



November 3, 2021

Biomonitoring Testing
for
Noland WR001
Paul R. Noland WWTP

Control No. 259818-1

Prepared for:

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Prepared by:

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ATTN: Ms. Donna McChristian
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Re: 7-day Renewal *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Noland WR001 - Paul R. Noland WWTP
NPDES Permit No. AR0020010 AFIN 72-00781

Dear Ms. Donna McChristian:


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 97 % effluent, which is equal to the critical dilution of 97 %. The NOEC for growth occurred at 97 % effluent, which is equal to the critical dilution of 97 %. **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 97 % effluent, which is equal to the critical dilution of 97 %. The NOEC for reproduction occurred at 97 % effluent, which is equal to the critical dilution of 97 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	97.5	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.514	PASS
Control Growth CV < or = 40%	13.0	PASS
Growth Minimum Significant Difference 12 to 30%	17.0	PASS
Critical Dilution CV < or = 40%	15.2	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	36.0	PASS
Control CV < or = 40% per Surviving Female	9.35	PASS
Reproduction Minimum Significant Difference 13 to 47%	21.9	PASS
Critical Dilution CV < or = 40%	35.8	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0020010 AFIN 72-00781
2. Test Requirements: TWO / YR
Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
 - a. Sampling Point: Noland WR001
 - b. Chemical Data:

Analysis	Sample 1	Sample 2	Sample 3
Dissolved oxygen (mg/l)	6.6	8.6	8.6
pH (standard units)	7.9	7.9	8.0
Alkalinity (mg/l as CaCO ₃)	160	150	150
Hardness (mg/l as CaCO ₃)	140	150	140
Conductivity (umhos/cm)	740	760	570
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	0.16	<0.1	0.29

2. Dilution Water Samples:
Moderately Hard

Analysis	259780-1	259815-1
Dissolved oxygen (mg/l)	6.1	7.6
pH (standard units)	8.0	8.0
Alkalinity (mg/l as CaCO ₃)	62	57
Hardness (mg/l as CaCO ₃)	81	81
Conductivity (umhos/cm)	350	360
Residual Chlorine (mg/l)	<0.05	<0.05

C. Test Methods

1. Test methods used:

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: October 26, 2021 at 1455
Date & Time Test Terminated: November 02, 2021 at 1303
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 26, 2021 at 1143
Date & Time Test Terminated: November 01, 2021 at 1330
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: In-house culture

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 and Bartlett's test. The survival data was then analyzed using Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and analyzed with Steel's Many-One Rank Test to determine the No Observable Effects Concentration (NOEC) for Reproduction. Dunnett's Test was used to calculate the PMSD.

IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on October 05, 2021 at 1504 to October 12, 2021 at 1450

The results were as follows: (Control No. 259170-1.)

Survival LC-50: 3649 mg/l

Growth IC-25: 2291 mg/l

Growth PMSD: 11.7

Ceriodaphnia dubia

A chronic reference test was performed on October 14, 2021 at 1338 to October 21, 2021 at 1255

The results were as follows: (Control No. 259534-2.)

Survival LC-50: 1772 mg/l

Reproduction IC-25: 1278 mg/l

Reproduction PMSD: 10.4

V. Organism History

Pimephales promelas (Fathead minnow)

Date: October 26, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: October 26, 2021

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

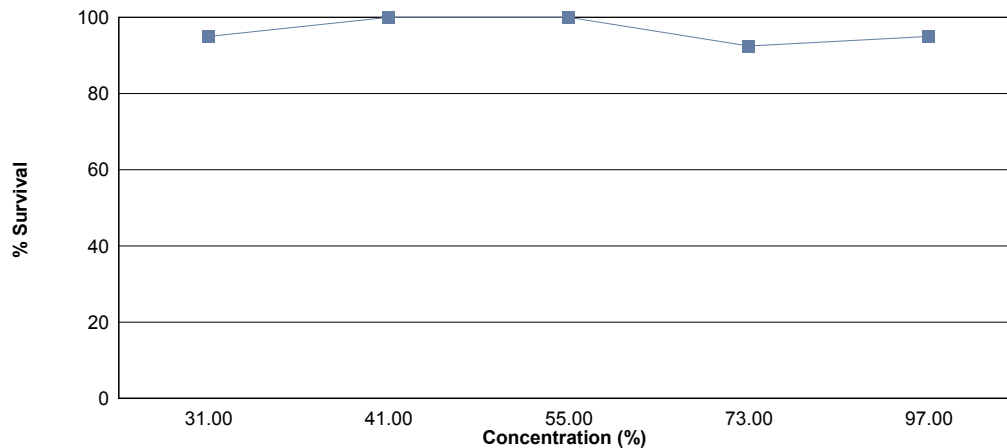
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 97 % effluent.

