Biomonitor

Permittee/Location	**Permit Number:**	**Outfall Number:**
Huntington WWTP Huntington, IN	IN0023132 Huntington 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	1.5	**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	1.5	**Pass**	Laboratory Report and **NetDMR** (Parameter Code 61428)

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

Whole Effluent Toxicity Test

HUNTINGTON
WASTEWATER TREATMENT PLANT

IN0023132

Huntington, Indiana

April 2024

GLP (Good Laboratory Practices)
COMPLIANCE STATEMENT

Project Name: Huntington Wastewater Treatment Plant

Project Date: April 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: 5/9/24

Michael Britton

Project Director
Date: 5/9/24

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

Three samples were collected April 21-25, 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) of 67% effluent (a TU_c not to exceed 1.5). 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:	IN0023132
Toxicity testing requirements:	Fathead minnow larval survival and growth test Ceriodaphnia survival and reproduction test
Plant location:	Huntington Wastewater Treatment Plant 20 Hitzfield Street Extended Huntington, Indiana 46750
Name of receiving water body:	Wabash 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:	7.5 – MGD	
Volume of wastewater flow during the sampling period:	April 21, 2024	5.25 MGD
	April 23, 2024	9.20 MGD
	April 25, 2024	6.72 MGD

Table III
Source of effluent and dilution water

I. Effluent samples

Sampling point:	Outfall 001	
Collection dates and times:	April 21, 2024	11:59 p.m.
	April 23, 2024	11:59 p.m.
	April 25, 2024	11:59 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:	April 23, 2024	10:30 a.m.
Date and time test terminated	April 29, 2024	10:17 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 surviving 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	16.3	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	3	2	0	3	3	0	2		10
	4	2	3	3	4	5	1	7	6	2	7		10
	5	6	5	7	8	0	6	0	0	4	0		10
	6	6	8	9	0	9	10	11	12	3	6		10
8.5%	1	0	0	0	0	0	0	0	0	0	0	18.0	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	2	0	0	4	2	0	4	4	2	4		10
	4	8	3	4	9	5	4	7	4	6	6		10
	5	0	7	6	0	6	7	0	0	0	0		10
	6	0	11	11	0	0	13	11	10	7	13		10
17%	1	0	0	0	0	0	0	0	0	0	0	20.8	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	2	4	3	3	3	0	4	4	3	4		10
	4	7	0	5	8	5	4	0	7	8	0		10
	5	0	10	0	8	0	8	7	0	0	7		10
	6	0	13	11	0	9	13	15	12	15	6		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		
33%	**1**	0	0	0	0	0	0	0	0	0	0	22.7	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	4	0	4	2	2	2	4	4	3	4		10
	4	7	3	8	8	7	4	5	0	6	9		10
	5	6	7	0	9	0	8	0	8	0	0		10
	6	0	11	12	0	11	13	12	12	16	16		10
67%	**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	4	0	2	4	0	4	4	0	5		10
	4	0	0	3	8	7	4	8	8	2	0		10
	5	7	9	9	8	0	9	0	0	7	7		10
	6	13	14	11	0	13	17	10	13	10	12		10
100%	**1**	0	0	0	0	0	0	0	0	0	0	24.0	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	4	4	4	4	3	0	5	4	4	4		10
	4	7	0	8	9	6	6	9	8	0	8		10
	5	6	8	0	9	0	6	0	0	6	0		10
	6	0	12	15	0	12	17	13	16	9	14		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	7.9 – 8.4	25	7.7 – 8.1	40-	100-120	320-350
8.5%	7.9 – 8.5	25	7.7 – 8.0			340-370
33%	7.8 – 8.7	25	7.6 – 8.0			450-460
100%	7.5 – 9.4	25	7.5 – 8.2	120-180	225-300	690-730

