

February 9, 2017

Test Results of  
Chronic 7-Day Renewal  
Biomonitoring Testing  
for  
Outfall 001

Control No. 209426-1

Prepared for:

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Prepared by:

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McClelland Consulting Engineers, Inc.  
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Re: Chronic 7-Day Renewal utilizing *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*  
Outfall 001  
NPDES Permit No. AR0021661 AFIN:43-00059

Dear Mr. Matt Bienvenu:

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: Statistically significant difference was noted at 100% effluent concentration for growth. However, the percent minimum significant (PMSD) is below the lower limit of 12. Following additional calculations provided in the EPA manual "Understanding and Accounting for Method Variability in Whole Effluent Toxicity Applications under the National Pollutant Discharge Elimination System Program", the 100% effluent concentration has a relative difference of 11%, which is below the PMSD limit of 12. **The sample, therefore PASSED both lethal and sub-lethal effects for the *Pimephales promelas* 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**

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	0.493	PASS
Control Growth CV < or = 40%	11.2	PASS
Growth Minimum Significant Difference 12 to 30%	10.8	BELOW
Critical Dilution CV < or = 40%	5.19	PASS

*Ceriodaphnia dubia* Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	21.8	PASS
Control CV < or = 40% per Surviving Female	21.3	PASS
Reproduction Minimum Significant Difference 13 to 47%	22.1	PASS
Critical Dilution CV < or = 40%	11.7	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0021661 AFIN:43-00059
2. Test Requirements: Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
  - a. Sampling Point: Outfall 001
  - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	8.8	8.5	8.7
pH (standard units)	6.7	6.8	7.6
Alkalinity (mg/l as CaCO <sub>3</sub> )	48	50	54
Hardness (mg/l as CaCO <sub>3</sub> )	53	66	67
Conductivity (umhos/cm)	280	400	370
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.30	0.17	<0.1

2. Dilution Water Samples:  
Moderately Hard

Analysis	209187
Dissolved oxygen (mg/l)	7.9
pH (standard units)	7.7
Alkalinity (mg/l as CaCO <sub>3</sub> )	57
Hardness (mg/l as CaCO <sub>3</sub> )	86
Conductivity (umhos/cm)	300
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: January 24, 2017 at 1650  
Date & Time Test Terminated: January 31, 2017 at 1055  
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: January 24, 2017 at 1640  
Date & Time Test Terminated: January 31, 2017 at 1500  
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. 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 January 5, 2017 at 1315 to January 12, 2017 at 11:30

The results were as follows: (Control No. 208852-1.)

Survival LC-50: 6392.6 mg/l

Growth IC-25: 5154 mg/l

Growth PMSD: 12.4

##### *Ceriodaphnia dubia*

A chronic reference test was performed on January 5, 2017 at 1100 to January 12, 1600 at 1600

The results were as follows: (Control No. 208852-2.)

Survival LC-50: 1825 mg/l

Growth IC-25: 1590 mg/l

Growth PMSD: 14.7

#### V. Organism History

##### *Pimephales promelas* (Fathead minnow)

Date: January 24, 2017

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

##### *Ceriodaphnia dubia*

Date: January 24, 2017

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

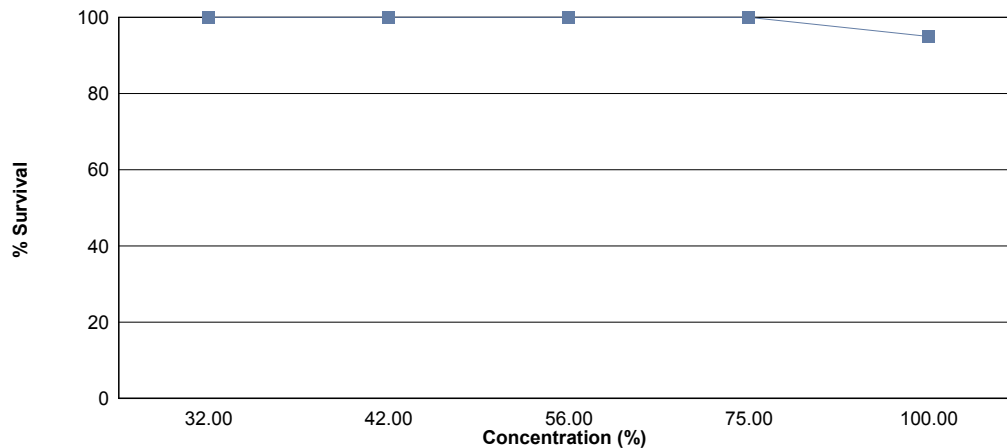
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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 January 24, 2017 at 1650 and continued through January 31, 2017 at 1055. 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 = 75 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	100	0.493
32 %	100	0.508
42 %	100	0.492
56 %	100	0.458
75 %	100	0.459
100 %	95.0	0.437 *

