Bio-Aquatic Testing, Inc.

TCEQ TNI Accredited

Pilgrim's Pride Corporation
Southwest WWP
OUTFALL 001

Client Address:
664 HIGHWAY 127 WEST
MOUNT PLEASANT, TX 75456

Chronic Biomonitoring Report

89242

Ceriodaphnia dubia
Pimephales promelas

December 03, 2024

Approved by: _____
Lab director

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***HAND-WRITTEN RAW DATA TABLES ARE AVAILABLE UPON REQUEST**

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TOXICITY TEST REPORT - Chronic

Client:	Pilgrim's Pride Corporation	Sample:	001
Facility:	Southwest WWP	Laboratory Number:	89242
Permit No.	WQ0003017000	Date:	December 03, 2024

Ceriodaphnia dubia **failed** survival and reproduction testing requirements. *Pimephales promelas* **passed** survival and growth testing requirements.

SAMPLE COLLECTION:

Composite effluent samples from Pilgrim's Pride Corporation, Southwest WWP, were received on December 02, 2024, December 04, 2024, and December 06, 2024. Effluent samples were collected from Outfall 001 by facility personnel.

The effluent samples were analyzed for total residual chlorine using the Hanna Ion Specific Meter #711 and contained <0.10 mg/L, <0.10 mg/L, and 0.97 mg/L, respectively. Effluent and laboratory dilution water pH, temperature, and dissolved oxygen data were collected daily.

TEST PROCEDURES:

EPA METHOD: 1002

Ceriodaphnia dubia

The seven-day (three brood) Chronic *Ceriodaphnia dubia* survival and reproduction test was initiated at 13:00 hours on December 03, 2024. Five effluent concentrations of 31%, 41%, 55%, 80%, and 98% were prepared using synthetic water as dilution water. The test was set up with 30mL plastic cups containing 15mL of test solution or control dilution water. Each effluent concentration or control dilution water included ten replicate cups with one organism in each cup. The control was conducted concurrently with the test. Test organisms were less than 24-hour old laboratory cultured neonates. Neonates were introduced into the test solutions using a blocking design. The test was renewed daily with newly prepared solutions. Food consisting of a half-milliliter suspension of the green algae, *Selenastrum capricornutum,* and YTC was added to the test solutions each day. The test proceeded for seven days or until 60% of the females in the control had three broods. Data on survival and number of young produced per female were collected daily. The test ended at 11:32 hours on December 11, 2024. Survival and reproduction data were statistically (p=0.05) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL:

Ceriodaphnia dubia

Fisher's Exact test on *Ceriodaphnia dubia* survival test data demonstrated statistically significant differences between the control and all the effluent concentrations tested.

LOEC: 31% Effluent
NOEC: 0% Effluent

REPRODUCTION:

Ceriodaphnia dubia

The *Ceriodaphnia dubia* reproduction data was not calculated due to statistically significant differences between the control and all the effluent concentrations tested for survival data.

LOEC: 31% Effluent
NOEC: 0% Effluent

TEST PROCEDURES:

EPA METHOD: 1000

Pimephales promelas

The seven-day Chronic *Pimephales promelas* survival and growth test was initiated at 14:49 hours on December 03, 2024. Five effluent concentrations of 31%, 41%, 55%, 80%, and 98% were prepared using synthetic water as dilution water. The test was set up with 450mL plastic cups containing 250mL of test solution as test chambers. Each concentration consisted of five replicate chambers containing eight organisms each, giving a total of 40 (forty) per treatment. The control test was conducted concurrently with the test. Test organisms were laboratory-cultured *Pimephales promelas* larvae less than 24-hours old. The number of surviving larvae and water quality parameters in the old test solutions were recorded after each 24-hour period. The test was renewed daily with fresh solutions. Surviving larvae in each test chamber were fed freshly hatched brine shrimp two times per day. The test proceeded for seven days.

At the end of the test, all organisms were sacrificed, dried, and weighed. Data on surviving organisms and water quality were collected. The test ended at 10:30 hours on December 10, 2024. Survival and growth (weight) were statistically ($p=0.05$) analyzed according to EPA procedures to determine the Lowest Observable Effect Concentration (LOEC) and the No Observable Effect Concentration (NOEC).

SURVIVAL:

Pimephales promelas

The non-parametric Steel's Many-One Rank test performed on *Pimephales promelas* survival data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 98% Effluent

GROWTH:

Pimephales promelas

The *Pimephales promelas* growth data were normally distributed at the alpha level of 0.01 (0.900) using Shapiro Wilk's test for normality. Growth data were shown to be homogeneous using Bartlett's test at the alpha level of 0.01 (15.09) without data transformations. Using ANOVA and Dunnett's test on *Pimephales promelas* growth data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)
NOEC: 98% Effluent

BIO-AQUATIC TESTING, INC.

TOXICITY TEST

Chronic ***Ceriodaphnia dubia***

Client: Pilgrim's Pride Corporation Southwest WWP

Lab ID: 89242

Permit Number: TPDES WQ0003017000

Test Temperature (oC): 25 ± 1

Sample Type: Composite

Photo Period: 16 hours light, 8 hours dark

Outfall Name: 001

Dilution Water: synthetic

Receiving Water Name: Tankersley Creek

Begin Date: 12/3/2024

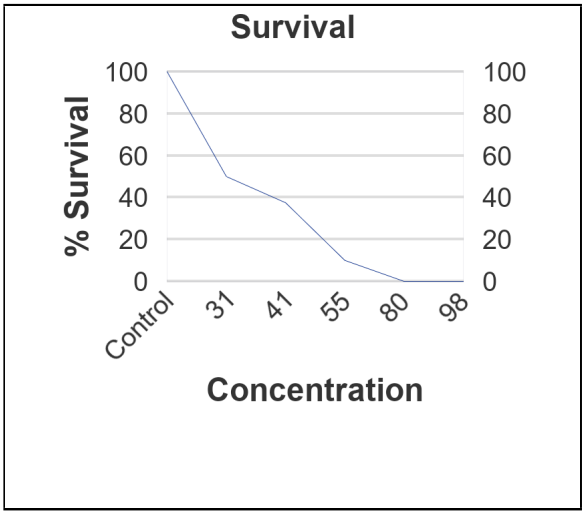
End Date: 12/11/2024

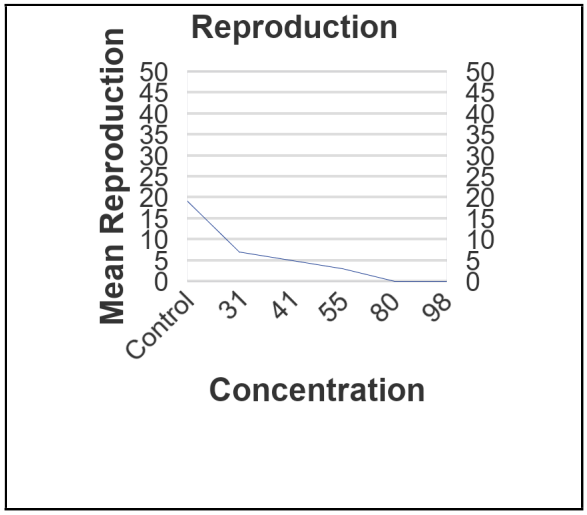
Test Start Time: 13:00 **Test End Time:** 11:32

SURVIVAL AND REPRODUCTION TABLE

FEMALE #	Control	31	%	41	%	55	%	80	%	98	%
1	18	D- 11		E		D- 0		D- 6		D- 0	
2	24	E		D- 2		D- 0		D- 9		D- 8	
3	28	D- 8		5		D- 7		D- 4		D- 4	
4	13	6		D- 0		D- 2		D- 0		D- 3	
5	29	2		D- 4		D- 0		D- 4		D- 2	
6	6	D- 13		6		D- 0		D- 0		D- 0	
7	14	E		4		D- 2		D- 0		D- 0	
8	17	D- 0		E		3		D- 7		D- 1	
9	E	7		D- 0		D- 0		D- 0		D- 0	
10	23	13		D- 0		D- 6		D- 4		D- 0	
Surv.Mean	19.1	7.0		5.0		3.0		0.0		0.0	
C.V%	39.5	64.9		20		?		D		D	
Total Mean	19.1	7.5		2.6		2.0		3.4		1.8	
Var	57.111	20.666		1		?		D		D	
Std.Dev.	7.557	4.546		1		?		D		D	
Max	29	13		6		7		D		D	
Min	6	2		4		3		D		D	

Concentration Response Relationships





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Survival and Reproduction

Control

Date	1	2	3	4	5	6	7	8	9	10
12/4	A	A	A	A	A	A	A	A	A	A
12/5	A	A	A	A	A	A	A	A	A	A
12/6	3	4	A	A	A	A	A	A	A	A
12/7	4	A	2	A	A	A	2	A	A	6
12/8	A	5	A	9	6	2	A	A	A	A
12/9	A	A	14	A	12	2	11	17	10	9
	7	9	16	9	18	4	13	17	10	15
12/10	11	A	A	1	A	2	A	A	E	A
	18	9	16	10	18	6	13	17		15
12/11	A	15	12	3	11	A	1	A	E	8
	18	24	28	13	29	6	14	17		23

Mean: 19.10 **CV%** 39.50
Var. 57.11 **Max** 29
Std.Dev. 7.56 **Min** 6

31

Date	1	2	3	4	5	6	7	8	9	10
12/4	A	A	A	A	A	A	A	A	A	A
12/5	A	A	A	A	A	A	A	A	A	A
12/6	4	A	A	A	A	A	A	A	A	3
12/7	A	E2	A	3	A	1	1	A	2	A
12/8	7	E	7	3	A	5	E	D	5	3
12/9	D	E	A	A	2	7	E	D	A	A
	11		7	6	2	13		0	7	6
12/10	D	E	D1	A	A	D	E	D	A	A
	11		8	6	2	13		0	7	6
12/11	D	E	D	A	A	D	E	D	A	7
	11		8	6	2	13		0	7	13