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:	April 23, 2024	10:00 a.m.
Date and time test terminated	April 30, 2024	10:00 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 Lab 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 assumption 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	100	100	100	100	240	340	340	370
8.5%	100	100	100	100	350	310	340	370
17%	100	100	100	100	290	320	330	320
33%	90	100	100	100	280	300	310	290
67%	100	100	100	100	360	320	350	320
100%	100	100	100	100	420	390	320	330

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	6.6 – 8.4	25	7.7 – 8.2	40-	100-120	350-
8.5%	6.5 – 8.5	25	7.7 – 8.2			360-370
33%	5.8 – 8.7	25	7.6 – 8.2			460-470
100%	4.8 – 9.4	25	7.5 – 8.1	120-180	225-300	710-750

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Huntington WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Huntington – 1 Monday Apr. 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 4-21-2024 Start Time 12:01AM

End Date 4-21-2024 End Time 11:59PM

NAME OF PERSON COLLECTING SAMPLE: Jerome w Livernois

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: 4 °C Refrigerated/iced

PRESERVATIVES: none

Relinquished by: _____

Date: 4/22/24 Time: 0800

Received by: RPola

Relinquished

Date: 4-22-24 Time: 10:20 c

Received by

~~Relinquished~~ by: _____

Date: 4/22/24 Time: 10:20 c

Received by: _____

Date: _____ Time: _____

TEMP: 9.2 °C

COMMENTS: Flow 5.25 MG

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Huntington WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Huntington – 2 Wednesday Apr. 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 4-23-2024 Start Time 12:01 AM

End Date 4-23-2024 End Time 11:59 PM

NAME OF PERSON COLLECTING SAMPLE: TONY ADAMS

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE FLOW 9.20 MG

SAMPLE STORAGE: 4°C (Refrigerated)/iced

PRESERVATIVES: none

Relinquished by: TL

Date: 4.24.24 Time: 8:00AM

Received by: RBola

Date: 4-24-2024 Time: 8:00 AM

Relinquished by: RBola

Date: 4/24/2024 Time: 10:45 a

Received by: QMC

Date: 4/24/24 Time: 10:45 a

TEMP: 6.9 °C

COMMENTS:

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SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: Huntington WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: Huntington – 3 Friday Apr. 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 4-25-2024 Start Time 12:01 AM

End Date 4-25-2024 End Time 11:59 PM

NAME OF PERSON COLLECTING SAMPLE: Angie Brubaker

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: 4°C (Refrigerated)/iced

Flow 6.72 MG

PRESERVATIVES: none

Relinquished by: Angie Brubaker

Date: 4-26-24 Time: 9:00AM

Received by: RBole

Date: 4-26-24 Time: 9:06 AM

Relinquished by: RBole

Date: 4-26-24 Time: 10:30AM

Received by: [signature]

Date: 4/26/24 Time: 10:30 a

TEMP: 8 °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: Huntington WWTP

Project #

Analysts: MMB, MN, AF

Test Dates

Start Date: 4/23/2024

Start Time: 1030

End Date: 4/29/2024

End Time: 1017

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	2	4	4	4	4+1	4
4	7	8	9	6	0	0
5	0	0	0	0	7	7
6	6	14	16	13	12	6
7						

Row 9

Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	4	3	0	2	0	3
4	0	5+1	2	6	2	8
5	6	0	7	0	4	0
6	9	16	10	9	3	15
7						

Row 8

Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	4	4	4	4	3	4
4	4	8	8	7	5+1	0
5	0	0	0	0	0	8
6	10	13	16	12	12	12
7						

Row 7

Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	4	4	3	5	4	4
4	5	7	7	9	0	8
5	0	0	0	0	7	0
6	12	11	11	13	15	10
7						

Row 6

Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	2	0	0	0	0	0
4	4	6	4	4	1	4
5	8	6	8	9	6	7
6	13	17	13	17	10	13
7						

Row 5

Day						
1	0	0	0	0	0	0
2	0	0	0	0	0	0
3	2	4	3	2	2	3
4	5	7	5	5	6+1	6
5	6	0	0	0	0	0
6	0	13	9	9	11	12
7						

