



CITY OF SOUTH BEND JAMES MUELLER, MAYOR
DEPARTMENT OF PUBLIC WORKS
ERIC HORVATH, DIRECTOR

June 27, 2024

Indiana Department of Environmental Management
Office of Water Quality – Mail Code 65-42
Compliance Data Section
100 N. Senate Ave.
Indianapolis, IN 46204-2251

Dear Sir or Madam,

Please find enclosed copies of the City of South Bend Wastewater Treatment Plant Whole Effluent Toxicity Test reports. This testing was done in accordance with NPDES Permit No. IN0024520, page 16, Whole Effluent Toxicity Testing Requirements.

Should you have any questions, please contact me at 574-235-5969

“I certify under penalty of law that this document and all attachments were prepared under my direction or supervision in accordance with a system designed to assure that qualified personnel properly gather and evaluate the information submitted. Based on my inquiry of the person or persons who manage the system or those directly responsible for gathering the information, the information submitted is, to the best of my knowledge and belief, true, accurate and complete. I am aware that there are significant penalties for submitting false information, including the possibility of fine and imprisonment.”

Sincerely,

A handwritten signature in black ink that reads 'Kim Thompson'.

Kim Thompson
Director of Wastewater
City of South Bend
3113 Riverside Drive
South Bend, IN 46628

Biomonitor

Permittee/Location South Bend WWTP South Bend, IN			Permit Number: IN0024520 St. Joseph Co.			Outfall Number: 047	
Laboratory Name and Contact: Biomonitor Michael Britton			Report Due Date:			Report Date: April 2024	
WETT Reporting Frequency or Type: (mark one)	Monthly	Quarterly	Semi-annual	Annual	TRE	Post TRE	First (per Reporting Frequency) 1/2
			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				4	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				4	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*

SOUTH BEND
WASTEWATER TREATMENT PLANT

IN0024520

South Bend, Indiana

April 2024

GLP (Good Laboratory Practices)
COMPLIANCE STATEMENT

Project Name: South Bend 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: 4/26/24

Michael Britton

Project Director
Date: 4/26/24

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.

<p style="text-align: center;">Section 1 Executive Summary</p>
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Biomonitor conducted whole effluent toxicity testing for the South Bend, IN Wastewater Treatment Plant in South Bend, IN during April 2024. The purpose of the testing was to fulfill the biomonitoring requirement for the NPDES permit.

Three samples were collected April 14-18, 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 25% effluent (a TU_c not to exceed 4.0). 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:	IN0024520
Toxicity testing requirements:	Fathead minnow larval survival and growth test Ceriodaphnia survival and reproduction test
Plant location:	South Bend Wastewater Treatment Plant 3113 Riverside Drive South Bend, Indiana 46628
Name of receiving water body:	St. Joseph River (Lake Michigan Drainage Basin)
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, chlorination/dechlorination, phosphorous removal
Design flow:	48 – MGD
Volume of wastewater flow during the sampling period:	April 14, 2024 - MGD April 16, 2024 - MGD April 18, 2024 - MGD

