

Biomonitor

Permittee/Location Kendallville WWTP Kendallville, IN			Permit Number: IN0020656 Noble Co.			Outfall Number: 003	
Laboratory Name and Contact: Biomonitor Michael Britton			Report Due Date:			Report Date: June 2024	
WETT Reporting Frequency or Type: (mark one)	Monthly	Quarterly	Semi-annual	Annual	TRE	Post TRE	<u>First</u> (per Reporting Frequency)
			X				

Test Organism	Test	Endpoint [1]	Units	Result	Compliance Value in TUs	Pass/Fail	Reporting			
<i>Ceriodaphnia dubia</i>	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 <u>and</u> NetDMR (Parameter Code 61425)
		Toxicity (chronic)	TU _c	1				1	Pass	Laboratory Report <u>and</u> NetDMR (Parameter Code 61426)

<i>Pimephales promelas</i>	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 <u>and</u> NetDMR (Parameter Code 61427)
		Toxicity (chronic)	TU _c	1				1	Pass	Laboratory Report <u>and</u> NetDMR (Parameter Code 61428)

Biomonitor

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

*Whole Effluent
Toxicity Test*

KENDALLVILLE UTILITIES
WASTEWATER TREATMENT PLANT

IN0020656

Kendallville, Indiana

June 2024

GLP (Good Laboratory Practices)
COMPLIANCE STATEMENT

Project Name: Kendallville Wastewater Treatment Plant

Project Date: June 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: 6/19/24

Michael Britton

Project Director
Date: 6/19/24

Other Participating Personnel:

Mukang'andu Ng'andwe
Arizona Fox
Melody Myers-Kinzie

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 Kendallville, IN Wastewater Treatment Plant during June 2024. The purpose of the testing was to fulfill the biomonitoring requirement for the NPDES permit.

Three samples were collected June 3-7, 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 LC₅₀ 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) of 100% effluent (a TU_c not to exceed 1.0). 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:	IN0020656
Toxicity testing requirements:	Fathead minnow larval survival and growth test Ceriodaphnia survival and reproduction test
Plant location:	Kendallville Wastewater Treatment Plant 501 W. Wayne Street Kendallville, Indiana 46656
Name of receiving water body:	Unnamed tributary to Henderson Lake
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:	Class III, Single-stage nitrification activated sludge treatment.
Design flow:	2.68 – MGD
Volume of wastewater flow during the sampling period:	June 3, 2024 -MGD June 5, 2024 -MGD June 7, 2024 -MGD

Table III
Source of effluent and dilution water

I. Effluent samples

Sampling point:	Outfall 003	
Collection dates and times:	June 3, 2024	6:00 a.m.
	June 5, 2024	6:00 a.m.
	June 7, 2024	6:00 a.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:	<i>Ceriodaphnia</i> 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 eight days because control animals did not produce an average of greater than 15 young per female by until day eight.	
Date and time test initiated:	June 4, 2024	1:00 p.m.
Date and time test terminated	June 12, 2024	2:00 p.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**

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

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

Raw Data:

See Table 8

LC₅₀ or NOEL obtained:48-hr LC₅₀ = greater than 100% effluent

NOEL for survival = 100% effluent

NOEL for reproduction = 100% effluent

Control survival was 90% after eight 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**