Mean: 7.00 **CV%** 64.90
Var. 20.67 **Max** 13
Std.Dev. 4.55 **Min** 2

41

Date	1	2	3	4	5	6	7	8	9	10
12/4	A	A	A	A	A	A	A	A	A	A
12/5	A	A	A	A	A	A	A	A	A	A
12/6	A	A	A	A	A	A	4	A	A	A
12/7	A	2	A	A	A	A	A	A	A	A
12/8	E1	D	A	D	2	3	A	E	A	A
12/9	E	D	5	D	A	3	A	E	D	D
		2	5	0	2	6	4		0	0
12/10	E	D	A	D	D2	A	A	E	D	D
		2	5	0	4	6	4		0	0
12/11	E	D	A	D	D	A	A	E	D	D
		2	5	0	4	6	4		0	0

Mean: 5.00 **CV%** 20.00
Var. 1.00 **Max** 6
Std.Dev. 1.00 **Min** 4

55

Date	1	2	3	4	5	6	7	8	9	10
12/4	A	A	A	A	A	A	A	A	A	A
12/5	A	D	A	A	A	A	A	A	A	A
12/6	A	D	A	A	A	A	A	A	A	4
12/7	A	D	A	A	A	A	1	A	A	A
12/8	D	D	1	D2	A	A	1	A	A	D2
12/9	D	D	6	D	A	A	D	3	D	D
	0	0	7	2	0	0	2	3	0	6
12/10	D	D	D	D	D	A	D	A	D	D
	0	0	7	2	0	0	2	3	0	6
12/11	D	D	D	D	D	D	D	A	D	D
	0	0	7	2	0	0	2	3	0	6

Mean: 3.00 **CV%** ?
Var. ? **Max** 7
Std.Dev. ? **Min** 3

80

Date	1	2	3	4	5	6	7	8	9	10
12/4	A	A	A	A	A	A	A	A	A	A
12/5	A	A	A	A	A	A	A	A	A	A
12/6	A	A	A	A	A	A	A	A	A	4
12/7	A	A	A	A	A	A	A	A	A	A
12/8	6	5	2	D	A	A	D	D7	D	D
12/9	D	4	2	D	2	A	D	D	D	D
	6	9	4	0	2	0	0	7	0	4
12/10	D	A	D	D	2	A	D	D	D	D
	6	9	4	0	4	0	0	7	0	4
12/11	D	D	D	D	D	D	D	D	D	D
	6	9	4	0	4	0	0	7	0	4

Mean: 0.00 **CV%**
Var. **Max**
Std.Dev. **Min**

98

Date	1	2	3	4	5	6	7	8	9	10
12/4	A	A	A	A	A	A	A	A	A	A
12/5	A	A	A	A	A	A	A	A	A	A
12/6	A	A	A	A	A	A	A	A	A	A
12/7	D	A	A	A	A	A	A	A	A	A
12/8	D	2	4	3	A	D	A	A	A	A
12/9	D	6	D	D	D2	D	A	1	D	D
	0	8	4	3	2	0	0	1	0	0
12/10	D	D	D	D	D	D	D	D	D	D
	0	8	4	3	2	0	0	1	0	0
12/11	D	D	D	D	D	D	D	D	D	D
	0	8	4	3	2	0	0	1	0	0

Mean: 0.00 **CV%**
Var. **Max**
Std.Dev. **Min**

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Pilgrim's Pride Corporation - Southwest WWP** Lab ID: **89242** Culture No.: Bio 112524A

TEST INSTRUCTIONS: NETDMR

ORGANISMS ADDED: Date: 12-3-24 Time: 1300 Technician: MH

Photo Period 16hr Light/8hr dark

RANDOMIZATION: SC-10 24

Dilution: Control

DATE/TIME/ TECHNICIAN	1	2	3	4	5	6	7	8	9	10
24Hr 12-04-24 MW 1158	A									A
48Hr 12-05-24 MW 1010	A									A
72Hr 12-06-24 1303 RA	3	4	A	A	A	A	A	A	A	A
96Hr 12-07-24 1510 RA	4	A	3/A	A	1/A	A	2/A	A	A	6
5 days 12-08-24 1622 RA	A	1/4	1/M	1/8	5	2/A	6	A	A	6
6 days 12-09-24 1436 RA	2/M	A	6	A	12	3/A	5	M	10	3
7 days 12-10-24 MH 0855	A2	2/A1	A	1/A	A	1/A	A	A	E*	A
8 days 12-11-24 MW 1132	10	12	12	3	11	A	A1	A	1	8

*Empty MH

Dilution: 31 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	4	A	A	A	A	A	A	A	A	3
96Hr	A	2/M	A	3	A	1/A	1/A	A	2/A	A
5 days	1/6		M	3	A	1/4	A	R	1/4	3
6 days	D		A	A	2/A	M			A	A
7 days			1/D	A	A	D			A	1/A
8 days				A	A				A	S

E(2) Empty RA

E(7) Empty RA

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Pilgrim's Pride Corporation - Southwest WWP** Lab ID: **89242** Culture No.:

TEST INSTRUCTIONS: NETDMR

Dilution: 41 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A									A
72Hr	A	A	A	A	A	A	A	A		A
96Hr	A	2 A	A	A	A	A				A
5 days	1 E*	D	A	D	2 A	3	A	E*	A	A
6 days			2 3		A	3	A		D	D
7 days			A		1 D	A	A			
8 days			A			A	A			

E(1) Empty; BA

E(8) Empty; BA

Dilution: 55 %

	1	2	3	4	5	6	7	8	9	10
24Hr	A									A
48Hr	A	D	A							A
72Hr	A		A			A	A	A	A	4
96Hr	A		A	A	A	A	1 A	A	A	A
5 days	D		1 A	2 D	A	A	1 A	A	A	3 A
6 days			2 4		A	A	D	3	D	
7 days			D		D	A		A		
8 days						D		A		

Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not

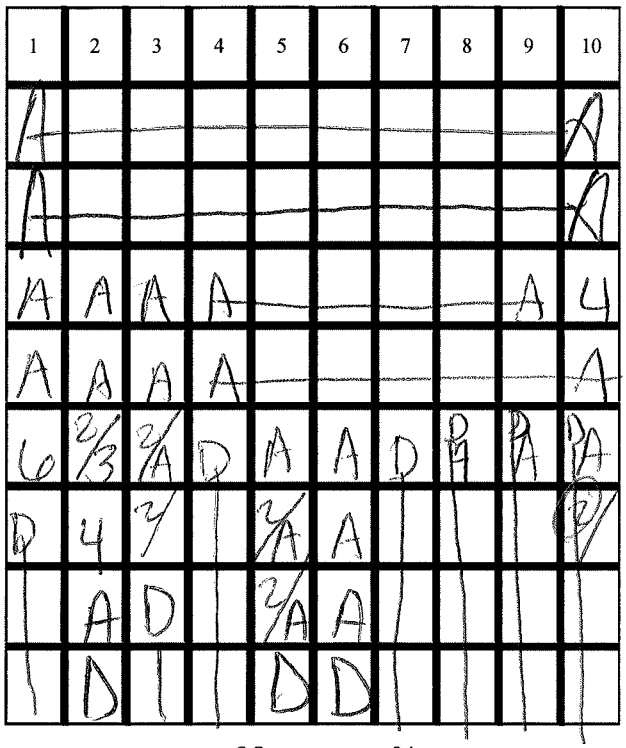
BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

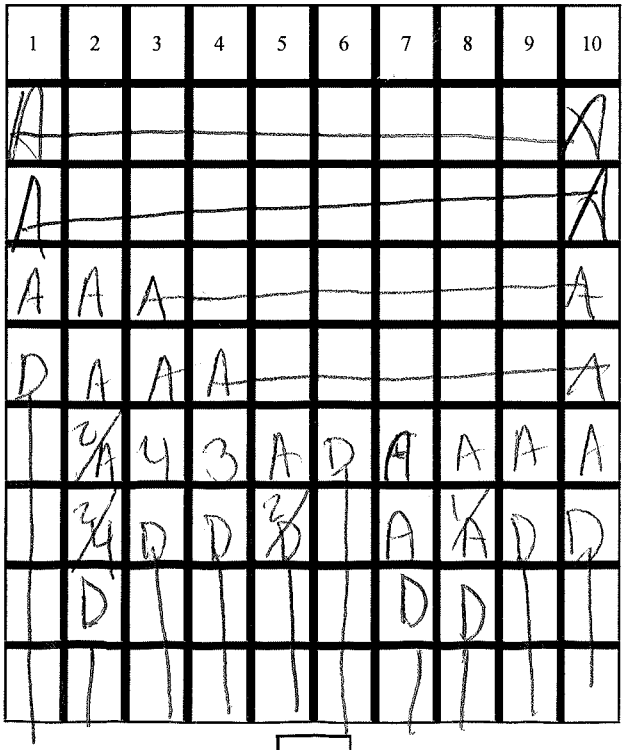
Client: **Pilgrim's Pride Corporation - Southwest WWP** Lab ID: **89242** Culture No.:

TEST INSTRUCTIONS: NETDMR

Dilution: 80 %



Dilution: 98 %



Code: Cells in numbered columns indicate daily survival and reproduction: "A" means adult alive and no young produced, a number means adult alive and that number of young produced, "D" followed by a zero means adult dead and no young produced, "D" followed by a number means adult dead and that number of young produced. "E" indicates toss out due to experimenter error. Lined through spaces preceded by a number or letter represent the same number. Lined spaces without a preceding number or letter indicate unused or not applicable spaces.

BIO-AQUATIC TESTING, INC.