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

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

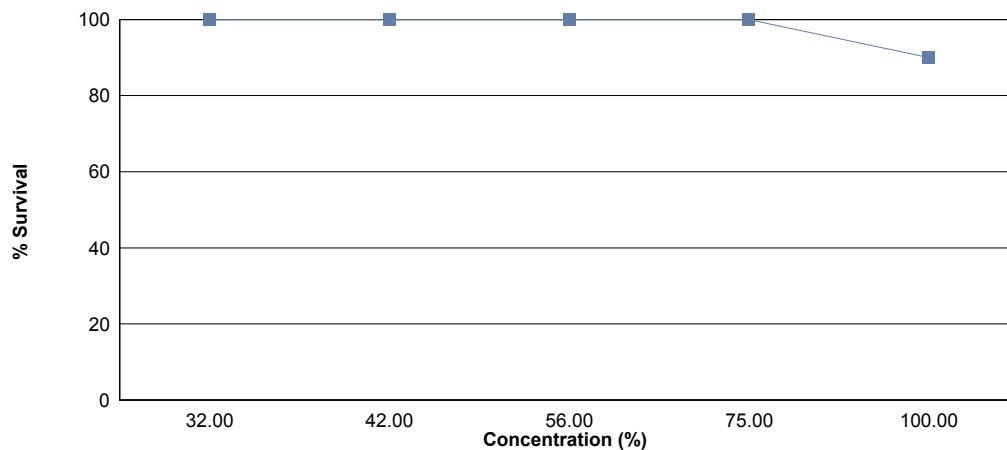
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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 January 24, 2017 at 1640 and continued through January 31, 2017 at 1500. 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 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	21.8
32 %	100	29.2
42 %	100	29.0
56 %	100	31.0
75 %	100	31.9
100 %	90.0	30.4



Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: January 24, 2017 at 1650

Date and Time Test Terminated: January 31, 2017 at 1055

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	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	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	7	7	7	7	7
	E	8	8	8	8	7	7	7

Appendix A1: Test 1000.0

*Pimephales promelas* (Fathead Minnow) 7-Day Growth

Test Initiated: January 24, 2017 at 1650

Test Terminated: January 31, 2017 at 1055

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.91944	.92269	0.00325	8	0.406
	B	.92145	.92552	0.00407	8	0.509
	C	.92425	.92857	0.00432	8	0.540
	D	.92827	.93254	0.00427	8	0.534
	E	.93152	.93531	0.00379	8	0.474
32 %	A	.92964	.93357	0.00393	8	0.491
	B	.92420	.92842	0.00422	8	0.528
	C	.92435	.92844	0.00409	8	0.511
	D	.92518	.92893	0.00375	8	0.469
	E	.92617	.93049	0.00432	8	0.540
42 %	A	.92683	.93061	0.00378	8	0.472
	B	.92844	.93199	0.00355	8	0.444
	C	.93132	.93550	0.00418	8	0.522
	D	.92208	.92637	0.00429	8	0.536
	E	.92275	.92663	0.00388	8	0.485
56 %	A	.92383	.92733	0.00350	8	0.438
	B	.92768	.93108	0.00340	8	0.425
	C	.92690	.93049	0.00359	8	0.449
	D	.92597	.92975	0.00378	8	0.472
	E	.93000	.93403	0.00403	8	0.504
75 %	A	.92134	.92480	0.00346	8	0.432
	B	.91699	.92043	0.00344	8	0.430
	C	.92011	.92370	0.00359	8	0.449
	D	.93371	.93767	0.00396	8	0.495
	E	.92306	.92696	0.00390	8	0.488
100 %	A	.92266	.92639	0.00373	8	0.466
	B	.92068	.92416	0.00348	8	0.435
	C	.93089	.93431	0.00342	8	0.428
	D	.92063	.92388	0.00325	8	0.406
	E	.92447	.92807	0.00360	8	0.450

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: January 24, 2017 at 1640

Date and Time Test Terminated: January 31, 2017 at 1500

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	0	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	4	4	4	4	4	6	3	3	0	38	10	3.80	
5	11	0	0	8	0	5	8	6	7	6	51	10	5.10	
6	0	8	7	0	10	0	0	0	0	0	25	10	2.50	
7	12	14	11	10	12	9	10	8	10	8	104	10	10.4	
8														
TOTAL	29	26	22	22	26	18	24	17	20	14	218	10	21.8	

Concentration: 32 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	6	5	5	5	6	1	1	4	6	45	10	4.50
5	9	0	0	11	0	9	10	8	11	0	58	10	5.80
6	0	15	12	0	12	0	1	1	0	11	52	10	5.20
7	12	15	14	14	16	13	11	12	15	15	137	10	13.7
8													
TOTAL	27	36	31	30	33	28	23	22	30	32	292	10	29.2

