

 **ANALYTICAL REPORT****PREPARED FOR**

Attn: Mr. John Davis
Malvern Water Works
506 Overman
Malvern, Arkansas 72104

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JOB DESCRIPTION

Biomonitoring

JOB NUMBER

192-4583-1

Job Notes

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Authorization



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Malvern Water Works
ATTN: Mr. John Davis
506 Overman
Malvern, AR 72104

Re: Chronic 7-Day Renewal *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
- Outfall 001
NPDES Permit No. AR0034126 AFIN 30-00040
Control No. 274651-1

Dear Mr. John Davis:

This report is the analytical results and supporting information for the samples submitted to Eurofins Arkansas. 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 Laboratory Manager 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. The supporting chemistry data included with this report is intended for accessing the basic water quality of the effluent as required by this test method and is not intended to be utilized for discharge monitoring reports. 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 16 % effluent, which is above the critical dilution of 12 %. The NOEC for growth occurred at 16 % effluent, which is above the critical dilution of 12 %. **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 16 % effluent, which is above the critical dilution of 12 %. The NOEC for reproduction occurred at 16 % effluent, which is above the critical dilution of 12 %. **The sample, therefore, PASSED both lethal and sub-lethal effects for the *Ceriodaphnia dubia* test.**

Table of Contents

I. Control Acceptance Criteria

II. Outlined Report

III. Data Analysis

IV. Standard Reference Toxicants

V. Organism History

VI. Results Summary

Pimephales promelas (Fathead minnow)

Ceriodaphnia dubia

Appendix (Data)

Test 1000.0

Pimephales promelas (Fathead minnow) Survival and Growth

Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Appendix (Statistics)

Appendix (Reference Toxicant)

Appendix (Completed Data Sheets for DEQ)

Pimephales promelas (Fathead minnow) Survival and Growth

Pimephales promelas (Fathead minnow) Chemical Parameters Chart

Ceriodaphnia dubia Survival and Reproduction

Ceriodaphnia dubia Chemical Parameters Chart

Appendix (Summary)

I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	95.0	PASS
Control Growth > or = 0.25 mg per Surviving minnow	0.528	PASS
Control Growth CV < or = 40%	11.4	PASS
Growth Minimum Significant Difference 12 to 30%	12.7	PASS
Critical Dilution CV < or = 40%	5.47	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	31.2	PASS
Control CV < or = 40% per Surviving Female	10.6	PASS
Reproduction Minimum Significant Difference 13 to 47%	17.9	PASS
Critical Dilution CV < or = 40%	8.11	PASS

II. Outlined Report

A. Introduction

1. Permit Number: AR0034126 AFIN 30-00040
2. Test Requirements: qtr
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)	7.6	8.7	7.8
pH (standard units)	6.9	6.9	6.9
Alkalinity (mg/l as CaCO ₃)	14	15	15
Hardness (mg/l as CaCO ₃)	18	14	17
Conductivity (umhos/cm)	130	130	140
Residual Chlorine (mg/l)	0.060	0.050	0.050
Ammonia as N (mg/l)	0.52	0.30	0.40

2. Dilution Water Samples:

Analysis	192-4263-A-1	192-4387-A-1	192-4545-A-1
Dissolved oxygen (mg/l)	8.3	8.1	8.0
pH (standard units)	7.6	7.6	7.5
Alkalinity (mg/l as CaCO ₃)	31	32	32
Hardness (mg/l as CaCO ₃)	46	44	43
Conductivity (umhos/cm)	160	160	170
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05

C. Test Methods

1. Test methods used:

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

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: August 22, 2023 at 1507
Date & Time Test Terminated: August 29, 2023 at 1550
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 22, 2023 at 1408
Date & Time Test Terminated: August 28, 2023 at 1605
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 Eurofins'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 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

Sodium chloride in synthetic moderately hard water.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on August 01, 2023 at 1110 to August 08, 2023 at 1111
The results were as follows: (Control No. 274631-1.)

Survival LC-50: 2244 mg/l

Growth IC-25: 1049 mg/l

Growth PMSD: 0

Ceriodaphnia dubia

A chronic reference test was performed on August 01, 2023 at 1105 to August 07, 2023 at 1300
The results were as follows: (Control No. 274631-2.)

