



August 18, 2021

Biomonitoring Testing
for
Noland WR001
Paul R. Noland WWTP

Control No. 257535-1

Prepared for:

Ms. Donna McChristian
Jacobs
1400 North Fox Hunter Road
Fayetteville, AR 72701

Prepared by:

AMERICAN INTERPLEX CORPORATION
8600 Kanis Road
Little Rock, AR 72204-2322

Jacobs
ATTN: Ms. Donna McChristian
1400 North Fox Hunter Road
Fayetteville, AR 72701

Re: 7-day Renewal *Pimephales promelas* (Fathead minnow) and *Ceriodaphnia dubia*
Noland WR001 - Paul R. Noland WWTP
NPDES Permit No. AR0020010 AFIN 72-00781

Dear Ms. Donna McChristian:

This report is the analytical results and supporting information for the samples submitted to American Interplex Corporation (AIC). The following results are applicable only to the sample identified by the control number referenced above. Accurate assessment of the data requires access to the entire document. Each section of the report has been reviewed and approved by the Chief Operating Officer or qualified designee.

Testing procedures and Quality Assurance were in accordance with "Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms" EPA-821-R-02-013, Fourth Edition, October 2002. Test results are summarized below:

The fathead minnow test concluded an interrupted concentration-response that appears to be related to pathogen interference.

Method 1000.0 Chronic *Pimephales promelas* (Fathead minnow) Survival and Growth Test: The No Observable Effects Concentration (NOEC) for survival occurred at 41 % effluent, which is below the critical dilution of 97 %. The NOEC for growth occurred at 41 % effluent, which is below the critical dilution of 97 %. **The sample, therefore, FAILED both lethal and sub-lethal effects for the Fathead minnow test.**

Method 1002.0 Chronic *Ceriodaphnia dubia* Survival and Reproduction Test: The No Observable Effects Concentration (NOEC) for survival occurred at 97 % effluent, which is equal to the critical dilution of 97 %. The NOEC for reproduction occurred at 73 % effluent, which is below the critical dilution of 97 %. **The sample PASSED lethal effects, however, FAILED sub-lethal effects for the *Ceriodaphnia dubia* test.**

AMERICAN INTERPLEX CORPORATION



John Overbey
Chief Operating Officer

PDF cc: Jacobs
ATTN: Mr. Thom Vinson
thom.vinson@jacobs.com

Jacobs
ATTN: Ms. Brandi Miller-Deweese
brandi.millerdeweese@jacobs.com

Jacobs
ATTN: Ms. Donna McChristian
donna.mcchristian@jacobs.com

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I. Control Acceptance Criteria

Pimephales promelas (Fathead minnow) Method 1000.0

| CRITERIA | RESULTS | PASS/FAIL |
|--|---------|-----------|
| Control Survival > or = 80% | 97.5 | PASS |
| Control Growth > or = 0.25 mg per Surviving minnow | 0.621 | PASS |
| Control Growth CV < or = 40% | 6.71 | PASS |
| Growth Minimum Significant Difference 12 to 30% | 28.4 | PASS |
| Critical Dilution CV < or = 40% | 5.65 | PASS |

Ceriodaphnia dubia Method 1002.0

| CRITERIA | RESULTS | PASS/FAIL |
|---|---------|-----------|
| Control Survival > or = 80% | 100 | PASS |
| Control Reproduction > or = 15 per Surviving Female | 30.5 | PASS |
| Control CV < or = 40% per Surviving Female | 18.8 | PASS |
| Reproduction Minimum Significant Difference 13 to 47% | 18.9 | PASS |
| Critical Dilution CV < or = 40% | 9.74 | PASS |

II. Outlined Report

A. Introduction

1. Permit Number: AR0020010 AFIN 72-00781
2. Test Requirements: TWO / YR
Test Methods 1000.0 and 1002.0

B. Source of Effluent/Dilution Water:

1. Effluent Samples:
 - a. Sampling Point: Noland WR001
 - b. Chemical Data:

| Analysis | Sample 1 | Sample 2 | Sample 3 |
|---|----------|----------|----------|
| Dissolved oxygen (mg/l) | 7.7 | 7.5 | 8.3 |
| pH (standard units) | 7.9 | 8.0 | 8.0 |
| Alkalinity (mg/l as CaCO ₃) | 180 | 160 | 180 |
| Hardness (mg/l as CaCO ₃) | 150 | 150 | 150 |
| Conductivity (umhos/cm) | 670 | 690 | 710 |
| Residual Chlorine (mg/l) | <0.05 | <0.05 | <0.05 |
| Ammonia as N (mg/l) | 0.15 | 0.17 | 0.22 |

2. Dilution Water Samples:
Moderately Hard

| Analysis | 257313-1 | 257486-1 |
|---|----------|----------|
| Dissolved oxygen (mg/l) | 7.0 | 7.2 |
| pH (standard units) | 7.8 | 7.9 |
| Alkalinity (mg/l as CaCO ₃) | 63 | 61 |
| Hardness (mg/l as CaCO ₃) | 83 | 80 |
| Conductivity (umhos/cm) | 330 | 340 |
| Residual Chlorine (mg/l) | <0.05 | <0.05 |

C. Test Methods

1. Test methods used:

Short Term Methods for Estimating the Chronic Toxicity of Effluents and Receiving Waters to Freshwater Organisms, EPA-821-R-02-013; test Methods 1000.0 and 1002.0, Fathead Minnow Survival and Growth and *Ceriodaphnia dubia* Survival and Reproduction.

2. Endpoint: No Observable Effects Concentration (NOEC)

3. Test Conditions:

Pimephales promelas (Fathead minnow) Survival and Growth Method 1000.0

Date & Time Test Initiated: August 3, 2021 at 1157
Date & Time Test Terminated: August 10, 2021 at 1138
Type & Volume of Test Chamber: 500 ml disposable beaker
Volume of Sample: 250 ml
Number of Organisms per replicate: 8
Number of Replicates per dilution: 5

Ceriodaphnia dubia Survival and Reproduction Method 1002.0

Date & Time Test Initiated: August 3, 2021 at 1132
Date & Time Test Terminated: August 10, 2021 at 1125
Type & Volume of Test Chamber: 30 ml disposable beaker
Volume of Sample: 15 ml
Number of Organisms per replicate: 1
Number of Replicates per dilution: 10

4. Source of test organisms: In-house culture

5. Test Temperature: 25 +/- 1 degree Celsius

D. Test Organisms

1. Scientific Name

a. Test 1000.0 *Pimephales promelas*

b. Test 1002.0 *Ceriodaphnia dubia*

III. Data Analysis

The data was analyzed using American Interplex Corporation's Laboratory Information Management Software based on Toxstat and following EPA method criteria.

Pimephales promelas (Fathead minnow) survival data was transformed using the Arc Sine transformation. Normality and homogeneity of variance were checked using Shapiro-Wilk's and Bartlett's test. The survival data was then analyzed using Dunnett's Test to determine the No Observable Effects Concentration (NOEC).

Fathead minnow growth data was analyzed for normality and homogeneity of variance using Shapiro-Wilk's and Bartlett's test. Dunnett's Test was used to determine the No Observable Effects Concentration (NOEC) for growth.

Ceriodaphnia dubia survival data was analyzed with Fisher's Exact Test. Reproduction data was analyzed using Kolmogorov's Test for Normality and Bartlett's test and analyzed with Dunnett's Test to determine the No Observable Effects Concentration (NOEC) for Reproduction.

IV. Standard Reference Toxicants

The sensitivity of the offspring is determined by performing a standard reference toxicant test monthly. Sodium chloride in synthetic moderately hard water is used as prescribed in EPA-821-R-02-013.

Pimephales promelas (Fathead minnow)

A chronic reference test was performed on July 01, 2021 at 1200 to July 08, 2021 at 1005

The results were as follows: (Control No. 256677-1.)

Survival LC-50: 3346 mg/l

Growth IC-25: 2302 mg/l

Growth PMSD: 18.4

Ceriodaphnia dubia

A chronic reference test was performed on July 01, 2021 at 1125 to July 07, 2021 at 1100

The results were as follows: (Control No. 256677-2.)