Chronic CERIODAPHNIA DUBIA SURVIVAL AND REPRODUCTION

Client: **Pilgrim's Pride Corporation - Southwest WWP** Lab ID: **89242** Culture No.:

TEST INSTRUCTIONS: NETDMR

Test Temperatures

	0Hr	24Hr		48Hr		72Hr		96Hr		5 days		6 days		7 days
	new	old	new	old	new	old	new	old	new	old	new	old	new	old
Control	25.0	25.6	25.3	25.5	25.3	25.3	24.1	24.3	24.3	24.8	25.1	25.5	24.1	25.5
31														
41														
55														
80														
98														
TIME/DATE TECH	12-3-24 MH 1300	12-04 MW	-24 1155	12-05 MW	-24 1010	12-06 ~~MW~~ am	-24 1303 RA	12-07 1500	-24 RA	12-08 1622	-24 RA	12-09 1436	-24 RA	12-10- MH 08
IR GUN ID #	012	012		012		~~012~~ 027 MW		027		027		027		012

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

Chronic *Pimephales promelas*

Client: Pilgrim's Pride Corporation Southwest WWP

Lab ID: 89242

Permit Number: TPDES WQ0003017000

Test Temperature (oC): 25 ± 1

Outfall Name: 001 **Sample Type:** Composite

Photo Period: 16 Hours Light 8 Hours Dark

Receiving Water Name: Tankersley Creek

Test Start Time: 14:49 Test End Time: 10:30

Begin Date: 12/3/2024

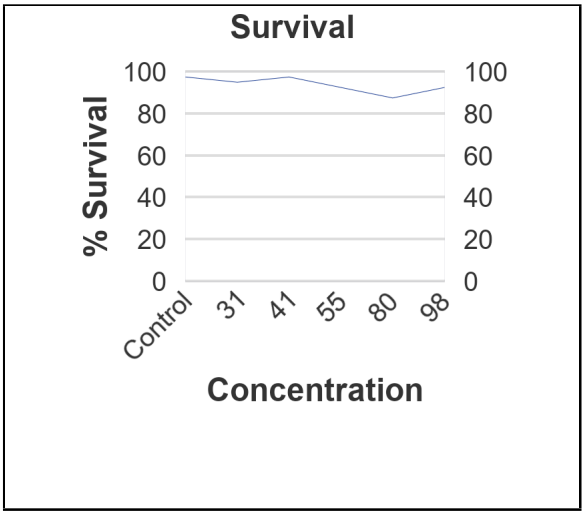
End Date: 12/10/2024

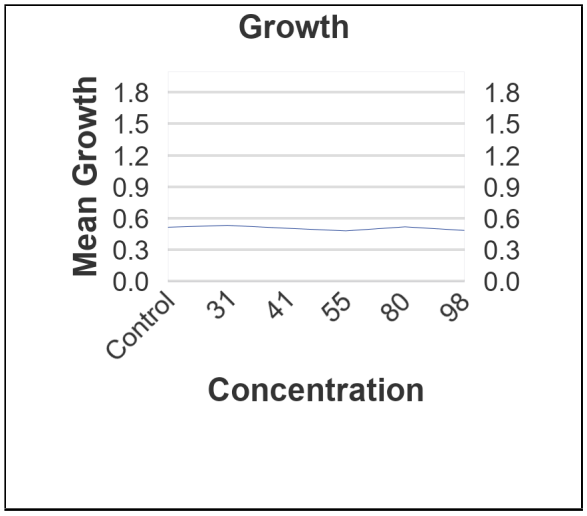
SURVIVAL

Effluent Concentration		Number Of Alive 12/3	12/4	12/5	12/6	12/7	12/8	12/9	12/10	Avg% Surv.
Control	A	8	8	8	8	8	8	8	8	97.5%
	B	8	8	8	8	8	8	8	7	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	8	8	8	8	8	
31	A	8	8	8	8	7	7	7	7	95.0%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	7	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	8	8	8	8	8	
41	A	8	8	8	8	8	8	8	8	97.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	7	7	7	
	E	8	8	8	8	8	8	8	8	
55	A	8	8	8	8	8	8	8	7	92.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	8	
	D	8	8	8	8	8	8	8	8	
	E	8	8	8	7	7	7	7	6	

Effluent Concentration		Number Of Alive								Avg% Surv.
		12/3	12/4	12/5	12/6	12/7	12/8	12/9	12/10	
80	A	8	8	8	8	8	8	8	8	87.5%
	B	8	8	8	8	8	8	8	8	
	C	8	8	8	8	8	8	8	6	
	D	8	8	8	8	7	7	7	6	
	E	8	8	8	8	8	8	8	7	
98	A	8	8	8	8	8	8	8	8	92.5%
	B	8	8	8	8	8	8	8	7	
	C	8	8	8	8	8	8	8	8	
	D	8	8	7	7	7	7	7	7	
	E	8	8	8	8	8	8	8	7	
	A									
	B									
	C									
	D									
	E									

Concentration Response Relationships





BIO-AQUATIC TESTING, INC.

Chronic Pimephales promelas SURVIVAL Lab ID: **89242**

Client: **Pilgrim's Pride Corporation** Facility **Southwest WWP** Outfall:001 Sample Type: Composite

TEST INSTRUCTIONS: NETDMR

Culture No. : PO-24-3370 Photo Period: 16hr light, 8hr dark **RANDOMIZATION:** SC-5 2

Dilution: Control 31 41 55

	DATE/TIME/ TECHNICIAN	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	12-3-24 1449 JS	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
24Hr	12-4-24 1149 JS	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
48Hr	12-5-24 0935 JS	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—
72Hr	12-6-24 1102 AR	8	—	—	—	—	8	—	—	—	—	8	—	—	—	—	8	—	—	—	7
96Hr	12-7-24 1152 AR	8	—	—	—	—	7	8	—	—	—	8	—	—	—	—	8	—	—	—	7
5 days	12-8-24 0957 AR	8	—	—	—	—	7	8	—	—	—	8	—	—	7	8	8	—	—	—	7
6 days	12-9-24 1023 AR	8	—	—	—	—	7	8	—	—	—	8	—	—	7	8	8	—	—	—	7
7 days	12-10-24 1030 MM	8	7	8	—	—	7	8	7	8	8	8	—	—	7	8	7	8	—	—	6

Dilution: 80 98

	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E	A	B	C	D	E
0Hr	8	—	—	—		8	—	—	—	—										
24Hr	8	—	—	—		8	—	—	—	—										
48Hr	8	—	—	—		8	—	—	7	8										
72Hr	8	—	—	—		8	—	—	7	8										
96Hr	8	—	—	7	8	8	—	—	7	8										
5 days	8	—	—	7	8	8	—	—	7	8										
6 days	8	—	—	7	8	8	—	—	7	8										
7 days	8	8	6	6	7	8	7	8	7	7										

BIO-AQUATIC TESTING, INC.

Chronic Pimephales promelas SURVIVAL Lab ID: **89242**

Client: **Pilgrim's Pride Corporation** Facility **Southwest WWP** Outfall:001 Sample Type: Composite

TEST INSTRUCTIONS: NETDMR

Test Temperatures

	0Hr	24Hr		48Hr		72Hr		96Hr		5 days		6 days		7 days
	new	old	new	old	new	old	new	old	new	old	new	old	new	old
Control	24.5	24.5	25.2	25.2	25.6	25.4	25.1	25.2	24.9	25.2	24.8	25.5	24.8	25.8
31														
41														
55														
80														
98														
TIME/DATE TECH	12-3-24 1409 JS	12-4	24 1149 JS	12-5	24 0925 JS	12-6	-24 1102 AR	12-7	-24 1152 AR	12-8	-24 0957 AR	12-9	-24 1023 AR	12-10- 1030
IR GUN ID #	020	020		020		028		020		020		020		020

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

Chronic *Pimephales promelas*

Client: Pilgrim's Pride Corporation Southwest WWP **Lab ID: 89242**

Permit Number: WQ0003017000

Sample Type: Composite **Outfall Name:** 001

Receiving Water Name: Tankersley Creek

Synthetic

	ON	SN	Wt.	Avg.	SN Avg.
A	8	8	4.491	0.561	0.561
B	8	7	4.555	0.569	0.651
C	8	8	3.279	0.410	0.410
D	8	8	4.268	0.534	0.534
E	8	8	4.075	0.509	0.509

Mean	C.V. %
0.517	12.4

SN Mean	SN C.V. %
0.533	16.4

31

	ON	Wt.	Avg.
A	8	4.400	0.550
B	8	3.605	0.451
C	8	4.576	0.572
D	8	3.824	0.478
E	8	4.969	0.621

Mean	C.V. %
0.534	13.0

41

	ON	Wt.	Avg.
A	8	4.448	0.556
B	8	3.617	0.452
C	8	3.848	0.481
D	8	4.806	0.601
E	8	3.550	0.444

Mean	C.V. %
0.507	13.6

55

	ON	Wt.	Avg.
A	8	3.997	0.500
B	8	4.159	0.520
C	8	3.331	0.416
D	8	5.319	0.665
E	8	2.468	0.309

Mean	C.V. %
0.482	27.4

80

	ON	Wt.	Avg.
A	8	5.309	0.664
B	8	4.490	0.561
C	8	4.338	0.542
D	8	3.018	0.377
E	8	3.651	0.456

Mean	C.V. %
0.520	20.9

98

	ON	Wt.	Avg.
A	8	4.283	0.535
B	8	4.078	0.510
C	8	4.071	0.509
D	8	2.894	0.362
E	8	4.112	0.514

Mean	C.V. %
0.486	14.5

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

	ON	Wt.	Avg.
A			
B			
C			
D			
E			

Mean	C.V. %

Note: ON stands for original number per replicate, while SN refers to the number surviving after test completion.

BIO-AQUATIC TESTING, INC. TOXICITY TEST

Chronic **Pimephales promelas** Lab ID: **89242**

Client: Pilgrim's Pride Corporation - Southwest WWP Balance: Radwag BAL-007

Begin Date: 12/3/2024 End Date: 12/10/2024 Organism: Pimephales promelas

Analyst: MS Date/Time placed in Oven: 12/10/2024 1544

Weigh Date: 12 - 11 - 2024 Date/Time removed from Oven: 12/11/2024 1415

Control

	Qty.	Wt.
A	8	4.491
B	7	4.555
C	8	3.279
D	1	4.268
E	1	4.075

31 %

	Qty.	Wt.
A	7	4.400
B	8	3.605
C	7	4.576
D	8	3.824
E	8	4.969

41 %

	Qty.	Wt.
A	8	4.448
B	1	3.617
C	1	3.848
D	7	4.806
E	8	3.550

55 %

	Qty.	Wt.
A	7	3.997
B	8	4.159
C	1	3.331
D	1	5.319
E	6	2.468

80 %

	Qty.	Wt.
A	8	5.309
B	8	4.490
C	6	4.338
D	6	3.018
E	7	3.651

98 %

	Qty.	Wt.
A	8	4.283
B	7	4.078
C	8	4.071
D	7	2.894
E	7	4.112

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

	Qty.	Wt.
A		
B		
C		
D		
E		

Lined through spaces preceded by a number represent the same number. Lined spaces without a preceding number indicate unused or not applicable spaces.