Survival LC-50: 1864 mg/l

Reproduction IC-25: 995.6 mg/l

Reproduction PMSD: 14

V. Organism History

Pimephales promelas (Fathead minnow)

Date: August 22, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: August 22, 2023

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

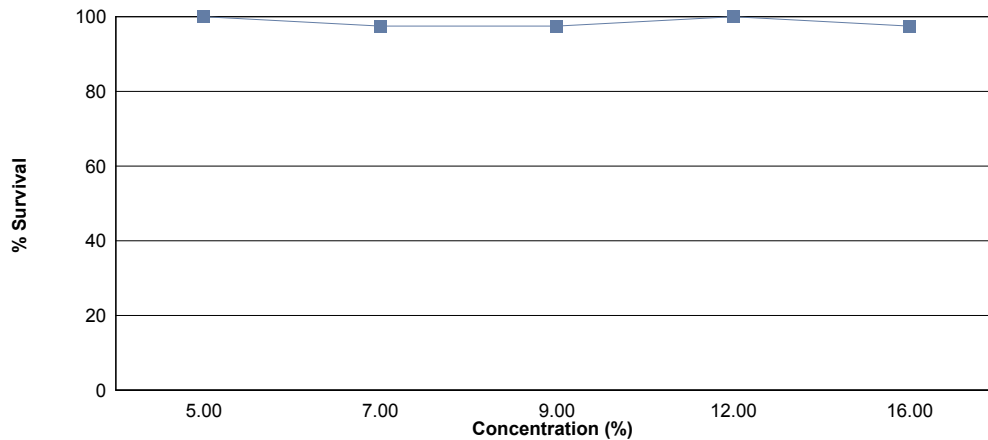
VI. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

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 concentrations for this test were 5 %, 7 %, 9 %, 12 %, 16 % in accordance with the NPDES permit.

The test was initiated on August 22, 2023 at 1507 and continued through August 29, 2023 at 1550. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 16 % effluent
- b.) NOEC growth = 16 % effluent



Summary of the 7-day Fathead Minnow Survival and Growth		
Concentration	Percent Survival	Mean Growth (mg)
Control	95.0	0.502
5 %	100	0.540
7 %	97.5	0.539
9 %	97.5	0.519
12 %	100	0.513
16 %	97.5	0.527

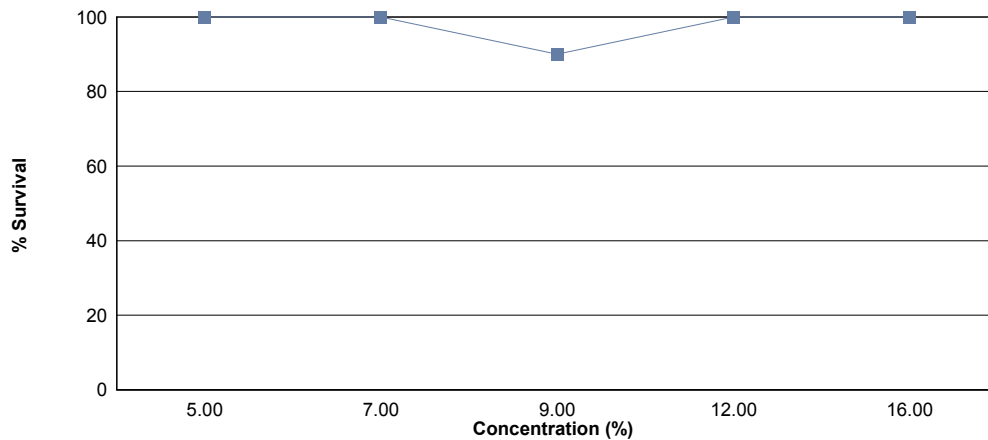
VI. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

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 concentrations for this test were 5 %, 7 %, 9 %, 12 %, 16 % in accordance with the NPDES permit.

The test was initiated on August 22, 2023 at 1408 and continued through August 28, 2023 at 1605. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 16 % effluent
- b.) NOEC reproduction = 16 % effluent



Summary of the 6-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	31.2
5 %	100	33.4
7 %	100	31.8
9 %	90.0	30.8
12 %	100	33.5
16 %	100	34.3

Appendix (Data): Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 22, 2023 at 1507

Date and Time Test Terminated: August 29, 2023 at 1550

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	7
	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	8	8	8	8	8
7 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	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	8	8	8	8	8
	C	8	8	8	8	8	8	7
	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	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
16 %	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 (Data): Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 22, 2023 at 1507