Concentration: 42 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	0	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	4	5	7	5	6	7	4	4	4	52	10	5.20
5	8	0	0	0	0	3	0	11	8	0	30	10	3.00
6	0	11	13	13	11	0	11	0	0	8	67	10	6.70
7	17	15	15	17	15	12	13	9	12	16	141	10	14.1
8													
TOTAL	31	30	33	37	31	21	31	24	24	28	290	10	29.0

Appendix A1: Test 1002.0

*Ceriodaphnia dubia* Survival and Reproduction

Date and Time Test Initiated: January 24, 2017 at 1640

Date and Time Test Terminated: January 31, 2017 at 1500

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	0	0	0	0	0	0	0	0	0	0	10	0.00
4	6	6	6	4	7	4	4	4	4	5	50	10	5.00	
5	10	0	0	0	0	8	11	10	9	0	48	10	4.80	
6	0	13	13	10	14	2	0	0	0	10	62	10	6.20	
7	13	18	14	13	18	14	16	16	15	13	150	10	15.0	
8														
TOTAL	29	37	33	27	39	28	31	30	28	28	310	10	31.0	

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	0	10	0.00
2	0	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	0	10	0.00
4	7	6	6	5	5	7	6	3	4	2	51	10	5.10	
5	11	0	0	13	0	13	10	10	11	0	68	10	6.80	
6	0	11	14	0	13	0	1	0	0	9	48	10	4.80	
7	18	18	14	15	21	16	14	12	11	13	152	10	15.2	
8														
TOTAL	36	35	34	33	39	36	31	25	26	24	319	10	31.9	

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	0	10	0.00
2	0	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	0	10	0.00
4	7	8	5	8	4	9	4	5	6	4	60	10	6.00	
5	10	0	6	12	0	12	12	0	13	1	66	10	6.60	
6	0	14	0	0	13	0	0	X	0	10	37	9	4.11	
7	18	15	15	19	16	14	14	X	12	18	141	9	15.7	
8														
TOTAL	35	37	26	39	33	35	30	5	31	33	304	10	30.4	

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	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	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	1.00000	1.39310
5	75 %	2	1.00000	1.39310
5	75 %	3	1.00000	1.39310
5	75 %	4	1.00000	1.39310
5	75 %	5	1.00000	1.39310
6	100 %	1	1.00000	1.39310
6	100 %	2	1.00000	1.39310
6	100 %	3	1.00000	1.39310
6	100 %	4	0.87500	1.20940
6	100 %	5	0.87500	1.20940

Appendix A2: Statistics

*Pimephales promelas* (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0.04049 W = 0.5593 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 %	27.50	16.00	5.00	
4	56 %	27.50	16.00	5.00	
5	75 %	27.50	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
<p>D = 0.03065 W = 0.9535 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 = 3.585 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	0.01837	0.003674	2.877	
Within (Error)	24	0.03065	0.001277		
Total	29	0.04902			
Critical F = 3.9 (alpha = 0.01, df = 5,24)					
2.62 (alpha = 0.05, df = 5,24)					
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	0.4926	0.4926			
2	32 %	0.5078	0.5078	-0.6725		
3	42 %	0.4918	0.4918	0.0354		
4	56 %	0.4576	0.4576	1.549		
5	75 %	0.4588	0.4588	1.496		
6	100 %	0.437	0.437	2.46	*	
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	0.05334	10.8	-0.0152		
3	42 %	5	0.05334	10.8	0.0008		
4	56 %	5	0.05334	10.8	0.035		
5	75 %	5	0.05334	10.8	0.0338		
6	100 %	5	0.05334	10.8	0.0556		



Appendix A2: Statistics

*Ceriodaphnia dubia* Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
32 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
42 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
56 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
75 %	10	0	10
Total	20	0	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

*Ceriodaphnia dubia* Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
100 %	9	1	10
Total	19	1	20

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Summary of Fisher's Exact Test				
Group	Identification	Exposed	Dead	Sig 0.05
0	Control	10	0	
1	32 %	10	0	
2	42 %	10	0	
3	56 %	10	0	
4	75 %	10	0	
5	100 %	10	1	

Appendix A2: Statistics

*Ceriodaphnia dubia* Reproduction

Kolmogorov Test for Normality	No Transformation
<p>D = 0.0833 D* = 0.6535 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 = 10.80 Critical B = 15.086 (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	661.7	132.3	3.956	
Within (Error)	54	1806	33.44		
Total	59	2468			
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	21.8	21.8			
2	32 %	29.2	29.2	-2.861		
3	42 %	29	29	-2.784		
4	56 %	31	31	-3.557		
5	75 %	31.9	31.9	-3.905		
6	100 %	30.4	30.4	-3.325		
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	5.974	27.4	-7.4	
3	42 %	10	5.974	27.4	-7.2	
4	56 %	10	5.974	27.4	-9.2	
5	75 %	10	5.974	27.4	-10.1	
6	100 %	10	5.974	27.4	-8.6	

