Biomonitor

Permittee/Location	**Permit Number:**	**Outfall Number:**
Connersville WWTP Connersville, IN	IN0032336 Fayette Co.	001
Laboratory Name and Contact:	**Report** Due Date:	**Report Date:**
Biomonitor Michael Britton		May 2024

WETT Reporting Frequency or Type:	Monthly	Quarterly	Semi-annual	Annual	TRE	Post TRE	First (per Reporting Frequency)
(mark one)				**X**			

Test Organism	**Test**	**Endpoint [1]**	**Units**	**Result**	**Compliance Value in TUs**	**Pass/Fail**	Reporting
Ceriodaphnia dubia	7-day Survival and Reproduction Definitive Static-Renewal	NOEC Survival	%	100			Laboratory Report
			TU_c	1			
		NOEC Reproduction	%	100			
			TU_c	1			
		IC25 Reproduction	%	100			
			TU_c	1			
		48 hr. LC50	%	>100			
			TU_a	<1			
		Toxicity (acute)	TU_a	<1	1	**Pass**	Laboratory Report and **NetDMR** (Parameter Code 61425)
		Toxicity (chronic)	TU_c	1	1.7	**Pass**	Laboratory Report and **NetDMR** (Parameter Code 61426)

Pimephales promelas	7-day Larval Survival and Growth Definitive Static-Renewal	NOEC Survival	%	100			Laboratory Report
			TU_c	1			
		NOEC Growth	%	100			
			TU_c	1			
		IC25 Growth	%	100			
			TU_c	1			
		96 hr. LC50	%	>100			
			TU_a	<1			
		Toxicity (acute)	TU_a	<1	1	**Pass**	Laboratory Report and **NetDMR** (Parameter Code 61427)
		Toxicity (chronic)	TU_c	1	1.7	**Pass**	Laboratory Report and **NetDMR** (Parameter Code 61428)

Biomonitor
8802 West Washington Street
Indianapolis, IN 46231
(317) 297-7713

Whole Effluent Toxicity Test

CONNERSVILLE WASTEWATER TREATMENT PLANT

IN0032336

Connersville, Indiana

May 2024

GLP (Good Laboratory Practices)
COMPLIANCE STATEMENT

Project Name: Connersville Wastewater Treatment Plant

Project Date: May 2024

This project has been conducted under GLP standards, as stated in 40 CFR Part 160, with the following exceptions:

Greg R. Bright

Quality Assurance Officer
Date: 5/21/24

Michael Britton

Project Director
Date: 5/21/24

Other Participating Personnel:

Mukang'andu Ng'andwe
Arizona Fox

Copies of the raw data and final report are maintained in the archives of Biomonitor for five years from the date of completion.

Section 1
Executive Summary

Biomonitor conducted whole effluent toxicity testing for the Connersville, IN Wastewater Treatment Plant during May 2024. The purpose of the testing was to fulfill the biomonitoring requirement for the NPDES permit.

Three samples were collected May 5-9, 2024. The water flea, *Ceriodaphnia dubia*, and Fathead minnow, *Pimephales promelas,* were used as the test organisms.

A total of six toxicity endpoints were measured. The following results were obtained:

Ceriodaphnia dubia test

48-hr LC_{50}	>	100% effluent	TU_a	<	1.0
NOEL for survival	=	100% effluent	TU_c	=	1.0
NOEL for reproduction	=	100% effluent	TU_c	=	1.0

Pimephales promelas test

48-hr LC_{50}	>	100% effluent	TU_a	<	1.0
NOEL for survival	=	100% effluent	TU_c	=	1.0
NOEL for growth	=	100% effluent	TU_c	=	1.0

The acute toxicity limits in the NPDES permit require the 48 and/or 96-hr LC_{50} to be greater than 100% effluent (a TU_a not to exceed 1.0). The effluent samples passed the acute toxicity limits during this testing period for both species.

The chronic toxicity limits in the NPDES permit require a NOEL (No Observable Effect Level) greater than 58.8% effluent (a TU_c not to exceed 1.7). According to the NPDES permit, there was not a “Demonstration of Toxicity” during this sampling period.