Test Terminated: August 29, 2023 at 1550

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.65573	.66052	0.00479	8	0.599
	B	.64772	.65180	0.00408	8	0.510
	C	.65649	.66016	0.00367	8	0.459
	D	.65904	.66280	0.00376	8	0.470
	E	.65434	.65813	0.00379	8	0.474
5 %	A	.65720	.66117	0.00397	8	0.496
	B	.65757	.66245	0.00488	8	0.610
	C	.66118	.66525	0.00407	8	0.509
	D	.65307	.65738	0.00431	8	0.539
	E	.66030	.66466	0.00436	8	0.545
7 %	A	.65009	.65443	0.00434	8	0.542
	B	.66010	.66497	0.00487	8	0.609
	C	.65794	.66246	0.00452	8	0.565
	D	.66769	.67167	0.00398	8	0.498
	E	.66256	.66640	0.00384	8	0.480
9 %	A	.64839	.65287	0.00448	8	0.560
	B	.66041	.66466	0.00425	8	0.531
	C	.65589	.65960	0.00371	8	0.464
	D	.66013	.66435	0.00422	8	0.528
	E	.65348	.65759	0.00411	8	0.514
12 %	A	.65834	.66281	0.00447	8	0.559
	B	.65454	.65851	0.00397	8	0.496
	C	.65060	.65472	0.00412	8	0.515
	D	.65804	.66212	0.00408	8	0.510
	E	.66563	.66952	0.00389	8	0.486
16 %	A	.66178	.66615	0.00437	8	0.546
	B	.66046	.66462	0.00416	8	0.520
	C	.65865	.66243	0.00378	8	0.472
	D	.65943	.66380	0.00437	8	0.546
	E	.65711	.66152	0.00441	8	0.551

Appendix (Data): Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2023 at 1408
 Date and Time Test Terminated: August 28, 2023 at 1605

Concentration: Control													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	4	4	3	4	3	7	6	4	2	3	40	10	4.00
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	12	9	10	10	9	13	10	11	9	10	103	10	10.3
6	18	19	17	16	15	17	15	19	15	18	169	10	16.9
7													
8													
TOTAL	34	32	30	30	27	37	31	34	26	31	312	10	31.2

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	5	5	3	4	3	4	5	5	4	6	44	10	4.40
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	12	13	10	9	12	13	10	11	10	8	108	10	10.8
6	22	20	17	17	16	19	21	18	15	17	182	10	18.2
7													
8													
TOTAL	39	38	30	30	31	36	36	34	29	31	334	10	33.4

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	4	4	3	5	3	5	4	5	4	4	41	10	4.10
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	11	9	10	11	9	10	11	12	10	9	102	10	10.2
6	22	20	19	4	16	19	20	19	18	18	175	10	17.5
7													
8													
TOTAL	37	33	32	20	28	34	35	36	32	31	318	10	31.8

Appendix (Data): Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 22, 2023 at 1408
 Date and Time Test Terminated: August 28, 2023 at 1605

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	5	3	4	4	5	5	4	4	4	38	10	3.80
4	3X	0	0	0	0	0	0	0	0	0	3	9	0.333
5	X	12	9	10	8	11	10	10	12	10	92	9	10.2
6	X	24	18	19	17	18	21	17	23	18	175	9	19.4
7													
8													
TOTAL	3	41	30	33	29	34	36	31	39	32	308	10	30.8

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	4	4	4	5	4	4	6	5	4	3	43	10	4.30
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	10	11	9	9	10	11	12	10	10	8	100	10	10.0
6	20	21	18	19	16	20	19	21	20	18	192	10	19.2
7													
8													
TOTAL	34	36	31	33	30	35	37	36	34	29	335	10	33.5

Concentration: 16 %													
Day	Replicate										No. of Young	No. of Adults	Young per Adult
	1	2	3	4	5	6	7	8	9	10			
1	0	0	0	0	0	0	0	0	0	0	0	10	0.00
2	0	0	0	0	0	0	0	0	0	0	0	10	0.00
3	5	6	3	4	3	4	4	5	4	4	42	10	4.20
4	0	0	0	0	0	0	0	0	0	0	0	10	0.00
5	10	9	10	9	10	13	11	10	12	11	105	10	10.5
6	24	22	18	18	17	19	21	20	19	18	196	10	19.6
7													
8													
TOTAL	39	37	31	31	30	36	36	35	35	33	343	10	34.3

Appendix (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	0.87500	1.20940
1	Control	5	0.87500	1.20940
2	5 %	1	1.00000	1.39310
2	5 %	2	1.00000	1.39310
2	5 %	3	1.00000	1.39310
2	5 %	4	1.00000	1.39310
2	5 %	5	1.00000	1.39310
3	7 %	1	1.00000	1.39310
3	7 %	2	1.00000	1.39310
3	7 %	3	0.87500	1.20940
3	7 %	4	1.00000	1.39310
3	7 %	5	1.00000	1.39310
4	9 %	1	1.00000	1.39310
4	9 %	2	1.00000	1.39310
4	9 %	3	0.87500	1.20940
4	9 %	4	1.00000	1.39310
4	9 %	5	1.00000	1.39310
5	12 %	1	1.00000	1.39310
5	12 %	2	1.00000	1.39310
5	12 %	3	1.00000	1.39310
5	12 %	4	1.00000	1.39310
5	12 %	5	1.00000	1.39310
6	16 %	1	1.00000	1.39310
6	16 %	2	1.00000	1.39310
6	16 %	3	0.87500	1.20940
6	16 %	4	1.00000	1.39310
6	16 %	5	1.00000	1.39310