Row 4	Day						
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	2	4	4	3	3	2
	4	8	9	9	4	4	9
	5	9	0	9	8	8	8
	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	3	4	4	0	0	0
	4	5	8	8	4	3	3
	5	0	0	0	6	9	7
	6	11	15	12	11	11	9
	7						

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

Row 1	Day						
	1	0	0	0	0	0	0
	2	0	0	0	0	0	0
	3	4	0	2	4	4	2
	4	0	2	6+2	7	7	5+2
	5	9	6	0	6	6	0
	6	13	6	0	0	0	0
	7						

Discharger:	Huntington WWTP	**Analyst:**	MMB, MN, AF
Location:	Huntington, IN	**Test Start- Date/Time:**	4/23/24 / 1030
Date Sample Collected:	4/21,23,25/24	**Test Stop- Date/Time:**	4/29/24 / 1017

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	0	0	0	3	2	0	3	3	0	2	13	10	1.3
	4	2	3	3	4	5	1	7	6	2	7	40	10	4.0
	5	6	5	7	8	0	6	0	0	4	0	36	10	3.6
	6	6	8	9	0	9	10	11	12	3	6	74	10	7.4
	Total	14	16	19	15	16	17	21	21	9	15	163	10	16.3

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
8.5%	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	0	0	4	2	0	4	4	2	4	22	10	2.2
	4	8	3	4	9	5	4	7	4	6	6	56	10	5.6
	5	0	7	6	0	6	7	0	0	0	0	26	10	2.6
	6	0	11	11	0	0	13	11	10	7	13	76	10	7.6
	Total	10	21	21	13	13	24	22	18	15	23	180	10	18.0

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
17%	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	4	3	3	3	0	4	4	3	4	30	10	3.0
	4	7	0	5	8	5	4	0	7	8	0	44	10	4.4
	5	0	10	0	8	0	8	7	0	0	7	40	10	4.0
	6	0	13	11	0	9	13	15	12	15	6	94	10	9.4
	Total	9	27	19	19	17	25	26	23	26	17	208	10	20.8

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of dults	Young per Adult
33%	[illegible]	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	4	2	2	2	4	4	3	4	29	10	2.9
	4	7	3	8	8	7	4	5	0	6	9	57	10	5.7
	5	6	7	0	9	0	8	0	8	0	0	38	10	3.8
	6	0	11	12	0	11	13	12	12	16	16	103	10	10.3
	Total	17	21	24	19	20	27	21	24	25	29	227	10	22.7

Conc.	Day	Replicate 1	2	3	4	5	6	7	8	9	10	No. of Young	No. of Adults	Young per Adult
67%	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	4	0	2	4	0	4	4	0	5	27	10	2.7
	4	0	0	3	8	7	4	8	8	2	0	40	10	4.0
	5	7	9	9	8	0	9	0	0	7	7	56	10	5.6
	6	13	14	11	0	13	17	10	13	10	12	113	10	11.3
	Total	24	27	23	18	24	30	22	25	19	24	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
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	4	4	4	4	3	0	5	4	4	4	36	10	3.6
	4	7	0	8	9	6	6	9	8	0	8	61	10	6.1
	5	6	8	0	9	0	6	0	0	6	0	35	10	3.5
	6	0	12	15	0	12	17	13	16	9	14	108	10	10.8
	Total	17	24	27	22	21	29	27	28	19	26	240	10	24.0

Huntington 4.24
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	6	11	22	19	2

Calculated Chi-Square goodness of fit test statistic = 4.2628
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Huntington 4.24
File: ceriorep Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 2.62
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	8.5%	10	0	
2	17%	10	0	
3	33%	10	0	
4	67%	10	0	
5	100%	10	0	

Huntington 4.24
File: ceriorep Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	10	9.000	21.000	16.300
2	8.5%	10	10.000	24.000	18.000
3	17%	10	9.000	27.000	20.800
4	33%	10	17.000	29.000	22.700
5	67%	10	18.000	30.000	23.600
6	100%	10	17.000	29.000	24.000