Section 2
Introductory Information

Table I
General

Permit number:	IN0032336
Toxicity testing requirements:	Fathead minnow larval survival and growth test Ceriodaphnia survival and reproduction test
Plant location:	Connersville Wastewater Treatment Plant 1000 South State Road 121 Connersville, Indiana 47331
Name of receiving water body:	West Fork of Whitewater River
Name of WET testing laboratory:	Biomonitor 8802 West Washington St. Indianapolis, IN 46231 (317) 297-7713

Table II
Plant Operations

Type of discharger:	Publicly owned treatment works Wastewater consists of treated sanitary and industrial wastes	
Type of waste treatment:	Activated sludge	
Design flow:	10.8 – MGD (average), MGD (peak)	
Volume of wastewater flow during the sampling period:	May 5, 2024	-MGD
	May 7, 2024	-MGD
	May 9, 2024	-MGD

Table III
Source of effluent and dilution water

I. Effluent samples

Sampling point:	Outfall 001	
Collection dates and times:	May 5, 2024	11:00 p.m.
	May 7, 2024	11:00 p.m.
	May 9, 2024	11:00 p.m.
Sample collection:	24-hour composite samples	
Physical and chemical data:	See Tables 9 and 15	

II. Dilution water samples

Source:	Moderately Hard Synthetic Water (MHSW)
	Collection date and time: N/A
Pretreatment:	None
Physical and chemical data:	See Tables 9 and 15

Section 3
Test Methods and Results

CERIODAPHNIA SURVIVAL AND REPRODUCTION TEST

Table IV
METHODOLOGY
***Ceriodaphnia* Survival and Reproduction Test**

Toxicity test method used:	*Ceriodaphnia* survival and reproduction test	
Endpoints of test:	Survival and reproduction (LC_{50}, NOEL, and LOEL)	
Reference method:	EPA-821-R-02-013	
Deviations from method:	Test was completed in six days because control animals produced an average of greater than 15 young per female by day six.	
Date and time test initiated:	May 7, 2024	10:15 a.m.
Date and time test terminated	May 13, 2024	10:20 a.m.
Type of test chambers:	Polyethylene	30 ml
Volume of solution used per chamber:	15 ml	
Number of organisms per chamber:	1	
Number of replicate chambers per treatment:	10	
Test temperature range:	25°C (no deviations)	

Table V
ORGANISMS USED
***Ceriodaphnia* Survival and Reproduction Test**

Scientific name:	*Ceriodaphnia dubia*
Age:	<24 hours
Life stage:	neonates
Mean length and weight:	Not applicable
Source	Laboratory culture in moderately hard reconstituted water
Diseases and treatment	Not applicable

Table VI
RESULTS
***Ceriodaphnia* Survival and Reproduction Test**

Raw Data:	See Table 8
LC_{50} or NOEL obtained:	48-hr LC_{50} = greater than 100% effluent NOEL for survival = 100% effluent NOEL for reproduction = 100% effluent Control survival was 100% after six days. Control reproduction averaged greater than 15 per female.
Methods used to calculate endpoints:	Fisher's Exact Test for the survival endpoint. Dunnett's Test for the reproduction endpoint. No calculations necessary for the acute endpoint.

Table VII
QUALITY ASSURANCE
***Ceriodaphnia* Survival and Reproduction Test**

Reference Toxicant used and source:	Copper chloride, reagent grade, from Carolina Biological
Date and time of most recent test:	April 23-30, 2024
Dilution water used in test:	Moderately hard synthetic water
Results:	48-hr LC_{50} = 80 μg/L as Cu NOEL (reproduction) = 40 μg/L as Cu LOEL (reproduction) = 80 μg/L as Cu
Comparison to recommended range:	Within the laboratory control range for both acute and chronic endpoints (see attachment)

Table VIII
TEST DATA
***Ceriodaphnia* Survival and Reproduction Test**

Effluent Concentration	Day No.	Number of Young Reproduced										Young Per Female	Total Live Breeders
		Replicate											
		A	B	C	D	E	F	G	H	I	J		
Control	1	0	0	0	0	0	0	0	0	0	0	23.6	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	4	0	0	4	4	3	3	2	3	5		10
	4	8	0	4	7	0	5	8	0	6	8		10
	5	0	9	10	10	10	0	0	8	0	12		10
	6	16	13	0	0	16	13	14	16	15	0		10
7.35%	1	0	0	0	0	0	0	0	0	0	0	26.5	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	4	3	0	3	4	2	3	3	3	3		10
	4	9	0	4	10	9	6	8	11	8	10		10
	5	0	7	10	14	0	0	0	0	0	0		10
	6	18	15	0	0	16	16	15	17	17	17		10
14.7%	1	0	0	0	0	0	0	0	0	0	0	23.0	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	2	3	0	0	4	2	3	3	3	5		10
	4	0	6	4	7	12	8	10	8	8	7		10
	5	4	0	3	12	0	0	0	0	0	0		10
	6	12	14	0	0	15	16	15	14	16	14		10