Appendix (Statistics)

Pimephales promelas (Fathead minnow) Survival

Shapiro - Wilk's Test for Normality		Transform: Arc Sin(Square Root(Y))
<p>D = 0.1215 W = 0.7519 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	5 %	32.50	16.00	5.00	
3	7 %	30.00	16.00	5.00	
4	9 %	30.00	16.00	5.00	
5	12 %	32.50	16.00	5.00	
6	16 %	30.00	16.00	5.00	
Critical values are 1 tailed (k=5)					

Appendix (Statistics)

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality	No Transformation
D = 0.04417 W = 0.9565 Critical W = 0.9 (alpha = 0.01, N = 30) Critical W = 0.927 (alpha = 0.05, N = 30) Data PASS normality test (alpha = 0.01).	

Bartlett's Test for Homogeneity of Variance	No Transformation
Calculated B1 statistic = 2.733 Critical B = 15.086 (alpha = 0.01, df = 5) Data PASS B1 homogeneity test at 0.01 level.	

Appendix (Statistics)

Pimephales promelas (Fathead minnow) Growth

ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	0.005401	0.00108	0.587	
Within (Error)	24	0.04417	0.00184		
Total	29	0.04957			
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	0.5024	0.5024			
2	5 %	0.5398	0.5398	-1.379		
3	7 %	0.5388	0.5388	-1.342		
4	9 %	0.5194	0.5194	-0.6266		
5	12 %	0.5132	0.5132	-0.3981		
6	16 %	0.527	0.527	-0.9068		
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	5 %	5	0.06403	12.7	-0.0374	
3	7 %	5	0.06403	12.7	-0.0364	
4	9 %	5	0.06403	12.7	-0.017	
5	12 %	5	0.06403	12.7	-0.0108	
6	16 %	5	0.06403	12.7	-0.0246	

Appendix (Statistics)

Ceriodaphnia dubia Survival

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 %	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
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.

Appendix (Statistics)

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
16 %	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	5 %	10	0	
2	7 %	10	0	
3	9 %	10	1	
4	12 %	10	0	
5	16 %	10	0	

Appendix (Statistics)

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality	No Transformation
D = 0.1422 D* = 1.116 Critical D* = 1.035 (alpha = 0.01, N = 60)	
Data FAIL normality test (alpha = 0.01).	

Steel's Many-One Rank Test					No Transformation
Ho:Control<Treatment					
Group	Identification	Rank Sum	Critical Value	DF	Sig 0.05
1	Control				
2	5 %	119.00	75.00	10.00	
3	7 %	117.50	75.00	10.00	
4	9 %	115.50	75.00	10.00	
5	12 %	124.50	75.00	10.00	
6	16 %	130.50	75.00	10.00	

Critical values are 1 tailed (k=5)

Appendix (Statistics)

Ceriodaphnia dubia Reproduction

Dunnett's Test for PMSD Calculation

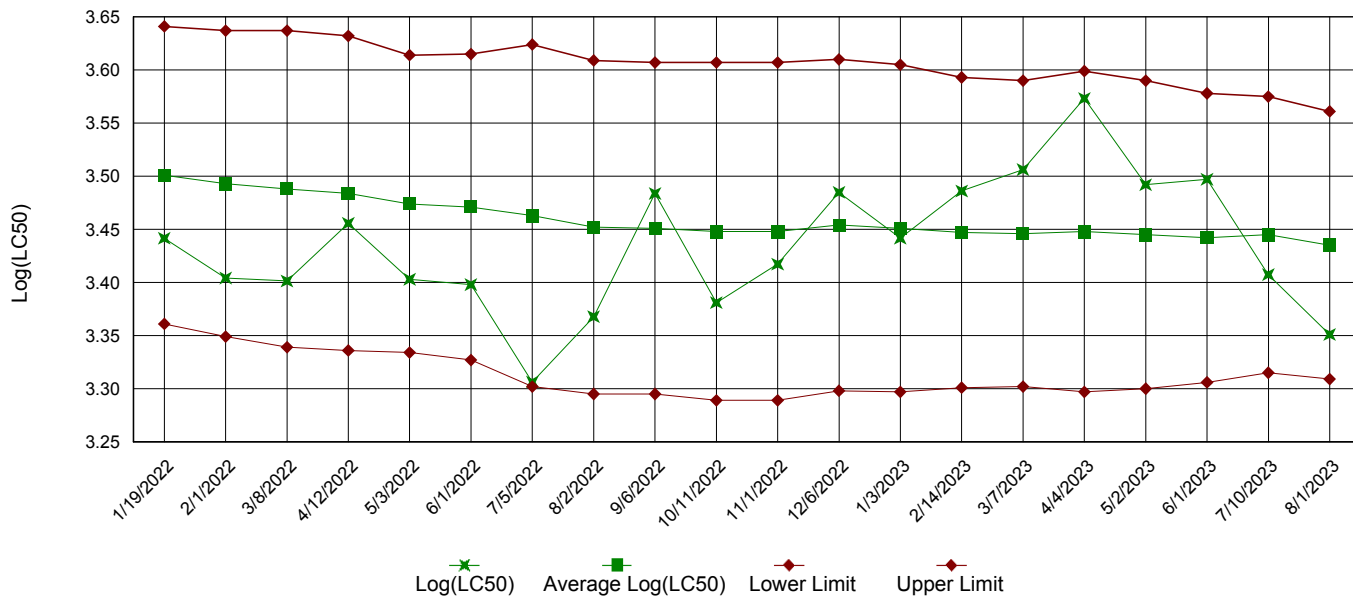
ANOVA Table				No Transformation	
SOURCE	DF	SS	MS	F	
Between	5	101.2	20.24	0.6963	
Within (Error)	54	1570	29.07		
Total	59	1671			
Critical F = 3.38 (alpha = 0.01, df = 5,54)					
2.38 (alpha = 0.05, df = 5,54)					
Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05)					