APPENDIX A

STATISTICS SUMMARY

Both the lethal and sub-lethal endpoints were statistically calculated according to their respective EPA guidelines. The Chronic Freshwater organisms were calculated according to EPA-821-R-02-013, October 2002 Fourth Edition. The Chronic Marine and Estuarine organisms were calculated according to EPA-821-R-02-014, October 2002 Third Edition. The Acute Freshwater and Marine organisms were calculated according to EPA-821-R-02-012, October 2002 Fifth Edition. The fertilization organisms were calculated according to EPA-600-R-95-136 or EPA-600-R-12-022, dependent upon the species. Listed below are the basic principles of these guidelines. If you would like a copy of the raw statistical calculations for your test then please contact us.

The chronic and acute *Pimephales promelas* and *Menidia beryllina* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts (parametric). If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test (non-parametric) is used. The chronic *Pimephales promelas* and *Menidia beryllina* growth data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Mysidopsis bahia* survival data is analyzed using Chi-square test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test or Bartlett's Test then Steels Many One Test is used. *Mysidopsis bahia* growth data is analyzed using Chi-square test and Bartlett's Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Mysidopsis bahia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shipiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The chronic *Ceriodaphnia dubia* survival data are analyzed using the Fisher's Exact Test. The chronic *Ceriodaphnia dubia* reproduction and are analyzed using the Chi-square test and Bartlett Test. If the data passes one of these tests then the data is run through ANOVA and Dunnetts. If the data fails Chi-square test and Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The acute *Daphnia pulex* and *Ceriodaphnia dubia* survival data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation may also be used.

The fertilization data is analyzed using Shapiro Wilks Test and Bartlett's Test. If the data passes both tests then the data is run through ANOVA and Dunnetts. If the data fails Shapiro Wilks Test or Bartlett's Test then Steels Many One Test is used. Point estimation or TST methodology may also be used.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	10	0	10
31	6	4	10
TOTAL	16	4	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 6.
Since b is less than or equal to 6 there is a significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

IDENTIFICATION	NUMBER OF ALIVE	NUMBER OF DEAD	TOTAL ANIMALS
CONTROL	10	0	10
41	5	5	10
TOTAL	15	5	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 5.
Since b is less than or equal to 6 there is a significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

NUMBER OF

IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
55	1	9	10
TOTAL	11	9	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 1.
Since b is less than or equal to 6 there is a significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS
CONTROL	10	0	10
80	0	10	10
TOTAL	10	10	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 0.
Since b is less than or equal to 6 there is a significant difference
between CONTROL and TREATMENT at the 0.05 level.

FISHER'S EXACT TEST

	NUMBER OF		
IDENTIFICATION	ALIVE	DEAD	TOTAL ANIMALS

	CONTROL	10	0	10
	100	0	10	10
	TOTAL	10	10	20

CRITICAL FISHER'S VALUE (10,10,10) (p=0.05) IS 6. b VALUE IS 0.
Since b is less than or equal to 6 there is a significant difference
between CONTROL and TREATMENT at the 0.05 level.

SUMMARY OF FISHER'S EXACT TESTS

GROUP	IDENTIFICATION	NUMBER EXPOSED	NUMBER DEAD	SIG (P=.05)
	CONTROL	10	0	
1	31	10	4	*
2	41	10	5	*
3	55	10	9	*
4	80	10	10	*
5	98	10	10	*

fathead growth
File: 89242.ppg Transform: NO TRANSFORMATION

Shapiro - Wilk's test for normality

D = 0.191

W = 0.966

Critical W (P = 0.05) (n = 30) = 0.927
Critical W (P = 0.01) (n = 30) = 0.900

Data PASS normality test at P=0.01 level. Continue analysis.

fathead growth
File: 89242.ppg Transform: NO TRANSFORMATION

Bartlett's test for homogeneity of variance
Calculated B1 statistic = 3.62

Table Chi-square value = 15.09 (alpha = 0.01, df = 5)
Table Chi-square value = 11.07 (alpha = 0.05, df = 5)

Data PASS B1 homogeneity test at 0.01 level. Continue analysis.

fathead growth
File: 89242.ppg Transform: NO TRANSFORMATION

ANOVA TABLE

SOURCE	DF	SS	MS	F
Between	5	0.010	0.002	0.260
Within (Error)	24	0.191	0.008	
Total	29	0.202		

Critical F value = 2.62 (0.05,5,24)
Since F < Critical F FAIL TO REJECT Ho: All equal

fathead growth
File: 89242.ppg Transform: NO TRANSFORMATION

DUNNETT'S TEST - TABLE 1 OF 2 Ho:Control<Treatment

GROUP	IDENTIFICATION	TRANSFORMED MEAN	MEAN CALCULATED IN ORIGINAL UNITS	T STAT	SIG
1	con	0.517	0.517		
2	31	0.534	0.534	-0.315	
3	41	0.507	0.507	0.174	
4	55	0.482	0.482	0.613	
5	80	0.520	0.520	-0.060	
6	98	0.486	0.486	0.542	

Dunnett table value = 2.36 (1 Tailed Value, P=0.05, df=24,5)

fathead growth
File: 89242.ppg Transform: NO TRANSFORMATION

DUNNETT'S 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	con	5			
2	31	5	0.133	25.8	-0.018
3	41	5	0.133	25.8	0.010
4	55	5	0.133	25.8	0.035
5	80	5	0.133	25.8	-0.003
6	98	5	0.133	25.8	0.031

89242

Bio-Aquatic Testing, Inc.

FRESH WATER TEST SETUP FORM

Client: Pilgrim's Pride Corporation

Permit WQ0003017000

Facility: Southwest WWP

Lab Number 89242

Outfall Name: 001

Number of samples 3

Dilution Water: Synthetic Lab

Receiving Water Name: Tankersley Creek

Dechlorinate Sample: No

Sx #	Rcvd Date	Rcvd Time	Sampling Dates		Sampling Times	
			Begin Date	End Date	Start	End
1	12/02/24	12:46	12/01/24	12/02/24	08:00	07:00
2	12/04/24	13:30	12/03/24	12/04/24	08:00	07:00
3	12/06/24	13:30	12/05/24	12/06/24	08:00	08:00

Type of Test(s)

Ceriodaphnia dubia	Chronic
Pimephales promelas	Chronic

Dilution Water

Sample #	Hardness As mg/L $CaCO_3$	Alkalinity as mg/L $CaCO_3$
1	136	61
2	130	68
3	130	68

Start Sx #	1	Date:	12/3/2024
Renew Sx #	1	Date:	12/4/2024
Renew Sx #	1	Date:	12/5/2024
Renew Sx #	2	Date:	12/6/2024
Renew Sx #	2	Date:	12/7/2024
Renew Sx #	3	Date:	12/8/2024
Renew Sx #	3	Date:	12/9/2024

Test Start Date: 12/3/2024

Test End Date: 12/10/2024

Ceriodaphnia dubia Test Set Up: 10 Reps & 1 Organisms per Rep

Pimephales Test Set Up: 5 Reps & 8 Organism per Rep

Concentrations: 31 41 55 80 98 %

Test Chemistry on these dilutions: 31 41 55 80 98

Samples received by: ○ Express Delivery ○ UPS Next Day ○ via Air Cargo ○ DHL ○ Federal Express ○ the Client ◉ Bio-Aquatic personnel

Other:

BIO-AQUATIC TESTING, INC.

Hardness, Alkalinity, Residual Chlorine, Specific Conductivity, and Salinity Analysis Data

Client: Pilgrim's Pride Corporation **Lab ID:** 89242

Facility: Southwest WWP **Outfall:** 001

Dilution Water(s): Synthetic Lab **Test Date:** December 3, 2024

EFFLUENT PARAMETERS

Effluent Sample #	Received		Residual Cl_2 (mg/L)	DeChlor (ml/L)[1]	Ammonia (mg/L)	Analyst Initials	Temp. Received
	Date	Time					
1	12/2/24	12:46	<0.10	N/A	<0.25	CAP	3.1
2	12/4/24	13:30	<0.10	N/A	<0.25	DT	3.1
3	12/6/24	13:30	0.97	N/A	<0.25	CAP	3.1

[1]**Dechlorination Reagent:** 0.025 N Sodium Thiosulfate

Effluent Sample #	pH	DO (mg/L)	Hardness (mg/L $CaCO_3$)	Alkalinity (mg/L $CaCO_3$)	Conductivity (umhos/cm)	Analyst Initials
1	6.6	9.1	466	209	1656	CAP
2	7.7	10.0	244	66	1639	DT
3	7.1	9.3	434	264	1600	CAP

DAILY RENEWAL CONDUCTIVITY**

Date		Sample #	Values are at Highest Dilution		Analyst
			Specific Conductivity as umhos/cm	Salinity (ppt)	
12/3	Lab H2O		479	0.3	CG
12/4	Lab H2O		404	0.2	GJ
12/5	Lab H2O		530	0.3	JR
12/6	Lab H2O		525	0.3	LH/MM
12/7	Lab H2O		411	0.2	AR/JS
12/8	Lab H2O		413	0.2	AR/CA
12/9	Lab H2O		429	0.2	CG/TM
12/3	OUTFALL*	1	1616	0.8	CG
12/4	OUTFALL*	1	1565	0.8	GJ
12/5	OUTFALL*	1	1670	0.9	JR
12/6	OUTFALL*	2	1747	0.9	LH/MM
12/7	OUTFALL*	2	1624	0.9	AR/JS
12/8	OUTFALL*	3	1485	0.8	AR/CA
12/9	OUTFALL*	3	1428	0.8	CG/TM

**Conductivity is taken on the highest remaining effluent concentration used for test renewal, not necessarily 100%

Analysis Methods: Chlorine: Hanna Colorimeter #HI711, Ammonia: Hanna Colorimeter #HI733, Hardness: Hanna Photometer #HI96735, Alkalinity: Hanna Colorimeter #HI775, pH, DO, Conductivity: Thermo Versa Star Benchtop Meter