Table VIII (cont.)
TEST DATA
***Ceriodaphnia* Survival and Reproduction Test**

Effluent Concentration	Day No.	Number of Young Reproduced										Young Per Female	Total Live Breeders
		Replicate											
		A	B	C	D	E	F	G	H	I	J		
29.4%	**1**	0	0	0	0	0	0	0	0	0	0	21.1	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	3	2	0	5	3	2	3	3	3	2		10
	4	9	0	4	9	0	4	11	7	8	10		10
	5	0	7	9	0	11	0	0	0	12	0		10
	6	14	8	0	0	0	13	17	17	0	15		10
58.8%	**1**	0	0	0	0	0	0	0	0	0	0	24.8	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	3	4	0	2	4	3	3	3	4	2		10
	4	9	7	0	7	11	5	9	9	8	8		10
	5	0	0	8	10	0	0	0	0	13	0		10
	6	15	14	0	0	16	19	16	19	0	17		10
100%	**1**	0	0	0	0	0	0	0	0	0	0	25.1	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	2	2	0	2	5	0	4	3	2	5		10
	4	0	8	1	4	0	5	8	8	11	7		10
	5	8	0	8	14	10	0	0	0	0	0		10
	6	14	16	0	0	17	14	18	19	18	18		10

Table IX
WATER CHEMISTRY
***Ceriodaphnia* Survival and Reproduction Test**

Effluent Concentration	D.O. Range mg/L	Temp. Range °C	pH Range S.U.	Alk. Range $CaCO_3$	Hardness Range $CaCO_3$	Cond. Range µS
CONTROL	7.8 – 8.4	25	7.5 – 7.9	40-	90-110	330-350
7.35%	7.8 – 8.4	25	7.5 – 7.9			330-350
29.4%	7.6 – 8.5	25	7.4 – 7.9			400-420
100%	7.5 – 9.4	25	7.3 – 7.9	110-120	200-325	580-680

FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

Table X
METHODOLOGY
Fathead Minnow Larval Survival and Growth Test

Toxicity test method used:	7-day fathead minnow larval survival and growth test	
Endpoints of test:	96-hr LC_{50} and no observable effect level (NOEL) for survival and growth. TU_c for survival and growth.	
Reference method:	EPA-821-R-02-013	
Deviations from method:	No Deviations	
Date and time test initiated:	May 7, 2024	10:30 a.m.
Date and time test terminated	May 14, 2024	10:30 a.m.
Type of test chambers:	Polyethylene	300 ml
Volume of solution used per chamber:	250 ml	
Number of organisms per chamber:	ten	
Number of replicate chambers per treatment:	four	
Test temperature range:	25°C (no deviations)	

Table XI
ORGANISMS USED
Fathead Minnow Survival and Growth Test

Scientific name:	*Pimephales promelas*
Age:	<24 hours
Life stage:	larvae
Mean length and weight:	Not applicable
Source	Biomonitor Laboratory Cultures
Diseases and treatment	Not applicable

Table XII
RESULTS
Fathead Minnow Larval Survival and Growth Test

Raw Data:	See Table 14
LC_{50} or NOEL obtained:	96-hr LC_{50} = >100% effluent NOEL for survival = 100% effluent NOEL for growth = 100% effluent Control survival and growth fell within the acceptable range
Methods used to calculate endpoints:	Dunnett's Test for the growth endpoint. Steel's Many-One Rank Test was required for the survival endpoint because the homogeneity of variance assumptions could not be met. No calculations needed for the acute endpoint.

Table XIII
QUALITY ASSURANCE
Fathead Minnow Larval Survival and Growth Test

Reference Toxicant used and source:	Potassium chloride, reagent grade, from Sigma-Aldrich
Date and time of most recent test:	April 23-30, 2024
Dilution water used in test:	Moderately Hard Synthetic Water
Results:	96-hr LC_{50} = 1189 mg /L as KCl
	NOEL (growth) = 1000 mg/L as KCl
	LOEL (growth) = 2000 mg/L as KCl
Comparison to recommended range:	Within the laboratory control range for both acute and chronic endpoints (see attachment)

Table XIV
TEST DATA
Fathead Minnow Larval Survival and Growth Test

Effluent Concentration	% Survival in Each Replicate				Average Dry Weight (µg) in Each Replicate			
	A	B	C	D	A	B	C	D
Control	90	100	100	100	300	300	450	340
7.35%	100	90	100	90	370	340	400	360
14.7%	100	100	100	100	390	410	430	370
29.4%	100	100	100	100	390	410	400	380
58.8%	100	90	100	100	400	350	370	340
100%	100	90	100	100	300	350	340	380

Table XV
WATER CHEMISTRY
Fathead Minnow Larval Survival and Growth Test

Effluent Concentration	D.O. Range mg/L	Temp. Range °C	pH Range S.U.	Alk. Range $CaCO_3$	Hardness Range $CaCO_3$	Cond. Range µS
CONTROL	5.5 – 9.0	25	7.3 – 7.9	40-	90-110	330-360
7.35%	5.5 – 9.1	25	7.3 – 7.9			340-360
29.4%	5.3 – 9.2	25	7.4 – 7.8			400-420
100%	5.2 – 9.8	25	7.4 – 7.7	110-120	200-325	600-690

Biomonitor

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Connersville WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Connersville - 1 Monday May 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 5-5-24 Start Time 12:00 Am