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	31.2	31.2			
2	5 %	33.4	33.4	-0.9124		
3	7 %	31.8	31.8	-0.2488		
4	9 %	30.8	30.8	0.1659		
5	12 %	33.5	33.5	-0.9539		
6	16 %	34.3	34.3	-1.286		
Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54)						

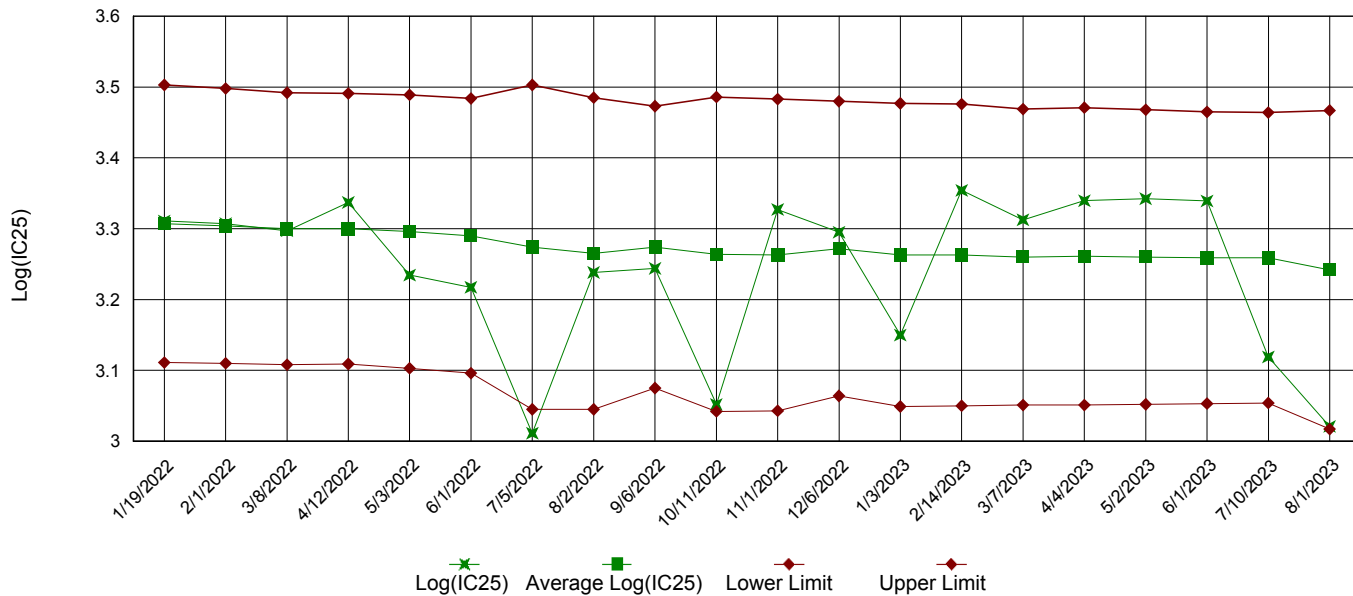
Dunnett's Test - Table 2 of 2					No Transformation	
Ho:Control<Treatment						
Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control	
1	Control	10				
2	5 %	10	5.57	17.9	-2.2	
3	7 %	10	5.57	17.9	-0.6	
4	9 %	10	5.57	17.9	0.4	
5	12 %	10	5.57	17.9	-2.3	
6	16 %	10	5.57	17.9	-3.1	

