March 10, 2023

Biomonitoring Testing
for
Outfall 001

Control No. 273622-1

Prepared for:

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

Prepared by:

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8600 Kanis Road
Little Rock, AR 72204-2322

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

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 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 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.**

Eurofins Arkansas

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	0.528	PASS
Control Growth CV < or = 40%	5.93	PASS
Growth Minimum Significant Difference 12 to 30%	18.5	PASS
Critical Dilution CV < or = 40%	9.18	PASS

Ceriodaphnia dubia Method 1002.0

CRITERIA	RESULTS	PASS/FAIL
Control Survival > or = 80%	100	PASS
Control Reproduction > or = 15 per Surviving Female	31.6	PASS
Control CV < or = 40% per Surviving Female	13.4	PASS
Reproduction Minimum Significant Difference 13 to 47%	22.2	PASS
Critical Dilution CV < or = 40%	10.6	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)	8.7	7.8	8.8
pH (standard units)	7.0	7.1	7.2
Alkalinity (mg/l as CaCO3)	19	18	17
Hardness (mg/l as CaCO3)	17	17	17
Conductivity (umhos/cm)	130	130	140
Residual Chlorine (mg/l)	<0.05	<0.05	<0.05
Ammonia as N (mg/l)	1.6	1.5	1.4

2. Dilution Water Samples:
   Soft

Analysis	273492-1	273575-1
Dissolved oxygen (mg/l)	7.8	7.0
pH (standard units)	7.6	7.7
Alkalinity (mg/l as CaCO3)	32	32
Hardness (mg/l as CaCO3)	42	42
Conductivity (umhos/cm)	160	170
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:	February 28, 2023 at 1313
Date & Time Test Terminated:	March 07, 2023 at 1331
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:	February 28, 2023 at 1305
Date & Time Test Terminated:	March 06, 2023 at 1454
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. Steel's Many-One Rank test was used to determine the No Observable Effects Concentration (NOEC) for growth. Dunnett's Test was used to calculate the PMSD.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and 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 February 14, 2023 at 0938 to February 21, 2023 at 0921
The results were as follows: (Control No. 272798-1.)

Survival LC-50: 3063 mg/l
Growth IC-25: 2260 mg/l
Growth PMSD: 13

Ceriodaphnia dubia

A chronic reference test was performed on February 14, 2023 at 0926 to February 20, 2023 at 1115
The results were as follows: (Control No. 272798-2.)

Survival LC-50: 1776 mg/l
Reproduction IC-25: 1199 mg/l
Reproduction PMSD: 16.4

V. Organism History

Pimephales promelas (Fathead minnow)

Date: February 28, 2023
Age: <24 hours
Source: In-house culture
Water: Moderately hard synthetic
Temperature: 25 deg.C

Ceriodaphnia dubia

Date: February 28, 2023
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

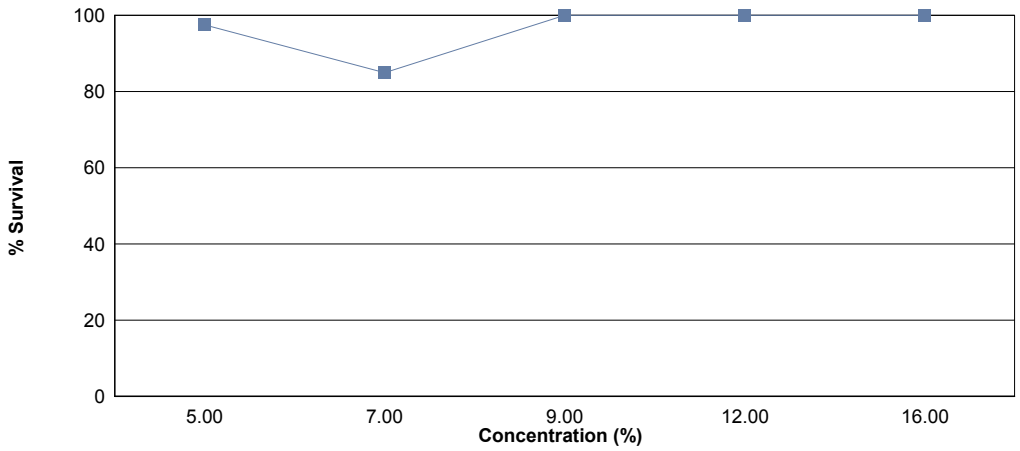
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

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

The low flow or 'critical' dilution is specified in the NPDES permit as 12 % effluent.