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Ceriodaphnia dubia**

Client: **Pilgrim's Pride Corporation** **Lab ID:** **89242**

Facility: **Southwest WWP** **Dilution Water(s):** **Synthetic Lab**

Outfall: **001** **Test Begin Date:** **December 3, 2024**

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	31	41	55	80	98		
CG	12/3	Start	1	pH	7.5	7.5	7.5	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.5	8.5	8.5		
GJ	12/4	24 Hr	1	pH	7.5	7.5	7.5	7.5	7.5	7.6		
		25 ± 1		DO (mg/L)	8.2	8.2	8.2	8.2	8.2	8.2		
		Renew	1	pH	7.7	7.8	7.9	7.9	8.0	8.0		
				DO (mg/L)	8.2	8.4	8.5	8.6	8.6	8.6		
JR	12/5	48 Hr	1	pH	8.3	8.2	8.2	8.2	8.2	8.2		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.4	8.3	8.4		
		Renew	1	pH	7.5	7.5	7.6	7.6	7.6	7.6		
				DO (mg/L)	8.1	8.3	8.5	8.5	8.6	8.6		
LH/MM	12/6	72 Hr	1	pH	8.5	8.4	8.4	8.4	8.4	8.4		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.4	8.4	8.4		
		Renew	2	pH	8.0	7.9	7.9	7.9	7.9	7.9		
				DO (mg/L)	8.1	7.9	8.0	8.1	8.2	8.2		
AR/JS	12/7	96 Hr	2	pH	7.9	8.0	8.1	8.2	8.3	8.5		
		25 ± 1		DO (mg/L)	8.8	8.8	8.8	8.8	8.8	8.8		
		Renew	2	pH	7.5	7.6	7.6	7.7	7.7	7.8		
				DO (mg/L)	8.4	8.4	8.4	8.3	8.3	8.3		
AR/CAP	12/8	120 Hr	2	pH	8.4	8.4	8.4	8.5	8.5	8.6		
		25 ± 1		DO (mg/L)	8.3	8.5	8.5	8.5	8.5	8.6		
		Renew	3	pH	7.5	7.6	7.6	7.8	7.8	7.9		
				DO (mg/L)	8.3	8.3	8.3	8.2	8.2	8.1		
AR/AK	12/9	144 Hr	3	pH	8.2	8.2	8.2	8.2	8.2	8.3		
		25 ± 1		DO (mg/L)	8.3	8.2	8.2	8.3	8.3	8.3		
		Renew	3	pH	7.8	7.7	7.7	7.7	7.7	7.7		
				DO (mg/L)	8.5	8.2	8.2	8.1	8.1	7.6		
CG/TM	12/10	168 Hr	3	pH	8.3	8.2	8.2	8.2	8.2	8.4		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.5	8.5	8.6		

BIO-AQUATIC TESTING, INC.

pH, Dissolved Oxygen

Chronic **Pimephales promelas**

Client: Pilgrim's Pride Corporation **Lab Number:** 89242

Facility: Southwest WWP **Dilution Water(s):** Synthetic Lab

Outfall: 001 **Test Begin Date:** December 3, 2024

NR indicates that the test is non-renewal.

					Concentration							
ANALYST	DATE	TIME	SX#	UNIT	Control	31	41	55	80	98		
CG	12/3	Start	1	pH	7.5	7.5	7.5	7.6	7.6	7.6		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.5	8.5	8.5		
GJ	12/4	24 Hr	1	pH	8.0	8.0	8.0	8.0	8.1	8.3		
		25 ± 1		DO (mg/L)	8.7	8.1	8.0	7.9	7.9	7.9		
		Renew	1	pH	7.7	7.8	7.9	7.9	8.0	8.0		
				DO (mg/L)	8.2	8.4	8.5	8.6	8.6	8.6		
JR	12/5	48 Hr	1	pH	7.7	7.7	7.7	7.7	7.7	7.7		
		25 ± 1		DO (mg/L)	8.3	8.2	8.0	8.0	7.8	7.8		
		Renew	1	pH	7.5	7.5	7.6	7.6	7.6	7.6		
				DO (mg/L)	8.1	8.3	8.5	8.5	8.6	8.6		
LH/MM	12/6	72 Hr	1	pH	8.1	8.0	8.0	7.9	7.9	8.0		
		25 ± 1		DO (mg/L)	8.1	8.0	8.0	7.9	8.0	7.9		
		Renew	2	pH	8.0	7.9	7.9	7.9	7.9	7.9		
				DO (mg/L)	8.1	7.9	8.0	8.1	8.2	8.2		
AR/JS	12/7	96 Hr	2	pH	7.4	7.5	7.5	7.7	7.7	8.0		
		25 ± 1		DO (mg/L)	8.4	8.4	8.4	8.1	8.1	8.0		
		Renew	2	pH	7.5	7.6	7.6	7.7	7.7	7.8		
				DO (mg/L)	8.4	8.4	8.4	8.3	8.3	8.3		
AR/CAP	12/8	120 Hr	2	pH	7.4	7.6	7.6	7.9	7.9	8.1		
		25 ± 1		DO (mg/L)	7.9	7.9	7.9	7.9	7.9	7.9		
		Renew	3	pH	7.5	7.6	7.6	7.8	7.8	7.9		
				DO (mg/L)	8.3	8.3	8.3	8.2	8.2	8.1		
AR/AK	12/9	144 Hr	3	pH	7.5	7.6	7.6	7.7	7.7	8.0		
		25 ± 1		DO (mg/L)	7.6	7.6	7.6	7.6	7.6	7.4		
		Renew	3	pH	7.8	7.7	7.7	7.7	7.7	7.7		
				DO (mg/L)	8.5	8.2	8.2	8.1	8.1	7.6		
CG/TM	12/10	168 Hr	3	pH	7.5	7.6	7.6	7.8	7.8	8.0		
		25 ± 1		DO (mg/L)	8.2	8.2	8.2	8.0	8.0	7.9		

Appendix B

Ceriodaphnia dubia

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

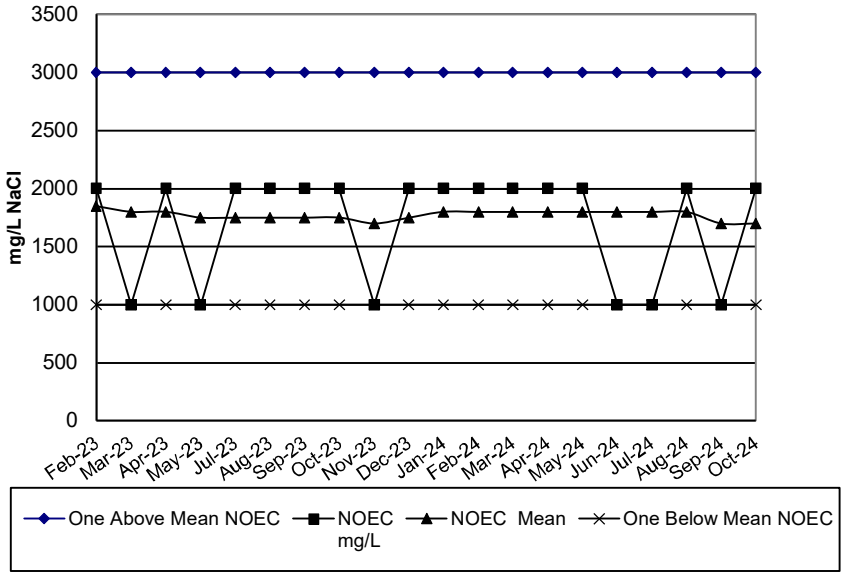
CHRONIC REFERENCE TOXICANT TEST RESULTS

DILUTION WATER:	Standard Synthetic Freshwater						
CHEMICAL:	Sodium Chloride						
DURATION:	3-Brood Chronic						
TEST NUMBER:	355						
PROJECT NUMBER:	93658						
START DATE:	10/29/2024						
START TIME:	17:04						
TOTAL NUMBER EXPOSED:	10 organisms per concentration						
CONCENTRATIONS (mg/L):	CON	250	500	1000	2000	3000	4000
NUMBER DEAD PER CONCENTRATION:	1	0	0	1	0	10	10
TEST METHODS:	As listed in EPA-821-R-02-013						
STATISTICAL METHODS:	SURVIVAL: Fisher's Exact Test REPRODUCTION: ANOVA-Dunnetts						

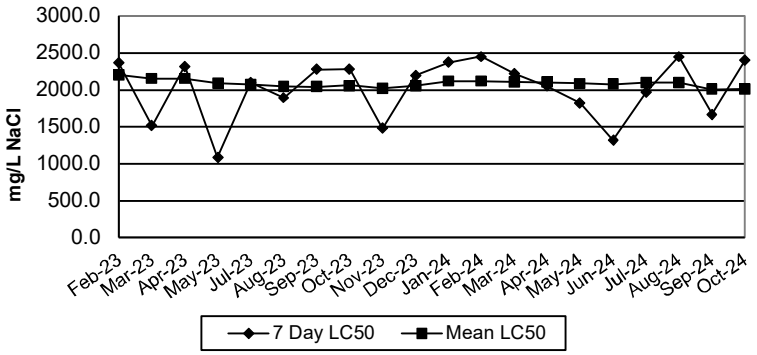
NOEC FOR SURVIVAL:	2000	mg/L
LOEC FOR SURVIVAL:	3000	mg/L
NOEC FOR REPRODUCTION:	1000	mg/L
LOEC FOR REPRODUCTION:	2000	mg/L

PMSD: 22.9

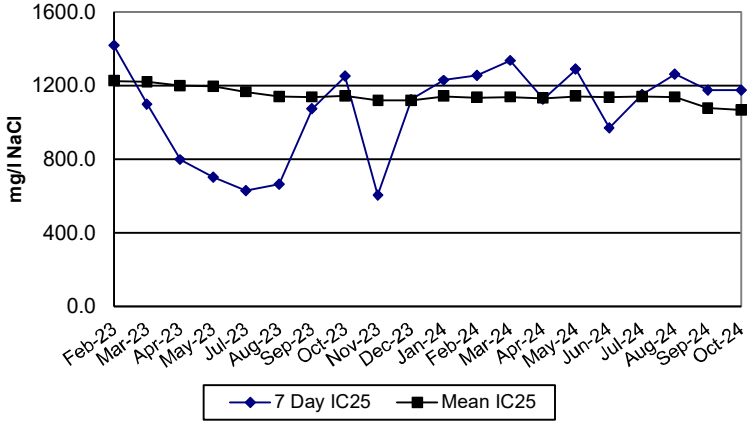
Ceriodaphnia Chronic Survival Control Chart