Appendix (Reference Toxicant): Test 1000.0
Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data

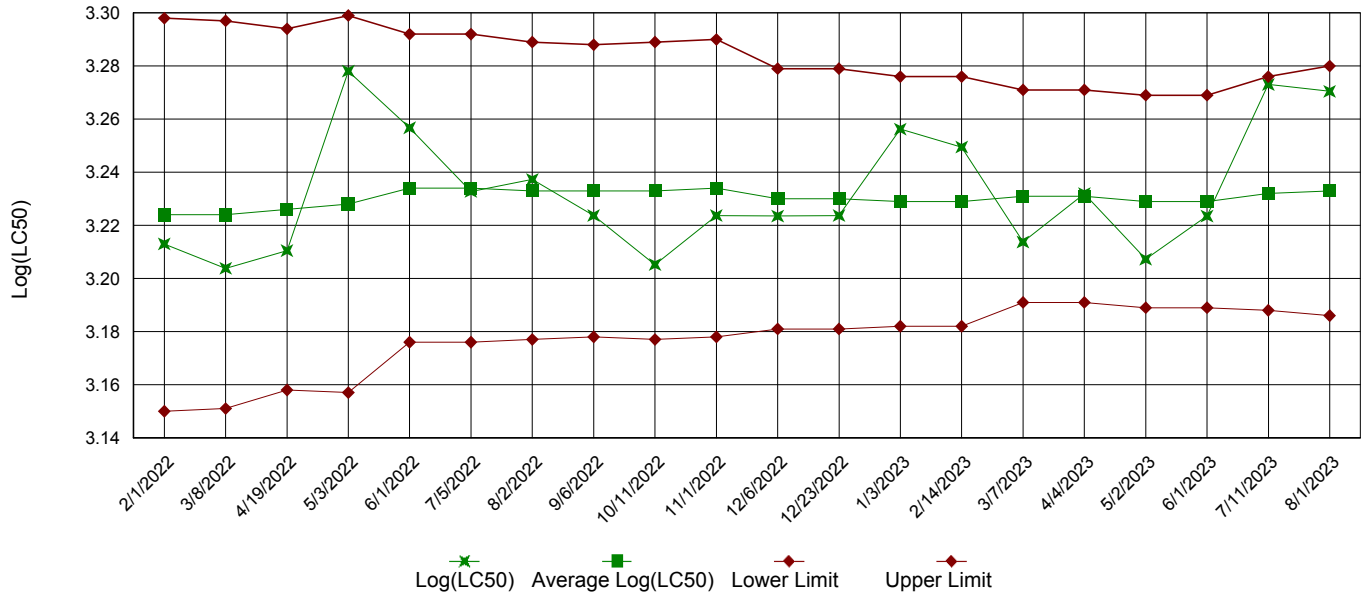


IC25 Growth Data

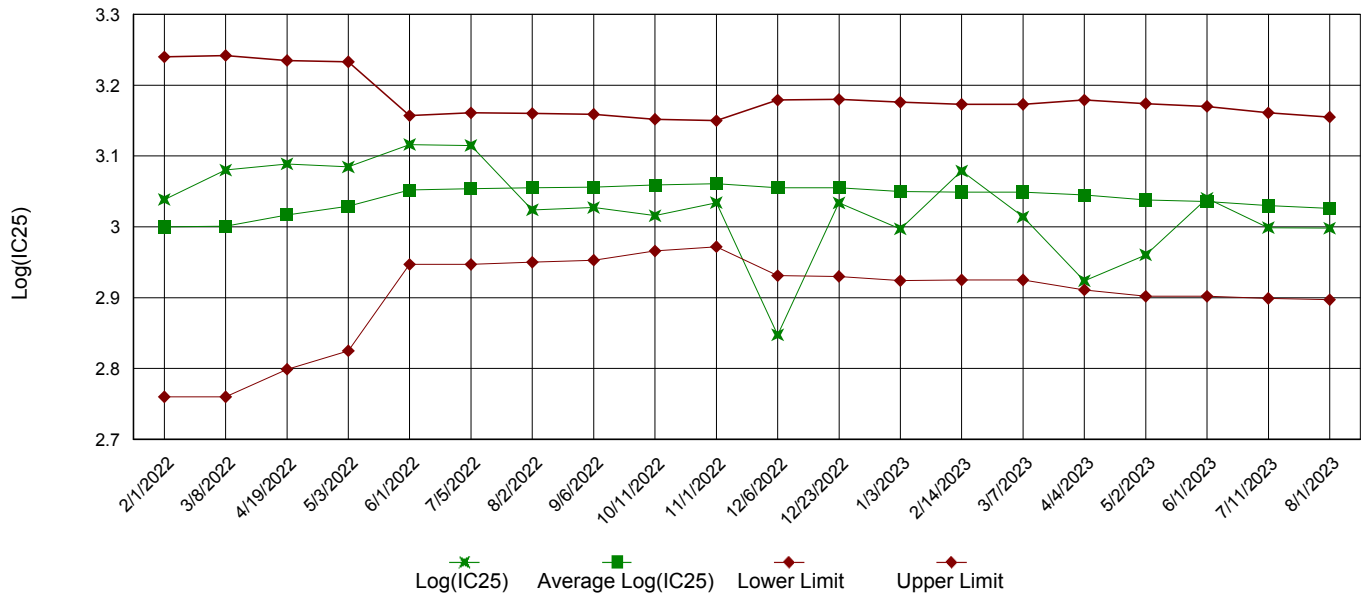


Appendix (Reference Toxicant): Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



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

Permittee: Malvern Water Works

NPDES No.: AR0034126 AFIN 30-00040

Date and Time Test Initiated: August 22, 2023 at 1507

Date and Time Test Terminated: August 29, 2023 at 1550

Dilution water used:

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	87.5	87.5	100	100	95.0	7.21
5 %	100	100	100	100	100	100	100	100	0.00
7 %	100	100	87.5	100	100	100	100	97.5	5.73
9 %	100	100	87.5	100	100	100	100	97.5	5.73
12 %	100	100	100	100	100	100	100	100	0.00
16 %	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	0.599	0.510	0.459	0.470	0.474	0.502	11.4
5 %	0.496	0.610	0.509	0.539	0.545	0.540	8.19
7 %	0.542	0.609	0.565	0.498	0.480	0.539	9.62
9 %	0.560	0.531	0.464	0.528	0.514	0.519	6.78
12 %	0.559	0.496	0.515	0.510	0.486	0.513	5.47
16 %	0.546	0.520	0.472	0.546	0.551	0.527	6.27

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix (Summary): 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: 16 % (TOP6C)
- 6. LOEC Pimephales Lethality: 16 % (TXP6C)
- 7. NOEC Pimephales Sublethality: 16 % (TPP6C)
- 8. LOEC Pimephales Sublethality: 16 % (TYP6C)
- 9. Coefficient of variation for Pimephales growth: 11.4 (TQP6C)
- 10. Sublethality for this test: 16 % (51714 or 51714S)

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

PERMITTEE: Malvern Water Works