The test was initiated on February 28, 2023 at 1313 and continued through March 07, 2023 at 1331. 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	100	0.528
5 %	97.5	0.498
7 %	85.0	0.420
9 %	100	0.507
12 %	100	0.453
16 %	100	0.486

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

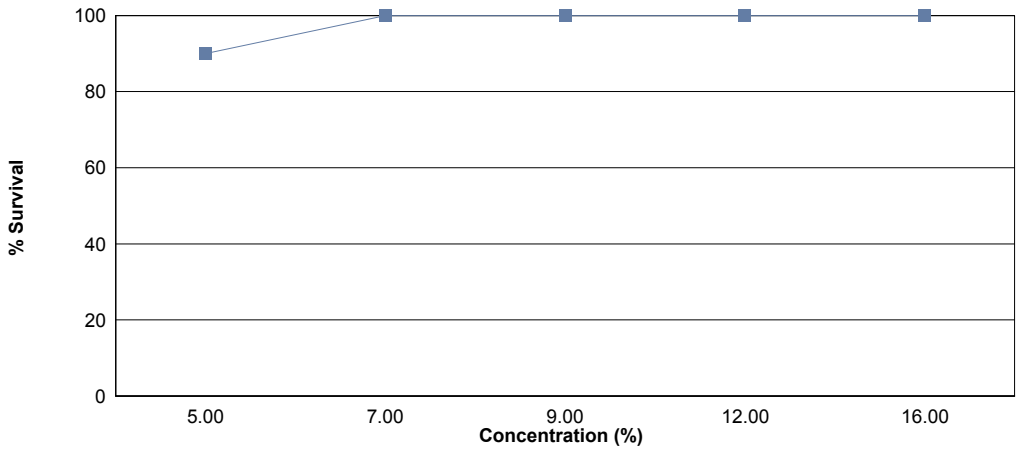
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

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

The low flow or 'critical' dilution is specified in the NPDES permit as 12 % effluent.

The test was initiated on February 28, 2023 at 1305 and continued through March 06, 2023 at 1454. 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 *Ceriodaphnia dubia* Survival and Reproduction Data		
Concentration	Percent Survival	Mean Reproduction
Control	100	31.6
5 %	90.0	29.1
7 %	100	28.6
9 %	100	29.7
12 %	100	32.5
16 %	100	31.8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: February 28, 2023 at 1313
Date and Time Test Terminated: March 07, 2023 at 1331

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
5 %	A	8	8	8	7	7	7	7
	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	7	7	7	7	7
	B	8	8	8	8	8	8	8
	C	8	8	8	8	8	8	8
	D	8	8	8	8	8	5	3
	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	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8
12 %	A	8	8	8	8	8	8	8
	B	8	8	8	8	8	8	8
	C	8	8	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	8	8	8	8	8
	D	8	8	8	8	8	8	8
	E	8	8	8	8	8	8	8

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: February 28, 2023 at 1313
Test Terminated: March 07, 2023 at 1331

Concentration	Replicate	Weight of pan	Weight of pan + fish	Total weight of fish (g)	Original # of fish	Mean dry weight (mg)
Control	A	.64990	.65398	0.00408	8	0.510
	B	.65167	.65598	0.00431	8	0.539
	C	.64986	.65372	0.00386	8	0.482
	D	.67585	.68027	0.00442	8	0.552
	E	.65491	.65936	0.00445	8	0.556
5 %	A	.66202	.66587	0.00385	8	0.481
	B	.66094	.66502	0.00408	8	0.510
	C	.65558	.65994	0.00436	8	0.545
	D	.66502	.66867	0.00365	8	0.456
	E	.66947	.67344	0.00397	8	0.496
7 %	A	.64160	.64532	0.00372	8	0.465
	B	.65168	.65594	0.00426	8	0.532
	C	.66025	.66409	0.00384	8	0.480
	D	.64032	.64191	0.00159	8	0.199
	E	.64261	.64599	0.00338	8	0.422
9 %	A	.65362	.65818	0.00456	8	0.570
	B	.64925	.65284	0.00359	8	0.449
	C	.65959	.66333	0.00374	8	0.468
	D	.66432	.66895	0.00463	8	0.579
	E	.64248	.64622	0.00374	8	0.468
12 %	A	.65263	.65628	0.00365	8	0.456
	B	.65863	.66209	0.00346	8	0.432
	C	.66660	.67015	0.00355	8	0.444
	D	.64690	.65107	0.00417	8	0.521
	E	.66758	.67087	0.00329	8	0.411
16 %	A	.65768	.66183	0.00415	8	0.519
	B	.65241	.65651	0.00410	8	0.512
	C	.65960	.66330	0.00370	8	0.462
	D	.66001	.66348	0.00347	8	0.434
	E	.65526	.65930	0.00404	8	0.505

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: February 28, 2023 at 1305
Date and Time Test Terminated: March 06, 2023 at 1454

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	5	5	5	6	5	6	7	4	4	7	54	10	5.40
4	10	10	11	0	11	12	12	11	10	12	99	10	9.90
5	0	0	0	15	0	16	0	0	0	16	47	10	4.70
6	16	18	16	0	16	0	17	18	15	0	116	10	11.6
7													
8													
TOTAL	31	33	32	21	32	34	36	33	29	35	316	10	31.6