Table III
Source of effluent and dilution water

I. Effluent samples

Sampling point:	Outfall 047	
Collection dates and times:	April 14, 2024	11:59 p.m.
	April 16, 2024	11:59 p.m.
	April 18, 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:	<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:	No Deviations.	
Date and time test initiated:	April 16, 2024	10:20 a.m.
Date and time test terminated	April 23, 2024	1:00 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 100% after seven 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>	March 19-27, 2024
<u>Dilution water used in test:</u>	Moderately hard synthetic water
<u>Results:</u>	48-hr LC ₅₀ = 80 µg/L as Cu NOEL (reproduction) = 40 µg/L as Cu LOEL (reproduction) = 80 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	15.4	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	0	0	0	3	0	0	0	0	0		10
	4	2	3	3	2	0	1	2	0	2	2		10
	5	6	4	6	5	6	3	6	4	0	2		10
	6	0	6	0	11	8	0	8	0	4	0		10
	7	10	4	6	0	10	7	0	3	6	9		10
6.25%	1	0	0	0	0	0	0	0	0	0	0	18.1	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	4	0	0	4	0	0	0	0	3		10
	4	3	0	3	2	4	0	2	2	2	4		10
	5	0	6	6	6	0	2	6	3	2	0		10
	6	7	0	9	10	9	9	0	0	0	8		10
	7	9	11	0	0	14	4	9	4	4	10		10
12.5%	1	0	0	0	0	0	0	0	0	0	0	17.6	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	3	4	0	3	0	0	0	0	0		10
	4	2	0	0	2	0	2	2	0	2	4		10
	5	4	6	8	8	4	6	6	0	4	6		10
	6	9	12	10	0	6	0	0	2	0	0		10
	7	0	0	0	10	9	12	10	3	8	9		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	19.6	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	4	0	0	0	0	0	0	0	3		10
	4	4	0	4	3	2	2	2	0	2	4		10
	5	4	6	5	6	6	6	6	2	4	0		10
	6	0	11	7	8	8	0	0	5	0	10		10
	7	12	0	0	0	13	11	7	9	9	11		10
50%	1	0	0	0	0	0	0	0	0	0	0	19.0	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	4	0	3	2	0	0	0	0	0		10
	4	2	0	3	0	0	1	2	2	2	4		10
	5	4	7	6	6	5	3	8	6	6	4		10
	6	6	13	9	12	6	0	0	0	0	0		10
	7	7	0	0	0	5	10	9	9	13	11		10
100%	1	0	0	0	0	0	0	0	0	0	0	16.6	10
	2	0	0	0	0	0	0	0	0	0	0		10
	3	0	4	4	3	2	0	0	0	0	0		10
	4	2	0	0	0	0	0	2	2	2	3		10
	5	0	6	5	6	6	4	6	2	4	6		10
	6	6	8	7	10	5	0	0	0	0	0		10
	7	9	0	0	0	8	10	11	7	8	8		10

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	7.5 – 8.5	25	7.5 – 8.0	40-50	100-130	320-330
6.25%	7.5 – 8.5	25	7.5 – 8.0	/	/	340-350
25%	7.5 – 8.5	25	7.5 – 7.9	/	/	450-470
100%	7.5 – 9.5	25	7.5 – 8.3	130-150	300-325	830-900

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>	April 16, 2024	10:30 a.m.
<u>Date and time test terminated</u>	April 23, 2024	10:30 a.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 Lab 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>	Steel's Many-One Rank Test was required for the survival endpoint because the homogeneity of variance assumptions could not be met. Dunnett's Test for the growth endpoint. 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>	March 19-26, 2024
<u>Dilution water used in test:</u>	Moderately Hard Synthetic Water
<u>Results:</u>	96-hr LC ₅₀ = 1189 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	400	450	400	430
6.25%	100	90	100	100	400	370	320	410
12.5%	90	100	90	100	370	430	360	400
25%	100	100	100	100	390	370	350	400
50%	90	100	100	100	400	410	430	460
100%	100	100	100	90	420	420	410	370

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	6.3 – 8.1	25	7.5 – 7.8	40-50	100-130	320-340
6.25%	6.3 – 8.1	25	7.5 – 7.8			340-350
25%	6.1 – 8.4	25	7.5 – 7.9			450-480
100%	5.8 – 9.3	25	7.4 – 8.1	130-150	300-325	870-940

Biomonitor

8802 W. Washington Street
Indianapolis, IN 46231
317-297-7713
www.biomonitor.com

SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: South Bend WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: S. Bend - 1 Monday Apr. 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 4-14-24 Start Time ~~1200~~ 1200am
End Date 4-14-24 End Time 11:59pm