 NPDES NO.: AR0034126 AFIN 30-00040
 CONTACT: Mr. John Davis
 ANALYST: 280, 343, 357, 358

Test Initiated: DATE: August 22, 2023 TIME: 1507
 Test Terminated: DATE: August 29, 2023 TIME: 1550

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	8.1	7.9	8.0	7.8	7.7
Final	6.1	5.5	5.5	5.9	5.1	5.0	6.3
pH Initial	7.6	7.5	7.6	7.5	7.5	7.6	7.6
Final	7.2	7.1	7.0	7.2	7.1	7.0	7.2

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.2	8.0	7.9	7.7	7.7
Final	6.0	6.2	5.7	5.8	4.8	5.6	5.9
pH Initial	7.6	7.5	7.5	7.4	7.5	7.6	7.6
Final	7.2	7.1	7.0	7.1	7.0	7.1	7.1

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	8.0	8.0	8.0	7.8	7.8	7.7
Final	5.8	5.6	5.7	5.6	5.1	5.1	5.8
pH Initial	7.6	7.5	7.6	7.5	7.5	7.6	7.5
Final	7.1	7.1	7.0	7.1	7.0	7.0	7.1

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.0	8.3	7.5	8.0	7.6	7.7
Final	5.7	5.2	5.7	5.9	4.9	5.0	5.9
pH Initial	7.6	7.5	7.6	7.5	7.5	7.6	7.5
Final	7.1	7.0	7.1	7.1	7.0	7.1	7.1

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.1	8.1	7.6	8.0	7.8	7.6
Final	5.6	5.6	5.4	5.5	4.8	4.9	6.0
pH Initial	7.6	7.4	7.5	7.4	7.5	7.5	7.5
Final	7.1	7.0	7.1	7.1	7.0	7.0	7.1

DILUTION 16 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	8.1	8.2	7.8	7.9	7.4	7.6
Final	5.6	5.7	5.3	5.9	4.6	5.0	5.9
pH Initial	7.6	7.4	7.5	7.4	7.5	7.5	7.5
Final	7.1	7.0	7.0	7.1	7.0	7.0	7.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
14	18	130	0.060	Bio #1 22-AUG-23
15	14	130	0.050	MWW Bio #2 23-AUG-23
15	17	140	0.050	MWW Bio #3 25-AUG-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	46	160	<0.05	192-4263-A-1
32	44	160	<0.05	192-4387-A-1
32	43	170	<0.05	192-4545-A-1