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	5	6	5	6	6	3	6	6	53	10	5.30
4	10	9	10	7X	10	10	11	11	9	10	97	9	10.8
5	0	13	0	X	0	0	14	0	0	15	42	9	4.67
6	19	0	15	X	18	17	0	16	14	0	99	9	11.0
7													
8													
TOTAL	34	27	30	13	33	33	31	30	29	31	291	10	29.1

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	3	6	0	6	6	6	6	6	5	8	52	10	5.20
4	10	9	1	10	8	10	10	10	10	10	88	10	8.80
5	0	0	0	15	0	0	16	0	0	15	46	10	4.60
6	16	16	0	0	17	17	0	19	15	0	100	10	10.0
7													
8													
TOTAL	29	31	1	31	31	33	32	35	30	33	286	10	28.6

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: February 28, 2023 at 1305
Date and Time Test Terminated: March 06, 2023 at 1454

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	3	6	0	7	4	5	6	6	6	8	51	10	5.10
4	11	12	0	11	10	11	12	11	11	10	99	10	9.90
5	0	0	2	14	0	0	16	0	0	17	49	10	4.90
6	17	18	0	0	15	17	0	18	13	0	98	10	9.80
7													
8													
TOTAL	31	36	2	32	29	33	34	35	30	35	297	10	29.7

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	5	6	7	5	6	6	6	6	6	8	61	10	6.10
4	9	10	11	10	9	10	9	10	10	12	100	10	10.0
5	0	0	0	15	0	0	16	0	0	16	47	10	4.70
6	15	18	20	0	14	13	2XE	18	19	0	117	10	11.7
7													
8													
TOTAL	29	34	38	30	29	29	31	34	35	36	325	10	32.5

E = Excluded fourth brood neonates

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	6	5	6	5	6	6	5	7	57	10	5.70
4	10	9	10	9	9	11	11	11	9	10	99	10	9.90
5	0	0	0	16	0	0	0	0	0	18	34	10	3.40
6	15	17	18	0	15	7	19	18	19	0	128	10	12.8
7													
8													
TOTAL	30	32	34	30	30	23	36	35	33	35	318	10	31.8

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	5 %	1	0.87500	1.20940
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	0.87500	1.20940
3	7 %	2	1.00000	1.39310
3	7 %	3	1.00000	1.39310
3	7 %	4	0.37500	0.65906
3	7 %	5	1.00000	1.39310
4	9 %	1	1.00000	1.39310
4	9 %	2	1.00000	1.39310
4	9 %	3	1.00000	1.39310
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	1.00000	1.39310
6	16 %	4	1.00000	1.39310
6	16 %	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))
D = 0.4311		
W = 0.5371		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

Data FAIL normality test (alpha = 0.01).

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 %	25.00	16.00	5.00	
3	7 %	22.50	16.00	5.00	
4	9 %	27.50	16.00	5.00	
5	12 %	27.50	16.00	5.00	
6	16 %	27.50	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Shapiro - Wilk's Test for Normality		No Transformation
D = 0.1032		
W = 0.8891		
Critical W = 0.9	(alpha = 0.01, N = 30)	
Critical W = 0.927	(alpha = 0.05, N = 30)	

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 %	20.50	16.00	5.00	
3	7 %	17.00	16.00	5.00	
4	9 %	25.00	16.00	5.00	
5	12 %	17.00	16.00	5.00	
6	16 %	20.00	16.00	5.00	

Critical values are 1 tailed (k=5)

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

Dunnett's Test for PMSD Calculation

ANOVA Table				No Transformation
SOURCE	DF	SS	MS	F
Between	5	0.03861	0.007722	1.796
Within (Error)	24	0.1032	0.0043	
Total	29	0.1418		

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	0.5278	0.5278		
2	5 %	0.4976	0.4976	0.7282	
3	7 %	0.4196	0.4196	2.609	*
4	9 %	0.5068	0.5068	0.5064	
5	12 %	0.4528	0.4528	1.808	
6	16 %	0.4864	0.4864	0.9982	

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	0.09788	18.5	0.0302
3	7 %	5	0.09788	18.5	0.1082
4	9 %	5	0.09788	18.5	0.021
5	12 %	5	0.09788	18.5	0.075
6	16 %	5	0.09788	18.5	0.0414

Appendix A2: Statistics

Ceriodaphnia dubia Survival

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
5 %	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
7 %	10	0	10
Total	20	0	20

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

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

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

Fisher's Exact Test			
Identification	Alive	Dead	Total Animals
Control	10	0	10
12 %	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.