Survival LC-50: 1772 mg/l

Reproduction IC-25: 1283 mg/l

Reproduction PMSD: 18.3

V. Organism History

Pimephales promelas (Fathead minnow)

Date: August 3, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

Ceriodaphnia dubia

Date: August 3, 2021

Age: <24 hours

Source: In-house culture

Water: Moderately hard synthetic

Temperature: 25 deg.C

VII. Results Summary *Pimephales promelas*, Fathead minnow Larval Survival and Growth Test -- Method 1000.0

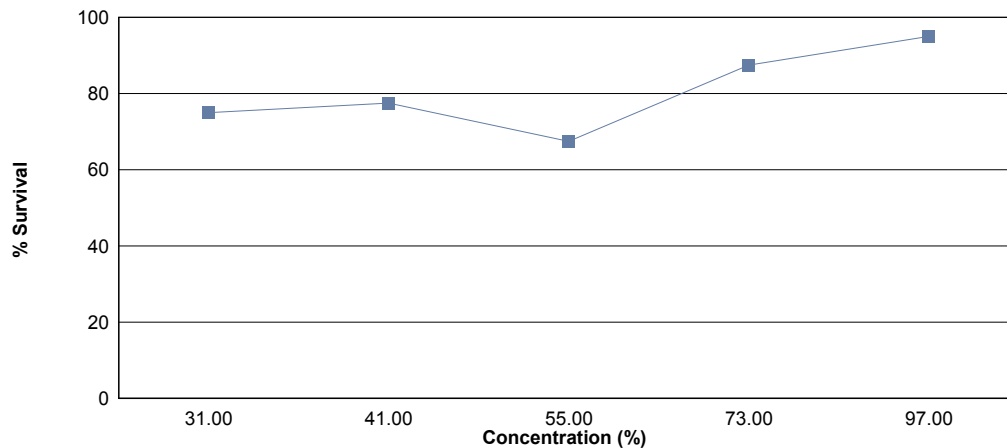
Larvae are exposed in a static renewal system for seven days to different concentrations of effluent with dilution water. Test results are based on the survival and growth (weight) of the larvae.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 97 % effluent.

The test was initiated on August 3, 2021 at 1157 and continued through August 10, 2021 at 1138. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 41 % effluent
- b.) NOEC growth = 41 % effluent



| Summary of the 7-day Fathead Minnow Survival and Growth | | |
|---|------------------|------------------|
| Concentration | Percent Survival | Mean Growth (mg) |
| Control | 97.5 | 0.605 |
| 31 % | 75.0 | 0.442 |
| 41 % | 77.5 | 0.437 |
| 55 % | 67.5 * | -- |
| 73 % | 87.5 | -- |
| 97 % | 95.0 | -- |

*Significant difference when compared to the control (p=0.05)

VII. Results Summary *Ceriodaphnia dubia*, Cladoceran Survival and Reproduction Test -- Method 1002.0

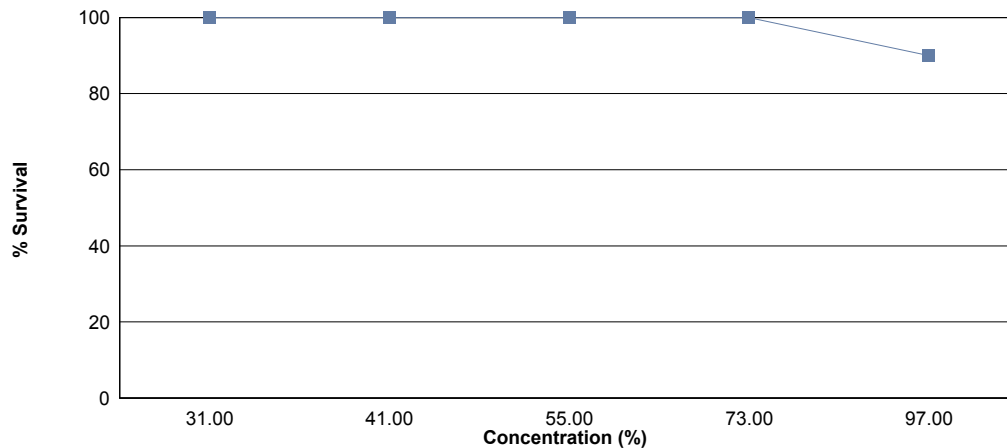
Neonates are exposed in a static renewal system to different concentrations of effluent with dilution water until 60% of surviving control organisms have three broods of offspring or a maximum of eight test days.

Effluent dilutions for this test were 31 %, 41 %, 55 %, 73 %, 97 % in accordance with the NPDES permit.

The low flow or 'critical' dilution is specified in the NPDES permit as 97 % effluent.

The test was initiated on August 3, 2021 at 1132 and continued through August 10, 2021 at 1125. Statistical analyses were performed on the observed data and the no observable effects concentrations (NOECs) were as follows:

- a.) NOEC survival = 97 % effluent
- b.) NOEC reproduction = 73 % effluent



| Summary of the 7-day <i>Ceriodaphnia dubia</i> Survival and Reproduction Data | | |
|---|------------------|-------------------|
| Concentration | Percent Survival | Mean Reproduction |
| Control | 100 | 30.5 |
| 31 % | 100 | 26.4 |
| 41 % | 100 | 26.4 |
| 55 % | 100 | 26.8 |
| 73 % | 100 | 26.7 |
| 97 % | 90.0 | 24.0 * |

*Significant difference when compared to the control (p=0.05)

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Survival

Date and Time Test Initiated: August 3, 2021 at 1157

Date and Time Test Terminated: August 10, 2021 at 1138

| Concentration | Replicate | Number of Survivors | | | | | | |
|---------------|-----------|---------------------|-------|-------|-------|-------|-------|-------|
| | | Day 1 | Day 2 | Day 3 | Day 4 | Day 5 | Day 6 | Day 7 |
| Control | A | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | B | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | C | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | D | 8 | 8 | 8 | 7 | 7 | 7 | 7 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 31 % | A | 8 | 8 | 8 | 8 | 7 | 4 | 4 |
| | B | 8 | 8 | 8 | 8 | 6 | 3 | 3 |
| | C | 8 | 8 | 8 | 8 | 7 | 7 | 7 |
| | D | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 41 % | A | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | B | 8 | 8 | 8 | 7 | 6 | 5 | 5 |
| | C | 8 | 8 | 8 | 8 | 6 | 6 | 6 |
| | D | 8 | 8 | 8 | 8 | 7 | 5 | 4 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 55 % | A | 8 | 8 | 7 | 6 | 6 | 3 | 3 |
| | B | 8 | 8 | 8 | 8 | 6 | 5 | 5 |
| | C | 8 | 8 | 8 | 8 | 8 | 6 | 6 |
| | D | 8 | 8 | 8 | 8 | 6 | 6 | 6 |
| | E | 8 | 8 | 8 | 8 | 7 | 7 | 7 |
| 73 % | A | 8 | 8 | 8 | 8 | 6 | 4 | 4 |
| | B | 8 | 8 | 8 | 8 | 7 | 7 | 7 |
| | C | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | D | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| 97 % | A | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | B | 8 | 8 | 8 | 8 | 8 | 7 | 7 |
| | C | 8 | 8 | 8 | 8 | 8 | 8 | 8 |
| | D | 8 | 8 | 8 | 8 | 8 | 7 | 7 |
| | E | 8 | 8 | 8 | 8 | 8 | 8 | 8 |

Appendix A1: Test 1000.0

Pimephales promelas (Fathead Minnow) 7-Day Growth

Test Initiated: August 3, 2021 at 1157
Test Terminated: August 10, 2021 at 1138

| Concentration | Replicate | Weight of pan | Weight of pan + fish | Total weight of fish (g) | Original # of fish | Mean dry weight (mg) |
|---------------|-----------|---------------|----------------------|--------------------------|--------------------|----------------------|
| Control | A | .65453 | .65951 | 0.00498 | 8 | 0.622 |
| | B | .65102 | .65625 | 0.00523 | 8 | 0.654 |
| | C | .65063 | .65554 | 0.00491 | 8 | 0.614 |
| | D | .66338 | .66774 | 0.00436 | 8 | 0.545 |
| | E | .66909 | .67381 | 0.00472 | 8 | 0.590 |
| 31 % | A | .66164 | .66362 | 0.00198 | 8 | 0.248 |
| | B | .65745 | .65945 | 0.00200 | 8 | 0.250 |
| | C | .65034 | .65434 | 0.00400 | 8 | 0.500 |
| | D | .63974 | .64448 | 0.00474 | 8 | 0.592 |
| | E | .66848 | .67342 | 0.00494 | 8 | 0.618 |
| 41 % | A | .66248 | .66685 | 0.00437 | 8 | 0.546 |
| | B | .66504 | .66770 | 0.00266 | 8 | 0.332 |
| | C | .64806 | .65115 | 0.00309 | 8 | 0.386 |
| | D | .65906 | .66170 | 0.00264 | 8 | 0.330 |
| | E | .65923 | .66397 | 0.00474 | 8 | 0.592 |
| 55 % | A | .64866 | .64994 | 0.00128 | 8 | 0.160 |
| | B | .66511 | .66784 | 0.00273 | 8 | 0.341 |
| | C | .65938 | .66281 | 0.00343 | 8 | 0.429 |
| | D | .65715 | .66043 | 0.00328 | 8 | 0.410 |
| | E | .66592 | .66955 | 0.00363 | 8 | 0.454 |
| 73 % | A | .65796 | .66066 | 0.00270 | 8 | 0.338 |
| | B | .66480 | .66846 | 0.00366 | 8 | 0.458 |
| | C | .64234 | .64723 | 0.00489 | 8 | 0.611 |
| | D | .66958 | .67433 | 0.00475 | 8 | 0.594 |
| | E | .66263 | .66771 | 0.00508 | 8 | 0.635 |
| 97 % | A | .65674 | .66106 | 0.00432 | 8 | 0.540 |
| | B | .66006 | .66414 | 0.00408 | 8 | 0.510 |
| | C | .64296 | .64755 | 0.00459 | 8 | 0.574 |
| | D | .64951 | .65400 | 0.00449 | 8 | 0.561 |
| | E | .66115 | .66588 | 0.00473 | 8 | 0.591 |