Appendix (Summary): Test 1002.0
 SUMMARY REPORTING FORMS
 CHRONIC BIOMONITORING
Ceriodaphnia dubia
 SURVIVAL AND REPRODUCTION

Permittee: Malvern Water Works

NPDES No.: AR0034126 AFIN 30-00040

Date and Time Test Initiated: August 22, 2023 at 1408

Date and Time Test Terminated: August 28, 2023 at 1605

Dilution water used:

PERCENT SURVIVAL

Time of Reading	Control	Percent Effluent				
		5 %	7 %	9 %	12 %	16 %
24 hour	100	100	100	100	100	100
48 hour	100	100	100	100	100	100
6 day	100	100	100	90.0	100	100

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

Replicates	Control	Percent Effluent				
		5 %	7 %	9 %	12 %	16 %
A	34	39	37	3	34	39
B	32	38	33	41	36	37
C	30	30	32	30	31	31
D	30	30	20	33	33	31
E	27	31	28	29	30	30
F	37	36	34	34	35	36
G	31	36	35	36	37	36
H	34	34	36	31	36	35
I	26	29	32	39	34	35
J	31	31	31	32	29	33
Mean per Adult	31.2	33.4	31.8	30.8	33.5	34.3
Mean per Surviving Adult	31.2	33.4	31.8	33.9	33.5	34.3
CV %	10.6	11.0	15.4	12.0	8.11	8.59

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

Appendix (Summary): 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: 16 % (TOP3B)
- 6. LOEC Ceriodaphnia Lethality: 16 % (TXP3B)
- 7. NOEC Ceriodaphnia Sublethality: 16 % (TPP3B)
- 8. LOEC Ceriodaphnia Sublethality: 16 % (TYP3B)
- 9. Coefficient of variation for Ceriodaphnia Reproduction: 10.6 (TQP3B)
- 10. Sublethality for this test: 16 % (51710 or 51710Q)

Appendix (Summary): Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Malvern Water Works
 NPDES NO.: AR0034126 AFIN 30-00040
 CONTACT: Mr. John Davis
 ANALYST: 280, 343, 357, 358

Test Initiated: DATE: August 22, 2023 TIME: 1408
 Test Terminated: DATE: August 28, 2023 TIME: 1605

DILUTION Control	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.2	8.1	7.9	8.0	7.8	7.7
Final	7.9	7.8	7.5	7.5	7.5	7.5	--
pH Initial	7.6	7.5	7.6	7.5	7.5	7.6	7.6
Final	7.7	7.6	7.6	7.8	7.8	7.7	--

DILUTION 5 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	7.9	8.2	8.0	7.9	7.7	7.7
Final	7.8	7.9	7.5	7.1	7.2	7.5	--
pH Initial	7.6	7.5	7.5	7.4	7.5	7.6	7.6
Final	7.7	7.6	7.6	7.8	7.8	7.7	--

DILUTION 7 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.0	8.0	8.0	8.0	7.8	7.8	7.7
Final	7.4	7.9	7.5	7.5	7.2	7.3	--
pH Initial	7.6	7.5	7.6	7.5	7.5	7.6	7.5
Final	7.7	7.6	7.6	7.8	7.8	7.7	--

DILUTION 9 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.2	8.0	8.3	7.5	8.0	7.6	7.7
Final	7.9	7.7	7.6	7.6	7.6	7.4	--
pH Initial	7.6	7.5	7.6	7.5	7.5	7.6	7.5
Final	7.7	7.6	7.6	7.7	7.8	7.6	--

DILUTION 12 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	8.3	8.1	8.1	7.6	8.0	7.8	7.6
Final	7.8	7.8	7.5	7.6	7.3	7.3	--
pH Initial	7.6	7.4	7.5	7.4	7.5	7.5	7.5
Final	7.7	7.6	7.6	7.7	7.8	7.6	--

DILUTION 16 %	DAY						
	1	2	3	4	5	6	7
D.O. Initial	7.8	8.1	8.2	7.8	7.9	7.4	7.6
Final	7.7	7.8	7.5	6.9	7.2	7.0	--
pH Initial	7.6	7.4	7.5	7.4	7.5	7.5	7.5
Final	7.7	7.6	7.6	7.8	7.8	7.7	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
14	18	130	0.060	Bio #1 22-AUG-23
15	14	130	0.050	MWW Bio #2 23-AUG-23
15	17	140	0.050	MWW Bio #3 25-AUG-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
31	46	160	<0.05	192-4263-A-1
32	44	160	<0.05	192-4387-A-1
32	43	170	<0.05	192-4545-A-1