Huntington 4.24
File: ceriorep Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	12.678	3.561	1.126
2	8.5%	24.222	4.922	1.556
3	17%	32.178	5.673	1.794
4	33%	14.011	3.743	1.184
5	67%	12.267	3.502	1.108
6	100%	16.667	4.082	1.291

Huntington 4.24
File: ceriorep Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	497.200	99.440	5.326
Within (Error)	54	1008.200	18.670	
Total	59	1505.400		

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

Huntington 4.24
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	16.300	16.300		
2	8.5%	18.000	18.000	-0.880	
3	17%	20.800	20.800	-2.329	
4	33%	22.700	22.700	-3.312	
5	67%	23.600	23.600	-3.778	
6	100%	24.000	24.000	-3.985	

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

Huntington 4.24
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	8.5%	10	4.464	27.4	-1.700
3	17%	10	4.464	27.4	-4.500
4	33%	10	4.464	27.4	-6.400
5	67%	10	4.464	27.4	-7.300
6	100%	10	4.464	27.4	-7.700

Discharger:	Huntington WWTP	**Test Dates:**	4/23/24 - 4/29/24
Location:	Huntington, IN	**Analysts:**	MMB, MN, AF

		Day							
Conc: Control		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	7.9	8.4	8.3	7.9	7.9		Template A
	Final	8.1	8.3	8.4	8.1	8.1	8.1		
pH	Initial	7.8	8.0	7.8	7.7	8.0	8.1		
	Final	7.7	7.8	7.7	7.8	7.8	8.0		
Alkalinity		40		40		40			
Hardness		120		100		100			
Conductivity		350		320		330			
Chlorine									

		Day							
Conc: 8.5%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.5	8.0	8.4	8.3	7.9	8.0		
	Final	8.1	8.3	8.4	8.0	8.1	8.1		
pH	Initial	7.8	8.0	7.8	7.7	7.9	8.0		
	Final	7.7	7.8	7.7	7.8	7.8	8.0		
Alkalinity									
Hardness									
Conductivity		370		340		350			
Chlorine									

		Day							
Conc: 17%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.6	8.0	8.4	8.3	8.0	8.1		
	Final	8.1	8.3	8.4	7.9	8.0	8.1		
pH	Initial	7.7	8.0	7.8	7.7	7.9	8.0		
	Final	7.7	7.8	7.7	7.8	7.8	8.0		
Alkalinity									
Hardness									
Conductivity		370		370		390			
Chlorine									

Discharger:	Huntington WWTP	**Test Dates:**	4/23/24 - 4/29/24
Location:	Huntington, IN	**Analysts:**	MMB, MN, AF

33% AZ

Conc: ~~34%~~		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	8.1	8.6	8.3	8.2	8.2		
	Final	8.0	8.2	8.3	7.8	7.9	8.0		
pH	Initial	7.7	7.9	7.7	7.6	7.8	7.9		
	Final	7.8	7.8	7.7	7.8	7.9	8.0		
Alkalinity									
Hardness									
Conductivity		460		450		450			
Chlorine									

67% AZ

Conc: ~~68%~~		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.9	8.4	8.6	8.5	8.6	8.4		
	Final	7.9	8.2	8.3	7.6	7.8	8.0		
pH	Initial	7.6	7.8	7.6	7.6	7.8	7.8		
	Final	7.8	7.9	7.7	7.9	7.9	8.1		
Alkalinity									
Hardness									
Conductivity		590		590		560			
Chlorine									

Conc: 100%		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	9.4	8.8	9.2	8.9	8.8	8.8		
	Final	7.8	8.2	8.2	7.5	7.7	7.9		
pH	Initial	7.6	7.7	7.5	7.5	7.7	7.7		
	Final	7.9	8.1	8.0	7.9	7.9	8.2		
Alkalinity		180		120		150			
Hardness		300		275		225			
Conductivity		710		730		690			
Chlorine		N.D.		ND		N.D			
Ammonia		N.D.		1.0		0.5			