End Date ~~5-6-24~~ 5-5-24 End Time ~~11:59 p~~ 11:00 ~~Am~~ pm

NAME OF PERSON COLLECTING SAMPLE: Marc Frank

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: Marc Frank

Date: 5-6-24 Time: 10:20 a

Received by: QGF

Date: 5/6/24 Time: 10:20 a

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: 7 °C

COMMENTS:

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Connersville WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Connersville - 2 Wednesday May 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 5-7-2024 Start Time 12:00 am

End Date 5-7-2024 End Time 11:00 pm

NAME OF PERSON COLLECTING SAMPLE: Matt Kendall

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: _____

Date: 5/8/2024 Time: 10:05 a

Received by: _____

Date: 5/8/24 Time: 10:05 a

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: 5.4 °C

COMMENTS:

Biomonitor

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Connersville WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Connersville - 3 Friday May 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 5/9/2024 Start Time 12:00 am

End Date 5/9/2024 End Time 11:00 pm

NAME OF PERSON COLLECTING SAMPLE: Matt Kendall

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: _____

Date: 5/10/2024 Time: 10:15 a

Received by: _____

Date: 5/10/24 Time: 10:15 a

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: 7 °C

COMMENTS:

Ceriodaphnia dubia

Reference Toxicant - Copper sulfate/chloride as Cu

Dilution Water - Moderately Hard Reconstituted Water

Date	LC_{50}	NOEL	LOEL	IC_{25}
mm/yy	48-hr µg/L	µg/L (repro.)	µg/L (repro.)	µg/L (repro.)
07/21	98	40	80	50
08/21	87	40	80	23
09/21	92	40	80	49
10/21	73	40	80	52
11/21	113	40	160	59
12/21	75	40	80	48
2/22	105	40	80	54
3/22	75	40	80	51
4/22	113	40	80	57
5/22	95	40	80	30
6/22	113	40	80	41
7/22	75	40	80	33
8/22	86	40	40	30
9/22	80	40	80	32
11/22	70	40	80	40
12/22	77	40	80	48
1/23	75	40	80	48
2/23	86	40	80	52
4/23	80	40	80	37
5/23	80	40	80	39
06/23	113	40	160	59
07/23	75	40	80	55
09/23	80	40	80	15
10/23	113	40	80	58
11/23	86	40	80	50
01/24	99	40	40	30
02/24	86	40	80	48
03/24	80	40	80	48
04/24	80	40	80	51
Average	88	Mode 40	80	44
St. Dev.	14			11
Upper Limit	116	80	160	67
Lower Limit	60	20	40	22

Pimephales promelas

Reference Toxicant - Potassium chloride

Dilution Water - Moderately Hard Reconstituted Water

Date	LC_{50}	NOEL	LOEL	IC_{25}
mm/yy	96-hr mg/L	mg/L (grwth)	mg/L (grwth)	mg/L (grwth)
11/21	1129	1000	2000	939
12/21	1129	500	1000	810
02/22	812	500	1000	612
03/22	946	500	1000	707
04/22	917	500	1000	703
05/22	1110	1000	2000	1223
06/22	856	500	1000	710
07/22	1130	500	1000	736
08/22	1093	500	1000	925
09/22	1278	1000	2000	950
11/22	1035	500	1000	684
12/22	1053	1000	2000	805
01/23	795	500	1000	664
02/23	1091	500	1000	741
04/23	1231	1000	2000	1121
05/23	1189	1000	2000	1110
06/23	951	500	1000	669
07/23	1091	500	1000	1091
09/23	1000	500	1000	702
10/23	1124	500	1000	768
11/23	1253	500	1000	849
01/24	1128	500	1000	699
02/24	952	1000	2000	798
03/24	1189	500	1000	908
04/24	1189	1000	2000	1037
Average	1067	Mode 500	1000	838
St. Dev.	131			166
Upper Limit	1328	1000	2000	1171
Lower Limit	806	250	500	506

Client: Connersville WWTP

Project #

Analysts: MMB, MN, AF, MMK

Test Dates

Start Date: 5/7/2024

Start Time: 1015

End Date: 5/13/2024

End Time: 1020

Template # A

Comments:

0 = Number of Live Young
/ = Test Organism Dead
y = Male
M = Lost or Missing

Row 10 Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	5	5	2	3	2	5
4	8	7	10	10	8	7
5	12	0	0	0	0	0
6	0	18	15	17	17	14
7						

Row 9 Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	2	3	4	3	3	3
4	11	8	8	8	6	8
5	0	12	13	0	0	0
6	18	0	0	17	15	16
7						

Row 8 Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	3	3	3	3	2	3
4	11	9	8	8	0	7
5	0	0	0	0	8	0
6	17	19	19	14	16	17
7						

Row 7 Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	3	3	3	4	3	3
4	11	8	8	8	10	9
5	0	0	0	0	0	0
6	17	15	14	18	15	16
7						