<u>Reference Toxicant used and source:</u>	Copper chloride, reagent grade, from Carolina Biological
<u>Date and time of most recent test:</u>	May 28 – June 3, 2024
<u>Dilution water used in test:</u>	Moderately hard synthetic water
<u>Results:</u>	48-hr LC ₅₀ = 87 µg/L as Cu NOEL (reproduction) = 20 µg/L as Cu LOEL (reproduction) = 40 µg/L as Cu
<u>Comparison to recommended range:</u>	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	14.1	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	0	0	0	0	0	0		10
	4	0	0	0	0	0	0	0	0	0	0		10
	5	0	0	2	0	2	2	2	2	2	3		10
	6	2	4	0	4	4	2	5	4	3	3		10
	7	3-	8	4	5	0	0	0	3	0	0		9
	8	-	10	9	11	6	7	5	7	9	8		9
6.25%	1	0	0	0	0	0	0	0	0	0	0	13.0	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	0	0	0	0	0	0		10
	4	0	0	0	0	0	1	0	0	0	0		10
	5	2	5	0	0	4	0	2	2	3	2		10
	6	0	0	2	2	0	5	3	2	0	0		10
	7	2	8	5	4	4	0	9	5	2	2		10
	8	5	9	7	6	5	11	0	0	4	7		10
12.5%	1	0	0	0	0	0	0	0	0	0	0	17.4	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	0	0	0	0	0	0		10
	4	0	0	0	1	0	1	1	2	1	0		10
	5	3	0	2	0	0	0	0	0	0	0		10
	6	0	2	4	4	2	2	3	4	4	4		10
	7	5	6	8	9	4	5	4	8	6	6		10
	8	8	8	0	9	6	8	14	9	7	4		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		
25%	1	0	0	0	0	0	0	0	0	0	0	19.8	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	0	0	0	0	0	0		10
	4	3	3	1	2	0	0	2	0	0	1		10
	5	0	3	6	0	3	0	0	2	0	0		10
	6	0	0	0	4	2	3	3	4	2	3		10
	7	4	8	7	7	8	7	7	10	5	7		10
	8	8	13	11	10	0	11	8	0	9	11		10
50%	1	0	0	0	0	0	0	0	0	0	0	22.1	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	0	0	0	0	0	0		10
	4	2	3	2	1	2	1	5	2	2	0		10
	5	3	5	0	0	0	0	0	0	4	2		10
	6	4	0	4	5	6	3	6	3	0	4		10
	7	0	10	8	12	7	8	7	8	8	6		10
	8	7	14	4	11	10	6	8	6	12	0		10
100%	1	0	0	0	0	0	0	0	0	0	0	15.1	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	0	0	0	0	0	0		10
	4	2	3	2	2	2	2	2	3	1	0		10
	5	3	5	4	0	0	0	0	0	0	0		10
	6	0	0	4	2	5	4	6	3	4	6		10
	7	6	6	0	4	6	5	3	6	6	-		9
	8	5	6	10	0	6	-	0	10	7	-		8

Table IX
WATER CHEMISTRY
Ceriodaphnia Survival and Reproduction Test

Effluent Concentration	D.O. <u>Range</u> mg/L	Temp. <u>Range</u> °C	pH <u>Range</u> S.U.	Alk. <u>Range</u> CaCO₃	Hardness <u>Range</u> CaCO₃	Cond. <u>Range</u> µS
CONTROL	7.7 – 8.6	25	7.4 – 8.0	30-40	100-110	320-
6.25%	7.7 – 8.6	25	7.4 – 8.0	/	/	350-360
25%	7.7 – 8.4	25	7.5 – 8.2	/	/	440-470
100%	7.6 – 8.5	25	7.5 – 8.4	160-180	250-275	810-860

FATHEAD MINNOW LARVAL SURVIVAL AND GROWTH TEST

Table X
METHODOLOGY
Fathead Minnow Larval Survival and Growth Test

<u>Toxicity test method used:</u>	7-day fathead minnow larval survival and growth test	
<u>Endpoints of test:</u>	96-hr LC ₅₀ and no observable effect level (NOEL) for survival and growth. TU _c for survival and growth.	
<u>Reference method:</u>	EPA-821-R-02-013	
<u>Deviations from method:</u>	No Deviations.	
<u>Date and time test initiated:</u>	June 4, 2024	2:00 p.m.
<u>Date and time test terminated</u>	June 11, 2024	2:00 p.m.
<u>Type of test chambers:</u>	Polyethylene	300 ml
<u>Volume of solution used per chamber:</u>	250 ml	
<u>Number of organisms per chamber:</u>	ten	
<u>Number of replicate chambers per treatment:</u>	four	
<u>Test temperature range:</u>	25°C (no deviations)	

