060](tel:501-224-5060) for a username and password. Once your login has been activated you will receive an email notification each time a new report is available.

Sincerely,
John Overbey
Laboratory Director

The documents with this transmission are only for the recipient(s) named therein, and they contain confidential information. Unauthorized disclosure, dissemination, or copying of this transmission is strictly prohibited. If received in error, please destroy.