NAME OF PERSON COLLECTING SAMPLE: C. Scott

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: *Sandra Montez*

Date: 4-15-24 Time: 11:40

Received by: *C. C. F.*

Date: 4/15/24 Time: 11:40c

Relinquished by: _____

Date: _____ Time: _____

Received by: _____

Date: _____ Time: _____

TEMP: 7 °C

COMMENTS:

Biomonitor

8802 W. Washington Street
Indianapolis, IN 46231
317-297-7713
www.biomonitor.com

SAMPLE SUMMARY AND CHAIN OF CUSTODY

CLIENT NAME: South Bend WWTP

PURPOSE OF SAMPLE: Whole Effluent Toxicity

SAMPLE IDENTIFICATION: S. Bend - 3 Friday Apr. 2024

DESCRIPTION: Outfall

DATE SAMPLE COLLECTED: Start Date 4-18-24 Start Time 1200am
End Date 4-18-24 End Time 1159pm

NAME OF PERSON COLLECTING SAMPLE: C. Scott

SAMPLE VOLUME: 8 Liters

NUMBER OF CONTAINERS: Two, HDPE

SAMPLE STORAGE: Refrigerated/iced

PRESERVATIVES: none

Relinquished by: [Signature]
Date: 4-18-24 Time: 11:18

Received by: [Signature]
Date: 4/19/24 Time: 11:48 a

Relinquished by: _____
Date: _____ Time: _____

Received by: _____
Date: _____ Time: _____

TEMP: 10 °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
<u>Average</u>	89	<u>Mode</u>	40	44
<u>St. Dev.</u>	14			11
<u>Upper Limit</u>	117		80	67
<u>Lower Limit</u>	60		20	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
<u>Average</u>	1062	<u>Mode</u>	500	830
<u>St. Dev.</u>	131			165
<u>Upper Limit</u>	1323		1000	1160
<u>Lower Limit</u>	800		250	501

Client: South Bend WWTP

Project # _____

Analysts: MMB, MN, AF, MMK

Start Date: 4/16/2024

Start Time: 1026

End Date: 4/23/2024

End Time: 1300

Template # B

Comments:

Test Dates

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

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

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

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

Row 7	Day	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
		4	2	2	2	2	2
		5	6	6	6	6	6
		6	0	0	5+3	0	0
		7	7	9	0	11	10

Row 6	Day	1	0	0	0	0	0
		2	0	0	0	0	0
		3	0	0	0	0	0
		4	2	0	2	1	1
		5	6	4	6	3	3
		6	0	0	0	0	0
		7	11	10	12	10	7

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

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

Discharger: South Bend WWTP Analyst: MMB, MN, AF, MMK
 Location: South Bend, IN Test Start- Date/Time: 4/16/2024 / 1020
 Date Sample Collected: 4/2, 4, 6, 7, 23 PM Test Stop- Date/Time: 4/23/2024 / 1300
4/14, 16, 18/24

Conc.	Day	Replicate										No. of Young 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	10	0	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	0	0.0
	3	0	0	0	0	3	0	0	0	0	0	0	3	10	0.3
	4	2	3	3	2	0	1	2	0	2	0	2	17	10	1.7
	5	6	4	6	5	6	3	6	4	0	2	42	10	4.2	
	6	0	6	0	11	8	0	8	0	4	0	37	10	3.7	
	7	10	4	6	0	10	7	0	3	6	9	55	10	5.5	
Total	18	17	15	18	27	11	16	7	12	13	154	10	15.4		

Conc.	Day	Replicate										No. of Young 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	10	0	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	0	4	0	0	4	0	0	0	0	0	11	10	1.1	
	4	3	0	3	2	4	0	2	2	2	4	22	10	2.2	
	5	0	6	6	6	0	2	6	3	2	0	31	10	3.1	
	6	7	0	9	10	9	9	0	0	0	8	52	10	5.2	
	7	9	11	0	0	14	4	9	4	4	10	65	10	6.5	
Total	19	21	18	18	31	15	17	9	8	25	181	10	18.1		