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 3, 2021 at 1132

Date and Time Test Terminated: August 10, 2021 at 1125

| Concentration: Control | | | | | | | | | | | | | | |
|------------------------|-----------|----|----|----|----|----|----|----|----|----|--------------|---------------|-----------------|------|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 4 | 3 | 39 | 10 | 3.90 |
| 5 | 11 | 8 | 9 | 0 | 12 | 12 | 11 | 12 | 10 | 11 | 96 | 10 | 9.60 | |
| 6 | 0 | 0 | 16 | 18 | 18 | 0 | 0 | 19 | 0 | 0 | 71 | 10 | 7.10 | |
| 7 | 12 | 10 | 0 | 18 | 0 | 18 | 19 | 0 | 10 | 12 | 99 | 10 | 9.90 | |
| 8 | | | | | | | | | | | | | | |
| TOTAL | 27 | 22 | 29 | 40 | 34 | 34 | 34 | 35 | 24 | 26 | 305 | 10 | 30.5 | |

| Concentration: 31 % | | | | | | | | | | | | | | |
|---------------------|-----------|----|----|----|----|----|----|----|----|----|--------------|---------------|-----------------|------|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 4 | 3 | 3 | 4 | 3 | 3 | 5 | 3 | 4 | 2 | 2 | 32 | 10 | 3.20 | |
| 5 | 11 | 10 | 13 | 12 | 12 | 14 | 7 | 10 | 13 | 11 | 113 | 10 | 11.3 | |
| 6 | 0 | 0 | 0 | 14 | 0 | 15 | 0 | 0 | 0 | 13 | 42 | 10 | 4.20 | |
| 7 | 10 | 10 | 14 | 0 | 10 | 0 | 10 | 11 | 12 | 0 | 77 | 10 | 7.70 | |
| 8 | | | | | | | | | | | | | | |
| TOTAL | 24 | 23 | 31 | 29 | 25 | 34 | 20 | 25 | 27 | 26 | 264 | 10 | 26.4 | |

| Concentration: 41 % | | | | | | | | | | | | | | |
|---------------------|-----------|----|----|----|----|----|----|----|----|----|--------------|---------------|-----------------|------|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 3 | 10 | 0.300 | |
| 4 | 3 | 4 | 4 | 4 | 3 | 0 | 3 | 4 | 0 | 4 | 29 | 10 | 2.90 | |
| 5 | 11 | 13 | 12 | 12 | 10 | 11 | 11 | 13 | 10 | 12 | 115 | 10 | 11.5 | |
| 6 | 0 | 0 | 10 | 0 | 14 | 13 | 0 | 0 | 10 | 0 | 47 | 10 | 4.70 | |
| 7 | 11 | 12 | 0 | 10 | 0 | 0 | 11 | 13 | 0 | 13 | 70 | 10 | 7.00 | |
| 8 | | | | | | | | | | | | | | |
| TOTAL | 25 | 29 | 26 | 26 | 27 | 27 | 25 | 30 | 20 | 29 | 264 | 10 | 26.4 | |

Appendix A1: Test 1002.0

Ceriodaphnia dubia Survival and Reproduction

Date and Time Test Initiated: August 3, 2021 at 1132

Date and Time Test Terminated: August 10, 2021 at 1125

| Concentration: 55 % | | | | | | | | | | | | | | |
|---------------------|-----------|----|----|----|----|----|----|----|----|----|--------------|---------------|-----------------|------|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult | |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 3 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 2 | 0 | 5 | 10 | 0.500 | |
| 4 | 0 | 5 | 6 | 5 | 2 | 5 | 4 | 5 | 4 | 2 | 38 | 10 | 3.80 | |
| 5 | 10 | 16 | 12 | 14 | 11 | 9 | 12 | 10 | 11 | 13 | 118 | 10 | 11.8 | |
| 6 | 11 | 0 | 0 | 0 | 4 | 0 | 13 | 0 | 11 | 0 | 39 | 10 | 3.90 | |
| 7 | 0 | 11 | 13 | 10 | 0 | 11 | 0 | 11 | 0 | 12 | 68 | 10 | 6.80 | |
| 8 | | | | | | | | | | | | | | |
| TOTAL | 24 | 32 | 31 | 29 | 17 | 25 | 29 | 26 | 28 | 27 | 268 | 10 | 26.8 | |

| Concentration: 73 % | | | | | | | | | | | | | |
|---------------------|-----------|----|----|----|----|----|----|----|----|----|--------------|---------------|-----------------|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 3 | 0 | 0 | 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 3 | 10 | 0.300 |
| 4 | 5 | 3 | 0 | 6 | 5 | 6 | 0 | 0 | 8 | 3 | 36 | 10 | 3.60 |
| 5 | 13 | 12 | 11 | 12 | 12 | 13 | 10 | 6 | 0 | 12 | 101 | 10 | 10.1 |
| 6 | 0 | 5 | 12 | 0 | 0 | 12 | 11 | 12 | 13 | 11 | 76 | 10 | 7.60 |
| 7 | 11 | 0 | 0 | 12 | 12 | 0 | 0 | 0 | 16 | 0 | 51 | 10 | 5.10 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 29 | 20 | 26 | 30 | 29 | 31 | 21 | 18 | 37 | 26 | 267 | 10 | 26.7 |

| Concentration: 97 % | | | | | | | | | | | | | |
|---------------------|-----------|----|----|----|----|----|----|----|----|----|--------------|---------------|-----------------|
| Day | Replicate | | | | | | | | | | No. of Young | No. of Adults | Young per Adult |
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 | 8 | 9 | 10 | | | |
| 1 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 2 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 3 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 10 | 0.00 |
| 4 | 0 | 3 | 0 | 5 | 3 | 3 | 4 | 4 | 3 | 3 | 28 | 10 | 2.80 |
| 5 | X | 12 | 9 | 12 | 13 | 12 | 10 | 12 | 11 | 12 | 103 | 9 | 11.4 |
| 6 | X | 13 | 17 | 0 | 15 | 12 | 8 | 0 | 11 | 0 | 76 | 9 | 8.44 |
| 7 | X | 0 | 0 | 12 | 0 | 0 | 0 | 11 | 0 | 10 | 33 | 9 | 3.67 |
| 8 | | | | | | | | | | | | | |
| TOTAL | 0 | 28 | 26 | 29 | 31 | 27 | 22 | 27 | 25 | 25 | 240 | 10 | 24.0 |

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

| Transformation of Data | | | Transform: Arc Sin(Square Root(Y)) | |
|------------------------|----------------|-----|------------------------------------|-------------|
| Group | Identification | Rep | Value | Transformed |
| 1 | Control | 1 | 1.00000 | 1.39310 |
| 1 | Control | 2 | 1.00000 | 1.39310 |
| 1 | Control | 3 | 1.00000 | 1.39310 |
| 1 | Control | 4 | 0.87500 | 1.20940 |
| 1 | Control | 5 | 1.00000 | 1.39310 |
| 2 | 31 % | 1 | 0.50000 | 0.78540 |
| 2 | 31 % | 2 | 0.37500 | 0.65906 |
| 2 | 31 % | 3 | 0.87500 | 1.20940 |
| 2 | 31 % | 4 | 1.00000 | 1.39310 |
| 2 | 31 % | 5 | 1.00000 | 1.39310 |
| 3 | 41 % | 1 | 1.00000 | 1.39310 |
| 3 | 41 % | 2 | 0.62500 | 0.91174 |
| 3 | 41 % | 3 | 0.75000 | 1.04720 |
| 3 | 41 % | 4 | 0.50000 | 0.78540 |
| 3 | 41 % | 5 | 1.00000 | 1.39310 |
| 4 | 55 % | 1 | 0.37500 | 0.65906 |
| 4 | 55 % | 2 | 0.62500 | 0.91174 |
| 4 | 55 % | 3 | 0.75000 | 1.04720 |
| 4 | 55 % | 4 | 0.75000 | 1.04720 |
| 4 | 55 % | 5 | 0.87500 | 1.20940 |
| 5 | 73 % | 1 | 0.50000 | 0.78540 |
| 5 | 73 % | 2 | 0.87500 | 1.20940 |
| 5 | 73 % | 3 | 1.00000 | 1.39310 |
| 5 | 73 % | 4 | 1.00000 | 1.39310 |
| 5 | 73 % | 5 | 1.00000 | 1.39310 |
| 6 | 97 % | 1 | 1.00000 | 1.39310 |
| 6 | 97 % | 2 | 0.87500 | 1.20940 |
| 6 | 97 % | 3 | 1.00000 | 1.39310 |
| 6 | 97 % | 4 | 0.87500 | 1.20940 |
| 6 | 97 % | 5 | 1.00000 | 1.39310 |