Discharger:	Huntington WWTP	**Test Dates**	4/23/24 -4/30/24
Location:	Huntington, IN	**Analysts:**	MMB, MN, AF

		No. Surviving Organisms							
		Day							
Conc:	Rep. #	1	2	3	4	5	6	7	Remarks
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	
8.50%	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	10	10	10	10	10	10	
17.0%	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	
MB 34% 3	A	10	9	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	
MB 68% 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	
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	10	10	10	10	10	

Comments: Start Time: 1000

FHM Source: L.C.

Huntington 4.24
File: fhmsurv Transform: ARC SINE(SQUARE ROOT(Y))

Shapiro Wilks test for normality

D = 0.020

W = 0.465

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.

Huntington 4.24
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.

Huntington 4.24
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	8.5%	1.412	18.00	10.00	4.00	
3	17%	1.412	18.00	10.00	4.00	
4	33%	1.371	16.00	10.00	4.00	
5	67%	1.412	18.00	10.00	4.00	
6	100%	1.412	18.00	10.00	4.00	

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

Discharge:	Huntington WWTP	Test Date(s) :	4/23-30/24	Drying Temp (°C):	100	
Location:	Huntington, IN	Weighing Date:	5/1/24	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	0.91320	0.91560	2.40	10	0.240	
	B	0.91800	0.92140	3.40	10	0.340	
	C	0.91360	0.91700	3.40	10	0.340	
	D	0.92350	0.92720	3.70	10	0.370	
Conc : 8.5%	A	0.91670	0.92020	3.50	10	0.350	
	B	0.91730	0.92040	3.10	10	0.310	
	C	0.92260	0.92600	3.40	10	0.340	
	D	0.92360	0.92730	3.70	10	0.370	
Conc : 17%	A	0.92310	0.92600	2.90	10	0.290	
	B	0.91230	0.91550	3.20	10	0.320	
	C	0.91470	0.91800	3.30	10	0.330	
	D	0.91380	0.91700	3.20	10	0.320	
Conc : 33%	A	0.91800	0.92080	2.80	9	0.280	
	B	0.92060	0.92360	3.00	10	0.300	
	C	0.92150	0.92460	3.10	10	0.310	
	D	0.91630	0.91920	2.90	10	0.290	
Conc : 67%	A	0.91870	0.92230	3.60	10	0.360	
	B	0.91290	0.91610	3.20	10	0.320	
	C	0.92180	0.92530	3.50	10	0.350	
	D	0.91320	0.91640	3.20	10	0.320	
Conc : 100%	A	0.91730	0.92150	4.20	10	0.420	
	B	0.91150	0.91540	3.90	10	0.390	
	C	0.91830	0.92150	3.20	10	0.320	
	D	0.91650	0.91980	3.30	10	0.330	

Huntington 4.24
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	8	8	0

Calculated Chi-Square goodness of fit test statistic = 5.0194
Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Huntington 4.24
File: fhm_grow Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 19.35
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).

Huntington 4.24
File: fhm_grow Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 1 of 2

GRP	IDENTIFICATION	N	MIN	MAX	MEAN
1	control	4	0.240	0.370	0.323
2	8.5%	4	0.310	0.370	0.343
3	17%	4	0.290	0.330	0.315
4	33%	4	0.280	0.310	0.295
5	67%	4	0.320	0.360	0.338
6	100%	4	0.320	0.420	0.365

Huntington 4.24
File: fhm_grow Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.003	0.057	0.028
2	8.5%	0.001	0.025	0.012
3	17%	0.000	0.017	0.009
4	33%	0.000	0.013	0.006
5	67%	0.000	0.021	0.010
6	100%	0.002	0.048	0.024

Huntington 4.24
File: fhm_grow Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.012	0.002	2.000
Within (Error)	18	0.021	0.001	
Total	23	0.033		