Conc.	Day	Replicate										No. of Young 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	10	0	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	0	3	4	0	3	0	0	0	0	0	10	10	1.0	
	4	2	0	0	2	0	2	0	2	0	4	14	10	1.4	
	5	4	6	8	8	4	6	0	4	0	6	52	10	5.2	
	6	9	12	10	0	6	0	0	2	0	0	39	10	3.9	
	7	0	0	0	10	9	12	10	3	8	9	61	10	6.1	
Total	15	21	22	20	22	20	18	5	14	19	176	10	17.6		

Conc.	Day	Replicate										No. of Young Adults	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	0	10	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	0	4	0	0	0	0	0	0	0	0	3	7	10	0.7
	4	4	0	4	3	2	2	2	0	2	4	4	23	10	2.3
	5	4	6	5	6	6	6	2	2	4	0	0	45	10	4.5
	6	0	11	7	8	8	0	5	0	0	10	10	49	10	4.9
	7	12	0	0	0	13	11	7	9	9	11		72	10	7.2
	Total	20	21	16	17	29	15	16	15	28		196	10	19.6	

Conc.	Day	Replicate										No. of Young Adults	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	0	10	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	0	4	0	3	2	0	0	0	0	0	0	9	10	0.9
	4	2	0	3	0	0	1	2	2	2	4	4	16	10	1.6
	5	4	7	6	6	5	3	8	6	6	4	4	55	10	5.5
	6	6	13	9	12	6	0	0	0	0	0	0	46	10	4.6
	7	7	0	0	0	0	5	10	9	13	11		64	10	6.4
	Total	19	24	18	21	18	14	19	17	21	19	190	10	19.0	

Conc.	Day	Replicate										No. of Young Adults	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	0	10	0.0
	2	0	0	0	0	0	0	0	0	0	0	0	0	10	0.0
	3	0	4	4	3	2	0	0	0	0	0	0	13	10	1.3
	4	2	0	0	0	0	0	2	2	2	3	3	11	10	1.1
	5	0	6	5	6	6	4	6	2	4	6	6	45	10	4.5
	6	6	8	7	10	5	0	0	0	0	0	0	36	10	3.6
	7	9	0	0	0	0	8	10	11	7	8	8	61	10	6.1
	Total	17	18	16	19	21	14	19	11	14	17	166	10	16.6	

South bend 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	4	14	28	9	5

Calculated Chi-Square goodness of fit test statistic = 3.4821

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

South bend 4.24

File: ceriorep

Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 6.55

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	6%	10	0	
2	12%	10	0	
3	25%	10	0	
4	50%	10	0	
5	100%	10	0	

South bend 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	7.000	27.000	15.400
2	6.25%	10	8.000	31.000	18.100
3	12.5%	10	5.000	22.000	17.600
4	25%	10	15.000	29.000	19.600
5	50%	10	14.000	24.000	19.000
6	100%	10	11.000	21.000	16.600

South bend 4.24

File: ceriorep

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	28.711	5.358	1.694
2	6.25%	46.544	6.822	2.157
3	12.5%	26.933	5.190	1.641
4	25%	26.267	5.125	1.621
5	50%	7.111	2.667	0.843
6	100%	8.711	2.951	0.933

South bend 4.24

File: ceriorep

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	119.683	23.937	0.995
Within (Error)	54	1298.500	24.046	
Total	59	1418.183		

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

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

South bend 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	15.400	15.400		
2	6.25%	18.100	18.100	-1.231	
3	12.5%	17.600	17.600	-1.003	
4	25%	19.600	19.600	-1.915	
5	50%	19.000	19.000	-1.642	
6	100%	16.600	16.600	-0.547	

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

South bend 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	6.25%	10	5.066	32.9	-2.700
3	12.5%	10	5.066	32.9	-2.200
4	25%	10	5.066	32.9	-4.200
5	50%	10	5.066	32.9	-3.600
6	100%	10	5.066	32.9	-1.200