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	798.4	159.7	7.764	
Within (Error)	53	1090	20.57		
Total	58	1888			
Critical F = 3.39 (alpha = 0.01, df = 5,53)					
2.39 (alpha = 0.05, df = 5,53)					
Since F > Critical F REJECT Ho: All equal (alpha = 0.05)					

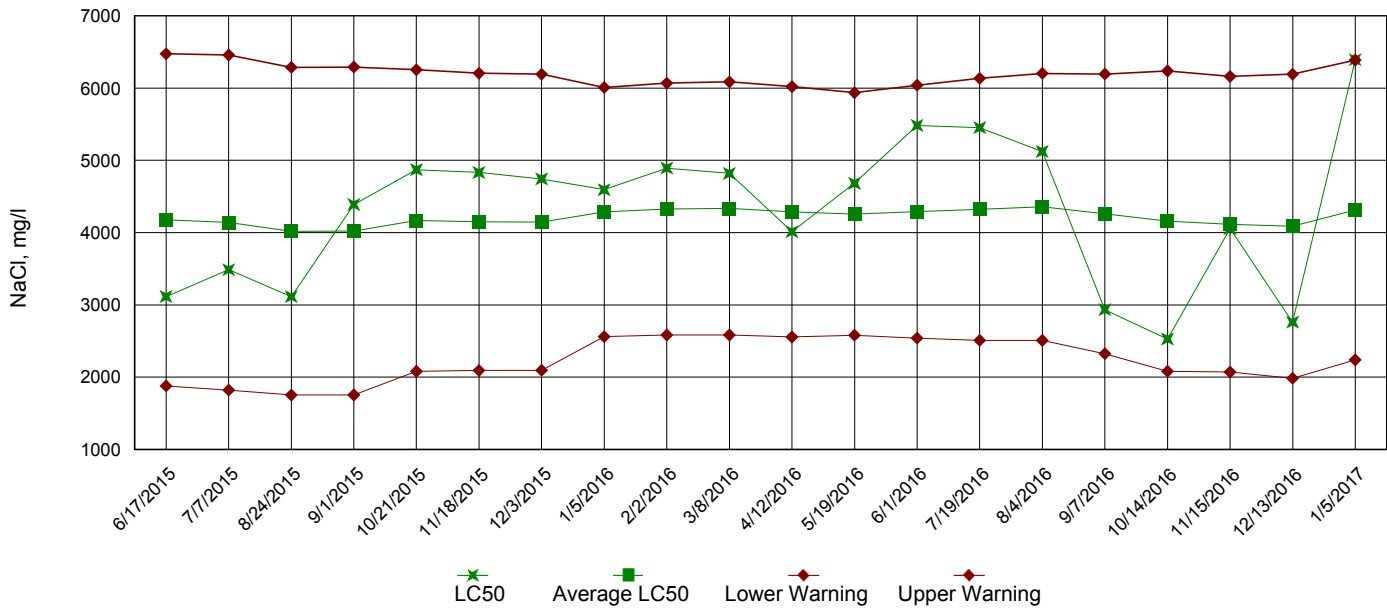
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	21.8	21.8			
2	32 %	29.2	29.2	-3.648		
3	42 %	29	29	-3.55		
4	56 %	31	31	-4.536		
5	75 %	31.9	31.9	-4.98		
6	100 %	33.222	33.222	-5.481		
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	4.685	21.5	-7.4	
3	42 %	10	4.685	21.5	-7.2	
4	56 %	10	4.685	21.5	-9.2	
5	75 %	10	4.685	21.5	-10.1	
6	100 %	9	4.814	22.1	-11.42	