Table XI
ORGANISMS USED
Fathead Minnow Survival and Growth Test

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

Table XII
RESULTS
Fathead Minnow Larval Survival and Growth Test

<u>Raw Data:</u>	See Table 14
<u>LC₅₀ or NOEL obtained:</u>	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
<u>Methods used to calculate endpoints:</u>	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

<u>Reference Toxicant used and source:</u>	Potassium chloride, reagent grade, from Sigma-Aldrich
<u>Date and time of most recent test:</u>	May 28 - June 4, 2024
<u>Dilution water used in test:</u>	Moderately Hard Synthetic Water
<u>Results:</u>	96-hr LC ₅₀ = 1169 mg /L as KCl NOEL (growth) = 500 mg/L as KCl LOEL (growth) = 1000 mg/L as KCl
<u>Comparison to recommended range:</u>	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	<u>% Survival in Each Replicate</u>				<u>Average Dry Weight (μg) in Each Replicate</u>			
	A	B	C	D	A	B	C	D
Control	100	100	100	100	280	350	390	340
6.25%	100	100	100	70	470	400	370	280
12.5%	100	100	60	100	310	250	160	280
25%	90	100	100	100	330	280	280	270
50%	100	100	100	80	310	350	340	320
100%	100	100	100	70	300	320	420	350

Table XV
WATER CHEMISTRY
Fathead Minnow Larval Survival and Growth Test

Effluent Concentration	D.O. <u>Range</u> mg/L	Temp. <u>Range</u> °C	pH <u>Range</u> S.U.	Alk. <u>Range</u> CaCO₃	Hardness <u>Range</u> CaCO₃	Cond. <u>Range</u> µS
CONTROL	5.5 – 8.5	25	7.4 – 7.9	30-40	100-110	300-330
6.25%	5.5 – 8.5	25	7.4 – 7.8	/	/	330-360
25%	5.0 – 8.6	25	7.5 – 7.7	/	/	440-460
100%	4.2 – 8.8	25	7.5 – 7.9	160-180	250-275	810-870

Biomonitor

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Indianapolis, IN 46231
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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Kendallville WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Kendallville - 1 Monday June 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 6-2-24 Start Time 8am

End Date 6-3-24 End Time 1pm

NAME OF PERSON COLLECTING SAMPLE: Jordan Underen

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: [Signature]

Date: 6.3.24 Time: 7:15am

Received by: [Signature]

Date: 6/3/24 Time: 11:55a

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: 11.1 °C

COMMENTS:

Biomonitor

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317-297-7713
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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Kendallville WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Kendallville - 2 Wednesday June 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 6.4.24 Start Time 8am

End Date 6.5.24 End Time 6am

NAME OF PERSON COLLECTING SAMPLE: Jordan Underen

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: [Signature]

Date: 6.5.24 Time: 7:15am

Received by: [Signature]

Date: 6/5/24 Time: 12:04 p

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: 14.3 °C

COMMENTS:

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Kendallville WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Kendallville - 3 Friday June 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 6.6.24 Start Time 8am

End Date 6.7.24 End Time 6am

NAME OF PERSON COLLECTING SAMPLE: Jordan Underen

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: Kristal Furlow

Date: 6.7.24 Time: 9am

Received by: Mr. Ree

Date: 6/7/24 Time: 12:18

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: _____ °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.)	
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	20	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	20	40	30	
02/24	86	40	80	48	
03/24	80	40	80	48	
04/24	80	40	80	51	
06/24	87	20	40	32	
<u>Average</u>	88	<u>Mode</u>	40	80	44
<u>St. Dev.</u>	14				11
<u>Upper Limit</u>	115		80	160	67
<u>Lower Limit</u>	60		20	40	21

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	
06/24	1169	500	1000	899	
<u>Average</u>	1071	<u>Mode</u>	500	1000	841
<u>St. Dev.</u>	130				164
<u>Upper Limit</u>	1330	1000	2000	1168	
<u>Lower Limit</u>	812	250	500	514	