Appendix A2: 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	1	
2	7 %	10	0	
3	9 %	10	0	
4	12 %	10	1	
5	16 %	10	0	

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

Kolmogorov Test for Normality		No Transformation
D = 0.212		
D* = 1.663		
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 %	86.00	75.00	10.00	
3	7 %	90.50	75.00	10.00	
4	9 %	105.00	75.00	10.00	
5	12 %	107.00	75.00	10.00	
6	16 %	106.00	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	131	26.19	0.568
Within (Error)	54	2490	46.11	
Total	59	2621		

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	31.6	31.6		
2	5 %	29.1	29.1	0.8232	
3	7 %	28.6	28.6	0.9879	
4	9 %	29.7	29.7	0.6257	
5	12 %	32.5	32.5	-0.2964	
6	16 %	31.8	31.8	-0.06586	

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

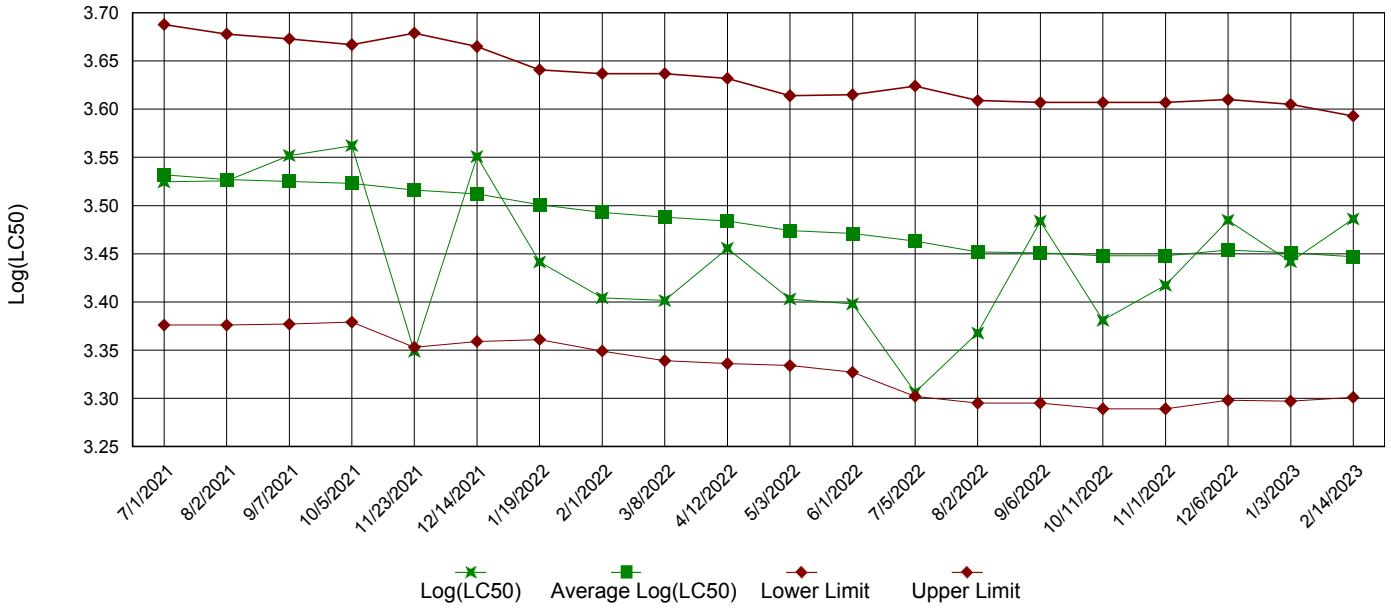
Ho:Control<Treatment

Group	Identification	Num of Reps	Min Sig Diff (In Orig. Units)	% of Control	Difference From Control
1	Control	10			
2	5 %	10	7.015	22.2	2.5
3	7 %	10	7.015	22.2	3
4	9 %	10	7.015	22.2	1.9
5	12 %	10	7.015	22.2	-0.9
6	16 %	10	7.015	22.2	-0.2