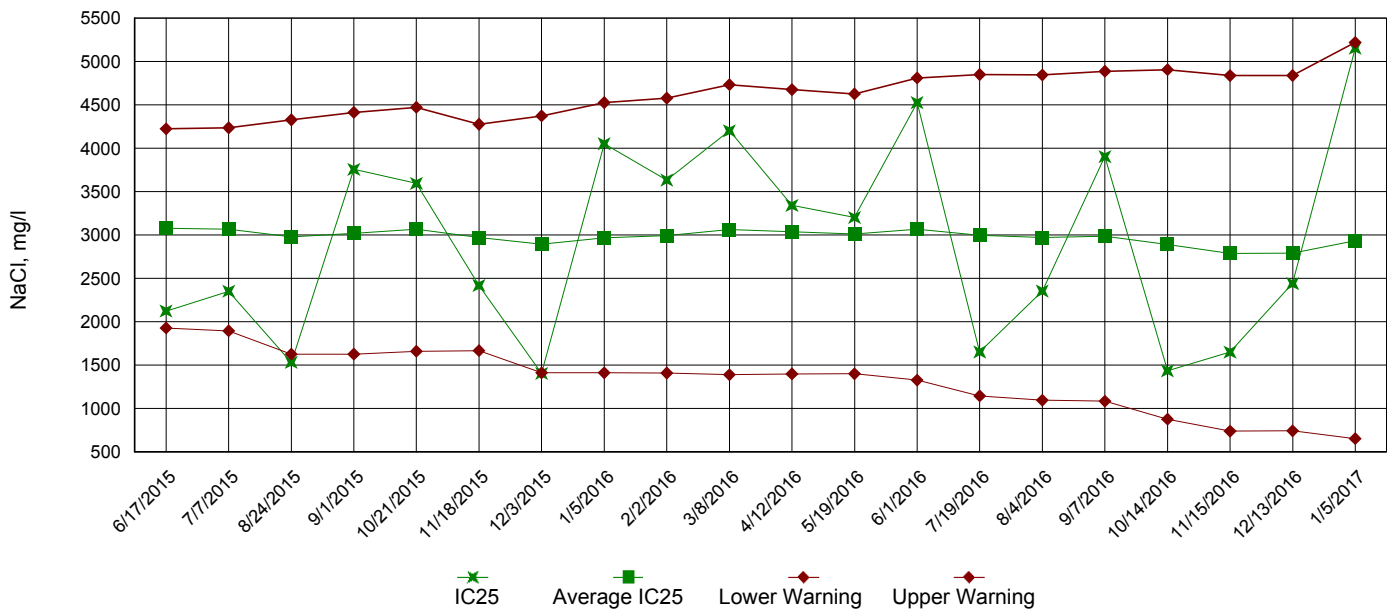
Appendix A3: Test 1000.0

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

LC50 Survival Data

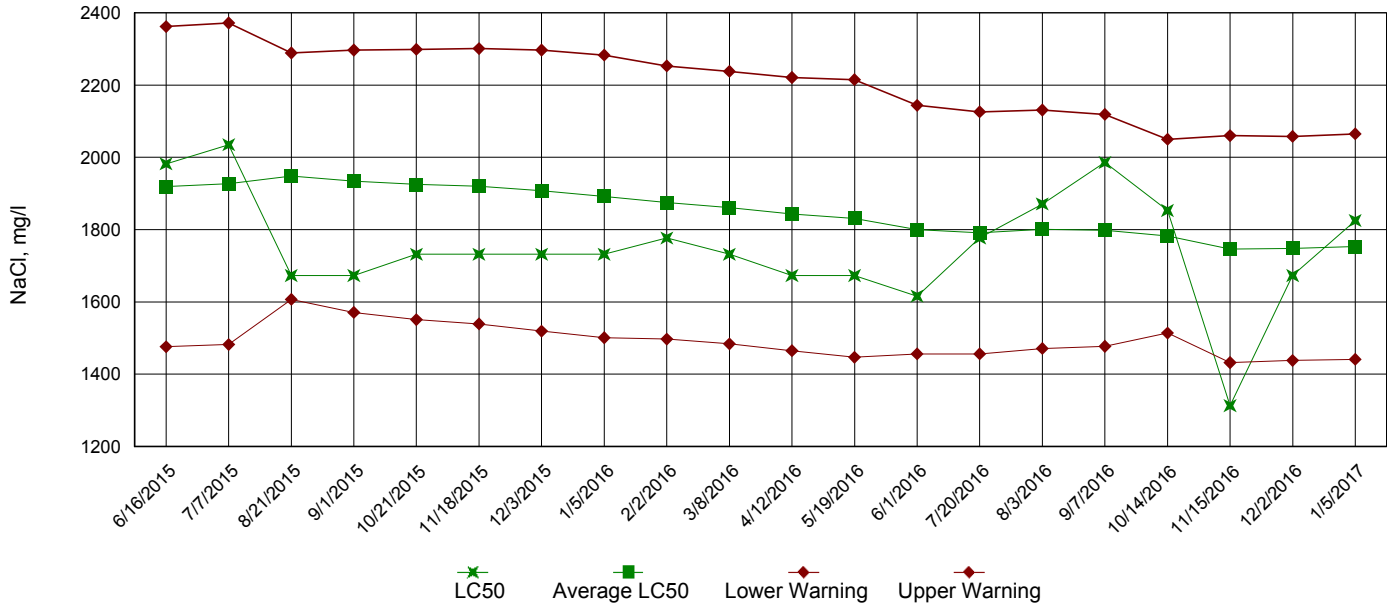


IC25 Growth Data

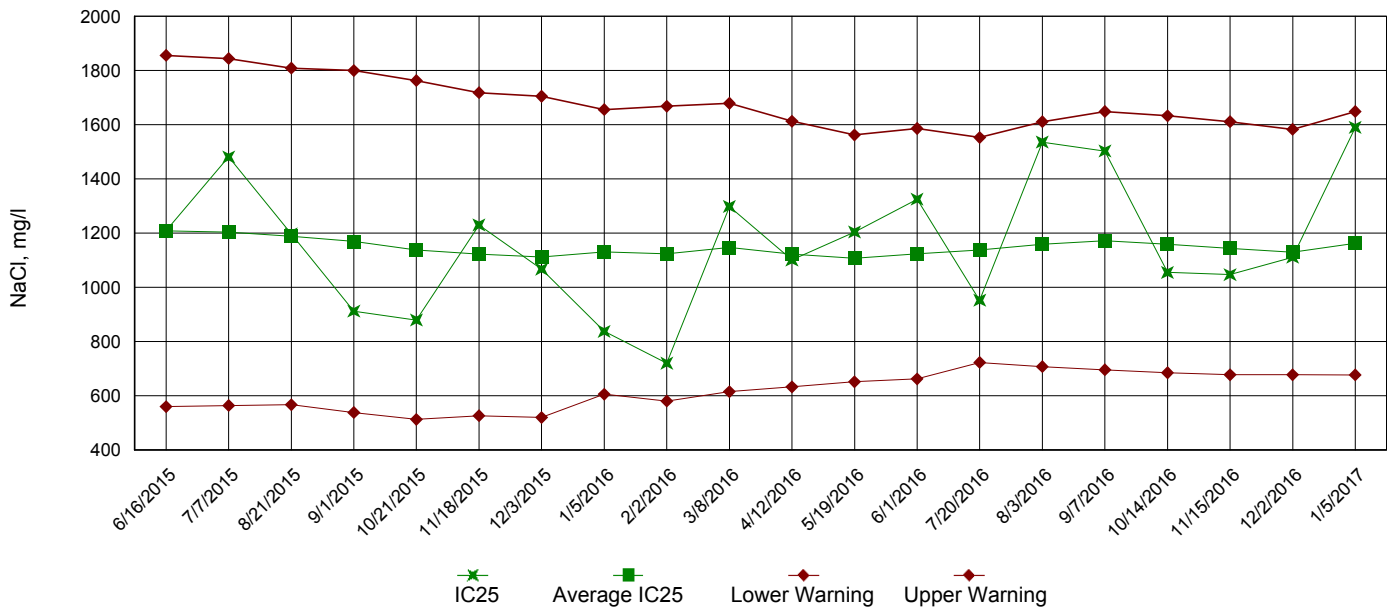


Appendix A3: Test 1002.0  
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: McClelland Consulting Engineers, Inc.

NPDES No.: AR0021661 AFIN:43-00059

Date and Time Test Initiated: January 24, 2017 at 1650

Date and Time Test Terminated: January 31, 2017 at 1055

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	100	100	100	100	0.00
56 %	100	100	100	100	100	100	100	100	0.00
75 %	100	100	100	100	100	100	100	100	0.00
100 %	100	100	100	87.5	87.5	100	100	95.0	7.21

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers					Mean dry weight, mg	CV%
	A	B	C	D	E		
Control	0.406	0.509	0.540	0.534	0.474	0.493	11.2
32 %	0.491	0.528	0.511	0.469	0.540	0.508	5.61
42 %	0.472	0.444	0.522	0.536	0.485	0.492	7.60
56 %	0.438	0.425	0.449	0.472	0.504	0.458	6.81
75 %	0.432	0.430	0.449	0.495	0.488	0.459	6.72
100 %	0.466	0.435	0.428	0.406	0.450	0.437	5.19

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]:   0   (TLP6C)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]:   0   (TGP6C)
5. NOEC *Pimephales* Lethality:  100 %  (TOP6C)
6. LOEC *Pimephales* Lethality:  100 %  (TXP6C)
7. NOEC *Pimephales* Sublethality:  100 %  (TPP6C)
8. LOEC *Pimephales* Sublethality:  100 %  (TYP6C)
9. Coefficient of variation for *Pimephales* growth:  11.2  (TQP6C)

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

PERMITTEE: McClelland Consulting Engineers,  
 NPDES NO.: AR0021661 AFIN:43-00059  
 CONTACT: Mr. Matt Bienvenu  
 ANALYST: 280, 310, 322

Test Initiated: DATE: January 24, 2017 TIME: 1650  
 Test Terminated: DATE: January 31, 2017 TIME: 1055