Row 6 Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	2	0	2	3	3	2
4	4	5	8	5	5	6
5	0	0	0	0	0	0
6	13	14	16	19	13	16
7						

Row 5 Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	4	4	4	4	3	5
4	9	11	12	0	0	0
5	0	0	0	10	11	10
6	16	16	15	16	0	17
7						

Row 4	Day						
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	5	3	2	4	0	2
	4	9	10	4	7	7	7
	5	0	14	14	10	12	10
	6	0	0	0	0	0	0
	7						

Row 3	Day						
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	0	0	0	0	0	0
	4	4	1	4	4	0	4
	5	3	8	9	10	8	10
	6	0	0	0	0	0	0
	7						

Row 2	Day						
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	3	4	2	2	0	3
	4	6	7	8	0	0	0
	5	0	0	0	7	9	7
	6	14	14	16	8	13	15
	7						

Row 1	Day						
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	3	4	4	3	2	2
	4	9	9	9	9	0	0
	5	0	0	0	0	8	4
	6	15	16	18	14	14	12
	7						

Discharger:	Connersville WWTP	**Analyst:**	MMB, MN, AF, MMK
Location:	Connersville, IN	**Test Start- Date/Time:**	5/7/24 / 1030
Date Sample Collected:	5/5,7,9/24	**Test Stop- Date/Time:**	5/13/24 / 1020

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
Control	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	4	0	0	4	4	3	3	2	3	5	28	10	2.8
	4	8	0	4	7	0	5	8	0	6	8	46	10	4.6
	5	0	9	10	10	10	0	0	8	0	12	59	10	5.9
	6	16	13	0	0	16	13	14	16	15	0	103	10	10.3
	Total	28	22	14	21	30	21	25	26	24	25	236	10	23.6

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
7.35% ~~6%~~	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	4	3	0	3	4	2	3	3	3	3	28	10	2.8
	4	9	0	4	10	9	6	8	11	8	10	75	10	7.5
	5	0	7	10	14	0	0	0	0	0	0	31	10	3.1
	6	18	15	0	0	16	16	15	17	17	17	131	10	13.1
	Total	31	25	14	27	29	24	26	31	28	30	265	10	26.5

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
14.7 ~~12%~~	1	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	2	3	0	0	4	2	3	3	3	5	25	10	2.5
	4	0	6	4	7	12	8	10	8	8	7	70	10	7.0
	5	4	0	3	12	0	0	0	0	0	0	19	10	1.9
	6	12	14	0	0	15	16	15	14	16	14	116	10	11.6
	Total	18	23	7	19	31	26	28	25	27	26	230	10	23.0

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
29.4 ~~25%~~	1	0	0	0	0	0	0	0	0	0	0	**0**	**10**	**0.0**
	2	0	0	0	0	0	0	0	0	0	0	**0**	**10**	**0.0**
	3	3	2	0	5	3	2	3	3	3	2	**26**	**10**	**2.6**
	4	9	0	4	9	0	4	11	7	8	10	**62**	**10**	**6.2**
	5	0	7	9	0	11	0	0	0	12	0	**39**	**10**	**3.9**
	6	14	8	0	0	0	13	17	17	0	15	**84**	**10**	**8.4**
	Total	**26**	**17**	**13**	**14**	**14**	**19**	**31**	**27**	**23**	**27**	**211**	**10**	**21.1**

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
58.8 ~~50%~~	1	0	0	0	0	0	0	0	0	0	0	**0**	**10**	**0.0**
	2	0	0	0	0	0	0	0	0	0	0	**0**	**10**	**0.0**
	3	3	4	0	2	4	3	3	3	4	2	**28**	**10**	**2.8**
	4	9	7	0	7	11	5	9	9	8	8	**73**	**10**	**7.3**
	5	0	0	8	10	0	0	0	0	13	0	**31**	**10**	**3.1**
	6	15	14	0	0	16	19	16	19	0	17	**116**	**10**	**11.6**
	Total	**27**	**25**	**8**	**19**	**31**	**27**	**28**	**31**	**25**	**27**	**248**	**10**	**24.8**

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
100%	1	0	0	0	0	0	0	0	0	0	0	**0**	**10**	**0.0**
	2	0	0	0	0	0	0	0	0	0	0	**0**	**10**	**0.0**
	3	2	2	0	2	5	0	4	3	2	5	**25**	**10**	**2.5**
	4	0	8	1	4	0	5	8	8	11	7	**52**	**10**	**5.2**
	5	8	0	8	14	10	0	0	0	0	0	**40**	**10**	**4.0**
	6	14	16	0	0	17	14	18	19	18	18	**134**	**10**	**13.4**
	Total	**24**	**26**	**9**	**20**	**32**	**19**	**30**	**30**	**31**	**30**	**251**	**10**	**25.1**