The test was initiated on October 26, 2021 at 1455 and continued through November 02, 2021 at 1303. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 97 % effluent
- b.) NOEC growth = 97 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	97.5	0.501
31 %	95.0	0.461
41 %	100	0.463
55 %	100	0.435
73 %	92.5	0.436
97 %	95.0	0.455

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

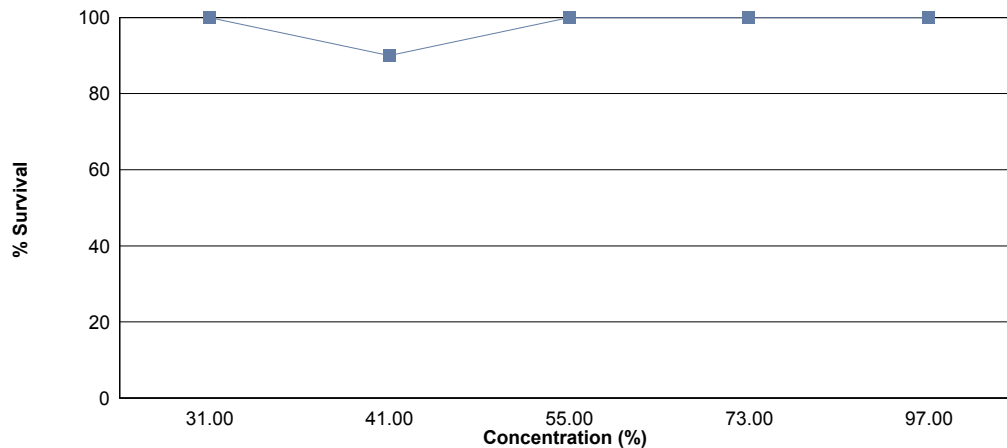
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 97 % effluent.

The test was initiated on October 26, 2021 at 1143 and continued through November 01, 2021 at 1330. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 97 % effluent
- b.) NOEC reproduction = 97 % effluent



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	36.0
31 %	100	36.5
41 %	90.0	32.4
55 %	100	36.6
73 %	100	34.4
97 %	100	30.7

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: October 26, 2021 at 1455

Date and Time Test Terminated: November 02, 2021 at 1303

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	7	7	7	7	7	7
31 %	A	8	8	8	8	7	7	7
	B	7	7	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
41 %	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
55 %	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
73 %	A	8	8	8	8	7	7	7
	B	8	8	8	8	7	7	7
	C	8	8	8	8	7	7	7
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
97 %	A	8	8	8	8	8	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	7	7	7
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: October 26, 2021 at 1455
Test Terminated: November 02, 2021 at 1303

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65338	.65787	0.00449	8	0.561
	B	.65373	.65695	0.00322	8	0.402
	C	.65111	.65529	0.00418	8	0.522
	D	.65181	.65621	0.00440	8	0.550
	E	.65536	.65914	0.00378	8	0.472
31 %	A	.65915	.66313	0.00398	8	0.498
	B	.65194	.65472	0.00278	8	0.348
	C	.65495	.65865	0.00370	8	0.462
	D	.66326	.66709	0.00383	8	0.479
	E	.65392	.65806	0.00414	8	0.518
41 %	A	.67012	.67393	0.00381	8	0.476
	B	.65646	.66024	0.00378	8	0.472
	C	.65151	.65503	0.00352	8	0.440
	D	.66017	.66398	0.00381	8	0.476
	E	.65674	.66033	0.00359	8	0.449
55 %	A	.65524	.65883	0.00359	8	0.449
	B	.66696	.66949	0.00253	8	0.316
	C	.66367	.66747	0.00380	8	0.475
	D	.66404	.66761	0.00357	8	0.446
	E	.65047	.65440	0.00393	8	0.491
73 %	A	.64276	.64595	0.00319	8	0.399
	B	.64826	.65177	0.00351	8	0.439
	C	.65569	.65921	0.00352	8	0.440
	D	.65737	.66072	0.00335	8	0.419
	E	.64983	.65369	0.00386	8	0.482
97 %	A	.66622	.66971	0.00349	8	0.436
	B	.65026	.65378	0.00352	8	0.440
	C	.66392	.66769	0.00377	8	0.471
	D	.65627	.65923	0.00296	8	0.370
	E	.64288	.64736	0.00448	8	0.560

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 26, 2021 at 1143
Date and Time Test Terminated: November 01, 2021 at 1330

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	4	0	4	10	0.400	
4	5	6	4	4	4	6	6	3	0	5	43	10	4.30	
5	13	12	10	10	13	13	13	12	9	11	116	10	11.6	
6	21	19	18	19	21	21	20	20	17	21	197	10	19.7	
7														
8														
TOTAL	39	37	32	33	38	40	39	35	30	37	360	10	36.0	

Concentration: 31 %													
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	3	0	3	10	0.300
4	4	4	5	4	5	4	6	6	0	5	43	10	4.30
5	14	10	12	10	12	13	13	13	11	15	123	10	12.3
6	22	21	18	20	21	19	20	20	21	14	196	10	19.6
7													
8													
TOTAL	40	35	35	34	38	36	39	39	35	34	365	10	36.5