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

| Shapiro - Wilk's Test for Normality | | Transform: Arc Sin(Square Root(Y)) |
|--|------------------------|------------------------------------|
| D = 1.3 | | |
| W = 0.9377 | | |
| Critical W = 0.9 | (alpha = 0.01, N = 30) | |
| Critical W = 0.927 | (alpha = 0.05, N = 30) | |
| Data PASS normality test (alpha = 0.01). | | |

| Bartlett's Test for Homogeneity of Variance | | Transform: Arc Sin(Square Root(Y)) |
|--|------------------------|------------------------------------|
| Calculated B1 statistic = 9.534 | | |
| Critical B = 15.086 | (alpha = 0.01, df = 5) | |
| Data PASS B1 homogeneity test at 0.01 level. | | |

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Survival

| ANOVA Table | | | Transform: Arc Sin(Square Root(Y)) | |
|--|----|--------|------------------------------------|-------|
| SOURCE | DF | SS | MS | F |
| Between | 5 | 0.5479 | 0.1096 | 2.023 |
| Within (Error) | 24 | 1.3 | 0.05417 | |
| Total | 29 | 1.848 | | |
| Critical F = 3.9 (alpha = 0.01, df = 5,24) 2.62 (alpha = 0.05, df = 5,24) | | | | |
| Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05) | | | | |

| Dunnett's Test - Table 1 of 2 | | | | Transform: Arc Sin(Square Root(Y)) | |
|---|----------------|------------------|------------------------|------------------------------------|----------|
| Ho:Control<Treatment | | | | | |
| Group | Identification | Transformed Mean | Mean In Original Units | T Stat | Sig 0.05 |
| 1 | Control | 1.3564 | 0.975 | | |
| 2 | 31 % | 1.088 | 0.75 | 1.823 | |
| 3 | 41 % | 1.1061 | 0.775 | 1.7 | |
| 4 | 55 % | 0.97492 | 0.675 | 2.592 | * |
| 5 | 73 % | 1.2348 | 0.875 | 0.8261 | |
| 6 | 97 % | 1.3196 | 0.95 | 0.25 | |
| Dunnett's critical value = 2.36 (1 Tailed, alpha = 0.05, df = 5,24) | | | | | |

| Dunnett's Test - Table 2 of 2 | | | | Transform: Arc Sin(Square Root(Y)) | |
|-------------------------------|----------------|-------------|----------------------------------|------------------------------------|----------------------------|
| Ho:Control<Treatment | | | | | |
| Group | Identification | Num of Reps | Min Sig Diff (In Orig. Units) | % of Control | Difference From Control |
| 1 | Control | 5 | | | |
| 2 | 31 % | 5 | 0.2385 | 25 | 0.225 |
| 3 | 41 % | 5 | 0.2385 | 25 | 0.2 |
| 4 | 55 % | 5 | 0.2385 | 25 | 0.3 |
| 5 | 73 % | 5 | 0.2385 | 25 | 0.1 |
| 6 | 97 % | 5 | 0.2385 | 25 | 0.025 |

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

| Shapiro - Wilk's Test for Normality | No Transformation |
|--|-------------------|
| <p>D = 0.1989 W = 0.9546 Critical W = 0.835 (alpha = 0.01, N = 15) Critical W = 0.881 (alpha = 0.05, N = 15)</p> <p>Data PASS normality test (alpha = 0.01).</p> | |

| Bartlett's Test for Homogeneity of Variance | No Transformation |
|---|-------------------|
| <p>Calculated B1 statistic = 6.149 Critical B = 9.21 (alpha = 0.01, df = 2)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p> | |

Appendix A2: Statistics

Pimephales promelas (Fathead minnow) Growth

| ANOVA Table | | | | No Transformation | |
|--|----|---------|---------|-------------------|--|
| SOURCE | DF | SS | MS | F | |
| Between | 2 | 0.09146 | 0.04573 | 2.758 | |
| Within (Error) | 12 | 0.1989 | 0.01658 | | |
| Total | 14 | 0.2904 | | | |
| Critical F = 6.93 (alpha = 0.01, df = 2,12) | | | | | |
| 3.88 (alpha = 0.05, df = 2,12) | | | | | |
| Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05) | | | | | |

| Dunnett's Test - Table 1 of 2 | | | | | No Transformation | |
|---|----------------|------------------|------------------------|--------|-------------------|--|
| Ho:Control<Treatment | | | | | | |
| Group | Identification | Transformed Mean | Mean In Original Units | T Stat | Sig 0.05 | |
| 1 | Control | 0.605 | 0.605 | | | |
| 2 | 31 % | 0.4416 | 0.4416 | 2.006 | | |
| 3 | 41 % | 0.4372 | 0.4372 | 2.06 | | |
| Dunnett's critical value = 2.11 (1 Tailed, alpha = 0.05, df = 2,12) | | | | | | |

| Dunnett's Test - Table 2 of 2 | | | | | | No Transformation | |
|-------------------------------|----------------|-------------|----------------------------------|--------------|----------------------------|-------------------|--|
| Ho:Control<Treatment | | | | | | | |
| Group | Identification | Num of Reps | Min Sig Diff (In Orig. Units) | % of Control | Difference From Control | | |
| 1 | Control | 5 | | | | | |
| 2 | 31 % | 5 | 0.1718 | 28.4 | 0.1634 | | |
| 3 | 41 % | 5 | 0.1718 | 28.4 | 0.1678 | | |

Appendix A2: Statistics

Ceriodaphnia dubia Survival

| Fisher's Exact Test | | | |
|---------------------|-------|------|---------------|
| Identification | Alive | Dead | Total Animals |
| Control | 10 | 0 | 10 |
| 31 % | 10 | 0 | 10 |
| Total | 20 | 0 | 20 |

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

| Fisher's Exact Test | | | |
|---------------------|-------|------|---------------|
| Identification | Alive | Dead | Total Animals |
| Control | 10 | 0 | 10 |
| 41 % | 10 | 0 | 10 |
| Total | 20 | 0 | 20 |

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

| Fisher's Exact Test | | | |
|---------------------|-------|------|---------------|
| Identification | Alive | Dead | Total Animals |
| Control | 10 | 0 | 10 |
| 55 % | 10 | 0 | 10 |
| Total | 20 | 0 | 20 |

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

| Fisher's Exact Test | | | |
|---------------------|-------|------|---------------|
| Identification | Alive | Dead | Total Animals |
| Control | 10 | 0 | 10 |
| 73 % | 10 | 0 | 10 |
| Total | 20 | 0 | 20 |

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 10. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

Appendix A2: Statistics

Ceriodaphnia dubia Survival

| Fisher's Exact Test | | | |
|---------------------|-------|------|---------------|
| Identification | Alive | Dead | Total Animals |
| Control | 10 | 0 | 10 |
| 97 % | 9 | 1 | 10 |
| Total | 19 | 1 | 20 |

Critical Fisher's value (10,10,10) (alpha=0.05) is 6. b value is 9. Since b is greater than 6 there is NO SIGNIFICANT DIFFERENCE between CONTROL and TREATMENT at the 0.05 level.

| Summary of Fisher's Exact Test | | | | |
|--------------------------------|----------------|---------|------|----------|
| Group | Identification | Exposed | Dead | Sig 0.05 |
| 0 | Control | 10 | 0 | |
| 1 | 31 % | 10 | 0 | |
| 2 | 41 % | 10 | 0 | |
| 3 | 55 % | 10 | 0 | |
| 4 | 73 % | 10 | 0 | |
| 5 | 97 % | 10 | 1 | |

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

| Kolmogorov Test for Normality | No Transformation |
|--|-------------------|
| <p>D = 0.123 D* = 0.965 Critical D* = 1.035</p> <p style="text-align: right;">(alpha = 0.01, N = 60)</p> <p>Data PASS normality test (alpha = 0.01).</p> | |

| Bartlett's Test for Homogeneity of Variance | No Transformation |
|---|-------------------|
| <p>Calculated B1 statistic = 12.71 Critical B = 15.086</p> <p style="text-align: right;">(alpha = 0.01, df = 5)</p> <p>Data PASS B1 homogeneity test at 0.01 level.</p> | |

Appendix A2: Statistics

Ceriodaphnia dubia Reproduction

| ANOVA Table | | | | No Transformation | |
|---|----|-------|-------|-------------------|--|
| SOURCE | DF | SS | MS | F | |
| Between | 5 | 218.6 | 43.72 | 1.409 | |
| Within (Error) | 54 | 1675 | 31.02 | | |
| Total | 59 | 1894 | | | |
| Critical F = 3.38 (alpha = 0.01, df = 5,54) 2.38 (alpha = 0.05, df = 5,54) | | | | | |
| Since F < Critical F FAIL TO REJECT Ho: All equal (alpha = 0.05) | | | | | |