Client: Kendallville WWTP

Project # _____

Analysts: MMB, MN, AF, MMK

Test Dates

Start Date: 6/4/2024

Start Time: 1300

End Date: 6/12/24

End Time: 1400

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	0	0	0	0	0	0
		4	0	0	1	0	0	0
		5	3	0	0	2	2	0
		6	3	6	3	0	4	4
		7	0	1	7	2	6	6
		8		-	11	7	0	4
Row 9	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	1	0	2	0	0	1
		5	0	0	4	3	2	0
		6	4	2	0	0	1+2	4
		7	6	5	8	2	0	6
		7	7	9	12	4	9	7
Row 8	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	0	2	3	2	0	0
		5	2	0	0	0	2	2
		6	2	3	3	4	4	4
		7	5	8	6	8	3	10
		0	0	6	10	9	7	0
Row 7	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	2	0	0	2	1	2+3
		5	0	2	2	0	0	0
		6	3	3	3+2	6	3	6
		7	7	9	0	3	4	7
		8	8	0	5	0	14	8
Row 6	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	0	2	1	1	0	1
		5	0	0	0	0	2	0
		6	3	4	2	3	2	4+1
		7	7	5	5	8	0	0
		11	1	8	6	7	11	
Row 5	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	0	2	0	0	0	2
		5	2+2	0	0	2	3	0
		6	0	6	2	2+2	2	5
		7	4	7	3+1	0	8	6
		5	5	10	6	6	0	6

Row 4	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	2	0	2	0	1	1
		5	0	0	0	0	0	0
		6	4	2	2	2+2	4	5
		7	7	4	4	5	9	12
			10	6	0	11	9	11
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	0	2	1	0	2	0
		5	2	4	6	0	0	2
		6	4	4	0	2	4	0
		7	8	0	7	5	8	4
			0	10	11	7	4	9
Row 2	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	0	3	3	3	0	0
		5	0	5	5	3	0	5
		6	2	0	0	0	4	0
		7	6	10	6	8	8	8
			8	14	6	13	10	9
Row 1	Day	1	0	0	0	0	0	0
		2	0	0	0	0	0	0
		3	0	0	0	0	0	0
		4	2	0	0	1+2	2	0
		5	3	0	2	0	3	3
		6	4	2	0	0	0	0
		7	0	3	2	4	6	5
			7	—	5	8	5	8

Discharger: Kendallville WWTP Analyst: MMB, MMK, MN, AF

Location: Kendallville, IN Test Start- Date/Time: 6/4/2024 / 1300

Date Sample Collected: 6/3,5,7/24 Test Stop- Date/Time: 6/12/2024 / 1400

Conc.	Day	Replicate										No. of Young Adults	No. of Adults	Young per Adult		
		1	2	3	4	5	6	7	8	9	10					
Control	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	4	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	5	0	0	2	0	2	2	2	2	2	2	2	2	3	15	1.5
	6	2	4	0	4	4	2	5	4	3	3	3	3	3	31	3.1
	7	3/	8	4	4	5	0	0	3	0	0	0	0	0	23	2.3
	8	0/	10	9	11	6	7	5	7	9	8	14	14	14	72	7.2
Total	5	22	15	20	12	11	12	16	14	14	14	14	14	141	9	14.1

> 15 per surviving female.

Conc.	Day	Replicate										No. of Young Adults	No. of Adults	Young per Adult			
		1	2	3	4	5	6	7	8	9	10						
6%	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0.0	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	
	4	0	0	0	0	0	1	0	0	0	0	0	0	0	1	0.1	
	5	2	5	0	0	4	0	2	2	3	2	2	2	2	20	10	2.0
	6	0	0	2	2	0	5	3	2	0	0	0	0	0	14	10	1.4
	7	2	8	5	4	4	0	9	5	2	2	2	2	2	41	10	4.1
	8	5	9	7	6	5	11	0	0	4	7	7	7	7	54	10	5.4
Total	9	22	14	12	13	17	14	9	9	9	9	9	11	130	10	13.0	