Concentration: 41 %													
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	6	0	6	10	0.600
4	4	5	4	3X	4	4	5	6	0	6	41	9	4.56
5	13	12	17	X	14	13	9	12	12	11	113	9	12.6
6	20	21	19	X	21	19	23	18	23	0	164	9	18.2
7													
8													
TOTAL	37	38	40	3	39	36	37	36	41	17	324	10	32.4

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: October 26, 2021 at 1143
Date and Time Test Terminated: November 01, 2021 at 1330

Concentration: 55 %														
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	4	0	4	10	0.400
4	4	3	6	4	5	6	5	5	0	6	44	10	4.40	
5	14	10	13	10	13	12	13	13	11	13	122	10	12.2	
6	14	19	22	23	20	22	23	20	19	14	196	10	19.6	
7														
8														
TOTAL	32	32	41	37	38	40	41	38	34	33	366	10	36.6	

Concentration: 73 %														
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	6	0	0	0	5	0	11	10	1.10	
4	3	6	6	4	0	5	6	5	0	5	40	10	4.00	
5	12	10	11	9	12	11	14	10	10	11	110	10	11.0	
6	22	19	20	20	23	19	19	20	21	0	183	10	18.3	
7														
8														
TOTAL	37	35	37	33	41	35	39	35	36	16	344	10	34.4	

Concentration: 97 %														
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	4	0	4	0	8	10	0.800	
4	3	5	4	5	5	6	0	6	0	7	41	10	4.10	
5	10	9	9	14	12	10	13	13	10	13	113	10	11.3	
6	0	21	0	17	22	22	20	23	20	0	145	10	14.5	
7														
8														
TOTAL	13	35	13	36	39	38	37	42	34	20	307	10	30.7	

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	0.87500	1.20940
2	31 %	1	0.87500	1.20940
2	31 %	2	0.87500	1.20940
2	31 %	3	1.00000	1.39310
2	31 %	4	1.00000	1.39310
2	31 %	5	1.00000	1.39310
3	41 %	1	1.00000	1.39310
3	41 %	2	1.00000	1.39310
3	41 %	3	1.00000	1.39310
3	41 %	4	1.00000	1.39310
3	41 %	5	1.00000	1.39310
4	55 %	1	1.00000	1.39310
4	55 %	2	1.00000	1.39310
4	55 %	3	1.00000	1.39310
4	55 %	4	1.00000	1.39310
4	55 %	5	1.00000	1.39310
5	73 %	1	0.87500	1.20940
5	73 %	2	0.87500	1.20940
5	73 %	3	0.87500	1.20940
5	73 %	4	1.00000	1.39310
5	73 %	5	1.00000	1.39310
6	97 %	1	0.87500	1.20940
6	97 %	2	1.00000	1.39310
6	97 %	3	1.00000	1.39310
6	97 %	4	0.87500	1.20940
6	97 %	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 = 0.1485 W = 0.9119 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		Transform: Arc Sin(Square Root(Y))
<p>Test can not be performed because at least one group has zero variance. Data FAIL to meet homogeneity of variance assumption.</p>		

Steel's Many-One Rank Test				Transform: Arc Sin(Square Root(Y))
Ho:Control<Treatment				
Group	Identification	Rank Sum	Critical Value	DF
1	Control			
2	31 %	25.00	16.00	5.00
3	41 %	30.00	16.00	5.00
4	55 %	30.00	16.00	5.00
5	73 %	22.50	16.00	5.00
6	97 %	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 = 0.07804 W = 0.9295 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 = 8.411 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	0.01461	0.002922	0.8985	
Within (Error)	24	0.07804	0.003252		
Total	29	0.09265			
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	0.5014	0.5014			
2	31 %	0.461	0.461	1.12		
3	41 %	0.4626	0.4626	1.076		
4	55 %	0.4354	0.4354	1.83		
5	73 %	0.4358	0.4358	1.819		
6	97 %	0.4554	0.4554	1.275		
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	31 %	5	0.08512	17	0.0404	
3	41 %	5	0.08512	17	0.0388	
4	55 %	5	0.08512	17	0.066	
5	73 %	5	0.08512	17	0.0656	
6	97 %	5	0.08512	17	0.046	

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
31 %	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
41 %	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
55 %	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
73 %	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
97 %	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	31 %	10	0	
2	41 %	10	1	
3	55 %	10	0	
4	73 %	10	0	
5	97 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
<p style="text-align: center;"> D = 0.1699 D* = 1.333 Critical D* = 1.035 (alpha = 0.01, N = 60) </p> <p style="text-align: center;">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	31 %	107.50	75.00	10.00	
3	41 %	107.00	75.00	10.00	
4	55 %	111.00	75.00	10.00	
5	73 %	100.00	75.00	10.00	
6	97 %	95.00	75.00	10.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	294.9	58.99	1.011	
Within (Error)	54	3152	58.37		
Total	59	3447			
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)					