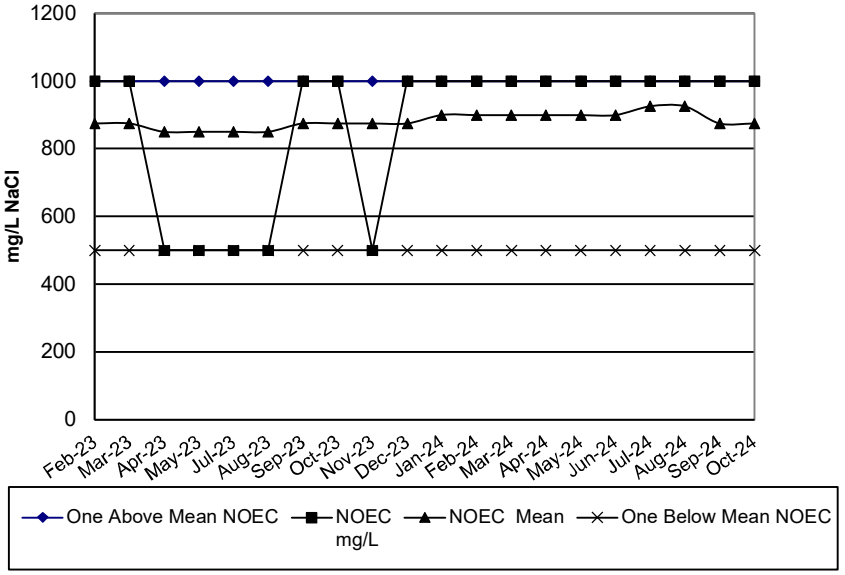
Ceriodaphnia 7-Day LC50



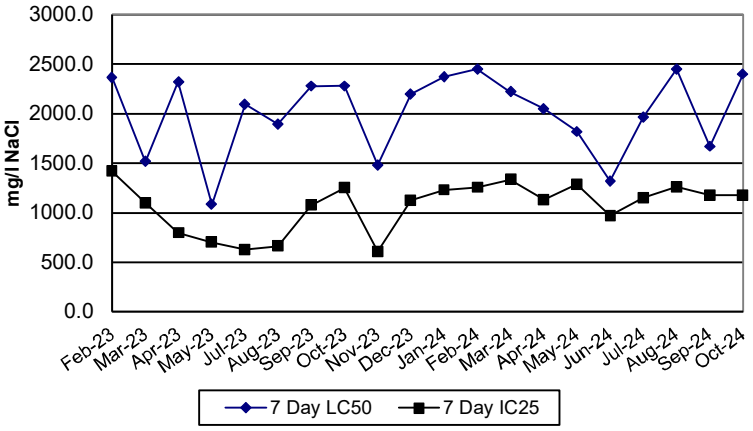
Ceriodaphnia 7-Day IC25



Ceriodaphnia Chronic Reproduction Control Chart



Ceriodaphnia 7-Day LC50 & IC25



Appendix B

Pimephales promelas

BIO-AQUATIC TESTING, INC.

Carrollton, TX

REFERENCE TOXICANTS

Bio-Aquatic Testing conducts reference toxicant testing monthly for organisms cultured in-house. For studies requiring purchased organisms, reference toxicant testing is performed simultaneously. Reference toxicant testing validates data and measures organism consistency. Only reagent grade chemicals are used of the following choices: sodium laurel sulfate (SLS), copper sulfate, copper chloride, potassium chloride, and sodium chloride. Organism responses are tracked with control charts for each reference toxicant/organism combination. The data are examined for sensitivity trends and to determine if results are within EPA described limits.

CHRONIC REFERENCE TOXICANT TEST RESULTS

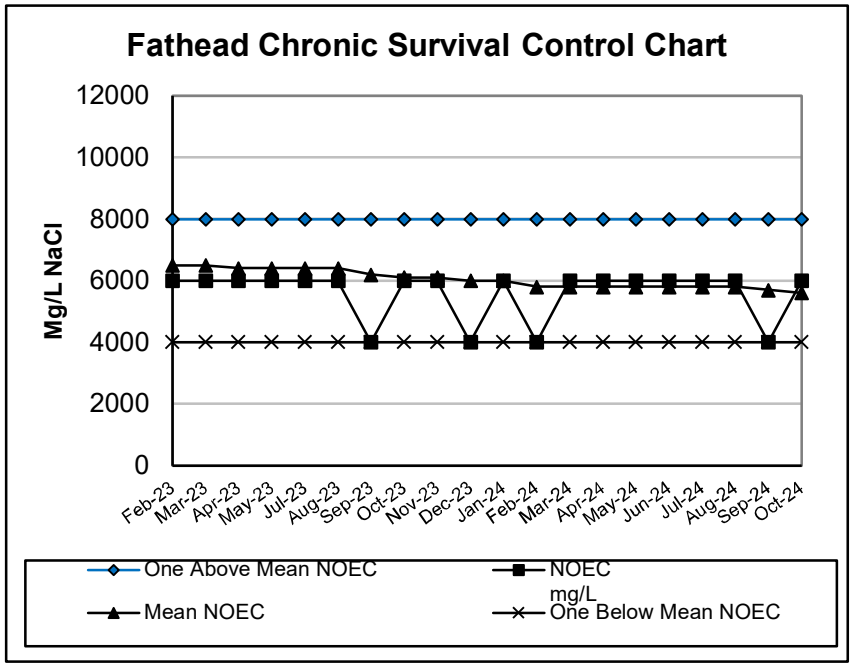
DILUTION WATER:	Standard Synthetic Freshwater
CHEMICAL:	Sodium Chloride
DURATION:	7 Days
TEST NUMBER:	395
PROJECT NUMBER:	93666
START DATE:	10/29/2024
START TIME:	17:10
TOTAL NUMBER EXPOSED:	40 organisms per concentration

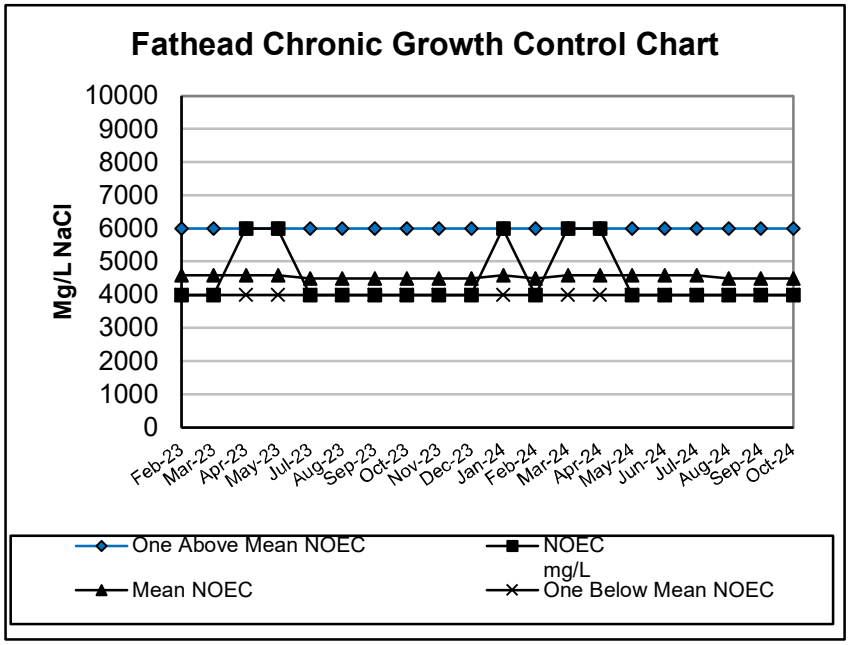
CONCENTRATIONS (mg/L):	CON	2000	4000	6000	8000	10000	12000
NUMBER DEAD PER CONCENTRATION:	1	0	1	2	15	38	40

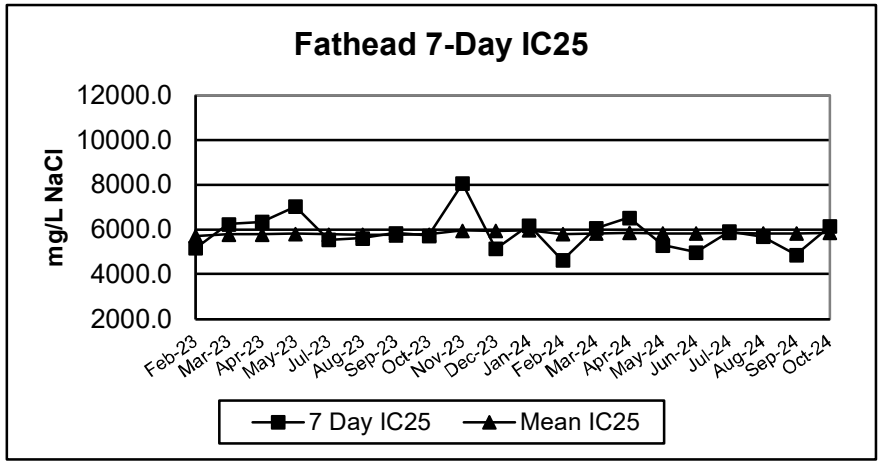
TEST METHODS:	As listed in EPA-821-R-02-013
STATISTICAL METHODS:	SURVIVAL: Steel's Many-One Rank Test GROWTH: ANOVA and Dunnett's Test