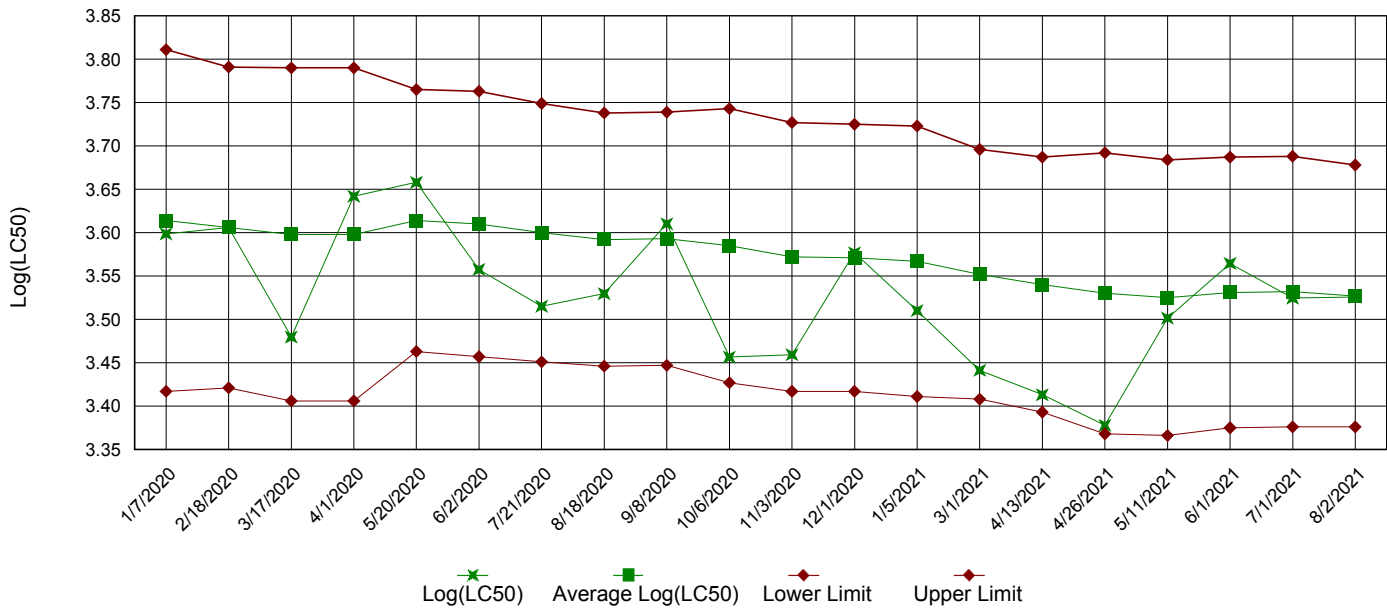
| Dunnett's Test - Table 1 of 2 | | | | | No Transformation | |
|---|----------------|------------------|------------------------|--------|-------------------|--|
| Ho:Control<Treatment | | | | | | |
| Group | Identification | Transformed Mean | Mean In Original Units | T Stat | Sig 0.05 | |
| 1 | Control | 30.5 | 30.5 | | | |
| 2 | 31 % | 26.4 | 26.4 | 1.646 | | |
| 3 | 41 % | 26.4 | 26.4 | 1.646 | | |
| 4 | 55 % | 26.8 | 26.8 | 1.485 | | |
| 5 | 73 % | 26.7 | 26.7 | 1.526 | | |
| 6 | 97 % | 24 | 24 | 2.61 | * | |
| Dunnett's critical value = 2.31 (1 Tailed, alpha = 0.05, df [used] = 5,40) (Actual df = 5,54) | | | | | | |

| Dunnett's Test - Table 2 of 2 | | | | | | No Transformation | |
|-------------------------------|----------------|-------------|----------------------------------|--------------|----------------------------|-------------------|--|
| Ho:Control<Treatment | | | | | | | |
| Group | Identification | Num of Reps | Min Sig Diff (In Orig. Units) | % of Control | Difference From Control | | |
| 1 | Control | 10 | | | | | |
| 2 | 31 % | 10 | 5.754 | 18.9 | 4.1 | | |
| 3 | 41 % | 10 | 5.754 | 18.9 | 4.1 | | |
| 4 | 55 % | 10 | 5.754 | 18.9 | 3.7 | | |
| 5 | 73 % | 10 | 5.754 | 18.9 | 3.8 | | |
| 6 | 97 % | 10 | 5.754 | 18.9 | 6.5 | | |

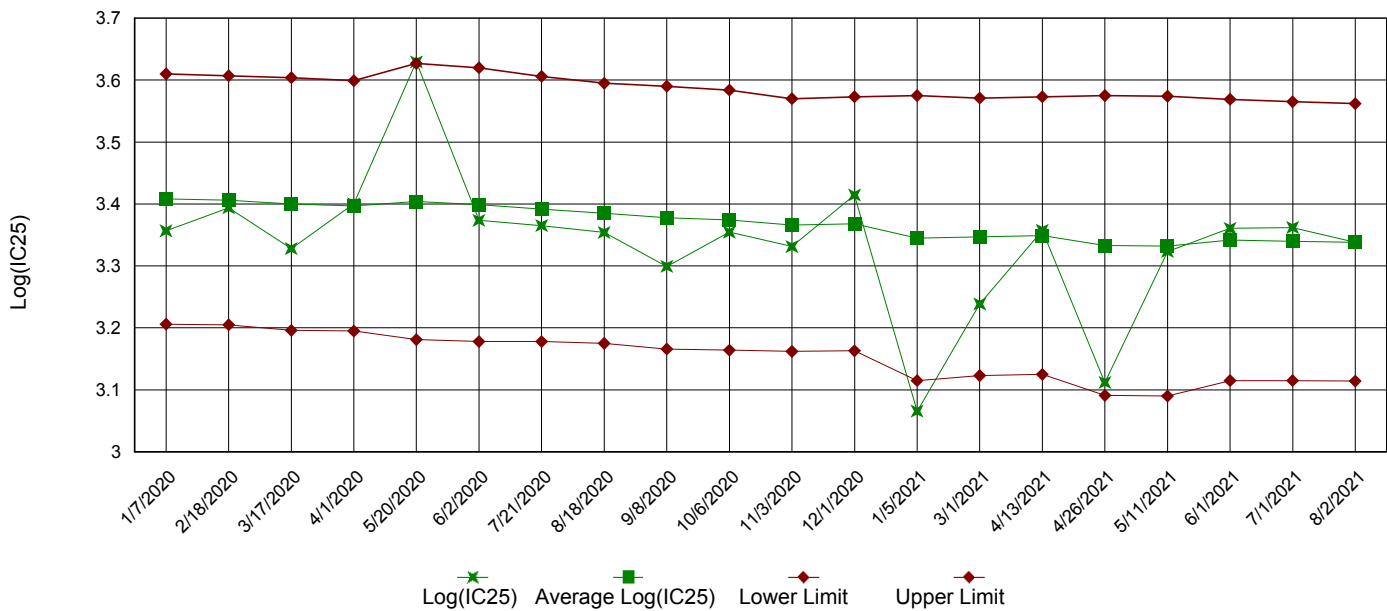
Appendix A3: Test 1000.0

Chronic Reference Toxicant, *Pimephales promelas* (Fathead Minnow)