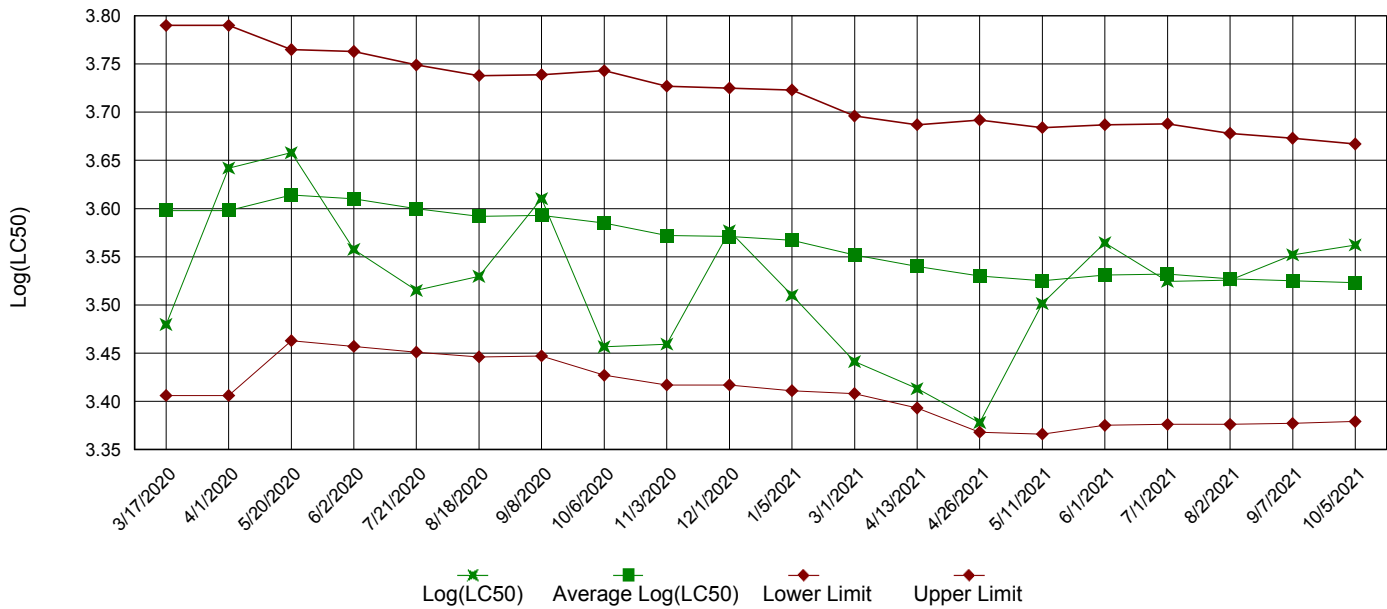
Dunnett's Test - Table 1 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Transformed Mean	Mean In Original Units	T Stat	Sig 0.05	
1	Control	36	36			
2	31 %	36.5	36.5	-0.1463		
3	41 %	32.4	32.4	1.054		
4	55 %	36.6	36.6	-0.1756		
5	73 %	34.4	34.4	0.4683		
6	97 %	30.7	30.7	1.551		
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	31 %	10	7.893	21.9	-0.5	
3	41 %	10	7.893	21.9	3.6	
4	55 %	10	7.893	21.9	-0.6	
5	73 %	10	7.893	21.9	1.6	
6	97 %	10	7.893	21.9	5.3	