Conc.	Day	Replicate										No. of Young Adults	No. of Adults	Young per Adult			
		1	2	3	4	5	6	7	8	9	10						
12%	1	0	0	0	0	0	0	0	0	0	0	0	0	10	0	0.0	
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	
	3	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0	
	4	0	0	0	1	0	1	1	2	1	0	0	0	0	6	10	0.6
	5	3	0	2	0	0	0	0	0	0	0	0	0	0	5	10	0.5
	6	0	2	4	4	2	2	3	4	4	4	4	4	4	29	10	2.9
	7	5	6	8	9	4	5	4	8	6	6	6	6	6	61	10	6.1
	8	8	8	0	9	6	8	14	9	7	4	4	4	4	73	10	7.3
Total	16	16	14	23	12	16	22	23	18	14	14	14	14	174	10	17.4	

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
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	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	4	3	3	1	2	0	0	2	0	0	0	1	12	1.2
	5	0	3	6	0	3	0	0	2	0	0	0	14	1.4
	6	0	0	0	4	2	3	3	4	2	3	21	10	2.1
	7	4	8	7	7	8	7	7	10	5	7	70	10	7.0
	8	13	11	11	10	0	11	8	0	9	11	81	10	8.1
Total	15	27	25	23	13	21	20	16	16	22	198	10	19.8	

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
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	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	4	2	3	2	1	2	1	5	2	2	0	20	10	2.0
	5	3	5	0	0	0	0	0	0	4	2	14	10	1.4
	6	4	0	4	5	6	3	6	3	0	4	35	10	3.5
	7	0	10	8	12	7	8	7	8	8	6	74	10	7.4
	8	7	14	4	11	10	6	8	6	12	0	78	10	7.8
Total	16	32	18	29	25	18	26	19	26	12	221	10	22.1	

Conc.	Day	Replicate										No. of Young	No. of Adults	Young per Adult
		1	2	3	4	5	6	7	8	9	10			
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	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	4	2	3	2	2	2	2	2	3	1	0	19	10	1.9
	5	3	5	4	0	0	0	0	0	0	0	12	10	1.2
	6	0	0	4	2	5	4	6	3	4	6	34	10	3.4
	7	6	6	0	4	6	5	3	6	6	0	42	9	4.2
	8	5	6	10	0	6	0	0	10	7	0	44	8	4.4
Total	16	20	20	8	19	11	11	22	18	6	151	8	15.1	

Kendallville 6.24
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	3	17	21	15	4

Calculated Chi-Square goodness of fit test statistic = 0.8592
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Kendallville 6.24
File: ceriorep Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 2.58
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	1	
1	6%	10	0	
2	12%	10	0	
3	25%	10	0	
4	50%	10	0	
5	100%	10	2	

Kendallville 6.24

File: ceriorep

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	10	5.000	22.000	14.100
2	6.25%	10	9.000	22.000	13.000
3	12.5%	10	12.000	23.000	17.400
4	25%	10	13.000	27.000	19.800
5	50%	10	12.000	32.000	22.100
6	100%	10	6.000	22.000	15.100

Kendallville 6.24

File: ceriorep

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	22.544	4.748	1.501
2	6.25%	16.889	4.110	1.300
3	12.5%	15.822	3.978	1.258
4	25%	21.511	4.638	1.467
5	50%	40.767	6.385	2.019
6	100%	31.878	5.646	1.785

Kendallville 6.24

File: ceriorep

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	619.883	123.977	4.979
Within (Error)	54	1344.700	24.902	
Total	59	1964.583		

Critical F value = 2.45 (0.05,5,40)

Since $F > \text{Critical } F$ REJECT H_0 :All groups equal

Kendallville 6.24

File: cericrep

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	14.100	14.100		
2	6.25%	13.000	13.000	0.493	
3	12.5%	17.400	17.400	-1.479	
4	25%	19.800	19.800	-2.554	
5	50%	22.100	22.100	-3.585	
6	100%	15.100	15.100	-0.448	

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

Kendallville 6.24

File: cericrep

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	6.25%	10	5.155	36.6	1.100
3	12.5%	10	5.155	36.6	-3.300
4	25%	10	5.155	36.6	-5.700
5	50%	10	5.155	36.6	-8.000
6	100%	10	5.155	36.6	-1.000