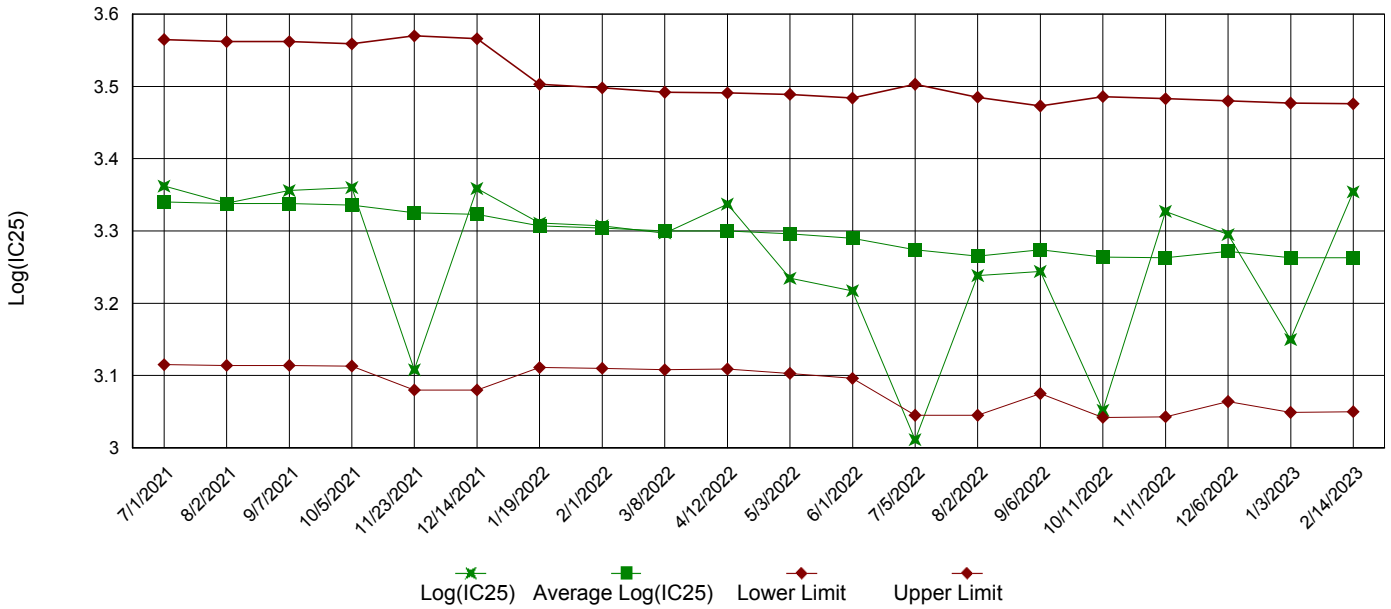
Appendix A3: Test 1000.0

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

LC50 Survival Data



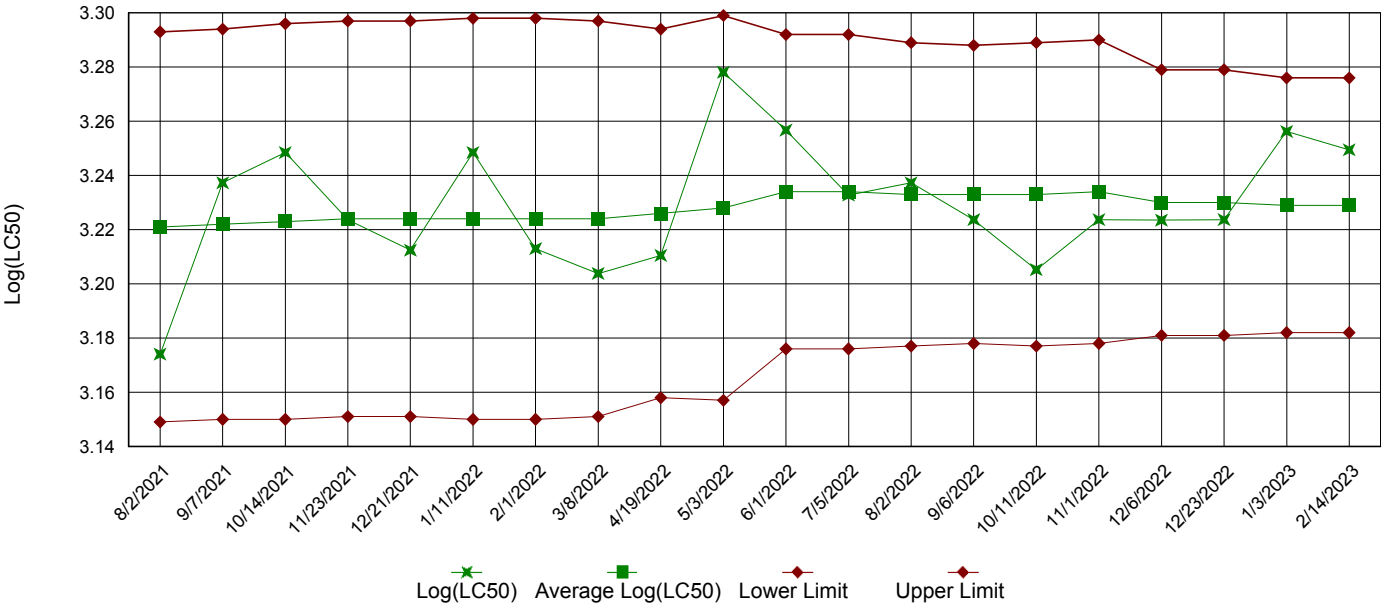
IC25 Growth Data



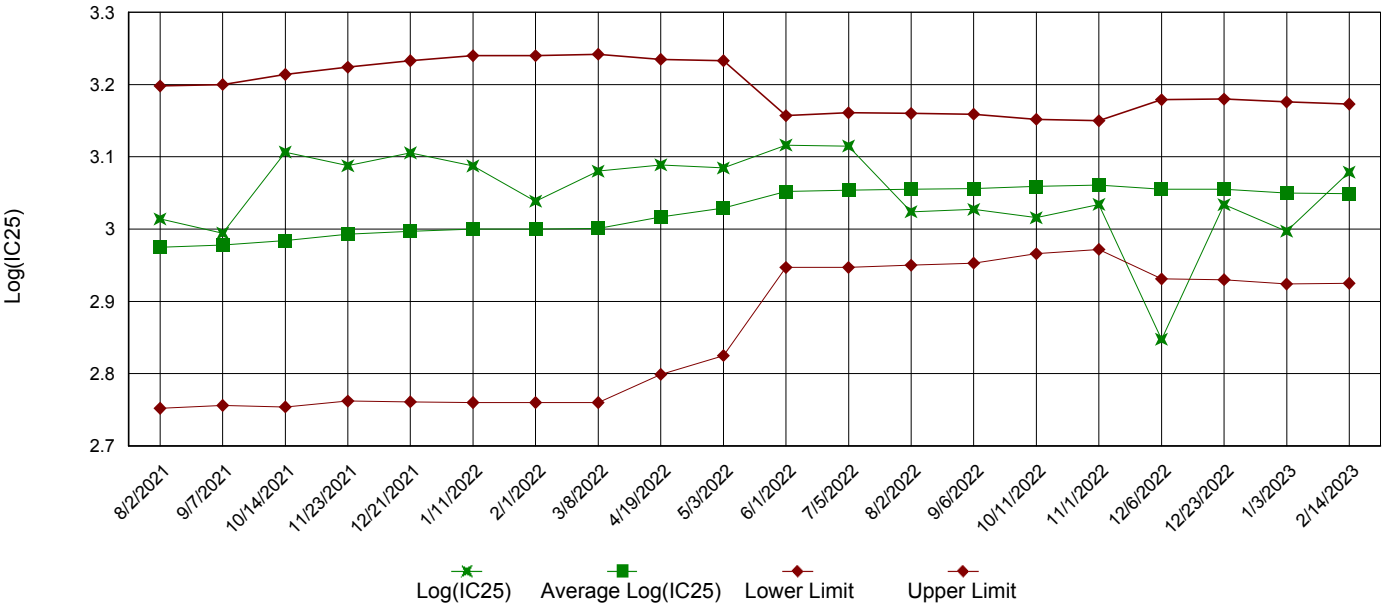
Appendix A3: Test 1002.0

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: Malvern Water Works

NPDES No.: AR0034126 AFIN 30-00040

Date and Time Test Initiated: February 28, 2023 at 1313
Date and Time Test Terminated: March 07, 2023 at 1331
Dilution water used: Soft

DATA TABLE FOR SURVIVAL

Effluent Conc. %	Percent Survival in replicate chambers A	B	C	D	E	Mean percent survival 24 hr	48 hr	7 days	CV%
Control	100	100	100	100	100	100	100	100	0.00
5 %	87.5	100	100	100	100	100	100	97.5	5.73
7 %	87.5	100	100	37.5	100	100	100	85.0	31.9
9 %	100	100	100	100	100	100	100	100	0.00
12 %	100	100	100	100	100	100	100	100	0.00
16 %	100	100	100	100	100	100	100	100	0.00

DATA TABLE FOR GROWTH

Effluent Conc. %	Average dry weight, mg replicate chambers A	B	C	D	E	Mean dry weight, mg	CV%
Control	0.510	0.539	0.482	0.552	0.556	0.528	5.93
5 %	0.481	0.510	0.545	0.456	0.496	0.498	6.67
7 %	0.465	0.532	0.480	0.199	0.422	0.420	30.8
9 %	0.570	0.449	0.468	0.579	0.468	0.507	12.3