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

Huntington 4.24
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	0.323	0.323		
2	8.5%	0.343	0.343	-0.894	
3	17%	0.315	0.315	0.335	
4	33%	0.295	0.295	1.230	
5	67%	0.338	0.338	-0.671	
6	100%	0.365	0.365	-1.901	

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

Huntington 4.24
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	8.5%	4	0.054	16.7	-0.020
3	17%	4	0.054	16.7	0.008
4	33%	4	0.054	16.7	0.027
5	67%	4	0.054	16.7	-0.015
6	100%	4	0.054	16.7	-0.043

Discharger:	Huntington WWTP	**Test Dates:**	4/23/24 -4/30/24
Location:	Huntington, IN	**Analysts:**	MMB, MN, AF

		Day							
Conc: Control		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.4	7.7	7.7	8.0	7.6	7.5	7.7	
	Final	7.0	7.0	6.8	6.9	7.1	6.6	7.2	
pH	Initial	7.8	8.0	8.0	8.2	8.0	7.9	8.1	
	Final	7.9	7.9	7.8	8.2	7.8	8.0	7.7	
Alkalinity		40		40		40			
Hardness		120		100		100			
Conductivity		350		350		350			
Chlorine									

		Day							
Conc: 8.5%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.5	7.8	7.8	8.0	7.7	7.6	7.8	
	Final	6.9	6.9	6.8	6.9	7.0	6.5	7.0	
pH	Initial	7.8	8.0	7.9	8.1	7.9	7.9	8.0	
	Final	7.9	7.9	7.8	8.2	7.8	8.0	7.7	
Alkalinity									
Hardness									
Conductivity		370		360		370			
Chlorine									

		Day							
Conc: 17%		1	2	3	4	5	6	7	**Remarks**
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.6	7.9	7.9	8.1	7.8	7.7	7.9	
	Final	6.9	6.8	6.7	6.8	6.9	6.4	6.7	
pH	Initial	7.7	7.9	7.8	8.1	7.9	7.8	7.9	
	Final	7.9	7.9	7.8	8.2	7.8	7.9	7.6	
Alkalinity									
Hardness									
Conductivity		370		390		400			
Chlorine									

Discharger:	Huntington WWTP	**Test Dates:**	4/23/24 -4/30/24
Location:	Huntington, IN	**Analysts:**	MMB, MN, AF

AZ

33%

Conc: ~~34%~~		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	8.0	8.1	8.2	8.0	7.8	8.0	
	Final	6.8	6.7	6.5	6.5	6.8	6.2	5.8	
pH	Initial	7.7	7.8	7.8	8.0	7.8	7.7	7.8	
	Final	7.9	7.8	7.7	8.2	7.7	7.9	7.6	
Alkalinity									
Hardness									
Conductivity		460		470		470			
Chlorine									

AZ

67%

Conc: ~~68%~~		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.9	8.4	8.2	8.3	8.2	7.9	8.1	
	Final	6.7	6.5	6.0	5.9	6.5	6.0	5.5	
pH	Initial	7.6	7.7	7.6	7.9	7.7	7.6	7.8	
	Final	7.8	7.8	7.7	8.1	7.7	7.8	7.5	
Alkalinity									
Hardness									
Conductivity		590		620		610			
Chlorine									

Conc: 100%		Day 1	2	3	4	5	6	7	Remarks
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	9.4	8.8	8.4	8.4	8.5	8.1	8.2	
	Final	6.6	6.3	5.0	5.7	5.8	5.6	4.8	
pH	Initial	7.6	7.6	7.6	7.8	7.6	7.6	7.7	
	Final	7.8	7.8	7.6	8.1	7.6	7.8	7.5	
Alkalinity		180		120		150			
Hardness		300		275		275			
Conductivity		710		750		750			
Chlorine		N.D.		ND		N.D.			
Ammonia		N.D.		1.0		0.5			