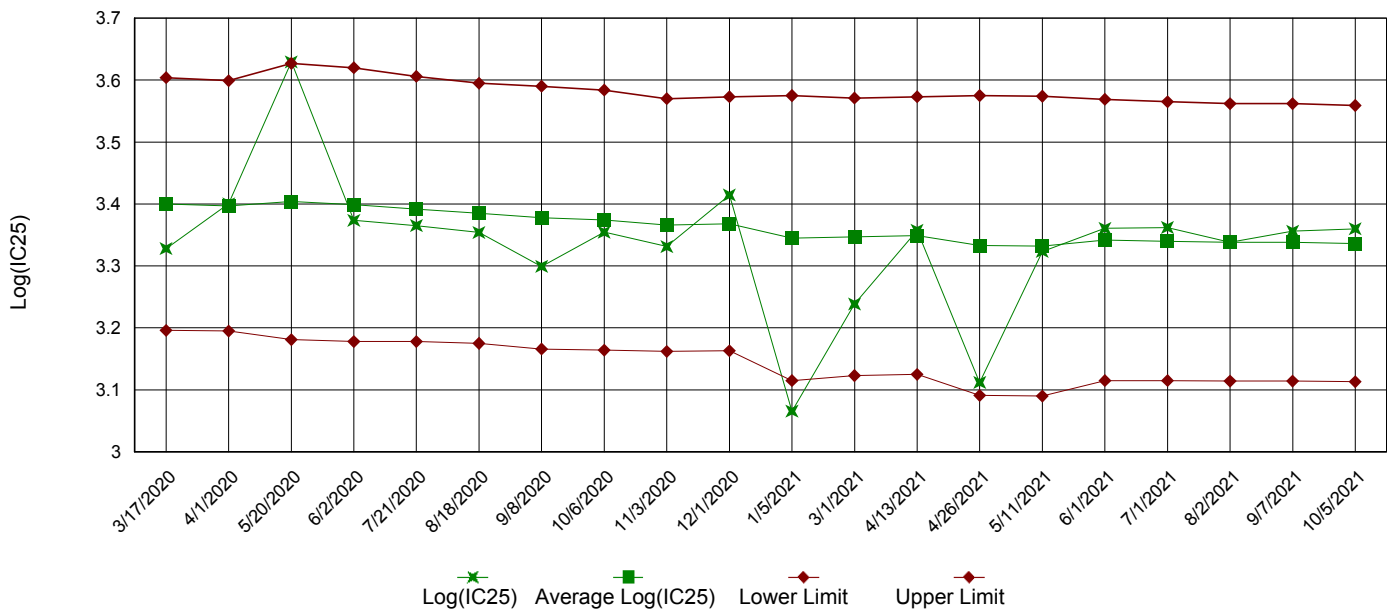
Appendix A3: Test 1000.0

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

LC50 Survival Data

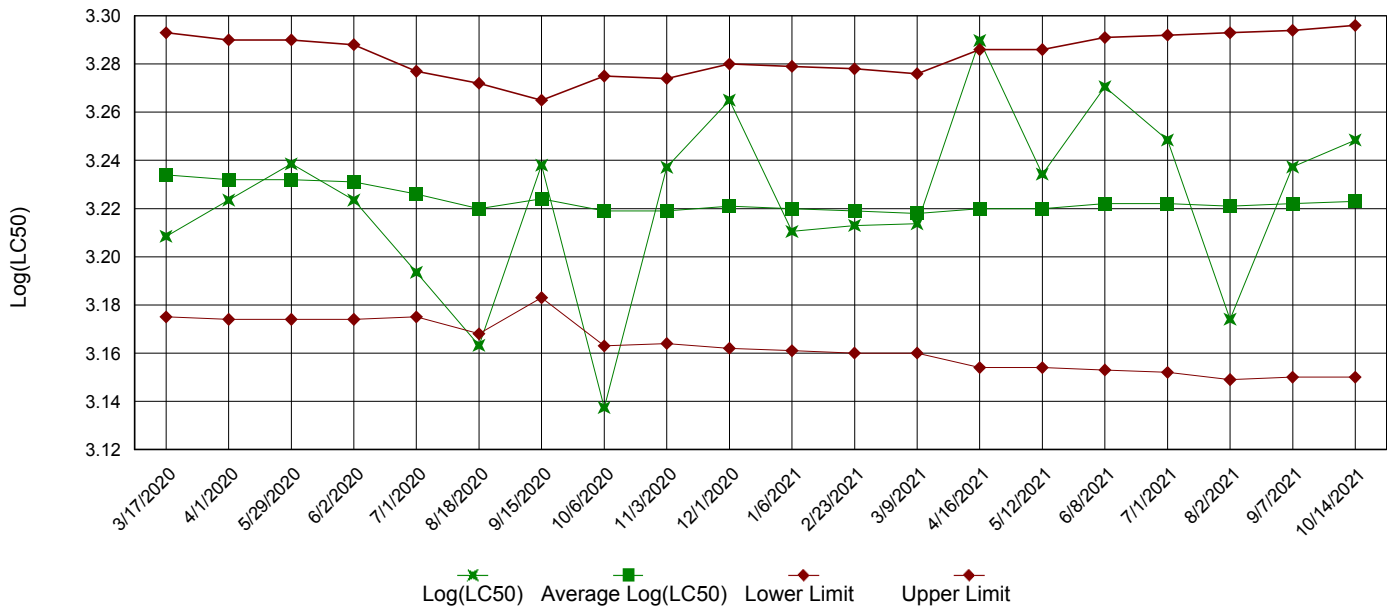


IC25 Growth Data

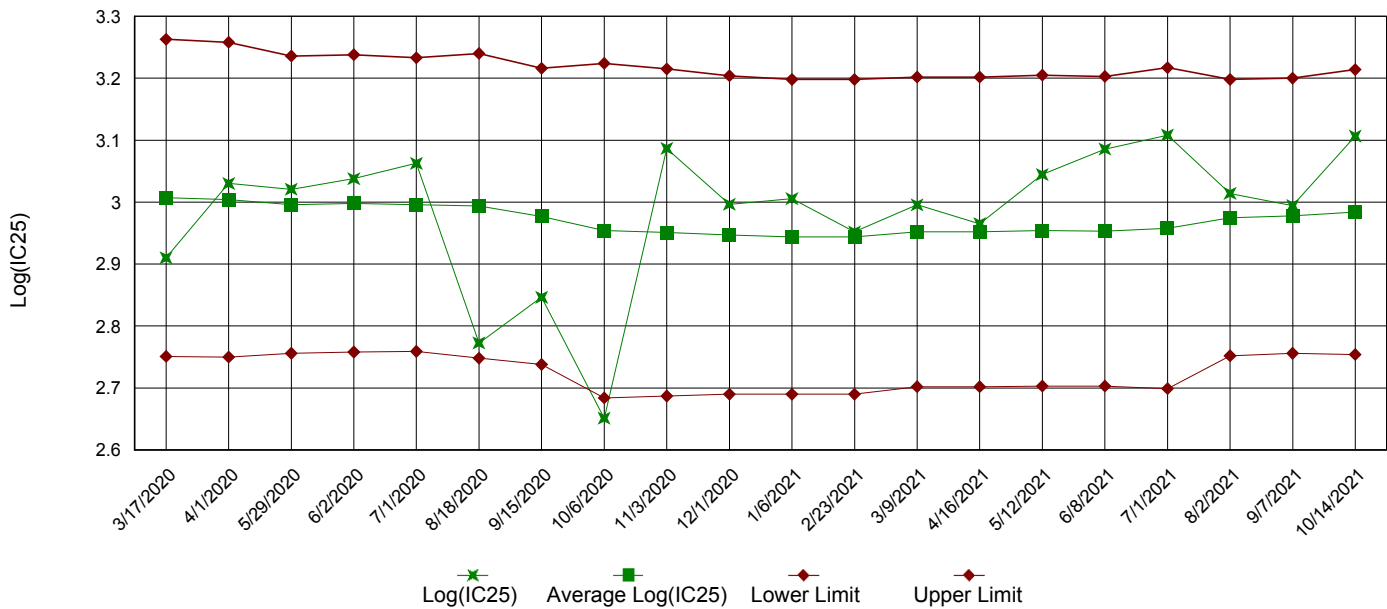


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Jacobs

NPDES No.: AR0020010 AFIN 72-00781

Date and Time Test Initiated: October 26, 2021 at 1455

Date and Time Test Terminated: November 02, 2021 at 1303

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	87.5	100	97.5	97.5	5.73
31 %	87.5	87.5	100	100	100	97.5	97.5	95.0	7.21
41 %	100	100	100	100	100	100	100	100	0.00
55 %	100	100	100	100	100	100	100	100	0.00
73 %	87.5	87.5	87.5	100	100	100	100	92.5	7.40
97 %	87.5	100	100	87.5	100	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	0.561	0.402	0.522	0.550	0.472	0.501	13.0
31 %	0.498	0.348	0.462	0.479	0.518	0.461	14.4
41 %	0.476	0.472	0.440	0.476	0.449	0.463	3.65
55 %	0.449	0.316	0.475	0.446	0.491	0.435	15.9
73 %	0.399	0.439	0.440	0.419	0.482	0.436	7.07
97 %	0.436	0.440	0.471	0.370	0.560	0.455	15.2

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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<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: 97 % (TOP6C)
6. LOEC *Pimephales* Lethality: 97 % (TXP6C)
7. NOEC *Pimephales* Sublethality: 97 % (TPP6C)
8. LOEC *Pimephales* Sublethality: 97 % (TYP6C)