12 %	0.456	0.432	0.444	0.521	0.411	0.453	9.18
16 %	0.519	0.512	0.462	0.434	0.505	0.486	7.56

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 _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ NO

2. Steel's Many-One Rank Test:

   Is the mean dry weight (growth) significantly different (p=0.05) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

   a.) LOW FLOW OR CRITICAL DILUTION _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP6C)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP6C)

5. NOEC Pimephales Lethality: 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: 9.18 (TQP6C)

10. Sublethality for this test: 16 % (51714 or 51714S)

Appendix B: 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: February 28, 2023 TIME: 1313
Test Terminated: DATE: March 07, 2023 TIME: 1331

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	8.0	7.0	7.9	8.5	8.4	8.4
	Final	6.2	5.2	5.5	6.4	6.3	5.8	6.1
pH	Initial	7.6	7.7	7.7	7.7	7.7	7.7	7.8
	Final	7.2	7.1	7.1	7.2	7.2	7.2	7.2

DILUTION 5 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.1	7.9	7.1	8.0	8.4	8.1	8.2
	Final	6.5	5.2	5.3	6.9	6.3	5.7	6.3
pH	Initial	7.6	7.6	7.6	7.7	7.7	7.8	7.7
	Final	7.3	7.1	7.1	7.3	7.2	7.1	7.2