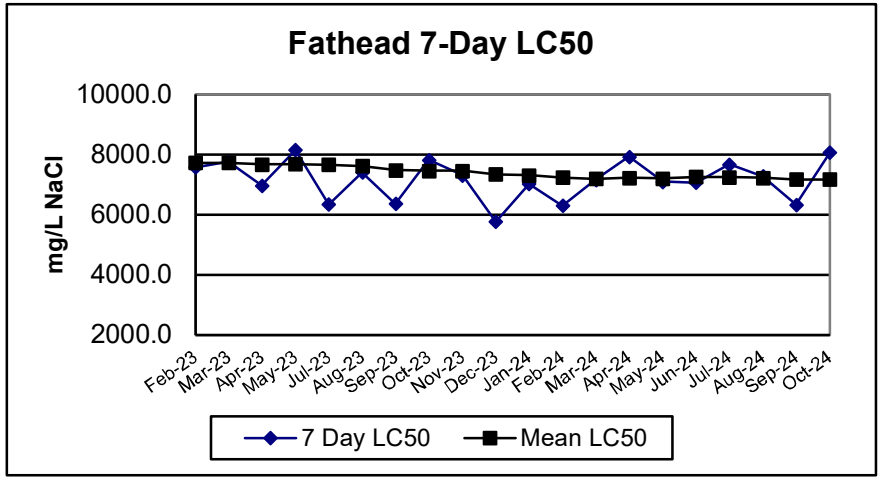
NOEC FOR SURVIVAL:	6000	mg/L
LOEC FOR SURVIVAL:	8000	mg/L
NOEC FOR GROWTH:	4000	mg/L
LOEC FOR GROWTH:	6000	mg/L

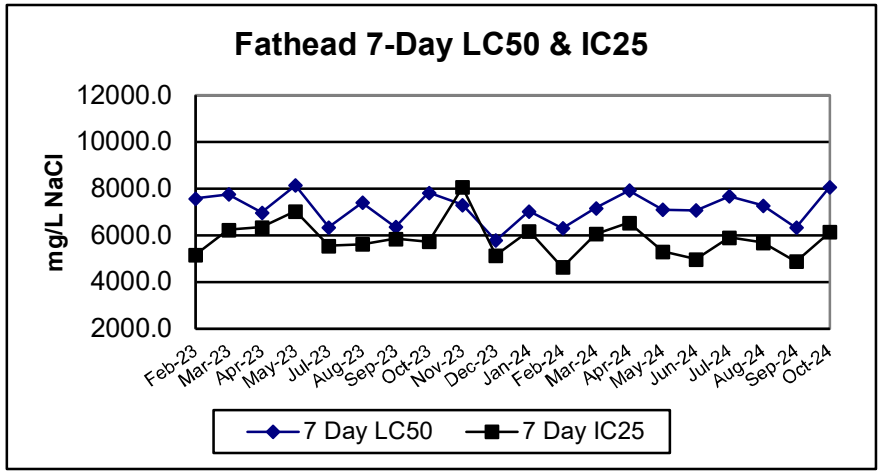
PMSD: 13.5


Fathead Chronic Survival Control Chart
Mg/L NaCl
12000
10000
8000
6000
4000
2000
0
Feb-23 Mar-23 Apr-23 May-23 Jul-23 Aug-23 Sep-23 Oct-23 Nov-23 Dec-23 Jan-24 Feb-24 Mar-24 Apr-24 May-24 Jun-24 Jul-24 Aug-24 Sep-24 Oct-24
One Above Mean NOEC
NOEC
Mean NOEC
One Below Mean NOEC
mg/L


Fathead Chronic Growth Control Chart
Mg/L NaCl
10000
9000
8000
7000
6000
5000
4000
3000
2000
1000
0
Feb-23 Mar-23 Apr-23 May-23 Jul-23 Aug-23 Sep-23 Oct-23 Nov-23 Dec-23 Jan-24 Feb-24 Mar-24 Apr-24 May-24 Jun-24 Jul-24 Aug-24 Sep-24 Oct-24
One Above Mean NOEC
NOEC
Mean NOEC
One Below Mean NOEC
mg/L


Fathead 7-Day IC25
mg/L NaCl
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
Feb-23 Mar-23 Apr-23 May-23 Jul-23 Aug-23 Sep-23 Oct-23 Nov-23 Dec-23 Jan-24 Feb-24 Mar-24 Apr-24 May-24 Jun-24 Jul-24 Aug-24 Sep-24 Oct-24
7 Day IC25
Mean IC25


Fathead 7-Day LC50
mg/L NaCl
10000.0
8000.0
6000.0
4000.0
2000.0
Feb-23 Mar-23 Apr-23 May-23 Jul-23 Aug-23 Sep-23 Oct-23 Nov-23 Dec-23 Jan-24 Feb-24 Mar-24 Apr-24 May-24 Jun-24 Jul-24 Aug-24 Sep-24 Oct-24
7 Day LC50
Mean LC50


Fathead 7-Day LC50 & IC25
mg/L NaCl
12000.0
10000.0
8000.0
6000.0
4000.0
2000.0
Feb-23 Mar-23 Apr-23 May-23 Jul-23 Aug-23 Sep-23 Oct-23 Nov-23 Dec-23 Jan-24 Feb-24 Mar-24 Apr-24 May-24 Jun-24 Jul-24 Aug-24 Sep-24 Oct-24
7 Day LC50
7 Day IC25

APPENDIX C

LITERATURE REFERENCES

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fifth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-012.

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents and Receiving Water To Marine And Estuarine Organisms (Third Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-014.

U.S.E.P.A., 2002. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To Freshwater Organisms (Fourth Edition) U.S. Environmental Protection Agency, Office of Water, Washington D.C., EPA-821-R-02-013.

U.S.E.P.A., 2012. Tropical Collector Urchin, *Tripneustes gratilla* (First Edition) U.S. Environmental Protection Agency, Office of Research and Development and Region 9, EPA-600-R-12-022.

U.S.E.P.A., 1995. Short-Term Methods For Estimating The Chronic Toxicity Of Effluents And Receiving Water To West Coast Marine and Estuarine Organisms (First Edition) U.S. Environmental Protection Agency, EPA-600-R-95-136.

U.S.E.P.A., 2010. National Pollutant Discharge Elimination System Test of Significant Toxicity Technical Document, U.S. Environmental Protection Agency, Office of Wastewater, Washington D.C., EPA-833-R-10-004.

U.S.E.P.A., 1991. Technical Support Document For Water Quality-Based Toxics Control, U.S. Environmental Protection Agency, EPA-505-2-90-001.

Zarr, Jerrold, H., 1984. Biostatistical Analysis, (Second Edition). Prentice-Hall, Inc., Englewood Cliffs, N.J.

CHAIN-OF-CUSTODY SHEETS

Appendix D

Report Date: 12/30/2024 Revision 1

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Bio-Aquatic Lab ID: 89242

BIO-AQUATIC TESTING, INC.
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 89242

Please Review & Complete Sections A, B, C, & D.

Sample No: 89242 – ____ (Revision 2, Effective Date 9/25/2017)

Check Sample No. : ____ First, ____ Second, or ____ Third.

P.O. No: 4515296162

Client: Pilgrim's Pride Corporation

Facility: Southwest WWP

Permit No: WQ0003017000

Outfall: 001

Client Contact: JAMES HOWELL

Client Phone: (903) 575-3931

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 31 41 55 80 98

(For TX) Setup separate 24hr Acute Test? No

To Ship the 1st Sample on: 12/2/2024

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex (water flea)*	*D. magna (water flea)*	*P. promelas (minnow)*	*Selenastrum (green algae)*	*M. beryllina (minnow)*	*Mysidopsis (shrimp)*
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: 4th Qtr

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	001	E	12/1/24	12/2/24	0800	0700	Comp	W/G Wayne Woodle	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	W/G Woodle	12/2/24	1246	RancePamott	12/2/24	1246
2	RancePamott	12/2/24	1600	Cody Goldman	12-3-24	0845
3						

Bio-Aquatic Sample Login

CAP
12.3.24
0950

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 12.3.24 **Time:** 0950 **By:** CAP **Temperature:** 3.1 (C) **IR#:** 026

Chlorine: <.10 mg/l	**Ammonia:** <.25 mg/l	**Int. Sal\Cond:** 1650 ppt/uS	**Adj. Salinity** ppt
pH: 6.6	**Hardness:** 466 mg/l		**Other**
DO: 9.1 mg/l	**Alkalinity:** 209 mg/l (OK)		**Condition:** Good

Report Date: 12/30/2024 Revision 1

Page 33 of 41

BIO-Aquatic Lab ID: 89242

BIO-AQUATIC TESTING, INC.
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 89242

Please Review & Complete Sections A, B, C, & D.

Sample No: 89242 – ____

Revision 2 Effective Date 9/25/2017

Check Sample No. : ____ First, ____ Second, or ____ Third.

P.O. No: 4515296162

Client: Pilgrim's Pride Corporation

Facility: Southwest WWP

Permit No: WQ0003017000

Outfall: 001

Client Contact: JAMES HOWELL

Client Phone: (903) 575-3931

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 31 41 55 80 98

(For TX) Setup separate 24hr Acute Test? No

To Ship the 1st Sample on: 12/2/2024

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex (water flea)*	*D. magna (water flea)*	*P. promelas (minnow)*	*Selenastrum (green algae)*	*M. beryllina (minnow)*	*Mysidopsis (shrimp)*
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: 4th Qtr

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	001	E	12/3/24	12/4/24	0800	0700	Comp	Wayne Woodke	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1		12/4/24	1330	RancaParrott	12/4/24	1330
2	RancaParrott	12/4/24	1600	DT	12-5-24	1332
3						

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ◉ No

Dilution Water: ○ Receiving Stream ◉ Synthetic Lab

Date: 12-5-24 Time: 1332 By: DT Temperature: 3.1 (C) IR#: 026

Chlorine: <0.1 mg/l	Ammonia: <0.25 mg/l	Int. Sal\Cond: 1639 ppt/uS	Adj. Salinity ppt		
pH: 7.7	Hardness: 244 mg/l (MR)	Other			
DO: 10.0 mg/l	Alkalinity: 66 mg/l (OK)	Condition: good			

Report Date: 12/30/2024 Revision 1

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Bio-Aquatic Lab ID: 89242

BIO-AQUATIC TESTING, INC.
2501 MAYES RD., STE. 100
CARROLLTON, TX 75006
PH: 972-242-7750 FAX: 972-242-7749

CHAIN OF CUSTODY

☐ Bio Only: No Sample Left

Lab Id : 89242

Please Review & Complete Sections A, B, C, & D.

Sample No: 89242 – ____

Revision 2 Effective Date 9/25/2017

Check Sample No. : ____ First, ____ Second, or ____ Third.