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.9	8.5	8.3	8.0	8.7	8.4	8.6
Final	8.0	8.3	8.3	8.8	7.5	7.8	7.5
pH Initial	7.7	7.7	7.2	7.9	7.9	7.8	7.2
Final	7.6	7.4	7.8	7.6	7.4	7.1	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	8.1	8.4	8.5	8.4	8.9	8.8	8.6
Final	8.0	8.5	8.5	8.5	7.4	7.7	7.4
pH Initial	7.3	7.5	7.1	7.7	8.0	7.7	7.0
Final	7.6	7.4	7.8	7.5	7.4	6.9	7.0

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	8.3	8.5	8.5	8.6	8.7	8.7	8.5
Final	7.9	8.6	8.6	8.5	7.3	7.6	7.4
pH Initial	7.2	7.5	7.1	7.6	7.8	7.6	7.0
Final	7.6	7.4	7.8	7.5	7.4	6.9	7.0

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	8.3	8.5	8.5	8.7	8.7	8.7	8.4
Final	7.9	8.6	8.6	8.7	7.3	7.6	7.6
pH Initial	7.1	7.4	7.1	7.6	7.6	7.5	6.8
Final	7.5	7.4	7.8	7.6	7.5	7.0	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
75 %							
D.O. Initial	7.5	8.4	8.5	8.8	8.6	8.7	8.4
Final	7.9	8.8	8.6	8.8	7.4	7.7	7.6
pH Initial	7.0	7.3	7.0	7.4	7.6	7.4	6.8
Final	7.5	7.4	7.8	7.6	7.4	6.9	7.1

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	8.8	8.3	8.5	8.8	8.7	8.7	8.5
Final	7.8	8.9	8.6	8.6	7.4	7.6	7.5
pH Initial	6.7	7.2	6.8	7.2	7.6	7.3	6.7
Final	7.4	7.4	7.8	7.5	7.5	6.9	7.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
48	53	280	<0.05	Cabot 24-JAN-17
50	66	400	<0.05	Cabot 26-JAN-17
54	67	370	<0.05	Cabot 26-JAN-17

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
57	86	300	<0.05	209187

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

Permittee: McClelland Consulting Engineers, Inc.

NPDES No.: AR0021661 AFIN:43-00059

Date and Time Test Initiated: January 24, 2017 at 1640

Date and Time Test Terminated: January 31, 2017 at 1500

Dilution water used: Moderately Hard

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
7 day	100	100	100	100	100	90.0

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

Replicates	Control	Percent Effluent				
		32 %	42 %	56 %	75 %	100 %
A	29	27	31	29	36	35
B	26	36	30	37	35	37
C	22	31	33	33	34	26
D	22	30	37	27	33	39
E	26	33	31	39	39	33
F	18	28	21	28	36	35
G	24	23	31	31	31	30
H	17	22	24	30	25	5
I	20	30	24	28	26	31
J	14	32	28	28	24	33
Mean per Adult	21.8	29.2	29.0	31.0	31.9	30.4
Mean per Surviving Adult	21.8	29.2	29.0	31.0	31.9	33.2
CV %	21.3	14.9	16.6	13.3	16.4	11.7

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

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

1. Fisher's Exact Test:

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

a.) LOW FLOW OR CRITICAL DILUTION	(100 %)	<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>      </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]:   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:  21.3  (TQP3B)

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

PERMITTEE: McClelland Consulting Engineers,  
NPDES NO.: AR0021661 AFIN:43-00059  
CONTACT: Mr. Matt Bienvenu  
ANALYST: 280, 310, 322

Test Initiated: DATE: January 24, 2017 TIME: 1640  
Test Terminated: DATE: January 31, 2017 TIME: 1500

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	7.9	8.5	8.3	8.0	8.7	8.4	8.6
Final	8.3	8.9	8.7	8.9	8.6	7.4	7.7
pH Initial	7.7	7.7	7.2	7.9	7.9	7.8	7.2
Final	7.8	7.7	7.8	8.0	8.0	7.4	7.3

DILUTION	DAY						
	1	2	3	4	5	6	7
32 %							
D.O. Initial	8.1	8.4	8.5	8.4	8.9	8.8	8.6
Final	8.4	9.0	8.8	8.6	8.6	7.4	8.5
pH Initial	7.3	7.5	7.1	7.7	8.0	7.7	7.0
Final	7.8	7.7	7.8	8.0	8.0	7.3	7.2

DILUTION	DAY						
	1	2	3	4	5	6	7
42 %							
D.O. Initial	8.3	8.5	8.5	8.6	8.7	8.7	8.5
Final	8.5	9.1	8.6	8.5	8.6	7.4	8.0
pH Initial	7.2	7.5	7.1	7.6	7.8	7.6	7.0
Final	7.8	7.8	7.8	8.0	8.0	7.3	7.3

DILUTION	DAY						
	1	2	3	4	5	6	7
56 %							
D.O. Initial	8.3	8.5	8.5	8.7	8.7	8.7	8.4
Final	8.5	9.2	8.6	8.5	8.7	7.3	7.7
pH Initial	7.1	7.4	7.1	7.6	7.6	7.5	6.8
Final	7.7	7.8	7.8	8.0	8.0	7.3	7.3