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	4.020	14.520	22.920	14.520	4.020
OBSERVED	5	11	24	19	1

Calculated Chi-Square goodness of fit test statistic = 4.7941
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 2.68
Closest, conservative, Table H statistic = 12.1 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 9
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 9.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

SUMMARY OF FISHERS EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	7.35%	10	0	
2	14.7%	10	0	
3	29.4%	10	0	
4	58.8%	10	0	
5	100%	10	0	

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	10	14.000	30.000	23.600
2	7.35%	10	14.000	31.000	26.500
3	14.7%	10	7.000	31.000	23.000
4	29.4%	10	13.000	31.000	21.100
5	58.8%	10	8.000	31.000	24.800
6	100%	10	9.000	32.000	25.100

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	19.822	4.452	1.408
2	7.35%	25.167	5.017	1.586
3	14.7%	47.111	6.864	2.171
4	29.4%	42.544	6.523	2.063
5	58.8%	46.400	6.812	2.154
6	100%	53.211	7.295	2.307

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	176.683	35.337	0.905
Within (Error)	54	2108.300	39.043	
Total	59	2284.983		

Critical F value = 2.45 (0.05,5,40)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	23.600	23.600		
2	7.35%	26.500	26.500	-1.038	
3	14.7%	23.000	23.000	0.215	
4	29.4%	21.100	21.100	0.895	
5	58.8%	24.800	24.800	-0.429	
6	100%	25.100	25.100	-0.537	

Dunnett table value = 2.31 (1 Tailed Value, P=0.05, df=40,5)

Connersville 5.24
File: ceriorep Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	10			
2	7.35%	10	6.455	27.4	-2.900
3	14.7%	10	6.455	27.4	0.600
4	29.4%	10	6.455	27.4	2.500
5	58.8%	10	6.455	27.4	-1.200
6	100%	10	6.455	27.4	-1.500

Discharger:	Connersville WWTP		**Test Dates:**	5/7/24 - 5/13/24			
Location:	Connersville, IN		**Analysts:**	MMB, MN, AF, MMK			

		Day							
Conc: Control		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.2	8.0	8.0	7.8	8.0	8.3		Template A
	Final	8.0	8.0	8.0	8.0	8.4	8.1		
pH	Initial	7.6	7.5	7.5	7.9	7.9	7.9		
	Final	7.5	7.5	7.8	7.6	7.7	7.9		
Alkalinity		40		40		40			
Hardness		100		110		90			
Conductivity		350		330		350			
Chlorine									

		Day							
Conc: 7.35%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.3	8.1	8.0	7.8	8.0	8.3		
	Final	7.8	8.0	8.0	8.0	8.4	8.1		
pH	Initial	7.6	7.5	7.5	7.9	7.9	7.9		
	Final	7.5	7.5	7.8	7.6	7.7	7.9		
Alkalinity									
Hardness									
Conductivity		350		330		350			
Chlorine									

		Day							
Conc: 14.7%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	8.2	8.1	7.8	8.1	8.4		
	Final	7.6	8.0	8.0	8.0	8.4	8.1		
pH	Initial	7.6	7.5	7.4	7.9	7.8	7.8		
	Final	7.4	7.5	7.8	7.6	7.7	7.8		
Alkalinity									
Hardness									
Conductivity		360		350		360			
Chlorine									

Discharger: Connersville WWTP
Location: Connersville, IN

Test Dates: 5/7/24 - 5/13 /24
Analysts: MMB, MN, AF, MMK

Conc: 29.4%		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.5	8.5	8.2	7.9	8.1	8.5		
	Final	7.6	7.8	7.9	8.0	8.3	8.1		
pH	Initial	7.5	7.5	7.4	7.8	7.8	7.8		
	Final	7.4	7.6	7.8	7.6	7.7	7.9		
Alkalinity									
Hardness									
Conductivity		420		410		400			
Chlorine									

Conc: 58.8%		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	8.6	8.3	8.0	8.3	8.7		
	Final	7.6	7.8	7.9	7.9	8.3	8.1		
pH	Initial	7.4	7.4	7.3	7.7	7.7	7.7		
	Final	7.5	7.6	7.8	7.6	7.7	7.9		
Alkalinity									
Hardness									
Conductivity		510		490		440			
Chlorine									

Conc: 100%		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	9.0	9.4	9.3	8.4	8.8	9.3		
	Final	7.5	7.8	7.9	7.8	8.2	8.1		
pH	Initial	7.4	7.3	7.3	7.5	7.5	7.5		
	Final	7.7	7.8	7.8	7.6	7.8	7.9		
Alkalinity		170		110		110			
Hardness		325		275		200			
Conductivity		640		680		580			
Chlorine		N.D.		ND		N.D.			
Ammonia		N.D.		ND		N.D			