Discharger: South Bend WWTP
 Location: South Bend, IN

Test Dates: 4/16/24 - 4/23/24
 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	7.9	7.9	8.3	8.5	8.2	8.3	8.2	
	Final	7.5	7.9	8.3	7.9	7.8	8.1	8.3	
pH	Initial	7.8	7.9	7.6	7.5	7.7	7.8	7.7	
	Final	7.8	7.9	7.9	7.6	7.7	7.9	7.9	
Alkalinity									
Hardness									
Conductivity		450		470		460			
Chlorine									

		Day							Remarks
Conc: 50%		1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	7.9	8.1	8.3	8.7	8.4	8.5	8.4	
	Final	7.5	7.9	8.3	7.9	7.8	8.1	8.3	
pH	Initial	7.7	7.8	7.7	7.5	7.7	7.8	7.6	
	Final	7.7	8.0	8.0	7.8	7.8	8.0	8.0	
Alkalinity									
Hardness									
Conductivity		580		640		580			
Chlorine									

		Day							Remarks
Conc: 100%		1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	8.6	8.8	9.5	9.3	8.7	9.3	
	Final	7.5	7.9	8.3	7.9	7.8	8.1	8.2	
pH	Initial	7.6	7.8	7.7	7.5	7.7	7.7	7.6	
	Final	8.3	8.2	8.2	8.1	8.2	8.1	8.1	
Alkalinity		130		150		130			
Hardness		325		300		325			
Conductivity		880		900		830			
Chlorine		ND		ND		ND			
Ammonia		ND		ND		ND			

Discharger: South Bend WWTP
 Location: South Bend, IN

Test Dates 4/16/24 - 4/23/24
 Analysts: MMB, MN, AF, MMK

		No. Surviving Organisms							
Conc:	Rep. #	Day							Remarks
		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	9	
	C	10	10	10	10	10	10	10	
	D	10	10	10	10	10	10	10	
12.5%	A	10	10	10	10	10	10	9	
	B	10	10	10	10	10	10	10	
	C	10	10	10	10	10	9	9	
	D	10	10	10	10	10	10	10	
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	10	10	10	10	10	
50%	A	10	10	10	10	10	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	
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	9	9	

Comments: Start Time: 1030

FHM Source: Biomonitor Lab Cultures

Southbend 4.24

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.

Southbend 4.24

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.

South Bend 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	6.25%	1.371	16.00	10.00	4.00	
3	12.5%	1.331	14.00	10.00	4.00	
4	25%	1.412	18.00	10.00	4.00	
5	50%	1.371	16.00	10.00	4.00	
6	100%	1.371	16.00	10.00	4.00	

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

Discharge: South Bend WWTP Test Date(s) : 4/16-23/24 Drying Temp (°C): 100
 Location: South Bend, IN Weighing Date: 4/24/24 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	0.92270	0.92670	4.00	10	0.400	
	B	0.92450	0.92900	4.50	10	0.450	
	C	0.91990	0.92390	4.00	10	0.400	
	D	0.91580	0.92010	4.30	10	0.430	
Conc : 6.25%	A	0.92110	0.92510	4.00	10	0.400	
	B	0.91760	0.92130	3.70	9	0.370	
	C	0.92450	0.92770	3.20	10	0.320	
	D	0.92260	0.92670	4.10	10	0.410	
Conc : 12.5%	A	0.91790	0.92160	3.70	9	0.370	
	B	0.92170	0.92600	4.30	10	0.430	
	C	0.91740	0.92100	3.60	9	0.360	
	D	0.91990	0.92390	4.00	10	0.400	
Conc : 25%	A	0.92010	0.92400	3.90	10	0.390	
	B	0.91390	0.91760	3.70	10	0.370	
	C	0.91860	0.92210	3.50	10	0.350	
	D	0.92240	0.92640	4.00	10	0.400	
Conc : 50%	A	0.92690	0.93090	4.00	9	0.400	
	B	0.92100	0.92510	4.10	10	0.410	
	C	0.92120	0.92550	4.30	10	0.430	
	D	0.92100	0.92560	4.60	10	0.460	
Conc : 100%	A	0.91760	0.92180	4.20	10	0.420	
	B	0.91870	0.92290	4.20	10	0.420	
	C	0.91780	0.92190	4.10	10	0.410	
	D	0.91650	0.92020	3.70	9	0.370	

Southbend 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	9	6	9	0

Calculated Chi-Square goodness of fit test statistic = 7.8193

Table Chi-Square value (alpha = 0.01) = 13.277

Data PASS normality test. Continue analysis.