DILUTION	DAY						
	1	2	3	4	5	6	7
75 %							
D.O. Initial	7.5	8.4	8.5	8.8	8.6	8.7	8.4
Final	8.4	9.0	8.7	8.5	8.7	7.5	8.6
pH Initial	7.0	7.3	7.0	7.4	7.6	7.4	6.8
Final	7.7	7.8	7.7	8.0	7.9	7.3	7.3

DILUTION	DAY						
	1	2	3	4	5	6	7
100 %							
D.O. Initial	8.8	8.3	8.5	8.8	8.7	8.7	8.5
Final	8.5	9.0	8.9	8.5	8.7	7.7	8.0
pH Initial	6.7	7.2	6.8	7.2	7.6	7.3	6.7
Final	7.7	7.8	7.7	7.8	7.9	7.4	7.3

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
48	53	280	<0.05	Cabot 24-JAN-17
50	66	400	<0.05	Cabot 26-JAN-17
54	67	370	<0.05	Cabot 26-JAN-17

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
57	86	300	<0.05	209187



LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: MCE	Project Cabot Interworks	Reference Tim J.	Manager Eric	Sample Identification Cabot	Date/Time Collected 1/23/17 0800
AIC Control No: 209426	AIC Proposal No:	Carrier:	Received Temperature C D-1	Remarks	
PO No.	MATRIX WATER	NO OF BOTTLES 3	ANALYSES REQUESTED	V	G R A B C O M P
Date/Time Relinquished By: EM	Date/Time Relinquished By: [Signature]	Date/Time Received By: [Signature]	Date/Time Received By: [Signature]	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:
Field pH calibration on _____ @ _____ Buffer:	Relinquished	Received	Date/Time	A = (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , NH <sub>4</sub> OH	Date/Time
Container Type Preservative	P	V	1/24/17		1/24/17
Comments:					

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: MCE  
 Project Reference: Cabot waste water  
 Project Manager: TIM J.  
 Sampled By: JAMES J.  
 AIC No. 28.0011014  
 Date/Time Collected 1/25/17 0800  
 Sample Identification 28.0011014  
 Container Type PE  
 Preservative None

PO No.	NO OF BOTTLES	ANALYSES REQUESTED	MATRIX	G R A B	C O M P	V O A	N	H	B	Date/Time Relinquished	Date/Time Received	By: <u>James J. Brown</u>	Date/Time Relinquished	Date/Time Received	By: <u>James J. Brown</u>
	3														

Field pH calibration on \_\_\_\_\_ @ \_\_\_\_\_ Buffer: \_\_\_\_\_  
 T = Sodium Thiosulfate  
 Z = Zinc acetate  
 A = (NH<sub>4</sub>)<sub>2</sub>SO<sub>4</sub>, NH<sub>4</sub>OH

Relinquished By: James J. Brown Date/Time: 1-26-17 1412  
 Received By: James J. Brown Date/Time: 1-26-17 0800

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: \_\_\_\_\_  
 Email Address: \_\_\_\_\_



CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: <u>ACE</u>		NO OF BOTTLES		ANALYSES REQUESTED												AIC CONTROL NO: <u>209426</u>
Project Reference:		PO No.														AIC PROPOSAL NO:
Project Manager: <u>Matt Brennan</u>		MATRIX														Carrier:
Sampled By: <u>Jesse James</u>		WATER														Received Temperature C <u>0.1</u>
AIC No. <u>3 Cabot</u>		GRA B														Remarks
Date/Time Collected <u>1/26-1/27</u>		COM P														
Container Type <u>P</u>		PRES														
G = Glass NO = none		P = Plastic S = Sulfuric acid pH2														Field pH calibration on _____ @ _____ Buffer:
Turnaround Time Requested: (Please circle) NORMAL or EXPEDITED IN _____ DAYS		V = VOA vials N = Nitric acid pH2														T = Sodium Thiosulfate Z = Zinc acetate A = (NH <sub>4</sub> ) <sub>2</sub> SO <sub>4</sub> , NH <sub>4</sub> OH
Expedited results requested by: _____		Relinquished		Date/Time		Received		Date/Time		By: <u>[Signature]</u>		Date/Time				
Who should AIC contact with questions: Phone: _____ Fax: _____		Relinquished		1-27-16/0350		1-27-17		1-27-17		By: <u>[Signature]</u>		1-27-17				
Report Attention to: _____		Comments:		1-27-17 1420		By: <u>[Signature]</u>		By: <u>[Signature]</u>		By: <u>[Signature]</u>		By: <u>[Signature]</u>				
Report Address to: _____																
Email Address: _____																