DILUTION 7 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.2	7.8	7.1	7.9	8.4	8.2	7.8
	Final	6.4	5.3	5.5	7.2	6.2	6.2	6.5
pH	Initial	7.6	7.6	7.6	7.6	7.7	7.7	7.7
	Final	7.2	7.1	7.2	7.3	7.3	7.2	7.2

DILUTION 9 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	8.0	7.2	8.0	8.4	8.3	8.3
	Final	6.2	5.2	5.8	6.6	6.1	6.2	5.8
pH	Initial	7.6	7.6	7.6	7.7	7.7	7.7	7.7
	Final	7.2	7.0	7.2	7.3	7.2	7.2	7.1

DILUTION 12 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	8.0	7.2	8.0	8.4	8.2	8.3
	Final	6.3	5.5	5.9	7.6	5.9	5.9	6.0
pH	Initial	7.6	7.6	7.6	7.6	7.6	7.6	7.7
	Final	7.2	7.1	7.2	7.4	7.1	7.1	7.1

DILUTION 16 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.9	7.2	7.8	8.5	8.2	8.2
	Final	6.4	5.5	5.5	6.6	5.8	6.0	6.0
pH	Initial	7.5	7.6	7.6	7.6	7.6	7.6	7.7
	Final	7.2	7.2	7.2	7.3	7.2	7.2	7.1

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
19	17	130	<0.05	MWW Bio #1 28-FEB-23
18	17	130	<0.05	MWW Bio #2 01-MAR-23
17	17	140	<0.05	MWW Bio #3 03-MAR-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	42	160	<0.05	273492-1
32	42	170	<0.05	273575-1

Appendix B: 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: February 28, 2023 at 1305
Date and Time Test Terminated: March 06, 2023 at 1454
Dilution water used: Soft

PERCENT SURVIVAL

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

NUMBER OF YOUNG PRODUCED PER FEMALE @ 6 DAYS

		Percent Effluent				
Replicates	Control	5 %	7 %	9 %	12 %	16 %
A	31	34	29	31	29	30
B	33	27	31	36	34	32
C	32	30	1	2	38	34
D	21	13	31	32	30	30
E	32	33	31	29	29	30
F	34	33	33	33	29	23
G	36	31	32	34	31	36
H	33	30	35	35	34	35
I	29	29	30	30	35	33
J	35	31	33	35	36	35
Mean per Adult	31.6	29.1	28.6	29.7	32.5	31.8
Mean per Surviving Adult	31.6	30.9	28.6	29.7	29.4	31.8
CV %	13.4	7.14	34.4	33.7	10.6	12.0

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 _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ NO

2. Steel's Many-One Rank Test:

   Is the mean number of young produced per female significantly different (p=0.05) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

   a.) LOW FLOW OR CRITICAL DILUTION _____YES __X__ NO
   b.) 1/2 LOW FLOW DILUTION _____YES _____ NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)

4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 0 (TGP3B)

5. NOEC Ceriodaphnia Lethality: 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: 13.4 (TQP3B)

10. Sublethality for this test: 16 % (51710 or 51710Q)

Appendix B: 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: February 28, 2023 TIME: 1305
Test Terminated: DATE: March 06, 2023 TIME: 1454

DILUTION Control		DAY 1	2	3	4	5	6	7
D.O.	Initial	7.8	8.0	7.0	7.9	8.5	8.4	8.4
	Final	7.6	7.2	7.9	8.0	8.0	7.7	--
pH	Initial	7.6	7.7	7.7	7.7	7.7	7.7	7.8
	Final	7.8	7.8	7.9	7.9	7.8	7.9	--

DILUTION 5 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.1	7.9	7.1	8.0	8.4	8.1	8.2
	Final	7.8	7.3	7.9	7.7	7.9	7.8	--
pH	Initial	7.6	7.6	7.6	7.7	7.7	7.8	7.7
	Final	7.8	7.8	7.9	7.9	7.8	7.8	--

DILUTION 7 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.2	7.8	7.1	7.9	8.4	8.2	7.8
	Final	7.7	7.1	8.0	8.1	8.0	7.7	--
pH	Initial	7.6	7.6	7.6	7.6	7.7	7.7	7.7
	Final	7.8	7.8	7.9	7.8	7.8	7.8	--

DILUTION 9 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	8.0	7.2	8.0	8.4	8.3	8.3
	Final	7.7	7.2	7.9	7.9	7.8	7.8	--
pH	Initial	7.6	7.6	7.6	7.7	7.7	7.7	7.7
	Final	7.8	7.8	7.9	7.9	7.9	7.9	--

DILUTION 12 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	8.0	7.2	8.0	8.4	8.2	8.3
	Final	7.6	7.1	7.9	8.1	8.0	7.8	--
pH	Initial	7.6	7.6	7.6	7.6	7.6	7.6	7.7
	Final	7.8	7.8	7.9	7.7	7.8	7.8	--