Discharger: Connersville WWTP
Location: Connersville, IN

Test Dates 5/7/24 -5/14/24
Analysts: MMB, MN, AF, MMK

No. Surviving Organisms

Conc:	Rep. #	Day 1	2	3	4	5	6	7	Remarks
Control	A	10	10	10	10	10	10	9	
	B	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	
7.35%	A	10	10	10	10	10	10	10	
	B	10	10	10	9	9	9	9	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	9	9	
14.7%	A	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	
29%	A	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	
59%	A	10	10	10	10	10	10	10	
	B	10	9	9	9	9	9	9	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	
100%	A	10	10	10	10	10	10	10	
	B	9*	9	9	9	9	9	9	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	

Comments: Start Time: 1030

FHM Source: Biomonitor Lab Cultures

Connersville 5.24
File: fhmsurv Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.086

W = 0.823

Critical W (P = 0.05) (n = 24) = 0.916
Critical W (P = 0.01) (n = 24) = 0.884

Data FAIL normality test. Try another transformation.

Warning - The two homogeneity tests are sensitive to non-normal data and should not be performed.

Connersville 5.24
File: fhmsurv Transform: ARC SINE(SQUARE ROOT(Y))

Hartley test for homogeneity of variance
Bartletts test for homogeneity of variance

These two tests can not be performed because at least one group has zero variance.

Data FAIL to meet homogeneity of variance assumption.
Additional transformations are useless.

Connersville 5.24
File: fhmsurv Transform: ARC SINE(SQUARE ROOT(Y))

STEELS MANY-ONE RANK TEST - Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	RANK SUM	CRIT. VALUE	df	SIG
1	control	1.371				
2	7.35%	1.331	16.00	10.00	4.00	
3	14.7%	1.412	20.00	10.00	4.00	
4	29%	1.412	20.00	10.00	4.00	
5	59%	1.371	18.00	10.00	4.00	
6	100%	1.371	18.00	10.00	4.00	

Critical values use k = 5, are 1 tailed, and alpha = 0.05

Discharge:	Connersville WWTP	Test Date(s) :	5/7 -14/24	Drying Temp (°C):	100
Location:	Connersville, IN	Weighing Date:	5/15/24	Drying Time (h):	6
Analyst:	MMB, MN, AF				

Conc :	Rep. No.	Wgt. of boat (g)	Dry wgt: foil and larvae (g)	Total dry wgt of larvae (mg)	No. of larvae	Mean dry wgt of larvae (g)	Remarks
Control	A	0.91790	0.92090	3.00	9	0.300	
	B	0.91470	0.91770	3.00	10	0.300	
	C	0.91560	0.92010	4.50	10	0.450	
	D	0.91950	0.92290	3.40	10	0.340	
Conc : 7.35%	A	0.91550	0.91920	3.70	10	0.370	
	B	0.92570	0.92910	3.40	9	0.340	
	C	0.92720	0.93120	4.00	10	0.400	
	D	0.91710	0.92070	3.60	9	0.360	
Conc : 14.7%	A	0.91520	0.91910	3.90	10	0.390	
	B	0.91480	0.91890	4.10	10	0.410	
	C	0.92120	0.92550	4.30	10	0.430	
	D	0.91250	0.91620	3.70	10	0.370	
[illegible] : 29%	A	0.92090	0.92480	3.90	10	0.390	
	B	0.92280	0.92670	4.10	10	0.410	
	C	0.92330	0.92730	4.00	10	0.400	
	D	0.91510	0.91890	3.80	10	0.380	
Conc : 59%	A	0.91600	0.92000	4.00	10	0.400	
	B	0.91600	0.91950	3.50	9	0.350	
	C	0.92180	0.92550	3.70	10	0.370	
	D	0.91070	0.91410	3.40	10	0.340	
[illegible] : 100%	A	0.92610	0.92910	3.00	10	0.300	
	B	0.90530	0.90880	3.50	9	0.350	
	C	0.91170	0.91510	3.40	10	0.340	
	D	0.91190	0.91570	3.80	10	0.380	

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

Chi-square test for normality: actual and expected frequencies

INTERVAL	<-1.5	-1.5 to <-0.5	-0.5 to 0.5	>0.5 to 1.5	>1.5
EXPECTED	1.608	5.808	9.168	5.808	1.608
OBSERVED	0	8	10	6	0

Calculated Chi-Square goodness of fit test statistic = 4.1251
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 30.15
Closest, conservative, Table H statistic = 184.0 (alpha = 0.01)

Used for Table H ==> R (# groups) = 6, df (# reps-1) = 3
Actual values ==> R (# groups) = 6, df (# avg reps-1) = 3.00

Data PASS homogeneity test. Continue analysis.

NOTE: This test requires equal replicate sizes. If they are unequal but do not differ greatly, the Hartley test may still be used as an approximate test (average df are used).