9. Coefficient of variation for *Pimephales* growth: 15.2 (TQP6C)
10. Sublethality for this test: 97 % (51714 or 51714S)

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

PERMITTEE: Jacobs
NPDES NO.: AR0020010 AFIN 72-00781
CONTACT: Ms. Donna McChristian
ANALYST: 280, 343, 357, 358

Test Initiated: DATE: October 26, 2021 TIME: 1455
Test Terminated: DATE: November 02, 2021 TIME: 1303

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.1	5.7	7.6	6.9	7.3	7.3	7.4
Final	5.4	6.0	5.9	6.3	6.4	6.2	6.6
pH Initial	8.0	8.0	8.0	7.9	8.0	8.0	7.9
Final	7.7	7.6	7.6	7.7	7.7	7.5	7.5

DILUTION 31 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	5.6	7.9	7.0	7.8	7.2	7.3
Final	5.2	6.0	6.0	6.0	6.2	6.2	6.6
pH Initial	7.9	8.0	7.9	7.9	8.0	8.0	7.9
Final	7.9	7.7	7.7	7.8	7.8	7.8	7.7

DILUTION 41 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.5	5.6	8.0	7.1	8.0	7.3	7.2
Final	5.1	6.0	5.6	6.0	6.3	6.2	6.6
pH Initial	7.9	8.0	7.9	7.8	8.0	8.0	7.9
Final	7.9	7.8	7.7	7.8	7.9	7.8	7.7

DILUTION 55 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.6	5.6	8.2	7.1	8.2	7.4	7.4
Final	5.0	6.0	6.1	6.3	6.0	6.3	6.7
pH Initial	7.9	8.0	7.9	7.8	7.9	7.9	7.8
Final	8.0	7.9	7.8	7.9	7.9	7.9	7.8

DILUTION 73 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	6.7	5.4	8.6	7.2	8.5	7.3	7.3
Final	5.1	6.1	5.8	6.2	6.0	6.3	6.6
pH Initial	7.9	8.0	7.9	7.8	7.9	7.9	7.8
Final	8.1	7.9	7.9	8.0	8.0	8.0	7.9

