



Bio-Aquatic Testing, Inc.



TCEQ TNi Accredited

City of Lawton
Outfall 001/003
OUTFALL 001/003

Client Address:
8104 SE 15TH STREET
LAWTON, OK 73501

Chronic Biomonitoring Report

90963

Ceriodaphnia dubia
Pimephales promelas

February 20, 2024

Approved by: Justin Reed
Lab director

Bio-Aquatic Testing, Inc. ♦ 2501 Mayes Rd. Ste. 100 ♦ Carrollton, Texas ♦ 75006

TABLE OF CONTENTS

TOXICITY TEST REPORT	3
SURVIVAL TEST SUMMARY	6
STATISTICAL & CHEMICAL ANALYSIS	Appendix A
REFERENCE TOXICANTS	Appendix B
LITERATURE REFERENCES	Appendix C
CHAIN-OF-CUSTODY SHEETS	Appendix D
REGULATORY AGENCY TABLES	Appendix E

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

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TOXICITY TEST REPORT - Chronic

Client:	Lawton, City of	Sample:	001/003
Facility:	Outfall 001/003	Laboratory Number:	90963
Permit No.	OK0035246	Date:	February 20, 2024

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

SAMPLE COLLECTION: Composite effluent samples from the City of Lawton, Outfall 001/003, were received on February 20, 2024, February 21, 2024, and February 22, 2024. Effluent samples were collected from Outfall 001/003 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 <0.10 mg/L, respectively. Effluent and laboratory dilution water pH, temperature, and dissolved oxygen data were collected daily. Sample one arrived with a DO outside of the organisms' tolerance range. The sample was aerated to bring the dissolved oxygen within tolerance.

TEST PROCEDURES:
Ceriodaphnia dubia

EPA METHOD: 1002

The seven-day (three brood) Chronic *Ceriodaphnia dubia* survival and reproduction test was initiated at 15:45 hours on February 20, 2024. Five effluent concentrations of 32%, 42%, 56%, 75%, and 100% 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 09:25 hours on February 28, 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).

OTHER REMARKS

-- TICKET _7_ OF _7_ --**MULTIPLE TICKETS ASSOCIATED WITH THIS JOB DUE TO SCOPE EXCEEDS 500FT, EACH TICKET HAS ENTIRE SCOPE OF JOB IN THE REMARKS SECTION**

GRIDS

12WI01N06NW;12WI02N31SW

MEMBERS NOTIFIED

Code	Name	Code	Name
T09627	Lawton/Sewer-Water T09627	T11158	AT&T Distribution T11158
S00153	Public Services of OK/A...	SUOLA03	Summit Utilities Lawton...

SURVIVAL:

Ceriodaphnia dubia

Fisher's Exact test on *Ceriodaphnia dubia* survival test data demonstrated no statistically significant differences between the control and any of the effluent concentrations tested.

LOEC: Not Calculable (Q)

NOEC: 100% Effluent

REPRODUCTION:

Ceriodaphnia dubia

The *Ceriodaphnia dubia* reproduction data were normally distributed at the alpha level of 0.01 (13.277) using the Chi-square test for normality. Reproduction 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 *Ceriodaphnia dubia* reproduction data demonstrated no statistically significant difference between the control and any effluent concentration tested.

LOEC: Not Calculable (Q)

NOEC: 100% Effluent

TEST PROCEDURES:

Pimephales promelas

EPA METHOD: 1000

The seven-day Chronic *Pimephales promelas* survival and growth test was initiated at 17:50 hours on February 20, 2024. Five effluent concentrations of 32%, 42%, 56%, 75%, and 100% 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 10:46 hours on February 27, 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: 100% 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 effluent concentrations tested.

LOEC: Not Calculable (Q)

NOEC: 100% Effluent