LC50 Survival Data

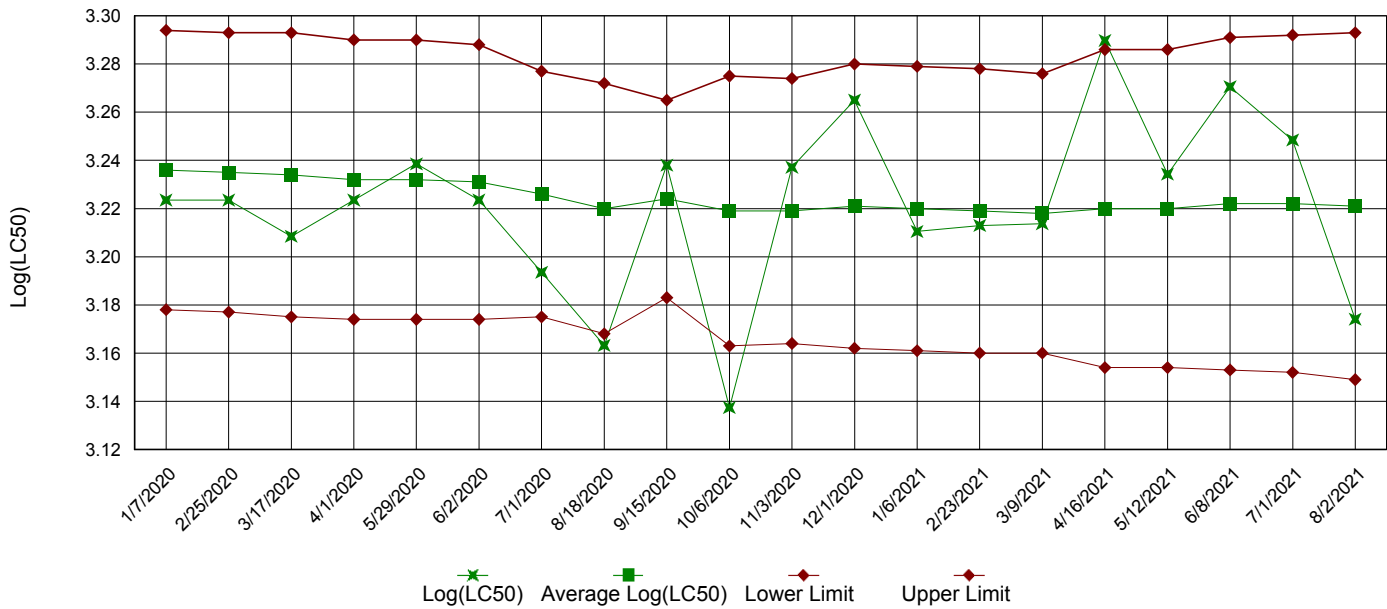


IC25 Growth Data

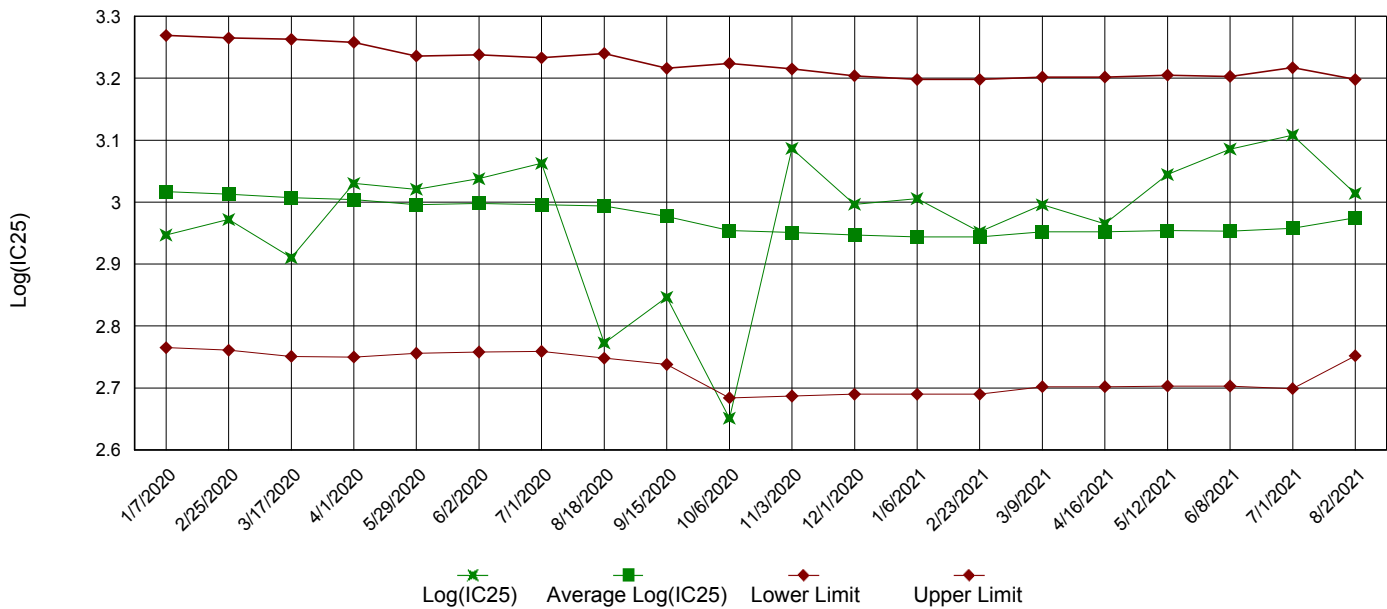


Appendix A3: Test 1002.0
Chronic Reference Toxicant, *Ceriodaphnia dubia*

LC50 Survival Data



IC25 Reproduction Data



Appendix B: Test 1000.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

Permittee: Jacobs

NPDES No.: AR0020010 AFIN 72-00781

Date and Time Test Initiated: August 3, 2021 at 1157

Date and Time Test Terminated: August 10, 2021 at 1138

Dilution water used: Moderately Hard

DATA TABLE FOR SURVIVAL

| Effluent Conc. % | Percent Survival in replicate chambers | | | | | Mean percent survival | | | CV% |
|---------------------|---|------|------|------|------|--------------------------|-------|--------|------|
| | A | B | C | D | E | 24 hr | 48 hr | 7 days | |
| Control | 100 | 100 | 100 | 87.5 | 100 | 100 | 100 | 97.5 | 5.73 |
| 31 % | 50.0 | 37.5 | 87.5 | 100 | 100 | 100 | 100 | 75.0 | 39.1 |
| 41 % | 100 | 62.5 | 75.0 | 50.0 | 100 | 100 | 100 | 77.5 | 28.9 |
| 55 % | 37.5 | 62.5 | 75.0 | 75.0 | 87.5 | 100 | 100 | 67.5 | 28.1 |
| 73 % | 50.0 | 87.5 | 100 | 100 | 100 | 100 | 100 | 87.5 | 24.7 |
| 97 % | 100 | 87.5 | 100 | 87.5 | 100 | 100 | 100 | 95.0 | 7.21 |

DATA TABLE FOR GROWTH

| Effluent Conc. % | Average dry weight, mg replicate chambers | | | | | Mean dry weight, mg | CV% |
|---------------------|--|-------|-------|-------|-------|------------------------|------|
| | A | B | C | D | E | | |
| Control | 0.622 | 0.654 | 0.614 | 0.545 | 0.590 | 0.605 | 6.71 |
| 31 % | 0.248 | 0.250 | 0.500 | 0.592 | 0.618 | 0.442 | 41.0 |
| 41 % | 0.546 | 0.332 | 0.386 | 0.330 | 0.592 | 0.437 | 28.2 |
| 55 % | 0.160 | 0.341 | 0.429 | 0.410 | 0.454 | 0.359 | 33.1 |
| 73 % | 0.338 | 0.458 | 0.611 | 0.594 | 0.635 | 0.527 | 23.9 |
| 97 % | 0.540 | 0.510 | 0.574 | 0.561 | 0.591 | 0.555 | 5.65 |

CV = Coefficient of variation = standard deviation * 100 / mean

Appendix B: Test 1000.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Pimephales promelas (Fathead Minnow)
SURVIVAL AND GROWTH

1. Dunnett's Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

a.) LOW FLOW OR CRITICAL DILUTION X YES NO
b.) 1/2 LOW FLOW DILUTION YES NO

2. Dunnett's Test:

Is the mean dry weight (growth) significantly different ($p=0.05$) than the control's dry weight (growth) for the % effluent corresponding to (significant non-lethal effects):

a.) LOW FLOW OR CRITICAL DILUTION X YES NO
b.) 1/2 LOW FLOW DILUTION YES NO

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 1 (TLP6C)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP6C)
5. NOEC Pimephales Lethality: 41 % (TOP6C)
6. LOEC Pimephales Lethality: 55 % (TXP6C)
7. NOEC Pimephales Sublethality: 41 % (TPP6C)
8. LOEC Pimephales Sublethality: 41 % (TYP6C)
9. Coefficient of variation for Pimephales growth: 6.71 (TQP6C)
10. Sublethality for this test: 41 % (51714 or 51714S)

Appendix B: Test 1000.0
 CHRONIC TOXICITY SUMMARY FORM
Pimephales promelas (Fathead minnow)
 CHEMICAL PARAMETERS CHART

 PERMITTEE: Jacobs
 NPDES NO.: AR0020010 AFIN 72-00781
 CONTACT: Ms. Donna McChristian
 ANALYST: 280, 343, 357, 358

 Test Initiated: DATE: August 3, 2021 TIME: 1157
 Test Terminated: DATE: August 10, 2021 TIME: 1138