Discharger: Kendallville WWTP
 Location: Kendallville, IN

Test Dates: 6/4/24 - 6/12/24
 Analysts: MMB, MN, AF, MMK

		Day							Remarks
Conc:	25%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	8.4	8.2	8.4	8.3	8.3	7.9	
	Final	8.1	8.2	8.4	8.2	8.1	7.7	7.7	
pH	Initial	7.6	7.5	7.7	7.6	7.6	7.6	7.8	
	Final	7.8	8.0	8.1	8.1	8.2	8.0	8.0	
Alkalinity									
Hardness									
Conductivity		440		460		470			
Chlorine									

		Day							Remarks
Conc:	50%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.3	8.4	8.2	8.5	8.3	8.3	8.0	
	Final	8.1	8.1	8.5	8.2	8.2	7.7	7.6	
pH	Initial	7.5	7.6	7.6	7.5	7.5	7.5	7.7	
	Final	7.9	8.2	8.1	8.2	8.3	8.1	8.0	
Alkalinity									
Hardness									
Conductivity		560		610		600			
Chlorine									

		Day							Remarks
Conc:	100%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	8.4	8.3	8.5	8.3	8.2	8.0	
	Final	8.1	8.2	8.5	8.2	8.3	7.7	7.6	
pH	Initial	7.5	7.6	7.6	7.5	7.5	7.5	7.7	
	Final	8.1	8.2	8.1	8.2	8.3	8.4	8.3	
Alkalinity		160		160		180			
Hardness		275		250		250			
Conductivity		810		860		840			
Chlorine		N.D.		N.D.		N.D.			
Ammonia		N.D.		N.D.		N.D.			

Discharger: Kendallville WWTP
 Location: Kendallville, IN

Test Dates 6/4/24 -6/11/24
 Analysts: MMB, MN, AF, MMK

		No. Surviving Organisms							Remarks
Conc:	Rep. #	Day							
		1	2	3	4	5	6	7	
Control	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	
6.25%	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	9	7	7	7	7	
12.5%	A	10	10	10	10	10	10	10	
	B	10	10	10	10	10	10	10	
	C	10	10	10	10	10	7	6	
	D	10	10	10	10	10	10	10	
25%	A	10	10	9	9	9	9	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	
50%	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	8	8	8	8	8	8	
100%	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	7	7	7	7	7	

Comments: Start Time: 1400

FHM Source: Biomonitor Lab Cultures

Kendallville 6.24

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

Shapiro Wilks test for normality

D = 0.563

W = 0.757

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.

Kendallville 6.24

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

Hartley test for homogeneity of variance

Bartlett's 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.

Kendallville 6.24

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	1.412				
2	6.25%	1.307	16.00	10.00	4.00	
3	12.5%	1.281	16.00	10.00	4.00	
4	25%	1.371	16.00	10.00	4.00	
5	50%	1.336	16.00	10.00	4.00	
6	100%	1.307	16.00	10.00	4.00	

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

Discharge: Kendallville WWTP Test Date(s) : 6/4-11/24 Drying Temp (°C): 100
 Location: Kendallville, IN Weighing Date: 6/12/24 Drying Time (h): 6
 Analyst: MMB, MIN, MMK

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	0.91670	0.92050	2.80	10	0.280	
	B	0.92870	0.93320	3.50	10	0.350	
	C	0.92110	0.92600	3.90	10	0.390	
	D	0.92450	0.92890	3.40	10	0.340	
Conc: 6.25%	A	0.92980	0.93550	4.70	10	0.470	
	B	0.91640	0.92140	4.00	10	0.400	
	C	0.92280	0.92750	3.70	10	0.370	
	D	0.92810	0.93190	2.80	7	0.280	
Conc: 12.5%	A	0.92420	0.92830	3.10	10	0.310	
	B	0.91900	0.92250	2.50	10	0.250	
	C	0.91330	0.91590	1.60	6	0.160	
	D	0.92490	0.92870	2.80	10	0.280	
Conc: 25%	A	0.91670	0.92100	3.30	9	0.330	
	B	0.91720	0.92100	2.80	10	0.280	
	C	0.91840	0.92220	2.80	10	0.280	
	D	0.92260	0.92630	2.70	10	0.270	
Conc: 50%	A	0.92030	0.92440	3.10	10	0.310	
	B	0.92690	0.93140	3.50	10	0.350	
	C	0.92360	0.92800	3.40	10	0.340	
	D	0.91900	0.92320	3.20	8	0.320	
Conc: 100%	A	0.92450	0.92850	3.00	10	0.300	
	B	0.91810	0.92230	3.20	10	0.320	
	C	0.91640	0.92160	4.20	10	0.420	
	D	0.91920	0.92370	3.50	7	0.350	