DILUTION 97 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.0	5.3	8.6	7.0	8.8	7.0	7.2
Final	4.9	6.1	5.6	6.0	6.0	6.0	6.6
pH Initial	7.9	8.0	7.8	7.8	7.9	7.9	7.9
Final	8.1	8.0	8.0	8.0	8.1	8.0	8.0

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
160	140	740	<0.05	Noland WR001 25-OCT-21
150	150	760	<0.05	Noland WR001 27-OCT-21
150	140	570	<0.05	Noland WR001 29-OCT-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	81	350	<0.05	259780-1
57	81	360	<0.05	259815-1

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

Permittee: Jacobs

NPDES No.: AR0020010 AFIN 72-00781

Date and Time Test Initiated: October 26, 2021 at 1143

Date and Time Test Terminated: November 01, 2021 at 1330

Dilution water used: Moderately Hard

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		31 %	41 %	55 %	73 %	97 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
6 day	100	100	90.0	100	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		31 %	41 %	55 %	73 %	97 %
A	39	40	37	32	37	13
B	37	35	38	32	35	35
C	32	35	40	41	37	13
D	33	34	3	37	33	36
E	38	38	39	38	41	39
F	40	36	36	40	35	38
G	39	39	37	41	39	37
H	35	39	36	38	35	42
I	30	35	41	34	36	34
J	37	34	17	33	16	20
Mean per Adult	36.0	36.5	32.4	36.6	34.4	30.7
Mean per Surviving Adult	36.0	36.5	35.7	36.6	34.4	30.7
CV %	9.35	6.23	20.2	9.83	19.9	35.8

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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<u> </u> YES	<u> </u> 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	<u> </u> YES	<u> X </u> NO
b.) 1/2 LOW FLOW DILUTION	<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: 97 % (TOP3B)
6. LOEC *Ceriodaphnia* Lethality: 97 % (TXP3B)
7. NOEC *Ceriodaphnia* Sublethality: 97 % (TPP3B)
8. LOEC *Ceriodaphnia* Sublethality: 97 % (TYP3B)
9. Coefficient of variation for *Ceriodaphnia* Reproduction: 35.8 (TQP3B)
10. Sublethality for this test: 97 % (51710 or 51710Q)

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

PERMITTEE: Jacobs
NPDES NO.: AR0020010 AFIN 72-00781
CONTACT: Ms. Donna McChristian
ANALYST: 280, 343, 357, 358

Test Initiated: DATE: October 26, 2021 TIME: 1143
Test Terminated: DATE: November 01, 2021 TIME: 1330

DILUTION	DAY						
	1	2	3	4	5	6	7
Control							
D.O. Initial	6.1	5.7	7.6	6.9	7.3	7.3	7.4
Final	5.5	7.5	7.6	7.5	7.8	7.5	--
pH Initial	8.0	8.0	8.0	7.9	8.0	8.0	7.9
Final	8.3	8.3	8.5	8.4	8.5	8.1	--

DILUTION	DAY						
	1	2	3	4	5	6	7
31 %							
D.O. Initial	6.5	5.6	7.9	7.0	7.8	7.2	7.3
Final	5.4	7.5	8.0	7.9	8.1	7.6	--
pH Initial	7.9	8.0	7.9	7.9	8.0	8.0	7.9
Final	8.6	8.6	8.7	8.6	8.7	8.3	--

DILUTION	DAY						
	1	2	3	4	5	6	7
41 %							
D.O. Initial	6.5	5.6	8.0	7.1	8.0	7.3	7.2
Final	5.5	7.7	8.1	7.9	7.9	7.5	--
pH Initial	7.9	8.0	7.9	7.8	8.0	8.0	7.9
Final	8.6	8.6	8.7	8.6	8.7	8.4	--

DILUTION	DAY						
	1	2	3	4	5	6	7
55 %							
D.O. Initial	6.6	5.6	8.2	7.1	8.2	7.4	7.4
Final	5.5	7.5	7.7	7.7	7.7	7.6	--
pH Initial	7.9	8.0	7.9	7.8	7.9	7.9	7.8
Final	8.6	8.6	8.7	8.7	8.7	8.5	--

DILUTION	DAY						
	1	2	3	4	5	6	7
73 %							
D.O. Initial	6.7	5.4	8.6	7.2	8.5	7.3	7.3
Final	5.6	7.6	7.8	7.8	8.0	7.4	--
pH Initial	7.9	8.0	7.9	7.8	7.9	7.9	7.8
Final	8.7	8.7	8.8	8.8	8.8	8.5	--

DILUTION	DAY						
	1	2	3	4	5	6	7
97 %							
D.O. Initial	7.0	5.3	8.6	7.0	8.8	7.0	7.2
Final	5.6	7.6	7.9	7.9	8.1	7.5	--
pH Initial	7.9	8.0	7.8	7.8	7.9	7.9	7.9
Final	8.7	8.8	8.8	8.8	8.8	8.6	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
160	140	740	<0.05	Noland WR001 25-OCT-21
150	150	760	<0.05	Noland WR001 27-OCT-21
150	140	570	<0.05	Noland WR001 29-OCT-21