P.O. No: 4515296162

Client: Pilgrim's Pride Corporation

Facility: Southwest WWP

Permit No: WQ0003017000

Outfall: 001

Client Contact: JAMES HOWELL

Client Phone: (903) 575-3931

A. REVIEW SCHEDULED TEST(s):

Chronic	Ceriodaphnia dubia
Chronic	Pimephales promelas

Concentration: 31 41 55 80 98

To Ship the 1st Sample on: 12/2/2024

(For TX) Setup separate 24hr Acute Test? No

B. Use area below to make changes, if the Scheduled Test(s) in "A" are incorrect:

Freshwater Species					Saltwater Species	
C. dubia (water flea)	*D. pulex (water flea)*	*D. magna (water flea)*	*P. promelas (minnow)*	*Selenastrum (green algae)*	*M. beryllina (minnow)*	*Mysidopsis (shrimp)*
☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour	☐Chronic ☐96 Hour ☐48 Hour ☐24 Hour

Notes: 4th Qtr

C.

	Sample ID or Location: (Outfall No. or Name)	Sample Type: E = Effluent RS = Rec. Stream S = Sediment	Sample Date From	Sample Date To	Sample Time (military) From	Sample Time (military) To	Grab or Composite	Sampled By: (Sign and Print Name)	Number Of Containers Shipped
1	001	E	12/5/24	12/6/24	08:00	08:00	Grab	Jeff Reed Jeff Reed	1
2									
3									

D.

	Relinquished By:	Date	Time	Received By:	Date	Time
1	Jeff Reed	12/6/24	8:00 AM	RameePamott	12/6/24	1330
2	RameePamott	12/6/24	1600	Christian Rose	12/7/24	1005
3						

Bio-Aquatic Sample Login

BAT sample personnel: ● Yes ○ No

Dechlorinate Sample: ○ Yes ● No

Dilution Water: ○ Receiving Stream ● Synthetic Lab

Date: 12.7.24	Time: 1605	By: CAP	Temperature: 2.1 (C)	IR#: 026			
Chlorine: 0.97 mg/l	Ammonia: <.25 mg/l	Int. Sal\Cond: 1600 ppt/uS	Adj. Salinity ppt				
pH: 7.1	Hardness: 434 mg/l (MR)	Other					
DO: 9.3 mg/l	Alkalinity: 264 mg/l (OK)	Condition: Good					

REGULATORY AGENCY TABLES

Appendix E

Table 1 (Sheet 1 of 4)

BIOMONITORING REPORT

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION TEST

Permittee: Pilgrim's Pride Corporation - Southwest WWP
Permit No.: WQ0003017000
Outfall No.: 001

		Date/Time		Date/Time
Dates and times Composites were collected:	FROM:	12/1/2024 @08:00	TO:	12/2/2024@ 07:00
	FROM:	12/3/2024 @08:00	TO:	12/4/2024@ 07:00
	FROM:	12/5/2024 @08:00	TO:	12/6/2024@ 08:00

Test Initiation: Time: 13:0 Date: 12/3/2024

Dilution Water Used: [ ] Receiving Water [X] Synthetic Dilution Water

NUMBER OF YOUNG PRODUCED PER ADULT AT TEST TERMINATION

	EFFLUENT CONCENTRATION (%)					
REPLICATE	0%	31 %	41 %	55 %	80 %	98 %
A	18	D- 11	E	D- 0	D- 6	D- 0
B	24	E	D- 2	D- 0	D- 9	D- 8
C	28	D- 8	5	D- 7	D- 4	D- 4
D	13	6	D- 0	D- 2	D- 0	D- 3
E	29	2	D- 4	D- 0	D- 4	D- 2
F	6	D- 13	6	D- 0	D- 0	D- 0
G	14	E	4	D- 2	D- 0	D- 0
H	17	D- 0	E	3	D- 7	D- 1
I	E	7	D- 0	D- 0	D- 0	D- 0
J	23	13	D- 0	D- 6	D- 4	D- 0
Surv. MEAN	19.1	7.0	5.0	3.0	0.0	0.0
Total MEAN	19.1	7.5	2.6	2.0	3.4	1.8
CV % [1]	39.5	64.9	20	?	D	D
PMSD	Acceptable Range 47 or Less					Q* %

[1] Coefficient of Variation = (standard deviation/mean) x 100) Calculations are based on young of the surviving females. Males are designated (M), and dead females are designated (D) along with the number of neonates released prior to death. (E) anomalous value, spilled cup, or technician error.

Table 1 (Sheet 2 of 4)

BIOMONITORING REPORT

Ceriodaphnia dubia SURVIVAL AND REPRODUCTION TEST

Permittee: Pilgrim's Pride Corporation - Southwest WWP

Permit No.: WQ0003017000

Outfall No.: 001

PERCENT SURVIVAL

	EFFLUENT CONCENTRATION (%)					
TIme of Reading	0%	31 %	41 %	55 %	80 %	98 %
24 HOURS	100.0	100.0	100.0	100.0	100.0	100.0
48 HOURS	100.0	100.0	100.0	90.0	100.0	100.0
7-DAY	100.0	50.0	37.5	10.0	0.0	0.0

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST
(with Bonferroni adjustment as appropriate for Sub-Lethality)
Is the mean number of young produced per adult significantly less (p=0.05) than the number of young per adult in the control for the % effluent corresponding to significant non-lethal effects?

CRITICAL DILUTION (98) : X YES _____ NO

*If you report NO, enter a '0' on the DMR form for Parameter **TWP3B**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Ceriodaphnia Sub-Lethal Pass/Fail.*

2. FISHER'S EXACT TEST (as appropriate for Lethality)

Is the mean survival at test end significantly less (p=0.05) than the control's survival for the % effluent corresponding to lethality?

CRITICAL DILUTION (98) : X YES _____ NO

*If you report NO, enter a '0' on the DMR form for Parameter **TLP3B**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Ceriodaphnia Lethal Pass/Fail.*

3. Enter the percent effluent corresponding to each NOEC/LOEC below:

a. NOEC Survival = 0 % Effluent (Parameter TOP3B)

b. LOEC Survival = 32 % Effluent (Parameter TXP3B)

c. NOEC Reproduction = 0 % Effluent (Parameter TPP3B)

d. LOEC Reproduction = 32 % Effluent (Parameter TYP3B)

Q* refers to a value that is not calculable

Table 1 (Sheet 3 of 4)
BIOMONITORING REPORT

Pimephales promelas SURVIVAL AND GROWTH TEST

Permittee: Pilgrim's Pride Corporation - Southwest WWP
Permit No.: WQ0003017000
Outfall No.: 001

		Date/Time		Date/Time
Dates and times Composites were collected:	FROM:	12/1/2024 @08:00	TO:	12/2/2024@ 07:00
	FROM:	12/3/2024 @08:00	TO:	12/4/2024@ 07:00
	FROM:	12/5/2024 @08:00	TO:	12/6/2024@ 08:00

Test Initiation: Time: 14:49 Date: 12/3/2024

Dilution Water Used: [ ] Receiving Water [X] Synthetic Dilution Water

DATA TABLE FOR GROWTH OF *Pimephales promelas*

Effluent Concentration	Average Dry Weight in milligrams (mg) per replicate					Mean Dry Weight (mg)	CV % [1]
	A	B	C	D	E		
0%	0.561	0.569	0.410	0.534	0.509	0.517	12.4
31 %	0.550	0.451	0.572	0.478	0.621	0.534	13.0
41 %	0.556	0.452	0.481	0.601	0.444	0.507	13.6
55 %	0.500	0.520	0.416	0.665	0.309	0.482	27.4
80 %	0.664	0.561	0.542	0.377	0.456	0.520	20.9
98 %	0.535	0.510	0.509	0.362	0.514	0.486	14.5
PMSD	Acceptable Range 30 or Less					25.8 %	

[1] Coefficient of Variation = (standard deviation/mean) x 100) ?= cannot be calculated due to 100% mortality or lab exception

DATA TABLE FOR SURVIVAL OF *Pimephales promelas*

Effluent Concentration	Percent Survival per replicate					Average % Survival			CV % [1]
	A	B	C	D	E	24 Hours	48 Hours	7-Day	
0%	100	87.5	100	100	100	100	100	97.5	5.7
31 %	87.5	100	87.5	100	100	100	100	95	7.2
41 %	100	100	100	87.5	100	100	100	97.5	5.7
55 %	87.5	100	100	100	75	100	100	92.5	12.1
80 %	100	100	75	75	87.5	100	100	87.5	14.3
98 %	100	87.5	100	87.5	87.5	100	97.5	92.5	7.4

Table 1 (Sheet 4 of 4)

BIOMONITORING REPORT

Pimephales promelas SURVIVAL AND GROWTH TEST

Permittee: Pilgrim's Pride Corporation - Southwest WWP

Permit No.: WQ0003017000

Outfall No.: 001

1. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST (with Bonferroni adjustment as appropriate for Sub-Lethality)

Is the mean dry weight at 7 days significantly less (p=0.05) than the control's mean dry weight for the % effluent corresponding to significant non-lethal effects?

CRITICAL DILUTION (98) : _____YES X NO

*If you report NO, enter a '0' on the DMR form for Parameter **TWP6C**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Pimephales Sub-Lethal Pass/Fail.*

2. DUNNETT'S PROCEDURE OR STEEL'S MANY-ONE RANK TEST OR WILCOXON RANK SUM TEST (as appropriate for Lethality) Is the survival at 7 days significantly less (p=0.05) than the control's survival for % effluent corresponding to lethality?

CRITICAL DILUTION (98) : _____YES X NO

*If you report NO, enter a '0' on the DMR form for Parameter **TLP6C**, other wise enter a '1'. This parameter is also referred to as the 7-DAY Pimephales Lethal Pass/Fail.*

3. Enter the percent effluent corresponding to each NOEC/LOEC below:

			For DMR Form:
a.	NOEC Survival =	98 % Effluent	(Parameter TOP6C)
b.	LOEC Survival =	Q* % Effluent	(Parameter TXP6C)
c.	NOEC Growth =	98 % Effluent	(Parameter TPP6C)
d.	LOEC Growth =	Q* % Effluent	(Parameter TYP6C)

Q* refers to a value that is not calculable