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | | | | | | | |
| D.O. Initial | 7.0 | 7.2 | 7.2 | 7.0 | 6.9 | 6.8 | 6.6 |
| Final | 6.6 | 7.0 | 6.4 | 6.5 | 6.4 | 5.8 | 6.1 |
| pH Initial | 7.8 | 7.4 | 7.9 | 7.5 | 8.0 | 7.6 | 7.6 |
| Final | 7.5 | 7.6 | 7.6 | 7.5 | 7.6 | 7.5 | 7.6 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 31 % | | | | | | | |
| D.O. Initial | 7.5 | 6.8 | 7.4 | 6.6 | 7.2 | 6.8 | 6.7 |
| Final | 6.5 | 6.8 | 6.1 | 6.2 | 5.7 | 5.8 | 6.3 |
| pH Initial | 7.8 | 7.5 | 7.8 | 7.5 | 8.0 | 7.6 | 7.7 |
| Final | 7.8 | 7.8 | 7.8 | 7.7 | 7.7 | 7.8 | 7.8 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 41 % | | | | | | | |
| D.O. Initial | 7.6 | 6.6 | 7.6 | 6.9 | 7.3 | 6.8 | 6.8 |
| Final | 6.3 | 6.9 | 6.0 | 5.8 | 5.9 | 5.8 | 6.3 |
| pH Initial | 7.8 | 7.6 | 7.9 | 7.6 | 8.0 | 7.7 | 7.8 |
| Final | 7.8 | 7.9 | 7.8 | 7.7 | 7.7 | 7.8 | 7.8 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 55 % | | | | | | | |
| D.O. Initial | 7.6 | 6.2 | 7.5 | 6.3 | 7.2 | 6.5 | 6.5 |
| Final | 6.4 | 6.8 | 6.3 | 6.2 | 6.5 | 6.0 | 6.2 |
| pH Initial | 7.9 | 7.6 | 7.9 | 7.6 | 8.0 | 7.8 | 7.9 |
| Final | 7.9 | 8.0 | 7.9 | 7.9 | 8.0 | 8.0 | 7.9 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 73 % | | | | | | | |
| D.O. Initial | 7.5 | 5.7 | 7.5 | 6.2 | 7.4 | 6.2 | 6.7 |
| Final | 6.2 | 7.0 | 6.4 | 6.1 | 6.5 | 5.7 | 6.4 |
| pH Initial | 7.8 | 7.6 | 7.9 | 7.6 | 8.0 | 7.8 | 7.9 |
| Final | 7.9 | 8.0 | 8.0 | 7.9 | 8.1 | 8.0 | 8.0 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 97 % | | | | | | | |
| D.O. Initial | 8.0 | 5.8 | 7.8 | 6.0 | 7.5 | 6.2 | 6.4 |
| Final | 6.0 | 6.9 | 6.2 | 6.3 | 6.0 | 5.8 | 6.4 |
| pH Initial | 7.8 | 7.6 | 7.8 | 7.6 | 8.0 | 7.7 | 7.9 |
| Final | 8.0 | 8.1 | 8.1 | 8.0 | 8.1 | 8.1 | 8.2 |

| Alkalinity | Hardness | Conductivity | Chlorine | Sample ID |
|------------|----------|--------------|----------|------------------------|
| 180 | 150 | 670 | <0.05 | Noland WR001 02-AUG-21 |
| 160 | 150 | 690 | <0.05 | Noland WR001 04-AUG-21 |
| 180 | 150 | 710 | <0.05 | Noland WR001 06-AUG-21 |

| Alkalinity | Hardness | Conductivity | Chlorine | Sample ID |
|------------|----------|--------------|----------|-----------|
| 63 | 83 | 330 | <0.05 | 257313-1 |
| 61 | 80 | 340 | <0.05 | 257486-1 |

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

Permittee: Jacobs

NPDES No.: AR0020010 AFIN 72-00781

Date and Time Test Initiated: August 3, 2021 at 1132

Date and Time Test Terminated: August 10, 2021 at 1125

Dilution water used: Moderately Hard

PERCENT SURVIVAL

| Time of Reading | Control | Percent Effluent | | | | |
|-----------------|---------|------------------|------|------|------|------|
| | | 31 % | 41 % | 55 % | 73 % | 97 % |
| 24 hour | 100 | 100 | 100 | 100 | 100 | 100 |
| 48 hour | 100 | 100 | 100 | 100 | 100 | 100 |
| 7 day | 100 | 100 | 100 | 100 | 100 | 90.0 |

NUMBER OF YOUNG PRODUCED PER FEMALE @ 7 DAYS

| Replicates | Control | Percent Effluent | | | | |
|--------------------------|---------|------------------|------|------|------|------|
| | | 31 % | 41 % | 55 % | 73 % | 97 % |
| A | 27 | 24 | 25 | 24 | 29 | 0 |
| B | 22 | 23 | 29 | 32 | 20 | 28 |
| C | 29 | 31 | 26 | 31 | 26 | 26 |
| D | 40 | 29 | 26 | 29 | 30 | 29 |
| E | 34 | 25 | 27 | 17 | 29 | 31 |
| F | 34 | 34 | 27 | 25 | 31 | 27 |
| G | 34 | 20 | 25 | 29 | 21 | 22 |
| H | 35 | 25 | 30 | 26 | 18 | 27 |
| I | 24 | 27 | 20 | 28 | 37 | 25 |
| J | 26 | 26 | 29 | 27 | 26 | 25 |
| Mean per Adult | 30.5 | 26.4 | 26.4 | 26.8 | 26.7 | 24.0 |
| Mean per Surviving Adult | 30.5 | 26.4 | 26.4 | 26.8 | 26.7 | 26.7 |
| CV % | 18.8 | 15.4 | 10.7 | 15.9 | 21.6 | 9.74 |

CV = Coefficient of variation = standard deviation * 100 / mean
(calculated based on young produced by surviving females)

Appendix B: Test 1002.0
SUMMARY REPORTING FORMS
CHRONIC BIOMONITORING
Ceriodaphnia dubia
SURVIVAL AND REPRODUCTION

1. Fisher's Exact Test:

Is the mean survival significantly different ($p=0.05$) than the control survival for the % effluent corresponding to (lethality):

| | | |
|-----------------------------------|-----------------------|----------------------|
| a.) LOW FLOW OR CRITICAL DILUTION | <u> </u> YES | <u> X </u> NO |
| b.) 1/2 LOW FLOW DILUTION | <u> </u> YES | <u> </u> NO |

2. Dunnett's Test:

Is the mean number of young produced per female significantly different ($p=0.05$) than the control's number of young per female for the % effluent corresponding to (significant non-lethal effects):

| | | |
|-----------------------------------|-----------------------|----------------------|
| a.) LOW FLOW OR CRITICAL DILUTION | <u> X </u> YES | <u> </u> NO |
| b.) 1/2 LOW FLOW DILUTION | <u> </u> YES | <u> </u> NO |

3. If you answered NO to 1.a) enter [0] otherwise enter [1]: 0 (TLP3B)
4. If you answered NO to 2.a) enter [0] otherwise enter [1]: 1 (TGP3B)
5. NOEC Ceriodaphnia Lethality: 97 % (TOP3B)
6. LOEC Ceriodaphnia Lethality: 97 % (TXP3B)
7. NOEC Ceriodaphnia Sublethality: 73 % (TPP3B)
8. LOEC Ceriodaphnia Sublethality: 97 % (TYP3B)
9. Coefficient of variation for Ceriodaphnia Reproduction: 18.8 (TQP3B)
10. Sublethality for this test: 73 % (51710 or 51710Q)

Appendix B: Test 1002.0
CHRONIC TOXICITY SUMMARY FORM
Ceriodaphnia dubia
CHEMICAL PARAMETERS CHART

PERMITTEE: Jacobs
NPDES NO.: AR0020010 AFIN 72-00781
CONTACT: Ms. Donna McChristian
ANALYST: 280, 343, 357, 358

Test Initiated: DATE: August 3, 2021 TIME: 1132
Test Terminated: DATE: August 10, 2021 TIME: 1125

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| Control | | | | | | | |
| D.O. Initial | 7.0 | 7.2 | 7.2 | 7.0 | 6.9 | 6.8 | 6.6 |
| Final | 6.9 | 7.2 | 7.0 | 7.2 | 6.9 | 6.7 | 6.7 |
| pH Initial | 7.8 | 7.4 | 7.9 | 7.5 | 8.0 | 7.6 | 7.6 |
| Final | 7.9 | 8.1 | 8.0 | 8.2 | 8.0 | 8.0 | 7.8 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 31 % | | | | | | | |
| D.O. Initial | 7.5 | 6.8 | 7.4 | 6.6 | 7.2 | 6.8 | 6.7 |
| Final | 7.2 | 7.5 | 7.2 | 7.7 | 7.4 | 6.8 | 7.2 |
| pH Initial | 7.8 | 7.5 | 7.8 | 7.5 | 8.0 | 7.6 | 7.7 |
| Final | 8.2 | 8.4 | 8.4 | 8.6 | 8.4 | 8.5 | 8.3 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 41 % | | | | | | | |
| D.O. Initial | 7.6 | 6.6 | 7.6 | 6.9 | 7.3 | 6.8 | 6.8 |
| Final | 7.1 | 7.7 | 7.3 | 7.9 | 7.2 | 6.8 | 7.1 |
| pH Initial | 7.8 | 7.6 | 7.9 | 7.6 | 8.0 | 7.7 | 7.8 |
| Final | 8.3 | 8.5 | 8.5 | 8.6 | 8.5 | 8.5 | 8.5 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 55 % | | | | | | | |
| D.O. Initial | 7.6 | 6.2 | 7.5 | 6.3 | 7.2 | 6.5 | 6.5 |
| Final | 7.1 | 7.5 | 7.2 | 7.4 | 7.0 | 6.8 | 7.0 |
| pH Initial | 7.9 | 7.6 | 7.9 | 7.6 | 8.0 | 7.8 | 7.9 |
| Final | 8.4 | 8.5 | 8.5 | 8.7 | 8.5 | 8.6 | 8.5 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 73 % | | | | | | | |
| D.O. Initial | 7.5 | 5.7 | 7.5 | 6.2 | 7.4 | 6.2 | 6.7 |
| Final | 7.0 | 7.6 | 7.2 | 7.7 | 7.1 | 7.0 | 6.8 |
| pH Initial | 7.8 | 7.6 | 7.9 | 7.6 | 8.0 | 7.8 | 7.9 |
| Final | 8.4 | 8.6 | 8.6 | 8.7 | 8.6 | 8.7 | 8.6 |