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
62	81	350	<0.05	259780-1
57	81	360	<0.05	259815-1



8600 Kanis Road
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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

Client: Jacobs		Task code: 659031CH.33N.WW.E1.03		PO # 148021330		NO OF		ANALYSES REQUESTED													
Project Reference: Fayetteville		Sample Matrix		W A T E R		S L U D G E		Chronic C. dubia		Chronic P. promelas											
Lab Manager: Donna McChristian		G R A B		C O M P		X		X		X											
Sampled By: Walter Chodor		Date/Time Collected		1400 - 1200		10/24/21 - 10/25/21															
AIC No. Noland WR001		Container Type Preservative		P = Plastic		S = Sulfuric acid pH 2															
		G = Glass		NO = none		V = VOA vials		H = HCl to pH 2		B = NaOH to pH 12											
		Turnaround Time Requested: (Please circle)		NORM		EXPEDITED		# of days =		Received											
		Expedited results requested by:		Who should AIC contact with questions: Donna McChristi		Phone: (479) 443-3292		Relinquished		Date/Time											
		Report Attention to: Donna McChristian, Matt Benton		Report Address to: 1400 N. Fox Hunter Rd.		Fayetteville, AR 72701		Relinquished		Date/Time											
		Comments: Please analyze and report samples according to units notated in Chain of Custody.						By: <i>Matt</i>		Date/Time: 10-25-21											
								By: <i>Heather Rupp</i>		Date/Time: 10-26-21											



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 2 OF 3

Client: Jacobs		PO # 148021330		NO OF	
Project Reference: Fayetteville		Task code: 659031CH.33M.WWLELO3		B O T T I E S	
Lab		Sample Matrix		C H R O N I C	
Manager: Donna McChristian		W A T E R L S		C O M P O S I T I O N	
Sampled By: <u>Matt Benton</u>		Date/Time Collected: 1400 - 1200		3	
AIC No. Noland WR001		10/26/21 - 10/27/21		X	
Remarks		Chronic C. dubia		X	
Remarks		Chronic P. promelas		X	
Field pH calibration on @		T = Sodium Thiosulfate		Z = Zinc acetate	
Buffer:		H = HCl to pH 2		B = NaOH to pH 12	
Container Type Preservative		V = VOA vials		N = Nitric acid pH 2	
G = Glass		P = Plastic		NO = none	
NO = none		S = Sulfuric acid pH 2		Relinquished Date/Time	
Turnaround Time Requested: (Please circle)		Relinquished By: <u>Matt Benton</u>		Date/Time	
[NORMAL] OR EXPEDITED # of days =		Relinquished Date/Time		Date/Time	
Expeditied results requested by:		By: <u>Matt Benton</u>		10-28-21	
Who should AIC contact with questions: Donna McChristi		Phone: (479) 443-3292		Received By: <u>D. Brown</u>	
Report Attention to: Donna McChristian, Matt Benton		Fayetteville, AR 72701		Date/Time: 0915	
Report Address to: 1400 N. Fox Hunter Rd.		Fayetteville, AR 72701		Comments:	
Fayetteville, AR 72701		Please analyze and report samples according to units notated in Chain of Custody.			

8600 Kanis Road
 Little Rock, AR 72204-2322
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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 3 OF 3

Client: Jacobs		PO # 148021330		NO OF	
Project Reference: Fayetteville		Task code: 659031CHJ33N.WW.EL03		B O T T I E S	
Lab Manager: Donna McChristian		Sample Matrix		S L U D G E S	
Sampled By: Walter Chodor		G R A B		C O M P	
Sample Identification: Noland WR001		Date/Time Collected: 1400 - 1200 10/28/21 - 10/29/21		X	
AIC No. 3		Container Type: Preservative		P	
AIC CONTROL NO: 259818		Carrier: FED EX		NO NO	
AIC PROPOSAL NO:		Received on Ice (4 C)? YES NO 0.1		NO NO	
Remarks:		Field pH calibration on @		P	
Buffer:		T = Sodium Thiosulfate Z = Zinc acetate		NO NO	
H = HCl to pH 2 B = NaOH to pH 12		Relinquished		Date/Time	
V = VOA vials N = Nitric acid pH 2		By: [Signature]		102921/1230	
Turnaround Time Requested: (Please circle) [NORMAL] OR EXPEDITED # of days =		Relinquished		Date/Time	
Expedited results requested by:		By: [Signature]		Received	
Who should AIC contact with questions: Donna McChristi		Phone: (479) 443-3292		By: JK358	
Report Attention to: Donna McChristian, Matt Benton		Report Address to: 1400 N. Fox Hunter Rd. Fayetteville, AR 72701		Date/Time 20 Oct 21 0840	
Comments: Please analyze and report samples according to units notated in Chain of Custody.					