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	0.300	0.450	0.348
2	7.35%	4	0.340	0.400	0.368
3	14.7%	4	0.370	0.430	0.400
4	29%	4	0.380	0.410	0.395
5	59%	4	0.340	0.400	0.365
6	100%	4	0.300	0.380	0.343

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.005	0.071	0.035
2	7.35%	0.001	0.025	0.012
3	14.7%	0.001	0.026	0.013
4	29%	0.000	0.013	0.006
5	59%	0.001	0.026	0.013
6	100%	0.001	0.033	0.017

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.011	0.002	2.000
Within (Error)	18	0.025	0.001	
Total	23	0.036		

Critical F value = 2.77 (0.05,5,18)
Since F < Critical F FAIL TO REJECT Ho:All groups equal

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	control	0.348	0.348		
2	7.35%	0.368	0.368	-0.894	
3	14.7%	0.400	0.400	-2.348	
4	29%	0.395	0.395	-2.124	
5	59%	0.365	0.365	-0.783	
6	100%	0.343	0.343	0.224	

Dunnett table value = 2.41 (1 Tailed Value, P=0.05, df=18,5)

Connersville 5.24
File: fhm_grow Transform: NO TRANSFORMATION

DUNNETTS TEST - TABLE 2 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	NUM OF REPS	Minimum Sig Diff (IN ORIG. UNITS)	% of CONTROL	DIFFERENCE FROM CONTROL
1	control	4			
2	7.35%	4	0.054	15.5	-0.020
3	14.7%	4	0.054	15.5	-0.052
4	29%	4	0.054	15.5	-0.048
5	59%	4	0.054	15.5	-0.017
6	100%	4	0.054	15.5	0.005

Discharger:	Connersville WWTP	**Test Dates:**	5/7/24 -5/14/24
Location:	Connersville, IN	**Analysts:**	MMB, MN, AF, MMK

		Day							
Conc: Control		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	7.6	7.3	7.7	7.6	7.9	9.0	
	Final	6.4	5.5	6.4	6.8	6.9	6.9	6.6	
pH	Initial	7.6	7.6	7.7	7.8	7.7	7.7	7.8	
	Final	7.6	7.7	7.6	7.6	7.7	7.9	7.3	
Alkalinity		40		40		40			
Hardness		100		110		90			
Conductivity		350		330		360			
Chlorine									

		Day							
Conc: 7.35%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.3	7.7	7.4	7.7	7.6	8.0	9.1	
	Final	6.4	5.5	6.4	6.8	6.9	6.8	6.6	
pH	Initial	7.6	7.6	7.7	7.8	7.7	7.7	7.7	
	Final	7.6	7.7	7.6	7.6	7.7	7.9	7.3	
Alkalinity									
Hardness									
Conductivity		350		340		360			
Chlorine									

		Day							
Conc: 14.7%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	7.8	7.5	7.6	7.7	8.1	9.2	
	Final	6.3	5.4	6.4	6.8	6.9	6.6	6.6	
pH	Initial	7.6	7.6	7.7	7.8	7.6	7.7	7.7	
	Final	7.6	7.7	7.6	7.6	7.7	7.8	7.3	
Alkalinity									
Hardness									
Conductivity		360		360		360			
Chlorine									

Discharger:	Connersville WWTP	**Test Dates:**	5/7/24 -5/14/24
Location:	Connersville, IN	**Analysts:**	MMB, MN, AF, MMK

		Day							
Conc: 29.4%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.5	7.9	7.6	7.7	7.8	8.3	9.2	
	Final	6.2	5.3	6.3	6.7	6.9	6.5	6.4	
pH	Initial	7.5	7.6	7.6	7.7	7.6	7.6	7.6	
	Final	7.6	7.6	7.6	7.6	7.7	7.8	7.4	
Alkalinity									
Hardness									
Conductivity		420		420		400			
Chlorine									

		Day							
Conc: 58.8%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	8.0	7.7	7.7	7.7	8.5	9.4	
	Final	6.1	5.3	6.3	6.7	6.9	6.7	6.5	
pH	Initial	7.4	7.5	7.5	7.6	7.5	7.5	7.5	
	Final	7.6	7.6	7.6	7.6	7.7	7.7	7.4	
Alkalinity									
Hardness									
Conductivity		510		530		490			
Chlorine									

		Day							
Conc: 100%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	9.0	8.3	8.1	8.1	8.3	9.0	9.8	
	Final	5.7	5.2	6.3	6.7	6.9	6.8	6.5	
pH	Initial	7.4	7.4	7.4	7.5	7.4	7.4	7.4	
	Final	7.5	7.5	7.6	7.7	7.6	7.7	7.4	
Alkalinity		120		110		110			
Hardness		325		275		200			
Conductivity		640		690		600			
Chlorine		N.D.		ND		N.D			
Ammonia		N.D.		ND		N.D			