| DILUTION | DAY | | | | | | |
|--------------|-----|-----|-----|-----|-----|-----|-----|
| | 1 | 2 | 3 | 4 | 5 | 6 | 7 |
| 97 % | | | | | | | |
| D.O. Initial | 8.0 | 5.8 | 7.8 | 6.0 | 7.5 | 6.2 | 6.4 |
| Final | 7.0 | 7.6 | 7.3 | 7.9 | 7.3 | 7.0 | 7.2 |
| pH Initial | 7.8 | 7.6 | 7.8 | 7.6 | 8.0 | 7.7 | 7.9 |
| Final | 8.5 | 8.7 | 8.7 | 8.8 | 8.7 | 8.8 | 8.7 |

| Alkalinity | Hardness | Conductivity | Chlorine | Sample ID |
|------------|----------|--------------|----------|------------------------|
| 180 | 150 | 670 | <0.05 | Noland WR001 02-AUG-21 |
| 160 | 150 | 690 | <0.05 | Noland WR001 04-AUG-21 |
| 180 | 150 | 710 | <0.05 | Noland WR001 06-AUG-21 |

| Alkalinity | Hardness | Conductivity | Chlorine | Sample ID |
|------------|----------|--------------|----------|-----------|
| 63 | 83 | 330 | <0.05 | 257313-1 |
| 61 | 80 | 340 | <0.05 | 257486-1 |



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 (501) 224-5072

CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 3

| | | | | | | | | | | | | | | | | | | | |
|--|--|---|--|------------------------|--|---------------------|--|--------------------|--|-----------|--|---------|--|--|--|--|--|--|--|
| Client: Jacobs | | Task code: 659031CH.33N.WW.ELD3 | | PO # 148021330 | | NO OF | | ANALYSES REQUESTED | | | | | | | | | | | |
| Project Reference: Fayetteville | | Sample Matrix | | W A T E R | | B O T T L E S | | | | | | | | | | | | | |
| Lab Manager: Donna McChristian | | G R A B | | C O P | | X | | | | | | | | | | | | | |
| Sampled By: <u>Walter Chedor</u> <u>Matt Benton</u> | | Date/Time Collected | | 1400 - 1200 | | 08/01/21 - 08/02/21 | | | | | | | | | | | | | |
| AIC No. 1 | | Sample Identification | | Noland WR001 | | X | | | | | | | | | | | | | |
| Carrier: FED EX | | Received on ice (4 C)? | | YES | | NO | | | | | | | | | | | | | |
| Remarks: | | Chronic C. dubia | | X | | X | | | | | | | | | | | | | |
| Chronic P. promelas | | X | | X | | X | | | | | | | | | | | | | |
| Field pH calibration | | on @ | | Buffer: | | | | | | | | | | | | | | | |
| T = Sodium Thiosulfate | | H = HCl to pH 2 | | B = NaOH to pH 12 | | Z = Zinc acetate | | | | | | | | | | | | | |
| Turnaround Time Requested: (Please circle) | | Relinquished | | Date/Time | | 08/22/20 | | Received | | Date/Time | | 7-23-21 | | | | | | | |
| [NORMAL] OR EXPEDITED # of days = | | By: <u>Matt Benton</u> | | By: <u>Flatt</u> | | | | | | | | | | | | | | | |
| Expedited results requested by: | | Who should AIC contact with questions: Donna McChristi | | Phone: (479) 443-3292 | | | | | | | | | | | | | | | |
| Report Attention to: Donna McChristian, Matt Benton | | Report Address to: 1400 N. Fox Hunter Rd. | | Fayetteville, AR 72701 | | | | | | | | | | | | | | | |
| Comments: | | Please analyze and report samples according to units noted in Chain of Custody. | | 7744 1886 | | 2839 | | | | | | | | | | | | | |



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

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| | | | | | | | | | | | | | |
|---|--|--|--|------------------------|--|---|--|--|--|------------------------|--|------------------------|--|
| Client: Jacobs | | Task code: 659031CHJ3N.WW.ELO3 | | PO # 148021330 | | NO | | ANALYSES REQUESTED | | AIC CONTROL NO: 257535 | | AIC PROPOSAL NO: | |
| Project Reference: Fayetteville | | Sample Matrix | | W | | A | | S | | L | | U | |
| Lab: Donna McChristian | | G | | R | | O | | M | | D | | I | |
| Manager: Donna McChristian | | A | | B | | E | | I | | G | | E | |
| Sampled By: Matt Benton | | C | | O | | M | | P | | L | | E | |
| Date/Time Collected: 08/03/21 - 08/04/21 | | X | | 3 | | X | | X | | X | | X | |
| Sample Identification: Noland WR001 | | NO = none | | V = VOA vials | | N = Nitric acid pH 2 | | H = HCl to pH 2 | | B = NaOH to pH 12 | | T = Sodium Thiosulfate | |
| Container Type: Preservative | | P = Glass | | S = Sulfuric acid pH 2 | | NO = none | | P = Plastic | | NO = NO | | Z = Zinc acetate | |
| Field pH calibration on @ | | Relinquished By: <i>Matt Benton</i> | | Date/Time: 080421 | | Date/Time: 1221 | | Received By: <i>Matt Benton</i> | | Date/Time: 8-5-21 | | Date/Time: 0928 | |
| Buffer: | | Relinquished By: | | Date/Time: | | Date/Time: | | Received By: <i>Matt Benton</i> | | Date/Time: 8-5-21 | | Date/Time: 0928 | |
| Comments: Please analyze and report samples according to units notated in Chain of Custody. | | Who should AIC contact with questions: Donna McChristi | | Phone: (479) 443-3292 | | Report Attention to: Donna McChristian, Matt Benton | | Report Address to: 1400 N. Fox Hunter Rd. Fayetteville, AR 72701 | | 7244 4401 9266 | | FORM 0060 | |



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CHAIN OF CUSTODY / ANALYSIS REQUEST FORM

PAGE 1 OF 1

| | | | | | | | |
|---|--|---------------------------------|--|------------------------------------|--|---------------------------|--|
| Client: Jacobs | | PO # 148021330 | | NO | | ANALYSES REQUESTED | |
| Project Reference: Fayetteville | | Task code: 659031CH-33N.WW/EL03 | | OF | | | |
| Lab: Fayetteville | | Sample Matrix | | B | | | |
| Manager: Donna McChristian | | W A S L U D I E S | | O | | | |
| Sampled By: Walter Chodor, Donna McChristian | | G R A B | | T | | | |
| Date/Time Collected: 1400 - 1200 | | C O M P | | I | | | |
| Sample Identification: Noland WR001 | | X | | E | | | |
| Date/Time Collected: 08/05/21 - 08/06/21 | | X | | S | | | |
| Remarks: Chronic C. dubia | | X | | 3 | | | |
| Remarks: Chronic P. promelas | | X | | | | | |
| Carrier: FED EX | | | | | | | |
| Received on Ice (4 C)? YES NO 0-6 | | | | | | | |
| Field pH calibration on @ | | | | | | | |
| Buffer: | | | | | | | |
| Turnaround Time Requested: (Please circle) [NORMAL] OR EXPEDITED # of days = | | H = HCl to pH 2 | | T = Sodium Thiosulfate | | Date/Time | |
| Expedited results requested by: | | V = VOA vials | | B = NaOH to pH 12 | | Received By: | |
| Who should AIC contact with questions: Donna McChristian | | N = Nitric acid pH 2 | | Date/Time: 080621/1221 | | Date/Time | |
| Report Attention to: Donna McChristian, Matt Benton | | | | Relinquished By: Donna McChristian | | Received By: [Signature] | |
| Report Address to: 1400 N. Fox Hunter Rd. Fayetteville, AR 72701 | | | | Date/Time | | Date/Time: 7/21/21 (0835) | |
| Comments: Please analyze and report samples according to units notated in Chain of Custody. | | | | | | | |