Kendallville 6.24
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	9	7	0

Calculated Chi-Square goodness of fit test statistic = 4.2910
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Kendallville 6.24
File: fhm_grow Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 18.60
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).

Kendallville 6.24

File: fhm_grow

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	0.280	0.390	0.340
2	6.25%	4	0.280	0.470	0.380
3	12.5%	4	0.160	0.310	0.250
4	25%	4	0.270	0.330	0.290
5	50%	4	0.310	0.350	0.330
6	100%	4	0.300	0.420	0.348

Kendallville 6.24

File: fhm_grow

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.002	0.045	0.023
2	6.25%	0.006	0.079	0.039
3	12.5%	0.004	0.065	0.032
4	25%	0.001	0.027	0.014
5	50%	0.000	0.018	0.009
6	100%	0.003	0.053	0.026

Kendallville 6.24

File: fhm_grow

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.042	0.008	2.667
Within (Error)	18	0.049	0.003	
Total	23	0.091		

Critical F value = 2.77 (0.05,5,18)

Since $F < \text{Critical } F$ FAIL TO REJECT H_0 :All groups equal

Kendallville 6.24

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	0.340	0.340		
2	6.25%	0.380	0.380	-1.033	
3	12.5%	0.250	0.250	2.324	
4	25%	0.290	0.290	1.291	
5	50%	0.330	0.330	0.258	
6	100%	0.348	0.348	-0.194	

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

Kendallville 6.24

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	6.25%	4	0.093	27.5	-0.040
3	12.5%	4	0.093	27.5	0.090
4	25%	4	0.093	27.5	0.050
5	50%	4	0.093	27.5	0.010
6	100%	4	0.093	27.5	-0.008

Discharger: Kendallville WWTP
 Location: Kendallville, IN

Test Dates: 6/4/24 -6/11/24
 Analysts: MMB, MN, AF, MMK

		Day							Remarks
Conc:	25%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	8.6	8.4	8.1	8.1	8.1	8.1	
	Final	7.1	7.2	7.1	5.8	6.4	5.0	6.6	
pH	Initial	7.6	7.5	7.6	7.6	7.6	7.7	7.6	
	Final	7.6	7.6	7.6	7.5	7.7	7.6	7.5	
Alkalinity									
Hardness									
Conductivity		440		460		460			
Chlorine									

		Day							Remarks
Conc:	50%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.3	8.6	8.5	8.0	8.0	8.2	8.1	
	Final	7.1	7.1	6.9	5.8	6.3	4.9	6.3	
pH	Initial	7.5	7.5	7.5	7.6	7.6	7.6	7.6	
	Final	7.7	7.7	7.6	7.6	7.7	7.7	7.6	
Alkalinity									
Hardness									
Conductivity		560		620		590			
Chlorine									

		Day							Remarks
Conc:	100%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	8.8	8.7	8.1	8.0	8.3	7.8	
	Final	7.1	7.1	6.5	5.9	5.3	4.2	6.1	
pH	Initial	7.5	7.5	7.5	7.5	7.6	7.6	7.6	
	Final	7.9	7.9	7.9	7.9	7.9	7.9	7.9	
Alkalinity		160		160		180			
Hardness		275		250		250			
Conductivity		810		870		850			
Chlorine		N.D.		N.D.		N.D.			
Ammonia		N.D.		N.D.		N.D.			