Southbend 4.24

File: fhm_grow

Transform: NO TRANSFORMATION

Hartley test for homogeneity of variance

Calculated H statistic (max Var/min Var) = 3.32

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).

Southbend 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	0.400	0.450	0.420
2	6.25%	4	0.320	0.410	0.375
3	12.5%	4	0.360	0.430	0.390
4	25%	4	0.350	0.400	0.378
5	50%	4	0.400	0.460	0.425
6	100%	4	0.370	0.420	0.405

Southbend 4.24
File: fhm_grow

Transform: NO TRANSFORMATION

SUMMARY STATISTICS ON TRANSFORMED DATA TABLE 2 of 2

GRP	IDENTIFICATION	VARIANCE	SD	SEM
1	control	0.001	0.024	0.012
2	6.25%	0.002	0.040	0.020
3	12.5%	0.001	0.032	0.016
4	25%	0.000	0.022	0.011
5	50%	0.001	0.026	0.013
6	100%	0.001	0.024	0.012

Southbend 4.24
File: fhm_grow

Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.0091	0.0018	2.250
Within (Error)	18	0.0150	0.0008	
Total	23	0.0241		

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

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

Southbend 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	0.420	0.420		
2	6.25%	0.375	0.375	2.250	
3	12.5%	0.390	0.390	1.500	
4	25%	0.378	0.378	2.125	
5	50%	0.425	0.425	-0.250	
6	100%	0.405	0.405	0.750	

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

Southbend 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	6.25%	4	0.048	11.5	0.045
3	12.5%	4	0.048	11.5	0.030
4	25%	4	0.048	11.5	0.043
5	50%	4	0.048	11.5	-0.005
6	100%	4	0.048	11.5	0.015

Discharger: South Bend WWTP
 Location: South Bend, IN

Test Dates: 4/16/24 -4/23/24
 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	7.9	8.2	8.2	7.9	8.4	7.7	8.1	
	Final	6.4	6.2	6.4	6.7	6.1	6.3	6.7	
pH	Initial	7.8	7.6	7.6	7.6	7.7	7.7	7.7	
	Final	7.9	7.6	7.5	7.6	7.6	7.7	7.5	
Alkalinity									
Hardness									
Conductivity		450		480		450			
Chlorine									

		Day							Remarks
Conc:	50%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	7.9	8.4	8.4	8.2	8.6	7.9	8.3	
	Final	6.3	6.2	6.3	6.7	5.9	6.2	6.6	
pH	Initial	7.7	7.6	7.6	7.5	7.6	7.7	7.6	
	Final	7.9	7.7	7.6	7.7	7.7	7.7	7.6	
Alkalinity									
Hardness									
Conductivity		580		640		590			
Chlorine									

		Day							Remarks
Conc:	100%	1	2	3	4	5	6	7	
Temp.		25	25	25	25	25	25	25	
D.O.	Initial	8.7	8.7	9.3	9.1	9.0	8.7	8.9	
	Final	6.1	6.3	6.3	6.6	5.8	6.0	6.6	
pH	Initial	7.6	7.7	7.6	7.6	7.6	7.4	7.5	
	Final	8.1	7.8	7.9	8.0	8.0	7.8	7.9	
Alkalinity		130		150		130			
Hardness		325		300		325			
Conductivity		880		940		870			
Chlorine		ND		ND		ND			
Ammonia		ND		ND		ND			