DILUTION 16 %		DAY 1	2	3	4	5	6	7
D.O.	Initial	8.0	7.9	7.2	7.8	8.5	8.2	8.2
	Final	7.7	7.1	7.9	7.9	7.9	7.8	--
pH	Initial	7.5	7.6	7.6	7.6	7.6	7.6	7.7
	Final	7.8	7.8	7.9	7.9	7.9	7.8	--

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
19	17	130	<0.05	MWW Bio #1 28-FEB-23
18	17	130	<0.05	MWW Bio #2 01-MAR-23
17	17	140	<0.05	MWW Bio #3 03-MAR-23

Alkalinity	Hardness	Conductivity	Chlorine	Sample ID
32	42	160	<0.05	273492-1
32	42	170	<0.05	273575-1

AMERICAN INTERPLEX CORPORATION LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Malvern Waste Water
Project Reference:
Project Manager:
Sampled By: Devan Baugh

PO No.

MATRIX

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL		NO OF BOTTLES	ANALYSES REQUESTED: BioMonitoring
1	MWW Bio #1	2/28/23 9:00AM-8:10AM		X				1	Y
		Container Type							
		Preservative							

AIC CONTROL NO: 2-28-23
AIC PROPOSAL NO:
Carrier:
Received on ice? (Yes) No Temp. 0.1 °C
Remarks
Field pH calibration on _____ @ _____
Buffer:

G = Glass, NO = none; P = Plastic, S = Sulfuric acid pH2; V = VOA vials, N = Nitric acid pH2; H = HCl to pH2, B = NaOH to pH12; T = Sodium Thiosulfate, Z = Zinc acetate; A=$(NH_4)_2SO_4$, NH_4OH

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Contact Phone: _____
Report Attention to: _____
Email Address:

Relinquished By:	Date/Time	Received By:	Date/Time
Devan Baugh	2/28/23 10:15AM		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		Luge Hoot	2-28-23 1015

Comments:

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE OF

Client: Malvern Waste Water
Project Reference:
Project Manager:
Sampled By: Devan Baugh
PO No.

MATRIX: W A T E R | S O I L

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	WATER	SOIL	NO OF BOTTLES	ANALYSES REQUESTED: Biomonitoring
2	MWW Bio #2	3/1/23 9:10Am		X			1	
		Container Type						
		Preservative						

AIC CONTROL NO: 273622
AIC PROPOSAL NO:
Carrier:
Received on ice? (Yes) No Temp. 2.4 °C
Remarks
Field pH calibration
on _____ @ _____
Buffer:

G = Glass, NO = none; P = Plastic, S = Sulfuric acid pH2; V = VOA vials, N = Nitric acid pH2; H = HCl to pH2, B = NaOH to pH12; T = Sodium Thiosulfate, Z = Zinc acetate; A=$(NH_4)_2SO_4$, NH_4OH

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Contact Phone: _____
Report Attention to: _____
Email Address:

Relinquished By:	Date/Time	Received By:	Date/Time
Devan Baugh	3/1/23 10:27Am		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		Paige Hopton	3-1-23 1627

Comments:

FORM 0060

AMERICAN INTERPLEX CORPORATION
LABORATORIES

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

Client: Malvern Waste Water
Project Reference:
Project Manager:
Sampled By: Devan Baugh
PO No.

AIC No.	Sample Identification	Date/Time Collected	GRAB	COMP	MATRIX WATER	MATRIX SOIL	NO OF BOTTLES	Biomonitoring	P, NO3+NO2-N
3	MWW Bio #3	3/3/2023 8:10 AM		X			1	X	
	MWW P, NO3+NO2-N	3/3/2023 8:15 AM	X				1		X
		Container Type							
		Preservative							

ANALYSES REQUESTED

AIC CONTROL NO: 273/22
AIC PROPOSAL NO:
Carrier:
Received on ice? Yes / No Temp. 0.1 °C
Remarks
AIC 273755
Field pH calibration on _____ @ _____ Buffer:

G = Glass, NO = none; P = Plastic, S = Sulfuric acid pH2; V = VOA vials, N = Nitric acid pH2; H = HCl to pH2, B = NaOH to pH12; T = Sodium Thiosulfate, Z = Zinc acetate; A=$(NH_4)_2SO_4$, NH_4OH

Turnaround Time Requested: (Please circle)
NORMAL or EXPEDITED IN ____ DAYS
Expedited results requested by: _____
Who should AIC contact with questions: _____
Contact Phone: _____
Report Attention to: _____
Email Address:

Relinquished By:	Date/Time	Received By:	Date/Time
Devan Baugh	3/3/2023 9:55AM		
Relinquished By:	Date/Time	Received in Lab By:	Date/Time
		D. BROWN	3·3·23 0955

Comments:

FORM 0060